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High-performance liquid chromatographic separation of a complex mixture of diuretics using a micellar mobile phase of sodium dodecyl sulphate
Application to human urine samples

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Abstract

A systematic optimization of the HPLC separation of a complex mixture containing 19 diuretics by micellar liquid chromatography using sodium dodecyl sulphate (SDS), a Hypersil (150 mm×3.0 mm I.D., 5 μm) C_{18} column, a flow-rate of 0.5 ml min^{-1} and UV absorbance detection has been carried out. Several mobile phases consisting of SDS and organic modifiers such as acetonitrile, tetrahydrofuran, propanol, butanol or pentanol, and the pH adjusted to 3.2, were tested. The effect of the organic modifier and SDS concentration on the retention behavior and separation of the diuretics was investigated. A mobile phase containing 40 m*M* SDS and 4% tetrahydrofuran was finally selected. Under these conditions, 14 out of 19 diuretics were separated in about 31 min. A bivariant optimization method for the mobile phase SDS–tetrahydrofuran corroborated the above results. The effect of temperature on the retention was also studied, and 50°C was selected. The optimized method was applied to human urine samples of subjects administered Diurex® (tablets containing 20 mg of the active ingredient xipamide) without sample preparation.

Keywords: Diuretics

1. Introduction

Diuretics (DIU) are therapeutic agents normally used to eliminate tissular liquids or to enhance renal excretion of salt and water. Their effects are not limited to sodium and chloride excretion, but may also influence the renal absorption and excretion of potassium, calcium, magnesium and other ions [1]. These compounds are also used in doping control to reduce weight before a competition or to deliberately dilute urine samples in an attempt to nullify a drug test, which is one of the most frequent adverse effects in the control of anabolic steroids. Some of these drugs have acid–base properties covering a wide range of pK_a values, e.g. 3.9 for furosemide, 4.8 for xipamide and 9.5 for polythiazide [2].

An alternative method to conventional liquid chromatography (CLC) developed in recent years is micellar liquid chromatography (MLC). The use of MLC for the separation of different samples is increasing due to some advantages with respect to

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CLC. For example, the low cost and low toxicity of the mobile phases due to the small amount of solvent employed in the mobile phases [3], the enhanced selectivity and rapid gradient elution capability and simultaneous separations of hydrophobic and hydrophilic compounds as a result of the large number of interactions of the solutes with the stationary and mobile phases [3,4]. The most important drawback of MLC is the poor chromatographic efficiency as compared to CLC [5–8] due to the poor wetting of the stationary phase and the low mass transfer of solutes between the mobile and stationary phases. This lack of efficiency can be improved, however, by adding small amounts of organic modifiers, which reduce the net charge density in the micelle surface, by keeping the same linear flow-rate (e.g., by using slower flow-rates and smaller column I.D.s instead of using slower flow-rates for conventional column I.D.s), or by increasing the column temperature. One of the main applications of MLC is the possibility of direct sample injection of biological material into the column due to the ability of micellar aggregates to dissolve sample proteins and other compounds [9–11].

Several HPLC procedures using CLC have been described for one DIU [12–19]. However, in recent years, only a few examples have been reported for complex mixtures. For this purpose, several detectors have been used. In routine analysis, UV absorbance detectors are most commonly used. However, for research work where compounds may need to be monitored at various wavelengths, a photodiode-array detector may be required [20,21]. Fluorescence and electrochemical detectors have also been employed [22–24]. These methods have been applied to the analysis of DIU in pharmaceutical preparations [25,26], human urine [27,28] and rat and human plasma [29], in many cases using gradient elution.

With regard to MLC, samples include one or two diuretics, and little attention has been payed to complex mixtures [30,31]. The majority of procedures have been applied to human urine [32,33], but there are also methods for DIU determination in plasma [34].

In this paper, a systematic optimization of the separation of a mixture of DIU (chemical structures are shown in Table 1) by MLC is described using a Hypersil (150 mm×3.0 mm I.D., 5 μm) C_{18} column and mobile phases containing SDS. 40 m*M* SDS was used to study the effect of several organic modifiers. After selection of tetrahydrofuran as organic modifier, the effects of the SDS concentration and temperature were also studied. The method was applied to urine samples, without sample preparation, of subjects administered Diurex® (tablets containing 20 mg of the active ingredient xipamide).

2. Experimental

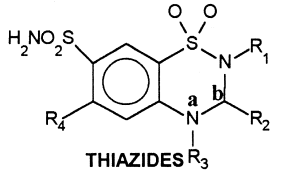
2.1. Chemicals

Althiazide (ALT), amiloride (AML), bendroflumethiazide (BND), benzthiazide (BNZ), bumetanide (BUM), canrenone (CAN), chlortalidone (CLR), clopamide (CLP), dichlorphenamide (DCP), ethacrynic acid (AET), furosemide (FRS), hydroflumethiazide (HFM), indapamide (IND), piretanide (PIR), polythiazide (POL), spironolactone (SPL), triamterene (TRI), trichlormethiazide (TCM) and xipamide (XIP) were supplied by Sigma (Alcobendas, Madrid). HPLC-grade 1-propanol (PrOH), 1-butanol (BuOH), 1-pentanol (PenOH), acetonitrile (ACN) and tetrahydrofuran (THF) were purchased from Promochem (Wesel, Germany), and sodium dodecyl sulphate and phosphoric acid were from Merck (Darmstadt, Germany). Water was purified with a Milli-Q system (Millipore, Molsheim, France). Millipore 0.45 μm Nylon filters (Bedford, MA, USA) were also used. Other chemicals were of analytical reagent grade.

