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UNCERTAINTY EVALUATION FROM DEVELOPED VALIDATION STUDIES. QUALITATIVE AND QUANTITATIVE ANALYSIS OF ETHANOL IN BLOOD BY HEADSPACE/GAS CHROMATOGRAPHY- FI D.

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ABSTRACT

A laboratory performing testing shall evaluate measurement uncertainty. A procedures that enable the use of all the measurand as a components contributions to measurement uncertainty of the developed method are useful for establishing a procedure for evaluation of measurement uncertainty. In the present study a guideline aimed at illustrate how the techniques use for evaluating uncertainty, can be applied to some typical chemical analyses was developed. The measurands contributing to measurement uncertainty were specified. All major uncertainty sources, taking into account all sources of error, of the developed method using cause and effect diagram were identified. The individual components of uncertainty were identified, combined them and quantified. The importance of proposed method is summarized simplified evaluation of measurement uncertainty, minimized and reduced the influence of random errors and increase the reliability of analytical method results.

المستخلص:

يلزم على معمل الاختبار الذي يجري المعايرات الخاصة به أن يطبق طريقة لتقدير اللايقين في نتائج القياس لكل المعايرات التي يجريها. تم وضع إجراء لتقييم والتعبير عن قيمة اللايقين في نتائج القياس عند إجراء المختبر تحليل كيميائي كمي. تم تحديد كل مكونات اللايقين التي تسهم في تقدير قيمة اللايقين للحالة. تم تحديد كل المصادر المساهمة في تقدير اللايقين باستخدام مخطط عظم السمكة. صممت دراسة مخطط يلخص مصادر اللايقين مع تقدير لقيمه. تأتي أهمية المخطط المقترح في تلخيص وتبسيط جميع خطوات اللايقين، التقييم المبسط لقيم اللايقين في القياس، تقليل تأثير الأخطاء العشوائية وزيادة موثوقية نتائج القياس. من الممكن تطبيق هذه الطريقة على بيانات طريقة التحليلية الكمية المتحقق منها.

Introduction

that describes “a range” (Miller and Miller, 2010) within which the value of the quantity being measured The uncertainty is the parameter associated with the result of a measurement ” (ISO Guide 98-3, 2008) is expected to lie, taking into account. A measurement uncertainty estimate takes into account the “precision of the method” (Thompson et al., 2002), statistical uncertainty involved in the bias measurements, (Eurachem, 1998) and the “reference material” (ILAC-G12, 2000) or method uncertainty. “Laboratory shall identify the contribution to measurement uncertainty” (ISO/IEC 17025:2017, 7.6.1). The guide (JCGM, 2008) uncertainty measurement differentiates between “statistical evaluations” (UKAS, 2012) and those using other methods. It categories them into two types “A” and “B” based on the evaluation method. In Type “A”, the uncertainty component is quantified in ideal conditions since all information for the reliable estimation of that effect on measurement uncertainty is available and in Type “B”

evaluations is the quantification of "systematic" (Miller and Miller, 2012) components of uncertainty, i.e. those that account for errors that remain constant while the measurement is made, (UKAS, 2012).

Keywords: uncertainty; measurand and cause and effect diagram.

MATERIALS AND METHODS

Material

- Human whole blood and post mortem samples used in evaluation of measurements uncertainty obtained from Forensic Laboratory Khartoum and verified to be negative for all analytes.

- The developed analytical method that use of human whole blood as an alternative matrix for the analysis of ethanol was chose as a study area for sets out guidelines and techniques for single-laboratory evaluating of measurement uncertainty.
- The ISO/IEC 17025:2017 standard, general requirements for the competence of testing and calibration laboratories was used as standards for establishing evaluating of measurement uncertainty procedure.

Reagents and standards

Ethanol absolute (CAS # 64-17-5), iso-propanol (CAS # 67-63-0), methanol absolute (CAS # 67-56-1) and acetone absolute (CAS # 67-64-1) were obtained from Dr. Ehren storfer GmbH. 200 mg 2-Propanol/100 ml water (internal standards) was obtained from Dr. Ehren storfer GmbH, 80 mg EtOH/ 100 ml blood (positive blood control sample) was obtained from Promochem . Resolution mixture (acetone, methanol, ethanol and 2-Propanol) and method blank control sample. Positive [EtOH] human whole blood obtained from Forensic Laboratory Khartoum, Sudan.

