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EVALUATION OF PHARMACOKINETIC PARAMETERS FOR PURE DRUG AND FAST DISSOLVING TABLETS OF MELOXICAM

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ABSTRACT

In the present investigation the pharmacokinetic parameters of pure drug and an optimized formulation of fast dissolving tablets of Meloxicam were evaluated and compared. The formulations were administered to 2 groups of white New Zealand rabbits (n=6) following cross over design pattern and the plasma levels were measured using LC-MS/MS method. Pharmacokinetic parameters were determined for each formulation. The comparison of the plasma time curves of the dosage forms showed that each dosage form caused significant differences in the drug plasma levels. The highest mean C_{max} value was observed for optimized Fast dissolving tablets (158.46 ± 0.15ng/ml) compared to pure drug (67.56 ± 0.43 ng/ml). The mean time taken to peak plasma concentration for *(Tmax)* following administration of pure drug was 11.42 ± 0.32 hours, while it was 6.15 ± 0.14 hour following administration of selected optimized fast dissolving tablets. The elimination rate constant (Kel) for pure drug and optimized fast dissolving tablets were found to be 0.96 ± 0.004h^{-1} and 0.82 ± 0.003h^{-1} respectively. The absorption rate constant (Ka) for pure drug and optimized Fast dissolving tablets were found to be 2.92 ± 0.01h^{-1} and 7.76 ± 0.01 h^{-1} respectively. The $AUC_{0-\alpha}$ values observed with optimized Fast dissolving tablets 564.9.± 1.36 ng hr/ml in compared to pure drug values 126 ± 1.23 ng hr/ml.Thus, the results of pharmacokinetic studies indicated rapid and higher oral absorption of Meloxicam when administered as its fast dissolving tablets. Both Ka and AUC were markedly increased by fast dissolving tablets.

KEYWORDS: LC-MS/MS, Meloxicam, Fast Dissolving, In-vivo Studies, Pharmacokinetic parameters.

INTRODUCTION

Meloxicam is a COX-2 inhibitor used to treat joint diseases such as osteoarthritis, rheumatoid arthritis and other musculoskeletal disorders. It is practically insoluble in water, leading to poor dissolution, variations in bioavailability, and gastric irritation on oral administration. Its solubility increases significantly with an increase in pH. It is a white crystalline powder to off-white crystalline powder and has poor flow properties and undesirable dissolution properties. As such it needs enhancement in the dissolution rate and bioavailability to derive its maximum therapeutic efficacy.[1-2] The major problem of Meloxicam is its very low water solubility, which results into poor dissolution rate.[3-4] The pharmacokinetic performance of Meloxicam fast dissolving tablets was studied in a comparison with that of pure drug in rabbits.

MATERIALS AND METHODS

The *in vivo* study of the optimized formulations were performed as per the guidelines approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of social Justice and Empowerment, Government of India. Prior approval by Institutional animal ethics committee was obtained for conduction of experiments (Ref: BCOP /IAEC/I-7/ 2015-2016, Dated 19-2-2016). Pure Meloxicam and optimized fast dissolving tablets were selected based on in-vitro release studies and stability conditions were chosen as dosage forms for administration.

Subject selection: The pharmacokinetic performance of Pure Meloxicam and optimized Meloxicam fast dissolving tablets were studied in a randomized crossover study design in rabbits. Twelve New Zealand healthy rabbits with a mean age of 10±2 weeks and with a mean body weight of 3±0.2 kg were used in this study. Each group consisted of six rabbits (n=6) each and were subjected for overnight fasting, it was taken care that there was no stress on the animals. Rabbits were randomly divided into two groups for different sampling time and each group was housed in one cage. Food and water were available ad libitum at all times during the experiment.[7-8] The study was conducted in a crossover design with 2 weeks washout periods in between the two experiments. The animal dose of Meloxicam was

calculated relevant to human dose by using the following formula.[5-6] The above dosage form was administered through gastric intubation method.

Human dose of Meloxicam = 7.5mg.

