

Determine the Content of Ethanol in Blood by Headspace Gas Chromatography

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Abstract *Objective* To establish a rapid detecting method for the determination of ethanol content in blood by headspace gas chromatography (HSGC). *Method* Blood samples were tested by HSGC with flame ionization detector. Compared with the standard controls operating parallelly, the retention time was used as the qualitative basis. Peak area ratio of ethanol to the internal standard substance was quantified ethanol concentration through the internal standard method. *Results* Under the optimized conditions of analysis, the coefficient of association was greater than 0.999 within the linearity range 10–300 mg/100 mL. The lowest minimum mass concentration of ethanol in blood was 1 mg/100 mL. The recovery rate was 97.76%–106.55% and the RSD of the degree of precision was less than 2%. *Conclusion* It was rapid, accurate and highly sensitive that the method could be used to detect ethanol content in blood.

Keywords: Ethanol, Headspace auto-injection, Gas chromatography, Internal standard method, Forensic science.

1 Introduction

Ethanol (common name is alcohol and molecular formula is C_2H_5OH) is widely used in daily life. However, drunk driving and alcoholism have been increasing in recent years. The determination of blood alcohol content, which therefore becomes a routine test of forensic identification and clinical diagnosis, is a critical evidence for traffic accidents and criminal cases. Now the methods to detect the content of ethanol in blood are gas chromatography, enzyme and chemical process, etc^[1–5]. All these methods have limitations^[6]. This article adopts HSGC (Headspace Gas Chromatography) for analysis and the accuracy and repeatability are favorable.

2 Materials and Methods

2.1 Instruments and Equipments

GC-2010 Plus; FID (Flame Ionization Detector); Quartz Capillary Column (Rtx-WAX, 30 m×0.25 mm×0.25 μ m); 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 50 mg/100 mL as working solution of the internal standard.

2.3 Instrumental Working Conditions

Headspace autosampler: oven temperature: 70°C; manifold temperature: 105°C; delivering tube temperature: 110°C; incubation time: 15.0 min.

Chromatographic column temperature: 40°C; flow velocity: 3.0 mL/min; purge flow velocity: 4.0 mL/min; H_2 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, 1600 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 0.1 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 0.1 mL the internal standard working solution in the vial, seal and blend it and wait for detection. Fetch 0.1

