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Analysis of pharmaceutical preparations containing antihistamine drugs by micellar liquid chromatography

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Abstract

Rapid chromatographic procedures for analytical quality control of pharmaceutical preparations containing antihistamine drugs, alone or together with other kind of compounds are proposed. The method uses C_{18} stationary phases and micellar mobile phases of cetyltrimethylammonium bromide (CTAB) with either 1-propanol or 1-butanol as organic modifier. The proposed procedures allow the determination of the antihistamines: brompheniramine, chlorcyclizine, chlorpheniramine, diphenhydramine, doxylamine, flunarizine, hydroxyzine, promethazine, terfenadine, tripelennamine and triprolidine, in addition to caffeine, dextromethorphan, guaifenesin, paracetamol and pyridoxine in different pharmaceutical presentations (tablets, capsules, suppositories, syrups and ointments). The methods require minimum handling sample and are rapid (between 3 and 12 min at 1 mL min^{-1} flow rate) and reproducible (R.S.D. values < 5%). Limits of detection are lower than 1 μg mL^{-1} and the recoveries of the analytes in the pharmaceutical preparations are in the range 100 ± 10%.
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Keywords: Micellar liquid chromatography; Antihistamine drugs; Pharmaceutical preparations; Cetyltrimethylammonium bromide

1. Introduction

Organisms produce histamine as a consequence of the decarboxylation of the histidine amino acid, which produces activation of the called histamine receptors. The major allergic responses are mediated through the called H_1 receptor. Effects on the called H_2 receptors include esophageal contraction, gastric acid secretion and increased lower airway secretion. In addition, histamine activates the specific receptors that are present in the nose, eyes, respiratory conducts and skin provoking allergic reactions [1].

Antihistaminic drugs act by competitive inhibition of the H_1 or H_2 histamine receptors reducing the allergic symptoms. Despite these beneficial effects, antihistamines provoke adverse reactions like somnolence, confusion, lack of coordination, etc. However, with the development of the called second generation of antihistamines, some of these problems are being solved [1].

The determination of antihistamines in pharmaceutical preparations for its quality control has been performed using several analytical techniques, such as volumetric analysis [2,3], voltametria [4], atomic absorption [5], fluorimetry [6,7], spectrophotometry [8–12], gas chromatography [13] liquid chromatography [14–17], capillary electrophoresis [18] and micellar electrokinetic chromatography [19,20].

The *United States Pharmacopeia* (USP) [21] recommends spectrophotometric and chromatographic methods. The spectrophotometric methods in the UV region require previous extractions with hexane, ether or chloroform, retroextractions using acid media, evaporation and reconstitution [21]. Liquid chromatography methods use alkylsylane, phenyl, cyano or porous silica columns and hydro-organic mobile phases with high organic solvent content (acetonitrile, methanol and tetrahydrofuran) [21]. Frequently, an ion-pair reagent such as sodium hexanesulfonate, octanesulfonate or lauryl sulphate and an alkyl-amine, i.e.

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N,*N*-dimethyloctylamine, triethylamine, trimethylamine are added to the mobile phase in RP-HPLC in order to improve the chromatographic peak characteristics (retention time and peak shape) [21].

Micellar liquid chromatography (MLC) is a mode of reversed phase liquid chromatography, which uses aqueous solutions of surfactants above the critical micellar concentration. This chromatographic system presents some differences with respect to the classical reversed phase chromatography because the stationary phase is modified by the absorption of surfactant and the mobile phase presents surfactant micelles. This system provides hydrophobic, electronic and steric sites of interaction for solutes that allows the effective separation of compounds of different nature [22,23]. In addition, the solubilization capability of the micellar solutions simplifies the sample preparation step and reduces the consumption of organic solvents. MLC analytical procedures to determine different kinds of drugs in pharmaceutical preparations have been reported [24–33].

The aim of this work was to develop simple and rapid methods for the analysis of pharmaceutical preparations containing the most used antihistamines (brompheniramine, chlorcyclizine, chlorpheniramine, diphenhydramine, doxylamine, flunarizine, hydroxyzine, promethazine, terfenadine, tripelennamine and triprolidine) and other active components such as caffeine, dextromethorphan, guaifenesin, paracetamol and pyridoxine in several pharmaceutical preparations (tablets, capsules, syrups and creams). In order to adjust the eluent strength of the micellar mobile phase and reduce the analysis time cetyltrimethylammonium bromide (CTAB) was used.

