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Optimization of selectivity in micellar chromatographic procedures for the determination of drugs in urine by direct injection

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Abstract: Selectivity was optimized for the determination of drugs in urine by direct injection micellar chromatography through changes in specific mobile phase parameters. The rôle of mobile phase pH and the type of surfactant used for mobile phase preparation was investigated. The retention of the urine matrix was found to be minimal between pH 5.5 and 7.5. The non-ionic surfactant, polyoxyethylene 23 lauryl ether (Brij 35), was found to be the surfactant of choice for the separation of drugs from urine. Favourable retention of both the urine background and the analyte was achieved with this surfactant. Micellar mobile phases of optimal composition were used to develop chromatographic procedures for the determination of furosemide, hydrochlorothiazide and propranolol in urine. Good accuracy (98–102% of drug recovered), precision (1–4% RSD) and linearity were obtained for all methods. Limits of detection for all drugs were adequate.

Keywords: *Direct urine injection; micellar liquid chromatography; hydrochlorothiazide; furosemide; propranolol.*

Introduction

Aqueous solutions of surfactants at concentrations above the critical micelle concentration (CMC) were first demonstrated by Armstrong and Henry [1] to be suitable mobile phases for reversed-phase chromatography with advantages over traditional hydro-organic mobile phases. These advantages include the ability to simultaneously chromatograph hydrophilic and hydrophobic compounds, the lower cost and greater safety of micellar mobile phases as compared with conventional mobile phases containing organic solvents, and the greater solubility of solutes that can be used to control ionic strength, pH and buffering capacity in micellar mobile phases. Since that first report, micellar chromatography has become a simple, rapid, accurate and precise method for the determination of drugs in various biological fluids by direct injection. The unique selectivity and solubilizing power of micellar mobile phases have resulted in the analysis of drugs in untreated serum [2–8] and urine [3, 8, 9]. Traditional methods for the determination of drugs in biological fluids typically include preliminary sample work-up steps. The advantages of direct injection techniques such as micellar chromatography over these procedures include reduced analysis time, increased sample throughput, improved accuracy and improved precision [10]. Direct injection is often accomplished by the automation of the sample pretreatment operations through the use of precolumns in conjunction with column-switching instrumentation. However, these automated direct injection procedures are more complicated than the micellar technique in that they require additional instrumentation in the form of precolumns, switching valves and HPLC pumps, and require accurate and precise timing of valve switching for the separation to be successful [10].

Previous work with micellar chromatography for the determination of drugs in urine [3, 8] focused on developing separations primarily on the basis of individual drug retention. In the present study, specific mobile phase parameters were identified and examined. When these parameters were properly chosen, the retention of the endogenous components of urine was minimized, and the selectivity of the separation between a drug and these components was improved. Optimal control of these parameters, which included pH and the type of surfactant used, can simplify the development of micellar chromatographic procedures for the determination of virtually any drug in urine.

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Three model compounds, furosemide, hydrochlorothiazide (HCT) and propranolol, were chosen for this investigation. These drugs were used as they are frequently monitored in urine using conventional liquid chromatographic procedures that require time-consuming sample pretreatment steps [11–17]. Additionally, they were expected to exhibit very different chromatographic behaviour. The structures of the three compounds are shown in Fig. 1. Furosemide has a carboxylic acid group that can ionize in the normal range of mobile phase pH used in reversed-phase chromatography. HCT contains polar functional groups including an ionizable sulphonamide that remains neutral in the normal pH range. Propranolol is a basic amine that will be positively charged over the entire usable pH range. Optimized micellar chromatographic procedures for the determination of the model compounds in urine were developed and pertinent analytical data that demonstrate the validity of these methods are presented.

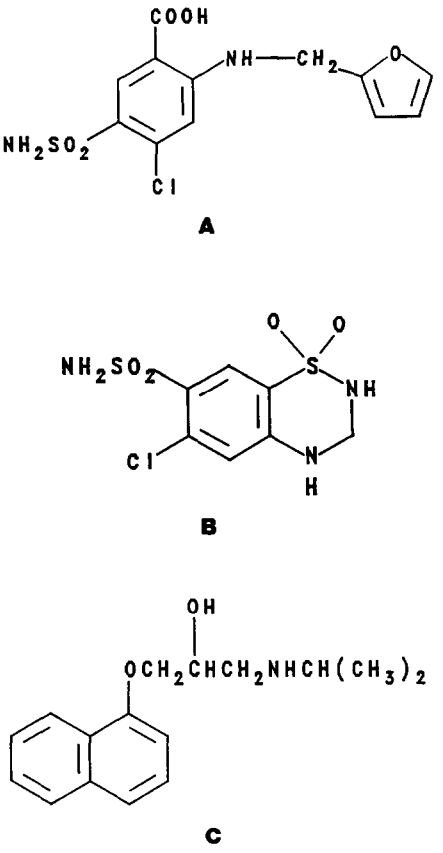

Figure 1
Structures of (A) furosemide, (B) hydrochlorothiazide and (C) propranolol.

