Talanta 138 (2015) 231–239

Contents lists available at ScienceDirect

Talanta

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

Development of liquid chromatography methods coupled to mass spectrometry for the analysis of substances with a wide variety of polarity in meconium

Marie Meyer-Monath [a], Claudine Chatellier [a], Deirdre Cabooter [c], Florence Rouget [d,e,f], Isabelle Morel [b], Francois Lestremau [a,*]

[a] INERIS, Direction des Risques Chroniques, Parc Technologique ALATA – BP 2, 60550 Verneuil-en-Halatte, France
[b] Laboratoire de Toxicologie Biologique et Médico-légale, CHU and INSERM, 991, F-35033 Rennes, France
[c] KU Leuven, Department for Pharmaceutical and Pharmacological Sciences, Pharmaceutical Analysis, Herestraat 49, Leuven, Belgium
[d] Brittany Registry of Congenital Malformations, Rennes, France
[e] Department of Pediatrics, Rennes University Hospital, France
[f] National Institute for Health and Medical Research (Inserm) U1085, IRSET, Rennes, France

ARTICLE INFO

Article history:
Received 21 October 2014
Received in revised form
24 February 2015
Accepted 28 February 2015
Available online 19 March 2015

Keywords:
Reversed phase
Ion exchange
HILIC
LC/MS/MS
Meconium
Micropollutants

ABSTRACT

Meconium is the first fecal excretion of newborns. This complex accumulative matrix allows assessing the exposure of the fetus to xenobiotics during the last 6 months of pregnancy. To determine the eventual effect of fetal exposure to micropollutants in this matrix, robust and sensitive analytical methods must be developed. This article describes the method development of liquid chromatography methods coupled to triple quadrupole mass spectrometry for relevant pollutants. The 28 selected target compounds had different physico-chemical properties from very polar (glyphosate) to non-polar molecules (pyrethroids). Tests were performed with three different types of columns: reversed phase, ion exchange and HILIC. As a unique method could not be determined for the simultaneous analysis of all compounds, three columns were selected and suitable chromatographic methods were optimized. Similar results were noticed for the separation of the target compounds dissolved in either meconium extract or solvent for reversed phase and ion exchange columns. However, for HILIC, the matrix had a significant influence on the peak shape and robustness of the method. Finally, the analytical methods were applied to "real" meconium samples.

1. Introduction

Like the general population, pregnant women are commonly exposed to many environmental pollutants (pesticides, solvents, etc.) which have been determined harmful to humans due to their carcinogenic properties and/or their effects on reproduction. To determine the exposure to micropollutants of women during pregnancy, the Perturbateurs Endocriniens: Étude Longitudinale sur les Anomalies de la Grossesse, l'Infertilité et l'Enfance (PELAGIE) project, wherein maternal urine and cord blood samples were analyzed, was carried out in Brittany, France [1]. The results of this study identified some important compounds of interest, such as organophosphorus pesticides and dialkylphosphates (metabolites of organophosphorus) [2]. The constant exposure of pregnant women to these compounds could affect the development of the fetus and especially induce the production of congenital malformations. To determine if an association exists between congenital malformations and fetal exposure to organic pollutants, a new project, the PENEW project (Pregnancy Environment and NEWborn malformations), has recently been launched by the Registry of congenital malformations in Brittany, France. This project includes the study of compounds quantified in the PELAGIE project (organophosphorus pesticides) and other relevant molecules widely used in Brittany and suspected to be toxic for the fetus such as other pesticides (triazoles, pyrethroids, glyphosate, etc.) and VOCs; with some associated metabolites.

