Chemical Research in Toxicology

Rapid Report

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Identification and Quantification of 1-Hydroxybutene-2-yl Mercapturic Acid in Human Urine by UPLC- HILIC-MS/MS as a Novel Biomarker for 1,3-Butadiene Exposure

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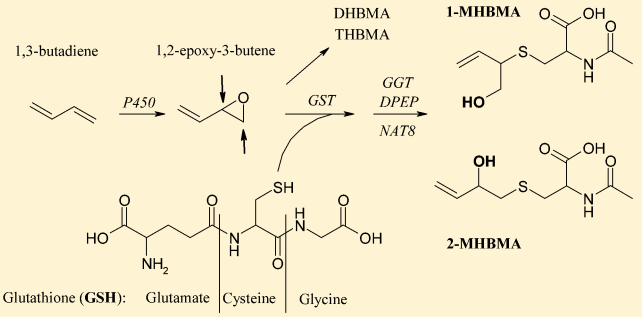
Supporting Information

ABSTRACT: 1,3-Butadiene (BD) is a Class 1 carcinogen present at workplaces, in polluted air, in automobile exhaust, and in tobacco smoke. 2-Hydroxybutene-1-yl mercapturic acid (2-MHBMA) is a urinary metabolite often measured as a biomarker for exposure to BD. Here, we show for the first time that an additional MHBMA isomer is present at significant amounts in human urine, 1-hydroxybutene-2-yl mercapturic acid (1-MHBMA). For its quantification, a highly sensitive UPLC-HILIC-MS/MS method was developed and validated. Analyzing urinary samples of 183 volunteers, we demonstrate that 1-MHBMA is a novel and potentially more reliable biomarker for BD exposure than the commonly analyzed 2-MHBMA.

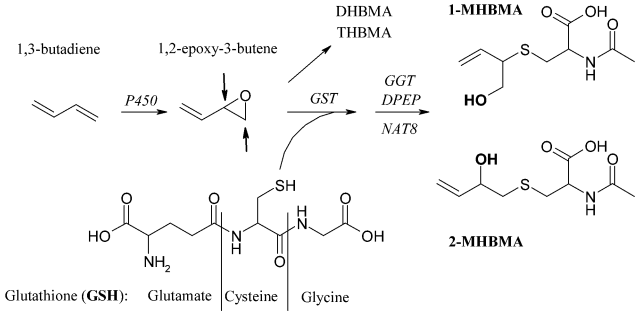


1,3-Butadiene (BD) ranks 36th in the list of most produced chemicals in the United States (three billion pounds per year; 12 billion globally). It is widely used by industries as a monomer to manufacture polymers and as a chemical intermediate to produce industrial chemicals.[1] BD is present at workplaces, in combustion- and automobile exhausts, polluted- and urban air, and tobacco smoke leading to broad exposure of the human population. It is classified as a Class 1 carcinogen ("carcinogenic to humans") by the International Agency for Research on Cancer.[2]

Mechanistically, BD is converted to 1,2-epoxy-3-butene by P450 cytochrome enzymes in mammals (Scheme 1).[3] Next, this monoepoxide is conjugated to glutathione (GSH, γ-L-glutamyl-L-cysteinylglycine) by glutathione *S*-transferases (GSTs) to yield the two isomeric intermediate compounds (1-hydroxy-3-butene-2-yl)-GSH and (2-hydroxy-3-butene-1-yl)-GSH. Further enzymatic reactions of γ-glutamyltranspeptidase (GGT), a cysteinylglycine dipeptidase (DPEP), and cysteine S-conjugate *N*-acetyltransferase (NAT8) stepwisely degrade these conjugates finally leading to two isomers of monohydroxybutenyl mercapturic acid, (1-hydroxy-3-butene-2-yl)-*N*-acetyl-L-cysteine (1-MHBMA) and (2-hydroxy-3-butene-1-yl)-*N*-acetyl-L-cysteine (2-MHBMA), which are excreted in urine.[3,4] Besides these metabolites, BD could also be converted to dihydroxybutyl mercapturic acid (DHBMA) and trihydroxybutyl mercapturic acid (THBMA) as well as form various hemoglobin and DNA adducts.[5−8] The genotoxic effects of BD are attributed to its DNA-reactive epoxide metabolites.[9]

For BD caused cancer risk assessment, as well as for prevention, and to study mechanisms of BD action, it is essential to identify and determine appropriate biomarkers. In the past years, urinary 2-MHBMA has turned out to be the most specific biomarker for quantifying 1,3-butadiene exposure.[10] Until now, 1-MHBMA was not identified in human urine, although both MHBMA isomers can be found in different proportions in mice and rats exposed to 1,2-epoxy-3-butene (Scheme 1) or BD.[3,11] With this study, we show for the first time that 1-MHBMA is present in human urine at levels somewhat similar to 2-MHBMA. For its quantification, a highly sensitive and specific LC-MS/MS method was developed. Furthermore, analyzing urinary samples of 183 volunteers, we show that 1-MHBMA might be a more reliable and specific

Scheme 1. Metabolism of 1,3-Butadiene to MHBMA



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biomarker for 1,3-butadiene exposure than the commonly used 2-MHBMA.

