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Extraction and Quantification of Lornoxicam in Human Plasma by Liquid Chromatography-Tandem Mass Spectrometry in Positive Ion Mode

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A novel liquid chromatography tandem mass spectrometry method is described for the quantitative determination of lornoxicam in human K_2 EDTA plasma in positive ion mode and validated using piroxicam as internal standard according to linearity, selectivity, precision, recovery and various stability studies. Sample preparation was accomplished by liquid liquid extraction technique. The eluted samples were chromatographed on ACE C18 (150 × 4.6 mm, 5 µ) column (agilent technologies) using a mobile phase consisting of HPLC grade acetonitrile: 0.3 % formic acid buffer (80:20 v/v) with injection volume of 15 µL and a run time of 3 min. The precursor to product ion transitions *m/z* 372.10 to 121.10 (lornoxicam) and *m/z* 332.10 to 95.20 (piroxicam, IS) were used for quantization. The calibration graph of lornoxicam was linear with $r^2 > 0.990$ over a concentration range of 5.086 ng/mL to 1518.325 ng/mL. CV % of intra- and inter-day precisions were found satisfactory and well within the limits. The drug was found to be stable for the studied parameters and found to be interference free for matrix effect with appreciable recovery. The novelty of the method makes it highly valuable, rapid, selective and sencitive for quantification of lornoxicam in human plasma and can be used in therapeutic drug monitoring of this drug.

Keywords: Lornoxicam, High-performance liquid chromatography, Mass spectrometry, Human plasma.

INTRODUCTION

Lornoxicam((3E)-6-chloro-3-[hydroxy(pyridin-2-ylamino)methylene]-2-methyl-2,3-dihydro-4H-thieno[2,3-e][1,2]thiazin-4-one 1,1-dioxide) is a non-steroidal antiinflammatory drug (NSAID)[1]. Lornoxicam is a compound in the same chemical class as piroxicam, meloxicam and tenoxicam with potent antiinflammatory, antipyretic and analgesic activity. Lornoxicam (chlortenoxicam), is a new nonsteroidal antiinflammatory drug (NSAID) of oxicam class. It is distinguished from established oxicams by a relatively short elimination half-life, lornoxicam inhibits the COX-1/COX-2 system, the production of interleukin-6 and the inducible NO synthase[2]. It may be applied by the intramuscular or intravenous route; its bioavailability after oral application is approximately 90 %. Although its elimination half-life is only about 4 h, the duration of effect is approximately 8 h, analogous to other acidic antipyretic analgesics. The analgesic potency of lornoxicam is remarkable. In doses of 16 mg (i.m.) its analgesic effect is comparable with that of 20 mg morphine (i.m.) or 50 mg tramadol (i.v.)[3]. It acts by nonselective inhibition of cyclooxygenase-1 and 2. It is prescribed for osteoarthritis, rheumatoid arthritis, acute lumbar sciatica conditions and postoperative pain management[4]. In the literatures a voltammetric[5], polarograhic[6], UV spectrophotmetric[7], LC/MS/MS[8,9], HPTLC[10] and high performance liquid chromatographic (HPLC)[11-13] methods were reported for the analysis of lornoxicam. Due to the increasing importance of speed and reliability of analysis in bioanalytical laboratories, a new method for determination of lornoxicam in human plasma with a short time of analysis (3 min) is described in this work. The LC-MS/MS technique was successfully employed to provide a satisfactory sensitivity and selectivity in a desirable time of chromatographic run.

EXPERIMENTAL

Lornoxicam (Fig. 1) was purchased from Cirex Pharmaceuticals Limited, India and Piroxicam, the internal standard (Fig. 2), from Mankind Pharma Limited (Calcutta, India). HPLC grade Acetonitrile and methanol were obtained from J.T. Baker. HPLC grade water was procured from Rankem pharma. Formic acid, ethyl acetate and ammonia solution (HPLC grade) were obtained from MERCK.

