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A Validated HPLC Method for the Determination of Meloxicam in Pharmaceutical Preparations

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Emirhan Nemutlu*°, Filiz Sayın*, Nursabah E. Başcı*, Sedef Kır*

Introduction

Meloxicam (MEL) (4-hydroxy-2-methyl-N-(5-methyl-2-thiazoly)-2H-1,2-benzo-thiazine -3-carboxamide-1,1dioxide) ($C_{14}H_{13}N_3O_4S_2$) (Figure 1) is a non-steroidal anti-inflammatory drug (NSAID) with a favorable COX-2 (cyclooxgenase-2): COX-1 (cyclooxgenase-1) selectivity has also been shown to have potent anti-inflammatory effects[1-4]. Because of very low solubility of MEL in acidic medium, it may cause local gastrointestinal adverse events[5].

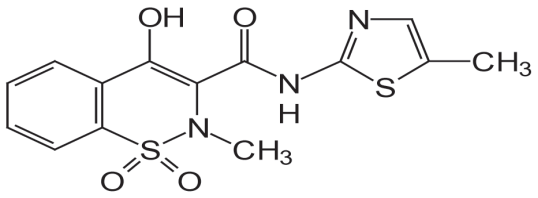

Figure 1
Chemical structure of MEL

In the literature, spectrophotometric[6-13], electrophoretic[14], chromatographic[9,10,15], polarographic[16-20] methods and a review[21] have been reported for the analysis of MEL in pharmaceuticals. HPLC is commonly used for the determination of MEL in plasma[22-23]. Joseph-Charles and

* Department of Analytical Chemistry, Faculty of Pharmacy, University of Hacettepe, 06100, Sıhhiye Ankara, TURKEY

° Corresponding Author: E-mail: enemutlu@hacettepe.edu.tr Fax: +90 312 311 47 77

Bertucat developed a HPLC for the determination of MEL in tablet formulations[9]. The HPLC method used a reversed-phase C18 column (Lichrocart Lichrospher 100-RP18, 125 x 4 mm i.d. and 5 µm particle size) with 0.05 M Tris acetic acid buffer - tetrabutylammonium reagent-acetonitrile (64:1:35, v/v/v) at a flow-rate 1.5 mL min^{-1}, and UV detection at 360 nm with isoxicam as the internal standard. The retention time of MEL was 6.21 under the chromatographic conditions. The method was linear over the concentration range of 1.5–3.5 µg mL^{-1} and the LOD of the method was 0.3 µg mL^{-1}. The method was not fully validated and the accuracy and precision studies have been performed at single concentration, although FDA or ICH regulations want to perform validation studies at three concentrations. Zawilla et al. presented a HPLC method for quantitative determination of MEL in pure form and pharmaceutical formulations[10]. The separation was performed on Spherisorb ODS (200 x 4.6 mm i.d. and 5 µm particle size) column with using MeOH : acetate buffer pH 4.3 (45:55, v/v) mixture as mobile phase. Under these conditions MEL was eluted at 13.8 min, but the analyte peak was not well defined because of its dramatic peak tailing. Vignaduzzo et al. developed a reversed-phase high-perfomance liquid chromatographic method for the simultaneous determination of MEL and pridinol mesylate in their synthetic mixtures and combined tablet formulations[15]. The drugs were separated on a 250×4.6 mm C18 analytical column packed with 5 µm particles. The mobile phase was a 51:9:40 (v/v/v) mixture of methanol, isopropanol and 50 mM potassium phosphate buffer (pH 5.9) at a flow rate of 1.0 mL min^{-1}. UV detection was performed at 225 nm. The method was validated in the concentration ranges of 33.7–61.8 µg mL^{-1} for MEL. The LOD and LOQ values were 0.22 and 1.7 µg mL^{-1}, respectively, while the method developed shows linear between 0.20-15.00 µg mL^{-1} with 0.02 µg mL^{-1} LOD and 0.20 µg mL^{-1} LOQ values.

Method validation is an important issue in drug analysis according to conventional regulations such as FDA, EMEA, and ICH. The process confirms that the analytical procedure employed for the analysis is suitable for its intended use and to show reliability of the results produced by any method. Therefore method validation is essential in drug analysis.

The main purpose of this study is to develop a simple, rapid, accurate, linear, sensitive, rugged and reproducible HPLC method for the determination of MEL. The developed HPLC method was validated with respect to linearity, accuracy, precision, sensitivity, and robustness.

