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Comparison of the performance of butanol and pentanol as modifiers in the micellar chromatographic determination of some phenethylamines

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Abstract

A procedure was developed for the determination of several phenethylamines (amphetamine, arterenol, ephedrine, phenylephrine, phenylpropanolamine, mephentermine, methoxyphenamine, pseudoephedrine and tyramine), using micellar mobile phases of sodium dodecyl sulfate (SDS), a C_{18} column and UV detection. The drugs were eluted at short retention times with conventional acetonitrile–water or methanol–water mobile phases. In contrast, in the micellar system, they were strongly retained due to association with the surfactant adsorbed on the stationary phase, and needed the addition of butanol or pentanol to be eluted from the column. These modifiers allowed a simple way of controlling the retention. The chromatographic efficiencies obtained with the hybrid mobile phases of SDS–butanol and SDS–pentanol were also very high, mostly in the N=3000–7000 range, significantly greater than those achieved with a conventional acetonitrile–methanol–water mobile phase. Butanol and pentanol yielded similar selectivities, but the latter modifier permitted significantly shorter retention times than butanol, and was preferred to expedite the analysis of the pharmaceuticals. Most binary combinations of the nine phenethylamines can be resolved with these mobile phases. A mobile phase of 0.15 M SDS–5% pentanol was used to assay five of the phenethylamines (amphetamine, ephedrine, phenylephrine, phenylpropanolamine and pseudoephedrine) in 22 pharmaceutical preparations, which contained diverse accompanying compounds. The results agreed with the declared compositions and with those obtained with a mobile phase of methanol–acetonitrile–0.05 M phosphate buffer (pH 3) 10:5:85, with no interferences and relative errors usually below 2%. However, with the aqueous–organic mobile phase, the retention time for phenylephrine was too low and could not be usually evaluated.

Keywords: Mobile phase composition; Phenethylamines; Butanol; Pentanol; Sodium dodecyl sulfate

1. Introduction

Phenethylamines, such as amphetamine, ephedrine, phenylpropanolamine and pseudoephedrine, are characterized by a phenyl ring having an alkylamine chain (Table 1). These compounds are adrenergics and stimulants of the central nervous system, and their use should be controlled. Many of them are

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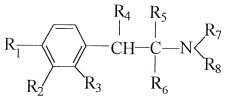
Table 1
Structures and acid–base constants of the phenethylamines



Compound	pK_a[a]	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
Amphetamine	10.0	H	H	H	H	H	CH_3	H	H
Arterenol	8.6, 9.8, 12.0	OH	OH	H	OH	H	H	H	H
Ephedrine	9.6	H	H	H	OH	H	CH_3	H	CH_3
Phenylephrine	8.9. 10.1	H	OH	H	OH	H	H	H	CH_3
Phenylpropanolamine	9.4	H	H	H	OH	H	CH_3	H	H
Mephentermine	10.4	H	H	H	H	CH_3	CH_3	H	CH_3
Methoxyphenamine	10.1	H	H	OCH_3	H	H	CH_3	H	CH_3
Pseudoephedrine	9.5	H	H	H	OH	H	CH_3	H	CH_3
Tyramine	9.3, 10.9	OH	H	H	H	H	H	H	H

[a] From Ref [1].

commercialized in pharmaceutical preparations due to their vasoconstrictor and bronchodilator effects [1]. Conventional HPLC with aqueous–organic mobile phases has been extensively studied for the determination of phenethylamines in pharmaceuticals. The reported procedures commonly employ C_{18}, C_8 and cyano columns, combined with binary isocratic mobile phases containing methanol–water [2–5], acetonitrile–water [6–12], and gradient elution with acetonitrile–water [13,14], or ternary mobile phases of acetonitrile–methanol–water [15,16], and acetonitrile–ethanol–water [17]. For most procedures, the detection is performed in the UV region, but electrochemical and fluorescence detection have also been utilized.

Micellar liquid chromatography (MLC) has demonstrated to be a useful technique in the determination of diverse groups of drugs in pharmaceutical preparations, such as diuretics [18–20], sulfonamides [21], steroids [22], β-blockers [23,24], and benzodiazepines [25]. A procedure has been developed to determine pseudoephedrine in cold tablets with an isocratic mixed mobile phase of sodium dodecyl sulfate (SDS), Brij 35 and 1-propanol [26]. The two surfactants were needed to separate pseudoephedrine from acetaminophen and chlorpheniramine.

One of the major advantages of MLC is the capacity of describing the retention behaviour of compounds eluted with hybrid micellar mobile phases of surfactant and organic modifiers, with high accuracy. This description allows the simple selection of mobile phase composition [27]. Propanol is commonly employed as organic modifier, the use of other alcohols such as butanol and pentanol is rather unusual. These two alcohols have been recommended to reduce the retention times of highly hydrophobic compounds, such as sulfonamide azodyes, steroids and benzodiazepines, in C_{18} columns [21,22,25].

The purpose of this work was to develop an MLC procedure for the analysis of pharmaceuticals containing several phenethylamines (amphetamine, arterenol, ephedrine, phenylephrine, phenylpropanolamine, mephentermine, methoxyphenamine, pseudoephedrine and tyramine), using UV detection. These compounds are eluted at short retention times with conventional acetonitrile–water or methanol–water mobile phases, using a C_{18} column. In contrast, in the micellar system, they are strongly retained due to association with the surfactant, and need the addition of butanol or pentanol to be eluted from the column. These alcohols also allow an adequate control of the retention. A comparison of their performance is next presented.

2. Experimental

2.1. Reagents

The reagents used in the mobile phases were the surfactant sodium dodecyl sulfate (99% purity,

Merck, Darmstadt, Germany), the modifiers 1-propanol, 1-butanol or 1-pentanol (Scharlau, Barcelona, Spain), the buffer salt sodium dihydrogenphosphate (Panreac, Barcelona) and HCl or NaOH (Probus, Badalona, Spain). Acetonitrile, methanol (Scharlau) and triethylamine (Fluka, Buchs, Switzerland) were used in the aqueous–organic mobile phase of the reference method.

The following phenethylamines (Table 1) were kindly donated by the pharmaceutical laboratories indicated: amphetamine (Miquel, Barcelona), ephedrine, phenylephrine (Fardi, Barcelona), phenylpropanolamine (Boehringer Mannheim, Terrassa, Barcelona), and pseudoephedrine (Lasa, Sant Feliu de Llobregat, Barcelona). Other phenethylamines were from Sigma (St. Louis, MO, USA): arterenol, mephentermine, methoxyphenamine and tyramine. Stock solutions containing 200 mg/l of the drugs were prepared in distilled-deionized water (Barnstead, Sybron, Boston, MA, USA), and conveniently diluted for analysis. The micellar mobile phases and phenethylamine solutions were filtered through 0.45 μm nylon membranes (Micron Separations, Westboro, MA). The micellar mobile phase recommended in this work for the analysis of the pharmaceutical preparations is: 0.15 M SDS–5% pentanol.

2.2. Apparatus

Absorbance measurements were obtained with a Perkin Elmer UV–Vis–NIR spectrophotometer (Model Lambda 19, Norwalk, CT, USA). The pH was measured with a Crison potentiometer (Model micropH 2001, Barcelona), provided with a combined Ag/AgCl/glass electrode.

