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Separation of ibuprofen, codeine phosphate, their degradation products and impurities by capillary electrophoresis I. Method development and optimization with fractional factorial design

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Abstract

A capillary electrophoresis method has been developed and optimized for the separation of ibuprofen, codeine phosphate and their main degradation products and impurities. In the course of developing the method, it was found that micellar electrokinetic capillary chromatography was necessary for the separation of the eleven peaks. A fractional factorial design was used for the optimization of the experiments. Six process parameters were varied at two levels: the concentration of sodium dodecyl sulfate (SDS), the pH, the concentration of acetonitrile, the concentration of boric acid, the field strength and the temperature. All these factors had a significant effect on the migration time and resolution. The optimum conditions were found to be a borate buffer of 40 m*M* H_3BO_3 at pH 10 with the addition of 40 m*M* SDS and 9% acetonitrile, a field strength of 515 V/cm and a temperature of 25°C. This resulted in baseline separation of the eleven peaks within 12 min. © 1998 Elsevier Science B.V.

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1. Introduction

Codeine phosphate hemihydrate is an opioid analgesic that is employed in combination with the non-steroidal anti-inflammatory drug (NSAID) ibuprofen, to relieve slight to moderate acute pain. Muhtadi and Hassan [1] reported a pK_a value of 8.2 for codeine.

Codeine phosphate is manufactured by methylation of morphine and its potential impurities are methylcodeine, dimethylpseudomorphine and thebaine. These are expected to be present in concentrations of about 0.1%. A possible degradation pathway is an oxidation to codeine N-oxide [2].

The racemic mixture of ibuprofen, (*R*,*S*)-2-(4-isobutylphenyl)propionic acid, was used. The compound is sensitive to oxidative and thermal decomposition and is known to degrade to 2-(4-isobutyrylphenyl)propionic acid and to 4-isobutylacetophenone. The latter may also be present as an impurity [3]. Other known impurities are 2-[4(1-hydroxy-2-methylpropyl)phenyl]propionic acid and 2-(4-isobutylphenyl)propion-amide. According to Albert and Serjeant [4], the pK_a of ibuprofen is around 5.

Further, an ibuprofen–codeine ester is known as a probable degradation product in a combination product of the two agents. Structures of codeine phosphate, ibuprofen and the related substances (im-

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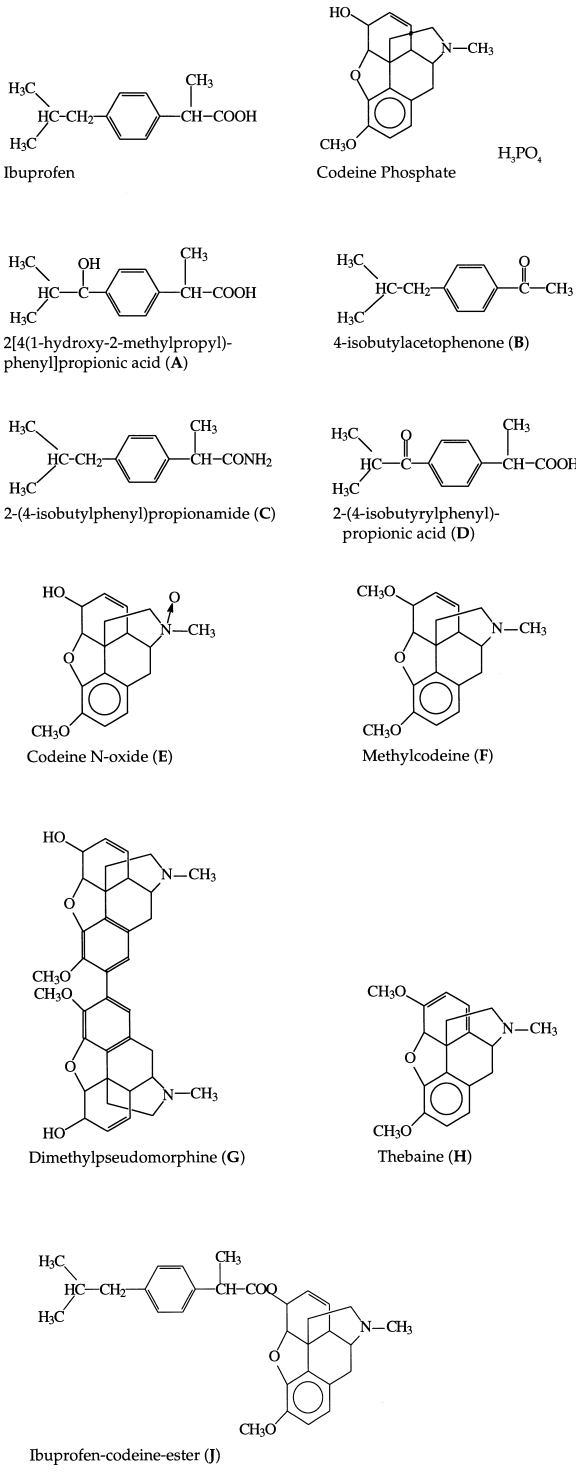