Experimental

Apparatus

The HPLC equipment comprised of a solvent delivery system (Shimadzu 10 ATVP) and a photodiode array detector (Shimadzu M 10VP). The separations were achieved by a reversed phase column (Nucleosil 100-5 C18 150 x 4.6 mm i.d., 5 µm particle size) at room temperature. The mobile phase was consisted of 50 mM phosphate buffer – MeCN – MeOH (50:15:35 v/v/v) (pH 5.5) at a flow-rate of 1.0 mL min^{-1}. UV detection was performed at 366 nm.

The capillary zone electrophoretic (CZE) analyses (comparison method) were performed on an Agillent 3D CE apparatus consisted of an automatic injector, an auto sampler, a variable wavelength diode array detector and a temperature controlling system. Electrophoretic separations were carried out using fused silica capillary having 50 µm i.d. and 44 cm total length (35.5 cm effective length), in a positive mode using constant voltage (20 kV). Injections were performed hydrodynamically at the anodic side by pressure (50 mbar) for 3 s and capillary temperature was set 25 ^{0}C. Electropherograms were recorded at 205 nm.

Chemicals and reagents

MEL and tenoxicam (IS) were kindly supplied by Drug Industry of Nobel Joint-Stock Company and Drug Industry of Mustafa Nevzat Joint-Stock Company, respectively. MeCN, MeOH and potassium dihydrogenphosphate were purchased from Sigma. The water was purified using a Milli-Q system and used for the preparation of buffer and other aqueous solutions.

Standard and sample solutions

Standard solutions

Standard stock solution of MEL (250 µg mL^{-1}) and IS (1000 µg mL^{-1}) were prepared in MeOH. This solution was kept at + 4 ^{0}C protecting from light for 2 months. Working standard solutions were prepared by diluting stock solution with mobile phase.

HPLC method : standard solutions were daily prepared by diluting stock solutions in mobile phase to the MEL concentrations of 0.2, 0.5, 1, 2, 5, 10, and 15 µg mL^{-1} containing IS (5 µg mL^{-1}). The calibration curve

was prepared by plotting the peak area ratio of MEL to IS against to the concentration of MEL.

CZE method (Comparison method) [14]: Various aliquots of standard stock solution of MEL were taken to give a final analyte concentration (1, 2.5, 5, 10, 20, 50, 100 and 150 μg mL^{-1}) of MEL. Before diluting to 5 mL with background electrolyte [100 mM borate buffer (pH 8.5) containing 5 % MeOH], the 100 μL of IS standard stock solution was added.

Sample preparation

Tablet solutions

For each dosage forms, ten tablets were weighed and powdered, separately. Equivalent amount to one tablet was weighed and transferred to a 50 mL volumetric flask. MeOH (30 mL) was added and the flask was sonicated for 15 min to complete dissolution and diluted to the mark with MeOH. Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant and diluting them with mobile phase for HPLC or background electrolyte for CZE to give final concentration. Then tablet sample solutions were analysed same as standard solutions.

Synthetic tablet preparations

Synthetic tablets were prepared by mixing excipients (25 mg sodium citrate dehydrate, 95 mg lactose monohydrate, 39 mg avicel, 0.5 mg aerosile, 3 mg magnesium stearate and 10 mg PVP) and 7.5 mg of MEL. Then mixture was transferred 50 mL volumetric flask and than dissolved and analysed as explained in tablet solution.

Results and Discussion

Optimization

In the presented method, MEL and IS are separated on Nucleosil 100-5 C18 analytical column (150 x 4.6 mm i.d., 5 μm particle size) with a mobile phase containing 50 mM phosphate buffer – MeCN – MeOH (50:15:35, v/v/v) (pH 5.5) at a flow rate of 1.0 mL min^{-1} and UV detection was performed at 366 nm. The retention times were 11.1 min for MEL and 5.6 min for IS. The theoretical plate numbers and peak symmetry for MEL were 48000 and 1.08, respectively. Under optimized chromatographic conditions MEL and IS were accurately resolved from baseline and separated each other.

Buffer pH has an influence on the degree of ionization of the solutes. Therefore pH of buffer effects the retention time of solutes and efficiency (the number of the theoretical plate) of the method. To find optimum pH of the mobile phase, MEL was analysed at pH of 4.5, 5.5 and 6.5. pH value of the mobile phase had dramatic effect on the retention time of MEL and the column efficiency (Figure 2). The retention time of MEL decreased by increasing of the pH and the efficiency was increased until pH 5.5. Therefore the pH 5.5 was selected as optimum pH to obtain short analyses time without any lack of in the column efficiency.