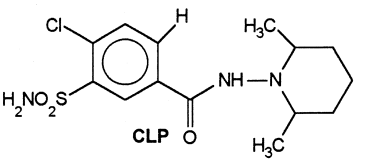
2.2. Apparatus

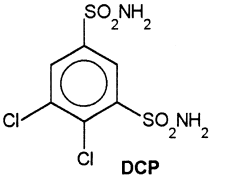
The chromatographic system consisted of the following components, all of them from TSP (FL, USA): ConstaMetric 4100 solvent delivery system, SpectroMonitor 5000 photodiode-array detector covering the range 190–360 nm and interfaced to a computer for data acquisition, and as recorder a Model CI 4100 data module. A Rheodyne 20 μL loop injector (Cotati, CA, USA) and a Jones-Chromatography block heated series 7960 for thermostating columns in the range 30–60°C (Seagate Technology, Scotts Valley, CA, USA) were also used. A reversed-phase Hypersil (150 mm×3.0 mm

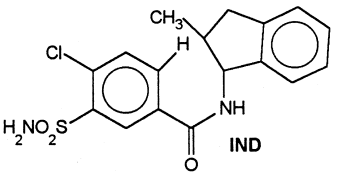
Table 1
Chemical structures of diuretics

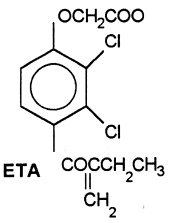


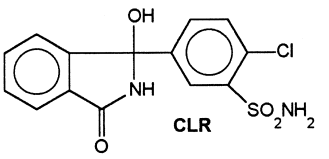
R_1	R_2	R_3	R_4	A_{a-b}	Name
H	$-CH_2SCH_2C_6H_5$	H	Cl	=	BNZ
H	$-CH_2SCH_2-CH_2$	-	Cl		ALT
$-CH_3$	$-CH_2SCH_2CF_3$	H	Cl		POL
H	$-CH_2C_6H_5$	H	CF_3		BND
H	H	H	CF_3		HFM
H	$-CHCl_2$	H	Cl		TCM

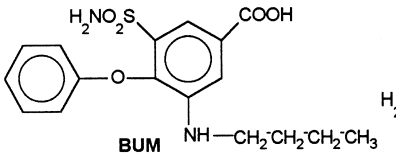


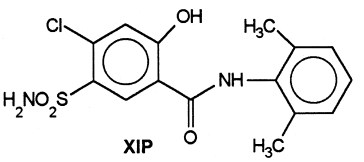


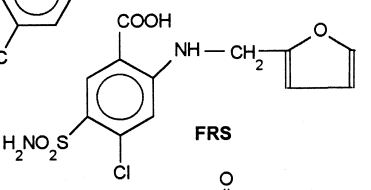


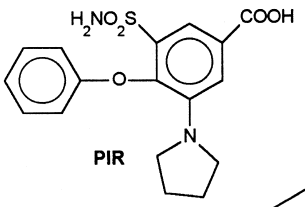


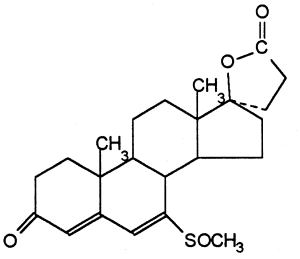


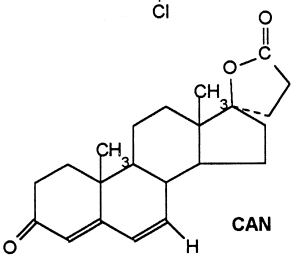


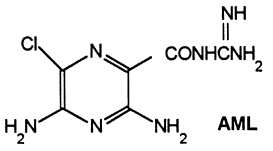


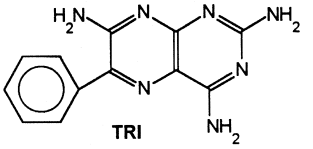












I.D., 5 μm) C_{18} column and a vortex mixer Mixo-Tub-30 from Crison (Barcelona, Spain) were also used.

2.3. Mobile phase

The micellar mobile phase was prepared daily by mixing aqueous SDS solutions of pH 3.2 (prepared with Milli-Q water) with solutions of PrOH, BuOH, PenOH, ACN or THF in SDS (adjusted to pH 3.2) at the required volume ratio by programming the pump. To obtain any solution with pH adjusted to 3.2, the required amount of phosphoric acid was added to all the above SDS solutions. All solvents and mobile phases were first filtered under vacuum through a 0.45 μm Nylon filter and degassed using helium sparge.

2.4. Drug administration and sample preparation

Three healthy volunteers aged 24, 25 and 45 years, having given informed consent and not receiving any medical treatment, received one tablet of Diurex which contains 20 mg of the active ingredient xipamide (Merck Farma). Before (blank samples) and after drug administration, several urine samples were collected from the volunteers during 24 h and stored at 4°C until analysis. Samples were then filtered through a 0.45 μm Nylon filter and injected into the HPLC system.

