DEVELOPMENT OF A METHOD FOR QUANTIFICATION OF TWO GENOTOXIC IMPURITIES IN LURASIDONE USING LC-MS/MS

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ABSTRACT

A sensitive and stability indicating liquid chromatography – tandem mass spectrophotometry method is developed for the simultaneous detection and quantification of 3-(1-piperazinyl)-1,2-benzisothiazole and 4-chloro-1,2-benzisothiazole potential treated as possible genotoxic impurities of lurasidone. The method utilizes Hypersil BDS C18 column (50 mm × 4.6 mm, 3.0 m) with electrospray ionization in multiple reaction monitoring mode for quantitation of two potential genotoxic impurities. The method is validated following the International Conference on Harmonization guidelines and is proficient to quantitate 3-(1-piperazinyl)-1,2-benzisothiazole at 0.3 ppm and 4-chloro-1,2-benzisothiazole at 0.3 ppm with respect to 5.0 mg/mL of lurasidone. The proposed method is specific, accurate and precise. The method is linear in the range of 0.3 ppm -4.5 ppm in respect to 3-(1-piperazinyl)-1,2-benzisothiazole and 4-chloro-1,2-benzisothiazole. The correlation coefficients are 0.9996 and 0.9998 in each case which matches the range of limit of quantification–150 % of predictable permitted level (3.0 ppm). The impurities are not present in the studied three pure and formulation batches of lurasidone. The accuracy of the method is ranged between 98.53 % and 102.93 % for both potential genotoxic impurities. This method can be further utilized as an excellent quality control tool for low level quantization of both 3-(1-piperazinyl)-1,2-benzisothiazole and 4-chloro-1,2-benzisothiazole as potential low level genotoxic impurities of lurasidone.

Keywords: lurasidone, 3-(1-piperazinyl)-1,2-benzisothiazole, 4-chloro-1,2-benzisothiazole, potential genotoxic impurities.

INTRODUCTION

The key task of the pharmaceutical industry is to separate and quantify the potential genotoxic impurities (GTIs) escalating from the process of drug production. Sensitive and selective analytical methods have to be developed for trace level quantification of these GTIs. The presence of (GTIs) can induce genetic mutations, chromosomal rearrangements and breaks. Furthermore, they have the potential to cause cancer in human [1, 2]. Even small amounts of GTIs may affect the efficacy and the safety of the final active pharmaceutical ingredients (API). The European Medicines Agency (EMEA) and the US Food and Drug Administration (US FDA) have recently proposed a regulatory guidance in respect to genotoxic impurities of the new commercial drugs. Ac-

cording to both agencies regalement analytical methods should be developed to meet the required limit of 1.5 µg/day of individual impurity. Based on the threshold of toxicological concern (TTC), it is accepted that the GTIs have to be limited to a daily dose of 1.0 µg/day - 1.5 µg/day [3].

Lurasidone (LSD), [(3a*R*,4*S*,7*R*,7a*S*)-2-{(1*R*,2*R*)-2-[4-(1,2-benzisothiazol-3-yl)piperazin-1-ylmethyl] cyclohexylmethyl}hexahydro-4,7-methano-2*H*-isoindole-1,3-dione hydrochloride] belongs to the class of benzothiazol derivatives. There are structure-activity relationship studies summarizing its efficacy and safety in treatment of schizophrenia and bipolar depression based on the results from both short-term and longer-term controlled clinical trials [4, 5]. Currently, only quetiapine, olanzapine-fluoxetine combination, and lurasidone refer to a new-generation of atypical antipsychotics approved by the US FDA for the treatment of schizophrenia and bipolar depression [4, 6]. 3-(1-piperazinyl)-1,2-benzisothiazole (PBI) and 4-chloro-1,2-benzisothiazole (CBI) are the most important intermediates during the synthesis of LSD, which are identified as GTIs in the finished pharmaceutical substances. However, no methods have been reported for the determination of PBI and CBI process related impurities in LSD. The chemical structures of PBI and CBI along with that of LSD are presented in Fig. 1. Several methods have been developed for the estimation of LSD in biological samples employing HPLC-UV, HPLC fluorescence, gas chromatography and mass spectrometric detection [7, 8]. According to the current regulatory guidance for GTIs, analytical methods should be developed to meet the required limit of 1.5 µg/day intake of an individual impurity [3]. Based on the TTC limit of 1.5 µg/day and on the maximum adult daily dose of LSD of 400 mg/person, its GTIs are required to be controlled at a concentration limit of 3.75 µg/g (ppm) in the drug substance. Due to its higher sensitivity and selectivity, liquid chromatography–tandem mass spectrophotometry (LC–MS/MS) has been applied for the quantification of process related impurities in drug substances [9, 10]. We have now developed a simple LC–MS/MS method that can quantify PBI and CBI at permitted levels in LSD. The developed method is fully validated as per ICH guidelines in terms of limit of detection (LOD), limit of quantification (LOQ), linearity, precision, accuracy, specificity and robustness.

