ELSEVIER

Journal of Chromatography A, 731 (1996) 346–350

JOURNAL OF CHROMATOGRAPHY A

Short communication

Simultaneous determination of chlorpheniramine and maleate by high-performance liquid chromatography using tetra-*n*-butylammonium phosphate as an ion-pair reagent

Susumu Yamato*, Masaharu Nakajima, Kenji Shimada

Department of Analytical Chemistry, Niigata College of Pharmacy, 5-13-2 Kamishin'ei-cho, Niigata 950-21, Japan

Received 10 July 1995; revised 24 October 1995; accepted 24 October 1995

Abstract

A rapid, convenient and precise chromatographic method for the simultaneous determination of chlorpheniramine and maleate is described. By the use of a Capcell Pak C_8 column and an isocratic mobile phase containing 50 m*M* KH_2PO_4, 5 m*M* tetra-*n*-butylammonium phosphate as an ion-pair reagent and 15% methanol (pH 2.6), maleate of a weak acidic moiety and chlorpheniramine of a weak basic moiety in chlorpheniramine maleate were separately eluted within 10 min, and both moieties were simultaneously determined. The present approach was a useful tool for the quality control of manufactured chlorpheniramine maleate. This method was also applicable to the determination of chlorpheniramine maleate in the ophthalmic solutions.

Keywords: Ion-pair agents; Pharmaceutical analysis; Chlorpheniramine maleate; Maleic acid; Antihistamines

1. Introduction

Various methods have been reported for the analysis of chlorpheniramine maleate (CPM), a representative antihistaminic drug [1–4]. In the Pharmacopoeia of Japan XII, a colorimetric method, measurement of melting range and infrared (IR) absorption spectrum analysis are employed for identification test, and TLC for purity test. Non-aqueous titration and UV spectrophotometric methods are utilized for the quantitative determination of CPM, which methods were also adopted in United States Pharmacopoeia and British Pharmacopoeia. On the other hand, a high-performance liquid chromatographic (HPLC) technique is a useful tool for the analysis of pharmaceutical preparations. Reversed-phase ion-pair HPLC using heptanesulfonic acid as an ion-pair reagent has been developed for the analysis of CPM [5]. An anionic counter ion such as 1-octanesulfonic acid or lauryl sulfate has been also used for the quality control of manufactured CPM in the pharmaceutical industry. These methods are in any event focused only on the analysis of chlorpheniramine (CP), a weak basic moiety. In a previous paper, we have carefully examined the elution behavior of dicarboxylic acids, i.e., malic, oxalic, malonic, succinic, glutaric, adipic, fumaric and maleic acid, based on reversed-phase ion-pair HPLC using various mobile phases containing tetra-*n*-butylammonium phosphate (TBAP) as an ion-pair reagent [6]. The interesting result that maleic acid (MA) was strongly retained on the column led us to

*Corresponding author.

0021-9673/96/$15.00
SSDI 0021-9673(95)01226-5

continue investigating the applicability of assaying the drugs containing MA. Here we report on a convenient technique for the simultaneous determination of MA of a weak acidic moiety and CP of a weak basic moiety in CPM. This method has been successfully applied to the determination of CPM in commercially available ophthalmic solutions.

2. Experimental

2.1. Materials and solutions

Chlorpheniramine maleate (CPM) was purchased from Sigma (St. Louis, MO, USA). *m*-Hydroxybenzoic acid (*m*-HOBA) and *p*-hydroxybenzoic acid (*p*-HOBA) were from Aldrich (Milwaukee, WI, USA) and Kanto Chemical (Tokyo, Japan), respectively. The ion-pair reagent, tetra-*n*-butylammonium phosphate (TBAP), was obtained from Nacalai Tesque (Kyoto, Japan). HPLC-grade methanol was purchased from Kanto Chemical. Ophthalmic solutions were from the market. Other reagents were of analytical-reagent grade. Stock standard solutions were prepared at a concentration of 5 m*M*. *m*-HOBA, *p*-HOBA and CPM were dissolved in 50% methanol, and working standard solutions were then diluted with doubly demineralized, distilled water to the required concentrations. Preparation of standard solutions of fumaric acid (FA) and maleic acid (MA) was described in the previous paper [6].