A chromatograph Hewlett-Packard (Model HP 1100, Palo Alto, CA, USA), equipped with a quaternary pump, an autosampler (20 μl injection volume), and a UV–Vis detector (190–700 nm range), was used. Monitoring was performed at 274 nm for arterenol, methoxyphenamine and tyramine, and at 256 nm for the other drugs. An ODS-2 column (5 μm particle size, 120 mm×4.6 mm i.d.) was used (Scharlau). Injection of the solutions into the chromatograph was made through a Rheodyne valve (Cotati, CA, USA). The flow-rate was 1.0 ml/min. The dead time was determined as the mean value of the first significant deviation of the base-line in the chromatograms of the analytes. The signal was acquired by a PC computer connected to the chromatograph, through an HP Chemstation. The chromatographic data were treated with MICHROM, an MS-DOS software developed in our laboratory [28].

2.3. Procedure

Most pharmaceuticals considered in this work were pills, tablets and cough syrups, other preparations were capsules, powders, and eye, nose and oral drops. For the pills, tablets and powder bags, 10 units were weighed, ground and homogenized, several portions were taken and weighed, and each one was dissolved with a small amount of methanol and diluted with distilled-deionized water. The capsules were weighed after being carefully emptied, to obtain the accurate mass of the capsule content. Subsequently, the same procedure was applied. Aliquots of the homogenized syrups and drops were taken and diluted with a small amount of methanol and water.

The excipients were not soluble in the methanol–water medium, hence the sample solutions should be filtered before injection into the chromatograph. The filtration was however always performed directly into the autosampler vials through 0.45 μm nylon membranes of 13 mm diameter.

2.4. Mathematical treatment

The retention of the phenethylamines was modeled according to [27]:

$$k = \frac{K_{AS}\dfrac{1 + K_{SD}\varphi}{1 + K_{AD}\varphi}}{1 + K_{AM}\dfrac{1 + K_{MD}\varphi}{1 + K_{AD}\varphi}[M]} \quad (1)$$

where $[M]$ and φ are the concentrations of surfactant and modifier, K_{AS} and K_{AM} correspond to the equilibria between solute in bulk water and stationary phase or micelle, respectively; K_{AD}, K_{SD}, and K_{MD} measure the relative variation in the concentration of solute in bulk water, stationary phase and micelles due to the presence of modifier, referred to a pure micellar solution (without modifier).

The optimization of the resolution of mixtures of compounds was made by measuring the overlapped fractions of each chromatographic peak:

$$O_i = 1 - \frac{w_i'}{w_i} \quad (2)$$

w_i being the total area of a given peak, and w_i', the area of the peak overlapped by the chromatogram formed by the remaining peaks. These values were combined as:

$$R = \prod_{i=1}^{p} O_i \quad (3)$$

where p is the number of peaks in the chromatogram. The shape of the chromatographic peaks (which were frequently asymmetrical) was also modeled to obtain the overlapped fractions and to predict chromatograms, according to:

$$h(t) = H \exp\left[-\frac{1}{2}\left(\frac{t - t_R}{s_0 + s_1(t - t_R)\cdot\cdot\cdot}\right)^2 \right]$$

where H is peak height, t_R the retention time, s_0 the standard deviation of a symmetrical peak that describes the central region of the skewed peak, and s_1 a coefficient that quantifies its skewness [29].

The efficiencies of the peaks were evaluated with the equation suggested by Foley and Dorsey [30]:

$$N = \frac{41.7\left(\frac{t_R}{A+B}\right)^2}{\frac{B}{A} + 1.25} \quad (5)$$

where B and A are the distance between the center and the tailing or leading edge of the peak, respectively, measured at 10% of peak height. The parameter B/A is the asymmetry factor.

3. Results and discussion

3.1. Elution strength and peak shape parameters in hybrid eluents

The equilibria between the monoprotonated (BH^+) and non-protonated (B) phenethylamines (acid–base constants, $pK_a = 8.5–12$, see Table 1) take place outside the working pH range of a C_{18} column (2.5–7.5). For these compounds, the retention was thus the same using mobile phases of SDS at pH 3 and 7. Following work was made at pH 7, that was buffered with phosphate.

The association of the protonated phenethylamines to an SDS-modified C_{18} column was too strong, as indicated by the long retention times obtained when eluted with pure micellar eluents of the surfactant (without organic modifiers), and with mobile phases containing also a weak modifier, such as propanol. The elution strength of alcohols increases with the length of its carbon chain. Two alcohols were then selected to expedite the elution of the studied compounds: butanol and pentanol. As shown below, the behaviour of both modifiers (i.e. changes in retention factors, efficiencies and asymmetries of the chromatographic peaks), at variable concentration of surfactant and modifier, is different. The concentration ranges studied for these modifiers were 0.05–0.15 M for SDS, 3–6% for butanol, and 2–5% for pentanol. The chromatographic data from four mobile phases at two concentrations of SDS and modifier are given as an example in Tables 2 and 3, for butanol and pentanol, respectively.

The usual behaviour in MLC with SDS is the achievement of decreased efficiencies with increased concentration of surfactant. In contrast, the efficiencies increase at larger concentrations of modifier. On the other hand, the retention factors are lower for both SDS and modifier at increasing concentrations. This behaviour was followed by the phenethylamines, except for the dependence of the efficiencies with the concentration of modifier, which for some compounds decreased at the largest percentage assayed (Tables 3 and 4). It should be noted that the efficiencies obtained with the hybrid mobile phases of SDS–butanol and SDS–pentanol for the studied compounds are very high, mostly in the $N = 3000–7000$ range, except for arterenol with $N = 1000–2000$. The upper reported values of efficiency for MLC are frequently below $N = 4000$ [31]. The efficiencies with butanol are somewhat greater than those achieved with pentanol. Otherwise, those obtained for an aqueous–organic mobile phase (methanol–acetonitrile–water 10:5:85), used in this work for comparison purposes, were $N = 1460$, 1275, 4640, 1550 and 1400, for amphetamine, ephedrine, phenylephrine, phenylpropanolamine and pseudoephedrine, respectively. The poor column efficiencies

Table 2
Chromatographic parameters for some SDS–butanol micellar mobile phases

Compound	SDS 0.05 M 3% butanol			SDS 0.05 M 6% butanol			SDS 0.15 M 3% butanol			SDS 0.15 M 6% butanol		
	k	*N*	*B*/*A*	*k*	*N*	*B*/*A*	*k*	*N*	*B*/*A*	*k*	*N*	*B*/*A*
Amphetamine	34.4	5310	1.14	27.2	5600	1.12	21.0	4090	11.13	12.8	4360	1.10
Arterenol	5.5	1400	2.21	4.8	1780	1.40	3.2	990	2.19	2.6	930	2.42
Ephedrine	23.2	5840	1.03	17.4	5200	1.10	11.8	3880	1.08	8.4	4000	1.17
Phenylephrine	10.5	6890	1.06	8.6	5930	1.08	5.7	4230	1.06	4.3	4470	1.12
Phenylpropanolamine	28.2	6910	1.10	22.8	5500	1.12	17.5	4290	1.14	11.0	3240	0.67
Mephentermine	27.9	6340	1.05	23.2	3090	0.64	18.3	3410	1.16	8.4	3980	1.19
Methoxyphenamine	27.8	5390	1.09	19.7	4600	1.07	15.5	3460	1.09	10.1	1550	1.90
Pseudoephedrine	22.2	6120	1.12	16.3	5040	1.13	12.0	4250	1.07	8.4	4580	1.10
Tyramine	9.8	6950	1.10	7.7	5940	1.07	5.3	4310	1.05	4.0	4550	1.12

