Journal of Chromatography, 545 (1991) 445–460
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 222

Analysis of barbiturates in human serum and urine by high-performance capillary electrophoresis–micellar electrokinetic capillary chromatography with on-column multi-wavelength detection

WOLFGANG THORMANN*, PETER MEIER, CLAUDIA MARCOLLI and FRANK BINDER
Department of Clinical Pharmacology, University of Berne, Murtenstrasse 35, CH-3010 Berne (Switzerland)

ABSTRACT

The analysis of barbiturates in human serum (or plasma) and urine by high-performance capillary electrophoresis–electrokinetic capillary chromatography with on-column fast-scanning multi-wavelength detection is discussed. The use of a buffer of *ca.* pH 8 and containing sodium dodecyl sulphate provides a medium suitable for fast and high-resolution separations of barbiturates. Seven barbiturates are characterized by their retention and absorption spectra between 195 and 320 nm. Comparison of these computer-stored data with those of unknown samples is shown to allow the identification of barbiturates in samples of patients undergoing pharmacotherapy and in toxicological urine and serum specimens. Three-dimensional electropherograms provide reliable information on the requirement and suitability of sample pretreatment procedures. With urine, extraction of barbiturates prior to analysis is necessary. With human serum several barbiturates, including phenobarbital, are shown to elute in an interference-free window in front of uric acid and the proteins, allowing these substances to be determined by direct sample injection. The need for multi-wavelength detection over a relatively wide wavelength range as a means of peak confirmation in electrokinetic capillary analyses is demonstrated and limitations of this technique for compounds with similar retention behaviour and absorption spectra are discussed.

INTRODUCTION

Screening and confirmation of drugs in body fluids, including barbiturates (Fig. 1) in serum (plasma) and urine, are important for the investigation of intoxication, for therapeutic drug monitoring and for pharmacokinetic and metabolic studies. Every laboratory involved with clinical toxicology should be prepared to analyse a biological specimen to determine (i) whether or not a barbiturate is present and (ii) its identity and in some instances also its concentration. Barbiturates have a low therapeutic index and are therefore prone to cause poisoning. Monitoring their concentrations in body fluids, especially in serum, is therefore essential to optimize pharmacotherapy [1]. As instrumental approaches for the analysis of barbiturates in body fluids, immunoassays [2–4] and many chromatographic methods [5–8] have been developed. Immunological techniques are very attractive because of their ease of performance, speed of analysis

0021-9673/91/$03.50

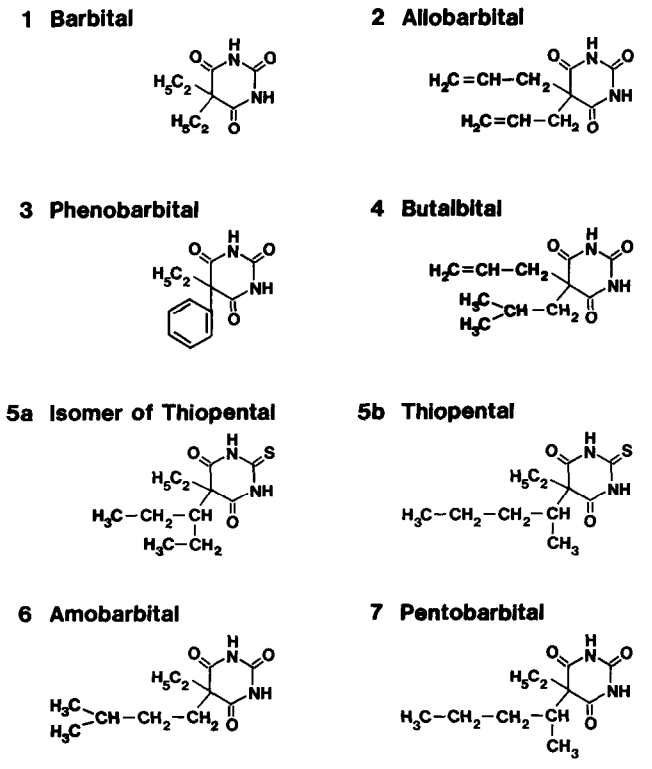


Fig. 1. Structures of the barbiturates investigated.

and sensitivity. However, with the exception of phenobarbital, these assays are not specific enough to monitor or identify a single compound. They simultaneously respond to different barbiturates at a characteristic specificity to each of them, providing their suitability to monitor the presence of barbiturates in a toxicological specimen. Chromatographic procedures have been applied for the determination of all common barbiturates. These methods separate and therefore provide data on multiple compounds in one run. They require, however, time-consuming sample pretreatments and are characterized by a low sample throughput.

Recently, high-performance capillary electrophoresis (HPCE) and micellar electrokinetic capillary chromatography (MECC, an interface between electrophoresis and chromatography) were found to be attractive approaches for the analysis of pharmaceuticals [9–15]. HPCE is a one-phase process with separation based on differences in electrophoretic mobilities. In MECC two distinct phases are used, an aqueous and a micellar phase or pseudo-stationary phase. These two phases are established by employing buffers containing surfactants [*e.g.*, sodium dodecyl sulphate (SDS)], which are added above their critical micellar concentration. Variation of the surfactant concentration has similar effects to changes in surface structure in HPLC (*e.g.*, change from C_8 to C_{18}). An MECC analysis is performed in equipment designed for HPCE, *i.e.*, in an open-tubular capillary of very small I.D. A high-voltage d.c.

electric field is applied along the column, thus causing both a movement of the entire liquid (the so-called electroosmotic flow) and migration of the charged micelles. Non-ionic solutes partition between the two phases and elute with zone velocities between those of the two phases. In this case separation is solely of chromatographic nature. For ionic solutes which are also differentially distributed between the two phases, separation is based on chromatography and electrophoresis. This was found to be the case with barbiturates having a buffer of *ca.* pH 8 and containing 50 m*M* SDS, hence the assay is referred to as an HPCE–MECC methodology.

