Application Note

Agilent
Trusted Answers

Hand Sanitizer Analysis Using the Agilent 8860 GC Configured with a Flame Ionization Detector

Author

Jie Zhang
Agilent Technologies, Inc.

Abstract

This application note describes the analysis of ethanol- and isopropanol-based hand sanitizer. The Agilent J&W DB-WAX column was used to optimize chromatographic resolution and peak shape. Flame ionization detection (FID) was used for detection. Internal calibration standard (IS) was used for quantitation based on US Pharmacopeia (USP) <611> method recommendation. The instrument suitability was investigated in terms of peak tailing factor, alcohol resolution to internal standard, and quantitation repeatability.

Introduction

Ethanol and isopropyl alcohol (IPA) are the main active components in hand sanitizer. To maintain effectiveness, the concentration of ethanol or isopropyl alcohol (IPA) should remain in an appropriate range, usually 65 to 75% as labeled on the sanitizer container. Because other alcohols can also be present in sanitizer products as impurities or improper additions as a means to make counterfeit products, measuring the correct alcohols at the proper concentration levels is important. Gas chromatography (GC) is one of the techniques recommended by USP for alcohol concentration determination. GC can also provide retention time information to verify the alcohol identity, ensuring that the hand sanitizer has not been contaminated. With the qualitative and quantitative analysis capability, gas chromatography will continue to play a key role in the quality control of hand sanitizer.

The methods for alcohol determination, such as USP <611>[1] and ASTM D3695,[2] recommend a mid-polar or polar column for good separation and response of alcohol components. Based on our previous work,[3] Agilent J&W DB-Wax or Agilent DB-624 Ultra Inert columns can deliver sharper and more symmetric peak shapes for alcohols. An FID is recommended for alcohol detection because it is easy to use and can generate a stable and adequate response within the tested concentration range after alcohols are diluted during the sample preparation process. In this work, a DB-WAX Ultra Inert column was used on an Agilent 8860 GC in conjunction with FID for alcohol analysis. The system performance was evaluated for retention time repeatability, peak resolution, peak tailing factor, and quantitation precision. This was done to demonstrate the suitability of the 8860 GC/FID system coupled with an Agilent Ultra Inert column for alcohol analysis in hand sanitizer.

Experimental

The Agilent 8860 GC is equipped with a split/splitless inlet and an FID. Liquid sample injection was done using an Agilent 7693A automatic liquid sampler (ALS) with a 5 µL syringe. The analytical parameters are shown in Table 1.

Chemicals and standards

The alcohol solvents and acetonitrile (IS) were purchased from ANPEL Scientific Instrument (Shanghai). The glycerin working solution was prepared by weighing 58.4 mg of glycerin (approximately 50 µL) and diluting it in distilled water to 1 mL. The calculated volume concentration for glycerin working solution was 4.6% (v/v). The alcohol calibration standards were prepared by adding aliquots of pure alcohols or its working solution to distilled water with a final volume at 1 mL. Ethanol, IPA, and glycerin are in the group 1 calibration solutions. The calibration range for ethanol and IPA are from 1 to 4% (v/v). Glycerin concentration is from 0.01 to 0.18% (v/v). Methanol and *n*-propanol (n-PA) were prepared in group 2 calibration solutions. The calibration range for methanol was from 1 to 4% (v/v), and 0.1 to 1.5% (v/v) for *n*-propanol. The internal standard (IS) acetonitrile (ACN) was added to the calibration solutions at a concentration of 5% (v/v). The calibration solutions were prepared as shown in Table 2. The QC sample was prepared by diluting 25 µL of ethanol, 25 µL of IPA, and 50 µL of acetonitrile to 1 mL in distilled water.

The hand sanitizer gel sample was too viscous for direct liquid injection and had to be diluted before injection by the 7693A auto liquid sampler. That is the reason the real samples and calibration standards are prepared with solvent dilution. The hand sanitizer sample of 50 µL was dispensed into a flask using a 1 mL gas-tight syringe. Next, 50 µL of acetonitrile were added as IS, and the sample was diluted to 1 mL by distilled water for later analysis.

Table 1. Analytical parameters of an Agilent 8860 GC-FID on alcohol standards.

