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Gas chromatographic–mass spectrometric screening procedure for the identification of formaldehyde-derived tetrahydroisoquinolines in human urine☆

F. Musshoff*, Th. Daldrup and W. Bonte

Institute of Legal Medicine, Heinrich-Heine-University Düsseldorf, Moorenstrasse 5, W-4000 Düsseldorf 1 (Germany)

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ABSTRACT

A gas chromatographic–mass spectrometric method has been developed for the identification of 1,2,3,4-tetrahydroisoquinoline and six metabolites extracted from urine in the picogram range. The derivatization procedure for the substances, formed by reaction of formaldehyde with biogenic amines, employs propionic anhydride and can take place in aqueous medium. In this way artificial formation of these compounds via condensation of biogenic amines with aldehydes or α-keto acids during the work-up procedure is eliminated. The procedure results in hydrophobic compounds, which are quantitatively extractable by liquid–liquid extraction with organic solvents. Further clean-up was performed by solid-phase extraction on C_{18} sample preparation columns.

INTRODUCTION

We recently reported a gas chromatographic–mass spectrometric (GC–MS) screening procedure for formaldehyde-derived tetrahydro-β-carbolines (THBC), which could be involved in the aetiology of chronic alcoholism [1]. Tetrahydroisoquinolines (TIQ), condensation products between catecholamines and aldehydes formed by Pictet–Spengler reaction (Fig.1), could be of comparable significance. The formation occurs readily under physiological conditions [2] and produces substances that can function as neurotransmitters and/or neuromodulators. Like THBC compounds, TIQs evoke neuropharmacological and psychopharmacological actions in humans and animals, including inhibition of tyrosine hydroxylase activity [3], inhibition of monoamine oxidase (MAO) [4–7] and stimulation of prolactine release [8]. They are substrates and competitive inhibitiors of catecholamine-O-methyl transferase (COMT) [5,9] as well as of catecholamine uptake in neurons [10,11]. TIQ compounds expel catecholamines out of vesicles in brain dopamine fibres [12], were released and can function as "false transmitters" [13,14], and act as agonists or antagonists at dopamine-stimulated adenylate cyclase [15–17]. During the past

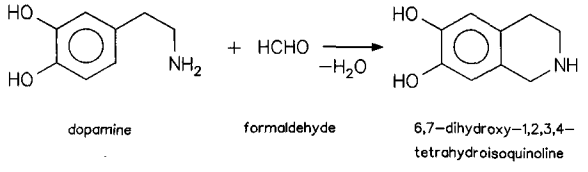

Fig. 1. The Pictet–Spengler reaction.

* Corresponding author.

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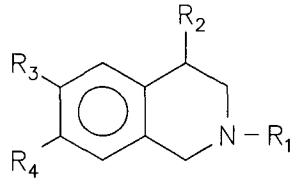
two decades interest has focused on the hypothesis that endogenously formed TIQ compounds similar to THBC compounds could contribute to psychotic effects observed in alcoholism, in schizophrenia or Parkinson's and Huntington's diseases [18–33]. Acute and chronic administration of selected TIQ compounds to rats has been reported to alter alcohol consumption significantly in the same way as THBC compounds [34–36].

The congener alcohol methanol, used as an alcoholism marker and discussed as a decisive factor in the research on the aetiology of chronic alcoholism [37–42], is regarded as an exogenous formaldehyde source. This oxidation product is a more potent reaction partner for TIQ formation than acetaldehyde [43,44]. There are some known metabolic pathways of formaldehyde synthesis, which may account for *in vivo* TIQ formation in tissues [45,46], but up to now nothing is known about how formation in humans depends on the oxidation product of ingested methanol. In our opinion, chronic consumption of alcoholic beverages coupled with methanol accumulation may cause an induction of TIQ- and THBC-forming mechanisms. So formaldehyde-derived TIQ and THBC compounds could be involved in the aetiology of alcoholism, and therefore could be considered as biological alcoholism markers. However, it is absolutely necessary to take dietary sources of tested persons into account, because exogenous TIQ compounds may be normal constituents of chocolate, bananas and other foods [47–49]. The aim of the present paper is to present a sensitive screening procedure for the measurement of formaldehyde-derived TIQ compounds in the urine of healthy volunteers and chronic alcoholics.

Various analytical methods have been developed to identify TIQ compounds [50–62]. Examination of these reports revealed a range of analytical methods with different sensitivities or specifities. It should be taken into consideration that analytical pitfalls produced by the work-up procedure, as described for the analysis of THBC compounds, can occur for TIQ compounds in the same way [63,64].

