Supplementary Information

Optimizing Extraction and Analysis of Pharmaceuticals in Human Urine, Struvite, Food Crops, Soil, and Lysimeter Water by Liquid Chromatography-Tandem Mass Spectrometry

Rachel A. Mullen [a], Krista R. Wigginton [b], Abraham Noe-Hays [c], Kim Nace [c], Nancy G. Love [b], Charles B. Bott [d], and Diana S. Aga [a, *]

[a] Department of Chemistry, University at Buffalo, The State University of New York, Buffalo NY 14260

[b] Department of Civil and Environmental Engineering, University of Michigan, Ann Arbor, MI

[c] Rich Earth Institute, Brattleboro, VT

[d] Hampton Roads Sanitation District, Virginia Beach, VA

*Email of corresponding author: dianaaga@buffalo.edu

Standard preparation and stability

Standard solutions of each individual analyte was prepared at 0.5 mg/mL in methanol and stored at -40°C in amber glass vials. An exception was ciprofloxacin, due to its limited solubility, stock standards were prepared in 1% acetic acid in methanol.

A standard mix at 1 µg/mL was prepared in methanol and stored at -40 °C. Quality control standards were prepared at 50 µg/L from the spiking solution in water/acetonitrile (9/1, v/v) to monitor for instrument drift or irregularities throughout an analysis. Additionally, seven-point calibration standard solutions from 100 µg/L to 1.5 µg/L were prepared. Stability of calibration solutions were tested against freshly prepared standards. All pharmaceutical standards are stable for at least 2 years.

Field plots were constrained to a 200L HDPE drum with the soil supported 15 cm from the bottom, allowing the lowest portion of the tank to act as a reservoir where leachate could accumulate and be stored out of contact with the soil

Glassware treatment

In this study all glassware was soaked for at least 12 hours in a 2% nitric acid bath to decrease analyte adsorption and remove residual chemicals. Glassware was baked prior to use to remove remaining moisture.

Figure S1. Lysimeter set-up

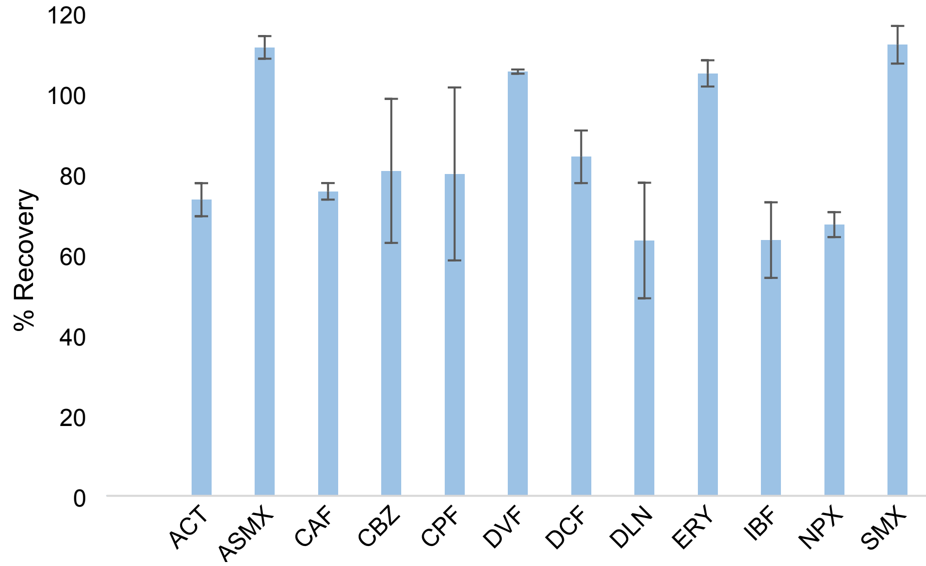

Figure S2. Solid phase extraction (SPE) recoveries of analytes spiked at 500 µg L^{-1} into nanopure water

Liquid chromatography tandem mass spectrometry (LC-MS/MS)

Prior to detection by mass spectrometry, separation was achieved using liquid chromatography. A chromatogram showing the separation can be seen in **Figure S3.**