EXPERIMENTAL

Chemicals and Reagents

All chemicals and solvents were of an analytical grade with purity higher than 99.0 %. Reference samples of PBI, CBI and LSD of the highest purity (> 99.0) were obtained from Sigma-Aldrich (St. Louis, MA, USA). HPLC grade acetonitrile, methanol and ammonium acetate were purchased from Merck (Mumbai, India). Formic acid, trifluoroacetic acid and all other chemicals were obtained in their highest grade from SD fine chemicals limited (Mumbai, India). High pure Milli-Q water was obtained using Millipore Milli-Q plus purification system (Bedford, MA, USA).

Preparation of Stock and Standard Solutions

A stock solution of a mixture of GTIs was prepared at 2.0 mg/mL concentration in acetonitrile-water (70:30 v/v) mixture after weighing the individual GTIs very ac-

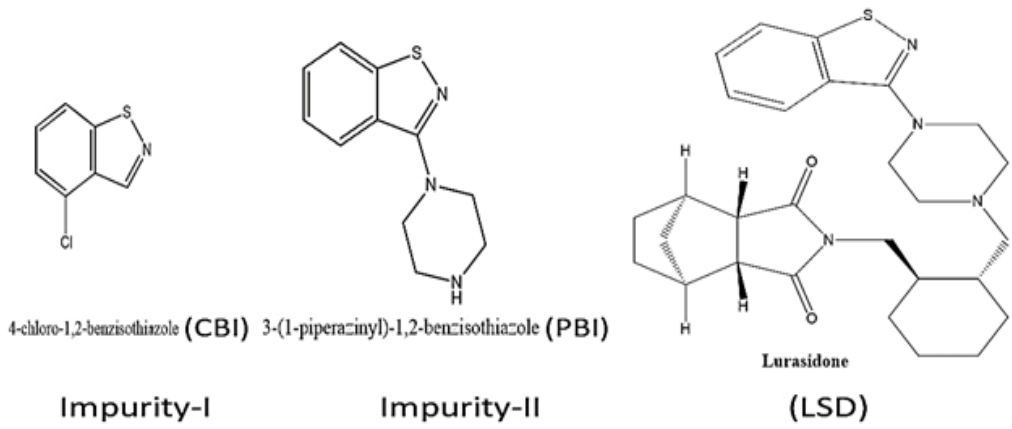

Fig. 1. Structures of CBI, PBI and LSD.

curately. Thereafter, a diluted stock solution of GTIs of a concentration of 0.1 mg/mL was prepared by adding 5.0 mL of the stock solution to 100 mL standard flask and made up to the mark with acetonitrile-water (70:30 v/v) mixture. The working standard solution was prepared by accurately weighing 50 mg of LSD in 10 mL volumetric flask and dissolving it in acetonitrile-water (70:30 v/v) mixture. The GTIs samples for validation at 0.3 ppm, 1.2 ppm, 1.8 ppm, 2.4 ppm, 3.0 ppm, 3.6 ppm and 4.5 ppm concentrations relative to the drug substance were prepared in the same manner using 0.5 µg/mL of the diluted stock solution. The concentration of the standard solutions and the samples was optimized to achieve a desired signal-to-noise ratio (S/N) and a good peak shape. All the standards were sonicated well and filtered through 0.22 µm membrane filters prior to the analysis.

Instrumentation

Applied Biosystems Sciex API 4000 model (Switzerland) MS system was used. It was coupled with HPLC consisting of LC-20AD binary gradient pump, SPD- 10AVP UV detector, SIL-10HTC auto sampler and a column oven CTO-10ASVP (Shimadzu Corporation, Kyoto, Japan). The data acquisition and processing were conducted using Analyst 1.5.1 software on a Dell computer (Digital equipment Co.)

