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# Determination of aromatic choline esters by micellar electrokinetic capillary chromatography

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ABSTRACT

Micellar electrokinetic capillary chromatography (MECC) using sodium dodecyl sulphate (SDS), alkyltrimethylammonium bromide ($C_{10}$ to $C_{18}$ TAB) or sodium cholate (NaCh) as the micellar phase has been investigated for separation and quantitation of individual aromatic choline esters. MECC based on NaCh was found to be suitable for determination of these choline esters. The influence of changes in separation conditions were evaluated according to migration times, peak areas, and separation efficiency for the compounds considered. Up to 485 000 theoretical plates per meter were obtained and relative standard deviations were 0.4–0.7% for relative migration times. Repeatability, linearity and detection limits for the developed method were determined with the detection limits found to be 25–60 pg. It is shown that efficient separations of structurally closely related compounds are possible within a few minutes in test solution as well as in samples prepared from plant material.

## INTRODUCTION

Choline is present in all living cells as phosphate and carboxylic acid esters of which aromatic choline esters form a well defined group of natural products occurring in plants [1]. These compounds are all derivatives of benzoic or cinnamic acids containing phenolic groups or other substituents on the aromatic ring [2–9]. Sinapine is the best known in *Brassica* [9], where it can accumulate to an appreciable level in the seeds and co-occur with several other aromatic choline esters [10]. These compounds call for special attention owing to their effects on rapeseed quality and the physiological effects they may have [10–12].

Methods of analyses for determination of individual aromatic choline esters are needed for efficient studies of the above mentioned problems. Available methods include high-performance liquid chromatography (HPLC), based on ion-pairing chromatography [13,14]. However, HPLC methods suffer from some disadvantages compared to the potential possibilities with use of high-performance capillary electrophoresis (HPCE) for determination of various plant constituents [15,16]. Recent developments indicate thus, that the HPCE technique is an attractive alternative to HPLC determination of aromatic choline esters.

Separation and determination of cations have been achieved in capillary zone electrophoresis of mono- and divalent metal ions, amines [17,18], oligopeptides [17] and ammonium salts [19]. Moreover, the technique of micellar electrokinetic capillary chromatography (MECC) [20] has proved to be successful for catecholamines [21–23] using sodium dodecyl sulphate (SDS) micelles. However, strong ionic interaction of cationic compounds to the polar group of the SDS micelles may constitute a problem for certain cationic species [21–24]. Bile salts, on the other hand, have proved to be applicable as

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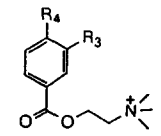
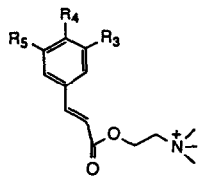
detergents in MECC for the separation of basic solutes as well as hydrophobic compounds [24–26].

This paper aims at an efficient HPCE method of analysis for the aromatic choline esters evaluated. Various types of detergents for MECC of these compounds have been tested. A method for qualitative and quantitative analysis, using an anionic bile salt surfactant as the micellar phase supplemented with a zwitterionic compound to prevent adsorption of analytes to the capillary wall, is presented. A test solution containing six structurally closely related compounds was used for evaluation of the influence of different separation conditions on various separation parameters. With the combined technique of group separation, purification, and MECC now developed, a rapid, simple and efficient method of analysis of naturally occurring aromatic choline esters is obtained as illustrated for the complex mixture of these compounds occurring in seeds of *Hesperis matronalis* L. and *Sinapis alba* L.



