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Short communication

Quantitative determination of organic solvents by capillary electrophoresis using indirect UV detection

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Abstract

A sodium dodecyl sulphate (SDS)-based micellar electrokinetic capillary chromatography method is reported for the quantitative determination of a range of simple organic solvents employing indirect UV absorbance detection. Veronal buffer is used to provide both pH buffering and the background UV signal for indirect detection. The concentrations of both SDS and veronal buffer were optimized using an experimental design approach.

The method was shown to give acceptable performance in terms of various performance parameters including selectivity, linearity, precision and detection limits. The method was succesfully applied to the determination of the ethanol content in pharmaceutical formulations and alcoholic beverages. Good correlation was obtained between the CE results and the label claims. The method has several features including simplicity, short analysis time and robustness.

1. Introduction

The levels of organic solvents in various samples may be quantified by a variety of analytical techniques [1]. The most widely used of these techniques is GC which is very sensitive and allows detection of trace levels. However, problems of capillary fouling and blockages can occur when samples are presented which have complicated matrices. Therefore, extensive sample pretreatment may be required prior to GC analysis.

The capillary electrophoresis technique of micellar electrokinetic capillary chromatography (MECC) has been shown to resolve mixtures of aliphatic alcohols using indirect UV detection [2] or indirect fluorescence detection [3]. These reports highlighted the potential application of CE to this area. However, the separations could not be routinely applied to quantitative solvent determinations as there was insufficient resolution of the solvents from each other and from the peak due to water.

In the method reported here solvents are detected by virtue of indirect UV absorbance. This is a commonly employed detection scheme in CE as analytes with little or no chromophore are often analysed. For instance, alkylsulphate surfactants have been determined by CE employing veronal (barbitone) buffer to maintain an appropriate high pH and to provide sufficient background UV absorbance to allow indirect detection [4].

The objectives of this work were to extend previous methodology to achieve increased resolution of the organic solvents and to assess the

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analytical performance of the method for quantitative analyses.

2. Experimental

A Beckman P/ACE 5000 (Fullerton, CA, USA) was employed for CE analyses, connected to a Hewlett-Packard HP1000 data handling system (Bracknell, UK). Capillaries were purchased from Composite Metal Services (Harlow, UK). All results were calculated using integrated peak areas. The polarity of the detector output was reversed to give apparently positive peaks making automated integration easier.

Both experimental designs were generated, and statistical analysis of the data was performed, using Design Ease (version 2.07) and Design Expert (version 3.05) software (Stat-Ease, Minneapolis, MN, USA).

2.1. Chemicals

Organic solvents were purchased from Rathburn (Walkerburn, UK) and BDH (Poole, UK). Barbitone buffer (5,5-diethylbarbituric acid) and sodium dodecyl sulphate (SDS) were obtained from Sigma (Poole, UK).

The separation conditions are as follows. Rinse 1: 0.5 min with 0.1 *M* NaOH; rinse 2: 0.5 min with electrolyte; set temperature: 30°C; detection: indirect UV at 230 nm; injection: 2 s pressure; separation: +5 kV; capillary: 27 cm × 50 μm.

The capillary was rinsed for 20 min with 0.1 *M* NaOH to rehydrate the surface prior to the first injection. The capillary was maintained only for use with this application.

3. Results and discussion

3.1. Method development and optimisation

The earlier reports of the separation of organic solvents by MECC [2,3] employed relatively high SDS concentrations in the range 100–150 m*M* and conventional length capillaries. Use of higher SDS concentrations would be impractical under these conditions due to excessive heating which would be generated within the capillary. In this study higher SDS concentrations, up to 300 m*M*, were employed as a combination of both a lower voltage (5 kV) and a shorter capillary (27 cm) were employed to minimise heating without unduly extending analysis time.

Barbitone (or veronal) buffer was employed to provide both pH buffering and high UV background. This buffer gives a high and reproducible electroendosmotic flow. The natural pH of this buffer is pH 9.5, no pH adjustment of any electrolyte solutions was performed. Therefore the effect of pH was not investigated in this study.

