Asian Journal of Chemistry; Vol. 33, No. 5 (2021), 1165-1168

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2021.23135

Method Development and Validation for Quantification of Potential Genotoxic Impurity, PyCl in Lansoprazole Hydrochloride using Liquid Chromatography Combined with Mass Spectrometry

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Received: 23 January 2021; *Accepted*: 30 March 2021; *Published online*: 16 April 2021; AJC-20340

A sensitive and robust high performance liquid chromatography combined with tandem mass spectrometry (LC-MS/MS) method was developed and validated for the determination of potential genotoxic impurity (PGI), 2-(chloromethyl)-3-methyl-4-(2,2,2-trifluoroethoxy)-pyridine hydrochloride (PyCl) in lansoprazole as per ICH Q2 guideline. In this method, PyCl and lansoprazole were well-separated from each other on Acquity UPLC BEH-C18 column (50 × 4.6 mm × 1.7 µ) in a gradient elution mode with the mobile phase consisting of 0.1% formic acid in water (mobile phase-A) and acetonitrile (mobile phase-B) at a flow rate of 0.4 mL/min. For the quantitation of Py-Cl, selective ion monitoring (SIM) mode was used with *m/z* 240 ion in LC-MS method. The validated method was found to be precise, accurate and linear from the range of LOQ level to 150% with respect to sample concentration and the correlation co-efficient was found to be 0.998. Limit of detection (LOD) and limit of quantifications (LOQ) were found to be 0.000012 and 0.000004 mg/mL, respectively. The validated method was found to be sensitive and the recoveries were found to be well within the range from 83.4% to 95.9% for Py-Cl. Further, the solution stability was also established as the same were found to be stable upto 24 h.

Keywords: Genotoxic impurity, Lansoprazole, Method development, LC-MS.

INTRODUCTION

Compounds that can induce chromosomal rearrangements and genetic mutations are considered as potential genotoxic impurities (PGIs). Potential genotoxic impurity (PGI) have the potential to damage DNA at any level of exposure and such damage may lead to tumor development. For these carcinogenic PGIs, it is predicted that there is no apparent threshold and any level of exposure carries a risk [1-3]. International Conference on Harmonization (ICH) and the European Medicines Agency (EMA) guidelines provided the limits for impurities in drug substances and drug products, however, these limits are not acceptable for PGIs due to their adverse effects. Hence, it is necessary to set up the limits based on the daily dose of the drug substance [4-6]. Therefore, it is essential to develop analytical methods and demonstrate the synthetic process controls. However, the relevant strategies are not readily available for all the drug substances. As per Q3A guidelines, most of the PGIs are likely to be aroused during synthesis, purification and storage of the new drug substance [7-9].

Lansoprazole is a popular proton-pump inhibitor (PPI) and exerts remarkable impacts on the treatment of acid-related disorders such as duodenal and gastric ulcers, reflux esophagitis and Zollinger-Ellison syndrome through interaction with the (H^+/K^+)-ATPase in the secretory membrane of the parietal cell [10]. Few reports available in the literature on determination of drug in bulk and capsule formulation [11]. Some stability indicating validated methods and degradation studies on lansoprazole are available in the literature [12,13]. Another LC-MS method available on the quantitation of lansoprazole in oral suspension by UPLC triple quadrupole-MS [14]. Quantitation of lansoprazole in human plasma was reported using liquid chromatography-tandem mass spectrometry (LC-MS/MS) method [15]. Wang *et al.* [16] reported a column-switching LC-MS/MS method for the quantification of lansoprazole enantiomers in dog plasma [16].

Global regulatory organizations have released guidelines on PGIs limits and suggested a highest daily exposure of 1.5 µg per day for PGIs. Based on the toxicological concern threshold, the impurity concentration limits in a drug substance/

product can be obtained depending on the maximum daily dose. The genotoxic evaluation is needed to check the presence of any PGIs throughout synthetic pathway of drug and duration of stability. It is of utmost significance for patient safety to demonstrate that PGIs are regulated to safe concentrations. From this threshold value, a permitted level in the active substance can be calculated based on the expected daily dose. Based on the maximum daily dosage 120 mg/day of lansoprazole and its PGI, PyCl (2-(chloromethyl)-3-methyl-4-(2,2,2-trifluoroethoxy)pyridine hydrochloride) are required to be controlled at a limit of 12.5 µg/g. The higher limits may be justified under certain conditions such as short-term exposure periods. Determination of these impurities at µg/mL levels requires highly sensitive analytical methodologies in pharmaceutical research and development. To the best of our knowledge, no method was reported for the quantitation of PyCl in lansoprazole using LC-MS. Hence, an attempt was made to overcome the shortcomings of the existing methods and in developing a highly sensitive, cost-effective, specific, direct and accurate LC-MS method.

