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High-performance liquid chromatographic method for an automated determination of local anaesthetics in human plasma

Jürgen Drewe[a,*], Stefan Rufer[a], Jörg Huwyler[a], Ernst Küsters[b]

[a]*Outpatient Clinic and Departments of Anaesthesia and Research, University Clinic, Kantonsspital Basel, CH-4031 Basel, Switzerland*
[b]*Chemical Development, Sandoz Ltd., Basel, CH-4002 Basel, Switzerland*

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Abstract

A method is described that allows the rapid and precise determination of the local anaesthetics bupivacaine and etidocaine from biological fluids. This method uses a fully automated system with solid-phase extraction in combination with a column-switching technique. Both sample extraction on a LiChrocart pre-column and elution onto the analytical LiChrospher column, were performed automatically and concomitantly using conventional HPLC equipment in conjunction with an OSP-2 on-line sample preparator from Merck combined with UV detection. Recoveries were found to be 96.7 and 96.4% for 2 μg/ml bupivacaine and etidocaine, respectively. Lower limits of quantification were found to be 0.05 μg/ml plasma for both of the compounds.

Keywords: Bupivacaine; Etidocaine

1. Introduction

A permanent matter of interest is the search for methods that allow rapid, sensitive, selective and reproducible determination of local anaesthetics from human plasma, urine and cerebrospinal fluid. An excellent overview of these efforts has recently been published by Yu et al. [1]. Whereas in the past pre-treatment of biological samples (e.g. solvent extraction) was mandatory, modern approaches use new techniques that allow direct injection into the chromatographic system. These approaches include new stationary phases, use of pre-columns and the development of column-switching techniques [2–6] and they allow sufficient recoveries obtained from human plasma but are still subject to further improvement.

This paper describes a method that allows the rapid and precise determination of the local anaesthetics bupivacaine and etidocaine (Fig. 1) from biological fluids. This method uses a fully automated system with solid-phase extraction in combination

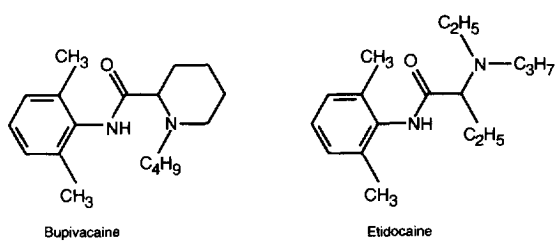

Fig. 1. Structures of (a) bupivacaine and (b) etidocaine.

*Corresponding author.

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with a column-switching technique. Both sample extraction on a LiChrocart pre-column and elution onto the analytical LiChrospher column, were performed automatically and concomitantly using conventional HPLC equipment in conjunction with an OSP-2 on-line sample preparator from Merck combined with UV detection.

2. Experimental

2.1. Materials

Pure drug substances of bupivacaine and etidocaine hydrochloride were a kind gift from Astra (Deggendorf, Switzerland). All other chemicals were obtained from commercial sources and were of analytical grade.

2.2. Instrumentation

The system consisted of two pumps (Models L-6200 and L-6000) combined with a solvent selector, an AS-2000 autosampler and an on-line sample preparator (Model OSP-2, all Merck, Darmstadt, Germany). The device, which is described in detail in Refs. [4] and [6], allows on-line sample extraction of a sample while the previously extracted sample on the previous cartridge is eluted onto the analytical column. The temperature of the analytical column was controlled by a column oven. UV detection of separated local anaesthetics was performed by a variable-wavelength UV detector (Model Spectroflow 773, Kratos, Ramsey, NJ, USA). Data acquisition and analysis were performed by a Tandon personal computer using Interfaces (Series 900) and software (Turbochrom, version 3.0), the latter two were purchased from Perkin-Elmer-Nelson (Cupertino, CA, USA).

2.3. Columns

LiChrospher 60 RP-select B cartridges (4×4 mm I.D.; particle size 10 μm, Darmstadt, Germany) were used for the OSP-2. LiChrospher 60 RP-select B (250×4 mm I.D.; particle size 5 μm) combined with a 60 RP-select B guard column (4×4 mm I.D.; particle size 5 μm; both Merck) was used as analytical column. The column temperature was kept at 50°C.

