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Novel SLE Prototype vs. Diatomaceous Earth: Evaluation of Phospholipid-Depletion, Matrix Effect and Recovery of Cortisol and 6β-Hydroxycortisol

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Overview

Purpose

- To compare an SLE prototype against diatomaceous earth for phospholipid depletion, matrix effect and recovery of cortisol and 6β-hydroxycortisol.

Method

- Cortisol and 6β-hydroxycortisol were fortified in human urine and human plasma (the latter matrix included lipemic and hemolyzed) and extracted with both types of SLE substrates.
- Phospholipid depletion, matrix effect and recovery were evaluated.

Results

- SLE prototype appears advantageous over the diatomaceous-earth based SLE sorbent in terms of recovery for 6β-hydroxycortisol in plasma, and comparable for the recovery of cortisol. In addition, the SLE prototype has shown to efficiently eliminate phospholipids, which otherwise might impact assay robustness due to ion suppression.

Introduction

Early commercial, supported liquid extraction (SLE) products, implementing diatomaceous earth, facilitated the adsorption of aqueous samples with subsequent analyte elution using water immiscible solvents. However, more polar elution solvents, such as ethyl acetate, resulted in the co-extraction of ion suppressors, such as phospholipids (*J. Chromatogr. B*, 2012, 891-892, p.71-80).

A novel sorbent prototype designed to trap phospholipids promises their elimination, even when using polar elution solvents. To evaluate the efficacy of phospholipid removal, in addition to matrix effect and recovery, the SLE prototype was compared with traditional diatomaceous earth using cortisol and 6β-hydroxycortisol (major metabolite of cortisol) as test compounds (Figure 1), since their optimal extraction requires ethyl acetate.

In previously reported work by our group, this evaluation methodology was used for the comparison of a commercially available SLE polymeric sorbent with diatomaceous earth. While using the polymeric sorbent eliminated significantly more phospholipids, it also resulted in lower recoveries for the test compounds, even in optimal elution conditions. A SLE product, which would provide both high recoveries and extensive phospholipid depletion, would be of great potential interest as an alternative to diatomaceous earth for routine SLE bioanalysis workflows.

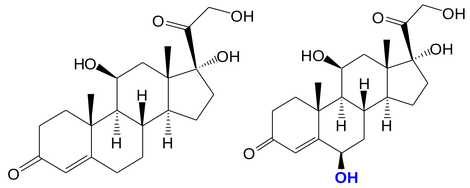

Figure 1. Structures of cortisol and its metabolite, 6β-hydroxycortisol.

Methods

Sample Extraction

Human plasma (200 µL) or urine (200 µL), fortified with 250 ng/mL of cortisol and 1600 ng/mL of 6β-hydroxycortisol, was supplemented with deuterated internal standard (50 µL) and mixed with 0.5M Na_2CO_3 (200 µL). A 200 µL aliquot was loaded onto Agilent Technologies' SLE prototype (250 mg) and Biotage ISOLUTE® diatomaceous earth (200 mg) 96-well plates. Samples were allowed to soak for five minutes and then eluted with five sorbent volumes (1.25 mL for the prototype and 1.00 mL for the diatomaceous earth) of organic solvent(s). Elution was finalized by centrifugation, and extracts evaporated (70°C) and reconstituted with a mobile phase compatible solution.

Chromatography and Detection

Cortisol (*m/z* 421 > 331) and 6β-hydroxycortisol (*m/z* 437>347) acetate adducts formed by (-) ESI were monitored using a SCIEX API 5000, along with 12 phospholipid MRM transitions forming the common fragment ion *m/z* 184 (Table 1). Chromatographic separation was achieved using a C_{18} column.

Table 1. Phospholipid transitions monitored.

