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Quantification of antihistamine acrivastine in plasma by solid-phase extraction and high-performance liquid chromatography

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Abstract

An automated solid-phase extraction method was developed for the determination of the H_1-antihistamine acrivastine in plasma samples. Acrivastine was analyzed at the wavelength of 254 nm using a reversed-phase HPLC assay. Both extraction procedure and analytical condition were optimized and validated for maximum recovery and resolution. The developed method was further applied to plasma samples collected from an *in vivo* pharmacokinetic study in rabbits. The assay was found to be simple, specific, accurate and reproducible.

Keywords: Antihistamine acrivastine; Solid-phase extraction; HPLC; Method optimization and validation

1. Introduction

The number of reported cases of allergic disorders has risen substantially during the past quarter of a century. Increased amounts of air pollutants as well as natural environmental allergens, xenobiotics and stress have all been attributed to the increase in incidence of allergic diseases [1,2]. The severity of an allergic disorder is highly variable among the individuals, and may range from mild discomfort to significant impairment of the quality of life, in some rare cases, even leading to life-threatening conditions. Increases in allergic disorders have already had, and will continue to have a considerable impact on the healthcare resources. Common treatment of allergic disorders, especially allergic rhinitis and uticaria, includes the administration of H_1-antihistamines, medications that reduce or inhibit the release of histamine through negative modulation of the histaminic receptors [3].

Acrivastine [(E)-3-(6-[3-pyrrolidino-1-(4-tolyl)-prop-1E-enyl]-2-pyridyl)-acrylic acid] (Fig. 1A), is a second-generation, non-sedating antihistamine that was derived from the first-generation compound triprolidine (Fig. 1B) [4]. Compared to the earlier antihistamines, the second-generation compounds have been significantly improved pharmacologically and pharmacokinetically, so that they have exhibited better patient compliance and fewer adverse effects, in particular, lower effects on the central nervous system (e.g., sedation, impairment of psychomotor functions) [3,5]. Various antihistamines have been commercially available for clinical applications for decades; acrivastine (Semprex® and Semprex®-D) is one of them that is specifically used for the treatment of allergic rhinitis [6,7].

Numerous analytical methods have been reported to determine acrivastine in both pharmaceutical preparations and biological specimens. These included derivative spectrophotometry [8], high-performance liquid chromatography with ultraviolet detection (HPLC-UV) [9], gas chromatography with mass spectrometric detection (GC–MS) [10,11], radioimmunoassay [12], and electrochemical measurement [13]. We have recently reported an HPLC-UV method to simultaneously analyze acrivastine and pseudoephedrine hydrochloride in Semprex®-D capsules [14]. The assay was found to be accurate, specific, selective, rapid and versatile for use in routine quality control of acrivastine and pseudoephedrine preparations.

For pharmacokinetic and pharmacodynamic evaluation of any medication, a reproducible and simple separation method is a prerequisite for the generation of accurate and reliable study data. Solid-phase extraction has been exten-

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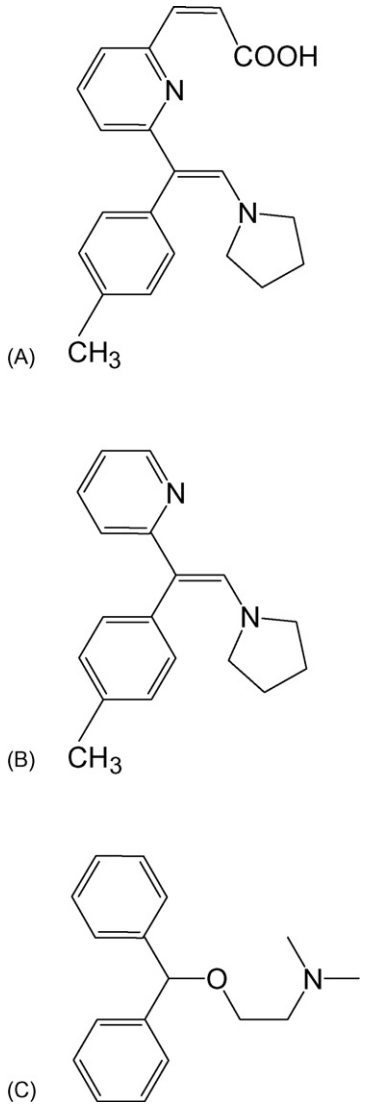

