Chromatographic Separation and Spectroscopic Characterization of the E/Z Isomers of Acrivastine

PRAKASH M. DAVADRA,[1]* BATUK DABHI,[1] MANOJ K. SINGH,[2] MUKUL R. JAIN,[3] HITENDRA S. JOSHI,[4] AND ATUL H. BAPODRA[5]

[1]*Department of Biopharmaceutics, Zydus Research Centre, Ahmedabad, Gujarat, India*
[2]*Department of Strategic Technology Development, Zydus Research Centre, Ahmedabad, Gujarat, India*
[3]*Department of Pharmacology and Toxicology, Zydus Research Centre, Ahmedabad, Gujarat, India*
[4]*Department of Chemistry, Saurashtra University, Rajkot, Gujarat, India*
[5]*Department of Chemistry, M. D. Science College, Porbandar, Gujarat, India*

ABSTRACT A reverse phase high performance liquid chromatography (HPLC) method has been developed for the separation of two geometric isomers of Acrivastine using crude reaction mixture. The resolution between two isomers was found more than 2.9. The geometric isomers have been isolated by preparative HPLC and characterized by spectroscopic techniques, such as NMR, infrared, and MS. The developed method has been validated for the determination of *Z*-isomer in Acrivastine. The limit of detection and limit of quantification of the *Z*-isomer were 0.05 and 0.2 μg/ml, respectively. The developed method is precise, linear, accurate, rugged and robust for its intended use. *Chirality 23:955–960, 2011.*

KEY WORDS: Acrivastine; geometric isomers; E/Z isomers; HPLC; validation

INTRODUCTION

Acrivastine, (*E,E*)-3-{6-[1-(4-methyl-phenyl)-3-(1-pyrrolidinyl)-1-propenyl]-2-pyridinyl}-2-propenoic acid (Fig. 1a), is a potent histamine H1-receptor antagonist derived from triprolidine.[1] It has rapid onset of action and has a low potential to penetrate the central nervous system.[1] Acrivastine has shown to be an effective and a well-tolerated antihistamine in the treatment of chronic urticaria and allergic rhinitis.[2]

A literature review revealed that Acrivastine has been determined and studied by several procedures; gas chromatography mass-spectrometric analysis,[3] a sensitive radioimunoassay to measure plasma levels,[4] nondirect spectrofluorimetric method[5] and several spectrophotometric procedures to measure acrivastine in urine and pharmaceuticals.[5–8] The high performance liquid chromatography (HPLC) methods have also been reported for the determination of acrivastine in capsules using ultraviolet detection.[8,9] There are reports for the determination of the Acrivastine in human urine and pharmaceutical by spectroflourimetric method[10] and by electrochemical method.[11]

Acrivastine (Fig. 1a) has two unsaturated bonds, one at the 2-position of propenoic acid and second at 1-position of the propene attached to 6-position of pyridine ring. All the four substituents at both double bonds are different, therefore four geometric isomers are possible, i. e., *(E, E)*, *(E, Z)*, *(Z, E)*, and *(Z, Z)*. Since, during the synthesis of Acrivastine the unsaturated bond at 2-position of propanoic acid is constructed by "Heck reaction" and the Heck reaction is known for the generation of trans geometry predominantly. Therefore, configuration of the unsaturated bond at 2-position of propenoic acid is "*E*", while the configuration of the unsaturated bond at 1-position of propene attached to 6-position of pyridine ring may "*E*" or "*Z*." The geometry of one of the double bond is fixed, because of extensive precedences and mechanism of the Heck reaction and the second double bond was formed in propane moiety due to dehydration (elimination reaction). Therefore, two geometric isomers *(E, E)* and *(E, Z)* are possible during synthesis, the scheme and mechanism for the formation of both the isomers are presented in Figure 2.

The isomer *(E, E)* is desired isomer (Acrivastine) as a drug, while *(E, Z)* isomer is undesired. Therefore, it is necessary to monitor the amount of undesired isomer present in Acrivastine, for which suitable, precise and accurate chromatographic method is needed. Now onward, we refer undesired *(E, Z)* isomer, as a "*Z*-isomer" of Acrivastine. The structure of the "*Z*-isomer" is shown in Figure 1b.