2.5. Chromatographic analysis

Once the column had been conditioned with the mobile phase, chromatograms were obtained at the programmed temperature (range 40–70°C). For optimization purposes based on the use of different mobile phases, a methanolic solution containing a single DIU or an appropriate mixture (5 μg ml^{-1}) was injected (20 μl) at a flow-rate of 0.5 ml min^{-1}. Peak identification was performed by comparison of the retention times and the UV absorbance spectra of the chromatographic peaks with those of reference compounds previously registered by injection of each compound individually. Analyses were carried out at 220 nm. An exception was made for SPL (245 nm) and CAN (275 nm).

3. Results and discussion

3.1. Preliminary conditions

Chromatographic analyses of complex mixtures of DIU are usually performed using mobile phases adjusted to acidic pH values due to the wide range of dissociation constants [20,21,30]. On these grounds, MLC was initiated using mobile phases of THF–SDS (pH 3.2), a Hypersil column (50°C) with 3.0 mm I.D. and a flow-rate of 0.5 ml min^{-1} (instead of those used in CLC, e.g. 4.6 mm I.D. and 1 ml min^{-1}). Working under these conditions, a linear flow-rate is practically maintained without losing efficiency. 40 m*M* SDS was initially selected since it is typically used in MLC [30,32]. In addition, this value assures a concentration over the critical micellar concentration (cmc 8.1 m*M*) [35]. UV absorption spectra obtained using these mobile phases did not show significant differences with respect to those obtained using THF–water.

3.2. Organic modifier optimization in MLC based on DIU separation characteristics

In order to optimize an adequate DIU separation in MLC, an evaluation of their characteristics using mobile phases containing 40 m*M* SDS and variable compositions of several organic modifiers (short chain alcohols such as PrOH, BuOH, PenOH and ACN or THF) was carried out. Table 2 shows the solvent concentration range studied (SCR) and the optimum composition (OPT) achieved according to

Table 2
DIU separation characteristics obtained using mobile phases containing 40 m*M* SDS and different solvents (pH 3.2)[a]

Solvent	SCR (%)	OPT (%)	NSC	RTA (min)
PrOH	2–4	2	15	30
BuOH	1–2	1.5	15	21
PenOH	0.3–0.5	0.3	13	25
THF	4–8	4	14	30
ACN	12–18	16	14	30

[a] SCR and OPT are the solvent concentration ranges used and the optimum selected; NSC is the number of separated compounds and RTA the run time analysis involved. Conditions: Hypersil C_{18} (150 mm×3.0 mm I.D., 5 μm) (50°C) and flow-rate 0.5 ml min^{-1}.

the number of separated compounds (NSC) and run time involved (RTA). As can be observed from these data, good results were obtained for THF and BuOH and the results were reasonable for the remaining solvents.

Table 3 lists the retention factors, *k*, for DIU under optimum solvent conditions. As can be observed, when PrOH, PenOH or ACN are used, HFM was separated from the other compounds. However, it was not detected because it coelutes with the system peak. Consequently, retention factors $k = 0$ for HFM were assigned in such cases. From these data, selectivity was examined for the solvent tested. As can be seen, selectivity is influenced by the organic modifier employed. In addition, different elution orders were achieved when comparing the retention factors for the organic modifiers and also for the short chain alcohols (PrOH, BuOH and PenOH) in spite of their structure, although ACN exhibits more differences. Moreover, different coelutions occurred: for THF, (IND, BNZ), (SPL, CAN, PIR) and (CLP, BUM, AML) coeluted; for PrOH, (FRS, BNZ), (POL, BND), (CAN, PIR) and (CLP, AML); for BuOH, (CLR, DCP), (FRS, IND, POL) and (BUM, AML); for PenOH, (TCM, DCP), (FRS, POL), (BND, IND), (PIR, AML), (BUM, CLP) and (AET, TRI); and for ACN, (CLR, DCP, TCM), (ALT, FRS), (IND, BNZ) and (SPL, XIP). Using the data of Table 2 and considering the different organic modifier performances (number and peak shape of separated compounds and run time analysis involved), 4% THF was finally selected (NSC = 14). The chromatograms obtained at 220, 245 and 275 nm for a standard mixture of DIU prepared in methanol using 4% THF and 40 m*M* SDS are shown in Fig. 1A, B and C. As can be seen, PIR (peak 13) can be detected in the presence of SPL and CAN at 220 nm, although SPL, CAN and PIR (peaks 11, 12 and 13) coeluted. In a similar manner, SPL (peak 11) can be detected at 245 nm in the presence of CAN and PIR, and CAN (peak 12) at 275 nm in the presence of PIR and SPL. In summary, 16 DIU are detected using these experimental conditions.