Animal dose = $\frac{\text{Human dose} \times \text{Animal weight}}{\text{Human weight}}$

= 7.5 x3/70= 0.3214mg = 0.4mg

Blood sampling: About 1 ml of blood samples were collected from the tracheal lobular vein of the rabbit using and the blood was stored in screw top heparinized plastic tubes, the sampling time for blood was done at 0 (before drug administration), 0.5, 1.0, 2.0, 3.0, 4.0 and 6.0 hrs after pure drug administration and at 0, 1, 2, 4, 6, 8, 12, 16, 20, and 24 hrs after administration of Meloxicam fast dissolving tablets. The plasma was immediately separated by aspiration after centrifugation at 4000 rpm for 5 minutes and frozen at -20 ºC until analyzed by LC-MS/MS method.[7-8]

Determination of Pharmacokinetic Parameters

Various pharmacokinetic parameters such as peak plasma concentration (C_{max}), time at which peak occurred (T_{max}), area under the curve (AUC), elimination rate constant (K_{el}), biological half-life ($t_{½}$) and mean residence time (MRT) were calculated using the noncompartmental pharmacokinetics data analysis software PK Solutions 2.0™(Summit Research Services, Montrose, CO, USA).[9] The pharmacokinetic parameters of the tested formulations were statistically analyzed using paired sample's t-test for normal distributed results of C_{max}, K_a, K_e, MRT and $AUC_{0-\alpha}$ value. All tests were performed at 0.001 level of significance.[10]

Estimation of Meloxicam in plasma (LC-MS/MS method)[11]

Chromatographic conditions

UPLC: Waters

Mass: API 2000

Ion Source: Heated Nebulizer

Polarity: Positive ion mode.

Detection Ions

Meloxicam: 352.1*amu (parent), 115.1* amu (product)

Piroxicam: 332.2*amu (parent), 121. 0 *amu (product)

Column: Agilent,Zorbax Eclipse –XDB, 4.6xl50mm, 5µ

Column oven temperature: 35.0 °C

Peltier temperature: 20 °C

Mobile phase: 5mM Ammonium formate: Acetonitrile (30:70)

Flow rate: 1.000ml/min.

Volume of Injection: 15 µl

Retention times: Meloxicam 0.80 to 1.50 minutes: ISTD: 0.80 to 1.50 minutes

Run time: 2.00 minutes

Preparation of working standard solutions

Preparation of Meloxicam standard stock solution

Meloxicam working standard equivalent to 5 mg of Meloxicam was weighed and transferred into a 5 ml volumetric flask and dissolved in 0.1 N hydrochloric acid in methanol. The solution was made up to the volume with methanol. The concentration of resulting solutions was calculated by considering the purity of Meloxicam. The solutions were labeled and stored in a cold store at 2-8°C.

Preparation of Piroxicam as internal standard stock solution

Piroxicam working standard equivalent to5 mg of Piroxicam was weighed and transferred in to a 5 ml volumetric flask and dissolved in methanol. The solution was made up to the volume with methanol. The concentration of resulting solution was calculated by considering the purity of Piroxicam. The solution was labeled and stored in a cold room at 2-8°C.

Calibration curve standards

Preparation of stock dilutions of standard Meloxicam solution

Stock dilutions of Meloxicam ranging from 0.200 µg/ml to 40.000 µg/ml were prepared with 60% Acetonitrile in water solutions using dilutions of main stock solution prepared for calibration curve standards.

Spiking of plasma for calibration curve standards

Concentrations of Meloxicam ranging from 10 ng/ml to 2000 ng/ml were prepared with plasma as showed in Table 5.6 and were labeled as CC1 to CC8. The calibration curve standards were prepared fresh for each validation run.

Sample preparation

Step 1: Blank, calibration curve standards and the subject samples were withdrawn from the deep freezer and allowed them to thaw. The thawed samples were vortexed to ensure complete mixing of the contents. To 0.2 ml of plasma sample in a ria vial, 20µl of Piroxicam(1µg/ml) was added. To plasma blank and pre-dose (0.0hr), 20 ul of 60% Acetonitrile in water solution was added. The samples were vortexed to ensure complete mixing of contents.

Step 2: Phenomenex strata ^{TM} – X 33 µm polymeric cartridge were taken, (new cartridge for each sample) on to a positive pressure processor and the following procedure was followed:

1. Conditioning: 1 ml methanol and followed by 1 ml of water was added. (Taken care not to dry the cartridge).

2. Application: The sample was applied and allowed it to dry for about 1 minute under positive pressure.

3. Rinsing: The cartridge was rinsed twice with 1ml of water and was allowed to dry under positive pressure for about 5 minutes.

