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Determination of celecoxib, a COX-2 inhibitor, in pharmaceutical dosage forms by MEKC☆

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Abstract

A micellar electrokinetic chromatographic (MEKC) method was developed for the quantification of celecoxib, a COX-2 inhibitor in pharmaceutical dosage forms within the total analysis time of 7 min. The method has been validated and proven to be rugged. The quantification was carried out at 35 °C and 25 kV, using a 25 mM borate buffer (pH 9.3), 25 mM sodium dodecyl sulphate with an extended light path capillary (48.5 cm × 50 μm I.D., 40 cm to detector). Calibration curves were constructed for celecoxib (0.2–0.6 mg/ml) by the internal standard method with 2-nitro aniline as an internal standard (coefficient of correlation greater than 0.999). The intermediate precision (between day precision) of migration times and peak area ratios of celecoxib to internal standard were 1.44 and 1.58% R.S.D., demonstrates good reproducibility of the method. The method was applied to a commercial celecoxib formulation (Revibra, 100 mg) and the percentage recoveries were ranged from 93.0 to 98.4%. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Celecoxib; MEKC; Dosage form; Validation; Stability; R.S.D.

1. Introduction

COX-2 inhibitors belong also to the group of NSAIDs used for the treatment of inflammation and pain.

Celecoxib, (4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl] benzenesulfonamide), is a COX-2 inhibitor that has been received marketing approval for the treatment of rheumatoid arthritis and osteo arthritis in adults in several countries [1].

In recent years some high performance liquid chromatographic methods were reported for the analysis of celecoxib in pharmaceutical formulations and in human plasma [2–5]. We focussed our interest on the use of capillary electrophoresis for the quantification of celecoxib in formulations due to several advantages such as high resolution, efficiency and short analysis time [6]. Till date no capillary electrophoresis (CE) methods were reported for the quantitative determination of celecoxib in pharmaceutical formulations. In this paper, a micellar electrokinetic chromatographic

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method (MEKC) was described using sodium dodecyl sulphate (SDS) as surfactant for the determination of celecoxib in pharmaceutical formulations. Compared with the reported HPLC procedures, the developed MEKC method was more environment friendly as it involves no organic modifiers like acetonitrile, methanol in the electrolyte. Therefore, the developed MEKC method can be used as a versatile alternative with a clear advantage over liquid chromatographic methods for the analysis of celecoxib in pharmaceuticals. 2-Nitro aniline was used as an internal standard for the quantitative determination of celecoxib.

2. Experimental

2.1. Chemicals

Samples of celecoxib (**I**), 2-nitro aniline (**II**), and synthetic impurities of celecoxib namely 4-hydrazino benzenesulfonamide (**III**) and 1-(4-methylphenyl)-4,4,4-trifluorobutan-1,3-dione (**IV**) were received from Process Research and Technology Development Department of Dr Reddy's Research Foundation, Hyderabad, India. The chemical structures of **I**, **II**, **III**, and **IV** were given in Fig. 1. Finished dosage of celecoxib (Revibra, 100 mg) was received from Dr Reddy's Laboratories, Hyderabad, India. Revibra is supplied as capsules containing 100 mg of active pharmaceutical ingredient (API) i.e. celecoxib and the rest excipients namely, lactose, SDS, povidone, croscarmellose sodium, and magnesium stearate. About 50 mM borate buffer solution pH 9.3 for HPCE (part No. 5062-8573) was purchased from Hewlett–Packard, Waldbronn, Germany. Ultrapure SDS (SDS electrophoresis) was purchased from Sisco-Research Laboratories PVT. LTD, Mumbai, India. HPLC grade acetonitrile was purchased from E. Merck (India) LTD, Mumbai, India and Milli-Q water was prepared by using Milli Q plus purification system (Millipore, USA).

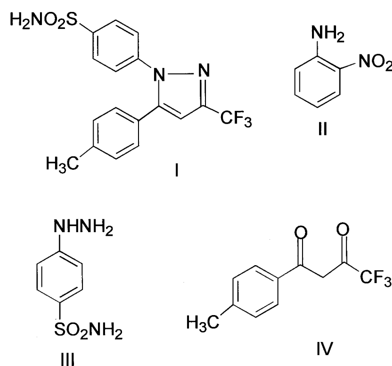

Fig. 1. Chemical structures of celecoxib (**I**), its related substances (**III** and **IV**) and internal standard (**II**).

