

# Rapid GC-MS assessment of hand sanitizers according to the U.S. FDA method

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## Goal

The aim of this application note is to demonstrate the performance of the Thermo Scientific™ ISQ™ 7000 single quadrupole mass spectrometer, coupled to a Thermo Scientific™ TRACE™ 1310 gas chromatograph, for the simultaneous determination of alcohol content and harmful impurities in finished hand sanitizer products in compliance with the United States Food & Drug Administration (U.S. FDA) published method.<sup>1</sup>

## Introduction

Hand washing is the single most important measure in reducing the spread of diseases and infections. It is also an essential part of the response to COVID-19 in preventing the spread of the virus. Therefore, the World Health

Organization and the Centers for Disease Control and Prevention (CDC) recommend to wash the hands often with soap and water for at least 20 seconds and to use alcohol-based hand sanitizers containing at least 60% alcohol when soap and water are not available.<sup>1,2</sup>

Testing ethanol for methanol contamination is a quality standard set by the United States Pharmacopeia (USP) for alcohol used in pharmaceuticals (including hand sanitizers) and enforced by U.S. FDA under the FD&C Act.<sup>3</sup> Recently, the U.S. FDA reported that some hand sanitizers labeled to contain ethanol or isopropyl alcohol have been tested positive for 1-propanol contamination.<sup>4</sup> Both methanol and 1-propanol impurities above certain limits are not acceptable, as these compounds are highly neurotoxic

and can cause skin irritation and in some cases allergic reactions. The U.S. FDA has recently published some guidelines communicating temporary policies for preparing alcohol-based hand sanitizer products<sup>5</sup> and developed a laboratory analytical procedure to assess the quality of finished hand sanitizer products based on a modification of the existing USP <467> method.<sup>1</sup> The analytical procedure instructs the use of gas chromatography coupled to mass spectrometry (GC-MS) for the screening of potentially harmful impurities and for the quantitative determination of the alcohol content (quality assurance) in products formulated with either ethyl alcohol (ethanol) or isopropyl alcohol (isopropanol, 2-propanol, or IPA) as active ingredients.<sup>1</sup>

## Experimental

In the experiments described here, a Thermo Scientific ISQ 7000 single quadrupole mass spectrometer equipped with Thermo Scientific™ NeverVent™ technology was coupled to a Thermo Scientific TRACE 1310 gas chromatograph and a Thermo Scientific™ AS 1310 liquid autosampler and used for the determination of impurities and alcohol content in finished hand sanitizer products in accordance to the U.S. FDA method. Chromatographic separation was achieved on a Thermo Scientific™ TraceGOLD™ TG-624 SiIMS 30 m × 0.25 mm × 1.4 μm column (P/N 26059-3320). Additional GC-MS parameters as well as a complete list of the target compounds and their interim limits established in the U.S. FDA guidances<sup>5</sup> are detailed in Table 1 and Table 2, respectively.

## Data acquisition, processing, and reporting

Data was acquired, processed, and reported using the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software, version 7.3. Integrated instrument control ensures 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. Simplified eWorkflows™ deliver effective data management, ensuring ease of use, data integrity, and traceability. They have been designed to guide the user through the entire analysis process from sequence set up to data reporting.<sup>6</sup>

**Table 1. GC-MS operating conditions for impurities and alcohol content determination in accordance with the U.S. FDA method**

<b>TRACE 1310 GC, AS 1310 autosampler and ISQ 7000 single quadrupole MS parameters</b>	
Inlet module and mode	SSL, split
Liner	Thermo Scientific™ LinerGOLD™ precision liner; quartz wool (P/N 453A1255-U)
Inlet temperature (°C)	250
Split ratio	50:1
Septum purge mode, flow (mL/min)	Constant, 5
Carrier gas, mode, flow (mL/min)	He, constant flow, 1.0
<b>Oven temperature program</b>	
Temperature 1 (°C)	40
Hold time (min)	5.0
Temperature 2 (°C)	240
Rate (°C/min)	30
Hold time (min)	4.0
GC total run time (min)	15.67
<b>AS 1310 autosampler</b>	
Injection volume (μL)	1.0
Syringe	10 μL syringe, fixed needle (P/N 36500525)
Draw speed	Slow
Fill strokes	10
Sample depth	Bottom
Cold needle injection	Enabled
<b>ISQ 7000 single quadrupole MS</b>	
Ion source	Thermo Scientific™ ExtractaBrite™
Transfer line temperature (°C)	280
Source temperature (°C)	300
Ionization mode	EI
Electron energy (eV)	70
Acquisition mode	Full Scan
Mass range (m/z)	25–110
Filament delay (min)	0
<b>Chromatographic separation</b>	
Column	TraceGOLD TG-624 SiIMS 30 m × 0.25 mm × 1.4 μm (P/N 26059-3320)

**Table 2. List of level 1 and level 2 impurities and their interim limits according to U.S. FDA guidance**

Compound	Concentration limit (NMT, ppm)
<b>Level 1 impurities</b>	
Methanol	630
Benzene	2
Acetaldehyde	50
1,1-Diethoxyethane (Acetal)	50
<b>Level 2 impurities</b>	
Acetone	4400
1-Propanol	1000
Ethyl Acetate	2200
2-Butanol	6200
Isobutanol	21700
1-Butanol	1000
3-Methyl-1-Butanol	4100
Amyl Alcohol	4100

\* NMT = not more than

### Standard and sample preparation

Ethanol (absolute, 99.9%, [P/N 13268633](#)), HPLC-MS grade isopropanol (99.9%, [P/N 10684355](#)), HPLC-MS grade methanol (99.9%, [P/N 10767665](#)), acetone (99.9%, [P/N 10449242](#)), ethyl acetate (99.9%, [P/N 10736305](#)), and 1-propanol (extra pure, 99+%, [P/N 10336550](#)) were purchased from Fisher Scientific. The other compounds listed in Table 2 were purchased from Sigma-Aldrich (see Appendix A for catalog numbers). HPLC-MS grade acetonitrile (Fisher Scientific, [P/N 10616653](#)) was used as diluent. Commercially available hand sanitizers, containing ethanol or isopropanol at 70% v/v, were purchased from local UK retailers.

Mixed stock standard solution and working standard solutions as well as hand sanitizer sample preparation for recovery and impurity/alcohol assessment were prepared according to the U.S FDA method and detailed in Appendix B. The density of the hand sanitizer sample was determined by weighting 1 mL of hand sanitizer on an analytical balance.