Phospholipid	Transition Monitored (Q1/Q3)
Lysophosphatidylcholine (18:2)	520.3/184.1
Lysophosphatidylcholine (18:1)	522.4/184.1
Lysophosphatidylcholine (18:0)	524.4/184.1
Lysophosphatidylcholine (20:4)	544.3/184.1
Phosphatidylcholine (30:1)	704.5/184.1
Phosphatidylcholine (34:2)	758.6/184.1
Phosphatidylcholine (34:1)	760.6/184.1
Phosphatidylcholine (36:3)	784.6/184.1
Phosphatidylcholine (36:2)	786.6/184.1
Phosphatidylcholine (38:6)	806.6/184.1
Phosphatidylcholine (38:5)	808.6/184.1
Phosphatidylcholine (38:4)	810.6/184.1

Results and Discussion

Recovery Evaluation

An in-house validated LC-MS/MS method for the quantitation of cortisol and 6β-hydroxycortisol was used for the evaluation of SLE substrates, which included determining the extent of phospholipid trapping, analyte recovery and matrix effect.

Recovery was evaluated in both human urine and plasma (the latter matrix included lipemic and hemolyzed) fortified with cortisol and 6β-hydroxycortisol. Five elution solvents were screened, including MTBE, diethylether, ethyl acetate, CH_2Cl_2 and $CHCl_3$:MTBE (1:4). The highest recoveries were achieved using ethyl acetate (Tables 2 and 3), consistent with analyte polarity. For cortisol, although recoveries obtained between matrix lots were comparable with the prototype substrate (85–94%) and diatomaceous earth (87–96%), recoveries in plasma were ca. 15% higher for the more polar 6β-hydroxycortisol when using the prototype (77–82% vs. 60–67%). In urine, comparable 6β-hydroxycortisol recoveries were obtained (ca. 90%).

Table 2. Cortisol average recovery (n = 2) comparison between diatomaceous earth and prototype SLE.

Solvent	Cortisol Recovery							
	Urine		Plasma		Lipemic Plasma		Hemolysed Plasma	
	Diatomaceous Earth	Prototype	Diatomaceous Earth	Prototype	Diatomaceous Earth	Prototype	Diatomaceous Earth	Prototype
MTBE	64.3%	54.6%	75.6%	70.1%	94.4%	80.2%	68.5%	80.9%
Diethylether	75.0%	72.6%	57.2%	68.0%	71.1%	64.4%	57.9%	66.1%
Ethyl Acetate	92.1%	90.3%	89.0%	84.7%	86.8%	93.1%	96.2%	93.7%
CH_2Cl_2	66.3%	16.8%	79.7%	59.7%	102.9%	76.8%	78.6%	35.6%
$CHCl_3$:MTBE (1:4)	64.8%	54.3%	74.7%	59.6%	56.6%	46.6%	52.0%	48.7%

Table 3. 6β-Hydroxycortisol average recovery (n = 2) comparison between diatomaceous earth and prototype SLE.

Solvent	6β-Hydroxycortisol Recovery							
	Urine		Plasma		Lipemic Plasma		Hemolysed Plasma	
	Diatomaceous Earth	Prototype	Diatomaceous Earth	Prototype	Diatomaceous Earth	Prototype	Diatomaceous Earth	Prototype
MTBE	49.6%	68.5%	65.1%	67.5%	61.1%	70.3%	45.1%	69.4%
Diethylether	26.0%	23.6%	31.1%	17.7%	26.5%	14.8%	36.5%	19.5%
Ethyl Acetate	90.4%	90.5%	67.2%	77.1%	59.8%	82.0%	58.8%	77.8%
CH_2Cl_2	8.6%	4.5%	23.1%	13.0%	41.3%	15.2%	36.9%	12.3%
$CHCl_3$:MTBE (1:4)	58.8%	46.0%	48.8%	44.0%	39.5%	32.4%	49.0%	34.8%

Table 4. Average phospholipid depletion (n = 2) comparison between diatomaceous earth and prototype SLE.

