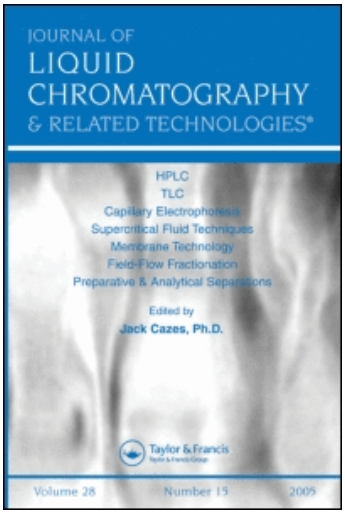

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G. Cavazzutti[a]; L. Gagliardi[a]; D. D. Orsi[a]; D. Tonelli[b]
[a] Laboratorio di Chimica del Farmaco Istituto Superiore di, Rome, Italy [b] Dipartimento di Chimica Industriale e dei Materiali, Università di Bologna Viale del Risorgimento, Bologna, Italy

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SIMULTANEOUS DETERMINATION OF BUZEPIDE, PHENYLPROPANOLAMINE, AND CLOCINIZINE IN PHARMACEUTICAL PREPARATIONS BY ION-PAIR REVERSED-PHASE HPLC

G. CAVAZZUTTI[1], L. GAGLIARDI[1], D. DE ORSI[1]*, AND D. TONELLI[2]

[1]*Laboratorio di Chimica del Farmaco*
Istituto Superiore di Sanità
Viale Regina Elena
299 - 00161 Rome, Italy

[2]*Dipartimento di Chimica Industriale e dei Materiali*
Università di Bologna
Viale del Risorgimento 4
40136 Bologna, Italy

ABSTRACT

A rapid, accurate and precise method for the determination of buzepide, phenylpropanolamine, and clocinizine in a cough-mixture is described. The method is based on reversed-phase ion-pair HPLC. The only sample preparation necessary for the analysis is its dilution with the mobile phase. The resulting solution is analyzed on a column packed with Lichrosorb RP-18 (10μm) with acetonitrile in aqueous 5 10-2M sodium perchlorate, pH 3.0 (15:85) as initial mobile phase (2 mL·min-1) and detection at 220 and 254 nm. Then a linear gradient up to 95% acetonitrile in 20 min, is applied. The detection limits are 500 ng injected for phenylpropanolamine, 40 and 5 ng for buzepide and clocinizine, respectively.

* Author to whom correspondence should be addressed

INTRODUCTION

Reversed-phase ion-pair liquid chromatography (RP-IPC) is a well established method for the separation of ionic or ionizable organic compounds. With proper us of the technique, complex separations can be readily achieved, since both ionized and un-ionized components are analyzed under the same chromatographic conditions. Recently, most publications have discussed the application of RP-IPC for the simultaneous determination of the active components in pharmaceutical formulations. The analysis time is short, and usually only a simple dilution of the sample is required prior to injection.

Buzepide (2,2-diphenyl-4-examethyleneiminobutyramidemethiodide), phenylpropanolamine hydrochloride, and clocinizine hydrochloride (1-(4-chlorobenzhydril)-4-cinnamylpiperazine-dihydrochloride) are active components employed in cold preparations. One commercial product (Denoral, Rhone-Poulenc, Milano, Italia) extensively used in Italy to relieve congestion of the nasal mucosa and sinuses in the treatment of colds, sinusitis, rhinitis, and hay fever, is a combination of the three drugs, which are present in other formulations sold on the European and U.S.A. markets. The dosage form (tablets) also contains excipients, some of which may interfere with the analysis of the active ingredients.

Phenylpropanolamine hydrochoride (I) is very often present as decongestant, in cough-cold formulations and many HPLC methods have been developed for its determination in pharmaceutical dosage forms and biological fluids (1-6). On the contrary, no information is appeared in literature on the analysis of buzepide (II) or clocinizine (III) alone or in combinations with (I) by HPLC.

This paper reports the simultaneous determination of (I), (II), and (III) by RP-IPC. Validation data are presented for the analysis of the drugs in commercial samples of cold formulations.

EXPERIMENTAL

Apparatus

The modular HPLC apparatus consisted of a single piston ternary gradient pump (Varian, Model 2510) equipped with a 10 μL sample loop, a Varian Polychrom 9065 photodiode-array detector, and a personal computer IBM PS/2. The analytical column was of stainless-steel (250 mm x 4.0 mm I.D.) packed with 10 μm Lichrosorb RP-18 (Merck,Darmstadt, Germany). The temperature of the column was maintained at 25 °C.