2.4. Sample preparation

Samples of blood (4 ml) or cerebrospinal fluid (1 ml) were drawn in EDTA-coated or native tubes, respectively. After immediate centrifugation at 4°C plasma was separated. Plasma or liquor samples were stored at −70°C pending analysis. During analysis, plasma or liquor samples (injection volume 100 μl for both organic matrices) were injected using an autosampler AS-2000 (Merck). Drugs were separated from the organic matrix by on-line solid-phase extraction using the on-line sample preparator OSP-2 (Merck). OSP-2 cartridges could be used up to five times.

2.5. HPLC conditions

Samples were loaded onto cartridges using 0.02 *M* diammonium hydrogenphosphate, adjusted for pH 8.5 by NaOH as mobile phase (solvent I). Washing of cartridges was performed by 100 m*M* sodium phosphate buffer, pH 7.0 (solvent II). Mobile phase for elution consisted of a linear gradient from 12 m*M* tetraethylammonium phosphate buffer, pH 3.0 to 1:9 (v/v) 12 m*M* tetraethylammonium phosphate–acetonitrile. After elution, cartridges were washed by 1:1 (v/v) acetonitrile–water (solvent III) and preconditioned subsequently by solvent I. Details are given in Table 1.

2.6. Detection

Local anaesthetics were detected by UV detection at 210 nm wavelength.

3. Results and discussion

3.1. Extraction

Spiked plasma samples for quality control were prepared using pooled human plasma obtained by the hospital blood donation centre. Recoveries were determined by comparing peak areas of directly

Table 1
Experimental details of extraction, elution and conditioning

Time (min)	OSP-2				Solvent sector solvent[c]	Pump L-6000 flow-rate (ml/min)	Pump L-6200		Comments
	Valve 1[a]	Valve 2[a]	Clamp[b]	Move			Eluent B[d] (% Flow)	Flow-rate (ml/min)	
0.0	ON	ON	ON	OFF	I	0.5	0	1.5	Loading of pre-column; equilibration of analytical column
6.0	ON	ON	ON	OFF	I	1.22	0	1.5	
8.0	ON	ON	ON	OFF	II	1.46	22	1.5	Pre-conditioning for elution of tubing of pre-column
10.0	OFF	ON	ON	OFF	II	1.7	22	1.5	
12.9	OFF	ON	ON	OFF	II	1.7	22	1.5	
13.1	ON	ON	ON	OFF	II	0.3	22	1.5	By-pass of pre-column
13.6	OFF	OFF	ON	OFF	II	0.3	22	1.5	Opening of clamp
13.7	OFF	OFF	OFF	OFF	III	0.5	22	1.5	
13.8	OFF	OFF	OFF	ON	III	1.0	22	1.5	Move of pre-column to analytical side of OSP-2
13.9	ON	OFF	ON	OFF	III	1.0	22	1.5	Washing of pre-column
14.0	ON	ON	ON	OFF	III	1.0	22	1.5	Start of analytical separation and data acquisition
18.9	ON	ON	ON	OFF	I	1.0	22	1.5	
31.0	ON	ON	ON	OFF	I	0.5	0	1.5	Start of next cycle

[a] OFF=bypass of cartridge.
[b] OFF=cartridge holding clamp open.
[c] I: 0.02 *M* diammonium hydrogenphosphate; adjusted for pH 8.5; II: 100 m*M* sodium phosphate buffer; III: 1:1 (v/v) acetonitrile–water.
[d] Linear gradient of eluent A and B: fractional flow of the eluent B. Difference to 100% is eluent B. A: 12 m*M* tetraethylammonium phosphate buffer, pH 3.0 and B: 1:9 12 m*M* tetraethylammonium phosphate–acetonitrile.

injected standards in water with peak areas of the respective compound after solid-phase extraction from spiked plasma samples. Recoveries±S.D. (n=13) were found to be 96.7±1.2 and 96.4±1.4% for 2 μg/ml bupivacaine and etidocaine, respectively. Matrix effects were assessed using spiked plasma samples (concentration of bupivacaine and etidocaine of 1 μg/ml) from six healthy human subjects (5 or 6 determinations each). Recoveries were 98.7±5.8 and 111±5.2% for 1 μg/ml bupivacaine and etidocaine, respectively. The influence of freeze–thaw cycles (n=2) on recovery was studied at concentrations of 0.05 and 2.0 μg/ml of bupivacaine and etidocaine (n=5–6). At bupivacaine concentrations of 0.05 and 2.0 μg/ml on average an increase of 16.0% and a loss of 1.96% was observed. For etidocaine there was a loss of 13.1 and 11.9% at these respective concentrations.