Multi-wavelength detection, such as the use of a photodiode-array detector, is a powerful technique for solute monitoring in liquid chromatography [16] and has recently also been applied to HPCE [17,18]. It allows the collection of absorbance *vs.* time (*i.e.*, registration of a chromatogram or electropherogram), absorbance *vs.* wavelength (spectrum) and absorbance *vs.* time *vs.* wavelength data for a three-dimensional depiction of the data (Fig. 2), this permitting peak confirmation and peak

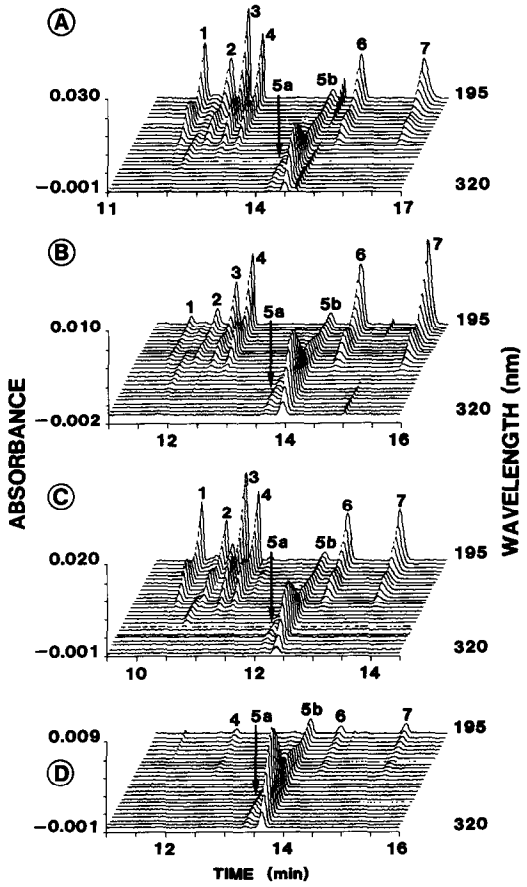


Fig. 2. Three-dimensional electropherograms of a model mixture of seven barbiturates in (A) buffer and (B) bovine plasma after Bond-Elut C_{18}, (C) chloroform and (D) pentane extraction. The applied voltage was a constant 20 kV in all instances and the currents were 60–63 μA. Barbiturates: 1 = barbital; 2 = allobarbital; 3 = phenobarbital; 4 = butalbital; 5a = isomer of thiopental; 5b = thiopental; 6 = amobarbital; 7 = pentobarbital. Sample concentrations and recoveries are summarized in Table I.

purity to be evaluated via comparison with absorption spectra. Using HPCE–MECC with a fast-scanning multi-wavelength detector [19], the objectives of the work described in this paper were (i) to investigate the suitability of direct sample introduction and different extraction procedures for barbiturates in human serum and urine, (ii) to measure barbiturates (parent drug and metabolites) in serum samples from patients undergoing barbiturate pharmacotherapy and (iii) to confirm the presence and determine the identity of barbiturates in urine specimens which were received in the departmental drug assay laboratory and were found to be barbiturate positive using immunoassays.

EXPERIMENTAL

Chemicals

The barbiturates, of European Pharmacopoeia quality, were purchased from Grogg Chemie (Stettlen, Switzerland), except thiopental, which was from Abbott Labs. (Cham, Switzerland). All other chemicals were of analytical-reagent or research grade. Bovine plasma was prepared by centrifugation of bovine blood (from the local slaughter house) and blank human serum was obtained by centrifugation of our own blood (500 *g* for 10 min).

Instrumentation and running conditions

Both laboratory-made and commercial HPCE instrumentation was used. The laboratory-made device 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 Model UVIS 206 PHD fast-scanning multi-wavelength detector with on-column capillary detector cell No. 9550-0155 (Linear Instruments, Reno, NV, USA) towards the capillary end. The effective separation distance was 70 cm. Two 50-ml plastic bottles served as electrode vessels and a VacTorr 150 vacuum pump (CGA/Precision Scientific, Chicago, IL, USA) was used to rinse the capillary with cleaning solution (0.1 *M* sodium hydroxide) and electrophoresis buffer. Current was applied at a constant voltage (20 kV) with an HCN 14-20000 power supply (FUG Elektronik, Rosenheim, Germany). The cathode was on the detector side. Sample application occurred manually via gravity through lifting the anodic capillary end, dipped into the sample vial, *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 Model 206 detector software package version 2.0 (Linear Instruments) with windows 286 version 2.1 (Microsoft, Redmont, WA, USA). Conditioning for each experiment was effected by rinsing the capillary with 0.1 *M* sodium hydroxide solution for 3 min and with buffer for 5 min. Throughout this work the Model 206 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 for each wavelength was 3.69 data points/s.

The commercial instrument was a Model 270A capillary electrophoresis system (Applied Biosystems, San Jose, CA, USA). This apparatus features automated capillary rinsing, sampling and execution of the electrophoretic run. For our experiments it was equipped with a 50-μm I.D. fused-silica capillary of effective separation length 44 cm. A Model D-2000 chromato-integrator (Merck–Hitachi,

Darmstadt, Germany) was used for recording the pherograms and for quantification by peak-area measurements. The integrator sampling period was set to one data point per 200 ms throughout this work. Before each run the capillary was rinsed with 0.1 *M* sodium hydroxide solution (1 min) and with buffer (2 min). Injection of sample occurred via vacuum suction for 2 s. In all experiments a constant voltage of 30 kV was applied and the temperature was set at 40°C. To obtain responses at different wavelengths, barbiturate samples were detected sequentially at 215 and 290 nm.