As, we can see in the preceding literature review, there is no HPLC method reported for the separation of isomeric impurity, i.e., *Z*-isomer of Acrivastine from the Acrivastine. The aim of this work was to devise a suitable separation method and subsequently validate it. This work deals with the systematic method development, isolation and characterization of the *Z*-isomer and Acrivastine, determination of relative response factor (RRF) of *Z*-isomer and method validation.

MATERIALS AND METHODS

Chemicals

Crude reaction mixture containing Acrivastine and its *Z*-isomer was supplied by Department of Strategic Technology Development of Cadila Healthcare, Ahmedabad, India. HPLC grade acetonitrile and methanol were purchased from S.D.Fine, India. AR grade Di-sodium hydrogen phosphate, Phosphoric acid and Potassium bromide were purchased from Merck, India. Triflouro acetic acid and Deuteriated methanol were purchased from Sigma Aldrich, Germany.

Analytical HPLC Method

Analytical HPLC method was developed using Agilent 1100 series (Germany) HPLC system equipped with degasser auto sampler, auto injector, thermostatic compartment, and photo diode array (PDA) detector.

*Correspondence to: Prakash Madhavjibhai Davadra, Department of Biopharmaceutics, Zydus Research Centre, Sarkhej-Bavla N. H. No. 8A, Moraiya, Ahmedabad 382 213, Gujarat, India.
E-mail: prakashdavadra@zyduscadila.com or prakashdavadra@yahoo.co.in
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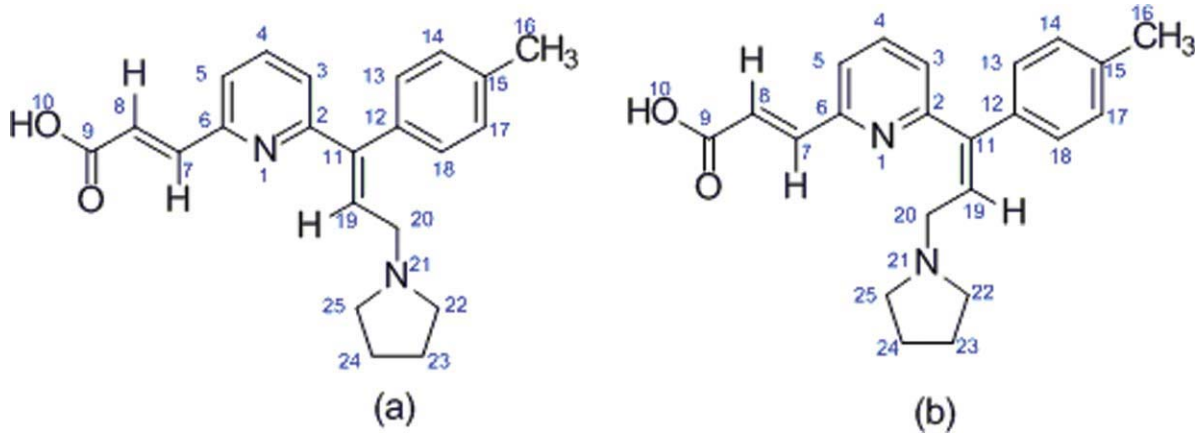

Fig. 1. Chemical structure (a) Acrivastine (b) *Z*-isomer of Acrivastine. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The out put signal was monitored and processed using Agilent Chemstation software. The Cosmosil packed column, 5C18-PAQ (250 × 4.6 mm, 5 μm) was used in isocratic mode using mobile phase consisting of 0.05% triflouroacetic acid in water-acetonitrile (72:28, v/v) for the separation. The flow rate was set at 1.0 ml/min. The column was maintained at 30°C, and the detection was carried out at a wavelength of 229 nm. The