As the first step, we developed a method allowing simultaneous quantification of 1-MHBMA and 2-MHBMA using LC-MS/MS. For this, we applied electrospray ionization in negative ion mode and acquired enhanced product ion spectra (Figure 1A,B). The most prominent product ion for 1-MHBMA was m/z 103 formed by fragmentation of the hydroxy-butene-thiol group followed by m/z 73 (~15% intensity compared to m/z 103). This fragment might be the butaneolate ion being formed by fragmentation of 3-butene-1-ol coupled to the sulfur atom of 1-MHBMA. By contrast, 2-MHBMA showed only one significant product ion at m/z 103 (no fragment at m/z 73). For analyte separation, a hydrophilic interaction liquid chromatography (HILIC) column with 1.7 μm particle size was chosen. These small particles strongly improve peak separation and lead to sharper and more narrow peaks (leading to improved S/N ratios) than conventional HPLC columns with 3 μm particles. For 1-MHBMA quantification, ion m/z 73 (m/z 232 → m/z 73) was chosen because m/z 103 was too unspecific (Figure 1C,D; Figure S1A–D, Supporting Information; and Table S1, Supporting Information). It strongly interfered with matrix components. For 2-MHBMA quantification, m/z 232 → m/z 103 was used.

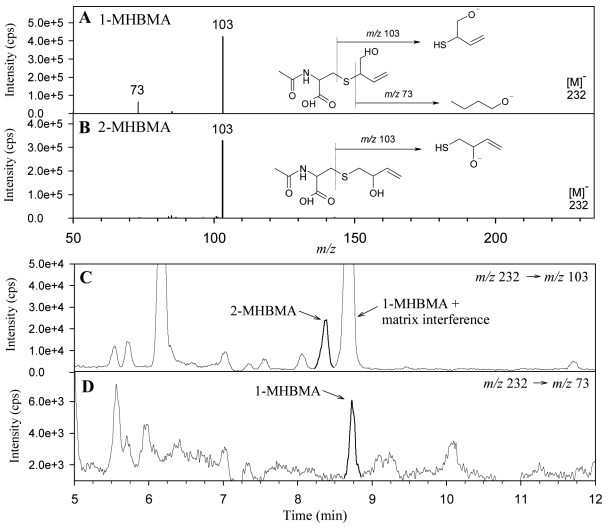

Figure 1. Enhanced product ion spectra of 1- and 2-MHBMA (A,B). MRM chromatograms for 1- and 2-MHBMA of an unspiked smoker urine sample (C,D).The matrix interference (C) might include (4-hydroxy-2-butene-1-yl)-*N*-acetyl-L-cysteine, a third MHBMA isomer, as described by Alwis et al.[12].

For sample preparation, 100 μL of urine was evaporated and reconstituted in methanol. This procedure is perfectly suited for high throughput applications. For quantification and to compensate for ionization efficiency variations, D_6-1- and D_6-2-MHBMA were added as internal standards (IS) before sample preparation (Table S2, Supporting Information). Calibration lines were generated by adding up to 6 levels of each analyte to a human urine pool (linear, $R^2 > 0.99$), and the ratio of analyte to IS was used for quantification (Figure S2A,B, Supporting Information). For urinary 2-MHBMA, we could achieve the lowest detection and quantification limits described until now, which are primarily due to improved peak shapes. More importantly, our method was even more sensitive to 1-MHBMA (LOD, 0.05 vs 0.24 ng/mL; LOQ, 0.15 vs 0.72 ng/mL; Table S2, Supporting Information). Although the absolute intensity of the 1-MHBMA (m/z 73) peak was lower than that for 2-MHBMA (m/z 103), its S/N ratio was higher due to lower background noise. To further evaluate LC-MS/MS method performance, we validated it according to the FDA guidelines.[13] The obtained data are summarized in Tables S3–5 (Supporting Information). Accuracies as well as coefficients of variation (CV) for intra- and interday precisions were improved for 1-MHBMA compared to 2-MHBMA (accuracy, 98.0–102.9% vs 85.3–107.0%; CVs intraday precision, 2.7–6.1% vs 3.9–7.4%; and CVs interday precision, 2.8–7.3% vs 4.4–19.7%). No significant matrix effect could be detected. Samples were stable for 24 h at RT, at 6 freeze/thaw cycles, and postpreparative for 3 days in the autosampler (10 °C).