Absorption spectra were measured with a Lambda 15 UV–VIS spectrophotometer (Perkin-Elmer, Überlingen, Germany).

Electrophoresis buffers and standard solutions

For monitoring of barbiturates a buffer composed of 50 m*M* sodium dodecyl sulphate (SDS), 9 m*M* $Na_2B_4O_7$ and 15 m*M* NaH_2PO_4 (pH *ca.* 7.8) was employed.

Standard solutions of single barbiturates (100 μg/ml) were prepared in methanol whereas a standard model mixture composed of seven barbiturates (barbital, allobarbital, phenobarbital, butalbital, thiopental, amobarbital and pentobarbital; 500 μg/ml each) was dissolved in the electrophoresis SDS buffer. Spiking of blank and patients' samples occurred through addition of known aliquots of these standard solutions to the body fluids prior to sample extraction. For quantification of barbiturates in serum, aliquots of the methanolic standard solutions were added to a glass test-tube with a conical bottom, evaporated to dryness under a stream of nitrogen (40°C) and reconstituted with either bovine plasma, blank human serum or patients' serum.

Sample preparation for barbiturates in urine

Extraction of barbiturates from urine was achieved using Bond-Elut Certify cartridges and the Vac-Elut set-up (both from Analytichem International, Harbor City, CA, USA). With minor alterations, the manufacturer's instructions for chromatographic analyses were followed. 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 6). The columns were then rinsed with 1 ml of 0.1 *M* phosphate buffer–methanol (80:20), dried under full vacuum for 5 min, rinsed with 1 ml of 1 *M* acetic acid, again dried under full vacuum (10 min) and rinsed with 1 ml of hexane. Elution was effected with 4 ml of methylene chloride into a test-tube before evaporation to dryness under a gentle stream of nitrogen at 40°C. The residue was dissolved in 100–200 μl of running buffer.

Sample preparation for barbiturates in serum (plasma)

Bond Elut C_{18}. Extraction of barbiturates was achieved using Bond-Elut C_{18} cartridges and the Vac-Elut set-up. The cartridges were conditioned immediately prior to use by drawing first methanol then water (both twice) through each column. For these steps the columns were filled completely with the two solvents. The columns were loaded by application of 0.6 ml of a mixture of 0.75 ml of serum (plasma) and 0.15 ml of 0.1 *M* Na_2HPO_4 (pH *ca.* 8.5). After an incubation period of several minutes the solutions were slowly drawn, the columns were rinsed three times with water and dried

under full vacuum for about 1 min. Elution was effected first with 0.2 ml (after a short incubation period) and continued with 0.25 ml of methanol into a test-tube before evaporation to dryness under a gentle stream of nitrogen at 40°C. The residue was dissolved in 200 μl of running buffer.

Chloroform extraction. Liquid–liquid extraction with chloroform from acidified serum was executed after the method of Shiu and Nemoto [7]. A 0.2-ml volume of serum (plasma), 0.1 ml of 1 *M* hydrochloric acid and 2 ml of chloroform were placed in an 11-ml screw-capped Sovirel glass tube. After vigorous shaking for 15 min and centrifugation at 500 *g* for 10 min, the lower (organic) layer was transferred into a centrifuge glass tube with a short conical bottom and evaporated to dryness under a gentle stream of nitrogen at 40°C. The residue was reconstituted in 200 μl of electrophoresis running buffer and shaken for about 60 s.

Pentane extraction. Liquid–liquid extraction with pentane at pH 6.4 was also investigated for feasibility with the seven barbiturates. A 0.5-ml volume of serum (plasma), 1 ml of phosphate buffer (pH 6.4) and 5 ml of *n*-pentane were placed in an 11-ml screw-capped Sovirel test-tube. After vigorous shaking for 10 min and centrifugation at 1000 *g* for 10 min, the upper (organic) phase was transferred into a centrifuge glass tube with a conical bottom and evaporated to dryness under a gentle stream of nitrogen at 40°C. The residue was dissolved in 200 μl of running buffer and vortexed for 30 s.

Sample preparation for direct injection

Biological fluids were filtered using 0.2-μm Nalgene (25 mm diameter) disposable syringe filters (Nalge, Rochester, NY, USA) prior to sample injection.

Recovery

The recovery after sample pretreatment was determined by comparing electrophoretic peak areas after extraction with peak areas obtained by direct injection of equal amounts of the drugs in buffer.

RESULTS AND DISCUSSION

Analysis of barbiturates in human serum (plasma)

Multi-wavelength detection allows the monitoring of components that do not absorb at equal wavelengths in a single experiment. The three-dimensional electropherograms depicted in Fig. 2 represent the absorbance *vs.* retention times *vs.* wavelength relationships for a model mixture of seven barbiturates (see Fig. 1). Peak 5a represents an isomer of thiopental found in commercial thiopental, which was previously detected by HPLC [8]. Each component is characterized by its retention/migration behaviour with barbital being the fastest and pentobarbital the slowest of the investigated components. Barbiturates have pK_a values between 7.3 and 8.1 and are therefore all partially ionized at pH 7.8, hence they are migrating against the electroosmotic flow (same direction as SDS micelles). Partitioning between the buffer and the micelles is also occurring, as there is evidence that they elute in order of increasing pK_a values (*e.g.*, the pK_a values of phenobarbital, thiopental, amobarbital and pentobarbital are 7.4, 7.5, 7.9 and 8.1, respectively [20]). This correlates with the decrease in negative charge with increasing pK_a. The absorption spectrum of each

