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A capillary zone electrophoretic method for the determination of nimesulide in pharmaceutical preparation and serum

A simple capillary zone electrophoretic (CZE) method was developed to determine nimesulide in pharmaceutical tablet formulations and serum. Method development was conducted in a fused-silica capillary using a background electrolyte of 10 mM borate buffer containing 10% ethanol at pH 8.1, injecting for 1 s by vacuum injection, applying 30 kV. Salicylic acid was found to be a good internal standard (IS) and detection was performed at 200 nm. Nimesulide and IS appeared at average migration times (RSD%) of 7.2 (0.75%) and 10.4 (0.91%) min, respectively. The limit of detection ($S/N = 3$) and limit of quantitation ($S/N = 10$) were 2.2×10^{-6} M and 6.7×10^{-6} M, respectively. The method was applied to the tablets containing 100 mg nimesulide and the results were compared to those of UV-spectrophotometry. The recoveries of methods (as mean (mg) ± RSD%) were found to be 99.6 ± 1.59 for CZE and 98.9 ± 1.04 for UV spectrophotometry. The proposed method was also applied for the determination of nimesulide in serum by the standard addition technique. The proposed method is sensitive, precise, and easy to use for the determination of nimesulide in tablets and serum.

Key Words: Nimesulide; Capillary zone electrophoresis; Pharmaceutical analysis

Ms received: January 31, 2001; revised: April 3, 2001; accepted: April 10, 2001

1 Introduction

Nimesulide (4-nitro-2-phenoxymethanesulphonanilide) (NMS, **Figure 1**) is a nonsteroidal anti-inflammatory drug (NSAID) of the sulfonanilide class with analgesic and antipyretic properties. It was reported that NMS is an effective and well tolerated alternative to other NSAIDs in the short term treatment of pain and inflammation of osteoarthritis and various other causes [1]. Further, NMS is a relatively weak inhibitor of prostaglandin synthesis in vitro and appears to exert its effects through a variety of mechanisms including free-radical scavenging, effects on histamine release, the neutrophil myeloperoxidase pathway, bradykinin activity, tumor necrosis factor α-release, cartilage degradation, metalloprotease synthesis, phosphodiesterase type IV inhibition, platelet aggregation, and synthesis of platelet activating factor. NMS is extensively metabolized to several metabolites which are excreted mainly in the urine or feces. The drug is almost completely transformed to 4-hydroxynimesulide in both free and conjugated forms and this metabolite appears to contribute to the anti-inflammatory activity of the compound [1].

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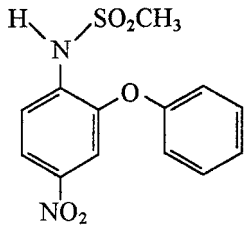

Figure 1. The chemical structure of nimesulide.

Several methods have been reported for the determination of NMS and its major metabolite 4-hydroxynimesulide in plasma and urine by HPLC [2–8] and HPTLC [9]. Several methods have been described for analysis of NMS in pharmaceutical tablet formulations including HPLC [10,11], voltammetry [12], polarography [13], HPTLC [14], spectrophotometry [15–20] and fluorometry [21]. pK_a determination of NMS has been reported using potentiometric [22] and spectrophotometric [23] methods.

The aim of this study is to develop a simple, rapid, and accurate capillary zone electrophoretic method for the determination of NMS in tablets and serum. The method was validated by comparing the results to those of UV spectrophotometry.

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 1615-9306/2001/0909–0743$17.50+.50/0

2 Experimental

2.1 Chemicals

Standard NMS was generously supplied by Pfizer Ilaçlari (Istanbul, Turkey) and it was used without further purification. All the other chemicals used in the experiments were the products of Merck Co. (Darmstadt, Germany) and they were all of analytical grade. Double distilled water and ethanol were used for the preparation of the solutions. The commercial preparation of NMS, Mesulid® tablets (containing 100 mg active material), was purchased from a local market.

2.2 Apparatus

The capillary zone electrophoretic experiments were conducted using a Spectrophoresis 100 system equipped with Modular Injector, a model Spectra FOCUS ultraviolet and visible scanning detector (Thermo Separation Products, San Jose, CA, USA) cabled to a Model Etacomp 486 DX4-100 computer. The data processing was done by using a PC 1000 (Version 2.6) working under OS/2 Warp (Version 3.0). The capillary was a 87.5 cm $\times$ 75 μm ID (57.5 cm to detector) fused silica capillary tube (Phenomenex, Torrance, CA, USA). All the solutions used during the experiments were filtered through a model Phenex microfilter (25 mm, 0.45 μm) (Phenomenex, Torrance, CA, USA) and degassed. The pH of the solutions was measured using a model P 114 pH meter (Consort, Belgium). Spectrophotometric studies were conducted using a model UV-2401PC spectrophotometer (Shimadzu, Japan).

