Performance Comparison of Bond Elut Plexa PCX and SampliQ SCX Mixed Mode Strong Cation Exchange Polymeric SPE Resins for the Separation of Acidic, Basic, and Neutral Drugs from Plasma

Technical Overview

Introduction

A study was conducted to determine if either of the two polymeric strong cation exchange SPE products, the Bond Elut PCX or the SampliQ SCX performed better in the retention of acidic, neutral, and basic components in human plasma.

After spiking three components into a solution of human plasma and 1% formic acid, the solution was then loaded onto the SPE cartridge. The SPE cartridge was washed with 2% formic acid, air dried under vacuum, eluted with methanol for the acidic and neutral components, and then eluted with 5% NH_4OH/methanol for the basic components. An internal standard was added to the eluents, followed by HPLC analysis.

HPLC analysis of the solid phase extraction (SPE) extracts was conducted using 30% methanol/70% potassium phosphate dibasic (pH 7.0) as the isocratic mobile phase, with a flow rate of 1.2 mL/min, and a diode array detector set at 237 nm. The column used was an Agilent ZORBAX Eclipse Plus C18, Rapid Resolution 4.6 mm x 75 mm, 3.5 µm, maintained at 35 °C.

Recoveries of neutral and basic components were the same for a SPE source, Bond Elut or SampliQ. Neutral and basic component recoveries for the Bond Elut PCX and SampliQ SCX were 94% and 91%, respectively. Recoveries for the acidic phenol, acetaminophen, were noticeably different for the PCX and SCX and were 77% and 40%, respectively.

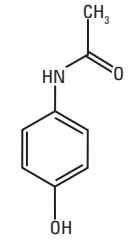
For the recovery of acidic compounds, the Bond Elut Plexa PCX was significantly better than the SampliQ SCX. In terms of the recovery of neutral and basic compounds, the Bond Elut Plexa PCX was equal to or slightly better than the SampliQ SCX. Excellent intraday and interday reproducibility (%RSD) for the recovery of neutral and basic compounds was observed for both products. For acidic compounds Bond Elut Plexa PCX showed better (lower) intraday reproducibility (%RSD) than that observed for SampliQ SCX.

Agilent Technologies

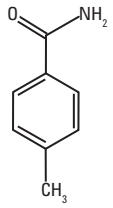
Study Purpose and Methodology

The purpose of the study was to determine if there were any performance differences between Bond Elut Plexa PCX and SampliQ SCX Strong Cation Exchange Polymeric SPE Resins.

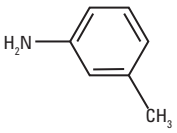
Acetaminophen, p-toluamide and m-toluidine were chosen as representative acidic (phenol), neutral and basic compounds, respectively. See Figure 1 for the chemical structures of the compounds.



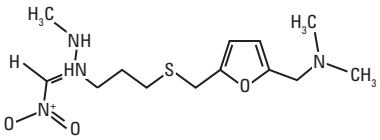
acid (phenol)
acetaminophen, APAP



neutral compound
p-toluamide



base
m-toluidine



internal standard base
ranitidine

Figure 1. Chemical structures.

Standard stock solutions were prepared at 4,000 µg/mL in methanol. The spike solution (acetaminophen, p-toluamide and m-toluidine, each 800 ug/mL), prepared from the stock standards solution, and the internal standard solution, ranitidine, (2,000 ug/mL) were also prepared in methanol. These solutions were stored in the freezer when not in use. Calibration solutions were prepared by combining the spike solution (0.25 mL) with the internal standard solution (0.25 mL) and diluting to 5.0 mL with methanol. The calibration solution was 40 ug/mL of each standard and 100 µg/mL of the internal standard.

A 6 mL amount of 1% formic acid (aq) was added to 2 mL of human plasma and the solution vortexed. The sample was then spiked with 100 µL of spiking solution and loaded onto Bond Elut Plexa PCX and SampliQ SCX SPE tubes (3 mL tube, 60 mg). The spiked components were 40 µg/mL with respect to the 2 ml of plasma. Samples were processed as illustrated in Figure 2.

