# Fractionation of Acidic, Neutral and Basic Drugs from Plasma with Polymeric SPE Cation Exchange, and an Agilent Bond Elut Plexa PCX

Application Note

BioPharma


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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 fractioned off the sorbent using organic solvents and changing the pH to elute the compounds of interest.

Agilent Bond Elut Plexa PCX is a member of the Agilent Plexa family of polymeric SPE products 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 Plexa PCX was developed for the extraction of acidic, neutral and basic drugs in human plasma.


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# Materials and Methods

Table 1. SPE reagents and solutions.

| Reagents | 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) |
| Agilent Bond Elut Plexa 10 mg 96 well plate (p/n A4968010) | |

Table 2. SPE method.

| | |
|---|---|
| Sample Pretreatment | 100 µL human plasma. Dilute 1:3 with 2% $H_3PO_4$ |
| Condition | 1. 500 µL $CH_3OH$<br>2. 500 µL DI $H_2O$ |
| 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) |

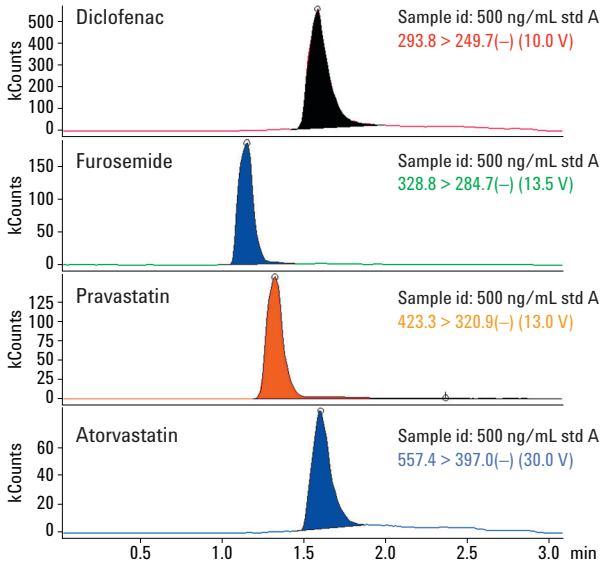
All samples evaporated to dryness and reconstituted in 100 µL of 5 mM ammonium formate (acids and neutrals), or 100 µL of 80:20 0.1% aqueous formic acid: $CH_3OH$ (bases).

# Results and Discussion

## Acids

| LC conditions | Acids and neutrals | |
|---|---|---|
| Mobile phase: | A: 5 mM Ammonium Formate<br>B: Methanol | |
| Gradient: | t = 0 min<br>t = 0-1 min<br>t = 2-3 min | 60% A: 40% B<br>20% A: 80% B<br>60% A: 40% B |
| Column: | Agilent Pursuit C18,<br>2.0 × 50 mm, 3 µm (p/n A3051050X020) | |

| MS conditions | Acids | | |
|---|---|---|---|
| Compound | Q1 | Q3 | CE |
| Atorvastatin | 557.4 | 397.0 | 30.0 V |
| Diclofenac | 293.8 | 249.7 | 10.0 V |
| Furosemide | 328.8 | 284.7 | 13.5 V |
| Pravastatin | 423.3 | 320.9 | 13.0 V |
| Capillary: | 80 V | | |
| Dry gas temperature: | 350 °C, 30 psi | | |
| CID: | Argon | | |
| Polarity: | Negative | | |




Chromatograms of a 50 ng/mL extract.

Acid analytes are retained on Plexa PCX through 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 first 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 three orders of magnitude from 1.0 ng/mL to 5.0 µg/mL with all correlation coefficients above 0.999.

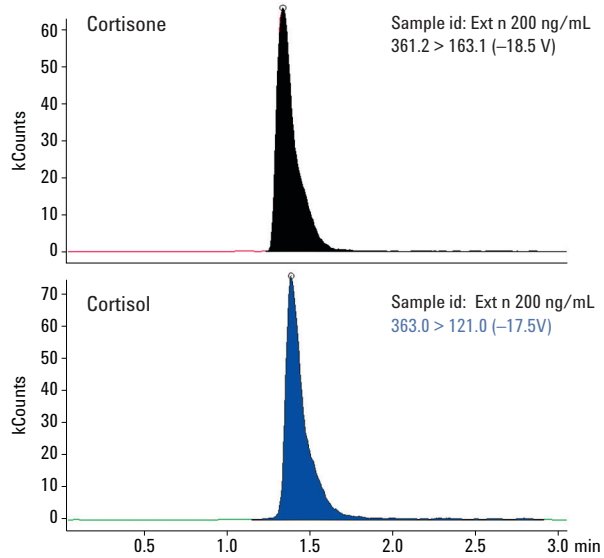
To demonstrate reproducibility, samples were analyzed at two concentrations (n = 6). Table 3 shows that the described generic SPE protocol yields reproducible, high recoveries.

