Research Paper

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Method Validation and Quantitative Determination of 2-Mercapto Benzimidazole in Lansoprazole by Lc/Ms/Ms

Chemistry

KEYWORDS : Lansoprazole, 2-Mercapto-benzimidazole, Method Validation, LC/MS/MS, Trace analysis

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ABSTRACT

A sensitive and selective liquid chromatographic-tandem mass spectrometric (LC/MS/MS) method was developed and validated for the trace analysis (>1 ppm level) of 2-Mercapto benzimidazole, a genotoxic impurity, in lansoprazole drug. The chromatographic separation was achieved on Hypersil BDS C8 (50 x 3 mm, 3 µm) column using a mobile phase consisting of 5mM ammoniumacetate, methanol and acetonitrile (60:20:20, v/v/v) at flow rate of 0.5 mL/min. The API-4000 LC/MS/MS was operated on an electrospray ionization equipped with an ESI interface operated in positive mode. The multiple-reaction monitoring (MRM) mode was used during the analytical run and it is able to quantitate up to 0.1ppm of 2-Mercapto benzimidazole. The newly developed method was validated as per achieved on International Conference on Harmonization (ICH) guidelines

INTRODUCTION

Pharmaceutical genotoxic impurities (PGIs) may induce genetic mutations, chromosomal breaks (rearrangements) and they have potential to cause cancer in human [1-2]. Therefore exposure to even low levels of such impurities present in final active pharmaceutical ingredient (API) may be of significant toxicol importance [3]. Hence it is significant for process chemists to avoid such genotoxic impurities in the manufacturing process [4]. However it would be difficult or impossible to eliminate PGIs completely from the synthetic scheme. Therefore it is a great challenge to analytical chemists to develop an appropriate analytical method to quantify the impurity accurately and control their levels in APIs. According to the European Medicines Evaluation Agency (EMEA) and feedback from US Food and Drug Administration (USFDA) the proposed use of a threshold of toxicological concern (TTC), it is accepted that genotoxic impurities will be limited to a daily dose of 1.0-1.5 µg/day [5-6].

Lansoprazole, chemically known as 2-[[3-methyl- 4-(2,2,2-trifluoroethoxy) pyridin-2-yl] ethylsulfinyl] -1H-benzimidazole. Lansoprazole, a member of the proton-pump-inhibitor class of gastric acid inhibitory agent, effectively raises intragastric pH and is indicated for the short-term treatment of active erosive reflux esophagitis, gastric ulcer, duodenal ulcer, and nonerosive gastroesophageal reflux disease. Lansoprazole is also indicated as a long-term maintenance therapy in patients with healed reflux esophagitis and healed duodenal ulcer and in the treatment of pathological hypersecretory conditions, such as Zollinger-Ellison syndrome. Lansoprazole as a proton-pump inhibitor, and is also a necessary component of dual- and triple therapy regimens for the eradication of Helicobacter pylori infection. The latest FDA-approved labeling for lansoprazole includes the indication of healing and risk reduction in nonsteroidal anti-inflammatory drug-associated gastric ulcers [7-9].

The potential presence of these genotoxic impurities has attracted the attention of regulatory authorities, draft guidelines from the European Medicines Agency and feedback from the US Food and Drug Administration (USFDA) to the pharmaceuticals industry via responses to drug applications have influenced the industry to establish interim strategies. It is a great challenge to both analytical and synthetic chemists to prepare a drug dose of 1.5 µg/day which does not cross 0.1 ppm of this genotoxic impurity. Though 2-Mercapto benzimidazole is a well known carcinogen, this data would ascertain that the regulatory authorities may be expected to control the levels of 2-Mercapto benzimidazole 0.1 ppm in the drug substance (assuming a 1.5 µg/day daily dose). A method capable of such a lower level of detection is great challenge for analytical method development for controlling these genotoxic impurities. It was deemed necessary to develop an assay method for simultaneous quantification of 2-Mercapto benzimidazole and lansoprazole in API by LC-MS/MS with positive mode. Some of the analytical methods have been reported for Lansoprazole by spectroscopic determination [10-14] and voltammetric [15]. Methods in biological fluids using LCMS/MS and validated LC method [16-25] for the estimation of Lansoprazole in bulk and tablet dosage form. In that HPLC by UV detection and UV spectroscopic methods were used, which were not suitable for clinical trials because for their low sensitivity [26-29]. To the best of our knowledge no published method is available for the simultaneous determination of 2-Mercapto benzimidazole and lansoprazole API using LC-MS/MS. This method provides high degree of precision, accuracy, sensitivity and stability by simple liquid – liquid extraction based on liquid chromatography separation and detection in positive ion mode by electro-spray tandem mass spectrometry.

