The use of mixed-mode SPE to minimize LC-MS matrix effects due to dosing vehicles

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Introduction

Sample preparation is essential when analyzing drugs in biological fluids, even when a selective detector such as MS-MS is used. Endogenous sample components (such as proteins or phospholipids), and exogenous compounds (for example drug dosing vehicles such as PEG 400 or Tween 80) are known to cause significant matrix effects (particularly ion suppression) in LC-MS analyses. Reduction of these effects is an important goal of the sample preparation procedure.

It has been suggested that improved sample clean-up[1] is an important approach to minimize matrix effects caused by polymeric dose vehicles.

This presentation describes the use of a simple, generic mixed-mode SPE procedure to minimize matrix effects caused by the presence of drug dosing vehicles. Data will be presented for a variety drugs, showing high, reproducible analyte recovery. Matrix effects will be investigated using post column infusion LC-MS/MS experiments.

Mixed-mode solid phase extraction

Mixed-mode SPE uses a dual retention mechanism to extract ionizable drugs from biological fluids. This approach allows a rigorous interference elution procedure to be used; selectively removing interfering compounds from the SPE column, prior to elution of drugs of interest (see **Figure 1**). Mixed-mode SPE significantly improves sample clean-up compared to SPE based on a single retention mechanism.

In this study we evaluate ISOLUTE® HCX, a silica-based mixed-mode sorbent consisting of non-polar (C8) and strong cation exchange functionality, for the ability to produce high recoveries of basic drugs from plasma, with minimal ion suppression from dose vehicles. Data is compared to that for a non-polar single extraction mode ISOLUTE C8 equivalent.

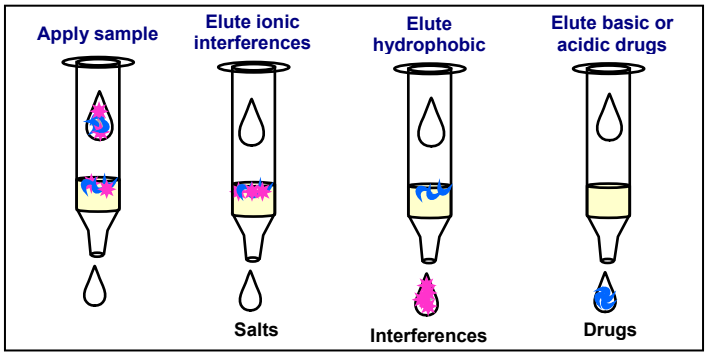

Figure 1. Representative procedure for mixed-mode SPE

Experimental

Sample preparation

Sample: Pooled human plasma
Recovery experiment: spiked at 5 ng/mL of 3 beta-blockers
Matrix effects experiment: spiked at 50 µg/mL with dose vehicle

SPE methods

Mixed-mode SPE (silica-based sorbent)

ISOLUTE Array HCX 25 mg/1 mL (p/n 902-0025-RP)
1. Condition each well with methanol (1 mL)
2. Equilibrate with NH_4OAc buffer (0.05M, pH 6, 250 µL)
3. Load plasma sample (100 µL diluted 1:1 (v/v) with 0.05M NH_4OAc buffer, pH 6)
4. Wash (i) NH_4OAc buffer (0.05 M, pH 6, 250 µL)
   (ii) acetic acid (1 M, 250 µL), dry for 30 s
   (iii) methanol (250 µL)
5. Elute with methanol:NH_4OH (95:5, v/v, 2 x 200 µL)
6. Evaporate and reconstitute in H_2O/MeOH (80/20, v/v, 200 µL)

Non-polar SPE ISOLUTE C8

ISOLUTE Array C8 25 mg/1 mL (290-0025-RP)
1. Condition each well with methanol (1 mL)
2. Equilibrate with NH_4OAc buffer (0.05M, pH 6, 250 µL)
3. Load plasma sample (100 µL diluted 1:1 (v/v) with 0.05M NH_4OAc buffer, pH 6)
4. Wash with 0.05M NH_4OAc buffer, pH 6:methanol (95:5, v/v, 1 mL)
5. Elute with MeOH/1 M NH_4OAc (99.5/0.5, v/v, 250 µL)
6. Evaporate and reconstitute in H_2O/MeOH (80/20, v/v, 200 µL)

LC Conditions

HPLC column: Zorbax Eclipse XDB-C18 (100 x 2.1 mm, 3.5 µm)
Guard column: Zorbax Eclipse XDB-C8. (12.5 x 2.1 mm, 5 µm) (Agilent)
Instrument: Waters 2795 Separations Module
Mobile phase: Isocratic 0.1% Formic acid(aq)/ACN (75/25, v/v)
Flow rate: 0.25 mL/min.
Injection volume: 25 µL
The entire column effluent was directed into the MS.

MS Conditions

Instrument: Waters Quattro Ultima Pt triple quadrupole equipped with an ESI source.
Source temp: 100°C
Desolvation Temp: 350°C
Collision cell pressure: 2.23 e^{-3} mbar

Analyte	MRM Transition	Collision Energy (eV)
Propranolol	260.1 > 116.1	17
Oxprenolol	266.2 > 72.1	18
Metoprolol	268.2 > 116.1	18

Post column infusion conditions: A 1µg/mL solution (methanol/water, 50:50 (v/v)) of each analyte was infused at flow rate of 5 µL/min.