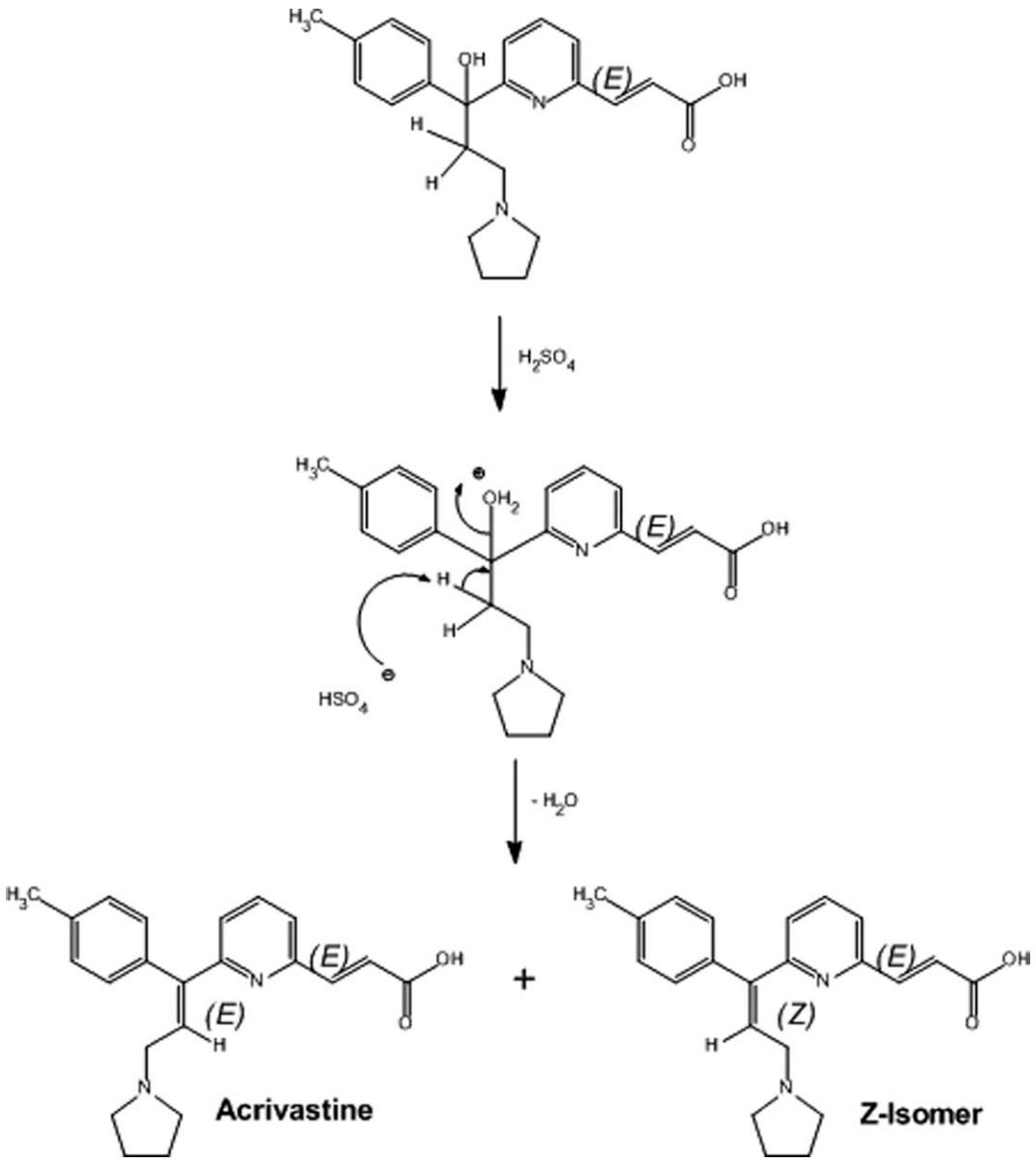

Fig. 2. Scheme and mechanism for the formation of both isomers.

injection volume was 5 μl. The sample was prepared in water-acetonitrile (70:30, v/v) at a concentration 0.5 mg/ml.

Preparative HPLC Method

Preparative HPLC system used was Shimadzu LC-8A with ultraviolet (UV) detector. Data was processed through Class-VP software. YMC make, DAC (250 × 50 mm) self-packed column and ODS-AQ media with 10 μm particle size were used for the preparative work.

The mobile phase consisting of 0.05% triflouroacetic acid in water-acetonitrile (80:20, v/v) was flowed at 80 ml/min for ~40 min. The detector was operated at 229 nm. Two to five ml of DMF was used to dissolve crude reaction mixture.

Mass Spectrometry

The isolated compound was dissolved (about 100 ppm) in methanol and injected (2 μl) through liquid chromatography with methanol-water (90:10, v/v) at a flow rate 0.3 ml/min. Waters Quatromicro MSMS instrument was operated in electrospray ionization (ESI) positive mode with capillary voltage 3.5 KV, cone voltage 20V, source temperature 120°C, desolvation temperature 400°C, and desolvation gas flow 800 l/h.

