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Journal of Chromatography A 826 (1998) 95–102

JOURNAL OF CHROMATOGRAPHY A

Separation of ibuprofen, codeine phosphate, their degradation products and impurities by capillary electrophoresis II. Validation

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Received 16 March 1998; received in revised form 20 August 1998; accepted 28 August 1998

Abstract

A micellar electrokinetic chromatography method for the determination of ibuprofen and codeine phosphate hemihydrate and their degradation products and impurities in a commercial tablet formulation has been validated. The validation has been performed according to the International Conference of Harmonisation's guidance on the validation of analytical methods, and selectivity, linearity, accuracy, precision, detection limit, quantitation limit, robustness and range tests were performed to determine the suitability of the method. It was possible to use the fractional factorial design model from the optimisation of the method to draw conclusions about its robustness. The results confirm that the method is highly suitable for its intended purpose.

Keywords: Validation; Pharmaceutical analysis; Ibuprofen; Codeine phosphate

1. Introduction

The United States Pharmacopoeia (USP) [1] defines validation of analytical methods as the process by which it is established, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical applications. Both the USP and the International Conference of Harmonisation (ICH) [2,3] have recommended a procedure for the validation of analytical methods.

Several papers describe the validation of quantitative capillary electrophoresis (CE) methods and micellar electrokinetic chromatography (MEKC) methods for the determination of degradation products and impurities in pharmaceutical products [4–8].

We have recently developed and optimised by fractional factorial design an MEKC method for the separation of ibuprofen, codeine phosphate hemihydrate, their degradation products and impurities [9]. The optimal conditions for separating these compounds were found to be a borate buffer of 40 m*M* H_3BO_3 at pH 10 with the addition of 40 m*M* sodium dodecyl sulfate (SDS) and 9% acetonitrile, a field strength of 515 V/cm and a temperature of 25°C. This resulted in baseline separation of the 11 peaks within 12 min.

Codeine phosphate hemihydrate is an opioid analgesic which is employed in combination with the non-steroidal anti-inflammatory drug ibuprofen to relieve slight to moderate acute pain.

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0021-9673/98/$ – see front matter
PII: S0021-9673(98)00711-0

The present method is intended to be used for quantification of the two main substances in a commercially available tablet consisting of 200 mg ibuprofen and 30 mg codeine phosphate hemihydrate and for the determination of degradation products and impurities in area% of each main peak. An internal standard (I.S.), benzoic acid, is used for the assay.

The aim of this work was to validate the CE method and to investigate whether it is suitable for its intended use. The validation was performed according to the ICH guidelines [2,3][1] and the following parameters were studied: selectivity, linearity, accuracy, precision, detection limit, quantitation limit, robustness and range. A system suitability test is recommended to check the performance of the system prior to use of the method.

2. Experimental

2.1. Equipment

CE was performed on a Hewlett-Packard ^{3D}CE instrument (Walbronn, Germany), with a built-in diode array detector tuned to 214 nm. The data were 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 and 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), came from Knoll Pharmaceuticals (Nottingham, UK). Codeine phosphate hemihydrate was purchased from Macfarlan Smith (Edinburgh, UK) and the impurities of codeine phosphate, methylcodeine (F) and dimethylpseudomorphine (G), were supplied at no cost by Tasmanian Alkaloids (Tasmania, Australia). Thebaine (H) came from Apoteket (Gothenburg, Sweden). The major formulation degradant, ibuprofen– codeine ester (J), was synthesised by Astra Production Chemicals (Södertälje, Sweden). Both ibuprofen and codeine phosphate hemihydrate are reference materials of documented purity. SDS was purchased from Sigma (St. Louis, MO, USA). Purified water was obtained from a Waters Milli-Q system (Watford, Herts., UK) and benzoic acid was used as an I.S. and came from Merck (Darmstadt, Germany). All the chemicals used for buffers, boric acid (H_3BO_3) (Sigma), sodium hydroxide (NaOH) and acetonitrile (ACN) (Merck), were of analytical grade. The ibuprofen–codeine tablets were produced by Astra Production Tablets (Södertälje, Sweden).

2.3. Procedures

The CE method development is described in Ref. [9].