2.2. Instrumentation and electrophoretic procedure

CE experiments were performed on an Agilent CE system (Agilent Technologies, Waldbronn, Germany) with built in diode array detector (from 190 to 600 nm), an auto injector and a power supply able to deliver up to 30 kV. A CE ChemStation software was used for instrument control, data acquisition, and data analysis. The capillary thermostatting system employed high velocity forced air circulating at 10 m/s. A peltier device was used to control the temperature between 10 °C below ambient and 60 °C, with a precision of 0.1 °C.

An extended light path capillary with a 50 μm inner diameter (I.D.) used was of 48.5 cm length (HP part No. G1600-60232). An alignment interface, containing an optical slit matched to the internal diameter was used and the detection wavelength was set at 252 nm. The background buffer consisted of 25 mM aqueous borate buffer at pH 9.3. Anionic detergent SDS as surfactant was used at a concentration of 25 mM. The electrolyte was filtered through 0.2 μm syringe filter prior to use.

All experiments were carried out in cationic mode. The capillary temperature was established at 35 °C. A constant voltage of 25 kV, was applied during analysis. Sample injections were achieved using the pressure mode for 5 s at 10 mbar.

Before use, the capillary was flushed with water for 10 min, followed by 0.1 M NaOH for 20 min and again with water for 5 min at a system default pressure 900 mbar. To achieve high migration

time reproducibility between analyses the capillary was equilibrated with the electrolyte for 8 min.

As electrolysis can change the electroosmotic flow (EOF) and can effect migration time, efficiency and selectivity and, therefore, cause problems of reproducibility which leads to poor quantitative assays in pharmaceutical analysis, the running electrolyte was replaced with a fresh electrolyte after four injections.

2.3. Electrolyte preparation

A 5.0 ml of electrolyte was prepared by dissolving 36.05 mg of SDS in 5.0 ml of 25 mM borate buffer pH 9.3. The borate buffer was prepared by diluting 2.5 ml of 50 mM borate pH 9.3 with 2.5 ml of highly pure water.

2.4. Sample preparation

Stock solutions of celecoxib and 2-nitro aniline were prepared by dissolving each compound in acetonitrile, in order to get a concentration of 4.0 mg/ml. Test solutions of celecoxib were prepared by taking 0.25, 0.375, 0.5, 0.625, and 0.75 ml of celecoxib stock solution in 5.0 ml volumetric flasks. To maintain uniform concentration of internal standard (0.4 mg/ml), 0.5 ml of 2-nitro aniline stock solution was added to each of the flasks and made up to the mark with the electrolyte. The sample solutions were filtered through 0.2 μm syringe filter prior to use so as to remove particles.

3. Results and discussion

3.1. Method development

To develop a CE method for the quantitative determination of celecoxib in pharmaceutical formulations the following parameters were consecutively optimised.

3.1.1. Influence of buffer pH and anionic detergent (SDS)

The first step in the method development process was the selection of optimum pH value and subsequent choice of the correct CE (capillary zone electrophoresis (CZE), MEKC etc). The optimisation was carried out with a buffer concentration of 25 mM, at a voltage of 25 kV and at a temperature of 35 °C. Celecoxib is weakly acidic in nature with a pK_a value 11.1 in water. Buffer solutions in the pH range 2.5–12 were studied in CZE mode. The retention of celecoxib was decreased with the increase in pH and its peak shape was very broad in the entire studied pH range. The introduction of anionic detergent SDS (25 mM) in the run buffer gave a significant effect on the peak shape of celecoxib. A similar retention phenomenon with improved peak shape was observed with MEKC compared with CZE. A 25 mM borate buffer pH 9.3 with 25 mM SDS was chosen for subsequent method development. 2-Nitro aniline was selected as an internal standard for the quantitative determination of celecoxib.

The electrophoretic mobility of the micelles during MEKC was measured by injecting Sudan III as marker substance. Methanol was used to measure the EOF. The electroosmotic mobility was found to be 1.48×10^{-5} cm^2/V s.

For the optimisation of the CE method the experiments were carried out with extended light path capillaries and with normal capillaries of the same dimension (48.5 cm length and 50 μm internal diameter). The results showed that the sensitivity of the method has increased twice using the capillaries with extended light path.

3.1.2. Influence of SDS concentration

The effect of surfactant concentration on the migration and peak shape of celecoxib was studied by varying the SDS concentration from 15 to 40 mM. The migration time of celecoxib was increased with the increase in SDS concentration. The peak shape of celecoxib was also improved with the increase in SDS concentration. About 25 mM SDS was selected for the experiments because it gave sharp peaks with an acceptable current (60 μA) and analysis time (7 min).

