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Journal of Pharmaceutical and Biomedical Analysis 37 (2005) 663–667
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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Simultaneous analysis of the H_1-antihistamine acrivastine and the decongestant pseudoephedrine hydrochloride by high-performance liquid chromatography

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Received 28 July 2004; accepted 30 October 2004
Available online 25 December 2004

Abstract

High-performance liquid chromatography (HPLC) was used for the simultaneous quantification of the H_1-antihistamine acrivastine and the decongestant pseudoephedrine hydrochloride. Both compounds were detected at the wavelength of 214 nm. The influence of the mobile phase and the detection wavelength was evaluated and optimized. This method was used to assay various samples from studies of the commercial preparation Semprex®-D capsules. The method was found to be accurate, specific, selective, rapid, and versatile for use in routine quality control analyses.

Keywords: Acrivastine; Pseudoephedrine hydrochloride; Simultaneous quantification; HPLC

1. Introduction

The incidence of allergic disorders has been increasing dramatically throughout the world for the past several decades. Natural environmental allergens, air pollutants, stress, and xenobiotics have all been implicated in the genesis of these disorders [1,2]. Although many allergic disorders are not considered life-threatening, they significantly reduce the quality of life for the patients and consume a great amount of healthcare resources. H_1-antihistamines have been extensively used for the treatment of allergic disorders, especially allergic rhinitis and urticaria [3].

Acrivastine, [(E)-3-(6-[3-pyrrolidino-1-(4-tolyl)-prop-1E-enyl]-2-pyridyl)-acrylic acid] (Fig. 1), is a second-generation, non-sedating antihistamine derived from the first-generation compound triprolidine [4]. It possesses specific antihistaminic activity for the treatment of allergic rhinitis with reduced potential for the sedative side effects that characterize triprolidine and other first-generation H_1-antihistamines [5]. Commercial acrivastine preparations are currently available in the US and Europe. Semprex®-D capsules contain acrivastine, and the decongestant pseudoephedrine hydrochloride (Fig. 1), a commonly used antihistamine–decongestant combination that relieves rhinorrhea, sneezing, itching, and nasal congestion.

Analytical methods have been developed to determine acrivastine and pseudoephedrine hydrochloride in pharmaceutical preparations and in biological samples. However, all of these methods were designed to measure acrivastine or pseudoephedrine hydrochloride separately. When both compounds must be quantitated, such as in the case of Semprex®-D capsules, repeated pretreatment of the test samples is normally involved. Methods used for acrivastine include gas chromatography–mass spectrometry [6] and radioimmunoassay [7], while methods used for pseudoephedrine hydrochloride are titrimetry [8], spectrophotometry [9] and high-performance liquid chromatography (HPLC) [8,10,11].

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doi:10.1016/j.jpba.2004.10.054

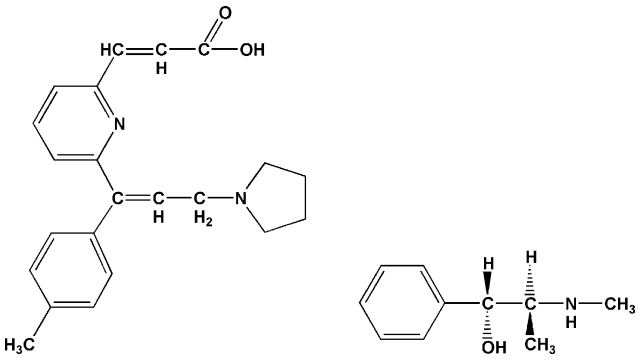

Fig. 1. Chemical structures of acrivastine (left) and pseudoephedrine (right).

In the only study in which acrivastine and pseudoephedrine hydrochloride were simultaneously detected in a pharmaceutical formulation, HPLC and derivative spectrophotometry were used [12]. The HPLC method required a high flow rate of 3 mL/min, yet the retention time for acrivastine was still relatively long at approximately 6.5 min.

For pharmaceutical quality control purposes, the main objectives for any assay development are to simplify sample preparation procedures and to achieve cost-effective sensitivity. In this study, we developed a simple and rapid HPLC assay that was able to determine acrivastine and pseudoephedrine hydrochloride simultaneously without any sample pretreatment. The influence of the mobile phase and the detection wavelength was evaluated in order to obtain optimal chromatographic conditions. This method was further used to analyze various samples from studies of the commercial preparation Semprex®-D capsules.

