# Naphthalene metabolites 1-naphthol and 2-naphthol

| | |
|---|---|
| **Application** | Determination in urine |
| **Analytical principle** | Capillary gas chromatography/mass spectrometric detection (GC/MS) |
| **Completed in** | November 2007 |

## Summary

The method described here permits the determination of 1-naphthol and 2-naphthol in urine following exposure to naphthalene in the range of relevance to both occupational and environmental medicine.
An isotope-labelled internal standard $D_7$-1-naphthol is added to the urine samples and they undergo enzymatic hydrolysis overnight at a pH of 5.0 using $\beta$-glucuronidase/arylsulphatase at 37 °C. Then the samples are acidified and extracted with hexane. After back-extraction in an alkaline potassium carbonate solution, derivatisation is performed with acetic anhydride to form the corresponding naphthyl acetates, which are extracted with hexane. 1-Naphthol and 2-naphthol are analysed by means of GC/MS in the SIM mode and quantified with respect to the internal standard. Calibration is carried out by processing spiked pooled urine samples.

## 1-Naphthol

| | | |
|---|---|---|
| Intra-assay repeatability: | Standard deviation (rel.) | $s_w = 1.8\%$ or 1.2% |
| | Confidence interval | $u = 4.1\%$ or 2.7% |
| | at a concentration of 3.0 or 41.8 µg 1-naphthol[1] per litre urine and where n = 10 determinations in each case | |
| Inter-day repeatability: | Standard deviation (rel.) | $s_w = 1.0\%$ |
| | Confidence interval | $u = 2.3\%$ |
| | at a concentration of 21.8 µg 1-naphthol per litre urine and where n = 10 determinations | |
| Accuracy: | Recovery rate (rel.) | $r$ = 98.4% at 4.2 µg/L[1] and 100.1% at 21.0 µg/L[1] |
| Detection limit: | 0.1 µg 1-naphthol per litre urine | |

[1] Spiked in the form of 1-naphthyl-$\beta$-D-glucuronide.

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## 2-Naphthol

| | | |
|---|---|---|
| Intra-assay repeatability: | Standard deviation (rel.) | $s_w = 1.6\%$ or 1.4% |
| | Confidence interval | $u = 3.6\%$ or 3.2% |
| | at a concentration of 3.4 or 52.2 µg 2-naphthol per litre urine and where n = 10 determinations in each case | |
| Inter-day repeatability: | Standard deviation (rel.) | $s_w = 3.0\%$ |
| | Confidence interval | $u = 6.8\%$ |
| | at a concentration of 21.3 µg 2-naphthol per litre urine and where n = 10 determinations | |
| Accuracy: | Recovery rate (rel.) | $r$ = 87.3% at 4.0 µg/L and 102.6% at 25.0 µg/L |
| Detection limit: | 0.1 µg 2-naphthol per litre urine | |

A general section on naphthalene can be found in the method to determine 1-naphthol and 2-naphthol by means of 3D high-performance liquid chromatography and fluorescence detection, which is also published in this volume. A toxicological evaluation of naphthalene is presented in [1, 2].

Author: *J. Hardt*
Examiners: *D. Barr, B. Roßbach*

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## Contents



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## 1 General principles

An isotope-labelled internal standard $D_7$-1-naphthol is added to the urine samples and they are hydrolysed by the enzymes $\beta$-glucuronidase/arylsulphatase overnight at a pH of 5.0 and a temperature of 37 °C. Then the samples are acidified and extracted with hexane. After back-extraction in an alkaline potassium carbonate solution, derivatisation is performed with acetic anhydride to form the corresponding naphthyl acetates, which are subsequently extracted with hexane. 1-Naphthol and 2-naphthol are analysed by means of GC/MS in the SIM mode and quantified with respect to the internal standard. Calibration is carried out by processing spiked pooled urine samples.

## 2 Equipment, chemicals and solutions

### 2.1 Equipment

Gas chromatograph with a split/splitless injector, mass-selective detector, data processing system, autosampler or 10 µL syringe for the gas chromatograph.

