LABORATORY INFORMATION BULLETIN

Quantitation of Methanol, Ethanol and Isopropanol in Gel Hand Sanitizer Product by Gas Chromatography-Flame Ionization Detection (GC-FID)

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ABSTRACT

The COVID-19 outbreak and pandemic resulted in an increase in the number of firms producing hand sanitizers and an increase in the numbers of hand sanitizers requiring analysis in U.S. FDA laboratories. The analysis was necessitated by initial screenings of imported hand sanitizers by Customs and Border Protection that tested positive for methanol. Due to the anticipated workload, the Office of Medical Products Specialty Laboratory Operations determined that a single method was needed to quantitate ethanol or isopropanol active ingredients as well as to quantitate methanol adulterant if present. The analytical procedure developed uses GC-FID for quantitation of ethanol, isopropanol and/or methanol in hand sanitizer from levels as low as 0.25% v/v up to 100% v/v. The limit of detection of methanol is 0.0625% v/v. The method is an extension of the USP <611> Alcohol Determination method for ethanol, and now includes methanol and isopropanol in the analysis. This method was successfully validated using a gel hand sanitizer matrix containing a label claim of 70% ethanol, with glycerin, propylene glycol and aloe among the inactive ingredients. The correlation coefficient for linearity (r^2) was ≥ 0.9997, spike recovery values were from 99.1-100.3%, and %RSDs were < 1% for methanol, ethanol and isopropanol. The validated method was used to successfully quantify the amounts of ethanol, isopropanol and methanol in 29 gel hand sanitizer products that had previously tested positive for methanol by Customs and Border Protection, and 31 other hand sanitizer products that did not previously test positive for methanol.

INTRODUCTION

Due to the COVID-19 outbreak, the CDC recommended the use of alcohol-based hand sanitizers that contain at least 60% alcohol, as a preventative measure in the spread of COVID-19 when soap and water are not available for proper hand hygiene. In response to the increased demand for these alcohol-based hand sanitizers, the FDA released a guidance communicating a temporary policy allowing for additional sources from industry to prepare certain alcohol-based hand sanitizer products for the duration of the COVID-19 public health emergency.[1] With this released guidance, an increase in the number of firms producing hand sanitizers has been observed. As such, the quality of these hand sanitizers has become an FDA priority and concern. One of the main potential impurities of interest is methanol (MeOH), which can be used as an economically motivated adulterant in place of ethanol (EtOH) or isopropanol (IPA). Recently, a large number of hand sanitizer samples analyzed by Customs and Border Protection (CBP) have tested positive for the presence of MeOH.[2,3] MeOH is toxic with many associated health hazards. The FDA guidance limits the amount of MeOH in hand sanitizer to 630 ppm.[1]

The USP <611> Alcohol Determination method IIB[4] uses Gas Chromatography with Flame Ionization Detection (GC-FID) for quantitation of EtOH in various drug products. This method can be used to quantify EtOH in alcohol-based hand sanitizer. However, to quantify IPA in hand sanitizers where that is the active ingredient, and to quantify MeOH if present as an adulterant, it is necessary to extend the method to add IPA and MeOH as analytes. Fortunately, the existing USP chromatographic method gives adequate peak separation between all three alcohols and acetonitrile (ACN) internal standard, such that no modification to chromatographic conditions is necessary.

This report details the validation of USP <611> Alcohol Determination Chromatographic Method IIb for analysis of MeOH, EtOH and IPA. The validation was performed according to USP <1225>[5] and evaluates the performance characteristics accuracy precision, specificity, detection limit, quantitation limit, linearity and range. Since the method described is a combination of a category I method (assay for active ingredient, i.e., EtOH or IPA) and a category II method (assay for impurity, i.e., MeOH), all of the aforementioned performance characteristics are required except for detection limit. The detection limit was evaluated to ensure that MeOH could be detected in hand sanitizer down to the 630 ppm limit specified in the FDA guidance.

