Received 2 October 2014
Received in revised form 5 November 2014
Accepted 29 November 2014
Available online 16 December 2014
Peer Reviewed
2157118X.4.1.R1

The Confirmation on Method GA/T 1073-2013

XU Pian [*, a], ***MENG Qingfang*** [a], ***ZHENG Jing*** [a]

a Chongqing Engineering Research Center for Criminal Investigation Technology, Institute of Forensic Science, Chongqing Public Security Bureau, Chongqing 400021, China.

* Corresponding author:XU Pian, Ph.D. Tel: +86-023-63751658. E-mail: xupian555@126.com

Abstract *Objective* To confirm whether the examination method GA/T 1073-2013 for determining the content of ethanol in blood by headspace gas chromatography is feasible under the condition of our laboratory and can cope with large batches of cases in practise. *Methods* This paper set up experiments about precision, accuracy and repeatability to test some samples with high, medium and low concentration in the range of calibration curve. Through the results from the aboved experiments, the relative standard deviations were caculated. *Results* The relative standard deviations of the precision, the accuracy and the repeatability are all less than 5%. The average recovery of standard addition was founded to be in the range of 96.50%-99.42%. *Conclusion* All the results indicated that the method was precise, accurate and repeatable.

Keywords: Forensic science, Confirmation, Headspace gas chromatography, ISTD, Blood alcohol content.

1 Introduction

Ethanol (common name is alcohol and molecular formula is C_2H_6O) is widely used in daily life. Therefore, the determination of blood alcohol content is a routine test of forensic indetification and clinical diagnosis. Now the methods to detect the content of ethanol in blood are gas chromatography, enzyme and chemical process, etc[1-3]. This research center has adopted the analysis method for ethanol concentration in blood, which is the professional standard of public security of the PRC published and carried out in 2009[4]. The method, short for GA/T 842-2009, is just aimed at checking the content of ethanol in blood, which is reliable and can meet requirement in practical work. While in 2013, another professional standard namely GA/T 1073-2013 was issued and implemented by the department of public security of the PRC[5]. Its complete title is HS-GC examination methods for ethanol, methanol, n-propanol, aldehyde, acetone, isopropanol and n-butanol in blood and urine. It is clearly that GA/T 1073-2013 has a wide range of detection and can test several compounds which include ethanol. So this paper set up a series of experiments about specificity, precision, repeatability and accuracy to demonstrate whether GA/T 1073-2013 is applicable to determine the content of ethanol in blood under the current condition of our laboratory and can deal with large quantities of cases with results accurate and stable.

2 Materials and Methods

2.1 Instruments and Equipments

GC-2010 Plus; FID (Flame Ionization Detector); Guartz Capillary Column (Rtx-WAX, 30 m×0.25 mm×0.25μm); GCsolution Ver 2.32 workstation; DANI HSS 86.50 Headspace Autosampler; Pipettor; Headspace vials with matched caps; Sealing Plier.

2.2 Reagents

The standard substances: 99.9% anhydrous ethanol (chromatographically pure), 99.5% tertiary-butanol (analytically pure) as the internal standard. Make the preparation of 8000 mg/100 mL

ethanol as stock solution, store it in the refrigerator of 4°C. Dissolve appropriate tertiary-butanol into ultrapure water in the volumetric flask, preparing 4 mg/100 mL as working solution of the internal standard.

2.3 Instrumental Working Conditions

Headspace autosampler: oven temperature: 65°C; manifold temperature: 105°C; delivering tube temperature: 110°C; incubation time: 10.0 min.

Chromatographic column temperature: 40°C; flow velocity: 4.0 mL/min; purge flow velocity: 4.0 mL/min; H2 flow velocity: 40.0 mL/min; Air flow velocity: 400.0 mL/min; make-up gas flow velocity: 30.0 mL/min; injection port temperature: 150°C; detector temperature: 250°C; split ratio: 20.0.

2.4 Experimental Methods

2.4.1 Drawing of calibration curve

Make a preparation of 100 mg/100 mL, 200 mg/100 mL, 500 mg/100 mL, 800 mg/100 mL, 1000 mg/100 mL, 2000 mg/100 mL, 3000 mg/100 mL ethanol standard series. Add 0.09 mL blank blood respectively to 0.01 mL standard series solution and 0.5 mL internal standard working solution in the headspace vials, seal and blend them and wait for determination.

