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Application News

No. GC-2107

Gas Chromatography

Organic Impurities in Ethanol for Alcohol-Based Hand Sanitizer Products

■ Introduction

The COVID-19, or coronavirus, pandemic has created an unprecedented demand for alcohol-based hand sanitizers. As a result of the pandemic, the United States Food and Drug Administration (FDA) has provided guidance[1,2] that expands the scope of manufacturing of hand sanitizers that use ethanol or isopropanol as their active ingredient. Regardless of the origins of the ethanol or IPA, organic contaminants may be incorporated during their production and could lead to toxicity and health concerns.

The USP monograph[3] for alcohol describes the method for analyzing organic impurities in ethanol and specifies a maximum concentration for four impurity compounds: acetaldehyde, methanol, acetal, and benzene. This method is commonly followed by ethanol manufacturers. The new FDA guidance[1,2,4] includes a list of impurities that manufacturers of ethanol or hand sanitizers need to screen. In addition to the four compounds specified in the USP monograph (level 1 impurity compounds), eight level 2 impurity compounds need to be monitored. In this application note, these 12 impurities as well as several additional compounds were screened using a Shimadzu Nexis GC-2030 gas chromatograph according to the method described in the USP monograph for alcohol.

■ Samples and Analytical Conditions/Experimental

Analytical standards for organic impurities in ethanol (LQC-GC-KUF, 10-100ppm) as well as two custom standards (USP mix and FDA mix, Table 1) were purchased from Bion Sciences. Ethanol (200 proof) was purchased from Sigma Aldrich.

Table 1: Custom Standards from Bion

Compound	Custom mix 1, USP mix (µL/L)	Custom mix 2, FDA mix (µg/mL)
acetaldehyde	10	117.8
methanol	200	1582
acetone	--	1580
isopropanol	--	3925
1-propanol	--	1608
ethyl acetate	--	1804
2-butanol	--	1616
benzene	2	4.4
2-methyl-1-propanol	--	1606
acetal	30	124.5
1-butanol	--	1620
toluene	--	1618
3-methyl-1-butanol	--	1618
2-methyl-1-butanol	--	1618
1-pentanol	--	1622
ethanol	--	3945
4-methylpentan-2-ol	300	--
solvent	Ethanol	Acetonitrile

A GC-2030 chromatograph equipped with split/splitless injector (SPL), flame ionization detector (FID) and AOC-20 Plus autosampler was used for this analysis and the data were acquired, analyzed, and reported using LabSolutions LCGC software. The method parameters are shown in Table 2 below.

Table 2: Instrument Configuration and Analysis Conditions

GC system	Shimadzu GC-2030 with SPL, FID and AOC-20 Plus autosampler
Column	ZB-624, 30 m x 0.32 mm x 1.8 µm
Injector Mode	Split at 1:20 ratio
Injection Volume	1.0 µL
Carrier Gas	Helium (He) or Hydrogen (H_2), constant linear velocity of 35 cm/sec
Column Temperature	40° C, 12 min – 10° C/min –240° C, 10 min
Injection Port Temperature	200° C
FID Temperature and Gases	280° C, Hydrogen 32 mL/min, Air 200 mL/min, Makeup (Nitrogen) 24 mL/min

■ Results and Discussion

Chromatographic Separation of Organic Impurities

Both the USP method and FDA guidance contain a list of organic impurities that must be monitored. FDA guidance categorizes the impurities into two different levels (shown in Table 3). The four regulated impurity compounds per USP method are the same as level 1 impurities per FDA guidance.

A mixture containing 20 organic impurities in ethanol ranging from 10 ppm to 100 ppm (w/w) purchased from Bion Sciences (Table 3, except acetone) was assayed on the GC-2030 FID. Both helium (He) and hydrogen (H_2) were tested as carrier gas. All compounds were successfully resolved using USP method parameters (Figure 1). Similar results were obtained using either carrier gas. As shown in Table 3, the retention times using He or H_2 are comparable.