Operating conditions of LC–MS/MS

Hypersil BDS C18 (50 mm x 4.6 mm, 3.0 µm) analytical column was used in an isocratic mode using 10 mM ammonium acetate and acetonitrile in the ratio of 70:30 (v/v). The flow rate was 0.7 mL/min, which decreased down to 0.2 mL/min in the MS source. The column oven temperature was maintained at 45ºC, while the sample cooler temperature was set at 10 ºC. The injection volume was 10 µL. The positive electro spray ionization (ESI) probe operating with a multiple reactions monitoring (MRM) mode was used for the quantification of both PBI and CBI PGIs. The two PGIs PBI were monitored at their molecular ions $[M+H]^+$ *m/z* of 220.1 (protonated) and daughter ion $[M+H]^+$ *m/z* of 134.1 (protanated); CBI were monitored at their molecular ions $[M+H]^+$ *m/z* of 170.1 (protonated) and daughter ion $[M+H]^+$ *m/z* of 134.1 (protanated). LSD was monitored at its molecular ion [M+H]+ *m/z* of 493.1 (protonated). The ion spray voltage (V), the declustering potential (DP) and the entrance potential (EP) were kept as 5000V, 60V and 10V, respectively. The nebulisation pressure of the curtain gas flow, the ion source gas 1 and the ion source gas 2 was maintained as 30 psi, 35 psi and 14 psi, respectively. All the parameters of LC and MS were controlled by the analyst software version 1.5.1.

RESULTS AND DISCUSSION

Method development and Optimization

The optimization of the chromatographic conditions is performed, particularly the composition of mobile phase, through several trials aiming to achieve symmetric peak shapes of the analytes peaks, as well as short run time and a low cost. A resolution positive mode LSD is achieved by using acetonitrile as an organic content of the mobile phase. Separation was attempted on different columns like C18 and C8 of different makes like zorbax column and symmetry C18 column using various combinations of acetonitrile and a buffer of varying contents of each component. Finally Hypersil BDS C18 50X4.6mm, 3.0µm column is found giving the best chromatographic resolution with a flow rate of 0.7 mL/min and total run time of 12 min. The PBI, CBI and LSD are eluted at 4.06 min, 7.07 min and 2.1 min with MRM mode.

METHOD VALIDATION

Specificity and selectivity

Specificity is the ability of the method to assess unequivocally the analyte response in presence of components that may be expected to be present in the sample. PBI, CBI and LSD compounds solutions are prepared individually at a concentration of about 0.01mg/mL in diluents. A solution of PBI, CBI and LSD is also prepared. The specificity is established through the interference observed in case of injecting LSD spiked with its impurities. The blank and the specificity chromatograms are shown in Fig. 2(a,b).

Robustness

The robustness of the developed method is studied in case of slight deliberate changes of the mobile phase and column tempareture. The effect of the changes in the mobile phase flow rate (-2 % to + 2 %) is studied, while the amounts of the other mobile phase components were held constant. The column oven temperature on resolution was studied at (-2°C to + 2 °C, i.e. at 43ºC and 47ºC buffer units). In all the deliber-ately varied chromatographic conditions, the performance as well as the selectivity of the method was unchanged, which proves the robustness of the method.