2.3 Preparation of solutions

Standard NMS solution was prepared by dissolving 10 mg of substance in 25 mL of ethanol. Salicylic acid was used as an internal standard (IS) and the solution was prepared by dissolving 13.5 mg salicylic acid in 100 mL of water and diluting to 4.8×10^{-5} M with background buffer in all the experiments. Background buffer was 10 mM borate buffer at pH 8.10 containing 10% (*v/v*) ethanol.

2.4 Capillary zone electrophoretic procedure

Fused silica capillary was conditioned by washing with 0.1 M NaOH and distilled water for 3 min and then with background buffer for 5 min before each run. After washing the instrument was set on 30 kV of applied voltage and 200 nm of detection wavelength. The samples were introduced by using 1 s of vacuum injection corresponding to 65 nL. The solutions of 7.0×10^{-5} M NMS were used for the optimization of CZE parameters.

2.5 Spectrophotometric procedure

A solution of 1×10^{-3} M NMS was prepared in ethanol and a series of standard solutions in the concentration range of 3×10^{-5} M to 9×10^{-5} M diluted from the stock solution to give a final ethanol concentration of 10% (*v/v*). Spectrophotometric measurements were performed at 300 nm using quartz cells against a solvent of 10% (*v/v*) ethanol in water as a blank.

2.6 Analysis of NMS in tablets

Fifteen Mesulid® tablets, each containing 100 mg NMS, were accurately weighed. The average weight of one tablet was calculated and the tablets were then finely powdered. A sufficient amount of tablet powder equivalent to the average weight of one tablet was accurately weighed, transferred to a 25 mL flask and ethanol was added to dissolve the active material. It was magnetically stirred for 10 min and made up to the final volume with ethanol. The solution was then centrifuged at 5000 *g* for 10 min. The supernatant was diluted with the appropriate solutions to carry out the assay by capillary zone electrophoresis or spectrophotometrically.

2.7 Analysis of NMS in human serum

Serum was supplied by healthy volunteers (after giving a signed written consent). NMS (0.46 mg/mL) was spiked with 1 mL of serum and 3 mL ethanol containing a fixed amount of IS (4.8×10^{-5} M). It was well mixed using a Vortex mixer and centrifuged at 5000 *g* for 5 min. The clear supernatant was directly injected into the capillary electrophoresis apparatus under the same experimental conditions as described above.

3 Results and discussion

NMS is a weakly acidic compound ($pK_a = 6.5$) due to the sulfonanilide group in its structure [22, 23]. Therefore, an aqueous and basic buffer system was chosen to carry out the experiments. Since a low buffer concentration provides a short analysis time, it would be appropriate to choose a background electrolyte containing 10% ethanol and 10 mM borate because of its low electrophoretic mobility. The maximum signal to noise was obtained at 200 nm and therefore the compound was detected at this wavelength.

The variations of migration time against pH were examined in the pH range of 7.7 and 10.2 applying 25 and 30 kV potential and sample injection of 1 s. The plots exhibited stability between pH 7.2 and 9 and the migration time gradually increased from pH 9 to pH 10.2, as shown in **Figure 2**.

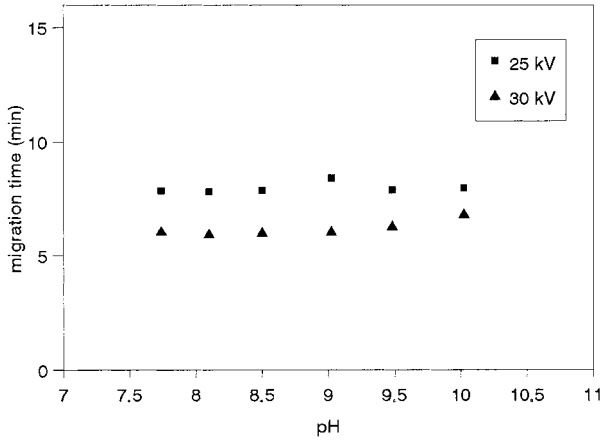

Figure 2. The variations of migration time against pH applying 25 and 30 kV.

