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Quantitative analysis of heterocyclic amines in urine by liquid chromatography coupled with tandem mass spectrometry

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ABSTRACT

A sensitive, reproducible, and rapid analytical method for the analysis of trace-level heterocyclic amines (HCAs) that are expected to have high levels of human exposure was developed. Liquid–liquid extraction (LLE) with dichloromethane (DCM) followed by solid-phase extraction (SPE) was carried out. Liquid extraction with DCM under basic conditions was efficient in extracting HCAs from urine samples. For further purification, mixed mode cationic exchange (MCX) cartridges were applied to eliminate the remaining interferences after liquid extraction. Separation and quantification were performed by liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) in selected reaction monitoring (SRM) mode. The overall recoveries ranged between 71.0% and 113.6% with relative standard deviations (RSDs) of 5.1% to 14.7% for the entire procedure. The limits of detection (LODs) and limits of quantification (LOQs) of the proposed analytical method were in the ranges of 0.04 to 0.10 ng/ml and 0.15 to 0.36 ng/ml, respectively. This method was applied to the analysis of monitoring in urine samples for Korean school children, and the results demonstrated that the method can be used for the trace determination of HCAs in urine samples.

Assessment of human exposure to heterocyclic amines (HCAs)[1] has been of increasing concern since the discovery of mutagenic activity in smoke particles from broiled fish [1]. These compounds are formed in thermally treated protein-rich food at the nanogram/gram (ng/g) level [2] but are also detected in several other environments, including cooking fumes, cigarette smoke, air, rain, and river water [3]. So far, the toxicity of HCAs related to carcinogenic and mutagenic response has been evaluated in experimental animal and cell models [4,5]. For an estimation of the overall risk to people, epidemiological studies depending on dietary habit, race, age, and gender should be investigated extensively [6,7]. However, the lack of information for quantitative data on HCA exposure limits the ability to evaluate the health risk and safety management. To integrate the data of human exposure, urine has been used as a biological fluid that has the advantage of simple and noninvasive collection. In spite of the useful matrix for monitoring, the parent HCAs of urinary excretion were detected at sub-nanogram levels because HCAs are rapidly metabolized by two different pathways: detoxification and activation [8,9]. During the metabolic process, hydroxylation, sulfation, glucuronidation, and adduct form with macromolecules such as protein and DNA to show that carcinogenic effects may also be involved [10–17]. Therefore, a highly sensitive, selective, and reliable method for the determination of HCAs is required due to their low concentrations and the high complexity of the matrix.

Previously, we reported that a multiple solid-phase extraction (SPE) method was used with liquid chromatography coupled with mass spectrometry (LC–MS) for the analysis of HCAs in human urine [18]. However, there are several drawbacks to determine HCAs in urine samples using the previous method with respect to achieving large-scale analysis for biomonitoring. Here, we developed a more sensitive, reproducible, and rapid method for the analysis of eight HCAs for biomonitoring study. The target analytes, which are expected to have high levels of exposure through the meat and dairy products of South Korea, are shown along with the precursor for the formation in Fig. 1. We employed simple liquid–liquid extraction (LLE), followed by one step (SPE) for the sample preparation and the selected reaction monitoring (SRM) technique with tandem mass spectrometry (MS/MS), for the quantification of HCAs. The method was validated with respect to precision, accuracy, linearity,

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[1] *Abbreviations used:* HCA, heterocyclic amine; SPE, solid-phase extraction; LC–MS, liquid chromatography coupled with mass spectrometry; LLE, liquid–liquid extraction; SRM, selected reaction monitoring; MS/MS, tandem mass spectrometry; LOD, limit of detection; LOQ, limit of quantification; IS, internal standard; MeOH, methanol; DCM, dichloromethane; HPLC, high-performance liquid chromatography; MCX, mixed mode cationic exchange; QC, quality control; QA, quality assurance; MS/MS, tandem mass spectrometry; CE, collision energy; LOD, limit of detection; LOQ, limit of quantification; RSD, relative standard deviation; UV, ultraviolet; ESI, electrospray ionization.