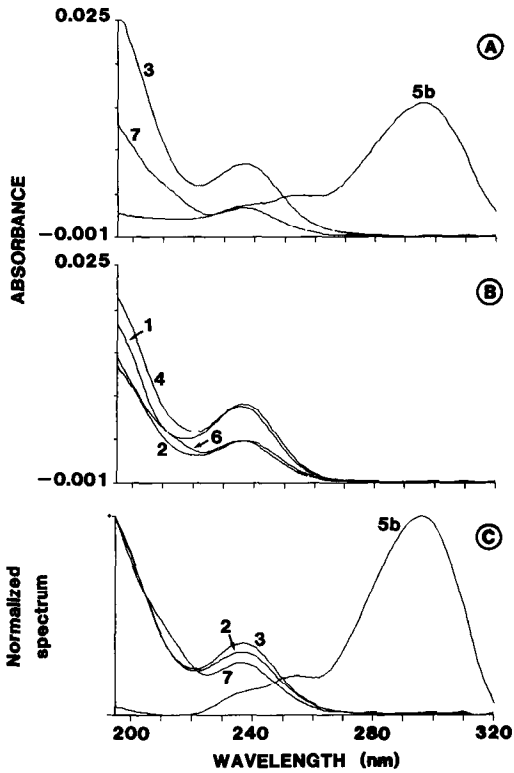


Fig. 3. (A, B) Absorption spectra and (C) normalized spectra obtained as time slices from data in Fig. 2A.

compound can be extracted from the gathered data points as so-called time slices (Fig. 3A and B). With the exception of thiopental, there is great similarity between these absorbance *vs.* wavelength relationships. The spectra were found to compare well with those measured on a regular spectrophotometer, with the exception that the absorbance with the Model 206 detector was higher at 195 nm. For the sake of comparison, normalized spectra, such as those shown for thiopental, pentobarbital, phenobarbital and allobarbital (Fig. 3 C), are employed.

For the monitoring of barbiturates in patients' samples, different extraction methods were investigated. In Fig. 2B–D three-dimensional pherograms of the seven barbiturates extracted from spiked bovine plasma using Bond-Elut C_{18} (B), chloroform (C) and pentane (D) pretreatment procedures are shown. The detected peaks could easily be identified by comparison of their spectra with those obtained from Fig. 2A and from their retention time (time of detection) relative to a known compound (*e.g.*, thiopental). Small peaks, such as that for butalbital after pentane extraction (peak 4 in Fig. 2D), were also verified by addition of a small amount of the drug to the reconstituted sample. It is clear from these results that (i) the early eluting barbiturates are poorly extracted by the Bond-Elut C_{18} procedure, (ii) all seven barbiturates are extracted well with acidified chloroform and (iii) the pentane extraction method is specific for thiopental. Recoveries, calculated from measured peak areas obtained with the automated apparatus (data not shown), are listed in Table I. It should be noted that

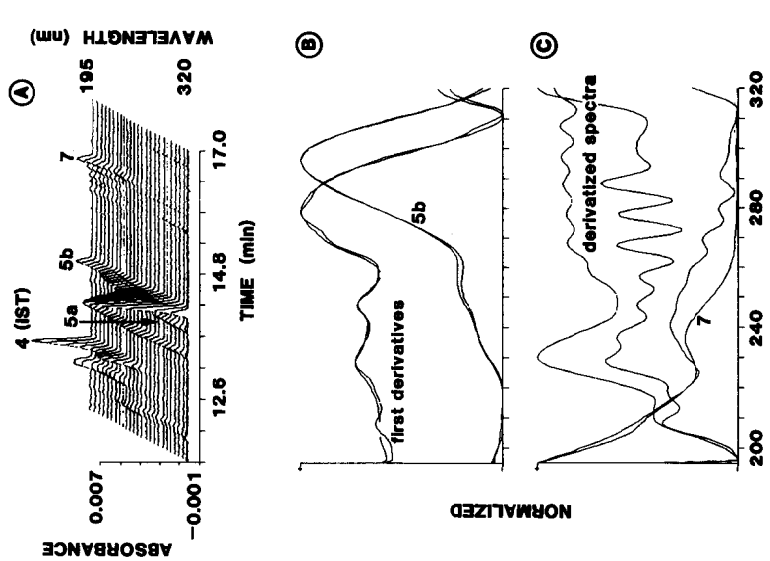


Fig. 5. (A) Three-dimensional data plot together with normalized time slices (spectra) of (B) thiopental and (C) pentobarbital of the serum sample in Fig. 4. Normalized and first derivatives are compared with those of the model compounds in Fig. 2.

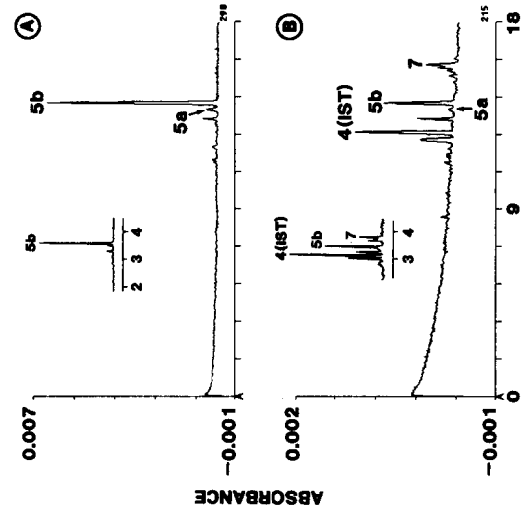


Fig. 4. Single-wavelength electropherograms (A, 290 nm; B, 215 nm) of a human serum sample after Bond-Elut C_{18} extraction. Conditions as in Fig. 2. The insets depict the corresponding pherograms obtained on the automated instrument with application of a constant 30 kV (70 μA) and having the plotting scale four-fold smaller for the 290-nm than the 215-nm data.