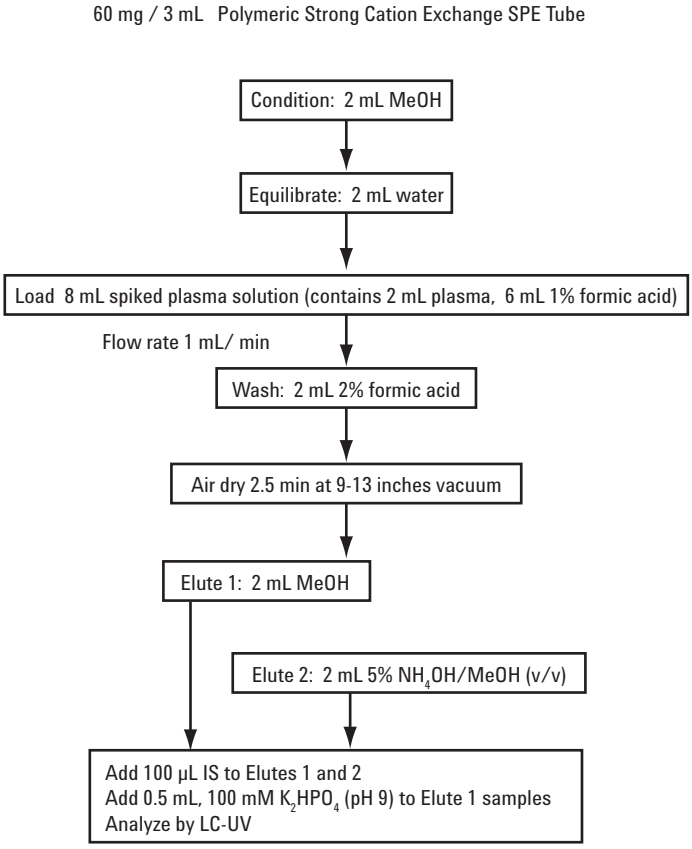


Figure 2. Sample cleanup scheme using Polymetric PCX and SCX SPE tubes.

A 100 µL spike of ranitidine solution (IS) (2,000 µg/mL) was added to SPE Eluents 1 and 2. In addition, 500 µL of buffer (100 mM K_2HPO_4, pH 9) were added to Eluent 1 to neutralize any residual traces of formic acid not removed in the SPE drying step.

The DAD was set at 237 nm for the detection of all components.

The properties and identification of the SPE tubes used are listed in Table 1.

Table 1. Properties and Identification of the SPE Tubes

Brand name	p/n	Nominal particle size (µm)	Volume (mL)	Bed mass (mg)
Bond Elut Plexa PCX	12108603	50	3	60
SampliQ SCX	5982-6236	60	3	60

Results and Discussion

Interday recoveries were determined for plasma samples with n=6. Table 2 lists the average recovery data and the respective % RSDs. Figure 3 is a bar graph of the recovery results.

In terms of the neutral compound, p-toluamide, and the base, m-toluidine, recoveries are about the same and in the 90-94% range, for both PCX and SCX SPE material. High reproducibility for both p-toluamide and m-toluidine, using both the PCX and SCX tubes, is evident by the low %RSD of 1.5% to 2.6%. However, the PCX tubes clearly have better retention and recovery for the acidic type of compounds represented by the phenol, acetaminophen. Acetaminophen recovery is about 77% with the PCX tubes, while only about 40% with the SCX tubes. The reproducibility for acetaminophen is noticeably better using the PCX material as evidenced by the PCX recovery's %RSD, which is about half of that observed using the SCX material.

