

Determination of benzene, toluene, ethylbenzene, *p*-, *m*-, *o*-xylene, and *n*-butyl acetate in urine by a validated gas chromatography method: Application to an occupational monitoring study

Emrah Dural^{1*}, Betül İşiner Kaya², Görkem Mergen³, Erhan Boran⁴,
Tülin Söylemezoğlu⁵

^{1,2,3,4,5}Toxicology Department, Institute of Forensic Sciences, Ankara University, Ankara, Turkey, ¹Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Cumhuriyet University, Sivas, Turkey, ²Department of Basic Sciences, Faculty of Dentistry, Mehmet Akif University, Burdur, Turkey

This study was aimed to determine occupational and non-occupational exposure to benzene, toluene *p*-*m*-*o*-xylene (BTEXs) and butyl-acetate (nBA). The aim of this work was to develop a simple, sensitive, and reliable chromatographic method using urine, a non-invasive human sample. The method was applied to samples collected from furniture spray workers (n=53) who are at risk of exposure to BTEXs and nBA and office workers (n=51) who have no known exposure risk. Method validation tests, include the sensitivity (LOD≤0.018 ng/mL), precision (RSD≤4.1), accuracy (RE% (-3.9)-4.7), recovery (96.1-103.8%) and linearity ($r^2 \geq 0.999$). Urinary benzene (1.77 vs 1.23 ng/mL, exposed-control, respectively), toluene (51.22 vs 0.77 ng/mL), ethylbenzene (9.25 vs 6.69 ng/mL), *para*-xylene (1.73 vs 0.62 ng/mL), *meta*-xylene (2.58 vs 1.20 ng/mL), *ortho*-xylene (1.61 vs 0.88 ng/mL), and butyl acetate (33.14 vs 1.63 ng/mL) concentrations were determined in the exposed and control group samples. Significant correlations were found between benzene ($p=0.286^*$), ethylbenzene ($p=0.552^{***}$) and *o*-xylene ($p=0.292^*$) levels and smoking status in samples belonging to the control group. The occupationally-exposure-risk group samples have significantly higher BTEXs and nBA concentrations than the control ($p<0.001$). It was determined that smoking was a significantly effective factor in exposure to benzene, ethylbenzene and *o*-xylene in the control group.

Keywords: BTEXs. *n*-Butyl acetate. Headspace-GC-FID. Urine. Occupational exposure. Smoking.

INTRODUCTION

Volatile organic compounds (VOCs) have been one of the major issues in the environmental sciences and their wide distribution has raised major concerns especially in public health. They were used as main components in the synthesis of chemicals and also manufacturing wide range commercial products. They are of prime concern due to their chemical and physical characteristics such as partial volatility, lipid solubility, and the ability to pass through biological membranes and accumulate in

fatty tissues (Chary, Fernandez-Alba, 2012). Although, studies have been carried out to clarify their toxicity and carcinogenic properties in the last 30 years have resulted in some decreases in indiscriminate use, both occupational and environmental exposure risk are still continuing to be a major concern (Calabrese, Kenyon, 1991; Alkalde *et al.*, 2004). Aromatic compounds are the most prominent group contributing to VOCs. Benzene, toluene, ethylbenzene, *para* (*p*)-xylene, *meta* (*m*)-xylene and *ortho* (*o*)-xylene (BTEXs) has great attention in the aromatic hydrocarbons, due to their widespread distribution in the tropospheric area along with the risks they pose to human health (Amini *et al.*, 2017).

BTEXs (Figure 1) are known as major representatives of VOCs (Yu *et al.*, 2022). These monoaromatic

*Correspondence: E. Dural Department of Pharmaceutical Toxicology Faculty of Pharmacy Cumhuriyet University, 58140-Sivas, Turkey Phone: +90 (346) 487 39 38 Fax: +90 (346) 487 16 34 E-mail: emrahdural@gmail.com <https://orcid.org/0000-0002-9320-8008>

hydrocarbon compounds have serious carcinogenic and mutagenic effects (AlSalka, Karabet, Hashem, 2010; Yu *et al.*, 2022). Exposure to BTEXs has adverse effect on many organs and systems including heart, kidneys, liver, nervous system and also developing risk of nonlymphocytic leukemia (Khajeh, Zadeh, 2012; Nagaraju, Vijayakumar, Ramana Reddy, 2019). Paint, varnish, thinner, vehicle gasoline, and other industrial raw materials are the main resource of BTEXs (Sarafraz-Yazdi, Khaleghi-Miran, Es'haghi, 2010; González *et al.*, 2017). In general, BTEXs

are released into the environment through three major sources. These are through combustion, petrogenic sources and processed products (Yu *et al.*, 2022). Vehicle exhaust and cigarette smoke contributes significantly to personal exposure, particularly indoor levels (Alexopoulos, Chatzis, Linos, 2006). Since, these compounds are omnipresent in environmental samples like air, water, and soil, humans are at risk of exposure via ingestion, inhalation or the absorption route to the skin (Yu *et al.*, 2022).

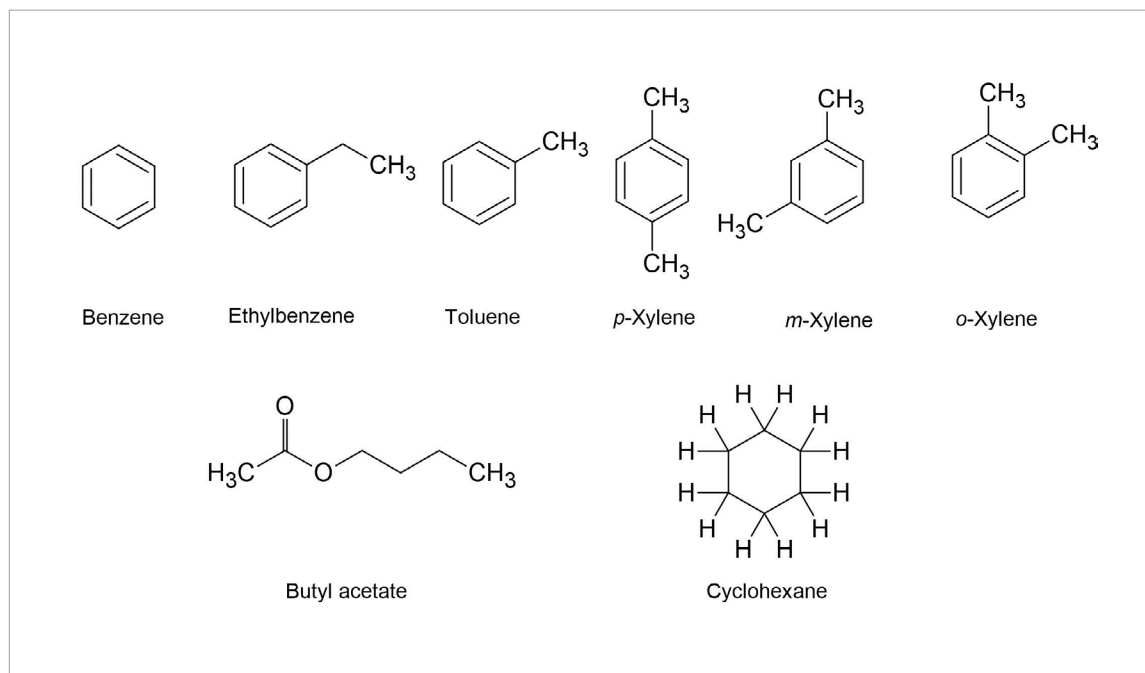


FIGURE 1 - Skeletal chemical structures of benzene, ethylbenzene, toluene, *p*-, *m*-, *o*-xylene, butyl acetate were used as the analytes and cyclohexane which was employed as the internal standard in the study.

Benzene is widely used as a starting chemical for the manufacturing of a lot of other chemicals that have industrial and medicinal importance. It has excellent solvent properties, resulting in wide use in various industries and products including dyes, varnish, lacquer, leather etc. It is found as a component of cigarette smoke and gasoline (Khan, 2007). Benzene has been linked with increased rates of solid tumors and leukemia in the occupational setting, although its role in public health

outcomes is unclear (Hinwood *et al.*, 2007). Toluene is used in the production of coatings and paints, inks, adhesives, resins, pharmaceuticals and as a cleaning solvent in the printing, leather and coating sectors (Duydu *et al.*, 1999). Since toluene vapour is heavier than air it may travel along the ground and be detrimental by causing air contamination levels to increase rapidly by the evaporation of toluene at 20°C (Alexopoulos, Chatzis, Linos, 2006). Occupational toluene exposure has adverse

effects on the liver, kidneys, central nervous system and mucous membranes (Ghittori *et al.*, 2004; Hinwood *et al.*, 2007) and it has been linked with teratogenic effects (Wilkins-Haug, 1997). Ethylbenzene, a colorless liquid with an aromatic odor, is primarily used as a solvent and in the styrene production. It is manufactured by the alkylation of benzene with ethylene (Jang, Droz, Kim, 2001). It is a constituent of gasoline and mixed xylenes (Saillenfait *et al.*, 2007). Commercial xylene has about 20% ethylbenzene (Jang, Droz, Kim, 2001). Ethylbenzene is irritating to eyes, skin and mucous membranes (Jang, Droz, Kim, 2001). Chronic ethylbenzene exposure has adverse effects on the nervous system, kidneys and the respiratory system (NTP, 1999). Xylene, a common lipophilic and highly volatile aromatic hydrocarbon, exists in three isomeric forms named para-, meta-, and ortho-xylene (Adams *et al.*, 2005). Xylene has been commonly used in the manufacture of chemicals, dyes, thinners, plastics, synthetic fibers and pharmaceuticals (Fay *et al.*, 1998). Central nervous system depression, disturbances in coordination, prolonged reaction time, hematologic diseases (e.g. anemia, leukopenia) and electrocardiogram abnormalities are the symptoms of the xylene toxicity (Langman, 1994; Hinwood *et al.*, 2007). *N*-butyl acetate, a colorless fruity-smelling liquid and a typical short-chain fatty acid ester, has both consumer and non-consumer uses (Lv *et al.*, 2021). *n*BA is mainly used as a solvent in the coating industry since it has excellent solubility properties (David *et al.*, 2001), along with its use in the food, cosmetics, pharmaceutical and biofuel industries (Lv *et al.*, 2021). It is reported that, *n*BA is a hepatotoxic agent as indicated by an increase in serum bile acids (Franco *et al.*, 1986). Its ocular, respiratory and neurotoxic effects are described in various study results (Bushy Run Research Center 1987; David *et al.*, 2001). Benzene is a confirmed animal and human carcinogen and it is classified in Group 1 by IARC (International Agency for Research on Cancer (IARC) 2021). Ethylbenzene is classified in Group 2B as a possible human carcinogen (International Agency for Research on Cancer (IARC) 2021). Toluene, and xylene are classified in Group 3 (International Agency for Research on Cancer (IARC) 2021).

As a result of furniture painting and varnishing applications being carried out in closed workplaces with insufficient ventilation and without personal protection measures, it is assumed that workers are at risk of exposure to BTEXs and *n*-butyl acetate greater than the legal limits reported in both national and international regulations (Tajik *et al.*, 2017). Since BTEXs and *n*BA have a high volatility and lipid-solubility properties, they can easily enter the circulation through respiration and then widely dissolved in the target organs (Sato, 1988). Quantitative detection of BTEXs and *n*BA solvents in human body fluids is of great importance because even if they are present in low concentrations, they are extremely harmful to human health, especially in the case of chronic exposure (Calabrese, Kenyon, 1991; Krämer Alkalde *et al.*, 2004). Urine is a more suitable biological sample for BTEXs and *n*BA analysis as it provides both chronic accumulation and stable chemical structure compared to blood and other biological samples.

