Extraction of Acidic Compounds From Human Plasma Using Plexa PAX

Application Note

Drug Discovery

Author

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Introduction

A significant number of bioanalytical compounds in the pharmaceutical industry are basic and can easily be extracted using hydrophobic or cation exchange sorbents. However, lipid lowering statins and anti-inflammatory drugs tend to be acidic in nature. These compounds can be problematic using traditional hydrophobic exchange sorbents. A new suitable polymeric strong anion exchange material is now available.

A simple generic method was developed for acidic compounds using Plexa PAX. Method performance was evaluated by measuring analyte recoveries from the sorbent bed, linearity of extracted sample responses, and accuracy and precision of spiked plasma samples.

Because the method validation process is time consuming and requires high quality data, Solid Phase Extraction (SPE) methods that are fast, while producing good recoveries with high reproducibility are desirable. Method validation can be simplified and shortened to the extent that the SPE process is streamlined without compromising data integrity. The Bond Elut Plexa family minimizes method development with general-purpose methods and improves analytical sensitivity and reproducibility.

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Materials and Methods

SPE Reagents and Solutions

2% NH_4OH	Add 20 µL of concentrated (28-30%) NH_4OH to 1 mL of DI water
5% Formic Acid MeOH	Add 0.050 mL of formic acid to 1 mL of methanol

Plexa PAX 30 mg 96 well plate (p/n A4967030)

SPE Method

	Plexa PAX (30 mg)
Sample	100 µL human plasma
Pretreatment	Dilute 1:3 with 2% NH_4OH
Conditioning	1. 500 µL MeOH 2. 500 µL H_2O
Washes	1. 500 µL H_2O 2. 500 µL MeOH
Elution	500 µL 5% Formic Acid MeOH

All samples evaporated to dryness and reconstituted in 100 µL of 80:20 5 mM aqueous ammonium formate: CH_3OH.

LC/MS performed on an Agilent 325 LC-MS/MS – vESI,

LC conditions

Mobile Phase	A: 5 mM Ammonium Formate B: Methanol
Gradient	t = 0 - 50% A : 50% B t = 2:0 - 2:59 min 20% A : 90% B t = 3:0 - 4:00 min 50% A : 50% B
Column	Pursuit XRs Diphenyl 2.0 × 50 mm, 3 µm

MS conditions

Compound	LogP	PKa
Atorvastatin	5.7	4.5
Diclofenac	4.2	4.2
Furosemide	1.5	4.7
Ketoprofen	3.2	5.2
Pravastatin	2.6	4.6

Compound	Q1	Q3	CE
Acetylsalicylic acid	136.0	136.0	5.0 V
Atorvastatin	557.4	397.0	30.0 V
Diclofenac	293.7	249.6	10.5 V
Furosemide	328.8	284.7	13.0 V
Ketoprofen	252.7	208.7	7.0 V
Pravastatin	423.3	320.1	13.0 V

Capillary	41 V
Dry gas Temp	285 °C, 30 psi
Vortex Gas	300 °C, 20 psi
Nebulizing Gas	50 psi
CID	Argon
Pol	Negative

Results and Discussion

Analyte recoveries

Analyte recoveries were determined by measuring analyte response compared to a spiked mobile phase standard. Any loss of analyte due to extraction inefficiency, ion suppression, or non-elution is measured in the recovery. Despite some of the recoveries being in the 50 to 60% range the responses are linear as demonstrated by the good curve linearity and accuracy of the method. Reproducible recoveries were achieved at better than 50% with RSDs less than 7% (Table 1).

Good linearity is achieved from 0.5 ng/mL to 200 ng/mL (Figure 2). A midpoint concentration of 50 ng/mL was chosen to measure the extract accuracy of the calibration curve (Table 2).

