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# Ibuprofen quality control by electrochromatography

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**Abstract**

The quality control of drugs is generally made by HPLC. This control could be made also by capillary electrochromatography (CEC). In this paper we report the analysis by CEC of ibuprofen, a well-known anti-inflammatory non steroidic drug, and some of its impurities. The analyses were performed in a 100-μm inner diameter (I.D.) fused silica capillary, packed with RP-18 stationary phase. The mobile phase was a mixture of 100 mM formic acid solution (pH 2.5), water and acetonitrile (ACN). The ACN percentage in the mobile phase and the applied voltage were carefully studied to well resolve the drug from each impurity. The results, obtained determining ibuprofen and related compounds by CEC, showed the selectivity and efficiency of the method.


*Keywords:* Ibuprofen; Quality control; Electrochromatography

## 1. Introduction

Capillary electrochromatography (CEC) is a powerful electrophoretic method that can be successfully applied to the analysis of both charged and uncharged compounds. The analytes separation is performed in a fused silica capillary either packed with chromatographic particles (p-CEC) or containing monolithic phases or functionalized on the wall (o-CEC). In CEC the separation of analytes is based on the combination of both chromatographic and electrophoretic theory, e.g. different partitioning between mobile and stationary phase (selectivity) and different electrophoretic mobility (efficiency). The main driving force, responsible of the transport of the mobile phase through the stationary phase, is the electro-osmotic flow (EOF). EOF is generated by the silanol groups, present in the packed particles and in the internal capillary wall, applying a relative high electric field across the column.

The experimental set-up of CEC is very similar to that used in capillary zone electrophoresis (CZE) with a difference in the capillary employed; in CEC the capillary is packed with a stationary phase. Therefore, in a certain sense, CEC can be considered as an electrically driven high performance liquid chromatography (HPLC) [1].

Several HPLC stationary phases can be successfully employed also in CEC and among them the RP18 is currently used [2].

In theory all compounds can be separated by CEC. In practice the separation of charged compounds is influenced by the applied electric field, interactions with stationary phase and differences in the electromigration. The separation of uncharged analytes instead is affected only by the differential interactions between the mobile and stationary phases. Many researchers used CEC to analyse chiral and non chiral pharmaceuticals [3–10].

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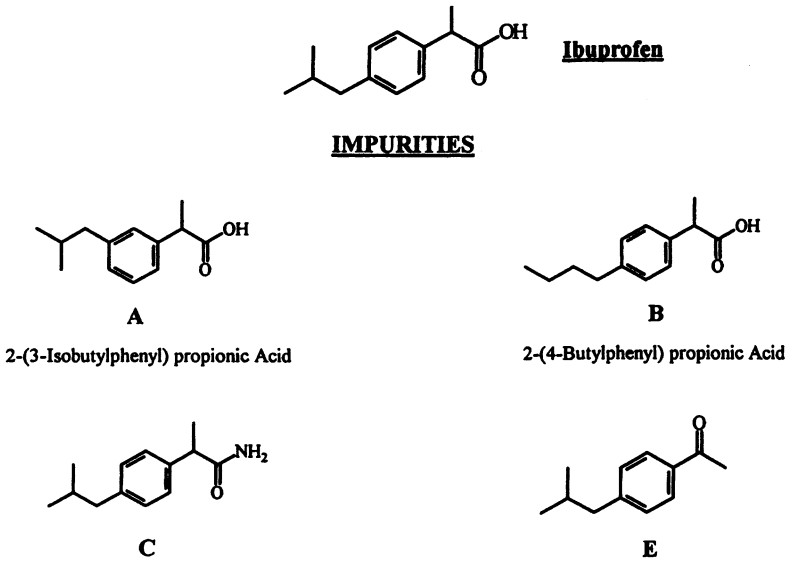


Fig. 1. Chemical structures of ibuprofen and impurities.

As it can see in the literature the drugs quality control is generally made using chromatographic techniques. In this paper we propose CEC as an alternative technique to control the quality of a drug, ibuprofen.

Ibuprofen, the (RS)-2-(4-isobutylphenyl)proprionic acid, is an effective cyclooxygenase inhibitor, being the first member of the propionic acid derivatives to come into general use as a non steroidal anti-nflammatory drug (NSAID).

