Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis
35 (2004) 593–598
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Determination of some local anesthetics in human serum by gas chromatography with solid-phase extraction

M. Baniceru [a,*], O. Croitoru [a], S.M. Popescu [b]

[a] *Faculty of Pharmacy, University of Medicine and Pharmacy, Peru Rares 4, 1100 Craiova, Romania*
[b] *Faculty of Dental Medicine, University of Medicine and Pharmacy, Petru Rares 4, 1100 Craiova, Romania*

Received 18 October 2003; received in revised form 31 January 2004; accepted 16 February 2004

Available online 13 April 2004

Abstract

A method of analysis based on solid-phase extraction coupled with capillary gas chromatographic system for determination of mepivacaine, bupivacaine and lidocaine from human serum was developed. As extraction sorbents were used Chromosorb 103, Tenax-GC and Chromosorb T. The best extraction sorbent proved to be Chromosorb 103. Their recoveries ranged from 91 to 94% at the target concentrations of approx. 1.5 $\mu g\,ml^{-1}$ in serum. Relative standard deviation of the recoveries ranged from 3.11 to 5.30 at these concentrations. As internal standard was used lidocaine. The chromatographic analysis was performed on a gas chromatograph equipped with a capillary column, HP-Innowax, and flame ionisation detector. Samples were injected in splitless mode. This method was applied in a stomatological clinic to healthy volunteers to whom superior–posterior alveolar nerve block anesthesia with mepivacaine was administered.

Keywords: Gas chromatography; Mepivacaine; Lidocaine; Bupivacaine; Ropivacaine; Human serum; Solid-phase extraction

1. Introduction

Mepivacaine, ropivacaine, bupivacaine and lidocaine are amino-amide type local anesthetics. Local anesthetics are utilised to produce anesthesia by blocking the conduction of impulses in nerve fibers. Since they are toxic, causing cardiovascular and central-nervous disorders, the therapeutic blood-plasma/serum concentrations should not exceed certain limits.

Absorption and distribution of amide local anesthetics depend on factors connected to location or method of administration and varies with blood flow characteristics, plasma protein binding, plasma pH and the physical properties of the local anesthetic. It is also worth mentioning that some commonly administered drugs such as opioids, cimetidine or propranolol may affect the metabolism or pharmacokinetics of the amide local anesthetics during medical treatment requiring local anesthesia.

Similarly to many other drugs, local anesthetics bind to plasma proteins to some extent. α_1-Acid glycoprotein and albumin [1] are the two serum proteins involved with amide local anesthetics. α_1-Acid

* Corresponding author. Tel.: +40-251-523929; fax: +40-251-523929.
E-mail address: mbaniceru@k.ro (M. Baniceru).

0731-7085/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2004.02.012

glycoprotein readily binds drugs but has a limited capacity for them, while albumin has a low affinity and a large capacity. The percentage bound to the two proteins decreases as the concentration of a local anesthetic in serum increases. The free fraction of a drug in serum influences its pharmacokinetics and toxicity and active uptake or diffusion into surrounding tissue [2].

The anesthetics have been analysed by gas chromatography [3–16], liquid chromatography [17–25] or capillary electrophoreses [26,27].

Many authors have attempted to determine the local anesthetic-free fraction from serum or plasma by using ultrafiltration [18,22–24] and microdialysis [18,20]. In some other works, the free fraction of the anesthetics was estimated by analysing the correlation between the degree of their protein binding, pH levels, temperature and solutes concentration [14,16,23,25].

As methods for sample preparation liquid–liquid extraction [6,8,9,21], solid-phase extraction [10–13,19] and solid-phase microextraction [3–5,7,16] have been used.

