Eurasian Journal of Analytical Chemistry

Eurasian J Anal Chem 6(3): 159-172, 2011

Validation and Uncertainty Estimation of Analytical Method for Determination of Benzene in Beverages

Dyah Styarini*, Oman Zuas and Nuryatini Hamim

Research Center for Chemistry, Indonesian Institute of Sciences (Pusat Penelitian Kimia – LIPI), Kawasan Puspiptek Serpong, Tangerang, Banten, Indonesia 15314

Received: *19/10/2010****; Accepted:*** *18/03/2011*

Abstract

Analytical method for the determination of benzene in beverages was developed using HS/GC-FID technique. Validation of the method was done for knowing the performance of the analytical method used. Linearity obtained r^2= 0.9971 was observed in the range from 5 to 100 ng mL^{-1} of benzene in sample. The method has good recoveries (average 101 %) and average of relative standar deviation for repeatability and intermediate precision at 3 level concentrations are less than 10 %. The limit of detection and limit of quantitation obtained from calculation are 0.90 and 2.86 ng mL^{-1} respectively. The expanded uncertainty of the method, U, is about 30 %.

Keywords:

Method validation; uncertainty estimation; headspace; GC-FID; benzene

1. Introduction

Benzene at the very low concentration (ng mL^{-1}) in food has been reported in early 1990s. Benzene could be formed in foods, particularly in softdrink, when both benzoates and ascorbic acid are present under the influence of heat, UV light and metals ions as catalysts [1]. Based on the 5-years study to determine the amount of volatile organics in food from 1996 to 2000 that was conducted by The U.S. Food and Drug Administration, the maximum concentration of benzene were found in ground beef (maximum 190 ppb), raw bananas (maximum 132 ppb), carbonated cola (maximum 138 ppb), and coleslaw with dressing (maximum 102 ppb) [2]. Eating foods or drinking liquids containing high levels of benzene can cause vomiting, irritation of the stomach, dizziness, sleepiness, convulsions, rapid heart rate, coma, and death. However, adverse health effects from eating foods or drinking liquids containing lower levels of benzene are still remains unknown [1]. There is no legal limit for benzene in softdrink although some institutions have drawn on the WHO guideline for save levels in drinking water (10 ppb) as an appropriate comparison. While removing the product from sale is requested when higher level of benzene (above 10 ppb) is found [3]**.** In Indonesia, benzene is allowed in drinking water to a maximum level of 10 μg L^{-1} [4].

With regard to very low level of benzene in beverage, development of sensitive and validated method for benzene is crucial. The most common method for benzene analysis is static headspace gas chromatography (HS/GC). In HS/GC the sample is prepared by using static headspace technique followed by identification and quantitation using gas chromatography. Headspace (HS) technique demonstrate its the best performance for

* **Corresponding Author**
E-mail: dyah_styarini@yahoo.com ; dyah_styarini@lipi.go.id
ISSN: 1306-3057,

compound having boiling point of less than 125 ^{o}C. Greatly reducing the complication associated with sample extraction and matrix effect is also disclosed by HS/GC techniques [5, 6]. While rapid with solvent free analysis is another advantage for this method.

In the present work, the known amount of benzene spiked in to drinking water and some fruit juice samples. Those samples then prepared by using static headspace technique before analyzed by using GC-FID for qualitative and quantitative measurement. The validation of method was performed based on important method validation criteria such as method detection limit (MDL), linearity, presicion and accuracy.

In the method validation procedure, the estimation of the uncertainty is one of the main focus of interest due to its importance in showing the data quality. ISO standard 17025 requires to present the uncertainty data of the analytical results. Since a typical chemical measurement consists of a number of measurements steps, it requires careful design of measurement procedure to keep the traceability chain to the SI unit.To make a measurement result traceable to the SI unit, it is also necessary to evaluate the uncertainty of every step in the measurement procedure and combined them to meet the principles of the internationally agreed guide [7, 8].

According to the requirement of ISO 17025, testing laboratories shall have and apply procedures for estimating uncertainties of measurement. When estimating the uncertainty of measurement, all uncertainty components which are of importance in the given situation shall be taken into account using accepted methods of analysis [8].

The purpose of this work was to develop simple and accurate of analytical method for determination of benzene in the beverages samples, to validate the method and estimating the measurement uncertainty.

2. Experimental

2.1. Chemicals

Benzene with purity min 99.7 %, Cat. No. 1.01783.2500 (Merck, Germany) was used in this study. Methanol with HPLC grade with min purity 99.8 %, Cat. No1.06018.2500 (Merck, Germany) was used as a solvent and aquadest was used as a sample matrix.

2.2. Instruments

A headspace auto sampler (Tekmar 7000) coupled with gas chromatography equipped with flame ionization detector (Hewlett Packard 6890) was used in this study. Platen, valve and line temperature in the automated headspace autosampler were kept at 60 ^{o}C. Equilibrium time of sample was 25 minutes. Sample loop was 1 mL.

A DB-5 capillary column (30 m x 0.25 mm I.D. x 0.25 μm) was used for separation. The helium was used as carrier gas with flow rate 1 mL min^{-1}. The injector port and detector temperature were kept at 200 ^{o}C and 250 ^{o}C, respectively. The oven temperature was programmed from 40 ^{o}C for 2 minutes and was then followed by 10 ^{o}C min^{-1} ramping to 200 ^{o}C held for 2 min. Split injection mode with split ratio 1:25 was used.

