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Chromatographic analysis of phenethylamine–antihistamine combinations using C_8, C_{18} or cyano columns and micellar sodium dodecyl sulfate–pentanol mixtures

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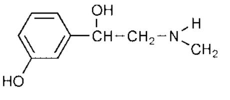
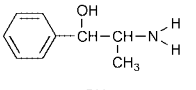
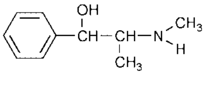
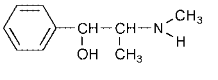
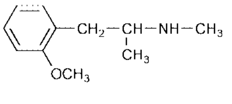
The chromatographic behaviour of binary and ternary mixtures of several phenethylamines (phenylephrine, phenylpropanolamine, ephedrine, pseudoephedrine and methoxyphenamine) and antihistamines (pheniramine, carbinoxamine, doxylamine, chlorpheniramine, dexchlorpheniramine, dexbrompheniramine, diphenhydramine, tripolidine, azatadine and phenyltoloxamine), found in cough–cold pharmaceutical preparations, was studied using C_8, C_{18} and cyano columns, micellar mobile phases of sodium dodecyl sulfate (SDS) and pentanol and UV detection. Using a C_8 column and mobile phases of 0.05 mol l^{-1} SDS–6% v/v pentanol or 0.15 mol l^{-1} SDS–2% v/v pentanol at pH 7, more than 30 different phenethylamine–antihistamine combinations can be resolved in <15 min. Intra- and inter-day repeatabilities and reproducibilities evaluated at three different drug concentrations (0.5, 5 and 25 μg ml^{-1}, $n = 10$) were below 1.6, 2.5 and 2.4%, respectively. The drug amounts found in 18 formulations agreed with those declared by the manufacturers within the tolerance limits, and with those obtained using a mobile phase of 55% v/v methanol at pH 7. No interference was observed from other accompanying drugs such as acetylsalicylic acid, ascorbic acid, betamethasone, bromhexine, caffeine, codeine, dextromethorphan, paracetamol, prednisolone, salicylamide and tartrazine. The proposed procedure has the advantage over the conventional aqueous–organic procedure of using a small amount of organic solvent, which is highly retained in the SDS solution. The efficiencies are also greater. On the other hand, in the micellar system, the retentions of phenethylamines and antihistamines are similar, although the compounds can be easily resolved. In contrast, using the methanol–water mobile phase, the phenethylamines are weakly retained, whereas the antihistamines usually show a high retention.

Binary or ternary combinations of phenethylamines and antihistamines are administered as decongestants for the treatment of coughs, colds and allergies. Phenethylamines act as oral vasoconstrictors and bronchodilators and antihistamines provide symptomatic relief of allergic symptoms caused by histamine release.[1] Most reversed-phase liquid chromatographic (RPLC) procedures proposed for the determination of these drugs alone or in mixtures utilise C_8,[2,3] C_{18},[4–6] or cyano[7–9] columns, with mobile phases of acetonitrile–water, methanol–water or acetonitrile–methanol–water.

In a previous paper, we reported a procedure for the determination of several phenethylamines in pharmaceuticals, using micellar mobile phases of sodium dodecyl sulfate (SDS), a C_{18} column and UV detection.[10] In contrast to conventional

Table 1 Structures, dissociation constants and octanol–water partition constants of the phenethylamines[a]

Compound	Structure	pK_1	pK_2	Log $P_{o/w}$
Phenylephrine (Fardi, Barcelona, Spain)		8.9	10.1	−0.09
Phenylpropanolamine (Boehringer Mannheim, Terrassa, Barcelona, Spain)		9.4	—	0.58
Ephedrine (Sigma, St. Louis, MO, USA)		9.6	—	0.89
Pseudoephedrine (Lasa, Sant Feliu de Llobregat, Barcelona, Spain)		9.5	—	0.89
Methoxyphenamine (Sigma)		10.1	—	1.66

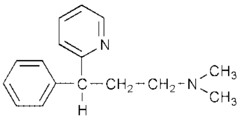
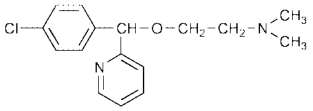
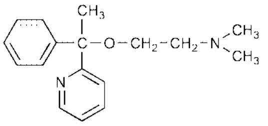
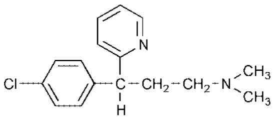
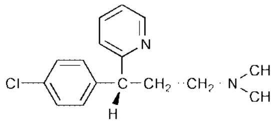
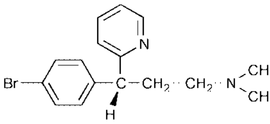
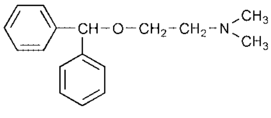
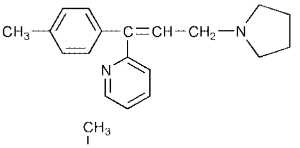
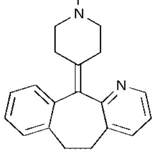
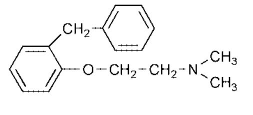
[a] From refs. 21–23.

acetonitrile–water or methanol–water mobile phases, which elute phenethylamines at short retention times, the micellar system retained the cationic species (which dominates in the working pH range of the column) strongly, by association with the anionic surfactant adsorbed on the stationary phase. The retention times of these drugs were decreased by adding pentanol to the SDS mobile phase. Noteworthily, the chromatographic efficiencies in the hybrid SDS–pentanol system were significantly greater than those achieved with conventional aqueous–organic mobile phases.