3.2. Chromatography

The selectivity of the chromatographic system is shown in Fig. 2 (typical chromatogram of a plasma sample spiked with etidocaine and bupivacaine). For all determinations an injection volume of 100 μl was used. Baseline separation of these two compounds was obtained with retention times of 12.2 and 15.4 min, respectively. Similar results can be obtained for determination in cerebrospinal fluid (Fig. 3a). Bupivacaine and etidocaine are metabolised extensively [12]. Due to the higher hydrophilicity of the known metabolites it is expected that coelution with the corresponding parent compounds will not occur. Unfortunately, these metabolites were not available to us. Therefore, the hypothesis could not be tested, nevertheless, the assumption is supported by the fact that the slightly less hydrophobic compound

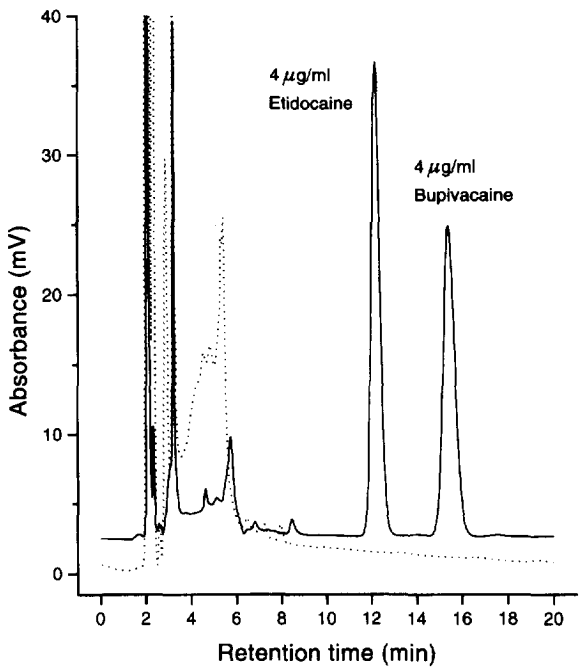

Fig. 2. Solid curve represents a chromatogram of 4 μg/ml etidocaine and 4 μg/ml bupivacaine in human plasma. The chromatogram of blank plasma is displayed for comparison by the dotted curve. UV detection was at 210 nm.

mepivacaine is eluted rapidly within 5 min after injection. However, a systematic evaluation was not possible, because these metabolites were not available.

Calibration curves showed a good linearity between peak areas and concentrations of the two drugs in plasma. Correlation coefficients (r^2) were always ($n=4$ or 5 experiments per dose level) greater than 0.998 for all compounds in the range of 0.025–4 μg/ml. This sensitivity could be increased by the use of 400-μl samples volumes, for which the chromatographic system showed still linearity.

3.3. Lower limit of quantification

The lower limit of quantification (LLQ) was defined according to Shah et al. [13] as the lowest concentration at which single measurements did not vary from mean values by more than 20% coefficient of variation (C.V.). Bupivacaine and etidocaine fulfilled this criterion at concentrations of ≥0.05 μg/ml within each measurement series and for accuracy (Table 2). Therefore the LLQ was set to be 0.05 μg/ml for both compounds, although variability between runs was slightly higher for bupivacaine at

Table 2
Within- and between-runs variabilities for bupivacaine and etidocaine

Concentration (μg/ml)	Assay variability (C.V. %)		Accuracy (%)[a] ($n=17$–26)
	Between-run ($n=4$)	Within-run ($n=4$–5)	
Bupivacaine			
0.025	30.7	6.9	62.1
0.05	25.2	3.7	9.8
0.1	13.8	8.5	7.1
1.0	3.9	8.3	2.2
2.0	3.8	5.1	3.0
4.0	4.0	4.4	0.1
Etidocaine			
0.025	20.3	5.1	31.3
0.05	18.3	9.3	10.7
0.1	13.7	8.7	6.8
1.0	12.7	6.9	0.9
2.0	6.5	7.6	3.6
4.0	4.9	7.1	0.1

[a] =Calculated as percent deviation from true value.