Agilent 8860 GC parameters	
S/SL Inlet	250 °C, split ratio 20:1
Injection Volume	0.2 µL
Carrier Gas	He
Column Flow Rate	7 mL/min, constant flow mode
Oven	50 °C (5 min), 30 °C/min to 230 °C (3 min)
FID	250 °C, air: 400 mL/min, fuel gas (H_2): 30 mL/min, constant make up (N_2): 18 mL/min
Column	Agilent J&W DB-WAX UI, 30 m, 530 µm, 1 µm (p/n 125-7032UI)
Inlet Liner	Agilent Ultra Inert, low pressure drop with glass wool (p/n 5190-2295)

Results and discussion

This application note focused on the analysis of the main alcohol components and possible methanol contamination in hand sanitizer. Five types of alcohols were included in the calibration standard. They were methanol, ethanol, IPA, *n*-propanol, and glycerin. Among them, the concentrations of ethanol, IPA, and *n*-propanol are usually labeled because they are closely related with the effectiveness of eliminating viruses. Glycerin and other types of alcohols can be detected in real samples, but there is no specific concentration labeled by producers for these alcohols. To include glycerin in the calibration standard aims to show how the described system can generate a decent peak and give adequate detection of late-eluting and sticky alcohols such as glycerin.

The separation of five alcohols and one internal standard in aqueous solution with component concentration varying from 1.0 to 4% (v/v), was performed on the Ultra Inert DB-WAX column. This sample was a mixture of the middle-level calibration standard of group 1 and group 2 with glycerin working standard. As shown in the chromatogram (Figure 1) and Table 3, the tailing factors of all peaks were less than 1.2. Methanol and IPA eluted before ethanol. The resolution of *n*-propanol to acetonitrile (IS) was 9, and the resolution of ethanol to acetonitrile was 12. The USP <611> standard requires alcohol peak tailing factors of less than 2.0 and peak resolutions to internal standard more than 4.0. The resolution and peak shape achieved on the 8860 GC, with the flow path starting from the S/SL inlet, passing through the analytical column, and ending at the FID, proved that the system was suitable for the targeted alcohol analysis.

The resolution of ethanol and IPA was 1.31. It is not a baseline separation but usually does not impact the accurate quantitation of ethanol or IPA considering they do not co-exist in the same hand sanitizer in most cases. Even

Table 2. Alcohol calibration standards preparation.

Level No.	Group One Calibration Standards			Group Two Calibration Standards	
	Ethanol (µL)	IPA (µL)	Glycerin Working Solution (µL)	Methanol (µL)	*n*-Propanol (µL)
1	40 (4.0%)	10 (1.0%)	2 (0.0092%)	49 (4.9%)	1 (0.1%)
2	35 (3.5%)	15 (1.5%)	5 (0.023%)	45 (4.5%)	5 (0.5%)
3	30 (3.0%)	20 (2.0%)	10 (0.046%)	40 (4.0%)	10 (1.0%)
4	25 (2.5%)	25 (2.5%)	20 (0.092%)	35 (3.5%)	15 (1.5%)
5	20 (2.0%)	30 (3.0%)	40 (0.184%)	30 (3.0%)	NA
6	15 (1.5%)	35 (3.5%)	NA	25 (2.5%)	
7	10 (1.0%)	40 (4.0%)		20 (2.0%)	
8	NA	NA		15 (1.5%)	
9				10 (1.0%)	

The final volume of calibration solution at different levels is 1 mL. The alcohol concentrations at different levels are shown as the values in the parentheses.