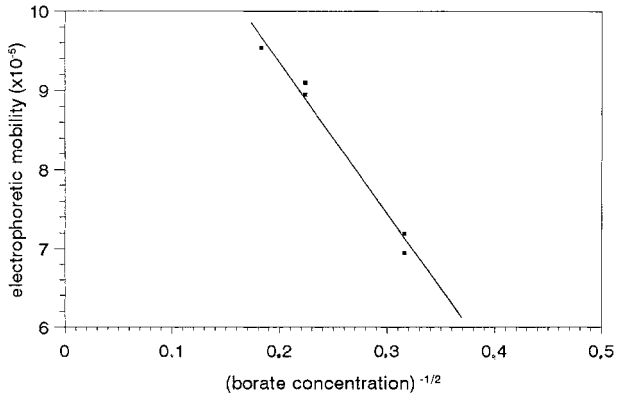

Figure 3. The relation of electrophoretic mobility of NMS against reciprocal square-root of borate concentration.

The effect of borate concentration was tested in the range of 5 and 30 mM at pH 8.1 with the application of 30 kV. The repeatability of the migration time was poor when 5 mM borate was used. The current went out of range on use of 30 mM borate, while the baseline noise increased when the borate buffer was 20 mM. Linearity was achieved when 10–30 mM borate was used as shown in **Figure 3**. The linearity fitted to the equation of $1.3 \times 10^{-4} - 1.9 \times 10^{-4}/C^{1/2}$.

Accordingly, the optimum analytical and instrumental conditions were determined to be as follows: use of the background electrolyte at pH 8 having 10 mM borate and 10% ethanol; applying 30 kV, sample injection for 1 s and detection at 200 nm. Mesityl oxide was used as t_o marker to detect the signal of electroosmosis.

Complete separation of NMS and salicylic acid as an internal standard (IS) was achieved within 12 min using the optimum analytical and instrumental conditions described above. Typical electropherograms of NMS and salicylic acid (IS) are shown in **Figure 4**. The migration time of

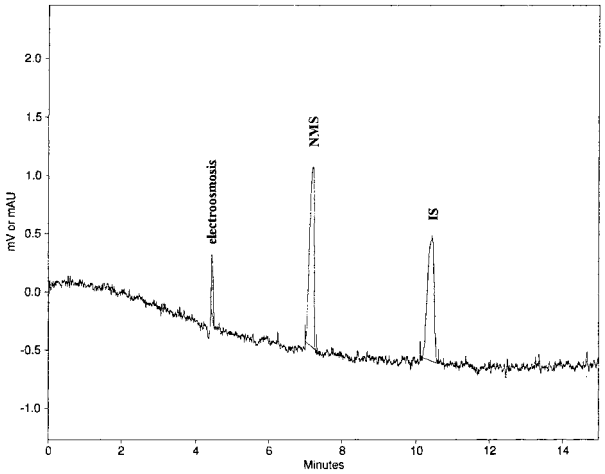

Figure 4. Typical electropherograms of NMS and IS. Conditions are: detection wavelength of 200 nm; capillary, fused silica 87.5 cm × 75 μm ID (57.5 cm to detector); vacuum injection of 1 s; applied voltage of +30 kV; running buffer of 10 mM borate containing ethanol 10% at pH 8.1

Table 1. Precision of peak areas of 6.9×10^{-5} M NMS and 4.8×10^{-5} M salicylic acid (IS). (day = 3; n = 6; RSD%).

	Repeatability	Intermediate precision
IS	2.36	2.86
PN	3.12	6.32
IS and PN	1.94	2.86

electroosmosis was observed at 4.4 min while NMS and internal standard appeared at 7.2 and 10.4 min, respectively, under the described experimental conditions. The resolution was 17.8 and 11.3 for NMS and for IS, respectively. Furthermore, the asymmetry factors of the peaks were 0.63 and 0.78 for NMS and IS, respectively.

Repeatability of peak area ratios depends generally on the conditioning of the inner side of capillary. To avoid adsorption of NMS on the capillary wall and to obtain better reproducibility, the capillary was washed with 0.1 M NaOH and distilled water for 3 min and then with background electrolyte for 5 min before each injection.

Six injections of 6.94×10^{-5} M NMS and 4.8×10^{-5} M salicylic acid (IS) were performed to examine intra-day and inter-day precision. They were evaluated as PN (peak normalization, ratio of NMS area to migration time), IS (ratio of NMS and IS as integrated areas), and PN and IS (ratio of peak normalization values of NMS and IS). The results are illustrated in **Table 1**.

