Extraction of Acidic Drugs from Plasma with Polymeric SPE

Application Note

Pharmaceuticals

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Introduction

Acidic drug extraction from biofluids often poses unique challenges for the bioanalytical chemist. While basic drugs are routinely extracted by means of cation exchange solid phase extraction (SPE), the related approach for acids (anion exchange) often fails. The reason is that naturally occurring ions (phosphate, citrate, various sulfates, and other larger anions) present in blood and other biofluids, are likely to retain on anion-exchange sorbents and interfere with extraction of acidic analytes. This effect is less pronounced in cation exchange SPE of basic analytes because endogenous cations are typically limited to Group 1 and 2 metals such as sodium and potassium, which are considerably smaller, more polar, and therefore less likely to retain by ion exchange, or interfere in the extraction.

An alternative to anion exchange of acidic analytes is a nonpolar extraction. For optimal extraction using this retention mode, the analytes should be neutralized (protonated) at the SPE load step by applying the sample under acidic conditions.

Because the nonpolar retention mode in SPE is less selective than ion-exchange, the possibility of interferences and ion suppression effects in LC/MS analysis should be considered for these types of extractions.

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Bond Elut Plexa, a unique polymeric SPE phase, is an alternative for the extraction of acidic analytes. A gradient of polarity on the polymer surface shunts small analytes, including neutralized acids, to the more hydrophobic center of the polymer bead where they are retained. Because the particle surface is highly polar and entirely amide-free, binding of proteins on the polymer surface is minimized, resulting in cleaner samples and reduced ion suppression. The procedure described here provides a simple and effective SPE method for the extraction of acidic drugs from human plasma.

Materials and Methods

SPE reagents and solutions

1% formic acid	Add 10 µL concentrated formic acid 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 1% formic acid
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 5 mM ammonium formate: CH_3OH.

LC/MS performed – ESI, drying gas @ 250 °C, 25 psi in negative ionization mode

LC conditions

Mobile phase

A	5 mM ammonium formate
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 A6001050X020)
Flow rate	0.2 mL/min

Results and Discussion

The Limit of Quantitation (LOQ) of the combined SPE and LC/MS/MS analysis was 5.0 ng/mL. The internal standard for the application was 100 ng/mL naproxen. Recoveries were calculated from a second order regression with RSD values based on a sampling of n = 6. Excellent recoveries were achieved (Table 1), demonstrating good retention and elution, as well as minimal ion suppression. Response for all compounds evaluated was linear up to 3 orders of magnitude from 5.0 ng/mL to 5.0 µg/mL with correlation coefficients all above 0.995. To demonstrate reproducibility, samples were analyzed at two concentrations (n = 6 at each concentration). Figure 1 shows the chromatograms of the extracts at 50 ng/mL. As shown in Table 1, the extractions produced reproducibly high recoveries.

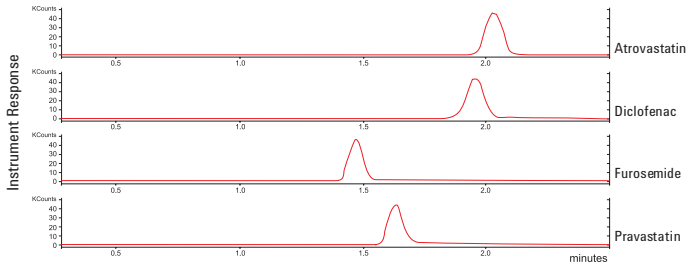

Figure 1. Chromatograms of a 50 ng/mL human plasma extract.

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

Drug	log P	pKa	2 µg/mL %Recovery	%RSD	5 µg/mL %Recovery	%RSD	R^{2*} 5.0 ng/mL to 5000 ng/mL
Atorvastatin	6.3	4.5	91	10	100	9	0.9967
Diclofenac	4.2	4.2	97	6	100	5	0.9995
Furosemide	1.5	4.7	95	5	100	2	0.9983
Pravastatin	2.6	4.6	95	8	100	7	0.9986

* Second-order regression used to calculate correlation coefficient (R^2)

Conclusions

As shown in Figure 1 and Table 1, extraction of acidic drugs on the general-purpose SPE product Bond Elut Plexa provides a viable alternative to mixed-mode and other complicated ion exchange sorbents. Using a simple method with no buffers in the eluant, good recoveries with high reproducibility are achieved for a variety of acidic compounds spanning a range of polarities from log P 1.5 to 6.3. Improved analytical sensitivity and reproducibility arise from the performance features built directly into the polymeric sorbent, so the SPE methodology can remain simple. Bond Elut Plexa is recommended for high-throughput assays where method development time must be minimized without compromising data quality or reproducibility.

For More Information

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Printed in the USA
March 21, 2011
5990-7684EN

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