The sample pretreatment is a fundamental step for method validation allowing the testing of the following parameters: sensitivity, accuracy, precision, reproducibility and recovery etc. A suitable pretreatment method removes potential interferences that could mask undesired objects or enrich target analytes from complicated matrices. It also allows extraction of the compounds of interest and any adaptation to the methods.

The most popular sample preparation method for the determination of BTEXs and *n*BA from the biological fluids is dispersive liquid–liquid microextraction (Assadi, Ahmadi, Hossieni, 2010), solid phase extraction (Yu *et al.*, 2022), solid phase micro extraction (Andreoli *et al.*, 1999; Yu *et al.*, 2022), purge and trap (Brčić Karačonji, Skender, Karačić, 2007; Lai *et al.*, 2022), headspace solid phase micro extraction (Fustinoni *et al.*, 1999; Karačonji, Skender, 2007; Tajik *et al.*, 2017) and head-space (Ketola *et al.*, 1997; Polkowska *et al.*, 2004).

Although, there are some alternative extraction methods in the literature, the headspace method conditions are fully optimized to present important advantages both liquid and solid samples. As a result it has been commonly used in the pretreatment of the BTEXs and *n*BA.

Ghanbarian *et al.* (2022) developed a GC-MS method based on the headspace pretreatment for the determination of BTEXs in urine. Rahimpoor *et al.* (2021) developed a new method of analysis for the extraction of the BTEX compounds from a urine matrix. They utilized the 3D Ni/Co-BTC bimetallic metal–organic framework acting as a solid absorbent packaged inside a Dynamic Headspace Needle Trap Device for the extraction of BTEXs. In this study, extraction time (35 min), temperature (35°C) and salt content (7.5%) values were optimized. Also, the thermal desorption temperature (275 °C) and time (3 min), belong to absorbent, were optimized. The method had a detection limit of 0.2–1.1 ng/mL and recovery analyses of 95–99%. The repeatability (1.9–5.8%) and reproducibility (5.5–8.2%) tests were also carried out. Fakhari *et al.* (2012) developed a headspace-based extraction method for the pretreatment of the BTEXs compounds in water and wastewater samples (Yu *et al.*, 2022). Satisfactory relative recoveries from wastewater samples (84–98%) and well water (88–105%) were observed after controlling for parameters affecting HS extraction efficiency, such as salt addition, sample volume, extraction time and mixing speed. In addition to this, headspace was used in the solid sample analysis. Zhou *et al.* (2013) used HS for field analysis of BTEX in soil. In the head-space sampling process, sodium chloride solution (26%) was added into a vial containing 5 g soil, and subsequently the gas from the HS was extracted and analyzed. The method precision was between 5.3 and 7.8% and also the recovery of different analytes ranged from 87.2 to 105.1%. These examples show that, the chromatographic methods based on HS extractions were present and there were sufficient validation test values for the determination of BTEXs in urine, for instance RSD <10%, percent recovery was between 88% and 106% and sensitivity was ≤ 0.2 ng/mL. Although other variables such as column, column program and detector affect this and other validation test values, the common feature of these studies is the use of headspace technique in extraction.

Headspace can be defined as one of the most suitable and preferred pretreatment/extraction techniques from past to present for the determination of BTEXs and nBA

in urine analysis. When considering the physical and chemical properties of BTEXs and nBA and reflecting on the target population, this agrees that headspace is the most suitable. In addition, not only liquid samples such as urine but also solid samples such as soil, can be applied to headspace for the analysis of BTEXs (Zhou *et al.*, 2013). It is a sensitive, reliable, repeatable and cost effective method. The headspace allows many changes that can be applied to improve analysis, and this extraction unit has more accessibility than other complex equipment in the scientific research center. The headspace sample pretreatment applications/technique is not complex and it does not need personal qualifications and experience, so it can be easily applied to quality control samples and real samples. Sample preparation is one of the main topics of analysis because of the high volatility of BTEXs and n-butyl acetate which can lead to loss of analyte from the matrix. Headspace sample bottles are suitable for use in the preparation of main and working stocks to prevent analyte loss from matrix. At this stage, a special GC needle that can puncture the septum at the tightly closed mouth of the vial can be used during the preparation of the samples.

The goal of this study was to develop, optimize and validate a simple, sensitive and reliable chromatographic method that enables the determination of BTEXs and nBA without using a time-consuming and complex extraction technique or large volumes of solvents. It is aimed to develop an analysis method that has low cost, provides fast results, does not require a solvent, is environmentally friendly, and does not require special experience and skill in the sample preparation. Once the validity of the method is determined in accordance with the ICH guidelines (ICH, 2005), it is planned to be applied to real urine samples to monitor BTEXs and nBA levels.

Here, we developed a simple, sensitive and reliable determination method from human urine based on headspace extraction, gas chromatography separation and flamed ionization detection. Once the suitable headspace extraction technique and its parameters that may affect the analysis were determined, tested and optimized, the gas chromatography oven program was chosen under those

conditions. Also, the flamed ionization detector analysis conditions were checked with the trial test applications. The method suitability parameters named as the retention time (t_R), determination coefficient (r^2), capacity factor (k), theoretical plate number (N), specificity factor (α), resolution (R_s) and symmetry factor were determined. Then, the method was validated in terms of linearity, accuracy, precision, sensitivity, and recovery. The method was applied to real human urine samples collected from volunteers who were under occupational exposure risk ($n=53$) and no known exposure risk ($n=51$). Before the study, a questionnaire including demographics, habits and professional information was taken from the participants. In order to determine the effect of smoking on BTEXs and nBA levels in the urine, the relationship between smoking frequency and BTEXs and nBA urinary levels were monitored. In addition, other statistical analyzes were performed by taking into account the factors that may affect BTEXs and nBA levels in the urine, such as age and gender. According to our research, this is the first validated method for the determination of urinary BTEXs and nBA based on the headspace extraction, GC-FID determination and the monitoring of these analytes from urine samples. These samples were collected from individuals who were under the occupational exposure risk and were also collected from the general population with no-known exposure risk to BTEXs and nBA chemicals. This is also the first study to investigate the relationship between urinary nBA concentration and smoking by including occupational exposure.

MATERIAL AND METHODS

Study population

Urine samples were collected from the participants who had occupational exposure risk to organic volatile compounds ($n=53$) named benzene, toluene, ethylbenzene, *p*, *m*, *o*-xylene, and butyl acetate and also individuals who had no known occupational exposure risk to these organic solvents ($n=51$). Exposure-risk group samples were collected from furniture painters working in Ankara-Siteler (Ankara-Turkey) which is one of the biggest

furniture production workplaces not only in this region but also in Turkey and control group samples were collected from office workers working in Ankara University. Both groups were composed of volunteers between the ages of 18 and 55, weighing 65-85 kg who have no known chronic disease. Written informed consent and permission to use their information for future studies of BTEXs and nBA exposure was obtained from each participant who was eligible for the study. The study design was approved by the Institutional Medical Ethics Committee in 2009 (Approval number: 152-4827). Samplings were performed in accordance with the principles of The Declaration of Helsinki and its subsequent revisions.

Sample collection

Urine sample, taken as the first-morning urine (10 mL), was placed in 20 mL sample bottles which have 60 mg NaF and 2.4 g NaCl and stored in the refrigerator at +4 °C until analyses were conducted (within two days of initial storage). Sample vial was capped firmly with PTFE/butyl septum coated with aluminium cap. The collection and analysis process of all samples was completed within two weeks.

Chemicals and reagents

Benzene ($\geq 99.9\%$), ethylbenzene (99.9%), toluene ($\geq 99.9\%$), *p*-($\geq 99.5\%$), *m*-xylene ($\geq 99.5\%$), *o*-($\geq 99.5\%$), butyl acetate ($\geq 99.5\%$) and cyclohexane ($\geq 99.5\%$), (Figure 1), were bought from Sigma-Aldrich (Steinheim, Germany). HPLC-grade methanol and gas chromatography grade hexane were purchased from Merck (Darmstadt, Germany). Nitrogen (99.999%), as the carrier and make-up gas; hydrogen (99.999%) and dry air (99.999%), as the fuel and the oxidising gas during the combustion process respectively, were supplied from Oksan (Ankara, Turkey).

Instrumentation

An Agilent 6890 series gas chromatograph (GC) was employed for chromatographic separation (Palo Alto, CA, USA). An Agilent HP 7694E Headspace (HS)

was used for the extraction of analytes from urine and then by automated sampling to the gas chromatograph from the inlet part which may use split or splitless. Chromatographic separation was carried out with an HP-INNOWax column (30 m x 0.32 mm x 0.25 mm). An Agilent 6890 flame ionization detector (FID) was

used for the determination and quantification of analytes. Pre-analysis test applications were applied, in order to avoid any condensation possibility and determination of optimum extraction capacity before validation application. The operating parameters of the headspace and gas chromatography parts were given in Table I.

TABLE I - The optimized operating parameters of the static headspace (HS) and gas chromatography (GC) parts belong to the chromatographic system

N°	Parameters	Values
1	HS-Vial temperature	70.0 °C
2	HS-Vial shaking time	2.0 min
3	HS-Vial equilibrium time	15.0 min
4	HS-Vial cycle time	13.0 min
5	HS-Loop temperature	80.0 °C
6	HS-Loop fill time	0.2 min
7	HS-Loop equilibrium time	0.05 min
8	HS-Pressure time	0.2 min
9	HS-Transfer line temperature	120.0 °C
10	GC-inlet-Injection type	Splitless
11	GC-inlet-Injection time	1.0 min
12	GC-Run time	15.7 min

The optimized oven programme was given in the following: This main separation module was initially maintained at 40 °C for 6 min and temperature was gradually increased to 140 °C with a rate of 15 °C/min and oven temperature was held at this level for 3 min. For further analysis to be performed, the column furnace temperature was again reduced to 40 °C. The analysis run time was 9.0 min, although the total run time was 15.7 min. The inlet part and detector part temperature were set at 280 °C and 250 °C, respectively. During the analyses, the HS-GC-FID analysis laboratory was ventilated to reach the optimum cycle time between analyses and reduce the cooling load of the column oven.

Preparation of analytical standard solutions

BTEXs and nBA main stock solutions and standard chemicals were prepared in the 20 mL headspace vial. Stock solutions were prepared in two steps in order to prevent the possible concentration error related to chemical vapour escaping. Initially, the required solvent (methanol) was added into the vials and then capped tightly with rubber septum and strengthened by aluminium caps. Following, BTEXs and nBA chemical standards, a required volume was injected into vials throughout a glass GC injector (100 µL). This methodology was maintained during the preparation of the analytical standard solutions.

The main stock solutions of BTEXs and nBA were prepared as 1 mg/mL concentrations in methanol. The main stock solutions were used in the preparation of the working solutions. Stock solutions were prepared separately for each of the chemicals assessed: benzene, ethylbenzene, *p*-, *m*-, *o*-xylene, and butyl acetate. Their concentrations were 5, 10, 20, 50, and 100 µg/mL. Concentrations of 5, 10, 20, 50, and 100 ng/mL benzene, ethylbenzene, *p*-, *m*-, *o*-xylene, and butyl acetate were obtained by dissolving 10 µL of the respective stock solutions in 10 mL of blank-urine. In addition to these chemical standard points, for toluene, a 500 µg/mL stock solution was prepared to establish a concentration of 500 ng/mL toluene in the urine.