The USP method requires resolution between acetaldehyde and methanol not less than 1.5 at 10 µL/L using a G43 column of 30 m x 0.32 mm with 1.8 µm film thickness. To achieve this separation, different columns were tested. Of the G43 columns tested, not all yielded satisfactory results.

Importantly, ZB-624 Plus (and similarly Rxi-624Sil MS, which has different backbone arrangement of the stationary phase) did not yield as much separation for acetaldehyde and methanol as the original ZB-624 phase column. A 1301 column was also tested (the other typical G43 phase) and it gave a resolution result in between ZB-624 and ZB-624 Plus. Therefore, ZB-624 column was chosen for this application work.

The inlet liner also plays an important role, as it affects the peak shape of acetaldehyde and methanol. Peak resolution is a function of both separation and peak width. An improperly deactivated liner will result in poor peak shape and greatly reduce resolution. A Restek Topaz split liner with wool was used in this work to achieve satisfactory resolution and repeatability (see Repeatability section below) results.

Using either He or H_2 carrier gas, the resolution between these two peaks meets USP criteria. As expected, using H_2 carrier gas gave better resolution results (R > 1.7, Figure 1) than He carrier gas (R > 1.5, data not shown), since H_2 has higher efficiency (smaller HETP) than He at the linear velocity of 35 cm/sec based on Van Deemter's plot.