Table 3
Chromatographic parameters for some SDS–pentanol micellar mobile phases

Compound	SDS 0.05 M 2% pentanol			SDS 0.05 M 5% pentanol			SDS 0.15 M 2% pentanol			SDS 0.15 M 5% butanol		
	k	*N*	*B*/*A*	*k*	*N*	*B*/*A*	*k*	*N*	*B*/*A*	*k*	*N*	*B*/*A*
Amphetamine	31.9	3250	1.27	9.4	4970	1.14	16.0	3930	1.11	7.4	4040	1.15
Arterenol	5.0	1680	1.65	2.9	1840	1.84	3.1	1390	2.40	2.4	1730	1.78
Ephedrine	20.3	5130	1.19	8.6	4810	1.19	9.5	3750	1.09	5.5	3790	1.12
Phenylephrine	9.3	5830	1.12	5.0	4770	1.18	5.0	4300	1.04	3.4	4370	1.12
Phenylpropanolamine	23.5	6520	1.14	9.0	5850	1.14	13.8	4240	1.16	6.7	4580	1.14
Mephentermine	24.0	5230	1.10	10.2	4340	1.25	13.6	3440	1.15	6.6	3600	1.18
Methoxyphenamine	20.6	4390	1.20	9.3	4280	1.26	11.4	3330	1.12	5.8	3540	1.13
Pseudoephedrine	17.5	5140	1.19	8.1	5340	1.13	9.4	3810	1.03	5.3	4030	1.17
Tyramine	10.0	5850	1.11	5.1	5470	1.12	4.5	4260	1.06	3.1	4380	1.04

Table 4
Limits of detection, and intra- and inter-day repeatabilities for the phenethylamines eluted with 0.15 M SDS–5% pentanol

Compound	LOD (μg/ml)	Intra-day[a] CV (%) ($n = 5$)	Inter-day[a] CV (%) ($n = 3$)
Amphetamine	0.24	2.7	1.8
Arterenol	0.031	1.4	1.1
Ephedrine	0.22	2.1	3.7
Phenylephrine	0.006	1.6	2.2
Phenylpropanolamine	0.26	4.3	8.1
Mephentermine	0.064	5.1	4.6
Methoxyphenamine	0.003	1.1	6.0
Pseudoephedrine	0.053	0.37	1.4
Tyramine	0.022	1.4	5.4

[a] Calculated for 20 μg/ml.

with aqueous–organic mobile phases are due to excessive peak tailing.

The hydrophilic layer formed by the sulfate head groups of SDS above the surface of the silica influences the retention of the compounds [32]. The hydroxyl groups on the silica surface play a less important role in the separation as a result of SDS adsorption. Since the hydrophilic layer exists above the silica surface, the association kinetics which is controlled primarily by the electrostatic interaction are more facile than ion exchange processes involving the silanol groups on the silica surface. Furthermore, the interaction of the protonated phenethylamines with the hydrophilic layer formed by SDS reduces the penetration depth of the compounds into

the bonded phase. The net effect is an improvement in efficiency when a micellar mobile phase is utilized since the role of the silanol groups on the silica surface have been diminished with respect to their participation in the retention mechanism.

As expected, the elution strength of pentanol was greater. Thus, for instance, for amphetamine $k=34.4$ for 0.05 M SDS–3% butanol, and decreased to $k=21.0$, when the concentration of surfactant was increased to 0.15 M, and $k=27.2$ when butanol was increased to 6%. For the same compound, $k=31.9$ for 0.05 M SDS–2% pentanol, and decreased to $k=16.0$, when the concentration of surfactant was increased to 0.15 M, and $k=9.4$ when pentanol was increased to 5%. It can be observed that, in the studied concentration ranges, the changes in the retention produced by a change in SDS were higher than those produced by the modifier when butanol was used, but for pentanol the behaviour was opposite. Pentanol wets better the bonded phase than butanol, thereby reducing to a greater degree the amount of SDS adsorbed on the bonded phase. Anyway, the strength of SDS shown in the elution of the phenethylamines was large, which suggests the large affinity of the compounds for the micelles. The strong retention of the compounds in the surfactant-modified stationary phase is also indicative of the strong association of the phenethylamines with the surfactant molecules.

The retention factors for the nine compounds are plotted in Fig. 1 for experimental mobile phases of pentanol and butanol of similar elution strength (0.05 M SDS–2% pentanol vs. 0.05 M SDS–3% butanol, 0.05 M SDS–5% pentanol vs. 0.05 M SDS–8% butanol, and 0.15 M–2% pentanol vs. 0.15 M SDS–3% butanol). It can be observed that, for the three pairs of mobile phases, the points align approximately along a straight-line of slope close to unity, which indicates that the relative interactions of all the compounds with the micelles modified with both butanol and pentanol are similar. Butanol and pentanol present a distinctive behaviour when dissolved in a micellar medium of SDS, in comparison with other alcohols (i.e. methanol and propanol) or other organic solvents (i.e. acetonitrile and tetrahydrofuran) [33]. These alcohols are inserted into the micellar assembly, owing to their low solubility in water and to their particular structure that combines a polar

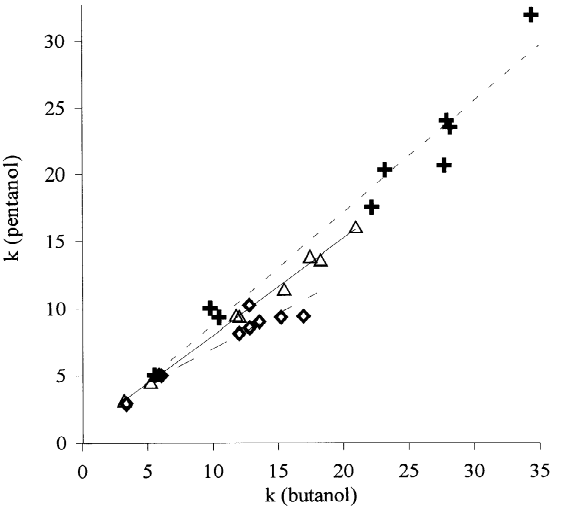


Fig. 1. Correlation of the retention factors of the nine phenethylamines eluted with 0.05 M SDS–2% pentanol and 0.05 M SDS–3% butanol (+), 0.05 M SDS–5% pentanol and 0.05 M SDS–8% butanol (◇), and 0.15 M SDS–2% pentanol and 0.15 M SDS–3% butanol (△).

group with a non-polar chain, similarly to the surfactant molecule. The alcohol and surfactant molecules align together in the micelle palisade, the polar hydroxyl group of the alcohol orientated towards the Stern layer and the alkyl chain located in the non-polar micelle core. This gives rise finally to swollen mixed micelles [34].

The elution order for both modifiers was essentially the same: arterenol, phenylephrine, tyramine, pseudoephedrine, ephedrine, methoxyphenamine, phenylpropanolamine, mephentermine, and amphetamine. Phenylephrine and tyramine changed their elution order at low percentage of pentanol, and methoxyphenamine, phenylpropanolamine and mephentermine, which eluted at close retention times, yielded frequent order reversals with both modifiers.

