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Strategies for the monitoring of drugs in body fluids by micellar electrokinetic capillary chromatography

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ABSTRACT

Electrokinetic capillary techniques can exploit numerous separation principles, making them flexible and easily applicable to a variety of separation problems. In recent publications, this emerging technology has been shown to be well suited for monitoring drugs and metabolites in body fluids, including serum, saliva and urine. Most attention has been focused on micellar electrokinetic capillary chromatography (MECC) because it permits the separation and determination of drugs with discrimination being largely based on differences in hydrophobicity. An overview of literature data on the MECC of drugs in body fluids and recent data obtained with antiepileptics in serum and saliva, with model mixtures of illicit drugs, and with extracts from urine specimens that tested positively for opiates and cocaine metabolites are presented. Emphasis is focused on buffer selection and simple sample preparation procedures, including direct injection of body fluids, ultrafiltration and solid-phase extraction.

INTRODUCTION

A knowledge of drug levels in body fluids, such as serum, saliva and urine, allows the optimization of pharmacotherapy and provides the basis for studies on patient compliance, bioavailability, pharmacokinetics and genetics, organ function and the influences of co-medication. Furthermore, screening and confirmation of drugs in body fluids are important for the investigation of intoxications, the detection of potential users of drugs and the control of drug addicts following withdrawal therapy. Currently used methods are based on the principles of spectrophotometry, immunoassays and chromatography (for reviews, see refs. 1–3). All of these techniques have advantages and disadvantages. The reagents for many of the immunological assays are available in kit form, together with highly automated instrumentation. This permits such analyses to be performed easily, efficiently and with high sensitivity and precision. They provide the most rapid, high-sample-throughput analytical procedures available to date. However, immunological techniques are prone to disturbances by molecules of similar structure (cross-reactivity). Many antibodies involved recognize not only the drug of interest, but also some of its metabolites. Moreover, these techniques are by nature unsuited to the simultaneous monitoring of several drugs and metabolites. Chromatographic assays, on the other hand, provide specific results for multiple compounds but typically require extensive sample preparation and/or derivatization, are characterized by a low sample throughput and are difficult to automate.

Recently, instrumentation for electrokinetic separations in capillaries of very small I.D. (25–75 μm) have become available [4–8] and some papers reporting its use for drug monitoring in body fluids have appeared (Table I) [9–27]. Electrokinetic separations/analyses in capillaries should be regarded as complementary or as attractive alternatives to other capillary separation techniques, such as gas chromatography (GC), high-performance liquid chromatography (HPLC), supercritical fluid chromatography and field flow fractionation. The advantages of electrokinetic capillary analyses are high resolution, efficiency and speed, automation,

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small sample size, rapid method development, use of small amounts of inexpensive and non-polluting chemicals and simple adaptation for micropreparative work. Electrokinetic capillary techniques can exploit numerous separation principles, making them flexible and easily applicable to a variety of separation problems. The specific techniques are capillary zone electrophoresis, capillary isotachophoresis, capillary isoelectric focusing and a range of electrokinetic capillary chromatography methods [*e.g.*, micellar electrokinetic capillary chromatography (MECC)]. The differentiating features of these methods lie in the initial and boundary conditions applied, which determine the character of the migrating sample zones [5,28]. The major disadvantage of most of these techniques performed in capillaries of very small I.D. is the relatively low concentration sensitivity.

MECC, an interface between electrophoresis and chromatography, is characterized by two distinct phases, an aqueous and a micellar phase or pseudostationary phase [27,29]. These two phases are established by employing buffers containing surfactants [*e.g.*, sodium dodecyl sulphate (SDS)], which are added above their critical micellar concentration. Micelles are dynamic structures that are in equilibrium with the monomer. A MECC analysis is performed in equipment designed for capillary electrophoresis, *i.e.*, in an open-tubular capillary of very small I.D. A high-voltage d.c. electric field is applied along the column, causing both a movement of the entire liquid (the so-called electroosmotic flow) and migration of the charged micelles. As a result, the two phases migrate at different velocities, permitting chromatographic separations [27,29]. Different advantageous aspects of MECC for the determination of drugs in body fluids have been reported (Table I), including the possibility of direct application of proteinaceous samples [10–12,18,19], simple calibration for quantification with and without extraction prior to analysis [19,30] and the attractiveness of multi-wavelength solute monitoring [18–22].

In this paper, important aspects, highlights and limitations of this methodology, ranging from sample pretreatment and application to solute monitoring and quantification, are discussed. Examples given illustrate the impact of buffer variations on solute separability and elution, as well as strategies for sample pretreatment which include direct sample application, ultrafiltration and solid-phase extraction.

TABLE I

SELECTED APPLICATIONS OF MECC DRUG DETERMINATIONS IN BODY FLUIDS

Abbreviations: THC = 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid; AFMU = 5-acetylamino-6-formylamino-3-methyluracil; AAMU = 5-acetylamino-6-amino-3-methyluracil; 1X = 1-methylxanthine; 1U = 1-methyluric acid; L–L = liquid–liquid extraction; S–L = solid-phase extraction; ABS = absorbance; MW-ABS = multi-wavelength absorbance; LIF = laser-induced fluorescence; None refers to direct sample injection, but may include simple fluid handling, such as dilution, centrifugation or filtering.