We have developed an analytical method that allows the determination of the following formaldehyde-derived TIQ compounds: N-methyl-1,2,3,4-tetrahydroisoquinoline (N-Me-TIQ), 1,2,3,4-tetrahydroisoquinoline (TIQ), 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (6,7-MeO-TIQ), 6-methoxy-7-hydroxy-1,2,3,4-tetrahydroisoquinoline (6-MeO-7-OH-TIQ), N-methyl-4,6,7-trihydroxy-1,2,3,4-tetrahydroisoquinoline (N-Me-4,6,7-OH-TIQ), 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (6,7-OH-TIQ) and 4,6,7-trihydroxy-1,2,3,4-tetrahydroisoquinoline (4,6,7-OH-TIQ) (Fig. 2). Glucuronidase is used to hydrolyse the samples, because TIQ compounds in urine may be excreted as glucuronides [51,59]. In analogy to our extraction procedure for THBC compounds, the procedure includes an initial chemical derivatization in aqueous solution to prevent artificial formation of TIQ compounds during the work-up procedure. The use of this kind of derivatization facilitates the isolation by liquid–liquid extraction. In a second step, a solid-phase extraction is used to eliminate most of the interfering matrix compounds. For separation and identification, a GC–MS method was developed.



	R_1	R_2	R_3	R_4
N-methyl-1,2,3,4-tetrahydroisoquinoline	$-CH_3$	–H	–H	–H
1,2,3,4-tetrahydroisoquinoline	–H	–H	–H	–H
6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline	–H	–H	$-OCH_3$	$-OCH_3$
6-methoxy-7-hydroxy-1,2,3,4-tetrahydroisoquinoline	–H	–H	$-OCH_3$	–OH
N-methyl-4,6,7-trihydroxy-1,2,3,4-tetrahydroisoquinoline	$-CH_3$	–OH	–OH	–OH
6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline	–H	–H	–OH	–OH
4,6,7-trihydroxy-1,2,3,4-tetrahydroisoquinoline	–H	–OH	–OH	–OH

Fig. 2. Structures of tetrahydroisoquinolines identified with the procedure described.

EXPERIMENTAL

Materials

Methanol, ethyl acetate (Uvasol, Merck, Darmstadt, Germany), acetonitrile (gradient grade, Merck), water (HPLC grade, Baker, Gross-Gerau, Germany), propionic anhydride, TIQ hydrochloride, β-glucuronidase–sulphatase type H-1 (all from Sigma, Deisenhofen, Germany), 3,4-dimethoxyphenylethylamine, N-methylphenylethylamine and 3-O-methyldopamine hydrochloride (Aldrich, Steinheim, Germany) were used. Inorganic chemicals used were acetic acid, hydrobromic acid, hydrochloric acid, paraformaldehyde, sodium hydrogencarbonate, semicarbazide (p.a., Merck). Worldwide Monitoring Clean Up C_{18} end-capped extraction columns (100 mg, 1 ml) were purchased from Amchro (Sulzbach/Taunus, Germany). Samples of 4,6,7-OH-TIQ and N-Me-4,6,7-OH-TIQ were purchased from Dr. Oebels. 6,7-OH-TIQ, 6,7-MeO-TIQ, 6-MeO-7-OH-TIQ and N-Me-TIQ were prepared by reaction of paraformaldehyde with 3,4-dimethoxyphenylethylamine, 3-O-methyldopamine and N-methylphenylethylamine, respectively, according to the method of Buck [65], with a slight modification according to the procedure of Barker *et al.* [52].

Instrumentation

A Model 5890A gas chromatograph (Hewlett-Packard) with a 5970A mass-selective detector (MSD) was used for analysis. Data acquisition and manipulation were performed using standard software supplied by the manufacturer. Perfluorotributylamine was used for a daily automatic tune. For sample analysis, the electron multiplier voltage of the detector was set in the range 200–400 V above the autotune voltage. A fused-silica capillary column OV1 (12 m × 0.2 mm I.D., d_f = 0.33 μm) was used. The temperature was programmed from an initial value of 60°C, held for 2 min, followed by a linear increase to 210°C at 30°C/min, then by a linear increase to 300°C at 10°C/min. The final temperature was held for 5 min. The injector (2 min split off) was maintained at a temperature of 260°C.

