ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
15 (1996) 287-293

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Simultaneous determination of bromhexine hydrochloride and methyl and propyl p-hydroxybenzoate and determination of dextromethorphan hydrobromide in cough-cold syrup by high-performance liquid chromatography

Jussi-Pekka Rauha[a,*], Hannele Salomies[a], Marja Aalto[b]

[a]*Department of Pharmacy, Pharmaceutical Chemistry Division, P.O. Box 56 (Viikinkaari 5), FIN-00014 University of Helsinki, Helsinki, Finland*

[b]*Association of Finnish Pharmacies, Pieni Roobertinkatu 14C, FIN-00120 Helsinki, Finland*

Received for review 27 February 1996; revised manuscript received 3 April 1996

Abstract

Liquid chromatographic methods were developed for the determination of bromhexine hydrochloride, methyl *p*-hydroxybenzoate and propyl *p*-hydroxybenzoate (method A) and dextromethorphan hydrobromide (method B) in cough-cold syrup formulations. Reversed-phase analytical columns (150 mm × 3.9 mm i.d.) were used with (A) C_{18} and (B) phenyl as stationary phases and mixtures of (A) acetonitrile and aqueous 15 mM triethylamine solution (43:57) and (B) methanol and aqueous 3% ammonium formate buffer solution (53:47) as mobile phases at a flow rate of 1.0 ml min^{-1}. Both aqueous components were adjusted to pH 3.9. UV detection of analytes was at (A) 245 nm and (B) 278 nm. In both methods, the time required for an HPLC run giving good separations and recoveries was less than 8 min.

Keywords: High-performance liquid chromatography; Bromhexine hydrochloride; Methyl *p*-hydroxybenzoate; Propyl *p*-hydroxybenzoate; Dextromethorphan hydrobromide; Cough-cold syrup formulation

1. Introduction

Bromhexine (**I**) is chemically a weak base with a pK_a value of 8.5 [1]. Because it is only slightly soluble in water, it is normally used in pharmaceuticals as the hydrochloride salt. The pharmacological effects of bromhexine are to increase the amount of bronchial mucus and decrease its viscosity. A combination cough-cold syrup newly proposed for preparation in Finnish pharmacies contains bromhexine hydrochloride as the active ingredient, methyl and propyl *p*-hydroxybenzoate (**II** and **III**) as preservatives and sorbitol and aniseed water as flavouring. High-performance

* Corresponding author.

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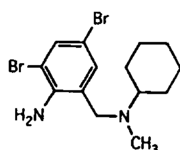
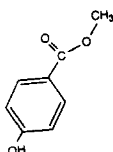
PII S0731-7085(96)01846-8

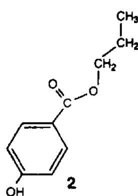
liquid chromatographic (HPLC) methods have been described for the determination of bromhexine hydrochloride in both tablets [2,3] and mixtures [4,5]. These methods are usually carried out using a reversed-phase C_{18} column with a mobile phase containing over 50% of organic solvent. However, none of the available methods was ideal for our purpose, and an improved method was sought through modification of the procedure described by Sane et al. [2]. The simple composition of the mixture offered an opportunity to develop a method for the simultaneous determination of both mucolyte and preservatives (method A).

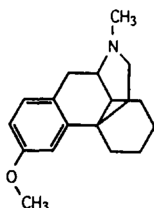




I

II





III

IV

Structure 1.

Dextromethorphan (**IV**), also a weakly basic compound ($pK_a = 8.3$), is a widely used antitussive which typically appears in pharmaceutical compositions as its hydrobromide salt. Numerous papers have described the determination of dextromethorphan hydrobromide by HPLC, with various chromatographic conditions recommended especially for the quantification of mixtures [6–10]. However, as none of the reported methods was suitable for our cough–cold syrup, we set out to modify a method normally used for tablets [11]. The objective in the method development was to separate dextromethorphan hydrobromide from the matrix components of a cough–cold mixture containing senega, liquorice and anis extracts as expectorants, methyl *p*-hydroxybenzoate as preservative and sorbitol as flavour, and then to quantify this compound (method B).