Fig. 1. Structure of relevant compounds: (A) acrivastine; (B) triprolidine; (C) diphenhydramine.

sively employed in the pharmaceutical and biomedical analysis, due largely to significant advantages and improvements in automation, reproducibility and high-throughput capability [15,16]. For acrivastine, however, previous extraction methods were mainly complex and time-consuming, which commonly involved several rounds of extraction and derivatization [11,12]. They are unable to meet the extensive requirements for investigational and regulatory purposes.

In this study, we developed a simple and reproducible solid-phase extraction procedure to separate acrivastine in the plasma and then used an HPLC-UV assay for the drug measurement. The method was optimized and validated to achieve high accuracy and reliability. The resultant approach was further employed to separate and analyze acrivastine concentrations in plasma samples collected from an *in vivo* pharmacokinetic study in a rabbit model.

2. Experimental

2.1. Chemicals

Acrivastine standard and Semprex®-D capsules were received as gifts from Celltech Pharmaceuticals (Rochester, NY, USA). Diphenhydramine (Fig. 1C), the internal standard, was obtained from Parke-Davis Co. Ltd. (Brockville, Ont., Canada). Concentrated acetic acid, acetonitrile, methanol and sodium acetate trihydrate were purchased from Fisher Scientific (Fair Lawn, NJ, USA). All chemicals and reagents used were either HPLC grade or AC grade. Deionized water was obtained from a Millipore® Milli-Q system (Bedford, MA, USA).

2.2. Standard solutions

Concentrated stock solutions containing 1 mg/mL of acrivastine and 1 mg/mL of diphenhydramine were accurately prepared using deionized water. Diphenhydramine was further diluted to a final concentration of 50 μg/mL, while acrivastine was diluted to make a series of standard solutions, at concentrations ranging between 0.4 and 25 μg/mL. All standard solutions were stored at 4 °C, and were replaced and recalibrated every 3 months. Calibration curves were obtained over the aforementioned range of concentrations for acrivastine by spiking blank plasma and correlating the linear relationship between the peak area ratio (acrivastine/diphenhydramine) and the concentration of acrivastine.

2.3. Extraction procedure

The previously-frozen plasma samples were thawed at the room temperature in a lukewarm water bath (30 °C) prior to the extraction. Once the samples had been fully thawed, 250 μL of plasma was pipetted into a test tube; added to this were 750 μL of deionized water and 100 μL of the internal standard. The spiked calibration curve contained acrivastine ranging between 0.4 and 25 μg/mL, by the replacement of 100 μL deionized water with the standard solutions. All samples were mixed for 10 min using a Baxter® multivortex shaker (Deerfield, IL, USA) to ensure the homogeneity of the solution.

The extraction procedure was completed with a Zymark® RapidTrace™ SPE Workstation (Hopkinton, MA, USA), controlled by RapidTrace™ Software in a notebook computer. The optimized automatic extraction procedure took 11 steps, including priming, extraction and separation, and required 12.4 min per extraction cycle. Briefly, Waters® Sep-Pak® Vac 3cc (500 mg) C_{18} cartridges (Milford, MA, USA) were first conditioned with 5 mL of methanol at a flow rate of 12 mL/min, followed by twice with 5 mL of deionized water at the same flow rate. After aspiration, the prepared samples (1.1 mL) were loaded separately onto each cartridge at a flow rate of 2 mL/min, allowing for partitioning of the compounds with the stationary phase. The cartridges were then consecutively washed with 2 mL of deionized water and 3 mL of 30% methanol solution at a flow rate of 8 mL/min. After complete drying of the cartridges, acrivastine and the internal standard were then eluted with 2 mL of methanol

at a flow rate of 2 mL/min, which was collected into a clean test tube.

