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Applikationsbericht

# Analysis of Residual Solvents in Hemp Oil Using Headspace Sampling and Atmospheric Pressure GC-MS/MS

Douglas M. Stevens

Waters Corporation

## Abstract

The extraction of active compounds from hemp plant material is achieved through various processes using a wide range of solvents. Because the extracts are commonly consumed directly, or used as ingredients in products consumed by humans, care must be taken to ensure the reduction or elimination of residual solvents that could cause negative effects on product quality or lead to unacceptable human exposure. In the past, gas chromatography (GC) flame ionization detection (FID) has been used for the analysis of residual solvents in plant extracts. However, as the ways in which hemp-derived products are consumed and the number of different regions in which those products have become legal has increased, there has been a trend towards lower reporting limits and longer lists of analytes than FID can achieve routinely. This has led to the increased use of GC mass spectrometry (MS) for this analysis. While traditional GC-MS employs electron ionization (EI), which occurs in a vacuum region of the mass spectrometer, this work describes an investigation of atmospheric pressure ionization GC-MS for the detection of residual solvents in hemp oil using an APGC (atmospheric pressure gas chromatography) source. The results obtained demonstrate the utility of headspace (HS) APGC-MS/MS for quantitative analysis of more than 20 common residual solvents using the same system also capable of analyzing for terpenes and pesticide residues in hemp samples.[1]


### Benefits

- By reducing the amount of matrix and analytes introduced to the system the sensitivity of APGC helps decrease maintenance frequency of both the MS and GC while still ensuring fit-for-purpose performance for the lowest reporting limits
- Automated HS sample extraction requires minimal sample preparation and also limits the amount of matrix loaded into the system
- A single MS is able to perform multiple quality and safety analyses for hemp-derived products minimizing operator training and bench space requirements

## Introduction

Around the world many government and professional organizations create regulatory and guidance methods for the analysis of residual solvents in plant material extracts with reporting limits from as low as 1 ppm and as high as 5000 ppm. The specific list of analytes in these methods varies based on the solvents commonly

used for extraction as well as analytes that may unintentionally be introduced to extracts as contaminants from the process equipment, packaging, storage, or the extraction solvent itself. It may also be necessary to monitor for residual solvents from the use of illicit extraction processes or solvents. For these reasons it is common for hemp oil and products containing hemp-derived ingredients to require quantitative screening for residual solvents.

The trend towards the use of GC-MS can be traced to the need for longer analyte lists and lower reporting limits. Those factors must be balanced against the need to keep the analysis time from becoming prohibitively long as well as the prospective need to incorporate additional compounds into the analysis as new regulations and guidelines are published.

Non-MS detection, such as GC-FID, is only able to distinguish between near eluting analytes based on limited criteria such as chromatographic separation and retention time while MS has the added potential of separating even co-eluting analytes in the mass (*m/z*) dimension. This property of MS allows the accurate and precise quantitative analysis of more analytes in the same or faster analysis time. The minimum headspace incubation time that achieves representative and reproducible quantitation of the entire analyte list is commonly the limiting consideration in analysis time rather than the speed of either the GC or MS. However, the use of headspace for the extraction of residual solvents from hemp oil samples has the advantages of limiting the amount of matrix loading into the GC-MS while efficiently extracting the target analytes and requiring minimal sample preparation. Previous work applying HS SPME (solid phase microextraction) and APGC-MS on QTof MS focused on qualitative identification of unknowns[2] rather than quantitative analysis. This work will evaluate the use of HS APGC-MS for the quantitative analysis of residual solvents in hemp oil. Figure 1 shows the chromatographic separation of the full analyte list for this method with analyte names appearing in Table 2.