Table 3
Retention factors, *k*, for DIU using different organic modifiers under optimum conditions[a]

DIU	THF, 4%	PrOH, 2%	BuOH, 1.5%	PenOH, 0.3%	ACN, 16%
HFM	2.29	0.00	1.78	0.00	0.00
CLR	3.97	4.39	2.79	4.40	2.36
DCP	4.57	3.46	2.71	3.65	2.28
TCM	5.03	3.10	2.48	3.27	2.36
ALT	6.53	4.80	3.46	4.72	3.48
FRS	7.40	5.79	4.78	6.63	3.78
IND	8.29	7.56	4.82	7.44	4.98
BNZ	8.50	5.79	4.24	5.63	5.04
POL	11.47	7.00	4.82	6.68	5.97
BND	12.14	7.10	5.64	7.64	6.55
SPL	13.45	12.19	7.10	9.50	14.40
CAN	13.47	14.56	7.90	11.38	16.93
PIR	13.71	14.56	9.04	13.42	13.34
BUM	17.50	17.65	11.01	16.10	17.74
CLP	17.70	16.56	11.95	16.80	19.48
AML	17.70	16.56	10.82	13.27	25.81
XIP	24.60	20.96	13.22	19.64	14.40
AET	26.45	19.23	15.54	21.85	21.17
TRI	30.50	29.04	17.50	21.84	>22
NSC	14	15	15	15	14
RTA	31	30	21	25	30

[a] SDS 40 m*M*. Other conditions as in Table 2.

3.3. Effect of SDS concentration

The SDS concentration was varied in the range 30–60 m*M* using 4% THF. Retention factors, *k*, for DIU were obtained at 50°C. As can be observed in Fig. 2 (plot of $\log k$ for DIU vs. log[SDS]), an increase of the SDS concentration produced shorter retention times for all DIU. In addition, the curves obtained for most of them tend to converge (for SDS 30 m*M*, NSC was 15 and RTA 45 min and for SDS 60 m*M*, NSC was 11 and RTA 15 min). Thus, not only the retention factors, *k*, but also the selectivity depend on the SDS concentration. 40 m*M* SDS was finally selected as a compromise between resolution and run time analysis.

3.4. Bivariant optimization method for the SDS–THF system

A bivariant optimization method using a continuous variation of the concentrations of the SDS–THF system was performed (SDS concentration was decreased when that of THF was increased). Two different solutions containing SDS–THF 20 m*M*: 8% and 80 m*M*: 2% were appropriately mixed by programming the pump to obtain adequate mobile phases covering SDS 20–80 m*M* and 2–8% THF

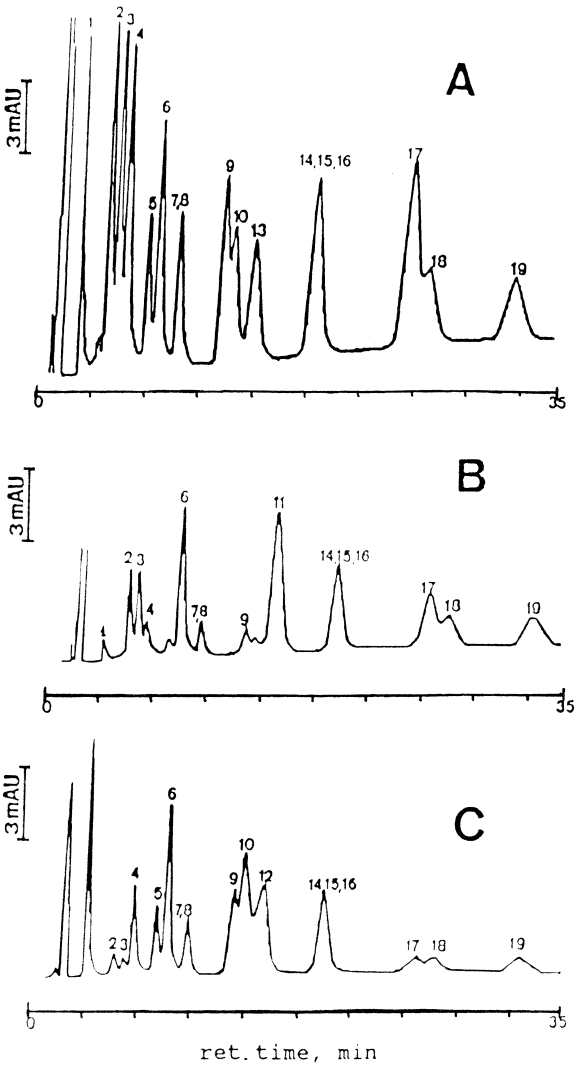


Fig. 1. Chromatograms obtained for a standard mixture of DIU prepared in methanol (5 μg $m1^{-1}$) with UV absorbance detection at different wavelengths: (A) 220 nm, (B) 245 nm and (C) 275 nm. Peaks: 1=HFM, 2=CLR, 3=DCP, 4=TCM, 5=ALT, 6=FRS, 7=IND, 8=BNZ, 9=POL, 10=BND, 11=SPL, 12=CAN, 13=PIR, 14=BUM, 15=CLP, 16=AML, 17=XIP, 18=AET, 19=TRI. Conditions: Hypersil C_{18} (150 mm×3.0 mm I.D., 5 μm) (50°C); mobile phase (SDS 40 m*M*, 4% THF) pH 3.2; flow-rate, 0.5 ml min^{-1}.