Drug/metabolite	Matrix	Sample preparation	Detection method	Ref.
B_6 vitamers	Urine	L–L	LIF	15
Cefpiramide	Plasma	None	ABS	10,11
Aspoxicillin	Plasma	None	ABS	12
Cicletanine enantiomers	Plasma	L–L	ABS	16
Antiepileptics	Plasma	L–L	MW-ABS	17
Barbiturates	Serum/urine	None, L–L, S–L	MW-ABS	18
Thiopental	Serum	L–L	ABS	30
Drugs of abuse	Urine	S–L (hydrolysis)	MW-ABS	20,22
THC	Urine	Hydrolysis, S–L	MW-ABS	21
Substituted purines	Serum/saliva/urine	None, L–L, S–L	MW-ABS	19
AFMU, 1X	Urine	None	ABS	23
AFMU, AAMU, 1X, 1U	Urine	None	MW-ABS	26
Cimetidine	Serum	Electrochromatography	ABS	24
Antiepileptics	Serum, saliva	None, ultrafiltration	MW-ABS	This work

EXPERIMENTAL

Chemicals, samples and immunoassays

All chemicals were of analytical-reagent or research grade. SDS and polyoxyethylene 23 lauryl ether (Brij 35, referred to here simply as Brij) were purchased from Sigma (St. Louis, MO, USA). Methanol, 2-propanol and acetonitrile (all of HPLC grade) were from Rathburn Chemicals (Walkerburn, UK).

The drugs employed as reference compounds were of European Pharmacopoeia quality. Blank human serum was obtained by centrifugation of our own blood (1500 *g* for 10 min) and Lyphocheck TDM Control Serum Level 3, consisting of human serum spiked with more than 30 drugs, was purchased from Bio-Rad (ECS Division, Anaheim, CA, USA). Our own saliva and urine were employed as blank matrices. Samples from patients were collected in our routine drug assay laboratory where they were received for therapeutic drug monitoring or drug screening. After centrifugation, serum and saliva samples were assayed for three antiepileptics (carbamazepine, phenytoin and phenobarbital) using enzyme immunoassay techniques (EMIT) (Syva, Palo Alto, CA, USA) on a Cobas Fara centrifugal analyser (Hoffmann-La Roche, Diagnostica, Basle, Switzerland) and stored at −20°C until further analysis. Valproate levels were determined by an automated fluorescence polarization immunoassay on a TDx analyser (Abbott Labs., Irving, TX, USA). These assays for carbamazepine, phenytoin, phenobarbital and valproate are designed to determine total serum concentrations in the ranges 8–85 μM (1.9–20 μg/ml), 5–119 μM (1.3–30 μg/ml), 20–345 μM (4.6–80 μg/ml) and 5–1041 μM (0.72–150 μg/ml) respectively. Urine samples were screened for the presence of opiates and cocaine metabolites by automated enzyme immunoassay techniques (EMIT-dau) (Syva) on a Cobas Fara centrifugal analyser and stored at 4°C until further analysis. The EMIT-dau tests contain morphine and benzoylecgonine, respectively, as calibrators with a cut-off level of 300 ng/ml each. Samples that gave a response equal to or higher than that of the the calibrator were interpreted as positive.

Electrophoretic instrumentation and running conditions

The instrument with multi-wavelength detection employed was described previously [18–22]. Briefly, it featured a 75 μm I.D. fused-silica capillary of about 90 cm length (Product TSP/075/375; Polymicro Technologies, Phoenix, AZ, USA) together with a UVIS 206 PHD fast-scanning multi-wavelength detector with No. 9550-0155 on-column capillary detector cell (both from Linear Instruments, Reno, NV, USA) towards the capillary end. The effective separation distance was 70 cm. A constant voltage of 20 kV was applied. The cathode was on the detector side. Sample application occurred manually through dipping the anodic capillary end into the sample vial and lifting it *ca.* 34 cm for a specified time interval (typically 5 s). Multi-wavelength data were read, evaluated and stored employing a Mandax AT 286 computer system and running the UVIS 206 PHD detector software package version 2.0 (Linear Instruments) with windows 286 version 2.1 (Microsoft, Redmont, WA, USA).

Conditioning for each experiment was done by rinsing the capillary with 0.1 *M* NaOH for 3 min and with buffer for 5 min. Throughout this work the UVIS 206 PHD detector was employed in the high-speed polychrome mode by scanning from 195 to 320 nm at 5-nm intervals (26 wavelengths). With these settings the sampling rate was 3.69 data points per second and per wavelength.

Electrophoresis buffers and standard solutions

Unless stated otherwise, a buffer composed of 75 m*M* SDS, 6 m*M* $Na_2B_4O_7$ and 10 m*M* Na_2HPO_4 (pH $\approx$ 9.1) was employed. All standard solutions were prepared in buffer or methanol at concentrations of 100–300 μg/ml. Blank and patient samples were spiked by addition of known aliquots of these standard solutions to the body fluids prior to sample injection or extraction.

Direct injection of body fluids and removal of proteins

Serum, saliva and urine samples were either injected as received or, prior to analysis, centrifuged at 1500 *g* for 10 min and/or filtered using 0.2-μm Nalgene (25 mm diameter) disposable syringe filters (Nalge, Rochester, NY, USA). For the removal of the proteins, selected samples were ultrafiltered at

1500 *g* for 20 min using a Centrifree Micropartition System (Grace, Amicon Division, Wallisellen, Switzerland).