Bonded silica phases are problematic from the point of view of pH stability and residual chemical activity of the unprotected silica support, which can induce tailing peaks and variable retention times for basic compounds.[11] The higher efficiencies achieved for the phenethylamines in the micellar system indicate that the residual silanol groups on the silica surface play a less important role in the separation with respect to conventional chromatography, as a result of the adsorption of SDS whose sulfate head groups form a hydrophilic layer above the silica surface.[12] The penetration depth of the analytes into the bonded phase is thus reduced. The modification of the stationary phase by adsorbed surfactant has profound implications not only with regard to retention and efficiency, but also selectivity.[13,14]

Most reported analytical procedures with micellar mobile phases employ C_{18} columns and only a few applications have been reported for C_8 and cyano columns. Some examples are the analysis of mixtures of proteins[15] and phenols[16] and the determination of chlorthalidone in plasma[17] and sunscreen agents in cosmetic products.[18] A degradation study was also reported with C_8 columns.[19] The amount of surfactant adsorbed on the stationary phase has important implications with respect to the retention behaviour of solutes. Adsorption isotherms of SDS have shown that the amount of adsorbed surfactant on C_8

Table 2 Structures, dissociation constants and octanol–water partition constants of the antihistamines[a]

Compound	Structure	pK_1	pK_2	Log $P_{o/w}$
Pheniramine (Pfizer, Madrid, Spain)		4.2	9.3	2.02
Carbinoxamine (ICN Biochemicals, Aurora, OH, USA)		8.1	—	2.17
Doxylamine (ICN Biochemicals)		4.4	9.2	2.28
Chlorpheniramine (Schering-Plough, Madrid, Spain)		9.2	—	2.73
Dexchlorpheniramine (Schering-Plough)		9.2	—	2.73
Dexbrompheniramine (Pfizer)		9.3	—	2.88
Diphenhydramine (Aldo Unión, Barcelona, Spain)		9.0	—	3.36
Triprolidine (ICN Biochemicals)		6.5	9.5	3.47
Azatadine (Schering-Plough)		9.3	—	3.60
Phenyltoloxamine (Pfizer)		9.1	—	3.90

[a] From refs. 21–23.

columns is lower than that in C_{18} columns.[20] The adsorbed surfactant is constant above the critical micellar concentration (8.1×10^{-3} mol l^{-1}) for C_{18} columns, but the plateau is only reached at 0.3 mol l^{-1} SDS for C_8 columns. The amount of SDS on the bonded phase is even lower for cyano columns, and increases with the concentration of surfactant in the mobile phase, at least up to 0.4 mol l^{-1} SDS.[20]

The aim of this work was to develop a rapid and simple procedure for the determination of the large number of phenethylamine–antihistamine combinations that are administered in different countries, using a limited number of mobile phases. For this purpose, the chromatographic behaviour of the drugs was examined in C_8, C_{18} and cyano columns. Mixtures of the phenethylamines phenylephrine, phenylpropanolamine,

Table 3 Analysis of pharmaceutical preparations

Compounds	Pharmaceutical (laboratory)	Composition	Found^a/ mg	RSD^a (%)	Found^b/ mg	RSD^b (%)
Phenylephrine–chlorpheniramine	Bisolgrip (Fher, Barcelona, Spain)	Per packet: phenylephrine hydrochloride (10), chlorpheniramine maleate (2), paracetamol (500), saccharose and excipients	9.71 1.92	0.8 0.9	9.80 1.95	0.8 1.3
	Rinomicine (Fardi, Barcelona, Spain)	Per packet: phenylephrine hydrochloride (6), chlorpheniramine maleate (4), ascorbic acid (300), caffeine (30), paracetamol (400), salicylamide (200) and excipients	5.89 4.05	0.4 0.6	5.91 4.08	0.4 0.7
	Propalgina plus (Roche, Barcelona, Spain)	Per packet: phenylephrine chlorhydrate (7.5), chlorpheniramine maleate (2), paracetamol (500), dextromethorphan hydrochloride (10), ascorbic acid (200), sodium cyclamate (180), saccharose (3670) and excipients	7.53 1.89	0.6 0.4	7.45 1.92	0.5 0.6
	Desenfriol D (Shering-Plough, Madrid, Spain)	Per tablet: phenylephrine hydrochloride (12.2), chlorpheniramine maleate (2), acetylsalicylic acid (390), caffeine (32.4), saccharose and excipients	11.8 1.93	0.9 0.7	12.1 1.89	0.9 1.3
	Desenfriol infantil (Shering-Plough)	Per chewable tablet: phenylephrine hydrochloride (2.5), chlorpheniramine maleate (0.5), acetylsalicylic acid (80), saccharose and excipients	2.41 0.51	0.8 1.4	2.45 0.48	0.9 0.9
Phenylpropanolamine–chlorpheniramine	Baby Rinol (Marion Merrell Dow, Alcalá de Henares, Madrid, Spain)	Per 5 ml syrup: phenylpropanolamine hydrochloride (50), chlorpheniramine maleate (3.75), paracetamol (600), saccharose, ethanol and excipients	47.8 3.77	0.9 0.4	48.5 3.75	0.8 0.7
	Durasina (Smith Kline & French, Madrid, Spain)	Per 5 ml syrup: phenylpropanolamine hydrochloride (50), chlorpheniramine maleate (4), saccharose and excipients	48.2 3.87	0.9 0.5	48.7 3.91	0.7 1.2
	Coricidin fuerte (Shering-Plough)	Per 5 ml syrup: phenylpropanolamine hydrochloride (25), chlorpheniramine maleate (4), paracetamol (500) and excipients	24.1 3.89	0.3 0.5	25.6 3.83	0.6 0.9
Phenylpropanolamine–pheniramine	Triominic gotas (Sandoz, Barcelona, Spain)	Per ml solution: phenylpropanolamine chlorhidrate (20), pheniramine maleate (10), pyrilamine maleate (10), sodium saccharin (1.6), saccharose (290), tartrazine and other excipients	18.9 9.73	1.0 0.9	19.2 9.87	0.7 0.5
Pseudoephedrine–carbinoxamine	Rondec (Abbott, Madrid, Spain)	Per tablet: pseudoephedrine hydrochloride (60), carbinoxamine maleate (2.5), saccharose and excipients	58.9 2.46	0.4 0.9	57.9 2.44	0.6 1.1
Pseudoephedrine–chlorpheniramine	Lasa con codeina (Lasa, Barcelona, Spain)	Per 5 ml syrup: pseudoephedrine hydrochloride (30), chlorpheniramine maleate (2), codeine phosphate (10) and excipients	29.3 1.88	0.7 1.1	29.5 2.02	0.4 0.6
Pseudoephedrine–dexchlorpheniramine	Polaramine expectorante (Shering-Plough)	Per 5 ml syrup: pseudoephedrine sulfate (20), dexchlorpheniramine maleate (2), saccharose, ethanol and excipients	20.9 1.91	0.6 0.6	19.7 2.03	0.8 0.9
Pseudoephedrine–dexbrompheniramine	Disofrol repetabs (Schering-Plough)	Per tablet: pseudoephedrine hydrochloride (60), dexbrompheniramine maleate (3) and excipients	59.1 2.85	0.6 1.2	58.5 2.87	0.4 0.9
Pseudoephedrine–tripolidine	Iniston jarabe (Gayoso Wellcome, Madrid, Spain)	Per 5 ml syrup: pseudoephedrine hydrochloride (30), tripolidine hydrochloride (1.25), saccharose (3.5) and excipients	28.5 1.19	0.7 0.6	28.8 1.27	0.6 0.8
Pseudoephedrine–azatadine	Atiramin jarabe (Juste)	Per 5 ml syrup: pseudoephedrine sulfate (30), azatadine maleate (1), saccharose (2000), ethanol and excipients	28.2 0.97	0.9 1.2	29.2 0.96	0.8 0.7
	Idulanex jarabe (Schering-Plough)	Per 5 ml syrup: pseudoephedrine sulfate (30), azatadine maleate (1), saccharose (2000), ethanol and excipients	28.5 0.98	0.8 0.7	29.3 0.94	0.5 1.0
Ephedrine–diphenhydramine	Bisolvon compositum (Fher)	Per 5 ml solution: ephedrine hydrochloride (7.5), diphenhydramine hydrochloride (7.5) bromhexine hydrochloride (2.5), codeine hydrochloride (10), ethanol and excipients	7.45 7.33	0.7 0.8	7.56 7.39	0.5 0.6
Phenylephrine–Chlorpheniramine–diphenhydramine	Paidoterin (Aldo-Union, Barcelona, Spain)	Per ml solution: phenylephrine hydrochloride (5), chlorpheniramine maleate (0.75), diphenhydramine (5), saccharose and excipients	4.87 0.74 4.91	0.7 0.8 0.7	4.91 0.77 4.89	1.1 0.7 0.8