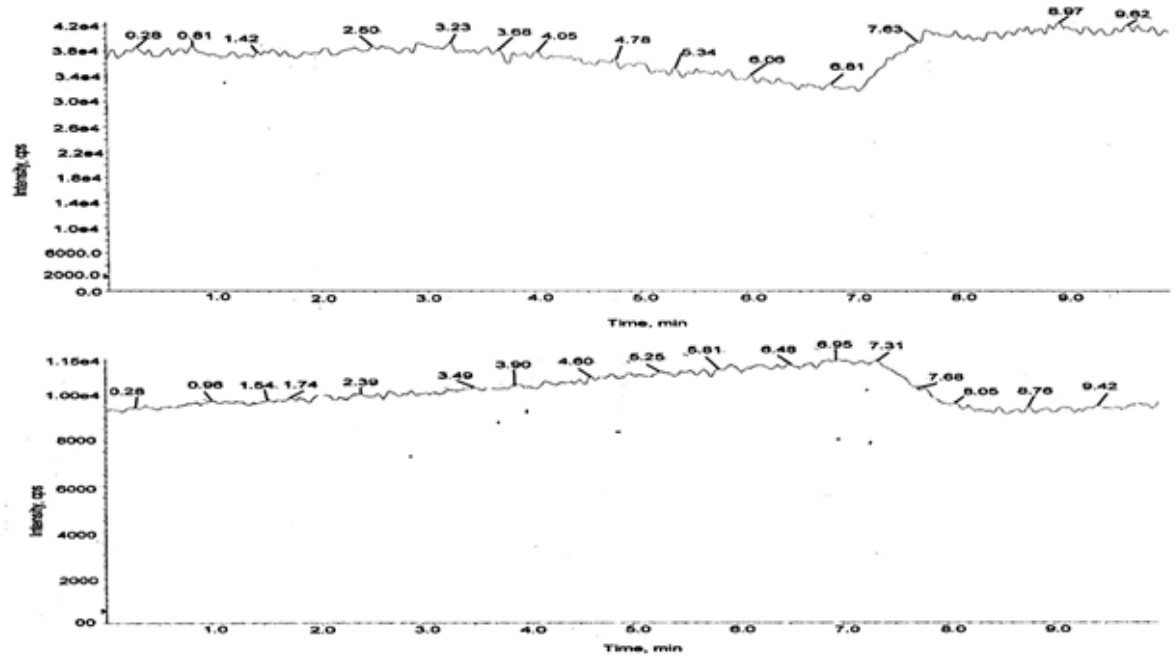

Fig. 2. (a) Blank chromatogram of PBI and CBI.

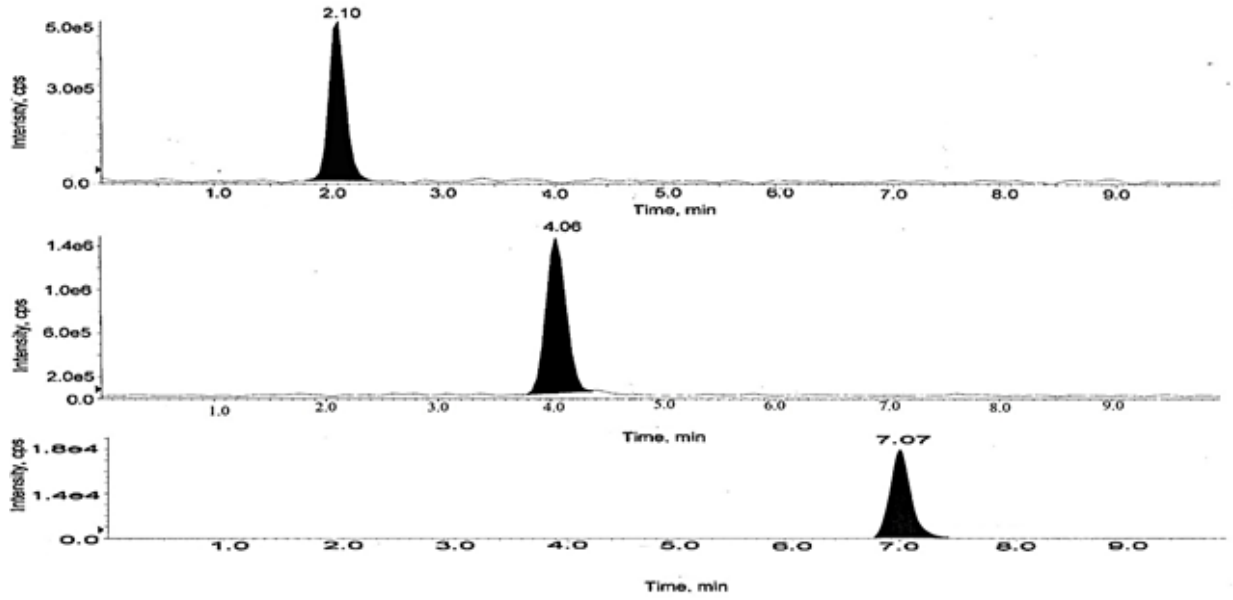

Fig. 2 (b) Specificity chromatogram of LSD, PBI and CBI.