Capillary gas chromatographic column:
Stationary phase: 35% diphenyl / 65% dimethylpolysiloxane; length: 60 m; inner diameter: 0.25 mm, film thickness: 0.25 µm (e.g. DB-35ms, Agilent Technologies No. 122-3862)

Sealable vessels (e.g. made of polypropylene) for collecting urine

Analytical balance

100 mL and 250 mL Glass bottles

250 mL Glass measuring cylinder

100 mL Plastic bottle

10 and 250 mL Volumetric flasks

Pipettes for adding volumes between 50 and 5000 µL (e.g. from Eppendorf)

Hand dispenser for adding volumes between 30 and 5000 µL (e.g. Multipette, Eppendorf)

24 mL Screw-capped glass jars with screw caps and PTFE-coated septa

If necessary, 8 mL screw-capped glass jars with screw caps and PTFE-coated septa

Fluted filters (e.g. from Schleicher & Schuell)

pH Meter

Vortex mixer

Thermostatically-controlled vibratory water bath (e.g. from GFL)

Horizontal shaker (e.g. from Gerhardt)

Laboratory centrifuge

Device for evaporation under a stream of nitrogen

1.8 mL Rolled-edge vials and crimp caps with PTFE-coated septa (e.g. from Agilent Technologies)

Microinserts for the rolled-edge vials, usable volume 200 µL (e.g. from Agilent Technologies)

## 2.2 Chemicals

Sodium acetate trihydrate, p.a. (e.g. Merck 1.06267.0500)

Bidistilled water

Acetic acid (100%), p. a. (e.g. Merck No. 1.00063.1000)

Potassium carbonate, p. a. (e.g. Merck No. 1.04928.0500)

Sodium hydroxide pellets, p. a. (e.g. Merck No. 1.06498.0500)

Hydrochloric acid (37%), p. a. (e.g. Merck No. 1.00317.1000)

1-Naphthol-2,3,4,5,6,7,8-$D_7$ (e.g. Aldrich No. 487538)

1-Naphthol (e.g. Dr. Ehrenstorfer No. C 15430000)

2-Naphthol (e.g. Dr. Ehrenstorfer No. C 15430500)

If required, 1-naphthyl-$\beta$-D-glucuronide sodium salt (e.g. Sigma No. N9013)

Methanol SupraSolv® (e.g. Merck No. 1.06011.2500)

$\beta$-Glucuronidase/arylsulphatase, *Helix pomatia,* $\beta$-glucuronidase activity: 100,000 Fishman units/mL, and arylsulphatase activity: 800,000 Roy units/mL (e.g. Roche Diagnostics No. 10127698001)

n-Hexane Unisolv® (e.g. Merck No. 1.04369.2500)

Acetic anhydride, p. a. (e.g. Fluka No. 45830)

Nitrogen 5.0 (e.g. from Linde)

Helium 5.0 (e.g. Linde)

## 2.3 Solutions

1 M Sodium hydroxide:
4.0 g of sodium hydroxide pellets are weighed into a 100 mL plastic bottle and then dissolved in 100 mL of bidistilled water.

0.28 M Acetate buffer (pH 5.0):
6.8 g of sodium acetate trihydrate are weighed into a 250 mL glass bottle and then dissolved in 250 mL of bidistilled water. Then 1.2 mL of concentrated acetic acid are added. The pH value is checked using a pH electrode and then adjusted to pH 5.0, if necessary, using a 50 µL pipette to add acetic acid or 1 M sodium hydroxide drop by drop.

0.1 M Potassium carbonate solution:
3.5 g of potassium carbonate are weighed into a 250 mL glass bottle and then dissolved in 250 mL of bidistilled water.

1 M Hydrochloric acid:
55 mL of bidistilled water are placed in a 100 mL glass bottle, 5 mL of hydrochloric acid (37%) are added, and the contents are mixed by shaking them cautiously.

## 2.4 Calibration standards

### 2.4.1 Internal standard (ISTD)

Stock solution of the ISTD:
Approx. 10 mg of $D_7$-1-naphthol are weighed into a 24 mL screw-capped glass jar and then dissolved in 10 mL of methanol. This stock solution has a concentration of approx. 1 g/L. It is stored at –18 °C.

Working solution of the ISTD:
100 µL of the stock solution are diluted with 19.9 mL of methanol in a 24 mL screw-capped glass jar. This resulting working solution has a concentration of approx. 5 mg/L. The screw-capped glass jar is sealed tightly and stored at 4 to 8 °C.

### 2.4.2 Calibration standards

1-Naphthol stock solution:
Approx. 10.0 mg of 1-naphthol are weighed exactly into a 10 mL volumetric flask. The volumetric flask is then filled to its nominal volume with methanol. The concentration of this solution is 1 g/L.

2-Naphthol stock solution:
Approx. 10.0 mg of 2-naphthol are weighed exactly into a 10 mL volumetric flask. The volumetric flask is then filled to its nominal volume with methanol. The concentration of this solution is 1 g/L.

Working solution:
100 µL each of the two stock solutions are pipetted into a 10 mL volumetric flask, into which approximately 5 mL of methanol have been previously placed. The volumetric flask is then filled to its nominal volume with methanol. The working solution contains 10 mg/L each of 1-naphthol and 2-naphthol.

