HYDROXYPROPYL-β-CYCLODEXTRIN DURING SOIL REMEDIATION

PERSISTENCE AND EFFECTIVENESS OF HYDROXYPROPYL-β-CYCLODEXTRIN DURING SOIL REMEDIATION

By

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ABSTRACT

Hydroxypropyl-β-cyclodextrin (HPβCD) is receiving increasing interest as an enhancing remediation agent used for soils contaminated with hydrophobic contaminants. While being used in remediation experiments, no previous study has investigated the fate of HPβCD under non-enhanced uncontrolled conditions in field soils. This study assesses the removal efficiency of DDT using 10%-HPβCD solutions within laboratory soil columns, and examines the persistence of HPβCD within uncontrolled un-enhanced field soil conditions. The bench scale soil column study was found to remove 19% and 20% of the initial DDT and DDE masses respectively after ten treatments of 10%-HPβCD solutions applied twice per day for one week. This definitively shows HPβCD does vertically mobilize DDT in the soil profile. Stable carbon isotopic measurements of technical grade HPβCD resulted in an expected $\delta^{13}C$ value of -16‰. The expected stable carbon isotopic value ($\delta^{13}C$) of the initial untreated soils was -24.5‰. The soils treated with 10%- and 20%-HPβCD solutions both indicated an increase in carbon isotopic values during and after the experiment, indicating retention of HPβCD within the soil. Measurements of HPβCD in soils using liquid chromatograph mass spectrometry (LCMS) indicated a smaller persistence of HPβCD within the soils during and shortly after HPβCD applications. The carbon isotopic measurements and LCMS concentration of HPβCD reflected similar short and long-term trends for both increasing and decreasing concentrations. Evidence from both stable carbon isotope analysis and LCMS analysis indicated that HPβCD persisted in the soils six weeks after applications; however, there was no evidence of HPβCD in soils eleven months after the treatments ceased.

ACKNOWLEDGEMENTS

I would like to dedicate this thesis to my parents, Marion and Murray Etherington and Phillip Solakov, without your combined support over the past two years; I don't know how I would have completed this degree. To my advisor Jim Smith, your continuous encouragement and support have been invaluable, you have helped me grow stronger as a researcher and I can honestly say that I am taking away so much more now than when I started: Thank You.

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PREFACE

This work has been written in the following style for the efficient submission of manuscripts to peer reviewed journals of chapters 2, 3, and 4. This style necessitates some repetition between chapters concerning the introduction, background and field site descriptions; however all results are unique.

While receiving significant feedback and assistance in the performance of the research, I am the sole author of this work.

CHAPTER 1
INTRODUCTION

1.1 CYCLODEXTRIN

An enzymatic degradation of starch with the enzyme cyclodextrin glycosyltransferase, (commonly referred to as CGTase) is responsible for the synthesis of cyclodextrins (CDs) (Schmid, 1996). Cyclodextrins are complex polycyclic oligosaccharides composed of several glucopyranose units. Three major cyclodextrins are industrially produced consisting of: eight (α-CD), seven (β-CD) and six (γ-CD) glucopyranose units, β-CD is shown in figure 1.1.

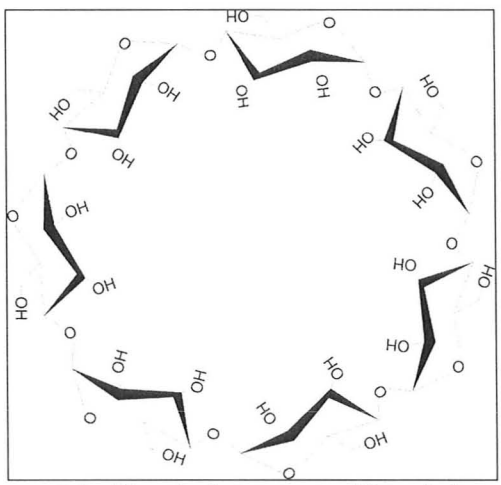

Figure 1.1. Chemical structure of β-cyclodextrin.

These cyclodextrins are often referred to as the parent cyclodextrins (Szejtli, 1998). These parent cyclodextrins have been chemically modified to create a large number of derivatives produced for a wide range of applications including drug carriers, separating agents, catalysts and additives (ie. detergents) (Szejtle, 1998). Some derivatives such as hydroxypropyl-β-cyclodextrin (HPβCD), and randomly methylated β cyclodextrin (RAMEB) are industrially produced by the tons often chosen over the parent CDs for

their improved solubility, interior cavity volume, and increased stability with guest molecules (Szejtle, 1998; Del Valle, 2004). The cyclodextrin ring is shaped as a conical cylinder and is commonly called a "doughnut or wreath shaped truncated cone" (Szejtli, 1998). Hydroxyl groups exist on the interior cavity and hydroxypropyl groups remain on the exterior shell resulting in a very unique structure creating a hydrophilic shell and hydrophobic slightly apolar cavity (Wang, 1993) shown in figure 1.2.

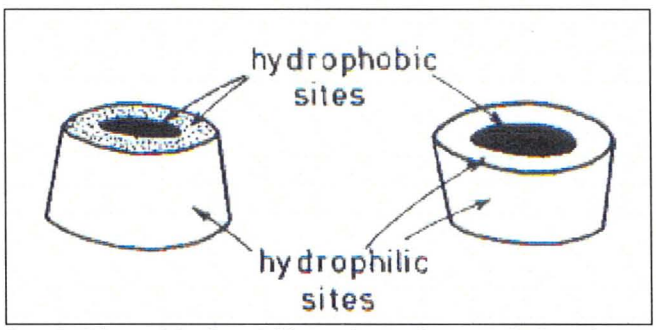

Figure 1.2. The hydrophobic and hydrophilic regions of the cyclodextrin structure a schematic representation (Szejtle, 1998).

All cyclodextrins have an ability to form water soluble inclusion complexes with non-polar, low solubility organic compounds. The cyclodextrin is referred to as the *host* and the molecule contained within the cavity is termed the *guest* molecule. Non-polar guest molecules can form hydrogen bonds or Van der Waal interactions (Szejtli, 1998). In aqueous solution the CD cavity contains water but it is more energetically favourable for cyclodextrins to form inclusion complexes with non-polar molecules than with water molecules, thus creating the most common inclusion complex ratio of 1:1 (guest:host) (Szejtli, 1998). This formation of inclusion complexes is described by a thermodynamic equilibrium given in equation 1 (Wang and Brusseau, 1993; Szejtli, 1998).

$$CD + S \longleftrightarrow CD \bullet S \tag{1.1}$$

Where CD is the uncomplexed host cyclodextrin molecule, S is the free compound and CD•S is the concentration of complexed solutes. When dissolved these compounds reach an equilibrium between the associated and disassociated compounds expressed by the complex stability constant K_a given in equation 1.2.

$$K_{1:1} = \frac{[CD \bullet S]}{[CD][S]} \tag{1.2}$$

Wang and Brusseau (1993) identified a linear partition relationship given by equation 1.3 to represent the solubilization of organic compounds by cyclodextrin.

$$S_t = S_o(1 + K_s C_o) \tag{1.3}$$

Where S_t is the total aqueous-phase concentration of cyclodextrin solution in which both free and complexed species exist, S_o is the aqueous solubility of the compound, K_s is the stability constant described by equation 1.2 also known as a partition coefficient of the compound between cyclodextrin and water, and C_o is the initial or free cyclodextrin concentration. Wang and Brusseau (1993) found that compounds such as trichloroethene, naphthalene, anthracene, chlorobenzene and DDT could be experimentally fit to this linear partition model. The experimental linear fit of HPβCD and DDT is shown in figure 1.3. Furthermore, several studies (Wang & Brusseau, 1993; Brusseau et al., 1994; Wang & Brusseau, 1995; McCray and Brusseau, 1998; Boving et al., 1999, 2000) have found that inclusion complexes formed between hydroxypropyl-β-cyclodextrin (HPβCD) and non-polar organic compounds lead to an increase in solubility of the organic contaminant.

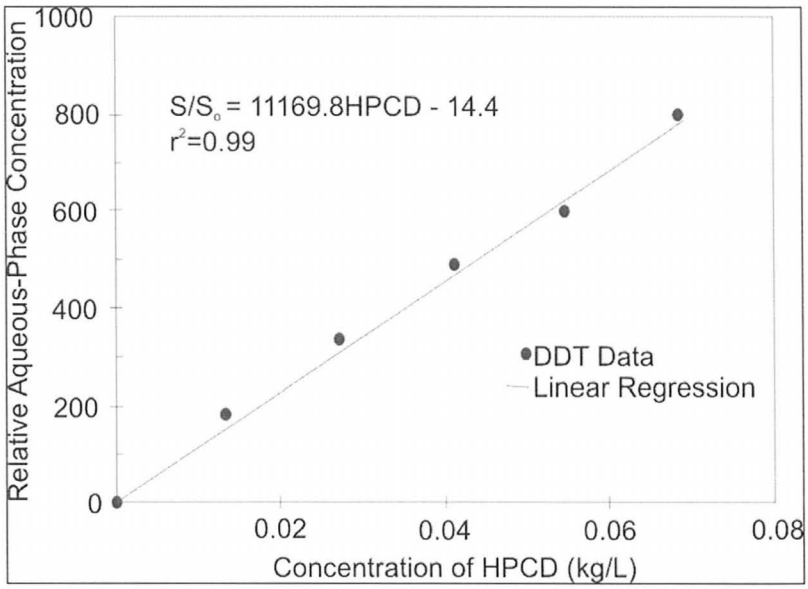

Figure 1.3. Plot of the relative aqueous-phase concentration versus the HPβCD concentration for DDT (Wang and Brusseau, 1998).

Inclusion complexes formed with non-polar compounds increase solubility of the guest molecule and therefore make it more available for transport and biodegradation (Szejtle, 1998; Wang et al., 1998). In many cases the guest molecule is stabilized and has reduced reactivity. Inclusion in the cavity can lead to modified reaction pathways and promote biologic and chemical reactions due to its increased solubility, increased bioavailability, and reduced sorption (Szejtli, 1996; Wang et al., 1998).

Hydroxypropyl-β-cyclodextrin (HPβCD) shown in figure 1.4 is a chemically modified cyclodextrin of the parent β-cyclodextrin molecule. A fully substituted molecule having seven exterior hydroxypropyl groups (DS=7) has a chemical formula of $C_{63}O_{42}H_{112}$. This modified cyclodextrin has an increased solubility from that of β-cyclodextrin and is therefore more useful from an aqueous a transport perspective of included guest molecules. Brusseau et al. (1994) reported no retardation of HPβCD in

saturated porous material and that the retardation of the included organic molecules such as anthracene and pyrene decreased resulting in enhanced transport.

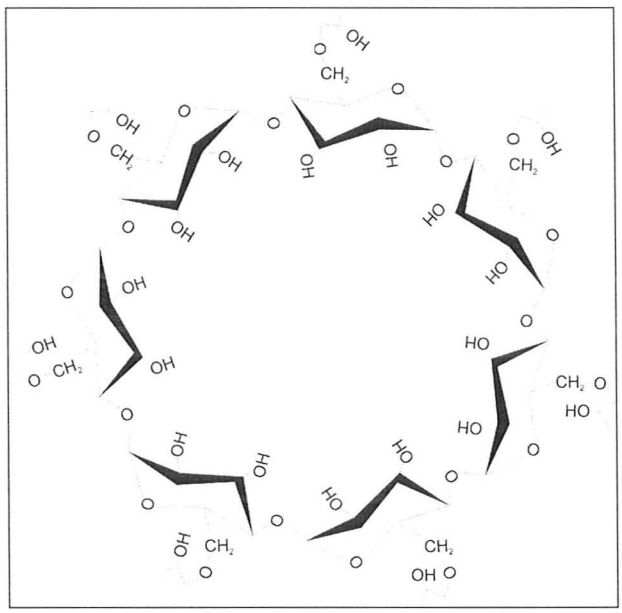

Figure 1.4. Chemical structure of hydroxypropyl-β-cyclodextrin with seven degrees of substitution.

Increasing interest for use of cyclodextrins as an enhancing solubilization agent for porous media contaminated with hydrophobic contaminants has been generated (Wang and Brusseau, 1993; Brusseau et al., 1998; McCray and Brusseau, 1998; Tick et al, 2003; Boving et al, 1999; Vigilanti et al, 2006). Furthermore interest in the enhanced biodegradation of included guest molecules within cyclodextrins have been recently studied (Molnar et al., 2005; Shao et al., 2003; Wang et al., 1998). Laboratory and field studies (Fenyvesi et al., 2005; Verstichel et al, 2004) have monitored cyclodextrin degradation in controlled enhanced soil conditions; however, the persistence of this compound in uncontrolled field soil conditions has not been examined. This study will examine the removal efficiency and persistence of technical grade HPβCD in unsaturated soils contaminated with aged DDT.

1.2. DDT AND DDE

1,1,1-Trichloro-2,2-di(4-chlorophenyl)ethane commonly known as DDT is an organochlorine compound widely used as an insecticide since the 1940s by industrialized countries as an agricultural pest control until it was widely banned in 1972 (Foght et al., 2001). Today DDT is still used in tropical countries to control mosquito populations that can bring malaria and dengue fever to humans. Despite discontinued use, DDT and its toxic derivatives DDE (1,1-dichloro-2,2-bis(p-dichlorodiphenyl)ethylene) and DDD (1,1-dichloro-2,2-bis(p-chlorophenyl)ethane) remain persistent in the environment posing human and animal health risks. Chemical structures of DDT, DDE and DDD are given in figure 1.5.

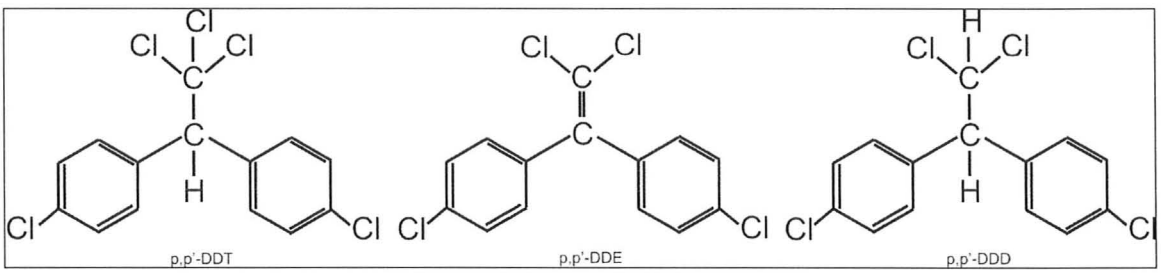

Figure 1.5. Chemical structure of DDT, DDE and DDD (reproduced from Aislabie et al., 1997).

In 1962, Rachel Carson's book *Silent Spring* identified the harmful effects of DDT. She brought attention to the thinning of bird egg-shells and possible carcinogenic properties of DDT leading to public and political debates over DDT use until its ban in the 1970s. In 2004, the World Health Organization reversed its position to support the use of DDT in malaria-endemic countries to combat against malaria until viable, affordable and effective alternatives are available (WHO, 2004).

DDT is recalcitrant, toxic and persistent because of its chlorinated aliphatic and aromatic structure (Corona-Cruz, 1999). Many important chemical properties given in

table 1.1 govern the behaviour of DDT in the environment including low solubility, increased resistance to degradation, low vapour pressure and partitioning into and onto organics. DDT has two common isomers: o,p-DDT and p,p-DDT. Often in the literature, no distinction is made between these isomers and the ∑DDT = o,p-DDT + p,p-DDT is referred to as DDT. The unbalanced electronegativity of the structure results in a non-polar compound that is very hydrophobic and very lipophilic. This nature allows DDT to preferentially accumulate in the fatty tissues of living organisms and sorb to organic matter in the soil and within water column. There are no known acute effects of DDT uptake to humans; however, the chronic or long-term effects remain an important concern (Boul, 1994; Aislabie et al., 1997).

Table 1.1. Chemical Properties of DDT, DDE, DDD. (a. Schwarzenbach, 2003, b. Spencer, c. Stiver, 1990, d. Sigma-Aldrich, 1996a, e. Sigma-Aldrich, 1996b, f. Pontilillo and Eganhouse, 2001, g. De Brujin, 1989, h. Boul, 1994, i. Kan et al., 1998).

	DDT	DDE	DDD
Formula	$C_{14}H_9Cl_5$	$C_{14}H_8Cl_4$	$C_{14}H_{10}Cl_4$
Molecular Weight	354.49	318.03_e	320.05_d
Boiling Point (°C)	260_d	336	350
Melting Point (°C)	109.0_a	89_e	76_d
Vapour Pressure (mmHG)	$55.3x10^{-7}{}_h$	$69.4x10^{-7}{}_h$	$10.2x10^{-7}{}_h$
Solubility (mg/L @25°C)	$0.00012\text{-}6.21_f$	$0.001\text{-}1.24_f$	
Log K_{ow}	$4.89\text{-}6.91_f$	$4.28\text{-}6.96_f$	6.22_g
Log K_{oc}	$4.58\text{-}4.84_i$		

The daughter products of DDT are formed by different transformation mechanisms. DDE is formed by abiotic photolysis and biotic microbial transformation by dehydrochlorination reactions occurring in aerobic environments. DDD is formed by

abiotic and biotic reductive dechlorination of DDT occurring in reducing anaerobic environments (Schwarzenbach et al., 2003; Foght et al., 2001).

DDT and its residues can strongly adsorb to the surface of soil particles and organic matter. DDT holds a slightly negative charge due to three Cl^- atoms, and it is attracted to the positively charged surfaces of clay minerals. Van der Waals forces have been identified as the major mechanism that holds DDT to positively charged mineral surfaces (Champion and Olsen, 1971; Gevao, 2000). DDT breaks down following first order kinetics, however, these kinetic rates can be impacted by adsorption, degradation and loss mechanisms (Boul, 1994). Nash and Woolson (1967) reported a mean half life of 10.5 years with a large time range of 2.5 - 35 years depending on the soil type and conditions. Losses of DDT from the soil are attributed to volatization, chemical decomposition, photodegradation, biological metabolism or removal by adsorption. Boul, (1994) report that DDT half-lives range from 2-10 years in temperate soils, and less than 1 year in tropical soils suggesting that both temperature and soil conditions can affect the loss of DDT from the soil. Warm temperatures and increased precipitation levels can cause flooding and reducing environments that promote an increase in biologic activity leading to biologically mediated reductive dechlorination of DDT to DDD (Aislabie et al., 1997). This biotransformation is co-metabolic and requires an alternative carbon source (Guenzi and Beard, 1967; Aislabie et al., 1997).

1.3 FIELD SITE: POINT PELEE NATIONAL PARK, CANADA

The soils at Point Pelee National Park, Canada (PPNP) have been contaminated with DDT and its derivatives DDE and DDE. The park was created for its biologic values in May of 1918 (Crowe and Smith, 2007). DDT was widely used from 1948-1970 to spray roadways, campgrounds, orchards and picnic areas for mosquito control until 1967 (Crowe and Smith, 2007). High levels of DDT found in the Park's amphibians and reptiles were traced to the DDT within the shallow surface soils of the Park (Crowe and Smith, 1997). Investigation of DDT, DDE and DDD within the soils of the park (Marenco, 2000; Badley, 2003; Mironov, 2004) revealed the highest concentrations in the former agricultural areas of the Park. DDT and DDE were found to comprise 58.5% and 36.9% of the ΣDDT present (Crowe and Smith, 2007).

Badley (2003) designed and implemented a remediation experiment using applications of technical grade HPβCD (DS=6.5) to remove DDT, DDE and DDD from surface soils within the former orchard area at PPNP. The remediation experiment consisted of a 3m x 3m square grid subdivided into nine treatment plots with dimensions of .49m x .49m x .15m. The average soil porosity was 0.49 and had an average bulk density of 1.0g/cm^3. There were two treatment solutions of HPβCD: three plots received 10%-HPβCD solution, three received 20%-HPβCD solutions and three control plots received no HPβCD treatment. One pore volume (33L) of HPβCD treatment solution was applied weekly for thirteen and nineteen weeks for the 20%- and 10%-HPβCD treatment plots, respectively. The 20%-HPβCD applications were discontinued after thirteen weeks (thirteen pore volumes) due to a reduced infiltration rate.

Soil samples were removed from the grid prior to the next weekly application of the HPβCD solutions. The upper thatch layer of the treatment plots was removed (2-3cm), and samples were collected from a depth of 2-7.5cm below ground surface. Samples were removed using a chromed steel garden trowel rinsed with 50:50 acetone:hexane solution prior to each sampling. Removed soil samples were promptly stored in amber glass jars, with aluminum foil between the sample and the lid. Sticks, large roots and leaves were removed from the sample as it was placed into the jar. All samples were placed in a cooler until return to the laboratory and then stored in the refrigerator until analysis for DDT, DDE and DDD.

The 20%-HPβCD treatments successfully resulted in a decrease of 90%, 77%, and 82% of the initial DDT, DDE, and DDD present in the soils. The 20%-HPβCD treatments resulted in a decrease of 90%, 74%, and 73% of the initial DDT, DDE and DDD masses, respectively. After approximately ten pore volumes of treatment the treatment removal reached a tailing effect, by which no further DDT, or DDE was removed from the soils.

Fundamental changes in soil properties resulted from the applications of the HPβCD solutions. An increase in organic matter content measured by loss on ignition found that the 20%-treated soils had an organic matter content of 19.9%, the 10%-treated soils was 17.4% in comparison with the control plots with an organic matter content of 7.05%. In addition, a continuous increase in the in-situ soil moisture content was observed measured with TDR (time domain reflectometry). This increase remained even after the 20%-HPβCD treatments discontinued. A decrease in the infiltration rate

accompanied the increase in moisture, accounted for by a decrease in the in-situ field saturated hydraulic conductivity. DAPI staining showed an increase in the number of bacterial cells within the treatment plots.

In 2003, Mironov (2004) sampled soils from the remediation grid to assess long term effectiveness of HPβCD as a remediation agent. This study sampled for DDT, DDE, DDD concentrations and measures of organic matter eleven months after the treatment discontinued. Using log transformed data from 2002 and 2003; the DDT and DDE were found to be reduced by 74% and 64% respectively. The organic matter content was found to be consistent with control soils. There was no analysis of HPβCD during the works of Badley (2003) and Mironov (2004), thus, the presence and persistence of HPβCD within the soils remains unknown.

1.4 OBJECTIVES AND HYPOTHESIS

The primary purpose of this study is to examine the efficiency and persistence of HPβCD within the soils of Point Pelee National Park, Canada. This topic is largely overlooked within the literature for its remediation use in natural environmental systems. An objective of this study was to use laboratory columns to assess the vertical mobilization of DDT from soils from Point Pelee National Park using a 10%-HPβCD solution applied twice per day for one week. It was expected that similar removal efficiency of DDT and DDE would be observed using shorter time periods in comparison with field results by Badley (2003) and Mironov (2004).

A second objective was to assess the persistence of HPβCD within soils from a field remediation experiment. To assess the persistence of HPβCD in the soils from a remediation experiment (Badley, 2003) two methods were used: stable carbon isotopic analysis as a measure of the contribution of HPβCD to the overall organic carbon in the soils, and liquid chromatography mass spectrometry to measure the molecular concentration of HPβCD in soils. It was expected that the stable carbon ($\delta^{13}C$) isotopic values of HPβCD and the natural soil at PPNP would reflect those of C_4 and C_3 vegetation carbon isotopic signatures ($\delta^{13}C$) respectively. It was anticipated that the stable carbon isotopic signature of the treated soils would increase (reflecting an increase in carbon-13) in response to the applications of HPβCD. The concentrations of HPßCD in the soil measured by liquid chromatography mass spectrometry were expected to identify that HPβCD was retained in the soil after applications in measurable concentrations. It was anticipated that concentrations of the technical grade HPβCD molecules comprised of different degrees of substitution (DS) would reveal a preference of removal of a particular degree of substitution over another.

CHAPTER 2
DDT REMOVAL FROM SOILS USING 10% HYDROXYPROPYL-β-CYCLODEXTRIN: A BENCH SCALE TREATABILITY STUDY

2.1 ABSTRACT

The soils at Point Pelee National Park, Canada have been contaminated with DDT and its daughter products DDE and DDD since its widespread use began in 1948. DDT use to control pest and mosquito populations in the Park discontinued in 1970. A bench scale soil column study was used to test if a short term use of 10%-hydroxypropyl-β-cyclodexterin (HPβCD) would remove DDT and DDE as effectively as a pilot-scale in-situ field remediation project reported by Badley (2003). The bench scale study was found to remove 19% and 20% of DDT and DDE masses respectively after 10 treatments of one pore volume per treatment. Therefore, while this study showed treatment with 10% HPβCD to be an effective remediation agent, the lab method was not as effective at leaching DDT and DDE from the soil as the same treatment extended over a longer period under field conditions which removed 90% and 74% of DDT and DDE respectively. The results of this study indicate that time between applications, stimulated microbial activity, and other environmental factors combined to apparently play first order roles during the field soil remediation study conducted by Badley (2003).

2.2 INTRODUCTION

DDT (dichloro-diphenyl-trichloroethane) (figure 2.1) and its daughter products DDE (dichloro-diphenyl-dichloroethylene), and DDD (dichloro-diphenyl-dichloroethane) (figure 2.2) have long been present in the soil at Point Pelee National Park, Ontario, Canada. DDT was applied at Point Pelee National Park from 1948 to 1970 to control

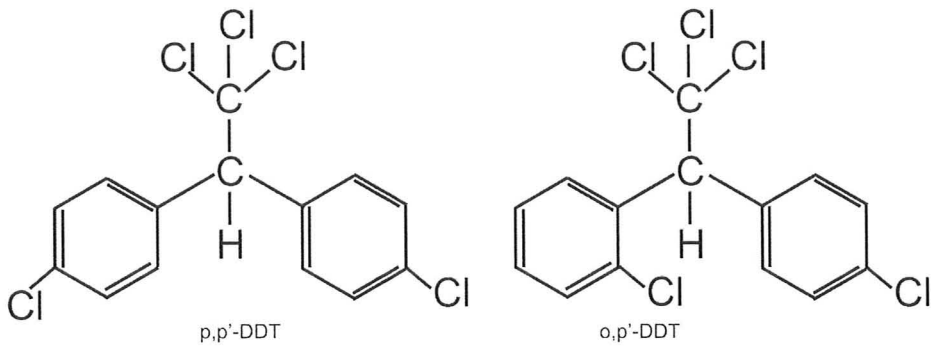

Figure 2.1. The chemical structures of DDT (reproduced from Aislabie et al., 1997).

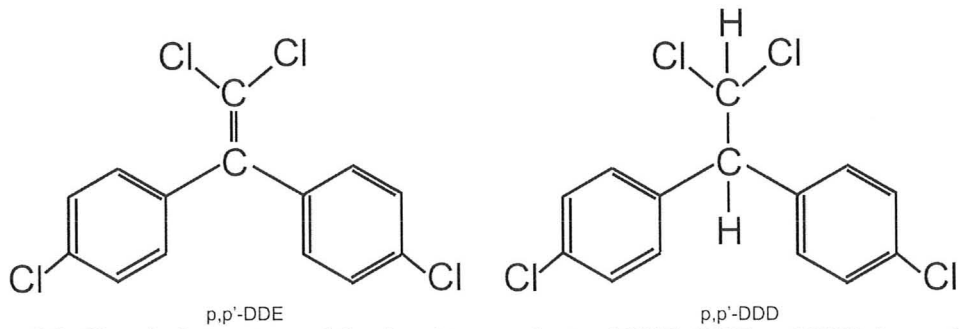

Figure 2.2. Chemical structure of the daughter products of DDT: DDE and DDD (reproduced Aislabie et al., 1997).

mosquitoes in recreational areas and pests in agricultural areas (Crowe and Smith, 2007). Previous studies by Marenco (2002) and Crowe (2007) have identified the high concentrations and variability of DDT and its daughter product persisting in the soils. Studies by Badley (2003) and Mironov (2004) at Point Pelee found that application of aqueous solutions of technical grade hydroxypropyl-β-cyclodextrin (HPβCD) (figure 2.3)

were effective for the removal of DDT, DDE, and DDD from the upper soil layer, i.e. Ah-horizon.

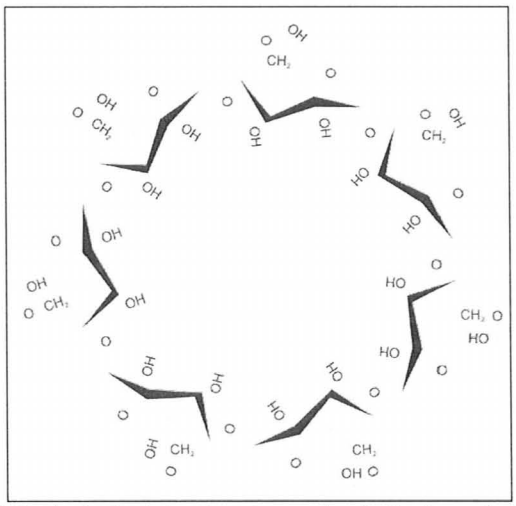

Figure 2.3. Hydroxypropyl-β-Cyclodextrin structure. Analytical grade HPβCD is shown, technical grade HPβCD may contain less hydroxyproyl groups on the exterior of the ring.

Cyclodextrins are formed by the enzymatic degradation of corn starch. They are conical in shape and have a hydrophobic inner cavity and a hydrophilic outer shell. It is known to form inclusion complexes and linearly enhance the solubility of non-polar compounds (Wang and Brusseau, 1993) (figure 2.4).

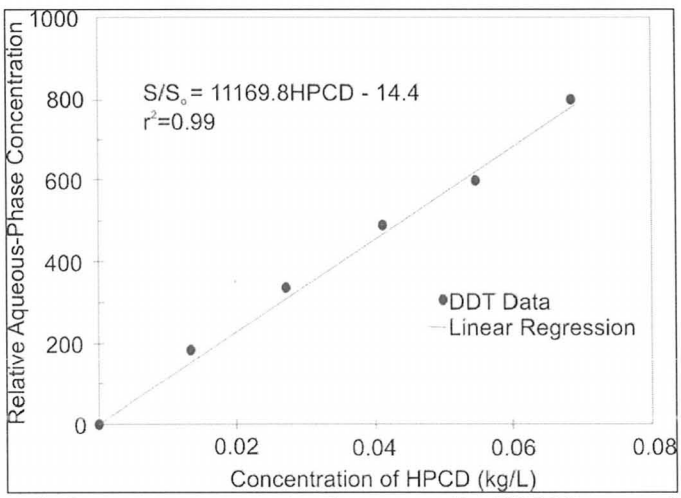

Figure 2.4. The relative aqueous concentration versus the HPβCD concentration for DDT (reproduced from Wang and Brusseau, 1993).

2.3 BACKGROUND

2.3.1 *DDT AND ITS DERIVATIVES*

DDT is recalcitrant, toxic and persistent because of its chlorinated aliphatic and aromatic structures (figure 2.2) (Corona-Cruz, 1999). Many important chemical properties govern the behaviour of DDT in the environment including low solubility, low vapour pressure, and partitioning into and onto organics (soil organic matter), all resulting in an increased resistance to degradation.

DDT was applied to many agricultural fields as a pest control. Due to its xenobiotic nature and persistence in the environment, many surface soils in Canada and the United States are still contaminated by this pesticide even though applications essentially discontinued after a ban in 1972. DDT usage discontinued in Point Pelee National Park between 1969 and 1970 (Crowe and Smith, 2007). The Canadian Environmental Quality Guidelines state that DDT must be below 0.7 μg/g in both agricultural fields and recreational/parklands (CCME, 2006). Guenzi and Beard (1967) found that DDT remained in the upper 3cm of all soils regardless of the volume of water applied in an effort to observe the effects of rainfall and fluctuations in the soil moisture content.

DDE and DDD are the transformation products or residues of DDT and are also toxic compounds. Their structures are similar to DDT however; they have one less chlorine atom as shown in figure 2.2. DDE contains double bonded carbon atoms, making this structure less susceptible to attack by degradation mechanisms. DDD is known to be less persistent in the environment than DDE as it is more susceptible to

microbial attack in reducing environments (Boul, 1994). DDD is formed by abiotic and biotic reductive dechlorination of DDT. DDE is formed by abiotic photolysis and biotic microbial transformation of DDT by dehydrodechlorination reactions (Foght et al., 2001).

The ageing of the soil and remaining DDT is a concern due to its recalcitrant properties. Unaided, these aged residues cannot be desorbed from the soil surfaces or organic matter. Evidence has been found to suggest that over time these residues can become biologically resistant and cannot be further degraded or extracted for analytical purposes (Gevao et al., 2000). Losses of DDT from the soil are attributed to volatization, chemical decomposition, photodegradation, biological metabolism or removal by adsorption. Nash and Woolson (1967) reported a mean half life of 10.5 years with a large time range of 2.5 - 35 years depending on the soil type and conditions. Boul (1994) reported that DDT half-lives range from 2-10 years in temperate soils, and less than 1 year in tropical soils suggesting that both temperature and soil conditions can affect the loss of DDT from the soil. The half life of DDT in Point Pelee soils has been reported to be 25-30 years (Crowe and Smith, 2007).

2.3.2 *CYCLODEXTRIN*

An enzymatic degradation of starch with the enzyme cyclodextrin glycosyltransferase, (commonly referred to as CGTase) is responsible for the synthesis of cyclodextrins. Cyclodextrins are macrocycles composed of several glucopyranose units to form a conical ring shape. Three major cyclodextrins are industrially produced and are often called the parent CDs: α-CD, β-CD, γ-CD. Hydroxyl groups in the interior cavity

and hydroxypropyl groups on the exterior result in a very unique structure creating a hydrophilic shell and hydrophobic apolar cavity (Wang, 1993). Hydroxypropyl-Beta-Cyclodextrin (HPβCD) has 7 glucopyranose units as shown in figure 2.3. All cyclodextrins have the ability to form water soluble inclusion complexes with non-polar, low solubility organic compounds. Several studies (Wang & Brusseau, 1993; Brusseau et al., 1994; Wang & Brusseau, 1995; Wang et al., 1999; Boving et al., 1999, 2000) have found that inclusion complexes (1:1) formed between HPβCD and non-polar organic compounds lead to an increase in solubility of organic contaminants. Using a linear partitioning model Wang and Brusseau (1998) were able to fit experimental data of the relative aqueous-phase concentration (S_t/S_o) of DDT plotted against the concentration of HPβCD, shown in figure 2.4. S_t is the total aqueous phase of the complexed and free solute, and S_o is the aqueous solubility of the free solute. This is evidence of the increased solubility of DDT in the presence of HPβCD solution. Further work by Brusseau et al. (1998) indicated that HPβCD did not interact with saturated porous materials and therefore showed no retardation and behaved as a conservative solute during transport.

2.4 *Field Location: Point Pelee National Park*

DDT was applied to recreational areas and apple orchards of Point Pelee National Park (PPNP) from 1948 to 1967 for pesticide control (Crowe et al., 2002). Marenco (2001) collected soil samples from areas including the former orchard area at Point Pelee National Park and observed a high degree of variation in DDT concentrations. This study found ranges of up to 100μg/g within the Park. Crowe and Smith (2007) report the

highest concentrations of DDT and DDE are within the former agricultural areas of the Park. It was also found that DDT and DDE were the most dominant of the DDT compounds present. The former orchard area is shown by the arrow in figure 2.5. At this same location, Badley (2003) successfully designed and implemented a 20 week pilot-scale remediation study using technical grade HPβCD. The design consisted of three control plots, three 10%-, and three 20%- HPβCD solution plots to move DDT, DDE and DDD concentrations away from the surface soils and into the deeper subsurface.

The grid was a random Latin squares design and is show below in figures 2.6a and 2.6b. One pore volume of 10% HPβCD solution (33L) was applied to each of three plots once per week for 19 weeks, and a 20% solution (33L) was applied to each of three additional plots for 13 weeks. The study successfully decreased the DDT and DDE concentrations by 90% and 74% respectively within the 10%-HPβCD treatment plots of over the course of four months.

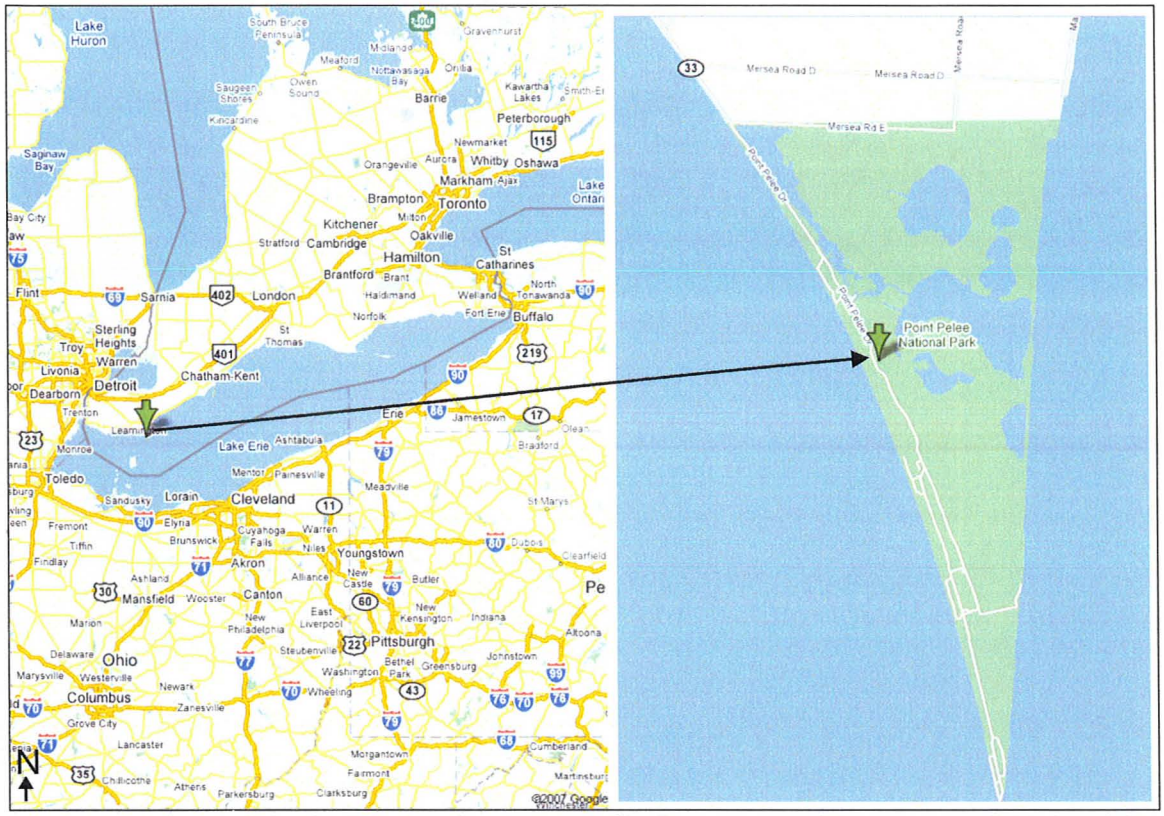

Figure 2.5. Location of Point Pelee National Park (left). Image to the right shows the field site of the remediation plots. (http://maps.google.ca)

20-1	10-1	20-2
20-3	C1	10-2
10-3	C2	C3

Figure 2.6. a. Design of the remediation grid implemented by Badley (2003) and re-sampled by Mironov (2004). The grid consisted of a 3m x 3m plot of soil subdivided into 1m x 1m squares; 20%-, 10% HPβCD solutions were applied 1 pore volume per week for 20 weeks. Control plots received no HPβCD applications. Note: figure b (left) is rotated 180°.

Additional soil samples and ground water samples were collected by Mironov (2004) from the same treatment plots and below the treatment plot grid. No statistically significant change was observed in DDT, DDE and DDD concentrations in the treatment plot soils from the values observed at the end of the study by Badley (2003), thereby indicating no additional losses. Concentrations of DDT in groundwater samples taken in 2003 and 2004 from below the treatment plots were 10-100 times above background levels, however, this was insufficient DDT mass to account for the total mass removed by the HPβCD treatments in 2003 (Mironov, 2004; Mills, 2004).

2.5 OBJECTIVES

1. To determine the effectiveness of the applications of ten pore-volumes of 10% HPβCD solution over a five days period to remove DDT and DDE from the soil in laboratory column treatability trials.
2. To assess if mobilization (translocation) was the primary mechanism of DDT and DDE removal from the upper soil horizon during treatment with 10% HPβCD in the field trials reported by Badley (2003).

2.6 HYPOTHESIS

1. Time between HPβCD applications will have no effect on the mass of DDT and DDE removed from the Ah horizon soil such that similar results will be obtained in laboratory columns over 5 days as field trials receiving similar treatment over 4 months.
2. Vertical mobilization by HPβCD applications to the soil is the primary mechanism for the decline in the DDT and DDE concentrations in Ah horizon soil.

2.7 METHODS

2.7.1 *FIELD METHODS: SOIL SAMPLING*

A plot 3m x 3m bordering the Northern edge of the Badley (2003) remediation plot was selected for removal of the Ah horizon. The UTM coordinates of NE border of the $9m^2$ plot was UTM: 17V 373418m E 4646532m N) (NAD 83). This area was selected as the soil was untreated and untouched by HPβCD. On October 17, 2006, the upper grass layer was removed and a 15cm depth of the Ah soil layer was removed and sifted through a ¼" sieve on site by a Parks Canada archaeologist as required. Samples removed were placed into 20L pails, which were immediately loosely covered with aluminum foil and transported back to McMaster University.

2.7.2 *COLUMN SETUP*

A bench scale column experiment was set up in the Soils Laboratory at McMaster Univeristy, shown in figure 2.7. Six columns were used: three columns received 10%-HPβCD treatment, and three columns received treatment of distilled water. The columns had a radius of 2.2cm and the top of the column was left open to the atmosphere. A larger rubber stopper with a drainage hole was inserted into the bottom opening of the column to allow for solution collection. Above the bottom rubber stopper a large metal mesh and a fine screen mesh were inserted to ensure no soil loss through the drainage hole.

Figure 2.7. Bench scale column setup for soils.

Soil from one 20L sample pail of Ah soil from Point Pelee was mixed with a shovel and a large mechanical splitter was used to fill twelve bags with 290g to 325g of soil each. Of these twelve bags, six were randomly chosen for use in the column study.

The Ah horizon of the soil at the sampling location in Point Pelee is 15cm. The volume of columns was 228cm^3. Badley (2002) reported an average bulk density of 0.97g/cm^3, therefore a bulk density of 1.0g/cm^3 was chosen for the column study to mimic the field soil physical properties. Columns were each packed with approximately 288g of soil to reach the 15cm height in order to achieve the target bulk density which had a porosity 0.52. Once the columns were packed they were covered with tinfoil to reduce exposure to light. A retort stand held an application funnel with tubing attached for flow control above each column.

2.7.3 *Solution Application*

Badley (2003) observed a tailing effect with no further decreases in DDT concentrations after 10 pore volumes of HPβCD solutions were applied. Consequently, in this study a total of 10 applications of one pore volume of 10%-HPβCD were applied

to each of three of the columns in the lab bench study. One pore volume (120ml) of 10%-HPβCD solution (w/w) was applied to each column at an approximate rate of 6ml/min. The three remaining columns received 10 applications of one pore volume of DI water.

2.7.4 *DDT ANALYSIS*

Column effluent from each treatment was collected in pre-rinsed (DCM) amber glass jars. Once the effluent was collected the jar was sealed and stored in the refrigerator at 4°C. At the end of the treatment period, each column had 10 glass sample jars containing samples of effluent solution containing either: 1. DDT, DDE and HPβCD or 2. DDT, DDE and water. These samples were then extracted for p,p'- and o,p'-DDT and p,p'- and o,p'-DDE analysis using the method described in section 5.5.6 below. In addition to this method for liquid effluent, the soil columns were disassembled at the end of the experiment. The soils from each column were homogenized and air dried in aluminum dishes, lightly covered with aluminum foil, to allow excess moisture to evaporate. Air dried soil from each column was removed from the aluminum dishes and placed into an amber glass sample jar that had been pre-rinsed with DCM. Soils were extracted for DDT and DDE using the method discussed in section 2.6.6 to analyze for p,p'-DDT and p,p'-DDE.

2.7.5 *Liquid-Liquid Extraction*

The liquid-liquid extraction used for solutions collected from the drainage port of each column is based on EPA method 3510C: Separatory Funnel Liquid-Liquid Extraction. The surrogate standards used were decachlorobiphenyl and 2,4,5,6-tetrachloro-M-xylene. Samples were filtered through No. 4 Watman filter papers filled with ~15g of anhydrous sodium sulphate. Extract solution was collected into an amber jar, tightly sealed and stored in the refrigerator until the evaporation stage. Based on the recommendation by the analyst, due to the large volume of the liquid samples, a Büchi Rotovapour was used to evaporate samples followed by the suggested Nitrogen blow down technique. Liquid samples were transferred into round bottom flasks which were placed onto the sample flask evaporation area; the instrument was then lowered into a 40°C water bath and set to rotate at level 7. The instrument was run until the liquid samples reached the volume of ~20 ml. The rest of the liquid sample was blow down to 5ml using the nitrogen blow down technique described by EPA method 3510C.

2.7.6 *Soil Extraction*

The DDT and DDE in the soil from each column was determined by solid phase extraction using a hexane:acetone (1:1) solution. A Mars 230/60 microwave was used for Microwave digestion to extract DDT and DDE from the soil. A weight of 1-2g of sample added to the vessel, each sample was spiked with 400µl of surrogate standard solution and then filled with 30ml of hexane:acetone, a magnetic stir bar was placed into each vessel before sealing them. Seven sealed Green Chem sample vessels per run were then

placed into the microwave. Using a 100% of 1200W power; the temperature was ramped to 115°C over 10 minutes and held for 15 minutes. The stir bar was set to low and Psi was 180. Once samples cooled, the samples were filtered through Watman #4 filter papers containing approximately 15g of anhydrous sodium sulphate into a 250ml round bottom flask. Similar to section 2.6.5, liquid extracts were evaporated down using a Büchi Rotovapour to approximately 20ml and then further reduced to 5ml using nitrogen blow down.

Concentrations measured from soils and effluents using the GCMS were summed together to report a total DDT and DDE concentration described by Equations 2.1 and 2.2. Thus, each concentration of "total" DDT and DDE was calculated using the sum of the o,p' and p,p' isomeric forms.

$$DDT = o,p'\text{–}DDT + p,p'\text{–}DDT \tag{2.1}$$

$$DDE = o,p'\text{–}DDE + p,p'\text{–}DDE \tag{2.2}$$

The masses of DDT and DDE measured in the soils were calculated using equation 2.3:

$$M_{DDX} = C_{DDX} \times M_S \tag{2.3}$$

where M_{DDX} is the mass of DDT or DDE in the soil (μg), C_{DDX} is the concentration of DDT or DDE measured (μg/g), and M_s is the mass of soil used to pack the column (g) to a bulk density of 1.0g/cm^3 and to a height of 15cm in the column with a radius of 2.2cm.

2.7.7 *GC/MS Analysis*

Once all samples were evaporated down to 5ml, 1ml of the liquid sample was removed and placed into a GC vial in preparation for analysis using a Hewlett Packard HP 6890 Series GC System with a Hewlett Packard 5973 Mass Selective Detector. EPA method 8081A for semivolatile organic compounds by GC/MS was followed for DDT and DDE analysis. Using an injection volume of 1μL, the front inlet was run in splitless mode with a purge flow of 40mL/min and a purge time of 1 minute. The oven was ramped to a temperature of 300°C and held for 5 minutes. Helium gas was used to flow through the GC column HP-5MS. Sample was run in constant flow mode with a flow rate of 1.2mL/min through a column length of 30m with a 250μm diameter and a .25m thickness. The transfer line between the GC and the Mass Spectrometer was maintained at a temperature of 300°C. The mass spectrometer used a scan acquisition mode and a 6 minute solvent delay. The scan parameters were a low mass of 40 and high mass of 550. The MS quad zone was 150°C and the source zone was 230°C.

DDT and DDE concentrations measured from the effluent liquid samples after each treatment provide values of contaminant removal per treatment. DDT and DDE concentrations measured from the soil samples from the destroyed columns provide end-time DDT and DDE concentrations in the soils after HPβCD treatment.

2.8 RESULTS AND DISCUSSION

2.8.1 *CONCENTRATION OF DDT AND DDE*

The extractions from both soil and liquid samples collected from the column experiment were analyzed for p,p'-DDT, o,p'-DDT, p,p'-DDE, and o,p'-DDE. Both isomers were present for all samples analyzed; however, p,p'-DDT and p,p'-DDE were consistently found in higher concentration than the o,p'-isomers for both DDT and DDE. Similar findings were reported by Badley (2003) from the Point Pelee remediation experiment field soils. The sum of the o,p'- and p,p'- isomeric forms of both DDT and DDE have been summed using equations 2.1 and 2.2 to provide values of total DDT and DDE respectively for the remaining discussion.

Columns that only received applications of water, did not show significant DDT or DDE removal; the concentrations within the water samples collected were less than the limit of detection (30ng/g). This indicates that the soils treated with water are no different than those not treated with water applications. Thus, the soils treated with water are an example of persistent recalcitrant DDT present in the soils.

Aqueous solutions of effluent containing 10%-HPβCD solution along with DDT and DDE were collected after each pore volume of HPβCD solution was applied. The concentrations represent the amount of DDT and DDE removed from the columns after each application. Table 2.1 gives the concentrations removed per each application of HPβCD. Each pore volume applied removed a similar amount of DDT and DDE, i.e. an average of 0.3μg/g of DDT and 0.2μg/g of DDE was removed per treatment with a variance of $0.00729(\mu g/g)^2$ for DDT and $0.0049(\mu g/g)^2$ for DDE.

Table 2.1. Concentrations of DDT and DDE measured by liquid-liquid extraction after each application of 10%-HPβCD.

Application No:	DDE Average (µg/g soil)	DDT Average (µg/g soil)
1	0.4	0.5
2	0.3	0.4
3	0.3	0.3
4	0.2	0.3
5	0.2	0.3
6	0.2	0.3
7	0.2	0.3
8	0.2	0.3
9	0.2	0.2
10	0.2	0.2
Average	**0.2**	**0.3**
Variance $(\mu g/g)^2$	0.0049	0.0072

Using untreated soils from the remaining soil splits not used to pack the columns , the arithmetical mean of the initial soil concentrations (C_i) of DDT was 6.2µg/g with a variance of 3.1$(\mu g/g)^2$ and for DDE was 3.9µg/g with a variance of 1.8$(\mu g/g)^2$. The final soil concentrations in the columns measured at the end of the study were 5.0µg/g ± 1.2 and 2.6µg/g ± 0.6 for DDT and DDE respectively. The average initial DDT and DDE concentrations measured by this study are lower than those reported by Badley (2003) and by Mironov (2004), but are well within the range of concentrations they observed (Marenco, 2002; Badley, 2003). All analyses of DDT were completed after the soil columns were destroyed; thus, further examination after 10 pore volumes was not possible.

2.8.2 *Mass of DDT and DDE removed from the soil*

The initial concentrations were used in equation 2.3 to calculate the initial mass of DDT and DDE within the soils and the subsequent masses removed from the soils with each application of HPβCD solution. Figure 2.8 shows the DDT and DDE masses removed after each pore volume of HPβCD solution was applied. The masses of initial and end-time DDT and DDE values are reported in summary tables 2.2 and 2.3. The initial masses of DDT and DDE were 1666μg ± 477 and 1059μg ± 372. The masses at the end of the study (10PV applied) measured from soils from the destroyed columns were 1240μg ± 276 and 686μg ± 155 of DDT and DDE respectively, plus or minus one standard deviation. These values indicate that there was a decrease in concentration due to HPβCD treatments measured between initial and final sampling. As the initial DDT and DDE values of the soils used to pack the columns were not measured directly, the measured values of DDT and DDE removed from the column per application were added to the final measured DDT values to create an initial value of the column soils to be 1556μg and 913μg for DDT and DDE respectively. These values agree with the measured initial values of the untreated soil splits within one standard deviation. The percentage of DDT and DDE mass removed after 10 pore volumes of treatment were 19% and 21% respectively.

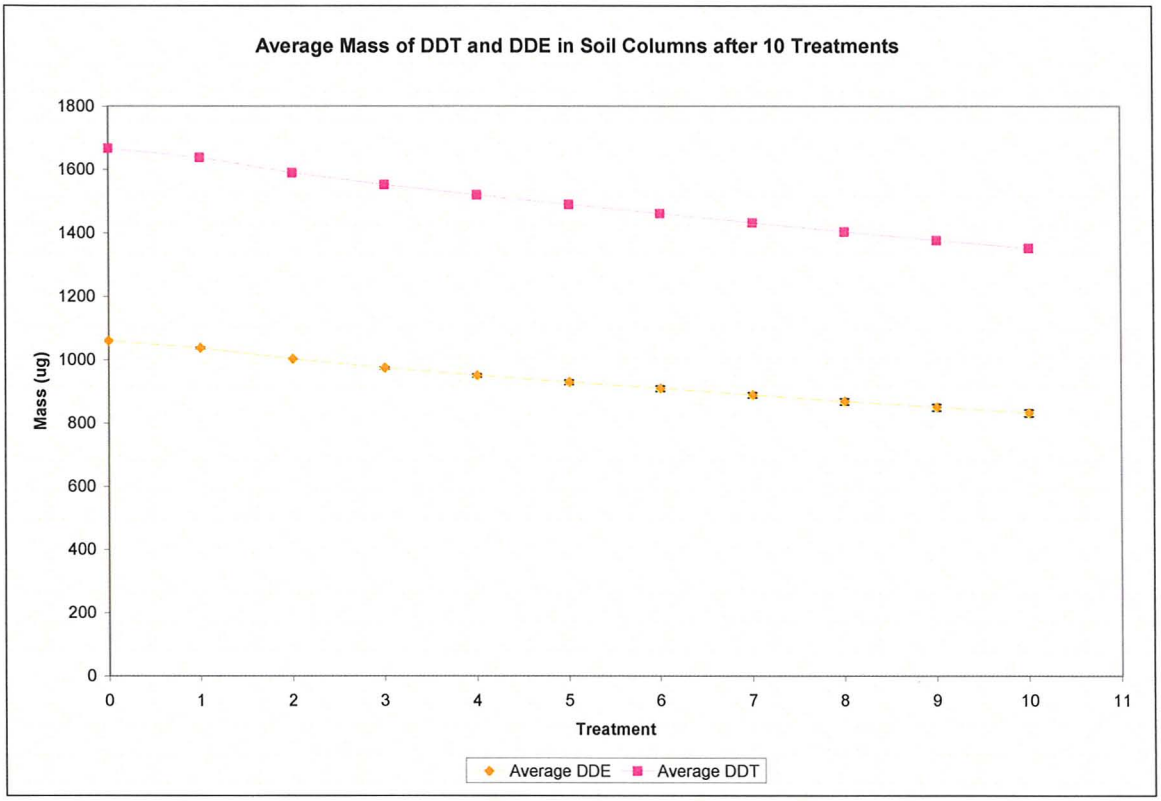

Figure 2.8. DDT and DDE masses after each pore volume of 10% HPβCD treatment is applied. Error bars represent a 90% confidence limit of 3 replicate samples for each date.

Table 2.2. Calculated Mass of DDT in columns with a depth of 15cm of soil using the arithmetical average concentration.

	Initial	End-time for 10% Applications
Average DDT Concentration, μg/g	6.2	5.0
Mass of DDT, g	$1.8x10^{-3}$	$1.4x10^{-3}$
Removed DDT mass, g		$3.2x10^{-4}$
Removed DDT mass, %		19

Table 2.3. Calculated mass of DDE in column with a depth of 15cm of soil using the arithmetical average concentration.

	Initial	End-time for 10% Applications
Average DDE Concentration, μg/g	3.9	2.6
Mass of DDE, g	$1.1x10^{-3}$	$8.3x10^{-4}$
Removed DDE mass, g		$2.3x10^{-4}$
Removed DDE mass, %		21

In comparison, Badley (2003) reported a decrease in DDT and DDE concentrations of 90% and 74% respectively using the arithmetical average for the 10%-HPβCD solution treatment soils. Using sample data from 2002 and additional data collected in 2003, Mironov (2004) reported a decrease in concentration of DDT and DDE in the 10%-HPβCD soils of 86% and 72% for DDT and DDE respectively using arithmetical data. Further analysis by Mironov (2004) using ln-transformed data shows decreases in the concentrations of DDT and DDE in 10%-HPβCD treated plots of 74% and 64% respectively. The ln-transformed data was reported to be more representative of the distribution of DDT and DDE concentrations measured at Point Pelee National Park (Marenco, 2002).

