IMPLEMENTATION, VALIDATION AND ANALYSIS OF THE PHENYLMERCAPTURIC ACID EXPOSURE BIOMARKER: DETERMINATION IN URINE OF GASOLINE EXPOSED FUEL POST WORKERS

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INTRODUCTION

According to the International Agency for Research on Cancer (IARC) about two million workers are constantly exposed to benzene, one of the toxic chemicals most present in industrial processes and in gasoline, so it is a Group 1 carcinogen.. Benzene workers in their daily activities is called occupational exposure and includes activities ranging from their synthesis process to activities that can release gases and vapors as fugitive emissions. The biological exposure indicator for monitoring worker exposures to regulated benzene through Portaria 34 of 12/20/2001 of MTE is trans-trans-muconic acid (ATTM). However, altered levels of ATTM may also be related to eating habits, since some foods containing sorbic acid generate a multitude of metabolites, including trans-trans-muconic acid. To overcome this limitation, it has been proposed to monitor these workers exposed to benzene through the determination of phenylmercapturic acid (AFM), a benzene-specific metabolite that does not suffer interference from ingested foods, even for exposures to benzene occurring in concentrations below 0.5 ppm.

OBJECTIVE

To implement, standardize and validate the methodology for the quantification of phenylmercapturic acid and to evaluate the level of exposure of workers from resellers of fuels in the city of Rio de Janeiro to the solvents present in gasoline through the use of biomarkers of exposure, trans-trans muconic acid (ATTM) and phenylmethyl mercapuric acid (AFM), in order to quantify and compare the presence of the two benzene metabolites in the workers' urine and thus characterize the risks related to the occupational exposure of these workers.

METHODOLOGY

EPIDEMIOLOGICAL STUDY

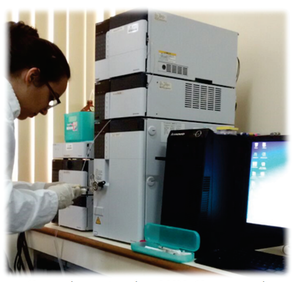
This is a cross-sectional epidemiological study of gas station workers located in the city of Rio de Janeiro (Zona Sul and Centro/RJ). The workers (exposed and control group) recruited formalized their acceptance by signing the free informed consent form and responding to two questionnaires (individual and clinical) with questions related to socio-demographic variables, activities performed, working hours, exposure of substances Chemicals, signs, symptoms, previous and life history.

GROUP OF EXPOSED WORKERS:
Fuel station workers
Working time ≥ 6 months
GROUP OF WORKERS NOT EXPOSED (CONTROL):
Office workers, that is, not occupationally exposed to solvents.

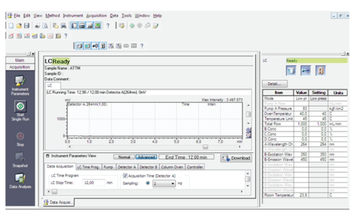
INCLUSION CRITERIA:
Age ≥ 18 years
Resident of the City of Rio de Janeiro;
Last image examination ≥3 months.
EXCLUSION CRITERIA:
Behavioral problems such as alcoholism, aggression and mental problems.

EVALUATION OF EXHIBITION BIOMARKERS

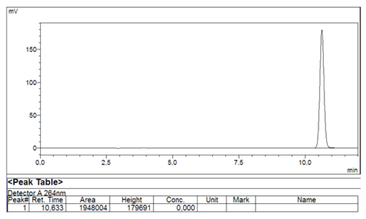
Urinary levels of ATTM were determined. The AFM assessment technique is in the implementation phase. Parameters for a determination of ATTM are described in TABLE 1 and its methodology involves a continuous phase extraction (SPE) stage. The details of the AFM evaluation technique are presented in TABLE 2 and the analytical method for its determination included five steps of preparation: acidification, AFM SPE present in the urine; Concentration of analyte at 60ºC per nitrogen flow; Alkaline hydrolysis and derivatization As urine samples collected after the end of the workday with the Environmental Mutagenesis Laboratory (LMA) where urine creatinine testes were performed using the Modified Jaffé Method. For an evaluation of ATTM and urinary AFM, how samples were processed and injected into HPLC, according to the scheme below.



Sample entry by HPLC manual injection



HPLC in operation, LabSolutions® integration software panel



Chromatogram indicating the peak and retention time of ATTM, for example, on the calibration curve.