Extraction

Drugs of abuse in urine were extracted as described previously [20] using Bond Elut Certify cartridges and a Vac Elut set-up (both from Analytichem International, Harbor City, CA, USA). The procedure used was that for cocaine and metabolites recommended by the manufacturer of the solid-phase extraction columns. Briefly, the cartridges were conditioned immediately prior to use by passing sequentially 2 ml of methanol and an equal volume of 0.1 *M* phosphate buffer (pH 6) through the columns. The vacuum was turned off to prevent column drying. The columns were loaded by slowly drawing of a mixture of 5 ml of urine and 2 ml of 0.1 *M* phosphate buffer (pH adjusted to 5). The columns were sequentially rinsed with 3 ml of deionized water, 3 ml 0.1 *M* HCl and 9 ml of methanol. Elution was effected with 2 ml of a mixture of methylene chloride and 2-propanol (80:20) containing 2% ammonia into a test-tube. The eluate was evaporated to dryness under a gentle stream of nitrogen at room temperature. The residue was dissolved in 100 μl of running buffer, providing a maximum concentration factor (100% recovery) of 50.

RESULTS AND DISCUSSION

Selectivity in MECC is reported to be dependent on the concentration of the micelle-forming agent, the buffer pH and the use of additives, including organic modifiers and salts [27,29,31]. For the monitoring of drugs and metabolites in body fluids, the selection of the buffer configuration is dependent on the substances to be analysed. The following dis-

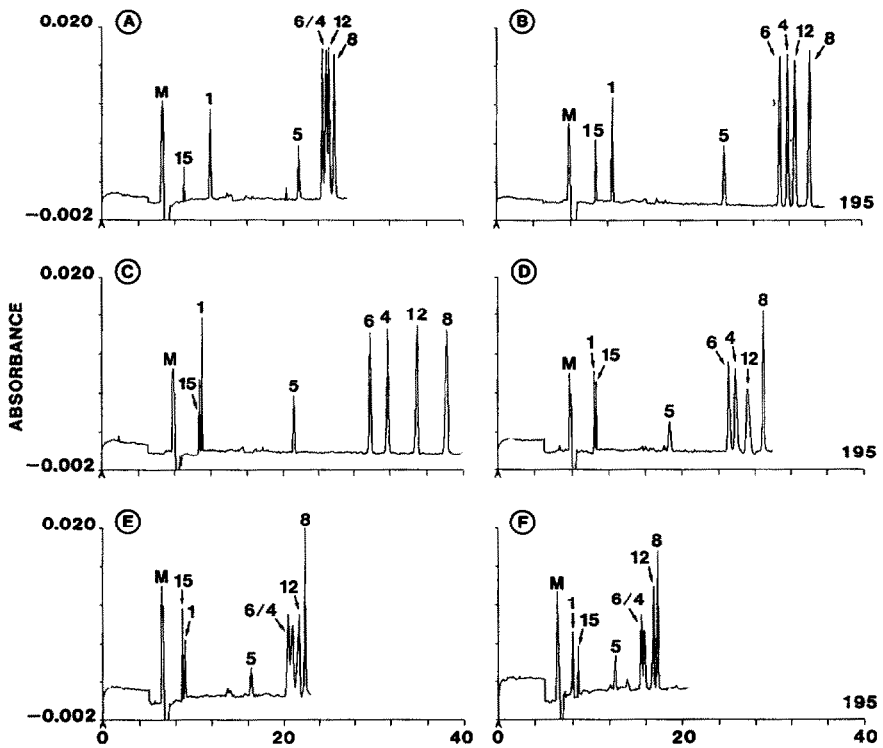

Fig. 1. Single-wavelength (195 nm) electropherograms of a model mixture (50 μg/ml of each compound) analysed in borate–phosphate buffers of pH 9.2 with (A) 75 m*M* SDS, (B) 71.25 m*M* SDS–5% methanol, (C) 71.25 m*M* SDS–5% 2-propanol, (D) 71.25 m*M* SDS–5 m*M* Brij–5% methanol, (E) 75 m*M* SDS–5 m*M* Brij and (F) 75 m*M* SDS–10 m*M* Brij. The applied voltage was a constant 20 kV in all instances (for currents, see Table II). Peaks: 1 = benzoylecgonine; 4 = methamphetamine; 6 = amphetamine; 8 = methadone; 12 = diazepam; 15 = morphine-3-glucuronide; M = methanol. The sample identification is the same as given previously [21,22].

TABLE II

IMPACT OF BUFFER MODIFICATIONS ON MECC ELUTION RANGE AND CAPACITY FACTORS OF SELECTED DRUGS

Systems 1–6 correspond to those shown in Fig. 1 and systems 7–9 are those given in [22]. Methanol and methadone served as markers for the electroosmotic flux (t_0) and micelle elution (t_{mc}), respectively. A borate–phosphate buffer with 75 m*M* SDS and pH 9.2 was employed. Buffers containing organic solvents were diluted by the content of the solvent (5 or 10%).

System	Additives	Current (μA)	t_0 (min)	t_{mc} (min)	Capacity factors of eluting compounds[a]					
					15	1	5	6	4	12
1	None	78	6.60	25.77	0.567	1.51	14.5	50.3	73.3	115
2	5% methanol	68	7.78	34.26	0.546	0.960	7.85	30.4	43.2	67.1
3	5% 2-propanol	70	7.84	38.16	0.517	0.570	3.83	12.8	18.1	40.8
4	5% methanol, 5 m*M* Brij	74	7.71	28.90	0.597	0.540	4.02	17.1	22.4	42.2
5	5 m*M* Brij	88	6.50	22.36	0.566	0.656	5.64	25.1	34.7	76.8
6	10 m*M* Brij	90	6.38	17.32	0.699	0.472	3.62	13.4	17.2	64.8
7	None	77	6.81	23.24	–	1.68	17.3	63.3	81.5	133
8	5% acetonitrile	72	7.69	33.70	–	0.854	6.86	27.0	36.8	58.9
9	10% acetonitrile	72	7.94	45.85	–	0.468	2.94	10.1	14.2	24.5

[a] Capacity factors were calculated by $k' = (t - t_0)/t_0(1 - t/t_{mc})$, where t is the elution time of the compound [27].

cussion is intended to illustrate the impact of buffer changes on drug separation and elution.

