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Research Article

DEVELOPMENT AND VALIDATION OF LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETER METHOD FOR THE ESTIMATION OF ACRIVASTINE IN HUMAN PLASMA

SNEHALATHA B[1]*, GOWRI SANKAR D[2]

[1]Department of Quality Assurance, Oriental College of Pharmacy, Sanpada, Navi Mumbai - 400 705, Maharashtra, India. [2]Department of Quality Assurance, Andhra University, Vishakhapatnam, Andhra Pradesh, India. Email: bslatha2003@gmail.com

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ABSTRACT

The objective of this study was to validate a simple, specific, accurate and precise solid phase high performance liquid chromatographic method with tandem mass spectrometry method for the determination of acrivastine (AC) in human plasma using pirfenidone as internal standard (ISTD). The precision and accuracy data have to fulfill the requirements for quantification of the analytes in biological matrices to generate data for bioequivalence and bioavailability investigations. A peerless basic C18, 5 µ column having 4.6 mm×100 mm internal diameter in binary gradient mode with flow rate was 0.8 mL/minutes of mobile phase containing methanol and 2 mm ammonium formate were used. The chromatographic separation was achieved by using elution solution consisting of methanol and 2 mm ammonium formate (70:30%, v/v)], diluent solution of methanol and water (50:50%, v/v)] were monitored on a triple quadrupole mass spectrometer, operating in the multiple reaction monitoring mode. The method was validated over the concentration range 0.5-205.170 ng/mL. Limit of detection and limit of quantification were found 0.18 ng and 0.536 ng, respectively. The retention time for AC and ISTD were 2 minutes and 2.4 minutes respectively and overall chromatography run time was 3.2 minutes. The mean recovery of AC (100.0%) and ISTD (102.5%) from spiked plasma samples was consistent and reproducible. The method was validated for linearity, accuracy, precision, specificity, limit of detection, limit of quantification and robustness. The intra- and inter-day precision and accuracy values were found to be within the assay variability limits as per the food and drug administration guidelines.

Keywords: Acrivastine, Pirfenidone, Liquid chromatography tandem mass spectrometer, Linearity, Validation.

INTRODUCTION

Acrivastine (AC) is used as antihistamine and antiallergic. AC is a histamine H1-receptor antagonist with a rapid onset of action and low sedative potential due to poor penetration into the central nervous system. AC chemical name is (E)-3-(6-((E)-3-(pyrrolidin-1-yl)-1-p-tolylprop-1-enyl) pyridine-2-yl) acrylic acid. Its molecular formula is $C_{22}H_{24}N_2O_2$and molecular weight is 348.44 g/mol. The molecular structure of AC is shown in Fig. 1. Literature survey reveals only a few analytical methods [1-8] for the determination of AC individually and in combination with other drugs in biological samples and pharmaceutical preparations. The purpose of the present study was to develop and validate an liquid chromatography tandem mass spectrometer (LC-MS-MS) [9,10] method as per USFDA bioanalytical method validation guideline, with simple sample preparation technique to determine AC concentration in human plasma. This assay method demonstrated acceptable sensitivity (lower limit of quantitation [LLOQ]: 0.536 ng/mL), precision, accuracy, selectivity, recovery and stability, and less absolute and relative matrix effect.

Instrumentation

Shimadzu (high-performance liquid chromatography) HPLC system and MS/MS API-3200.

Reagents/materials

Methanol (HPLC grade), water (HPLC grade), acetic acid (AR grade), formic acid (AR grade), ammonium formate (GR grade), AC working standard and pirfenidone (PIR) internal standard (ISTD).

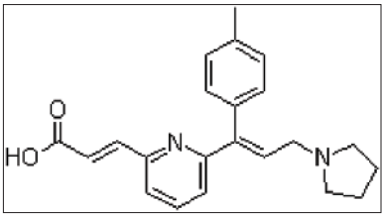

Fig. 1: Chemical structure of acrivastine

Stock solutions

AC stock solutions and PIR stock solutions were prepared in methanol.

Biological matrix

Human plasma is containing K2 ethylenediamine tetraacetic acid as anticoagulant was used as a biological matrix during method validation. Selectivity and sensitivity tests were performed before bulk spiking.

