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# Determination of the major mercapturic acids of 1,3-butadiene in human and rat urine using liquid chromatography with tandem mass spectrometry

Michael Urban [a], Gerhard Gilch [a], Georg Schepers [b], Erik van Miert [c], Gerhard Scherer [a,*]

[a] *ABF Analytisch-biologisches Forschungslabor GmbH, Goethestrasse 20, 80336 Munich, Germany*
[b] *PHILIP MORRIS Research Laboratories GmbH, Fuggerstrasse 3, 51149 Cologne, Germany*
[c] *PHILIP MORRIS Research Laboratories bvba, Grauwmeer 14, 3001 Leuven, Belgium*

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**Abstract**

The major urinary metabolites of 1,3-butadiene are monohydroxybutenyl-mercapturic acids (MHBMA) and dihydroxy-butyl-mercapturic acid (DHBMA). These metabolites can be used as biomarkers of exposure to this diene. In order to determine the smoking-related exposure to 1,3-butadiene, we have developed a rapid LC-MS/MS method for the determination of MHBMA and DHBMA in urine of humans and rats. The method requires 2–5 ml of urine which is solid phase extracted prior to LC-MS/MS analysis. Precision for MHBMA is ≤11.2% for human and ≤17% for rat urine. Corresponding values for DHBMA are ≤7.2 and ≤19%, respectively. Recovery rates are approximately 100% for both analytes in human urine and about 115% in rat urine. Limits of detection (LOD) are for humans 0.9 and 23 ng/ml and for rats 1.5 and 33 ng/ml for MHBMA and DHBMA, respectively. Application of the method to urine of humans and rats showed a significant effect of tobacco smoke exposure on the urinary excretion of MHBMA and the metabolic ratio DHBMA/(DHBMA + MHBMA).
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*Keywords:* Mercapturic acids; 1,3-Butadiene

## 1. Introduction

1,3-Butadiene is mainly used for the production of synthetic rubber alone or as a copolymer with styrene [1]. Environmental sources of 1,3-butadiene are automobile exhaust and exhaust from heating. In mainstream smoke of cigarettes, 1,3-butadiene levels of 16–75 and in sidestream smoke levels of 205–361 μg per cigarette have been reported [2]. Environmental tobacco smoke (ETS)-related concentrations of this compound range from 3 to 19 $\mu g/m^3$ [1–3].

The International Agency for Research on Cancer (IARC) has classified 1,3-butadiene as "probable carcinogenic to humans" (Group 2A) [1]. The German Commission for maximal workplace concentrations

* Corresponding author. Tel.: +49-89-535395; fax: +49-89-5328039.
*E-mail address:* gerhard.scherer@abf-lab.com (G. Scherer).

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(MAK) has classified 1,3-butadiene as "human carcinogen" (Category 1) [4].

The above mentioned properties require a reliable assessment of the human exposure to 1,3-butadiene by suitable biomarkers. The initial steps in the metabolism of 1,3-butadiene are cytochrome P450-catalyzed oxidation processes to highly reactive epoxides, which have the potential to react with cellular macromolecules such as DNA and proteins [5]. The epoxides can be deactivated by enzymatic hydrolysis to corresponding hydroxy-metabolites and conjugation with glutathione leading to mercapturic acids which are excreted in urine. In principle, protein and DNA adducts of 1,3-butadiene as biomarkers for the biologically effective dose as well as its mercapturic acids as biomarkers for the internal dose have been used for biomonitoring purposes, particularly in occupationally-exposed humans or in experimentally-exposed animals [5–9].

The major 1,3-butadiene-derived mercapturic acids are monohydroxybutenyl-mercapturic acids (MHBMA, also termed MII) and dihydroxy-butyl-mercapturic acid (DHBMA, MI) [5,6]. Two isomeric forms of MHBMA have been reported: *R*,*S*-1-hydroxy-2-(*N*-acetylcysteinyl)-3-butene (**1**) and *R*,*S*-2-hydroxy-1-(*N*-acetylcysteinyl)-3-butene (**2**). The chemical structures of MHBMA isomeric forms (**1**, **2**) and of DHBMA (**3**) are shown in Fig. 1.

DHBMA is suggested to indicate hydrolysis of 1,2-epoxy-3-butene before glutathione (GSH) conjugation at the double bond, whereas MHBMA should indicate the detoxification of this epoxide via the GSH pathway [6]. The metabolic ratio DHBMA/(DHBMA + MHBMA), after 1,3-butadiene exposure, has been shown to be species-specific and was found to be about 0.98 for humans [6,7], 0.25–0.5 for rats [7] and 0.2–0.4 for mice [7].

The objective of our work was to develop a robust and rapid method for the quantification of the major mercapturic acids of 1,3-butadiene in urine of humans and rats. Special emphasis was given to the rapidity of the method so that larger series of urine samples from humans and rats can be analyzed in a reasonable time.