From the synthetic process many are the impurities which can remain in the ibuprofen raw material. In this paper we cannot examine all impurities, but only four of them among the more frequent (Fig. 1): 2-(3-isobutylphenyl)propionic acid (impurity A), 2-(4-butylphenyl)propionic acid (impurity B), 3-(4-isobutylphenyl)propionic amide (impurity C), 4-isobuthyl-acetophenone (impurity E).

The simultaneous separation of ibuprofen and its impurities (A, B, C, E) was then performed by CEC using a RP-18 packed capillary eluted with a mobile phase containing ammonium formate (pH 2.5), water and acetonitrile. The effect of mobile phase composition (% of organic modifier) and applied voltage on the resolution and selectivity was studied.

## 2. Experimental

### 2.1. Chemicals

Ibuprofen was supplied by Sigma (St. Louis, MO). The standards of impurities A, B, C and E were purchased from PromoChem GmbH (Wesel, Germany). The CEC capillary was filled with Lichrosphere 100 RP-18 (5 μm) obtained from Merck (Darmstadt, Germany). Formic acid (85%) was purchased from Carlo Erba (Milan, Italy). All other chemicals used were provided by VWR International (Milan, Italy) and were all of analytical or HPLC grade, water included.

### 2.2. Apparatus and electrochromatographic conditions

The electrochromatographic analyses were carried out with a Hewlett–Packard$^{3D}$ CE apparatus (Waldbronne, Germany) equipped with a linear UV–Vis diode array detector and an autosampler. The instrument was controlled and the data were evaluated by a ChemStation and a computer HP KAYAK XM 600 Pentium 3. CEC experiments were carried out in a fused silica capillary (total length 33 cm, effective length 24.5 cm, I.D. 100 μm) packed with Lichrosphere 100 RP-18 (5 μm) particles (VWR International, Milano-Italia,). The capillary was packed in our laboratory for the whole length and the modified silica particles were blocked by two retaining frits at the inlet and outlet ends of the capillary.

The mobile phase was a mixture of 100 mM formic acid solution, titrated to pH 2.5 with ammonia solution, water and acetonitrile (ACN) in the ratio of 10/40/50.

The packed capillary was equilibrated with the mobile phase for 30 min applying a pressure of 12 bar at the inlet end of the capillary. CEC experiments were carried out applying 25 kV and 10 bar pressure at both ends of the capillary. During the analysis a constant voltage (25 kV) and pressure (12 bar) was maintained up to obtain a stable current and baseline signal (about 15 min). The injection was done at the anodic end of the capillary by

high pressure application (12 bar for 24 s).The capillary temperature was maintained at 25 °C.

### 2.3. Analytical procedures

The analyses were carried out, as below described, using only standard materials.

#### 2.3.1. Standard solutions

Methanolic solutions of ibuprofen and related impurities A,B,C and E were separately prepared by dissolving ibuprofen (about 0.5 mg/ml) and each individual impurities (about 5 mcg/ml), exactly weighed, in a 10 ml volumetric flask

#### 2.3.2. Working standard solution

One milliliter of each standard solution have been transferred in a 10-ml volumetric flask and diluted up to mark with mobile phase.

#### 2.3.3. Calibration curve

For quantitative application a linear relationship between ibuprofen peak area and concentration (0.05–0.15 mg\ml) was verified. The same relationship between each impurity peak area and concentration (0.05–0.15 mcg\mml) was also tested.

## 3. Results and discussion

Although CEC is suitable for uncharged compounds, nevertheless it allows a good separation also of the charged substances. Therefore a wide number of pharmaceutical compounds, belonging to different therapeutic classes, can be analyzed by CEC.

Several NSAIDs, including ibuprofen [11], were examined by CEC. We applied this technique to the analysis of ibuprofen and four of its impurities. As we described in the experimental procedures the analyses were carried out in a fused silica capillary, packed with RP-18 stationary phase.

Ibuprofen and related compounds (Fig. 1) have acidic properties. Consequently an ammonium formate buffer solution at pH 2.5, containing water and an organic modifier seemed to be suitable as mobile phase. Preliminary experiments were carried out using different organic modifier added to the mobile phase. Among them ACN resulted to be the best because allowed a relatively high EOF, even at low pH, useful to have a reasonable analysis time. Therefore, acetonitrile was selected as organic modifier. These analytical conditions reduce the analytes dissociation, increase their hydrophobicity useful for the selective interactions with the stationary phase [12].

