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# Aromatic Compounds in Blood – Determination using Headspace Gas Chromatography with Mass Spectrometric Detection

## Biomonitoring Method – Translation of the German version from 2018

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# Aromatic Compounds in Blood – Determination using Headspace Gas Chromatography with Mass Spectrometric Detection

## Biomonitoring Methods

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### Abstract

The working group „Analyses in Biological Materials" of the Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area verified the presented biomonitoring method.
The here described analytical method enables the simultaneous determination of benzene, toluene, chlorobenzene, ethylbenzene, o-xylene, m-xylene, p-xylene, styrene, n-propylbenzene, isopropylbenzene (cumene), 1,2,3-trimethylbenzene (hemimellitene), 1,2,4-trimethylbenzene, 1,3,5-trimethylbenzene (mesitylene) and 1,2,3,5-tetramethylbenzene (isodurene) in blood. For determination, the blood samples are introduced into headspace vials. The vials are sealed and heated to 50 °C in the autosampler. Subsequently, an aliquot of the vapour phase is injected into the GC system and analysed using mass spectrometry. Calibration standards are prepared in ovine blood and processed in the same way as the samples to be analysed.



### Keywords

benzene; toluene; chlorobenzene; ethylbenzene; o-xylene; m-xylene; p-xylene; styrene; n-propylbenzene; isopropylbenzene; cumene; 1,2,3-trimethylbenzene; hemimellitene; 1,2,4-trimethylbenzene; 1,3,5-trimethylbenzene; mesitylene; 1,2,3,5-tetramethylbenzene; isodurene; blood; biomonitoring; Analyses in Biological Materials; gas chromatography; mass spectrometry; headspace; GC-MS



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# Aromatic Compounds in Blood – Determination using Headspace Gas Chromatography with Mass Spectrometric Detection

| | |
|---|---|
| **Matrix:** | Blood |
| **Hazardous substances:** | Aromatic compounds |
| **Analytical principle:** | Headspace gas chromatography with mass spectrometric detection (GC/MS) |
| **Completed in:** | November 2016 |

Overview of the parameters that can be determined with this method and the corresponding hazardous substances:

| Hazardous substance | CAS | Parameter | CAS |
|---|---|---|---|
| Benzene | 71-43-2 | Benzene | 71-43-2 |
| Toluene | 108-88-3 | Toluene | 108-88-3 |
| Chlorobenzene | 108-90-7 | Chlorobenzene | 108-90-7 |
| Ethylbenzene | 100-41-4 | Ethylbenzene | 100-41-4 |
| m-Xylene | 108-38-3 | m-Xylene | 108-38-3 |
| p-Xylene | 106-42-3 | p-Xylene | 106-42-3 |
| o-Xylene | 95-47-6 | o-Xylene | 95-47-6 |
| Styrene | 100-42-5 | Styrene | 100-42-5 |
| Isopropylbenzene (Cumene) | 98-82-8 | Isopropylbenzene | 98-82-8 |
| n-Propylbenzene | 103-65-1 | n-Propylbenzene | 103-65-1 |
| 1,3,5-Trimethylbenzene (Mesitylene) | 108-67-8 | Mesitylene | 108-67-8 |
| 1,2,4-Trimethylbenzene | 95-63-6 | 1,2,4-Trimethylbenzene | 95-63-6 |
| 1,2,3-Trimethylbenzene (Hemimellitene) | 526-73-8 | Hemimellitene | 526-73-8 |
| 1,2,3,5-Tetramethylbenzene (Isodurene) | 527-53-7 | Isodurene | 527-53-7 |

## Summary

The analytical method described hereinafter enables the simultaneous determination of benzene, toluene, chlorobenzene, ethylbenzene, o-xylene, m-xylene, p-xylene, styrene, n-propylbenzene, isopropylbenzene (cumene), 1,2,3-trimethylbenzene (hemimellitene), 1,2,4-trimethylbenzene, 1,3,5-trimethylbenzene (mesitylene) and 1,2,3,5-tetramethylbenzene (isodurene) in blood. For determination, the blood samples are introduced into headspace vials. The vials are sealed and heated to 50 °C in the autosampler. Subsequently, an aliquot of the vapour phase is injected into the GC system and analysed using mass spectrometry. Calibration standards are prepared in ovine blood and processed in the same way as the samples to be analysed.

### Reliability data of the method

**Benzene**

| | | |
|---|---|---|
| Within-day precision: | Standard deviation (rel.) | $s_w$ = 1.3% or 1.8% |
| | Prognostic range | $u$ = 2.9% or 4.1% |
| | at a spiked concentration of 14 µg or 56 µg benzene per litre blood and where n = 10 determinations | |
| Day-to-day precision: | Standard deviation (rel.) | $s_w$ = 6.7% |
| | Prognostic range | $u$ = 14.0% |
| | at a spiked concentration of 10 µg benzene per litre blood and where n = 20 determinations | |
| Accuracy: | Recovery rate (rel.) | $r$ = 90% |
| | at a spiked concentration of 14 µg benzene per litre blood and where n = 10 determinations | |
| Detection limit: | 0.7 µg benzene per litre blood | |
| Quantitation limit: | 2.1 µg benzene per litre blood | |

**Toluene**

| | | |
|---|---|---|
| Within-day precision: | Standard deviation (rel.) | $s_w$ = 1.7% or 3.6% |
| | Prognostic range | $u$ = 3.8% or 8.1% |
| | at a spiked concentration of 87 µg or 348 µg toluene per litre blood and where n = 10 determinations | |
| Day-to-day precision: | Standard deviation (rel.) | $s_w$ = 8.0% |
| | Prognostic range | $u$ = 16.7% |
| | at a spiked concentration of 60 µg toluene per litre blood and where n = 20 determinations | |
| Accuracy: | Recovery rate (rel.) | $r$ = 92% |
| | at a spiked concentration of 87 µg toluene per litre blood and where n = 10 determinations | |
| Detection limit: | 0.7 µg toluene per litre blood | |
| Quantitation limit: | 2.1 µg toluene per litre blood | |

