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Analytical Method Development and Validation of Different Marketed Omperazole Tablets by LC-MS/MS

***Amit M. Parekh[1]; Anil B. Shrirao[1]; Pritesh V. Thakkar[2]**

1. New Jersey Institute of Technology, Newark, New Jersey;

2. Bharati Vidyapeeth College of Pharmacy, Navi Mumbai, India.

Amp49@njit.edu; abs25@njit.edu; thakkarpriteshv@gmail.com;

*Corresponding author: Amit M. Parekh email id: amp49@njit.edu

ABSTRACT:

A Simple, high speed, sensitive and suitable analytical method for validation of Omeprazole (OME) by liquid chromatography-Tandem mass spectrometric (LC-MS/MS) assay method has been developed for the determination of omeprazole (OME) in different marketed tablets. Loperamide (LOP) was used as an internal standard (IS). The standard solutions and samples from different marketed tablets of omeprazole were chromatographed using reverse phase high performance liquid chromatography (RP-HPLC). The MS/MS detection was set at mass transitions of 346.24/197.9 m/z for Omeprazole and 477.3/266.0 m/z for Loperamide (IS) in positive ion mode. The standard curve obtained for Omeprazole was linear (R2= 0.9994) over the concentration range of 15.25-3906.25pg/ml. The results of intra- and inter-day precision studies were all within the acceptable limits (Branch, 2005). The overall average recoveries of analytes and IS were found approximately between 99% and 103 %. The high throughput LC-MS/MS method was validated for an accuracy, precision, sensitivity, recovery, and calibration range. The method has been successfully applied to the evaluation of existing marketed tablets containing omeprazole.

Keywords: Omeprazole, LC-MS/MS, analytical method, positive ion mode

INTRODUCTION

Gastroesophageal reflux disease (GERD) affects 25-40% of the adult population globally(Bough Jr et al., 1995). It occurs when stomach acid or stomach content flows back into esophagus. This phenomenon irritates esophagus lining causing GERD. Omeprazole, a proton pump inhibitor suppresses stomach acid section (Puscas, Coltau, Baican & Domuta, 1999) by specific inhibiting H+/K+ ATPase system irreversibly. Thus this action inhibits Hydrogen ions release and prevents back flow of stomach content in esophagus. Omeprazole is used in treatment of many other indications such as dyspepsia, peptic ulcer disease and Zollinger-Ellison syndrome (Falk, 1991). In Pharmacokinetic studies, it is a probe substrate for evaluating CYP 2C9 activity (Yamazaki et al., 1997).

As per the literature search, several LC-MS/MS methods have been reported for the determination of omeprazole individually or with other drugs in biological samples and as standards alone(Sivasubramanian & Anilkumar, 2007; Zhang et al., 2010; Vyas, Patel, Ladva, Joshi & Bapodra, 2011; Ahmad et al., 2015). It has also been used as internal standard (IS) in many studies. The objective of project was to

develop and validate suitable LC-MS/MS method for estimation of concentration of drug in marketed formulations.

2. EXPERIMENTAL

2.1 Chemicals and Reagents

Following items were ordered from Sigma-aldrich, St Loius, MO: Ammonium acetate(Cat#17836-50G), Methanol(34860-4L-R), Water (Cat#W3500-1L), Formic acid (399388-100ml), Dimethyl sulfoxide (DMSO) (D8418-250ml), Loperamide (IS)(Cat#34014-100MG), Omeprazole (Cat#19329).

2.2 Instrumentation and Chromatographic Conditions

An HPLC system (Shimadzu, Kyot, Japan) consisting of binary LC-AD prominence pump, an auto sampler (SIL-HTc) and a solvent degasser (DGU-20A3) was used for the study. The samples were mixed with equal volume of IS and 10uL were injected into the column. The analytical column was a Phenomenx 50 x 2.1mm, 4u was kept at 40^{o}C . The mobile phase A consisted of 0.1% Formic acid with 2mM Ammonium acetate in water and mobile phase B made up of 0.1% formic acid with 2mM ammonium acetate in Methanol. The injector wash solvent was 0.1% formic acid in 1:2:1 Acetonitrile/Methanol/ water (Parekh & Jadhav, 2009; Atienzar et al., 2014) .

