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High performance liquid chromatographic determination of ambroxol in the presence of different preservatives in pharmaceutical formulations

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Abstract

A high-performance chromatographic method is described for simultaneous determination of ambroxol in the presence of different preservatives in syrups. The method separates ambroxol from methyl- ethyl-, propyl- and butyl paraben and from other multi-component mixtures. The retention behaviour of ambroxol and parabens as a function of both pH and mobile phase composition was investigated. The eluents were monitored with a UV detector at 247 nm. Linear relationships between the amount of pharmaceutical compounds and peak heights were confirmed at the concentrations of 0.74–14.08 μg ml^{-1}. The high recovery (no extraction of the samples is required) and the low %RSD confirm the suitability of the proposed method for the determination of ambroxol in different pharmaceutical preparations.

Keywords: Ambroxol; Parabens; Sodium benzoate; Syrup; Expectorant; Reverse-phase liquid chromatography

1. Introduction

Ambroxol (*trans*-4-[(2-amino-3,5-dibromophenyl-methyl-amino] cyclohexanol) is used as a bronchosecretolysis mucolytic expectorant ingredient in different pharmaceutical preparations. It is administered as hydrochloric salt in daily doses of 30–120 mg using mostly oral formulations like tablets and syrups.

Several spectrophotometric methods have been employed for the qualitative and quantitative determination of ambroxol. These include among others simple UV spectrophotometry [1] and flow injection [2,3]. In the latter, a continuous liquid–liquid extractor coupled on-line to a spectrophotometer or an atomic absorption spectrometer is described for the determination of ambroxol using orange IV and bromothymol blue dyes, the inorganic complexes BiI_4^-, $Co(SCN)_4^{2-}$ and Reinecke's salt to form ion-pairs with the drug [2–5]. Capillary zone electrophoresis combined with photodiode-array detection for the drug assay in dosage forms and biological fluids is also reported [6].

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Currently, ambroxol is assayed in human plasma, urine and pharmaceutical formulations by gas chromatography with electron capture detection [7,8] and by high-performance liquid chromatography with UV and amperometric detection [1,9–12] using different chromatographic systems. In most pharmaceutical preparations, especially in syrups, preservation is essential because the excipients, and sometimes the drug itself, may be destroyed by different micro-organisms and consequently the formulation breaks down. Synthetic preservatives constitute the largest and most commonly used group in the preservation of pharmaceutical products. The esters of *p*-hydroxybenzoic acid with different alcohols, known as hydroxybenzoates or parabens, and sodium benzoate are widely used as preservatives [13]. However in the HPLC methods reported in the literature for the determination of ambroxol in different pharmaceutical preparations, co-elution of ambroxol with parabens or other preservatives is not considered possible. The described procedure allows the simultaneous determination of ambroxol with different preservatives, in the presence of a sweetening agent, without sample pretreatment.

2. Experimental

2.1. Chemicals and solutions

Ambroxol hydrochloride was kindly donated by Boehringer Ingelheim while sodium benzoate, saccharine methyl-, ethyl-, propyl-, and butyl-paraben were obtained from Sigma (St Louis, MO) and used without further purification. HPLC-grade methanol and water were bought from Riedel-de Haen and used throughout. All other chemicals were of analytical reagent grade and were used as received.

Stock solutions were prepared by accurately weighing the appropriate amounts of ambroxol hydrochloride, parabens and sodium benzoate and dissolving each separately in methanol in a 50-ml volumetric flask to prepare standard stock solutions. Working standard solutions of ambroxol and other analytes were prepared from the stock solutions by sequential dilutions with mobile phase to give concentrations in the range, as indicated in Table 1.

Samples from the syrups were also dissolved appropriately in mobile phase and portions of the sample solutions were analysed directly.

2.2. Instrumentation

The HPLC apparatus consisted of a Shimadzu Series LC-6A, a SPD-6AV UV spectrophotometric detector controlled by a SCL-6B system programmer module and was operated at 247 nm. Chromatograms were recorded on chart paper with a Shimadzu Chromatopac Model C-R 6A thermal printer-plotter at a speed of 2 mm min^{-1}. The Alltech (Rosil C_{18} 5 μm, 150 mm × 4.6 mm i.d.) reversed phase column with a guard cartridge precolumn was placed in a Model CTO-6A oven at 40°C, and equipped with a Rheodyne Model 7167 injector with a 20-μl loop. The mobile phase was delivered to the column isocratically.

