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Short communication

# Determination of meloxicam in human plasma by liquid chromatography–tandem mass spectrometry following transdermal administration

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**Abstract**

A highly sensitive and selective liquid chromatography–tandem mass spectrometry (LC–MS–MS) method was developed to determine meloxicam of low concentration in human plasma. After a simple sample preparation procedure by one-step protein precipitation with methanol, meloxicam and the internal standard piroxicam were chromatographed on a Zorbax SB $C_{18}$ column. The mobile phase consisted of acetonitrile–water–formic acid (80:20:0.2, v/v/v). Detection was performed on a triple quadrupole tandem mass spectrometer by selected reaction monitoring (SRM) mode via eletrospray ionization (ESI) source. The method had a lower limit of quantification of 0.10 ng/ml. The calibration curve was demonstrated to be linear over the concentration range of 0.10–50.0 ng/ml. The assay was specific, accurate (percentage deviations from nominal concentrations were within ±2.5%), precise (intra- and inter-day relative standard deviation (R.S.D.) <7%). The validated method was successfully applied to the determination of meloxicam in human plasma collected up to 180 h after a transdermal administration of 30 mg meloxicam for evaluation of the pharmacokinetics.


*Keywords:* Meloxicam; LC–MS–MS; Pharmacokinetics; Transdermal

## 1. Introduction

Meloxicam, 4-hydroxy-2-methyl-*N*-(5-methyl-2-thiazolyl)-2*H*-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]. Though a good gastrointestinal tolerability has been well-documented compared with other NSAIDs, bellyache and indigestion are the common side effects. In order to avoid the irritation of gastrointestinal tract, minimize systemic toxicity and achieve a better therapeutic effect, meloxicam was developed as a topical transdermal formulation [2,3].

A number of analytical methods exist for the determination of meloxicam in biological fluids, including reversed phase high-performance liquid chromatography (HPLC) equipped with ultraviolet (UV) [4,5] or mass spectrometric detection [6,7]. The low plasma concentration and long resident time after topical application of the drug justify the necessity of developing a sensitive LC–MS–MS method which could present a more accurate description for the pharmacokinetic profile of meloxicam after the application of a transdermal formulation.

In the present study, a more sensitive LC–MS–MS method was to be developed and applied to the pharmacokinetic study after a transdermal administration of 30 mg meloxicam to 10 healthy volunteers. The calibration range was between 0.1 and 50 ng/ml, and the LLOQ of this method was 0.1 ng/ml.

## 2. Experimental

### 2.1. Materials

Meloxicam (99% purity) and piroxicam (internal standard, 99% purity) were supplied by Taiyang Pharmaceutical Co.,

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Ltd. (Beijing, China). Methanol and acetonitrile (HPLC-grade) were purchased from Kangkede Chemical (Tianjin, China). Formic acid (analytical grade) was from Shenyang Chemical Co. (Shenyang, China).

### 2.2. Instrumentation and LC–MS–MS conditions

A Shimadzu LC-10AD system with a SIL-HT auto-sampler was used in the LC–MS–MS system. Isocratic chromatographic separation was performed on a Zorbax SB $C_{18}$ column (150 mm × 4.6 mm I.D., 5 μm, Agilent, USA), with a Security Guard $C_{18}$ guard column (4 mm × 3.0 mm I.D., Phenomenex, Torrance, CA, USA), using the mobile phase of acetonitrile–water–formic acid (80:20:0.2, v/v/v) which was delivered at a flow rate of 0.5 ml/min. The column temperature was maintained at room temperature (25 °C). The sample injection volume was 20 μl.

A Thermo Finnigan TSQ Quantum Ultra triple-quadrupole mass spectrometer (San Jose, CA, USA), equipped with an eletrospray ionization (ESI) source, was used in positive ion mode with selected reaction monitoring (SRM) for the quantitative analysis. The spray voltage was set to 3.5 kV, and the capillary temperature was maintained at 320 °C. Nitrogen was used as the sheath gas (30 arb) and auxillary gas (8 arb) for nebulization. For collision-induced dissociation (CID), argon was used as the collision gas at a pressure of 1.2 mTorr. In the experiment, the collision energy in the SRM mode was set at 25 eV for meloxicam and piroxicam. Quantification was performed using the transitions *m/z* 352 → 115 for meloxicam and *m/z* 332 → 95 for piroxicam, with a scan time of 0.3 s per transition. Analytical data were acquired using Xcalibur 1.4 software, and quantification processing was performed using LCquan software (Finnigan).

