Journal of Chromatography B, 934 (2013) 53–59

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography B

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

High-throughput method for the analysis of ethylenethiourea with direct injection of hydrolysed urine using online on-column extraction liquid chromatography and triple quadrupole mass spectrometry☆

Eva Ekman*, Margaretha Maxe, Margareta Littorin, Bo A.G. Jönsson, Christian H. Lindh

Division of Occupational and Environmental Medicine, Department of Laboratory Medicine, Faculty of Medicine, Lund University, SE-221 85 Lund, Sweden

ARTICLE INFO

Article history:
Received 12 April 2013
Accepted 30 June 2013
Available online 5 July 2013

Keywords:
Biomarkers
Dermal
Ethylenethiourea
High-throughput
LC/MS/MS
Urine

ABSTRACT

Ethylenethiourea (ETU) is of major toxicological concern, since in experimental animal studies, ETU has shown a large spectrum of adverse effects. High occupational exposure can be found among agricultural workers or during manufacturing of ethylenbisdithiocarbamates (EBDC). For the general public, sources of environmental exposure may be residues of ETU in commercial products, food and beverages. For the determination of ETU in human urine we present a high-throughput online on-column extraction liquid chromatography triple quadrupole mass spectrometry method using direct injection of hydrolysed urine samples. This method is simple, user- and environmentally friendly and all sample preparation is performed in 96-well plates. A labelled ETU internal standard was used for quantification. The method showed a good sensitivity with a limit of quantification (LOQ) of 0.5 ng ETU/mL urine and the calibration curve was linear in the range 0.25–200 ng ETU/mL urine. The within-run, between-run and between-batch precision was between 6% and 13%. Alkaline hydrolysis considerably increased the levels of ETU indicating a potential conjugate. The method was applied in an experimental dermal exposure study in humans, with sample concentrations ranging from 0.4 to 5.0 ng ETU/mL urine. The excretion in urine was 10% of the applied dose. The elimination profile seemed to differ between the two individuals. The results show an estimated half-life of ETU between 34 and 72 h. Although the experiment is limited to two individuals, the data provide valuable and new information regarding the toxicokinetics of ETU after dermal exposure.

© 2013 The Authors. Published by Elsevier B.V. All rights reserved.

1. Introduction

Ethylenbisdithiocarbamates (EBDCs) is a group of fungicides of which ethylenethiourea (ETU) is an environmental degradation product as well as a metabolite and impurity. Potential sources of ETU exposure to humans may be occupational or environmental. Occupational exposure may be high among agricultural workers and among workers manufacturing EBDCs [1–9]. Further, ETU is used as a vulcanization agent in the production of polychloroprene (neoprene) and polyacrylate rubbers and in several other products such as dyes. In the general population, residues of ETU in products can be one of many sources of environmental exposure to ETU. Associations have been observed between ETU, and smoking, wine drinking and consumption of fruit and vegetables [4,10]. ETU is of major toxicological concern. In animal studies, ETU has caused a large spectrum of adverse effects, mainly concerning mutagenic, teratogenic, carcinogenic and hepatogenic effects [11–13]. However, the evidence for such effects in humans is less well founded. ETU has been classified to be "reasonably anticipated to be a human carcinogen" based on sufficient evidence of carcinogenicity from animal experiments [14]. Some data suggest that ETU affects the lymphocyte genome and the thyroid gland among heavily exposed workers [1,2,15]. Both for EBDCs and ETU, there is a need for large scale epidemiological studies of exposure-effect relationships. In such studies, an accurate exposure assessment is required. Biomarkers have many advantages in comparison with other methods of exposure assessment, but reliable analytical methods are needed. Many analytical methods for measurement of ETU in biological samples have been presented [16]. Several mass spectrometry (MS) based analytical methods for quantification of the low levels of ETU present in human urine after occupational or environmental exposures, using gas chromatography/mass

Abbreviations: ADI, acceptable daily intake; b.w., body weight; CID, collision induced dissociation; EBDC, ethylenbisdithiocarbamates; ETU, ethylenethiourea; IS, internal standard; LC/MS/MS, liquid chromatography triple quadrupole mass spectrometry; LOD, limit of detection; LOQ, limit of quantification; QC, quality control.

