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

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INTRODUCTION

Currently, in Brazil, there are about 59,000 fuel stations, 1,368 of which are located in the city of Rio de Janeiro, where they operate under risk conditions, further exposing workers. Benzene present in gasoline is classified as carcinogenic to humans (IARC, Group 1), being capable of causing genetic alterations, hematological alterations and medullary aplasia. In Brazil, Portaria 34 (MTE - 12/20/01) establishes the monitoring of workers exposure to benzene through the analysis of trans-trans-muconic acid (AttM) in the urine, which acts as a biological indicator of exposure. However, the AttM is influenced by some factors that can change its concentration in the urine, such as the diet containing sorbic acid and smoking. Due to these limitations, monitoring by phenylmercaptoric acid (AFM), a very specific biomarker, even for exposure to benzene occurs at concentrations below 0.5 ppm, since it is not influenced by other factors such as AttM .

The study aimed to validate the technique for AFM analysis; determine AFM in gas station workers and in non-exposed workers; determine the AttM of the same study group and compare it with the AFM results.

MATERIAL AND METHODS

- **Collection of data and samples**

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) Biological samples (urine) were collected in plastic containers, homogenized and filtered. They were then transferred to Falcon tubes with a capacity of 50 mL, capped and stored under refrigeration at -20 °C.

- **AFM implementation, validation and analysis**

The technique was implemented and validated in mobile phase (acetonitrile-acetic acid 0.5% (50:50 v/v) and biological matrix (urine pool). The calibration curve in the mobile phase was defined by the preparation of a solution the AFM model in the mobile phase and serial dilutions were made at 10, 20, 30, 70, 140, 270, 550, 1100 and 2190 µg/L. In relation to the calibration curve in urine, the pool was prepared with urine samples of both sexes (n=20). The samples were homogenized and filtered, and later enriched with standard solutions of the biomarkers analyzed in different concentrations 10; 20; 40; 80; 160 and 200 µg/L. After preparation of all samples, aliquots were injected into HPLC coupled with fluorescence detector.

- **Validation and analysis of AttM**

The validation of the technique for the determination of AttM was performed by determining the calibration curve in urine made from a standard stock solution of AttM in methanol (100 µg/mL), which was used for the preparation of intermediate solutions in urine, at concentrations 7.5; 75; 750; 1250 and 2500 µg/L. After preparation of all the samples, aliquots were injected into the HPLC coupled with UV/visible detector.
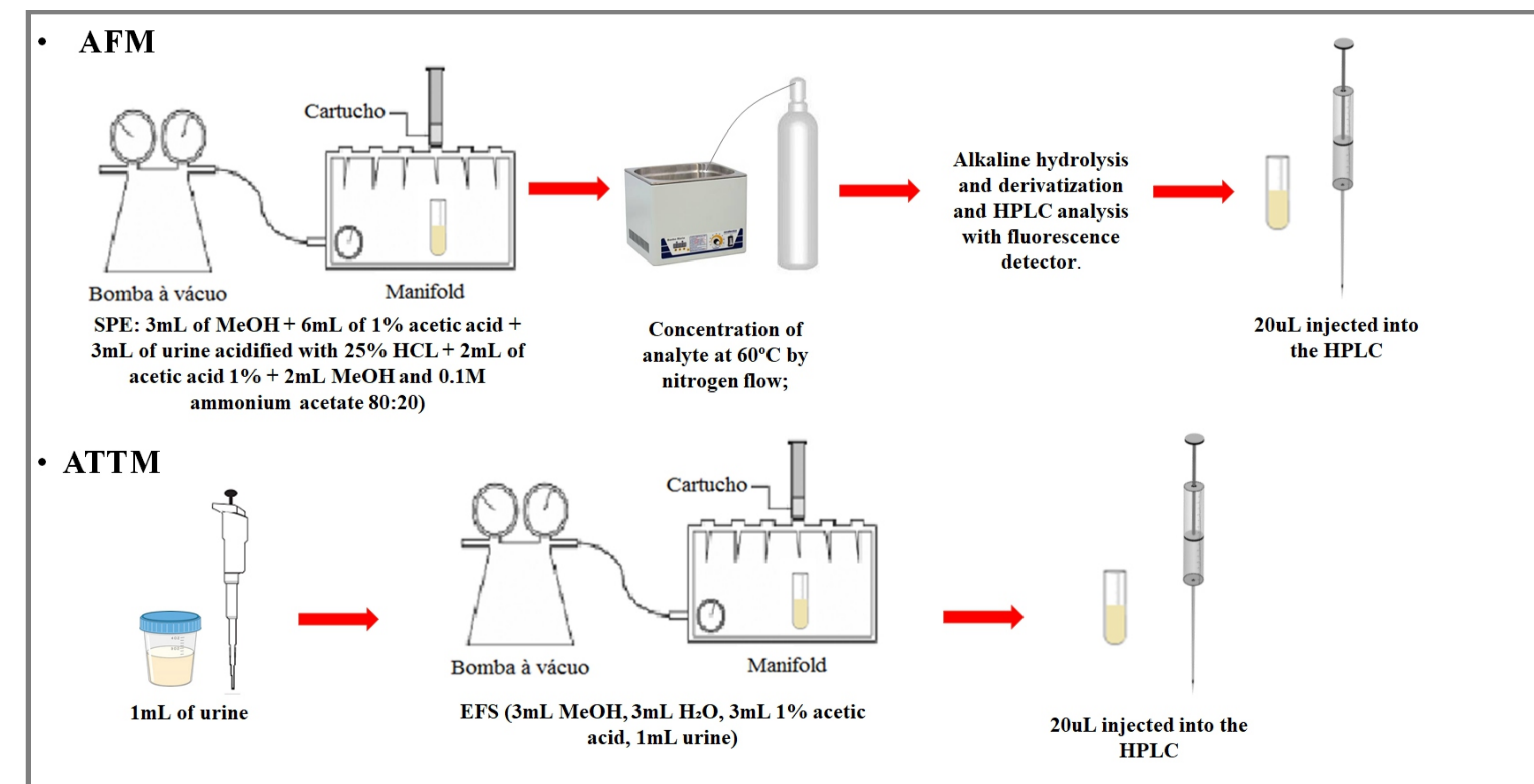
PARTIAL RESULTS

