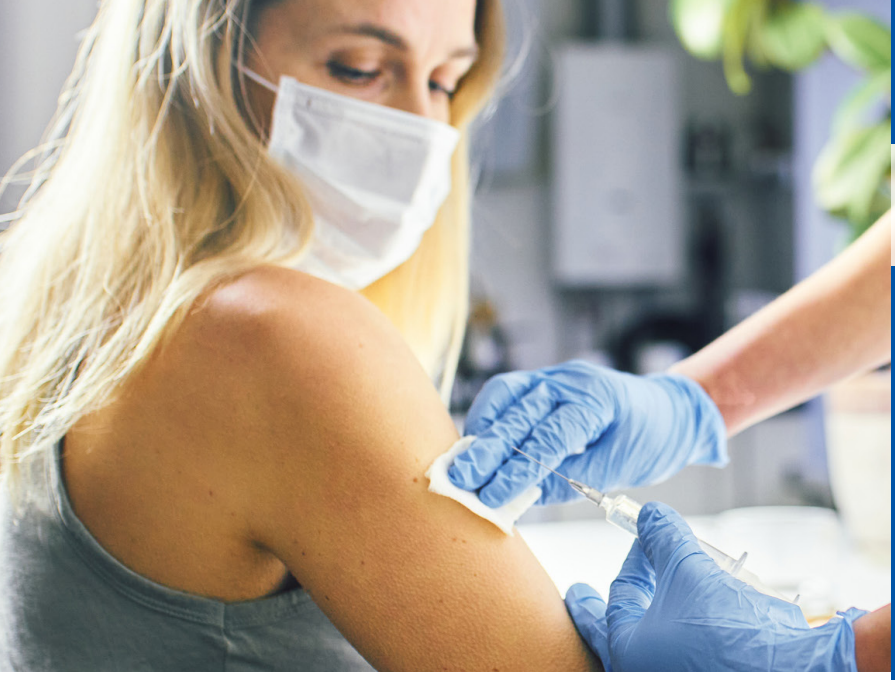

APPLICATION NOTE

Gas Chromatography

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Analysis of United States Pharmacopeia (USP) Grade Isopropyl Alcohol Impurities

Introduction

Isopropyl alcohol, also known as Isopropanol or IPA, is commonly used as a sanitizer especially on skin surfaces such as prior to an injection of a vaccine or medicine. It is imperative this solution does not contain any impurities that are known to be a health hazard thus, the requirement for quality testing. The USP has published specifications for the maximum levels of known IPA targeted impurities including ethyl ether, acetone, diisopropyl ether, 1-propanol, and 2-butanol at 0.1% or less. This is explored in two parts; the first, the USP recommend conditions producing a 42-minute analysis time. The second section contains a speed optimized method for rapid, accurate and precise result in less than 6 minutes.

Instrumentation

The PerkinElmer Clarus® 690 GC with integrated autosampler was used to perform these experiments. The Clarus 690 GC was configured with a capillary injector and dual wide range flame ionization detector (FID). A PerkinElmer Elite 624 and BAC-1 columns were installed in the injector via a two-hole ferrule or a "Y" splitter. The GC conditions required for the analysis are listed in Table 1.

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Experimental

Sample Preparation

An aliquot of the IPA sample was added to a 2 mL autosampler vial and placed into the integrated autosampler of the Clarus 690 Gas Chromatograph (GC).

Standard Preparation

The method of calibration of Isopropanol is the common weight percent method and utilizes the Not Less Than (NLT) technique.

Table 1: Chromatography conditions.

GC Parameters	
Instrument	PerkinElmer Clarus 690 GC
Carrier Gas	Helium
Columns	Elite-624 30 m 0.32 mm 1.8 µm N9316203 Elite-BAC1 30 m 0.32 mm 1.8 µm N9315071
Column Pneumatics	LCV: 35 cm/sec Split flow: 20 mL/min
Autosampler Parameters	
Syringe size	5 µL
Injection volume	1 µL
Injection speed	Normal
# of plunges	8
Pre-washes	0
Sample washes	2
Post washes	4
Viscosity delay	0
Injector Parameters	
Injector	Type: CAP Temp: 200° C
Detector Parameters	
Type	WR-FID
Temperature	230° C
Range	20
Att	0
Hydrogen	30 mL/min
Air	450 mL/min
Data rate	12.5 pt/sec
Oven Parameters	
Oven initial temperature	40° C
Oven initial hold	12 minutes
Ramp Rate	10 °C/minute
Final Temperature	240 °C
Final Time	10 minutes
Oven maximum	260° C
Equilibration time	0