The liquid chromatographic system consist of LC Shimadzu LC10 from Shimadzu, an auto sampler of Shimadzu (SIL-HTc) coupled with an applied Bio systems SCIEX a triple quadrupole mass spectrometer (API 4000) with electro spray ionization

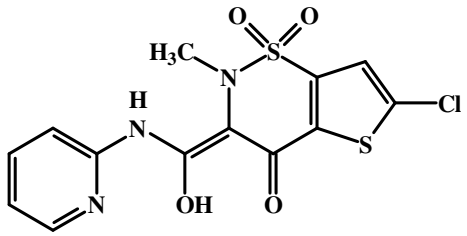

Fig. 1. Chemical structure of lornoxicam

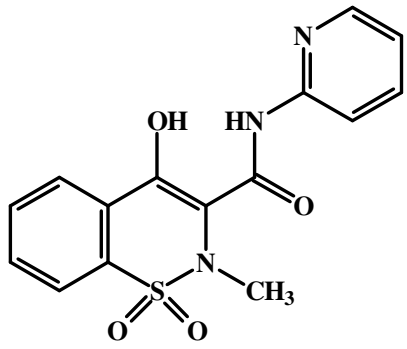

Fig. 2. Chemical structure of piroxicam

(ESI) used for analysis. Date of acquisition and processing were controlled by applied bio systems/MDS SCIEX analyst software (version 1.4.2) with ACE 3 C18 column (150 × 4.6 mm, 5 µ).

The chromatographic analysis was performed by using a mobile phase of HPLC grade acetonitrile: 0.3 % formic acid buffer (80:20 v/v) with flow rate 1 mL/min by positive ion mode (API 4000). Detection is performed by atmospheric pressure electro spray ionization (ESI) tandem mass spectrometry in positive ion mode. The chromatograms were recorded. Detection of the ions were performed by multiple reaction monitoring (MRM) of the transitions *m/z* 372.10 and 121.10 for lornoxicam and *m/z* 332.10 and 95.20 for the internal standard.

Preparation of stock standard and working solutions of lornoxicam: The stock solution of lornoxicam was prepared by dissolving 5 mg of lornoxicam in 0.25 % ammonia solution in methanol and made up the volume with the same in a 5 mL volumetric flask to produce a solution of 1000000 ng/mL. This solution was kept in refrigerator at 2- 8 °C. The stock solutions were diluted to suitable concentrations using diluent for spiking into plasma to obtain calibration curve (CC) standards, quality control (QC) samples for further use. All other dilutions were made in mobile phase.

Preparation of stock solution of piroxicam (internal standard): A stock solution of internal standard (IS) was prepared by dissolving 5 mg of piroxicam in diluent (mixture of HPLC grade acetonitrile and water in a ratio (60:40) and made up the volume with the same in a 5 mL volumetric flask to produce a solution of 1000000 ng/mL. This solution was kept in refrigerator at 2- 8 °C. Working internal standard solutions were prepared by suitably diluting the above mentioned stock solution afresh before use.

Preparation of calibration curve standards and quality control (QC) samples: Calibration curve standard consisting of a set of nine non-zero concentrations ranging from 5.086 to 1518.325 ng/mL of lornoxicam was prepared. Prepared quality control samples consisted of concentrations of 5.123 ng/mL (lower limit of quantification quality control sample), 15.069 ng/mL (lower quality control sample), 251.148 ng/mL (middle quality control sample-1), 751.940 ng/mL (middle quality control sample-2) and 1303.189 ng/mL (higher quality control sample) for lornoxicam. These samples were stored at -70 ± 10 °C until use. Twelve sets of LQC and HQC samples were stored at deep freezer to check stability.

Sample preparation procedure: After bulk spiking, aliquots of 200 µL for CCs and 200 µL for QCs of spiked plasma samples were pipetted out into a prelabelled polypropylene micro centrifuge tubes and then all the bulk spiked samples were stored to deep freezer at -70 ± 10 °C, except twelve replicates each of LQC and HQC, which were stored in deep freezer for generation of stability data. The thawed samples were vortexed to ensure complete mixing of the contents. 100 µL of the plasma sample was pipetted into stoppered test tubes, 20 µL (2012.760 µg/mL of piroxicam) internal standard spiking solution were added to it and vortexed, except in blank plasma samples where 20 µL diluent was added to it and vortexed. Then 25 µL of 10 % formic acid buffer was added to it and vortexed. Followed by addition of 5 mL of ethyl acetate and shaken for 20 min on reciprocating shaker at 200 rpm. Samples were centrifuged at 4000 rpm for 5 min at 4 °C. Then supernatant organic layer (4 mL) was transferred to prelabelled glass dry test tubes and evaporated to dryness in turbovap at 40 °C. The samples were reconstituted in 1000 µL of mobile phase and 15 µL sample was injected to HPLC with MS-MS detection.