2.3. Preparing of standard solution

Stock standard solution with concentration 2.2 mg mL^{-1} was prepared by adding 50 μL of benzene into headspace vial 22 mL containing 20 mL of methanol [9]. An intermediate solution (54 μg mL^{-1}) was prepared by diluting 0.5 mL of stock standard solution into headspace vial containing 20 mL of methanol. The working standard solution of 0.5 μg mL^{-1} was prepared daily by diluting an appropriate volume of intermediate solution.

2.4. Sample Preparation

A known concentration of benzene in 10 mL of aquadest or softdrink sample was transferred into 22 mL sealed headspace vial. The sample was then heated in the headspace auto sampler oven at 60 ^{o}C for 25 min. Some volume of gas phase from vial was transferred to the GC-FID to be analyzed.

3. Result and Discussion

3.1. Method Validation

Before applying the method to the analysis of real samples, the method is validated. During the validation procedure, the following parameters were evaluated: linearity, limit of detection and limit of quantitation, repeatability and intermediate presicion, trueness [7, 10, 11, 12, 13].

By using the condition of GC-FID described above, aquadest sample containing benzene was analyzed, and benzene eluted from the column at 3.70 minutes.

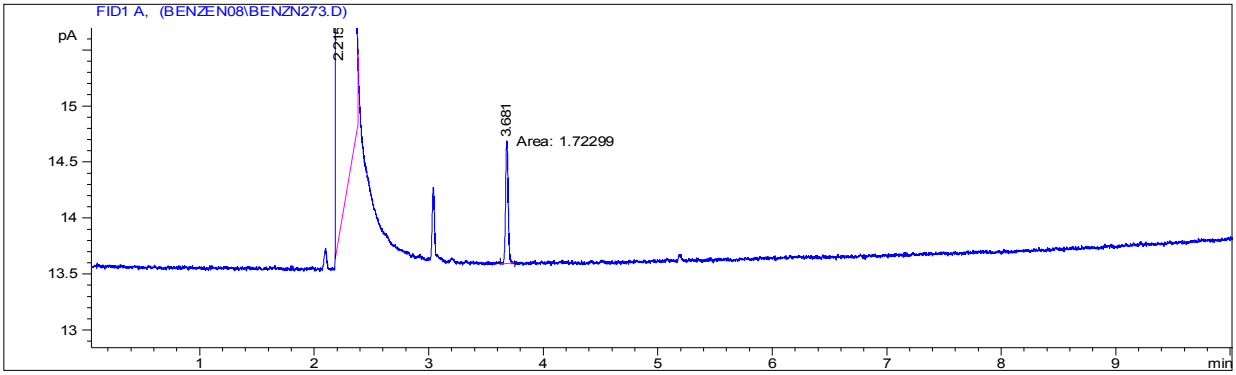

Fig. 1. Chromatogram of standard benzene in the aquadest sample.

3.1.1. Linearity

The linearity of benzene obtained from sample preparation in destilled water as a blank matrix was studied by evaluating the calibration curve at six level of concentrations, 5; 20; 40; 60; 80 and 100 ng mL^{-1}. This calibration curve serves as a graphical representation of measuring signal as a function of quantity of analyte. The result shows a good linearity because the r^2 value obtained from the calibration curve is greater than 0.990 (Fig. 2).

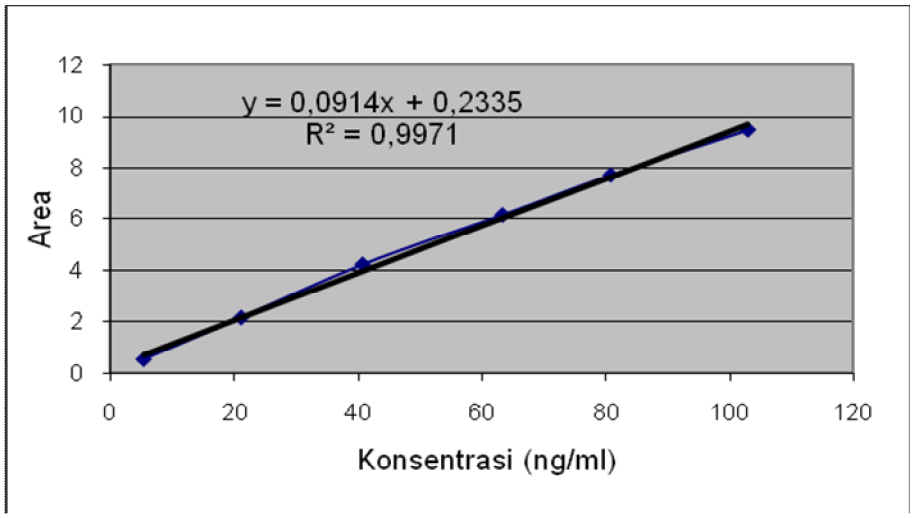

Fig. 2. Calibration curve of benzene in aquadest with the linear range from 5-100 ng mL^{-1}.