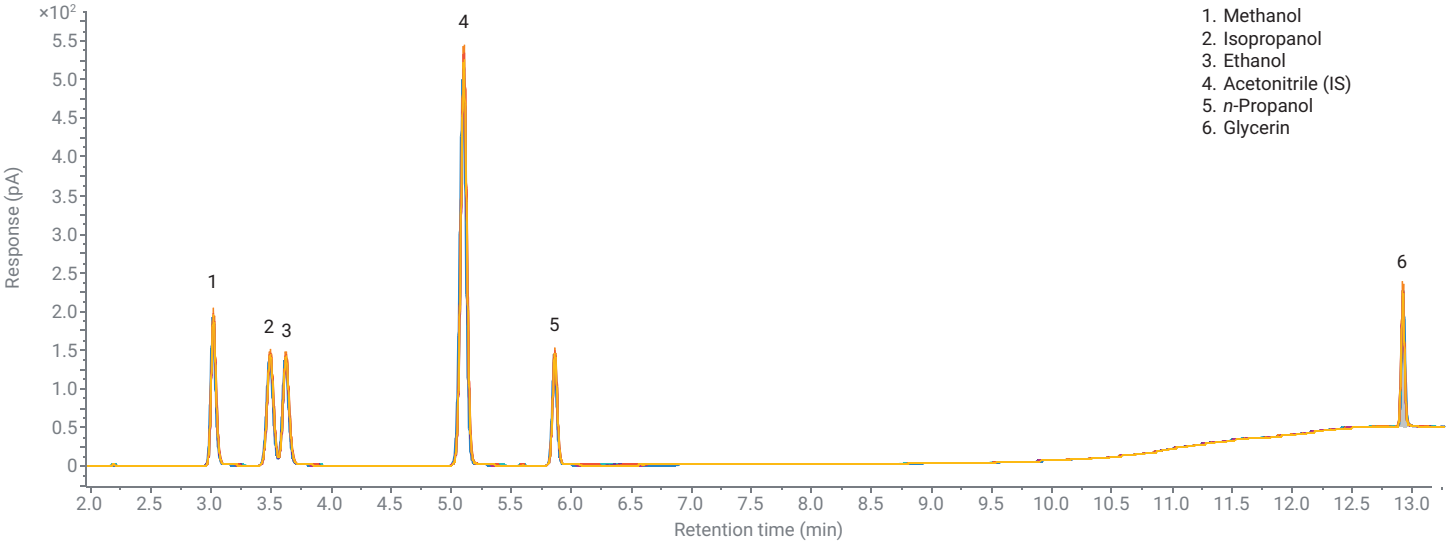


Figure 1. Chromatogram overlay of alcohol standards analyzed on an Agilent 8860 GC-FID installed with an Agilent DB-WAX Ultra Inert column.

if they are co-existent, the resolution of 1.31 is sufficient to give an accurate quantitation on the principal alcohol, which can be shown in the following analysis result on a hand sanitizer gel sample.

The alcohol calibration curves were developed using an internal standard calibration method. As shown in Figure 2 and Table 3, the correlation coefficients (R^2) for the alcohols across their calibration range are from 0.997 to 0.999.

The quantitation precision was evaluated by seven injections of alcohol mixture with each alcohol ranging from 1 to 2% (v/v). Their overlaid chromatograms are shown in Figure 1. The quantitation precisions were assessed based on the relative standard deviation (RSD) of the response ratio of alcohols to acetonitrile during the seven injections. Most of the quantitation precision was better than 1%, except glycerin, with RSD% at 1.7%. The USP <611> standard requires an RSD of no more than 4.0% in the ratio of the peak of alcohol to the peak of the internal standard. The precision performance on the tested platform exceeded the USP <611> requirement.

The alcohol analysis in two hand sanitizer gels and in one sanitizer spray from different vendors was performed. The chromatograms of the three products are shown in Figure 3. The test results of the main alcohols in each sample are tabulated in the inset table. For sanitizer gel sample 1, ethanol, *n*-propanol, and glycerin are identified. The tested concentrations of ethanol, *n*-propanol, and glycerin are 61.0% (v/v), 10.4% (v/v), and 0.3% (v/v). The ethanol and *n*-propanol concentration labeled by the producer was 54 to 66% (v/v) and 9 to 11% (v/v). The concentration measured and labeled for gel sample 1 matched quite well with each other.