Table 1
Concentration range, linear regression and correlation data of calibration curves for the compounds (standards) determined at 247 nm

Compound	Concentration (μg ml^{-1})	Height (mm)	t_R (min)	k'	Slope	Intercept	r
Ambroxol	0.74–5.88	10–80	6.16	1.4	13.64	−0.54	0.9993
Methyl paraben	0.13–1.02	16–121	3.72	0.3	118.21	−1.71	0.9999
Ethyl paraben	0.16–1.28	17–132	4.39	0.4	102.46	0.61	0.9989
Propyl paraben	0.16–1.28	12–90	5.00	0.8	68.38	−0.36	0.9997
Butyl paraben	0.29–2.31	19–125	5.92	1.0	53.12	6.04	0.9988
Sodium benzoate	1.76–14.08	11–91	3.97	0.5	6.50	−0.52	0.9999

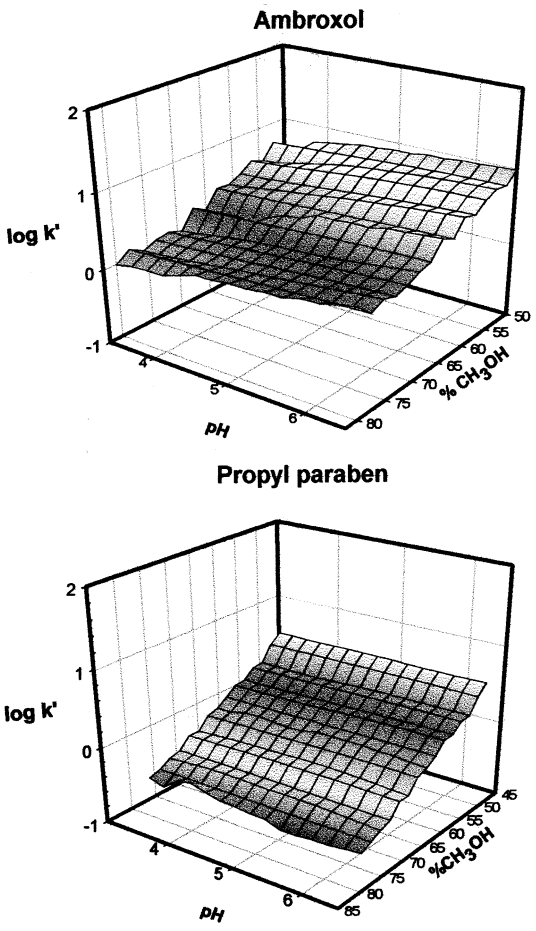

Fig. 1. Predicted retention behavior of ambroxol hydrochloride and propyl paraben as functions of pH and methanol ratio in mobile phase.

2.3. Chromatographic conditions

The mobile phase was 0.05 M ammonium acetate buffer, adjusted to pH 3.45 with glacial acid and methanol, 30:70 v/v. The mobile phase was filtered, degassed and pumped at a flow rate of 0.6 ml min^{-1}, whereas detection was performed at the appropriate wavelength with a sensitivity of 0.004 Aufs. The column was previously equilibrated with the eluting mobile phase by pumping the mobile phase at a rate 0.2 ml min^{-1} and degassing continued by slowly bubbling helium gas.

3. Results and discussion

3.1. Optimum conditions for chromatographic procedure

Initial attempts to develop a reversed phase chromatographic system with methanol and phosphate buffer or with methanol, acetonitrile and the same buffer solution as eluents yielded an assay where ambroxol and different preservatives were co-eluted. Moreover, formation of precipitates was observed in the mobile phase in cases where the amount of organic solvent was increased in the mobile phase. Therefore ammonium acetate buffer was used which was easily miscible with the organic solvents and improved peak symmetry.

In the present study, the chromatographic behaviour of ambroxol together with parabens and sodium benzoate has been investigated in order to assess the relationships between capacity factor, mobile phase properties, like methanol content, pH and solute properties. It is accepted that basic compounds are difficult to elute and determine in their un-ionized form. In contrast, at low pH, the proportion of protonated species is increased and as a consequence the magnitude of the ionic interaction between the solute and solvent molecules increases. Moreover protonated molecules tend to partition as an ion pair, depending on the nature of the available anion in the mobile phase. In case of ambroxol, low pH values considerably affect the chromatographic behaviour and separation of the compound whereas for other analytes their chromatographic behaviour remains almost intact.

Fig. 1 shows the predicted retention behaviour of ambroxol and paraben as a function of both pH and mobile phase composition. The effect of methanol content was studied at proportions from 50 to 80% and the effect of pH was tested in the range 3.35–6.50. A response surface method based on non-linear multiple regression analysis was used to illustrate the log k' values for all combinations of pH values (3.35, 3.45, 4.00, 4,50, 5.00, 5.50, 6.00, 6.50) and different compositions of methanol–water ratios (50, 55, 60, 65, 70, 75, 80 v/v) with a total number of numerical values of 56. The correlation coefficient of the predicted networks for ambroxol and paraben was 0.9609, 0.9904, respectively. The

method was applied by using the SigmaPlot® 4.0, a technical graphing computer program converting unordered xyz triplet data to mesh format.

