Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2011: 3 (5) 138-145
(http://scholarsresearchlibrary.com/archive.html)

Der Pharmacia Lettre
Scholars Research Library
ISSN 0974-248X
USA CODEN: DPLEB4

Quantification of Esomeprazole in Human plasma by Liquid Chromatography Tandem Mass Spectrometry and its Application to Bioequivalence Study

Ramakotaiah.Mogili[1,3*], Kanchanamala.Kanala[1]. Chandrasekhar.Kottapalli.Bannoth[1], Babu Rao.Chandu[4], Bala sekhara Reddy.Challa[2]

[1]*Jawaharlal Nehru Technological University, Anantapur, Andhra Pradesh, India*
[2]*Nirmala College of Pharmacy, Madras Road, Kadapa, Andhra Pradesh, India*
[3]*Siddhartha Institute of Pharmaceutical sciences, Jonnalagadda, Narasaraopet, Guntur, Andhra Pradesh, India*
[4]*Donbosco PG College of Pharmacy,Guntur, Andhra Pradesh, India*

ABSTRACT

The present study aims at developing a simple, sensitive and specific liquid chromatography–tandem mass spectrometry (LC-MS/MS) method for the quantification of Esomeprazole (EO) in human plasma using Omeprazole-d3 (OMD3) as an internal standard (IS). Chromatographic separation was performed on Xbridge C18, 50 x 4.6 mm, 5 µm column with an isocratic mobile phase composed of 5mM Ammonium formate (pH 9.0) : Acetonitrile (70:30 v/v) at a flow-rate of 0.6 mL/min. EO and OMD3 were detected with proton adducts at m/z 346.1→198.0 and 349.0→197.9 in multiple reaction monitoring (MRM) positive mode respectively. EO and OMD3 were extracted by Precipitation method. The method was validated over a linear concentration range of 5.0-2000.0 ng/mL with a correlation coefficient of (r^2) ≥ 0.9989. This method demonstrated intra and inter-day Precision within 1.6 to 2.3 and 2.0 to 2.2 % and Accuracy within 97.9 to 100.7 and 98.0 to 99.3 %. EO was found to be stable throughout freeze-thawing cycles, bench top and postoperative stability studies. This method was utilized successfully for the analysis of plasma samples following oral administration of EO (40 mg) in 27 healthy Indian male human volunteers under fasting conditions.

Keywords: Mass spectrometry; Precipitation method; Bioequivalence; Esomeprazole.

INTRODUCTION

Esomeprazole Magnesium trihydrate is chemically described as, bis (5- methoxy-2-[(S) - [(4-methoxy -3,5- dimethyl -2- pyridnyl) methyl] sulphinyl] - H - benzimidazole -1-yl) magnesium trihydrate compound. The molecular formula is $(C_{17}H_{18}N_3O_3S)_2$ Mg X 3 H_2O which corresponds

to a molecular weight of 767.2 and 713.1 on anhydrous basis. Esomeprazole is the S-enantiomer of Omeprazole. Esomeprazole is a proton pump inhibitor which reduces gastric acid secretion through inhibition of H^+/K^+-ATPase in gastric parietal cells by inhibiting the functioning of this enzyme, the drug prevents formation of gastric acid. Esomeprazole is used in the treatment of dyspepsia, peptic ulcer disease (PUD), gastroesophageal reflux disease (GORD/GERD) and Zollinger-Ellison syndrome. (1).

