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Short communication

Method development and validation for the analysis of meloxicam in tablets by CZE☆

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Abstract

A capillary zone electrophoresis assay for the analysis of meloxicam has been developed and validated. The influence of buffer concentration, buffer pH, methanol as organic modifier, capillary temperature, applied voltage and injection time was systemically investigated in a fused silica capillary (i.d. 50 μm, total length 44 cm and effective length 35.5 cm). Optimum results were obtained with a 100 mM borate buffer (pH 8.5) containing 5% methanol, capillary temperature 25 °C, applied voltage 20 kV and injection time 3 s hydrodynamic injection. The detection wavelength was set to 205 nm. Diflunisal was used as internal standard. The method showed good selectivity, accuracy, precision, linearity and sensitivity according to the evaluation of the validation parameters. The method was applied to the determination of six pharmaceutical preparations including two dosage forms. The relative standard deviation of 7 replicate analyses for each sample was less than 0.66%. The results were compared with a spectrophotometric method reported in literature and no significant difference was found statistically.

Keywords: Meloxicam; Capillary zone electrophoresis; Validation; Tablet analysis

1. Introduction

Meloxicam (MEL) (4-hydroxy-2-methyl-*N*-(5-methyl-2-thiazoly)-2H-1,2-benzo-thiazine -3-carboxamide-1,1dioxide) ($C_{14}H_{13}N_3O_4S_2$) 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].

The application of capillary zone electrophoresis (CZE) by the pharmaceutical industry is due mainly to the wide range of possible benefits that may be obtained, when compared to well established and widely used technique of high performance liquid chromatography (HPLC). Principal advantages, which are likely to be obtained, include improvements in cost efficiencies, avoidance of solvent purchase and disposal, and method simplicity. These benefits are obtained in particu-

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lar when performing simple assay or identity confirmation testing, as many compounds can often be analyzed using a single set of operating conditions [6].

In the references, spectrophotometric [7–10], densitometric [8], HPLC [9], flow injection [7,11] and polarographic [12–16] methods are reported for the analysis of MEL in pharmaceuticals. HPLC is the technique most commonly used for the determination of MEL in plasma [17–19] and capillary electrophoretic analysis of MEL has not been reported in the literature in any matrix.

The aim of this study was the development and validation of CZE method for the determination of MEL. For this purpose, the influence of buffer concentration, buffer pH, methanol (MeOH) as organic modifier, capillary temperature, applied voltage and injection time was systemically investigated and the method validation studies were performed.

2. Material and methods

2.1. Apparatus

The CZE analyses were performed on an Agilent 3D CE (Agilent, Waldbornn, Germany) apparatus consisted of an automatic injector, an auto sampler, a variable wavelength diode array detector (DAD) (190–600 nm) and a temperature controlling system (15–60 °C). A CE Chemstation software was used for instrument control, data acquisition and data analysis. The spectrophotometric measurements were carried out using an Agilent 8453 model UV–VIS spectrophotometer with a DAD (190–800 nm). The pH of solutions was measured by a pH meter (Orion Model 420 A). All solutions utilized during the experiments were filtered (0.45 μm) and degassed before use (Bandelin, Snorex Super RK 154 BH).

2.2. Chemicals and reagents

MEL was kindly supplied by Nobel (Turkey). Diflunisal (IS), MeOH, Fe(III)$Cl_3 \cdot 6H_2O$ 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.

2.3. Standard and sample solutions

2.3.1. Standard stock solutions

Standard stock solutions of MEL (250 μg ml^{-1}) and IS (1000 μg ml^{-1}) were prepared in MeOH. These solutions were kept at +4 °C maximum for 2 months and MEL and IS stock solutions were stable during this period. Standard stock solutions of Fe(III)$Cl_3 \cdot 6H_2O$ was prepared 2×10^{-2} M in MeOH.