The calibration standards are prepared in pooled urine. Spontaneous urine samples are collected from non-smokers and mixed to prepare the pooled urine. The pooled urine is stored at –18 °C and then passed through a fluted filter before use. An analysis to check the suitability of the pooled urine is recommended before the calibration standards are prepared. The background content of 1-naphthol and 2-naphthol in the pooled urine should be less than 5 µg/L in each case in order to ensure that the accuracy of the calibration is not adversely affected.
The calibration standards are prepared in 250 mL volumetric flasks in accordance with the pipetting scheme shown in Table 1. For this purpose about half the volume of pooled urine is always placed in the flask, the working solution is added, and then the flask is filled to its nominal volume with pooled urine. The calibration standards are spiked with the analytes 1-naphthol and 2-naphthol, in each case with the concentration given in Table 1.

**Table 1.** Pipetting scheme for the preparation of the calibration standard solutions in pooled urine.

| Volume of the working solution [µL] | Final volume of the calibration standard solution [mL] | Spiked concentration in the calibration standard solution [µg/L] |
|---|---|---|
| 0 | 250 | 0 |
| 50 | 250 | 2 |
| 250 | 250 | 10 |
| 500 | 250 | 20 |
| 1000 | 250 | 40 |
| 2500 | 250 | 100 |

All the calibration standards are divided into aliquots in screw-capped glass jars. The stock solutions, working solutions and calibration standard solutions are stored at –18 °C. They are stable for at least one year under these conditions.

## 3 Specimen collection and sample preparation

The urine samples are collected in sealable vessels (e.g. made of polypropylene). They can be stored at –18 °C for at least one year before processing.

### 3.1 Sample preparation

The urine samples are thawed and brought to room temperature. They can be placed in a refrigerator on the previous day so that they thaw overnight, or the thawing process can be accelerated in a water bath at room temperature. The urine sample is mixed, then 5 mL is pipetted into a 24 mL screw-capped glass jar, and a pH meter is used to measure the pH value while it is being adjusted to between 4.8 and 5.2 by adding 1 M hydrochloric acid or 1 M sodium hydroxide drop by drop. After each sample has been adjusted, the pH electrode has to be rinsed carefully with bidistilled water to avoid carryover of the analytes. 5 mL of acetate buffer (pH 5.0) and 50 µL of the working solution of the ISTD are each added, then the sample is mixed briefly using a vortex mixer. Then 50 µL of the enzymatic suspension of $\beta$-glucuronidase/arylsulphatase are added, and the sample is briefly mixed again. The sample is subsequently hydrolysed overnight at 37 °C in a vibratory water bath for at least 16 hours. When the sample has cooled to room temperature, 1 mL of hydrochloric acid (37%) and 4 mL of n-hexane are added, the sample is shaken for five minutes on a horizontal shaker and subsequently centrifuged for 10 minutes at approx. 1900 g. Then 3 mL of the supernatant organic phase are pipetted into a new 24 mL or 8 mL screw-capped glass jar, in which 2 mL of 0.1 M potassium carbonate solution has been previously placed. The sample jars are shaken for two minutes on a horizontal shaker and then centrifuged for three minutes. The supernatant organic phase is withdrawn completely with a pipette and discarded. 1 mL of hexane and 30 µL of acetic anhydride are added to the lower aqueous phase, then the samples are shaken for 20 minutes on a horizontal shaker and subsequently centrifuged for 5 minutes. About 0.6 mL of the upper hexane phase are pipetted into a 1.8 mL rolled-edge vial and evaporated to approx. 100 µL in a stream of nitrogen. The samples may not be evaporated to dryness. Each of the solutions is transferred to a microinsert, which is sealed in a rolled-edge vial with a crimp cap.

## 4 Operational parameters

### 4.1 Operational parameters for gas chromatography

| | | |
|---|---|---|
| Capillary column: | Material: | Fused silica |
| | Stationary phase: | DB-35ms |
| | Length: | 60 m |
| | Inner diameter: | 0.25 mm |
| | Film thickness: | 0.25 µm |
| Temperatures: | Column: | Initial temperature 60 °C, 0.2 min isothermal, then increase at a rate of 20 °C/min to 90 °C, 15 min isothermal, then increase at a rate of 3 °C/min to 170 °C, 5 min isothermal, then increase at a rate of 10 °C/min to 270 °C, 15 min at final temperature. |
| | Injector: | 250 °C |
| | Transfer line: | 280 °C |
| Carrier gas: | Helium 5.0 at a constant flow rate of 1.5 mL/min | |
| Split: | Splitless, split on after 1.5 min | |
| Injection volume: | 1 µL pulsed splitless | |

### 4.2 Operational parameters for mass spectrometry

| | |
|---|---|
| Ionisation type: | Electron impact ionisation (EI) |
| Ionisation energy: | 70 eV |

All other parameters must be optimised in accordance with the manufacturer's instructions.