METHODS AND MATERIALS

Equipment

a. Agilent Technologies GC 7890B Series with 7693A autosampler and FID (Flame Ionization Detector), or equivalent
b. Restek Rtx-1301 Column, Catalog number: 16085, Nominal Dimensions: ID: 0.53 mm; Film Thickness: 3.0 microns; Length 30 m (USP phase G43)
c. Positive Displacement Pipette – Eppendorf Repeater E3x, 1 µL – 50 mL, or equivalent
   Note: due to the high viscosity of the gel sample, the pipette used for transferring the sample must be a positive displacement pipette to obtain accurate volumes
d. Pipette tips – Eppendorf Combitips® Advanced, 1 mL, 5 mL, and 10 mL volumes
e. Class A volumetric flasks

Sample Matrix Used for Validation

Hand sanitizer gel
Active ingredient: ethyl alcohol, 70% v/v
Inactive ingredients: water, glycerin, propylene glycol, isopropyl myristate, aloe barbadensis leaf juice, tocopheryl acetate, isopropyl alcohol, carbomer, triisopropanolamine

Reagents and Standards

a. DI Water (NLT resistivity 18 MΩ·cm)
b. Methanol (MeOH), USP reference standard, catalog number: 1424109

c. Ethanol (EtOH), "Alcohol Determination—Alcohol" USP reference standard, catalog number 1012688 (approximately 2% v/v ethanol in water)
d. Isopropanol (IPA), USP reference standard, catalog number: 1570428
e. Acetonitrile (ACN), Fisher, catalog number: A955 (used as internal standard)

Solutions

a. MeOH and IPA Stock Standard Solution (2 % v/v)
Pipette 1 mL of each alcohol to a 50 mL volumetric flask and dilute to volume with water.

b. EtOH Stock Standard Solution (2 % v/v)
Use the Alcohol Determination USP standard.

c. Internal Standard Stock Solution (2% ACN)
Pipette 10 mL of ACN to a 500 mL volumetric flask and dilute to volume with water.

d. Standard Solution (0.4% MeOH, EtOH, IPA & ACN)
Pipette 5 mL of each Stock Standard Solution and 5 mL of Internal Standard Stock Solution to a 25 mL volumetric flask; dilute to volume with water.

e. Sample stock solution preparation
Pipette 0.5 mL of gel hand sanitizer sample using a positive displacement pipette to a 25 mL volumetric flask. Dilute to volume with water and mix well.

f. Sample solution and spike sample solutions – see Table 1 below.

Table 1: Preparation of Sample and Spike Sample

Solution Name	Volume of Sample Stock Added (mL)	Volume of Each* Stock Standard Solution Added (mL)	Volume of Internal Standard Stock Added (mL)	Dilute to Final Volume (mL) with Water	Spike Concentration Level (%v/v)
Sample Solution	4	0	4	20	0%
75% Spike Sample Solution	4	3	4	20	0.3%
100% Spike Sample Solution	4	4	4	20	0.4%
125% Spike Sample Solution	4	5	4	20	0.5%

* MeOH and IPA Stock Standard Solution, and EtOH Stock Standard Solution are both added

Validation Solutions for Linearity, LOD and LOQ Determination

a. Internal Standard Diluent Solution (0.4% ACN)
Pipette 50 mL of Internal Standard Stock Solution to a 250 mL volumetric flask; dilute to volume with water.

b. Linearity 1 Solution (1% MeOH, EtOH & IPA, 0.4 % ACN)
Pipette 5 mL of Alcohol Determination USP standard, 0.10 mL of MeOH, 0.10 mL of IPA and 2 mL Internal Standard Stock Solution (2% ACN) to a 10 mL volumetric flask; dilute to volume with water.

c. Other linearity, LOQ and LOD solutions – see Table 2 below for preparation. See Method Validation discussion section for how LOD and LOQ concentrations were set.

Table 2: Preparation of Linearity, LOQ and LOD Solutions for Validation

Solution Name	Stock Solution Used	Volume of Stock Added (mL)	Dilute to Final Volume (mL) with Internal Standard Diluent	Alcohols Final Concentration Level (%v/v)
Linearity 2	Linearity 1	4	10	0.4%
Linearity 3	Linearity 1	2	10	0.2%
Linearity 4	Linearity 1	1	10	0.1%
Linearity 5	Linearity 1	1	25	0.04%

Solution Name	Stock Solution Used	Volume of Stock Added (mL)	Dilute to Final Volume (mL) with Internal Standard Diluent	Alcohols Final Concentration Level (%v/v)
Linearity 6	Linearity 4	1	10	0.01%
Linearity 7 (LOQ)	Linearity 6	1	10	0.001%
LOD Solution	Linearity 6	0.25	10	0.00025%

Instrumentation

An Agilent Technologies 7890B Series GC-FID, along with a Restek Rtx-1301 Column was used for the method validation and sample analysis. Table 3 on the next page describes the GC-FID conditions used.