Infrared Spectroscopy

The infrared (IR) spectra were recorded in solid state as KBr dispersion using Shimadzu 8400S FT-IR, Japan.

Nuclear Magnetic Resonance

^{1}H and ^{13}C NMR spectra of isolated Acrivastine and its *Z*-isomer was recorded on 400MHz NMR spectrometer, Bruker (Germany). The probe was a 5 mm PABBO *z*-gradient probe. Spectra were recorded in deuteriated chloroform (5-mm tubes) at 298 K. Sample concentration was 20 mg in 0.5 ml. The residual protonated resonance of the solvent (deuteriated chloroform) was used as an internal chemical shift standard, which was related to tetramethylsilane with chemical shifts of 7.2 and 77 ppm, respectively, for ^{1}H and ^{13}C. Processing of the raw data were performed using Topspin NMR software. The pulse conditions were 12.75μs (attenuation 0 db) for ^{1}H and 10.20μs (attenuation −1.0db) for ^{13}C.

RRF

The standard sample solution having 5 ppm concentration of Acrivastine and its *Z*-isomer was used to determine RRF. The average area of five replicate injections was used for calculation.

Method Validation

The mixture containing equal quantity of Acrivastine and its *Z*-isomer was injected in the equilibrated chromatographic system. The system suitability parameters such as, resolution (Rs), symmetry (S), retention factor (k), separation factor (α), and number of theoretical plates (N) were obtained. By injecting both the isomers separately, specificity and peak purity of the method were also confirmed. The precision of the method was checked by analyzing six replicate samples of Acrivastine (at 0.5 mg/ml) spiked with 0.2% of *Z*-isomer and calculating the percentage relative standard deviation (RSD) of retention time and peak area. The intermediate precision was also evaluated at different day by performing six successive injections.

Limit of detection (LOD) and Limit of quantification (LOQ) were determined at a signal-to-noise ratio of 3 and 10 respectively. LOD and LOQ were achieved by injecting a series of dilute solutions of *Z*-isomer. The precision and accuracy for *Z*-isomer at LOQ was checked.

Detector response linearity was assessed by six-point calibration of *Z*-isomer covering from 0.2 (LOQ level) to 3.0 ppm (0.2, 0.5, 1.0, 1.5, 2.0, and 3.0). The regression curve was obtained by plotting peak area versus concentration, using the least squares method. Linearity was checked for three consecutive days in the same concentration range from the same stock solution. The percentage RSD of the slope and Y-intercept of the calibration curve was calculated.

The standard addition and recovery experiments were conducted to demonstrate accuracy of the method. The study was carried out in triplicate for the determination of recovery of *Z*-isomer at 50, 100, and 150% level with respect to the specification limit for *Z*-isomer, i.e., 0.2%. The recovery of *Z*-isomer was calculated from the slope and Y-intercept value obtained in the linearity study.

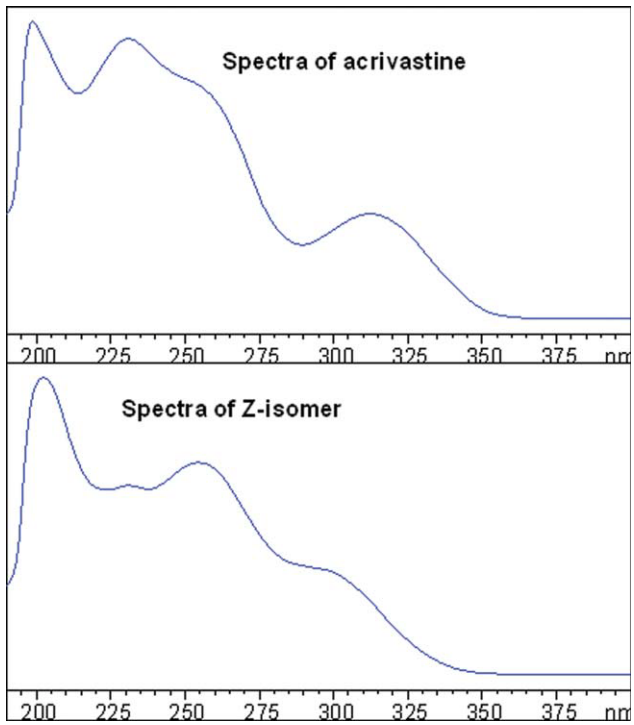

Fig. 3. UV spectra obtained from PDA detector. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The robustness of a method was determined by altering experimental conditions and chromatographic resolution between Acrivastine and its *Z*-isomer was evaluated. To study the effect of flow rate, mobile phase composition and column temperature on the resolution, it was changed by varying 0.1 units, −1 to +1%, and −2 to +2 °C, respectively while the other chromatographic parameters were held constant as stated in section "Analytical HPLC method".