Methods

A number of protocol and guideline applied as guide to the expression of uncertainty in measurement (Eurachem /CIATC, 2012; JCGM, 2008; JRC, 2012; UKAS, 2012; NIST, 1994). Among the above guidelines (NIST, 1994 and Eurachem /CIATC, 2012) is the more convenient guideline and illustrate how the techniques for evaluating uncertainty, can be applied to some typical chemical analyses. That is why the present study had been design according to Eurachem and NIST guideline (NIST, 1994 and Eurachem /CIATC, 2012) to mapping and evaluating of measurement uncertainty. The individual component of uncertainty identified and combined them statistically using "root sum square process (RSS)" (Thompson et al., 2010; Miller and Miller, 2010) to evaluate the uncertainty of measurement.

Evaluation of measurement uncertainty

The process of the evaluation of measurements uncertainty was performed (NIST, 1994 and Eurachem /CIATC, 2012) using a developed validation method, ethanol analysis in blood by Headspace /Gas Chromatography / Flame-Ionization detector as a study area.

Specification

The general procedure for measurements of blood alcohol concentrations was shown in Table 1. The calculation of an alcohol concentrations are based on linearity of the calibration curve based on peak area.

Table 1: General procedure

Step	Description
1	Preparing of the internal standards solution.
2	Preparing of positive blood control sample.
3	Preparing of method blank control sample.
4	Preparing the resolution mixture control sample.
5	Each sample was analyze on the gas chromatograph (alcohol reference solution, standards and controls).
6	Measuring blood alcohol concentrations.

Specify the measurand

The measurands of the developed method was specifying. The measurement procedure and measurement function, f, (JCGM, 2008) were specified. The model equation/ modelling the measurement is built as follows:

Measurement function: $Y = f(x1, x2, x3 \ldots xn)$

Where: Y is the output quantity/ measurand; X1, X2... XN is the input quantities.

Identification of the uncertainty sources

To identify all major uncertainty sources of the developed method, the proposed method based on previously method (Ellison and Barwich, (1998) was used "cause and effect diagram" (Ishikawa, 1986). The parameters in the equation of the developed method results, considering each step of the developed method, formed the main branches of the diagram. Contributory factors for each branch were added.

Quantification of the uncertainty components

The uncertainty evaluation process will encompass a number of influence quantities/input, that affect the result obtained for the output quantity/ measurand, (Maurice Cox, 2003). The uncertainty arising from each individual of method data source was quantifying. A measurement uncertainty "budget" (Paul and Helmut, 2003; Colin and Bridget, 2015) was "created". The standard uncertainty was estimated (Halawa, 2014) as follows:

Ustandard = √U2A+U2B

Where: Ustandard is the standard uncertainty; U2A is the random uncertainties "Type A"; U2B is the systematic uncertainties "Type B".

The combine standard uncertainty was calculating using the "root sum square process (RSS)" (Thompson et al., 2010; Eurachem /CIATC, 2012) as follows:

$$uc(y(x1,x2...)) = \sqrt{\sum_{i=1,n} ci^2\, u(xi)^2}$$

Where: uc standard uncertainty; y (x1,x2...xn) is a function of several independent variables x1,x2,...; ci is a sensitivity coefficient evaluated as $ci = \frac{\delta y}{\delta xi}$ the partial differential of y with respect to xi; u (xi) and u(y) are standard uncertainties, that is, measurement uncertainties expressed in the form of standard deviations.

Determination of confidence

To cover a larger fraction of likely values than those covered in the range of one standard uncertainty, the "expanded uncertainty Uexpanded" (JRC, 2012), was determined according to (Miller and Miller, 2010; JRC, 2012) as follows:

$$Uexp = uc \times k$$

Where: Uexp is the expanded uncertainty; uc is the standard uncertainty; k is the coverage factor.