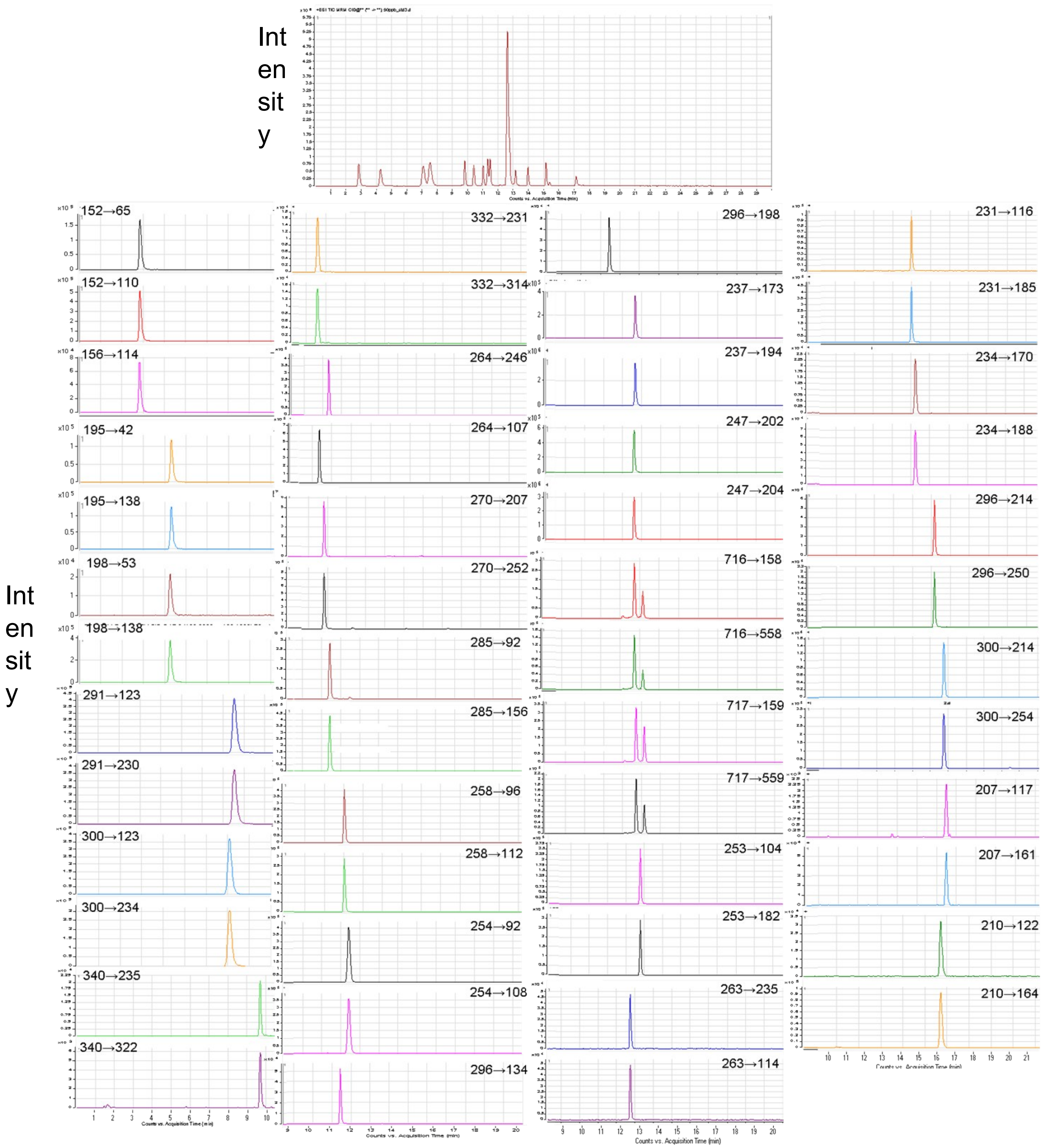

Figure S3. Total ion chromatogram (top) and individual chromatograms of each target analyte under multiple reaction monitoring (MRM) mode. Co-eluting peaks are distinguishable from one another due to different MRM transitions, as shown for individual analytes below the total ion chromatogram (top-most chromatogram).

Urine method optimization

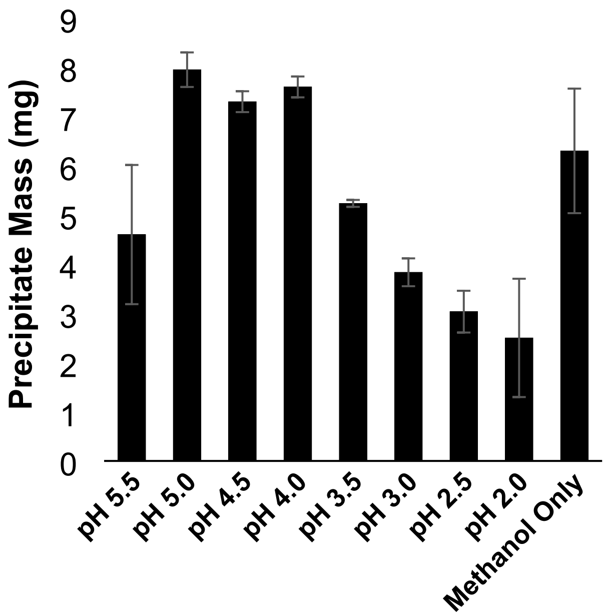

Figure S4. Impact of pH on the amount of urine precipitate recovered. Replicate experiments were conducted at each tested pH (n = 3); error bars represent standard deviation in the recovered precipitate mass. Highest mass was observed when urine was adjusted at pH 5. Unadjusted urine had a pH of 9.

