thermo scientific

TECHNICAL NOTE 10654

Negative Ion Chemical Ionization (NCI) studies using the Thermo Scientific ISQ 7000 single quadrupole GC-MS system

Author
Amit Gujar
Thermo Fisher Scientific,
Austin, TX, USA

Keywords
Negative ion chemical ionization (NICI or NCI), IDL, Octafluoronaphthalene, ISQ 7000, gas chromatography-mass spectrometry

Goal

To demonstrate NCI capabilities of the Thermo Scientific™ ISQ™ 7000 single quadrupole GC-MS system with methane reagent gas, attainable typical instrument detection limits (IDLs), and the effect of the ion source temperature and reagent gas flow rates on NCI mass spectra.

Introduction

Negative ion chemical ionization (NICI or NCI) is a popular ionization technique for gas chromatography-mass spectrometry (GC-MS) that is frequently used for analyzing electrophilic molecules of environmental concern such as brominated flame retardants (BFR)[1] and chlorinated pesticides.[2] It is also a popular technique in steroid and drug analysis after derivatization of the analyte with compounds that add electronegative atoms to the molecule.[3] The advantages the NCI technique offers are high sensitivity and selectivity for electrophilic molecules in complex matrices.

Electron capture (as opposed to true negative ionization involving high energy electron-molecule reaction) is the main mechanism in formation of negative ions in the mass spectrometry technique as shown below.[4]

$$AB + e^- (\sim 0.1\ eV) \rightarrow AB^{-\cdot}\ \text{(Resonance electron capture)}$$

$$AB + e^- (0\text{-}15\ eV) \rightarrow A^{\cdot} + B^- \text{(Dissociative electron capture)}$$

$$AB + e^- (>10\ eV) \rightarrow A^- + B^+ + e^- \text{(Ion-pair formation)}$$

ThermoFisher SCIENTIFIC

This is why NCI is also referred to as electron capture negative ionization (ECNI). ECNI results from resonance capture of low-energy (thermalized) secondary electrons formed as a result of electron ionization (EI) of a reagent gas such as methane contained in a closed ion source. Other examples of commonly used reagent gases include isobutane and ammonia.

NCI is considered to be a soft ionization technique yielding a mass spectral pattern with less fragmentation and where the molecular or pseudo-molecular ions are easily identifiable. A comparison of mass spectra for EI and NCI of octafluoronaphthalene (OFN) is shown in Figure 1. NCI shows significantly less fragmentation than EI with a dominant molecular ion peak ($M^{-\cdot}$) of 272.

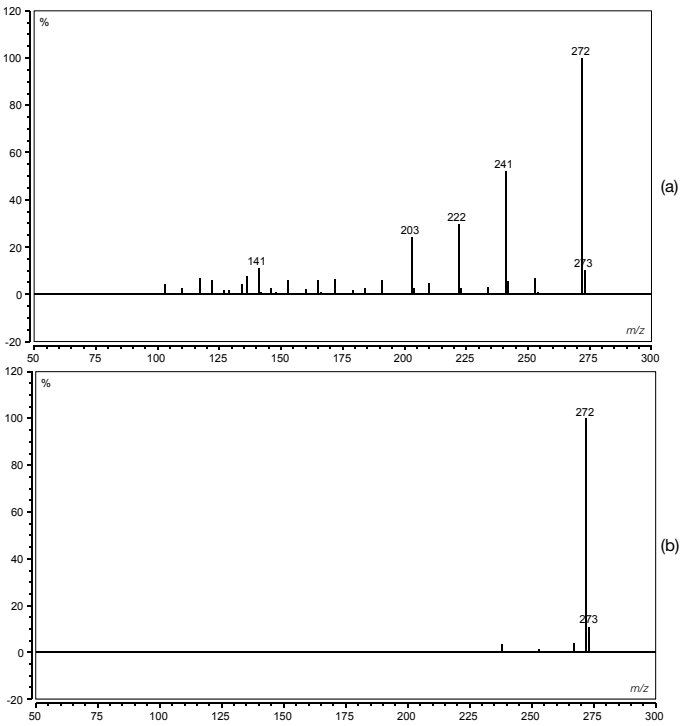

Figure 1. (a) EI mass spectrum of Octafluoronaphthalene (b) NCI mass spectrum of Octafluoronaphthalene