To evaluate direct fetal exposure to xenobiotics throughout pregnancy, fetal matrix was considered. Commonly used fetal matrices, such as cord blood, urine and newborn blood, only reflect the last days of exposure. Therefore, the meconium matrix was selected to represent a larger exposure window. Meconium is the first stool of a newborn. It starts forming during the 12th–13th week of gestation in intestinal compartments and accumulates until birth [3–5]. It is a very complex matrix [6] composed of water

* Corresponding author. Tel.: +33 3 44 55 65 20; fax: +33 3 44 55 68 72.
E-mail address: francois.lestremau@ineris.fr (F. Lestremau).

http://dx.doi.org/10.1016/j.talanta.2015.02.058
0039-9140/

(~70%) and lipophilic compounds (~30%): lipids, proteins, bile acids, enzymes, lanugo, etc. Usually, it is expelled by the newborn within 24 h after birth. The collection of meconium is non-intrusive for the newborn and simple, unlike some other fetal matrices (urine, blood).

Meconium was already used to quantify fetal exposure to drugs and metabolites [7,8], alcohol metabolites [9,10] or pesticides [11,12] with the analysis performed by liquid chromatography coupled with tandem mass spectrometry LC/MS/MS. Usually these analytical methods were focused only on restricted classes of compounds and eventually their metabolites [8,9,13]. To determine a possible link with malformations, the target compounds of the PENEW project regrouped a large variety of pollutants within several families of pesticides, their metabolites, VOCs [14] and metabolites. Compared to a previous study carried out by our group where the analysis of certain micropollutants was performed on a C_{18} column [12], the current list of compounds included additional pesticides (epoxiconazole, tebuconazole, 2, 4-D, etc.) and also, notably, substances with stronger polarity (glyphosate) or different polar functional groups (acid compounds with the metabolites of VOCs). As the considered molecules encompassed a much larger variety in physico-chemical properties, ranging from very polar to non-polar, the previously developed method was inadequate for the extended list of substances.

Analytical method development was therefore performed for the 28 substances in meconium. Extraction methods for these compounds have been investigated and described elsewhere [15]. The aim of the method development by LC/MS/MS was to obtain one or several methods suited for all target substances with acceptable retention times (not eluted in the void volume), peak shapes and sensitivity with the fewest constraints. Analysis time was also considered due to the large number of samples to be analyzed (around 235 meconium samples). Tests on several columns with different properties, buffer optimization and matrix effects are described in this article. Chromatographic tests with meconium matrix were carried out after the sample preparation optimized in this matrix [15]. The final analytical method was applied to real PENEW "cases".

2. Experimental

2.1. Chemicals and materials

o-Cresol (o-c, purity: 99.9%), hippuric acid (HA, 98.0%), 2-methylhippuric acid (2-MHA, 98.0%), 3-methylhippuric acid (3-MHA, 98.0%), 4-methylhippuric acid (4-MHA, 98.0%), phenylglyoxylic acid (PGA, 98.0%), S-phenylmercapturic acid (S-PMA, 99.0%), diethylthiophosphate (DETP, 98.0%), 2,4-dichlorophenoxyacetic acid (2,4-D, ≥98.0%), diazinon (purity: 98.3%), cypermethrin (98.5%), cyfluthrin (99.8%), deltamethrin (99.7%), clopyralid (99.3%), glyphosate (99.2%), tebuconazole (99.5%), propoxur (99.8%), dichlorvos (99.9%) and benzoic acid-d_5 (BA-d_5, 99.0%) were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). S-benzylmercapturic acid (S-BMA, purity: 98.0%) was obtained from Sigma-Aldrich (Milwaukee, WI, USA). Chlorpyrifos (purity: 99.9%), malathion (97.2%), epoxiconazole (99.2%) were purchased from Riedel-de Haën (Seelze, Germany). Dimethylthiophosphate (DMTP, 99.2%), dimethyldithiophosphate (DMDTP, 99.4%), 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid (DCCA, purity: ≥95.0%, at 100 μg mL^{-1} in acetonitrile), diethylthiophosphate-d_{10} (DETP-d_{10}, purity: 98.0%, at 100 μg mL^{-1} in methanol) were obtained from Cambridge Isotope Laboratories Inc. (Andover, MA, USA). Dimethylphosphate (DMP, 98.0%) was obtained from Acros Organics (Geel, Belgium). Permethrin (99.8%) was purchased from Ultra Scientific (Rhode Island, USA). Mandelic acid (MA, 99.0%) was obtained from ChromaDex (Irvine, CA, USA). Diethylphosphate (DEP, 99.5%) was obtained from Chem Service Inc. (West Chester, PA, USA). 3-(2,2-Dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (Br_2CA, 99.0%, at 10 μg mL^{-1} in methanol), diazinon-d_{10} (96.5%, at 100 μg mL^{-1} in acetonitrile), propoxur-d_3 (99.5%, at 100 μg mL^{-1} in acetonitrile), dichlorvos-d_6 (96.0%, at 100 μg mL^{-1} in cyclohexane), trans-cypermethrin-d_6 (98.5%, at 100 μg mL^{-1} in acetonitrile), chlorpyrifos-d_{10} (98.0%, at 100 μg mL^{-1} in acetonitrile), tebuconazole-d_6 (95.0%, at 100 μg mL^{-1} in acetonitrile), 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid-d_6 (DCCA-d_6, 96.0%, at 100 μg mL^{-1} in acetonitrile), 2,4-dichlorophenoxyacetic acid-d_3 (2,4-D-d_3, 97.0%, at 100 μg mL^{-1} in acetonitrile) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Hippuric acid-d_5 (HA-d_5, 98.0%) was obtained from Toronto Research Chemicals Inc. (North York, Canada).

