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A micellar liquid chromatographic procedure for the determination of amiloride, bendroflumethiazide, chlorthalidone, spironolactone and triamterene in pharmaceuticals

E. BONET-DOMINGO, M.J. MEDINA-HERNANDEZ and M.C. GARCIA-ALVAREZ-COQUE*

Departamento de Química Analítica, Facultad de Química, Universitat de València, 46100-Burjassot, València, Spain

Abstract: A procedure for the determination of amiloride, bendroflumethiazide, chlorthalidone, spironolactone and triamterene in pharmaceutical preparations (tablets) by micellar liquid chromatography, using a 0.07 M SDS–0.5% pentanol mobile phase, is proposed. Recoveries were found in the 100–108% range, with relative standard deviations of 0.4–3.1%. Elution of the most retained diuretics occurred in less than 18 min (at a 1 ml min^{-1} flow rate). The change in the values of the solute–micelle binding constants and the partition coefficients of the diuretics between the stationary phase and water, upon addition of pentanol, was also studied.

Keywords: *Diuretics; pharmaceuticals; HPLC; micellar hybrid mobile phase.*

Introduction

Diuretics are a group of compounds that are extensively used owing to their therapeutic importance in the treatment of congestive heart failure and hypertension, among other diseases. Unfortunately these compounds are also misused to quickly reduce weight. In the literature, several reversed-phase liquid chromatographic procedures have been reported to evaluate the content of diuretics in pharmaceuticals, the mobile phases being mainly methanol–water and acetonitrile–water [1–4].

In the last decade, some procedures have been reported for the determination of drugs by micellar liquid chromatography [5–9]. The use of micellar mobile phases has some advantages over the conventional hydro-organic mobile phases, they are less expensive, less flammable, non-toxic and are able to separate solutes of different nature because of the large number of interactions (electrostatic, hydrophobic and steric). Recently, we described a procedure to determine diuretics of different types by micellar liquid chromatography, using a 0.05 M sodium dodecyl sulphate (SDS)–3% propanol mobile phase and a C_{18} column. Amiloride, triamterene and spironolactone showed long retention times (18, 29 and 43.5 min, respectively) [10], therefore for these diuretics a mobile phase of higher eluent strength should be more appropriate.

The retention of many solutes with purely micellar eluents is frequently excessive and a short-chain alcohol must be added to achieve adequate retention times. The decrease in the capacity factors is larger as the concentration and hydrophobicity of the alcohol increases. The addition of an alcohol also leads to improvements in the efficiency [11].

In this work, a 0.07 M SDS–0.5% pentanol mobile phase is recommended for the determination of amiloride, bendroflumethiazide, chlorthalidone, spironolactone and triamterene in tablets, which reduced the elution of the most retained diuretics to less than 18 min (at a 1 ml min^{-1} flow rate). The change in the values of the solute–micelle binding constant, K_{AM}, and the stationary phase–water partition coefficient, P_{SW}, when pentanol is added to a SDS micellar phase, is also studied.

* Author to whom correspondence should be addressed.

Experimental

Reagents

Mobile phases were prepared by mixing aqueous solutions of sodium dodecyl sulphate (99%, Merck, Darmstadt, Germany) and a small amount of pentanol (analytical-reagent grade, Merck). The pH was 6.9. The mobile phases were vacuum-filtered through 0.47 μm Nylon membranes (Micron-Scharlau, Barcelona, Spain).

Stock solutions of 100 μg ml^{-1} of the diuretics in 0.07 M SDS were prepared. A small volume of methanol was added to facilitate dissolution. Triamterene was obtained from Sigma (Buchs, Switzerland). The other diuretics were kindly donated by several pharmaceutical laboratories: amiloride (Ici Farma, Madrid, Spain), bendroflumethiazide (Davur, Madrid, Spain), chlorthalidone (Ciba–Geigy, Barcelona, Spain), hydrochlorothiazide (Galloso Wellcome, Madrid, Spain) and spironolactone (Searle, Madrid, Spain). No decomposition was observed in the diuretic stock solutions for at least 1 month, except for bendroflumethiazide, which was prepared daily. The decomposition of this diuretic gave a peak close to the dead volume, which increased with age of the solution. Nanopure deionized water (Barnstead Sybron, MA, USA) was used to prepare the mobile phases and diuretic solutions.