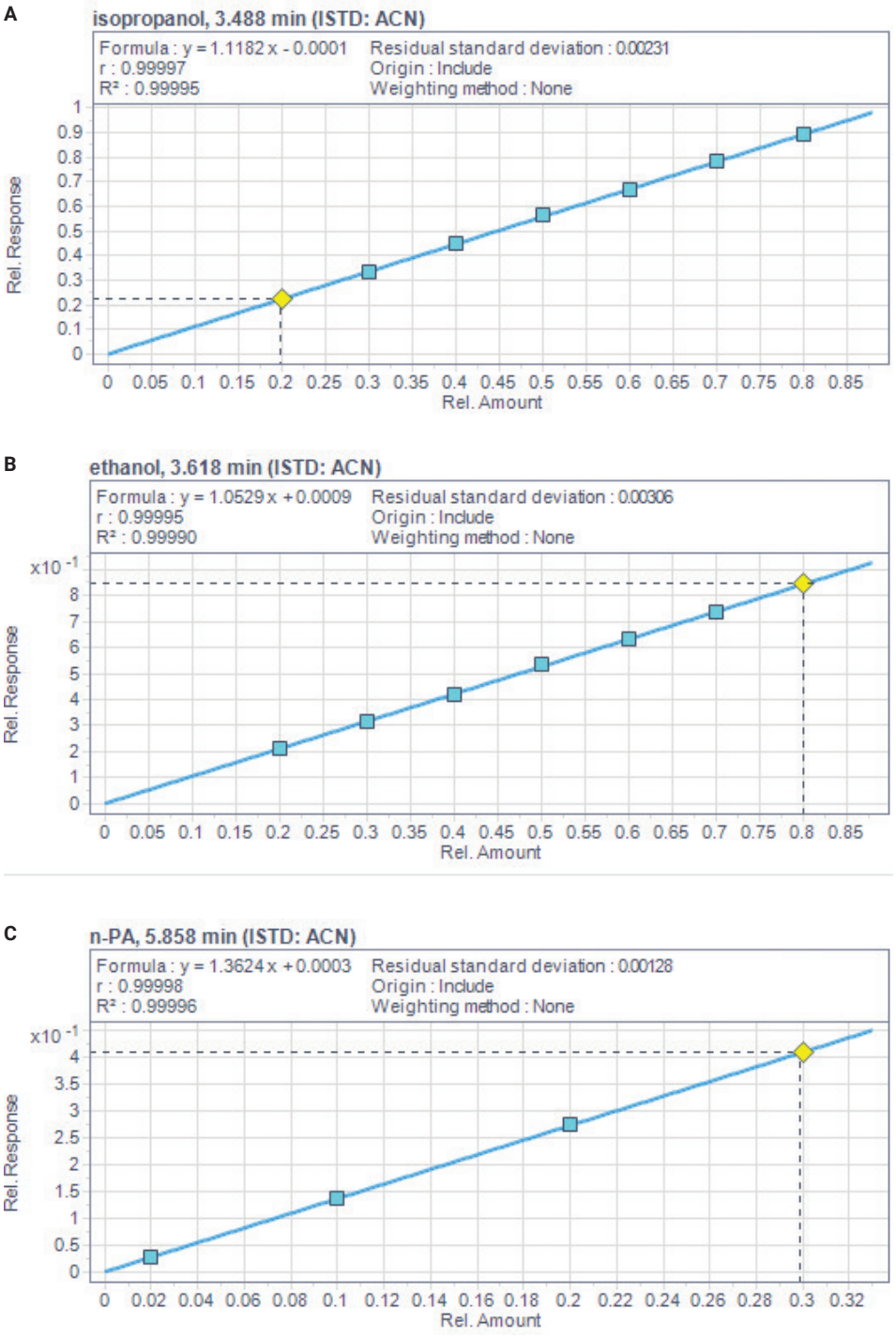
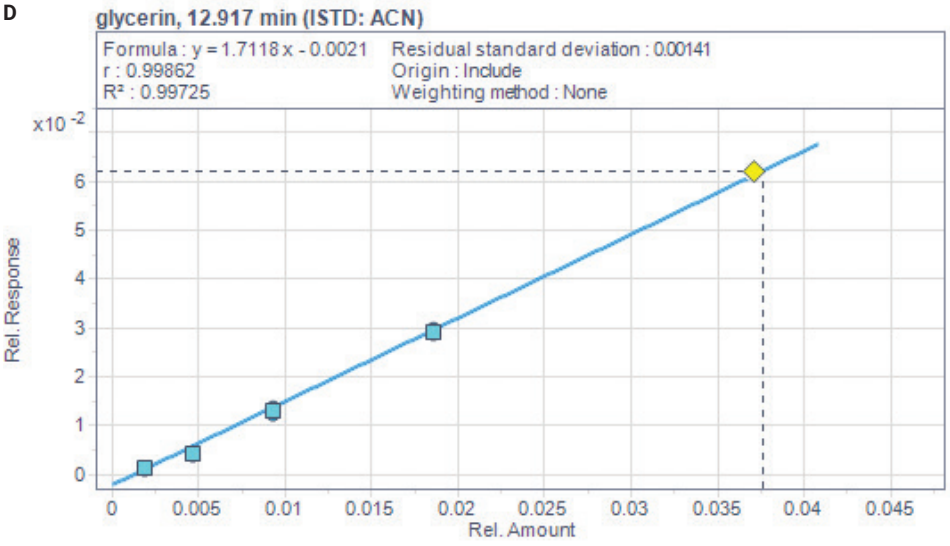