mL blank blood and 0.1 mL 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

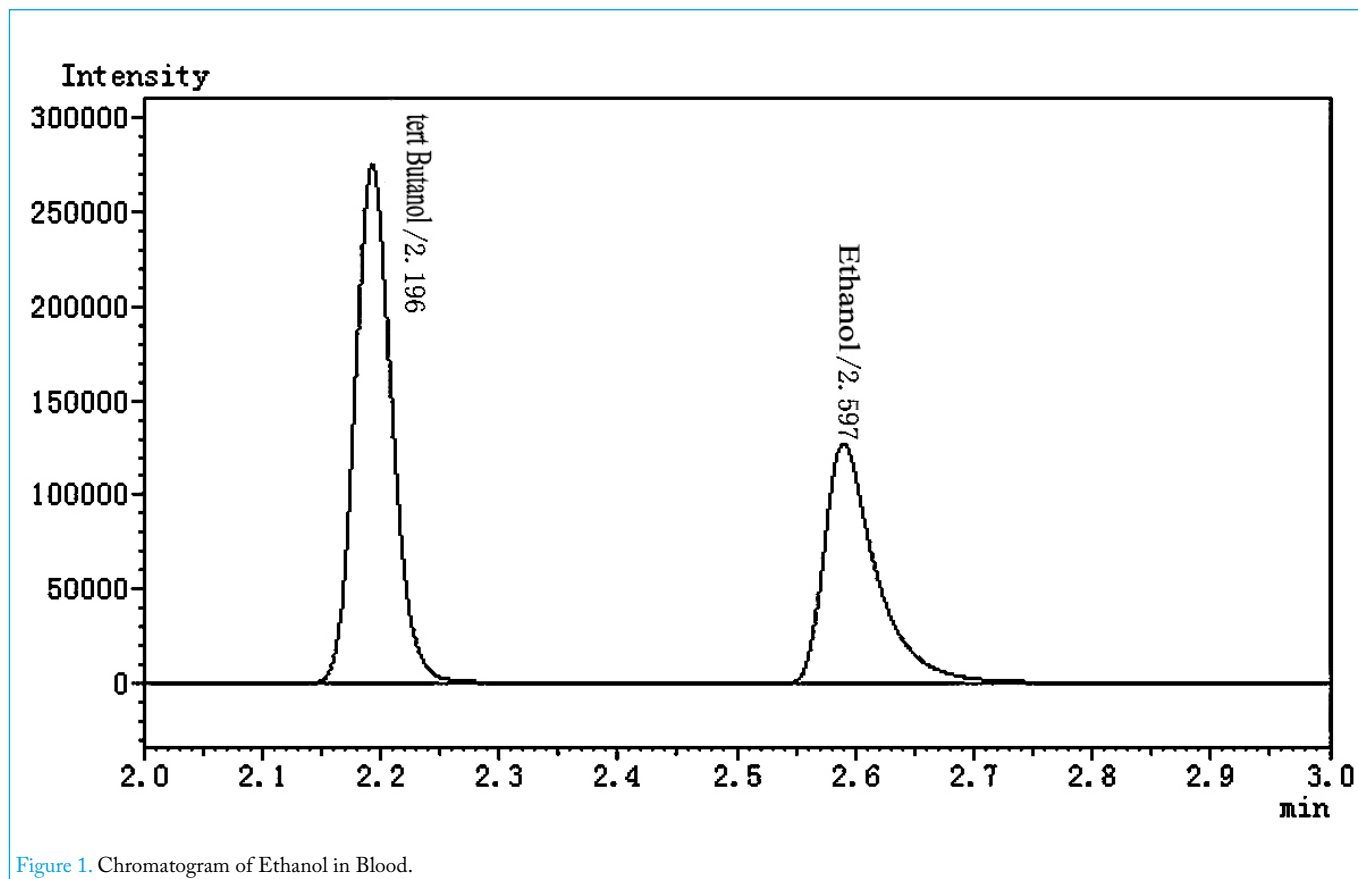


Figure 1. Chromatogram of Ethanol in Blood.

