Appendix E
Guidelines of the GTFCh for Quality Assurance in Forensic Toxicology. Version 02

ANALYSIS OF CONGENER SUBSTANCES IN BIOLOGICAL MATERIALS AND BEVERAGE SAMPLES BY HEADSPACE GAS-CHROMATOGRAPHY

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Amendment to the general guidelines

Contents

1. General

Most alcoholic beverages, particularly those involving fermentation, contain not only ethanol but also methanol and other higher aliphatic alcohols which in forensic terms, are called 'congener substances'. There are, however, high-proof alcoholic beverages containing congener substances in concentrations of no practical relevance.

The concentration profile of congener substances in blood depends upon the amount ingested and the period of time after ingestion. Knowing the theoretical blood or serum concentrations for congener substances over time, values determined by headspace gas-chromatography (HS-GC) in case samples can be compared with those calculated. The rapid ingestion of a high-proof alcoholic drink shortly before a blood sample is drawn is often a post-offence drinking claim which would explain completely, mainly, or in part the resulting blood alcohol level.

Analysis of alcohols and congener substances by HS-GC was developed by MACHATA (1). In 1979, congener substance analysis was first introduced in a court of law by BONTE and in 1983 this test was recognised by supreme court jurisdiction (State Supreme Court Celle) as an unbiased method for the qualitative and quantitative investigation of suspected post-offence drinking (2).

In the course of a survey (3), guidelines for the forensic analysis of congener substances in whole blood, serum and plasma were harmonised. The present guidelines do not refer to the expert assessment of analytically confirmed concentrations of congener substances in a particular sample. Despite this and in order to guard against false interpretation, an evaluation of the results of congener substance analysis cannot be recommended without comprehensive information and understanding of the fundamentals of the analytical procedure.

In general, the guidelines of the GTFCh are applicable (4).

2. Objective and area of application

The aim of this test is to provide a report of concentrations of congener substances in a suitable whole blood, serum or plasma sample or in an alcoholic beverage for use as evidence in a court of law. The test is performed on behalf of public authorities or institutions on samples obtained according to paragraph 81a of the German Code of Criminal Procedure.

Separate guidelines apply to the forensic analysis of ethanol in blood (5).

3. Scope of investigation

The analysis of congener substances should contain the following: methanol, 1-propanol, 2-butanone (methyl-ethyl-ketone), 2-butanol, 2-methyl-1-propanol (isobutanol), 1-butanol as well as 2-methyl-1-butanol and 3-methyl-1- butanol (alternatively as total). Further substances may be added if required.

4. Instrumentation

Analysis of congener substances is usually performed by HS-GC with either flame ionisation (FID) or mass-spectrometric (MS) detection. Two columns of differing polarity may be used. To introduce the sample, headspace sampling, syringe injection, valve loop injection or pressure balance injection may be used. For sample enrichment suitable methods (e. g. cryofocussing or adsorption trapping) may also be used.

5. Sample material

5.1. Overview

The forensic analysis of congener substances in biological material is usually carried out long after that of ethanol in blood. Therefore, it is vital to confirm that the available sample is suitable for analysis (sample volume, sample storage effects). If serum or plasma is available, then these must be used for analysis.

As a basis for interpreting the levels of congener substances in serum, plasma or whole blood samples, the concentrations of these substances in the suspected alcoholic beverage are required. For this purpose, comparable data (2,6) or concurrently measured values of the beverage in question are appropriate.

When beverage samples are analysed and the results are aimed to be added to congener substance libraries, rigorous documentation is required for e.g. exact name of beverage, manufacturer, batch number, date and condition (original or opened).

5.2. Sample storage

The conditions for the storage of samples are consistent with those laid down in the guidelines for blood alcohol analysis (5). Alcoholic beverages may be stored at ambient room temperature.

6. Staff and laboratory requirements

6.1. Staff

The regulations covering personnel requirements correspond to those laid down in the GTFCh guidelines (4).

6.2. Laboratory

The forensic analysis of congener substances may only be performed in especially designated laboratories; e.g. in a laboratory for blood alcohol testing.

Contamination of blood, serum or plasma samples, standards, reagents and instruments with volatile compounds, e.g. ethanol or other substances, must be strictly avoided.

7. Analysis

7.1. Sample preparation

The sample volume required depends upon the size of the instrument vial used for the analysis. For 20 mL headspace vials, a sample volume of at least 200 µL serum is recommended.

Addition of anhydrous sodium sulphate is required to increase the headspace pressure. The amount added depends upon the fluid volume in the vial; e.g. for a total volume of 400 µL (serum sample + internal standard), at least 0.3 g of anhydrous sodium sulphate (Na_2SO_4) are deemed sufficient.

Other sample preparation methods such as deproteinisation or ultrafiltration are allowed.

Analogous to the gas-chromatographic determination of blood alcohol, tertiary butanol (*tert*-butanol) may be used (e.g. 1 mg/L) as the internal standard. Other suitable alternatives are possible. When using mass spectrometry, deuterated analogues may be used as internal standards provided the isotopic purity is guaranteed.

Additional glucuronide digestion is permitted and this must be documented and considered separately.

