

Short Communication

High-performance liquid chromatographic determination of ambroxol in pharmaceuticals

VITTORIO BRIZZI*† and UMBERTA PASETTI‡

† Dipartimento Farmaco Chimico Tecnologico, Università di Siena, Banchi di Sotto 55, 53100 Siena, Italy
‡ Istituto Chimico Internazionale "Dr G. Rende", Via Salaria 1240, 00100 Roma, Italy

Keywords: High-performance liquid chromatography; expectorants; assay of ambroxol.

Introduction

Ambroxol [1], trans-4-(2-amino-3,5-dibromo-benzyl)-aminocyclohexanol, a metabolite of bromhexin [2], as the hydrochloride, is used as a bronchosecretolytic and expectorant drug.

Ambroxol hydrochloride is found in pharmaceutical preparations mainly as injectable solutions in ampoules or as granules for instant preparations. The ampoule solutions contain ambroxol hydrochloride and sodium phosphate, whilst the granules contain, in addition to ambroxol hydrochloride, a number of excipients, stabilizers and flavouring agents.

Prior to developing a system for the quantitative analysis of ambroxol in pharmaceuticals it was noticed that, at present, only reversed-phase high-performance liquid chromatographic (RP-HPLC) methods for the determination of ambroxol in biological fluids have been published [3-5]. The HPLC methods make use of ternary solvent mixtures, 5 μ m stationary phases, relatively high flow-rates and in one case of an ion-pairing reagent. A gas-liquid chromatographic method also has been proposed [6]. The aim of the present

work was to find out if a simpler method could be developed for the determination of ambroxol in pharmaceuticals, particularly in ambroxol preparations in which only a few known compounds are present.

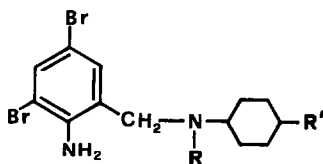
Experimental

Materials and reagents

Acetonitrile and water were Farmitalia-Carlo Erba, HPLC grade solvents; ammonium acetate and triethylamine (TEA) were Merck analytical grade reagents; sodium dodecylsulphate (SDS) was BDH analytical grade; ambroxol hydrochloride and commercial preparations were from ICI "Dr G. Rende".

Apparatus

The experiments were performed on a Perkin-Elmer system comprising a Model LC-10 pump fitted with a Model 7125 Rheodyne valve (6 μ l loop); a Model LC-90 absorbance detector, operated at 248 nm, and a Model LCI-100 integrator. The column used was a Perkin-Elmer 10 μ m, RP 18 LiChrosorb (250 \times 4.6 mm i.d.), $V_0 = 2.0$ ml (NaNO_2).



Ambroxol R = H R' = OH

Bromhexin R = CH₃ R' = H

* Author to whom correspondence should be addressed.

Mobile phase composition

The following mobile phases were used, all of which contained 80% (v/v) acetonitrile as the organic modifier: (i) 10 mM ammonium acetate adjusted to pH 4.0 with acetic acid; (ii) 10 mM ammonium acetate, 10 mM SDS, adjusted to pH 4.0 with acetic acid; (iii) 10 mM ammonium acetate, 10 mM triethylamine, adjusted to pH 4.0 with acetic acid. A flow-rate of 1.5 ml min⁻¹ was used throughout the work.

Analysis of commercial preparations

The quantitative analysis of ambroxol in pharmaceuticals was carried out using mobile phase (iii). After dilution of the liquid preparation with water or after dissolution of the granulate powder in water, up to the desired concentration of the active principles, the samples were filtered through a 0.2 μm filter prior to injection.

Results and Discussion

In preliminary experiments a standard sample of ambroxol was analysed using a Partisil RP 18, 10 μm, column with an acetonitrile–10 mM ammonium acetate buffer, pH 4.0 (8:2, v/v) eluent at a constant flow-rate of

1.5 ml min⁻¹. Following this a sample, prepared from the granulate, was analysed. Under these conditions ambroxol was eluted after 5.2 min, ($k' = 2.4$), as a single peak, well resolved from the other components present in the granulate, but the peak symmetry was very poor as illustrated in Fig. 1a.

