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Evaluation of Extraction Methods and Third Generation HPLC columns for Analysis of Diuretics

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Introduction

The analysis of diuretic drugs has been carried out for many years. The drugs are somewhat problematic since they are relatively water soluble and carry a number of different polar and ionic functional groups. We were interested in developing a single or two column solid phase extraction (SPE) method for both the acidic and basic diuretics. In addition, HPLC reversed phase column technology has advanced to a third generation of phases. The first was controlled totally porous particles (ex., Hypersil) with chemically bonded phases. These materials had variable surface chemistry, and often gave rise to very tailed peaks for basic compounds. The second was characterized by better surface chemistry and end-capping procedures (ex., Supelcosil LC-18). With increased understanding of the types of surface groups which cause tailing and with surface blocking bonding chemistries, the third generation columns have emerged (ex., Zorbax SB-18). We report here on a systematic investigation of these columns for diuretic analysis.

Experimental

A number of solid phase extraction materials were used, including Certify, Certify II, and CBA-COOH (Varian), XAD (Supelco), and Clean Screen DAU (WorldWide Monitoring).
The diuretics studied were polar (acetazolamide; amiloride(AMI); chlorothiazide(CZ); hydrochlorthiazide(HCZ)) non-polar (bendroflumethazide(BFZ); benzthiazide(BZZ); cyclothiazide(CYZ); methychlothiazide(MTZ); polythiazide; trichloromethiazide; spirononlactone(SPL); canrenone (CAN); pemoline(PEM); caffeine; triamterene(TRM); dichlophenamide; quinethazone(QUZ); metolazone; chlorthalidione(CHD)), and acidic (ethacrynic acid(EA); furoseimde; butmetanide; probenecid(PRB)).
Three analytical columns were evaluated: Hypersil ODS (150x4.6 mm, Hewlett Packard); AsahiPak ODP-18 (250x4.6 mm; Hewlett Packard); Zorbax C-18 SB (150x4.6, MacMod). The Asahipak material is a polyvinyl alcohol-based support with bonded C-18 groups. The Zorbax C-18 SB phase makes use of the surface preparation procedure, then uses a di-*t*-butyl-octadecyl silane bonding chemistry to protect the surface.

Results and Discussion

We attempted to develop an SPE extraction method using the chemical functionalities on the various diuretics as a guide. Thus, Certify II, a mixed function non-polar and cation exchange SPE, could be used at alkaline pH to trap hydrophobic and basic diuretics. The poor extraction efficiency on silica-gel based C-18 phases prompted us to look at XAD resins, and although the extraction efficiency was much higher, the diuretics could not be adequately separated from pigments in the urine. The recoveries of groups of diuretics are summarized in Table 1. Given this data, identifying even a two column SPE method was ruled out. Nevertheless, we combined a procedure using an SPE column and a liquid-liquid extraction which gave excellent recovery of all compounds from a single procedure, which is summarized in Figure 1.

The column comparison was more productive. Hypersil ODS columns, operated at pH 4.5, showed extreme peak tailing for amiloride and triamterene, and moderate tailing for acetazolamide, caffeine, hydrochlorthiazide, and probenecid. In addition, two poorly resolved peaks were observed for cyclothiazide. Asahipak ODP-18 had less chromatographc efficiency than either of the silica-based columns, and most peaks had an asymmetric shape. We attribute this to slow solvent equilibration with the polymer column. The Zorbax SB C-18 column showed excellent peak shape for all peaks, with only slight tailing for amilioride and triamterene (See Figure 2 and 3). Interestingly, the cyclothizaide was resolved into three well-resolved peaks on this column (Figure 3). Although this was initially of some concern, cyclothiazide has been shown to have eight possible stereoisomers which form four separate racemates (1).

Conclusions

We were unable to develop a one or two column SPE method which gave acceptable recovery for all of the diuretics. By combining an carboxylate SPE method with a basic liquid-liquid extraction employing ethylacetate and n-butylchloride, we were able to achieve improved recoveries of all diuretics. Excellent chromatographic performance was achieved with a Zorbax SB C-18 column.

References

1. Nusser E, Banerjee A, Gal J. Excavations in drug chirality: 1. Cyclothiazide. ***Chirality*** 1991; 3:.2-13.

Table 1.

