Journal of Chromatography B, 870 (2008) 203–208

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Determination of heterocyclic aromatic amines in human urine by using hollow-fibre supported liquid membrane extraction and liquid chromatography-ultraviolet detection system

Faiz Ullah Shah [a], Thaer Barri [a], Jan Åke Jönsson [a,*], Kerstin Skog [b]

[a] Department of Analytical Chemistry, Lund University, P.O. Box 124, SE-221 00 Lund, Sweden
[b] Department of Food Engineering, Technology and Nutrition, Lund University, P.O. Box 124, SE-221 00 Lund, Sweden

ARTICLE INFO

Article history:
Received 20 September 2007
Accepted 5 June 2008
Available online 11 June 2008

Keywords:
Heterocyclic aromatic amines
Urine sample
Hollow-fibre supported liquid membrane
High performance liquid chromatography with ultraviolet detector

ABSTRACT

A hollow-fibre supported liquid membrane (HF-SLM) extraction method has been developed for determination of 11 heterocyclic aromatic amines (HCAs) in human urine samples by using high performance liquid chromatography (HPLC) equipped with an ultraviolet (UV) absorbance detector. These compounds were extracted from an alkaline urine sample (donor phase) into the organic solvent residing in the pores of a polypropylene hollow fibre and then back extracted into an acidic solution (acceptor phase) inside the lumen of the hollow fibre. After extraction, HCAs were analyzed by injecting the analyte enriched acceptor phase into the HPLC. The analyte enrichment factors ranged between 241 and 339 obtained in a 90 min extraction time, and method detection limits (MDL) ranged between 0.1 and 0.5 $\mu g\,L^{-1}$ with relative standard deviation (RSD) values between 3.4% and 11%. The extraction technique employed in this work is easy to use and rapid as it involves only a few minutes manipulation of each sample. It is the most economical sample preparation/preconcentration technique to our knowledge as compared to other microextraction techniques.

© 2008 Elsevier B.V. All rights reserved.

1. Introduction

In the late 1970s, Japanese scientists reported high mutagenic activity on the surface of grilled meat and fish [1]. This finding led to the discovery of a series of mutagenic and carcinogenic heterocyclic aromatic amines (HCAs) that are formed at ng/g levels during cooking of meat and fish [2]. Today, there are several literature reports where more than 20 HCAs have been isolated and identified from cooked foods [3–5]. Some of these HCAs have also been found in cigarette smoke, soy sauce, wine, vinegar, beer, river water and human urine [6–11].

These compounds (see Table 1) have high mutagenic and carcinogenic properties. It has been observed that the rising cancer incidences in human beings in the developed countries, especially the diseases of colon, large intestine, prostate, liver and kidneys are related to a large extend to the greater consumption of meat as well as preserved meat. The concentration of HCAs in meat depends on cooking temperature and duration of heating. Under normal conditions, the HCAs are formed at very low concentrations but if the cooking temperature is increased above 150 °C the formation of these compounds increases significantly [12].

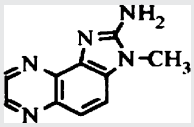
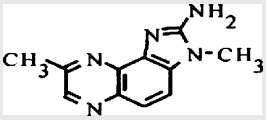
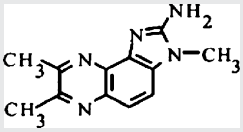
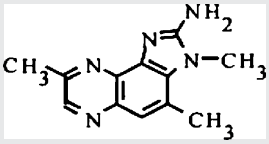
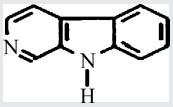
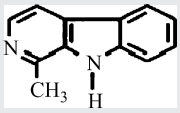
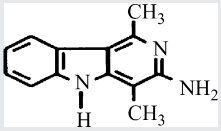
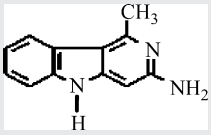
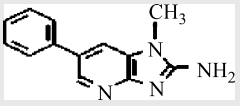
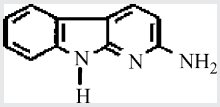
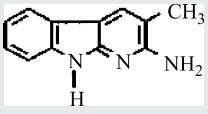
These amines are formed from free amino acids, creatine/creatinine, and carbohydrates [13,14] during cooking of food, particularly when proteinaceous food is heated at moderate to high temperatures. Their content varies greatly with cooking conditions, so it is difficult to obtain an accurate estimation of the exposure. Thus, methods have been developed to determine these compounds in biological samples (mainly urine samples) [15–19] to reflect recent HCAs exposure. These methods of analysis are usually based on liquid chromatography (LC) [20–24], but gas chromatography (GC) [25,26] and capillary electrophoresis (CE) [27] have also been used. For sample extraction, solid-phase extraction (SPE) is the most commonly used technique [17,20,22–25], but also liquid–liquid extraction (LLE) [16,18,19] and solid-phase microextraction (SPME) [28] have been applied.

