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Quantitation and validation of *cis*-camphoric acid 3-methyl ester and *cis*-camphoric acid 1-methyl ester using CE

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Abstract

The 1- and 3-methyl esters of *cis*-camphoric acid, the active agents of a mild laxative (Flubilar™) have been simultaneously assayed using capillary electrophoresis (CE). The compounds are completely separated using a sodium acetate buffer pH 4.0, 40 mmol/l. In order to obtain reproducible results, [+]-naproxen has been used as internal standard (IS). Initially migration times changed over 50% within a series of 20 runs. This problem has been overcome by using an overnight capillary preconditioning (1 mol/l NaOH, 1.5 h) and subsequent equilibrating (running buffer, 12 h). Thereby a precision corresponding to a CV % of about 1.17 and 1.42 for the *cis*-camphoric acid methyl esters has been obtained (six series of $n = 10$ runs each). The method has been validated regarding specificity, accuracy, precision, linearity and robustness. In order to test robustness, all key parameters have been considered. The result of the validation is given in nine tables. In the case under investigation, lamp age and wavelength accuracy are the most critical parameters. Therefore, the lamp age should be limited to about 1000 h. The wavelength accuracy can be indirectly controlled using quality assurance samples. According to the fundamental mechanisms in CE, changes in the voltage and in the temperature influence migration times and peak areas. However, these effects are very well compensated using an IS. Slight variations of the parameters buffer pH and molarity rinsing times, storage conditions of buffers and samples as well as the capillary material had little or no influence on the analytical results.

Keywords: Capillary electrophoresis; *cis*-Camphoric acid 1-methyl ester; *cis*-Camphoric acid 3-methyl ester

1. Introduction

cis-Camphoric acid 3-methyl ester and *cis*-camphoric acid 1-methyl ester (Fig. 1) are the active ingredients of a laxantic drug. The pharmaceutical preparation is filled into ampoules. These are opened prior to use. The solution is then orally applied. Except the active ingredients, the recipe also contains a large amount of sucrose, sour cherry flavor, sodium benzoate as preservative, and cochenille-red as dye (E 124). A method had to be found to separate the esters and determine

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them quantitatively, and which is further applicable to routine analysis. This method had to be validated.

2. Experimental

2.1. Instrumentation

The CE experiments were carried out using a Spectraphoresis 1000 instrument (Thermo Separation Products, Fremont, CA, USA). This instrument is equipped with an autosampler tray (82 positions) and a peltier element for capillary thermostatting. The injection pressure has been set to a vacuum of 1.5 psi.

2.2. Materials

For the separation CElect-FS50 fused silica columns (Supelco, Deisenhofen, Germany) with an O.D. of 363 μm and an I.D. of 50 μm were used. The total length of the column was 43 cm; the effective length was 35 cm.

As running buffer a 40 mmol solution (pH 4.0) of sodium acetate trihydrate (Fluka, Deisenhofen, Germany; MW 136.08) was prepared:

Sodium acetate trihydrate (5.4432 g) were dissolved in about 100 ml of HPLC-grade water. This solution was adjusted to pH 4.0 with about 85 ml of acetic acid using a freshly calibrated pH-electrode. The solution was made up to 1000 ml with HPLC-grade water.

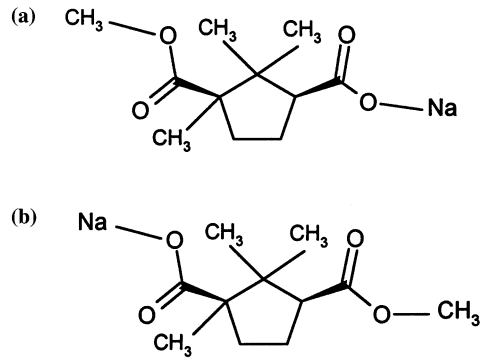

Fig. 1. (a) *cis*-Camphoric acid-sodium-3-methyl ester; (b) *cis*-camphoric acid-sodium-1-methyl ester.

The buffer for preparing the samples and the calibrator solutions contains 20 mmol of sodium acetate trihydrate.