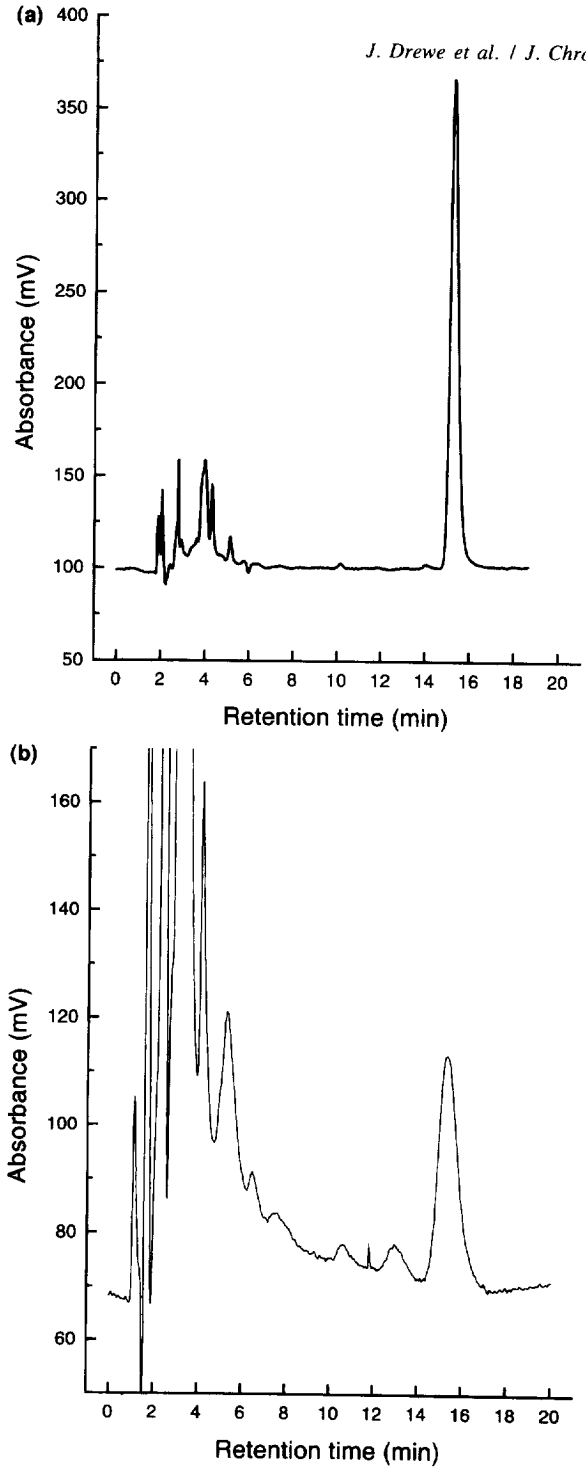

Fig. 3. (a) Representative chromatogram of bupivacaine in human (patient) cerebrospinal fluid after failed spinal anaesthesia. Cerebrospinal fluid sample was obtained by second puncture. Bupivacaine concentration was 26.36 μg/ml. (b) Representative chromatogram of bupivacaine in human plasma after axillary plexus blockade. Bupivacaine concentration was 1.38 μg/ml.

this concentration. This appears to be justified, since within each run, results were calibrated and accuracy was acceptable at this concentration of bupivacaine.

The sensitivity of the present method is in the same order as that of other methods recently published [14,15]; however our method is fully automated and allows rapid determinations, making it especially suitable for therapeutic drug monitoring.

The clinical applicability of the method is demonstrated by two examples: (1) in Fig. 3a the cerebrospinal fluid concentration of bupivacaine was determined after a failed spinal anaesthesia. Cerebrospinal fluid was obtained after a second puncture; (2) in Fig. 3b the plasma concentration of bupivacaine was determined in a patient who underwent axillary plexus blockade.

4. Conclusion

This paper describes a fast chromatographic method with on-line sample extraction to determine bupivacaine and etidocaine from a small sample of biological matrix. The sensitivity allows for determination of the range of plasma concentrations that is observed after administration of clinically relevant doses of bupivacaine intramuscularly [7], epidurally [8], intrathecally [9,10] and intrapleurally [11]. Since this method does not require additional (manual) sample extraction it is especially useful for therapeutic drug monitoring purposes in cases of potential toxic drug reactions.

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