[a] 0.05 M SDS–6% pentanol at pH 7, except for phenylpropanolamine–pheniramine and phenylephrine–chlorpheniramine–diphenhydramine, for which 0.15 M SDS–2% pentanol at pH 7 was used. [b] 55% methanol at pH 7.

ephedrine, pseudoephedrine and methoxyphenamine (Table 1) and the antihistamines pheniramine, carbinoxamine, doxylamine, chlorpheniramine, dexchlorpheniramine, dexbrompheniramine, diphenhydramine, tripolidine, azatadine and phenyltoloxamine (Table 2) were considered. Mobile phase selection was made with the aid of an optimisation strategy based on the modelling of the retention and peak shape. Initial experimental runs were made in the isocratic mode, followed by computer simulation and mapping of the resolution as a function of mobile phase composition.[24] It is shown that acceptable separations are obtained for most combinations with only two micellar mobile phases of SDS and pentanol and a C_8 column, which is advantageous over the other two stationary phases. The results are compared with those using an optimised mobile phase of methanol–water.

Experimental

Reagents and columns

The drugs analysed (Tables 1 and 2) were kindly donated by pharmaceutical companies, except those from Sigma and ICN Biochemicals. Stock standard solutions containing 200 μg ml^{-1} of each compound were prepared in 0.15 mol l^{-1} sodium dodecyl sulfate (99% purity, Merck, Darmstadt, Germany). The solutions were conveniently diluted for analysis.

Micellar mobile phases were prepared with SDS and pentan-1-ol (Scharlab, Barcelona, Spain). Methanol (Scharlab) was utilised in the aqueous–organic mobile phase. Disodium hydrogenphosphate (Panreac, Barcelona, Spain), HCl and NaOH (Probus, Badalona, Spain) were used to buffer the pH of the solutions. Distilled, de-ionised water (Barnstead, Sybron, Boston, MA, USA) was used throughout. The drug solutions and the mobile phases were filtered through 0.45 μm nylon membranes of 12 and 45 mm diameter (Micron Separations, Westboro, MA, USA), respectively.

The chromatographic columns were C_8 (Eclipse XDB-C, 150 × 4.6 mm id; Agilent, Palo Alto, CA, USA), C_{18} (Kromasil, 120 × 4.6 mm id; Scharlab) and cyano (Nucleosil 100-5 CN, 125 × 4 mm i.d.; Agilent). The particle size was 5 μm for all three columns.

