Fast, cost-effective quantification of alcohol content in hand sanitizers by direct injection GC-FID

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Goal

The aim of this application note is to demonstrate the suitability of the Thermo Scientific™ TRACE™ 1310 Gas Chromatograph coupled with flame ionization detection (FID) for the determination of ethanol (in compliance to the USP <611> method) and isopropanol content in hand sanitizers.

Introduction

COVID-19 is an infectious disease caused by a newly discovered coronavirus involving mild to moderate respiratory illness. According to the World Health Organization, washing the hands with soap or using an alcohol based hand sanitizer when soap and water are not available is the best way to prevent the spread of the virus and to slow down its transmission.[1] Hand sanitizers together with face masks have, therefore, become the first safety measures to prevent the spread of COVID-19. Hand sanitizers contain ethanol or isopropanol (IPA) in high concentrations (usually 65–80%) to be effective against germs and bacteria. The United States Pharmacopeia (USP) has published an analytical procedure (USP <611>)[2] for the determination of alcohol content that can be applied to evaluate products formulated with ethanol. In this procedure, ethanol can be determined either by distillation (USP <611> Method I) or gas chromatography coupled with FID and either packed (USP <611> Method IIa) or capillary columns (USP <611> Method IIb).

In this application note, the gas chromatographic method coupled to capillary column was applied but the suggested oven ramp was shortened in compliance with the USP General Notices and Requirements[3] resulting in a time and cost-effective alternative for pharmaceutical laboratories dealing with high sample throughput. Since isopropanol can be used as a main ingredient of hand sanitizers as well as ethanol, it was added to the standard solution to broaden the scope of the analysis for hand sanitizer testing.

Experimental

In the experiments described here, a Thermo Scientific TRACE 1310 gas chromatograph equipped with a Thermo Scientific™ Instant Connect™ Split/Splitless injector (iC-SSL) and a Thermo Scientific™ Instant Connect™ Flame Ionization Detector (iC-FID) was coupled to a Thermo Scientific™ AS 1310 liquid autosampler. Chromatographic separation was achieved on a Thermo Scientific™ TraceGOLD™ TG-624 SilMS 30 m × 0.53 mm × 3.0 µm column (P/N 26059-3960) in accordance with USP <611> Method IIb. Since water was used as a diluent, a Thermo Scientific™ ultra-inert LinerGold™ Precision liner with quartz wool (P/N 453A1255-UI) and a Thermo Scientific™ HydroGOLD Column Guard (P/N 26H50-0553) were used to improve water vaporization and to extend the lifetime of the chromatographic column by reducing the potential degradation of the stationary phase caused by repeated water injections. Additional GC-FID and autosampler parameters are detailed in Table 1.

Data acquisition, processing, and reporting

Data was acquired, processed, and reported using Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software, version 7.3. Chromeleon CDS allows full automation from instrument setup to data processing, reporting, and storage in compliance with Title 21 of the Code of Federal Regulations Part 11 (Title 21 CFR Part 11), which defines the rules of the Food and Drug Administration.

Standard and sample preparation

A USP alcohol determination reference standard at 2% v/v was purchased from Sigma-Aldrich (P/N USP-1012688). HPLC-MS grade acetonitrile (P/N 10001334) and HPLC-MS grade isopropanol (IPA, P/N 10684355) were purchased from Fisher Scientific. Two commercially available ethanol (label 70% v/v) and one commercially available isopropanol-based (label 70% v/v) hand sanitizers were purchased at local retailers. Acetonitrile (used as Internal standard, IS), IPA, and samples were diluted using HPLC-MS grade water (Fisher Scientific, P/N 10777404) to an approximate concentration of 2% v/v as per USP <611> Method IIb.

Standards and samples for analysis were prepared following the Test and Standard Preparation procedures reported in the USP <611> Method IIb.

Test preparation: 5 mL of sample (2% v/v) and 5 mL of IS (2% v/v) were further diluted using HPLC-MS grade water to a final volume of 25 mL.

Table 1. GC-FID operating conditions according to USP <611> Method IIb. A carrier flow of 4.55 mL/min corresponds to a linear velocity of 34 cm/s as per USP method requirements.