Stability of solution at analyte concentration was studied by keeping the solution in tightly capped volumetric flask at room temperature on a laboratory bench for 2 days. Area of *Z*-isomer was checked for 12 h interval upto the study period.

RESULTS AND DISCUSSION

Method Development

The crude reaction mixture containing both isomers was used for method development. To develop a suitable and precise LC method for the separation of Acrivastine and its *Z*-isomer, different mobile phases and columns were employed to achieve adequate separation and resolution. Finally, the mobile phase consisting of 0.05% triflouroacetic acid in water-acetonitrile (72:28, v/v) at a flow rate 1.0 ml/min using Cosmosil, 5C18-PAQ (250 × 4.6 mm, 5 μm) column was found to be appropriate, allowing good separation of Acrivastine and its *Z*-isomer. The wavelength of detection was selected 229 nm based on the UV profile (Fig. 3) of both the isomers. In the present method, selectivity was found 1.13 with resolution more than 2.9. The chromatogram is shown in Figure 4c. The columns were tried namely, J'sphere-ODS, YMC-C_8, Kromasil C_{18} and YMC, ODS-AQ, also showed separation of Acrivastine and its *Z*-isomer but peak shape, peak symmetry, sharpness, ideal retention and resolution were found to be optimal in selected column, i.e., Cosmosil, 5C18-PAQ.

