Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography A, 1131 (2006) 58–66
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Quantitation of mercapturic acids from acrylamide and glycidamide in human urine using a column switching tool with two trap columns and electrospray tandem mass spectrometry

Marco Kellert, Karoline Scholz, Silvia Wagner, Wolfgang Dekant, Wolfgang Völkel *

Department of Toxicology, University of Würzburg, Versbacher Strasse 9, 97078 Würzburg, Germany

Received 3 April 2006; received in revised form 4 July 2006; accepted 7 July 2006
Available online 1 August 2006

Abstract

A sensitive and specific electrospray tandem mass spectrometry method using a column switching unit with two trap columns was established to quantify the mercapturates (MAs) of acrylamide (AA) and glycidamide (GA) in human urine. A specially endcapped material was applied for trapping the hydrophilic MAs and a pre-trap column was used to remove lipophilic compounds from the directly injected urine to protect the trap column. The limits of quantitation for AA–MA and GA–MA in urine were 0.5 μg/L and 1 μg/L, respectively. Urine was spiked with deuterated internal standards and injected directly into LC–MS/MS. Urine of smokers ($n = 13$) revealed the highest concentrations of AA–MA and GA–MA in the range of 61–706 μg/L and 5–54 μg/L, respectively. Lower levels for AA–MA (14–102 μg/L) and GA–MA (1–11 μg/L) were detected in non-smokers ($n = 13$).

Keywords: LC–ESI-MS/MS; Column switching; Human urine; Acrylamide; Glycidamide; Mercapturic acids

1. Introduction

Foodstuffs such as french fries, gingerbread or crispbread were identified as a possible source for exposure, and nowadays, it is known that acrylamide (AA) is formed in food by the well-known Maillard reaction [1–5]. AA is also used as an industrial chemical with a worldwide production volume of 2×10^8 kg/year [6]. AA is neurotoxic [7,8] with a no observed effect level (NOEL) of 0.5 mg/kg body weight [9] and causes tumors in different tissues (testes, thyroid, and mammary gland) of rats at a dose of 1–2 mg/kg body weight [10]. Hemoglobin adducts of AA were detected in humans without intentional exposure to AA [11,12].

After oral administration to rats, AA is rapidly absorbed from the intestine and 60% of the dose is conjugated with glutathione (GSH) [13]. To some extent AA is transformed to the highly reactive epoxide glycidamide (GA) by cytochrome P 450 2E1 enzymes (CYP 2E1) [14–16]. GA is able to react with nucleophilic sites of proteins and with glutathione [17]. Degradation of these GSH conjugates by transpeptidases and subsequent acetylation leads to the corresponding mercapturic acids (MAs) that are excreted with urine (Fig. 1) [18].

MAs, in general, are important degradation products of reactive exogenous and endogenous electrophiles and thus, constitute a major tool in human biomonitoring approaches [19,20]. In contrast to former research studies using HPLC–UV, HPLC-fluorescence, or GC–MS for detection of MAs, nowadays almost exclusively, LC–MS/MS-based methods are used for quantitation of MAs [19,21–24]. In literature, several LC–MS/MS-based methods are described using a mercapturate-specific constant neutral loss of 129 Da for the detection of more than 50 signals in both human and rat urine specimen [25,26].

Concerning AA and GA, several methods exist for quantitation of the corresponding renally excreted MAs in urine samples from unintentionally exposed humans [24,27,28]. For AA–MA, the limit of detection (LOD) of these methods is relatively high (<5 μg/L [27], 1 μg/L [28], and 2 μg/L [24]), and a complex sample workup using solid phase extraction for the more sensitive methods is necessary [24]. The poor LOD for AA–MA and GA–MA prevents their quantitation in some urine samples

* Corresponding author. Tel.: +49 931 201 48432; fax: +49 931 201 48865.
E-mail address: voelkel@toxi.uni-wuerzburg.de (W. Völkel).

