Journal of Chromatography, 308 (1984) 181—187
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2105

MONITORING OF *S*- AND *R*-TOCAINIDE IN HUMAN PLASMA AFTER HEPTAFLUOROBUTYRYLATION, SEPARATION ON CHIRASIL-VAL® AND ELECTRON-CAPTURE DETECTION*

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(First received December 28th, 1983; revised manuscript received January 31st, 1984)

SUMMARY

The conditions for the heptafluorobutyrylation of tocainide have been studied. An almost instantaneous reaction was obtained with 0.01% of heptafluorobutyric anhydride in toluene at 40°C. Higher anhydride concentration caused degradation of the initially formed derivative, mainly by the loss of water, as shown by mass spectral analysis.

Tocainide was isolated from plasma by extraction into dichloromethane at alkaline pH. Gas chromatographic separation was performed with a fused-silica capillary column coated with a methyl silicone gum. The enantiomers were separated on a glass capillary column coated with Chirasil-Val®.

Upon analysing 0.1 ml of plasma eight times the precision was 4.7% at the 10 μmol/l level for the *S*-form of tocainide.

INTRODUCTION

Tocainide [2-amino-N-(2,6-xylyl)propanoic acid amide] is an orally active antiarrhythmic drug (Fig. 1). Its determination in biological samples has been performed by liquid chromatographic [1—6] and gas chromatographic [7—13]

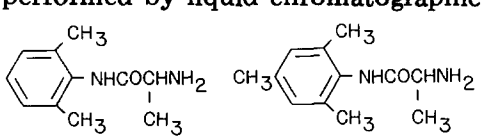

Fig. 1. Chemical structures of tocainide (left) and the internal standard (H 155/73, right)

*Presented at the Annual Meeting of The Swedish Academy of Pharmaceutical Sciences, 1982, and The 1st International Symposium on Drug Analysis, Brussels, 1983.

0378-4347/84/$03.00

methods. Under certain conditions the steady-state concentration of tocainide in plasma is high enough to allow the use of gas chromatography with flame-ionization detection of the free base [10]. Normally, the primary amino group is blocked. This can be accomplished by perfluoroacylation [7, 9, 11, 12] or by Schiff base formation [13]. Flame-ionization [9, 12], electron-capture [8, 11] and nitrogen-selective [12, 13] detection have all been used.

As an alternative to the liquid chromatographic [2] and Schiff base [13] methods developed in our laboratories we have now investigated the possibility of using a method based on the heptafluorobutyryl derivative. The aim was also to reduce the sample volume required and to lower the detection limit. Moreover, it was of interest to obtain a derivative suitable for the chiral resolution of the enantiomers instead of using an optically active derivatizing agent [12].

We report here conditions for the acylation of tocainide with heptafluorobutyric anhydride and a complete method for the determination of the individual enantiomers by electron-capture gas chromatography after separation on Chirasil-Val®. Detailed studies of the acylation reaction and the degradation when an excess of anhydride is used will be published elsewhere [14].

EXPERIMENTAL

Chromatography

A Varian 3700 gas chromatograph equipped with a nitrogen-selective detector was used with a glass column (120 × 0.2 cm I.D.) filled with 3% OV-17 on Gas-Chrom Q, 100—120 mesh. The nitrogen carrier gas flow-rate was 30 ml/min. Hydrogen and air flow-rates were 5 and 175 ml/min, respectively. The temperatures of the injector, oven and detector were 250° C, 200° C and 300° C, respectively.

Electron-capture gas chromatography was performed with a Hewlett-Packard 5730 instrument with a glass column (200 × 0.2 cm I.D.) filled with the same packing as above. Argon—methane (95:5) was used as carrier gas with a flow-rate of 20 ml/min. The temperatures were as above.

The tocainide racemate was also chromatographed on a fused-silica capillary column (20 m × 0.32 mm I.D., siloxane-deactivated) coated with a methyl silicone gum, in the Hewlett-Packard instrument with an electron-capture detector. The inlet pressure was 80 kPa (helium). The split flow-rate was 10 ml/min, and that of the argon—methane used for the electron-capture detector make-up gas was 20 ml/min. The column was maintained isothermally at 170° C. Samples were injected automatically (Hewlett-Packard Model 7671) every fifth minute. Peaks were evaluated by a Spectra Physics integrator Model 4270.