Sample preparation

The sample preparation procedure is outlined in Fig. 3. The sample consisted of 10 ml of urine adjusted to pH 4.5 with acetic acid, to which were added 0.1 ml of 1 *M* semicarbazide and β-glucuronidase (2500 U). For enzymic hydrolysis, the sample was incubated at 55°C for 2 h and then saturated with sodium hydrogencarbonate to adjust the pH to 9.0. Finally, 20 ml of 5% propionic anhydride in ethyl acetate were added. The sample was shaken for 30 min and centrifuged (5 min, 1000 *g*), and the organic supernatant was transferred to a glass tube. This procedure was repeated, and the combined organic layers were evaporated. To derivatize the rest of the free TIQ compounds, the residue was reconstituted in 50

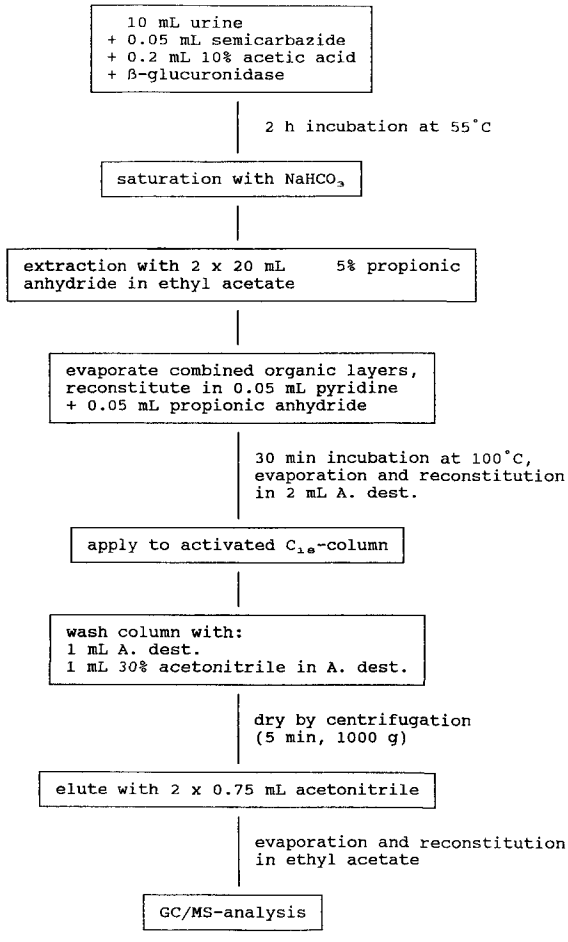


Fig. 3. Sample preparation procedure.