2.2. Apparatus and analytical procedures

The HPLC system consisted of a Shimadzu (Kyoto, Japan) LC-6A pump, a spectrophotometer (SPD-6AV, Shimadzu) equipped with an 8-μl flow cell and a C-R4A integrator (Shimadzu) for recording the chromatographic data. A Capcell Pak C_8 (150×6 mm I.D., SG type, 5-μm particle; Shiseido, Tokyo, Japan) was used as the analytical column. The column temperature was maintained at 30°C with a Shimadzu CTO-6A column oven. The mobile-phase solvents consisted of the ion-pair reagent (TBAP), potassium dihydrogenphosphate (KH_2PO_4) and methanol, buffered with 5% orthophosphoric acid (H_3PO_4). The mobile phase was pumped at a flow-rate of 1.0 ml/min and sample solutions were injected through a Rheodyne Model 7125 injector (Cotati, CA, USA) with a 100-μl sample loop. The column effluent obtained with isocratic elution was monitored by UV absorption at 215 nm. After one week of use, the analytical column was washed with water and then 70% methanol at a flow-rate of 1.0 ml/min for at least 30 min to keep the column efficiency.

Table 1
Linearity of assay

Analyte	Slope (a)	*y*-Intercept (*b*)	Correlation coefficient
Method 1			
MA	0.654	0.049	0.9995
CP	0.534	0.119	0.9991
Method 2			
MA	639.1	54.0	0.9992
CP	519.0	89.7	0.9991

$y=ax+b$, where y means peak area of sample per peak area of the internal standard and x means amount of injection (Method 1), and where y means peak area (mV s) of sample and x means amount of injection (Method 2).

3. Results and discussion

3.1. Optimization of chromatographic conditions

Reversed-phase ion-pair chromatography has become an important technique for the analysis of polar ionized solutes [7]. The pK_a-values of organic acids and CP used in this study are as follows: MA (pK_{a1}=1.75, pK_{a2}=5.83); FA (pK_{a1}=2.85, pK_{a2}=4.10); *m*-HOBA (pK_{a1}=4.08, pK_{a2}=9.96); *p*-HOBA (pK_{a1}=4.58, pK_{a2}=9.46) [8]; and CP (pK_{a1}=9.2, pK_{a2}=4.0) [9,10]. MA, a divalent acid, is dissociated even at low pH. The capacity factor (k') was determined from the retention time relative to the dead time, i.e., t_0, of a non-retained compound as described in the previous paper [6]. Briefly, two compounds, sodium nitrite and oxalate, as candidates for a non-retained standard were examined under the mobile phases buffered to various pH values. The elution times (min) for sodium nitrite were: 4.18 (pH 2.0), 3.57 (pH 3.5), 3.14 (pH 5.0), 3.10 (pH 6.0) and 3.09 (pH 7.0); and for oxalate: 3.15 (pH 2.0), 3.06

Table 2
Determination of chlorpheniramine and maleate in commercial ophthalmic solutions

Sample	Composition	Labeled content (mg/ml)	Found content (mg/ml)	
			Maleic acid	Chlorpheniramine
I	Chlorpheniramine maleate Taurine Dipotassium glycyrrizate Pyridoxine hydrochloride	0.1	0.102	0.104
II	Chlorpheniramine maleate Dipotassium glycyrrizate Tetrahydrozoline hydrochloride	0.3	0.293	0.284
III	Chlorpheniramine maleate Neostigmine methylsulfate Tetrahydrozoline hydrochloride ε-Aminocaproic acid	0.1	0.100	0.104
IV	Chlorpheniramine maleate Neostigmine methylsulfate Taurine Dipotassium glycyrrizate Sodium chondroitin sulfate Magnesium potassium L-aspartate	0.2	0.195	0.194
V	Chlorpheniramine maleate Neostigmine methylsulfate Magnesium potassium L-aspartate Dipotassium glycyrrizate Cyanocobalamin Pyridoxine hydrochloride	0.2	0.198	0.198