The initial stage of the method development was to optimise both the SDS and barbitone concentrations and the operating temperature. Given the number of combinations of buffer and SDS concentrations and temperature, it was decided to employ an experimental design procedure to reduce the number of experiments. A number of reports [5–10] have shown use of experimental designs on the optimisation of CE methods. The designs reported to-date include Plackett–Burman [5,6] and overlapping resolution mapping [7–10]. Another suitable design is a central composite [11] which allows evaluation of a method over a range to produce appropriate response surfaces. Central composite designs are particularly useful in the robustness of methods to small deliberate changes in method parameters. These designs are widely used in evaluation of HPLC methods [12] and have been used in an evaluation of the robustness of a CE method [11]. A central composite design was used to evaluate the effect of temperature and both electrolyte and SDS concentration during the optimisation of the separation.

The method conditions were explored in the ranges of 25–35°C for temperature, 12–28 m*M* barbitone and 150–250 m*M* SDS. The central composite design required 20 experiments, conducted in duplicate, covering a range of [SDS], [barbitone] and temperature combinations. The flexibility of controlling the entire CE instrument using a personal computer allowed this to be

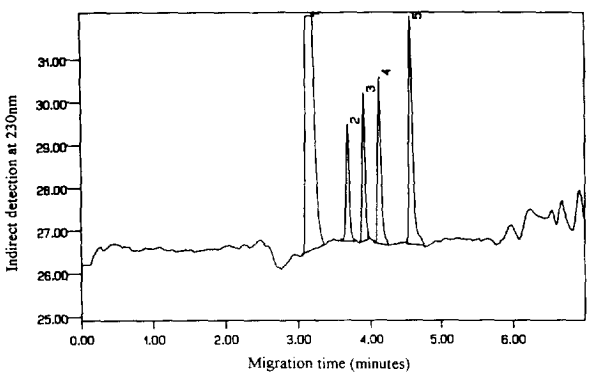

Fig. 1. Separation of 1% (v/v) test mixture of methanol, ethanol, acetone and 2-propanol. Separation conditions: as in the Experimental section using 200 m*M* SDS with 20 m*M* barbitone, 30°C. Peaks: 1 = water; 2 = methanol; 3 = ethanol; 4 = acetone; 5 = 2-propanol.

conducted in an overnight sequence. The test sample employed was a 1% (v/v) aqueous solution of methanol, ethanol, 2-propanol and acetone. Fig. 1 shows the separation achieved at the mid-point value of the ranges explored i.e. 30°C, 20 m*M* barbitone and 200 m*M* SDS. Peak 1 is due to water and is also obtained from analysis of a blank solution. A minimum resolution criteria of 2.0 was applied to ensure baseline resolution of all components. Statistical analysis showed that temperature had no significant effect on resolution in the range evaluated. Fig. 2 shows a response surface for the resolution between methanol and ethanol to be most critical to [SDS] requiring SDS concentration of greater than 180 m*M* to achieve the resolution requirement of 2.0.

The following limits were applied to the method: SDS concentration 200 m*M* ± 10; barbitone buffer concentration 20 m*M* ± 2; temperature 30°C ± 5. Higher SDS concentrations resulted in improved resolutions but with longer analysis times. Variation in buffer temperature, or SDS concentrations, had no significant effect on either peak height or areas.

3.2. Detector wavelength

The detection wavelength was varied and assessed at 200, 214, 230, 254 and 280 nm (being