☆ This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

* Corresponding author. Tel.: +46 46 2221645; fax: +46 46 173180.
E-mail address: eva.ekman@med.lu.se (E. Ekman).

1570-0232/$ – see front matter © 2013 The Authors. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jchromb.2013.06.035

spectrometry (GC/MS) [17], and liquid chromatography/tandem mass spectrometry (LC/MS/MS) have been developed [18–21]. However, these methods are all laborious, time-consuming and not suitable for epidemiological studies.

Dermal exposure is thought to play an important role in the risk assessment of pesticides [22]. However, the knowledge of dermal exposure to pesticides is limited. Moreover, there is only little knowledge of exposure assessment techniques for quantification of dermal exposure. For data of biomarker levels to be useful, supporting toxicokinetic data are needed. Knowledge of the parent compound and the major metabolites excreted in urine, including the excretion half-life, is important [23]. In guinea pigs, dermally exposed to ETU, 14% of the applied dose was absorbed after 24 h [24]. There are several occupational studies addressing dermal EBDC and ETU exposure. Dermal exposure to ETU is related to the presence of ETU as contaminant in EBDC-based formulation, or in re-entry workers that come in contact with treated crops where EBDC degradation products may be present. In studies of agricultural workers exposed to EBDC, dermal exposure to ETU was measured using filter pads [8,25]. A significant correlation was found between end-shift ETU levels in urine and the measured levels on pads [25]. This result is in agreement with a study of workers at a production plant where ETU levels in urine correlated with EBDC contamination on the hands [5]. Earlier, no studies of experimental dermal exposure to ETU have been performed in humans. On the other hand very few pesticide dermal exposure studies have been performed in humans [22,26–29].

The aim of this study was to develop a high-throughput online on-column extraction LC/MS/MS method for analysis of ETU in human urine. The method was applied in a human experimental dermal exposure study of ETU.

2. Materials and methods

2.1. Chemicals and materials

The internal standard (IS) [$^2H_4$]-ETU was purchased from Dr. Ehrenstorfer (Augsburg, Germany). Hexane was from Lab-Scan (Dublin, Ireland). Sodium hydroxide (NaOH), hydrochloric acid (HCl) and methanol (hyper grade for LC–MS) were from Merck (Darmstadt, Germany). ETU was a PESTANAL® analytical standard, formic acid (FA), pentafluorobencyl bromide (PFBr) and tetrabutylammonium hydrogen sulphate (TBA) were from Sigma–Aldrich Inc. (St. Louis, MO, USA). Purified water from a Millie-Q Integral 5 system (Millipore, Billerica, MA, USA) was used.

Plastic 96-well-plates SQW block with clear glass insert vials 1.5 mL, SQW 45 × 7.7 mm, sealed with a welled sealmat block cover, was from La-Pha-Pack®GmbH (Langerwehe, Germany) and 96-well-aluminium plates with1.5 mL aluminium block cover, was from J.G. Finneran Associates, Ltd (Surrey, United Kingdom).

2.2. Instrumentation

Quantitative analysis was conducted using a triple quadrupole linear ion trap mass spectrometer, equipped with TurboIonSpray source (QTRAP 5500; AB Sciex, Foster City, CA, USA) coupled to a liquid chromatography system with four pumps (UFLC^{RX}, Shimadzu Corporation, Kyoto, Japan). The MS/MS analyses were carried out using selected reaction monitoring (SRM) in positive atmospheric pressure chemical ionization (APCI) mode. Air was used as nebulizer spraying gas. Pure nitrogen was used as curtain gas and collision gas. The APCI temperature was set at 450 °C. The instrument was tuned to a peak resolution of 0.5 ± 0.1 Da at half the peak height in high resolution mode. To establish the appropriate SRM conditions, standard solutions were infused into the MS/MS for optimization. Collision-induced dissociation (CID) of each $[M+H]^+$ was performed and the product ions giving the best signal to noise ratio were selected for the SRM analysis. All data acquisition and processing was performed using the Analyst 1.5.1 application software (Applied Biosystems, Foster City, CA, USA).