Table 2. Sample Recovery Data

	Acetaminophen (acidic phenol)		p-toluamide (neutral compound)		m-toluidine (base)	
	% Recovery	% RSD (n=6)	% Recovery	% RSD (n=6)	% Recovery	% RSD (n=6)
PCX						
Day 1	79	3.8	95	1.5	93	1.8
Day 2	74	4.6	93	2.6	92	1.6
Average	77		94		93	
SCX						
Day 1	39	7.6	90	2.1	90	1.7
Day 2	40	8.2	91	1.9	90	1.5
Average	40		91		90	

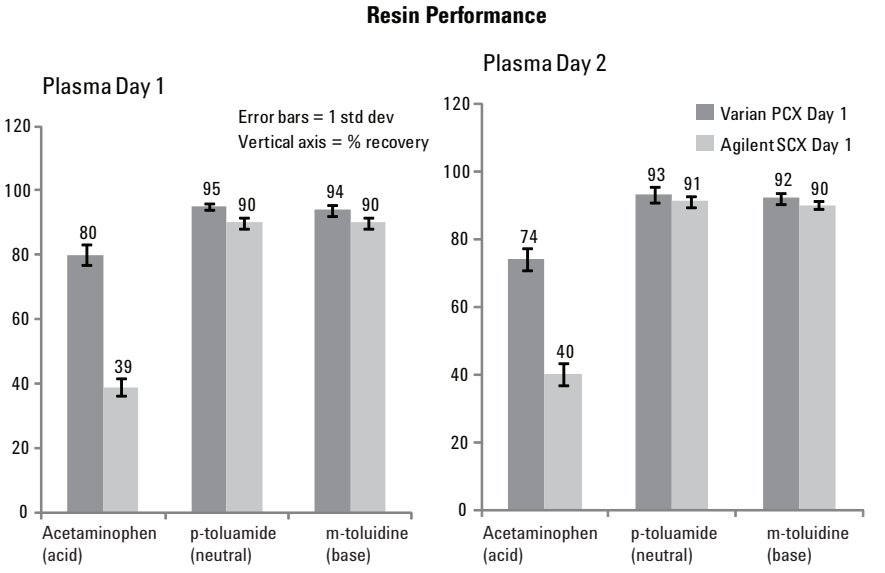


Figure 3. Average sample recoveries from polymeric PCX and SCX SPE tubes.

Figure 4 shows all standards are well separated and eluted in less than 5 minutes. All components are quantified by comparison to ranitidine (IS).

The chromatograms (not presented) of the SPE processed blank plasma eluents from both PCX and SCX materials are very clean with no impurities interfering with the standards.

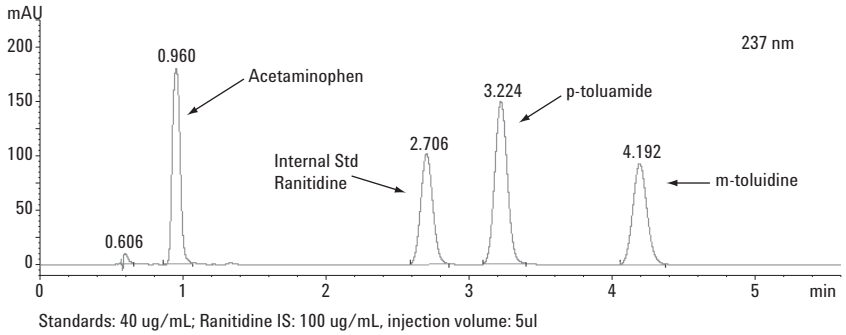


Figure 4. Chromatogram of standards.

Figure 5 shows representative chromatograms used in quantification of recoveries in the fortified plasma samples for both PCX and SCX SPE tubes. The peaks are well resolved with clean chromatograms. Acetaminophen and p-toluamide are quantified in Eluent 1, and m-toluidine is quantified in Eluent 2.

