*Hacettepe University, Journal of Faculty of Pharmacy*
Volume 24 / Number 1 / January 2004 / pp. 13-24

# Validated Determination of Meloxicam in Tablets by Using UV Spectrophotometry

Received : 27.09.2004
Revised : 04.11.2004
Accepted : 08.11.2004

**Emirhan Nemutlu* / 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) [1,2]. MEL is a novel NSAIDs with a favorable COX-2 (cyclooxgenase-2): COX-1 (cyclooxgenase-1) selectivity has also been shown to have potent anti-inflammatory effects [3,4]. Because of very low solubility of MEL in acidic medium, it may cause local gastrointestinal adverse events [5].

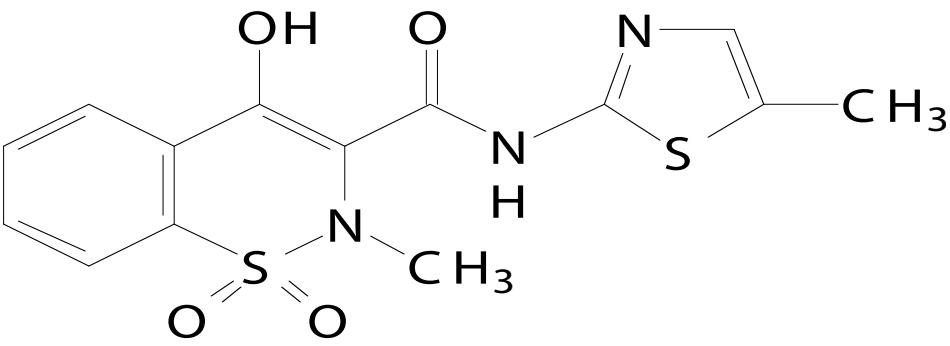


**Figure 1**
Chemical structure of MEL

In the references, spectrophotometric[6-12], electrophoretic[13], chromatographic[9,10] and polarographic[14-18] methods are reported for the analysis of MEL in pharmaceuticals. HPLC is the technique that most commonly used for the determination of MEL in plasma[19-21].

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

Method validation is an important issue in pharmaceutical analysis. The process confirm that the analytical procedure employed for the analysis is suitable for its intended use and to show reliability of the method. Therefore method validation is necessary in pharmaceutical analysis. In this study, all validation parameters for quantitative analysis of MEL in tablets were tested and validation data were evaluated according to their acceptance criteria.

The main purpose of this study is to develop a simple, rapid, accurate, linear, sensitivity, robust and reproducible spectrophotometric method for the determination of MEL in tablets. Some spectrophotometric methods have also been reported. Most of them are either time consuming or tedious or involve the use of organic solvent, furthermore are not completely validated for quantitative analysis.

The proposed method is very simple application and less expensive in comparison to the above mentioned techniques but at the same time offering a high degree of accuracy, precision and sensitivity when compared to the other specrophotometric methods in the literature and could be used simply to determine the shelve-stability time of the studied drug.

UV analysis of MEL was performed in 100 mM borate buffer (pH 8.5). The spectrum were recorded from 200 nm to 450 nm. The quantitative analysis was carried out at 363 nm.

The method was validated and applied for the determination of MEL in six pharmaceutical preparations including two dosage forms. The data from developed method was statistically compared with capillary electrophoretic (CE) method in the literature[13].

## *Material and Methods*

### *Apparatus*

The spectrophotometric measurements were carried out using an Agilent 8453 model UV-VIS spectrophotometer with a diode array detector (DAD) (190-1100 nm). A CE Chemstation software was used for instrument control, data acqusition and data analysis.

The CZE analyses were performed on an Agilent 3D CE capillary electrophoresis system.

The pH of solutions was measured by a pH meter (Orion Model 420 A).

*Chemicals and reagents*

MEL was kindly supplied by Nobel İlaç A.Ş. (Turkey). It was tested for purity by measuring its melting point and IR spectra and no impurities were found. Diflunisal [Internal standard (IS)], MeOH, NaOH and boric acid were purchased from Sigma. Milli-Q water was used for the preparation of buffer and other aqueous solutions. Pharmaceutical preparations of MEL were obtained from local pharmacies.

*Standard solutions*

Standard stock solution of MEL (250 µg/mL) and diflunisal (IS) (1000 µg/mL) were prepared in MeOH. This solution was kept at + 4 $^{0}$C maximum for 2 month and the stock solution was stable during this period.

UV method : Various aliquots of standard stock solution were taken and diluted to 5 ml with 100 mM borate buffer (pH 8.5) to give a final analyte concentration of wanted volume (0.5, 1, 2.5, 5, 10, 15, 20, 25 and 30 µg/mL). Then the absorbance of these solutions was measured.