Table 3: GC-FID Parameters

Flows and pressure	
Average linear velocity	34 cm/sec
Mode	Constant flow
Injection Parameters	
Mode	Split
Volume	0.5 µL
Temperature	210°C
Split ratio	5:1
GC Parameters	
Oven initial	50°C
Initial time	5.0 min
Ramp rate	10°C/min
Final temperature after ramp	200°C
Final time after ramp	4.0 min
Run time	24 min
FID Parameters	
Heater Temp	280°C
H_2 Flow	30 mL/min
Air Flow	350 mL/min

Quantitation of MeOH, EtOH and IPA is performed by single point calibration using the peak area ratio of each alcohol to acetonitrile internal standard. The following formula is used:

$$\text{Result (\%v/v)} = CD(R_U/R_S)$$

C = Concentration of Standard Stock Solution
D = Dilution factor
R_U = Peak response ratio obtained from the Sample Solution preparation
R_S = Peak response ratio obtained from the Standard Solution preparation (average from 6 system suitability injections)

RESULTS and DISCUSSION

Analysis of Sample Matrix Used for Validation

A hand sanitizer gel with a formulation generally representative of hand sanitizer gels on the market was chosen as the sample matrix for the validation (see "Sample Matrix Used for Validation" earlier in this report). Three preparations of the hand sanitizer gel sample were performed and analyzed according to the method. The results are in Table 4. Although USP <611> itself does not provide any assay acceptance criteria,[4] the general expectation based on USP <2> is that the result will be within 90-110% of the label claim,[6] which would mean 63 – 77% v/v EtOH for this particular sample. The amount of EtOH found, 73.7%, was indeed within this range. IPA was also detected at below the LOQ level. This result is consistent with the presence of isopropyl alcohol on the product label as an inactive ingredient, though the amount was not provided. Figures 1-3 show the chromatograms of standard solution, sample solution and scaled-up sample solution to show more clearly the trace isopropanol peak.

Table 4. Unspiked sample matrix, measured %v/v in sample

	MeOH	EtOH	IPA
Prep 1	0	73.5	< 0.25*
Prep 2	0	73.7	< 0.25*
Prep 3	0	73.9	< 0.25*
Average	0	73.7	< 0.25*
%RSD	N/A	0.23	N/A
Label Claim (%v/v)	N/A	70	N/A

* Amount less than method LOQ

Figure 1: GC-FID Chromatogram of Standard Solution (0.4% v/v MeOH, EtOH & IPA)