TABLE I

RECOVERIES OF BARBITURATES

The values given represent percentage recovered, expressed in 5% increments. ND denotes that, under the described conditions, the component was not detectable with the ABI 270A. VL means that the component was detected as a very small peak which could not be reliably quantified. The investigated sample concentrations are listed under the matrix labels and calculated concentrations in case of 100% recovery (maximum concentrations after sample pretreatment) are given under the extraction procedures. The ratio of the two values provide a maximum concentration factor associated with an extraction procedure.

No.	Barbiturate	Matrix			
		Bovine plasma (33.3 μg/ml)	Bovine plasma (50 μg/ml)	Bovine plasma (62.5 μg/ml)	Human urine (10 μg/ml)
		Extraction			
		Bond-Elut C_{18} (82.5 μg/ml)[a]	Pentane (125 μg/ml)[a]	Chloroform (125 μg/ml)[a]	Bond-Elut Certify (250 μg/ml)[a]
1	Barbital	10	ND	60	VL
2	Allobarbital	20	ND	55	15
3	Phenobarbital	25	ND	75	65
4	Butalbital	65	VL	80	90
5b	Thiopental	85	45	80	75
6	Amobarbital	95	5	70	90
7	Pentobarbital	95	5	70	95

[a] Maximum concentration.

in all these investigations high barbiturate concentrations were employed. Concentrations in spiked bovine serum were between 33.3 and 62.5 μg/ml. Depending on the extraction procedure and assuming a 100% recovery, maximum barbiturate concentrations after sample pretreatment were therefore in the range 82.5–125 μg/ml (Table I). It was interesting to find that reconstituted samples after the extraction with chloroform had to be measured within 1–2 days, whereas the other samples appeared to be stable for more than 1 week when stored at 4°C.

On measuring blank human serum or blank bovine plasma, no peaks were found with all three extraction procedures when analysed in the range 195–320 nm (data not shown). However, investigation of serum samples from patients undergoing thiopental pharmacotherapy for several days or from toxicological specimens revealed bands that could be identified as one of the seven barbiturates. Using a serum sample from a patient under thiopental pharmacotherapy, spiked with butalbital (20 μg/ml) as internal standard and extracted with Bond-Elut C_{18}, single-wavelength data at 290 nm suggested the presence of thiopental (Fig. 4A). From the data obtained at 215 nm one could assume the presence of up to four or five barbiturates (Fig. 4B). Data obtained at two wavelengths are clearly insufficient for proper assignment of the peaks, but it is pleasing to note that the patterns obtained with the two instruments agree very well. By scanning from 195 to 320 nm while the zones were transported through the detector cell, the data depicted in Fig. 5A were obtained. Analysis of this run reveals that the time slice at 14.1 min, and also its first derivative, agree extremely well with those of

thiopental (Fig. 5B). The same is true for the internal standard, butalbital, at 12.7 min (data not shown). According to the retention, the peak at 15.95 min was assumed to be pentobarbital, a metabolite of thiopental. Graphical presentation of the time slice and its first derivative with standard data for pentobarbital does not prove the identity of the two compounds (Fig. 5C). The peak here is unfortunately too small for proper assignment by spectral comparison. The sample had to be spiked with pentobarbital and re-run for proper analysis. This example reveals that the signal should be larger than 0.001 absorbance for sample verification by spectral information obtained with the UVIS 206 detector. Linear regression of the detection times of butalbital, thiopental and pentobarbital from this run with the corresponding values from the experiment depicted in Fig. 2A revealed a correlation coefficient of 0.9999.

Preliminary attempts at the quantification of pentobarbital and thiopental in human plasma were performed by the internal standard method using butalbital (20 μg/ml, Figs. 4 and 5A) as reference compound, the Bond-Elut C_{18} extraction procedure and the automated instrument. Peak-area ratios were employed as the basis for data evaluation. Calibration graphs were constructed with spiked bovine plasma in the concentration range 1–60 μg/ml (eight data points). Pentobarbital was monitored at 215 nm whereas thiopental was measured at 215 and 290 nm. All three graphs showed good linearities with correlation coefficients of 0.994, 0.977 and 0.988,

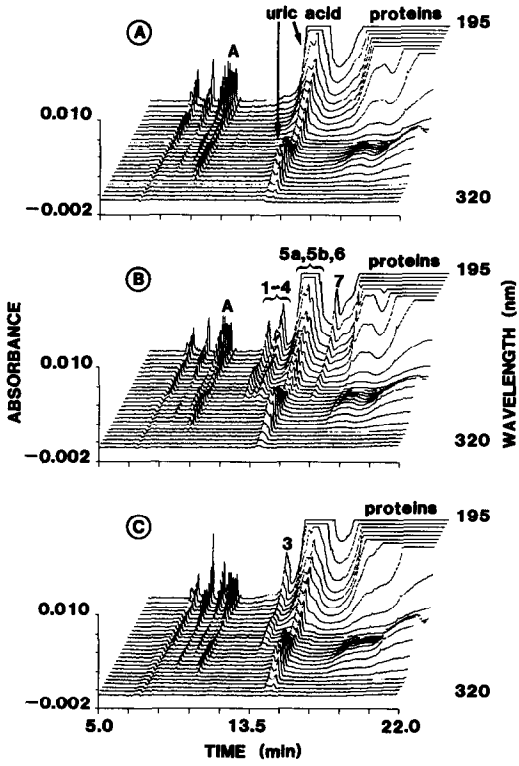


Fig. 6. Three-dimensional electropherograms obtained with direct injection of human serum having (A) blank serum, (B) blank serum spiked with barbiturates and (C) serum from a patient undergoing phenobarbital pharmacotherapy. A represents a group of three resolved peaks, the centre one being caffeine.

respectively, and passed through the origins. For the example presented in Figs. 4 and 5A the pentobarbital concentration was determined to be 4.9 μg/ml and the thiopental content was found to be 21.2 and 18.5 μg/ml for the calibrations at 215 and 290 nm, respectively, representing pharmacologically meaningful data [1–4].