This technical note provides details on the methane-NCI capability of the ISQ 7000 single quadrupole GC-MS system. It shows the IDL for the system using octafluoronapthalene (OFN) and the effect of ion source temperature and methane gas flow rates on the mass spectral pattern and chromatographic peak shape for 4TMS-derivative of benzo[a]pyrene-7,8,9,10-tetrol (BaPT) and PFPA-HFIP-derivatized 11-nor-9-carboxy-THC. BaPT is an important metabolite in toxicokinetic studies of benzo[a]pyrene, a potent carcinogenic polycyclic aromatic hydrocarbon (PAH).[5] BaPT is routinely monitored in blood and urine, and due to being nonvolatile, it is often derivatized in order to make it appropriate for GC analysis. 11-nor-9-carboxy-THC is an important metabolite of tetrahydrocannabinol (THC) which is formed in the body and is regarded as an indicator of cannabis consumption.[6] It is routinely monitored in various biological matrices like plasma, urine and hair. Hair matrix is especially challenging because of the low concentration of the metabolite in hair and small amounts available. However, GC-MS/MS in NCI mode has been demonstrated to be suitable.[7]

Experimental

Sample and sample preparation: 1 fg/µL OFN in isooctane (Thermo Scientific, catalog #1R6310-0101K) was used, as is, in IDL studies. Other concentrations shown in these studies were prepared by diluting the 1 fg/µL OFN solution by isooctane.

Benzo[a]pyrenetetrol (BaPT, catalog # B205850) was purchased from Toronto Research Chemicals, Inc. (Canada). As received, BaPT (0.125 mg) was derivatized by the addition of 1 mL Thermo Scientific™ MSTFA + 1% TMCS Silylation Reagent (catalog # TS-48915) and heating at 60 °C for 40 hours in a sealed vial. After derivatization the sample was directly injected into the GC-MS system without further dilution. Figure 2 shows the molecular structure of BaPT and its TMS-derivatized form.

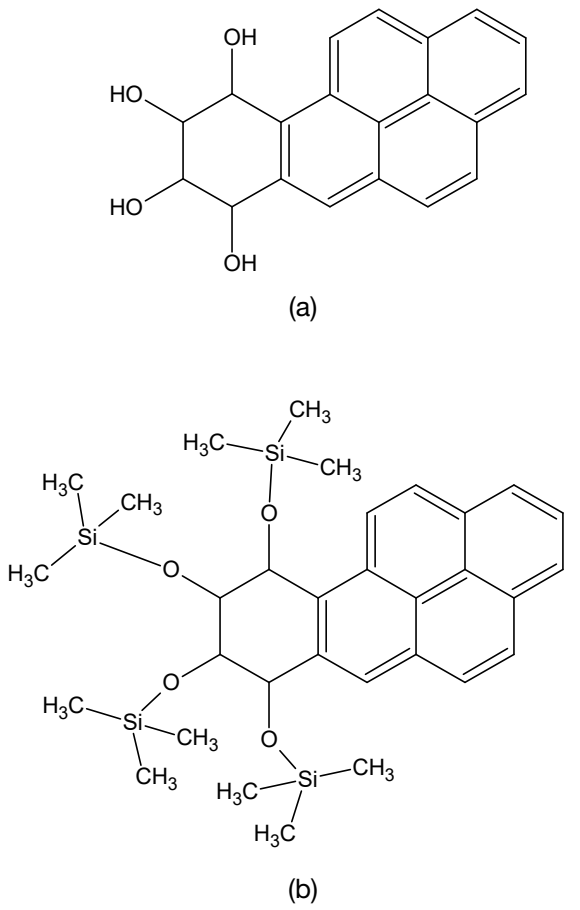

Figure 2. Molecular structures of (a) Benzo[a]pyrene-7,8,9,10-tetrol (BaPT) and (b) 4TMS-BaPT

11-nor-9-Carboxy-Δ^9-THC (catalog # T-010) was purchased from Cerilliant corporation (Round Rock, Texas) and derivatized with perfluoropropionic anyhydride (PFPA) and hexafluoroisopropanol (HFIP) using a procedure similar to that in Reference 7. The chemical reaction is shown in Figure 3. Notice that the derivatized product is highly fluorinated and hence is ideal for analysis by GC-MS in NCI mode. Figure 4 shows comparison of the EI and NCI mass spectra for the compound. Even though the EI spectrum gives a distinguishable molecular ion (*m/z* 640) the compound fragments into many low mass ions while the NCI spectrum shows prominent M-HF (*m/z* 620) ions with noticeably less fragmentation.