$$\text{Result} = (R_U/R_T) \times 100$$

R_U = Peak response of isopropyl alcohol
R_T = Sum of all the peak responses

Acceptance criteria: NLT 99.0%

The GC conditions, following the USP protocol, are found in Table 1. A faster method was developed by PerkinElmer as an alternative using the same columns as shown later in this document.

Data Acquisition

Data acquisition and data processing was performed using TotalChrom™ chromatography data system (CDS) software.

Calibration

The target analytes for impurities in IPA are ethyl ether, acetone, diisopropyl ether, 1-propanol and 2-butanol at 0.1% each in IPA. (RS system suitability solution)

$$\text{Result} = (R_U/R_T) \times 100$$

R_U = Peak response of each individual impurity in the Sample Solution
R_T = Sum of all the peak responses in the Sample Solution

Suitability requirements

Resolution: NLT 1.5 between acetone and isopropyl alcohol

Relative standard deviation (% RSD): NMT 2.0% for the isopropyl alcohol peak

Tailing factor: NMT 2.0 for the isopropyl alcohol peak

Signal-to-noise ratio: NLT 10 for any of the following peaks:

ethyl ether, acetone, diisopropyl ether, 1-propanol, and 2-butanol

IPA RS impurities standards.

Results and Discussion

Since there are many organic compounds, and several co-elute, dual column confirmation was used to help prevent the reporting of false negatives. The forensic industry uses this technique for blood alcohol investigation.

The USP method uses a G43 (common name 624) phase as the reporting column for results and allows for a column of different polarity stationary phase for the confirmatory column. The BAC 1 phase was chosen for its chromatographs and ability to effectively separate these compounds. In addition, it is well vetted by the forensics industry as the reporting column for blood alcohol investigation.

The IPA sample and RS System suitability sample are shown below in figure 1 and 2.

Figure 1: HPLC grade IPA sample.