2. Experimental

2.1. Reagents and standards

Micellar mobile phases were prepared using cetyltrimethylammonium bromide (99%, Acros Organics, Geel, Belgium) as surfactant. CTAB was dissolved in different buffered solutions depending on the required working pH for the analysis: (i) for pHs 3, 6 and 7, aqueous solutions of 0.05 M phosphate buffer were prepared with sodium dihydrogen phosphate (analytical reagent, Panreac, Barcelona, Spain) and (ii) for pH 5, aqueous solutions of 0.05 M citric buffer were prepared with trisodium citrate (analytical reagent, Guinama, Valencia, Spain). Appropriate amount of 2 M solutions of sodium hydroxide (for analysis, Panreac) or hydrochloric acid (for analysis, Merck, Darmstadt, Germany) were added to adjust the pH. After that, adequate volumes of 1-propanol or 1-butanol (both HPLC grade, Scharlab, Barcelona, Spain) were added to obtain the working concentration.

The antihistamine drugs and the other compounds involved in this work were obtained from several sources: brompheniramine, chlorcyclizine, chlorpheniramine, doxylamine, flunarizine, guaifenesin, promethazine, terfenadine and triprolidine from Sigma–Aldrich, S.A. (Madrid, Spain); caffeine, dextromethorphan, diphenhydramine, hydroxyzine, paracetamol, pyridoxine and tripelennamine from Guinama.

Stock standard solutions of the antihistamine drugs were prepared by dissolving the compounds in 0.02 or 0.04 M CTAB solutions, depending on the surfactant concentration in the mobile phase. Working solutions were prepared by dilution of the stock standard solutions with mobile phase.

Barnstead E-pure, deionized water (Sybron, Boston, MA) was used throughout. The mobile phases and the solutions injected into the chromatograph were vacuum-filtered through 0.45 μm nylon membranes (Micron Separations, Westboro, MA, USA). The solutions were stored in the refrigerator at 4 °C.

2.2. Instrumental and measurement

An Agilent 1100 chromatograph with an isocratic pump, an UV–vis detector was used (Palo Alto, CA, USA). Data acquisition and processing were performed on a HP Vectra XM computer (Amsterdam, The Netherlands) equipped with HP-Chemstation software from Agilent (A0402, 1996).

The solutions were injected into the chromatograph through a Rheodyne valve (Cotati, CA, USA) with a 20 μL loop. A Spherisorb octadecyl-silane column (5 μm, 250 mm × 4.6 mm i.d.) from Scharlau (Barcelona, Spain) was used. The mobile phase flow rate was 1.0 mL min^{-1}. UV detection was performed using wavelength values close to the maximum absorption ones of the compounds. All the assays were carried out at room temperature.

In order to obtain the absorption spectra of the compounds, an Agilent 8452A Spectrophotometer with diode array and equipped with Hewlett-Packard computer, model Vectra ES/12 (Palo Alto, CA, USA) was used.

A microph 2000 pH-meter (Crison, Barcelona, Spain) was used for pH adjustment and an ultrasonic bath (Ultrasons Selecta, Barcelona, Spain) was used to remove the air from the mobile phases.

2.3. Sample preparation

Pharmaceuticals of antihistamines are commercialized under different presentations, such as tablets, capsules, ointments, suppositories and syrups.

For the analysis of tablets, 10 units were weighed, ground in a mortar and finally, an adequate amount of the solid (50 mg) was taken and dissolved in 0.02 M CTAB solution, buffered at pH 3, using an ultrasonic bath (10 min). In the case of the pharmaceutical presentations Ilvico and Delor, after grinding the tablets, the powder obtained was dissolved in methanol. After that, an adequate volume of aliquot was taken and diluted with mobile phase. The resulting solution was centrifuged and finally, an aliquot of the clean solution was injected into the chromatograph. For the analysis of capsules, three units were taken and dissolved in 0.02 M

Table 1
Structure, log *P*, p*K* and detection wavelength (nm) of the antihistamine drugs and the other compounds determined

