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應用手冊

Exact Molecular Mass Determination of Polar Plant Metabolites Using GCT with Chemical Ionization

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Abstract

This application note describes the use of chemical ionization on a Waters Micromass GCT orthogonal acceleration time of flight (oa-TOF) mass spectrometer for the exact mass determination of a range of non-volatile polar compounds.

Benefits

The oa-TOF GCT Mass Spectrometer allows routine exact mass measurements of <5 ppm RMS to be readily obtained

Introduction

Due to the large diversity of physical and chemical properties of phytochemicals in the plant kingdom, a number of techniques are required for their analysis. GC-MS has traditionally been associated with the analysis of volatile species. Non-volatiles need to be derivatized, however, derivatization may not achieve volatility for all compounds (e.g., flavonoidglycosides), introducing additional complexity. Metabolite identification by GC-MS is routinely carried out by the comparison of EI spectra to spectral libraries such as NIST. However, many components in GC-MS plant extracts are either absent from the library, or the EI spectra are dominated by derivatized groups, thus making de novoidentification of unknown peaks difficult. If derivatization is successful and the analyte is volatilized, it must remain energetically stable enough to be detected, otherwise the compound will fragment and molecular weight information may be lost, thereby complicating identification.

Chemical ionization (CI) is an alternative mode of detection and is a less energetic process. CI often results in the formation of species with reduced fragmentation, allowing access to molecular ion information.

This Application Note describes the use of CI on a Waters Micromass GCT orthogonal acceleration time of flight (oa-TOF) Mass Spectrometer for the exact mass determination of a range of non-volatile polar compounds.

GC-MS with Trimethylsilylation

Plant metabolites such as sugars, amino acids and hydroxyacids are generally not directly amenable to GC-MS and require derivatization prior to analysis. The method used here is a two-stage derivatization process whereby carbonyl groups are protected by methoximation and acidic protons silylated with MSTFA. The derivatization scheme outlined was used for the analysis of a QC standard sample and the methodology was then applied to the analysis of a dried aqueous polar extract from tomato fruit.

Experimental

Sample Derivatization

Three dried QC samples and a dried polar extract from a tomato fruit were each derivatized with 20 µL methoxyaminehydrochloride (40 mg/mL in pyridine) at 28 °C for 90 minutes, followed by the addition of 180 µL of N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) at 37 °C for 30 minutes.

GC Conditions

GC:	HP 6890
Column:	J&W Scientific, DB5-MS 20 m x 180 µm ID x 0.18 µm film
Carrier gas:	Constant flow 0.8 mL/min. helium
Injection volume:	1 µL, split 10:1
Injector temp.:	250 °C
Temperature Ramp:	85 °C for 2 min., ramp to 320 °C at 15 °C/min., hold for 5 min.
Solvent delay:	4.3 min.

MS Conditions

Ionization:	CI positive (ammonia reagent gas)
Acquisition range:	85 to 700 Da
Scan time:	0.25 s

Inter-scan delay:	0.05 s
Lock reference:	Chloropentafluorobenzene
Lock mass:	*m/z* 201.9609

Results and Discussion

QC Standards

A representative TIC chromatogram from a QC standard is shown in Figure 1 and the exact mass measurements obtained from the standards are tabulated below in Figure 2.

The GCT is a time-of-flight (TOF) mass detector and provides an elevated resolution of 7,000 (FWHM). The system is capable of mass measurement accuracies of <5 ppm RMS in both EI and CI modes of operation. The results below show that this mass accuracy can be readily achieved with RMS errors of <3 ppm being observed for the pseudo-molecular ions of the polar compounds present in the QC standard mix samples.

