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Quantitative determination of acrivastine and pseudoephedrine hydrochloride in pharmaceutical formulation by high performance liquid chromatography and derivative spectrophotometry

T.G. Altuntas *, S.S. Zanooz, D. Nebioglu

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Ankara, 06100 Tandogan, Ankara, Turkey

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Abstract

In this study, fourth derivative spectrophotometry and high performance liquid chromatography (HPLC) have been used and described for the quantitative determination of acrivastine (**I**) and pseudoephedrine hydrochloride (**II**) in their pharmaceutical capsules form (Duact®). In the former method, $d^4A/d\lambda^4$ values were measured in methanol at 315 and 269 nm for (**I**) and (**II**) respectively. The relative standard deviations (RSD) for the method were found to be 1.16% for (**I**) and 0.94% for (**II**). The latter method based on reversed phase HPLC system using LiChrosorb C18 analytical column. The mobile phase used for separation of (**I**), (**II**) and internal standard (*p*-hydroxymethylbenzoate) were the water/ acetonitrile/methanol/perchloric acid/*n*-octylamine (500:130:25:13:0.3 v/v) and the detection of the compounds in the capsules were at 260 nm using UV detector. The RSD for the HPLC method were determined to be 0.79 and 0.88% for (**I**) and (**II**) respectively. The proposed methods, which give thoroughly comparable data, are simple, rapid, and allow precise and accurate results and could be used for commercial formulations containing acrivastine and pseudoephedrine hydrochloride in combination.

Keywords: Acrivastine; Pseudoephedrine hydrochloride; Derivative spectrophotometry; High performance liquid chromatography; Pharmaceutical form

1. Introduction

Spectrophotometry is a common technique in the field of pharmaceutical and biomedical analysis. Direct UV-absorbance measurement is subject to interference from co-formulated drugs, excipients and/or degradation products. Derivative spectrophotometry [1–6] is an analytical technique of great utility for extracting both qualitative and quantitative information from spectra composed or unresolved bands. It tends to emphasize subtle spectral features by representing them in a new and visually more accessible way, allowing the resolution of multi-component elements and reducing the effect of spectral background interferences. In pharmaceutical

* Corresponding author.

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application, derivative spectrophotometry has led to significant developments in the analysis of drugs in the presence of their degradation products or in multicomponent mixtures [7–9].

High performance liquid chromatography (HPLC) has also application in pharmaceutical analysis [10–13].

Acrivastine (**I**) and pseudoephedrine hydrochloride (**II**) (Fig. 1) are used in capsules form (Duact®) as antihistaminic-decongestant in Turkey.

Various methods including titrimetry [14,15], spectrophotometry [16–20], derivative spectrophotometry [21–23], colorimetry [24,25], HPLC [26–30], gas chromatography-mass spectrometry (GC-MS) [31–33] and capillary electrophoresis [34,35] have been used for the determination of acrivastine, pseudoephedrine hydrochloride and ephedrine in pharmaceutical preparations, biological fluids and plants either separately or in combination with other drugs. However, no methods have been reported for their simultaneous quantification in two component (**I** and **II**) mixtures.

This paper describes a procedure for the quantitation of acrivastine and pseudoephedrine hydrochloride in pharmaceutical capsule form by derivative UV-spectrophotometry and reversed phase high performance liquid chromatography (RP-HPLC) providing accurate and precise results.

2. Experimental

2.1. Material and reagents

Acrivastine, pseudoephedrine hydochloride and a commercial preparation (Duact® capsule) were gifts from GlaxoWellcome Co. (Istanbul, Turkey). The labelled content in one capsule was as follows: acrivastine (8.0 mg) and pseudoephedrine hydrochloride (60.0 mg).

HPLC grade acetonitrile, methanol, perchloric acid and *n*-octylamine were purchased from J.T. Baker Inc. (Phillipsburg, NJ, USA). All other solvents and chemicals (analytical grade) were obtained from Merck Co. (Darmstadt, Germany).

2.2. Spectrometric equipment and conditions

A Shimadzu UV-160 double beam spectrophotometer with a fixed slit with 2 nm was used. The derivative UV spectra of standard and test solutions were recorded in 1 cm quartz cells over the range 240–380 nm ($\Delta\lambda = 12.6$ nm). The scan speed was 20 nm min^{-1}.

