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Determination of meloxicam in human plasma using a HPLC method with UV detection and its application to a pharmacokinetic study

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Abstract

A simple and sensitive high performance liquid chromatography method using UV detection (HPLC-UV) for the determination of meloxicam in human plasma was developed and validated. After extraction with diethyl ether, the chromatographic separation of meloxicam was carried out using a reverse phase Sunfire C_{18} column (150 mm × 4.6 mm, 5 μm) with a mobile phase of acetonitrile–20 mM potassium hydrogen phosphate (40:60, v/v, pH 3.5) and UV detection at a wavelength of 355 nm. The flow rate of mobile phase was 1.2 ml/min and the retention time of meloxicam and internal standard, piroxicam, was found to be 11.6 and 6.3 min, respectively. The calibration curve was linear within the concentration range, 10–2400 ng/ml ($r^2 > 0.9999$). The lower limit of quantification was 10 ng/ml. This method improved the sensitivity for the quantification of meloxicam in plasma using a HPLC-UV. The mean accuracy was 98–114%. The coefficient of variation (precision) in the intra- and inter-day validation was 1.6–4.3 and 2.4–7.3%, respectively. The pharmacokinetics of meloxicam was evaluated after administering an oral dose of 15 mg to 11 healthy Korean subjects. The AUC_{inf}, C_{max}, t_{max} and $t_{1/2}$ were 42.4 ± 13.2 μg h/ml, 1445.7 ± 305.5 ng/ml, 4.1 ± 0.3 h and 22.0 ± 4.9 h, respectively.

Keywords: Meloxicam; HPLC; Pharmacokinetics

1. Introduction

Meloxicam [4-hydroxy-2-methyl-*N*-(5-methyl-2-thiazolyl)-*2H*-1,2-benzothiazine-3-carboxamide-1,1-dioxide] is a nonsteroidal anti-inflammatory drug (NSAID) that preferentially inhibits cyclooxygenase-2 (COX-2), imparting analgesic, antipyretic and anti-inflammatory effects [1]. The oral meloxicam can be almost completely absorbed with an absolute bioavailability of 89% [2]. Meloxicam binds strongly to serum albumin (>99%) and reaches a maximum concentration in 4–5 h after oral administration. Meloxicam is metabolized extensively in the liver into four pharmacologically inactive metabolites that are excreted in both the urine and feces. The apparent oral clearance (CL/F) of meloxicam ranges from 0.42 to 0.7 l/h [3].

A number of analytical methods for the determination of meloxicam in biological samples were reported. They include the methods using high performance liquid chromatography with UV detector (HPLC-UV) [4,5], HPLC with diode array detector [6] and LC–tandem mass spectroscopy (LC–MS–MS) [7–9]. Although LC–MS–MS has higher detection sensitivity, it is expensive and not readily available. HPLC-UV is a readily available and more economic instrument for measuring drug concentration in biological samples. Velpandian et al. [4] and Dasandi et al. [5] developed HPLC-UV methods for the determination of meloxicam in biological samples, and the lower limits of quantification (LOQ) of their methods were 100 and 50 ng/ml, respectively. These LOQ values were not enough for pharmacokinetic studies of oral single dose of 15 mg meloxicam.

In this study, the more sensitive HPLC-UV method was developed for a pharmacokinetic study of meloxicam. The HPLC-UV method was validated by evaluating the time profile of plasma concentrations of meloxicam in humans and the pharmacokinetic parameters.

2. Experimental

2.1. Reagents and chemicals

Meloxicam, piroxicam (I.S.), potassium dihydrogen phosphate, hydrochloric acid and *ortho*-phosphoric acid were

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purchased from Sigma–Aldrich (St. Louis, MO, USA). HPLC grade of acetonitrile and diethyl ether were purchased from J.T. Baker (Mallinckrodt Baker Inc., Phillipsburg, NJ, USA) and Fisher Scientific (Waltham, MA, USA), respectively. HPLC grade water was obtained using a Milli-Q water purification system (Millipore, Milford, MA, USA) and was used throughout the study.

