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DETERMINATION OF TRIMECAINE METABOLITES IN BLOOD PLASMA BY CAPILLARY ISOTACHOPHORESIS

ZDENĚK STRÁNSKÝ*

Department of Analytical and Organic Chemistry, Faculty of Natural Sciences, Palacký University, 771 46 Olomouc (Czechoslovakia)

ZDENĚK CHMELA

Department of Pathological Physiology, Faculty of Medicine, Palacký University, 771 46 Olomouc (Czechoslovakia)

PAVEL PEČ

Department of Analytical and Organic Chemistry, Faculty of Natural Sciences, Palacký University, 771 46 Olomouc (Czechoslovakia)

and

LUDĚK ŠAFAŘÍK

State Institute for Drugs Control, Prague (Czechoslovakia)

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SUMMARY

A method is proposed for the determination of trimecaine (diethylglycylmesidide) and its de-ethylated metabolites (monoethylglycylmesidide and glycylmesidide) in blood plasma by capillary isotachophoresis. The deproteinated plasma is extracted into chloroform after alkalinization and the total solids in the organic layer are dissolved in acidified 25% 2-propanol. Subsequent isotachophoretic analysis is performed in an operational system consisting of potassium acetate buffer (pH 4.75) as the leading and β-alanine as the terminating electrolyte. The order of the zones corresponds to the molecular weights of the separated compounds. The recovery of all substances of interest is 55% and the limit of determination is 0.05 μg of each substance in 1 ml of plasma.

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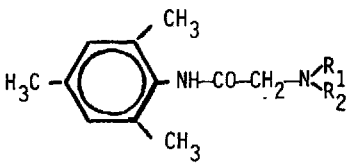
INTRODUCTION

Lidocaine (diethylglycylxylidide, DEGX) and trimecaine (diethylglycylmesidide, DEGM, Fig. 1) are used as heart antiarrhythmics. The origination and behaviour of the metabolites have been studied in detail for lidocaine [1—6]. Biotransformation proceeds in the liver by successive N-de-ethylation and by subsequent hydrolysis to 2,6-xylidine. In the next or parallel steps, hydroxylation to 4-hydroxy-2,6-xylidine, N-hydroxylation and cyclization of monoethylglycylxylidide (MEGX) to imidazolidinones occur. Nelson et al. [1] determined six metabolites in addition to lidocaine by mass spectrometry (MS) with chemical ionization. In some instances it is necessary with intravenous application to observe the parent compound and de-ethylated metabolites, which also have an antiarrhythmic effect, convulsant effect and CNS toxicity. For lidocaine metabolites, about 85% antiarrhythmic activity for monoethylglycylmesidide (MEGM, Fig. 1) and 10% activity for glycylxylidide (GX) in comparison with the parent compound have been found [2].



Compound	R_1	R_2
Trimecaine (DEGM)	C_2H_5	C_2H_5
Monoethylglycylmesidide (MEGM)	C_2H_5	H
Glycylmesidide (GM)	H	H

Fig. 1. Chemical structures of trimecaine, monoethylglycylmesidide and glycylmesidide.

With accumulation of metabolites, the CNS toxicity increases, which may be caused by GX [3]. In most patients the concentrations of metabolites in blood plasma are lower than that of lidocaine, but the opposite situation has also been found [4—6]. In such instances the antiarrhythmic activity may be sufficient even below the therapeutic level of lidocaine and good analytical control is necessary.

The determination of lidocaine and its metabolites has most often been carried out by gas—liquid chromatography (GLC) [3, 7—10], in which case the metabolites require preliminary derivatization [3, 9, 10]. High-performance liquid chromatography [6], MS [1] and combined gas chromatography—mass spectrometry (GC—MS) [11] have also been used.

Capillary isotachophoresis has been used only for the determination of lidocaine in drug form and for its separation from other local anaesthetics [12]. Paper isotachophoresis has been applied for the same purposes [13].

The precise analytical control of trimecaine metabolites has not been described. A course of the transformation analogous to that of lidocaine can be expected, simplified by the fact that with trimecaine hydroxylation of the aromatic nucleus in position 4 cannot occur. Paper chromatography has been used for the separation of the products of trimecaine biotransformation in the

liver homogenate of a rabbit [14]. The determination of trimecaine by GC and thin-layer chromatography has been described [15].

This paper describes the determination of trimecaine and its de-ethylated metabolites in blood plasma. Isotachophoresis, chosen for the final determination, does not require derivatization of the metabolites and it is a more selective method than GLC or HPLC. The disadvantage of the method is its lower sensitivity.