The percent mass of DDT and DDE removed from the columns do not reflect values or trends similar to the masses removed under field conditions by Badley (2003)

shown in figure 2.9 and 2.10. This comparison indicates that the in the lab the DDT and DDE were less efficiently removed by HPβCD applications. These graphs demonstrate that the application of HPβCD in the field was approximately three times more effective than the laboratory column study at removing DDT and DDE after 10 pore volumes of HPβCD application. This indicates that mobilization (translocation) by 10%-HPβCD is not likely the sole primary mechanism for DDT and DDE removal from soil in the field remediation trials. DDT and DDE are recalcitrant in the soil at Point Pelee National Park, and thus rain effects alone cannot be responsible for additional flushing of DDT and its constituents (Aislabie et al., 1997). It was estimated by Crowe and Smith (2007) that the half-live of DDT in Point Pelee soils was between 20-30 years. The hydrophobic nature of the DDT and DDE chemical structures result in very low solubility in water. Photolysis is a degradation pathway transforming DDT to DDE and only occurs during the first few years until it has adsorbed onto organic matter in the soil (Aislabie et al., 1997). Once adsorbed to organic matter DDT is recalcitrant to rain and photolysis degradation mechanisms (Boul, 1994; Aislabie et al., 1997).

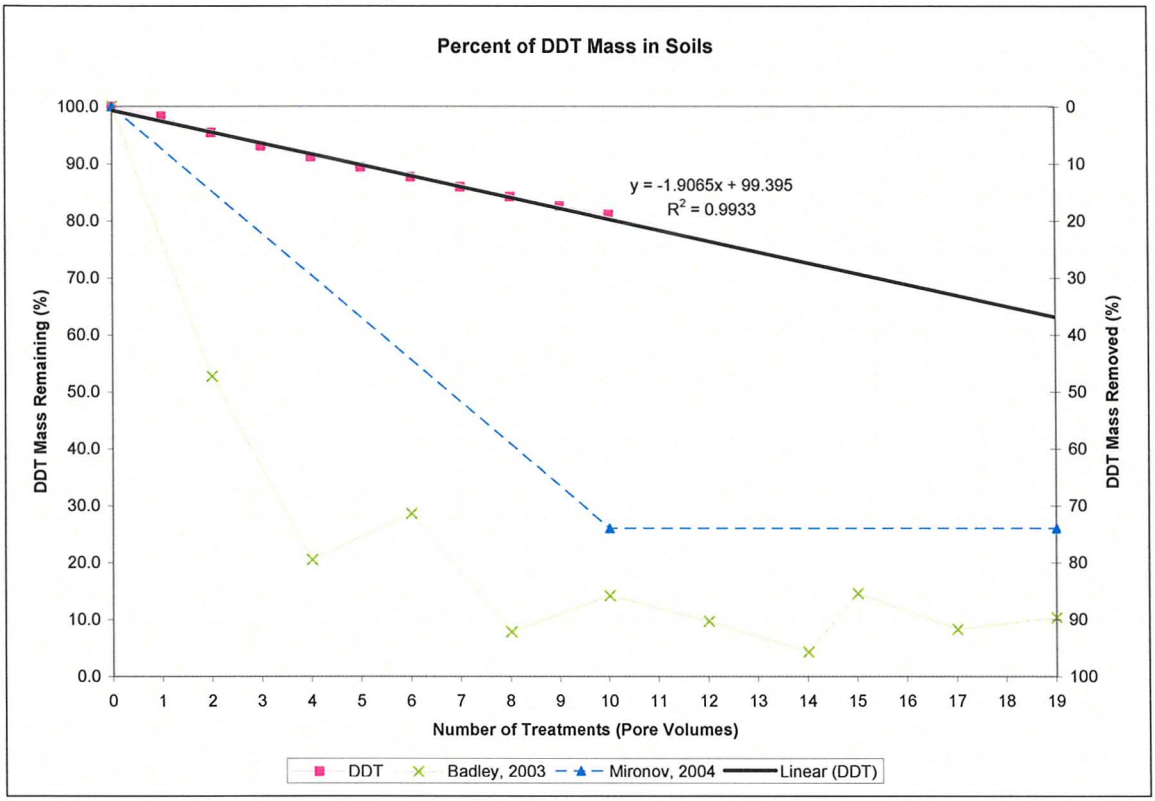

Figure 2.9. Comparison of the percentage of DDT mass removed by three studies. This study removed 19% of DDT mass in the laboratory, the field study by Badley (2002) removed an average of 86% DDT mass, with the ln-transformed average of this value of 74% removal demonstrated by Mironov (2004).

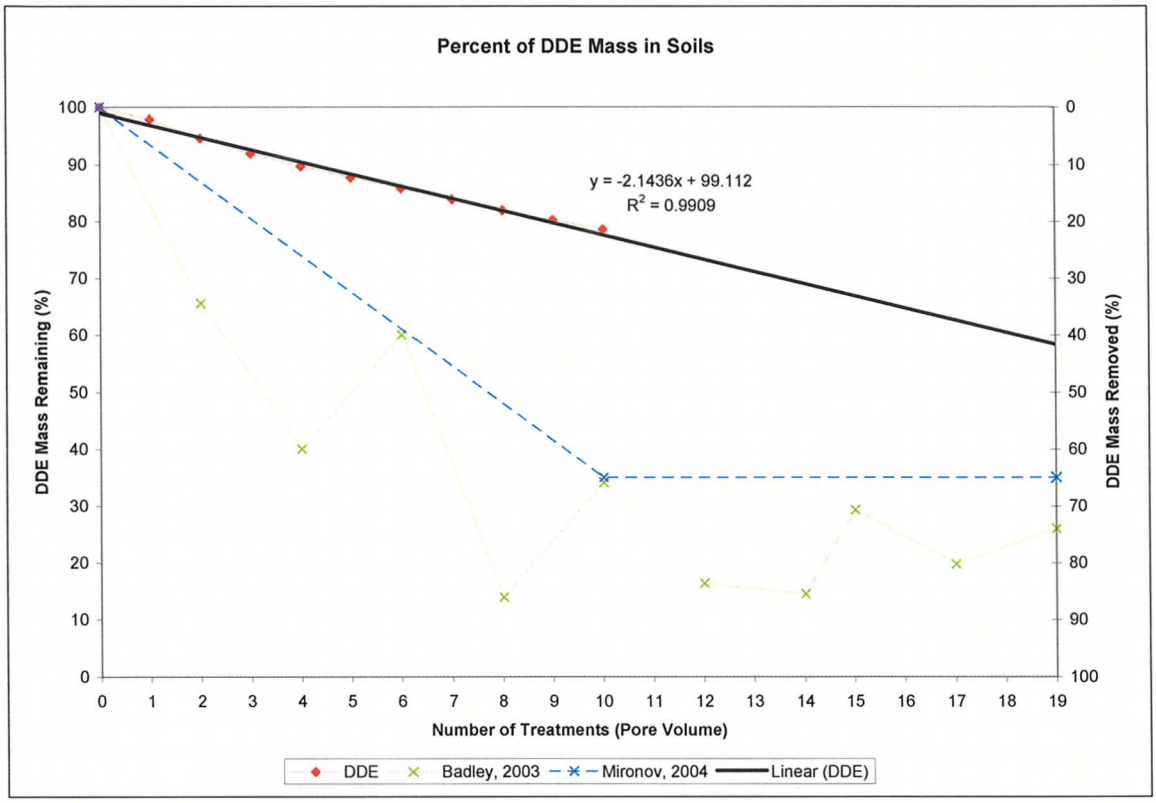

Figure 2.10. Comparison of the percentage of DDE mass removed by three studies. This study removed 21% of DDE mass in the laboratory, the field study by Badley (2002) removed an average of 72 % DDE mass, with the ln-transformed average of this value of 65% removal demonstrated by Mironov (2004).

Using both field sampling and numerical modeling Mironov (2004) and Mills (2004) concluded the mass of DDT, DDE and DDD removed by the flushing with HPβCD solution (Badley, 2003) was not present in the ground water below the remediation plots and could not have flowed completely outside the sampling area. The conclusion was suggested that microbial transformations in the presence of HPβCD were likely responsible for much of the mass loss. It is reported in the literature (Shao et al., 2003) that HPβCD reduces the toxicity of the contaminant contained within the HPβCD cavity by removing the direct exposure of the bacteria to the contaminant. Thus, there is potential that this reduced toxicity may allow microbes to degrade DDT and DDE in-situ at Point Pelee in the presence of HPβCD solution. HPβCD can result in an increased bioavailability of DDT and its derivatives and enhance the biodegradation of hydrophobic soil contaminants (Fava et al., 1998; Wang et al., 1998).

Evidence for microbial activity in PPNP soils was demonstrated in the treatment plots by Badley (2003) and Essa (2004). An increased microbial cell count using DAPI staining was observed by Badley (2002) for both 10%- and 20% HPβCD soil remediation plots. Badley (2002) also observed a clogging effect and decreased infiltration. In-situ measurements of moisture contents with TDR suggested increased saturation of the soils. Ten months after the discontinuation of the HPβCD treatments Essa (2003) identified anoxic conditions that were allowing indigenous bacteria to complete sulphate reduction in the same 20%-HPβCD plots. Anoxic conditions are favourable for the formation of DDD from DDT (Aislabie et al., 1997).

A laboratory study with Pelee soils from the same area was treated with 20%-HPβCD solution once per week for four months (Smith, 2004). This study observed a bioclogging effect and increased moisture contents of the biotic columns with no observed clogging effect within abiotic columns. It was suggested that HPβCD could act as a carbon source to promote microbial growth; however, this was not the primary focus of this study. These studies suggest that microbial activity in the presence of HPβCD could be a primary mechanism of DDT degradation and may be the reason why the field trials were substantially more effective at removal of DDT and DDE than the laboratory column study reported herein.

2.8.3 *Pore Volume Half-Life*

Further comparison to the field results can be done with the analysis of the pore volume half life. DDT is known to follow a first-order kinetic degradation reaction (Aislabie et al., 1997; Crowe and Smith, 2007). Thus, the half life can be calculated using the following equations:

$$\frac{C_t}{C_o} = e^{-kt} \tag{2.4}$$

$$t_{1/2} = \frac{\ln 2}{k} \tag{2.5}$$

Where C_t is the concentration at time t, C_o is the initial concentration, k is the decay constant and t is the time. Badley (2003) observed a tailing effect and used a first-order kinetic reaction to estimate the desorption pore volume half-life ($pv_{1/2}$), shown in equations 2.6 and 2.7.

$$\frac{C_{pv}}{C_o} = e^{-kpv} \tag{2.6}$$

$$pv_{1/2} = \frac{\ln 2}{k} \tag{2.7}$$

where C_{pv} is the concentration after a number of pore volumes is applied (pv), C_o is the initial concentration (pv=0), and k is the decay constant. The exponential first order reaction indicates that a portion of DDT will not desorb from the organic and clay mineral surfaces, however, some portion of DDT will desorb into the HPβCD cavity, thus increasing the DDT solubility and mobility (Wang et al., 1994). To make a further assessment of the removal efficiency in the laboratory, the natural logarithm of the average C_{pv}/C_o was plotted against the number of pore volumes (figures 2.11 and 2.12). Furthermore, the comparison of the field and laboratory data of $\ln(C/C_o)$ against the number of pore volumes applied is also demonstrated in figures 2.11 and 2.12. The least squares best-fit function in Microsoft Excel was used to fit the data and to provide the equation below:

$$\ln \frac{C_{pv}}{C_o} = -kpv \tag{2.8}$$

The trend line generated was forced through the origin of (0,0) to indicate initial conditions. The slope of the line is the decay constant k. The trend line of the laboratory data was extrapolated to match the nineteen pore volumes that were applied in the field. This extrapolation assumes that the removal of DDT and DDE would be continuous and show no tailing effects.

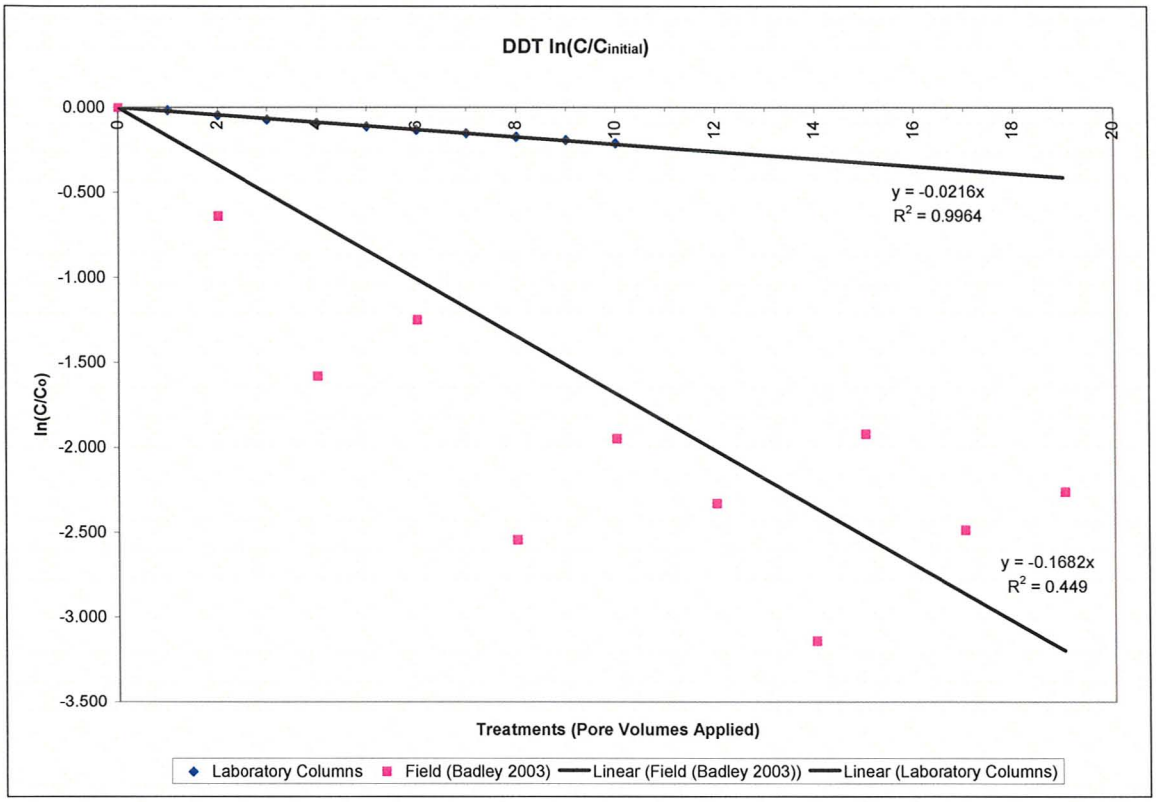

Figure 2.11. Plot of $\ln(C/C_0)$ of DDT vs. the number of pore volume treatments applied. Both laboratory column data and field data (Badley, 2003) are shown.

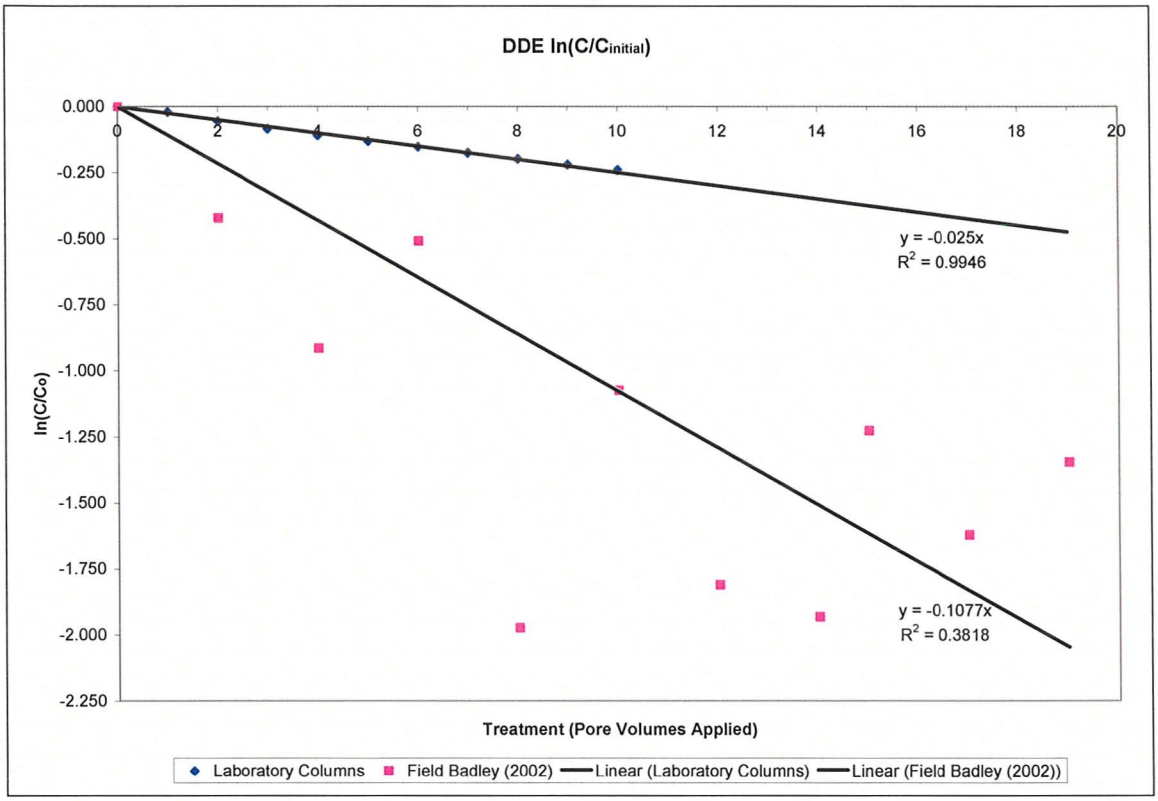

Figure 2.12. Plot of $\ln(C/C_0)$ of DDE vs. the number of pore volume treatments applied. Both laboratory column data and field data (Badley, 2003) are shown.

The fit values of k were used to calculate $pv_{1/2}$ using the equation 2.8. The pore volume half life calculated for laboratory conditions was 32.1 for DDT and 27.7 for DDE. A comparison of these values with values from the field reported by Badley (2003) is given in table 2.4.

Table 2.4. Calculated values of pore volume half-lifes: comparing field $pv_{1/2}$ (Badley, 2003) to lab $pv_{1/2}$. The laboratory data was only completed for a total of 10pv as was the early time field data.

	10% Lab Data	10% Field Data (early)	10% Field Data (all)
$pv_{1/2}$ for DDT	32.1	2.8	4.1
$pv_{1/2}$ for DDE	27.7	4.5	6.4

Badley (2003) reported a tailing effect after 10 pore volumes of treatment, thus, the least squares best-fit function creates a skewed effect of the $pv_{1/2}$ calculation resulting in a representation of a conservative pore volume half-life. The skew resulted in a decrease of the trend line slope. To correct for this, the pore volume half-life of the early time data (≤10 PV) was calculated and is also reported in table 2.4. The pore volume half lives ($pv_{1/2}$) calculated demonstrate that in comparison between the lab and all field data that the half-life of DDE in the lab was approximately four times longer than in the field. The DDT pore volume half life in the lab was significantly longer than in the field, requiring almost thirty two pore volumes of 10%-HPβCD treatment rather than the four pore volumes used in the field. This suggests that the field was more successful at remediating DDT and its derivatives from the soil over a longer wait time between applications. A comparison against the early time field data further indicates that the field was more successful at remediating DDT and DDE with only three pore volumes required for half of the DDT mass to be removed and four and a half pore volumes to

remove the DDE mass from the soils. A comparison of the R^2 values demonstrates that there is as expected a reduced variability in the lab dataset (R^2=0.99) compared with the low R^2 values observed in field (R^2=0.45) results which measured a high variability in DDT and DDE concentrations. Large R^2 values for the laboratory data indicate that the linear least squares best fit function generated provides a good representation of the DDT and DDE removal and further indicates there was no measurable tailing effect during laboratory treatments.

Marenco (2002) reported an estimated half-life for DDT at Point Pelee National Park between 25-30 years. Badley (2003) reduced the DDT concentration by half within 4 pore volumes of treatment over 4 weeks. Schepanow (2002) reported no difference in reduction of DDT between different waiting times between applications. This study shows that flushing DDT and DDE with HPβCD is not the sole primary mechanism in DDT reduction and indicates that the longer wait time of one week between applications of 10%-HPβCD solution in addition to unidentified field conditions may be necessary to reduce the concentration DDT and its derivatives in field soils.

2.9 CONCLUSIONS

Hydroxypropyl-β-cyclodextrin provides real promise as a new remediation technology for organic contaminants in soils. It has been shown that applications of 10%-HPβCD solutions are successful at removing DDT and its constituents from shallow soil. This laboratory column soil treatability study showed after ten pore-volumes of 10%-HPβCD application 19% of the DDT mass and 20% of the DDE mass was removed from

the soil. This reduction was due to vertical mobilization. In contrast, the efficiency of DDT and DDE removal in the field under similar application of 10%-HPβCD solution over a four month period resulting in removal of 90% of DDT mass and 74% of DDE mass (Badley, 2003). Laboratory treatments of 10%-HPβCD were applied twice per day over a 5 days period, whereas field applications were once per week. The field remediation experiment resulted in a greater removal of DDT. Thus, it is apparent that time and alternative degradation mechanism under field conditions contributed to the increased removal efficiencies.

It has been shown by this laboratory study that mobilization by HPβCD solution is not the sole primary mechanism in DDT degradation or removal. Significantly less DDT and DDE masses were removed in comparison with field removals. A comparison of pore volume half-lives of HPβCD with the work of Badley (2003) indicates that it is important to allow sufficient time between pore volume applications for the most efficient removal of DDT from contaminated soils. Using a first order decay constant reaction, the pore volume application half life approximately ten times as large as that of the field was found by this study. Thus, indicating that the field conditions were more favourable to DDT and DDE removal and/or degradation. Further investigation is required to determine optimal field conditions for maximum DDT, DDE and DDD removal at aged contaminated field sites when using HPβCD solution as a remediation agent.

Evidence by previous studies (Badley, 2003; Essa, 2003; Smith, 2003; Mironov, 2004; Mills, 2004) further indicates that mobilization by HPβCD is not the sole primary

mechanism for DDT removal. It is suggested that in addition to mobilization, HPβCD increases bioavailability of DDT and is derivatives by reducing its toxicity and enhances their degradation by the microbial communities in the soil.

2.10 RECOMENDATIONS FOR FUTURE WORK

It is expected from this study, that the environmental conditions such as microbe communities present play a pivotal role in accelerating DDT and DDE degradation in the presence of HPβCD. Further study is required to investigate this relationship and the optimal HPβCD concentrations to succeed at equally effective contaminant removal from unsaturated soils. In addition to this it is recommended that further work focus on the concentration of HPβCD applied for remediation of DDT contaminated soils.

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CHAPTER 3
USING STABLE CARBON ISOTOPES TO INVESTIGATE HYDROXYPROPYL-β-CYCLODEXTRIN AS A SOIL REMEDIATION AGENT

3.1 ABSTRACT

Stable carbon isotope analysis has been used as a part of a larger study to examine the persistence of hydroxypropyl-β-cyclodextrin (HPβCD) in soils at Point Pelee National Park, Ontario. This is the first study known to monitor carbon contributions due to HPβCD applications in soils using stable carbon isotopes. Technical grade HPβCD was found to have a $\delta^{13}C$ value of -16‰ reflecting that of a C_4 carbon isotopic signature. The carbon isotopic value of soils sampled the former orchard area Point Pelee was -24.5‰ reflecting a C_3 plant. Using these values as source end members, the isotopic shifts measured in Point Pelee soils from the applications of 10%- and 20%-HPβCD solutions was monitored. Weekly applications of one pore volume (33L) were applied to the test plots of the soils for nineteen and thirteen weeks for the 10%- and 20%-HPβCD solutions respectively. HPβCD was found to contribute to the total soil organic carbon values, with a maximum contribution of 50% observed in the 20%-HPβCD treated soils after receiving nine HPβCD treatments. Carbon isotopic values returned to initial carbon isotopic values in the soils within approximately 44 and 50 weeks after HPβCD applications ceased. It has been identified that HPβCD did contribute to the total organic carbon pool in the soils for a short-term period, however, did not persist under long-term conditions, i.e. on the order of one year.

3.2 INTRODUCTION

Cyclodextrins have received increased use in laboratory and field studies to remediate soils contaminated with hydrophobic compounds. Hydroxypropyl-β-cyclodextrin (HPβCD) in particular has been used to remediate compounds such as polyaromatic hydrocarbons, pesticides, polychlorinated biphenyls, and chlorinated hydrocarbons from the saturated zone (Wang and Brusseau, 1995; McCray and Brusseau, 1998; Boving et al., 1999, 2000). Cyclodextrins are naturally produced by the enzymatic degradation of cornstarch using the enzyme cyclodextrin glucanotransferase (CGTase) (Szejtli, 1998). The HPβCD consists of a ring shaped structure with a hydrophilic exterior and an apolar interior cavity, and can act as a host molecule to form inclusion complexes with hydrophobic molecules. Chemically modified cyclodextrins such as hydroxypropyl-β-cyclodextrin have been found to linearly enhance solubility of non-polar guest molecules (Wang and Brusseau, 1993). Previous studies have focused on the removal of contaminants from soils using HPβCD (Viglianti et al., 2006; Badley, 2003), the enhanced biodegradation of contaminants in the presence of HPβCD (Molnar et al., 2005), and the biodegradation of HPβCD in the presence of contaminants (Vertischel et al., 2004; Fenyvesi et al., 2005). While a useful remediation agent for mobilizing organic contaminants in soils, the long-term effects of cyclodextrins on the local soil organic carbon pool remain unknown.

Cyclodextrins are complex macromolecules and can consist of different degrees of substitution (DS) of hydroxypropyl groups (C_3H_7O) on the exterior of the carbon ring result in differing molecular masses of HPβCD molecules. A fully substituted HPβCD

molecule has seven hydroxypropyl groups on the exterior of its structure (DS=7), resulting in a chemical formula of $C_{63}O_{42}H_{112}$ and a molecular weight of 1540. Thus, an HPβCD molecule with six degrees of substitution (DS=6) has a chemical formula of $C_{59}O_{40}H_{110}$, and five degrees of substitution (DS=5) has a formula of $C_{54}O_{38}H_{108}$.

Stable carbon isotopic analysis of soil organic matter has been used to investigate source identification regarding changes in vegetation, land use practices and turnover rates of soil organic carbon (Boutton, 1996). Soil organic matter is primarily composed of oxygen, hydrogen and carbon arising from living and decaying plant material, animals and microbes. Carbon accounts for 52-58% of soil organic matter (Sparks, 2003). The carbon stored in soils is of great interest due to its large significance to the global carbon cycle as soil respiration is a significant flux of CO_2 to the atmosphere (Schlesinger, 1997). Carbon is fractionated differently by C_3 plants using the Calvin-Benson photosynthetic pathway and by C_4 plants using the Hatch-Slack photosynthetic pathway. This different fractionation results in different $^{13}C/^{12}C$ ratios that can be used to distinguish C_3 from C_4 plant organic matter. Particularly, the average $\delta^{13}C$ value obtained from C_3 plants is -27‰ and from C_4 plants is -13‰ (Cerling, 1996). Undecomposed organic carbon in soils retains the carbon isotopic composition of its parent material (plant biomass) and does not exhibit the small isotopic changes resulting from soil carbon decay (Balesdent, 1996; Wynn et al., 2005). Work by Wynn and Bird (in review) has suggested that C_4 derived active soil organic carbon decomposes twice as fast as that of the total organic carbon pool presenting implications for paleoecological studies. To date, using the change from one photosynthetic pathway such as C_3 (low

$^{13}C/^{12}C$) to the other C_4 (high $^{13}C/^{12}C$) pathway permits the use of the isotopic composition of the soil organic matter and is an acceptable method to trace past land use changes.

Cyclodextrin is produced from the enzymatic degradation of corn starch. Corn is a C_4 plant, thus, exhibits a carbon isotopic value ($\delta^{13}C$) of -12‰ (Bird and Gröcke, 1997). In this study, a natural ^{13}C-labelling method was used during a field experiment of HPβCD applications to soils contaminated with aged DDT. Using measurements of technical grade cyclodextrin $\delta^{13}C$ value as a C_4 end-member and the measurements of $\delta^{13}C$ value of the untreated soil organic matter as a C_3 end-member the persistence of HPβCD in soils is identified. Carbon isotopic values reveal the effects of the HPβCD applications to the soil on the soil organic carbon pool.

3.3 BACKGROUND

Marenco (2001) collected soil samples from the former orchard area at Point Pelee National Park, Canada, to determine DDT concentrations. This former orchard area is known to have grown apple and peach trees (use C_3 photosynthetic pathways). At this same location, Badley (2002) successfully designed and implemented a pilot-scale remediation plot applying aqueous solutions of technical-grade HPβCD. The design consisted of nine treatment plots: three control plots, three 10%-HPβCD, and three 20%-HPβCD solution plots to reduce DDT concentrations from the surface soils. Untreated soils from the control plots were sampled once per week for nineteen weeks. A total of nineteen pore volumes (PV=33L) of 10%-HPβCD solution were applied to three plots

once per week, and a total of thirteen pore volumes (PV=33L) of 20%-HPβCD solution were applied to the 20%-treated soil plots once per week. The 20%-HPβCD treatments were discontinued after an observed clogging effect ie. reduced infiltration rates (Badley, 2002). Additional soil samples were collected by Mironov (2004) from the same treatment plots. All soil samples removed were stored in the refrigerator at 4°C. The remediation study successfully removed 90%, 77% and 82% of the initial DDT, DDE and DDD concentrations in the soils using a 20%-HPβCD solution and removed 90%, 74%, and 73% of the initial DDT, DDE and DDD concentrations using the 10%-HPβCD solutions.

3.4 METHODS

In 2006, soil samples were collected from the Badley (2003) remediation plots. Soil samples from 2001, 2002, 2003, and 2006 were stored in the dark at 4°C (Marenco, 2001; Badley, 2003; Mironov, 2004). Three replicates of the soil samples from both the untreated and treated plots from all years were used for stable isotope analysis of organic carbon. Representative samples of soil including all organic material <2mm was milled to a fine powder. Analysis was done by continuous flow-isotope ratio mass spectrometry using a Costech Elemental Analyzer to combust samples into CO_2 and N_2 gases, which were then measured on a Finnegan Delta Plus XP mass spectrometer. All samples were analyzed at the Stable Isotope Biogeochemistry Laboratory at McMaster University. Stable isotopic carbon values are calculated using equation 3.1 and are expressed using

delta notation. The international standard Vienna Pee Dee Belemnite (VPDB) was used as a reference for the samples. The uncertainty of isotopic measurements was ±0.1‰.

$$\delta^{13}C(‰) = \left(\frac{\frac{^{13}C}{^{12}C}_{sample} - \frac{^{13}C}{^{12}C}_{standard}}{\frac{^{13}C}{^{12}C}_{standard}} \right) \times 1000 \quad (3.1)$$

Where $\delta^{13}C$ is the ratio of the difference between the $^{13}C/^{12}C$ ratios of the sample to that of the $^{13}C/^{12}C$ ratio of the standard (VPDB), although it is dimensionless, it is assigned a unit of parts per thousand or per mil (‰) because it is multiplied by 1000. The isotopic carbon content given is a bulk carbon isotopic value that accounts for organic carbon from soils and any possible calcium carbonate. Preliminary tests to remove carbonates from the soils using 1M hydrochloric acid treatments resulted in no bubbling or sample weight loss that would indicate the presence of carbonates (Dane and Topp, 2002). This acid treatment removed the cyclodextrin isotopic value of interest from the soils and therefore as the carbonate composition was identified to be very low, all samples discussed further were not pretreated with HCl. Thus, the bulk isotopic value given is assumed to be due only to organic materials present in the soil.

3.5 RESULTS AND DISCUSSION

Preliminary testing determined that the carbon isotopic value ($\delta^{13}C$) of technical grade hydroxypropyl-β-cyclodextrin used in the study was -16‰. This $\delta^{13}C$ value reflects a C_4 plant carbon isotopic signature and is representative of the enzymatic degradation of cornstarch (a C_4 plant) by which cyclodextrins are produced. The average

value of all untreated soil samples collected in 2001, 2002, 2003 and 2006 resulted in $\delta^{13}C = -24.5 \pm 1.2‰$ and reflects the C_3 plant signature known to most soils within Southern Ontario, Canada. Twenty-nine samples were analyzed for the initial $\delta^{13}C$ values. All other $\delta^{13}C$ values reported for treated soil samples are an average $\delta^{13}C$ value using three samples (n=3) all treated with the same HPβCD solution on a given date. Uncertainty values reported are equal to one standard deviation of the samples used for the average carbon isotopic value ($\delta^{13}C$) and reflect the inherent natural heterogeneity in soils. All samples have been stored in the refrigerator and were analyzed for $\delta^{13}C$ during the same sample run in 2007. Values of $\delta^{13}C$ for all samples analyzed are reported in Appendix C.

Figure 3.1 demonstrates the long-term $\delta^{13}C$ value after applications of one pore volume of 20%-HPβCD solutions to the soils once per week. Once treatments began (June 18, 2002), the isotopic values measured show an increase in $\delta^{13}C$ values shifting towards the isotopic value of technical grade HPβCD (-16‰). Further examination of figure 3.1 demonstrates fifty weeks after the final 20%-HPβCD solution was applied, the $\delta^{13}C$ value of the soil returned to the initial carbon isotopic composition of the soils ($-24.5 \pm 1.2‰$). Additional samples collected approximately two hundred weeks after the last treatment remained at initial $\delta^{13}C$ values of soil conditions, thus suggesting that HPβCD does not remain in the soil organic matter.

An enhanced view of the short-term changes in the carbon isotopic values during the application period of HPβCD solutions is shown by figure 3.2.

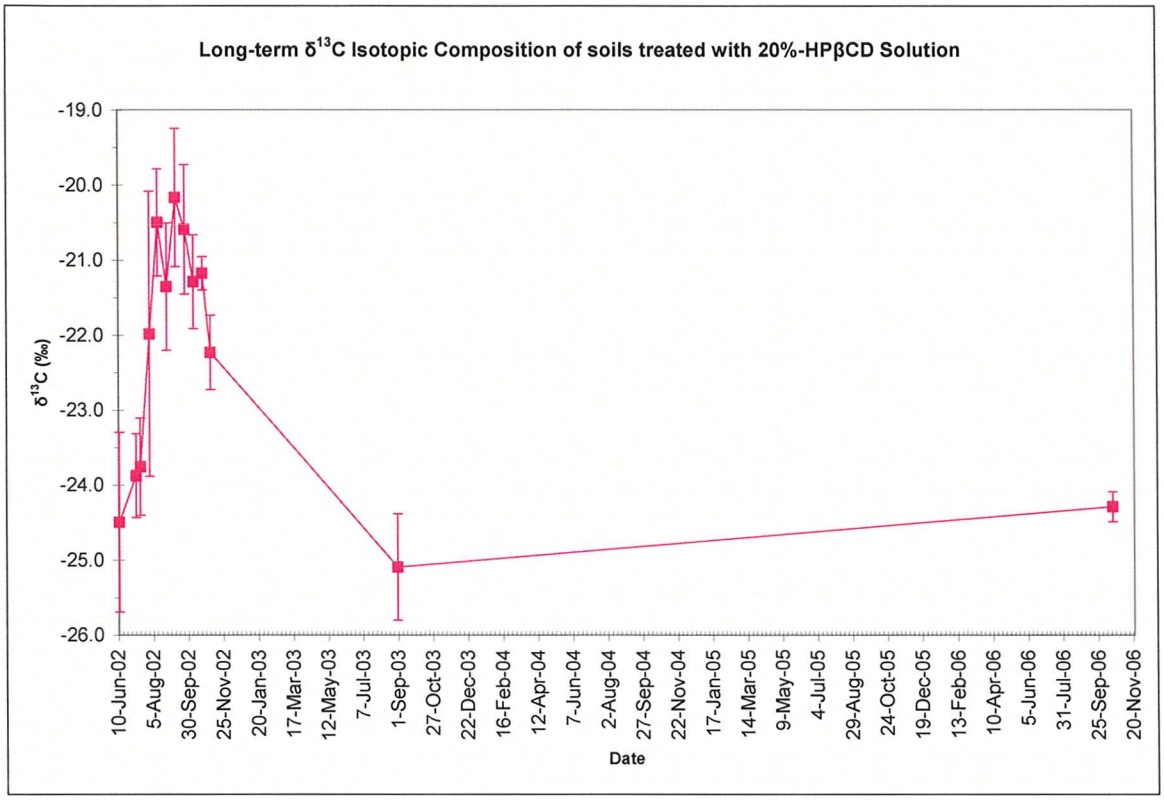

Figure 3.1. Long-term of $\delta^{13}C$ isotopic composition of soils after 13 pore volumes of 20%-HPβCD treatment were applied once per week. Treatments began July 8th, 2002 and were discontinued after the last treatment was applied September, 15th, 2002 due to a clogging effect that reduced infiltration. Error bars indicate one standard deviation of three soil replicates used to make the average $\delta^{13}C$ shown.

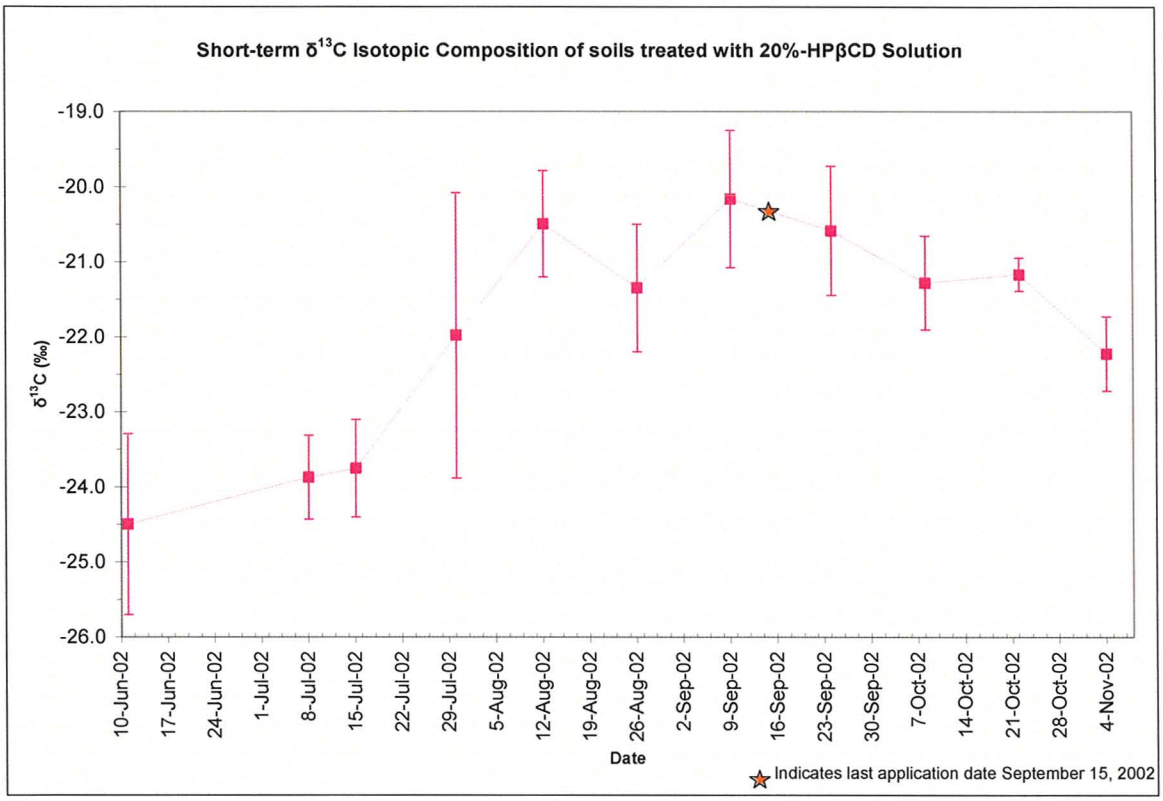

Figure 3.2. Short-term view of the $\delta^{13}C$ isotopic composition of soils after 13 pore volumes of 20%-HPβCD treatments were applied. Initial samples were collected June 11th, 2002 and treatments begun July 8th, 2002 and continued once per week until September 15th, 2002. Note the slow return of $\delta^{13}C$ towards the initial $\delta^{13}C$ of -24.5‰ after HPβCD applications ceased. Error bars indicate one standard deviation of three soil replicates used to make the average $\delta^{13}C$ shown.

September 10th, 2002, was the thirteenth and final HPβCD treatment applied to the 20%-treated soil plots, due to a clogging effect that reduced the infiltration rate of the HPβCD solution (Badley, 2003). The largest $\delta^{13}C$ value obtained was -20.2 ± 0.9‰ on September 9th, 2002, which had received twelve pore volumes of HPβCD solution. After treatments discontinued there is a clear decrease observed in $\delta^{13}C$ values in the 20%-HPβCD treated soils from -20.2‰ to -24.5‰ from September 23rd, 2002 to November 4th, 2002, (6 weeks) indicating a slow return to the initial soil $\delta^{13}C$ value.

Similar to figure 3.1, the long-term $\delta^{13}C$ values of the soil measured after weekly treatments of 10%-HPβCD solutions are given in figure 3.3. The last 10%-HPβCD treatment was applied on October 22nd concluding nineteen weekly of treatments applied to the soils. The 10%-HPβCD treatments did not result in a large enrichment of $\delta^{13}C$ value seen by the 20%-HPβCD treatments. An enhanced view of the 10%-HPβCD application time period is shown in figure 3.4. The 10%-HPβCD treated soils do show a small enrichment reaching a maximum value of approximately $\delta^{13}C = -23 \pm 1.2‰$. Carbon isotopic compositions of soils shown in figure 3.3 were analyzed forty-four (September 30, 2003) and two hundred weeks (October 17, 2006) after the last application date. These dates are considered to be 'late-time' conditions. On September 30th, 2003, the $\delta^{13}C$ values were -24.0 ± 0.4‰ and -25.1 ± 0.7‰ for the 10%- and 20%-HPβCD treated soils respectively. At October 17, 2006, the $\delta^{13}C$ values were -23.3 ± 0.4‰ and -24.3 ± 0.2‰ for the 10%- and 20%-HPβCD treated soils. These values reflect the initial soil conditions of $\delta^{13}C = -24.5 \pm 1.2‰$ shown by figures 3.1 through 3.4.

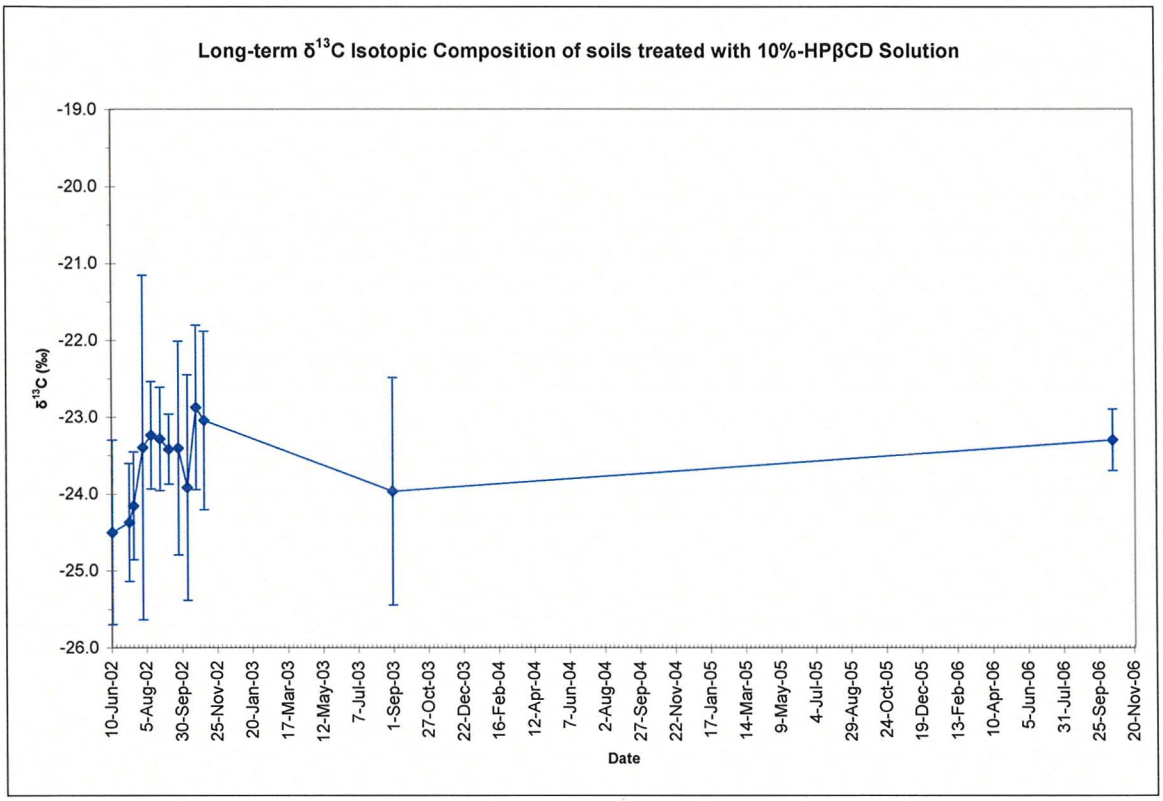

Figure 3.3. Long-term of $\delta^{13}C$ isotopic composition of soils after 19 pore volumes of 10%-HPβCD treatments were applied once per week. Treatments began July 8th, 2002 and were discontinued after the last treatment was applied October 22nd, 2002. No clogging effect was observed. Error bars indicate one standard deviation of three soil replicates used to make the average $\delta^{13}C$ shown.

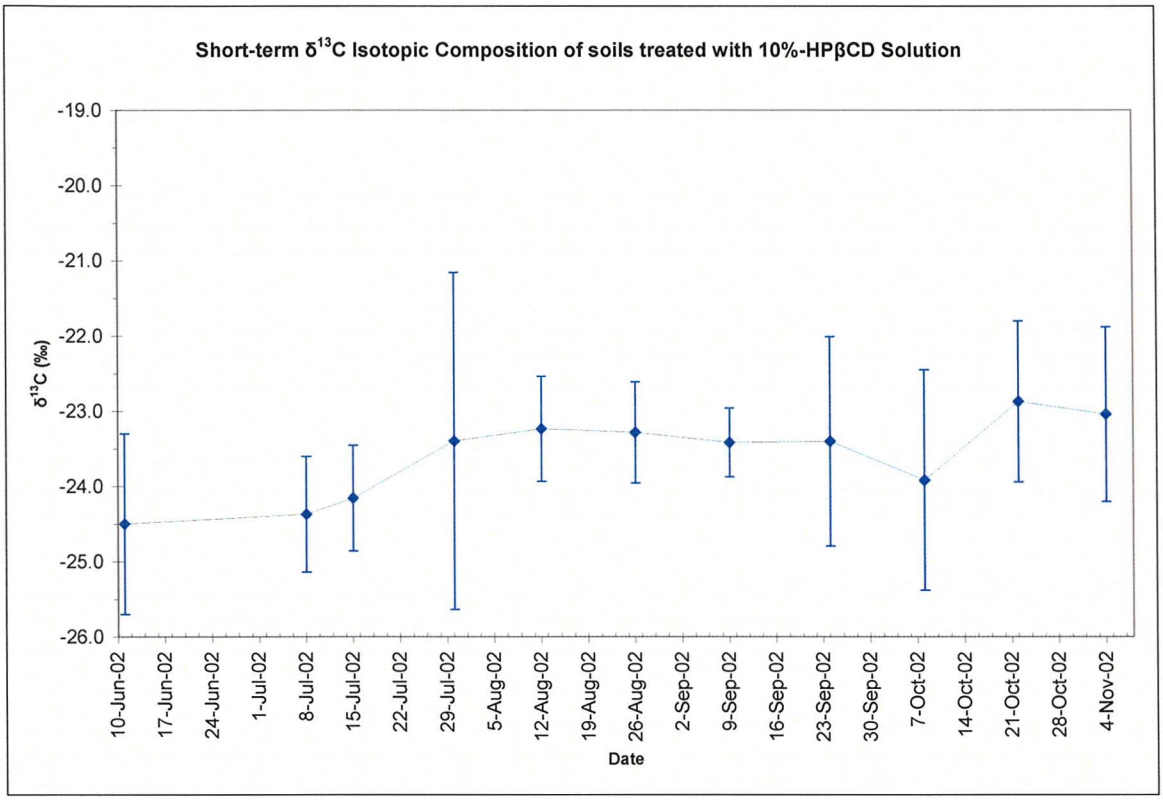

Figure 3.4. Short-term of the $\delta^{13}C$ isotopic composition of soils after 19 pore volumes of 20%-HPβCD treatments were applied. Initial samples were collected on June 11th, 2002, and treatments of HPβCD solution begun on July 8th, 2002 and continued once per week until October 22, 2002. Error bars indicate one standard deviation of three soil replicates used to make the average $\delta^{13}C$ shown.

Thus, the HPβCD does contribute to the organic carbon in the soils for a short time period during the thirteen and nineteen weeks of applications, however, it does not persist for more than approximately six weeks after applications cease, reflected by the decrease shown in the 20%-HPβCD treated soils given in figure 3.2. It was shown by this study that although HPβCD does remain in the soil for short term periods after applications cease (six weeks), HPβCD does not persist for more than one year.

3.5.1 *Statistical Analysis*

Statistical analyses were performed to determine that the shifts in carbon isotopic values ($\delta^{13}C$) measured were in fact different than initial $\delta^{13}C$ values of the soils. The data groups are identified as untreated samples representing 'initial' conditions, treated samples from July 8th to November 4th as mid-time data, and only the August 30, 2003 and October 17, 2007 data points representing late-time conditions. The sample groups were tested for statistical significance using an analysis of variance (ANOVA). This technique separates the total variance in a group of measurements into various sources and considers both the differences in means and variances. A one-way analysis of variance described by Davis (2002) was used to determine the equivalency of the sample groups. This test has a null hypothesis that all sample means are equal, whereas the alternative hypothesis is that at least one mean is different. The total sum of squares (SS_T) described by equation 3.2 is the sum of the squared differences between observations and the overall grand mean (X) and indicates the total variation in the data.

$$SS_T = \sum_{j=1}^{m} \sum_{i=1}^{n} \left(x_{ij}^2 - \bar{X} \right)^2 = \sum_{j=1}^{m} \sum_{i=1}^{n} x_{ij} - \frac{\left(\sum_{j=1}^{m} \sum_{i=1}^{n} x_{ij} \right)^2}{N} \quad (3.2)$$

Where m is the number of samples, n is the number of replicates per sample, x_{ij} is the i^{th} replicate of the j^{th} sample, N is the total number of observations where N = n x m and the last term in the equation is a correction term. The sum of squares among the samples (SS_A) indicates the variation attributed to the differences between samples and is given by equation 3.3.

$$SS_A = \sum_{j=1}^{m} \left(\bar{X}_j - \bar{X} \right)^2 = \sum_{j=1}^{m} \frac{(\sum_{j=1}^{m} \sum_{i=1}^{n} x_{ij})^2}{N} \quad (3.3)$$

The source of variation within the samples is described by the error sum of squares (SS_E) and is given by equation 3.4.

$$SS_E = \sum_{j=1}^{m} \left(\sum_{i=1}^{n} x_{ij} - \bar{X}_j \right)^2 = \sum_{j=1}^{m} \sum_{i=1}^{n} - \sum_{j=1}^{m} \frac{(\sum_{i=1}^{n} x_{ij})^2}{n} \quad (3.4)$$

The statistical analyses described below were completed using the Analysis Toolpak software provided with Microsoft Excel ©. All tests were completed to determine statistical differences between samples at a 95% confidence interval. The Bonferroni tests were completed using the Analysis Toolpak software, however, the α value was manually adjusted according to the number of intended comparisons.

Initial analysis using ANOVA indicated that there was a statistical difference between the three groups identified as initial, mid-time and late-time data within a 95% confidence interval ($P<0.05$) within the 20%-HPβCD treated soils. This analysis did not provide any indication of which mean was not equal within the set. Initial analysis of the 10%-HPβCD treated soils using ANOVA indicated no statistical difference between the

initial, mid-time, and late-time data within a 95% confidence interval ($P<0.05$). Further analysis of the 20%-HPβCD treated soils was done using both ANOVA with a 95% confidence interval and a Bonferroni adjustment to further constrain the P value by dividing the acceptable α value (0.05) by the number of comparisons. The Bonferroni adjustment using an alpha value of 0.017 further indicates that the initial, mid- and late-time data groups are statistically significantly different ($P<0.017$) for the 20%-soils.

Early vs. late-time data for both the 10%- and 20%-HPβCD treated soil was found not to be statistically significant (F less than F_{crit}, $P>0.05$), suggesting that over the 227 week study that the carbon contributed by HPβCD has been flushed away or degraded in-situ. Thus, the additional carbon-13 from HPβCD has not been incorporated into the soil organic matter. Initial vs. mid-time values for both 10%- and 20%-treated soils were found to be statistically significant (F exceeds F_{crit}, $P<0.05$), suggesting that the addition of HPβCD did alter the total soil organic carbon pool during the HPβCD treatment period. The mid-time to late-time data was found to be statistically significant (F exceeded F_{crit}, $P<0.05$) for the 20%-HPβCD treated soils, but was found not to be statistically significant (F less than F_{crit}, $P>0.05$) for the 10%-HPβCD treated soils. The 20%-HPβCD solution application was discontinued at after thirteen pore volumes had been applied weekly. This is reflected in the $\delta^{13}C$ values (figure 3.2) and shows a slow return to initial $\delta^{13}C$ value conditions once the HPβCD applications were discontinued. The slight change in $\delta^{13}C$ values between the initial and mid-time data in the 10%-HPβCD treated soils was considered to be more rigorously represented by the ANOVA test of the initial, mid- and late-time data. Thus, the changes in $\delta^{13}C$ values measured in

the 10%-HPβCD treated soils are not statistically significant (P>0.05) at any time during the remediation experiment. The differences between datasets indicate that the 10%- and 20%-HPβCD treated soils are behaving differently during the remediation experiment, further work is required to investigate this behaviour.

This evidence shows that 20%-HPβCD solutions do alter the carbon isotopic signature of soils for a short time during the HPβCD applications and remain in the soil for a few weeks after the last application. The carbon isotopic values ($\delta^{13}C$) measured in soils during the HPβCD treatments that reflected the addition of a C_4 plant material are not retained in the isotopic values fifty weeks after the applications discontinued.

3.5.2 *Proportion of HPβCD in Soil Organic Matter Contributing to $\delta^{13}C$ Values*

The analysis completed thus far has only resolved the shifts in isotopic values of $\delta^{13}C$ after applications of HPβCD solutions to the soils. In addition to this we address the proportion of the reported carbon isotopic composition ($\delta^{13}C$ value) that is due to the addition of HPβCD. This proportion was determined using a mass balance approach described by Boutton (1996) to trace the carbon source and is given by equation 3.5.

$$\delta^{13}C_{soil} = (\delta^{13}C_{Isoil})(x) + (\delta^{13}C_{HP\beta CD})(1-x) \quad (3.5)$$

Where $\delta^{13}C_{soil}$ is the $\delta^{13}C$ measured of the organic matter from the soil samples collected by the 2002, 2004, and 2007 studies, $\delta^{13}C_{HP\beta CD}$ is the measured carbon isotopic value for technical grade HPβCD, $\delta^{13}C_{Isoil}$ is the average initial carbon isotopic value measured of the soil organic matter from Pelee, x is equal to the proportion of carbon from the

undisturbed soil organic matter ($\delta^{13}C_{Isoil}$) source, and (1-x) was the proportion of carbon contributed by the HPβCD ($\delta^{13}C_{HP\beta CD}$) source. This equation assumes that only the sources of carbon contributing to the total soil organic carbon value are from HPβCD carbon and the existing soil organic carbon.

The shift in isotopic composition ($\delta^{13}C$) observed in the 20%-HPβCD treated soils observed each week reaches a maximum at the mid-time date of September 9th 2002 of -20.2 ± 0.9‰ and indicates that 51% of the organic carbon in the soil was sourced from HPβCD, shown in figure 3.5. Once applications discontinue after September 13, 2002, the proportion of HPβCD contributing to the total organic carbon slowly decreases. In contrast, the late-time $\delta^{13}C$ value reported for November 4th, 2002, was -22.2 ± 0.5‰, which is only 27% sourced from HPβCD with regards to organic matter measured. This change in proportion of the carbon source further indicates that applications of HPβCD did alter the carbon isotopic composition of the soil organic matter. Thus, not all HPβCD solution was immediately flushed through the system as would be expected from a non-sorbing agent. Furthermore, approximately eleven months after the last application of HPβCD solutions (10%- and 20%-) there is no proportion of the organic carbon attributed to HPβCD. Therefore, while maintaining a short-term contribution to the soil organic matter, the HPβCD is not fully incorporated into soil organic matter for long periods of time (less than one year). This agrees with findings by Wynn and Bird (in review) that C_4–derived organic carbon decomposes two times faster than that of the total active soil organic carbon pool.