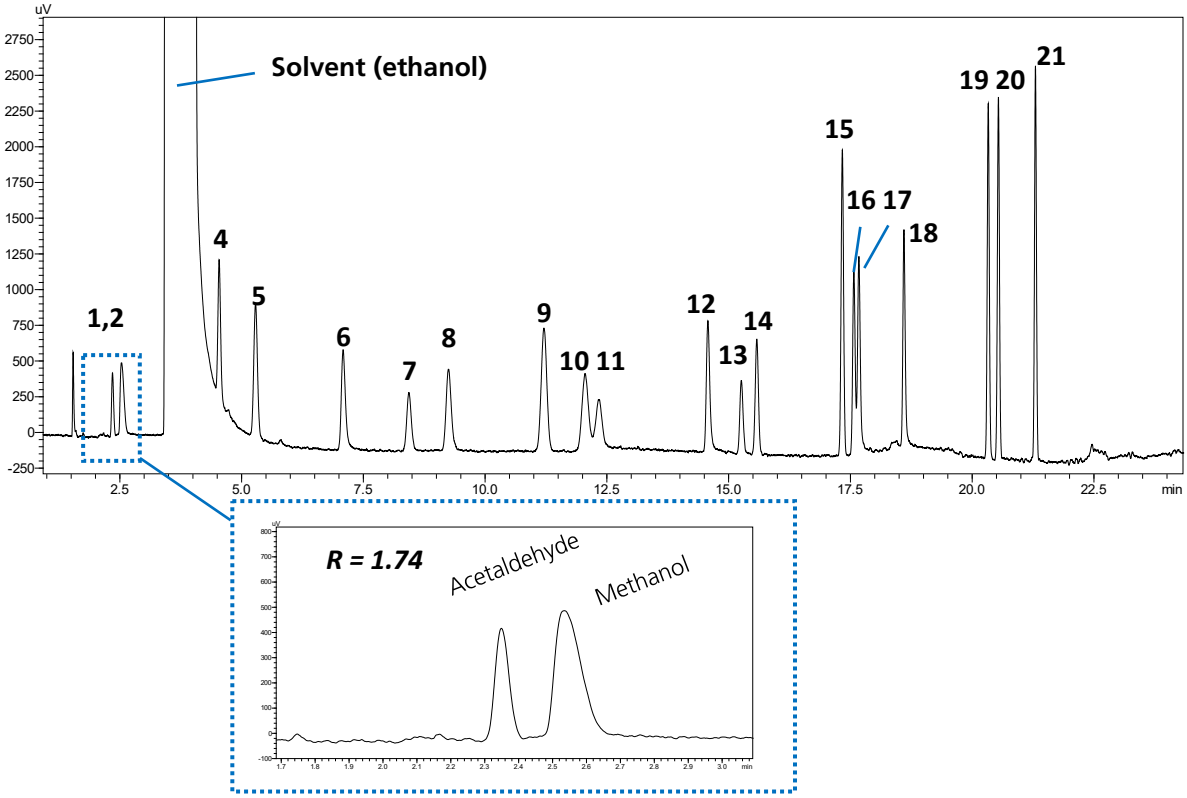

Figure 1: Chromatograms of 10 ppm ethanol impurity standard. See Table 3 for peak identity. H_2 carrier gas was used in this chromatogram. For acetaldehyde and methanol, 10 ppm (w/w) is equivalent to 10 µL/L.

Table 3: List of organic impurity compounds in ethanol and hand sanitizer products.

Peak no.	Compound Name	Impurity Level (FDA guidance)	Ret. Time (min)		r^2 values	
			He	H_2	He	H_2
1	Acetaldehyde	1	2.321	2.348	0.9992	0.9982
2	Methanol	1	2.499	2.533	0.9997	0.9992
3	Acetone *	2	3.981	3.991	*n.d.*	*n.d.*
4	Isopropanol	*n.a.*	4.506	4.538	0.9998	0.9999
5	2-methyl-2-propanol (*tert*-butanol)	*n.a.*	5.240	5.287	0.9998	0.9999
6	1-propanol (*n*-propanol)	2	7.018	7.087	0.9998	0.9998
7	Ethyl acetate	2	8.336	8.435	0.9998	0.9998
8	2-butanol	2	9.168	9.252	0.9995	0.9999
9	Benzene	1	11.066	11.207	0.9996	0.9999
10	2-methyl-1-propanol (*iso*-butanol)	2	11.947	12.064	0.9999	0.9999
11	Isovaleraldehyde (3-methylbutanal)	*n.a.*	12.225	12.337	0.9994	0.9984
12	1-butanol (*n*-butanol)	2	14.526	14.578	0.9998	0.9999
13	1,4-Dioxane	*n.a.*	15.209	15.263	0.9999	0.9999
14	Acetal (1,1-diethoxyethane)	1	15.532	15.578	0.9999	0.9999
15	Toluene	*n.a.*	17.290	17.333	0.9999	0.9999
16	3-methyl-1-butanol (isoamyl alcohol)	2	17.546	17.574	0.9997	0.9996
17	2-methyl-1-butanol	*n.a.*	17.649	17.675	0.9999	0.9999
18	1-pentanol (amyl alcohol)	2	18.580	18.601	0.9999	0.9999
19	Ethyl benzene	*n.a.*	20.307	20.328	0.9998	0.9999
20	Xylene	*n.a.*	20.518	20.538	0.9999	0.9999
21	Styrene	*n.a.*	21.281	21.297	0.9998	0.9999

* not included in Bion calibration standard set
n.a. not applicable
n.d. not determined

Although the standards from Bion contain an extensive list of impurity compounds (Table 3), they do not contain acetone. The new FDA guidance specifies acetone as an impurity in hand sanitizer products and defines a maximum acceptable level of 4400 ppm. Furthermore, acetonitrile was used as a diluent for hand sanitizer.

To investigate if acetone and acetonitrile can be separated from other impurities using the current setup, a mixture of compounds corresponding to the FDA guidance[4] was also assayed. As shown in Figure 2, these compounds, including acetone and acetonitrile (solvent), can be successfully separated using the setup and parameters described.

