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Short communication

# Determination of ibuprofen and flurbiprofen in pharmaceuticals by capillary zone electrophoresis

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**Abstract**

Capillary zone electrophoresis with spectrophotometric detection was used for the determination of ibuprofen (IB) and flurbiprofen (FL) in pharmaceuticals. The separation was carried out in a fused silica capillary (60 cm × 100 μm i.d. effective length 45 cm) at 30 kV with UV detection at 232 nm. The optimized background electrolyte was 20 mM *N*-(2-acetamido)-2-aminoethanesulfonic acid (ACES) with 20 mM imidazole and 10 mM α-cyclodextrin of pH 7.3. 2-Naphthoxyacetic acid was used as internal standard. A single analysis took less than 5 min. Rectilinear calibration ranges were 2–500 mg $l^{-1}$ for IB and 1–60 mg $l^{-1}$ for FL. The relative standard deviations (R.S.D.) values ($n = 6$) were 1.53% for IB and 1.29% for FL (for 200 mg $l^{-1}$ IB and 10 mg $l^{-1}$ FL). This validated method has been successfully applied for the routine analysis of 10 commercially available pharmaceutical preparations (syrup, tablets, cream and gel).


*Keywords:* Capillary electrophoresis; Flurbiprofen; Ibuprofen; Pharmaceutical analysis

## 1. Introduction

Ibuprofen [(±)-2-(*p*-isobutylphenyl) propionic acid] and flurbiprofen [2-(2-fluorobiphenyl-4-yl)propionic acid] are nonsteroidal antiinflammatory drugs with good analgetic, antiinflammatory and antipyretic effects. Their pharmacological effect is related to the supression of prostaglandin synthesis. Their common side effects are gastrointestinal haemorrhage and ulceration. Other side effects, such as nephrotoxicity are less frequent. Flurbiprofen has more pronounced antiinflammatory effect than ibuprofen and is mainly used in treatment of rheumatoid arthritis [1]. Various methods have been used for the determination of these substances both in pharmaceuticals and biological samples. Until now, chromatographic methods (HPLC, GC, HPTLC, TLC) [2–10], electrophoretic methods [11–16], spectrophotometric methods (UV, IR) [17–19] and titrimetric methods with visual and potentiometric indications [20–22] are the major techniques used for the determination of IB and FL. Some articles dealing with spectrofluorometric determination of these compounds are also published [23,24]. The combination of chromatographic and electrophoretic techniques with liquid–liquid extraction (LLE) and solid phase extraction (SPE) is usually used for the determination of these compounds in biological samples [25–28]. CE–MS, HPLC–MS and GC–MS have been used for the direct determination of selected antiflogistics and their metabolites in urine, river sediments and wastewater samples [29–31]. The enantiomeric separations of these chiral pharmaceuticals were investigated using CZE with various cyclodextrin derivatives and glycosidic antibiotics as the appropriate chiral selectors [32–34]. Recently, an RP-HPLC method for the analysis of FL in rat plasma, excised skin extract and transdermal patch formulations has been published. Ibuprofen was used as internal standard [35]. The pharmaceutical quality control of acid and neutral drugs based on competitive self-assembly in amphiphilic system has also been recently presented [36].

The current Czech Pharmacopoeia 2005 [20] and the European Pharmacopoeia, 5th ed. [22] specify an alkalimetric titration with visual indication for the determination of IB. In USP 28 [21], the LC method with UV detection at 254 nm and valerophenon as internal standard is described. For the determination of FL in Czech Pharmacopoeia 2005 and European Pharmacopoeia, 5th ed., an alkalimetric titration with potentiometric indication is used; in USP 28 an alkalimetric titration with visual indication is specified. The described pharmacopoeial meth-

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ods are used for the quality control of analytes in the form of substances. Because of the presence of several interfering compounds in the pharmaceutical preparations, these methods are not suitable for their analysis. That is why selective and sensitive analytical methods have to be developed for the analysis of pharmaceuticals.

The aim of this work was the development and validation of simple and fast electrophoretic method for the analysis of IB and FL in pharmaceuticals.