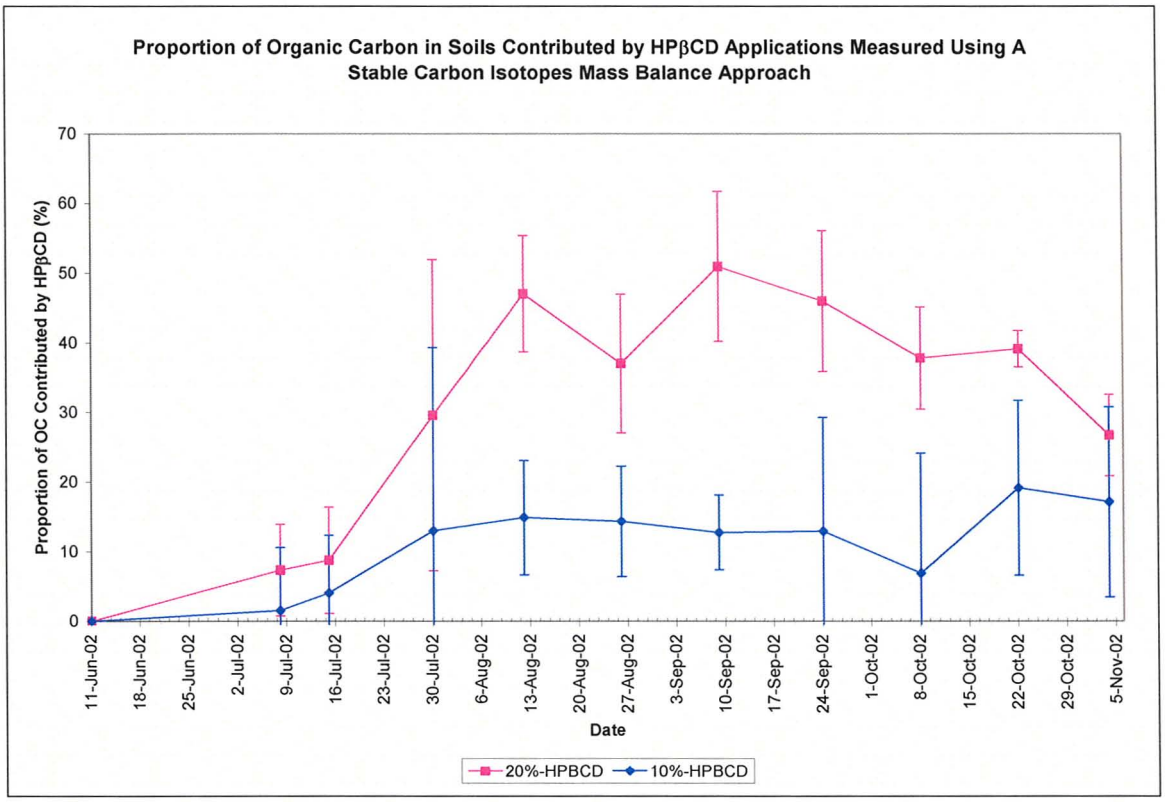

Figure 3.5. Proportion of organic carbon contributed by HPβCD applications per date of the total organic carbon isotopic value ($\delta^{13}C$) measured in soils. Proportion determined using mass balance equation described by Boutton (1996). Error bars indicate ± one standard deviation.

3.6 CONCLUSIONS

Hydroxypropyl-β-cyclodextrin provides real promise as a new remediation technology for organic contaminants in soils. This is the first study known to use stable carbon isotopes to monitor HPβCD impacts on the total organic carbon pool in soils. It has been shown by this study that stable carbon isotopic analysis can be used as an effective evaluation tool for cyclodextrins in soils over time. Stable carbon isotopic values measured of soils treated with 10%-HPβCD and 20%-HPβCD solutions were a useful indicator of HPβCD retention and persistence in the soil. The untreated soil of the former orchard area was measured to have a carbon isotopic value of $\delta^{13}C$ of -24.5 ± 1.2‰. Technical grade HPβCD was measured to have an carbon isotopic value of -16‰.

Stable carbon isotope ($\delta^{13}C$) values of the soil samples removed from the Badley (2003) remediation experiment soil plots were used to monitor the contribution of carbon to the soil from the HPβCD applications. HPβCD is produced from the enzymatic degradation of cornstarch, thus, resulting in a carbon isotopic signature of a C_4 photosynthetic pathway. Point Pelee soils were measured to have an initial untreated carbon isotopic value of -24.5 ± 1.2‰. Figures 3.1 to 3.4 display the shift in carbon isotopic values towards the HPβCD carbon source isotopic value (-16‰). These figures show that HPβCD does alter the isotopic value of the soil organic matter for short periods of time (during applications) and last for up to six weeks after applications discontinue. A statistically significant effect is seen within the $\delta^{13}C$ values of soils treated by 20%-HPβCD solutions; however, the $\delta^{13}C$ values of the soils treated with 10%-HPβCD solutions were not statistically significant. Approximately 44 and 50 weeks after

applications discontinued in the 10%- and 20%-HPβCD soils there is no evidence of the HPβCD within in the carbon isotopic signature. Therefore it is concluded that HPβCD does alter soil carbon values, however, this effect is not persistent in the soil environment at Point Pelee. It is suggested that HPβCD degrades in-situ or is flushed through the soil profile to groundwater within one year.

To further assess the impact of HPβCD on the soil carbon values, a mass balance approach described by Boutton (1996) was used to calculate the contribution of HPβCD to the soil organic carbon content. It was shown that during the application time period, HPβCD contributed up to 50% of the carbon contained within the organic carbon measured by August 13th, 2002, after eight pore volumes of 20%-HPβCD solution had been applied. Once applications of the 20%-HPβCD solution discontinued (September 15, 2002) the proportion of HPβCD decreased slowly over six weeks and had returned to initial carbon isotopic values fifty weeks after the last application was applied. The 10%-HPβCD treated soils did not show such as slow decrease as applications were continued for the full nineteen weeks of sampling. The 10%-HPβCD treated soils increased in proportions to a maximum average of 14 ± 4% by July 30th, 2002. The proportion of HPβCD contributing to the total carbon isotopic value returned to 0% approximately 44 weeks after solutions discontinued. Therefore, it has been shown that using stable carbon isotopic measurements of soils treated with HPβCD solutions is an effective tool for monitoring changes to soil organic carbon values. Furthermore, it has been shown that HPβCD is not retained within the soil after approximately fourty four weeks (less than one year) and does not have a lasting impact on the overall organic carbon pool in soil.

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CHAPTER 4
AN INVESTIGATION OF THE FATE OF HYDROXYPROPYL-β-CYCLODEXTRIN IN SOIL UNDER NATURAL FIELD CONDITIONS WITH IMPLICATIONS FOR TDR MEASURES OF SOIL WATER

4.1 ABSTRACT

Hydroxypropyl-β-Cyclodextrin (HPβCD) has recently been used and is receiving increasing interest as an enhancing remediation agent used for soils contaminated with aged hydrophobic contaminants. While being used in remediation experiments, no study has investigated the fate of HPβCD using unaided natural conditions in field soils. Hence, this paper will address the fate of HPβCD in natural field conditions of Point Pelee soils (Ah horizon of Melanic Brunisols) contaminated with aged DDT and its residues. Concentrations of HPβCD were measured over a four month pilot-scale remediation experiment using both 10%- and 20%-HPβCD solutions applied to field soils once per week for 19 and 13 weeks respectively. Concentrations of HPβCD were found to be less than 2% retained in the soils after applications ceased, thus indicating that the vast majority of the HPβCD applied passed through the soil. Data on the relationship between TDR measured water contents and microbial communities present are also presented which indicates that TDR measurements represent both free water and the water within extracellular polymeric substances (EPS) produced by microbial and bacterial communities within soils. Implications of this relative to system analyses are presented.

4.2 INTRODUCTION

The use of cyclodextrins (CDs) has received increasing attention and show promise for effective and economic methods to remediate soils contaminated with hydrophobic contaminants. Cyclodextrins are commonly used in the pharmaceutical, medical and food industries. More recently cyclodextrins have been investigated as a useful remediation technology for soils and ground water systems contaminated with hydrocarbons, PAHs, PCBs and pesticides. These non-toxic organic compounds (Saenger, 1980) are formed by an enzymatic degradation of corn starch using the enzyme cyclodextrin glycosyltransferase, commonly referred to as CGTase (Schmid, 1996). Cyclodextrins are cyclic oligosaccharides composed of several glucopyranose units. Three major cyclodextrins are industrially produced and are often referred to as the parent CDs known as: α-CD, β-CD, γ-CD. Each cyclodextrin compound has a different sized inner cavity and consists of different amounts of glucopyranose units: α-CD has 6, β-CD has 7 and γ-CD has 8 units. The cyclodextrin ring is shaped as a truncated conical cylinder similar to a lamp shade (Wang, 1993). Hydroxyl groups exist on the interior and hydroxypropyl groups on the exterior of the CD ring resulting in a very unique structure creating a hydrophilic outer shell and hydrophobic inner cavity (Wang and Brusseau, 1993). Consequently, they have an ability to form water soluble inclusion complexes with non-polar, low solubility organic compounds, therefore increasing the included contaminant solubility and bioavailability. Brusseau et al., (1994) identified that cyclodextrins behave as conservative solutes within saturated soils, i.e. do not adsorb to soil particles.

The cyclodextrin-derivative known as hydroxypropyl-beta-cyclodextrin (HPβCD) is produced industrially and consists of 7 glucopyranose units, with a fully substituted compound having 7 hydroxypropyl groups on the exterior of the CD ring (figure 4.1). It has a solubility in water of 500g/L (CTD, 2007).

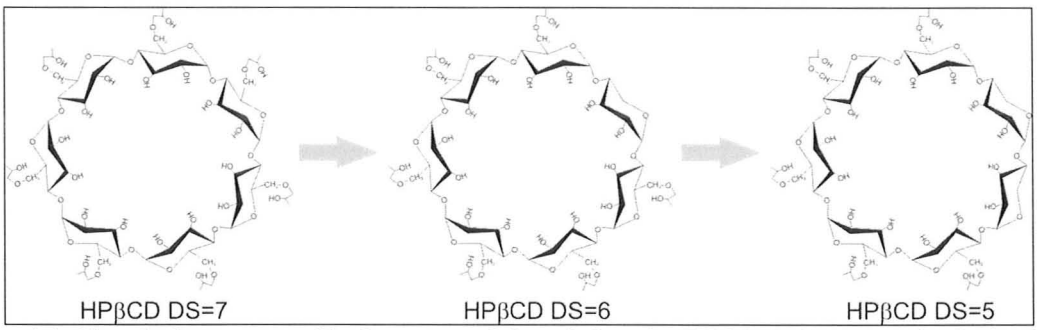

Figure 4.1. Chemical structure of hydroxypropyl-β-cyclodextrin (HPβCD). Degrees of substitution (DS) of the exterior hydroxypropyl groups are shown. Left: full substitution (DS=7), middle: loss of 1 hydroxypropyl group (DS=6), right: loss of 2 hydroxypropyl groups (DS=5). Losses of hydroxypropyl groups can occur from any glucopyranose unit thus, images shown are example only.

Several studies (Wang & Brusseau, 1993; Brusseau et al., 1994; Wang & Brusseau, 1995; Wang et al., 1999, Boving et al., 1999, 2000, Badley, 2003, Wang, 2005) have found that inclusion complexes formed between HPβCD and non-polar organic compounds leads to an increase in the solubility and mobilization of organic contaminants in soils. Aged contaminants in soils pose problems due to their lower solubility, recalcitrant properties and high toxicity. In addition to increasing solubility, CD inclusion complexes enhance the biodegradation of hydrophobic contaminants by carrying the contaminants into aqueous solutions thus increasing their bioavailability and allowing them to become more susceptible to microbial attack (Shao et al., 2003). Cyclodextrin in the soil has been shown to have no deleterious effects on present microbial communities (Alter et al., 2003) and can be used as a carbon source (Fava et al., 1998). Using the additional carbon

as a food source, microbes can increase the biomass yield and degrade organic soil contaminants (Bardi, 2000). A number of studies have addressed the enhanced bioremediation and increased mass removal of non-polar contaminants such as PAHs, PCBs and pesticides using HPβCD (Molnar et al., 2002, Wang et al., 1993, 1994, 1998, McCray and Brusseau, 1998, Fava et al., 1998, 2002).

It has been shown that microbial communities that can degrade organic contaminants in the presence of HPβCD can also degrade the HPβCD itself (Shao et al., 2003, Fenyvesi et al., 2005, Verstichel et al., 2004, Bardi et al., 2000). Slower biodegradation rates of HPβCD were found compared with unmodified-CDs after 45 days under controlled composting conditions at 58°C (Verstichel et al., 2004). HPβCD was found to fully biodegrade after 280days in controlled reactor experiments at 20°C. Bardi et al., (2000) reported an enhanced microbial population in the presence of HPβCD but no measurable HPβCD loss. To our knowledge the fate of HPβCD under natural uncontrolled field conditions has not been assessed before or reported in previous studies, nor has the efficiency of contaminant removal under natural field conditions.

This work will address the fate of technical grade hydroxypropyl-beta-cyclodextrin in field soils at Point Pelee National Park, ON, contaminated with aged DDT, DDE and DDD. A pilot-scale remediation study by Badley (2003) at the site showed the promising efficiency of HPβCD to remediate recalcitrant DDT, DDE and DDD from the soils. Given the structure of HPβCD and previous laboratory studies (Verstichel et al., 2004; Fenyvesi et al., 2005) is expected that HPβCD is readily available, and degrades in-situ in the soils

4.3 METHODS

4.3.1 *Remediation Plots*

Badley (2003) successfully designed and implemented a 20 week pilot-scale remediation grid of 3m x 3m that was subdivided into nine plots. The remediation site was located within a former orchard area in Point Pelee National Park, Ontario, Canada. The design consisted of three control plots receiving no treatments, three plots were treated with 10%-HPβCD solutions in water, and the remaining three plots were treated with 20%-HPβCD solutions to reduce DDT concentrations from the soils. Each soil plot had dimensions of 0.49m x 0.49m x 0.15m and an average porosity of 0.49 with an average bulk density of 1.0g/cm^3. Technical grade HPβCD (C*Cavitron 82006) was selected due to its substantially lower cost and was obtained from Cerestar Inc. USA.

Samples from each soil plot were collected prior to the application of one pore volume (33L, i.e. equal to soil saturated to 15cm depth) of HPβCD solution. Three replicate samples from each treatment (10% and 20% HPβCD) were collected each week. The 10%-treated soils received a total of nineteen pore volumes of HPβCD solution, whereas the 20%-treated soils received thirteen pore volumes after a substantial clogging effect, i.e. reduced infiltrability was observed. Soils were sampled and analyzed by Badley (2003) and Mironov (2004) for DDT, DDE and DDD concentrations. Soils were stored in a refrigerator at 4°C to limit microbial activity and preserve the condition of the soil. A detailed list of application scheduling can be found in Appendix 1. DDT, DDE and DDD concentrations arithmetically decreased by 90%, 77%, and 82% (Badley, 2003) and were log normally decreased by 74%, 64% and 61% (Mironov, 2004). During the

work of Badley (2003) and Mironov (2004) there was no examination of the fate of HPβCD.

4.3.2 *Analysis of HPβCD*

Chemical analysis of soils containing hydroxypropyl-β-cyclodextrin was completed by the McMaster Regional Centre for Mass Spectrometry using the following method that was developed. Samples collected by Badley (2003), Mironov (2004) and this study were extracted using standard solid phase extraction procedures. Sub-samples of the soil were milled using a mortar and pestle with ethanol washing of all equipment between samples to ensure no cross contamination. Subsequently, 500mg of soil was weighed into a 1.5ml Eppendorf tube with 1ml of distilled deionized water added. The solution was vortexed for 10 minutes and then centrifuged for an additional 10 minutes. The aqueous layer was removed with a pipette tip and placed into another 1.5ml Eppendorf tube. A vacuum manifold fitted with a Waters Oasis HLB 1cc (10mg) extraction cartridge that was flushed with 1ml methanol and then with 1ml of distilled deionized water. The aqueous layer was then loaded onto the cartridge and washed with 1ml of distilled deionized water. The 250μL of aqueous layer was then loaded onto the conditioned cartridge and washed with 1ml of distilled deionized water. The cartridge was moved to a new manifold position containing a new sample vial followed by an elution of the aqueous layer containing HPβCD with 2ml of 25%methanol:75% distilled deionized water solution. The sample was analyzed by a Waters-Micromass Quattro Ultima with Waters HPLC 2695.

4.4 RESULTS AND DISCUSSION

Soils were analyzed for technical grade hydroxypropyl-beta-cyclodextrin (HPβCD) concentrations in soils from Point Pelee National Park, Canada. A sample spectra of the technical grade HPβCD from the LCMS analysis is shown in figure 4.2. The ion peaks at 1558, 1500 and 1442 mass units had the greatest response and have been selected to represent the bulk "total" HPβCD measured. Each of these ion peaks represents a loss of a hydroxypropyl group (58 mass units) from the HPβCD structure. The position on the ring of removed hydroxypropyl groups has not been identified; rather the removal is identified through the mass units lost. The ion peak of 1558 is the fully substituted technical grade HPβCD structure with seven hydroxypropyl groups attached to the exterior of the cyclodextrin structure as shown in figure 4.1. The ion peaks ranging from full a full degree of substitution (DS=7) to a lower degree of substitution of 5 hydroxypropyl groups will hereafter be referred to as DS7, DS6, and DS5. The less substituted structures of DS4 and DS3 hydroxypropyl groups were identified during analysis, but appeared intermittently as very low concentrations and were not consistent between replicates; therefore they have been disregarded for this discussion. A spectra from a soil sample from the remediation experiment is given in figure 4.3.

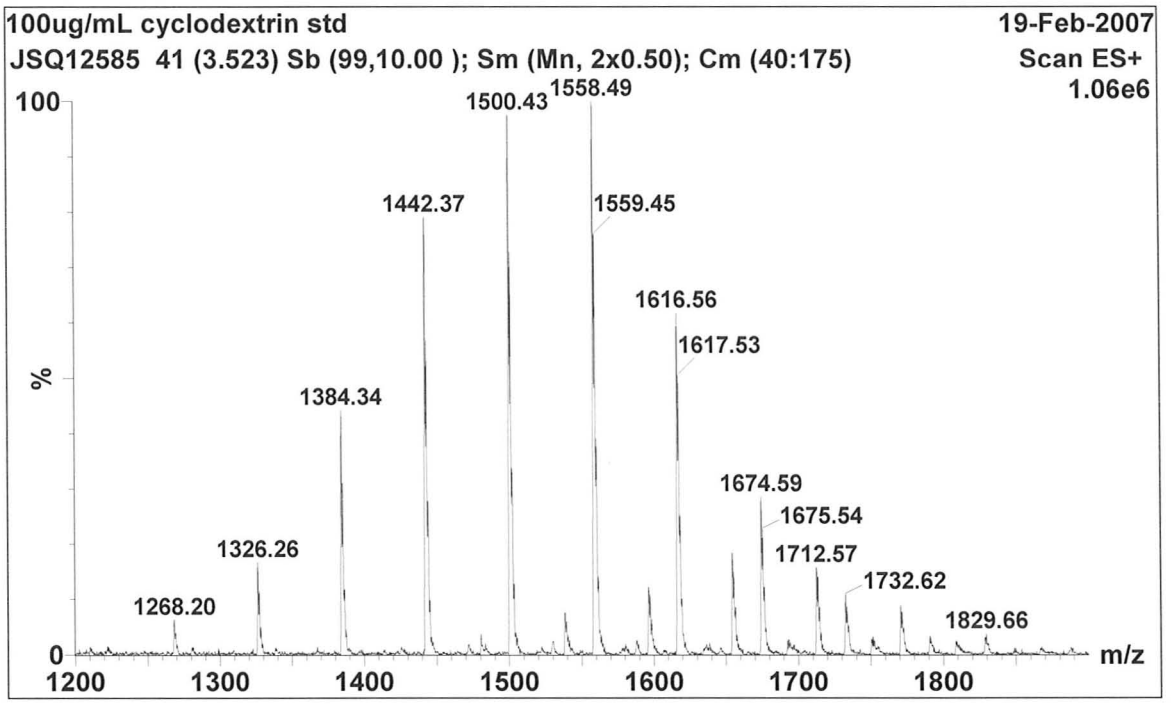

Figure 4.2. Example spectra from LCMS analysis for technical greade hydroxypropyl-β-cyclodextrin.

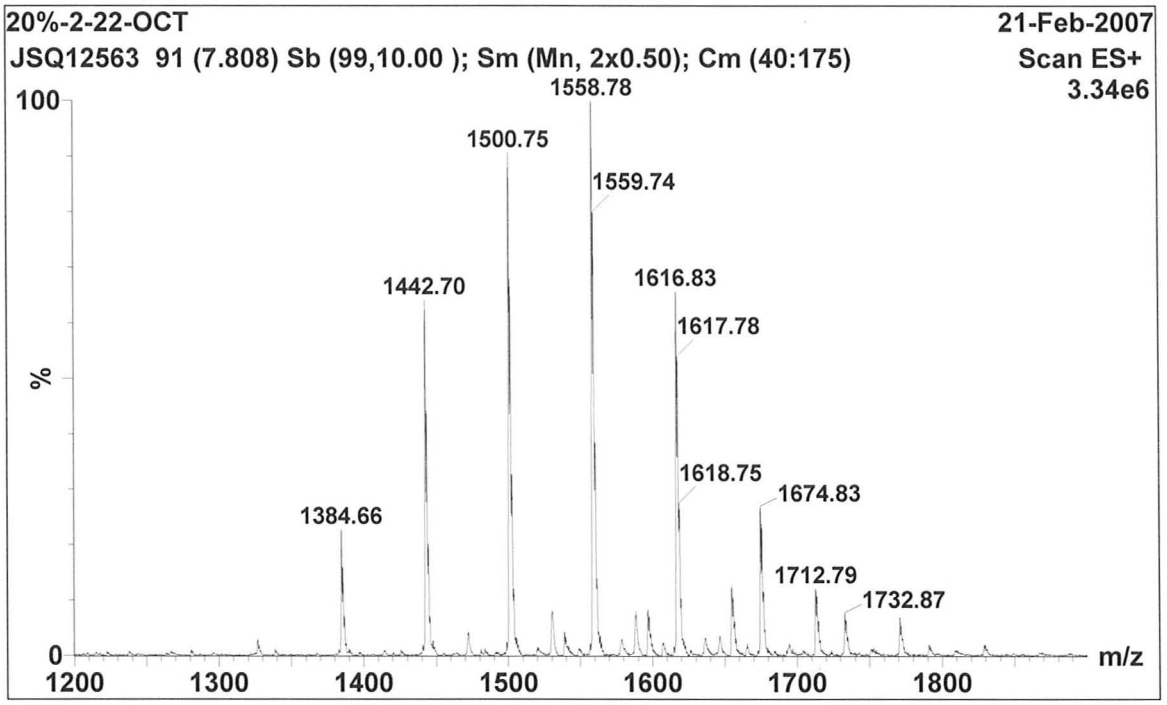

Figure 4.3. Example spectra from LCMS analysis of soil sample treated with 20%-HPβCD solution.

Consequently this research will deal with DS7, DS6, DS5 concentrations which behaved similarly to each other and showed no systematic trends to indicate that one was higher or lower than the others. Fenyvsi et al., (2005) found that more substituted forms of CDs were more resistant to degradation such that the parent CDs were degraded faster than HPβCD and RAMEB (randomly methylated cyclodextrin). This trend was not observed for different degrees of substitution for HPβCD in this study (data not shown). Table 4.1 identifies the ions observed and associated names and masses for each.

Table 4.1. Summary table describing ions measured using LCMS and the corresponding degree of substitution (DS) of hydroxypropyl groups to the exterior of the HPβCD molecule.

Ion (mass units)	I.D.	Hydroxypropyl Groups
1558	DS7	7
1500	DS6	6
1442	DS5	5
1384	DS4	4
1326	DS3	3

Three replicate soil samples were collected from each treatment plot once per week prior to the next treatment application of 20%-, 10%-, or 0%-HPβCD solution. A total of nine soil samples were collected each week, even after 20%-HPβCD treatments were discontinued after 14 weeks. The concentration of ions DS7, DS6, DS5 were standardized relative to technical grade HPβCD then averaged to obtain the measured total HPβCD concentration for that date. Using the total HPβCD concentration per sample, the average of the three samples receiving the same treatment was taken to represent the total HPβCD concentration on a given sample date. It is noted that soil samples from plot 10%-2 were substantially inconsistent with plots 10%-1 and 10%-3 and interpreted as outliers and therefore not included in the average calculation, resulting

in the 10%-treated soils as a duplicate sample average. The average total HPβCD concentration will be referred to as the HPβCD concentration for the remaining discussion.

Figure 4.4 shows the HPβCD concentrations in soil over time for both 10%- and 20%-HPβCD treated soils including the 95% confidence intervals. The 10%-HPβCD soils do not exhibit a substantial change or clear systematic trend with a mean concentration of 600μg/g soil after two pore volumes of treatment were applied. Similar evidence is shown by figure 4.5, indicating that the 10%-HPβCD soils exhibit variability but do not accumulate HPβCD mass with each additional application of HPBCD solution. Mass of HPβCD shown in figure 4.5 was calculated by multiplying the volume of soil in the plot, the bulk density and HPβCD concentration measured after each application. In contrast the 20%-HPβCD treatment plot soils do show a systematic increase in concentration over 15 weeks (figure 4.4b). The last application of 20%-HPβCD solution was applied in week 14, totaling 13 pore volumes of HPβCD applied to the soil. After treatments were ceased, the HPβCD concentration remained relatively constant for 5 weeks after which there was a significant decrease between week 19 and week 21.

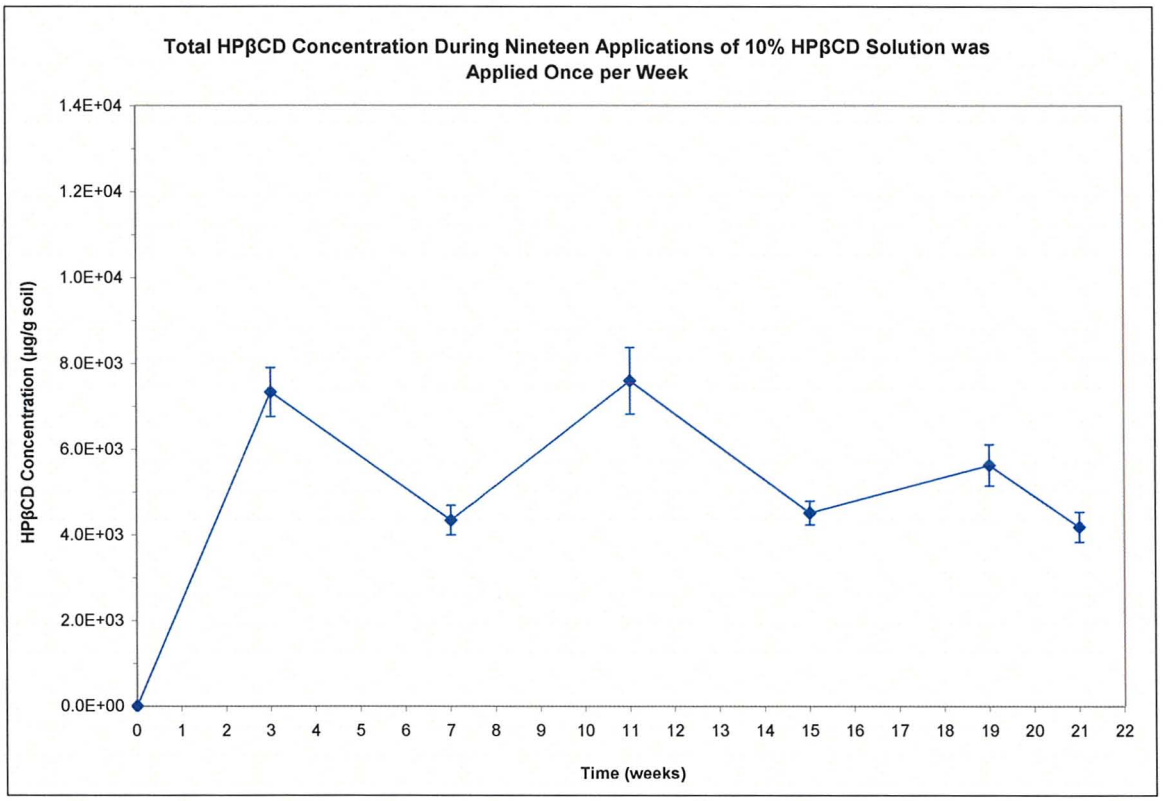

Figure 4.4a. Total HPβCD concentration in soils after 20 weeks of 10%-HPβCD solution was applied once per week with a total of 19 treatments (beginning in week 3). Error bars indicate a 95% confidence interval of total HPβCD concentrations.

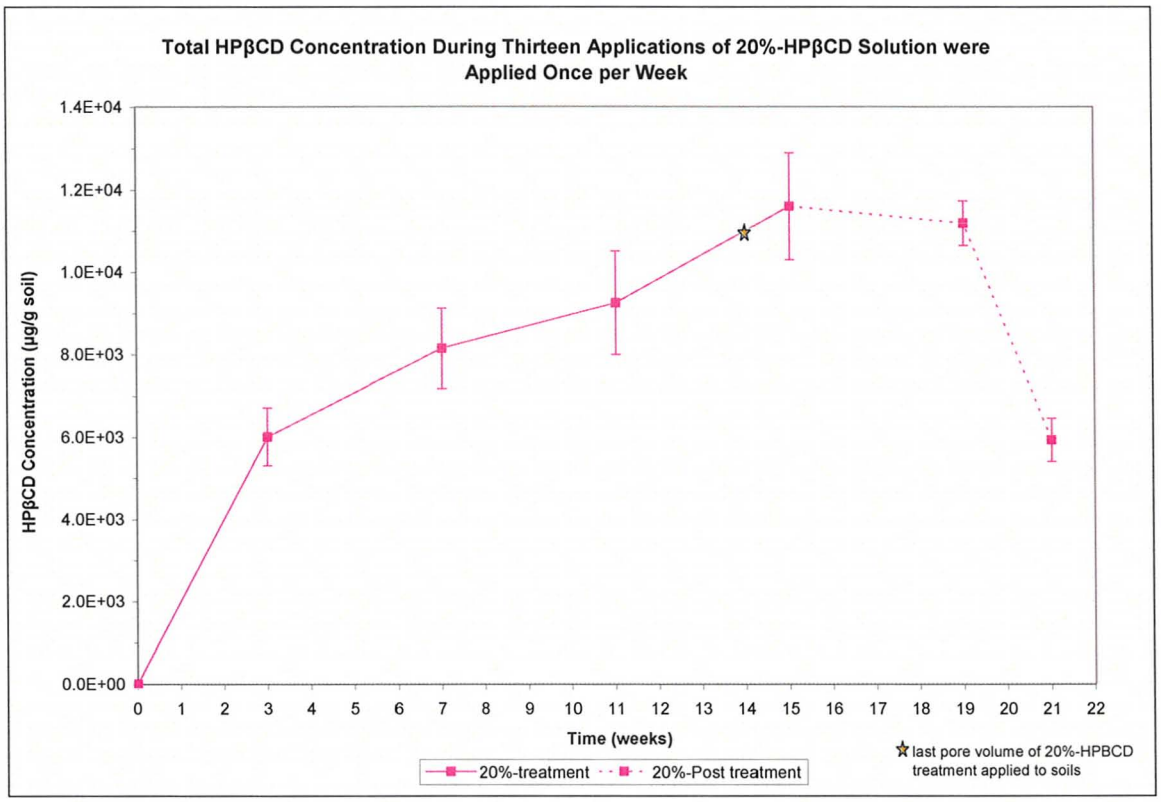

Figure 4.4b. Total HPβCD concentration in soils after 13 pore volumes of 20%-HPβCD solution was applied once per week beginning in week 3 and ending in week 15. Error bars indicate a 95% confidence interval of total HPβCD concentrations.

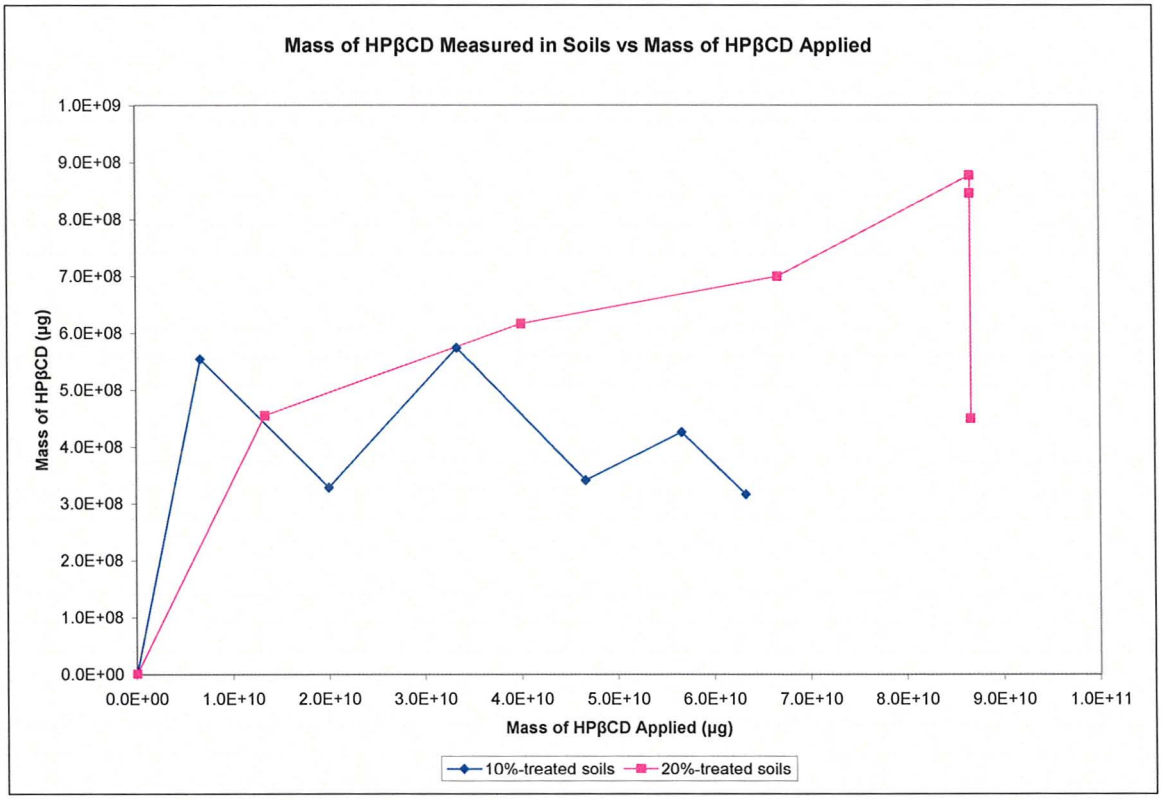

Figure 4.5. Mass of HPβCD measured from soil samples removed every two weeks in comparison with the mass of HPβCD applied in solution.

Samples were also collected after 63 weeks which is 44 weeks after the last application to the 10%-HPβCD soil plots and 50 weeks after the last application to the 20%-HPβCD soil plots. These samples showed no evidence of the technical grade HPβCD in the soil samples. This data provides evidence that HPβCD does not persist in the soil 44 weeks after the last application.

The HPβCD was applied as an aqueous solution of HPβCD. The moisture content of the soil treatment plots was measured in-situ weekly using a 20cm long Time Domain Reflectometry (TDR) probe prior to the applications of HPβCD to the soils by Badley (2003). The moisture contents shown in figure 4.6 were measured for 21 weeks including the period of the thirteen (20%-) and nineteen (10%-) pore volumes of HPβCD solutions applied. The moisture content shows a clear systematic trend of larger volumetric moisture content in the 20%-treatment plots than in that of the 10%- or control-treatment soils. Infiltration of precipitation events could play a role by displacing the HPβCD solutions downward through the soil profile, thereby decreasing the HPβCD concentrations in soil waters. This could be expected particularly since Brusseau et al., (1994) has shown HPβCD to behave as a conservative solute. The precipitation data for the study site are displayed in figure 4.6 (Environment Canada, 2007). It is noteworthy that the precipitation even on September 20th 2002 of 49mm was sufficient to flush 0.05m depth of the soil which is 33% of the depth profile of interest (0.15m). The HPβCD concentration did not drop after this precipitation event as expected with an increased volume of water to transport the HPβCD through the profile, rather the concentration increased over the previous treatment. If HPβCD was behaving like a conservative solute

within the soil profile then the precipitation event of September 20th could have had enough time to drain, yet the sampling date of September 24th still shows a small concentration of HPβCD (figure 4.7). This is the first field evidence to suggest that HPβCD may not behave as a conservative solute in unsaturated soils contrary to the work of Brusseau et al. (1994) in saturated soils. In addition to not fully displacing HPβCD the precipitation event did not result in significant moisture content increase or decrease in the soils. The 20%-HPβCD soils received no further treatments after this precipitation event; however, the 10%-treated soils did show a significant increase in moisture even after the next treatment on September 23rd, 2002.

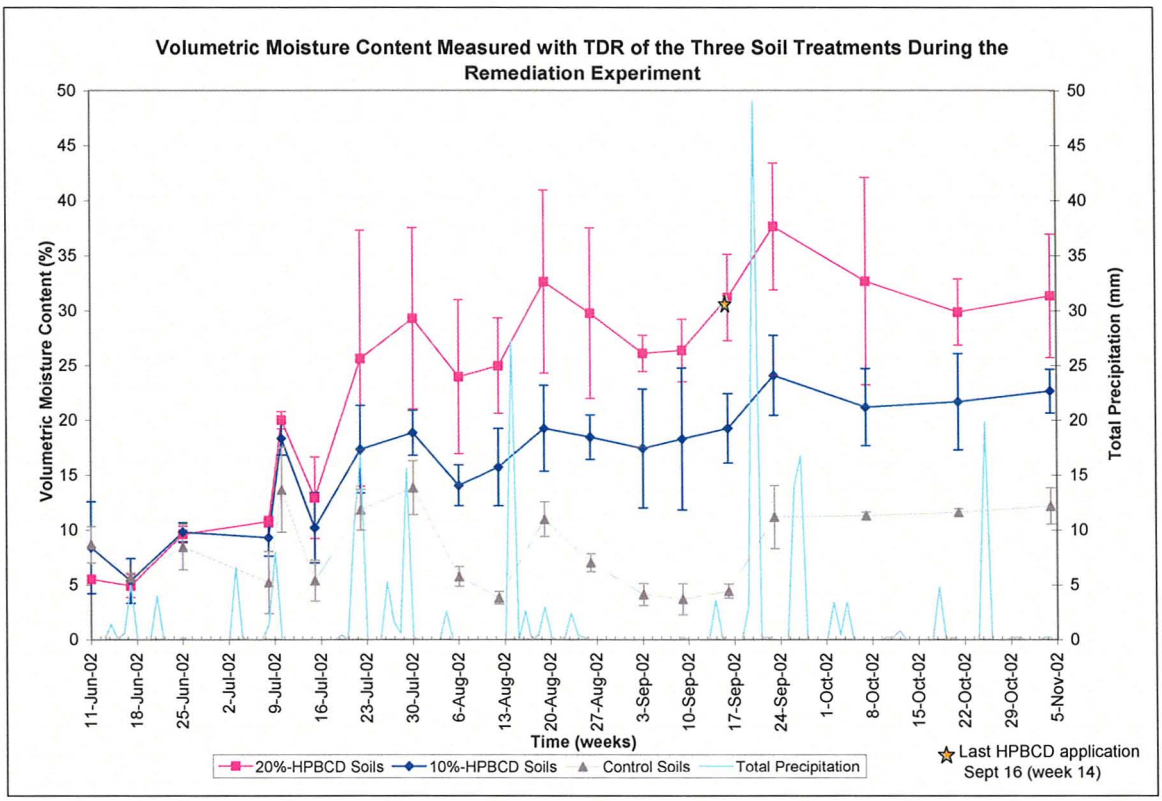

Figure 4.6. Volumetric moisture content measured by Badley (2003) in-situ using TDR. Star indicates the last application date of 20%-HPβCD solution at September 16th, 2002 (week 14). Precipitation data shown was provided online by Environment Canada (2007).

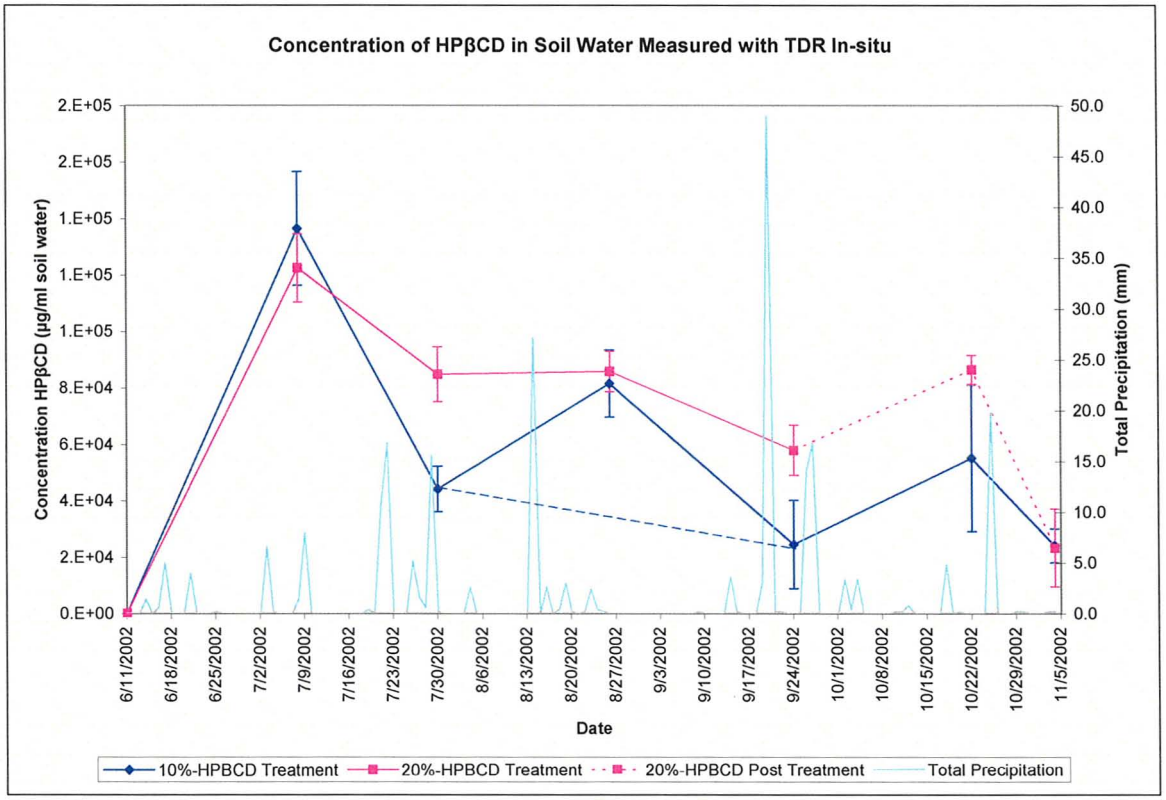

Figure 4.7. The concentration of HPβCD in soil water during the remediation experiment (applications started June 18th). Error bars indicate ± one standard deviation (three samples).

Further investigation of the HPβCD concentration in soil waters shown in figure 4.7 reveals that at no point do the 20%-HPβCD treated soils suggest that flushing by precipitation has played a significant role. Concentrations of HPβCD in the 20%-treated soils increase between the last application date (September 15th, 2002) and the sample date of October 22, 2002. During this time small precipitation events did occur, but did not have great flushing potential. After the precipitation events of July 22nd and 29th, 2002, the 20%-HPβCD treated soil shows a substantial increase in moisture content on July 30th, 2002, whereas the 10%-HPβCD treated soil shows only a relative increase compared to the control-soils moisture content. During this time, the HPβCD concentrations in both the 20%- and 10%-treated soils are maintained or increase, prior to the next HPβCD application. This again does not reflect conservative solute behaviour. By July 30th, 2002, the 20%-HPβCD treated soils hold three times as much water as the control soils and the 10%-HPβCD treated soils hold approximately twice as much water, however, the HPβCD concentration remains in the soils. Therefore, there is no significant decrease in HPβCD concentration to reflect a dilution effect due to precipitation.

Evapotranspiration (ET) is an important part of the water cycle and occurs from bare soils and soil covered with grasses such as the Point Pelee remediation site. Mills (2004) estimated that daily evapotranspiration from the surface of the remediation plots was equal or less than 6.5mm/day at peak times during the summer months. The average evapotranspiration value was approximately 4mm/day. The ET values were calculated using the Thornthwaite (1948) method for a grass covered field, which uses the mean

monthly air temperature, the annual heat index and assuming sunshine for a 12 hour period each day. There is no evidence in the HPβCD masses of a concentrating effect due to evapotranspiration. Also, this water loss is not enough to substantially affect the potential HPβCD flushing abilities of the recorded precipitation events. Therefore, evapotranspiration from the soil is considered to play a negligible role relative to the fate of HPβCD.

In addition to the increase in moisture content in the 10%- and 20%-treatment plots, there was a noted decrease in field saturated hydraulic conductivity (K_{fs}). The decrease in hydraulic conductivity measured in-situ with a Guelph permeameter and observed increases in infiltration times of applied solutions are an indication of blocked pores and were attributed to the clogging the soil pores by HPβCD enhanced bio-clogging caused by increased biologic activity (Badley, 2003; Smith, 2004). In addition, DAPI staining of the soils revealed an increase in microbial counts for both the 20%- and 10% treated soils. Further work by Smith (2004) showed similar trends in laboratory columns with similar Point Pelee soils receiving one pore volume of 20%-HPβCD treatment once per week. The abiotic soil columns were treated with a sodium azide solution along with HPβCD treatments and did not show an increase in moisture content or decreased infiltration rates. The columns which were treated only with HPβCD solution (no biocide) did show a significant moisture increase and decreased infiltration rates thereby establishing that microbial activity played a significant role in the soil profile when treated with HPβCD solution. It has been identified in literature that extracellular polymeric substances (EPS) excreted by microbes to protect against

desiccation can increase the water-retention capacity of soils while decreasing the hydraulic conductivity (Vandevivere and Baveye, 1992; Chenu, 1993; Rockhold et al., 1992; Wiebe et al., 1996; Or et al., 2007). Microbial communities grow at the air-liquid interface and attach onto mineral particles, thus, blocking formerly open pore spaces and reducing the hydraulic conductivity resulting in reduced flow paths for water to move freely in the soil profile and cause reduced infiltration rates (Yarwood et al., 2006). The reduced hydraulic conductivity and infiltration noted in the field and laboratory conditions along with the increase in moisture content can be attributed to microbial communities stimulated by HPβCD treatments (Smith, 2004). The TDR measured higher moisture content retained due to the presence of a microbial community has reduced the flushing efficiency of the HPβCD solution. That is, in this study the HPβCD solution did not behave as a conservative solute as reported in previous studies (Brusseau et al., 1994). The microbially mediated hydraulic properties of the soil played a significant role in retaining HPβCD within the targeted depth interval.

Time domain reflectometry (TDR) is a geophysical method used to measure the apparent dielectric permittivity in soils which is well correlated to soil water content. Each component in soils: air, mineral particles and water have a different dielectric permittivty (ε^{β}_{b}). The dielectric permittvity of air (ε^{β}_{a}) is ~1, mineral matter (ε^{β}_{s}) varies between 3 and 5, and water (ε^{β}_{w}) ~80. As soil wets and dries the bulk apparent permittivity of the soil changes. Using the universal relationship described by Topp (1980) the apparent dielectric permittivity can be related to the volumetric water content (θ) of the soil:

$$\theta v = - 5.3 \times 10^{-2} + 2.92 \times 10^{-2}\varepsilon_b - 5.5 \times 10^{-4}\varepsilon_b + 4.3 \times 10x^{-6}\, \varepsilon^3{}_b \qquad (4.1)$$

TDR was used by Badley (2003) to measure the water content of the soil plots during the remediation study. The HPβCD was applied as a water solution to the soils and is measured by TDR as free water. Smith (2004) measured the apparent dielectric permittivity of a 20%-HPβCD solution using a two-probe TDR setup in each column and monitoring it with TACQ software. A correction factor was calculated for the differences observed in TDR measured dielectric permittivity values of water and cyclodextrin solution. Using the modified Topp (1980) equation the correction factor was calculated by dividing the pure water equation (4.2) by the cyclodextrin solution equation (4.3) as both permittivity values were measured under controlled conditions. A correction factor of 1.11 was calculated for cyclodextrin solutions by Smith (2004).

$$\theta v = [\varepsilon_\beta{}^{1/2}\text{-}(2\text{-}n)/(\varepsilon^\beta{}_w\text{-}\varepsilon^\beta{}_a)] \qquad (4.2)$$

$$\theta v = [\varepsilon_\beta{}^{1/2}\text{-}(2\text{-}n)/(\varepsilon^\beta{}_{CDw}\text{-}\varepsilon^\beta{}_a)] \qquad (4.3)$$

This correction factor cannot be applied to TDR moisture contents measured in the field as the conditions were not controlled and other unknown factors cannot be accounted for by this study. It is important to note that increased moisture contents measured in the laboratory controlled biotic columns reflect the measured increased moisture contents under field conditions whereas the abiotic columns did not reflect the field study conditions.

Since the HPβCD was applied as an aqueous solution and HPβCD does not adsorb onto mineral particles or soil organic matter, it is expected that the HPβCD should reside in the soil water phase. The mass of HPβCD measured in the soil (μg/g soil)

shown in figure 4.5 was converted to mass per volume of soil water by dividing the concentration in soils with the moisture contents measured by TDR in the field. The HPβCD expressed as held in the soil water is shown in figure 4.7. This reveals an increase in HPβCD concentration (μg/ml), followed by a systematic decrease in both 10%- and 20%-HPβCD treated soil waters over time. Since the concentration of 20%-HPβCD applied to the soils was double that of the 10%-solution the concentration as expressed in soil water for the 20%-treated soils should exhibit twice that of the 10%-HPβCD treated soils. This was not consistently the case; the relationship between the 10%- and 20%-HPβCD treated soils in figure 4.7 suggests that other processes (factors) are significant. The behaviour displayed in figure 4.7 further suggests that the TDR measurement of soil moisture content (figure 4.6) is not only representing the solution of HPβCD applied.

Water in soils is considered to be free water or water that is able to move unimpeded through the system and can be expressed by the soil moisture characteristic function curves. Work by Smith (2004) indicated that the additional water observed in the presence of HPβCD solution is a direct consequence of the presence of a significant and substantial microbial community. The TDR moisture contents are addressed relative to the presence of a microbial community by applying a first order approximation that the average moisture content of the control soils represents the free water content in all soil plots. This approximation makes the explicit assumption that the difference between TDR measures of water content of the control soils and the treatment soils is entirely due to relatively immobile water held with in the EPS or closely associated with the microbial

community. This strongly suggests that the moisture contents of the 10%- and 20%-treated soils shown in figure 4.6 do not only represent free water within the soil. After applications discontinued at week 14 for the 20%-HPβCD soils, the moisture contents remain at a steady value of approximately 31%. Figure 4.7 exhibits approximately twice as much HPβCD held in the 20%-treatment soils compared to the 10%-treatment soils with the exception of a single data point. Therefore, the HPβCD content held in soil water does not support that TDR is providing a direct measure of the free soil water.

There is no increase in HPβCD concentrations even after continuous applications over 21 weeks. There is no indication that HPβCD is substantially accumulating in the soils over time (figures 4.5 and 4.7) and therefore it must be passing through the soil profile. Figure 4.8 shows the concentration is approximately 10% per application concentration ($1.0x10^5$µg/ml). However, the 20%-HPβCD soils are not at a relative expected HPβCD concentration level of $2.0x10^5$µg/ml. Not all the soil water is free water but may include water within EPS (Rockhold et al., 2002; Smith, 2004; Or et al., 1997). Rockhold et al., (2002) used a simple composite media model to represent systems containing both free water and EPS held water. Using a sand media similar to that of Pelee soils, the porosity of a porous sand medium with attached biomass phase (θ_s^{comp}) can be calculated from equation 4.4:

$$\theta_s^{comp} = \theta_s^{sand} - n_f(1 - \vartheta_s^{bio}) \tag{4.4}$$

where n_f is equal to the volume of attached biomass divided by the volume of porous media and θ_s^{bio} is the assumed porosity of the attached biomass. Using equation 4.4, the volumetric moisture content (θ_w^{comp}) of the composite porous medium can be calculated

as a function of pressure head (Ψ), where θ_w^{sand} and θ_w^{bio} are the volumetric moisture contents of the sand and biomass medias respectively. For the system studied here, we make the inherent approximation that θ_w^{comp} equals θ_w^{TDR}, i.e. the water content measured with TDR is the total composite water content. While this does not take into account that the HPβCD solutions have slightly lower dielectric permittivity discussed earlier, it is a valid first order approximation. Using equation 4.5, the hydraulic conductivity of the media can be calculated from equation 4.6 using an empirical parameter *b* to indicate relative saturation conditions.

$$\vartheta_w^{TDR}(\psi) = \vartheta_w^{sand}(\psi) - n_f\left[\left(\frac{\vartheta_w^{sand}(\psi)}{\vartheta_s^{sand}}\right) - \vartheta_w^{bio}(\psi)\right] \tag{4.5}$$

$$K^{TDR}(\theta_w^{TDR}) = K^{sand}(\vartheta_w^{sand})\left[1-\left(\frac{n_f}{\vartheta_s^{sand}}\right)\right]^b + K^{bio}(\vartheta_w^{bio})n_f \tag{4.6}$$

Use of equation 4.6 requires knowledge of the hydraulic conductivities of both the original porous media and the attached biomass phases.

To further assess the TDR measured water content, figure 4.8 shows that the relative percent of applied HPβCD present in the soil does not vary from approximately 10% even though the moisture contents increase substantially. If TDR measured water content was correct, the amount of HPβCD mass content present in the 20%-treated soils should be four times greater than the 10%-treated soils when there is twice as much water.

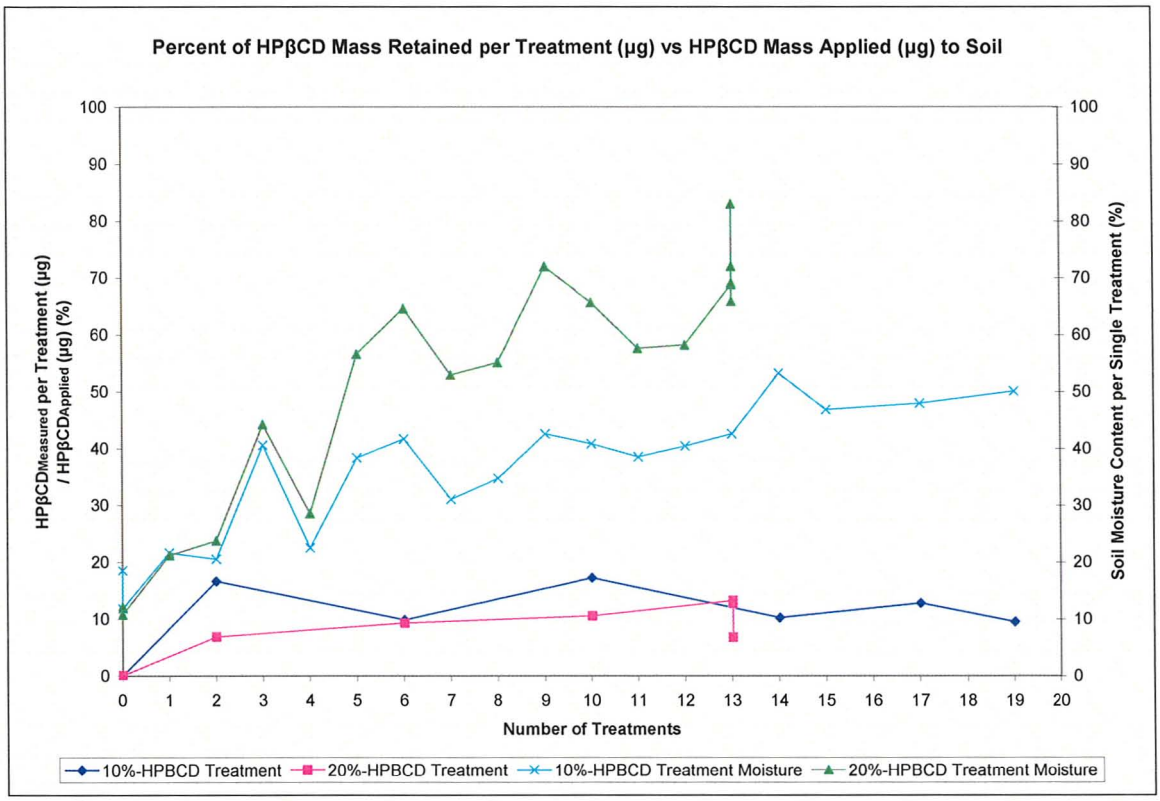

Figure 4.8. The percentage of HPβCD mass retained per HPβCD solution treatment applied for the number of treatments indicated. The moisture per treatment at that time of HPβCD solution is given (right). The 10%-HPβCD soils received 19 pore volume treatment of HPβCD solution, and the 20%-HPβCD soils received 13 pore volume treatments of HPβCD solution.

