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An optimized method for determination of benzene in exhaled air by gas chromatography–mass spectrometry using solid phase microextraction as a sampling technique

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ABSTRACT

The determination of benzene in exhaled air has contributed for the increase in the use of breath analysis in biological monitoring. This paper describes SPME as a sampling technique for determining benzene in exhaled air by GC–MS. A system was developed to generate a gaseous benzene standard by a permeation method to accomplish the breath analyses. The method presented good resolution, repeatability (the mean of %RSD values for intra-day measurements was 6.3), sensitivity (2.4 and 3.1 ppb for LOD and LOQ, respectively), and linearity of response (R^2 = 0.994). After optimizing the conditions, analyses of real samples were performed on two groups (exposed and not exposed to benzene). The results presented an average of 8.2 ppb for the control group and 25.3 ppb for the exposed group.

1. Introduction

Benzene is a ubiquitous pollutant and an important organic compound present in the steel and petroleum industries. It is a natural product of petroleum refining and is used as an intermediate in the production of a wide variety of chemical substances. It is a by-product of the production of coke for steel manufacturing [1]. Its toxicological importance is a result of having been classified as a member of group I – carcinogenic to humans – by the International Agency on Cancer Research since the 1980s [2]. However, the risk of exposure to benzene is not only an occupational risk; the general population is involved. A significant contribution to non-occupational exposure from tobacco smoke exists [3,4], as well as the significant emission from engine exhausts, it being an important component in gasoline.

Gasoline contains 1–5% benzene, the amount varying in different countries [5]. As a result, workers from the petrochemical industry, automobile mechanics and other occupational groups exposed to automobile emissions run a higher risk of contracting leukemia [6]. In addition, the risk is amplified when gasoline is used without exposure control in homes, as a solvent and within many occupational places. Some workers are exposed by multiple routes; some wash their hands with gasoline and even siphon gasoline by mouth. Dermal routes may be the source, since as much as 80% of the benzene levels measured in blood following repair work involves direct contact with gasoline [7]. Therefore, it is necessary to determine the risk by environmental and biological monitoring. Several biomarkers of benzene exposure are sufficiently specific and sensitive for routine use among low-exposure subjects, including non-metabolized benzene in the exhaled breath [5].

In humans, a spectrum of blood dyscrasias, including pancytopenia, aplastic anemia, thrombocytopenia, granulocytopenia, lymphocytopenia, myeloid leukemia and acute leukemia, can result from exposure to benzene. The level, timing and pattern of exposure are extremely important factors in determining the incidence and severity of hematological and bone marrow changes. Furthermore, the stage of stem cell development affected will determine which effects to observe [1].

Benzene has become one of the most intensely regulated occupational agents in the world. With a rapidly increasing number of reports of its hematological effects since 1930, there has been a reduction in exposure limits. These effects have been identified at ever-lower levels, accompanied by a societal concern for improved standards of occupational health. Over the past 25 years, benzene exposure limits have been extensively revised and reduced to the point that, currently, most developed countries have full-shift exposure limits in the range of 0.5–1.0 ppm [8]. The American Conference of Governmental Industrial Hygienists (ACGIH-USA) has established benzene occupational exposure limits of 100, 50, and 25 ppm since 1946. The limit was reduced to 10 ppm in 1977. After 20 years (1997), the threshold limit value (TLV) recommended was

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reduced to 0.5 ppm, and this limit holds to the present day [9]. Many other countries either used or followed the ACGIH TLV and have been influential in setting the standards for exposure controls worldwide.

The analysis of benzene in exhaled air has been proposed and used as a biomarker for the assessment of occupational exposure and has been the object of several studies. A correlation between the levels in exhaled air and the exposure in the workplace atmosphere was observed [3,4,6,10–17]. Benzene present in exhaled breath is related to the blood concentration and the absorbed dose. This biomarker can provide direct information about the body burden, and inferences can be made from occupational exposure.