## 2. Experimental

### 2.1. Chemicals

MHBMA, as a mixture of *R*,*S*-1-hydroxy-2-(*N*-acetylcysteinyl)-3-butene (**1**) and *R*,*S*-2-hydroxy-1-(*N*-acetylcysteinyl)-3-butene (**2**); $D_6$-MHBMA, as a mixture of *R*,*S*-1-hydroxy-2-(*N*-acetylcysteinyl)-3-butene-[$D_6$] and *R*,*S*-2-hydroxy-1-(*N*-acetylcysteinyl)-3-butene-[$D_6$] (isomers (**1**) and (**2**) are present at a molar ratio of about 1:1); DHBMA, *R*,*S*-1,2-dihydroxy-4-(*N*-acetylcysteinyl)-butane (**3**); $D_7$-DHBMA, *R*,*S*-1,2- dihydroxy-4-(*N*-acetylcysteinyl)-butane-[$D_7$] were obtained from Toronto Research Chemicals, Ontario, Canada. The purity of DHBMA and $D_7$-DHBMA was >98%. MHBMA and MHBMA-$D_6$ standards contained an unidentified impurity of <5%. All other reagents are of analytical grade.

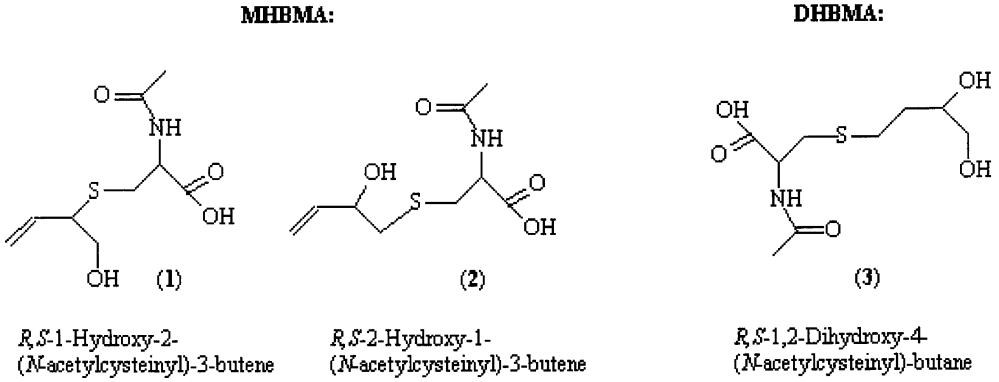


Fig. 1. Structures of 1,3-butadiene mercapturic acids under investigation.

### 2.2. Instrumentation

The LC-MS/MS system consisted of an HPLC system Model HP 1100 (Agilent Technologies, Waldbronn, Germany) with the following components: Binary pump (G1312A), column oven (G1316A), degasser (G1322), thermostated autosampler (G1329A). The HPLC was directly coupled to a triple quadrupole mass spectrometer (Model API 2000, Applied Biosystems, Langen, Germany) equipped with an atmospheric pressure chemical ionization source. An electrospray ionization (ESI) source is used for tuning purposes. Nitrogen was supplied by a system consisting of a compressor (Jun-Air Model 4000, Ahrensburg, Germany), membrane air dryer (Whatman Model 64-01, Maidstone, UK) and a nitrogen generator (Whatman Model 75-72).

### 2.3. Sample preparation and LC-MS/MS analysis

Frozen urine samples were thawed at room temperature, centrifuged (2000 × *g*, 10 min, 10 °C) and adjusted to pH 2.0 with 4 and 1N HCl. After transfer of 5 ml human urine (2.5 ml rat urine) to polyethylene tubes, 100 μl of each $D_6$-MHBMA and $D_7$-DHBMA (10 μg/ml, aqueous solutions) were added. The sample was applied to a StrataX cartridge (200 mg, 6 ml, Phenomenex, Aschaffenburg, Germany) preconditioned with 6 ml methanol and 6 ml 0.01N HCl. The cartridge was washed with 3 ml 0.01N HCl and 1 ml 0.01N HCl containing 2% acetonitrile, sucked to dryness (550 mbar, 3 min), centrifuged (3300 × *g*, 10 min, 20 °C) and completely dried in a stream of nitrogen (grade 5.0). Elution was performed with 4 ml ethyl acetate saturated with gaseous ammonia. The eluate was evaporated to dryness in a speedvac concentrator (Jouan, Unterhaching, Germany). The residue was dissolved in 50 μl 0.01N HCl/methanol (70:30). Ten microliters of the solution were injected into the LC-MS/MS system equipped with a C18(2) column (Synergi Luna, 5 μm particle size, mesh 100 Å, dimensions 100 mm × 4.6 mm) and a corresponding pre-column (4 mm × 3 mm i.d., 4 μm particle size, Phenomenex, Aschaffenburg, Germany). The column was kept at 50 °C. The mobile phase consisted of 10 mM ammonium acetate pH 4.3 (A) and methanol (B). Gradient elution with a flow rate of 1.0 ml/min without splitting was applied as follows: 5% B (1 min); 5–50% B in 3 min; 50% B (2 min); back to 5% B in 4 min. The MS/MS system was operated in the negative atmospheric pressure chemical ionization mode (APCI, heated nebulizer source). The nebulizer heater was maintained at 495 °C with the nebulizer current at 2 μA. Nitrogen was used as nebulizing, auxiliary, and curtain gas at 70, 20, and 50 psi, respectively. Retention times and ion transitions of analytes in the multiple reaction monitoring (MRM) mode were as follows: $D_7$-DHBMA: 1.97 min, 257.2 → 128.0 *m/z*; DHBMA: 2.03 min, 250.0 → 121.0 *m/z*; $D_6$-MHBMA: 3.92 min, 238.2 → 109.0 *m/z*; MHBMA: 3.93 min, 232.1 → 103.0 *m/z*.