2.3. Chromatographic system and conditions

The HPLC (Jasco International Co. Ltd., Tokyo, Japan) consisted of a model PU-980 solvent delivery system, and a model 970/975 UV-VIS detector connected to a Panasonic model KX-P1150 multimode integrator (Matsushita Electric Industrial Co. Ltd., Japan). A model 7125 sample injector (Rheodyne, Cotati, CA, USA) equipped with a 20 ml loop was used.

The separation was performed on a reversed phase LiChrosorb RP-C18 analytical column (200 × 4.6 mm i.d., 5 μm particle size) (Hichrome Ltd., Berkshire, UK). The mobile phase consisted of a mixture of water/acetonitrile/methanol/perchloric acid/*n*-octylamine (500:130:25:13:0.3 v/v). The mobile phase was prepared daily, filtered, sonicated before use, and delivered at a flow rate of 3 ml min^{-1}. The detector wavelength was set at 260 nm.

2.4. Preparation of calibration curves

2.4.1. Derivative spectrophotometric method

2.4.1.1. Acrivastine. A stock solution of acrivastine was prepared by dissolving approximately 100 mg, accurately weighed, in 100 ml of

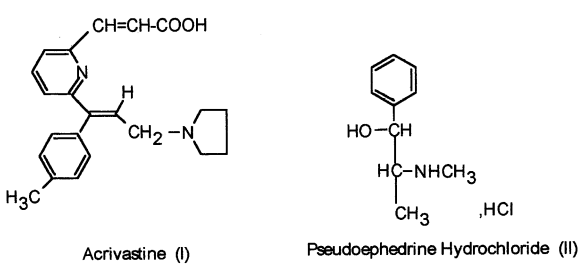

Fig. 1. Chemical structures of compounds.

methanol (solution **I**). A 25 ml aliquot of solution **I** was transferred into a 100 ml calibrated flask and diluted to volume with methanol (solution **II**). Standard solutions were prepared from solution **II** in methanol with concentrations of acrivastine in the range 10–20 mg ml^{-1}. The calibration curve for fourth derivative spectrophotometry was constructed by plotting the drug concentration versus the absorption of $d^4A/d\lambda^4$ at 315 nm.

2.4.1.2. Pseudoephedrine hydrochloride. A stock solution of pseudoephedrine hydrochloride was prepared by dissolving approximately 150 mg in 100 ml of methanol. Dilutions from the stock solution were prepared in methanol in the range 75–150 μg ml^{-1}. The calibration curve for fourth derivative spectrophotometry was costructed by plotting the drug concentration versus the absorption of $d^4A/d\lambda^4$ at 269 nm.

To study the accuracy and pecision of the proposed methods, recovery experiments were carried out by standard addition technique. Working standard solutions of acrivastine-pseudoephedrine hydrochloride mixtures in methanol (containing 100 μg ml^{-1} of pseudoephedrine hydrochloride and increasing concentrations of acrivastine ranging from 12 to 20 μg ml^{-1}; and containing 10 μg ml^{-1} of acrivastine and increasing concentrations of pseudoephedrine hydrochloride ranging from 80 to 160 μg ml^{-1}) were prepared. The fourth derivative spectra of these solutions were recorded at 269 and 315 nm for pseudoephedrine hydrochloride and acrivastine respectively. Above solutions were also used for HPLC analysis.

2.5. HPLC method

Stock solutions of acrivastine (**I**) (150 μg ml^{-1}) in methanol, and pseudoephedrine hydrochloride (**II**) (350 μg ml^{-1}) in water were prepared. Mixtures containing **I** and **II** were prepared by dilution with mobile phase. The concentrations of **I** and **II** were in the range 5–45 and 50–150 μg ml^{-1}, respectively. A constant concentration of the internal standard (*p*-hydroxymethylbenzoate) (2 μg ml^{-1} in methanol) was added into the mixture of **I** and **II**. The mixtures (20 ml) were then chromatographed on the C18 column. The calibration curve was obtained by plotting the peak area ratio of the drug to the internal standard against the drug concentration.