Both cough–cold syrups were prepared in a Finnish pharmacy. The cough–cold syrup containing bromhexine·HCl as active substance is of a new composition and has not been assayed earlier. The medicine containing dextromethorphan·HBr in the other syrup has been quantified earlier by a spectrophotometric method. There was a need, however, for a more selective method in which the dextromethorphan·HBr could easily be separated from the complex matrix before quantification. HPLC proved to be the method of choice for the analysis of both medicines.

2. Materials and methods

2.1. Reagents and materials

HPLC-grade methanol and acetonitrile (ACN) were obtained from Rathburn Chemicals (Walkerburn, UK), pro analysi grade phosphoric acid and formic acid from Merck (Darmstadt, Germany), pro analysi grade ammonia solution from Riedel-de Haën (Seelze, Germany) and triethylamine (TEA) from Fluka Chemie (Buchs, Switzerland) and bromhexine hydrochloride (BRHX), methyl *p*-hydroxybenzoate (MPHB), propyl *p*-hydroxybenzoate (PPHB) and dextromethorphan hydrobromide (DX) standards from the University Pharmacy (Helsinki, Finland).

2.2. Equipment

The HPLC system consisted of two Waters Model 501 pumps, a Waters Model 680 automated gradient controller (Waters, Milford, MA, USA) and a Waters Model 991 photodiode-array detector (Waters Associates, Milford, MA, USA). Injection was performed manually with a Rheodyne Model 7125 injector (Rheodyne, Cotati, CA, USA) with a 20 μl loop. Integration was accomplished with a NEC Powermate 386/25 computer (NEC Technologies, Boxborough, MA, USA). pH measurements were carried out with a PHM 83 Autocal pH meter (Radiometer, Copenhagen, Denmark).

2.3. Chromatographic conditions

The chromatographic columns (150 mm × 3.9 mm i.d.) were packed with Nova-Pak C_{18} (A) or phenyl (B) 4 μm particles (Waters). In method A the mobile phase consisted of 43% (v/v) of ACN and 57% of 15 mM aqueous TEA solution adjusted to pH 3.9 with orthophosphoric acid. The flow rate was set at 1.0 ml min^{-1} with a typical back-pressure of 1100 psi. Exactly 20 μl of the standard and sample solutions were injected on to the column and UV detection of analytes was applied at 245 nm. Under these conditions, typical retention times were ca. 1.8, 2.9 and 4 min for MPHB, PPHB and BRHX, respectively. In method B the mobile phase consisted of 53% (v/v) of methanol and 47% (v/v) of aqueous buffer (pH 4.1) containing 3.0% of ammonium formate [8] adjusted to pH 3.9 with formic acid. The flow rate was 1.0 ml min^{-1} with a typical back-pressure of ca. 1850 psi, bringing DX to the detector with a retention time of 6 min. UV detection of dextromethorphan·HBr in 20 μl standard and sample preparations was applied at 278 nm.

2.4. Preparation of standards (A)

Stock solutions of each analyte separately were prepared by dissolving the analytes in methanol at concentrations of 10 mg ml^{-1} for MPHB and BRHX and 2.5 mg ml^{-1} for PPHB. Stock standard solutions were pipetted into five separate volumetric flasks in amounts required to give concentrations of 0.015, 0.03, 0.06, 0.09 and 0.12 mg ml^{-1}. Each flask was diluted to volume with mobile phase and mixed well. For HPLC, portions of the samples were filtered through nylon bulk membrane filters (pore size 0.45 μm).

2.5. Preparation of samples (A)

A 1 ml volume of cough–cold syrup was carefully measured with a 10 ml burette into a 20 ml volumetric flask and diluted to volume with mobile phase. The solution was mixed well and a portion was filtered through a nylon bulk membrane filter (pore size 0.45 μm), and was then ready for HPLC injections.

