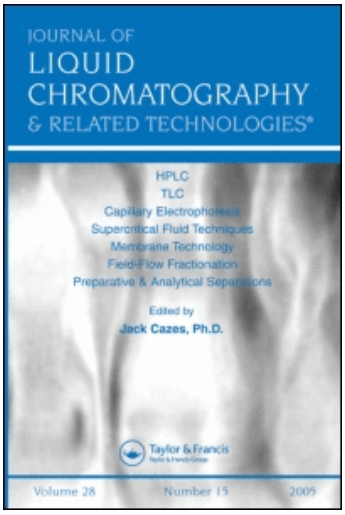

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DETERMINATION OF AMBROXOL HYDROCHLORIDE BY HPLC

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DETERMINATION OF AMBROXOL HYDROCHLORIDE BY HPLC

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ABSTRACT

A rapid, specific, and reliable technique has been developed for the determination of ambroxol hydrochloride in solution by high performance liquid chromatography. To do so, reverse phase liquid chromatography was employed, using methanol-0.01 M diammonium phosphate buffer, pH=6, (70:30 v/v) and a detector wavelength of 247 nm, the concentration range employed being 90–120 μg/mL.

The technique was seen to have excellent linearity, accuracy, and intra- and inter-day precision for the concentration range studied, both when the response was measured by peak areas and when measured by peak heights.

INTRODUCTION

Ambroxol hydrochloride is an active principle with expectorant and secretolytic properties that is used in solid and liquid preparations for chronic treat-

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ments.[1,2] It is an active metabolite of bromhexidrin, which acts by decreasing the viscosity of bronchial solutions and is, therefore, used as a coadjuvant therapy for treating bronchitis, pulmonary emphysema, pneumonia, tuberculosis, cystic fibrosis. Despite its widespread clinical use, it is only described in the Italian and German[3,4] Pharmacopoeias, and there are no monographic works concerning it in the USP, the BP, or the European Pharmacopoeias.

In the present work, we validate a reverse phase chromatographic technique on the basis of reliability parameters[5–9] (selectivity and specificity, linearity, accuracy, and precision) for the analysis of ambroxol hydrochloride in solution, a technique that can be applied to quantitative and qualitative analysis of the active principle in studies aimed at the development of pharmaceutical preparations and in the finished product.

EXPERIMENTAL

HPLC Instrumentation and Conditions

The chromatographic equipment consisted of a Varian pump (mod. 420) coupled to a Varian ultraviolet detector (mod. 2050). Data collection was accomplished with a Varian integrator (mod. 4270).

Reverse phase Nucleosil LiChrospher RP-18 columns (12.5 × 0.4 cm i.d.) (Merck. Darmstadt, Germany) of 5 μm particle size were used. The mobile phase was composed of a mixture (70:30, v/v) of acetonitrile and 0.01 M diammonium phosphate buffer, adjusted to pH = 6 with H_3PO_4 of 85% purity, in a Crison pH-meter (mod. 2001). This mobile phase was prepared daily, filtered in a Supelco vacuum system (mod. 5-8068) with 0.45 mm nylon filters (Whatman. Malstone, U.K.), and degassed in a P-Selecta ultrasound bath (mod. M-515). The flow rate during the assays was 1.5 mL/min and $\lambda_{detection}$ at 247nm. The process was carried out at room temperature (20 ± 5°C).

Chemicals and Reagents

Ambroxol hydrochloride (Lab. Erregeirre, Italy), acetonitrile (Fisher-Scientific, Leicestershire, U.K.), diammonium hydrogen phosphate, 85% orthophosphoric acid (Panreac, Barcelona, Spain), saccharine sodium, sorbitol, citric acid, sodium bisulfite (Sigma Chemical Co., St.Louis, MO, USA), hydroxyethylcellulose (Aqualon, Barcelona), methylparaben and propylparaben (Nipa Laboratories Ltd., U.K.).

Selectivity

For the study of selectivity,[6] a simple syrup was prepared with the excipients normally used with ambroxol hydrochloride in commercial preparations: sorbitol (18 mg/mL), citric acid (60 μg/mL), methylparaben (40 μg/mL), propylparaben (8 μg/mL), saccharine sodium (10 μg/mL), sodium bisulfite (10 μg/mL) and hydroxyethylcellulose (80 μg/mL).

Quantification

The concentration of ambroxol hydrochloride in the problem samples was determined from the following equation:

$x=(response-a)/b$

where x is the concentration of ambroxol in μg/mL, a is the ordinate at the origin of the calibration straight line, and *response* is the peak height or area of the chromatogram (Figure 1).