Lettuce and carrot method optimization

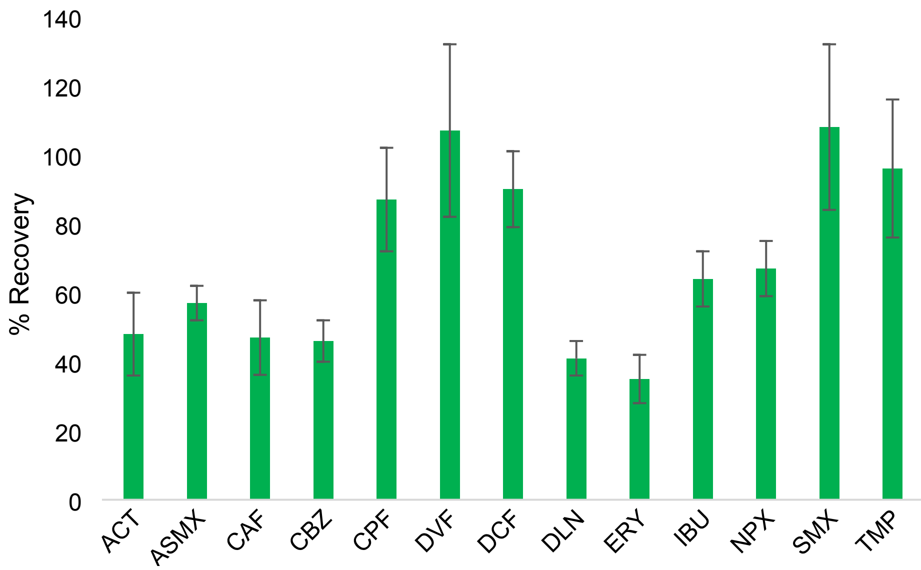

Figure S5. Recoveries of pharmaceuticals spiked in lettuce after methanol extraction (n=3); error bars are standard deviation.

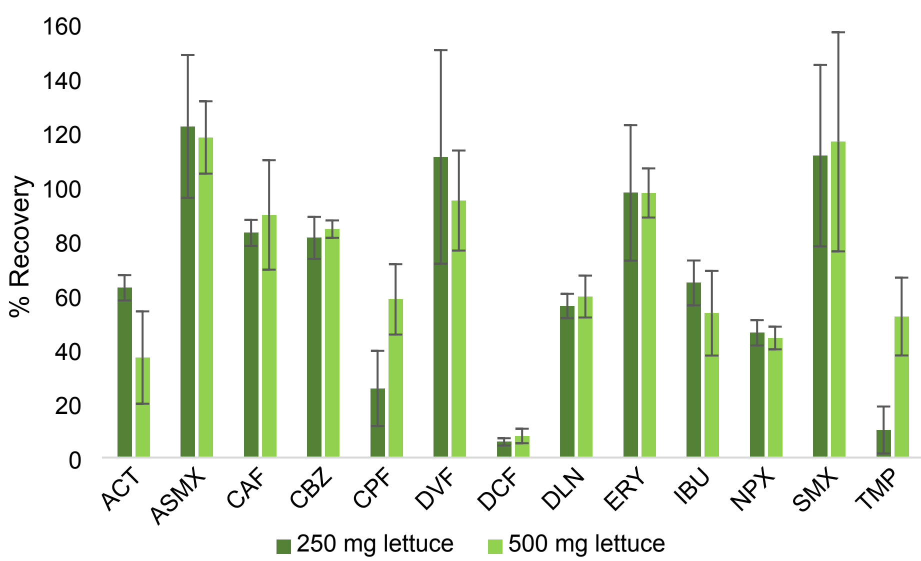

Figure S6. Recoveries of pharmaceuticals spiked in 250-mg and 500-mg freeze-dried lettuce after sonication (n=3); error bars are standard deviation.

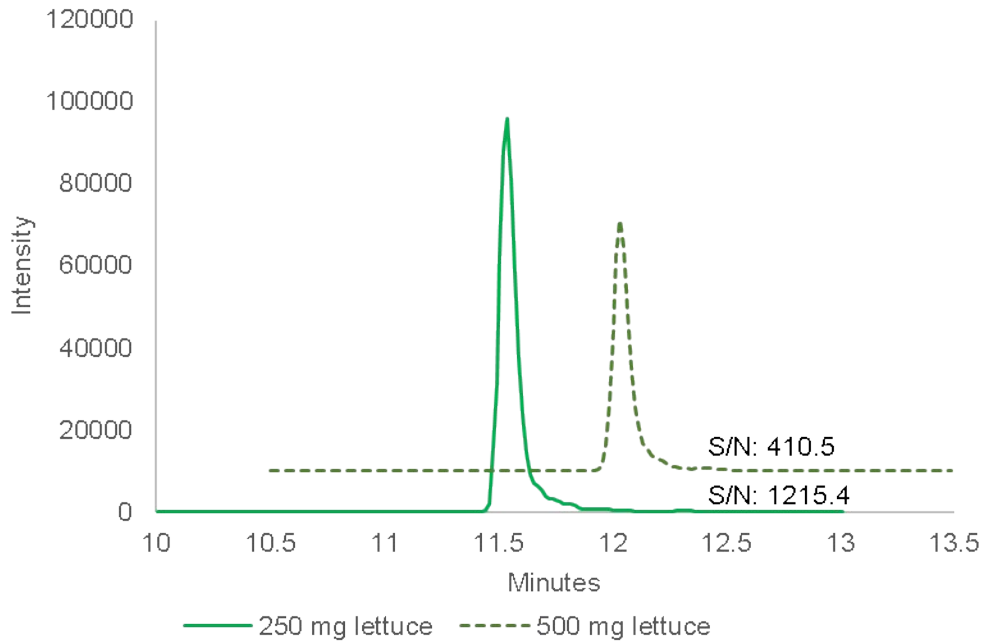

Figure S7. Matrix suppression of d10-carbamazepine in lettuce.

