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Research Article

EXTRACTION LESS HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR DETERMINATION OF LORNOXICAM IN HUMAN PLASMA

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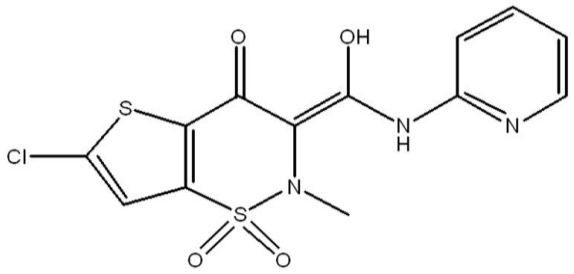
ABSTRACT
A simple and rapid (extraction less) high-performance liquid chromatographic method with UV detection, at 375 nm, was developed for the determination of Lornoxicam in human plasma. Piroxicam is added as internal standard. The separation is performed at 40°C on a C_{18} HiQ Sil column with 0.1 M sodium dihydrogen o-phosphate: methanol (45:55, v/v, pH 4.3) as mobile phase. The retention time for Lornoxicam is 7.64 min. & for Piroxicam 5.96 min**.** The lower limit of detection is 0.1 µg/ml.

Keywords: High-performance liquid chromatography, Lornoxicam, Trichloroacetic acid, Piroxicam.

INTRODUCTION

Lornoxicam ((3E)-6-chloro-3-[hydroxy(pyridin-2-ylamino) methyl ene]-2-methyl-2,3-dihydro-4H-thieno[2,3-e][1,2]thiazin-4-one 1,1-dioxide) [1]is a non-steroidal anti-inflammatory drug (NSAID). Lornoxicam is a compound in the same chemical class as piroxicam, meloxicam and tenoxicam, with potent anti-inflammatory, antipyretic and analgesic activity.



Lornoxicam (chlortenoxicam), is a new nonsteroidal anti-inflammatory drug (NSAID) of oxicam class. It is distinguished from established oxicams by a relatively short elimination half-life[2], Lornoxicam inhibits the COX-1/COX-2 system, the production of interleukin-6, and the inducible NO synthase[3]. 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 four hours, the duration of effect is approximately eight hours, analogous to other acidic antipyretic analgesics. The analgesic potency of Lornoxicam is remarkable. In doses of 16mg (i.m.) its analgesic effect is comparable with that of 20mg morphine (i.m.) or 50mg Tramadol (i.v.) [4].

Lornoxicam readily penetrates into synovial fluid. Lornoxicam synovial fluid: plasma AUC ratio is 0.5 after administration of 4mg twice daily[5]. In elderly patients the clearance of Lornoxicam is reduced by about 30% to 40%; thus the half-life is somewhat longer. Even in the presence of impaired kidney and liver function, no major differences in pharmacokinetics have been observed. On account of its short half-life, no accumulation is likely to occur even in cases of repeated administration – in contrast to NSAID with a longer half-life. Like other Oxicams and Diclofenac, Lornoxicam is metabolised via Cytochrome P450 (CYP-2C9). Due to a genetic polymorphism some individuals may metabolise slowly and therefore have elevated levels of Lornoxicam. Lornoxicam's potency of effect on the two COX isoenzymes in vitro is similar to that of Diclofenac and about two powers of ten stronger than that of Tenoxicam. Lornoxicam is an active substance from the group of acidic anti-pyretic analgesics. The accumulation of acidic analgesics in the inflamed tissue is considered to be a significant aspect of their anti-inflammatory effect. In cases of painful inflammatory reactions, the capillaries in the inflamed tissue are damaged and plasma proteins along with bound pharmaceutical substances are discharged into the extra vascular space. On account of the reduced pH value in inflamed tissue, analgesic acids are able to move from the extracellular space and enter the cells more easily. This also explains why the duration of action of acidic substances is generally longer than one would expect in consideration of their plasma half-life. The inflamed tissue probably behaves like a deep compartment whose filling and depletion adjust to the plasma concentrations with substantial delay. Like all other NSAID Lornoxicam's mechanism of action is based on the inhibition of Cyclo-oxygenase (COX); an almost equivalent inhibition of COX-1 and COX-2 is achieved. [4]

Literature survey reveals several HPLC methods for the determination of Lornoxicam in pharmaceutical formulation[6]. Determination of Lornoxicam in pharmaceutical preparation by using spectrophotometric and chromatographic methods[7], Polarographic determination of Lornoxicam in pharmaceutical formulation is also reported[8]. Reports are available for determination Lornoxicam and its metabolites in plasma & synovial fluid[9] and LC-MS/MS method with electro spray ionization for quantization of Lornoxicam in human plasma[10]. No work has been reported for the determination of the Lornoxicam in plasma by HPTLC method. The new quantitative HPLC method described below, for which excellent accuracy and precision are demonstrated, is faster and successfully validated.