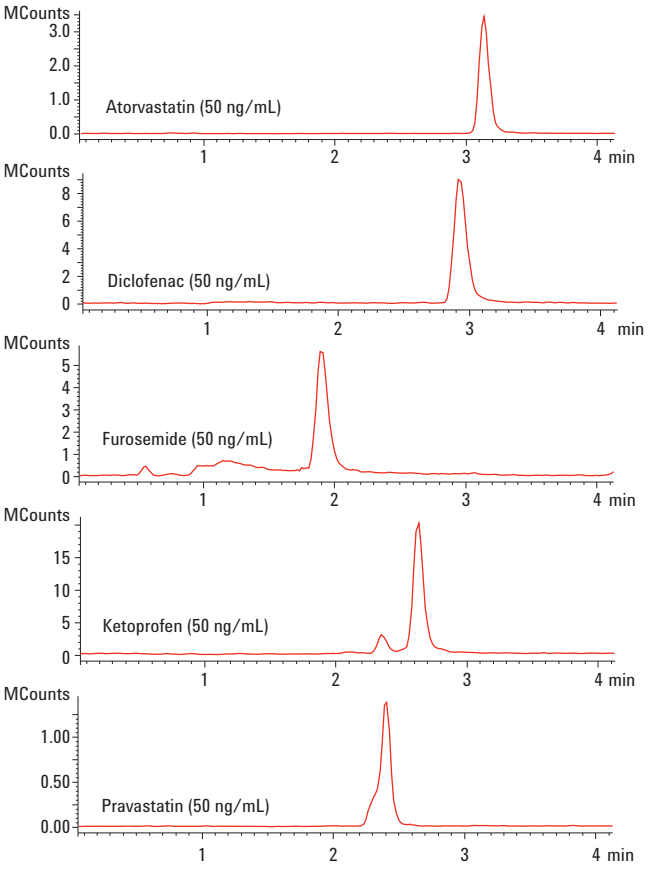

Figure 1. Sample chromatograms of 50 ng/mL extracts from human plasma.

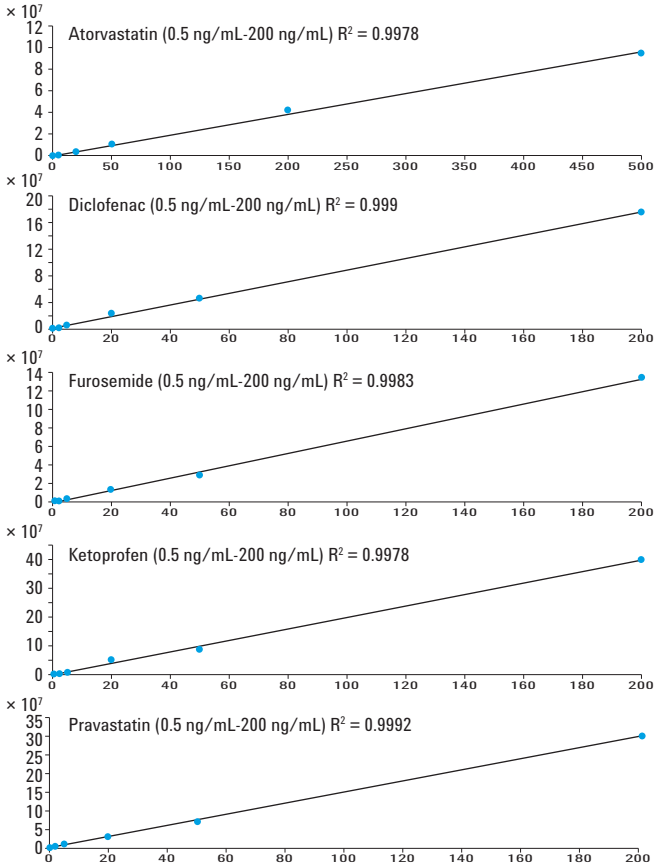

Figure 2. Calibration curves.

Table 1. Analyte Recoveries

	PAX % Rec	RSD (n=6)
Atrovastatin	62%	4.4%
Diclofenac	52%	3.7%
Furosemide	96%	6.1%
Ketoprofen	67%	2.3%
Pravastatin	95%	3.4%

Table 2. Accuracies

	0.5 ng/mL Accuracy	RSD (n=8)	50 ng/mL Accuracy	RSD (n=8)
Atorvastatin	90%	11%	106%	11%
Diclofenac	116%	14%	109%	6%
Furosemide	99%	14%	95%	9%
Ketoprofen	104%	13%	108%	4%
Pravastatin	108%	9%	96%	8%

Conclusion

A simple, easy, method for extracting acidic compounds using Plexa PAX was developed. Good recoveries for all compounds was achieved and these recoveries were linear over a range of 0.5 – 200 ng/mL in human plasma. The calibration was linear achieving correlation coefficients better than 0.995 for a single order regression. The method demonstrated accuracy and reproducibility with most results within 10% of the true value and RSDs below 15% for all compounds.

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