Soil Method Optimization.

Extractions of 250 mg vs. 500 mg vs. 1 g samples using the same extraction method as is used for lettuce and carrots. The recoveries were lower than what was observed in lettuce and carrots (see Figure S8).

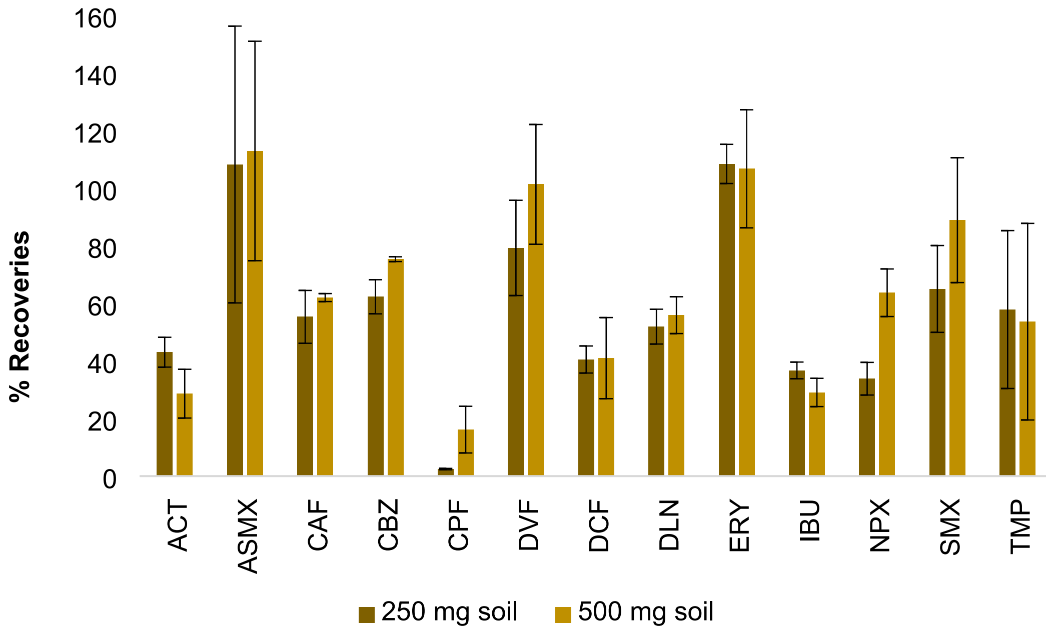

Figure S8. Percent Recoveries of 250 mg and 500 mg soil (n=3) error bars are standard deviation.

Sample Size	AVG Area	% RSD	N
250 mg	7200000	12	7
500 mg	7000000	10	7
1 g	7700000	4	7

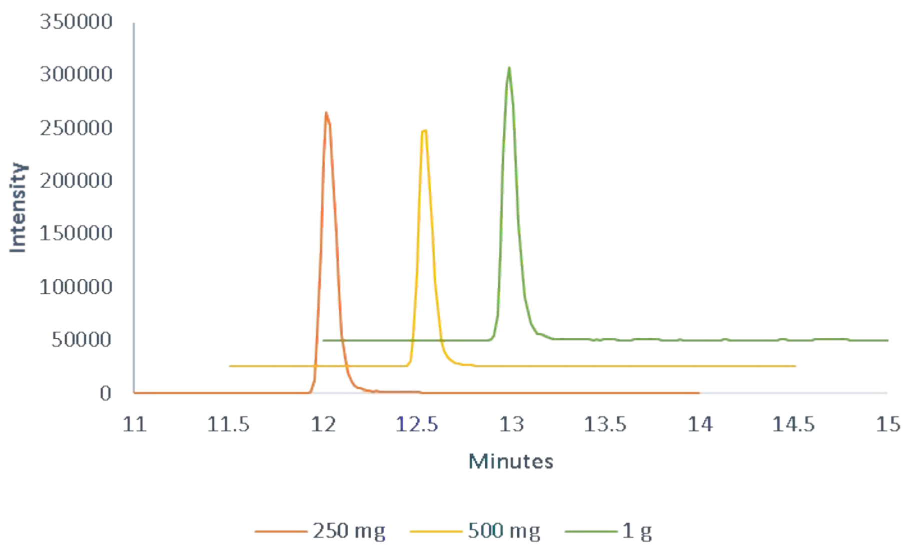

Figure S9. Matrix suppression of d10-carbamazepine in soil sample sizes of 250 mg, 500 mg, and 1 g.

Table S1. Squared correlation coefficient (r^2) of seven-point calibration curves (n=3).