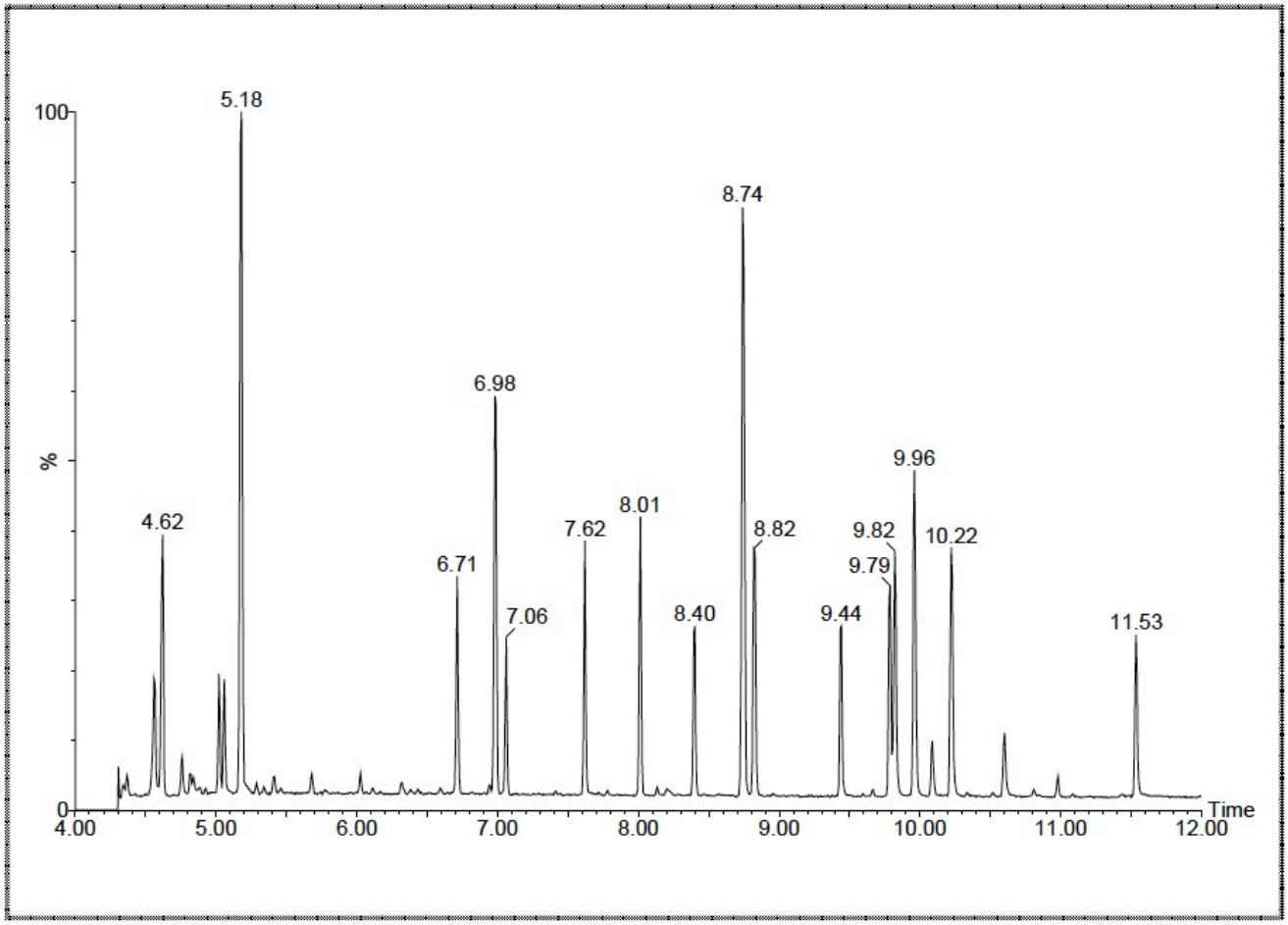

Figure 1. TIC from CI analysis of QC standard sample.

