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Low volume liquid-liquid extraction for the determination of benzene, toluene, and xylene in water by GC-FID and HPLC-UV

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Abstract. A simple sample preparation method based on green analytical chemistry was proposed to measure benzene, toluene, and xylene (BTX) in water. The method implemented low volume liquid-liquid extraction for extraction of BTX from water using acetonitrile without any solid phase extraction (SPE) clean-up to cut the chemical consumption during the sample preparation. The quantification of BTX was done by GC-FID and HPLC-UV for comparison. The result showed that the method in combination with GC-FID was able to detect toluene and xylene in spiked water samples below the limit regulated by Ministry of Health of Indonesia (MRL toluene=0.7 mg/L; MRL xylene 0.5 mg/L) but not for benzene (MRL benzene=0.01 mg/L).

1. Introduction

Benzene, toluene, and xylene (BTX) are known as toxic chemicals. The international agency for research on cancer (IARC) has classified benzene as group 1 because it has been proved to be carcinogenic to humans [1]. Toluene and xylene, although were classified as group 3 [2], had been shown to be toxic with some adversity effects on humans, especially for human kidneys and liver. As major volatile contaminants in indoor air pollution, their occurrences have been associated with fatigue, nausea, skin and eye irritation, and influenza [3]. Moreover, human exposure may cause drowsiness and dizziness, rapid or irregular heartbeat, and even death if breathing in a high concentration of benzene [4-6].

BTX are volatile compounds thus easily emitted to the atmosphere and becoming major contaminants in the atmosphere. Therefore many research has focused on the analysis of BTX in the atmosphere [7-11] in which analysis of BTX usually performed by applying porous materials as adsorbents to adsorb BTX before submitting to instruments for quantification [10,12].

Similar to the atmosphere, research on BTX analysis in water also utilize adsorbent to adsorb BTX [13,14]. Likewise, liquid-liquid extraction was also applied for the analysis of BTX in river water but with the utilization of solid phase extraction afterwards. The previously mentioned techniques are considered costly since they are needed SPE or adsorbents that had to be synthesized. Moreover, direct analysis of BTX without extraction was recently implemented for the surface waters [15] but with an additional instrument which had to be built specifically for the analysis.

Our study was aimed to develop and validate a simple low volume liquid-liquid extraction, based on green analytical chemistry, as a sample preparation for the determination of BTX in water followed by



quantification by gas chromatography–flame ionization detector (GC-FID) and high-performance liquid chromatography–ultraviolet detector (HPLC-UV).

2. Experimental setup

2.1. Chemicals and reagents

Benzene, toluene, xylene, n-hexane, methanol (HPLC grade), and magnesium sulfate were sourced from Merck. The stock solution was prepared in n-hexane (for standard) and methanol (for spike experiment) at 1000 mg.L⁻¹. Standard solutions were prepared by diluting the stock solution in n-hexane.

2.2. Apparatus

GC-FID analysis was carried out with an Agilent 7890B fitted with an Agilent HP-5 column while HPLC-UV analysis was carried out with an HPLC Hitachi-7000 series fitted with a μ -Bondapak C18 column.

2.3. Water samples

The water sample was collected from the Campus of LIPI, Bandung, Indonesia and stored at 4°C before being used.

2.4. Sample extraction

Low volume liquid-liquid extraction was performed in a rotary agitator. For the spike experiment, 30 mL of water sample was spiked with stock solution of BTX and then extracted with 3 mL of n-hexane. The extraction was done twice. The control samples were conducted similarly but without the BTX spiking. The n-hexane phase was then quantified either by HPLC-UV or GC-FID. For GC-FID quantification, the n-hexane was passed through a column containing magnesium sulfate to remove the water residue before being injected. A clean-up step using silica gel was also applied after the liquid-liquid extraction for some samples to see the effect of the clean-up.

2.5. Method evaluation

The method was evaluated through the limit of detection, linearity, precision, percent recovery, and matrix effect.

3. Results and discussion

Quantification of BTX was done by GC-FID and HPLC-UV for comparison. GC-FID was optimized for the relative lower concentrations while HPLC-UV for the higher concentrations.