Apparatus

A Hewlett–Packard HP 1050 chromatograph with an isocratic pump, a UV–vis detector and an HP 3396A integrator was used (Palo Alto, CA, USA). Data acquisition was made with the Peak-96 software from Hewlett–Packard (Avondale, PA, USA). The sample was injected through a Rheodyne valve (Cotati, CA, USA) with a 20 μl loop. A Spherisorb octadecyl silane ODS-2 C_{18} column (5 μm, 120 × 4.6 mm) was used. A guard column of similar characteristics (35 × 4.6 mm) (Sharlau, Barcelona, Spain) was also used.

Analysis of the tablet formulations

The tablets were pulverized and an adequate amount was weighed out and dissolved in 0.07 M SDS. The sample was immersed for 5 min in an ultrasonic bath to facilitate dissolution. Any solid particles in the sample were eliminated by first filtering through sintered glass and then vacuum-filtering through the 0.47 μm Nylon membrane.

Results and Discussion

Amiloride, chlorthalidone, bendroflumethiazide, triamterene and spironolactone showed high retention in a mobile phase of SDS without alcohol. These diuretics were protonated at the pH of the mobile phase, 6.9, and had a great affinity for the modified stationary phase, where monomers of the anionic surfactant were adsorbed. The addition of a moderate amount of methanol or propanol to the mobile phase was not enough to decrease the capacity factors to practical values. For chlorthalidone, bendroflumethiazide, amiloride, triamterene and spironolactone, k' was 10.7, 15.4, 33.7, 78 and >80 in a 0.05 M SDS mobile phase; 8.7, 12.5, 29.5, 60 and >60 in 0.05 M SDS–5% methanol, and 6.0, 9.9, 22.4, 37.1 and 55.5 in 0.05 M SDS–3% propanol, respectively [10].

The values of the capacity factors and plate counts, N, for the five diuretics are indicated in Table 1 for several micellar mobile phases containing different concentrations of SDS and pentanol. The detection was performed at 254 nm. The efficiency was usually higher for the mobile phases containing a lower surfactant concentration. The low efficiencies found in micellar liquid chromatography have been attributed to the slow mass transfer into the modified stationary phase [12]. Because of the asymmetry of the peaks, the efficiency was calculated by using the equation of Foley and Dorsey [13]. In this equation B/A is the asymmetry factor (Table 1), where B and A are the distance between the centre of the peak and the trailing or leading edge of the peak, respectively, measured at 10% of peak height. The peaks of bendroflumethiazide and chlorthalidone were in most cases almost symmetrical, whereas the peaks of spironolactone were highly asymmetrical.

For a constant SDS concentration in the mobile phase, when pentanol was increased from 0.5 to 3%, the diminution in the capacity factors of the diuretics was around 80% for 0.05 M SDS, 55% for 0.1 M SDS and 50% for 0.15 M SDS. Thus, the eluent strength of pentanol decreased at increasing SDS concentration, as has been observed for other compounds [14, J.R. Torres-Lapasió, unpublished results]. When the concentration of SDS in the

Table 1
Effect of the addition of pentanol on the values of capacity factors (k'), efficiencies (N) and asymmetries (B/A) of the chromatographic peaks