Benzoic acid derivatives

| No. | $R_3$ | $R_4$ | Name |
|---|---|---|---|
| 2 | - H | - OH | 4 - Hydroxybenzoylcholine |
| 3 | - $OCH_3$ | - OH | Vanillylcholine |
| 9 | - OH | - $OCH_3$ | Isovanillylcholine |
| 10 | - $OCH_3$ | - $OCH_3$ | Hesperaline |



Cinnamic acid derivatives

| No. | $R_3$ | $R_4$ | $R_5$ | Name |
|---|---|---|---|---|
| 4 | - H | - OH | - H | p - coumaroylcholine |
| 5 | - $OCH_3$ | - OH | - H | Feruloylcholine |
| 6 | - $OCH_3$ | - OH | - $OCH_3$ | Sinapine |
| 7 | - $OCH_3$ | - $OCH_3$ | - H | 3,4 - dimethoxycinnamoylcholine |
| 8 | - OH | - $OCH_3$ | - H | Isoferuloylcholine |

Fig. 1. Structures and names of benzoic and cinnamic acid derivatives used in MECC analyses. Numbers indicated are used in connection with the other figures and tables. Trigonelline amide serves as internal standard and is numbered 1.

## EXPERIMENTAL

### *Apparatus*

The apparatus used was an ABI Model 270A capillary electrophoresis system (Applied Biosystems, Ramsey, NJ, USA), with a 760 mm × 0.05 mm I.D. fused-silica capillary tube. Detection was performed by on-column measurements of UV absorption at a position 530 mm from the injection end of the capillary. For data processing, a Shimadzu (Kyoto, Japan) Chromatopac C-R3A was used.

### *Samples and reagents*

Aromatic choline esters studied were isolated or synthesized in our laboratory according to methods described elsewhere [13,27].

The names and structures of the benzoic- and cinnamic acid derivatives are presented in Fig. 1, together with numbers used in the text as well as in the other figures and tables.

Disodium hydrogenphosphate, disodium tetraborate, taurine, SDS, alkyltrimethylammonium bromide ($C_{10}$ to $C_{18}$ TAB) and sodium cholate (NaCh) were obtained from Sigma (St. Louis, MO, USA). 2-Propanol was from Merck (Darmstadt, Germany). All chemicals were of analytical-reagent grade.

### *Procedure*

The separation buffer in the SDS system was prepared with 50 m*M* SDS, 100 m*M* disodium hydrogenphosphate, 10% 2-propanol and, if added, 1 *M* taurine. Investigations with different chain lengths of alkyltrimethylammonium bromide (50 m*M*) were performed with a buffer containing 30 m*M* disodium hydrogenphosphate, 18 m*M* disodium tetraborate and 5% 2-propanol in addition to the detergent. pH was adjusted to 7.0 in all systems.

The different separation buffers tested in the NaCh system were prepared with variations in concentrations of 2-propanol, NaCh, taurine and

phosphate. pH of the buffers was 7.3, except when the pH dependence was investigated.

Common for all systems was filtration of buffers through a 0.20 $\mu$m membrane filter prior to use. Washing of the capillary was performed with 1.0 *M* NaOH for 2 min and with buffer for 5 min before each analysis. When buffer composition was changed, the washing procedure was extended to 4 min with 1.0 *M* NaOH and 10 min with the new buffer. Buffers were changed manually.

In the SDS and NaCh systems, the samples were introduced from the positive end of the capillary and, in the $C_{10}$ to $C_{18}$ TAB systems, from the negative end by vacuum for 1 s. On-column detection at 235 nm was applied. Unless otherwise stated, the separations were performed at 30°C and 20 kV (SDS and NaCh) or 40°C and 18 kV ($C_{10}$ to $C_{18}$ TAB).

Calculations of relative migration times (*RMT*), normalized area (*NA*), theoretical plates (*N*) and resolution ($R_s$) were performed as described by Michaelsen *et al.* [15]. Repeatabilities were estimated from the means and relative standard deviations (R.S.D.). The linearity of the method was determined from linear regression analysis based on least-squares estimates. Approximate detection limits for the compounds investigated were determined from the linearity analysis and a signal-to-noise ratio of 2:1.

## RESULTS AND DISCUSSION

Preliminary trials with SDS as a surfactant in MECC resulted in long migration times (*MT*) and poor separation of the aromatic choline esters studied (Fig. 1). Strong ionic interactions between the positively charged analytes and the anionic part of the surfactant seemed to be the dominating factor, as taurine addition were unable to shorten *MT*. Taurine is a zwitterionic compound, reducing interactions of cations with the negatively charged capillary wall by simple competition mechanism [28].