Component Name	RT mins	TMS Derivative $[M+H]^+$	Calculated Mass $[M+H]^+$	Standard Sample QC1			Standard Sample QC2			Standard Sample QC3		
				Measured Mass $[M+H]^+$	Error mDa	Error ppm	Measured Mass $[M+H]^+$	Error mDa	Error ppm	Measured Mass $[M+H]^+$	Error mDa	Error ppm
Urea	4.62	$C_7H_{21}N_2OSi_2$	205.1192	205.1191	-0.1	-0.7	205.1197	0.5	2.2	205.1194	0.2	0.8
Isoleucine	5.02	$C_{12}H_{30}NO_2Si_2$	276.1815	276.1823	0.8	2.9	276.1824	0.9	3.2	276.1825	1.0	3.6
Leucine	5.06	$C_{12}H_{30}NO_2Si_2$	276.1815	276.1822	0.7	2.5	276.1809	-0.6	-2.2	276.1824	0.9	3.2
Glycine	5.18	$C_{11}H_{30}NO_2Si_3$	292.1584	292.1583	-0.1	-0.5	292.1589	0.5	1.6	292.1586	0.2	0.5
Alanine	5.68	$C_{12}H_{32}NO_2Si_3$	306.1741	306.1748	0.7	2.3	306.1750	0.9	3.0	306.1733	-0.8	-2.6
Citramalate	6.71	$C_{14}H_{33}O_5Si_3$	365.1636	365.1641	0.5	1.4	365.1647	1.1	3.1	365.1642	0.6	1.7
Threitol	6.98	$C_{16}H_{43}O_4Si_4$	411.2238	411.2238	0.0	0.0	411.2239	0.1	0.1	411.2224	-1.0	-3.5
Alpha-Ketoglutarate Meox	7.62	$C_{12}H_{26}NO_5Si_2$	320.1350	320.1349	-0.1	-0.2	320.1353	0.3	1.1	320.1353	0.3	1.1
Benzoate 4-Hydroxy	8.01	$C_{13}H_{23}O_3Si_2$	283.1186	283.1193	0.7	2.6	283.1186	-0.8	-2.7	283.1188	0.2	0.8
Ribitol	8.74	$C_{20}H_{53}O_5Si_5$	513.2739	513.2762	2.3	4.4	513.2743	0.4	0.7	513.2748	0.9	1.7
Putrescine	8.82	$C_{16}H_{45}N_2Si_4$	377.2660	377.2671	1.1	2.9	377.2669	0.9	2.4	377.2662	0.2	0.6
Citrate	9.44	$C_{18}H_{41}O_7Si_4$	481.1929	481.1952	2.3	4.7	481.1935	0.6	1.2	481.1944	1.5	3.0
Sorbose Meox1	9.79	$C_{22}H_{56}NO_6Si_5$	570.2954	570.2964	1.0	1.7	570.2968	1.4	2.4	570.2964	1.0	1.7
Sorbose Meox2	9.82	$C_{22}H_{56}NO_6Si_5$	570.2954	570.2964	1.0	1.7	570.2955	0.1	0.2	570.2972	1.8	3.1
Glucose Meox1	9.96	$C_{22}H_{56}NO_6Si_5$	570.2954	570.2973	1.9	3.3	570.2961	0.7	1.2	570.2963	0.9	1.6
Glucose Meox2	10.09	$C_{22}H_{56}NO_6Si_5$	570.2954	570.2966	1.2	2.1	570.2969	1.5	2.6	570.2972	1.8	3.1
Sorbitol	10.22	$C_{24}H_{63}O_6Si_6$	615.3240	615.3250	1.0	1.6	615.3246	0.6	0.9	615.3273	3.3	5.3
Pantothenate	10.60	$C_{18}H_{42}NO_5Si_3$	436.2371	436.2386	1.5	3.5	436.2374	0.3	0.7	436.2375	0.4	1.0
Caffeate	11.53	$C_{18}H_{33}O_4Si_3$	397.1687	397.1693	0.6	1.6	397.1693	0.6	1.6	397.1684	-0.3	-0.7
				Std Dev	**0.7**	**1.5**	**Std Dev**	**0.6**	**1.6**	**Std Dev**	**1.0**	**2.1**
				RMS Error		**2.3**	**RMS Error**		**1.9**	**RMS Error**		**2.3**

Figure 2. Table of exact mass measurement results from the analysis of the QC standards.

Analysis of Tomato Fruit Extract

The TIC chromatogram obtained from the analysis of the polar fraction of a tomato fruit extract is given in Figure 3. This shows a complex trace of major, minor, and trace components.