2.6. Preparation of standards (B)

About 125 mg of DX analytical standard was accurately weighed into a 100 ml volumetric flask and dissolved in and diluted to volume with water. The solution was mixed well and 1, 2, 4, 6 and 8 ml portions were pipetted into five 50 ml volumetric flasks. Each flask was diluted to volume with 0.1 M hydrochloric acid and the solution was mixed. Concentrations of the working standard mixture solutions were 0.025 to 0.20 mg ml^{-1}. Portions of these samples were filtered through

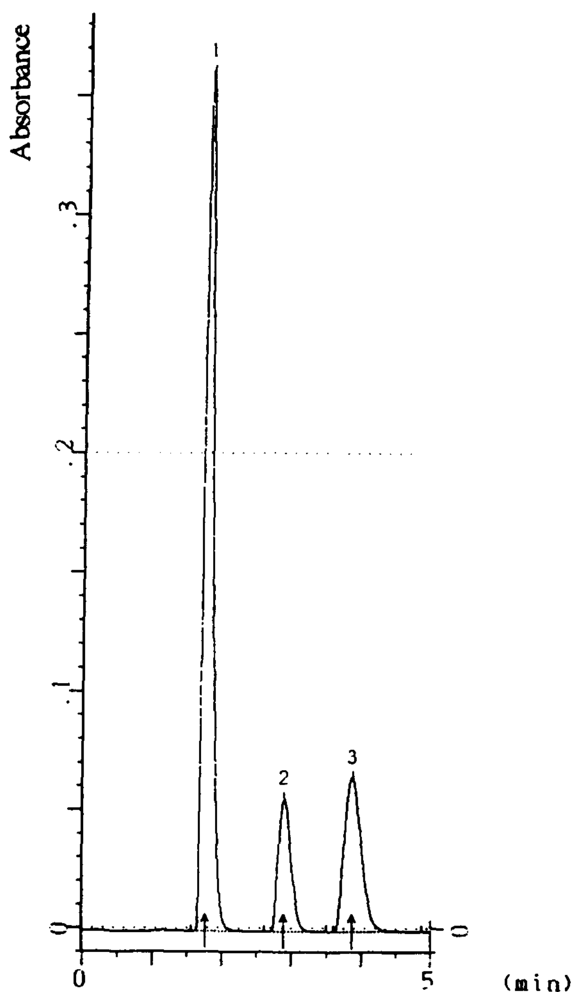


Fig. 1. Chromatogram from a typical HPLC run with the expectorant syrup. Peaks (1) methyl p-hydroxybenzoate; (2) propyl p-hydroxybenzoate; (3) bromhexine hydrochloride.

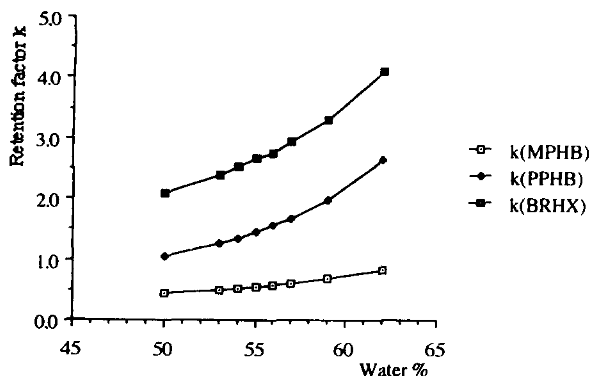


Fig. 2. Relationship between retention factor and water content of the mobile phase at pH 3.9.

hydrophilic membrane filter (pore size 0.45 μm) for HPLC.

2.7. Preparation of samples (B)

A 2 ml volume cough–cold syrup was measured with a 10 ml burette into a 50 ml volumetric flask and diluted to volume with 0.1 M HCl. For HPLC injections a portion of the sample was filtered through a hydrophilic membrane filter (pore size 0.45 μm).