Main stock and working solutions were stable at -18 °C and +4 °C for a month and week, respectively. Working solutions of BTEXs and nBA were prepared daily from the stock solution given above.

Preparation of validation and real samples to the chromatographic analysis

Validation test samples and real urine samples were prepared for analysis using the protocol described above, except that the step of adding BTEXs and nBA analytes was not performed on real urine samples. In brief, a fresh 10 mL human urine sample was transferred to a sample vial containing 60 mg sodium fluoride used as an antimicrobial and 2.4 gr sodium chloride used for increasing the BTEXs and nBA vapour. Vial was sealed with a rubber septum coated with an aluminium cap, following 10 µL of internal standard (100 µg/mL), named cyclohexane (CH) was added into the vial through a glass GC injector and the final internal standard concentration was 100 ng/mL.

In order to prepare the validation test samples, BTEXs, nBA and the internal chemical standards were added into the GC vials containing urine sample through an injection from the rubber septum by a glass GC injector after being sealed to prevent possible chemical escape.

In the preliminary laboratory trials, it was detected that a large amount of BTEXs and nBA could be lost from the real urine samples depending on the freezing application which was carried out for the storage of urine samples for an extended period. As a result, after urine samples were taken from volunteers, specimens were placed in the head-space sample vials contain 60 mg sodium fluoride and 2.4 gr sodium chloride and stored at +4 °C and then they were subjected to the chromatographic analysis detailed above within a period of two days to minimize the risk of analyte loss.

Validation test applications

The method was validated in terms of linearity, sensitivity, accuracy, precision and recovery in accordance with International Conference on Harmonization guideline coded as Q2R1 (ICH, 2005). An intra-day and inter-day validation protocol was applied considering the reproducibility of the method to obtain accurate and precise measurements.

Validation tests were performed using real urine samples to observe matrix effects in the analysis. A urine pool was created with real urine samples taken from volunteers who were known not to be exposed to BTEXs, nBA and CH (ISTD) at the preliminary stage of the laboratory application. Also, the urine pool was subjected to GC analysis to ensure that it was free of BTEXs, nBA and CH before being used in the validation test application. In order to prevent microbial reproduction, sodium fluoride was added to the urine pool at a concentration of 3 mg/mL.

In order to simultaneously determine BTEXs, nBA and CH analytes, the head-space-GC-FID analysis system was utilized under the optimized conditions explained above, Table I and in the Figure 2. The retention times of analytes named, benzene, toluene, butyl acetate, ethylbenzene, *p*-, *m*-, *o*-xylene were 2.7, 4.5, 5.5, 7.2, 7.7, 8.1 and 8.7 minutes, respectively.

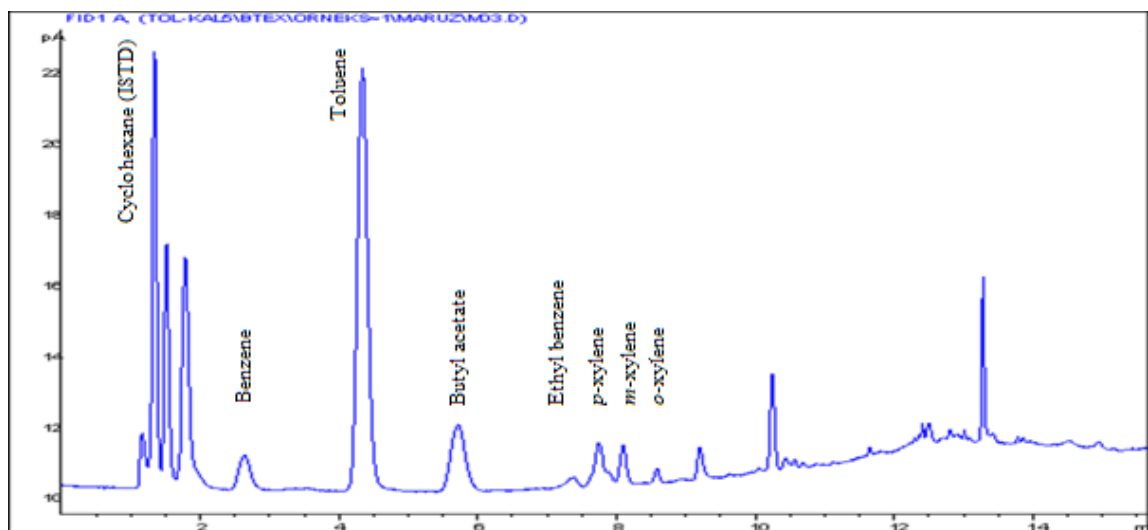


FIGURE 2 - A real urine sample chromatogram belongs to a participant who works in a furniture maker as a spray painter for these reasons he has occupationally exposure risk to the BTEXs and butyl acetate. The chromatogram contains benzene, toluene, ethylbenzene *p*-, *m*-, *o*-xylene, and butyl acetate, suggesting that it is related to the occupation of the person from whom the urine was drawn.

Linearity

After chromatographic conditions were optimized, calibration curves of benzene, toluene, butyl acetate, ethylbenzene and *p*-, *m*-, *o*-xylene were plotted against the internal standard. Calibration curves of all analytes except toluene were drawn by adding the standard to the urine at concentrations of 5, 10, 20, 50 and 100 ng/mL. In addition to these calibration points given above for toluene and butyl acetate, two more calibration points, 500 and 1000 ng/mL, were added to calculate and plot the calibration curve. The linearity study was performed with triplicate analyzes for each calibration point in the calibration curves of BTEXs and nBA standard chemicals.

Repeatability

Precision and accuracy tests were carried out under the sub-headings of the repeatability test. Precision, calculated as relative standard deviation (RSD%), is a measure of closeness between data from a series of experiments under similar conditions. The experiment

was performed with five individual/independent implementations at each of three different concentration points, low (5 ng/mL), medium (20 ng/mL), and high (100 ng/mL) concentrations ($n=5$) for the BTEXs and nBA analytes. Accuracy calculated by the relative error (RE%) means the degree of closeness between the expected and the observed value determined by the method. It was calculated as the percentage difference between the added and found BTEXs and nBA quantity by 5 separate replicates both intra-day and inter-day.

These test applications were carried out both on the same day (intra-day) and on five consecutive days (inter-days).

Sensitivity

The concentration of 2.5 ng/mL and half of the lowest calibration values of the all analytes, was used in the sensitivity tests of BTEXs. 10 quality control samples were individually prepared, for all analytes, on the same day and applied to the GC analyses with sequential analyses. All samples were prepared to the

matrix, therefore, each sample was analyzed after its preparation in its own matrix. The BTEXs and nBA peak areas in the chromatograms were calculated according to the data obtained from the linearity test.

Recovery

The recovery experiment was carried out in three experimental groups for all analytes, each containing five blank-urine (BTEXs free) samples. First of all, BTEXs and nBA chemicals were at a concentration of 5 ng/mL in the urine samples of all groups. The analyzes of the first group was performed according to the preferred method and the BTEXs concentrations observed in the chromatograms were calculated. The urine samples of the second group contain 5 ng/mL BTEXs chemicals, another 25 ng/mL BTEXs and nBA was added with the standard addition method and was then analysed. Finally, the urine samples of the third group containing 5 ng/mL BTEXs were analyzed by adding another 100 ng/mL concentration of BTEXs, and nBA. The analyses of the three groups were carried out according to the stated order. Thus, in the recovery calculation, the theoretically expected (5, 25 and 105 ng/mL) and observed BTEXs, and nBA concentrations of three different groups were compared.

Statistical analysis

Statistical Package for Social Sciences (SPSS) version 23.0 software for Windows 10 was utilized for the statistical analyses. The Shapiro–Wilk test was used

to examine the normality of numerical variables. Analysis data did not provide parametric test assumption. The urinary BTEXs and nBA concentration comparisons of the group were carried out by the Mann-Whitney U test. All data were shown as median and quartile range (IQR) due to the non-normal distribution of numerical data. The Spearman's test was employed for the correlation analysis between cigarette consumption and seven (7) volatile organic compounds. The statistical significance level was accepted as $p < 0.05$ and marked with a star (*). When statistical significances were found as $p < 0.01$ and $p < 0.001$, two (**) and three star (***) abbreviations were used, respectively.

RESULTS

At the beginning of the study, A HS-GC-FID analysis method conditions were developed and the chromatographic parameters and variables belong to the headspace part. The GC-inlet and FID detector were determined with individually performed test applications. According to the preliminary test applications, the optimum gas chromatography oven programme with maximum efficiency, was detected.

At the end of this part of the study, this chromatographic method was established and the main analysis conditions determined. Chromatographic parameters which are retention times, calibration equations and determination coefficients were calculated. The system suitability parameters capacity factor (k'), theoretical plate number (N), specificity factor (α), resolution (R_s), and symmetry factor were calculated. Results were detailed in Table II.

TABLE II - System suitability parameters of the recommended method calculated in accordance with The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guideline

Analyte	Retention time (tR)	Calibration equation (y=bx±a)	Determination coefficient (r2)	Capacity factor (k)	Theoretical plate number (N)	Specificity factor (α)	Resolution (Rs)	Symmetry factor
Benzene	2.7	y = 0.0196x + 0.0388	0.9996	1.5	638.4	2.3	2.2	1.0
Toluene	4.5	y = 0.0178x + 0.0337	0.9993	3.1	1296.0	2.2	3.8	1.0
Butyl acetate	5.5	y = 0.0028x + 0.0052	0.9999	4.0	1444.0	1.4	21.7	1.0
Ethylbenzene	7.2	y = 0.0229x + 0.0796	0.9997	5.5	9460.8	1.4	5.9	1.0
<i>p</i> -Xylene	7.7	y = 0.0216x - 0.0221	0.9999	6.0	3794.6	1.1	1.2	1.1
<i>m</i> -Xylene	8.1	y = 0.021x + 0.0066	0.9998	6.4	26244	1.1	3.2	1.0
<i>o</i> -Xylene	8.7	y = 0.0172x - 0.0007	0.9993	6.9	29584	1.1	3.4	1.0

Notes: Capacity factor (k') = $\frac{tR - t_0}{t_0}$; Theoretical plate number (N) = $16\left(\frac{tR}{W_t}\right)^2$; Resolution (R_s) = $\frac{\sqrt{N}(\alpha - 1)}{4\alpha} \frac{k}{(k + 1)}$; Specificity factor (α) = $\frac{k_2}{k_1}$; $k_1 = tR_1 - tR_0$; $k_2 = tR_2 - tR_0$ Abbreviations: t_k : retention time of the analyte peak; t_0 : retention time of mobile phase peak; WT: peak width; b: slope; a: intercept.

The linearity test implementations were performed between 5 ng/mL and 100 ng/mL in five calibration points, excluding the toluene and butyl acetate chemicals. For these two analytes, since their observed peaks in the real human samples were higher than other analytes, three more calibration points, 200, 500 and 1000 ng/mL, were added in the establishment of their calibration curves.

The determination coefficient values were detected in the range of 0.9993 and 0.9999. The individual calibration curves for all analytes were drawn and their calibration equations were determined by considering the internal standard peak area. The obtained results were presented in Table II and Table III.