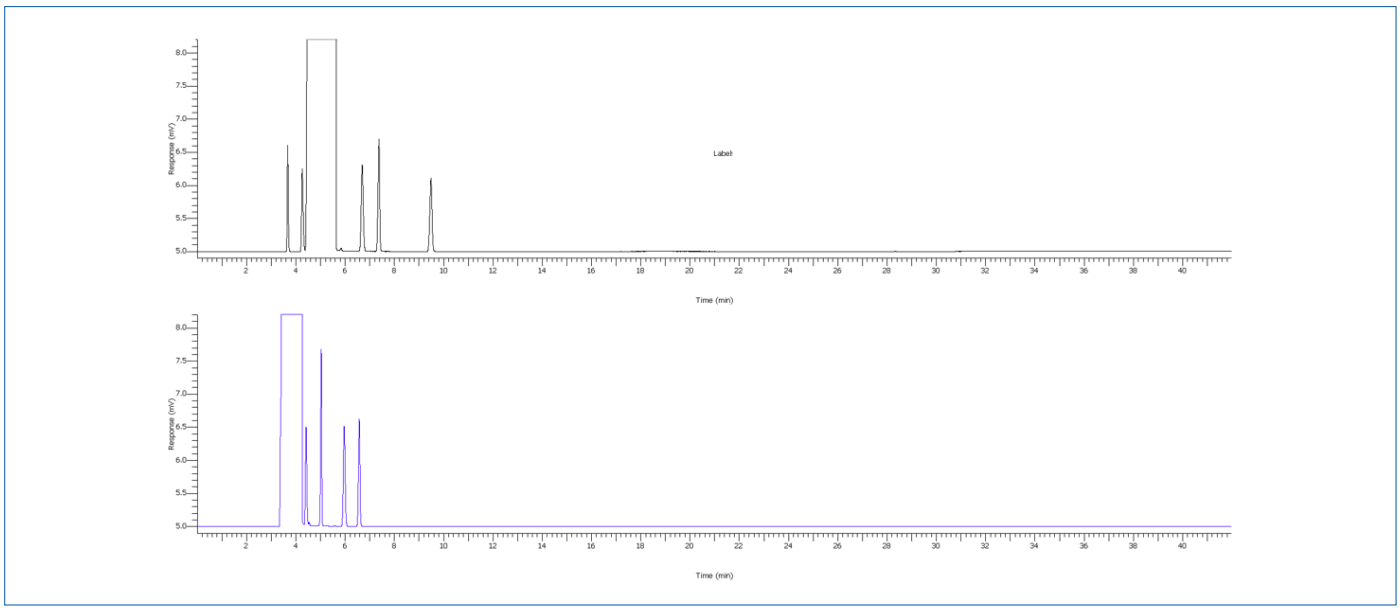


Figure 2: IPA RS System suitability standard at 0.1%.

Sample results

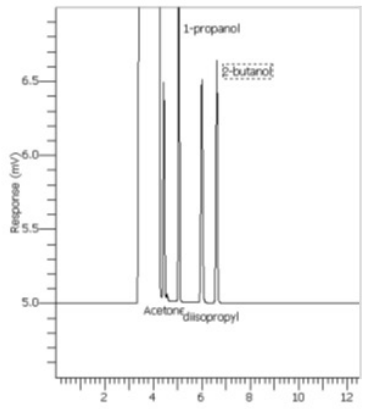
An IPA sample of unknown origin was analyzed to determine if it meets USP criteria of all target must be less than 0.1%. The sample passed with ease and the results/calculations are shown in Table 2.

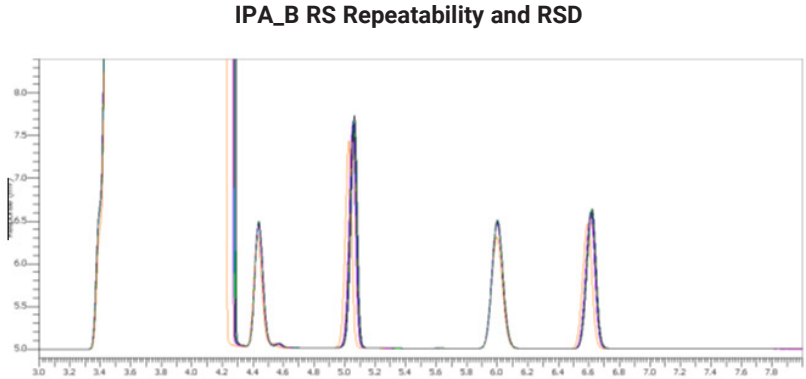
A set of 8 replicates were run to determine the repeatability as shown in Table 3.

Table 2: Calculation of impurities in IPA.

Target	Area	Area %	Spec
Ethyl Ether	0		0.10%
Acetone	293	0.007499	0.10%
Diisopropyl	0		0.10%
1-Propanol	390	0.009982	0.10%
2-Butanol	0		0.10%
Total area of all peaks	3906982		

Table 3: 8 replicates of IPA results.