TRACE 1310 GC and AS 1310 autosampler parameters	
Inlet module and mode	SSL, split
Liner	LinerGOLD Precision Liner; Quartz Wool (P/N 453A1255-UI)
Inlet temperature (°C)	210
Split ratio	5:1
Septum purge mode, flow (mL/min)	Constant, 5
Carrier gas, mode, flow (mL/min)	He, constant flow, 4.55
Oven temperature program	
Temperature 1 (°C)	50
Hold time (min)	1.0
Temperature 2 (°C)	250
Rate (°C/min)	70
Hold time (min)	5.1
GC total run time (min)	9.00
FID	
Temperature (°C)	280
Air flow (mL/min)	350
H_2 flow (mL/min)	35
N_2 flow (mL/min)	40
Acquisition rate (Hz)	25
AI/AS 1310 autosampler	
Injection volume (µL)	0.2
Syringe	5 µL syringe, fixed needle (P/N 36500505)
Draw speed	Slow
Fill strokes	10
Sample depth	Bottom
Cold needle injection	Enabled
Chromatographic separation	
Column	TraceGOLD TG-624 SilMS 30 m x 0.53 mm x 3.0 µm (P/N 26059-3960)

Standard preparation: 5 mL of USP alcohol determination reference standard (2% v/v), 5 mL of IPA (2% v/v), and 5 mL of IS (2% v/v) were diluted using HPLC grade water to a final volume of 25 mL.

1 mL of the Test and Standard preparations were then transferred in 2 mL screw top amber vials (P/N 60180-561, caps P/N 60180-729), and 0.2 µL were injected into the chromatographic system to assess instrument suitability and quantitative performance.

Results and discussion

Chromatography

Chromatographic performance was assessed by applying the system suitability test (SST) criteria reported in the USP <611> Method IIb: peak asymmetry (A_s) for ethanol <2.0, resolution (R_s) between ethanol and IS >4, and peak area ratio (ethanol/IS) %RSD <4 using n=6 replicate injections of the standard solution. These criteria were extended to isopropanol with the exception of the resolution; however, a resolution greater than 1.5 was considered acceptable. Peak asymmetry and resolution were automatically calculated in Chromeleon CDS by applying the formulae reported in the USP <621> system suitability section.[4] Gaussian peak shape was obtained for both ethanol and IPA with average A_s factors of 1.07 and 1.02, respectively, demonstrating the inertness of the system and the effective water sample transfer during injection. Average chromatographic resolution with respect to the IS resulted to be 7.05 for ethanol and 2.19 for IPA.

Peak area ratio %RSD (ethanol/IS and IPA/IS) was calculated using n=10 repeated injections of the standard preparation and resulted to be 0.47 and 0.16, respectively.

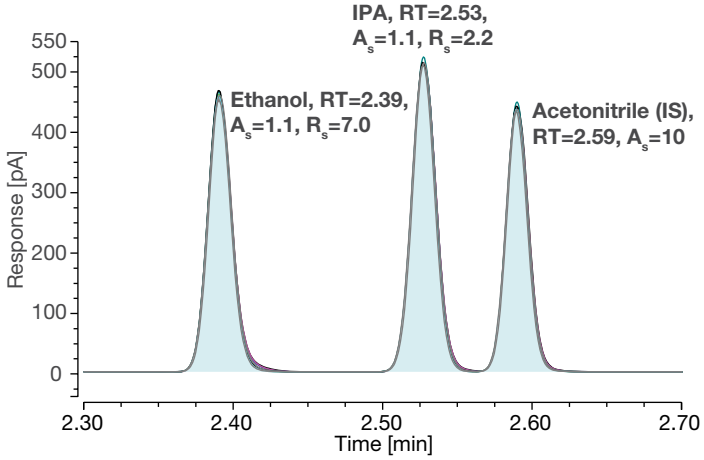


Figure 1. Overlaid chromatograms (n=10) showing chromatographic separation and peak area repeatability for ethanol, IPA, and IS in solvent standard. USP <611> Method IIb SST criteria were met with Gaussian peak shape for both ethanol and IPA with average A_s factors of 1.07 and 1.02, respectively, and average R_s with respect to the IS of 7.05 for ethanol and 2.19 for IPA. Peak area ratio (ethanol/IS and IPA/IS) %RSD was 0.47 for ethanol and 0.16 for IPA.

An example of overlaid chromatograms for n=10 standard injections is shown in Figure 1; average A_s and R_s are annotated. The SST results are summarized in Table 2.

Table 2. SST results obtained for n=10 consecutive injections of standard preparation. USP <611> method IIb system suitability requirements were met for both ethanol and IPA with peak area ratio (ethanol/IS and IPA/IS) %RSD of 0.47 and 0.16, average A_s factors of 1.07 and 1.02 and average R_s with respect to the IS of 7.05 and 2.19, respectively.

