Fractionation of Acidic, Basic and Neutral Drugs from Urine with an SPE Mixed Mode Strong Anion Exchange Polymeric Resin (Agilent SampliQ-SAX)

Application Note

Drug Discovery

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Abstract

A polymeric mixed mode strong anion exchange resin, Agilent SampliQ SAX, was evaluated in terms of its ability to extract acidic, basic and neutral drugs from urine. An SPE procedure was applied whereby acidic drugs were eluted in the acidic fraction while the neutral and basic drugs were eluted in the neutral fraction. High recoveries (79.6 – 109%) and high reproducibilities (RSDs ranged from 0.06 – 1.12%) were obtained. The calibration curves were linear for nortriptyline, ketoprofen and naproxen ($r^2 > 0.999$) in the 0 to 10 µg/mL concentration range. Secobarbital was, however, linear from 0 – 25 µg/mL. The limits of detection were 0.21 µg/mL, 0.04 µg/mL, 0.03 µg/mL, 0.02 µg/mL and quantification values were 0.81 µg/mL, 0.12 µg/mL, 1.04 µg/mL, 2.74 µg/mL for secobarbital, nortriptyline, ketoprofen and naproxen, respectively.

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Introduction

Various domains, such as forensic science, toxicology, doping control and therapeutic drug monitoring employ solid phase extraction (SPE) prior to chromatographic analysis. In bioanalysis, urine and blood present a very complex matrix for the determination of drugs and their metabolites. Therefore, sample preparation for cleanup and preconcentration of analytes to improve their detection is very important.

The fractionation of different classes of drugs (acidic, basic and neutral) in biological fluids has been reported in a number of studies [1- 4]. Protein precipitation, liquid-liquid extraction (LLE) and SPE are among the most popular sample preparation techniques. The versatility of SPE allows for the preferential use of the technique, as it is not only employed for class fractionation but also for trace enrichment and purification. Commercial sorbents such as chemically-modified silica gel, polymer and graphitized or porous carbon are available [5]. These offer interactions based on normal phase, reversed phase, ion exchange and mixed mode ion exchange (combination of reversed phase and ion exchange) mechanisms. The mixed mode sorbents have proven to give cleaner extracts and better separations than standard reversed phase or ion exchange sorbents since they take advantage of both the ion exchange and hydrophobic interactions [6].

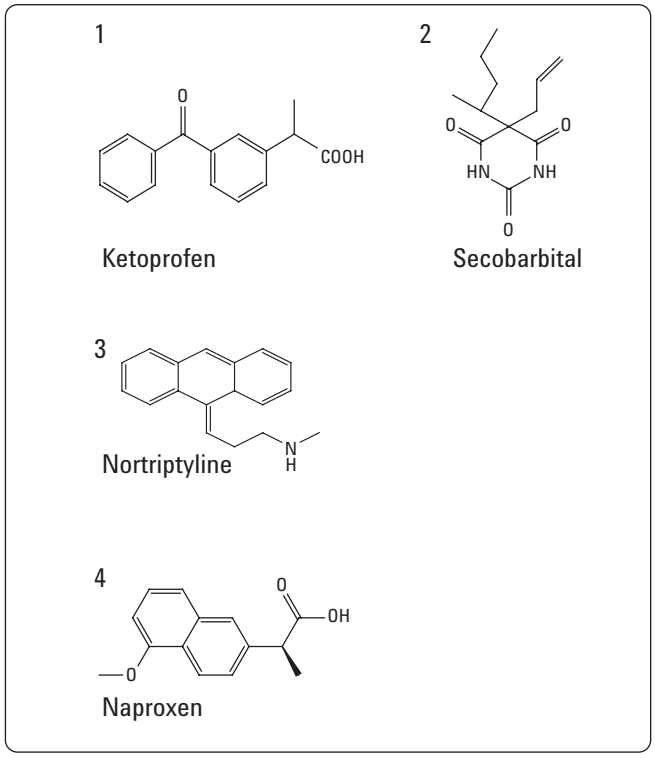


Figure 1. Structures of the drugs used: ketoprofen and naproxen (acidic), secobarbital (neutral) and nortriptyline (basic).

In the present study, a method based on SPE was developed for the fractionation of acidic, basic and neutral drugs in urine with Agilent SampliQ-SAX, a mixed mode strong anion exchange polymer. The resin is a tertiary amine-modified divinylbenzene polymer that exhibits both anion exchange and reversed phase behavior. In addition, it provides excellent reproducibility and enables a simple extraction protocol. Specific drugs (Figure 1) were used as representatives of the three classes of drugs (acidic, basic and neutral).

