SUPPLEMENTAL INFORMATION

A Robust and High Throughput Method for Anionic Metabolite Profiling: Preventing Polyimide Aminolysis and Capillary Breakage under Alkaline Conditions in CE-MS

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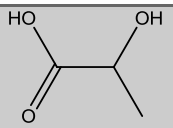
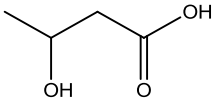
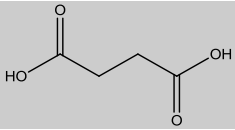
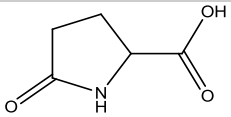
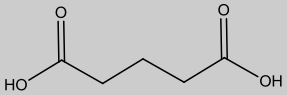
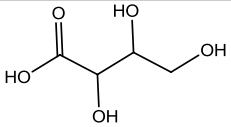
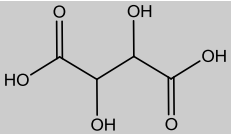
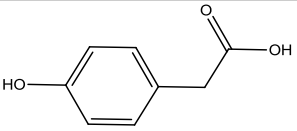
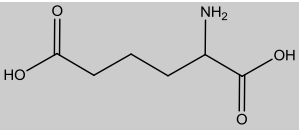
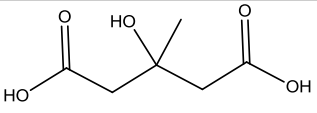
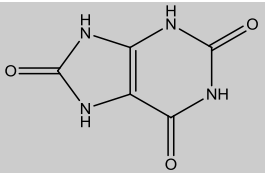
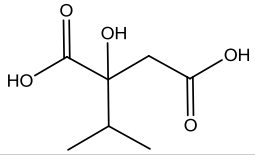
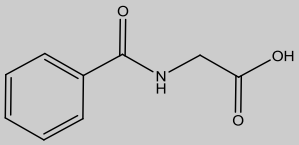
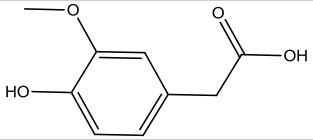
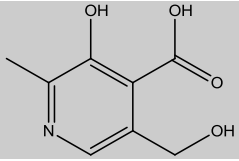
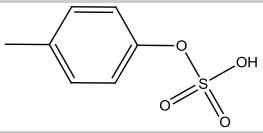
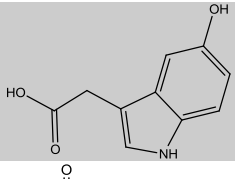
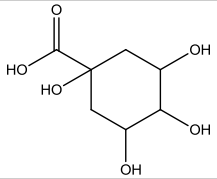
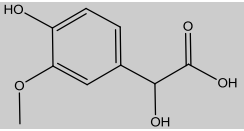
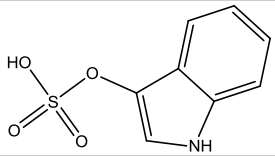
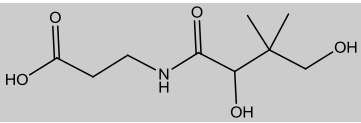
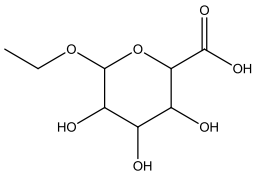
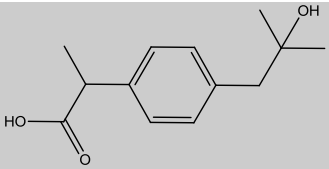
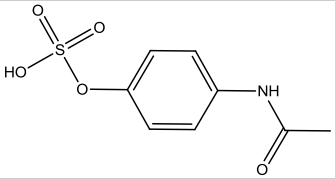
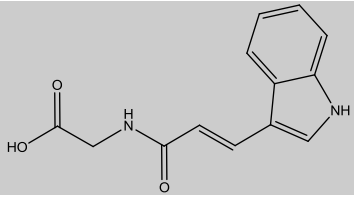
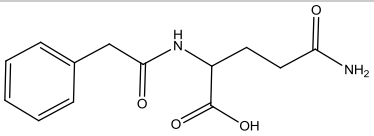
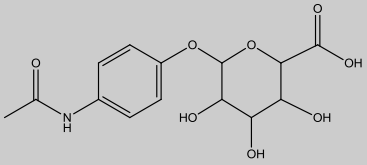
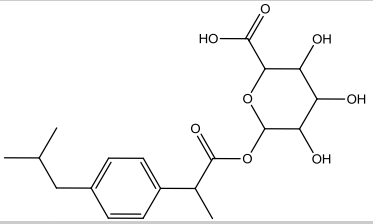
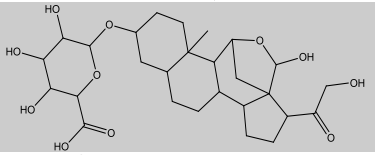
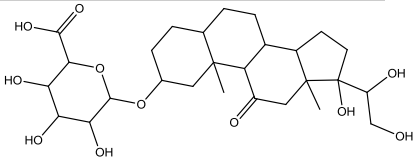
Supplemental Materials: Supplemental Figures S1-S5. Table S1