Calibration curve (CC) standards and quality control (QC) sample concentrations

The standards (CC) were prepared at ranges from 0.536 to 205.170 ng/mL concentrations for AC. The QC samples for AC were prepared at concentrations of 0.536 ng/mL (LLOQ QC), 1.530 ng/mL low QC, 101.999 ng/mL medium QC and 175.860 ng/mL high QC.

Buffer (2 mm ammonium formate)

Weigh accurately around 63 mg of ammonium formate and transfer it to 500 mL volumetric flask. Make up the volume up to the mark with Milli-Q water.

Mobile phase

Take methanol and 2 mm ammonium formate buffer in the ratio 70:30 v/v. Mix well, sonicate and degas in an ultrasonicator.

Diluent

Take methanol and Milli-Q Water in a ratio of 50:50 v/v. Mix well, sonicate and degas in an ultrasonicator (Table 1).

Sample preparation

Take out required numbers of CC/QC and/or subject samples from freezer and thaw in the water bath maintained at room temperature. Vortex for proper mixing. Aliquot 100 mL from each sample into prelabeled ria vials. Add 500 mL of ISTD solution (100 ng/mL of PIR in methanol) to each tube (Except Blank) and mix for 2 minutes on vortexer. For "Blank" sample add 500 mL methanol instead of ISTD dilution and vortex for 2 minutes. Centrifuge the samples at 4600 rpm for 15 minutes at 5°C. Separate supernatant layer in prelabeled HPLC vials and inject.

Data processing

The chromatograms have to be acquired using the computer-based analyst 1.4.2. The concentration of the unknown has to be calculated from the area ratio versus spiked plasma concentration regression equations with reciprocate of the drug concentration, as weighting factor is $1/x^2$ for AC.

$$y=mx+c$$

Where,

y=Ratio of analyte peak area and ISTD peak area (drug area/ISTD area)

x=Ratio of analyte concentration and ISTD concentration (drug concentration/ISTD concentration)

m=Slope of the **CC**

c=y-axis intercept value

RESULTS AND DISCUSSION

LC-MS-MS method for the estimation of AC in human plasma was developed and validated using PIR as an ISTD. Sample preparation was accomplished by liquid-liquid extraction method. The processed samples were chromatographed on peerless basic C18 (100 mm×4.6 mm), 5 µ columns using a mobile phase consisting a mixture of methanol and 2 mm ammonium formate buffer in the volume ratio of 70:30 v/v at a flow rate of 0.8 mL/minutes. The method was validated over a concentration range of 0.536-205.170 ng/mL concentrations for AC. During validation selectivity, sensitivity and recovery exercise was carried out. Precision and accuracy exercise was carried out by processing four precision and accuracy batches. Results of various stabilities, reinjection reproducibility and ruggedness were carried out.

Table 1: Summary of bioanalytical chromatographic conditions

Parameter	Conditions
System	LC-MS/MS API-3200
Detector	MS
Software	Analyst software 1.4.2
Column	Peerless basic C_{18} (100 mm×4.6 mm), 5 µ
Mobile phase	Methanol: 2 mm ammonium formate buffer (70:30 v/v)
Flow rate	0.8 mL/minutes
m/z	Acrivastine-349.1/278.3 PIR-186.1/92
Injection volume	10 µl
Column oven temperature	40°C±1°C
Diluent	Methanol:water (50:50 v/v)
Autosampler temperature	6°C±1°C

LC-MS/MS: Liquid chromatography tandem mass spectrometer, PIR: Pirfenidone

Table 2: CC details of acrivastine from one batch of validation

Spiked plasma concentration (ng/mL)	Mean concentration (ng/mL)±SD	RSD (%) N=5	Accuracy (%)
0.516	0.50±0.0015	0.3	97.9
1.032	1.07±0.009	0.8	104.0
10.32	10.39±0.25	2.5	100.8
30.806	31.87±0.16	0.5	103.5
102.686	104.68±2.34	2.2	101.9
153.262	147.216±0.58	0.4	96.1
184.653	180.755±1.90	1.1	97.9
205.170	200.98±2.80	1.4	98.0

RSD: Relative standard deviations, SD: Standard deviation, CC: Calibration curve

Selectivity and specificity

The analysis of AC and PIR using multiple reaction monitoring function was highly selective with no interfering compounds. Specificity was performed by using six different lots of human plasma.