The determination of benzene in the exhaled air offers some advantages by being a selective and sensitive biomarker for evaluating recent exposure. It is easily accepted by the workers because it is not an invasive sampling method. In addition, the matrix (air) is extremely simple, compared to other biological fluids. On the other hand, there is a lack of data on which to base the analysis of exhaled air; mainly because it is not a common practice in biological monitoring. In spite of the fact that the toxickinetics of a large part of those substances are known, they provide little information about the relationship between the concentration in the exhaled air and exposure. This fact is especially true for the normal variation of the concentration of contaminants in the workplace environment [18]. The analysis of exhaled air also presents a challenge because of the low concentrations of the chemical substances present, which require a highly sensitive analytical technique. The results can also be affected by habitual smoking.

The sampling can be performed during the workday, at the end of the day (30 min after termination of the work shift) and the following morning to evaluate the occupational exposure. The standardization and the interpretation of the results should take the collection schedule into account because the time of collection is critical for analytical reliability, especially because the benzene in exhaled air has a short half-life. The concentration at the end of the day is greatly affected by the variations in exposure during work. The concentration of benzene in exhaled air during the work period contemplates the moment of sampling. Some authors suggest that the determination of benzene in exhaled air that was collected on the morning following the exposure reflects the integral exposure of the previous day and displays a better correlation with occupational exposure [19].

Interest in the VOC analysis of exhaled air, be it clinical interest or interest in the biomonitoring of occupational exposure to chemical substances, has grown in recent years. However, the concentration of foreign compounds in human breath is extremely low, a fact that explains why they are not detected. The SPME technique has demonstrated an enormous potential in the VOC analysis of exhaled air, and it has been applied for analysis of chemical substances present in human expiration in the nanomolar range [20]. The technique is extremely attractive because it combines the sampling and pre-concentration of the analyte in a single process and permits the direct desorption in a chromatography system. SPME is a fast, selective and relatively inexpensive method for sample preparation [21].

The present study sought to optimize a simple and sensitive method for determination of benzene by gas chromatograph–mass spectrometry using SPME for active sampling. Active sampling involves the collection of exhaled air as the individual expels breath over a fiber that is attached to a simple mouthpiece [20]. An SPME device, modified as described by Grote and Pawliszyn [22], was used. The SPME fiber, with a protective Teflon tube, was inserted directly into the mouth of the subject. A homemade permeation device was developed and used to generate the gaseous benzene standard.

2. Experimental

2.1. GC–MS system

The chromatographic system used was a thermo electron trace gas chromatograph (GC), equipped with a POLARIS Q model ion trap mass spectrometer with EI and CI ionization modes; a 30 m × 0.25 mm i.d. × 0.25 μm film capillary column containing 5% diphenyl, 95% dimethylpolysiloxane-HP-5 MS (Hewlett-Packard) as the stationary phase and *X CALIBUR* data acquisition. The oven temperature was programmed for 30 °C for 1 min, 10 °C/min to 60 °C, then 30 °C/min to 150 °C, and 220 °C for 10 min after the run to clean any contaminants that might be released by the SPME fiber from the column. The flow rate of the helium carrier gas was 1.0 mL/min.

The mass spectrometer was operated in the electron impact (EI) mode with ionization energy of 70 eV. The ion source was set to 200 °C and the GC–MS interface to 275 °C. In addition to analyses in the scan mode, full scan (mass range 50–90 m/z) and selected ion monitoring (SIM) was applied to quantitative analysis.

2.2. Permeation device to generate benzene standard

The gaseous benzene standard was generated by a permeation device designed to continuously release material at a fixed rate at 35.0 °C, the temperature being precisely controlled (±0.1 °C). An accurately metered dilution flow (Fig. 1) was provided. Synthetic air was used as a diluent in the permeation system for generation of the gaseous benzene standard, and the flow measurements were performed in quintuplicate. The pressure was maintained at 5.0 (±0.2) psi during all the experiments.

The standard emission device is an inert polymeric tube that contains the analyte in its liquid form. This permeation tube for benzene was purchased from VICI Metronics, Inc. and was certified traceable to N.I.S.T. standards with the following characteristics: 3.5 cm long; permeation rate of 19.8 ± 2.0 ng/min at 35.0 °C.

