Development and validation of a stability indicating HPLC method for the analysis of lornoxicam in powder for injection

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Abstract: A rapid, isocratic stability indicating high performance liquid chromatographic method was developed and validated for the estimation of lornoxicam in its powder for injection. The analysis was performed on a Shimadzu VP-ODS (4. 6mm×15cm, 5μm) column. The mobile phase consisted of sodium acetate (pH 5.8; 0.05M) and methanol (45:55) flowed at 1.0 ml/min. Detection was carried out at 290 nm. The developed method had the good ability to separate lornoxicam well from the degradation products. The regression data showed good linear relationship at the concentration range of 4.04–20.20 μg/ml with r^2=0.9999. Specificity, linearity, accuracy, precision and robustness of the method were evaluated to validate the proposed method. Stressed degradation studies were conducted to provide an indication of its stability indicating property. The limits of detection and quantitation were 9.70 and 33.94 ng /ml, respectively. Lornoxicam was found to be stable in the mobile phase in 24 h. The co-existed excipients had no interference with the analytical procedure. Additional peaks appeared in the chromatograms of five kinds of forced degraded samples (light, heat, acid, base and oxidation degradation). Mean recovery assessed at three levels was from 99. 7 to 100.3%, indicating the good accuracy of the method. Repeatability and inter-day RSD of the method was determined to be 0. 38% and 0.81%, respectively. The HPLC method was demonstrated to be robust for intentional minor changes of ratio, pH change, salt concentration and column temperature. The method should be utilized as the routine analysis and quality control of lornoxicam in injectable formulation.

Keywords: Lornoxicam; HPLC; stability indicating.

INTRODUCTION

Lornoxicam [6-chloro-4-hydroxy-2-methyl-N-2-pyridyl-5H-thieno[2,3-e]-thiazine- 3-carboxa mide -1,1-dioxide] (fig. 1) is a nonsteroidal anti-inflammatory drug of the oxicam class, with analgesic and anti-inflammatory activities (Balfour *et al*., 1996). Due to its short half-life compared to other oxicams, lornoxicam has both high therapeutic potency of oxicams and improved gastrointestinal profile (Radhofer-Welte *et al*., 2000).

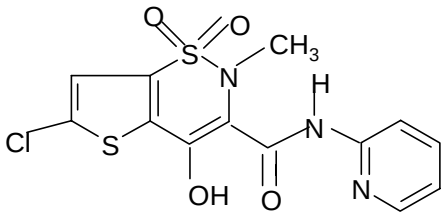

Fig.1: Structural formulae of lornoxicam

Several literatures reported the quantitation of lornoxicam in biological fluids by HPLC method (Hitzenberger *et al*., 1990; Bareggi *et al*., 1997; Radhofer-Welte *et al*., 1998; Bozal *et al*., 2010). Suwa *et al*. (1993) reported a HPLC method with electrochemical detection to determine lornoxicam and its metabolite, 5'- hydroxylornoxicam, in human plasma simultaneously. However, none of them was validated to be stability indicating for the assay of lornoxicam in its pharmaceutical products.

The objective of the present study was to develop and validate a stability indicating RP-HPLC method for the assay of lornoxicam in pharmaceutical powder for injection. It was a simple, economical analytical method that can be used for the quantitative assay of lornoxicam co-existing with the excipients and the potential degradation products.

MATERIALS AND METHODS

Materials and reagents

Lornoxicam reference standard was kindly provided by Zhenyuan Pharmaceutical Co. Ltd. (Zhejiang, China). Pharmaceutical injectable powder for reconstitution (Dalu®, Batch No. 101011, 101021, 101026) was obtained commercially and was claimed to contain 8.6mg of lornoxicam in each vial. Methanol (Tedia Company Inc., USA) was HPLC grade. All other analytical chemicals and reagents were purchased from Shanghai Chemicals Co. Ltd. (Shanghai, China). Distilled and deionized water was used through the study.