The sensitivity of the multiple reaction monitoring (MRM) was optimized by testing with an infusion of 0.4ug/ml of analyte and 10ng/ml internal standard in mobile phase. The turbo gas temperature was 550C and the auxillary gas flow setting was 70. Nebulizing gas, curtain gas, collision gas flows were at instrument settings of 80, 50 and 50, respectively. The declustering potentials (DP) were 46V for omeprazole and 90V for Loperamide. The entrance potential (EP) were 10V for omeprazole and 10V for Loperamide. The mass spectrometer was operated in MRM mode with collision energy (CE), Collision cell exit potential (CXP) of 21eV and 4V for Omeprazole and 31eV and 24V for Loperamide, respectively. As described in Figure 1 and 2 the transitions (precursor to product) monitored were m/z 346.24-197.9 for Omeprazole and m/z 466.3-266.0 for Loperamide in Positive ion mode. The dwell time was 200ms for both. Both Q1 and Q3 quadrapoles were maintained at unit resolution.

2.3 Preparation of Standard solution

Omeprazole stock solution: Approximately 25 mg of OME was weighed and transferred to 50 mL volumetric flask containing 10µl Formic acid (Sivasubramanian & Anilkumar, 2007; Shrirao, Hussain, Cho & Perez-Castillejos, 2012). Then Methanol was dissolved so that the volume reaches the mark to make approximately 1000µg/ml stock solution. This stock solution was transferred in a reagent bottle with appropriate label and stored at 2-8 °C. Further dilutions of OME for spiking were prepared in dilution solution consisting of 0.1% Formic acid in 1:1 solution of DI water and methanol.

Loperamide (Internal standard) stock solution: Approximately 25 mg of LOP was weighed and transferred to 25 mL volumetric containing 10µl of Liquor Ammonia to get 1000µg/mL stocks with methanol. Stock solution was transferred in a reagent bottle with appropriate label and stored at 2-8 °C. Further dilution of internal standard was made in 0.1% Formic acid dissolved in Methanol/water in 1:1 ratio. The Concentration of internal standard used for the analysis was 10ng/ml throughout the analysis (Atienzar et al., 2014).

2.5 Assay procedure

Twenty tablets, each containing OME (20.6 mg) tablets weighed, finely powdered and weighed accurately about powder equivalent of 20mg of OME sample and transfer it into a 50ml volumetric flask. The sample was extracted with 1:1 methanol/water and volume was adjusted into 50ml. The solution was filtered through 0.45µ membrane filter and sonicated before

use. From the filtrate 0.5ml was transferred into volumetric flask and make up the volume with mobile phase. The above indices procedure was followed for all marketed products.

For capsules, all the powder from twenty capsules were collected and solubilized with Methanol/water 1:1.
The final concentration of both Tablet and capsules were made as such that it fits the standard curve and were back calculated.

3. RESULTS AND DISCUSSION

3.1 Method development

The goal of this work was to develop and validate a simple, rapid, selective, and sensitive assay method for the quantitation of OME in marketed formulations. To achieve the goal during method development, different options were evaluated to optimize detection parameters, and chromatography. It was found that the best signal was achieved with in positive ion mode using gradient mobile phase. The gradient phase comprises of 0.1% Formic acid and 2mM ammonium acetate in water for Mobile phase A and 0.1% Formic acid and 2mM ammonium acetate in methanol for Mobile phase B. With this optimized mobile phase, the m/z value of Omeprazole and Loperamide were 346.24/197.9 and 466.3/266.0 respectively. The different concentrations of both mobile phases A and B were analyzed in order to develop the LC method. The optimized injection timing of mobile phases is described in Table1 where the injection cycle time was 2 minutes. Omeprazole is polar while Loperamide is relative hydrophobic (Ray & Yaksh, 2008). The best separation was achieved with 2 minutes cycle and variant concentration with time is plotted in the chart described in table. Good separation of Omeprazole and Internal standard Loperamide was achieved. In addition, it maintained good shapes with the retention times at ~0.74 min and ~0.71 for Omeprazole and Loperamide. Analyte and internal standard were well retained and were well separated indicating the method is well-suited for simultaneous analysis of analytes possessing diverse polarities.