A traditional method for testing the linearity of calibration functions after linear regression is to compute the correlation coefficient, r (or the similar coefficient of determination, r^2). But further comments have been made on the miss-use of r for testing the linearity. The value of r correctly describes a correlation between two files; it describes the quality of the fit only poorly and its linearity not at all. Since equal results can have different meanings, depending on the number of the degrees of freedom, the use of r is not reliable measure of linearity. [14, 15]

The virtue of any calibration can be very well characterised by the standard deviation ($S_{\Delta y/\hat{y},n-2}$) of the relative residuals (residuals/predicted $\Delta y_i = y_i - \hat{y}$; $Y_i = \Delta y_i / \hat{y}$), which is calculated with n-2 degrees of freedom by applying following Eq. [1]. [16, 17]

$$s_{\Delta y/\hat{y}} = \sqrt{\frac{\sum (Y_i - \overline{Y})^2}{n-2}} \qquad (1)$$

where;

y_i is the response obtained from spotting/injecting analytical standard.
$\hat{y}_i$ is the point corresponding with analytical standard on the regression line.
n is the total number of standard spots/injections e.g. when the calibration is made at three level with duplicate injections, then n is equal to 6.

The standard deviation of relative residuals obtained from the calibration curve above is 0.1. A good calibration curve has a standard deviation of relative residuals less than 0.1. Since the standard deviation of the relative residuals is not constant but generally proportional to the injected analyte, standard deviation of the relative residuals reflect the average variability of the calibration points even if unweighted regression equation is used for the estimation of the calibration relationship [16]. The standard deviation of the relative residuals clearly better to interpret than r, because of their linear response to the random errors of the signals combined with possible systematic errors produced by non-linearity of the real calibration function. By using this concept, problems due to different numbers of degrees of freedom between calibration and analytical data could be avoided.[14]

3.1.2. Instrument Detection Limit (IDL)

The IDL is treated as the minimum concentration of pure standard solution that can be reliably detected by the instrument system used in the study under the stated condition of analysis. IDL can be determined by injecting the standard solution of the analyte several times and then can be estimated by using these equations below:

$$\text{IDL } (\mu g\ mL^{-1}) = SD \times t_{95} \qquad (2)$$

Where SD is the standard deviation of peak areas analytes for the replicate injections and t_{95} is the student's t at 95 % level of confidence. [18, 19]

According to te NATA Technical Note 17 (2006), Limit of Detection (LoD) of a method is the smallest amount or concentration of an analyte that can be reliably distinguished from zero with a specified level of confidence. In otherway MDL can be defined as statistically determined values that define how easily measurements of a substance by a specific analytical protocol can be distinguished from measurements of blank (background noise). There are several approaches in estimating the MDL value, for example:

3.1.3. Method Detection Limit (MDL)

The estimated MDL (EMDL) can be estimated from the IDL as follow: [18, 19]

$$EMDL(\mu g/g) = \frac{IDL \times 1 \times 100}{M \times \%\,\mathrm{Re}\,c} \quad (3)$$

Where: M is the mass of the sample (g) and % Rec is the average percent recovery of analyte in the method. The 1 term in the equation referes to the 1 mL fixed volume sample loop of the HS-GC/FID system.

In this experiment, MDL was calculated using water that is spiked with the analyte of interest, although they can also be determined in specific matrices using the same procedure [20]. In this study, the GC was externally calibrated using four standards of benzene at 3; 10; 15 and 20 ng/mL and 10 ml of aquadest were fortified with 5.375 ng mL^{-1} of benzene. The results (in ng mL^{-1}) are listed in Table 1.

Table 1. Measurement results from the analysis of aquadest containing benzene at concentration level of 5.375 ng mL^{-1}.

Sample #	Result	Recovery %
Sample 1	5.215	97
Sample 2	4.456	83
Sample 3	4.969	92
Sample 4	5.266	98
Sample 5	5.290	98
Sample 6	5.037	94
Sample 7	5.004	93
Mean	5.034	94
Std. Dev	0.286	

The number of observation is equal to seven replicates with six degrees of freedom. The student's *t* value for seven replicates and six degrees of freedom is 3.143. The MDL was calculated as follows:

MDL = (s) (*t*-value) (4)

By using the equation 4 above, the calculated MDL obtained is 0.8987 ng mL^{-1}. The following requirements are useful for evaluating a calculated MDL:

Calculated MDL < Spike Level < 10 x Calculated MDL (5)

3.1.4. Limit of Quantitation (LOQ)

Based on the result, the conditions above are met so it means that an appropriate spike level has been attained and the calculated MDL could be accepted. The Limit of Quantitation (LOQ), 2.86 ng mL^{-1}, was calculated using equation 6.

LOQ = 10 x (s) (6)

The values obtained are lower than the respective maximum level of benzene in the drink water. After getting the LOD and MDL value then aquadests containing benzene at level concentration of 1 and 2 ng mL^{-1} (more or less match with the level concentration of MDL and LOQ) were analyzed. The result can be seen in Fig.3. Sample containing benzene at concentration of 1 ng mL^{-1} still can be detected by the method which the peak has S/N ≥3. This means that the calculated value of MDL, ±1 ng mL^{-1}, can be accepted. The chromatogram at a concentration between the MDL and LOQ values of benzene is shown in Fig. 3b.