Experimental

Chemicals

Ketoprofen, secobarbital, nortriptyline and naproxen were purchased from Sigma-Aldrich Chemicals (St. Louis, MO, USA). Phosphoric acid, formic acid and potassium hydroxide were purchased from Merck Chemicals (Gauteng, South Africa) while the HPLC-grade methanol (MeOH) was from Merck KGaA (Darmstadt, Germany) and potassium dihydrogen phosphate was purchased from Saarchem Analytical (Krugersdorp, South Africa).

The mobile phase was prepared with ultrapure water (18.2 MΩcm) from a MilliQ system by Millipore (Milford, Mass, USA) and filtered through a Whatman membrane filter (47 mm diameter and 2 µm pore size). The stock solutions (1000 ppm) of the four analytes were prepared in methanol and kept at 4 °C while the working solutions were prepared daily by diluting the stock solutions, to appropriate concentrations, in methanol. The urine was from a donor who was not using or has not used the drugs investigated in this study.

Instrumental

The analysis was performed on an Agilent 1200 Series High Performance LC System (HPLC) equipped with a binary pump and a diode array detector (DAD) set at λ = 222 nm. Separation of the compounds was achieved on an Agilent ZORBAX Eclipse Plus C18 column (4.6 mm × 75 mm, 3.5 µm, Agilent p/n 959933-902). The data was processed by Agilent ChemStation HPLC-2D software. The SPE cartridges were Agilent SampliQ SAX, 1-mL/30 mg containing a polymeric anion exchanger with 25-35 µm average particle size (Agilent p/n 5982-3313). A Jenway 3510 pH meter (London, UK) was employed for pH adjustments.

Sample pretreatment: SPE procedure

A 5-mL amount of urine was hydrolyzed with 1 M KOH at 60ºC for 15 min and diluted with 10 mM CH_3COONa (1:1 v/v). The pH was then adjusted to 2 with phosphoric acid. The urine sample, unspiked (blank) and spiked with drugs, was loaded onto the SampliQ SAX cartridges using the conditions shown in Figure 2. This SPE procedure was optimized for maximum recovery and reproducibility of experimental results.

Separation and Analysis

The HPLC conditions are shown in Table 1.

Table 1. HPLC Conditions

Column	Agilent ZORBAX Eclipse Plus C18, 4.6 mm × 75 mm, 3.5 µm
Flow rate	1.5 mL/min
Column temperature	30 °C
Injection volume	5 µL
Mobile phase	Isocratic elution
	A: 55% CH_3OH
	B: 45% 25 mM KH_2PO_4, pH 7
Run time	8 min
Post time	1 min
Detection:	DAD @ 222 nm

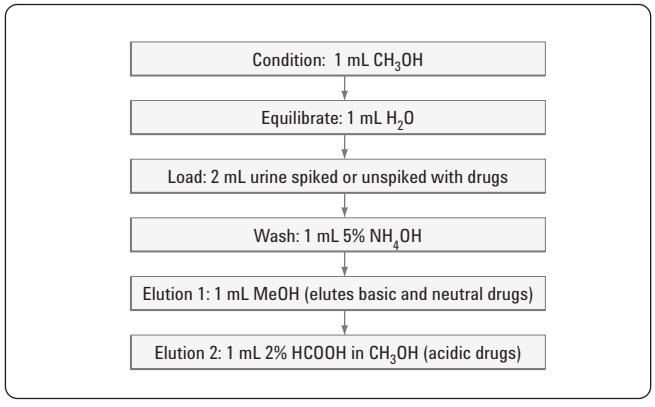


Figure 2. SPE procedure for acidic, basic, and neutral drugs using SampliQ SAX.

Results and Discussion

Separation

The chromatogram of a standard solution containing secobarbital, nortriptyline, naproxen and ketoprofen is shown in Figure 3. A baseline separation of these standards was obtained. Under the conditions used in Table 1, all analytes were eluted within 9 min.