EXPERIMENTAL

Reagents and Chemicals

Methanol and water were of HPLC Grade. While Sodium Hydroxide, Sodium Dihydrogen Orthophosphate, Trichloroacetic acid were all AR grade chemicals. Standard bulk drug samples of Lornoxicam and Piroxicam supplied by Glenmark Pharmaceutical Ltd (Baddi, HP) and Wockhardt Research Center (Aurangabad), respectively.

Instrumentation and Chromatographic Conditions

Jasco HPLC system (Japan) consisting of Jasco PU-2080 plus HPLC pump and MD-2010 Plus PDA detector equipped with HiQ Sil C_{18} (250 x 4.6 mm i.d.) column was used in analysis. A Rheodyne injector with 50 µL loop was used for injecting the sample. BORWIN 1.50 software was used to collect and process the analytical data. All Weighing were done on Shimadzu balance (Model AY-120).

Separation and analysis was carried out on HiQ Sil C_{18} (250 x 4.6 mm i.d.) column with UV detector. Mobile phase consisting of Sodium dihydrogen o-phosphate (pH-4.3): Methanol in ratio of (45:55,v/v) was filtered through a 0.2 µm membrane filter, degassed, sonicated and used with flow rate of 1.0 ml min^{-1}. The column temperature was maintained 40 ^{0}C. Quantization of both drugs was carried out at 375 nm.

Preparation of Standard Stock Solutions

A Standard stock solution of Lornoxicam was prepared by dissolving 5 mg drug in 0.3 ml of 0.1 M NaOH and then diluted with mobile

phase to final volume of 10 ml in volumetric flask to get concentration 500 µg/ml. To prepare the calibration plot the stock solution of Lornoxicam was diluted to 0.2, 0.5, 1, 2, 3, 4 and 5 µg/ml with mobile phase.

Preparation of ISTD stock solution of PR (500 µg/ml):

5 mg of Piroxicam was dissolved in 0.3 ml of 0.1 M NaOH and then diluted with mobile phase to final volume of 10 ml in volumetric flask to get concentration of 500 µg/ml. Using a calibrated pipette, 0.8 ml of ISTD stock solution (500 µg/ml) was pipette into a 10.0 ml volumetric flask and made up the volume with the mobile phase to get concentration of 40 µg/ml.

Preparation of plasma sample solution

To 0.5 ml of plasma, 50 µl of an I.S. solution (Piroxicam, 40 µg/ml), 0.5 ml of Lornoxicam calibration solutions, 0.5 ml methanol and 0.5 ml of trichloroacetic acid (10 % w/v) were added to a glass tubes. Each sample was vortex mixed for 3 min and centrifuged (2500 rpm for 20 min). After centrifugation 50 µl aliquots of supernatant of each concentration were injected into the HPLC system.

VALIDATION

The method was validated in accordance with FDA guidelines [11]

Calibration Plot

The calibration plot for the HPLC method was constructed by analysis of six solutions containing different concentrations of Lornoxicam (0.2, 0.5, 1, 2, 3, 4 and 5 µg/ml). In the range 0.2-5 µg/ml the data were best fitted by a linear equation mx + b = y, the coefficient of determination (R^2) was 0.993 (Table 1 & Figure 1).

Selectivity

Six blank plasma samples were tested for interferences by of R_F values obtained from human plasma samples spiked with Lornoxicam. Result is given in Figure 2.

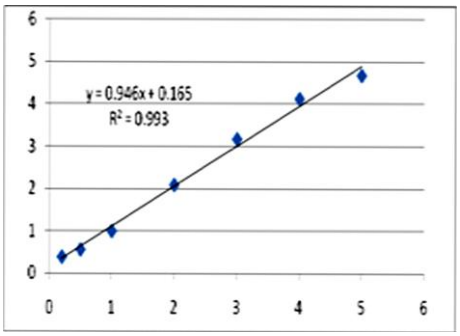


Fig 1: Calibration curve of Lornoxicam in plasma.

Recovery

Recovery from human plasma samples was evaluated in triplicate for each of three concentrations of Lornoxicam; the response for each level being compared with that from the corresponding standard solution. Recovery was from 87.05 to 80.30 % (Table 2).