3.2 Validation

ICH guidelines and USFDA guidelines were followed for method validation (Branch, 2005). The method was validated for its selectivity, stability, linearity, accuracy, precision and robustness.

3.2.1 Selectivity: The selectivity of the method was assessed by comparing chromatogram of negative controls (blank which is methanol) and samples (drug and IS). The retention times of drug and internal standard were observed at 0.74 and 0.71. It was observed from figure 3 and 4 there were no interferences in the peak shape and retention times.

3.2.2 Linearity: The standard curve was plotted using the peak area ratio versus the concertation of the analytes. The standard curve was found to be linear over the concentration range from 15.25pg/ml to 3906.25pg/ml. The linearity graph and peak area are shown in table 2 and figure 5. The linearity was represented by a linear regression equation as follows.

$Y= 0.000114x+0.0014$ ($R^2 = 0.9997$)

The sample solutions prepared from marketed formulation were injected and plotted on the standard curve as final confirmation of method.

3.2.3 Precision : Precision studies were carried out to assure the reproducibility of the proposed method . The reproducibility was determined by preparing and measuring six

identical concentration of the standard solutions. The intraday precision study was carried out by preparing drug solution of identical concentrations and analyzing it at three different times in a day. The same procedure was followed for three different days to determine interday precision (Halima, Aneesh, Ghosh & Thomas, 2012). The results of intraday and interday precision studies are shown in table 3 and table 4. These results showed a good reproducibility with recovery ranging from 99% to 103% of the actual concentrations.

3.2.4 Accuracy: The accuracy of the developed method was determined by calculating recoveries of OME by method of standard additions(Shah, Suthar, Baldania, Chhalotiya & Bhatt, 2012). Equal volumes of known amounts of OME were added to a pre-quantified sample solution, and the amount of OME was estimated by measuring the peak areas and by fitting these values to the straight-line equation of standard curve.

3.2.5 Robustness: Analysis was carried out at two different temperatures, room temperature and at 4ºC to determine the robustness of the method. The results indicates that method is robust with less than 2% standard deviation. The results are described in table 6.

3.2.6 LOQ AND LOD: Limit of detection (LOD) is the lowest amount of analyte in the sample that can be detected. Limit of quantification (LOQ)is the lowest amount of analyte in the sample that can be quantitatively determined by suitable precision and accuracy (Shrirao, Hussain, Cho & Perez-Castillejos). The values of LOQ and LOD were found to be 7.81 and 3.4 pg/ml respectively.

4. Quantification in marketed formulation:

After developing and validating the method market formulations were evaluated. From the stock solution of tablets, appropriate dilutions were made so that the quantification value is within standard curve. The dilution was made in methanol: water 1:1 containing 0.1% Formic acid. The results obtained from analysis are given in Table 7.

5. Conclusion:

The LC-MS/MS method reported in this paper was validated according to internationally accepted criteria. This method can be considered reliable and feasible on the basis of validation data. ESI technique has proven effective in generating ions closed to the protonated molecule with sufficient intensity to be monitoring quantitatively, accurately and selectively. The method is highly specific and precise with run time of 2 min allows the analysis of a large number of samples in a short period of time. The method was applied successfully to the analysis of OME tablet dosage form so it can be easily and conveniently adopted for routine QC analysis of raw materials, formulations, pharmacokinetic studies and also for dissolution studies.

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Tables

Time in minutes	Mobile Phase B in %	Event
0.01	2	Start
0.10	2	
0.30	90	
1.31	90	
1.50	2	
2.00	2	Stop

Table 1. Gradient elution of OME

Sample conc(pg/ml)	Analyte peak Area	IS peak Area	Calculated value (pg/ml)	Accuracy (%)
15.25	1350	442000	14.39	94.4
30.50	2150	442000	30.80	101
61.00	3830	442000	62.83	103
122.00	7350	433000	136.64	112
244.00	13600	442000	258.64	106
488.00	25800	432000	512.4	105
976.00	48900	442000	951.60	97.5
1952.00	95500	442000	1883.68	96.5
3904.00	199000	442000	3943.04	101

Table 2. Results from linearity plot of Omeprazole using Loperamide as IS.

