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Application Note SI-01013

Fractionation of Acidic, Neutral, and Basic Drugs from Plasma with Polymeric SPE cation exchange, Bond Elut™ Plexa™ PCX

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Introduction

Bioanalytical SPE has been dominated by polymeric sorbents in recent years. The ease-of-use, good flow, and resistance to effects of drying relative to silica-based sorbents make polymeric sorbents an obvious choice for high volume, high throughput assays requiring quick validation and minimal method development. Mixed mode polymers are often preferred among polymeric sorbents for basic drugs which take advantage of the cation exchange properties for an efficient extraction. In some drug studies the analyst may need to extract multiple drug classes in a single extract due to limited sample size. A mixed mode polymer is an effective way to analyze multiple drug classes in a single plasma sample. Acidic and neutral drugs can be retained on the hydrophobic portion while basic drugs interact with the sorbent's cation exchange properties. Each drug class can then be fractionated off the sorbent using organic solvents and changing the pH to elute the compounds of interest.

Bond Elut™ Plexa™ PCX is a new addition to the Plexa family and uses a mixed mode polymer cation exchange technique. This advanced SPE sorbent retains neutral and acidic compounds from biofluids via hydrophobic interactions and concentrates basic analytes due to ion-exchange capabilities. A single method is sufficient to fractionate different classes of compounds at high recoveries in clean extracts. Acidic and neutral compounds are eluted in a neutral fraction, while basic compounds elute in a basic fraction.

Plexa PCX significantly reduces ion suppression because its highly polar, hydroxylated surface is entirely amide-free. The particle exterior minimizes protein access to the pore structure and avoids strong binding of phospholipids ensuring reduced ion suppression. A simple method utilizing the new Plexa PCX was developed for the extraction of acidic, neutral and basic drugs in human plasma.

Materials and Methods

Table 1: SPE Reagents and Solutions

2% Phosphoric Acid	Add 20 μL of concentrated H_3PO_4 to 1 mL of DI water
Methanol	Reagent grade or better
2% Formic Acid	Add 20 μL of concentrated formic acid to 1 mL of DI water
Methanol:Acetonitrile (1:1, v/v)	Add 1 mL of methanol to 1 mL of acetonitrile
5% NH_3 Methanol:acetonitrile (1:1, v/v)	Add 50 μL of concentrated ammonia to 1 mL of methanol:acetonitrile (1:1, v/v)
Bond Elut Plexa 10 mg 96 well plate, (Varian Part no. A4969010)	

Table 2: SPE Method

Sample Pretreatment	100 μL human plasma. Dilute 1:3 w/ 2% H_3PO_4
Condition	1. 500 μL CH_3OH 2. 500 μL H_2O
Load	Sample with the drug mixture at the flow rate of 1 mL/min
Wash	500 μL 2% formic acid
Elution 1 (acids, neutral)	500 μL methanol:acetonitrile (1:1, v/v)
Elution 2 (bases)	500 μL 5% NH_3 methanol:acetonitrile (1:1, v/v)

All samples evaporated to dryness and reconstituted in 100 μL of 5mM ammonium formate (acids and neutrals), or 100 μL of 80:20 0.1% Aq formic acid: CH_3OH (bases). LC/MS performed on a Varian 320 instrument – ESI.

Results and Discussion

Acids

LC conditions – Acids and Neutrals

Mobile Phase: A: 5 mM Ammonium Formate
B: Methanol

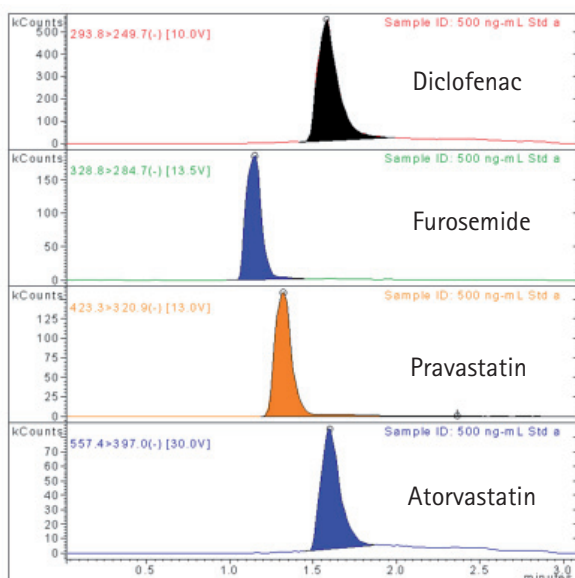
Gradient: t = 0 min 60% A: 40% B
t = 0 – 1:0 min 20% A: 80% B
t = 2:00 – 3:0 min 60% A: 40% B