4. Rinsing: The cartridge was rinsed with 1ml of water solution for 2 minutes and was allowed to dry under positive pressure for about 5 minutes.

5. Elution: The drug was eluted into 1 ml of methanol and was allowed to dry under positive pressure for approximately 2 minutes. The organic layer was evaporated under a stream of nitrogen gas at 50°C. The residue was reconstituted with 0.3ml of mobile phase and vortexed. The samples were transferred in to auto-injector vials and loaded the vials in to auto sampler. 15 ul of sample was injected in to LC-MS/MS system.

Data processing

The chromatograms were obtained by using the computer-based Analyst 1.4.2 version software supplied by the Applied Biosystems, Canada. The concentrations of the unknown samples have to be calculated from the equation using regression analysis of spiked plasma calibration standard with $1/x^2$ as weighting factor. y = mx + c;Where,y = Ratio of Meloxicam peak area and ISTD peak area (analyte area / ISTD area); x = concentration of Meloxicam;m = slope of the calibration curve; c = y-axis intercept value.Linear regression analysis equation of stock dilutions of standard Meloxicam solution with plasma is y = 0.00232x – 0.00134.

RESULTS AND DISCUSSION

The *in vivo* experiments were conducted as per the protocol and procedure described earlier. Bio analytical methods employed for the quantitative determination of drugs and their metabolites in biological matrix (plasma, urine, saliva, serum etc) play a significant role in evaluation and interpretation of pharmacokinetic data. For the successful conduct of pharmacokinetic study, the development of selective and sensitive bioanalytical methods plays an important role for the quantitative evaluation of drugs and their metabolites (analytes). The LC-MS/MS methods were highly sensitive and suitable for the detection of drug in plasma even in low concentrations. Calibration curves were constructed from blank sample (plasma sample processed without IS), blank+IS samples and eight point calibration standards for Meloxicam in plasma. Plasma concentrations of Meloxicam at different times were calculated and are shown in Table 2 and in Fig 2. Pharmacokinetic parameters such as absorption rate constant, elimination rate constant, half life, AUC, and MRT were calculated from the plot of time versus plasma concentration and subjected to statistical analysis and the results were shown in Table 3.

The results indicated that the parameters significantly differed following optimized fast dissolving tablets administration, compared to pure drug administration. The highest mean C_{max} value was observed for optimized fast dissolving tablets (158.46 ± 0.15ng/ml) compared to pure drug (67.56 ± 0.43 ng/ml). The mean time taken to peak plasma concentration for *(Tmax)* following administration of pure drug was 11.42 ± 0.32 hours, while it was 6.15 ± 0.14 hour following administration of selected optimized fast dissolving tablets .The elimination rate constant (Kel) for pure drug and optimized fast dissolving tablets were found to be 0.96 ± 0.004h^{-1} and 0.82 ± 0.003h^{-1} respectively. The absorption rate constant (Ka) for pure drug and optimized Fast dissolving tablets were found to be 2.92 ± 0.01h^{-1} and 7.76 ± 0.01 h^{-1} respectively. The $AUC_{0-\alpha}$ values observed with optimized fast dissolving tablets 564.9.± 1.36 ng hr/ml in compared to pure drug values 126 ± 1.23 ng hr/ml. Thus, the results of pharmacokinetic studies indicated rapid and higher oral absorption of Meloxicam when administered as its fast dissolving tablets. Both Ka and AUC were markedly increased by fast dissolving tablets.

Table. 1: Analyte Concentrations of Stock Dilutions of Standard Meloxicam Solution with Plasma.

S. No	Sample Name	Analyte Concentration (ng/ml)	Analyte peak area	IS Peak Area	Area Ratio	Calculated Concentration (ng/ml)	Accuracy (%)
1	Plasma blank	0	0	0	0	N/A	N/A
2	Blank+ISTD	0	0	82223	0	N/A	N/A
3	CC1	10	2159	100545	0.02	9.846	98.46
4	CC2	20	4280	92612	0.05	20.524	102.62
5	CC3	50.050	11618	100613	0.12	50.413	100.73
6	CC4	150.100	35542	100599	0.35	153.056	101.97
7	CC5	500.350	111504	98127	1.14	490.984	98.13
8	CC6	1000.750	224913	95369	2.36	1018.374	101.76
9	CC7	1501.100	318307	95018	3.35	1446.321	96.35
10	CC8	2001.500	415353	89597	4.64	2001.259	99.99

Table. 2: Plasma Concentrations of Meloxicam following oral administration of Pure Meloxicam and optimized fast dissolving tablets.