## 2. Experimental

*Instruments*: The experiments were performed with PrinCE capillary electrophoresis system (Prince Technologies B.V., The Netherlands) equipped with UV–vis HPLC spectrophotometric detector LAMBDA (Leonberg, Germany) and WinPrinCE software. The separation capillary used was a fused silica capillary with 60 cm total length (45 cm to the detector) and 100 μm i.d., maintained at 25 °C. Samples were injected hydrodynamically at a pressure of 5 kPa for 6 s. A constant voltage of 30 kV was applied and the UV detector was set at 232 nm. Before the beginning of the measurements, the capillary was rinsed with 1 M NaOH for 20 min, ultrapure water for 10 min and running buffer for 20 min. Between analyses the capillary was rinsed with 0.1 M NaOH for 2 min, ultrapure water for 2 min and running buffer for 3 min.

*Chemicals*: The standards of ibuprofen and flurbiprofen, ACES and α-cyclodextrin were obtained from Sigma-Aldrich (Milwaukee, WI, USA). Imidazole, potassium hydrogenphosphate, sodium tetraborate and 2-naphthoxyacetic acid were obtained from Fluka (Buchs, Switzerland); methanol and sodium hydroxide were purchased from Lachema (Brno, Czech Republic). A Millipore Milli-Q RG ultra pure water (Millipore, Bedford, MA, USA) was used for the preparation of the solutions. Commercially available drugs containing ibuprofen [Brufen syrup, Brufen 400 tablets (Knoll Pharmaceuticals, Nottingham, UK); Apo-ibuprofen tablets (Apotex Inc., Toronto, Canada); Ibalgin 200 tablets, Ibalgin cream (Zentiva, Prague, Czech Republic); Nurofen tablets (Boots Healthcare International, Nottingham, UK); Dolgit gel, Dolgit cream (Dolorgiet, St. Augustin/Bonn, Germany)] and flurbiprofen [Flugalin tablets (Knoll Pharmaceuticals, Nottingham, UK); Ansaid tablets (Upjohn, Crawley, UK)] were used for the assay.

*Running buffer*: 20 mM ACES with 20 mM imidazole with an addition of α-cyclodextrin was used as running buffer. It was prepared by dissolving a calculated amount of ACES, imidazole and α-cyclodextrin in ultrapure water. The pH was adjusted with 1 M NaOH.

*Internal standard solution*: The solution was prepared by dissolving 50 mg of 2-naphthoxyacetic acid in methanol, adding 0.5 ml 1 M NaOH and diluting to 100 ml with ultrapure water.

*Standard solutions*: Stock solutions were prepared by weighing an appropriate amount of the corresponding standard substance, dissolving in 50 ml methanol, adding 0.5 ml 1 M NaOH and diluting to 100.0 ml with water. Before analysis, a calculated amount of stock solution and of internal standard solution were diluted by 50% (v/v) methanol and injected for the analysis.

*Sample preparation*: Brufen syrup: 5.0 ml of the syrup (corresponding to 100 mg of ibuprofen) were mixed with 0.5 ml of 1 M NaOH and diluted with 50% aqueous methanol to 100.0 ml.

Ibalgin 200 tablets, Nurofen tablets, Brufen 400 tablets, Apo-Ibuprofen tablets, Dolgit gel, Dolgit cream, Ibalgin cream, Flugalin tablets, Ansaid tablets: An amount of triturated tablets (gel, cream) corresponding to 100 mg of ibuprofen (or 20 mg of flurbiprofen), was weighed and mixed with 0.5 ml of 1 M NaOH and 50 ml of 50% aqueous methanol. This mixture was stirred intensively for 15 min (tablets) or 25 min (gel, creams) and consequently diluted with 50% aqueous methanol to 100.0 ml.

Before analysis, 10.0 ml of the filtered sample solution were mixed with the internal standard solution (1.0 ml for preparations containing IB, 4.0 ml for preparations containing FL) and diluted with 50% aqueous methanol to 100.0 ml.