Referring back to figure 4.5 recall that the systematic increase in moisture content relative to the applied amount does not see a systematic increase in HPβCD retained in the soil. This is consistent with the condition that the TDR measured water content is expressing both free water and bio-water (EPS), not only soil free water containing HPβCD.

The 10%-treated soils shown in figure 4.8 reach a steady HPβCD mass at early times and do not accumulate HPβCD mass greater than 12% of the mass applied per a single treatment. The 20%-treated soils reached a similar steady state of approximately 10%. There is evidence of the microbial community shift and/or growth by a measured increase in organic matter, measured by Badley (2003) using loss on ignition. The concentration of HPβCD in soils remains relatively constant over time ~10-12% which indicates that a small amount of HPβCD solution is being retained in the soil. To further examine the fate of HPβCD in the soil, figure 4.9 shows the relative amount of HPβCD measured in the soil samples (μg) per total HPβCD mass applied per treatment (μg) in comparison to the total HPβCD mass applied (μg) after 19 and 13 pore volumes respectively. Data shown in figure 4.9 reflects the HPβCD mass as a total whereas the data presented in figures 4.7 and 4.8 is representative of one application. This figure further indicates that HPβCD did not remain in the soil profile. The time between sampling and the early time applications is considered to be too short for significant microbial degradation of HPβCD. Fenyvesi et al., (2005) reported a slow degradation of HPβCD with less than 20% degradation after 25 days. Therefore it is suggested that the HPβCD mass was translocated through the soil profile and was not contributing

significantly to increased moisture contents. From this it is concluded that of the total mass of HPβCD applied only 0.6% remained in the 20%-treated soils. After 21 weeks, the proportion of HPβCD remaining in the soil is ≤1%, and ~99.5% of the mass applied was no longer in the soil. Figure 4.9 supports that initially approximately 8% of the HPβCD mass applied remains in the soil, therefore most of the HPβCD applied passed through the soil profile. When expressed as a proportion of the total amount applied there is an observed decrease in mass overtime. After the initial first 2 treatments there is less than 2% at any time with a decreasing trend. This clearly indicates that applying more to the soil does not lead to an accumulating trend or increased retention. Rather, the soil is retaining less than 2% of what is applied. Although there is a continuous application once per week, there is no proportional observed accumulation of HPβCD over time. The vast majority of HPβCD solution applied was not retained in the soil therefore it is reasonable to conclude that a greater portion of HPβCD solution applied was drained from the soil profile to ground water at the water table which was approximately 2.5m (Mills, 2004) below ground surface.

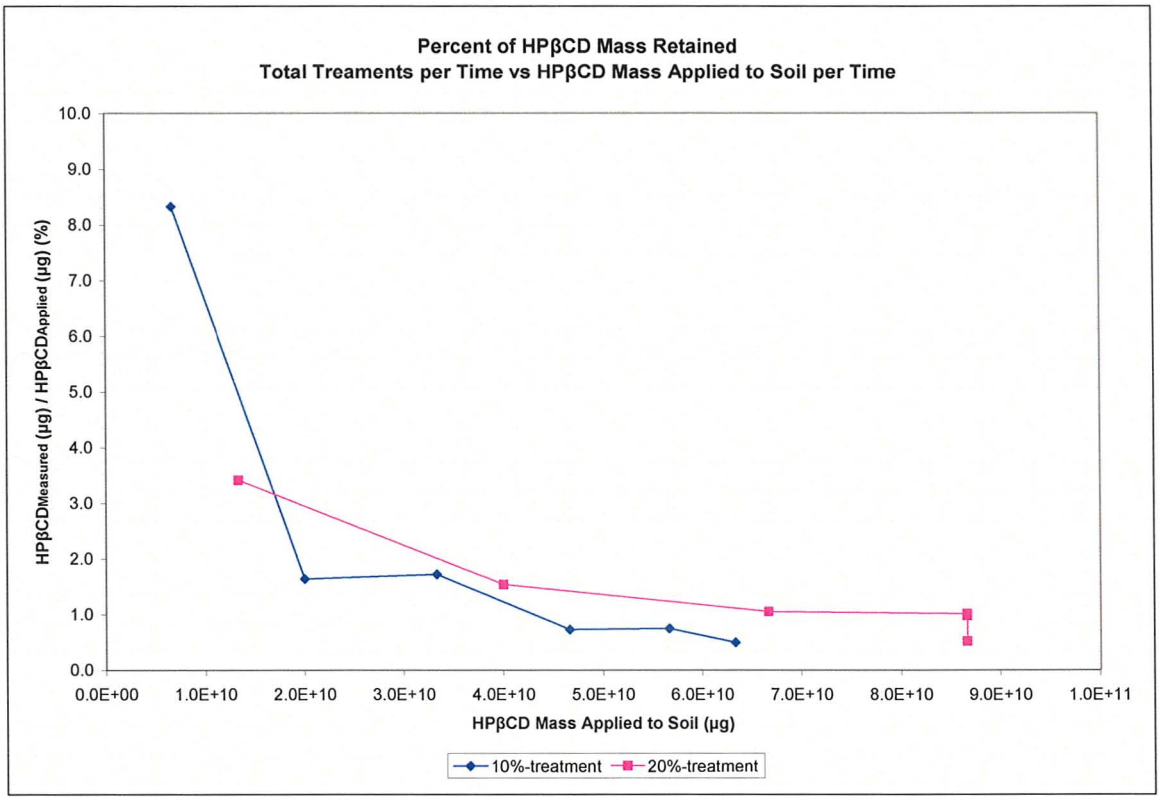

Figure 4.9. The percent of HPβCD mass retained in soils after cumulate total treatment per date for the HPβCD mass applied to the soils per date.

4.5 CONCLUSIONS

Hydroxypropyl-β-cyclodextrin (HPβCD) is a promising remediation agent to remove hydrophobic contaminants from the upper soil layer and thus away from the most biologically active depths in soils. HPβCD has been found to be an effective remediation agent for compounds such as DDT and other hydrophobic compounds such as PAHs, and hydrocarbons. This study expected that hydroxypropyl-β-cyclodextrin was degraded in-situ within the soils at Point Pelee National Park, ON and samples collected approximately 11 months post treatment had no residual HPβCD.

It has been identified that LCMS analysis of cyclodextrins can be used to estimate the concentration of hydroxypropyl-β-cyclodextrin in soils. This study has found that HPβCD does not persist in the soil at Point Pelee and therefore is biodegraded or translocated in the soil profile relatively soon after application ceases. There is less than 2% of the HPβCD mass applied retained in the soil profile after nineteen weeks of weekly application for the 10%-HPβCD solutions and thirteen weeks of 20%-HPβCD solutions to the soils. The effects of precipitation and evapotranspiration on HPβCD concentrations have been identified as negligible; therefore the majority of the HPβCD solution has drained to ground water or been biodegraded in-situ. The microbial communities could not have been stimulated enough in the short time period (one week) between treatment and sampling to degrade such a large amount of HPβCD and therefore the HPβCD losses are attributable to drainage through the soil profile.

This work has shown that while the proportion of HPβCD retained is small, that which is retained is more persistent than a non-sorbing "conservative" solute. That

persistence is at least in part due to the bio-clogging caused by the associated stimulated microbial community.

In addition to HPβCD concentrations an unanticipated conclusion of this work is that TDR measures of soil water content are not first order accurate measures of free soil water. While it is often assumed that TDR can represent free water it has been determined by this along with other studies (Rockhold et al., 2002; Smith, 2004; Or et al., 2007) that a composite media model is needed to better represent soils when significant microbial growth is present or stimulated. It has been determined that the TDR measured moisture in the soil profile of the remediation plot soils does not represent a useful expression of the actual free water. This study has identified that the TDR moisture content is not only measuring free mobile water but includes that of the water held by or associated with the EPS produced by microbial communities.

4.6 FURTHER WORK

From this study it is apparent that LCMS analysis is a useful technique for quantifying cyclodextrins in soils. Further method development in this area, will allow for more exact quantification and monitoring of cyclodextrins in soils. This study has identified that only a small proportion of the hydroxypropyl-beta-cyclodextrin applied is retained in the soil or soil waters even with continuous weekly applications. Thus, further investigation into the concentration of HPβCD solutions applied ie. 1% w/w would be more cost effective with similar expected remediation effects. Given the small proportion retained in the surface soils, an investigation into the fate of HPβCD in the

ground water system will provide further information regarding the fate of HPβCD in the subsurface. Given that both TDR and gravimetric techniques provide measures of "total" soil water, it is highly desirable that further work be conducted to investigate and develop a new method to measure free water within the soil profile in the presence of a significant microbial community.

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CHAPTER 5
DISCUSSION OF THREE METHODS USED TO ANALYZE HPβCD IN SOILS

5.1 INTRODUCTION

This study has reported on stable carbon isotopic analysis and liquid chromatograph mass spectrometry (LCMS) as two methods of analysis to assess the persistence of hydroxypropyl-β-cyclodextrin in treated soils. Loss on ignition was used by Badley (2003) to assess the changes in organic matter content due to the applications of HPβCD. Each of these methods of analysis has resulted in unique results of HPβCD retained in the soil and will be further discussed in comparison with each other. Figures 5.1 and 5.2 demonstrate the percent of HPβCD within the soil organic matter at an early time of July 8, 2002. These soils had received two pore volumes of both 20%- and 10%-HPβCD solutions. Figures 5.3 and 5.4 demonstrate the percent of HPβCD within the soil organic matter at a mid-time of September 24, 2002. This time reflects the highest amounts of HPβCD within the soil. These soils had received thirteen and fourteen pore volumes of 20%- and 10%-HPβCD. This sample date is one week after the last application of HPβCD solution to the 20% soil plots. The late-time data of November 4, 2002 is given in figures 5.5 and 5.6; these soils had received thirteen and nineteen pore volumes of 20%- and 10%-HPβCD solutions.

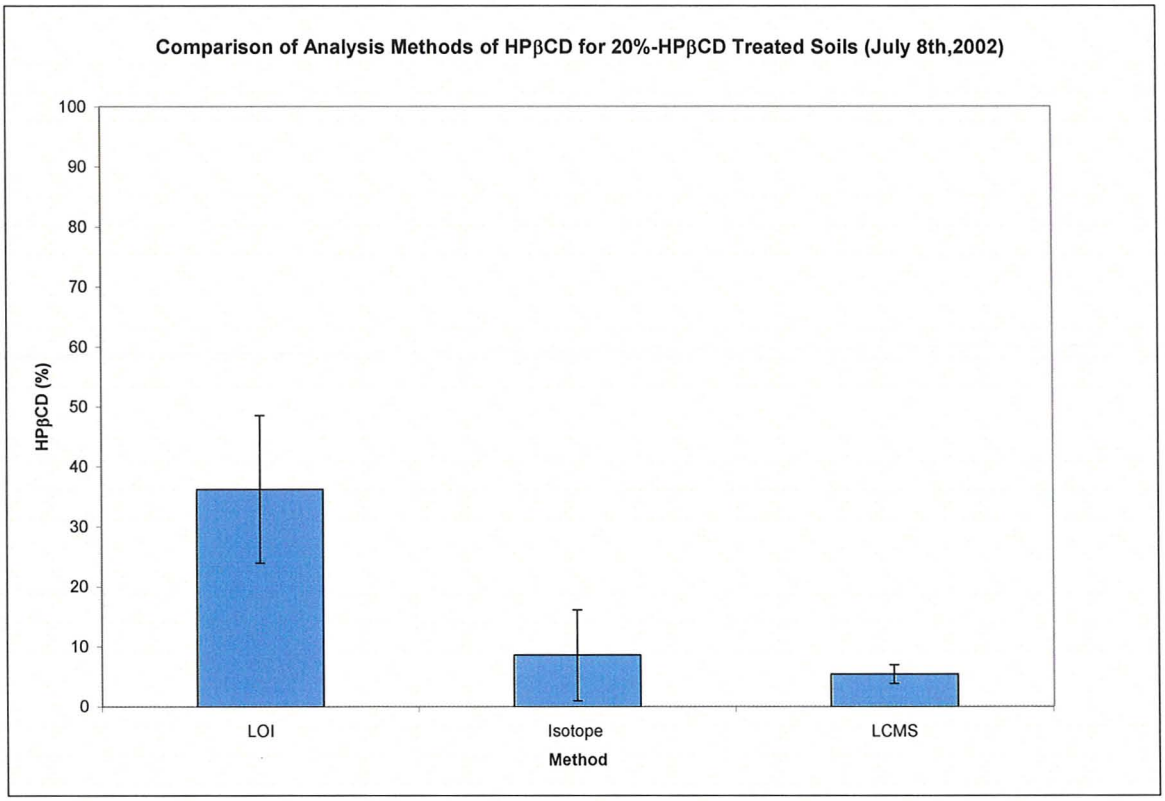

Figure 5.1. Percent of HPβCD retained in the 20%-HPβCD treated soils on July 8, 2002.

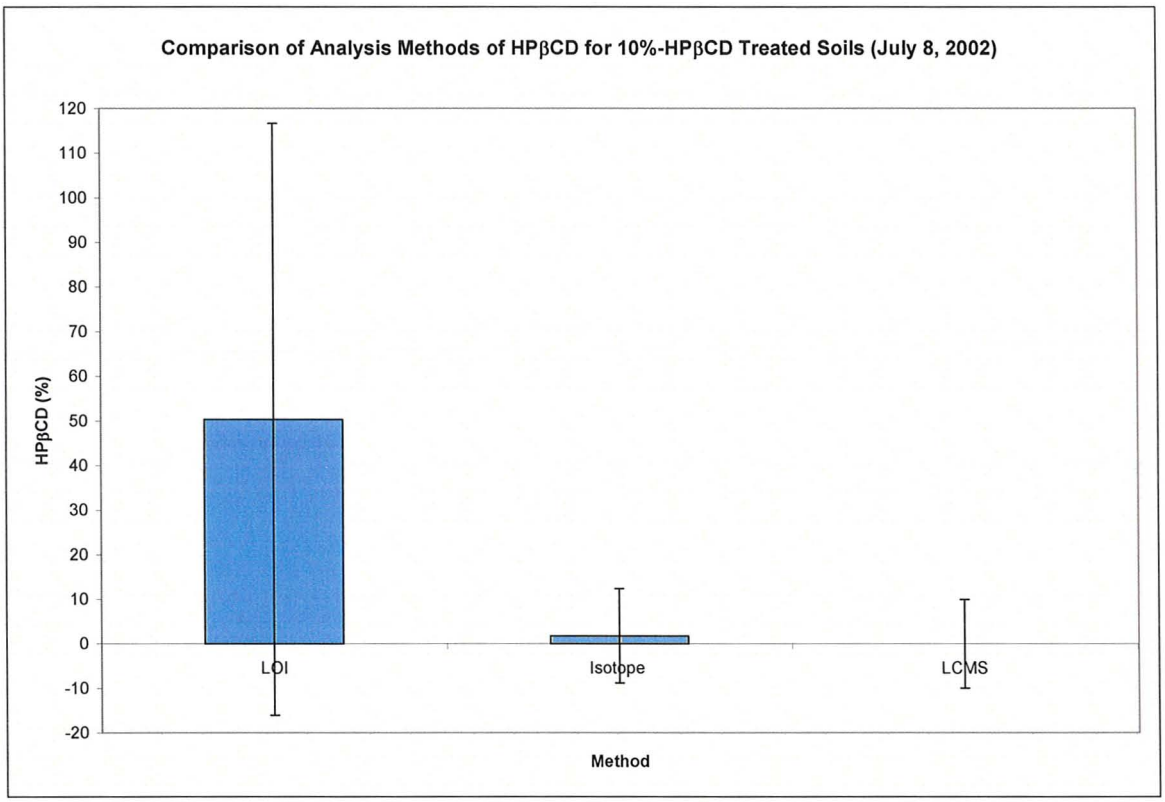

Figure 5.2. Percent of HPβCD retained in the 10%-HPβCD treated soils on July 8, 2002.

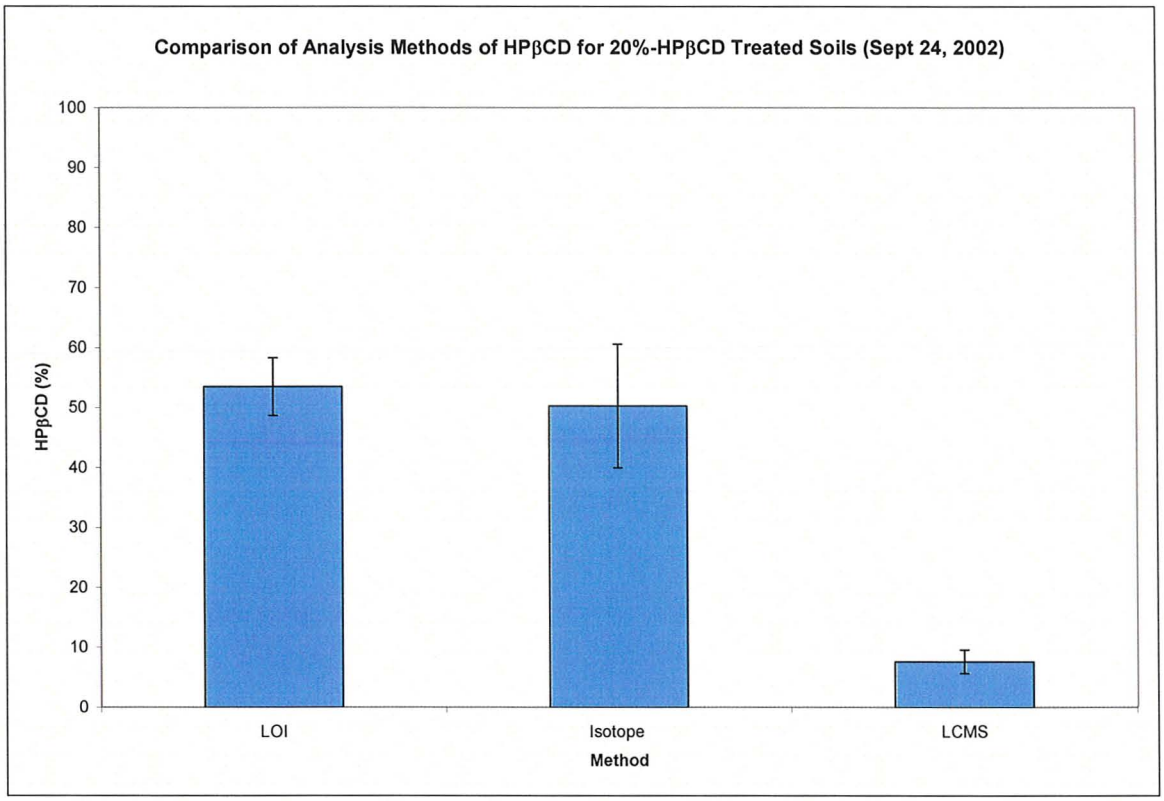

Figure 5.3. Percent of HPβCD retained in the 20%-HPβCD treated soils on September 24, 2002.

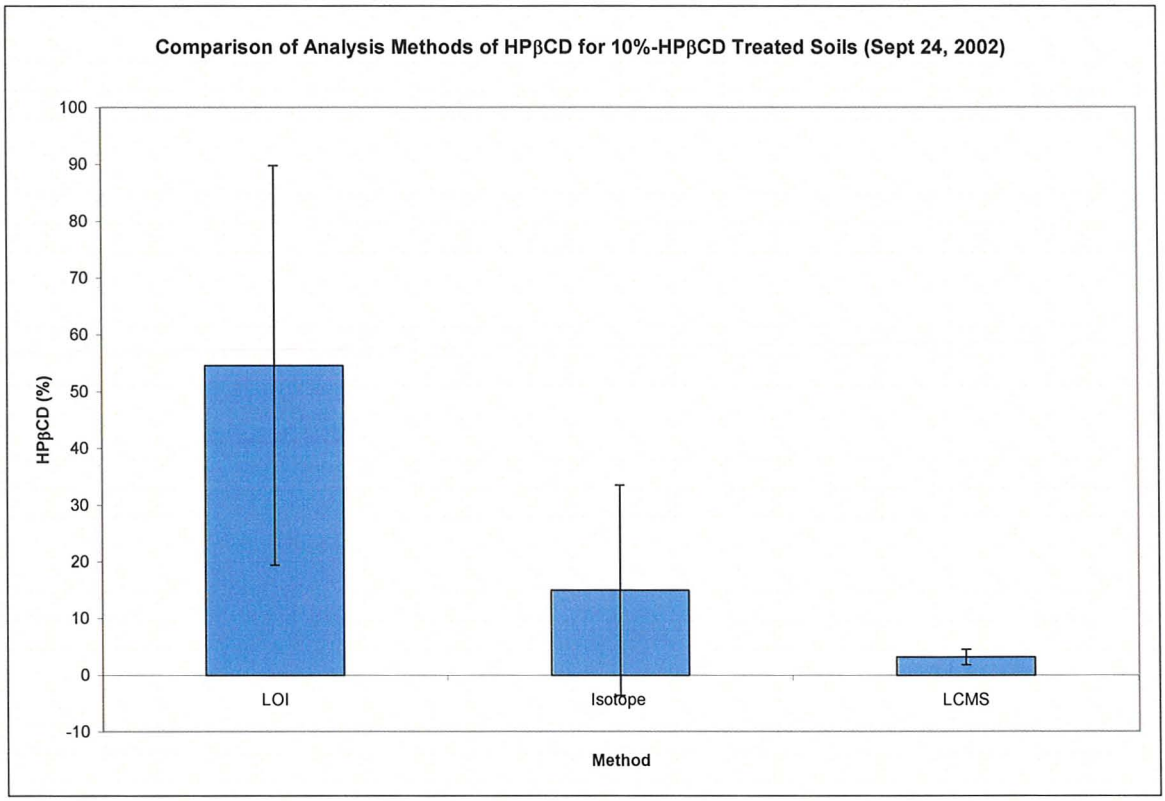

Figure 5.4. Percent of HPβCD retained in the 10%-HPβCD treated soils on September 24, 2002.

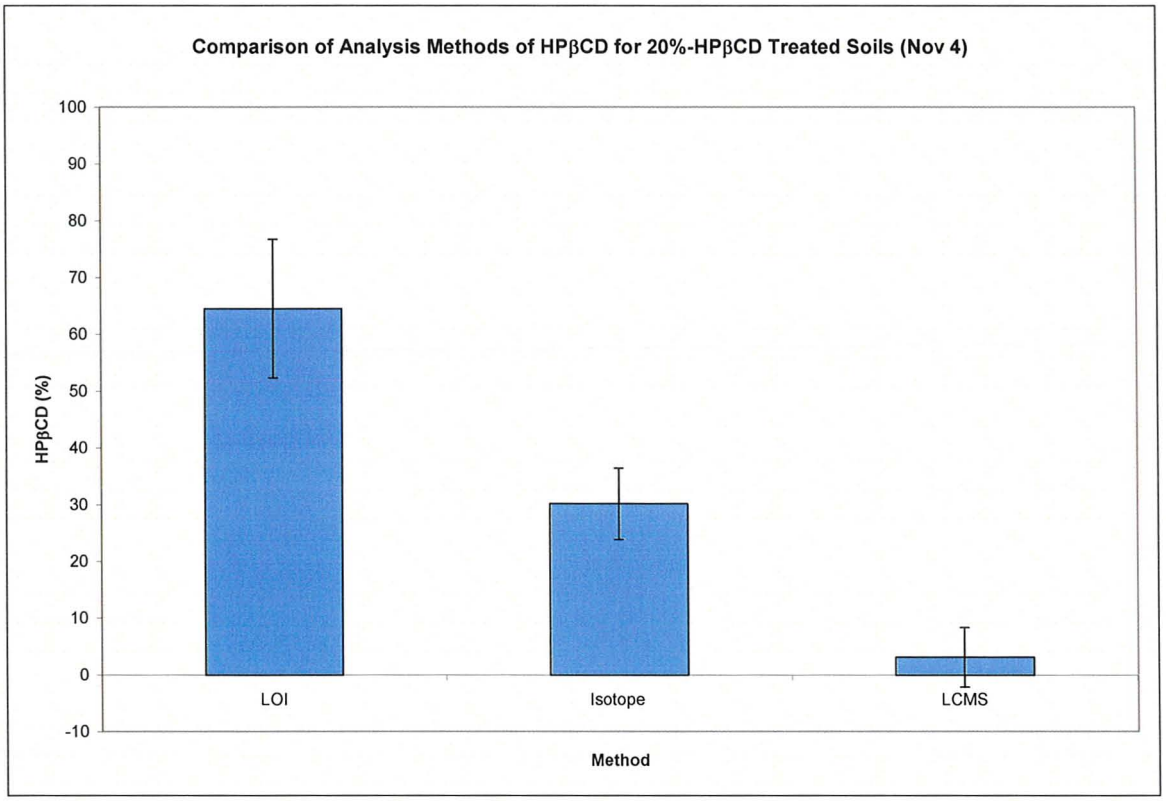

Figure 5.5. Percent of HPβCD retained in the 20%-HPβCD treated soils on November 4, 2002.

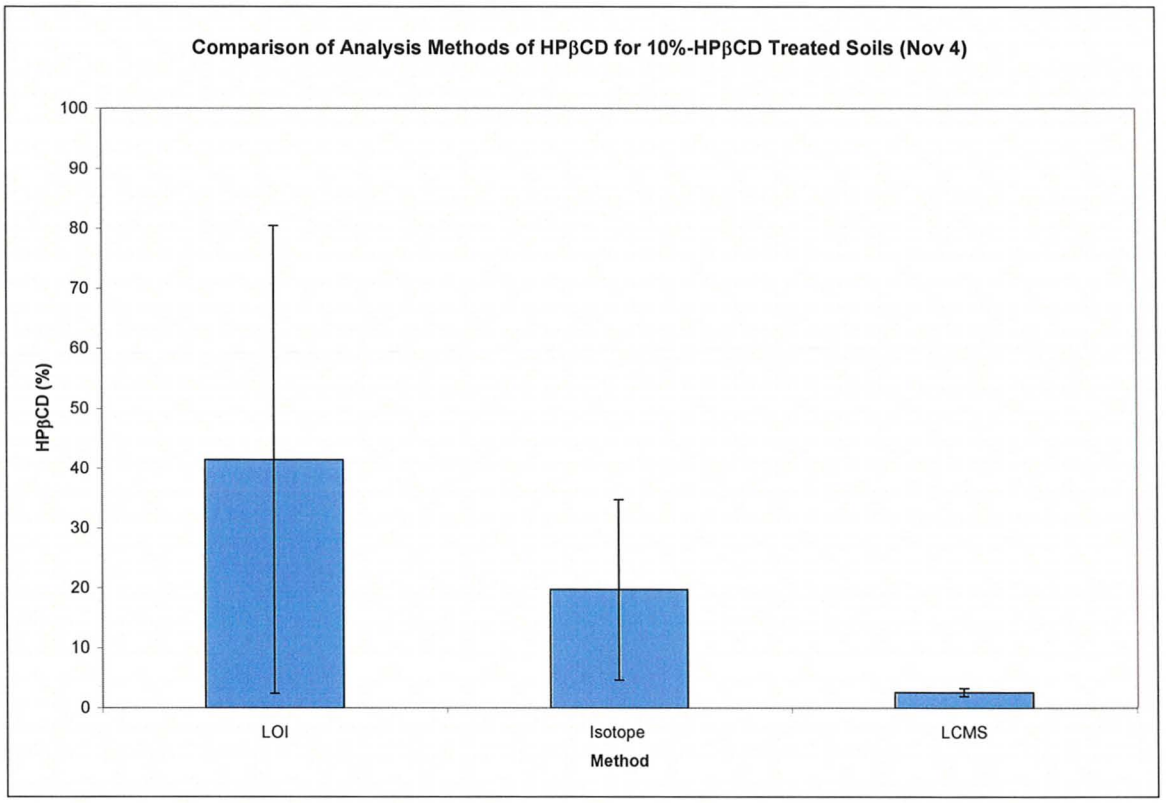

Figure 5.6. Percent of HPβCD retained in the 10%-HPβCD treated soils on November 4, 2002.

5.2. ISOTOPES VERSUS LCMS

LCMS was used to quantify the HPβCD within the soils (μg/g soil) at a molecular compound specific level. Technical grade HPβCD is a complex mixture of HPβCD molecules with differing degrees of substitution. The technical grade HPβCD used in this study had a degree of substitution of 6.5 provided by Cerestar Inc. This method showed increases in HPβCD in both the 10%- and 20%-HPβCD treated soils. The 20%-HPβCD treated soils showed a large increase in HPβCD concentrations over thirteen weeks of treatment; however, only a slight increase in concentration was seen in the 10%-HPβCD treated soils which reached a plateau concentration after treatments began. Treatments were discontinued in the 20%-treatment soils after September 16, 2002 due to an observed clogging effect (reduced infiltration). Once treatment was stopped there was a slow decrease in HPβCD concentrations. LCMS provided unquestionable information regarding the presence and relative trends of increase and decrease in HPβCD concentrations. However, further method development regarding analysis is required for improved quantitative results (outside the scope of this study).

Stable carbon isotopic analysis was used to assess the proportion of carbon from HPβCD applications that contributed to the total soil organic carbon. This proportion was assessed using a mass balance approach described by Boutton (1996). The carbon isotopic signature of technical grade HPβCD was measured to be a $\delta^{13}C$ value of -16‰, whereas, the $\delta^{13}C$ value of the untreated Point Pelee soil was -24.5‰. It was shown that the applications of HPβCD did alter the carbon isotopic values of the soil shifting them to a larger $\delta^{13}C$ value, indicating a change in the carbon source of the soils was occurring

during HPβCD applications. The 20%-HPβCD treated soils reflected a decrease in $\delta^{13}C$ values once applications discontinued after thirteen weeks of application.

Both carbon isotopic values and LCMS measured HPβCD concentrations show the same trends shown when comparing figure 3.2 with figure 4.4b and figure 3.4 with figure 4.4a. Trends of increase and decrease in HPβCD values are particularly evident in the 20%-HPβCD treated soils (figures 3.3 and 4.3b). For both methods of analysis the HPβCD concentration and carbon inputs increase and both reach maximum values near to the last application date of September 10, 2002. The $\delta^{13}C$ value reaches a peak value of -20.2‰ at September 9, 2002, whereas the LCMS concentration data reaches maximum value of $1.16x10^4$µg/g soil by September 24, 2002. In addition to mimicking the trend of increase of HPβCD retained within the soils, both methods reflect similar decreasing trends of HPβCD within the soils for six weeks after applications discontinued and showed no evidence of the compound eleven months after applications discontinued. Both methods of analysis provide similar results indicating that HPβCD is retained within the soil for a short-term period during applications of HPβCD, however, the HPβCD does not persist long-term leaving no trace after approximately six weeks after the last application. If both methods were measuring the HPβCD retained in the soil equally then when plotted against each other they should provide a ratio of 1:1. This trend is not observed shown in figures 5.7 and 5.8. These figures demonstrate that for both 10%- and 20%-HPβCD treated soils the LCMS analysis greatly underestimates the HPβCD retained with in the soil in comparison to the carbon isotopic analysis.

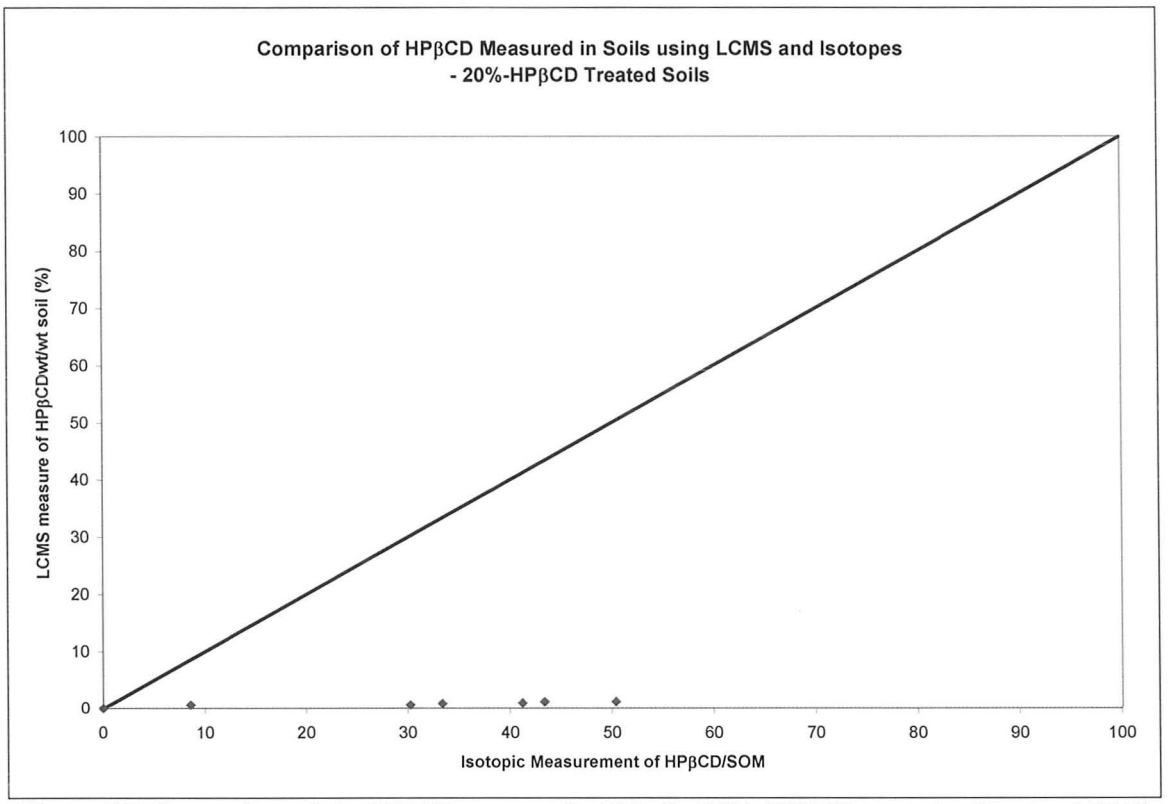

Figure 5.7. Comparison of the HPβCD measured within the 20%-HPβCD treated soils using LCMS and isotopes. LCMS measurements are underestimating the HPβCD in soils compared with stable carbon isotopic measurements.

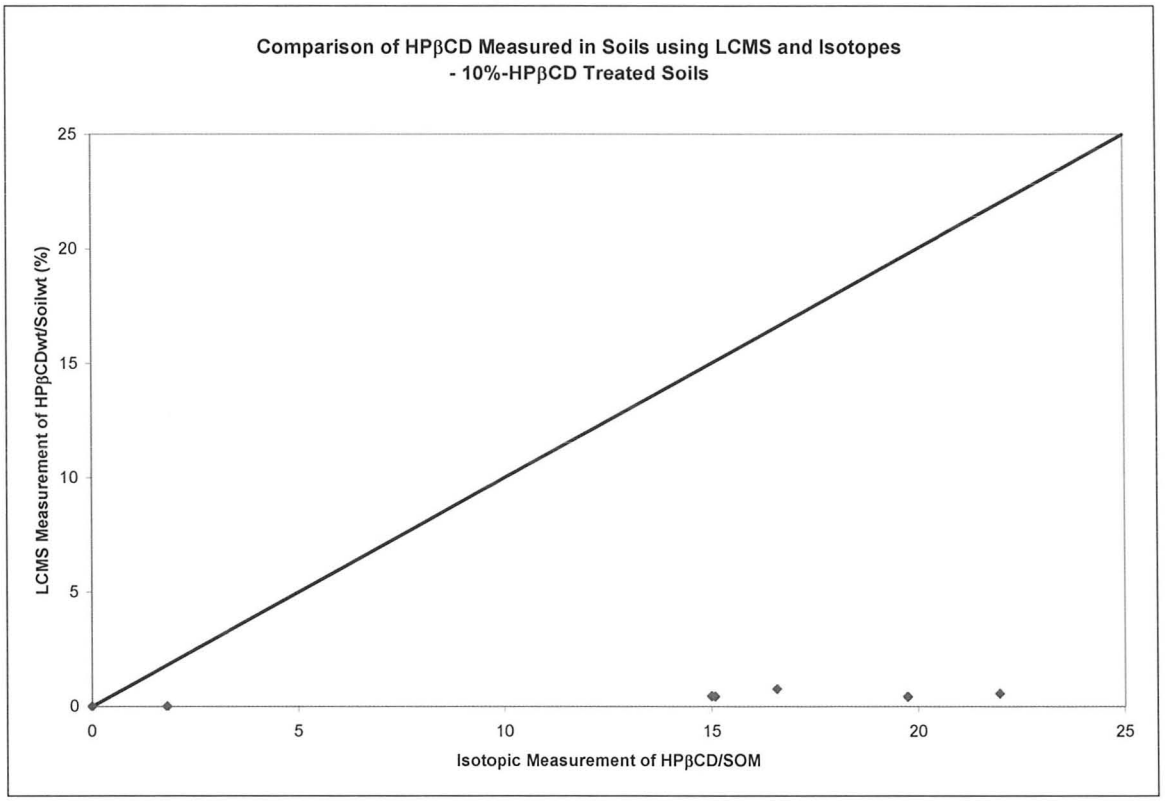

Figure 5.8. Comparison of the HPβCD measured within the 10%-HPβCD treated soils using LCMS and isotopes. LCMS measurements are underestimating the HPβCD in soils compared with stable carbon isotopic measurements.

In regards to the 10%-HPβCD treated soils, LCMS analysis provides definitive evidence that that HPβCD persists in the soils in low concentrations. An ANOVA test of the stable carbon isotopic values during the remediation experiment indicate that the change in $\delta^{13}C$ values due to HPβCD treatments are not statistically different ($P>0.05$). Early time in the 10%-HPβCD treated soils (figures 5.2 and 5.8) does suggest similar amounts, however, beyond this there is no agreement between the datasets.

With regards to the two methods of analysis, the stable isotopic method of analysis is a well defined method of sample preparation and analysis; there is a known distinction between the carbon isotopic signatures of Point Pelee soils (C_3 vegetation) and HPβCD (C_4 vegetation). Using stable carbon isotope analysis it has been identified that HPβCD did result in changes of the organic carbon in the soil within the 20%-HPβCD treated soils. The LCMS sample preparation and method of extractions were developed for this particular HPβCD analysis. Isotopic measurements of the technical grade HPβCD were used in both analyses, however, the LCMS method did not use a standard pure grade reference material but rather a calibration curve of the technical grade HPβCD itself, whereas the isotopic measurements used an international standard of Vienna PeeDee Belemnite (VPDB) as a reference material. Thus, results provided by stable carbon isotopes as to the proportion of HPβCD added to the soils provide a better quantitative method.

It is suggested that the difference in proportions of HPβCD observed between the two methods may reflect transformation products of HPβCD within the soil that are retaining the carbon-13 contributed by the initial HPβCD applied. Microbial

transformation preferentially uses the lighter isotope carbon-12, thus, increasing the carbon-13 of the remaining product (Faure, 1986). This retention of carbon-13 in the soil organic matter could result in the enriched $\delta^{13}C$ value observed. These unidentified transformation products were not measured using this particular LCMS analysis method. Thus, stable carbon isotope measurements provide an excellent indicator of the persistence of HPβCD and its possible transformation products in the soil.

5.3 ISOTOPES VERSUS LOSS ON IGNITION

In particular both stable carbon isotopic analysis, and loss on ignition (LOI) performed by Badley (2003) can be used to examine the changes in organic matter of the soil. Stable carbon isotope analysis was used to interpret the proportion of carbon contributed to the soils treated with 10%- and 20%-HPβCD solutions once per week for nineteen and thirteen weeks respectively. A weight conversion factor was formulated to asses the contribution of carbon in HPβCD molecules to the soils. An HPβCD molecule with a degree of substitution of 6.5 has a chemical formula of $C_{61}O_{41}H_{108}$ and a molecular mass of 1500. Using the mass of 61 carbon molecules equal to 732g/mol and a molecular mass of HPβCD equal to 1500g/mol provides a conversion factor of 2.05. This conversion factor can be applied to the proportion of carbon reported in section 3.5.2 thus converting the value into a percent weight of carbon contributed by HPβCD to the soil. This conversion factor assumes that 48.8% of the HPβCD molecule is comprised of carbon.

The same soil samples used for isotopic analysis were analyzed for organic matter content using loss on ignition by Badley (2003). Loss on ignition was used as a measure of organic material that was burned off at a temperature of 400°C; this includes both existing soil organic matter and HPβCD. Badley (2003) observed an increase in organic matter from 7% in the control soils to 17% in the 10%-HPβCD treated soils and 20% in the 20%-HPβCD treated soils and was attributed to the HPβCD applications. Organic matter can be converted to organic carbon using equation 5.1,

$$\%OM = \%OCx1.724 \tag{5.1}$$

Where %OM is the organic matter, %OC is the organic carbon and 1.724 is an acceptable conversion factor for converting %OC to %OM (Brady and Weil, 1999). This factor assumes that 58% of the organic matter is comprised of organic carbon.

Using (x-1) value from equation 2.4, the conversion factor discussed above was used to asses the percentage of organic carbon in the soil contributed by the HPβCD source. The percent of HPβCD sourced carbon contributing to the $\delta^{13}C$ values was multiplied by 2.05, to create a percent of the HPβCD as a weight. This value provides a better assessment of how much HPβCD applied and not just carbon alone is retained within the soil. Furthermore, this percentage of organic carbon was used in equation 5.1 to determine the percent organic matter resulting from HPβCD applications. A ratio of the HPβCD weight (%) to the total soil organic matter weight (%) indicates whether the HPβCD is contributing more or less than the soil organic matter to the total organic carbon in the soil. This ratio is demonstrated in figure 5.9.

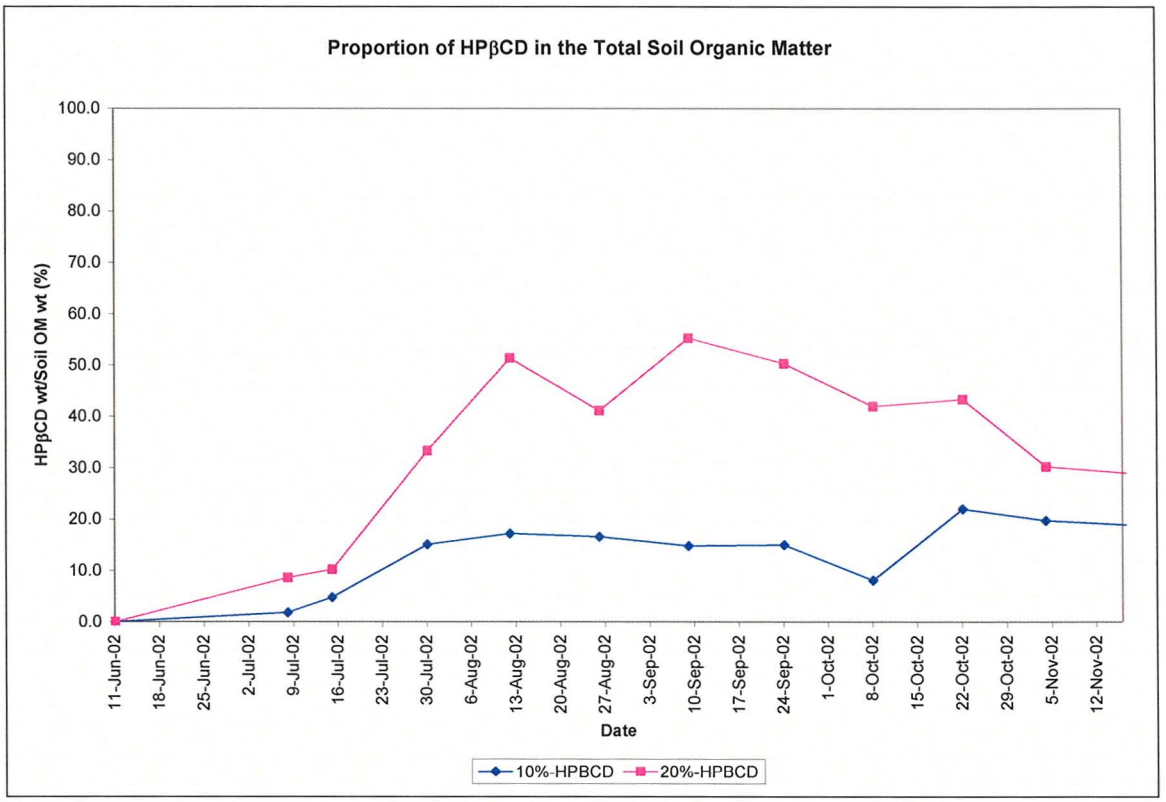

Figure 5.9. The proportion of HPβCD within the organic matter measured using stable carbon isotope analysis of $\delta^{13}C$ values. Percentages greater than 100 indicate that HPβCD is largely contributing to the soil organic matter.

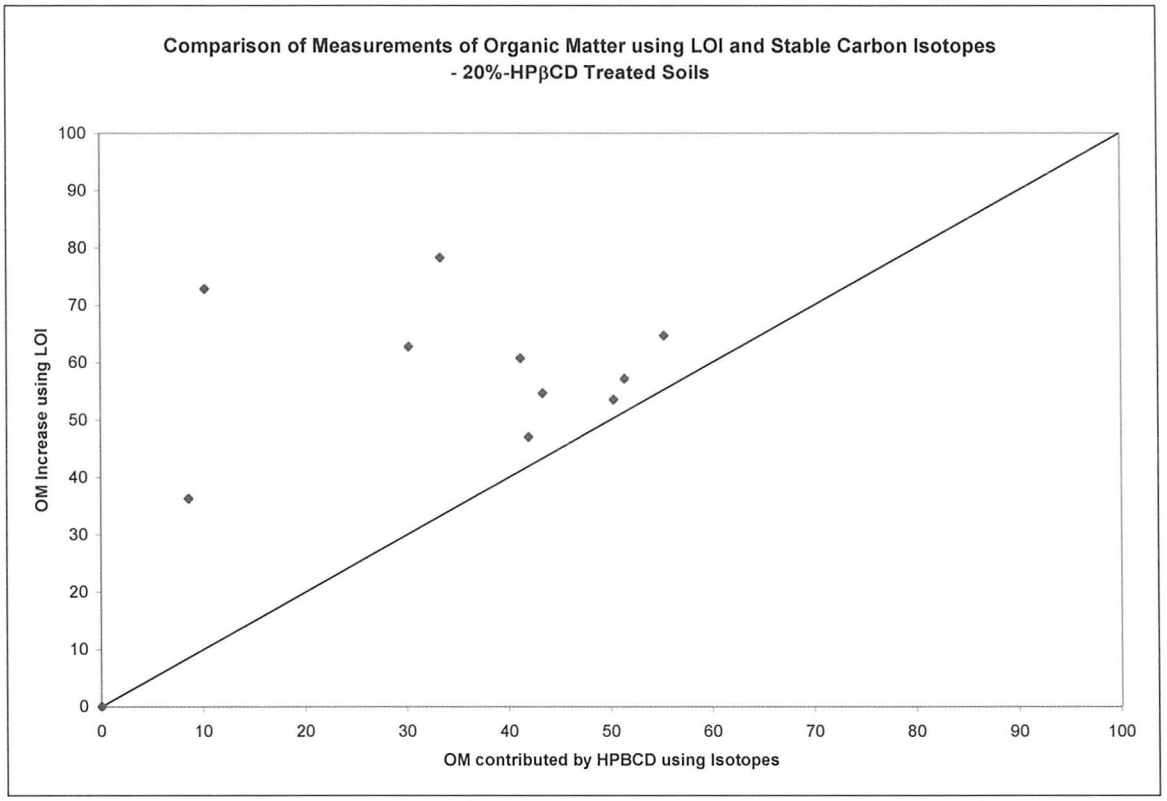

Figure 5.10. Comparison of the HPβCD measured within the 20%-HPβCD treated soils using loss on ignition (LOI) and stable carbon isotopic measurements. LOI measurements are overestimating the HPβCD in soils compared with stable carbon isotopic measurements.

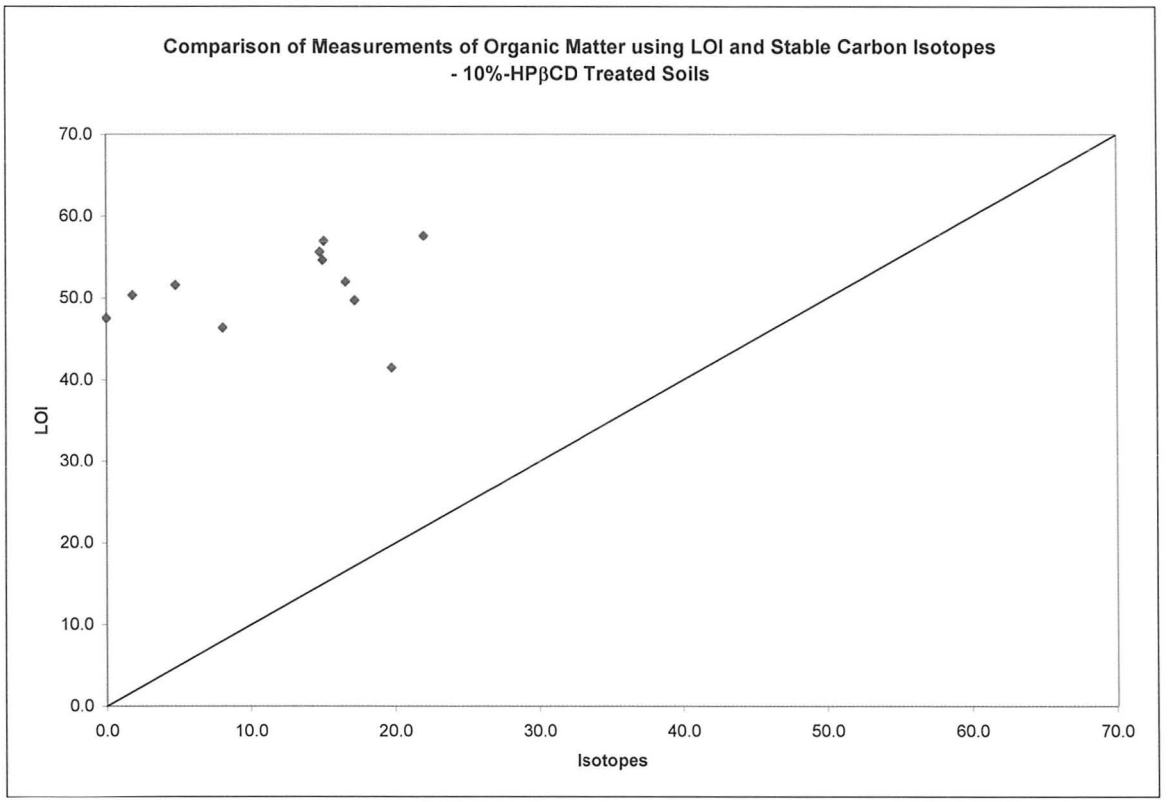

Figure 5.11. Comparison of the HPβCD measured within the 10%-HPβCD treated soils using loss on ignition (LOI) and stable carbon isotopic measurements. There is no clear trend between the measurements observed.

Using this ratio, the methods used to measure organic matter in soils can be compared. Loss on ignition measures the weight of the soil lost when the soil is heated to a temperature of between 400°C thus a measure of HPβCD along with other organic materials in the soil, whereas, the isotopic value is a measure of a known source of carbon, thus, the ratio calculated is a measure of only the carbon contributed by HPβCD. This comparison of methods is reflected in figure 5.10 and 5.11, indicating that LOI measures more organic matter than the isotopic measurement of HPβCD itself. If the organic matter increases observed by Badley (2003) were only contributed by the HPβCD applications then the lines would match. Figure 5.10 shows more clearly the trend in the 20%-treated soils that LOI overestimates the organic matter in the soil contributed by HPβCD applications. This trend is more scattered within the 10%-HPβCD treated soils shown by figure 5.11, indicating a fundamental difference in the behavior of the 10%- and 20%-HPβCD treated soils. This figure also demonstrates that there is no good correlation between the methods of LOI and stable carbon isotopes as measurements of soil organic matter for soils treated with HPβCD.

The difference between measurements indicates that the measure of the total organic matter by loss on ignition is representing the soil organic matter, the HPβCD present and an additional unknown organic material. Thus, loss on ignition is not a direct measure of the amount of HPβCD in the soil and is only representative of the percent increase in organic matter observed ie. combustible components. The isotopic measurements are a direct measure of the carbon contributed by HPβCD applications to the soil. This is a known value as both the carbon $\delta^{13}C$ value of technical grade HPβCD

and the initial $\delta^{13}C$ of the untreated soils were measured as source end members. Using a mass balance approach (equation 3.4) these known end members allow for a good representation of the HPβCD present in the soil.

There is a fundamental difference in the behaviour of the 10%- and 20%-treated soils. The large difference between LOI and isotopic measurements are clearly observed in the 20%-HPβCD treated soils. It is suggested that this increase may be due to an increase in microbial populations within the 20%-HPβCD soil plots. Evidence of this was measured using DAPI staining by Badley (2003) who observed a larger increase in bacterial counts within 20%-treated soils in comparison to the 10%-HPβCD and control soils. Additionally, Essa (2004) observed anoxic conditions within the treatment plot soils eleven months after applications ceased and found evidence of sulphate reduction thought to be from sulphate reducing bacteria. Smith (2004) identified that Point Pelee soils treated with 20%-HPβCD solution in laboratory columns resulted in an increase in a bioclogging effect in biotic columns, and no bioclogging in abiotic columns, thus suggesting that HPβCD treatments stimulate microbial growth in soils. Thus it is expected that the difference in organic matter reported between the two measurements of the same soil samples is due to additional biomass stimulated by HPβCD treatments, particularly in the 20%-HPβCD treated soils. Further investigation is necessary to confirm this hypothesis.

5.4 SUMMARY AND CONCLUSIONS

Three methods used to analyze the percent of HPβCD in the soils each method had definitive limitations and resulted in differing quantities of HPβCD, however, the methods did show similar trends of increase and decrease. A summary of the percent HPβCD within the soil measured using each of the three analytical methods indicated that isotopic measures of $\delta^{13}C$ have the greatest detail concerning HPβCD and its transformation products retained in the soil at mid-time. The comparison of the three methods further demonstrates that although trends may be similar between analysis methods, the quantification of HPβCD retained in the soil is not equally measured. Loss on Ignition is a routine available inexpensive analysis, while it has value; it has been proven not to be a reliable indicator of the amount of HPβCD retained within the soil. LCMS data is a useful tool to identify the presence of the original form of HPβCD within the soil, however, may not be reflecting differing forms of HPβCD present ie. degradation products. Using LCMS to analyze samples of soils treated with HPβCD solutions is a useful method to determine relative changes in HPβCD concentration; however requires further method development to improve quantification. LCMS does not provide a direct measure of the change in organic matter content caused by HPβCD applications. In comparison with LOI and LCMS methods, stable carbon isotopic analysis is considered to be a more reliable indicator of both the presence and total amount of HPβCD retained within the soil. This method is a measure of the natural stable carbon isotope ratio ($^{13}C/^{12}C$) indicating the presence of the untreated soil (low $^{13}C/^{12}C$) or HPβCD (high $^{13}C/^{12}C$) and their relative contribution to the soil organic

matter over the time of interest. This method is also capable of measuring the transformation products of HPβCD retaining carbon-13 remaining in the soil.

CHAPTER 6
SUMMARY AND CONCLUSIONS

Hydroxypropyl-β-cyclodextrin (HPβCD) demonstrates real promise as an effective remediation agent in the field to remove hydrophobic contaminants away from the surface soils. This large study has complied data from a laboratory column leaching experiment, stable carbon isotope values, and liquid chromatography mass spectrometry measurements of HPβCD in soils to assess the effectiveness and persistence of hydroxypropyl-β-cyclodextrin as a remediation agent used in soils historically contaminated with DDT. Prior to this study it was unknown if the HPβCD applied to soils in efforts to remove DDT, DDE and DDD from the surface soils at Point Pelee National Park, Canada remained in the soil itself. This knowledge is critical to understanding the true effectiveness and persistence of this chemical compound in use.