Time (h)	Plasma concentration (ng/ml) (Mean ± s.d)	
	Pure drug	Meloxicam fast dissolving tablets
0	0	0
0.5	08.51 ±1.86	28.23 ±1.36
1	11.35±1.74	36.44±1.78
1.5	12.51±1.52	42.20±1.56
2	14.61±1.14	45.24±1.24
3	16.75±1.64	49.62±1.12
4	18.81±1.35	54.12±1.65
5	19.62±1.43	61.18±1.67
6	21.93±1.24	68.33±1.85
8	22.76±1.43	62.15±1.72
10	24.90±1.24	57.42±1.22
12	27.72±1.27	49.23 ±1.36
14	24.64±1.36	42.20±1.43
16	21.96±1.53	35.24±1.64
18	18.84±1.32	28.53±1.18
20	15.92±1.21	23.12±1.62
24	12.28±1.39	17.18±1.47

Table. 3: Statistical Treatment of Pharmacokinetic Parameters (Mean ± S.D.) of following oral administration of Pure Meloxicam and optimized fast dissolving tablets.

Pharmacokinetic parameter	Pure Drug	Optimized fast disolving tablets	Calculated value of 't'
C_{max} (ng/ml)	67.56 ± 0.43	158.46 ± 0.15	12.53***
$t_{1/2}$ (h)	11.42 ± 0.32	6.15 ± 0.14	8.96***
K_{el} (h^{-1})	0.96 ± 0.004	0.82 ± 0.003	5.70***
K_a (h^{-1})	2.92 ± 0.01	7.76 ± 0.01	85.68***
$AUC_{0-\square}$ (ng h/ml)	126 ± 1.23	464.9.± 1.36	146.40***
Null hypothesis (H_o): There is no significant difference between the pharmacokinetic parameters of Meloxicam obtained with pure drug and optimized fast dissolving tablets. Table value of 't' with 10 DF at the 0.001 level is 4.587.			
Result: H_o is not accepted as the calculated't' value more than the table Value of 't' with 10 DF at 0.001 levels of significance. It was therefore concluded that there was significant difference between the pharmacokinetic parameters of obtained with pure drug and optimized fast dissolving tablets.			

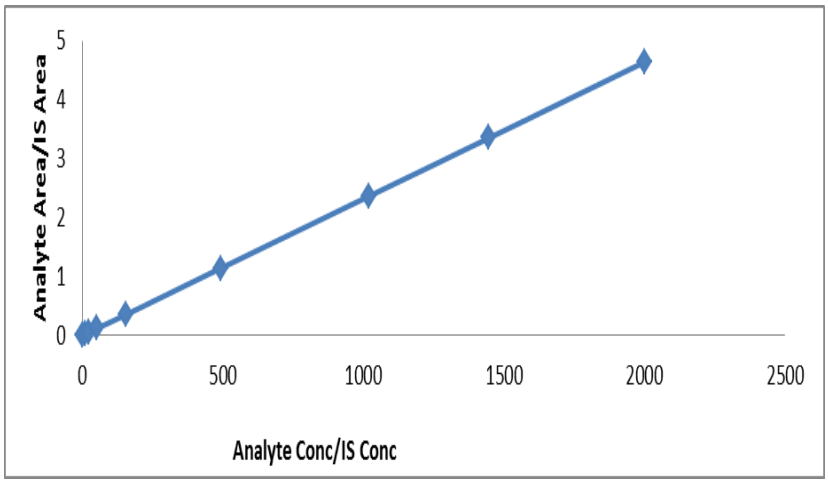


Figure. 1: Calibration Curve for Estimation of Meloxicam in Plasma.