Standards and reagents

All reagents used were of analytical-reagent grade. The drug standards were supplied by Rhone-Poulenc (France) and were used as obtained. Acetonitrile was of HPLC grade. Water was deionized and doubly distilled from glass apparatus. All solvents and solutions for HPLC analysis were filtered through a Millipore filter (pore size 0.45 μm) and vacuum degassed by sonication before use.

HPLC conditions

The mobile phase was prepared by mixing acetonitrile with $5 \cdot 10^{-2}$M solution of sodium perchlorate in water, pH 3.0, adjusted with 70% perchloric acid. Flow-rate was 2.0mL·min^{-1} and detection was performed at 220 and 254 nm. A gradient elution was applied, consisting of a linear increase of acetonitrile from 15 to 95% (V/V)in 20 min.

At the end of the elution, the initial mobile phase was passed through the column for 10 min to allow a good re-equilibration of the chromatographic system.

Calibration standard solutions

Stock solutions of compounds I-III were prepared by dissolving weighed

amounts in the mobile phase containing 50% acetonitrile to make 100 mL. A set of standard solutions was prepared by diluting aliquots of the stock solutions to give concentrations ranging from 50 to 1000 $\mu g \cdot mL^{-1}$ for (I), from 1 to 15 $\mu g \cdot mL^{-1}$ for (II) and from 1 to 100 $\mu g \cdot mL^{-1}$a for (III). The calibration graphs were constructed by plotting the peak areas obtained at the wavelength of 254 nm for (I) and (III), and at 220 nm for (II), versus the amounts (μg) injected.

Sample preparation

25 tablets were weighed and ground to a fine powder. Enough powder to represent ten tablets was accurately weighed and mixed with 15 mL of the mobile phase containing 50% acetonitrile. The mixture was immersed in a ultrasonic bath for 5 min, brought to volume (20 mL) and filtered.

10 μL-aliquots were injected into the liquid chromatograph, setting the detector at 220 nm, for the analysis of buzepide. The solution was subsequently diluted ten times and injected with the detector settled at 254 nm, for the analysis of clocinizine and phenylpropanolamine.

RESULTS AND DISCUSSION

Figures 1A and 1B show the chromatograms of a standard solution of phenylpropanolamine, buzepide and clocinizine, recorded at 220 and 254 nm, respectively. The detection wavelength selected depended on the molar absorptivity of the compound concerned. In particular, 254 was chosen for I and III, whereas 220 was chosen for buzepide since this compound possesses insignificant UV absorption at wavelengths higher than 230 nm. When injecting standard solutions of buzepide a trace impurity was found to elute immediately after peak III. It could be observed only at 220 nm, and therefore the presence of

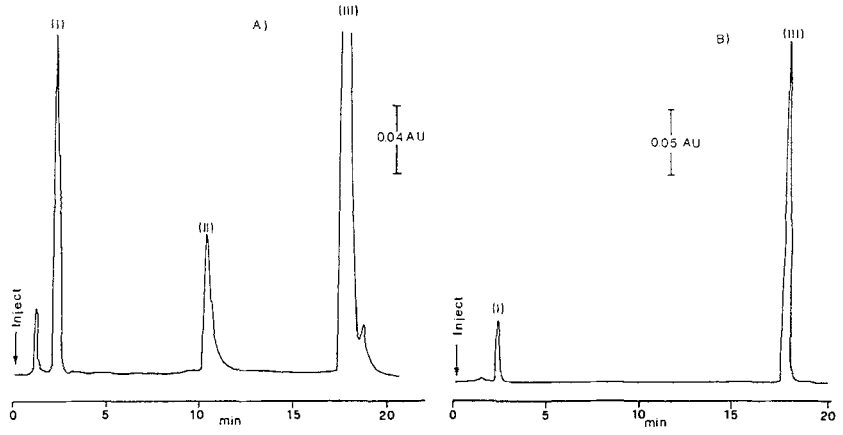

Figure 1 Typical chromatograms obtained at 220 nm (A) and 254 nm (B) for a standard solution containing 1 mg mL^{-1} of phenylpropanolamine (I) and buzepide (II), and 8 mg mL^{-1} of clocinizine (III).

this impurity did not interfere in the measurement of the peak area corresponding to clocinizine.

The photodiode-array detector allowed the estimation of the purity parameter format values (7) which are very useful in the analysis of a pharmaceutical preparation both in confirming peak purity and peak identification. The values were calculated over the range 220-367 nm and resulted 233.185 $\pm$ 0.011 for I, 223.075 $\pm$ 0.009 for II, and 241.171 $\pm$ 0.010 for III.

