Altasciences CLINICAL RESEARCH ALGORITHME PHARMA

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Polymeric *vs.* Diatomaceous Earth SLE Sorbents: A Comparison of Phospholipid Depletion, Matrix Effect and Recovery for Cortisol and 6b-Hydroxycortisol

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Overview

Purpose

- To compare polymeric and diatomaceous earth SLE sorbents for phospholipid depletion, matrix effect and recovery of cortisol and 6b-hydroxycortisol.

Method

- Cortisol and 6b-hydroxycortisol were fortified in urine and plasma (3 donors, including lipemic and hemolyzed) and extracted with both SLE sorbents.
- Phospholipid depletion, matrix effect and recovery were evaluated.

Results

- While the diatomaceous earth SLE sorbent proved advantageous in terms of recovery for cortisol and 6b-hydroxycortisol, the polymeric sorbent was deemed potentially beneficial for applications requiring the elimination of phospholipids, which otherwise might impact assay robustness due to ion suppression.

Introduction

Solid phase-supported liquid extraction (SLE) is a process wherein liquid extractions are aided by a solid media to which an aqueous sample is adsorbed and analytes eluted using a water-immiscible solvent. Early commercial SLE products had diatomaceous earth as their sorbent, a naturally-occurring sedimentary rock (e.g. ISOLUTE®). However, more polar elution solvents, such as ethyl acetate, could result in the co-extraction of ion-suppressing phospholipids.

The recent introduction of a polymeric sorbent designed to trap phospholipids (Novum™) promises their elimination, even when using polar elution solvents. A previously validated SLE-LC-MS/MS method for cortisol and 6b-hydroxycortisol in urine demonstrated ethyl acetate to be the optimal elution solvent. To gauge the efficacy of phospholipid removal, in addition to matrix effect and recovery, the polymeric resin was compared with traditional diatomaceous earth using cortisol and 6b-hydroxycortisol as test compounds.

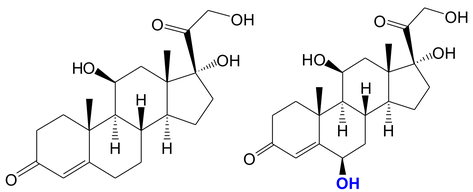

Figure 1. Structures of cortisol and its major metabolite, 6b-hydroxycortisol.

Methods

Sample Extraction

Human plasma or urine (200µL) was fortified with stable-label internal standard (50 µL) and mixed with 0.5 M Na_2CO_3 (200 µL). A 200 µL aliquot was loaded onto Phenomenex Novum™ or Biotage ISOLUTE® SLE 96-well plates (200 mg bed mass). Samples were allowed to soak for five minutes and then eluted with 1 mL of organic solvent. Elution was finalized by centrifugation, and extracts were evaporated (70 °C) with reconstitution in a mobile phase compatible solvent.

Chromatography and Detection

Cortisol (*m/z* 421 > 331) and 6b-hydroxycortisol (*m/z* 437 > 347) acetate adduct derived MRM transitions were monitored using a SCIEX API 5000 in negative ESI mode. Twelve phospholipid MRM transitions were monitored for the common fragment ion *m/z* 184 (Table 1) with a declustering potential of 60 V and a collision energy of 50 eV. Chromatographic separation was achieved using a Waters XBridge C_{18} column with an acidified MeOH/H_2O mobile phase. Different elution conditions were used for the cortisol and 6b-hydroxycortisol method and the semi-quantitative phospholipid method.

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

A previously validated SLE-LC-MS/MS method for cortisol and 6b-hydroxycortisol for urine was used as a starting point for the evaluation of SLE sorbent types, which included determining the extent of phospholipid removal, recovery and matrix effect.

Recovery was evaluated in one human urine lot and three plasma lots (including one lipemic and one hemolyzed) fortified with 250 ng/mL of cortisol and 1600 ng/mL 6 β -hydroxycortisol. Five elution solvents were screened, including methyl *tert*-butyl ether (MTBE), diethyl ether, ethyl acetate (EtAc), dichloromethane (DCM) and $CHCl_3$:MTBE (1:4). Recoveries obtained for both cortisol and 6b-hydroxycortisol were lower in urine and plasma for the polymeric sorbent compared to diatomaceous earth (Tables 2 and 3). Highest recoveries were achieved using ethyl acetate, consistent with previous work and the polarity of cortisol/ 6 β -hydroxycortisol. For cortisol, recovery between matrix donors ranged from 93 to 100% and 43 to 77% for ISOLUTE® and Novum™, respectively; recovery for 6b-hydroxycortisol ranged from 88 to 94% (ISOLUTE®) and 59 to 80% (Novum™).