Table 1
Current versus borate buffer concentration

Buffer concentration (mM)	Current produced (μA)
10	35
15	42
25	60
40	104
50	138

3.1.3. Influence of buffer concentration

The ionic strength of buffer has significant effects on solute mobilities and separation efficiency. This effect was studied by varying the borate buffer concentration from 10 to 50 mM. The migration of celecoxib was decreased with the increase in buffer concentration. The current produced at different concentrations of the borate buffer was given in Table 1. About 25 mM borate buffer was selected as a compromise between peak shape and current generated in the capillary.

3.1.4. Influence of capillary temperature and applied voltage

A temperature lower than 20 °C was not considered for the following reason. The surfactant has sufficient solubility to form micelles only at temperatures above the Kraft point (16 °C for SDS) [7]. The effect of temperature on the migration of celecoxib between 20 and 45 °C was investigated. The migration of celecoxib was increased with the increase in capillary temperature and 35 °C was selected for the experiments. The applied voltage was varied from 15 to 30 kV. A decrease in migration time was observed for celecoxib with increasing applied voltage. Since high voltages give higher efficiencies, a potential of 25 kV was selected.

3.1.5. Optimised conditions

The optimised conditions consisted of electrolyte containing of 25 mM aqueous borate buffer, pH 9.3 and 25 mM SDS. The separation and quantification were carried out at 35 °C and 25 kV.

3.2. Method validation

3.2.1. Specificity

Specificity of the method was checked using the above conditions. System suitability tests were performed with the impurities **III**, **IV** and celecoxib (Fig. 2). The data is presented in Table 2. Peaks at migration time 2.7 and 3.5 min are the impurities present in **III** and **IV**, respectively. In a separate experiment, assay of celecoxib (0.4 mg/ml) was determined in presence of the impurities **III** and **IV** at 50% concentration (0.2 mg/ml) of the analyte. The results did not change in the presence of the impurities (Table 3). Peak purity test was performed using photo diode array detector (PDA). It confirmed the non-interference of any other impurities with celecoxib. Hence this method is a stability indicating method.

3.2.2. Reproducibility

Method reproducibility was determined by measuring repeatability and intermediate precision (between day precision) of migration times of celecoxib and peak area ratios of celecoxib versus internal standard using 0.4 mg/ml solutions.

The repeatability was better than 0.7% R.S.D. for the migration times and 0.6% R.S.D. for the peak area ratios. The intermediate precision was also evaluated over 3 days by performing six successive injections on each day. The intermediate precision was better than 1.5% R.S.D. for the migration times and 1.6% R.S.D. for the peak area ratios exhibiting good reproducibility of the method.

3.2.3. Linearity

The target analyte concentration of celecoxib was fixed as 0.4 mg/ml. The calibration curve for celecoxib was drawn by plotting the peak area ratio of celecoxib/internal standard versus concentration of celecoxib yielded coefficient of regression (r^2) 0.9995 over the concentration range of 0.2–0.6 mg/ml. The regression equation for celecoxib was $y = 7.3632x + 0.02385$. Linearity was checked for three consecutive days for the same concentration range from the same stock solutions. The R.S.D. values of the slope, intercept and the coefficient of regression of the calibration curve were 1.6, 38 and 0.005%, respectively.

3.2.4. Assay of celecoxib in formulation sample

The contents of four capsules of celecoxib (Revibra, 100 mg) were finely ground using agate mortar and pestle. The ground material equivalent to 40 mg of celecoxib (API) was weighed and transferred in a 25 ml volumetric flask and extracted into acetonitrile by vortex mixing followed by ultrasonication. The resultant mixture was filtered through 0.45 μm membrane filter. The filtrate was used as a stock solution for preparing test solutions. Test solutions in triplicate were prepared by mixing 1.0 and 5.0 ml of celecoxib and internal standard stock solutions and diluted to 5.0 ml with the electrolyte. The concentration of each of these solution was 0.32 mg/ml. Two more test solutions containing API at concentrations 0.36 and 0.4 mg/ml were prepared under identical conditions. The percentage recoveries