Experimental

Reagents

Polyoxyethylene 23 lauryl ether (Brij 35) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Sodium dodecyl sulphate (SDS), electrophoresis purity, was obtained from Bio Rad Laboratories (Richmond, CA, USA). Furosemide, hydrochlorothiazide and propranolol were USP Reference Standards obtained from the United States Pharmacopeial Convention, Inc. (Rockville, MD, USA). All reagents were used as received.

Mobile phase compositions

Mobile phases were prepared by dissolving the appropriate quantity of surfactant with any indicated buffer or organic additive in distilled water. Mobile phases specifically for pH studies were prepared by dissolving the appropriate quantity of surfactant in a 0.01 M Na_2HPO_4 solution. Any adjustments in mobile phase pH were made with a 50% sodium hydroxide solution or with phosphoric acid (85%) as required. All mobile phases were filtered through a 0.45-μm nylon-66 membrane filter and degassed under vacuum before use. The mobile phase composition employed for the determination of each drug in urine is presented in Table 1.

Table 1
Chromatographic parameters for the analysis of drugs in urine by micellar direct injection

Drug	Injection volume (μl)	Type of detection	Mobile phase composition
Furosemide	20	Fluorescence (λ_{ex} = 235 nm)	0.02 M Brij 35 and 0.01 M Na_2HPO_4, pH 3.0
HCT	10	Ultraviolet (λ = 271 nm)	0.02 M Brij 35, 0.004 M SDS and 0.01 M Na_2HPO_4, pH 6.5
Propranolol	50	Fluorescence (λ_{ex} = 215 nm)	0.08 M Brij 35 and 2.0% Triethylamine, pH 5.0

Apparatus

An HPLC system consisting of a Perkin–Elmer Series 100 high pressure pump, a Rheodyne Model 7010 fixed loop sample injection valve, a Schoeffel Spectroflow Monitor SF 770 variable wavelength UV–vis detector, a Schoeffel FS970 fluorometric detector and a Perkin–Elmer R50 strip chart recorder was used in conjunction with a 5-μm Hypersil C_{18} column (Hewlett–Packard, Avondale, PA, USA) [100 × 4.6 mm i.d.]. A Rheodyne Model 7335 high pressure filter containing a 0.5-μm filter element, 3 mm dia, was placed between the injection valve and the column.

A 10-μl injection volume was used for all investigative studies. The flow rate was 1.0 ml min^{-1} throughout. A 320 nm emission wavelength cutoff filter and a photomultiplier tube voltage setting of 350 V were used for the detection of all fluorescent compounds. The injection volume, type of detection and the detection or excitation wavelength used in the specific method developed for each drug are summarized in Table 1.

Procedures

The time equivalent, t_m, of the void volume was determined for the column by injecting distilled water and measuring the time of the first deviation from the baseline. The value obtained for t_m was used to calculate capacity factors, k'. Column efficiencies, N, were calculated using the asymmetry based equation derived by Dorsey and Foley [18],

$$N = \frac{41.7(t_R/W_{0.1})^2}{B/A + 1.25},$$

where t_R is the retention time of the peak, $W_{0.1}$ is the peak width at 10% of peak height, B is the distance between the centre of the peak and the trailing edge of the peak measured at 10% of peak height, and A is the distance between the leading edge of the peak and the centre of the peak measured at 10% of peak height. The peak asymmetry factor, A_s, was calculated with the equation $A_s = B/A$, where B and A are defined previously.

The working concentrations used for the investigative studies were 25 μg ml^{-1} furosemide, 13 μg ml^{-1} hydrochlorothiazide and 155 μg ml^{-1} propranolol. The recovery of each drug from urine was determined at two concentration levels: furosemide, 1 and 10 μg ml^{-1}; hydrochlorothiazide, 1 and 25 μg ml^{-1}; and propranolol, 0.4 and 5 μg ml^{-1}. The peak heights of six urine samples spiked with drug at the indicated concentrations were compared with the peak heights of standards prepared with distilled water at the same concentrations to determine the amount of drug recovered and the precision of the method. All urine samples were filtered through a 0.45-μm nylon-66 membrane filter before use and injected directly into the chromatographic system without further treatment.

The relative limits of detection (LOD) were determined by extrapolating the plot of peak height versus standard concentration used to construct the linear calibration curve to where the peak height equalled three times the baseline noise. The standard deviation of the response from six injections of the blank urine measured under the appropriate chromatographic conditions at the retention time for each respective drug was used to quantify the baseline noise. Absolute limits of detection were calculated by multiplying the relative LOD times the volume injected.