Sources and values of uncertainty

To identify all major sources and its values of uncertainty, of the proposed method, sources and values of uncertainty diagram was creating. The study was analyzing relevant uncertainty sources by representing cause of uncertainty by a horizontal arrows and method of determination of uncertainty by vertical arrows.

RESULTS AND DISCUSSION

Evaluating of measurement uncertainty

Specify the measurand

In order to ensure the uncertainty is appropriate it is important to "specify" (JRC, 2012) what is being measured by the method. The measurement procedure is illustrated schematically in table 1. The separate stages are:

Homogenization

The complete sample is mix in the tube (approx. 2 ml) to ensure the homogeneity.

Preparing internal standards solution

Iso-Propanol in µl and distilled water in ml.

Preparing controls, positive blood control sample

Absolute ethanol in ml and negative whole blood in ml using Calibrated fixed Micropipette in µl and volumetric flask in ml. Calculate the desired volume of absolute ethanol to prepare blood control sample as follows:

$$VEtOH = \frac{CBCS \times VBCS}{d\ EtOH \times 103} \qquad \text{(equation 1)}$$

Where: VEtOH is the Volume of absolute ethanol (ml); CBCS is the Conc. of blood control sample (mg EtOH /100 ml blood); VBCS is the volume of blood control sample (ml); d EtOH is the density of absolute ethanol (g/ml).

Method Blank control sample

Internal standard in ml and distilled water in ml using Calibrated Hamilton Micro lab 500A Series Digital Diluter with Hand Prop.

Resolution mixture control sample

Distilled water in ml using graduated cylinder in ml and volumetric flask in ml. Ethanol in µl using calibrated micropipette in µl. 2 -Propanol in µl using calibrated micropipette in µl. Acetone in µl using calibrated micropipette in µl. Methanol in µl using calibrated micropipette in µl

Sample preparation

All samples [alcohol reference solution, standards and controls] are at room temperature in ^{o}C when prepared for the analysis. Sample volume in ml.

Analyzing on the gas chromatograph

Standards and sample in ml; Retention time in minute; Calibration curve using EtOH standards in mg and CRM in mg.

Measurements of blood alcohol concentrations

The blood alcohol concentrations (C) as mg/100ml of sample by weight is given by:

$$C = \frac{\left[\frac{A_{EtOH}}{A_{isoPropand}}\right]}{S} \times 200 - \frac{Y}{S} \qquad \text{(equation 2)}$$

Where: C is the concentration of alcohol in mg/100ml; AEtOH: Area of ethanol peak ; AisoPropanol: Area of 2-propanol peak; Y: The intercept from the calibration curve; S: The slope from the calibration curve.

Measurement of blood alcohol mass concentration

Injection and GC measurement of 5 µL of sample to give the peak intensity Iop. Preparation of an approximately 5 µg mL-1 standard (actual mass concentration cref). GC calibration using the prepared standard and injection and GC measurement of 5 µL of the standard to give reference peak intensity Iref. The mass concentration cop in the final sample is given by:

$$cop = cref \frac{Iop}{Iref} \ \mu gml\text{-}1 \qquad \text{(equation 3)}$$

Where: cop is the mass concentration of ethanol in the sample [mg kg-1]; Cref is the mass concentration of the reference standard [µg mL-1]; Iop is the peak intensity of the sample; Iref is the peak intensity of the reference standard.

Identifying and analyzing uncertainty sources

The relevant uncertainty sources are shown in cause and effect diagrams figures 1, 2 and 3. The present study considered each step in the analytical procedure (table 1) and all the parameters in basic expression used to calculate the measurand (equations 1, 2 and 3), since all expression may have an uncertainty associated with their value and are their fore potential uncertainty sources. These parameters are represented by main branches of the diagram. The present study was considering all parameters that do not appear in expression, e.g. reagent purity (the concentration of solution will not be known exactly even if the parent material has been assayed, since some uncertainty related to assaying procedure remains; sample preparation (dilution error); calibration of instruments and equipments (that are used for analysis, preparation procedures or control of environmental and storage condition). For the qualitative methods many guidelines, (Meinrath, 2007; Brynn, 2007; Donald, 2015) suggest the use of cause and effect diagram to identify all major uncertainty sources. This diagram is more convenient for avoid double counting, analyzing the reasons of occurrence of both systematic and random errors and possibilities of reducing influence of or eliminating the uncertainty components.