**Chlorobenzene**

| | | |
|---|---|---|
| Within-day precision: | Standard deviation (rel.) | $s_w$ = 2.6% or 3.0% |
| | Prognostic range | $u$ = 6.0% or 6.7% |
| | at a spiked concentration of 53 µg or 213 µg chlorobenzene per litre blood and where n = 10 determinations | |
| Day-to-day precision: | Standard deviation (rel.) | $s_w$ = 6.9% |
| | Prognostic range | $u$ = 14.4% |
| | at a spiked concentration of 50 µg chlorobenzene per litre blood and where n = 20 determinations | |
| Accuracy: | Recovery rate (rel.) | $r$ = 87% |
| | at a spiked concentration of 53 µg chlorobenzene per litre blood and where n = 10 determinations | |
| Detection limit: | 0.9 µg chlorobenzene per litre blood | |
| Quantitation limit: | 2.7 µg chlorobenzene per litre blood | |

**Ethylbenzene**

| | | |
|---|---|---|
| Within-day precision: | Standard deviation (rel.) | $s_w$ = 2.0% or 3.7% |
| | Prognostic range | $u$ = 4.5% or 8.4% |
| | at a spiked concentration of 86 µg or 344 µg ethylbenzene per litre blood and where n = 10 determinations | |
| Day-to-day precision: | Standard deviation (rel.) | $s_w$ = 7.0% |
| | Prognostic range | $u$ = 14.7% |
| | at a spiked concentration of 60 µg ethylbenzene per litre blood and where n = 20 determinations | |
| Accuracy: | Recovery rate (rel.) | $r$ = 87% |
| | at a spiked concentration of 86 µg ethylbenzene per litre blood and where n = 10 determinations | |
| Detection limit: | 0.9 µg ethylbenzene per litre blood | |
| Quantitation limit: | 2.7 µg ethylbenzene per litre blood | |

**m-Xylene and p-xylene**

| | | |
|---|---|---|
| Within-day precision: | Standard deviation (rel.) | $s_w$ = 2.1% or 2.9% |
| | Prognostic range | $u$ = 4.8% or 6.6% |
| | at a spiked concentration of 85 µg or 341 µg m-xylene/p-xylene per litre blood and where n = 10 determinations | |
| Day-to-day precision: | Standard deviation (rel.) | $s_w$ = 7.2% |
| | Prognostic range | $u$ = 15.1% |
| | at a spiked concentration of 130 µg m-xylene/p-xylene per litre blood and where n = 20 determinations | |

| | | |
|---|---|---|
| Accuracy: | Recovery rate (rel.) | $r$ = 88% |
| | at a spiked concentration of 85 µg m-xylene/p-xylene per litre blood and where n = 10 determinations | |
| Detection limit: | 0.9 µg m-xylene/p-xylene per litre blood | |
| Quantitation limit: | 2.7 µg m-xylene/p-xylene per litre blood | |

**o-Xylene**

| | | |
|---|---|---|
| Within-day precision: | Standard deviation (rel.) | $s_w$ = 1.8% or 3.1% |
| | Prognostic range | $u$ = 4.0% or 7.0% |
| | at a spiked concentration of 87 µg or 348 µg o-xylene per litre blood and where n = 10 determinations | |
| Day-to-day precision: | Standard deviation (rel.) | $s_w$ = 7.2% |
| | Prognostic range | $u$ = 15.1% |
| | at a spiked concentration of 130 µg o-xylene per litre blood and where n = 20 determinations | |
| Accuracy: | Recovery rate (rel.) | $r$ = 89% |
| | at a spiked concentration of 87 µg o-xylene per litre blood and where n = 10 determinations | |
| Detection limit: | 0.9 µg o-xylene per litre blood | |
| Quantitation limit: | 2.7 µg o-xylene per litre blood | |

**Styrene**

| | | |
|---|---|---|
| Within-day precision: | Standard deviation (rel.) | $s_w$ = 2.1% or 4.0% |
| | Prognostic range | $u$ = 4.6% or 9.1% |
| | at a spiked concentration of 88 µg or 354 µg styrene per litre blood and where n = 10 determinations | |
| Day-to-day precision: | Standard deviation (rel.) | $s_w$ = 8.1% |
| | Prognostic range | $u$ = 17.0% |
| | at a spiked concentration of 60 µg styrene per litre blood and where n = 20 determinations | |
| Accuracy: | Recovery rate (rel.) | $r$ = 70% |
| | at a spiked concentration of 88 µg styrene per litre blood and where n = 10 determinations | |
| Detection limit: | 1.0 µg styrene per litre blood | |
| Quantitation limit: | 3.0 µg styrene per litre blood | |

**Isopropylbenzene (cumene)**

| | | |
|---|---|---|
| Within-day precision: | Standard deviation (rel.) | $s_w$ = 2.1% or 3.3% |
| | Prognostic range | $u$ = 4.8% or 7.6% |
| | at a spiked concentration of 86 µg or 345 µg cumene per litre blood and where n = 10 determinations | |

| | | |
|---|---|---|
| Day-to-day precision: | Standard deviation (rel.) | $s_w$ = 9.0% |
| | Prognostic range | $u$ = 18.8% |
| | at a spiked concentration of 60 µg cumene per litre blood and where n = 20 determinations | |
| Accuracy: | Recovery rate (rel.) | $r$ = 86% |
| | at a spiked concentration of 86 µg cumene per litre blood and where n = 10 determinations | |
| Detection limit: | 1.0 µg cumene per litre blood | |
| Quantitation limit: | 3.0 µg cumene per litre blood | |