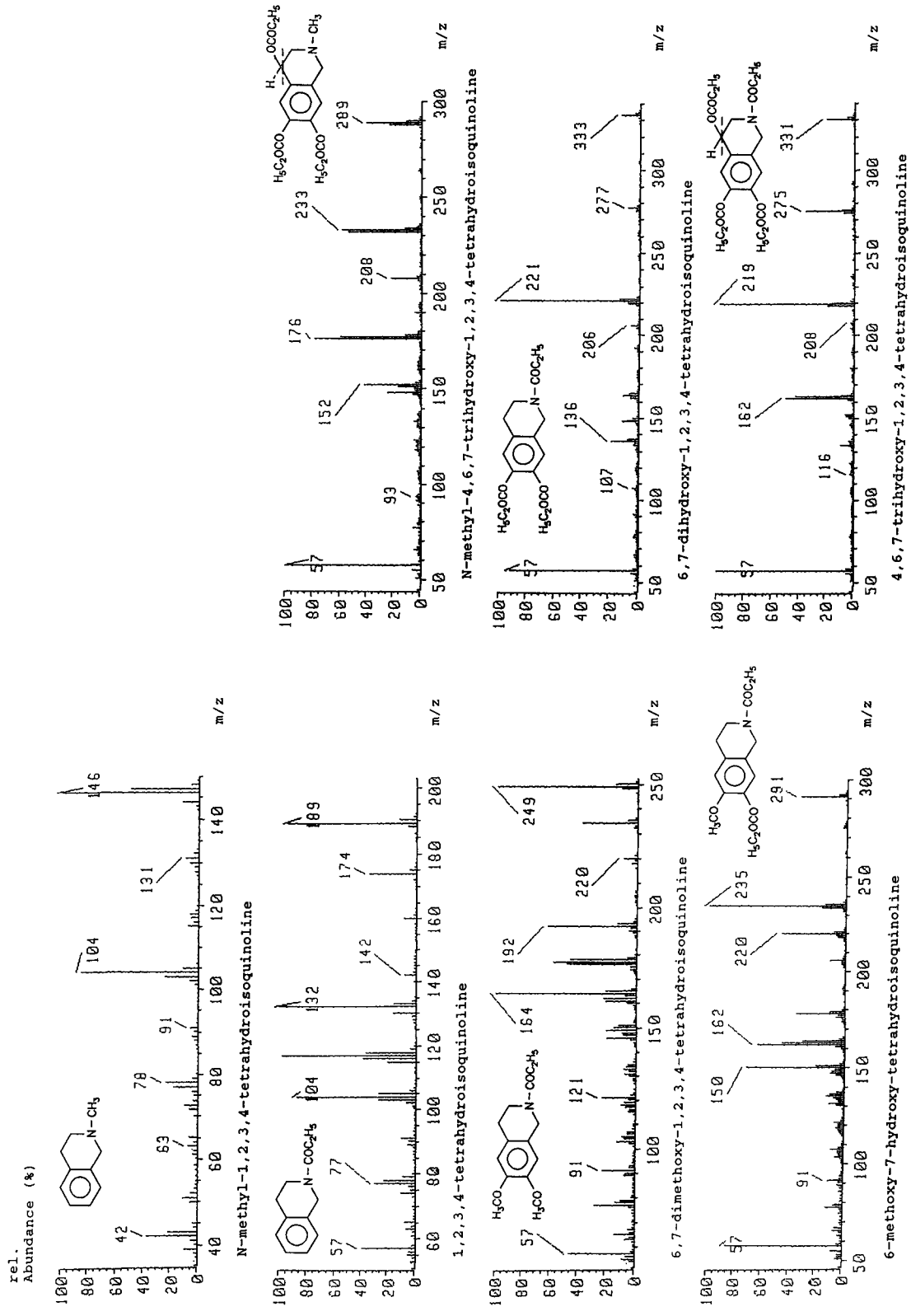


Fig. 4. Mass spectra of derivatized tetrahydroisoquinolines identified with this procedure.

μl of propionic anhydride–pyridine (1:1) and incubated for 30 min at 80°C. After cooling to room temperature, for solid-phase extraction 2 ml of water were added.

Before application of a sample, the C_{18} extraction columns were conditioned by washing with 2 ml of methanol followed by 2 ml of water. Prepared samples were applied to the columns under vacuum at a flow-rate of *ca.* 1 ml/min. The columns were washed with 1 ml of water, followed by 1 ml of 30% acetonitrile in water, and dried by centrifugation of the columns (5 min, 1000 *g*). The TIQ compounds were eluted with two 0.75-ml volumes of acetonitrile and collected in a vial. The eluate was evaporated to dryness at 50°C under a stream of nitrogen. The residue was dissolved in 20 μl of ethyl acetate, and a 2-μl aliquot was subjected to GC–MS analysis.

RESULTS AND DISCUSSION

Electron impact (EI) mass spectra of the compounds were recorded by total-ion monitoring. The mass spectra of the propionyl derivatives are shown in Fig. 4. Both 4-hydroxy metabolites yielded no molecular ion, because propionic acid is eliminated.

Each compound was characterized with respect to its base peak (normalized to 100%) and other prominent secondary mass fragments. The retention times were recorded, and the chosen diagnostic mass fragments were monitored for each compound in the selected-ion monitoring (SIM) mode in several acquisition groups (Table I). The mass fragments *m/z* 42, 104 and 146 were chosen to monitor for the presence of N-Me-TIQ, *m/z* 104, 117, 132 and 189 were used for TIQ, *m/z* 164, 192 and 249 were used for monitoring of 6,7-MeO-TIQ, *m/z* 150, 162, 235 and 291 for 6-MeO-7-OH-TIQ, the mass fragments *m/z* 152, 176, 233 and 289 for N-Me-4,6,7-OH-TIQ, *m/z* 57, 221 and 333 for 6,7-OH-TIQ, and the mass fragments selected for 4,6,7-OH-TIQ were *m/z* 162, 219, 275 and 331. Reference standards were examined in the SIM mode, with retention times and ion ratios being recorded, prior to and following injection of the samples obtained from urine extracts.

Fig. 5 shows a gas chromatogram of a reference standard with 100 pg per substance. Experiments with spiked urine samples (100 pg/ml) showed that the peaks observed had the same retention times, mass fragments and ion mass ratios as those observed for the external standards. The biological matrix did not produce any shift in the three identification marks. Recoveries determined with external standard range from 69.9 ± 7.1 to 93.7 ± 8.7% (n = 5). Using the routine method described above, the minimum detectable concentrations of the TIQ compounds were 50–100 pg/ml urine.

