Asian Journal of Chemistry; Vol. 28, No. 8 (2016), 1783-1789
ASIAN JOURNAL OF CHEMISTRY
http://dx.doi.org/10.14233/ajchem.2016.19822

Tandem Mass Spectrometric Method for the Estimation of Meloxicam in Plasma Samples: Application to Pharmacokinetic Studies

M. NAGESWARA RAO[1], K. VIJAY[2], P.R.K. VENI[3], K. GEETHA BHAVANI[4], K. BALA MURALI KRISHNA[1] and B. HARI BABU[1,*]

[1]Department of Chemistry, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur-522510, India
[2]University College of Pharmaceutical Sciences, Acharya NagarjunaUniversity, Nagarjuna Nagar, Guntur-522 510, India
[3]Sasi Institute of Technology and Engineering, Tadepalligudem-534 101, India
[4]J.M.J. College for Women, Tenali-522 202, India

*Corresponding author: E-mail: dr.b.haribabu@gmail.com

Received: 5 January 2016; *Accepted*: 14 March 2016; *Published online*: 30 April 2016; AJC-17891

A simple sensitive and specific tandem mass spectrometric (LC-MS/MS) method for the determination of meloxicam in human plasma using Phenomenex Gemini C_{18} column (50 mm × 4.6 i.d, 5 µm) was developed. The analyte and the internal standard repaglinide were extracted from plasma by liquid-liquid extraction and a mixture of 5 mM ammonium acetate (pH 5.5 ± 0.3) and acetonitrile in the ratio of 10:90 (v/v) was used as mobile phase. The retention times of meloxicam and internal standard were 0.96 and 1.52 min, respectively. Detection was carried out using API 3200 (MDS Sciex) with a mass spectrometer operating in selected reaction monitoring mode. The flow rate maintained was 0.800 mL/min with a run time of 2.2 min. The method had a lower limit of quantification of 1 ng/mL. The calibration curve was demonstrated to be linear over the concentration range of 1.00 to 2503.85 ng/mL. The within-batch and between batch precision values for meloxicam at lower limit of quantification are 5.8 to 7.9 % and 7.9 % and accuracy are 93.6 to 103.8 % and 97.9 %, respectively. The pharmacokinetic parameters for meloxicam was found to be T_{max}-4.6 h, C_{max}-1014.2 ng/mL, $t_{1/2}$-18.8 h, AUC_{0-T}-10142.0 ng/mL and $AUC_{0-\infty}$-10333.5 ng/mL. The entire results obtained in the study were well acceptable to a pharmacokinetic study in human volunteers.

Keywords: Meloxicam, LC-MS, Human plasma, Selected reaction monitoring mode.

INTRODUCTION

Meloxicam (MX, Fig. 1), 4-hydroxy-2-methyl-N-(5-methyl-2-thiazole)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide, is a potent non-steroidal anti-inflammatory drug (NSAID) of the oxicam derivatives which shows preferential inhibition of cyclo-oxygenase-2 (COX-2) and prostaglandin synthesis. It has definite activity in treating rheumatoid arthritis, osteoarthritis and other joint diseases [1]. Meloxicam binds strongly to serum albumin (> 99 %) and reaches a maximum concentration at 4.5 h after oral administration. Meloxicam is metabolized extensively in liver into four pharmacologically inactive metabolites that are excreted through urine and feces [2] with an absolute bioavailability of 89 %.

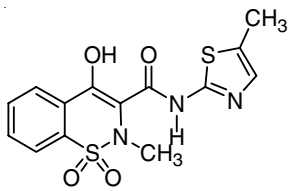

Fig. 1. Structure of meloxicam

Literature survey reveals that only a few HPLC [3-6] and LC-MS [7-13] were reported for the determination of meloxicam from biological fluids either singly or with their degradation products. Among the above mentioned methods of estimation for meloxicam in biological matrix, HPLC methods possesses low sensitivity due to high linear range, which are unsuitable for estimating the nanogram (ng) level of meloxicam while the LC-MS/MS methods are sensitive enough to estimate meloxicam in ng level. Though the reported LC-MS/MS methods are advantageous, it suffers drawbacks such as, use of guard column and high plasma volume for sample preparation [9] and high ammonium formate concentration (15 mM) and a less commonly available instrumentation which is costly [12].

The present work describes a simple, rapid, sensitive and selective method for the determination of meloxicam using commonly available LC-MS/MS system. The current method offers a number of advantages over existing methods: shorter run time, less sample volume, simple sample clean up procedure and cost effectiveness. This method can be applied to pharmacokinetic study of plasma meloxicam concentration after oral administration.

EXPERIMENTAL

The working standard samples of meloxicam and internal standard were gifted by M/s Hetro drug Ltd., (Hyderabad, India). HPLC grade acetonitrile and ethyl acetate were purchased from Merck® Ltd (Mumbai, India). Analytical grade ammonium acetate and acetic acid (GR grade) were purchased from Merck® Ltd (Mumbai, India). The water used for the analysis was prepared by Milli-Q® water purification system (Bangalore, India). Human plasma was obtained from Jeevan-Dhara Blood Bank, Hyderabad, India.