Method validation: The method was validated for selectivity, linearity, accuracy, precision, recovery, stability and carry over test according to the principles of the FDA industry guidance.

Sensitivity: The lowest limit of reliable quantification for lornoxicam was set at the concentration of the LLOQ *i.e.*, 5.071 ng/mL. The precision and accuracy for lornoxicam at this concentration was estimated.

Linearity: The linearity of calibration curve for lornoxicam was assessed at nine concentration levels in the range of 5.086 ng/mL to 1518.325 ng/mL in plasma samples. Peak area ratios for each solution against its corresponding concentration were measured and the calibration curve was obtained from the least squares linear regression presented with their correlation coefficient.

Extraction recovery: Twenty four blank matrix samples were processed and six sets of each blanks samples were re-constituted with the aqueous QC dilutions at low, middle and high concentration without internal standard, which represents 100 % extraction of analyte(s) (non-extracted samples). Six blanks were reconstituted with the internal standard solution, which represents 100 % extraction of internal standard. (Non-extracted sample). The non-extracted samples were injected. The recovery comparison samples of lornoxicam were compared against extracted samples of LQC, MQC2 and HQC of PA BATCH-I (precission and accuracy). The recovery comparison samples of internal standard were compared against the response of internal standard in MQC2 level.

$$R\ (\%) = (Psbe/Psae) \times 100$$

where: R is extraction recovery, Psbe is the mean value of the peak area responses obtained from plasma samples spiked with analyte before extraction and Psae is the mean value of the peak area responses obtained from plasma samples spiked with analyte after extraction.

Accuracy and precision: Intra assay precision and accuracy were determined by analyzing six replicates at five different QC levels in two runs on the same day. Inter-assay precision and accuracy were determined by analyzing six replicates at five different QC levels on five different runs. The acceptance criteria included accuracy within =15 % deviation (SD) from the nominal values, except LLOQ QC, where it should be ≤ 20 % and a precision of ≤ 15 % relative standard deviation (RSD), except for LLOQ QC, where it should be ≤ 20 %.

Stability: Stability of lornoxicam in plasma was performed using six replicates of two QC samples at low and high levels. Samples were prepared by spiking drug-free plasma with appropriate volumes of standard solutions of lornoxicam. The stability was evaluated with different studies such as room temperature stock solution stability, refrigerated stock solution stability, room temperature spiking solution stability, refrigerated spiking solution stability, freeze-thaw, short term stability, bench top stability *etc*. Stability tests were conducted to evaluate the analyte stability in stock solutions and in plasma samples under different conditions. The stock solution stability at room temperature and refrigerated conditions (2 - 8 °C) was performed by comparing the area response of the analytes (stability samples) with the response of the sample prepared from fresh stock solution. Bench top stability (6 h), processed sample stability (auto sampler stability for 32 h), freeze thaw stability (four cycles), reinjection stability (24 h), wet extract stability (30 h) and plasma samples stability at -20 °C were performed at LQC and HQC levels using six replicates at each level. Samples were considered to be stable if assay values were within the acceptable limits of accuracy (≤ 15 % SD) and precision (≤ 15 % RSD).

Matrix effect test of lornoxicam: Two sets of extracted blank plasma samples each containing six tubes (plasma taken from six different lots) are taken. One set of tubes are reconstituted with equivalent aqueous concentration of LQC and the other set of tubes are reconstituted with equivalent aqueous concentration of HQC. These samples are known as post spiked samples. These samples are analyzed along with equivalent aqueous LQC and HQC samples. The matrix effect is evaluated by determining the % response ratio using the formula.

$$\%\ \text{Response ratio} = \frac{\text{Mean area ratio of post spiked samples}}{\text{Mean area ratio of equivalent aqueous samples}} \times 100$$