CZE method (Comparison method): Various aliquots of standard stock solution of MEL were taken, the 100 µl of IS standard stock solution was added from and then diluted to 5 ml with background electrolyte [100 mM borate buffer (pH 8.5) containing 5 % MeOH], to give a final analyte concentration (1, 2.5, 5, 10, 20, 50, 100 and 150 µg/mL) [13].

Preparation of 100 mM borate buffer (pH 8.5) solution; 0.620 g boric acid was weighed and transferred 100 ml volumetric flask and than 85 ml deionised water was added and the flask was sonicated for 15 min to complete dissolution. Before diluted to constant volume, the desired pH was adjusted with 1 N NaOH.

*Tablet solutions*

Ten tablets were weighed from each dosage forms and powdered. 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 100 mM borate buffer (pH 8.5) to give final concentration (20 µg/mL). Then the absorbance of these solutions was measured.

*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 labeled amount (7.5 and 15 mg) of MEL. Then mixture was transferred 50 ml volumetric flask and than dissolved and analyzed as explained in tablet solution.

*UV Procedure*

Before the analysis of solutions containing MEL, the spectrophotometry was adjusted with 100 mM borate buffer pH 8.5. The spectrums were recorded from 200 nm to 450 nm. Appropriate dilutions were made in a linear range (1 – 150 µg/mL) from stock solution or samples with 100 mM borate buffer (pH 8.5). The quantitative analysis was performed at 363 nm.

*Electrophoretic Procedure*[13]

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). At the beginning of each working day, the capillary was rinsed with 0.1 N NaOH solution for 10 min. Between each injection, the capillary was rinsed with 0.1 N NaOH solution (2 min), water (2 min) and background electrolyte (3 min), respectively. 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 (band width 10 nm) using diode array detector.

## *Results and Discussion*

The UV spectra of standard solutions of MEL in MeOH and 0.1 N NaOH solution were found different from the spectra of tablet solutions. Therefore the pH of standard and tablet solutions was measured and the pH of standard solutions was found different from the tablet solutions. This situation was considered that pH was caused shift of the absorption band. As a result buffer solution (100 mM borate buffer) was chosen as a solvent to prevent changing of the pH consequently to shift of maximum wavelength. To determine the pH of buffer solution, the UV spectrum of MEL was measured at different pH and pH 8.5 was chosen as pH of 100 mM borate buffer to eliminate distinct between sample and standard solutions.

At the end of these studies, 100 mM borate buffer (pH 8.5) was chosen, because of time gain to prepare of solution and cost saving the eliminations of purchase and disposal of organic solvents. At this condition UV spectrum of MEL (15 µg/mL) was illustrated in Figure 2.

The UV spectrum of MEL in 100 mM borate buffer (pH 8.5) has two maximum absorption band at 205 and 363 nm. The absorbance of excipient in synthetic and tablet solution interfered with MEL at 205 nm (Figure 3). As a result 363 nm was selected for quantitative analysis to prevent excipient interference.