## Results and discussion

### Chromatography

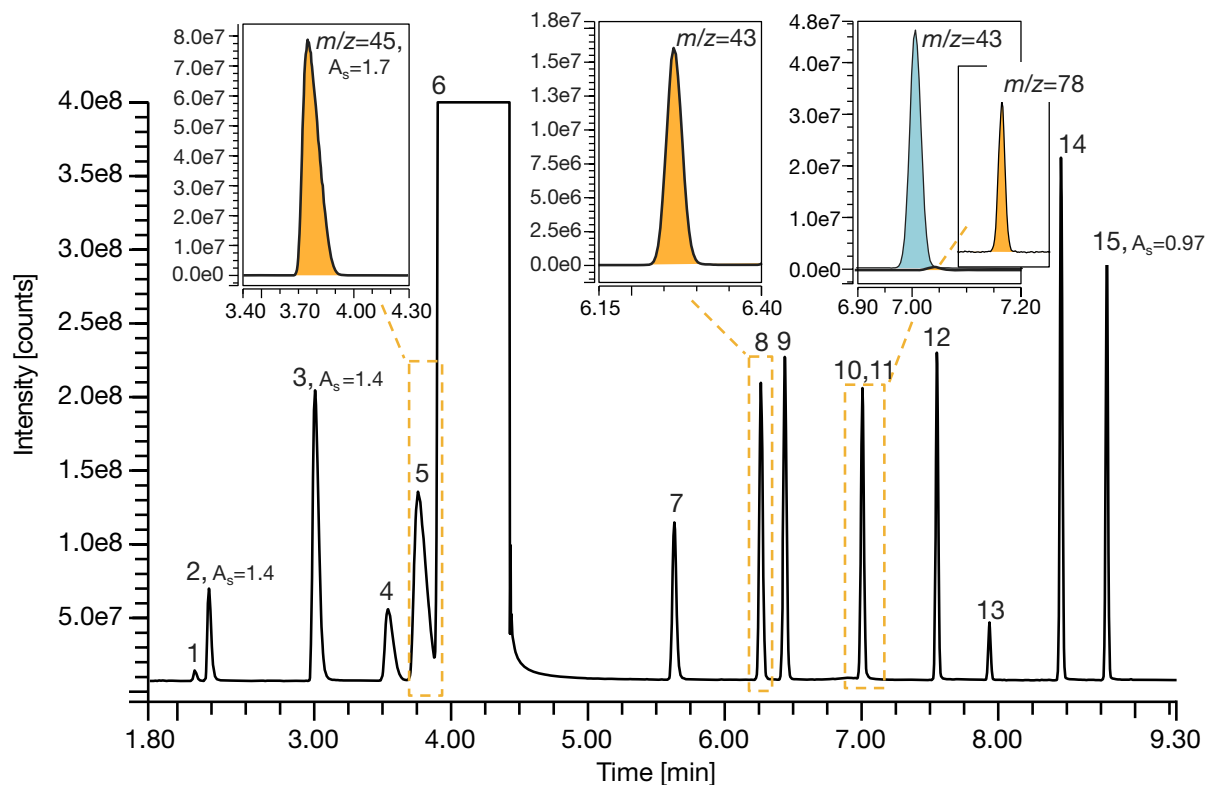
Chromatographic peaks were assigned to target compounds following the U.S. FDA method. The total ion chromatogram (TIC) was used to assign peak areas to the investigated impurities, with the exception of isopropanol (RT = 3.76 min,  $m/z$  45), ethyl acetate (RT = 6.26 min,  $m/z$  43), isobutanol (RT = 6.98 min,  $m/z$  43), and benzene (RT = 7.04 min,  $m/z$  78), for which the extracted ion chromatograms (XIC) were used. As highlighted in the method, co-elution between benzene and isobutanol occurred, but the compounds could be easily separated by extracting characteristic ions.

The chromatographic resolution ( $R_s$ ) in the TICs and the peak asymmetry ( $A_s$ ) factors were automatically calculated using Chromeleon software, applying the USP suggested equation.<sup>7</sup> Baseline resolution ( $R_s > 1.5$ ) was achieved for the investigated compounds with the exception of benzene and isobutanol, which co-elute in the total ion chromatogram. Gaussian peak shapes were obtained for all the compounds, with average  $A_s$  factor of  $< 1.2$ , indicating a high inertness of the system towards the most polar analytes and an efficient chromatographic process. A typical example of chromatographic separation for a standard solution is reported in Figure 1, with data acquired in full-scan mode ( $m/z$  25–110). The insets in Figure 1 show the extracted ion traces for the selected compounds.

### Recovery

Analyte recovery was assessed according to the U.S. FDA method. A sample sequence was prepared according to the method template recommended by the U.S. FDA methods:

- An initial blank (diluent) injection was followed by  $n=6$  repeated injections of the standard solution.
- Another blank injection was followed by un-spiked hand sanitizer samples, spiked hand sanitizer samples and the spiking solution.



1=Acetaldehyde, 2=Methanol, 3=Ethanol, 4=Acetone, 5=Isopropanol, 6=Acetonitrile, 7=1-Propanol, 8=Ethyl Acetate, 9=2-Butanol, 10=Isobutanol, 11=Benzene, 12=1-Butanol, 13=Acetal, 14= 3-Methyl-1-butanol, 15=Amyl Alcohol

Figure 1. Chromatographic separation for the investigated impurities in a solvent standard acquired in full scan ( $m/z$  25–110), along with extracted ion peaks for selected compounds highlighted in the insets. Asymmetry ( $A_s$ ) values are annotated as an example for some of the compounds.

Table 3. Peak area %RSD for n=6 repeated injections of standard solution and calculated recoveries for ethanol or isopropanol-based hand sanitizers spiked with the spiking solution. %RSDs <5.5% and %recovery within 80 to 120% showed full compliance with the U.S. FDA method.

Compound	Standard peak area %RSD (n=6)	Recovery (%)	
		Sample 1	Sample 2
<b>Level 1 impurities</b>			
Methanol	1.3	107	105
Benzene	5.3	96	114
Acetaldehyde	2.6	114	105
1,1-Diethoxyethane (Acetal)	3.3	104	95
<b>Level 2 impurities</b>			
Acetone	2.1	106	98
1-Propanol	2.6	98	107
Ethyl Acetate	3.9	100	99
2-Butanol	3.9	100	102
Isobutanol	3.6	99	100
1-Butanol	2.8	98	98
3-Methyl-1-Butanol	2.2	99	98
Amyl Alcohol	2.0	99	99

System suitability (SST) criteria were met with calculated peak area %RSD for n=6 standard injections <5.5% for all the target impurities, exceeding the U.S. FDA method requirements of %RSD <10%. Percentage recovery was calculated using Equation 1 and results were compliant with the method requirements, with average recovery values between 80 and 120%.

Details of SST and recovery results are reported in Table 3.

#### Equation 1

$$\%Recovery = \frac{(Peak Area_{Spiked} - Peak Area_{Unspiked})}{Peak Area_{SpikingSolution}} \times 100$$

#### Where:

$Peak Area_{Spiked}$  = peak area of target compound in the spiked sample  
 $Peak Area_{Unspiked}$  = peak area of target compound in the un-spiked sample  
 $Peak Area_{SpikingSolution}$  = peak area of target compound in the spiking solution

Overlaid chromatograms of un-spiked and spiked ethanol-based hand sanitizer sample are reported as an example in Figure 2.