### 2.4. Calibration and recovery

The method was calibrated by spiking 5 ml of a nonsmoker pool urine (or 2.5 ml of a urine pool from unexposed rats) with 5–2500 ng of MHBMA reference compound and with 250–5000 ng of DHBMA reference compound. Calibration samples were processed as described in Section 2.3. The linear regression between the analyte/internal standard ratios (minus the ratio of the unspiked urine) and concentrations was calculated.

Recovery rates were determined by comparing the signals of internal standards of nonsmoker pool urine samples (urine of unexposed rats) which have been spiked with 200 ng/ml each of $D_6$-MHBMA and $D_7$-DHBMA either before or after (100%) the solid phase extraction (SPE) step.

### 2.5. Human urine samples

Twenty-four hours urine samples of 10 adult smokers (having smoked 6–38 cigarettes per day) and 10 adult nonsmokers were used for the determination of MHBMA and DHBMA. Aliquots of 10 μl were stored at −24 °C for about 12–18 months in polystyrene tubes prior to analysis. Experiments on the stability of the MHBMA and DHBMA which now ran for 9 months showed no loss of the analytes under these conditions. Carbon monoxide in exhalate was determined in all subjects by means of a hand-held instrument (electrochemical CO gas sensor, Bedfont, UK) [11]).

Cotinine in saliva was determined by LC-MS/MS [12] in order to correlate the MHBMA and DHBMA data with specific biomarkers for tobacco smoke exposure.

### 2.6. Urine samples of rats

Male Sprague–Dawley rats were nose-only exposed to cigarette mainstream smoke from the University of Kentucky reference cigarettes 1R4F and 2R4F with similar 1,3-butadiene yields of about 35 μg per cigarette or to filtered, conditioned air (sham) for 2 or 6 h per day. Total particulate matter (TPM) exposure concentrations were 750 μg/l (2 h per day) and 75 or 150 μg/l (6 h per day). Urine was collected on ice over a 24 h period starting with the daily exposure. Aliquots of the urine samples were stored at approximately −20 °C. Analysis was performed on samples from five rats per group for seven different groups: sham-exposed for 2 and 6 h per day (two groups), exposed to smoke from 1R4F cigarettes for 6 h per day at 75 and 150 μg/l (two groups), exposed to smoke from 2R4F cigarettes for 6 h per day at 75 and 150 μg/l (two groups), exposed to smoke from 1R4F cigarettes for 2 h per day at 750 μg/l (one group).

## 3. Results

### 3.1. Precision, recovery, linearity and detection limits

The most intensive product ions of MHBMA and DHBMA in the MS/MS mode are *m/z* 103.0 and 121.0, respectively. The product ion spectra and the fragmentation hypotheses are shown in Fig. 2. Typical chromatograms of urine samples from a smoker and a nonsmoker are shown in Fig. 3.

Table 1 summarizes the characteristics of the method for human urine. The intra-day precision was determined by analyzing a pool urine sample six times within 1 day. The inter-day precision was determined by analyzing the same sample on 12 different days. The recovery rate was determined by spiking a nonsmoker pool urine sample with two concentrations of MHBMA (1 and 5 ng/ml) and DHBMA (100 and 500 ng/ml). The limits of detection (LOD) for MHBMA and DHBMA were determined as the three times standard deviation ($3\sigma$) of the noise determined with a nonsmoker pool urine which has been measured 10 times. The limit of quantification was

Table 1
Characteristics of the described LC-MS/MS method with SPE for the determination of 1,3-butadiene-derived mercapturic acids MHBMA and DHBMA in human urine

| | MHBMA | DHBMA |
|---|---|---|
| Linearity after subtraction of blank level, 3.7 ng/ml for MHBMA and 178 ng/ml for DHBMA (ng/ml) | 1–500 ($R^2 = 0.9972$) | 50–1000 ($R^2 = 0.9990$) |
| Intra-day precision, $N = 6$ (%) | | |
| Nonsmoker urine | 11.2 | 7.2 |
| Smoker urine | 5.9 | 7.1 |
| Inter-day precision, $N = 12$ (%) | | |
| Nonsmoker urine | 8.9 | 6.4 |
| Smoker urine | 9.4 | 6.2 |
| Recovery, $N = 6$ (%) | | |
| Low spike[a] | 99.8 (91–104) | 104.0 (98–116) |
| High spike[b] | 98.2 (81–120) | 100.4 (94–110) |
| Limit of detection (ng/ml) | 0.9 | 23 |
| Limit of quantification (ng/ml) | 2.7 | 76 |

[a] 1 ng/ml MHBMA, 100 ng/ml DHBMA.
[b] 5 ng/ml MHBMA, 500 ng/ml DHBMA.