The drugs and many of their related compounds generally have very similar structures. Therefore the separation system needs a close study of the parameters influencing the retention factor and consequently the resolution. Afterwards we carefully studied the influence of the ACN concentration in the mobile phase and the applied voltage on the analytes separation.

The ACN concentration ratio in the mobile phase was carefully studied because influenced, as we said before, some parameters like EOF, migration time and retention factor. The increase of ACN concentration in the running buffer has, as consequence, the increase of EOF velocity [13], but caused a general decrease in the analytes resolution (Fig. 2). Therefore to optimize the analytical conditions we carried out the analyses using as mobile phase a formate buffer solution containing different amount of ACN in the range of 50–80% (v/v). As we can see in Fig. 2 the mobile phase containing 80% of ACN reduced the analysis time, because of the increasing of electro-osmotic flow mobility, but didn't allow the separation of all analytes. Two impurities, C and E, were very well resolved, but A, ibuprofen and B were unresolved. Decreasing the ACN concentration in the mobile phase from 80 to 60% the resolution of ibuprofen from impurities A and B increases. The separation of each impurities from the drug became satisfactory when the percentage of ACN was reduced to 50%. Fig. 3 shows the correlation between ACN concentration in the mobile phase and logarithmic function of the retention factor ($K'$). Actually the electrochromatograms showed that the ACN concentration has an evident effect on the analytes resolution, but did not influence their migration order. In our experiments the best mobile phase resulted to be a mixture of 100 mM ammonium formate buffer at pH 2.5, water and ACN in the ratio of 10/40/50.

In order to reduce the analysis time, we studied the influence of the applied voltage on this parameter. A mixture of ibuprofen and impurities was analyzed applying different voltage values in the range of 20–30 kV (Fig. 4). The increase of the applied voltage reduced the migration time of impurity E (the last peak in the electropherogram), but the analysis time remain still long. It is evident that the experimental conditions, above reported, allowed a satisfactory resolution of the analytes, but didn't allow to have a short analysis time. Therefore we inverted the polarity and the injection side. In this way we obtained a capillary with an effective length of 8.5 cm instead of 33 cm. In these conditions the analysis time was manifestly reduced (Fig. 5). The impurities C and E were very well-resolved, but the resolution of impurity A, ibuprofen and impurity B were not perfectly on line, but is quite good. The optimum of working temperature was 20 °C.

Ibuprofen and its considered impurities need separate methods particularly selective, efficient and sensitive. The described analytical conditions allowed to have a good selectivity and efficiency. The sensitivity of the