LC-MS/MS conditions: Chromatographic separation was performed by using an isocratic, Shimadzu HPLC equipment consisting of two LC10AT VP pumps, VP CTO-10AS VP column oven, a Phenomenex Gemini C_{18} column (50 mm × 4.6 i.d, 5 µm), with a mobile phase consisting of mixture of 5 mM ammonium acetate (pH 5.5 ± 0.3) and acetonitrile in the ratio of 10:90 v/v at a flow rate of 0.8 mL/min.

Mass spectrometric detection was performed using an API 3200 (MDS Sciex) equipped with an electrospray ion (ESI) source. The mass spectrometer was operated in selected reaction monitoring mode. The spray voltage and source temperature were 5200 V and 450 °C, respectively. Detection was carried at unit resolution for both Q1 and Q3 with scan time 200 ms per channel. Nebulizer gas, curtain gas and gas for collision activated dissociation (CAD) were kept 45, 40 and 6 Psi, respectively for both analyte and internal standard. The mass transition ion pair was selected as 352.0→115.1 for meloxicam and 453.3→230.3 for internal standard. The parent and product ion spectrum for meloxicam are represented in Figs. 2 and 3. The data acquisition software, analyst version 1.4.2 was used for quantification. Method of least squares with weighting $1/X^2$ was used to calculate the peak area ratio of the target ions of the drugs to those of the internal standard and the calibration curve was constructed by plotting peak area ratios of meloxicam against its concentration.

Preparation of standard and quality control solutions: A standard stock solution of meloxicam was prepared by dissolving 5.046 mg of meloxicam in acetonitrile and transferred into 5 mL volumetric flask. The volume was made upto the mark to get a concentration of 1 mg/mL. The solution was then further diluted to achieve standard working solutions of desired concentrations. Internal standard working solution (1 mg/mL) was prepared by dissolving 5.982 mg of repaglinide in acetonitrile, transferred into 5 mL volumetric flask and made upto the mark with acetonitrile. All the working solutions were kept in refrigerator at 1-10 °C and brought to room temperature before use.

Preparation of calibration curve for standard and quality control standards: The standard solutions were used to spike in 100 µL blank plasma samples either for calibration curve or quality control standards of meloxicam during the validation. Calibration curve spiking solutions and quality control spiking solutions were used to spike the screened blank plasma matrix to prepare plasma calibration curve standards ranging from 1.00 to 2503.85 ng/mL and plasma quality control sample ranging from 1.01 to 1935.21 ng/mL. 0.2 mL aliquots of the above plasma calibration curve standards and plasma quality control samples were taken in pre-labelled polypropylene vials, capped tightly and stored in a freezer at -70 °C.

Extraction process of plasma samples for drying: 100 µL plasma samples (calibration curve standards and quality control samples) were transferred to a set of pre-labelled