RESULTS AND DISCUSSION

The main aim of this work was to develop a rapid and selective analytical method including an efficient and reproducible sample clean-up step for quantitative analysis of lornoxicam in human plasma. Subsequently, a simple and inexpensive extraction procedure that could be implemented in monitoring laboratories provided an assay well suited for real time analysis. In optimizing the chromatographic conditions, the formic acid solution was adopted in the mobile phase of the HPLC in order to suppress the tailing phenomena of chromatographic peaks of lornoxicam and piroxicam. Besides ethyl acetate buffer was investigated and the inclusion made the chromatographic peaks sharp and symmetric. Further experimental results showed that acidifying the mobile phase with formic acid also contributed to improve peak shapes of lornoxicam and piroxicam. Therefore, a concentration of 0.3 % formic acid was used in mobile phase. The acceptable retention and separation of lornoxicam and piroxicam was obtained by using an elution system of acetonitrile: 0.3 % formic acid 80:20, v/v) as the mobile phase. The LC/MS/ MS method described here satisfies the requirement of routine analyses since it has a short run time (3 min), which has advantages over other methods described in the literature. The MS optimization was performed by direct injection of lornoxicam and piroxicam into the mass spectrometer. The mass parameters were optimized to obtain better ionization of lornoxicam and piroxicam molecules. The full scan spectrum was dominated by protonated molecules *m/z* 372.10 and 332.10 for lornoxicam and piroxicam and the major fragment ions observed in each product spectrum were at *m/z* 121.10 and 95.20 respectively. The retention times obtained for lornoxicam and piroxicam were 1.98 and 2.14 min, respectively. Representative chromatograms of an aqueous standard with internal standard mixture, blank plasma and plasma samples spiked with lornoxicam and IS were shown in Fig. 3-5.

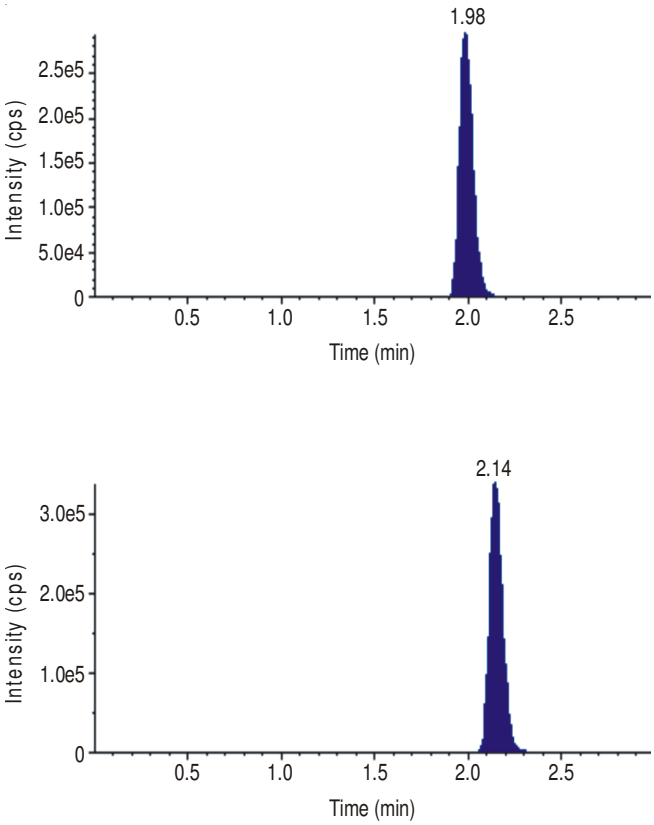

Fig. 3. Representative chromatogram of an aqueous standard and internal standard mixture

Linearity: The correlation coefficient for lornoxicam over the concentration range of 5.086 to 1518.325 ng/mL was 0.9991. The average slope and intercept of regression equations were 0.0001 and 0.0034, respectively. Linearity was found to be quite satisfactory and reproducible and represented by (Fig. 6).