3.1. Instrument optimization and qualitative analysis

Before the extraction, the GC-FID and HPLC-UV were optimized for good separation of BTX using mix standard and individual standard solutions. The separation of BTX by GC-FID was achieved at these following conditions. The injector and detector temperatures were 200°C and 250°C, respectively. The oven temperature was programmed from 50°C (2 min) to 100°C at 20°C min⁻¹, then to 150°C at 30°C min⁻¹, and finally to 225°C at 40°C min⁻¹ and was held at 225°C for 4 min. Samples for analysis were injected (1 μ L) on split mode (1:1) by an automatic liquid sampler. Helium was used as the carrier (1 mL min⁻¹) and make up (25 mL min⁻¹). The flow of helium gas and air were programed at 60 and 400 mL min⁻¹, respectively.

The separation of BTX by HPLC-UV was performed at ambient temperature and the detector was set at 254 nm. The mobile phase was methanol-water (7:3) at 0.8 mL min⁻¹ on isocratic mode. The injection volume was 20 μ L.

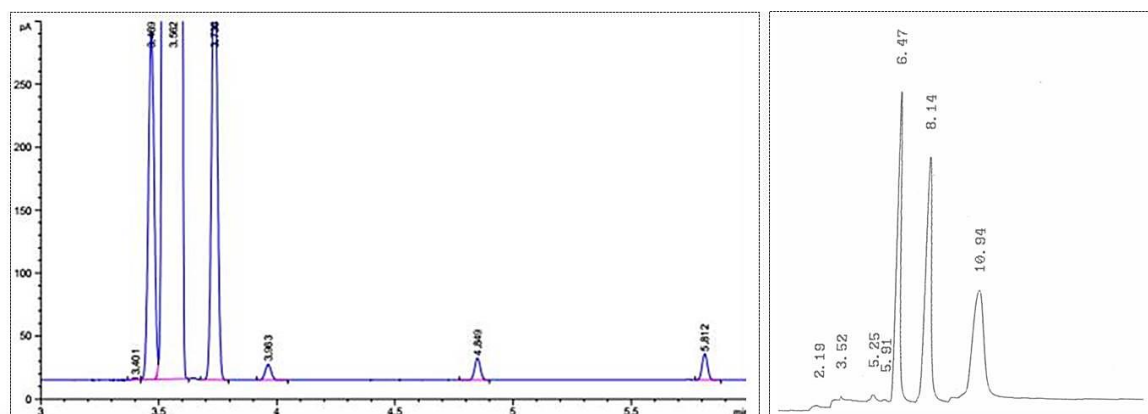


Figure 1. GC-FID (left) and HPLC-UV (right) chromatogram of BTX.

The chromatograms for BTX analysis by GC-FID and HPLC-UV is shown in Figure 1. It can be seen from the chromatograms that GC-FID at the above-mentioned conditions was able to separate benzene, toluene and xylene. Similarly, HPLC-UV was also able to separate benzene, toluene, and xylene.

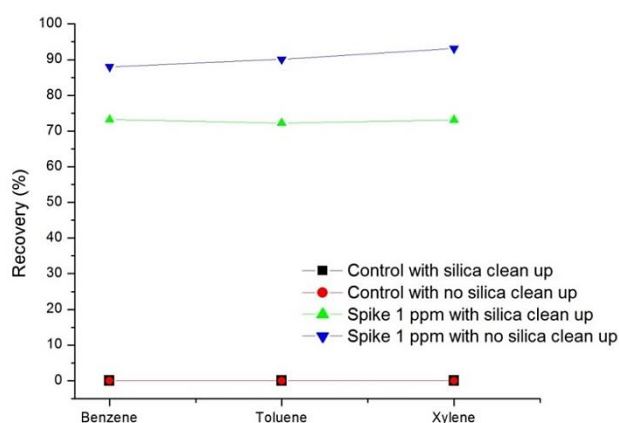


Figure 2. Recovery of BTX with and without silica clean-up.