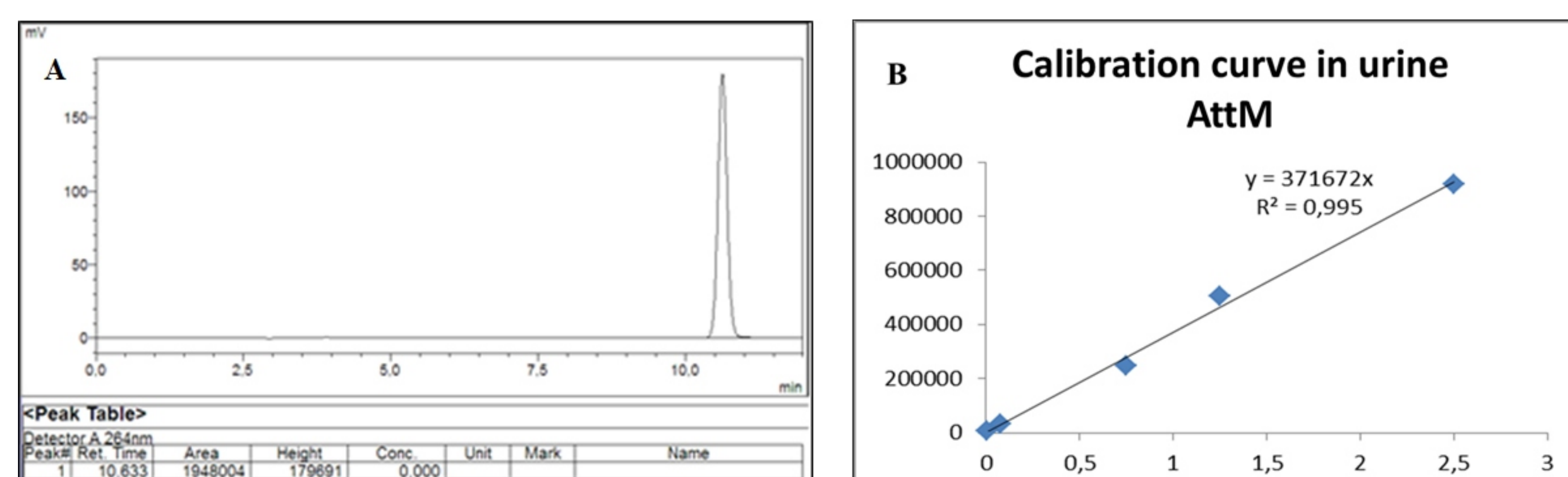- **AttM validation: calibration curve in urine**

FIGURE 1. (A) Chromatogram obtained during one of the chromatographic runs, where the pattern can be observed at the peak obtained; (B) AttM calibration curve.