Figure 5: Until, now we identified in 5 HSCT HLA- identical patients and 1 patient who received an Haplo-identical HSCT that NK cells at the D+30 post-HSCT were increased while CD4+T cells were decreased. Additionality, there were an absence of B cells in both HLA-identical and Haplo-identical HSCT patients

Table 1: Chromatographic Conditions (ATTM)

PARAMETERS	SPECIFICATION
SPE	(SAX), 500 mg/3mL
Column	Phenomenex® 5µ C18(250x4,6mm)
Column temperature	40°C
Mobile Phase	1% acetic acid / methanol (pH 2,72)
Wavelength (λ)	264 nm
Pumping	Isocratic
Race time	13 min
Flow	1,0 mL/min
Detection	UV

Table 2: Chromatographic conditions (AFM)

PARAMETERS	SPECIFICATION
SPE	(C18), 500 mg/3mL
Column	Reverse Phase
Column temperature	35°C
Mobile Phase	Acetronile/ 0,5% acetic acid
Wavelength (λ)	375 nm (excitation) 480 nm (emission)
Pumping	Isocratic
Race time	15 min
Flow	2,0 ml/min
Detection	Fluorimetry

PARTIAL RESULTS

- **ATTM laboratory analyzes**

In total, 436 urine samples were analyzed in the laboratory, of which 118 were from the control group and 317 from occupationally exposed workers. For each urine sample the following analyzes were performed: urinary creatinine and ATTM. Acocording to table 3, t can be noticed that the mean (ATTM) of the occupationally exposed workers was practically double (0.29 mg/g creatinine) of the average found in the control group (0.15 mg/g creatinine).

Table 3 - Urinary ATTM analysis (non-exposed control group and occupationally exposed group).

	Control (n)	Exposed(n)
Numeric values	118	317
Mean	**0,15**	**0,29**
(IC 95%)	(0,08-0,22)	(0,22-0,36)
Median	**0,05**	**0,09**
Standard error	0,03	0,03
Shapiro-Wilk (Normality test)	Não	Não
P value	< 0,0001	< 0,0001

Observing the percentage of compliance for the established reference values for ATTM (0.5mg ATTM / g creatinine), it was possible to notice that 2.5% (three people) of the non-exposed workers had ATTM value above that recommended in Ordinance 34/2001, compared to 15.8% of changes observed in the occupationally exposed group (50/317), as observed in table 4.

Table 4 - comparison of ATTM values obtained in the groups.

GROUP CONTROL			EXPOSED GROUP		
	N=	%		N=	%
ATTM ≤ 0,1	78	66,10	ATTM ≤ 0,1	168	53,0
0,1 < ATTM ≤ 0,2	17	14,41	0,1 < ATTM ≤ 0,2	50	15,8
0,2 < ATTM ≤ 0,3	10	8,47	0,2 < ATTM ≤ 0,3	32	10,1
0,3 < ATTM ≤ 0,4	5	4,24	0,3 < ATTM ≤ 0,4	15	4,7
0,4 < ATTM ≤ 0,5	5	4,24	0,4 < ATTM ≤ 0,5	2	0,6
ATTM > 0,5	3	2,54	ATTM > 0,5	50	15,8
TOTAL	118	100	TOTAL	317	100

- **AFM Validation: Mobile phase calibration curve**

The mobile phase calibration curve (acetonitrile-acetic acid 0.5% (50:50 v / v)) was the first step developed in the design for the identification of the characteristic AFM peak and the retention time in the chromatogram. The curve was constructed with 9 points (10 µg / L, 20 µg / L, 30 µg / L, 70 µg / L, 270 µg / L, 550 µg / L, 1100 µg / L, Without samples passing through the stages of the technique (Figure 1).

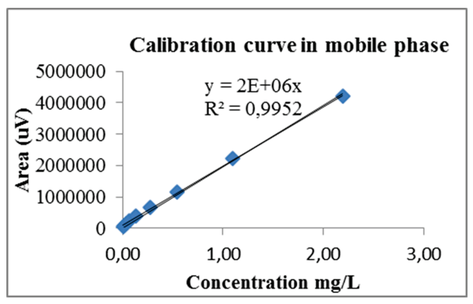


Figure 1 - Calibration curve in mobile phase, relating the area of the peak found in a certain concentration.

- **AFM validation: calibration curve in biological matrix**

The analytical curve is being constructed with six points (10 µg / L; 20 µg / L; 40 µg / L ; 60 µg / L, 80 µg / L and 100 µg / L) for the quantification of AFM in the urine samples, the construction of the calibration curve is still in progress using the matrix matching method. This method consists in adding known amounts of the AFM standard solution in the biological matrix; For this, known volumes of the standard AFM intermediate solution at 25 µg / mL were transferred to 25 mL volumetric flasks containing urine pool. According to the chromatograms obtained, the retention time of the analyte was approximately 9 minutes (Figure 2).

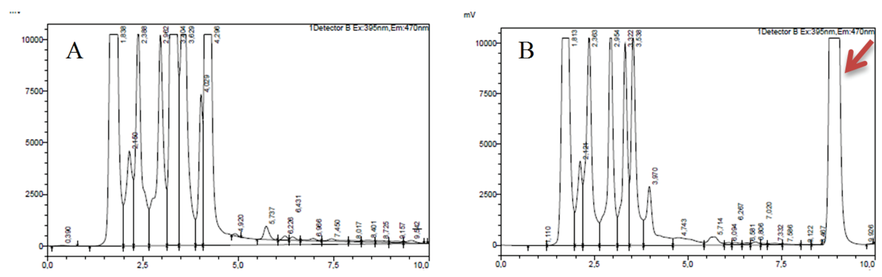


Figure 2 - chromatograms obtained, where A) characteristic peaks of the compounds present in the biological matrix without addition of devivatizante and buffer; B) urine + derivatizing + buffer + AFM standard (retention time ~ 9,0 min).

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