2.3.2. Standard solutions for CZE analysis

Various aliquots of standard stock solution of MEL were taken, the IS added and then diluted to 5 ml with 100 mM borate buffer (pH 8.5) containing 5% MeOH, to give a final analyte concentration (0.5, 1, 2.5, 5, 10, 20, 50, 75, 100 and 150 μg ml^{-1}).

2.3.3. Standard solutions for spectrophotometric analysis (Comparison method)

Fe(III)$Cl_3 \cdot 6H_2O$ solution (2 ml) was transferred into 10 ml of volumetric flask. Various aliquots of standard stock solution of MEL was added then completed to the volume with MeOH (2–200 μg ml^{-1}). The absorbance of MEL–Fe(III) (2:1) complex was measured at 600 nm against 2×10^{-3} M Fe(III) solution in 1 h [7].

2.3.4. Running buffer

Five ml of 0.5 M boric acid and 1.25 ml MeOH were transferred into 25 ml volumetric flask and diluted to a constant volume with water. pH was adjusted to desired value with 0.1 N NaOH. This solution freshly prepared each day. The final buffer solution consisted of 100 mM borate (pH 8.5) and 5% (v/v) MeOH.

2.3.5. Tablet sample 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 effect 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) containing 5% MeOH. Then tablet sample solutions were analyzed same as CZE and spectrophotometric standard solutions.

All solutions were filtered through a 0.45 μm filter and degassed with ultrasonic bath for 5 min before injection to the CZE system.

2.4. Electrophoretic technique

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 for 10 min. Between each injection, the capillary was rinsed with 0.1 N NaOH (2 min), water (2 min) and running buffer (3 min). Injections were performed hydrodynamically at the anodic side by pressure (50 mbar) for 3 s and capillary temperature was 25 °C. MEL and IS were detected using a DAD at 205 nm (band width 10 nm).

3. Results and discussion

3.1. Optimization of electrophoretic conditions

Several studies have shown that the use of internal standard is crucial for reproducibility in CZE in order to compensate injection errors and minor fluctuations of the migration time [20]. In this study diflunisal was used as an internal standard.

Buffer pH has an influence on the degree of ionization of the solutes and their electrophoretic mobility. MEL has two pK_a values as 1.08 and 4.18. MEL is cationic form below pH 1.08, zwitterionic form between pH 1.08 and 4.18 and anionic form above pH 4.18 [21,22]. Therefore working pH has to be below 1.08 or above 4.18, so that MEL can be ionized and analyzed using CZE. MEL is very low soluble at low pH values and its solubility comes better with increasing pH. Because of that the effect of pH was investigated in the range from 7.5 to 9.0 (100 mM borate and 5% MeOH). So pH 8.5 was chosen as the optimum pH value of the running buffer for suitable migration time (t) and efficiency (Fig. 1).

The effect of borate concentration of running buffer was examined by varying the concentration from 50 to 150 mM (pH 8.5 and 5% MeOH). As the buffer concentration increase the analyte peak were narrower and sharper because of the difference in ionic strength between the running buffer and the analyte solution, which has been reported as a cause of increasing efficiency [23]. When the borate concentration increased, the migration times of MEL and IS were increased but the resolution between MEL and IS remained without any change. Meanwhile the peak area was increased until 100 mM borate concentration then peak area was slightly changed. So 100 mM was selected as an optimum borate concentration for running buffer (Fig. 2).

Addition of an organic solvent is affected migration time, peak symmetry and resolution [24]. In this study, MeOH was chosen in order to get an enhanced resolution and peak symmetry. Five percentage MeOH was chosen as optimal percentage of organic solvent because of good peak symmetry (0.97) and resolution (9.5).

Applied voltage was studied between 15–30 kV. Then 20 kV was chosen as separation voltage for suitable migration time and good selectivity (1.17) between MEL and IS.