EXPERIMENTAL

An instrument for capillary isotachophoresis (Research Laboratories and Workshops of Palacký University) with coupled columns [16] was used. The pre-separation PTFE capillary was of 0.8 mm I.D. and the analytical capillary was of 0.3 mm I.D. The length of each capillary was 230 mm, and in both instances a conductivity detector was used. The current was 150 μA in the pre-separation and 50 μA in the analytical capillary. Samples were injected using Hamilton microsyringes (1—50 μl).

All the chemicals were of analytical-reagent grade. Chloroform was redistilled before use. Hydrochlorides of DEGM (Mesocain Spofa), MEGM and glycylmesidide (GM, Fig. 1) (prepared in our laboratory) were used as standards.

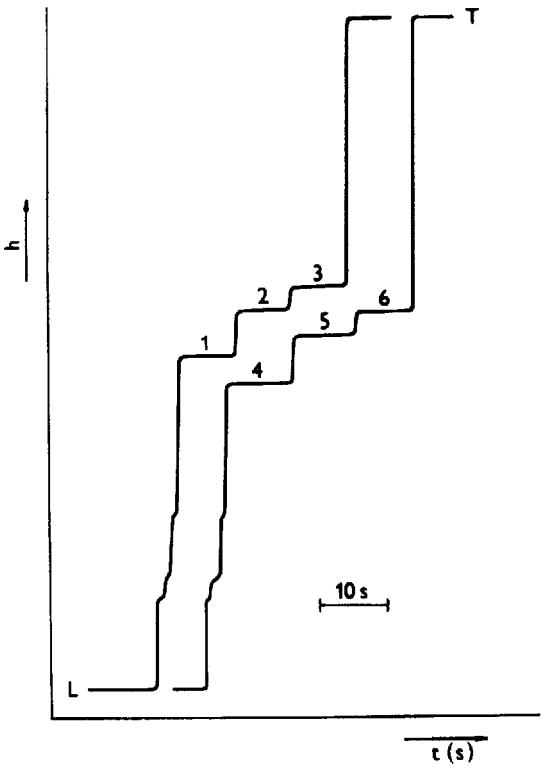


Fig. 2. Separation of model mixtures of trimecaine, lidocaine and their metabolites. 1 μg of each substance was injected. 1 = GM; 2 = MEGM; 3 = DEGM; 4 = GX, 5 = MEGX, 6 = DEGX.

The optimal leading electrolyte was found to be acetate buffer (pH 4.75) containing 0.05% of poly(vinyl alcohol) (Gohsenol GM-14L). The concentration of the leading ion (K^+) was 0.01 mol/l. β-Alanine (0.05 mol/l) was used as terminating electrolyte. The order of zones in this system corresponds to the order of molecular weights. The time required for one analysis was about 30 min. Fig. 2 shows the separation of model mixtures of trimecaine, lidocaine and their metabolites.

For the construction of calibration graphs, 10% (w/w) solutions of dried human plasma in water with contents of 0.33—5.0 μg of DEGM, MEGM and GM were used.

Isolation procedure

A 2-ml volume of human plasma was homogenized in an all-glass Potter—Elvehjem homogenizer with 70 μl of 70% perchloric acid. The deproteinized homogenate was transferred into a 10-ml polypropylene (PP) test-tube, the homogenizer was rinsed with 1 ml of 0.4 *M* perchloric acid and the combined volumes were centrifuged at 5000 *g* for 10 min. The transparent supernatant was transferred into another PP test-tube containing 0.7 g of sodium chloride and 250 μl of 5 *M* sodium hydroxide saturated with sodium chloride and 3 ml of chloroform were added. After intensive shaking (5 min), both phases were separated by centrifugation (15 min) in the cooled centrifuge at 5000 *g*. A 2-ml volume (66.7%) of the chloroform phase was transferred into a conical glass test-tube, chloroform was evaporated at 55°C using a flow of nitrogen, the walls were rinsed with 0.5 ml of chloroform and the solution was again evaporated. The residue in the tip of the test-tube was dissolved in 50 μl of a 0.01 *M* solution of hydrochloric acid in 2-propanol—water (1:1) and finally 50 μl of 0.01 *M* hydrochloric acid were added. A 50-μl volume of this solution was injected.

RESULTS

The proposed method was applied to the determination of trimecaine and its metabolites in a set of patients. The results of the determination in lyophilized plasma samples are summarized in Table I. Lyophilization was performed for safe transportation from the hospital to a distant laboratory. The results of the isotachophoretic determination were evaluated by the absolute calibration method and the calibration graphs, which were rectilinear up to 5.0 μg/ml of any component, were constructed from model samples of dried human plasma.

