# ASMS 2013

# MP-684

# Unknowns Analysis of Natural Products Using GC/Q-TOF and GC/IonTrap in Positive Electron Impact and Positive Chemical Ionization Modes with MS/MS

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## Introduction

Unknowns analysis of natural products is of interest not only for the elucidation of novel therapeutic compounds, but also for the identification of place of origin and potentially harmful contaminants. The use of Solid Phase Micro Extraction (SPME) and Large Volume Injection (LVI) will aid in sample preparation and facilitate an environmentally friendly approach that will eliminate non volatile matrix while providing maximum selective efficiency for the analytes of interest, which include aromatic compounds and flavonoids. A novel approach using high resolution GC/MS positive chemical ionization (PCI) and MS/MS will be shown. A GC/EI/CI-Q-TOF and GC/Ion Trap method was used for non-targeted analysis of natural products.

## Experimental

Various tea samples from India, South Africa and the United States were analyzed for unknown components. The samples were prepared in a vial for temperature programmed large volume injection (LVI) solvent vent, or Solid Phase Micro Extraction (SPME) using a selective adsorption fiber for potential analytes of interests including but not limited to flavonoids, polyphenols, environmental contaminants and pollutants. Employing a workflow of electron impact ionization after gas chromatographic separation, a preliminary identification via reference mass spectrum libraries was achieved. For analytes of interest tentatively identified by these libraries, positive chemical ionization was applied to obtained molecular weight information. High resolution and accurate mass spectra were obtained for identification of unknowns. The accurate mass GC/QTOF provided mass accuracies of less than 5ppm, which facilitated generating excellent molecular formula information about the unknown components. The workflow described provides a guideline for scientist interested in performing unknowns analysis using a GC/QTOF or GC/Ion Trap. Having the capabilities of high resolution mass spectrometry and accurate mass is essential for confidently identifying targeted and non-targeted unknowns.

The MS/MS capabilities of the instrument can be utilized to perform molecular structure elucidation using Agilent MassHunter Molecular Structure Correlator. The high resolving power of the time-of-flight (TOF) mass spectrometer was essential in being able to isolate analytes of interest from any additional potential matrix interference. From the performed experiments terpenes, flavonoids and polyphenols were identified in terms of molecular weight and structure to a very high degree of confidence.

## Experimental

### Method
The analytical method was developed on the Agilent 7890A GC and 7200 Q-TOF and 240 Ion Trap in positive electron impact and positive chemical ionization modes. Methane was used as the reagent gas on the GC/Q-TOF and acetonitrile on the GC/Ion Trap. A mid column backflush configuration was employed using a 5 m HP-5MS pre-column followed by a 20 m HP-5MS column connected via a helium purged ultimate union. The analysis time was less than 25 minutes. All of the analytes were monitored in their underivatized form after SPME or water LVI solvent vent. The extracted samples were analyzed by desorbing the SPME fiber onto the 260°C multi-mode inlet of the GC/Q-TOF and GC/Ion Trap systems in the pulsed splitless mode.

### Unknowns Analysis Software Workflow
• Analyze and perform deconvolution to create components
• Perform library matching on components, view component and library spectra
• View molecular structure vs. spectrum
• View ion peak shapes vs. component peak shape



**Figure 1.** Unknowns Analysis in India Tea via GC/Ion Trap
Top Sample was prepared with SPME. The screen capture shows the identification of Humulene, or α-caryophyllene, a naturally occurring monocyclic sesquiterpene. A flavoring agent which has an woody type odor.
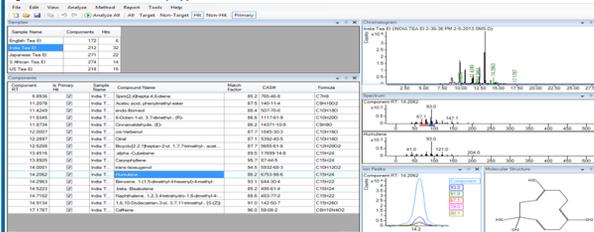