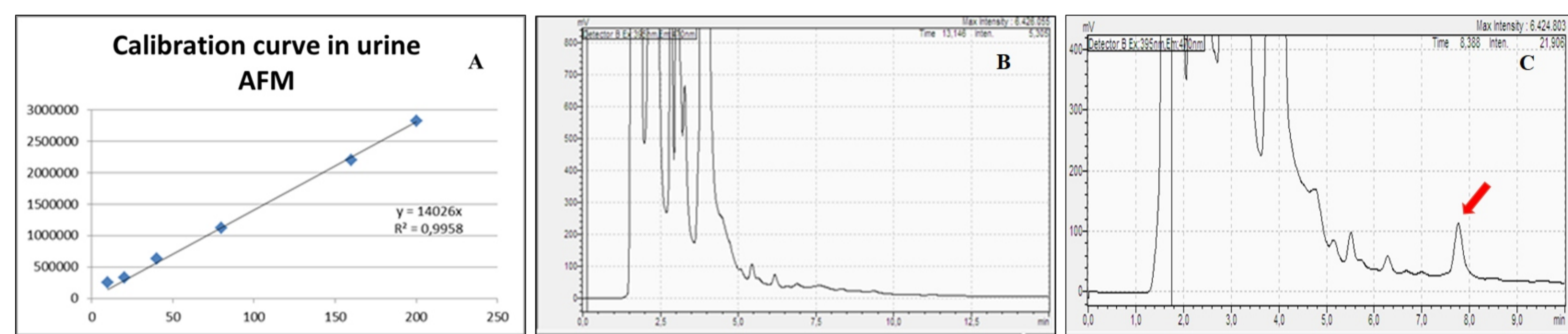- **AFM validation: calibration curve in biological matrix**

FIGURE 2. (A) AFM calibration curve in urine; (B) Chromatogram with characteristic peaks of the compounds present in the biological matrix without addition of AFM; (C) Chromatogram with characteristic peaks of the compounds present in the biological matrix with addition of AFM (retention time =7.7 min).

- **Analysis of AFM and urinary AttM of samples**

For each urine sample the following analyzes were performed: urinary creatinine, trans, trans-muconic acid and phenylmercaptúric acid. Up to the present moment, 11 samples from the occupationally exposed group to benzene and 19 samples from the control group (not occupationally exposed to benzene).

According to Table 1, after the analysis of the urinary AttM of the 11 workers exposed in 2018, it was observed that no worker presented values above the Biological Limit established for the AttM by Portaria 34/2001 (0.5mg AttM/g creatinine), a result different from that found in 2015, where 1 worker had a value of urinary AttM greater than double (1.22mg AttM/g creatinine) of the value recommended by Portaria 34. It is worth noting that this limit does not exclude the worker from the risk, since there is no safe limit to exposure to carcinogenic substances, such as benzene. Regarding the samples from the control group, all 19 samples analyzed showed values below the Detection Limit (0.0075 mg/L) (TABLE 1).

TABLE 1 - Results of the analyzes of AttM (2015/2018) and AFM (2018) in workers exposed and not occupationally exposed to benzene.

Identification of the worker	Type of exposure	AttM/g creatinine (2015)	AttM/ g creatinine (2018)	AFM ug/g creatinine (2018)
Workers occupationally exposed to benzene				
E010	Inhalation	0,09	0,12	2,88
E005	Inhalation/Dermal	0,26	0,13	2,96
E007	Inhalation/Dermal	0,36	0,28	17,47
E008	Inhalation/Dermal	0,37	0,13	2,73
E013	Inhalation	0,41	0,26	3,75
E020	Inhalation/Dermal	0,15	0,31	<LD
E021	Inhalation/Dermal	0,18	0,46	4,45
E036	Inhalation/Dermal	1,22	0,37	35,85
E039	Inhalation	0,09	0,00	<LD
E040	Inhalation	0,08	0,14	3,76
E023	Inhalation	0,04	0,20	31,88
Workers in the control group (not occupationally exposed to benzene)				
C002	-	0,06	0,04	<LD
C006	-	<LD	<LD	<LD
C013	-	-	0,08	<LD
C033	-	0,02	<LD	<LD
C043	-	<LD	<LD	<LD
C045	-	<LD	0,01	<LD
C067	-	0,09	<LD	<LD
C069	-	<LD	0,09	<LD
C073	-	<LD	0,26	<LD
C083	-	<LD	<LD	<LD
C088	-	<LD	<LD	<LD
C099	-	<LD	0,19	<LD
C104	-	<LD	<LD	<LD
C108	-	0,04	<LD	<LD
C109	-	<LD	<LD	<LD
C122	-	0,08	<LD	<LD
C124	-	0,19	0,03	<LD
C125	-	0,15	<LD	<LD
C139	-	<LD	<LD	<LD

After analyzing the AFM of the 11 occupationally exposed workers to benzene in 2018, it was observed that 2 workers presented values above the Biological Limit of exposure established by the ACGIH (25 µg AFM/g creatinine), not excluding the other workers from the risk, since there is no safe limit of exposure to carcinogenic substances. Regarding the samples from the control group, all 19 samples analyzed showed values below the Detection Limit (LD) (7.5 µg/L).

CONCLUSION

As seen previously, when comparing the values of urinary AFM and AttM between control group (workers not exposed) and workers exposed to solvents occupationally, it was possible to observe that the levels were higher among the exposed group, where 2 workers had AFM values above the limit allowed by ACGIH (25µg AFM/g creatinine) in 2018 and 1 worker had a urinary AttM level above that allowed by Portaria 34/2001 (0.5mg AttM/g creatinine) in 2015.

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