2.3. SPME method

The solid phase microextraction (SPME) was performed with a manual holder with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS)-50/30 μm fiber (purchased from Supelco, PA, USA). The fiber was conditioned with an injector temperature of 250 °C for 4 h to remove fiber contaminants. A blank of the SPME fiber was analyzed by GC before each standard extraction and sample analysis to check the memory effect and also to condition the SPME fiber for the next injection. After sampling and extraction, the SPME fiber was desorbed in the hot injection port at 250 °C during 3.0 min. The split ratio was 1:20.

The extraction procedure for the gaseous benzene standard consisted of: (1) introduction of the SPME device into the sampling bulb as shown in Fig. 1 and (2) subsequent exposure of the fiber during a period of 30 s. An extraction time of 30 s was determined as a function of the concentration range of interest for construction of the analytical curve.

2.4. Analytical curve

The concentration was changed by varying the diluent flow rate to create a range of concentrations while the device was kept at a constant temperature set point. The concentration of benzene obtained in ppm by volume was computed using the following formula:

$$C = \frac{KP}{F}$$

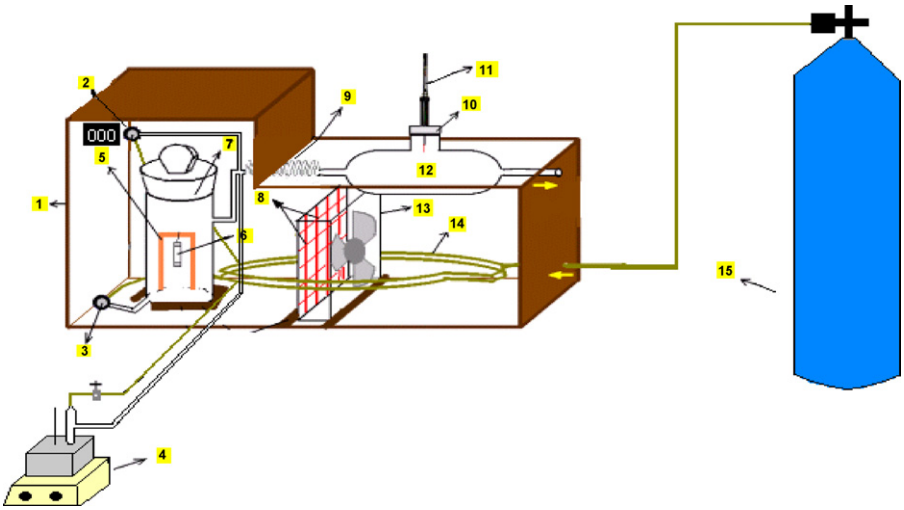

Fig. 1. Steams generation system for the permeation method. The arrows indicate the gas flow sense: (1) wood box; (2) dilution valve; (3) control flow valve of the gas of the permeation camera; (4) generating system of humidity; (5) metallic supports; (6) permeation tube; (7) permeation camera; (8) resistance; (9) serpentine; (10) Teflon septum with sealing wax and aluminum; (11) SPME device; (12) sampling bulb; (13) mini fan; (14) copper tube and (15) synthetic air.

where: C is the concentration in ppm by volume, F the dilution flow rate in mL/min, P the permeation rate in ng/min, K the molar constant = 24.46/MW and MW is the molecular weight of benzene.

The analytical curve was constructed for a certain concentration range as a function of its applicability for biological monitoring of occupational exposure. Concentration levels for the environmental presence of benzene were included. The standard flow rate for the gaseous generation system for a concentration range of 6.0–53.0 ppb was adjusted and measured in quintuplicate.

2.5. Sampling procedure

The sampling of exhaled air was based on the procedure described by Grote and Pawliszyn [22], which consisted of the direct exhalation onto the SPME fiber inserted into a Teflon tube. The Teflon tube was made for that purpose from a compact piece of Teflon. The subjects were trained to perform a slow exhalation to completely empty the lungs so as to obtain the same amount of sample from all the subjects.

A small opening was made near the end of the tube to allow the substitution of the air present in the tube before the individual exhaled and to facilitate exhalation during the sampling procedure. The internal diameter on the right side of the tube was smaller than that on the left side so as to permit the perfect fit of the SPME (Fig. 2).