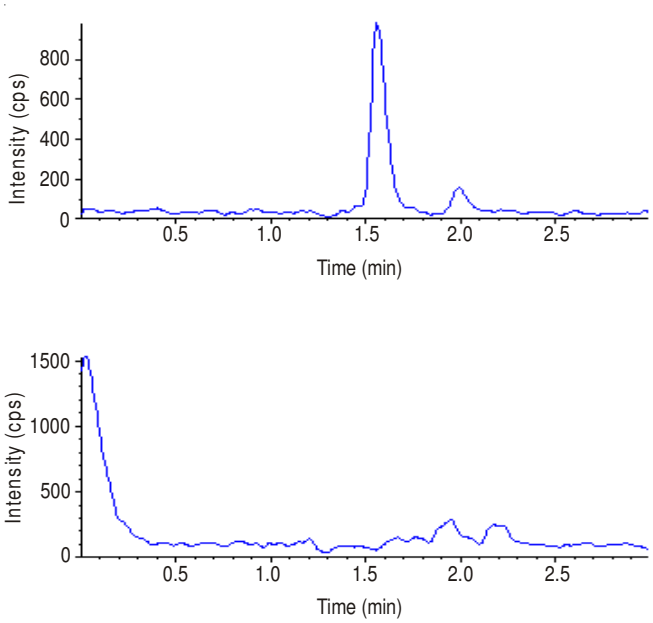

Fig. 4. Representative chromatogram of blank plasma sample

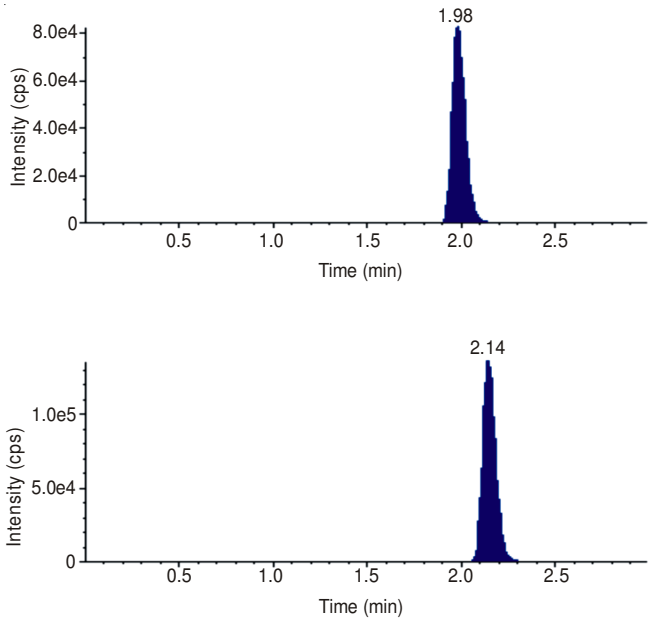

Fig. 5. Representative chromatogram of MQC2 sample of lornoxicam with internal standard

Selectivity: There was no significant interference from endogenous components observed at the mass transitions of lornoxicam and internal standard.

Sensitivity: The lowest limit of reliable quantification for lornoxicam was set at the concentration of the LLOQ, 5.071 ng/mL and the results for sencitivity are shown in Table-1. The precision and accuracy for lornoxicam at this concentration was found to be 5.18 and 112.34 %, respectively.

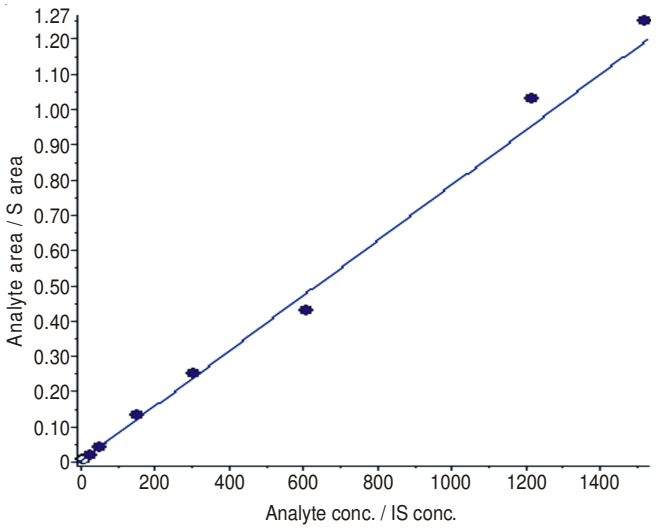

Fig. 6. Representative calibration curve for regression analysis of lornoxicam

TABLE 1
WITHIN BATCH PRECISION AND ACCURACY
FOR SENSITIVITY OF LORNOXICAM

Sample ID	Concentration (ng/mL)
	LLOQ
	5.071
1	5.800
2	5.491
3	5.506
4	5.474
5	5.671
6	6.240
Mean	5.6970
S.D (+/-)	0.29488
C.V. (%)	5.18
% Nominal	112.34
N	6

Extraction recovery: The percent recoveries of lornoxicam and piroxicam are shown in Tables 2 and 3, respectively. The extraction recoveries determined were found to be 70.98 % with a precision ranging from 0.76 to 7.91 % for lornoxicam and 71.02 % with a precision ranging from 1.18 to 1.78 % for piroxicam. The results are well within the limits.