A series of soil columns were set up in the laboratory packed with soils historically contaminated with DDT from Point Pelee National Park, Canada. The soil columns received ten pore volumes of 10%-HPβCD solutions twice per day for five days. The analysis of soils and effluents from the treatments determined that 19% and 20% of the DDT and DDE masses were removed by the end of the study. In contrast, under similar pore volume applications of 10%-HPβCD solution in the field demonstrated 90% and 74% of DDT and DDE mass removal (Badley, 2003). Application schedules differed between studies; the laboratory columns received two pore volumes per day for one week, whereas field treatments were applied once per week for four months. The field remediation experiment resulted in greater removal of DDT and DDE from the soil. It

has been shown by the laboratory column study that mobilization of DDT and DDE is not the sole primary removal mechanism, thus it is apparent that alternative degradation mechanisms were contributing to mass removal efficiency in the field.

Furthermore, a comparison of pore volume half life determined using a first order decay constant resulted with a pore volume half life approximately ten times larger in the laboratory conditions than in the field. This indicates that field conditions were more favourable for DDT and DDE removal from the upper soil layers. Further work is necessary to investigate optimal field conditions and remediation solution concentrations to promote maximum removal efficiency.

Previous studies (Badley, 2003; Essa, 2003; Smith, 2003; Mironov, 2004; Mills, 2004) have provided evidence to further indicate that HPβCD is not the sole primary mechanism for DDT removal. It is suggested that HPβCD increases bioavailability of DDT and is derivatives and enhances their degradation by the microbial communities in the soil (Shao et al., 2003).

This is the first study known to use stable carbon isotopic analysis to assess the changes in organic carbon composition in HPβCD treated soils. It has been shown by this study that stable carbon isotope ($\delta^{13}C$) values can be used as a useful indicator of the retention and persistence of HPβCD and carbon-13 contributed by HPβCD within the soil. The carbon isotopic composition of technical grade HPβCD was -16‰ reflecting that of a C_4 vegetation source, in contrast the untreated soils from Point Pelee, Canada, had a $\delta^{13}C$ value of -24.4‰, reflecting that of a C_3 vegetation source. Although receiving the same remediation conditions (except the HPβCD concentration), there was a

statistically significant change ($P<0.05$) in the $\delta^{13}C$ values of the 20%-HPβCD treated soils during the remediation experiment, however, the 10%-HPβCD treated soils showed no statistically significant change, indicating a different behaviour between soils treated with different HPβCD solutions. Using a mass balance approach, the changes in soil organic carbon due to the applications of HPβCD to the soils was monitored. It was identified that HPβCD does alter the organic carbon source $\delta^{13}C$ value, thus, leaving a footprint (additional ^{13}C) on the organic carbon of the soil. This change in $\delta^{13}C$ value was found to be a short-term effect only lasting up to six weeks after applications discontinued as shown by the 20%-HPβCD treated soil. No carbon isotopic evidence of HPβCD was found eleven months after treatments discontinued.

Liquid chromatograph mass spectrometry (LCMS) was a useful measurement tool to assess the presence and concentration of technical grade HPβCD in the soil. This method identified the presence of HPβCD in the soils exhibiting a wide range of degrees of substitution (of exterior hydroxypropyl groups) ranging from a DS of 7 to a DS of 3. The hydroxypropyl group substitutions of DS7, DS6, and DS5 showed the highest response and were considered by this study to represent the bulk HPβCD in the soils. No trends between the different degrees of substitution were identified over the four months of the field remediation study. No conclusion could be made concerning a preference of degradation in the soil regarding the differing chemical structures of HPβCD identified.

The LCMS data showed that HPβCD was not retained in the soil at amounts greater than 2% of the mass applied after nineteen and thirteen weeks of weekly application for the 10%- and 20%-HPβCD solutions respectively. In addition no

evidence of the HPβCD chemical structure was identified in the soil eleven months after applications ceased. This evidence is supported by the findings of the stable carbon isotopic analysis: that HPβCD is a persistent compound short-term during applications of thirteen (10%-HPβCD) and nineteen weeks (20%-HPβCD), but begins to slowly decrease in concentrations after applications are discontinued and showed no evidence of persistence one year later. Thus, indicating that all the HPβCD had been removed/degraded from the soil.

In addition, LCMS data also identified that the behavior of HPβCD is not the same in unsaturated systems as that reported for saturated systems by Wang and Brusseau (1994). This is the first field study to identify that HPβCD is not acting conservatively and exhibits short-term persistence.

An unanticipated conclusion of this work is that TDR measures of soil water content are not first order measures of free water. TDR is often used to measure soil free water, however, this study among others (Rockhold et al., 2002; Smith, 2004; Or et al., 2007) identified that a composite media model is required to provide a better representation of soils when a significant microbial growth is present or stimulated. This study has identified that TDR measurements of the soil water are not only representing the free soil water but include the water held by or associated with the EPS produced by microbial communities stimulated by the applications of HPβCD during the field treatment study.

Each of the three methods (LCMS, LOI, stable isotopes) used to assess the amount of HPβCD retained in the soils were able to successfully confirm a 'temporary'

effect of HPβCD on the soils. All methods agree that there was no persistence of HPβCD one year after treatments. Each of these methods has definitively different limitations. LCMS measurements were a useful indicator of the presence of HPβCD in soils, however, the exact quantification of this mass requires further method development with regards to reference materials beyond this study. Increases observed in organic matter measured by loss on ignition were attributed to only HPβCD (Badley, 2003). Loss on ignition is not a direct measure of HPβCD (such as LCMS or stable carbon isotopes) but rather a measure of all organic material combusted at 400°C including HPβCD. This study and others (Badley, 2003; Essa, 2004; Smith, 2004) have shown evidence of microbial growth within the treatments plots that may have contributed to the increase in organic matter that was measured. Isotopic analysis provided results that indicated only the carbon contributed from the HPβCD carbon source when mixed with the initial soil carbon $\delta^{13}C$ values. Thus, stable carbon isotopic analysis is an excellent indicator of the presence and persistence of HPβCD and its transformation products (unidentified) within the soil both during and after applications of HPβCD solutions to the soil.

CHAPTER 7
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APPENDIX A:
HPβCD APPLICATION DATES

Application schedule of HPβCD solutions designed by Badley (2003). Soil samples were collected prior to HPβCD applications (often day before).

Sampling Date	Week (beginning-end of sampling)	# of Pore Volumes of HPBCD solution applied
11-Jun-02	0	0
18-Jun-02	1	0
25-Jun-02	2	1
9-Jul-02	4	2
10-Jul-02	4	3
16-Jul-02	5	4
23-Jul-02	6	5
31-Jul-02	7	6
7-Aug-02	8	7
13-Aug-02	9	8
20-Aug-02	10	9
27-Aug-02	11	10
4-Sep-02	12	11
10-Sep-02	13	13
17-Sep-02	14	14/13
24-Sep-02	15	15/13
8-Oct-02	17	17/13
22-Oct-02	19	19/13
4-Nov-02	21	-
30-Aug-03	63	-
17-Oct-06	227	-

APPENDIX B:
DDT RESULTS

Results of o,p'-DDE and p,p'-DDE removal from columns after each application of HPβCD solution (measurements using GCMS).

Sample ID	o,p DDE (ng/g ppb)	p,p DDE (ng/g ppb)	Total DDE (ng/g soil ppb)	Total DDE (ug/gsoil ppm)
col 1 app 1 CD	80	290	370	0.37
col 3 app 1 CD	70	280	350	0.35
col 5 app 1 CD	70	290	360	0.36
col 1 app 2 CD	40	260	300	0.3
col 3 app 2 CD	50	270	320	0.32
col 5 app 2 CD	40	260	300	0.3
col 1 app 3 CD	40	200	240	0.24
col 3 app 3 CD	50	230	280	0.28
col 5 app 3 CD	40	200	240	0.24
col 1 app 4 CD	10	180	190	0.19
col 3 app 4 CD	40	180	220	0.22
col 5 app 4 CD	40	160	200	0.2
col 1 app 5 CD	40	150	190	0.19
col 3 app 5 CD	40	160	200	0.2
col 5 app 5 CD	40	120	160	0.16
col 1 app 6 CD	40	140	180	0.18
col 3 app 6 CD	40	140	180	0.18
col 5 app 6 CD	40	120	160	0.16
col 1 app 7 CD	40	130	170	0.17
col 3 app 7 CD	40	140	180	0.18
col 5 app 7 CD	40	150	190	0.19
col 1 app 8 CD	40	130	170	0.17
col 3 app 8 CD	50	140	190	0.19
col 5 app8 CD	50	120	170	0.17
col 1 app 9 CD	40	120	160	0.16
col 3 app 9 CD	40	120	160	0.16
col 5 app 9 CD	40	110	150	0.15
col 1 app 10 CD	40	120	160	0.16
col 3 app 10 CD	40	110	150	0.15
col 5 app 10 CD	40	100	140	0.14
col 2 app 1 H2O	< 30	< 30	ND	ND
col 2 app 2 H2O	< 30	< 30	ND	ND
col 4 app 2 H2O	< 30	< 30	ND	ND
col 6 app 2 H20	< 30	< 30	ND	ND
col 2 app 6 H20	< 30	< 30	ND	ND
col 4 app 6 H20	< 30	< 30	ND	ND
col 6 app 6 H20	< 30	< 30	ND	ND
col 2 app 10 H20	< 30	< 30	ND	ND
col 4 app 10 H20	< 30	< 30	ND	ND
col 6 app 10 H20	< 30	< 30	ND	ND

Results of o,p'-DDT and p,p'-DDT removal from columns after each application of HPβCD solution (measurements using GCMS).

Sample ID	o,p DDT (ng/g ppb)	p,p DDT (ng/g ppb)	Total DDT (ng/g soil ppb)	Total DDT (ug/g soil ppm)
col 1 app 1 CD	170	290	460	0.46
col 3 app 1 CD	160	290	450	0.45
col 5 app 1 CD	170	320	490	0.49
col 1 app 2 CD	120	290	410	0.41
col 3 app 2 CD	130	300	430	0.43
col 5 app 2 CD	120	300	420	0.42
col 1 app 3 CD	100	220	320	0.32
col 3 app 3 CD	130	250	380	0.38
col 5 app 3 CD	100	230	330	0.33
col 1 app 4 CD	100	190	290	0.29
col 3 app 4 CD	90	210	300	0.3
col 5 app 4 CD	80	170	250	0.25
col 1 app 5 CD	90	170	260	0.26
col 3 app 5 CD	90	190	280	0.28
col 5 app 5 CD	90	150	240	0.24
col 1 app 6 CD	90	170	260	0.26
col 3 app 6 CD	90	170	260	0.26
col 5 app 6 CD	80	150	230	0.23
col 1 app 7 CD	90	160	250	0.25
col 3 app 7 CD	90	160	250	0.25
col 5 app 7 CD	90	170	260	0.26
col 1 app 8 CD	90	160	250	0.25
col 3 app 8 CD	100	160	260	0.26
col 5 app8 CD	100	150	250	0.25
col 1 app 9 CD	90	140	230	0.23
col 3 app 9 CD	90	140	230	0.23
col 5 app 9 CD	90	140	230	0.23
col 1 app 10 CD	90	140	230	0.23
col 3 app 10 CD	80	130	210	0.21
col 5 app 10 CD	80	120	200	0.2
col 2 app 1 H2O	< 30	< 30	ND	ND
col 2 app 2 H2O	< 30	< 30	ND	ND
col 4 app 2 H2O	< 30	< 30	ND	ND
col 6 app 2 H20	< 30	< 30	ND	ND
col 2 app 6 H20	< 30	< 30	ND	ND
col 4 app 6 H20	< 30	< 30	ND	ND
col 6 app 6 H20	< 30	< 30	ND	ND
col 2 app 10 H20	< 30	< 30	ND	ND
col 4 app 10 H20	< 30	< 30	ND	ND
col 6 app 10 H20	< 30	< 30	ND	ND

Initial DDE and DDT concentrations in soils, measured in initial soil splits used to pack the columns.

Sample ID	o,p DDE (ug/g)	p,p DDE (ug/g)	DDE (ug/g soil ppm)	o,p DDT (ug/g)	p,p DDT (ug/g)	DDT (ug/g soil, ppm)
control a	< 2	3.1	3.1	2.3	2.9	5.2
b	< 2	2.6	2.6	1.9	2.2	4.1
c	< 2	3.6	3.6	3.2	3.4	6.6
s-control a	< 2	6.5	6.5	4.0	5.5	9.4
	< 2	6.9	6.9	4.2	5.9	10.1
b	< 2	6.8	6.8	3.5	5.4	8.9
S 10 1a	< 2	3.6	3.6	2.9	3.2	6.1
b	< 2	3.6	3.6	2.7	3.0	5.7
c	< 2	4.0	4.0	2.8	3.3	6.1
S 20 1a	< 2	4.0	4.0	3.0	3.3	6.3
	< 2	4.0	4.0	3.0	3.3	6.3
1b	< 2	3.4	3.4	2.4	2.8	5.2
col 2a H20	< 2	4.2	4.2	3.5	3.9	7.4
b	< 2	2.6	2.6	1.8	2.2	4.0
col 4a H20	< 2	3.2	3.2	2.2	2.8	5.0
b	< 2	3.2	3.2	2.4	2.9	5.3
col 6a H20	< 2	3.0	3	2.3	2.7	5.0
b	< 2	2.8	2.8	2.1	2.5	4.6
AVERAGES			**3.94**			**6.19**
Variance			1.8			3.1

Final DDE and DDT concentrations in soils. Values measured from destroyed columns at the end of study.

Sample ID	o,p DDE (ug/g)	p,p DDE (ug/g)	DDE (ug/g soil ppm)	o,p DDT (ug/g)	p,p DDT (ug/g)	DDT (ug/g soil, ppm)
col 1a CD	< 2	2.5	2.5	2.3	2.5	4.8
b	< 2	1.8	1.8	1.7	1.8	3.5
col 3a CD	< 2	2.4	2.4	2.2	2.4	4.6
b	< 2	3.0	3	2.9	3.2	6.1
c	< 2	2.9	2.9	2.6	2.8	5.4
col 5a CD	< 2	3.5	3.5	3.3	3.5	6.8
b	< 2	2.0	2	1.6	1.9	3.5
AVERAGES			**2.59**			**4.96**
Variance			0.4			1.5

Three methods used to check calculations between initial soils measured from soil splits and DDT and DDE.

Method 1: Using Initial soils and calculated removed with DDT/DDE measured from each effluent solution.

	DDE	Standard Deviation	DDT	Standard Deviation
Cinitial (ug)	1059.1	372.0	1666.4	477
Cremoved (ug)	227.0	9.6	316.3	9.6
Cfinal (Ci-Cr) (ug)	832.1	9.6	1350.1	9.6
% Remaining	**78.6**		**81.0**	
% Removed	21.4		19.0	

Method 2: Using Initial soil and measured end soil (microwaved)

	DDE	Standard Deviation	DDT	Standard Deviation
Cinitial (ug)	1059.1	372.0	1666.4	477
Cfinal (meas) (ug)	685.6	155.2	1240.0	276
Cremoved (Ci-Cf) (ug)	373.5	155.2	426.4	276
% Remaining	**64.7**		**74.4**	
% Removed	35.3		25.6	

Method 3: Using final soils and adding back in the removed masses of DDT/DDE from effluent solutions collected after each application

	DDE	Standard Deviation	DDT	Standard Deviation
Cinitial (Cf+Cr) (ug)	912.7		1556.4	
Cremoved (meas) (ug)	227.0	9.6	316.3	9.6
Cfinal (meas) (ug)	685.6	155.2	1240.0	276
Remaining	**75.1**		**79.7**	
Removed	24.9		20.3	

APPENDIX C:
ISOTOPE DATA

Initial soil isotopic carbon value ($\delta^{13}C$) measured by SIBL, McMaster University.

Sample Date	10% Control $\delta^{13}C$	20% Control $\delta^{13}C$
11-Jun-02 'C1	-24.6	-24.6
11-Jun-02C2	-25.4	-25.4
11-jun-02 'C3	-24.0	-24.0
11-Jun-02 S8	-25.7	-25.7
10-1-11JUN	-24.1	-24.1
10-2-11JUN	-24.6	-24.6
10-3-11JUN	-23.8	-23.8
20-1-11JUN	-25.2	-25.2
20-2-11JUN	-25.9	-25.9
20-3-11JUN	-25.2	-25.2
30-sep-03 C1	-24.6	-24.6
30-sep-03 C2	-23.2	-23.2
30-sep-03 C3	-24.4	-24.4
C1 P5S16	-23.1	-23.1
C1 P5S23	-25.2	-25.2
C1 P5S5	-25.3	-25.3
C2 P8S12	-21.8	-21.8
C2 P8S2	-24.3	-24.3
C2 P8S25	-23.4	-23.4
C3 P9S17	-25.7	-25.7
C3 P9S24	-22.7	-22.7
C3 P9S5	-24.9	-24.9
C 17-oct-06	-24.9	-24.9
11-Jun-02 S1	-26.2	-26.2
11-Jun-02 S2	-26.1	-26.1
11-Jun-02 S3	-23.3	-23.3
11-Jun-02 S4	-22.6	-22.6
11-Jun-02 S5	-25.7	-25.7
11-Jun-02 S6	-26.1	-26.1
10-1-8JUL	-24.8	-24.0
10-2-8JUL	-24.9	-24.4
10-3-8JUL	-23.5	-23.3
10-1-15JUL	-25.0	-24.5
10-2-15JUL	-23.6	-23.6
10-3-15JUL	-23.9	-23.2

ANOVA single factor test for 20%-treated soils comparing three groups: untreated initial soils conditions, all treated soils with 20%-HPβCD solutions, and late-time soils (α=0.05)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Initial	35	-854.8	-24.4	1.3
20% Mid	24	-507.9	-21.2	1.1
20% late	13	-316.9	-24.4	1.9

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	169.0	2	84.5	63.7	0.000	3.1
Within Groups	91.5	69	1.3			
Total	260.5	71				

ANOVA single factor test for 20%-treated soils comparing three groups: untreated initial soils conditions, all treated soils with 20%-HPβCD solutions, and late-time soils (Bonferroni adjustment: α=0.017)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Initial	35	-854.8	-24.4	1.3
20% Mid	24	-507.9	-21.2	1.1
20% late	13	-316.9	-24.4	1.9

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	169.0	2.0	84.5	63.7	0.000	4.3
Within Groups	91.5	69.0	1.3			
Total	260.5	71.0				

ANOVA single factor test for 10%-treated soils comparing three groups: untreated initial soils conditions, all treated soils with 20%-HPβCD solutions, and late-time soils (α=0.05)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Initial	35	-857.5	-24.5	1.2
10% Mid	24	-559.7	-23.3	1.2
10% late	10	-239.0	-23.9	2.0

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	19.9	2	10.0	7.5	0.0	3.1
Within Groups	87.2	66	1.3			
Total	107.1	68				

ANOVA single factor test for 20%-treated soils comparing groups of untreated initial soils conditions and eleven months after HPβCD treatments discontinued. (α=0.05)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Untreated	29	-711.9	-24.5	1.4
Sep30/03	9	-225.9	-25.1	0.5

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2.1	1	2.1	1.7	0.2	4.1
Within Groups	43.2	36	1.2			
Total	45.3	37				

ANOVA single factor test for 10%-treated soils comparing groups of untreated initial soils conditions and eleven months after HPβCD treatments discontinued. (α=0.05)

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Untreated	29	-711.9	-24.5	1.4
Sep30/03	10	-239.0	-23.9	2.0

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3.13237	1	3.1	2.0	0.2	4.1
Within Groups	57.08697	37	1.5			
Total	60.21934	38				

ANOVA single factor test for 20%-treated soils comparing groups of untreated initial soils conditions and mid-time HPβCD treated soils (α=0.05).

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Initial	35	-854.8	-24.4	1.3
20% Mid	24	-507.9	-21.2	1.1

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	151.5	1.0	151.5	126.2	0.0000	4.0
Within Groups	68.4	57.0	1.2			
Total	220.0	58.0				

ANOVA single factor test for 10%-treated soils comparing groups of untreated initial soils conditions and mid-time HPβCD treated soils (α=0.05).

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Initial	35	-857.5	-24.5	1.2
10% Mid	24	-559.7	-23.3	1.2

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	19.8	1	19.8	16.3	0.0002	4.0
Within Groups	69.3	57	1.2			
Total	89.1	58				

ANOVA single factor test for 20%-treated soils comparing groups of untreated mid-time HPβCD treated soils and late-time previously treated soils (α=0.05).

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
20% Mid	24	-507.9	-21.2	1.1
20% late	13	-316.9	-24.4	1.9

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	87.2	1.0	87.2	63.3	0.0000	4.1
Within Groups	48.2	35.0	1.4			
Total	135.3	36.0				

ANOVA single factor test for 10%-treated soils comparing groups of untreated mid-time HPβCD treated soils and late-time previously treated soils (α=0.05).

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
10% Mid	24	-559.7	-23.3	1.2
10% late	10	-239.0	-23.9	2.0

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2.4	1.0	2.4	1.7	0.2	4.1
Within Groups	45.4	32.0	1.4			
Total	47.8	33.0				

Results of calculations for the 10%-HPβCD treated soils of the proportion of HPβCD measured using the mass balance equation described by Boutton (1996) using $\delta^{13}C$ values reported. Using the percentages of HPβCD and soil the calculated contributions of HPβCD to the soil organic carbon.

	$\delta^{13}C$	Application week	% Soil	% HPBCD	HPBCD Carbon wt (%)	Soil OC using $\delta^{13}C$ (%)	HPβCD /SOC
10-1-30JUL	-25.7	6	222	-122	-249.80	382.48	-65.31
10-2-30JUL	-21.2	6	-288	388	794.66	-495.89	-160.25
10-3-30JUL	-23.2	6	-62	162	331.94	-106.75	-310.95
10-1-12AUG	-24.0	8	25	75	154.67	42.32	365.44
10-2-12AUG	-23.1	8	-74	174	357.00	-127.83	-279.28
10-3-12AUG	-22.6	8	-133	233	477.54	-229.20	-208.35
10-1-26AUG	-24.1	10	32	68	138.70	55.75	248.78
10-2-26AUG	-22.8	10	-109	209	428.78	-188.19	-227.84
10-3-26AUG	-23.0	10	-89	189	388.28	-154.13	-251.91
10-1-9SEP	-22.9	12	-99	199	407.07	-169.94	-239.54
10-2-9SEP	-23.6	12	-23	123	252.55	-39.99	-631.54
10-3-9SEP	-23.8	12	1	99	202.03	2.50	8076.69
10-1-24SEP	-24.8	14	115	-15	-29.87	197.52	-15.12
10-2-24SEP	-23.4	14	-38	138	282.38	-65.08	-433.92
10-3-24SEP	-22.0	14	-202	302	619.98	-348.99	-177.65
10-1-8OCT	-25.5	16	192	-92	-189.04	331.38	-57.05
10-2-8OCT	-23.8	16	-2	102	208.37	-2.83	-7352.90
10-3-8OCT	-22.5	16	-140	240	492.95	-242.15	-203.57
10-1-22OCT	-24.1	18	34	66	134.86	58.99	228.61
10-2-22OCT	-22.5	18	-141	241	493.95	-243.00	-203.27
10-3-22OCT	-22.0	18	-200	300	614.77	-344.61	-178.40
10-1-4NOV	-23.6	20	-22	122	250.71	-38.44	-652.24
10-2-4NOV	-23.8	20	8	92	187.58	14.65	1280.21
10-3-4NOV	-21.7	20	-235	335	685.82	-404.36	-169.61
10-1 P2S1	-25.8	63	227	-127	-260.18	391.21	-66.51
10-1 P2S15	-23.9	63	9	91	185.71	16.22	1144.93
10-1 P2S17	-26.1	63	264	-164	-335.41	454.47	-73.80
10-2 P6S10	-23.4	63	-43	143	293.79	-74.67	-393.46
10-2 P6S25	-24.8	63	119	-19	-39.95	206.00	-19.40
10-2 P6S6	-24.3	63	63	37	75.14	109.21	68.81
10-3 P7S11	-23.7	63	-12	112	230.12	-21.13	-1089.28
10-3 P7S14	-21.9	63	-218	318	651.55	-375.53	-173.50
10-3 P7S24	-21.9	63	-208	308	630.93	-358.20	-176.14
10-2 17-oct-06	-23.3	227	-54	154	314.86	-92.39	-340.80

Results of calculations for the 20%-HPβCD treated soils of the proportion of HPβCD measured using the mass balance equation described by Boutton (1996) using $\delta^{13}C$ values reported. Using the percentages of HPβCD and soil the calculated contributions of HPβCD to the soil organic carbon.

	$\delta^{13}C$	Application week	% Soil	% HPBCD	HPBCD Carbon wt (%)	Soil OC using $\delta^{13}C$ (%)	HPβCD /SOC
20-1-30JUL	-24.0	6	94	6	12.93	162	8
20-2-30JUL	-20.2	6	49	51	104.47	85	124
20-3-30JUL	-21.8	6	68	32	64.60	118	55
20-1-12AUG	-20.8	8	57	43	88.13	98	90
20-2-12AUG	-19.7	8	43	57	116.23	75	156
20-3-12AUG	-21.0	8	58	42	85.13	101	84
20-1-26AUG	-22.3	10	74	26	53.03	128	41
20-2-26AUG	-20.7	10	55	45	92.64	94	98
20-3-26AUG	-21.1	10	60	40	81.94	103	79
20-1-9SEP	-20.1	12	48	52	105.98	83	127
20-2-9SEP	-19.3	12	39	61	125.85	67	189
20-3-9SEP	-21.1	12	60	40	81.67	104	79
20-1-24SEP	-20.0	14	47	53	108.23	81	133
20-2-24SEP	-20.2	14	49	51	104.21	85	123
20-3-24SEPT	-21.6	14	66	34	70.39	113	62
20-1-8OCT	-20.9	16	58	42	86.19	100	86
20-2-8OCT	-20.9	16	58	42	86.16	100	86
20-3-8OCT	-22.0	16	71	29	60.02	122	49
20-1-22OCT	-21.4	18	64	36	74.79	110	68
20-2-22OCT	-21.0	18	58	42	85.47	101	85
20-3-22OCT	-21.2	18	61	39	80.25	105	77
20-1-4NOV	-21.7	20	67	33	67.31	116	58
20-2-4NOV	-22.7	20	79	21	43.45	136	32
20-3-4NOV	-22.3	20	74	26	53.31	128	42
20-1 P1S15	-25.9	63	116	-16	-32.69	200	-16
20-1 P1S17	-25.5	63	112	-12	-25.29	194	-13
20-1 P1S7	-25.4	63	111	-11	-22.53	191	-12
20-2 P3S12	-24.6	63	101	-1	-1.87	174	-1
20-2 P3S18	-25.4	63	110	-10	-20.78	190	-11
20-2 P3S24	-25.5	63	112	-12	-24.97	193	-13
20-3 P4S15	-24.8	63	103	-3	-6.15	178	-3
20-3 P4S24	-23.5	63	89	11	23.40	153	15
20-3 P4S8	-25.3	63	109	-9	-19.33	189	-10
20-2 17-oct-06	-24.3	227	98	2	4.83	168	3

APPENDIX D:
LCMS DATA

Concentration HPBCD in control soils measured by MRCMS, McMaster University. Each ion peak represents a different degree of substitution of hydroxypropyl groups on the exterior HPBCD chemical structure: 1558 = Ds7, 1500 = DS6, 1442 = DS5, 1384 = DS4, 1326 = Ds3, 1268 = DS2.

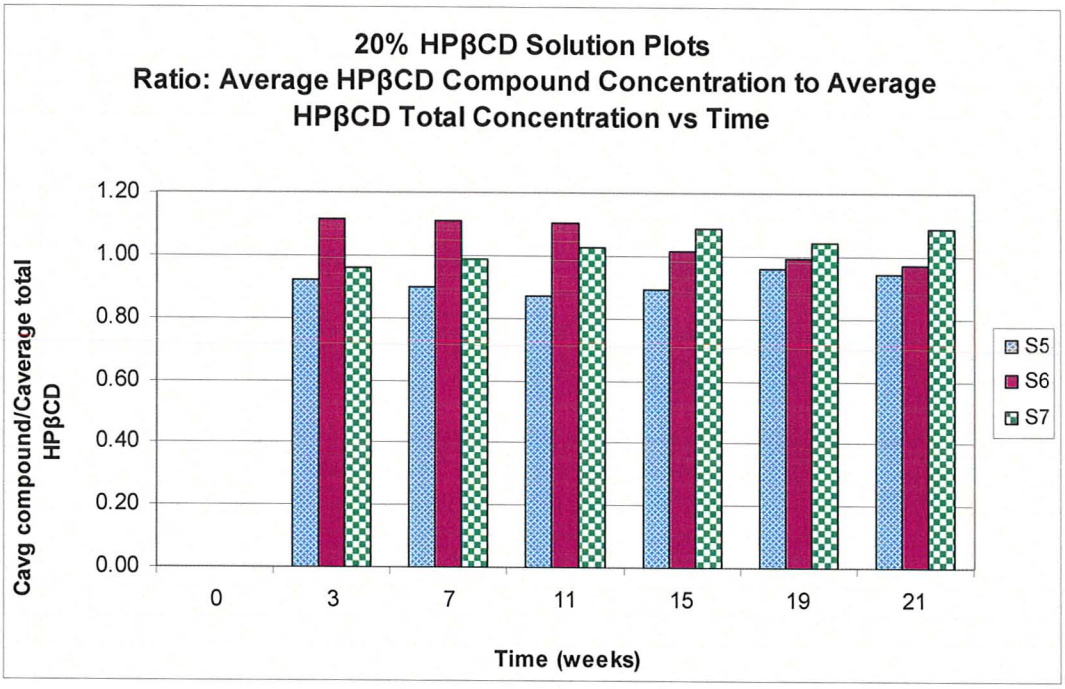
Samplelist Text	Pore Volume of HPBCD Applied	Peak 1268 (ug/g soil)	Peak 1326 (ug/g soil)	Peak 1384 (ug/g soil)	Peak 1442 (ug/g soil)	Peak 1500 (ug/g soil)	Peak 1558 (ug/g soil)	AVERAGE Total Concentration (1558,1500,1442)
C1	12575	0	0	0	0	0	0	**0**
C3	12577	0	0	0	0	0	0	**0**
C2	12576	0	0	0	0	0	0	**0**

Concentration HPBCD in 10%-HPBCD treated soils measured by MRCMS, McMaster University. Each ion peak represents a different degree of substitution of hydroxypropyl groups on the exterior HPBCD chemical structure: 1558 = Ds7, 1500 = DS6, 1442 = DS5, 1384 = DS4, 1326 = Ds3, 1268 = DS2.

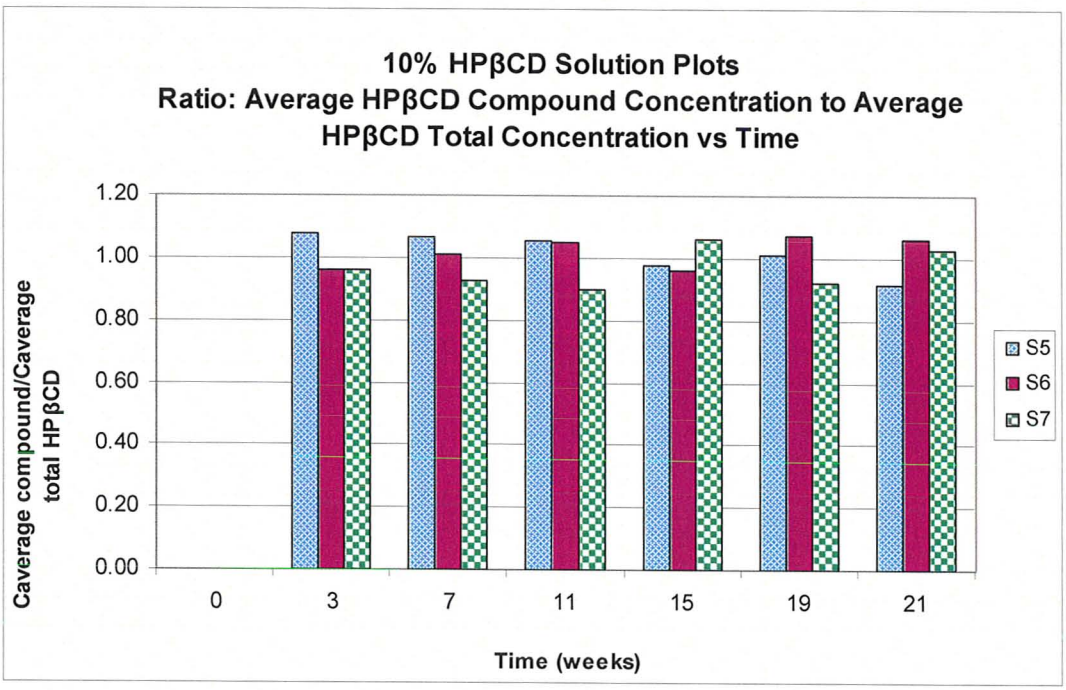
Samplelist Text	Pore Volume of HPBCD Applied	Peak 1268 (ug/g soil)	Peak 1326 (ug/g soil)	Peak 1384 (ug/g soil)	Peak 1442 (ug/g soil)	Peak 1500 (ug/g soil)	Peak 1558 (ug/g soil)	AVERAGE Total Concentration (1558,1500,1442)
10%-2-11-JUN shallow	0	0	0	0	0	0	0	**0**
10%-1-8-JUL	2	0	0	6942	7553	8086	8212	**7951**
10%-3-8-JUL	2	0	0	14598	11060	9336	9050	**9815**
10%-APR 20/06 (8-JUL)	2	0	5612	3383	4763	4739	5009	**4837**
10%-2-30-JUL	6	0	0	2884	3195	2895	2975	**3021**
10%-3-30-JUL	6	0	0	8952	6022	5884	5034	**5647**
10%-1-30-JUL	6	0	0	64	64	61	0	**41**
10%-3-26-AUG	10	0	0	17228	10170	10508	8478	**9719**
10%-1-26-AUG	10	0	0	7976	6288	8394	6952	**7211**
10%-2-26-AUG	10	0	0	4811	5841	5419	5122	**5461**
10%-APR 20/06-SHORT WAIT (sep24)	14	0	8172	3897	6518	7206	8060	**7261**
10%-3-24-SEP	14	0	0	2372	2304	1455	1513	**1758**
10%-1-24-SEP	14	0	0	61	13	0	29	**14**
10%-2-22-OCT	17	0	0	16060	10433	11016	8943	**10131**
10%-3-22-OCT	17	0	0	62	903	1036	1413	**1117**
10%-1-22-OCT	17	0	0	53	394	425	535	**452**
10%-3-4-NOV	19	0	0	3396	2422	3014	2451	**2629**
10%-1-4-NOV	19	0	0	3851	3407	4062	3800	**3757**
10%-APR 20/06-LONG WAIT	19	0	9890	3121	5236	5830	6114	**5727**

Concentration HPBCD in 20%-HPBCD treated soils measured by MRCMS, McMaster University. Each ion peak represents a different degree of substitution of hydroxypropyl groups on the exterior HPBCD chemical structure: 1558 = Ds7, 1500 = DS6, 1442 = DS5, 1384 = DS4, 1326 = Ds3, 1268 = DS2.

Samplelist Text	Pore Volume of HPBCD Applied	Peak 1268 (ug/g soil)	Peak 1326 (ug/g soil)	Peak 1384 (ug/g soil)	Peak 1442 (ug/g soil)	Peak 1500 (ug/g soil)	Peak 1558 (ug/g soil)	AVERAGE Total Concentration (1558,1500,1442)
20%-3-8-JUL	2	0	0	7790	5955	7784	6367	**6702**
20%-1-8-JUL	2	0	0	5163	3906	5116	4164	**4395**
PLOT 3-20%-2-JULY 8/06	2	0	11324	5941	6744	7233	6796	**6924**
20%-3-30-JUL	6	0	0	10180	10173	12140	10597	**10970**
20%-2-30-JUL	6	0	0	7666	8137	10630	9479	**9416**
20%-1-30-JUL	6	0	0	3929	3677	4358	4132	**4056**
20%-3-26-AUG	10	0	0	7269	7805	10031	10174	**9337**
20%-2-26-AUG	10	0	0	8062	8589	11147	10698	**10145**
20%-1-26-AUG	10	0	0	9356	7694	9409	7670	**8258**
20%-1-24-SEP	13	0	0	8877	8798	11192	9655	**9881**
20%-2-24-SEP (short wait	13	0	0	8342	11948	14251	16567	**14255**
20%-3-24-SEP	13	0	0	6971	10327	9878	11661	**10622**
20%-1-22-OCT	13	0	0	8322	12339	10856	11279	**11492**
20%-2-22-OCT	13	0	0	7968	11496	11299	13704	**12166**
20%-3-22-OCT	13	0	0	8272	8425	11089	10101	**9872**
20%3-4-NOV	13	0	0	1321	2050	2597	3912	**2853**
20%-1-4-NOV	13	0	0	10465	11949	11496	11923	**11789**
PLOT 3 -20%-2-NOV 4/02	13	0	4886	1697	2743	3151	3538	**3144**



Plot of Average HPβCD concentration in 10%-HPβCD treated soils over nineteen pore volumes applied once per week. No trend among the ion peaks is evident. Note: S5, S6, S7 represent the degrees of substitution of hydroxypropyl groups on the exterior HPβCD structure.



Plot of Average HPβCD concentration in 20%-HPβCD treated soils over nineteen pore volumes applied once per week. No trend among the ion peaks is evident. Note: S5, S6, S7 represent the degrees of substitution of hydroxypropyl groups on the exterior HPβCD structure.

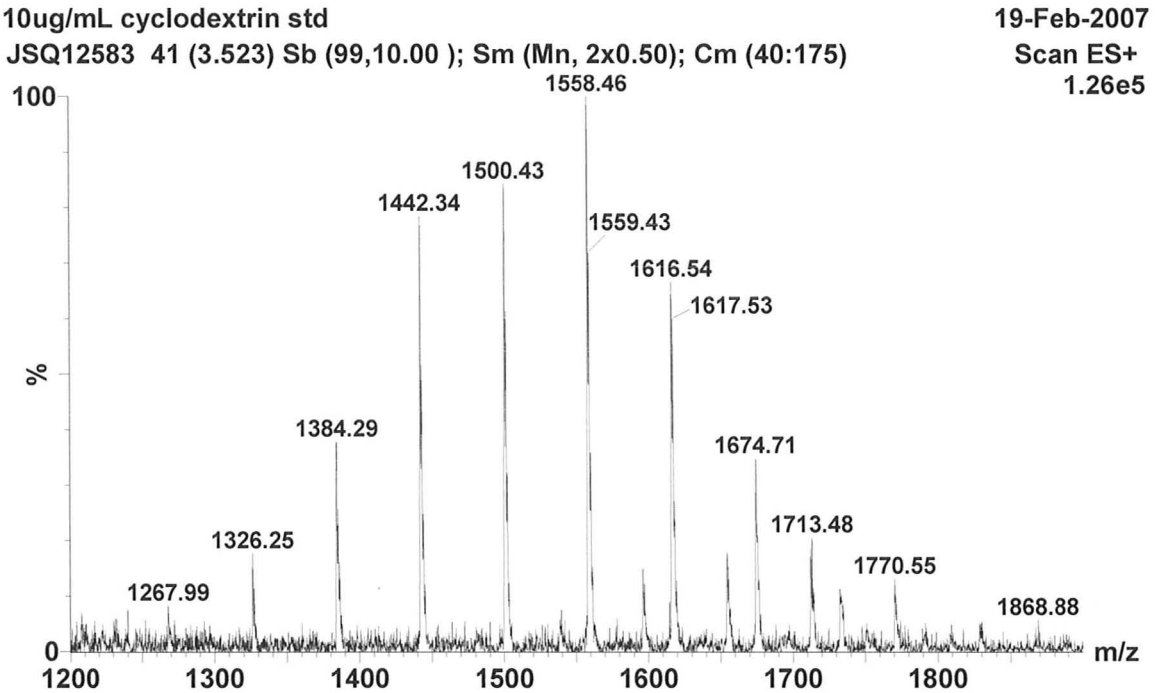
10%-HPβCD soils: data concerning the percent of mass retained per applications per individual treatments, total treatment mass and the concentration of HPβCD retained in soil water.

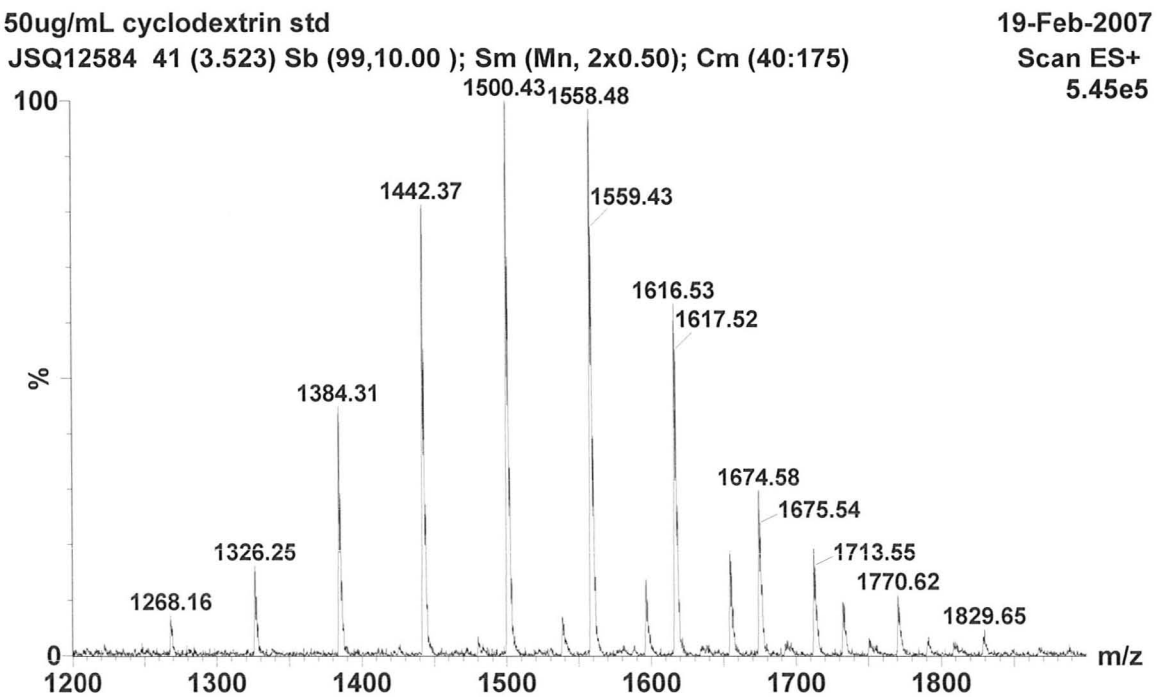
Date	Week	% Retained per application (HPBCD Measured/Applied per treatment) (%)	% Retained per applications (HPBCD mass soil/total mass applied)	Conc. HPBCD in soil water using VWCplot (ug/ml soil water)
6/11/2002	0			0
7/8/2002	3	16.66	8.3	136511
7/30/2002	7	9.85	1.6	44226
8/26/2002	11	17.25	1.7	81609
9/24/2002	15	10.25	0.7	24552
10/22/2002	19	12.79	0.8	55136
11/4/2002	21	9.50	0.5	24057

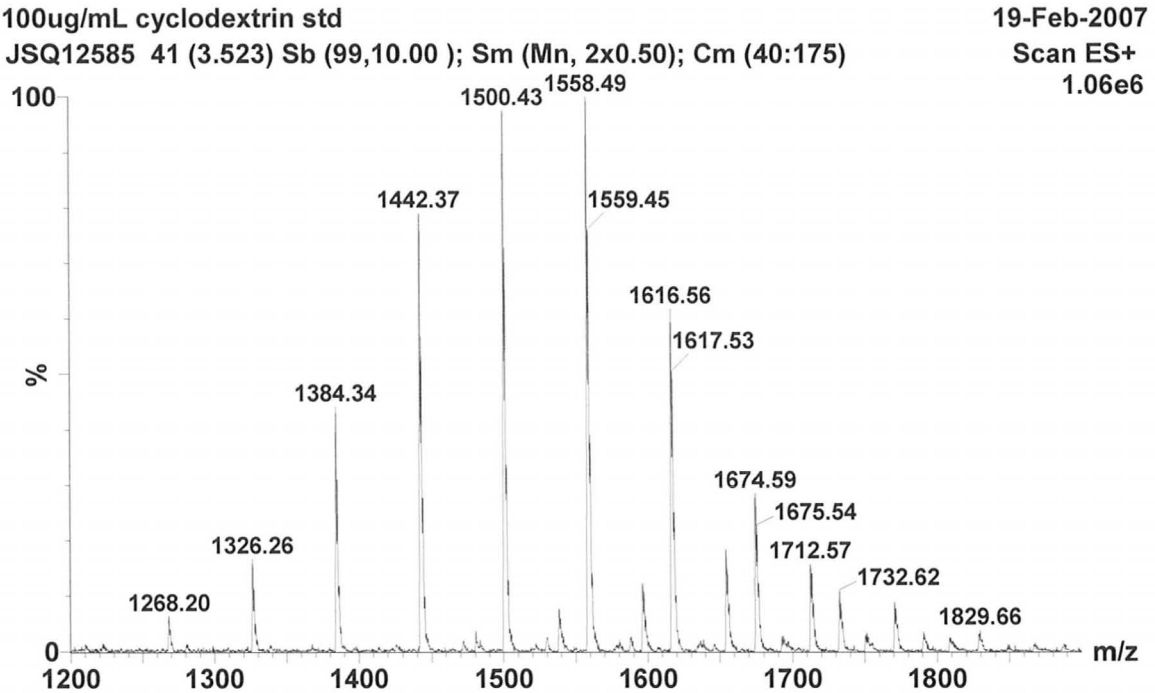
20%-HPβCD soils: data concerning the percent of mass retained per applications per individual treatments, total treatment mass and the concentration of HPβCD retained in soil water.

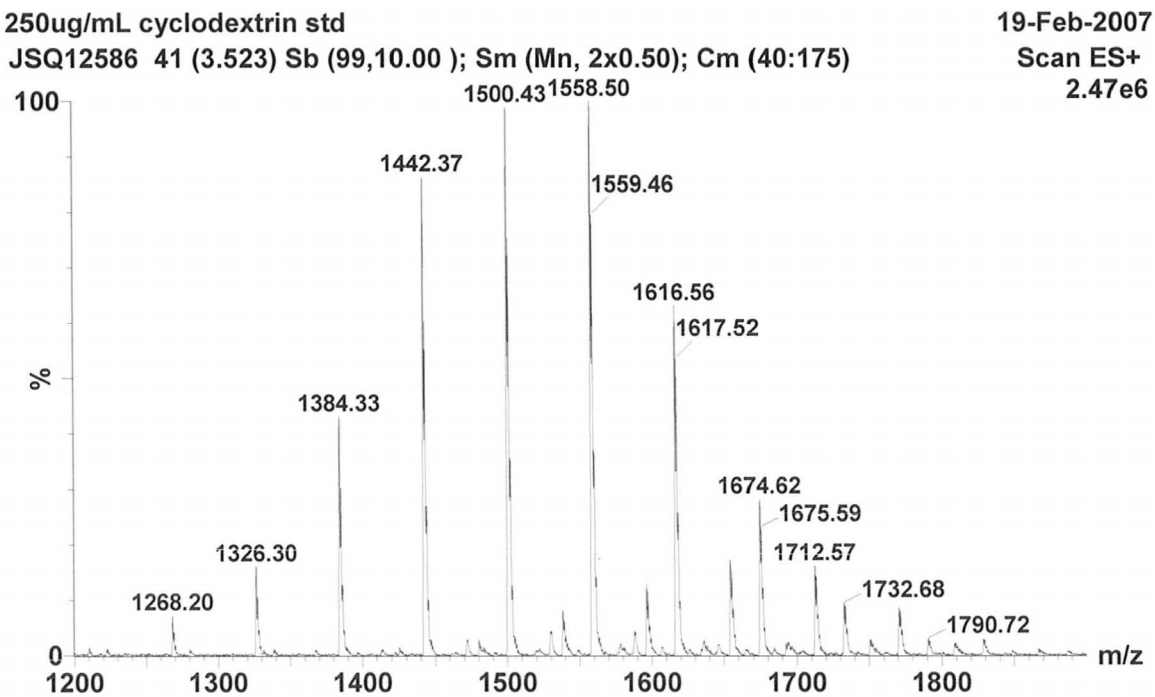
Date	Weeks	% Retained per application (HPBCD Measured/Applied per treatment) (%)	% Retained per applications (HPBCD mass soil/total mass applied)	Conc HPBCD in soil water using VWCplot (ug/ml soil water)
6/11/2002	0			0
7/8/2002	3	6.83	3.4	122596
7/30/2002	7	9.26	1.5	84865
8/26/2002	11	10.51	1.1	85879
9/24/2002	15	13.17	1.0	57835
10/22/2002	19	12.70	1.0	86416
11/4/2002	21	6.74	0.5	23129

APPENDIX E: CHEMICAL SPECTRAS FOR EACH SOIL SAMPLE PROVIDED BY MCMASTER REGIONAL CENTRE FOR MASS SPECTROMETRY


10ug/mL cyclodextrin std
JSQ12583 41 (3.523) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
19-Feb-2007
Scan ES+
1.26e5
100
%
0
1558.46
1500.43
1442.34
1559.43
1616.54
1617.53
1384.29
1674.71
1713.48
1326.25
1770.55
1267.99
1868.88
1200
1300
1400
1500
1600
1700
1800
m/z


50ug/mL cyclodextrin std
JSQ12584 41 (3.523) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
19-Feb-2007
Scan ES+
5.45e5
100
%
0
1500.43
1558.48
1442.37
1559.43
1616.53
1617.52
1384.31
1674.58
1675.54
1326.25
1713.55
1770.62
1268.16
1829.65
1200
1300
1400
1500
1600
1700
1800
m/z


100ug/mL cyclodextrin std
JSQ12585 41 (3.523) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
19-Feb-2007
Scan ES+
1.06e6
100
%
0
1500.43
1558.49
1442.37
1559.45
1616.56
1617.53
1384.34
1674.59
1675.54
1326.26
1712.57
1732.62
1268.20
1829.66
1200
1300
1400
1500
1600
1700
1800
m/z


250ug/mL cyclodextrin std
JSQ12586 41 (3.523) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
19-Feb-2007
Scan ES+
2.47e6
100
%
0
1500.43
1558.50
1442.37
1559.46
1616.56
1617.52
1384.33
1674.62
1675.59
1326.30
1712.57
1732.68
1268.20
1790.72
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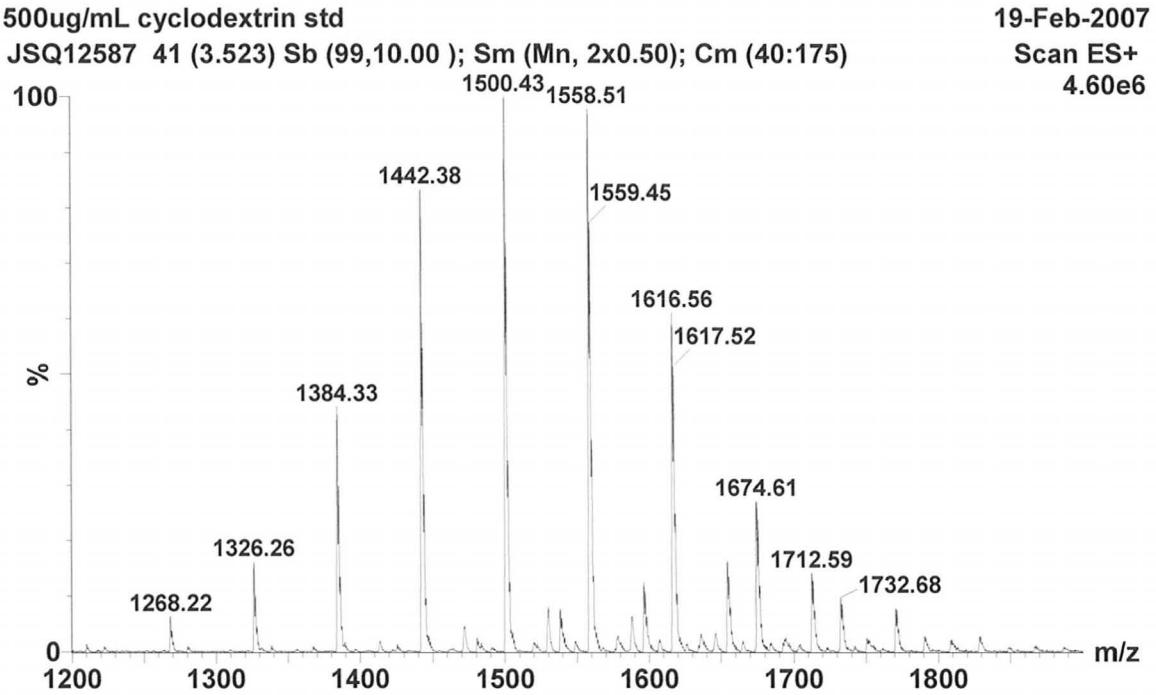
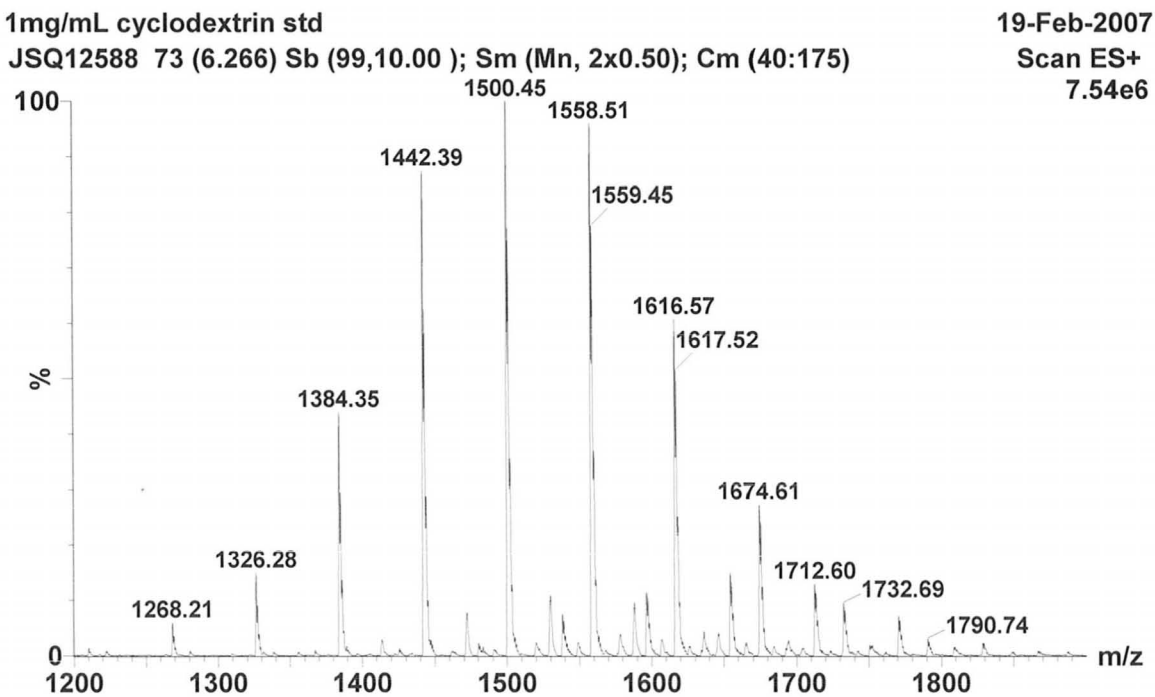
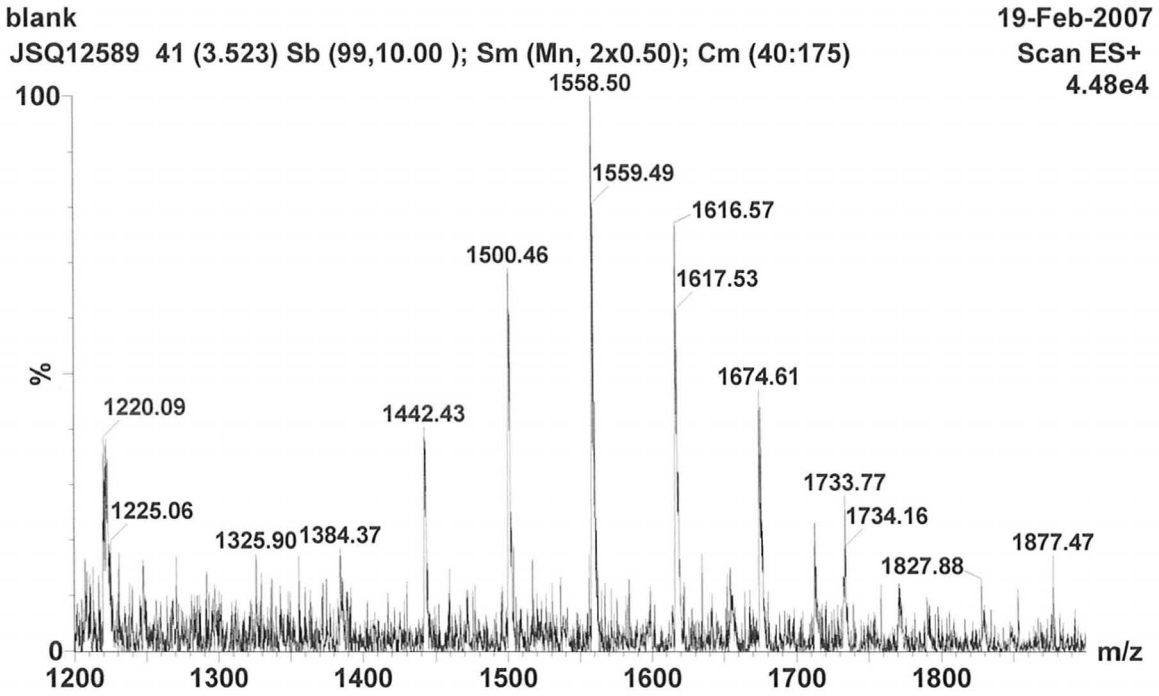
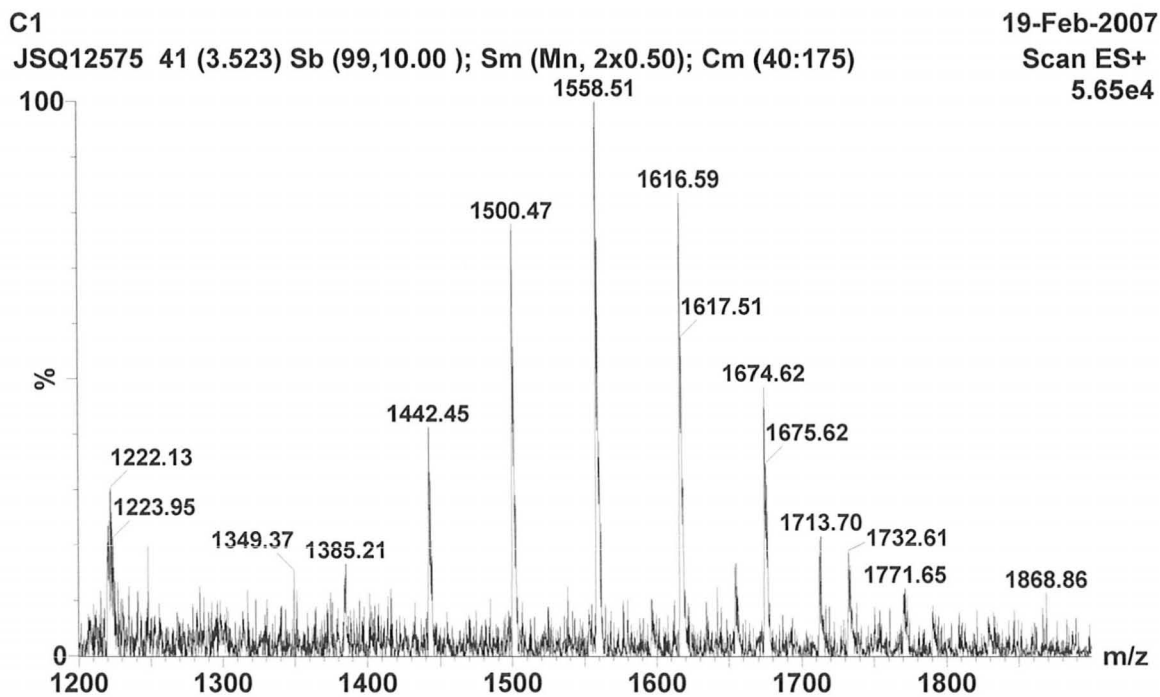
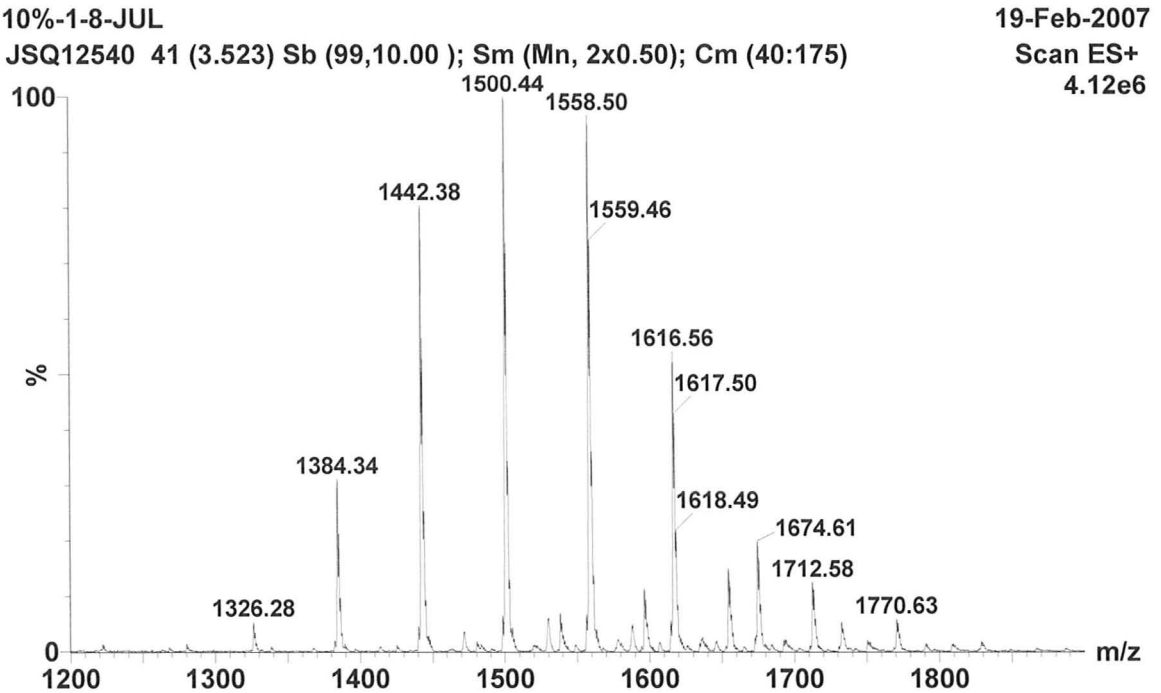
1300
1400
1500
1600
1700
1800
m/z