2.2. Chromatographic conditions and instruments

The HPLC system consisted of a Waters 515 HPLC pump, a Waters 717 Plus Autosampler and a Waters 2487 Dual λ Absorbance Detector (Waters, Milford, MA, USA). An Autochro Data Module and Autochro Win-Chromatography Data System (Young Lin Instruments, Korea) were used as the data module and software, respectively. The analytic column was a Sunfire C_{18} column (particle size: 5 μm, 150 mm × 4.6 mm, Waters). The detection wavelength, 355 nm, was determined by scanning the maximum absorbance wavelength of meloxicam and piroxicam in the mobile phase using an UV spectrophotometer (Agilent 8453, Agilent Technologies Inc., DE, USA). The column temperature was 30 °C. The mobile phase contained a mixture of 20 mM potassium monophosphate-acetonitrile (60:40, v/v) and was adjusted to pH 3.5 with *ortho*-phosphoric acid, at a flow rate 1.2 ml/min. The mobile phase was filtered through a 0.2 μm pore size membrane filter (Millipore) and degassed ultrasonically after mixing.

2.3. Preparation of standard solutions and quality control samples

A stock solution of meloxicam (0.5 mg/ml) and piroxicam (I.S., 0.5 mg/ml) was prepared using 50% methanol. The secondary standard solutions of meloxicam were prepared by diluting the stock solutions with 50% methanol. The working standard solutions of meloxicam were produced by diluting the secondary standard solutions with blank human plasma. The seven calibration standards of meloxicam (final concentrations: 10, 40, 200, 600, 1200, 1800 and 2400 ng/ml) were prepared independently. The working I.S. solution (8 μg/ml) was prepared by diluting the stock solution with water. All solutions were stored at 4 °C until needed. The quality control (QC) samples at a concentration 40 ng/ml (low) and 1800 ng/ml (high) were made by diluting the secondary standard solution with blank human plasma.

2.4. Preparation of samples

Five hundred microliters of plasma or the calibration standards, 50 μl of an I.S. solution (piroxicam, 8 μg/ml) and 100 μl of 5 M HCl were added to a glass tube. After brief vortex mixing (Genie 2, Scientific Industries Inc., Bohemia, NY, USA), 6 ml of diethyl ether was added and the mixture was vortex mixed for 30 s. Each sample was centrifuged (2500 rpm for 10 min), and the organic layer was transferred to a new glass tube and evaporated to dryness under a gentle stream of nitrogen at 40 °C. The residue was reconstituted with 500 μl of the mobile phase and a 65 μl aliquot was injected into the HPLC system.

2.5. Validation of method

2.5.1. Calibration curve

The linearity of the method was evaluated by a calibration curve in the range of 10–2400 ng/ml meloxicam. The calibration curve was obtained by plotting the area ratios of meloxicam and I.S. as a function of the meloxicam concentration using least-squares linear regression analysis. The LOQ was defined as a reproducible lowest possible concentration, linear with the calibration curve having a coefficient of variation (CV) below 20% and accuracy between 80 and 120%. The LOQ was analyzed five times for conformation.

The matrix effect (co-eluting, undetected endogenous matrix compounds that may influence the chromatography) was investigated by extracting "blank" normal human plasma from 10 different sources, reconstituting the final extract in injecting solvent containing a known amount of the meloxicam and I.S., analyzing the reconstituted extracts and then comparing the peak areas of the meloxicam and I.S.

2.5.2. Accuracy and precision

The intra-day and inter-day accuracy and precision were determined by replicate analysis of five sets of samples spiked with seven different concentrations of meloxicam (10, 40, 200, 600, 1200, 1800 and 2400 ng/ml) within 1 day or on 5 consecutive days. The precision was determined to be the coefficient of variation (CV), and the accuracy is expressed as the relative standard error (R.S.E. (%) = measured concentration/targeted concentration × 100)).