0021-9673/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2006.07.026

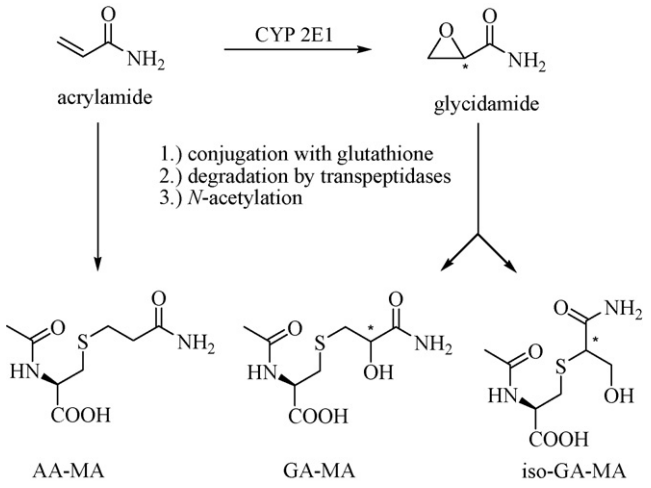

Fig. 1. Metabolic pathway of acrylamide with respect to the formation of mercapturic acids. CYP 2E1: Cytochrome P 450 isoform 2E1; AA–MA: mercapturic acid of acrylamide; GA–MA: mercapturic acid of glycidamide.

[24,28]. However, due to the fact that the highly reactive GA seems to play a major role in the carcinogenic effects of AA [14,15,29,30], specific and sensitive methods are necessary for its biomonitoring throughout the population.

To reduce the sample workup to a minimum, different column switching methods have been developed [31,32]. In general, this technique enables the injection of large volumes (>100 μL) combined with a reduction of ion suppression, resulting from the high content of salts in urine. Quantitation of AA–MA by a column switching method was described by Li et al. [27]. However, this method only allows the measurement of AA–MA.

The method presented here enables the quantitation of both AA–MA and GA–MA, as well as the detection of iso-GA–MA. It is based on a novel column switching technique consisting of two trap columns, i.e. a pre-trap column and a conventional trap column (Fig. 2). The injection volume is 125 μL of urine fortified with $^{2}H_{3}$-labelled internal standards of AA–MA, GA–MA, and iso-GA–MA. By this method, GA–MA and AA–MA were quantified in 38 urine samples from not intentionally exposed humans.

2. Experimental

2.1. Chemicals

Water was purchased from Roth (Karlsruhe, Germany). All other chemicals were from Sigma/Fluka (Taufkirchen, Germany). All solvents used were HPLC grade.

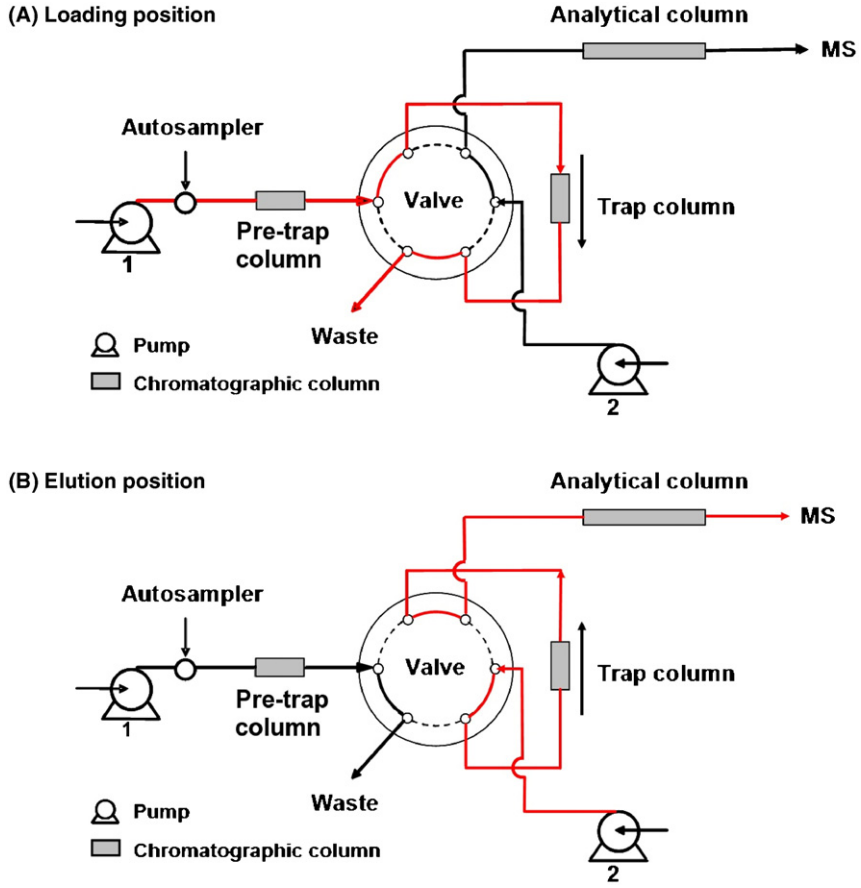

Fig. 2. Assembly of the column switching unit. Loading of the sample on the trap column in position A. Elution of the analytes from trap column over the analytical column in position B.