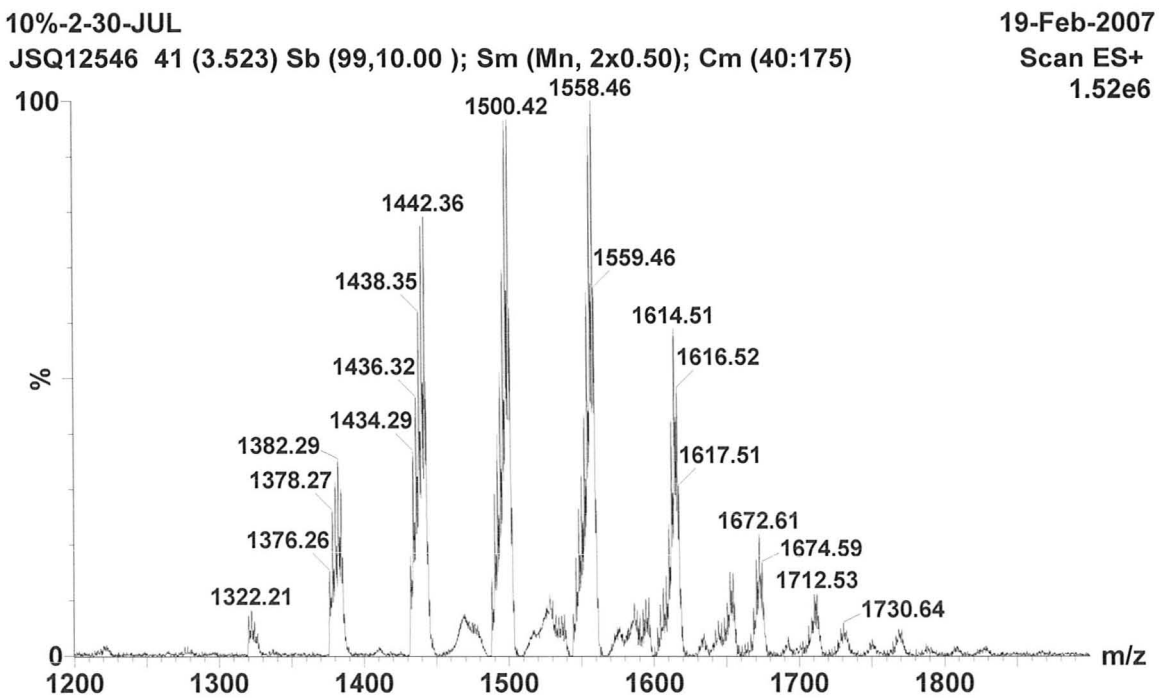

500ug/mL cyclodextrin std
JSQ12587 41 (3.523) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
19-Feb-2007
Scan ES+
4.60e6
100
%
0
1500.43
1558.51
1442.38
1559.45
1616.56
1617.52
1384.33
1674.61
1326.26
1712.59
1732.68
1268.22
1200
1300
1400
1500
1600
1700
1800
m/z

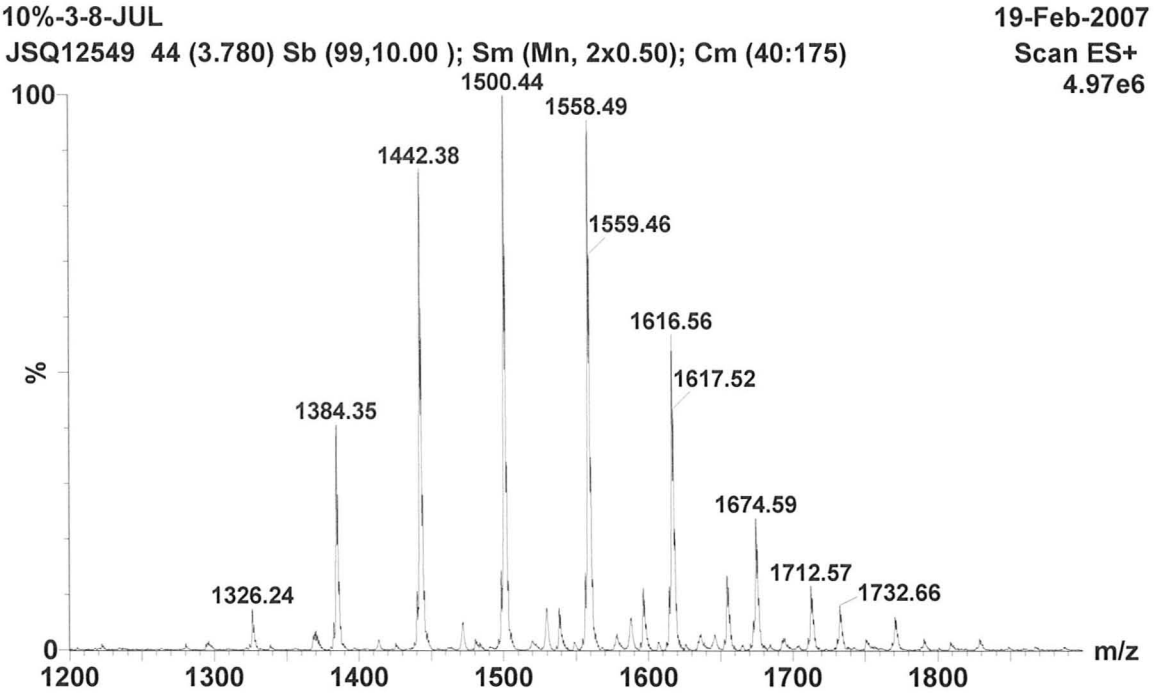

1mg/mL cyclodextrin std
JSQ12588 73 (6.266) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
19-Feb-2007
Scan ES+
7.54e6
100
%
0
1500.45
1558.51
1442.39
1559.45
1616.57
1617.52
1384.35
1674.61
1326.28
1712.60
1732.69
1268.21
1790.74
1200
1300
1400
1500
1600
1700
1800
m/z

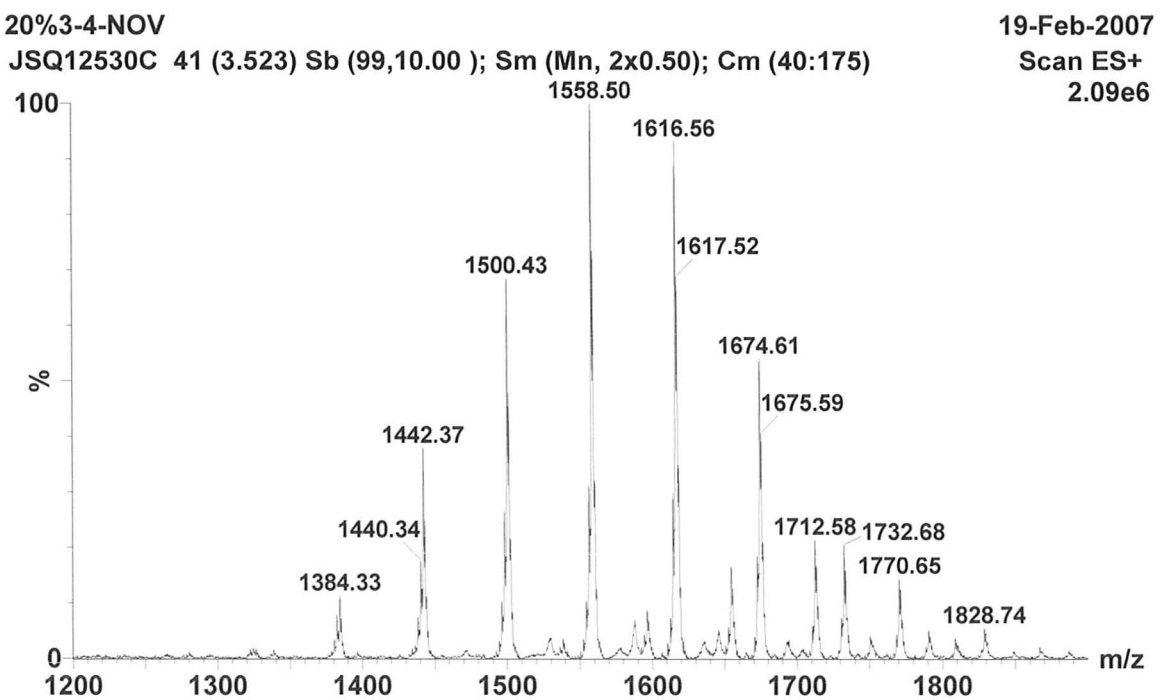

blank
19-Feb-2007
JSQ12589 41 (3.523) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
Scan ES+
4.48e4
100
%
0
1200
1300
1400
1500
1600
1700
1800
m/z
1558.50
1559.49
1616.57
1500.46
1617.53
1674.61
1220.09
1442.43
1733.77
1734.16
1225.06
1325.90
1384.37
1877.47
1827.88

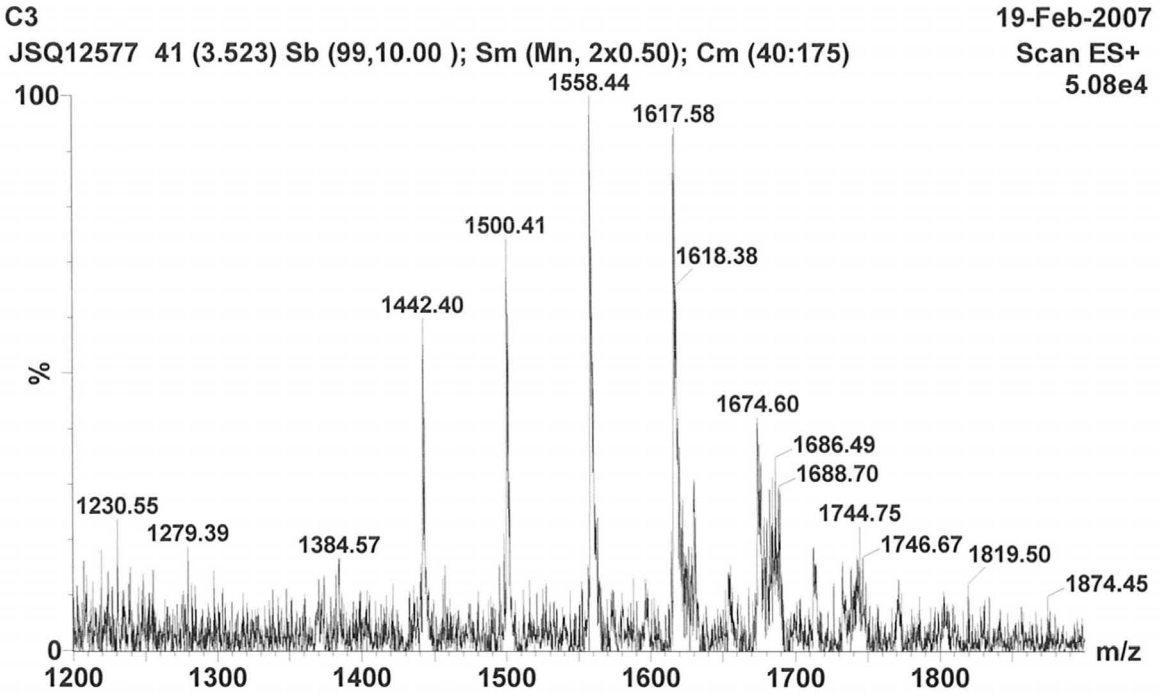

C1
19-Feb-2007
JSQ12575 41 (3.523) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
Scan ES+
5.65e4
100
%
0
1200
1300
1400
1500
1600
1700
1800
m/z
1558.51
1616.59
1500.47
1617.51
1674.62
1442.45
1675.62
1222.13
1223.95
1713.70
1732.61
1349.37
1385.21
1771.65
1868.86

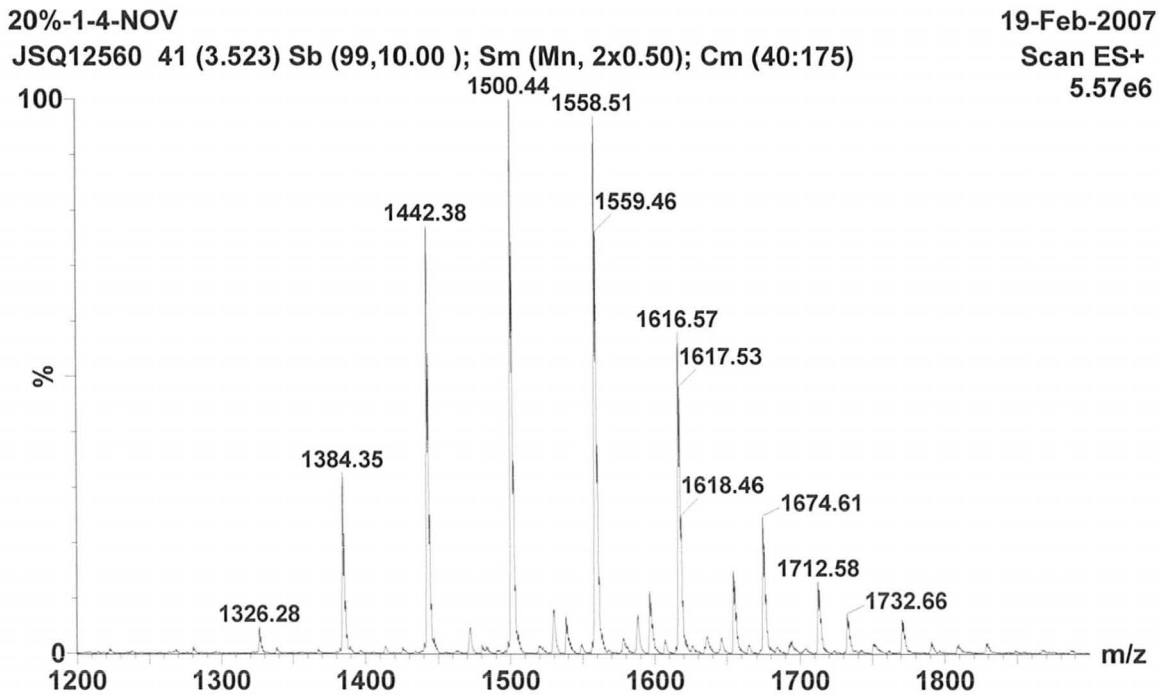

10%-1-8-JUL
19-Feb-2007
JSQ12540 41 (3.523) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
Scan ES+
4.12e6
100
%
0
1500.44
1558.50
1442.38
1559.46
1616.56
1617.50
1384.34
1618.49
1674.61
1712.58
1326.28
1770.63
1200
1300
1400
1500
1600
1700
1800
m/z

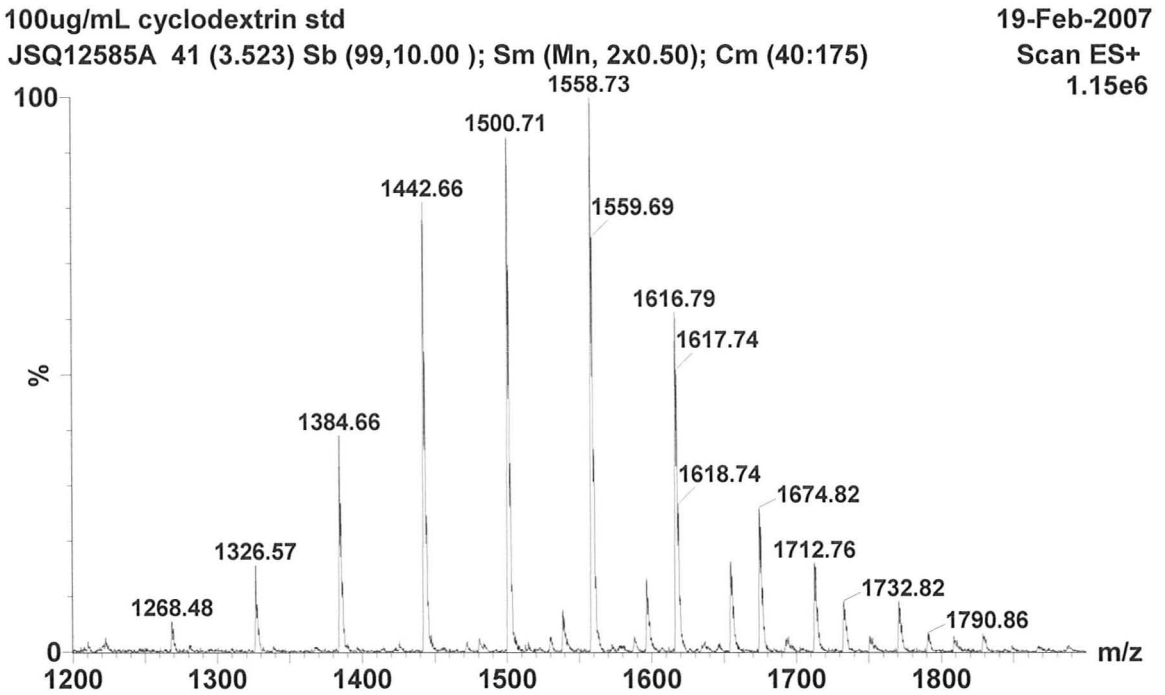

10%-2-30-JUL
19-Feb-2007
JSQ12546 41 (3.523) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
Scan ES+
1.52e6
100
%
0
1558.46
1500.42
1442.36
1559.46
1438.35
1614.51
1616.52
1436.32
1434.29
1382.29
1617.51
1378.27
1672.61
1376.26
1674.59
1712.53
1322.21
1730.64
1200
1300
1400
1500
1600
1700
1800
m/z

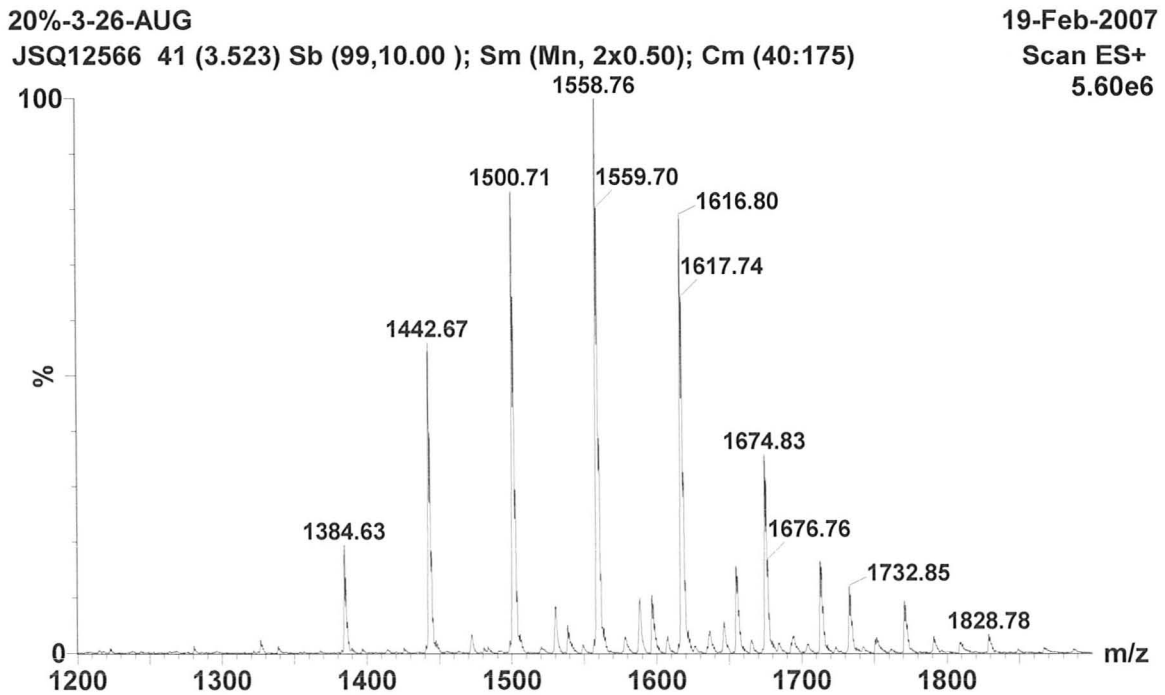

10%-3-8-JUL
19-Feb-2007
JSQ12549 44 (3.780) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
Scan ES+
4.97e6
100
%
0
1500.44
1558.49
1442.38
1559.46
1616.56
1617.52
1384.35
1674.59
1712.57
1732.66
1326.24
1200
1300
1400
1500
1600
1700
1800
m/z

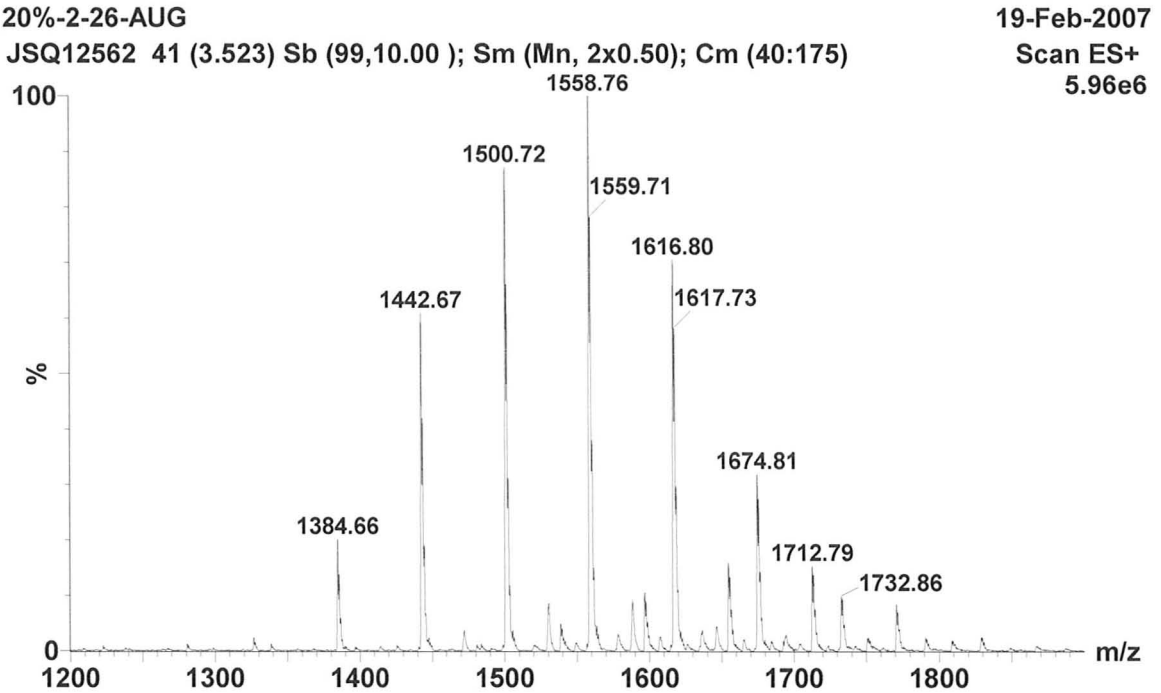

20%3-4-NOV
19-Feb-2007
JSQ12530C 41 (3.523) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
Scan ES+
2.09e6
100
%
0
1558.50
1616.56
1617.52
1500.43
1674.61
1675.59
1442.37
1440.34
1712.58
1732.68
1770.65
1384.33
1828.74
1200
1300
1400
1500
1600
1700
1800
m/z

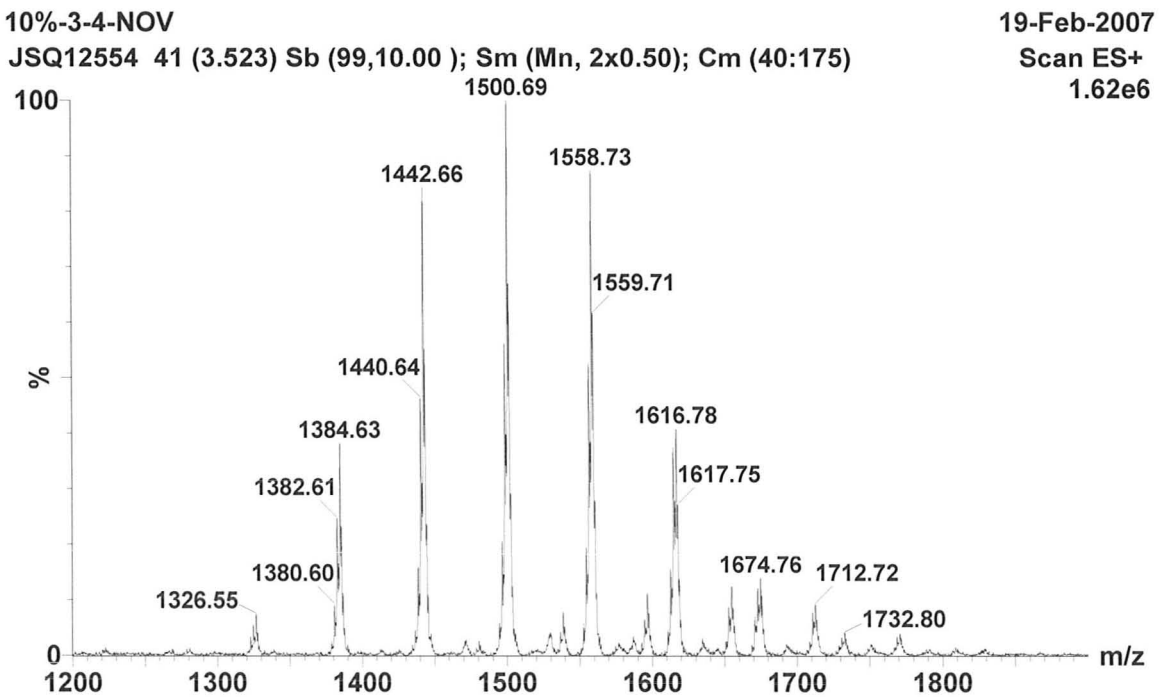

C3
19-Feb-2007
JSQ12577 41 (3.523) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
Scan ES+
5.08e4
100
%
0
1200
1300
1400
1500
1600
1700
1800
m/z
1558.44
1617.58
1500.41
1618.38
1442.40
1674.60
1686.49
1688.70
1230.55
1279.39
1384.57
1744.75
1746.67
1819.50
1874.45

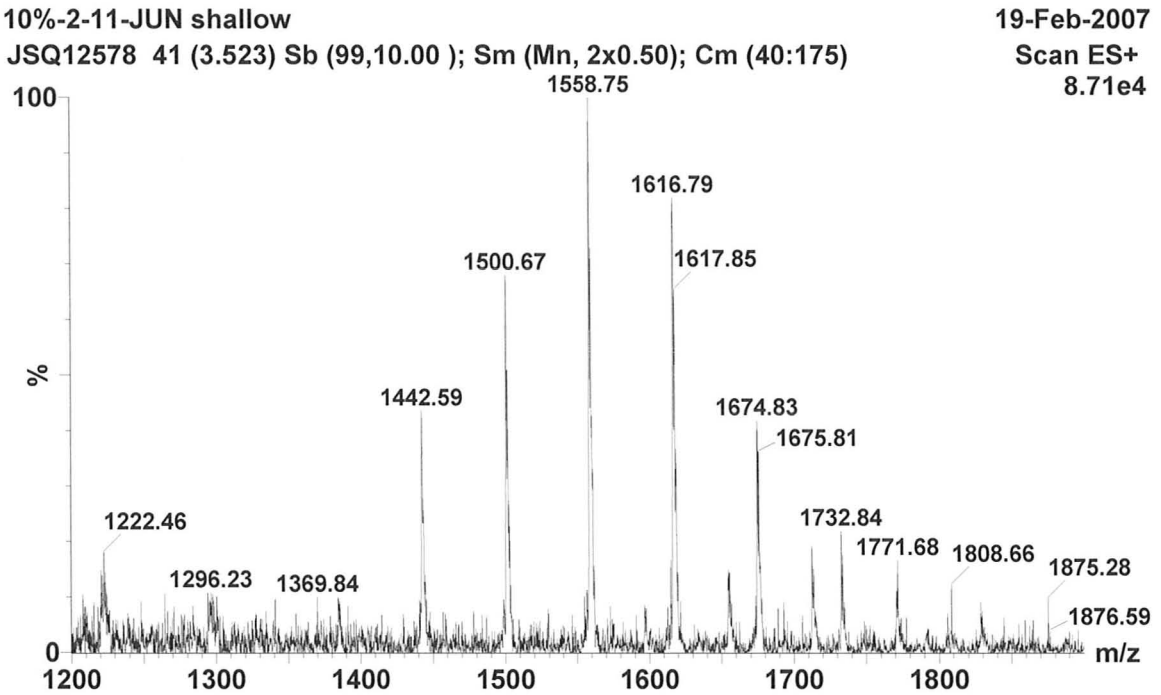

20%-1-4-NOV
19-Feb-2007
JSQ12560 41 (3.523) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
Scan ES+
5.57e6
100
%
0
1200
1300
1400
1500
1600
1700
1800
m/z
1500.44
1558.51
1442.38
1559.46
1616.57
1617.53
1384.35
1618.46
1674.61
1712.58
1732.66
1326.28

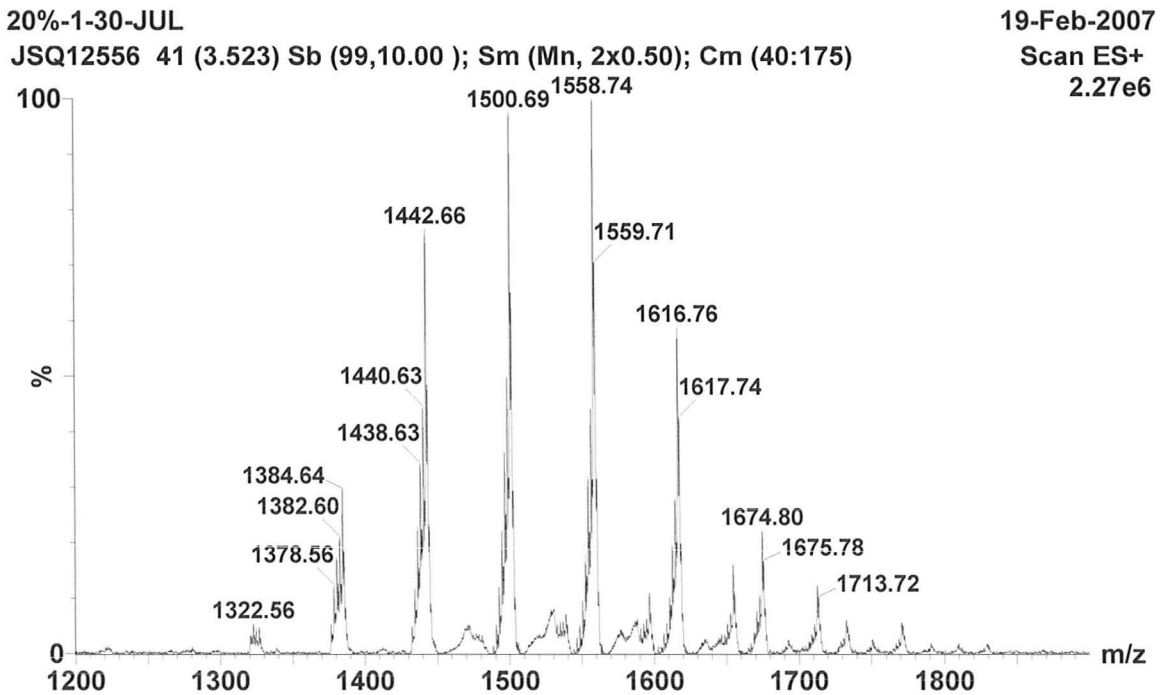

100ug/mL cyclodextrin std
JSQ12585A 41 (3.523) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
19-Feb-2007
Scan ES+
1.15e6
100
%
0
1200
1300
1400
1500
1600
1700
1800
m/z
1558.73
1500.71
1442.66
1559.69
1616.79
1617.74
1384.66
1618.74
1674.82
1326.57
1712.76
1732.82
1268.48
1790.86

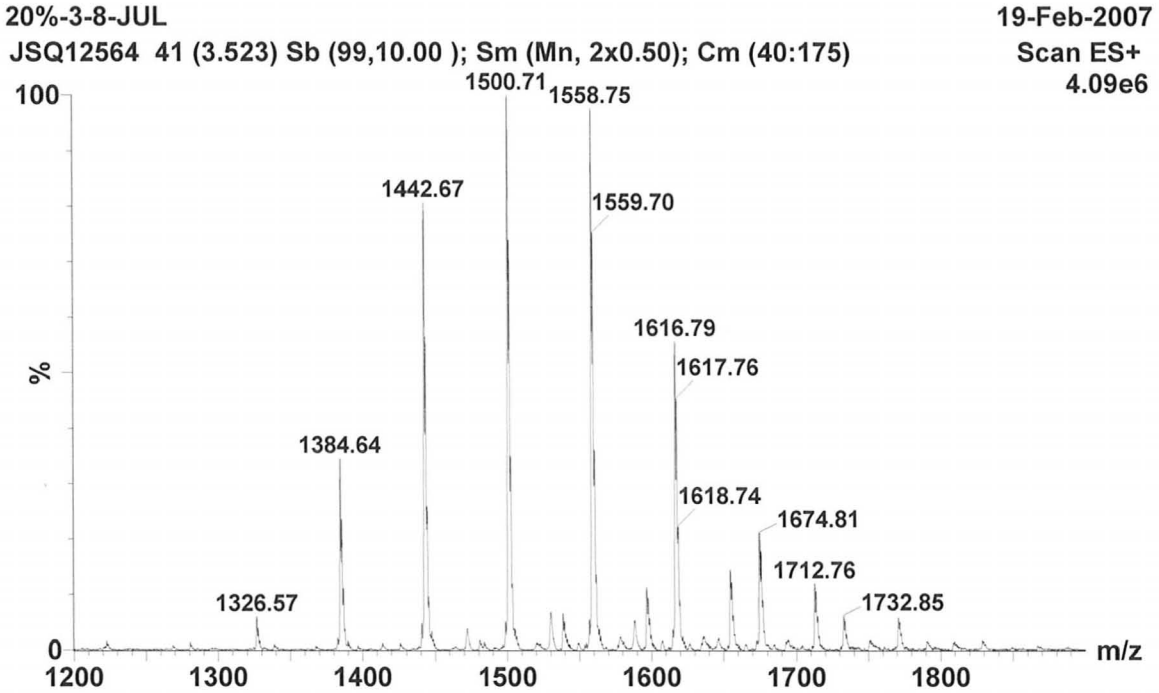

20%-3-26-AUG
JSQ12566 41 (3.523) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
19-Feb-2007
Scan ES+
5.60e6
100
%
0
1200
1300
1400
1500
1600
1700
1800
m/z
1558.76
1500.71
1559.70
1616.80
1617.74
1442.67
1674.83
1384.63
1676.76
1732.85
1828.78

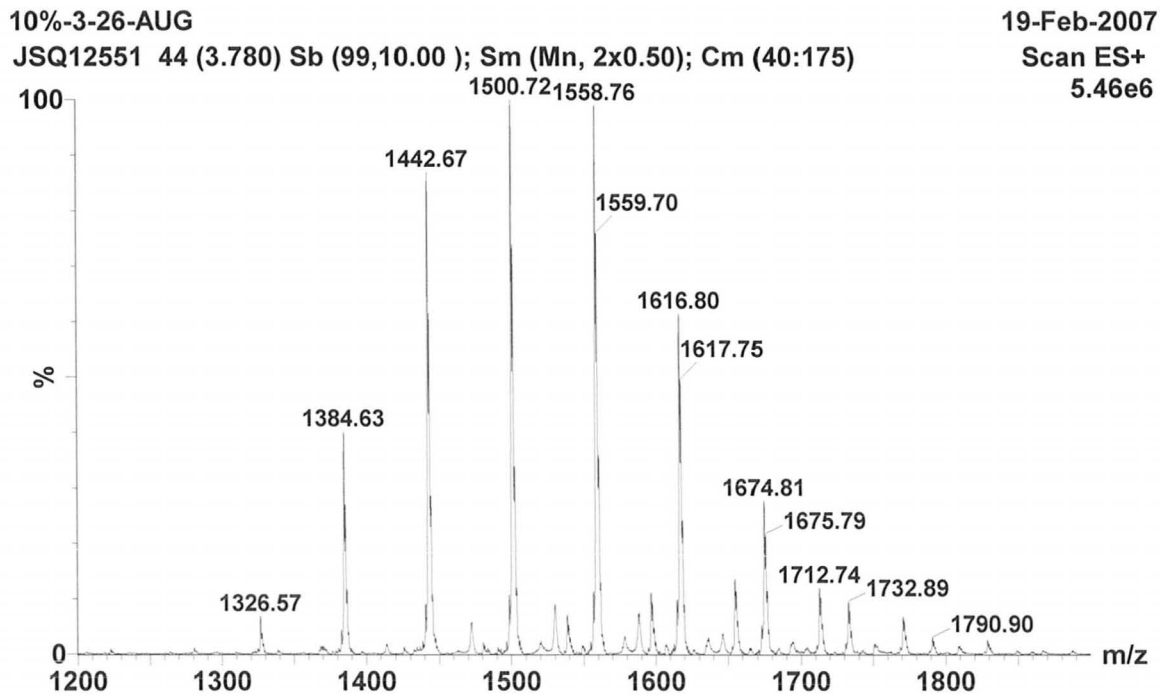

20%-2-26-AUG
19-Feb-2007
JSQ12562 41 (3.523) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
Scan ES+
5.96e6
100
%
0
1558.76
1500.72
1559.71
1616.80
1442.67
1617.73
1674.81
1384.66
1712.79
1732.86
1200
1300
1400
1500
1600
1700
1800
m/z

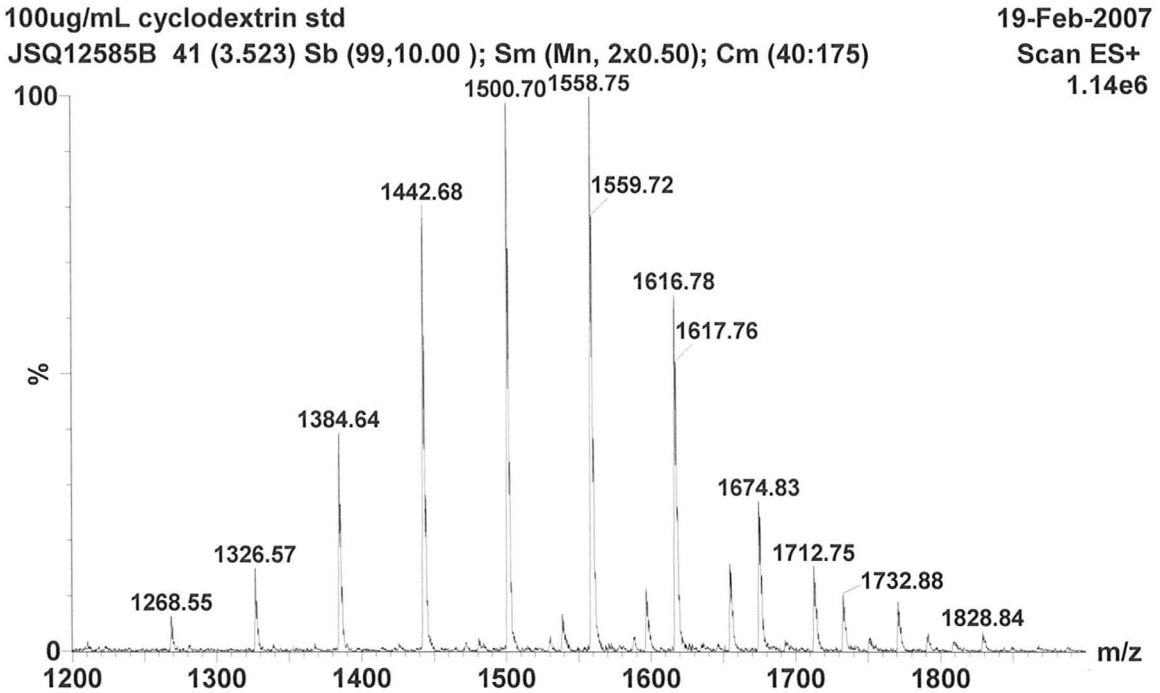

10%-3-4-NOV
19-Feb-2007
JSQ12554 41 (3.523) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
Scan ES+
1.62e6
100
%
0
1500.69
1558.73
1442.66
1559.71
1440.64
1616.78
1384.63
1617.75
1382.61
1674.76
1712.72
1380.60
1326.55
1732.80
1200
1300
1400
1500
1600
1700
1800
m/z

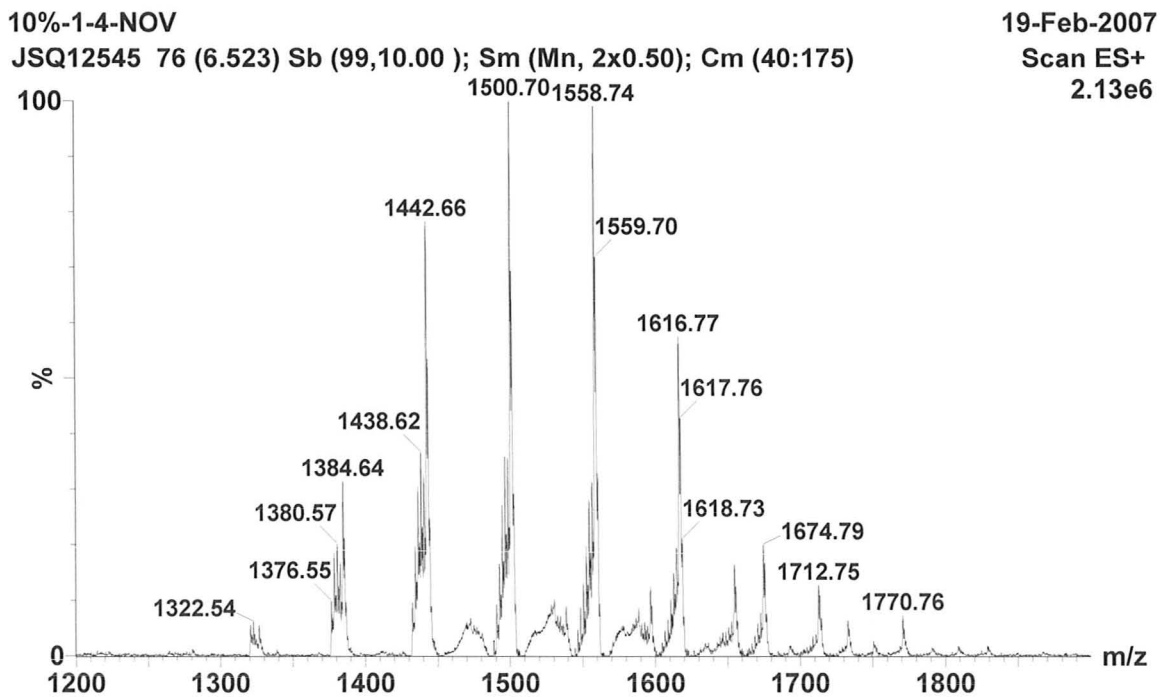

10%-2-11-JUN shallow
19-Feb-2007
JSQ12578 41 (3.523) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
Scan ES+
8.71e4
100
%
0
1200
1300
1400
1500
1600
1700
1800
m/z
1558.75
1616.79
1500.67
1617.85
1442.59
1674.83
1675.81
1222.46
1732.84
1771.68
1808.66
1875.28
1296.23
1369.84
1876.59

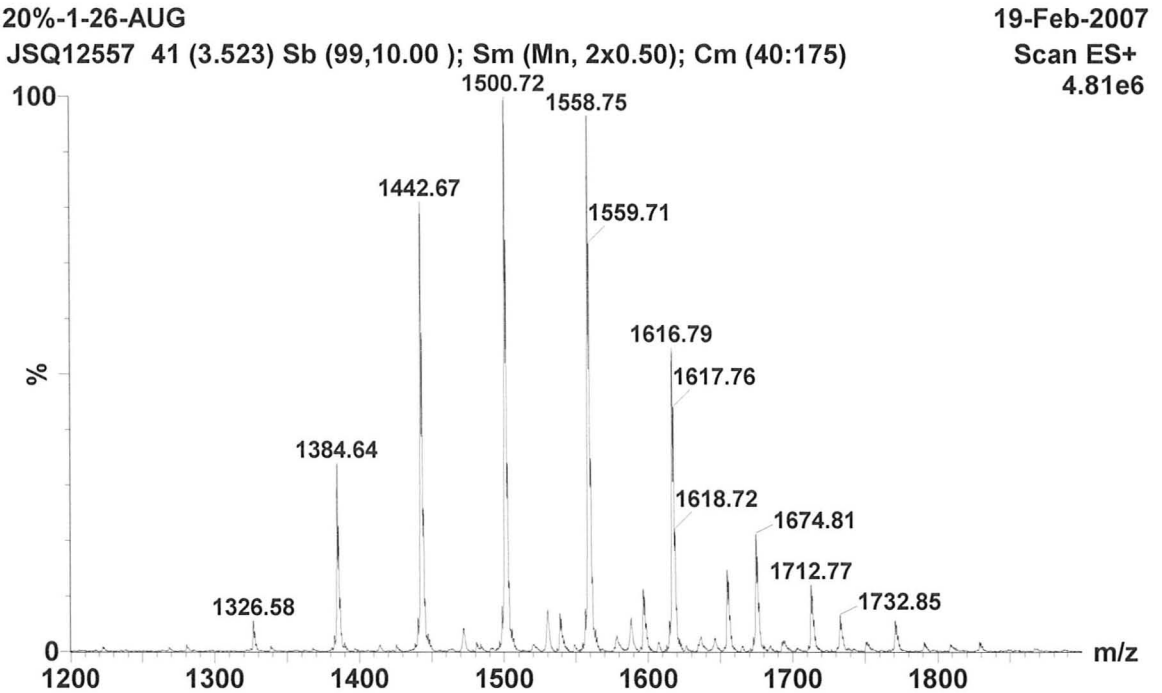

20%-1-30-JUL
19-Feb-2007
JSQ12556 41 (3.523) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
Scan ES+
2.27e6
100
%
0
1200
1300
1400
1500
1600
1700
1800
m/z
1500.69
1558.74
1442.66
1559.71
1616.76
1440.63
1617.74
1438.63
1384.64
1382.60
1674.80
1675.78
1378.56
1713.72
1322.56

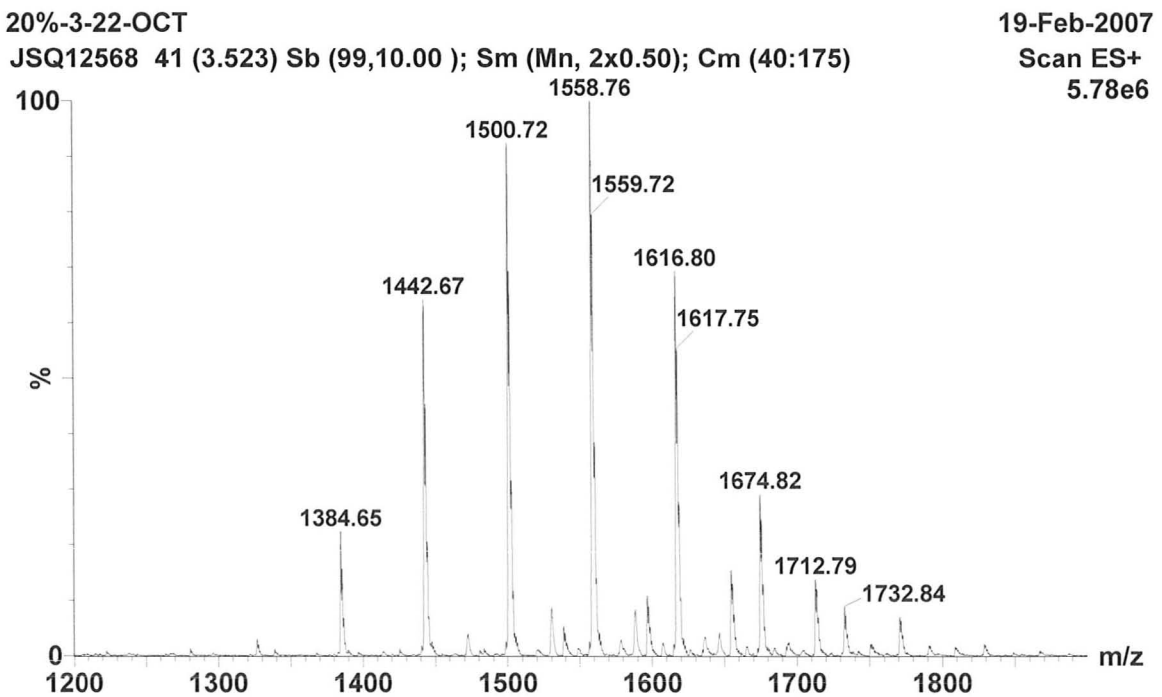

20%-3-8-JUL
19-Feb-2007
JSQ12564 41 (3.523) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
Scan ES+
4.09e6
100
%
0
1500.71
1558.75
1442.67
1559.70
1616.79
1617.76
1384.64
1618.74
1674.81
1712.76
1326.57
1732.85
1200
1300
1400
1500
1600
1700
1800
m/z

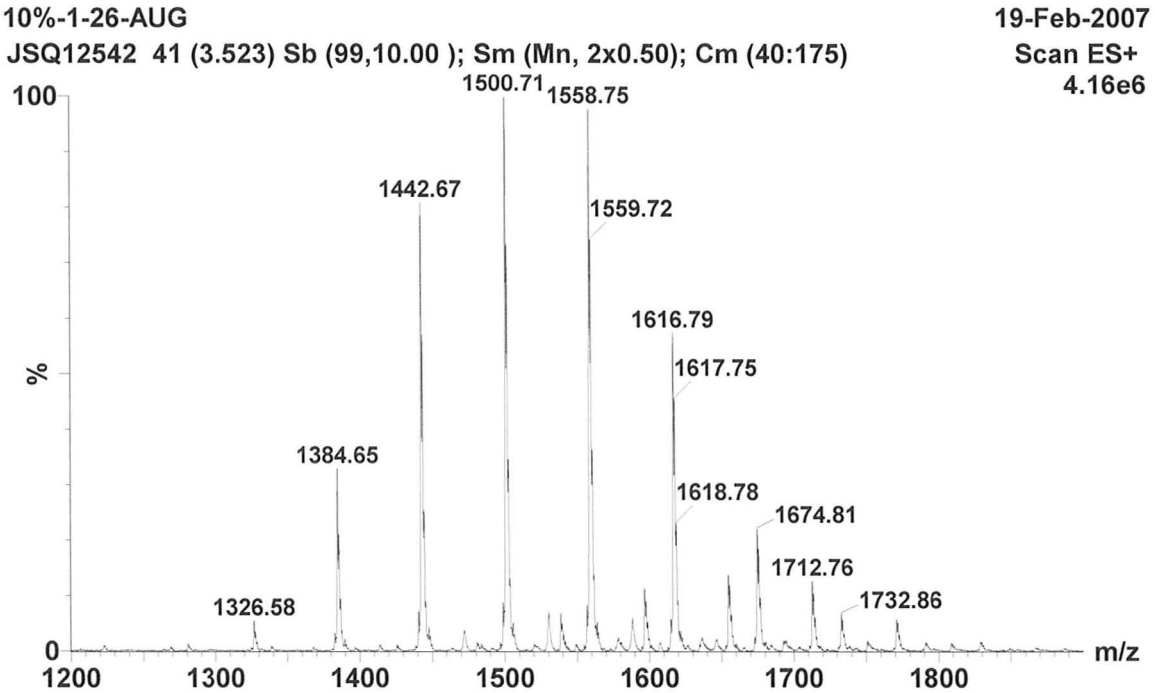

10%-3-26-AUG
19-Feb-2007
JSQ12551 44 (3.780) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
Scan ES+
5.46e6
100
%
0
1500.72
1558.76
1442.67
1559.70
1616.80
1617.75
1384.63
1674.81
1675.79
1712.74
1732.89
1326.57
1790.90
1200
1300
1400
1500
1600
1700
1800
m/z