Since the values of PN for inter-day and intra-day were higher than the IS values, due to the shifting of the migration times which increased the RSD values, it was decided that the most appropriate evaluation would be when IS and PN values were used. Employing the integration and

Table 2. Results of inter-day and intra-day calibration studies for linearity and accuracy of the method in the concentration range of 2.2×10^{-5} and 1.1×10^{-4} M.

	Intra-day (day = 1; $n = 5$)	Inter-day (day = 3; $n = 15$)
r^2	0.9996	0.9988
intercept	-5.23×10^{-3}	-9.52×10^{-3}
slope	18823	18878
slope ± CL	18823 ± 742	18878 ± 407
accuracy	mean recovery ± CL (%)	
50%	99.37 ± 2.39	
100%	100.55 ± 2.09	
150%	100.63 ± 2.33	

evaluation results, the electrical field strength was calculated to be 343 $V \cdot cm^{-1}$. Considering the effective and total length of capillary, electroosmotic (μ_{eo}) and electrophoretic mobility (μ_{ep}) for NMS were found to be 6.3×10^{-4} and 2.5×10^{-4} $cm^2 \cdot V \cdot s^{-1}$ respectively. The results indicate that all conditions for the quantititation of NMS are convenient. The capacity factor of the compounds is 13.45 and 19.87 for NMS and IS, respectively.

Linearity of intra-day and inter-day conditions was tested by using IS and PN values versus the standard NMS concentrations. A well-correlated standard curve of NMS was obtained by plotting the peak normalization ratios against concentration over the range of 2.2×10^{-5}–1.1×10^{-4} M, as shown in **Table 2**. The results indicate that the determination of NMS can be practically performed by the use of the proposed method.

The elements of the calibration equation and accuracy of the method was investigated by analyzing a mixture of excipients (cornstarch, magnesium stearate, lactose, and talc) spiked with NMS at various concentration. Square-root of correlation coefficient, slope, intercept of the calibration coefficient and the percentage of the recovery with their 95% confidence interval (CL) are given in Table 2.

The method precision was examined in order to measure the repeatability using three concentrations for intra-day and inter-day. These concentrations correspond to 50, 100, and 150% of tablet contents and they were spiked onto the placebo tablets and analyzed by CE. The RSD% values of intra-day and inter-day assays were found to be lower than 3% (**Table 3**). The limit of detection (LOD) at $S/N = 3$ and limit of quantitation (LOQ) at $S/N = 10$ values were 2.21×10^{-6} M (6.7 RSD) and 6.69×10^{-6} M (6.7 RSD), respectively.

3.1 Determination of NMS in tablets

The determination of NMS in tablets (Mesulid®, containing 100 mg NMS) was carried out by the method developed in this study. Tablets were processed as described in the experimental section. Optimum electrophoretic conditions were employed for the analysis. The migration time values were found to be identical for tablets and for NMS standard. Furthermore, there was no interfering peak from the tablet (**Figure 5**).

Table 3. The precision of the method for the determination of intra-day and inter-day at three different concentrations.

	Intra-day (day = 1; $n = 6$)	Inter-day (day = 3; $n = 18$)
50% (3.27×10^{-5} M)	2.29	2.86
100% (6.54×10^{-5} M)	1.98	2.05
150% (9.81×10^{-5} M)	2.20	2.42

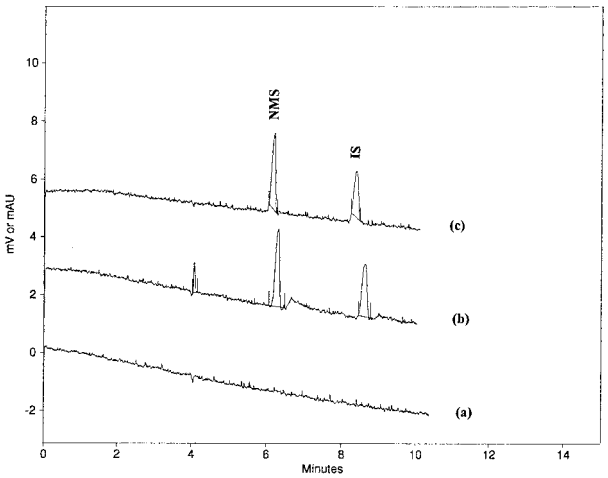

Figure 5. Typical electropherograms of placebo tablet (a), a 7.5×10^{-5} M solution of NMS (b) and Mesulide tablet solution (c). Conditions are the same as shown in Figure 4.