## 5 Analytical determination

To analyse the urine samples processed as described in Section 3.1, in each case 1 µL of the organic phase is injected into the gas chromatograph and analysed by gas chromatography/mass spectroscopy under the stated conditions. At least one quality control sample is analysed with each analytical series. The retention times of the analytes and the recorded masses are summarised in Table 2.

**Table 2.** Retention times and recorded masses.

| Analyte | Retention time [min] | Recorded mass [m/z] |
|---|---|---|
| $D_7$-1-Naphthol (ISTD) | 51.3 | 193*, 151 (151*, 193)** |
| 1-Naphthol | 51.4 | 144*, 115 or 186***, 145 |
| 2-Naphthol | 52.0 | 144*, 115 or 186***, 145 |

* Ion trace for the quantitative evaluation
** These ion traces were used by one of the examiners
*** This ion trace was used by one of the examiners as the qualifier

These retention times serve only as guidelines. Users of the method must satisfy themselves of the separation power of the separation column used and the resulting retention behaviour of the substances. Figure 1 shows an example of a chromatogram of a processed native urine sample from a non-smoker. Figure 2 shows the EI mass spectrum of acetylated 1-naphthol.
If the measurement result of a sample is higher than the calibration range (> 100 µg/L), then the urine sample is diluted in the ratio of 1 : 10 with bidistilled water, processed, and measured anew.

## 6 Calibration

The calibration standard solutions prepared as described in Section 2.4.2 are processed in the same manner as the urine samples (described in Section 3.1) and analysed by gas chromatography/mass spectrometry as stipulated in Sections 4 and 5. The peak areas of the analytes (1-naphthol and 2-naphthol) are divided by the peak area of the internal standard, and the resulting quotients are plotted as a function of the spiked concentrations. The gradient of the calibration function is calculated by linear regression. The background value of the pooled urine used to prepare the calibration standards can be read off from the corresponding intercept of the calibration graph with the y axis.
The calibration functions for 1-naphthol and 2-naphthol are linear up to a concentration range of 100 µg/L (see Figure 3). It is not necessary to plot a complete calibration graph for every analytical series. It is sufficient to process a calibration standard solution with a concentration of 40 µg/L as well as the unspiked pooled urine and include them in each analysis series (two-point calibration).

## 7 Calculation of the analytical results

The quotients obtained for the peak areas of the analytes in the analysed samples with the peak area of the internal standard are divided by the gradient of the relevant linear calibration graph to give the concentrations of 1-naphthol and 2-naphthol.

## 8 Standardisation and quality control

Quality control of the analytical results is carried out in accordance with the guidelines of the *Bundesärztekammer* [German Medical Association] [3] and the special preliminary remarks in this series. An aliquot of a urine control sample is analysed in each analytical series to check the precision. Insofar as material for quality control is not commercially available for this purpose, it must be prepared in the laboratory. This is achieved by spiking pooled urine that is not identical to that used for the calibration standards with defined amounts of 1-naphthol and 2-naphthol. The control material is divided into aliquots in suitable glass vessels and stored in the deep-freezer at –18 °C.
The theoretical value and the tolerance range of this quality control material are ascertained in a pre-analytical period (one analysis of the control material on each of 15 different days) [3–5].

## 9 Evaluation of the method

### 9.1 Precision

The intra-assay repeatability was determined by ten-fold analysis of two samples of pooled urine spiked with different amounts of the analytes. Whereas pooled urine sample 1 was spiked with 2.5 µg/L each of 1-naphthol and 2-naphthol, pooled urine sample 2 was spiked with 100 µg/L of 1-naphthyl-$\beta$-D-glucuronide (equivalent to 42 µg/L of 1-naphthol) and 50 µg/L of 2-naphthol. The intra-assay repeatability shown in Table 3 was determined.

**Table 3.** Intra-assay repeatability for the determination of 1-naphthol and 2-naphthol in urine determined using spiked pooled urine (n = 10).

| Parameter | Spiked pooled urine | Pooled urine 1 | Pooled urine 2 |
|---|---|---|---|
| | Creatinine concentration [g/L] | 1.2 | 1.0 |
| 1-Naphthol | Measured concentration [µg/L] | 3.0 | 41.8 |
| | Standard deviation (rel) $s_w$ [%] | 1.8 | 1.2 |
| | Confidence interval $u$ [%] | 4.1 | 2.7 |
| 2-Naphthol | Measured concentration [µg/L] | 3.4 | 52.2 |
| | Standard deviation (rel) $s_w$ [%] | 1.6 | 1.4 |
| | Confidence interval $u$ [%] | 3.6 | 3.2 |

The inter-day repeatability was determined by means of a pooled urine sample that had been spiked with 20 µg each of 1-naphthol and 2-naphthol per litre and subsequently divided into aliquots. This urine was processed and analysed on ten different days. The precision results are shown in Table 4.