**n-Propylbenzene**

| | | |
|---|---|---|
| Within-day precision: | Standard deviation (rel.) | $s_w$ = 2.8% or 3.3% |
| | Prognostic range | $u$ = 6.2% or 7.5% |
| | at a spiked concentration of 84 µg or 337 µg n-propylbenzene per litre blood and where n = 10 determinations | |
| Day-to-day precision: | Standard deviation (rel.) | $s_w$ = 8.8% |
| | Prognostic range | $u$ = 18.4% |
| | at a spiked concentration of 60 µg n-propylbenzene per litre blood and where n = 20 determinations | |
| Accuracy: | Recovery rate (rel.) | $r$ = 87% |
| | at a spiked concentration of 84 µg n-propylbenzene per litre blood and where n = 10 determinations | |
| Detection limit: | 1.0 µg n-propylbenzene per litre blood | |
| Quantitation limit: | 3.0 µg n-propylbenzene per litre blood | |

**1,3,5-Trimethylbenzene (mesitylene)**

| | | |
|---|---|---|
| Within-day precision: | Standard deviation (rel.) | $s_w$ = 2.1% or 3.2% |
| | Prognostic range | $u$ = 4.8% or 7.2% |
| | at a spiked concentration of 85 µg or 339 µg mesitylene per litre blood and where n = 10 determinations | |
| Day-to-day precision: | Standard deviation (rel.) | $s_w$ = 9.9% |
| | Prognostic range | $u$ = 20.7% |
| | at a spiked concentration of 60 µg mesitylene per litre blood and where n = 20 determinations | |
| Accuracy: | Recovery rate (rel.) | $r$ = 85% |
| | at a spiked concentration of 85 µg mesitylene per litre blood and where n = 10 determinations | |
| Detection limit: | 1.5 µg mesitylene per litre blood | |
| Quantitation limit: | 4.5 µg mesitylene per litre blood | |

**1,2,4-Trimethylbenzene**

| | | |
|---|---|---|
| Within-day precision: | Standard deviation (rel.) | $s_w$ = 3.3% or 4.6% |
| | Prognostic range | $u$ = 7.5% or 10.3% |
| | at a spiked concentration of 86 µg or 342 µg 1,2,4-trimethylbenzene per litre blood and where n = 10 determinations | |
| Day-to-day precision: | Standard deviation (rel.) | $s_w$ = 9.1% |
| | Prognostic range | $u$ = 19.0% |
| | at a spiked concentration of 60 µg 1,2,4-trimethylbenzene per litre blood and where n = 20 determinations | |
| Accuracy: | Recovery rate (rel.) | $r$ = 90% |
| | at a spiked concentration of 86 µg 1,2,4-trimethylbenzene per litre blood and where n = 10 determinations | |
| Detection limit: | 1.5 µg 1,2,4-trimethylbenzene per litre blood | |
| Quantitation limit: | 4.5 µg 1,2,4-trimethylbenzene per litre blood | |

**1,2,3-Trimethylbenzene (hemimellitene)**

| | | |
|---|---|---|
| Within-day precision: | Standard deviation (rel.) | $s_w$ = 1.8% or 4.9% |
| | Prognostic range | $u$ = 4.0% or 11.0% |
| | at a spiked concentration of 87 µg or 348 µg hemimellitene per litre blood and where n = 10 determinations | |
| Day-to-day precision: | Standard deviation (rel.) | $s_w$ = 8.4% |
| | Prognostic range | $u$ = 17.6% |
| | at a spiked concentration of 60 µg hemimellitene per litre blood and where n = 20 determinations | |
| Accuracy: | Recovery rate (rel.) | $r$ = 90% |
| | at a spiked concentration of 87 µg hemimellitene per litre blood and where n = 10 determinations | |
| Detection limit: | 1.5 µg hemimellitene per litre blood | |
| Quantitation limit: | 4.5 µg hemimellitene per litre blood | |

**1,2,3,5-Tetramethylbenzene (Isodurene)**

| | | |
|---|---|---|
| Within-day precision: | Standard deviation (rel.) | $s_w$ = 4.5% or 6.4% |
| | Prognostic range | $u$ = 10.3% or 14.5% |
| | at a spiked concentration of 87 µg or 347 µg isodurene per litre blood and where n = 10 determinations | |
| Day-to-day precision: | Standard deviation (rel.) | $s_w$ = 9.8% |
| | Prognostic range | $u$ = 20.5% |
| | at a spiked concentration of 60 µg isodurene per litre blood and where n = 20 determinations | |

| | | |
|---|---|---|
| Accuracy: | Recovery rate (rel.) | $r$ = 93% |
| | at a spiked concentration of 87 µg isodurene per litre blood and where n = 10 determinations | |
| Detection limit: | 3.0 µg isodurene per litre blood | |
| Quantitation limit: | 9.0 µg isodurene per litre blood | |

## General information on the hazardous substances

In chemical terms, most industrially used aromatic solvents are mixtures of various aromatic compounds, so that persons subjected to aromatic solvent exposure are often exposed to a number of the aforementioned aromatic compounds at the same time. The Commission has already classified most of the aromatic compounds that can be determined by this method. Table 1 gives an overview of the individual toxicological classifications. Please refer to the respective MAK and BAT Value Documentations of the individual substances for details on the toxicological classifications.