Apparatus

The absorbance was measured with a Perkin-Elmer (Norwalk, CT, USA) Lambda 19 spectrophotometer and pH with a Crison (Barcelona, Spain) MicropH 2001 potentiometer provided with a combined Ag/AgCl/glass electrode. Chromatographic work was performed with an Agilent HP 1100 chromatograph, equipped with a quaternary pump, an autosampler with a Rheodyne (Cotati, CA, USA) valve, and a UV–visible detector (190–700 nm range). The flow rate, injection volume and detection wavelength were 1.0 ml min^{-1}, 20 μl and 260 nm, respectively. The chromatographic runs were made at 25 ± 0.2 °C. The signal was acquired by a PC connected to the chromatograph through an HP Chemstation. The software MICHROM was used for data treatment.[25]

Analytical procedures

The pharmaceuticals (Table 3) were presented as conventional tablets (Desenfriol D and Rondec), chewable tablets (Desenfriol infantil), film coated tablets (Disofrol repetabs), powders (Bisolgrip, Rinomicine and Propalgina plus), solutions (Bisolvon compositum, Triominic gotas and Paidoterin) and syrups (Baby Rinol, Durasina, Coridin fuerte, Lasa con codeina, Polaramine expectorante, Iniston jarabe, Atiramin jarabe and Idulanex jarabe).

For the analyses, 10 tablets were weighed, ground to a fine powder and homogenised. Several portions were taken and weighed, dissolved in 0.15 mol l^{-1} SDS solution containing a small amount of methanol and diluted to a suitable concentration with 0.15 mol l^{-1} SDS. The powders were treated similarly. An aliquot of the solutions and syrups was mixed with a small amount of methanol and diluted with 0.15 mol l^{-1} SDS. All sample solutions were filtered into the autosampler vials through 0.45 μm nylon membranes of 12 mm diameter.

The analyses were carried out using a C_8 column and mobile phases of 0.05 mol l^{-1} SDS–6% v/v pentanol or 0.15 mol l^{-1} SDS–2% v/v pentanol (see Table 4), both at pH 7. The results were compared with those obtained using 55% v/v methanol at pH 7, using the same column and chromatographic conditions.

Results and discussion

Optimisation of mobile phase composition

Phenethylamines are weakly acidic with pK = 8.9–10.1 (Table 1). Consequently, their retention did not change in the working pH range of conventional alkyl bonded stationary phases (pH 3–7), where the protonated species dominates. Most antihistamines studied in this work are also weakly acidic with pK = 8.1–9.3 (Table 2). However, pheniramine, doxylamine and tripolidine exhibit an additional equilibrium with p$K \approx$ 4–6.5. For these compounds, the cationic monoprotic species probably dominates at neutral pH, but at lower pH the equilibrium is shifted towards the diprotic species. In a micellar chromatographic system with the anionic SDS, the stronger attraction of the doubly charged cationic drugs to the surfactant adsorbed on the stationary phase, with respect to the micelles in the mobile phase, increases their retention at decreasing pH. The following series and the determination of the drugs in the pharmaceuticals were performed at pH 7, because of the shorter retention of the antihistamines that show a second acid–base equilibrium.

Good correlations have been reported between the retention factors (k or log k) in a micellar chromatographic system and the logarithm of the octanol–water partition constants (log $P_{o/w}$).[26] Antihistamines are low polarity compounds (log $P_{o/w}$ = 2.02–3.90), and phenethylamines are polar or moderately polar (log $P_{o/w}$ = −0.09–1.66), so the retentions of the two groups of compounds should be significantly different. Surprisingly, the elution strength of SDS micellar mobile phases was similar in both cases. It should be noted that, in a micellar system, the elution behaviour is governed by a combination of hydrophobic and electrostatic interactions.[27] The retention of phenethylamines should be explained by the strong association of the protonated species with the monomers of surfactant adsorbed on the stationary phase.

The retention of phenethylamines and antihistamines was high in a pure micellar chromatographic system (without modifier). A strong organic modifier added to the SDS mobile phase was therefore needed to elute the drugs from the columns. In previous work, a small amount of pentanol was shown to decrease the retention times of strongly retained solutes in C_{18} columns to adequate values.[10,28] This alcohol was selected for analysing the phenethylamine–antihistamine combinations. The concentration of SDS and volume fraction of pentanol in the mobile phase were optimised using a strategy based on the description of the retention and peak shape of the analytes by model equations. This strategy has been detailed elsewhere,[24] and was applied with the aid of MICHROM,[25] software that facilitates mobile phase selection on the basis of maximum separation and minimum analysis time.

The same experimental design consisting of five mobile phases buffered at pH 7 was used to examine the chromatographic behaviour of the 15 drugs (five phenethylamines and 10 antihistamines), in C_8, C_{18} and cyano columns: 0.05 mol l^{-1} SDS–2% v/v pentanol, 0.05 mol l^{-1} SDS–6% pentanol, 0.15 mol l^{-1} SDS–2% pentanol, 0.15 mol l^{-1} SDS–6% pentanol and 0.10 mol l^{-1} SDS–4% pentanol. Retention factors, efficiencies (*N*, measured at 10% of the peak height according to Foley and Dorsey[29]) and asymmetry factors (*B/A*, with *B* and *A* defined as the distance between the centre and the tailing edge and that between the centre and the leading edge of the chromatographic peak, respectively) were obtained. Fig. 1 compares the retention factors of the drugs for two mobile phases located in opposite corners of the experimental design, 0.05 mol l^{-1} SDS–6% pentanol and 0.15 mol l^{-1} SDS–2% pentanol. Fig. 2 shows the efficiencies and asymmetry factors of the drugs in these mobile phases.

The retention factors decreased at increasing concentrations of SDS and volume fraction of pentanol for the C_8 and C_{18} columns, at a greater rate for the latter for which the solutes were strongly retained in the absence of modifier. The retention decreased also at greater concentration of the surfactant for the cyano column, but increased with the addition of more modifier. This column was soon discarded owing to the low efficiencies (in the range 100–1500) and high asymmetry factors (often > 5). For the C_{18} column, the efficiencies and asymmetries were usually in the range 3000–5000 and < 1.3 for the phenethylamines and 500–1500 and < 2.1 for the antihistamines, respectively. The efficiencies were larger and the asymmetries were similar for the C_8 column (4000–6000 and < 1.4 for the phenethylamines and 2000–2500 and < 1.9 for the antihistamines). For this reason, and also because of the lower retention, this column was selected to separate the phenethylamine–antihistamine mixtures in the pharmaceuticals.