Sodium acetate trihydrate (2.7216 g) (Fluka, Deisenhofen, Germany; MW 136.08) were dissolved in about 100 ml of HPLC-grade water and adjusted to pH 4.0 with about 42 ml of 1 M acetic acid (again checked with a calibrated pH-electrode). The solution was made up to 1000 ml with HPLC-grade water.

2.3. Settings and parameters

Outlet side of the capillary	Cathode side
Voltage	27.5 kV
Current	56 μA
Temperature	25°C
Injection time	6 s
Sample injection	Hydrodynamic injection with 1.5 psi ($\approx$ 50 mbar)
Buffer rinse	2 min after each run, $p = 1$ bar
Prewash	6 min with 40 mmol sodium acetate buffer at 25°C
Postwash	None
Detection	UV detection at 210 nm

2.4. Capillary pretreatment

Prior to first use, every capillary has to be conditioned. Therefore, the capillary was rinsed with 1 M NaOH at 50°C during 90 min followed by washing the capillary with the running buffer for 5 min at 25°C. The capillary was equilibrated with this buffer applying voltage (27.5 kV) for at least 12 h. The column is ready for use if there is no visible trend in the migration time of the analytes during ten subsequent injections of a standard solution. If there should remain any trend, the column will not stable and has to be equilibrated again for at least 1 h. After 200–300 runs the capillary might show clear ascendant migration times; then the capillary must be replaced.

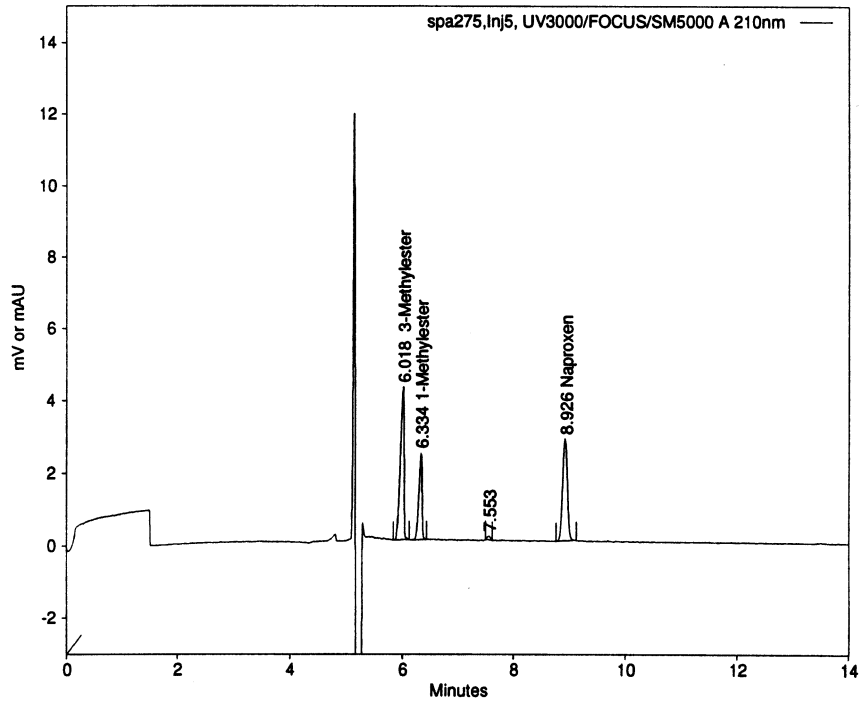

Fig. 2. Typical electropherogram from the two methyl esters of camphoric acid at 210 nm. Peak no. 1, *cis*-camphoric acid 3-methyl ester (6.018 min); peak no. 2, *cis*-camphoric acid 1-methyl ester (6.334 min); peak no. 3, naproxen as IS (8.926 min).

2.5. Solutions

2.5.1. Stock solution for the internal standard

Naproxen (8–9 mg) exactly weighted, was dissolved in a 100 ml measuring flask with HPLC-grade methanol and made up to 100 ml. This master solution (3 ml) was pipetted to the reference solution and to the sample solutions.

2.5.2. Sample solution

Methanol (10 ml) and 3 ml of the internal standard (IS) solution (equivalent to 0.24–0.27 mg naproxen) was pipetted into a 100 ml measuring flask, then the content of 1 ampoule Flubilar, exactly weighted, was made up with 20 mM sodium acetate buffer, pH 4, to 100 ml.