The enantiomers were separated on a Chirasil-Val (Applied Science Labs., PA, U.S.A.; No. 13815) glass capillary column (25 m × 0.3 mm I.D.). The column was connected to a Varian 3700 gas chromatograph by a Dani PC-IN 68/156 injector (Monza, Italy) and to the detector by a coupling from Gerstel (Mülheim an der Ruhr, F.R.G.). The all-glass injectors were high-temperature silanized and filled with 1-cm long silanized glass wool plugs. The inlet pressure was 140 kPa and the split flow-rate 10 ml/min. The injector and the detector were maintained at 250° C. The column was kept at 110° C for 1 min after the

injection and then taken to 210°C by increasing the oven temperature by 25°C/min. Samples were introduced every 18 min by a Varian 8000 autosampler unit.

Reagents and chemicals

Tocainide hydrochloride, internal standard as the hydrochloride [H 155/73, 2-amino-N-(2,4,6-mesityl)propanoic acid amide, Fig. 1], *S*- and *R*-tocainide hydrochloride and the heptafluorobutyric derivative of tocainide (H 191/12) were from the department of Organic Chemistry, AB Hässle. The following metabolites were also from the same source: the lactic xylidide (H 170/82), the pyruvic xylidide (H 170/83), the oxime (H 170/84) and the 3-(2,6-xylyl)-5-methyl hydantoin.

p-Bromobenzophenone was from BDH (Poole, U.K.). The methyl carbamate of dibenzylamine was prepared as previously described [15].

Heptafluorobutyric anhydride (Regis, Morton Grove, IL, U.S.A.) was stored in small glass bottles with PTFE-lined screw caps. A 0.5% solution in toluene was prepared and used the same day. Care should be taken that the solution is homogeneous. The corresponding imidazol was from Pierce (Rockford, IL, U.S.A.).

HPLC grade dichloromethane and glass-distilled toluene were obtained from Rathburn (Walkerburn, U.K.) and ethyl acetate p.a. from Merck (Darmstadt, F.R.G.).

A 1.25% solution of hydroxylamine hydrochloride (Merck p.a.) was prepared with deionized water.

Methods

Studies on the acylation of tocainide. Acylation studies of tocainide were performed with 10 μg of tocainide (5.2 nmol) and a marker (4-bromobenzophenone for electron-capture detection, and methyl carbamate of dibenzylamine for nitrogen selective detection) in 2 ml of toluene. After addition of the acylating agents aliquots were withdrawn and washed with buffer pH 7.4. Analysis with electron-capture detection necessitated dilution of the solution, and analysis by nitrogen-selective detection required the elimination of toluene as solvent. This was accomplished by evaporation and reconstitution in ethyl acetate.

Determination of tocainide in human plasma. A 0.1-ml plasma sample was mixed with 50 μl of the internal standard solution (H 155/73, 25 μmol/l) and 50 μl of 1.25% aqueous hydroxylamine hydrochloride solution. After 30 min at room temperature the mixture was made alkaline by the addition of 0.1 ml of 1 *M* sodium hydroxide and extracted with 5 ml of dichloromethane for 10 min. After centrifugation and aspiration of the aqueous phase, the organic phase was decanted into a new tube and evaporated to dryness at 30°C under a stream of dry nitrogen. The residue was dissolved in 0.5 ml of toluene, and 10 μl of the heptafluorobutyric solution, 0.5% in toluene (or 50 μl of 0.1% solution), were added. The reaction was allowed to proceed at 40°C for 10 min or at room temperature for 30 min, and was taken to dryness as above. The remainder was dissolved in 0.2—1 ml of toluene. A 3-μl volume was injected into the gas chromatograph.

RESULTS AND DISCUSSION

Heptafluorobutyrylation of tocainide

Several authors have gas chromatographed tocainide after heptafluorobutyrylation [8, 9, 11, 12]. Both the anhydride [8, 11, 12] and the imidazol [9] have been used. In our hands acylation with the imidazol was slower than with the anhydride. By increasing the concentration of the imidazol the reaction time could be reduced to 10 min (0.05%). However, interfering peaks appeared in the electron-capture gas chromatograms [16] and the yield was 10% lower than with the anhydride. Also, the reagent itself was labile once the ampoule had been opened although the contents were protected against moisture. For these reasons heptafluorobutyric anhydride was preferred as acylating reagent.