The collection of exhaled human breath involved the following procedure:

- The volunteers inhaled through their noses and held their breath for about 5 s.
- They exhaled for approximately 5 s without the presence of the SPME fiber.
- They exhaled as slowly as possible directly onto the exposed SPME fiber during the total 30-s extraction time with a clip compressing the nose.

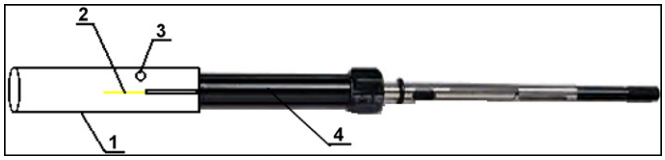

Fig. 2. Adaptation of SPME device goes breath sampling: (1) Teflon tube; (2) exposed fiber; (3) hole and (4) SPME device [22].

3. Study protocol and volunteers

Two groups of 25 subjects, “not exposed” and “exposed to benzene from gasoline”, participated in this study. There were subjects in the exposed group who worked at gasoline stations and laboratories for the quality control of gasoline. The non-exposed or control group included volunteers among employees, teachers and students from the Federal University of Minas Gerais. Non-smokers were selected for both groups. All the samples were collected at the end of morning or in the middle of the work shift.

4. Results and discussion

Some SPME parameters were studied. The choice of an appropriate coating is essential for the SPME method. The sensitivity of each type of fiber varies according to the molecular weight and the polarity of the analytes to be extracted [22]. Four SPME fibers with different polymeric phases were investigated to optimize the method for determination of benzene: (1) 100 μm polydimethylsiloxane (PDMS); (2) 70 μm carbowax/divinylbenzene (CW/DVB); (3) 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB); and (4) 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS). The fiber chosen was the 50/30 μm DVB/CAR/PDMS, which is specific for gaseous samples; it presented a larger area (response) than the other phases.

The development of SPME methods requires a study to establish optimal analyte extraction conditions. For this purpose, other SPME parameters were studied in addition to the type of fiber. Another parameter determined was the effect of the length of the fiber inside of the tube. The SPME device allows the adjustment of the length of the needle where the fiber is collected. This test assumed that the efficiency of extraction of the analytes depends on the distance to which the fiber is inserted into the mouth. Moderate exhalations of 20 s were made using 2.0 and 4.0 cm extensions of exposed fiber. This experiment showed that the length of the exposed fiber in the tube affects the degree of extraction of the ana-