Table S1. *In silico* modeling of the relative migration time (RMT) of 30 different anionic metabolites in urine based on two fundamental properties of an ion using the Hubbard-Onsager equation.

Assignment	*m/z:*RMT	*V* (Å^3)	*pK$_a$*	z_{eff} (pH 8.5)	RMT (pred)	% error	Chemical Structure
Lactic Acid	89.024:1.154	71.8	3.78	-1.0	1.13	-2.48	
3-Hydroxybutyric Acid	103.040:1.026	88.9	4.44	-1.0	1.04	1.34	
Succinic acid	117.019:1.950	88.2	4.16 5.61	-2.0	1.98	1.71	
Oxoproline	128.035:1.020	98.4	3.61	-1.0	1.01	-1.47	
Glutaric Acid	131.035:1.700	105.7	4.31 5.41	-2.0	1.81	6.30	
Threonic Acid	135.030:0.993	102.2	3.40	-1.0	0.99	-0.04	
Tartaric Acid	149.009:2.019	99.5	2.98 4.34	-2.0	1.86	-7.74	
Hydroxyphenyl acetic Acid*	151.040:0.932	118.3	4.00	-1.0	0.95	1.77	
2-Aminoadipic Acid	160.062:0.898	135.3	2.14 4.21 9.77	-1.1	0.95	5.35	
3-Hydroxymethyl-glutaric Acid	161.046:1.602	129.8	3.68 4.44	-2.0	1.65	2.92	

Uric Acid	167.020:0.959	90.6	7.61	-1.0	1.03	7.64	
2-Isopropylmalic Acid	175.060:1.456	147.5	3.63 5.57	-2.0	1.57	7.48	
Hippuric Acid	178.051:0.905	135.4	3.59	-1.0	0.91	0.74	
Homovanillic Acid	181.051:0.880	143.7	3.74	-1.0	0.90	1.89	
4-Pyridoxic Acid	182.046:0.936	134.0	2.55	-1.0	0.92	-2.29	
p-Cresol Sulfate	187.007:1.068	109.6	10.36	-1.0	0.97	-9.09	
5-Hydroxyindoleacetic Acid	190.051:0.880	142.2	10.30	-1.0	0.90	2.18	
D-Quinic Acid	191.055:0.881	152.1	3.46	-1.0	0.88	0.20	
Vanillylmandelic Acid	197.055:0.876	150.8	3.11	-1.0	0.89	1.02	
Indoxyl Sulfate	212.002:1.027	121.6	8.38	-1.0	0.94	-8.40	
Pantothenic Acid	218.103:0.816	198.3	4.35	-1.0	0.82	0.92	