EXPERIMENTAL

Lansoprazole and 2-(chloromethyl)-3-methyl-4-(2,2,2-trifluoro-ethoxy)pyridine hydrochloride (PyCl) were procured from local manufacturing drug unit, Hyderabad, India. HPLC grade acetonitrile was procured from Merck, India and Water was purified by using a Milli-Q water system (Merck, India).

Instrumentation, HPLC conditions and sample preparation: The LC-MS analysis was performed using waters UPLC (Waters Corporation, USA) system with quaternary pump connected to degasser unit, Photodiode array detector, column cooler and heater compartment and auto sampler with loop volume 50 µL.

The chromatographic separation was done on Acquity UPLC BEH-C18 (50 mm × 4.6 × 1.7 µm) column in a gradient elution mode using the mobile phase composed of 0.1% formic acid in water and acetonitrile. The gradient program was set as follows: (Tmin/%solution of B): 0.0/10, 0.5/10, 3.5/90, 5/10, 8/10. The injection volume was 2 µL, the flow rate was 0.4 mL/min and the column temperature was maintained at 30 ºC. The samples were prepared in diluent, which is composed of acetonitrile and water (50:50; v/v) and injected into LC-MS system for analysis.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions: During method development, used different columns and made several trials to achieve the good separation of PGI from Lansoprazole. First, used X-bridge C18 (100 × 4.6 mm) 3.5 µ column with the mobile phase comprising of 0.1% formic acid in water: acetonitrile (20:80; v/v) and the column temperature 30 ºC was maintained as the starting conditions for method development. When these conditions are used, both the sample lansoprazole and standard PyCl were co-eluted (merged) as a single peak. Secondly, mobile phase composition was changed to 0.1% formic acid in water: methanol, but no separation was observed. Then the column was changed to X-select CSH (150 mm × 4.6 mm × 3.5 µ) with the same mobile phase and observed some sort of separation, which is not good enough. Finally, Acquity UPLC BEH-C18 (50 mm × 4.6 mm × 1.7 µm) column and with the same mobile phase consisting of 0.1% formic acid in water (mobile phase A) and acetonitrile (mobile phase B) was used and observed good separation with good peak shape. The gradient program was set as follows: (Tmin/%solution of B): 0.0/10, 0.5/10, 3.5/90, 5/10, 8/10. The injection volume was 2 µL, the flow rate was 0.4 mL/min and the column temperature was maintained at 30 ºC.

Method validation: The method was validated with respect to specificity, precision, accuracy, linearity, sensitivity, robustness and ruggedness/intermediate precision as per ICH Q2 guideline.

Specificity: The method specificity was assessed by comparing the sample chromatogram with blank chromatogram. The blank solution was acetonitrile, which has been used as diluent (blank) to dissolve the drug. The data of specificity is provided in Table-1. From chromatograms (Fig. 1), it can be seen that the method was found to be specific.

Precision studies: The precision of the method was studied in terms of repeatability (intra-day precision) and intermediate

TABLE-1
DATA OF SPECIFICITY

Injection	Retention time (min)
Blank	NA
Py-Cl	2.2
Sample	NA
Spiked sample	2.2

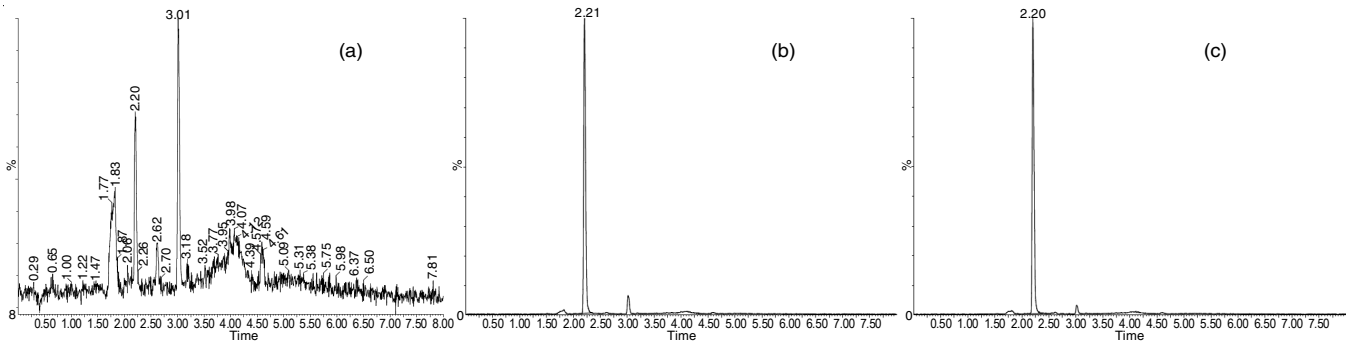

Fig. 1. LC-MS SIM chromatogram of (a) blank, (b) Py-Cl and (c) spiked sample

precision (inter day precision) by preparing five individual sample solutions on different day, different analyst, different instrument and different column. The standard deviation and %RSD calculated values are provided in Tables 2 and 3. The data of precision studies indicate that the method was found to be precise and rugged. Precision study was also performed at LOQ level and %RSD was well within the acceptance criteria. The LOQ precision data are provided in Table-4.

