Headspace GC-MS Methods and Sample Preparation for Hand Sanitizers Liquid, Gel, and Wipe Products: Method Development, Validation, and Application

Diaa Shakleya, Nicolas Abrigo, Connie Ruzicka, and Patrick J. Faustino
CDER/Office of Pharmaceutical Quality/Office of Testing and Research

Abstract

PURPOSE: To assist the Agency with the hand sanitizer product quality testing, FDA scientists developed and validated headspace GC-MS methods to test hand sanitizer products for ethanol, isopropanol, and impurity concentrations. The developed methods were designed to best ensure the effectiveness and safety of hand sanitizer products. **METHODS**: Headspace GC-MS methods were developed for the evaluation of alcohol-based hand sanitizer products that include liquid, gel, and wipe formulations. The methods were validated according to ICH Q2 (R1) guidelines for specificity, linearity, range, limit of quantitation, accuracy, precision, robustness, spike recovery, and stability for ethanol, isopropanol and 12 impurities. Sample preparation for liquid and gel products consisted of diluting the hand sanitizer products in DMSO for analysis whereas wipe products required a liquid extraction step in DMSO. **RESULTS**: Headspace GC-MS methods for liquid, gel, and wipe hand sanitizer products were each individually validated according to the ICH Q2 (R1) guidelines and passed all the set criteria. All validated analytes maintained their reported linearity with coefficients of determination (R2) greater than 0.99. Inter-day percent accuracy and precision of the tested analytes for the four levels all met the specifications within 80-120 % accuracy and ≤ 5% RSD. All of the tested analytes demonstrated % recovery within the allowable limits (80%-120%). Application of the methods has resulted in the testing of numerous samples collected under a domestic surveillance assignment to monitor product quality. **CONCLUSIONS**: The testing of hand sanitizer products provided scientific information to support CDER regulators to take regulatory actions to help to ensure the safety and efficacy of these products for use by the American public.

Methods

Headspace Gas Chromatography Mass Spectrometry (GC-MS) methods were developed for alcohol-based hand sanitizer products in liquid, gel, and wipe formulations. The methods were validated according to ICH Q2 (R1) for specificity, linearity, range, limit of quantitation, accuracy, precision, robustness, spike recovery, and stability. Two active ingredients (ethanol and isopropanol) and 12 impurities (methanol, benzene, acetaldehyde, acetal, acetone, 1-propanol, ethyl acetate, 2-butanol, Isobutanol, 1-butanol, 3-methyl-1-butanol, and amyl alcohol) were evaluated using the respective analytical methods. Sample preparation for liquid and gel products consisted of diluting the hand sanitizer products in dimethylsulfoxide (DMSO) for analysis. Sample preparation for wipe products consisted of a liquid extraction step in DMSO. A calibration curve was used for determining the concentration of analytes based on the relative response of analyte to internal standard. A spike recovery assay was performed by spiking a known concentration of standard analytes into the hand sanitizer samples and determining the percent recovery relative to the known amount spiked into the diluent.

Table 1: Active ingredient content recommendations for alcohol-based hand sanitizers

Active Ingredient	FDA Guidance (WHO recommendation)	Minimum Limit (CDC recommendation)
Ethanol	80% (v/v)	60% (v/v)
Isopropanol	75% (v/v)	70% (v/v)

Materials and Methods

Figure 1: Chromatogram of analytes

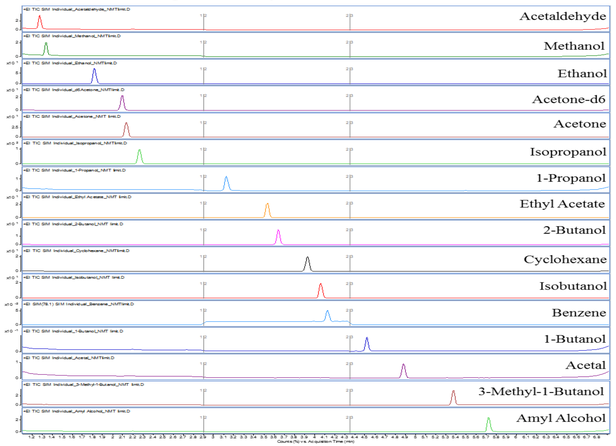

Table 2: Level 1 impurities

Impurity	Interim Limit Under CDER Guidance (ppm)
Methanol	NMT 630
Benzene	NMT 2
Acetaldehyde	NMT 50
Acetal	NMT 50