3. Results and discussion

3.1. Optimization of method A

The expectorant cough–cold syrup was colourless and clear. No preparation of samples or standards was required except dilution with mobile phase and filtration.

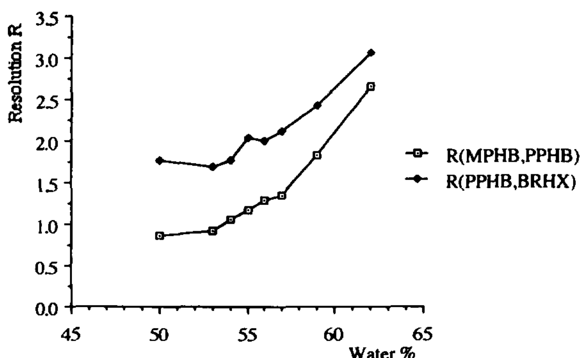


Fig. 3. Relationship between resolution and water content of the mobile phase at pH 3.9.

Table 1
Equations of calibration curves and correlation coefficients for BRHX, MPHB and PPHB

Compound	Equation of calibration curve	Correlation coefficient
BRHX	$y = 06181x - 0.0004$	0.9991
MPHB	$y = 1.6792x - 0.0028$	0.9988
PPHB	$y = 1.5866x - 0.0002$	0.9985

The column (C_{18}), solvents (ACN and water) and flow rate (1 ml min^{-1}) were selected according to the literature [2]. The mobile phase contained 15 mM TEA in the aqueous phase, as described [2], but the acidity was adjusted with phosphoric acid instead of acetic acid because phosphoric acid absorbs more weakly at the detection wavelength of 245 nm.

The influence of the organic to aqueous ratio on the separation of MPHB, PPHB and BRHX was investigated at acidic pH. At pH 3.9 the retention times were moderate and the separation good (Fig. 1). At lower pH the retention times were shorter and the separation inadequate. At higher pH the retention times were longer and peaks broader, but there was little improvement in separation. pH 3.9 was accordingly selected.

An increase in the polarity of the mobile phase led to longer retention times, as can be seen in Fig. 2. The more polar the mobile phase, the better was the resolution, both between MPHB and PPHB and between PPHB and BRHX, as shown in Fig. 3. The ACN:water ratio of 43:57 (v/v) was a compromise between short retention times and sharp peaks, and good resolution and somewhat wider peaks.

3.2. Equations and repeatability (A)

The calibration equations were calculated on the basis of concentration versus peak area. No

Table 2
Recovery data for the simultaneous determination of BRHX, MPHB and PPHB in a pharmacy-formulated cough–cold syrup and for the determination of BRHX in a commercially available mixture

Analysis	Compound	Theory (mg ml^{-1})	Found (mg ml^{-1})	Recovery (%)	SD (%)	n
1	BRHX	0.8	0.826	103.3	1.10	6
	MPHB	0.8	0.862	107.8	0.89	6
	PPHB	0.2	0.208	103.9	0.90	6
2	BRHX	0.8	0.830	103.7	0.46	6
	MPHB	0.8	0.860	107.6	0.40	6
	PPHB	0.2	0.208	104.0	0.38	6
3	BRHX	0.8	0.755	94.4	0.35	3

deviation from linearity was observed over the range 0.015–0.12 mg ml^{-1} for BRHX and MPHB and 0.00375–0.03 mg ml^{-1} for PPHB. The equations and correlation coefficients are presented in Table 1. The response was also linear over a wider range, but there was no need for these additional concentrations in method A.

Relative standard deviations (RSDs) were calculated from six injections each of the calibration standards of highest and lowest concentrations. The values were between 0.2 and 0.4% for all analytes, which can be considered good repeatability of injections.