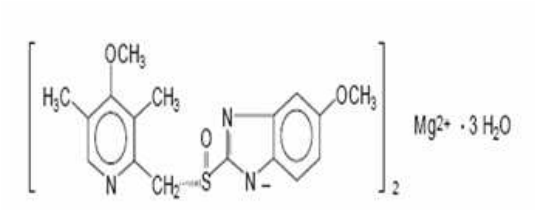
Several techniques such as, Liquid chromatography (LC) (2-8, 14), Supercritical fluid chromatography (13,16), Capillary electrophorcis (15) Preparative chiral chromatography (21), NMR (18) methods have been reported in the literature for the quantitative estimation of EO in biological fluids (2-14) and pharmaceutical (15-21) compounds. Moreover, Capillary electrophorcis, Preparative chiral chromatography, Supercritical fluid chromatography involves a tedious extraction procedure involving too many steps. Quantification of EO in human plasma by using LC-MS/MS (2-4) and HPLC (6-8, 14) were reported. Authors (2-4) could not achieve better results for quantification of EO in terms of Sensitivity, ruggedness, Extraction, runtime and recovery.

The Aim of present research is to develop and validate the simple, sensitive, selective, rugged and reproducible analytical method for quantification of EO in Human plasma samples by LC-MS/MS. Moreover, the analyte is to be compare with deuterated internal standard, which is most useful in selectivity and matrix effect experiments by using LC-MS/MS.

MATERIALS AND METHODS

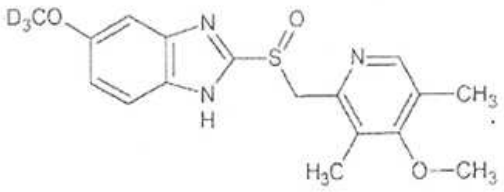
Chemicals and Reagents

Esomeprazole (Fig.1a) and Omeprazole-d3 (Fig.1b) obtained from Dr.Reddy's Labs, Hyderabad. All other chemicals and solvents were purchased from S.D fine chemicals Mumbai. Human plasma was obtained from Navjeevan blood blank, Hyderabad.



Esomeprazole Magnesium trihydrate

Fig.1a



Omeprazole d3

Fig.1b

Fig.1. Chemical structures of Esomeprazole (Fig.1a) and Omeprazole-d3 (Fig.1b)

Instrumentation

HPLC system (1200 series model, Agilent Technologies, Waldbronn, Germany), Mass spectrometry API 4000 triple quadrupole instrument (ABI-SCIEX, Toronto, Canada) using multiple reaction monitoring(MRM).

Detection

Detection was performed by turbo ion spray positive mode with Unit resolution. For EO the MH^+ (m/z: 346.1) was monitored as the precursor ion and fragmented at m/z 198.0 as product ion. For internal standard, the MH^+ m/z 349.0 was monitored as the precursor ion and fragmented at m/z 197.9 as the product ion. Mass parameters were optimised as Source temperature 500 °C, heater gas 30 psi (nitrogen), nebulizer gas 40 psi (nitrogen), Curtain gas 20 psi (nitrogen), CAD gas 3 psi(nitrogen), Ion Spray (IS) voltage 5500 volts, Source flow rate 600 µL/min without split, Entrance potential(EP) 10 V, Declustering potential (DP) 45 V for Analyte and 45V for IS, Collision energy(CE) 20 V for Analyte and 20 V for IS, Collision cell exit (CXP) potential 10 V for both Analyte and I.S .

Chromatographic conditions

Xbridge C18, 50x 4.6 mm 5 µm was selected as the analytical column at 40°C. The mobile phase composition was 5mM Ammonium formate (pH 9.0) : Acetonitrile (70:30 v/v). at 0.6 mL/min flow. Omeprazole-d3 was found to be appropriate internal standard in terms of chromatography and extractability. The retention time of EO, OMD3 was found to be 2.7 ± 0.2 min with a total run time of 4.0 min.

Preparation of Standards and Quality control (QC) Samples

Standard stock solutions of EO (100.0µg/mL) and OMD3 (100.0µg/mL) were prepared in methanol. The IS spiking solution (250.0 ng/mL) was prepared in 20% methanol from OMD3 stocksolution. Standard stock solutions and IS spiking solutions were stored in refrigerator (2 - 8 °C) until analysis. Standard stock solutions were added to drug-free human plasma to obtain EO concentration levels of 5.0, 10.0, 50.0, 100.0, 200.0, 400.0, 800.0, 1200.0, 1600.0 and 2000.0 ng/mL for analytical standards and 5.0, 15.0, 700.0 and 1400.0 ng/mL for Quality control standards and stored in freezer at -30°C until analysis. The Aqueous standards were prepared in reconstitution solution (30% Acetonitrile in 5mM Ammonium formate (pH-9.0)) and stored in fridge at 2-8°C until analysis.