Ion pairing between analytes and surfactants was prevented by use of long chain cationic detergents as $C_{10}$ to $C_{18}$ alkyltrimethylammonium bromides. These detergents also create a double layer on the capillary wall changing the negatively charge here to a positive double layer [15]. Different chain lengths were tested ($C_{10}$ to $C_{18}$), with tetradecyltrimethylammonium bromide (TTAB) giving the best separations (Fig. 2). However, the separations obtained were not considered satisfactory and changes of temperature, voltage and concentration of 2-propanol did not lead to sufficient improvements.

The separation using NaCh as pseudo-stationary phase is, as for SDS, based on the hydrophobic and ion pairing interaction of the positively charged aromatic choline esters and the negatively charged micelles. However, micellar structure of NaCh compared to SDS are thought to prevent too strong ionic bonds, and NaCh, at concentrations higher than the critical micelle concentration (CMC), has been suggested to form rod-like or cylindrical micelles

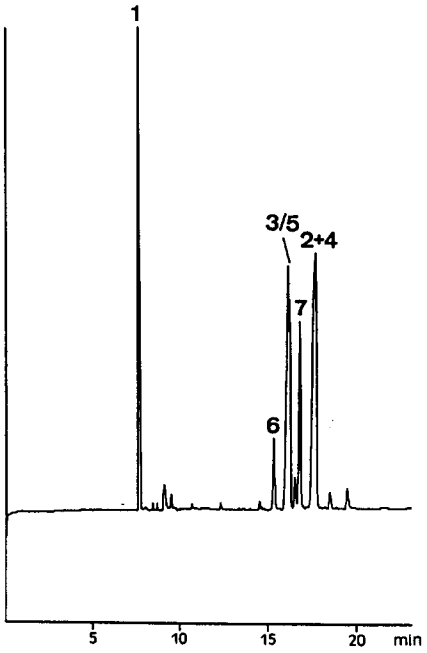



Fig. 2. Electropherogram of the mixture of aromatic choline esters separated by the MECC method (TTAB). Numbers as in Fig. 1. Separation conditions: buffer composition, 30 m*M* disodium hydrogenphosphate–18 m*M* disodium tetraborate–50 m*M* TTAB–5% 2-propanol; temperature, 40°C; voltage, −18 kV; total length of capillary, 760 mm; detection 530 mm from injection end; UV detection at 235 nm. Vacuum injection for 1 s.

with the hydrophobic part situated on the surface and the hydrophilic portions turned inward [26,29]. This should be compared to the likely spherical form of alkyltrimethylammonium bromide or SDS micelles with the charged groups situated on the surface of the micelles and having a hydrophobic core [15,20,30]. Addition of taurine to the buffer is thought to reduce interactions between the negatively charged capillary wall and the positively charged analytes.

Under the separation conditions applied, micelles formed by NaCh move toward the anode, whereas the direction of electroosmotic flow (EOF) will be toward the cathode. Injection of the positively charged aromatic choline esters at the positive end of the capillary results in an increase of analyte speed due to EOF, whereas micelles act retardingly. The selective retention obtained is thus a result of differential partitioning of the aromatic choline esters between the aqueous buffer and the hydrophobic micellar phase.

The electropherogram in Fig. 3 shows the elution order of the aromatic choline esters investigated. Identification of the peaks corresponding to the individual compounds was done from mixing of the authentic compounds [13,27], and according to their UV spectroscopic properties with use of detection at various wavelengths in MECC. In addition, *RMT* under the given separation conditions were determined for the individual compounds when required. *MT* for benzoic acid derivatives is seen to be less than *MT* for cinnamic acid derivatives, indicating less interaction of the benzoic acid derivatives with the micellar phase at the applied conditions. The internal standard appears as peak no. 1, whereas the unnumbered peaks late in the electropherogram are due to the solvent front. The first peak in the solvent front represents EOF as determined by the injection of methanol.

