Journal of Chromatography, 204 (1981) 355–360
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,162

SPECIFIC AND SENSITIVE METHOD FOR THE DETERMINATION OF TOBANUM® USING GAS CHROMATOGRAPHY WITH ELECTRON-CAPTURE AND MASS FRAGMENTOGRAPHIC DETECTION

EVA TOMORI* and ISTVAN ELEKES
Institute for Drug Research, P.O. Box 82, 1325 Budapest (Hungary)

SUMMARY

Quantitative gas–liquid chromatographic (GLC) and GLC–mass spectrometric (MS) methods for the determination of Tobanum®, a new β-blocking agent, in human plasma have been developed. After solvent extraction, a bis-trifluoroacetyl derivative is formed which is measured by an electron-capture detector. The quantitation is controlled by using an internal standard, propranolol hydrochloride, which is added to all samples. The electron-capture detector response is linear in the concentration range used, 5–150 ng/ml. The identity and quantity of the GLC peaks is confirmed by GLC–MS. The minimum detectable concentration of Tobanum® is 1 ng using 3-ml plasma samples.

INTRODUCTION

The determination of Tobanum® in plasma is important for the understanding of the mechanism of action of this reagent. Tobanum hydrochloride [1-(*tert.*-butylamino)-3-(2,5-dichlorophenoxy)-propanol-2 hydrochloride] is a new β-adrenergic receptor antagonist having antihypertensive, antiarrhythmic and anticonvulsant activity[1,2].

Several methods have been reported for quantitative determinations of β-blocking drugs. Propranolol and sotalol have been determined fluorimetrically[3,4], practolol colorimetrically[5] and alprenolol by gas–liquid chromatography (GLC)[6]. An improved method based on GLC of a fluorinated derivative has been developed for determination of propranolol and its metabolite[7]. A gas chromatography–mass spectrometry (MS)–computer technique[8] has been employed for assay of urinary propranolol and its metabolites.

Our aim was to combine specificity with high sensitivity; therefore two methods have been developed based on GLC and GLC–MS–computer techniques. This study presents, for the first time, methods for the quantitative determination of Tobanum in plasma. Conditions for extraction, derivatization and detection are described.

0021-9673/81/0000–0000/$02.50

EXPERIMENTAL

Standard and reagents

Propranolol hydrochloride was kindly supplied by ICI (Macclesfield, Great Britain). Tobanum hydrochloride was prepared in our laboratories. The following reagents were used: freshly distilled *n*-hexane and nanograde benzene (Mallinckrodt, St. Louis, MO, U.S.A.); trifluoroacetic anhydride as the derivatization reagent (Pierce, Rockford, IL, U.S.A.); 6 *N* sodium hydroxide, 1.25 *N* sulphuric acid, 0.5 *M* phosphate buffer, pH 6.0, prepared with glass-distilled water and stored in glass bottles.

After cleaning with chromic acid, all glassware was silanized with dimethyldichlorosilane (Pierce) and rinsed with methanol and toluene.

Apparatus

For GLC, a Hewlett-Packard 5710A gas chromatograph with a frequency-modulated ^{63}Ni electron-capture detector was used. The glass chromatographic columns (3 ft. × 4 mm I.D.) were packed with 3% OV-17 and 3% OV-1 stationary phases on Gas-Chrom Q (100–120 mesh) (Applied Science Labs., State College, PA, U.S.A.). The carrier gas was argon–methane (95:5) at a flow-rate of 50 ml/min. Temperatures: injection port, 250°C, column, 180°C and 170°C and detector, 250°C.

For GLC–MS, a Hewlett-Packard 5992A instrument was used. The mass spectrometric conditions were: ionization beam energy, 70 eV; electron multiplier, 2800 V. The glass column (3 ft. × 2 mm I.D.) was packed with 2% OV-101 on Gas-Chrom Q (100–120 mesh) and operated at 170°C with a helium gas flow-rate of 25 ml/min.