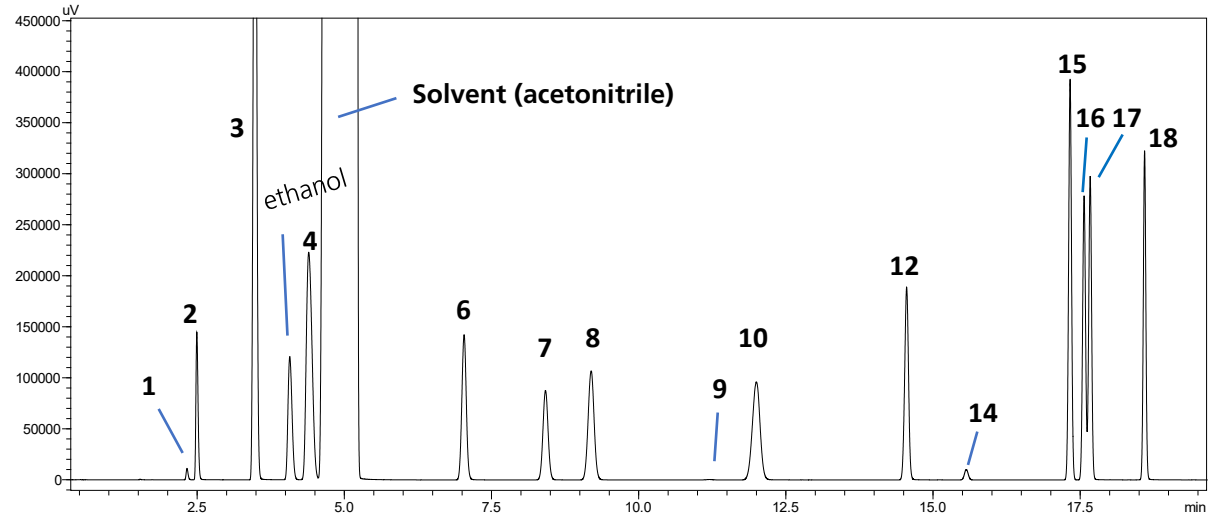

Figure 2: Chromatogram of components in the mixtures per FDA guidance. See Table 3 for peak identity. H_2 carrier gas was used in this chromatogram. Similar chromatograms were obtained using He carrier gas.

Calibration Curves

The calibration standard set from Bion contains five standards ranging from 10 ppm to 100 ppm (w/w) for 20 impurity compounds (Table 2). Five-point calibration curves were generated for each compound using these standards except for acetone, methanol, and benzene. Acetone was not included in the Bion calibration set and was not calibrated in this study. For methanol and benzene, six-point calibration curves were generated (methanol, 10 µL/L to 200 µL/L and benzene, 2 µL/L to 90 µL/L) using both the Bion calibration set and the USP custom mix.

The additional calibration point was included so that the maximum concentration allowed by USP method could be encompassed in the calibration range. Example calibration curves for level 1 impurities are shown in Figure 3. The curves were fitted to linear regression without forcing through zero. All compounds showed r^2 value > 0.998 (Table 3).

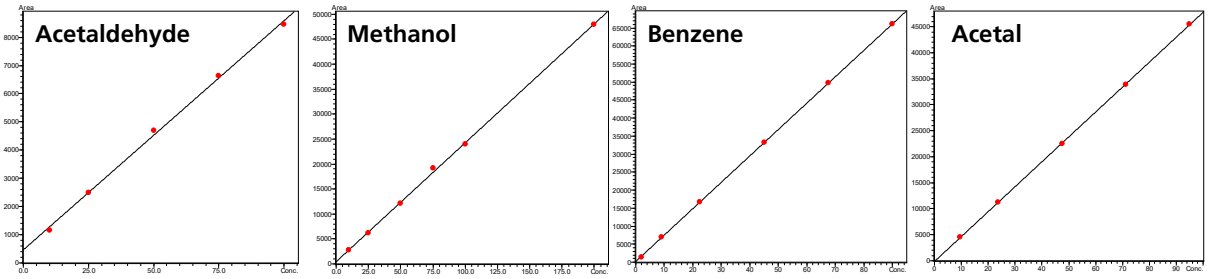

Figure 3: Calibration curves for level 1 organic impurities.