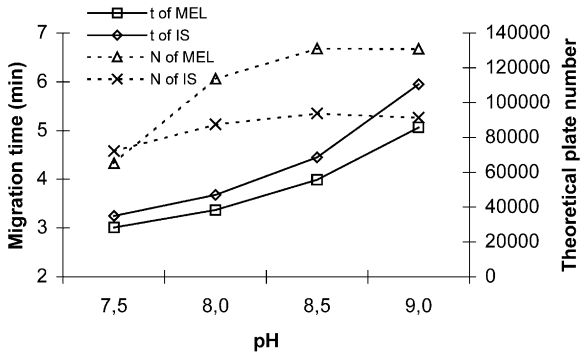

Fig. 1. Effect of buffer pH on migration time (t) and theoretical plate number (N). *Operating conditions:* 100 mM borate, 5% MeOH, hydrodynamic injection (3 s at 50 mbar) 20 kV, 25 °C, 205 nm (band width 10 nm) (MEL and IS 20 μg ml^{-1}).

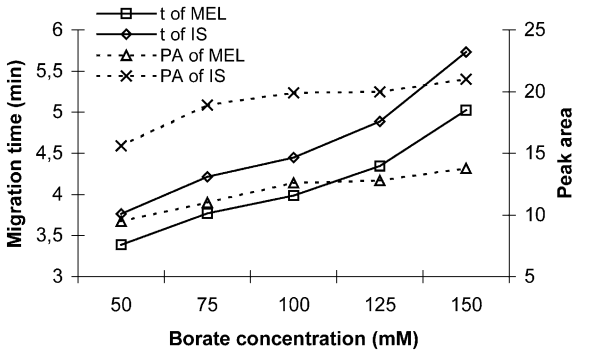

Fig. 2. Effect of borate concentration on peak area (PA) and migration time (t). *Operating conditions:* pH 8.5, 5% MeOH, hydrodynamic injection (3 s at 50 mbar), 20 kV, 25 °C, 205 nm (band width 10 nm) (MEL and IS 20 μg ml^{-1}).

Injection time affects on the peak width and peak height. Analysis was performed in changing injection times from 1 to 5 s at 50 mbar. Injection time up to 3 s increased peak height without any difference in the peak width. At the injection times above 3 s, the peak widths of MEL and IS were increased and the peak shapes were deformed. Therefore 3 s was chosen as the optimum injection time.

Moreover the effect of temperature on analysis was investigated for 20, 25 and 30 °C. Baseline separation of MEL and IS was achieved in all temperatures, 25 °C was chosen as the optimum temperature which is close to the room temperature.

The detection wavelength was selected as 205 nm (band width 10 nm) in which MEL and IS is showed the maximum absorption.

Through the experiments above, the optimum conditions for the determination of MEL were decided. The typical electropherogram of a standard solution of MEL is given in Fig. 3a.

3.2. Validation

The assay of MEL was validated with respect to stability, linearity, precision, accuracy, selectivity and recovery [25–27].

3.2.1. Stability

The standard stock solutions of MEL were stored, in two different conditions, as +4 °C

Table 1
Precision of peak area, peak height, peak normalization, ratio of peak area and ratio of peak normalization values of MEL and IS[a] ($n = 10$)

	MEL			IS			PN (MEL)/PN (IS)	Peak area (MEL)/Peak area (IS)
	Peak area	Peak height (mA)	PN[b]	Peak area	Peak height (mA)	PN[b]		
$\bar{x}$	12.56 ± 0.11	8.39 ± 0.14	3.20 ± 0.03	19.59 ± 0.16	8.92 ± 0.10	4.48 ± 0.04	0.71 ± 0.00	0.64 ± 0.00
SD	0.34	0.43	0.11	0.51	0.32	0.14	0.01	0.01
RSD (%)	2.74	5.10	3.41	2.59	3.58	3.20	0.98	1.02

$\bar{x}$, mean ± standard error; SD, standard deviation; RSD, relative standard deviation.
[a] MEL and IS (20 μg ml^{-1}).
[b] PN (Peak normalization), peak area/migration time.