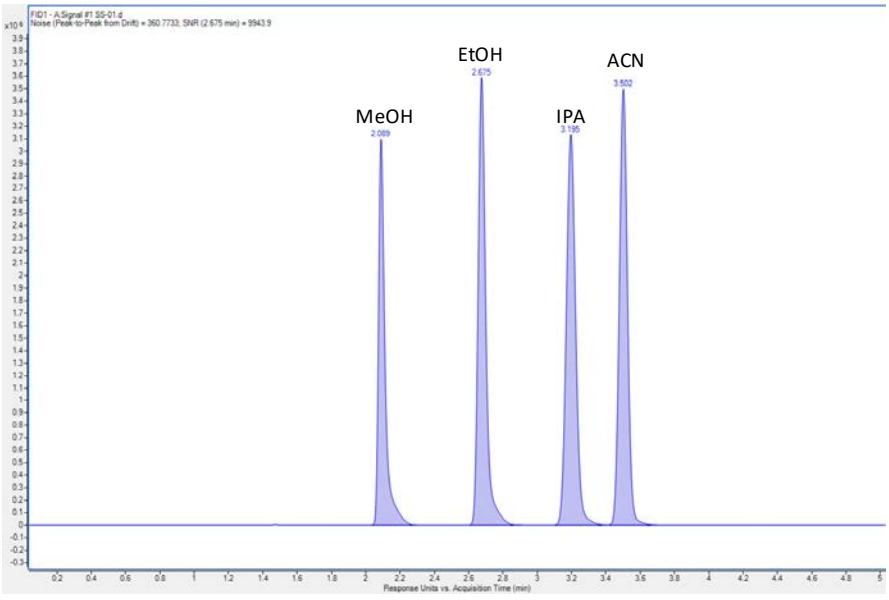


Figure 2: GC-FID Chromatogram of Hand Sanitizer Gel Sample

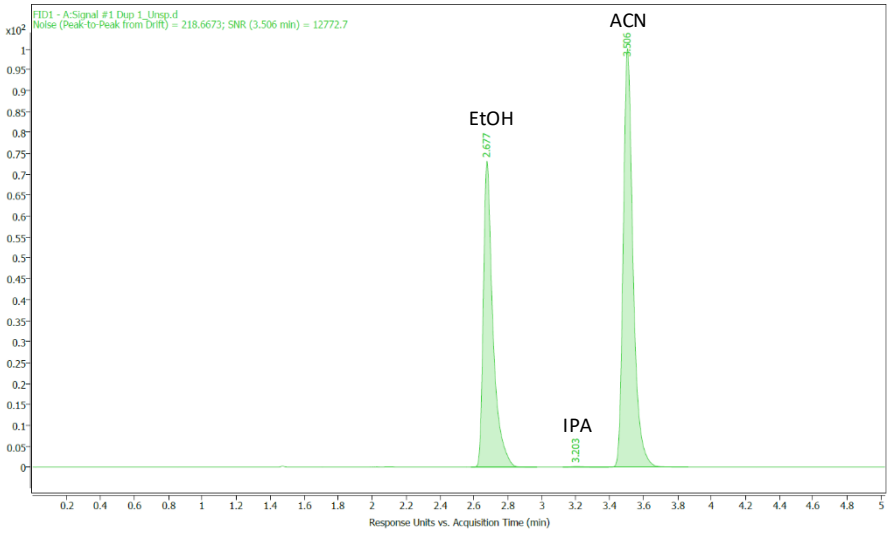
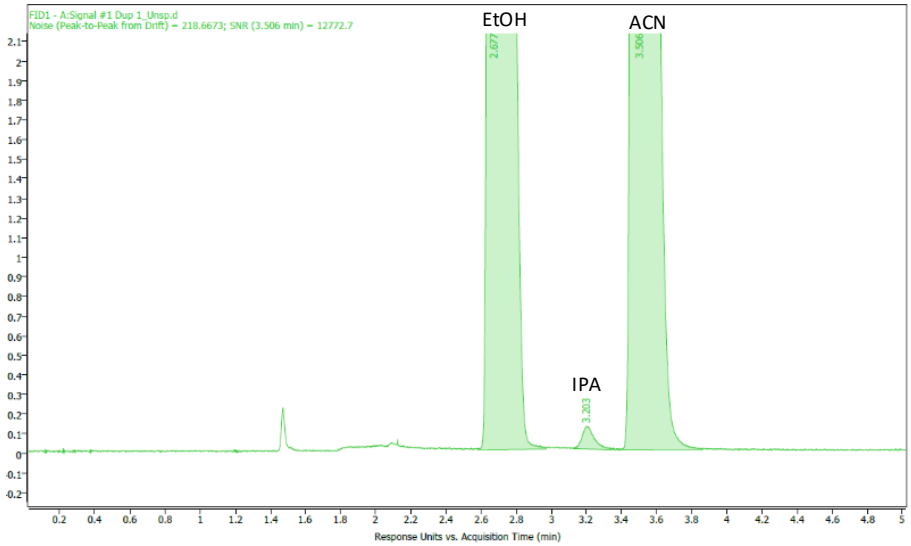