Detection Limit for Benzene

Per the USP method, benzene should not exceed 2 µL/L in ethanol. The FDA guidance has a similar requirement. To determine the detection limit for benzene, the USP custom mix (Table 1) containing 2 µL/L benzene was assayed. As shown in Figure 4, benzene can be easily detected at this concentration and the peak has a signal-to-noise (S/N) ratio of at least 11 using either H_2 carrier gas or He carrier gas.

When the instrument was well stabilized, the S/N also improved (Figure 4 inset, S/N for benzene > 17). Based on this, the detection limit for benzene is about 0.6 µL/L.

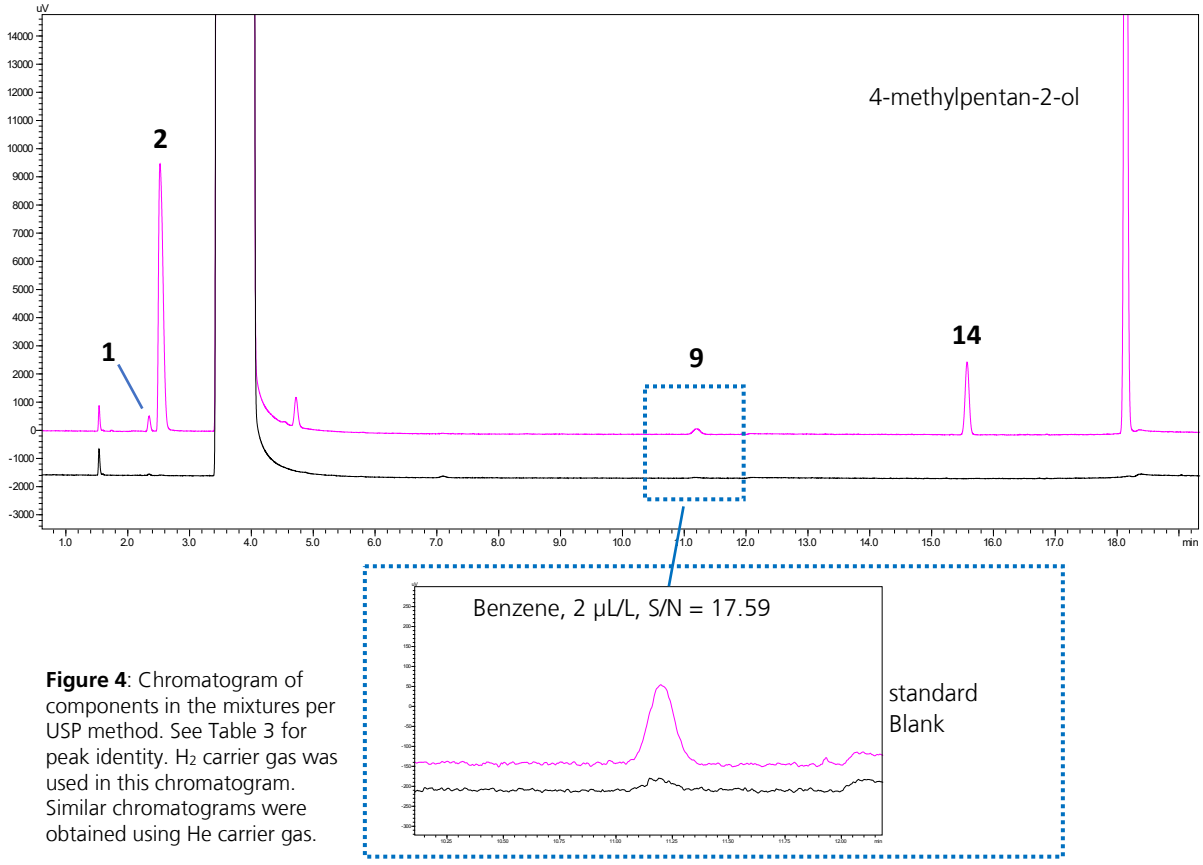

Figure 4: Chromatogram of components in the mixtures per USP method. See Table 3 for peak identity. H_2 carrier gas was used in this chromatogram. Similar chromatograms were obtained using He carrier gas.