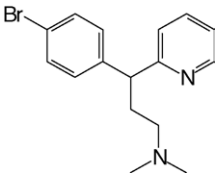
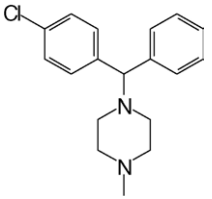
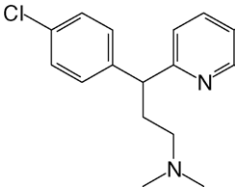
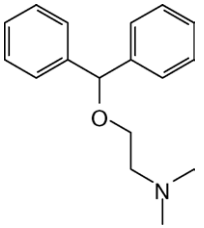
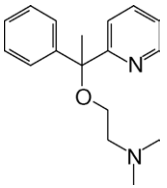
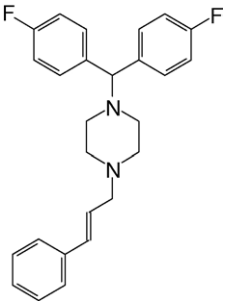
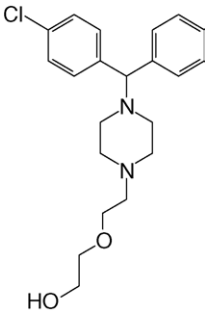
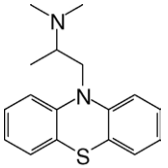
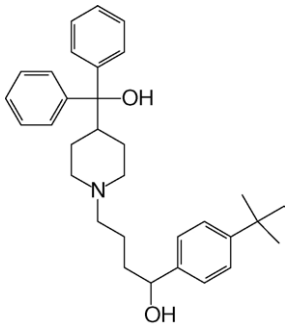
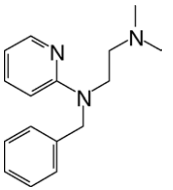
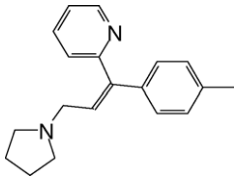
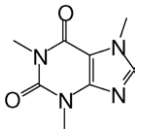
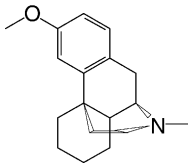
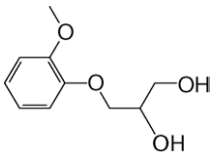
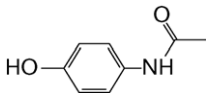
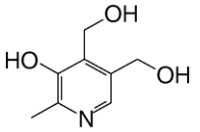
Compound	Structure	log *P*	p*K*	λ, nm	Compound	Structure	log *P*	p*K*	λ, nm
Bromfeniramine		4.06	3.90 9.79	225	Chlorcyclizine		4.68	2.12; 8.15	231
Chlorpheniramine		3.38	4.00; 9.16	225	Diphenhydramine		3.36	9.00	225
Doxylamine		2.28	4.40; 9.20	260	Flunarizine		6.42	2.10; 7.80	254
Hydroxyzine		4.16	2.13; 7.13	231	Promethazine		4.65	9.10	254

Table 1 (*Continued*)

Compound	Structure	log *P*	p*K*	λ, nm	Compound	Structure	log *P*	p*K*	λ, nm
Terfenadine		6.09	9.50	225	Tripelennamine		2.85	4.20; 8.71	240
Triprolidine		3.47	6.50; 9.50	232	Caffeine		0.07	0.6; >14	273
Dextromethorphan		3.99	8.3	232	Guaifenesin		−0.11	–	232
Paracetamol		0.49	9.71	240	Pyridoxine		−0.69	5.00; 8.96	225

CTAB solution by immersion in an ultrasonic bath (1 h), then, an adequate aliquot was taken an dissolved in mobile phase.

Ointments and suppositories were completely dissolved in 1-propanol using a magnetic stirrer with gentle heating. Then, an aliquot was taken and diluted with mobile phase. At this stage, a precipitate could be obtained when the mobile phase is added. In this case, the solution was centrifuged and filtered before injecting into the chromatograph. Finally, in the case of syrups, an adequate aliquot was taken and dissolved in mobile phase.

In all cases, after the appropriated dilution with mobile phase, working solutions were injected into the chromatographic system through 0.45 μm nylon membrane. Finally, for each pharmaceutical presentation, three independent sample solutions were prepared and for each sample solution, three injections into the chromatograph were carried out (nine injections per pharmaceutical).

3. Results and discussion

3.1. Retention behaviour of antihistamines with CTAB micellar mobile phases

Table 1 shows the structures, the octanol water partition coefficient (log *P*), the protonation constant in aqueous medium (log *K*) and the detection wavelength in nm ($\lambda_{detection}$) of the antihistamines and the other drugs of interest determined. In order to select the detection wavelength, the absorption spectra of compounds in CTAB micellar medium were obtained.

As can be observed in Table 1, antihistamines are hydrophobic compounds with log *P* ranged between 2.28 for doxylamine and 6.42 for flunarizine. They are basic compounds with one or two ionisable groups and log *K* values between 8 and 10 for the first protonation constant (tertiary amine group) and between 2 and 6 for the second protonation (nitrogen heterocyclic).

In order to select the nature of surfactant for preparing micellar mobile phases it is important to take into account the interactions that could take place in the chromatographic system between the solutes and the surfactants. At the usual working pH range in RPLC, antihistamines are positively charged. If an anionic surfactant (as SDS) is used to prepare the micellar mobile phases, high retention times of compounds could be expected by the electrostatic attractions between analytes and the surfactant adsorbed onto the stationary phase, in addition to the hydrophobic component [14]. Therefore, large amount of organic solvents with high eluent strength had to be added to the mobile phase in order to adjust the elution time of analytes (i.e. pentanol; [14]).