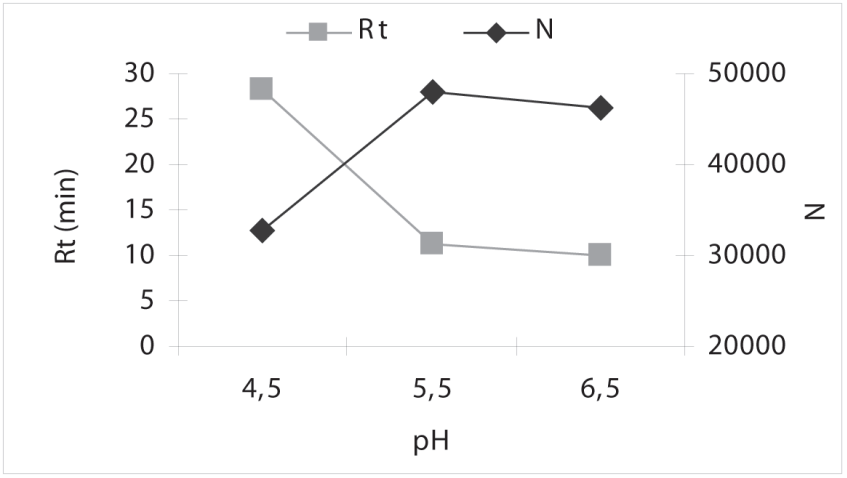

Figure 2
The effect of pH on retention time (Rt) and efficiency (N) in HPLC

Validation

The use of IS is an important parameter for reproducibility in HPLC in order to compensate the errors from injection and extraction process. In this study, tenoxicam was selected as an IS. The method was tested with respect to validation parameters such as stability, selectivity, linearity, precision, accuracy and ruggedness [26].

Stability

In previous study [13], the stability of stock solutions of MEL was evaluated in two different conditions, at +4 ^{0}C and at ambient temperature for 2 months and the stability of stock solutions of MEL in both

conditions was found stable at least two months. In this study the auto sampler stability of MEL (2 µg mL^{-1}) in mobile phase was evaluated for 24 hours. During this period, samples were analysed at 0, 2, 4, 6, 12, and 24 hours and the concentration of MEL was calculated with calibration curve. The relative standard deviation (RSD) of samples was 1.51 %. It is indicated that MEL is stable in the mobile phase at least 24 hours.

Selectivity/Specificity

The chromatograms obtained from tablet and synthetic tablet solutions (Figures 3c and 3d) were identical with that obtained chromatogram from standard solution containing an equivalent concentration of MEL (Figure 3a). There was no peak observed when the analyses of placebo solution without MEL (Figure 2c). In addition peak purity index for the MEL and IS were investigated and found 0.999 and 0.999, respectively in chromatograms of the tablets. These results suggested that the method presented in this study was specific.

Linearity range

Developed method was linear in the range of 0.20 - 15.00 µg mL^{-1}. The equation for the calibration curve obtained with the least square regression was y = 0.6229x + 0.0438 (n=6) where y is the peak area MEL