Figure 2A,B,C. Calibration curves of IPA (A), ethanol (B), and *n*-propanol (C), with correlation coefficients more than 0.999.

D



E

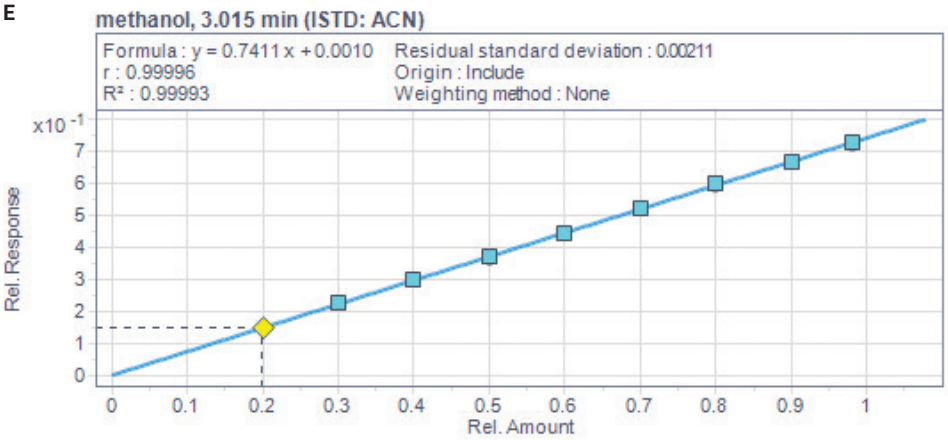


Figure 2D,E. Calibration curves of glycerin (D) and methanol (E), with correlation coefficients more than 0.995.

Table 3. Demonstration of system suitability for alcohol analysis.

Compound	Average RT (min)	RT RSD%	Quantitation Precision (%)	Calibration Correlation Coefficient	Calibration Range (v/v)	Peak Tailing Factor	Resolution with IS
Methanol	3.015	0.025	0.3	0.9999	1 to 4.9%	1.2	>15
IPA	3.488	0.028	0.41	0.9999	1 to 4%	1.1	>15
Ethanol	3.618	0.026	0.16	0.9999	1 to 4%	1.0	15
n-Propanol	5.858	0.008	0.29	0.9999	0.1 to 1.5%	1.1	9
Glycerin	12.917	0.006	1.67	0.9970	0.0092 to 0.184%	1.0	>9