Kakinohana and Okuda [25] found out that local anesthetic-protein binding is hydrophobic and Taheri et al. [2] hydrophobic and electrostatic. The later concluded that the hydrophobic bindings were much stronger than the ionic ones, after estimating the affinity of neutral lidocaine to be at least eight times the one of protonated lidocaine. But is well known that ionic forces are stronger than the hydrophobic ones. That is why we consider that at an alkaline pH—which is necessary to pass the drug in a neutral base in view of performing chromatographic analysis—the protein–drug bindings are only through the hydrophobic forces and the determined quantity stands for the entire quantity of drug in plasma or serum. The bound fraction is in fact the one existing in ionised state (bound by the protein through ionic and hydrophobic forces), at the physiological pH and 37 °C, according to pK_a values of the anesthetics.

Since any dosing method modifies the existing equilibrium between the free and the bound fraction in serum or plasma, we consider that the free fraction cannot be determined precisely.

Our analysis method is based on solid-phase extraction. As solid phases Chromosorb 103 (cross-linked polystyrene), Tenax-GC (2,6-diphenyl-*p*-phenylene oxide) and Chromosorb T were used. This type of sorbents are much more lipophilic than surface-modified silica gels (C_{18} and cyanopropyl, Bond-Elut, C_{18} Sep-Pak [10,12,19]) and they also have a higher capacity of sample loading due to their polymeric structure. The analytes are stronger adsorbed on these types of sorbents due to orientation and induction intermolecular forces than with C_{18}-silica in which only dispersion forces occurred. The best results concerning recoveries of mepivacaine were obtained on Chromosorb 103.

This method was applied to healthy volunteers to whom an anesthesia with mepivacaine was administered. The precision (defined as the relative standard deviation, CV, of replicate analysis) and the accuracy (defined as the recovery %) of the assay were evaluated.

2. Experimental

2.1. Chemicals and reagents

Chromosorb 103 and Chromosorb T were purchased from Merck; Tenax GC from Applied Science Laboratories Inc.; lidocaine, mepivacaine hydrochloride, bupivacaine hydrochloride from Sigma Aldrich Co., Germany; ropivacaine hydrochloride from Pharm. Chemical Corporation, Shanghai; acetonitrile from Riedel de Haën.

2.2. Instrumentation and chromatographic conditions

The gas chromatographic analysis was performed using Hewlett-Packard model HP 6890 equipped with split-splitless injector and flame ionisation detector. The column was a capillary column HP Innowax 30 m × 0.25 mm, ID ×0.25 μm film thickness, purchased from Hewlett-Packard. The column flow rate of the hydrogen carrier gas was 2 ml min^{-1}. Samples were injected in splitless mode. Injector and detector temperature was 240 °C. In order to avoid interference of the analytes with endogenous compounds, the oven temperature was programmed in different conditions, depending on serum composition. The chromatograms from Figs. 1 and 2 were performed in the following conditions: after initial hold of 1 min at 50 °C, the temperature was increased at 25 °C min^{-1} to 150 °C, then another increase at 5 °C min^{-1} to 240 °C where