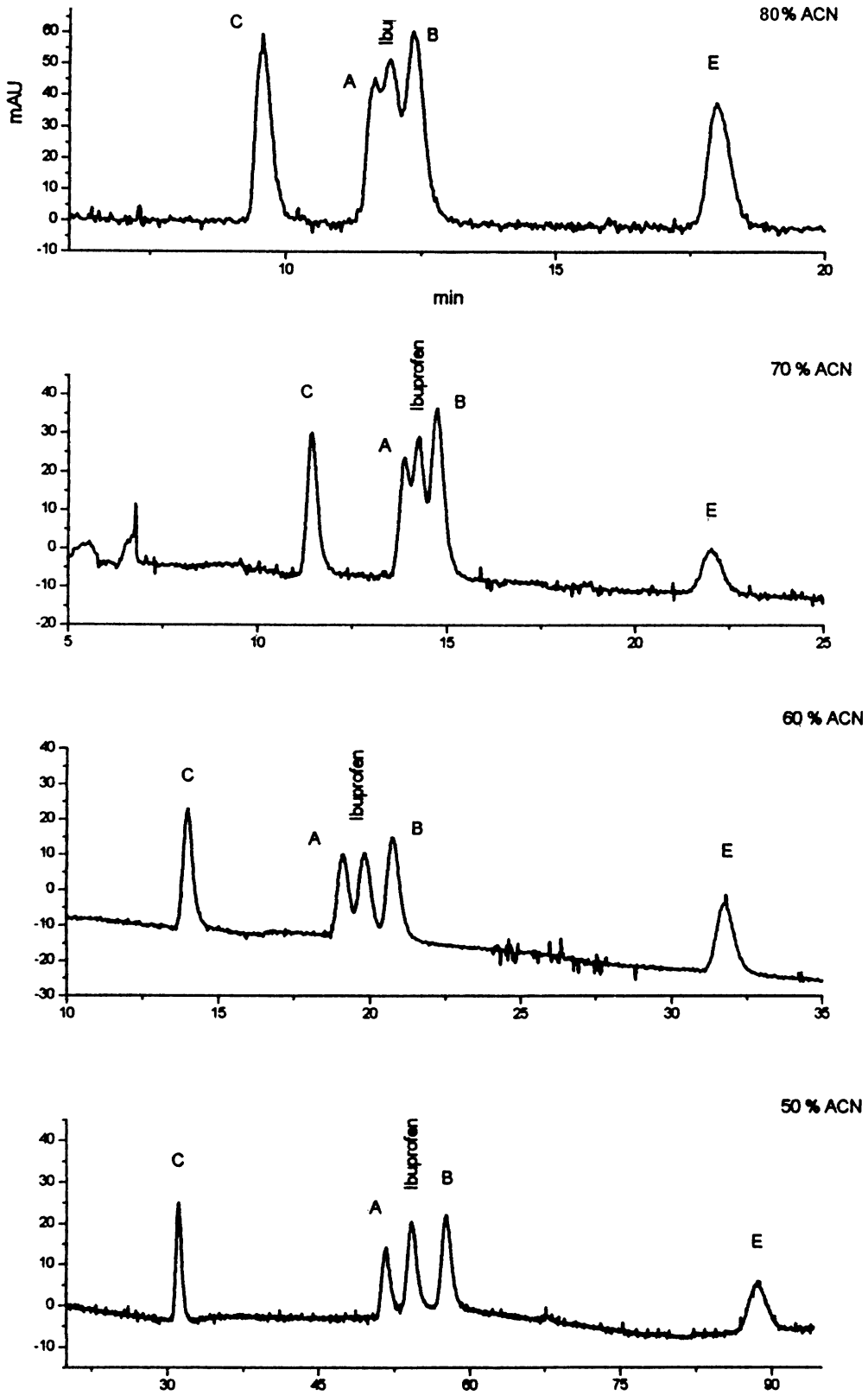


Fig. 2. Influence of ACN concentration in the running buffer on the selectivity. Capillary length 33 cm (effective length 24.5, I.D 100 μm) Stationary phase: LiChrospher 100 RP-18 (5 μm). Mobile phase: 5 mM ammonium formate pH 2.5/ACN. Applied voltage 25 kV. Applied pressure (both sides) 10 bar, injection 12 bar for 24 s.

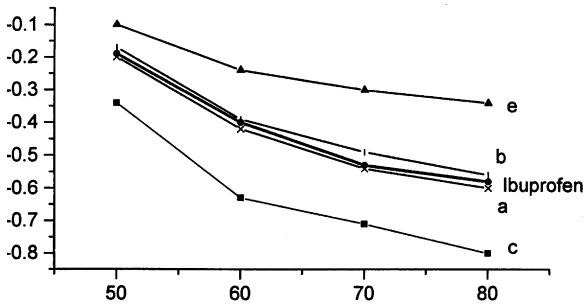


Fig. 3. Dependence of ACN concentration in the mobile phase versus logarithmic function of the retention factor ($K'$).

method was checked by determining the limits of detection (LOD) and quantitation (LOQ). The sensitivity was emphasized using a low UV wavelength (195 nm) for the analysis. The found value of LOD, determined considering the lowest concentration at which drug and impurities can be detected, was 0.05 ng. The LOD corresponds with an electrophoretic peak which is three times upper the baseline noise level. The LOQ, corresponding with an electrophoretic peak which was ten times upper the baseline noise level, corresponded to the lowest concentration in the calibration curve.