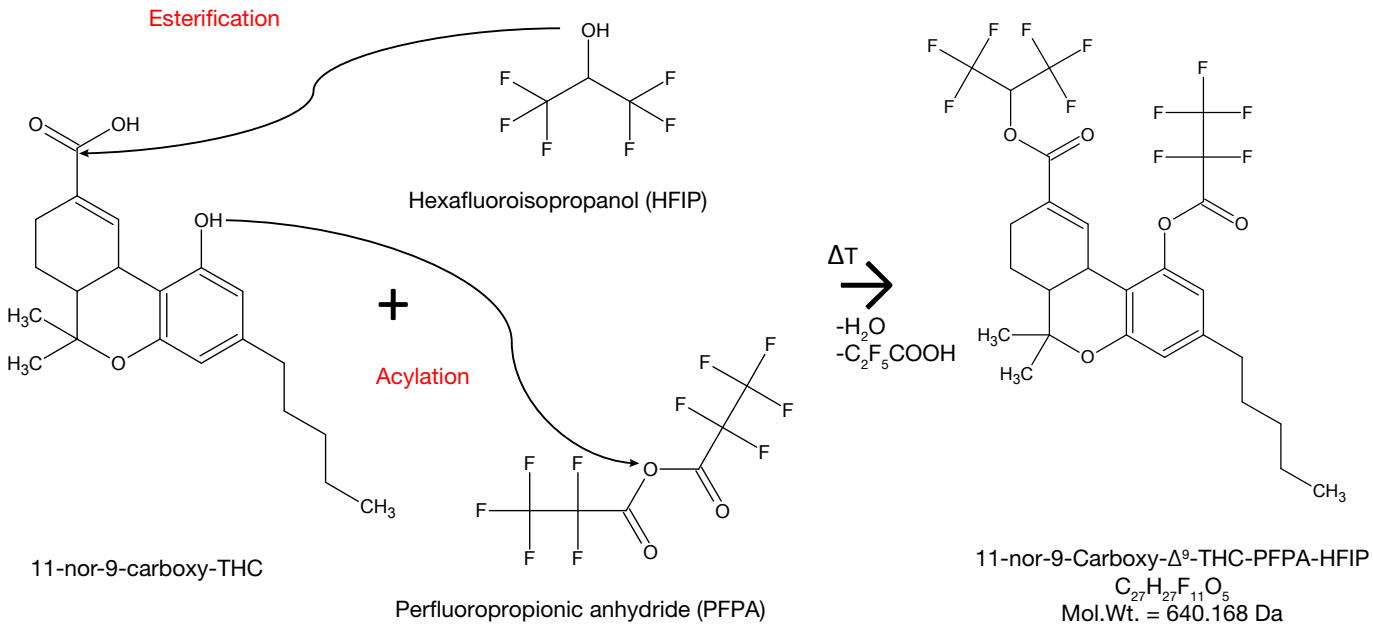

Figure 3. Chemical reaction for derivatization of 11-nor-9-Carboxy-Δ^9-THC leading to highly fluorinated analyte suitable for GC-MS NCI analysis