Analyte	Urine	Struvite	Lettuce	Carrot	Soil	Lysimeter
ACT	0.9831	0.9984	0.9989	0.9899	0.9836	0.9999
ASMX	0.9809	0.9882	0.9845	0.9962	0.9921	0.9996
CAF	0.9638	0.9958	0.9971	0.9941	0.9659	0.9995
CBZ	0.9770	0.9986	0.9919	0.9967	0.9839	0.9999
CPF	0.9750	0.9696	0.9864	0.9894	0.9567	0.9989
DVF	0.9618	0.9774	0.9913	0.9884	0.9540	0.9994
DCF	0.9653	0.9971	0.9792	0.9872	0.9718	0.9995
DLN	0.9764	0.9920	0.9951	0.9989	0.9618	0.9994
ERY	0.9775	0.9935	0.9856	0.9958	0.9685	0.9999
IBF	0.9728	0.9910	0.9912	0.9952	0.9665	0.9998
NPX	0.9730	0.9841	0.9986	0.9881	0.9897	0.9960
SMX	0.9862	0.9959	0.9968	0.9981	0.9725	0.9997
TMP	0.9785	0.9976	0.9897	0.9889	0.9744	0.9999

Table S2. Average retention times of analytes at 50 µg L^{-1} ± the standard deviation (n=3).

Analyte	Urine	Struvite	Lettuce	Carrot	Soil	Lysimeter
ACT	2.916 ± 0.006	2.746 ± 0.006	2.89 ± 0.01	2.79 ± 0.01	2.76 ± 0.01	2.848 ± 0.004
ASMX	11.491 ± 0.005	12.64 ± 0.04	11.85 ± 0.02	12.790 ± 0.005	13.0 ± 0.3	11.43 ± 0.06
CAF	4.48 ± 0.02	3.94 ± 0.01	4.59 ± 0.02	4.207 ± 0.006	3.98 ± 0.04	4.36 ± 0.01
CBZ	12.795 ± 0.004	12.720 ± 0.006	12.676 ± 0.007	12.566 ± 0.005	12.783 ± 0.009	12.653 ± 0.004
CPF	9.64 ± 0.02	9.88 ± 0.02	9.769 ± 0.002	9.78 ± 0.06	9.95 ± 0.02	9.756 ± 0.004
DVF	10.68 ± 0.01	11.40± 0.01	10.73 ± 0.03	10.8 ± 0.7	11.448 ± 0.02	10.552 ± 0.003
DCF	15.161 ± 0.004	15.200 ± 0.006	15.188 ± 0.006	15.095 ± 0.003	15.240 ± 0.02	15.166 ± 0.005
DLN	12.867 ± 0.005	12.853 ± 0.008	12.813 ± 0.005	12.697 ± 0.005	12.923 ± 0.008	12.818 ± 0.005
ERY	12.176 ± 0.001	13.154 ± 0.005	12.649 ± 0.003	12.574 ± 0.006	12.769 ± 0.008	12.591 ± 0.001
IBF	15.355 ± 0.004	15.450 ± 0.004	15.437 ± 0.007	16.419 ± 0.002	15.49 ± 0.02	15.407 ± 0.007
NPX	13.762 ± 0.004	14.040 ± 0.003	14.010 ± 0.004	13.905 ± 0.005	14.10 ± 0.02	13.995 ± 0.006

SMX	11.851 ± 0.002	11.96 ± 0.01	12.03 ± 0.09	12.73 ± 0.04	11.24 ± 0.02	12.28 ± 0.06
TMP	6.13 ± 0.02	6.15 ± 0.08	6.18 ± 0.06	7.14 ± 0.02	5.9 ± 0.4	6.84 ± 0.09

Table S3. Intraday relative standard deviation of analyte peak areas observed in sample matrices. The areas were obtained from a spiked concentration of 50 µg L^{-1} (n=3).

Analyte	Standard solution	Urine	Struvite	Lettuce	Carrot	Soil	Lysimeter
ACT	0.27	1.22	0.67	6.87	5.88	4.41	1.57
ASMX	4.66	1.36	1.70	1.03	2.81	1.28	2.88
CAF	2.39	2.45	1.08	4.27	3.79	2.60	1.66
CBZ	4.04	0.70	1.60	4.31	9.68	1.29	4.28
CPF	5.50	3.56	4.31	1.72	2.80	1.40	3.81
DVF	1.19	2.71	6.05	3.13	4.19	3.58	6.63
DCF	2.41	3.44	1.22	1.76	2.15	1.51	2.36
DLN	5.12	2.55	4.53	4.31	5.25	4.02	4.38
ERY	3.18	3.45	1.32	4.48	3.18	3.37	4.74
IBU	3.91	4.45	1.95	5.84	4.83	2.74	1.88
NPX	3.71	7.55	4.55	5.46	5.30	4.60	6.21
SMX	2.27	1.65	3.70	2.40	4.31	1.39	5.52
TMP	0.84	0.79	2.97	2.20	1.28	1.41	2.50

Table S4. Interday relative standard deviation of analyte peak areas observed in sample matrices. The areas were obtained from a spiked concentration of 50 µg L^{-1} (n=3).