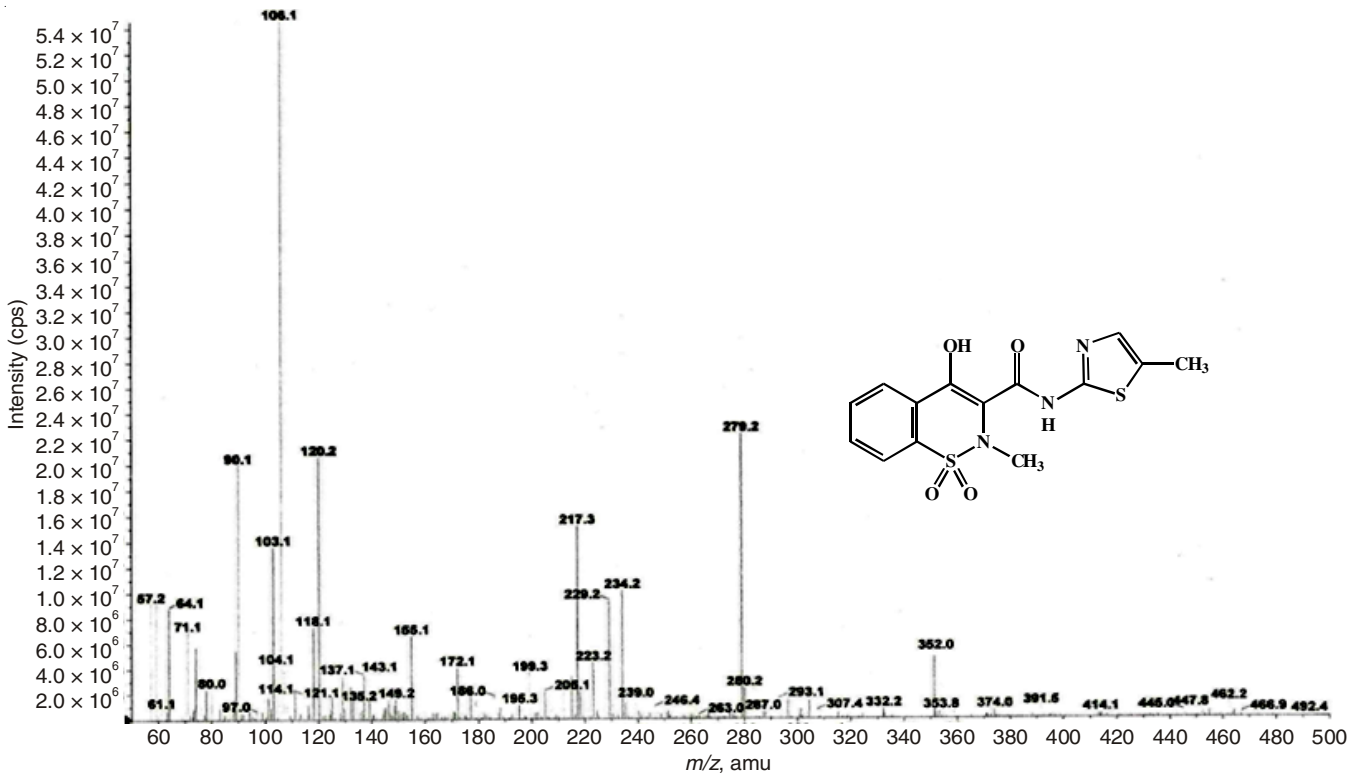

Fig. 2. Mass spectra of meloxicam parent (Q1) masses

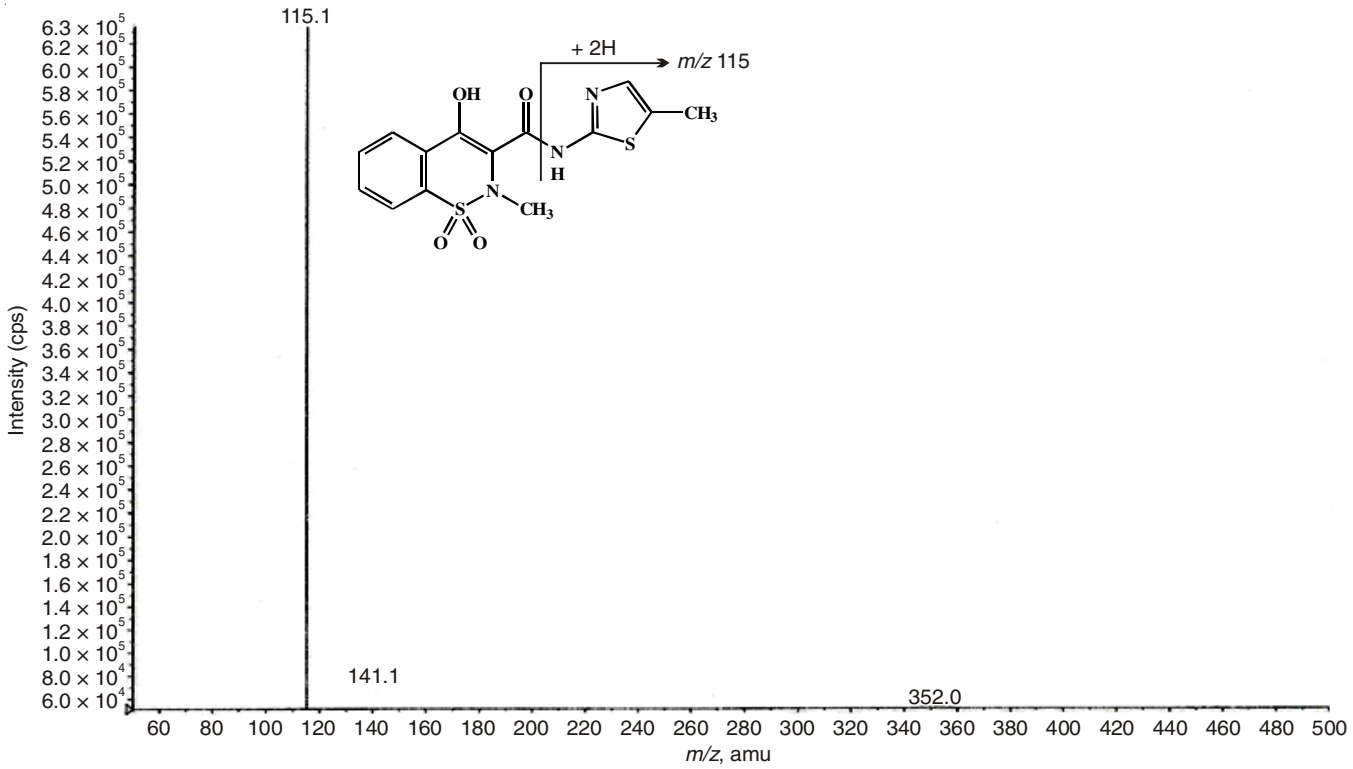

Fig. 3. Mass spectra of meloxicam product ion (Q3) masses

polypropylene tubes containing 50 µL of repaglinide dilution (internal standard; 500 ng/mL) which were vortexed for 10 seconds. All the tubes were pre-treated with 200 µL of 1 % formic acid in water and vortexed. To each of the tubes 2.5 mL of ethyl acetate was added and were further vortexed for 10 min at 2500 rpm on a vibramax unit and then were centrifuged at 4500 rpm for 5 min in a refrigerator centrifuge at 10 °C. From these tubes approximately 2 mL of the supernatant layer was transferred to each of a new set of polypropylene tubes. The contents of the tubes were evaporated in a stream of nitrogen at 50 °C for 15 min and the residues of the dried tubes were reconstituted with 0.4 mL of the mobile phase. The contents of the tubes were vortexed and transferred into auto-sampler vials and then analyzed by Tandem mass spectrometer. An aliquot of 10 µL of the sample was drawn each time from the vials in the auto sampler.