2.5.3. Reference solution

The reference substances were kindly provided by Byk Gulden.

About 150 mg *cis*-camphoric acid 3-methyl ester and about 50 mg *cis*-camphoric acid 1-methyl ester, exactly weighted, were dissolved in 10 ml methanol. Then 5.0 ml (6.256 g) placebo solution was added, and the solution was made up to 100 ml. This solution, with respect to the concentration of the esters, was equivalent to the sample solutions. This solution can be used for at least 1 month if they are stored at 4°C.

3. Results and discussion

3.1. Separation conditions and EOF stability

In order to find the best separation conditions, several standard buffers of pH between 2 and 12 have been tested (compare [1]). The separation conditions had been optimized to an acetate buffer pH 4.0, 40 mmol/l (compare Fig. 2).

The separation had to be carried through at pH 4. Insufficient selectivity has been found using other pH values or when SDS was added (MEKC mode). It is well known that the EOF is less stable

in the pH range between 4 and 7. However, a stable EOF is necessary to correctly estimate sample mobility and sample amount. Therefore, it is a crucial factor that should be carefully considered for validated CE methods. Thus, buffers of weakly acidic pH should be avoided whenever possible [1].

Consequently, other separation conditions have been tried including various MEKC approaches. Still, the selectivity was by far inferior to the above conditions. Instead, the difficulties could be overcome by improving the capillary equilibration procedure.

EOF instability may be explained by incomplete reaction of siloxane to silanol groups during the preconditioning process. This is less critical when an alkaline buffer is used. Here the hydroxide concentration may be sufficient to complete the reaction during the equilibration with the running buffer. Therefore, longer preconditioning times (e.g. 2–10 h) with higher NaOH concentrations (e.g. 1 M) are recommended when weakly acidic pH buffers are used, in order to achieve a complete hydrolysis of siloxane [2]. The temperature had been set at 50°C during preconditioning to further accelerate the preconditioning process.

Even after this improved preconditioning, minor but perceptible long-term changes were observed. Nevertheless, results can be well compared from day-to-day. Furthermore, these long-term changes can be mostly compensated by the use of ISs.

3.2. Quantitation

3.2.1. Matrix interferences

At first reference substances were dissolved in water. The concentration was chosen as the nominal concentration of the drug sample. Surprisingly, the peak areas corresponding to the camphoric acid methyl esters in the reference electropherograms were about 10% higher then the peak areas in the sample electropherograms. This was possibly due to the different viscositiesH of the solutions, which could lead to alterations in the injected volume and the EOF.

All sample constituents were well known and purely available. Therefore, this matrix interference was successfully avoided when preparing standard solutions containing all excipients such as sugar, color and flavor in relevant concentrations.

3.2.2. Internal standard

In order to compensate for dosage errors (e.g. injection errors, volume dilution errors, etc.), the method of the IS is usually utilized. Thereby the precision of analytical results in CE is essentially improved [1]. The same amount of IS is added to all samples and standards. The sample must not contain the IS. Moreover, the IS must completely separate from all other substances of the analysis. Hence, all samples and standards will contain the IS in the same concentration c_i. Further, in all obtained electropherograms the related peak area A_i of the IS was determined. Instead of comparing directly the peak areas of analysis and reference solution (method of external standard), the area ratio A/A_i is considered as signal. If no dosage error occurs, the area corresponding to the IS is equal for all sample and reference solutions. Thus, the area of the IS can be cancelled in Eq. (1). In this case the calculation is identical to the external standard method. On the contrary, if too much or not enough volume is injected, the amount of IS increases or decreases as well. Consequently, the dosage error is compensated.

$$c_a = c_s \frac{A_a/A_{i(a)}}{A_a/A_{i(s)}} \quad (1)$$

Here, $A_{i(a)}$ means the peak area of the IS in the electropherogram of the analysis and $A_{i(s)}$ the peak area of the IS in the electropherogram of the standard solution. Usually the analytical result is calculated from multiple measurements. Here the ratios $A_a/A_{i(a)}$ and $A_s/A_{i(s)}$ are individually determined for each electropherogram, then the respective means of the ratios are calculated. These means are finally inserted into Eq. (1) to obtain the analytical result C_a.