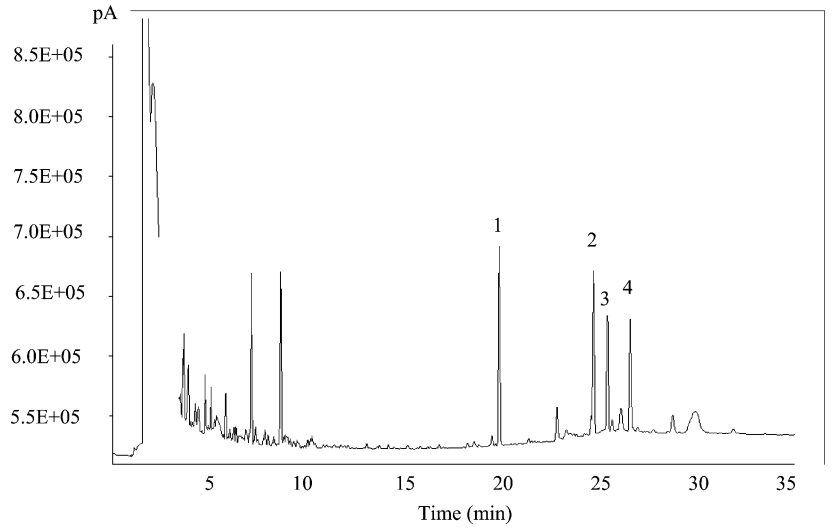

Fig. 1. Chromatogram for spiked serum with local anesthetic 1.56 μg ml^{-1} mepivacaine, 1.485 μg ml^{-1} ropivacaine, 1.56 μg ml^{-1} bupivacaine and 1.6 μg ml^{-1} lidocaine (IS). Condition: column HP Innovax 50 °C (1 min) $\xrightarrow{25\,^{\circ}\mathrm{C\,min^{-1}}}$ 150 °C $\xrightarrow{5\,^{\circ}\mathrm{C\,min^{-1}}}$ 240 °C (12 min); flow rate 2 ml min^{-1} H_2; detector FID; sample size 1 μl. Peaks: (1) lidocaine; (2) mepivacaine; (3) ropivacaine; (4) bupivacaine.

it was kept for 12 min. Gas make up flow (nitrogen) 35 ml min^{-1}. The injected volume was 1 μl.

2.3. Sample preparation

The stock solution of the analytes was prepared by adding 4 mg mepivacaine, 3.8 mg ropivacaine, 4 mg bupivacaine in approx. 6 g (5.9755 g) acetonitrile. Lidocaine was used as internal standard (4.1 mg lidocaine in 5.9832 g acetonitrile).

Serum standard was prepared by adding at 1 ml of serum, 3 μl stock solution and 3 μl internal standard solution. The pH of the serum standard was adjusted to 12 by adding 100 μl of 30% NaOH.

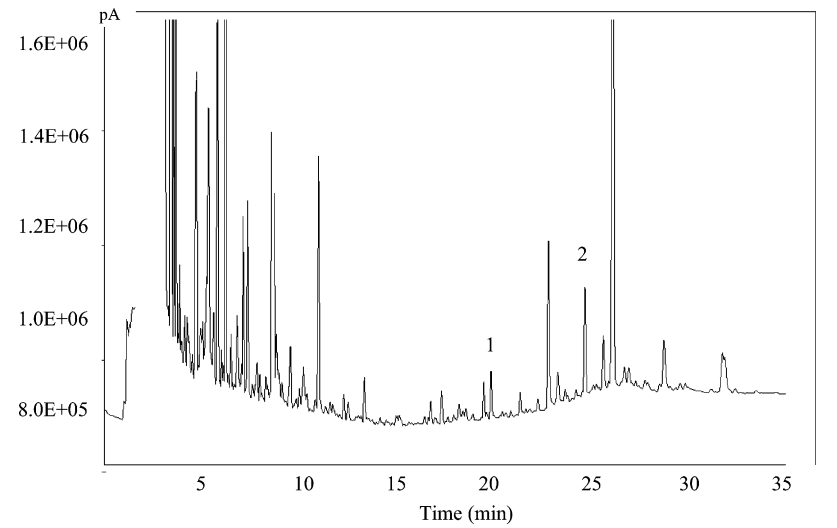

Fig. 2. Chromatogram for volunteer 3 serum, 15 min after injection. Condition: column HP Innovax 50 °C (1 min) $\xrightarrow{25\,^{\circ}\mathrm{C\,min^{-1}}}$ 150 °C $\xrightarrow{5\,^{\circ}\mathrm{C\,min^{-1}}}$ 240 °C (12 min); flow rate 2 ml min^{-1} H_2; detector FID; sample size 1 μl. Peaks: (1) lidocaine (IS); (2) mepivacaine.