	Peak area ratio (%RSD)		Asymmetry (A_s)		Resolution (R_s)	
	Ethanol	IPA	Ethanol	IPA	Ethanol	IPA
Std 1	1.13	1.27	1.10	1.03	6.97	2.18
Std 2	1.11	1.26	1.11	1.03	7.05	2.17
Std 3	1.12	1.27	1.10	1.00	7.03	2.16
Std 4	1.13	1.27	1.06	1.02	7.07	2.20
Std 5	1.12	1.27	1.06	1.05	6.95	2.18
Std 6	1.12	1.27	1.04	1.03	7.09	2.21
Std 7	1.13	1.27	1.10	1.02	7.09	2.19
Std 8	1.13	1.27	1.06	1.05	6.99	2.21
Std 9	1.12	1.27	1.05	1.01	7.06	2.17
Std 10	1.12	1.27	1.04	0.99	9.96	2.16
Average	1.12	1.27	1.07	1.02	7.05	2.19
Std. dev.	0.005	0.002	0.023	0.019	0.054	0.016
%RSD	**0.47**	**0.16**	**2.16**	**1.90**	**0.76**	**0.74**

Quantitative analysis

Standard and test preparations were injected in triplicate, and the percentage (% v/v) of alcohol (ethanol or IPA) was calculated using Equation 1. The advanced reporting capability of Chromeleon CDS allowed for automatic calculation and easy data review as shown in Figure 2. Quantitative results were in accordance with the label claim with calculated concentrations ±2% of the expected values as reported in Table 3.

Equation 1

$$\text{Alcohol content (as \%v/v)} = CD(R_U / R_S)$$

Where:

C = labeled concentration of USP alcohol determination standard

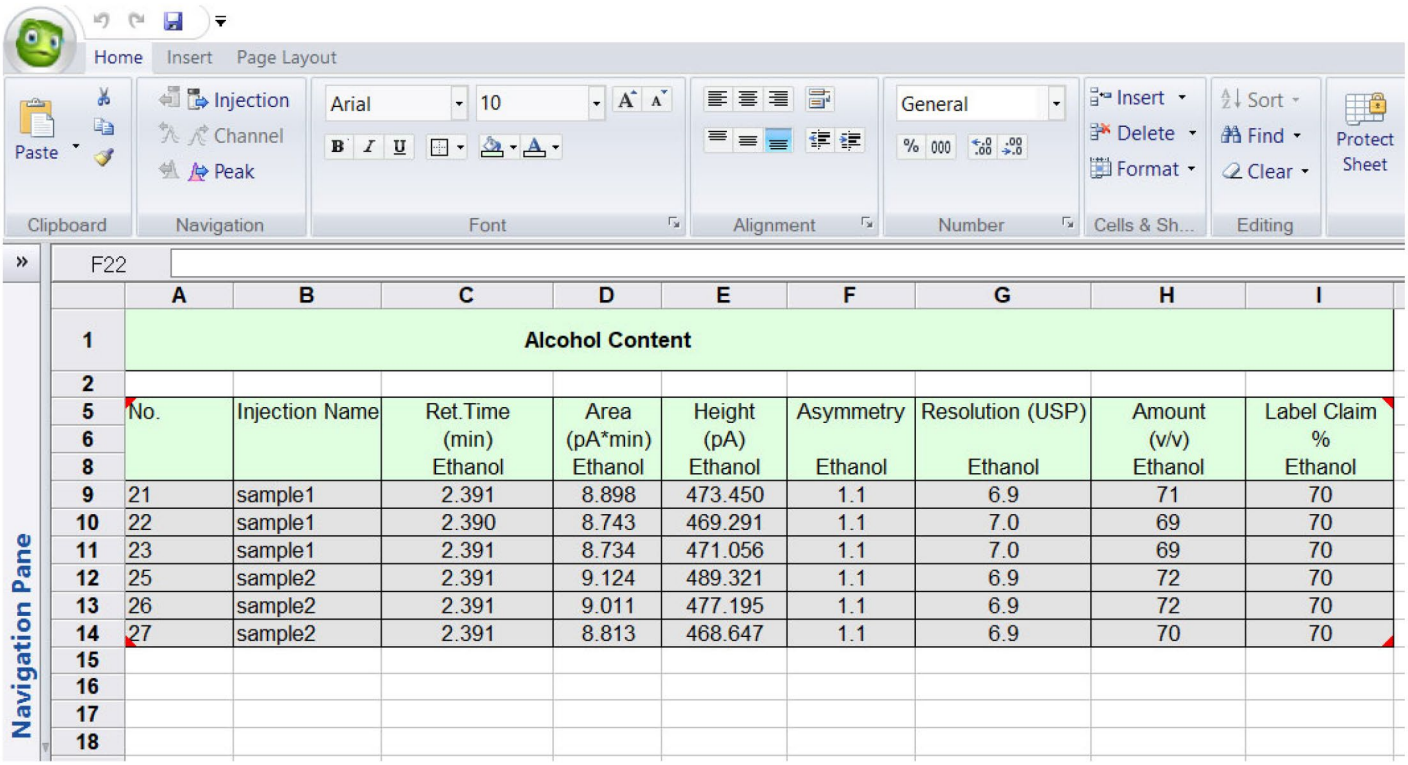
D = dilution factor (volume of test stock/volume of spiked sample)