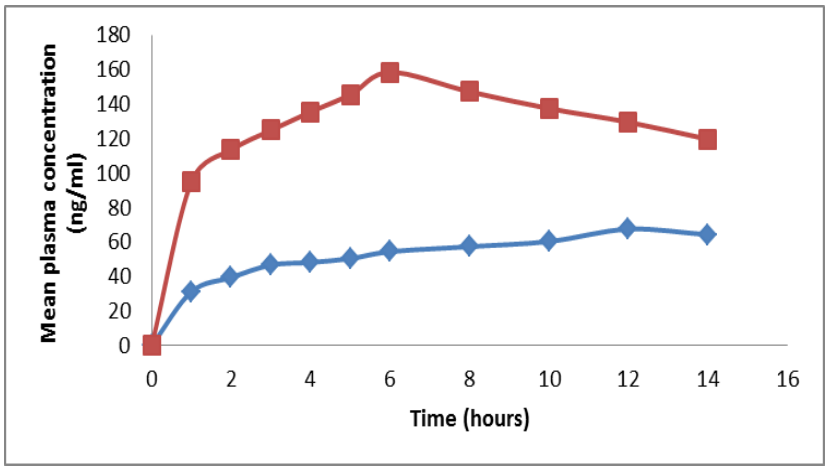


Figure. 2: Concentration-Time Curve of Meloxicam following oral administration of Pure Meloxicam and optimized fast dissolving tablets.

(-♦-)plasma Concentration -Time Curve of Meloxicam following pure drug administration

(-■-)plasma Concentration -Time Curve of Meloxicam following optimized fast dissolving tablets administration

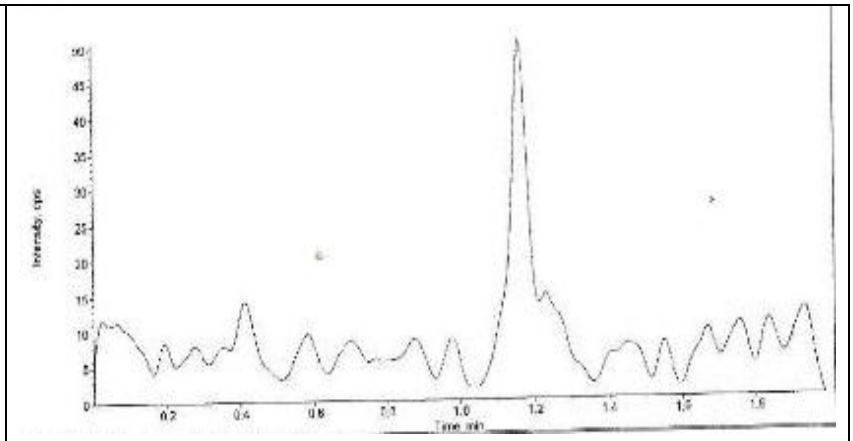
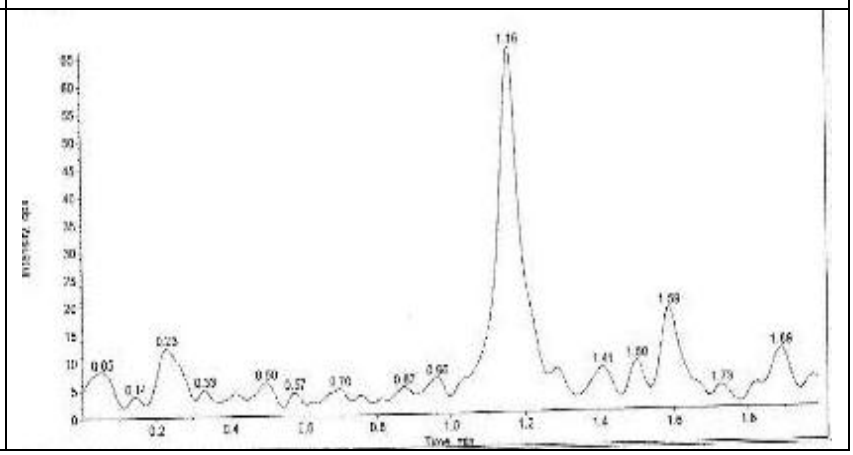
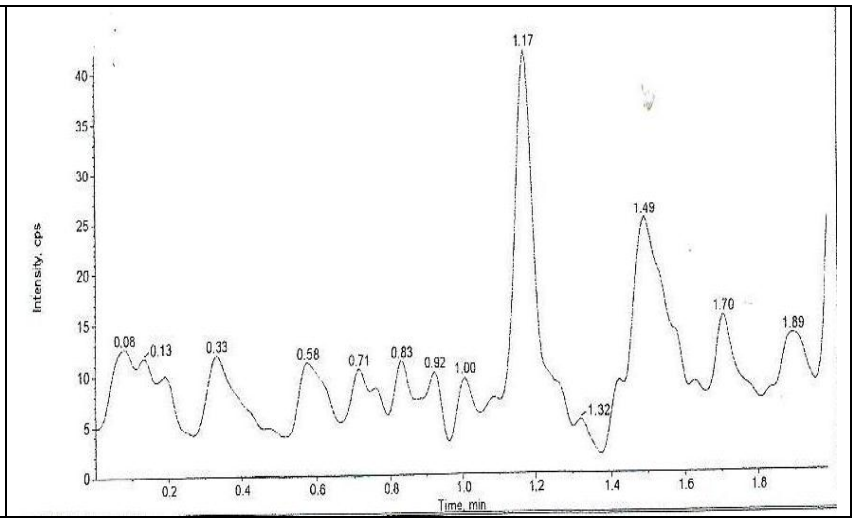
Sample Name: Plasma blank Peak Name: Meloxicam Mass(es): 352.1/115.1 amu Concentration:0.000ng/ml Calculated conc:N/A Proc.Algorithm: Analyst classic	
Sample Name: Plasma blank Peak Name: Piroxicam Mass(es): 332.2/121.0 amu Concentration:1.000 ng/ml Calculated conc :N/A Proc. Algorithm: Analyst classic	