On the other hand, due to the hydrophobic character of antihistamines, the use of non-ionic surfactants also provides high retention times (*k* values ranged between 17 and 120 for doxilamine and flunarizine, respectively, using 0.06 M Brij35, pH 7.4 micellar mobile phase; [34]). Therefore, micellar mobile phases of a cationic surfactant seem to

Table 2
Retention factors (*k*) of the compounds in different CTAB mobile phases

	Retention factor (*k*)								
	0.02 (M)[a] 1-Propanol[b]				0.04 (M)[a] 1-Butanol[b]				
	3 (%)[c]			3 (%)[c]	1 (%)[c]	3 (%)[c]	10 (%)[c]	10 (%)[c]	3 (%)[c]
	7[d]	6[d]	3[d]	3[d]	3[d]	3[d]	3[d]	6[d]	5 (citrate)[e]
Brompheniramine	–	60.3	40.3	15.1	12.9	10.7	–	–	–
Chlorcyclizine	>100	75.2	7.91	5.10	3.70	3.60	–	–	8.70
Chlorpheniramine	–	60.4	39.7	15.8	12.3	10.8	–	–	–
Diphenhydramine	17.5	3.50	1.21	1.10	0.95	0.90	–	–	–
Doxylamine	2.00	0.50	0.20	0.20	0.17	0.10	–	–	–
Flunarizine	48.6	–	–	41.0	34.3	26.7	9.63	48.8	–
Hydroxyzine	>100	68.5	4.15	2.90	2.10	2.60	–	–	–
Promethazine	91.1	49.1	9.25	5.70	4.20	4.00	–	–	–
Terfenadine	–	–	–	37.5	25.2	24.1	11.1	16.1	–
Tripelennamine	9.90	1.70	0.26	0.20	0.21	0.10	–	–	–
Triprolidine	–	5.40	0.37	0.30	0.25	0.10	–	–	–
Caffeine	–	–	–	1.10	1.10	0.86	–	–	–
Dextromethorphan	–	4.00	–	–	–	–	–	–	–
Guaifenesin	–	8.14	–	5.80	5.80	5.30	–	–	3.60
Paracetamol	–	–	–	3.40	3.20	2.60	–	–	–
Pyridoxine	0.86	–	–	–	–	–	–	–	–

[a] CTAB (M).
[b] Modifier.
[c] Concentration of modifier.
[d] pH (phosphate).
[e] pH (citrate).

be more appropriate in order to reduce the retention of compounds due to the expected electrostatic repulsions between the analyte and the surfactant monomers adsorbed on the stationary phase and CTAB was selected for further studies.

The influence of the mobile phase pH, surfactant concentration and nature and concentration of organic modifiers on the retention of antihistamines was studied. Table 2 shows the retention factors obtained using different CTAB mobile phases. In all cases, the decrease of the mobile phase pH in the range 6–3 produced a decrease in the retention, especially for clorcyclizine and hydroxycine. The behaviour observed can be explained taking into account that the decrease of the mobile phase pH produces an increase of the cationic molar charge of antihistamines so the electrostatic repulsions between solutes and the CTAB monomers absorbed on the stationary phase will be stronger. A mobile phase pH 3 was selected for further studies.

As can be observed in Table 2, an increase in CTAB concentration from 0.02 to 0.04 M (at pH 3 and 3% 1-propanol) produced a decrease in k, especially for the most retained antihistamines (brompheniramine and chlorpheniramine). The presence of organic modifiers like alcohols in micellar chromatography is usual because their addition improves the retention and the peak efficiency. The use of 1-butanol instead of 1-propanol (at pH 3, 0.04 M CTAB and 3% alcohol) decreases the retention of the compounds. In the same way, when the 1-butanol concentration increase the retention of the antihistamines decrease.

3.2. Mobile phase selection for pharmaceutical analysis

Some of the pharmaceutical preparations available in Spain contain other active components besides the antihistamines. Some of these compounds were also determined. Therefore, additional studies were performed in order to select the adequate composition of the mobile phase. Table 3 shows the mobile phase composition selected for the analysis of these pharmaceutical preparations.

For Ilvico preparation, that contains brompheniramine, caffeine and paracetamol, a good resolution between compounds was obtained for all the mobile phases assayed (see Table 2). Therefore, the mobile phase with the highest eluent strength for these compounds (CTAB 0.04 M, 3% 1-butanol and pH 3) was selected. The same mobile phase was adequate to determine chlorpheniramine and paracetamol in the pharmaceutical Delor.