SDS (M)		0.05			0.05	
Pentanol (v/v)		0.5%			1%	
Compound	k'	N	B/A	k'	N	B/A
Amiloride	11.4	1400	1.6	8.6	1620	1.5
Bendroflumethiazide	7.3	2370	1.2	5.7	1760	1.0
Chlorthalidone	4.0	1975	1.1	2.9	1790	1.3
Spironolactone	13.4	280	4.1	8.8	450	3.0
Triamterene	19.6	1080	1.4	13.4	1350	1.5
SDS (M)		0.05			0.05	
Pentanol (v/v)		1.7%			3%	
Compound	k'	N	B/A	k'	N	B/A
Amiloride	7.2	1465	1.8	6.8	1070	1.6
Bendroflumethiazide	4.2	405	4.3	4.2	6700	1.0
Chlorthalidone	2.0	2020	1.2	1.6	1980	1.3
Spironolactone	6.6	825	2.4	5.7	1130	1.6
Triamterene	10.1	1200	2.0	8.8	740	2.3
SDS (M)		0.05				
Pentanol (v/v)		4%				
Compound	k'	N	B/A			
Amiloride	2.7	230	6.0			
Bendroflumethiazide	2.0	1900	1.2			
Chlorthalidone	0.8	1270	1.5			
Spironolactone	2.7	540	1.3			
Triamterene	3.8	1210	1.7			
SDS (M)		0.1			0.1	
Pentanol (v/v)		0.5%			1%	
Compound	k'	N	B/A	k'	N	B/A
Amiloride	6.0	1270	1.5	4.6	1300	1.4
Bendroflumethiazide	4.1	1530	1.2	3.4	1450	1.2
Chlorthalidone	2.6	1350	1.2	2.0	1230	1.2
Spironolactone	9.3	160	4.0	5.9	280	2.8
Triamterene	10.7	940	1.4	7.5	1200	1.3
SDS (M)		0.1			0.1	
Pentanol (v/v)		2%			3%	
Compound	k'	N	B/A	k'	N	B/A
Amiloride	3.1	1035	1.1	2.8	580	0.8
Bendroflumethiazide	2.2	1700	1.2	2.1	1775	1.2
Chlorthalidone	1.1	1270	1.3	0.9	1290	1.4
Spironolactone	3.4	530	2.3	2.9	690	1.8
Triamterene	4.6	1350	1.5	4.1	1260	1.6
SDS (M)		0.15			0.15	
Pentanol (v/v)		0.5%			1%	
Compound	k'	N	B/A	k'	N	B/A
Amiloride	4.4	1430	0.9	2.9	1350	0.8
Bendroflumethiazide	3.0	1200	1.2	2.1	1130	1.2
Chlorthalidone	2.0	1080	1.2	1.3	970	1.3
Spironolactone	8.0	115	4.1	4.2	215	3.1
Triamterene	8.0	870	1.4	4.9	1060	1.3
SDS (M)		0.15				
Pentanol (v/v)		3%				
Compound	k'	N	B/A			
Amiloride	2.0	1425	1.7			
Bendroflumethiazide	1.6	1270	1.3			
Chlorthalidone	0.8	970	1.5			
Spironolactone	2.4	540	1.9			
Triamterene	3.1	1225	1.4			

mobile phase increased, for a given pentanol concentration, the capacity factors decreased. These values were in the $4 < k' < 20$ range in a 0.05 M SDS–0.5% pentanol mobile phase, and in the $2 < k' < 8$ range in a 0.15 M SDS–0.5% pentanol mobile phase.

The best shaped peaks were obtained in the mobile phases containing 3% pentanol in all cases. However, the elution of the diuretics in these mobile phases occurred in an excessively narrow range of k' values. Although a mobile phase containing a relatively high amount of pentanol (e.g. 3%) may be adequate for the determination of a given diuretic, in this work a mobile phase of lower eluent strength, that is, with a lower concentration of pentanol, such as 0.5%, was preferred.

The retention of triamterene in a 0.05 M SDS–0.5% pentanol mobile phase was too long, and in a 0.1 M SDS–0.5% pentanol mobile phase, spironolactone and triamterene eluted at too close k' values (the efficiency of the peaks should be also considered). Therefore, a mobile phase of intermediate SDS concentration was chosen for the determinations: 0.07 M SDS–0.5% pentanol. In this eluent the capacity factors (and plate counts) for chlorthalidone, bendroflumethiazide, amiloride, triamterene and spironolactone were 3.7 (1700), 6.0 (2700), 9.6 (800), 15.8 (700) and 17.9 (650), respectively. Figure 1 shows chromatograms of mixtures of these diuretics.

Partitioning behaviour of the diuretics

The retention behaviour of a solute in a purely micellar mobile phase is adequately described by:

$$\frac{1}{k'} = \frac{K_{AM}}{\phi P_{SW}}[M] + \frac{1}{\phi P_{SW}}, \qquad (1)$$

where $[M]$ is the total concentration of surfactant in the mobile phase minus the cmc, ϕ is the phase ratio, where $\phi = V_S/V_M$, V_S being the volume of the stationary phase and V_M the volume of the mobile phase in the column, P_{SW} is the partition coefficient of the solute between the stationary phase and water, and K_{AM} the solute–micelle binding constant [12]. This equation seems to be also valid for mobile phases with the same alcohol concentration and varying micelle concentration [15].