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Optimized chromatographic conditions

The isocratically reversed-phase chromatographic separation was performed on a Shimadzu VP-ODS column (4.6×150 mm, 5 μm) using the Shimadzu HPLC (Kyoto, Japan) consisted of a LC-10AT pump, a SPD-10A UV-VIS detector. The column temperature was maintained at a constant of 30ºC. The mobile phase comprising a mixture of methanol and 0.05mol/L sodium acetate buffer (pH 5.8) (55:45, v/v) was pumped at a flow rate of 1.0ml/min. The mobile phase was prepared freshly, filtered through a 0.45 μm Millipore filter. The elute absorbance was monitored at 290 nm with 20 μl injection volume. The detection sensitivity was set at 0.02 AUFS. To obtain good resolution between lornoxicam and the potential degradation products, the composition of buffer-methanol mixtures, pH value and salt concentration of buffer were adjusted and optimized, before an optimum chromatographic condition was achieved.

Preparation of solutions

Lornoxicam stock solution of 80.8 μg/ml was prepared using mobile phase as the solvent. Standard solutions were then prepared by appropriately diluting the stock solution with mobile phase. Lornoxicam concentration was at the range of 4.04 to 20.20 μg/ml.

Preparation of sample solutions

Each vial of lornoxicam injectable powder for reconstitution was quantitatively transferred to 50 ml volumetric flask and dissolved in mobile phase to keep the final concentration to 172 μg/ml. Aliquot of this solution were diluted in mobile phase at about 13.76 μg/ml.

Stressed degradation

Stressed degradation studies were conducted to check the ability of the method to separate lornoxicam from its degradation products and provide the proof of the stability indicating ability and specificity of the reported method. In this study, intentional degradation was accomplished by exposing the injectable powder for reconstitution to stressed conditions of heat, acid, base, light and oxidation. Briefly, appropriate amounts of powder (about 8 mg lornoxicam) was mixed with 0.5ml of acid (1mol/L of HCl), base (1mol/L of NaOH) or peroxide solution (5% H_2O_2) and allowed to stand at 80ºC for appropriate period according to the extent of degradation. Thermal and photodegradation were attempted by exposing the product to high temperature at 100ºC for 1h or direct sunlight for 3 days. Samples were all filtered before analysis.

Validation of the assay method

The method was validated by evaluating the linearity, recovery, accuracy, precision, limit of quantification, limit of detection and ruggedness. Stability of lornoxicam in test solution was also evaluated. Linearity the calibration curve of peak area versus concentration was obtained with six standard solutions with concentrations ranging from 4.04 to 20.20 μg/ml. Least-square linear regression method was used to perform the linear regression analysis and linearity evaluation. The accuracy at three levels (80%, 100% and 120%) was determined by recovery experiments. Solutions of three levels were prepared in triplicate and assayed. An accurately weighed amount of lornoxicam (about 20, 25 and 30 mg lornoxicam, respectively) and corresponding formulated amounts of excipients present in injectable powder for reconstitution were transferred into 100 ml volumetric flask and completely dissolved in mobile phase. Aliquots of 2 .0 ml of the solution were transferred into 25 ml volumetric flask and mobile phase was added to makeup the volume.

Precision

The method precision was evaluated by both intra-day (repeatability) and inter-day precision and was denoted as RSD of a series of measurements. Repeatability was assessed by conducting six consecutive injections of 12.12 μg/ml lornoxicam standard solution. Inter-day RSD was calculated from analytical results from 5 days.

Limit of detection (LOD) and limit of quantitation (LOQ)

To estimate the LOD and LOQ of the method, mobile phase was injected three times to determine the noise level. Then the LOQ and LOD were calculated by stepwise dilution of 12.12 μg/ml lornoxicam standard solution at a signal-to-noise ratios of 3:1 and 10:1, respectively.

Specificity

To confirm the specificity of the proposed method, placebo solution containing all inactive excipients was prepared and injected into the chromatographic system to examine the existence of the interference of excipients.