The analysis of Iniston preparations was performed using mobile phases of CTAB 0.02 M, 3% 1-propanol and pH 6 (see Table 3). With this mobile phase, a good resolution for triprolidine and guaifenesin and for triprolidine and dextromethorphan was obtained. The content of doxylamine and pyridoxine in the pharmaceutical preparation Cariban were determined using 0.02 M CTAB, 3% 1-propanol and pH 7 ($k_{\text{doxylamine}} = 2.00$ and $k_{\text{pyridoxine}} = 0.86$).

Finally, for the determination of chlorcyclizine and guaifenesin present in Diminex preparation, the resolution was achieved changing the mobile phase pH from 3 to 5. As can be observed in Fig. 1, the retention of guaifenesin was scarcely modified by the pH due to its non-ionic nature, while the retention time for chlorcyclizine increases when the mobile phase pH increases. An adequate resolution was achieved using 0.04 M CTAB, 3% 1-butanol at pH 5 mobile phase. In order to achieve an adequate buffer capacity at this pH, the phosphate buffer system was changed by citrate system. In this situation, a slight change in the retention of the compounds was observed ($k_{\text{chlorcyclizine}} = 9.90$; $k_{\text{guaifenesin}} = 5.20$

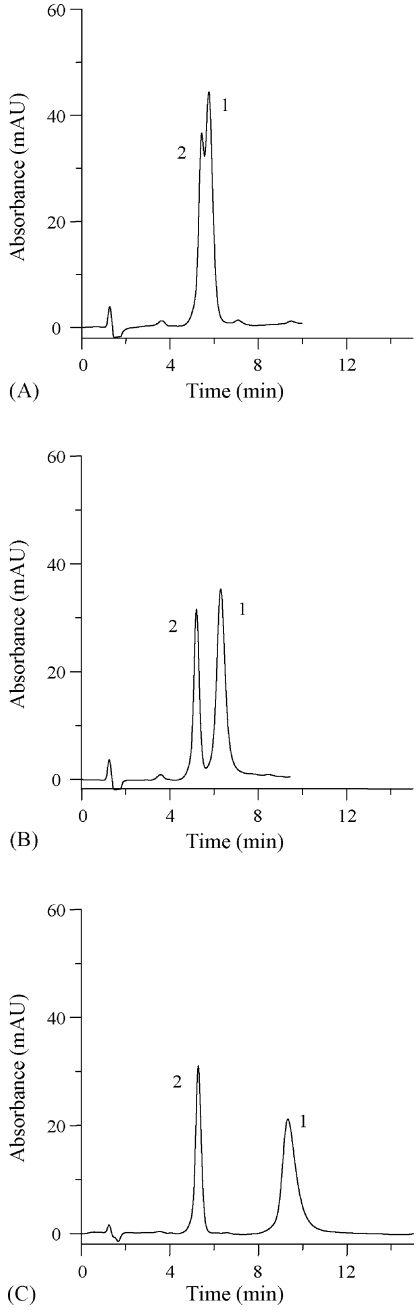


Fig. 1. Influence of the mobile phase pH (CTAB 0.04 M, 3% 1-butanol) on the resolution of chlorcyclizine (1) and guaifenesin (2) in the pharmaceutical preparation Diminex: (A) pH 3; (B) pH 4; (C) pH 5.

Table 3
Composition of the pharmaceutical preparations, recoveries and mobile phases used