Analyte concentration (pg/ml)	Analyte peak area	IS peak area	Calculated concentration (pg/ml)	
50	3130	442000	49.80	%Nominal=99.63
50	3110	442000	49.50	%CV= 2.49
50	2990	442000	47.60	
50	3210	442000	51.10	Mean= 49.816
50	3190	442000	50.80	SD= 1.241
50	3150	442000	50.10	

Table 3. Precision results showing repeatability

DAYS	Analyte concentration (pg/ml)	Intra-day Precision				Inter-Day precision			
		% Nominal	% CV	Mean found (pg/ml)	±SD	% Nominal	%CV	Mean found (pcg/ml)	±SD
1	50	100.63	1.775	50.316	0.893	102.96	4.541	51.483	2.337
2	50	100.23	1.500	50.116	0.752	100.60	4.107	50.301	2.065
3	50	101.33	3.235	50.667	1.639	101.42	3.952	50.716	2.004

Table 4. Precision of method for determining OME in Quality control sample

Labelled concentration (pg/ml)	Amount added (pg/ml)	Theoretical Final value (pg/ml)	Calculated value (pg/ml), n=3	%Nominal
100	20	60	61.20 ± 1.323	102 ± 2.161
100	50	75	75.42 ± 0.876	100.56 ± 1.161
100	100	100	99.32 ± 2.843	99.32 ± 2.863

Table 5. Accuracy reading of Omeprazole

Temperature	Concentration (pg/ml)	Calculated value (pg/ml)	% Recovered
Room temperature	50	50.22 ± 1.100	100.44 ±2.190
4ºC	50	51.34 ± 1.431	102.68 ± 2.787

Table 6. Results showing robustness of method.

Name of the Brand	Claimed value (mg)	Mean Calculated value (mg) , n=3	Mean% of labeled amount, (n=3)	%RSD
Equate	20.6	20.533 ± 0.2516	99.67	1.225
Prilosec	20.6	20.642 ± 0.079	100.19	0.384
Zegerid	20.6	20.54 ± 0.0854	102.7	0.415