Typical electropherograms obtained with a model mixtures composed of morphine-3-glucuronide, benzoylecgonine, codeine, amphetamine, methamphetamine, diazepam and methadone are presented in Fig. 1 and corresponding characterizing data with a range of modifiers are summarized in Table II. In all instances, methanol ($k' = 0$) and methadone ($k' = \infty$) were employed as marker substances for the determination of the electroosmotic flux (t_0) and micelle elution (t_{mc}), respectively. With the experimental configuration (instrument used, pH = 9.2, SDS concentration = 75 m*M*), the separation of the last four, highly hydrophobic substances is incomplete (peaks 6, 4, 12 and 8 in panel A). However, dilution of that buffer with methanol (panel B), 2-propanol (panel C) or acetonitrile (see also data in [22]) provides complete resolution and improved separation with increasing content of the organic buffer modifier. This is at the expense of increased run times, however, which is even more pronounced when the pH is lowered to 8.5 or below (data not shown). The run times can be reduced with the use of neutral co-micelles formed by Brij in addition to the organic solvent modifier (panel D). In this configuration, elution of hydrophilic substances (peaks 1 and 15) is significantly altered compared with the data presented in panel B. Addition of Brij to the buffer without the use of an organic solvent is demonstrated to reduce retention times (compare panels E and F with panel A in Fig. 1), to change the relative elution behaviour and even the order of hydrophilic compounds, and not to provide significant improvement in resolution for the hydrophobic compounds. Brij concentrations higher than 10 m*M* provided electropherograms with decreased resolution and partly ill-shaped zones (data not shown).

The data presented in Fig. 1 and elsewhere [22] illustrate the difficulty in establishing optimized MECC conditions for a range of substances and having reasonable elution time intervals. Employing small amounts of methanol, 2-propanol or acetonitrile as organic modifiers, the impact of solubilization changes on the hydrophobic side of the elution range is strongest with 2-propanol (panel C in Fig. 1). However, the changes occurring on the hydrophilic side of the elution range, *i.e.*, the range with morphine-3-glucuronide and benzoylecgonine as probes, are different. These two compounds are demonstrated to be best separated without any addition of an organic modifier (panel A), well separated with methanol (panel B) and hardly separat-

ed with 2-propanol (panel C) and the use of Brij (panels D and E). Hence optimized MECC conditions are typically established for a few compounds of interest only, a situation that typically requires a selective extraction of the drug prior to analysis or, in a general screening, the stepwise extraction followed by sequential analysis of each eluate [22].

From the data presented in Table II, it is interesting that with the addition of organic modifiers strong changes in the solubilization of the compounds but only small changes in the electroosmotic flux (characterized by t_o) are observed. Compared with the plain SDS buffer (systems 1 and 7), electroosmosis is decreased in presence of organic solvents and increased with Brij. Further, the data in Table II reveal the impact of differences in capillary conditions and buffer preparations. The two sets of data, systems 1–6 and 7–9, were obtained in different capillaries and buffer batches. Comparison of systems 1 and 7, without the addition or organic modifiers, reveals small differences in electroosmotic flow (t_o) and solute partitioning (t_{mc} and capacity factors).

On receipt of a sample, typically it has to be prepared for analysis. This stage is intended to improve the specificity of the assay by removing interfering matrix compounds whilst concentrating the analyte, to stabilize the analyte and to remove matrix particles that would block instrumental parts (*e.g.*, syringe, tubing, column). Sample preparation methods include simple liquid handling procedure (*e.g.*, centrifugation, dilution, filtering), release of the analyte from the biological matrix (*e.g.*, hydrolysis, sonication), the removal of endogenous compounds (*e.g.*, precipitation, ultrafiltration and extraction) and the enhancement of selectivity and sensitivity by analyte derivatization (a general comprehensive review of the preparation of biological samples was given by McDowall [32]). For MECC of drugs in body fluids, most of these techniques have been applied. Selected examples are listed in Table I.

Most intriguingly, MECC with dodecyl sulphate micelles was shown to allow the direct injection of proteinaceous fluids, such a serum, an approach which bears similarity to HPLC with micellar mobile phases [33]. The three-dimensional data depicted in Fig. 2A were obtained with a serum blank that was applied without any particular sample pretreatment. In that approach, the proteins are solubilized