Validation: The validation for the determination of meloxicam in plasma samples was performed in accordance with food and drug administration (FDA) guidelines for bioanalytical method validation [14,15]. The method was validated for its selectivity, linearity, precision, accuracy, recovery and stability. Selectivity was performed by control concentration for meloxicam and at one concentration for internal standard. The % recovery was evaluated by comparing the areas of the extracted quality control samples with equivalent aqueous samples.

The assessment of matrix effect (co-eluting, undetected endogenous matrix compounds that may influence the analyte ionization) constitutes an important and integral part of validation for quantitative LC-MS/MS method for supporting pharmacokinetic studies. It was performed by processing six lots of different normal controlled plasma samples in six replicates (n = 6). Lower quality control (LQC) and higher quality control (HQC) working solutions were spiked with post extraction in duplicate for each lot. The co-efficient of variation (% C.V.) for six values at each level was calculated by taking the mean of the 12 different blank plasma samples (containing K_2EDTA as anti coagulant) to test for interference at retention time of meloxicam and internal standard.

The intra- and inter-run accuracy was determined by replicate analysis of the three quality control levels along with the lower limit of quantification and higher quality control levels. In each of the precision and accuracy batches, six replicates at each quality control level inclusive of the lower limit of quantification and higher quality control levels were analyzed.

Assay precision was calculated by using the formula:

$$CV\ (\%) = (SD/M) \times 100$$

where M is the mean of the experimentally determined concentrations and SD is the standard deviation of M.

The extraction efficiencies of meloxicam and internal standard were determined from the analysis of six replicates at low, medium and high quality value obtained by injecting the post extracted samples prepared in duplicate from each plasma lot. Moreover, the minor enhancement of analyte signal due to endogenous matrix interferences does not affect the quantification of analyte and internal standard peak which was confirmed by the post-column infusion techniques.

As a part of the method validation, stability was evaluated. Analytes were tested using the quality control samples wherever appropriate. The bench top stability values of meloxicam in human plasma was kept at room temperature of about 25 °C, was evaluated for 9-10 h and were 99.9 and 97.4 % at lower quality control and higher quality control samples, respectively.

The freeze thaw stability was conducted by comparing the stability of the samples which was been frozen and thawed over 6 cycles with freshly spiked quality control samples. Six aliquots of each low and high concentration were used for the freeze thaw stability evaluation. For long term stability six replicates of lower quality control and higher quality control samples were spiked in plasma, kept at -70 °C for 51 days. The dry extract stability was conducted by comparing the dried stability samples that had been stored at 10 °C. The dry residue samples were then reconstituted after 47.60 h and compared with freshly spiked quality control samples at low and high concentrations. In addition long-term stability of meloxicam in human plasma was carried by storing the stability samples at a temperature of -70 °C for 51 days and was analyzed along with six replicates of freshly prepared quality control samples at low and high concentrations. The long term stability values obtained for meloxicam were 98.6 % and 101.6 % at low and high concentrations, respectively.

Pharmacokinetic study design: The study was performed in screened male healthy volunteers (n = 6). The ethics committee approved the protocol and the volunteers provided with informed written consent. The 7.5 mg of meloxicam was orally administered. Blood samples were collected after oral administration at a fixed time points of pre dose 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 7, 8, 10, 12, 16, 24, 36, 48, 72, 96 and 120 h in K_2EDTA vacutainer collection tubes (BD Vacutainer®, NJ, USA). The tubes were centrifuged for 20 min at 3000 rpm on a refrigerated centrifuge (Eppendorf® Model 5810 R; USA) at 4 °C and the plasma was collected and were stored at -70 °C until their use. These subject samples were analyzed along with calibration curve and quality control samples. The lower quality control, medium quality control and higher quality control were well distributed along between the subject samples in the analytical batch run; individually 50 % at each quality control level and overall 67 % of all the quality control samples utilized in the analytical batch should pass. The plasma concentration *vs.* time profile of each analyte was analyzed by non-compartmental method using WinNonlin® Version 5.2 (Pharsight Corporation, Mountain View CA, USA).