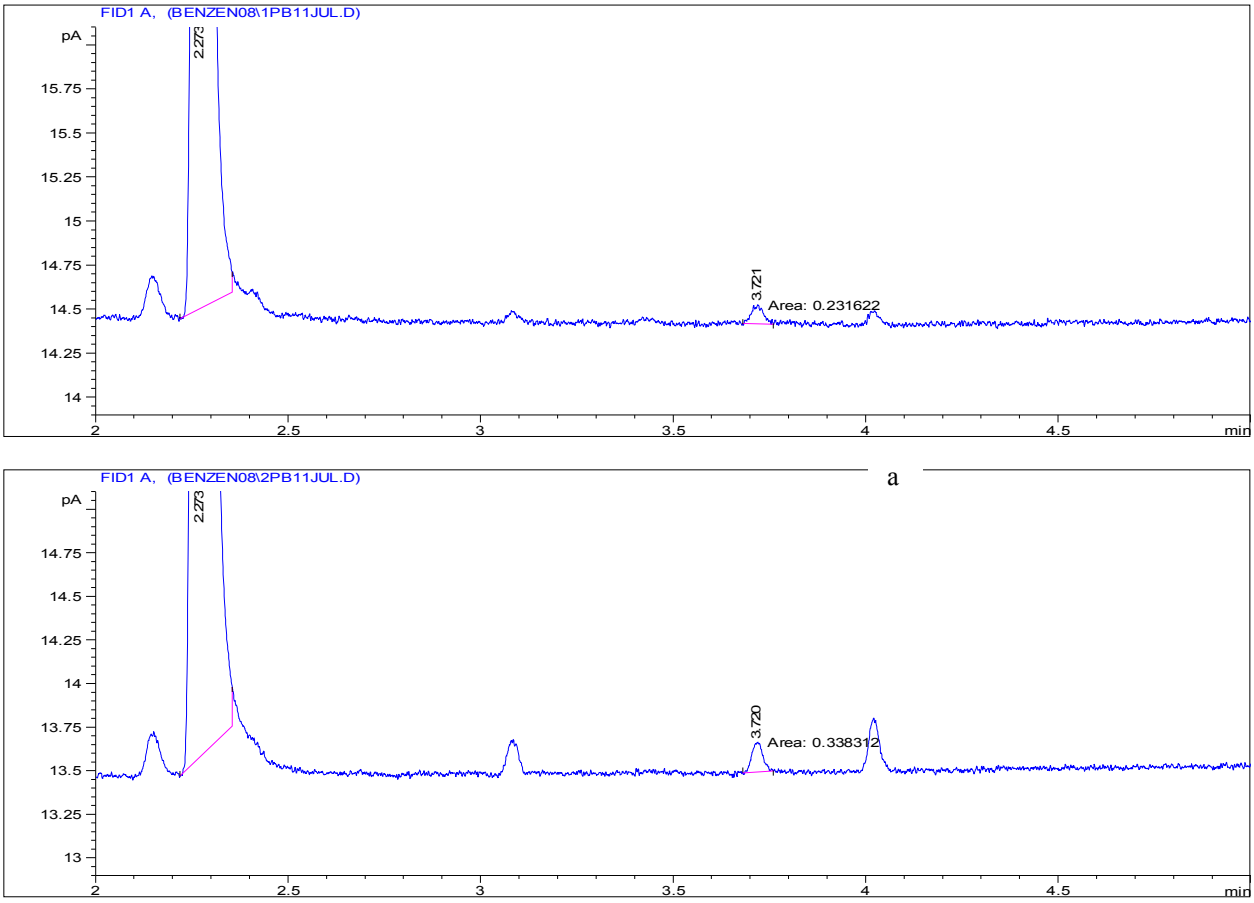

Fig.3 A chromatogram of benzene in the aquadest at concentration of a) 1 ng mL^{-1}; b) 2 ng mL^{-1}

The analysis of the real uncontaminated beverages sample then spiked with benzene at 3 ng mL^{-1} level, gave the result shown at Fig 4. The peak of benzene looks high enough to be seen and to be detected.

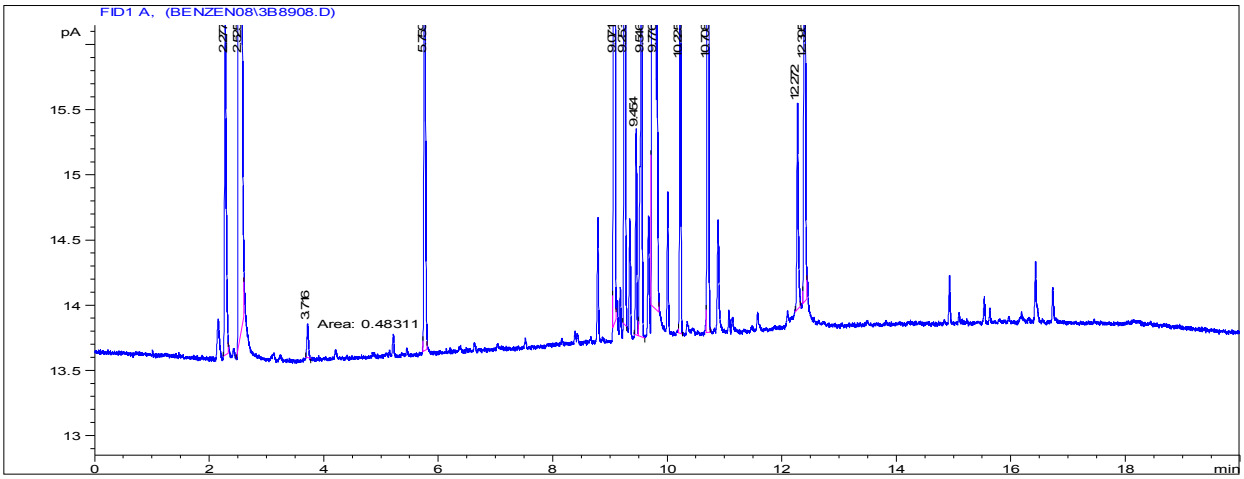

Fig. 4. The chromatogram resulted from the analysis of benzene at the concentration level of 3.23 ng mL^{-1} in the beverage sample.

