ANALYSIS OF BENZENE EXPOSURE BIOMARKERS: TRANS, TRANS-MUCONIC ACID AND PHENYLMERCAPTURIC ACID

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Financial Support: PPSUS/Faperj; OPAS; INCA/MS.

INTRODUCTION

Most cancer cases are related to exposure to carcinogenic agents such as benzene (IARC, Group I) present in gasoline. 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 smoking and 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. The objective of the present study was to perform AttM analyzes on urine samples from workers at gas stations in the city of Rio de Janeiro and to implement, standardize and validate the AFM biomarker technique.

MATERIALS AND METHODS

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.

Urine samples were collected after the end of the workday and sent to the Laboratory of Environmental Mutagenesis (LMA), where urine creatinine tests were performed using the Jaffé Modified method. Urinary levels of AttM were determined by the parameters described in TABLE 1 and the methodology involving the solid phase extraction (SPE) process and HPLC analysis with UV detector. Urinary levels of AFM were determined by the parameters described in TABLE 2 and the methodology for evaluating the implemented, validated and standardized AFM includes five stages of preparation: acidification of urine; SPE; Concentration of analyte at 60ºC by nitrogen flow; Alkaline hydrolysis and derivatization and HPLC analysis with fluorescence detector.

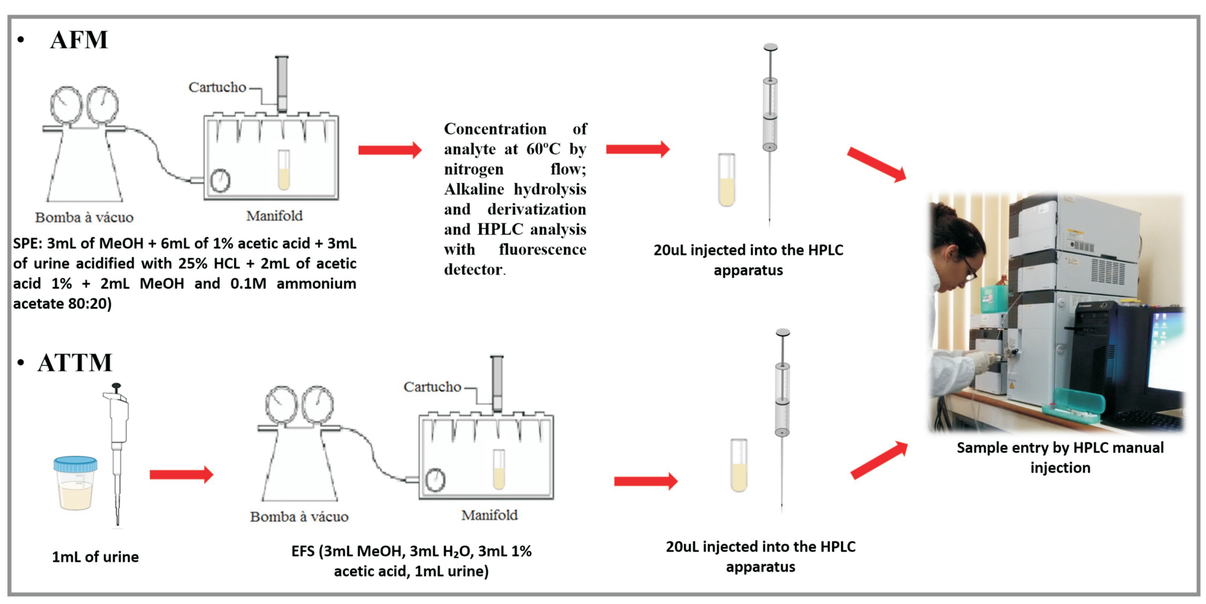

Table 1: Chromatographic conditions (AttM)

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 (ʎ)	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 (ʎ)	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	N=	%	EXPOSED GROUP	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: calibration curve in biological matrix

The AFM methodology was implemented, standardized and validated, with the calibration curve in biological matrix (urine) made in 6 concentrations (10, 20, 40, 80, 160 and 200 µg/L), obtaining an R^2=0.9958 (FIGURE 1).

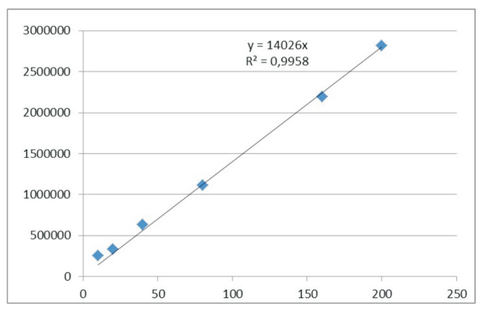

Figure 1: AFM calibration curve in biological matrix (urine)

As observed in the following chromatogram (FIGURE 2) the retention time of the compound was approximately 7.5 min.

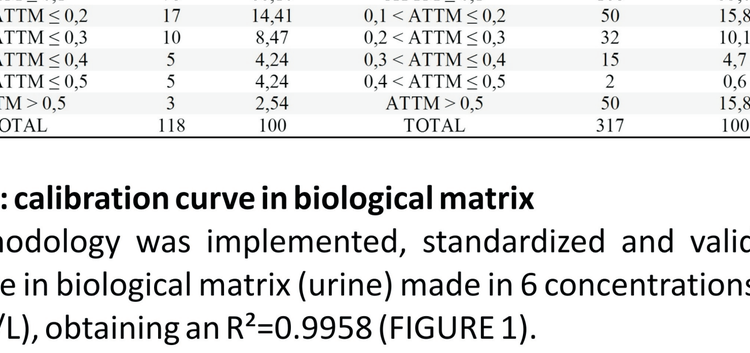

Figure 2: Chromatogram of AFM

CONCLUSION

In relation to AttM, it was observed that exposed workers obtained higher levels of urinary AttM than the control group and that smoking influences the levels of this biomarker. It was not possible to compare urinary AttM levels with AFM, because the study is in the collection phase for determination of the latter. It is concluded that the implementation, standardization and validation of the AFM methodology was performed and finalized.

Projeto Gráfico: Área de Edição e Produção de Materiais Tecnico-Científicos/INCA