Ethyl Glucuronide	221.075:0.828	176.3	3.45	-1.0	0.85	2.49	
Hydroxy Ibuprofen	221.118:0.799	155.1	4.63	-1.0	0.88	9.89	
Acetaminophen Sulfate	230.013:0.921	179.5	9.46	-1.0	0.85	-8.29	
Indolylacryloylglycine	243.077:0.868	155.4	3.95	-1.0	0.88	1.10	
Phenylacetylglutamine	263.104:0.805	226.7	3.90	-1.0	0.80	-1.01	
Acetaminophen Glucuronide	326.088:0.757	246.7	3.17	-1.0	0.78	3.16	
Ibuprofen Glucuronide	381.156:0.735	340.8	3.41	-1.0	0.73	-1.28	
Tetrahydroaldosterone 3-Glucuronide	539.250:0.704	481.9	3.47	-1.0	0.67	-4.20	
Cortolone 3-Glucuronide	541.265:0.700	489.4	3.56	-1.0	0.67	-3.96	

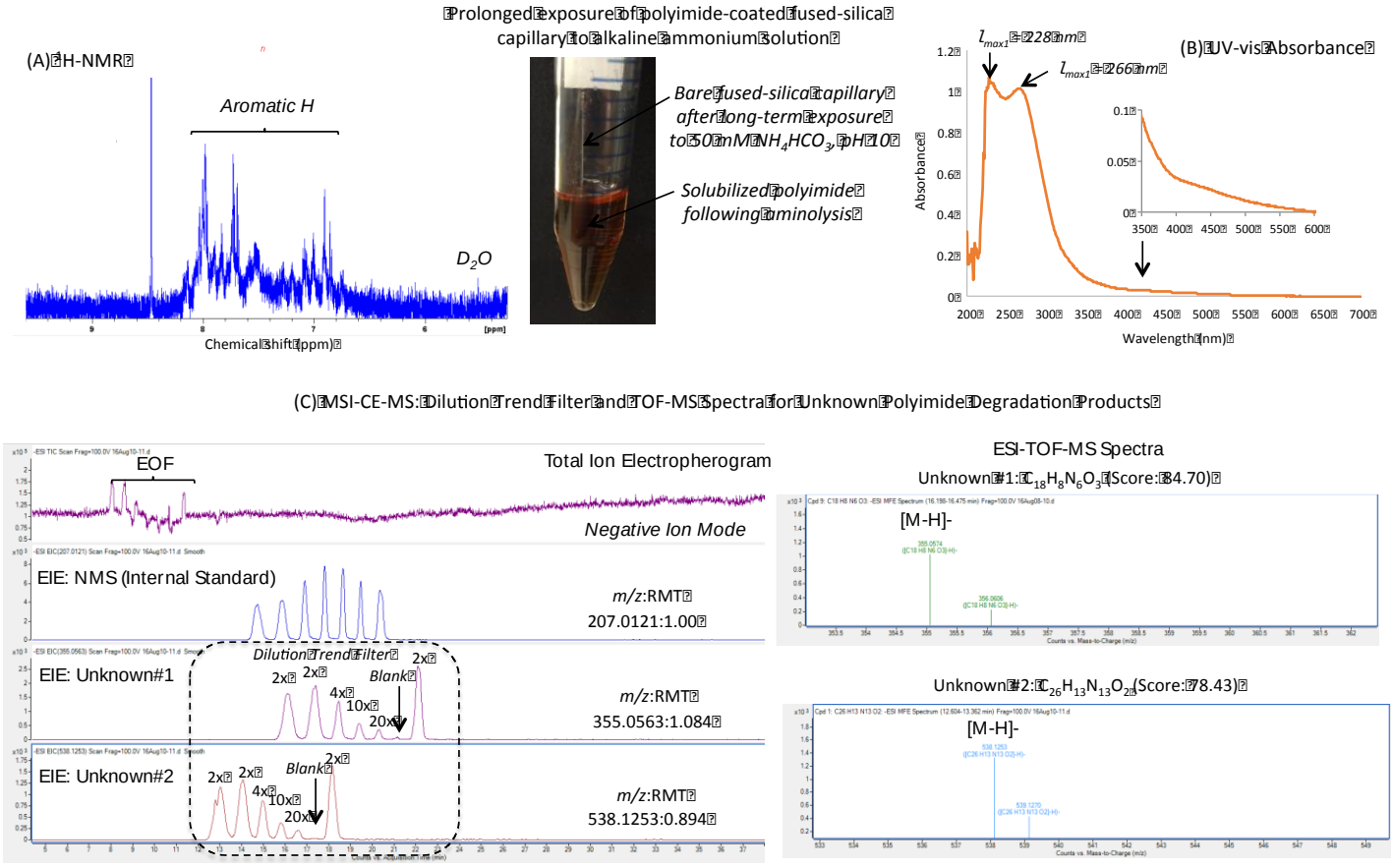


Figure S1. Characterization of aminolysis by-products of the outer polyimide coating of fused-silica capillaries following complete dissolution of polymer into solution after prolonged exposure to 50 mM ammonium bicarbonate, pH 10 at room temperature. (A) ^{1}H-NMR spectra of the solubilized polyimide product following evaporation and reconstitution in D_2O shows a series of distinctive set of aromatic proton resonances (6.7-8.2 ppm). (B) Blank-corrected UV-vis absorbance spectra of 5-fold diluted orange-brown polymer solution showing two distinctive absorbance bands indicative of a highly unsaturated/aromatic chromophore with weak visible absorbance in blue spectrum. IR absorbance spectra of polyimide solution also confirmed that lack of distinctive aromatic imides stretches at 1780 cm^{-1} [*C=O*, sym.], 1720 cm^{-1} [*C=O*, asym.] and 1380 cm^{-1} [*C-N*] for the degraded polymer in solution following aminolysis (data not shown). (C) Chemical analysis of polymer degradation products in solution using MSI-CE-MS with a dilution trend filter under negative and ion mode conditions following use of molecular feature extractor. Two unknown anions [M-H$^-$] were identified based on their characteristic *m/z*:RMT together with their corresponding top-ranked molecular formula. A Pubchem search using molecular formula generated no known chemical structures in the database. A similar strategy was also performed by MSI-CE-MS under strongly acidic conditions (1 M formic acid, pH 1.8) under positive ion mode detection as described in our previous study [1], which revealed four unknown cations [MH$^+$] as defined by their *m/z*:RMT (4-chlorotyrosine as IS), including 116.9848:0.481, 211.0975:0797, 212.0829:0818, and 316.0939:0856. Thus, a total of six discrete unsaturated, nitrogen-rich, aromatic ions were identified as polyimide aminolysis by-products in solution.

