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Forensic Toxicological Analysis of Tetrahydrofuran in Body Materials

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Summary. Routine analysis of tetrahydrofuran (THF) in biologic materials has become feasible using GC and GC/MS and the headspace method. Problems of the headspace method and this substance which has a high water- and lipid-solubility were overcome by using the salting-out technique.

Identification was made by mass spectral examination, in case of concentrations over 5 μg per sample. For quantitative determinations, tetrahydropyran (pentamethylene oxide) was used as an internal standard in GC, and a stable isotopic substance, octadeuterated THF (TDF) in GC/SIM. THF was detected in 1 μg per sample by GC, and 0.1 μg per sample by GC/SIM.

THF blood levels in laboratory animals reached their highest values about 1 h after the oral administration, and the half-life was about 5 h. Ratios of tissue levels to blood were ca. 1.5–2 in the adipose tissue and kidney, and fairly equal in the brain, liver, spleen, and muscle.

Key word: Tetrahydrofuran, in histologic materials

Zusammenfassung. Der Einsatz und die Kombination von GC, GC/MS und Headspace-Variante ermöglicht die Routine-Analyse von Tetrahydrofuran (THF) in biologischem Material. Probleme mit der Headspace-Methode, die auf die beträchtliche Wasser- und Lipidlöslichkeit dieser Substanz zurückgehen, konnten durch eine Aussalz-Technik gelöst werden.

Bei Konzentrationen von über 5 μg pro Probe wurde die Identifizierung massenspektrometrisch durchgeführt. Im Rahmen quantitativer Bestim-

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Abbreviations: GC, gas chromatography; MS, mass spectrometry; SIM, selected ion monitoring; IS, internal standard; THF, tetrahydrofuran; TDF, octadeuterated THF; THP, tetrahydropyran

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mungen kam bei der GC Tetrahydropyran (Pentamethylen-oxid) bzw. bei der GC/SIM octadeuteriertes THF (TDF) als innerer Standard zum Einsatz. THF konnte bis zu 1 µg pro Probe mittels GC und bis zu 0,1 µg pro Probe mittels GC/SIM nachgewiesen werden.

Bei Labortieren wurden Maximalspiegel von THF im Blut ungefähr 1 h nach oraler Applikation beobachtet; die Halbwertszeit lag bei etwa 5 h. Das Verteilungsverhältnis zwischen Gewebe und Blut war etwa 1,5–2 bei Fettgewebe und Niere bzw. praktisch übereinstimmend bei Gehirn, Leber, Milz und Muskulatur.

Schlüsselwort: Tetrahydrofuran, Nachweis in biologischem Material

Introduction

Volatile substances, such as alcohols and hydrocarbons in the blood and urine are generally analyzed by the simple headspace method of GC or GC/SIM [1–5]. This technique is, however, often difficult for analyzing unhomogeneous samples consisting of various chemicals and body tissue components. Tetrahydrofuran (THF), of which a fatal case of poisoning was reported by the authors [6], belongs to one of the untoward events with this method because of its high lipid- and water-solubilities [7]. This paper deals with improved techniques where the THF concentration in the headspace was increased and tetrahydropyran (THP) and stable isotopical octadeuterated THF (TDF) were introduced as internal standards.

THF distribution after oral administration to laboratory animals was also investigated.

Materials and Methods

Reagents

THF including 0.025% di-*tert*-butyl-*p*-cresol purchased from Nakarai Chemicals, Ltd. was used in a 1% aqueous solution. TDF from E. Merck Co. was used in a 1% aqueous solution, as the internal standard (IS) for GC/SIM.

THP, pentamethylene oxide, obtained from Tokyo Kasei Kogyo Co. Ltd. was used in a 2% aqueous solution as IS for GC.

Preparation of Sample

Each 0.5 g of specimen and 10 µl of IS solution were placed into a 15-ml glass bottle, including 2 ml of cold distilled water, and then cooled in an ice bath. The contents were homogenized in a Polytron homogenizer. After addition of 2 g sodium chloride for salting-out, the homogenates were incubated at 40°C for 1 h in a water bath, and finally 0.5 ml of the headspace vapor was injected through a glass syringe into a GC or GC/MS instrument.

Conditions of GC and GC/MS

GC: Shimadzu Gas Chromatograph GC-6AM equipped with flame ionization detector. Column: 1 m × 3 mm i.d. glass tube packed with Porapak P, 80/100 mesh. Temperature:

190°C at injection and detector block, and 140°C in column oven. Carrier gas: nitrogen at a flow rate of 40 ml/min.