R_U = peak area (ethanol or IPA) in the test preparation

R_S = peak area (ethanol or IPA) in the standard preparation

Table 3. Calculated concentrations for ethanol and IPA in hand sanitizer samples according to USP <611> method IIb. Calculated concentrations were ± 2% of the label claim (70% v/v).

	Ethanol	
	Calculated concentration (%v/v)	**Labeled concentration (%v/v)**
Sample 1	70	70
Sample 1	69	70
Sample 1	69	70
Sample 2	72	70
Sample 2	71	70
Sample 2	70	70
	IPA	
	Calculated concentration (%v/v)	**Labeled concentration (%v/v)**
Sample 3	72	70
Sample 3	72	70
Sample 3	72	70



	A	B	C	D	E	F	G	H	I
1	Alcohol Content								
2									
5	No.	Injection Name	Ret.Time (min) Ethanol	Area (pA*min) Ethanol	Height (pA) Ethanol	Asymmetry Ethanol	Resolution (USP) Ethanol	Amount (v/v) Ethanol	Label Claim % Ethanol
6									
8									
9	21	sample1	2.391	8.898	473.450	1.1	6.9	71	70
10	22	sample1	2.390	8.743	469.291	1.1	7.0	69	70
11	23	sample1	2.391	8.734	471.056	1.1	7.0	69	70
12	25	sample2	2.391	9.124	489.321	1.1	6.9	72	70
13	26	sample2	2.391	9.011	477.195	1.1	6.9	72	70
14	27	sample2	2.391	8.813	468.647	1.1	6.9	70	70
15									
16									
17									
18									

Figure 2. Chromeleon CDS report browser showing an example of a customized report for automatic calculation of alcohol content (% v/v) and fast data review in several ethanol-based hand sanitizer samples

Robustness

Instrument robustness for analytical testing analysis was evaluated for n=200 repeated injections of ethanol and IPA test preparations (at 0.4% v/v), water blanks, and standard preparation (at 0.4% v/v) over three days of unattended continuous operation. Peak area ratio (ethanol/IS and IPA/IS) was consistently stable across the entire analytical sequence, with %RSD=1.93 as reported in Figure 2A. Retention time %RSDs were 0.04 for both ethanol and IPA and 0.03 for IS (Figure 3B); average asymmetry factors were 1.17 for ethanol and 1.07 for IPA with resolution between ethanol-IS and IPA-IS of 6.72 and 2.12, respectively, for n=160 injections. An example of overlaid chromatograms for n=100 sample injections is reported in Figure 4 with peak area %RSDs annotated. No inlet, guard column, or chromatographic column maintenance were performed during the three days of continuous sample acquisition, demonstrating robust system performance for continuous analyses of water-based sample injections.