Table 3: Level 2 impurities

Impurity	Interim Limit Under CDER Guidance (ppm)
Acetone	**NMT 4400**
1-Propanol	**NMT 1000**
Ethyl Acet.	**NMT 2200**
2-Butanol	**NMT 6200**
Isobutanol	**NMT 21700**
1-Butanol	**NMT 1000**
3-Methyl-1-Butanol	**NMT 4100**
Amyl Alc.	**NMT 4100**

Table 4: Linearity validation results for active ingredients and level 1 impurities. The linear regression line for all analytes resulted in $R^2 > 0.99$.

Range of Compound (µg/mL)	Validation Day	Equation	R2
Acetaldehyde (0.265-4.25)	Validation Day 1	y=0.026885*x+0.000006	0.9972
	Validation Day 2	y=0.026836*x-0.000426	0.9951
	Validation Day 3	y=0.025028*x+0.000546	0.9991
Methanol (3.34-53.4)	Validation Day 1	y=0.009561*x+0.005666	0.9960
	Validation Day 2	y=0.009483*x+0.004019	0.9954
	Validation Day 3	y=0.008795*x+0.001703	0.9996
Ethanol (98.6-1578)	Validation Day 1	y=0.007237*x+0.115075	0.9961
	Validation Day 2	y=0.007259*x+0.102598	0.9956
	Validation Day 3	y=0.006469*x+0.136147	0.9978
Isopropanol (98.1-1570)	Validation Day 1	y= 0.026679*x+0.879872	0.9926
	Validation Day 2	y=0.026658*x+0.809167	0.9932
	Validation Day 3	y=0.024935*x+0.805491	0.9949
Benzene (0.0107-0.171)	Validation Day 1	y=0.091878*x-0.000072	0.9945
	Validation Day 2	y=0.095407*x-0.000148	0.9950
	Validation Day 3	y=0.127246*x+0.000073	0.9997
Acetal (0.264-4.22)	Validation Day 1	y=0.031232*x-0.002264	0.9931
	Validation Day 2	y=0.034845*x-0.002868	0.9916
	Validation Day 3	y=0.043868*x-0.001841	0.9992

Results and Discussion

Table 5: Accuracy and precision validation results for the quality control standard concentrations. The accuracy for all analytes was between 90-110% and the precision was below 10%.

	QC LLOQ	QC Low	QC Mid	QC High
Acetaldehyde				
Nominal Concentration (µg/mL)	0.265	0.796	1.59	3.19
Calculated Concentration (µg/mL)	0.276	0.806	1.59	3.01
Accuracy (%)	104.0	101.2	99.9	94.4
Precision (Standard Deviation)	0.015	0.014	0.042	0.057
Precision (%RSD)	5.4	1.7	2.6	1.9
Methanol				
Nominal Concentration (µg/mL)	3.34	10.0	20.0	40.1
Calculated Concentration (µg/mL)	3.55	10.3	20.2	40.3
Accuracy (%)	106.3	102.5	100.9	100.7
Precision (Standard Deviation)	3.55	10.27	20.21	40.33
Precision (%RSD)	4.8	2.4	1.3	1.9
Ethanol				
Nominal Concentration (µg/mL)	98.6	296	592	1184
Calculated Concentration (µg/mL)	96.6	313	622	1186
Accuracy (%)	97.9	105.7	105.1	100.2
Precision (Standard Deviation)	96.6	312.8	622.0	1186.3
Precision (%RSD)	4.6	2.6	1.4	2.0
Isopropanol				
Nominal Concentration (µg/mL)	98.1	294	589	1178
Calculated Concentration (µg/mL)	90.2	320	636	1177
Accuracy (%)	91.9	108.8	108.1	99.9
Precision (Standard Deviation)	90.200	320.180	636.379	1176.648
Precision (%RSD)	4.5	3.1	1.7	2.0
Benzene				
Nominal Concentration (µg/mL)	0.0107	0.0321	0.0643	0.129
Calculated Concentration (µg/mL)	0.0108	0.0330	0.0645	0.128
Accuracy (%)	101.2	102.9	100.3	99.7
Precision (Standard Deviation)	0.011	0.033	0.064	0.128
Precision (%RSD)	6.3	2.6	2.0	1.1
Acetal				
Nominal Concentration (µg/mL)	0.264	0.792	1.58	3.17
Calculated Concentration (µg/mL)	0.292	0.765	1.52	3.06
Accuracy (%)	110.5	96.6	95.8	96.7
Precision (Standard Deviation)	0.292	0.765	1.517	3.062
Precision (%RSD)	4.2	2.7	1.6	1.9