2.5.3. Recovery

The absolute recovery of the meloxicam were determined in triplicate in normal plasma by extracting the blank human plasma samples spiked with meloxicam (10, 40, 200, 600, 1200, 1800 and 2000 ng/ml). The recovery was calculated by comparing the peak areas of the samples extracted with those obtained from a mobile phase with the same concentration.

2.5.4. Stability

The stability of meloxicam in human plasma was tested using low-quality (40 ng/ml) and high-quality control samples (1800 ng/ml) for three freeze–thaws, short-term, long-term and post-preparative stabilities. For the short-term stability, the frozen plasma samples (−70 °C) were kept at room temperature for 6 h before sample preparation. The freeze–thaw stability of the meloxicam was determined over three freeze–thaw cycles within 3 days. In each freeze–thaw cycle, the spiked plasma samples were frozen for at −70 °C 24 h and thawed at room temperature. The long-term stability was evaluated after keeping the plasma samples frozen at −70 °C for 1 month. The stability of the prepared plasma samples was tested after keeping the samples in an autosampler at 18 °C for 12 h. The samples were analyzed and the results were compared with those obtained for the freshly prepared samples.

2.5.5. Pharmacokinetic application

Eleven healthy Korean subjects (10 males and 1 female), a mean age of 24.3 ± 2.0 years and a mean body mass index (BMI) of 22.8 ± 1.5 kg/m^2, were enrolled in this study. All subjects were healthy, as defined by their medical history, a physical examination and routine laboratory tests, and the woman was not pregnant. The subjects were restricted from taking any medication, caffeine, grapefruit products and alcoholic beverages for at least 1 week before and throughout the study period. All the subjects provided informed consent, both verbally and in writing, to participate in the study. The study was performed according to the Declaration of Helsinki, and was approved by the Institutional Ethics Committee of College of Pharmacy, Sungkyunkwan University.

On the day of the study, each subject received a 15 mg oral dose of meloxicam (Mobic®, Boehringer Ingelheim Korea, Seoul, Korea) with 240 ml of water, after an overnight fast. The subjects were maintained in the fasting state for 4 h after administering the drug. Venous blood samples (10 ml) were obtained before and at 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, 48 and 72 h after meloxicam administration. The blood samples were immediately centrifuged, and the plasma samples were stored at −70 °C until needed.

The pharmacokinetic parameters of meloxicam were estimated using noncompartmental methods with BA calc 2002 [10]. The actual blood sampling times were used, and the maximum plasma concentration (C_{max}) and the time to reach C_{max} (t_{max}) were the observed values. The area under the plasma concentration–time curve (AUC) was calculated using the linear trapezoidal rule. The elimination rate constant (k_e) was estimated from the least-squares regression slope of the terminal plasma concentration. The AUC from 0 to infinity (AUC_{inf}) was calculated as the $AUC_{inf} = AUC + C_t/k_e$ (C_t is the last plasma concentration measured). The half-life ($t_{1/2}$) was calculated as $\ln 2/k_e$.

3. Results and discussion

3.1. Specificity

No matrix effect for meloxicam and I.S. was observed for 10 different plasma pools tested. The peak areas in the chromatograms of the 10 reconstituted samples had a CV 3.8% for meloxicam and 3.7% for I.S. No endogenous interference was found at the retention times of meloxicam and the I.S. Fig. 1 shows representative chromatograms for human blank plasma (Fig. 1A), human plasma spiked with meloxicam (10 ng/ml) and the I.S. (piroxicam 400 ng/ml) (Fig. 1B), and plasma sample obtained from a healthy volunteer at 10 h after an oral administration of 15 mg meloxicam (Fig. 1C). The LOQ was defined as a reproducible lowest possible concentration, linear with the calibration curve. The LOQ was found to be 10 ng/ml and a signal-to-noise ratio of LOQ was above 10. The intra- and inter-day CV were found to be 4.3 and 7.3%, respectively (Table 2). The LOQ value of our analytical method was the same as that reported by Medvedovici et al. using HPLC-diode array detector [6], 5 times lower than that reported by Dasandi et al. [5] and 10 times lower than that reported by Velpandian et al. [4]. And, it can be comparable with LOQ (8.96 ng/ml) of LC–MS–MS method [7].