Fig. 1. Structure of ibuprofen, codeine phosphate and their degradation products and impurities.

purities and degradation products) mentioned above are shown in Fig. 1.

The separation of NSAIDs, among them ibuprofen, by capillary electrophoresis (CE) and micellar electrokinetic capillary chromatography (MECC) has been reported by several groups [5–7]. Also, chiral separations of ibuprofen by using different chiral selectors has been described [8–11] and capillary zone electrophoresis (CZE) has been used for the determination of codeine [12–14] in forensic studies. Korman et al. [15] have further used both CZE and MECC to separate codeine from its by-products in different pharmaceutical formulations. The utilisation of chemometrics for optimization and robustness testing of CE based methods has e.g., been described by Altria et al. [16] and Morris et al. [17].

The main aim of the present study was to develop a method based on CE for the separation of ibuprofen and codeine phosphate from their degradation products and impurities and to optimize the process by using chemometrics. A secondary aim was to achieve a separation of the components that corresponded to a resolution (R_s) of at least 1.5 at the shortest possible migration time.

A fractional factorial design based on systematic multivariate schemes was used to identify critical parameters, and more experiments were then added to make an optimization of the separation possible.

2. Experimental

2.1. Equipment

CE was performed on a Hewlett-Packard ^{3D}CE instrument (Walbronn, Germany), with a built-in diode-array detector. The data was recorded with the matching ^{3D}CE ChemStation software.

Fused-silica (FS) capillaries from Hewlett-Packard with a total length (L_t) of 48.5 cm and a length to the detector (L_d) of 40 cm were used. The outer diameter was 365 μm and the inner diameter was 50 μm.

2.2. Chemicals

Ibuprofen was purchased from Jeil Moolsan (Seoul, South Korea) and codeine phosphate

hemihydrate from Macfarlan Smith (Edinburgh, UK).

The impurities of ibuprofen, i.e., 2-[4-(1-hydroxy-2-methylpropyl)phenyl]propionic acid (A), 4-isobutylacetophenone (B), 2-(4-isobutylphenyl)-propionamide (C) and 2-(4-isobutyrylphenyl)-propionic acid (D) and the degradation product of codeine phosphate, codeine N-oxide (E), were kindly provided by Knoll Pharmaceuticals (Nottingham, UK). The impurities of codeine phosphate, methylcodeine (F) and dimethylpseudomorphine (G), were donated by Tasmanian Alkaloids (Tasmania, Australia) and thebaine (H) from Apoteksbolaget (Gothenburg, Sweden). The major formulation degradation product, ibuprofen–codeine ester (J), was synthesized by Astra (Södertälje, Sweden). Sodium dodecyl sulfate (SDS), hexadecyltrimethylammonium bromide (HTAB) and Triton X-100 were purchased from Sigma (St. Louis, MO, USA). Purified water was obtained from a Waters Milli-Q system (Watford, UK). All the chemicals used for buffers, boric acid (Sigma), NaOH and acetonitrile (ACN) (Merck, Darmstadt, Germany) were of analytical grade.

2.3. Procedures

Ibuprofen was dissolved in ACN and its related substances in ACN–water (4:1). Codeine phosphate hemihydrate and codeine N-oxide were dissolved in water, while 0.1 *M* HCl was used as a solvent for the impurities of codeine [15]. To obtain a realistic method, the concentrations of the two active agents were chosen to be higher than those of the related substances. This was done to prepare for future work on a commercially available tablet consisting of 200 mg ibuprofen and 30 mg codeine phosphate hemihydrate.

