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Comprehensive profiling of mercapturic acid metabolites from dietary acrylamide as short-term exposure biomarkers for evaluation of toxicokinetics in rats and daily internal exposure in humans using isotope dilution ultra-high performance liquid chromatography tandem mass spectrometry

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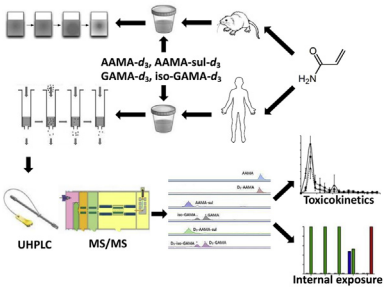
HIGHLIGHTS

- Mercapturic acid metabolites from acrylamide were comprehensively profiled.
- Baseline separation between two oxidative metabolites was achieved.
- Mercapturic acid metabolites were quantified within only 8 min per run.
- Current simultaneous analysis was used for toxicokinetics of acrylamide in rats.
- This method was used for internal exposure evaluation of acrylamide in humans.

GRAPHICAL ABSTRACT



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ABSTRACT

Mercapturic acid metabolites from dietary acrylamide are important short-term exposure biomarkers for evaluating the *in vivo* toxicity of acrylamide. Most of studies have focused on the measurement of two metabolites, *N*-acetyl-S-(2-carbamoylethyl)-L-cysteine (AAMA) and *N*-acetyl-*S*-(2-carbamoyl-2-hydroxyethyl)-L-cysteine (GAMA). Thus, the comprehensive profile of acrylamide urinary metabolites cannot be fully understood. We developed an isotope dilution ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) method for the simultaneous determination of all four mercapturic acid adducts of acrylamide and its primary metabolite glycidamide under the electroscopy ionization negative (ESI-) mode in the present study. The limit of detection (LOD) and limit of quantification (LOQ) of the analytes ranged 0.1–0.3 ng/mL and 0.4–1.0 ng/mL, respectively. The recovery rates with low, intermediate and high spiking levels were calculated as 95.5%–105.4%, 98.2%–114.0% and 92.2%–108.9%, respectively. Acceptable within-laboratory reproducibility (RSD < 7.0%) substantially supported the use of current method for robust analysis. Rapid pretreatment procedures and short run time (8 min per sample) ensured good efficiency of metabolism profiling, indicating a wide application for investigating short-term internal exposure of dietary acrylamide. Our proposed UHPLC-MS/MS method was successfully applied to the toxicokinetic study of acrylamide in rats. Meanwhile, results

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of human urine analysis indicated that the levels of *N*-acetyl-*S*-(2-carbamoylethyl)-L-cysteine-sulfoxide (AAMA-sul), which did not appear in the mercapturic acid metabolites in rodents, were more than the sum of GAMA and *N*-acetyl-*S*-(1-carbamoyl-2-hydroxyethyl)-L-cysteine (iso-GAMA). Thus, AAMA-sul may alternatively become a specific biomarker for investigating the acrylamide exposure in humans. Current proposed method provides a substantial methodology support for comprehensive profiling of toxicokinetics and daily internal exposure evaluations of acrylamide *in vivo*.

1. Introduction

Acrylamide as an industrial chemical has been widely used in the manufactures of polymer and resin, and also can be generated in heat processing food through the Maillard reaction [1,2]. Acrylamide contaminant in food raises considerable concerns due to its probable neurotoxicity, genotoxicity and reproductive toxicity for humans [3]. High exposure of acrylamide in general populations mainly derives from dietary intake, including potato-based food, bakery products, fast snacks, cookies, coffee, etc. Besides, acrylamide exposure to humans also originates from occupational exposure, migration of polyacrylamide-based packaging materials or polyacrylamide gel electrophoresis (PAGE), environmental drinking water exposure, smoking, cosmetics, etc [4]. Thus, acrylamide has been classified as a probable carcinogen to humans (Group 2A) by the International Agency for Research on Cancer [5].

The metabolism and toxicokinetics of acrylamide has been reported in both rodent and human studies. Both *in vivo* and *in vitro* studies revealed acrylamide could be converted into glycidamide in the presence of cytochrome P450 2E1 [6,7], while a portion of glycidamide could be further converted into non-toxic 2,3-dihydroxy-propionamide [8,9]. Furthermore, both acrylamide and glycidamide were capable of conjugating with DNA, hemoglobin (Hb) or glutathione (GSH). As an important category of exposure biomarkers, four DNA adducts of acrylamide have been identified [10]. Among these, the efficiency of glycidamide-DNA conjugation was much higher than the conjugating efficiency between acrylamide and DNA [11]. The Hb adducts are selected as alternative biomarkers for the exposure assessment in epidemiological studies of dietary acrylamide. Two adducts, *N*-carbamoylethylvaline (AAVal) and *N*-(*R*,*S*)-2-hydroxy-2-carbamoylethylvaline (GAVal), have been identified and regarded as important biomarkers for evaluating the *in vivo* exposure dose of acrylamide [12,13]. The DNA and Hb adducts of acrylamide have been regarded as long-term and mid-term exposure biomarkers in toxicokinetic and epidemiologic studies, respectively.