Robustness

The optimized parameters for mobile phase were intentionally changed to examine their effect on retention time, drug recovery and peak parameters and then to evaluate the method ruggedness. The mild changes made included: pH value (from 5.5 to 6.2), salt concentration of sodium acetate (from 0.045 to 0.055M), the methanol ratio in mobile phase (from 53% to 57%), the flow rate (from 0.98 to 1.02), the detection wavelength (from 288 to 292) and column temperature (from 29.5 to 30.5ºC).

Stability of analyte in test solution

The stability of lornoxicam in test solution was determined by injecting the 12.12 μg/ml lornoxicam standard solution every 2h till 24h. The stability was studied by checking the change in 5 lornoxicam peak area and the appearance of new peak. RSD of the data gave an indicative judgment of the stability test.

Analysis of the formulation

To determine the content of lornoxicam in three batches of injectable powder for reconstitution, solid powders existed in 10 vials of each batch were taken out, powdered and mixed thoroughly. An accurately weighed amount of yellow powder (equivalent to 8.6 mg of lornoxicam) were then put into 100 ml volumetric flask and completely dissolved in mobile phase. Aliquots of 2.0 ml of this solution were transferred into 10 ml measuring flask and mobile phase was then added to makeup the volume.

RESULTS

Optimization of chromatographic conditions

The orientation in developing the HPLC technique was setting up an optimum chromatographic condition, which can elute and resolve lornoxicam and its related substances while complying with the general requirements for system suitability. So some main chromatographic parameters, including the wavelength for detection, the pH value of the aqueous phase and the composition of mobile phase were assessed. Two maximum absorption wavelengths, 290 nm and 378 nm, were found in UV spectrum. However, degradation products cannot be efficiently detected in chromatograms at 378 nm. On the other hand, excellent detectability and peak resolution between the chromatographic peaks of lornoxicam and the related substances can be achieved at the wavelength of 290 nm, as shown as below.

In optimizing the composition of mobile phase, firstly, the simple mixture of 50% methanol in water was used as the mobile phase, but no desirable peak shape was achieved. So sodium acetate buffer (0.5 M) was used as the aqueous phase to enhance the lornoxicam peak symmetry successfully.

Volume ratio of methanol and sodium acetate buffer was optimized in the next step to achieve satisfactory separation and appropriate retention time. Methanol-0.05 M sodium acetate (40:60, v/v) system was found to be able to elute lornoxicam but it increased column 6 pressure and lengthened the retention time significantly (>15 min). While large methanol proportion (>70%) created very short retention time (<4.5min) with weak peak separation. Good separation and excellent peak characteristics were obtained meanwhile when the proportion of methanol in mobile phase was adjusted to about 55%.

Finally, variations in pH from 5.8 to 3 resulted in disappearance of some peaks present in aforementioned chromatograms and the sharp increase of tailing factor of lornoxicam peak, perhaps due to the weak solubility of lornoxicam and related substances in excessive acidic medium. Thereby, a representative chromatogram obtained in the optimum chromatographic conditions mentioned previously was shown in fig. 2, which showed the significant separation of lornoxicam and its related substances.

Stability indicating properties

Chromatograms of the degradation samples of lornoxicam by stressed heat, light, base, acid and oxidation were shown in fig. 3, demonstrating that the degradation products could be detected and well separated from lornoxicam within 10 min. The retention time for lornoxicam was about 7.5 min.