1. Analyte Recovery

Analyte recovery from blank plasma spiked at 5 ng/mL with each beta-blocker was determined by LC-MS.

Results

SPE retention mechanism	Analyte recovery (rsd, n=6)		
	Metoprolol	Oxprenolol	Propranolol
ISOLUTE HCX	92 (7)	90 (8)	94 (13)
ISOLUTE C8	94 (8)	95 (5)	105 (6)

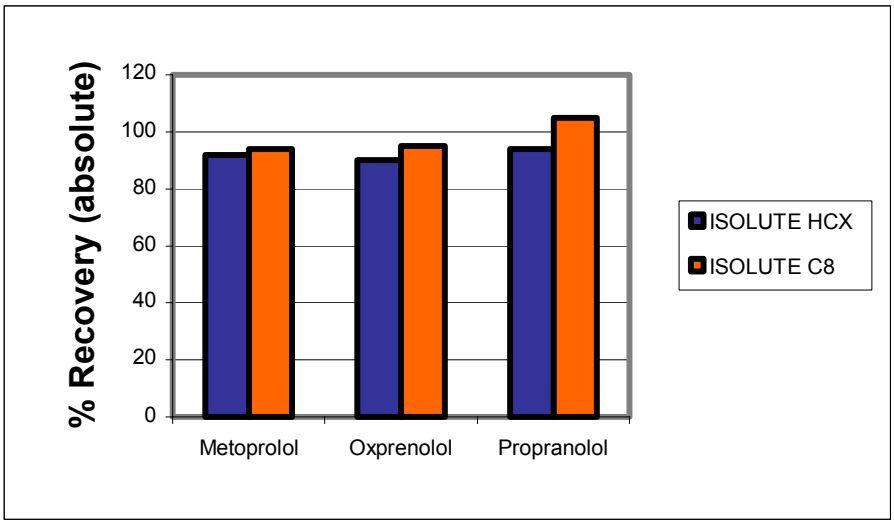

Figure 2. Recovery of beta blockers, using mixed-mode SPE (ISOLUTE HCX) and non-polar SPE (ISOLUTE C8)

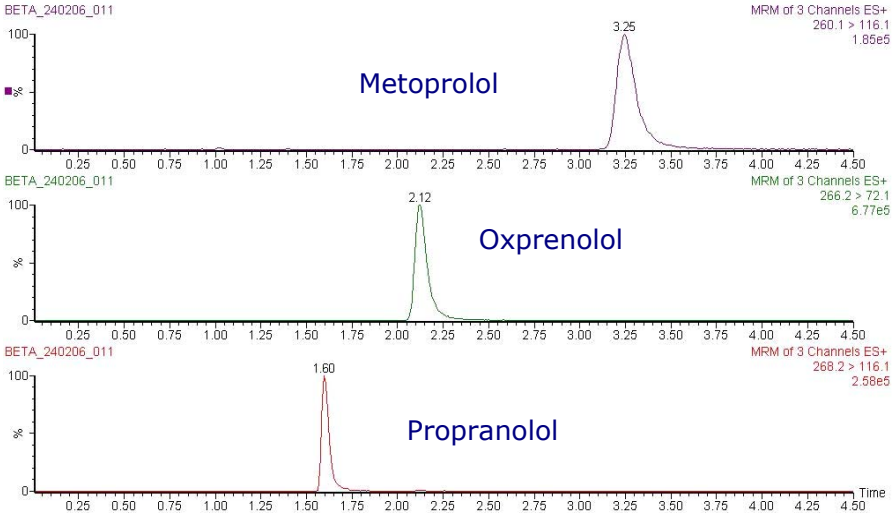

Figure 3: Mass chromatogram for three beta-blockers

2. Matrix Effects due to Dose Vehicles

In order to quantify the effects of different SPE retention mechanisms on the reduction of matrix effects in LC-MS, blank pooled plasma samples were spiked at 50 µg / mL with a range of dose vehicles. Samples were extracted as described in the experimental section using ISOLUTE HCX and ISOLUTE C8.

Samples were fortified after evaporation with beta-blockers (5 ng/mL equivalent), and analyte response compared to those achieved using standards to show suppression effects due to residual dose vehicles in the SPE extracts only.