(pH 3.5), 2.92 (pH 5.0), 2.90 (pH 6.0) and 2.89 (pH 7.0). We confirmed that high polar oxalate was not retained to the column in the absence of TBAP as a counter ion, and then the apparent t_0 of 2.89 min for this column was obtained. The k' value of FA, MA, chlorpheniramine (CP), and *m*-HOBA and *p*-HOBA as candidates for an internal standard was examined with mobile phases with different concentrations of methanol, TBAP and pH values. As expected, the retention of acids was enhanced with increasing TBAP-concentration, whereas a decrease in retention for CP with increasing concentration of TBAP, having the same charge as CP, was observed (data not shown). This decrease would be mainly explained by an electrostatic repulsion model [11]. Another explanation for this effect is that TBAP masks the accessible silanols, which contribute to retention of amines. As a result of careful examination, a mobile phase of 5 m*M* TBAP, 50 m*M* KH_2PO_4 and 15% (v/v) methanol, buffered to pH 2.6 with H_3PO_4 was the best choice for the separation of the compounds. MA was ion-paired with TBAP and retained to the column, whereas CP, a divalent amine, is protonated at pH 2.6. Under the chromatographic conditions used, MA of a weak acidic moiety and CP of a weak basic moiety in CPM were separately eluted within 10 min, and both moieties were simultaneously determined. FA, a candidate of an impurity and/or a by-product in pharmaceutical preparations of CPM, was also detectable simultaneously. The retention times (min) were 4.91, 7.45, 9.05, 13.46 and 17.33 for FA, CP, MA, *p*-HOBA and *m*-HOBA, respectively. *p*-HOBA with a faster elution was better suited as an internal

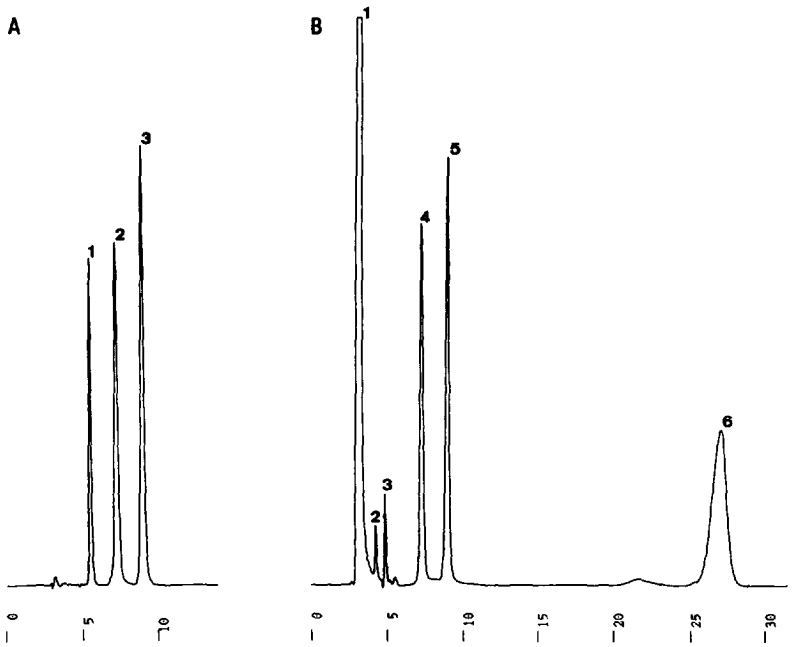

Fig. 1. Chromatograms of commercial opthalmic solutions. Conditions: stationary phase, Capcell Pack C_8; mobile phase, 5 m*M* TBAP, 50 m*M* KH_2PO_4, 15% MeOH (pH 2.6); flow-rate, 1.0 ml/min; temperature, 30°C; injection volume, 20 μl. (A) Solution obtained from sample II in Table 2. Peaks: 1=tetrahydrozoline hydrochloride; 2=chlorpheniramine; 3=maleic acid. (B) Sample V shown in Table 2. Peaks: 1=pyridoxine hydrochloride; 2=neostigmine methylsulfate; 3=dipotassium glycyrrizate; 4=chlorpheniramine; 5=maleic acid; 6=cyanocobalamin.

standard than *m*-HOBA when the present method was used for the quality control of manufactured CPM.