Sample preparation

Precipitation extraction method was used to isolate EO and OMD3 from human plasma. For this, 50 µL of OMD3 (250.0 ng/mL) and 850 µL of plasma sample (respective concentration) was added into labeled polypropylene tubes and vortexed briefly about 5 minutes followed by centrifuge at 14000 rpm for approximately 2 min at ambient temperature. From this, 100µL of supernatant sample was transferred into labeled polypropylene tubes containing 400µL of 15% Acetonitrile in 5mM Ammonium formate (pH 9.0) and vortexed briefly. Finally, transferred the sample into auto sampler vials for injection.

Analysis of patient samples

The bioanalytical method described above was used to determine EO concentrations in plasma following oral administration of healthy human volunteers. These volunteers were contracted in APL Research Centre, Hyderabad, India. Each volunteer was administered 40 mg dose (one 40mg capsules) in 27 healthy volunteers by oral administration with 240 mL of drinking water. The reference product Nexium capsules (Astrazenica) 40 mg, USA and Test product EO capsules (Test capsules) 40 mg was used. Study protocol was approved by IEC (Institutional Ethical committee) as per ICMR (Indian council of medical research). Blood samples were

collected as pre-dose (0) hr 5 minutes prior to dosing followed by further samples at 0.75, 1, 1.333, 1.667, 2, 2.333, 2.667, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10.5 and 12 hours. After dosing 4 ml blood was collected each time in vaccutainers containing K_2EDTA. A total of 38 (19 time points for test and 19 time points for reference) time points were collected by using centrifugation at 3200 rpm, 10°C, 10 min and stored at -30 °C until sample analysis. Test and reference formulations were administered to same human volunteers under fasting conditions separately with proper washing periods as per approved protocol by IEC.

RESULTS AND DISCUSSION

Method development and validation

The goal of this work was to develop and validate a simple, rapid and sensitive assay method for the quantitative determination of EO from plasma samples. LC-MS/MS has been used as one of the most powerful analytical tool in clinical pharmacokinetics for its selectivity, sensitivity, reproducibility and rapid analysis.

The MS optimization was performed by direct infusion of solutions of both EO and OMD3 into the ESI source of the mass spectrometer. Other parameters, such as gas parameters (Nebulizer gas, heater gas, Curtain gasses) and compound parameters (Declustering potential(DP), Entrance Potential (EP), Focusing potential(FP), Collision cell exit potential (EXP), Collision energy (CE)) were optimized through several trails to obtain a better spray shape and better ionization to form the protonated ions of EO *m*/*z* 198.0 and OMD3 *m*/*z* 197.9.

Chromatographic optimization

Initially, we tried with different extraction techniques like, SPE, Precipitation techniques. Finally, Precipitation was selected as suitable extraction for drug and IS interms of recovery and reproducibility. Chromatographic conditions especially, the composition and nature of the mobile phase, different columns were optimized through several trials to achieve best resolution to increase the signal of EO and OMD3. A good separation and elution were achieved with Xbridge C18, 50x 4.6 mm 5 µm, 5 mM Ammonium formate (pH 9.0): Acetonitrile (70:30v/v) as the mobile phase at a flow-rate of 0.6 mL/min with 10 µL of injection volume.

Selectivity& Specificity

Selectivity was performed by using six different lots of human plasma. The analysis of EO and OMD3 using MRM function was highly selective with no interfering compounds Specificity was performed by screened blank plasma. Chromatograms obtained (LOQ) from plasma spiked with EO (5.0 ng/mL) and OMD3 (250.0 ng/mL) are shown in (Fig. 2).