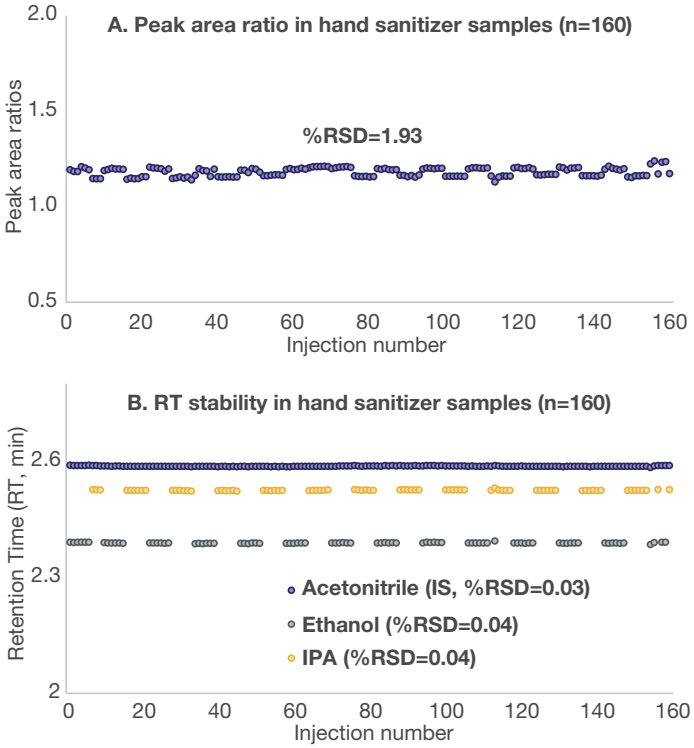


Figure 3. Peak area ratio (ethanol/IS and IPA/IS) %RSDs (A) and RT stability (B) for n=160 sample injections (blanks and standard injections not included) at 0.4% v/v obtained over three days of unattended operation. The peak area ratio (ethanol/IS and IPA/IS) %RSD for the hand sanitizer samples across the sequence was 1.93% and the RT %RSD was 0.04 for ethanol and IPA and 0.03 for acetonitrile.

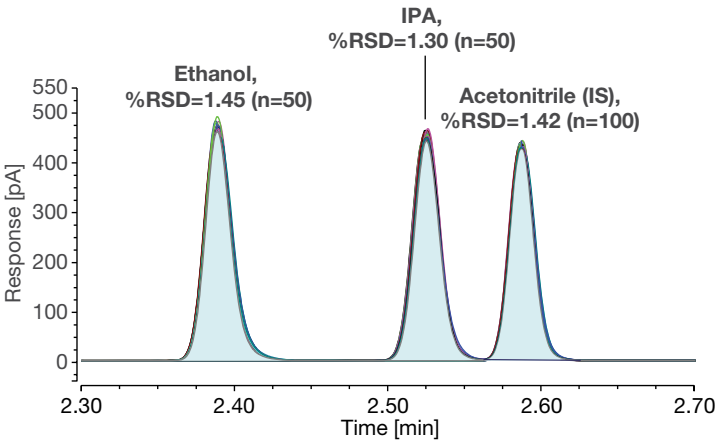


Figure 4. Overlaid chromatograms for ethanol (n=50) and IPA (n=50) test preparations at 0.4% v/v. Peak area %RSDs are annotated.

Conclusions

The results obtained in these experiments demonstrate that the TRACE 1310 GC-FID coupled to the AS 1310 autosampler allows for fast, robust, and cost-effective determination of alcohol content in hand sanitizers.

- The USP <611> Method IIb was modified in compliance with the USP General Notices and Requirements resulting in ~2.7 times improvement in analysis speed. Moreover, the scope of the analysis was broadened by adding isopropanol to the standard solution as it is used as main ingredient of hand sanitizers besides ethanol.
- The shorter method offered a cost-effective alternative to QA/QC laboratories dealing with high sample throughput. About 144 samples can be run every day compared to the classical approach (around 50 samples/day).
- Fast chromatographic separation of target analytes was achieved in <3 minutes with chromatographic resolution between ethanol and IS of 7.05 and ethanol peak asymmetry factor of 1.07, indicating high inertness of the system, correct sample transfer during injection, and effective column refocusing.
- Peak area ratio (ethanol/IS) %RSD from n=10 repeated standard injections was 0.47, demonstrating adequate repeatability of measurement.
- SST criteria were extended to IPA with the exception of the resolution requirement. Peak asymmetry and peak area ratio (IPA/IS) %RSD using n=10 repeated standard injections were 1.02 and 0.16, respectively. Baseline resolution was achieved between IPA and IS with average R_s value of 2.19.

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- The instrument ease of use combined with the advanced reporting features of Chromeleon CDS allowed for fast data acquisition, reliable quantitative analysis, and immediate review and interpretation of the results.
- The high inertness of the liner and the capillary GC column combined with the ruggedness of the AS 1310 autosampler allowed for a large number of direct, water-based hand sanitizer injections with no system maintenance.
- Chromeleon CDS (compliant with the FDA 21 CFR part 11 requirements) ensured data integrity, traceability, and effective data management, allowing for easy and fast data processing, quantitation, and reporting.

References

1. https://www.who.int/health-topics/coronavirus#tab=tab_1
2. USP <611> Alcohol Determination, Monograph, United States Pharmacopeia, 2020.
3. General Notices and Requirements USP <38>, United States Pharmacopeia, 2015.
4. General Chapter USP <621> Chromatography, Physical Tests, United States Pharmacopeia, First Supplement to USP 40-NF 35, 2017.

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