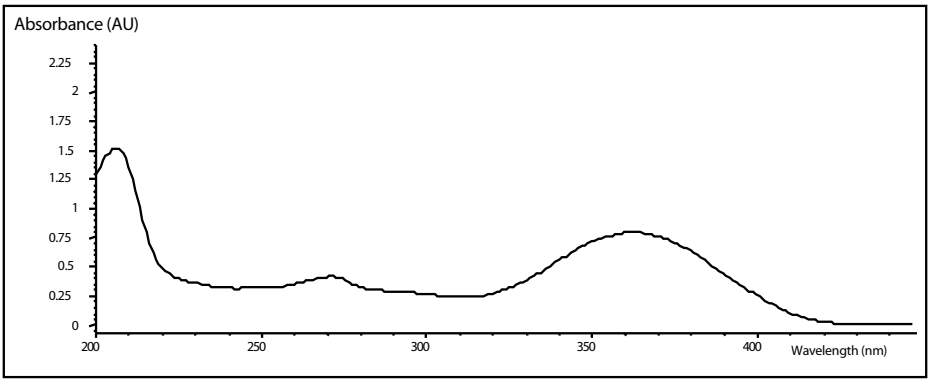


**Figure 2**
UV spectrum of MEL (15 mg/mL) in 100 mM borate buffer (pH 8.5)

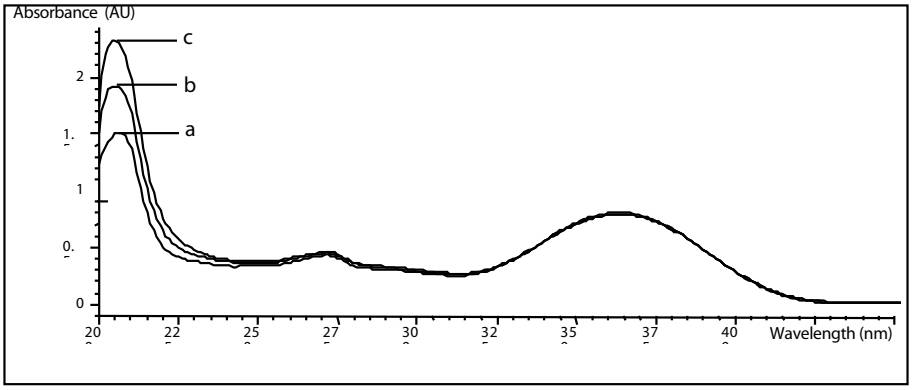


**Figure 3**
The UV spectrum of MEL (15 mg/mL) a) standard solution b) synthetic solution and c) tablet solution

*Validation*

The assay of MEL was validated with respect to stability, linearity, precision, accuracy, selectivity / sensitivity, robustness and ruggedness[22].

*Stability*

The standard stock solutions of MEL were stored, in two different conditions, at +4 $^0$C and at ambient temperature for 2 months. During this period, the solutions were analyzed with UV spectrophotometric method, the spectrum was compared with the spectrum of daily prepared standard solution, and no difference was obtained between them. It is decided that MEL is highly stable in the mentioned conditions.

*Linearity and range*

In developed UV method, calibration curve was linear in the range from 0.5 to 30 µg/mL MEL. Calibration curve was constructed with 9 different concentrations. Each concentration was analysed 6 times. The regression equation was y = (0.054 ± 0.001) x - (0.008 ± 0.003) (n = 10), where y is the absorbance and x is the concentration in µg/mL (r=0.9999). The r value was found to be significant ($t_{Calculated} = 263.13 < t_{Tabulated} = 2.36$ p < 0.05) and the intercept was not significantly different from zero ($t_{Calculated} = 0.01 < t_{Tabulated} = 2.45$ p > 0.05).

*Limit of Detection and Quantification*

A signal to noise ratio (S/N) of approximately 3:1 is generally considered to be acceptable for estimating the limit of detection (LOD), which is the lowest concentration that can be detected. LOD was obtained with 0.05 µg/mL for MEL.

Limit of quantification (LOQ) is generally determined by the analysis of samples with known concentration of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision. The precision for MEL (RSD : 8.49, Bias : 2.54%) was performed by analyzing six different standard solutions containing the lowest concentration on the calibration graph (0.5 µg/mL). This value was lower than acceptance criteria of 10 % [23,24].

*Precision*

Repeatability is the results of the method operating over a short time interval under the same conditions. The low RSD % values of intra-day precision (Table I), recoveries assays (Table II), and tablet solutions assays (Table III) showed that the method have high repeatability.

Intermediate precision: Three different concentration of MEL (1, 5 and 25 µg/mL) in the linear range were analyzed in six independent series on the same day (intra-day precision) and six consecutive days (inter-day precision) from three measurements of every sample in each series (Table I). The RSD % values varied from 0.16 to 1.22 for intra-day and from 0.28 to 1.36 for inter-day precision. Intra-day precision was better than inter-day precision as expressed in the lower RSD % values (Table I). The low RSD % values of intra-day and inter-day and also the low RSD % values obtained from the analyses of pharmaceutical formulations (Table III) indicated that the method has high precision.

*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 and added concentrations of MEL. The obtained results for intra-day inter-day accuracy were ≤ 1.00 (Table I).

TABLE I
Precision and Accuracy of the Developed UV Method for the Analysis of MEL (n=6)

| | **Intra-day** | | | **Inter-day** | | |
|---|---|---|---|---|---|---|
| **Added µg/mL** | **Found $\bar{x}$ (µg/mL) ± SE** | **Accuracy Bias %[α]** | **Precision RSD %** | **Found $\bar{x}$ (µg/mL) ± SE** | **Accuracy Bias %[α]** | **Precision RSD %** |
| 1 | 0.99 ± 0.01 | -1.00 | 1.22 | 1.01 ± 0.01 | 1.00 | 1.36 |
| 5 | 5.01 ± 0.01 | 0.20 | 0.46 | 5.01 ± 0.02 | 0.20 | 0.84 |
| 25 | 24.98 ± 0.02 | -0.08 | 0.16 | 25.01 ± 0.03 | 0.04 | 0.28 |

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

The determination of MEL in a synthetic tablet samples [the mixture of excipients and labeled amount (7.5 and 15 mg) of MEL] were done. The recovery percentages for MEL in synthetic tablets were 99.69 ± 0.13 % (RSD % : 0.33) for 7.5 mg and 99.93 ± 0.07 % (RSD % : 0.19) for 15 mg (Table II ).

