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QUANTITATIVE ANALYSIS OF TRIFLUOROACETIC ACID IN BODY FLUIDS OF PATIENTS TREATED WITH HALOTHANE

LUDGER WITTE and HEINZ NAU*

Gesellschaft für Biotechnologische Forschung, Mascheroder Weg 1, 3300 Braunschweig-Stöckheim (G.F.R.)

JÜRGEN-HINRICH FUHRHOP**

Geschellschaft für Biotechnologische Forschung, Mascheroder Weg 1, 3300 Braunschweig-Stöckheim, and Institut für Organische Chemie A der Technischen Universität Braunschweig, Schleinitzstrasse, 3300 Braunschweig (G.F.R.)

and

ALFRED DOENICKE and BERNHARD GROTE

Poliklinik München, Pettenkoferstrasse, 8000 München 2 (G.F.R.)

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SUMMARY

A simple procedure for the quantitative analysis of trifluoroacetic acid (TFA) in urine and serum from patients narcotized with halothane is described. This involves addition of sodium hydroxide to the body fluid, evaporation of the aqueous phase and esterification of TFA in concentrated sulphuric acid with 2,2,2-trichloroethanol. The gaseous phases above the reaction mixture were then analyzed by gas chromatography with a nickel-63 electron-capture detector. The detection limit was 1 μg of TFA per millilitre of body fluid (200 μg of body fluid are analysed) and the relative standard deviation was ±6%. Patients treated with ethrane, another commercial anaesthetic, did not produce any detectable TFA.

INTRODUCTION

The narcotic halothane (2-bromo-2-chloro-1,1,1-trifluoroethane) is partly metabolized in man to trifluoroacetic acid (TFA) [1, 2]. As TFA could be responsible for the observed toxicity of halothane [3], it is desirable to develop methods for the quantitative analysis of this highly water-soluble metabolite.

*Present address: Pharmakologisches Institut der Freien Universität Berlin, Thielallee 69/73, 1000 Berlin 33, G.F.R.
**To whom correspondence should be addressed at the following address: Gesellschaft für biotechnologische Forschung, Mascheroder Weg 1, 3300 Braunschweig-Stöckheim, G.F.R.

Earlier attempts to detect TFA in urine involved formation of the methyl ester, extraction with diethyl ether and final gas chromatographic (GC) [4] or infrared [5] analysis or paper chromatography and colorimetric analysis of fluorine in the combusted sheets [6]. We report here a simple and more sensitive procedure in which the 2,2,2-trichloroethyl ester of TFA is quantified by head-space analysis and by gas chromatography with electron-capture detection. No extraction, separation, sample transfer or other isolation procedures are needed owing to the specifity of detection.

EXPERIMENTAL

Materials

Urine and serum were collected from 35 individuals at various time intervals after anaesthesis with halothane. Details of the medical treatments and results will be reported elsewhere [7]. Halothane (Hoechst, Frankfurt/M, G.F.R.), ethrane (Abbot, S. Pasadena, Calif., U.S.A.) and TFA (Merck, Darmstadt, G.F.R.) were used without further purification. 2,2,2-Trichloroethanol (zur Synthese, Merck) and heptafluorobutyric acid (HFB) (Pierce, Rockford, Ill., U.S.A.) were distilled before use. Trifluoroacetyl-2,2,2-trichloroethyl ester was prepared by adding TFA anhydride to 2,2,2-trichloroethanol. The product was distilled three times.

Gas chromatography

A Perkin-Elmer F 20 FE gas chromatograph equipped with a nickel-63 electron-capture detector (ECD) (10 mCi) was used throughout. The injection port temperature was 150°, the column temperature 80° and the detector temperature 260°. The carrier gas nitrogen flow-rate was 20 ml/min, the purge gas flow-rate 40 ml/min, the pulse interval 10 μsec, the attenuation × 8 and the range 25. The column employed in all studies was a 2 m × 2 mm I.D. glass column packed with 4% OV-17 on Chromosorb W HP, 100—120 mesh (Perkin-Elmer, Norwalk, Conn., U.S.A.).