### 2.3. Preparation of standard and quality control samples

Stock solution of meloxicam was prepared in methanol at the concentration of 400 μg/ml. Calibration curves were prepared by spiking 50 μl of the appropriate working solutions into 0.25 ml of blank human plasma. Effective concentrations in plasma samples were 0.10, 0.25, 0.80, 2.0, 4.0, 10, 20 and 50 ng/ml for meloxicam. The quality control (QC) samples used in the validation and during the pharmacokinetic studies were prepared in the same way as the calibration standard at the concentration of 0.25, 4.0 and 45 ng/ml, and then treated as described below.

### 2.4. Sample preparation

To a 0.25 ml aliquot of plasma sample, 50 μl of internal standard (50 ng/ml piroxicam in methanol) and 0.50 ml of methanol were added. The mixture was vortex-mixed for 30 s, followed by centrifugation for 5 min at 3000 × *g*, then the supernatant was transferred to a clean glass tube and a 20 μl aliquot of the resulting solution was injected into the LC–MS–MS system for analysis.

### 2.5. Method validation

Plasma samples were quantified using the ratio of the peak area of meloxicam to that of I.S. as the assay parameter. Peak area ratios were plotted against meloxicam concentrations and standard curves in the form of $y = A + Bx$ were calculated using weighted ($1/x^2$) least squares linear regression.

To evaluate linearity, plasma calibration curves were prepared and assayed in duplicate on three separate days. The accuracy and precision were assessed by determining six replicates of QC samples at 0.25, 4.0 and 45 ng/ml of meloxicam on the three different validation days. The lower limit of quantification (LLOQ) was assessed by analyzing six plasma samples spiked with 0.1 ng/ml of meloxicam. The matrix effect was evaluated by comparing the response of the solution spiked with the blank processed matrix with the solution at the same concentration.

Absolute recoveries of meloxicam at three QC levels were determined by assaying the samples as described above and comparing the peak areas of both meloxicam and I.S. with those obtained from direct injection of the compounds dissolved in the supernatant of the processed blank plasma.

Meloxicam was reported stable for three freeze–thaw cycles in human plasma and the analyte was stable in the supernatant after protein precipitation in the autosampler for 8 h [5]. Stability of meloxicam in plasma was further assessed by analyzing replicates ($n = 3$) of QC samples at 0.25 and 45.0 ng/ml stored for 24 h at ambient temperature after preparation and following under storage conditions (−20 °C) for 30 days. The results were compared with those QC samples freshly prepared, and the percentage concentration deviation was calculated.

### 2.6. Application in the pharmacokinetic study

The method described above was used to determine meloxicam concentrations in plasma samples after giving each of the 10 healthy male volunteers 30 mg meloxicam transdermally in a gel (3 g) in a pharmacokinetic study approved by the Ethics Committee of Liaoning People's Hospital, China. The gels were evenly applied to the back of the volunteers on two defined areas each of 225 $cm^2$ (15 cm × 15 cm), one on either side of the spine. The gels were not cleaned up until 12 h after application. Serial blood samples (4 ml) were withdrawn into heparinized tube from antecubital vein at 0, 4, 8, 12, 24, 36, 60, 72, 84, 108, 132, 156 and 180 h post dose. Plasma was separated by centrifugation at 3000 × *g* for 10 min and kept frozen at −20 °C until analysis. Pharmacokinetic parameters were calculated with non-compartmental model of TOPFIT 2.0 program.

