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Application Data Sheet

No.13

GC
Gas Chromatography

Analysis of Alcohol Compounds in Blood (2)

Measurements of oxygenated compounds and alcohols, primarily ethanol, in blood, are frequently performed in forensic medicine, emergency medicine, and other fields. In forensic medicine, such measurements are utilized to determine levels of intoxication from alcohol consumption and to evaluate criminality. In emergency medicine, they are utilized to distinguish between alcohol consumption and other medical cases. Application Data Sheet No. 12 introduced results for the repeatability of ethanol and the separation of standard solutions of oxygenated compounds using the HS-20 and the Rtx-BAC Plus series of columns specifically designed for alcohol analysis.

This report introduces the results of an investigation of linearity and repeatability for blood spiked with ethanol.

Analysis Conditions

HS-20
Shared Conditions

Oven Temp.:	85 °C	Vial Agitation:	Off
Vial Warming Time:	15 min.	Vial Pressurization:	100 kPa
Vial Pressurization Time:	1 min.	Load Time:	0.5 min.
Injection Time:	0.5 min.	Needle Flash Time:	0.5 min.
Sample Line Temp.:	150 °C	Transfer Line Temp.:	150 °C
Vial Volume:	20 mL		

GC-2010 Plus AF + LabSolutions LC/GC

Column:	Rtx-BAC Plus 2, 0.32 mm × 30 m, d.f. 0.6 μm		
Column Temp.:	40 °C		
Carrier Gas Pressure:	100 kPa (helium pressure mode)	Split Ratio:	1:20
FID Temp.:	250 °C	Hydrogen:	40 mL/min.
Makeup Gas:	30 mL/min. (helium)	Air:	400 mL/min.
Sample:	Ethanol (EtOH) added to sterilized sheep blood		
Internal Standard Solution (IS):	200 mg/100 mL aqueous t-butanol solution		

Measurement Sequence

Fig. 1 shows the measurement sequence for the blood samples. The pretreatment method was as per "GA/T 842-2009 Analysis Method for Ethanol Concentration in Blood." Sterilized sheep blood was used for the blood samples.

0.5 mL sample
↓
0.1 mL internal standard solution added to the sample
(IS: 200 mg/100 mL t-butanol in H_2O)
↓
Warmed at 85 °C for 15 min
↓
Headspace gas injected into the GC

Fig. 1 Measurement Sequence

Results

Fig. 2 shows overlapping chromatograms for a blank blood sample and blood spiked with the equivalent of 8 mg/100 mL to 160 mg/100 mL of EtOH. Fig. 3 shows the linearity obtained when blood was spiked with the equivalent of 8 mg/100 mL to 160 mg/100 mL of EtOH, and Table 1 shows the concentration ratios and area ratios. As indicated, a favorable linearity of R = 0.9999 was obtained.
Tables 2 and 3 show the repeatability of retention times, area values, and area ratios for blood spiked with the equivalent of 40 mg/100 mL of EtOH. Favorable repeatability was obtained, as the retention time RSD% was 0.096 % for EtOH and 0.088 % for the IS; the area value RSD% was 0.83 % for EtOH and 1.18 % for the IS; and the area ratio RSD% was 0.68 %.

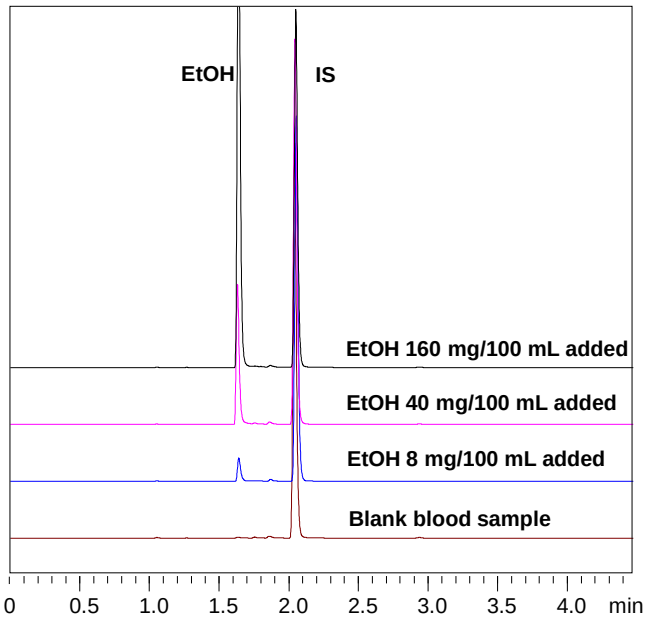

Fig. 2 Chromatograms for a Blank Blood Sample and Blood Spiked with the Equivalent of 8 mg/100 mL to 160 mg/100 mL of EtOH

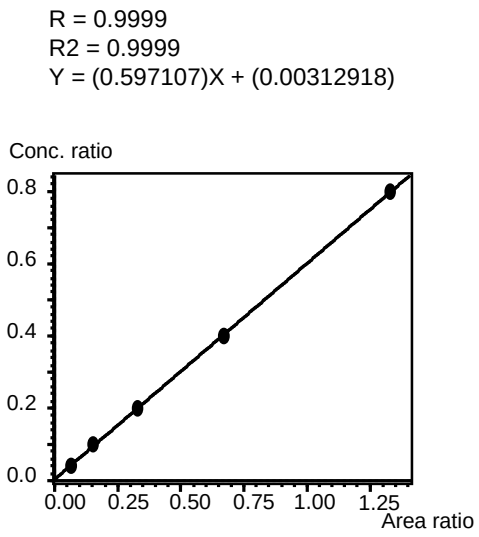

Fig. 3 Linearity of Blood Spiked with the Equivalent of 8 mg/100 mL to 160 mg/100 mL of EtOH

Table 1 Concentration Ratios and Area Ratios

Concentration ratio (EtOH/IS)	0.04	0.10	0.20	0.40	0.80
Area ratio (EtOH/IS)	0.06632	0.15329	0.33028	0.67099	1.3320

Table 2 Retention Time (min) Repeatability (40 mg/100 mL)

	1	2	3	4	5	6	mean	SD	RSD%
EtOH	1.640	1.641	1.642	1.643	1.643	1.644	1.642	0.0016	0.096
IS	2.054	2.055	2.055	2.057	2.057	2.058	2.056	0.0018	0.088

Table 3 Area Value (μV*s) and Area Ratio Repeatability (40 mg/100 mL)

	1	2	3	4	5	6	mean	SD	RSD%
EtOH	384101	374675	376905	378761	377604	378506	378425	3142.8	0.830
IS	1158476	1126253	1135762	1125928	1121669	1130570	1133110	13314.8	1.175
EtOH/IS	0.3316	0.3327	0.3319	0.3364	0.3366	0.3348	0.3340	0.0023	0.679

Reference: Chinese National Standards: GA/T 842-2009 Analysis Method for Ethanol Concentration in Blood

Notes: This product has not been approved or certified as a medical device under the Pharmaceutical and Medical Device Act of Japan. It cannot be used for the purpose of medical examination and treatment or related procedures.

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