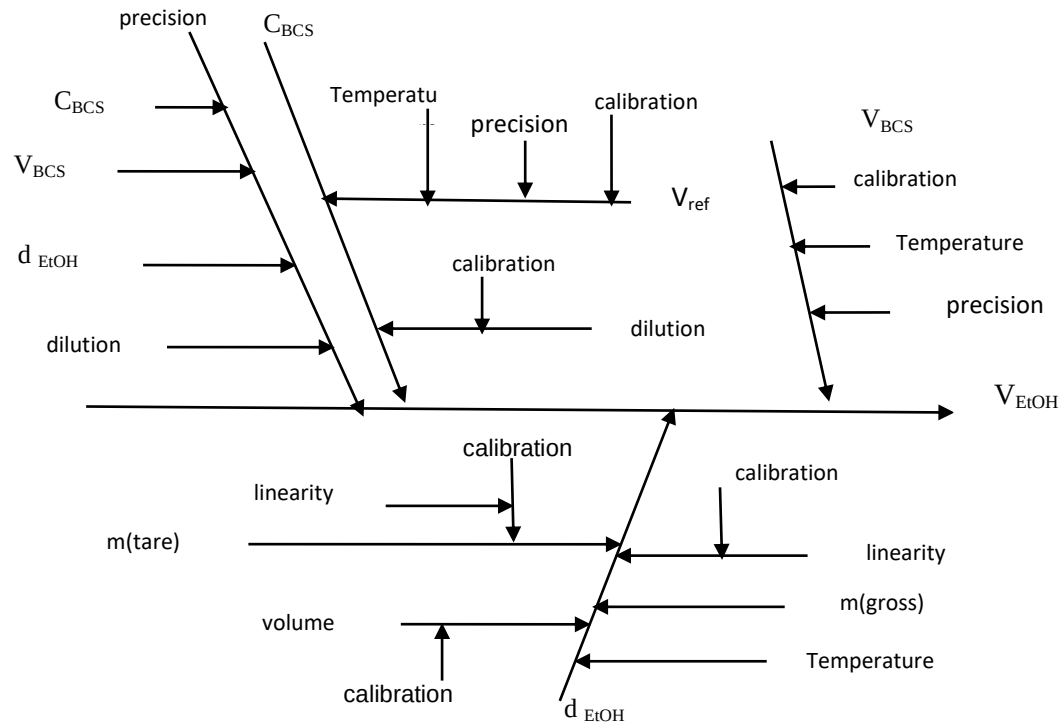

Fig.1: Cause and effect diagram with added uncertainty sources for calculation the desired volume of ethanol to prepare blood control sample (V EtOH).