3.2. Optimization of mobile phase composition

Combinations of two phenethylamines are usually administered in several countries [35]. Some examples are the mixtures of phenylephrine with ephedrine, phenylpropanolamine or pseudoephedrine, or

methoxyphenamine with pseudoephedrine. We considered the possibility of using the same mobile phase to carry out these analyses. We performed, therefore, an optimization study for mixtures of all the drugs included in this work. Adequate control of the concentrations of surfactant and modifier can lead to chromatograms showing good resolution and sufficient elution strength.

In order to optimize the mobile phase composition, the retention equations (Eq. (1)) of the nine phenethylamines were obtained using a reduced (six) and selected number of mobile phases. The errors in

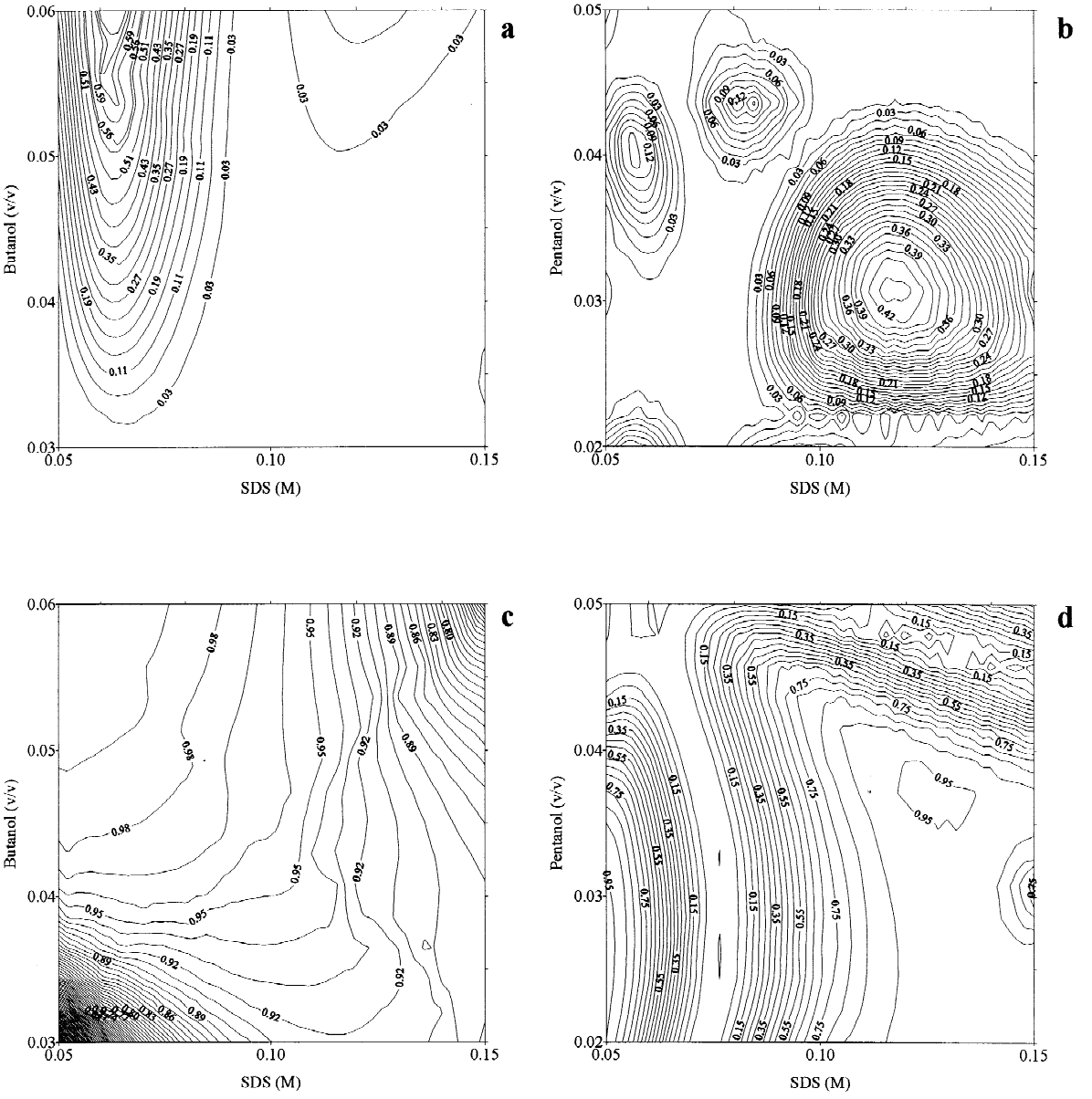


Fig. 2. Contour maps of resolution for: (a) and (b) nine phenethylamines, and (c) and (d) seven phenethylamines, eluted with SDS micellar mobile phases containing butanol or pentanol.

the retention factors predicted with these equations were below 2%, for most compounds. The contour maps in Fig. 2 were drawn assisted by the MICHROM software [28], using the unnormalized product of overlapped fractions as resolution criterium (Eqs. (2) and (3)). Simultaneously, the evolution of the shape of the chromatograms with mobile phase composition was followed using the same software.

The contour maps in Fig. 2a and b correspond to the separation of the nine phenethylamines examined in this work. The values of resolution indicate that baseline separation is not possible. Maximum resolution was achieved with 0.065 M SDS–6% butanol ($R = 0.650$) and 0.115 M SDS–3% pentanol ($R = 0.443$). The chromatograms for these maxima are given in Fig. 3. The elution order of the compounds was the same for both modifiers, but the resolution was poorer for pentanol, although the analysis time was shorter.

The resolution of overlapped peaks in the chromatograms was still possible. This was revealed by carrying out new optimizations which considered only these peaks. For butanol, only the peaks of pseudoephedrine and ephedrine improved their resolution (Fig. 4a), using 0.05 M SDS–6% butanol. The chromatogram in Fig. 3a already shows the maximum resolution that can be achieved for methoxyphenamine, mephentermine and phenylpropanolamine. For pentanol, optimal separation for the three pairs of overlapped peaks (tyramine–phenylephrine, pseudoephedrine–ephedrine, and mephentermine–phenylpropanolamine) was reached for 0.15 M SDS–3.8% pentanol (Fig. 4b), 0.05 M SDS–2% pentanol (Fig. 4c), and 0.05 M SDS–5% pentanol (Fig. 4d), respectively.

We also examined the separation of seven phenethylamines (arterenol, phenylephrine, tyramine, pseudoephedrine, methoxyphenamine, phenylpropanolamine, and amphetamine). The corresponding contour maps are depicted in Fig. 2c and d, for butanol and pentanol, respectively. For the first modifier, a wide region of good resolution was observed in the upper left corner of the factor space. The retention times for amphetamine with the mobile phase of minimum (0.05 M SDS–4.5% butanol) and maximum (0.083 M SDS–6.0% butanol) elution strength, corresponding to the isoline of $R = 0.98$ in Fig. 2c, were 34 and 23 min, respectively. The