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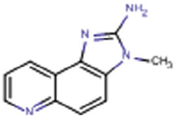
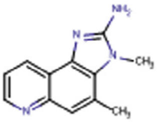
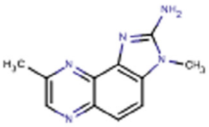
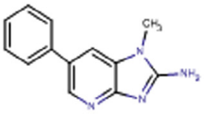
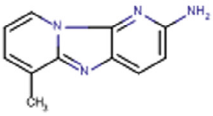
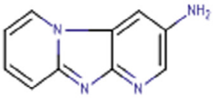
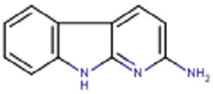
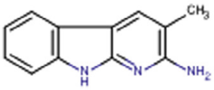
Chemical name	Abbreviation	Structure	Molecular mass and properties	Precursor
Amino-imidazo-azzarene 2-amino-3-methylimidazo [4,5-f]quinoline	IQ		198.2239 pKa=3.5	Creatine, Gly, Phe, Ser, Pro, Fructose, Glucose
2-amino-3,4-dimethylimidazo [4,5-f]quinoline	MeIQ		212.2505	Creatine, Ala, Fructose
2-amino-3,8-dimethylimidazo [4,5-f]quinoxaline	MeIQx		213.2385	Creatine, Gly, Ala, Thr, Lys, Fructose, Glucose, Ribose
2-amino-1-methyl-6-phenylimidazo [4,5-b]pyridine	PhIP		224.2612 pKa=5.6	Creatine, Phe, Glucose
Amino-carboline 2-amino-6-methyldipyrido [1,2-a:3',2'-d]imidazole	Glu-P-1		198.2239 pKa=6.0	Glutamic acid
2-amino-dipyrodo[1,2-a:3',2'-d]imidazole	Glu-P-2		184.1973 pKa=5.9	Glutamic acid Casein
2-amino-9H-pyrodo[2,3-b]indole	AαC		183.2093 pKa=4.4	Soybean Globulin
2-amino-3-methyl-9H-pyrodo[2,3-b]indole	MeAαC		197.2358	Soybean Globulin

Fig.1. Abbreviations, structures, and properties of target HCAs.

limit of detection (LOD), and limit of quantification (LOQ). The proposed method was applied successfully to the analysis of monitoring in urine samples for Korean school children and can offer the assessment of human health risk of HCA exposure.

Materials and methods

Reagents and chemicals

Neat standards of eight HCAs (IQ, Glu-P-1, Glu-P-2, MeIQx, MeIQ, PhIP, AαC, and MeAαC; see Fig. 1 for full chemical names) were purchased from Toronto Research Chemicals (Toronto, Canada). 1-Naphthyl amine as the internal standard (IS) and β-glucuronidase were purchased from Sigma (St. Louis, MO, USA). Methanol (MeOH), Acetonitrile, dichloromethane (DCM), ammonium acetate, and acetic acid were of high-performance liquid chromatography (HPLC) grade and were purchased from Fisher Scientific (Pittsburgh, PA, USA). Water was purified using a Milli-Q system (Millipore, Bedford, MA, USA). An Oasis MCX (mixed mode cationic exchange) cartridge (30 mg, 1 ml) was obtained from Waters (Milford, MA, USA). Ammonia solution (25%) and hydrochloric acid (37% fuming) were obtained from Merck (Darmstadt, Germany).

Samples

A 500-ml pooled urine sample that did not contain the eight HCAs was prepared and used in spiking experiments for quality control (QC) and quality assurance (QA). For the stability test, an individual 2-ml aliquot of a pooled urine sample was spiked with 5 ng of HCAs and then stored at −18 °C until batch analysis of samples. All urine samples from Korean school children were provided by the Korea Food and Drug Administration (KFDA).

Sample preparation

All samples were thawed and centrifuged before analysis. For the enzymatic deglucuronidation and hydrolysis, 50 μl of β-glucu-