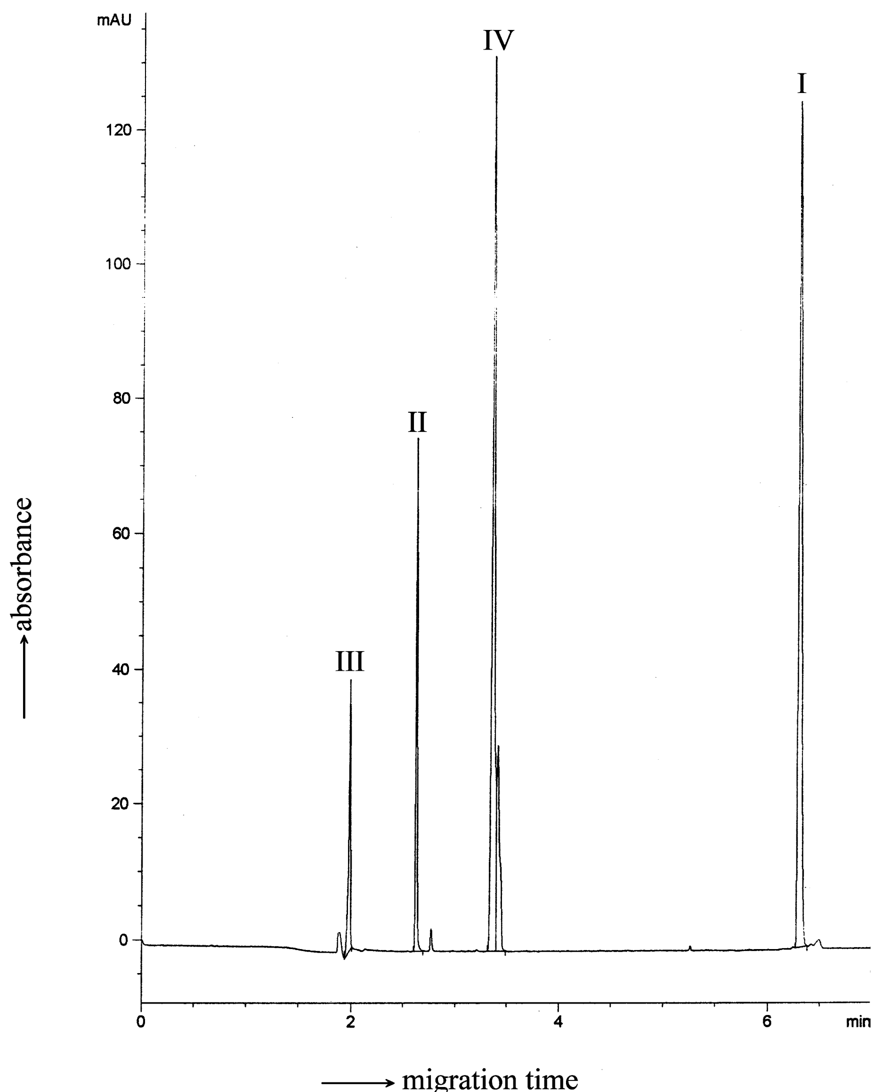

Fig. 2. Micellar electrokinetic chromatogram of celecoxib (**I**) 0.4 mg/ml spiked with internal standard (**II**) and its related substances (**III** and **IV**). Operating conditions: uncoated extended light path fused silica capillary L 48.5 and L 40 cm, I.D. L 50 μm; 35 °C; 25 kV; 252 nm; running buffer 25 mM borate buffer pH 9.3 containing 25 mM SDS.

Table 2
System-suitability report

Compound ($n=3$)	Migration time (min)	USP resolution (R)	USP tailing factor (t)	Number of theoretical plates (N) tangent method
III	2.0	–	1.6	95 000
II	2.6	25.4	0.8	200 000
IV	3.3	22.0	1.2	98 000
I	6.2	55.5	0.6	250 000

n, number of determinations.

were ranged from 93.0 to 98.4 (Table 4). The micellar electrokinetic chromatogram of celecoxib formulation (0.4 mg/ml) was shown in Fig. 3.

3.2.5. Limit of detection and limit of quantitation

The limit of detection (LOD) represents the concentration of analyte that would yield a signal-to-noise ratio of 3 [8]. LOD for **III** and **IV** were 3.5 and 2.2 μg/ml, respectively.

The limit of quantitation (LOQ) represents the concentration of analyte that would yield a signal-to-noise ratio of 10 [8]. LOQ for **III** and **IV** were12.0 and 7.5 μg/ml, respectively.

3.2.6. Stability

Solution stability of celecoxib in electrolyte was checked by storing them on a laboratory bench and in the refrigerator [9]. They were tested for 2 days in 24 h interval. The solution placed on the laboratory bench was found to be stable for the study period whereas the precipitation occurred for the solution placed in the refrigerator after 24 h. On the other hand, there was no precipitation for the celecoxib stock solution prepared in acetonitrile and stored in refrigerator. The solutions prepared on each day from the stock solution were analysed and found to be stable during the study period. This indicates the stability of celecoxib in acetonitrile when it was stored in refrigerator.