TABLE III - Validation test parameters of the developed method, including linearity, sensitivity, and recovery test results calculated for each analyte in accordance with the ICH guideline

Analytes	Linear range (ng/mL)	LOD (ng/mL)	LOQ (ng/mL)	Recovery (%)		
				5 ng/mL	25 ng/mL	105 ng/mL
Benzene	5-100	0.009	0.030	96.7±3.5	96.8±3.6	97.8±2.7
Toluene	5-1000	0.014	0.046	96.1±2.1	96.5±3.7	102.6±1.9
Butyl acetate	5-1000	0.011	0.036	96.3±3.2	103.6±2.7	98.5±2.4
Ethylbenzene	5-100	0.012	0.040	103.8±3.6	96.8±3.2	97.5±2.9
<i>p</i> -Xylene	5-100	0.013	0.043	103.0±3.0	99.6±3.9	103.4±3.8
<i>m</i> -Xylene	5-100	0.018	0.060	96.7±3.8	98.4±3.1	101.9±2.1
<i>o</i> -Xylene	5-100	0.012	0.040	97.5±3.5	98.8±2.4	102.6±1.5

Abbreviations: LOD: Limit of detection, LOQ: Limit of quantification; Equations: $LOD=3.3\sigma/S$; $LOQ=10\sigma/S$ (σ : The SD of the response; S : The slope of the calibration curve).

Sensitivity testing was performed at a concentration of 1 ng/mL, which is one-fifth of the lowest concentration points for all analytes. Limit of detection (LOD) values were ≤ 0.26 ng/mL (0.09 - 0.26 ng/mL) and limit of quantification (LOQ) values were ≤ 0.86 ng/mL (0.30 - 0.86 ng/mL). Recovery test applications were carried out at 5, 25 and 105 ng/mL values. Recovery test results ranged between 96.1% to 103.8% and 96.5% to 103.6% for 5 and 25 ng/mL concentrations, respectively. Another recovery test result, carried out at 105 ng/mL, which is the highest calibration point for all analytes, was between 97.5% and 103.4%. Sensitivity and recovery test results were detailed in Table III.

The repeatability test applications were carried out as intra-day and inter-day precision and accuracy tests at 5,

20 and 100 ng/mL values. At each of these concentrations, five independent samples were individually prepared in blank urine and then they were applied to the gas chromatography system under the given chromatographic conditions. Relative standard deviation (RSD%) values of the obtained results were used in the determination of precision and relative error (RE%) values were used in the calculation of the accuracy results. Considering all carried-out repeatability implementation results, intra-day precision (RSD%) values were ≤ 4.1 and inter-day precision (RE%) values were detected as ≤ 4.7 . Intra-day accuracy (RE%) values were between (-3.6) and 3.2. Inter-day accuracy values were between (-3.9) and 4.7. The results were given in detail in Table IV.

TABLE IV - The repeatability parameters of the method including precision and accuracy values for both intra-day and inter-day applications

Analyte	Intra-day						Inter-day					
	Precision (RSD%)			Accuracy (RE%)			Precision (RSD%)			Accuracy (RE%)		
	5 ng/mL	20 ng/mL	100 ng/mL	5 ng/mL	20 ng/mL	100 ng/mL	5 ng/mL	20 ng/mL	100 ng/mL	5 ng/mL	20 ng/mL	100 ng/mL
Benzene	3.6	3.2	2.9	-3.5	-2.9	-2.0	3.8	3.5	3.4	-3.9	-3.0	-3.1
Toluene	3.9	3.6	1.4	-2.4	-3.1	2.9	3.5	3.1	3.9	-2.8	-3.5	3.0
Butyl acetate	3.5	2.5	3.3	-3.6	2.5	-3.0	4.2	2.7	3.2	-3.9	3.3	-3.2
Ethylbenzene	2.4	3.5	3.1	2.4	-3.5	-3.6	3.7	3.8	4.0	3.7	-3.8	-2.9
<i>p</i> -Xylene	3.9	3.2	3.5	3.2	-0.9	2.8	4.1	4.7	3.9	4.1	-3.2	3.7
<i>m</i> -Xylene	4.1	3.5	2.1	3.1	2.7	1.0	3.9	3.7	3.3	4.2	3.4	1.5
<i>o</i> -Xylene	3.3	3.2	2.8	-3.0	-2.5	1.8	3.9	1.9	3.9	-3.4	-3.1	3.7

A total of 104 real urine samples were analysed, 53 of them belonged to people who had occupational exposure risk and the remaining 51 of them obtained from the people who had no known exposure risk. The samples were successfully analysed under given conditions. The observed results for those with occupational exposure risk were 0.85 - 8.75 ng/mL for benzene, 2.31 - 351.49 ng/mL for toluene, 5.84 - 56.44 ng/mL for ethylbenzene, 0.44

– 9.45 for *p*-xylene, 1.23 and 5.53 ng/mL for *m*-xylene, 0.44 - 12.05 ng/mL for *o*-xylene and 1.05 and 762.10 ng/mL for. In the group who had no known exposure risk, the data were 0.30 - 2.47 for benzene, 0.55 – 3.29 ng/mL for toluene, 2.38 – 14.73 ng/mL for ethylbenzene, 0.56 – 0.96 for *p*-xylene, 0.89 – 2.61 ng/mL for *m*-xylene, 0.73 to 1.26 for *o*-xylene and 1.20- 15.70 nBA. Both groups data were given in Table V and Table VI.

TABLE V - Urine benzene, toluene, ethylbenzene, *p*-, *m*-, *o*-xylene and butyl acetate concentrations of furniture painters at risk of occupational exposure to BTEXs and nBA

Sample number	Benzene (ng/mL)	Toluene (ng/mL)	Ethylbenzene (ng/mL)	<i>p</i> -Xylene (ng/mL)	<i>m</i> -Xylene (ng/mL)	<i>o</i> -Xylene (ng/mL)	Butyl acetate (ng/mL)
ES1	8.75	351.49	5.84	1.47	4.78	7.37	56.14
ES2	2.19	5.85	56.44	1.80	5.32	12.05	29.58
ES3	3.14	191.11	21.13	9.41	2.30	5.69	292.56
ES4	3.20	8.65	52.45	9.45	2.30	2.49	762.10
ES5	3.67	123.38	13.36	4.70	5.53	2.54	56.57
ES6	7.92	129.21	10.25	1.37	3.89	3.45	42.47
ES7	3.34	73.30	8.25	1.43	5.48	1.84	37.00
ES8	8.54	127.28	8.48	1.73	4.73	2.38	44.95
ES9	2.96	132.55	8.26	0.96	3.49	1.56	43.92
ES10	4.69	31.67	7.40	1.17	4.19	1.65	32.13
ES11	6.56	11.24	26.41	8.59	2.30	2.44	104.78
ES12	2.38	5.97	16.29	5.47	4.19	1.61	56.41
ES13	2.24	10.90	17.96	0.96	1.98	2.62	126.46
ES14	1.05	2.31	6.89	0.69	1.32	0.44	1.05
ES15	1.89	94.23	7.49	0.75	2.21	0.64	3.00
ES16	1.21	11.84	6.90	0.98	1.32	0.76	5.51
ES17	1.63	8.22	6.82	0.73	1.37	0.59	5.34
ES18	1.20	81.26	7.58	1.08	2.42	0.95	32.29
ES19	0.92	14.57	6.87	0.44	2.93	1.10	4.78
ES20	1.80	14.37	6.87	4.14	1.52	0.80	4.54
ES21	2.12	12.40	6.80	0.55	1.86	0.60	4.28
ES22	1.26	56.20	51.26	5.32	2.51	0.96	28.49
ES23	1.55	41.23	6.89	7.95	5.12	0.85	56.31
ES24	1.89	84.69	9.32	0.98	3.26	1.26	1.24
ES25	1.63	42.32	7.15	0.95	2.89	2.94	6.32
ES26	0.85	59.79	10.25	8.36	5.18	1.52	48.63
ES27	0.99	21.34	18.26	9.26	2.57	1.25	64.10
ES28	1.96	12.57	21.36	7.14	2.01	0.74	236.26
ES29	1.32	8.39	18.34	6.39	4.94	6.56	1.98
ES30	1.58	9.56	7.69	2.03	5.26	2.39	300.23
ES31	1.89	78.52	12.25	1.26	3.48	1.59	48.54
ES32	2.97	124.63	15.69	3.47	1.26	.75	33.98

Sample number	Benzene (ng/mL)	Toluene (ng/mL)	Ethylbenzene (ng/mL)	<i>p</i> -Xylene (ng/mL)	<i>m</i> -Xylene (ng/mL)	<i>o</i> -Xylene (ng/mL)	Butyl acetate (ng/mL)
ES33	2.87	65.32	7.89	5.69	1.89	2.12	201.36
ES34	3.12	96.74	6.91	0.69	1.57	2.58	24.58
ES35	1.41	102.36	8.65	0.84	1.32	1.25	38.41
ES36	1.56	52.36	9.41	0.77	2.58	1.36	232.98
ES37	.89	2.71	17.36	1.98	1.36	1.52	20.10
ES38	1.42	14.23	11.47	0.55	2.78	1.36	14.63
ES39	1.69	18.65	43.84	0.85	3.59	1.98	2.36
ES40	1.71	156.31	6.25	7.49	3.60	1.47	21.98
ES41	1.42	88.69	15.36	5.36	4.69	2.89	37.56
ES42	1.78	21.39	6.32	1.29	1.23	2.49	145.61
ES43	1.56	71.59	11.25	2.85	1.79	8.69	3.65
ES44	1.64	84.64	8.36	3.47	2.91	1.25	7.35
ES45	1.98	51.22	7.59	5.26	2.04	1.47	9.21
ES46	1.06	96.57	6.64	7.61	4.48	1.59	142.32
ES47	1.65	21.87	9.25	8.36	1.25	2.39	2.36
ES48	1.81	33.58	7.89	0.56	1.78	4.26	0.00
ES49	1.66	74.02	8.56	0.47	2.96	3.32	2.69
ES50	1.77	47.69	40.69	1.52	1.51	0.84	2.94
ES51	1.63	32.56	18.26	1.69	3.74	5.18	123.58
ES52	1.56	89.25	9.64	4.89	4.19	2.69	25.48
ES53	2.28	52.58	14.25	7.06	1.47	1.68	34.75
Min	0.85	2.31	5.84	0.44	1.23	0.44	0.00
Max	8.75	351.49	56.44	9.45	5.53	12.05	762.10
Median	1.77	51.22	9.25	1.73	2.58	1.61	33.14
Mode	1.56	2.31	6.87	0.55	1.32	1.25	2.36
Percentile-25	1.49	13.40	7.28	0.96	1.79	1.18	5.38
Percentile-50	1.77	51.22	9.25	1.73	2.58	1.61	33.14
Percentile-75	2.63	88.97	16.83	5.58	4.19	2.60	56.53

TABLE VI - Benzene, toluene, ethylbenzene, *p*-, *m*-, *o*-xylene, and butyl acetate concentrations in urine samples of participants who may be at risk of environmental exposure but not at risk of occupational exposure