In order to check whether this approach is reasonable and whether it gives accurate results, the results for trimecaine were compared with those obtained by GC (Table I). The GC determination was performed according to Hawkins et al. [17] with lidocaine as the internal standard. According to the analysis of variance, GC gives less precise results than isotachophoresis.

The isolation procedure described gives a 55% recovery of the amount of DEGM and of both of its metabolites originally present. The remainder stays in the centrifugation pellet after deproteinization. The portion isolated is, however, reproducible and in the given concentration range (0.33—5.0 μg/ml)

TABLE I

CONTENT OF HYDROCHLORIDES OF TRIMECAINE AND METABOLITES (μg/ml) IN PLASMA

A 100-mg amount of trimecaine was administered to a patient during 1 h and the sample was taken 30 min later.

Sample	DEGM (ITP*)	DEGM (GLC)	MEGM (ITP)	GM (ITP)
1	1.3	1.35	0.1	0
2	1.4	1.4	11.3	0.35
3	1.35	1.2	0.05	0.05
4	1.3	1.4	0.1	0.05
5	1.0	—	0	0
6**	5.3	—	0.2	0.1
7**	2.8	—	0.15	0
8	1.05	—	0	0
9	0.4	0 3	0	0
10	0.95	1.08	0	0
11	1.05	1.15	0	0
12	0.6	0.5	0	0

*ITP = isotachophoresis.
**Samples from patients after anaesthesiological application of trimecaine.

it does not change. The relative standard deviation of the method is 5% in the range 0.5—5.0 μg/ml in plasma and the limit of determination is 0.05 μg/ml.

In this study, one result with an extremely high level of MEGM (11.3 μg/ml) was noted whereas the levels of DEGM and GM were in normal ranges. This case confirms the conclusions about different rates of metabolism in various patients [4—6] and draws attention to the possibility of the individual toxicity of trimecaine or lidocaine.

DISCUSSION

Model experiments showed that on direct injection of blood plasma only samples containing μg/ml levels of DEGM and its metabolites could be analysed by isotachophoresis. In addition to its low sensitivity, the direct analysis would be impractical with regard to the high content of Na^+, which increases the analysis time disproportionately even when using coupled columns. Therefore, extraction concentration of the sample was necessary.

The measurement of distribution ratios between an alkaline aqueous solution and an organic solvent (toluene, ethyl methyl ketone, tetrachloromethane, chloroform, dichloromethane and diethyl ether) revealed that only the last three organic solvents permitted nearly quantitative one-step extractions with the same volumes of the phases. From solutions of dried plasma the recovery of DEGM and its metabolites was, however, much lower and strongly dependent on the conditions, as illustrated in Fig. 3.

Even though the recovery gradually increased with increasing pH and ionic strength of the "aqueous phase", the reproducibility of the results remained very poor. An optimization test revealed that four significant factors affect

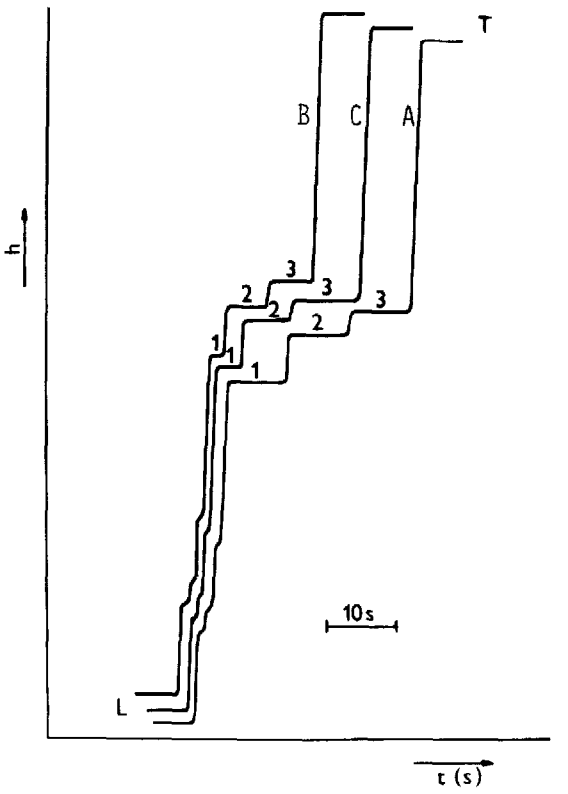


Fig. 3. Effect of extraction conditions on recovery. (A) Extraction of an equimolar mixture of DEGM, MEGM and GM from an aqueous solution into diethyl ether. (B) Extraction from a 10% solution of plasma containing the same amount of mixture as in A; the sample was extracted 24 h after preparation and before extraction was rendered alkaline with 1 ml of 2 *M* sodium hydroxide. (C) Sample as in B, saturated with sodium chloride before extraction.

the reproducibility: deproteinization, choice of the solvent, dissolution of the residue and the age of plasma solution at GM.