The low bias values and high recovery percentages indicated that the developed method is highly accurate.

TABLE II
Recovery Results of MEL in Synthetic Tablets Obtained with UV Method (n=7)

| | **7.5 mg MEL** | | **15 mg MEL** | |
|---|---|---|---|---|
| | **Found amount (mg)** | **Recovery %** | **Found amount (mg)** | **Recovery %** |
| Mean ± SE | 7.48 ± 0.01 | 99.69 ± 0.13 | 14.99 ± 0.01 | 99.93 ± 0.07 |
| SD | 0.02 | 0.33 | 0.03 | 0.18 |
| RSD | 0. 28 % | 0.33 % | 0.20 % | 0.19 % |

*Specificity and selectivity*

The spectra obtained from tablet and synthetic tablet solution (Figure 3b and 3c) were identical with that obtained spectrum from standard solution containing an equivalent concentration of MEL (Figure 3a). In addition the standard addition technique was applied to the same preparations which were analysed by standard curves. The regression equation of standard addition curve were found to be y = 0.054 x + 0.256 (r = 0.9999). There was not any difference between slopes of standard and standard addition techniques. This results show that there was no any interference from matrix components. Therefore it could be said that developed method are highly selective.

*Robustness and Ruggedness*

Robustness and ruggedness show the reliability of an analyte with respect to deliberate variations in method parameters. Robustness test was performed with deliberate small changes at buffer pH (pH 8.4 and 8.6), borate buffer concentration (90 and 110 mM) and detection wave-

length (361 and 365 nm). For ruggedness test of MEL analysis was performed by different analyst and different device in another laboratory (Agilent 8453 UV spectrophotometer in Hacettepe University, Faculty of Pharmacy, inter discipliner laboratory). Only one parameter was changed in the each experiments. Each deliberate small changes was analyzed 6 independent series containing 15 µg/mL MEL. These results were compared statistically (Friedman analysis) and there was no difference between results (p=0.138 > p=0.050). Therefore the method is robust and rugged to the small changes in experimental conditions.

*Analysis of Tablets*

MEL in six different tablets containing two dosage forms was analyzed through the procedure as explained in the tablet solution. Analysis was performed under optimum conditions. Each tablet was analyzed seven independent determinations and each series were analysed 3 times. The obtained results for MEL were compared with reference CZE method [13]. The statistical comparison of two methods was done by Wilcoxon paired test. The results showed that there was no significant difference between UV and reference CZE methods (Table III).

TABLE III
Comparison of the Results Obtained by UV and CE [13] Methods for the Assay of Tablets Containing 7.5 and 15 mg MEL (n=7)

| | **UV method** | **CE method** |
|---|---|---|
| Mobic® 7.5 (7.5 mg MEL) | $\bar{x}$ : 7.55 ± 0.02<br>SD :0.06<br>RSD : 0.79 % | $\bar{x}$ : 7.57 ± 0.01<br>SD : 0.04<br>RSD : 0.53 % |
| | p= 0.249 > p = 0.05 | |
| Mobic® 15 (15 mg MEL) | $\bar{x}$ : 15.04 ± 0.02<br>SD : 0.04<br>RSD : 0.27 % | $\bar{x}$ : 15.06 ± 0.01<br>SD : 0.02<br>RSD : 0.13 % |
| | p= 0.271 > p = 0.05 | |
| Melox® (7.5 mg MEL) | $\bar{x}$ : 7.50 ± 0.02<br>SD : 0.05<br>RSD : 0.66 % | $\bar{x}$ : 7.57 ± 0.02<br>SD : 0.05<br>RSD : 0.66 % |
| | p= 0.176 > p = 0.05 | |
| Melox® Fort (15 mg MEL) | $\bar{x}$ : 15.09 ± 0.05<br>SD : 0.14<br>RSD : 0.93 % | $\bar{x}$ : 15.10 ± 0.03<br>SD : 0.08<br>RSD : 0.53 % |
| | p= 0.866 > p = 0.05 | |

| | **UV method** | **CE method** |
|---|---|---|
| Exen® 7.5 (7.5 mg MEL) | $\bar{x}$ : 7.51 ± 0.03<br>SD : 0.09<br>RSD : 1.20 % | $\bar{x}$ : 7.56 ± 0.01<br>SD : 0.02<br>RSD : 0.26 % |
| | p= 0.176 > p = 0.05 | |
| Exen® Fort (15 mg MEL) | $\bar{x}$ : 15.03 ± 0.04<br>SD : 0.09<br>RSD : 0.60 % | $\bar{x}$ : 15.08 ± 0.01<br>SD : 0.03<br>RSD : 0.20 % |
| | p= 0.128 > p = 0.05 | |

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

*Conclusion*

In this study a simple and rapid UV spectrophotometric method for the determination of MEL in pharmaceutical formulation has been developed and validated.