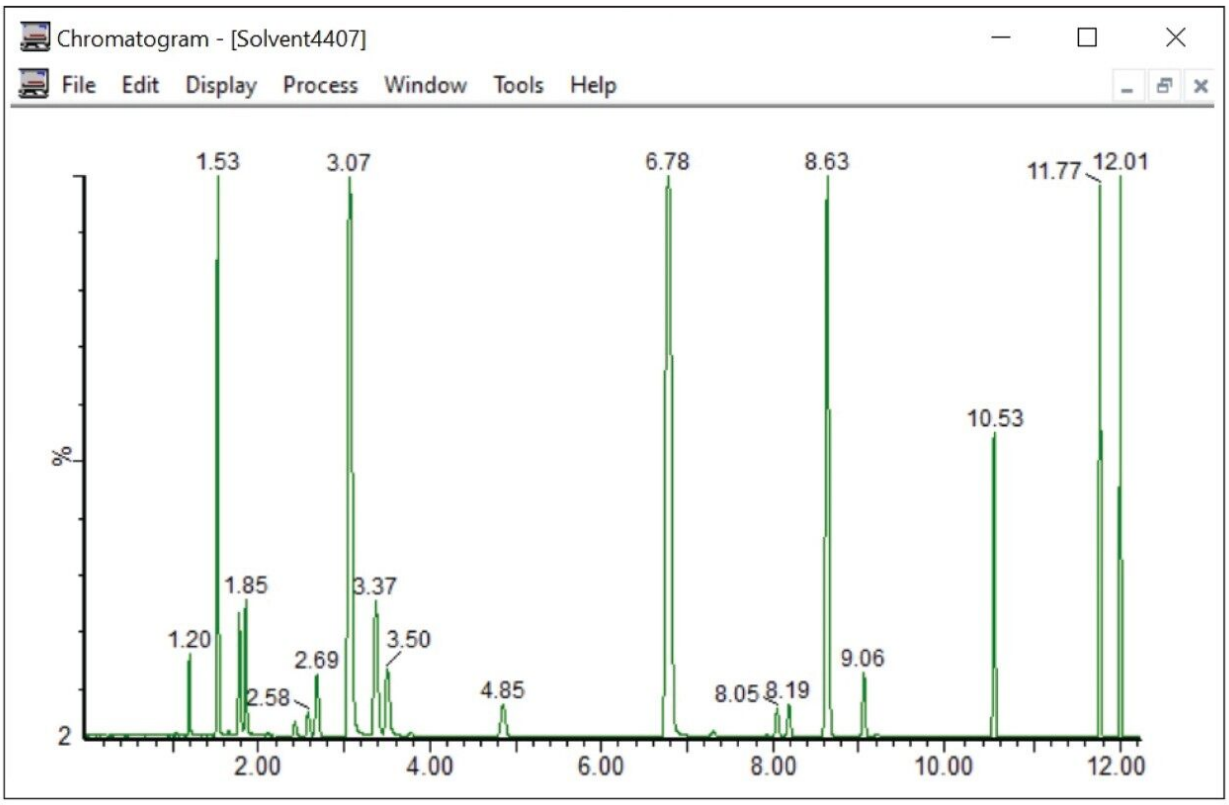



*Figure 1. Overlayed MRM chromatograms of residual solvents analyzed by HS APGC-MS/MS.*

## Experimental

### Sample Description

Hemp seed oil and a proficiency test (PT) sample, parts 54996 and 38554, were purchased from Absolute Standards (Hamden, CT USA). The standards, CA Residual Solvents Category I and CA Residual Solvents Category II (Z-G34-115300-03-5PAK, Z-G34-115301-03-5PAK) were purchased from CPI International (Santa Rosa, CA USA). The standards were initially combined into a single 1:10 dilution in n,n-dimethylacetamide (DMA, 38840-1L-F, Millipore Sigma, St. Louis, MO USA) followed by serial dilutions to produce a calibration curve at 0.32, 1.6, 8, 40, 200, and 1000 ppm for the Category II analytes and 0.0032 0.016, 0.08, 0.4, 2, and 10 ppm for the Category I analytes. Samples and standards were analyzed using 50 µL loaded directly into a 20 mL headspace vial. The proficiency test sample was diluted 1:10 in DMA prior to analysis.