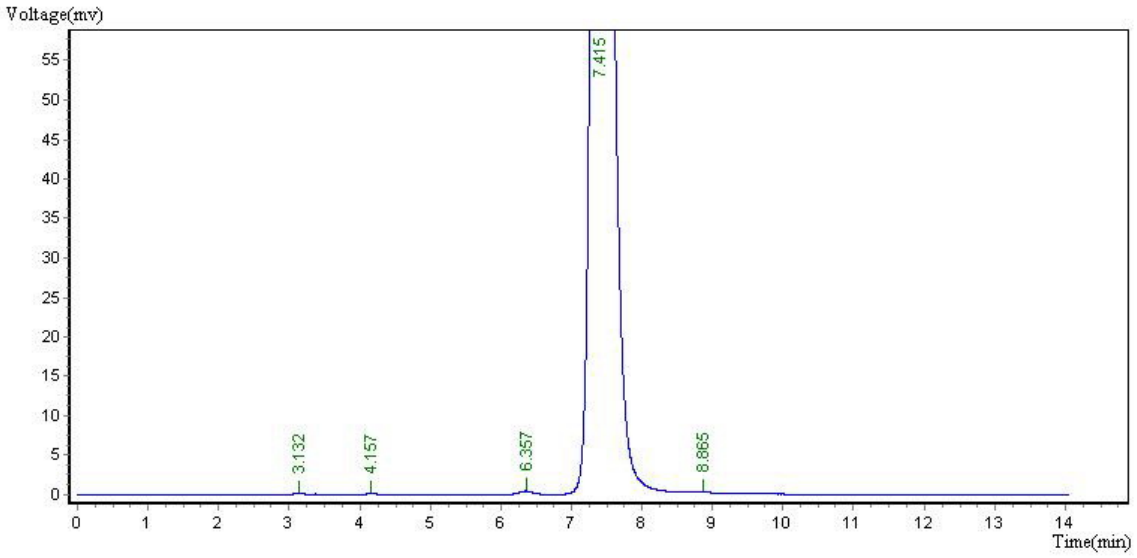
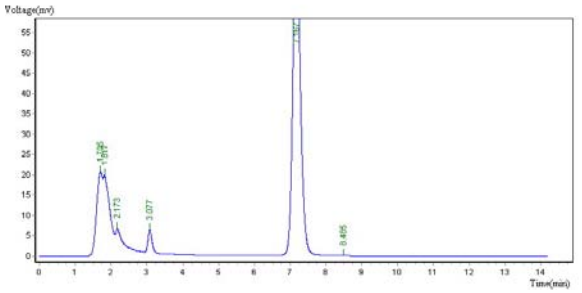


Fig. 2: Representative chromatogram of lornoxicam.



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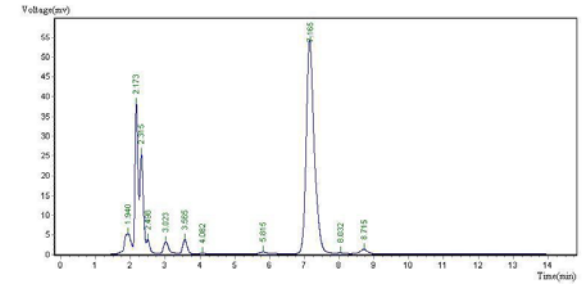

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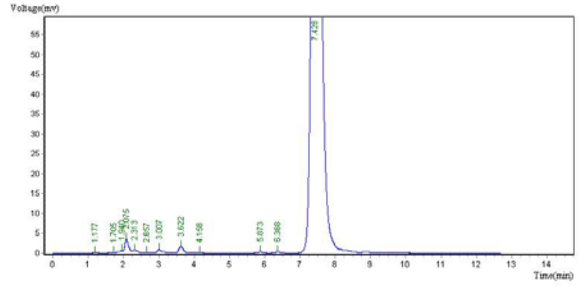

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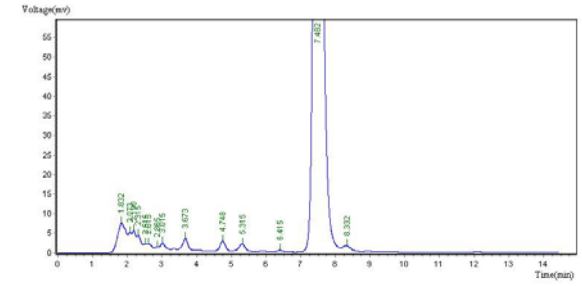

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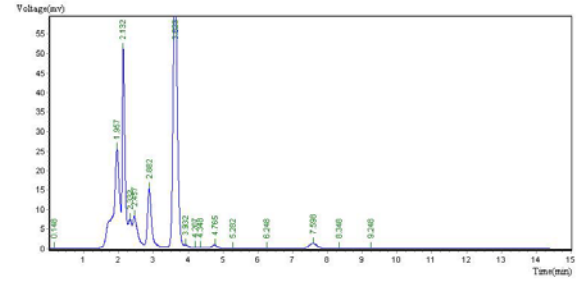

V

Fig. 3: HPLC chromatograms of lornoxicam and its stressed degraded products by: (I) acid degradation; (II) base degradation; (III) photodegradation; (IV) thermal degradation; (V) oxidative degradation.