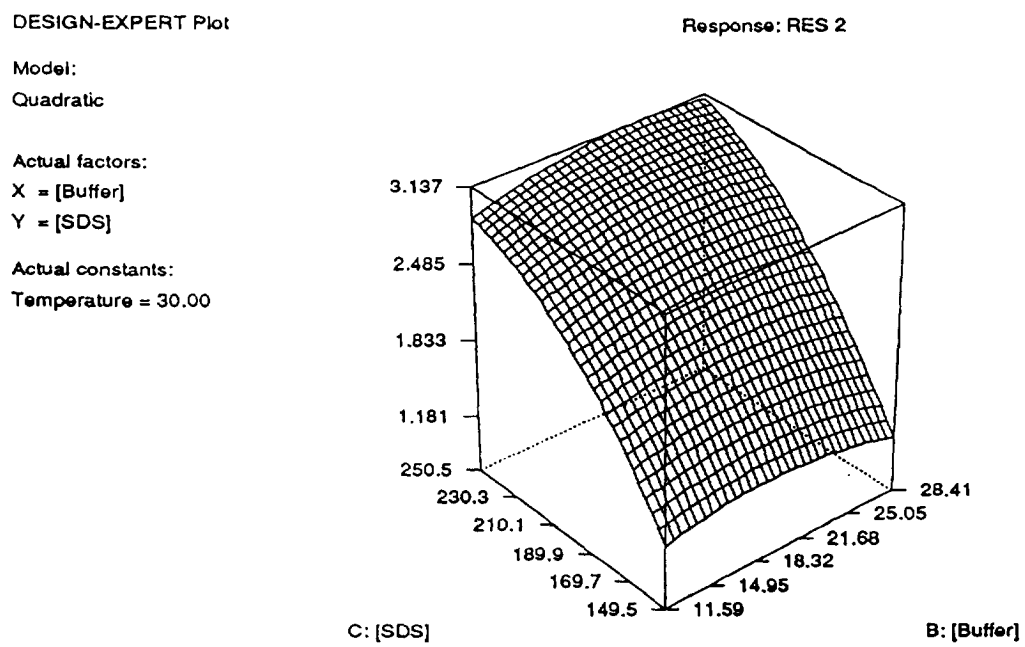

Fig. 2. Response surface plot for the resolution of methanol and ethanol with changes in [SDS] and [buffer].

the wavelength filters available on the particular CE instrument employed). No peaks were observed at 280 nm, whilst smaller peaks were seen at 200, 214 and 254 nm. Therefore 230 nm was selected as the wavelength for indirect detection in the final method.

3.3. Operating voltage

Voltage was assessed between +3 to +7 kV. Operation at +7 kV produced a considerably faster analysis (peak 5, 3.3 min) at the expense of reduced resolution and increased baseline noise. Operation at +3 kV produced increased resolution with longer analysis times (peak 5, 7.8 min). Therefore, the earlier value of +5 kV was employed in all further studies.

3.4. Peak identification

The range of solvents of interest was extended to methanol, ethanol, 2-propanol, 1-propanol and acetone. It is also necessary to confirm the migration position of butan-1-ol which is a likely interferent. A test mixture containing 1% (v/v) of each of the solvents in water was prepared and analysed (Fig. 3). Aliquots of this test mixture were spiked with 1% (v/v) of each individual solvent to confirm identity. The migration order was confirmed as water > methanol > ethanol > acetone > 2-propanol > 1-propanol > butan-1-ol (last).