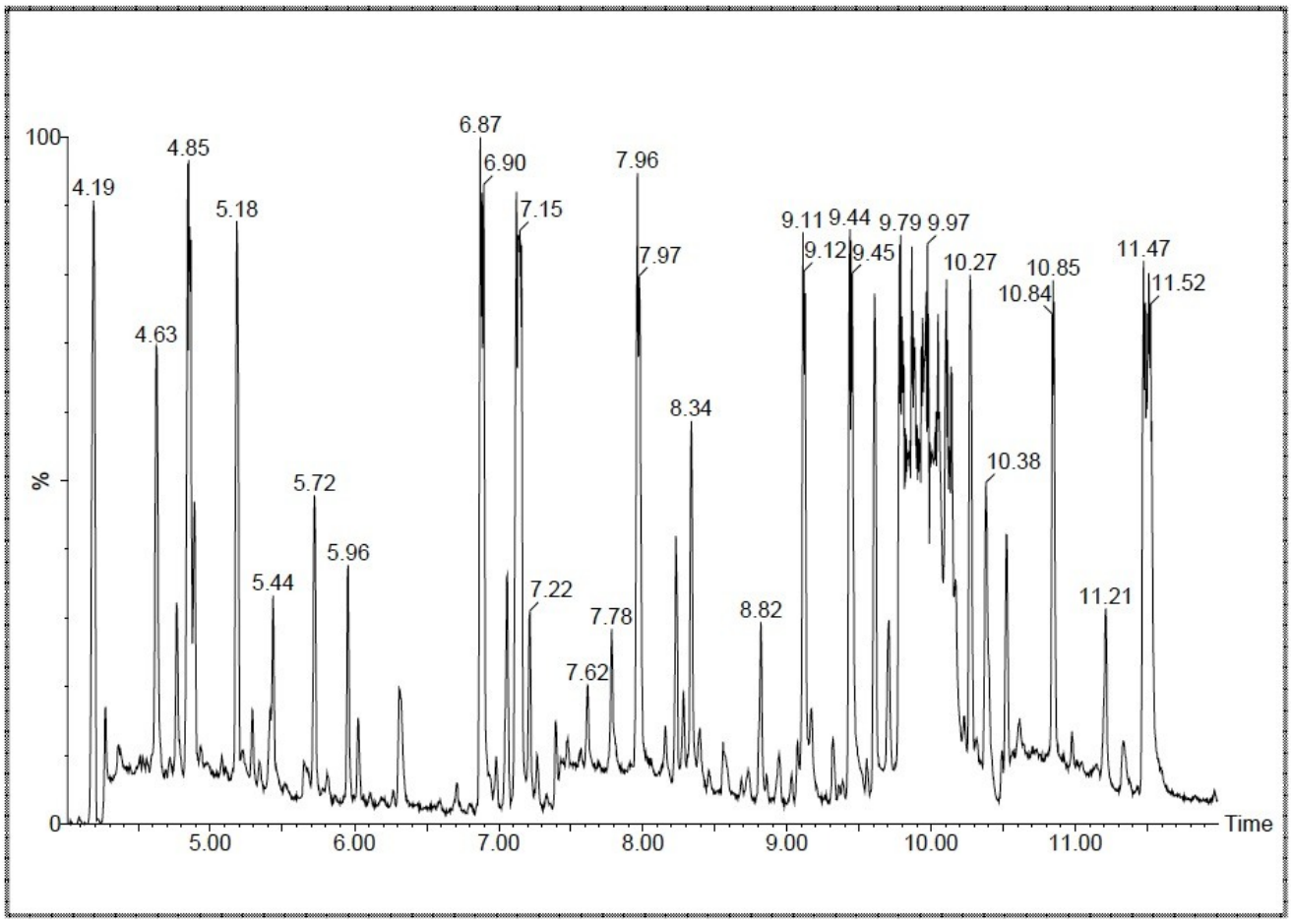

Figure 3. TIC from CI analysis of tomato fruit extract.

The spectrum of the minor component eluting at 8.40 minutes can be seen in Figure 4 and demonstrates a molecular ion cluster and minimal fragmentation. The pseudo-molecular ion observed at *m/z* 468.2458 corresponds to an elemental composition of $C_{18}H_{46}NO_5Si_4$ (error 0.5 mDa, 1.1 ppm) and is from a monosaccharide ($C_5H_{10}O_5$). The elemental composition report within a 3 ppm tolerance is displayed in Figure 5.