Table 6: Testing results for liquid hand sanitizers

Active Ingredients	Limit	LA	LB	LC	LD	LE	LF
Ethanol % (v/v)	*60%*	72%	87%	0%	0%	0%	0%
Isopropanol % (v/v)	*70%*	0%	0%	88%	77%	80%	84%
Measured pH	*N/A*	7.8	8.3	7.4	8.4	6.6	7.5
Impurities	**Limit (ppm)**						
Acetaldehyde	*50*	**67 ppm**	**99 ppm**	< LLOQ	< LLOQ	< LLOQ	< LLOQ
Methanol	*630*	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	486 ppm
Benzene	*2*	ND	< LLOQ	ND	ND	ND	ND
Acetal	*50*	18 ppm	< LLOQ	< LLOQ	ND	ND	ND
Level 2 Impurities	*No level 2 impurities detected at or above the concentration limit in the products*						

Table 7: Testing results for gel hand sanitizers

Active Ingredients		GA	GB	GC	GD	GE	GF
Labeled Active Ingredient		Ethanol	Ethanol	Ethanol	Isopropanol	Isopropanol	Isopropanol
Labeled Content (%)		62.5%	70%	70%	70%	70%	70%
Determined Content (%)		70%	68%	71%	65%	77%	**0%***
Impurities	**Limit (ppm)**						
Acetaldehyde	*50*	**146 ppm**	< LLOQ	< LLOQ	< LLOQ	< LLOQ	30 ppm
Methanol	*630*	< LLOQ	< LLOQ	ND	< LLOQ	< LLOQ	**845 ppm**
Benzene	*2*	**15 ppm**	**2 ppm**	ND	ND	ND	ND
Acetal	*50*	**459 ppm**	< LLOQ	< LLOQ	**57 ppm**	ND	39 ppm
Level 2 Impurities		*No level 2 impurities detected at or above the concentration limit in the products*					

** Product GF was labeled as 70% isopropanol but was determined to contain 35% isopropanol and 34%% ethanol.*

Table 8: Testing results for wipe hand sanitizers

Active Ingredients		WA	WB	WC	WD	WE	WF
Labeled Active Ingredient		Ethanol	Ethanol	Ethanol	Isopropanol	Isopropanol	Isopropanol
Labeled Content (%)		75%	75%	75%	70%	70%	75%
Determined Content (%)		**46%**	62%	64%	70%	69%	**0%****
Impurities	**Limit (ppm)**						
Acetaldehyde	*50*	< LLOQ	28 ppm	< LLOQ	< LLOQ	< LLOQ	< LLOQ
Methanol	*630*	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
Benzene	*2*	ND	ND	ND	ND	ND	ND
Acetal	*50*	< LLOQ	< LLOQ	< LLOQ	ND	ND	ND

Conclusion

- FDA scientists have developed methods for hand sanitizer products using headspace GC-MS. The methods have been validated following ICH Q2 (R1) and have been tested using marketed products.
- These methods were used for surveillance testing of hand sanitizer products.
- The analytical methods provide the Agency with regulatory tools to monitor hand sanitizer products manufactured as liquids, gels, or wipes.

Disclaimer

This poster reflects the views of the authors and should not be construed to represent FDA's views or policies.