Metabolism of the individual substances varies quite significantly. Benzene is primarily ring-oxidized to phenol, which can be further oxidized to hydroquinone and catechol or other reactive products. These oxidation products can be excreted after conjugation or they may react with glutathione or with nucleophilic macromolecules in the cell [Henschler 1992]. In contrast, the aliphatic side chain of the alkylbenzenes is predominantly oxidized to form carboxylic acids, which are excreted in the urine either in the free form or as conjugates, e.g. xylene in the form of methylhippuric acids [Greim 1998a, *translated*], toluene also as hippuric acids or as o-cresol and p-cresol [Greim 1993, *translated*], chlorobenzene as sulphate and glucuronic acid conjugates of the chlorophenols and chlorocatechols [Greim 1995], and styrene in the form of mandelic acid, phenylglyoxylic acid, benzoic acid or hippuric acid [Henschler 1987]. However, this method only allows the analysis of unmetabolised aromatic compounds in blood. Therefore, this method is predomi-

**Table 1** Classification of aromatic compounds by the Commission [List of MAK and BAT Values 2017][a].

| Substance | Designation H or S | Carcinogen category | Germ cell mutagen category | Assessment value in blood | Occupational exposure limit (MAK) |
|---|---|---|---|---|---|
| Benzene | H | 1 | 3A | – | – |
| Toluene | H | – | – | BAT: 600 µg/L | 50 mL/m³ |
| Chlorobenzene | – | – | – | – | 5 mL/m³ |
| Ethylbenzene | H | 4 | – | – | 20 mL/m³ |
| Xylene (all isomers) | H | – | – | – | 100 mL/m³ |
| Styrene | – | 5 | – | – | 20 mL/m³ |
| Isopropylbenzene | H | 3B | – | – | 10 mL/m³ |
| Trimethylbenzene (all isomers) | – | – | – | – | 20 mL/m³ |

[a] n-Propylbenzene and isodurene have not been classified by the Commission yet.

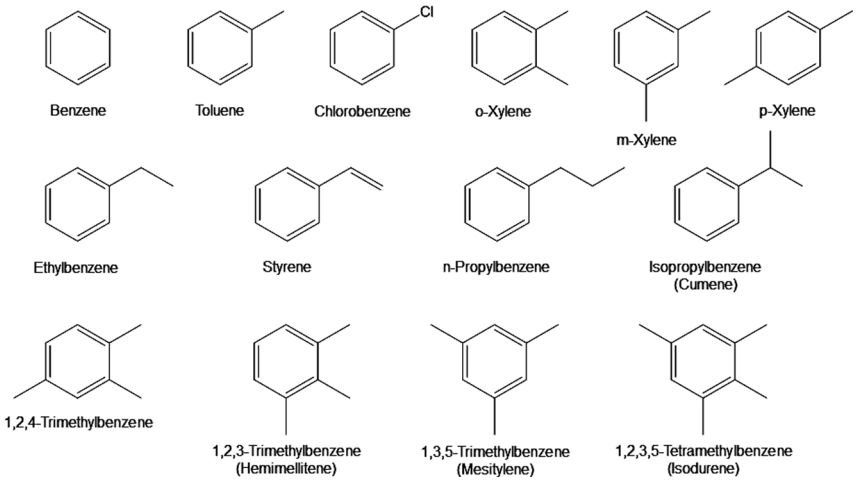


**Figure 1** Structures of the analytes.

nantly suitable for the determination of acute exposure that took place only a few hours before specimen collection. Figure 1 shows the structures of the analytes that can be determined by this method.

Benzene is used as a starting material for the synthesis of many benzene derivatives such as styrene, phenol, cyclohexane, aniline, etc. Due to its carcinogenicity, benzene is no longer used as a solvent [Römpp 2017]. Benzene is well absorbed after oral or inhalative exposure. A substantial portion of benzene is exhaled unchanged. It accumulates in fatty tissues and can be absorbed in the placenta. Percutaneous absorption of benzene is also possible [Henschler 1992, 1988].

Toluene is primarily employed as a solvent for paints, resins, varnishes and adhesives as well as for the extraction of natural substances. Besides, toluene is a fuel additive. Furthermore, it is an important starting material for chemical syntheses [Römpp 2017; Henschler 1986]. Apart from inhalation [Greim 1993, *translated*], dermal absorption [Greim 1998b] is one major route of exposure to toluene.

Technical xylene occurs as a mixture of the isomers o-xylene, m-xylene and p-xylene, which is why in practice there is often exposure to all three isomers at once. Xylenes are used as solvents for oils, fats, resins, varnishes and paints as well as to remove lubricating grease from metals. Furthermore, o-xylene and p-xylene are starting materials for the production of phthalic anhydride and terephthalic acid [Weissermehl and Arpe 1978; Römpp 2017]. Inhalation is the main intake route, however, also dermal absorption is possible.

Ethylbenzene is produced by alkylating benzene with ethylene and is used both as a solvent or diluent and as a starting material for styrene synthesis [Weissermehl and Arpe 1978; Römpp 2017]. It is readily absorbed through the lungs and, to a smaller extent, through the skin.

Trimethylbenzene is a natural component of petroleum. It can be found in fuel and in mixtures of aromatic solvents. Technical trimethylbenzene usually occurs as a mixture of the isomers 1,2,3-trimethylbenzene (hemimellitene), 1,2,4-trimethyl-

benzene (pseudocumene) and 1,3,5-trimethylbenzene (mesitylene), with the ratios of the individual isomers varying considerably. Solvents containing trimethylbenzene are particularly used in the paint, printing and plastics industries. Additional applications include the production of surface coatings, adhesives, rubber and solvents for the chemical industry [Drexler and Greim 2008].