concentration ranges. Mobile phases of SDS–THF (20 m*M*: 8.0%), (35 m*M*: 6.5%), (50 m*M*: 5%), (65 m*M*: 3.5%), (80 m*M*: 2.0%) allowed the separation of 10, 13, 14, 14 and 12 compounds involving RTA close to 42, 37, 25, 19 and 16 min, respectively. As expected, changes in selectivity were observed for the mobile phases assayed (e.g., different coelutions occurred). Moreover, mobile phases SDS–THF (50 m*M*: 5%) and (65 m*M*: 3.5%) yielded similar results to those obtained before (40 m*M*: 4%) based on NSC and RTA. This behavior corroborates the univariant method and guarantees the SDS concentration over its cmc. Obviously, the optimal conditions can be achieved by decreasing the SDS concentration from 65 m*M* and increasing THF from 3.5%. In summary, the bivariant design seems to be adequate for optimizing micellar mobile phase composition and consequently for the separation of complex mixtures.

3.5. Effect of temperature

The effect of temperature on DIU retention was studied in the 40–70°C range under the optimum working conditions. An improvement of the chromatographic resolution and a decrease of retention was observed with increasing temperature. An exception to this behavior was found for SPL, AML and TRI, where the retention remained practically constant when the temperature was increased (RTA was always about 34 min, however at 40 and 70°C, NSC was 13 and 12, respectively). The linear behavior observed in Fig. 3 (Van't Hoff plots) shows that the integrity of the micelle structure is maintained over the temperature range studied [36–38]. Enthalpy values, ΔH, were derived from the slopes (Fig. 3) and were found to be in the range -1.33 to -12.71 kJ $mo1^{-1}$. A temperature of 50°C was finally chosen taking into account NSC and peak shape.

3.6. Calibration graphs

Standards containing mixtures of DIU were prepared by adding to blank urine samples five different concentrations in the range 0.5–20 μg ml^{-1} using 5 μg ml^{-1} of XIP (for HFM, TCM, IND, POL, PIR, BUM and TRI), ALT (for CLR, BNZ, BND, AML and XIP), BNZ (for DCP, ALT, CAN, CLP and ETA) or TCM (for TRS and SPL) as internal

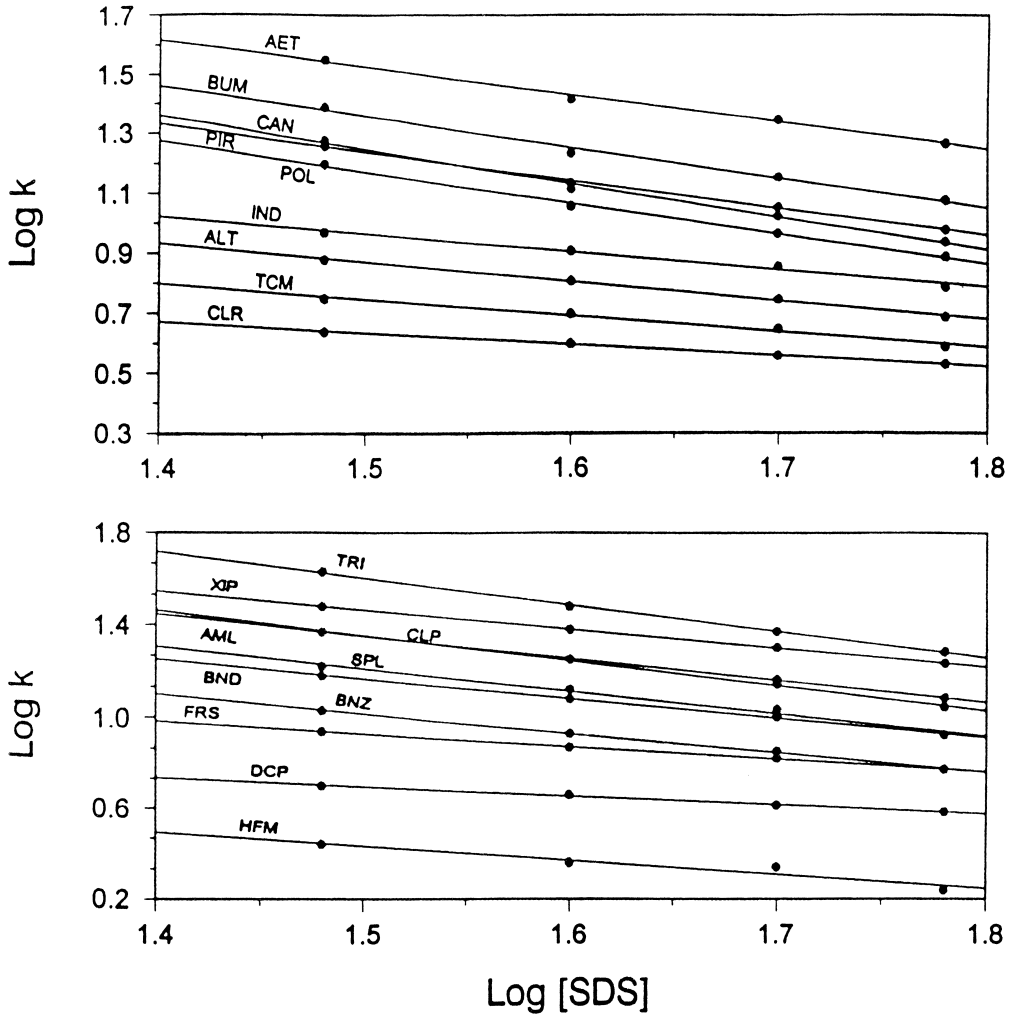


Fig. 2. Effect of SDS concentration on DIU retention.