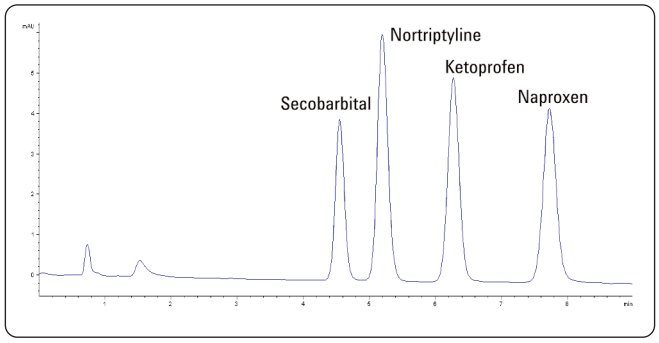


Figure 3. Chromatogram of a standard solution (5 µL) containing 1) secobarbital (10 µg/mL), 2) nortriptyline (5 µg/mL), 3) ketoprofen (5 µg/mL) and 4) naproxen (2 µg/mL).

Analysis of standard solutions

Calibration curves were constructed in the concentration range 0.0-8.0 µg/mL for nortriptyline and ketoprofen, 0-7 µg/mL for naproxen and 0-35 µg/mL for secobarbital as shown in Figure 4. Good linearity was obtained with $r^2 > 0.999$. Due to the diverse polarities and pH characteristics of the compounds tested each one was monitored at its maximum absorption wavelength (Table 2). It can be seen that secobarbital gave a weak response compared to the other drugs in the standard mix. Therefore, in later experiments, the concentration of this drug was adjusted upward to provide a stronger signal.

Table 2. Chemical and Physical Characteristics of the Studied Drugs

Drug	Classification	Log P	pKa	λmax (nm)
Secobarbital	Neutral	1.97	7.90	222
Nortriptyline	Basic	4.28	9.70	242
Ketoprofen	Acidic	0.97	5.94	258
Naproxen	Acidic	3.18	4.53	230

