Determination of ten steroid hormones in animal waste manure and agricultural soil using inverse and integrated clean-up pressurized liquid extraction and gas chromatography tandem mass spectrometry

Supplementary Information

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Supplementary Information Abstract

The absolute recoveries of the steroid hormones and the surrogate interference compounds (SIC) from 22 mL PLE cells packed with 0.5+2.0 g diatomaceous earth (DE) are found in **Table S-1**. The cells were extracted in two separate cycles with *n*-heptane in one group and a heptane:acetone (97:3) mixture in the last group. This experiment clearly shows that the SIC are flushed from the cells with n-heptane, while adding just few per cent of a more polar solvent such as acetone, will also elute the steroid hormones.

In **Table S-2** the absolute cycle cumulative recoveries are shown. In these experiment different amounts of DE was tested to find the required mass of DE to retain the steroid hormones, while flushing the SIC with *n*-heptane (during the i-PLE). From the table it is clear that 1.0 and 2.0 g DE are unable to retain the steroid hormones while eluting with *n*-heptane (i-PLE). In contrary, 4.0 g DE was shown to be sufficient to retain the steroid hormones, while the SIC were completely eluted with 2 cycles of *n*-heptane. After the *n*-heptane cycles, quantitative recoveries of the steroid hormones were obtained with 2 cycles of acetone:methanol (1:1).

Photographs of the PLE cells, collection vials, and used and non-used internal clean-up materials from a 0.50 g manure extraction are shown in Figure S-1. The ic-PLE extracts (in photograph 'B') was visually compared to i-PLE following extraction of the steroid hormones with acetone:methanol (1:1) without additional clean-up materials (in photograph 'A'). Naturally, the *n*-heptane extracts (left collection vial) between A and B are similar. However, the acetone:methanol extracts (right collection vial) are very different, demonstrating the clean-up

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ability of the ic-PLE modification. The remaining fatty residues (not possible to evaporate by nitrogen), from 0.50 g manure sample, in the displayed extracts were around 40 mg in the *n*-heptane fractions, less than 20 mg in the ic-PLE acetone:methanol fraction, while the acetone:methanol fraction without internal clean-up contained ca. 70 mg. The ca. 20 mg fatty residues in the ic-PLE extracts are possible to remove with the external clean-up procedure (Figure 2).

Table S-1. Absolute recoveries (%) of steroid hormones from 22 mL PLE cells using two different eluents in two consecutive cycles (n=2). The cells were packed with 0.5 g DE pre-spiked with analytes placed on top of another 2.0 g DE. Spike levels; 50 ng steroid hormones and 200 ng sterols and stanols. Extraction parameters; 5 min static cycles, 1500 psi, 80 °C, 60% flush and 120 s purge. Deviations between duplicates were removed for simplicity and were below 22% in all cases.

	Heptane		**Heptane:acetone (97:3)**	
	Cycle 1	Cycle 2	Cycle 1	Cycle 2
Progestagens				
PRE	NA	NA	NA	NA
PRO	ND	ND	54	0.4
Androgens				
DHEA	NA	NA	NA	NA
AN	ND	ND	70	ND
TS	2.5	3.3	89	0.9
DHT	ND	ND	64	ND
Estrogens				
E1	0.1	0.2	52	0.2
αE2	ND	ND	51	ND
βE2	0.5	0.5	60	0.8
EE2	0.9	0.3	58	0.4
Surrogate interference compounds				
CHOL	100	9.1	103	6.7
COPs	101	7.7	102	3.1
SITO	98	28	124	37

NA, not applied in study. ND, not detected.

Table S-2. Absolute cumulative fractionation recoveries (%) of steroid hormones and surrogate interference compounds (SIC) by varying the DE content in a 22 mL cell. Steroid hormones and SIC were spiked on top of DE as 50 ng and 100 ng, respectively. The class wise averaged data of 2.0 and 4.0 g DE are presented in Figure 4. Cycles 1 to 4 utilized *n*-heptane as solvent, while cycles 5 to 8 utilized acetone:methanol (1:1). DHEA was not implemented in the experiments with 1.0 g and 2.0 g DE.

	Progestagens			Androgens			Estrogens				SIC		
Cycle	PRE	PRO	DHEA	AN	TS	DHT	E1	αE2	βE2	EE2	CHOL	COPs	SITO
						1.0 g DE (n =3)							
1	99±16	90±10	NA	94±13	85±5	88±19	66±11	49±13	59±5	69±7	109*	106*	**
2	99*	90*	NA	94*	93*	88*	81*	49*	62*	81*	111*	106*	**
3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	114*	106*	**
4	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	118*	106*	**
5	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	124*	114*	**
6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	124*	120*	**
7	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	125*	126*	**
8	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	128*	126*	**
						2.0 g DE (n =3)							
1	35±26	13±15	NA	1±1	2±2	26±28	**	5±4	10±8	18±14	96±5	88±7	**
2	36±23	13±15	NA	1±1	4±3	28±25	**	5±4	11±8	19±14	97±5	90±6	**
3	37±22	13±15	NA	1±1	4±3	28±25	**	5±4	11±9	19±14	97±5	91±7	**
4	38±21	13±15	NA	1±1	4±3	28±25	**	5±4	11±8	19±14	98±5	91±7	**
5	72±10	13±15	NA	71±8	82±19	94±10	**	57±12	68±10	86±8	99±5	92±6	**
6	72±10	15±13	NA	71±8	82±19	94±10	**	57±12	69±10	86±8	99±5	92±6	**
7	72±10	15±13	NA	71±8	82±19	94±10	**	57±12	69±10	86±8	99±5	92±6	**
8	72±10	15±13	NA	71±8	82±19	94±10	**	57±12	69±10	88±8	99±5	92±6	**
						4.0 g DE (n =2)							
1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	122±30	97±9	109±52
2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	130±26	106±7	114±59
3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	130±26	106±7	114±59
4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	133±22	106±7	114±59
5	105±43	111±2	101±38	103±30	109±9	88±15	76±22	44±10	50±3	70±10	133±22	106±7	114±59
6	106±45	111±2	105±36	103±30	134±26	89±17	77±22	44±10	51±3	72±8	133±22	106±7	114±59
7	107±44	111±2	105±36	103±30	135±26	91±20	78±20	44±10	59±12	72±8	133±22	106±7	114±59
8	107±45	111±2	105±36	103±30	136±26	91±20	79±20	44±10	59±12	72±8	133±22	106±7	114±59