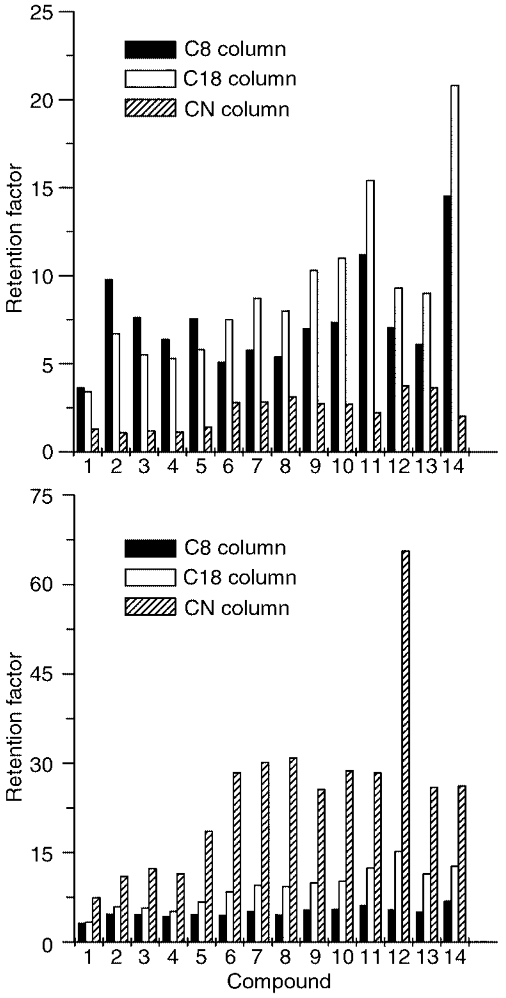


Fig. 1 Retention factors for the phenethylamines and antihistamines chromatographed with three stationary phases. Compounds: (1) phenylephrine; (2) phenylpropanolamine; (3) ephedrine; (4) pseudoephedrine; (5) methoxyphenamine; (6) pheniramine; (7) carbinoxamine; (8) doxylamine; (9) chlorpheniramine–dexchlorpheniramine; (10) dexbrompheniramine; (11) diphenhydramine; (12) tripolidine; (13) azatadine and (14) phenyltoloxamine. Mobile phases: 0.05 mol l^{-1} SDS–6% pentanol (top) and 0.15 mol l^{-1} SDS–2% pentanol (bottom).

A comparison of the chromatographic parameters in the C_8 column for 0.05 mol l^{-1} SDS–6% pentanol and 0.15 mol l^{-1} SDS–2% pentanol with those obtained in an aqueous–organic mobile phase, used below as reference in the analysis of the pharmaceuticals (55% v/v methanol), was also made. The retention times (min) for these three mobile phases were phenylephrine 4.8, 5.9, 2.2, phenylpropanolamine 7.0, 13.7, 3.0, ephedrine 6.4, 10.2, 3.5, pseudoephedrine 6.5, 8.7, 3.4, pheniramine 6.3, 8.1, 7.5, carbinoxamine 7.6, 8.0, 13.9, chlorpheniramine 8.2, 10.4, 15.2, dexbrompheniramine 7.5, 9.8, 18.0, diphenhydramine 8.3, 16.0, 25.1, tripolidine 8.0, 9.5, 25.9 and azatadine 6.9, 8.4, 19.0. As observed, the retention of the groups of phenethylamines and antihistamines differ less in the micellar system. In 55% methanol, the phenethylamines were weakly retained, whereas the antihistamines usually showed a high retention. A greater amount of methanol decreased even more the retention times of the phenethylamines, which eluted with the void volume. A smaller amount of organic solvent yielded very long retentions for the antihistamines. On the other hand, for 55% methanol, the efficiencies were in the ranges 1300–2800 and 300–1000 for the phenethylamines and antihistamines, respectively, and the asymmetry factors were in the ranges 1.2–1.9 and 2.2–3.7, respectively.

Martindale indicates the binary, ternary and quaternary combinations of the studied phenethylamines and antihistamines that are marketed throughout the world.[1] An example of the administered combinations is shown in Table 4 (about 40 combinations), which comprise one or two phenethylamines or antihistamines. The resolution of these combinations in the C_8 column was obtained at various mobile phase compositions (in the selected 0.05–15 mol l^{-1} SDS and 2–6% pentanol ranges), using the product of free peak areas.[24] This is a good measurement of peak purity and indicates reliably the best conditions of separation. In order to measure the peak purities, the retention behaviour of the drugs was modelled according to an equation proposed by Arunyanart and Cline-Love,[30] modified to consider the displacement of the micelle–water and stationary phase–water equilibria in the presence of an organic solvent.[31] This retention model has been checked previously for many different compounds eluted using C_{18} columns. The results in this work indicate that the model is also valid for C_8 columns. The prediction errors for the retention times were $< 5\%$ for most compounds.

Fig. 3 shows some examples of surface resolution diagrams for phenethylamine–antihistamine combinations. In all cases, wide regions of baseline resolution ($R \approx 1$) were observed in the whole experimental domain, except for the ternary mixtures pseudoephedrine–dexchlorpheniramine–methoxyphenamine and pseudoephedrine–ephedrine–dexchlorpheniramine. When possible, the selected mobile phase was 0.05 mol l^{-1} SDS–6% pentanol [Fig. 3(a)–(d)] because of the shorter analysis times. However, for some combinations, the resolution with this mobile phase was poor, but satisfactory for 0.15 mol l^{-1} SDS–2% pentanol [Fig. 3(e) and (f)]. On the basis of these observations, we recommend these two mobile phases for the analysis of the different combinations (Table 4), except for the two ternary combinations mentioned, for which 0.05 mol l^{-1} SDS–2% pentanol (pseudoephedrine–dexchlorpheniramine–methoxyphenamine), and 0.05 mol l^{-1} SDS–3% pentanol (pseudoephedrine–ephedrine–dexchlorpheniramine) are better choices.