Matrix effect

The overall precision of the matrix factor is expressed as Coefficient of Variation (CV%) and was determined to be 2.3 for EO and 1.4 for OMD3

Linearity, Precision and Accuracy

Calibration curves were plotted as the peak area ratio (EO/OMD3) versus EO concentration. Calibration was found to be linear over the concentration range of 5.0-2000.0 ng/mL. The %CV was less than 5% and the accuracy ranged from 97.8 to 102.6 %. The correlation

coefficients (r^2) were greater than 0.9992 for all curves (Table 1). Precision and accuracy for this method was controlled by calculating the intra and inter-batch variations at three concentrations (15.0, 700.0 and 1400.0 ng/mL) of QC samples in six replicates. As shown in (Table 2), the intra-batch %CV was less than 2.3 % and the accuracy ranged from 97.9 to 100.7 %. Inter-batch %CV was less than 2.2 % and the accuracy ranged from 98.0 to 99.3 %. These results indicate the adequate reliability and reproducibility of this method within the analytical range.

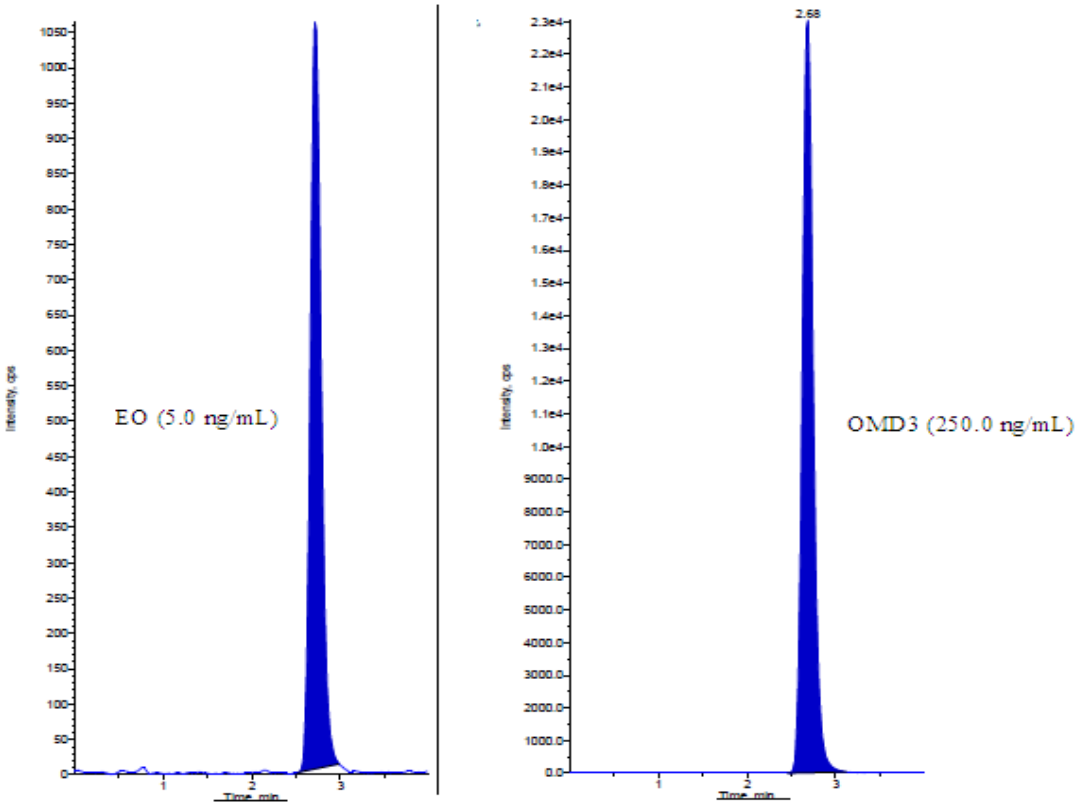


Fig: 2.Chromatogram of LOQ

Table 1: Calibration curves details

Spiking plasma concentration (ng/mL)	Concentration measured(mean) (ng/mL)±SD	CV (%) (n = 5)	Accuracy %
5.0	4.9 ± 0.05	1.0	98.6
10.0	10.2 ± 0.2	2.0	102.6
50.0	49.5± 0.5	1.0	99.2
100.0	101.2 ± 0.9	0.9	101.3
200.0	204.7± 2.9	1.4	102.4
400.0	402.2 ± 10.6	2.7	100.6
800.0	782.1 ± 21.3	2.7	97.8
1200.0	1181.5 ± 26.0	2.2	98.5
1600.0	1604.7 ± 30.2	1.9	100.3
2000.0	1976.5 ± 38.7	2.0	98.8

Table 2: Precision and accuracy (analysis with spiking plasma samples at three different concentrations)

Spiked plasma concen-tration (ng/mL)	Within-run			Between-run		
	Concentration measured (*n*=6) (ng/mL) (mean±S.D.)	(%)CV	Accuracy %	Concentration measured (*n*=30) (ng/mL) (mean±S.D.)	(%)CV	Accuracy %