* no replicate. ** unknown interference, excluded from the data set. NA, not analyzed. ND, not detected.

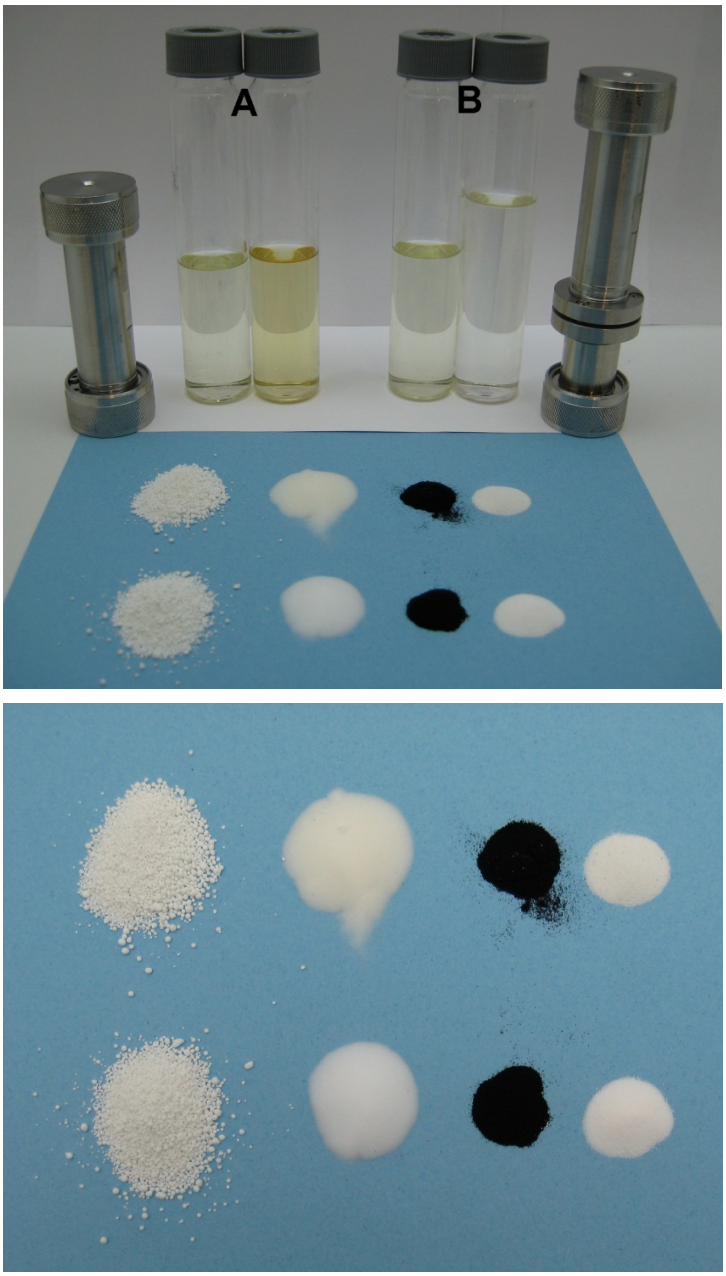

Figure S-1. Photographs of PLE extracts with and without integrated clean-up, and the non-used and used clean-up materials. Photographs of plain i-PLE extracts (A) and i-PLE combined with ic-PLE (B) of the same homogenized manure sample. The i-PLE extract was obtained by a 22 mL cell packed according to Figure 3A, flushed with *n*-heptane (left collection vial A) and extracted with acetone:methanol (1:1, right collection vial A). The ic-PLE extract was obtained by a 22 mL cell packed according to Figure 3A, flushed with *n*-heptane (left collection vial B) and an 11 mL cell packed according to Figure 3B and extracted with acetone:methanol (1:1, right collection vial B). In front (and bottom picture) of the cells and collection vials the used (top row) and non-used (bottom row) clean-up materials are shown. Order of materials; DE, silica, graphite and basic alumina (left to right). The remaining fatty residues (not possible to evaporate by nitrogen) were around 7% in the two *n*-

heptane extracts, 4% in the ic-PLE acetone:methanol fraction (B), while the acetone:methanol fraction without internal clean-up (A) contained more than 13% of fatty residues relative to the manure sample mass.