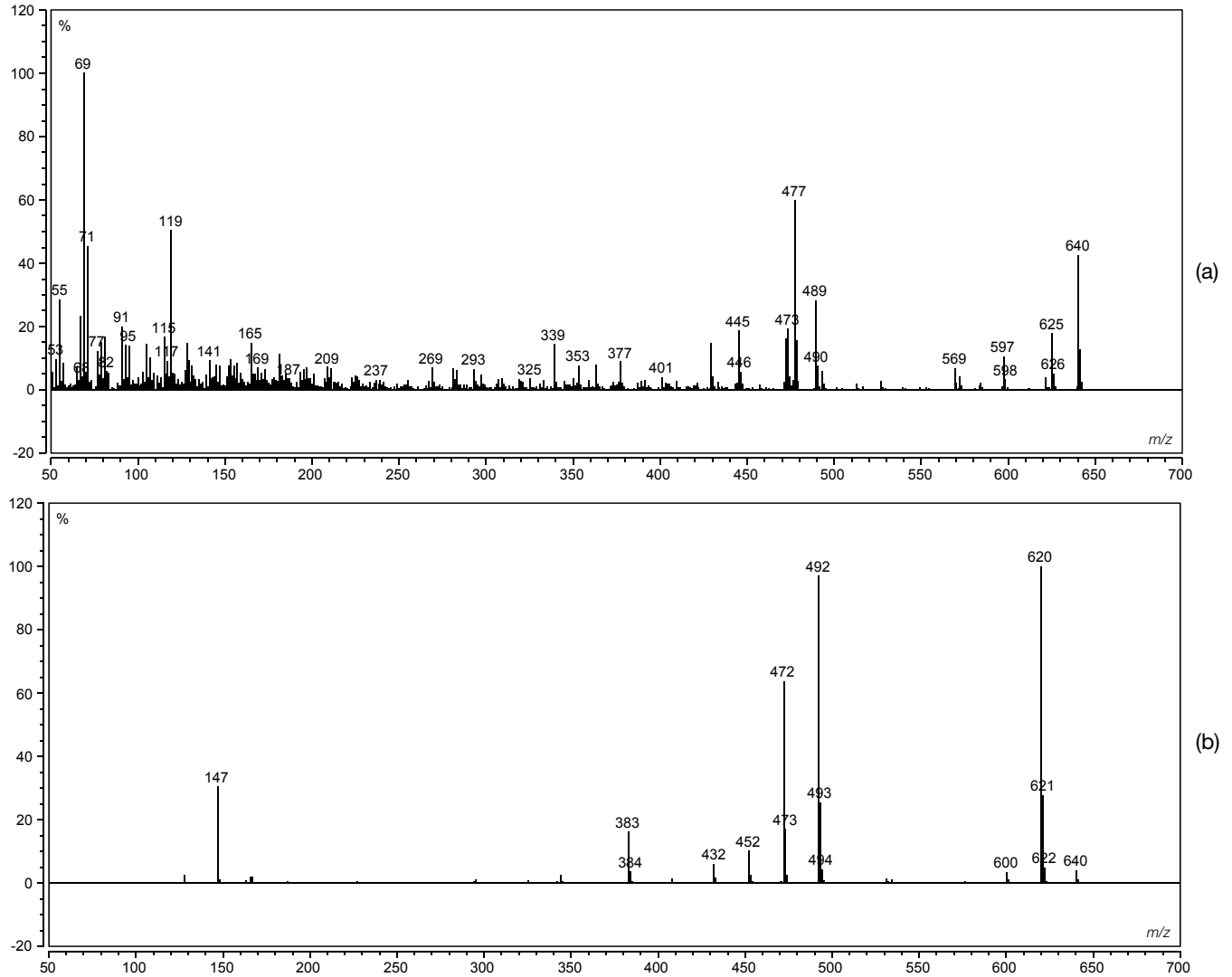

Figure 4. Comparison of the EI (a) and NCI (b) mass spectrum of PFPA-HFIP-11-nor-THC-COOH at ion source temperature of 200 °C

GC-MS conditions

The ISQ 7000 mass spectrometer coupled with a Thermo Scientific™ TRACE™ 1310 Gas Chromatograph and a Thermo Scientific™ AI/AS 1310 Series Autosampler was used for the experiments. Typical autosampler, GC, and MS method parameters used for the OFN detection limit studies are shown in Table 1. The Thermo Scientific™ ExtractaBrite™ source with CI ion volume was used. One important point to note is that electron energy of 70 eV is used. It has been traditionally regarded that high electron energy (~500 eV) is necessary to get good chemical ionization.[8,9] This was because older filament designs used an electron trap collector where higher electron energies were necessary for the electrons to travel through the reagent gas to get to the collector. The modern hairpin filament design of the ISQ 7000 GC-MS system and the Thermo Scientific™ TSQ™ 9000 triple quadrupole GC-MS/MS system does not require a separate electron collector and has an integrated electron lens that draws the electrons out and into the ion volume. High electron energies are not optimal for this design.