Test tubes containing the methanol eluent were transferred to a heated water bath (50 °C) and dried under a gentle stream of nitrogen. The evaporation process required about 15–20 min to complete. The residue was reconstituted using 250 μL of mobile phase, vigorously votexed for 1 min, and then centrifuged at 13,800 rpm for 10 min (Hettich Zentrifugen Mikro 22R, Tuttlingen, Germany). The supernatant was transferred to an HPLC vial and an aliquot of 50 μL was injected for drug analysis. The final concentration of the internal standard in the assay was 20 μg/mL.

2.4. HPLC condition

The HPLC assay was modified from a previous assay method for simultaneous determination of acrivastine and pseudoephedrine developed in our laboratory [14]. Briefly, the Waters® component system (Milford, MA, USA) was comprised of a 600 S Controller, a 616 Solvent Delivery Pump, a 717 Autosampler, a 996 Photodiode Array Detector, and a C_{18} Nova-Pak® column (4 μm, 3.9 mm × 150 mm). The data were acquired and processed using the Millennium® 32 operating software. The mobile phase was a mixture of acetate buffer (0.12 M sodium acetate trihydrate, pH adjusted to 4.0 using concentrated acetic acid)/acetonitrile/methanol at a ratio of 45:47:8 (v/v/v), and was delivered at a flow rate of 0.75 mL/min. Both acrivastine and diphenhydramine were detected at the wavelength of 254 nm. Under the chromatographic condition, acrivastine and diphenhydramine were eluted from the column at 2.9 and 5.5 min, respectively; the quantification limit of acrivastine was 5 ng.

2.5. In vivo animal study

An *in vivo* animal study was carried out to examine the pharmacokinetic and pharmacodynamic effects of acrivastine, as well as to test the developed method for acrivastine extraction and quantification. The Animal Use Protocol was approved by the University of Manitoba, and the study was conducted according to current guidelines published by the Canadian Council for Animal Care (CCAC). Seven New Zealand white rabbits were each orally administered one Semprex®-D capsule (8 mg acrivastine and 60 mg pseudoephedrine hydrochloride) after a fast of 12 h overnight. Blood samples were collected from a venous catheter on the ear at 0, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 27 and 30 h after the administration. Plasma samples were separated from the blood, and acrivastine was extracted and analyzed using the method described above.

2.6. Data analysis

The extraction recovery and assay variation were calculated for the solid-phase extraction and the HPLC assay. The pharmacokinetic parameters of acrivastine were obtained by subjecting the plasma concentrations of acrivastine to a one-compartmental model using WinNonlin® software (Pharsight, Mountain View, CA, USA).

3. Results and discussion

3.1. Optimization of SPE procedure

To allow for high-throughput in pharmaceutical and biomedical analysis in support of pharmacokinetic evaluation or clinical trials of new chemical entities, a developed extraction method has to exhibit sufficient robustness and flexibility in its application. The advent of solid-phase extraction has greatly evolved in the past several years, gradually replacing traditional extraction approaches with liquid–liquid manipulation. In addition, solid-phase extraction is particularly advantageous and efficient over other separation techniques in automation, reproducibility and reliability. Currently, most solid-phase extraction procedures could be modified from three major steps; that is, conditioning of the cartridge, cleaning of the interfering biological components and eluting of the test compounds [17,18].

The extraction method used in this study focused optimization on cleaning of unwanted biological components in the plasma. Acrivastine is slightly soluble in water (0.70 mg/mL at pH 6.8); use of water as cleaning liquid should not significantly influence its partition between the stationary phase and the liquid phase. In order to separate lipophilic components in plasma from the test target, various combinations of methanol and water solution were evaluated for their extraction recovery and chromatogram cleanness. Table 1 lists results from four different combinations of methanol and water solution. It was found that optimal extraction recovery resulted from the combination of methanol and water at a ratio of 30:70 (v/v). The extraction also allowed for clean separation of acrivastine from pseudoephedrine hydrochloride present in Semprex®-D capsule, which was likely eluted during the cleaning procedures. The internal standard was also an antihistamine; its chromatographic profile was therefore compatible with that of acrivastine. No any other interference was found with the chromatographic properties. Because acrivastine is readily soluble in methanol, an absolute methanol wash was used to elute acrivastine and the internal standard. Methanol was also appropriate for evaporation and concentration at lower temperature, which did not compromise stability of the test compounds. Overall, the extraction was simple, straightforward, with only two solvent involvement and complete automation. In addition, this method could also