On the basis of the chromatographic behavior of the compounds, optimum conditions were found where good peak symmetry and acceptable separation among ambroxol and other compounds was achieved. In fact, ionization of the drug enhances the elution ability of mobile phases at low pH values and improves its separation mode. At neutral pH, where ionization is considerably reduced, excessive retention and broadness of late eluting peak were observed.

Although no more than two parabens co-exist in the same formulation, simultaneous separation and determination of five analytes reflect the potential of the developed method and make it most suitable for screen testing. Also, another preservative, sodium benzoate, separates from the compound.

The retention time of ambroxol with the rest of the preservatives was found to be reproducible under the described chromatographic conditions. Representative HPLC chromatograms showing successful separation of the combinations of the pharmaceutical compounds are shown in Fig. 2.

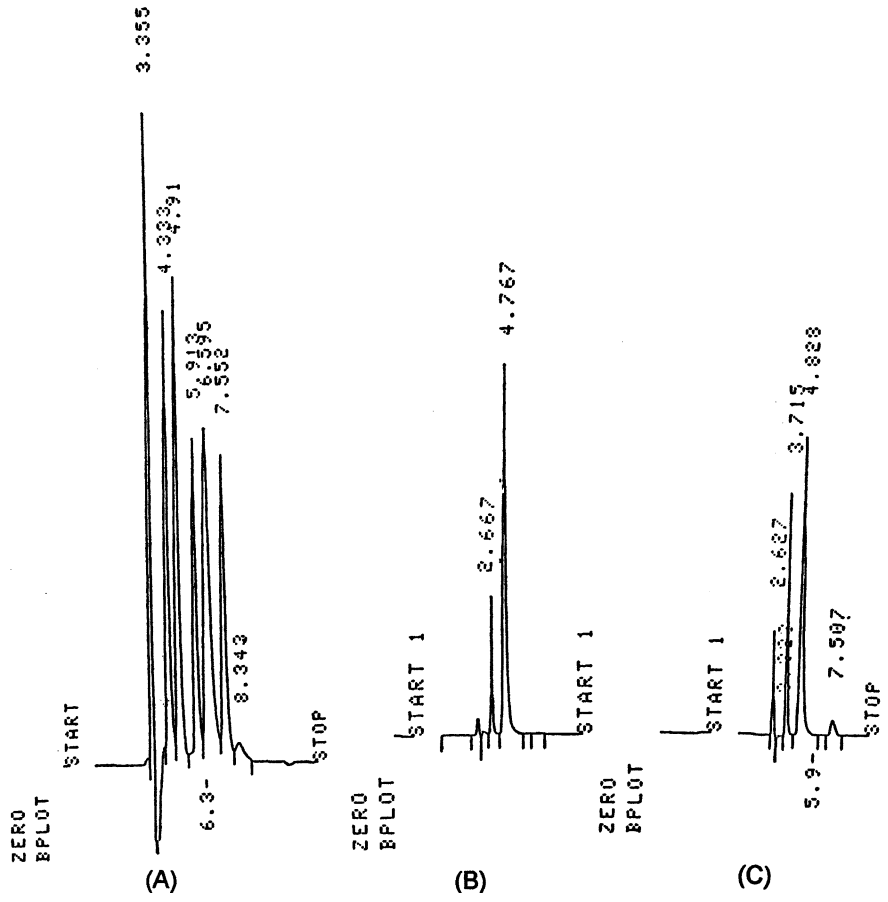

Fig. 2. Typical chromatograms of (A) ambroxol (t_R 7.55, 3.68 μg ml^{-1}), methyl- (t_R 4.33, 0.64 μg ml^{-1}), ethyl- (t_R 4.91, 0.8 μg ml^{-1}), propyl- (t_R 5.91, 0.4 μg ml^{-1}), and butyl-paraben (t_R 6.59, 1.44 μg ml^{-1}), standards in mobile phase consisting of ammonium acetate buffer (pH 3.45; 0.05 M)–methanol (30:70, v/v), $\lambda = 247$ nm, flow rate 0.6 ml min^{-1}. (B) Ambroxol (t_R 4.76, 3.68 μg ml^{-1}), sodium benzoate (t_R 2.66, 32 μg ml^{-1}) standards and saccharine. (C) Ambroxol syrup samples with sodium benzoate.