The addition of an alcohol to the eluent solvates the hydrocarbonaceous bonded phase and reduces the amount of sorbed surfactant on the stationary phase. This effect is larger with increasing concentration and hydrophobicity of the alcohol [12]. In a purely micellar mobile phase, the most retained diuretics, amiloride, triamterene and spironolactone, showed intercepts close to zero in the plot of $1/k'$ vs $[M]$, indicating that P_{SW} must

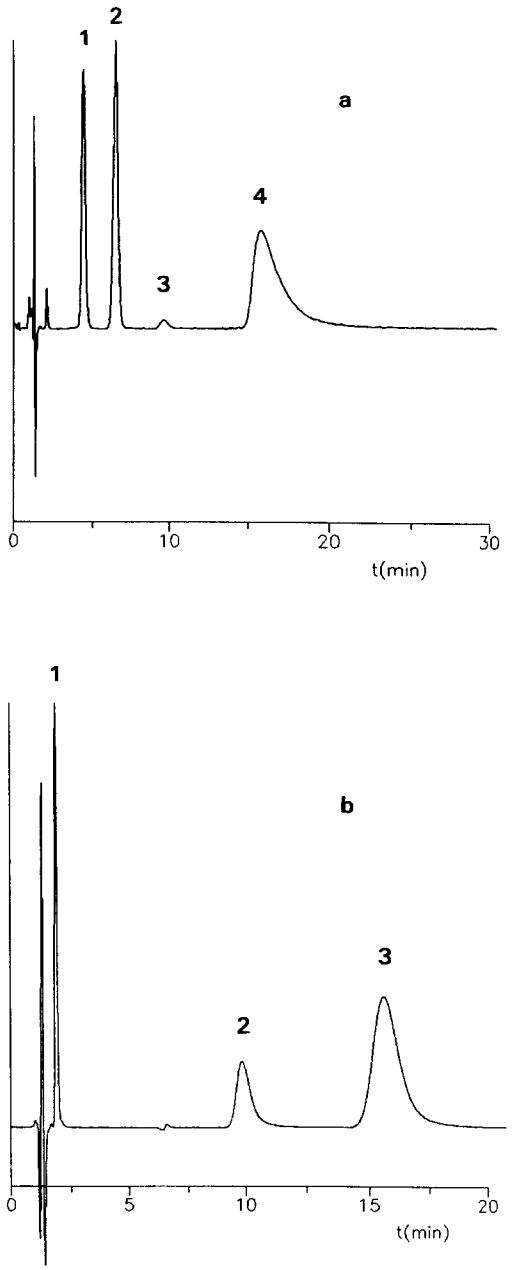

Figure 1
Chromatogram of (1a) 10.1 μg ml^{-1} chlorthalidone, (2a) 10.2 μg ml^{-1} bendroflumethiazide, (3a) 10.6 μg ml^{-1} atenolol, (4a) 10.4 μg ml^{-1} spironolactone, (1b) 10.6 μg ml^{-1} hydrochlorothiazide, (2b) 10.2 μg ml^{-1} amiloride, (3b) 11.2 μg ml^{-1} triamterene. Mobile phase: SDS 0.07 M–0.5% pentanol.

be very large. For bendroflumethiazide and chlorthalidone ϕP_{SW} was 177 and 56, respectively [10]. Table 2 shows the values of ϕP_{SW} for SDS mobile phases containing 0.5 and 3% pentanol. The value of ϕP_{SW} decreased for bendroflumethiazide, chlorthalidone, spironolactone and triamterene in mobile phases containing 0.5% pentanol with respect to purely micellar eluents, and a further decrease was observed in mobile phases with 3% pentanol. For amiloride ϕP_{SW} decreased when 0.5% pentanol was present, but it increased with 3% pentanol.