**Table 4.** Inter-day repeatability for the determination of 1-naphthol and 2-naphthol in urine (n = 10).

| Parameter | | Spiked pooled urine |
|---|---|---|
| | Creatinine concentration [g/L] | 0.8 |
| 1-Naphthol | Measured concentration [µg/L] | 21.8 |
| | Standard deviation (rel) $s_w$ [%] | 1.0 |
| | Confidence interval $u$ [%] | 2.3 |
| 2-Naphthol | Measured concentration [µg/L] | 21.3 |
| | Standard deviation (rel) $s_w$ [%] | 3.0 |
| | Confidence interval $u$ [%] | 6.8 |

In addition, the recovery rate (see also Section 9.2) was determined at two different concentrations by analysing ten and nine spiked individual urine samples respectively. The precision of the recovery rate shown in Table 5 was then calculated from the difference in the contents of the spiked and unspiked urine samples. The creatinine levels of the individual urine samples were between 0.3 and 2.8 g/L.

**Table 5.** Precision of the recovery rate for the determination of 1-naphthol and 2-naphthol in urine obtained by analysis of spiked individual urine samples (n = 10 and 9 respectively).

| Parameter | Spiked individual urine sample | Series 1 | Series 2 |
|---|---|---|---|
| | Number | 10 | 9 |
| 1-Naphthol | Spiked concentration [µg/L] | 4.2* | 21* |
| | Recovered concentration | 4.1 | 21.0 |
| | Standard deviation (rel) $s_w$ [%] | 7.2 | 2.6 |
| | Confidence interval $u$ [%] | 16.3 | 6.0 |
| 2-Naphthol | Spiked concentration [µg/L] | 4.0 | 25.0 |
| | Recovered concentration | 3.5 | 25.7 |
| | Standard deviation (rel) $s_w$ [%] | 11.2 | 5.5 |
| | Confidence interval $u$ [%] | 25.3 | 12.7 |

* Spiked as 1-naphthyl-$\beta$-D-glucuronide (10 µg/L and 50 µg/L respectively)

## 9.2 Accuracy

The accuracy of the method was checked as part of the recovery tests to determine the precision of the recovery. The relative recovery rates that were obtained are summarised in Table 6. No influence on the recovery due to the different composition of the individual urine samples was observed.

**Table 6.** Relative recovery rates for 1-naphthol and 2-naphthol in spiked individual urine samples.

| Parameter | Individual urine sample n | Spiked concentration [µg/L] | Mean relative recovery *r* [%] | Range [%] |
|---|---|---|---|---|
| 1-Naphthol | 10 | 4.2* | 98.4 | 90.3–113.8 |
| | 9 | 21.0* | 100.1 | 94.4–103.4 |
| 2-Naphthol | 10 | 4.0 | 87.3 | 75.1–97.8 |
| | 9 | 25.0 | 102.6 | 92.4–111.3 |

* Spiked as 1-naphthyl-$\beta$-D-glucuronide (10 µg/L and 50 µg/L respectively)

In addition, the accuracy of the method was checked by comparison with another independent method (HPLC with fluorescence detection) that is also published in this method collection. For this purpose 15 urine samples with different naphthol concentrations were investigated in two laboratories (for the results see the method for the determination of 1-naphthol and 2-naphthol by means of 3D-LC/FLD). Identical results were obtained (R = 0.97) for the analyte 2-naphthol in all the samples with regard to measurement accuracy. In the case of 1-naphthol too there was good overall correlation of the values (R = 0.96; n = 14) with the exception of one pair of values. In the case of this sample the analytical value obtained by the comparative method was distinctly higher (34.5 µg/L vs. 80.8 µg/L).

## 9.3 Detection limits

Under the conditions stated for sample preparation and for gas chromatographic/mass spectrometric analysis, the detection limit was 0.1 µg/L for both 1-naphthol and 2-naphthol. This detection limit was estimated as three times the signal/background noise ratio of ion trace 144 that was used for quantification. If the criterion of three times the signal-background noise ratio is applied to ion trace 115, which is used as a qualifier, then the detection limit is 0.5 µg/L for both isomers. One of the examiners of the method used ion trace 186 as a qualifier and obtained a detection limit of 0.4 µg/L in this case.