3.3. Recoveries A

The precision and accuracy of method A were investigated in parallel determinations carried out on different days (see Table 2). A paired Student's t-test was applied to calculate the differences between the results. There was no significant deviation in recoveries for the same samples analysed on the same day (analysis 1) or for different samples analysed on different days (analysis 2) ($p = 0.05$). In addition, a commercially available BRHX mixture was assayed using the proposed method (analysis 3).

3.4. Optimization of method B

In all runs, a strong pattern of non-separated peaks, evidently due to the syrup matrix, was eluted just after the solvent. The pattern was the same irrespective of the bonded phase. The chromatographic behaviour of DX was different, and because we were only interested in separating DX from the other ingredients and then quantifying it, the elution of these compounds all together did not disturb the assay. The most promising results were obtained with a relatively polar reversed-phase column packed with phenyl-bonded RP material. The separation factor between DX and the nearest peak of the other compounds was 2.0, which showed DX to be totally separated (Fig. 4). In practice, optimization was done according to the visual appearance of the peak. The best separation of DX as judged by peak sharpness was achieved with a ratio of methanol to aqueous buffer of 53:47 (v/v). As regards the acidity of the aqueous component of the mobile phase, pH 3.9 was low enough to prevent the deprotonation of the free silanol groups and thus the tailing of peaks. On the basis of the area to height ratio of the DX peaks, it was decided that the concentration of buffer in the aqueous phase should be 3%. Further optimization was investigated by varying the concentration between 1 and 5% while the pH, methanol to water ratio and flow rate were held constant. The concentration of buffer had no particular effect on the retention of DX, although it did affect the peak shape. Increasing the buffer concentration over 3% did not produce any significant improvement in peak shape.

The solvent used to dilute the samples had a considerable effect on the analysis time. If the sample was diluted with organic solvent, the anethol (AN) present because of the anise drops