Table 1
Extraction recovery of acrivastine at different methanol percentages

	Methanol:water (v/v)			
	20:80	30:70	40:60	50:50
Recovery (%)	84.2	90.3	86.9	80.4
S.D.	0.51	0.57	0.59	0.39
C.V.	2.41	2.53	2.74	1.94

Test concentration: 25 μg/mL; S.D.: standard deviation; C.V.: coefficient of variation (%); $n = 18$.

be used for the extraction of diphenhydramine from biological specimens.

3.2. Assay calibration and validation

The HPLC assay that was previously developed for simultaneous determination of acrivastine and pseudoephedrine was found to be satisfactory for both acrivastine and diphenhydramine. Slight modification was needed to further improve the resolution and sharpness of the peaks in this study. Fig. 2 shows typical chromatograms of a blank plasma extract and a spiked sample of acrivastine and diphenhydramine. Both peaks were well separated, while retention time for the compounds also allowed for quick run of each injection. The detection wavelength was set at 254 nm to increase analytical sensitivity. The quantification limit was 5 ng for acrivastine, which was able to measure low drug concentrations found in pharmacokinetic and pharmacodynamic studies.

The linear concentration range of the assay was found to be 0.4–25 μg/mL for acrivastine. Using this selected calibration concentration range, the calibration curve was $Y = 3.14 \times 10^3 X + 3.77 \times 10^4$ ($R^2 = 0.996$, Y: peak area ratio, X: concentration of acrivastine, $n = 9$). The calibration curve proved to be robust and stable; no changes were found over the duration of the study. Assay validation was carried out by spiking blank plasma samples at six different concentrations across the calibration range and checking for reproducibility of the extraction procedure and the assay. Table 2 lists the results from the validation study. The range of accuracy of the method was found to be between 91% and 106% for acrivastine, which was considered acceptable for the assay. No significant variation among injections was found on the same test day (coefficient of variation 0.011) and between 2 different days (coefficient of variation 0.024), indicating satisfactory stability and reproducibility of the SPE extraction and the HPLC analysis.

Table 2
Results of assay validation

	Test concentration					
	3.0	4.2	8.4	12.0	16.2	24.0
Mean	2.78	3.84	8.90	11.88	15.54	22.65
S.D.	0.32	0.12	0.20	0.42	0.29	0.53
C.V.	11.34	3.15	2.23	3.51	1.86	2.34
Accuracy (%)	92.7	91.4	105.9	99.0	95.9	94.4

Concentration: μg/mL; S.D.: standard deviation; C.V.: coefficient of variation (%); $n = 6$.

3.3. Assay application

Seven rabbits (2.7 ± 0.1 kg) were used in the animal studies, and 12 blood samples were collected from each animal. Concentrations of acrivastine from these plasma samples were extracted and analyzed using the described method. Fig. 3 shows the plasma–time curve of acrivastine in the rabbits. Table 3 lists primary pharmacokinetic parameters of acrivastine in rabbits from a one-compartmental model. Acrivastine exhibited a fast onset of action as well as a relatively short elimination half-life in the animals. However, its skin wheel suppression effect lasted for up to 12 h after a single dose of oral administration (data not shown). The pharmacokinetic parameters obtained from this