## 9.4 Sources of error

The naphthalene metabolites 1-naphthol and 2-naphthol are excreted as glucuronide and sulphate conjugates in urine. The individual conjugates are cleaved at different rates by the enzymatic hydrolysis carried out as part of the sample work-up. As the control material was spiked with 1-naphthyl-$\beta$-D-glucuronide during the validation of the method, it was possible to check for the complete cleavage of this conjugate in the hydrolysis step. However, this does not apply to 1-naphthyl sulphate, which accounts for approximately 20% of the excreted 1-naphthyl conjugates and which is

cleaved more slowly. The hydrolysis time should be at least 16 hours to ensure complete cleavage of the conjugates of 1-naphthol. The use of smaller amounts of enzymes also poses a risk of incomplete hydrolysis of 1-naphthyl sulphate. The cleavage of the conjugates of 2-naphthol proceeds more rapidly and is therefore less problematic [6].
The pH value of each urine sample must be adjusted individually to 5.0 in order to ensure an effective enzymatic hydrolysis with $\beta$-glucuronidase/arylsulphatase. The addition of the acetate buffer (pH 5.0) alone is insufficient in many urine samples. However, in contrast to the sample processing described in Section 3.1, this pH adjustment can also be performed after addition of the buffer.
During extraction of the hydrolysed urine samples, to which concentrated hydrochloric acid has been added, it is important to ensure that no trace of the acidic aqueous phase is carried over when the hexane phase is withdrawn. The acid otherwise lowers the pH value of the alkaline potassium carbonate solution, which may cause the back-extraction and the subsequent derivatisation of the deprotonated naphthols to fail. One of the examiners successfully used anhydrous sodium sulphate to dry the hexane phase in order to make the sample preparation less susceptible to interference at this point.
The samples may on no account be evaporated to dryness when they are concentrated in a stream of nitrogen, as otherwise losses due to processing that adversely affect the reproducibility are to be expected. It would also be conceivable to use a keeper (e.g. decane or toluene) to prevent excessive evaporation of the samples.
On principle, 1-naphthol reacts under the influence of light. However, analytically relevant problems were only to be observed in the case of exposure to extremely strong sunlight. Therefore exposure to strong, direct sunlight should be avoided as far as possible and amber glass vessels should be used if necessary.

## 10 Discussion of the method

This analytical method enables reliable quantitative determination of the naphthalene metabolites 1-naphthol and 2-naphthol in urine in the concentration range of relevance to both environmental and occupational medicine. It has already proved successful in investigations of groups from the general population [7]. The analytical procedure represents a further development of the method devised by Bouchard et al. (2001) [8] and uses the extraction and derivatisation procedure published by Hoppe (1999) [9] for the analysis of pentachlorophenol. Very good results with regard to precision and accuracy are achieved due to the use of an isotope-labelled standard ($D_7$-1-naphthol). As expected, these are somewhat better for the 1-naphthol analyte than for 2-naphthol.
The influence of the urine matrix on the analytical results is compensated by the internal standard used. For this reason it is also possible to analyse urine samples with naphthol concentrations above the measurement range (>100 µg/L) when they are diluted with bidistilled water. Calibration using calibration standards that had been prepared with bidistilled water instead of pooled urine resulted in almost identical gradi-

ents of the calibration graphs. Although this was not comprehensively tested, these results indicate that matrix effects are not to be expected as a consequence of the sample work-up that was used. Furthermore, the experience of the authors and examiners to date show that a reagent blank value is not to be expected.

In addition to the high sensitivity of the method, it is remarkable in that it does not require technically complex detection methods, such as triple-quadrupole mass spectrometry used in the method developed by Hill et al. (1995) [10] or high-resolution mass spectrometry in the case of Li et al. (2006) [6].

During development of the method hydrolysis using hydrochloric acid was tested as well as enzymatic hydrolysis. Individual urine samples were analysed using both hydrolysis alternatives to compare the hydrolysis methods. The results were strikingly different in many cases [11]. Analytical problems arising from the use of acidic hydrolysis during the analysis of naphthols are also described in the literature [10, 12]. Therefore consistent use of enzymatic hydrolysis is necessary to obtain comparable results. As part of the method validation the author spiked the control material with 1-naphthyl-$\beta$-D-glucuronide instead of 1-naphthol in order to determine the effectiveness of the enzyme suspension used for the sample work-up.

This method was also compared with an independent method (HPLC with fluorescence detection) when the accuracy of the method was investigated. Comparison showed a good overall correlation of the values, both for 1-naphthol and for 2-naphthol. Only in the case of one urine sample was the naphthol value measured with the comparative method distinctly higher (GC/MS: 34.5 µg/L vs. 3D-LC/FLD: 80.8 µg/L). The cause of this could not be clarified. However, it was notable that this sample exhibited an extreme ratio of the concentrations of 1-naphthol to 2-naphthol (GC/MS: 0.8 µg/L and 3D-LC/FLD: 1.1 µg/L), which is atypical for exposure to naphthalene. The person concerned may have been exposed to 1-naphthol or carbaryl.