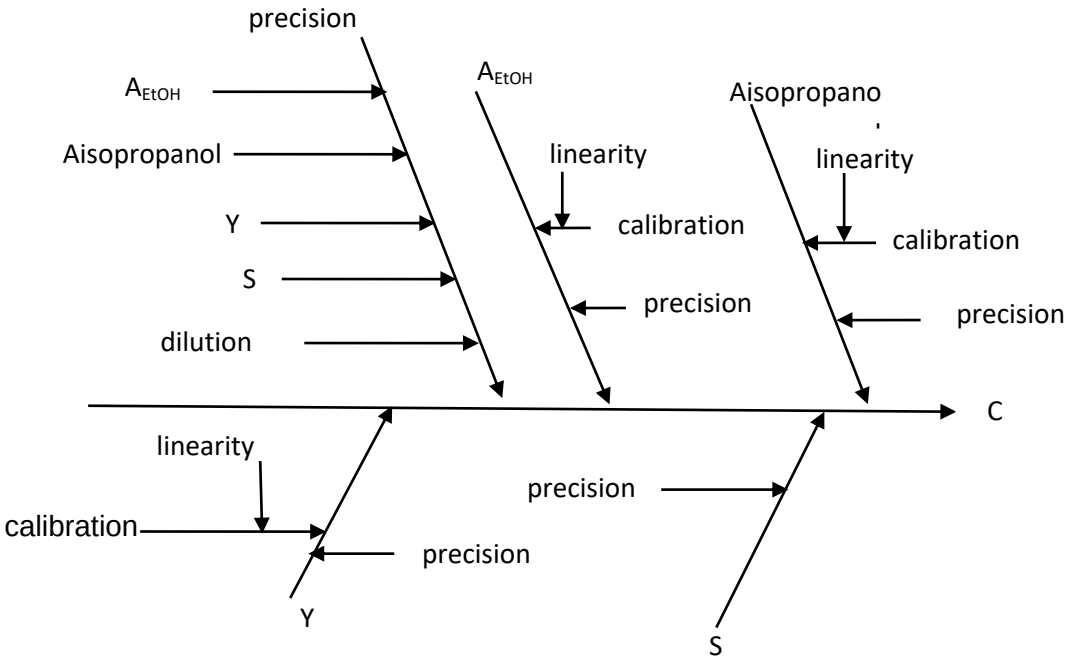

Fig.2: Cause and effect diagram with added uncertainty sources for calculation the blood alcohol concentrations (C).

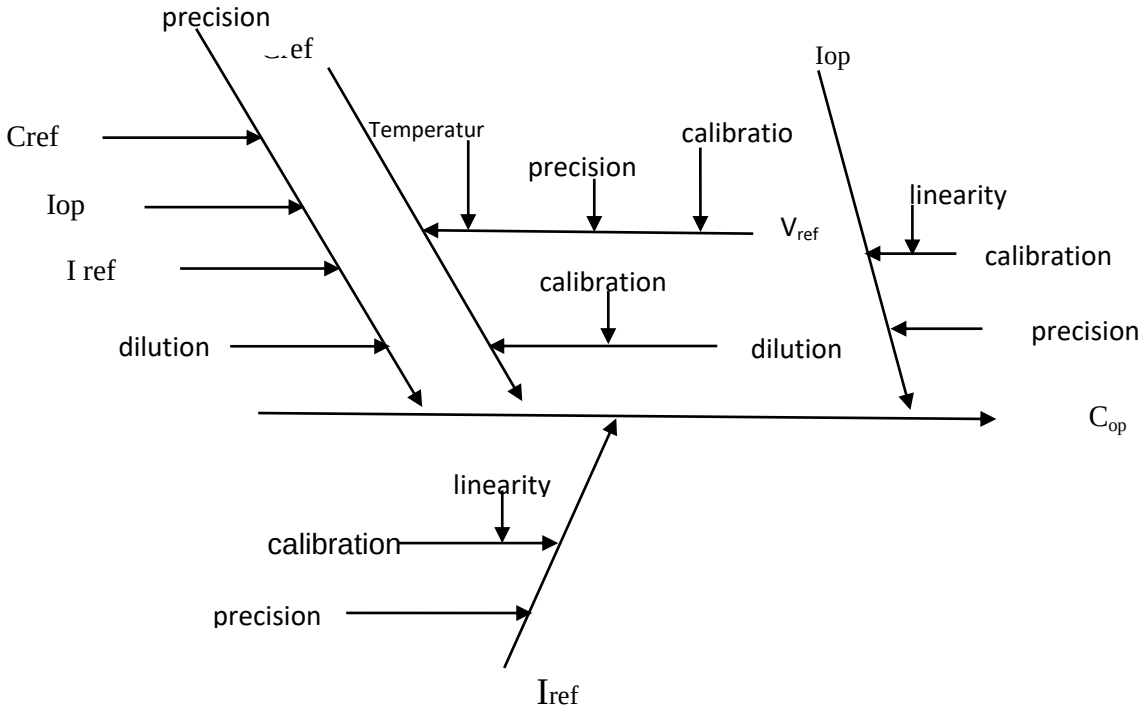

Fig.3: Cause and effect diagram with added uncertainty sources for calculation the blood alcohol mass concentration (Cop).