Column: Pursuit C18 3 μ 50 x 2.0 mm
(Varian Part no. A3051050X020)

MS conditions - Acids

Compound	Q1	Q3	CE
Atorvastatin	557.4	397.0	30.0V
Diclofenac	293.8	249.7	10.0V
Furosemide	328.8	284.7	13.5V
Pravastatin	423.3	320.9	13.0V

Capillary = 80 V, Dry gas Temp = 350 °C, 30 psi, CID = Argon
Pol: Negative



Chromatograms of a 50 ng/mL extract

Acid analytes are retained on Plexa™ PCX via hydrophobic interaction at a pH below their pKa values. The Limit of Detection (LOD) of the combined solid phase extraction and LC-MS-MS analysis was 1.0 ng/mL. Recoveries were calculated from a 1st order regression with RSD values based on a sampling of n = 6. Excellent absolute recoveries were achieved demonstrating good retention and elution, as well as minimal ion suppression. Response for all the compounds evaluated was linear up to 3 orders of magnitude from 1.0 ng/mL to 5.0 μ g/mL with correlation coefficients all above 0.999. To demonstrate reproducibility, samples were analyzed at two concentrations (n = 6). As shown in Table 3, the described generic SPE protocol yields reproducibly high recoveries.

Table 3: Analyte Relative Recoveries – Acids

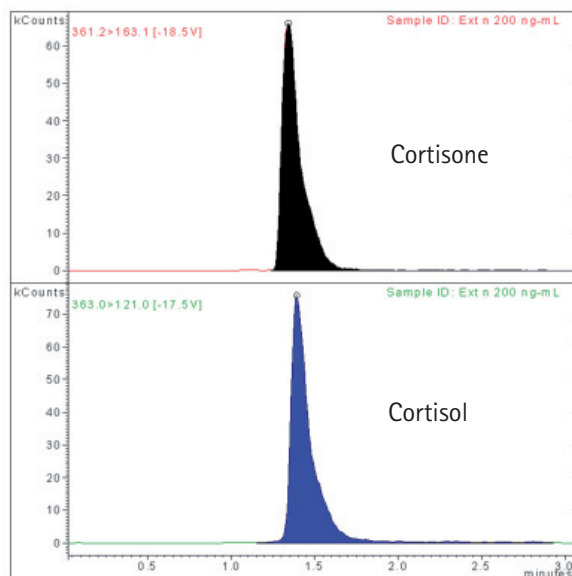
			0.5 μ g/mL		1.0 μ g/mL	
	log P	pKa	Rec %	RSD	Rec %	RSD
Diclofenac	4.2	4.2	101	4	103	6
Furosemide	1.2	3.9	104	3	96	2
Pravastatin	2.6	4.7	95	4	106	6
Atorvastatin	6.3	4.5	100	4	103	5

Neutrals

MS conditions - Neutrals

Compound	Q1	Q3	CE
Cortisone	361.2	163.1	-18.5V
Cortisol	363.2	121.0	-17.5V

Capillary = 80 V, Dry gas Temp = 350 °C, 30 psi, CID = Argon
Pol: Positive



Chromatograms of a 50 ng/mL extract

Neutral compounds have a similar retention behavior as non-dissociated acid compounds and are therefore eluted in the neutral fraction. The Limit of Detection (LOD) of the combined solid phase extraction and LC-MS-MS analysis was 1.0 ng/mL. Recoveries were calculated from a 2nd order regression with RSD values based on a sampling of n = 6. Excellent absolute recoveries were achieved demonstrating good retention and elution, as well as minimal ion suppression. Response for all the compounds evaluated was linear up to 3 orders of magnitude from 1.0 ng/mL to 5.0 μ g/mL with correlation coefficients all above 0.998. To demonstrate reproducibility, samples were analyzed at two concentrations (n = 6). As shown in Table 4, the extractions according to the generic protocol with Plexa PCX produced reproducibly high recoveries.