Sample number	Benzene (ng/ mL)	Toluene (ng/ mL)	Ethylbenzene (ng/mL)	<i>p</i> -Xylene (ng/ mL)	<i>m</i> -Xylene (ng/ mL)	<i>o</i> -Xylene (ng/ mL)	Butyl acetate (ng/mL)
CS1	1.32	0.89	2.17	0.55	0.90	1.52	1.63
CS2	0.35	0.54	7.05	0.78	2.61	1.30	1.25
CS3	1.80	0.63	7.43	0.62	1.02	1.19	3.56
CS4	1.11	0.58	7.36	0.49	0.95	0.89	2.68
CS5	1.23	0.74	10.99	0.51	0.63	0.96	3.51
CS6	1.23	0.55	6.74	0.62	1.16	0.94	2.95
CS7	1.88	0.49	2.38	0.52	1.30	0.74	1.63
CS8	1.71	0.74	3.28	0.58	1.41	0.96	2.48
CS9	1.26	0.54	7.97	0.45	1.62	0.78	2.51
CS10	1.5	0.63	1.29	0.49	0.85	0.82	1.26
CS11	1.33	0.71	8.13	0.56	1.56	0.89	0.89
CS12	0.41	0.84	7.00	0.69	1.02	0.74	1.36
CS13	1.73	0.67	7.03	0.48	1.88	0.85	0.51
CS14	1.30	0.78	7.19	0.74	0.95	1.13	1.05
CS15	2.47	3.29	11.62	0.85	1.05	0.93	15.7
CS16	0.79	0.50	6.77	0.62	1.23	0.86	0.75
CS17	1.03	0.78	7.09	0.67	1.42	0.70	1.61
CS18	1.04	0.69	8.77	0.76	1.26	0.69	2.25
CS19	0.97	0.85	9.85	0.59	1.15	0.79	1.20
CS20	1.03	0.96	7.17	0.53	0.96	0.94	1.65
CS21	1.52	0.79	7.82	0.84	0.91	0.71	1.21
CS22	0.8	0.56	6.58	0.51	0.90	0.73	1.37
CS23	0.95	0.51	1.59	0.82	1.03	0.76	1.49
CS24	0.63	0.89	2.74	0.56	1.10	0.97	0.64
CS25	1.20	0.70	6.28	0.71	1.26	0.81	0.79
CS26	0.32	0.96	5.87	0.48	0.97	0.69	0.81
CS27	0.41	0.98	3.97	0.52	0.99	0.72	0.94
CS28	0.71	0.59	2.59	0.57	1.05	0.79	0.84
CS29	1.56	0.96	6.32	0.59	1.25	1.02	1.54
CS30	1.74	2.21	14.73	0.96	2.29	1.26	1.67
CS31	0.55	0.78	4.69	0.62	0.89	1.54	2.84
CS32	0.41	0.93	3.79	0.67	0.95	1.02	2.69

Sample number	Benzene (ng/mL)	Toluene (ng/mL)	Ethylbenzene (ng/mL)	<i>p</i> -Xylene (ng/mL)	<i>m</i> -Xylene (ng/mL)	<i>o</i> -Xylene (ng/mL)	Butyl acetate (ng/mL)
CS33	0.58	0.65	7.48	0.58	2.36	1.63	4.78
CS34	0.73	0.91	3.95	0.79	1.25	1.18	2.47
CS35	1.28	0.48	7.56	0.52	1.20	0.89	1.56
CS36	0.58	0.91	8.25	0.59	1.51	0.81	7.89
CS37	2.21	0.77	2.78	0.84	1.07	0.96	6.32
CS38	1.26	0.53	6.87	0.56	1.23	0.88	1.59
CS39	1.36	0.79	4.41	0.84	1.58	0.74	2.67
CS40	2.01	2.45	4.97	0.71	1.45	0.94	3.49
CS41	1.18	0.78	6.39	0.62	1.60	0.74	1.84
CS42	1.25	1.26	8.26	0.69	1.18	0.77	1.65
CS43	1.89	0.63	3.59	0.57	2.21	0.91	1.48
CS44	0.51	0.71	2.71	0.59	2.15	0.74	1.91
CS45	0.45	0.69	2.05	0.75	1.25	0.61	1.75
CS46	0.85	0.81	8.26	0.99	1.38	0.84	1.49
CS47	2.27	0.96	6.69	1.02	0.91	0.82	5.69
CS48	1.69	0.81	4.18	0.57	1.74	0.93	1.14
CS49	2.02	0.58	8.96	0.79	0.90	0.95	1.56
CS50	1.57	0.96	2.10	0.61	1.41	0.74	1.94
CS51	0.42	0.62	3.26	0.87	0.92	0.93	3.26
Min	0.32	0.48	1.29	0.45	0.63	0.61	0.51
Max	2.47	3.29	14.73	1.02	2.61	1.63	15.70
Median	1.23	0.77	6.69	0.62	1.20	0.88	1.63
Mode	0.41	0.96	8.26	0.62	0.90	0.74	1.49
Percentile-25	0.71	0.62	3.59	0.56	0.96	0.74	1.25
Percentile-50	1.23	0.77	6.69	0.62	1.20	0.88	1.63
Percentile-75	1.57	0.91	7.56	0.76	1.45	0.96	2.68

DISCUSSION

In the study, it was observed that the occupational exposure risk to BTEXs and nBA compared the urinary BTEXs and nBA concentrations with a control group who have no known exposure risk to these volatile compounds. For this purpose, a new method was developed, optimised, and also validated according to the ICH guideline

(ICH, 2005) and also it was successfully applied to the real samples. The analysis method was based on the headspace extraction, gas chromatographic separation and flamed ionization detection. The headspace and gas chromatograph application parameters were optimized and presented in Table I. After the method optimisation application, validation tests were carried out and the sample pre-treatment steps and details were determined

before the real sample analysis applications began. Although the exposure time and conditions are different, samples taken from furniture painting workers known to be exposed to BTEXs and *n*BA were preferred for the determination of occupational exposure. As the control group, urine samples were taken from people who did not have any information about their occupational exposure to BTEXs and *n*BA. Thus, urine samples were taken from two groups consisting of the office workers who

work at the different departments of Ankara University and the furniture painters work in Ankara-Siteler which is the biggest furniture manufacture site of the Middle Anatolian, Turkey. Additionally, the effect of smoking (pocket/day) on the concentration of the BTEXs and *n*BA in the urine samples was investigated. A sample urine chromatogram represented exposure to BTEXs compounds was given in Figure 3.

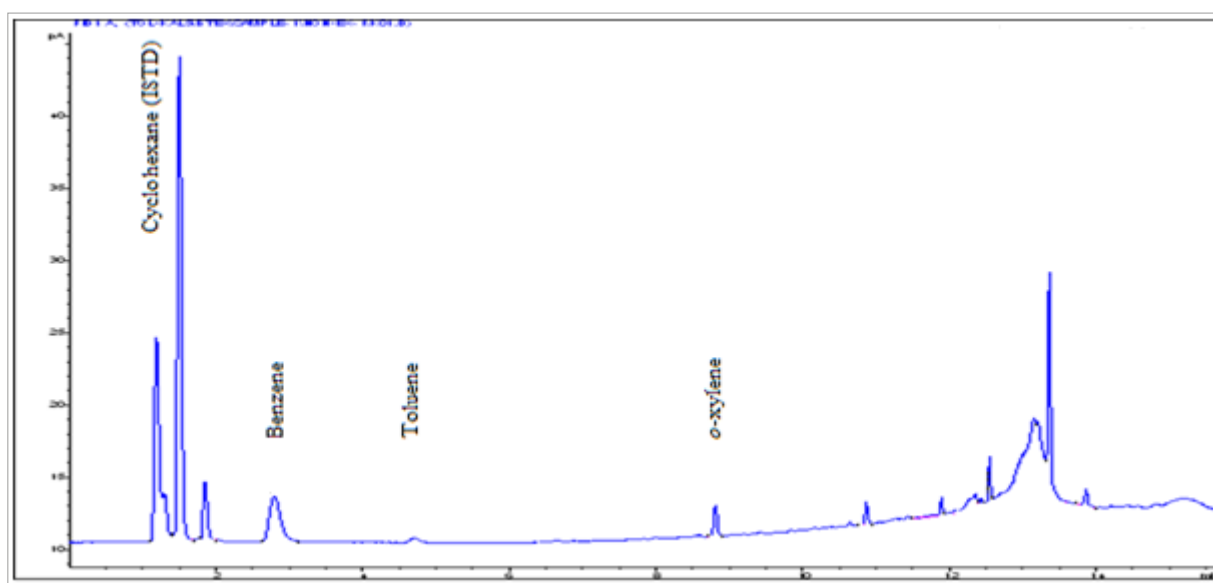


FIGURE 3 - A urine sample chromatogram which belongs to an officer who works at Ankara University. Although it is not known that the volunteer whose urine sample was taken was exposed to volatile solvents from a known source, significant amounts of benzene, toluene and *o*-xylene are detected in the chromatogram. This sample belongs to the control group.

Since BTEXs and *n*BA chemicals likely to be present in the urine are highly volatile, samples were analyzed as soon as possible after collection. Urine samples (10 mL) were transferred to the headspace vials, containing 2.4 g NaCl and 60 mg NaF, from the urine specimen flasks which were filled with samples and then tightly capped. The experiences obtained from the pre-trial studies clearly showed that the analytes, BTEXs and *n*BA, escape from the urine matrix if the urine specimen flasks were not fully filled with the urine sample, so if

any headspace was left in the flask, BTEXs and *n*BA can evaporate and escape from the urine matrix when the flask lid is opened and sample was conveyed to the headspace flask. The real sample results belonging to “occupational exposure risk” group and “no know exposure risk” control group, were given deeply in the Table V and Table VI, respectively. These tables include individual statistically descriptive information belonging to groups data which are min and max values, mode, median, percentile-25 (Q1), percentile-50 (Q2) and percentile-75th (Q3) data.

Are there not any risks for those not occupationally exposed to BTEXs and nBA?

In this study data, it was observed that the occupational exposure risk group's urinary benzene ($p<0.001$), toluene

($p<0.001$), ethylbenzene ($p<0.001$), *p*- ($p<0.001$), *m*- ($p<0.001$), *o*- xylene ($p<0.001$) and butyl acetate ($p<0.001$) concentrations were statistically higher than the no-known exposure group's (control group) values. Results were presented in Figure 4 and Figure 5 as bar charts.

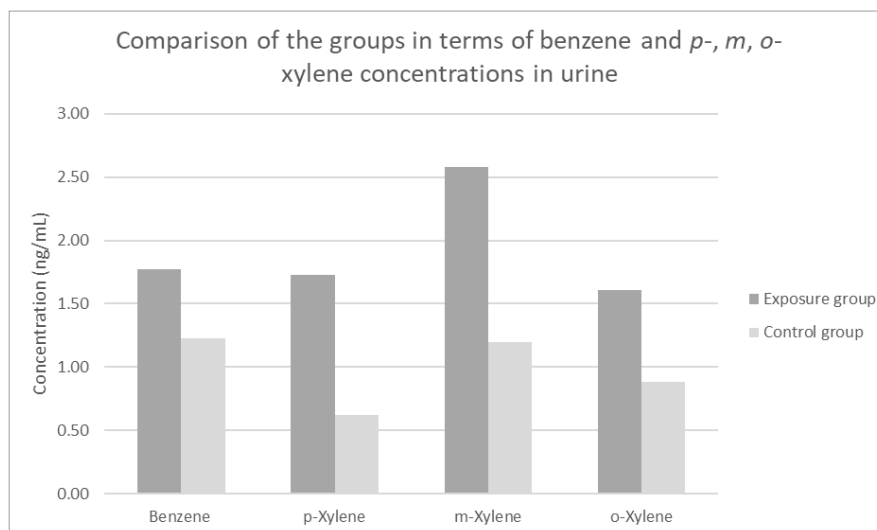


FIGURE 4 - Comparative representation of benzene, *m*-xylene, *p*-xylene and *o*- xylene levels in the urine samples from the occupationally exposed and the general population group with no known exposure to these chemicals for occupational reasons. The three-stars symbol, ***, means $p<0.001$.