Results and Discussion

Effect of pH

The influence of pH on the retention of a biological matrix has not been examined to any great extent for the direct injection technique. Most reports of micellar direct injection for the analysis of drugs in serum or urine use mobile phases at their native pH [2–5]. Separations that require pH adjustment do so to change the ionization state of the analyte of interest [2, 6–8].

The effect of pH was examined in this study by measuring the retention of the last peak of the urine matrix as a function of mobile phase pH with the surfactant concentration held constant. The variation in k' of this last eluting component with changing pH is shown in Fig. 2 for both SDS and Brij 35 mobile phases. Retention with both surfactants remained essentially constant as pH was decreased from 7.5 to 5.5. A sharp increase in k' was measured when the pH was further lowered below pH 5.5 to 3.0. The value of k' increased approximately 2.5 times with the Brij 35 mobile phase and 3 times with the SDS mobile phase between pH 5.5 and 3.0.

The relationship between surfactant concentration, pH and the retention of urine is presented for SDS mobile phases in Fig. 3A

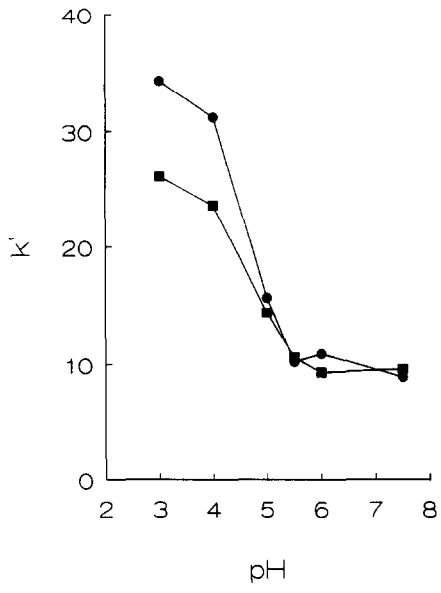

Figure 2
Change in the capacity factor, k', of the last eluting component of the urine matrix as a function of mobile phase pH. ●, 0.06 M SDS in 0.01 M Na_2HPO_4 mobile phases. ■, 0.01 M Brij 35 in 0.01 M Na_2HPO_4 mobile phases.

and Brij 35 mobile phases in Fig. 3B. The data demonstrate that retention of the urine matrix is minimized at pH 7.5, particularly with SDS mobile phases, for all surfactant concentrations studied. As the surfactant concentration of the Brij 35 mobile phase was increased, the difference between k' for the pH 3.0 and 7.5 mobile phases decreased markedly. In fact, k' at these pHs approached the same value ($k' = 10$) as the surfactant concentration neared 0.10 M. The retention of the urine matrix was prohibitively long ($k' > 100$) with the 0.01 M SDS mobile phase at pH 3.0. Even at the fairly high SDS concentration of 0.06 M, the retention of the last component of the matrix was much higher at pH 3.0 ($k' = 30$) than at pH 7.5 ($k' = 9$). Based on these results, the use of SDS mobile phases having a pH much below the optimum range established (pH 5.5–7.5) would not be feasible, even at high SDS concentrations. The surfactant concentrations examined encompass those successfully employed in the application of micellar chromatography to the direct injection of biological fluids [2–9].

Surfactant selection

The retention of the last eluting peak of the urine matrix as a function of surfactant type is compared at pH 3 in Fig. 4A, and at pH 5 and 7.5 in Fig. 4B. The retention of the urine components was markedly longer with SDS mobile phases than with Brij 35 mobile phases at pH 3 and 5, even at higher surfactant concentrations. The differences between the two surfactant types were less significant at pH 7.5. At this pH, the retention was longer with SDS mobile phases having lower surfactant