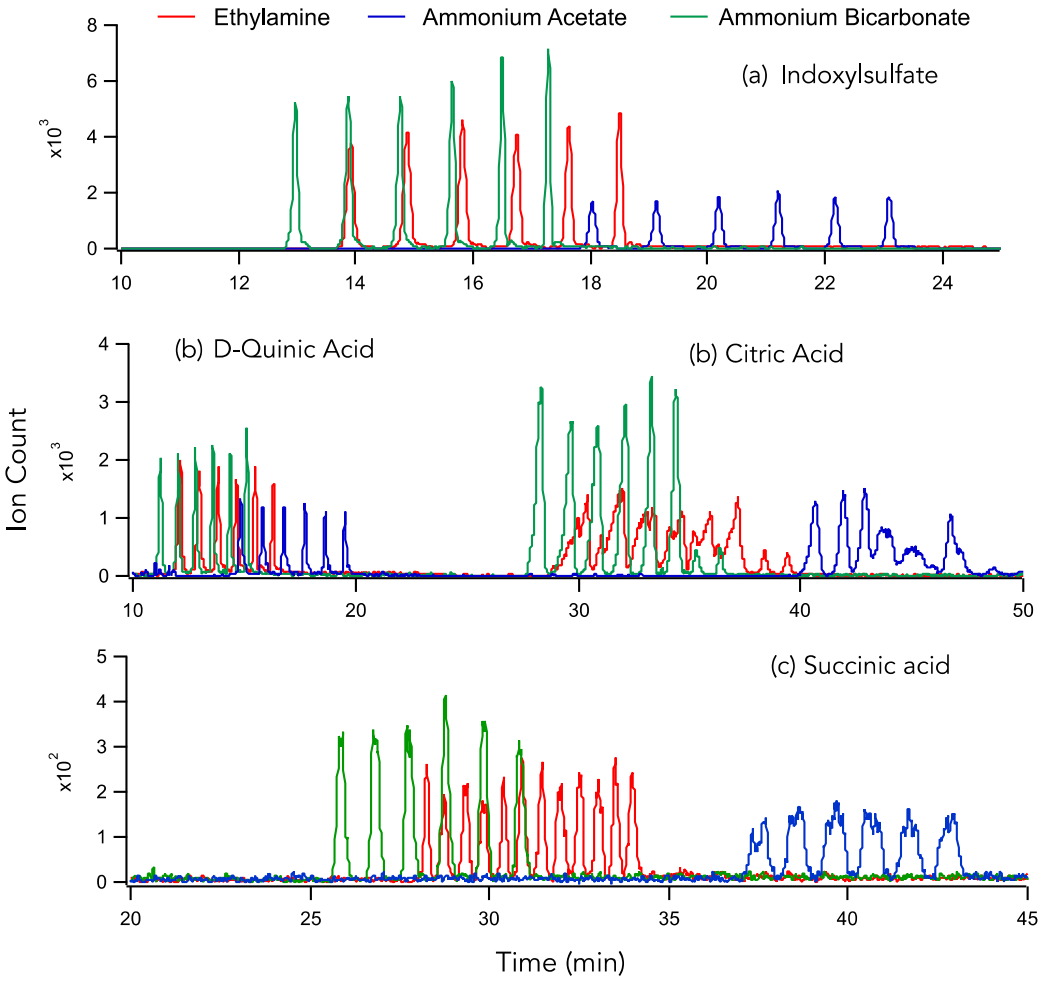


Figure S2. Overlay of extracted ion electropherograms comparing the separation efficiency and ionization responses for representative acidic urinary metabolites as a function of background electrolyte (BGE) composition, including 50 mM ethylamine (pH 10), 50 mM ammonium acetate (pH 8.5) and 50 mM ammonium bicarbonate (pH 8.5). Overall, ammonium bicarbonate was found to provide shorter migration times, sharper peaks with greater ion responses for diverse classes of strong acids (*e.g.*, indoxylsulfate), weakly acidic organic acids (*e.g.*, *D*-quinic acid) in human urine samples, including tricarboxylic acids without deleterious peak splitting or band dispersion, such as citric acid and succinic acid. As a result, ammonium bicarbonate was used as BGE for subsequent long-term stability studies when analyzing human urine samples by MSI-CE-MS at a pH (8.5) that prevents polyimide degradation. Note that *D*-quinic acid and citric acid are isobaric anions [M-H-] readily resolved by CE when extracted within 10 ppm. Also, in many cases later migrating samples migrate as sharper peaks without increased band dispersion as a result of diffusion due to likely sample self-stacking (*i.e.*, transient isotachophoresis) during zonal electropherotic separation when analyzing highly saline urine samples.