## AS Conditions

| | |
|---|---|
| Autosampler: | CTC PAL3 RSI |
| Syringe: | 2.5 mL Smart Headspace Syringe at 105 °C |
| Incubation time: | 15 min at 250 rpm, On 5 s, Off 2s |
| Incubation temp.: | 80 °C |
| Injection volume: | 500 µL |
| GC cycle time: | 22 min |

## GC Conditions

| | |
|---|---|
| Gas chromatograph: | Agilent 7890B |
| Column: | Restek Rxi-624Sil MS, 30 m x 0.25 mm I.D. x 1.4 µm film |
| Column outlet: | 14 psi |
| Injection: | SSL at 225 °C, Split 50:1, Restek Topaz 1 mm I.D. liner |
| Carrier gas: | Helium at 1.5 mL/min, 25 cm/s linear velocity at 30 °C |
| Temperature program: | 30 °C for 6 min, ramp to 85 °C at 15°C/min, ramp to 260 °C at 35 °C/min and hold for 1.5 min |

## MS Conditions

| | |
|---|---|
| Mass spectrometer: | Xevo TQ-S micro |

| | |
|---|---|
| Source type: | APGC, dry source mode |
| Source temp.: | 120 °C |
| Transfer line temp.: | 260 °C |
| Corona current: | 2.0 µA |
| Auxiliary gas: | 100 L/hr |
| Cone gas: | 45 L/hr |
| Detector gain: | 0.3 |
| Cone voltage and collision energy: | See Table 2 |

### Data Management

| | |
|---|---|
| MS acquisition software: | MassLynx v4.2 SCN 1017 |
| Quantitation software: | TargetLynx XS |

## Results and Discussion

Due to the low molecular weight of the analytes in this method, it was anticipated that not all would generate a charge retaining fragment as is used in a typical MRM transition. Nine analytes yielded traditional precursor > fragment MRM transitions while the remaining 13 analytes required the use of precursor > precursor MRM transitions. An example of the sensitivity achieved for methanol, which was monitored using a precursor > precursor MRM transition is shown in Figure 2. All analytes, regardless of the type of transition used, were able to achieve fit-for-purpose sensitivity for reporting limits outlined in multiple methods.[3,4,5] Note that due to the coelution of para- and meta- xylene, the 22 analytes elute in 21 chromatographic peaks. Because xylene isomers are commonly reported as total xylenes, it is unnecessary to chromatographically

resolve the para and meta isomers. All analytes except ethylene oxide and acetonitrile had secondary MRM transitions available.