The addition of pentanol modifies some micellar properties, such as the critical micellization concentration and the aggregation number of the surfactant. Pentanol incorporates in the micelle and increases the hydrophobic character of the bulk aqueous phase. Tomasella *et al.* [15] reported the diminution of the solute–micelle binding constant for different compounds, in SDS mobile phases containing an increasing propanol concentration. This effect was larger for more hydrophobic solutes. The same behaviour was observed for bendroflumethiazide and chlorthalidone when 0.5% pentanol was added to the SDS eluents. In a purely micellar eluent K_{AM} was 249 and 101, respectively, for these diuretics (because of high retention, the binding constants of amiloride, spironolactone and triamterene in an eluent without alcohol could not be obtained). When a larger amount of pentanol was added (3% pentanol), K_{AM} increased with respect to 0.5% pentanol for all diuretics, except chlorthalidone. The incorporation of pentanol into the micelles may have changed the affinity of the solutes towards the micelle, which decreased when a small amount of pentanol was added, and increased with a larger amount of pentanol. In spite of these changes, the addition of pentanol led to a diminution of the capacity factors of the diuretics, since these values depended inversely on the $K_{AM}/\phi P_{SW}$ ratio (slope of the $1/k'$ vs $[M]$ plot) see Table 2). In purely micellar eluents this ratio was 0.75, 1.40, 1.80, 0.28 and 0.35 for amiloride,

Table 2
Solute–micelle binding constants (K_{AM}) and partition coefficients between stationary phase and water multiplied by the phase ratio (ϕP_{SW})

Compound	0.5% Pentanol K_{AM}	ϕP_{SW}	$K_{AM}/\phi P_{SW}$
Amiloride	43.8	31.2	1.4
Bendroflumethiazide	35.3	17.9	2.0
Chlorthalidone	16.9	6.8	2.5
Spironolactone	9.0	17.9	0.5
Triamterene	34.6	46.5	0.7
Compound	3% Pentanol K_{AM}	ϕP_{SW}	$K_{AM}/\phi P_{SW}$
Amiloride	269.8	78.2	3.4
Bendroflumethiazide	49.0	12.2	4.0
Chlorthalidone	15.5	2.4	6.3
Spironolactone	29.0	11.7	2.5
Triamterene	65.8	30.9	2.1

Table 3
Nominal contents, recoveries and reproducibility for the drugs in the pharmaceutical compounds

Formulation	Content	Recovery (%)	RSD (%)
Aldactone-A	25 mg spironolactone and excipient	100.3	0.4
Aldoleo	50 mg chlorthalidone	104.1	0.9
	50 mg spironolactone and excipient	108.6	0.7
Ameride	5 mg amiloride chlorhydrate	104.0	1.1
	50 mg hydrochlorothiazide lactose and other excipients	101.0	3.1
Neatenol	5 mg bendroflumethiazide	108.0	0.5
	100 mg atenolol and excipient		
Normopresil	25 mg chorthalidone	104.3	0.5
	100 mg atenolol and excipient		
Triniagar	50 mg triamterene	101.6	1.5
	50 mg mebuticine lactose and other excipients		
Salidur	77.6 mg furosemide-xantinol		
	25 mg triamterene and excipient	103.4	1.0
Spirometon	2.5 mg bendroflumethiazide	105.8	0.4
	50 mg spironolactone and excipient	102.4	1.7

bendroflumethiazide, chlorthalidone, spironolactone and triamterene, respectively.

Analysis of the diuretics in tablets

Calibration curves were obtained for each diuretic after triplicate injection of nine solutions of different concentration (0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 15.0, 20.0 and 25.0 μg ml^{-1}), prepared in a 0.07 M SDS medium. Correlation coefficients of the fitted straight-lines were in all cases $r > 0.999$. Limits of detection were estimated for all diuretics to be lower than 0.2 μg ml^{-1}.

Table 3 shows the compositions, recoveries and reproducibilities for the pharmaceuticals analysed in this work. Well resolved peaks were obtained in all cases, even for the tablets containing atenolol, a β-blocker. When solutions were injected into the column, a peak was always observed at the dead volume of the system, which probably corresponded to the excipient.

The recoveries with respect to the composition given by the manufacturers, calculated from the calibration curves, were usually in the 100–108% range. Relative standard deviations (RSDs) of five replicate injections were in the 0.4–3.1% range. In one of the pharmaceuticals (Ameride), the diuretic hydrochlorothiazide was also determined with good results. This diuretic showed a low retention time in 0.07 M SDS–0.5% pentanol ($k' = 1.0$, $N = 1500$).

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