**Figure 2.** Unknowns Analysis in India Tea via GC/Ion Trap
Bottom Sample was prepared with LVI. The screen capture shows the identification of Eugenol a phenylpropene, an allyl chain-substituted guaiacol. It can be extracted from cinnamon and other aromatic spices.
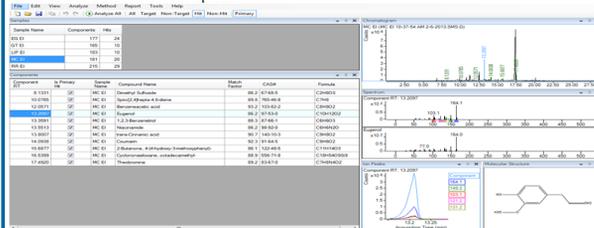


**Figure 3.** MassHunter Qualitative results of the peak at 13.2 min shown above with CI confirmation and library search by GC/Ion Trap

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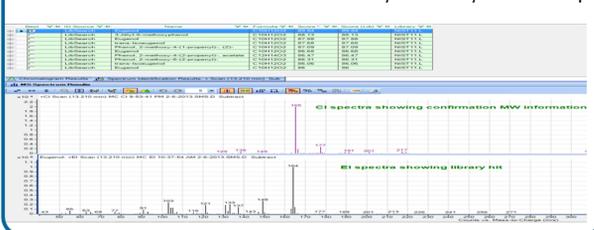
# Results and Discussion

**Figure 4.** Unknowns Analysis in S. African Tea via GC/Ion Trap
Top Sample was prepared with SPME. The screen capture shows the identification of Alpha terpineol, a kind of terpene alcohol of mixed of isomeric terpineol. Terpineol is usually a mixture of isomers with alpha-terpineol as the major constituent. Terpineol is a naturally occurring monoterpene alcohol that has been isolated from a variety of sources such as cajuput oil, pine oil, and petitgrain oil.
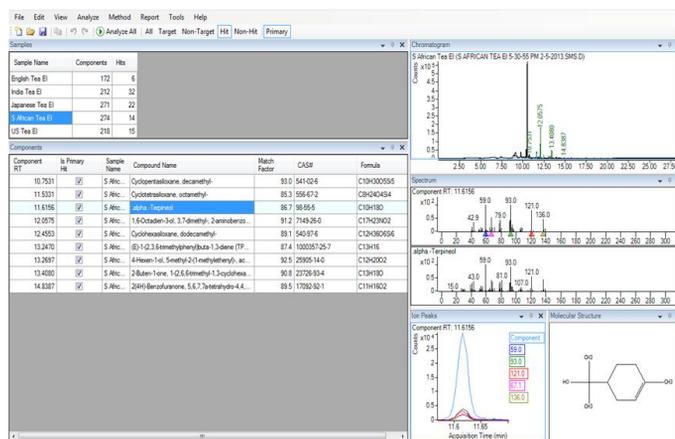


**Figure 5.** Unknowns Analysis in S. African Tea via GC/Ion Trap
Bottom Sample was prepared with LVI. The screen capture shows the identification of Vanillin a phenolic aldehyde, one of the most important aromatic flavor compounds used in foods.
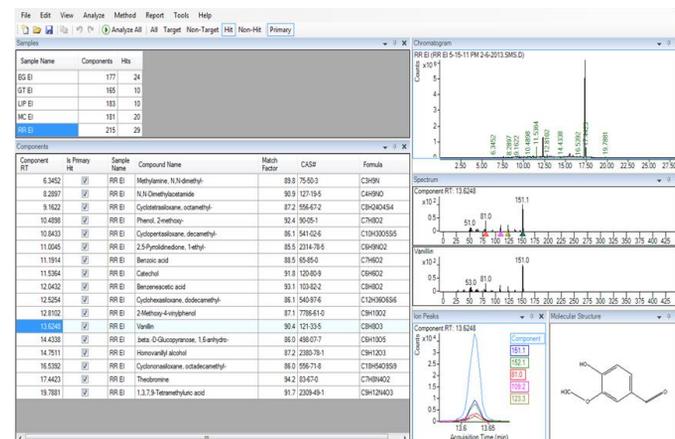