### Level 1 and level 2 impurity assessment

The assessment of level 1 and level 2 impurities followed the sequence template reported in the method: a blank run (diluent) followed by triplicate injections of the standard solution, then a second blank (diluent) followed by the samples. As required, the standard solution was injected at least once every six samples and at the end of the sequence. The standard solution was injected as many times as necessary to reach a total number of six injections at the end of the sequence. Samples (un-spiked and spiked) were injected in triplicate and bracketed within repeated injections of the standard solution (n=2). The peak area %RSD for all standard injections across the sequence (n=7) was <10% for all the investigated compounds with average value of 5.7%, meeting the method SST requirement.

### Quantitation of level 1 impurities

Quantitation of level 1 impurities was performed by comparing the peak areas of the detected impurities in the sample chromatogram to the peak areas of the same impurities in the standard solution by using Equation 2. No level 1 impurities were found in the un-spiked samples. Calculated amounts of level 1 impurities for spiked samples are reported in Table 4 and were within 20% the spiked concentrations.

#### Equation 2

$$\text{Impurity (ppm)} = \frac{\text{Peak Area}_{\text{Sample}}}{\text{Peak Area}_{\text{Std}}} \times C_S \times V_{\text{Extract}} \times V_{\text{Container}} \div 0.3 \text{ mL} \div W$$

#### Where:

$\text{Peak Area}_{\text{Sample}}$  = area of the impurity peak in the sample solution

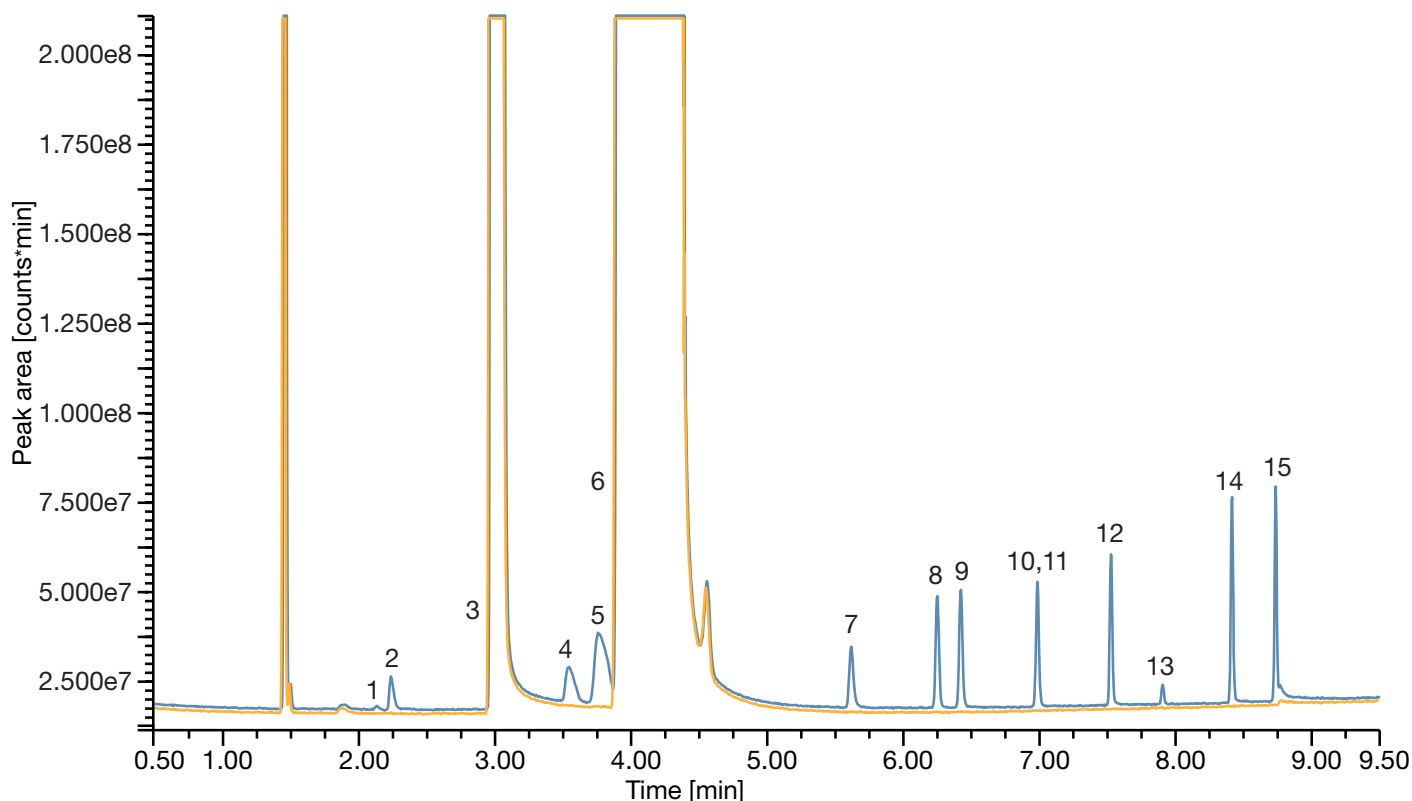
$\text{Peak Area}_{\text{Std}}$  = average area (n=2) of the impurity peak from the standards bracketing the sample

$C_S$  = concentration of the impurity in the standard solution in  $\mu\text{g/mL}$

$W$  = weight of total volume of hand sanitizer in the original container (g)

$V_{\text{Extract}}$  = volume of sample dilution (mL)

$V_{\text{Container}}$  = total volume of hand sanitizer in the original container (mL)



1=Acetaldehyde, 2=Methanol, 3=Ethanol, 4=Acetone, 5=Isopropanol, 6=Acetonitrile, 7=1-Propanol, 8=Ethyl Acetate, 9=2-Butanol, 10=Isobutanol, 11=Benzene, 12=1-Butanol, 13=Acetal, 14=3-Methyl-1-butanol, 15=Amyl Alcohol

Figure 2. Overlaid chromatograms of un-spiked (yellow) and spiked (light blue) ethanol-based hand sanitizer sample

The weight of total volume of hand sanitizer in the container (g) was calculated applying Equation 3:

**Equation 3**

$$W = V_L \times \text{Density}$$

**Where:**

$V_L$  = labeled volume (mL)

Density = calculated density for the hand sanitizer (g/mL)

**LOD determination**

Matrix-matched calibration curves were obtained by sequentially diluting (1:2) the mixed stock solution with one of the samples. Each calibration level (n=6) was injected in triplicate. The limits of detection for level 1 and level 2 impurities were determined using n=10 repeated injections of un-spiked and spiked sample at the lowest calibration level and calculated by applying Equation 4.<sup>8</sup> Calibration

ranges, coefficient of correlation (R), and calculated LODs are reported in Table 5. Calibration curves obtained for level 1 impurities are reported in Figure 3.