Table 2
Characteristics of the described LC-MS/MS method with SPE for the determination of 1,3-butadiene-derived mercapturic acids MHBMA and DHBMA in rat urine

| | MHBMA | DHBMA |
|---|---|---|
| Linearity after subtraction of blank level, 7.0 ng/ml for MHBMA and 211 ng/ml for DHBMA (ng/ml) | 2–1000, $R^2 = 0.9855$ | 100–2000, $R^2 = 0.9932$ |
| Intra-day precision, $N = 5$ (%) | | |
| Urine of control rats | 17.1 | 5.9 |
| Inter-day precision, $N = 6$ (%) | | |
| Urine of control rats | 12.1 | 19.4 |
| Urine of smoke-exposed rats | 12.6 | 10.6 |
| Recovery, $N = 5$ (%) | 115.6 (109–122) | 112.9 (106–118) |
| Limit of detection (ng/ml) | 1.5 | 33 |
| Limit of quantification (ng/ml) | 4.5 | 100 |

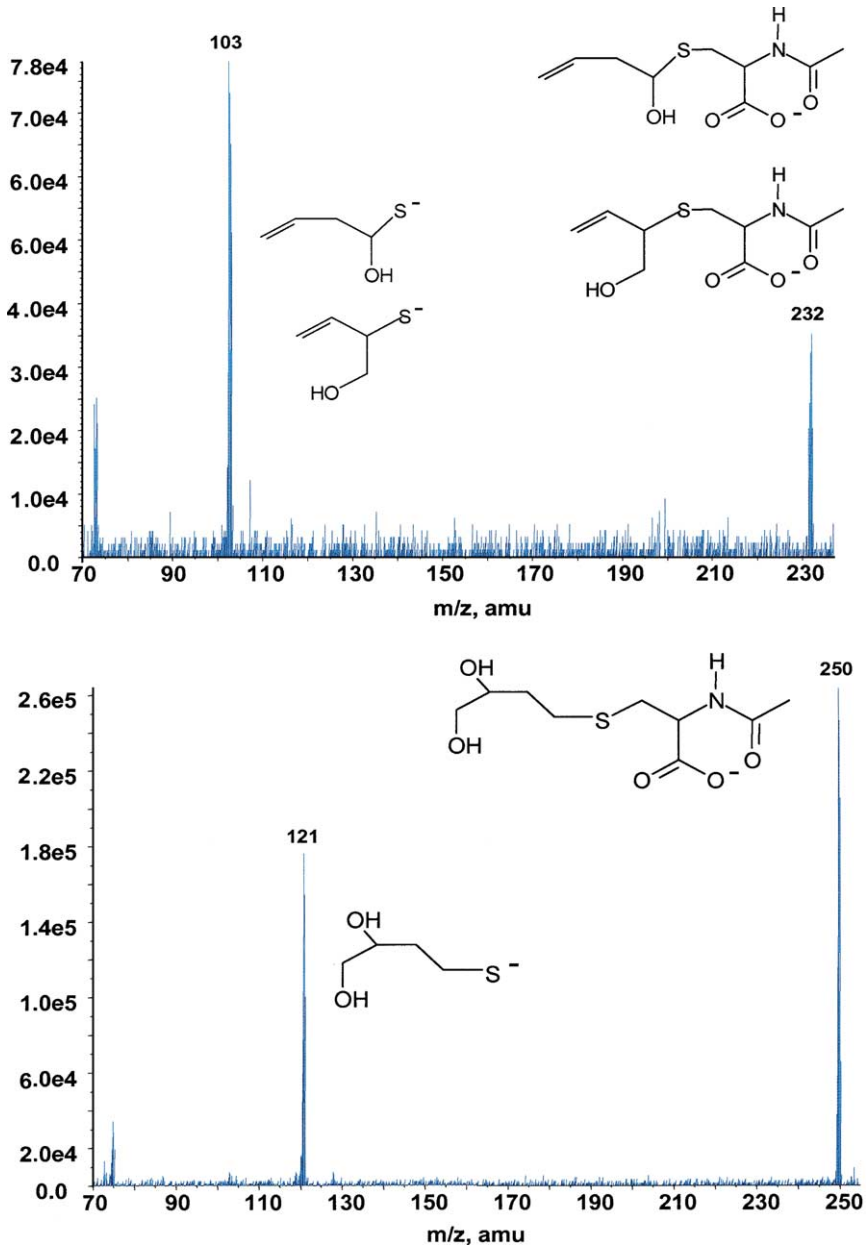


Fig. 2. Product ion spectrum and fragmentation hypotheses for MHBMA (top) and DHBMA (bottom) in negative ion electrospray ionisation (ESI) mode.

defined as $10\sigma$. Method validation data for rat urine were determined correspondingly and are shown in Table 2. For the determination of the recovery rate, pooled urine of control rats was spiked with 2.5 ng/ml MHBMA and 25 ng/ml DHBMA.