RESULTS AND DISCUSSION

The aim of this work was to develop and validate a simple, rapid and sensitive assay method for the extraction and quantitation of meloxicam for its pharmacokinetics studies. To achieve this during the method development, different trails were carried out to optimize sample extraction, detection parameters and chromatography. As the pKa of meloxicam is 1.1, in order to maintain it in unionized form and to prevent it from ionization before extraction, the plasma samples were treated with 1 % formic acid and extracted by liquid-liquid extraction. By acidification with 1 % formic acid, the response increased and the best signal with symmetrical peaks were achieved with positive ion electrospray (ESI) mode. A mobile phase containing 5 mM ammonium acetate (pH 5.5 ± 0.3) and acetonitrile in the ratio of 10:90 v/v resulted in improved signal. As a part of method development, matrix effect was determined by post column infusion technique. There was no ion suppression or enhancement observed at the RT of analyte and internal standard. Use of Phenomenex Gemini C_{18} column (50 mm × 4.6 mm i.d, 5 µm) resulted in reduced flow rate and run time of 2.2 min. With the above mentioned optimized parameters, the retention time of meloxicam and internal standard were observed at 0.96 and 1.52 min, respectively.

Selectivity: Meloxicam, chromatogram of the extracted blank, blank + internal standard (ISTD), STD1 (lower limit of quantification) and STD8 (upper limit of quantification) plasma sample are shown in Fig. 4. The standard meloxicam eluted at RT 0.96 min with a sharp and symmetrical peak, while internal standard eluted at 1.52 min. Hence, in the developed method, the RT of both meloxicam and internal standard are significantly less when compared to the methods reported by other researchers [9,10,12]. Further, no interfering peaks of endogenous compounds were observed at RT of analyte or internal standard in blank human plasma containing K_2EDTA as anticoagulant from six different lots.

Linearity: The linearity of the method was determined by a weighted ($1/X^2$, where × is concentration) least square regression analysis of the standard plots associated with the eight point standard curve for meloxicam. The calibration line was linear in the range of 1.00 to 2503.85 ng/mL of the drug as shown in Fig. 5. A straight-line fit made through the data points by least square regression analysis showed a constant proportionality with minimal data scattering. The correlation coefficient (r^2) ranged from 0.9976 to 0.9999 for meloxicam (Table-1).

Lower limit of quantification: The lower limit of quantification is defined as the lowest concentration that could be analyzed with acceptable accuracy and precision of 20 %. The lower limit of quantification of meloxicam was found to be 1 ng/mL, emphasizing its usage in pharmacokinetic study after oral administration.

TABLE-1
BACK CALCULATED CONCENTRATIONS OF MELOXICAM (MX) AND CALIBRATION CURVE (CC) PARAMETERS

STD ID	Meloxicam								Slope (m)	Intercept (c)	r^2
	STD1	STD2	STD3	STD4	STD5	STD6	STD7	STD8			
Conc. (ng/mL)	1.00	2.00	10.02	75.34	251.14	1101.49	1953.00	2503.85			
MX CC1	1.03	1.94	10.11	74.59	244.58	1141.74	1969.30	2431.52	0.0085	0.0258	0.9999
MX CC2	0.94	2.18	9.86	72.63	255.62	1156.50	1962.41	2598.61	0.0082	0.0244	0.9996
MX CC3	1.04	1.89	9.84	77.65	255.74	1199.63	1904.52	2408.62	0.0088	0.3340	0.9999
MX CC4	1.05	1.98	10.44	77.29	269.37	1087.50	1892.49	2551.62	0.0079	0.0273	0.9976
Mean	1.015	1.998	10.063	75.540	256.328	1146.343	1932.180	2497.593			
± SD	0.0506	0.1271	0.2800	2.3724	10.1482	46.2830	39.2943	92.0239			
CV (%)	5.0	6.4	2.8	3.1	4.0	4.0	2.0	3.7			
Nominal (%)	101.5	99.9	100.4	100.3	102.1	104.1	98.9	99.8			