Determination of LOD and LOQ

The LOD and LOQ, as a measure of method sensitivity, are calculated from S/N (signal to noise) ratios. To determine LOD and LOQ values for PBI and CBI the concentration is sequentially decreased to reach S/N ratio of 2.8 and 3.2 referring to LOD, as well as 10.1 and 10.2 in case of LOQ. The LOD and LOQ chromatograms obtained are shown in Fig. 3 (a,b). The data is generated from six injections (without API) containing 0.3 ppm of each PBI and CBI with respect to API sample concentration of 5 mg/mL. The LOQ of 0.3 ppm is typical for PBI and CBI, with LOD approximately ten times less than LOQ. In addition, the relative efficiency of MRM modes in respect to sensitivity improvement is also evaluated.

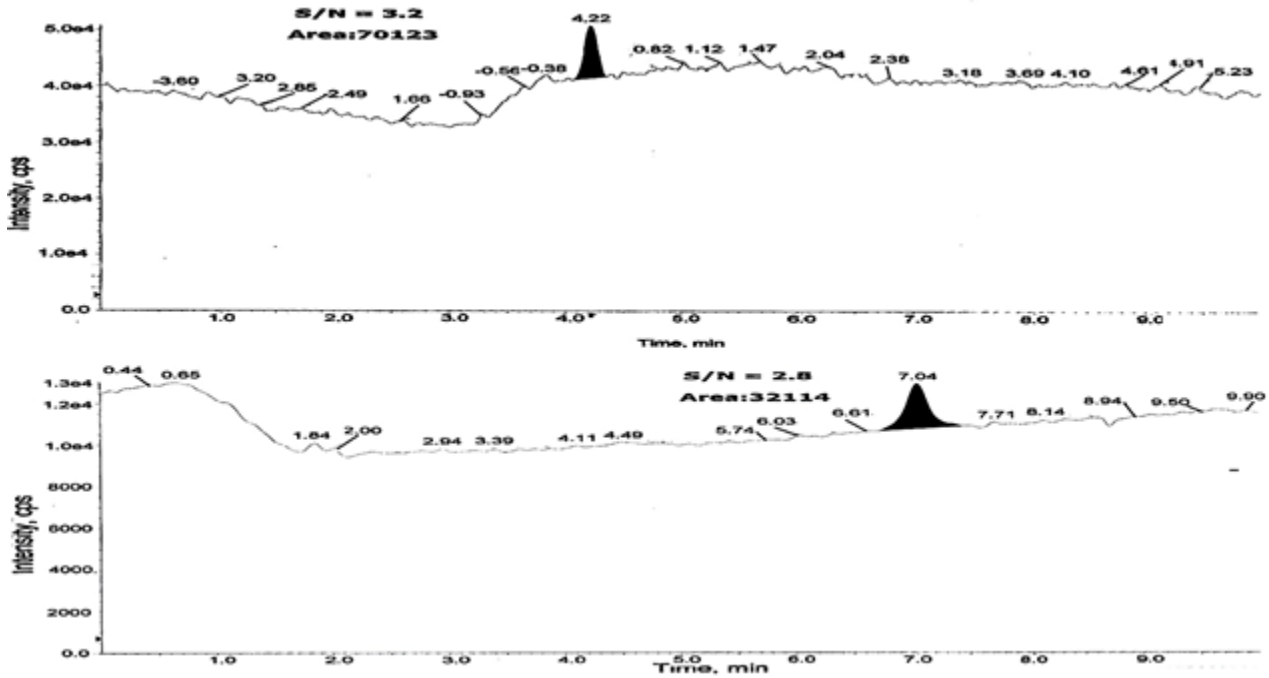

Fig. 3. (a) LOD chromatogram of PBI and CBI.