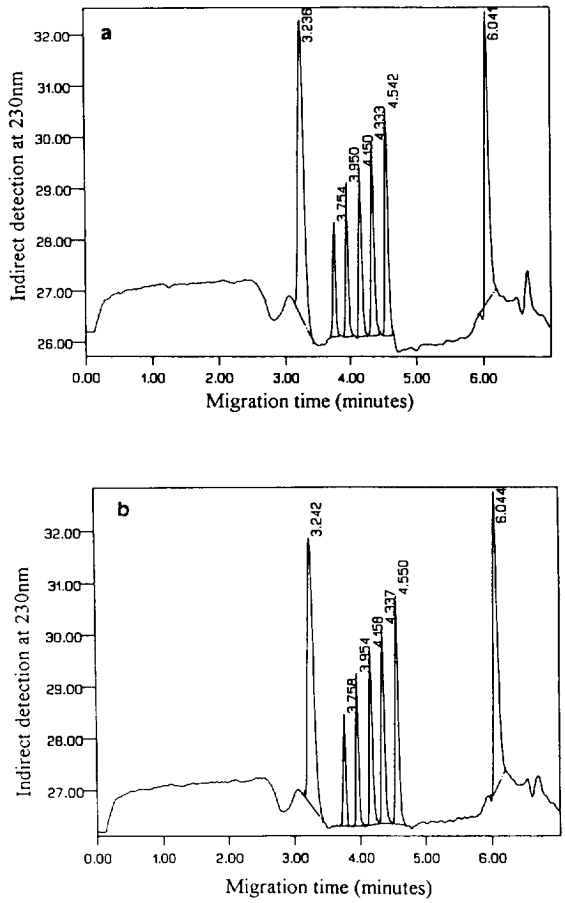

Fig. 3. Injection repeatability. (a) First injection; (b) tenth injection. Separation conditions as in Fig. 1. Migration order: water, methanol, ethanol, acetone, 2-propanol, 1-propanol, butan-1-ol.

3.5. Assessment of analytical performance

The validation criteria commonly employed in the evaluation of a CE method are similar to those tested for in HPLC [13]. Preliminary evaluation of this method included assessments of linearity, precision and detection limits.

Precision

A 1% (v/v) mixture of the six solvents in water was injected using the method settings given in the experimental section. Fig. 3 shows the first (Fig. 3a) and the tenth (Fig. 3b) injection. Table 1 shows that the migration time precision was excellent, R.S.D. values of <0.1% being obtained for all solvents, indicating good performance of the method for qualitative analysis. Peak area precision for the solvents was in the region of 2–3%. However, when using peak area ratios, precision was improved to 0.7–2.8% which supports the use of an internal standard [14]. Precision was worse for methanol as it had the smallest peak area and precision in CE is highly dependent on concentration and peak area [15,16]. This performance suggests, however, that the method is entirely suitable for quantitative determination of solvents.

Table 1
Precision of injection

	Area (R.S.D., %)	PAR (R.S.D., %)	Height (R.S.D., %)	Relative height (R.S.D., %)
Methanol	4.8	2.8	2.3	0.9
Ethanol	3.0	2.9	2.1	1.4
Acetone	2.9	1.8	2.0	1.0
2-Propanol	2.8	0.7	2.1	0.8
1-Propanol	2.4	0.7	1.6	0.8

Relative height and peak-area ratio (PAR) with respect to butan-1-ol; $n = 10$.

Linearity

Detector response was measured over the range 0.05–5% (v/v) spiking of the various solvents. Seven solutions were prepared within this range which were analysed in duplicate. Peak area linearity correlation coefficients over this range were typically 0.996. The correlation coefficients of peak height over the range 0.05–2.5% (v/v) spiking were typically 0.997. Higher spiking levels did not lead to linear increases in peak height due to peak broadening, as has been established previously [17].

Detection limits

Peaks at three times the signal-to-noise were obtained for 0.05% (v/v) standards which represents an approximate limit of detection. A 0.1% (v/v) standard was injected ten times (Fig. 4) giving average peak area ratios in the region of 5–10% R.S.D. This level represents a limit of quantitation for the solvents. It is noted that these figures are not as sensitive as GC and other techniques [1].

3.6. Applications

Many liquid pharmaceutical formulations contain levels of ethanol as a preservative. These formulations also contain a range of other excipients such as sugars and colouring agents as well as the active ingredients. These components can foul GC columns and may require sample pre-treatment. A syrup sample containing drug (at 10 mg/ml) and ethanol at 6.0% (v/v) was tested. An internal standard (2-propanol) was employed at 1% (v/v) for increased precision and to compensate for any viscosity differences between sample and standard solutions (as the injection volume in CE is related to solution viscosity). The syrup was appropriately diluted with internal standard solution and then injected directly (Fig. 5a) onto the capillary. Other peaks observed for the injection of the syrup samples are attributed to excipients in the syrup formulation. The peak due to drug migrated considerably later than the peaks of interest and did not interfere. The rinse steps between injections

