Isolation of Basic Amines in the Presence of Acidic and Neutral Impurities from Human Plasma Using Silica-Based SCX SPEs

Technical Overview

Introduction

A comparison study involving two silica-based, strong cation exchange (SCX) solid phase extraction (SPE) products was conducted, focusing on the recovery of two basic amines. In addition, one acid and two neutral impurities were spiked into human plasma to evaluate the selectivity for amines only.

A five-component solution was spiked into human plasma containing 2% H_3PO_4. The sample was then loaded onto a preconditioned SPE cartridge. The SPE cartridge was washed with 2% formic acid, then with methanol/acetonitrile (1:1) to remove any organic, nonbasic components. The basic components were then eluted with 5% NH_4OH/(methanol/acetonitrile (1:1)).

HPLC analysis of the SPE extracts used an Agilent ZORBAX Rapid Resolution Eclipse Plus C18 column with a gradient of 20 to 90% methanol/10 mM ammonium formate (pH 3.8).

Both Agilent Bond Elut SCX and SampliQ Si-SCX showed comparable and consistent recoveries for the basic compounds used in this study.

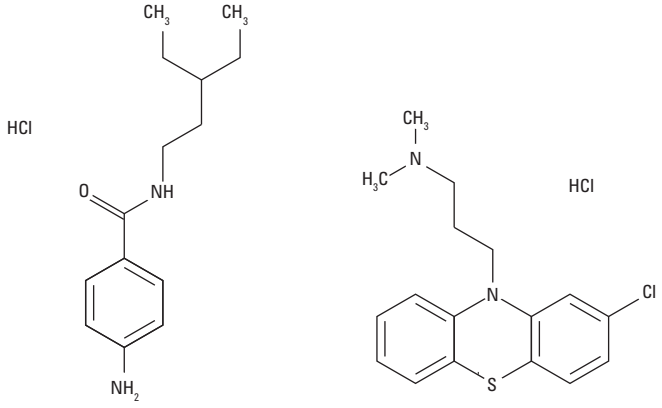
The extractions presented in this technical note suggest that the use of Bond Elut SCX (silica based SCX) gives a clean extract and high recovery of basic compounds, such as amines.

Agilent Technologies

Study Purpose and Methodology

The purpose of the study was to evaluate performance differences between Agilent Bond Elut SCX and Agilent SampliQ Si-SCX, which are silica-based strong cation exchange SPE products, with respect to their retention of basic amines. The recoveries of two basic components and the chromatographic profile of the eluents are evaluated to determine performance differences.

Procainamide and chlorpromazine were chosen as representative bases. One acid and two neutral compounds were added to the base spiking solution as representative acid and neutral impurities. Figure 1 shows the chemical structures of the bases.



Procainamide HCl Chlorpromazine HCl

Figure 1. Structures of compounds.

Individual compound solutions were prepared in methanol at 5 mg/mL. A solution containing the five components was prepared by combining 2 mL of each individual compound solution, resulting in a concentration of 1 mg/mL for each compound. The working solution was prepared by combining 1 mL of the five-component solution with 1 ml of methanol in a 5 mL volumetric flask, and filling to volume with Milli Q water. This results in a 200 µg/mL concentration of each compound in 40/60 methanol/water. Solutions were stored at –20 °C. The working solution was prepared fresh daily.

The calibration standard solution was prepared daily by diluting the 200 µg/mL of working solution with mobile phase to a calibration solution concentration of 2.75 µg/mL. Subsequently, serial dilutions with mobile phase were made for the calibration curve.

The SPE cartridges were conditioned prior to sample loading with 3 mL of methanol and 3 mL of water.

Three mL of 2% H_3PO_4 (aq) were added to 1 mL of human plasma and vortexed. The sample was then spiked with 200 µL of the working solution (200 µg/mL), vortexed and loaded onto preconditioned Bond Elut SCX and SampliQ Si-SCX SPEs (100 mg/1mL). The spiked components were 40 µg/mL with respect to the 1 mL of plasma. Any retained acid and neutral components were eluted with Wash 2, methanol/acetonitrile (1:1). Retained bases were eluted with 5% NH_4OH/(methanol/acetonitrile, 1:1). Since the eluent was essentially methanol/acetonitrile, aliquots of each eluent were diluted with buffer (1:4) giving an 80/20 buffer/organic solution prior to analysis. Samples were processed as illustrated in Figure 2.