Figure 3: Scaled GC-FID Chromatogram of Hand Sanitizer Gel Sample



Method Validation

The method was validated using the same hand sanitizer gel sample used for sample analysis. The results and acceptance criteria are given in Tables 5-10. System suitability criteria for %RSD and tailing factor were taken from USP <611>[4]. System suitability was determined using 6 injections of Standard Solution (0.4% MeOH, EtOH, IPA & ACN). Linearity was determined by injecting standard solutions at seven concentration levels, from 0.001% to 1.0% v/v MeOH, EtOH and IPA. LOD and LOQ were determined by 3 injections each of 0.00025% and 0.001% v/v alcohol solutions, respectively. The LOD concentration was set sufficiently low to detect MeOH in hand sanitizer sample at the FDA guidance limit of 630 ppm, taking into account the dilution of the sample, and where signal-to-noise (S/N) values were consistently ≥ 3. LOQ was set a level where the S/N values were consistently ≥ 10. Accuracy and precision were determined using 3 preparations of un-spiked hand sanitizer gel sample (Table 4) and 3 preparations each at 3 different spike concentrations. No interfering peaks were observed on the sample chromatograms. All acceptance criteria were met.

Table 5. System suitability results

Sequence #	Test	MeOH	EtOH	IPA	Criteria	Result
1	%RSD peak area ratio with ACN (n = 6)	0.10	0.06	0.03	≤ 4.0	Pass
	Tailing Factor	1.43	1.18	1.07	≤ 2.0	
	Resolution*			3.28	≥ 1.5	
2	%RSD peak area ratio with ACN (n = 6)	0.14	0.10	0.05	≤ 4.0	Pass
	Tailing Factor	1.65	1.29	1.10	≤ 2.0	
	Resolution*			3.20	≥ 1.5	

*Resolution between IPA and ACN in 1st standard solution injection

Table 6. Linearity results; coefficient of determination (R^2)

	MeOH	EtOH	IPA	Criteria	Result
slope	1.9675	2.5625	2.8842	N/A	Pass
y-intercept	-0.0086	-0.0112	-0.0109	N/A	
R^2	0.9997	0.9997	0.9998	≥ 0.995	
Range (%v/v)	0.001-1.0	0.001-1.0	0.001-1.0	N/A	

Table 7. Signal-to-Noise (S/N) of LOD and LOQ solutions

		MeOH	EtOH	IPA	Criteria	Result
LOD (0.00025% v/v solution)	Inj. #1	3.68	3.64	3.73	≥ 3	Pass
	Inj. #2	3.45	3.37	3.42		
	Inj. #3	4.30	4.06	4.05		
	Average	3.81	3.69	3.73		
LOQ (0.001% v/v solution)	Inj. #1	12.58	13.52	13.34	≥ 10	Pass
	Inj. #2	9.97	10.42	10.26		
	Inj. #3	14.64	15.31	15.08		
	Average	12.40	13.08	12.89		

Table 8. %Recovery and %RSD for LOQ solution

	MeOH	EtOH	IPA	Criteria	Result
Inj. #1	97.1	101.0	108.9	80-120%	Pass
Inj. #2	112.9	96.7	110.6		
Inj. #3	114.3	100.8	107.8		
Average %Recovery	108.1	99.5	109.1		
%RSD	8.8	2.4	1.3	≤ 20%	Pass

Table 9. Method LOD and LOQ values (%v/v)

		MeOH	EtOH	IPA
LOD	In test solution	0.00025	0.00025	0.00025
	In hand sanitizer sample	0.0625	0.0625	0.0625
LOQ	In test solution	0.001	0.001	0.001
	In hand sanitizer sample	0.25	0.25	0.25

Table 10. Accuracy and Precision, Spike %Recovery

Spike Level		MeOH	EtOH	IPA	Criteria	Result
0.3% v/v	Prep #1	99.4	99.9	99.6	90-110%	Pass
	Prep #2	99.6	100.1	99.6		
	Prep #3	99.6	100.3	99.8		
	Average	99.5	100.1	99.7		
	%RSD	0.11	0.21	0.12	≤ 10%	Pass
0.4% v/v	Prep #1	99.6	100.2	99.6	90-110%	Pass
	Prep #2	99.3	99.6	99.3		
	Prep #3	99.5	100.1	99.6		
	Average	99.5	100.0	99.5		
	%RSD	0.18	0.36	0.18	≤ 10%	Pass
0.5% v/v	Prep #1	99.4	99.4	99.6	90-110%	Pass
	Prep #2	99.6	99.3	99.4		
	Prep #3	99.1	99.1	99.1		
	Average	99.3	99.3	99.4		
	%RSD	0.27	0.16	0.22	≤ 10%	Pass

Unspiked results from this sample are provided earlier in Table 4.