Table 7. Results of evaluation of marketed formulations containing omeprazole

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Figures

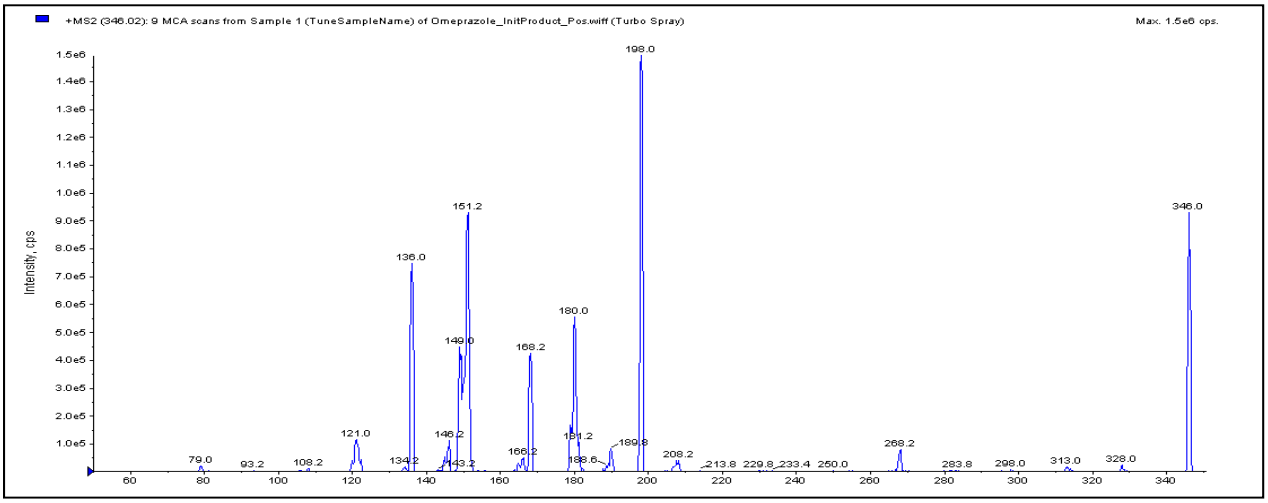

Figure 1. Mass Spectrum of Omeprazole having ion transitions of m/z 346-198

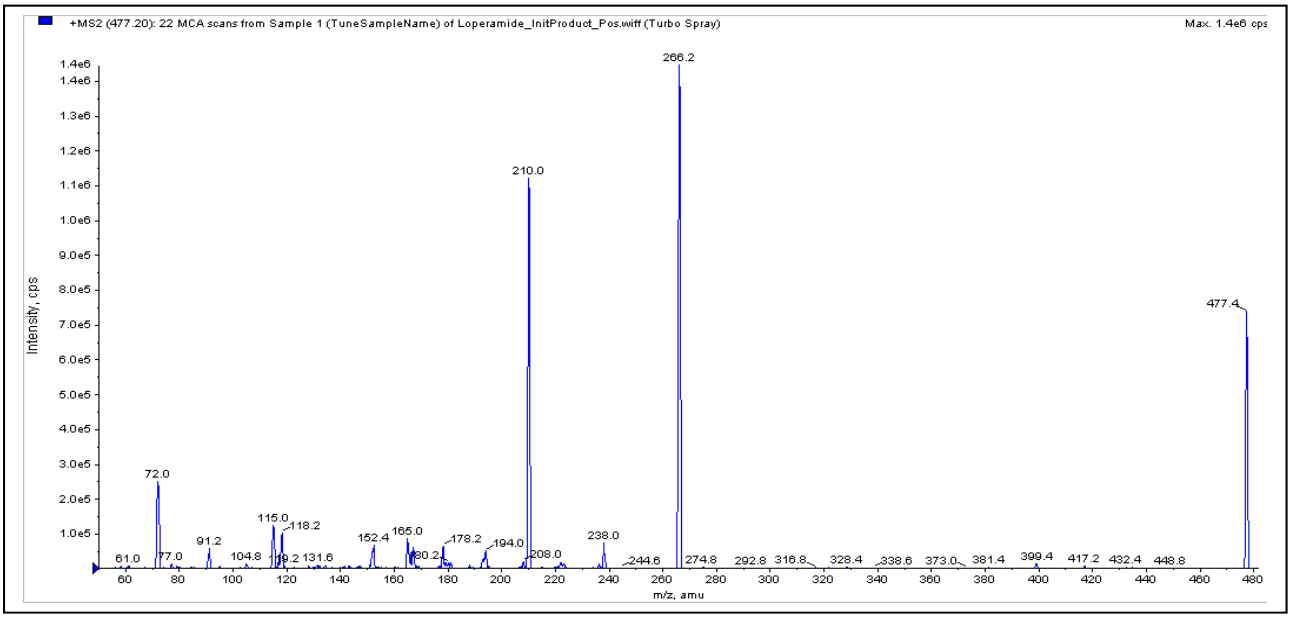

Figure 2. Mass Spectrum of Internal standard(IS) Loperamide having ion transitions of m/z 477.3-266.0

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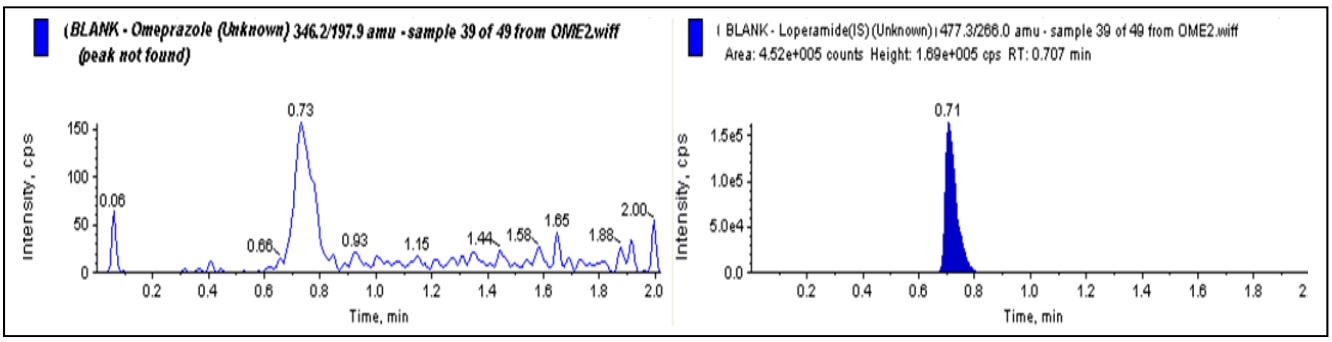

Figure 2. Blank + Internal standard.