## 3. Results and discussion

### 3.1. LC–MS–MS optimization

The LC–MS–MS method for the determination of meloxicam and piroxicam in human plasma was investigated. The soft ionization process in the ESI source produced the protonated molecules $[M + H]^+$ of the analytes. The protonated molecules at *m/z* 352 and *m/z* 332 represented meloxicam and piroxicam,

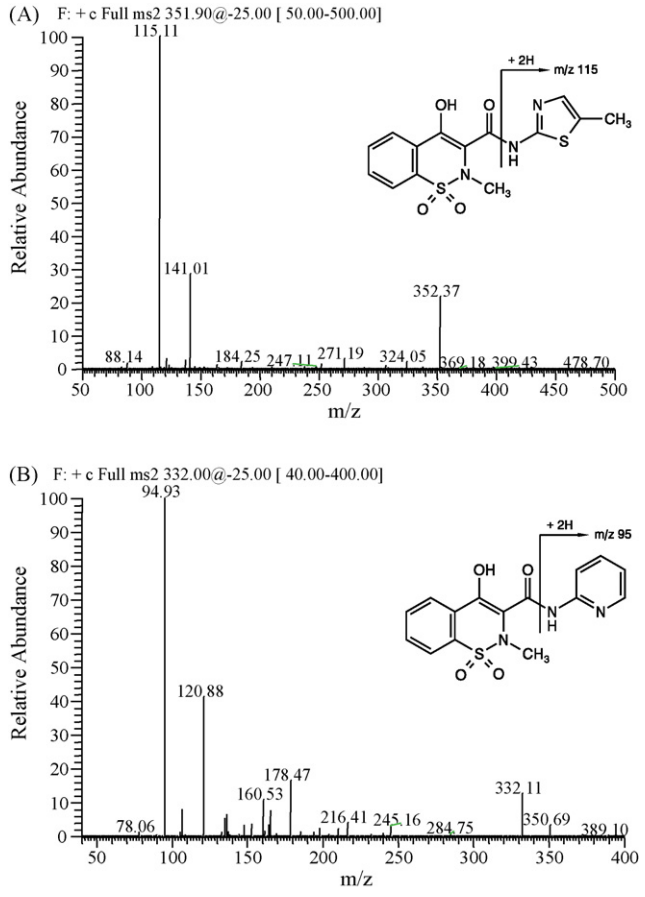


Fig. 1. Full scan product ion mass spectra of $[M+H]^+$ ions of meloxicam (A) and piroxicam (B).

respectively. Under the experimental conditions, the product ion mass spectrum of meloxicam showed an intense fragment at *m/z* 115 formed by the cleavage of an amide bond. Piroxicam showed an intense product ion at *m/z* 95 resulting from the similar fragmentation pathway with meloxicam by the cleavage of amide bond. The major product ions at *m/z* 115 and *m/z* 95 were selected for the sensitive quantification of meloxicam and piroxicam (Fig. 1), respectively.

The chromatographic conditions were investigated to optimize sensitivity, speed and peak shape. The mobile phase composition was optimized by flow injection of analytes while varying the percentage of organic solvent. It was found that high organic solvent contents (about 80%) in the HPLC system decreased the background noise and provided rapid separation and stable MS signal throughout an analytical run, allowing the enhancement of sensitivity. Acetonitrile was chosen as the organic solvent for its lower background noise than methanol. It was also found that the addition of acidic modifiers (formic acid) to the mobile phase could improve the sensitivity by promoting the ionization of the analytes. A mobile phase consisting of acetonitrile–water–formic acid (80:20:0.2, v/v/v) was used in the experiment provided symmetrical peak shapes, meanwhile, under the present chromatographic condition, the retention times for both meloxicam and I.S. were less than 3.8 min.

### 3.2. Sample preparation

Two sample preparation techniques were tried including liquid–liquid extraction and protein precipitation. Interfering peaks were found after liquid–liquid extraction, perhaps due to the impurity of the organic solvents. The protein precipitation technique employed was simple and effective which allowed high throughput analysis. Methanol was chosen as the protein precipitation solvent because it was less toxic and cheaper than acetonitrile with the same protein precipitation effect.

### 3.3. Method validation

#### 3.3.1. Linearity of calibration standards

The plotted calibration curves and correlation coefficients >0.99 confirmed that the calibration curves were linear over the concentration range 0.10–50.0 ng/ml for the analyte. A typical standard curve was as follows: $y = 0.2143x + 2.913 \times 10^{-2}$, $r = 0.9978$, where $y$ represents the ratios of meloxicam peak area to that of I.S. and $x$ the plasma concentrations of meloxicam.