Table 4: Analyte Relative Recoveries – Neutrals

			0.5 μ g/mL		1.0 μ g/mL	
	log P	pKa	Rec %	RSD	Rec %	RSD
Cortisone	1.5	N/A	93	4	97	6
Cortisol	1.5	N/A	101	4	101	4

Bases

LC conditions – Bases

Mobile Phase: A: 0.1% Formic Acid
B: Methanol

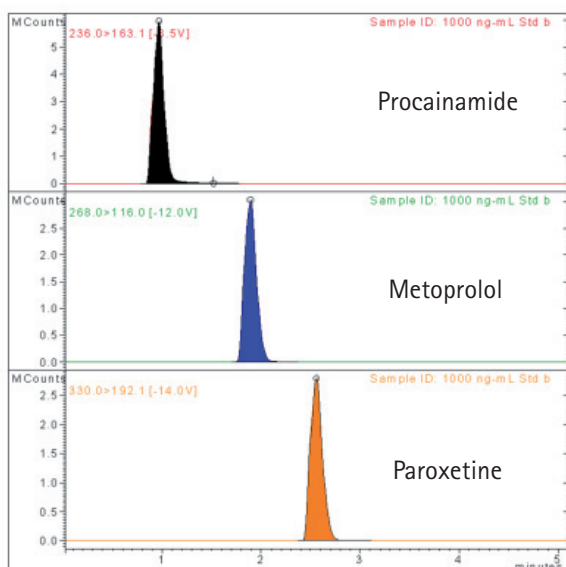
Gradient
t = 0 min 80% A : 20% B
t = 0 – 2:0 min 20% A : 80% B
t = 3:30 – 5:0 min 80% A : 20% B

Column: Pursuit C18 3 μ 50 x 2.0 mm
(Varian Part no. A3051050X020)

MS conditions - Bases

Compound	Q1	Q3	CE
Procainamide	236.0	163.1	-8.5V
Metoprolol	268.0	116.0	-12.0V
Paroxetine	330.0	192.1	-14.0V

Capillary = 25 V, Dry gas Temp = 400 °C, 30 psi, CID = Argon
Pol: Positive



Chromatograms of a 50 ng/mL extract

Basic analytes from human plasma samples are retained by the cation exchange interactions with the sorbent and elute separately utilizing an ammoniated solvent system. The Limit of Detection (LOD) of the combined solid phase extraction and LC-MS-MS analysis was 1.0 ng/mL. Recoveries were calculated from a 2nd order regression with RSD values based on a sampling of n = 6. Excellent absolute recoveries were achieved demonstrating good retention and elution, as well as minimal ion suppression. Response for all the compounds evaluated was linear up to 3 orders of magnitude from

1.0 ng/mL to 5.0 μ g/mL with correlation coefficients all above 0.999. To demonstrate reproducibility, samples were analyzed at two different concentrations (n = 6). As shown in Table 5, reproducibly high recoveries were obtained according to the generic standard protocol.

Table 5: Analyte Relative Recoveries – Bases

			0.5 μ g/mL		1.0 μ g/mL	
	log P	pKa	Rec %	RSD	Rec %	RSD
Procainamide	1.3	9.2	100	5	98	3
Metoprolol	1.9	9.6	94	4	92	6
Paroxetine	3.4	9.9	94	5	99	4

Conclusions

With Bond Elut™ Plexa™ PCX, a generic protocol for drug extraction from plasma can be applied to analytes which belong to different chemical classes of drugs. Under acidic conditions, charged basic analytes bind to the cation exchange groups of the sorbent whereas the neutralized acidic and neutral compounds are retained in the more hydrophobic center of the polymer bead. As the non-polar retention mode in SPE is less selective than ion exchange, the polar interferences and proteins as well as ion suppression effects in LCMS analysis must be minimized by a wash step with an acidic, aqueous solution. An elution with 50% methanol:acetonitrile is sufficient to achieve high recoveries and a clean extract for the acidic and neutral compounds. Finally, a mixture of organic solvents with ammonia is used to disrupt the cation exchange interaction, resulting in the elution of the basic drugs.

Plexa PCX particles have much narrower particle size distribution creating more consistent interstitial paths. The consistent Plexa particle size results in superior flow characteristic across the 96-well plate and excellent well-to-well reproducibility. Automated 96-well technology is simplified opening new opportunities to maximize efficiency. Bond Elut Plexa PCX is a useful tool for high-throughput SPE applications which require analysis at low concentration levels, validated reproducibility, and quick implementation. Minimal method development is needed with a wide range of different compounds. Plexa PCX is highly recommended for multiple compounds in bioanalytical work and systematic toxicological analysis.

These data represent typical results.

For further information, contact your local Varian Sales Office.

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