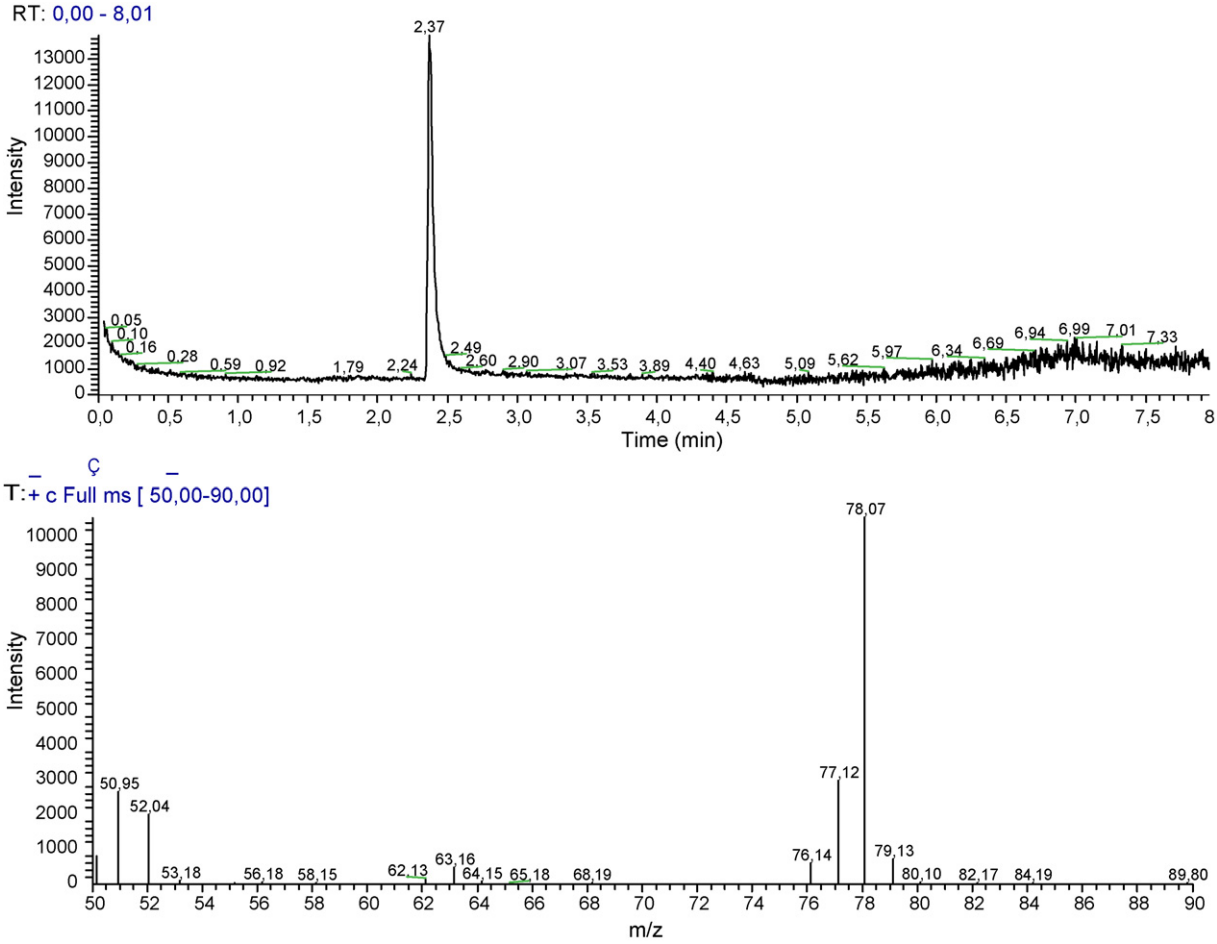

Fig. 3. GC–EI-MS selected-ion-monitoring (*m*/*z* 51 and 78) chromatogram and mass spectrum of benzene for gaseous standard (30.8 ppb).

lytes. The standard deviation obtained from the repetitions with the fiber having a length of 4.0 cm and closer of the mouth was larger (20%) than the standard deviation obtained for the repetitions with the 2.0 cm fiber. Thus, the 2.0-cm-long fiber was chosen.

The extraction and desorption times were evaluated by monitoring the peak areas observed at 20, 30, 45 and 60 s for absorption and 0.5, 1.0, 2.0 and 3.0 min for desorption. The optimum times were 30 s for extraction and 3.0 min for desorption and were considered to be the most reasonable with regard to the sampling of the breath and the sensitivity of standard gas analysis. Method validation was then performed. Chromatograms of gaseous benzene standard and exhaled air from workers exposed to benzene are shown in Figs. 3 and 4.

Knowledge of the equilibration time for the permeation system was important for the construction of the analytical curve. The study evaluated the stability of the system after the adjustment of the flow rate, which was necessary for construction of the analytical curve. The flow rate was measured five times (%RSD = 0.98). Fig. 5 presents the study for establishing the equilibration time of the permeation system for the construction of the analytical curve. A 50/30-μm DVB/CAR/PDMS fiber was used to extract benzene every 0.5–3.5 h. The procedure was repeated for three consecutive days. According to the data, the system reached equilibrium in approximately 1 h, which was considered to be a satisfactorily short time frame.