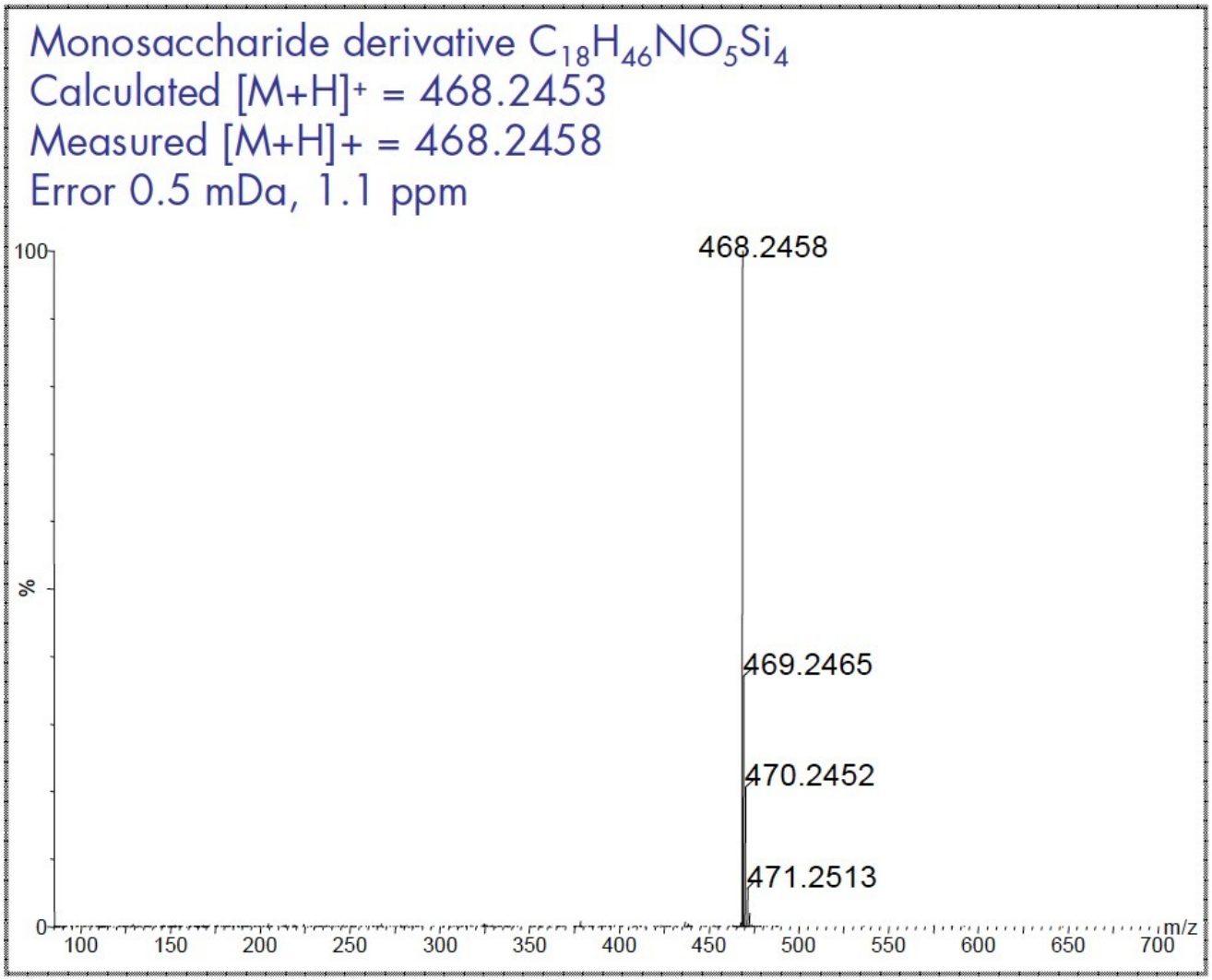

Figure 4. Spectrum from peak at RT 8.40 minutes.