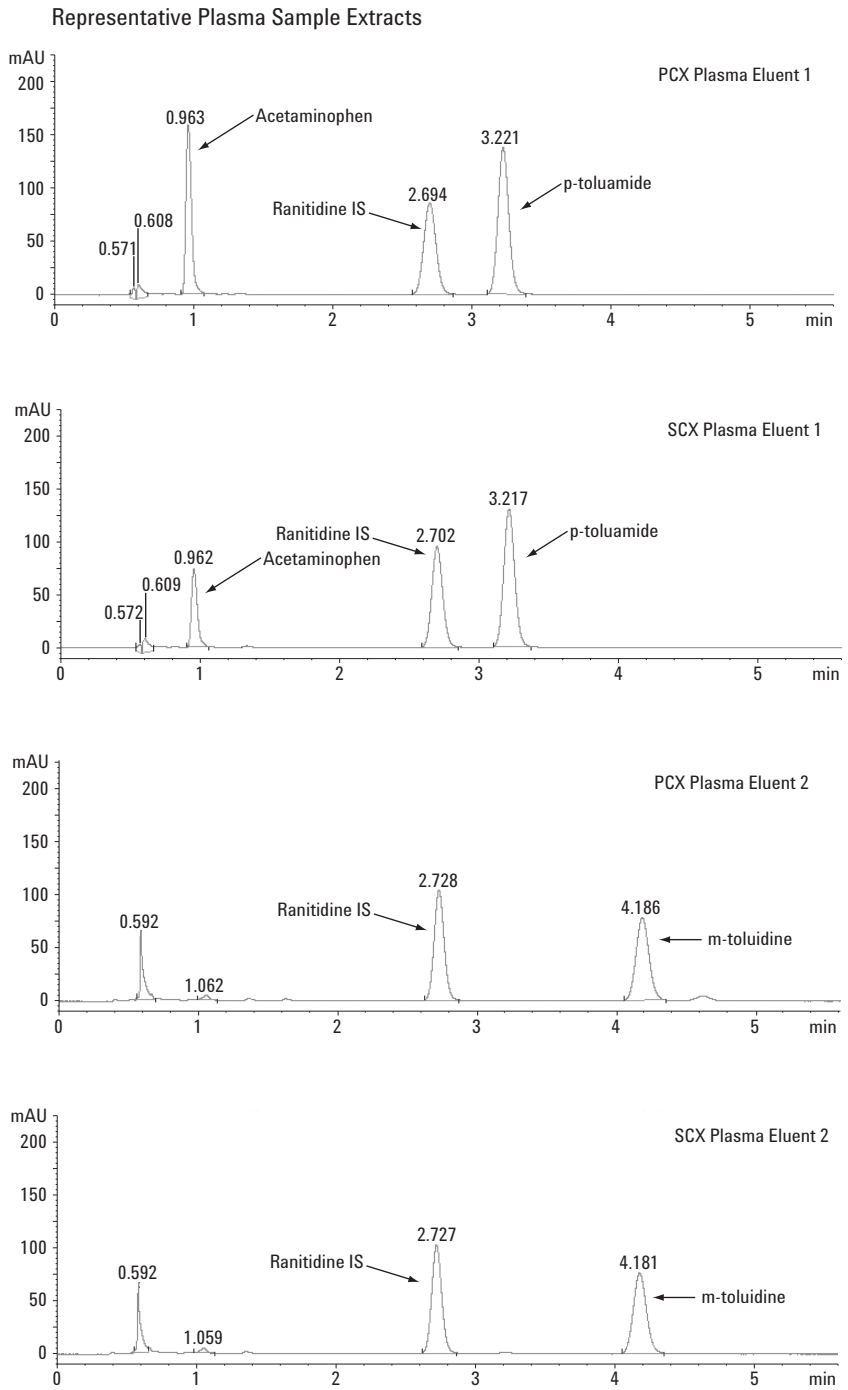


Figure 5. Chromatograms of extracts of plasma samples: Eluent 1 (acids and neutrals), Eluent 2 (bases).

Figure 6 is a calibration curve showing linearity from the spiking level of 40 µg/mL down to 4 µg/mL with CVs greater than 0.999, for each component. The calibration curves for acetaminophen and m-toluidine were essentially the same and overlapped each other.