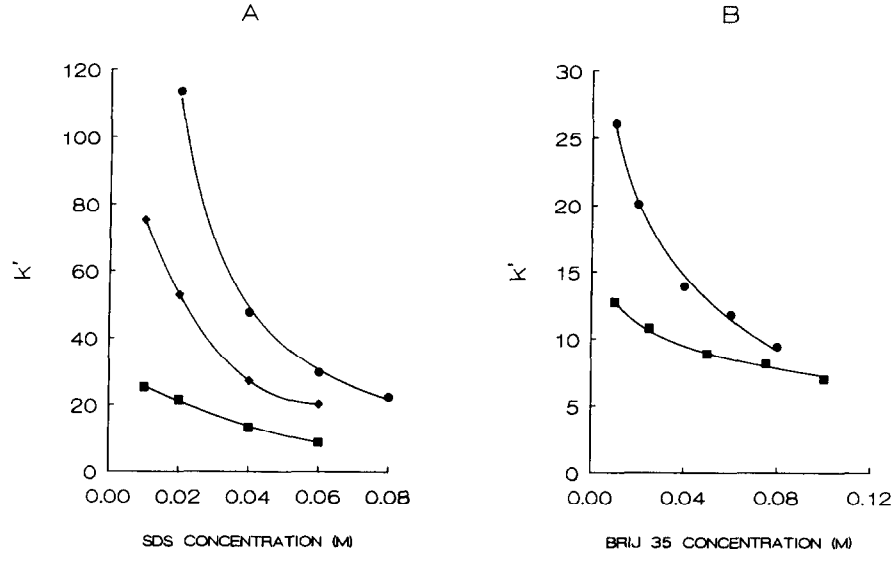

Figure 3
Change in the capacity factor, k', of the last eluting component of the urine matrix as a function of mobile phase surfactant concentration at various pHs. (A) SDS mobile phases at pH 3.0 (●), 5.0 (◆) and 7.5 (■). (B) Brij 35 mobile phases at pH 3.0 (●) and 7.5 (■).

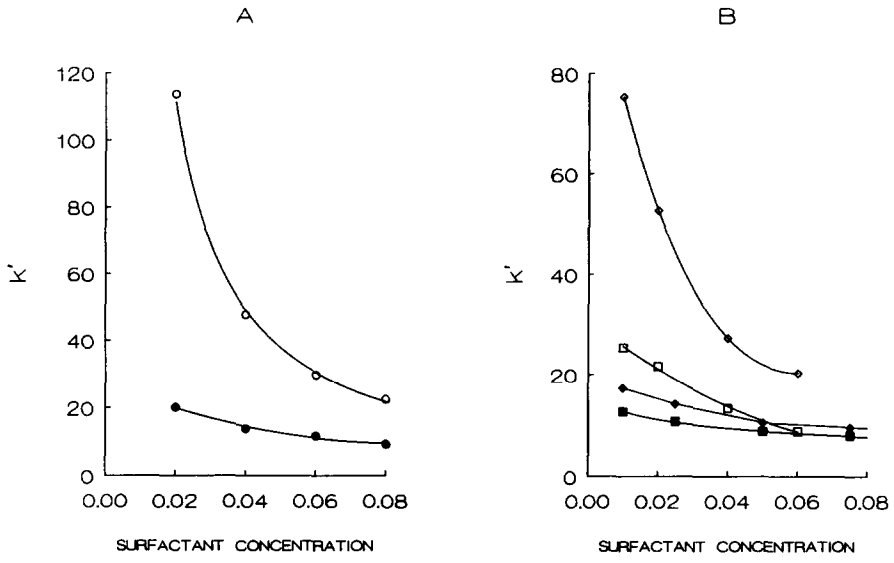

Figure 4
Comparison of the capacity factor, k', of the last eluting component of the urine matrix as a function of surfactant type. (A) SDS (○) and Brij 35 (●) mobile phases at pH 3.0. (B) Mobile phases of SDS at pH 5.0 (◇) and 7.5 (□), and Brij 35 at pH 5.0 (◆) and 7.5 (■).

concentrations, but approached the retention obtained with Brij 35 at the higher surfactant concentrations. Also, as previously shown in Figs 3A and 3B, the retention of the urine matrix was much less pH dependent for Brij 35 mobile phases than for SDS mobile phases. These results indicate that Brij 35 is the surfactant of choice if the objective is to minimize the retention of the urine matrix. The retention of the matrix, however, must be considered in conjunction with that of the analyte if a separation is to be achieved.

Hydrochlorothiazide. The separation of HCT from urine, even with mobile phases of optimal pH, could only be accomplished with a Brij 35 mobile phase. The required resolution (Fig. 5) was obtained between HCT and urine with this surfactant type since retention of the HCT was much longer with Brij mobile phases than with SDS mobile phases (Fig. 6), even though Brij minimized the retention of the urine matrix.

Furosemide. The separation of furosemide from urine was difficult because furosemide had minimal retention in the optimal pH range (pH 5.5–7.5) due to the ionized carboxylic acid group (pK_a = 3.9 [19]). Maximal retention of furosemide was obtained at low pH. Resolution between the urine components and furosemide was achieved with a 0.02 M Brij 35, pH 3.0 mobile phase (Fig. 7). The retention of furosemide was sufficiently longer than that of urine components at this Brij 35 concentration, even though the retention of the urine matrix was at its maximum value at this pH. No separation could be accomplished with SDS mobile phases at higher pH values due to the minimal furosemide retention, or at lower pH values due to the long retention of the urine matrix. Unlike HCT, the capacity factor of furosemide was similar with both surfactant types (Fig. 6). The separation obtained with Brij was due only to the significantly shorter retention of urine at pH 3 with this surfactant than with SDS, as illustrated in Fig. 4A.