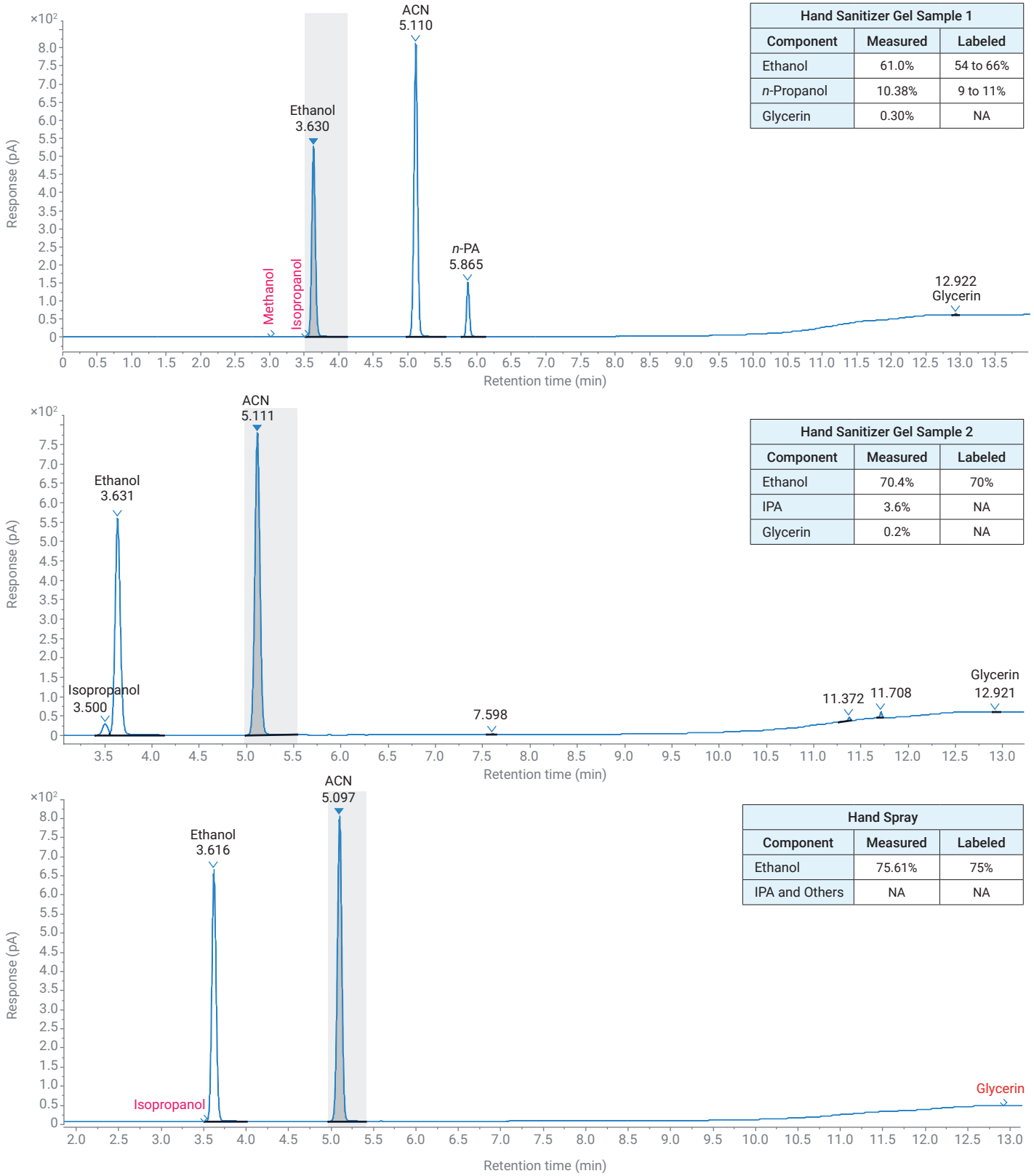


Figure 3. Chromatograms of hand sanitizer gels and spray and their identification results.

Gel sample 2 was identified with 70.4% (v/v) ethanol, 3.6% (v/v) IPA and 0.2% (v/v) glycerin. It was labeled with ethanol concentration at 70% (v/v), another good match with the test results. For gel sample 2, IPA and ethanol did not separate at the baseline level, but the quantitation of ethanol is still accurate. The ethanol concentration in alcohol spray is labeled at 75% (v/v) and determined at 75.6%. No glycerin was detected in the alcohol spray. There are other peaks detected in sanitizer gel sample 2, which eluted at 7.6, 11.4, and 11.7 minutes. It is likely that these components are added by producers to the sanitizer as antimicrobial ingredient or humectant, etc. Their concentration was estimated less than 1% (v/v) by using the relative response factor of glycerin. There is no methanol detected in the three hand sanitizers samples.