TABLE-2
DATA OF METHOD PRECISION

Number of injections	Area of Py-Cl
Preparation-1	56189.46
Preparation-2	54810.77
Preparation-3	56194.06
Preparation-4	55571.20
Preparation-5	57402.59
Preparation-6	55845.71
Average	56002.3
Standard deviation	855.88
%RSD	1.5

TABLE-3
DATA OF INTERMEDIATE PRECISION

Number of injections	Area of Py-Cl
Preparation-1	55512.23
Preparation-2	54886.37
Preparation-3	55822.73
Preparation-4	54965.42
Preparation-5	56796.74
Preparation-6	54975.36
Average	55493.1
Standard deviation	737.987
%RSD	1.3

TABLE-4
DATA OF LOQ PRECISION

Number of injections	Area of Py-Cl
Injection-1	12497.83
Injection-2	12593.06
Injection-3	12618.46
Average	12569.78
Standard deviation	63.594
%RSD	0.5

Linearity and range: Linearity was determined by a series of injections of five standards whose concentrations are ranging from LOQ (0.03%) to 150% of the specified concentration. The response was directly proportional to the concentrations of the analytes. A linear regression equation applied and a good linearity was found and the correlation coefficient (R) was found to be greater than 0.999. The data of linearity are provided in Table-5.

Limit of detection (LOD) and limit of quantitation (LOQ): The LOD and LOQ values were estimated at a signal-to-noise ratio of 3:1 and 10:1, respectively. The LOD and LOQ values were found to be 0.000004 and 0.000012 mg/mL, respectively (Table-6).

Accuracy: Accuracy of the method was evaluated by standard addition method. The known amount of PyCl spiked in the range of LOQ to 150% of specified level and the sample solutions were analyzed in triplicate as per proposed method. The recovery was found to be well within the range from 83.4% to 95.9% for PyCl (Table-7).

TABLE-5
DATA OF LINEARITY

Concentration (ppm)	Area of Py-Cl
1.6	12545.45
4.1	31707.83
6.6	48075.03
8.2	58345.06
9.9	68670.45
12.3	87990.88
Correlation coefficient	0.9991
Slope	6888.9757
Y-Intercept	2195.9047
R^2	0.998

TABLE-6
DATA OF LOD AND LOQ

Parameter	Conc. (ppm)	Conc. (mg/mL)	RT	Area	USP S/N
LOD	0.5	0.000004	2.21	–	5.95
LOQ	1.6	0.000012	2.20	12497.83	20.41

TABLE-7
DATA OF RECOVERY

Accuracy conc. (%)	Area of Py-Cl + sample	Py-Cl area in sample	Py-Cl area in standard
Accuracy at 1.6 ppm (LOQ)	12343.11	0	64370.8
Accuracy at 4.1 ppm	31711.92		
Accuracy at 8.2 ppm	55731.60		
Accuracy at 12.3 ppm	85843.48		
% Recovery at 1.6 ppm (LOQ)		95.9	
% Recovery at 4.1 ppm		98.1	
% Recovery at 8.2 ppm		86.4	
% Recovery at 12.3 ppm		83.4	

Robustness: To determine the robustness of the method, experimental conditions were purposely altered. The flow rate of mobile phase is 0.4 mL/min. The effect of flow rate on the resolution was studied by changing from 0.35 to 0.45 mL/min while the other mobile phase components were held constant. The effect of column temperature on resolution was also studied at 25 and 35 ºC, instead of 30 ºC while the other mobile phase components were held constant. As no significant changes in content of PyCl were observed by changing these chromatographic conditions (flow rate and column temperature), confirms that the robustness of the method.

Solution stability of samples: Solution stability was also established for the samples and the solutions were found to be stable up to 24 h.

Conclusion

Quantitation of genotoxic impurity and 2-(chloromethyl)-3-methyl-4-(2,2,2-trifluoroethoxy)pyridine hydrochloride (PyCl) in lansoprazole was carried out using selective ion monitoring (SIM) LC-MS method with *m/z* 240 ion according to

ICH Q2 guideline. The validated method was found to be precise, accurate and linear from the range of LOQ level to 150% with respect to sample concentration and the correlation co-efficient was found to be 0.998. Limit of detection (LOD) and limit of quantifications (LOQ) were found to be 0.000012 and 0.000004 mg/mL, respectively. The validated method was found to be very sensitive and the recoveries were found to be well within the range from 83.4% to 95.9% for Py-Cl. Further, the solutions were found to be stable upto 24 h.

ACKNOWLEDGEMENTS

The authors thank the Management of GVK Biosciences Pvt. Ltd., Hyderabad, India for providing the research facilities, and support during the study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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