Variation of the separation parameters in the NaCh system implies changes of temperature, voltage and composition of the separation buffer including pH and concentrations of 2-propanol, NaCh, taurine and phosphate. A systematic investigation of the influence of those parameter changes on *MT*, *RMT*, *NA*, $R_s$ and *N*, as an expression for efficiency, have been carried out.

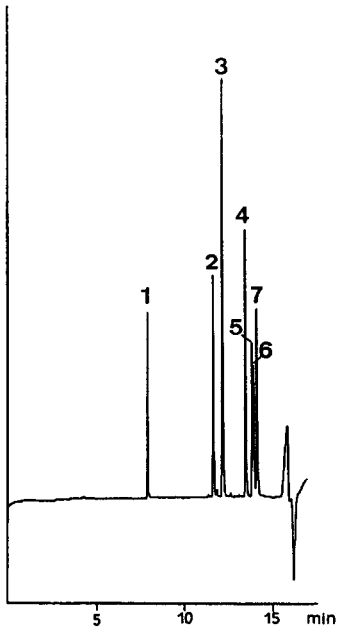



Fig. 3. Electropherogram of the mixture of aromatic choline esters separated by the MECC method (NaCh). Numbers as in Fig. 1. Separation conditions: buffer composition, 100 m*M* disodium hydrogenphosphate–600 m*M* taurine–50 m*M* NaCh–10% 2-propanol; temperature, 30°C; voltage, 20 kV; total length of capillary, 760 mm; detection 530 mm from injection end; UV detection at 235 nm. Vacuum injection for 1 s.

*2-Propanol concentration*

An increase in the concentration of 2-propanol in the separation buffer from 0 to 12% considerably increased *MT* for all of the solutes. *RMT* values, with trigonelline amide (**1**) as the reference compound, were nearly unaffected or only slightly decreased. A reduction in *NA* was observed for **3** with increasing concentration of 2-propanol in the buffer. For the other compounds, only a little decrease was observed with higher concentration of the organic modifier. $R_s$ values were unaffected by the content of 2-propanol in the buffer, whereas *N* increased for all of the aromatic choline esters tested.

Presence of an organic modifier in the buffer affects EOF, due to reductions in the zeta potential [31,32]. This reduction in EOF correlated well with the observed increase in *MT* for the compounds analysed.

NaCh micelles tolerate high concentrations of organic modifier, which is probably due to the bile salt micelle structure, having the hydropho-

bic portion of the monomers turned outward the micelle (see above). A more stable micelle [26,29] may thus be the result, when an organic modifier such as 2-propanol is added to the buffer. This may again affect partitioning of aromatic choline esters between the aqueous and micellar phase, leading to the observed changes in *NA* as well as separation efficiency. It is thus seen, that the modifier increases the rate of association/dissociation between micelles and analytes.

Addition of 2-propanol was shown to lower the solubility of taurine, resulting in precipitation of taurine crystals in the buffer at high concentrations of 2-propanol. As a relatively high concentration of taurine was necessary in obtaining a satisfactory separation (see below), it was only possible to test up to 12% 2-propanol. For further studies, a concentration of 10% 2-propanol was chosen, as good separation here was obtained within a reasonable period of time and coelution of the last analytes with the peak caused by buffer constituents was avoided.

*NaCh concentration*

The increase in *MT* and *RMT* with increasing NaCh concentration from 10 to 75 m*M* was most pronounced for the compounds appearing late in the electropherogram. *NA* was nearly unaffected. Appreciable improvement of $R_s$ was seen for **3–4** with increasing NaCh concentration, whereas *N* changed in a non-systematic way.

An increase in *MT* values of the compounds late in the electropherogram with increasing NaCh concentration is anticipated due to increasing the phase ratio, *i.e.* the ratio of the volume of micellar phase to that of the aqueous phase. Moreover, alterations in the EOF may contribute to the effect observed [24]. With a CMC of NaCh at 13 m*M* [33], the coelution of analytes found when 10 m*M* NaCh was used, indicates the necessity of micelles for separation of aromatic choline esters. Increasing concentration to 25 m*M* NaCh gave a good baseline separation but too narrow separation between the two groups of benzoic and cinnamic acid choline esters. Additional increase of NaCh concentration gave improved separation of the benzoic acid derivatives from the cinnamic acid derivatives as also reflected in $R_s$ for **3–4** and for further studies 50 m*M* NaCh was chosen. The influence of NaCh concentration on *N* and $R_s$ was probably due to changes in the micellar concentration and structure, the interaction of compounds depending on the properties of the individual aromatic choline esters.