The precision of the method was evaluated in three different concentrations using optimized conditions. Seven replicate extractions were performed with benzene concentration levels of 6.0, 19.0 and 41.0 ppb. The relative standard deviations (%RSD) observed

Table 1
Results of breath analysis of benzene from the non-exposed volunteers and workers exposed to gasoline

Sample	Benzene concentration (ppb)	
	Control group	Exposed group
1	6.4	30.7
2	9.4	71.2
3	9.9	14.9
4	15.0	15.8
5	9.6	18.7
6	5.3	11.8
7	9.5	20.9
8	8.3	26.6
9	4.9	11.5
10	13.5	18.3
11	7.3	26.5
12	12.5	10.9
13	<LOD	13.5
14	2.3	15.2
15	2.8	11.6
16	<LOD	15.8
17	3.7	22.2
18	8.5	22.7
19	7.8	22.3
20	11.5	35.3
21	14.6	52.2
22	3.2	49.4
23	11.5	39.2
24	8.0	28.4
25	4.2	28.1
Minimum concentration	2.3	10.9
Maximum concentration	15.0	71.2
Media	8.2	25.3

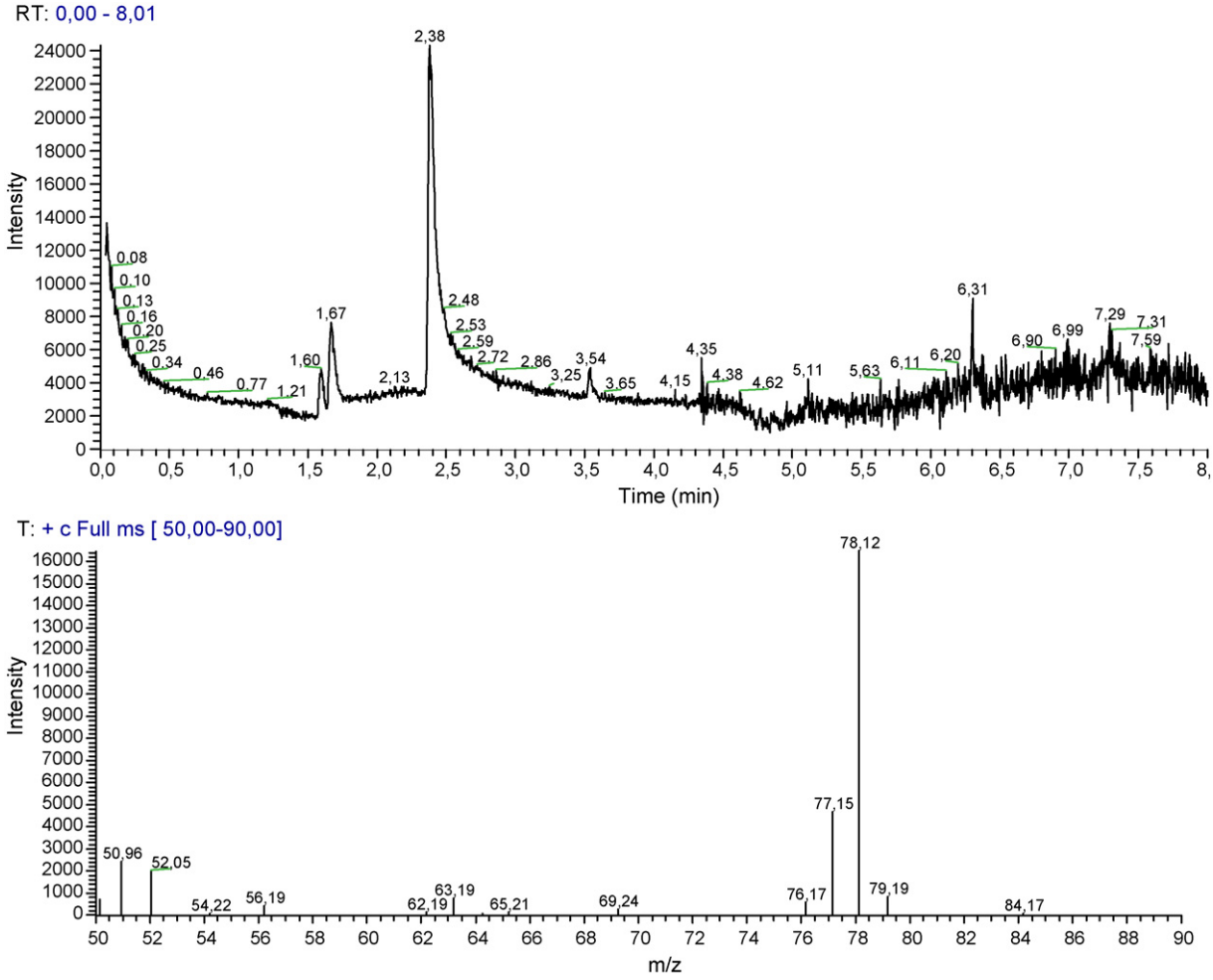

Fig. 4. GC–EI-MS selected-ion-monitoring (*m*/*z* 51 and 78) chromatogram and mass spectrum of benzene in exhaled air from worker exposed to gasoline (52.2 ppb).

were 11.0, 4.6 and 3.2, respectively. The mean of the %RSD values for intra-day measurements (6.3) were satisfactory when compared to other studies with SPME [22–24].

As for sensitivity, the limit of detection (LOD) and the limit of quantification (LOQ) were calculated according to the recommendations of the EURACHEM Guide (2002) [25]. This method used the equations LOD = mean of sample blank + 3*S*, and LOQ = mean of sample blank + 10*S*, where *S* is the standard deviation for 10 repetitions of the extraction procedure with the sample blank. The generation system was maintained for 24 h without a benzene permeation tube prior to the experiment. The results obtained were 2.4 and 3.1 ppb for LOD and LOQ, respectively.

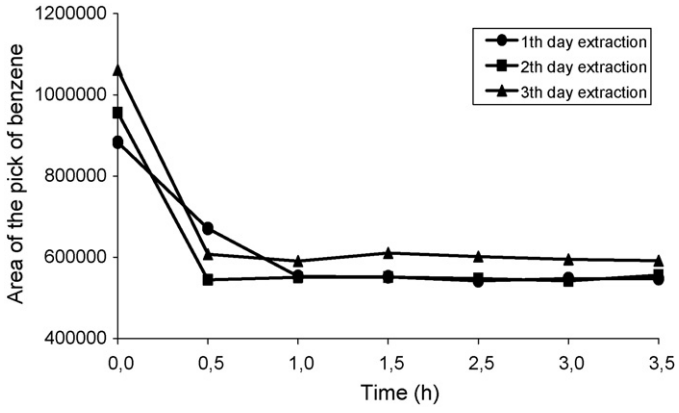

Fig. 5. Study of benzene flow rate equilibrium time of the permeation system.

The application of the SPME method to real samples was achieved through the analysis of workers exposed to gasoline and volunteers who were not exposed, as described in Item 3. This study has approved by the Ethical Committee of the Federal University from Minas Gerais, in accordance with the World Medical Association's "Ethical Principles for Medical Research Involving Human Subjects". The results for the control group and the exposed group are presented in Table 1.