EXTRACTION OF DIURETICS USING SOLID PHASE EXTRACTION

RECOVERY OF DIURETICS:

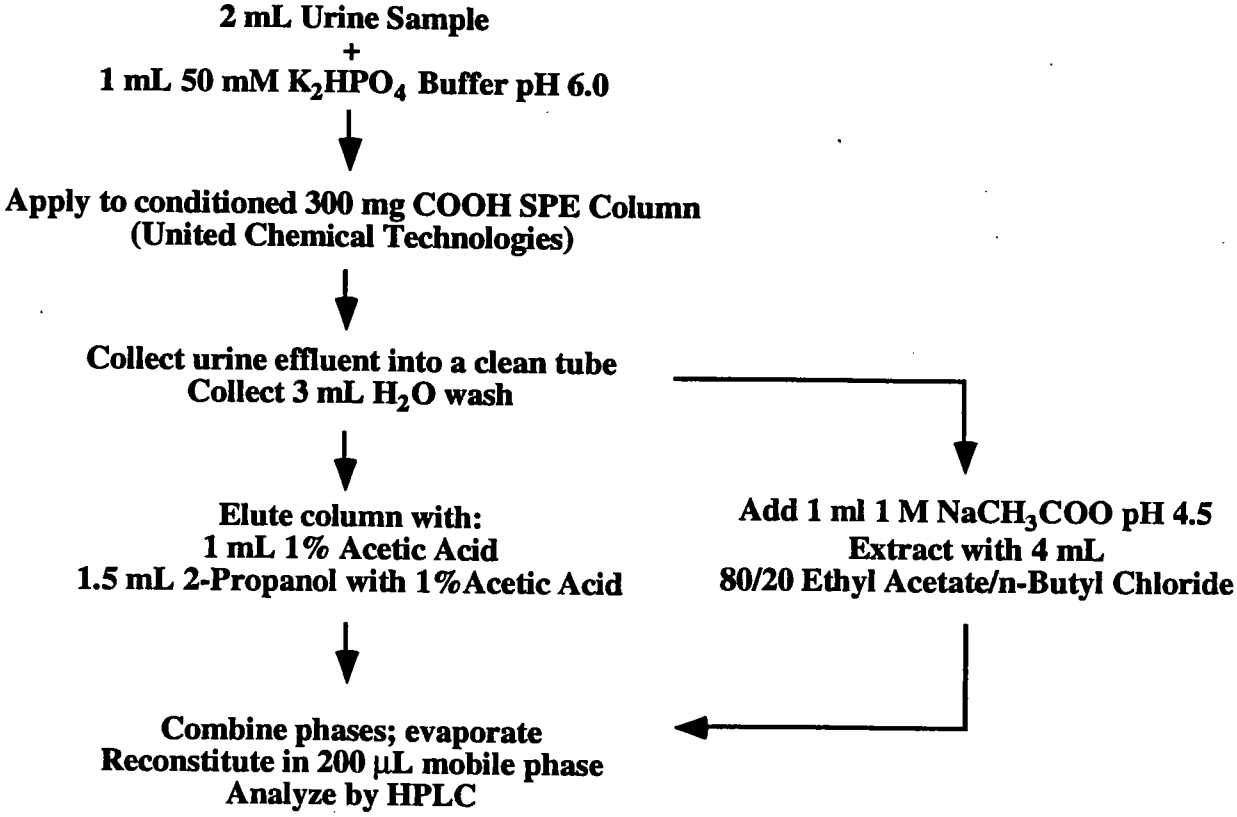
COLUMN	DIURETIC POLARITY				
	Polar	**Nonpolar**	**Acidic**	**Other**	**Comments**
Certify	Not retained	≥ 75%	≥ 75%	ND	
Certify II	Not retained	ND	≥ 75%	ND	
Clean Screen DAU	Not retained	≥ 75%	≥ 75%	ND	
XAD	≥ 98%	≥ 98%	≥ 98%	ND	No separation from pigment
CBA-COOH	Not retained	ND	Not retained	≥ 45%	