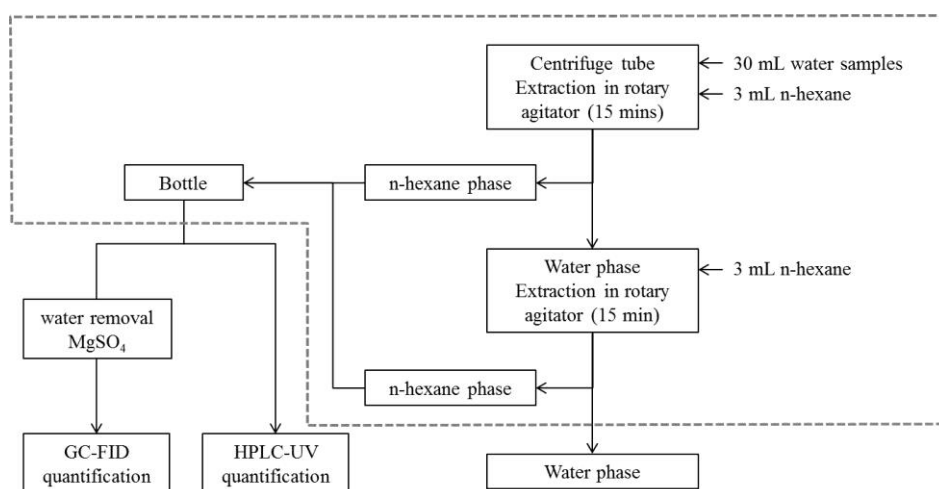


Figure 3. Extraction procedure of the method (inside the dashed area).

3.2. Extraction

A silica clean-up was applied to the n-hexane phase in order to remove interferences. Silica gel, which is a polar compound, was expected not to retain BTX, non-polar compounds. However, the result (Figure 2) shows that silica clean-up reduces the recovery of BTX thus implying that some of the BTX was retained in the silica. This probably due to the molecule structures of BTX that were sterically hindered when passing through the silica gel. Therefore for further experiment, the silica clean-up was removed from the procedure.

The step-by-step extraction procedure is given in Figure 3. The step was simple and utilizing no absorbent or clean-up procedure, therefore, was cheaper compared to methods which utilized absorbent or clean-up procedure as mentioned in the Introduction.

3.3. Evaluation of the method

The result of the method evaluation is shown in Table 1.

Table 1. Evaluation of the method.

Instrument	GC-μECD			HPLC-UV		
Compound	Benzene	Toluene	Xylene	Benzene	Toluene	Xylene
MRL ^a	0.01	0.7	0.5	0.01	0.7	0.5
LOD	0.1	0.1	0.1	0.3	0.1	0.3
Linearity Standard						
R	0.9976	0.9979	0.9987	0.9989	0.9992	0.9985
Slope	0.3170	0.3424	0.3458	1200.3389	1308.1609	1005.2225
Intercept	0.1751	0.0823	0.1345	1495.0000	761.7000	342.8000
Linearity of method						
R	0.9999	0.9958	0.9983	0.9991	0.9379	0.8452
Slope	0.9698	1.4425	1.4896	5247.7572	9479.9157	6724.1484
Intercept	0.1266	0.0042	0.1032	347.8145	2753.2412	1435.3356
Precision (n=6)						
%RSD	8.0549	2.4232	6.5246	3.3144	6.4986	11.9012
2/3 CV Horwitz	10.2611	10.2317	10.1443	8.5872	8.3705	8.4799
% Recovery						
Average	85.98	87.86	93.29	84.20	100.06	92.06
Deviation (\pm)	6.93	2.13	6.09	2.79	6.50	10.96
One-way ANOVA						
F _{calculation}	0.006	1.662	0.038	0.706	1.061	1.256
F _{critical}	4.965	4.965	4.965	4.965	4.965	4.965

^a Permenkes [16]