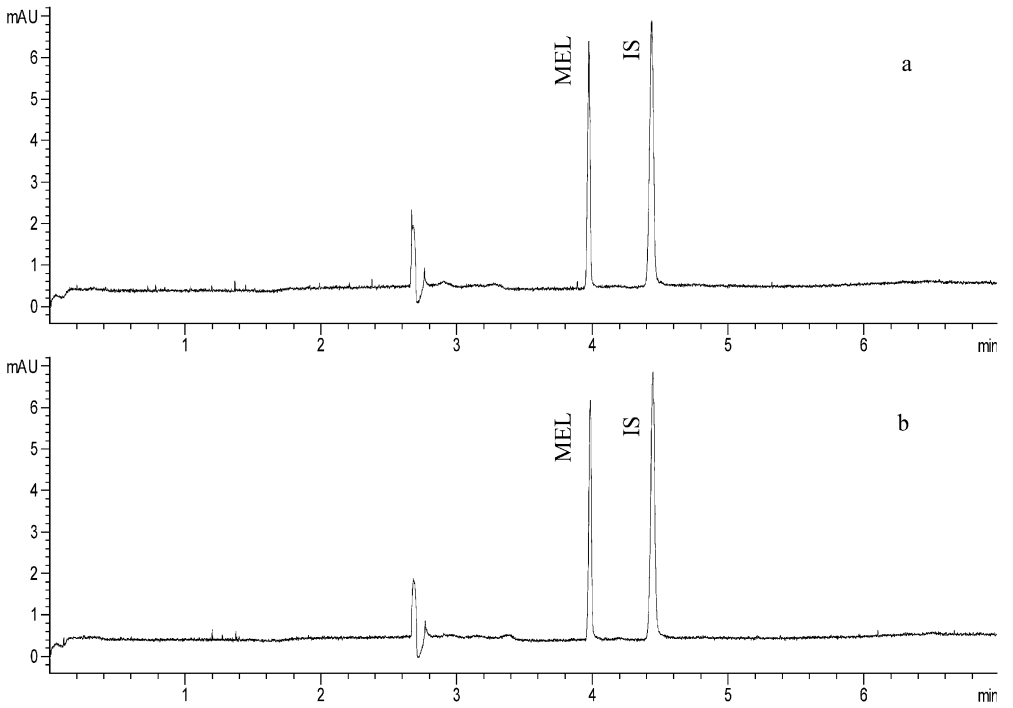

Fig. 3. The electropherograms of MEL and IS. Operating conditions: 100 mM borate, pH 8.5, 5% MeOH, hydrodynamic injection (3 s at 50 mbar), 20 kV, 25 °C, 205 nm (band width 10 nm). (a) In the standard solutions (MEL and IS 20 μg ml^{-1}); (b) in the tablet solutions (MEL and IS 20 μg ml^{-1}).

and room temperature for 2 months. During this period, the solutions were analyzed with UV spectrophotometry, 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.

3.2.2. Linearity

In developed CZE method, calibration curve was linear in the range from 0.5 to 150 μg ml^{-1} MEL. The ratio of peak area technique was chosen for plotting calibration curve because of providing lower RSD (1.02%) and the best linearity ($r = 0.9999$).

The regression equation of calibration curve was $y = 0.677 \pm 0.005x + 0.009 \pm 0.001$ where y is the ratio of peak area (MEL/IS) and x is the ratio of concentration (MEL/IS), ($r = 0.9999$, $n = 10$). The r value was found to be significant ($t_{Calculated} = 263.132 > t_{Tabulated} = 2.36$, $P < 0.05$) and the intercept was not significantly different from zero ($t_{Tabulated} = 2.45 > t_{Calculated} = 0.03$, $P > 0.05$) [28]. All raw data were calculated using calibration curve.

Limit of detection is the lowest concentration that can be distinguished from the noise level, the concentration of MEL at a signal-to-noise ratio of 3:1 was 0.3 μg ml^{-1}.