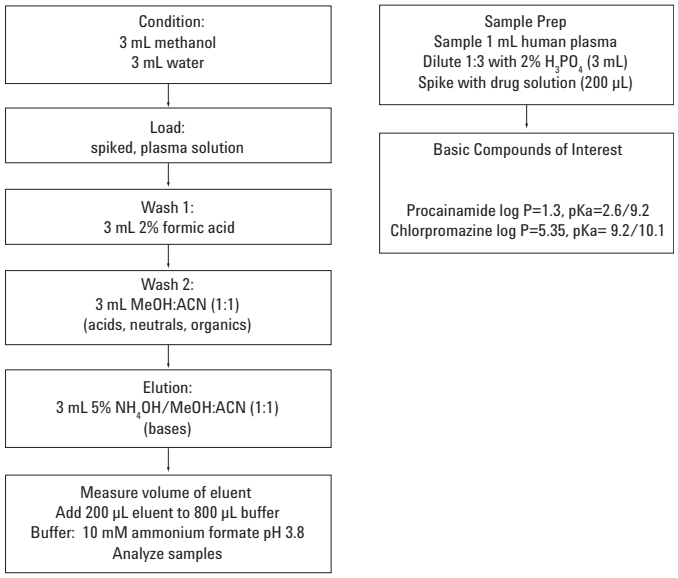


Figure 2. Sample processing scheme, and compound log P and pKa.

Table 1 lists the physical properties for both the Bond Elut and the SampliQ SPE.

Table 1. Properties and Identification of the SPE Cartridges

Brand name	Part No.	Nominal particle size (microns)	Nominal capacity meq/g	Volume (mL)	Bed mass (mg)
Bond Elut SCX	12102013	55	0.83	1	100
SampliQ Si-SCX	5982-2111	45	0.6	1	100

Results and Discussion

Table 2 lists the results for Bond Elut and SampliQ silica-based SCX, showing intra- and interday recovery and % RSDs. Figure 3 is a graphical representation of the basic amine recovery results from plasma.

Similar recoveries for the basic amines are observed for the Bond Elut and SampliQ, at 90–98% (Table 2, Figure 3).

Table 2. Sample Recovery Data from Plasma

	Procainamide		Chlorpromazine	
n = 6	% Recovery	%RSD	% Recovery	%RSD
BE-SCX				
Day 1	93	1.6	91	1.6
Day 2	98	1.1	96	1.4
Average	96		94	
Si-SCX				
Day 1	92	0.7	90	0.7
Day 2	96	1.6	94	1.5
Average	94		92	