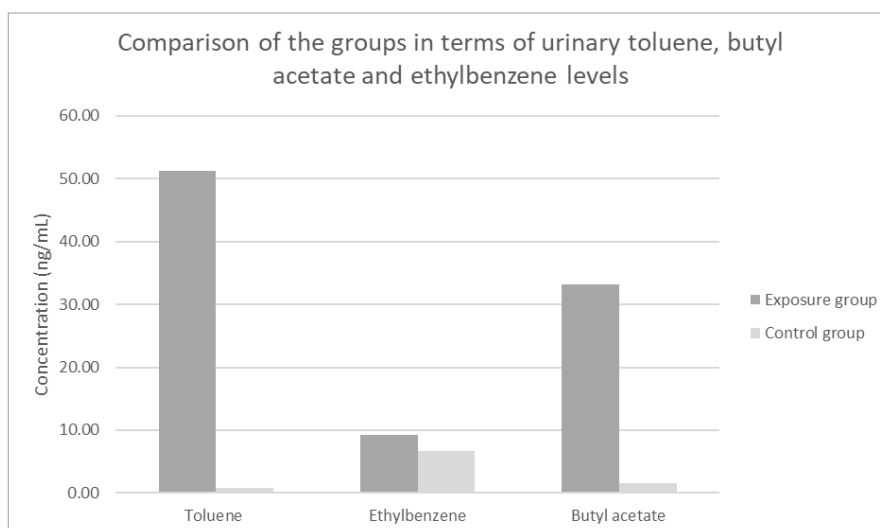


FIGURE 5 - Comparative representation of toluene, ethylbenzene, and butyl acetate levels in the urine samples collected from the occupationally exposed and the general population group with no known exposure to these chemicals for occupational reasons.

The results demonstrating the urinary BTEXs and nBA concentrations of the occupational exposure group were higher than the control group was predicted after receiving information from the workers including observations about the working conditions during the collection of the samples from the workplaces. However, when the BTEXs and nBA concentrations detected in the urine of the exposed group were compared with the control group, the high significance levels ($p < 0.001$) were remarkable in terms of showing the severity of the risk. It has been observed that none of the employees whose urine was taken in the workplaces do not use masks, their daily working hours are over 10 hours on average, and the ventilation in the workplaces is insufficient. Working 6 days a week also caused an increase in the load of volatile organic solvents, especially BTEXs and nBA taken into the body by inhalation, and the main substance-metabolites removed from the body by metabolism activities were insufficient.

Although there was no risk of occupational exposure to BTEXs and nBA in the control group or a known risk to those from whom the samples were taken, surprisingly, urine samples showed high concentrations of BTEXs and nBA chemicals, and in some individuals even higher urinary BTEXs and nBA concentrations similar to those of occupationally exposed individuals. This situation has brought to mind the question of what could be such important sources of exposure to BTEXs and nBA.

High BTEXs and nBA load detected in the urine of a female volunteer from the control group were associated with conditions that changed in a short time in the life of that individual. It was concluded that the person from whom the sample was taken was exposed to organic solvents more than the other control group members due to the fact that she moved to a newly painted house a week ago and the furniture, carpet, curtains, etc. were newly purchased. In other words, the fact that the house where the person lived was newly painted and the presence of products using volatile organic solvents in the production of the house and therefore the indoor air inhaled in the house affected the urinary BTEXs and nBA concentrations.

On the other hand, it was reported that another control group member, whose urine had high concentrations of benzene, toluene, ethylbenzene, and butyl acetate, had just painted his office and bought a new car. It was discovered that another control group member with high BTEXs in his urine had the internal doors of his house painted around 15 days ago. BTEXs and nBA chemicals were used in the preparation of the dye type used by these people. This leads to the conclusion that people have high occupational exposure to these chemicals, but there are also confounding risks of non-occupational exposure. Items such as furniture, carpets and curtains used in daily life in the house are associated with an increase in the load of volatile organic compounds in the indoor air. This situation shows that these chemicals are inevitable substances in terms of exposure.

Ghanbarian *et al.* (2022) evaluated to the BTEXs urinary concentration in the general population who lived in Tehran. In these as biomarkers of exposure BTEXs non metabolized BTEXs compounds in urine were used. The investigation was carried out with total of 76 urine samples. The chromatographic separation was performed with a gas chromatograph and in the quantitative determination a mass selective detector was used. Median urinary levels of benzene, toluene, ethylbenzene, *m* + *p* xylene, *o* xylene were 87.5, 60.5, 98.0, 65.6, and 47.0 ng/mL, respectively. In our study, which we carried out with 54 urinary samples representing the general population, we observed benzene, toluene, ethylbenzene, *p*-xylene, *m*-xylene, *o*-xylene, as 1.23, 0.77, 6.69, 0.62, 1.20 and 0.88 ng/mL respectively. Our urinary benzene, toluene, ethylbenzene, *m* + *p* xylene, *o* xylene concentrations are higher than 14.1, 12.7, 110.6, 27.7 and 18.7 times reported in the Ghanbarian *et al.* (2022) study. The urinary BTEXs concentrations detected in the two studies appear to be significantly different. It is thought that the observed situation may be related to the group members and the number of samples included in the study. In our study, control group samples were collected from people working in the city center. BTEXs and nBA are included in many different substances such as paint, thinner, fuel oil and cigarette smoke. These

types of products are also consumed in high amounts in areas where city life is most intense. On the other hand, the geographical structure of Ankara, the capital city of Turkey, resembles a bowl, and it is one of our provinces where air pollution and particles suspended in the air are the highest from past to present. In the study of Ghanbarian *et al.* (2022) it is seen that urine samples were collected from very large rural areas of Tehran. The differences observed in the two studies were thought to be strongly related to these reasons.

Is smoking an effective exposure source on the urinary BTEXs and nBA levels?

There is various information in the literature about the effect of smoking cigarettes on urinary BTEXs and nBA levels. In a study (Ghanbarian *et al.*, 2022) conducted on the general population (n=76) on this subject, a smoking habit and its effect on urinary BTEXs levels were investigated. The relationship between age, body mass index, gender, job status, and traffic location with urinary BTEXs concentrations was also investigated in the study. In the evaluation, the correlation was observed between urinary benzene ($p < 0.001$), toluene ($p < 0.001$), ethylbenzene ($p < 0.001$), $p + m$ xylene ($p < 0.001$), o -xylene ($p < 0.001$) concentrations and smoking habit. In the study,

it was determined that the urine benzene, ethylbenzene, $p + m$ and o -xylene levels were related to the occupations of the people whose urine samples were taken. However, in that study, p -xylene and m -xylene could not be separated from each other chromatographically. Therefore, p - and m -xylene urine concentrations were determined and evaluated together. In the study, the correlation between the BTEXs concentrations investigated in the urine content was also investigated. The highest correlation was observed between $p + m$ -xylene and benzene ($r = 0.820$) and ethylbenzene ($r = 0.800$). Correlation was observed between benzene and ethylbenzene ($r = 0.750$), benzene and o -xylene ($r = 0.630$), also $p + m$ -xylene and o -xylene concentrations ($r = 0.680$).

In our study, we evaluated the relationship between urinary BTEXs and nBA concentrations and smoking data (paket/per year) of two groups which composed aspect to exposing to volatile organic compounds. Correlations were observed between the annual cigarette consumption data and urine benzene ($r = 0.286^*$), ethylbenzene ($r = 0.552^{***}$), o -xylene ($r = 0.292^*$) concentration in the control group. Also, a correlation was detected between urinary p -xylene and butyl acetate ($r = 0.289^*$) concentrations from subjects belonging to the same group (Table VII).

TABLE VII - Correlation test results of the control group who have no known BTEXs and nBA exposure

		Benzene	Toluene	Ethylbenzene	p-Xylene	m-Xylene	o-Xylene	Butyl acetate	Cigarette (per-year)
Benzene	Correlation coefficient	1.000							
	Sig. (2-tailed)	0.000							
Toluene	Correlation coefficient	0.056	1.000						
	Sig. (2-tailed)	0.696	0.000						
Ethylbenzene	Correlation coefficient	0.128	0.091	1.000					
	Sig. (2-tailed)	0.370	0.527	0.000					
p -Xylene	Correlation coefficient	0.095	0.242	0.115	1.000				
	Sig. (2-tailed)	0.506	0.087	0.421	0.000				

		Benzene	Toluene	Ethylbenzene	<i>p</i> -Xylene	<i>m</i> -Xylene	<i>o</i> -Xylene	Butyl acetate	Cigarette (per-year)
<i>m</i> -Xylene	Correlation coefficient	0.082	-0.038	0.064	0.038	1.000			
	Sig. (2-tailed)	0.566	0.791	0.657	0.790	0.000			
<i>o</i> -Xylene	Correlation coefficient	0.183	0.057	0.096	0.079	-0.061	1.000		
	Sig. (2-tailed)	0.198	0.689	0.504	0.581	0.669	0.000		
Butyl acetate	Correlation coefficient	0.184	0.142	0.105	0.289	-0.030	0.257	1.000	
	Sig. (2-tailed)	0.195	0.320	0.464	p<0.05	0.837	0.068	0.000	
Cigarette (per-year)	Correlation coefficient	0.286	0.191	0.552	0.195	0.079	0.292	0.227	1.000
	Sig. (2-tailed)	p<0.05	0.180	p<0.001	0.170	0.580	p<0.05	0.110	0.000

In the group of the occupationally exposed to BTEXs and nBA, the significant correlations were detected between the urinary benzene and toluene ($r=0.283^*$), benzene and *o*-xylene (0.319^*). Also, correlations were detected between the data of *p*-xylene and ethylbenzene (0.370^{**}), *p*-xylene and butyl acetate (0.430^{**}), *m*-xylene and *o*-xylene ($r=0.373^{**}$). However, there was not any correlation observed between the annual cigarette consumption data and BTEXs and nBA values of the group occupational exposed to the organic solvents (Table VIII). Unmetabolized BTEXs and nBA concentrations in the urine of individuals occupationally exposed to BTEXs and nBA are significantly higher than all BTEXs and nBA values of the control group (Figures 4 and 5). This indicates that the amount of BTEXs and nBA that the control group members were exposed to through inhalation was higher than the control group. The correlation observed between the urine levels of

benzene, ethyl benzene and nBA of the control group and smoking (pack/year) values were not observed in the group members who were occupationally exposed to BTEXs and nBA, possibly because the amount of BTEXs and nBA exposed by smoking was occupationally is associated with much lower than the exposure amount. While a relationship was found between cigarette smoking and benzene, ethyl benzene and n-butyl acetate concentrations in the control group, due to the high level of organic solvent exposure in the occupationally exposed group, although members of this group were also exposed to BTEXs and nBA through cigarette smoke, this effect was suppressed and probably therefore it was not observed. It is thought that increasing the number of control group members, who are closer to the general population, will contribute positively to obtaining more statistical power and more reproducible data in determining the effect of smoking on BTEXs and nBA.