A long sequence was run to test the stability of the system. A total of 40 injections of hand sanitizer gel samples and five QC samples were run. The sequence started from a QC sample, followed by five injections of sanitizer gel sample 1 and five injections of gel sample 2. This was followed by another three rounds of one QC sample injection and 10 real sample injections, ending with a fifth injection of QC sample. The nominal concentration of ethanol and IPA in the QC sample was 50.0% (v/v). The overlaid chromatograms of five QC samples are shown in Figure 4. RT and quantitation results for the five QC samples are listed in Table 4. The average measured concentration for ethanol and IPA were 50.2% (v/v) and 51.2% (v/v). The quantitation accuracy of QC samples was 101% and 102%. The quantitation precision for IPA and ethanol were 0.715% and 1.11%. The RT repeatabilities were 0.03% and 0.04%.

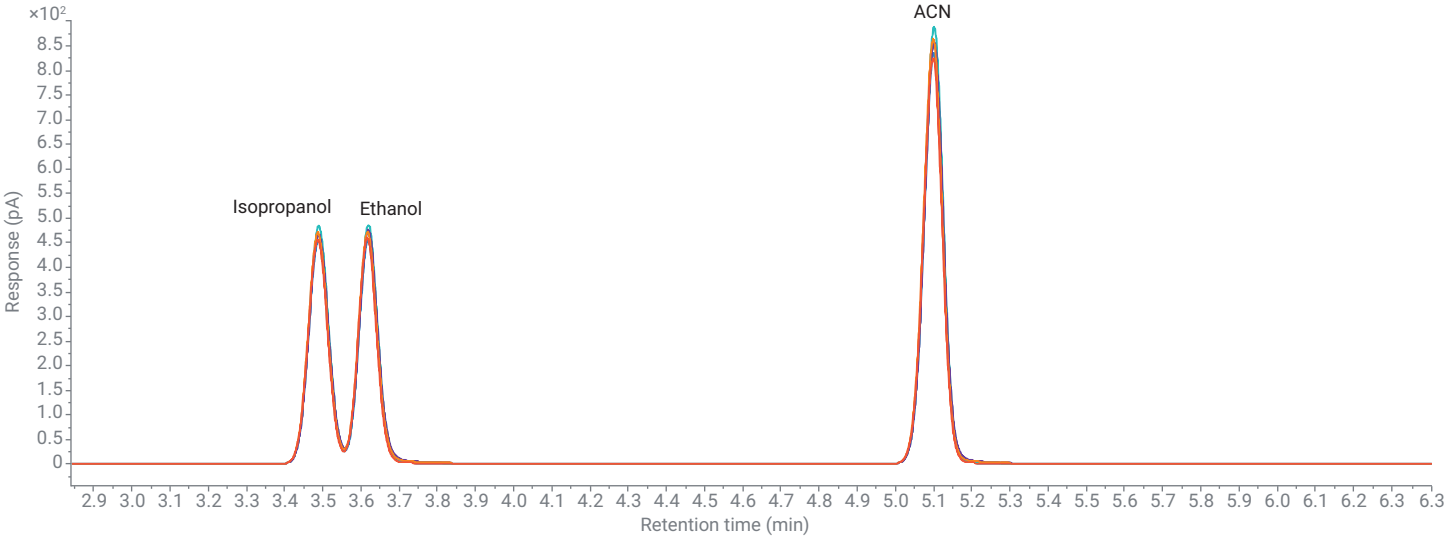


Figure 4. The overlaid chromatograms of five QC samples.

Table 4. Quantitation and RT precision of five QC samples during 40 runs of real sample analysis.

Quantitation (Vol%)	QC1	QC2	QC3	QC4	QC5	Quan. RSD%
IPA	50.363	50.038	49.958	50.045	50.821	0.71%
Ethanol	50.544	51.061	52.067	51.473	51.051	1.11%
Retention Time (min)	**QC1**	**QC2**	**QC3**	**QC4**	**QC5**	**RT RSD%**
IPA	3.487	3.488	3.488	3.486	3.486	0.03%
Ethanol	3.617	3.618	3.618	3.616	3.615	0.04%

The result summary for 20 injections of gel samples 1 and 2 is tabulated in Table 5. The concentration measured for the 40 injections of real hand sanitizer samples matched well with the labelled concentration range. The quantitation precision for ethanol and *n*-propanol in sample 1 was 0.45% and 0.38%, respectively, and the quantitation precision for IPA and ethanol in sample 2 was 0.55% and 0.31%. The RT repeatability for ethanol, IPA and *n*-propanol during 20 injections ranged from 0.02 to 0.04%, comparable to the RT RSD% of 0.01 to 0.03% generated in seven consecutive injections shown in Table 3. The quantitation accuracy and precision of the QC and real samples demonstrated that the 8860 GC-FID system, coupled with the Ultra Inert DB-WAX column, can provide reliable and accurate alcohol analysis.