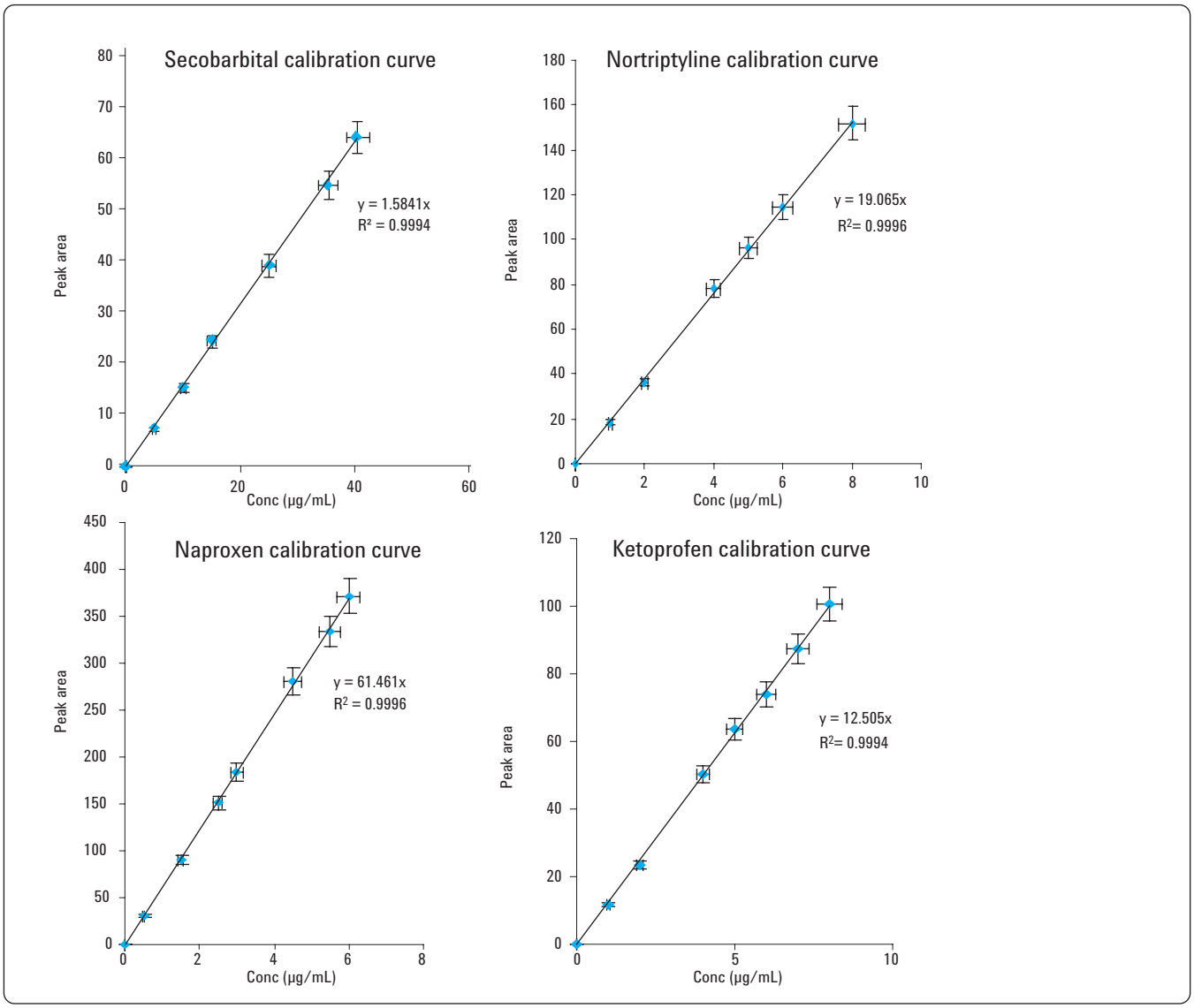


Figure 4. Calibration curves (at the λ max of each) for secobarbital, nortriptyline, ketoprofen and naproxen.

Solid phase extraction procedure for drugs in urine

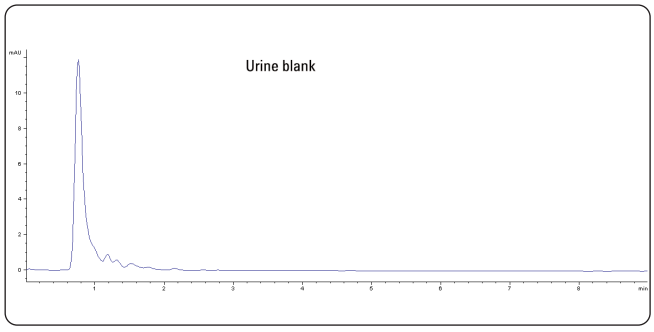
Agilent SampliQ SAX, a polymeric mixed-mode, strong anion exchange SPE sorbent was successfully used to simultaneously extract acidic, basic and neutral drugs from a spiked urine sample using the SPE procedure depicted in Figure 2. First, blank urine containing no drugs was treated using the SPE method. Figures 5a for the basic and neutral elution conditions showed nothing eluting in the region of the acidic and neutral drugs in the standards. Figure 5b, which depicts a blank urine using the acidic elution conditions, also showed nothing eluting in the region of the acidic drugs. For the spiked urine samples, the neutral (secobarbital) and basic (nortriptyline) drugs were eluted in the neutral fraction (Figure 6a) since they were retained through hydrophobic interactions. The acidic drugs (naproxen and ketoprofen), retained by the strong anion exchange functionalities of the sorbent, eluted separately in the acidic fraction, as shown in Figure 6b. A small amount (< 10%) of the neutral/basic drugs were also found in the acidic fraction. A larger volume of methanol in the prior step could have been used to improve extraction efficiency.

Recovery and reproducibility

The recoveries were calculated by comparing the peak area of the analyte concentration in the spiked urine after SPE to that of the standard solution at the same concentration level. To demonstrate reproducibility the samples were analyzed at three different concentration levels (n = 6). As indicated in Table 3, high recoveries (> 85%) were obtained except for the lowest level of secobarbital. The RSD values were excellent and ranged from 0.06 to 1.12 for n = 6 runs.

Table 3. Recoveries for Secobarbital, Nortriptyline, Ketoprofen and Naproxen from Urine

Drug	SPE fraction	Spike level (µg/ml)	Recovery	%RSD` (n=6)
Secobarbital	Neutral	5	79.63	1.12
		10	92.70	0.78
		15	86.47	0.31
		1	91.20	1.04
Nortriptyline	Neutral	2.5	86.48	0.47
		4	85.32	0.12
		1	109.34	0.54
Ketoprofen	Acidic	2.5	99.18	0.58
		4	85.88	0.16
		0.5	106.97	0.18
Naproxen	Acidic	1	87.66	0.63
		2.5	83.41	0.06