2. Experimental

2.1. Materials

Acrivastine pure compound and Semprex®-D capsules were obtained as a gift from Celltech Pharmaceuticals (Rochester, New York, USA). Pseudoephedrine hydrochloride was purchased from Medisca Pharmaceutique (Montreal, Quebec, Canada). Concentrated acetic acid, acetonitrile, hydrochloric acid, methanol, phosphoric acid, dipotassium hydrogen phosphate and sodium acetate trihydrate were purchased from Fisher Scientific (Fair Lawn, New Jersey, USA). All chemicals and reagents used were either HPLC grade or AC grade. Deionized water was obtained using a Millipore® Milli-Q system (Bedford, Massachusetts, USA).

2.2. HPLC conditions

A Waters® component system (Milford, Massachusetts, USA) was used for the assay, which included a 600S Controller, a 616 Solvent Delivery Pump, a 717 Autosampler, and a 996 Photodiode Array Detector. The data was acquired and processed using Millennium® 32 operating software.

A Waters® C_{18} Nova-Pak® column (4 μm, 3.9 mm × 150 mm) was used for the analysis. The mobile phase was composed of acetate buffer (0.12 M sodium acetate trihydrate, pH adjusted to 4.0 using concentrated acetic acid)/acetonitrile/methanol at a volumetric ratio of 45:47:8. The mobile phase was delivered at a flow rate of 0.8 mL/min. Both acrivastine and pseudoephedrine hydrochloride were detected at the wavelength of 214 nm.

2.3. Standard solutions

Stock solutions containing 0.5 mg/mL of acrivastine or 1.0 mg/mL of pseudoephedrine hydrochloride were prepared in deionized water. They were further diluted in deionized water to make a series of standard calibration solutions. The concentration range of acrivastine for calibration was 0.4–4.0 μg/mL, while that of pseudoephedrine hydrochloride was 1.0–20.0 μg/mL. Standard solutions were stored at 4 °C, and were prepared and recalibrated every 3 months. Calibration curves were obtained over the ranges of stated concentrations by linearly correlating the relationship between compound peak area readings and compound concentrations.

2.4. Assay optimization and validation

Method optimization was carried out by focusing on two major chromatographic parameters, component separation/retention time and sensitivity, by adjusting the mobile phase composition and the detection wavelength. The initial mobile phase was composed of acetonitrile and acetate buffer (50:50). By modifying the proportion of organic and aqueous phase by 5% volume increments from 30:70 to 70:30, respectively, chromatographic resolution and detection limits were calculated and compared under each condition. When methanol was introduced into the mobile phase, its proportion was increased by 2% volume increments from 2 to 10%. The effect of pH of acetate buffer on chromatographic separation and detection was also evaluated at 0.5 pH increments from 4.0 to 5.5. Detection sensitivity was measured by scanning the absorbance of the compounds between 200 and 400 nm with a photodiode array detector. Criteria for method optimization were to achieve the highest detection sensitivity as well as maximal peak separation, but to keep the chromatographic runtime within 3 min.

Once the optimal chromatographic conditions were determined, the assay was validated by checking the accuracy, precision, detection limit, quantitation limit, and linearity of the method. Both acrivastine and pseudoephedrine hydrochloride at three different concentrations were repeatedly injected during one test day and on different test days. The three concentrations of acrivastine for assay validation were 0.4, 1.6, and 4.0 μg/mL, and the three concentrations of pseudoephedrine hydrochloride for assay validation were 1.0, 6.0, and 20.0 μg/mL. A total 36 sets of

injection data were collected for each concentration. The average and variation of the results were calculated and compared.

2.5. Acrivastine solubility

The solubility of acrivastine was measured at room temperature (25 °C) under three different pH conditions: pH 1.0 (0.1 M hydrochloric acid), pH 4.5 (0.05 M sodium acetate trihydrate, pH adjusted to 4.5 using concentrated acetic acid), and pH 6.8 (0.05 M dipotassium hydrogen phosphate, pH adjusted to 6.8 using concentrated phosphoric acid). A saturated acrivastine solution was prepared by mixing excessive acrivastine pure compound in the solution and stirring the resultant solid–liquid mixture constantly for 16 h. The saturated solution was filtered using a 0.2 μm Nalgene® membrane filter (Rochester, New York, USA) and diluted appropriately to obtain a suitable concentration for the measurement of acrivastine using the HPLC.