Table 2. Cortisol recovery comparison between Isolute® and Novum™ sorbents

Cortisol Recovery								
Solvent	Urine		Plasma		Lipemic Plasma		Hemolysed Plasma	
	Isolute	Novum	Isolute	Novum	Isolute	Novum	Isolute	Novum
MTBE	81.3%	63.7%	78.5%	57.2%	89.3%	54.3%	78.5%	46.3%
Diethylether	85.0%	57.8%	51.4%	56.1%	55.8%	73.2%	48.2%	51.0%
EtAc	93.4%	73.8%	95.2%	66.5%	99.6%	77.1%	96.0%	43.0%
DCM	78.6%	54.1%	95.0%	26.6%	98.8%	38.3%	86.6%	29.0%
$CHCl_3$:MTBE 2:8	85.0%	59.4%	82.8%	72.4%	60.2%	65.3%	61.1%	51.3%

Table 3. 6b-Hydroxycortisol recovery comparison between Isolute® and Novum™ sorbents

6β-Hydroxycortisol Recovery								
Solvent	Urine		Plasma		Lipemic Plasma		Hemolysed Plasma	
	Isolute	Novum	Isolute	Novum	Isolute	Novum	Isolute	Novum
MTBE	86.9%	53.9%	71.2%	41.9%	65.9%	42.9%	64.4%	41.1%
Diethylether	37.2%	16.7%	22.7%	12.7%	22.3%	14.9%	21.4%	12.4%
EtAc	88.7%	70.9%	93.9%	76.4%	89.6%	79.7%	88.2%	58.9%
DCM	17.0%	3.5%	15.1%	2.4%	16.9%	3.1%	12.9%	2.0%
$CHCl_3$:MTBE 2:8	82.6%	41.0%	68.2%	36.0%	64.9%	35.5%	60.9%	29.4%

Table 4. Phospholipid depletion comparison between Isolute® and the Novum™.

Matrix	Solvent	Isolute			Novum			% Phospholipd Depletion Novum vs. Isolute
		Total LPC Area	Total PC Area	Total Phospho. Area	Total LPC Area	Total PC Area	Total Phospho. Area	
Plasma	MTBE	990	1933948	1934938	0	0	0	-100%
	Diethylether	4123	2532211	2536334	0	0	0	-100%
	EtAc	4268762	84089176	88357938	7603	1731572	1739175	-98%
	DCM	903	1324677	1325580	0	0	0	-100%
	$CHCl_3$:MTBE 2:8	91	1288	1379	0	0	0	-100%
Lipemic Plasma	MTBE	567436	24070969	24638405	0	0	0	-100%
	Diethylether	145055	14490571	14635626	0	0	0	-100%
	EtAc	5367173	102165695	107532868	0	181261	181324	-100%
	DCM	1725	1128368	1130092	0	0	0	-100%
	$CHCl_3$:MTBE 2:8	51	2406	2457	0	0	0	-100%
Hemolyzed Plasma	MTBE	441989	20391844	20833833	0	0	0	-100%
	Diethylether	269606	12465324	12734930	0	0	0	-100%
	EtAc	6708697	169646092	176354790	48786	7560112	7608898	-96%
	DCM	5108	10456624	10461733	0	4251	4331	-100%
	$CHCl_3$:MTBE 2:8	75	3071	3146	0	0	0	-100%

Results and Discussion (Continued)

Matrix Effect & Phospholipid Depletion

Chromatographic conditions for cortisol/6b-hydroxycortisol were optimized to separate multiple interfering peaks co-extracted from matrix. Under these conditions, ion suppression was not observed and all phospholipids were resolved from cortisol/6 β -hydroxycortisol (Figure 1). Nonetheless, for the majority of elution solvents examined, phospholipid profiling revealed a much higher trapping efficiency for these potential ion suppressors when using the Novum™ polymeric sorbent when compared to diatomaceous earth. Results obtained using a semi-quantitative phospholipid method (Figure 2) indicate that 96 - 100% of phospholipids were in fact depleted from Novum™ extracts (Table 4).

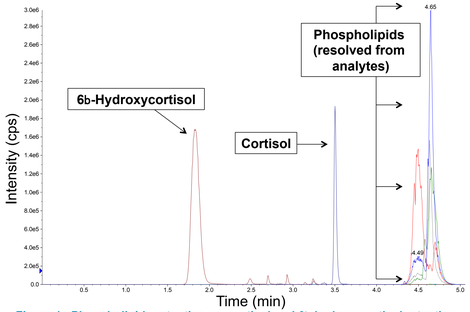

Figure 1. Phospholipids retention vs. cortisol and 6b-hydroxycortisol retention.

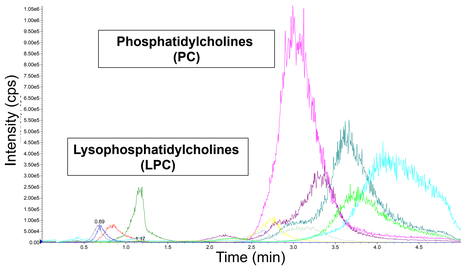

Figure 2. Exemplary chromatogram for the semi-quantitative assessment of phospholipid content in extracts. Note that elution conditions differed from those used for the separation of cortisol and 6b-hydroxycortisol.

Conclusion

Results demonstrate that while the diatomaceous-earth based SLE sorbent boasts higher recovery for cortisol and 6b-hydroxycortisol, the Novum™ polymeric sorbent is of potential benefit for applications requiring the elimination of phospholipids which otherwise might impact assay robustness due to ion suppression.