*Taurine concentration*

*MT* and *RMT* were slightly decreased with increasing taurine concentration (200–600 m*M*). *NA* for **3** and **7** was largely reduced, when taurine content in the buffer was increased from 200 to 400 m*M*, whereas only weak decreases were seen for the other compounds. From 400 to 600 m*M*, *NA* was nearly unaffected. Changes in *N* with increasing taurine concentration was remarkable for **7**, which more than doubled from 200 to 400 m*M* taurine. Other changes were only small and non-systematic. $R_s$ only increased for **3–4** with increasing taurine concentration, indicating a more effective separation between cinnamic and benzoic acid derivatives without changing the separation of compounds within each group.

The importance of having a zwitterion included in the system was shown from results without taurine added to the buffer, giving very late elution and poor separation of analytes. The effect on *MT* with increasing concentration of taurine is probably a combination of reduced association of the positively charged aromatic choline esters with the negatively charged capillary wall and decreased EOF due to the zwitterion associated with the capillary wall and to some extent higher ionic strength of the buffer [33]. A possible explanation for the large reduction in *NA* for **3** and **7** could be decreasing response factors at low taurine concentration. The response factors of aromatic choline esters vary also in HPLC according to the individual compounds and separation conditions [34]. The effect of taurine on the viscosity seem not to be appreciable, as no general effect on the *NA* values was found.

Separation efficiency are affected by changes in the interaction between solutes and micellar phase, which are thought to occur as a result of altered micellar properties, and taurine may affect the binding of counterions to the cholate micelles and hereby change CMC. Moreover,

the CMC value as well as the aggregation number of micelles may be affected by the high ionic strength caused by the added zwitterion [30,35]. 600 m*M* taurine was chosen for further studies due to the short *MT* and high *N* and $R_s$.

*Phosphate concentration*

A positive relationship was found between *MT* for compounds late in the electropherogram and increasing concentration of phosphate in the buffer (pH = 8.0). *RMT* was nearly unaffected by increased phosphate concentration. The *NA* values remained relatively constant for the different aromatic choline esters except for **3** which got greatly reduced *NA* by increasing phosphate concentration from 100 to 150 m*M*. *N* and $R_s$ were improved with increasing electrolyte concentration except for **5**, where *N* decreased a little.

A change in buffer composition to 50 m*M* phosphate plus 50 m*M* borate giving 100 m*M* of electrolyte resulted in poorer baseline separation, especially for the cinnamic acid derivatives. It was furthermore observed, that less efficient separation was obtained when adjusting the pH to 8.0, instead of using an unadjusted buffer with a pH around 7.3. To avoid heat damage caused by a high electrical current when 150 m*M* phosphate was used, the following separations took place with 100 m*M* phosphate in the buffer.

*pH in separation buffer*

Changing the pH from 6.0 to 8.0 resulted in faster migration of analytes. The decrease in *MT* was highest for the cinnamic acid derivatives, indicating that altered interaction with the micellar phase was of importance. Differences in *RMT* for the two groups of derivatives were reduced as the pH was increased. *NA* was unaffected except for **3**, which showed a considerably increase when going from pH 7.0 to 8.0. An increase in pH resulted in increased *N* although the number of theoretical plates for **6** decreased strongly when changing pH from 7.0 to 8.0. On the other hand, the best $R_s$ was achieved at the lowest pH, where the separation gave complete baseline separation for all of the compounds considered. However, the separation buffer with low pH was unstable, resulting in precipitations of cholic acid after only few runs.