	Acetone				1-propanol				diisopropyl				2-butanol			
File	Time	Area	Adjusted	Height	Time	Area	Adjusted	Height	Time	Area	Adjusted	Height	Time	Area	Adjusted	Height
Name	[min]	[µV·s]	Amount	[µV]	[min]	[µV·s]	Amount	[µV]	[min]	[µV·s]	Amount	[µV]	[min]	[µV·s]	Amount	[µV]
10212020_IPA_RS_Repeatability_B001.rst	4.44	5783.6	0.01	1480.68	5.06	8233.1	0.01	2722.41	6	7792.2	0.01	1503.62	6.62	7073.6	0.01	1637.41
10212020_IPA_RS_Repeatability_B002.rst	4.44	5660.5	0.01	1442.16	5.06	8056.9	0.01	2657.9	6	7631.2	0.01	1478.05	6.61	6919.6	0.01	1611.53
10212020_IPA_RS_Repeatability_B003.rst	4.44	5607.8	0.01	1430.42	5.05	8006.7	0.01	2637.49	6	7556	0.01	1470.7	6.61	6879.6	0.01	1601.36
10212020_IPA_RS_Repeatability_B004.rst	4.44	5701.9	0.01	1464.25	5.06	8127.4	0.01	2671.78	6	7675.7	0.01	1474.86	6.62	6982.1	0.01	1614.2
10212020_IPA_RS_Repeatability_B005.rst	4.44	5620.7	0.01	1445.08	5.06	8015.1	0.01	2630.04	6	7565.5	0.01	1466.79	6.61	6884.7	0.01	1594.57
10212020_IPA_RS_Repeatability_B007.rst	4.44	5563.9	0.01	1427.99	5.05	7932.1	0.01	2607.81	6	7488.8	0.01	1455.61	6.61	6812.8	0.01	1573.69
10212020_IPA_RS_Repeatability_B008.rst	4.44	5650.9	0.01	1436.02	5.06	8100.1	0.01	2674.54	6	7592.1	0.01	1468.18	6.62	6959.4	0.01	1608.04
10212020_IPA_RS_Repeatability_B009.rst	4.44	5749.1	0.01	1465.32	5.06	8162.9	0.01	2683.41	6	7731.7	0.01	1502.71	6.62	7009.9	0.01	1625
10212020_IPA_RS_Repeatability_B010.rst	4.44	5693.8	0.01	1457.63	5.06	8127	0.01	2676.76	6	7651.5	0.01	1480.61	6.62	6976.6	0.01	1615.45
Averages	4.44	5670.3	0.01	1449.95	5.06	8084.6	0.01	2662.46	6	7631.6	0.01	1477.9	6.62	6944.3	0.01	1609.03
%RSD	0.02	1.2	1.23	1.24	0.08	1.1	1.13	1.27	0.02	1.2	1.23	1.09	0.05	1.1	1.13	1.13

Table 4: Precision of Impurities in IPA.

Isopropanol		
Compound	**Action Limit (µL/L)**	**Precision% RSD**
Ethyl Ether	1000	1.0
Acetone	1000	1.7
Diisopropyl	1000	1.0
1-Propanol	1000	1.0
2-Butanol	1000	1.0

Summary part one

It was demonstrated that the PerkinElmer dual column FID Clarus 690 GC was able to easily analyze impurities in IPA using the USP method with excellent accuracy and selectivity for alcohols used for manufacturing hand sanitizer by USP methodology.

Alternate Method

Alternate Introduction

The analysis of isopropanol by the USP method as described above works well with good results. There are a few items that could be improved upon to increase lab productivity. In the analysis of isopropanol, all the target compounds elute within the first 10 minutes of a 42-minute run time. The function of the temperature ramp at the end of the run is to clean up the column for the next run. If we raise the oven ramp rate from 10 to 50C/min this will significantly reduce the total analysis time. Also, the oven ramp could start just after the elution of isopropanol as we have significant resolution of the remaining target analytes. The analysis can be reduced as much 7 times.

Alternate Experimental

The Instrument configuration is the same as described above.

Sample/Standard Preparation

Sample and Standard preparation was identical to the procedure above.

Chromatography Conditions

The chromatography column and condition are similar to those in Table 1 and only modified for improved speed or data quality. Changed parameters as compared to Table 1 are highlighted below in Table 5.

Table 5: Alternate instrument parameters for impurities in Isopropanol.

GC Parameters	
Instrument	PerkinElmer Clarus 690 GC
Carrier Gas	Helium
Columns	Elite-624 30 m 0.32 mm 1.8 µm N9316203 Elite-BAC1 30 m 0.32 mm 1.8 µm N9315071
Column Pneumatics	LCV: 40 cm/sec Split flow: 20 mL/min
Autosampler Parameters	
Syringe size	5 µL
Injection volume	1 µL
Injection speed	Normal
# of plunges	6
Pre-washes	2
Sample washes	2
Post washes	2
Viscosity delay	0
Injector Parameters	
Injector	Type: CAP Temp: 200° C
Detector Parameters	
Type	WR-FID
Temperature	230° C
Range	20
Att	-6
Hydrogen	30 mL/min
Air	450 mL/min
Data rate	12.5 pt/sec
Oven Parameters	
Oven initial temperature	40° C
Oven initial hold	5 minutes
Ramp Rate	70 °C/minute
Final Temperature	230 °C
Final Time	1 minute
Oven maximum	260° C
Equilibration time	0

Results and Discussion

The overall w/result and discussion of the alternate method is similar to the classic method as shown above. Most notable is the greatly reduced analysis time thus allowing greater laboratory sample throughput.