2.3.1. Electrolyte preparations

Borate buffer 40 m*M* H_3BO_3 was adjusted to pH 10.0 with 1.0 *M* NaOH. A solution consisting of 9% (v/v) ACN in the above mentioned buffer was used as solvent for both the standards and the samples. The background electrolyte (BGE) consisted of 40 m*M* SDS dissolved in the borate buffer containing 9% ACN.

2.3.2. Solution preparation

The I.S. benzoic acid (0.48 mg/ml dissolved in 50% ACN) was used to compensate for the injection error, one of three major error sources in CE besides detection and integration. Benzoic acid was chosen since it migrates between the two main peaks. It was also found to be stable both in the standard and sample preparations. The solutions were prepared by weighing 35±0.5 mg ibuprofen and 5±0.5 mg codeine phosphate hemihydrate for standard solution, Std 1 and 20±0.5 mg ibuprofen and 3±0.5 mg codeine phosphate hemihydrate for standard solution, Std 2. These standard solutions were dissolved in 5.0 ml 50% ACN and diluted by taking 2.0 ml of each standard solution into a 25.0-ml volumetric flask, 5.0

[1]The term ''specificity'' has been replaced by ''selectivity''.

ml of the I.S. solution was added and filled to volume with borate buffer pH 10.0 (40 m*M* H_3BO_3) containing 9% ACN. Five tablets were shaken with approx. 100 ml 50% ACN in a 200-ml volumetric flask for 20 min and thereafter filled to volume with 50% ACN. Approx. 20 ml of this solution was centrifuged at 2000 rpm for 3 min and the supernatant was diluted, for the assay sample, by placing 2.0 ml into a 25.0-ml volumetric flask, adding 5.0 ml I.S. solution and filling to volume with borate buffer, pH 10.0 (40 m*M* H_3BO_3) containing 9% ACN. For the degradation product and impurity sample the supernatant was diluted by placing 2.0 ml in a 5.0-ml volumetric flask and filling to volume with the same solution as mentioned above.

The standards and samples were injected hydrodynamically towards the cathode for 3 s at a pressure of 5 kPa, which corresponds to approx. a volume of 5 nl, and a plug of BGE was injected under the same conditions immediately after each injection.

Between runs, the capillary was flushed for 7 min with 0.1 *M* NaOH, 3 min with Milli-Q water and 5 min with BGE.

2.3.3. Analytical procedure

Two injections of BGE were made before starting the analysis to stabilise the system. For the HP^{3D} system, the waste vial was half-filled with the buffer containing 9% ACN to rinse the capillary at the outside. Vials of 0.1 *M* NaOH, water, waste and BGE were replaced approximately every fifth injection to keep the same levels in the vials during the whole run and to avoid evaporation.

The standards and samples were made in duplicate.

2.3.4. Calculations

2.3.4.1. Response factor

An average response factor ratio, $R_f/R_{I.S.}$, was calculated for ibuprofen and codeine from the standards according to the following formulas:

$$R_{f,ibu}/R_{I.S.}, \text{ ibuprofen} = (A_{I.S.}C_{ibu})/(C_{I.S.}A_{ibu})$$

$$R_{f,cod}/R_{I.S.}, \text{ codeine} = (A_{I.S.}C_{cod})/(C_{I.S.}A_{cod})$$

where: $A_{I.S.}$ = corrected area of I.S., C_{ibu} or C_{cod} = standard concentrations of ibuprofen and codeine phosphate hemihydrate, respectively, $C_{I.S.}$ = concentration of I.S. and A_{ibu} or A_{cod} = corrected areas, standard, of ibuprofen and codeine, respectively.

2.3.4.2. Assay

The amounts in mg/tablet of ibuprofen and codeine phosphate hemihydrate were calculated according to the following formulas:

mg/tablet of ibuprofen:

$$(R_{f,ibu}/R_{I.S.}C_{I.S.}A_{ibu}200m_{av})/(A_{I.S.}0.08m_{tot})$$

mg/tablet of codeine phosphate hemihydrate:

$$(R_{f,cod}/R_{I.S.}C_{I.S.}A_{cod}200m_{av})/(A_{I.S.}0.08m_{tot})$$

where: A_{ibu} or A_{cod} = corrected areas, sample, of ibuprofen and codeine, respectively, m_{av} = average mass of the tablets and m_{tot} = total mass of five tablets.