15.0	14.6±0.3	2.3	97.9	14.7± 0.31	2.1	98.0
700.0	705.2±15.1	2.1	100.7	695.3± 13.6	2.0	99.3
1400.0	1398.2±22.0	1.6	99.9	1377.1±30.8	2.2	98.4

Limits of Quantification (LOQ) and Limit of Detection (LOD)

The limit of quantification, Limit of detection were proved as 5.0 (ng/mL) and 0.05 pg respectively.

Stability (Freeze-thaw, Auto sampler, Bench top, Long term)

Quantification of the EO in plasma subjected to 3 freeze-thaw (-30^{o}C to room temperature) cycles showed the stability of the analyte. No significant degradation of the EO was observed even after 50.5h storage period in the auto sampler tray and the final concentrations of EO was between 97 to 102.0 %. In addition, the long-term stability of EO in QC samples after 55 days of storage at -30^{o}C was also evaluated. The concentrations ranged from 98.0 to 104.0%. These results confirmed the stability of EO in human plasma for at least 55 days at -30^{o}C. (Table 3)

Table 3: Stability of the Analyte in human plasma

Spiking plasma concentration (ng/mL)	Room temperature stability		Processed sample stability		Long term stability		Freeze and thaw stability	
	24.5 h		50.5 h		55 days		Cycle 3 (48 h)	
	Concentration measured (*n*=6) (ng/mL) (mean±S.D)	(%) CV (*n*=6)	Concentration measured (*n*=6) (ng/mL) (mean±S.D)	(%)CV (*n*=6)	Concentration measured (*n*=6) (ng/mL) (mean±S.D)	(%)CV (*n*=6)	Concentration measured (*n*=6) (ng/mL) (mean±S.D)	(%)CV (*n*=6)
15.0	14.3 ± 0.4	3.2	14.4 ± 0.2	1.5	14.6 ± 0.3	2.0	14.3 ± 0.16	1.1
1400.0	1346.6 ± 20.6	1.5	1378.3 ± 24.8	1.8	1365.1 ± 23.4	1.7	1355.2 ± 20.7	1.5

Recovery

The recovery of EO was determined at three different concentrations 15.0, 700.0 and 1400.0 ng/mL were found to be 94.5, 94.2 and 96.6 %, respectively. The overall average recovery of EO and OMD3 were found to be 95.1 and 95.1% respectively.