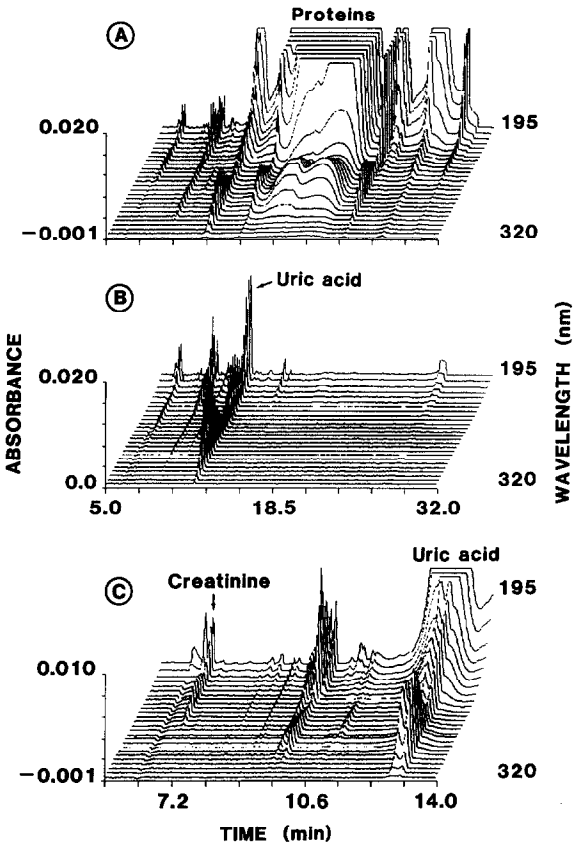

Fig. 2. Three-dimensional MECC data for blank serum obtained (A) by direct serum injection and (B) injection of the ultrafiltrate. Both electropherograms are drawn on the same time axis. (C) Section of the data in (A) with expanded time and absorbance scales. The applied voltage for this and all subsequent figures was a constant 20 kV (current *ca.* 80 μA).

by SDS and elute (essentially as a very broad zone) after uric acid. Ultrafiltration prior to sample application removes the proteins and provides a much simplified electropherogram which is shown in panel B. An almost clear electropherogram at elution time intervals after uric acid is obtained, whereas no substantial change of the pattern in front of uric acid is observed. Fig. 2C depicts an expanded section of the data of panel A, providing an improved insight into the zones of endogenous substances in front of uric acid and revealing essentially two interference-free or analytical windows bracketed by creatinine and uric acid. Serum (or plasma) levels of

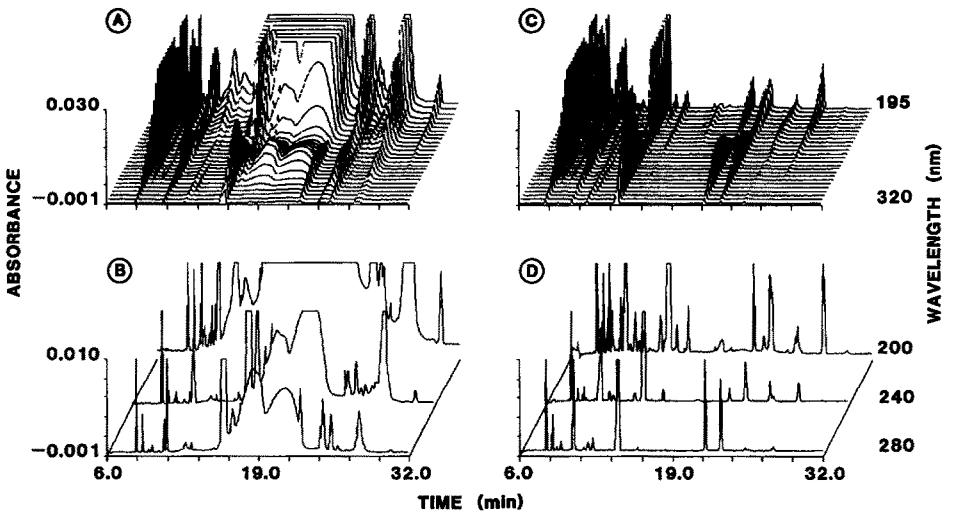

Fig. 3. Three-dimensional MECC data for a commercial blank serum spiked with more than 30 drugs obtained (A and B) with direct injection and (C and D) after ultrafiltration. Other experimental conditions as in Fig. 2.

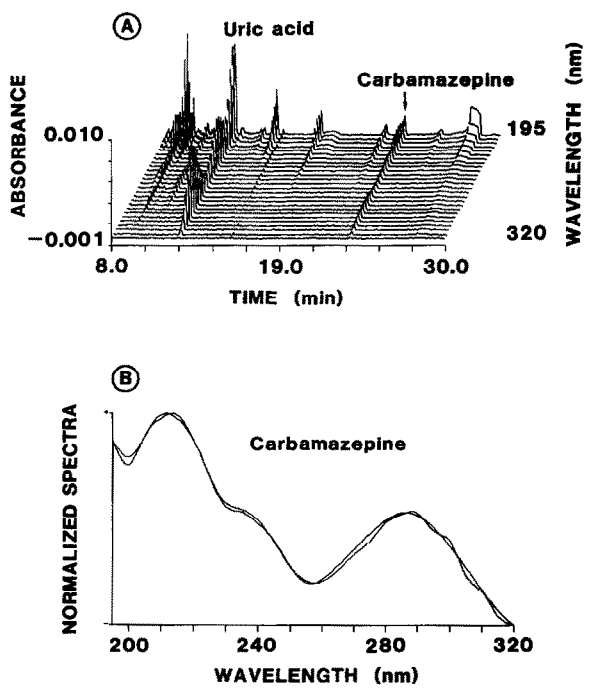

Fig. 4. (A) Three-dimensional MECC data for an ultrafiltered serum from a patient undergoing carbamazepine pharmacotherapy and (B) spectral identity proof of carbamazepine zone (for explanations, see text). Other experimental conditions as in Fig. 2.

different drugs that elute within these interference-free sections, including cefpiramide [10,11], aspoxicillin [12], phenobarbital [18], caffeine and theophylline [19], have been determined by direct sample injection.