**Figure 6.** MassHunter Qualitative results of the peak at 13.624 min Vanillin shown above with CI confirmation and library search by GC/Ion Trap
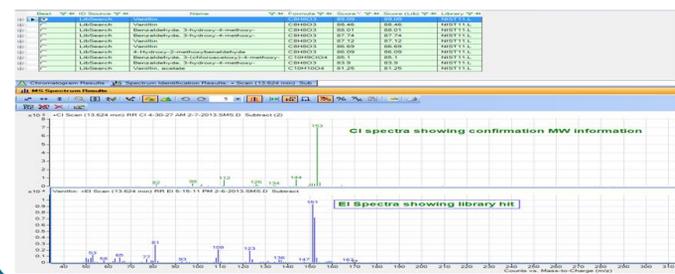


**Figure 7.** MassHunter Mass Calculator shown below allows for easy calculations of theoretical mass from proposed molecular formula of $C_{12}H_8O_4$ for Bergapten shown in Figure 9.
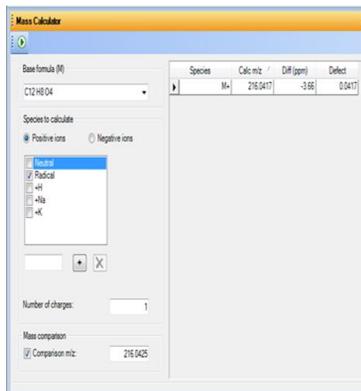


*Mass Accuracy 3.66ppm for Bergapten without IRM Correction*

**Figure 8.** MassHunter Formula Calculator shown below facilitates determination of molecular formula based on obtained accurate mass of 152.0471 for Vanillin shown in Figure 10.
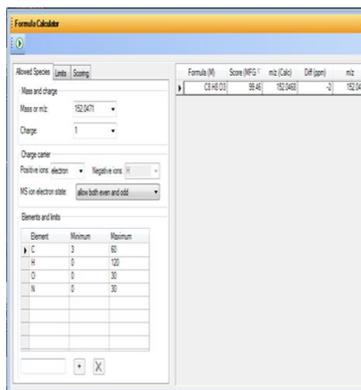


*Mass Accuracy 2.00ppm for Vanillin without IRM Correction*

It was interesting to see that all teas regardless of origin contained detectable levels of vanillin. However, only the tea From US origin contained traces of Bergaptan.

# Results and Discussion

**Figure 9.** Unknowns Analysis in US Tea via GC/QTOF (Non Polar Extraction) sample prepared by LVI

*Agilent MassHunter Unknowns Analysis - Unk2.uat*

File   Edit   View   Analyze   Method   Report   Tools   Help

Analyze All   All   Target   Non-Target   Hit   Non-Hit   Primary

**Samples**

| Sample Name | File Name | Components | Hits | Non-Target |
|---|---|---|---|---|
| EG_Hex | EG_Hex.D | 776 | 555 | 645 |
| EG_MeOH | EG_MeOH.D | 594 | 675 | 721 |