3.1.5. Injection repeatability

The closeness of agreement between independent test results obtained under stipulated condition, so called precision [21], was determined by analyzing the concentration of benzene

in aquadest at concentration level of 5 and 27 ng mL^{-1} with five replications for each. The result showed good repeatability for quantification with percentage of relative standard deviation (RSD %) values for retention time and peak area are less than 1 and 10, respectively (Table 2).

Table 2. Precision of sample preparation method expressed in percentage of RSD for retention times and peak area at concentration of 5 and 27 ng mL^{-1}.

No	Concentration (ng ml^{-1})			
	5		27	
	Rt	Area	Rt	Area
1	3.720	0.704287	3.720	2.79759
2	3.719	0.715753	3.721	3.04637
3	3.720	0.726517	3.721	2.78327
4	3.719	0.794929	3.721	2.93032
5	3.720	0.701725	3.721	3.07232
Average	3.7196	0.728642	3.7208	2.925974
sd	0.000548	0.038348	0.000447	0.134887
RSD%	0.01472	5.26295	0.012019	4.60999

3.1.6. Recovery, Repeatability and Intermediate Precision

A fruit juice sample was used as a matrix for the recovery test. According to the expected levels of real concentrations, the spiking was performed at three fortification levels (3, 10 and 50 ng mL^{-1}). Evaluation of recovery and repeatability in the same day and in the different day (intermediate precision) was carried out. Recovery is expressed as the amount/weight of the compound of interest analyzed as a percentage to the theoretical amount present in the medium. Determination of analysis repeatability, express as the RSD, consist of multiple measurements of a sample by the same analyst under the same analytical conditions. It is often combined with accuracy and carried out as a single study. Intermediate precision was previously known as part of ruggedness. Depending on time and resources, the method can be tested on multiple days, analysts, instrument, etc. Mean recovery data and R.S.D. obtained from replication in the same day are given in Table 3 and from replication in the different day are given in Table 4. [7, 21]

Table 3. Mean recovery and RSD data obtained from five replications in the same day at several spiking level.

No of replication	Spiking level (ng mL^{-1})		
	3	10	50
1	87.613	98.556	101.127
2	87.402	108.440	121.368
3	74.018	109.981	96.495
4	78.671	96.657	111.399
5	71.511	101.453	112.443
Mean of Recovery	79.843	103.017	108.567
SD	7.454	5.931	9.849
RSD %	9.336	5.757	9.072

Table 4. Mean recovery and RSD data obtained from five replications in the different day at several spiking level.

Day	Spiking level (ng mL^{-1})		
	3	10	50
1	118.793	89.938	83.981
2	71.037	92.406	93.879
3	93.542	92.909	95.069
4	80.079	92.787	96.112
5	96.337	111.807	93.019
Mean of Recovery	94.457	91.751	90.976
SD	18.170	8.936	4.857
RSD %	19.236	9.739	5.338

3.1.7. Accuracy and Traceability to the SRM 3000 (Benzene in Methanol)

The Standard Reference Material (SRM) 3000 is gravimatically prepared single compound solution (benzene) in methanol intended primarily for the calibration of instrumentation and validation of methods for volatile organic compound (VOC) determinations. The certified concentration value for benzene, reported as a mass fraction is given below:

Benzene (mass fraction): 0.01001 g g^{-1} ± 0.00007 g g^{-1} [22]. Dilution of this SRM is made by gravimatically (weighed) and not by volumetric means (volume can be calculated for transfer purpose only). The amount of benzene added to the diluent can then be determine from the mass added and the certified value. The dilution of methanol p.a. that is used for preparing the calibration curve then prepared by gravimatically also. In this accuracy and traceability test using SRM 3000 NIST, a known amount of SRM was spiking in to the five aquadest matrix so that it contain 16 - 18 ng g^{-1} of benzene. The samples were analyzed and plotted to the 4 point calibration curve which has squared correlation coefficient, r^2, 0.996. The result of accuracy test by using the SRM can be seen in table 5. The mean of % recovery that can be reached was 101 % . This result is satisfy.