Analyte	Standard solution	Urine	Struvite	Lettuce	Carrot	Soil	Lysimeter
ACT	2.34	2.73	4.93	1.82	1.83	6.01	6.26
ASMX	1.75	4.34	4.78	2.20	4.94	1.82	2.05
CAF	3.45	6.61	4.42	2.28	4.77	4.62	2.23
CBZ	1.75	4.18	5.27	3.08	5.08	1.46	5.39
CPF	3.87	24.90	3.36	5.12	5.60	1.33	4.78
DVF	2.48	3.82	3.35	5.43	6.31	6.94	1.77
DCF	2.20	2.96	5.76	2.82	5.46	5.34	5.95
DLN	5.35	14.65	5.58	3.90	6.42	1.91	3.07
ERY	5.64	2.86	6.60	3.93	5.40	6.70	2.06
IBU	4.49	1.39	5.06	7.11	2.78	3.23	4.05
NPX	5.30	2.25	6.27	7.63	4.03	1.76	4.74
SMX	3.71	0.44	4.26	5.74	5.74	4.69	3.02
TMP	1.75	5.35	5.29	2.22	2.70	3.83	3.50

Table S5. Quantification and qualification ion ratios in standards and spiked matrix (RSD (n=3)

Sample	ACT	ASMX	CAF	CBZ	CPF	DVF	DCF	DLN	ERY	IBU	NPX	SMX	TMP
Standard 1.56 µg L^{-1}	1.90 (9)	<LOD	0.85 (17)	8.48 (8)	1.13 (8)	17.23 (9)	2.83 (20)	1.12 (6)	2.18 (17)	22.18 (10)	<LOD	1.43 (1)	1.24 (5)
Standard 3.13 µg L^{-1}	2.19 (11)	1.07 (10)	0.94 (17)	8.84 (7)	1.10 (4)	16.46 (11)	2.97 (8)	1.10 (8)	2.50 (13)	23.24 (5)	<LOD	1.41 (3)	1.13 (4)
Standard 6.25 µg L^{-1}	2.51 (6)	0.99 (19)	1.05 (7)	9.15 (5)	1.13 (6)	20.03 (10)	3.10 (9)	1.07 (5)	2.18 (19)	20.59 (8)	2.46 (13)	1.39 (5)	1.09 (3)
Standard 12.5 µg L^{-1}	2.50 (3)	0.99 (9)	1.06 (2)	9.10 (3)	1.09 (3)	16.94 (10)	2.89 (3)	1.10 (4)	1.96 (9)	23.76 (1)	2.49 (7)	1.45 (13)	1.06 (2)
Standard 25 µg L^{-1}	2.61 (3)	0.92 (4)	1.05 (2)	9.01 (2)	1.10 (1)	16.72 (17)	3.02 (4)	1.09 (3)	1.77 (2)	22.48 (2)	1.58 (10)	1.30 (9)	1.05 (1)
Standard 50 µg L^{-1}	2.65 (3)	0.90 (6)	1.07 (2)	8.91 (2)	1.12 (5)	16.86 (3)	3.17 (2)	1.10 (1)	1.76 (1)	23.08 (4)	2.28 (6)	1.43 (6)	1.06 (1)
Standard 100 µg L^{-1}	2.68 (1)	0.89 (2)	1.09 (2)	9.01 (1)	1.9 (2)	16.94 (10)	3.19 (1)	1.10 (2)	1.76 (0.4)	23.76 (1)	2.61 (4)	1.32 (2)	1.05 (1)
Urine 1.56 µg L^{-1}	2.18 (1)	<LOD	<LOD	6.31 (11)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Urine 3.13 µg L^{-1}	2.22 (2)	<LOD	1.53 (0.5)	7.11 (6)	<LOD	<LOD	<LOD	0.49 (22)	<LOD	19.65 (8)	2.90 (2)	1.34 (4)	3.14 (25)
Urine 6.25 µg L^{-1}	2.12 (6)	1.15 (26)	1.53 (0.3)	8.19 (4)	1.73 (25)	<LOD	<LOD	0.66 (19)	<LOD	22.02 (9)	1.71 (38)	1.34 (14)	2.41 (29)
Urine 12.5 µg L^{-1}	2.27 (17)	1.07 (20)	1.54 (0.4)	8.26 (6)	1.40 (11)	17.23 (9)	1.76 (2)	0.70 (22)	2.27 (17)	20.35 (14)	2.13 (10)	1.27 (4)	1.74 (35)
Urine 25 µg L^{-1}	2.08 (1)	1.10 (16)	1.54 (0.9)	8.52 (1)	1.18 (16)	18.13 (8)	2.08 (6)	0.90 (19)	2.21 (4)	21.77 (7)	2.47 (5)	1.38 (2)	1.29 (12)
Urine 50 µg L^{-1}	2.14 (4)	1.12 (9)	1.56 (0.1)	8.33 (2)	1.14 (5)	16.72 (18)	3.10 (12)	0.91 (4)	2.23 (5)	21.78 (4)	2.83 (11)	1.37 (0.3)	1.09 (7)
Urine 100 µg L^{-1}	2.23 (3)	0.98 (1)	1.55 (0.3)	8.01 (1)	1.06 (2)	16.67 (3)	3.23 (2)	1.06 (7)	2.18 (6)	23.68 (9)	2.79 (1)	1.49 (17)	0.95 (8)
