Journal of Pharmaceutical & Biomedical Analysis
Vol. 8, Nos 8–12, pp. 1101–1103, 1990
Printed in Great Britain

0731–7085/90 $3.00 + 0.00

Short Communication

Gas chromatographic analysis, with nitrogen detection, of tocainide in plasma*

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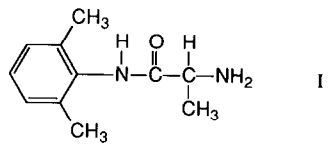
Keywords: *Tocainide; plasma assay; gas chromatography; nitrogen detection.*

Introduction

The primary amine analogue of lidocaine, tocainide (Fig. 1), is a class I anti-arrhythmic drug and is used in the treatment of ventricular arrhythmias. The drug is active when administered orally. Unfortunately, adverse effects are common during tocainide therapy. Tocainide plasma levels are related to anti-arrhythmic activity and toxicity. Concentrations within the range 4–10 μg ml^{-1} represent the desirable therapeutic range while the frequency of side effects increases at plasma levels >10 μg ml^{-1}. Pharmacokinetic variations between patients warrant individualization of the dosage in tocainide therapy on the basis of plasma levels [1]. Reported methods for the analysis of tocainide in plasma involve high-performance liquid chromatography (HPLC) [2–7] and gas chromatography (GC) [8–14] using flame ionization [11, 14], electron capture [8, 10, 12, 13] or nitrogen detection [9]. Most GC methods are laborious and appeared to be not suitable in the author's laboratory for routine analysis in therapeutic drug monitoring.

This paper describes a new sensitive GC method for tocainide using a nitrogen selective detector. The assay has proved to be very suitable for routine analysis.



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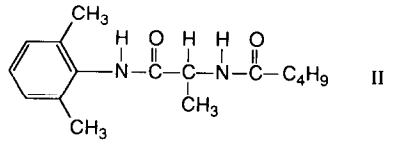

II

Figure 1
Structures of tocainide (**I**) and the acylated derivatives (**II**).

Experimental

Chemicals

Tocainide hydrochloride originated from Astra Pharmaceutica BV (Rijswijk, The Netherlands) and methaqualone was purchased from Brocacef (Maarssen, The Netherlands). *n*-Valeric acid anhydride came from Merck (Darmstadt, FRG). All other chemicals and reagents were of analytical grade.

Sample preparation

To 0.5 ml of plasma (unknown plasma sample or calibration samples spiked with an aqueous solution of tocainide) 0.5 ml of the internal standard solution (methaqualone; 2.5 μg/ml^{-1} in water), 0.5 ml of 5 M sodium hydroxide solution and 5.0 ml of diethylether were added. These mixtures were shaken for

*Presented at the "Second International Symposium on Pharmaceutical and Biomedical Analysis", April 1990, York, UK.

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10 min, centrifuged at 3000 rpm for 5 min and then placed in the refrigerator (−25°C) for 15 min. Subsequently, the organic layer was separated from the frozen aqueous phase and transferred to a conical tube. Next, 20 μl of valeric acid anhydride reagent was added and the solution evaporated to dryness at 60°C. The dry extracts were kept for 20 min at 60°C to complete the derivatization reaction. The resulting residue was reconstituted with 50 μl of ethanol, and 1 μl was injected into the chromatograph.

Gas chromatography

A HP-5710 gas chromatograph (Hewlett Packard, Avondale, PA, USA) equipped with a model 18789A dual nitrogen-phosphorus (NP) flame ionization detection system (Hewlett Packard) was used. The glass column (6 ft long) was packed with 10% OV-1 on 80–100 mesh Chromosorb WHP (Chrompack, Middelburg, The Netherlands). The flow rate of the carrier gas helium was 45 ml min^{-1}, the hydrogen flow rate was 3 ml min^{-1} and the air flow rate was 100 ml min^{-1}. Injector and detector temperatures were 250°C. The analytes were chromatographed isothermally at 210°C. Peak areas or heights were computed with a SP 4270 integrator (Spectra Physics, Santa Clara, CA, USA).

Validation

Linearity of the method was investigated by analysing spiked plasma samples. Precision, accuracy and recovery were determined by repeatedly assaying plasma samples with known concentration of the drug on several days in comparison to aqueous solutions of known concentrations of the drug. For the establishment of the detection limit the detector was set at a response equal to three times the average noise level. Spiked plasma samples (10 μg ml^{-1}) were stored at 20, 4 and −25°C to check for tocainide stability.

