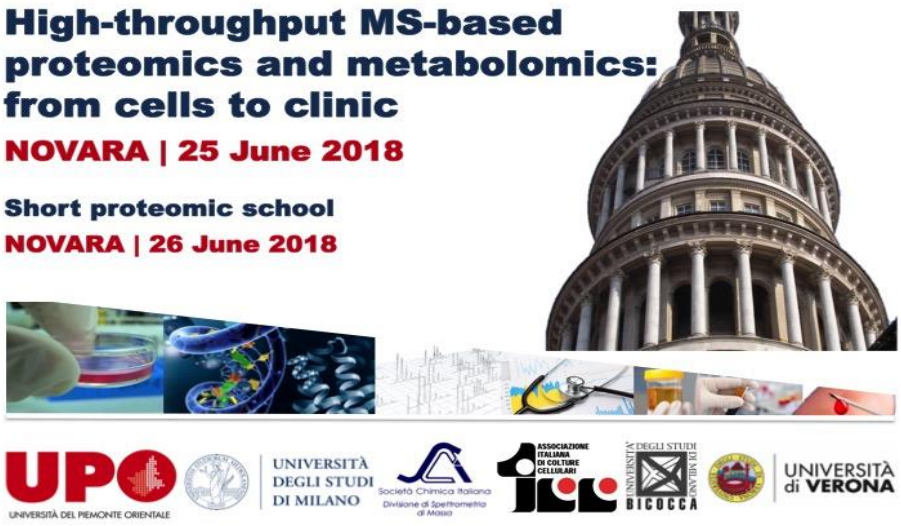

POSTER ABSTRACT SUBMISSION FORM

To be sent by *May 5, 2018* *to*

http://www.spettrometriadimassa.it/Congressi/proteomics-metabolomics-novara-june-2018/prot-met-novara_SubmissionForm.php

Presenting Author

Surname and name Frigerio Gianfranco		
Company or Organization Università degli Studi di Milano		
Address Via S. Barnaba, 8		*Zip code – Town – Country* 20122 - Milan - Italy
Telephone +39 0250320110	*Fax*	*E-mail* gianfranco.frigerio@unimi.it

Poster

Title Development, validation and application of an HPLC-MS/MS method to quantify urinary mercapturic acids
Authors Gianfranco Frigerio, Rosa Mercadante, Laura Campo, Elisa Polledri, Luca Olgiati, Pasquale Missineo, Luca Boniardi, Silvia Fustinoni
Company(es) or Organization(s) Università degli Studi di Milano

Development, validation and application of an HPLC-MS/MS method to quantify urinary mercapturic acids

Gianfranco Frigerio[1], Rosa Mercadante[1], Laura Campo[1], Elisa Polledri[1], Luca Olgiati[1], Pasquale Missineo[1], Luca Boniardi[1], Silvia Fustinoni[1]

[1] Laboratorio di Tossicologia Ambientale e Industriale, Dipartimento di Scienze Cliniche e di Comunità, Università degli Studi di Milano e Fondazione Cà Granda, IRCCS Ospedale Maggiore Policlinico, Via San Barnaba, 8, 20122 Milano

Summary: *In this work we present a method suitable to quantify 17 mercapturic acids, which are urinary metabolites of toxicants. This method was subjected to a complete validation and a first application was carried out on urine samples derived from petrochemical workers.*

Keywords: *Urinary metabolites, biomarkers, LC-MS/MS*

Introduction

Mercapturic acids are metabolic end products of some occupational and environmental toxicants such as volatile organic compounds. They are metabolites formed by the conjugation of an electrophilic compound with glutathione. These electrophilic metabolic intermediates are believed to be the active species able to react with DNA and responsible for the genotoxicity associated with parent compounds [1].

Mercapturates can be found in urine and, therefore, they can be considered useful non-invasive biomarkers of exposure. Although several analytical methods were reported for the analysis of single or small groups of mercapturates [2], only two papers describes the analysis of several mercapturates [3,4]. The aim of this work was to set up a LC-MS/MS method able to determine mercapturic acids derived from different toxicants.

Experimental

For the preparation of standard solution, the majority of standard compounds were purchased from Toronto Research Chemicals (Ontario, Canada), along with relative isotopically labelled standards. The complete list of analytes is reported in Table 1. The simple sample preparation developed includes dilution with formic acids (0.2 M), addition of an internal standard mixture of 16 deuterated analogs and filtration with 0.45 µm regenerated cellulose membrane filter (Agilent Technologies, Santa Clara, California).