Naproxen can only be used in this high dilution because it dissolves sparsely in water. Sorbic acid is also suitable as IS. However, naproxen has been preferred, because it is stable in aqueous solutions for several weeks.

3.3. Validation

3.3.1. General

Before a method is routinely used, it must be validated. Validation is the process of proving that a method is acceptable for its intended purpose. This is decided by using a number of performance characteristics. These are specificity, linearity, accuracy, precision, range, limit of detection, limit of quantitation and robustness [1,3]. Definitions of these terms based on the recommendations of the International Conference of Harmonisation (ICH) [4].

3.3.2. Precision

The precision describes the closeness of agreement between a series of measurements (general term, includes repeatability, intermediate precision and reproducibility) [4].

It is the most important target value for quantitations. The precision of the peak area ratio (Eq. (1)) is the most important parameter because it corresponds to the precision of the analytical result. However, the precision of further parameters such as absolute peak areas and migration times have also been investigated.

Changes in these parameters are of interest for the properties of a method. Such changes, however, are not critical for the application of this method for quality control, because within an analysis series they influence all analytical values in the same way.

The precision of separation efficiency and peak resolution has not been considered in detail, since the separation was excellent in all cases. In a short series, all peak pairs exceeded resolution values of two (compare Table 1).

The high precision of the method has been confirmed determining the intermediate precision, which is related to day-to-day variations of experimental conditions in one laboratory. Here six series of $n = 10$ values each have been performed during 72 h (Table 1).

3.3.3. Selectivity

Next the selectivity of the method has been tested. All sample constituents were well known and purely available. No peak interferences have been noted.

3.3.4. Accuracy

The accuracy is the closeness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value, and the value found [4].

In this case the accepted reference values have been obtained weighing highly pure standard substances characterized by various analytical techniques like GC–MS and IR. The weighing error is usually considered as being very small. This method to determine accuracy is often referred to as recovery. The recovery is then the ratio of measured and weighed amount [4]. A series was done with $n = 10$ runs. An average recovery of 100.80% *cis*-camphoric acid 3-methyl ester and 99.82% *cis*-camphoric acid 1-methyl ester has been found, respectively. The method works accurately (Table 2).

3.3.5. Range

The range is the interval between the upper and the lower concentration [4].

In doing so, it represents the upper and the lower limit of that concentration of analyte, for which precision, correctness, and linearity have been proven. In the present case, this range is chosen as 80–120% of the specified content, like suggested in [5]. The linearity is then checked by the 'three times eight' design using an ANOVA analysis [5].

3.3.6. Linearity

For three different concentrations, eight measurements at a time were performed. The concentrations are chosen at the two endpoints and in the middle of the measuring range (80–120%). The order of measurements followed a randomized scheme.

The linearity of the method is unambiguous. This statement best becomes clear looking at the residual plot (Fig. 3). The visual impression has been confirmed by the ANOVA-lack-of-fit method.

3.3.7. Robustness

Robustness is the capacity to remain unaffected by small, but deliberate, variations in method parameters [4].

Table 1
Peak resolution, repeatability and intermediate precision[a]

Cis-camphoric acid-3-methylester						Cis-camphoric acid-1-methylester						Naproxen (internal standard)			
Peak resolution															
Peaks of analytes ($n = 10$)															
Plates	Plates (R.S.D.%)	Resolution				Plates	Plates (R.S.D.%)	Resolution				Plates	Plates (R.S.D.%)	Resolution	
29321	1.93	2.266				49689	0.749	2.266				30380	0.616	7.915	
Repeatability ($n = 10$)															
Precision ($n = 10$)															
t_M	t_M (R.S.D.%)	A	A (R.S.D.%)	R	R (R.S.D.%)	t_M	t_M (R.S.D.%)	A	A (R.S.D.%)	R	R (R.S.D.%)	t_M	t_M (R.S.D.%)	A	t_M (R.S.D.%)
5.53	0.089	21729	0.148	1.9547	0.335	5.76	0.09	10364	1.07	0.932	0.975	7.61	0.26	11113	0.521
Intermediate precision ($n = 60$)															
Day-to-day precision ($n = 6 \times 10$)															
t_M	t_M (R.S.D.%)	A	A (R.S.D.%)	R	R (R.S.D.%)	t_M	t_M (R.S.D.%)	A	A (R.S.D.%)	R	R (R.S.D.%)	t_M	t_M (R.S.D.%)	A	t_M (R.S.D.%)
5.62	1.288	11800	1.17	1.3	1.66	5.84	1.63	9172	1.42	0.36	1.96	7.72	4.6	9172	1.42

[a] t_M, Migration time; A, area; R, relative area $A_{analyte}/A_{IS}$; R.S.D., relative standard deviation.