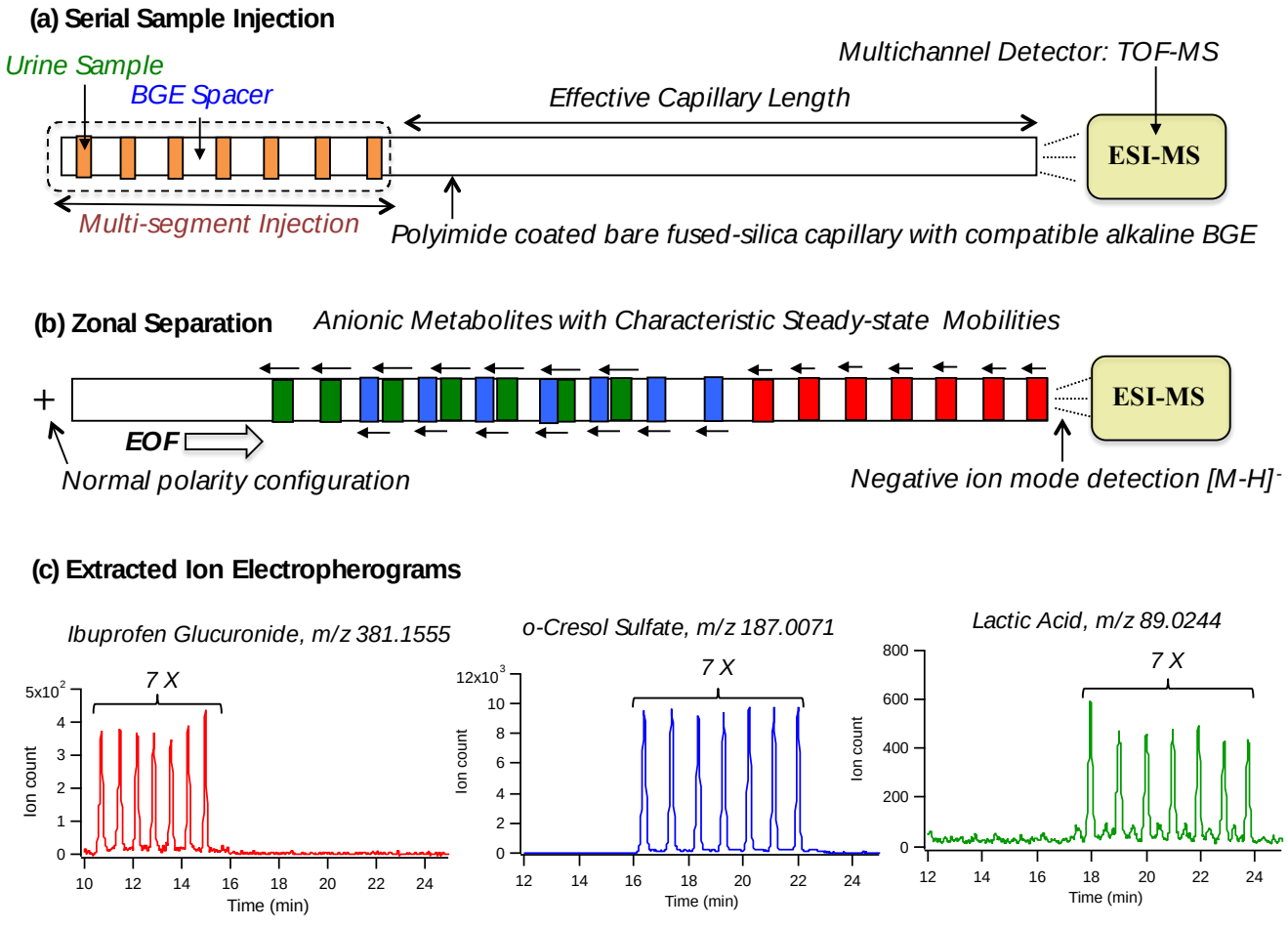


Figure S3. Schematic depicting the operation of MSI-CE-MS as a multiplexed separation method for high throughput profiling of anionic metabolites in human urine while using a compatible alkaline buffer to prevent polyimide degradation (50 mM ammonium bicarbonate, pH 8.5) with negative ion mode detection. (a) Serial injection of seven or more sample segments is performed alternating with BGE as spacer plugs that overall fills approximately one third of the total capillary length; (b) Voltage application with zonal separation of acidic urinary metabolites from within each sample plug, where anionic metabolites and their isomers are separated based on differences in their electrophoretic mobility (*i.e.*, charge density) that migrate counter to the EOF while using a bare fused-silica capillary under normal polarity configuration; (c) Extracted ion electropherograms for three representative acidic metabolites as defined by their characteristic *m/z*:RMT highlighting the high peak capacity and separation efficiency of MSI-CE-MS for resolving complex and highly saline biological samples, such as human urine.