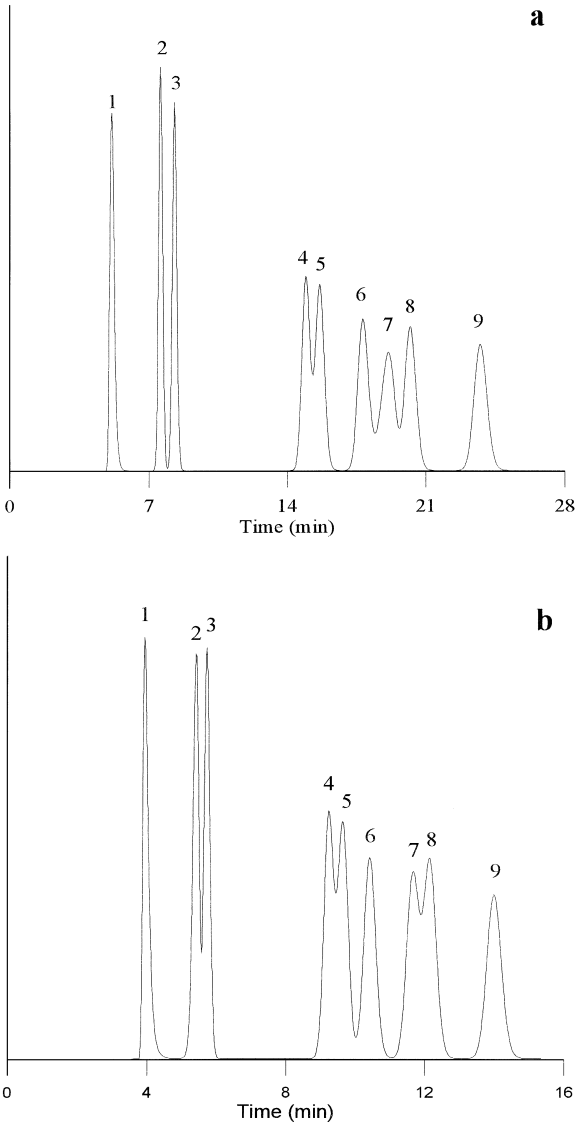


Fig. 3. Chromatograms of a mixture of nine phenethylamines for the optimal mobile phase compositions (see Fig. 2a and b): (a) 0.065 M SDS–6% butanol, and (b) 0.115 M SDS–3% pentanol. Compounds: (1) arterenol, (2) tyramine, (3) phenylephrine, (4) pseudoephedrine, (5) ephedrine, (6) methoxyphenamine, (7) mephentermine, (8) phenylpropanolamine, and (9) amphetamine.

chromatogram for the optimum composition at 0.05 M SDS–6% butanol ($R = 0.9993$) is shown in Fig. 5a. For pentanol, the contour map showed three narrow regions of maximum resolution, with optima at 0.05 M SDS–2.6% pentanol ($R = 0.969$), 0.133 M SDS–3.6% pentanol ($R = 0.948$), and 0.15 M SDS–

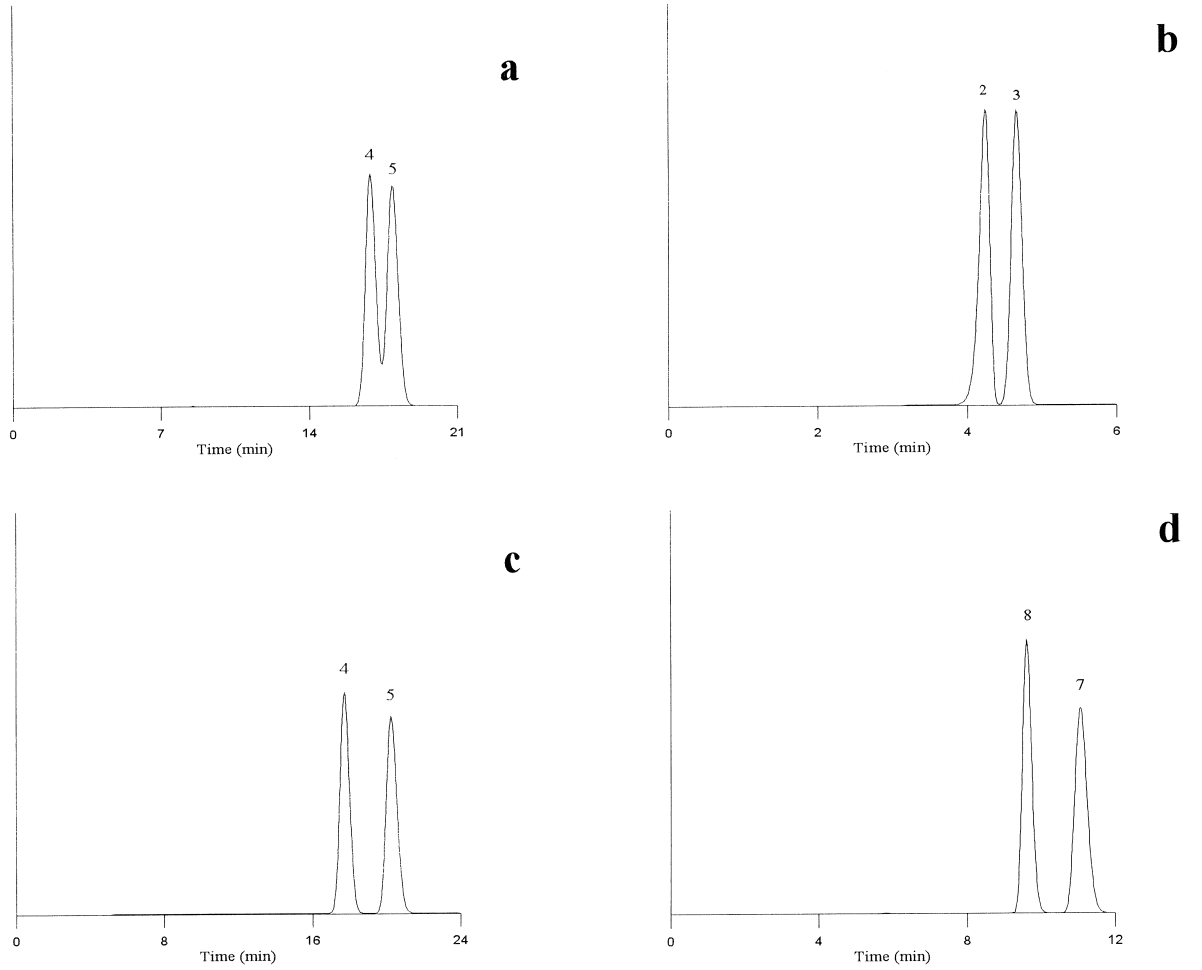


Fig. 4. Resolution of the overlapped peaks in Fig. 3. Mobile phases: (a) 0.05 M SDS–6% butanol, (b) 0.15 M SDS–3.8% pentanol, (c) 0.05 M SDS–2% pentanol, and (d) 0.05 M SDS–5% pentanol. See Fig. 3 for peak identification.

2.2% pentanol ($R = 0.927$) (see also chromatograms in Fig. 5b, c and d).

In conclusion, both modifiers gave rise to similar selectivities, but the resolutions were slightly poorer for pentanol. However, this modifier yielded significantly shorter retention times than butanol, and was preferred in this work to expedite the analysis of the pharmaceuticals. The resolution study shown above indicates that most binary combinations of the nine phenethylamines can be resolved. The results can also be useful to examine the possible screening of the phenethylamines in physiological fluids.