Table 2
Recovery (t_M, migration time; A, area; R, relative value $A_{Analyte}/A_{IS}$; R.S.D. %, relative standard deviation in percent)

	Recovery even $n = 10$															
	cis-Camphoric acid-3-methyl ester						*cis*-Camphoric acid-1-methyl ester						Naproxen (IS)			
	t_M	t_M (RSD%)	A	A (RSD%)	R	R (RSD%)	t_M	t_M (RSD%)	A	A (RSD%)	R	R (RSD%)	t_M	t_M (RSD%)	A	T_M (RSD%)
Sample	5.523	0.185	16 494	0.938	1.31	0.938	5.79	0.21	6038	1.55	0.48	1.54	8.06	0.5	12 591	0.66
Spiced sample	5.556	0.62	22 380	1.768	1.78	1.76	5.84	0.68	10 645	1.31	0.86	1.79	8.15	1.1	12 550	1.3

It is an important indicator for the reliability of a method in normal application.

3.3.7.1. Temperature (24, 25, 26°C). The temperature is the critical parameter in transferring a method between instruments of different manufacturers. This investigation has been performed twice because of its outstanding importance. Both experimental series led to the same result. Though migration times are influenced by temperature (a change in temperature influences the viscosity and thus the electroosmotic flow) and also the absolute peak areas change (compare voltage), neither the absolute values nor the RSD % of the peak area ratios have been affected. All temperature effects are effectively compensated using the IS. The temperature is not a critical parameter for this method (Table 3).

3.3.7.2. pH values of the buffer (3.95, 4.00, 4.05). Here the pH was varied in the order of magnitude that could be expected from minor preparation errors. No significant change in any of the observed parameters was found (Table 4).

3.3.7.3. Buffer molarity (38, 40, and 42 mM). Here the molartiy was varied in the order of magnitude that could be expected from minor preparation errors. No significant change in any of the observed parameters was found.

3.3.7.4. Stability of buffer- and sample-solution (0, 6, 11 weeks). Simultaneous experiments were carried through using freshly prepared solutions as well as buffer solutions and sample solutions that had been prepared 6 and 11 weeks before and stored in measuring flasks. This experimental design was chosen to estimate the influence of the age of these solutions but to avoid apparatus- and capillary-dependant variations.

Hardly any significant change in the target parameters was observed, though all solutions were stored at room temperature. Solely after 11 weeks the RSD % value of the measured parameters increased, but it was not clear, if the increase was statistically significant. This increase, however, was without practical importance. The used solutions were very stable (Table 5).

3.3.7.5. Capillaries. The investigations regarding the long time reproducibility were performed with five different capillaries from two different lots. Variations due to differences in the capillary material were included in the overall variation of less than 1.5%, in contrast to earlier investigations where much larger variations were found [6,7]. Obviously differences in the capillary material were very well compensated by the developed equilibration procedures.

3.3.7.6. Length of rinsing cycles (3, 4, 5 and 6 min). Significant changes of the parameters could not be stated as a result of the rinsing time, when values between 4 and 6 min were used. By further shortening to 3 min slight but perceptible changes occurred. A rinsing time of 5 min ensured robust results (Table 6).