Figure. 3: Chromatograms of reserve stock solution of Standard Meloxicam Solution with Plasma.

Sample Name: Blank+ISTD Peak Name: Meloxicam Mass(es): 352.1/115.1 amu Concentration: 0.000ng/ml Calculated conc:N/A Proc.Algorithm: Analyst classic	

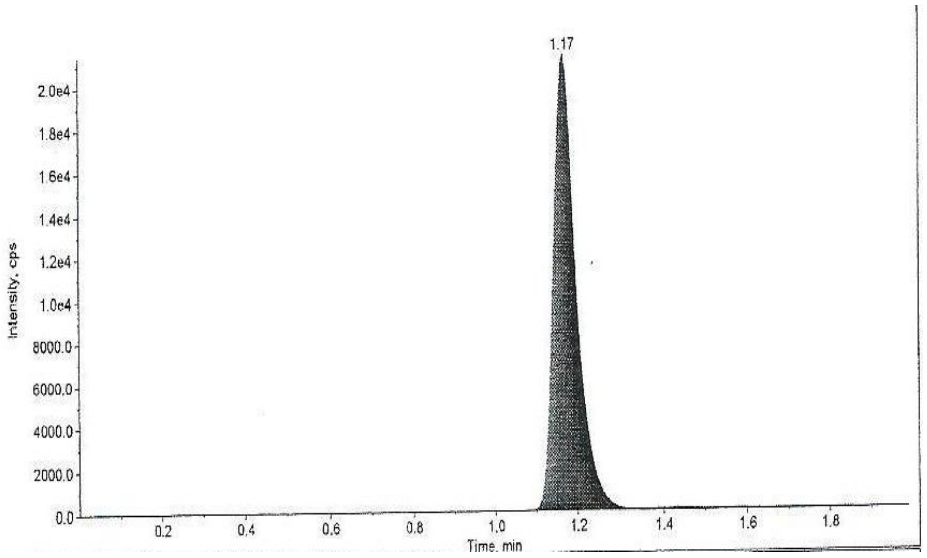
Sample Name: Blank+ISTD Peak Name: Piroxicam Mass(es): 332.2/121.0 amu Concentration:1.000 ng/ml Calculated conc:N/A Proc.Algorithm: Analyst classic Bunching factor:2 Noise Threshold:10.00 Area Threshold :100.00 Num Smooths :10 Sep .Width:0.20 Sep. Height:0.01 Exp .Peak Ratio:5.00 Exp.Adj Ratio:4.00 Exp .Val Ratio :3.00 RT Window :30.0sec Expected RT:1.03min Use Relative RT: No Int. Type:Base To Base Retention Time:1.17 min Area: 82223 counts Height:2.14+004 cps Start Time:1.07 min End Time:1.33min	

Figure. 4: Chromatograms of Plasma blank and internal standard (Meloxicam-d4).

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