Until now, 1-MHBMA was not identified in human urine. Although a few groups have published methods to separate both MHBMA isomers (all using m/z 232 → m/z 103), 1-MHBMA could not be detected or quantified in human samples.[10,12,14] Both isomers are present in rodent urine.[3,11] Therefore, we analyzed urinary samples from 183 volunteers using our novel UPLC-HILIC-MS/MS method. One hundred forty-one persons were smokers and thus were significantly exposed to BD. We could determine significant amounts (>LOQ) of 1-MHBMA in 127 samples, generally at about equal levels as those of 2-MHBMA [1-MHBMA, 0.53 ng/mL (mean) and 0–5.82 ng/mL (range); 2-MHBMA, 0.52 ng/mL and 0–1.84 ng/mL]. In mice treated with 1,2-epoxy-3-butene or BD, the major urinary isomer is 2-MHBMA (2-MHBMA/1-MHBMA, ~2:1), whereas in rats 1-MHBMA can be found predominantly (1-MHBMA/2-MHBMA, ~3:1).[3,11] To further confirm the occurrence of 1-MHBMA, its exact mass was analyzed in the reference substance and confirmed in human urine samples using a time-of-flight mass spectrometer (resolution: 12,000 fwhm). It was m/z = 232.0644, which is consistent with the elemental composition. We assume that 1-MHBMA was not yet identified in human urine because most groups might have tried to determine it using the peak obtained with the mass transition m/z 232 → m/z 103. As shown before, this mass transition dramatically interferes with matrix components and might lead to a massive overestimation of potential urinary 1-MHBMA levels.

As discussed before, 2-MHBMA is an established and valuable urinary biomarker of BD exposure.[10] To test if 1-MHBMA could be used as additional or even alternative biomarker of exposure to BD, we first compared urinary creatinine-corrected MHBMA levels in smokers (n = 142) and nonsmokers (n = 41). Smokers had 8.1 times higher 1-MHBMA concentrations and 4.6 times higher 2-MHBMA levels than nonsmokers (1-MHBMA (mean), 663.o2 vs 82.35 ng/g crea; 2-MHBMA (mean), 779.04 vs 169.44 ng/g crea; $p <$ 0.0001 for both isomers using the Mann–Whitney U test). To further compare the performance of both MHBMA isomers as biomarkers for exposure to BD, we correlated the levels of 1- or 2-MHBMA to other smoking-related biomarkers in urine and serum. As indicated in Table 1, we found that all correlations with 1- MHBMA revealed significantly different and higher correlation coefficients for all analyzed biomarkers than correlations with 2- MHBMA ($p < 0.01$). For example, the correlation coefficient of 4-aminobiphenyl correlated with 1-MHBMA was 0.821, whereas with 2-MHBMA the coefficient was 0.592; the difference between these correlations was highly significant ($P = 2.78 \times 10^{-7}$). The significance of correlation differences was calculated by a specific statistical test designed

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Table 1. Pearson Correlation Coefficients (r) of 1-MHBMA or 2-MHBMA and Other Biomarkers of Exposure ($n = 183$)[a]

	1-MHBMA (r)	2-MHBMA (r)	sig. of difference for r
nicotine (U)	0.603	0.469	1.19×10^{-2}
cotinine (S, nicotine)	0.499	0.354	3.85×10^{-3}
thiocyanate (S, hydrogen cyanate)	0.753	0.577	2.44×10^{-5}
1-OH-pyrene (U, pyrene)	0.597	0.461	1.08×10^{-2}
S-phenyl mercapturic acid (U, benzene)	0.869	0.687	1.30×10^{-6}
4-aminobiphenyl (U)	0.821	0.592	2.78×10^{-7}

[a]Significance of difference between the correlations was calculated as described in the Supporting Information. For correlation with serum (S) parameters, the urine (U) levels were corrected for creatinine. Biomarker (matrix, precursor).

for dependent samples.[15] Together, these results show that 1-MHBMA is a more specific biomarker for smoking related exposure to BD than 2-MHBMA. We would assume that it is also a more specific marker for sole BD exposure. However, this has to be confirmed, e.g., with samples from workers with occupational exposure to BD.

In conclusion, our data show for the first time that 1-MHBMA does occur at significant levels in human urine. Since its determination is more sensitive, specific, and accurate, we propose that 1-MHBMA might be a more suitable and reliable biomarker for exposure to BD than the commonly used 2-MHBMA.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, additional MRM chromatograms, and method calibration data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

BD, 1,3-butadiene; CV, coefficient of variation; DHBMA, dihydroxybutyl mercapturic acid; DPEP, cysteinylglycine dipeptidase; GGT, γ-glutamyltranspeptidase; GSH, glutathione; HILIC, hydrophilic interaction liquid chromatography; HPLC, high performance liquid chromatography; IS, internal standard; LC-MS/MS, liquid chromatography coulped to tandem mass spectrometry; LOD, limit of detection; LOQ, limit of quantification; 1-MHBMA, 1-hydroxybutene-2-yl mercapturic acid, (1-hydroxy-3-butene-2-yl)-*N*-acetyl-L-cysteine; 2-MHBMA, 2-hydroxybutene-1-yl mercapturic acid, (2-hydroxy-3-butene-1-yl)-*N*-acetyl-L-cysteine; MRM, multireaction monitoring; NAT8, cysteine S-conjugate *N*-acetyltransferase; r, Pearson correlation coefficient; S, serum; THBMA, trihydroxybutyl mercapturic acid; U, urine; UPLC, ultra performance liquid chromatography

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