2.6. Drug content in Semprex®-D capsules

The contents of 20 Semprex®-D capsules were emptied onto a clean sheet of wax paper and mixed thoroughly. An accurately weighed portion of the mixture, equivalent to 8 mg of acrivastine and 60 mg of pseudoephedrine hydrochloride, was transferred to a 100 mL volumetric flask. Deionized water was added to dissolve the material completely under agitation. The solution was filtered using a 0.2 μm membrane filter, and 1.0 mL of the filtrate was transferred to another 100 mL volumetric flask and diluted with deionized water. An aliquot of 25 μL solution was injected to the HPLC for content measurement.

2.7. Dissolution tests

Dissolution tests were carried out on a VanKel® 600 Dissolution Apparatus (Palo Alto, California, USA) using USP Apparatus 1 (basket method) [13]. The dissolution medium was 500 mL of 0.1 M hydrochloric acid (pH 1.0), the rotation speed was 50RPM and the temperature was maintained at 37 ± 0.5 °C. Two milliliters of dissolution sample was collected at 3, 5, 10, 15, 20, 25, 30, 40, 50, and 60 min. The dissolution volume was maintained by replenishing 2.0 mL of fresh dissolution medium at each sampling interval. Six replicates were tested for the dissolution tests.

Dissolution samples were filtered through a 0.2 μm membrane filter before appropriate dilution was carried out. The extent of sample dilution was adjusted to ensure that the concentrations of acrivastine and pseudoephedrine hydrochloride were always within the range of the calibration curves. Drug dissolution was calculated as the percentage dissolved at each sampling time by dividing the amount of each compound present in each sample by the average amount of each compound measured in the original capsules.

3. Results and discussion

3.1. Optimization of HPLC conditions

When the mobile phase was composed of only acetonitrile and acetate buffer, separation of both compounds was not adequately resolved, as acrivastine was eluted from the column too close to pseudoephedrine hydrochloride. The relative retention (α, acrivastine/pseudoephedrine hydrochloride) was less than 1.2 and the resolution (R) was less than 0.6. The addition of methanol to the mobile phase increased the retention time of acrivastine significantly, resulting in satisfactory separation of the two compounds. Changes in the pH of the acetate buffer were found to have no significant influence on chromatographic separation and peak detection. Using the current composition of the mobile phase (pH 4.0 acetate buffer–acetonitrile–methanol at 45:47:8) and a flow rate of 0.8 mL/min, the retention times for pseudoephedrine hydrochloride and acrivastine were 2.0 and 2.5 min, respectively. Both compounds eluted well and chromatographic separation was complete ($\alpha = 1.6$, $R = 1.5$). These conditions also yielded rapid analysis of the compounds, meeting the high-throughput requirement for routine daily quality control tests of acrivastine and pseudoephedrine hydrochloride. A typical chromatogram of acrivastine and pseudoephedrine hydrochloride is shown in Fig. 2A.

Various ultraviolet (UV) wavelengths had been used for the detection of acrivastine and pseudoephedrine hydrochloride. Using the photodiode array detector, it was possible to scan the entire UV spectrum of both test compounds from 200 to 400 nm. Acrivastine had absorbance peaks at 214, 260, and 320 nm, while pseudoephedrine hydrochloride has absorbance peaks close to 210 and 260 nm. The peak area of pseudoephedrine hydrochloride at 260 nm was only 5% of that at 214 nm. The peak area of acrivastine at 260 nm was 40% of that at 214 nm (Fig. 2B). Considering the fact that the amount of pseudoephedrine hydrochloride in the capsule preparation is significantly higher than that of acrivastine (60 mg pseudoephedrine hydrochloride versus 8 mg acrivastine), selecting optimal detection wavelength was mainly based on the detection sensitivity of acrivastine. Therefore, the wavelength of 214 nm provided significantly better detection sensitivity than 260 nm wavelength for both compounds. At this wavelength, the detection limit was 0.1 μg/mL for acrivastine and 0.2 μg/mL for pseudoephedrine hydrochloride, and the lowest quantitation limit was 0.2 μg/mL for acrivastine and 0.4 μg/mL for pseudoephedrine hydrochloride, respectively. No interference was found from any other formulation excipients or additives.