Matrix	Solvent	Diatomaceous Earth			Prototype			% Phospholipid Depletion Agilent vs. Isolute
		Total LPC Area	Total PC Area	Total Phospholipid Area	Total LPC Area	Total PC Area	Total Phospholipid Area	
Plasma	MTBE	24247	2878008	2902255	117	294	411	**-100%**
	Diethylether	13092	1595491	1608583	180	28943	29123	**-98%**
	Ethyl Acetate	87245	4393819	4481065	9961	733233	743194	**-83%**
	CH_2Cl_2	80952	2402105	2483057	114	41798	41913	**-98%**
	$CHCl_3$:MTBE (1:4)	71499	2643636	2715135	87	153	240	**-100%**
Lipemic Plasma	MTBE	25121	4629571	4654693	123	57338	57461	**-99%**
	Diethylether	6669	2100359	2107028	66	180	246	**-100%**
	Ethyl Acetate	202830	5537145	5739975	74646	1635036	1709682	**-70%**
	CH_2Cl_2	149074	5622875	5771949	150	80251	80401	**-99%**
	$CHCl_3$:MTBE (1:4)	63442	3796066	3859507	75	135	210	**-100%**
Hemolyzed Plasma	MTBE	12756	2466302	2479057	63	3107	3170	**-100%**
	Diethylether	15955	1802356	1818311	48	147	195	**-100%**
	Ethyl Acetate	161744	4964330	5126073	67802	3717714	3785516	**-26%**
	CH_2Cl_2	115276	3902939	4018215	105	20719	20824	**-99%**
	$CHCl_3$:MTBE (1:4)	33662	2186651	2220313	60	120	180	**-100%**

Results and Discussion (Continued)

Matrix Effect & Phospholipid Depletion

Chromatographic conditions for cortisol and 6β-hydroxycortisol were optimized as part of method development to separate multiple interfering peaks co-extracted from the matrix. Under these conditions, ion suppression/enhancement was not observed and all phospholipids were resolved from analytes of interest (Figure 2). Nonetheless, for all elution solvents examined, phospholipid profiling revealed a much higher trapping efficiency for these potential ion suppressors when using the prototype substrate. Results obtained using a semi-quantitative method (Figure 3) suggest that 26 to 100% fewer phospholipids were co-extracted using the prototype sorbent (Table 4). In addition, visual inspection of the hemolyzed samples extracts revealed that those obtained with the prototype were clearer (no coloration), suggesting that less blood components were co-extracted with the compounds of interest.

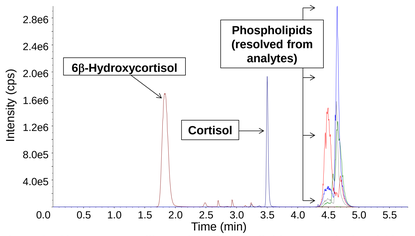

Figure 2. Chromatogram illustrating phospholipids vs. cortisol and 6β-hydroxycortisol retention. Results presented are for a plasma sample extracted with ethyl acetate and the diatomaceous earth sorbent.

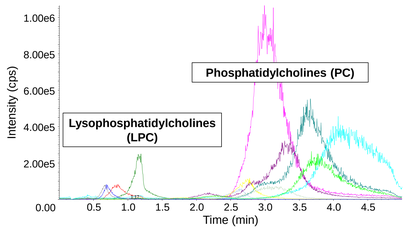

Figure 3. Chromatogram example for the semi-quantitative assessment of phospholipid content in extracts. Note that elution conditions differed from those used for the separation of cortisol and 6β-hydroxycortisol.

Conclusion

Results obtained in this study demonstrate that the SLE prototype evaluated appears advantageous over diatomaceous earth based SLE in terms of recovery for 6β-hydroxycortisol in plasma, and comparable for the recovery of cortisol. In addition, the SLE prototype has shown to efficiently eliminate phospholipids, which otherwise might impact assay robustness due to ion suppression.