UV spectrophotometry was used as a comparison method. Calibration studies were performed by preparing standard solutions in the range of 3×10^{-5} to 9×10^{-5} M NMS. A calibration plot was drawn and no deviation was observed. The equation was computed as [$A = 7.5 \times 10^{-3} + 7767.1\ C$ (M); $r = 0.9998$] at 300 nm, where A is the absorbance and C(M) is the molar concetration of NMS.

The results of the methods were compared to each other by common statistical tests at the 95% probability level which are shown in **Table 4**. According to the results of the t- and F-tests insignificant differences were observed between the two method ($1.06 < t_{0.05}$ and $2.40 < F_{0.05}$). In addition, the content of one tablet satisfies the USP requirements [24].

The proposed CZE method for the determination of NMS in tablets is simple, cost effective, and rapid. Solvent consumption is less and no extraction process is required. Therefore, it can be used practically in quality control laboratories.

Table 4. The statistical evaluations of assay of Mesulid® tablet containing 100 mg NMS using capillary zone electrophoresis (CZE) and UV-spectrophotometry.

Technique	CZE	UV
mean (n = 8)	99.6	98.9
SD	1.59	1.02
RSD%	1.59	1.04
CL	99.6 ± 1.3	98.9 ± 0.9
t-value	1.06	Table $t_{0.05}$ = 2.36
F-value	2.40	Table $F_{0.05}$ = 3.79

Table 5. The assay of nimesulide at three concentrations (μg/mL) in human serum by CE.

Amount added (μg/mL)	Amount found (μg/mL)	Recovery[a] %	RSD %
46	36.3	78.9 ± 0.81	1.03
92	73.8	80.2 ± 1.51	1.88
138	111.6	80.9 ± 1.40	1.73

[a] Mean ± SD (n = 3).

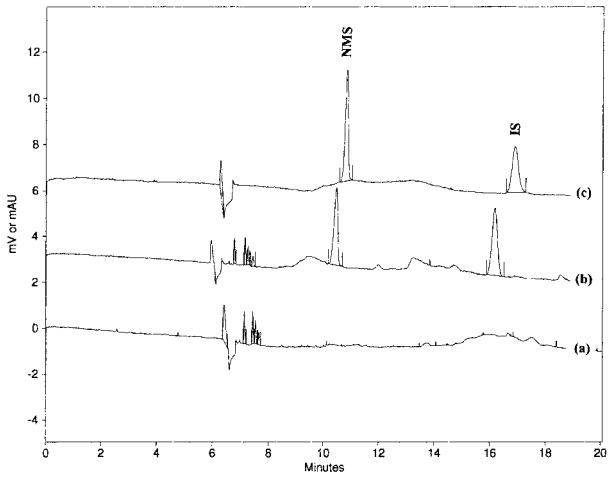

Figure 6. Typical electropherograms of blank serum (a), human serum spiked with NMS equivalent to 92 μg/mL (b) and water spiked with NMS at the same amount for serum. Conditions are the same as shown in Figure 4.

3.2 Determination of NMS in human serum

The determination of NMS in serum was investigated by applying the standard addition method. Three different concentrations of NMS were added to 1 mL serum and then 3 mL ethanol containing a fixed amount of IS was added to a tube. The contents of the tubes were centrifuged at 5000 *g* and precipitated proteins were discarded. The clear supernatant was injected into the CE instrument. No interfering peaks from endogenous material in the serum appeared at the migration time. **Figure 6** shows typical electropherograms of blank serum (a), serum spiked with NMS (b), and water spiked with NMS (c). The migration time of NMS in the serum application was different from that obtained under the tablet conditions. It may be due to high ethanol contents of samples since ethanol has the diffusion coefficient of 1.00 cm^2/s [25].

Values for the spiked serum with NMS in the amounts of 46 μg/mL, 92 μg/mL, and 138 μg/mL were plotted against the corresponding peak normalization ratios. There was a linearity with a high correlation coefficient and the calibration equation was found to be (PN and IS) = $-0.0681 + 9.51 \times 10^{-3}$ C (μg/mL) ; r = 0.9997.

The limit of detection (S/N = 3) and limit of quantitation (S/N = 10) were found to be 0.69 μg/mL and 2.12 μg/mL, respectively. Therefore, the proposed method can be used for the determination of nimesulide in the serum studies.

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