Chlorobenzene is an important industrial solvent for oils, fats, resins, rubber and ethyl cellulose. Furthermore, it serves as a heat transfer medium and as an intermediate in the industrial synthesis of insecticides, pigments and other chemicals [Römpp 2017]. Inhalation is the primary route of exposure to chlorobenzene at the workplace.

Styrene, n-propylbenzene, isopropylbenzene (cumene) serve as starting materials, intermediates and end products in major industrial processes.

Styrene is primarily used in the production of polymers (polystyrenes). The main route of exposure is inhalation. Dermal absorption plays only a minor role [Henschler 1987].

n-Propylbenzene is often used as a solvent for cellulose acetate and in the textile dyeing industry. Cumene, however, is mainly used as an intermediate in the synthesis of acetone, phenol and α-methylstyrene. From an occupational medical point of view, the focus of attention is on inhalation and percutaneous absorption [Lehnert and Greim 2001].

## Contents

# 1 General principles

The analytical method described hereinafter enables the simultaneous determination of benzene, toluene, chlorobenzene, ethylbenzene, o-xylene, m-xylene, p-xylene, styrene, n-propylbenzene, isopropylbenzene (cumene), 1,2,4-trimethylbenzene, hemimellitene (1,2,3-trimethylbenzene), mesitylene (1,3,5-trimethylbenzene) and isodurene (1,2,3,5-tetramethylbenzene) in blood. For determination, the blood samples are introduced into headspace vials. The vials are sealed and heated to 50 °C in the autosampler. Subsequently, an aliquot of the gas phase is injected into the GC system and analysed using mass spectrometry. Calibration standards are prepared in ovine blood and processed in the same way as the samples to be analysed. Quantification is performed without the use of an internal standard.

# 2 Equipment, chemicals and solutions

## 2.1 Equipment

- Gas chromatograph with mass spectrometric detector (e.g. Agilent 5890 A with Agilent 5975 C)
- Headspace autosampler (e.g. Perkin Elmer TurboMatrix HS 40 Trap)
- Capillary gas chromatographic column: stationary phase: 6% cyanopropyl-phenyl-methylpolysiloxane, length: 60 m; inner diameter: 0.32 mm; film thickness: 1.8 µm; (e.g. VF 624 ms, Agilent, No. CP9105)
- 20-mL Headspace vials (e.g. Agilent, No. 5183-4474)
- Aluminium crimp caps with Teflon-coated butyl rubber septa (e.g. Agilent, No. 5183-4479)
- Crimping tool (e.g. Agilent, No. 5190-3189)
- 25-µL Microlitre syringe (e.g. Hamilton SYR 25 µL, 702 N)
- Microlitre pipettes with variable volumes, ranging from 10 to 100 µL and from 100 to 1000 µL with suitable pipette tips (e.g. Eppendorf Multipette M4; Eppendorf Reference 10–100 µL and 100–1000 µL)
- 25-mL and 50-mL volumetric flasks (e.g. VWR)
- Roll mixer (e.g. VWR)
- 10-mL EDTA blood collection tubes (e.g. Sarstedt S-Monovette®)

## 2.2 Chemicals

Unless otherwise specified, all chemicals must be at least p.a. grade.

- Benzene (e.g. Merck, No. 101783)
- Chlorobenzene, ≥ 99.5% (e.g. VWR, No. 319996)
- Ethanol, absolute (e.g. Merck, No. 1.00983)
- Ethylbenzene (e.g. Merck, No. 801372)
- Isopropylbenzene (Cumene) (e.g. Sigma-Aldrich, No. 36698)
- n-Propylbenzene (e.g. Sigma-Aldrich, No. 82118)
- Styrene (e.g. Sigma-Aldrich, No. 45993)

- 1,2,3,5-Tetramethylbenzene (Isodurene), 95% (e.g. Alfa Aesar, No. L19756)
- Toluene (e.g. Merck, No. 1.00849)
- 1,2,3-Trimethylbenzene (Hemellitene) (e.g. Sigma-Aldrich, No. 45935)
- 1,2,4-Trimethylbenzene (e.g. Sigma-Aldrich, No. 45996)
- 1,3,5-Trimethylbenzene (Mesitylene) (e.g. Sigma-Aldrich, No. 63908)
- m-Xylene, 99% (e.g. Alfa Aesar, No. L03788)
- p-Xylene, 99% (e.g. Alfa Aesar, Nr. A10534)
- o-Xylene, 99% (e.g. Alfa Aesar, No. A11358)
- Ovine blood (with EDTA, e.g. Fiebig)

### 2.3 Calibration standards

**Stock solution.**

After placing 10 mL ethanol into a 25-mL volumetric flask, 10 µL benzene, 50 µL chlorobenzene, 100 µL each of o-xylene, m-xylene and p-xylene as well as 300 µL each of the remaining aromatic compounds are pipetted into the flask. The flask is then filled to the mark with ethanol and the solution is mixed thoroughly.

The stock solution can be stored in the refrigerator at +7 °C for at least two years.

**Spiking solution.**

250 µL of the stock solution are pipetted into a 50-mL volumetric flask, which is then filled to the mark with ethanol. The analyte concentration levels in the spiking solution are as follows: 1.8 mg/L benzene, 11.1 mg/L chlorobenzene, 17.6 mg/L o-xylene, 17.2 mg/L m-xylene, 17.3 mg/L p-xylene, 54.6 mg/L styrene, 51.6 mg/L each of cumene, n-propylbenzene and mesitylene, 52.8 mg/L 1,2,4-trimethylbenzene, 53.4 mg/L hemimellitene and 54.0 mg/L isodurene.

The spiking solution can be stored in the refrigerator at +7 °C for at least two years.