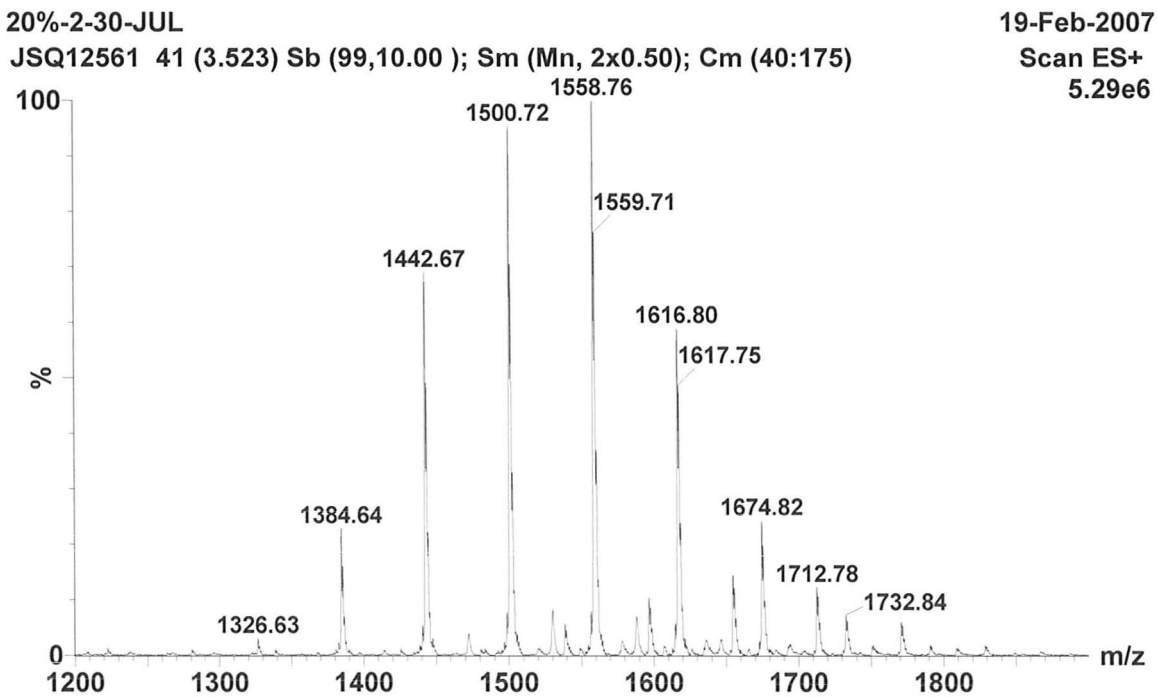

100ug/mL cyclodextrin std
JSQ12585B 41 (3.523) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
19-Feb-2007
Scan ES+
1.14e6
100
%
0
1500.70
1558.75
1442.68
1559.72
1616.78
1617.76
1384.64
1674.83
1326.57
1712.75
1732.88
1268.55
1828.84
1200
1300
1400
1500
1600
1700
1800
m/z

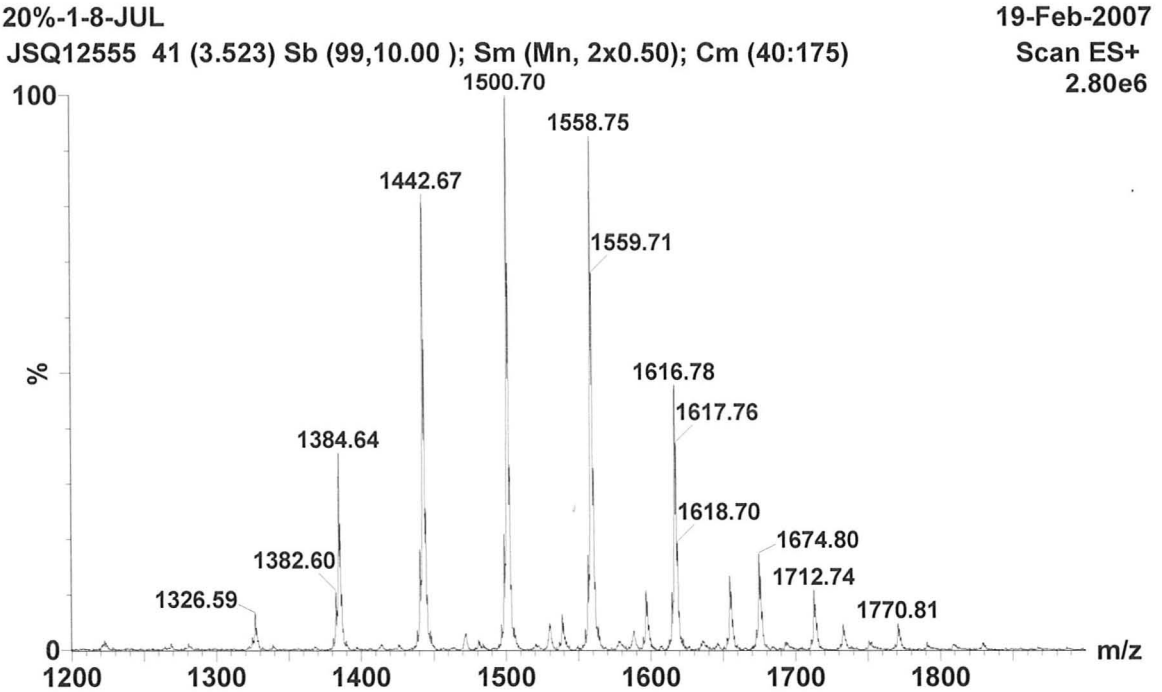

10%-1-4-NOV
JSQ12545 76 (6.523) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
19-Feb-2007
Scan ES+
2.13e6
100
%
0
1500.70
1558.74
1442.66
1559.70
1616.77
1617.76
1438.62
1384.64
1380.57
1618.73
1674.79
1376.55
1712.75
1322.54
1770.76
1200
1300
1400
1500
1600
1700
1800
m/z

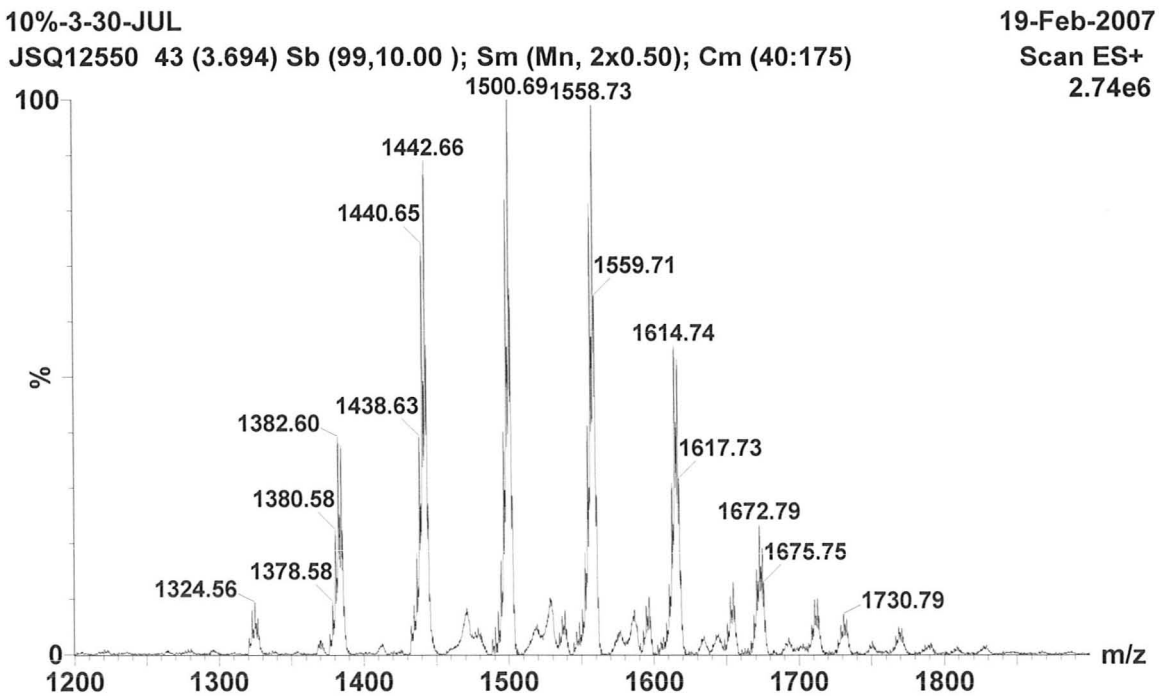

20%-1-26-AUG
JSQ12557 41 (3.523) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
19-Feb-2007
Scan ES+
4.81e6
100
%
0
1500.72
1558.75
1442.67
1559.71
1616.79
1617.76
1384.64
1618.72
1674.81
1712.77
1326.58
1732.85
1200
1300
1400
1500
1600
1700
1800
m/z

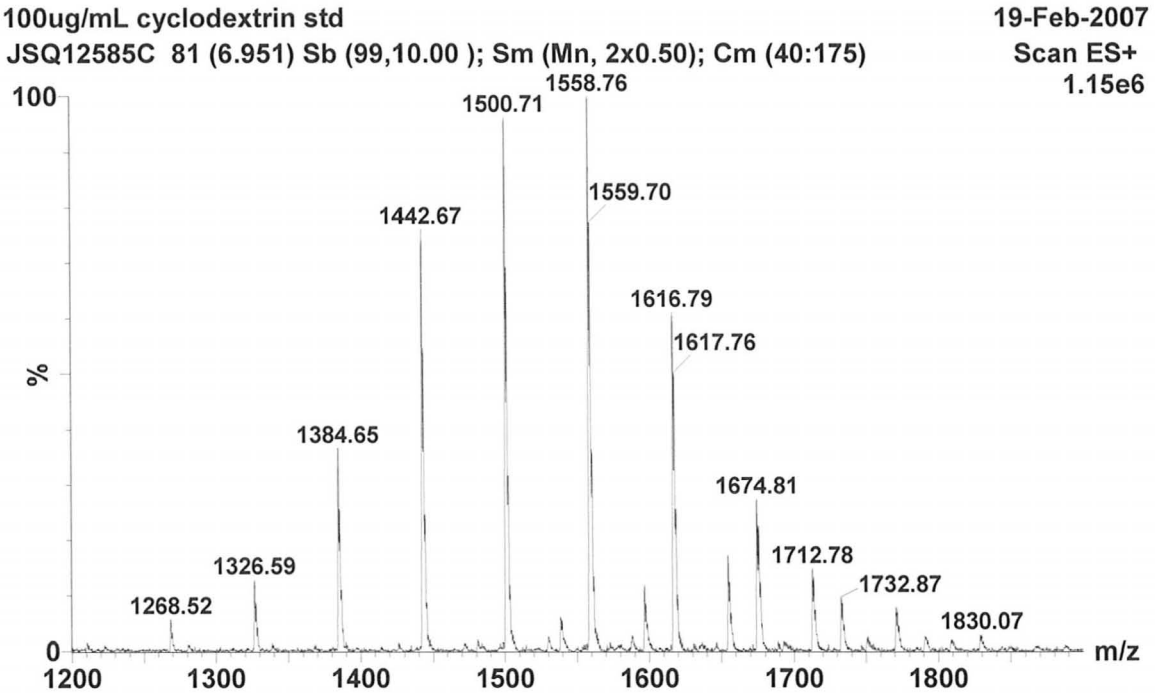

20%-3-22-OCT
JSQ12568 41 (3.523) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
19-Feb-2007
Scan ES+
5.78e6
100
%
0
1558.76
1500.72
1559.72
1616.80
1442.67
1617.75
1674.82
1384.65
1712.79
1732.84
1200
1300
1400
1500
1600
1700
1800
m/z

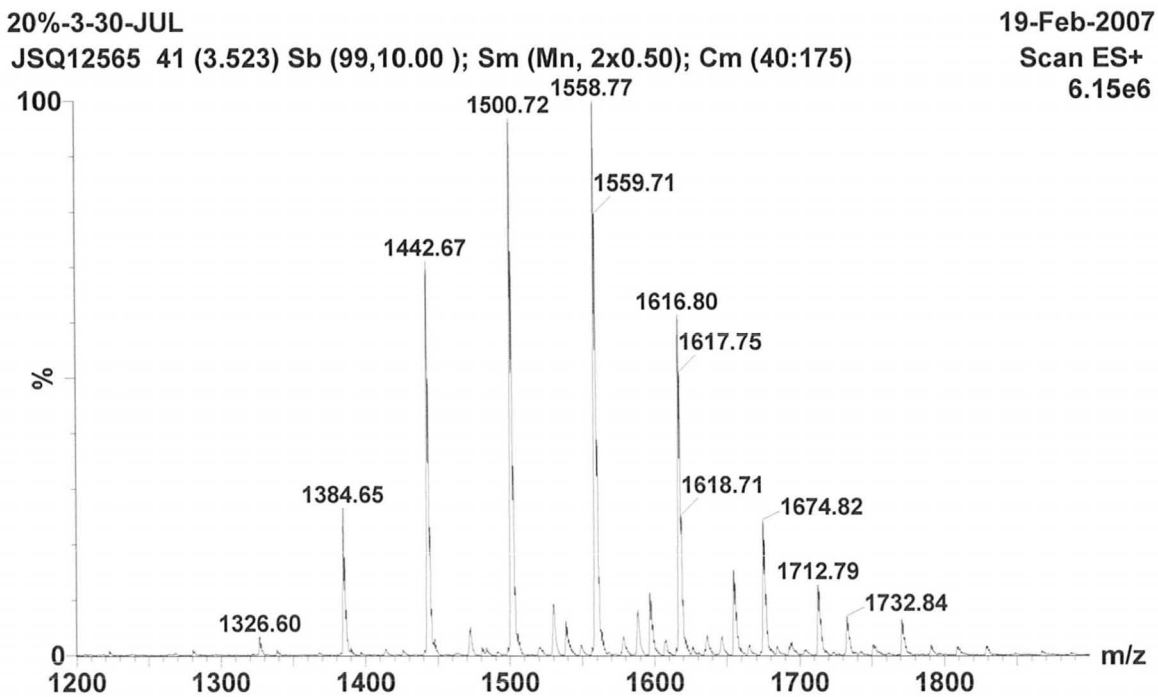

10%-1-26-AUG
19-Feb-2007
JSQ12542 41 (3.523) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
Scan ES+
4.16e6
100
%
0
1500.71
1558.75
1442.67
1559.72
1616.79
1617.75
1384.65
1618.78
1674.81
1712.76
1732.86
1326.58
1200
1300
1400
1500
1600
1700
1800
m/z

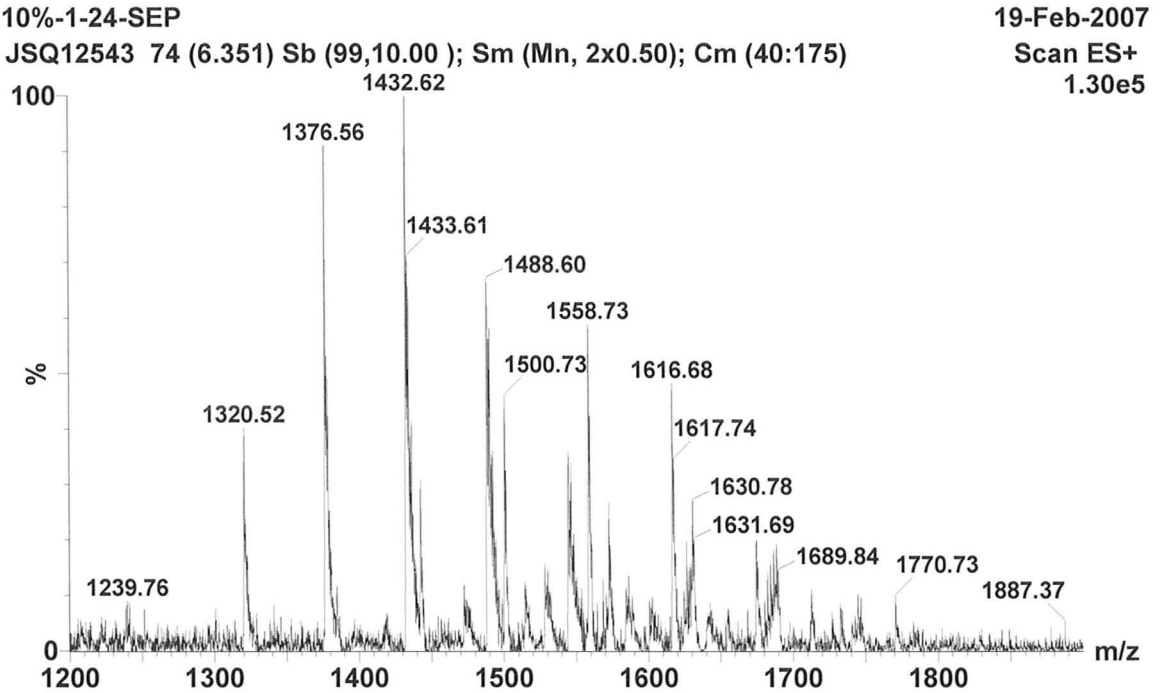

20%-2-30-JUL
19-Feb-2007
JSQ12561 41 (3.523) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
Scan ES+
5.29e6
100
%
0
1558.76
1500.72
1559.71
1442.67
1616.80
1617.75
1674.82
1384.64
1712.78
1732.84
1326.63
1200
1300
1400
1500
1600
1700
1800
m/z

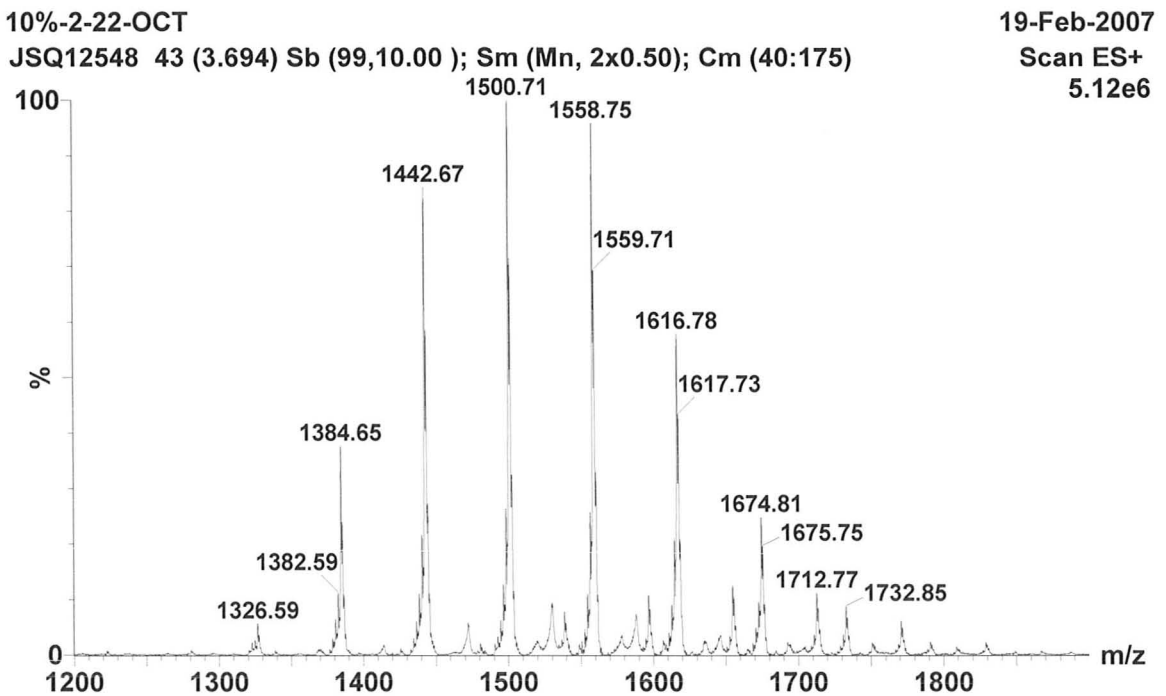

20%-1-8-JUL
19-Feb-2007
JSQ12555 41 (3.523) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
Scan ES+
2.80e6
100
%
0
1500.70
1558.75
1442.67
1559.71
1616.78
1617.76
1384.64
1618.70
1674.80
1382.60
1712.74
1326.59
1770.81
1200
1300
1400
1500
1600
1700
1800
m/z

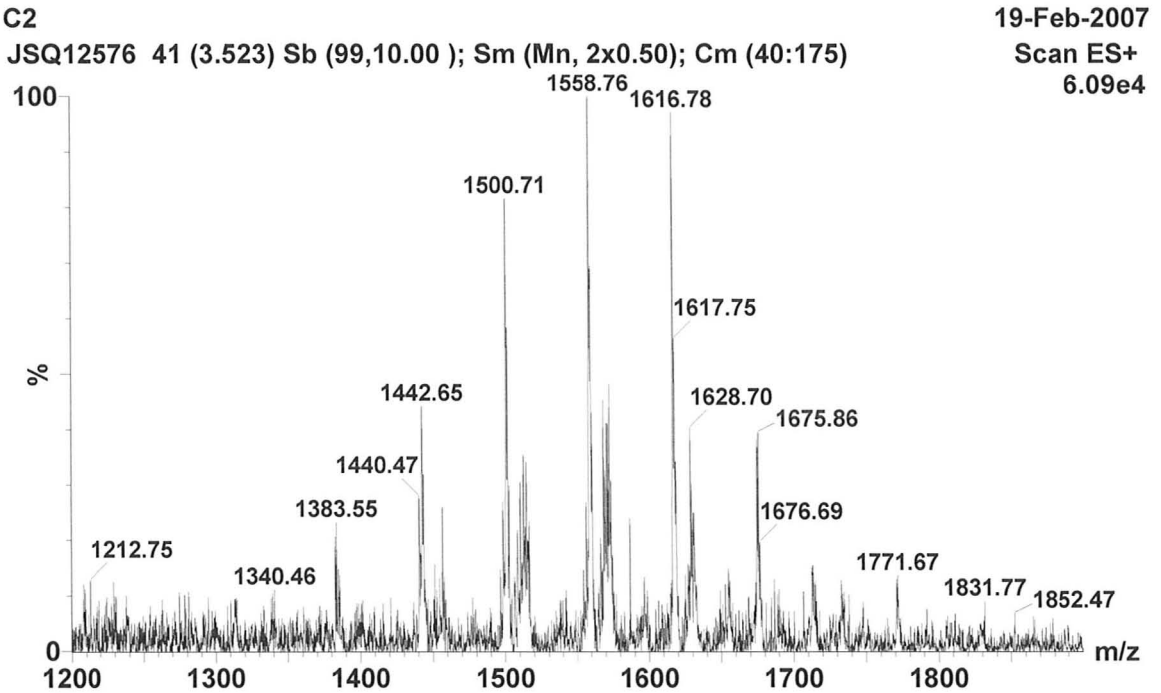

10%-3-30-JUL
19-Feb-2007
JSQ12550 43 (3.694) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
Scan ES+
2.74e6
100
%
0
1500.69
1558.73
1442.66
1440.65
1559.71
1614.74
1438.63
1382.60
1617.73
1380.58
1672.79
1675.75
1378.58
1324.56
1730.79
1200
1300
1400
1500
1600
1700
1800
m/z

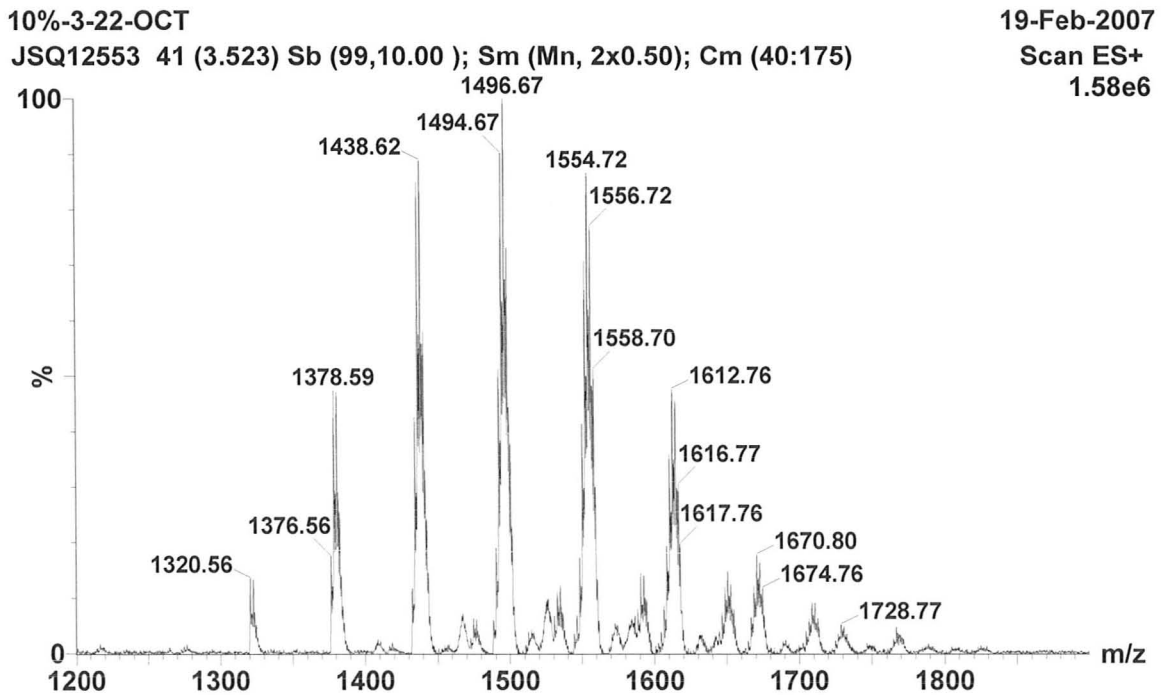

100ug/mL cyclodextrin std
JSQ12585C 81 (6.951) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
19-Feb-2007
Scan ES+
1.15e6
100
%
0
1558.76
1500.71
1559.70
1442.67
1616.79
1617.76
1384.65
1674.81
1712.78
1326.59
1732.87
1268.52
1830.07
1200
1300
1400
1500
1600
1700
1800
m/z

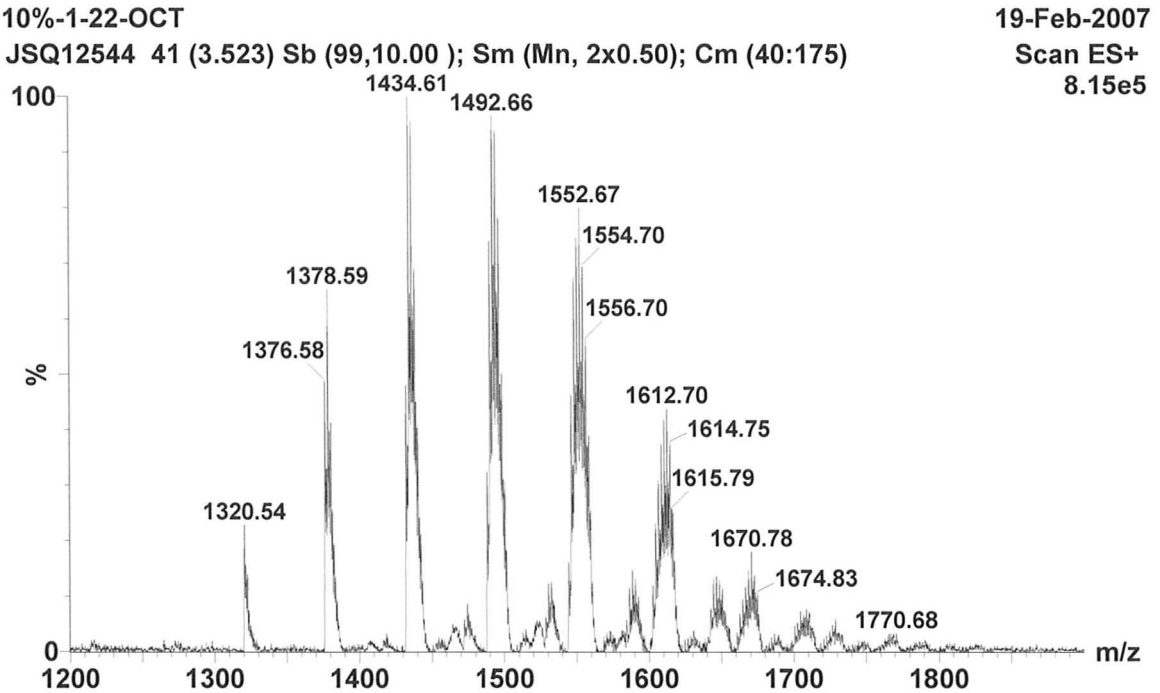

20%-3-30-JUL
JSQ12565 41 (3.523) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
19-Feb-2007
Scan ES+
6.15e6
100
%
0
1558.77
1500.72
1559.71
1442.67
1616.80
1617.75
1618.71
1384.65
1674.82
1712.79
1732.84
1326.60
1200
1300
1400
1500
1600
1700
1800
m/z

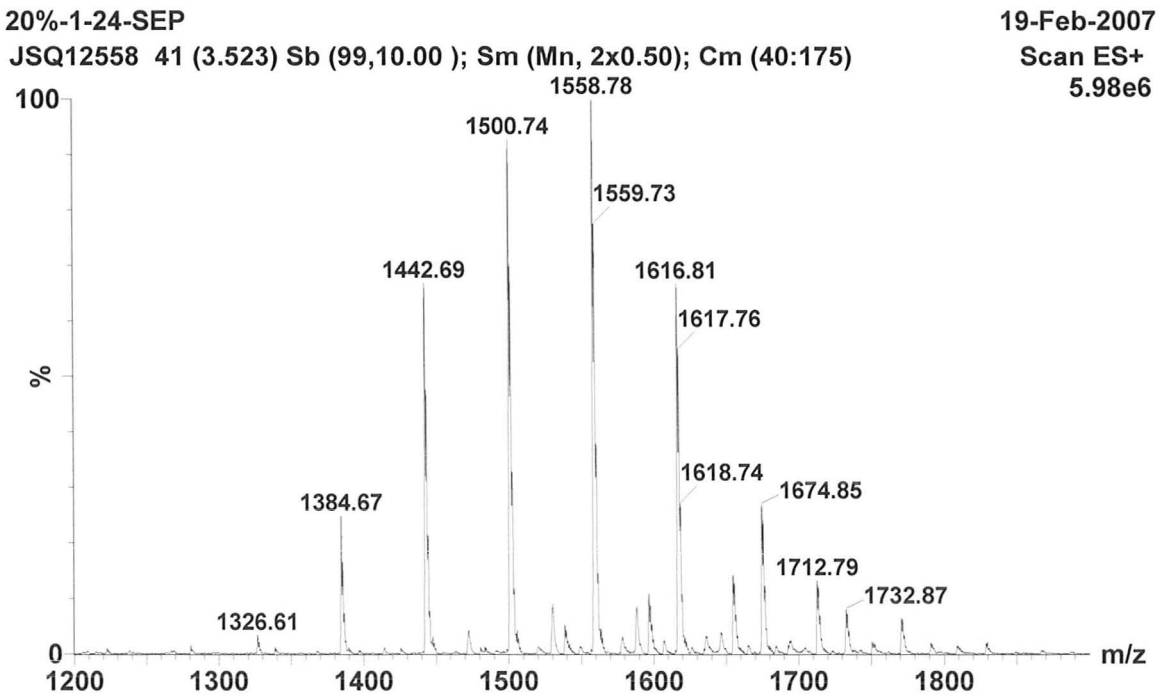

10%-1-24-SEP
JSQ12543 74 (6.351) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
19-Feb-2007
Scan ES+
1.30e5
100
%
0
1432.62
1376.56
1433.61
1488.60
1558.73
1500.73
1616.68
1320.52
1617.74
1630.78
1631.69
1689.84
1770.73
1239.76
1887.37
1200
1300
1400
1500
1600
1700
1800
m/z

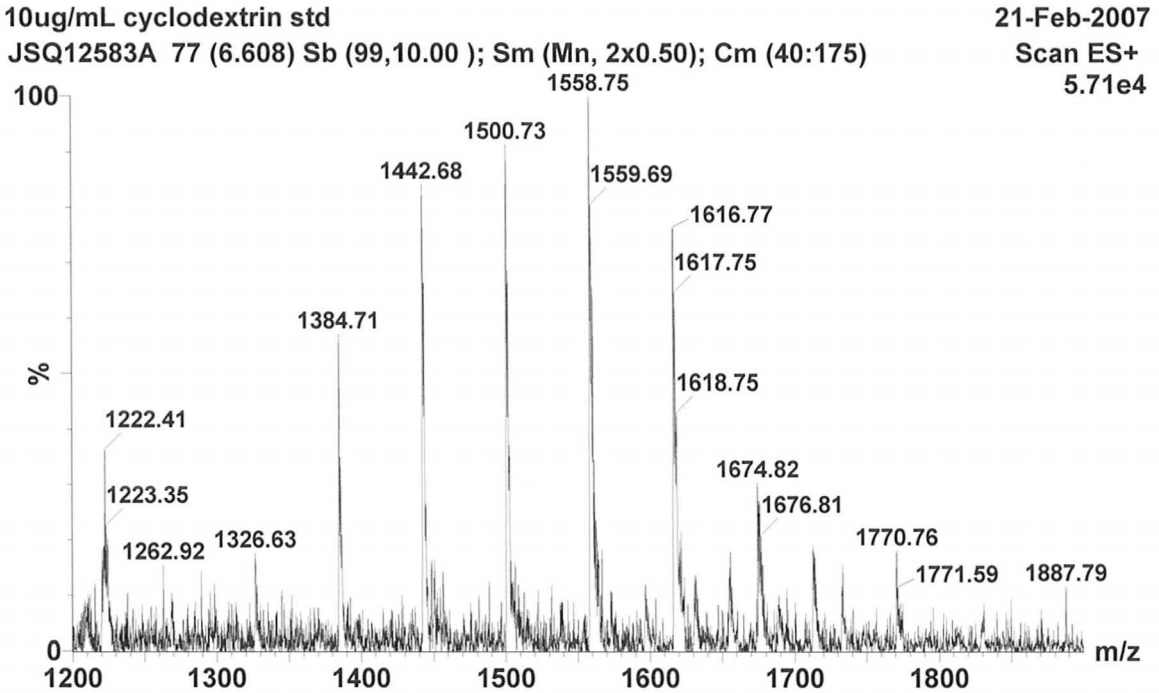

10%-2-22-OCT
JSQ12548 43 (3.694) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
19-Feb-2007
Scan ES+
5.12e6
100
%
0
1500.71
1558.75
1442.67
1559.71
1616.78
1617.73
1384.65
1674.81
1675.75
1382.59
1712.77
1732.85
1326.59
1200
1300
1400
1500
1600
1700
1800
m/z

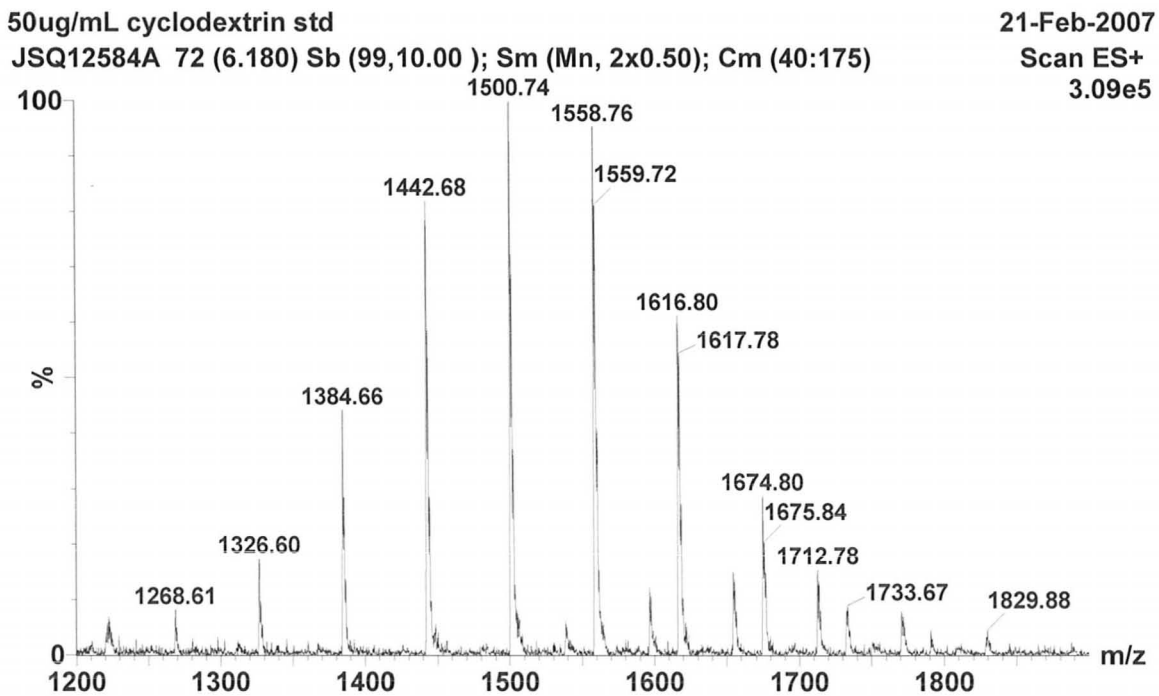

C2
19-Feb-2007
JSQ12576 41 (3.523) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
Scan ES+
6.09e4
100
%
0
1558.76
1616.78
1500.71
1617.75
1442.65
1628.70
1675.86
1440.47
1383.55
1676.69
1212.75
1340.46
1771.67
1831.77
1852.47
1200
1300
1400
1500
1600
1700
1800
m/z

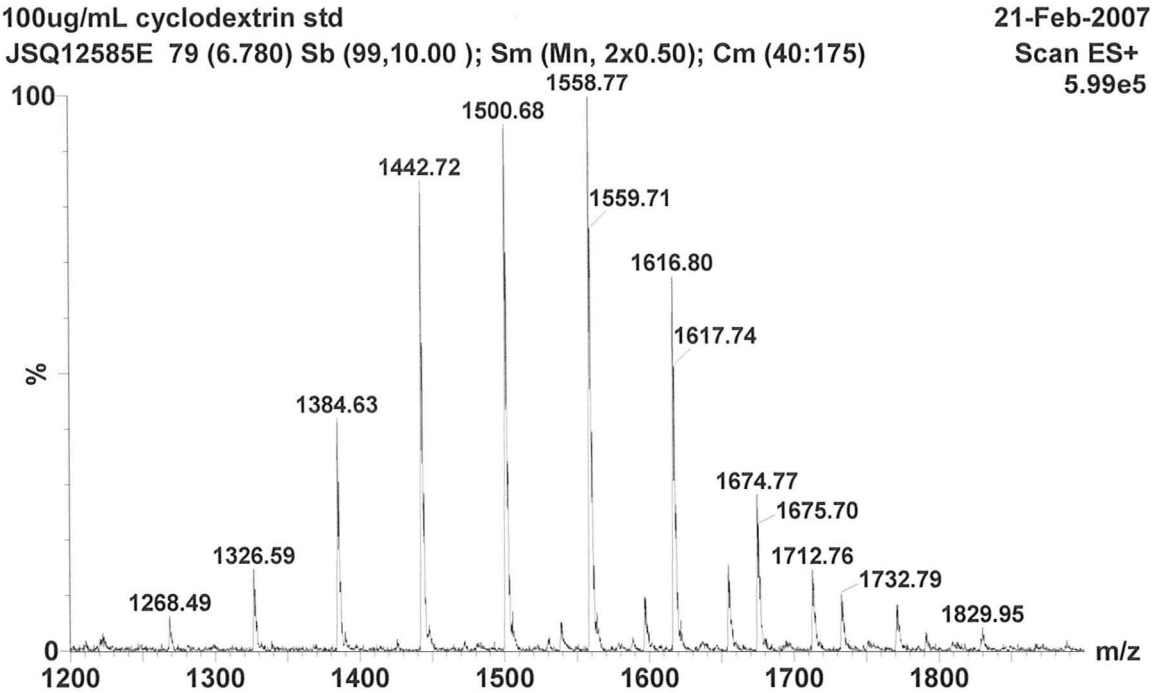

10%-3-22-OCT
19-Feb-2007
JSQ12553 41 (3.523) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
Scan ES+
1.58e6
100
%
0
1496.67
1494.67
1438.62
1554.72
1556.72
1558.70
1378.59
1612.76
1616.77
1617.76
1376.56
1670.80
1320.56
1674.76
1728.77
1200
1300
1400
1500
1600
1700
1800
m/z

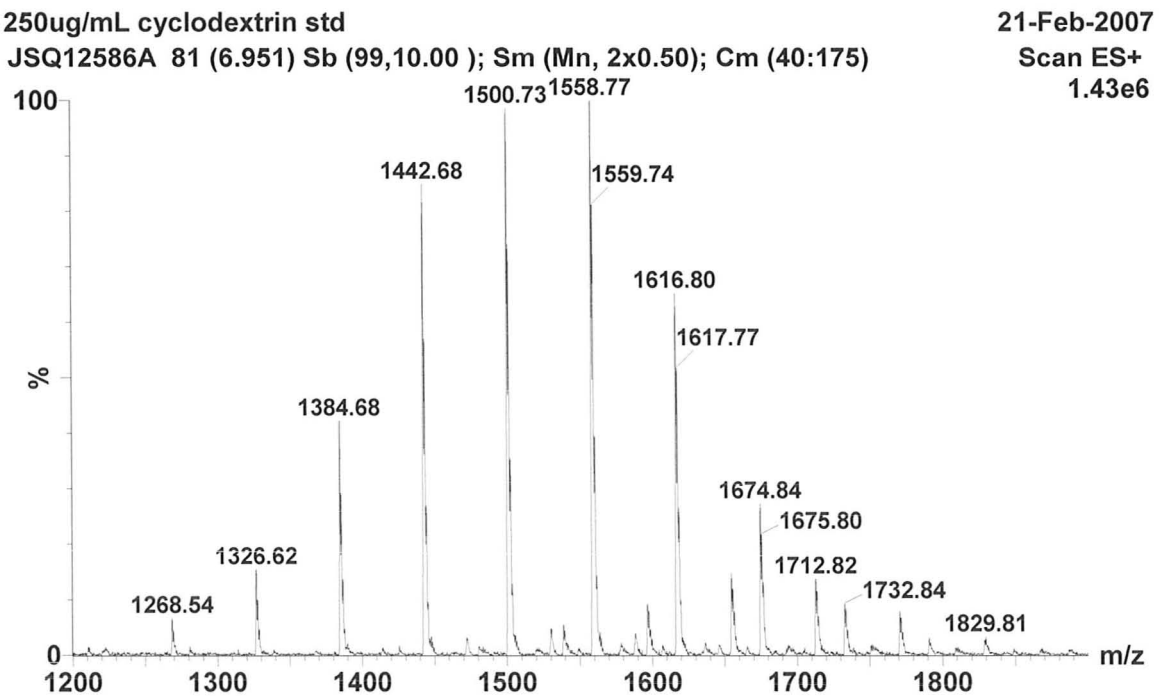

10%-1-22-OCT
19-Feb-2007
JSQ12544 41 (3.523) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
Scan ES+
8.15e5
100
%
0
1434.61
1492.66
1552.67
1554.70
1378.59
1556.70
1376.58
1612.70
1614.75
1615.79
1320.54
1670.78
1674.83
1770.68
1200
1300
1400
1500
1600
1700
1800
m/z

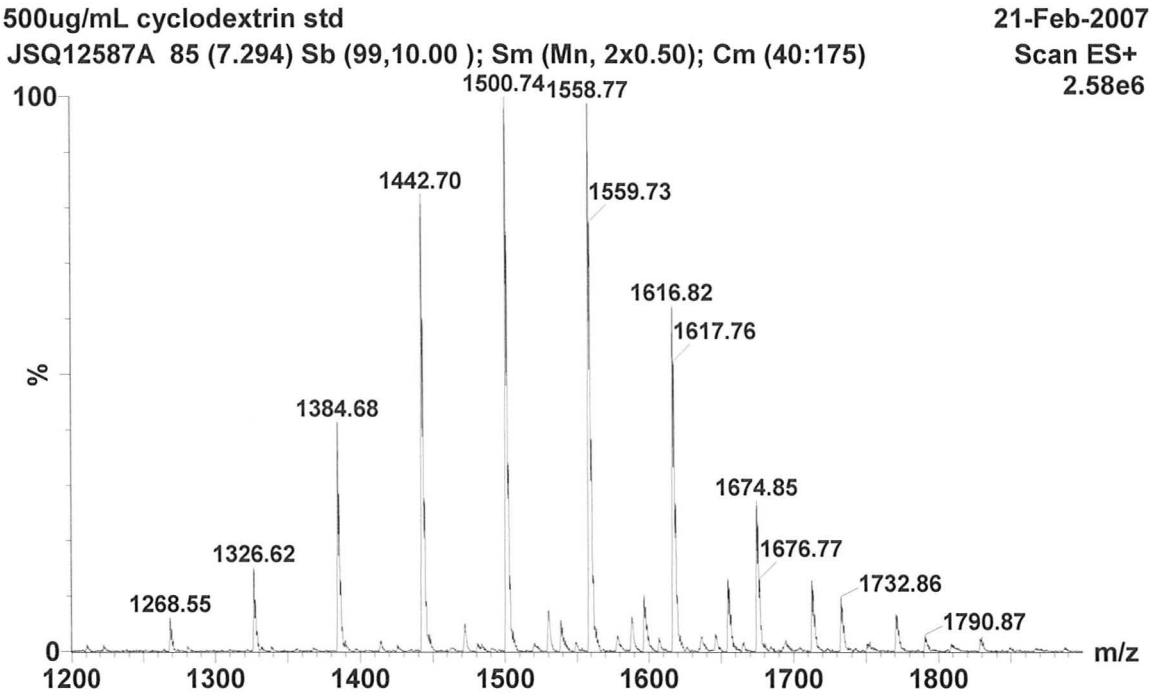

20%-1-24-SEP
19-Feb-2007
JSQ12558 41 (3.523) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
Scan ES+
5.98e6
100
%
0
1558.78
1500.74
1559.73
1442.69
1616.81
1617.76
1618.74
1384.67
1674.85
1712.79
1732.87
1326.61
1200
1300
1400
1500
1600
1700
1800
m/z

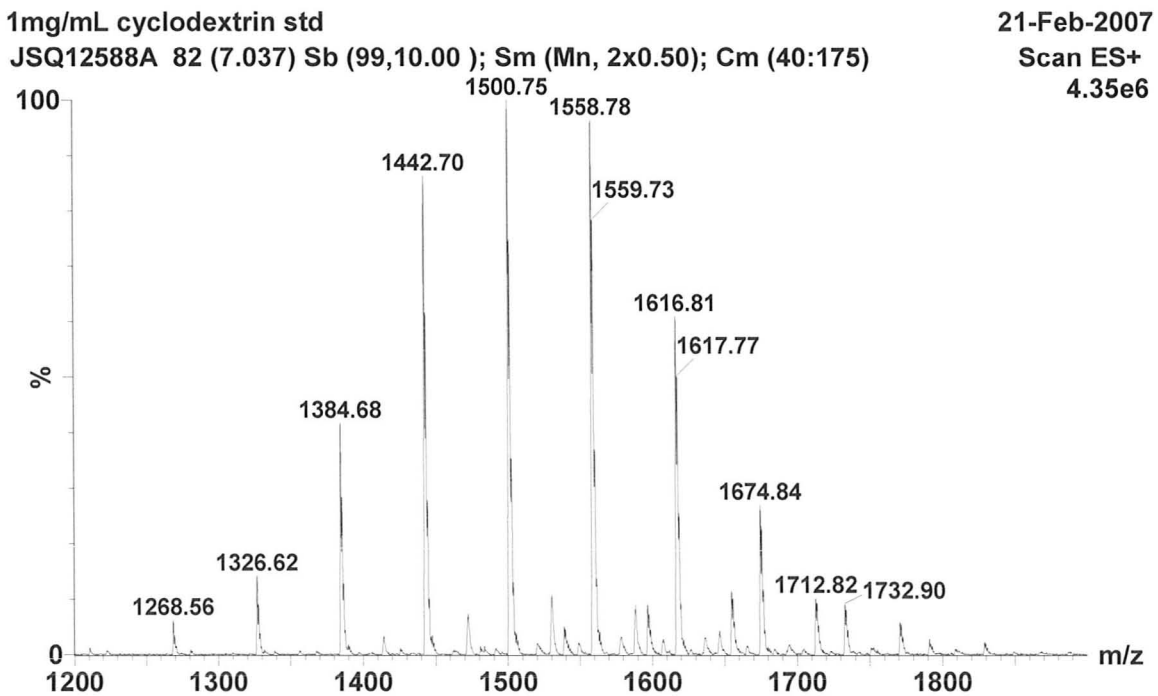

10ug/mL cyclodextrin std
JSQ12583A 77 (6.608) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
21-Feb-2007
Scan ES+
5.71e4
100
%
0
1200
1300
1400
1500
1600
1700
1800
m/z
1558.75
1500.73
1442.68
1559.69
1616.77
1617.75
1384.71
1618.75
1222.41
1674.82
1223.35
1676.81
1262.92
1326.63
1770.76
1771.59
1887.79

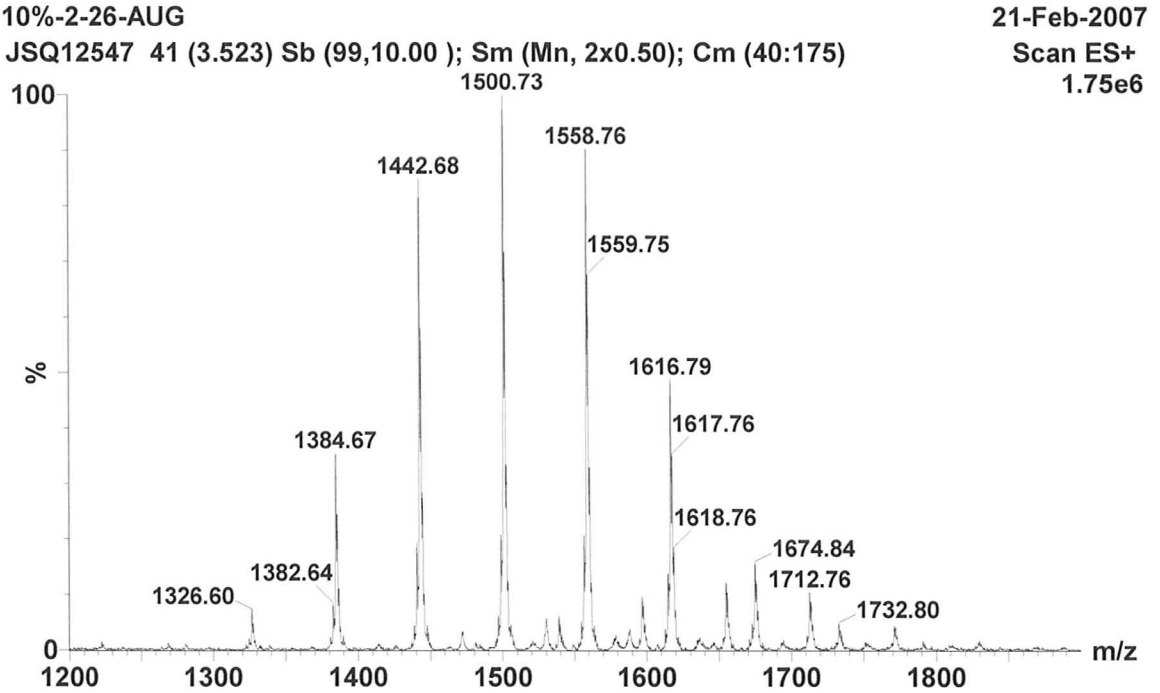

50ug/mL cyclodextrin std
JSQ12584A 72 (6.180) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
21-Feb-2007
Scan ES+
3.09e5
100
%
0
1200
1300
1400
1500
1600
1700
1800
m/z
1500.74
1558.76
1559.72
1442.68
1616.80
1617.78
1384.66
1674.80
1675.84
1326.60
1712.78
1268.61
1733.67
1829.88

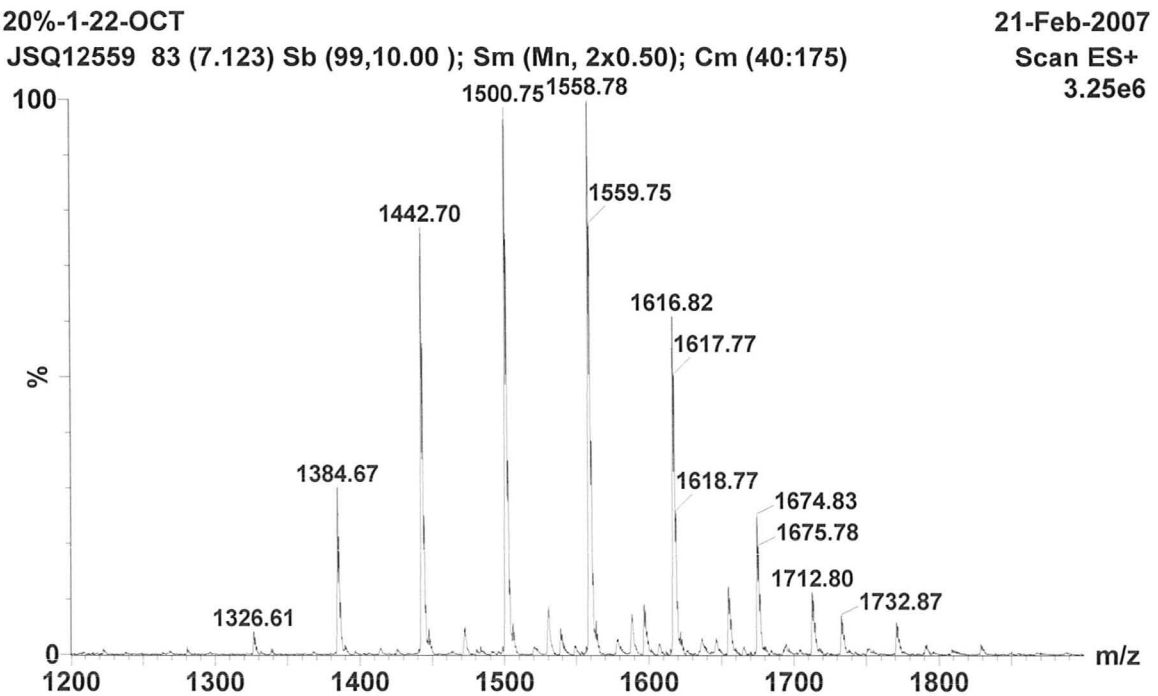

100ug/mL cyclodextrin std
JSQ12585E 79 (6.780) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
21-Feb-2007
Scan ES+
5.99e5
100
%
0
1200
1300
1400
1500
1600
1700
1800
m/z
1558.77
1500.68
1442.72
1559.71
1616.80
1617.74
1384.63
1674.77
1675.70
1326.59
1712.76
1732.79
1268.49
1829.95