One of the examiners was able to replicate the method and its reliability data immediately without any problems. However he recommends the use of 8 mL instead of 24 mL screw-capped glass jars for the back-extraction of the naphthols from hexane in alkaline potassium carbonate solution in order to facilitate the complete withdrawal of the supernatant organic phase. The second examiner did not have the GC column intended for use at his disposal. The use of a shorter GC column (30 m) led to a poorer separation of the peaks from the interfering background, resulted in higher detection limits, and it is therefore not to be recommended. In contrast, the temperature program for the gas chromatographic separation with the intended DB-35ms column (60 m) can be shortened to permit a higher sample throughput. One of the examiners worked successfully using the following program for the GC column oven: initial temperature 60 °C, 0.2 minutes isothermal, then increase at a rate of 20 °C/min to 90 °C, 2 min isothermal, then increase at a rate of 5 °C/min to 230 °C, then increase at a rate of 20 °C/min to 270 °C, 5 min at the final temperature. The following retention times were obtained for the acetylated analytes: $D_7$-1-naphthol 24.9 min, 1-naphthol 25.0 min, 2-naphthol 25.5 min.

Instruments used:
Gas chromatograph 6890 plus with mass selective detector 5973, data system ChemStation and autosampler 7673, from Agilent Technologies/Hewlett Packard.

## 11 References

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Author: *J. Hardt*
Examiners: *D. Barr, B. Roßbach*