Results

Dose vehicle	Ion suppression					
	Metoprolol		Oxprenolol		Propranolol	
	HCX	C8	HCX	C8	HCX	C8
Blank	12	12	8	-15	40	-6
PEG	31	76	1	17	27	17
Tween	52	77	35	44	25	16
Cremophore	-14	43	1	62	23	45
CMC	-12	3	-21	-23	18	-4
CH_3-C	-2	10	-14	-15	19	3

CMC = carboxymethylcellulose *CH_3-C = methyl cellulose*

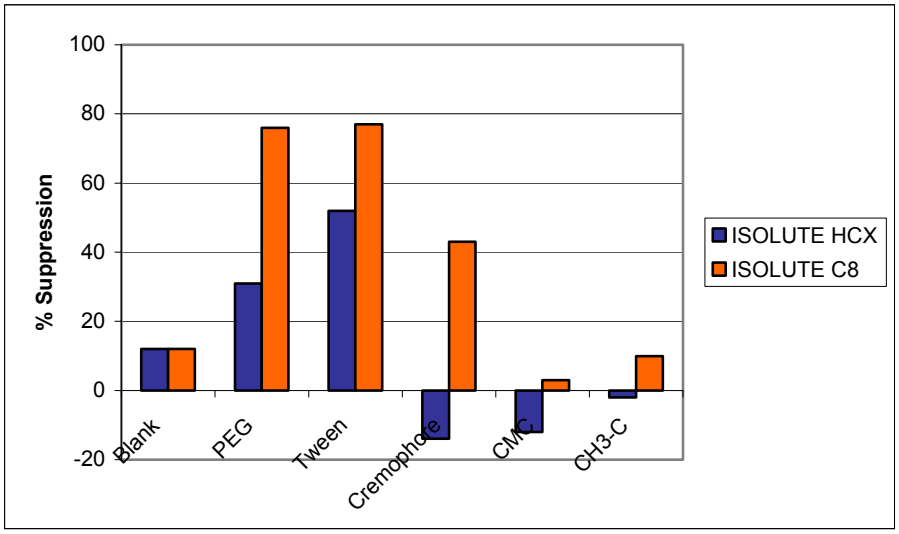

Figure 4. Comparison of ion suppression / enhancement for metoprolol from residual dose vehicles in plasma, when extracted using mixed mode (ISOLUTE HCX) and non-polar (ISOLUTE C8) SPE methods

Post-column infusion

Post-column infusion of beta blockers was then used to show the areas of suppression in the chromatogram due to various dose vehicles in plasma. **Figure 5** shows the relative suppression of metoprolol due to PEG, spiked at a concentration of 50 µg/mL in plasma and extracted with ISOLUTE HCX and ISOLUTE C8 respectively.

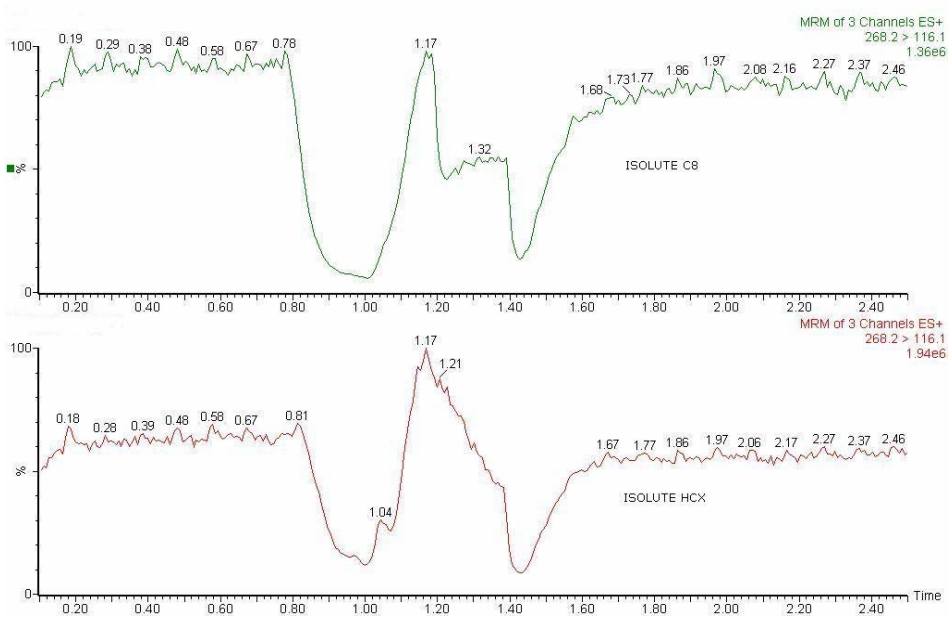

Figure 5. Plasma containing 50 µg/mL PEG (post column infusion of metoprolol)

Discussion

Ion suppression data for beta blockers generally shows less suppression / enhancement due to a range of dose vehicles using mixed-mode SPE (ISOLUTE HCX) compared to non-polar SPE (ISOLUTE C8). This is because dose vehicles are retained on the SPE column using non-polar retention mechanisms only, whereas basic analytes are retained by both non-polar and cation exchange interactions. Dose vehicles can therefore be selectively removed from ISOLUTE HCX using the interference elution regime (methanol wash) possible with mixed-mode SPE, without loss of basic analyte. A 100% methanol wash is not possible with ISOLUTE C8 (non-polar retention only) without loss of analyte.

Overall Conclusions

1. Both mixed-mode (ISOLUTE HCX) and non-polar (ISOLUTE C8) SPE provide high analyte recoveries with low RSDs.
2. In general, improved clean-up (reduced ion suppression/enhancement) can be achieved using mixed-mode SPE.

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