The absolute yield with toluene as solvent was found to be 92% as determined with the synthetic derivative as reference. The actual concentration of the reagent was only 0.01% since higher concentrations of the anhydride resulted in degradation of the derivative (Fig. 2). Upon temperature programming of the column it was revealed that at least three new compounds with shorter retention times than the heptafluorobutyryl tocainide derivative were present. Preliminary results indicate that two of the compounds have lost water. The instability of the derivative with excess anhydride was also apparent with other solvents such as hexane, diethyl ether, ethyl acetate, dichloromethane and acetonitrile. Other losses of the derivative were also observed with hexane.

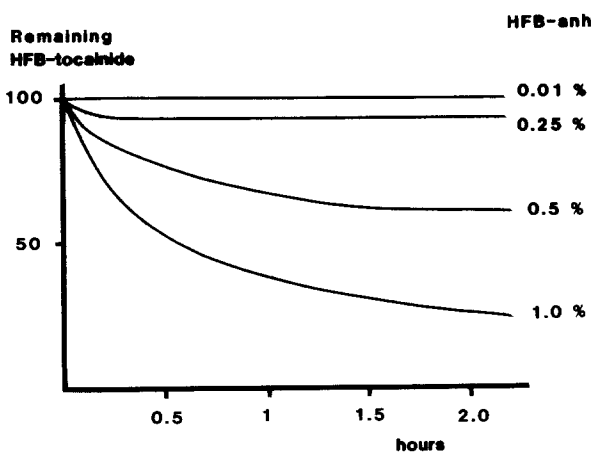

Fig. 2. Stability of the heptafluorobutyryl (HFB) derivative of tocainide in toluene in the presence of excess anhydride (anh) at 40° C.

The heptafluorobutyrylation was also studied after isolation of tocainide and the internal standard from plasma to ensure that co-extracted components did not reduce the yield. A pooled plasma sample (60 μmol/l) was studied. The concentration of the anhydride could be reduced from 0.01% to 0.003% before the yield of tocainide and the standard became irregular.

Due to varying quality of the anhydride from batch to batch, we recommend a check of its potency when a new batch is used or when the method is started

up anew. A slight increase in the anhydride will only have a minor influence on the yield, which will be compensated for by the internal standard. At present we use 0.03%.

Isolation of tocainide from plasma

Tocainide was extracted from alkaline human plasma into dichloromethane. The extraction coefficient is 8 [2, 17] and the high organic-to-aqueous-phase ratio (> 15) will give a near complete extraction. The addition of hydroxylamine prior to extraction improves the yield and precision [13] of the method.

Purification of the reaction mixture

Although a low concentration of heptafluorobutyric anhydride is used in this method, it was not possible to inject aliquots of the reaction solution directly without any disturbance to the electron-capture gas chromatographic system. The racemate of tocainide eluted on the tail of the solvent front. The excess of anhydride was thus always removed and this is also a prerequisite for the use of the Chirasil-Val column. Evaporation of the anhydride was preferred to the buffer wash as it is less time-consuming. The yields were equivalent with both methods and no sign of degradation was observed either by hydrolysis or by dehydration.

The mild heptafluorobutyrylation conditions, i.e. low concentration of the reagent, short reaction time and low temperature, have a positive effect on the chromatographic background noise caused by endogenous compounds (Fig. 3) [18, 19]. If the anhydride concentration was raised to 10% the baseline increased considerably, but few new peaks were observed.

Gas chromatography of heptafluorobutyric tocainide

Heptafluorobutyric tocainide can be gas chromatographed on a Carbowax 20M fused-silica capillary column [11]. When using a methyl silicone as stationary phase we found adequate separation from the methyl homologue used as internal standard. The peaks were symmetrical and the standard devia-