2.6. Procedure for pharmaceutical capsule form

Five capsules were emptied and the contents were transferred into a 100 ml volumetric flask, then 50 ml of methanol was added. After sonicating for 30 min in the ultrasonic bath, the volume made up to 100 ml with water. The contents of the flask were filtered using Whatman No. 1 filter paper. Then, 20 ml of filtrate was trasferred to a 100 ml of volumetric flask (solution T) and 2 μg ml^{-1} of internal standard (*p*-hydroxymethylbenzoate) were added. The contents were diluted to volume with water. The solution (20 μl) was chromatographed by HPLC. The amounts of acrivastine and pseudoephedrine hydrochloride were calculated from the linear regression equations of the calibration curves or using reference standard solution injected under the same conditions.

For the fourth derivative spectrophotometric method, the solution T was diluted to 1:5 with methanol. The contents of acrivastine and pseudoephedrine hydrochloride were calculated using the corresponding calibration curves.

3. Results and discussion

The purpose of this study was to determine a mixture of acrivastine and pseudoephedrine hydrochloride in pharmaceutical capsule form in a reasonable time and to quantitate the analysis with a suitable level of precision by derivative spectrophotometry and HPLC.

Fig. 2 shows the zero-order UV spectra of acrivastine (**I**), pseudoephedrine hydrochloride (**II**) and mixture of (**I**) and (**II**). Due to the extensive overlap of the spectral bands of two drugs, conventional UV spectrophotometry cannot be used for their individual determination in a binary mixture. Derivative spectra of different orders were obtained using smoothed spectra with acrivastine, pseudoephedrine hydrochloride and

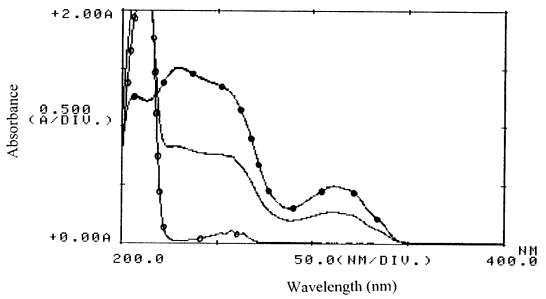

Fig. 2. Zero-order UV spectra of 16 mg μl^{-1} acrivastine (●), 120 μg ml^{-1} pseudoephedrine hydrochloride (○), and its binary mixture (—) in methanol.

their binary solutions (Fig. 3). First, second and third derivative spectra were not found to be resolved as can be seen in Fig. 3. However, zero-crossing fourth derivative spectrophotometry permits a more selective identification and determination of the two drugs in a mixture comparing first, second and third derivative spectrophotometry (Fig. 3). The zero-crossing method involves measurement of the absolute value of the total derivative spectrum at an abscissa value corresponding to the zero-crossing wavelengths of the derivative spectra of the individual component. The fourth derivative spectrum of acrivastine exhibits a maximum at 315 nm, while pseudoephedrine hydrochloride reads zero and pseudoephedrine hydrochloride exhibits an absorption at 269 nm while acrivastine reads zero (Fig. 3D).

Quantitative investigations using regression analysis have established that the concentration of acrivastine and pseudoephedrine hydrochloride correlates very well with the measured fourth derivative peaks. The regression equations were $y = 0.0004 + 0.079x$ ($r^2 = 0.9997$) for acrivastine at 315 nm and $y = 0.0013 - 1.116x$ ($r^2 = 0.9982$) for pseudoephedrine hydrochloride at 269 nm (where y is the $d^4A/d\lambda^4$ value, x is the concentration of drug in μg ml^{-1}).

The recovery test was performed from synthetic mixtures containing various amount of acrivastine and pseudoephedrine hydrochloride (Table 1). The results show that the mean recovery is 100.54% for acrivastine and 100.28% for pseudoephedrine hydrochloride using proposed fourth derivative spectrophotometric method. The relative standard deviation (RSD) for acrivastine and pseudoephedrine hydrochloride is 1.16 and 0.94% respectively. The results are reproducible and precise as the RSD values are very low. This method has been successfully applied to a commercial pharmaceutical capsule form and the results obtained from commercial capsule form were shown