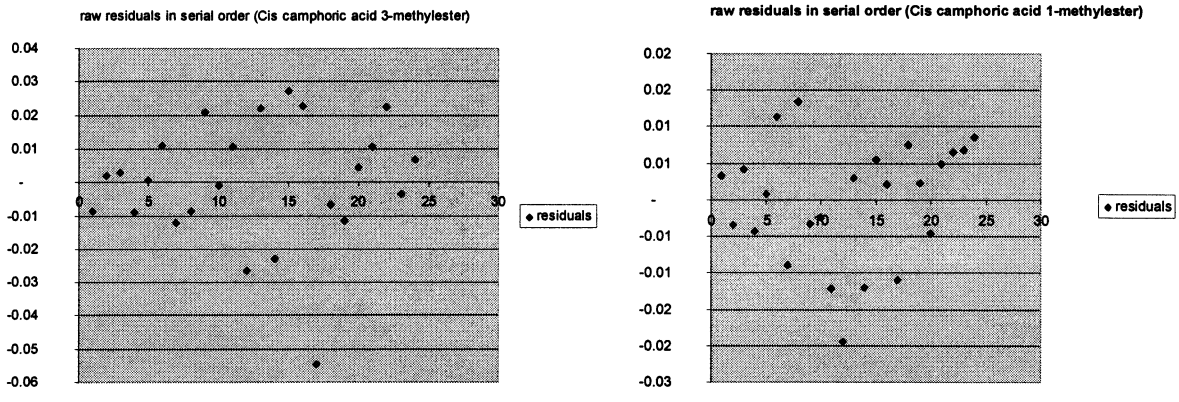

Fig. 3. Plot of the raw residuals.

Table 3
Temperature values (t_M, migration time; A, area; R, relative value $A_{Analyte}/A_{IS}$; R.S.D. %, relative standard deviation in percent)

Temperature (°C)	1 series $n = 10$															
	cis-Camphoric acid-3-methyl ester						*cis*-Camphoric acid-1-methyl ester						Naproxen (IS)			
	t_M	t_M (RSD%)	A	A (RSD%)	R	R (RSD%)	t_M	t_M (RSD%)	A	A (RSD%)	R	R (RSD%)	t_M	t_M (RSD%)	A	A (RSD%)
24	7.97	0.247	18 276	0.573	1.208	0.325	8.38	0.338	6699	1.047	0.4428	0.733	12.19	0.874	15 126	0.497
25	7.76	0.193	18 265	0.824	1.214	0.824	8.14	0.218	7025	1.262	0.4412	1.581	11.6	0.658	15 045	0.655
26	7.82	1.26	19 127	1.093	1.212	1.311	8.17	1.311	6892	1.596	0.4369	1.031	11.36	2.039	15 774	1.595
	2 series $n = 10$															
24	5.63	0.716	16 816	1.22	1.319	1.176	5.89	0.711	6073	1.435	0.476	1.816	8.16	0.739	12 746	0.913
25	5.61	0.48	17 144	1.187	1.331	1.6	5.88	0.455	6182	1.563	0.479	1.503	8.1	0.388	12 885	0.63
26	5.53	0.181	17 095	0.388	1.333	0.535	5.78	0.212	6197	0.744	0.483	0.7788	7.9	0.461	12 926	0.703

Table 4
pH values (t_M, migration time; A, area; R, relative value $A_{Analyte}/A_{IS}$; R.S.D. %, relative standard deviation in percent)

PH value	$n = 10$															
	cis-Camphoric acid-3-methyl ester						*cis*-Camphoric acid-1-methyl ester						Naproxen (IS)			
	t_M	t_M (RSD%)	A	A (RSD%)	R	R (RSD%)	t_M	t_M (RSD%)	A	A (RSD%)	R	R (RSD%)	t_M	t_M (RSD%)	A	A (RSD%)
3.95	6.97	0.399	21 577	0.614	1.1079	0.68	7.14	0.377	10 630	0.56	0.545	0.49	10.21	0.35	19 476	0.609
4.0	6.76	1.38	20 847	1.27	1.1003	2.09	7.14	1.5	10 224	0.85	0.54	1.8	10.4	2.19	18 951	1.74
4.05	6.74	0.623	21 031	0.36	1.0891	1.21	7.17	0.71	10 427	0.803	0.54	0.97	10.94	1.44	19 312	1.077

Table 5
Stability of buffer values (t_M, migration time; A, area; R, relative value $A_{Analyte}/A_{IS}$; R.S.D. %, relative standard deviation in percent)