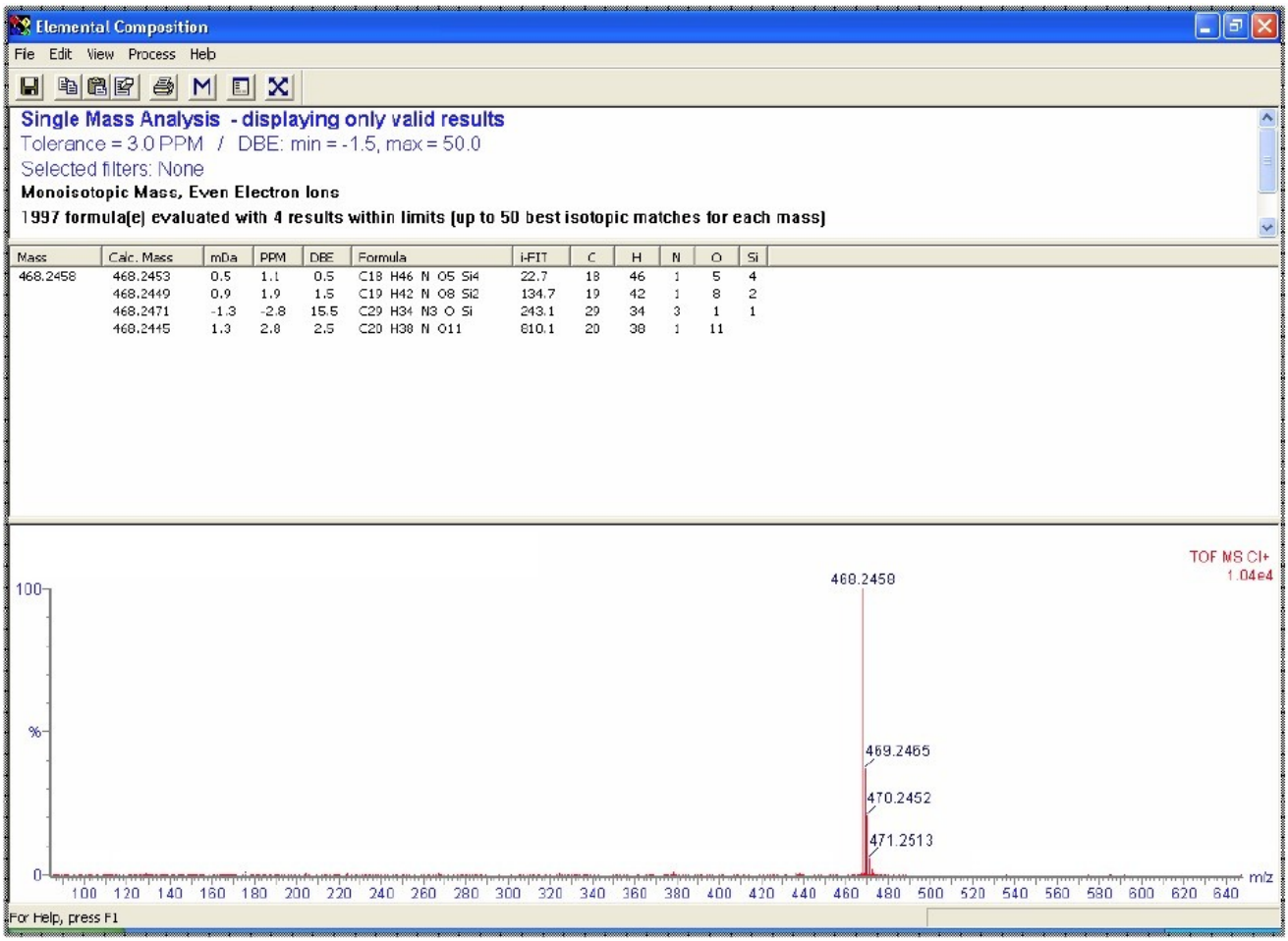

Figure 5. Elemental composition report for peak at RT 8.40 minutes within 3 ppm tolerance.

The elemental composition report shows four hits within 3 ppm, with $C_{18}H_{46}NO_5Si_4$ giving the best fit using the i-FIT elemental composition calculator for Waters MassLynx Software (the lower the number the better the fit). This is a probability fit based on both exact mass and intensity of the peaks in the isotope cluster.

An elemental composition report is shown in Figure 6 for the same ion at *m/z* 468.2458, but with the mass tolerance set to 250 mDa. In this case, the number of potential hits rises to 605 but due to the measured exact mass and isotopic pattern, the best i-FIT prediction is still for $C_{18}H_{46}NO_5Si_4$, demonstrating the benefits of exact mass in identifying unknown components.