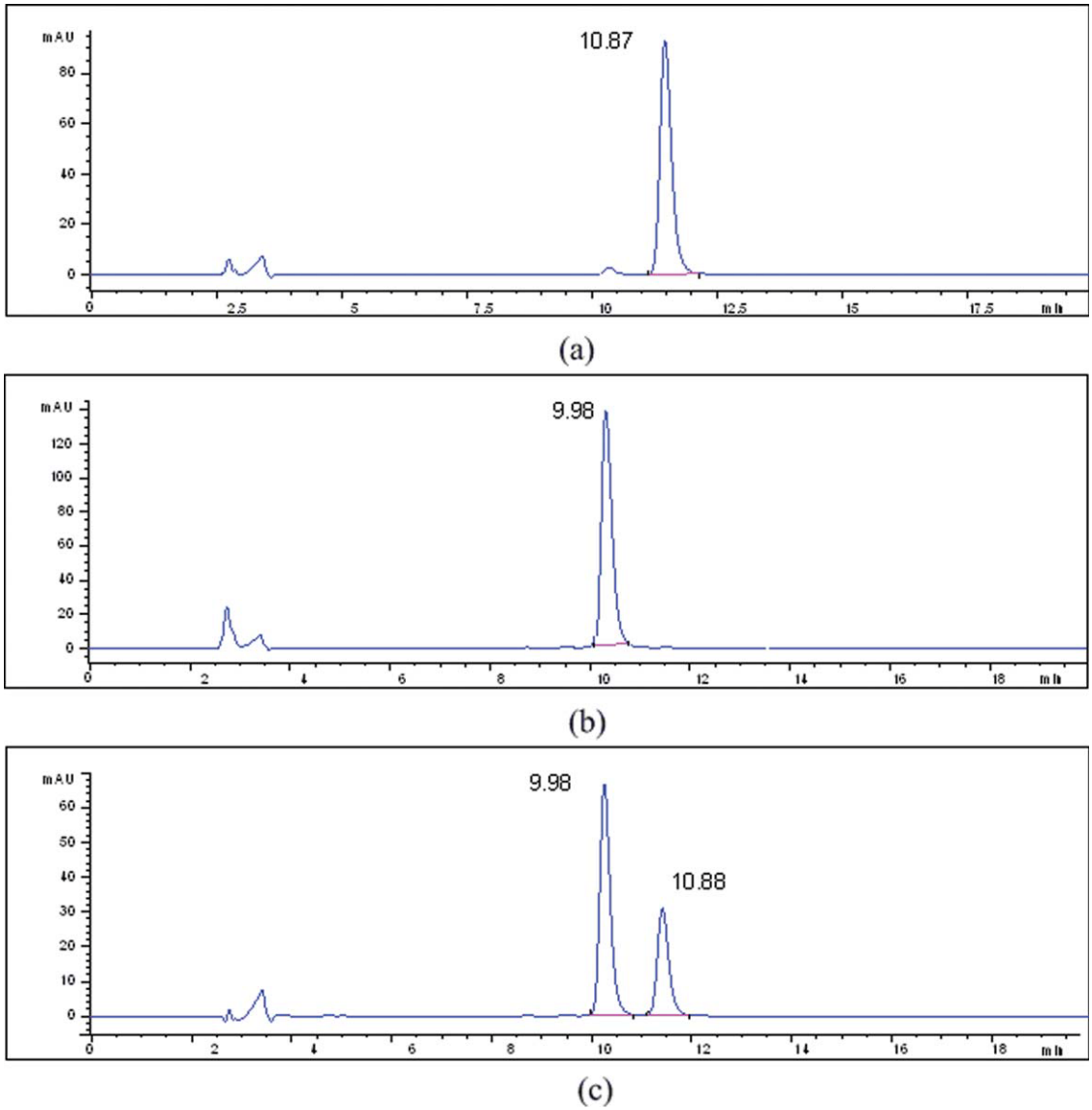

Fig. 4. Chromatogram of specificity (a) Acrivastine (b) *Z*-isomer of Acrivastine, and (c) mixture of both isomers. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Preparative HPLC Isolation

Acrivastine and its *Z*-isomer were isolated by preparative HPLC using the conditions described in experimental section. Purity of the collected fractions was monitored by analytical HPLC, the fractions having purity <99% were repurified. All the fractions with purity >99% were mixed and concentrated separately for both the isomers under vacuum on a rotavapor and the organic solvent was stripped off. The aqueous was subjected to lyophilization separately. After 2 days drying white solid material with purity, >99% was obtained for both the isomers. The isolated compounds were subjected to spectroscopic characterization.

Characterization

Acrivatine and its *Z*-isomer were analyzed by HPLC and the retention time and UV spectrum obtained in the PDA detection was matched with that of crude reaction mixture for identification. The purity of the Acrivatine and its *Z*-isomer was found to be 99.26 and 99.02% respectively. The mass spectrum showed peak at 349.56 m/z corresponds to the M+1 in ESI positive mode, which confirmed molecular weight of both isomers.

The presence of the different functional group was confirmed by IR spectroscopy. Thus, α,β-unsaturated carboxylic acid appeared at 3412 cm^{-1} in Acrivastine as broad band, whereas in its *Z*-isomer, it appeared at 3452 cm^{-1}. Similarly, the carbonyls in both cases appeared at 1682 and 1693 cm^{-1} respectively. The IR bands observed at different wave number are presented in Table 1.

The widely used and acceptable technique for the structure elucidation and confirmation is NMR. The signals observed in ^{1}H and ^{13}C NMR are complied in Tables 2 and 3, respectively for both isomers and assignment was validated by 2D NMR data of Heteronuclear Multiple Quantum Correlation (HMQC) and ^{1}H-^{1}H COSY. Being regioisomers, Acrivastine and its *Z*-isomers, by and large show similar ^{1}H and ^{13}C-NMR patterns. Both provide complete accountability for

TABLE 1. FT-IR spectral data

Compound	IR (KBr, cm^{-1})
Acrivastine	3412, 2916, 1682, 1639, 1564, 1444, 1346, 1211, 1155
Z-isomer	3452, 2922, 1693, 1632, 1566, 1435, 1302, 1200, 1134

every proton and carbon via their δ values, types of protons, carbons, multiplicity patterns, integration, and nature of carbons (methyl, methylene, methane, and quaternary) via DEPT experiments. The differences are due to their geometric isomer nature in ^{1}H-NMR, deshielding of H_{19} at 7.61 δ ppm is obvious in *E*-isomer, i.e., Acrivastine due to intra molecular H-bonding, whereas it is shielded at 6.15 δ ppm in *Z*-isomer. Similar effects of shielding (3.55 δ ppm for CH_2, H_{20}) in *E*-isomer and deshielding (4.26 δ ppm) of *Z*-isomer are noteworthy.