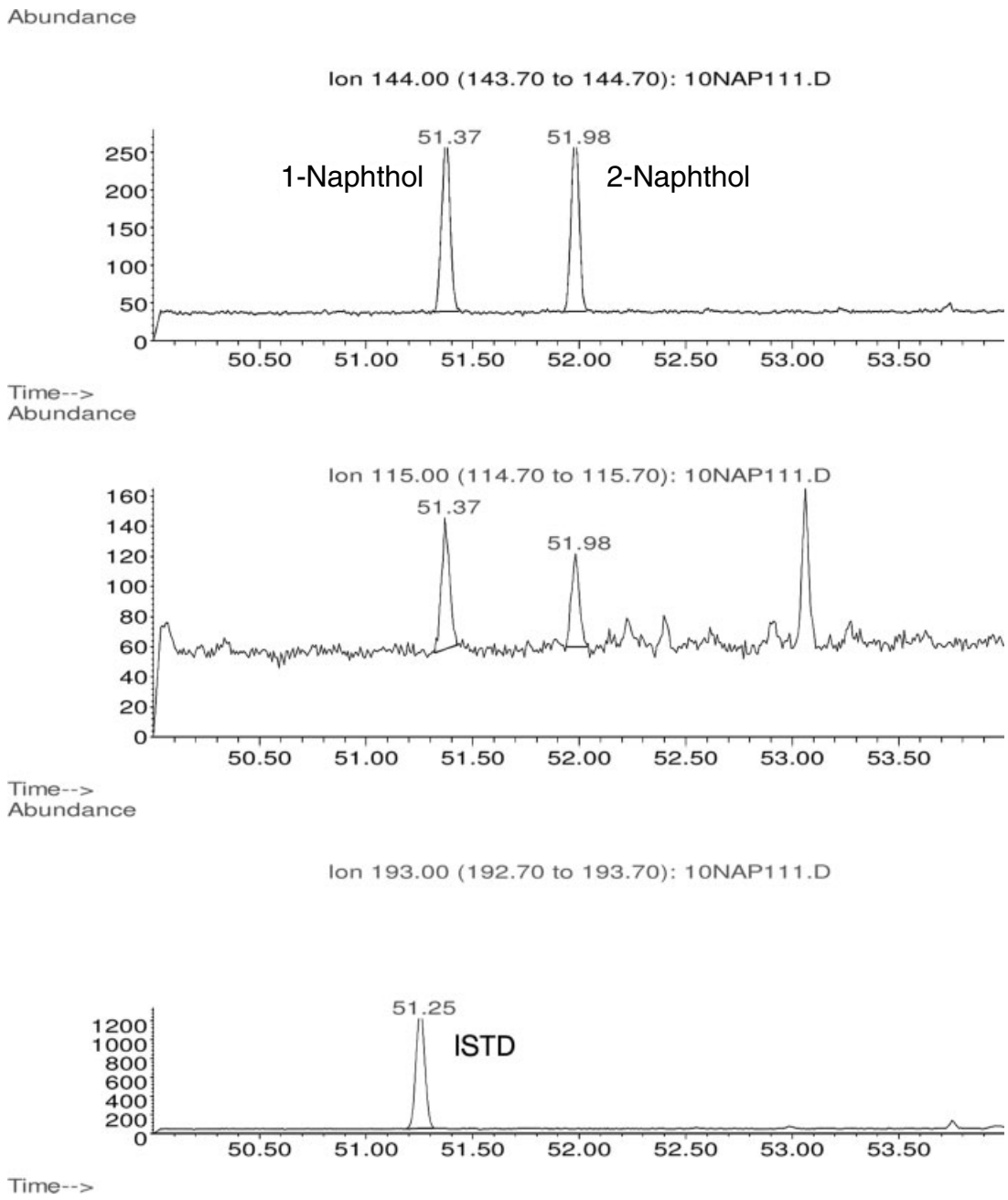


**Fig. 1.** GC/MS chromatogram in the EI-SIM mode of a processed urine sample from a non-smoker containing 0.72 µg/L 1-naphthol and 0.85 µg/L 2-naphthol (creatinine: 1.0 g/L).

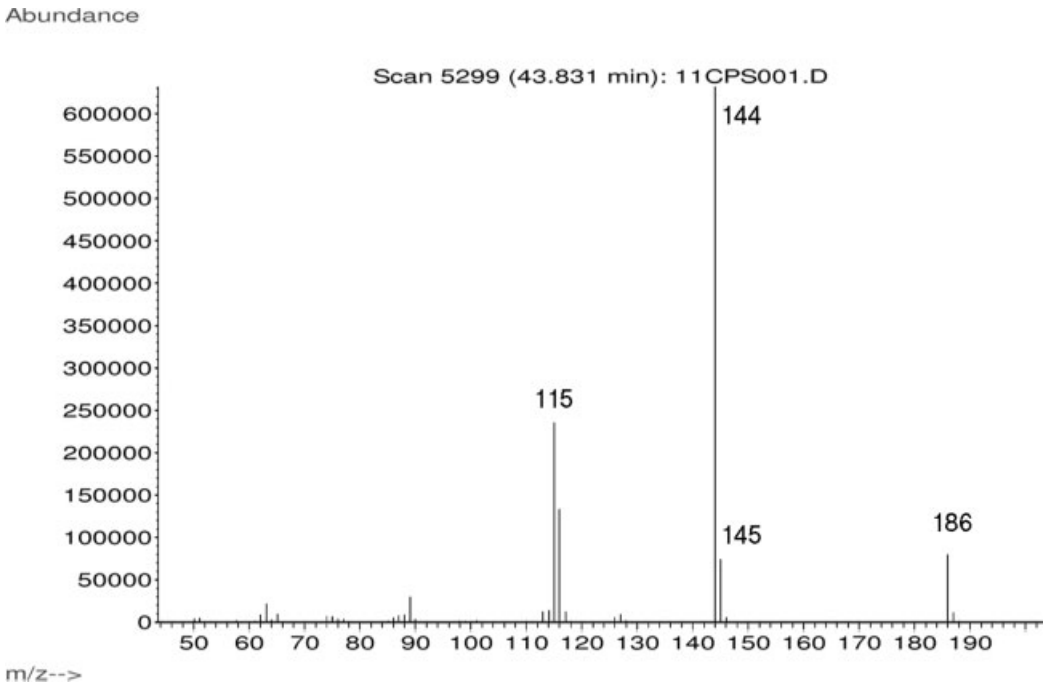


**Fig. 2.** EI mass spectrum of acetylated 1-naphthol.

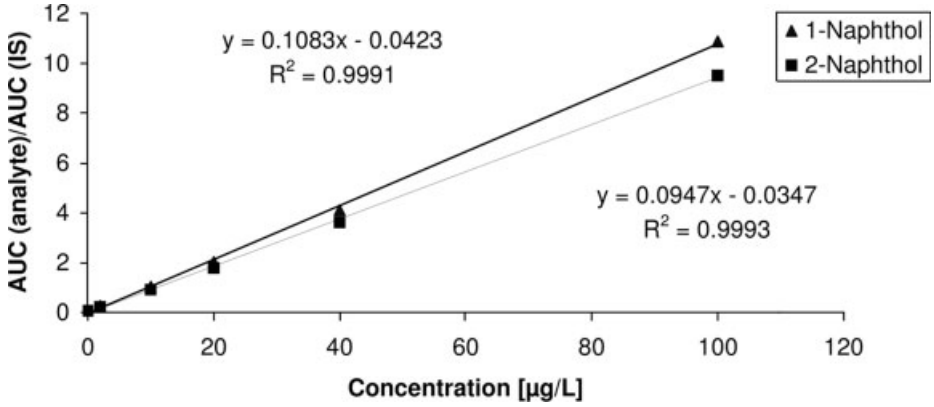


**Fig. 3.** Calibration curves for 1-naphthol and 2-naphthol in pooled urine.