Struvite 1.56 µg kg^{-1}	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	2.53 (4)	19.74 (3)	<LOD	<LOD	1.12 (4)
Struvite 3.13 µg kg^{-1}	<LOD	1.08 (21)	0.92 (8)	9.48 (6)	1.13 (7)	18.42 (29)	2.33 (9)	1.09 (3)	2.55 (3)	20.44 (13)	<LOD	<LOD	1.09 (6)
Struvite 6.25 µg kg^{-1}	2.25 (4)	1.04 (11)	1.01 (11)	9.27 (7)	1.14 (10)	17.23 (9)	2.42 (1)	0.98 (8)	2.38 (4)	17.46 (6)	1.47 (10)	1.35 (2)	1.10 (7)
Struvite 12.5 µg kg^{-1}	2.30 (1)	0.97 (25)	0.89 (4)	9.23 (1)	1.12 (5)	18.14 (8)	2.79 (5)	1.08 (6)	2.23 (3)	18.64 (9)	2.11 (14)	1.34 (2)	1.12 (7)
Struvite 25 µg kg^{-1}	2.28 (2)	0.93 (15)	0.95 (2)	8.93 (3)	1.08 (2)	16.72 (18)	2.81 (2)	1.05 (4)	2.17 (0.5)	19.88 (10)	2.56 (4)	1.33 (4)	1.10 (2)
Struvite 50 µg kg^{-1}	2.32 (1)	0.91 (11)	0.94 (2)	8.81 (2)	1.10 (1)	16.67 (3)	2.74 (2)	1.08 (1)	2.09 (2)	19.55 (5)	2.89 (7)	1.43 (4)	1.09 (0.2)
Struvite 100 µg kg^{-1}	2.32 (1)	0.94 (4)	0.91 (1)	8.92 (1)	1.08 (1)	15.07 (4)	2.76 (1)	1.08 (2)	2.12 (0.3)	19.07 (5)	3.04 (4)	1.41 (2)	1.09 (1)
Lettuce 1.56 µg kg^{-1}	<LOD	<LOD	<LOD	8.96 (15)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Lettuce 3.13 µg kg^{-1}	2.37 (22)	<LOD	<LOD	9.20 (16)	<LOD	<LOD	<LOD	<LOD	2.39 (6)	18.74 (2)	3.11 (8)	<LOD	<LOD
Lettuce 6.25 µg kg^{-1}	2.73 (7)	1.05 (10)	1.00 (4)	10.02 (5)	1.14 (31)	<LOD	3.12 (5)	1.12 (12)	2.20 (2)	18.94 (2)	2.58 (49)	1.38 (3)	1.13 (3)
Lettuce 12.5 µg kg^{-1}	2.93 (3)	1.10 (5)	0.99 (3)	9.72 (8)	1.14 (10)	16.20 (2)	3.31 (1)	1.11 (2)	2.17 (6)	24.59 (3)	2.12 (58)	1.33 (1)	1.15 (2)
Lettuce 25 µg kg^{-1}	2.93 (3)	1.10 (5)	1.00 (2)	9.38 (2)	1.04 (2)	17.25 (4)	3.24 (2)	1.14 (2)	2.14 (3)	25.72 (1)	2.74 (8)	1.35 (4)	1.13 (0.4)
Lettuce 50 µg kg^{-1}	3.08 (9)	1.46 (58)	1.03 (2)	9.26 (4)	1.52 (24)	18.69 (2)	2.69 (18)	1.25 (10)	2.10 (4)	25.51 (1)	2.06 (54)	1.37 (14)	1.18 (1)
Lettuce 100 µg kg^{-1}	3.00 (2)	1.35 (34)	1.06 (2)	9.88 (10)	1.29 (6)	21.10 (8)	2.97 (19)	1.14 (3)	2.14 (11)	24.32 (3)	2.40 (62)	1.32 (4)	1.13 (3)
Carrot 1.56 µg kg^{-1}	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	25.49 (2)	1.18 (13)	<LOD	<LOD
Carrot 3.13 µg kg^{-1}	2.96 (4)	<LOD	<LOD	<LOD	<LOD	<LOD	1.29 (14)	<LOD	<LOD	24.26 (10)	1.18 (14)	<LOD	<LOD
Carrot 6.25 µg kg^{-1}	2.94 (9)	<LOD	1.03 (4)	<LOD	<LOD	<LOD	2.27 (16)	1.27 (8)	<LOD	21.77 (13)	2.06 (11)	1.33 (1)	1.08 (2)
Carrot 12.5 µg kg^{-1}	2.79 (20)	1.41 (24)	1.00 (2)	8.80 (0.5)	3.26 (21)	15.52 (6)	1.39 (12)	1.06 (13)	2.30 (3)	20.39 (15)	3.01 (9)	1.49 (18)	1.14 (1)
Carrot 25 µg kg^{-1}	2.88 (5)	1.24 (6)	1.02 (3)	8.36 (3)	1.90 (44)	17.89 (7)	3.02 (5)	1.20 (3)	2.47 (17)	17.47 (7)	2.77 (9)	1.36 (14)	1.08 (3)