The mercapturic acid adducts of acrylamide and glycidamide, including *N*-acetyl-*S*-(2-carbamoylethyl)-L-cysteine (AAMA), *N*-acetyl-*S*-(2-carbamoylethyl)-L-cysteine-sulfoxide (AAMA-sul), *N*-acetyl-*S*-(2-carbamoyl-2-hydroxyethyl)-L-cysteine (GAMA) and *N*-acetyl-*S*-(1-carbamoyl-2-hydroxyethyl)-L-cysteine (iso-GAMA), have been recognized as short-term exposure biomarkers [14,15]. The chemical structures of these metabolites and their generation from acrylamide are shown in Fig. 1. AAMA and GAMA are currently considered as main urine biomarkers, while the ratio of AAMA to GAMA is employed for comparing the metabolism of acrylamide between rodents and humans [16]. Previous toxicokinetic study used this ratio to indirectly investigate the conversion rate from acrylamide into the more reactive glycidamide [17]. Besides, the epidemiologic study took the excretion amounts of AAMA and GAMA as internal-dose exposure biomarkers for the carcinogenic risk assessment of dietary acrylamide [18]. The excretion of iso-GAMA in human urine has been found via a single oral administration of deuterium-labeled acrylamide in further metabolic investigation, indicating dietary doses of acrylamide cause an overload of detoxification via AAMA and induce the generation of carcinogenic glycidamide in humans [19]. A further metabolite AAMA-sul from the oxidization of AAMA was found in humans but not in rodents [16,19]. Taken together, simultaneous monitoring of the above four mercapturic acid metabolites of acrylamide and glycidamide could comprehensively profile the short-term internal-dose exposure of acrylamide especially in humans. Unfortunately, such simultaneous analysis has not been established so that the acrylamide urinary metabolites could not be fully understood.

Current analytical methods for the determination of mercapturic acid metabolites include high-performance liquid chromatography (HPLC) with ultraviolet or fluorescence detection [20,21], gas chromatography mass spectrometry (GC–MS) [22], liquid chromatography mass spectrometry (LC-MS) [23] and liquid chromatography tandem mass spectrometry (LC-MS/MS) [24,25]. Among these, the LC-MS/MS method is preferred for the quantification of acrylamide

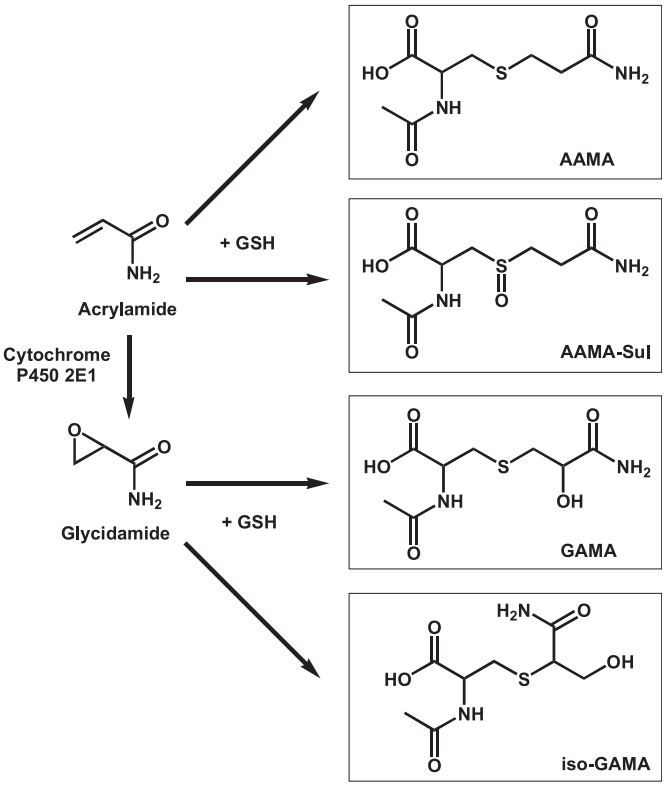

Fig. 1. The metabolic pathway of acrylamide and glycidamide in urine. Four mercapturic acid metabolites were regarded as short-term exposure biomarkers and analyzed in this study. AAMA, *N*-acetyl-*S*-(2-carbamoylethyl)-L-cysteine; AAMA-sul, *N*-acetyl-*S*-(2-carbamoylethyl)-L-cysteine-sulfoxide; GAMA, *N*-acetyl-*S*-(2-carbamoyl-2-hydroxyethyl)-L-cysteine; iso-GAMA, *N*-acetyl-*S*-(1-carbamoyl-2-hydroxyethyl)-L-cysteine. GSH, glutathione.