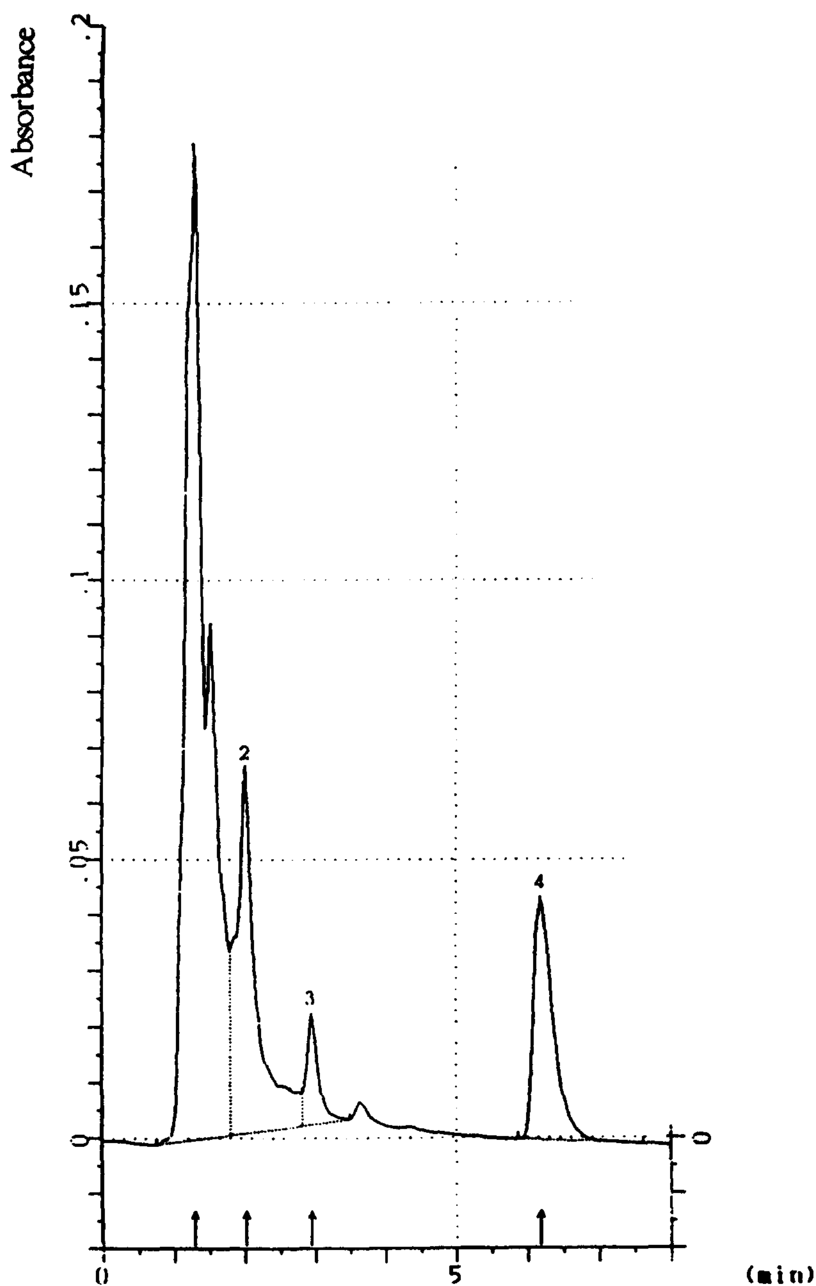


Fig. 4. Chromatogram from a typical HPLC run with the antitussive syrup. Excipients eluted before the dextromethorphan hydrochloride (peak 4).

dissolved very well and was injected on to the column. With a retention time of nearly 11 min, it heavily tailed the other compounds on elution from the column. Injection of AN on to the column could be prevented by diluting samples with water instead of organic solvent. Being insoluble in water, AN was then filtered off with the precipitate of the mixture. The use of dilute hydrochloric acid instead of water increased the recovery of DX dramatically.

Table 3
Recovery data for parallel determinations of DX

Analysis	Theory (mg ml^{-1})	Found (mg ml^{-1})	Recovery (%)	SD (%)	n
1	3.0	2.552	85.1	4.23	6
2	3.0	2.897	96.6	0.70	6

3.5. Equations and repeatability B

The peak area of DX was directly proportional to concentration over the range 0.025–0.20 mg ml^{-1}. The equations of the calibration curve was $y = 0.1308x - 4.7 \times 10^{-5}$ and the correlation coefficient was 1.0000. A study of the repeatability of injection with standard solutions ($n = 6$) gave RSD values varying between 0.2 and 1.0%. Subsequently, a blank solution containing all other ingredients of the syrup except DX was prepared. Exact amounts of DX were then mixed with the blank solution and the sample was prepared and chromatographed in the same way as real samples of the cough–cold syrup. Recoveries calculated with the equation of the calibration curve ($y = 0.1303x - 6.5 \times 10^{-5}$) obtained with this solution were very similar to those calculated with the first equation.

3.6. Recoveries B

When the recovery of DX in the cough–cold syrup was investigated with samples diluted with water, the results were about 15% below the declared amount of DX (analysis 1 in Table 3). The reason for the low concentrations was found to be the high pH of the samples (8.9). Under these conditions only about 20% of DX, a base, would be in water-soluble ionized form and presumably a considerable part of the DX was removed with the precipitate, which was filtered off before injection on to the column. When the sample was diluted instead with 0.1 M HCl, before filtration the pH was 1.3. Presumably all the DX would be dissolved under such acidic conditions (analysis 2 in Table 3). According to the paired Student's t-test, there was no difference in the results of intra-day determinations ($p = 0.05$).

4. Conclusions

The HPLC methods we have described for the analysis of cough–cold mixtures are simple and rapid. Retention times can be varied through adjustment of the ratio of organic solvent to water in the mobile phase. Such adjustments should allow easy adaptation of the method for other BRHX and DX formulations.

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