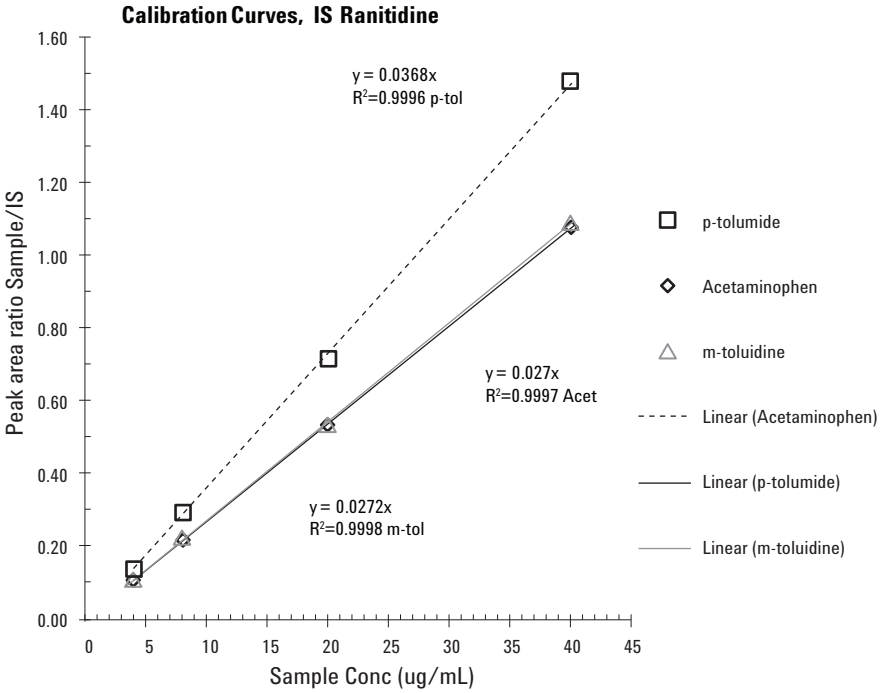


Figure 6. Calibration curves of the standards (acetaminophen and m-toluidine overlap).

HPLC Chromatographic conditions are listed in Table 3.

Table 3. HPLC Chromatographic Conditions

HPLC Analysis	
Column	Agilent ZORBAX Eclipse Plus C18, Rapid Resolution 4.6 mm x 75 mm, 3.5 µm Agilent p/n 959933-902
Flow rate	1.2 mL/min
Column temperature	35 °C
Injection volume	5 µL
Mobile phase	Isocratic elution: 70/30 A/B A: 25 mM K_2HPO_4, pH 7.0 B: methanol
Flow cell	10 mm, 13 µL
Diode array detector (DAD)	237 nm

Conclusion

For the recovery of acidic compounds, the Bond Elut Plexa PCX was significantly better than the SampliQ SCX. The recovery of neutral and basic compounds with Bond Elut Plexa PCX was equal to or slightly better than that for the SampliQ SCX. Excellent intraday and interday reproducibility (%RSD) for the recovery of neutral and basic compounds was observed for both products. The intraday reproducibility of acidic compounds (%RSD) was notably better (lower) with the Bond Elut Plexa PCX, than with SampliQ SCX.

Agilent SPE and column part numbers

Description	Part number
Bond Elut Plexa PCX	12108603
SampliQ SCX SPE tube, 3 mL tube, 60 mg	5982-6236
ZORBAX Eclipse Plus C18, Rapid Resolution 4.6 mm x 75 mm, 3.5 µm	959933-902

Author/Contact

Joan Stevens is a sample preparation applications scientist at Agilent Technologies, Inc., Wilmington, DE, USA.

For More Information

For more information on our products and services, visit our Web site at www.agilent.com/chem

www.agilent.com/chem

Printed in the USA
October 22, 2010
5990-6440EN

Agilent Technologies