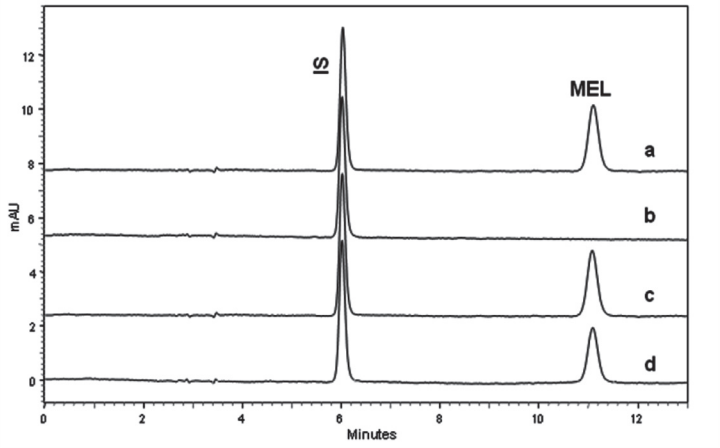

Figure 3

The chromatograms of MEL and IS. Operating conditions: 50 mM phosphate buffer – MeCN – MeOH (50:15:35 v/v/v) (pH 5.5) at a flow-rate of 1.0 mL min^{-1} and UV detection was performed at 366 nm. a) In the standard solutions (MEL 2.00 µg $mL^{-1}$and IS 5.00 µg mL^{-1}); b) Placebo solution without MEL (IS 5.00 µg mL^{-1}); c) In the synthetic solution (MEL= 2.00 µg $mL^{-1}$and IS 5.00 µg mL^{-1}); d) In the tablet solutions (IS 5.00 µg mL^{-1}).

to IS and x is the MEL concentration. The linearity was expressed by the linear correlation coefficient of 0.9999.

Limit of detection and quantitation

Limit of detection (LOD) of the method was 0.020 μg mL^{-1} at a signal-to-noise ratio of 3:1. The limit of quantitation (LOQ) for MEL was found to be 0.20 μg mL^{-1} (RSD: 5.04 %, n=6).

Precision

Precision studies of the method were performed at three different concentrations of MEL in the linear range by using six independent series in the same day (intra-day precision) and six consecutive days (inter-day precision). The RSD values of intra-day and inter-day studies varied from 0.33 % to 3.25 % showing that the intermediate precision of the method was satisfactory (Table I).

TABLE I

Precision and accuracy of the developed method (n=6).

	Intra-day			Inter-day		
Added μg mL^{-1}	Found $\bar{x}$ (μg mL^{-1}) ± SE	Accuracy Bias %	Precision RSD %	Found $\bar{x}$ (μg mL^{-1}) ± SE	Accuracy Bias %	Precision RSD %
0.50	0.49 ± 0.01	-2.00	2.05	0.51 ± 0.01	2.00	2.24
2.00	2.05 ± 0.02	2.50	0.88	2.06 ± 0.06	3.00	3.25
10.00	9.99±0.03	-0.10	0.33	10.10 ± 0.23	1.00	2.52

$\bar{x}$: Mean, SE : standard error, Bias % : [(found – added) / added] x 100, RSD : Relative standard deviation

Accuracy

The accuracy of a method is expressed as the closeness of agreement between the found value and reference value. It is determined by calculating the percentage relative error between the measured mean concentrations and added concentrations at the same concentration level of MEL. The results obtained for intra and inter day accuracy were ≤ 2.50 and ≤3.00 %, respectively (Table I).

Recovery

The recovery studies of MEL were performed in synthetic tablet samples prepared according to Section 2.3.2 (synthetic tablet preparations). The percentage recovery for MEL was found as 100.9 ± 1.22 % (RSD = 2.99 %, n=6).

Ruggedness

Ruggedness test of MEL analysis were performed by different analyst. 6 independent series containing 15 µg mL^{-1} MEL were analysed. The result was compared statistically (Wilcoxon paired test) and there was no difference between results (p=0.238 > p=0.050). Therefore the method is rugged.

Analysis of tablets

Tablets containing two dosage forms of MEL were analysed through the procedure as explained in the tablet solution. Analysis was performed under optimum conditions. Each tablet was analysed seven independent determinations and each series were analysed 3 times. The obtained results for MEL were compared with CZE method [14]. The statistical comparison of two methods was done by Wilcoxon paired test and there was no significant difference between HPLC and CZE methods (Table II).

Conclusion

In this study, a simple, efficient and reliable HPLC method was developed and fully validated for the analysis of MEL in tablets. The linearity range, limit of detection and quantification, precision, accuracy, specificity, selectivity, and ruggedness were performed to determine the suitability of the method. These full validation assays have been concluded that the developed HPLC method is linear, sensitive, accurate, precise, selective and rugged for the determination of MEL. As this method has the lowest LOD value is more sensitive than the other published HPLC methods. These advantages encourage the application of this method in routine analysis of MEL in pharmaceutical formulations.