standard (I.S.), under sample preparation conditions (Section 2.4). These solutions were analyzed with a mobile phase composed of 40 m*M* SDS containing 4% THF, a flow-rate of 0.5 ml min^{-1}, and UV absorbance detection at 220 nm, with the exception of SPL and CAN which were detected at 245 and 275 nm, respectively. The results were analyzed by linear regression. Plotting each DIU peak area to I.S. ratio (PAR) versus the concentration (x) of each diuretic, the calibration equation $PAR = A + Bx$ (μg ml^{-1}) was obtained. Table 4 shows parameters A (intercept), B (slope) and r (regression coefficient). In all cases the intercepts were not significantly different from zero.

3.7. Precision and detection limits

The precision was examined by analyzing five different samples of DIU containing 5 μg ml^{-1} each using the calibration graphs. The C.V. for each DIU is shown in Table 4. The detection limits (LODs) for each DIU were assessed for a signal-to-noise ratio (S/N) of 3 ($n = 10$) by means of the calibration graphs (Table 4).

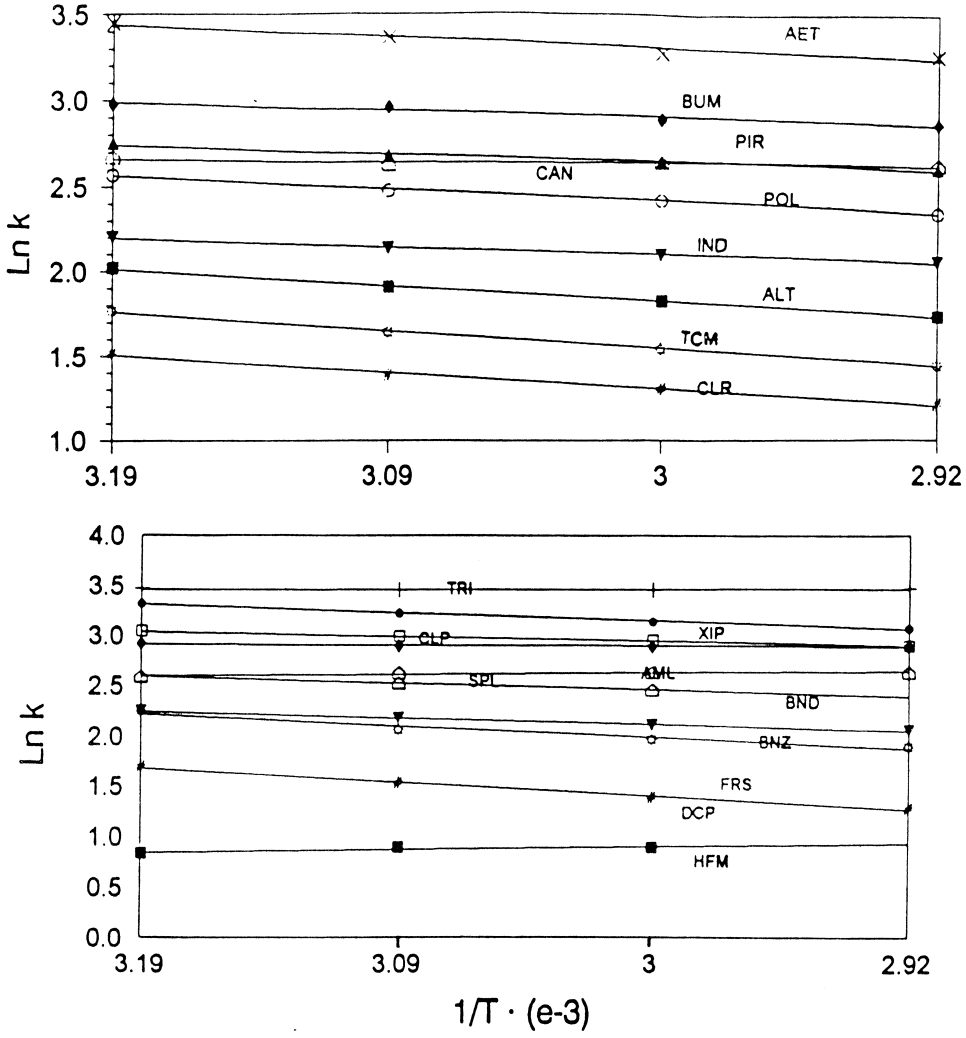


Fig. 3. Effect of temperature on the separation of DIU.