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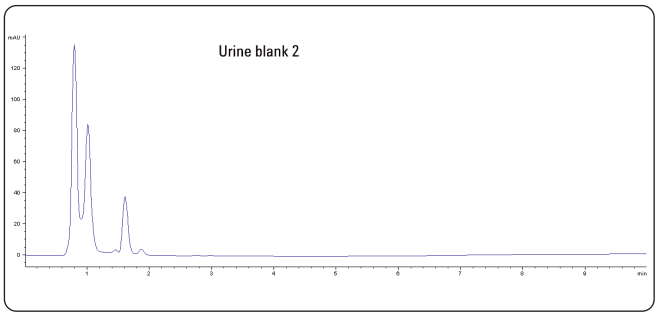
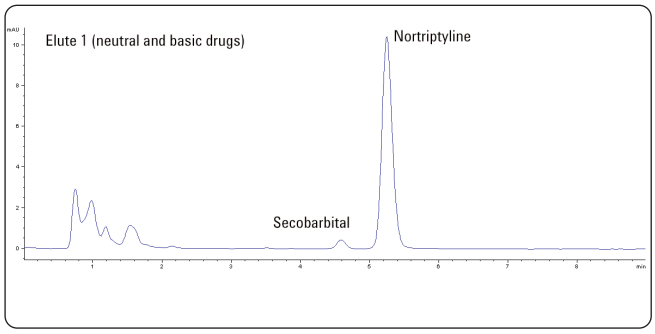


b.

Figure 5a. Chromatograms of blank urine extract by SPE method using Elution 1 for neutral and basic compounds (see Figure 2).

Figure 5b. Chromatograms of blank urine extract by SPE method using Elution 2 for acidic compounds (see Figure 2).



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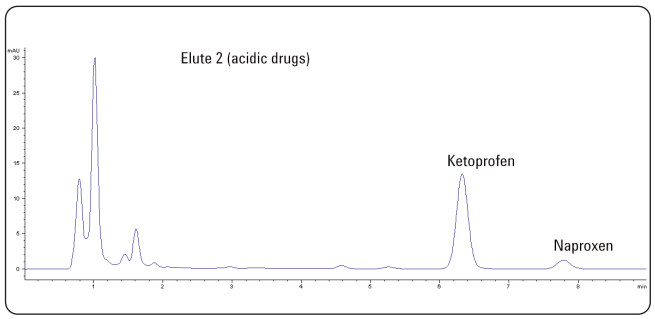

b.

Figure 6a. Chromatograms of neutral and basic drugs (Elution 1) extracted from spiked urine: 1) secobarbital and 2) nortriptyline.

Figure 6b. Chromatograms of acidic drugs (Elution 2) extracted from spiked urine: 3) ketoprofen and 4) naproxen.

Linearity, limits of detection and limits of quantification

After SPE was performed, the method linearity as well as the limits of detection (LOD) and quantification (LOQ) were determined. Linearity was determined in the concentration range 0-25 µg/mL for secobarbital and 0 – 10 µg/mL for nortriptyline, ketoprofen, and naproxen. Secobarbital and nortriptyline were linear in the chosen range while ketoprofen and naproxen showed linearity from 0 – 4.5 µg/mL. Table 4 shows the linearity equations and correlation coefficients.

Table 4. Linearity after SPE

	Concentration range (0 – 8 µg/ml)	
Drugs	**Linear equation**	**Correlation coefficient (r^2)**
Secobarbital	$y = 1.3325x$	$r^2 = 0.9993$
Nortriptyline	$y = 17.595x$	$r^2 = 0.9991$
Ketoprofen	$y = -1.2748x^2 + 17.896x$	$r^2 = 0.9991$
Naproxen	$y = -1.9003x^2 + 33.527x$	$r^2 = 0.9993$

The LOD and LOQ results are shown in Table 5. The following equations 1 and 2 were used to calculate LOD and LOQ, where Syx = standard error of the regression line and b = gradient.

$$LOD = \frac{3.3 \times Syx}{b} \qquad \text{Equation 1}$$

$$LOQ = \frac{10.0 \times Syx}{b} \qquad \text{Equation 2}$$

Table 5. LOD and LOQ for the Analytes

Drug	LOD (µg/ml)	LOQ (µg/ml)
Secobarbital	0.21	0.81
Nortriptyline	0.04	0.12
Ketoprofen	0.03	1.04
Naproxen	0.03	2.74

Conclusion

The SPE method employed is relatively simpler than other protocols reported in literature. With the strong anionic exchange polymer, Agilent SampliQ SAX, the simultaneous extractions of acidic drugs, a basic drug and a neutral drug from a spiked urine matrix were obtained. High recoveries and good reproducibilities were achieved for extraction of all drugs from the urine.

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