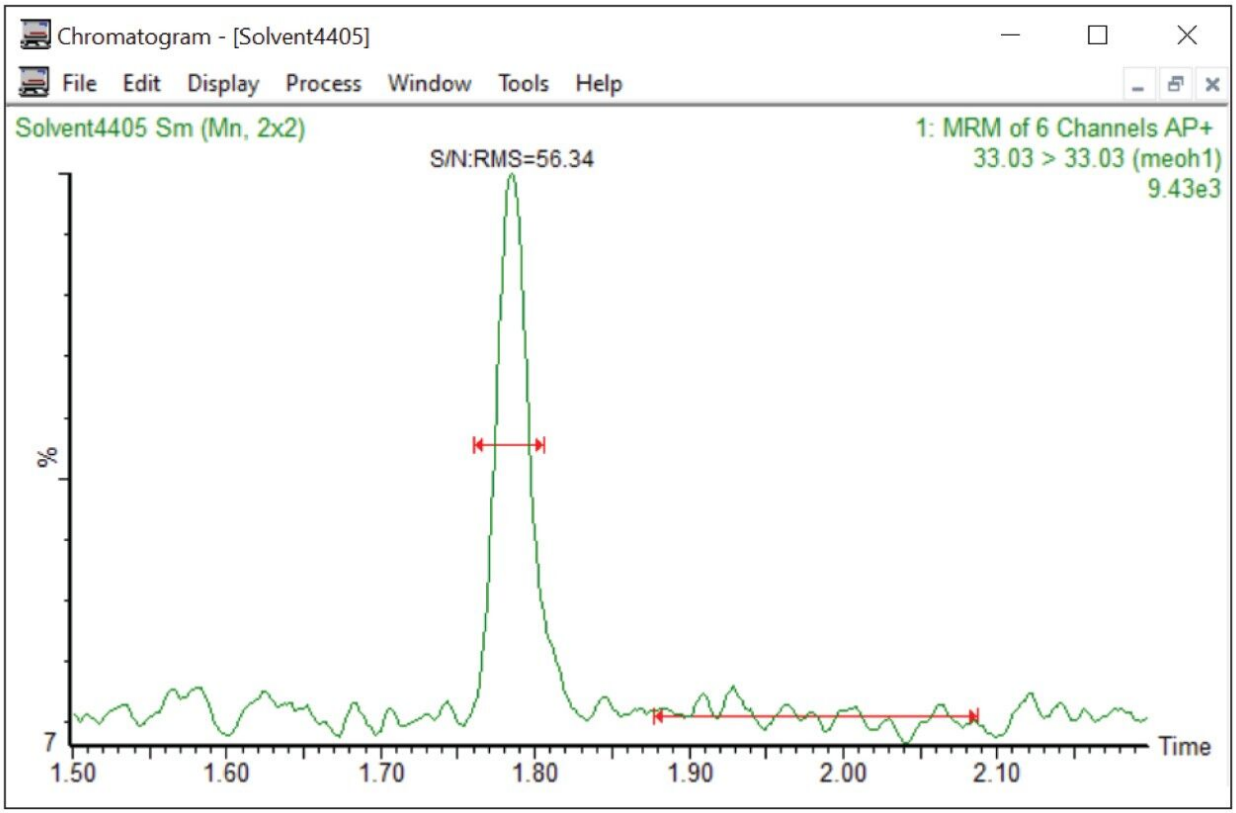



*Figure 2. Sensitivity evaluation for methanol at 40 ppm.*

Compounds targeted using precursor > product MRM transitions were quantified using the most intense transition with the secondary transition serving as a confirmatory ion as in Figure 3A. For compounds targeted based on precursor > precursor MRM transitions, the two most intense transitions were summed for quantitation as shown in Figure 3B. It is possible to sum these transitions and to also use one of the values as a qualifying ion with the corresponding ratio serving as an additional confirmatory measure.

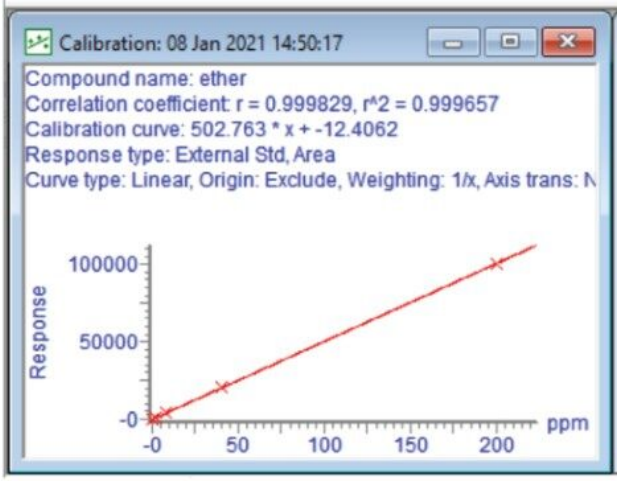
TargetLynx - Solventsfinal5

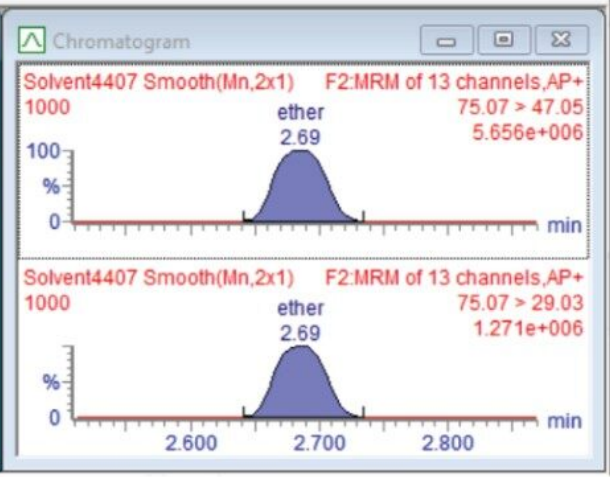
File Edit View Display Processing Window Help

ether

| | Name | Type | Std. Conc | RT | Area | Flags | ppm | %Dev |
|---|---|---|---|---|---|---|---|---|
| 2 | Solvent4402 | Standard | 0.3200 | 2.69 | 164.7 | bb | 0.35 | 10.1 |
| 3 | Solvent4403 | Standard | 1.6000 | 2.69 | 705.9 | bb | 1.43 | -10.7 |
| 4 | Solvent4404 | Standard | 8.0000 | 2.69 | 3912.7 | bb | 7.81 | -2.4 |
| 5 | Solvent4405 | Standard | 40.0000 | 2.69 | 20818.0 | bb | 41.43 | 3.6 |
| 6 | Solvent4406 | Standard | 200.0000 | 2.69 | 99987.1 | bb | 198.90 | -0.5 |