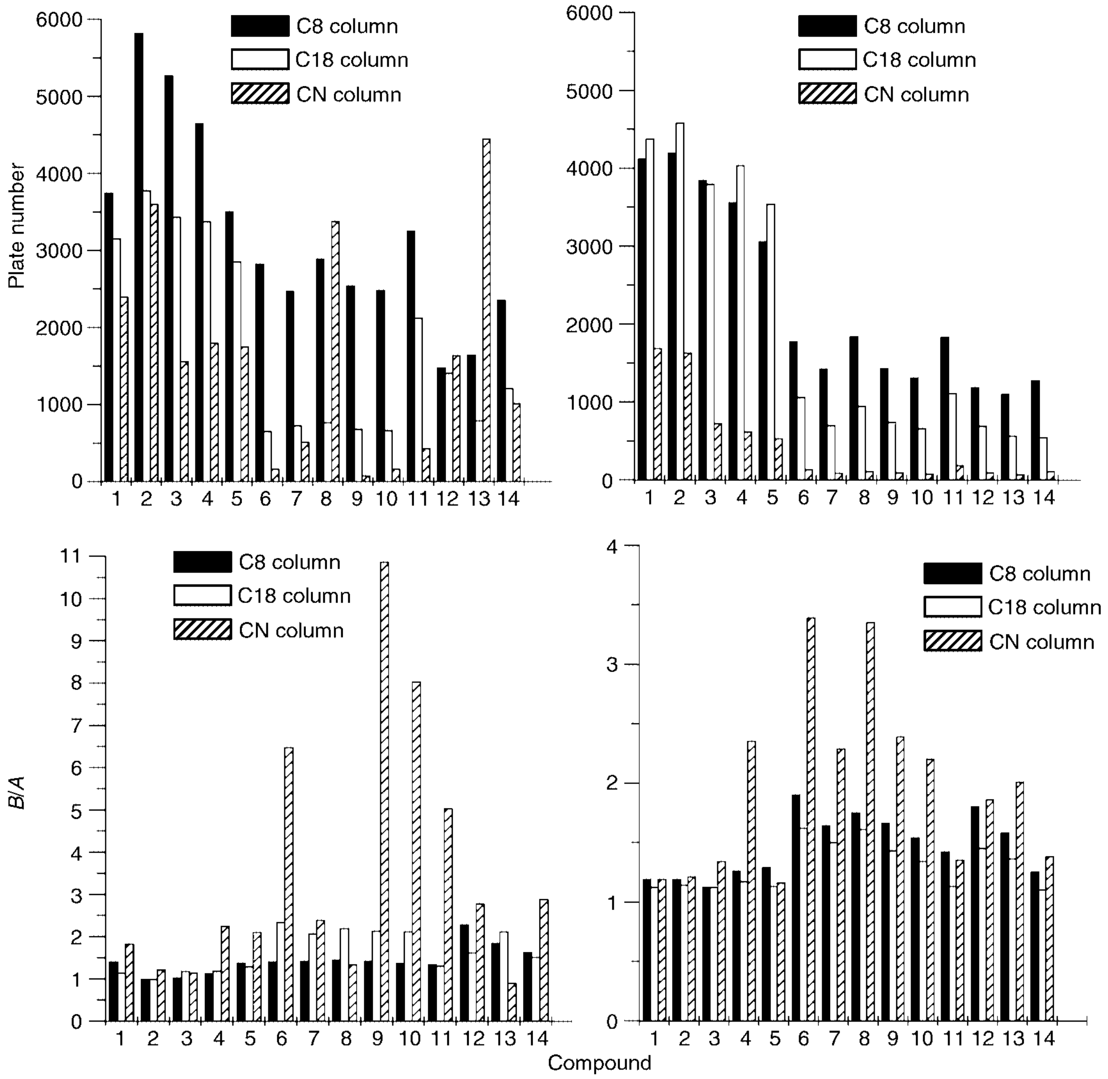


Fig. 2 Plate numbers and asymmetry factors for the phenethylamines and antihistamines chromatographed with three stationary phases. Mobile phases: 0.05 mol l^{-1} SDS–6% pentanol (left) and 0.15 mol l^{-1} SDS–2% pentanol (right). See Fig. 1 for compound identity.

Table 4 Administered combinations of antihistamines and phenethylamines, and recommended mobile phases

0.05 M SDS–6% pentanol	0.15 M SDS–2% pentanol
Phenylephrine–phenylpropanolamine	Phenylephrine–doxylamine
Phenylephrine–ephedrine	Phenylephrine–phenylpropanolamine–pheniramine
Phenylephrine–pseudoephedrine	Phenylephrine–phenylpropanolamine–chlorpheniramine
Phenylephrine–carbinoxamine	Phenylephrine–chlorpheniramine–diphenhydramine
Phenylephrine–chlorpheniramine	Phenylephrine–chlorpheniramine–phenyltoloxamine
Phenylephrine–dexbrompheniramine	Phenylephrine–phenylpropanolamine–carbinoxamine–phenyltoloxamine
Phenylephrine–diphenhydramine	
Phenylephrine–phenylpropanolamine–dexbrompheniramine	
Phenylephrine–ephedrine–dexchlorpheniramine	
Phenylpropanolamine–carbinoxamine	Phenylpropanolamine–ephedrine
Phenylpropanolamine–chlorpheniramine	Phenylpropanolamine–pheniramine
Phenylpropanolamine–diphenhydramine	Phenylpropanolamine–pseudopheniramine–dexchlorpheniramine
Phenylpropanolamine–phenyltoloxamine	
Phenylpropanolamine–dexchlorpheniramine–phenyltoloxamine	
Ephedrine–carbinoxamine	Ephedrine–pheniramine
Ephedrine–chlorpheniramine	Ephedrine–doxylamine
Ephedrine–diphenhydramine	
Ephedrine–dexchlorpheniramine–phenyltoloxamine	
Pseudoephedrine–carbinoxamine	Pseudoephedrine–methoxyphenamine
Pseudoephedrine–chlorpheniramine	Pseudoephedrine–pheniramine
Pseudoephedrine–dexbrompheniramine	
Pseudoephedrine–diphenhydramine	
Pseudoephedrine–tripolidine	
Pseudoephedrine–azatadine	
Methoxyphenamine–chlorpheniramine	
Methoxyphenamine–diphenhydramine	