It is known that the analysis of basic compounds by reversed-phase LC is complicated by poor peak shape, leading to errors in quantitative analysis.

Nonetheless, this problem can be solved by the addition of appropriate modifiers to the mobile phase [7].

In order to improve the peak shape 10 mM SDS was added to the mobile phase. The addition of the ion-pair reagent brought about a reasonable improvement of the peak symmetry and reduced the retention time ($Rt = 4.4$ min), as shown in Fig. 1b.

Furthermore it was found possible to determine ambroxol in acetonitrile–10 mM ammonium acetate, (8:2, v/v), by simply adding 10 mM TEA to the aqueous phase, and adjusting to pH 4.0. This resulted in a further improvement in peak symmetry and in a further reduction of the retention time ($Rt = 4.0$ min). The improvement generated by TEA

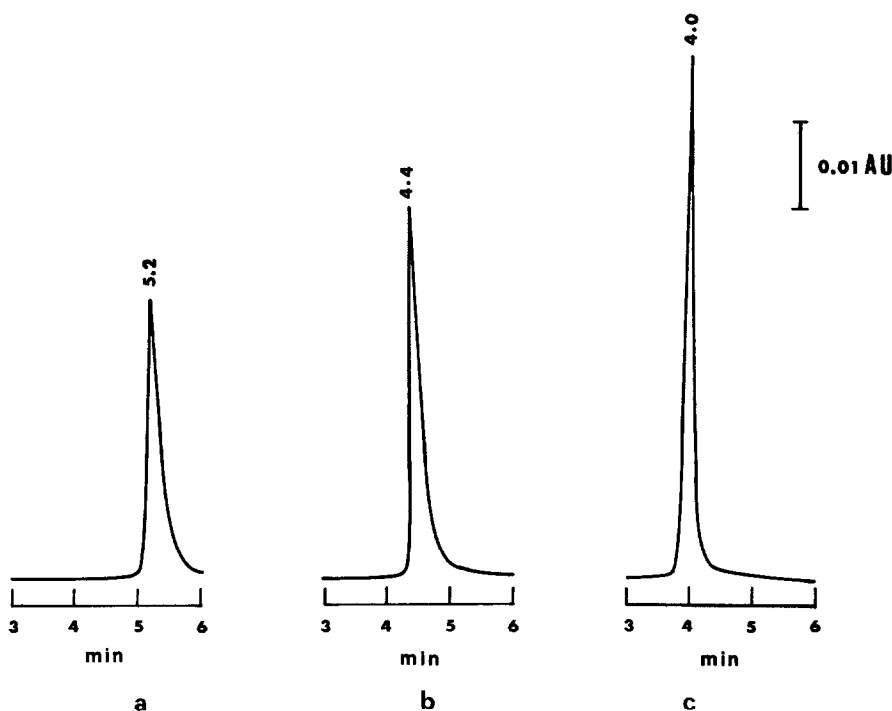


Figure 1
Elution profiles for ambroxol hydrochloride on a column RP 18-Partisil, 10 μm (250 × 4.6 mm i.d.). Flow-rate 1.5 ml min⁻¹; λ = 248 nm. Eluent (8:2, v/v): (a) acetonitrile–10 mM ammonium acetate, pH 4.0; (b) acetonitrile–10 mM ammonium acetate, 10 mM SDS, pH 4.0; (c) acetonitrile–10 mM ammonium acetate, 10 mM TEA, pH 4.0.