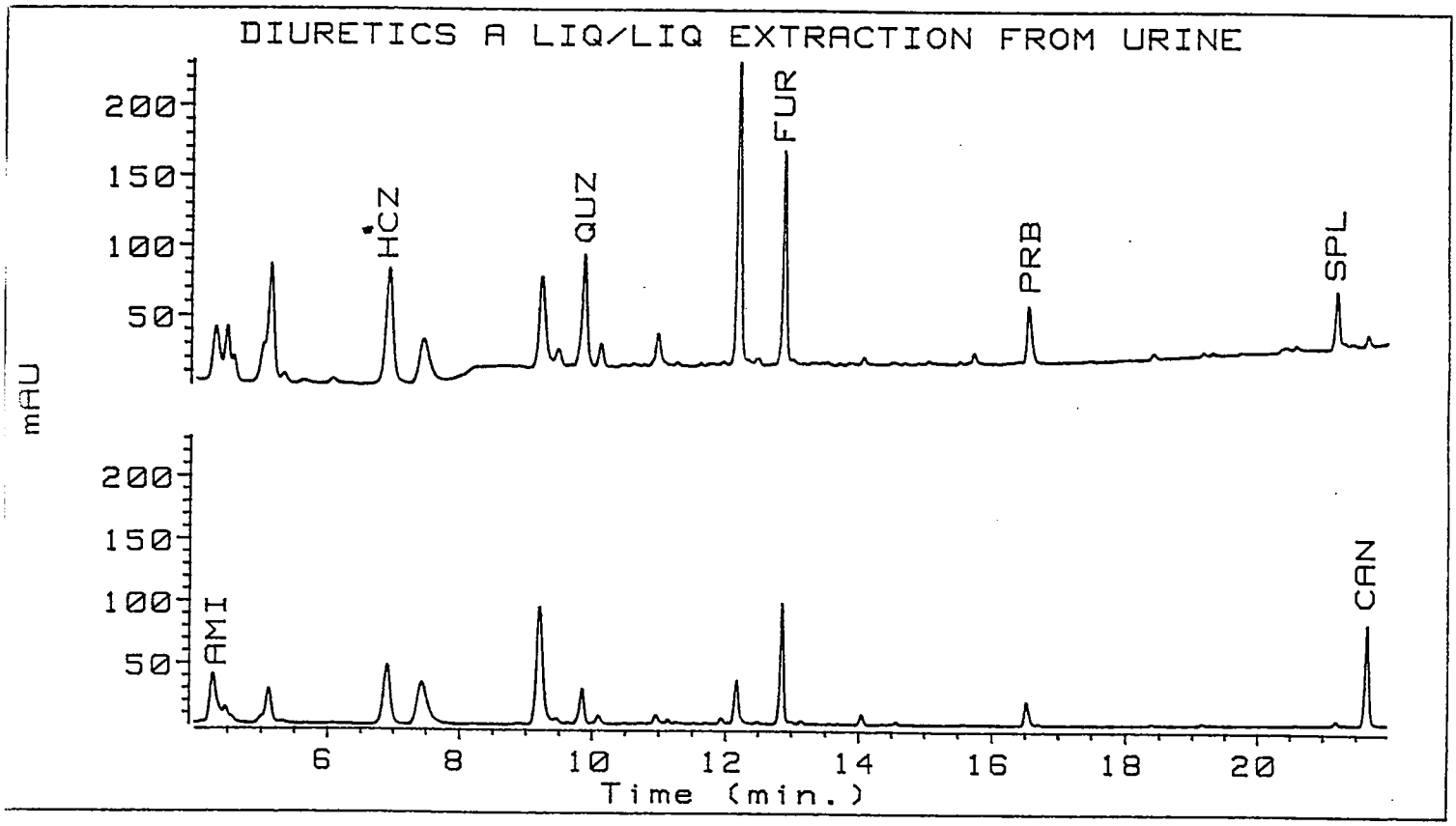
Figure Captions

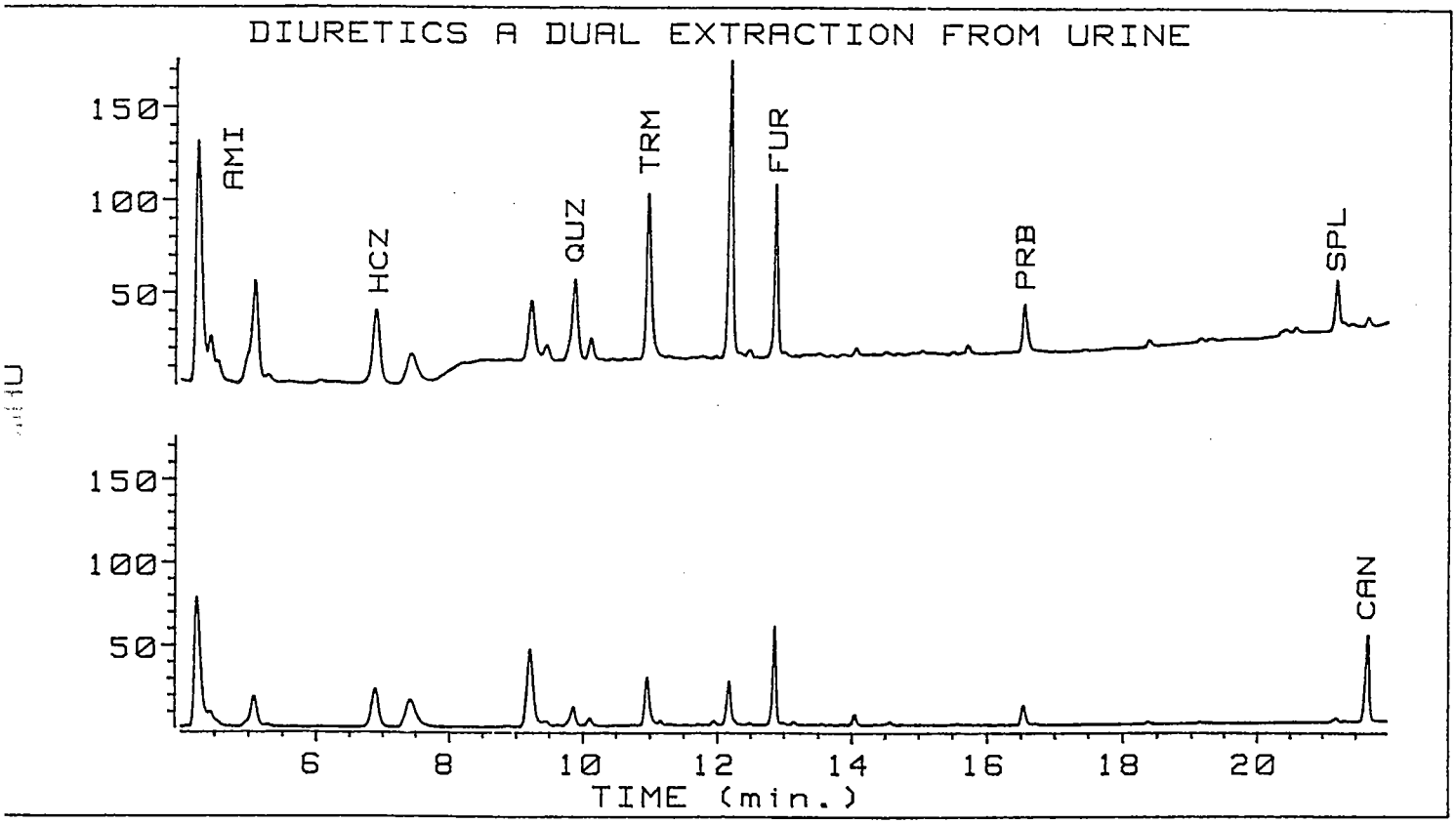
Figure 1. Sample preparation scheme for urine diuretic procedure.