The IDL was calculated by statistical analysis using the one-tailed Student's t-distribution at a 99% confidence interval for eight sequential injections. The equation used is:

$$IDL = t * Amount * \%RSD,$$

where:
t = one-tailed Student's *t*-value
Amount = amount of analyte (injected on-column)
RSD = relative standard deviation for chromatographic peak areas

Using one-sided Student's *t*-distribution for 99% confidence interval for eight injections (n, degrees of freedom = 7) we have t = 2.998. Thus the IDL equation becomes

$$IDL = 2.998 * Amount * \%RSD$$

The IDL for OFN was demonstrated in a SIM (selected ion monitoring) mode, monitoring *m/z* 272.

Software

Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software, version 7.2 was used for data acquisition and analysis. The Cobra peak detection algorithm was used and the peak asymmetry (also known as tailing factor) calculation mentioned in the results below are based on the USP formula considering the peak width measurements at 5% of the height.

Table 1. Autosampler, GC, and MS parameters used for the OFN detection limit experiments.

AI/AS 1310 Series Autosampler	
Syringe	10 µL, 25 gauge, 50 mm length, cone tip (P/N 36500525)
Injection volume	1 µL
Plunger strokes	3
Draw speed	Slow
Sampling depth	Bottom
Pre-/post-injection dwell time	0.0 s
Pre-injection solvent and cycles	0
Sample rinses	1
Post-injection solvent and cycles	0

TRACE 1310 Gas Chromatograph	
Column	TG-SQC 15m × 0.25mm × 0.25µm (P/N 26070-1300)
Liner	LinerGOLD liner, splitless single taper with quartz wool, 4mm × 6.5mm × 76.5mm (453A1925-UI)
SSL mode	Splitless
Inlet temperature	220 °C
Splitless time	0.5 min
Split flow	50 mL/min
Septum purge flow	Constant flow of 5.0 mL/min
Carrier flow	Constant He flow of 1.2 mL/min
Oven program	45 °C (0.5 min), 40 °C/min to 190 °C (0 min)

ISQ 7000 GC-MS system	
Method type	Acquisition – General
MS transfer line temperature	250 °C
Ion source temperature	200 °C
Ionization mode	Negative CI, 70 eV
CI gas	Methane
CI gas flow rate	1.25 mL/min
Emission current	50 µA
Scan start	2.4 min
Scan	SIM *m/z* 272
Dwell time	0.1 s
Tuning used	Autotune_NCI

Results and Discussion

Detection limit study for OFN

Figure 5 shows the time and signal offset overlaid chromatograms of eight 1 µL sequential injections of 1 fg/µL OFN for *m/z* 272. The calculated IDL was 0.1 fg. For comparison, the average calculated OFN IDL in EI mode using the Thermo Scientific™ Advanced Electron Ionization (AEI) source was 0.64 fg.[10] Figure 6 shows the area count trend for over 100 sequential injections of 1 fg/µL OFN demonstrating the stability of the system. The %RSD of the area counts was 4%. Figure 7 shows comparison of the chromatogram at various low levels of OFN ranging from 1 fg to 0.05 fg on-column. Also shown is the blank iso-octane chromatogram. Notice the peak-to-peak signal to noise ratio on the peak label. We observe that one can easily detect OFN in NCI mode at levels as low as 100 ag on-column with signal to noise ratio of >3, thus confirming the calculated IDL. This equates to detecting approximately 200,000 molecules.