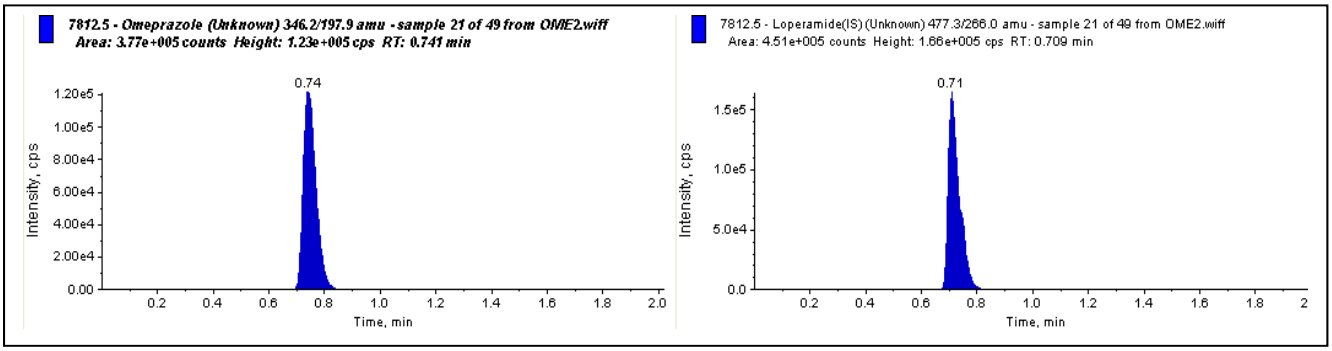

Figure 3. Representative Chromatograms for standard solution OME and LOP(IS).

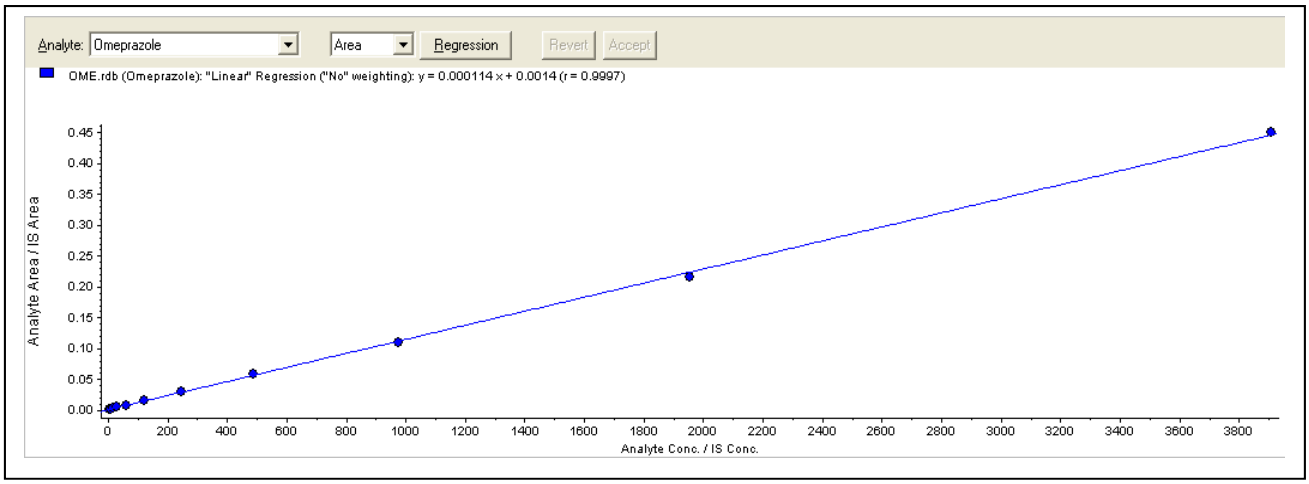

Figure 4. Standard curve plot of Omeprazole.