2 mL of ovine blood are each transferred into 20-mL headspace vials, which are then sealed with aluminium crimp caps. The volumes of the spiking solution listed in Table 2 are added to the ovine blood through the septum using a microlitre syringe. Afterwards, the prepared calibration standards are put on the roll mixer for 1 h and can then be directly used for analysis.

## 3 Specimen collection and sample preparation

Blood samples of occupationally exposed workers should preferably be taken at the workplace immediately after exposure. Approx. 5 mL whole blood are taken from the arm vein using EDTA blood collection tubes. The contents of the EDTA containing monovettes are mixed thoroughly. Afterwards, 2 mL of the blood sample are injected into a 20-mL headspace vial, which is immediately sealed. The samples are stored at −18 °C until analysis. Prior to analysis, the samples are thawed at room temperature and mixed thoroughly.

**Table 2** Pipetting scheme for the preparation of calibration standards to determine aromatic compounds in blood.

| Calibration solution | Volume of the spiking solution [µL] | Concentration of the calibration standard [µg/L] | | | |
|---|---|---|---|---|---|
| | | Benzene | Chlorobenzene | Xylenes | remaining aromatic compounds |
| K0 | – | 0 | 0 | 0 | 0 |
| K1 | 1 | 0.9 | 5.6 | 8.6–8.8 | 25.8–27.3 |
| K2 | 2 | 1.8 | 11.1 | 17.2–17.6 | 51.6–54.6 |
| K3 | 4 | 3.6 | 22.2 | 34.4–35.2 | 103.0–109.0 |
| K4 | 7 | 6.3 | 38.9 | 60.2–61.6 | 181.0–191.0 |
| K5 | 10 | 9.0 | 55.5 | 86.0–88.0 | 258.0–273.0 |
| K6 | 15 | 13.5 | 83.3 | 129.0–132.0 | 387.0–410.0 |
| K7 | 20 | 18.0 | 111.0 | 172.0–176.0 | 516.0–546.0 |

## 4 Operational parameters

Analysis is performed using a gas chromatograph coupled to a headspace injector, a mass selective detector (MSD) and a data processing system.

### 4.1 Headspace autosampler

| | |
|---|---|
| Equilibration time: | 60 min at 50 °C |
| Transfer line: | 110 °C |
| Pressure generation: | 114 kPa for 0.5 min |
| Injection time: | 0.08 min |
| Needle temperature: | 70 °C |

### 4.2 Gas chromatography

| | | |
|---|---|---|
| Capillary column: | Stationary phase: | VF 624 ms (6%-cyanopropyl-94%-phenyl-methylpolysiloxane) |
| | Length: | 60 m |
| | Inner diameter: | 0.32 mm |
| | Film thickness: | 1.8 µm |
| Detector: | Mass selective detector (MSD) | |
| Temperatures: | Headspace oven: | 50 °C (60 min) |
| | Column: | Initial temperature 45 °C, 10 min hold time, increase at a rate of 5 °C/min to 110 °C, 5 min hold time, then increase at a rate of 10 °C/min to 220 °C, then 11 min at the final temperature |
| | Injector: | 230 °C |
| | Transfer line: | 280 °C |

| | | |
|---|---|---|
| Carrier gas: | Helium 5.0 | |
| | Flow rate: | 1.2 mL/min |
| Injection: | Split | 1:5 |

### 4.3 Mass spectrometry

| | |
|---|---|
| Ionisation mode: | Electron impact ionisation (EI) |
| Ionisation energy: | 70 eV |
| Source temperature: | 230 °C |
| Quadrupole temperature: | 150 °C |
| Dwell time: | 50 ms |
| Detection mode: | Single Ion Monitoring (SIM) |

All parameters serve as rough guidelines only and may have to be optimised in accordance with the manufacturer's specifications.

## 5 Analytical determination

For analytical determination of the blood samples prepared as described in Section 3, an aliquot of the headspace phase is injected into the GC/MS system after heating the samples at 50 °C for 1 h in the headspace oven. Identification of the analytes is based on retention times and characteristic ion traces. The temporal profiles of the ion traces shown in Table 3 are recorded in SIM mode. A quality con-

**Table 3** Retention times and detected ion traces of the analytes.

| Analyte | Retention time [min] | Ion trace [*m/z*] | |
|---|---|---|---|
| | | Quantifier | Qualifier |
| Benzene | 17.2 | 78 | 77 |
| Toluene | 23.3 | 91 | 92 |
| Chlorobenzene | 28.6 | 112 | – |
| Ethylbenzene | 28.9 | 91 | 106 |
| m-Xylene/p-xylene | 29.5 | 91 | 106 |
| o-Xylene | 30.8 | 91 | 106 |
| Styrene | 30.8 | 104 | 78 |
| Isopropylbenzene | 32.1 | 105 | 120 |
| n-Propylbenzene | 33.3 | 91 | 120 |
| 1,3,5-Trimethylbenzene (Mesitylene) | 33.8 | 105 | 120 |
| 1,2,4-Trimethylbenzene | 34.8 | 105 | 120 |
| 1,2,3-Trimethylbenzene (Hemimellitene) | 35.8 | 105 | 120 |
| 1,2,3,5-Tetramethylbenzene (Isodurene) | 38.2 | 134 | 120 |

trol sample and a reagent blank value consisting of double-distilled water are included in each analytical series.

The retention times given in Table 3 are intended to be a rough guide only. Users of the method must ensure proper separation performance of the column used influencing the resulting retention behaviour of the analytes. Figure 2 (in the Appendix) shows a GC/MS chromatogram of an aromatic compound standard.