Deproteinization leads to a marked decrease in the recovery (to the mentioned 55%), but at the same time it levels the recoveries of single components and increases the reproducibility. The use of chloroform increased the reproducibility compared with diethyl ether. In the evaporation residue after isolation, some lipids are present that prevent the dissolution of isolated bases. Their influence was removed by addition of 2-propanol.

The time factor is significant only with GM.

The amount of GM found decreases if the sample of plasma solution is allowed to stand for a long period. Such losses of GM may be caused by irreversible conjugation to protein components of the plasma and/or by degradation through the effect of aminooxidases.

To investigate this aspect, the activity of serum monoaminooxidase in the solution of dried plasma used (0.014 I.U./ml) and the activity of plasma benzylaminooxidase (0.22 I.U./ml, i.e., 3.6 nkatal) were determined. We further compared the decrease in GM content with samples in the presence and absence of inhibitors of aminooxidases (10^{-4} mol/l semicarbazide, potassium

TABLE II

LOSS OF GM IN A 10% SOLUTION OF PLASMA AFTER 24 h AT 25° C

Composition of sample	GM present (μg/ml)	GM found (μg/ml)	Loss (%)
GM	5.0	2.9	42
GM + SC*	5.0	3.6	28
GM, DEGM, MEGM	5.0	2.8	44
GM, DEGM, MEGM + SC*	5.0	3 7	26
GM	5.0	2.8	44
GM + KCN**	5.0	2.8	44
GM, DEGM, MEGM	5.0	3.0	40
GM, DEGM, MEGM + KCN**	5.0	3.0	40
GM	5.0	2.9	42
GM + DABY***	5.0	2.8	44
GM, DEGM, MEGM	5.0	3.1	38
GM, DEGM, MEGM + DABY***	5.0	3.1	38

*In the presence of 10^{-4} mol/l of semicarbazide (SC).
**In the presence of 10^{-4} mol/l of potassium cyanide (KCN).
***In the presence of 10^{-4} mol/l of 1,4-diaminobutyne (DABY).

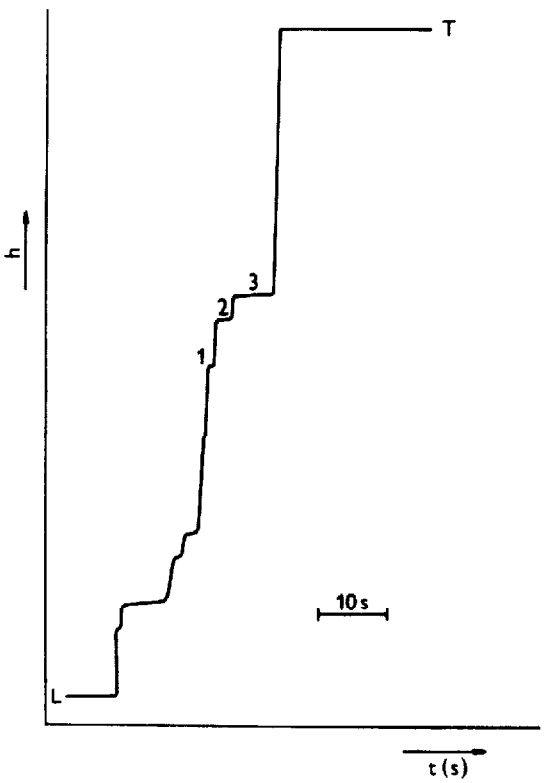


Fig. 4. Record of isotachophoresis analysis of a patient's plasma after 6 h of trimecaine infusion. The total amount of trimecaine administered to the patient was 360 mg. The sample was taken 4 h after the occlusion of infusion; 30 μg of isolated solution were injected. Amounts found per 1 ml of plasma: 1, 0.22 μg of GM; 2, 0.63 μg of MEGM; 3, 1.88 μg of DEGM.

cyanide and 1,4-diaminobutyne [18]), for GM alone and in the presence of DEGM and MEGM. Only semicarbazide led to the reduction in the decrease in GM (Table II). From the results, it is evident that the GM losses are probably caused by chemical rather than enzymatic reactions and that semicarbazide acts as a competitive reagent rather than as an inhibitor. In any event, GM must be a very poor substrate for aminooxidases.

Fig. 4 gives a typical isotachophoresis record for a plasma sample from a patient treated by trimecaine infusion and demonstrates the clinical application of the method.

It can be concluded that the described determination of trimecaine and its metabolites is a sufficiently sensitive and precise method for the needs of clinical analysis.

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