Gas chromatography—mass spectrometry (GC—MS)

The GC separation conditions were identical with those described above, but helium was used as the carrier gas. The glass column was coupled by a 1/4-in. Swagelok union drilled to 1/4 in. via a membrane separator to an AEI MS 30 mass spectrometer [8]. This was coupled with a DS-50 computer system (AEI) to obtain mass spectra continuously at intervals of 7 sec. The separator was kept at 230° and the temperature of the source was about 240°. The accelerating voltage was 4 kV and the electron energy 24 eV.

Derivatization of urine and serum samples

Samples of 200 μl of serum or urine were mixed with 100 μl of HFB standard (100 mg in 100 ml of water) and 200 μl of 0.1 *N* sodium hydroxide solution in 30-ml Minnert vials (Precision Sampling Corp., Baton Rouge, La., U.S.A.). The samples were frozen with liquid nitrogen and lyophilized in a desiccator (sodium hydroxide pellets) for ca. 4 h at room temperature. Then 200 μl of trichloroethanol were added and the inside of the upper part of the

vessel was wetted with six drops of concentrated sulphuric acid and quickly closed before the acid reached the bottom. The reaction mixture was equilibrated at 50° for 15 min and 200 µl of the vapour phase were injected into the port of the gas chromatograph.

Calibration

Various amounts of TFA and HFB standard were added to urine and serum blanks to yield TFA solutions with 13 concentrations in the range from 1 µg/ml to 2 mg/ml. These samples were esterified with trichloroethanol in a procedure identical with that described above. The relative standard deviations of the peak heights in various samples of identical TFA concentrations were 6%. Using the calibration graph, TFA concentrations in urine and serum samples from narcotized patients were determined graphically from the ratio of the heights of TFA and HFB peaks. Owing to the non-linear detector response, a linear relationship between peak height and sample size was not obtained.

A graph of recovery for the esterification was also determined by comparison of urines with known TFA concentrations with samples of pure TFA 2,2,2-trichloroethyl ester in diethyl ether solution. It was found that the yields were highly dependent on concentration (Table I). Measurements should be taken in the linear range from 0 to 100 µg/ml. In the higher concentration range of trifluoroacetic acid (starting at ca. 150 µg/ml), appreciable amounts (up to 10%) of trifluoroacetic anhydride were formed during the derivatization procedure. All effects that had an influence on the yield of the trichloroethyl ester and therefore also an quantification of trifluoroacetic acid were eliminated by the calibration method described above.

RESULTS AND DISCUSSION

We chose the ECD for quantitative analysis of TFA in the GC peaks because of its high sensitivity and selectivity for the detection of halogenated carbon compounds. However, the electron affinity of the trifluoromethyl group was not sufficient for the determination of TFA in serum and urine without elaborate work-up procedures. Therefore, we prepared esters of TFA with the following

TABLE I

RECOVERY OF THE 2,2,2-TRICHLOROETHYL ESTER AT VARIOUS CONCENTRATIONS OF TRIFLUOROACETIC ACID IN THE SAMPLES

TFA in urine (µg/ml)	Yield (%)	TFA in urine (µg/ml)	Yield (%)
5	12	145	30
40	12	160	38
70	12	175	45
110	18	200	49
		225	57

halogenated alcohols: 2,2,2-trichloroethanol, 1,1,1,3,3,3-hexafluoroisopropanol, 1,1,2,2-tetrahydroperfluorohexanol and 1,1-dihydroperfluoroheptanol. Esterification with 2,2,2-trichloroethanol yielded the best results and we used that derivatization method throughout the study. 2,2,2-Trichloroethanol has also been used by other workers for the determination of aromatic and aliphatic acids other than TFA [9,10].