In agreement with Kauert *et al.* [66], this method is based on the known chemical reactivity of acyl anhydrides, especially propionyl anhydride,

TABLE I

CHARACTERISTIC MASS FRAGMENTS, RETENTION TIMES AND RECOVERIES DETERMINATED WITH EXTERNAL STANDARD

Substance	*m/z*	Retention time (min)	Recovery (mean ± S.D., n = 5) (%)
N-Me-TIQ	42, 104, 146	5.52	93.7 ± 8.7
TIQ	104, 117, 132, 189	7.94	93.5 ± 8.0
6,7-MeO-TIQ	164, 192, 249	10.79	89.5 ± 6.3
6-MeO-7-OH-TIQ	150, 162, 235, 291	12.43	80.9 ± 7.1
N-Me-4,6,7-OH-TIQ	152, 176, 233, 289	12.61	74.3 ± 6.2
6,7-OH-TIQ	57, 221, 333	14.02	76.3 ± 6.8
4,6,7-OH-TIQ	162, 219, 275, 331	15.51	69.9 ± 7.1

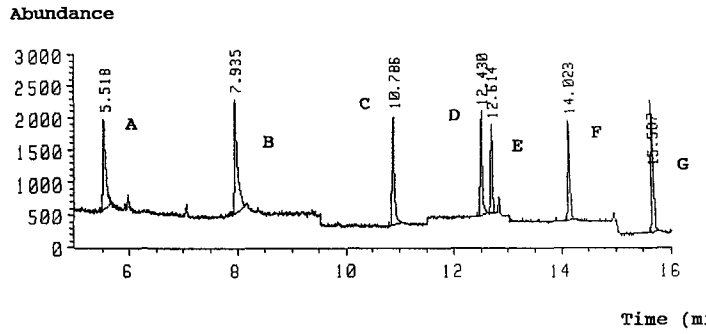


Fig. 5. Gas chromatogram obtained with 100 pg of each substance. Peaks: A = N-Me-TIQ; B = TIQ; C = 6,7-MeO-TIQ; D = 6-MeO-7-OH-TIQ; E = N-Me-4,6,7-OH-TIQ; F = 6,7-OH-TIQ; G = 4,6,7-OH-TIQ.

with catecholamines, phenylethylamines, indolalkylamines and with the quite similar TIQ compounds producing stable and hydrophobic acyl derivatives. The procedure can take place in an aqueous medium initially to decrease or eliminate the potential for artificial formation of TIQ compounds, because biogenic amines are no longer free and available to react with an aldehyde. Furthermore, derivatization results in products that are readily extractable by organic solvents. Additionally, semicarbazide was added to the sample to remove any formaldehyde present or arising during the work-up procedure. The following extensive clean-up by solid-phase extraction, including a washing step with 30% acetonitrile in water, produced highly purified extracts, and so the derivatives with relatively low mass are not obscured by "background" ions from other biological compounds in the matrix. These derivatives possess good GC properties. Therefore the retention times, together with the diagnostic mass fragments (at least three) and the specific ion ratios, can be used to identify TIQ compounds in human urine (Fig. 6). The procedure described can be considered as an effective screening method and should facilitate the study of *in vivo* formation of TIQ compounds.

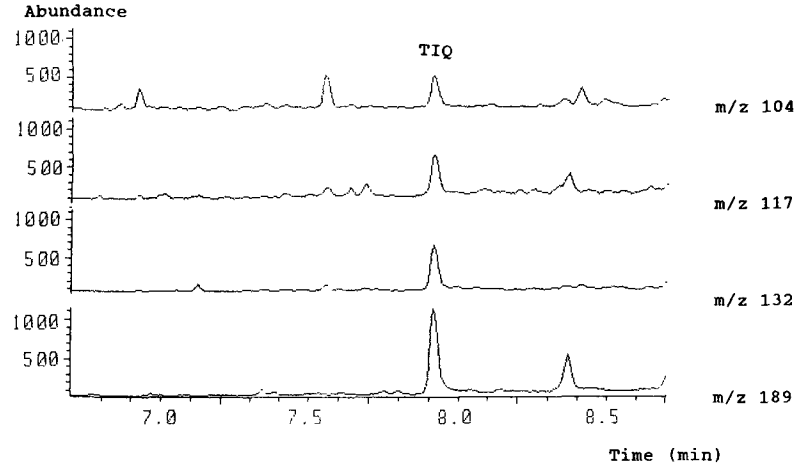


Fig. 6. TIQ detected in an urine sample from a chronic alcoholic.

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