3A



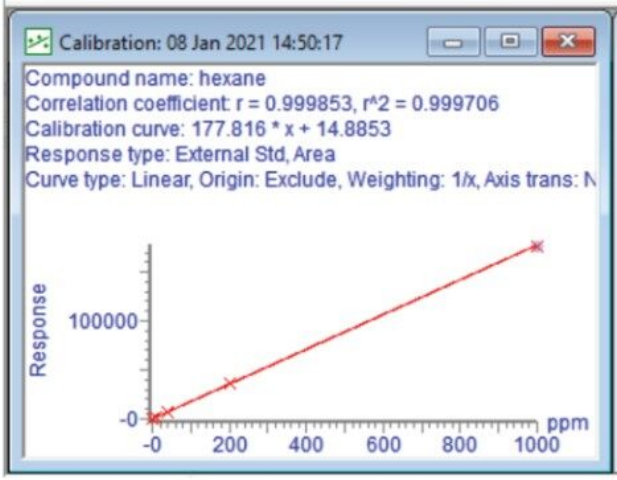

TargetLynx - Solventsfinal5

File Edit View Display Processing Window Help

hexane

| | Name | Type | Std. Conc | RT | Area | Flags | ppm | %Dev |
|---|---|---|---|---|---|---|---|---|
| 3 | Solvent4403 | Standard | 1.6000 | 4.86 | 267.8 | bb | 1.42 | -11.1 |
| 4 | Solvent4404 | Standard | 8.0000 | 4.85 | 1516.2 | bb | 8.44 | 5.5 |
| 5 | Solvent4405 | Standard | 40.0000 | 4.85 | 7347.7 | bb | 41.24 | 3.1 |
| 6 | Solvent4406 | Standard | 200.0000 | 4.85 | 36734.0 | bb | 206.50 | 3.3 |
| 7 | Solvent4407 | Standard | 1000.0000 | 4.85 | 176407.1 | bb | 991.99 | -0.8 |

3B



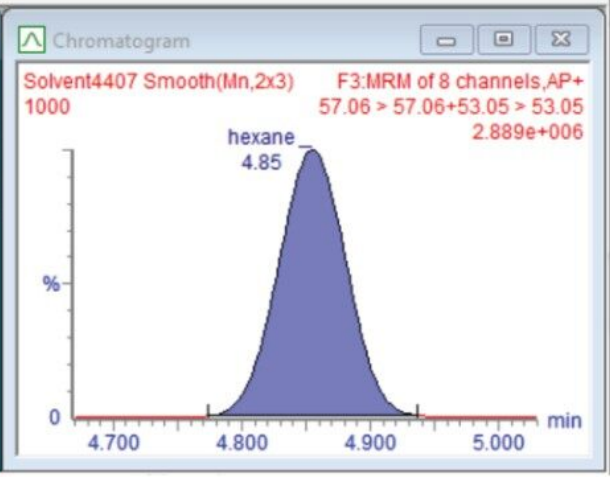



*Figure 3. Example calibration curves for diethyl ether (3A) and hexane (3B).*

All analytes were quantified using a linear calibration curve fit with 1/x weighting applied except for propane which had a response factor fit applied. A minimum $r^2$ value of 0.990 was achieved for all compounds with an average $r^2$ value of 0.9975 for all linear curves and 12% RSD for propane.