An investigation was made on this stability problem, using a buffer system containing 50 m*M* NaCh, 600 m*M* taurine, 100 m*M* phosphate and 10% 2-propanol with different pH increasing 0.2 units from 6.0 to 6.8 (both included). The results showed that only buffers with pH above 6.8 were stable. The explanation for this has to be found in the $pK_a$ value of 6.4 for NaCh. As pH in the buffer approaches $pK_a$ for NaCh, protonization of the carboxyl groups will occur. Cholate hereby becomes uncharged and hence more hydrophobic resulting in precipitation. To avoid this problem, pH of the buffer has to be above 6.8.

*Voltage*

In accordance with theory, increasing the voltage from 15 to 25 kV reduced *MT* considerably, but this had only little effect on *RMT*. *NA* was nearly unaffected. *N* changed in a non-systematic way, though giving the highest values at 15 kV. $R_s$ was only affected for separation of **3–4** and **5–6** with the best results at 20 kV. The influence of voltage on *N* and $R_s$ values has been discussed further in Bjergegaard *et al.* [16]. An acceptable separation was obtained using a voltage of 20 kV.

*Temperature*

*MT* was reduced in a non-linear way as the temperature increased, the reduction in *MT* being lowest at high temperatures. The same phenomenon was obtained analysing flavonoids in a similar buffer system containing NaCh [36]. *RMT* remained unchanged. A reduced viscosity of the solvent in the capillary caused by higher temperature explains the overall faster analysis. Although attempts were made to avoid evaporation, *NA* increased as temperature rose (Fig. 4). Changes in *NA* for cinnamic and benzoic acid derivatives were as discussed previously [16]. *N* was increased for **6** at temperatures above 30°C, whereas a slight decrease was seen for the other compounds tested. $R_s$ was unaffected except for **3–4**, where a decrease was seen with increasing temperatures. Changed separation efficiency at higher temperatures may be explained by

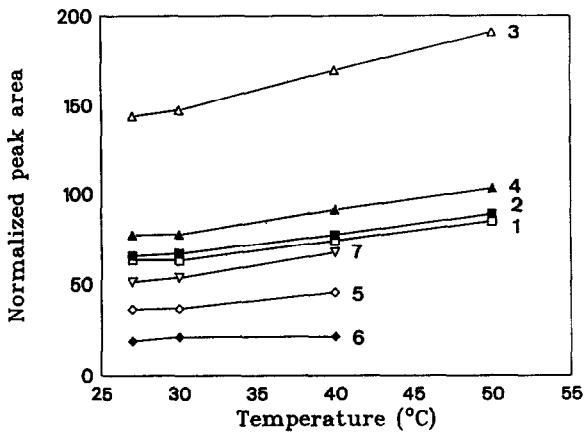



Fig. 4. Relationship between temperature and normalized peak areas of aromatic choline esters. Numbers as in Fig. 1. Other conditions as in Fig. 3.

changed interactions of analytes with the micellar phase [30]. A temperature of 30°C was chosen for the separation of aromatic choline esters, as it resulted in an acceptable separation and total time of analysis.

*Adjustment of the buffer system*

As a final adjustment of the buffer system, small changes in combinations of NaCh and 2-propanol concentration were performed. Moreover, taurine concentration was lowered from 600 to 500 m*M*. With negligible variations, it was possible to affect separation efficiency markedly. The optimal buffer solution found gave baseline separation of the six structural closely related aromatic choline esters in the test mixture within a detection range of less than 2 min and a total time of analysis about 10 min (Fig. 5). The numbers of theoretical plates per meter of capillary under the chosen separation conditions were 287 000 (**2**), 233 000 (**3**), 403 000 (**4**), 485 000 (**5**), 388 000 (**6**) and 281 000 (**7**), respectively. $R_s$ values for **2–3**, **3–4**, **4–5**, **5–6** and **6–7** were calculated to be 5.0, 8.0, 6.7, 3.3 and 1.5, respectively.