Stability	Even $n = 10$															
	cis-Camphoric acid-3-methyl ester						*cis*-Camphoric acid-1-methyl ester						Naproxen (IS)			
	t_M	t_M (RSD%)	A	A (RSD%)	R	R (RSD%)	t_M	t_M (RSD%)	A	A (RSD%)	R	R (RSD%)	t_M	t_M (RSD%)	A	A (RSD%)
0 Weeks	4.81	0.41	13 333	1.283	1.395	0.671	5.03	0.423	4780	1.083	0.500	0.885	6.65	0.52	9557	0.959
6 Weeks	4.82	0.51	12 804	0.9537	1.404	1.002	5.03	0.89	4526	0.8959	0.496	0.896	6.59	0.53	9117	1.243
11 Weeks	4.95	1.13	14 241	0.8456	1.433	1.071	5.15	1.17	5063	1.397	0.509	1.656	6.66	1.47	9937	1.332

3.3.7.7. Voltage (26.5, 27.5, 28.5 kV). The voltage clearly influences the migration behavior of the substances. This is expected, since the movement of the substances is a result of the applied voltage. However, the effects of small changes in voltage were still minor. For naproxen, the migration time even increased about 30 s when the voltage was increased by 1 kV, due to minor changes in the EOF during the measurement series.

The absolute values of the peak areas were influenced as well by changing migration times. Faster migrating substances move more quickly through the detector and lead to a peak area with the same height but smaller width. However, this effect influences the analyte and the IS to the same extent. Thus, the absolute value and the RSD % of the peak area ratios are not affected. Further, severe voltage fluctuations are not expected during a CE experiment (Table 7).

3.3.7.8. Detection wavelength (208, 210, 212 nm). The most important target value, which is the precision of the peak area ratios, remains unaffected by wavelength changes, whilst all other values change. The peak area of the analyte diminishes, while the peak area of the IS increases with increasing wavelength.

This is not problematic, because changes of the wavelength during the experiment are not to be expected in the case of the used reliable measuring apparatuses. Furthermore, a verification of the correctness of the wavelength was performed during the instrument qualification. Nevertheless, the use of quality assurance samples is recommended in order to quickly identify any variations (Table 8).

3.3.7.9. Lamp age (about 50 and 1000 operating h). These examinations were performed using a new and an older deuterium lamp, which was kept for this purpose. Whereas the absolute values of the investigated parameters are, as expected, barely influenced by a change of the lamp, the scattering of the measured values increased. The reproducibility for the older lamp is less favorable, but within the set specifications (<2 RSD %). As a result of this investigations it is recommended to limit the usage of deuterium lamps (to 1000 h in the case under investigation) (Table 9).

4. Conclusions

4.1. Method development

The shown method is now used in routine analysis. Reproducibility and accuracy data in routine correspond to the data given in Tables 1 and 2. A good baseline separation of the esters is obtained. The RSD % of 1.22 and 1.11 for the peak areas of *cis*-camphoric 3-methyl ester and *cis*-camphoric 1-methyl ester, respectively, are acceptable. In order to obtain robust results, an IS was used.

The developed preconditioning procedure allowed working with a stable EOF despite the unfavorable buffer pH. Possibly this procedure is generally helpful, if buffers with a pH between 4 and 7 are used. If this pH range could successfully be utilized again, this would facilitate method development.

4.2. Validation

The method has been validated with respect to the ICH guidelines. The experimental design for this test has been described in detail. The parameters rinsing time, pH, molarity, storage and capillary material proved to be uncritical. In the case of changes, no significant differences were observed.

According to the separation mechanism, migration times and peak areas change with voltage and temperature. However, these changes are mostly compensated by using an IS. Thus, these parameters can be rated as uncritical as well.

Changes in the detection wavelength and the lamp age can influence analytical results. Although changes in the detection wavelength are not expected during the course of an experimental series, carefully designed experimental schemes should provide the measuring of quality assurance standards in order to allow the quick identification of an instrumental failure or breakdown.