Analysis of Import Samples Using Validated GC-FID Method

A variety of imported hand sanitizer gel samples that tested positive for the presence of MeOH by CBP[2] were sent to the Pacific Southwest Medical Products Laboratory to be tested for MeOH, EtOH and IPA content using the validated GC-FID method (Table 11). The results from these samples are contrasted with imported hand sanitizer samples that did not test positive for the presence of MeOH (Table 12). Each of the hand sanitizer gel samples were prepared twice in the same manner as described earlier in Table 1. Each sample preparation was injected twice and the results of all injections averaged to produce the values reported in Tables 11-12. To verify the accuracy of results, each sample was also spiked at the 100% level as also described earlier in Table 1, and obtained MeOH, EtOH and IPA spike recoveries (not shown) were within the 90-110% criterion for all samples. No interfering peaks were observed on the chromatograms for any of the samples.

Each of the analyzed hand sanitizer gel samples declared ethyl alcohol as the active ingredient on the label, with claimed concentrations ranging from 62-80% v/v, except sample #41, which claimed 70% isopropyl alcohol. All samples that previously tested positive for MeOH by CBP were found to have MeOH by the GC-FID method with a very wide range of concentrations across the samples, from 1.6-79.3% v/v (Table 11). Except for #19, the samples with MeOH were also found to have well below claimed EtOH concentrations, with two of the samples not having any detectable amounts of EtOH. These sample results, where EtOH content was low and MeOH content high, were consistent with economically motivated partial or complete

substitution of EtOH with MeOH in the product. The samples that did not contain MeOH, in contrast (Table 12), generally had close to the expected amount of EtOH based on the label claim, with only 3 out of the 30 of such samples having less than 90% of the claimed amount. The one sample, #41, that claimed IPA as the active ingredient was found to have IPA at 61.3% v/v, slightly less than 90% of the amount claimed. Sample #40 claimed IPA as an inactive ingredient without declaring the amount, and was found in that sample at a level of 3.7% v/v.

Table 11. MeOH, EtOH and IPA Concentrations in Hand Sanitizer Gel Samples Previously Tested Positive for MeOH by CBP

Sample #	EtOH Label Claim (%v/v)	EtOH Content (%v/v)	MeOH Content (%v/v)	IPA Content (%v/v)	EtOH % of Label Claim	EtOH Content Within ±10% Label Claim?
1	62	32.3	31.7	N.D.	52	N
2	80	0.9	14.6	N.D.	1	N
3	70	4.7	66.3	N.D.	7	N
4	80	1.2	67.3	N.D.	2	N
5	70	0.8	54.3	N.D.	1	N
6	70	37.5	24.9	N.D.	54	N
7	70	21.5	10.0	N.D.	31	N
8	70	1.1	64.8	N.D.	2	N
9	70	19.2	2.1	N.D.	27	N
10	70	1.4	71.5	N.D.	2	N
11	70	0.8	77.3	< 0.25*	1	N
12	70	< 0.25*	63.0	N.D.	0	N
13	70	< 0.25*	66.0	N.D.	0	N
14	75	39.6	28.3	N.D.	53	N
15	80	33.9	24.1	N.D.	42	N
16	70	< 0.25*	73.2	N.D.	0	N
17	70	50.9	18.4	N.D.	73	N
18	70	1.0	61.5	N.D.	1	N
19	70	67.0	1.6	N.D.	96	Y
20	70	0.4	75.2	N.D.	1	N
21	70	26.5	37.6	N.D.	38	N
22	70	31.4	2.3	N.D.	45	N
23	70	18.8	47.9	N.D.	27	N
24	70	N.D.	79.3	N.D.	0	N
25	70	< 0.25*	70.2	N.D.	0	N
26	70	20.3	4.7	N.D.	29	N
27	75	63.3	6.0	N.D.	84	N
28	70	N.D.	63.2	N.D.	0	N
29	70	3.6	59.1	N.D.	5	N