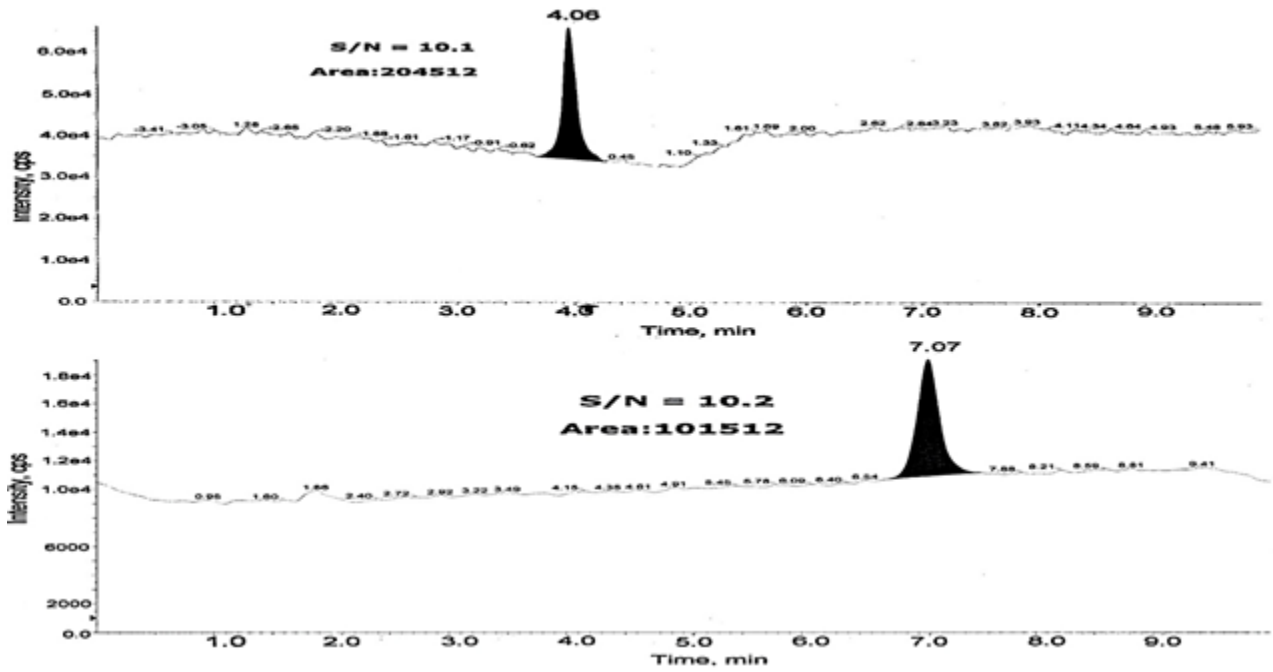

Fig. 3. (b) LOQ chromatogram of PBI and CBI.

Recovery studies

The recovery studies are performed by the standard addition method to evaluate the accuracy and specificity. The accuracy of the method is determined in triplicate at LOQ level in a bulk drug sample. The recoveries are calculated. Excellent recovery values of PBI and CBI referring to 99.73 % compared to 102.93 %, and to 98.53 % compared to 101.11 %, respectively, are obtained. At such a low levels these recoveries and % RSD lower than 3.0 are satisfactory. LOQ chromatograms of the