**Equation 4**

$$MDL = t_{(n-1, 1-\alpha=0.99)} * S$$

**Where:**

$t$  = Student's t-value appropriate for the single-tailed 99th percentile  $t$  statistic and a standard deviation estimate with  $n-1$  degrees of freedom, for  $n = 10$  injections:  $t = 2.821$

$S$  = standard deviation of the replicate analysis

**Level 2 impurities limit test**

Samples investigated for level 1 impurities were also assessed for level 2 impurities using a limit test approach. The isopropanol-based hand sanitizer sample was found to contain small traces of 1-propanol, while the ethanol-based hand sanitizer sample resulted free from

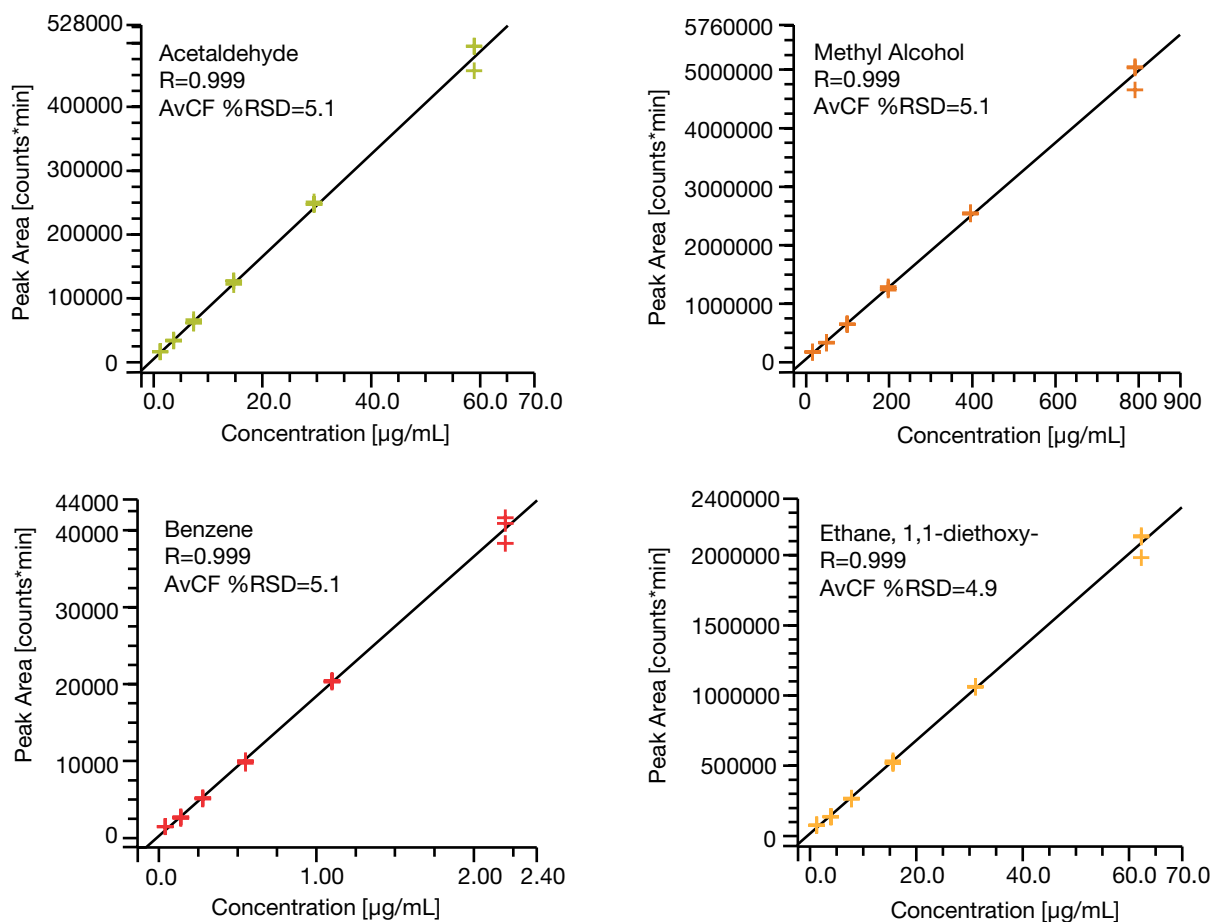
**Table 4. Calculated amounts for spiked hand sanitizer samples. Calculated concentrations (ppm) were within 20% of the spiked concentration. Calculated amounts are expressed to one decimal figure as per U.S. FDA method requirements.**

Compound	Spiked concentration (ppm)	Calculated amount (ppm)							
		Sample 1				Sample 2			
Level 1 impurities		Unspiked	Spiked			Unspiked	Spiked		
Methanol	581.6	< LOD	590.2	588.5	572.6	< LOD	568.0	558.1	578.9
Benzene	2.0	< LOD	1.6	1.7	1.7	< LOD	1.7	1.8	1.8
Acetaldehyde	53.1	< LOD	49.3	54.1	57.5	< LOD	49.9	46.0	48.2
Acetal	47.4	< LOD	50.9	49.9	51.5	< LOD	46.4	44.7	45.3

**Table 5. Calibration range, coefficient of correlation (R), average calibration factor (AvCF) %RSD, and calculated LOD (ppm) for level 1 and level 2 impurities**

Compound	Calibration range (µg/mL)	Correlation coefficient (R)	AvCF %RSD	Calculated LOD (ppm)
<b>Level 1 impurities</b>				
Methanol	16–791	0.999	5.1	10.3
Benzene	0.04–2	0.999	5.1	0.03
Acetaldehyde	10–59	0.999	5.1	2.3
1,1-Diethoxyethane (Acetal)	10–62	0.999	4.9	1.2
<b>Level 2 impurities</b>				
Acetone	15–790	0.999	5.3	10.4
1-Propanol	16–804	0.999	5.1	10.0
Ethyl Acetate	18–902	0.999	5.2	11.6
2-Butanol	16–646	0.999	4.9	2.3
Isobutanol	16–803	0.999	4.8	9.7
1-Butanol	16–810	0.999	4.8	10.1
3-Methyl-1-Butanol	16–809	0.999	4.9	10.2
Amyl Alcohol	16–811	0.999	4.8	8.3





**Figure 3. Matrix-matched calibration curves for level 1 impurities obtained by sequentially diluting (1:2) the mixed stock solution with one of the samples.** Each calibration level (n=6) was injected in triplicate. Coefficient of correlation (R) and AvCF %RSD are annotated.

impurities. The limit test for 1-propanol was done using a simple calculation and comparison. The peak area of the impurity in the standard solution was multiplied by its limit conversion factor (Equation 5) and then compared with the peak area of the impurity in the hand sanitizer sample. According to the reporting guidelines for level 2 impurities, if the peak area of the impurity in the sample is greater than the peak area of the same impurity in the standard multiplied by the limit conversion factor, then the test must be considered “Failed.” If it is less, the test is “Passed.” For the investigated sample, the test passed as reported in Figure 4. As an example, the overlaid chromatogram for isopropanol-based hand sanitizer sample and the standard solution is shown in Figure 5.