Limit of quantification is generally determined by the analysis of samples with known concentration of analyte and by establish the minimum level at which the analyte can be quantified with acceptable accuracy and precision. The precision for MEL was performed by analyzing six different standard solutions containing the lowest concen-

Table 2
Accuracy and precision data obtained by CZE method for MEL

Added[a] (μg ml^{-1})	Intra-day		Inter-day	
	Found (μg ml^{-1})	% Relative error[b]	Found (μg ml^{-1})	% Relative error[b]
5.00	4.93	1.40	4.87	2.60
5.00	5.12	−2.40	5.00	0.00
5.00	5.00	0.00	5.11	−2.20
5.00	5.03	−0.60	4.95	1.00
5.00	5.06	−1.20	4.86	2.60
5.00	4.95	1.00	4.97	0.60
	$\bar{x}$: 5.02±0.03		$\bar{x}$: 4.96±0.04	
	SD: 0.07		SD: 0.09	
	RSD: 1.41%		RSD: 1.86%	
	CI: 4.94–5.10		CI: 4.86–5.06	
20.00	19.90	0.50	19.51	2.45
20.00	19.78	1.10	20.47	−2.35
20.00	19.71	1.45	19.68	1.60
20.00	19.66	1.70	19.92	0.40
20.00	20.03	−0.15	20.06	−0.30
20.00	20.38	−1.90	20.19	−0.95
	$\bar{x}$: 19.91± 0.11		$\bar{x}$:19.97± 0.14	
	SD: 0.27		SD: 0.35	
	RSD: 1.34%		RSD: 1.74%	
	CI: 19.51–20.31		CI: 19.61–20.33	
100.00	100.52	−0.52	102.00	−2.00
100.00	99.96	0.04	99.81	0.19
100.00	99.85	0.15	100.23	−0.23
100.00	100.47	−0.47	98.23	1.77
100.00	100.03	−0.03	99.23	0.77
100.00	99.84	0.16	100.09	−0.09
	$\bar{x}$: 100.11±0.12		$\bar{x}$: 99.93±0.51	
	SD: 0.31		SD: 1.25	
	RSD: 0.31%		RSD: 1.25%	
	CI: 99.80–100.42		CI: 98.62–101.24	

$\bar{x}$, mean ± standard error; SD, standard deviation; RSD, relative standard deviation; CI, confidence intervals ($\alpha = 0.05$).
[a] Each series was injected 3 times.
[b] % Relative error = [(added − found)/added] × 100.

tration on the calibration graph (0.5 μg ml^{-1}). The RSD was 5.3% and was lower than acceptance criteria of 10% [26].

3.2.3. Precision

The assay was investigated with respect to repeatability and inter-day precision. The repeatability of the system (while keeping the operating conditions identical) was examined by injecting 20 μg ml^{-1} of MEL and 20 μg ml^{-1} of IS with 10 replicate injections and they were evaluated by considering migration time, peak height, peak area, ratio of peak normalization and ratio of peak area values of MEL and IS. The precision values with their RSD are shown in Table 1.

Three different concentrations of MEL (in the linear range) were analyzed in six independent series in the same day (intra-day precision) and three consecutive days (inter-day precision) within each series every sample was injected 3 times. Intra-day precision was better than inter-day precision as expressed in the lower RSDs (Table

Table 3
Comparison of the results obtained by CZE and UV [7] methods for the assay of tablets containing of 7.5 and 15 mg MEL ($n = 7$)