Accuracy and precision: The intra-day precision and accuracy data and inter day precision and accuracy for QCs are summarized in Tables 4 and 5. The international acceptance criteria were met in each case[14].

Stability: The processing and storage conditions of clinical samples need to maintain the integrity of a drug or at least keep the variation of pre-analysis as minimal as possible[15]. For this reason, stability studies play an important role in a bio analytical method development. In this study, the stability was assessed by considering different studies such as room temperature stock solution stability, refrigerated stock solution stability, room temperature spiking solution stability, refrigerated spiking solution stability, freeze-thaw, short term stability, bench top stability *etc*. The results presented in Table-6 shows that lornoxicam is stable under the studied conditions, since in all cases the international acceptance criteria (variation values for area smaller than 15 %) were met[16].

Matrix effect: There is no significant matrix effect was observed in all the eight batches including hemolysis and lipemic (batch no. P040310-253, P050510-287, P050510-288, P240610-309, P240610-313, P050510-290, P070310-254 (lipemic) and P070310-255 (hemolysis)) plasma for lornoxicam at low (LQC) and high (HQC) concentrations. The precision and accuracy for lornoxicam at LQC concentration was found to be 1.13 and 96.29 %, respectively and at HQC concentration was found to be 5.17 and 94.93 %, respectively and given in Table-7.

TABLE-2
RECOVERY OF LORNOXICAM FROM HUMAN PLASMA

	LQC Response		MQC2 Response		HQC Response	
	Extracted QC	Non Extracted QC	Extracted QC	Non Extracted QC	Extracted QC	Non Extracted QC
Sample ID	LQC (07-12)	LQC (1-6)	MQC-2 (07-12)	MQC-2 (1-6)	HQC (07-12)	HQC (1-6)
1	13779	23423	755694	1083876	1406681	1871379
2	16174	22584	735315	1071141	1402984	1846192
3	15923	23506	752772	1089191	1383853	1843838
4	17193	22874	742229	1079307	1380593	1887485
5	17286	22441	748231	1093403	1413012	1858840
6	16435	22701	751970	1090403	1406291	2093833
Mean	16131.7	22921.5	747701.8	1084553.5	1398902.3	1900261.2
SD	1275.25	444.70	7634.97	8269.42	13360.81	96218.74
CV (%)	7.91	1.94	1.02	0.76	0.96	5.06
N	6	6	6	6	6	6
Recovery (%)	70.38		68.94		73.62	
Overall recovery	70.98					

TABLE-3
RECOVERY OF PIROXICAM FROM HUMAN PLASMA

Extracted QC ID	IS Response in extracted samples (area)	Non-Extracted QC ID	IS Response in Non-Extracted Samples (Area)
MQC2-7	1130117	NON EXTRACTED-MQC-2-1	1596096
MQC2-8	1090395	NON EXTRACTED-MQC-2-2	1576012
MQC2-9	1125548	NON EXTRACTED-MQC-2-3	1583342
MQC2-10	1151154	NON EXTRACTED-MQC-2-4	1548819
MQC2-11	1130426	NON EXTRACTED-MQC-2-5	1591354
MQC2-12	1116161	NON EXTRACTED-MQC-2-6	1599503
Mean	1123966.8	Mean	1582521.0
SD	20045.88	SD	18598.69
CV (%)	1.78	CV (%)	1.18
N	6	N	6
Recovery (%)	71.02		

TABLE-4
INTRADAY PRECISION AND ACCURACY FOR LORNOXICAM

	Concentration (ng/mL)				
	LLOQ QC	LQC	MQC1	MQC2	HQC
QC#	5.123	15.069	251.148	751.940	1303.189
1	4.692	12.794	245.650	705.433	1319.298
2	4.798	15.290	228.544	711.415	1330.641
3	4.982	14.816	223.461	705.559	1329.086
4	4.836	15.806	226.286	680.202	1278.680
5	4.948	16.111	229.365	698.276	1314.472
6	4.802	15.110	228.477	710.734	1300.038
7	5.508	16.368	247.731	744.581	1379.190
8	5.017	15.463	244.620	762.928	1389.626
9	5.341	15.701	247.917	744.434	1383.311
10	5.310	17.615	247.317	741.560	1390.452
11	5.506	15.692	237.419	718.190	1361.198
12	5.340	16.519	240.629	749.606	1382.868
Mean	5.09000	15.60708	237.28467	722.74317	1346.57167
S.D.	0.293872	1.154418	9.470377	25.107946	39.118333
C.V.%	5.77	7.40	3.99	3.47	2.91
Nominal (%)	99.36	103.57	94.48	96.12	103.33
N	12	12	12	12	12