TABLE VIII - Correlation test results belong to the occupationally exposed group to BTEXs and nBA

		Benzene	Toluene	Ethylbenzene	p-Xylene	m-Xylene	o-Xylene	Butyl acetate	Cigarette (per-year)
Benzene	Correlation coefficient	1.000							
	Sig. (2-tailed)	0.000							
Toluene	Correlation coefficient	0.283*	1.000						
	Sig. (2-tailed)	0.040	0.000						
Ethylbenzene	Correlation coefficient	0.083	-0.182	1.000					
	Sig. (2-tailed)	0.556	0.193	0.000					
p-Xylene	Correlation coefficient	0.061	0.045	0.370**	1.000				
	Sig. (2-tailed)	0.666	0.749	0.006	0.000				
m-Xylene	Correlation coefficient	0.102	0.217	0.084	0.188	1.000			
	Sig. (2-tailed)	0.467	0.118	0.548	0.177	0.000			
o-Xylene	Correlation coefficient	0.319*	0.156	0.264	0.157	0.373**	1.000		
	Sig. (2-tailed)	0.020	0.265	0.056	0.263	0.006	0.000		
Butyl acetate	Correlation coefficient	0.273*	0.120	0.186	0.430**	0.241	0.254	1.000	
	Sig. (2-tailed)	0.048	0.397	0.187	0.001	0.086	0.069	0.000	
Cigarette (per-year)	Correlation coefficient	-0.101	0.000	-0.127	-0.142	-0.139	-0.203	-0.251	1.000
	Sig. (2-tailed)	0.470	0.997	0.365	0.310	0.322	0.144	0.073	0.000

While there was a statistically significant relationship between cigarette consumption in the control group and benzene, ethylbenzene, and o-xylene concentrations in the urine, no correlation was observed with cigarette consumption in the group exposed to organic solvents. When both groups are evaluated in terms of all descriptive statistics data (median, min-max) for BTEXs and nBA, it is seen that the control group has significantly lower algebraic values. Although this group is not exposed to organic solvents professionally, volunteers in this group are at risk of being exposed to these solvents from various sources such as gas stations, paint products, tobacco use, etc. in their daily lives. Increasing tobacco use and secondary exposure in the community results in its emergence as an important source that has the power to change the amount of BTEXs and nBA exposed and urine concentrations. Therefore, it is significant that the concentrations of benzene, ethylbenzene and butyl

acetate in urine change as a biomarker related to cigarette consumption (pocket/year). On the other hand, the lack of a relationship between cigarette consumption and urinary BTEXs and nBA levels in the exposed group is related to the fact that the organic solvent load in the body due to occupational BTEXs and nBA exposure is too high to be changed by BTEXs and nBA exposure through smoking. Although not significant, the correlation trend between nBA and cigarette consumption was thought to be related to this situation.

According to the method sensitivity tests, the LOQ values were found to be between 0.30 ng/mL and 0.86 ng/mL, and the recovery test results were found to be between 96.1% and 103.4%, considering all the tested concentration values of BTEXs and nBA analytes. Precision test results range from 1.4 to 4.7 (RSD%). Accuracy test data ranges from (-3.9) to 4.7 RE%.

Linearity test data is ≥ 0.9993 (r^2) when all analytes are taken into account. The obtained validation data proved that the method is at a level to enable detection and quantification of benzene, toluene, ethylbenzene, *p*, *m*, *o*-xylene and butyl acetate in urine samples of groups with occupational exposure and no known risk of exposure to organic solvents.

Ghanbarian *et al.* (2022) were monitored benzene, toluene, ethylbenzene, *p* + *m* and *o*-xylene levels in the urine samples belonging to the general population. It was observed that the study results' median values were 87.5, 60.5, 98.0, 65.6 and 47.0 pg/mL. They utilised a head space solid phase micro extraction method in the sample pre-treatment and a gas chromatograph coupled with a mass spectrometer for separation and quantitation of the analytes (Ghanbarian *et al.*, 2022). In this study, since *p*+*m* xylene could not be separated chromatographically, the amounts in the urine were calculated and given together. As can be clearly seen from the real sample result in our study (Table V and VI), *p* and *m* xylene have different concentrations in the urine. Successful separation of these two xylene derivatives and calculation of their respective concentrations are important, as they are used to calculate xylene exposure. In this study, we optimized the oven temperature for the analysis. Although, they were not given any validation result in their study, considering the real sample results the LOD of the method can be assumed as ≤ 47 pg/mL since the lowest result was given as is. This result clearly pointed out higher sensitivity values than our suggested method. However, they were using a head space solid phase extraction (HS-SPME) method and a mass selective detector (MS) in their research. As clearly known, the MS detector depending on the used ion-monitoring mode has a higher sensitivity compared to the flamed ionisation detector (Dinh, Thompson, 2015). GC systems with MS detectors require some special conditions, such as the inability to turn off the device, the need for special high-purity gases, apart from the analyses processes, which cause an increase in analysis costs compared to FID systems. The sensitivity ability of the HS-SPME technique is higher than the classical headspace system. However, the system requires special

equipment for extraction and experience and skill for successful application. The successful results obtained from the study we applied to the analysis of real samples showed that the proposed method in the analysis of unmetabolized BTEXs and *n*BA levels from urine has appropriate repeatability, sensitivity and recovery values. This method gave successful results in both occupational and general population analyses of BTEXs and *n*BA.

Analyte standards were prepared in headspace sample vials. The master stock and working solutions of BTEXs and *n*BA analytes were prepared in sample containers sealed with a rubber septum. The use of 10, 50, 100, 250 μ L GC injectors during the process of establishing the standards significantly increased the sensitivity during the development of the method. Due to the fact that BTEXs and *n*BA are volatile organic solvents, their vapor pressures are quite high and this causes their rapid loss from the environment when they come into contact with open air. In the preliminary tests of the method development, as stated in various publications, the freezing and re-thawing of urine samples caused the loss of BTEXs and *n*BA in its content.

For this reason, in the developed method, urine samples were not frozen, instead, if the urine samples were taken in the laboratory, they were placed as 10 mL in the HS vial without waiting, and the mouth was tightly closed with a sealant. It was stored at $+4^\circ\text{C}$ until analysis, which was to be carried out within two days at the latest. If the sample was taken at the workplace, taking into account the hygiene rules, after the relevant sample container was completely filled with urine, its mouth was tightly closed without leaving any upper space, and it was cooled with ice batteries and delivered to the laboratory, and transferred to headspace vials within 2 hours at the latest.

The present study showed that not only furniture staining workers are at risk of occupational exposure but also city dwellers are at inevitable exposure risk to BTEXs and butyl acetate chemicals. Although urine data obtained from the study show that non-occupational exposure to organic solvents is significantly lower than occupational exposure, exposure to BTEXs and *n*BA,

especially benzene, with their carcinogenic power, poses a serious risk for public health.

In individuals who are occupationally exposed to organic solvents, smoking habitually cannot change their urinary BTEXs and nBA concentrations. However, individuals who smoke without active exposure to organic solvents from a known source can significantly alter urine benzene, toluene, ethylbenzene, and n-butyl acetate levels as a biomarker.

The analysis method includes advantages such as low analysis cost for the detection of a total of 7 volatile compounds, including six analytes and one internal standard, from urine, having a practical sample preparation protocol, and high analysis efficiency by determining the optimum method conditions in a 9-minute analysis period. The data obtained from the accuracy and precision validation tests quantitatively show the agreement of the expected and observed data in the preparation of the quality control samples. The validation of the method by performing validation tests in accordance with the ICH guidelines is also of great importance in terms of the reliability of the real human urine data obtained. Loss of volatile organic solvents during experimental applications is the most important challenge of the application. Placing

the urine samples directly into the headspace vials and performing the analysis within two days at the latest is effective in preventing the escape of BTEXs and nBA chemicals from the sample matrix. In this respect, the observations obtained from the study showed that storing the urine samples in a separate urine container at +4°C after collection from the human or freeze-thawing for long-term storage application causes significant loss of analytes. Although if necessary, it contains hygienic risks, urine can be analyzed by placing it in headspace vials, after being completely filled with the sample so that there is no space on the screw cap test tubes and stored at +4°C for a maximum of one day. The greenness of the method was evaluated with an online software called AGREEprep. In this method, the highest value “1” represents the highest greenness of the method and the lowest value is 0, which means to it doesn't have any greenest properties. The calculated value of the method is 0.68 means that the suggested method has acceptable, good greenness properties and it can be used for many types of purposes (Figure 6). So, the developed method can be used reliably in accredited toxicology laboratories also occupational medicine laboratories.

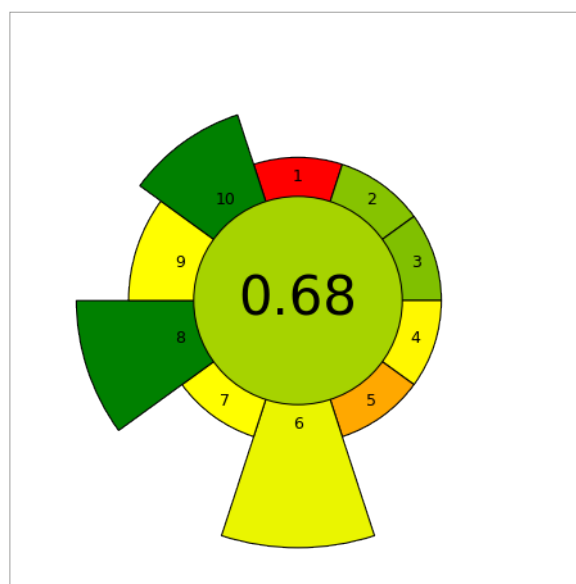


FIGURE 6 - The AGREEprep report that developed the demonstrate the greenness of the developed method.

In this study, we developed a determination method for the un-metabolised BTEXs and nBA in human urine then it was validated by ICH guidelines. Following, to determination of the exposure levels of VOCs, urinary un-metabolized BTEXs and nBA concentrations were analysed in the real human urine samples. The comparison of some highlighted results observed from the suggested method and also the comparisons of the methods are given in Table IX. In this study, a GC (FID) system coupled with a static headspace was employed although the compared other studies used more sophisticated extraction and analysis systems for example; HS-SPME/GC-MS, HS-SPME/GC-FID, HS/GC-MS, Dynamic HS-PT/GC-PID,

Cooling/heating-assisted HS-SPME/GC-FID, Dynamic HS/ GC-PID, Dynamic HS-needle trap/GC-FID (Table IX). Determination coefficient (r^2) value of our suggested study was the 0.9993 which is the highest values in one of two values of these studies. The highest accuracy values were observed in the suggested method when compared with other studies (Table IX). In this study, precision values were equal and below 4.7 (RSD%). This is the highest value observed from the all studies in Table IX. A significant recovery values were observed. Suggested method has excellent sensitivity compared other studies. The lowest LOD (0.018 ng/L) value were detected from the method.

TABLE IX - The validation parameters and chromatographic properties of BTEXs and nBA determination methods from urine samples in literature

Technique	Instrumentation	Linearity (ng/mL)	Accuracy (RE%)	Precision (RSD%)	Recovery (%)	LOD (ng/L)	References
HS	GC-FID	ND	ND	ND	ND	ND	Ghanbarian <i>et al.</i> (2022)
Dynamic HS-needle trap	GC-FID	ND	ND	1.9 – 5.6	95.0-99.0	≤1.10	Rahimpoor <i>et al.</i> (2021)
Dynamic HS	GC-PID	≥0.9993	(-20) - 0	0.2 – 10.0	80-100	0.02-0.04	Brčić, Skender, (2003)
Cooling/heating-assisted HS-SPME	GC-FID	≥0.995	ND	4.8 – 10.6	92 - 102	≤0.02	Tajik <i>et al.</i> (2017)
Dynamic HS-PT	GC-PID	≥0.998	(-20) - 0	0.2 – 10.0	ND	≤0.03	Karaconji, Skender, (2007)
HS-SPME	GC-MS	≥0.998	(-9.0) - 0	2.0 – 11.0	ND	≤0.05	Karaconji, Skender, (2007)
HS-SPME	GC-FID	≥0.9981	ND	3.1 – 5.2	ND	≤0.071	Hrivnák, Královicová, (2009)
HS	GC-MS		(-6.8) – (10.0)	0.8 - 13.4	>90	≤0.052	Perbellini <i>et al.</i> (2002)
Our proposed method (HS)	GC-FID	≥0.9993	(-3.9)-4.7	1.4 – 4.7	96.1 – 103.4	0.018	-

Abbreviations: ND: No data, PID: photoionization detector, HS: headspace, SPE: solid phase microextraction, SPME: solid phase microextraction, PT: Purge and trap

A novel, sensitive, practical, reliable and reproducible HS-GC-FID assay was developed for the determination of BTEXs and butyl acetate. This method was successfully validated in terms of linearity, sensitivity, recovery,

and repeatability test including intra-day and inter-day precision and accuracy. The suitability of the standard addition procedure performed with the help of a GC injector in closed containers with rubber septum, which

was preferred in the development of the method, was determined with the high determination coefficients obtained in the linearity data.