The results for the real samples indicated a significant difference between the groups. Application of the one-way ANOVA test, Brown–Forsythe's Test for equal variance, the Bonferroni Test and the Scheffe Test showed that the population means were significantly different at the 0.05 level. However, both groups presented a large variability in benzene levels of the breath, indicating that the exposed group was not homogeneous. However, it is consistent with findings in other occupational studies [6]. The control group could be considered to be an environmentally exposed group similar to those studied by Perbellini et al. [12].

5. Conclusions

This work describes an alternative method for analysis of benzene and other volatile organic compounds (VOC) using SPME for active sampling of exhaled air. The method proposed in this study was proven to be suitable for evaluating occupational and envi-

ronmental exposure through biological monitoring. This procedure was validated and was found to be precise, linear and sensitive in the range of concentrations of interest to the occupational and environmental fields.

The advantage of this method is the short time in which an analysis can be completed. It requires approximately 10 min, after which more samples can be analyzed. In addition, the active sampling onto SPME fiber decreases contamination and loss of sample, thereby permitting the determination of low levels of benzene. Finally, this method presents the advantages of being solvent-free, of low cost and fast. However, this method requires that the sample not be collected in the workplace because of contamination by the work environment. If the analysis requires, the sample is stable for 30 min when absorbed on the SPME fiber [26]. The applicability of this method to determine other VOC at low concentrations needs to be evaluated.

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