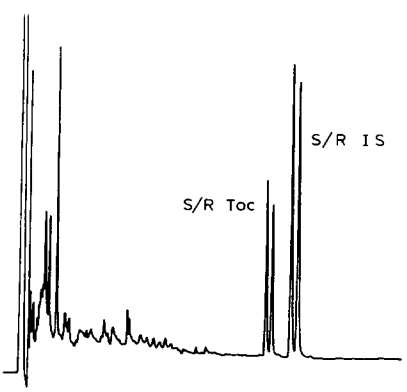

Fig. 3. Separation of the enantiomers of tocainide (Toc) and the internal standard (I.S.) as heptafluorobutyryl derivatives. Sample: 0.1 ml of plasma from a male volunteer taken 0.5 h after a 400-mg oral dose of tocainide hydrochloride, with 1.25 nmol of the internal standard added. Found levels: 2.7 and 2.3 μmol/l of *S*- and *R*-tocainide, respectively. Column: Chirasil-Val. Temperature: 110°C for 1 min and then taken to 210°C at 25°C/min. Retention time of *R*-internal standard: 8 min.

tion upon repeated injection was < 5%. No signs of adsorption or degradation compared with an inert marker could be observed [20]. As methyl silicone capillary columns are used routinely for many other drug determinations in our laboratories there was no need to investigate other stationary phases.

In order to separate the enantiomers of tocainide, capillary columns coated with Chirasil-Val were investigated. A column from Applied Science Labs. gave adequate separation of the enantiomers (Fig. 3). The resolution of the enantiomers as their heptafluorobutyryl derivatives was marginally inferior to that of the pentafluoropropionyl derivative (Table I) and superior to that obtained with the trifluoroacetyl derivatives. The responses of the pentafluoropropionyl and heptafluorobutyryl derivatives were about eight times greater than that of the trifluoroacetyl derivative. Recently a method was published describing the enantiomeric separation of heptafluorobutyric tocainide on Chirasil-Val [21]. Optically active derivatizing agents such as *S*-α-methoxy-α-trifluoromethylphenylacetyl chloride [12] can also be used but such methods demand a high optical purity of the reagent.

TABLE I

RESOLUTION OF PERFLUOROACYL DERIVATIVES OF TOCAINIDE ON CHIRASIL-VAL

Conditions: 190° C, split flow-rate 20 ml/min, inlet pressure 100 kPa.

Derivative	k'_S*	k'_R	α
Trifluoroacetyl	3.49	3.68	1.055
Pentafluoropropionyl	3.09	3.31	1.071
Heptafluorobutyryl	3.46	3.70	1.070

*k' = capacity factor

Selectivity of the present method towards metabolites

Possible interferences from four metabolites [22] were investigated. Plasma was spiked to a concentration of 200 μmol/l of the pyruvic and lactic xylidides, the oxime and the hydantoin. Only the hydantoin, which is formed as an artefact at alkaline pH from the N-carboxytocainide glucuronide, was a potential interference. It elutes just after *R*-tocainide, but was not observed in any of the experimental plasma samples analysed.

Application to plasma samples

Standard curves were prepared by analysing plasma spiked with tocainide in the range 0.3—20 μmol/l. The precision upon repeated analysis of plasma samples is presented in Table II. For routine quantitative determinations five

TABLE II

PRECISION DATA FROM THE REPEATED ANALYSIS OF TOCAINIDE IN PLASMA

Conditions. 0.1 ml of plasma, n = 8

	μmol/l	R.S.D. (%)
Tocainide (racemate)	10	3.3
	0.3	7.7
R-Tocainide	10	4.5
	0 3	9 5
S-Tocainide	10	4 7
	0 3	6 0

reference samples were prepared by adding 50 μl of a stock solution to 0.1 ml of plasma (1.3 μmol/l). The minimum determinable concentration is about 0.3 μmol/l.

Pharmacokinetic data of *R*- and *S*-tocainide in healthy subjects obtained by this method will be published elsewhere [23].

CONCLUSIONS

We have developed an electron-capture gas chromatographic method for the simultaneous determination of *S*- and *R*-tocainide in minute plasma samples. By careful control of the heptafluorobutyrylation conditions a low concentration of the reagent can be used. A methyl analogue is used as internal standard and the detection limit is 0.3 μmol/l (58 ng/ml) with a precision $< 10\%$. A Chirasil-Val capillary column is used for separation of the enantiomers and a methyl silicone capillary column for the racemate. No interference was observed from four metabolites. The present method offers significant improvements over other methods [13, 21].

ACKNOWLEDGEMENTS

We are greatly indebted to Ms. Gunilla Edvardsson and Mr. Torsten Eklund for skilful technical assistance.

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