Examination of this risk with living cells in a special experimental setup, taking into account the physical properties of BTEXs and nBA, is necessary for the quantitative determination of the toxicological risks of the relevant chemicals at these concentrations.

In the continuation of this study, it is necessary to reevaluate the BTEXs and nBA concentrations detected in the urine contents of the exposed and control groups and to apply them to lymphoblastoid-derived special cell lines in an *in vitro* cell culture-based study, and to investigate the cytotoxicity and genotoxicity effects with RPD% and micronucleus tests. Exposure to BTEXs and nBA chemicals observed in the control group of the study makes it necessary and important in terms of public health to determine the sources of this inevitable condition, which is thought to take place in daily life, or to minimize exposure.

ACKNOWLEDGEMENTS

In this study, Ankara University Institute of Forensic Sciences research laboratories were used. The authors would like to thank the institute management and laboratory staffs for the open collaboration and support presented in the implementation of this research.

Funding

The authors declare that no funds, grants, or other support were received during the carry-out of this study and preparation of this manuscript.

Declaration of competing interest

The author declares that they have no-known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

- Adams JC, Dills RL, Morgan MS, Kalman DA, Pierce CH. A physiologically based toxicokinetic model of inhalation exposure to xylenes in Caucasian men. *Regul Toxicol Pharmacol.* 2005;43(2):203-214.
- Alexopoulos EC, Chatzis C, Linos A. An analysis of factors that influence personal exposure to toluene and xylene in residents of Athens, Greece. *BMC Public Health.* 2006;6:50
- Alkalde TK, Peralba MdCR, Zini CA, Caramão EB. Quantitative analysis of benzene, toluene, and xylenes in urine by means of headspace solid-phase microextraction. *J Chromatogr A.* 2004;1027(1-2):37-40.
- Alsalka Y, Karabet F, Hashem S. Development and optimisation of quantitative analytical method to determine BTEX in environmental water samples using HPLC-DAD. *Anal Methods.* 2010;2(8):1026-1035.
- Amini H, Hosseini V, Schindler C, Hassankhany H, Yunesian M, Henderson SB, et al. Spatiotemporal description of BTEX volatile organic compounds in a Middle Eastern megacity: Tehran Study of Exposure Prediction for Environmental Health Research (Tehran SEPEHR). *Environ Pollut.* 2017;226:219-229.
- Andreoli R, Manini P, Bergamaschi E, Brustolin A, Mutti A. Solid-phase microextraction and gas chromatography-mass spectrometry for determination of monoaromatic hydrocarbons in blood and urine: Application to people exposed to air pollutants. *Chromatographia.* 1999;50(3):167-172.
- Assadi Y, Ahmadi F, Hossieni MRM. Determination of BTEX Compounds by Dispersive Liquid-Liquid Microextraction with GC-FID. *Chromatographia.* 2010;71(11):1137-1141.
- Brčić Karačonji I, Skender L, Karačić V. Determination of nicotine and cotinine in urine by headspace solid phase microextraction gas chromatography with mass spectrometric detection. *Acta Chim. Slov.* 2007;54(1):74-78.

- Bushy Run Research Center (Union Carbide Corporation). *n*-Butyl Acetate: Acute 346 Vapor Inhalation Toxicity Test in Rats. Project no. 50-135. Pennsylvania; 1987.
- Calabrese EJ, Kenyon EM. *Air Toxics and Risk Assessment*. Chelsea, Michigan, Lewis Publishers, 1991.
- Chary NS, Fernandez-Alba AR. Determination of volatile organic compounds in drinking and environmental waters. *TrAC - Trends Anal Chem*. 2012;32:60-75.
- David RM, Tyler TR, Ouellette R, Faber WD, Banton MI. Evaluation of subchronic toxicity of *n*-butyl acetate vapor. *Food Chem Toxicol*. 2001;39(8):877-886.
- Duydu Y, Süzen S, Erdem N, Uysal H, Vural N. Validation of hippuric acid as a biomarker of toluene exposure. *Bull Environ Contam Toxicol*. 1999;63(1):1-8.
- Fakhari AR, Hasheminasab KS, Baghdadi M, Khakpour A. A simple and rapid method based on direct transfer of headspace vapor into the GC injector: application for determination of BTEX compounds in water and wastewater samples. *Anal. Methods*. 2012;4(7):1996-2001.
- Fay M, Eisenmann C, Diwan S, de Rosa C. ATSDR evaluation of health effects of chemicals. V. Xylenes: health effects, toxicokinetics, human exposure, and environmental fate. *Toxicol Ind Health*. 1998;14(5):571-781.
- Franco G, Fonte R, Tempini G, Candura F. Serum bile acid concentrations as a liver function test in workers occupationally exposed to organic solvents. *Int Arch Occup Environ Health*. 1986;58(2):157-164.
- Fustinoni S, Giampiccolo R, Pulvirenti S, Buratti M, Colombi A. Headspace solid-phase microextraction for the determination of benzene, toluene, ethylbenzene and xylenes in urine. *J Chromatogr B Biomed Sci Appl*. 1999;723(1-2):105-115.
- Ghanbarian M, Nazmara S, Masinaei M, Ghanbarian M, Mahvi AH. Evaluating the exposure of general population of Tehran with volatile organic compounds (BTEX). *Int J Environ Anal Chem*. 2022;102(16):4261-4271.
- Ghittori S, Alessio A, Negri S, Maestri L, Zadra P, Imbriani M. A Field Method for Sampling Toluene in End-Exhaled Air, as a Biomarker of Occupational Exposure: Correlation with Other Exposure Indices. *Ind Health*. 2004;42(2):226-234.
- González JL, Pell A, López-Mesas M, Valiente M. Simultaneous determination of BTEX and their metabolites using solid-phase microextraction followed by HPLC or GC/MS: An application in teeth as environmental biomarkers. *Sci Total Environ*. 2017;603-604:109-117.
- Hinwood AL, Rodriguez C, Runnion T, Farrar D, Murray F, Horton A et al. Risk factors for increased BTEX exposure in four Australian cities. *Chemosphere*. 2007;66(3):533-541.
- Hrivnák J, Král'ovicová E, Tölgyessy P, Ilavský J. Analysis of unmetabolized VOCs in urine by headspace solid-phase microcolumn extraction. *J. Occup. Health*. 2009;51(2):173-176.
- International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonised Tripartite Guideline. Validation of Analytical Procedures: Text and Methodology ICH Q2(R1).2005.
- International Agency for Research on Cancer (IARC). *Agents Classified by the IARC Monographs, Volumes 1-134*.;2021.
- Jang J, Droz PO, Kim S. Biological monitoring of workers exposed to ethylbenzene and co-exposed to xylene. 2001;74(1):31-37.
- Karačonji IB, Skender L. Comparison between dynamic headspace and headspace solid-phase microextraction for gas chromatography of BTEX in urine. *Arh Hig Rada Toksikol*. 2007;58(4): 421-427.
- Ketola RA, Virkki VT, Ojala M, Komppa V, Kotiaho T. Comparison of different methods for the determination

of volatile organic compounds in water samples. *Talanta*. 1997;44(3):373-382.

Khajeh M, Zadeh FM. Response surface modeling of ultrasound-assisted dispersive liquid-liquid microextraction for determination of benzene, toluene and xylenes in water samples: Box-behnken design. *Bull Environ Contam Toxicol*. 2012;89(1):38-43.

Khan HA. Benzene's toxicity: a consolidated short review of human and animal studies. *Hum Exp Toxicol*. 2007;26(9):677-685.

Lai JQ, Lai SY, Ye XL, Wang C, Ye M. Determination of 6 BTEXs in urine by purge and trap with gas chromatography-mass spectrometry. *Chinese J Ind Hyg Occup Dis*. 2022;40(8):619-622.

Langman JM. Xylene: Its toxicity, measurement of exposure levels, absorption, metabolism and clearance. *Pathology*. 1994;26(3):301-309.

Lv Y, Jiang Y, Lu J, Gao H, Dong W, Zhou J et al. Comprehensive evaluation for the one-pot biosynthesis of butyl acetate by using microbial mono- and co-cultures. *Biotechnol Biofuels*. 2021;14:203.

Nagaraju P, Vijayakumar Y, Ramana Reddy M V. Room-temperature BTEX sensing characterization of nanostructured ZnO thin films. *J Asian Ceram Soc*. 2019;7(2):141-146.

NTP Toxicology and Carcinogenesis Studies of Ethylbenzene (CAS No. 100-41-4) in F344/N Rats and B6C3F1 Mice (Inhalation Studies). *Natl Toxicol Program Tech Rep Ser*. 1999;466:1-231.

Perbellini L, Pasini F, Romani S, Princivale A, Brugnone F. Analysis of benzene, toluene, ethylbenzene and m-xylene in biological samples from the general population. *J. Chromatogr. B Biomed. Appl*. 2002;778(1-2):199-210.

Polkowska Z, Kozłowska K, Namieśnik J, Przyjazny A. Biological fluids as a source of information on the exposure of man to environmental chemical agents. *Crit Rev Anal Chem*. 2004;34(2):105-119.

Rahimpour R, Firoozichahak A, Nematollahi D, Alizadeh S, Alizadeh PM, Langari AAA. Bio-monitoring of non-metabolized BTEX compounds in urine by dynamic headspace-needle trap device packed with 3D Ni/Co-BTC bimetallic metal-organic framework as an efficient absorbent. *Microchem J*. 2021;166:106229.

Saillenfait AM, Gallissot F, Sabaté JP, Bourges-Abella N, Muller S. Developmental toxic effects of ethylbenzene or toluene alone and in combination with butyl acetate in rats after inhalation exposure. *J Appl Toxicol*. 2007;27(1):32-42.

Sarafraz-Yazdi A, Khaleghi-Miran SH, Es'haghi Z. Comparative study of direct immersion and headspace single drop microextraction techniques for BTEX determination in water samples using GC-FID. *Int J Environ Anal Chem*. 2010;90(14):1036-1047.

Sato A. Toxicokinetics of benzene, toluene and xylenes. *IARC Sci Publ*. 1988;85: 47-64.

Tajik L, Bahrami A, Ghiasvand A, Shahna FG. Determination of BTEX in urine samples using cooling/heating-assisted headspace solid-phase microextraction. *Chem Pap*. 2017;71(10):1829-1838.

Wilkins-Haug L. Teratogen update: Toluene. *Teratology*. 1997;55(2):145-151.

Yu B, Yuan Z, Yu Z, Xue-song F. BTEX in the environment: An update on sources, fate, distribution, pretreatment, analysis, and removal techniques. *Chem Eng J*. 2022;435:134825.

Zhou YY, Yu JF, Yan ZG, Zhang CY, Xie YB, Ma LQ, et al. Application of portable gas chromatography-photo ionization detector combined with headspace sampling for field analysis of benzene, toluene, ethylbenzene, and xylene in soils. *Environ Monit Assess*. 2013;185(4):3037-3048.

Received for publication on 22nd January 2024

Accepted for publication on 15th May 2024

Associated Editor: Marcos Bruschi