From the spectroscopic data of Mass, IR, and NMR (^{1}H, ^{13}C, and DEPT, HMQC and ^{1}H-^{1}H COSY) confirmed the structure of both the isomers and the values for ^{1}H-NMR, ^{13}C-NMR and Mass agree are with assigned chemical structure.

Result of RRF

Since, two different compounds may have different UV profile, therefore, detection of them at single wavelength will not give equal response. To calculate exact amount of impurity, RRF is required. Here, response factor of *Z*-isomer relative to Acrivastine was found to be 0.57. The weight percentage of the *Z*-isomer present in Acrivastine sample was calculated using its RRF value and peak response.

TABLE 2. ^{1}H and ^{13}C assignments for Acrivastine

Position[a]	^{1}H	δ (ppm)	J (Hz)	^{13}C	DEPT
1	–	–	–	–	–
2	–	–	–	155.83	
3	1H	7.18/d or 6.64/d	7.2	121.72	CH
4	1H	7.44/t	7.6	136.80	CH
5	1H	7.18/d or 6.64/d	7.2	122.59	CH
6	–	–	–	153.91	–
7	1H	7.59/d	15.6	139.24	CH
8	1H	7.39/d	15.6	129.19	CH
9	–	–	–	172.81	–
10	–	–	–	–	–
11	–	–	–	143.94	–
12	–	–	–	134.41	–
13	1H	7.07/d	8.0	129.56 or 129.58	CH
14	1H	7.26/d	7.6	129.56 or 129.58	CH
15	–	–	–	137.62	–
16	3H	2.42/s	–	21.33	CH_3
17	1H	7.26/d	7.6	129.56 or 129.58	CH
18	1H	7.07/d	8.0	129.56 or 129.58	CH
19	1H	7.61/t	7.2	124.97	CH
20	2H	3.55/d	7.2	53.81	CH_2
21	–	–	–	–	–
22	2H	3.07/bs	–	52.50	CH_2
23	2H	1.94/bs	–	23.41	CH_2
24	2H	1.94/bs	–	23.41	CH_2
25	2H	3.07/bs	–	52.50	CH_2

[a]Refer structural formula (Fig. 1a) for numbering; s, singlet; d, doublet; t, triplet; q, quartet; m, multiple; b, broad.

TABLE 3. ^{1}H and ^{13}C assignments for *Z*-isomer

Position[a]	^{1}H	δ (ppm)	J (Hz)	^{13}C	DEPT
1	–	–	–	–	–
2	–	–	–	157.49	–
3	1H	7.08/bd or 7.59/bd	7.6 or 15.6	126.35 or 123.93	CH
4	1H	7.70/t	7.6	137.69	CH
5	1H	7.08/bd or 7.59/bd	7.8	126.35 or 123.93	CH
6	–	–	–	152.36	–
7	1H	7.75/d	15.6	143.92	CH
8	1H	7.02/d	15.6	122.97	CH
9	–	–	–	168.86	–
10	–	–	–	–	–
11	–	–	–	147.81	–
12	–	–	–	137.42	–
13	1H	7.18–7.14/m	–	129.51 or 128.31	CH
14	1H	7.18–7.14/m	–	129.51 or 128.31	CH
15	–	–	–	139.62	–
16	3H	2.37/s	–	21.34	CH_3
17	1H	7.18–7.14/m	–	129.51 or 128.31	CH
18	1H	7.18–7.14/m	–	129.51 or 128.31	CH
19	1H	6.15/t	7.2	119.95	CH
20	2H	4.26/d	7.2	53.19	CH_2
21	–	–	–	–	–
22	2H	3.84/bs and 3.12/bs	–	52.83	CH_2
23	2H	2.11/bd	–	23.87	CH_2
24	2H	2.11/bd	–	23.87	CH_2
25	2H	3.84/bs and 3.12/bs	–	52.83	CH_2

[a]Refer structural formula (Fig. 1b) for numbering; s, singlet; d, doublet; t, triplet; q, quartet; m, multiple; b, broad.

Validation Results of the Method

The system suitability results are summarized in Table 4, which showed that resolution between acrivastine and its *Z*-isomer was not less than 2.9. Peak purity of acrivastine and its *Z*-isomer was not less than 0.999/990 using PDA detector. The chromatograms for specificity of the peak of acrivastine and its *Z*-isomer were shown in Figure 4.