2.4.2 Sample preparation

Add 0.1 mL blood to be measured to 0.5 mL the internal standard working solution in the vial, seal and blend it and wait for detection. Fetch 0.1 mL blank blood and 0.5 mL

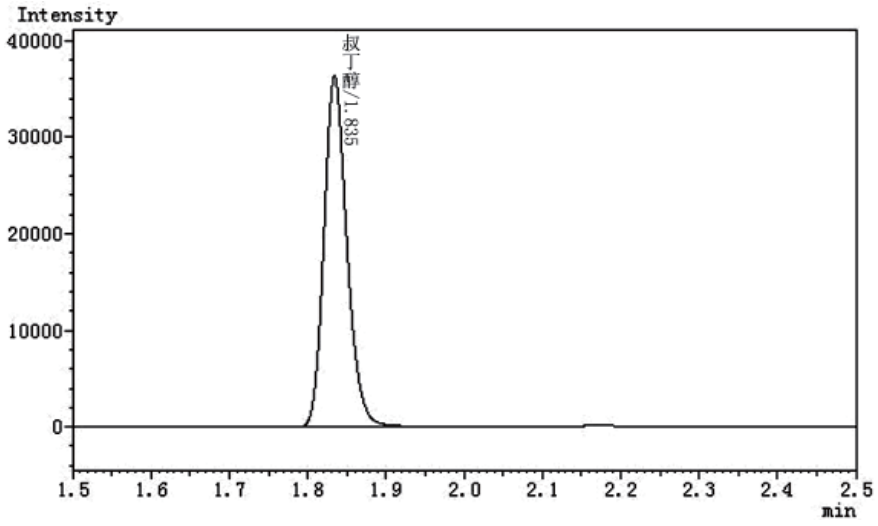

Fig 1. Chromatogram of Blank Control.

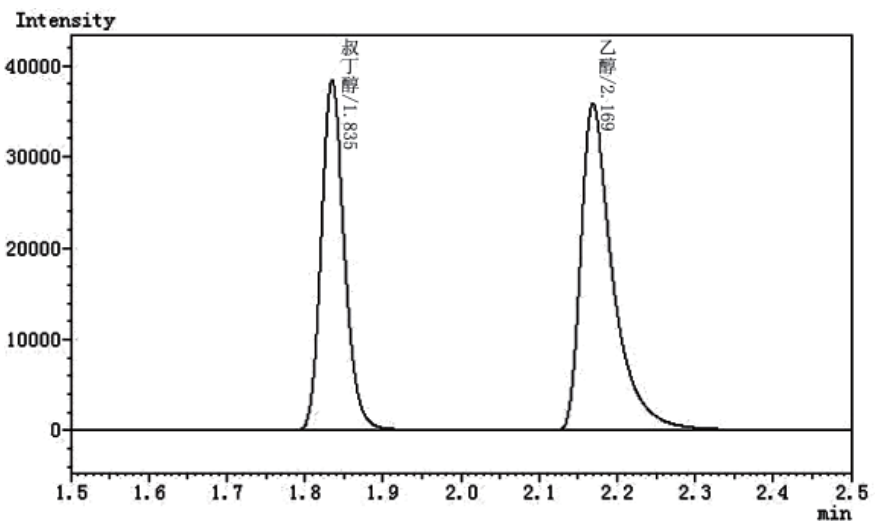

Fig 2. Chromatogram of Ethanol Standard.