Carrot 50 μg kg⁻¹	3.00 (4)	1.20 (7)	1.04 (2)	8.42 (1)	1.31 (8)	17.11 (7)	3.32 (3)	1.15 (5)	2.16 (1)	18.80 (3)	2.88 (2)	1.37 (0.2)	1.08 (1)
Carrot 100 μg kg⁻¹	2.96 (4)	1.12 (4)	1.05 (3)	9.07 (3)	1.53 (51)	17.88 (10)	3.37 (1)	1.18 (2)	2.17 (1)	16.83 (0.3)	2.63 (5)	1.36 (3)	1.08 (1)
Soil 1.56 μg kg⁻¹	2.38 (0.1)	0.90 (22)	0.88 (8)	<LOD	<LOD	<LOD	2.14 (10)	<LOD	<LOD	21.58 (13)	0.69 (28)	1.36 (3)	1.09 (3)
Soil 3.13 μg kg⁻¹	2.19 (0.2)	1.19 (15)	0.91 (20)	9.27 (5)	1.44 (13)	<LOD	2.17 (8)	1.11 (15)	2.07 (0.4)	21.87 (12)	1.64 (33)	1.31 (3)	1.12 (7)
Soil 6.25 μg kg⁻¹	2.35 (0.1)	1.11 (20)	0.87 (9)	9.13 (3)	1.62 (53)	14.92 (6)	2.46 (5)	0.98 (7)	1.96 (1)	21.93 (8)	1.25 (27)	1.32 (2)	1.06 (4)
Soil 12.5 μg kg⁻¹	2.35 (0.1)	1.15 (16)	0.92 (3)	9.61 (4)	2.87 (36)	17.34 (8)	2.83 (9)	1.09 (3)	1.94 (2)	19.15 (5)	1.63 (6)	1.33 (4)	1.06 (4)
Soil 25 μg kg⁻¹	2.42 (0.04)	1.18 (11)	0.92 (2)	9.31 (0.2)	1.38 (25)	17.19 (8)	2.63 (3)	1.10 (3)	2.12 (6)	20.35 (14)	2.60 (15)	1.36 (14)	1.08 (1)
Soil 50 μg kg⁻¹	2.37 (0.1)	1.12 (11)	0.95 (2)	9.52 (3)	1.26 (13)	17.67 (17)	2.64 (4)	1.11 (1)	2.31 (7)	2.78 (6)	3.23 (4)	1.37 (0.3)	1.06 (1)
Soil 100 μg kg⁻¹	2.33 (0.03)	1.00 (13)	0.90 (6)	8.87 (10)	1.22 (11)	19.24 (15)	2.27 (15)	1.05 (8)	2.44 (11)	20.88 (11)	2.65 (40)	1.36 (2)	1.06 (4)

Table S6. Percent matrix effects calculated by dividing the area of the analyte in matrix divided by the area of the analyte in a standard both at 50 μg L^{-1} (n=3).

Analyte	Urine	Struvite	Lettuce	Carrot	Soil	Lysimeter
ACT	74.2	89.6	83.2	96.4	86.6	97.3
ASMX	49.2	82.4	76.1	100.4	67.2	95.1
CAF	64.3	89.8	73.9	78.8	84.7	104.7
CBZ	21.4	70.0	98.1	84.2	74.5	107.0
CPF	53.5	29.4	56.8	68.7	16.6	76.5
DVF	72.4	88.9	73.7	66.3	63.5	97.1
DCF	30.9	67.2	74.0	80.4	67.3	83.7
DLN	55.9	73.9	78.1	82.5	82.9	87.7
ERY	40.8	75.5	83.3	51.7	71.6	98.2
IBF	78.9	96.7	60.1	79.9	77.2	98.1
NPX	48.7	95.8	69.2	67.5	92.2	102.8
SMX	30.5	65.5	112.4	76.6	55.8	93.1
TMP	72.5	87.5	69.6	87.3	78.1	96.8

Table S7. Limits of quantification (LOQs)

Compound	Urine (μg/L)	Struvite (μg/kg)	Lettuce (μg/kg)	Carrot (μg/kg)	Soil (μg/kg)	Lysimeter (μg/L)
ACT	0.09	11	3.1	4.7	9.0	0.0030
ASMX	0.25	8.0	9.0	10.7	4.3	0.011
CAF	0.15	6.3	7.3	5.7	4.3	0.0077
CBZ	0.08	10	2.9	6.7	6.7	0.0077
CPF	0.33	5.7	11	23	15	0.031
DVF	0.83	3.2	16	17	5.0	0.023
DCF	0.23	8.7	6.3	4.3	8.3	0.018
DLN	0.40	9.0	13	8.3	12	0.019
ERY	0.53	4.7	5.3	11	11	0.013
IBU	0.11	2.9	5.0	2.1	4.7	0.0050
NPX	0.18	13	4.7	2.6	7.7	0.018
SMX	0.19	11.67	6.00	8.00	7.0	0.014
TMP	0.19	5.33	10.67	7.00	7.67	0.0053