## 3. Results and discussion

### 3.1. Method optimization

The scope of this work was to find a suitable electrolyte system for the determination and quantification of IB and FL in pharmaceuticals. The p$K_a$ value of IB is 4.31 and for FL 4.33 [37]. In the published papers dealing with capillary electrophoresis, fused-silica capillaries (50–75 μm i.d.) and borate or phosphate buffers are usually used. In this work, the 100 μm i.d. untreated capillary was used for analyses of IB and FL for the first time. The advantage of the 100 μm i.d. capillary is the higher sensitivity obtained and lower adsorption of IB and FL on the capillary wall, which led to the improvement of repeatability of the results obtained. In this case, borate and phosphate buffers were unsuitable for the CZE analysis. When a 20 kV voltage was applied, high current (above 100 μA in 10 mM phosphate buffer) was generated and the peaks obtained were asymmetric due to the electromigration dispersion. When the voltage was decreased, the analysis time significantly prolonged and no improvement in the results was observed. For this reason, a biological buffer with lower co-ion effective mobility was examined. ACES with its effective mobility of $-31.3.10^{-9}\ m^2\ V^{-1}\ s^{-1}$, effective pH range 6.1–7.5 and p$K_a$ = 7.84 was chosen as co-ion (the $\mu_{eff}$ of phosphate is $-54.3.10^{-9}\ m^2\ V^{-1}\ s^{-1}$). Imidazole (p$K_a$ 6.95, useful pH range 6.2–7.8) was tested as counterion [38]. In this electrolyte system, following parameters were optimized.

*pH*: The buffer pH affects the ionization and the effective mobility of the analytes. Thus, the effect of pH was examined in buffers containing 20 mM ACES and 20 mM imidazole adjusted to the pH values ranging between 6.0 and 8.0. At lower pH values, the analytes were not separated and only one peak appeared on the electrophoreogram. With the increasing pH partial separation of the analytes occurred. Best results were achieved at pH 7.3, but the peaks were not fully separated. With the increasing pH value the quality of separation deteriorated and at higher pH values no separation of the analytes was observed.

Therefore, running buffer with pH 7.3 was used for the next measurements.

*Buffer concentration*: Keeping other parameters constant (pH 7.3, 25 kV, 25 °C), different concentrations (20–40 mM) of ACES were tested. An increase in migration time and current intensity was observed when the concentration of the buffer increased, but no improvement in the quality of separation was achieved. The best results – considering also the time of analysis and the current generated – were obtained with 20 mM ACES.

*Addition of methanol*: The addition of 5–20% of methanol only increased the migration times and had no positive effect on the quality of separation. Therefore, BGE without the addition of methanol was used.

*Addition of α-cyclodextrin*: Since no satisfactory separation of the analytes under study was achieved, the effect of addition of cyclodextrins was examined. It was supposed, that selective interactions of the analytes with α-cyclodextrin could contribute to the improvement of their separation. The tested molarity of the α-cyclodextrin varied from 2.5 to 20 mM. At 2.5 mM concentration level no full separation was achieved. At 5 mM of α-cyclodextrin the peaks were partially separated. When the concentration of α-cyclodextrin was increased to 10 mM, full separation of the analytes was observed. The peaks were symmetric and the resolution was satisfactory. The results with the addition of 20 mM α-cyclodextrin were similar to those when 10 mM α-cyclodextrin was used. For this reason, BGE with an addition of 10 mM α-cyclodextrin was used for the analysis (Fig. 1).

For the analysis of chiral drugs by CE, a suitable chiral selector is essential for obtaining good resolution. It is noted that no resolution of enantiomers of drugs tested was observed in the optimized electrolyte system. Mixtures of cyclodextrin derivatives consisting of highly-sulfated cyclodextrin and neutral cyclodextrin or glycosidic antibiotic have been used for separation of arylpropionic acids such as FL and IB recently [32,34].