Table 3. Analyte relative recoveries, acids.

| | | | 0.5 µg/mL | | 1.0 µg/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 | -18.5 V |
| Cortisol: | 363.2 | 121.0 | -17.5 V |
| Capillary: | 80 V | | |
| Dry gas temperature: | 350 °C, 30 psi | | |
| CID: | Argon | | |
| Polarity: | Positive | | |




Chromatograms of a 50 ng/mL extract.

Neutral compounds have a similar retention behavior as nondissociated acid compounds and are, therefore, eluted in the neutral fraction. The LOD of the combined solid phase extraction and LC/MS/MS analysis was 1.0 ng/mL. Recoveries were calculated from a second 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 three orders of magnitude from 1.0 ng/mL to 5.0 µg/mL with all correlation coefficients above 0.998.

To demonstrate reproducibility, samples were analyzed at two concentrations (n = 6). Table 4 shows that the extractions according to the generic protocol with Plexa PCX produced reproducible high recoveries.

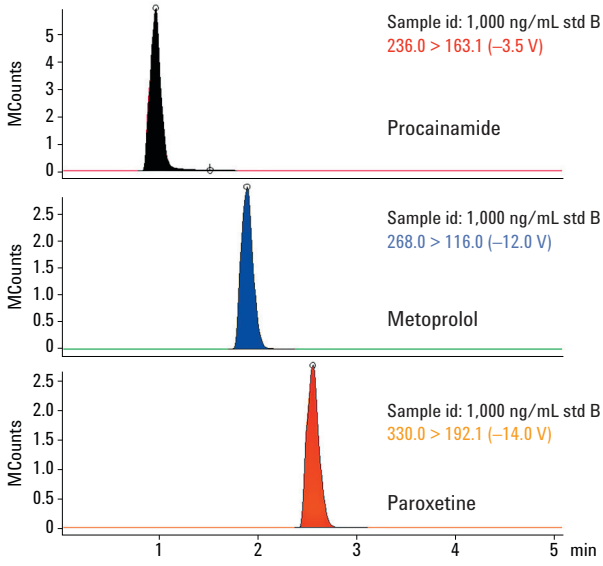
Table 4. Analyte relative recoveries, neutrals.

| | | | 0.5 µg/mL | | 1.0 µg/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 min | 20% A : 80% B |
| | t = 3.5-5 min | 80% A : 20% B |
| Column: | Pursuit C18 3 µm, 2.0 × 50 mm (p/n A3051050X020) | |

| MS conditions | Bases | | |
|---|---|---|---|
| **Compound** | **Q1** | **Q3** | **CE** |
| Procainamide: | 236.0 | 163.1 | -8.5 V |
| Metoprolol: | 268.0 | 116.0 | -12.0 V |
| Paroxetine: | 330.0 | 192.1 | -14.0 V |
| Capillary: | 25 V | | |
| Dry gas temperature: | 400 °C, 30 psi | | |
| CID: | Argon | | |
| Polarity: | 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 LOD of the combined solid phase extraction and LC/MS/MS analysis was 1.0 ng/mL. Recoveries were calculated from a second 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 three orders of magnitude from

1.0 ng/mL to 5.0 µg/mL with correlation all coefficients above 0.999. To demonstrate reproducibility, samples were analyzed at two different concentrations (n = 6). Table 5 shows that reproducible high recoveries were obtained according to the generic standard protocol.

Table 5. Analyte relative recoveries, bases.

| | | | 0.5 µg/mL | | 1.0 µg/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 Agilent 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 nonpolar retention mode in SPE is less selective than ion exchange, the polar interferences and proteins as well as ion suppression effects in LC/MS 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.

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