*Repeatability*

Determination of the repeatabilities of *MT*, *RMT*, *NA* and relative normalized peak area (*RNA*) values (trigonelline amide as reference compound) were performed in the adjusted

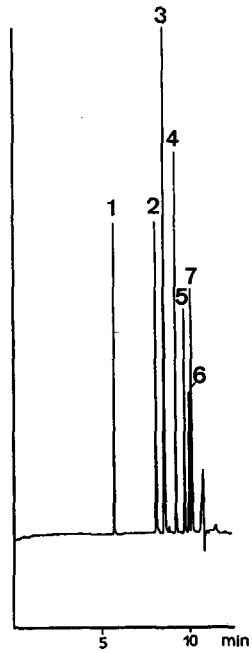



Fig. 5. Electropherogram of the mixture of aromatic choline esters separated by the MECC method (NaCh). Numbers as in Fig. 1. Buffer composition, 100 m*M* disodium hydrogenphosphate–500 m*M* taurine–35 m*M* NaCh–2% 2-propanol. Other separation conditions as in Fig. 3.

buffer system (see above) with the test solution used for variation of separation parameters. Results are shown in Table I.

The experiments were done by changing the buffer at the inlet side between each analysis and at the outlet side after five analyses. Uncertainty caused by evaporation from sample vials during the test [15] was minimized using anti-evaporation septa on the vials. Under the conditions mentioned, the instrument performed very well with respect to repeatabilities. When *RMT*, *NA* and *RNA* values were used compared to *MT* and uncorrected peak areas, the repeatabilities expressed as R.S.D. values were reduced considerably.

*Linearity*

The linearity was determined from 2 different tests. Test 1 determined the correlation between injection time of a mixture of six different aromatic choline esters and the corresponding *NA* values. Test 2 determined the correlation between increasing concentrations of the same

TABLE I

RELATIVE STANDARD DEVIATION (R.S.D.) OF MIGRATION TIMES (*MT*), RELATIVE MIGRATION TIMES (*RMT*), NORMALIZED PEAK AREAS (*NA*) AND RELATIVE NORMALIZED PEAK AREAS (*RNA*) FOR AROMATIC CHOLINE ESTERS

Separation conditions as in Fig. 5. Numbers in bold are aromatic choline ester numbers (see Fig. 1). For all calculations $n = 8$.

| Aromatic choline ester | Relative standard deviation (%) | | | |
|---|---|---|---|---|
| | *MT* | *RMT* | *NA* | *RNA* [a] |
| **2** | 1.44 | 0.38 | 1.68 | 1.02 |
| **3** | 1.51 | 0.45 | 1.73 | 0.64 |
| **4** | 1.59 | 0.54 | 1.67 | 0.66 |
| **5** | 1.67 | 0.63 | 1.31 | 1.11 |
| **6** | 1.71 | 0.66 | 1.60 | 2.43 |
| **7** | 1.73 | 0.68 | 1.55 | 1.03 |

[a] Relative to trigonelline amide (**1**)

solution as used in test 1 and the corresponding *NA* values. The results from both tests are shown in Table II.

Both tests showed good linearity with correlation coefficients ranging from 0.9950 to 0.9984. The linear increase in *NA* with increasing injection time as well as increasing concentrations of aromatic choline esters injected shows, that the method now developed may be used to quantitate aromatic choline esters. However, this provides use of an internal standard as trigonelline amide and response factors determined from the results obtained when testing the linearity.

*Detection limits*

Approximate detection limits have been determined from a signal-to-noise ratio of 2:1 using various dilutions of the aromatic choline ester stock solution. The detection limits found here correspond to the conditions applied, which were a 1-s sample injection into a 760 mm long capillary with an internal diameter of 50 $\mu$m. Detection limits were between 16 and 49 $\mu M$ of each aromatic choline ester in the sample. According to Harbaugh *et al.* [37] and Vinther [38], the injected volume was calculated to be 4.28 nl, assuming a viscosity in the buffer and sample identical to water and with a capillary temperature of 30°C. This results in detection limits between 69 and 282 fmol for each of the aromatic choline esters, which again corresponds to 26–60 pg.