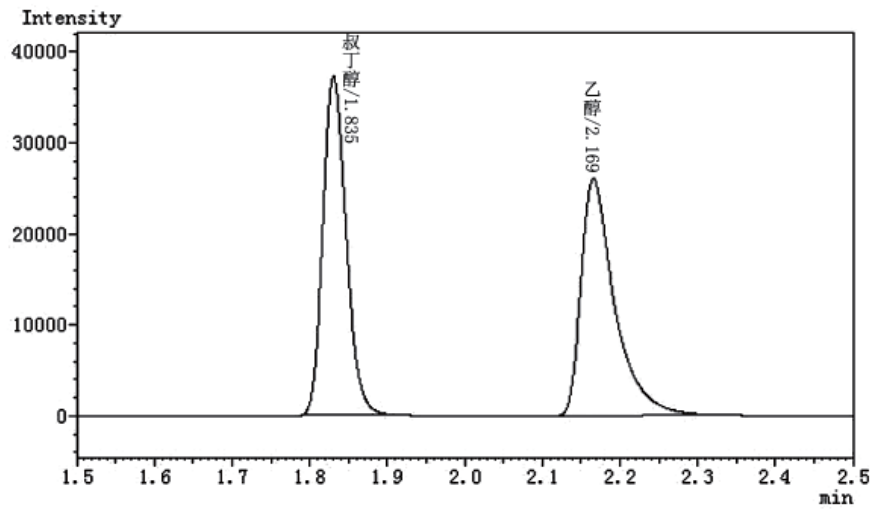

Fig 3. Chromatogram of Blood Sample.

internal standard working solution in the vial, as blank control.

2.4.3 Detection

Place vials of blank control, standard series and sample to be tested into HS autosampler, under the set condition of instrumental working conditions, get these samples injection. The retention times of ethanol and tertiary-butanol are 2.169 mins and 1.835 mins.

3 Results and Discussion

3.1 The Specificity of Method (Figs. 1-3)

Determine blank control, ethanol standard and blood sample successively, thus get chromatograms of them. From these graphs below, it is obvious that the qualification of the method is accurate.

3.2 Linear Regression Equation and Correlation Coefficient of Method

Use peak area ratio of ethanol to the internal standard as ordinate, the concentration of ethanol as abscissa, and get the linear regression equation $Y = 1.287\ X\text{-}1.061\times10^{-2}$. The correlation coefficient is $R^2 = 0.9997$, and the good linear range for ethanol concentration is 10 mg/100 mL-300 mg/100 mL. See ethanol standard series adjusting graph in Figure 4.

3.3 The Lowest Limit of Detection and Quantification Limit of Method

Make an attenuation of ethanol standard solution 100 mg/100 mL to 50 mg/100 mL, 25 mg/100 mL, 20 mg/100 mL, 15 mg/100 mL, 10 mg/100 mL and 5 mg/100 mL solutions. Add 0.09 mL blank blood respectively to 0.01 mL standard solution above and 0.5 mL internal standard working solution in the vials, seal and blend them and wait for determination. According to S/N≥ 3, the lowest minimum mass concentration of ethanol in blood is 1 mg/100 mL. According to S/N≥ 10, the limit of quantification is 5 mg/100 mL.

3.4 The Degree of Precision of Method

Prepare low, medium and high mass concentration of ethanol, and each concentration get 5 paralleled samples introduction. The results in Table 1, it is clear that the degree of precision is 1.96%-3.06%, which meets the requirement.

3.5 The Repeatability of Method

Prepare low, medium and high mass concentration of ethanol, and each concentration get 6 paralleled samples introduction. The results in Table 2 show the repeatability of method is favorable.

3.6 The Recovery Test of Method

Get blank blood prepared to 3 groups of solution in which ethanol concentration are 20 mg/100 mL, 80 mg/100 mL and 160 mg/100 mL, respectively. Each group has 3 paralleled samples tested to calculate the recovery rate of standard addition. The results in Table 3 obviously show that the recovery is 96.50%-99.42%, and the RSD is far lower than 2%, both which indicate the method is accurate and reliable.

3.7 Samples Stability Test

Prepare low, medium and high mass concentration of ethanol, store them in the refrigerator of 4°C. Each concentration check 6 paralleled samples on the first day, the third day, the seventh day and the fourteenth day. Separately compare the averages of 3rd day, 7th day and 14th day with that of 1st day. The results in Table 4 display the relative deviations are all less than 10%, which proves the blood samples can be preserved at least 2 weeks time in the refrigerator of 4°C.