The data presented in Fig. 6 were obtained with human serum samples which were only passed through a 0.2-μm syringe filter prior to sample injection. The data for the blank serum shown in Fig. 6A reveal that only components eluting between caffeine (here the peak at 9.54 min) and uric acid (the so-called analytical or separation window [14]) have a chance to be analysed with direct sample application, *i.e.*, without extraction. Analysis by an enzyme-multiplied immunoassay technique (EMIT) revealed a caffeine concentration of 4.43 μg/ml (22.8 μ*M*). Serum proteins are solubilized by the micelles and elute (as a very broad zone) after uric acid. The data depicted in Fig. 6B were obtained with the blank human serum which was spiked with the barbiturates in Fig. 1. The first four drugs were found to form their zones in front of uric acid, thiopental and amobarbital to coelute with uric acid and pentobarbital to be located in the "valley" between the broad uric acid band and the proteins. The suitability of direct sample introduction for drug monitoring is demonstrated with the example shown in Fig. 6C. A serum sample from a patient undergoing phenobarbital pharmacotherapy was injected, producing a clear phenobarbital zone within the analytical window. Its normalized spectrum was in good agreement with that given in Fig. 3 (data not shown). The serum concentration of phenobarbital was determined to be 20.4 μg/ml (88 μ*M*) using an EMIT. Hence HPCE–MECC permits the determination of serum phenobarbital at the pharmacologically interesting concentration level (the therapeutic range of this barbiturate is 10–30 μg/ml) and without elaborate sample pretreatment. It is interesting that bovine plasma does not exhibit a characteristic analytical window (data not shown).

Identification of barbiturates in human urine

Direct injection of urine provides very complex pherograms which precludes the determination of barbiturate concentrations at the μg/ml (μ*M*) level (see also ref. 10; three-dimensional data not shown). Fig. 7 depicts data obtained with a blank urine (A and B), illustrating that very few peaks are detected after extraction with Bond-Elut Certify at pH 6. One zone could be assigned to caffeine. The response of the seven investigated barbiturates after the extraction is depicted in Fig. 7C and calculated recoveries are listed in Table I. Similarly to the Bond-Elut C_{18} treatment, barbital and allobarbital are shown to be poorly extracted. Good recoveries were obtained for the other five barbiturates.

A urine specimen obtained from the emergency care unit was found to be markedly positive for benzodiazepines, cocaine, opiates and barbiturates using EMIT drug-screening procedures and for cannabinoids using a fluorescence polarization immunoassay. This very interesting, complex sample was pretreated with Bond-Elut Certify as described above and analysed with both instruments. Single-wavelength data for 290 and 215 nm obtained on the two instruments are depicted in Fig. 8A and B, respectively, and three-dimensional multi-wavelength data are presented in Fig. 9. Analysing this sample at one or two wavelengths is clearly insufficient for identification of the unknown peaks. Knowing the behaviour of barbiturates in that system, and looking at the data obtained at 290 nm, the presence of thiopental can be suspected.

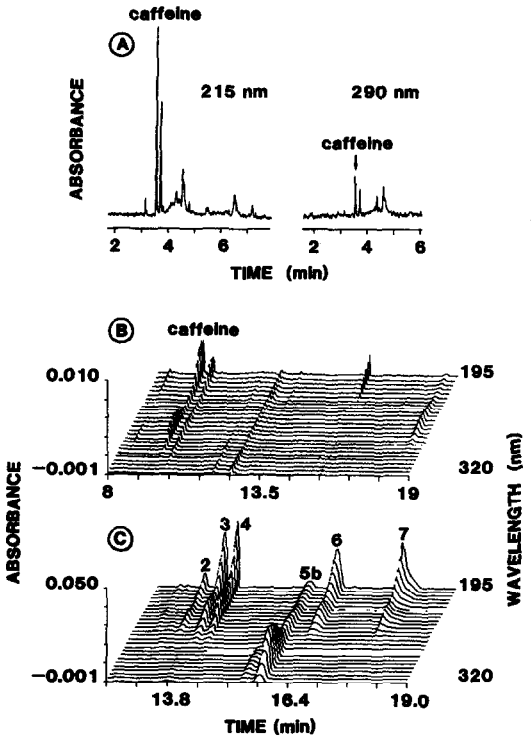
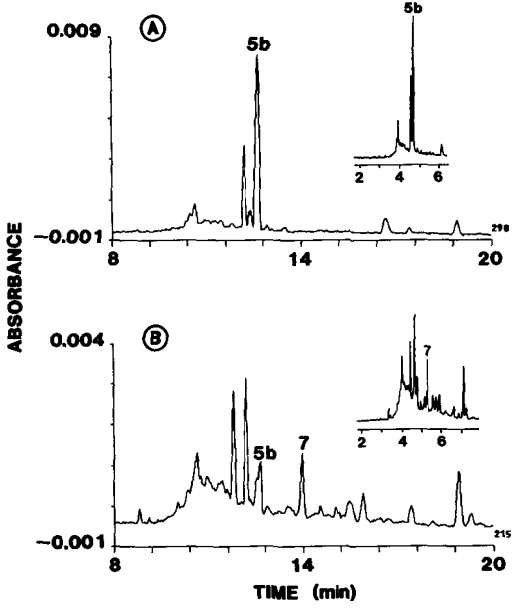


Fig. 7. Data for (A, B) blank urine and (C) blank urine spiked with seven barbiturates after Bond-Elut Certify extraction. Single-wavelength data (A) were obtained on the automated instrument with a constant 30 kV (70 μA). The three-dimensional data (B, C) were collected as described for Fig. 2. Barbiturate concentrations and recoveries are listed in Table I.