The relevant uncertainty sources are shown in cause. The present study considered each uncertainty components in cause and effect diagrams (fig. 1, 2 and 3). These components are represented by the main branches of the diagram. The horizontal arrow represent cause of uncertainty and vertical arrow represent method of determination of uncertainty. Extend of horizontal arrow mean that the uncertainty not determines.

For the qualitative methods many guidelines, (Meinrath, 2007; Brynn, 2007; Donald, 2015) suggest the use of cause and effect diagram to identify all major uncertainty sources. This diagram is more convenient for avoid double counting, analyzing the reasons of occurrence of both systematic and random errors and possibilities of reducing influence of or eliminating the uncertainty components.

Quantifying the sources of uncertainty:

The results are shown in table 2, utilises data from the developed and validation studies (ISO/IEC 17025:2017, 7.6.3) to quantify of the different uncertainty components. Type "A" evaluated from "sample data" (Ronald, 2007; Bob, 2013) "all points for the instrument" (Gupta, 2012), "by statistical analyses of a series of repeated observation" (Maalouf, 2014) and Type "B" "for measurement process" (Ronald, 2007) and "corresponding to variance" (Taylor and Kuyatt, 1994; Gupta, 2012). All uncertainty values was expressing in % to eliminate the necessity to convert measurements to the same units. In historical data, an

uncertainty was making from repeated observation of randomly varying process so an uncertainty was gave in the form of 95% confidence interval (Eurachem/CITAC, 2012). The limits values of uncertainty are likely so the study assume a rectangular distribution, with a standard deviation of a/√3, (Eurachem/CITAC, 2012). There is a large amount of historical data (n > 20), so the coverage factor for 95% confidence level is k = 2, (Eurachem/CITAC, 2012; Halawa, 2014).

Table 2: Prepare uncertainty budget report

Source of Uncertainty	Value (units)	Distribution	Divisor	Uncertainty	K or K_{corr}
Historical data (n>20), SD expressed as percent, 3,6%	3,60%	Normal	1	3.6/√2 = 2.55%	
Type B-Calibration uncertainty					
Accuracy of CRM control			1	1.54/√3 = 0.88%;	2
Volume of sample (precision of Hamilton diluter)	0.2%	rectangular	√3	0.11%	
Accuracy of Cerilliant control		rectangular	√3	1.55/2=0.7750=0.0154=1.54%	2
Volume of calibrators (precision of Hamilton diluter)	0.2%	rectangular	√3	0.11%	
Volume of internal standard (precision of Hamilton diluter) rectangular	0.2%	rectangular	√3	0.11%	2
				Combined uncertainty (type A and B) = √(2.552 + 0.882 +0.112 + 0.112 + 0.112) = 2.7	
				Uex = Combined uncertainty x k (2) = 5.4	

Determination of confidence

distribution applies In general, the value of the coverage factor k was chose according to the degree of the confidence required for the range, i.e. U = u × k. typically, k is in the range (2 to 3). When the normal and uc is a reliable estimate of the standard deviation of result, Uexpanded = 2 × uc (i.e. k = 2) defines an interval having 95% of confidence level, and Uexpanded = 3 × uc (i.e. k = 3) defines an interval having 99% of confidence level. Since u is analogous to a standard deviation, if k is 2 (this is generally taken as the default value if no other information is given), then U gives approximately one-half of the 95% confidence interval, (Miller and Miller, 2010).

Sources and values of uncertainty

Sources and values of uncertainty of the developed method are shown in fig. 4. The diagram summarized of a particular measurand or experimental procedure (determining mass, volume, concentration and density) that affect the validity of developed method results, represented of the main components and sources of uncertainty, considering each uncertainty components in cause and effect diagrams (fig. 1, 2 and 3) and suggested method of determining the uncertainty arising from each source , these values used for help in deciding whether a particular component is significant, which indicates good diagram for evaluation of measurement uncertainty.