Figure 4 shows a clean IPA blank of both FID channels with interfering sample carryover for improved confidence of the reported results. While Figure 5 show excellent separation of the targeted IPA impurities in less than 5.8 minutes to allow a seven-fold decrease in the analysis run time.

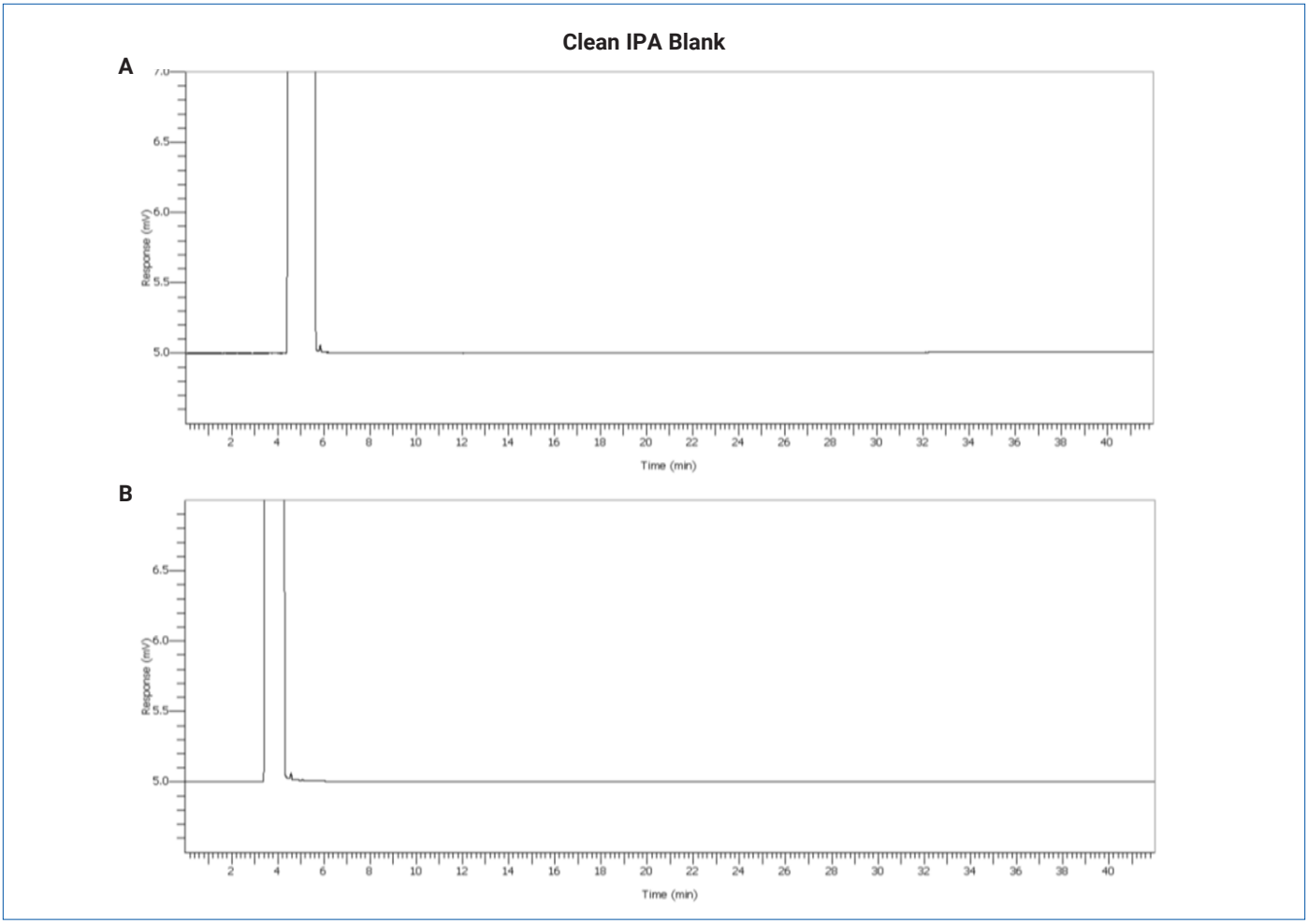


Figure 3: Chromogram of IPA blank.

