MERCAPTURIC ACIDS AS BIOMARKERS FOR PROFILING HUMAN EXPOSURE TO ENVIRONMENTAL AND OCCUPATIONAL POLLUTANTS

R. Mercadante, G. Frigerio, E. Polledri, L. Campo, S. Fustinoni

EPIGET - Epidemiology, Epigenetics, and Toxicology Lab, Department of Clinical Sciences and Community Health, Università degli Studi di Milano, and Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy Italy

Background: Mercapturic acids (MA) are final urinary metabolic products of some environmental and occupational toxicants, formed by the conjugation of glutathione and electrophilic compounds. These electrophilic compounds are believed to be active species able to react with DNA and responsible for the genotoxicity associated with parent compounds. The aim of this work was the development and validation of an analytical assay for the determination of several urinary mercapturic acids to be used as biomarkers for profiling human exposure.

Method: An isotope dilution tandem mass spectrometry method, coupled with reversed-phase liquid chromatography, was developed for the analysis of eighteen urinary MA from acrolein (3-HPMA), acrylamide (AAMA, GAMA), acrylonitrile (CEMA, HEMA), benzene (SPMA), 1,3-butadiene (DHBMA, MHBMA), crotonaldehyde (CMEMA, HMPMA), 4-chloronitrobenzene (NANPC), N,N-dimethylformamide (AMCC), ethylene oxide (HEMA), propylene oxide (2-HPMA), styrene (PHEMA1 e 2), toluene (SBMA), methylating (MMA) and ethylating agents (EMA). Samples were prepared by simple filtration after dilution. Calibration curves, sensitivity, accuracy, precision, selectivity, process efficiency, and stability were evaluated for method validation. Moreover an external validation was performed. The assay was applied to the analysis of 46 end of shift urine samples from non-smoker workers of 6 different workplaces: refinery workers, coke oven workers, traffic policemen, rotogravure printing workers, gasoline station attendants, asphalt workers, and workers not occupationally exposed to chemicals.

Result: Limits of quantitation ranged from 0.01 to 3.2 µg/L, precision assays yielded optimal values for all compounds, with all RSDs < 10%, and accuracy values ranging from 93.4% to 114.9% of theoretical value. The use of deuterated internal standards was suitable to control for matrix effect. The assay allowed the simultaneous quantitation of urinary mercapturic acids at different ranges of concentration. The external validation exercise was positive for all compounds. The application of the assay to urine samples of workers highlighted differences in mercapturic acid profiles among groups for: CEMA, DHBMA, MHBMA, PHEMA and SPMA, in agreement with the expected patterns of exposure.

Conclusion: This high-throughput method is a valid and useful tool for the determination of urinary mercapturic acids, suitable for human biomonitoring of occupational and environmental exposure.