**Equation 5**

$$\text{Level 2 Impurity} = \text{Peak Area Impurity} \times \text{Limit Conversion Factor}$$

**Where:**

Peak Area Impurity = peak area from the impurity in the standard solution  
 Limit Conversion Factor = value for each impurity shown in Appendix C

**Alcohol content assessment**

Ethanol and isopropanol content of commercial hand sanitizer samples were assessed on the same day of the impurities testing. According to the U.S. FDA method, as the peak areas for ethanol and isopropanol in the sample were more than 5x greater than in the standard, the samples were diluted so that the resulting peak areas were approximately 0.5x the standard peak areas for those alcohols. Examples of chromatograms for a standard solution, samples, and diluted samples for ethanol and isopropanol-based hand sanitizers are reported in Figure 6.

The alcohol content (reported as % assay) was calculated by applying Equations 6 and 7. Calculated amounts were consistent with the manufacturer’s specification, with % assays of 101% as the label claim for the ethanol-based hand sanitizer and 89% as the label claim for the isopropanol-based hand sanitizer.

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	A	B	C	D	E	F
1	No.	Injection Name	Ret.Time min	Area counts*min	Limit_Value	Test Result
2			TIC	TIC	TIC	TIC
3			1-Propanol	1-Propanol	1-Propanol	1-Propanol
4						
5	22	std	5.612	6754872	1080780	
6	27	sample5	5.618	45447		Passed
7	28	sample5	5.622	47601		Passed
8	29	sample5	5.625	46661		Passed

Figure 4. Chromeleon browser showing an example of customized report for level 2 impurity test results for the isopropanol-based hand sanitizer sample. Sample was injected in triplicate.

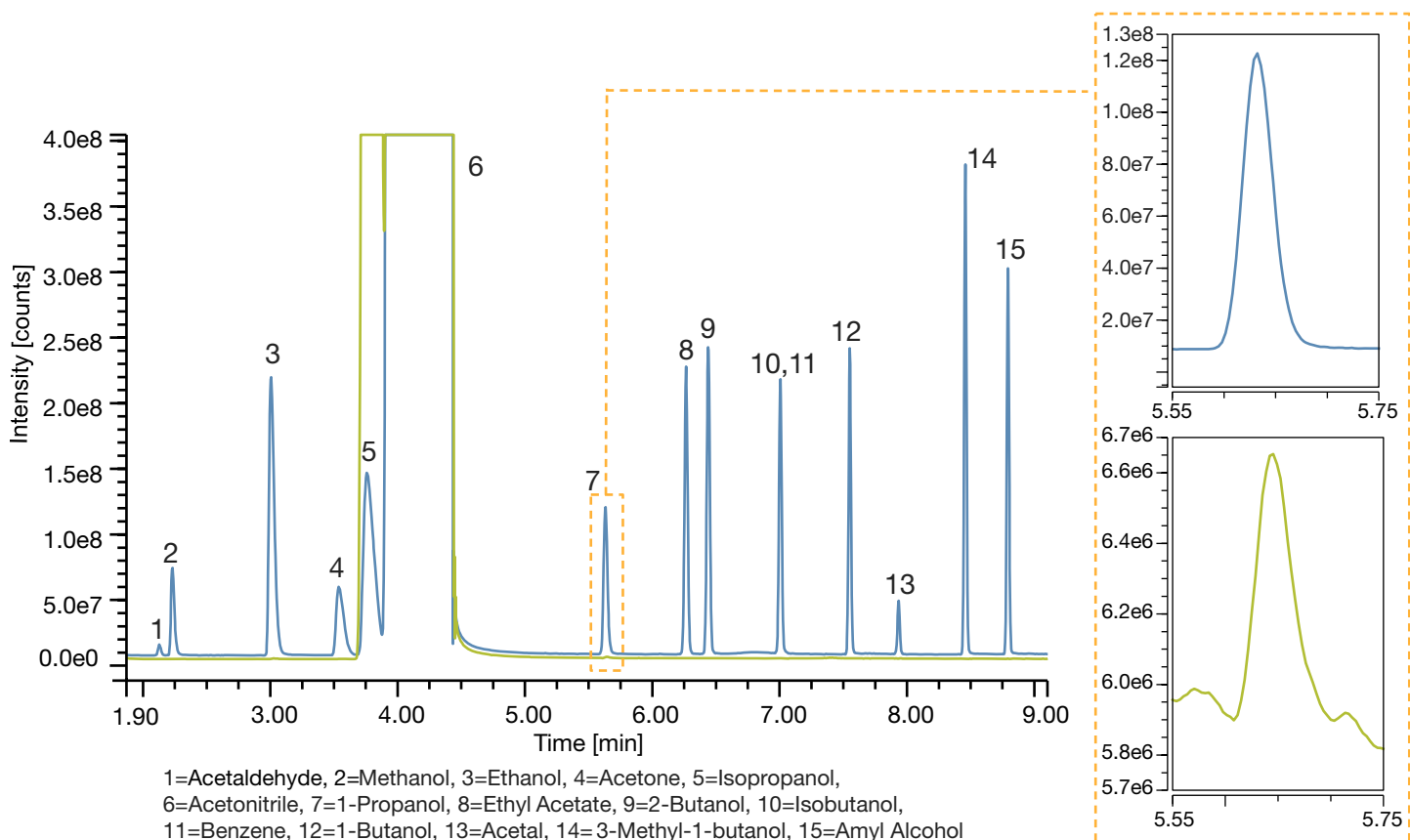


Figure 5. Overlaid chromatograms of an un-spiked isopropanol-based hand sanitizer (green) and the standard solution (blue). The sample was found to contain small traces of 1-propanol. The calculated amount for the impurity was well within the interim limits established by the U.S. FDA guidance.