3.2. Assay calibration and validation

The linear concentration range of the assay was found to be 0.2–10.0 μg/mL for acrivastine and 0.4–50.0 μg/mL for pseudoephedrine hydrochloride, respectively. Based on the actual drug contents and optimal dilution pro-

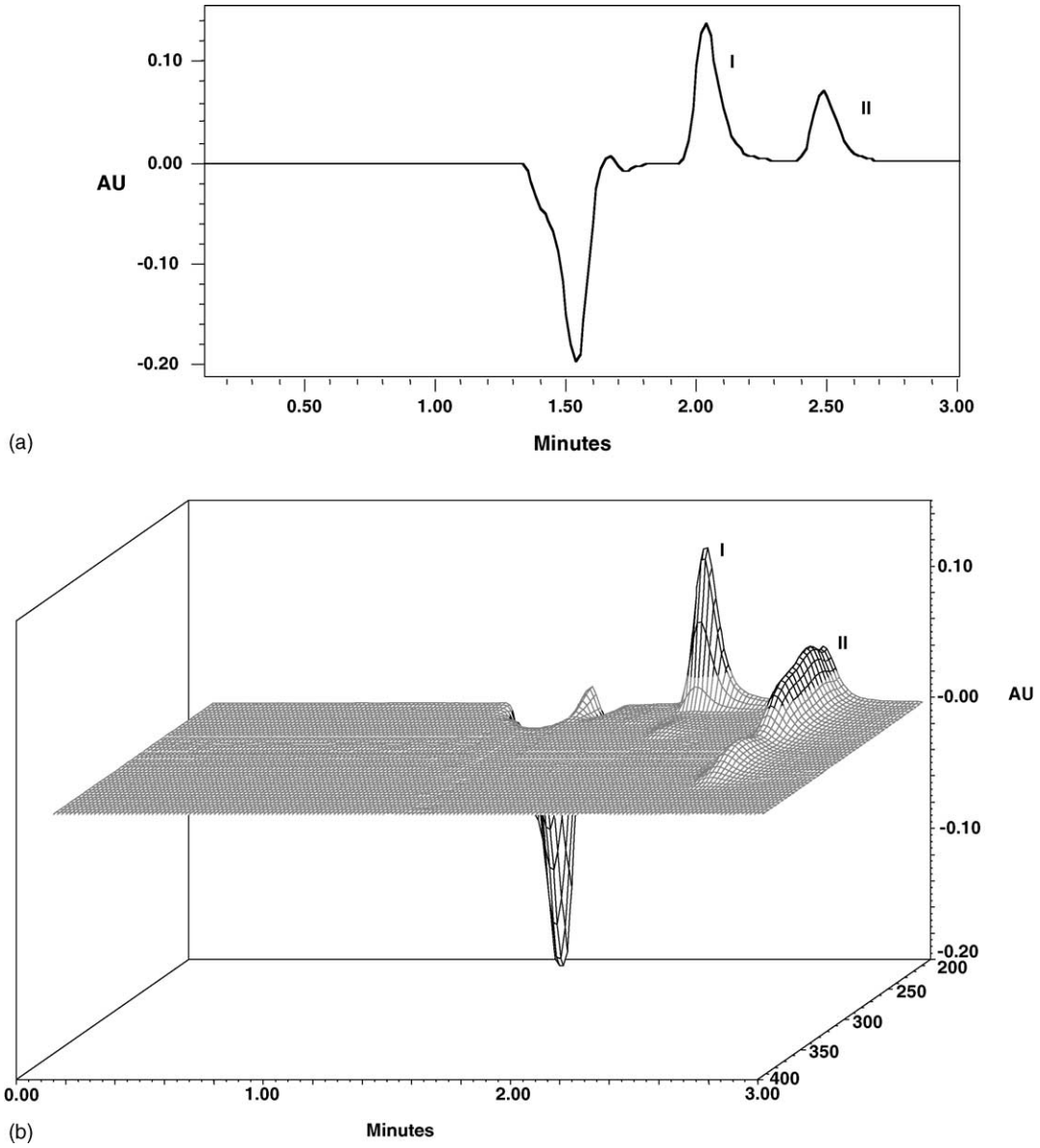

Fig. 2. Chromatograms and spectra of pseudoephedrine hydrochloride (I) and acrivastine (II).

cedures, the calibration concentration range was set at 0.4–4.0 μg/mL for acrivastine and 1.0–20.0 μg/mL for pseudoephedrine hydrochloride. Using this selected calibration concentration range, both compounds showed satisfactory linear correlations between the peak areas and the drug concentrations. The calibration curves for acrivastine and pseudoephedrine hydrochloride were $Y = 1.29 \times 10^5 X - 9.41 \times 10^3$ ($R^2 = 0.9993$, Y: concentration in μg/mL, X: peak area) and $Y = 4.79 \times 10^4 X - 4.19 \times 10^3$ ($R^2 = 0.9998$), respectively. No changes in the calibration equations were found over the course of 3 months.