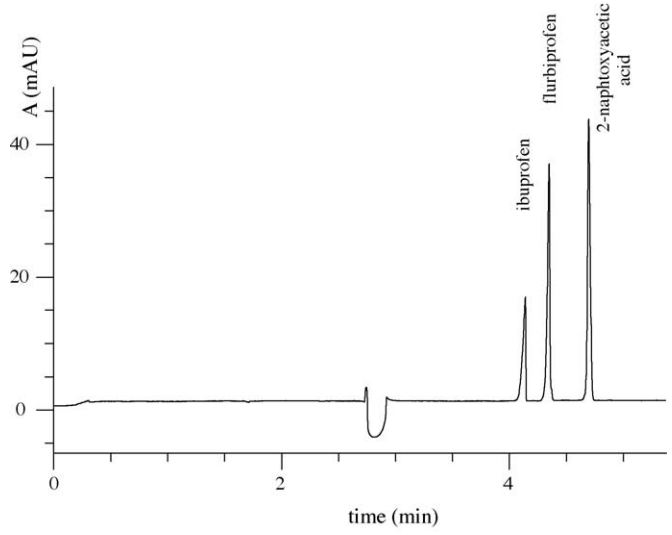


Fig. 1. Electropherogram of standard solutions of ibuprofen (80 mg $l^{-1}$), flurbiprofen (80 mg $l^{-1}$) and internal standard 2-naphthoxyacetic acid. Conditions: 20 mM ACES with 20 mM imidazole and with the addition of 10 mM α-cyclodextrin, pH 7.3. UV detection at 232 nm.

Table 1
Quantitative parameters of the analysis

| Analyte | Ibuprofen | Flurbiprofen |
|---|---|---|
| Calibration range (mg $l^{-1}$) | 2–500 | 1–60 |
| Slope ± S.D. | 0.01397 ± 0.00026 | 0.2006 ± 0.0015 |
| Intercept ± S.D. | 0.115 ± 0.054 | 0.084 ± 0.048 |
| Correlation coefficient | 0.99914 | 0.99986 |
| R.S.D. (%) area[a] | 1.53 | 1.29 |
| R.S.D. (%) migration time[a] | 1.30 | 1.21 |
| LOD (mg $l^{-1}$) | 0.5 | 0.1 |

[a] $n = 6$, for concentrations: 200 mg $l^{-1}$ for IB and 10 mg $l^{-1}$ for FL.

*Voltage*: The applied voltage ranged from 20 to 30 kV. With the increasing voltage migration times – as expected – decreased while the current intensity increased. The resolution was unaffected. For the analysis 30 kV voltage was used. This value permitted shorter analysis time while the current intensity was still suitable.

We mention here, that IB–FL separation problem is just theoretically interesting fact concerning the migration of these two drugs in ACES/imidazole running buffer and it does not bring any contradiction between the aim and outcome of this paper. It is evident that from the pharmaceutical standpoint it would be purposeless to combine both IB and FL as active principles of the same pharmacological effect in a single preparation (in fact there is no such a pharmaceutical preparation available on the market).

The final optimum conditions for the measurements were: BGE: 20 mM ACES with 20 mM imidazole, 10 mM α-cyclodextrin, pH 7.3. The separations were carried out at 30 kV, 25 °C and with UV detection at 232 nm.

*Method validation*: The optimized method was validated by standard procedure. The linearity of the method was tested in the concentration range 2–500 mg $l^{-1}$ for ibuprofen and 1–60 mg $l^{-1}$ for flurbiprofen with 5 mg $l^{-1}$ of 2-naphthoxyacetic acid as internal standard (each concentration level in triplicate). Regression analysis data for the calibration curves were calculated using peak areas. The detector responses were found to be linear in the tested concentration range—the linear regression equations $A = ac + b$ (where $A$ is the peak area, $c$ = the analyte concentration in mg $l^{-1}$, $a$ = the slope and $b$ = the intercept) and correlation coefficients are given in Table 1.

The repeatability was evaluated by performing six successive injections with the concentrations 200 mg $l^{-1}$ for IB and 10 mg $l^{-1}$ for FL and expressed as R.S.D. (%) for both migration times and peak areas (Table 1). Method precision was determined by the total analysis of six replicate preparation samples under the same operating conditions. The R.S.D. values are given in Table 2. For the unavailability of an appropriate placebo, the accuracy of the method had to be checked by the standard addition technique in accordance with the recommendation of Pharmaceutical Authorities of Czech Republic [39]. Each sample preparation was measured in six replicate determinations and the results are given in Table 2. Detection limits of the analytes