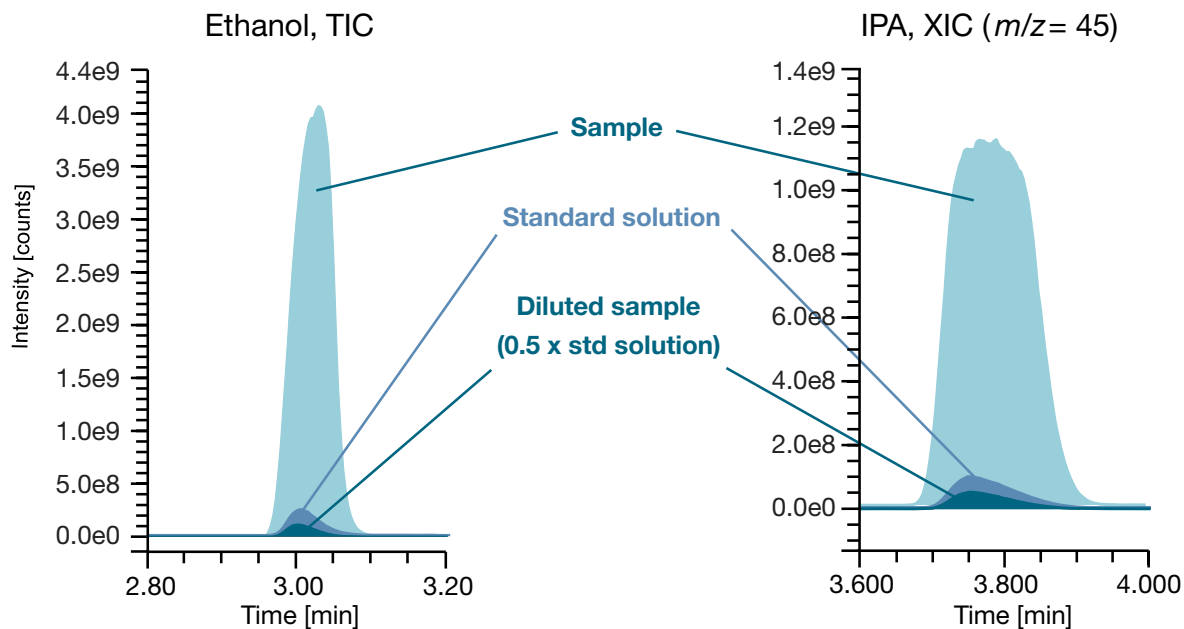


Figure 6. Overlaid TIC (ethanol) and XIC (IPA) chromatograms in hand sanitizer samples, standard solution, and diluted (approx. 0.5x standard solution) hand sanitizer samples. Calculated % assay was consistent with the label claim with a value of 101% for ethanol and 89% for isopropanol.

#### Equation 6

$$\% \text{ Alcohol (v/v)} = \frac{\text{Peak Area}_{\text{Sample}}}{\text{Peak Area}_{\text{Std}}} \times C_s \times \text{Dilution Factor} \times V_{\text{Extract}} \div 1 \times 10^6 \div \text{Density}_{\text{Alcohol}} \div 0.3 \text{ mL} \times 100$$

#### Where:

$\text{Peak Area}_{\text{Sample}}$  = peak area of the alcohol (ethanol or isopropanol) in the sample

$\text{Peak Area}_{\text{Std}}$  = average (n=2) of the alcohol peak areas in the standard solutions bracketing the samples

$C_s$  = concentration of the alcohol (ethanol or isopropanol) in the standard solution ( $\mu\text{g/mL}$ )

$\text{Dilution Factor}$  = dilution applied to obtain an alcohol (ethanol or isopropanol) peak area in the sample approx. 0.5x the peak area of the alcohol (ethanol or isopropanol) in the standard solution

$V_{\text{Extract}}$  = volume of sample dilution (mL)

$\text{Density}_{\text{Alcohol}}$  = 0.789 g/mL for ethanol and 0.785 g/mL for isopropanol

#### Equation 7

$$\% \text{ Assay} = \% \text{ Alcohol} / \text{Label Claim} * 100$$

#### Where:

$\text{Label Claim}$  = v/v% alcohol content in hand sanitizer

### Method robustness

Consistency of results using the proposed GC-MS method was evaluated over five days of laboratory work. The spiked recovery test, the impurities (level 1 and level 2), and the alcohol content determination were assessed on the first day (day 1). Sequences of eight hours in duration, including blanks and spiked samples bracketed by standard

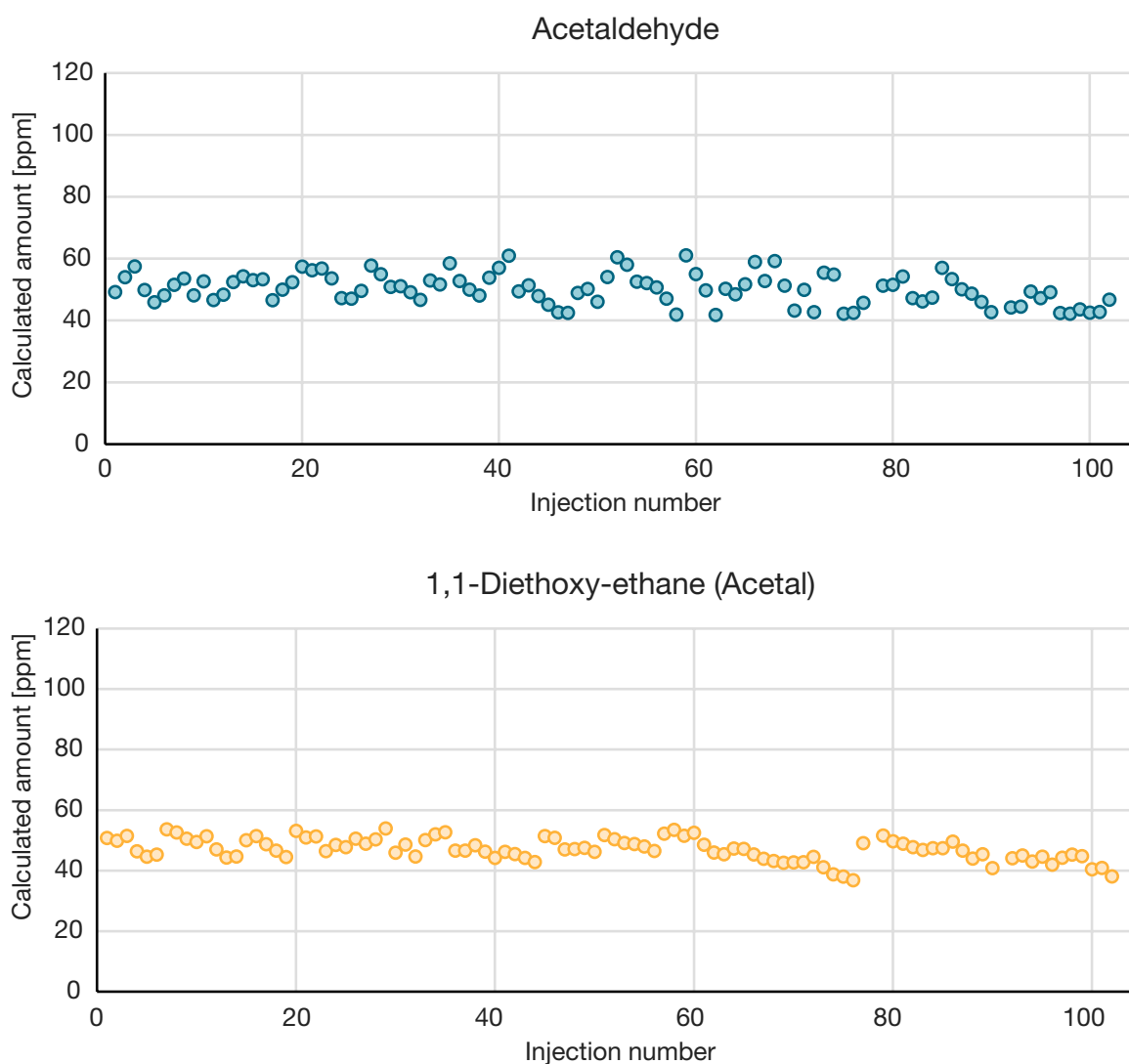
solutions (injected twice every six samples), were run on the following days (days 2–5) and used to monitor the instrument performance (total number of injections n=120).