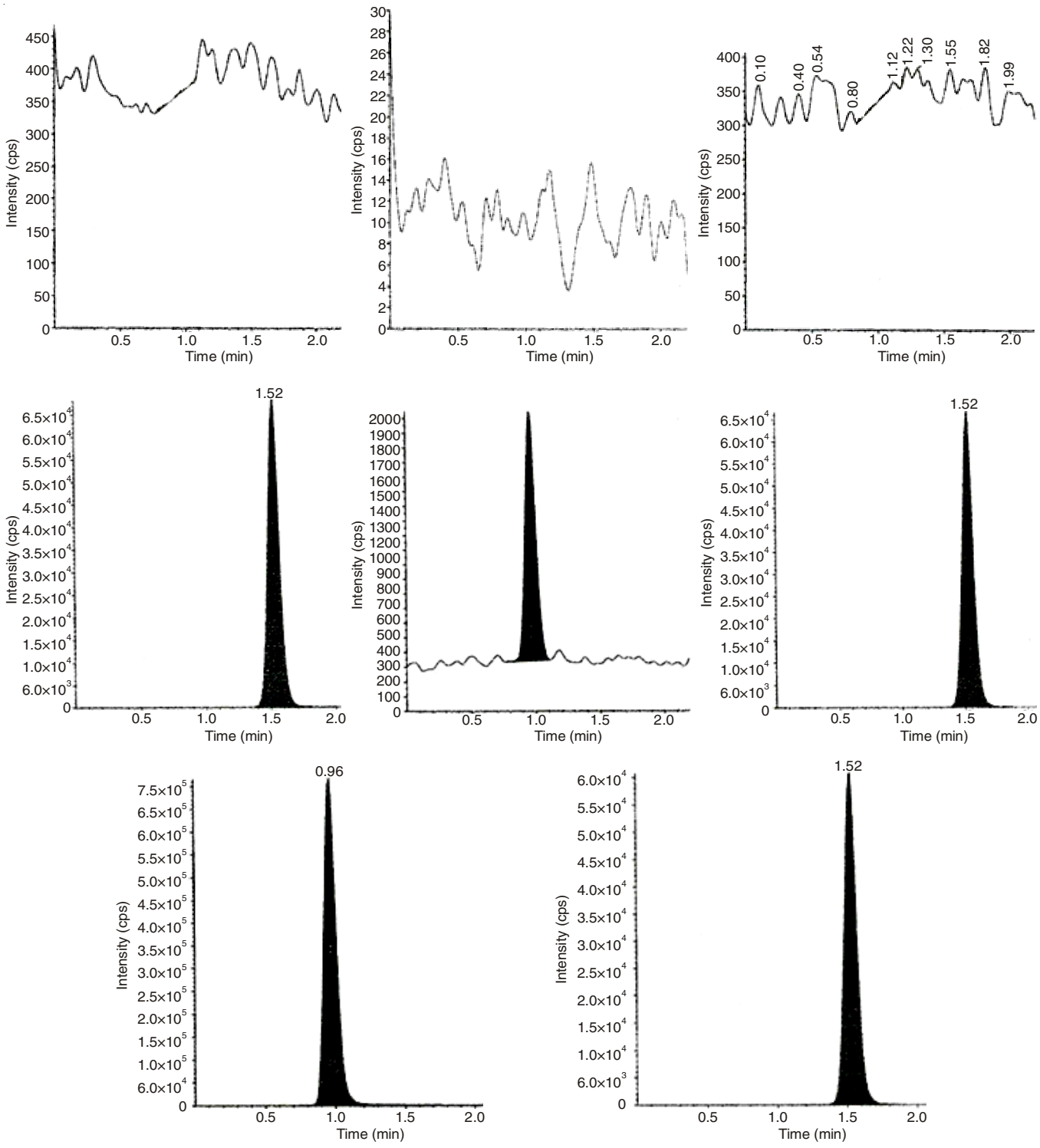

Fig. 4. Meloxicam chromatogram of the extracted Blank, Blank + Internal standard (ISTD), STD1 (lower limit of quantification) and STD8 (upper limit of quantification) plasma sample

Precision and accuracy: The intra-run precision at lower quality control, medium quality control and higher quality control ranged from 2.4 to 7.5 (CV, %) for meloxicam whereas at the lower limit of quantification level it was ranged from 5.8 to 7.9 % and the accuracy was within the range of 93.6-105.2 % for meloxicam across all the four levels tested (Table-2). The inter-run precision and accuracy were determined by pooling all individual assay results of replicate (n = 6) quality control samples over five separate batch runs. The inter-run precision at lower quality control, medium quality control and higher quality control ranged from 3.8 to 5.7 % for meloxicam where as at the lower limit of quantification level it was 7.9 %. The inter run accuracy was within the range of 97.9 to 103.3 % for meloxicam (Table-3).

Recovery: Six replicates at lower quality control, medium quality control and higher quality control concentrations for meloxicam were prepared for recovery determination. The mean recovery for meloxicam was 89.29 %. The % CV obtained

TABLE-2
ACCURACY AND PRECISION (INTRA-DAY) OF MELOXICAM IN HUMAN PLASMA (n = 6)

Batch ID	QC ID	MX LLOQ QC	MX LQC	MX MQC	MX HQC
Nominal conc. (ng/mL)		1.01	2.93	1045.02	1935.21
PA 01	Mean	1.048	2.965	1097.943	2035.738
	SD	0.0828	0.1431	27.4350	87.2554
	CV (%)	7.9	4.8	2.5	4.3
	Nominal (%)	103.8	101.2	105.1	105.2
PA 02	Mean	1.008	2.932	1060.272	2014.288
	± SD	0.0763	0.1350	52.7966	79.8461
	CV (%)	7.6	4.6	5.0	4.0
	Nominal (%)	99.8	100.1	101.5	104.1
PA 03	Mean	0.953	3.083	1024.538	1972.375
	SD	0.0638	0.0829	49.0599	47.8877
	CV (%)	6.7	2.7	4.8	2.4
	Nominal (%)	94.4	105.2	98.0	101.9
PA 04	Mean	0.945	2.828	1058.862	1973.573
	SD	0.0550	0.2124	39.2298	79.3259
	CV (%)	5.8	7.5	3.7	4.0
	Nominal (%)	93.6	96.5	101.3	102.0