Accuracy and Precision

The mean values for accuracy were within 15 % of the actual value (except at the LLOQ where 20 % is allowed) whereas for precision the relative standard deviation (RSD) values did not exceed the limit of 15% (except at the LLOQ where 20% is allowed).

Repeatability

The repeatability (intra-assay precision) of the method was evaluated in triplicate on the same day for three different concentrations performing of Lornoxicam. The results, expressed as mean amount of drug found, are shown in Table 3.

Reproducibility

The reproducibility (inter-assay precision) was evaluated in triplicate for three different concentrations of Lornoxicam on three consecutive days (fresh samples were prepared every day). The results, expressed as mean amounts of drug found, are shown in Table 4. **Stability**

Samples of Lornoxicam at three concentrations were tested in triplicate through three freeze–thaw cycles and during storage at – 5 ± 0 °C. The stability of samples in the bench top and the stability of the stock solution were also tested.

Results and Discussion

Recovery of Lornoxicam from human plasma was from 87.05 to 80.30 % compared with the standard solution, and the variability (RSD) on the same day and on different days was <15%. The range quantified in human plasma using linear regression was from 0.2 to 5 µg/ml. The mobile phase resolved the drug efficiently. The Rt values of Lornoxicam (Rt-7.64 min) & Piroxicam (Rt-5.96 min).

Lornoxicam was shown to be stable through three freeze–thaw cycles, during storage for 3 weeks at –5 ± 0 °C, during storage in bench top for 6 h, and in the stock solution for 5hr. 30 min; the results obtained were precise and accurate.

CONCLUSION

This HPLC method for quantification of Lornoxicam in human plasma is accurate, precise, rapid, and selective. It is a simple, practical, and economical alternative for studies of the bioavailability, bioequivalence, and pharmacokinetics of this drug in human plasma

Acknowledgments

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Table 1: Method validation data of quantification for Lornoxicam by HPLC.

Method characteristic	Value
Range	0.2-5 µg/ml
Coefficient of determination (R^2)	0.993
Lower limit of quantification (LLOQ)	0.2 µg/ml
System suitability	RSD = 5%

Table 2: Recovery of Lornoxicam.

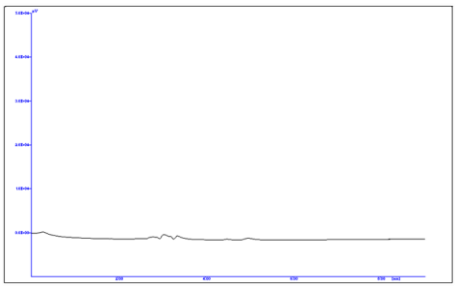
Level	Concentrations [µg/ml]	Mean amount [µg/ml]		Mean Recovery [%]	RSD [%]
		Plasma	Solution		
1	0.5	0.411± 0.017	0.511± 0.010	80.30	4.016
2	2	1.879±0.031	2.240±0.050	83.88	1.611
3	4	3.823± 0.078	4.392± 0.143	87.05	2.032
Average Mean Recovery			83.74		

Table 3: Repeatability of analysis of Lornoxicam.

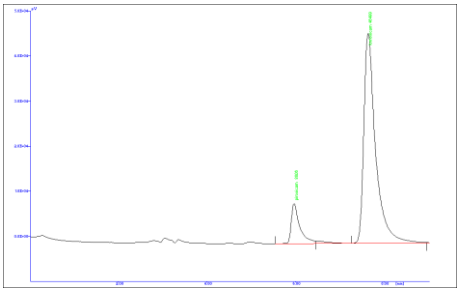
Concentration of Drug [µg/ml]	Mean amount of Drug found +SD [µg/ml]	RSD [%]
1	0.825± 0.0440	5.34
3	2.944± 0.108	3.67
4	3.727± 0.1213	3.25

Table 4: Reproducibility of analysis of Lornoxicam

Concentration of Drug [µg/ml]	Mean amount of Drug found ± SD [µg/ml]			Mean from 3 days ± SD [µg/ml]	RSD [%]
	Day 1	Day 2	Day 3		
0.5	0.396± 0.0078	0.397± 0.0216	0.407± 0.0134	0.4 ± 0.006	1.52
2	1.957± 0.1022	1.887± 0.0754	2.039± 0.1204	1.961± 0.076	3.88
4	3.793± 0.0980	3.754± 0.1177	3.739± 0.1736	3.762±0.028	0.74



Blank Plasma



Lornoxicam & Piroxicam peaks

Fig.2. Chromatograph of Plasma spiked with Lornoxicam (Rt-7.64 min) & Piroxicam (Rt-5.96 min)

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