Repeatability

To evaluate the repeatability of impurity concentration, a sample of denatured alcohol was assayed. As expected, the major impurity component is 2-Methyl-2-propanol (*tert*-butanol), which is the denaturant used in this denatured alcohol. All the other impurities are either not detected or present at very low levels (< 5.5 µL/L), below the maximum allowed concentrations specified by both the FDA guidance and USP alcohol monograph.

The relative standard deviation (RSD) was < 5.5% for all detected compounds, except for methanol, which had RSD of 13.4%. Methanol was also detected at the lowest concentration, less than 1 µL/L, which accounted for its large % RSD value. There is a caveat that the concentrations measured are outside of the calibration range. Despite the fact that the concentrations determined may not be accurate, they do fall below the maximum concentrations allowed by the regulations.

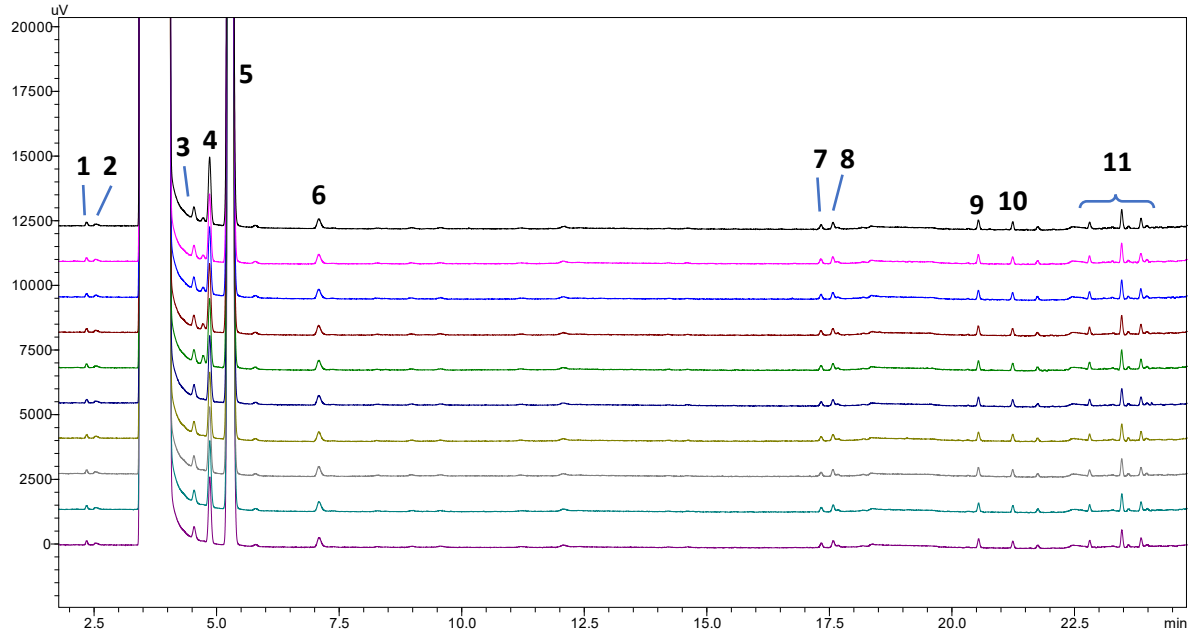

Figure 5: Chromatograms of repeated runs (n=10) of a denatured alcohol sample. H_2 carrier gas was used in this chromatogram. Identification of the peaks are shown in Table 4.

Table 4: Average concentration and % RSD (n=10) of impurity compounds in denatured alcohol sample calibrated using Bion standard set. Compounds not shown are not detected.