3.8. Application to urine samples

The proposed method was applied to the analysis of urine samples containing xipamide for screening purposes. Samples were collected at appropriate time intervals post-dose from three healthy volunteers administered Diurex® (see Section 2.4). Blank samples from different sex and age subjects were also collected before drug administration. Selectivity was assessed using the above blank urine samples spiked with standard solutions of XIP. The chromatograms obtained were free of interferences, allowing XIP detection. The LOD value (Table 4) was corroborated using four samples spiked with XIP at this concentration, obtaining a signal-to-noise ratio of >3. As an example, Fig. 4 shows the chromatograms obtained by direct injection of urine samples from a 24 year old volunteer, before (Fig. 4A) and after oral administration of a Diurex tablet (Fig. 4B). As can be seen from Fig. 4B, xipamide was identified. A comparison of Fig. 4A and Fig. 4B indicates that the proposed method is adequate for analysis of urine samples containing xipamide without matrix interferences [C.V. ($n = 6$) of the retention factors for

Table 4
Linear regression equations (PAR = $A + Bx$), detection limits (LODs) and C.V. for DIU[a]

DIU	*A*	*B*	*r*	C.V. (%)	LOD ($\mu g\ ml^{-1}$)
HFM	0.038	0.053	0.991	3.16	0.015
CLR	0.018	0.169	0.994	9.15	0.001
DCP	0.052	0.208	0.996	1.40	0.009
TCM	0.025	0.064	0.995	5.24	0.013
ALT	0.029	0.115	0.994	2.76	0.017
FRS	0.022	0.091	0.998	5.77	0.015
IND	0.014	0.037	0.998	3.71	0.022
BNZ	0.030	0.090	0.998	1.86	0.001
POL	0.001	0.055	0.991	0.75	0.015
BND	0.025	0.077	0.996	0.80	0.001
SPL	0.007	0.055	0.997	7.22	0.026
CAN	0.018	0.124	0.998	0.54	0.016
PIR	0.006	0.038	0.993	1.06	0.021
BUM	0.007	0.038	0.996	3.80	0.021
CLP	0.017	0.097	0.998	3.60	0.020
AML	0.011	0.123	0.997	3.58	0.001
XIP	0.012	0.220	0.994	1.23	0.001
AET	0.032	0.121	0.998	2.10	0.016
TRI	0.003	0.020	0.995	3.36	0.039

[a] PAR is the peak area ratio of DIU to 5 $\mu g\ ml^{-1}$ I.S. (XIP, ALT, BNZ or TCM); *x* is in $\mu g\ ml^{-1}$ of DIU and *r* is the correlation coefficient.

xipamide was <1%]. In other words, the detected endogenous compounds (EC) present in the urine matrix did not interfere with xipamide detection. Peak purity was investigated further using a diode-array detector (DAD) by displaying the spectra obtained at different points across the peak and comparing them with that obtained previously for xipamide (see Section 2.5). The possible impurities in each peak detected were negligible [39]. Thus, this separation showed the possibility of detecting/determining xipamide in human urine samples (over detection limits) without sample preparation. In a similar way, when comparing the chromatograms in Fig. 1 and Fig. 4A, this separation could also be applied to the detection of any of the diuretics under study for screening purposes.

4. Conclusions

Several micellar mobile phases were prepared with different organic modifiers and SDS. They were used for the separation of a complex sample containing DIU using a 3.2 mm inner diameter Hypersil C_{18}

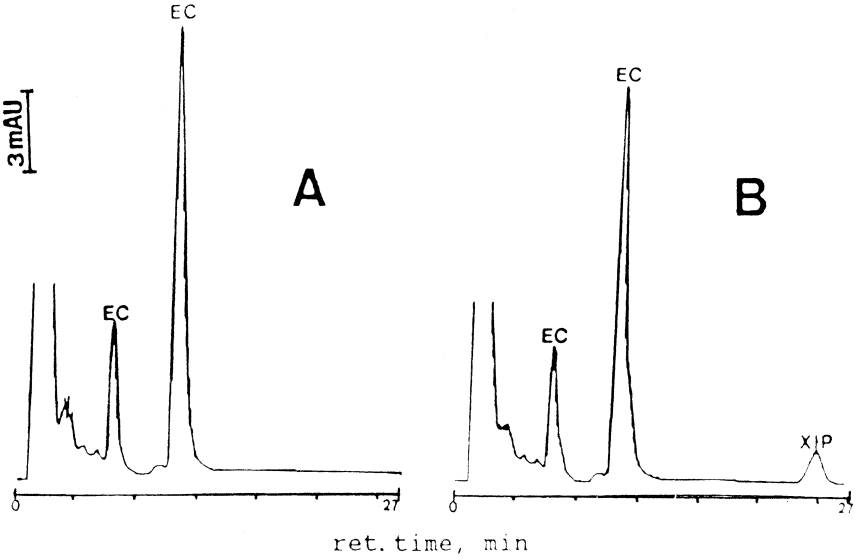


Fig. 4. Chromatograms obtained with UV detection at 220 nm under optimal conditions from: (A) a blank urine sample and (B) a urine sample of a subject administered Diurex (tablets containing 20 mg XIP) with direct injection. EC are endogenous compounds.

column. Fourteen of 19 DIU were separated in 31 min using a SDS–THF mobile phase. The coelution of SPL, CAN and PIR can be resolved by monitoring these compounds at 200, 245 or 275 nm, respectively. The proposed method is sensitive and reproducible, and has been shown to be simple and rapid since it does not require sample preparation. The samples were injected directly onto the column and endogenous compounds present in the urine sample did not cause any interference with xipamide. This separation method can potentially be applied for the detection of any diuretic under study for screening purposes. However, the risk of interferences is clear since they can vary from sample to sample.