A better understanding of the chromatographic interactions that lead to the observed retention of HCT and furosemide with Brij 35 and SDS can be obtained with the three-phase equilibrium model [20]. The capacity factor is related to micellar mobile phase concentration through the equation

$$1/k' = \frac{[M_m]K_2}{\phi[L_s]K_1} + \frac{1}{\phi[L_s]K_1},$$

where k' is the chromatographic capacity factor, $[M_m]$ is the concentration of surfactant in the micelle in the mobile phase, $[L_s]$ is the concentration of stationary sites, ϕ is the

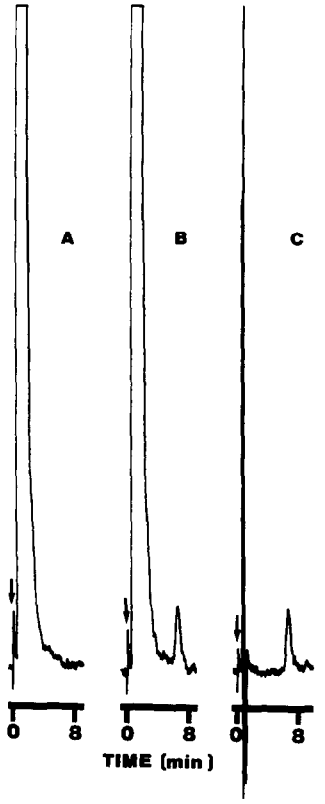

Figure 5
Chromatograms of (A) urine matrix, (B) urine spiked with 1.0 μg ml^{-1} of hydrochlorothiazide and (C) 1.0 μg ml^{-1} of hydrochlorothiazide in water, all recorded at a range of 0.01 AUFS; see Table 1 for chromatographic conditions.

chromatographic phase ratio, K_1 is the equilibrium constant for the transfer of solute in the bulk solvent mobile phase to the stationary phase and K_2 is the equilibrium constant for the transfer of solute in the bulk solvent mobile phase to the micelle per monomer of surfactant. The equation can be solved graphically by plotting $1/k'$ vs $[M_m]$ which gives the value of K_2 as the slope–intercept ratio and the value of $\phi[L_s]K_1$ as the reciprocal of the intercept. The equilibrium for the transfer of solute to the micelle per micelle, K_{eq}, is obtained by multiplying K_2 by the aggregation number of the surfactant. The equilibrium expressions obtained for HCT and furosemide are presented in Table 2. Both SDS and Brij 35 have been shown to adsorb to C_{18} ligands in micellar chromatography in amounts approximating the bonded phase itself [21].

The $\phi[L_s]K_1$ term can be used to compare the magnitude of solute-stationary phase interactions for the two types of surfactants without solving for K_1, since the same C_{18} bonded phase was used with both mobile phases. K_{eq}

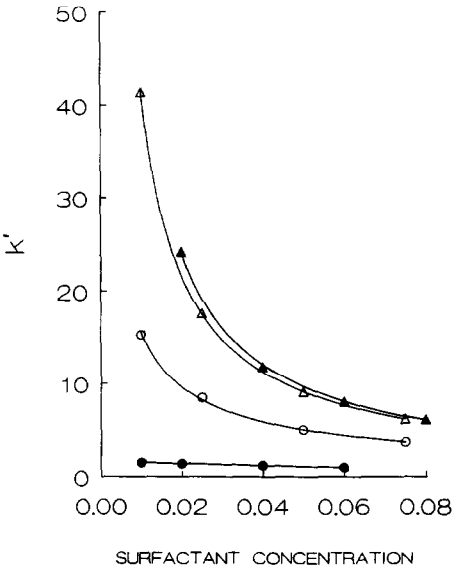

Figure 6
Change in the capacity factors of furosemide and hydrochlorothiazide as a function of surfactant type and concentration. △, Furosemide with a Brij 35 mobile phase at pH 3.0; ▲, furosemide with a SDS mobile phase at pH 3.0; ○, hydrochlorothiazide with a Brij 35 mobile phase at pH 7.5; and ●, hydrochlorothiazide with a SDS mobile phase at pH 7.5.