**Components**

| Component RT | Sample Name | Compound Name | Match Factor | CAS# | Formula |
|---|---|---|---|---|---|
| 15.8767 | EG_Hex | 2-Pentadecanone, 6,10,14-trimethyl- | 83.9 | 502-69-2 | C18H36O |
| 15.9178 | EG_Hex | 1H-Indene, 2,3-dihydro-1,1,3-trimethyl-3-phenyl- | 76.0 | 3910-35-8 | C18H20 |
| 15.9917 | EG_Hex | Cyclobutanol, 1-butyl- | 72.6 | 20434-34-8 | C8H16O |
| 16.0166 | EG_Hex | Caffeine | 91.5 | 58-08-2 | C8H10N4O2 |
| 16.1311 | EG_Hex | 1-(Hexahydropyridazin-3-ylidene)-3,3-dimethyl-butan-2-one | 52.2 | 1000194-96-3 | C13H21N2O |
| 16.1325 | EG_Hex | 2-deoc-Butoxycarbonylbenzoic acid | 75.6 | 1000371-67-0 | C12H14O4 |
| 16.1893 | EG_Hex | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | 77.7 | 102608-53-7 | C20H40O |
| 16.5428 | EG_Hex | Hexadecanoic acid, methyl ester | 80.4 | 112-39-0 | C17H34O2 |
| 16.7855 | EG_Hex | 2-Butanone | 55.3 | 78-93-3 | C4H8O |
| 16.8052 | EG_Hex | Pentanoic acid, cyclobutyl ester | 63.2 | 1000282-85-7 | C9H16O2 |
| 16.9181 | EG_Hex | Phthalic acid, 2-chloropropyl isobutyl ester | 77.8 | 1000356-82-4 | C15H19ClO4 |
| 16.9877 | EG_Hex | (-)-cis-2-Benzylaminocyclohexane-methanol | 52.2 | 71581-93-6 | C14H21NO |
| 17.0649 | EG_Hex | Isophthalic acid, di(2-fluorophenyl) ester | 67.5 | 1000344-66-4 | C20H12F2O4 |
| 17.1702 | EG_Hex | 2H-1-Benzopyran-2-one, 5,7-dimethoxy- | 93.5 | 487-06-9 | C11H10O4 |
| 17.2123 | EG_Hex | 4-Octanone | 71.3 | 589-63-9 | C8H16O |
| 17.6286 | EG_Hex | 2-Methyloxycarbonyl-6-methoxyanthracene | 53.2 | 297144-04-8 | C17H14O3 |
| 17.6912 | EG_Hex | s-Indacene-1,7-dione, 2,3,5,6-tetrahydro-3,3,5,5-tetramethyl- | 56.1 | 55591-17-8 | C16H18O2 |
| 17.8256 | EG_Hex | 7H-Furo[3,2-g][1]benzopyran-7-one, 4-methoxy- | 95.5 | 484-20-8 | C12H8O4 |
| 17.9076 | EG_Hex | Fluoranthene | 85.0 | 206-44-0 | C16H10 |
| 17.9217 | EG_Hex | Cyclohexene, 1-nonyl- | 66.4 | 15232-88-9 | C15H28 |
| 17.9600 | EG_Hex | trans-13-Octadecenoic acid, methyl ester | 82.6 | 1000333-61-3 | C19H36O2 |
| 17.9745 | EG_Hex | 1,3-Cyclohexadiene, 5-butyl- | 64.8 | 30168-57-1 | C10H16 |
| 18.0602 | EG_Hex | Phytol | 83.2 | 150-86-7 | C20H40O |
| 18.1388 | EG_Hex | Methyl stearate | 58.3 | 112-61-8 | C19H38O2 |
| 18.3368 | EG_Hex | Pyrene | 56.7 | 129-00-0 | C16H10 |
| 18.9538 | EG_Hex | 2,8-Dislatricyclo[7.3.0.0(3,7)]dodeca-4,6,10,12-tetraene, 2,2,8... | 57.5 | 1000163-56-4 | C20H26S2 |
| 19.1584 | EG_Hex | 7H-Furo[3,2-g][1]benzopyran-7-one, 4,9-dimethoxy- | 69.9 | 482-27-9 | C13H10O5 |
| 19.1908 | EG_Hex | 3,5-Dimethyldodecane | 63.3 | 107770-99-0 | C14H30 |
| 19.3939 | EG_Hex | Sulfurous acid, 2-ethylhexyl hexyl ester | 61.4 | 1000309-20-2 | C14H30O3S |
| 21.1378 | EG_Hex | Phthalic acid, octyl 2-propylpentyl ester | 79.5 | 1000377-92-4 | C24H38O4 |
| 21.2540 | EG_Hex | Hexadecanoic acid, cyclohexyl ester | 67.4 | 1673-08-1 | C22H42O2 |
| 22.1366 | EG_Hex | Cyclobutanol, 1-butyl- | 58.0 | 20434-34-8 | C8H16O |
| 23.3423 | EG_Hex | Squalene | 90.6 | 111-02-4 | C30H50 |
| 23.9649 | EG_Hex | 3-Bromo-dodecane | | | |