2.3.4.3. Degradation products and impurities

The content of degradation products and impurities is given as area% and was determined by adding the corrected areas of the peaks originating from ibuprofen and codeine, respectively, and dividing the total by the corrected area from each main peak.

3. Results and discussion

3.1. Selectivity

A standard, a sample, a mixture of the inactive ingredients of the tablet, and a standard with all the degradation products, impurities and with the I.S. were analysed according to the proposed method. The representative electropherograms in Fig. 1a–c show the identity of each separate peak and the separation between the main peaks and the I.S. The latter are well separated and the tablet excipients do not disturb the separation. All the degradation products and impurities are baseline-separated within 12 min.

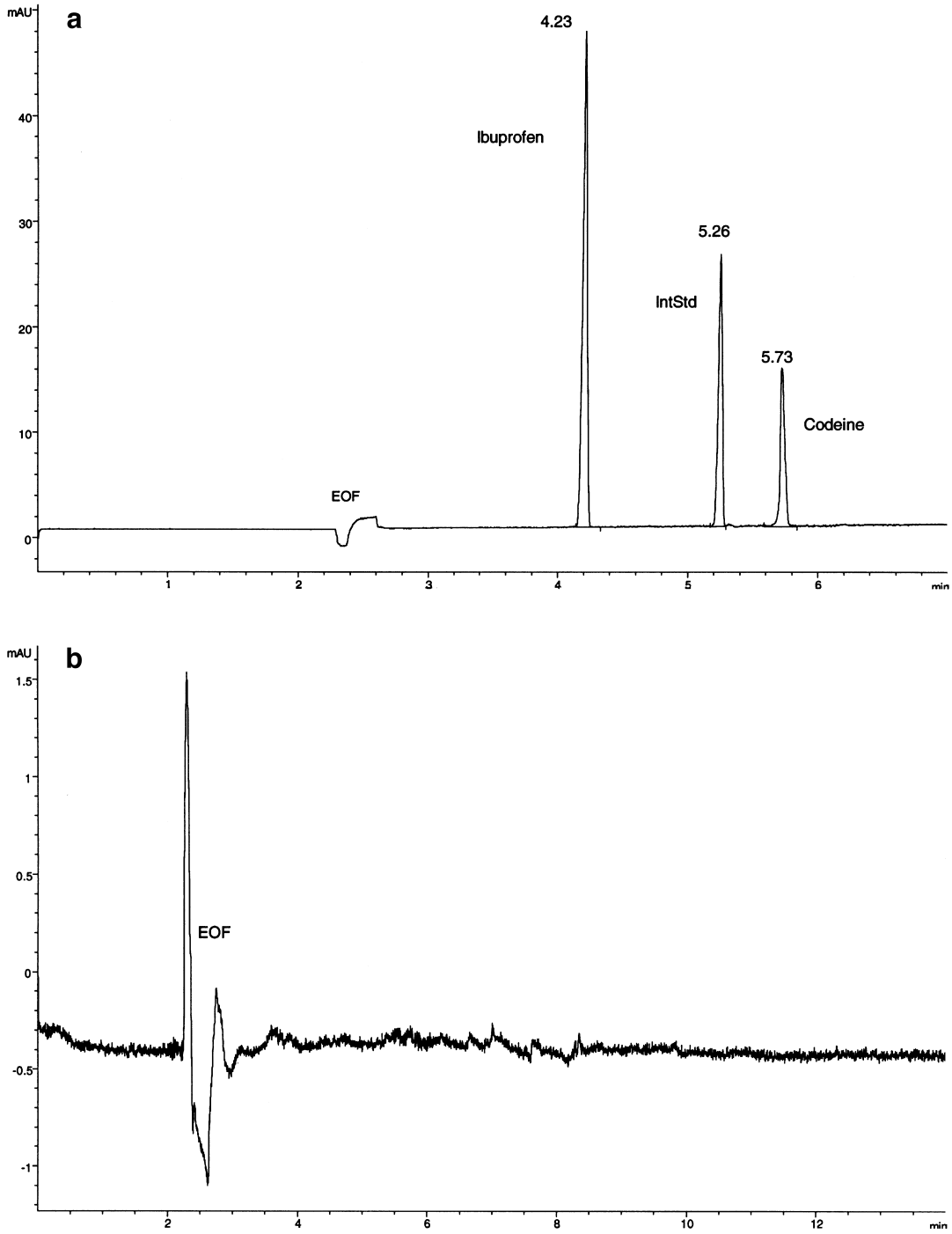

Fig. 1. Specificity/selectivity. (a) Standard solution. (b) Excipient solution. (c) A standard solution with all the degradation products and impurities added in the concentrations 33–75 μg/ml. The peaks are identified in Section 2.2.

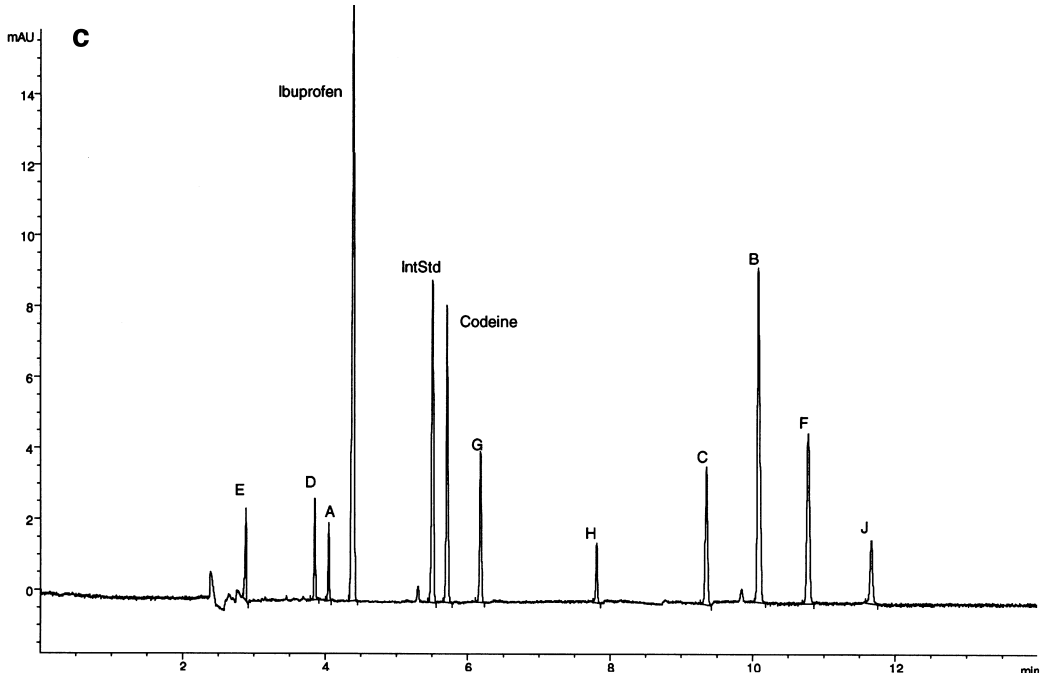

Fig. 1. (*continued*)

3.2. Linearity

Five standards, including the I.S., were diluted to cover 50–150% of the expected assay concentration of ibuprofen and codeine phosphate. Plots of the ratio of the corrected area of the standard to the corrected area of the I.S. versus the different standard concentrations resulted in the equations $y = 7.45x + 0.02$ and $y = 12.56x - 0.0003$ for ibuprofen and codeine, respectively. The linear regression correlation coefficients, R^2, were 0.999 in both cases. The corrected area is defined as the ratio of the measured area to the migration time of the peak.

The linear relationships confirm that the test results are directly proportional to the concentrations.

3.3. Accuracy

The accuracy of a method expresses the closeness between the theoretical value and the determined value and was tested in two different ways. Three different amounts of ibuprofen and codeine phosphate hemihydrate were added to a constant mixture of excipients. The three concentrations covered an 80–120% interval of the expected assay concentrations. The solutions were replicated three times each and the amounts determined were compared to the theoretical amounts. The recoveries ranged from 98.4 to 99.9% for ibuprofen and from 97.6 to 99.8% for codeine, which means that the method thus gives sufficient accuracy.