Table 5. Accuracy data by using SRM 3000 from NIST

Calculated concentration (ng/g)	Area	Observed concentration (ng/g)	Recovery %
18.6507	2.60891	17.95987	96.29596
18.6627	2.61034	17.96999	96.28826
16.4331	2.46375	16.92932	103.0196
15.5527	2.39856	16.45667	105.8123
16.0149	2.46375	16.92932	105.7098
Average	17.24903		101.4252
SD	0.681421		4.81778
RSD %	3.95049		4.750082

3.2. Estimation of Uncertainty

The estimation of uncertainty, nowadays, become an integral part of quantitative analysis. Several approaches were developed for the estimation of uncertainty related to the analytical measurements and two of best known, namely, "bottom-up" and "top-down". According to the EURACHEM / CITAC document, "bottom-up" approach can be used for estimation of combined standard uncertainty. This strategy splits the analytical process in single steps, estimating the individual contribution of each one to the uncertainty of the final results. The steps involved are specify measurand, identify uncertainty sources, quantify uncertainty components and calculate combined uncertainty. In the 'bottom-up' approach, the random error components (as repeatability) and the systematic components of uncertainty (as recoveries) were estimated.[23,24,25]

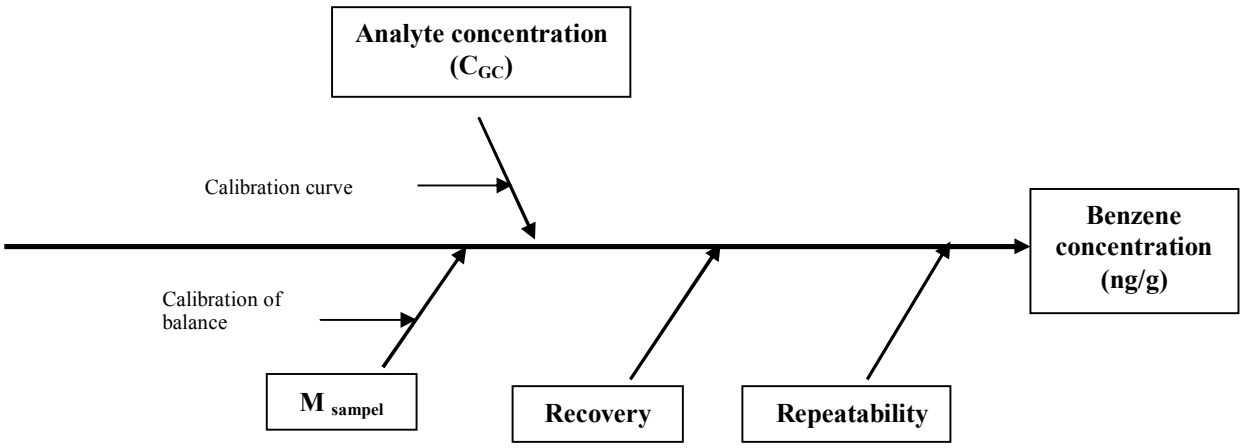

Fig.5 Cause and effect diagram for determination of Benzene in drinking water and beverages samples.

3.2.1. Identification of uncertainty sources

The analyte concentration in the sample, expressed in ng g^{-1} , is obtained from the equation

$$C_s = \frac{C_{GC}}{M_{sampel}} x \frac{1}{\mathrm{Re}\,c} \qquad (7)$$

where C_{GC} is the analyte concentration obtained from the calibration (in ng); Rec is the recovery and *m* sample is the weight of sample (g). For the identification of the uncertinty sources, the use of the so- called "cause and effect diagram" (also known as Ishikawa or fishbone diagrams) is suggested. The diagram can be seen in fig. 3. The concentration results of benzene were mainly affected by the following sources:

1. Analyte concentration (C_{GC}) that is affected by calibration curve
2. Sample weight (M_{sample})
3. Recovery (Rec)
4. Repeatability

3.2.2. Estimation of uncertainty derived from Analyte concentration (C_{GC})

C_{GC} is affected by calibration curve that used for the analysis. From a calibration curve, an linear equation can be obtained as follow:

$$Y = bX + a$$

The standard uncertainty derived from linear equation can be obtained by using following equation:

$$rsd(S_{y/x}) = \sqrt{\frac{\Sigma(Y_i - Y_c)^2}{(n-2)}} \quad (8)$$

Where: Yi = Area obtained from GC
Yc=Area obtained from calculation using the linear equation of calibration curve.
n= The number of standard using in calibration curve.

Then, standard uncertainty derived from concentration of analyte can be obtained as follow:

$$S_x = \frac{S_{Y/X}}{b}\sqrt{1+\frac{1}{n}+\frac{(Y_{sampel} - Y_{average})^2}{b^2\sum[X_i - X_{average}]^2}} \quad (9)$$

Where; b=slope in the linear equation
Y_{sample} = Area of the sample
$Y_{average}$ = Average area of sample
X_i= Concentration of the sample
$X_{average}$= Average concentration of the sample
n = the number of replication

The example of calculation of Sx for SJb-8 is shown below:

$$S_x = \frac{0{,}24}{0{,}016}\sqrt{1+\frac{1}{3}+\frac{\left(2{,}677-2{,}57\right)^2}{0{,}016^2\times 15728{,}7}} = 17{,}34$$

3.2.3. Estimation of uncertainty derived from Msample

The balance uncertainty is obtained from certificate of calibration (Group B). The value of expanded uncertainty at level confidence of 95 % is 0.0003. Standard uncertainty, (u_{sample}) is 0.0003/2. This source of uncertainty is concidered twice because the weighing process involves a difference.