TABLE II
The results obtained by HPLC and CZE methods for the assay of tablets containing 7.5 and 15 mg MEL (n=7)

	Mobic® 7.5 (7.5 mg MEL)		Mobic® 15 (15 mg MEL)	
	HPLC	CZE	HPLC	CZE
$\bar{x}$ (mg) ± SE	7.56 ± 0.92	7.57 ± 0.01	15.11 ± 0.52	15.04 ± 0.02
SD	2.25	0.04	1.28	0.02
RSD%	2.23	0.50	1.27	0.16

$\bar{x}$: Mean, SE : standard error, SD : Standard deviation, RSD : Relative standard deviation

Summary

A Validated HPLC Method for the Determination of Meloxicam in Pharmaceutical Preparations

A reversed-phase high-performance liquid chromatographic method is developed for the determination of meloxicam (MEL) in pharmaceutical preparations (tablets containing 7.5 and 15 mg MEL). MEL and the internal standard tenoxicam (IS) were analysed on a reversed-phase column (Nucleosil 100-5 C18 150 x 4.6 mm i.d., 5 µm particle size) with a mobile phase containing 50 mM phosphate buffer – MeCN – MeOH (50:15:35 v/v/v) (pH 5.5) at a flow-rate of 1.0 mL min^{-1} and UV detection was performed at 366 nm. The retention times for MEL and IS were 11.1 and 5.6 min, respectively. The linearity range was found to be 0.20-15.00 µg mL^{-1}. LOD and LOQ were found to be 0.02 µg mL^{-1} and 0.20 µg mL^{-1}, respectively. The method was validated and it was concluded that the developed method was accurate, sensitive, precise, rugged and useful for the quality control of MEL in pharmaceutical preparations. The tablet results were compared with a validated capillary zone electrophoretic method and there was no statistically difference between the results at the 95 % confidence level.

Keywords: Meloxicam; HPLC; Validation; Pharmaceuticals

Özet

Meloksikamın Farmasötik Preparatlardan Analizi İçin Valide Edilmiş HPLC Yöntemi

Meloksikamın (MEL) farmasötik preparatlardan (7,5 ve 15 mg içeren tabletler) tayini için ters faz yüksek performanslı sıvı kromatografisi yöntemi geliştirilmiştir. MEL ve tenoksikam (IS) ters faz kolon üzerinde (Nucleosil 100-5 C18 150 x 4.6 mm i.ç., 5 µm partikül çapı) 50 mM fosfat-asetonitril-metanol hareketli fazı ile 1 mL dak^{-1} akış hızında analiz edilmiştir ve UV tayin 366 nm'de gerçekleştirilmiştir. MEL ve IS'nin alıkonma süreleri sıra ile 11,1 ve 5,6 dakikadır. Doğrusallık aralığı 0,20-15,00 µg mL^{-1} olarak bulunmuştur. Yöntem kesinliğe, doğruluğa, hassaslığa ve tutarlılığa göre valide edilmiştir. Geliştirilen yöntemin doğru, kesin, hassas, tutarlı ve MEL farmasötik preparatlardan tayini için kullanışlı olduğuna karar verilmiştir.

Anahtar Kelimeler: Meloksikam, HPLC, Validasyon, Farmasötikler

REFERENCES

1. Noble, S., Balfour, J.A.: Meloxicam, Drugs, 51(3), 424 (1996).
2. Türck, D., Roth, W., Busch, U.: A Review of the Clinical Pharmacokinetics of Meloxicam, Brit. J. Rheumatol., 35(suppl.1), 13 (1996).
3. Engelhardt, G., Homma, D., Schlegel, K., Utzmann R., Schnitzler C.: Anti-Inflammatory, Analgesic, Antipyretic and Related Properties of Meloxicam, A New Non-Steroidal Anti-Inflammatory Agent with Favourable Gastrointestinal Tolerance, Inflamm. Res., 44, 423 (1995).
4. Davies, N.M., Skjodt, N.M.: Clinical Pharmacokinetics of Meloxicam. Clin. Pharmacokinet., 36(2), 115 (1999).
5. Martindale The Extra Pharmacopoeia, 32nd ed.: The Pharmaceutical Press, London, England, (1999), page. 52.
6. Garcia, M.S., Pedreno, C.S., Albero, M.I., Marti, A.: Spectrophotometric Methods for Determining Meloxicam in Pharmaceuticals Using Batch and Flow-Injection Procedures, Eur. J. Pharm. Sci., 9, 311 (2000).