Preparation (presentation), source	Composition	Recovery ± S.D. (%)	Mobile phase[a]
Ilvico (tablets), Merck Pharma and Chemistry, S.A.	Brompheniramine maleate 3 mg Caffeine 30 mg Paracetamol 325 mg Excipients cs	102.3 ± 0.7 102 ± 3 100.5 ± 0.5	(A)
Delor (tablets), Viñas Laboratory, S.A.	Chlorpheniramine maleate 2 mg Paracetamol 500 mg Phenilephrine hydrochloride 5 mg Excipients cs	96 ± 3 99.4 ± 0.4	(A)
Diminex (suppositories), Viñas Laboratory, S.A.	Chlorcyclizine hydrochloride 2.5 mg Guaifenesin 25 mg Codeine fosfato 2.5 mg Eucaliptol 50 mg	70 97.0	(B)
Benadryl (capsules), Warner Lambert	Diphenhydramine hydrochloride 50 mg Excipients cs	100.1 ± 0.4	(C)
Benadryl (syrup), Warner Lambert	Diphenhydramine hydrochloride 12.5 mg/5 mL Excipients cs	100.39 ± 0.08	(C)
Difenhidramina (tablets), Stein Laboratory	Diphenhydramine hydrochloride 50 mg Excipients cs	102 ± 1	(C)
Dormidina 25 (tablets), PENSA Laboratory	Doxylamine succinate 25 mg Excipients cs	100 ± 2	(D)
Cariban (capsules), Inibsa Laboratory, S.A.	Doxylamine succinate 10 mg Pyridoxine hydrochloride 10 mg Excipients cs	95 ± 5 101 ± 2	(D)
Sibelium (tablets), Esteve Laboratory, S.A.	Flunarizine hydrochloride 5 mg Excipients cs	99.7 ± 0.3	(E)
Atarax (tablets), UCB Pharma, S.A.	Hydroxyzine dihydrochloride 25 mg Excipients cs	96 ± 1	(A)
Atarax (syrup), UCB Pharma, S.A.	Hydroxyzine dihydrochloride 10 mg/5 mL Excipients cs	97 ± 3	(A)
Actithiol Antihistaminico (syrup), Almirall Prodesfarma, S.A.	Prometazine hydrochloride 2.5 mg/5 mL Carbocysteine 100 mg/5 mL Excipients cs	100.5 ± 0.3	(A)
Rapidal (tablets), Bial Industrial Pharmaceutical, S.A.	Terfenadine 60 mg Excipients cs	102 ± 3	(E)
Rapidal (syrup), Bial Industrial Pharmaceutical, S.A.	Terfenadine 30 mg/5 mL Excipients cs	101 ± 2	(E)
Azaron (ointment), Spanish Chefaro Laboratory, S.A.	Tripelennamine hydrochloride 20 mg Excipients cs	91 ± 3	(C)
Iniston Antitusivo (syrup), Pfizer Laboratory	Triprolidine hydrochloride 1.25 mg/5 mL Dextromethorphan hydrobromide 10 mg/5 mL Pseudoephedrine hydrochloride 30 mg/5 mL Excipients cs	90.9 ± 0 1 100.3 ± 0.7	(C)
Iniston Expectorante (syrup), Pfizer Laboratory	Triprolidine hydrochloride 1.25 mg/5 mL Guaifenesin 100 mg/5 mL Pseudoephedrine hydrochloride 30 mg/5 mL Excipients cs	92.1 ± 0.3 93.4 ± 0.3	(C)
Iniston (tablets), Pfizer Laboratory	Triprolidine hydrochloride 2.5 mg Pseudoephedrine hydrochloride 60 mg Excipients cs	102.1 ± 1.5	(C)

[a] Mobile phases used: (A) CTAB 0.04 M, 3% 1-butanol, pH 3, (B) CTAB 0.04 M, 3% 1-butanol, pH 5, (C) CTAB 0.02 M, 3% 1-propanol, pH 6, (D) CTAB 0.02 M, 3% 1-propanol, pH 7, (E) CTAB 0.04 M, 10% 1-butanol, pH 3.

and $k_{chlorcyclizine} = 8.70$; $k_{guaifenesin} = 3.60$ using phosphate and citrate buffer, respectively). For the rest of compounds, the mobile phase selected for their determination is indicated in Table 3.

3.3. Analytical data

In the selected chromatographic conditions, the calibration curve for each compound studied was obtained by triplicate injections of standard solutions containing analyte concentrations in the range 5–50 $\mu g\,mL^{-1}$. Table 4 shows the regression statistics of the calibration curves for each compound using peak areas as dependent variable, the relative standard deviations (R.S.D.) and the limits of detection (LOD). As can be observed in Table 4, adequate regression coefficients ($r > 0.999$) were obtained in all cases.

The repeatability of the methods (expressed as relative standard deviation) was evaluated at two concentration levels: 1 and 10 $\mu g\,mL^{-1}$ for hidroxyzine; 1 and 20 $\mu g\,mL^{-1}$ for brompheniramine, chlorpheniramine, paracetamol, prometazine and tripelennamine; and 5 and 20 $\mu g\,mL^{-1}$ for caffeine, dextromethorphan, diphenhydramine, doxylamine, flunarizine, guaifenesin, terfenadine, triprolidine and pyridoxine. For this purpose, 10 independent solutions for each concentration were injected. As can be observed in Table 4, the relative standard deviation values obtained were lower than 10% for the lowest concentration assayed and ranged between 1 and 5% for the highest concentration level studied.

The limits of detection were calculated according to the 3s criterion from the peak area standard deviation corresponding to 10 independent solutions injected into the chromatograph, with an analyte concentration close to its LOD value. In concrete, the concentrations for each compound assayed were: 1 $\mu g\,mL^{-1}$ for brompheniramine, chlorpheniramine, hydroxyzine, paracetamol, prometazine and tripelennamine, and 5 $\mu g\,mL^{-1}$ for caffeine, dextromethorphan, diphenhydramine, doxylamine, flunarizine, guaifenesin, pyridoxine, terfenadine and triprolidine. As can be observed in Table 4, the limits of detection obtained for the compounds studied were, in general, ranged between 0.03 and 1.8 $\mu g\,mL^{-1}$.