The retention time of meloxicam and I.S. was approximately 11.6 and 6.3 min, respectively, and the total running time for each sample was 13 min. Although the retention time of meloxicam in our analytical method was longer than that (2.7 min) reported by Velpandian et al. [4], the LOQ value of our analytical method was 10 times lower than that reported Velpandian et al. [4].

3.2. Linearity and sensitivity

The standard calibration curves were linear over the meloxicam concentration ranges of 10–2400 ng/ml in human plasma with a mean correlation coefficient of 0.9998. Table 1 shows the

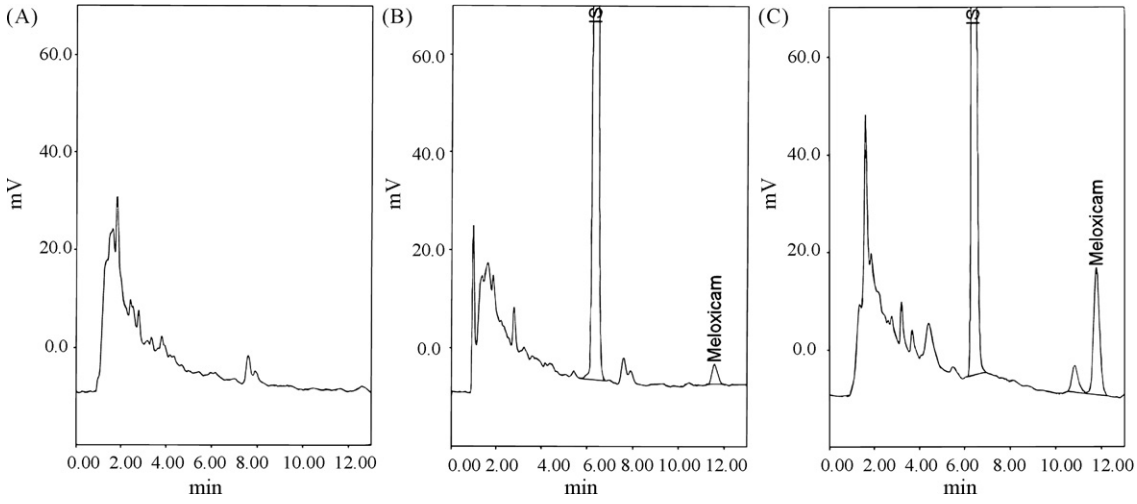

Fig. 1. HPLC chromatograms of meloxicam and the internal standard (I.S., piroxicam) in human plasma. (A) Blank human plasma, (B) blank human plasma spiked with meloxicam (10 ng/ml) and I.S. (piroxicam 400 ng/ml) and (C) human plasma sample collected at 72 h after administration of meloxicam 15 mg (meloxicam concentration: 76 ng/ml).

Table 1
Calibration curve data of meloxicam in human plasma

Number	Slope	Intercept	r^2
1	0.0016	−0.0014	0.9999
2	0.0016	−0.0033	0.9998
3	0.0016	−0.0027	0.9996
4	0.0016	0.0002	0.9999
5	0.0017	−0.0086	0.9996
Mean	0.0016	−0.0032	0.9998
S.D.	0.0000	0.0033	0.0002

individual calibration equations for meloxicam from five replicate experiments. The equation of the curve, which was obtained by a least-squares fit, was $y = 0.0016x - 0.0029$, where y represents the area ratios of the meloxicam peak ratio to that of I.S. and x is the plasma concentration of meloxicam.

3.3. Accuracy and precision

Table 2 gives a summary of the accuracy and precision at meloxicam concentrations of 10–2400 ng/ml. The intra-day accuracy and precision varied from 98.9 to 106.8%, and between 1.6 and 4.3%, respectively. The inter-day accuracy and precision ranged from 98.3 to 114.0% and from 2.4 to 7.3, respectively. This suggests that the present method has a satisfactory accuracy, precision and reproducibility.