7. Bebewy, L.I.: Stability-Indicating Method for the Determination of Meloxicam and Tetracaine Hydrochloride in Presence of their Degradation Products, Spectrosc. Lett., 31(4), 797 (1998).
8. Hassan, E.M.: Spectrophotometric and Fluorimetric Methods for the Determination of Meloxicam in Dosage Forms, J. Pharm. Biomed. Anal., 27, 771(2002).
9. Joseph-Charles, J., Bertucat, M.: Determination of Meloxicam in Tablets Formulations by Ultraviolet Spectrophotometry and High-Performance Liquid Chromatography., Anal. Lett., 32(10), 2051 (1999).
10. Zawilla, N.H., Abdul-Azim Mohammad, M., El Kousy, N.M., El-Moghazy Aly, SM.: Determination of Meloxicam in Bulk and Pharmaceutical Formulations, J. Pharm. Biomed. Anal., 32(6), 1135 (2003).
11. You W.W., Liu Y., Wang Z.B.: Determination of Meloxicam by Ultraviolet Spectrophotometry, Chinese J. Anal. Chem., 27(7), 841 (1999).
12. Taha E.A., Salama N.N. Fattah L.S.A., Stability-Indicating Methods for the Determination of Meloxicam and Tenoxicam in the Presence of their Degradation Products, Spectr. Lett. 35(4), 501 (2002).
13. Nemutlu E., Kır S., Validated Determination of Meloxicam in Tablets by Using UV Spectrophotometry, Hacettepe University Journal of The Faculty of Pharmacy, 24 (1) 13-24, 2004.
14. Nemutlu, E., Kır S.: Method Development and Validation for the Analysis of Meloxicam in Tablets by CZE, J. Pharm. Biomed. Anal., 31(2), 393 (2003).
15. Vignaduzzo, S.E., Castellano, P.M., Kaufman, T.S.: Method Development and Validation for the Simultaneous Determination of Meloxicam and Pridinol Mesylate Using RP-HPLC and its Application in Drug Formulations, J. Pharm. Biomed. Anal., Corrected Proof (2007).
16. Altıokka, G., Atkosar, Z., Tuncel, M.: Pulse Polarographic Determination of Meloxicam, Die Pharmazie, 56(2), 184 (2000).
17. Radi, A., El-Ries, M.A., El-Anwar, F., El-Sherif, Z.: Electrochemical Oxidation of Meloxicam and its Determination in Tablet Dosage Form, Anal. Lett., 34(5), 739 (2001).
18. Altınöz, S., Nemutlu, E., Kır, S.: Polarographic Behaviour of Meloxicam and its Determination in Tablet Preparations and Spiked Plasma, Il Farmaco, 57, 463 (2002).
19. Radi, A.E., Ghoneim, M., Beltagi, A., Cathodic Adsorptive Stripping Square-Wave Voltammetry of the Anti-Inflammatory Drug Meloxicam, Chem. Pharm. Bull., 49(10), 1257 (2001).
20. Rao, R. N., Meena, S., Rao A.R.: An Overview of the Recent Developments in Analytical Methodologies for Determination of COX-2 Inhibitors in Bulk Drugs, Pharmaceuticals and Biological Matrices, J. Pharm. Biomed. Anal., 39(3-4), 349 (2005).
21. Beltagi, A.M., Ghoneim. M.M., Radi, A.: Electrochemical Reduction of Meloxicam at Mercury Electrode and its Determination in Tablets, J. Pharm. Biomed. Anal., 27, 795 (2002).
22. Velpandian, T., Jaiswal, J., Bhardwaj, R.K., Gupta, S.K.: Development and Validation of a New High-Performance Liquid Chromatographic Estimation Method of Meloxicam in Biological Samples, J. Chromatogr.B, 738, 431 (2000).
23. Dasandi B., Saroj, S.H., Bhat, K.M.: LC Determination and Pharmacokinetics of Meloxicam, J. Pharm. Biomed. Anal., 28, 999 (2002).
24. Wiesner, J.L., De Jager, A.D., Sutherland, F.C.W., Hundt, H.K.L., Swart, K.J., Hundt, A.F., Els, J.: Sensitive and Rapid Liquid Chromatography–Tandem Mass Spectrometry Method for the Determination of Meloxicam in Human Plasma, J. Chromatogr. B, 785(1), 115 (2003).

25. Bae, J.W., Kim, M.J., Jang, C.G., Lee, S.Y.: Determination of Meloxicam in Human Plasma Using a HPLC Method with UV Detection and its Application to a Pharmacokinetic Study J. Chromatogr. B, 859 (1), 69 (2007).

26. ICH Topic Q2A, Validation of Analytical Procedures: Methodology, CPMP/ICH/281/95.