Extraction procedure and preparation of derivatives

To samples of 3 ml of plasma were added 300 ng of internal standard, propranolol hydrochloride, and 0.25 ml of 6 *N* NaOH in a glass-stoppered 10-ml centrifuge-tube. The samples were shaken with 3 ml of benzene for 5 min and centrifuged at 3000 *g* for 5 min. The benzene phase was transferred to a tube containing 2 ml of 1.25 *N* H_2SO_4. The tube was shaken for 5 min and centrifuged. The acidic aqueous phase was transferred to another tube, made alkaline (pH 12) with 0.75 ml of 6 *N* NaOH and shaken with 3 ml of benzene for 5 min. The benzene phase was transferred to another tube with a PTFE screw cap and evaporated to a small volume (50 μl) with a gentle stream of nitrogen at 60°C. A 70-μl volume of 1 *M* trimethylamine in benzene and 25 μl of trifluoroacetic anhydride were added. The tubes, after being tightly stoppered, were heated for 10 min at 60°C in a sand-bath. After cooling the reaction mixtures were shaken vigorously for 30 sec with 1 ml of 0.5 *M* phosphate buffer, pH 6.0, and centrifuged. Part of the benzene phase was taken for electron-capture GLC and GLC–MS assay.

Construction of standard calibration curves

Aliquots (3 ml) of control plasma samples were spiked with Tobanum hydrochloride in the range 5–150 ng and extracted according to the back-extraction procedure using 300 ng of propranolol hydrochloride as internal standard. In the case of GLC, peak area ratios of Tobanum to propranolol were measured and plotted as a

function of Tobanum concentration. In the case of GLC–MS the calibration curve was obtained from intensity ratios for the ion at *m/z* 266 in the spectra of Tobanum and propranolol.

RESULTS AND DISCUSSION

Fig. 1 shows a typical gas chromatogram obtained from plasma extract (pH 12) on the apolar silicon phase OV-1; a good separation is achieved ($\alpha = 1.57$).

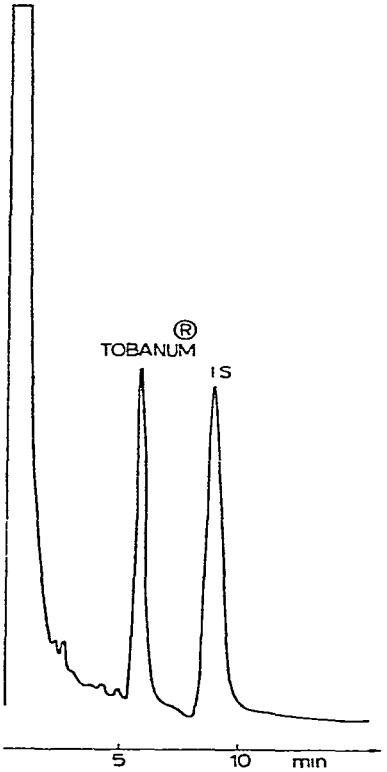

Fig. 1. Gas chromatogram of a plasma extract obtained from a patient 4 h after administration of 3 × 5 mg Tobanum. The column was OV-1 and the temperature was 170°C. Conditions as in Experimental.

The acylation of Tobanum was studied between 5 and 60 min at 30°C and 60°C. The relative peak size appeared to be independent of the acylation conditions investigated; hence, both Tobanum and I.S. are converted into their derivatives at the same rate and to the same extent. The use of propranolol as an internal marker was found to be satisfactory. The physico-chemical properties of this compound are closely related to those of Tobanum. Standard curves obtained from plasma B in Table I do not significantly differ from that obtained from water (A), indicating no significant interference from plasma binding.

TABLE I

STANDARD CURVES FOR TOBANUM

Curves: A = extraction from distilled water; B and C = extractions from plasma. A and B were measured by electron-capture GLC and C by GLC–MS. Conditions as in Experimental.