The concentrations of the samples corresponded to 0.33 mg/ml of ibuprofen and 0.26 mg/ml of codeine phosphate hemihydrate. For the degradation products and impurities, the concentrations were varied between 33–75 μg/ml. Borate buffer (120 m*M* H_3BO_3) of pH 10.2 was added to the sample vial to keep the pH above the pK_a value. SDS was also added to the vial in the same concentration as the background electrolyte (BGE). Borate buffers were prepared by mixing a known amount of H_3BO_3 with 1.0 *M* NaOH to the desired pH.

The buffers were filtered through a membrane with a pore size of 0.5 μm (Micro Filtration Systems, Sierra Court Dublin, CA, USA). The samples were injected hydrodynamically towards the cathode for 3 s at a pressure of 5 kPa and the detection was made at a wavelength of 214 nm. Between the runs, the capillary was flushed for 5 min with 0.1 *M* NaOH, 2 min with Milli-Q water and 5 min with BGE.

2.4. Experimental design

2.4.1. Factors

Six experimental parameters (factors) were varied at two levels: concentration of SDS (m*M*), pH, concentration of ACN (%, v/v), concentration of boric acid (m*M*), field strength (V/cm) and temperature (°C), Table 1. The levels were chosen based on some pre-experiments and knowledge about the system. The factors were varied at the same time, thereby making it possible to distinguish between the effects, e.g., the responses, of a single variable or of interacting variables. Replicating center points are added to check for curvature (quadratic effects), interactions (cross-product terms) in the model, obtain an independent estimate of the error and also to illustrate the reproducibility of the method. By focusing on the main effects it was possible to reduce the number of experiments and to run just a

Table 1
Experimental design

Level	SDS (m*M*)	pH	ACN (%, v/v)	H_3BO_3 (m*M*)	Field strength (V/cm)	Temperature (°C)
High (+1)	80	10.5	11	70	515	25
Center point (0)	60	9.75	9	45	412	20
Low (−1)	40	9.0	7	20	309	15

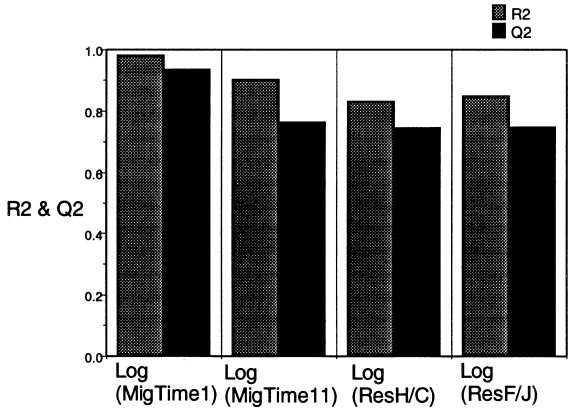

Fig. 2. Variations of the responses explained by the experimental design. The light shaded bars, R^2, denote the fraction of variation of the responses explained by the model and the dark shaded bars, Q^2, denote the fraction of variation of the responses that can be predicted by the model. Responses: MigTime1=migration time for the first peak, MigTime11=migration time for the last peak, ResH/C=resolution between peaks H and C, ResF/J=resolution between peaks F and J.

fraction of the complete factorial experiment [18]. A $2^{(6-2)}$ fractional factorial design was chosen, giving 16 experiments. The center point was replicated three times. With this reduced design, called resolution IV, it is possible to differentiate between the main effects and the two-factor interaction, however the two-factor interactions are aliased with each other. To exclude this and allow only for an alias of a two-factor interaction with the three-factor interactions, the design was expanded to a $2^{(6-1)}$ fractional factorial design with 16 more experiments, called resolution V.

2.4.2. Responses

The migration times for the first (E) and the last peak (J) and the resolutions between peaks H and C and between peaks F and J were chosen as responses in the design. These parameters were chosen to control the speed of the first peak and, in particular, to control the total time to reach a fast, but well-separated, system. The resolutions between peaks H and C and between peaks F and J were chosen as parameters because these were, on average, the most difficult peaks to separate in the previous experiments performed.