Analysis of pharmaceutical preparations

The optimised procedure was applied to the analysis of several combinations of the studied phenethylamines and antihistamines administered in Spain (see Table 3). Most pharmaceuticals were analysed using 0.05 mol l^{-1} SDS–6% pentanol, with analysis times < 10 min. The analysis times for the binary and ternary combinations using 0.15 mol l^{-1} SDS–2% pentanol, which is a weaker mobile phase, were < 17 min. The results were compared with those obtained with an RPLC procedure using 55% methanol at pH 7. This mobile phase was optimised in this work for these separations.

Calibration curves were constructed from triplicate injections of five solutions of each drug at increasing concentrations in the ranges 5–50 μg ml^{-1} for phenylpropanolamine and pseudoephedrine (with a lower sensitivity) and 0.5–25 μg ml^{-1} for the other drugs. Peak areas were measured. The linear regression coefficients (r) were always > 0.999. Table 5 lists the sensitivities (slopes of the calibration curves) and intercepts for 0.05 mol l^{-1} SDS–6% pentanol and 55% methanol. In all cases, the LODs were well below those required for the analysis of the pharmaceuticals, in the range 4–225 ng ml^{-1}.

Table 6 summarises the intra-day repeatabilities (average of 10 measurements made on the same day), inter-day repeatabilities (average of 10 intra-day values taken during five consecutive days) and reproducibilities (average of 10 intra-day values taken on 10 days during 3 months) at three different drug concentrations (0.5, 5 and 25 μg ml^{-1}). The relative standard deviations (RSDs) were below 1.6, 2.5 and 2.4%, respectively.

The results obtained in the analysis of 18 pharmaceutical preparations are given in Table 3. The recoveries were in the ranges 94–102 and 94–103% for the micellar–organic and aqueous–organic mobile phases, respectively. No interference

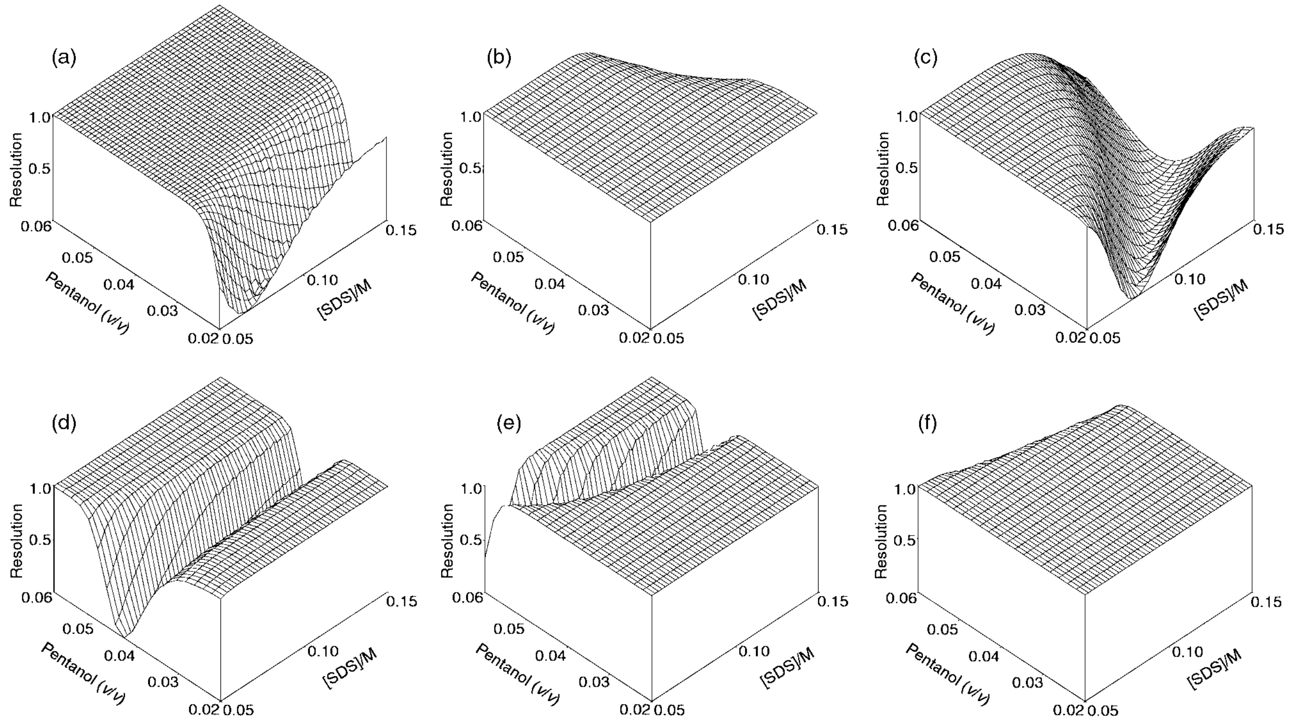


Fig. 3 Resolution diagrams for mixtures: (a) pseudoephedrine–azatadine; (b) pseudoephedrine–dexbrompheniramine; (c) pseudoephedrine–carbinoxamine; (d) phenylpropanolamine–chlorpheniramine; (e) phenylpropanolamine–pheniramine; and (f) phenylephrine–chlorpheniramine–diphenhydramine. The mobile phases contained SDS and pentanol at different concentrations and pH 7.