	CZE method	UV method
Mobic® 7.5 (7.5 mg MEL)	$\bar{x}$: 7.57 ± 0.01	$\bar{x}$: 7.54 ± 0.02
	SD: 0.04 RSD: 0.50% t_C: 9.5, t_T: 2, $t_C > t_T$ $P > 0.05$	SD:0.05 RSD: 0.68%
Mobic® 15 (15 mg MEL)	$\bar{x}$: 15.06 ± 0.01	$\bar{x}$: 15.02 ± 0.01
	SD: 0.02 RSD: 0.16% t_C: 8, t_T: 2 ,$t_C > t_T$, $P > 0.05$	SD: 0.03 RSD: 0.20%
Melox® (7.5 mg MEL)	$\bar{x}$: 7.57 ± 0.02	$\bar{x}$: 7.57 ± 0.03
	SD: 0.05 RSD: 0.66% t_C: 11.5, t_T: 2, $t_C > t_T$, $P > 0.05$	SD: 0.07 RSD: 0.98%
Melox® Fort (15 mg MEL)	$\bar{x}$: 15.10 ± 0.03	$\bar{x}$: 15.25 ± 0.06
	SD: 0.08 RSD: 0.53% t_C: 6, t_T: 2, $t_C > t_T$, $P > 0.05$	SD: 0.15 RSD: 0.97%
Exen® 7.5 (7.5 mg MEL)	$\bar{x}$: 7.56 ± 0.01	$\bar{x}$: 7.53 ± 0.02
	SD: 0.02 RSD: 0.31% t_C: 7, t_T: 2, $t_C > t_T$, $P > 0.05$	SD: 0.05 RSD: 0.63%
Exen® Fort (15 mg MEL)	$\bar{x}$: 15.08 ± 0.01	$\bar{x}$: 15.12 ± 0.04
	SD: 0.03 RSD: 0.19% t_C: 8, t_T: 2, $t_C > t_T$, $P > 0.05$	SD: 0.11 RSD: 0.71%

$\bar{x}$, mean ± standard error; SD, standard deviation; RSD, relative standard deviation; t_C, $t_{Calculated}$; t_T, $t_{Tabulated}$.

2). The RSD values varied from 0.31 to 1.86% showed that the inter-day precision of the method was satisfactory.

3.2.4. 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 of MEL. The results obtained for intra- and inter-day accuracy were $\leqslant 2.40$ and $\leqslant 2.60\%$, respectively (Table 2).

3.2.5. Selectivity

A standard and a tablet sample solution were analyzed by proposed method. The representative electropherograms in Fig. 3 show the separation between MEL and IS.

In order to investigate interactions of excipients in this method, the standard addition technique was applied to preparation, which were analyzed by calibration curve. The regression equation of standard addition curve was found to be $y = 0.677x + 0.170$. y is the ratio of peak area (MEL/IS) and x is the ratio of concentration (MEL/IS). There was no difference between the slopes of two methods. In addition the peak purity index were found 0.99 for MEL and 0.89 for IS by using Chemstation software.

All of these results showed that there was no interaction of excipients in the analysis of MEL.

3.2.6. Recovery

The excipients were sodium citrate dihydrate, lactose monohydrate, avicel, aerosile, magnesium stearate, PVP, which are commonly formulated in a tablet dosage form. 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 percentage recoveries for MEL in synthetic tablets were 99.72% (RSD%: 0.29) for 7.5 mg and 99.67% (RSD%: 0.12) for 15 mg in CZE method.

3.3. Analysis of tablets

MEL in six different tablets containing two dosage forms was analyzed through the procedure as given in the Section 2.3.5. Analysis was performed under optimum conditions. Each tablet was analyzed seven independent determination and each series were injected 3 times. The results obtained for MEL were favorably compared with reference UV method [7]. The statistical comparison of two methods was done by Wilcoxon paired test ($t_{Calculated} > t_{Tabulated}$, $P > 0.05$). The results showed that there was non-significant difference

between CZE and comparison UV methods (Table 3).

4. Conclusion

This study has demonstrated that the CZE is suitable for determination of MEL in the pharmaceutical tablets. The CZE method is linear, precise, accurate, sensitive and selective according to the evaluation of the validation parameters. Meanwhile the CZE method is rapid, cheap and easy to use. Therefore it offers a good alternative to published chromatographic, spectrophotometric and electrochemical methods.

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