Results and Discussion

The reaction of a primary amine with an acid anhydride is expected to yield an amide [15]. For the reaction of tocainide with valeric acid anhydride this will lead to the formation of the *N*-acyl derivative of tocainide (Fig. 1). Acetic acid anhydride and butyric acid anhydride were also tested as acylation reagents but valeric acid anhydride was selected as the derivatization reagent as it provided a product with optimal gas chromatographic properties. Reaction conditions such as temperature, reaction time and amount of anhydride reagent have been optimized and resulted in the proposed working method. Methaqualone was used as internal standard as it could be extracted very effectively with diethyl ether and possesses good chromatographic properties in the isothermal GC system with nitrogen detection used in this study. Under the experimental conditions methaqualone is not acylated. Representative GC chromatograms of a blank sample, a spiked sample and a patient's sample are shown in Fig. 2. The detection limit is 0.1 μg ml^{-1} and the assay is linear for at least up to 75 μg ml^{-1}. The recovery of the assay was 97.4 ± 3.4% and independent of the tocainide plasma concentration in the range 1–20 μg ml^{-1}. A typical equation for a calibration curve in the working concentration range (1–20 μg ml^{-1}) is $y = 0.989(\pm 0.05)x - 0.02(\pm 0.06)$ ($r^2 = 0.999$; $n = 6$), where x is tocainide concentration in μg ml^{-1} and y is the peak height ratio between the acylated tocainide product and methaqualone. Accuracy has been measured at therapeutic relevant levels (Table 1). The stability data of tocainide in plasma (Table 2) demonstrate that in this medium the drug degrades at room temperature, is only stable for 2 days in the refrigerator and can be stored for 1 week at −25°C.

In conclusion, a simple, rapid and sensitive GC method with selective NP detection is

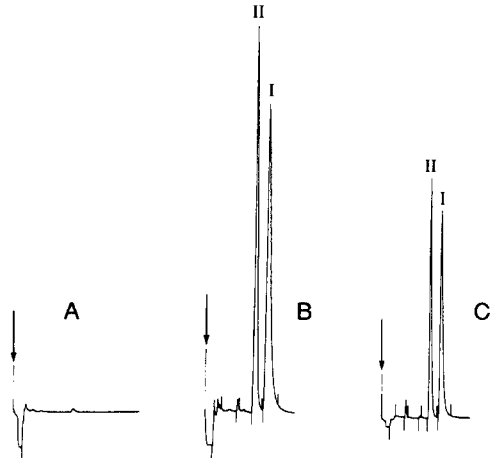

Figure 2
GC chromatograms of a blank plasma (A), plasma spiked with tocainide (B) and a patient sample (C). Retention time of methaqualone (**II**) is 2.8 min and the retention time of the tocainide derivative (**I**) is 3.4 min. Conditions as in text.

Table 1
Precision and accuracy for the analysis of tocainide in plasma

Concentration added*	Concentration found*	*n*	Accuracy (%)	RSD (%)
1.001	0.987	5	97.6	9.8
3.033	2.978	5	98.1	3.1
5.055	5.015	5	99.2	5.8
7.583	7.477	4	98.6	2.3
10.110	10.057	4	99.5	1.7
15.650	15.437	4	98.6	1.7

*Concentration in $\mu g\ ml^{-1}$.

Table 2
Stability of tocainide in plasma ($10.1\ \mu g\ ml^{-1}$), presented as percentages of the initial concentration

Storage time	20°C	4°C	−25°C
Day 0	100	100	100
1 Day	96.7	98.9	99.8
2 Days	92.7	99.3	99.7
1 Week	80.1	91.0	100.5
2 Weeks	54.8	73.4	89.6
3 Weeks	41.5	61.2	70.7
4 Weeks	33.0	50.0	74.5
5 Weeks	11.5	42.3	53.4

presented for the bio-analysis of tocainide, utilizing a pre-column acylation derivatization step with valeric acid anhydride. The method has been found to be suitable for routine analysis in therapeutic drug monitoring.

Acknowledgement — The authors thank Mr R. Bas for technical assistance.

References

[1] B. Holmes, R.N. Brogden, R.C. Heel, T.M. Speight and G.S. Avery, *Drugs* **26**, 93–123 (1983).
[2] P.J. Meffin, S.R. Harapat and D.C. Harrison, *J. Pharm. Sci.* **66**, 583–586 (1977).
[3] E.M. Wolshin, M.H. Cavanaugh, C.V. Manion, M.B. Meyer, E. Milano, C.R. Reardon and S.M. Wolshin, *J. Pharm. Sci.* **67**, 1692–1695 (1978).
[4] P.A. Reece and P.E. Stanley, *J. Chromatogr.* **183**, 109–114 (1980).
[5] A.J. Sedman and J. Gal, *J. Chromatogr.* **232**, 315–326 (1982).
[6] L. Conings and N. Verbeke, *Pharm. Res.* **6**, 311–313 (1985).
[7] R.N. Gupta and M. Lew, *J. Chromatogr.* **344**, 221–230 (1985).
[8] R. Venkataramanan and J.E. Axelson, *J. Pharm. Sci.* **67**, 201–205 (1978).
[9] L. Johansson and J. Vessman, *J. Chromatogr.* **239**, 323–334 (1982).
[10] G.K. Pillai, J.E. Axelson and K.M. McErlane, *J. Chromatogr.* **229**, 103–109 (1982).
[11] P.J. Smith, *Ther. Drug Monitor.* **8**, 361–364 (1986).
[12] A.J. Sedman and J. Gal, *J. Chromatogr.* **306**, 155–164 (1984).
[13] A.M. Antonsson, O. Gyllenhaal, K. Kylbergh-hanssen and L. Johansson, *J. Chromatogr.* **308**, 181–187 (1984).
[14] S.D. Gettings, R.J. Flanagan and D.W. Holt, *J. Chromatogr.* **225**, 469–475 (1981).
[15] J.D. Roberts, R. Stewart and M.C. Caserio, in *Organic Chemistry*, pp. 421–453. Benjamin, Menlo Park (1973).

[*Received for review 5 April 1990; revised manuscript received 23 April 1990*]