### 3.2. Application to human urine samples

The method was applied to 20 human urine samples (10 nonsmokers, 10 smokers). Means, standard errors of the means (S.E.) and ranges for MHBMA

and DHBMA as well as some smoking dose-related parameters of the two groups are shown in Table 3. Smokers excreted significantly higher amounts of MHBMA compared to nonsmokers. The difference in the excretion of DHBMA between smokers and nonsmokers was not significant. The metabolic ratio DHBMA/(DHBMA + MHBMA) was significantly lower in smokers than in nonsmokers.

In smokers, the correlation of both 1,3-butadiene-derived mercapturic acids and the metabolic ratio with biomarkers of the smoking dose such as carbon monoxide in exhaled air and salivary cotinine are weak ($r = 0.4$–$0.8$) and do not reach statistical significance. This is probably due to the low number of smokers investigated ($N = 10$). In all subjects, MHBMA and DHBMA are significantly correlated ($r = 0.53$, $N = 19$, $P < 0.05$).

### 3.3. Application to urine samples of rats

The method was applied to urine samples of rats exposed to filtered air and to various doses of mainstream smoke from reference cigarettes. Linear regression analysis for all rats ($N = 35$) revealed a significant increase of urinary excretion of MHBMA ($r^2 = 0.582$, $P < 0.001$) and DHBMA ($r^2 = 0.393$, $P < 0.001$) with the daily dose as well as a dose-dependent decrease of the metabolic ratio DHBMA/(DHBMA + MHBMA) ($r^2 = 0.216$, $P < 0.01$) (Fig. 4). The correlation between MHBMA and

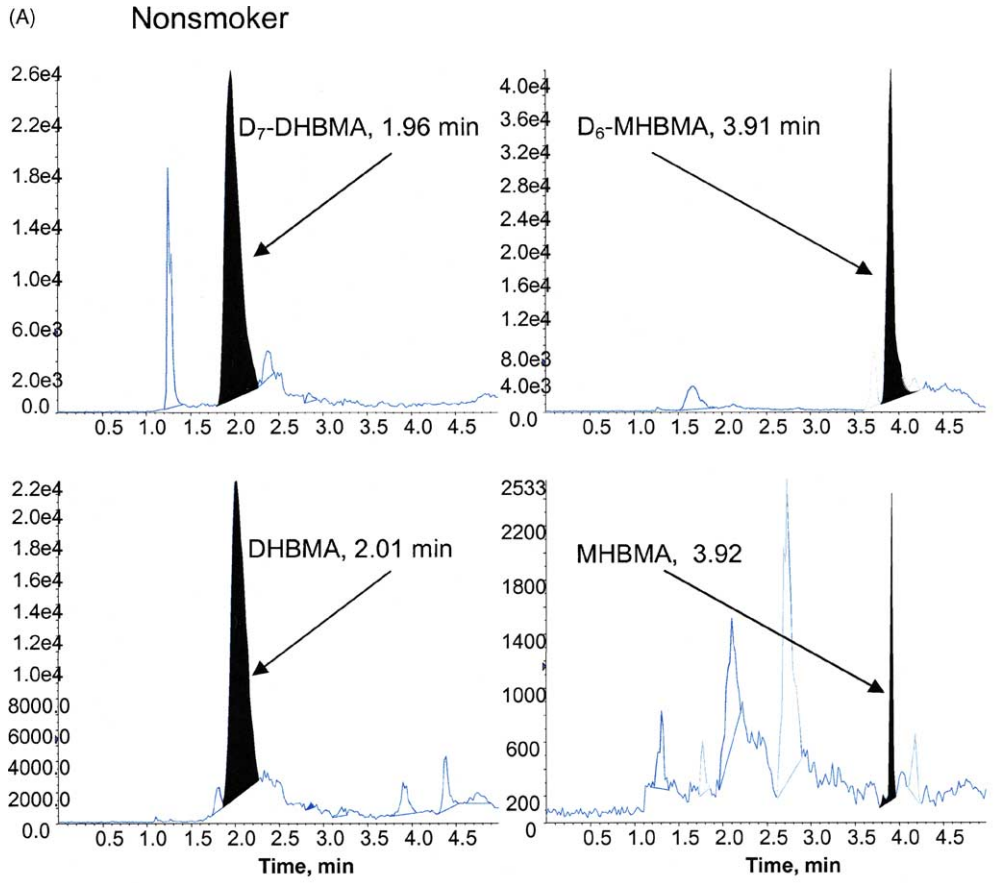


Fig. 3. Chromatograms in the negative multiple reaction monitoring (MRM) mode for MHBMA (*m/z* 232.0 → 103.0 and DHBMA (*m/z* 250.0 → 121.0) in urine samples of an adult nonsmoker (A) and an adult smoker (B). The traces of the mass chromatograms for the corresponding internal standards ($D_6$-MHBMA: *m/z* 238.0 → 109.0 and $D_7$-DHBMA: *m/z* 257.0 → 128.0) are also shown.