Ensuring that the mass spectrometer is performing as expected every single day is critical to obtain consistent results. The Thermo Scientific™ SmartTune™ wizard was used daily to check if corrective actions were necessary and to ensure consistency of mass spectrometer response over the time. The SmartTune is an intuitive easy-to-use tuning software with troubleshooting feedback capability that improves the usability of the instrument especially for new users as it is designed to have minimal user input.<sup>9</sup>

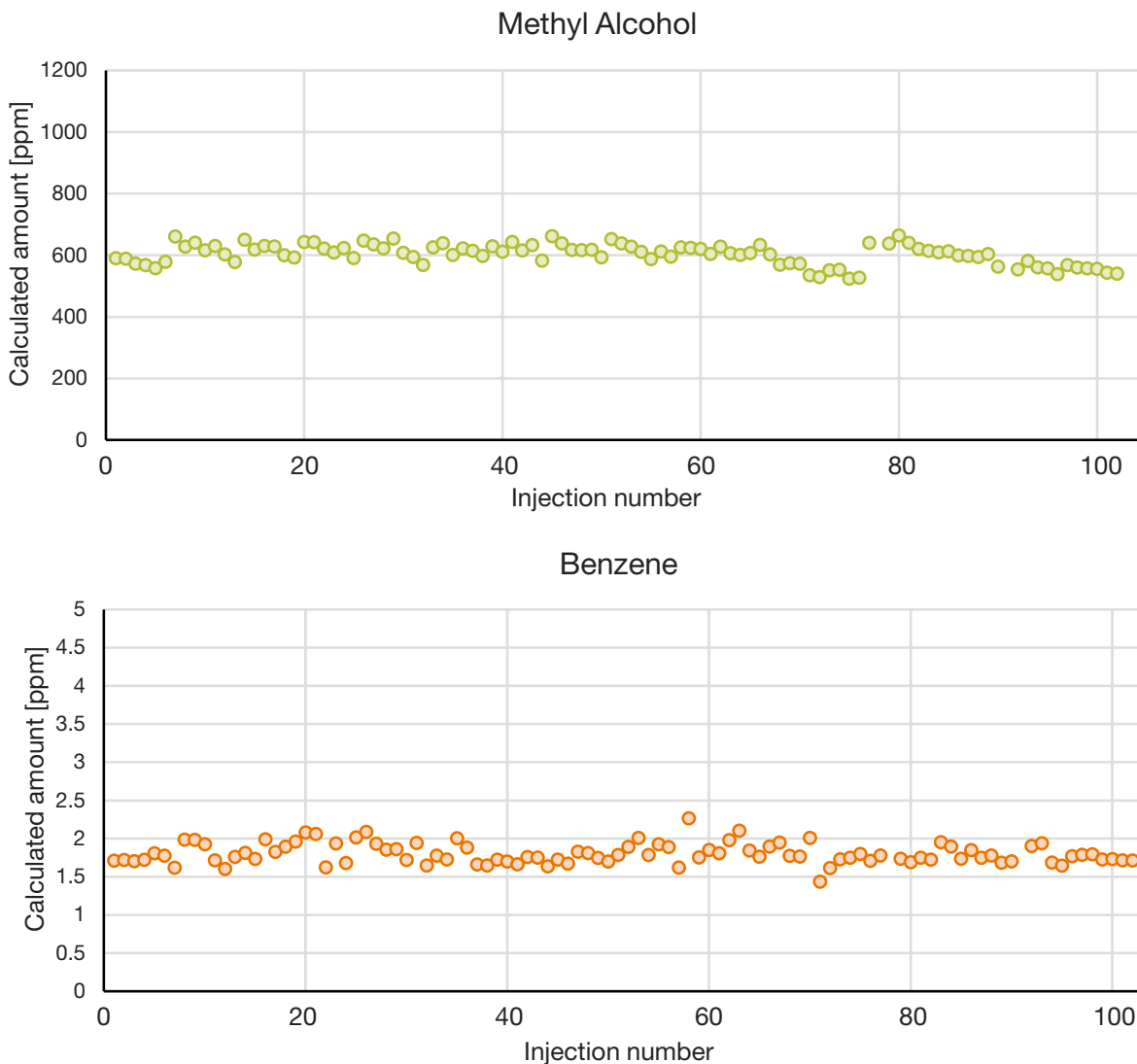
As the U.S. FDA method requires the quantitative assessment of level 1 impurities (while level 2 impurities are evaluated based on a limit test approach), quantitative performance over time was evaluated based on level 1 impurities only. As demonstrated in Table 6 and Figure 7, consistency of results was obtained across the sequences with peak area %RSD for all the standard injections in each sequence <10% and calculated amounts for spiked samples within 20% of the expected values. Retention times stability, as well as peak shape and resolution, were also very stable (Appendix D).

**Table 6. Quantitative performance was evaluated for level 1 impurities across four working days.** The spiked recovery test, the impurities (level 1 and level 2), and alcohol content determination were assessed on day 1. Sequences of eight-hour duration (days 2–5) including blanks and spiked samples bracketed by standard solutions (injected twice every six samples) were run on the following days and used to monitor the instrument performance (total number of injections = 120). Absolute peak area %RSD for all the standard injections in each sequence was <10% and calculated amounts for sample spiked with level 1 impurities (n=102) were within 20% the expected values.