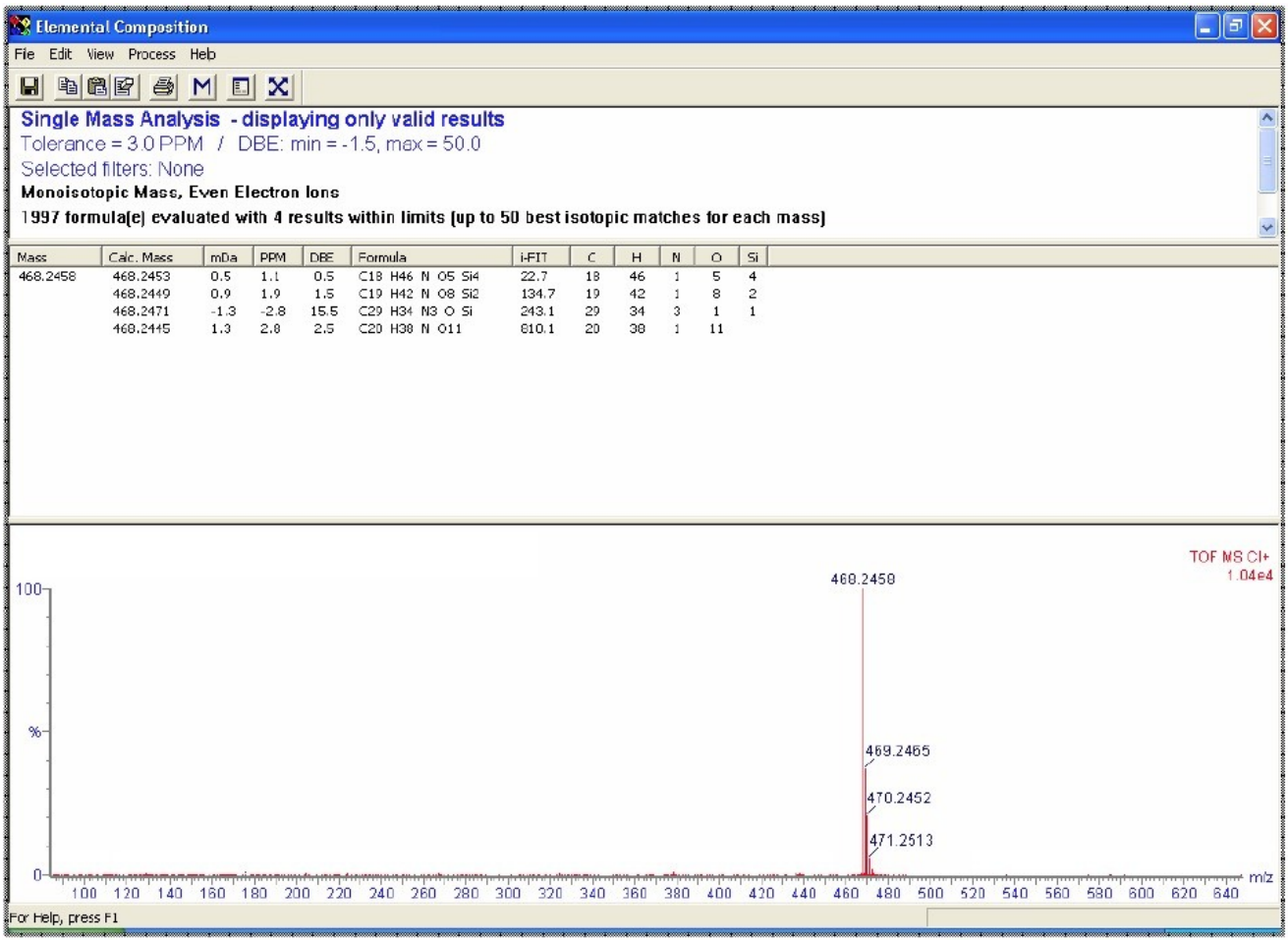

Figure 6. Elemental composition report for peak at RT 8.40 minutes within 250 mDa tolerance.

The spectrum from the component eluting at 9.44 minutes is shown in Figure 7. This component shows both a protonated and an ammoniated molecular ion but again, very little fragmentation is observed. The ion observed at *m/z* 481.1940 corresponds to an elemental composition of $C_{18}H_{41}O_7Si_4$ (error 1.1 mDa, 2.2 ppm) and was assigned as citric acid ($C_6H_8O_7$).