Peak No.	Compound	Average Concentration (µL/L)	% RSD	FDA max concentration	USP max concentration
1	Acetaldehyde	4.495	5.4	50	10
2	Methanol	0.936	13.4	630	200
3	isopropanol	3.965	1.6	*n.a.*	*n.a.* *
4	Other **	16.301	1.8	*n.a.*	*n.a.* *
5	*tert*-butanol	1332.271	0.5	*n.a.*	*n.a.*
6	n-propanol	5.483	3.0	1000	*n.a.* *
7	toluene	1.518	4.2	*n.a.*	*n.a.* *
8	isoamyl alcohol	3.079	3.0	4100	*n.a.* *
9	xylene	2.101	3.8	*n.a.*	*n.a.* *
10	styrene	1.724	3.7	*n.a.*	*n.a.* *
11	Other **	3.406	4.9	*n.a.*	*n.a.* *

n.a. not applicable

* subject to other impurity criteria per USP method

** unidentified compound, concentration determined using detector response factor of 4-methylpentan-2-ol according to USP method

■ Conclusion

An extended list of organic impurities was analyzed using Shimadzu Nexis GC-2030 on a ZB-624 column. Excellent separation, linearity, and repeatability were observed. The careful selection of a column and inlet liner is instrumental in achieving these results. Hydrogen was tested as the carrier gas and showed improved resolution to He carrier gas, as well as comparable sensitivity and linearity. Considering the high cost of He gas, H_2 may be the preferable carrier gas to use for this assay.

Furthermore, the integrated hydrogen sensor on GC-2030 improves safety when using H_2 carrier gas, so it may be used without safety concerns. Last, an integrated gas selector can be added to GC-2030 to allow the system to switch to nitrogen carrier gas during GC idle time, to minimize potential hazard as well as protect the column with inert gas.

■ References

1. *Policy for Temporary Compounding of Certain Alcohol-Based Hand Sanitizer Products During the Public Health Emergency, U.S. Food and Drug Administration, 2020*
2. *Temporary Policy for Manufacture of Alcohol for Incorporation into Alcohol-Based Hand Sanitizer Products During the Public Health Emergency (COVID-19), U.S. Food and Drug Administration, 2021*
3. *USP, Alcohol. The United States Pharmacopeia Convention, 2015*
4. *Direct Injection Gas Chromatography Mass Spectrometry (GC-MS) Method for the Detection of Listed Impurities in Hand Sanitizers, U.S. Food and Drug Administration, 2020*

■ Consumables

Part Number	Description	Unit	Instrument
221-76650-01	Septa, Green, Premium Low Bleed	Pk of 25	GC-2030
REST-23320	Restek Topaz Precision Split Liner with Wool	Pk of 5	
221-75597-03	FID jet for GC-2030	each	
221-81162-02	ClickTek Ferrule 0.5 mm	Pk of 6	
221-77155-41	ClickTek Column Connector	each	
221-74469-00	Syringe, 10 µL Teflon tip, fixed needle	each	AOC-20i/s
221-75173-01	Replacement plunger, for 221-74469-00	Pk of 2	
221-75174-00	Syringe, 10 µL Teflon tip, removable needle	each	
221-75174-01	Replacement needles, for 221-75174-00	Pk of 2	
221-75174-02	Replacement plungers, for 221-75174-00	Pk of 2	
220-97331-31	Sample Vials, 1.5 mL Amber Glass with Caps & Septa	Pk of 100	
220-97331-47	Sample Vials, 1.5 mL Amber Glass with Caps & Septa	Pk of 1000	
220-97331-62	200µL Glass Inserts for 1.5 mL Vials	Pk of 100	
220-97331-23	Wash Vials, 4 mL Amber Glass with Caps & Septa	Pk of 100	
220-94528-11	ZB-624 Capillary Column, 0.32 x 1.8 x 30	each	Column

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