3.3.1. Limit of detection. The limit of detection (LOD) was measured by spiking BTX to the water samples at a low concentration and then analysing the result. If the BTX was detected, the experiment was repeated at a lower concentration until the BTX was not detected by the instrument. It was shown from Table 1 that for GC-FID quantification, the LODs for toluene and xylene are below the maximum residue limit (MRL) for drinking water in Indonesia [16]. Similarly, for HPLC-UV quantification, the LODs for toluene and xylene were below the MRLs. Therefore, this method can be applied to detect toluene and xylene in drinking water samples in order to meet the MRLs from the Indonesian

Government. However, this method cannot be applied for benzene since it required a lower LOD that cannot be achieved by the method. This may due to the reason that benzene is more volatile than toluene and xylene thus easily release and enter the atmosphere during the extraction.

3.3.2. Linearity. Linearity was measured by analysing the standard solutions and the method at several concentrations. Good linearity with a correlation coefficient higher than 0.997 was observed for the standard solutions (calibration curve) for both GC-FID and HPLC-UV as shown in Figure 4 and Figure 5. The linearity of the method was good for GC-FID quantification (>0.995) but relatively not for HPLC-UV quantification especially for toluene and xylene ($r < 0.995$).

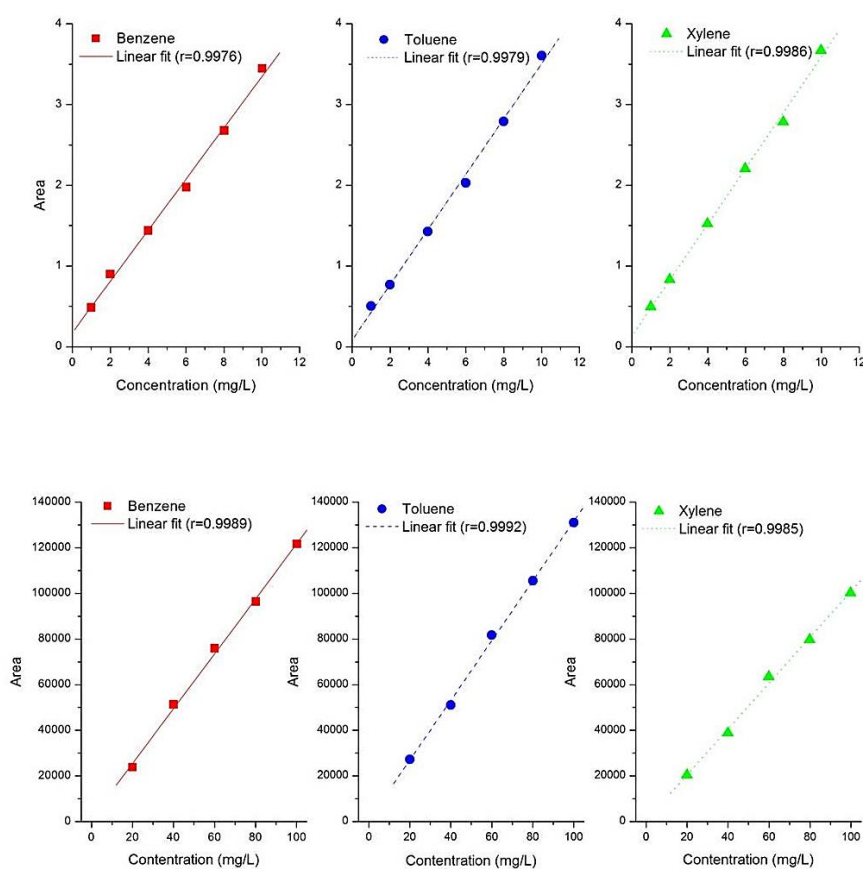


Figure 4. Calibration curve of standard solutions for GC-FID (above) and HPLC-UV (below).

3.3.3. Precision. Precision was examined by spiking BTX in water samples at 0.3 ppm and 1 ppm for GC-FID and HPLC-UV quantification respectively in six replicates. The calculated percent relative standard deviation (%RSD) was then compared to the value of two-thirds of Horwitz coefficient of variation (CV Horwitz). Table 1 shows that the obtained %RSD values are all lower compared to the values of two-thirds of CV Horwitz suggesting that the precision of the method is good. The %RSD values are also compared to the guideline by AOAC International [17]. The %RSD values for GC-FID quantification were all below the AOAC guideline (11% at 1 ppm).