The present study was undertaken to develop a sensitive and rapid LC/MS/MS method for the determination of 2-Mercapto benzimidazole in lansoprazole API. Due to its higher selectivity and sensitivity LC/MS/MS has been adopted for quantification of 2-Mercapto benzimidazole in lansoprazole which is used for the prevention and treatment of gastric ulcer disease, gastroesophageal reflux disease related diseases (GERD).

EXPERIMENTAL

Chemicals and Reagents

Methanol and acetonitrile of HPLC grade were purchased from Merck (Mumbai, India). Analytical grade ammonium acetate, HPLC grade water were purchased from Merck, (Mumbai, India). Water used for the LC-MS/MS analysis was prepared from Milli Q water purification system procured from Millipore (Bangalore, India). Reference substance of 2-Mercapto benzimidazole was obtained from Sigma- Aldrich (St. Louis,USA).

Preparation of stock and standard solutions

Primary stock solutions of 2-Mercapto benzimidazole was prepared in 5 mg/mL of impurities in 100ml of diluent. Further dilution 0.001mg/mL with diluents further achieved on 0.00001mg/mL with diluent. Diluted final concentration 0.1 ppm to get working solutions for obtaining calibration curve.

HPLC operating conditions

A Shimadzu LC-20 AD Series HPLC system (Shimadzu Corporation, Kyoto, Japan) was used to inject 5 µL aliquots of the processed samples on a Hypersil BDS column C8 (50 x 3 mm, 3 µm), which was kept at 40 ± 2°C temperature. The mobile phase, a mixture of 5 mM ammonium acetate : methanol: acetonitrile (60:20:20 v/v/v) was filtered through a 0.45 µm membrane filter

(Millipore, USA or equivalent), then degassed ultrasonically for 5 min and delivered at a flow rate of 0.5 mL/min into the mass spectrometer electrospray ionization chamber.

Mass spectrometry operating conditions

Quantitation was achieved with MS-MS detection using a Applied bio system (AB SCIEX) API-4000 mass spectrometer (Foster City, CA, USA) equipped with Turboionspray ™ interface at 400ºC. The MS/MS method consists of positive mode. The ion spray voltage was set at 4500 V. The source parameters viz., the ion source gases GS1, GS2, Nebulizer gas and Collision energy were set at 30, 35, 14 psi and 35 V respectively. The compound parameter viz. the declustering potential (DP) and entrance potential were set at 50 and 10V. Detection of the ions was carried out in the multiple reaction monitoring mode (MRM) by monitoring the Parent ion of *m/z* 151.1, Daughter ion to the *m/z* 93.1 for 2-Mercapto benzimidazole. The analytical data obtained were processed by Analyst software™ (version 1.5.1).

RESULTS AND DISCUSSION

Method development and optimization

Optimization of chromatographic conditions was performed, particularly the composition of mobile phase, through several trials to achieve symmetric peak shapes of the analytes peaks, as well as short run time and low cost. Resolution positive mode lansoprazole was achieved by using acetonitrile as an organic content in the mobile phase. Separation was attempted using various combinations of acetonitrile and buffer with varying contents of each component on different columns like C_{18} and C_8 of different makes like Cyano, Chromosil and Hypersil BDS columns. Finally Hypersil BDS column was found to give the best chromatographic resolution with a flow rate of 0.5 mL/min and total run time of 5 min. The 2-Mercapto benzimidazole and lansoprazole were eluted at 0.7 and 1.8 min with multiple reaction monitoring (MRM) mode. The inclusion of 5mM ammonium acetate instead of pure water enhanced the response and improved the reproducibility.

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. Lansoprazole and 2-Mercapto benzimidazole compounds solutions were prepared individually at a concentration of about 0.01mg/mL in the diluents and a solution of Lansoprazole spiked with 2-Mercapto benzimidazole were also prepared. Specificity was established by injecting lansoprazole spiked with its impurity where in no interference was observed. Blank and specificity chromatograms are shown in Fig. 1.