Curve	$y = a + bx$		
	a	*b*	*Correlation coefficient*
A	+0.02	0.009	0.997
B	+0.04	0.0073	0.994
	+0.03	0.0068	0.996
	+0.18	0.009	0.997
C	+0.006	0.0066	0.996

According to Table I, there are daily variations in the slope of the standard curves. Over a 2-month period, during which more standard curves were run, the slopes for Tobanum ranged from 0.006 to 0.009. All curves, however, passed through the origin and correlation coefficients were all high. The relative recovery from human plasma was constant in the range examined with a mean of 47 ± 0.05% ($n = 5$), and from water was 76 ± 0.02% ($n = 5$). The minimum detectable amount is about 1 ng using 3 ml of plasma, by both methods.

The GLC–MS assay confirmed the identity and the quantity of the GLC peaks. GLC peaks of Tobanum bis-trifluoroacetate should give a molecular ion at m/z 483 (0%). The mass spectrum (Fig. 2) exhibited an ion at m/z 468 (2%) corresponding to the loss of a methyl group from the molecular ion. The ion at m/z 162 (14%) is formed by loss of the side-chain after H-rearrangement, and the fragment ions at m/z 266 (55%) and m/z 152 (5%) arise from cleavage of the side-chain at various positions.

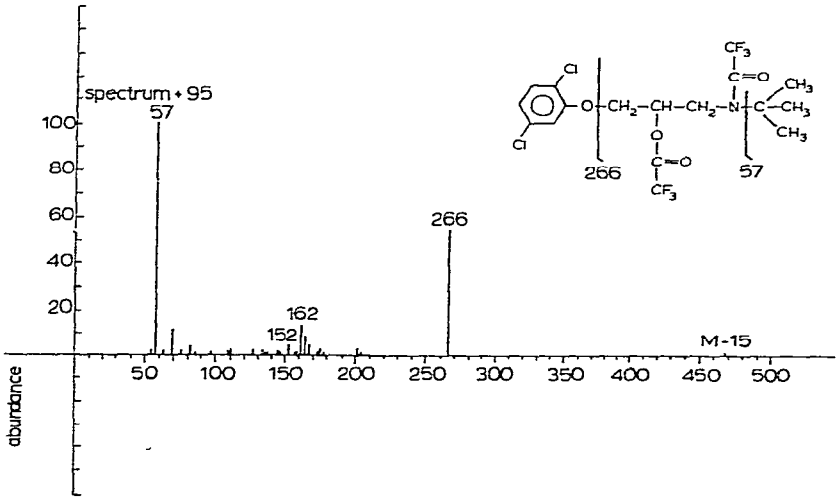

Fig. 2. Low-resolution mass spectrum of Tobanum bis-trifluoroacetate. Conditions as in Experimental.

The base peak at *m/z* 57 (100%) corresponds to the *tert.*-butyl group. The intense fragment ion at *m/z* 266 is characteristic of aryloxy β-blocking drugs[9].

For the quantitative determination of Tobanum the ion at *m/z* 266 was monitored. Typical single-ion recordings of plasma extracts are shown in Fig. 3.

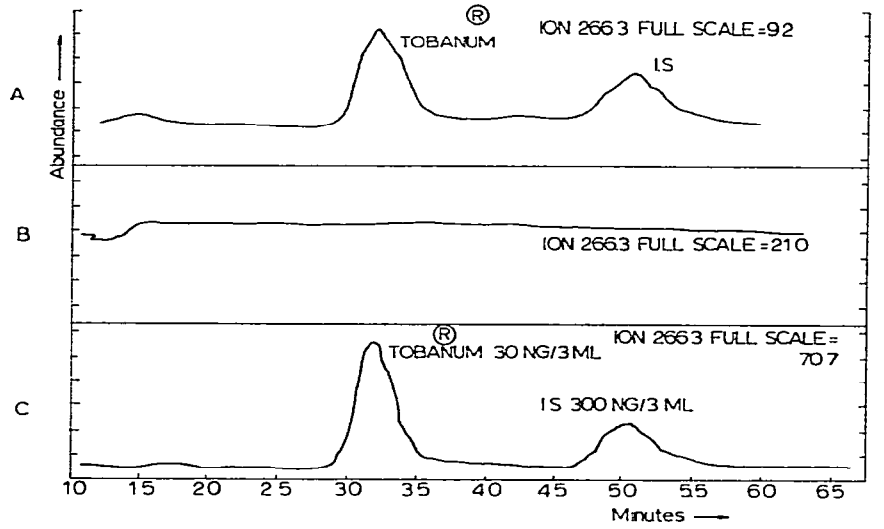

Fig. 3. Single-ion (*m/z* 266) recordings of plasma extracts at pH 12. Conditions as in Experimental. Curves: A = plasma from a patient treated with Tobanum; B = blank plasma; C = blank plasma spiked with known quantities of Tobanum and propranolol.