QC = Quality control; MX LLOQ QC = Meloxicam lower limit of quantification quality control; MX LQC = Meloxicam lower quality control; MX MQC = Meloxicam medium quality control; MX HQC = Meloxicam high quality control

TABLE-3
ACCURACY AND PRECISION (INTER-DAY) OF MELOXICAM IN HUMAN PLASMA (n = 6)

QC ID	MX LLOQ QC	MX LQC	MX MQC	MX HQC
Nominal conc. (ng/mL)	1.01	2.93	1045.02	1935.21
Mean	0.9888	2.9521	1060.4038	1998.9938
± SD	0.07843	0.16837	48.28257	75.32731
CV (%)	7.9	5.7	4.6	3.8
Nominal (%)	97.9	100.8	101.5	103.3

QC = Quality control; MX LLOQ QC = Meloxicam lower limit of quantification quality control; MX LQC = Meloxicam lower quality control; MX MQC = Meloxicam medium quality control; MX HQC = Meloxicam high quality control

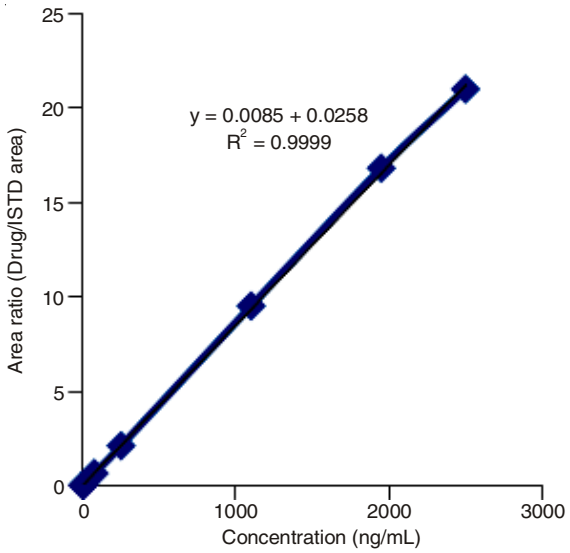

Fig. 5. Calibration curve (linearity) of meloxicam

was within the range of 1.3-7.8 % for meloxicam. The mean recovery for internal standard was 88.8 % with a % CV of 4.6 %.

Stability: The results on stability of meloxicam are presented in Table-4. Bench top, dry extract and process stabilities for meloxicam were investigated at lower quality control and higher quality control levels. The results revealed that meloxicam was stable in plasma for at least 9.10 h at room temperature of about 25 °C and 46.30 h in the auto sampler at 10 °C. It was confirmed that repeated freeze thawing (three cycles) of plasma spiked with meloxicam at lower quality control and higher quality control level did not affect the stability of meloxicam. Dry extract stability was also evaluated for a period of 47.60 h at lower quality control and higher quality control levels. The long term stability results also indicated that the analyte meloxicam was stable in the matrix up to 51 days at the storage temperature of -70 °C. Study on working solution stability proved that they were stable for more than 18.35 h at room temperature (about 25 °C) and stable up to 24.6 days under refrigeration (10 °C).

Pharmacokinetic study: In order to verify the sensitivity and selectivity of this method in a real-time analysis, the present method was used to test for meloxicam in human plasma samples collected from healthy male volunteers (n = 6) in between the age 18-45 years. Institutional review board approval was obtained before study start and all subjects given written informed consent before participation. Each subject received single oral dose of meloxicam 7.5 mg tablets and plasma samples obtained were analyzed for meloxicam. The mean plasma concentration verses time profiles of meloxicam is shown in Fig. 6. The plasma concentration–time data were analyzed by non-compartmental analysis method using the WinNonlin® Software (Version 5.0.1 from Pharsight Corporation, USA) for estimation of pharmacokinetic parameters. The results obtained were shown in Table-5 and matching with the published data.

TABLE-4
STABILITY DATA

Nominal conc. (ng/mL)	Stability	Mean ± S.D, n = 6 (ng/mL)	Precision (CV, %)	Accuracy (%)
2.93 (lower quality control)	Injector stability (10 °C, 46.30 h)	2.912 ± 0.0595	2.0	99.4
	Bench top stability (25 °C, 9.10 h)	3.023 ± 1.1089	3.6	103.2
	Dry extract stability (10 °C, 47.60 h)	2.903 ± 0.1541	5.3	99.1
	Freeze thaw stability (6 cycles)	3.003 ± 0.1663	5.5	102.5
	Long term stability (-70 °C, 51 days)	3.032 ± 0.1270	4.2	103.5
1934.06 (high quality control)	Injector stability (10 °C, 46.30 h)	1947.170 ± 43.7240	2.2	100.6
	Bench top stability (25 °C, 9.10 h)	1931.978 ± 105.945	5.5	99.8
	Dry extract stability (10 °C, 47.60 h)	2096.180 ± 76.0293	3.6	108.3
	Freeze thaw stability (6 cycles)	1992.627 ± 63.0538	3.2	103.0
	Long term stability (-70 °C, 51 days)	2043.380 ± 69.2258	3.4	105.6