Application to biological samples

The above validated method was used in the determination of EO in plasma samples for establishing the bioequivalence of a single 40 mg dose (one 40 mg capsule) in 27 healthy volunteers. Typical plasma concentration versus time profiles is shown in (Fig. 3). All the plasma concentrations of EO were in the standard curve region and retained above the 5.0 ng/mL (LOQ) for the entire sampling period. The observed maximum plasma concentration (C_{max}) for the standard and test were 1453.151±514.82 and 1287.51±478.76 ng/mL, respectively. The corresponding time of maximum concentration (T_{max}) for reference and test were found to be 2.667 and 3.5 hr, respectively. The value of area under the curve from time 0 to the last sampling time (AUC_{0-t}) for the standard and test were found to be 6396.45±196.92 and 6075.16

± 213.61 ng hr/mL, respectively. And the area under the curve from 0 to ∞ ($AUC_{0-\infty}$) was 6621.34 ng hr/mL for the standard and 6294.91 ng hr/mL for the test. The elimination half-life ($t_{1/2}$) was 2.12 hr for the reference drug and 2.08 hr for generic drug. In addition, the mean ratio of $AUC_{0-t}/AUC_{0-\infty}$ was higher than 90% with following the Food and Drug Administration Bioequivalence Guideline. The ratio test/reference (T/R) and 90% confidence intervals (90 CIs) for overall analysis were comprised within the previously stipulated range (80-125%). The ratio T/R and 90 CIs (in parenthesis) were 88.6 % for Cmax, 94.9 % for AUC_{0-t} and 95.1 % for $AUC_{0-\infty}$. Therefore, it can be concluded that the two esomeprazole formulations (reference and test) analyzed are bioequivalent in terms of rate and extent of absorption.

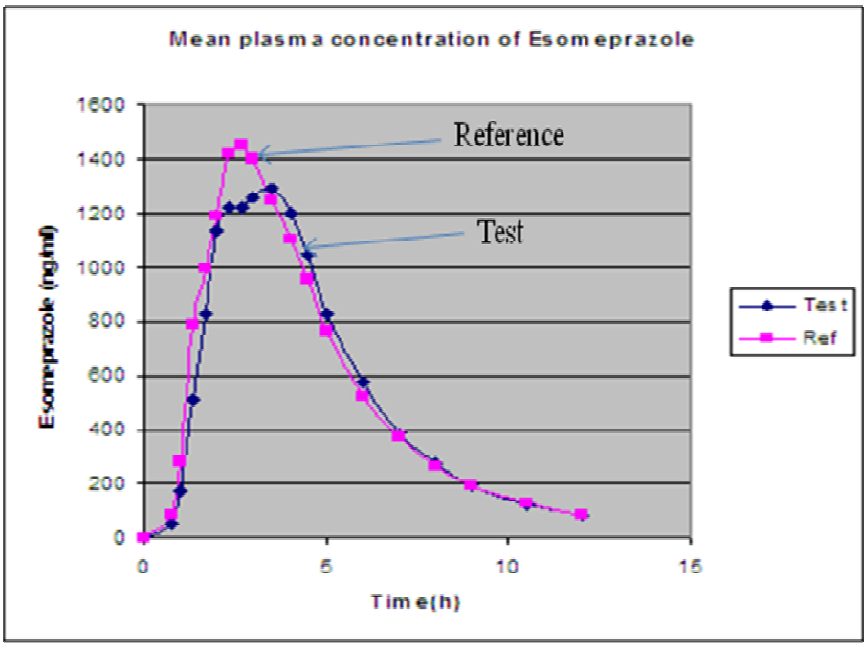


Fig: 3. Mean plasma concentrations of test vs. reference after a 200 mg dose (one 200mg Tablet) single oral dose (25 healthy volunteers).

CONCLUSION

The method described here is simple, sensitive, selective, rugged, reproducible and fast. Each sample requires less than 4.0 min of analysis time. The sensitivity of the assay is sufficient to follow accurately the pharmacokinetics of Esomeprazole following oral administration.

Acknowledgements
Authors wish to thank the support received (for providing Literature survey) from IICT (Indian institute of chemical technology) Hyderabad India., APL Research Pvt. Ltd Hyderabad, India to carry out this Research work.