Alcoholic beverages must be diluted appropriately with water so that the measurements lie within the respective calibration ranges. In order to detect low concentrations of congeners, a 1:10 dilution of the sample should be analysed. Analysis of further dilutions may be helpful in certain cases.

7.2. Chromatography

Medium and high polarity capillary GC columns may be used. When using standard injection techniques, wide-bore and thick-film columns are preferred because of the large vapour volume; however, if concentration techniques such as cryofocussing or trap are used, such columns are not required.

Gas-chromatographic separation is usually performed with a temperature program starting at a low initial temperature (e.g. 40°C). The program must be chosen to achieve the complete separation of methanol/acetaldehyde, 2-methyl-1-propanol (isobutanol)/3-methylbutanal (isovaleraldehyde), 2-methyl-1-butanol/3-methyl-1-butanol (either single or as total) and ethylacetate/2-butanol.

Mass-spectrometric detection must conform with the current guidelines of the GTFCh (4).

7.3. Calibration

For the quantitation of congener substances in body fluids, aqueous calibrators (standard solutions) or such prepared in serum are used and the respective concentrations must be guaranteed. According to the general guidelines, a calibration using aqueous solutions must conform with a calibration using identical standards prepared in a matrix.

For methanol, a range of 1.0 to 20 mg/L is recommended and for other congeners, between 0.1 and 2.0 mg/L.

The lower limit of detection for methanol has to be $\leq$ 1.0 mg/L and $\leq$ 0.1 mg/L for other congeners.

Analysis of congener substances in alcoholic beverages requires a calibration using aqueous solutions. The current guidelines of the GTFCh apply (4).

7.4. Procedure

If two blood samples are drawn, then both samples are to be tested. Analysis in duplicate with two separate sample preparation steps is preferred. To eliminate sample carry-over, the inclusion of a blank sample (water) between the individual test samples is mandatory.

7.5. Quality assurance

The method for analysis of congener substances must be validated according to the current guidelines of the GTFCh.

With regard to method specificity and selectivity, the following possible interfering substances are to be monitored: ethanol, acetaldehyde, ethyl acetate, methyl acetate, propyl acetate, isopropanol, acetone, propionylaldehyde, isobutylaldehyde and isovalerylaldehyde.

In addition, other volatiles may elute with similar retention times to those congeners to be assayed (e.g. hydrogen cyanide, ether, chloroform, halothane, benzene, *o*-, *m*-, and *p*-xylene, toluene, hexane, heptane, dichloromethane, cyclohexane, tricholorethane, trichloroethylene, carbon tetrachloride, trichloroacetic acid, benzylalcohol, methanethiol).

The possible effects of butyl-rubber stoppers or additional enzyme preparations should be considered. Should previously unidentified interferences occur, then this must be documented and should be reported to the appropriate scientific committee of the GTFCh.

Internal quality control consists of the analysis of at least two different QC samples, handled identically to test samples, in each run. Of these QC samples, at least one certified control sample - in a matrix identical to the test samples - must be analysed.

Results (data, date and laboratory analyst) have to be documented in an appropriate QC chart including target level, confidence intervals, manufacturer and batch number. The limits to be complied with correspond to those in the guidelines to quality assurance in forensic toxicological investigations compiled by the GTFCh.

Quality control of the method in use must be regularly checked. All variations must be documented, commented and corrective measures undertaken. Regular participation in external proficiency testing is mandatory where available.

7.6. Reporting

The report should contain the results of each single measurement given in mg/L or as the arithmetic mean in mg/L.

Each analysis result must be reported after cutting (not rounding) and should involve a maximum of two significant digits (e. g. one further digit after the first digit different from Zero), except when otherwise required.

Levels of 2- and 3-methyl-1-butanol may be given as total. All deviations from the guidelines must be commented and reported to the customer.

8. References and further documents

1. Machata G. Über die gaschromatographische Blutalkoholbestimmung. Blutalkohol 4 (1967):252-260
2. Bonte W (1987) Begleitstoffe alkoholischer Getränke. Verlag Max Schmidt-Römhild Lübeck
3. Schulz K, Teske J, Gilg T, Aderjan R, Herbold M, Bestandsaufnahme der Begleitstoffanalyse und Ergebnisse erster Ringversuche. Blutalkohol 43 (2006):269-276
4. Richtlinie der GTFCh zur Qualitätssicherung bei forensisch-toxikologischen Untersuchungen. Toxichem Krimtech 76 (2009):142-176
5. Richtlinien zur Bestimmung der Blutalkoholkonzentration im Blut (BAK) für forensische Zwecke. Blutalkohol 48 (2011):137-143
6. Arbeitskreis „Alkoholkonsum und Nachtrunk“,
7. http://www.gtfch.org/php/index.php/alkohol-und-nachtrunk

8. Richtlinie der GTFCh zur Qualitätssicherung bei forensisch-toxikologischen Untersuchungen. Anhang E - Begleitstoffuntersuchungen mit Dampfraum-Gaschromatographie im biologischen Material. Toxichem Krimtech 78 (2011):16-22

9. Effect

This amendment was ratified by the executive committee of the GTFCh on 8th December 2015 and is effective as of publication in Toxichem Krimtech.

Previous guidelines are no longer valid (7).

Only the original German version of these guidelines is applicable for forensic purposes.