3.4. Analysis of pharmaceutical preparations

Table 3 shows the composition of the pharmaceutical preparations analyzed, their presentation, manufacturing source, and the recoveries obtained using the chromatographic conditions selected for each compound. The content of each analyte in the pharmaceutical formulations was determined by triplicate injections of three independently prepared solutions. In the case of Ilvico and Delor, different dilutions for the determination of each active chemical were necessary due to different amount of compounds in the pharmaceutical preparation. Fig. 2 shows the chromatograms obtained for the pharmaceutical preparations analysed in the conditions selected previously. In all cases, an adequate resolution for the pharmaceutical preparations with different active chemicals was obtained.

As can be observed in Table 3, the recoveries obtained for all compounds studied in different preparations were close to 100%, with values ranged between 91 and 110%, which agree with the tolerances indicated by the USP pharmacopeia

Table 4
Regression statistics of the calibration curves for the antihistamines and the other chemicals, relative standard deviations (R.S.D.) and limits of detection (LOD) (peak areas as dependent variable)

Compounds	$m \pm ts$	$n \pm ts$	r	S.E.	R.S.D. (%)[c]	R.S.D. (%)[d]	LOD ($\mu g\,mL^{-1}$)
Brompheniramine	0.224 ± 0.007	0.0 ± 0.2*	0.9997	0.11	1.2	1.8	0.03
Chlorcyclizine	0.75 ± 0.13	−0.3 ± 1.7*	0.9969	0.3	2.0	1.9	0.34
Chlorpheniramine	0.249 ± 0.007	0.0 ± 0.2*	0.9998	0.09	7.8	0.3	0.2
Diphenhydramine	0.442 ± 0.008	−0.1 ± 0.2*	0.9999	0.11	10.3	1.5	1.2
Doxylamine	0.195 ± 0.006	−0.2 ± 0.2*	0.9998	0.08	5.5	3.1	0.65
Flunarizine	0.36 ± 0.02	0.0 ± 0.5*	0.9996	0.17	1.3	3.8	0.2
Hydroxyzine	0.63 ± 0.02	0.1 ± 0.7*	0.9996	0.3	4.7	4.1	0.2
Prometazine	1.59 ± 0.09	2 ± 3*	0.9991	1.3	1.1	3.3	0.03
Terfenadine	0.259 ± 0.006	0.0 ± 0.2*	0.9998	0.09	9.4	7.23	1.8
Tripelennamine	1.00 ± 0.04	1 ± 1*	0.9996	0.6	4.2	2.5	0.3
Triprolidine	0.85 ± 0.03	−0.5 ± 0.8*	0.9998	0.4	8.4	5.7	0.8
Caffeine	0.57 ± 0.03	1.0 ± 0.9	0.9992	0.4	3.1	1.5	0.64
Dextromethorphan	0.20 ± 0.01	0.0 ± 0.4*	0.9991	0.18	6.6	1.1	1.7
Guiafenesin[a]	0.43 ± 0.02	−0.8 ± 0.7*	0.9993	0.3	1.9	0.7	0.2
Guiafenesin[b]	0.350 ± 0.008	0.0 ± 0.2*	0.9998	0.08	1.1	0.5	0.15
Paracetamol	0.87 ± 0.01	0.5 ± 0.4	0.9999	0.16	3.7	0.9	0.1
Pyridoxine	0.30 ± 0.01	−0.2 ± 0.4*	0.9995	0.19	2.7	2.4	0.35

m: slope; n: intercept ((*) means statistically non-significant); ts: confidence interval at the 95% level; r: correlation coefficient; S.E.: standard error; R.S.D.: relative standard deviation; LOD: limit of detection.

[a] CTAB 0.02 M, phosphate buffer 0.05 M, pH 6, 3% 1-propanol.

[b] CTAB 0.04 M, citrate buffer 0.05 M, pH 5, 3% 1-butanol.

[c] Values for the lowest concentration used to determine R.S.D. (see Section 3.3).

[d] Values for the highest concentration used to determine R.S.D. (see Section 3.3).