$$u_M = \sqrt{0.00015^2 + 0.00015^2} = 0.000212$$

3.2.4. Estimation of uncertainty derived from Recovery

When recovery test has been done by using the uncontaminated sample and spiking by using CRM, the standard uncertainty derived from recovery can be calculated by the following equation (10):

$$uRm = Rm \times \sqrt{\left(\frac{s^2{}_{obs}}{n \times c^2{}_{obs}}\right) \times \left(\frac{u(C_{CRM})}{C_{CRM}}\right)^2} \quad (10)$$

Where:

S_{obs} = SD from replication (0.681421)
C_{obs}= concentration of analyte derived from analysis (17.24903 ng g^{-1})
uC_{CRM}=Standard uncertainty of C_{CRM} (0.00007/2=0.000035 g g^{-1})
C_{CRM}=concentration of CRM (0.01001 g g^{-1})
n=number of replication (5 replication)
Rm= Recovery (1.014252)

then:

$$uR_m = 1.014252 \times \sqrt{\left(\frac{0.681421^2}{5 \times 17.24903^2}\right) + \left(\frac{0.000035}{0.01001}\right)^2} = 0.0182353$$

3.2.5. Estimation of uncertainty derived from Repeatability

The repeatability value was obtained from analytical method validation data.

u(Rep) = RSD

= 0.0475

The combined uncertainty then can be calculated using the equation

$$u(C_s) = C_s \times \sqrt{\frac{u(C_{GC})^2}{C_{GC}{}^2} + \frac{u(\mathrm{Re}\,p)^2}{\mathrm{Re}\,p^2} + \frac{u(\mathrm{Re}\,c)^2}{\mathrm{Re}\,c^2} + \frac{uM^2}{M^2}}$$

The calculation of combined uncertainty for SJb-8 is shown below:

$$u(C_s) = 12{,}54 \times \sqrt{\left(\frac{17{,}34}{155{,}69}\right)^2 + \left(\frac{0{,}0475}{1}\right)^2 + \left(\frac{0{,}075}{1{,}014}\right)^2 + \left(\frac{0{,}000212}{10{,}73}\right)^2} = 1{,}779$$

Then, the expanded uncertainty at level confidence of 95 % is 1,779 x 2 = 3,56.

3.3. Aplication to the real samples

The validated method was applied to the analysis of aproximately 24 real beverages samples (from several brand) from a market. The result showed that most of samples gave negative result which means that most samples didn't contaminate with benzene. In the several samples there were very small peaks of benzene, since the concentration were far below the LOQ and MDL so that we reported benzene in the samples as not detectable (nd). In the five beverage samples from the same brand, we found benzene at level concentration higher than 10 ng g^{-1} which means overcame the levels established by Indonesian government [4]. The result of analysis of beverages samples and the value of the expanded uncertainty with coverage factor value is k=2 (at 95 % confidence level) can be seen at table 6 below. The combined uncertainty obtained for those five samples containing benzene range between 13-22 % (Only sample SJb-7which has the combined uncertainty more than 20 %). According to the hierarchy of RMs for content of trace elements or compounds in matrix, the accepted value for relative combined standard uncertainty obtained using the analytical techniques which have been validated is 5 % $< u <$ 20 % [26]. Uncertainty derived from analyte concentration became the main source of the relative high value of the combined uncertainty. This can be explained that the slope of the calibration curve at level concentration of ppb is low, so that it contributes to the high value of S_x, then the combined uncertainty become high also.

Table 6. The result of analysis of several beverage products from markets

No	Sample code	Conc. of benzene ± U (ng g^{-1})	Rt (min)	Remarks
1	SA-1	nd		Apel juice
2	SA-2	nd		
3	SA-3	nd		
4	SAg-1	nd		Grape Juice
5	SAg-2	nd		
6	SB-1	nd		Tea with blackcurrent falvour
7	SJb-1	nd		Guava Juice
8	SJb-2	nd		
9	SJb-3	nd		
10	SJb-4	nd		Tea with guava flavour
11	SJb-5	13,91 ± 3,85	3,722	Guava Juice (all from the same product)
12	SJb-6	12,59 ± 4,07	3,724	
13	SJb-7	9,44 ± 4,16	3,722	
14	SJb-8	12,54 ± 3,56	3,721	
15	SJb-9	12,30 ± 3,66	3,723	
16	SJ-1	nd		Orange Juice
17	SJ-2	nd		
18	SJ-3	nd		
19	SJ-4	nd		
20	SJ-5	nd		
21	SM-1	nd		Manggo Juice
22	SS-1	nd		Sirsak Juice
23	SS-2	nd		
24	SSt-1	nd		Strawberry Juice

To make sure the result we found in the samples contain benzene, then we analyze the samples by using GC-MS/EI with scan mode to make a confirmation. The result showed that the peaks detected in the five beverages samples at the retention time 3.7 min were benzene.

4. Conclusion

The result of validation shows that the HS/GC-FID method used is simple and reliable, and appropriate for the analysis of benzene in drinking water and also other beverages sample like fruit juice. The average value of expanded uncertainty for benzene using coverage factor 2 was about 30 %. Estimated value of limit of detection and limit of quantitation lower than the maximum concentration of benzene that is allowed in the drinking water makes the method suitable for routine analytical in evaluating the presence of benzene in the beverages and quality control assay of benzene in drinking water and other beverages. Measurement uncertainty of the method was estimated using the data obtained from method validation.