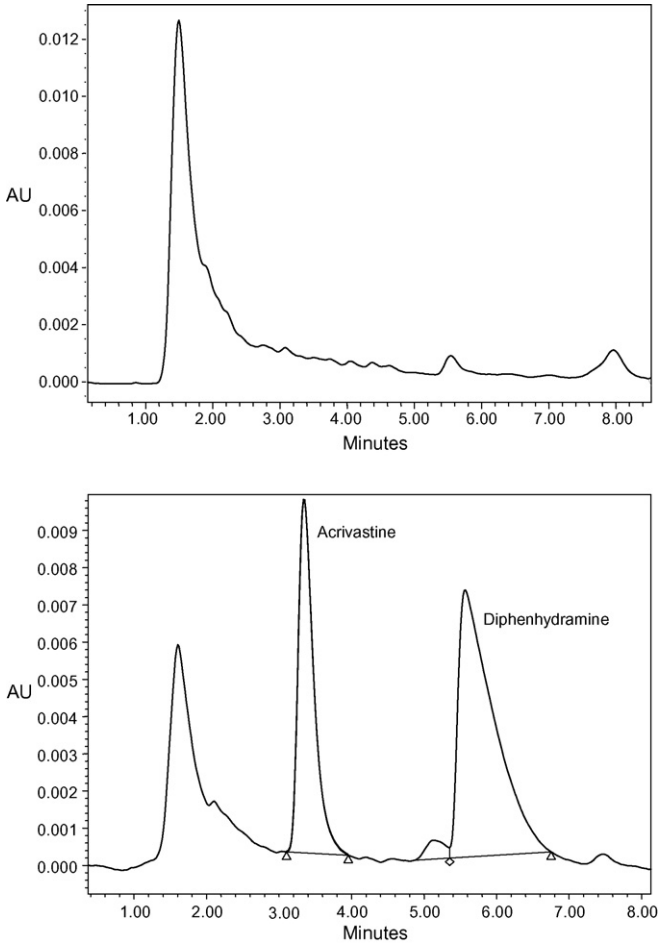

Fig. 2. Representative chromatograms of the blank extract (top) and the test compounds in spiked plasma (5.0 μg/mL) (bottom).

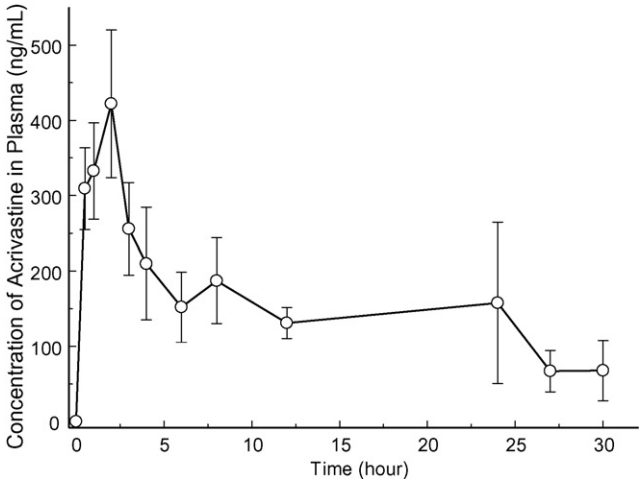

Fig. 3. Plasma concentration–time curve of acrivastine in rabbits (mean ± S.E.M., $n = 7$).

Table 3
Pharmacokinetic parameters of acrivastine in rabbits

$t_{1/2}$ (h)	6.08 ± 1.38
C_{max} (ng/mL)	479.53 ± 110.73
T_{max} (h)	1.19 ± 0.20
Cl (mL/min kg)	16.09 ± 2.51
V_d (L/kg)	7.51 ± 1.64
AUC (μg h/mL)	4.1 ± 1.2

Mean ± S.E.M., $n = 7$.

study were comparable to those published in other references [9,12]. Nevertheless, the solid-phase extraction and the HPLC assay described in this study were significantly improved to allow for simple, automatic and reliable analysis of acrivastine in large numbers of biological samples.

4. Conclusion

The automated solid-phase extraction process that was developed in this study was simple, accurate and reproducible. With the help of an automated computer-controlled program, the extraction process required only minimal sample pretreatment and achieved satisfactory extraction recovery. The method was capable of eliminating unintentional human errors in the operation and facilitating high throughput with a large number of biological samples.

The HPLC assay that was validated for the measurement of acrivastine in plasma samples was also accurate, specific, selective and reproducible. The method was able to aid in understanding the pharmacokinetics and pharmacodynamics of the antihistamine acrivastine in an animal model.

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