To well determine ibuprofen and impurities concentration, the high–low detection (Fig. 6) was used. A first injection of a suitable volume of Ibuprofen solution, allowing to maintain the electrophoretic peak of drug on scale, was made. In these conditions ibuprofen concentration can be determined, but not always the determination of impurities is possible if their concentration is less than LOQ. Therefore the volume of a second injection was increased; in these conditions the ibuprofen peak will be at full scale and the impurity peaks, increased, can be determined. The impurities concentra-

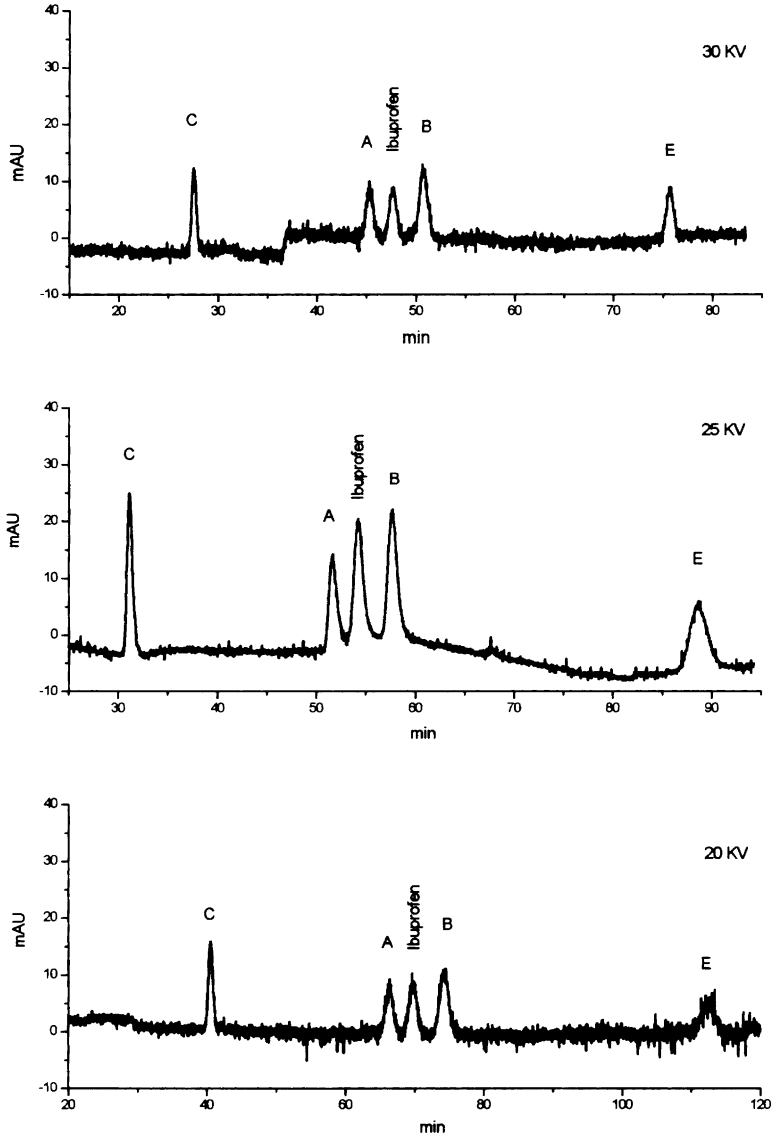


Fig. 4. Influence of the applied voltage on the ibuprofen and impurities separation.