With the detector used, 1 μg/ml of TFA in urine could still be detected in a sample volume of 200 μl; larger samples yielded higher sensitivity. The only limiting factors were the large detector noise level produced in the detector, its non-linear response and the presence of negative peaks. Preliminary measurement with a later gas chromatograph (Perkin-Elmer F22) equipped with a frequency-modulated ECD yielded higher sensitivity and better reproducibility. For our purposes, however, the accuracy obtained was sufficient to allow the use of the less sensitive detector which we had available at that time. Measurements with the GC–MS–computer system led to approximately the same results.

The conditions used in the esterification procedure proved to be critical. After salt formation with TFA and evaporation of water, the esterification with 2,2,2-trichloroethanol takes place in concentrated sulphuric acid. This acid should come into contact with the TFA–trichloroethanol mixture only after the reaction vessel has been closed. The reaction mixture has to be equilibrated at 50° for at least 15 min; longer reaction times (up to a few hours) did not change the results, but lower temperatures led to a decrease in reproducibility. We found it unnecessary to separate the residual trichloroethanol

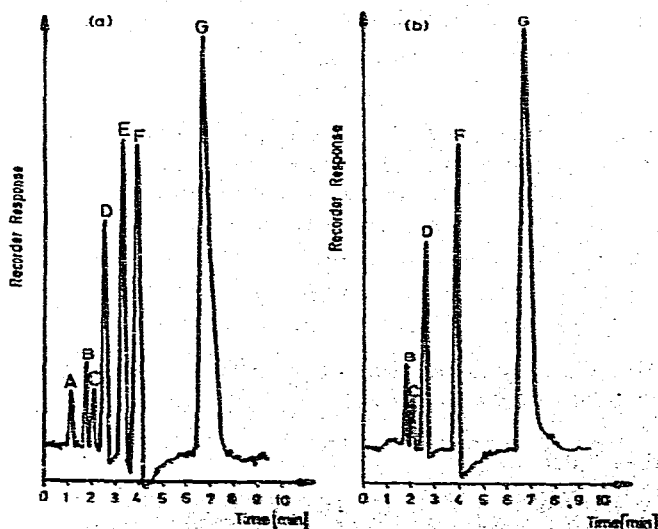

Fig. 1. (a) Gas chromatogram of 200 μl of the head-space of 200 μl of lyophilized urine from a halothane-treated patient, reacted with 200 μl of 2,2,2-trichloroethanol and approximately 70 μl of concentrated sulphuric acid. Peaks: A = TFA anhydride; B = $CHCl_3$; C = unknown; D = CCl_3CHO; E = 2,2,2-trichloroethyl trifluoroacetate; F = 2,2,2-trichloroethyl heptafluorobutyrate (internal standard); G = 2,2,2-trichloroethanol. (b) Blank; identical experiment as in (a), but with urine from an untreated patient.

and other compounds from the other constituents. We rather used the total mixture and found the following components (in order of their elution times): trifluoroacetic anhydride, chloroform, chloral (or its hydrate), a product from 2,2,2-trichloroethanol that could not be identified, 2,2,2-trichloroethyl trifluoroacetate, 2,2,2-trichloroethyl heptafluorobutyrate and 2,2,2-trichloroethanol (Fig. 1).

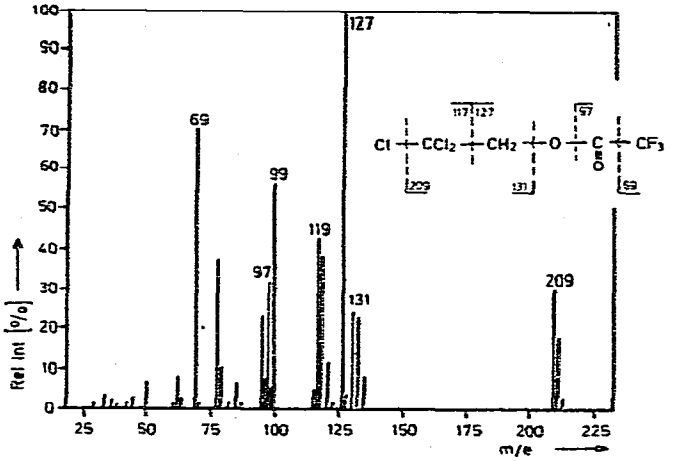

Fig. 2. Mass spectrum of 2,2,2-trichloroethyl trifluoroacetate.