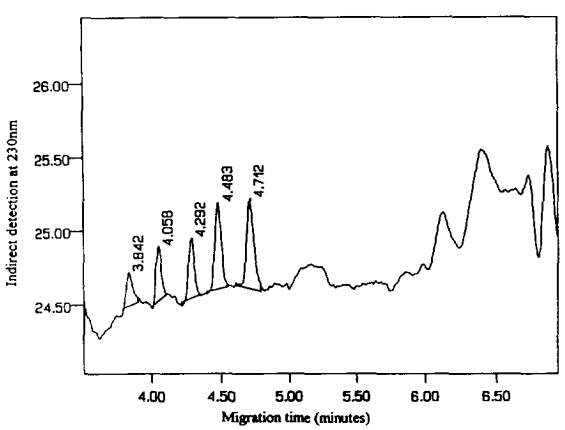

Fig. 4. Limit of quantitation (0.1%, v/v, for each solvent). Separation conditions as in Fig. 1. Migration order: methanol, ethanol, acetone, 2-propanol, 1-propanol.

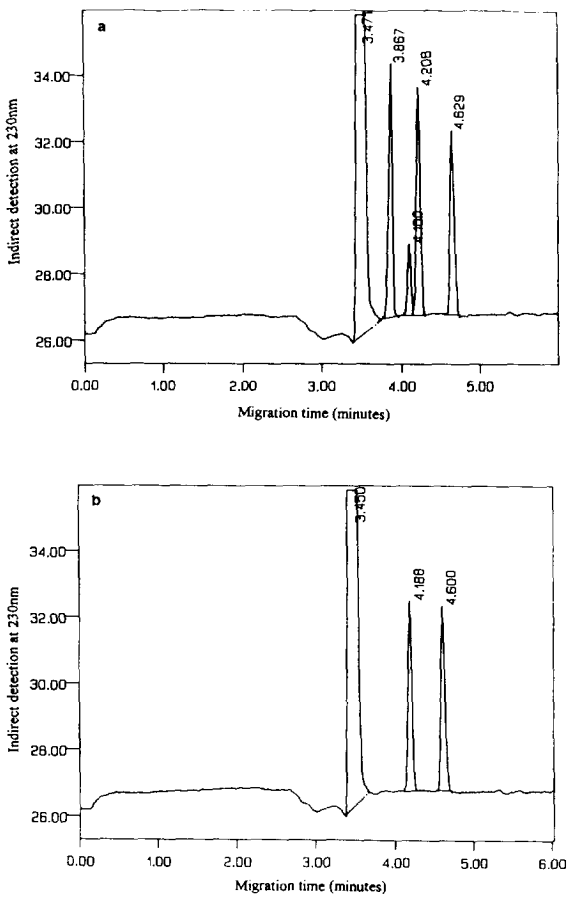

Fig. 5. Quantitation of ethanol content in syrup sample. (a) Injection of diluted syrup sample; (b) injection of a calibration solution containing 1% (v/v) of both 2-propanol and ethanol.

removed the drug and other unquantified material from within the capillary. The ethanol content was directly quantified by employing standards (Fig. 5b) containing both ethanol and 2-propanol at 1% (v/v). Results were calculated employing peak area ratios. Table 2 shows that the method gave results in line with the 6.0% ethanol label claim with good precision for migration times, relative migration times and response factors.

A further quantitative application of alcohol determination is shown in Fig. 6 which shows the analysis of alcoholic beverages. A sample of whisky (label claim of 40%, v/v, ethanol) was diluted with internal standard solution and directly injected (Fig. 6a), and no interfering peaks were observed. The results obtained (Table 2) were in good agreement with the label claim. An alcohol-free beer (<0.05% ethanol) was directly injected and confirmed as containing <0.05% alcohol (Fig. 6b), the origin of the early migrating peak(s) being unknown. This direct injection illustrates the robustness of the CE method to sample composition.