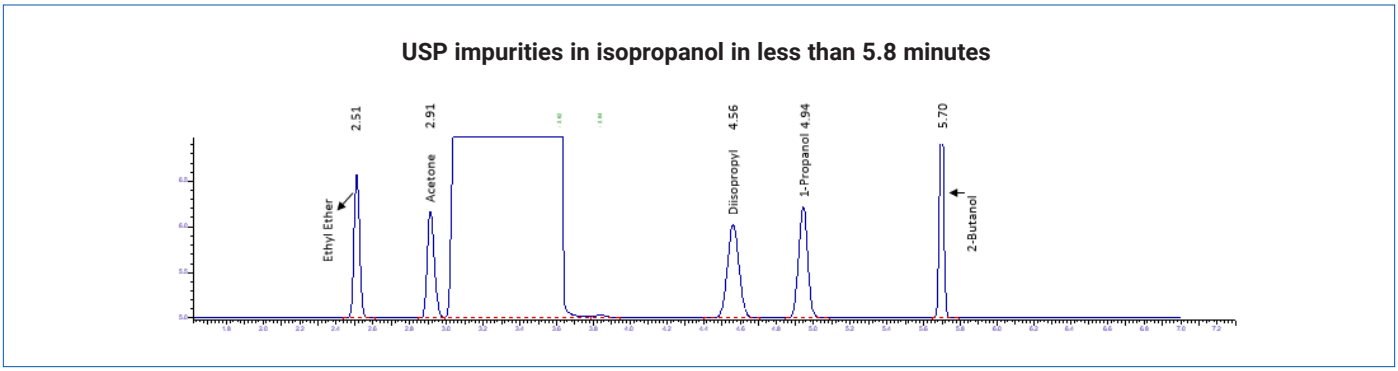
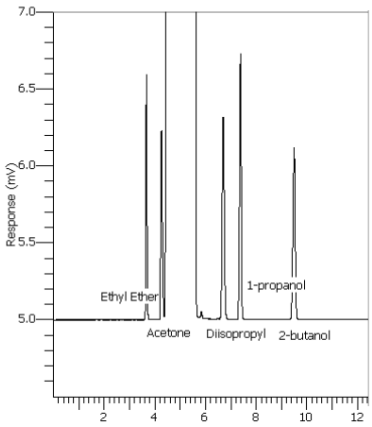
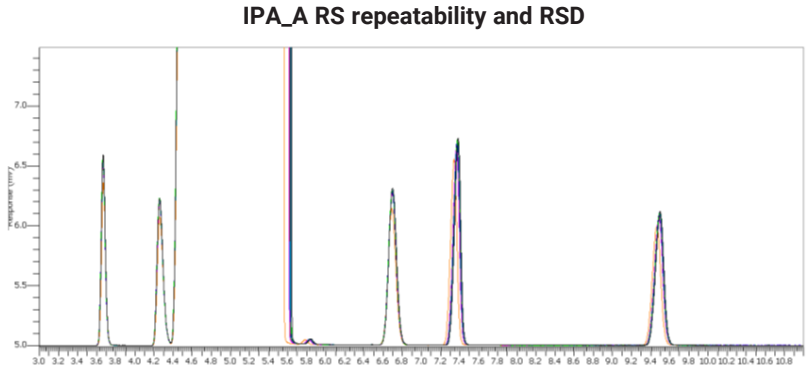


Figure 4: IPA impurities in less than 5.8 minutes using alternate analysis method.

Table 6 and 7 show excellent performance of this fast alternate analysis method on both instrument channels. The excellent repeatability is summarized in Table 8.

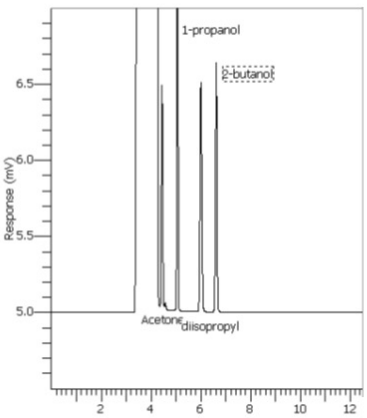
Table 6: Fast analysis method performance using column A.