For the determination of drugs that elute after uric acid (Fig. 2), sample pretreatment is required. The effect of the removal of proteins by ultrafiltration and MECC analysis of the filtrate is reported here for the first time. The electropherograms presented in Fig. 3 illustrate the impact of ultrafiltration employing a commercial control serum spiked with more than 30 drugs covering antiarrhytmics, anticonvulsants, antiasthmatics, antineoplastics, antibiotics, antidepressants, antipsychotics, analgesics and an immunosuppressant compound (cyclosporin) [34]. Comparison of panels A and B, obtained without any sample pretreatment, with the corresponding graphs C and D, for which ultrafiltration was applied prior to analysis, reveals the expected differences, *e.g.*, the formation of clearly formed MECC peaks after removal of the proteins. These peaks represent the analysis of free, unbound drugs.

The three-dimensional data depicted in Fig. 4A were obtained with an ultrafiltered serum from a patient undergoing carbamazepine pharmacothera-

py. A clear zone of the antiepileptic drug is produced within an elution area typically covered by interferences. Excellent agreement between the extracted normalized absorbance spectrum and that of a model run is obtained (Fig. 4B), revealing the unambiguous identification and purity of that zone. The total serum concentration of carbamazepine was determined to be 49 μM employing EMIT. Thus, MECC of ultrafiltered serum has the potential to analyse for free serum carbamazepine at a pharmacologically interesting concentration level (the therapeutic range of this compound is 15–40 μM and protein binding is about 75%) and without elaborate sample pretreatment.

The data depicted in Fig. 5, which represent the MECC serum analysis of a patient under multiple antiepileptic drug pharmacotherapy, provide, further insight into the effects of ultrafiltration of serum samples. Using the immunoassays, the investigated serum was found to contain 103 μM phenobarbital, 16 μM carbamazepine, 21 μM phenytoin and 496 μM valproate. With direct serum injection (panels A and B), the MECC data reveal a zone for phenobarbital and peaks for the two endogenous marker substances creatinine and uric acid (panel B). After ultrafiltration, carbamazepine is also monitored (panel C). Phenytoin and valproate are not detected in this assay, presumably owing to their high protein-binding capacities and hence removal through ultrafiltration.

Comparing the electropherograms in Fig. 5B and D reveals two interesting matrix effects. First, there is a change in peak shape for phenobarbital, the peak with direct serum injection being much broader than that obtained after injection of the ultrafiltrate. The zone shapes for the other compounds appear not to be affected by the pretreatment procedure. Second, the elution time intervals of all zones with serum injection are higher than those with the ultrafiltrate. These data clearly underline the need for multi-wavelength detection for identification purposes. Thus, retention (detection) times are unreliable parameters for zone assignment when samples with different matrices are considered.

Particularly in children, the requirement for venipunctures has remained a serious deterrent to routine drug monitoring. Therefore, the determination of levels of different antiepileptics in saliva has been promoted as an attractive alternative to the monitoring in blood [35,36]. As with serum, the possibilities of determining drugs by MECC employing untreated, filtered or ultrafiltered saliva was investi-

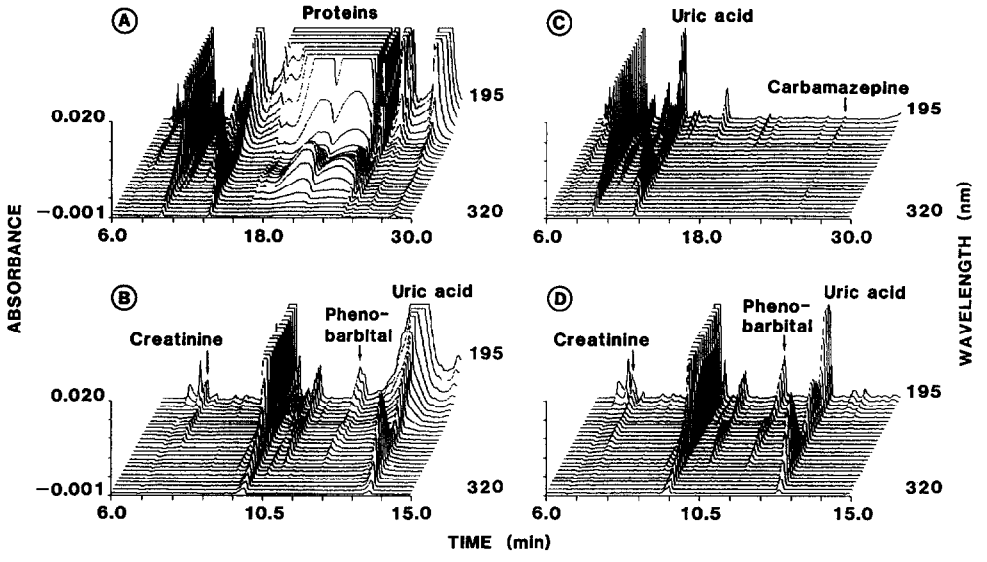

Fig. 5. Three-dimensional MECC data for a serum from a patient under multiple anticonvulsant drug therapy obtained (A and B) with direct injection and (C and D) after ultrafiltration. Panels B and D represent sections of the data depicted in panels A and C, respectively. Other experimental conditions as in Fig. 2.