4. Conclusion

A MEKC method was developed for the quantitative determination of celecoxib in pharmaceutical formulations. The method was validated showing satisfactory data for selectivity, linearity, precision and accuracy. Results obtained for celecoxib determination in commercial pharmaceutical formulations attest the precision and accuracy of the method.

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Table 3
Recovery results from specificity experiment

Pure celecoxib sample	Celecoxib spiked with the impurities **III** and **IV**
100.4	99.4
100.5	99.3
100.1	99.9
Mean 100.3	Mean 99.5
S.D. 0.2	S.D. 0.3
% R.S.D. 0.2	%R.S.D. 0.3

Table 4
Assay results from celecoxib formulation

Serial number	Taken	Recovery	%Recovery	%R.S.D.
I	0.3260	0.3032	93.0	1.2
		0.3106	95.3	
		0.3084	94.6	
II	0.3554	0.3496	98.4	0.7
		0.3476	97.8	
		0.3445	96.9	
III	0.4025	0.3924	97.5	1.0
		0.3871	96.2	
		0.3845	95.5	

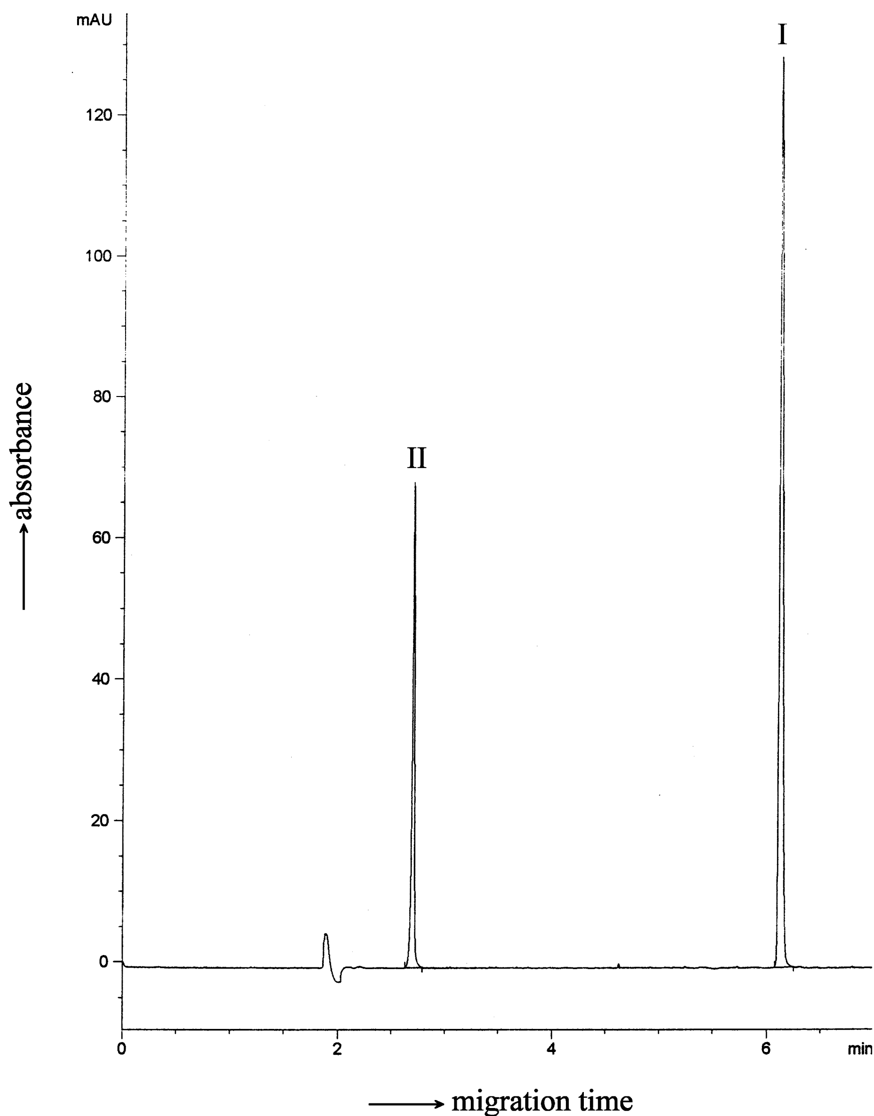

Fig. 3. Micellar electrokinetic chromatogram of celecoxib formulation (**I**) 0.4 mg/ml with internal standard (**II**). Operating conditions: uncoated extended light path fused silica capillary L 48.5 and 40 cm, I.D. 50 μm; 35 °C; 25 kV; 252 nm; running buffer 25 mM borate buffer pH 9.3 containing 25 mM SDS.

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