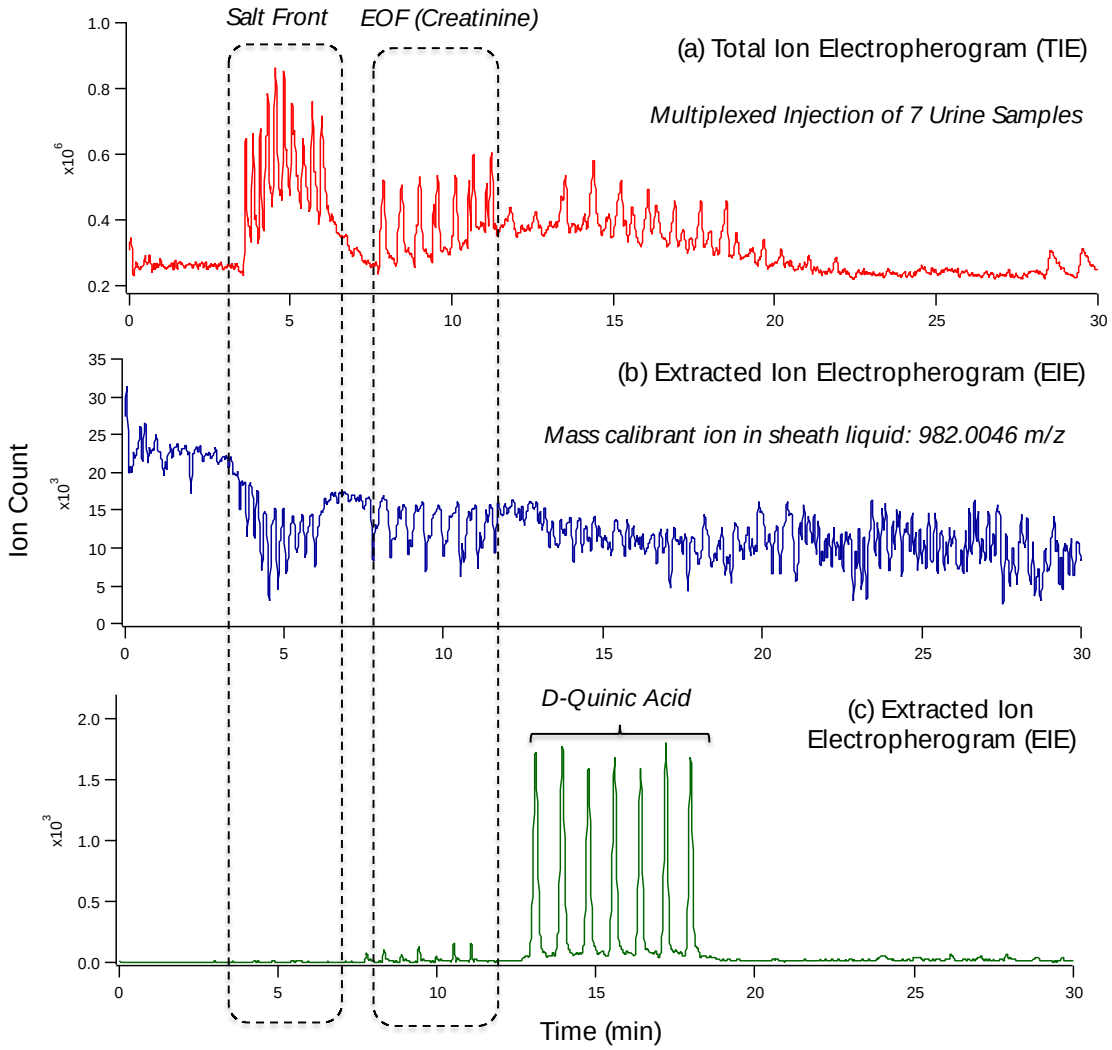


Figure S4. Spectral overlay for the multiplexed separation of 7 human urine samples using MSI-CE-MS when using 50 mM ammonium bicarbonate, pH 8.5 as BGE with polyimide coated fused silica capillaries, which highlights (a) total ion electropherogram and two early migrating zones associated with salt/electrolyte front (Na+) and EOF (co-migration of creatinine), (b) extracted ion electropherogram for the mass calibrant ion (982.0046 *m/z*) that is added into sheath liquid solution and continuously infused into ion source to improve mass accuracy while also enabling the monitoring of time regions susceptible to ion suppression (*i.e.*, attenuated signal) during separation, and (c) extracted ion electrophergram for *D*-quinic acid based on its characteristic *m/z*:RMT (191.055:0.881). In the latter case, there is no evidence of significant ion suppression in time region near migration of *D*-quinic acid and other acidic urinary metabolites due to the effective desalting and high separation efficiency of CE.