3.4. Recovery

Methyl-*t*-butyl ether (MTBE), diethyl ether, dichloromethane and chloroform were tested as a solvent for extracting meloxicam from plasma. When MTBE, diethyl ether or chloroform was used as an extracting solvent, unknown peaks superposed on the peak of meloxicam or I.S.. In addition, the extraction with dichloromethane or chloroform showed a lower yield compared with extraction with diethyl ether (data were not shown). Thus, diethyl ether was selected as a solvent for extraction.

Table 3
Recovery of HPLC assay method for plasma meloxicam ($n = 3$ for meloxicam, $n = 21$ for I.S.)

Concentration (ng/ml)	Recovery (%, mean ± S.D.)	C.V. (%)
Meloxicam		
10	86.7 ± 4.5	5.1
40	83.1 ± 2.6	3.1
200	80.2 ± 5.7	7.1
600	77.2 ± 5.9	7.6
1200	78.6 ± 5.1	6.5
1800	80.0 ± 2.5	3.1
2400	83.8 ± 1.7	2.0
IS		
8000	67.5 ± 3.0	4.4

The recovery of meloxicam in the liquid–liquid extraction procedure using diethyl ether from 0.5 ml of plasma was measured at seven different concentrations over the calibration range used. Table 3 shows the absolute recovery, which is expressed as a percentage, obtained for both meloxicam and I.S. Regardless of the drug concentration, the recovery ranged from 77.2 to 86.7% with a C.V. between 2.0 and 7.6%. A recovery of 67.5% was obtained for the I.S.

3.5. Stability

Plasma samples of meloxicam at two concentrations (40 and 1800 ng/ml) were used for the stability experiments. The stability was assessed under a variety of conditions and the data is shown in Table 4. Three freeze–thaw cycles of the quality control samples did not appear to affect the quantification of the meloxicam. The quality control samples stored in a freezer at −70 °C remained stable for at least 1 month. Thawing the frozen samples and keeping them at room temperature for 6 h had no effect on quantification. The extracted samples were ana-

Table 2
Precision and accuracy of HPLC assay method for plasma meloxicam ($n = 5$ for precision and accuracy)

Targeted concentration (ng/ml)	Measured concentration (ng/ml; mean ± S.D.)	Accuracy (%)	Precision (C.V.%)
Intra-day			
10	10.7 ± 2.5	106.8	4.3
40	40.6 ± 1.7	101.5	2.6
200	200.7 ± 8.8	100.3	3.3
600	608.3 ± 15.6	101.4	2.3
1200	1187.1 ± 20.5	98.9	2.2
1800	1785.1 ± 52.8	99.2	2.2
2400	2410.2 ± 54.3	100.4	1.6
Inter-day			
10	11.4 ± 1.5	114.0	7.3
40	41.4 ± 1.9	103.6	5.5
200	206.2 ± 5.9	103.1	3.2
600	605.2 ± 8.0	100.9	2.4
1200	1179.8 ± 25.3	98.3	2.6
1800	1798.6 ± 36.5	99.9	3.8
2400	2414.6 ± 45.1	100.6	2.9

Table 4
Stability of HPLC assay method for meloxicam in human plasma ($n = 3$)

Stability	40 ng/ml		1800 ng/ml	
	R.S.E. (%)	C.V. (%)	R.S.E. (%)	C.V. (%)
Short-term stability	96.5 ± 9.4	9.7	98.2 ± 3.8	3.9
Freeze–thaw stability	101.8 ± 8.9	8.8	109.4 ± 2.9	2.7
Long-term stability	99.1 ± 8.3	8.4	104.0 ± 2.3	2.2
Post-preparative stability	98.8 ± 1.6	3.0	100.8 ± 1.0	3.0

R.S.E.: Relative standard error.