Table 2
Results for ambroxol hydrochloride obtained from different pharmaceutical formulations

Commercial preparation	Labelled amount	% Found
Abrolen®	30 mg/5 ml	99.50
Afrodor®	30 mg/5 ml	98.90
Apochralen®	30 mg/5 ml	98.37
Ebertuss®	30 mg/5 ml	99.63
Fluibrox®	30 mg/5 ml	99.36
Grenovix®	30 mg/5 ml	99.43
Hivotex®	30 mg/5 ml	98.90
Mucosolvan®	30 mg/5 ml	100.10
Mucosolvan®	15 mg/5 ml	99.66
Nibren®	30 mg/5 ml	99.30
Provixen-n®	30 mg/5 ml	101.80
Tussefar®	30 mg/5 ml	98.83
Zyrantol®	30 mg/5 ml	100.20

3.2. Quantitative determinations

The specificity of the method was investigated by observing potential interferences in sample chromatographs. No interfering peaks were present. Eluted samples and standard peaks were collected and a complete UV spectrum was obtained for each peak. In all cases they were found to be identical. Therefore the method allows the direct determination of ambroxol in commercial dosage forms in the presence of excipients and preservatives. In syrups, saccharine is eluted very near the solvent front.

The linearity of the relationship between peak height and concentration was determined by analysing seven standard solutions for ambroxol and other preservatives. The concentration range and other parameters of the linear regression equations were calculated for each analyte and are presented in Table 1. For ambroxol the equation is $y = 13.64x - 0.54$ with 95% confidence intervals for slope 13.13 to 14.14 and for intercept -2.41 to 1.34 [14].

The t_R values of ambroxol are slightly increased when a mixture of parabens is analysed simultaneously with the drug. For all components, the relationship between peak height and concentration was highly linear over the entire concentration range. However, since all methods have near-zero intercepts, a single-point calibration is expected to give equivalent results. Assay values for the compound determined in different pharmaceutical formulations (syrups) available in the free market are shown in Table 2. These were in good agreement with the labelled amount. The %RSD ranged from 1.15 to 1.58. The high recovery (no extraction of the samples is required) and the low %RSD confirm the suitability of the proposed method for the determination of ambroxol in different pharmaceutical preparations.

3.3. Accuracy and precision

The accuracy and precision of the presented method were assessed by fortifying placebo syrup with known amounts of ambroxol HCl. Since all expectorant syrups available in the free market are sorbitol-based, glycerol sol. 85% USP, sorbitol sol. 70% USP, propylene glycol, saccharine sodium, benzoic acid and essence orange were used to prepare the syrup. Also, a stock solution of ambroxol HCl was prepared using methanol as solvent. Aliquots from this were transferred into a series of different tubes and evaporated to dryness at ambient temperature under a stream of nitrogen. To the residues, a portion of the placebo syrup was added, vortexed, placed in hot water and ultrasonicated for 5–10 min. Then appropriate dilution(s) were made and injected onto the HPLC column. The repeatability of the results was studied using three concentrations (2, 10 and 30 mg ml^{-1}) of the compound over 6 h of operating the system. The mean measured concentration was found to be 100.3 with %RSD 1.66. Similarly, the reproducibility of the method was carried out by two operators repeating the same experiment on two different days. The mean measured concentration was 101.04 with %RSD 1.82. The grand mean for 3 days was 100.8 with %RSD 1.76. Additional information is shown on Table 3.

4. Conclusion

The described method provides a convenient and efficient method for the determination of ambroxol in the presence of different preservatives in dosage forms. There were no interference peaks in the

Table 3
Precision and accuracy data for ambroxol HCl in spiked syrup samples

Concentration added (mg ml^{-1})	Day	Analyst	Measured concentration (mg ml^{-1})	Bias (%)
Within-day (repeatability)				
2	1	1	1.96	−2.0
			1.98	−1.0
10	1	1	9.96	−0.4
			10.19	+1.9
30	1	1	30.55	+1.8
			30.44	+1.5
Mean ($n=6$)	100.30			
%RSD	1.66			
Day-to-day (reproducibility)				
2	2	2	2.07	+3.5
			2.05	+2.5
10	2	2	9.82	−1.8
			9.93	−0.7
30	2	2	30.85	+2.8
			30.78	+2.6
2	3	2	2.02	+1.0
			1.97	−1.5
10	3	2	9.91	−0.9
			10.20	+2.0
30	3	2	30.36	+1.2
			30.54	+1.8
Mean ($n=12$)	101.04			
%RSD	1.82			
Grand mean ($n=18$)	100.8			
%RSD	1.76			

chromatograms, therefore no additional extractions or separations are required. The method is rapid and sensitive enough to be used for single tablet analysis.

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