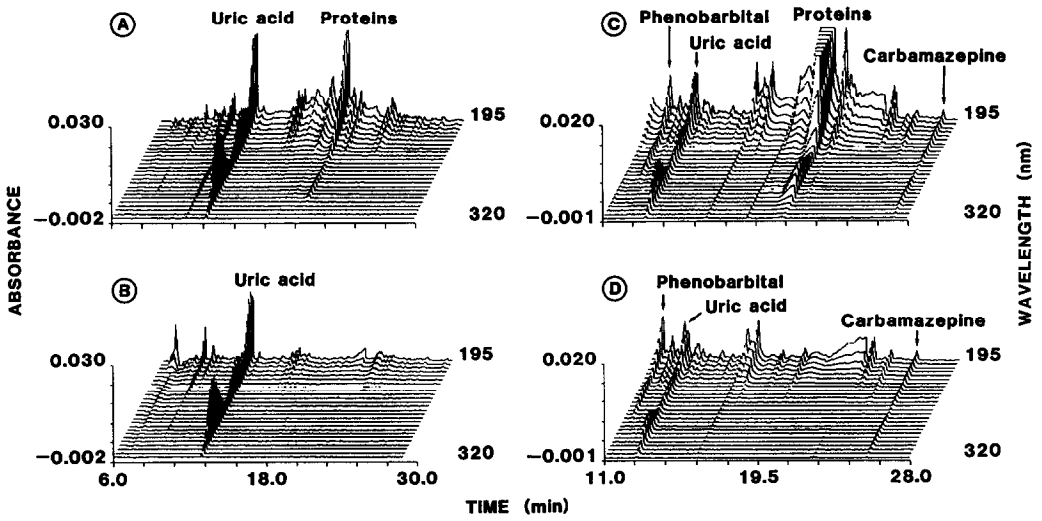

Fig. 6. Three-dimensional MECC data for a saliva blank which was (A) directly injected and (B) ultrafiltered, and (C and D) corresponding saliva data for the patient whose serum data are shown in Fig. 5. Other experimental conditions as in Fig. 2.

gated. Multi-dimensional electropherograms obtained with directly injected and ultrafiltered mixed saliva are presented in Fig. 6 A and B, respectively. The first pattern shows a much reduced protein content of saliva compared with that of serum. Again, with removal of the proteins, a simpler electropherogram is obtained, with shorter elution time intervals (matrix effect, see above). The three-dimensional electropherograms of a mixed saliva from a patient undergoing pharmacotherapy with four anticonvulsants (the same patient as for the serum data in Fig. 5) are shown in Fig. 6C and D. These data were obtained with a filtered and ultrafiltered sample, respectively. Both penobarbital and carbamazepine are monitored in both instances. Saliva proteins and carbamazepine were found not to interfere, which makes ultrafiltration for the determination of this drug in saliva unneccessary. Employing the immunoassays developed and calibrated for serum samples, the drug levels of phenobarbital, carbamazepine, phenytoin and valproate were determined to be 38, <8, 0 and 5 μM, respectively. Thus, MECC of directly injected saliva has the potential to analyse for phenobarbital and carbamazepine at pharmacologically interesting concentration levels.

Similar findings were previously found to be true for caffeine [19]. It is generally known, however, that saliva drug levels are lower than those in serum [35,36], which limits the widespread use of this approach. It should be clearly noted that with on-column UV absorption detection, direct injection of a body fluid requires that drug concentrations be at or higher than the μg/ml (μM) level.

Direct injection of urine has been shown to provide complex electropherograms within the first half of the elution range [19], making unambiguous identification of zones and complete separation difficult. Careful selection of the buffer allowed the determination of metabolites of caffeine by MECC with direct injection [23,26]. However, for most drug determinations within the first half of the elution range, and for all compounds at the lower μM (or below) level, sample extraction is required. Both liquid–liquid and solid-phase (solid–liquid) extraction schemes have been applied to a number of drug determinations (see Table I). These two approaches are attractive for two reasons. First, they are typically selective, thereby greatly simplifying the sample matrix, and second, the analyte can simultaneously be concentrated by one to two orders of magnitude. Owing to the relatively high sample concentrations required for electrokinetic capillary analysis, the latter effect is very important. On the other

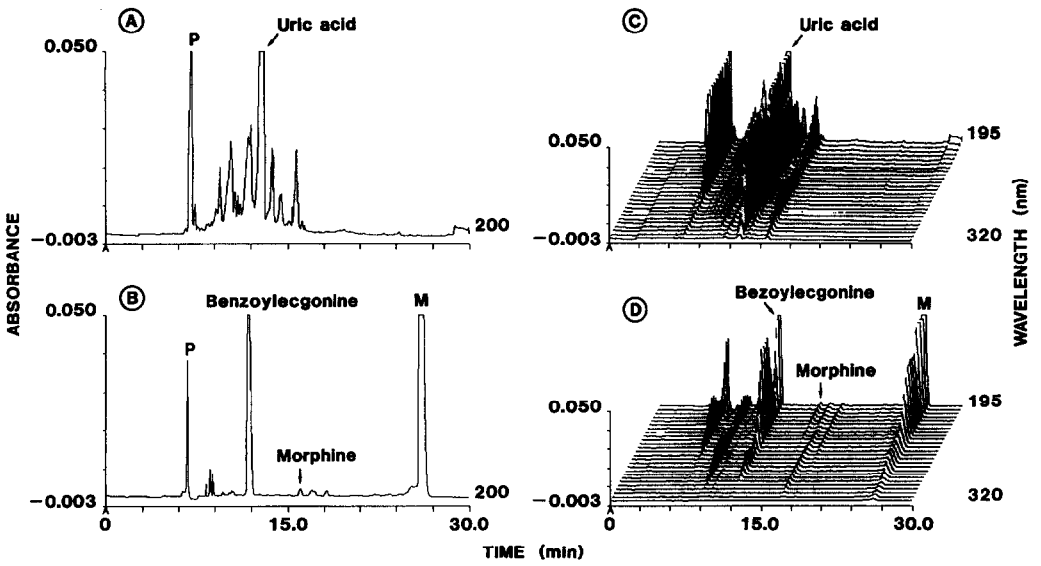

Fig. 7. MECC analysis of an opiate and cocaine-positive urine specimen after (A and C) direct injection and (B and D) solid-phase extraction. P and M refer to initial and micelle peaks, respectively. Other experimental conditions as in Fig. 2.

hand, liquid–liquid and sometimes also solid-phase extractions are time consuming. In most of the applications reported so far, standard approaches were employed. Liquid–liquid extractions were executed in glass tubes and solid-phase extractions with disposable cartridges, processes which are standard practice in chromatography [32]. Recent investigations mention the in-column use of solid-phase material for the on-line preconcentration of the analyte [37] and the application of solid-phase cartridges for electrochromatographic extraction [24].