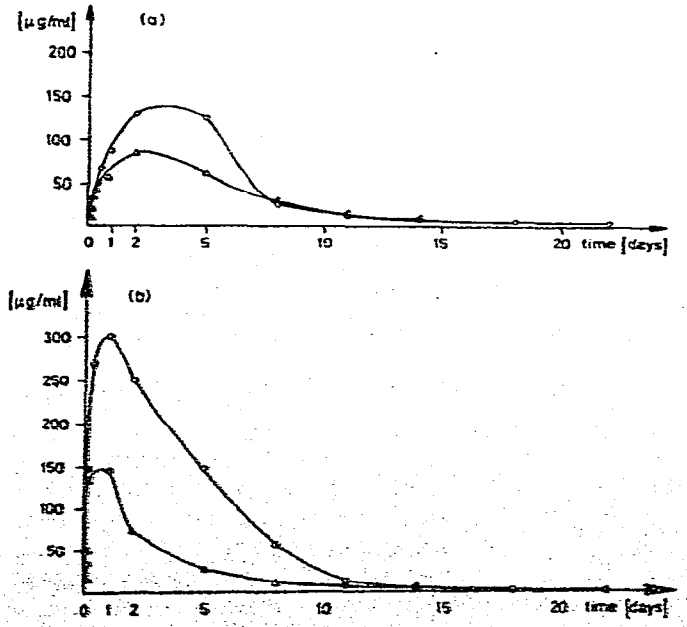

Fig. 3. Time-dependent increase of concentrations of trifluoroacetic acid in the urine (o) and serum (•) of halothane-treated patients. The curves represent approximately the upper (b) and lower (a) limits of TFA in the body fluids of 16 patients.

The mass spectrum of the 2,2,2-trichloroethyl ester of TFA is shown in Fig. 2. The spectrum does not contain a molar peak (M^+; *m/e* 244), but a peak at *m/e* 209 that corresponds to a loss of one chlorine atom. More important peaks are observed for the CF_3 group (*m/e* 69) and for fragments that are formed by loss of CCl_3 (*m/e* 127 base peak) and CCl_3—CH_2O (*m/e* 97). The *m/e* 99 peak ($C_2H_2F_3O$) corresponds to a loss of CCl_3 and of C=O by skeletal rearrangement [4]. This fragmentation pattern is in accordance with those of other highly halogenated carbon compounds [11]. All but one GC peak have been identified by mass spectrometry and by comparison with pure compounds, and originate from solvolytic and oxidation reactions of TFA and trichloroethanol in warm sulphuric acid. Quantitative analysis of the TFA trichloroethylester was not hampered by any of the other components. The total mixture can be analyzed and pure GC fractions resulted for the TFA and HFB derivatives owing to the specificity of the ECD. An even more specific method was MS single-ion recording at *m/e* 69, which detected only TFA and HFB derivatives.

Typical experimental results are displayed in Fig. 3, and agree with earlier paper chromatographic results [6]. Patients were narcotized with halothane and both urine and serum showed a considerable rise in TFA concentration for a period of about 8 h. After 2 days a maximum of ca. 130—300 μg/ml of TFA in urine had been reached. In the body fluids of patients treated with ethrane under otherwise identical conditions, no TFA or other acids [12] were found, down to the detection limit of about 1 μg/ml.

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