BE = Bond Elut Si-SCX = SampliQ

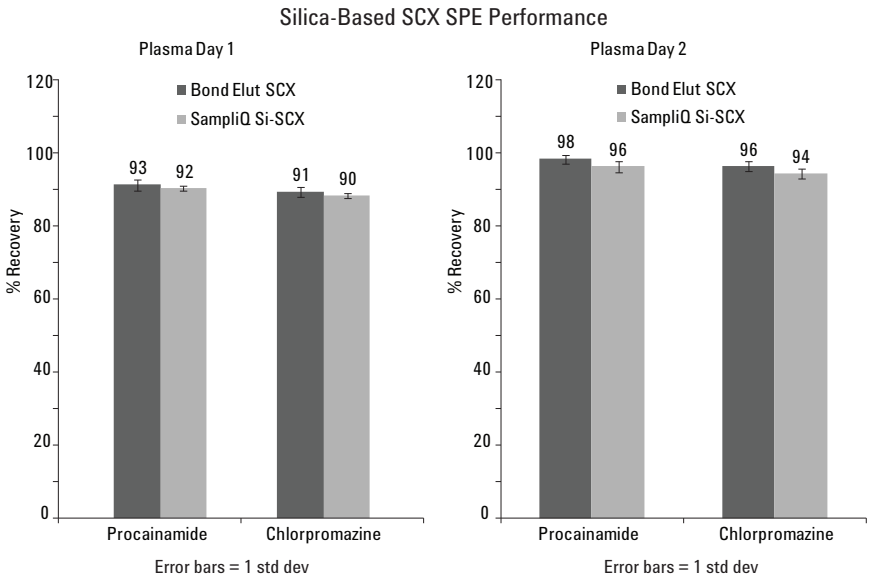


Figure 3. Sample recoveries from silica-based SCX SPE, Bond Elut SCX and SampliQ Si-SCX.

Figure 4 shows representative chromatograms for control plasma eluent for both SampliQ and Bond Elut. The Bond Elut SCX offers a cleaner extract relative to SampliQ Si-SCX, under these conditions.

Representative Plasma Control Eluent

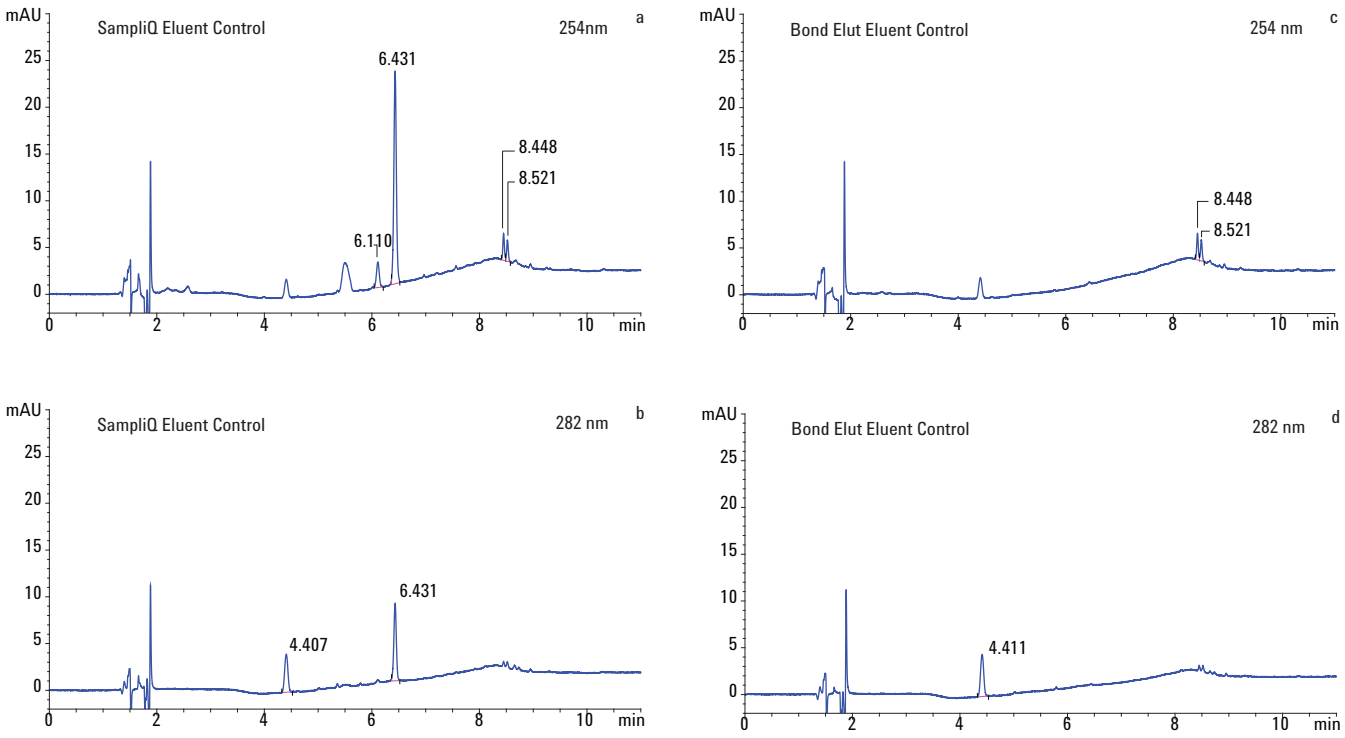


Figure 4. Chromatograms after SPE of the control plasma samples at the two representative wavelengths used to evaluate the recovery of the basic amines after SPE.