3.3. Figures of merit and analysis of pharmaceutical formulations

Only five of the nine phenethylamines are currently administered in our country: amphetamine, ephedrine, phenylephrine, phenylpropanolamine and pseudoephedrine. As shown above, mobile phases of SDS–pentanol have a large elution strength, therefore, the excipients and many accompanying compounds in pharmaceuticals containing the drugs should elute at the head of the chromatograms, without interfering their determination. For this

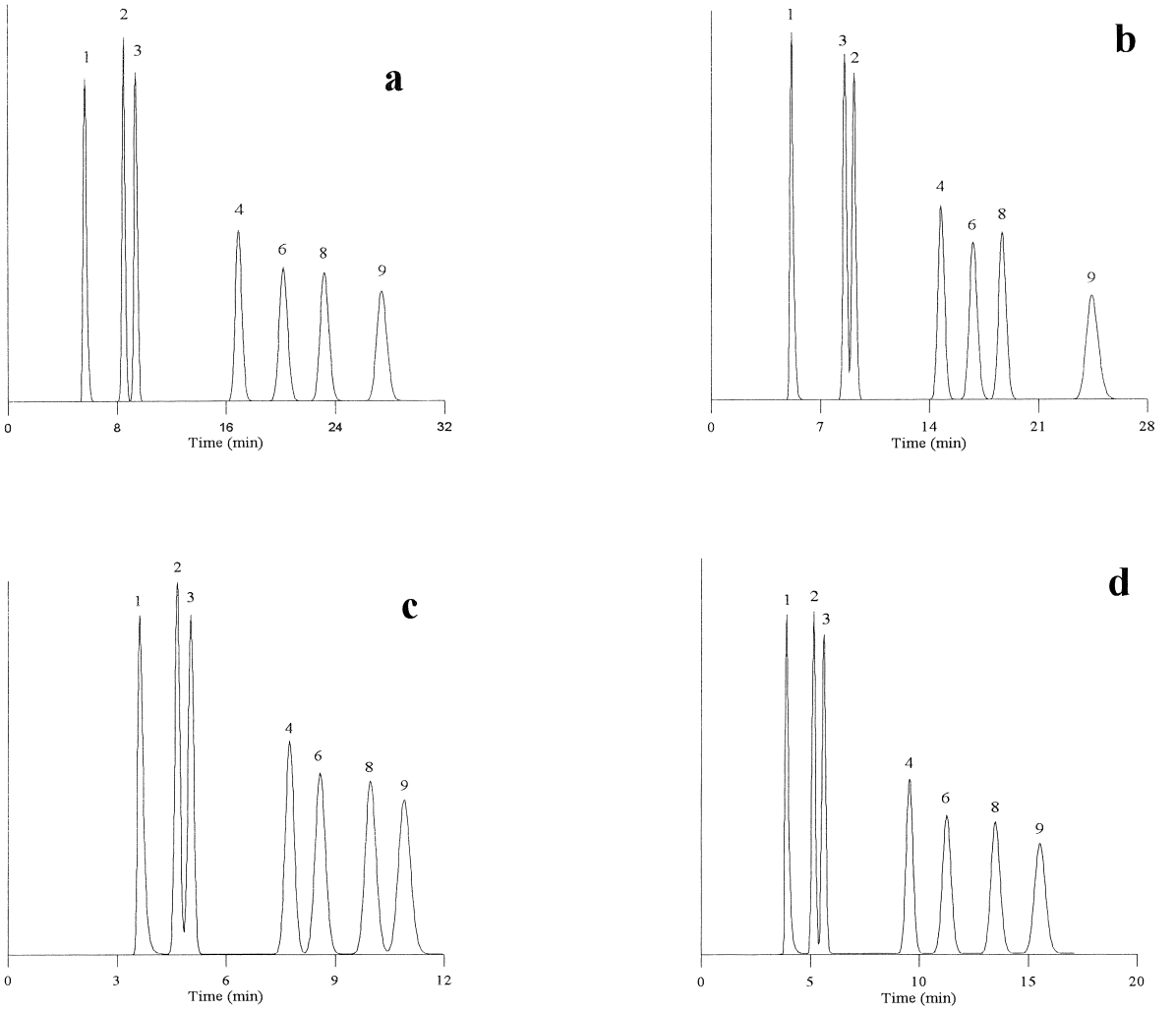


Fig. 5. Chromatograms of a mixture of seven phenethylamines for the optimal mobile phase compositions (see Fig. 2c and d): (a) 0.05 M SDS–6% butanol, (b) 0.05 M SDS–2.6% pentanol, (c) 0.133 M SDS–3.6% pentanol, and (d) 0.15 M SDS–2.2% pentanol. See Fig. 3 for peak identification.

reason, we next considered the possibility of using again the same mobile phase for the analysis of a wide group of pharmaceuticals containing the drugs, but now we reduced the analysis times as much as possible. The most rapid mobile phase in the studied concentration range, 0.15 M SDS–5% pentanol, was adequate since it yielded analysis times below 9 min. Achievement of similar retention times would need a high percentage of butanol. A theoretical calculation using Eq. (1) indicated that this percentage should be in the 10–16% butanol range. Such an amount of butanol cannot be dissolved in the SDS micellar medium.

The analytical figures of merit were, however, obtained for the nine phenethylamines. Calibration curves were constructed in the 20–100 μg/ml range for amphetamine, ephedrine, pseudoephedrine and mephentermine, 20–200 μg/ml for phenylpropanolamine, 10–50 μg/ml for phenylephrine and methoxyphenamine, 5–50 μg/ml for tyramine, and 20–50 μg/ml for arterenol. Triplicate injections of five solutions were made at increasing concentrations of

Table 5
Analysis of pharmaceutical preparations containing phenethylamines

Compound	Pharmaceutical (laboratory)	Composition/mg per capsule, tablet, pill, powder or ml syrup or drops	Found[a] (mg)	CV (%) ($n = 5$)	Found[b] (mg)	CV (%) ($n = 5$)
Amphetamine	Centramina (Miquel, Barcelona, Spain	**Amphetamine sulfate (10)**, lactose and other excipients	9.7	1.4	9.5	0.5
Ephedrine	Bucodrín (Fardi, Barcelona)	**Ephedrine ricinoleate (3)**, sulfathiazol (100), ethacrydine (2), saccharose, polyethylenglycol and other excipients	2.9	0.8	3.0	4.3
	Amidrín (Fardi)	**Ephedrine chlorhydrate (8)**, sulfanilamide (4), chlorbutanol (5) and excipients	7.7	5.2	7.5	6.8
	Bisolvon compositum (Fher, Barcelona)	**Ephedrine chlorhydrate (1.5)** bromhexine chlorhydrate (0.5), diphenhydramine chlorhydrate (1.5), codeine chlorhydrate (2), ethanol and other excipients	1.4	4.3	1.4	2.3
Phenylephrine	Desenfriol D (Schering Plough, Madrid)	**Phenylephrine chlorhydrate (12.2)** chlorpheniramine maleate (2), acetylsalycilic acid (390), caffeine (32.4), saccharose and other excipients	11.8	2.8	–	–
	Mirazul (Fardi)	**Phenylephrine chlorhydrate (1.25)**, ethanol, chlorbutanol and other excipients	1.3	0.8	–	–
	Rinomicine (Fardi)	**Phenylephrine chlorhydrate (6)**, chlorpheniramine maleate (4), salicylamide (200), paracetamol (400), caffeine (30), vitamin C (300), saccharose and other excipients	5.8	1.0	–	–
	Bisolgrip (Fher)	**Phenylephrine chlorhydrate (10)**, paracetamol (500), chlorpheniramine maleate (2), saccharose and other excipients	9.6	2.0	9.4[c]	1.8
	Paidoterín (Aldo-Union, Barcelona)	**Phenylephrine chlorhydrate (1)**, diphenhydramine chlorhydrate (1), chlorpheniramine maleate (0.15) and excipients	1.04	0.10	–	–
	Desenfriol infantil (Schering Plough)	**Phenylephrine chlorhydrate (2.5)**, acetylsalycilic acid (80), chlorpheniramine maleate (0.5) saccharine and excipients	2.4	0.19	–	–
Phenylpropanolamine	Coricidín F (Schering Plough)	**Phenylpropanolamine chlorhydrate (25)**, chlorpheniramine maleate (4), paracetamol (500), starch and magnesium estearate	23.7	0.9	34.1	0.8
	Mucorama (Boehringer Mannheim, Barcelona)	**Phenylpropanolamine chlorhydrate (2.2)**, IPG (12), saccharose and other excipients	2.2	3.1	2.11	0.6
	Baby Rinol (Marion Merrell Dow, Madrid)	**Phenylpropanolamine chlorhydrate (2)**, chlorpheniramine maleate (0.15), paracetamol (24), saccharose and other excipients	1.9	4.0	1.9	1.2
	Triominic drops (Sandoz Pharma, Barcelona)	**Phenylpropanolamine chlorhydrate (20)**, pheniramine maleate (10), pirylamine maleate (10), saccharose and other excipients	20.3	1.2	19.2	1.0
Pseudoephedrine	Narine repetabs (Schering Plough)	**Pseudoephedrine sulfate (120)**, loratadine (5), lactose, saccharose and other excipients	121	2.0	116	3.9
	Idulanex repetabs (Schering Plough)	**Pseudoephedrine sulfate (120)**, azatadine maleate (1), saccharose and other excipients	125	1.0	114	1.8
	Lasa with codeine (Lasa, Barcelona)	**Pseudoephedrine chlorhydrate (6)**, codeine phosphate (2), chlorpheniramine maleate (0.4) and excipients	6.3	3.2	5.6	7.0