Table 6
Rinsing cycle values (t_M, migration time; A, area; R, relative value $A_{Analyte}/A_{IS}$; R.S.D. %, relative standard deviation in percent)

	Prewash-procedure even $n = 10$															
	cis-Camphoric acid-3-methyl ester						*cis*-Camphoric acid-1-methyl ester						Naproxen (IS)			
	t_M	t_M (RSD%)	A	A (RSD%)	R	R (RSD%)	t_M	t_M (RSD%)	A	A (RSD%)	R	R (RSD%)	t_M	t_M (RSD%)	A	A (RSD%)
6 min	5.55	0.17	21 833	1.12	1.955	1.78	5.78	0.16	10 374	0.43	0.929	1.29	7.58	0.15	11 166	1.07
5 min	5.53	0.09	21 729	0.31	1.955	0.335	5.77	0.1	10 364	1.08	0.932	0.974	7.61	0.26	11 113	0.52
4 min	5.61	0.08	23 557	0.38	1.931	0.922	5.82	0.09	11 224	0.43	0.9206	0.749	7.44	0.76	12 196	0.76
3 min	5.69	0.4	24 114	0.48	1.924	0.829	5.89	0.44	11 478	0.74	0.9159	0.9006	7.41	0.82	12 533	0.82

Table 7
Voltage values (t_M, migration time; A, area; R, relative value $A_{Analyte}/A_{IS}$; R.S.D. %, relative standard deviation in percent)

Voltage (kV)	$n = 10$															
	cis-Camphoric acid-3-methyl ester						*cis*-Camphoric acid-1-methyl ester						Naproxen (IS)			
	t_M	t_M (RSD%)	A	A (RSD%)	R	R (RSD%)	t_M	t_M (RSD%)	A	A (RSD%)	R	R (RSD%)	t_M	t_M (RSD%)	A	A (RSD%)
26.5	7.25	0.124	23 321	0.463	1.115	0.776	7.65	0.121	11 524	1.690	0.551	1.620	11.09	0.447	20 905	0.637
27.5	6.06	0.831	18 599	0.433	1.114	1.468	6.38	0.894	9115	1.246	0.546	1.143	8.98	1.350	16 703	1.634
28.5	6.04	0.209	20 345	0.536	1.114	0.565	6.70	0.199	9990	0.888	0.546	0.895	9.46	0.370	18 266	0.392

Table 8
Wavelength values (t_M, migration time; A, area; R, relative value $A_{Analyte}/A_{IS}$; R.S.D. %, relative standard deviation in percent)

Wave length (nm)	$n = 10$															
	cis-Camphoric acid-3-methyl ester						*cis*-Camphoric acid-1-methyl ester						Naproxen (IS)			
	t_M	t_M (RSD%)	A	A (RSD%)	R	R (RSD%)	t_M	t_M (RSD%)	A	A (RSD%)	R	R (RSD%)	t_M	t_M (RSD%)	A	A (RSD%)
208	5.492	0.8	19 743	0.773	1.75	0.659	5.701	0.702	7117	0.517	0.631	0.683	7.47	0.607	11 282	1.001
210	5.479	0.589	18 085	0.398	1.385	0.902	5.704	0.654	6511	0.779	0.498	0.779	7.55	0.987	13 058	0.804
212	5.5677	0.397	16 456	0.88	1.102	1.29	5.782	0.443	5876	0.753	0.393	0.753	7.53	0.767	14 937	0.914

Table 9
Detector lamp age values (t_m, migration time; A, area; R, relative value $A_{Analyte}/A_{IS}$; R.S.D. %, relative standard deviation in percent)

Age of detector lamp	$n = 10$															
	cis-Camphoric acid-3-methyl ester						*cis*-Camphoric acid-1-methyl ester						Naproxen (IS)			
	t_M	t_M (RSD%)	A	A (RSD%)	R	R (RSD%)	t_M	t_M (RSD%)	A	A (RSD%)	R	R (RSD%)	t_M	t_M (RSD%)	A	A (RSD%)
Lamp (1000 h)	5.61	0.47	17 124	1.74	1.33	1.72	5.88	0.455	6146	1.02	0.48	1.058	8.11	0.38	12 854	0.775
New lamp (50 h)	5.48	0.6	18 071	0.338	1.38	0.763	5.7	0.653	6519	0.389	0.5	0.502	7.55	0.987	13 083	0.776

In addition, two interesting details for the performance of a method emerged from the examinations of the robustness:

The usage of deuterium lamps should be limited (to about 1000 operating h in this case). Moreover, sample and buffer solutions can often be used for 1 month or possibly even longer, if they are properly stored.

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