Venous blood was collected at a stomatologic clinic from three healthy volunteers who were previously anesthetised with 1.7 ml 3% mepivacaine. The samples have been collected at 5, 15, 30 and 60 min from injection. The serum was obtained after blood centrifugation, 2 h after sample collection. At 1 ml serum, 1.5 μl internal standard solution (lidocaine in acetonitrile) and 50 μl of 30% NaOH were added.

2.4. Solid-phase extraction

Solid-phase extraction of sample was made on Chromosorb 103, 80–100 mesh (0.5 g sorbent), Tenax GC, 80–100 mesh (0.23 g sorbent) and Chromosorb T 40–60 mesh (0.8 g sorbent).

The extraction was performed according to the following steps:

1. Sorbent conditioning was performed with 10 ml acetonitrile and 10 ml methanol;
2. The sorbent was washed with 5 ml buffer solution, pH 10;
3. The sample was loaded into the cartridge;
4. As washing phase 2× 2.5 ml of buffer solution pH 10 were passed through the cartridge;
5. For the elution of the anestethics elution 4 ml acetonitrile was utilised.

The concentration of mepivacaine in human serum was determined using Chromosorb 103.

3. Results and discussion

3.1. Optimization of separation conditions

The use of Chromosorb 103 and Tenax GC as adsorbents in gas–solid chromatography separation of amines, amides, aldehydes, alcohols and other compounds has leaded us to the idea of testing them as extraction sorbents for the above studied compounds, that have an amino-amide structure. Not only are these sorbents completely lipophilic, but they also retain the polar and polarisable analytes by intermolecular forces stronger than with C_{18}-silica or cyanopropyl-silica. C_{18}-silica and cyanopropyl-silica include residual sylanol groups that strongly absorb the water (sample solvent). If the sample solvent is strongly retained, the analytes retention will be weak. Observing the procedure steps used at SPE is very important. In paper [10] 1 ml of blood sample, with addition of drugs, was diluted with 10 ml distilled water. At pH 7 of sample, a fraction from local anesthetics was found in ionised state and was eluted by the rinsing solvent (water), before the elution of the analytes with

Table 1
Within-run accuracy and precision of serum sample with added local anesthetics

Substance	MV[a] (μg ml^{-1})	TV[b] (μg ml^{-1})	S.D.[c]	CV (%)[d]	RE (%)[e]
Extraction sorbent: Chromosorb 103					
Mepivacaine	1.513	1.560	0.030	2.01	96.99
Ropivacaine	1.327	1.485	0.081	6.14	89.36
Bupivacaine	1.436	1.560	0.070	4.87	92.03
Extraction sorbent: Chromosorb T					
Mepivacaine	1.260	1.560	0.055	4.36	80.79
Ropivacaine	1.390	1.485	0.011	0.80	93.60
Bupivacaine	1.372	1.560	0.044	3.22	87.95
Extraction sorbent: Tenax GC					
Mepivacaine	1.199	1.560	0.018	1.51	76.90
Ropivacaine	1.206	1.485	0.073	6.03	81.19
Bupivacaine	1.092	1.560	0.132	12.10	70.00

[a] Mean value of three determinations.
[b] True value.
[c] Standard deviation.
[d] Relative standard deviation.
[e] Recovery.

chloroform–methanol occurred. Similar situations are found in papers [12,19]. Also, in acidic or neutral solutions, local anesthetics are weakly absorbed by the hydrophobe sorbent because they exist in a great extend in ionised state. In this work, after conditioning of the sorbent, its pH was brought to the one of the sample (alkaline).

In order to get rid of endogenous compounds a pH 10 buffer solution was used. Because the analytes are soluble in both polar and nonpolar organic solvents, the cartridge should not be washed with any of them, when aiming to purify the sample of endogenous compounds.

For the elution of analytes the following solvents have been tested: diethyl ether, acrylonitrile, methanol, acetonitrile. The best recoveries were obtained with acetonitrile. Evaporation to dryness was not necessary.