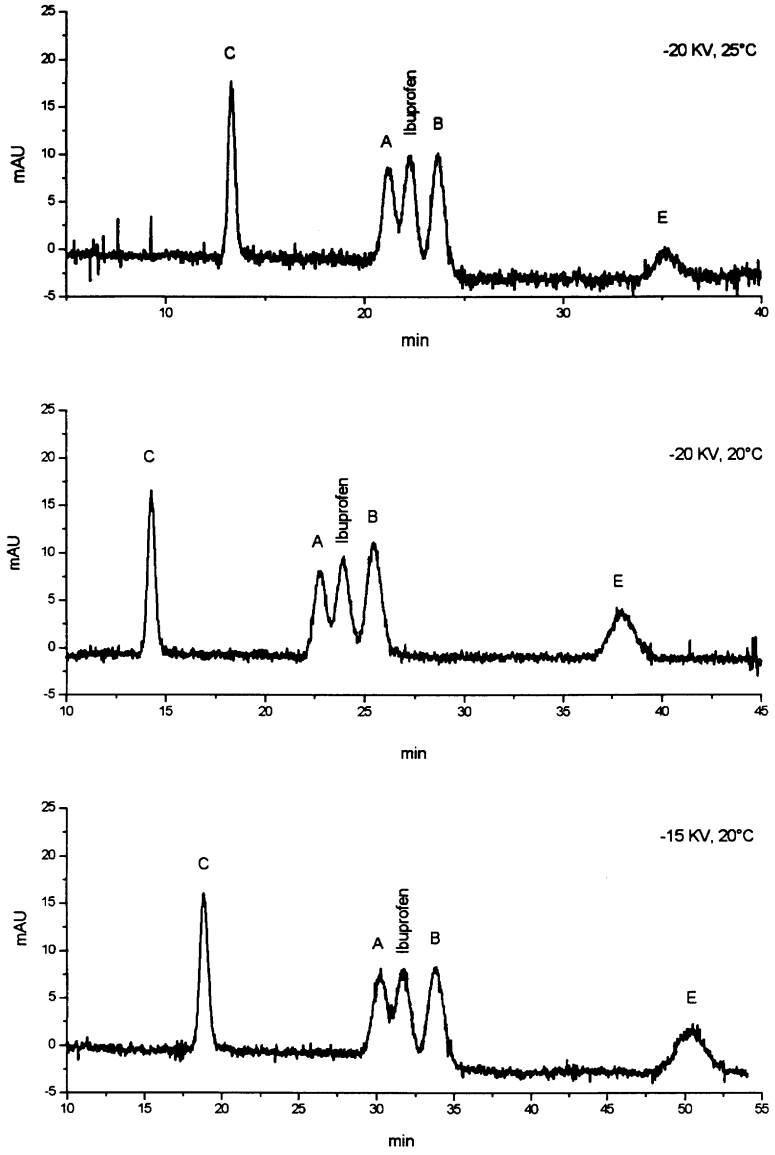


Fig. 5. Electropherograms obtained by inverting the polarity and the injection side (effective capillary length 8.5 cm). Applied voltages and temperatures from 25 kV 25 °C to 15 kV 20 °C.

tion were determined considering their peak areas as percentage of ibuprofen peak area. Using this approach a LOQ of 0.15 ng was found for all impurities. The verified linear relationship between ibuprofen peak area and concentration gave a RSD% = 0.92

The accuracy of the method, was determined by recovery studies. Known quantities of ibupofen, or impurities, were added to the standard and working standard solutions to obtained a fortified solutions. Quantitative recovery of 98.4–99.2% was obtained with a good intra-day precision (RSD% = 0.97; $n = 5$).

Repeatability was evaluated by injecting six times the working standard solution into CEC apparatus. The results were expressed as the ratios between the drug and the individual impurity areas.

## 4. Conclusions

The main objective of this paper was to verify if the CEC can be an HPLC alternative technique. The analysis of ibuprofen and its impurities by CEC seems to confirm that both techniques are suitable in the quality control. In fact CEC efficiency and selectivity are evident, although the CEC analysis time is longer (32 min) in comparison with HPLC (20 min) reported in the

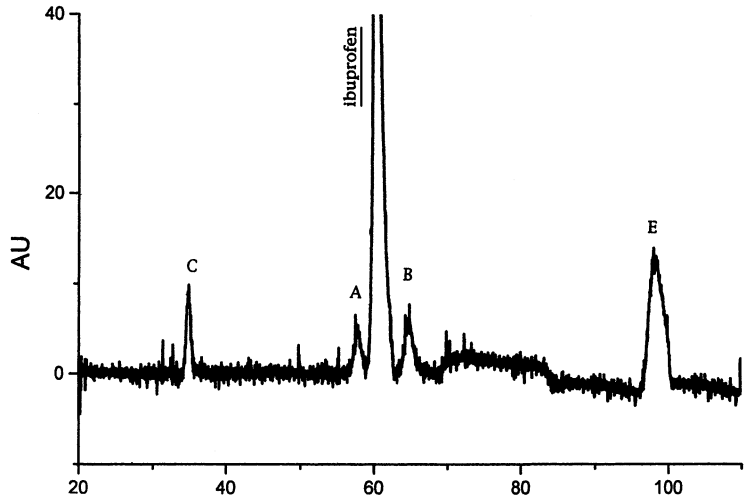


Fig. 6. Electropherogram obtained by injecting a working standard mixture containing ibuprofen with 1% of impurities A, B, C and E.

Italian Pharmacopoea [14]. In any case we can conclude that CEC and HPLC can successfully applied in the same pharmaceutical analysis topics.

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