Títle: A BTEX MULTIANALYTE VALIDATED HPLC-DAD METHOD

Authors: Ana Paula Sousa Macedo[1]; Vanessa Emídio Dabkiewicz[1]; Beatriz Campos Senna da Cruz[1]; Letícia Silva Braga Pereira[1,2]; Jaqueline Vitória do Amaral Melo[1,2]; Liliane Reis Teixeira[1]; Thelma Pavesi[1]

1 Center for the Study of Workers' Health and Human Ecology (CESTEH), National School of Public Health Sergio Arouca (ENSP)/ Oswaldo Cruz Foundation (FIOCRUZ), Rio de Janeiro, RJ

2 Federal University of Rio de Janeiro (UFRJ), Rio de Janeiro, RJ

INTRODUCTION: The volatile organic compounds benzene, toluene, ethylbenzene, and xylene isomers (BTEXs) are oil-derived substances with toxicity well-established. BTEXs present high neurotoxicity due to lipid tissues affinity. Benzene is a carcinogenic and myelotoxic agent. Toluene and ethylbenzene have been associated with hearing loss, while xylenes are irritants of the respiratory system. BTEXs are also linked to tissue inflammation, oxidative stress, and impaired immune function. Workers in the oil and gas industry are among the most affected, as they frequently handle crude oil and its derivatives (natural sources of BTEXs). **OBJECTIVE:** To optimize and validate a chromatographic method for the simultaneous determination of 9 BTEX exposure biomarkers, allowing the exposure assessment for more than one BTEX compound in one single analysis. **METHOD:** The analysis employed high-performance liquid chromatography (HPLC) with a diode array detector (DAD), a C18 column and gradient mobile phase. Method validation followed the guidelines for analytical method validation (DOQ-CGCRE-008/2020) established by INMETRO. The method optimization included a sample preparation study using solid phase extraction (SPE) C18 with different carbon content. **RESULTS AND CONCLUSION:** The validated multi-analyte method was suitable for the intended purpose. Evaluated exposure biomarkers were the following: for benzene, trans,trans-muconic acid (t,t-MA) and S-phenylmercapturic acid (SPMA); for toluene, hippuric acid (HA) and benzylmercapturic acid (SBMA); for ethylbenzene, phenylglyoxylic acid (PGA) and mandelic acid (MA); and for xylenes, the sum of 2-, 3-, and 4-methylhippuric acids (2-MHA, 3-MHA, and 4-MHA). Only SPE carbon content of 14% presented adequate extraction for all analytes. Due to observed matrix effects, validation parameters (linearity, linear working range, limits of detection and quantification, recovery, precision, and sensitivity) were evaluated using urine matrix. All analytes presented linearity curves, recoveries ranging from 80% to 110%, and both repeatability and intermediate precision with HORRAT values < 2. Detection and quantification limits were adequate to the measurement of the BTEXs latest established limits of the Brazilian law of the occupational health medical control program (PCMSO) and the American Conference of Governmental and Industrial Hygienists (ACGIH). The validated method was successfully applied in a preliminary exposure assessment of 10 oil and gas industry workers.

Keywords: BTEXs Exposure; Biological Assessment; Occupational Health.