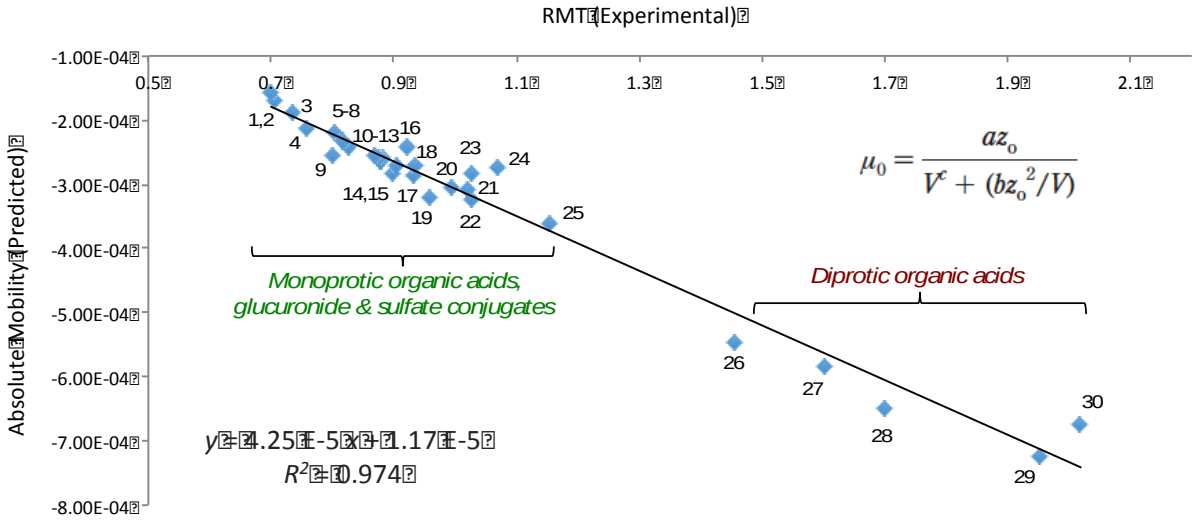


Figure S5. Modeling of the absolute electrophoretic mobility of weakly acidic urinary metabolites based on two intrinsic solute properties using the Hubbard-Onsager equation, namely pK_a or effective charge (z_o at pH 8.5) and molecular volume (*MV*) summarized in **Table S1**. Overall, there is a good correlation in measured relative migration times (RMTs) with predicted absolute mobility for acidic metabolites (n= 30) that supports identification of putative ions based on their accurate mass or elemental formula in cases when commercial standards or MS/MS spectra are unavailable. Urinary metabolites are 1: cortolone 3-glucuronide, 2: tetrahydroaldosterone 3-glucuronide, 3: ibuprofen 3-glucuronide, 4: acetominophen glucuronide, 5: hydroxyibuprofen, 6: pantothenic acid, 7: ethylglucuronide, 8: phenylacetylglutamine, 9: indoylacroylglutamine, 10: vanillylmandelic acid, 11: *D*-quinic acid, 12: 5-hydroxyindoleacetic acid, 13: homovannilic acid, 14: 2-aminoadipic acid, 15: hippuric acid, 16: acetominophen sulfate, 17: hydroxyphenylacetic acid, 18: 4-pyridoxic acid, 19: uric acid, 20: threonic acid, 21: oxoproline, 22: 3-hydroxybutyric acid, 23: indoxylsulfate, 24: cresol sulfate, 25: lactic acid, 26: 2-isopropylmalic acid , 27: 3-hydroxymethylglutaric acid , 28: glutaric acid, 29: succinic acid, 30: tartaric acid. Experimental migration times were normalized to a single internal standard in MSI-CE-MS, namely naphthalene monosulfonic acid (NMS) in order to derive improved precision (*CV* < 1%) based on solute relative migration times (RMT).