2.4.3. Statistical treatment

All the experiments were carried out in duplicate and in a randomized order.

The multivariate method partial least squares, PLS [19], of the program Modde 3.0 (Umetri, Umeå, Sweden) was used to try to find quantitative relations between the responses and the factors. There were a few so-called missing data in one of the 35 experiments and then it is preferable to use PLS instead of multiple linear regression (MLR). The statistical significance of the variables and interaction terms was tested at a significance level of $\alpha=0.05$. Fig. 2 shows the fraction of variation of the responses explained by the model, R^2, and the fraction of variation of the responses that can be predicted by the model, Q^2. The possible values are in the range 0–1.0, with 1.0 revealing the existence of a model with an excellent predictive power.

3. Results and discussion

3.1. Method development

The separation of the two main components, i.e., ibuprofen and codeine phosphate, was explored at three different pH values chosen to give different charges on the analytes, Table 2.

Although the two compounds could be separated in all three buffers, none of them could separate all of the related substances. Micellar agents with different charges, SDS (negative), Triton X-100

Table 2
The charges on ibuprofen and codeine phosphate at different pH values

Buffer type	pH	Ibuprofen	Codeine phosphate
Phosphate (H_3PO_4–NaOH)	3.0	Uncharged	Positive
Phosphate (H_3PO_4–NaOH)	6.0	Negative	Positive
Borate (H_3BO_3–NaOH)	10.0	Negative	Uncharged

(uncharged) and HTAB (positive), were therefore added which gave varying results. The concentrations of the micellar agents were around 10 times their critical micellar concentration (CMC) which are 8.27 m*M*, 0.24 m*M* and 0.026 m*M*, respectively. A high pH with the addition of SDS seemed the most promising, particularly in regard to the migration time. This system could not, however baseline separate the related substances from each other without the addition of an organic modifier, e.g., ACN or methanol (MeOH). Organic solvents are known to reduce the electroosmotic flow (EOF), which results in better resolution but also a longer analysis time. Since ACN gave shorter migration times than MeOH due to a smaller effect on the EOF [20], it was chosen as the organic modifier.

3.2. Method optimization

3.2.1. Experimental design

Performing the experiments according to a $2^{(6-2)}$ design, Table 1, produced the conclusion that all factors had a significant effect on the separation of one or more responses at a 95% confidence interval. Consequently, it was not possible to reduce the number of factors or to further optimize the separation without a new full factorial design. To allow the identification of two-factor interaction effects