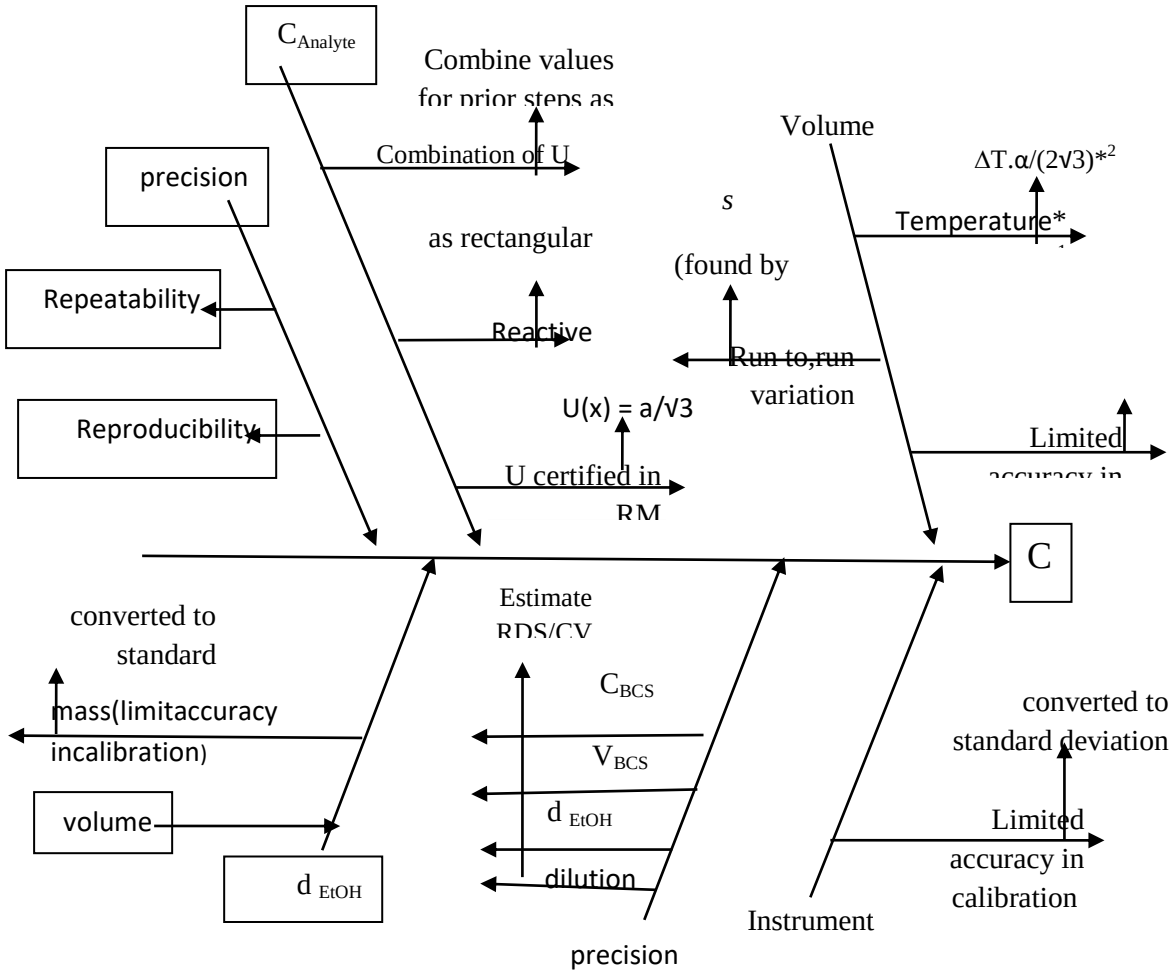

Fig.4: Uncertainty cause and method of determination of uncertainty diagram for calculation the blood alcohol concentrations (C).

*1 Temperature variation from the calibration temperature causes a difference in the volume at the standard temperature.

* ΔT is the possible temperature range and α is the coefficient of volume expansion of the liquid.

α is approximately 1×10-3 K-1 for organic liquids,(Eurachem /CIATC, 2000).

Conclusion

Appropriate method was establishing for the evaluation and expression of uncertainty in quantitative chemical analysis. In addition, it was possible to apply this method to validated data from quantitative analytical method. We created sources and values of uncertainty diagram, which summarized and simplified all steps of evaluation of measurement uncertainty procedure. This observation demonstrated the importance of diagram in documenting procedure. Finally, the proposed procedure can be easily implemented routine analysis and basic researc

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