TABLE-5
BETWEEN BATCH/INTER DAY PRECISION AND ACCURACY FOR LORNOXICAM

	LLOQ QC	LQC	MQC1	MQC2	HQC
QC#	5.123	15.069	251.148	751.940	1303.189
1	4.692	12.794	245.650	705.433	1319.298
2	4.798	15.290	228.544	711.415	1330.641
3	4.982	14.816	223.461	705.559	1329.086
4	4.836	15.806	226.286	680.202	1278.680
5	4.948	16.111	229.365	698.276	1314.472
6	4.802	15.110	228.477	710.734	1300.038
7	5.508	16.368	247.731	744.581	1379.190
8	5.017	15.463	244.620	762.928	1389.626
9	5.341	15.701	247.917	744.434	1383.311
10	5.310	17.615	247.317	741.560	1390.452
11	5.506	15.692	237.419	718.190	1361.198
12	5.340	16.519	240.629	749.606	1382.868
13	3.540	15.872	270.250	761.213	1355.549
14	5.999	13.361	272.662	744.291	1398.254
15	4.708	15.004	279.129	754.584	1390.328
16	6.064	13.724	280.046	753.255	1345.827
17	5.596	14.454	276.266	738.031	1369.179
18	5.168	14.613	270.271	732.451	1405.912
19	5.175	16.177	236.053	732.589	1300.137
20	4.887	15.657	244.948	704.179	1330.932
21	4.741	15.239	237.631	720.495	1329.011
22	4.895	14.141	230.177	721.362	1352.084
23	4.598	15.441	233.303	745.112	1350.150
24	4.802	15.443	231.339	691.000	1311.715
25	3.965	15.291	235.114	712.092	1348.022
26	4.374	13.818	244.893	717.474	1309.503
27	4.796	15.122	232.814	726.189	1366.995
28	4.654	15.437	243.953	751.561	1350.567
29	4.775	17.162	238.631	737.446	1362.908
30	4.831	16.559	243.632	724.380	1384.400
Mean	4.9549	15.3267	244.9509	728.0207	1350.6778
S.D.	0.51671	1.06994	16.69199	21.55574	33.47698
C.V.%	10.43	6.98	6.81	2.96	2.48
% Nominal	96.72	101.71	97.53	96.82	103.64

TABLE 6
STABILITY RESULTS OF LORNOXICAM AND PIROXICAM

Analyte	Lornoxicam		Piroxicam	Acceptance Criteria	
Stability method	Nominal (%)	Precision		Nominal (%)	Precision
Room temperature stock solution stability (0 & 6 h)	102.81 (6 h)		97.81 (6 h)	Comparison Response: 90-110	
Refrigerated stock solution Stability (4 days)	99.71		102.09		
Room temperature spiking Solution stability (6 h)	99.29 (6 h)		97.02 (6 h)	Comparison Response: 90-110	
Refrigerated spiking solution stability (3 days)					
LQC	98.37		……..	Comparison Response: 90-110	
HQC	98.46		……..	Comparison Response: 90-110	
Bench-top stability (6 h)	91.51-97.16	1.75-3.01	……..	85-115	≤ 15
Auto sampler stability (32 h)	91.60-97.98	1.23-2.93	……..	85-115	≤ 15
Freeze thaw stability (IV cycle)	91.21-97.74	1.51-2.92			
Reinjection stability (24 h)	104.14-108.50	1.89-3.44	……..	85-115	≤ 15
Wet extract stability (30 h)	98.05-102.16	3.92-4.53			
Plasma samples stability at –20 °C (2 days)	98.01-99.35	1.42-2.05			

Conclusion

An alternative HPLC/ESI/MS/MS method for quantification of lornoxicam in human plasma has been successfully developed and validated. A simple and inexpensive liquid liquid extraction procedure and an isocratic chromatography condition using a reversed-phase column provided an assay well suited for real time analysis. The method exhibited excellent performance in terms of selectivity, linearity, accuracy, precision, recovery, stability and matrix effect test. In addition, the reported method has a short analysis run time, an advantage over previously reported methods. Therefore, this method is suitable for therapeutic drug monitoring of lornoxicam.