Figure 5 shows the chromatograms of the eluent from plasma samples, which contain the basic compounds. Procainamide was quantified using the Diode Array Detector (DAD) set at 282 nm. Chlorpromazine was quantified at 254 nm.

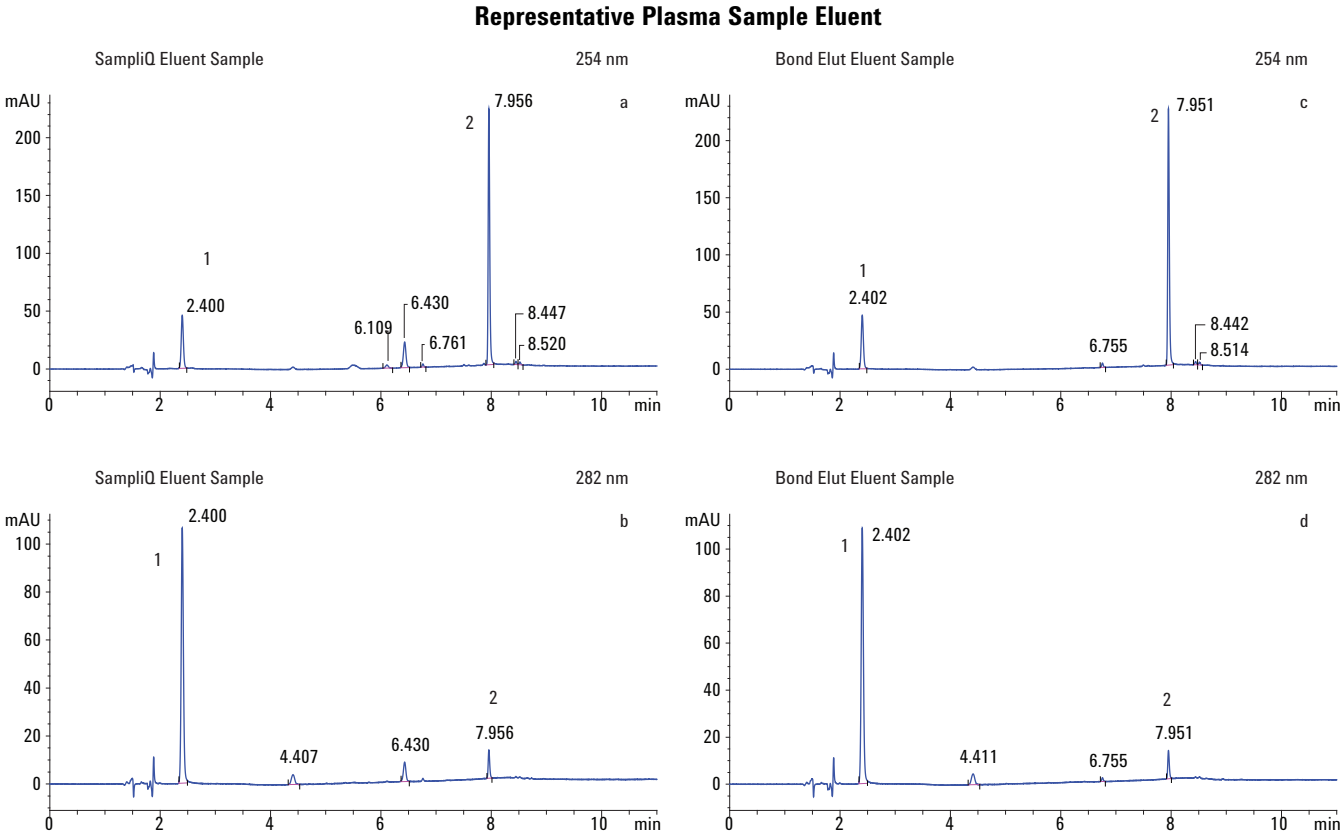


Figure 5. Chromatograms after SPE for the basic amines; 1. procainamide, 2. chlorpromazine at the two chosen wavelengths: 254 and 282 nm.