Qualitative identity confirmation is often required for input solvents in a manufacturing environment. For this testing, simple quantitative confirmation of the identity of solvent is required, and may be conventionally performed by IR and/or GC. This CE method is extremely suitable for such purposes given the good migration time and peak area precisions.

Table 2
Quantitative analysis results

	Result
Response factor ($n = 8$)	0.47% R.S.D.
MT of water ($n = 20$) (min)	3.48 (0.9% R.S.D.)
MT of 2-propanol ($n = 18$) (min)	4.65 (1.0% R.S.D.)
RMT of 2-propanol ($n = 18$)	0.35% R.S.D.
Syrup (6.0% nominal ethanol content)	(6.0, 6.1) (6.1, 6.1) average = 6.1
Whisky (40% nominal ethanol content)	(40.8, 4.12) average = 41.0%
Alcohol-free beer (<0.05% ethanol)	ND (less than 0.05%)

MT = Migration time; RMT = relative migration time; ND = not detected.

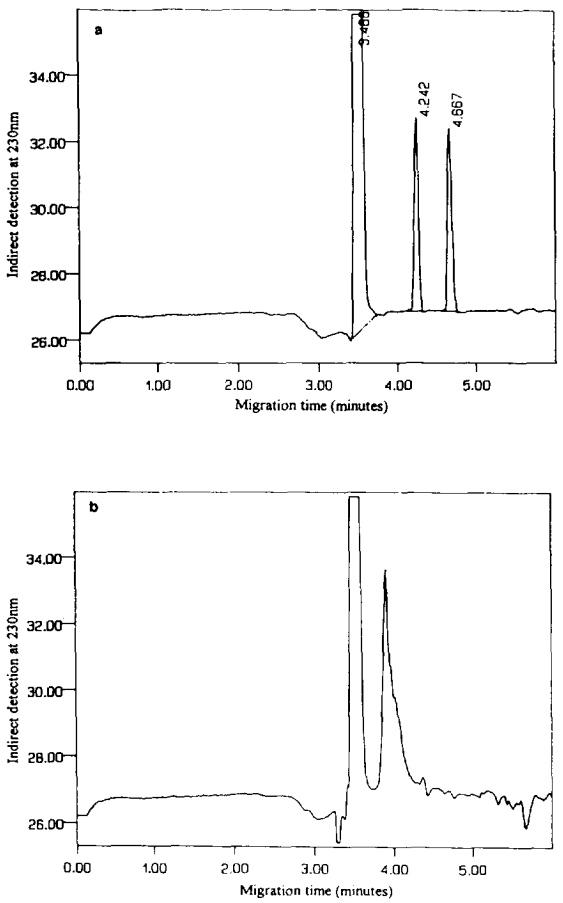

Fig. 6. Quantitation of ethanol content in alcoholic beverages. (a) Injection of diluted whisky sample; (b) direct injection of an alcohol-free beer.

3.7. Features of the method

The method is simple and robust, and the buffer can be prepared and stored for several weeks. The set-up time for the method is a matter of a few minutes resulting in quicker overall analysis times. Expenses are minimal compared to many alternatives as the reagents and consumables are very inexpensive. A particular feature is that samples of considerable complexity may be analysed with no fouling of the capillary, whereas extensive sample pretreatment may be required for other separative techniques. In addition, the testing is performed on conventional CE instrumentation using standard, inexpensive capillaries.

4. Conclusions

A MECC method has been developed and optimised for the quantitative determination of a range of organic solvents. A central composite experimental design and appropriate statistical analysis were employed in the optimisation of electrolyte composition and temperature. Acceptable performance of the method has been demonstrated in terms of precision, linearity and limits of detection. Features of the method compared to other analytical test procedures include robustness, simplicity and reductions in both overall analysis time and expense. The major disadvantage is reduced sensitivity which limits the scope of current applications.

Possible applications of the method include semi-quantitative identity confirmation of input solvents and determination of alcohol content in both liquid pharmaceutical formulations and alcoholic beverages. Undoubtedly further improvements in detector sensitivity and methodology will considerably improve the applicability and sensitivity of the method.

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