## 6 Calibration

The calibration standards (see Section 2.3) and the blood samples are analysed in the same way according to Sections 4 and 5. Calibration graphs are obtained by plotting the peak areas of the analytes against the spiked concentration. The calibration curve is linear for all analytes between the detection limit and the highest calibration point.

## 7 Calculation of the analytical results

The analyte concentration in the blood samples is calculated using the calibration function of the respective analyte (Section 6). In order to determine the analyte concentration in a blood sample, the peak area of each analyte is evaluated and entered in the calibration curve of Section 6. After any reagent blank values have been subtracted, the analyte concentration in µg/L is obtained.

If the analytical result is outside the calibration range, the respective sample is diluted appropriately and reanalysed.

## 8 Standardisation and quality control

Quality control of the analytical results is carried out as stipulated in the guidelines of the Bundesärztekammer (German Medical Association) and in a general chapter of the MAK-Collection for Occupational Health and Safety Part IV: Biomonitoring Methods [Bundesärztekammer 2008; Bader et al. 2010]. To check precision, at least one quality control sample with a known and constant analyte concentration is analysed within each analytical series. As material for quality control is not commercially available, it must be prepared in the laboratory. To this end, ovine blood is spiked with a standard solution containing all analytes. The concentration of the quality control material should lie within the relevant concentration range. After thorough mixing, the quality control material thus obtained is aliquoted and transferred to headspace vials (2 mL each) and stored at –18 °C. The nominal value and the tolerance ranges of the quality control material are determined in a pre-analytical period [Bader et al. 2010]. The concentration level of the quality control samples should also lie within the tolerance ranges obtained.

## 9 Evaluation of the method

The reliability of the method was proven by comprehensive validation and by implementation and validation of the procedure in a second independent laboratory.

### 9.1 Precision

The prepared quality control material (cf. Section 8) was used to determine within-day precision. To this end, the quality control material was processed and analysed ten times each. The obtained within-day precision data are presented in Table 4.

Day-to-day precision was determined by processing and analysing aliquots of a spiked blood sample on twenty days in a row. The obtained day-to-day precision data are presented in Table 5.

**Table 4** Within-day precision for the determination of aromatic compounds in blood (n = 10).

| Analyte | Spiked concentration [µg/L] | Within-day precision | |
|---|---|---|---|
| | | Standard deviation (rel.) $s_w$ [%] | Prognostic range $u$ [%] |
| Benzene | 14 | 1.3 | 2.9 |
| | 56 | 1.8 | 4.1 |
| Toluene | 87 | 1.7 | 3.8 |
| | 348 | 3.6 | 8.1 |
| Chlorobenzene | 53 | 2.6 | 6.0 |
| | 213 | 3.0 | 6.7 |
| Ethylbenzene | 86 | 2.0 | 4.5 |
| | 344 | 3.7 | 8.4 |
| m-Xylene/p-xylene | 85 | 2.1 | 4.8 |
| | 341 | 2.9 | 6.6 |
| o-Xylene | 87 | 1.8 | 4.0 |
| | 348 | 3.1 | 7.0 |
| Styrene | 88 | 2.1 | 4.6 |
| | 354 | 4.0 | 9.1 |
| Isopropylbenzene | 86 | 2.1 | 4.8 |
| | 345 | 3.3 | 7.6 |
| n-Propylbenzene | 84 | 2.8 | 6.2 |
| | 337 | 3.3 | 7.5 |
| 1,3,5-Trimethylbenzene | 85 | 2.1 | 4.8 |
| (Mesitylene) | 339 | 3.2 | 7.2 |
| 1,2,4-Trimethylbenzene | 86 | 3.3 | 7.5 |
| | 342 | 4.6 | 10.3 |
| 1,2,3-Trimethylbenzene | 87 | 1.8 | 4.0 |
| (Hemimellitene) | 348 | 4.9 | 11.0 |
| Tetramethylbenzene | 87 | 4.5 | 10.3 |
| (Isodurene) | 347 | 6.4 | 14.5 |

**Table 5** Day-to-day precision for the determination of aromatic compounds in blood (n = 20).

| Analyte | Spiked concentration [µg/L] | Day-to-day precision | |
|---|---|---|---|
| | | Standard deviation (rel.) $s_w$ [%] | Prognostic range $u$ [%] |
| Benzene | 10 | 6.7 | 14.0 |
| Toluene | 60 | 8.0 | 16.7 |
| Chlorobenzene | 50 | 6.9 | 14.4 |
| Ethylbenzene | 60 | 7.0 | 14.7 |
| m-Xylene/p-xylene | 130 | 7.2 | 15.1 |
| o-Xylene | 130 | 7.2 | 15.1 |
| Styrene | 60 | 8.1 | 17.0 |
| Isopropylbenzene | 60 | 9.0 | 18.8 |
| n-Propylbenzene | 60 | 8.8 | 18.4 |
| 1,3,5-Trimethylbenzene (Mesitylene) | 60 | 9.9 | 20.7 |
| 1,2,4-Trimethylbenzene | 60 | 9.1 | 19.0 |
| 1,2,3-Trimethylbenzene (Hemimellitene) | 60 | 8.4 | 17.6 |
| Tetramethylbenzene (Isodurene) | 60 | 9.8 | 20.5 |

## 9.2 Accuracy

Recovery tests were performed to determine the accuracy of the method. To this end, ten blood samples (aliquots) were spiked with the aromatic compounds and analysed. The results are presented in Table 6.

## 9.3 Limits of detection and limits of quantitation

The limits of detection were estimated based on the 3-fold signal-to-noise ratio. The limits of quantitation were determined analogously (9-fold signal-to-noise ratio). The obtained limits of detection and limits of quantitation are presented in Table 7.