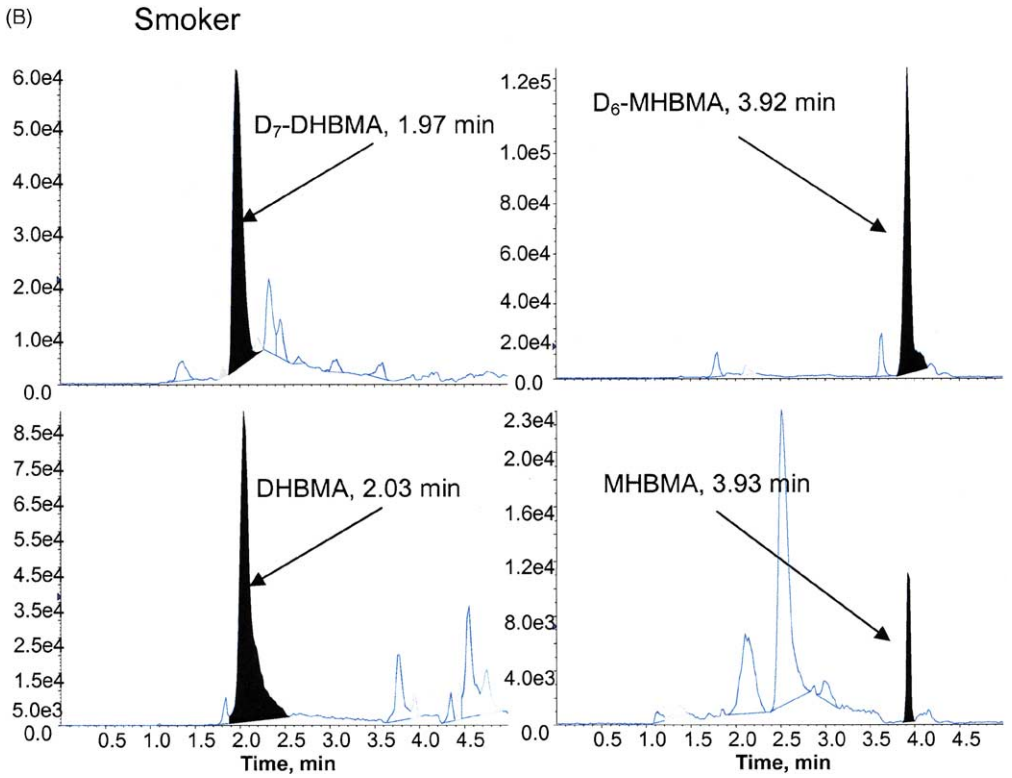


Fig. 3. (*Continued*).

Table 3
Mean ± standard error of the mean (range) of MHBMA and DHBMA and other variables in nonsmokers and smokers

| Parameter | Nonsmokers | Smokers |
|---|---|---|
| Number (*N*) | 10 | 10[a] |
| Cigarettes smoked per day | – | 16.3 ± 9.7 (6–38) |
| Carbon monoxide in exhalate (ppm) | 1.60 ± 0.41 (0.0–3.0) | 25.40 ± 5.45*** (7.0–56.0) |
| Cotinine in saliva (ng/ml) | 0.77 ± 0.23 (0.20–2.30) | 388 ± 69*** (117–893) |
| MHBMA (μg/24 h) | 12.5 ± 1.0 (7.0–18.0) | 86.4 ± 14.0*** (15.2–145.1) |
| DHBMA (μg/24 h) | 459 ± 72 (209–898) | 644 ± 90 (116–1084) |
| Metabolic ratio: DHBMA/(DHBMA + MHBMA) | 0.970 ± 0.003 (0.95–0.98) | 0.859 ± 0.026*** (0.69–0.98) |

[a] DHBMA values are only available for nine smokers.
*** Statistical significance: $P < 0.001$ (smokers vs. nonsmokers).

DHBMA in the urine of all 35 rats was statistically significant ($r = 0.60$, $P < 0.001$).

## 4. Discussion

An LC-MS/MS method has been developed for the determination of the 1,3-butadiene-derived mercapturic acids MHBMA and DHBMA in urine of humans and rats. The method is reproducible and shows good recovery rates. Sensitivity is high enough to quantify levels of both analytes in 5 ml of human urine (virtually unexposed or exposed to active smoking) and 2–3 ml of rat urine (sham-exposed to conditioned air or exposed to tobacco smoke).