3.3.4. Percent recovery. From Table 1, it was shown that the average values of percent recovery were all fall in the range of the AOAC guideline [17] which is from 80% to 100%. This suggests that the percent recovery values obtained by the method are acceptable for the BTX analysis.

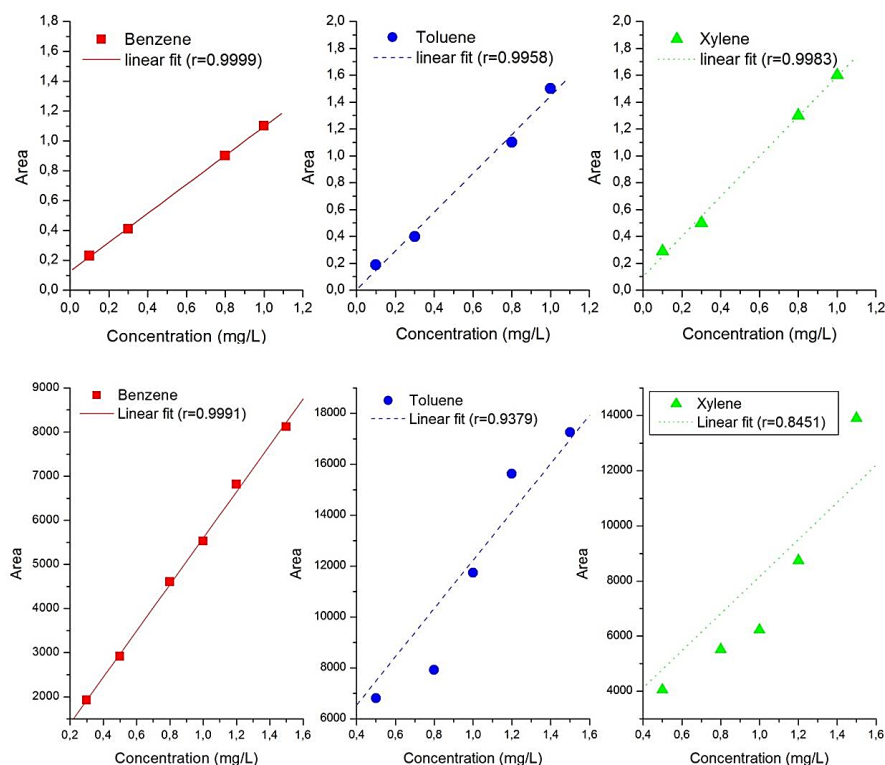


Figure 5. Linearity of the method for GC-FID (above) and HPLC-UV (below) quantification.

3.3.5. Matrix effect. One of the ways to evaluate the effect of the matrix is by comparing the result of the standard solution in pure solvent and the extracts of the spike samples at an exact series of concentrations (the method). In this study, a one-way analysis of variance (ANOVA) was applied to examine the matrix effect to the standard solution. The result showed that the method was statistically insignificant compared to the standard solution for both quantifications (GC-FID and HPLC-UV).

4. Conclusion

The proposed method which utilized low volume liquid-liquid extraction was able to analyze BTX in water. The method, which can be combined with GC-FID and HPLC-UV quantification, was simple, utilize no clean-up step or additional absorbent, use a low volume of the sample thus cheaper compare to other reported methods. For quantification by GC-FID, the LOD for toluene and xylene are below the MRL regulated by Indonesian Government, the linearity is good with correlation coefficients higher than 0.995, the precision is acceptable with %RSD values below the CV Horwitz and AOAC guideline, the percent recoveries are in the range of AOAC guideline, and the matrix effect is statistically insignificant therefore the GC-FID quantification can be applied for xylene and toluene analysis in drinking water to meet the regulation. However, for GC-HPLC quantification, the result was not as good as the GC-FID quantification.

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