## 9.4 Sources of error

The two analytes m-xylene and p-xylene cannot be quantified separately by this method as they are not separated chromatographically and as they show the same mass fragments. However, a chromatographic separation and a separate quantification of m-xylene and p-xylene are not strictly required as a mixed exposure to all three xylene isomers is commonplace in occupational settings.

**Table 6** Relative recovery rates for the determination of the aromatic compounds in blood (n = 10).

| Analyte | Spiked concentration [µg/L] | Mean rel. recovery rate [%] | Range [%] |
|---|---|---|---|
| Benzene | 14 | 90.3 | 88–92 |
| Toluene | 87 | 92.2 | 89–94 |
| Chlorobenzene | 53 | 86.5 | 83–90 |
| Ethylbenzene | 86 | 86.8 | 83–90 |
| m-Xylene/ p-xylene | 85 | 88.4 | 84–91 |
| o-Xylene | 87 | 88.8 | 85–92 |
| Styrene | 88 | 70.0 | 66–73 |
| Isopropylbenzene | 86 | 86.3 | 83–89 |
| n-Propylbenzene | 84 | 86.5 | 81–90 |
| 1,3,5-Trimethylbenzene (Mesitylene) | 85 | 84.7 | 81–87 |
| 1,2,4-Trimethylbenzene | 86 | 89.7 | 84–94 |
| 1,2,3-Trimethylbenzene (Hemimellitene) | 87 | 89.8 | 86–93 |
| 1,2,3,5-Tetramethylbenzene (Isodurene) | 87 | 93.0 | 85–99 |

**Table 7** Limits of detection and limits of quantitation of the analytes.

| Analyte | Limit of detection [µg/L] | Limit of quantitation [µg/L] |
|---|---|---|
| Benzene | 0.7 | 2.1 |
| Toluene | 0.7 | 2.1 |
| Chlorobenzene | 0.9 | 2.7 |
| Ethylbenzene | 0.9 | 2.7 |
| m-Xylene/p-xylene | 0.9 | 2.7 |
| o-Xylene | 0.9 | 2.7 |
| Styrene | 1.0 | 3.0 |
| Isopropylbenzene | 1.0 | 3.0 |
| n-Propylbenzene | 1.0 | 3.0 |
| 1,3,5-Trimethylbenzene (Mesitylene) | 1.5 | 4.5 |
| 1,2,4-Trimethylbenzene | 1.5 | 4.5 |
| 1,2,3-Trimethylbenzene (Hemimellitene) | 1.5 | 4.5 |
| Tetramethylbenzene (Isodurene) | 3.0 | 9.0 |

Calibration can be carried out with a specific xylene mixture (m-xylene : o-xylene : p-xylene 20:20:60) that reflects the common composition of xylene mixtures at the workplace. Calibration with this mixture leads – in laboratory practice – to negligible errors as there are only minor differences in the slopes of the calibration curves of m-xylene and p-xylene.

## 10 Discussion of the method

The method described above enables the simultaneous determination of 14 aromatic compounds in blood. The method is both sensitive (LOD 0.7–3.0 µg/L) and characterised by a high precision, with relative standard deviations ranging between 1.8% and 6.4% (within-series precision). Even without using an internal standard, relative recovery rates of between 81% and 99% were achieved for the individual analytes. The method's working range is linear in the validated concentration range for all analytes.

The method was validated using ovine blood. During the verification process of the method it was observed that recovery rates were 10–25% higher when using pooled human blood for calibration. These findings indicate matrix effects due to species differences. However, when evaluating these facts, it must be taken into consideration that blood in general is a matrix that may vary considerably, especially regarding several main components like lipid content or haematocrit that may differ individually.

The users of this method have to ensure adequate recovery and accuracy of their analysis for the calibration matrix used by comparative measurements. If necessary, a species-specific correction factor has to be applied.

**Instruments used:**

Gas chromatograph 5890 A with mass spectrometric detector MSD 5975 C (Agilent, Santa-Clara, USA) and automatic headspace injection system TurboMatrix HS 40 Trap (Perkin Elmer, Waltham, USA).

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# 12 Appendix

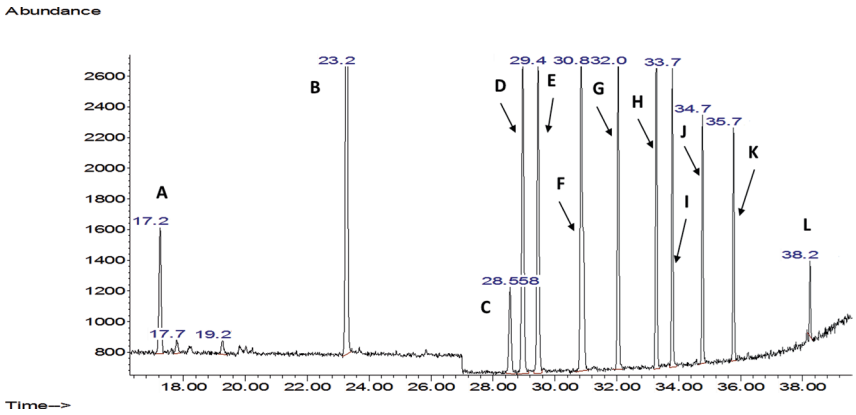


**Figure 2** Chromatogram of a standard solution containing 14 µg/L benzene and 80 to 90 µg/L of each of the other analytes. A – benzene, B – toluene, C – chlorobenzene, D – ethylbenzene, E – m-/p-xylene, F – styrene and o-xylene, G – isopropylbenzene, H – n-propylbenzene, I – mesitylene, J – 1,2,4-trimethylbenzene, K – hemimellitene, L – isodurene.