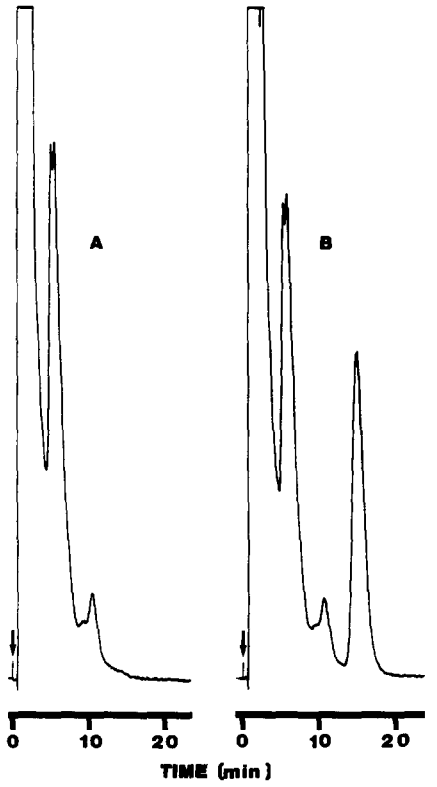

Figure 7
Chromatograms of (A) urine matrix and (B) urine spiked with 4.8 μg ml^{-1} of furosemide, recorded at a range of 0.1 μA; see Table 1 for chromatographic conditions.

Table 2
Equilibrium expressions calculated from chromatographic retention data

Drug	Mobile phase	pH	K_{eq} (l mol^{-1})	$\phi[L_s]K_1$
Furosemide	SDS	3.0	$(5.6 \pm 0.3) \times 10^3$	47 ± 3
Furosemide	Brij 35	3.0	$(1.5 \pm 0.2) \times 10^4$	180 ± 30
HCT	SDS	7.5	$(6.5 \pm 0.3) \times 10^2$	1.6 ± 0.03
HCT	Brij 35	7.5	$(3.4 \pm 0.2) \times 10^3$	27 ± 1
Propranolol	Brij 35/2% Triethylamine	5.0	$(5.2 \pm 0.5) \times 10^3$	92 ± 8

and $\phi[L_s]K_1$ were larger for both HCT and furosemide in a Brij 35 mobile phase than in an SDS mobile phase. The increases in the equilibrium expressions indicate an increased partitioning of the solute from the bulk solvent mobile phase to both the micelle and the stationary phase with Brij 35 mobile phases. The capacity factor of HCT was larger with the Brij 35 mobile phase than with the SDS mobile phase (Fig. 6). The values for the equilibrium expressions indicate that HCT partitioning from the mobile phase to the stationary phase increased to a greater extent than HCT partitioning to the micelle in the Brij 35 mobile phase. Partitioning to the micelle, as indicated by K_{eq}, increased approximately five times, while partitioning to the stationary phase, as indicated by $\phi[L_s]K_1$, increased approximately 17 times. The retention of furosemide remained approximately the same with the two surfactant types (Fig. 6) because although the partitioning of the solute to both the micelle and stationary phase increased with the Brij 35 mobile phase as compared with SDS, both K_{eq} and $\phi[L_s]K_1$ increased by approximately the same magnitude.

Propranolol. The separation of propranolol from the urine matrix was easily accomplished with either SDS or Brij 35 due to the long retention of propranolol with both surfactant types. However, the optimization of the determination in terms of sample detectability and total elution time was complicated by the fact that propranolol is an amine. The amine group of this compound, which has a pK_a of 9.5 [22], is positively charged in the pH range typically used for reversed-phase chromatography. The anionic SDS monomers in the bulk mobile phase and adsorbed to the stationary phase will interact electrostatically with the positively charged propranolol leading to the formation of ion pairs of greater hydrophobicity than propranolol alone. The retention obtained with SDS mobile phases was prohibitively long, even at a high surfactant concentration. The retention time was 54 min and the capacity factor was 68 with 0.1 M SDS. The peak height obtained at this long retention time did not provide the required detectability for the analysis of this drug in urine.

Retention of propranolol with Brij 35 mobile phases was significantly shorter because this surfactant is non-ionic and cannot interact electrostatically. The chromatographic efficiency with these mobile phases, however, was extremely poor ($N = 7.4$) due to excessive peak tailing (asymmetry factor = 5.5). The detectability requirements for the desired analysis were not met because of this poor efficiency. The peak asymmetry was due to the mixed retention mechanism that occurred during the separation. Propranolol will partition to the surfactant modified C_{18} ligands, and will also be electrostatically attracted to the residual silanols of the silica support since the non-ionic Brij 35 surfactant will not interact with the positive charge of propranolol. The significant contribution of surface silanols to the retention of organic amines including propranolol has been previously confirmed [23]. Examination of this separation with the three-phase equilibrium model also supports a mixed retention mechanism. The plot of $1/k'$ vs $[M_m]$, where k' is the capacity factor of propranolol and $[M_m]$ is the concentration of Brij 35 in the micelle, did not exhibit the linear relationship and positive y-intercept predicted by the model [20]. Instead, a shallow curve ($r = 0.9952$, RSD of slope = 2.5%) with a negative intercept (−0.006) was obtained. This indicates deviation from the model, expected in this case since the model and the equation derived from it are based on only one stationary phase equilibrium. The model does not account for both the hydrophobic and electrostatic stationary phase equilibria of propranolol.