**Chromatogram** — EG_Hex (EG_Hex.D)

**Spectrum** — Component RT: 17.8256

216.0425 ; 145.0289 ; 89.0391

7H-Furo[3,2-g][1]benzopyran-7-one, 4-methoxy- : 216.0 ; 38.0 ; 63.0 ; 89.0 ; 145.0 ; 173.0 ; 201.0

**Ion Peaks** — Component RT: 17.8256
216.0425 ; 173.0239 ; 145.0289 ; 201.0188 ; 188.0469

**Molecular Structure**

Bergapten (5-methoxypsoralen) is a psoralen/furocoumarin found in bergamot essential oil, citrus essential oils, and in grapefruit juice.

**Figure 10.** Unknowns Analysis in US Tea via GC/QTOF (Polar Extraction) sample prepared by LVI

*Agilent MassHunter Unknowns Analysis - Unk2.uat*

File   Edit   View   Analyze   Method   Report   Tools   Help

Analyze All   All   Target   Non-Target   Hit   Non-Hit   Primary

**Samples**

| Sample Name | File Name | Components | Hits | Non-Target |
|---|---|---|---|---|
| EG_Hex | EG_Hex.D | 776 | 555 | 645 |
| EG_MeOH | EG_MeOH.D | 594 | 675 | 721 |