#### 3.3.2. Assay specificity and LLOQ

Three typical SRM chromatograms from the study of meloxicam in human plasma are shown in Fig. 2. No interfering peak was observed in blank plasma (Fig. 2A). The SRM chromatograms of blank plasma spiked with meloxicam (0.1 ng/ml), and piroxicam (I.S., 10.0 ng/ml) are shown in Fig. 2B. A sample from a volunteer 108 h after a topical administration of 30 mg meloxicam is shown in Fig. 2C. For both the drug and the internal standard, the chromatograms were free from endogenous matrix interference at their respective retention times.

The present LC–MS–MS method offered an LLOQ of 0.1 ng/ml with an accuracy of −3.9% in terms of RE and a precision of 8.1% in terms of R.S.D. ($n = 6$).

#### 3.3.3. Precision and accuracy

The intra- and inter-day precision and accuracy were calculated by analysis of variances based on replicate analyses (3 days, 3 concentrations, each $n = 6$) of QC samples. The results are summarized in Table 1. In this study, the intra- and inter-day precisions were less than 7.0% for each QC level. The rela-

Table 1
Accuracy and precision for the analysis of meloxicam in human plasma (3 days, 6 replicates per day)

| Nominal plasma concentration (ng/ml) | Mean measured concentration (ng/ml) | Relative error (%) | Intra-day R.S.D. (%) | Inter-day R.S.D. (%) |
|---|---|---|---|---|
| 0.25 | 0.25 | −0.9 | 7.0 | 5.7 |
| 4.00 | 3.90 | 2.5 | 6.0 | 4.4 |
| 45.0 | 44.1 | 1.9 | 3.0 | 2.7 |

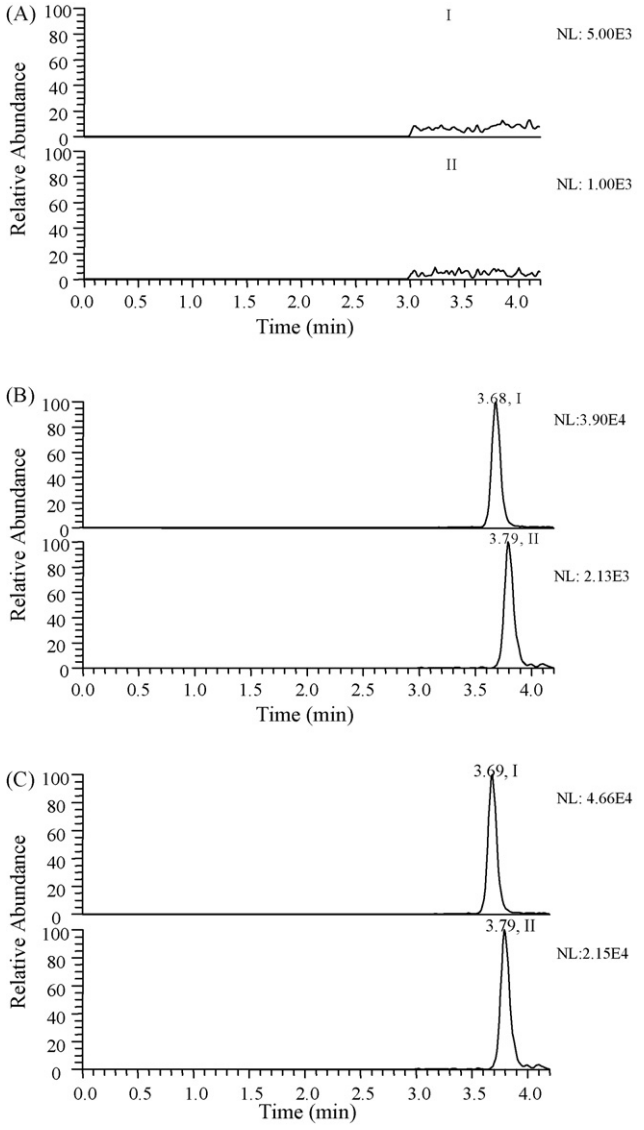


Fig. 2. Representative SRM chromatograms of piroxicam (I) and meloxicam (II) in human plasma samples. (A) Blank plasma sample; (B) blank plasma sample spiked with II (0.1 ng/ml) and I (10.0 ng/ml) and (C) volunteer plasma sample 108 h after a transdermal administration of 30 mg of II.

tive error was within ±2.5%. These data indicated reproducible LC–MS–MS results, and that the assay was accurate and reliable.