The linearity range, limit of detection and quantification, precision, accuracy, specificity, selectivity, robustness and ruggedness were performed to determine the suitability of the method.

In this study, the developed UV spectrophotometric method for the determination of MEL in pure and pharmaceutical forms has the advantage of being fast, simple, inexpensive and applicable over a wide concentration range with high precision and accuracy.

As this method has the lowest LOD value and wider linear range is more sensitive than the other published spectrophotometric methods.

These full validation assays have been concluded that the developed UV method is linear, sensitive, accurate, precise, selective robust and rugged for the determination of MEL.

The developed UV spectrophotometric method is cheaper, simpler and faster than CE, LC and GC methods for analysis of MEL in the pharmaceutical preparations. These advantages encourage the application of this method in routine analysis of MEL.

*Acknowledgement*

The authors thank Nobel İlaç A.Ş. for supporting to supply the MEL.

## *Summary*

A simple, accurate and sensitive UV spectrophotometric method was developed for the analysis of MEL in tablets. The analyses were performed in 100 mM borate buffer (pH 8.5). The measurement of UV absorbance

was done at 363 nm. The developed method was validated respect to stability, linearity, precision, accuracy, selectivity / sensitivity, robustness and ruggedness and applied to the determination of MEL in six pharmaceutical preparations including two dosage forms. The obtained data from developed method was compared with the CE method in the literature. It was concluded that the developed method was suitable for the quality control of MEL in pharmaceuticals.

*Keywords:* Meloxicam, UV spectrophotometry, validation, tablet analysis.

*Özet*

**Meloksikamin UV Spektrofotometresi Kullanılarak Tabletlerden Valide Edilmiş Tayini**

Meloksikamın tabletlerden analizi için basit, kesin ve duyarlı bir UV spektrofotometrik yöntem geliştirilmiştir. Analizler 100 mM borat tamponunda (pH 8.5) gerçekleştirilmiştir. UV absorbans ölçümleri 363 nm'de yapılmıştır. Geliştirilen yöntem dayanıklılık, doğrusallık, kesinlik, doğruluk, seçicilik/özgünlük, tutarlılık ve sağlamlılığa göre valide edilmiş ve iki dozaj formuna sahip altı farmasötik preparatta meloksikam tayini için uygulanmıştır. Geliştirilen yöntemde elde edilen veriler literatürdeki kapiler elektroforez yöntemi ile karşılaştırılmıştır. Geliştirilmiş yöntemin farmasötiklerdeki meloksikamın kalite kontrolü için uygun olduğu sonucuna varılmıştır.

*Anahtar Kelimeler:* Meloksikam, UV spektrofotometri, validasyon, tablet analizi

*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.: Method Development and Validation for The Analysis of Meloxicam in Tablets by CZE, J. Pharm. Biomed. Anal., 31(2), 393 (2003).
14. Altıokka, G., Atkosar, Z., Tuncel, M.: Pulse Polarographic Determination of Meloxicam, Die Pharmazie, 56(2), 184 (2000).
15. 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).
16. Altınöz, S., Nemutlu, E., Kır, S.: Polarographic Behaviour of Meloxicam and its Determination in Tablet Preparations and Spiked Plasma, Il Farmaco, 57, 463 (2002).
17. 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).
18. 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).
19. 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).
20. Dasandi B., Saroj, S.H., Bhat, K.M.: LC Determination and Pharmacokinetics of Meloxicam, J. Pharm. Biomed. Anal., 28, 999 (2002).
21. 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).
22. ICH Topic Q2A, Validation of Analytical Procedures: Methodology, CPMP/ICH/281/95.
23. Fabre, H., Altria, K.D.: Validating CE Methods for Pharmaceutical Analysis, LC-GC, 14 (5), 302 (2001).
24. Braggio, S., Barnaby, R.J., Grossi, P., Cugola, M.: A Strategy for Validation of Bioanalytical Methods, J. Pharm. Biomed. Anal., 14(4), 375 (1996).