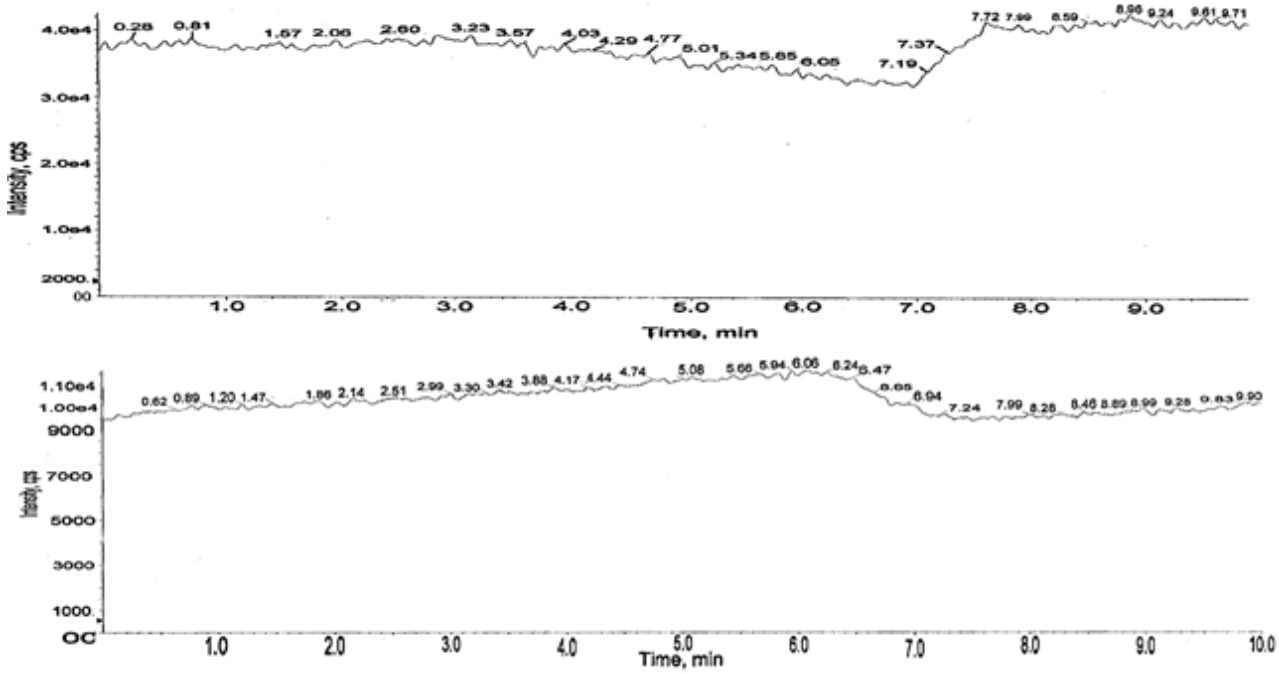

Fig. 4. (a) Sample chromatogram of PBI and CBI.

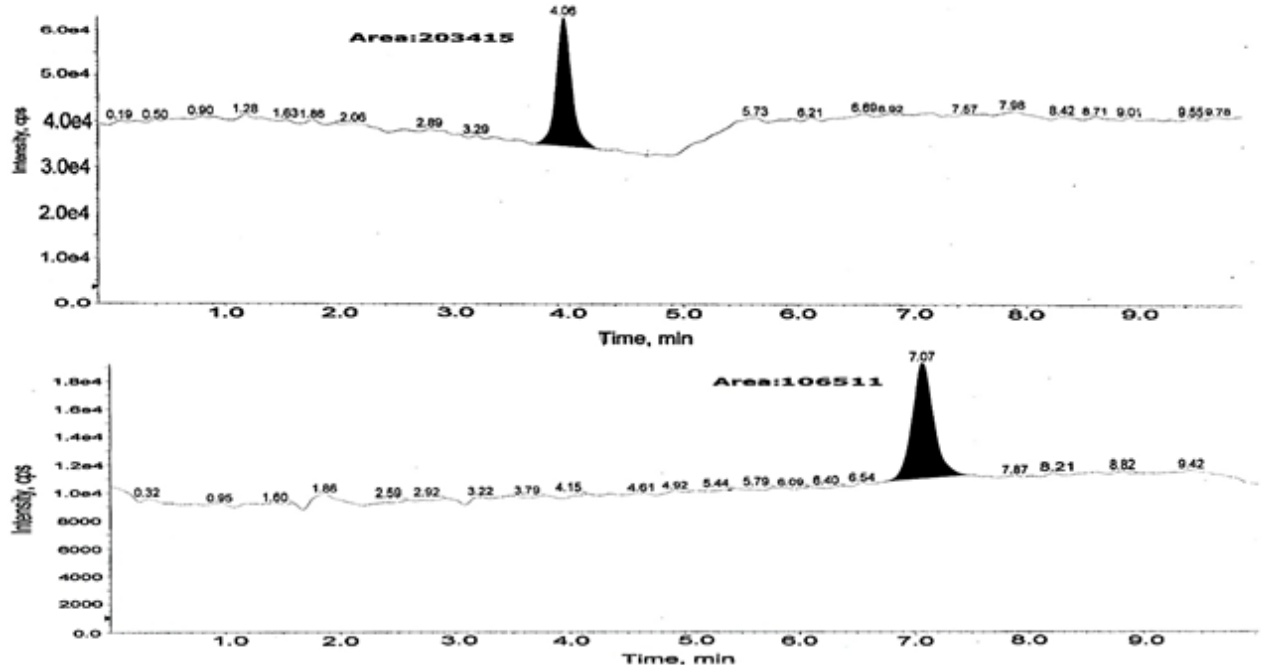

Fig. 4. (b) Spiked chromatogram of PBI and CBI.

sample and spiked mixtures are shown in Fig. 4(a,b). The relative standard deviation, % RSD is calculated from the average of triplicate analysis (Table 1). Further, the stability of PBI and CBI are studied at 0 h, 12 h and 24 h (Table 2).