**Components**

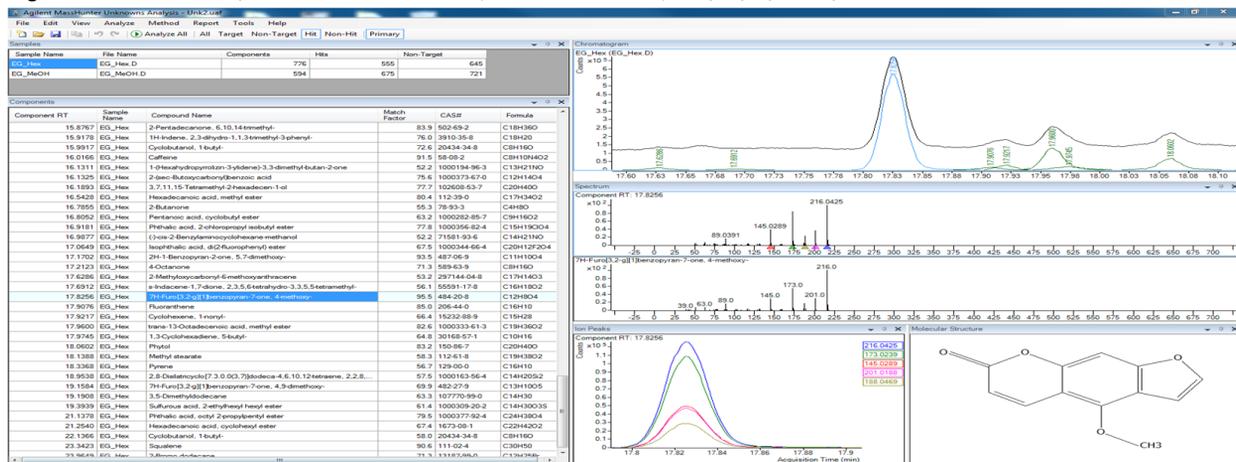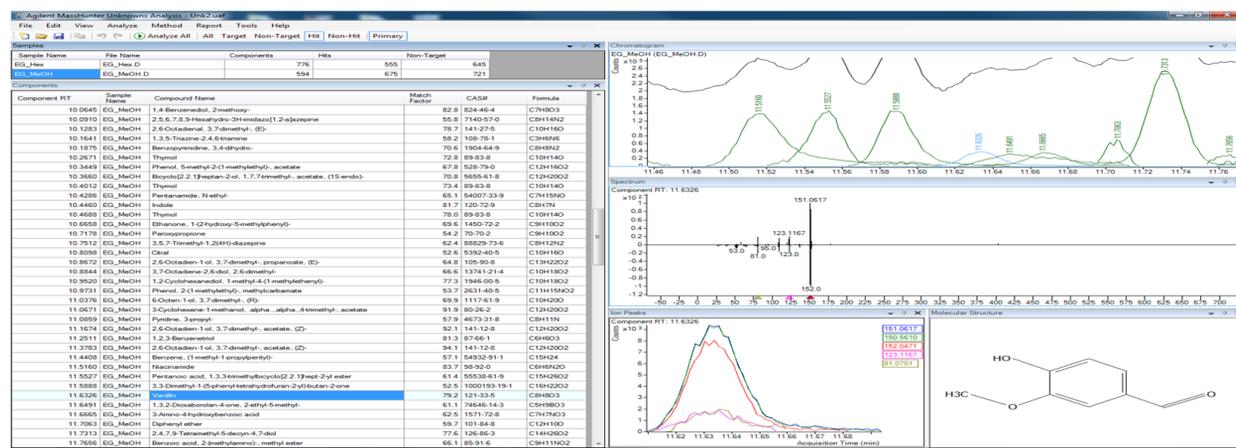| Component RT | Sample Name | Compound Name | Match Factor | CAS# | Formula |
|---|---|---|---|---|---|
| 10.0645 | EG_MeOH | 1,4-Benzenediol, 2-methoxy- | 82.8 | 824-46-4 | C7H8O3 |
| 10.0910 | EG_MeOH | 2,5,6,7,8,9-Hexahydro-3H-imidazo[1,2-a]azepine | 55.8 | 7140-57-0 | C8H14N2 |
| 10.1283 | EG_MeOH | 2,6-Octadienal, 3,7-dimethyl-, (E)- | 78.7 | 141-27-5 | C10H16O |
| 10.164 | EG_MeOH | 1,3,5-Triazine-2,4,6-triamine | 58.2 | 108-78-1 | C3H6N6 |
| 10.1875 | EG_MeOH | Benzopyrimidine, 3,4-dihydro- | 70.6 | 1904-64-9 | C8H8N2 |
| 10.2671 | EG_MeOH | Thymol | 72.8 | 89-83-8 | C10H14O |
| 10.3449 | EG_MeOH | Phenol, 5-methyl-2-(1-methylethyl)-, acetate | 67.8 | 528-79-0 | C12H16O2 |
| 10.3660 | EG_MeOH | Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate, (1S-endo)- | 70.8 | 5655-61-8 | C12H20O2 |
| 10.4012 | EG_MeOH | Thymol | 73.4 | 89-83-8 | C10H14O |
| 10.4286 | EG_MeOH | Pentanamide, N-ethyl- | 65.1 | 54007-33-9 | C7H15NO |
| 10.4460 | EG_MeOH | Indole | 81.7 | 120-72-9 | C8H7N |
| 10.4688 | EG_MeOH | Thymol | 78.0 | 89-83-8 | C10H14O |
| 10.6658 | EG_MeOH | Ethanone, 1-(2-hydroxy-5-methylphenyl)- | 69.6 | 1450-72-2 | C9H10O2 |
| 10.7178 | EG_MeOH | Paroxypropione | 54.2 | 70-70-2 | C9H10O2 |
| 10.7512 | EG_MeOH | 3,5,7-Trimethyl-1,2(4H)-diazepine | 62.4 | 88829-73-6 | C8H12N2 |
| 10.8098 | EG_MeOH | Citral | 52.6 | 5392-40-5 | C10H16O |
| 10.8672 | EG_MeOH | 2,6-Octadien-1-ol, 3,7-dimethyl-, propanoate, (E)- | 64.8 | 105-90-8 | C13H22O2 |
| 10.8844 | EG_MeOH | 3,7-Octadiene-2,6-diol, 2,6-dimethyl- | 66.6 | 13741-21-4 | C10H18O2 |
| 10.9520 | EG_MeOH | 1,2-Cyclohexanediol, 1-methyl-4-(1-methylethenyl)- | 77.3 | 1946-00-5 | C10H18O2 |
| 10.9731 | EG_MeOH | Phenol, 2-(1-methylethyl)-, methylcarbamate | 53.7 | 2631-40-5 | C11H15NO2 |
| 11.0376 | EG_MeOH | 6-Octen-1-ol, 3,7-dimethyl-, (R)- | 69.9 | 1117-61-9 | C10H20O |
| 11.0671 | EG_MeOH | 2-Cyclohexene-1-methanol, .alpha.,.alpha.,4-trimethyl-, acetate | 91.9 | 80-26-2 | C12H20O2 |
| 11.0859 | EG_MeOH | Pyridine, 3-propyl- | 57.9 | 4673-31-8 | C8H11N |
| 11.1674 | EG_MeOH | 2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)- | 92.1 | 141-12-8 | C12H20O2 |
| 11.2511 | EG_MeOH | 1,2,3-Benzenetriol | 81.3 | 87-66-1 | C6H6O3 |