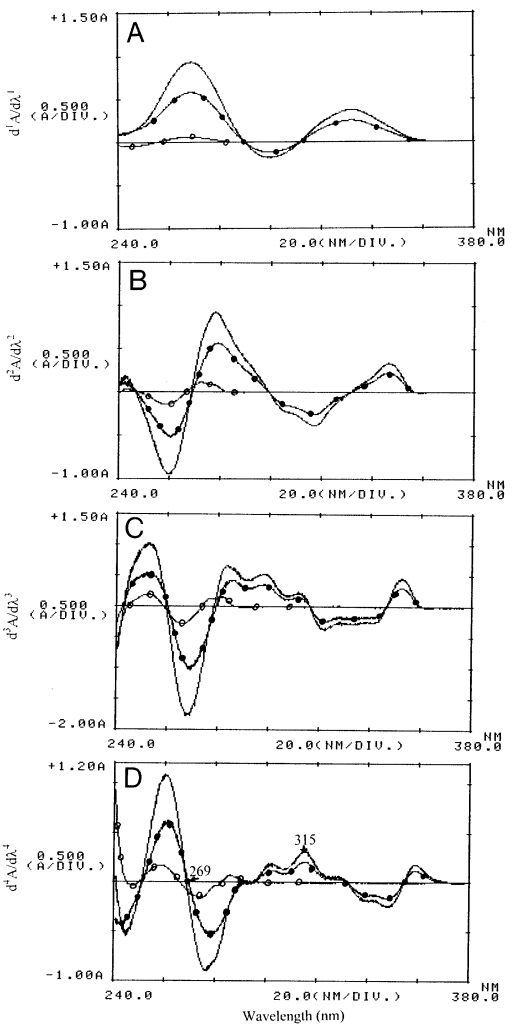

Fig. 3. First-order (A), second-order (B), third-order (C) and fourth-order (D) derivative spectra of 16 μg ml^{-1} acrivastine (●), 120 μg ml^{-1} pseudoephedrine hydrochloride (○), and its binary mixture (—), in methanol ($\Delta\lambda = 12.6$ nm).

Table 1
Recovery of acrivastine and pseudoephedrine hydrochloride in synthetic mixtures by proposed fourth derivative spectrophotometric and HPLC methods

Amount added (μg ml^{-1})		Found (μg ml^{-1})				Recovery (%)			
		I		**II**		**I**		**II**	
I	**II**	A	B	A	B	A	B	A	B
10	80	9.87	9.90	79.92	79.60	98.70	99.00	99.90	99.50
10	100	10.15	9.92	99.10	99.60	101.50	99.20	99.10	99.60
16[a]	120[a]	15.89	15.96	120.90	118.73	99.31	99.75	100.75	98.94
10	140	9.98	9.93	141.68	138.88	99.80	99.30	101.20	99.20
10	160	9.99	10.05	162.40	158.40	99.90	100.50	101.50	99.00
12	100	12.01	12.12	99.00	100.50	100.08	101.00	99.00	100.50
14	100	14.11	14.16	99.43	100.71	100.79	101.14	99.43	100.71
16	100	16.26	16.16	100.12	101.06	101.62	101.00	100.12	101.06
18	100	18.44	18.04	101.50	101.39	102.44	100.22	101.50	101.39
20	100	20.25	20.03	100.35	100.25	101.25	100.15	100.35	100.25
						$\bar{x}$ = 100.54	$\bar{x}$ = 100.13	$\bar{x}$ = 100.28	$\bar{x}$ = 100.01
						RSD = 1.16	RSD = 0.79	RSD = 0.94	RSD = 0.88

I, acrivastine; **II**, pseudoephedrine hydrochloride; A, fourth derivative spectrophotometric method; B, HPLC method.
[a] The amount of drug (at appropriate dilution) in the commercial pharmaceutical capsule form.

in Table 2. There was no interference observed with the excipients in the capsule.

For HPLC analysis, initially various mobile phase compositions were tried in attempts to separate drugs and internal standard. RP-HPLC system using C18 analytical column and water/acetonitrile/methanol/perchloric acid/*n*-octylamine (500:130:25:13:0.3 v/v) gave good separation of drugs and internal standard (*p*-hydroxymethylbenzoate). Small increase in the water content of the eluent can increase the retention time of acrivastine, but not significantly affect pseudoephedrine hydrochloride. A small increase in the acid concentration of the eluent can decrease the retention time of acrivastine. It has no affect on pseudoephedrine hydrochloride. Small variations in the *n*-octylamine content of the mobile phase have little or no affect on the separation. Omission of *n*-octylamine however, could lead to poor column performance. Small variations in the methanol/acetonitrile ratio of the eluent cause selectivity changes and peaks can co-elute.