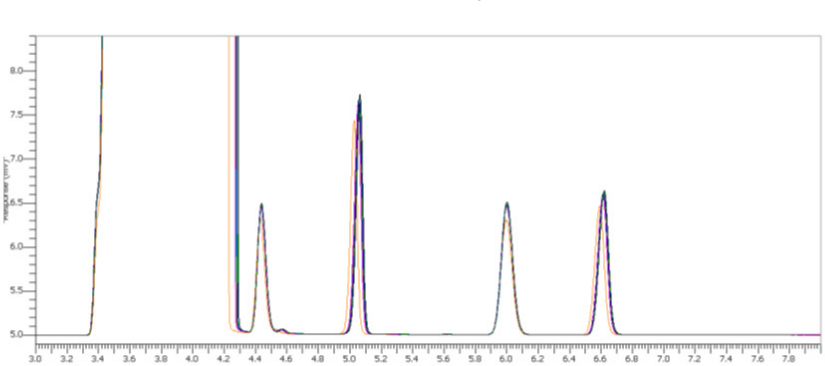




	Ethyl Ether				Acetone				Diisopropyl				1-propanol				2-butanol			
File	Time	Area	Adjusted	Height	Time	Area	Adjusted	Height	Time	Area	Adjusted	Height	Time	Area	Adjusted	Height	Time	Area	Adjusted	Height
Name	[min]	[µV·s]	Amount	[µV]	[min]	[µV·s]	Amount	[µV]	[min]	[µV·s]	Amount	[µV]	[min]	[µV·s]	Amount	[µV]	[min]	[µV·s]	Amount	[µV]
10212020_IPA_RS_Repeatability_A001.rst	3.67	4790.2	1006.21	1593.45	4.26	5504.3	1003.54	1227.65	6.7	7693.3	1009.69	1310.65	7.38	8125.8	1006.36	1727.27	9.5	6990.1	1013.41	1116.51
10212020_IPA_RS_Repeatability_A002.rst	3.67	4685.7	984.26	1561.69	4.26	5391.9	983.05	1209.58	6.7	7536.9	989.16	1280.31	7.37	7946.1	984.11	1686.05	9.49	6845.1	992.38	1075.55
10212020_IPA_RS_Repeatability_A003.rst	3.67	4645.4	975.79	1541.75	4.26	5341	973.76	1200.81	6.7	7468.1	980.14	1273.66	7.37	7903.2	978.8	1673.39	9.48	6803.6	986.37	1073.73
10212020_IPA_RS_Repeatability_A004.rst	3.67	4723.8	992.26	1563.23	4.26	5435.2	990.93	1217.92	6.7	7591.6	996.34	1287.04	7.38	8017.9	993	1697.06	9.5	6895.9	999.76	1092.55
10212020_IPA_RS_Repeatability_A005.rst	3.67	4650.5	976.86	1543.96	4.26	5347.3	974.91	1208.27	6.7	7480.6	981.77	1276.67	7.37	7917.5	980.56	1676.79	9.49	6803.7	986.38	1073.91
10212020_IPA_RS_Repeatability_A006.rst	3.67	4111.3	863.59	1360.6	4.26	4790.9	873.46	1071.93	6.69	6680.6	876.78	1141.47	7.34	7286.3	902.4	1554.62	9.46	6255.3	906.88	992.31
10212020_IPA_RS_Repeatability_A007.rst	3.67	4597.2	965.66	1531.02	4.26	5295.1	965.4	1195.55	6.7	7403.1	971.61	1265.94	7.37	7839.7	970.92	1671.34	9.49	6742.5	977.51	1081.14
10212020_IPA_RS_Repeatability_A008.rst	3.67	4658.8	978.6	1545.86	4.26	5376.5	980.24	1206.83	6.69	7501.4	984.5	1276.33	7.37	7981.2	988.45	1695.72	9.49	6863.8	995.1	1105
10212020_IPA_RS_Repeatability_A009.rst	3.67	4754.9	998.78	1588.01	4.26	5465.6	996.48	1233.25	6.7	7637.6	1002.38	1295.26	7.38	8054.4	997.52	1721.55	9.49	6926.8	1004.23	1103.21
10212020_IPA_RS_Repeatability_A010.rst	3.67	4675	982.02	1544.18	4.26	5401.6	984.81	1208.42	6.7	7554.5	991.47	1293.21	7.38	8014.5	992.58	1697.77	9.5	6889.9	998.88	1099.36
Averages	3.67	4629.3	972.4	1537.37	4.26	5334.9	972.66	1198.02	6.7	7454.8	978.38	1270.06	7.37	7908.7	979.47	1680.16	9.49	6801.7	986.09	1081.33
%RSD	0.03	4.1	4.12	4.25	0.03	3.8	3.76	3.82	0.04	3.8	3.82	3.7	0.15	3	2.95	2.85	0.11	3	3	3.2

Table 7: Fast analysis method performance using column B.