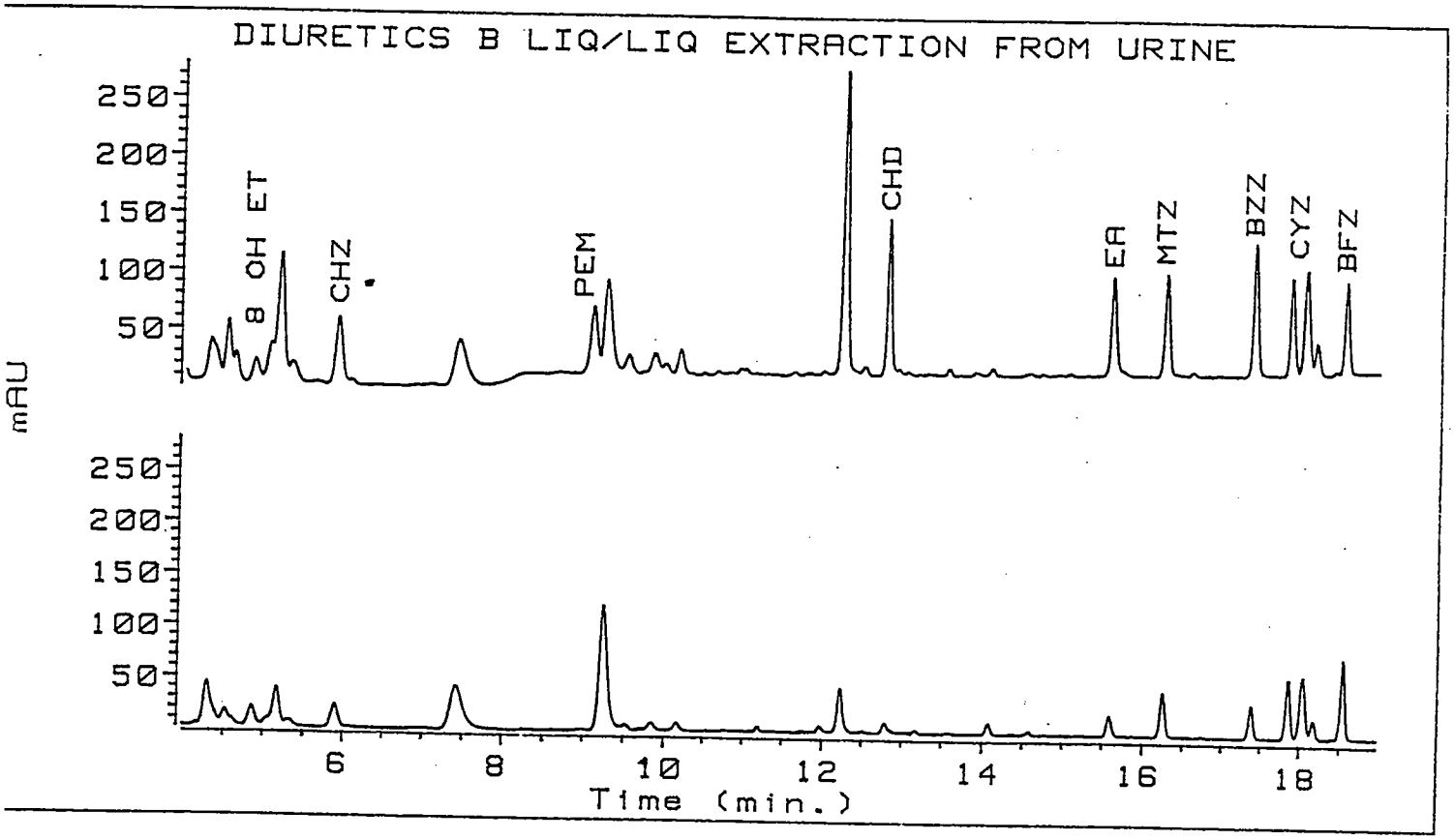
Figure 2. Chromatogram of urine sample spiked with diuretics noted by the letters above the peaks. Both a standard liquid-liquid extraction (top) and the extraction scheme in Figure 1 (bottom) are shown with separation on the Zorbax SB C-18 column. Note the improved extraction of amilioride (AMI) in the two column extraction.