Figure 6 is a calibration curve showing linearity from the 100% recovery level of 2.75 µg/mL to 0.14 ug/mL and forced through the origin for the two basic components. The coefficients of determination (r^2) are at least 0.9999.

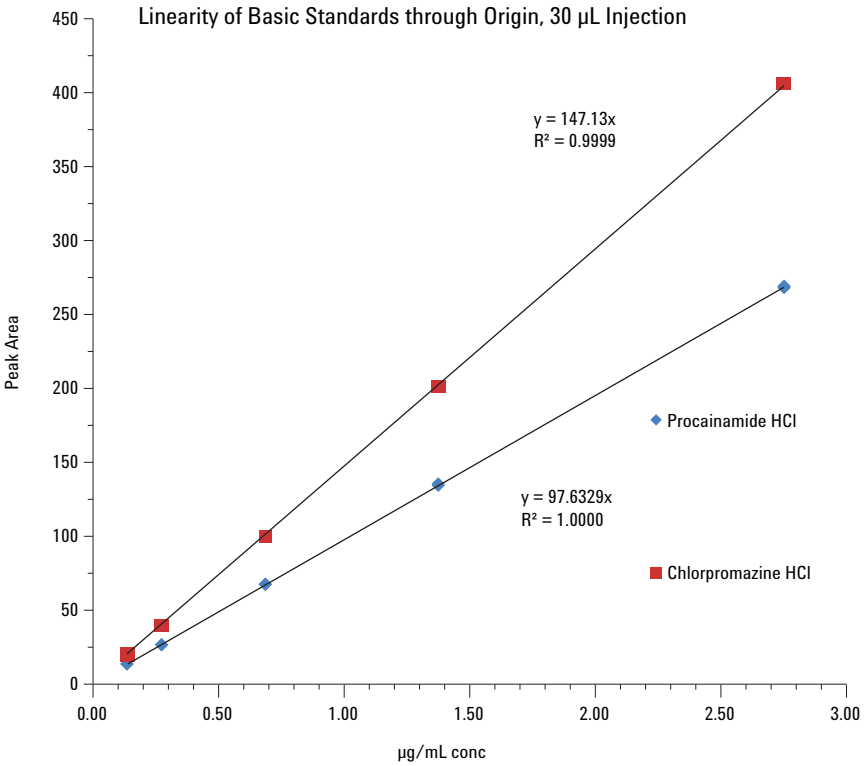


Figure 6. Linearity of the amines calibration curves for procainamide and chlorpromazine.

HPLC chromatographic conditions are listed in Table 3.

Table 3. HPLC Chromatographic Conditions

HPLC analysis

Column	Agilent ZORBAX Rapid Resolution Eclipse Plus C18, 4.6 mm × 150 mm, 3.5 µm Agilent (p/n 959963-902)
Flow rate	1.0 mL/min
Column temperature	25 °C
Injection volume	30 µL
Mobile phase	Gradient elution A = 10 mM ammonium formate, pH 3.8 B = methanol

Time	%B
0.0	20
1.0	20
6.0	90
9.5	90
11	20
18	20

Flow cell	10 mm, 13 µL
Diode array detector (DAD)	254 nm chlorpromazine 282 nm procainamide

Conclusion

The Bond Elut and the SampliQ SCX performed well in terms of the recovery of the two amine components. The Bond Elut SCX offered cleaner extracts than that of SampliQ Si-SCX.

The results presented have shown that the Bond Elut silica-based strong cation exchange SPE provides a cleaner sample, with high recovery of basic amines.

Table 4. Agilent SPE and column part numbers

Description	Part number
Bond Elut SCX	12102013
SampliQ Si-SCX	5982-2111
ZORBAX Rapid Resolution Eclipse Plus C18, 4.6 mm × 150 mm, 3.5 µm	959963-902

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