



# VARIAN

## Extraction of Polar Basic Drugs from Plasma with Polymeric SPE Cation Exchange, Bond Elut™ Plexa™ PCX

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### Introduction

Basic pharmaceutical drugs are ideal for a cation exchange sorbent. Analytes are easily charged in an acidic solution and readily interact with the ion exchange function of the sorbent. Polar basic compounds can be problematic for reverse phase sorbents due to their poor hydrophobic interaction and water solubility.

Bond Elut™ Plexa™ PCX is a new addition to the Plexa family and uses a polymeric cation exchange technique. Plexa PCX uses a generic and simplified method to remove neutral and acidic interferences from the matrix and concentrate basic analytes, resulting in improved analytical performance and sensitivity in the quantification of basic compounds.

In addition, Plexa PCX offers faster and highly reproducible flow rates, resulting in excellent tube-to-tube and well-to-well performance. Plexa PCX significantly reduces ion suppression because its highly polar, hydroxylated surface is entirely amide-free. The particle exterior minimizes strong binding of proteins and phospholipids. Efficient removal of phospholipids from plasma is ensured. A simple generic method was developed for the extraction of polar basic drugs in human plasma.

### Materials and Methods

Table 1. SPE Reagents and Solutions

A Bond Elut Plexa 10 mg 96 well plate (PN A4969010) has been used for the solid phase extraction of the target compounds.

2% Phosphoric acid	Add 20 µL of concentrated H <sub>3</sub> PO <sub>4</sub> 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 <sub>3</sub> in Methanol: Acetonitrile (1:1, v/v)	Add 50 µL of concentrated ammonia to 1 mL of methanol: acetonitrile (1:1, v/v)

Table 2. SPE Method

Sample pre-treatment	Spike 100 µL human plasma with basic drugs and internal standard. Then dilute 1:3 with 2% H <sub>3</sub> PO <sub>4</sub>
Condition	1. 500 µL CH <sub>3</sub> OH 2. 500 µL DI H <sub>2</sub> O
Load	Sample containing basic drugs at a flow rate of 1 mL/min
Wash 1 Wash 2	500 µL 2% formic acid 500 µL acetonitrile/methanol (1:1, v/v)
Elution	500 µL 5% NH <sub>3</sub> methanol: acetonitrile (1:1, v/v)

All samples are evaporated to dryness and reconstituted in 100 µL of 80:20 0.1% aq. Formic acid:CH<sub>3</sub>OH.

LC/MS is performed on a Varian 320-MS LC/Triple Quadrupole mass spectrometer - ESI.

### LC Conditions

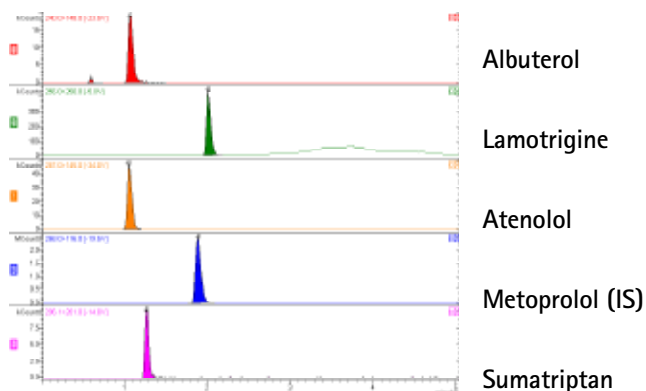
Mobile Phase	A. 0.1% Formic acid B. Methanol
Gradient:	t = 0 min                      80% A : 20% B t = 0-2:0 min                20% A : 80% B t = 3:30-5:0 min             80% A : 20% B
Column:	Pursuit C18 3 µm 50 x 2.0 mm (PN A3051050X020)

Table 3. MS Conditions

Transition ions and collision energy were:

Compound	Q1	Q3	CE [V]
Albuterol	240.1	148.0	-23.5
Lamotrigine	256.0	256.0	-5.0
Atenolol	267.0	145.0	-34.0
Sumatriptan	296.1	201.1	-14.0

Capillary voltage at 25 V; Drying gas temperature 400 °C and 30 psi; CID=Argon; ESI:Positive.



Chromatograms of a 50 ng/mL extract.

### Results and Discussion

This LC/MS method describes the quantitative determination of polar basic compounds in human plasma using Bond Elut Plexa PCX for SPE. The Limit of Detection (LOD) of the 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 recoveries were achieved which demonstrated 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 1.0 µg/mL with correlation coefficients all above 0.999. To demonstrate reproducibility, samples were analyzed at two different concentrations (n = 6). As shown in Table 4, reproducibly high recoveries were obtained according to the generic standard protocol.

Table 4. Recoveries of Polar Basic Compounds from Human Plasma

Analyte	pK <sub>a</sub>	logP	% Recovery <sup>1</sup> (500 ng/mL)	% RSD <sup>2</sup>	% Recovery <sup>1</sup> (1000 ng/mL)	% RSD <sup>2</sup>
Sumatriptan	9.6	0.96	95	5	97	4
Atenolol	9.6	1.30	94	3	91	2
Albuterol	10.3	1.30	95	5	100	7
Lamotrigine	5.7	1.50	92	3	97	4

<sup>1</sup>Recoveries calculated as % of signal intensity of an extracted sample compared to that of the calibration curve. <sup>2</sup>RSD = standard deviation/average recovery x 100; n = 6.

### Conclusion

With Bond Elut Plexa PCX, a generic drug extraction protocol from plasma can be applied to polar analytes with basic amino functional groups. Under acidic conditions, the charged analyte binds to the cation exchange groups of the sorbent (see Table 4 for pK<sub>a</sub>). Polar interferences and proteins are washed away with an acidic, aqueous solution. A neutral wash with relatively strong solvents, such as 50% methanol:acetonitrile, is possible without any loss of analyte. The wash elutes neutral compounds retained in the hydrophobic cores of the sorbent. Finally, a mixture of organic solvents with ammonia is used to disrupt the cation exchange interaction, resulting in the elution of the basic drugs.

Flow rate all over the 96 well plate is fast because Plexa PCX particles have much narrower particle size distribution with no fines to cause blockages, thus resulting in excellent well-to-well reproducibility. Automated 96 well technology is easily possible, which opens up new opportunities to maximize efficiency. Bond Elut Plexa PCX is therefore a useful tool for high throughput SPE applications which require analysis at low analyte levels, validated reproducibility, and quick implementation, with minimal method development. It is therefore highly recommended for bioanalytical work in pharmaceutical clinical trials, including contract research.

*These data represent typical results.*

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