GC/MS: Shimadzu-LKB 9000 (EI mode) controlled through a data system GCMSPAC 500D. Column: the same as in GC. Temperature: 140°C in column oven, 250°C at separator and 270°C at ion source. Carrier gas: helium at a flow rate of 30 ml/min. Electron energy: 20 eV. Selected ions for GC/SIM: m/z 72 for THF, m/z 80 for TDF.

Procedures of Analysis

Qualitative analysis was based on the retention time in the GC and the mass spectrum in the MS. Quantitative analysis was carried out by GC or GC/MS. Calibration curves were prepared from water samples with THF and IS.

Procedures of Animal Experiments

Male Wistar rats weighing 300 g were used for the study of THF distribution. The animals were given THF at 0.3 g/kg p.o. with 10% aqueous solution and killed by pithing at ⅙, ½, 1, 2, 3, and 5 h after administration. Three rats were used at every time of killing. The collected blood and tissues were placed into sample bottles and submitted to analysis. When examination was not immediately made after the necropsy, the following care was paid with the sample preservation, since THF in the blood sample decreased during the storage [6]. The collected blood was filled in a small glass bottle with a screw cap with a silicon seal, and other solid materials were tightly wrapped with a film of polyvinylidene chloride and additionally put into a small plastic bag to minimize the space where THF was supposedly released. All the samples were kept at −20°C. When stored by the method, little decrease of THF was observed in 3 months. Blood levels of two male rabbits (2.5 kg) given 0.7 g THF per kg were recorded during 7 or 8.5 h and tissue levels at the end of periods were measured for a comparison with the data obtained in rat experiments.

Results

Qualitative Analysis

Qualitative analysis of THF was made by examining both the retention index on gas chromatogram, 642, and the mass spectrum, as shown in Fig. 1. The main ions on the mass spectrum are m/z 72 (molecular ion: M^+), m/z 71 (M − H) and m/z 42 (M − CH_2O) [8]. The mass spectrum of TDF is shown in Fig. 1, in parallel with that of THF. The molecular ion was observed at m/z 80, and the base peak appeared at m/z 48, when CD_2O was removed from the TDF molecule.

The minimum amount of THF required for identification by MS was about 5 μg per sample.

The peak height ratio of m/z 72 to m/z 71, 1.16 with the same retention index, was found to be characteristic of THF and advantageous to detect very small amounts of THF.

Quantitative Analysis

Quantitative determination was carried out by either GC or GC/SIM using IS, THP, or TDF, of which chromatograms are shown in Fig. 2. Both molecular ions m/z 72 of THF and m/z 80 of TDF were monitored in GC/SIM. No interfer-

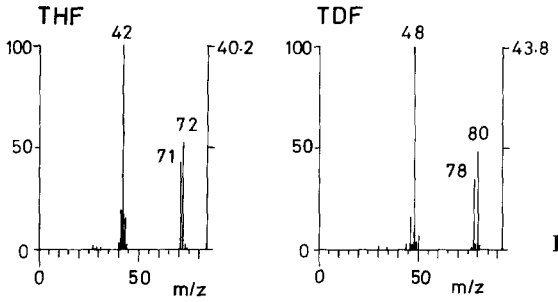

Fig. 1. Mass spectra of THF and TDF

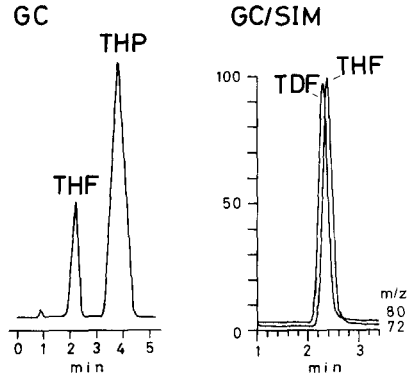

Fig. 2. Chromatograms from liver samples with THF and IS (THP or TDF) in GC and GC/SIM

ing background peak derived from body materials appeared on the chromatograms.

The calibration curves for GC and GC/SIM were prepared from 2.5 ml water samples with 2, 5, 10, 15, 20, and 25 µl THF solution, each containing 0.02–0.25 mg, and 10 µl IS solutions. Linear relationships were observed between peak area ratios of THF to ISs, that is THP in GC and TDF in GC/SIM, and amounts of THF.

The lower limit of THF detection was 1 µg per sample in the GC and 0.1 µg per sample in the GC/SIM, respectively.