As expected, no analytes from this method were detected in the hemp oil. Therefore, in order to test the quantitative capability of the method, a hemp oil PT sample was also analyzed. All five analytes contained in the PT sample were detected using this method. Furthermore, the calculated concentrations were all within the reported Low and High Acceptance Limits. An example for trichloroethene is shown in Figure 4 where the average calculated concentration of 31.3 ppm is within 18% of the Assigned Value of 38.2 ppm for this compound. The acceptable range for this work is for calculated concentration to be within 40% of the Assigned Value leading to an acceptable reporting range of 22.9 to 53.5 ppm for trichloroethene. See Table 1 for all reported concentrations determined using this method as well as the upper and lower acceptable reporting values.

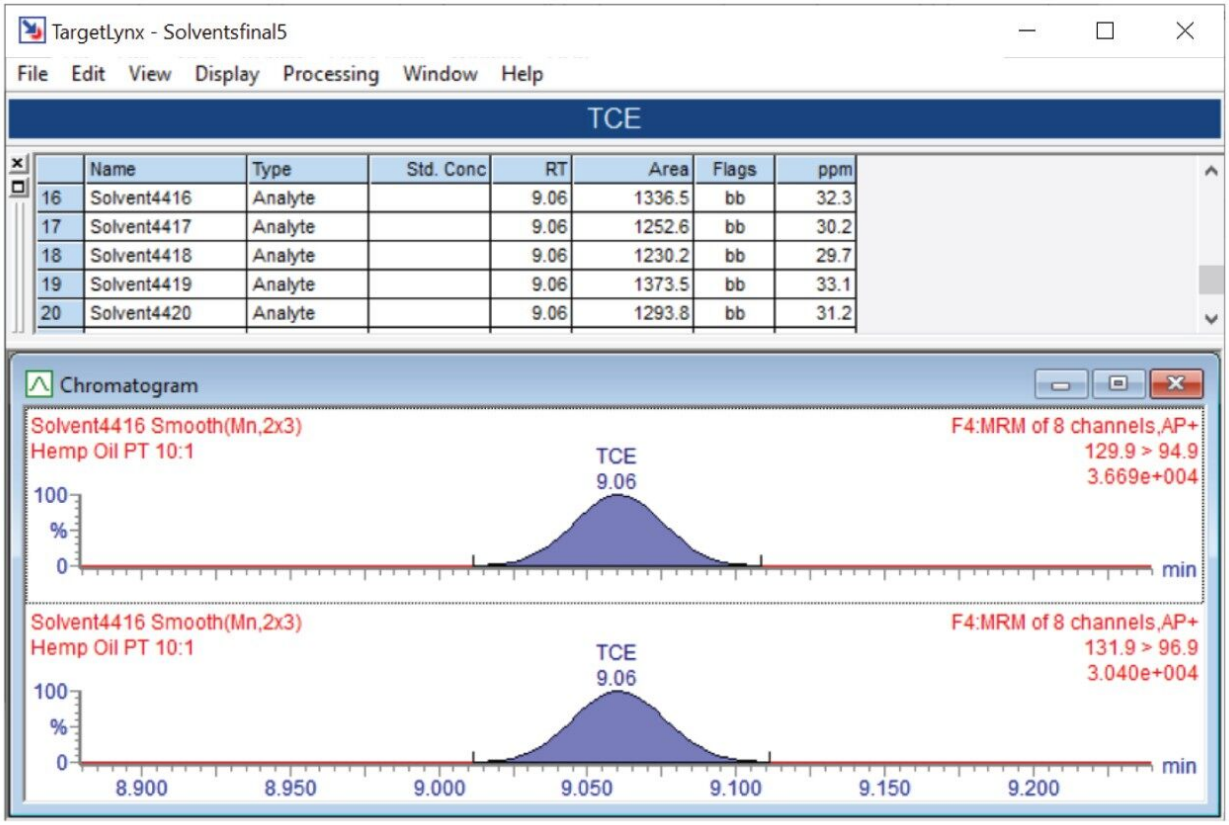



*Figure 4. Quantitation results for trichloroethene in hemp oil PT sample.*

## Table 1. Concentration Reporting Information

| Analyte | Study mean, assigned value | Lower acceptance limit | Upper acceptance limit | Calculated concentration |
|---|---|---|---|---|
| Trichloroethene | 38.2 | 22.9 | 53.5 | 31.3 |
| Methylene chloride | 12.0 | 7.2 | 16.8 | 9.3 |
| Ethylene oxide | 22.2 | 13.3 | 31.1 | 18.1 |
| 1,2-Dichloroethane | 28.2 | 16.9 | 39.5 | 20.7 |
| Chloroform | 17.2 | 10.3 | 24.1 | 11.2 |