Assay validation was carried out at three different levels to reflect the concentration range of the test compounds. Table 1 lists the accuracy and reproducibility results of the method validation. The range of accuracy of the assay was between 95 and 105% for both test compounds, which was considered acceptable for the assay. In addition, no significant variation among injections was found on the same test day and between two different test days, indicating satisfactory stability and reproducibility of the assay and the instrument.

3.3. Assay application

Acrivastine is only slightly soluble in water, while pseudoephedrine hydrochloride is readily soluble in water. Since drug dissolution and subsequent absorption take place in different sections of the gastrointestinal tract with variable pH values, the solubility of acrivastine at three different pH values was measured using this developed assay. Solubility of acrivastine in pH 1.0 medium (11.34 ± 0.11 mg/mL, mean ± S.D., $n = 4$) was significantly larger ($p < 0.05$) than that in pH 4.5 (0.85 ± 0.01 mg/mL) and

Table 1
Results of method validation

	Acrivastine			Pseudoephedrine hydrochloride		
Test concentrations	0.4	1.6	4.0	1.0	6.0	20.0
Mean	0.41	1.54	4.02	1.05	6.04	20.03
S.D.	0.01	0.01	0.01	0.02	0.05	0.08
C.V.	0.95	0.73	0.31	2.25	0.85	0.39
Accuracy (%)	102.5	96.3	100.5	105.0	100.7	100.2

Concentration: μg/mL; S.D.: standard deviation; C.V.: coefficient of variation (%); $n = 36$.

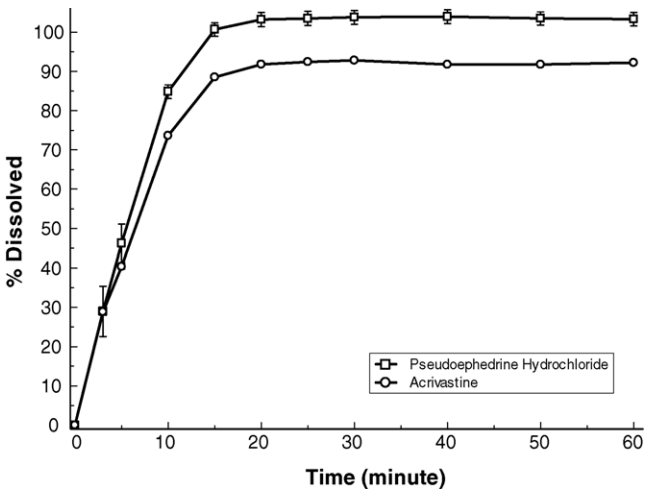

Fig. 3. Dissolution results of Semprex®-D in 0.1 M hydrochloric acid (pH 1.0).

pH 6.8 (0.70 ± 0.01 mg/mL) at room temperature. The assay was able to measure acrivastine accurately without pseudoephedrine hydrochloride. The presence of different buffer salts has no influence on the measurement of acrivastine.

Content of acrivastine and pseudoephedrine hydrochloride in Semprex®-D capsules was found to be $95.80 \pm 0.10\%$ and $97.30 \pm 0.50\%$ of the labeled drugs (mean ± S.D., $n = 6$), respectively. No interference was found from any excipients or additives present in the preparation.

Drug dissolution was carried out in simulated gastric fluid, as Semprex®-D is an immediate-release capsule preparation. Drug dissolution was complete within 30 min ($92.83 \pm 0.10\%$ dissolved for acrivastine and $103.81 \pm 1.75\%$ dissolved for pseudoephedrine hydrochloride at 30 min, based on the average drug content in the original capsules, mean ± S.D., $n = 6$), and the dissolution profiles of both compounds are shown in Fig. 3. Using this technique, sample preparation involved only direct dilution procedures and there was no interference from excipients or dissolution medium in the HPLC assay.

4. Conclusion

The HPLC method described for the simultaneous measurement of acrivastine and pseudoephedrine hydrochloride required no special reagents, and no pretreatment or extraction of the samples. Acrivastine and pseudoephedrine hydrochloride were well separated and the chromatographic runtime was 3 min, permitting a fast throughput of a large number of test samples. In conclusion, this method was accurate, specific, selective, rapid, and versatile for the simultaneous quantification of acrivastine and pseudoephedrine hydrochloride and would be suitable for use in routine quality control analyses.

Acknowledgement

The authors acknowledge the generous supply of acrivastine pure compound and Semprex®-D capsules by Celltech Pharmaceuticals.

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