Fig. 4A and 4B shows a typical HPLC chromatogram of standard compounds and extracts from pharmaceutical capsule form respectively. Chromatographic investigations revealed that a mixture of acrivastine and pseudoephedrine hydrochloride could be resolved from the co-formulated excipients using C18 stationary phase and a mixture of water/acetonitrile/methanol/perchloric acid/*n*-octylamine (500:130:25:13:0.3 v/v). The separations could be obtained in less than 7 min. The retention times for pseudoephedrine hydrochloride, internal standard, and acrivastine were found to be 1.4, 3.2 and 6.6 min, respectively (Fig. 4A and 4B).

The peak area ratios of acrivastine and pseudoephedrine hydrochloride to internal standard exhibit linear relationship with their concentrations. The regression equations for acrivastine and pseudoephedrine hydrochloride were $y = 0.196 + 0.104x$ and $y = 0.014 + 0.55x$, respectively (where y is peak area ratio and x is the concentration of drug in μg ml^{-1}). The correlation coefficient of the calibration curves were found to be 0.9999 for

Table 2
Results of the simultaneous determination of acrivastine and pseudoephedrine hydrochloride in pharmaceutical capsule form

Drug	Labelled (mg capsule^{-1})	n	Found ($\bar{x}$) (mg capsule^{-1})		Recovery (%)		RSD (%)	
			A	B	A	B	A	B
I	8	10	7.97	7.97	99.62	99.62	1.32	0.72
II	60	10	60.56	60.78	100.93	101.30	1.59	0.61

I, acrivastine; **II**, pseudoephedrine hydrochloride; A, fourth derivative spectrophotometric method; B, HPLC method.

acrivastine and 0.9998 for pseudoephedrine hydrochloride.

In the HPLC method, the RSD was found as 0.79% for acrivastine and 0.88% for pseudoephedrine hydrochloride (Table 1). Commercially available capsules were analysed by the HPLC method (Table 2). No significant differences were found between the results obtained by the HPLC from synthetic mixtures and the commercial capsule form for the RSD of the method.

It can be concluded that the reported methods for the determination of acrivastine and pseudoephedrine hydrochloride together in pharmaceutical capsule form (Duact®) are simple and rapid. Although there was no significant differences between the methods applied, the results indicate that the HPLC method could be considered for the routine analysis of commercial formulations containing these active ingredients. The method is accurate, precise, stability indicating and reproducible. Additionally, the sensitivity and reliability of the HPLC method over a wide range of the concentrations will extend the use of this method.

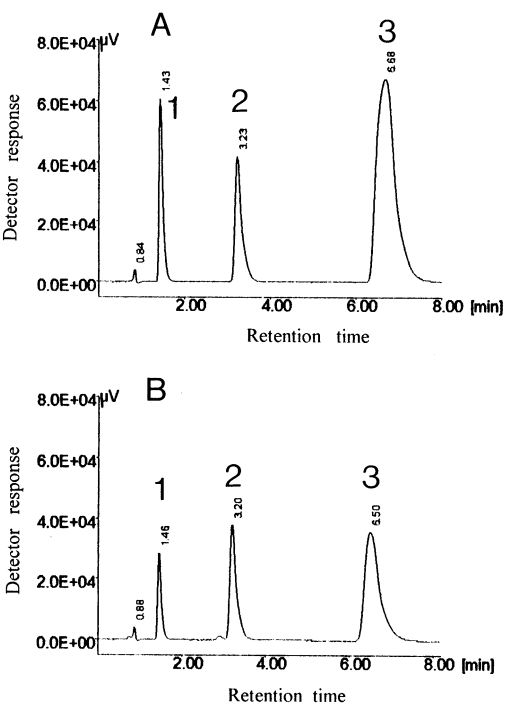

Fig. 4. Typical chromatogram obtained by RP-HPLC analysis of the standard (A) and pharmaceutical capsule form (B): (1) pseudoephedrine hydrochloride (120 μg ml^{-1}); (2) internal standard (*p*-hydroxymethylbenzoate) (3 μg ml^{-1}); (3) acrivastine (16 μg ml^{-1}) in synthetic mixture. Column: 5 mm LiChrosorb RP-C18, 200 × 4.6mm i.d.; mobile phase: water/acetonitrile/methanol/perchloric acid/*n*-octylamine (500:130:25:13:0.3 v/v); flow rate: 3 ml min^{-1}; detector wavelength, 260 nm; injection volume: 20 μl.

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