Sodium acetate (NaOAc), ammonium hydroxide solution (NH_4OH) (99.9%), and LC–MS grade methanol (MeOH) and acetonitrile (ACN) (≥99.9%) were obtained from Sigma-Aldrich (Steinheim, Germany). Ammonium acetate (>98%) was obtained by Merck (Darmstadt, Germany). Ultrapure water was supplied by a Mili-Q water purifier system from Millipore (Bedford, MA, USA). Acetic acid (100%) and sodium sulfate anhydrous ($NaSO_4$) were purchased from VWR BDH Prolabo (Leuven, Belgium).

2.2. Meconium

For method development, a pool of meconium was obtained from meconium samples collected from autopsied fetus and autopsied newborns by the anatomical pathology service of the University Hospital of Rennes, France. When tested, some of these meconium did not contain any of the analytes. These "blank" meconium were pooled to form a representative pool of "blank" meconium for use in all subsequent experiments. Meconium samples were kept at −80 °C until analysis to prevent bacteriological developments.

2.3. Sample preparation

The extraction procedure of meconium specimens for quantification using the LC/MS/MS was the same as described previously [15].

Briefly, 1 g of meconium sample was diluted with 8 mL of water. Ammonium hydroxide and the internal standards were added to 6 mL of the diluted sample. After vortex and centrifugation, 4 mL of supernatant were filtered. To the remaining meconium phase, ACN, ammonium sulfate and ammonium acetate were added. The supernatant was evaporated at 500 μL. The two supernatants were pooled together before performing purification steps with a Bond Elut SAX cartridge (Agilent). The elutions of loading and washing steps of the Bond Elut SAX cartridge were purified with a Strata-X cartridge (Phenomenex). The elutions of the two SPE cartridges were pooled together before LC/MS/MS analysis.

Pyrethroids are not soluble in water. To avoid precipitation of pyrethroids or glyphosate in a unique solution, standard solutions were prepared in 100 μL of water/ACN (50/50, v/v) for analysis with Ascentis Express RP-Amide and Acclaim Trinity P1 columns (one injection of 10 μL for each column). For analysis with LUNA HILIC column, the 80 μL of remaining solvent (mixture of ACN and water) in vial were evaporated to dryness and reconstituted in 80 μL of ACN.

2.4. Liquid chromatography columns tests

The meconium and calibration samples were analyzed on an Acquity UPLC H-Class from Waters (Milford, MA, USA). Chromatographic development was accomplished with six columns: Ascentis Express C18, 150 × 2.1 mm, 2.7 μm from Supelco (Bellefonte, USA);