There are several emerging alternative extraction techniques based on membrane extraction. Such techniques are typically very simple and cheap and can provide efficient clean-up and high enrichment factors. They are divided into two groups: those based on porous membranes such as Donnan dialysis, microdialysis and electrodialysis, and those based on non-porous membranes, such as supported liquid membrane (SLM) extraction, microporous membrane liquid–liquid extraction (MMLLE), polymeric mem-

* Corresponding author. Tel.: +46 462228169; fax: +46 462224544.
E-mail address: jan_ake.jonsson@analykem.lu.se (J.Å. Jönsson).

1570-0232/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2008.06.006

Table 1
Structures of the HCAs studied in this work

Name	pK_a	Structure
IQx	–	
MeIQx	5.94	
7,8-MeIQx	6.45	
4,8-MeIQx	6.25	
Norharman	–	
Harman	–	
Trp-P-1	8.55	
Trp-P-2	8.5	
PhIP	5.6	
AαC	4.6	
MeAαC	4.9	

brane extraction (PME) and membrane extraction with a sorbent interface (MESI) [29].

SLM extraction, either using flat membranes or in hollow-fibre configurations, is based on a three-phase (aqueous–organic–aqueous) system, where the organic solvent is held in the pores of a porous membrane supported by capillary forces. It is in contact with two aqueous phases, the donor phase, that is the aqueous sample, and the acceptor phase, usually an aqueous buffer. In the hollow-fibre configuration the latter is placed in the lumen of the fibre [30].

Only a very small amount of organic solvent is used for impregnating the membrane pores. The analytes are extracted from the aqueous donor phase into the organic membrane and then back-extracted to the second aqueous phase, the acceptor. This process is usually driven by differences in pH between the two aqueous phases. For basic analytes such as HCAs, the pH in the aqueous donor needs to be above the pK_a values of the analytes rendering them uncharged. The analytes are then in extractable form and are extracted into the membrane liquid. The analytes diffuse across the membrane and are back-extracted into the acceptor, where the pH is kept below the pK_a value. The basic analytes will thus be charged, non-extractable, and thereby trapped in the acceptor [29].

Recently, hollow-fibre supported liquid membrane extraction has been successfully applied to the determination of 2-amino-1-methyl-6-phenylimidazo [4,5-*b*] pyridine (PhIP) [31] in urine and blood plasma. This compound (see below) is the most abundantly found HCA arising from consumption of cooked meat-containing food. In the present work, a similar approach has been developed for extraction/pre-enrichment of 11 HCAs from human urine samples. The main aim of the study was to examine if the developed procedure [31] could be applied to the extraction of other HCAs. In order to detect all the relevant compounds an ultraviolet (UV) detector was used, limiting the detection limits that can be obtained. Using a more sensitive detection system such as LC–MS would help to detect HCAs in unspiked urine samples.

2. Experimental

2.1. Chemicals

The following HCAs (see Table 1 for their structures) were used in this work as model compounds:

2-Amino-3-methylimidazo[4,5-*f*]quinoxaline (IQx), 2-amino-3, 8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), 2-amino-3, 4,8-trimethylimidazo[4,5-*f*]quinoxaline (4,8-DiMeIQx), 2-amino-3, 7,8-trimethylimidazo[4,5-*f*]quinoxaline (7,8-DiMeIQx), 3-amino-1,4-dimethyl- 5*H*-pyrido[4,3-*b*]indole (Trp-P-1), 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2), 2- amino-1-methyl-6-phenylimidazo [4,5-*b*] pyridine (PhIP), 2-amino-9*H*-pyrido [2,3-*b*]indole (AαC), 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole (MeAαC), 2-methyl-β-carboline (Harman), (9Hpyrido[3,4-*b*] indole (Norharman). These HCAs were supplied by Toronto Research chemicals (Toronto, Canada).

High performance liquid chromatography (HPLC)-grade acetonitrile was supplied by Merck (Darmstadt, Germany). Sodium hydroxide was obtained from Scharlan Chemie (Barcelona, Spain). Sulphuric acid and 1-octanol were obtained from Sigma–Aldrich (Steinheim, Germany). All aqueous solutions were prepared by using reagent water purified by a Milli-Q Gradient system (Millipore, Bedford, MA, USA).

The Accurel® PP Q3/2 polypropylene hollow-fibre membranes (200 μm wall thickness, 600 μm inner diameter and 0.2 μm pore size) were obtained from Membrana GmbH (Wuppertal, Germany).

Microsyringes (0.8 mm outer needle diameter and 10–100 μL volume) were purchased from SGE (Australia) and Hamilton Bonaduz AG (Switzerland). The solution pH was measured by a 211 microprocessor pH-meter (Hanna instruments, Rhode Island, USA). A 10-position magnetic stirring device (RO 10 power, IKA-Werke GmbH & Co.KG, Staufen, Germany) was used for sample stirring during extraction.

Download English Version:

https://daneshyari.com/en/article/1216770

Download Persian Version:

https://daneshyari.com/article/1216770

Daneshyari.com