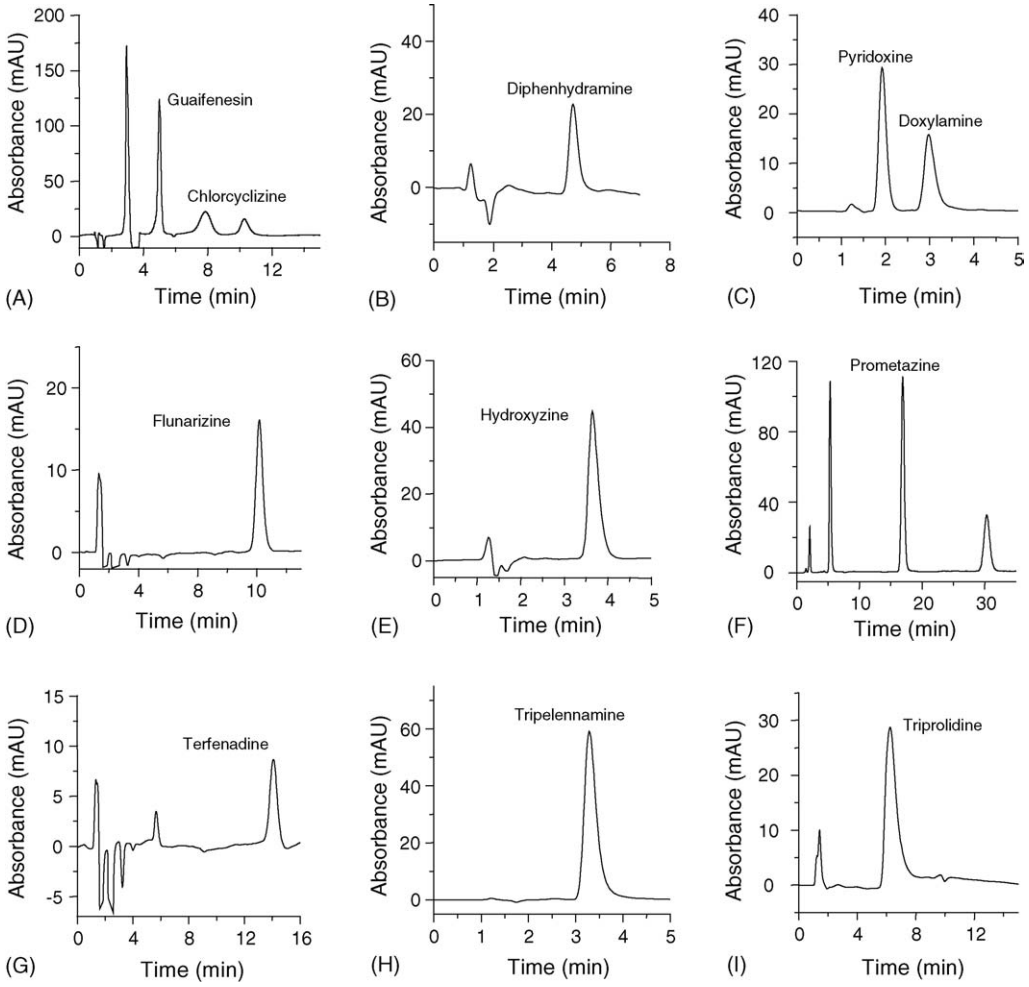


Fig. 2. Chromatograms obtained for some of the pharmaceutical presentations analysed: (A) Diminex (suppositories); (B) Benadryl (capsules); (C) Cariban (capsules); (D) Sibelium (tablets); (E) Atarax (syrup); (F) Actithiol Antihistamínico (syrup); (G) Rapidal (tablets); (H) Azaron (Ointment); (I) Iniston (tablets).

except for chlorcyclizine (70%) in Diminex (suppositories). On the other hand, the standard deviations for the recoveries are, in general, lower than 3, thus showing a good repeatability.

For the pharmaceutical Diminex a recovery study for chlorcyclizine was made in order to evaluate the existence of systematic errors in the method. For this purpose, four independent samples (suppositories) were prepared. The recovery for chlorcyclizine was 99.7% that indicates the absence of systematic errors at least at the concentration level assayed. Therefore, the low recovery obtained for this compound in the pharmaceutical preparation could be due to an error in the formulation or to a degradation of the chlorcyclizine.

4. Conclusions

The methods described allow a rapid, simple and reproducible determination of the antihistamine drugs and other compounds that are also presented in the pharmaceutical preparations. The LOD and R.S.D. values are sufficiently good for the applicability of this method in the quality control of these pharmaceutical formulations. Due to the versatility of the interactions in micellar liquid chromatography, it is possible to determine a great variety of compounds including those with high hydrophobicity in adequate times of analysis. Moreover, the micellar solutions possess a high solvent capability so the sample preparation step is very simple. On the other hand, this methodology reduces the use of organic solvents, so it is less contaminant than the traditional HPLC.

Therefore, the use chromatographic systems based on micellar mobile phases of CTAB surfactant can be a good alternative for the determination of antihistamine drugs in pharmaceutical preparations.

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