The methods described above have been applied to the determination of Tobanum levels in human plasma. Fig. 4 shows the plasma Tobanum levels in patients administered orally with 3 × 5 mg of Tobanum in tablets. The plasma levels were determined over a period of 24 h. Tobanum was still detectable (5 ng/ml) 24 h following administration. The calculated plasma half-life, $t_{\frac{1}{2}}$, was 6 h. The kinetic parameters calculated from the concentration in plasma were: $k_1 = 0.5577\ h^{-1}$

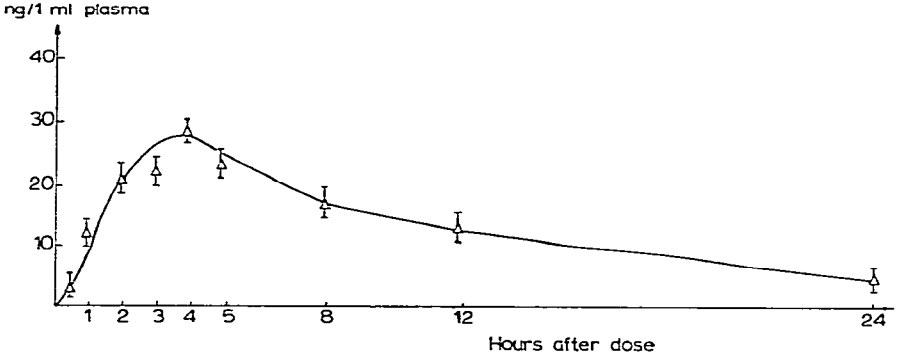

Fig. 4. Curve of Tobanum blood level *vs.* time in ten patients receiving 3 × 5 mg orally. Conditions as in Experimental.

(invasion constant); $k_2 = 0.0845\ h^{-1}$ (elimination constant). The value of k_2 shows that the elimination of the agent from the plasma is very slow. A concentration maximum of 28 ng/ml was observed in the fourth hour following administration. The absorption from the gastrointestinal tract was very rapid, resulting in high and lasting serum levels. The plasma levels obtained by GLC (Fig. 4) showed good agreement with results obtained by GLC–MS. This means that interfering compounds were not likely to be present in the samples.

REFERENCES

1 J. Borvendég, J. Eggenhoffer, E. Török, I. Tényi and J. Szám, *7th International Congress of Pharmacology, Paris, 1978.*
2 I. Tényi, M. Németh, T. Jávor, I. Nemes, L. Bódis, J. Borvendég and J. Eggenhoffer, *Curr. Ther. Res.*, 21 (1977) 823.
3 D. G. Shand, E. M. Nuckolls and J. A. Oates, *Clin. Pharmacol. Ther.*, 11 (1970) 112.
4 E. R. Garrett and K. Schnelle, *J. Pharm. Sci.*, 60 (1971) 833.
5 J. D. Fitzgerald and B. Scales, *Int. J. Clin. Pharmacol.*, 16 (1968) 467.
6 M. Ervik, *Acta Pharm. Suecica*, 6 (1969) 393.
7 E. Di Salle, K. M. Baker, S. R. Bareggi, W. D. Watkins, C. A. Chidsey, A. Frigerio and P. L. Morselli, *J. Chromatogr.*, 84 (1973) 347.
8 V. T. Vu and F. P. Abramson, *Biomed. Mass Spectrom.*, 5 (1978) 686.
9 Gy. Horváth, in E. Jucker (Editor), *Progress in Drug Research*, Vol. 18, Birkhäuser, Basel, 1974, pp. 399–473.