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Determination of acrylamide and glycidamide in various biological matrices by liquid chromatography–tandem mass spectrometry and its application to a pharmacokinetic study

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ABSTRACT

Acrylamide (AA) is a heat-generated food toxicant formed when starchy foods are fried or baked. This study describes a simple and sensitive liquid chromatography–tandem mass spectrometry assay for the simultaneous quantification of AA and its active metabolite, glycidamide (GA) in rat plasma, urine, and 14 different tissues. The assay utilized a simple method of protein precipitation and achieved a lower limit of quantification of 5, 10 and 25 ng/mL of AA and 10, 20 and 100 ng/mL of GA for plasma, tissues and urine, respectively. The assay was fully validated to demonstrate the linearity, sensitivity, accuracy, precision, process recovery, and stability using matrix matched quality control samples. The mean intra- and inter-day assay accuracy was 91.6–110% for AA and 92.0–109% for GA, and the mean intra- and inter-day assay precisions were $\leq$10.9% for AA and $\leq$8.60% for GA. The developed method was successfully applied to a pharmacokinetic study of AA and GA following intravenous and oral administration of AA in rats. Tissue distribution characteristics of AA and GA were also determined under steady-state conditions.

1. Introduction

Acrylamide (AA) is a white and highly water-soluble chemical that has been used in various fields such as oil, cosmetic, water treatment, and textile industries since mid-1950s [1]. AA has shown to cause a variety of tumors in experimental animals. The carcinogenicity of AA has been extensively studied and increased incidences of tumors in mammary gland, thyroid, and skin have been documented [2–4]. Based on the animal studies, AA is classified as a probable human carcinogen by the United States Environmental Protection Agency [1] and International Agency for Research on Cancer [5]. Various other toxicities have been reported, including neurotoxicity in animals [6,7] and humans [8–10], reproductive toxicity in rodents [11,12], and mutagenicity in somatic cells in vitro [13] and in vivo [14], as well as in germ cells in vivo [15].

In 2002, it has been reported that AA is formed by the Maillard reaction during cooking of carbohydrate-rich foods at high temperature [16,17]. Significant levels of AA were found in certain fried, baked, and deep-fried foods such as potato chips (318 ppb), ground coffee (205 ppm), crackers and snack (169 ppb), and bakery products (34 ppb) [16–19]. Since the hazardous effects of AA as an industrial chemical are well known, the discovery of AA in the daily diet renewed the interests in its potential health effects and raised considerable concern [20].

AA toxicities are known to be mediated by its epoxide metabolite, glycidamide (GA). GA is predominantly formed by CYP2E1 [21–24]. GA-induced genotoxicity and mutagenicity have been reported in rodents [14,25], rodent germ cells [15,26], and *Salmonella typhimurium* [27]. GA reacts with DNA to form DNA adducts with even higher affinity than AA [28–30], which appears to be the major cause of mutagenicity and carcinogenicity by AA exposure [5,31,32].

To assess the potential risks of AA exposure on human health, it is essential to determine AA and GA concentrations in biological fluids. While many analytical methods have been described for the determination of AA in drinking water [33–36] and food stuffs [37–39], few bioanalytical methods are available that can be used

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for in vivo pharmacokinetic or toxicokinetic studies. The first reported method to determine AA in biological samples was the radioactivity assay of labeled AA (2,3-^{14}C AA) [40]. Later, assays utilizing high pressure liquid chromatography (HPLC) and liquid chromatography–tandem mass spectrometry (LC–MS/MS) have been developed. These assays include an HPLC/UVD with protein precipitation for determination of AA in rat plasma [41], an LC–MS/MS assay with solid phase extraction (SPE) for determination of AA and GA in mouse serum [42] and an LC–MS/MS assay with protein precipitation to determine AA and GA in human placenta [43]. However, the HPLC/UVD and LC–MS/MS methods utilizing protein precipitation lack sufficient sensitivity (LLOQ=500 ng/mL) [41,43]. The only bioanalytical method which achieves sufficient sensitivity is the LC–MS/MS assay with SPE sample preparation (LLOQ=0.71 ng/mL for AA, 8.7 ng/mL for GA) [42]. To date, most of the pharmacokinetic studies of AA [31,32,44,45] rely on this LC–MS/MS assay with SPE which is complicated, time consuming, and less cost efficient. A simple and rapid assay which is adaptable to various biological samples with high sensitivity is not yet available.

In the present study, a simple and sensitive LC–MS/MS method using single-step protein precipitation sample preparation was developed and validated for the simultaneous determination of AA and GA in biological matrices including plasma, urine, and various tissues. The method was applied to an in vivo study to characterize the absorption, tissue distribution and disposition pharmacokinetics of AA and GA after intravenous (i.v.) injection, i.v. infusion, and oral administration in rats.