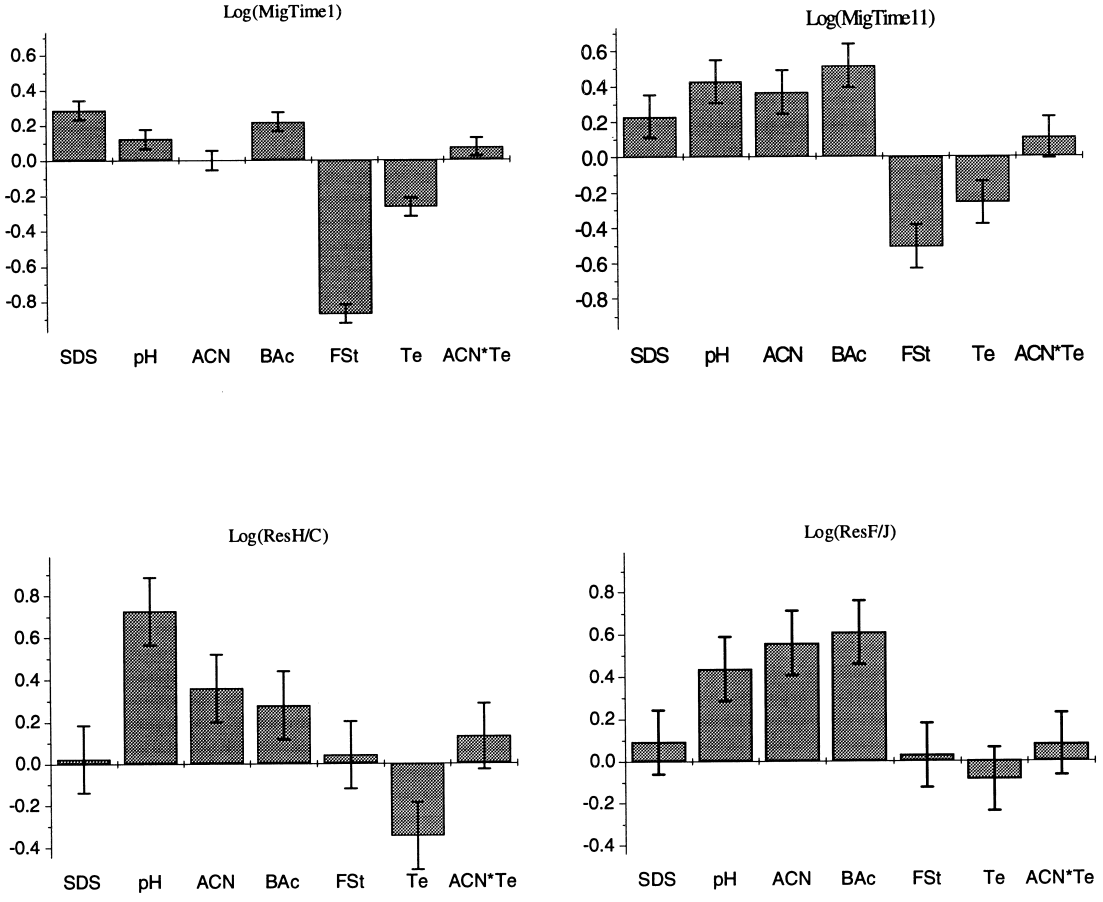

Fig. 3. Coefficient values in the experimental design for the responses at a 95% confidence level. The coefficients are divided by the standard deviation of their respective response. For explanation, see Section 3.2.2. Factors: SDS=concentration of SDS, pH=pH, ACN=concentration of acetonitrile, BAc=concentration of boric acid, FSt=field strength, Te=temperature and ACN*Te=concentration of acetonitrile*temperature.

containing just negligible three-factor or higher interactions, the design was expanded to a $2^{(6-1)}$ fractional factorial design by adding 16 more experiments to the design. A mathematical model was created, based on the total of 35 experiments. The variances of the responses were stabilized by a logarithmic transformation, which improved the model. R^2 in our model was found to be between 0.83 and 0.98, and the values for Q^2 between 0.75 and 0.94, Fig. 2. The standard deviation of the center points was lower than the standard deviation for all experiments, which also indicates a good model. No further improvement in the model was achieved by adding a quadratic term. However, one two-factor interaction, concentration of acetonitrile*temperature (ACN*Te), was observed to have a slightly significant effect on one of the responses and was therefore included in the model.

The evaluation of the model shows that it is almost linear, with only a slight curvature. This makes it hard to find an optimum within the examined area, as it is possible to improve the method by small changes in the parameters. A compromise is needed to reach a fast separation with complete resolution between all peaks.

3.2.2. Separation process

In Fig. 3 the shaded bars show half the effect of each of the factors on the separate responses, i.e., the extent to which a response changes if the factor is varied from the low level (−1) to the center point (0), or from the center point (0) to the high level (+1). The confidence interval is shown as error bars and the effect is not significant if the error bar crosses the *x*-axis. Considering, for example, the migration time 1 (MigTime1) coefficients, a change