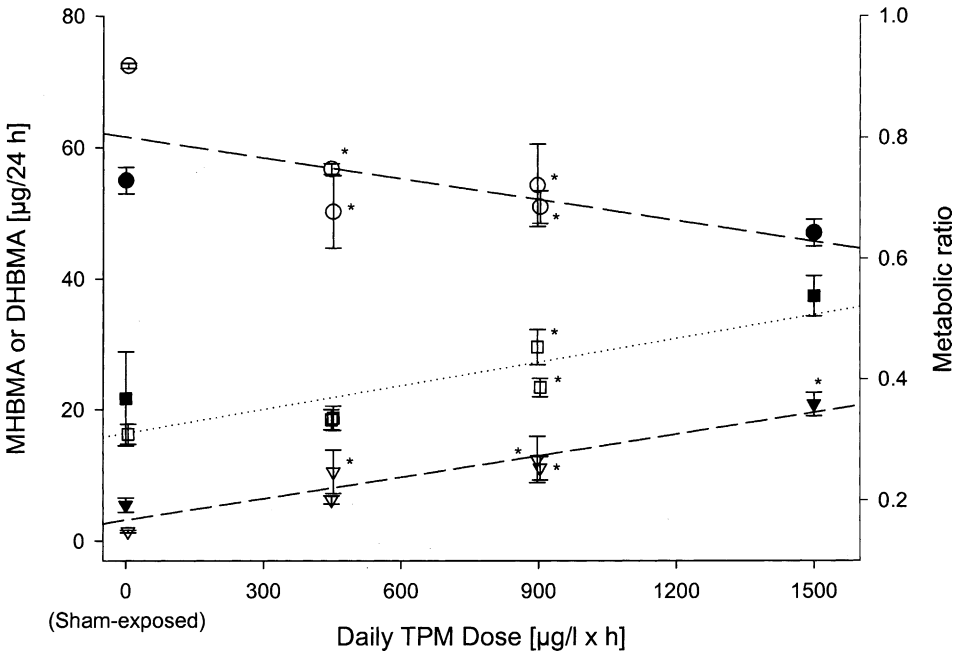


Fig. 4. Urinary excretion of MHBMA (▽), DHBMA (□) and metabolic ratio DHBMA/(DHBMA + MHBMA) (○) in rats exposed for 2 h per day (filled symbols) and 6 h per day (open symbols) to tobacco smoke (means ± S.E., $N = 5$). Asterisks indicate statistically significant differences to respective sham-exposed group. Lines indicate linear regressions between daily dose (expressed as total particulate matter (TPM), µg/l h) and excreted mercapturic acids or metabolic ratio.

MHBMA and DHBMA were first identified in urine of mice, rats, hamsters and monkeys experimentally exposed to radiolabeled 1,3-butadiene by Sabourin et al. [9]. Identification was performed by HPLC separation of the urinary metabolites and subsequent GC–MS analysis. These authors could show that the metabolic ratios DHBMA/(DHBMA + MHBMA) increased from mice (0.2) to rats (0.25–0.4) to hamsters (0.4) to monkeys (0.9). Epoxide hydrolase activity increases in the same order in these species. Based on these data, the authors proposed that DHBMA is formed by reaction of 1,2-dihydroxy-3-butene (the hydrolysis product of 1,3-butadiene monoexpoxide) with glutathione, whereas MHBMA is directly formed by the reaction of the monoepoxide with glutathione. The same working group developed a GC–MS method for the determination of the 1,3-butadiene-derived mercapturic acids in urine of workers exposed to 1,3-butadiene [7]. Since their method had a LOD of about 100 ng/ml, they were able to quantify DHBMA (but not MHBMA) in exposed (average: 3200 ng/ml), intermediate exposed (1390 ng/ml) and unexposed subjects (320–630 ng/ml). Based on the limit of sensitivity, the authors estimated a metabolic ratio of about 0.97 for humans. More recently, van Sittert et al. [5] developed a more sensitive GC method with negative electron capture ionisation tandem mass spectrometry (GC–NECI–MS/MS). Sample preparation included extraction with ethyl acetate and derivatisation by methylation and pentafluorobenzoylation. The method required 1 ml of urine and had a LOD (in methanol) of 0.1 ng/ml for MHBMA and 0.5 ng/ml for DHBMA. Medians (range) of MHBMA concentrations in urine of two unexposed control groups were 1.6 and 2.0 ng/ml (<0.1–8.2), in two groups of butadiene (monomer) workers 2.9 and 3.6 ng/ml (<0.1–44) and in two groups of styrene–butadiene–rubber (SBR) workers 4.4 and 20 ng/ml (<0.1–962). The corresponding levels for DHBMA were: controls: 355 and 524 ng/ml (197–1211); monomer workers: 484 and 508 ng/ml (52–3522); SBR workers: 600 and 1479 ng/ml (60–26 207). The metabolic ratio in these studies was 0.996 (0.984–1.000) for controls, 0.987 (0.946–1.000) for monomer workers, 0.981

(0.928–0.997) for SBR workers. The metabolic ratios for the 1,3-butadiene-exposed groups were significantly lower than that of the unexposed controls. The correlation between MHBMA and DHBMA was found to be significant ($r = 0.84$, $P < 0.001$). The authors did not find an influence of smoking on either urinary MHBMA or DHBMA.