Robustness

The robustness of the developed method was studied with slight and deliberate changes in experimental conditions. The effect of changes in flow rate of mobile phase (-2% to +2%) while the amounts of the other mobile phase components were held constant, column oven temperature (-2°C to +2°C).i.e at 38ºC and 42ºC buffer units was studied.

Determination of LOD and LOQ

The LOD and LOQ, as a measure of method sensitivity, were calculated from S/N (signal to noise) ratios. To determine LOD and LOQ values for a 2-Mercapto benzimidazole concentration were reduced sequentially such that they yield S/N ratio as 3.2 and 10.1 respectively. The determined LOD and LOQ chromatograms were shown in Fig.2. Data generated from six injections of (without API) containing 0.1 ppm of each 2-Mercapto benzimidazole with respect to an API sample concentration 5 mg/mL. The LOQ of 0.1 ppm is typical for the 2-Mercapto benzimidazole, with a LOD approximately three times less than LOQ. In addition, the relative efficiency of MRM modes in sensitivity improvement was also evaluated.

Recovery studies

The recovery studies by the standard addition method were performed to evaluate accuracy and specificity, accordingly the accuracy of the method was determined in triplicate at LOQ level in bulk drug sample. The recoveries were calculated. Excellent recovery values of 2-Mercapto benzimidazole 97.25 - 101.00 percentage were obtained. At such a low levels these recoveries and %RSD is <1.0 was satisfactory.Sample and accuracy at LOQ chromatograms are shown in Fig.3, and the relative standard deviation, %RSD were calculated from the average of triplicate analysis, which were shown in Table1. Further, the stability of 2-Mercapto benzimidazole was found as 48 hr and the stability of this impurity at different time intervals is presented in Table 3.

Linearity and range

The linearity test for the method was performed according to the guidelines laid by ICH. This method was evaluated at six different concentrations of analytes with in the range of 0.1-6 ng/mL. These standard solutions were prepared by suitable dilution of stock solution with mobile phase. The linearity of the plot was evaluated using least squares linear regression analysis by multiple reaction monitoring (MRM). The linearity of 2-Mercapto benzimidazole was 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 was produced by plotting the average of triplicate of 2-Mercapto benzimidazole injections against the concentrations expressed in percentage. The slope, intercept and correlation coefficient values were derived from linear least-square regression analysis and the data were presented in Table 2. It reveals that good correlation existed between the peak areas concentration of 2-Mercapto benzimidazole. Repeatability was checked by calculating the relative standard deviation (%RSD) of six determinations by injecting six freshly prepared solutions containing and 0.1 ppm of 2-Mercapto benzimidazole on the same day. The low %RSD values confirm the good precision of the developed method.

CONCLUSION

The present development study is based on validation of a highly sensitive, specific, reproducible and high-throughput LC-MS/MS method to quantification of 2-Mercapto bezimidazole in APIs. It has been established that it is highly sensitive with a limit of detection (LOD) of 0.033 ppm Trace level ammonium acetate is added to the mobile phase to enhance ionization and detection. Selected sample solvents were assessed for the effect on standard stability with and without 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 continuous understanding of the API manufacturing process. The method which is able to quantify them at ppm level is developed and validated. We can conclude that the developed method could be very useful for monitoring of 2-Mercapto benzimidazole in lansoprazole in its pure and tablet form.

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TABLES

Table 1

Accuracy/recovery of 2-Mercapto benzimidazole

S.No	Compound Name	Level	Sample area	Standard area	Spiked area	Theoretical concentration	Measured Concentration	%Recovery
1	2-MCB	LOQ	0	5796	5854	0.1	0.1010	101.00
		50%	0	112011	112414	2	2.0072	100.36
		100%	0	214524	212215	4	3.9566	98.92
		150%	0	328922	319878	6	5.8350	97.25

Table 2

Linearity plot of 2-Mercapto benzimidazole the concentration range of 0.1- 6 ppm level.

Concentration (ppm)	Peak Area
0.1	5746
1.6	91250
2.4	137452
3.2	184521
4	224512
4.8	273413
6	334587
Correlation	0.9998
Slope	55953.63119
Intercept	2129.392964

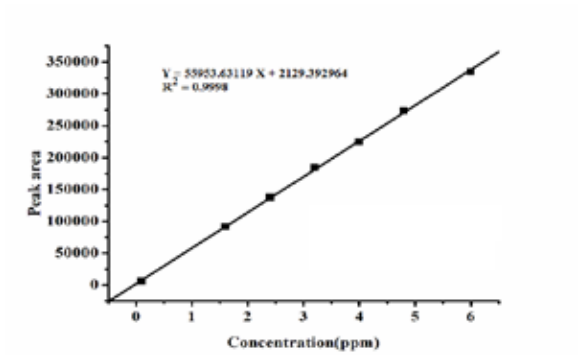

Table 3

Solution stability data of 2-Mercapto Benzimidazole indilent .