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TABLE-7
MATRIX EFFECT OF LORNOXICAM

Plasma (Batch no.)	M-163 ME QC#	Concentration (ng/mL) LQC 15.148			Mean	M-163 ME QC#	Concentration (ng/mL) HQC 1310.071			Mean
P040310-253	1, 2, 3	14.910	14.422	14.197	14.5097	1, 2, 3	1099.892	1159.416	1138.214	1132.5073
P050510-287	1, 2, 3	15.067	14.916	14.156	14.7130	1, 2, 3	1358.429	1254.845	1255.788	1289.6873
P050510-288	1, 2, 3	15.221	13.962	15.007	14.7300	1, 2, 3	1233.895	1178.383	1184.380	1198.8860
P240610-309	1, 2, 3	13.860	14.649	14.432	14.3137	1, 2, 3	1303.283	1285.072	1297.793	1295.3827
P240610-313	1, 2, 3	14.768	13.927	14.522	14.4057	1, 2, 3	1271.901	1220.394	1298.659	1263.6513
P050510-290	1, 2, 3	15.241	14.103	14.357	14.5670	1, 2, 3	1169.777	1216.058	1218.243	1201.3593
P070310-254 (Lipemic)	1, 2, 3	14.645	14.060	15.350	14.6850	1, 2, 3	1206.111	1300.321	1204.120	1236.8507
P070310-255 (Hemolytic)	1, 2, 3	14.767	14.890	14.625	14.7607	1, 2, 3	1330.702	1313.952	1346.859	1330.5043
Mean					14.5856					1243.6036
S.D.					0.16477					64.28803
C.V. (%)					1.13					5.17
Nominal (%)					96.29					94.93
N					8					8

REFERENCES

1. P. Kumar, S. Shukla, B.B. Subudhi and A.L. Ganure, *Int. J. Pharm. Pharm. Sci*., **4**, 1 (2012).
2. J. Berg, H. Fellier, T. Christoph, J. Grarup and D. Stimmeder, *Inflamm. Res*., **48**, 369 (1999).
3. N. Khisti and S. Bhandari, *Asian J. Pharm. Clin. Res*., **5**, 1 (2012).
4. J.A. Balfour, A. Fitton and L.B. Barradell, *Drugs*, **51**, 639 (1996).
5. M.M. Ghoneim, A.M. Beltagi and A. Radi, *Anal. Sci*., **18**, 183 (2002).
6. I. Cetin, N. Koçak and S. Aycan, *C.B.U. J. Sci*., **5**, 11 (2009).
7. L. Sivasubramanian, K.S. Lakshmi and T. Tintu, *Int. J. Pharm. Pharma. Sci*, **2**, 166 (2010).
8. Y.H. Kim, H.Y. Ji, E.-S. Park, S.-W. Chae and H.S. Lee, *Arch. Pharm. Res*., **30**, 905 (2007).
9. Y.L. Zeng, X.Y. Chen and Y.F. Zhang, *Yao Xue Xue Bao*, **39**, 132 (2004).
10. S. Singh, D.K. Jain and N. Dubey, *Int. J. Pharm. Biom. Anal*., **5**, 57 (2011).
11. W.S. Radhofer and P. Dittrich, *J. Chromatogr. B*, **707**, 151 (1998).
12. V.G. Kulkarni, S.V. Gandhi, P.B. Deshpande and P.H. Chaube, *Int. J. Pharm. Pharm. Sci*, **3**, 225 (2011).
13. A. Nakamura, M.N. Nakashima, M. Wada and K. Nakashima, *Bunseki Kagaku*, **54**, 755 (2005).
14. V.P. Shah, K.K. Midha, S. Dighe, I.J. McGilveray, J.P. Skelly, A. Yacobi, T. Layloff, C.T. Viswanathan, C.E. Cook, R.D. McDowall, K.A. Pittman and S. Spector, *J. Pharm. Sci*., **81**, 309 (1992).
15. E. Mikami, T. Goto, T. Ohno, H. Matsumoto and M. Nishida, *J. Pharm. Biomed. Anal*., **23**, 917 (2000).
16. L. Du, D.G. Musson and A.Q. Wang, *J. Pharm. Biomed. Anal*., **42**, 556 (2006).