Table 5. The result summary of sanitizer gel samples during stability test.

Gel 1					Gel 2				
Injection No.	RT (min)	Quan. (Vol%)	RT (min)	Quan. (Vol%)	Injection No.	RT (min)	Quan. (Vol%)	RT (min)	Quan. (Vol%)
	Ethanol		*n*-Propanol			IPA		Ethanol	
2	3.618	61.59	5.858	10.48	7	3.49	3.63	3.62	69.93
3	3.62	61.55	5.856	10.48	8	3.491	3.63	3.621	70.39
4	3.618	61.54	5.858	10.49	9	3.49	3.65	3.619	70.38
5	3.618	61.02	5.857	10.41	10	3.491	3.65	3.623	70.45
6	3.619	61.56	5.856	10.48	11	3.493	3.63	3.623	70.40
13	3.62	61.59	5.856	10.48	18	3.49	3.65	3.621	70.02
14	3.618	61.57	5.856	10.48	19	3.492	3.61	3.622	70.29
15	3.62	61.85	5.858	10.53	20	3.492	3.62	3.623	70.39
16	3.619	61.73	5.857	10.51	21	3.492	3.65	3.622	70.56
17	3.618	61.75	5.856	10.52	22	3.492	3.63	3.622	70.30
24	3.619	61.42	5.856	10.47	29	3.491	3.65	3.622	70.40
25	3.617	61.76	5.855	10.51	30	3.49	3.61	3.621	70.34
26	3.619	61.74	5.857	10.51	31	3.49	3.62	3.62	70.41
27	3.619	61.89	5.856	10.53	32	3.49	3.63	3.62	70.36
28	3.619	61.80	5.856	10.53	33	3.491	3.66	3.622	70.60
35	3.618	61.93	5.856	10.55	40	3.489	3.64	3.619	70.97
36	3.617	62.02	5.855	10.55	41	3.49	3.64	3.62	70.49
37	3.618	62.28	5.857	10.60	42	3.49	3.64	3.62	70.58
38	3.619	62.09	5.857	10.55	43	3.487	3.70	3.617	70.52
39	3.617	62.07	5.856	10.55	44	3.488	3.61	3.619	70.71
Mean	3.62	61.74	5.86	10.51		3.49	3.64	3.62	70.42
RSD%	0.03%	0.45%	0.02%	0.38%		0.04%	0.55%	0.04%	0.31%

Conclusion

This work used the 8860 GC system in conjunction with FID and an Ultra Inert DB-WAX column for alcohol analysis in hand sanitizers. The inert gas flow path contributed by the Ultra Inert liner and Ultra Inert column helped generate a sharp and symmetrical peak for the targeted alcohols, with peak tailing factors less than 1.2. The resolution of alcohol to internal standard exceeded the requirement in the USP <611> method. The average quantitation precision on the volatile alcohols are lower than 1%. The calibration performance for all five alcohols are excellent with linearity correlation coefficients better than 0.995. The analysis on the real hand sanitizer gels and sanitizer spray delivered accurate quantitation results. The system performance demonstrated how the 8860 GC/FID with the Ultra Inert column system is useful for alcohol analysis in hand sanitizers.

References

1. USP <611> Alcohol Determination, Monograph US Pharmacopeia, **2020**.
2. ASTM D3695-95(2013), Standard Test Method for Volatile Alcohols in Water by Direct Aqueous-Injection Gas Chromatography, ASTM International, West Conshohocken, PA, **2013**.
3. Abercrombie, V. *et al.* Analysis of Ethanol and Isopropyl Alcohol in Alcohol-Based Hand Sanitizers by Direct Injection GC/FID, *Agilent Technologies application note*, publication number 5994-1923EN.

www.agilent.com/chem

DE.5224768519

Printed in the USA, June 29, 2020
5994-2089EN

Agilent
Trusted Answers