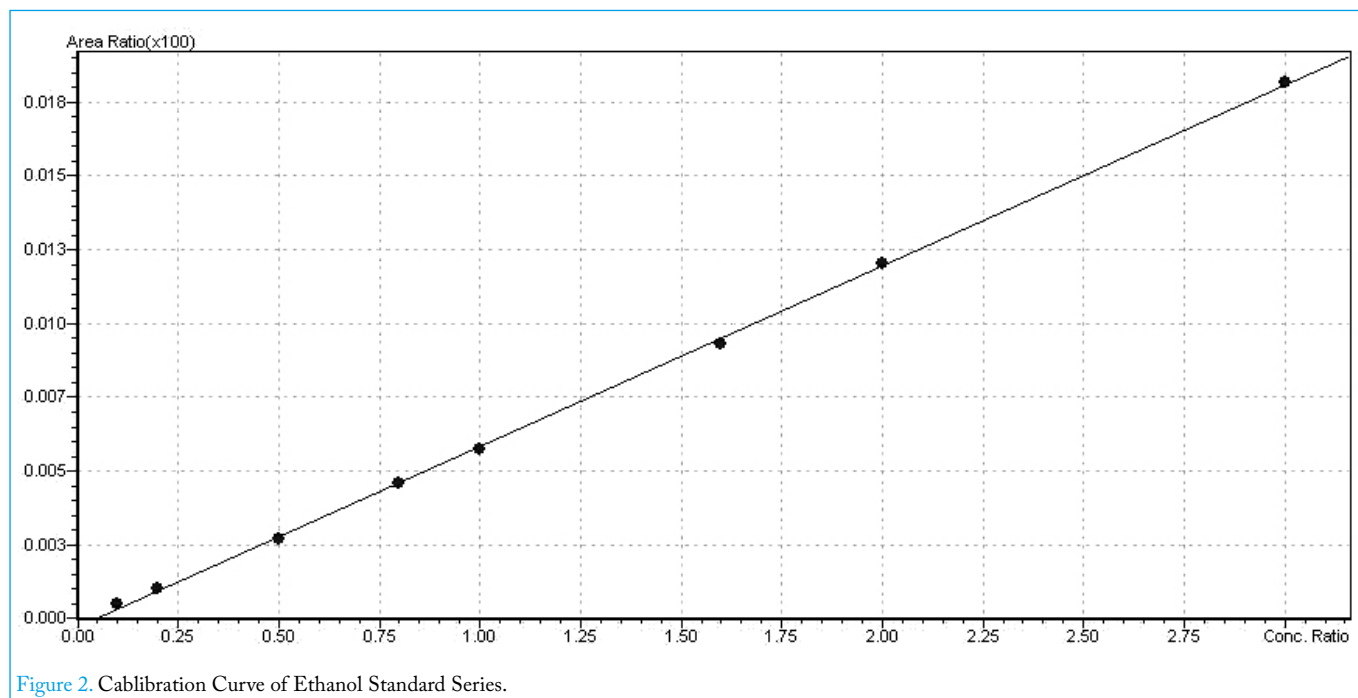


Figure 2. Cabliration Curve of Ethanol Standard Series.

Table 1. Method Degree of Precision Test.

Sample Concentration		Measured quality (mg/100 mL)					Average (mg/100 mL)	RSD (%)
Low	21.87	21.93	21.93	22.17	22.41	21.85	22.03	0.9985
Medium	78.82	80.87	81.34	80.64	81.34	80.46	80.58	1.158
High	156.39	161.70	161.58	163.67	165.28	162.32	161.82	1.858

Table 2. Method Degree of Accuracy and Recovery Test.

Number	Addition (mg/100 mL)	Measured quality (mg/100 mL)	Recovery rate (%)	Average recovery rate (%)	RSD (%)
1	20	21.30	106.50		
2	20	21.57	107.85	106.55	1.197
3	20	21.06	105.30		
4	80	78.52	98.15		
5	80	79.52	99.40	99.29	1.099
6	80	80.26	100.33		
7	160	158.09	98.81		
8	160	154.90	96.81	97.76	1.024
9	160	156.24	96.81		

conditions, get these samples injection. The retention times of ethanol and tertiary-butanol are 2.597 mins and 2.196 mins.

3 Results and Discussion

3.1 Determining of Blood Sample (Fig. 1)

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 $Y = 0.6118 X - 2.698 \times 10^{-2}$. The correlation coefficient is $R^2 = 0.9996$, 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 2.

3.3 The Lowest Limit of Detection

Make an attenuation of ethanol standard solution 100 mg/100 mL to 50 mg/100 mL, 25 mg/100 mL, 10 mg/100 mL and 5 mg/100 mL solution. Add 0.09 mL blank blood respectively to 0.01 mL standard solution above and 0.1 mL internal standard working solution in the vials, seal and blend them and wait for determination.

According to $S/N \geq 3$, the lowest minimum mass concentration of ethanol in blood is 1 mg/100 mL.

3.4 The Degree of Precision of Method

Prepare low, medium and high mass concentration of ethanol, and each concentration get 6 paralleled samples introduction. The results in Table 1, it is clear that the degree of precision is 0.9985%–1.858%, which meets the requirement.

3.5 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 2 obviously show that the recovery is 97.76%–106.55%, and the RSD is far lower than 2%, both which indicate the method is accurate and reliable.

4 Conclusion

The method is quick and simple, merely needing a small volume of blood, while the result is accurate. We do think it has practical value of the content check of ethanol in blood.

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