Matrix effect

The overall precision of the matrix factor was determined to be 7.7 at the low concentration and 3.6 at the high concentration for AC.

Linearity

CC were plotted at the peak area ratio (AC/PIR) versus (AC) concentration. CC was found to be linear over the concentration range of 0.536-205.170 ng/mL. The % relative standard deviations (RSD) was <3% and the accuracy ranged from 96.1 to 104%. The correlation coefficients (r^2) were >0.999 for all curves (Table 2).

Precision and accuracy

Precision and accuracy for this method was controlled by calculating the intra- and inter-batch variations at four concentrations (0.536, 1.530, 101.999 and 175.860 ng/mL) of QC samples in six replicates. As shown in Table 3, the intra-day RSD was <10.85% and the accuracy ranged from 90.1 to 95.5%. Inter-day RSD was <7.3% and the accuracy ranged from 93.6 to 96%. These results indicate the adequate reliability and reproducibility of this method within the analytical curve range.

Stability

Quantification of AC in plasma subjected to three freeze thaw (−30°C to room temperature) cycles showed the stability of the analyte. No significant degradation of AC was observed even after 23 hrs storage period in the auto sampler tray, and the final concentrations of AC was between 97.8% and 99.9% of the theoretical values. In addition, the long-term stability of AC in QC samples after 55 days of storage at −30°C was also evaluated. The concentrations ranged from 96.7 to 98.8% of the theoretical values (Table 4). These results confirmed the stability of AC in human plasma for at least 55 days at −30°C.

Recovery

The recovery following the sample preparation using the liquid-liquid extraction method with methanol was calculated by comparing the peak area ratios of AC in plasma samples with the peak area ratios of solvent samples and estimated at control levels of AC. The recovery of AC was determined at three different concentrations 1.53, 101.99 and 175.86 ng/mL and found to be 99%, 97% and 104%, respectively. The overall average recovery of AC and PIR was found to be 100% and 102.5%, respectively.

LOQ and LOD

The LOQ for this method was proven as the lowest concentration of the CC, which was proven as 0.536 ng/mL. The LOD was determined using aqueous solutions and found to be 0.18 ng/mL.

Table 3: Precision and accuracy of AC from one batch of validation

Spiked plasma concentration (ng/mL)	Intra-day			Inter-day		
	Concentration (ng/mL) *n*=6 mean±SD	RSD (%)	Accuracy	Concentration (ng/mL) *n*=18 mean±SD	RSD (%)	Accuracy
0.536	0.483±0.02	3.9	90.1	0.513±0.04	7.3	95.8
1.530	1.431±0.04	3.1	93.5	1.457±0.05	3.2	95.2
101.999	94.44±2.23	2.4	92.6	95.51±1.6	1.7	93.6
175.860	165.89±2.65	1.6	95.5	168.87±2.73	1.6	96

AC: Acrivastine, RSD: Relative standard deviations, SD: Standard deviation

Table 4: Stability of AC in plasma samples

Stability	Spiked plasma concentration (ng/mL)	Concentration measured (ng/mL) (mean±SD; *n*=6)	RSD (%) *n*=6
Room temp stability (6 hrs)	1.53	1.453±0.12	8.8
	175.86	172.38±1.24	0.7
Autosampler stability (23 hrs)	1.53	1.529±0.05	3.3
	175.86	171.93±2.59	1.5
Freeze thaw stability (cycle 348 hrs)	1.53	1.44±0.1	7
	175.86	172.11±3.19	1.9
Long term stability (55 days, −30°C)	1.53	1.48±0.02	1.35
	175.86	173.82±3.23	1.85

AC: Acrivastine, RSD: Relative standard deviations, SD: Standard deviation

CONCLUSIONS

The developed method is rapid, sensitive, rugged and reproducible with high recovery. Each sample requires <3.2 minutes of analysis time. Drug and ISTD were extracted with the simplest liquid-liquid extraction method with less matrix effect. The developed method can be successfully applied in the pharmacokinetic study to evaluate human plasma conc.

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