S.No	Compound Name	Injection time(hr)	Sample area	Standard area	Spiked area	Theoretical concentration	Measured concentration	%Recovery
1.	2-MCB	0	0	5796	5712	0.1	0.0986	98.55
		12	0	5897	5845	0.1	0.0991	99.12
		24	0	5548	5512	0.1	0.0994	99.35
		48	0	5945	5878	0.1	0.0989	98.87

Legends to Figures

Fig.1. (a) Specificity and (b) blank chromatograms of 2-Mercapto benzimidazole

Fig.2. (a) LOD and (b) LOQ chromatograms of 2-Mercapto benzimidazole

Fig.3. (a) Sample and (b) Spiked chromatograms of 2-Mercapto benzimidazole

Figures:

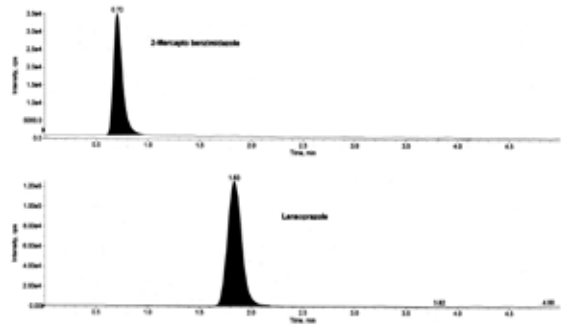

Fig.1. (a) Specificity chromatogram of 2-Mercapto benzimidazole

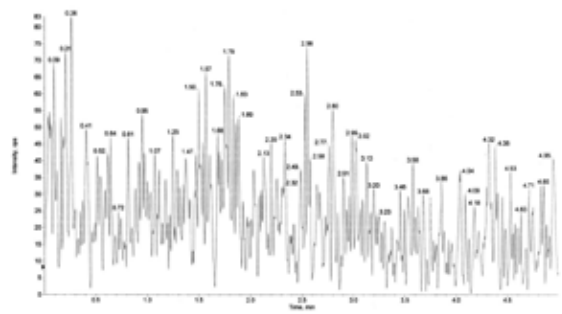

Fig.1. (b) Blank chromatogram of 2-Mercapto benzimidazole

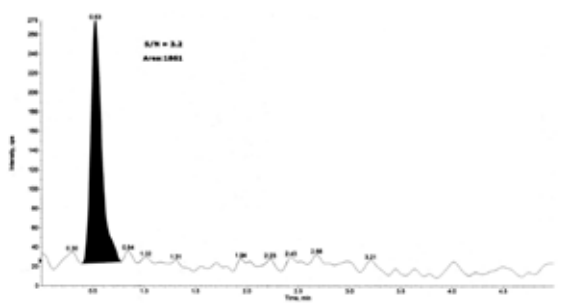

Fig.2. (a) LOD chromatogram of 2-Mercapto benzimidazole

Fig.2. (b) LOQ chromatogram of 2-Mercapto benzimidazole

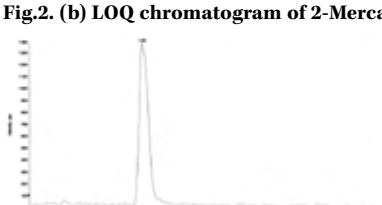

Fig.3. (a) Sample chromatogram of 2-Mercapto benzimidazole

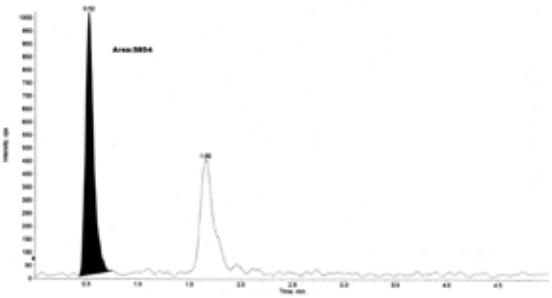

Fig.3. (b) Spiked chromatogram of 2-Mercapto benzimidazole

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