The results, based on the CE method, obtained from three different production batches were compared to the results obtained by a validated reversed-phase high-performance liquid chromatography (HPLC) method, Table 1. A statistical test, a *t*-test, shows that the codeine results from the two methods do not differ at a 95% confidence level, but the ibuprofen results are higher from the HPLC method. This is probably due to a too high ibuprofen recovery with the HPLC method.

3.4. Precision

3.4.1. Repeatability

The precision over a short time while keeping the operating conditions identical was checked by making six separate injections of a standard solution. The

Table 1
Accuracy, the HPLC method compared with the CE method, ibuprofen

Batch no.	Ibuprofen (mg/tablet)		Codeine phosphate hemihydrate (mg/tablet)	
	HPLC method	CE method	HPLC method	CE method
122	202.8	200.4	29.7	29.5
123	202.5	198.1	29.3	30.3
124	205.4	199.1	30.8	30.4

relative standard deviation, R.S.D., for the migration time of each active substance was found to be 0.34% for ibuprofen, 1.02% for codeine and 0.16% for the I.S. The ratio of the corrected area of the standard to the corrected area of the I.S. resulted in an R.S.D. of 0.35% for ibuprofen and 1.13% for codeine, which means that the repeatability of the method is good. The R.S.D. results from the HPLC method mentioned earlier were 0.7% for ibuprofen and 0.6% for codeine.

3.4.2. Intermediate precision

The within-laboratory variations were investigated by studies covering different days as well as different equipment. Five sample preparations of the same tablet batch were analysed on three different days to explore the variation between days and within days. The mean values for ibuprofen, with the R.S.D. values in parentheses, at three different days were 200.3 mg/tablet (0.3%), 199.2 mg/tablet (0.3%) and 200.1 mg/tablet (1.2%), respectively. For codeine phosphate hemihydrate the results were 29.5 mg/tablet (2.5%), 29.6 mg/tablet (2.2%) and 30.2 mg/tablet (2.7%), respectively. The eventual variations between tablets will affect the variations between and within days and are not taken into consideration. The results were evaluated by analysis of variance, ANOVA, which partitions the total variation of a set of data into the different sources of variation. The results show that there were no significant difference in the variation between days.

The variation between different equipment were evaluated by comparing the results with those from another Hewlett-Packard ^{3D}CE instrument and a fused-silica capillary from Polymicro (Phoenix, AZ, USA) with the same dimensions as described earlier. The results were shown statistically to be the same.

3.4.3. Reproducibility

The variations between laboratories, including different analysts, different instruments and equipment, were evaluated by comparing the results from a quality control laboratory which repeated the analyses with the same sample batch as the one used in the present experiments. The mean values for ibuprofen and codeine phosphate hemihydrate, with the R.S.D. values in parentheses, from the quality control laboratory were 202.3 mg/tablet (0.4%) and 29.5 mg/tablet (1.3%), respectively. The results from the research laboratory were 200.1 mg/tablet (0.7%) and 29.7 mg/tablet (2.1%), respectively. The evaluation by ANOVA showed that the variations between laboratories did not differ at a 95% confidence level.

3.5. Detection limit

A signal-to-noise ratio of approximately 2–3 is generally considered to be acceptable for estimating the detection limit, which is the lowest concentration that can be detected. This was obtained with solutions of 1 μg/ml of the degradation products codeine *N*-oxide (E) (the main degradation product of codeine) and 2-(4- isobutyrylphenyl)propionic acid (D) (the main degradation product of ibuprofen), and with a 3 μg/ml solution of the ibuprofen–codeine ester (J) (the main formulation degradation product), which are acceptable results. For E this corresponds to 0.3% of codeine when the degradation products and impurities sample is injected. For D this is 0.05% of ibuprofen.

3.6. Quantitation limit

The quantitation limit is the lowest concentration of a substance that can be quantified with acceptable

precision and accuracy. A typical signal-to-noise ratio is 10:1. The quantitation limits for the three degradation products, E, D and J, were 6 μg/ml, 3 μg/ml and 10 μg/ml, respectively. This is approximately 10-times the signal-to-noise ratio.