*Analyte detected, concentration below LOQ level N.D. = Not detected

Table 12. MeOH, EtOH and IPA Concentrations in Other Hand Sanitizer Gel Samples

Sample #	EtOH Label Claim (%v/v)	EtOH Content (%v/v)	MeOH Content (%v/v)	IPA Content (%v/v)	EtOH (or IPA) % of Label Claim	EtOH (or IPA) Content Within ±10% Label Claim?
30	65	68.1	N.D.	N.D.	105	Y
31	75	70.0	N.D.	N.D.	93	Y
32	70	72.9	N.D.	N.D.	104	Y
33	75	57.6	N.D.	N.D.	77	N
34	75	72.7	N.D.	N.D.	97	Y
35	75	65.6	N.D.	N.D.	88	N
36	75	71.3	N.D.	N.D.	95	Y
37	75	74.9	N.D.	N.D.	100	Y
38	70	75.4	N.D.	N.D.	108	Y
39	75	71.4	N.D.	N.D.	95	Y
40	72	71.3	N.D.	3.7	99	Y
41	70 (IPA*)	0.7	N.D.	61.3	88	N
42	75	74.4	N.D.	N.D.	99	Y
43	65	64.7	N.D.	N.D.	100	Y
44	74	76.3	N.D.	N.D.	103	Y
45	75	71.1	N.D.	N.D.	95	Y
46	75	78.5	N.D.	N.D.	105	Y
47	75	70.0	N.D.	N.D.	93	Y
48	68	70.9	N.D.	N.D.	104	Y
49	75	76.6	N.D.	N.D.	102	Y
50	75	73.4	N.D.	N.D.	98	Y
51	75	78.5	N.D.	N.D.	105	Y
52	72	77.8	N.D.	N.D.	108	Y
53	75	71.4	N.D.	N.D.	95	Y
54	75	73.8	N.D.	N.D.	98	Y
55	75	62.9	N.D.	N.D.	84	N
56	75	79.6	N.D.	N.D.	106	Y
57	75	75.8	N.D.	N.D.	101	Y
58	70	74.2	N.D.	N.D.	106	Y
59	80	78.2	N.D.	N.D.	98	Y
60	70	76.1	N.D.	N.D.	109	Y

*Label claim 70% isopropyl alcohol

N.D. = Not detected

CONCLUSION

The USP <611> Alcohol Determination method for quantification of EtOH has been successfully expanded to include determination of MeOH and IPA in gel hand sanitizer. The method validation study showed that the GC-FID method in gel hand sanitizer sample is specific, accurate, precise, and linear within a range of 0.001-1.0% v/v concentration for the analysis of MeOH, EtOH and IPA. LOD was established at 0.00025% v/v in solution, corresponding to 0.0625% v/v in test sample where a 250× dilution was performed, as described in this report. This LOD level is approximately the same as the limit of MeOH in the FDA guidance, insuring that MeOH can be detected at levels just above the limit. LOQ was established at 0.001% v/v concentration in solution, corresponding to 0.25% v/v in test sample with a 250× dilution. If a sample is found to contain MeOH but below the 0.25% v/v LOQ level, it should still be possible to quantify the MeOH in it by diluting the sample less, but that possibility was not explored in this report.

The validated GC-FID method was used to successfully quantify the MeOH and EtOH content in a variety of imported hand sanitizer gel samples that previously tested positive for the presence of MeOH by CBP, as well as other imported samples that did not previously test positive for MeOH. Most of the results for the MeOH positive samples were consistent with economically motivated partial or complete substitution of EtOH with MeOH in the product. The non-MeOH positive samples, on the other hand, did not show any MeOH in the product by GC-FID, and generally gave EtOH results consistent with the product label claim.

REFERENCES

1. “Temporary Policy for Preparation of Certain Alcohol-Based Hand Sanitizer Products During the Public Health Emergency (COVID-19) Guidance for Industry” published March 2020; updated August 7, 2020; U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER).
2. Hand sanitizer samples were tested by Customs and Border Protection using a Gemini hand-held FTIR/Raman spectrometer.
3. https://www.fda.gov/drugs/drug-safety-and-availability/fda-updates-hand-sanitizers-consumers-should-not-use
4. USP43-NF38 <611> “ALCOHOL DETERMINATION” method IIB
5. USP43-NF38 <1225> “VALIDATION OF COMPENDIAL PROCEDURES”
6. USP43-NF38 <2> section “ASSAY”