Table 5 Parameters of the calibration curves ($y = ac + b$; c in μg ml^{-1}) for the phenethylamines and antihistamines[a]

	SDS–pentanol			Methanol–water		
Compound	Slope	Intercept	r	Slope	Intercept	r
Phenylephrine	4.3 ± 0.2	0.11 ± 0.08	0.9990	4.0 ± 0.1	0.23 ± 0.06	0.9994
Phenylpropanolamine	0.95 ± 0.06	0.21 ± 0.06	0.9997	1.0 ± 0.1	0.11 ± 0.01	0.9997
Ephedrine	3.6 ± 0.1	0.30 ± 0.14	0.9990	3.4 ± 0.2	0.26 ± 0.08	0.9991
Pseudoephedrine	0.80 ± 0.03	0.18 ± 0.02	0.9992	1.1 ± 0.1	0.10 ± 0.03	0.9998
Methoxyphenamine	3.0 ± 0.3	0.41 ± 0.04	0.9998	3.2 ± 0.4	0.21 ± 0.11	0.9996
Pheniramine	13.3 ± 0.1	0.41 ± 0.12	0.9962	14.0 ± 0.2	0.15 ± 0.07	0.9994
Carbinoxamine	12.9 ± 0.2	3.2 ± 0.1	0.9991	12.5 ± 0.3	0.09 ± 0.02	0.9992
Doxylamine	39.0 ± 0.1	0.23 ± 0.05	0.9999	33.3 ± 0.7	0.14 ± 0.05	0.9995
Chlorpheniramine	13.5 ± 0.1	0.31 ± 0.04	0.9995	14.3 ± 0.3	0.05 ± 0.01	0.9991
Dexchlorpheniramine	13.3 ± 0.2	0.74 ± 0.12	0.9990	12.5 ± 0.3	0.17 ± 0.06	0.9992
Dexbrompheniramine	12.4 ± 0.1	0.10 ± 0.06	0.9988	12.0 ± 0.2	0.09 ± 0.02	0.9998
Diphenhydramine	11.5 ± 0.1	0.26 ± 0.11	0.9998	12.3 ± 0.1	0.27 ± 0.11	0.9992
Triprolidine	21.7 ± 0.2	0.22 ± 0.06	0.9993	24.6 ± 0.3	0.19 ± 0.07	0.9995
Azatadine	12.4 ± 0.1	0.50 ± 0.16	0.9990	13.6 ± 0.7	0.31 ± 0.14	0.9994
Phenyltoloxamine	32.4 ± 0.3	0.41 ± 0.03	0.9994	39.6 ± 0.5	0.17 ± 0.04	0.9997

[a] The mobile phases were 0.05 M SDS–6% pentanol and 55% v/v methanol at pH 7.

Table 6 Repeatabilities and reproducibilities [RSD (%), $n = 10$] for the phenethylamines and antihistamines[a]

	Intra-day repeatability			Inter-day repeatability			Reproducibility		
Compound	c_1	c_2	c_3	c_1	c_2	c_3	c_1	c_2	c_3
Phenylephrine	1.1	1.3	0.5	1.5	1.8	1.4	2.2	2.1	0.7
Phenylpropanolamine	1.9	1.1	0.7	2.5	1.4	1.7	2.4	2.0	1.2
Ephedrine	1.2	0.9	1.1	2.2	1.9	1.4	1.9	1.2	1.6
Pseudoephedrine	1.0	1.1	0.9	1.1	1.5	1.8	1.5	1.4	1.3
Methoxyphenamine	1.1	1.2	1.1	1.4	1.4	0.8	1.7	1.7	1.1
Pheniramine	0.9	1.9	1.0	1.2	1.1	1.4	1.4	1.3	1.6
Carbinoxamine	1.2	1.1	0.8	1.2	2.0	1.3	1.4	1.5	1.0
Doxylamine	1.3	2.2	0.4	1.4	1.2	2.3	2.0	1.5	0.6
Chlorpheniramine	1.4	0.9	0.6	1.3	1.2	1.2	1.9	1.4	1.3
Dexchlorphenirmine	1.2	0.5	1.0	1.6	1.5	0.9	2.0	1.3	1.1
Dexbrompheniramine	1.6	1.0	0.9	1.9	0.9	1.0	1.7	1.1	1.5
Diphenhydramine	1.0	0.6	0.8	1.1	1.9	0.9	1.4	1.0	0.7
Tripolidine	1.4	1.1	0.8	1.7	1.0	0.8	1.9	2.1	0.9
Azatadine	1.2	0.7	0.7	2.1	0.8	1.9	2.0	1.3	1.2
Phenyltoloxamine	1.3	1.6	0.4	1.6	1.2	0.7	2.5	1.7	1.2

[a] Drug concentrations were ($c_1 = 0.5$, $c_2 = 5$ and $c_3 = 25$ μg ml^{-1}). The mobile phase was 0.05 mol l^{-1} SDS–6% pentanol at pH 7.

was found from other compounds that accompany the assayed drugs in cold–cough preparations, such as acetylsalicylic acid, ascorbic acid, bromhexine, caffeine, codeine, dextromethorphan, paracetamol and salicylamide.

The proposed procedure has the advantage of using a small amount of organic solvent (2–6% pentanol against 55% methanol in the aqueous–organic procedure). Pentanol is also less toxic than methanol and is highly retained in the SDS micellar solution, which reduces the risk of evaporation. Shorter analysis times are also achieved. This work demonstrates that a large number of phenethylamine–antihistamine combinations can be analysed with only two different mobile phases of SDS and pentanol.

Acknowledgements

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