RESULTS AND DISCUSSION

Selectivity

Figure 2 shows a chromatogram of ambroxol hydrochloride (120 μg/mL) in aqueous solution (a) and of the same active principle in a solution containing sorbitol, citric acid, methylparaben, propylparaben, saccharine sodium, and hydroxyethylcellulose (b). The good selectivity and specificity of the analytical technique used for the determination of ambroxol hydrochloride can be seen, there being no interference with any other compound that might be present in the samples analyzed ($tr_{ambroxo}$ = 4.7 min).

Linearity

Over the range of 90–150 μg/mL (the dose range usually used in commercial formulations), a linear fit was used with satisfactory results. The data were fitted to a line by the equation y = a + bx, where y = response (height or area), b = slope, a = intercepts, and x is the concentration of ambroxol hydrochloride in μg/mL of the standard samples. Claims of linearity are supported by regression data that include correlation coefficient (r) and r-squared values (determination coefficient). Table 1 shows the parameters of the equations obtained in the regression study of each concentration in a intra-day (5 times) follow-up.

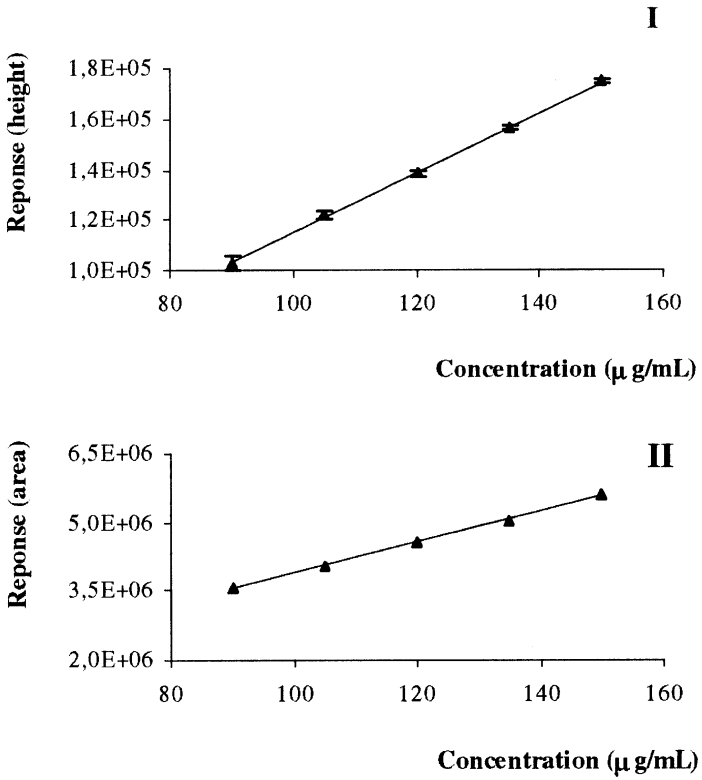

Figure 1. Calibration line of ambroxol hydrochloride in aqueous solution in the 90–150 μg/mL concentration range. (I, peak height; II, peak area).

Analysis of the response factor (fr) served to check the good linearity of the calibration. Table 2 shows the C.V. (%) of the response factor in an intra-day study. As may be seen, the C.V. (%) in the study by heights is lower than that obtained by areas, and in both cases is less than 3%.

The test comparing the observed variances shows that the linearity is correct for a very high degree of significance both by heights and by areas, and that the slope of the straight line is significantly different from zero in both cases (Tables 3 and 4).

Accuracy

The technique was found to be accurate when measuring the response by both heights and areas. In the first case, mean recovery was 99.84% ± 0.62, with a C.V.(%) = 0.62. The true value and the value found do not show statistically dif-