Triethylamine is frequently added to conventional reversed-phase mobile phases to bind to the available silanols to reduce the tailing of basic compounds. The utility of using this mobile phase additive in conjunction with Brij 35 mobile phases to improve propranolol peak symmetry was investigated. Table 3 shows the effect of increasing triethylamine concentration on the propranolol peak, with surfactant concentration and pH held constant. As the amount of triethylamine added to the mobile phase was increased, the capacity factor of propranolol decreased while peak symmetry and efficiency increased. The largest change in these values occurred upon the initial addition of triethylamine. Further increases in the triethylamine content produced smaller changes. The significant decrease in retention and peak tailing with the initial addition of triethylamine is further evidence of electrostatic interactions occurring between propranolol and the silanols when Brij 35 mobile phases are used. The smaller changes at the higher triethylamine concentrations are likely due to organic modifier effects rather than masking of the acidic silanols.

Although the capacity factor of propranolol decreased upon the addition of triethylamine to a Brij 35 mobile phase, good separation from the urine blank was achieved (Fig. 8). The improved peak symmetry and efficiency obtained provided sufficient limits of detection for the desired analysis. An optimum mobile phase composition of 0.08 M Brij 35 with 2.0% of triethylamine adjusted to pH 5 was employed for the determination of propranolol in urine. A mobile phase pH of 5 was selected because at this pH the urine matrix exhibited minimal retention and it was sufficiently below the pK_a of silica, which has been reported to be approximately 6.5 [24], to preclude ionization of the silanols. The retention behaviour of propranolol conformed to the three-phase model when Brij 35 mobile phases containing 2% triethylamine at pH 5 were employed. A linear relationship ($r = 0.9990$, RSD of slope = 1.1%) and a positive y-intercept (1.41) were obtained when $1/k'$ was plotted vs $[M_m]$. Values calculated for K_{eq} and $\phi[L_s]K_1$ from this graphical analysis are included in Table 2. Compliance to the model indicates that the addition of triethylamine to the Brij 35 mobile phase has shifted stationary phase interactions primarily to the partitioning of propranolol to the surfactant modified C_{18} ligands.

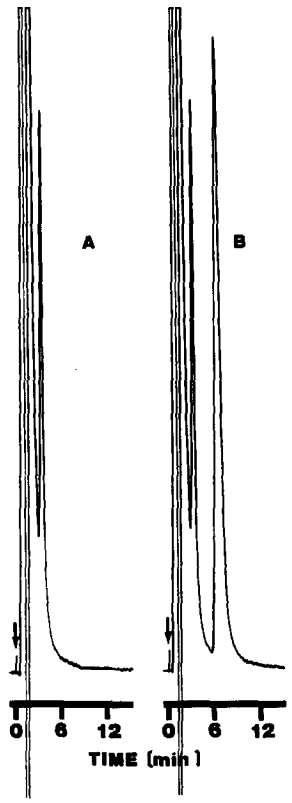

Figure 8
Chromatograms of (A) urine matrix and (B) urine spiked with 5.0 μg ml^{-1} of propranolol, recorded at a range of 0.1 μA; see Table 1 for chromatographic conditions.

Table 3
Effect of triethylamine on the separation of propranolol with a Brij 35 mobile phase*

Triethylamine concentration		k' of propranolol	Asymmetry factor	Theoretical plates (N)
%	mM			
0	0	16.0	5.5	7.4
0.25	18	12.3	2.9	73
0.5	36	11.8	2.5	109
1.0	72	10.4	2.2	146
2.0	140	8.8	1.9	218
3.0	220	7.6	1.8	240

*Mobile phase composition: 0.075 M Brij 35 with triethylamine, pH 5.0.