Compound	Standard peak area %RSD					Expected amounts (ppm)	Average calculated amount (ppm)				
	Day 1 (n=7)	Day 2 (n=9)	Day 3 (n=9)	Day 4 (n=9)	Day 5 (n=9)		Day 1	Day 2	Day 3	Day 4	Day 5
Methanol	5.3	9.7	9.1	7.8	7.6	581.6	576.1	623.8	616.5	588.1	584.3
Benzene	5.3	8.7	8.8	6.5	9.5	2.0	1.7	1.9	1.8	1.8	1.8
Acetaldehyde	6.1	8.9	8.2	8.2	6.7	53.1	50.8	51.7	52.5	47.4	50.1
1,1-Diethoxyethane (Acetal)	6.4	8.9	8.6	7.3	8.7	47.4	48.1	47.0	47.7	45.4	45.3



**Figure 7A. Consistency of results evaluated over four days (days 2–5) of sample measurements (spiked sample injections are reported, n=102).** Calculated amount for sample spiked with level 1 impurities were all within 20% of the expected values.



**Figure 7B. Consistency of results evaluated over four days (days 2–5) of sample measurements (spiked sample injections are reported, n=102).** Calculated amount for sample spiked with level 1 impurities were all within 20% of the expected values.

## Conclusion

The obtained results demonstrate that the ISQ 7000 single quadrupole mass spectrometer with NerverVent technology in combination with the TRACE 1310 GC and the AS 1310 liquid autosampler offers a robust solution for analytical and QA/QC manufacturer laboratories requiring reliable testing of finished hand sanitizer products for impurities and alcohol content, in compliance with the existing U.S. FDA method.

- The short GC cycle time allowed for high sample throughput. All target compounds were separated in <9 min with Gaussian peak shape and chromatographic resolution. As expected, benzene co-eluted with isobutanol, but peaks could be easily identified by extracting the characteristic ions as per U.S. FDA method requirements.
- System suitability criteria were met with peak area %RSDs (n=6) <10 and calculated recoveries for spiked samples between 80 and 120% for all the investigated impurities.
- Linearity was assessed using six calibration levels injected in triplicate. Correlation coefficient R was >0.999 and AvCF %RSD <5.2.
- No level 1 impurities were found in the commercial hand sanitizer samples, but small traces of 1-propanol were found in the isopropanol-based hand sanitizer. However, this impurity was below the interim limits established by the current U.S. FDA guidance and therefore considered harmless.
- The alcohol content (ethanol or isopropanol) was confirmed to be consistent with the manufacturer's claim.

- The ruggedness of the AS 1310 liquid autosampler combined with the stability of the ISQ 7000 system and the SmartTune wizard ensured consistency over time, allowing for peak area %RSD <10 for all the standard injections in multiple sequences and the calculated amount for spiked sample consistently within  $\pm 20\%$  of the spiked concentrations.
- Chromeleon CDS ensured ease of use for method development, quantitative analysis, and reporting in compliance with environments governed by current Good Manufacturing Practice (cGMP).

## References

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## Appendix A

**Table A1. Catalog numbers for impurities purchased at Sigma-Aldrich**

Compound	P/N
<b>Level 1 impurities</b>	
Benzene	270709-100mL
Acetaldehyde	00070-100mL
1,1-Diethoxyethane (Acetal)	A902-100mL
<b>Level 2 impurities</b>	
1-Propanol	34871-1L
2-Butanol	B85919-1L
Isobutanol	270466-1L
1-Butanol	281549-1L
3-Methyl-1-Butanol	M32658-1L
Amyl Alcohol	76929-1L

## Appendix B

### Mixed stock standard preparation

Mixed stock standard solution was prepared as reported in the Table 1 using HPLC-MS grade acetonitrile as diluent. Benzene (10  $\mu\text{L}$ ) was first diluted in 10 mL acetonitrile to a concentration of 874  $\mu\text{g/mL}$  and 0.5 mL of this solution were used to prepare the mixed stock standard.

**Table B1. Mixed stock standard preparation**

Compound	Spiked volume ( $\mu\text{L}$ )	Final volume (mL)	Concentration ( $\mu\text{g/mL}$ )
Methanol	200	100	1582
Benzene	500		4.4
Acetaldehyde	15		117.8
Acetal	15		124.5
Ethanol	500		3945
Isopropanol	500		3925
Acetone	200		1580
1-Propanol	200		1608
Ethyl Acetate	200		1804
2-Butanol	200		1616
Isobutanol	200		1606
1-Butanol	200		1620
3-Methyl-1-Butanol	200		1618
Amyl Alcohol	200		1622

### Standard preparation

Mixed stock standard (1 mL) was transferred to a 10 mL volumetric flask containing approximately 8 mL HPLC-grade acetonitrile, diluted to volume with HPLC-grade acetonitrile, and mixed well. This solution was prepared fresh daily.

### Spiked recovery standard preparation

Mixed stock standard (1 mL) was transferred to a 100 mL volumetric flask containing approximately 80 mL HPLC-grade acetonitrile, diluted to volume with HPLC-grade acetonitrile, and mixed.

### Hand sanitizer sample preparation

Hand sanitizer (0.3 mL) was transferred to a 10 mL volumetric flask containing 8 mL HPLC-grade acetonitrile, diluted to volume with HPLC-grade acetonitrile, and mixed.

### Spiked recovery hand sanitizer sample preparation

Hand sanitizer sample (0.3 mL) was transferred to a 15 mL conical centrifuge tube, spiked recovery standard (10 mL) was added, and the sample was mixed well.

### Un-spiked sample preparation

Hand sanitizer sample (0.3 mL) was transferred to a 15 mL conical centrifuge tube, HPLC-grade acetonitrile (10 mL) was added, and the sample was mixed well.

### Spiking solution preparation

HPLC-grade acetonitrile (0.3 mL) was transferred to a 15 mL conical centrifuge tube, spiked recovery standard (10 mL) was added, and the sample was mixed well.

## Appendix C

Table C1. Limit conversion factors for level 2 impurities

Compound	Limit conversion factor
Level 2 impurities	
Acetone	0.71
1-Propanol	0.16
Ethyl Acetate	0.31
2-Butanol	0.98
Isobutanol	3.45
1-Butanol	0.16
3-Methyl-1-Butanol	0.65
Amyl Alcohol	0.64

## Appendix D

Table D1. Retention times, peak asymmetry, and resolution across five working days

Compound	RT (%RSD)					Asymmetry factor average value					Resolution average value				
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 1	Day 2	Day 3	Day 4	Day 5	Day 1	Day 2	Day 3	Day 4	Day 5
Methanol	0.2	0.05	0.05	0.2	0.2	1.6	1.4	1.6	1.7	1.6	11	10	10	10	10
Benzene	0.2	0.1	0.1	0.05	0.05	1.0	0.9	1.0	1.0	1.0	*	*	*	*	*
Acetaldehyde	0.1	0.1	0.1	0.2	0.2	1.3	1.2	1.3	1.3	1.3	2	2	2	2	2
1,1-Diethoxyethane (Acetal)	0.05	0.05	0.05	0.05	0.05	1.0	0.9	1.0	1.0	1.0	15	14	15	15	15

\*= not applicable being extracted ion chromatogram

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