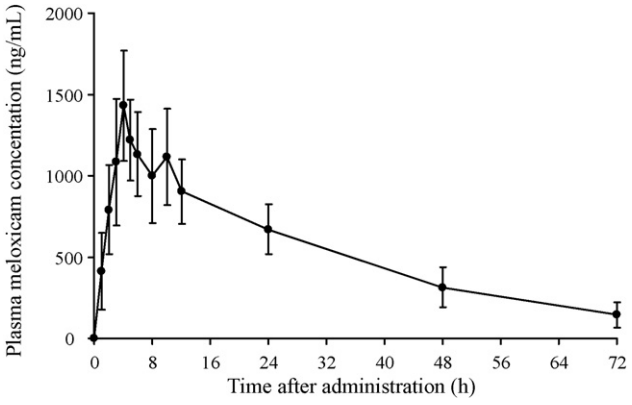

Fig. 2. Plasma concentration–time profile after the single oral administration of 15 mg meloxicam in healthy subjects (mean ± S.D., $n = 11$).

lyzed after at least 12 h at room temperature. This suggests that human plasma samples containing meloxicam can be handled under normal laboratory conditions without any significant loss of compound.

3.6. Pharmacokinetic application

This method was used to examine the pharmacokinetics of meloxicam after administering a single oral dose of 15 mg meloxicam. Fig. 2 shows the mean plasma concentration–time profile. Table 5 shows the mean values of the pharmacokinetic parameters for the volunteers. These pharmacokinetic parameters values are similar to those reported from India [5], Brazil [8] and Romania [6].

Table 5
The pharmacokinetic parameters of meloxicam in 11 volunteers after single oral dose of meloxicam 15 mg (mean ± S.D.)

Pharmacokinetic parameters	Mean ± S.D.
C_{max} (ng/ml)	1445.7 ± 305.5
t_{max} (h)	4.1 ± 0.3
$t_{1/2}$ (h)	22.0 ± 4.9
AUC_t (μg h/ml)	37.5 ± 9.8
AUC_{inf} (μg h/ml)	42.4 ± 13.2

In addition, this analytical method can be applied for the measurement of other oxicam NSAIDs (lornoxicam, piroxicam and tenoxicam) in human plasma.

4. Conclusion

In conclusion, a simple, accurate and sensitive reversed-phase HPLC method using UV detection was developed to measure the level of meloxicam in plasma. The method showed sufficient sensitivity for analyzing meloxicam in human plasma up to 72 h after administration of a single oral dose of 15 mg meloxicam.

References

[1] G. Engelhardt, D. Homma, K. Schlegel, R. Utzmann, C. Schnitzler, Inflamm. Res. 44 (1995) 423.
[2] N.M. Davies, N.M. Skjodt, Clin. Pharmacokinet. 36 (1999) 115.
[3] J. Schmid, U. Busch, G. Heinzel, G. Bozler, D. Kaschke, M. Kummer, Drug Metab. Dispos. 23 (1995) 1206.
[4] T. Velpandian, J. Jaiswal, R.K. Bhardwaj, S.K. Gupta, J. Chromatogr. B Biomed. Sci. Appl. 738 (2000) 431.
[5] B. Dasandi, Shivaprakash, H. Saroj, K.M. Bhat, J. Pharm. Biomed. Anal. 28 (2002) 999.
[6] A. Medvedovici, F. Albu, C. Georgita, C. Mircioiu, V. David, Arzneimittelforschung 55 (2005) 326.
[7] J.L. Wiesner, A.D. de Jager, F.C. Sutherland, H.K. Hundt, K.J. Swart, A.F. Hundt, J. Els, J. Chromatogr. B 785 (2003) 115.
[8] H.M. Rigato, G.D. Mendes, N.C. Borges, R.A. Moreno, Int. J. Clin. Pharmacol. Ther. 44 (2006) 489.
[9] Y. Yuan, X. Chen, D. Zhong, J. Chromatogr. B 852 (2007) 650.
[10] Y.J. Lee, Y.G. Kim, M.G. Lee, S.J. Chung, M.H. Lee, C.K. Shim, Yakhakhoeji 44 (2000) 308.