Table 2. MS Experiment File Summary

| Function 1 10.00 to 2.20 min | Dwell (s) | Cone (V) | Collision energy (eV) | Analyte |
|---|---|---|---|---|
| 1 : 33.03 > 33.03 | 0.007 | 15 | 3 | methanol |
| 2 : 39.04 > 39.04 | 0.007 | 15 | 3 | propane |
| 3 : 45.04 > 45.04 | 0.007 | 15 | 3 | ethylene oxide |
| 4 : 53.05 > 53.05 | 0.007 | 15 | 3 | propane/butane |
| 5 : 57.06 > 57.06 | 0.007 | 15 | 3 | butane |
| | | | | |
| **Function 2 2.20 to 4.20 min** | | | | |
| 1 : 42.04 > 42.04 | 0.010 | 15 | 3 | acetonitrile |
| 2 : 45.04 > 45.04 | 0.010 | 15 | 3 | ethanol |
| 3 : 53.05 > 53.05 | 0.010 | 15 | 3 | pentane/ethanol/isopropanol |
| 4 : 59.06 > 31.03 | 0.010 | 15 | 6 | acetone |
| 5 : 59.06 > 43.04 | 0.010 | 15 | 10 | acetone |
| 6 : 59.06 > 59.06 | 0.010 | 15 | 3 | isopropanol |
| 7 : 71.07 > 71.07 | 0.010 | 15 | 3 | pentane |
| 8 : 75.07 > 29.03 | 0.010 | 15 | 8 | ether |
| 9 : 75.07 > 47.05 | 0.010 | 15 | 5 | ether |
| 10 : 82.90 > 82.90 | 0.010 | 15 | 3 | methylene chloride |
| 11 : 84.90 > 84.90 | 0.010 | 15 | 3 | methylene chloride |
| | | | | |
| **Function 3, 4.20 to 7.75 min** | | | | |
| 1 : 53.05 > 53.05 | 0.020 | 15 | 3 | hexane |
| 2 : 57.06 > 57.06 | 0.020 | 15 | 3 | hexane |
| 3 : 61.06 > 43.04 | 0.020 | 15 | 7 | ethyl acetate |
| 4 : 61.06 > 61.06 | 0.020 | 15 | 3 | ethyl acetate |
| 5 : 82.90 > 82.90 | 0.020 | 15 | 3 | chloroform |
| 6 : 84.90 > 84.90 | 0.020 | 15 | 3 | chloroform |
| | | | | |
| **Function 4, 7.75 to 10.00 min** | | | | |
| 1 : 39.04 > 39.04 | 0.020 | 15 | 3 | heptane |
| 2 : 57.06 > 57.06 | 0.020 | 15 | 3 | heptane |
| 3 : 61.90 > 61.90 | 0.020 | 15 | 3 | dichloroethane |
| 4 : 63.90 > 63.90 | 0.020 | 15 | 3 | dichloroethane |
| 5 : 78.08 > 52.05 | 0.020 | 20 | 12 | benzene |
| 6 : 78.08 > 63.06 | 0.020 | 20 | 15 | benzene |
| 7 : 129.90 > 94.90 | 0.020 | 15 | 16 | trichloroethene |
| 8 : 131.90 > 96.90 | 0.020 | 15 | 16 | trichloroethene |
| | | | | |
| **Function 5, 10.00 to 16.00 min** | | | | |
| 1 : 93.09 > 51.05 | 0.025 | 20 | 22 | toluene |
| 2 : 93.09 > 77.08 | 0.025 | 20 | 12 | toluene |
| 3 : 107.10 > 65.06 | 0.025 | 20 | 22 | xylenes |
| 4 : 107.10 > 91.09 | 0.025 | 20 | 12 | xylenes |
| 5 : 107.10 > 105.10 | 0.025 | 20 | 10 | xylenes |

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