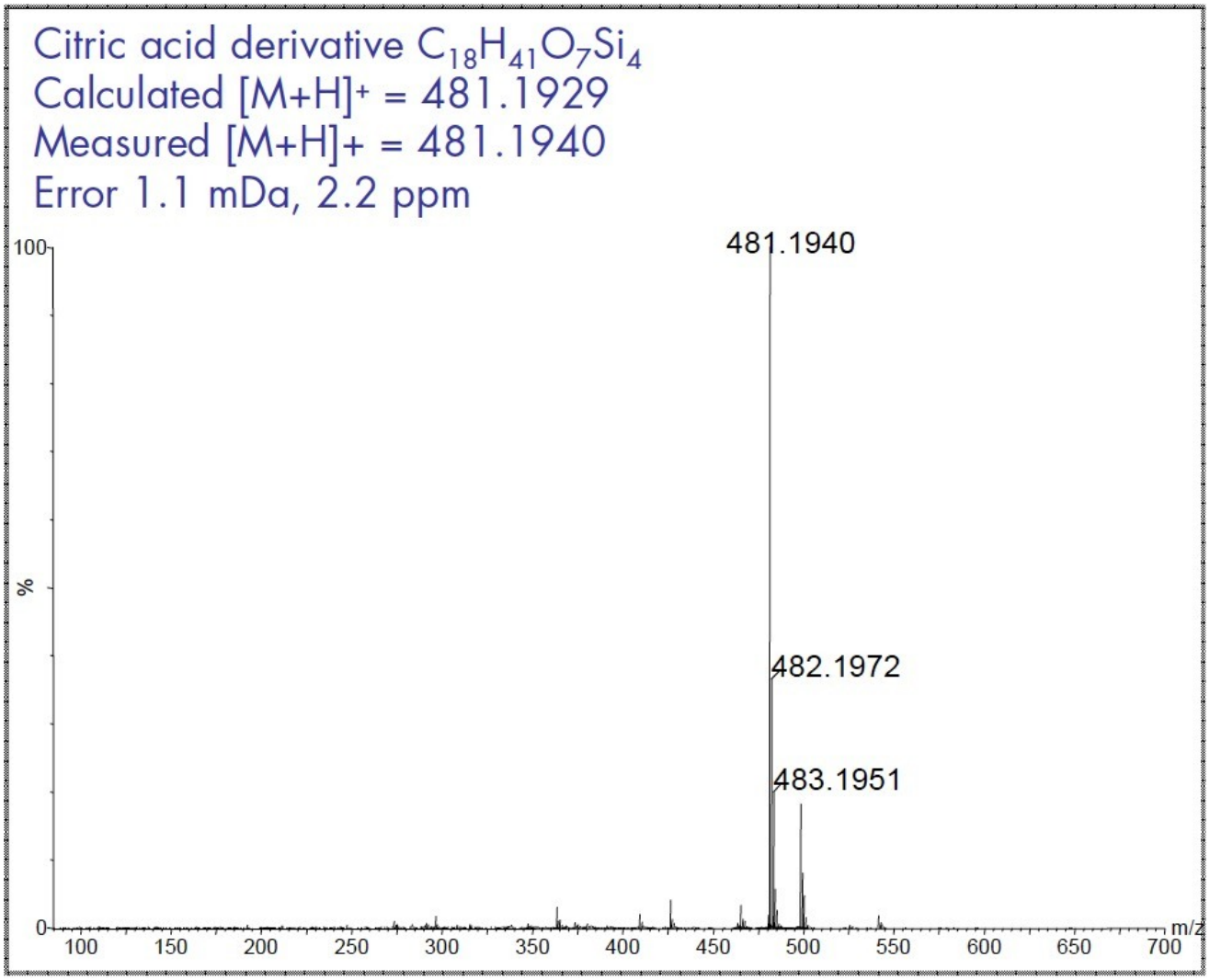

Figure 7. Spectrum from peak at RT 9.44 minutes.

Conclusion

The oa-TOF GCT mass spectrometer allows routine exact mass measurements of <5 ppm RMS to be readily obtained.

Operation in CI mode provides useful data by the generation of pseudo-molecular ions. This facilitates the exact mass determination of the molecular masses and hence the elemental compositions of the intact derivatized polar compounds can be derived.

The use of CI for the generation of exact mass pseudo-molecular ions, in conjunction with EI, can be a powerful tool in the identification of plant metabolites of unknown structure.

Acknowledgements

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