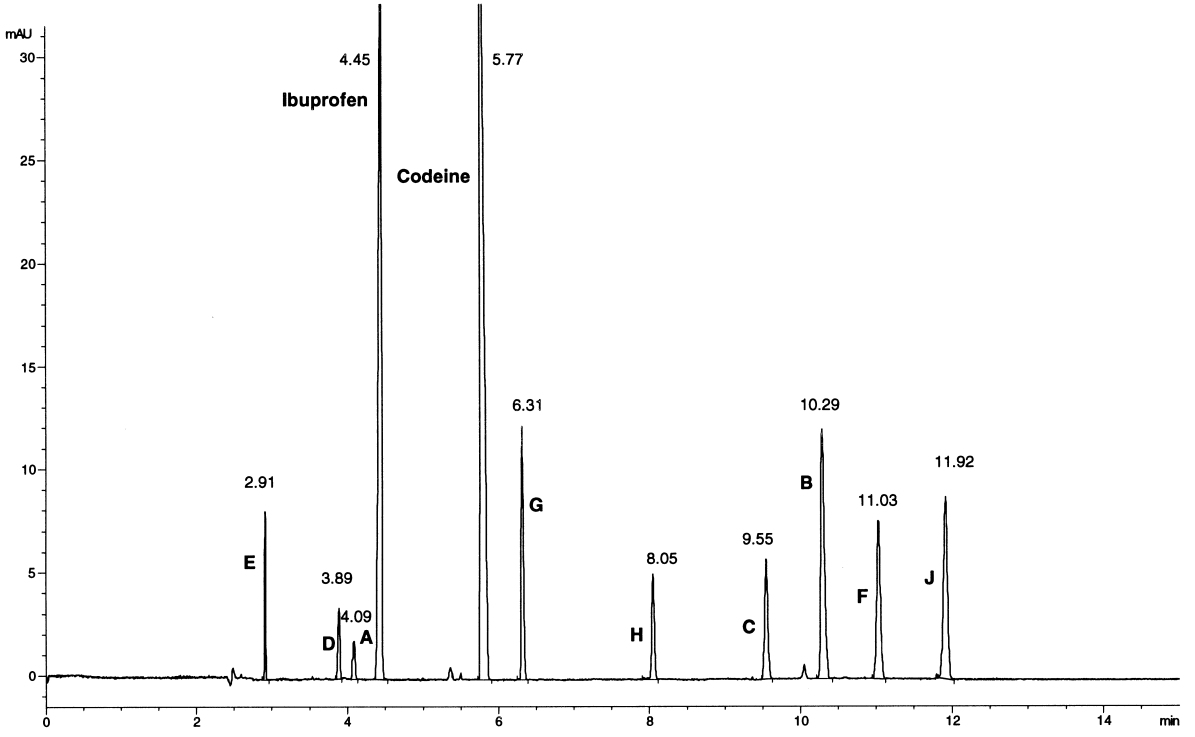

Fig. 4. Optimal separation of ibuprofen, codeine phosphate hemihydrate and their degradation products and impurities. Capillary, 40.0 cm (L_d), 48.5 cm (L_t), 50 μm I.D., 365 μm O.D. FS; BGE, borate buffer made from 40 m*M* H_3BO_3 and adjusted with NaOH to pH 10.0, 40 m*M* SDS, 9% ACN; field strength 515 V/cm; temperature 25°C; wavelength 214 nm; injection: pressure (3 s: 5 kPa); sample concentration, see Section 2.3. The current is 44 μA. A=2-[4-(1-hydroxy-2-methylpropyl)phenyl]propionic acid, B=4-isobutylacetophenone, C=2-(4-isobutylphenyl)propionamide, D=2-(4-isobutyrylphenyl)propionic acid, E=codeine N-oxide, F=methylcodeine, G=dimethylpseudomorphine, H=thebaine and J=ibuprofen–codeine ester.

in the field strength from 309 to 412 V/cm, or from 412 to 515 V/cm, will reduce the migration time remarkably.

The field strength and the concentration of SDS had significant effects on the migration times for the first and the last peak, but not on the resolution of the other peaks investigated. A high field strength, which produces a fast EOF, and a low concentration of SDS, which results in weaker interaction of the analytes with the micelles, will thus produce a fast migration time without affecting the resolution. A high temperature reduces the viscosity and gives a faster system, however, at the same time it will reduce the resolution between peaks H and C. A low level of the concentration of both boric acid and ACN results in a higher EOF, but reduces the resolution. The factors were therefore set to just below the center point value for the concentration of boric acid and to the center point value for the concentration of acetonitrile. The pH was set to just above the center point level to compromise between the migration time and the resolution.

Based on those considerations, we define an optimal separation when the factors are 40 m*M* H_3BO_3 at a pH of 10, an addition of 40 m*M* SDS and 9% ACN, a field strength of 515 V/cm and a temperature of 25°C. An electropherogram of a separation obtained by using these conditions is shown in Fig. 4. The experimental and the predicted values from the software (Modde 3.0) are very close, e.g., the migration times for the last peak are 11.92 min and 11.29 min, respectively.