Spiking of the sample with this compound and re-running the experiment would indeed show an increase in the size of the larger peak in this pattern. This, however, would not provide complete proof but only confirmation that the presumption could be correct. Having data between 195 and 320 nm, as shown in Fig. 9A and B, and also reference spectra of barbiturates (Fig. 3) permitted a quick and reliable confirmation of the presence of thiopental and its metabolite, pentobarbital, in that sample. As is shown in Fig. 9C, there is excellent agreement between the time slices at 12.67 and 13.99 min with those for thiopental and pentobarbital, respectively.

Single- and multi-wavelength data obtained from a barbiturate-positive (benzodiazepine-negative) patient's sample (screening with EMIT; patient from the department's out-patient clinic) are presented in Figs. 10 and 11, respectively. Comparison with blank data reveals the appearance of one major peak which could be a barbiturate. Again, with detection at 215 and 290 nm (Fig. 10), identification is not possible. According to its spectrum and retention (Fig. 11A), one can conclude that it represents either allobarbital (Fig. 11B) or phenobarbital (Fig. 11C). However, the absorption spectra of these two compounds are very similar, which makes proper identification difficult. Also, the retention time intervals in our instrumental set-up



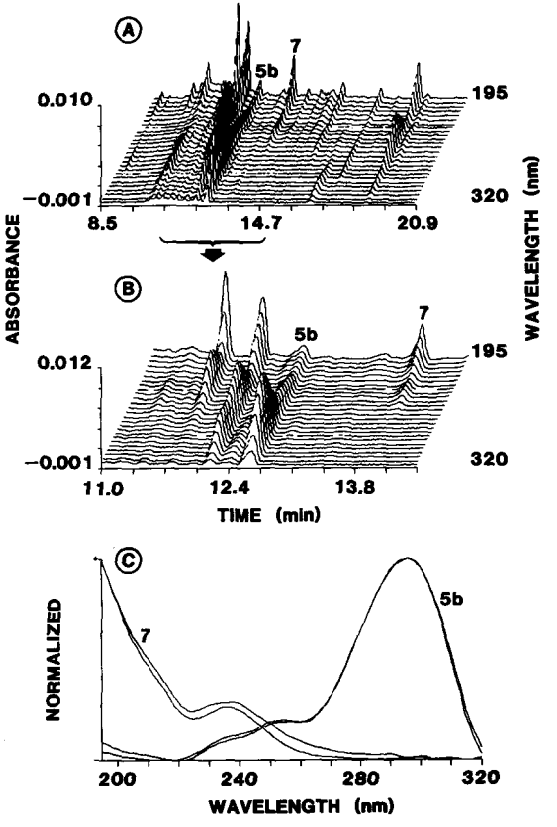


Fig. 8. Analysis of a Bond-Elut Certify pretreated toxicological urine specimen with detection at (A) 290 and (B) 215 nm. The insets depict the data obtained on the automated instrument. Conditions as in Fig. 7.

Fig. 9. (A) Three-dimensional data plot, (B) plot with expanded time and absorbance scales and (C) normalized spectra of thiopental and pentobarbital of the urine specimen in Fig. 8. There is excellent agreement between the normalized spectra of the two barbiturates from that experiment with those in Fig. 7C.

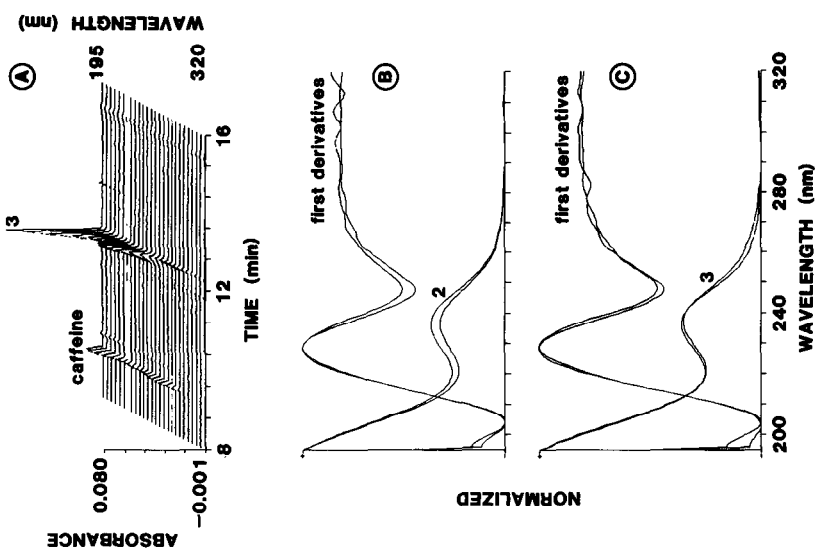


Fig. 11. (A) Three-dimensional electropherogram together with data analysis by normalized time slices for (B) allobarbital and (C) phenobarbital of the urine sample in Fig. 10.