In Fig. 1 chromatogram for spiked serum with local anesthetic: 1.56 μg ml^{-1} mepivacaine, 1.485 μg ml^{-1} ropivacaine, 1.56 μg ml^{-1} bupivacaine and 1.6 μg ml^{-1} lidocaine (IS), in the above-mentioned conditions is displayed. Fig. 2 presents a chromatogram for volunteer 3 serum, 15 min after injection.

3.2. Precision and accuracy

In Table 1 accuracy and precision values for the three extraction sorbents are presented. From this table one can notice that the best recovery was obtained for mepivacaine on Chromosorb 103. Good recoveries were also obtained for ropivacaine and bupivacaine on Chromosorb T. Since extraction is obviously a very important step, three extraction have been performed on Chromosorb 103. Each extract was then three times chromatographied. The results are mentioned in Table 2. The average of the three recoveries was 93.95 for mepivacaine, 91.09 for ropivacaine and 92.20 for bupivacaine. Standard deviation values were 3.11 for mepivacaine, 5.30 for ropivacaine and 5.19 for bupivacaine.

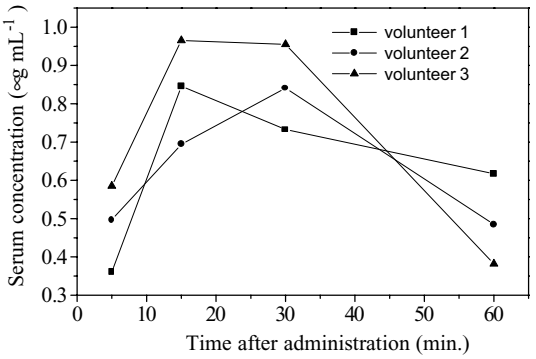

Fig. 3. Mean concentration of mepivacaine in serum versus time plot. Volunteer 1, 69-year-old female; volunteer 2, 32-year-old female; volunteer 3, 61-year-old female.

3.3. Method validation

The limits of quantification determined in this study were approx. 50 ng ml^{-1} for all the studied anesthetics. At drug concentration values in serum the detector response (FID) is linear. The precision and accuracy of the assay were previously presented in Tables 1 and 2.

3.4. Application of the method to biological samples

The present method has been applied for the determination of mepivacaine in serum (total amount of this

Table 2
Inter-extraction accuracy and precision of serum sample with added local anesthetics (extraction sorbent: Chromosorb 103)

Substance	EMV[a] (μg ml^{-1})	MV[b] (μg ml^{-1})	TV[c] (μg ml^{-1})	S.D.[d]	CV[e] (%)	RE[f] (%)
Mepivacaine	1.462, 1.422, 1.513	1.466	1.560	0.0456	3.11	93.95
Ropivacaine	1.347, 1.284, 1.427	1.353	1.485	0.0717	5.30	91.09
Bupivacaine	1.523, 1.382, 1.410	1.438	1.560	0.0746	5.19	92.20

[a] Per extraction mean value.
[b] Mean value of three extraction.
[c] True value.
[d] Standard deviation.
[e] Relative standard deviation.
[f] Recovery.

drug), at different time intervals from injection. After solid-phase extraction (on Chromosorb 103) each serum sample was three times chromatographied and the results are presented in Fig. 3.

The free fraction is the one existing in nonionised state, at physiologic pH and according to pK_a value, representing 33.38% of the total amount. One can notice that the highest mepivacaine concentration value occurs 15–30 min after injection.

4. Conclusion

A solid-phase extraction method and gas chromatographic analysis of some local anesthetics from human serum was presented. Good recoveries were obtained with Chromosorb 103 as extraction sorbent and acetonitrile as elution solvent. Since this method showed good precision and sensitivity it was successfully used in determination of mepivacaine from human serum.