The capacity factors (reported in Table 1) were reproducible under the experimental conditions used, the coefficient of variation (C.V.) ranging from 1.2 to 1.9 for within-day and from 2.3 to 3.9% for between-day studies. The average analysis time was about 30 min.

The calibration graphs were constructed from five consecutive injections over the covered range of concentration, as indicated in the experimental section. The least square regression fit showed good linearity, passing through the origin. The

TABLE 1
Analytical Parameters for Compounds I-III

Compound	Capacity Factor	Detection Wavelength (nm)	Detection limit (ng injected)
I	1.6	254	500
II	8.3	220	40
III	15.4	254	5

TABLE 2
Calibration Curves for Compounds I-III: linear regression of the amount injected (x) versus the peak area (y); mean value ± standard deviation at 95% confidence interval (t=3.18; n=5)

Compound	Intercept	Slope	R^2
I	(-0.30 ± 0.02)E3	(0.205 ± 0.003)E3	0.9996
II	(-0.31 ± 0.02)E3	(2.54 ± 0.04)E3	0.9996
III	(-6.8 ± 0.2)E3	(20.3 ± 0.2)E3	0.9975

data obtained for the calibration lines are shown in Table 2. The detection limits, calculated as a signal-to-noise ratio of 2:1, are reported in Table 1.

Within-day precision was analyzed using standard solutions containing varying concentrations of each compound. The sample was then run on five separate occasions over the course of the day. The within-day variation of the determination was minimal, with a CV of 0.9%. Between-day precision involved the analysis of a particular standard solution each day for five consecutive days. Between-day precision was also good, with a CV of 1.5%.

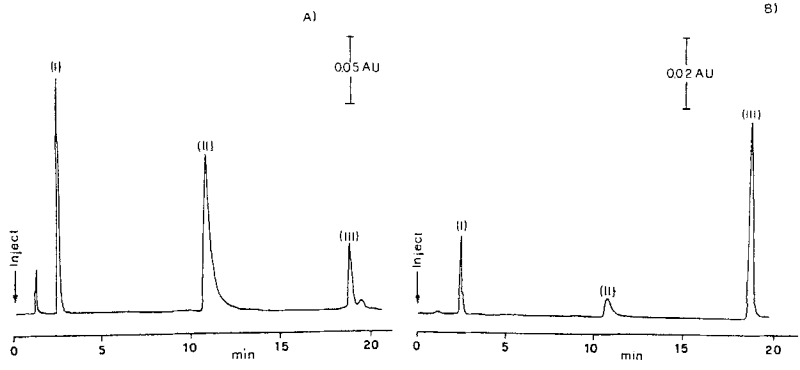

Figure 2 (A) Chromatogram recorded at 220 nm after injecting a sample of Denoral, (B) Chromatogram recorded at 254 nm, after a tenfold dilution.

TABLE 3
Analysis of a Pharmaceutical Formulation

Active principle	Label, mg/tablet	Assay results, mg/tablet	CV
Phenylpropanolamine	25.0	24.3	1.1
Buzepide	1.0	0.98	1.2
Clocinizine	5.0	4.92	0.9

* mean of five determinations.

The procedure was applied to the analysis of commercial samples of a pharmaceutical formulation containing all the three active principles, in the dosage form of tablets. Figure 2A and 2B show the chromatogram obtained from the analysis of the tablets. No problems were encountered with interfering compounds, since the impurity deriving from buzepide does not absorb at 254 nm, the wavelength at which chromatogram 2B was recorded.

The results obtained are shown in Table 3. The quantities found was in conformity with the values claimed by the manufacturer. Therefore, we can conclude that the developed method can be successfully adopted for the quantitation of I-III in pharmaceutical formulations.

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REFERENCES

1. V.D. Gupta, A.R. Heble, J. Pharm. Sci., 73: 1553-1556 (1984)

2. P.Taylor, P.D. Braddock, S. Ross, Analyst, 109: 619-621 (1984)

3. R. Dowse, J.M. Haigh, I. Kanfer, J. Pharm. Sci., 72 1018-1020 (1983)

4. C.S. Stockley, L.M.H. Wing, J.O. Miners, Ther. Drug Monit., 13: 332-338 (1991)

5. A-E-H N. Ahmed, Anal Lett., 26: 1153-1162 (1993).

6. T.D. Wilson, W.G. Jump, W.C.Neunamm, T. San Martin, J. Chromatogr., 641: 241-248 (1993)

7. T. Alfredson, T. Sheehan, T. Lenert, S. Aamodt, L. Correia, J. Chromatogr., 385: 213-217 (1987)

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