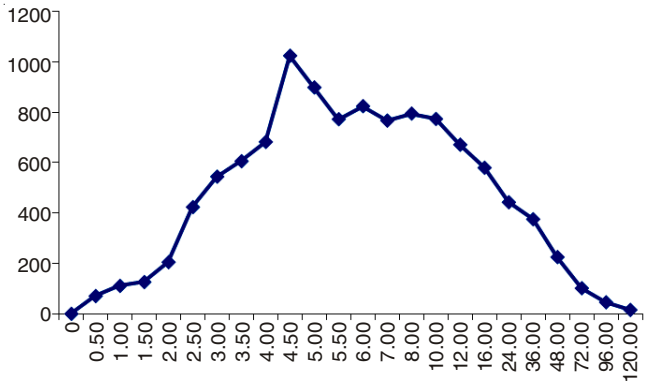

Fig. 6. Mean concentration-Time curve of the 6 volunteers for meloxicam

TABLE-5
PHARMACOKINETIC DATA FOR MELOXICAM

C_{max} (ng/mL)	1014.2
T_{max} (h)	4.6
$t_{1/2}$ (h)	18.8
$AUC_{(0\text{-}T)}$ (ng h/mL)	10142.0
$AUC_{(0\text{-}\infty)}$ (ng h/mL)	10333.5

Conclusion

A simple, selective, rapid and sensitive LC-MS/MS method for the determination of meloxicam in human plasma has been developed. The present method is advantageous over the reported method with a short run time of only 2.2 min as total run time with analyte eluting at 0.96 min for meloxicam and internal standard at 1.52 min, when compared to [10] with total run time of only 3 min and analyte (meloxicam) eluting at 2.06 and internal standard (piroxicam) at 1.82 min. In addition, the developed method makes use of commonly available and cost-effective LC-MS/MS instrument. Excellent specificity and linearity with a lower limit of quantification of 1 ng/mL for meloxicam is an added advantage in this method. As the developed method is both cost and time effective, it is suggested that it can be applied for the routine pharmacokinetic evaluation of meloxicam in human subjects after oral administration of the same.

ACKNOWLEDGEMENTS

The authors are thankful to Acharya Nagarjuna University, Guntur, India for constant support and Laila Impex R&D Centre, Vijayawada, India for providing the instrumental support.

REFERENCES

1. N.M. Davies and N.M. Skjodt, *Clin. Pharmacokinet.*, **36**, 115 (1999).
2. J. Schmid, U. Busch, G. Heinzel, G. Bozler, D. Kaschke and M. Kummer, *Drug Metab. Dispos.*, **23**, 1206 (1995).
3. J.-W. Bae, M.-J. Kim, C.-G. Jang and S.-Y. Lee, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, **859**, 69 (2007).
4. I. Madhusudhanareddy, R.M. Bhagavan, P.Y. Rajendra, R.K. Pavankumar, A. Meechel and B. Rajkumar, *Trop. J. Pharm. Res.*, **10**, 475 (2011).
5. F.S. Bandarkar and P.R. Vavia, *Trop. J. Pharm. Res.*, **8**, 257 (2009).
6. E. Csifo, T. Katona, J. Arseni, E. Nagy, I. Gergely and Ö. Nagy, *Acta Medica Marisiensis*, **60**, 142 (2014).
7. J. Shirako, M. Kawasaki, K. Komine, Y. Kunisue, M. Terada, C. Sasaki, W. Irie, C. Murakami, K. Tonooka, K. Tomobe and T. Shinozuka, *Forensic Sci. Int.*, **227**, 100 (2013).
8. H.Y. Ji, H.W. Lee, Y.H. Kim, D.W. Jeong and H.S. Lee, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, **826**, 214 (2005).
9. Y. Yuan, X. Chen and D. Zhong, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, **852**, 650 (2007).
10. M. Ganesh, P. Hemalatha, M.M. Peng, K. Sakthimanigandan and H.T. Jang, *J. Liq. Chromatogr. Rel. Technol.*, **36**, 867 (2013).
11. J.L. Wiesner, A.D. de Jager, F.C.W. Sutherland, H.K.L. Hundt, K.J. Swart, A.F. Hundt and J. Els, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, **785**, 115 (2003).
12. H.W. Lee, H.Y. Ji, H.Y. Kim, K.C. Lee and H.S. Lee, *Bioanalysis*, **1**, 63 (2009).
13. H.M. Rigato, G.D. Mendes, N.C. Borges and R.A. Moreno, *Int. J. Clin. Pharmacol. Ther.*, **44**, 489 (2006).
14. Food and Drug Administration, Guidance for Industry: Bioanalytical Method Validation. US Department of Health and Human Services, FDA, Center for Drug Evaluation and Research: Rockville, MD (2001).
15. C.T. Viswanathan, S. Bansal, B. Booth, A.J. DeStefano, M.J. Rose, J. Sailstad, V.P. Shah, J.P. Skelly, P.G. Swann and R. Weiner, *AAPS J.*, **9**, E30 (2007).