Reliability of this Technique

Recovery. The procedures of salting-out increased the THF peak height about 4 times higher than in case of no addition of salt. The relative recovery rates of THF from body materials vs from water samples were 100% in case of blood, 77% in liver and muscle, 46% in adipose tissue. The loss of THF due to homogenizing procedures was below 3%.

Reproducibility. The reproducibility of the assay was checked. Samples in five series were prepared from each 0.5 g blood, liver, muscle, and adipose tissue with 10 µl THF solution and 10 µl IS solutions, and the results are shown in

Table 1. Reproducibility of THF assay of body material

Material	GC	GC/SIM
Blood	105 (7.3)	101 (1.1)
Liver	112 (3.5)	101 (1.1)
Muscle	113 (3.3)	97.5 (1.9)
Fat	—	94.6 (2.9)

Main value is apparent recovery (%) to the standard value from a water sample and the value in parenthesis is its coefficient of variation (%)

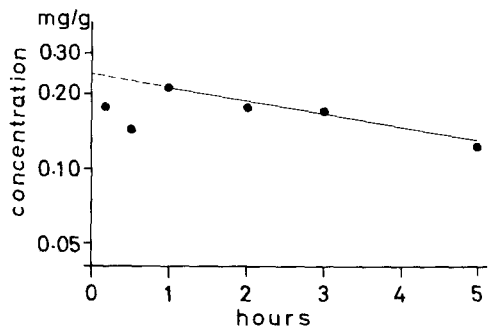

Fig. 3. Changes in blood levels vs time lapse in rats given THF orally

Table 2. Half-life and apparent distribution of THF

Species	Observation (h)	Half-life (h)	Apparent distribution (l/kg)
Rats	1–5	5.2	1.25
Rabbit 1	0.83–7	4.2	0.94
Rabbit 2	2–8.5	6.0	0.98

Table 1. Concentrations of THF in body materials were expressed in mg/g as unit, calculated by the following formula:

$$C = A\,d\,F/W$$

C = concentration (mg/g), A = value (μl) in calibration curve, d = specific density (0.889 mg/μl at 20°C), F = correction factor (100/apparent recovery), and W = amount (g) of body material in sample.

The coefficients of variation in the samples ($n = 5$) from animal experiments revealed 2.2% for blood and 9.1% for liver in the GC, and 1.1% for blood, 6.9% for liver, and 6.5% for adipose tissue in the GC/SIM.

Animal Experiments. THF concentrations in body materials were measured by GC. GC/SIM was used in case of trace analysis and particularly for analyzing adipose tissue samples. The change of average blood level vs time lapse,

Table 3. Ratio of tissue level to blood level of THF

Species	Measured time	Ratio (tissue level/blood level)						
		Liver	Kidney	Brain	Spleen	Muscle	Fat	Urine
Rats (average)	1–5 h	0.98 (13)	1.3 (20)	0.99 (12)	0.94 (16)	0.92 (12)	1.4 (13)	–
Rabbit 1	7 h	0.89	0.85	0.75	0.49	0.58	1.4	1.2
Rabbit 2	8.5 h	1.2	0.94	0.89	1.2	0.77	–	1.3

The values in parentheses are coefficients of variation

semilogarithmically plotted, is shown in Fig. 3. The blood level showed a tendency to decrease at a constant rate. The half-life and apparent volume of distribution, the dose per the extrapolated initial concentration, are listed in Table 2 together with the data on two rabbits, for comparison. The ratios of tissue levels of THF to blood in animals are given in Table 3. No significant differences were found in the results between two animals. Adipose tissue and kidney levels were slightly higher than levels in the other materials examined.

Discussion

Addition of NaCl after homogenization of a sample in water led to a higher sensitivity. As shown in Table 1, the slight variation in the data suggests that the analysis was carried out under the condition of a well-homogenized sample. This technique should be applicable to solid materials.

The introduction of ISs, THP for GC and TDF for GC/SIM, improved the accuracy, on both qualitative and quantitative determination processes. Although the recovery of each IS was much the same as that of the THF, THP could not be used for analysis of adipose tissue samples owing to the high lipid-solubility. On the other hand, TDF showed good results, even in case of adipose tissue.

Although quantitative determinations of THF from body materials is feasible with GC, GC/SIM is preferred to obtain highly reliable data and to eliminate the disadvantageous conditions of fatty materials.

From the data obtained in animal experiments, the blood level shows a positive relation to the dose, and the tissue level vs blood level is equivalent to the apparent distribution volume. As information on human materials is not presently available, our data should prove useful as reference standards.

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