The linearity around the quantitation limits was also studied and the concentrations were between 1–10 μg/ml for E, 1–11 μg/ml for D and 3–15 μg/ml for J. The correlation coefficients were 0.978, 0.998 and 0.992, respectively, which is a good result throughout.

3.7. Robustness

3.7.1. Stability of standard, sample solutions and BGE

Standard, including the internal standard, and sample solutions were found to be stable for ≥15 days when stored in laboratory glass and polypropylene vials at room temperature and in a refrigerator protected from light. Freshly prepared standards were compared as reference. The BGE is stable for ≥two days in laboratory glass at room temperature.

3.7.2. Robustness of the method

A robustness test is expected to confirm the reliability of an analysis to deliberately made variations in method parameters. A fractional factorial design was used for the optimisation and six factors were varied at two levels in a total of 35 experiments [9]. The process parameters were the concentration of SDS, the pH, the concentration of ACN, the concentration of boric acid, the field strength and the temperature. The migration times for the first and the last peak and the resolution between two peak pairs, peaks H and C (H/C) and peaks F and J (F/J), were chosen as responses for the optimisation. These responses were chosen to give a fast and well-separated system. The resolution between peaks H/C and F/J were chosen because these were, on average, the most difficult peaks to separate. However, the I.S. was not included in the optimisation studies. It was shown that all factors had a significant effect on the responses migration time and resolution.

The evaluation of the model made by the 35 experiments from the optimisation showed a good model which was capable of explaining and results satisfactorily. The model was applied to small and realistic variations around the defined optimum and predicted the responses with reliable results. The factorial variations and the maximum and minimum results predicted for the responses are shown in Table 2, and the conclusion is that the method is robust for small changes in the parameters.

3.8. Range

The range of a method is the interval in which it has a suitable level of precision, accuracy and linearity. The validation tests performed show that the range for this method is 80–120% of the expected main component assay.

3.9. System suitability

System suitability testing is based on the concept that the equipment, electronics, analytical operations and samples to be analysed constitute an integral system that can be evaluated as such [3]. For this method, the following system suitability test has been used.

The present method was judged to be suitable when a standard solution following two injections of BGE showed a resolution >1.5 between the I.S. and the codeine peak.

Table 2
Ruggedness test of the method

Factor	High level[a]	Low level[a]
SDS concentration (m*M*)	40.5	39.5
pH	10.2	9.8
ACN concentration (%)	9.1	8.9
H_3BO_3 concentration (m*M*)	40.5	39.5
Field strength (V/cm)	530	500
Temperature (°C)	26	24
Response	**Maximum[b]**	**Minimum[b]**
t_m, first peak (min)	3.0	2.5
t_m, last peak (min)	14.0	9.1
Resolution H/C	8.7	4.3
Resolution F/J	5.5	3.8

[a] The factorial variations around the defined optimum.
[b] The predicted maximum and minimum results for the responses from the factorial variations made above (t_m = migration time).

4. Conclusions

A MEKC method for the assay of ibuprofen and codeine phosphate hemihydrate and their degradation products and impurities in a commercial formulation has been validated. The validation was performed according to the ICH guidelines, and selectivity, linearity, accuracy, precision, detection limit, quantitation limit, robustness and range tests were performed to determine the suitability of the method. The results confirm that the method is selective, linear and highly accurate. The precision was determined with different instruments, equipment, laboratories and analysts and on different days, with satisfactory results. The detection limit is 1–3 μg/ml for the main degradation products and the quantitation limit is 3–10 μg/ml for the same substances.

The validation clearly shows the usefulness and advantages of experimental design, since it was possible to use the fractional factorial design model from the optimisation to evaluate the robustness of the method.

The results confirm that the method is suitable for its intended purpose and is useful in pharmaceutical analyses. The method is submitted to the regulatory authorities.

Acknowledgements

The authors wish to thank Mrs. Gerd Dall and Dr. Ludmila Westermark at the quality control laboratory, TQA, for their assistance in this work. We also wish to thank Professors Christina Graffner and Douglas Westerlund for valuable comments on the manuscript.

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