	Acetone				1-propanol				diisopropyl				2-butanol			
File	Time	Area	Adjusted	Height	Time	Area	Adjusted	Height	Time	Area	Adjusted	Height	Time	Area	Adjusted	Height
Name	[min]	[µV·s]	Amount	[µV]	[min]	[µV·s]	Amount	[µV]	[min]	[µV·s]	Amount	[µV]	[min]	[µV·s]	Amount	[µV]
10212020_IPA_RS_Repeatability_B001.rst	4.44	5783.6	0.01	1480.68	5.06	8233.1	0.01	2722.41	6	7792.2	0.01	1503.62	6.62	7073.6	0.01	1637.41
10212020_IPA_RS_Repeatability_B002.rst	4.44	5660.5	0.01	1442.16	5.06	8056.9	0.01	2657.9	6	7631.2	0.01	1478.05	6.61	6919.6	0.01	1611.53
10212020_IPA_RS_Repeatability_B003.rst	4.44	5607.8	0.01	1430.42	5.05	8006.7	0.01	2637.49	6	7556	0.01	1470.7	6.61	6879.6	0.01	1601.36
10212020_IPA_RS_Repeatability_B004.rst	4.44	5701.9	0.01	1464.25	5.06	8127.4	0.01	2671.78	6	7675.7	0.01	1474.86	6.62	6982.1	0.01	1614.2
10212020_IPA_RS_Repeatability_B005.rst	4.44	5620.7	0.01	1445.08	5.06	8015.1	0.01	2630.04	6	7565.5	0.01	1466.79	6.61	6884.7	0.01	1594.57
10212020_IPA_RS_Repeatability_B006.rst	4.43	5126.5	0.01	1298.3	5.03	7372.7	0.01	2429.13	6	6751.5	0.01	1303.99	6.59	6324.4	0.01	1461.77
10212020_IPA_RS_Repeatability_B007.rst	4.44	5563.9	0.01	1427.99	5.05	7932.1	0.01	2607.81	6	7488.8	0.01	1455.61	6.61	6812.8	0.01	1573.69
10212020_IPA_RS_Repeatability_B008.rst	4.44	5650.9	0.01	1436.02	5.06	8100.1	0.01	2674.54	6	7592.1	0.01	1468.18	6.62	6959.4	0.01	1608.04
10212020_IPA_RS_Repeatability_B009.rst	4.44	5749.1	0.01	1465.32	5.06	8162.9	0.01	2683.41	6	7731.7	0.01	1502.71	6.62	7009.9	0.01	1625
10212020_IPA_RS_Repeatability_B010.rst	4.44	5693.8	0.01	1457.63	5.06	8127	0.01	2676.76	6	7651.5	0.01	1480.61	6.62	6976.6	0.01	1615.45
Averages	4.44	5615.9	0.01	1434.78	5.06	8013.4	0.01	2639.13	6	7543.6	0.01	1460.51	6.61	6882.3	0.01	1594.3
%RSD	0.05	3.3	3.28	3.55	0.2	3	3.01	3.05	0.03	3.9	3.87	3.91	0.12	3	3.04	3.11

Table 8: Alternate results for Precision of Impurities in IPA.

Isopropanol	
Compound	**Precision% RSD**
Ethyl Ether	1.95
Acetone	1.69
Diisopropyl	1.42
1-Propanol	1.33
2-Butanol	1.31

Summary Part 2

The modified oven parameters enabled run times to be reduced from 42 minutes down to 5.8 minutes with great accuracy and precision. The GC system can run either the official extended method or the improved throughput method with a simple software method selection providing the best performance based on the laboratory current needs.

Reference

https://www.perkinelmer.com/libraries/APP-Hand-Sanitizer-LabelClaim

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