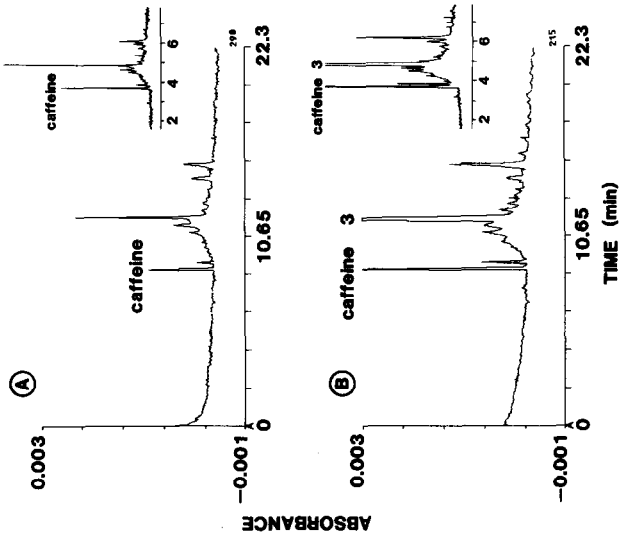


Fig. 10. Single-wavelength analysis of a Bond-Elut Certify pretreated urine sample containing one major peak with possible barbiturate identity. Conditions as in Fig. 8.

with manual sample introduction vary too much for this parameter to be used reliably for identification purposes. For complete confirmation this sample had to be spiked with phenobarbital. The example demonstrates that even with multi-wavelength monitoring zone assignment can be difficult when the retention behaviour and spectra are similar. This calls for the use of a combined technique, such as the on-line coupling of HPCE–MECC with mass spectrometry (MS). It is interesting to add that there is a small background interference at the location of phenobarbital in all pherograms in Figs. 7, 10 and 11.

CONCLUSIONS

These experiments have demonstrated the feasibility of monitoring barbiturates in human serum (plasma) and urine by HPCE/MECC with on-column multi-wavelength absorbance detection between 195 and 320 nm and using a phosphate–borate buffer of pH 7.8 containing 50 m*M* SDS. This approach is attractive for (i) drug and metabolite investigations, (ii) barbiturate identification in toxicological samples and (iii) efficient characterization of sample pretreatment schemes and the suitability of direct injection of urine and serum. The data indicate that some (including phenobarbital) but not all of the serum barbiturates investigated can be analysed without extraction. The preliminary quantitative results suggest the suitability of using HPCE–MECC as a therapeutic drug monitoring method for serum barbiturates. Extraction prior to analysis is necessary for urine barbiturates.

Characterization of sample zones by their retention behaviour and absorption spectra is a powerful approach for solute identification. In fact, having HPCE technology with multi-wavelength detection (as described here), its combination with MS [21,22] or HPLC [23] and together with the distinct advantages of electrokinetic capillary analyses (automation, small sample size, ease of buffer change and speed of analysis), HPCE–MECC may well become the most important analytical and confirmation method for drugs and metabolites in body fluids.

ACKNOWLEDGEMENTS

The authors acknowledge the valuable contributions of Dr. V. Purghart during the assembly phase of the laboratory-made instrument and Dr. P. Gebauer for performing the analyses with direct sample injection. The generous loan of the UVIS 206 detector by Linear Instruments, and of the HCN power supply by Mettler–Toledo (Greifensee, Switzerland) is gratefully acknowledged. This work was partly sponsored by the Research Foundation of the University of Berne and the Swiss National Science Foundation.

REFERENCES

1 R. F. Shaw, *Barbiturates (Abused Drugs Monograph Series)*, Abbott Laboratories, Diagnostics Division, Irving, TX, 1988; and references cited therein.
2 B. E. Pape, P. L. Cary, L. C. Clay and W. Godolphin, *Ther. Drug Monit.*, 5 (1983) 467.
3 A. T. Watson, J. E. Manno and B. R. Manno, *J. Anal. Toxicol.*, 7 (1983) 257.
4 D. L. Colbert, D. S. Smith, J. Landon and A. M. Sidki, *Clin. Chem.*, 30 (1984) 1765.
5 D. Berry, *J. Chromatogr.*, 86 (1973) 89.

6 C. Salvadori, R. Farinotti, Ph. Duvaldestin and A. Dauphin, *Ther. Drug Monit.*, 3 (1981) 171.
7 G. K. Shiu and E. M. Nemoto, *J. Chromatogr.*, 227 (1982) 207.
8 D. R. Stanski, P. G. Burch, S. Harapat and R. K. Richards, *J. Pharm. Sci.*, 72 (1983) 937.
9 M. C. Roach, P. Gozel and R. N. Zare, *J. Chromatogr.*, 426 (1988) 129.
10 Y. Tanaka and W. Thormann, *Electrophoresis*, 11 (1990) 760.
11 T. Nakagawa, Y. Oda, A. Shibukawa and H. Tanaka, *Chem. Pharm. Bull.*, 36 (1988) 1622.
12 T. Nakagawa, Y. Oda, A. Shibukawa, H. Fukuda and H. Tanaka, *Chem. Pharm. Bull.*, 37 (1989) 707.
13 H. Nishi, T. Fukuyama, M. Matsuo and S. Terabe, *J. Chromatogr.*, 513 (1990) 279.
14 H. Nishi, T. Fukuyama and M. Matsuo, *J. Chromatogr.*, 515 (1990) 245.
15 H. Nishi and S. Terabe, *Electrophoresis*, 11 (1990) 691.
16 M. Hayashida, M. Nihira, T. Watanabe and K. Jinno, *J. Chromatogr.*, 506 (1990) 133.
17 S. Kobayashi, T. Ueda and M. Kikumoto, *J. Chromatogr.*, 480 (1989) 179.
18 J. Vindevogel, P. Sandra and L. C. Verhagen, *J. High Resolut. Chromatogr.*, 13 (1990) 295.
19 K. Weinberger, *Am. Lab.*, December (1989) 12.
20 V. Dinnendahl and U. Fricke, *Arzneistoff-Profile*, Govi-Verlag, Frankfurt, 1987.
21 E. D. Lee, W. Mueck, J. D. Henion and T. R. Covey, *J. Chromatogr.*, 458 (1988) 313.
22 C. G. Edmonds, J. A. Loo, C. J. Barinaga, H. R. Udseth and R. D. Smith, *J. Chromatogr.*, 474 (1989) 21.
23 M. M. Bushey and J. W. Jorgenson, *Anal. Chem.*, 62 (1990) 978.