The results obtained by applying our optimal conditions to a commercially available tablet containing 200 mg ibuprofen and 30 mg codeine phosphate hemihydrate are presented in Fig. 5.

Since ibuprofen is negatively charged at a basic pH, it will not interact into the anionic SDS micelle. That theory is confirmed by measuring the apparent mobility of ibuprofen which is almost the same, irrespective of the presence of SDS in the system, which indicates that it migrates independently of the micelles. Codeine is uncharged at a basic pH and will easily partition into the micelle. The apparent mobility of codeine is $6.4 \cdot 10^{-4}$ $cm^2/V\,s$ in a system without SDS and $2.2 \cdot 10^{-4}$ $cm^2/V\,s$ when SDS is

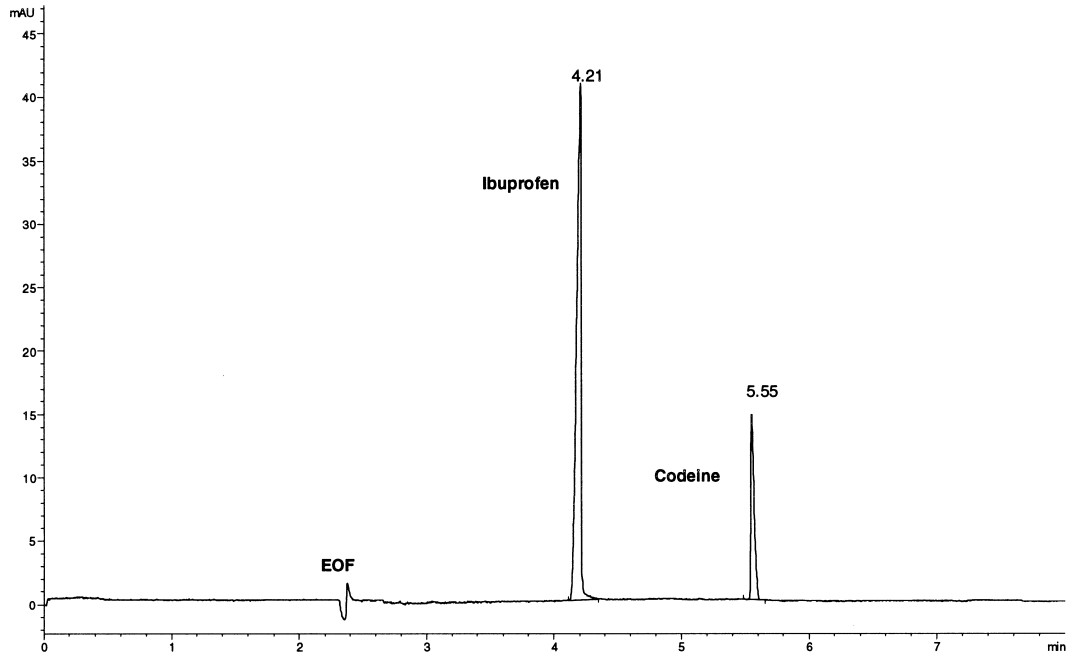

Fig. 5. A commercially available tablet analyzed according to the optimal conditions used in Fig. 4. Sample preparation: 1 tablet dissolved in 25 ml borate buffer (40 m*M* H_3BO_3) pH 10+9% ACN. The sample is centrifuged and filtered through a glass-fibre filter and a membrane filter, 0.5 μm, and diluted 18 times with BGE.

present. This result shows that codeine interacts strongly with the micelles and therefore migrates more slowly than ibuprofen.

4. Conclusions

A MECC method for the separation of ibuprofen, codeine phosphate hemihydrate, their nine potential degradation products and impurities has been developed and optimized using experiments with a fractional factorial design. Such an approach enables a method to be obtained which offers a fast separation with complete resolution between the eleven peaks investigated. The optimal system separates ibuprofen and codeine phosphate from their related substances within 12 min.

A commercially available tablet containing 200 mg ibuprofen and 30 mg codeine phosphate hemihydrate was analyzed in the optimal system conditions and it was found that the excipients in the formulation did not affect the separation. A validation of the method will be performed and published later.

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