Acknowledgement

The authors would like to extend their thanks to the Indonesian Government for funding support of this project and Ms. Sajekti Eka for her generous assistance during the laboratory experiment is highly appreciated.

References

1. Indications of the possible formation of benzene from benzoic acid in foods, BfR Expert Opinion No. 013/2006, 1 December 2005.
2. Fleming-Jones ME, Smith RE (2003) Volatile Organic Compounds in foods: A Five Year Study, J Agr Food Chem 51: 8120-8127.
3. Benzene in Soft Drinks, March 2006 available at: http://www.food.gov.uk/science/surveillance/fsisbranch2006/fsis0606
4. Syarat-syarat dan Pengawasan Kualitas Air Minum http://persembahanku.files.wordpress.com/2007/03/kepmenkes_no_907_2002_kualitas_air_minum_.pdf downloaded on 27 Juni 2007 at 09.45
5. US. US. EPA (1986) Headspace, Method 3810, it can be downloaded at http://www.caslab.com/EPA-Methods/PDF/EPA-Method-3810.pdf.
6. A Technical Guide for Static Headspace Analysis Using GC, Restek corp., 2000.
7. Serpil Y (2006) Validation and Uncertainty Assessment of Rapid Extraction and Clean-up Methods for the Determination of 16 Organochlorine Pestisicide Residues in Vegetables, Anal Chim Acta 571: 298-307.
8. ISO/IEC 17025 (Versi Bahasa Indonesia), 2005, Persyaratan umum Kompetensi Laboratorium Pengujian dan laboratorium kalibrasi, second edition.
9. CFSAN, 2006, Determination of Benzene in Soft Drinks and other Beverages. http://www.cfsan.fda.gov/~dms/benzmeth.html
10. Center for Drug Evaluation and Research (CDER), 1994, Reviewer Guidance "Validation of Chromatographic methods". Available at http://www.fda.gov/CDER/GUIDANCE/cmc3.pdf downloaded on 31 December 2008.
11. Michulec M, Konieczka P and Namieśnik J (2007) Validation of the HS-GC-FID method for the determination of ethanol residue in tablets. Accreditation and Quality Assurance: Journal for Quality, Comparability and Reliability in Chemical Measurement 12(5): 257-262, DOI: 10.1007/s00769-006-0252-3.
12. Magdalena M, Piotr K, Jacek N (2007) Validation of SPME-GC and HS-GC procedures for the determination of selected solvent residues in edible oil matrice. Journal of Accreditation and Quality assurance: Journal of Quality, Comparability and Reliability in Chemical Measurement , Volume 12 No. 2, Springer, Berlin.
13. Tiryaki O (2006) Method validation for the analysis of pesticide residues in grain by thin-layer chromatography. Accreditation and Quality Assurance 11 (10): 506-514.
14. Huber W (2004) On the use of the correlation coefficient r for testing the linearity of calibration functions. Accredit Qual Assur 9:726.
15. Hibbert D B (2005) Further comments on the (miss)-use of r for testing the linearity of calibration function. Accredit Qual Assur 10: 300-301.

16. Miller JC, Ambrus A (2005) Statistics in calibration analyses.Basic Statistics Chapter: 9 In *Lectures Database* FAO/IAEA Workshop on Introduction to QC/QA Measures in Pesticide Residue Analytical Laboratories, Seibersdorf, Vienna Austria, September 12 – October 7.
17. Tiryaki O (2006) Method validation for the analysis of pesticide residues in grain by thin-layer chromatography. Accreditation and Quality Assurance 11(10): 506-514.
18. Singh S B, Foster G D, Khan S U (2007) Determination of Thiophanate methyl and Carbendazim residues in Vegetable Samples Using Microwave-assisted Extraction, J Chromatogr A 1148: 152-157.
19. Singh S B, Foster G D, Khan S U (2004) Microwave-Assisted Extraction for the Simultaneous Determination of Thiamethoxam, Imidacloprid, and Carbendazim Residues in Fresh and Cooked Vegetable Samples. J Agric Food Chem 52: 105 – 109.
20. Ripp J (1996) Analytical Detection Limit Guidance & Laboratory Guide for Determining Method Detection Limits, Wisconsin Department of natural Resources laboratory certification Program.
21. The Fitness for Purpose of Analytical Methods, A Laboratory Guide to Method Validation and Related Topics, English Edition 1.0, LGC (Teddington) Ltd. 1998
22. National Institute of Standards and Technology (2005) Certificate of Analysis of Standard Reference material 3000 Benzene in Methano.
23. Ellison S L R, Rosslein M, Williams A (2000) Quantifying Uncertainty in Analytical Measurement, Second Edition, EURACHEM/CITAC, ISBN 0 948926 15 5.
24. Stepan R, Hajslova J, Kocourek V, Ticha J (2004) Uncertainties of Gas Chromatographic measurement of troublesome pesticide residues in apples employing conventional and mass spectrometric detectors. Anal Chim Acta 520: 245-255.
25. Tiryaki O, Baysoyu D (2008) Estimation of the efficiencies and uncertainties of the extraction and clean up steps of pesticide residue determination in cucumber using ^{14}C-Carbaryl. Accred Qual Assur 13: 91-99.
26. Pan X R (1997) Hierarchy of Reference Materials Certified for Chemical Composition. Int J Pure Appl Met 34: 35-39.