REFERENCES

[1] http://en.wikipedia.org/wiki/Esomeprazole.

[2] Martens-Lobenhoffer J, Reiche I,Troger U, Monkemuller K, Malfertheiner P, Bode Boger SM. (**2007**) *Chromatogr B Analyt Technol Biomed Life Sci*,;857(2):301-307.
[3] Hultman.Ia, Stenhoff.H, Liljeblad. M. (**2007**) *J Chromatogr B Analyt Technol Biomed Life Sci,;* 848(2):317-322.
[4] Stenhoff.H, Blomqvist.A, Lagerstrom.P.O (**1999**). *J Chromatogr B Biomed Sci Appl*,;734(2):191-201.
[5] Zhou G, Tan ZR, Zhang W, Ou-Yang DS, Chen Y, Guo D, Liu YZ, Fan L, Deng HW(**2009**). *Acta Pharmacol Sin*, ; 30(9):1330-1336.
[6] Orlando.R.M, Bonato P.S. (**2003**) *J.Chromatogr B Analyt Technol Biomed Life Sci* ,; 795(2): 227-235.
[7] Cairns AM, Chiou RH, Rogers JD, Demetriades JL.(**1995**). *J Chromatogr B Biomed Appl*; 666(2):323-328.
[8] Rhim S.Y , Park J.H, Park Y.S, Lee M.H, Hwang K.G, Kim Y.S, Shaw L.M, Lee Y.S, Kang J.S.(**2009**) *Int J Clin Pharmacol Ther, ;*47(1):23-29.
[9] Baldwin RM,Ohisson S, Pedersen RS, Mwinvi J, Ingelman-Sundberg M,Eliasson E, Bertilsson L(**2008**). *Br J Clin Pharmacol* ;65(5):767-774.
[10] Hassan-Alin M, Andersson T, Niazi M, Rohss K(**2005**). *Eur J Clin Pharmacol*;60(11):779-784.
[11] Hassan-Alin M, Andersson T, Bredberg E, Rohss K(**2000**). *Eur J Clin Pharmacol*;56(9-10):665-670.
[12] Tonini M, Vigneri S, Savarino V, Scarpignato C(**2001**). *Dig Liver Dis* , 2002; 34(1): 87.
[13] Bhoir SI,Bhoir IC, Bhagwat AM, Sundaresan M, (**2001**). *J Chromatogr B Analyt Technol Biomed Sci Appl*,;757(1):39-47.
[14] Tamminga WJ, Wemer J, Oosterhuis B,Brakenhoff JP, Gerrits MG, de Zeeuw RA, deLeijLF, Jonkman JH (**2001**). *Eur J Clin Pharmacol*,;57(2):143-146.
[15] OIsson J , Stegander F, Marlin N, Wan H, Blomberg LG(**2006**). *J Chromatogr A;* 1129(2):291-295.
[16] Toribio L, Alonso C, del Nozal MJ, Bernal JL, Martin MT (**2006**). *J Chromatigr A,;*1137(1):30-35.
[17] Kanazawa H, Okada A, Higaki M, Yokota H, Mashige F, Nakahara K (**2003**). *J Pharm Biomed Anal,;* 30(6):1817-1824.
[18] Redondo J, Capdevila A, Latorre I,(**2010**) *Chirality*,;22(5):472-478.
[19] Li XQ, Weidolf L, Simonsson R, Andersson TB,(**2005**) *J Pharmacol Exp Ther*,;315(2):777-787.
[20] Mihara K, Svensson US, Tybring G, Hai TN, Bertilsson L.A, Shton M, (**1999**). *Fundam Clin Pharmacol;;*13:671-5.
[21] Andersson S, Nelander H, Ohlen K, (**2007**). *Chirality* ;19(9):706-715.
[22] Guidance for industry: bioanalytical method validation, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), May **2001**.