Table 1
Assay results of ambroxol hydrochloride formulations by HPLC method

Formulations	Ambroxol hydrochloride		Added	Recovery (%)
	Claimed	Found* \pm SD		
Ampoules				
1	7.5 mg ml ⁻¹	7.356 \pm 0.084 mg	0.75 mg	99.21
2	7.5 mg ml ⁻¹	7.380 \pm 0.17 mg	1.5 mg	99.37
Mean \pm SD				99.29 \pm 0.11
Granulate				
3	6.0 (mg g ⁻¹)	5.860 \pm 0.24 mg	0.6 mg	99.15
4	6.0 (mg g ⁻¹)	5.950 \pm 0.26 mg	1.2 mg	99.91
Mean \pm SD				99.53 \pm 0.53
Standard solution				
5	75 mg ml ⁻¹	74.736 \pm 0.37 mg ml ⁻¹	—	—
6	75 mg ml ⁻¹	74.718 \pm 0.44 mg ml ⁻¹	—	—

* Average of five determinations.

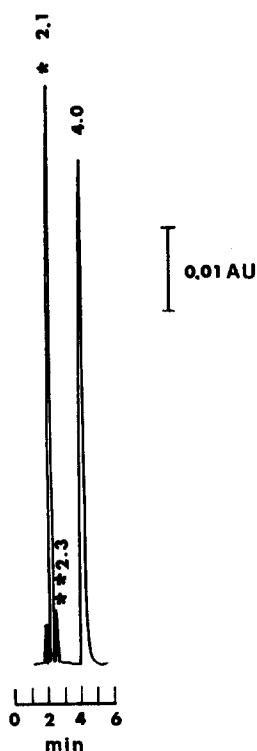


Figure 2
Elution profile for ambroxol as present in the powder for instant preparations after dilution with water up to a concentration of 60 mg ml⁻¹. HPLC conditions identical to Fig. 1c. The first two peaks are due to *methyl- and **ethyl-*p*-hydroxybenzoate.

is almost certainly due to the blocking of the residual silanol groups of the stationary phase [8]. Under the latter conditions the peak was also sharper than in the other two cases, as shown in Fig. 1c.

A value for repeatability of the assay was obtained by injecting the same standard sample 10 times (60 mg ml⁻¹) giving a RSD = 0.88.

An appropriate calibration curve was established over the range 0–100 mg ml⁻¹ using standard samples: the response was linear in this range ($r = 0.9979$).

The results of a recovery and accuracy study are summarized in Table 1.

No problems were encountered due to the presence of the other components in the powder, the most abundant of which were methyl and ethyl *p*-hydroxybenzoate, since these components were well resolved from the ambroxol peak, as shown in Fig. 2.

In conclusion, the RP-HPLC method described previously, provides a simple quantitative analysis for ambroxol in pharmaceuticals, the method is precise and accurate.

Furthermore, the use of TEA in the eluent mixture, eliminates the silanol effects and reduces the chance of column-to-column variation in peak shape and retention.

References

- [1] E. Schraven, F.W. Koss, J. Keck and G. Beisenherz, *Eur. J. Pharmac.* **1**, 445–451 (1967).
- [2] F. Arch, *Arzneim. Forsch. Drug Res.* **13**, 480–485 (1963).
- [3] R. Jauch, G. Bozler, R. Hammer, F.W. Koss, M. Karlsson, E. Vitek, I. Haering, K. Beschke, S. Hadamovsky, D.N. Maass and R. Wollmann, *Arzneim. Forsch. Drug Res.* **28**, 904–911 (1978).
- [4] M. Nieder and H. Jaefel, *J. High. Resolut. Chromat., Chromat. Commun.* **9**, 561–565 (1986).
- [5] M.H.A. Botterblom, T.J. Janssen, P.J.M. Guelen and T.B. Vree, *J. Chromat.* **421**, 211–215 (1987).
- [6] J. Schmid, *J. Chromat.* **414**, 65–75 (1987).
- [7] A. Sokolowski and K.G. Wahlund, *J. Chromat.* **189**, 299–316 (1980).
- [8] R.W. Roos and C.A. Laucam, *J. Chromat.* **370**, 403–418 (1986).

[Received for review 6 October 1988;
revised manuscript received 13 June 1989]