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References

[1] R. Herráez-Hernándex, P. Campíns-Falcó, A. Sevillano-Cabeza, Chromatographia 33 (1992) 177.
[2] A.I. Gasco-López, R. Izquierdo-Hornillos, J. Chromatogr. (submitted for publication).
[3] J.S. Landy, J.G. Dorsey, J. Chromatogr. Sci. 22 (1984) 68.
[4] D.W. Armstrong, S.J. Henry, J. Liq. Chromatogr. 3 (1981) 657.
[5] A. Berthod, L.G. Dorsey, Analusis 16 (1988) 75.
[6] D.W. Armstrong, Sep. Purif. Methods 14 (1985) 213.
[7] J.G. Dorsey, Adv. Chromatogr. 27 (1987) 167.
[8] A. Berthod, J.M. Asensio, J.J. Laserna, J. Liq. Chromatogr. 12 (1989) 2621.
[9] P.N. Fraga, E.B. González, A. Sanz-Medel, Anal. Chim. Acta 219 (1988) 181.
[10] A. Arunyanart, L.J. Cline Love, J. Chromatogr. 342 (1985) 293.
[11] M.G. Khaledi, J. Chromatogr. A 664 (1994) 1.
[12] Q.C. Meng, Y.F. Chen, S. Oparil, J. Chromatogr. 529 (1990) 201.
[13] C.T. Azumaya, J. Chromatogr. 532 (1990) 168.
[14] S. Bodenan, M. Paillet, M.O. Christen, J. Chromatogr. 533 (1990) 275.
[15] C. March, D. Farthing, B. Wells, E. Besenfelder, H.T. Karnes, J. Pharm. Sci. 79 (1990) 453.
[16] Y. Pramar, V. Das Gupta, T. Zerai, Drug Dev. Ind. Pharm. 17 (1991) 747.
[17] R.P. Haribhakti, K.S. Alexander, G.A. Parker, Drug Dev. Ind. Pharm. 17 (1991) 805.
[18] F. Varin, T. Minh Tu, F. Benoît, J. Chromatogr. 574 (1992) 57.
[19] R. Herráez-Hernández, P. Campíns-Falcó, A. Sevillano-Cabeza, J. Chromatogr. 582 (1992) 181.
[20] M. Saarinen, H. Sirén, M.L. Riekkola, J. Liq. Chromatogr. 16 (1993) 4063.
[21] R. Ventura, T. Nadal, P. Alcalde, J.A. Pascual, J. Segura, J. Chromatogr. A 655 (1993) 233.
[22] T.G. Wells, I.R. Hendry, G.L. Kearns, J. Chromatogr. 570 (1991) 235.
[23] P. Campíns-Falcó, R. Herráez-Hernández, M.D. Pastor-Navarro, J. Liq. Chromatogr. Relat. Technol. 20 (1997) 1867.
[24] W.J. Bachman, J.T. Stewart, J. Chromatogr. 612 (1993) 161.
[25] F. De Croo, W. Van Den Bossche, P. De Moerloose, J. Chromatogr. 325 (1985) 395.
[26] R. Herráez-Hernández, P. Campíns-Falcó, A. Sevillano-Cabeza, J. Liq. Chromatogr. 15 (1992) 2205.
[27] R.O. Fullinfaw, R.W. Bury, R.F.W. Moulds, J. Chromatogr. 415 (1987) 347.
[28] P. Campíns-Falcó, R. Herráez-Hernández, A. Sevillano-Cabeza, Chromatographia 35 (1993) 317.
[29] M.A. Alliegro, K.D. Dyer, E.J. Cragoe Jr., B.M. Glaser, M.C. Alliegro, J. Chromatogr. 582 (1992) 217.
[30] R. Herráez-Hernández, P. Campíns-Falcó, A. Sevillano-Cabeza, Chromatographia 33 (1992) 177.
[31] E. Bonet-Domingo, M.J. Medina-Hernández, G. Ramis-Ramos, M.C. García Alvarez-Coque, Analyst 117 (1992) 843.
[32] D. Dadgar, M.T. Kelly, Analyst 113 (1988) 1223.
[33] E. Bonet-Domingo, J.R. Torres Lapasió, M.J. Medina-Hernández, M.C. García Alvarez-Coque, Anal. Chim. Acta 287 (1994) 201.
[34] E. Bonet-Domingo, M.J. Medina-Hernández, G. Ramis-Ramos, M.C. García Alvarez-Coque, J. Chromatogr. 582 (1992) 189.
[35] C. Tandford, Hydrophobic Effect. Formation of Micelles and Biological Membranes, 2nd ed., Wiley, New York, 1980.
[36] J.R. Torres Lapasió, J.J. Baeza-Baeza, M.C. García Alvarez Coque, J. Chromatogr. A 769 (1997) 155.
[37] F.P. Tomasella, J. Fett, L.J. Cline Love, Anal. Chem. 63 (1991) 474.
[38] J.G. Dorsey, M.T. DeEchegaray, J.S. Landy, Anal. Chem. 55 (1983) 924.
[39] B.K. Logan, Anal. Chim. Acta 288 (1994) 111.