Linearity and Range

The linearity test for the method is performed according to the guidelines laid by ICH. This method is evaluated at six different concentrations of the analytes within the range of 0.3 ng/mL - 4.5 ng/mL. These

Table 1. Evaluation of accuracy and specificity data of two Lurasidone impurities.

Amount spiked[a]	% recovery of impurities[b]	
	PBI (mean ± %RSD)	CBI (mean ±%RSD)
LOQ	100.06 ± 1.1	100.44 ± 1.8
1.5 ppm (50%)	102.93 ± 1.9	98.53 ± 1.1
3.0 ppm (100%)	100.82 ± 1.9	100.89 ± 1.1
3.6 ppm (120%)	101.71 ± 2.5	98.08 ± 2.9
4.5 ppm (150%)	99.73 ± 1.4	101.11 ± 2.9

[a] *Amount of impurities spiked with respect to 10.0 mg/mL of Lurasidone.*

[b] *Mean±% RSD for three determinations.*

Table 2. The solution stability data of PBI and CBI at different time intervals.

Time (hr)	theoretical conc. (ppm)		% recovery[a] (mean ± %RSD)	
	PBI	CBI	PBI	CBI
Intial (0-hrs)	3.0	3.0	99.90±0.50	100.50±1.48
12 hrs	3.0	3.0	99.80±0.40	100.20±1.33
24 hrs	3.0	3.0	99.80±0.80	98.80±0.66
48 hrs	3.0	3.0	100.30±1.41	98.60±0.64

[a] *mean value of three determinations*

Table 3. Linearity plots of PBI nad CBI the concentration range of 0.3-4.5 ppm level.

	PBI		CBI	
Levels	Concentration (ppm)	Peak area	Concentration (ppm)	Peak area
LOQ	0.3	201450	0.3	101544
40%	1.2	801200	1.2	421500
60%	1.8	1204711	1.8	601440
80%	2.4	1601200	2.4	821400
100%	3.0	2045150	3.0	1045333
120%	3.6	2457100	3.6	1234500
150%	4.5	3145200	4.5	1544110
	Correlation	0.9996	Correlation	0.9998
	Slope	698326.763	Slope	343906.9565
	Intercept	-39411.2319	Intercept	-1115.695652

standard solutions are prepared by a suitable dilution of the stock solution with the mobile phase. The linearity of the plot is evaluated using the least squares linear regression analysis by multiple reactions monitoring (MRM). The linearity of PBI and CBI are satisfactorily established with a six point calibration curve between LOQ and 150 % of analyte concentrations (40 %, 60 %, 80 %, 100 %, 120 % and 150 %). The calibration curve is produced by plotting the average of triplicate PBI and CBI injections against the concentrations expressed in percentage. The slope, the intercept and the correlation coefficient values are derived from the linear least-square regression analysis and the data is presented in Table 3. It reveals that a good correlation exists between the peak areas concentration. The repeatability is checked by calculating the relative standard deviation (% RSD)

of six determinations by injecting six freshly prepared solutions containing 0.3 ppm of PBI and CBI on the same day. The low % RSD values confirm the good precision of the method developed.

CONCLUSIONS

The present development study is based on the validation of a highly sensitive, specific, reproducible and high-throughput LC-MS/MS method for the quantification of PBI and CBI in APIs. It is found that it is highly sensitive with a limit of detection (LOD) of 0.3 ppm. Trace level ammonium acetate is added to the mobile phase to enhance the ionization and the detection. Selected sample solvents are assessed for the effect on the standard stability in absence and presence of API. As a systematic approach, it is very important to utilize the comprehensive chromatographic knowledge gained throughout the lifecycle of the development of a drug candidate based on the continuous understanding of the API manufacturing process. A method providing their quantification at ppm level is developed and validated. It can be concluded that the developed method could be very useful for monitoring of PBI and CBI in LSD in its pure and tablet forms.

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