As an example, MECC confirmation analysis data for a urine specimen that tested markedly positive for cocaine and opiates using EMIT-dau procedures are depicted in Fig. 7. Direct injection of the urine provided an electropherogram in which uric acid could be identified only (panels A and C). After solid-phase extraction as described previously [20], the MECC determination of benzoylecgonine and morphine in that sample was possible (panels B and D). Comparison of the electropherograms in panels A and B reveals the much reduced matrix is obtained through extraction. The presence of benzoylecgonine (cocaine metabolite) and morphine was unambiguously confirmed by spectral comparison of the background-corrected, normalized absorbance spectra of the peaks eluting after 11.8 and 16.0 min, respectively, with the computer-stored spectra of a model run (data no shown). Solid-phase extraction (time required *ca.* 0.5 h) followed by evaporation of the elution medium and reconstitution with running buffer (time required *ca.* 1 h) was found to be a rapid and efficient sample preparation step for the MECC determination and confirmation of acidic, neutral and basic illicit drugs in urine samples [20–22].

CONCLUSIONS

Monitoring drugs in body fluids using MECC has several areas of interest, the most important being the determination of specific drugs or metabolites for therapeutic or research reasons [10–12, 15–19,30], the rapid determination of intoxications and the confirmation of drugs of abuse and metabolites in specimens that tested positively employing routine immunological screening procedures [20–22] and the determination of metabolic ratios for pharmacogenetic purposes [23,26]. HPLC and MECC data for thiopental in 66 samples from patients [30], and MECC and immunoassay data for theophyl-

line and caffeine [19] were found to compare well based on linear regression analysis. The graphs deviated from the line of equality, however. The elucidation of the reasons for this behaviour is the subject of continuing investigations. For the purpose of therapeutic drug monitoring, however, such differences are relatively insignificant.

The value of combining solid-phase extraction and MECC analysis with fast scanning multi-wavelength detection for the confirmation of opioids (heroin metabolites), the cocaine metabolite benzoylecgonine, amphetamines, barbiturates and hypnotics [18,20,22], and also 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH, metabolite of the psychotropic drug Δ^9-tetrahydrocannabinol present in marijuana [21], in human urine has been reported. Most importantly, this new methodology was found not to require sample hydrolysis (except for THC-COOH) and derivatization for monitoring these compounds at concentrations equal to or lower than those used as cut-off levels in routinely applied immunoassays. Reliable confirmation testing of drugs of abuse in a single aliquot of urine was efficiently accomplished by simultaneous adsorption of most illicit drugs and metabolites on a mixed-mode bonded-phase matrix and sequential elution in two or three steps for subsequent analysis by MECC [22]. Lloyd *et al.* [23] were able to demonstrate the value of MECC with direct injection of the body fluid for the determination of the acetylator phenotype by simply measuring peak-height ratios of two urinary metabolites of caffeine, 5-acetylamino-6-formylamino-3-methyluracil (AFMU) and 1-methylxanthine. The simultaneous analysis of AFMU, its decomposition product 5-acetylamino-6-amino-3-methyluracil (AAMU), 1-methylxanthine (1X) and 1-methyluric acid (1U) by MECC with multi-wavelength detection was investigated by Guo and Thormann [26] and shown to provide reliable MECC data for the determination of the phenotype status in man.

Using MECC instead of GC or HPLC for therapeutic drug monitoring has several important advantages: high efficiency and degree of automation, small sample size, potential for direct sample injection, no requirement of large amounts of organic solvents and rapidity of analysis. Further, an electrokinetic capillary set-up permits a rapid change from one buffer configuration to another, making method development simple and cost-effective. The direct injection of proteinaceous fluids, such as plasma and serum, in MECC provides a special feature of electrokinetic capillary analyses. It allows rapid investigations and can be performed on very small sample volumes (a few μl), such as serum samples of prematurely born infants [19], saliva, droplets of tears and sweat, samples that are typically too small to be pretreated. The data presented in this paper show that ultrafiltration, a simple sample preparation technique for removal of proteins and drugs bound to these macromolecules, extends the analytical opportunities in comparison with direct injection of samples. Detection limits are primarily dependent on the type of detector used and the analyte concentration in the applied sample. With UV absorption detection and using samples without preconcentration, the limit of detection is in the low μg/ml (μM) range. Using preconcentration via sample extraction, concentration as low as a few tens of ng/ml could be unambiguously monitored [21].

All the data obtained so far are very encouraging and demonstrate the high potential of MECC. However, further investigations are needed for the adoption of MECC as a routine method in drug assay laboratories. Important areas of research include the application of reliable automated operation over long periods of time (overnight), the establishment of automated data evaluation, recognition of unexpected interferences and capillary fouling, and the elucidation of optimized protocols for the determination of specific drugs.

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