3.2. Calibration graphs and reproducibility

Two kinds of calibration graphs were studied by measuring the peak area for ten different amounts of CPM. One is an internal standard method, which sample solutions were prepared by adding *p*-HOBA as the internal standard in the final concentration of 0.2 m*M* to the different concentrations of working standard of CPM. A 10-μl aliquot was injected onto the chromatographic system. The other method is an absolute calibration curve method, where 20-μl aliquots of sample solutions containing different amounts of CPM were injected through a Rheodyne injector. Linearity over the range 0.5–10 nmole (0.195–3.9 μg) per injection was observed for both methods. The calibration data are summarized in Table 1. The calibration curves obtained by injecting the working standard solutions of MA are in close agreement with those of MA in CPM described above. The relative standard deviation (R.S.D.) for five replicate injections of CPM (1.5 nmole per 20 μl with a 50-μl microsyringe) was 3.1% and 2.3% for MA and CP, respectively.

3.3. Application to the commercial ophthalmic solutions

The proposed method was applied to the determination of CPM in five different ophthalmic solutions on the market. Prior to the assay, we have confirmed that this method was not affected by chondroitin sulfate C sodium salt (10 mg/ml) and lysozyme (10 mg/ml), whereas these compounds

interfered the capillary electrophoretic assay of CPM [12].

The composition of the pharmaceutical preparations and the results obtained for the labeled content of CPM are shown in Table 2. The labeled content of CPM in the commercially available ophthalmic solutions, in most cases, corresponds to the directly measurable amount. The assay was carried out by the absolute calibration method, which precision is equivalent to that of the internal standard method. Chromatograms of samples II and V are shown in Fig. 1. All components are eluted within 10 min, except cyanocobalamin (vitamin B_{12}) which appeared at 26.98 min.

The proposed method is simple, rapid and convenient, and offers not only quantitative analysis but also identification and purity tests for a product of CPM. Simultaneous determination of CP and MA has enabled the true quality control of manufactured CPM. Simultaneous determination of antihistamines analogous to CPM and the assay of CPM in cough and cold drug should also be achievable by controlling the mobile phase.

Acknowledgments

We thank Miss Hiromi Kaneko and Miss Yuki Kimura for their technical assistance at an early stage of this experiment.

References

[1] C.G. Eckhart and T. McCorkle, in K. Florey (Editor), Analytical Profiles of Drug Substances, Academic Press, New York, 1978, Vol. 7, p. 43.
[2] R. Onishi, T. Morita and K. Kawamura, Yakugaku Zasshi, 90 (1970) 46.
[3] H.N. Al-Kaysi and M.S. Salem, Anal. Lett., 19 (1986) 915.
[4] H.S.I. Tan and G.C. Salvador, Anal. Chim. Acta, 188 (1986) 295.
[5] T.R. Koziol, J.T. Jacob and R.G. Achari, J. Pharm. Sci., 68 (1979) 1135.
[6] S. Yamato, H. Wakabayashi, M. Nakajima and K. Shimada, J. Chromatogr., 656 (1994) 29.
[7] M.T.W. Hearn (Editor), Ion-pair Chromatography: Theory and Biological and Pharmaceutical Applications, Chromatographic Science Series, Vol. 31, Dekker, New York, 1985.
[8] A.E. Martell and R.M. Smith, Critical Stability Constants, Vol. 3, Plenum Press, New York, 1977.
[9] D.D. Perrin (Editor), Dissociation Constants of Organic Bases in Aqueous Solution, Butterworths, London, 1965.
[10] T. Higuchi and K. Kato, J. Pharm. Sci., 55 (1966) 1080.
[11] B.A. Bidlingmeyer, S.N. Deming, W.P. Price, Jr., B. Sachok and M. Petrusek, J. Chromatogr., 186 (1979) 419.
[12] N. Ishii, M. Kuroki and Y. Akada, Proceedings of the 43th Annual Meeting of the Japan Society for Analytical Chemistry, Kyushu, 1994, p. 2.