In the precision study, the percentage RSD for retention time and peak area were presented in Table 5. In the intermediate precision study, results showed that % RSD values were in the same order of magnitude than those obtained for precision study (Table 5). The percentage bias between original conditions and changed conditions was less than 1% for average retention time and average peak area of both isomers (Table 5).

The LOD and LOQ concentration were estimated to be 0.05 and 0.2 ppm for *Z*-isomer, when the signal-to-noise ratio of 3 and 10 were used as the criteria. The method precision for *Z*-isomer at LOQ was 2.7% RSD. The recovery of *Z*-isomer at LOQ was 106.5% in the spiked Acrivastine sample. It can be said that LOQ value of the *Z*-isomer is quite law than the specification limit.

TABLE 4. System suitability data

System suitability parameters	*Z*-isomer (n =3)	Acrivastine (*n* = 3)
Retention factor (k)	2.54	2.87
Selectivity (α)		1.13
Resolution (Rs)	2.98	
Symmetry (S)	0.78	0.80
Theoretical plates (N)	10766	12297

TABLE 5. Results of precision and intermediate precision study

Validation parameter	Results	
	Acrivastine	*Z*-isomer
Precision (n = 6, % RSD)		
Retention time	0.08	0.07
Peak area	0.32	1.29
Intermediate precision (n = 6, % RSD)		
Retention time	0.09	0.08
Peak area	0.19	1.12
% Bias (average retention time)	−0.10	0.00
% Bias (average peak area)	0.14	0.92

The described method was linear in the range of 0.2 (LOQ level) to 3.0 ppm for *Z*-isomer. Plotting the peak area versus its corresponding concentration with a correlation coefficient of 0.999 drew the calibration curve. The equation of the calibration curve for *Z*-isomer was $y = 12.1937x + 1.1019$. Linearity was checked for *Z*-isomer over the same concentration range for three consecutive days. The percentage RSD of the slope and Y-intercept of the calibration curve were 1.08 and 7.79, respectively.

To determine the accuracy of the method standard addition and recovery experiments were conducted in triplicate at 50, 100, and 150% level of *Z*-isomer with respect to specification limit, i.e., 0.5, 1, and 1.5 ppm of *Z*-isomer. The recovery was calculated from slope and Y-intercept of the calibration curve obtained in linearity study and percentage recovery was ranged from 97 to 100 (Table 6).

The chromatographic resolution between both isomers was used to evaluate the method robustness under modified conditions. The resolution between acrivastine and its *Z*-isomer was found 2.98 in original condition. There was no major effect observed on resolution, under all the deliberately changed conditions (Table 7), demonstrating sufficient robustness.

No significant change in the content of *Z*-isomer was observed during solution stability experiments. Hence, sample solution is stable for at least 2 days.

CONCLUSIONS

A simple, rapid, robust, suitable, precise, and an accurate HPLC method has been developed for the determination of *Z*-isomer in Acrivastine. The RRF was determined for *Z*-isomer. The method was extended to preparative HPLC isolation, and both the isomers were isolated with HPLC purity more than 99%. The isolated compounds were completely characterized by ^{1}H, ^{13}C-NMR and DEPT, HMQC, ^{1}H-^{1}H COSY, IR, UV, Mass, and HPLC. The spectroscopic difference between acrivastine and its *Z*-isomer was discussed.

TABLE 6. Recovery results of *Z*-isomer

Added (ng)	Recovered (ng)	% Recovery	% RSD
500	486	97.2	2.58
1000	976	97.6	0.84
1500	1493	99.5	1.10

n = 3 determinations.

TABLE 7. Results of robustness study

Parameter	Resolution between Acrivastine and its *Z*-isomer and impurity-B (n = 3)
Flow rate (ml/min)	
0.9	3.09
1.0	2.98
1.1	2.83
Mobile phase composition (0.05% TFA:Acetonitrile, v/v)	
71:29	2.79
72:28	2.98
73:27	3.21
Column temperature (°C)	
28	3.05
30	2.98
32	2.84

The developed method was completely validated for the determination of the *Z*-isomer in Acrivastine with respect to specificity, system suitability, linearity, LOD and quantitation, accuracy, precision, robustness, intermediate precision, and solution stability. The result of validation showed satisfactory data for all the method validation parameters tested. The developed method can be used for the determination of *Z*-isomer in Acrivastine with LOD 0.05 ppm level.

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