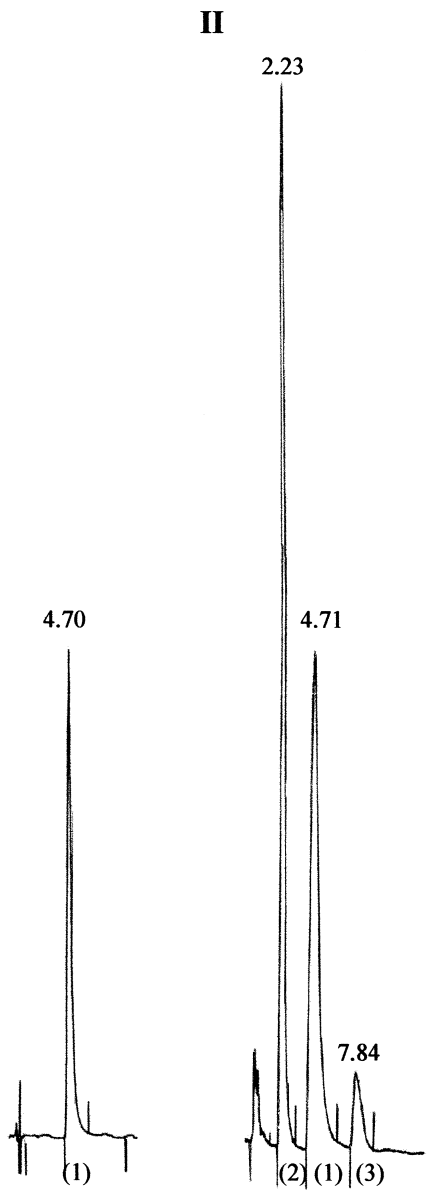

Figure 2. Chromatogram of ambroxol hydrochloride (120 μg/mL) in aqueous solution (I), and in a simple syrup (II). (1) ambroxol hydrochloride; (2) methylparaben; (3) propylparaben.

Table 1. Regression Linearity Fit by Equation (y=a+bx)*

Response	a	b	r	r^2
Heights	−3927.81	1189.42	0.999	0.998
Areas	−471987.12	34127.53	0.999	0.997

*a=intercepts, b=slope, r=correlation coefficient, r^2=determination coefficient

Table 2. Study of Relative Response Factor (fr) in an Intra-Day Study*

Response	x	σ_{n-1}	% C.V.
Heights	1153.12	20.64	1.78
Areas	38191.62	867.89	2.27

*x=mean value, σ_{n-1}=standar desviation, %C.V.=variation coefficient

Table 3. Analysis of Variance for the 90-150 μg/mL Concentration Range (Response=Height)

Source of Variation	SS	Degrees of Freedom	MS	DF	Probability
Regression	11194.95	1	11194.95	4677.01	10E-16
Residual	55.05	23	2.39		
Total	11250.00	24			

Table 4. Analysis of Variance for the 90-150 μg/mL Concentration Range (Response=Area)

Source of Variation	SS	Degrees of Freedom	MS	DF	Probability
Regression	11206.83	1	11206.83	5970.02	10E-16
Residual	43.18	23	1.88		
Total	11250.00	24			

Table 5. Results of Study of Precision of a 90-150 μg/mL Concentration Range of Ambroxol Hydrochloride in the Intra-Day Study

Concen. (μg/mL)	Mean Height	C.V.(%)	Mean Area	C.V.(%)
90	102774.81 ± 3128.80	3.04	3551229.39 ± 48102.22	1.35
105	121891.22 ± 2080.13	1.70	4049235.42 ± 43483.59	1.07
120	138329.01 ± 1353.79	0.98	4570587.81 ± 23059.11	0.51
135	156118.41 ± 909.13	0.58	5034602.02 ± 41582.10	0.83
150	174863.22 ± 869.19	0.50	5605423.23 ± 50130.81	0.89

ferent differences, as seen on applying a Student's t test: t_{exp} (0.219) < t_{tab} (2.064), $p > 0.05$ and 24 degrees of freedom.

In the second case, mean recovery was 100.03% ± 0.65, with a C.V.(%) = 0.65. Likewise, the true and observed values did not show statistically significant differences: t_{exp} (0.794) < t_{tab} (2.064), $p > 0.05$, 24 degrees of freedom.

Precision

To study the precision of this analytical method, standard solutions at the same concentrations as those used in previous studies were used. Reproducibility or intra-day studies were carried out, analysing the samples five times on the same day, and reproducibility studies, analysing five samples on five different days (inter-day precision) were conducted. The mean values of the results obtained and their coefficients of variation are shown in Tables 5 and 6.

In the study of both intra-day and inter-day precision, the variation coefficients of the means by areas and by heights were, in all cases, lower than 5%, indicating the good repeatability and reproducibility of the analytical technique employed.

Table 6. Results of Study of Precision of a 90-150 μg/mL Concentration Range of Ambroxol Hydrochloride in the Inter-Day Study

Concen. (μg/mL)	Mean Height	C.V.(%)	Mean Area	C.V.(%)
90	121334.42 ± 3066.81	2.52	4157840.01 ± 92326.12	2.22
105	140979.81 ± 4710.64	3.34	4841425.12 ± 98834.49	2.09
120	163970.23 ± 2664.79	1.62	5507607.22 ± 94680.12	1.71
135	184213.81 ± 3624.78	1.96	6199239.43 ± 72464.68	1.16
150	203877.62 ± 3290.10	1.61	6871009.24 ± 112027.01	1.64

CONCLUSIONS

A method for the quantitation of ambroxol hydrochloride in samples in solution has been developed and validated. The advantage of this assay lies in its simplicity. Our results show the excellent linearity, precision, and accuracy of the analytical technique, which should allow its implementation as a standard quantification method of the active principle in the pharmaceutical industry.

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