| 11.3783 | EG_MeOH | 2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)- | 94.1 | 141-12-8 | C12H20O2 |
| 11.4408 | EG_MeOH | Benzene, (1-methyl-1-propylpentyl)- | 57.1 | 54932-91-1 | C15H24 |
| 11.5160 | EG_MeOH | Nacinamide | 83.7 | 98-92-0 | C6H6N2O |
| 11.5527 | EG_MeOH | Pentanoic acid, 1,3,3-trimethylbicyclo[2.2.1]hept-2-yl ester | 61.4 | 55538-61-9 | C15H26O2 |
| 11.5888 | EG_MeOH | 3,3-Dimethyl-1-(5-phenyl-tetrahydrofuran-2-yl)-butan-2-one | 52.5 | 1000193-19-1 | C16H22O2 |
| 11.6326 | EG_MeOH | Vanillin | 79.2 | 121-33-5 | C8H8O3 |
| 11.6491 | EG_MeOH | 1,3,2-Dioxaborolan-4-one, 2-ethyl-5-methyl- | 61.1 | 74646-14-3 | C5H9BO3 |
| 11.6665 | EG_MeOH | 3-Amino-4-hydroxybenzoic acid | 62.5 | 1571-72-8 | C7H7NO3 |
| 11.7063 | EG_MeOH | Diphenyl ether | 59.7 | 101-84-8 | C12H10O |
| 11.7313 | EG_MeOH | 2,4,7,9-Tetramethyl-5-decyn-4,7diol | 77.6 | 126-86-3 | C14H26O2 |
| 11.7656 | EG_MeOH | Benzoic acid, 2-(methylamino)-, methyl ester | 66.1 | 85-91-6 | C9H11NO2 |

**Chromatogram** — EG_MeOH (EG_MeOH.D)

**Spectrum** — Component RT: 11.6326
151.0617 ; 123.1167 ; 152.0

**Ion Peaks** — Component RT: 11.6326
151.0617 ; 150.5610 ; 123.1167 ; 81.0701

**Molecular Structure**

Vanillin is a phenolic aldehyde, an organic compound with the molecular formula $C8H8O3$. Its functional groups include aldehyde, ether, and phenol. It is the primary component of the extract of the vanilla bean. Synthetic vanillin, instead of natural vanilla extract, is sometimes used as a flavoring agent in foods, beverages, and pharmaceuticals.

# Conclusions

The analysis of natural products using these various GC/MS techniques allows for the identification of unknown components using Agilent MassHunter Unknowns Analysis software. The flexibility of LVI and SPME facilitates sample preparation prior to analysis in the mass spectrometer. Employing the additional capabilities of the Agilent 7200 Accurate Mass Q-TOF GC/MS including fast data acquisition, Chemical Ionization and MS/MS generates greater confidence in the results obtained when analyzing unknown components in natural products. MassHunter Workstation software is a complete package that allows for chromatographic and spectral deconvolution, library identification and fast analysis of results via its intuitive interface across all GC/MS platforms.