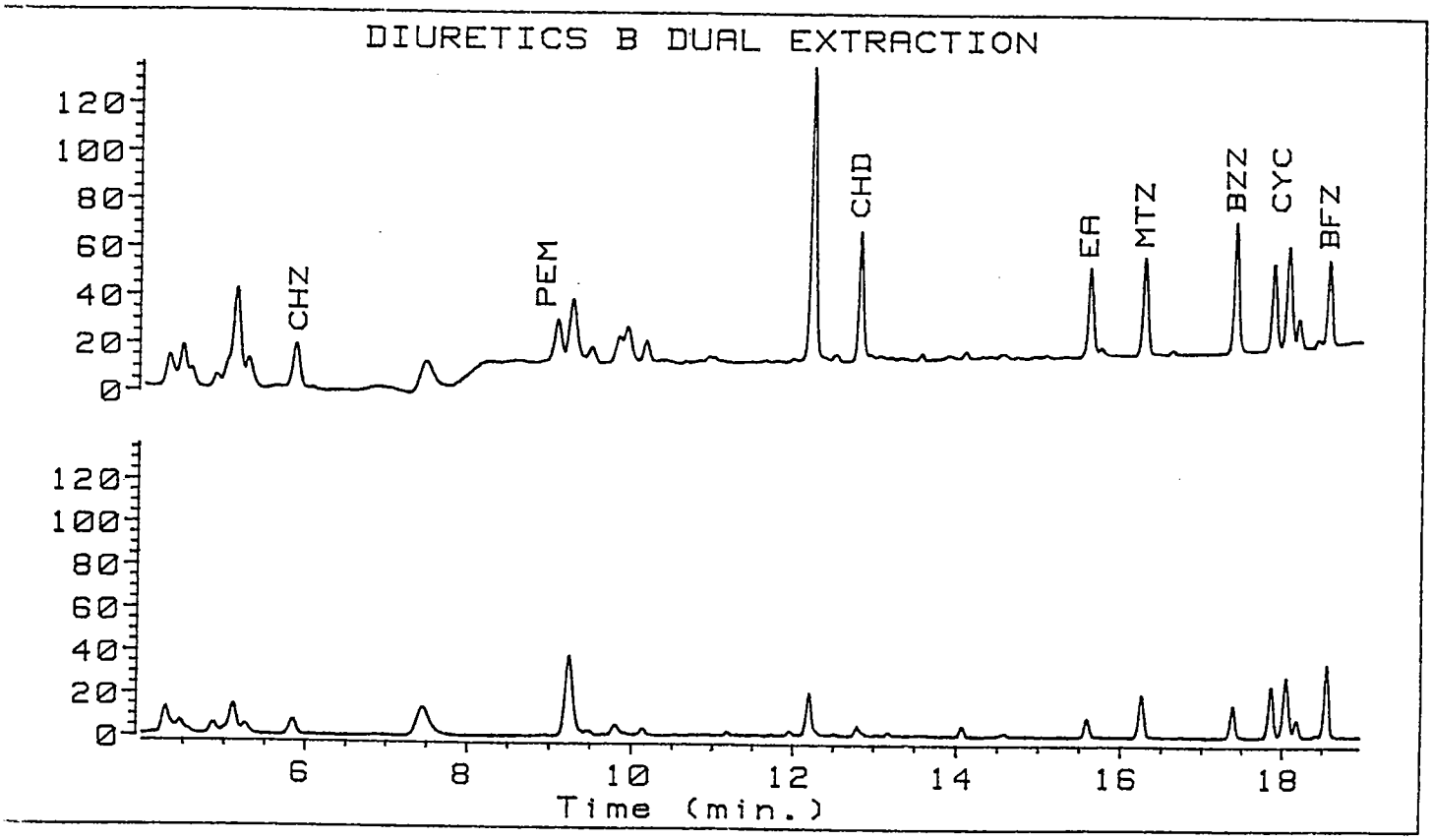
Figure 3. Chromatogram of urine sample spiked with diuretics noted by the letters above the peaks. Both a standard liquid-liquid extraction (top) and the extraction scheme in Figure 1 (bottom) are shown with separation on the Zorbax SB C-18 column. Note the resolution of cyclothiazide (CYZ) into three peaks on this column.


2 mL Urine Sample
+
1 mL 50 mM K_2HPO_4 Buffer pH 6.0
Apply to conditioned 300 mg COOH SPE Column
(United Chemical Technologies)
Collect urine effluent into a clean tube
Collect 3 mL H_2O wash
Elute column with:
1 mL 1% Acetic Acid
1.5 mL 2-Propanol with 1%Acetic Acid
Add 1 ml 1 M $NaCH_3COO$ pH 4.5
Extract with 4 mL
80/20 Ethyl Acetate/n-Butyl Chloride
Combine phases; evaporate
Reconstitute in 200 µL mobile phase
Analyze by HPLC


DIURETICS A LIQ/LIQ EXTRACTION FROM URINE
mAU
200
150
100
50
HCZ
QUZ
FUR
PRB
SPL
AMI
CAN
6
8
10
12
14
16
18
20
Time (min.)


DIURETICS A DUAL EXTRACTION FROM URINE
150
100
50
AMI
HCZ
QUZ
TRM
FUR
PRB
SPL
CAN
6
8
10
12
14
16
18
20
TIME (min.)


DIURETICS B LIQ/LIQ EXTRACTION FROM URINE
mAU
250
200
150
100
50
8 OH ET
CHZ
PEM
CHD
EA
MTZ
BZZ
CYZ
BFZ
6
8
10
12
14
16
18
Time (min.)


DIURETICS B DUAL EXTRACTION
120
100
80
60
40
20
0
CHZ
PEM
CHD
EA
MTZ
BZZ
CYC
BFZ
6
8
10
12
14
16
18
Time (min.)