*MECC of samples from plant materials*

Aromatic choline esters were isolated from seeds of *Hesperis matronalis* L. and *Sinapis alba* L. by the procedure described elsewhere [10], but with the use of a vacuum system (Supelco) for the group separation. Thereby, the group

TABLE II

LINEARITY TESTS

Correlation coefficients ($r^2$) from linear regression analyses by least squares method of normalized peak areas (*NA*) for various injection times and various concentrations of aromatic choline esters, respectively. Conditions as in Table I and Fig. 5.

| Aromatic choline ester | Test 1 | | Test 2 | |
|---|---|---|---|---|
| | Injection time (s) | $r^2$ | Concentration range (m$M$) [a] | $r^2$ |
| **2** | 1–9 | 0.9970 | 0.049–1.977 | 0.9968 |
| **3** | 1–9 | 0.9979 | unknown | 0.9971 |
| **4** | 1–9 | 0.9968 | 0.023–0.921 | 0.9982 |
| **5** | 1–9 | 0.9970 | 0.017–0.663 | 0.9984 |
| **6** | 1–9 | 0.9950 | 0.008–0.325 | 0.9976 |
| **7** | 1–9 | 0.9957 | 0.022–0.863 | 0.9980 |

[a] $n = 9$.

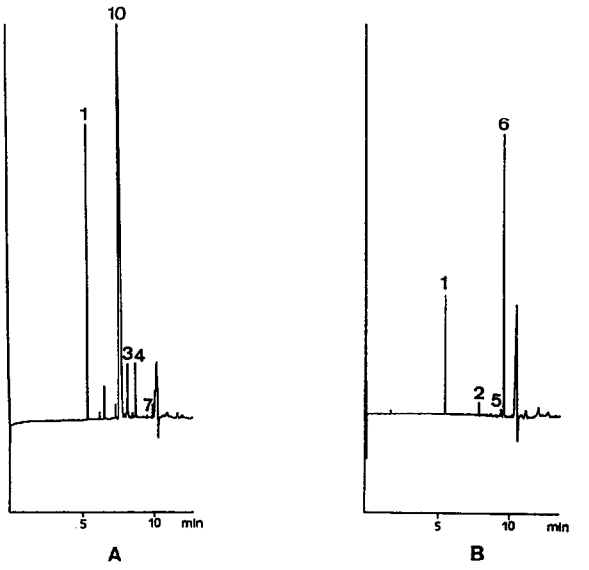



Fig. 6. Electropherogram of the aromatic choline esters accumulated in seeds of *Hesperis matronalis* L. (A) and *Sinapis alba* L. (B). Numbers as in Fig. 1. Separation conditions as in Fig. 5.

separation and purification were reduced in time to only a few minutes. A sample of the eluate (500 $\mu$l) was evaporated to dryness and redissolved in water. This solution was used directly for the MECC, resulting in separations of individual aromatic choline esters as shown in Fig. 6. The individual aromatic choline esters in the sample have been identified previously (see above), and as revealed from the electropherograms, it is easy to detect both the quantitatively dominating and minor components in the samples by the applied method.

## CONCLUSIONS

Analysis of aromatic choline esters with SDS as the detergent was unsuccessful, probably because of too strong ionic interaction between the positively charged analytes and the negatively charged capillary wall. Use of a positive detergent system, here TTAB, reduced these problems and provided a method which, however, showed much poorer and longer lasting separations compared to the NaCh system tested. Therefore, the MECC technique performed with a bile salt as the surfactant and taurine as the zwitterion is recommended. This system was proven to be highly effective for the separation of the individual aromatic choline esters, having a polar as well as an apolar portion.

The method now developed can be used for qualitative as well as quantitative analysis. Relative migration times are to be known for identification, providing an internal standard to be present in the samples. For quantitation, internal standard and normalized peak areas shall be used in combination with the use of an anti-evaporator on the samples and changing of the buffer at the inlet side preferably after each analysis and at the outlet side after 5 analyses. Taking these precautions, a very reproducible, effective (up to 485 000 theoretical plates per meter) and quick (10 minutes) analysis is provided.

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