Validation of the method

Specificity

The chromatogram of placebo formulation consisting of all inactive ingredients in the injectable powder showed no any peak under current HPLC conditions. The capacity of the method to separate lornoxicam from its degradation products and the non-interference with lornoxicam indicates the specificity of the method.

Linearity

The calibration curve for lornoxicam was constructed by plotting peak area (A) versus concentration (C, µg/ml). Statistics analysis was performed with the aid of SAS 6.12 software and results were listed in table 1. It was demonstrated that an excellent correlation existed between the peak area and lornoxicam concentration at the tested concentration range.

Table 1: Linear regression: Least-squares fit data

Parameters	Results
Range of linearity	4.04~20.20 µg/ml
Regression equation	A=26769×C-7607.80
Regression coefficient	R =0.9999
Standard error of intercept	2723.28
Standard error of slope	203.24
Limit of detection	9.70 ng/ml
Limit of quantification	33.94 ng/ml

Limit of detection and limit of quantitation

The LOQ and LOD were listed in table 1, indicating the high sensitivity of the proposed method.

Accuracy

Recovery data was listed in table 2. The result demonstrated the good accuracy of the method for assay of lornoxicam in injectable powder.

Table 2: Method validation and application

Parameters	Mean ± SD	RSD
Accuracy		
Low level (80%) recovery	99.73±0.46	0.46
Middle level (100%) recovery	100.3±0.38	0.38
High level (120%) recovery	99.89±0.56	0.56
Precision		
Repeatability	100.8±0.38	0.38
Inter-day precision	99.24±0.80	0.81
Application		
Content determination	101.8%; 98.65%; 100.9%	1.62

Precision

The precision of the method was evaluated by both intra-day and inter-day and was denoted as RSD of a series of measurements. The results in table 2 indicated the high precision of the method.

Robustness

Intentional chromatographic change was made to evaluate the method ruggedness. It was shown that retention time of lornoxicam shifted slightly with the adjustment of the proportion of methanol and the flow rate. But the final assay did not show a significant change. The tailing factor and theoretical plates for lornoxicam was always less than 1.1 and close to 6000, respectively. The components were also well separated. Considering the stability in the system suitability parameters, the method conditions would be concluded to be robust.

Stability of the analyte in solution

Lornoxicam was demonstrated to be stable in mobile phase during the period of 24 h since the change in the peak was not significant with the RSD value being 0.57% and the peaks corresponding to the degradation products were also not observed.

Method application for assay of lornoxicam in formulation

The validated HPLC method was applied for all measurements to determine the content and related substances in injectable powders. The amounts of lornoxicam in three batches of products were determined, expressed as the percentage of the label claim and listed in table 2, demonstrating that the contents of the lornoxicam in the injectable powder met the requirement (95-105% of the label claim). Related substances in three batches of products were also determined to be lower than 1.0%.

DISCUSSION

The developed isocratic stability indicating high performance liquid chromatographic method was rapid and suitable for the estimation of lornoxicam in its powder for injection. It had the good ability to separate lornoxicam well from the degradation products. Specificity, linearity, precision, accuracy and robustness were demonstrated by method validation. Stressed degradation studies were conducted to provide an indication of its stability indicating property. The stability of analytical solutions was sufficient for the whole analytical process. Using the established method, the amount of lornoxicam in the powder for injection was determined to be within the USP requirements of 95%-105% of the label claims.

CONCLUSIONS

The developed and validated HPLC method is specific, precise, accurate and also stability indicating. It should be practicably advantageous and can be utilized as the routine analysis and quality control of lornoxicam in powder for injection.

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