References

[1] G.R. Arthur, J.A.W. Wildsmith, G.T. Tucker, in: J.A.W. Wildsmith, E.N. Armitage (Eds.), Principles and Practice of Regional Anesthesia, 2nd ed., Churchill Livingstone Publishers, New York, 1993, pp. 29–44.
[2] S. Taheri, L.P. Cogswell, A. Gent, G.R. Strichartz, J. Pharmacol. Exp. Therap. 304 (2003) 71–80.
[3] G.R. Strichartz, C.B. Berde, in: Miller, R.D. (Ed.), Anesthesia, 4th ed., vol. 1, Churchill Livingstone Inc., New York, 1994, pp. 489–521.
[4] M. Abdel-Rehim, M. Bielenstein, T. Arvidsson, J. Microcolumn Sep. 12 (2000) 308–315.
[5] M. Abdel-Rehim, M. Bielenstein, T. Arvidsson, J. Microcolumn Sep. 10 (1998) 589–596.
[6] Y. Ding, X. Zhu, B. Lin, Electrophoresis 21 (1999) 1890–1894.
[7] M. Abdel-Rehim, M. Andersson, E. Portelius, L.G. Blomberg, J. Microcolumn Sep. 13 (2001) 313–321.
[8] M. Abdel-Rehim, J. Sep. Sci. 25 (2002) 252–254.
[9] T. Watanabe, A. Namera, M. Yashiki, M. Iwasaki, T. Kojima, J. Chromatogr. B 709 (1998) 225–232.
[10] S. Cherkaoui, J.L. Veuthey, J. Pharm. Biomed. Anal. 27 (2002) 615–626.
[11] R.J.E. Grouls, E.W. Ackerman, H.H.M. Korsten, L.J. Hellebrekers, D.D. Breimer, J. Chromatogr. B 694 (1997) 421–425.
[12] N. Jitsufuchi, K. Kudo, T. Imamura, K. Kimura, N. Ikeda, Forensic Sci. Int. 90 (1997) 103–109.
[13] P. Koivisto, S.K. Bergstrom, E. Markides, J. Microcolumn Sep. 13 (2001) 197–201.
[14] K. Kudo, Y. Hino, N. Ikeda, H. Inoue, S. Takahashi, Forensic Sci. Int. 116 (2001) 9–14.
[15] H. Hattori, S. Yamamoto, T. Yamada, O. Suzuki, J. Chromatogr. B 564 (1991) 278–282.
[16] M. Siluveru, J.T. Stewart, J. Chromatogr. B 690 (1997) 359–362.
[17] T. Ohshima, T. Takayasu, J. Chromatogr. B 726 (1999) 185–194.
[18] N. Laroche, A. Leneveu, A. Roux, B. Flouvat, J. Chromatogr. B 716 (1998) 375–381.
[19] A.G.L. Burm, et al., Anesth. Analg. 84 (1997) 85–89.
[20] M.W.J. Van Hout, W.M.A. Van Egmond, J.P. Franke, R.A. Zeeuw, G.J. Jong, J. Chromatogr. B 766 (2002) 37–45.
[21] A.A. Vletter, W. Olieman, A.G.L. Burm, K. Groen, J.W. Van Kleef, J. Chromatogr. B 678 (1996) 369–372.
[22] M. Abdel-Rehim, G. Carsson, M. Bielenstein, T. Arvidsson, L.G. Blomberg, J. Chromatogr. Sci. 38 (2000) 458–464.
[23] B. Bachmann-Mennenga, J. Biscoping, R. Schurg, E. Sinning, G. Hempelmann, Arzneimittelforschung 41 (1991) 520–524.
[24] B. Bachmann, J. Biscoping, E. Sinning, G. Hempelmann, Acta Anaesthesiol Scand. 34 (1990) 311–314.
[25] K. Lau Chan, Y.C. Wong, Meth. Find Exp. Clin. Pharmacol. 7 (1991) 475–481.
[26] T. Arvidsson, E. Eklund, J. Chromatogr. B 668 (1995) 91–98.
[27] O. Kakinohana, Y. Okuda, Masui 42 (1993) 1488–1496.