Extraction of Basic Drugs from Plasma with Polymeric SPE

Application Note

Pharmaceuticals

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Introduction

Bioanalytical solid phase extraction (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.

Because the method validation process is time consuming and requires high quality data, SPE methods that are fast, yet produce good recoveries with high reproducibility, are desirable. To the extent that the SPE process is streamlined without compromising data integrity, method validation can be simplified and shortened. Bond Elut Plexa minimizes method development with simple and effective methods and improves analytical sensitivity and reproducibility with an advanced polymeric structure that minimizes binding of large biomolecules to the surface, with the end result of simplifying and streamlining the SPE process.

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Materials and Methods

SPE reagents and solutions

2% ammonium hydroxide	Add 20 µL concentrated ammonium hydroxide to 1 mL DI H_2O
Methanol	Reagent grade or better
5% methanol	Add 5 mL methanol to 95 mL DI H_2O
Bond Elut Plexa	10 mg 96 well plate (p/n A4969010)

SPE method

Sample	100 µL human plasma
Pretreat	Dilute with 300 µL 2% NH_4OH
Condition	1. 500 µL CH_3OH 2. 500 µL H_2O
Wash	500 µL 5% CH_3OH in H_2O
Elute	500 µL CH_3OH

All samples evaporated to dryness and reconstituted in 100 µL of 80:20 0.1% formic acid: CH_3OH aq.

LC/MS performed – ESI, drying gas @ 400 °C, 30 psi

LC conditions

Mobile phase

A	0.1% Formic acid
B	Methanol

LC gradient program

Time (min)	%B
0:00	40
0:15	40
1:00	80
3:00	80
4:30	40

Column

Type	Pursuit XRs C18 3 µm, 50 × 2.0 mm (p/n A3001050X020)
Flow rate	0.2 mL/min

Results and Discussion

The procedure described provides a simple and effective SPE method for the extraction of basic or neutral drugs from human plasma. The Limit of Quantitation (LOQ) of the combined SPE and LC/MS/MS analysis was 1.0 ng/mL. The internal standard for the application was 50 ng/mL quetiapine.

Recoveries were calculated from a second order regression with RSD values based on a sampling of n = 6. Excellent recoveries were achieved demonstrating good retention and elution, as well as minimal ion suppression. Response for all the compounds evaluated was linear up to three orders of magnitude from 1.0 ng/mL to 1.0 µg/mL with correlation coefficients all above 0.995 (n = 6). To demonstrate reproducibility, samples were analyzed at two concentrations (n = 6). Figure 1 shows the chromatograms of the extractions at 100 ng/mL. As shown in Table 1, the extractions produced reproducibly high recoveries.

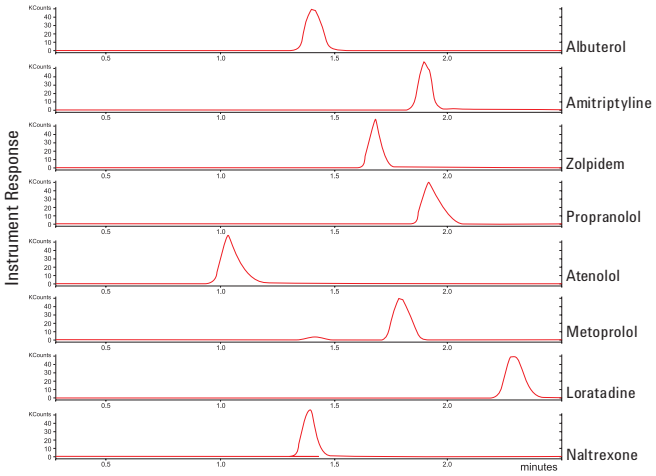

Figure 1. Chromatograms of a 100 ng/mL human plasma extract.

Table 1. High Recoveries of Basic Drugs with Bond Elut Plexa

Drug	log P	pKa	0.5 µg/mL %Recovery	%RSD	1.0 µg/mL %Recovery	%RSD
Albuterol	1.3	10.3	95	5	100	2
Amitriptyline	4.6	9.4	100	10	100	4
Zolpidem	3.9	6.2	100	8	103	2
Propranolol	3.6	9.5	102	6	101	6
Atenolol	1.3	9.6	97	4	101	4
Metoprolol	1.3	10.8	100	5	100	5
Loratadine	5.2	4.9	97	5	95	3
Naltrexone	1.8	9.2	103	11	100	4

Conclusions

Bond Elut Plexa is a useful tool for high-throughput SPE applications that require analysis at low analyte levels, need validated reproducibility, and must be quickly implemented with minimal method development. A single method for basic analytes covers a broad range of analyte polarites and delivers reproducibly high recoveries. Bond Elut Plexa is therefore highly recommended for bioanalytical work in pharmaceutical clinical research trials, including contract research.

For More Information

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