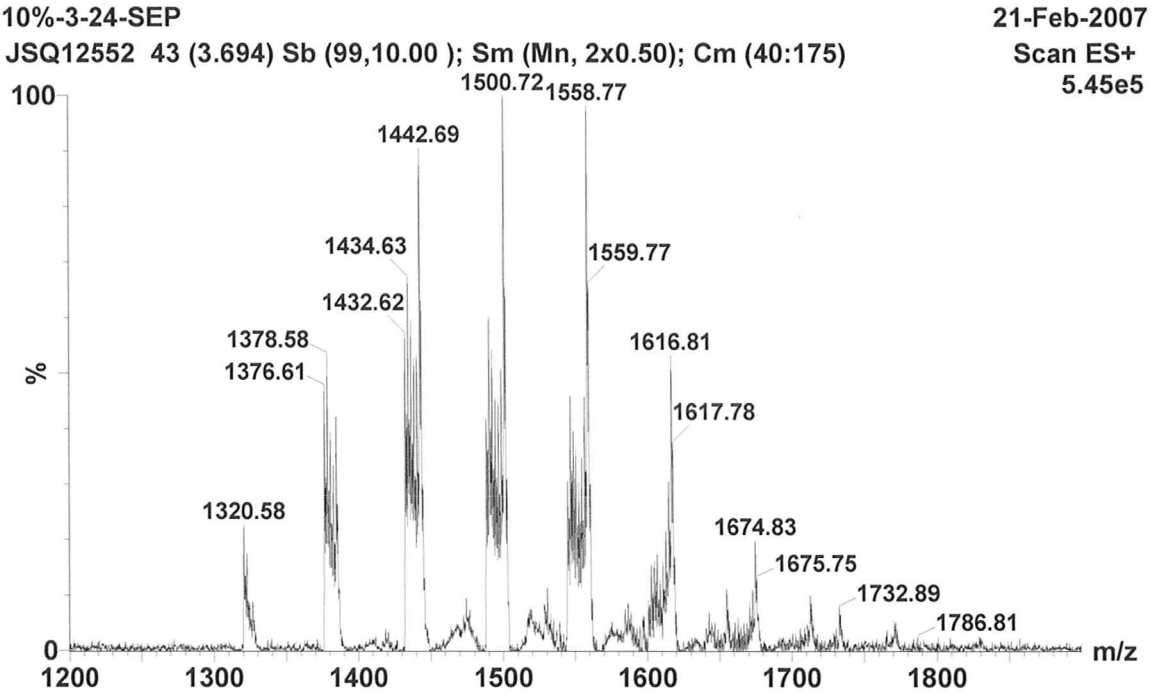

250ug/mL cyclodextrin std
JSQ12586A 81 (6.951) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
21-Feb-2007
Scan ES+
1.43e6
100
%
0
1200
1300
1400
1500
1600
1700
1800
m/z
1500.73
1558.77
1442.68
1559.74
1616.80
1617.77
1384.68
1674.84
1675.80
1326.62
1712.82
1732.84
1268.54
1829.81

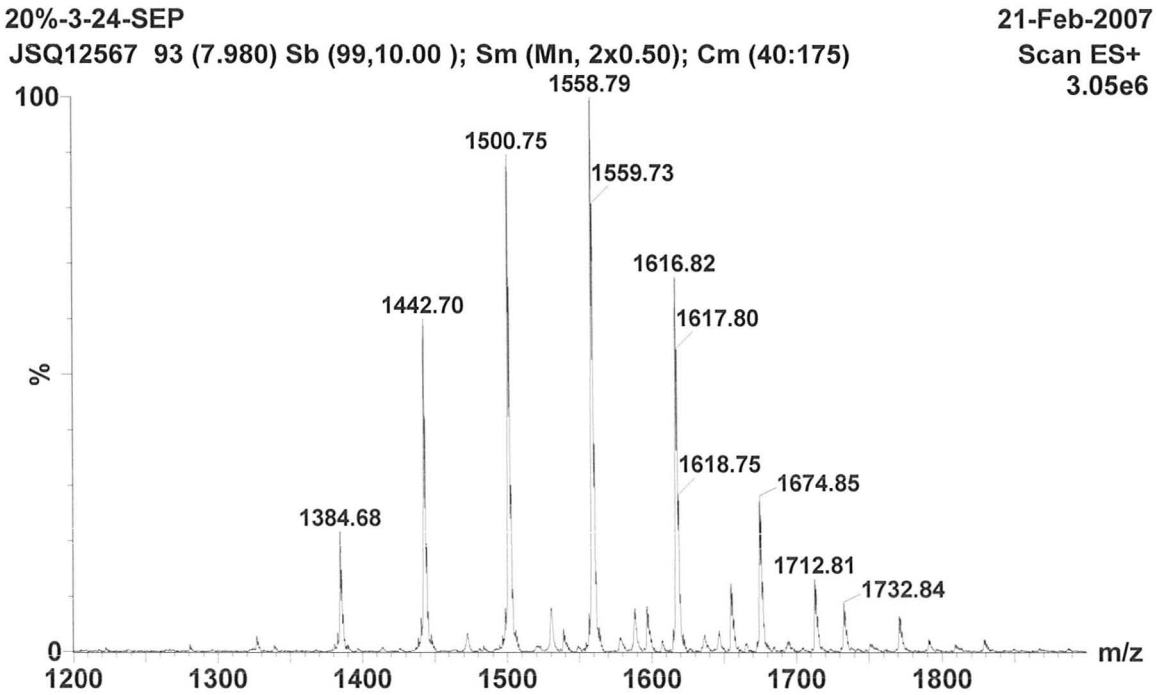

500ug/mL cyclodextrin std
21-Feb-2007
JSQ12587A 85 (7.294) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
Scan ES+
2.58e6
100
%
0
1500.74
1558.77
1442.70
1559.73
1616.82
1617.76
1384.68
1674.85
1326.62
1676.77
1732.86
1268.55
1790.87
1200
1300
1400
1500
1600
1700
1800
m/z

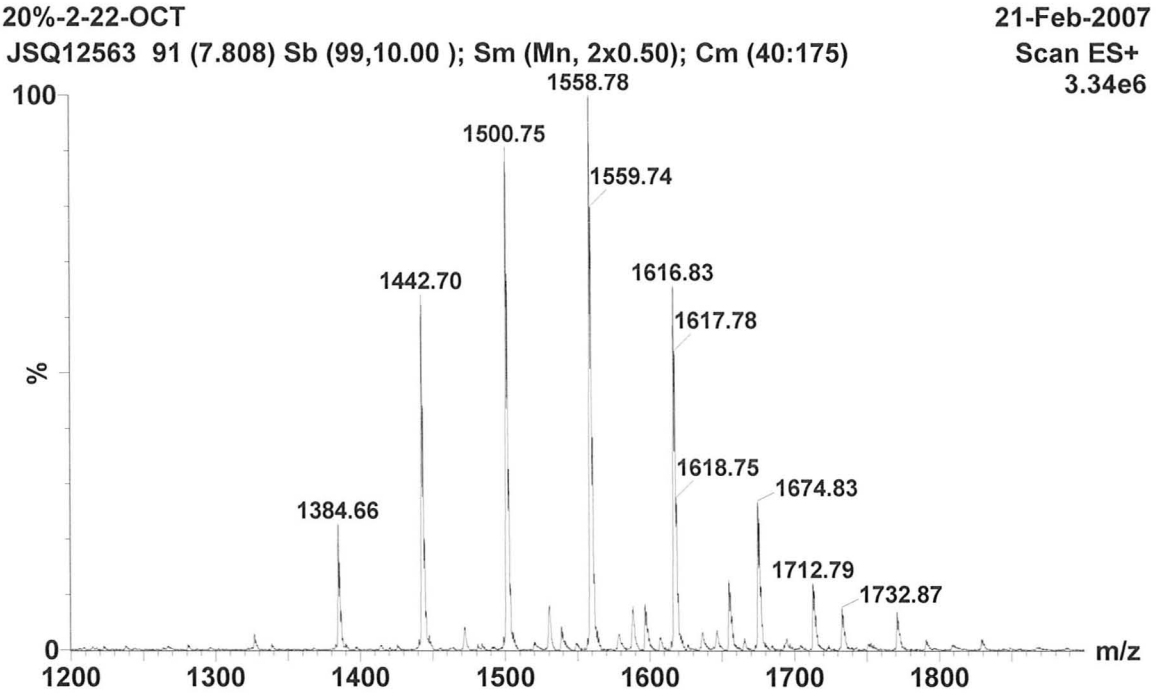

1mg/mL cyclodextrin std
21-Feb-2007
JSQ12588A 82 (7.037) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
Scan ES+
4.35e6
100
%
0
1500.75
1558.78
1442.70
1559.73
1616.81
1617.77
1384.68
1674.84
1326.62
1712.82
1732.90
1268.56
1200
1300
1400
1500
1600
1700
1800
m/z

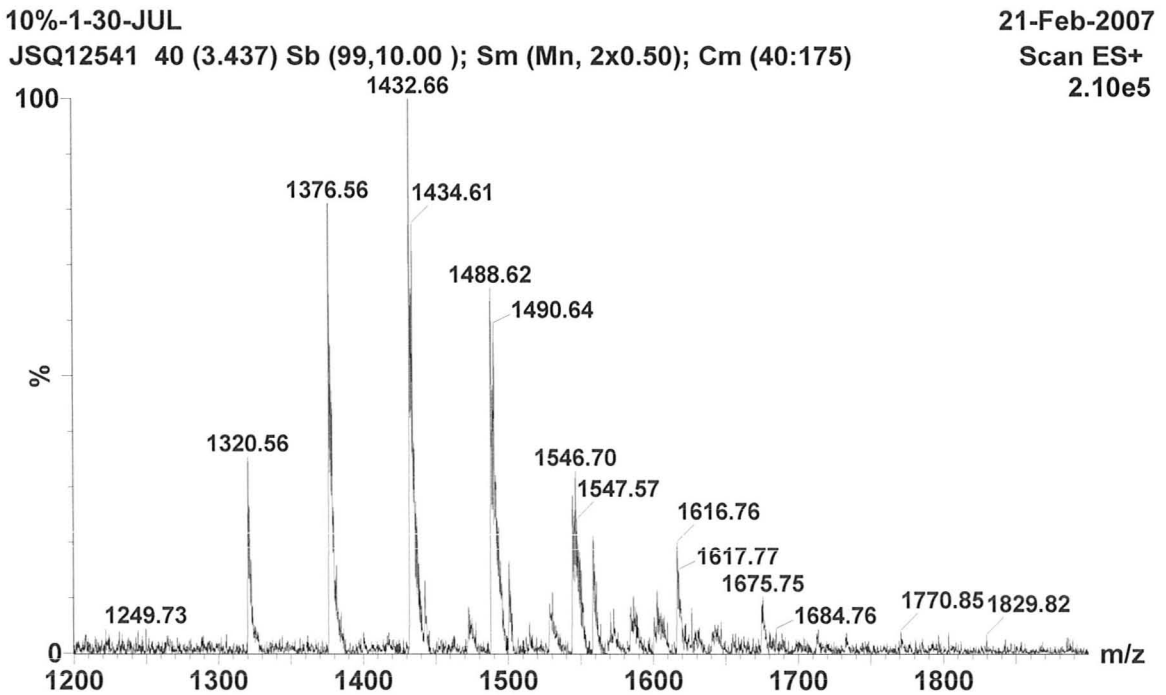

10%-2-26-AUG
JSQ12547 41 (3.523) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
21-Feb-2007
Scan ES+
1.75e6
100
%
0
1500.73
1558.76
1442.68
1559.75
1616.79
1617.76
1384.67
1618.76
1674.84
1712.76
1382.64
1326.60
1732.80
1200
1300
1400
1500
1600
1700
1800
m/z

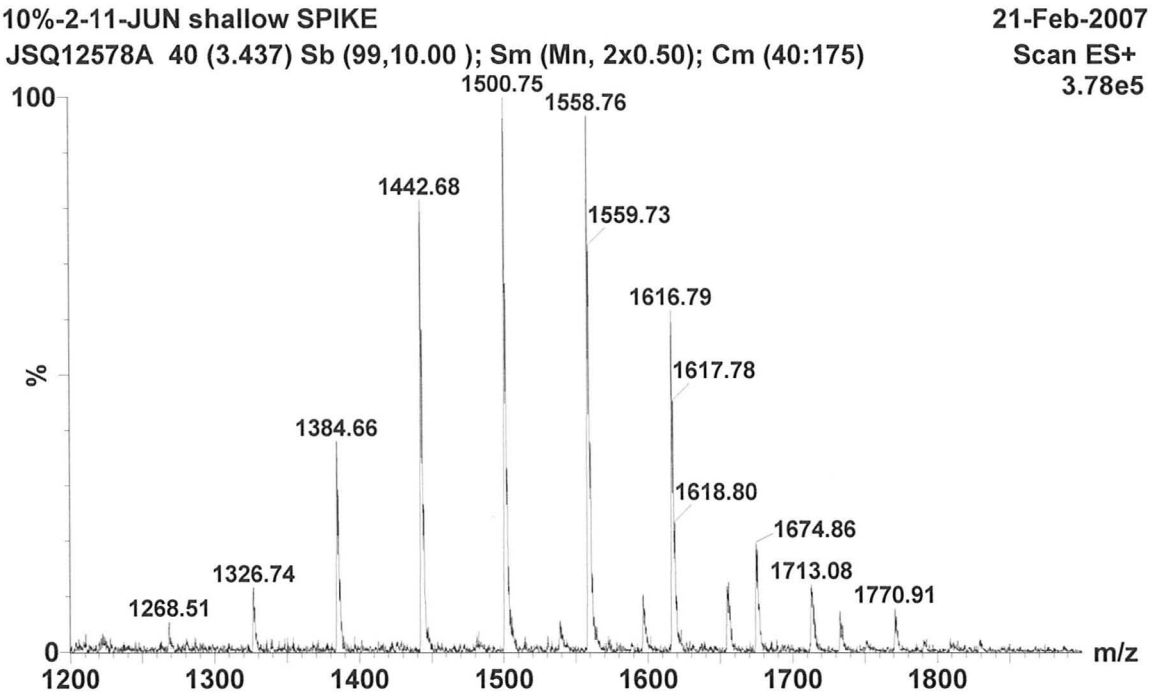

20%-1-22-OCT
JSQ12559 83 (7.123) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
21-Feb-2007
Scan ES+
3.25e6
100
%
0
1500.75
1558.78
1559.75
1442.70
1616.82
1617.77
1384.67
1618.77
1674.83
1675.78
1712.80
1732.87
1326.61
1200
1300
1400
1500
1600
1700
1800
m/z

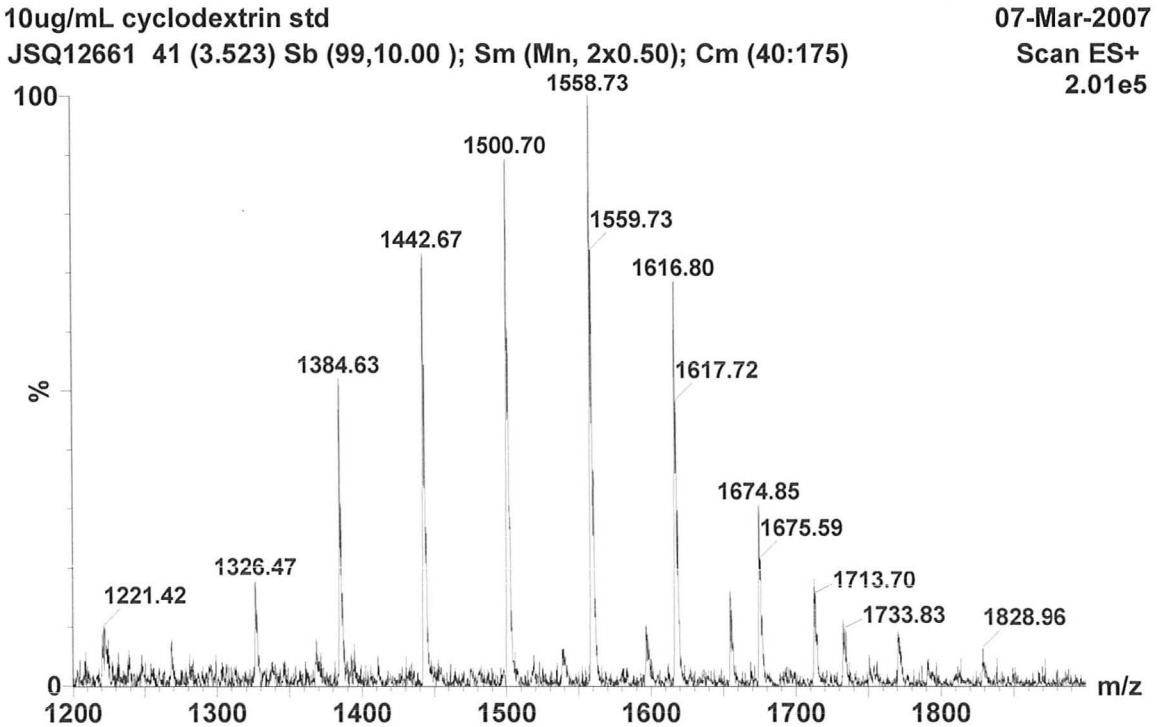

10%-3-24-SEP
21-Feb-2007
JSQ12552 43 (3.694) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
Scan ES+
5.45e5
100
%
0
1500.72
1558.77
1442.69
1434.63
1559.77
1432.62
1378.58
1376.61
1616.81
1617.78
1320.58
1674.83
1675.75
1732.89
1786.81
m/z
1200
1300
1400
1500
1600
1700
1800

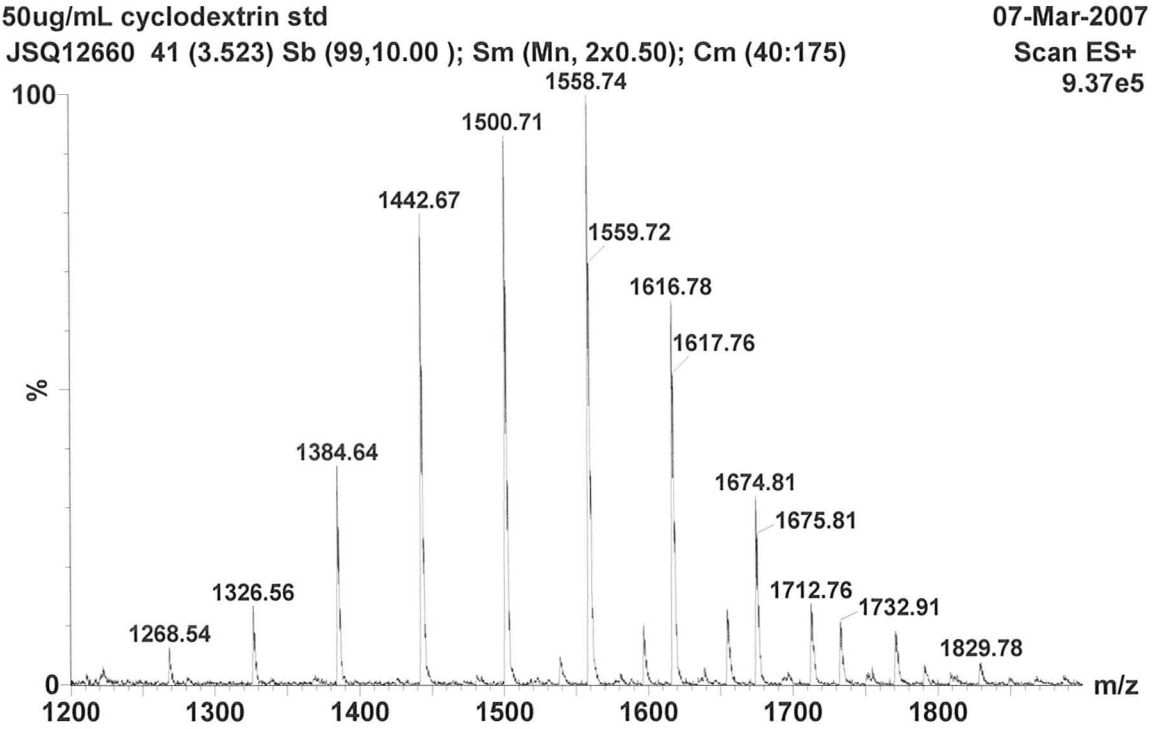

20%-3-24-SEP
21-Feb-2007
JSQ12567 93 (7.980) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
Scan ES+
3.05e6
100
%
0
1558.79
1500.75
1559.73
1616.82
1442.70
1617.80
1618.75
1674.85
1384.68
1712.81
1732.84
m/z
1200
1300
1400
1500
1600
1700
1800

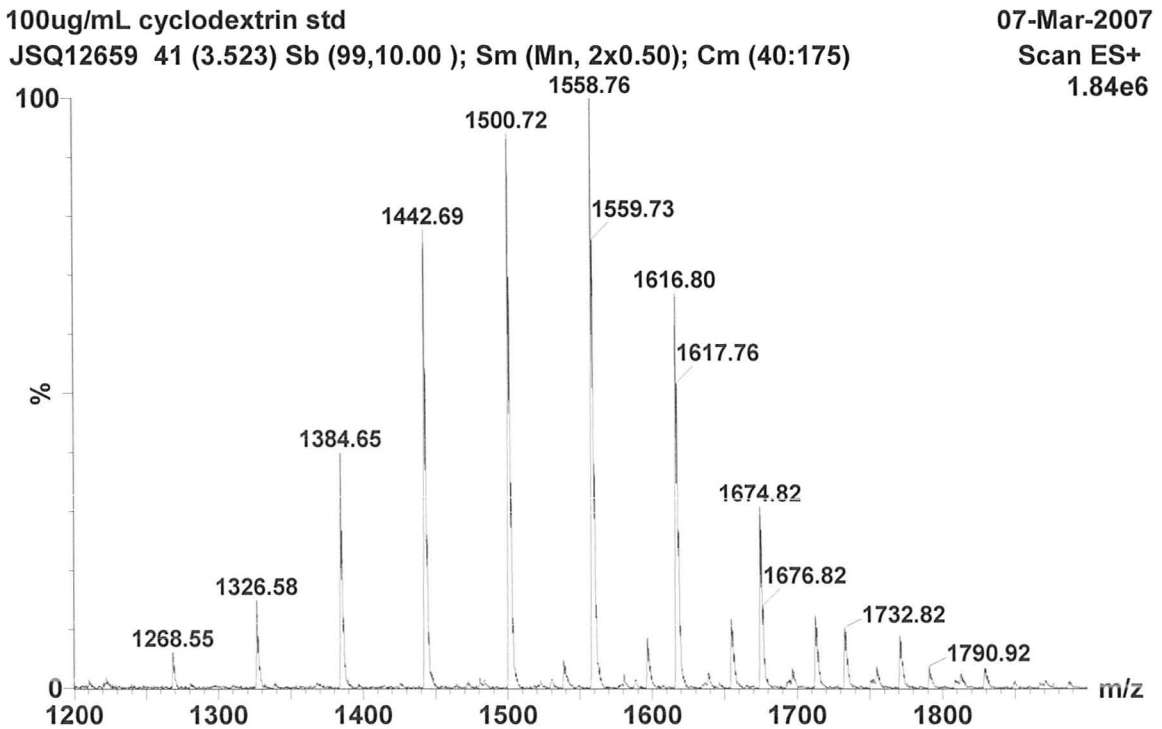

20%-2-22-OCT
21-Feb-2007
JSQ12563 91 (7.808) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
Scan ES+
3.34e6
100
%
0
1200
1300
1400
1500
1600
1700
1800
m/z
1558.78
1500.75
1559.74
1442.70
1616.83
1617.78
1618.75
1674.83
1384.66
1712.79
1732.87

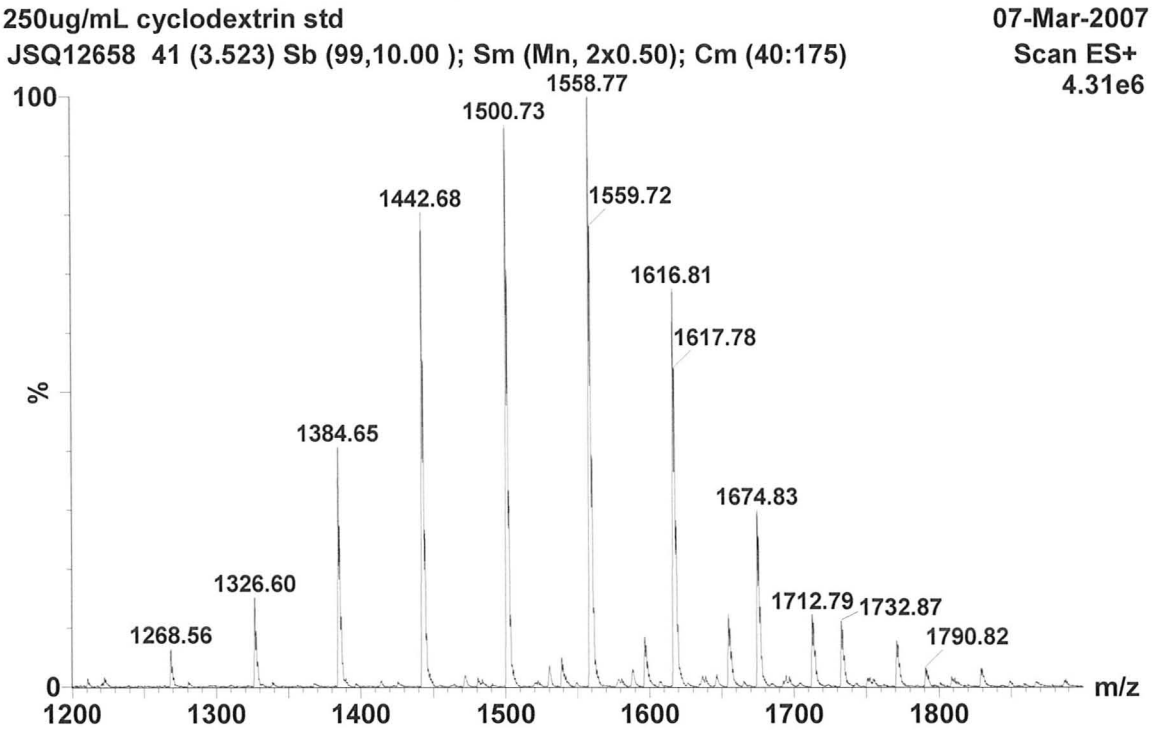

10%-1-30-JUL
21-Feb-2007
JSQ12541 40 (3.437) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
Scan ES+
2.10e5
100
%
0
1200
1300
1400
1500
1600
1700
1800
m/z
1432.66
1376.56
1434.61
1488.62
1490.64
1320.56
1546.70
1547.57
1616.76
1617.77
1675.75
1249.73
1684.76
1770.85
1829.82

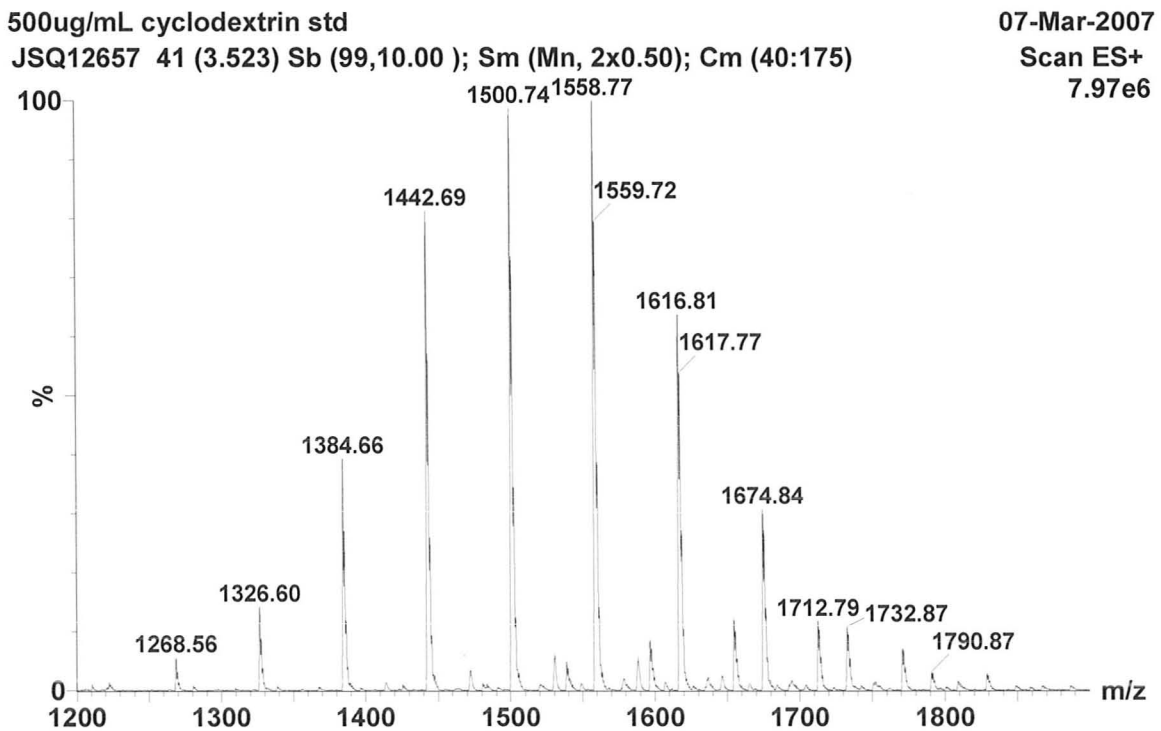

10%-2-11-JUN shallow SPIKE
21-Feb-2007
JSQ12578A 40 (3.437) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
Scan ES+
3.78e5
100
%
0
1500.75
1558.76
1442.68
1559.73
1616.79
1617.78
1384.66
1618.80
1674.86
1326.74
1713.08
1770.91
1268.51
1200
1300
1400
1500
1600
1700
1800
m/z

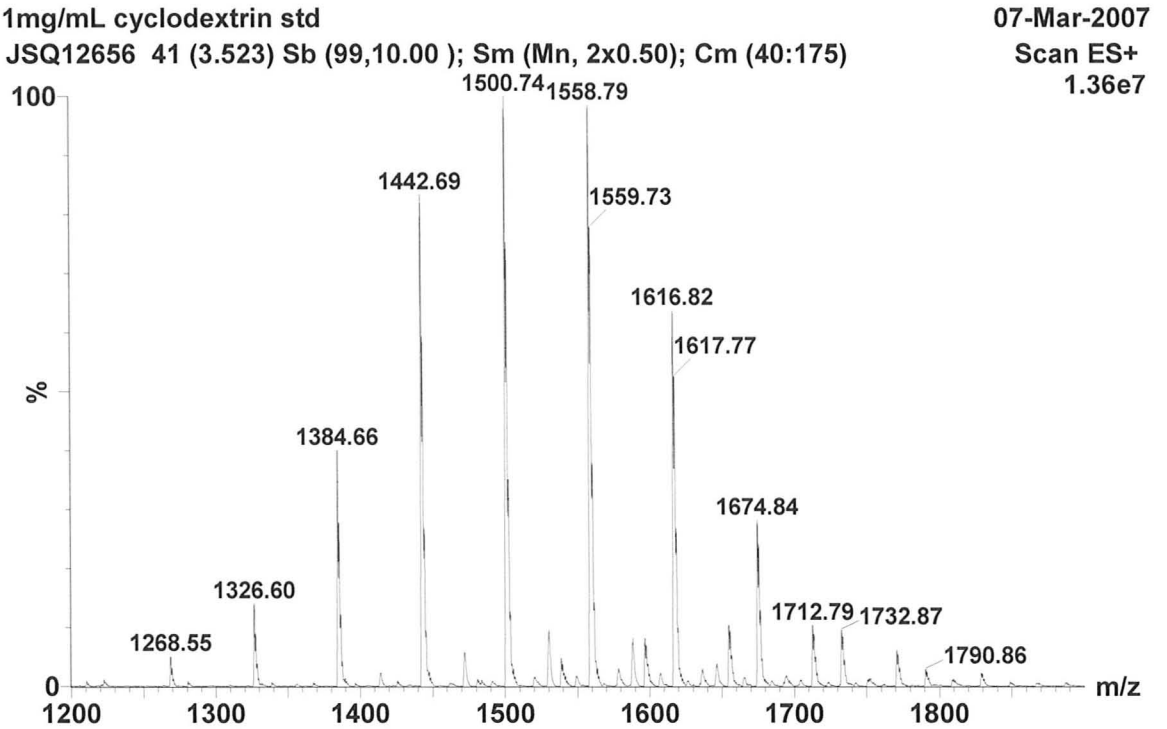

10ug/mL cyclodextrin std
07-Mar-2007
JSQ12661 41 (3.523) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
Scan ES+
2.01e5
100
%
0
1558.73
1500.70
1559.73
1442.67
1616.80
1384.63
1617.72
1674.85
1675.59
1326.47
1713.70
1221.42
1733.83
1828.96
1200
1300
1400
1500
1600
1700
1800
m/z

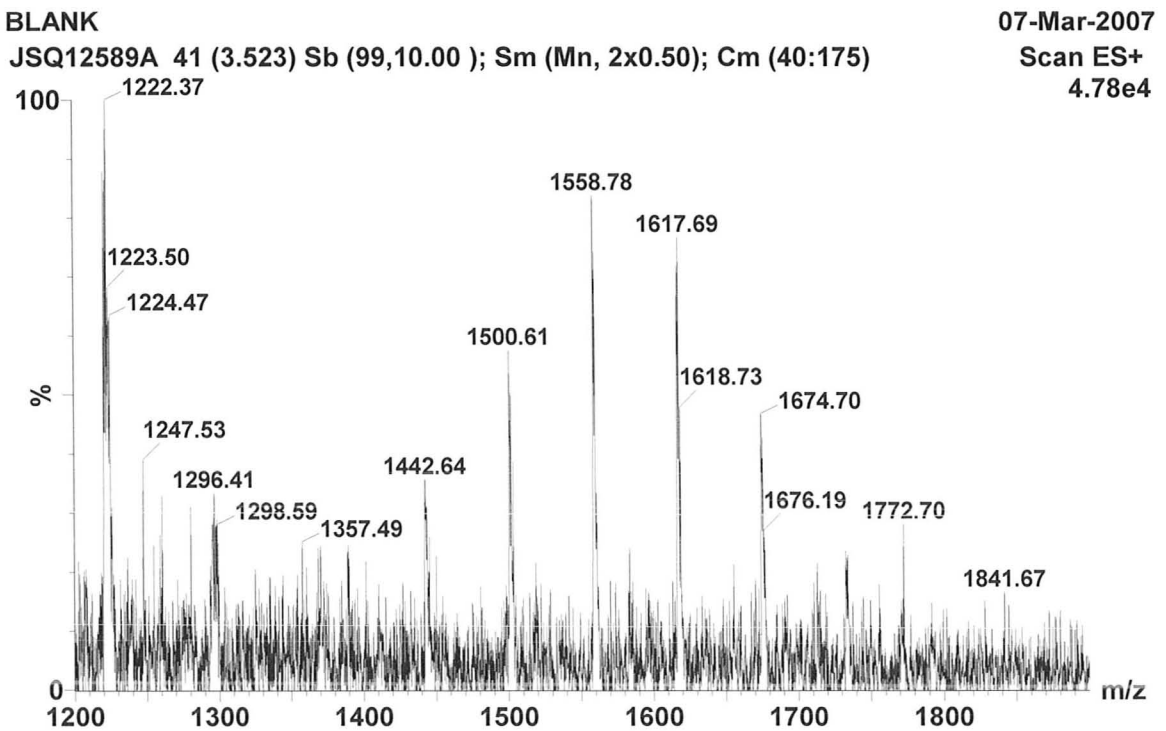

50ug/mL cyclodextrin std
07-Mar-2007
JSQ12660 41 (3.523) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
Scan ES+
9.37e5
100
%
0
1200
1300
1400
1500
1600
1700
1800
m/z
1558.74
1500.71
1442.67
1559.72
1616.78
1617.76
1384.64
1674.81
1675.81
1326.56
1712.76
1732.91
1268.54
1829.78

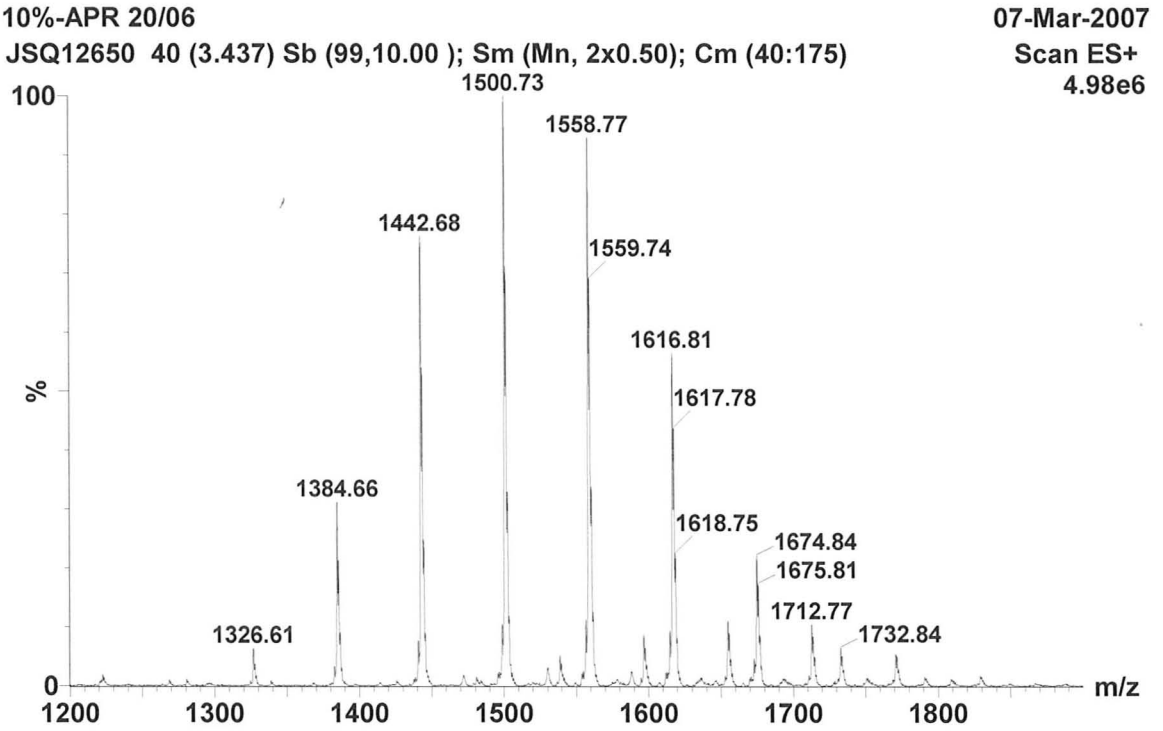

100ug/mL cyclodextrin std
07-Mar-2007
JSQ12659 41 (3.523) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
Scan ES+
1.84e6
100
%
0
1200
1300
1400
1500
1600
1700
1800
m/z
1558.76
1500.72
1442.69
1559.73
1616.80
1617.76
1384.65
1674.82
1676.82
1326.58
1732.82
1268.55
1790.92

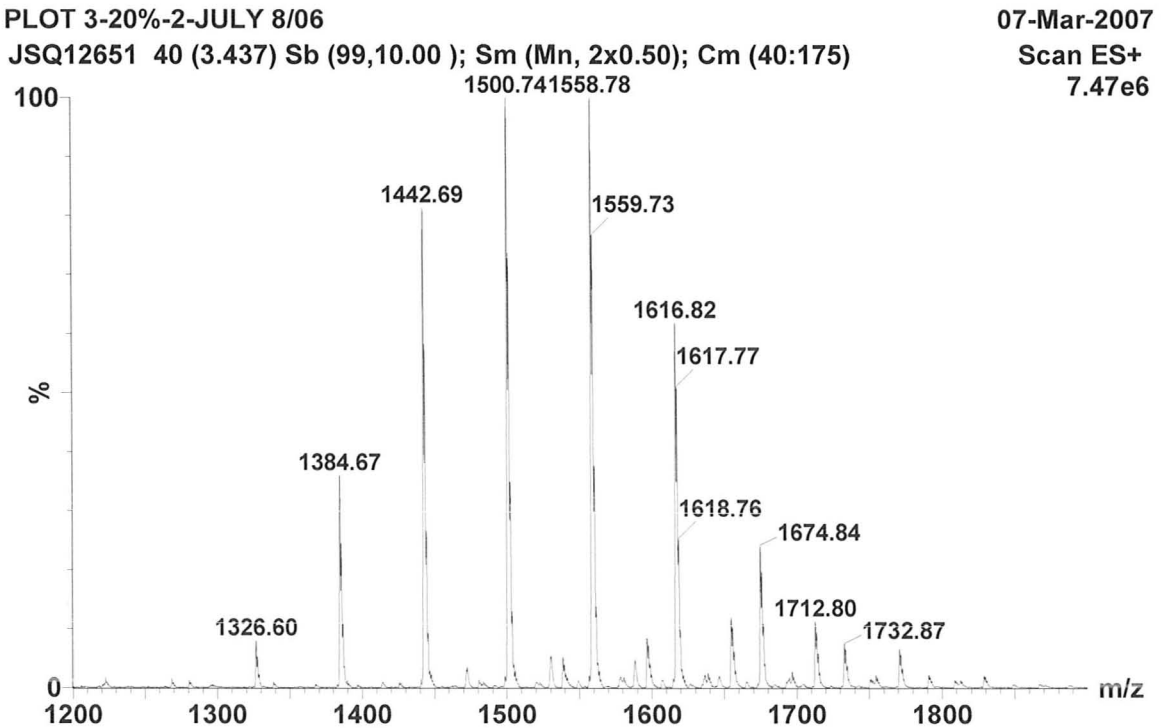

250ug/mL cyclodextrin std
07-Mar-2007
JSQ12658 41 (3.523) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
Scan ES+
4.31e6
100
%
0
1268.56
1326.60
1384.65
1442.68
1500.73
1558.77
1559.72
1616.81
1617.78
1674.83
1712.79
1732.87
1790.82
m/z
1200
1300
1400
1500
1600
1700
1800

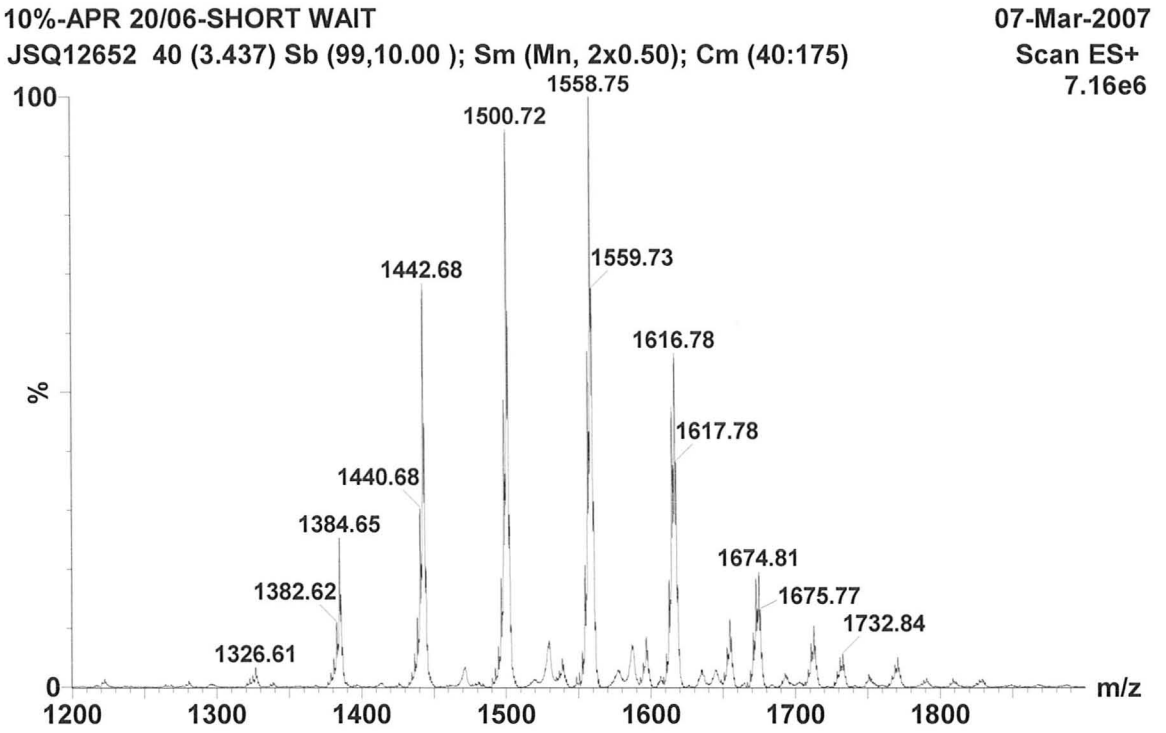

500ug/mL cyclodextrin std
07-Mar-2007
JSQ12657 41 (3.523) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
Scan ES+
7.97e6
100
%
0
1268.56
1326.60
1384.66
1442.69
1500.74
1558.77
1559.72
1616.81
1617.77
1674.84
1712.79
1732.87
1790.87
m/z
1200
1300
1400
1500
1600
1700
1800

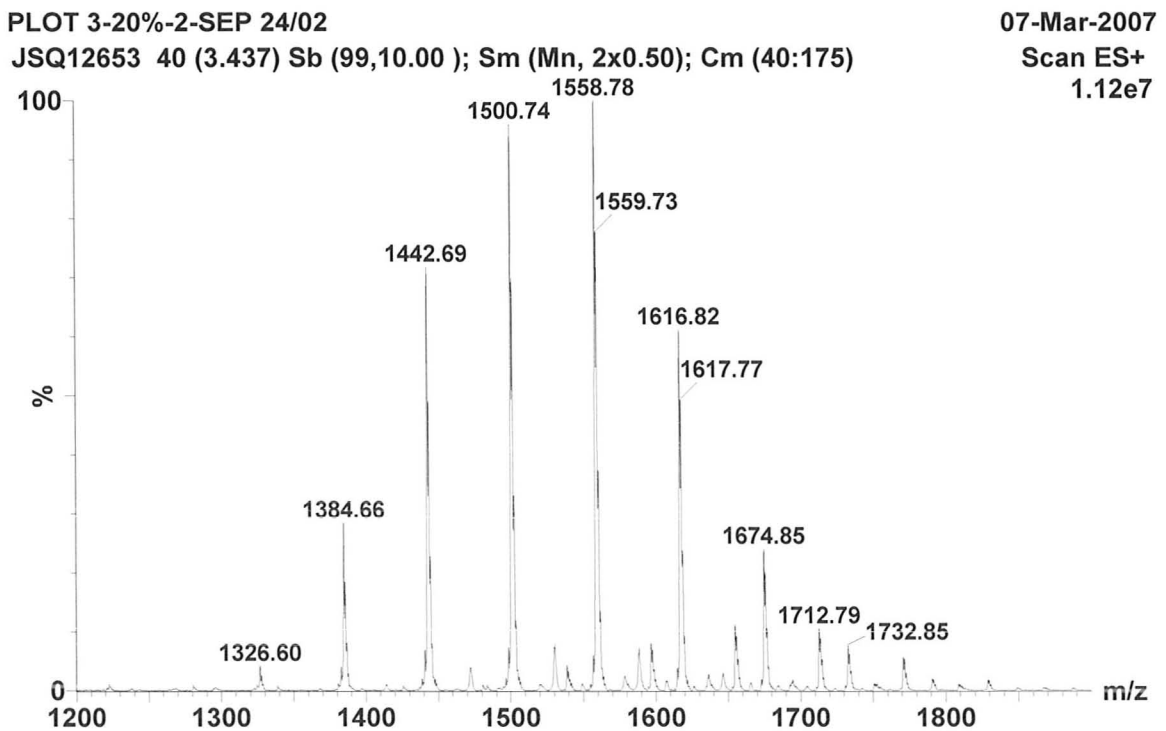

1mg/mL cyclodextrin std
JSQ12656 41 (3.523) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
07-Mar-2007
Scan ES+
1.36e7
100
%
0
1500.74
1558.79
1442.69
1559.73
1616.82
1617.77
1384.66
1674.84
1326.60
1712.79
1732.87
1268.55
1790.86
m/z
1200
1300
1400
1500
1600
1700
1800


BLANK
JSQ12589A 41 (3.523) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
07-Mar-2007
Scan ES+
4.78e4
100
%
0
1222.37
1558.78
1617.69
1223.50
1224.47
1500.61
1618.73
1674.70
1247.53
1442.64
1296.41
1298.59
1357.49
1676.19
1772.70
1841.67
m/z
1200
1300
1400
1500
1600
1700
1800


10%-APR 20/06
07-Mar-2007
JSQ12650 40 (3.437) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
Scan ES+
4.98e6
100
%
0
1500.73
1558.77
1442.68
1559.74
1616.81
1617.78
1384.66
1618.75
1674.84
1675.81
1712.77
1326.61
1732.84
1200
1300
1400
1500
1600
1700
1800
m/z


PLOT 3-20%-2-JULY 8/06
07-Mar-2007
JSQ12651 40 (3.437) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
Scan ES+
7.47e6
100
%
0
1500.741558.78
1442.69
1559.73
1616.82
1617.77
1384.67
1618.76
1674.84
1712.80
1326.60
1732.87
1200
1300
1400
1500
1600
1700
1800
m/z


10%-APR 20/06-SHORT WAIT
07-Mar-2007
JSQ12652 40 (3.437) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
Scan ES+
7.16e6
100
%
0
1200
1300
1400
1500
1600
1700
1800
m/z
1558.75
1500.72
1559.73
1442.68
1616.78
1617.78
1440.68
1384.65
1674.81
1382.62
1675.77
1732.84
1326.61


PLOT 3-20%-2-SEP 24/02
07-Mar-2007
JSQ12653 40 (3.437) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
Scan ES+
1.12e7
100
%
0
1200
1300
1400
1500
1600
1700
1800
m/z
1558.78
1500.74
1559.73
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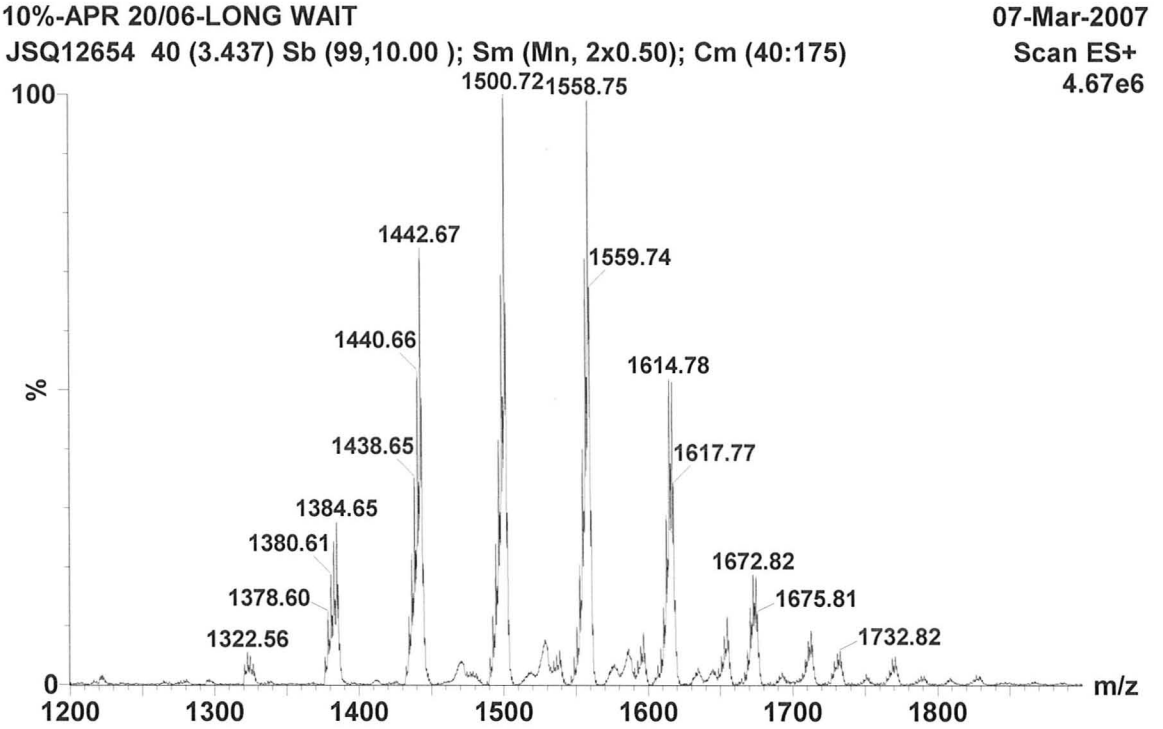
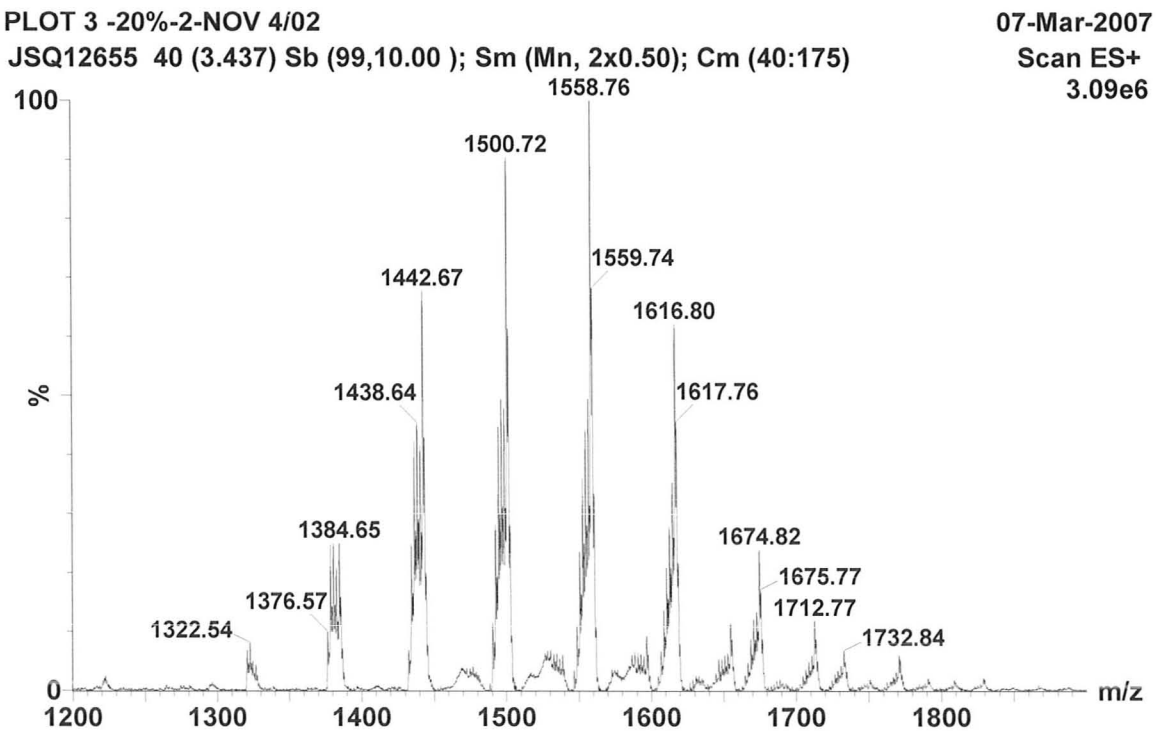
1616.82
1617.77
1384.66
1674.85
1712.79
1732.85
1326.60


10%-APR 20/06-LONG WAIT
07-Mar-2007
JSQ12654 40 (3.437) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
Scan ES+
4.67e6
100
%
0
1500.72
1558.75
1442.67
1559.74
1440.66
1614.78
1438.65
1617.77
1384.65
1380.61
1672.82
1378.60
1675.81
1322.56
1732.82
1200
1300
1400
1500
1600
1700
1800
m/z


PLOT 3 -20%-2-NOV 4/02
07-Mar-2007
JSQ12655 40 (3.437) Sb (99,10.00); Sm (Mn, 2x0.50); Cm (40:175)
Scan ES+
3.09e6
100
%
0
1558.76
1500.72
1559.74
1442.67
1616.80
1438.64
1617.76
1384.65
1674.82
1675.77
1376.57
1712.77
1322.54
1732.84
1200
1300
1400
1500
1600
1700
1800
m/z

APPENDIX F:
DATA OF METHOD COMPARISON

Comparison of each method of the HPβCD in soils for the 10%-HPβCD treated soil.

Date	LCMS HPBCD/OM (%)	Isotopic Measurement of HPBCD wt /Soil wt (%)	LOI Measurement of Increase in OM (%)
6/11/2002	0.00	0.0	47.5
7/8/2002	0.00	1.8	50.3
7/30/2002	2.98	15.1	56.96
8/26/2002	4.63	16.6	51.95
9/24/2002	3.22	15.0	54.60
10/22/2002	3.83	22.0	57.57
11/4/2002	2.63	19.7	41.46

Comparison of each method of the HPβCD in soils for the 20%-HPβCD treated soil.

Date	LCMS HPBCD/OM (%)	Isotopic Measurement of HPBCD wt /Soil wt (%)	LOI Measurement of Increase in OM (%)
6/11/2002	0.00	0.00	0.0
7/8/2002	5.43	8.58	36.3
7/30/2002	2.51	33.32	78.3
8/26/2002	5.15	41.13	60.72
9/24/2002	7.64	50.31	53.5
10/22/2002	7.19	43.30	54.6
11/4/2002	3.13	30.20	62.8