2. Experimental

2.1. Materials

AA, acrylamide-D_3 standard solution (500 mg/L, internal standard, IS) and formic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). GA was purchased from Toronto Research Chemicals (Toronto, ON, Canada). Acetonitrile, methanol, and distilled water (all HPLC grade) were purchased from Mallinckrodt Baker (Phillipsburg, NJ, USA).

2.2. Preparation of standard solutions

Primary stock solutions of AA and GA were prepared by dissolving 10 mg each of AA and GA separately in 10 mL of distilled water. The working standard solutions were prepared by further dilution of the primary stock solutions with acetonitrile. The acrylamide-D_3 standard solution (100 μL) was diluted to 10 mL with acetonitrile.

2.3. Calibration standards and quality control (QC) samples

All calibration curves consisted of at least seven calibrator concentrations, a blank sample (without IS) and a zero sample (with IS). The calibrator and QC samples were prepared by spiking 5 μL of the working standard solution to 45 μL of the blank rat biological matrices. Various blank biological samples were obtained from at least 10 rats by collecting blood, urine, and tissues of liver, kidney, spleen, lung, heart, testis, stomach, small intestine, large intestine, fat, skin, muscle, brain, and thyroid. Tissue samples were homogenized in isotonic saline. Four different levels of low QC, medium QC, high QC, and lower limit of quantification (LLOQ) samples were prepared for AA and GA in each biological matrix. Different calibration ranges were constructed depending on the biological matrices of plasma, urine, and tissue samples. The calibration standards and QC samples for AA and GA in different biological matrices are presented in Table 1. The QC samples were prepared once, and aliquots (50 μL each) were stored at −20 °C until analysis.

2.4. Sample preparation

The working standard solutions of IS (500 ng/mL for plasma and tissue samples, and 2000 ng/mL for urine samples) were added to 50 μL of the biological samples. Blank acetonitrile (50 μL for plasma and tissue samples and 650 μL for urine samples) was used as the protein precipitating solvent. The mixture was mixed on a vortex mixer for 1 min and centrifuged at 16,060*g* for 10 min (Biofuge Fresco; Kendro, Osterode, Germany). For all tissue samples except thyroid, the supernatant (75 μL) was diluted with the same volume of acetonitrile and centrifuged at 16,060*g* for 10 min. Finally, 75 μL of the diluted supernatant was mixed with 25 μL of distilled water, and a portion (7.5 μL) was injected into the LC–MS/MS.

2.5. LC/MS/MS instruments and conditions

A model 1200 HPLC coupled with a model 6430 LC–MS/MS system (Agilent, Santa Clara, CA, USA) was used for sample analysis. Samples were separated on a dC_{18} column (150 × 2.10 mm^2 i.d., 3 μm; Atlantis, Milford, MA, USA) with a Security Guard column (Phenomenex, Torrence, CA, USA). The mobile phase was a mixture of acetonitrile and 0.05% of formic acid (10:90 v/v). The flow rate was 0.1 mL/min, and the column oven temperature was 30 °C for all samples.

The electro spray ionization source was operated in a positive mode and samples were detected in the multiple reaction monitoring (MRM) mode with a dwell time of 200 ms per MRM channel. Gas temperature, gas flow rate, and nebulizer gas pressure was set at 300 °C, 10 L/min, and 20 psi, respectively. The MRM transition of precursor to product ion pairs were m/z 71.9→55.0 for AA, m/z

Table 1
Concentrations of calibrators and QC samples for acrylamide and glycidamide in various biological samples.

Compound	Matrix	Concentration (ng/mL)	
		Calibrator	QC samples
Acrylamide	Plasma	5, 10, 50, 100, 500, 1000, 2000, and 5000	5, 20, 500, and 4500
	Urine	25, 50, 100, 500, 1000, 2000, 5000, and 10,000	25, 300, 1000, and 9000
	All tissues*	10, 20, 50, 100, 500, 1000, 2000, and 5000	10, 40, 500, and 4500
Glycidamide	Plasma	10, 50, 100, 500, 1000, 2000, and 5000	10, 20, 500, and 4500
	Urine	100, 150, 500, 1000, 2000, 5000, and 10,000	100, 300, 1000, and 9000
	All tissues*	20, 50, 100, 500, 1000, 2000, and 5000	20, 40, 500, and 4500

* The lowest concentration in the calibrator and QC samples for thyroid was 5 ng/mL for acrylamide and 10 ng/mL for glycidamide."