### 3.3.4. Recovery and stability

The recovery of meloxicam, determined at three concentrations (0.25, 4.0, 45 ng/ml), were 94.4 ± 5.7%, 97.2 ± 3.5% and 92.8 ± 4.9% ($n = 6$), respectively. The recovery of piroxicam was investigated as 90.6 ± 4.0% ($n = 6$).

The results of stability experiments showed that meloxicam was stable for 24 h after preparation at ambient temperature, and for at least 30 days in plasma at −20 °C, the mean relative errors (RE%) were within ±6.0% and ±9.0%, respectively.

### 3.3.5. Pharmacokinetic study

The method has been successfully applied to the pharmacokinetic study of meloxicam after transdermal application of meloxicam gel (30 mg meloxicam) for 180 h to 10 healthy volunteers. The mean plasma concentration–time profile of meloxicam is shown in Fig. 3. The main pharmacokinetic parameters of meloxicam in 10 volunteers were calculated. After topical administration of 30 mg meloxicam, $T_{max}$ and $C_{max}$ values were found to be 74 ± 33 h and 11.0 ± 10.4 ng/ml, respectively. Plasma concentrations declined with $t_{1/2}$ of 60.7 ± 45.3 h. The $AUC_{0-t}$ values were 1061 ± 1141 ng h/ml, which was about 1.21% compared with that (44 ± 7 μg h/ml) of reported after an oral dose of 15 mg meloxicam to 17 healthy male volunteers [4]. The low exposure indicated that the topical formulation could avoid gastrointestinal irritation and decrease the systemic toxicity. The results were consistent with a previously reported transdermal application of piroxicam patches (48 mg) for 24 h to the upper arms of four healthy male volunteers, $C_{max}$ and $T_{max}$ were 6.1 ± 1.4 ng/ml and 33 h, respectively [7]. It was also reported that after the topical application of diclofenac cream, the drug was absorbed slowly with 1% systemic bioavailability [8].

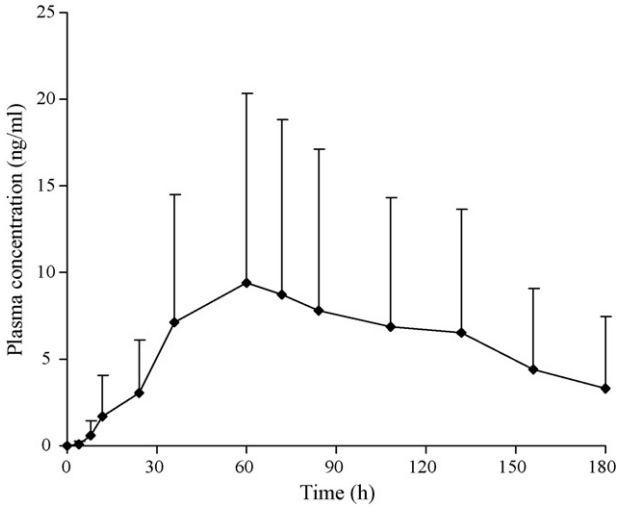


Fig. 3. Mean plasma concentration–time curve of meloxicam after a transdermal administration of 30 mg meloxicam to 10 healthy volunteers (each point represents mean ± S.D.).

## 4. Conclusion

An LC–MS–MS method was developed and validated for the determination of meloxicam in human plasma following transdermal administration. The method is rapid, sensitive and specific with a LLOQ of 0.1 ng/ml using 0.25 ml human plasma. The method was successfully applied to the pharmacokinetic evaluation of meloxicam after a transdermal administration of 3 g topical gel containing 30 mg meloxicam to 10 healthy volunteers.

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