Additional considerations. A mobile phase composition that is optimal for the separation of a drug of interest from the urine matrix may not always provide adequate separation between the drug and related compounds. Further compound-specific optimization may be required after the desired separation from the matrix is achieved. The results obtained for the two surfactants studied indicate that the non-ionic surfactant, Brij 35, is the surfactant of choice for the analysis of drugs in urine by micellar chromatography. However, for example, although HCT was well separated from the urine matrix with a Brij 35 mobile phase, no resolution could be achieved between HCT and its hydrolysis product, 5-chloro-2,4-disulphamoylaniline, with mobile phases containing only Brij 35. The hydrolysis product can form in aqueous solutions of HCT upon standing at room temperature [25]. Baseline separation could be achieved between the two compounds with SDS mobile phases. However, as previously discussed, the SDS mobile phases did not provide separation of HCT from urine. Unique and improved selectivity can sometimes be obtained through the use of mixed surfactant systems. The final optimized mobile phase employed for the determination of HCT in urine contained 0.004 M SDS, a concentration that is below the CMC of SDS, in addition to 0.02 M Brij 35. The small amount of SDS was added to obtain resolution (R_s = 0.8) between HCT and 5-chloro-2,4-disulphamoylaniline. This resolution was adequate to detect hydrolysis in standards and samples undergoing analysis. The separation of HCT from urine was not compromised with the use of this mobile phase. Mixed surfactant mobile phases have been used previously to obtain the selectivity required to achieve difficult separations [26].

Summary

Micellar chromatographic methods were successfully developed for the analysis of furosemide, HCT and propranolol in urine. Optimization of these methods was based on the knowledge of the control of selectivity gained in the studies discussed above. Excellent specificity was obtained in each case. No interference from the urine peaks was observed at the retention time of any of the drugs as demonstrated by the chromatograms of the HCT, furosemide and propranolol separations presented in Figs 5, 7 and 8, respectively. Good accuracy, precision and linearity were obtained with each of the methods as shown by the data in Table 4. Limits of detection (LODs) for each method are presented in Table 5. The LODs obtained are above the lowest LODs reported in the literature for the liquid chromatographic analysis of the three

Table 4
Retention time, accuracy, precision, and linearity data for the analysis of drugs in urine by micellar direct injection

Drug (retention time, min)	Concentration (μg ml^{-1})	% Recovered (RSD, %)*	Instrumental precision†	Linear range (RSD of the slope, %)‡
Furosemide	1	99.4 (3.3)	1.0	0.3–52
(15.9)	10	101.6 (2.0)	0.9	(0.5)
HCT	1	98.8 (3.9)	3.2	0.3–110
(7.0)	25	98.2 (3.6)	0.3	(0.4)
Propranolol	0.4	102.0 (3.9)	1.2	0.1–20
(6.7)	5	101.4 (0.8)	0.7	(0.4)

*n = 6.
†RSD, %, n = 6.
‡Eight to nine different concentrations were used to determine the linear calibration range; all ranges are in μg ml^{-1}.

Table 5
Limits of detection of selected drugs in urine

Drug	Relative LOD (μg ml^{-1})	Absolute LOD (ng)	Literature LOD* (μg ml^{-1})	Typical concentration range monitored in urine (μg ml^{-1})
Furosemide	0.03	0.5	0.02 [11]	0.1–20 [12, 13]
HCT	0.28	2.8	0.05 [14]	0.5–50 [15]
Propranolol	0.03	1.5	0.001 [16]	0.1–1 [16]

*Lowest LOD value found in the literature for the analysis of the indicated drug in urine using an LC method.

Table 6
Capacity factors of selected drugs and common over-the-counter drugs

Drug	Capacity factor using various optimized mobile phases		
	Furosemide mobile phase	HCT mobile phase	Propranolol mobile phase
Furosemide	21.7	—	—
HCT	—	9.0	—
Propranolol	—	—	8.6
Acetominophen	ND	2.4	ND
Caffeine	ND	1.0	ND
Ibuprofen	ND	16.6	12.2
Salicylic acid	17.0	0.4	1.2

ND = none detected.

compounds. However, each is low enough to cover the concentration range of drug typically expected in urine (Table 5).

The micellar chromatographic methods optimized for the separation of the three model compounds from the urine matrix also provided selectivity for the separation of these compounds from common drug substances. This is demonstrated by the data presented in Table 6. The capacity factors (k') of some common over-the-counter drugs obtained using the various optimized mobile phases are compared in this table to the k' of the model compounds. Such over-the-counter drugs could be present in urine and potentially interfere with the chromatographic separation.

Conclusions

Retention of the sample matrix rather than that of specific drugs has been examined for the first time in the optimization of micellar chromatographic procedures for the determination of drugs in urine. The type of surfactant used in the mobile phase and the pH of the mobile phase have been identified as two key parameters that can be varied to obtain the required resolution between a drug and the components of urine. Retention of the urine matrix could best be minimized with the use of the non-ionic surfactant Brij 35 in mobile phases adjusted between pH 5.5 and 7.5. The retention of the urine components was minimized with Brij 35 and the selectivity offered by this surfactant often lead to favourable retention of the analyte. Proper control of the parameters identified offers a systematic and generalized approach for the development of simple, accurate and reproducible methods for the determination of structurally different drugs in urine.

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