2.3. Preparation of calibration standards, quality control and samples

Stock solutions were prepared in duplicates by dissolving accurately weighed amounts of [$^2H_4$] ETU (IS) and ETU in methanol. The IS and ETU standard stock solutions were diluted further in methanol and stored at −20 °C. Urine samples for the calibration standards and for quality control samples were obtained from healthy volunteers at our laboratory.

For the calibration curve, 475 μL blank urine was spiked with 25 μL of the standard solutions and 25 μL of the IS solution, giving a urinary concentration between 0.25 and 200 ng ETU/mL and 5 ng [$^2H_4$] ETU/mL urine. The calibration curve was corrected with the amount found in the urine. As quality control (QC) urine samples naturally containing 2 and 7 ng ETU/mL and the 7 ng/mL QC spiked to 32 ng/mL were used. The low, medium and high QC-samples were divided into several aliquots and stored at −20 °C. The chemical blank was prepared from Millie-Q water and thereafter treated like the other samples. The urine samples and QC-samples were vortex-mixed after thawing and aliquots of 500 μL were transferred into 1.5 mL glass vials and placed in an aluminium 96-well plate, and 25 μL of IS solution was added.

For the hydrolysis, 20 μL of 2.5 M NaOH was added to the samples, standards, QC-samples and chemical blanks, giving a final concentration of 0.09 M NaOH. To prevent evaporation during hydrolysis the glass-vials were sealed with a sealmat and a cover was screwed on. After sealing, the samples were mixed thoroughly for 1 min and then transferred to a heating oven. Hydrolysis was performed for 1 h at 100 °C. The aluminium 96-well-plates was used in the hydrolysis step, because of the possibility to secure the cover of the vials with four screws and the capacity of fast temperature transfer throughout the plate. After hydrolysis, the samples were cooled to room temperature and the glass vials were moved to plastic 96-well-plates compatible with the autosampler. Aliquots of 15 μL of 5 M HCl were added to acidify the samples. The samples were mixed thoroughly and centrifuged for 10 min at 2600 × *g* before analysis.

2.4. Analysis

The two mobile phases used consisted of 0.1% (v/v) FA in water (mobile phase A) and 0.1% (v/v) FA in methanol (mobile phase B). The two dimensional separation was carried out, using two identical analytical columns Genesis® Lightn AQ (C18, 4.6 × 100 mm, 4 μm, Grace Vydac, Hesperia, CA, USA) and two sets of LC pumps, each set containing two pumps. The columns and LC pumps were connected through a diverter valve. An aliquot of 20 μL of the sample was injected on the first column and the separation was carried out by isocratic elution, using 100% mobile phase A and a flow rate of 0.7 mL/min. After 2.5 min, the diverter valve switched over and the effluent was diverted into the second column during 1 min and thereafter the diverter valve switched over again. The second set of pumps continued the isocratic elution of the analytes on the second column, using 100% mobile phase A. A diverter valve on the MS diverted the column effluent to the MS between 4.6–6.9 min. The first column was cleaned with 95% mobile phase B at a flow rate of 1.2 mL/min for 1 min, followed by equilibration with 100% mobile phase A for 2.5 min, during the time ETU was eluting on the second column. The second column was reconditioned with 95% mobile phase B for 0.5 min in the end of the analytical run and then

Download English Version:

https://daneshyari.com/en/article/7618278

Download Persian Version:

https://daneshyari.com/article/7618278

Daneshyari.com