Our findings on MHBMA and DHBMA in urine of rats and humans are consistent with most of the data reported earlier. Levels of DHBMA in human urine determined in our study (10 adult smokers, 10 adult nonsmokers, not occupationally exposed to 1,3-butadiene) are comparable with those of unexposed subjects in earlier studies [5,7]. Levels of MHBMA determined for the subjects in our study are higher than those determined for unexposed controls in the study of van Sittert et al. [5]. We have no explanation for this discrepancy. As a result, metabolic ratios for our subjects are somewhat lower. In contrast to the study of van Sittert et al. [5], we were able to differentiate between smokers and nonsmokers in terms of their urinary excretion of MHBMA (significantly higher in smokers compared to nonsmokers) and DHBMA (trend for increased levels in smokers, not significant). Number of smokers and strength of smoking in the van Sittert study was not reported [5]. It is, therefore, difficult to compare the results of both studies with respect to the influence of smoking on the urinary excretion of MHBMA and DHBMA. It is interesting to note that we observed a significantly lower metabolic ratio of smokers compared to nonsmokers. Van Sittert et al. [5] reported a significantly lower metabolic ratio in 1,3-butadiene-exposed workers compared to unexposed controls. These findings may indicate an induction of the glutathione-related pathway of the detoxification of 1,2-epoxy-3-butene in both smokers and 1,3-butadiene-exposed workers. Our results with humans are based on a limited number of subjects and, therefore, need confirmation.

Background levels for MHBMA and DHBMA in urine of untreated rats (i.e. rats not exposed to 1,3-butadiene) have not been reported previously. Metabolic ratios reported for rats range from 0.25 to 0.5 [7,9,10]. The metabolic ratios that we observed in our study were higher and amounted to 0.73–0.92 (sham-exposed rats) as well as to 0.64–0.75 (rats exposed to tobacco smoke). A possible explanation for the higher metabolic ratios in our study might be that our rats, in general, were held at much lower exposure levels (<1 ppm 1,3-butadiene in the tobacco smoke test atmosphere) compared to animals in other studies [7,9]. Again, it is worth noting that the metabolic ratio of smoke-exposed rats tended to be lower than that of unexposed rats. This finding confirms earlier results showing that rats exposed to 8000 ppm 1,3-butadiene had a lower metabolic ratio (0.35) compared to rats exposed to 11.7 ppm (0.52) [7].

As in previous studies [5,7], we also observed substantial urinary background levels of the 1,3-butadiene-derived mercapturic acids, in particular of DHBMA, in humans and rats. The sources of these background levels are still unknown.

In conclusion, the LC-MS/MS method for urinary MHBMA and DHBMA that we have developed is rapid and leads to reproducible results. The sensitivity is high enough to determine background levels in unexposed humans and rats. The major advantage compared to earlier reported GC methods is its rapidity allowing a higher sample throughput. Active human smoking and experimental exposure of rats to tobacco smoke has been shown to significantly increase the urinary excretion of MHBMA and decrease the metabolic ratio DHBMA/(MHBMA + DHBMA).

## References

[1] International Agency for Research on Cancer, Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: 1,3-Butadiene, World Health Organization, Lyon, 1992.
[2] K.D. Brunnemann, M.R. Kagan, J.E. Cox, D. Hoffmann, Carcinogenesis 11 (1990) 1863.
[3] G. Löfroth, R.M. Burton, L. Forehand, S.K. Hammond, R.L. Seila, R.B. Zweidinger, J. Lewtas, Environ. Sci. Technol. 23 (1989) 610.
[4] Deutsche Forschungsgemeinschaft, MAK-und BAT-Werte-Liste 1998, Senatskommission zur Prüfung gesundheitsschädlicher Arbeitsstoffe, Wiley/VCH, Weinheim, 1998.
[5] N.J. van Sittert, H.J.J.J. Megens, W.P. Watson, P.J. Boogaard, Toxicol. Sci. 56 (2000) 189.
[6] P.J. Boogaard, N.J. van Sittert, H.J.J.J. Megens, Chem. Biol. Interact. 135–136 (2001) 695.
[7] W.E. Bechtold, M.R. Strunk, I.Y. Chang, J. Ward, R.F. Henderson, Toxicol. Appl. Pharmacol. 127 (1994) 44.
[8] S. Osterman-Golkar, K. Peltonen, T. Anttinen-Klemetti, H.H. Landin, V. Zorcec, M. Sorsa, Mutagenesis 11 (1996) 145.
[9] P.J. Sabourin, L.T. Burka, W.E. Bechtold, A.R. Dahl, M.D. Hoover, I.Y. Chang, R.F. Henderson, Carcinogenesis 13 (1992) 1633.

[10] K.A. Richardson, M.M. Peters, B.A. Wong, R.H. Megens, P.A. van Elburg, E.D. Booth, P.J. Boogaard, J.A. Bond, M.A. Medinsky, W.P. Watson, N.J. van Sittert, Toxicol. Sci. 49 (1999) 186.
[11] M.J. Jarvis, M. Belcher, C. Vesey, D.C.S. Hutchison, Thorax 41 (1986) 886.
[12] M. Meger, I. Meger-Kossien, A. Schuler-Metz, D. Janket, G. Scherer, J. Chromatogr. B 778 (2002) 251.