(*continued on next page*)

Table 5. (*Continued*)

Compound	Pharmaceutical (laboratory)	Composition/mg per capsule, tablet, pill, powder or ml syrup or drops	Found[a] (mg)	CV (%) ($n = 5$)	Found[b] (mg)	CV (%) ($n = 5$)
Pseudoephedrine	Polaramine expectorant (Schering Plough)	**Pseudophedrine sulfate (4)**, dexchlorpheniramine maleate (0.4), guayacolate glyceryl (20) and excipients	4.0	0.9	3.8	8.0
	Iniston (Gayoso Wellcome, Madrid)	**Pseudoephedrine chlorhydrate (60)**, tripolidine chlorhydrate (2.5), lactose and other excipients	57.5	0.2	57.3	1.1
	Iniston syrup (Gayoso Wellcome)	**Pseudoephedrine chlorhydrate (6)**, tripolidine chlorhydrate (0.25), saccharose and other excipients	5.6	1.9	5.7	1.3
	Iniston antitusive (Gayoso Wellcome)	**Pseudoephedrine chlorhydrate (6)**, tripolidine chlorhydrate (0.25), dextrometorphane bromhydrate (2), saccharose, sorbitol and other excipients	6.1	0.9	6.0	0.3
	Iniston expectorant (Gayoso Wellcome)	**Pseudoephedrine chlorhydrate (6)**, tripolidine chlorhydrate (0.25), guaiphenesine (20), saccharose and other excipients	5.9	1.0	5.9	1.0

[a] Micellar mobile phase: 0.15 M SDS–5% pentanol.
[b] Aqueous–organic mobile phase: methanol–acetonitrile–phosphate buffer 10:5:85.
[c] Aqueous mobile phase: methanol–triethylamine–phosphate buffer 5:0.1:95.

the drugs. The regression coefficients were always better than $r > 0.999$. Table 4 shows the limits of detection (LODs) (3s criterium), and the inter- and intra-day repeatabilities.

The results obtained in the analysis of 22 pharmaceutical preparations presented as pills (Idulanex and Narine), tablets (Bucodrin, Centramina, Desenfriol D, Desenfriol infantil and Iniston), capsules (Coricidin F), powders (Bisolgrip and Rinomicine), cough syrups (Baby Rinol, Bisolvon compositum, Iniston syrup, Iniston antitusive, Iniston expectorant, Lasa with codeine, Mucorama, Paidoterin and Polaramine expectorant), and eye (Mirazul), nasal (Amidrin) or oral drops (Triominic drops), are given in Table 5. A literature survey of the reported reversed-phase chromatographic procedures was made in order to select a suitable aqueous–organic mobile phase to validate these analyses. A mobile phase of methanol–acetonitrile–0.05 M phosphate buffer (pH 3) 10:5:85 was used, which has been recommended for amphetamine [16]. The results are also given in Table 5.

Fig. 6 shows the chromatograms of four pharmaceuticals: Bisolvon compositum (containing ephedrine), Centramina (amphetamine), Lasa with codeine (pseudoephedrine) and Mucorama (phenylpropanolamine), analyzed with both micellar and conventional mobile phases. Peaks corresponding to other accompanying drugs were observed in these and other chromatograms, which did not interfere with the analyses.

The repeatabilities were usually below 2% and the recoveries agreed with the declared contents inside the tolerance limits of 92–106%, for both micellar and aqueous–organic mobile phases, except for phenylephrine with the latter one. The retention time for phenylephrine was too low with the aqueous–organic mobile phase (1.4 min). The peak of this drug was overlapped with the peaks of other accompanying compounds or eluted very close to the dead volume, for the assayed pharmaceuticals. This made its quantification difficult. A new mobile phase was then used, which has been reported as adequate for phenylephrine (methanol–triethylamine–phosphate 5:0.1:95 [5]), but the retention time was even shorter. The results in Table 5 for Bisolgrip (which are acceptable) were obtained with this mobile phase. The recoveries for the other pharmaceuticals containing phenylephrine (not given) were, however, above 140%.

The elution order and retention times (min) for the selected micellar mobile phase (0.15 M SDS–5% pentanol) were: phenylephrine (4.3), pseudoephedrine (6.2), ephedrine (6.8), phenylpropanolamine (7.5). and amphetamine (8.3), and for the aqueous–organic mobile phase (methanol–acetonitrile–0.05 M