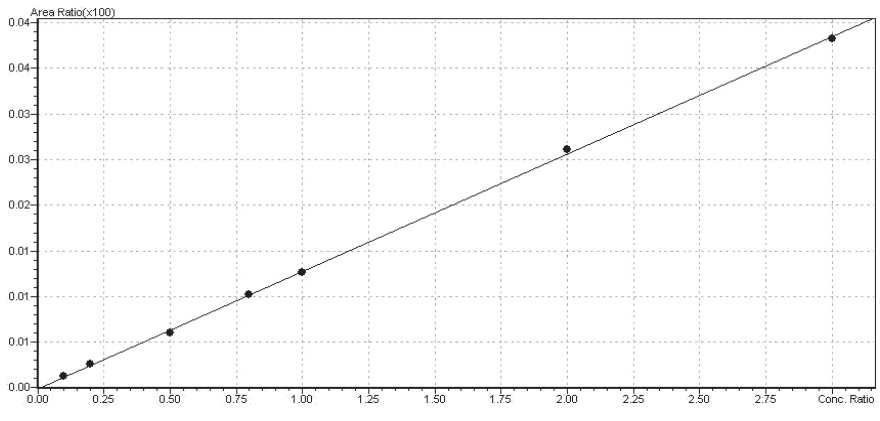

Fig 4. Cablibration Curve of Ethanol Standard Series.

4 Conclusion

It is feasible that the method can be used to detect the content of ethanol in blood, which has a high sensitivity, a good linearity of calibration curve. Moreover, the test results of precision, accuracy and repeatability show it meets requirement.

Acknowledgement

We appreciate the excellent technical assistance provided by SHI Yintao, DUAN Jie, SONG Shengli. We also thank TAO Daiqin for polishing the article.

References

[1] DAI Juan, CHEN Man, LAI Yi, et al. Study for Directly Injected Sample Gas Chromatography Capillary Column Determination of Ethanol Content in Whole Blood [J]. Chinese Journal of Health Laboratory Technology, 2010, 20(2): 283-284. (In Chinese)
[2] LUO Yun, YUE Yunyao, LI Dachun. A Study on Determination of Ethanol in Blood [J]. Chinese Journal of Health Laboratory Technology, 2010, 20(8): 1897-1898. (In Chinese)
[3] LIU Yingchun, CHEN Jie, JI Huagui, et al. Direct Dilution Gas Chromatographic Method for The Determination of Ethanol Content in Blood [J]. West China Medical Journal, 2011, 26(4): 572-575. (In Chinese)
[4] The Department of Public Security of The PRC. The Professional Standard of Public Security of The PRC GA / T 842-2009 [S]. Beijing: Standards Press of China. 2009. (In Chinese)
[5] The Department of Public Security of The PRC. The Professional Standard of Public Security of The PRC GA / T 1073-2013 [S]. Beijing: Standards Press of China. 2013. (In Chinese) ■

Table 1. Method Degree of Precision Test.

Sample Concentration	Measured quality (mg/100 mL)					Average (mg/100 mL)	RSD (%)
Low	16.30	15.70	15.60	15.80	16.20	15.92	1.96
Medium	74.90	72.00	72.70	74.90	76.30	74.16	2.38
High	150.0	142.9	142.5	147.4	152.9	147.14	3.06

Table 2. Method Repeatability Test.

Sample Concentration	Measured quality (mg/100 mL)						Average (mg/100 mL)	RSD (%)
Low	20.50	19.20	19.30	18.80	19.40	19.00	19.37	3.07
Medium	78.70	78.50	80.60	77.60	78.80	78.30	78.75	1.27
High	157.9	158.4	164.2	157.9	160.6	158.2	159.5	1.57

Table 3. Method Recovery Test.

Number	Addition (mg/100 mL)	Measured quality (mg/100 mL)	Recovery rate (%)	Average recovery rate (%)	RSD (%)
1	20	19.20	96.00		
2	20	19.30	95.00	96.50	0.518%
3	20	19.40	97.00		
4	80	78.70	98.38		
5	80	78.50	98.13	98.34	0.194%
6	80	78.80	98.50		
7	160	158.4	99.00		
8	160	160.6	100.38	99.42	0.837%
9	160	158.2	98.88		

Table 4. Method Degree of Precision Test.

Sample Concentration	Measured Average (n=6) (mg/100 mL)				Relative Deviation (%)		
	1st Day	3rd Day	7th Day	14th Day	3rd Day	7th Day	14th Day
Low	20.41	19.85	19.42	18.95	2.78	4.97	7.42
Medium	79.00	77.93	78.03	76.90	1.36	1.24	2.69
Medium	158.78	155.28	156.81	154.90	2.23	1.25	2.47