Table 2
Method precision and accuracy

| Validation step | Parameter | Flugalin tablets | Ansaid tablets | Brufen syrup | Ibalgin 200 tablets | Nurofen tablets | Apo-Ibuprofen tablets | Brufen 400 tablets | Dolgit gel | Dolgit cream | Ibalgin cream |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Method precision | R.S.D. (%)[a] | 1.32 | 2.02 | 3.87 | 2.00 | 1.16 | 1.58 | 0.31 | 2.18 | 0.97 | 1.57 |
| Accuracy | Spike recovery (%)[a] | 100.35 | 95.53 | 98.10 | 99.70 | 102.78 | 98.13 | 101.21 | 97.50 | 103.40 | 101.32 |
| | Recovery R.S.D. (%) | 2.60 | 3.06 | 4.12 | 2.07 | 2.44 | 1.28 | 3.18 | 1.06 | 1.53 | 2.03 |

[a] $n = 6$.

Table 3
Determination of the studied compounds in pharmaceutical preparations ($n = 6$)

| | Declared | Found | % |
|---|---|---|---|
| Preparations with flurbiprofen | | | |
| Flugalin tablets | 50 mg/tablet | 50.40 mg/tablet | 100.80 |
| Ansaid tablets | 50 mg/tablet | 49.87 mg/tablet | 99.72 |
| Preparations with ibuprofen | | | |
| Brufen syrup | 100 mg/5 ml | 97.75 mg/5 ml | 97.75 |
| Ibalgin 200 tablets | 200 mg/tablet | 198.13 mg/tablet | 99.06 |
| Nurofen tablets | 200 mg/tablet | 209.32 mg/tablet | 104.66 |
| Apo-Ibuprofen tablets | 400 mg/tablet | 392.59 mg/tablet | 98.15 |
| Brufen 400 tablets | 400 mg/tablet | 390.59 mg/tablet | 97.65 |
| Dolgit gel | 50 mg/g | 51.15 mg/g | 102.30 |
| Dolgit cream | 50 mg/g | 51.12 mg/g | 102.24 |
| Ibalgin cream | 50 mg/g | 51.09 mg/g | 102.18 |

were determined as a signal-to-noise ratio of three and were 0.5 mg $l^{-1}$ for IB and 0.1 mg $l^{-1}$ for FL.

## 4. Determination of ibuprofen or flurbiprofen in pharmaceuticals

The developed method was used for the assay of these analytes in 10 pharmaceutical preparations. All formulations analysed are simple pharmaceutical preparations containing only one active principle. Other constituents present in the matrix are generally electrophoretically indifferent and do not interfere with the analysis. The compounds used as conservants are not ionized at pH 7.3 and do not interfere with the analysis. The results of the determination of IB or FL were in good agreement with their nominal content (Table 3).

Both compounds investigated are chiral anti-inflammatory drugs and a special attention should be paid to the control of their enantiomeric purity. The chiral resolution of these pharmaceuticals by HPLC, GC and CE has also been reported in literature [40]. As a rule only one of two or more possible optical isomers exhibits desirable therapeutic effect. *R*-enatiomer of flurbiprofen is less effective than the *S*-enantiomer, but it causes low toxicity in comparison to the other enantiomer [41]. Although only the *S*-enantiomer of ibuprofen exhibits therapeutic activity, it is (and also some other non-steroidal anti-inflammatory drugs) still marketed as a racemic mixture. Therefore, the purpose of the present investigation was not to develop a chiral electrophoresis system allowing the determination of the enantiomeric purity of drugs. In this sense, the proposed CZE method is fully applicable for the assay of given active pharmaceutical ingredients in pharmaceutical dosage forms.

## 5. Conclusion

The proposed CZE method with UV detection permits qualitative control of pharmaceutical preparations containing ibuprofen and flurbiprofen as active substances. The total analysis time was within only 5 min using 20 mM ACES buffer with 20 mM imidazole and 10 mM α-cyclodextrin. The method has been validated and the results obtained were precise and accurate. The lower sensitivity (higher detection limits) of the proposed method compared to the HPLC method published earlier is fully sufficient for the analysis of pharmaceutical preparations.

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