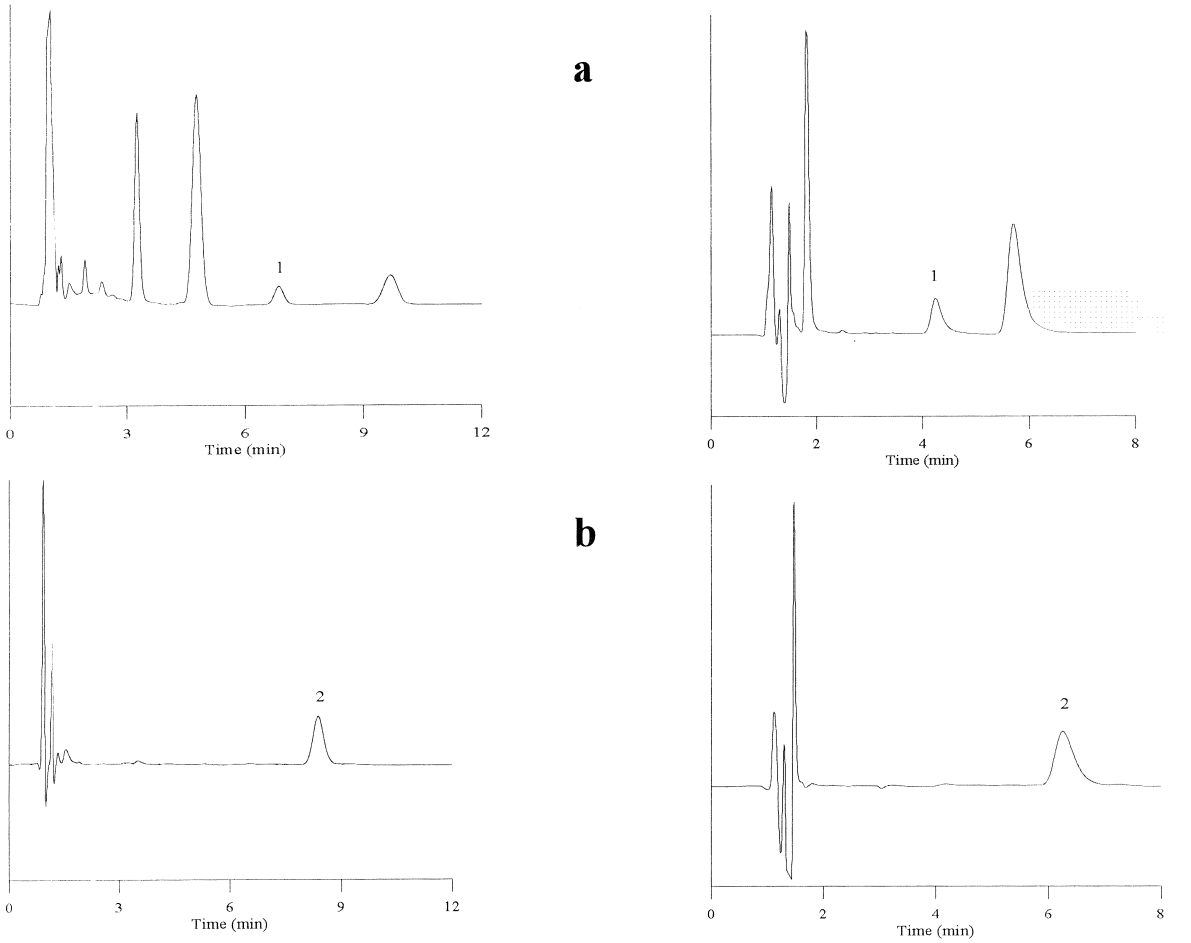


Fig. 6. Chromatograms of several pharmaceuticals with 0.15 M SDS–5% pentanol (left side), and methanol–acetonitrile–0.05 M phosphate buffer (pH 3) 10:5:85 (right side): (a) Bisolvon compositum, (b) Centramine, (c) Lasa with codeine, and (d) Mucorama. Compounds: (1) ephedrine chlorhydrate, (2) amphetamine sulfate, (3) pseudoephedrine chlorhydrate, and (4) phenylpropanolamine chlorhydrate.

phosphate 10:5:85): phenylephrine (1.4), phenylpropanolamine (3.4), pseudoephedrine (4.4), ephedrine (4.4), and amphetamine (6.5). Note that the latter retention times were obtained with a very weak aqueous–organic mobile phase. In contrast, in the micellar chromatographic system, the phenethylamines showed a strong retention, which was produced by the presence of the surfactant as monomers adsorbed on the stationary phase. The increased retention allowed a better control of the retention of the drugs, and the easy resolution of close compounds as ephedrine and pseudoephedrine. In the Spanish market, binary combinations of phenethylamines are not commercialized at the present time and could not be assayed, but as demonstrated, the SDS–pentanol system is able to resolve these mixtures.

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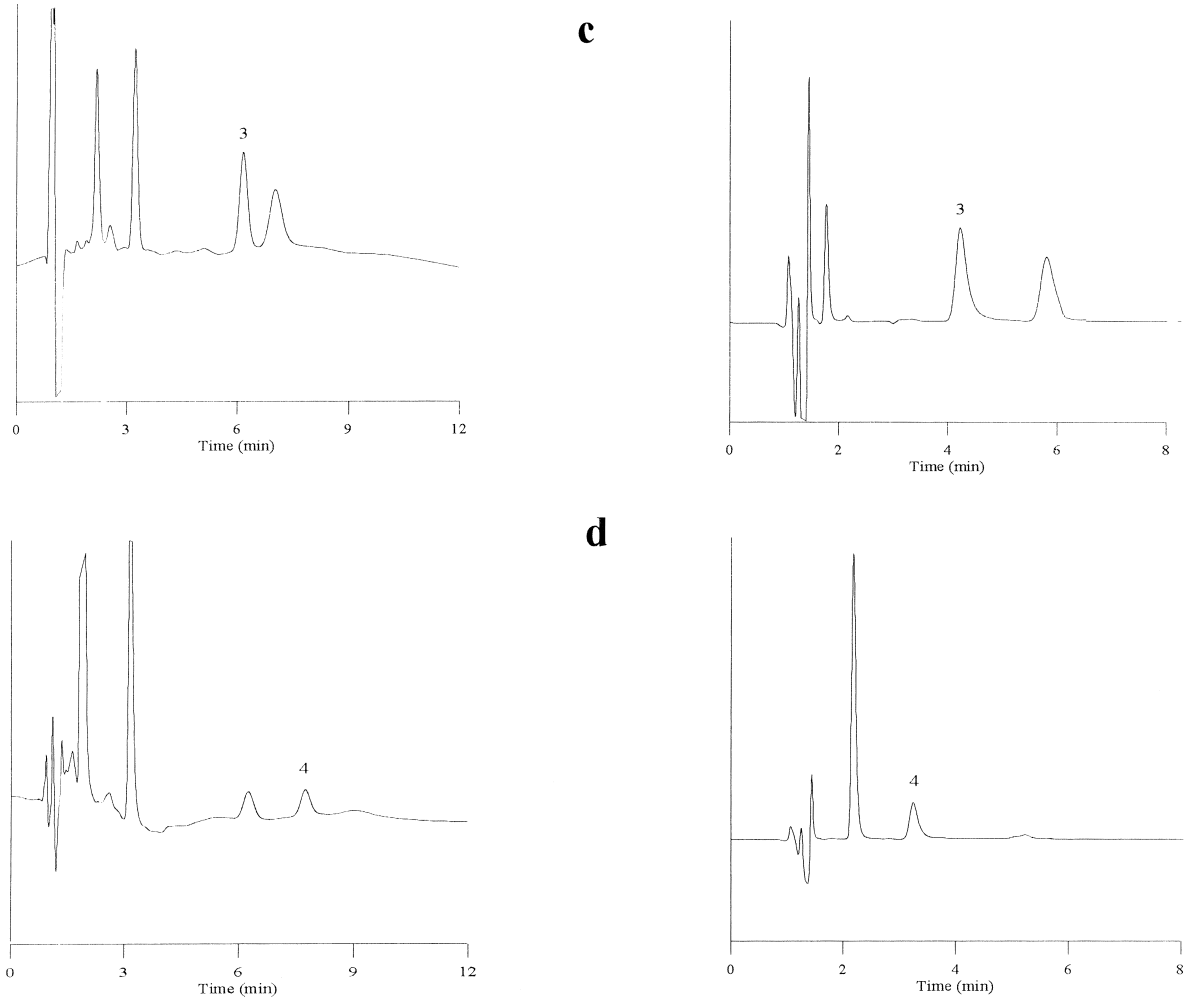

Fig. 6. (*continued*)

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