Table 1. List of considered analytes

Investigated analytes	Parent compounds
2-HPMA	propylene oxide
3-HPMA	acrolein
AAMA	acrylamide
AMCC	N,N-dimethylformamide
CEMA	acrylonitrile
CMEMA	crotonaldehyde
EMA	ethylating agents
GAMA	acrylamide
HEMA	alkylating agents
HMPMA	crotonaldehyde
M1	1,3-butadiene
M2	1,3-butadiene
MMA	Methylating agents
NANPC	4-chloronitrobenze
PHEMA1+2	styrene
SBMA	toluene
SPMA	benzene

Analysis were carried out using a hybrid triple quadrupole/linear ion trap mass spectrometer (QTRAP 5500, AB Sciex, Monza, Italy) interfaced with an ultrahigh pressure liquid chromatograph (UHPLC, Agilent 1220, Cernusco sul Naviglio, Italy) equipped with a Betasil C18 column (150 x2.1 mm, 5 µm; Thermo Fisher Scientific, Rodano, Italy) and a pre-column BETASIL C18 (10 x 2,1 mm, 5µ; Thermo Fisher Scientific, Rodano, Italy). Chromatographic separation was performed using a linear gradient with an aqueous mobile

phase composed by an aqueous solution of ammonium formiate 5 mM and 0.1% formic acid and an organic mobile phase composed by acetonitrile.
A complete validation was carried out: linearity, sensitivity, accuracy, precision, selectivity, matrix effect, recovery and process efficiency were evaluated according to both FDA guidelines and the considerations reported in the review written by González and co-workers [5,6].
The method was then applied to the analysis of urine samples from adult subjects with different smoking habits: non-smokers, electronic cigarette smokers, and traditional tobacco smokers.

Results

Results from linearity assays showed that correlation coefficients (R^2) were close to 1 for most of compounds, demonstrating optimal linear responses for the considered concentrations ranges, although a polynomial regression was necessary for AAMA since it showed a saturation at high concentrations. Limits of quantitation (LOQ) values were between 0.15 and 1 µg/L, except for HEMA and AAMA (1.93 and 1.30 µg/L respectively). Precision, evaluated as relative standard deviations (RDS), was below 15% for most analytes in both intra-day and inter-day tests. Accuracy was between 85 and 110 % of expected values, with few exceptions exceeding 120% at the lowest concentrations. Selectivity was verified by injection of a blank sample (synthetic urine) showing no chromatographic peak having an area at 20% of LOQ at the relative retention time and mass transition of compounds of interest. The same condition was verified analysing a blank sample immediately after the injection of the standard mixture at the highest concentration of the calibration curve, indicating the absence of carry-over. Results from the matrix effect, recovery and process efficiency tests were suitable in most of the cases, with some exceptions that were partially corrected using the internal standards.
Results from urine samples of individuals with different smoking habit showed significant differences between smokers and non-smokers: 11 different mercapturic acids were significantly higher (P-value ≤0.005) in traditional tobacco smokers than in non-smokers (an example is illustrated in Figure 1).

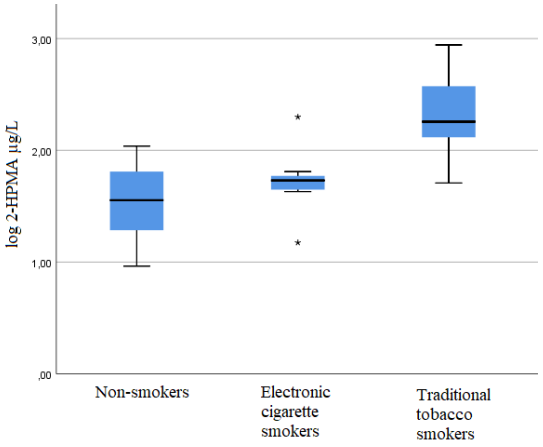

Fig. 1. Box-plot illustrating differences between non-smokers, electronic cigarette smokers and traditional tobacco smokers for 2-HPMA (mercapturic acid of propylene oxide).

Conclusion

In this work, we developed a method useful to quantify mercapturic acids in urine samples. The method was subjected to a complete validation and showed to be suitable for most of the considered analytes. Despite some critical issues with some analytes (in particular HEMA), it demonstrated to be an useful tool for fast determination of mercapturates. The first application carried out using human urine samples suggests that mercapturic acids are suitable biomarkers for toxicants in tobacco smoke.

References

1. B.M. De Rooij, J.N.M. Commandeur, N.P.E; *Biomarkers*, 3 (1998), pp 239-303.
2. P.I. Mathias, C. B'hymer; *Biomarkers*, 21 (2016), pp 293-315.
3. K.U. Alwis, B.C. Blount, A.S. Britt, D. Patel, D.L. Ashley; *Analytica Chimica Acta*, 750 (2012), pp 152-160.
4. N. Pluym, G. Gilch, G. Scherer, M. Scherer; *Analytical and Bioanalytical Chemistry*, 407 (2015), pp 5463-5476.
5. FDA. Guidance for Industry - Bioanalytical Method Validation. (2013) Available at: https://www.fda.gov/downloads/Drugs/Guidances/ucm368107.pdf
6. O. González, M.E. Blanco, G. Iriarte, L. Bartolomé, M.I. Maguregui, R.M. Alonso; *Journal of Chromatography A*, 1353 (2014), pp10-27.