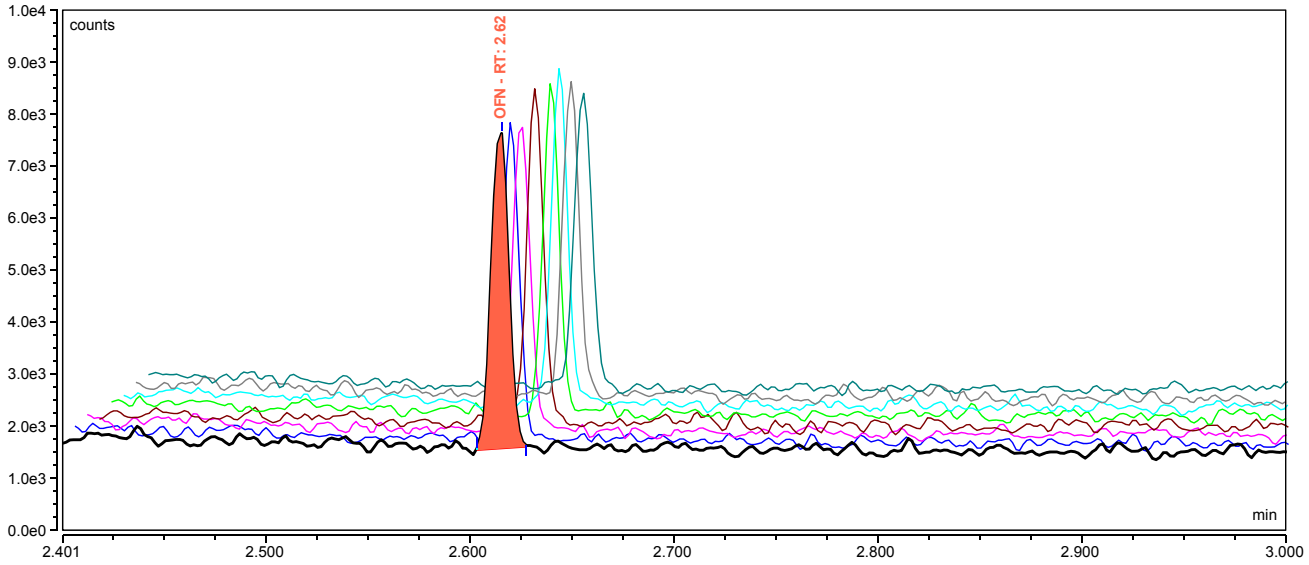

Figure 5. Chromatogram showing eight sequential 1 µL injections of 1 fg/µL OFN for SIM of *m/z* 272

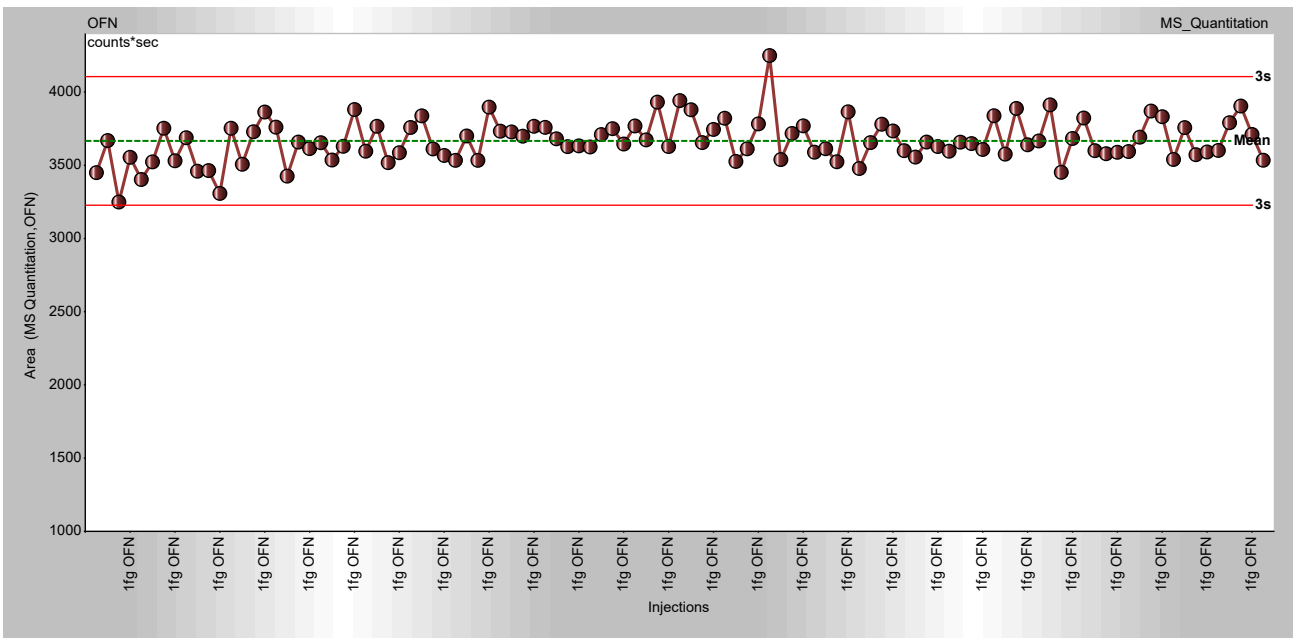

Figure 6. Area counts trend for over 100 sequential injections of 1 fg/µL OFN for SIM of *m/z* 272 with mean and +/- 3 standard deviations

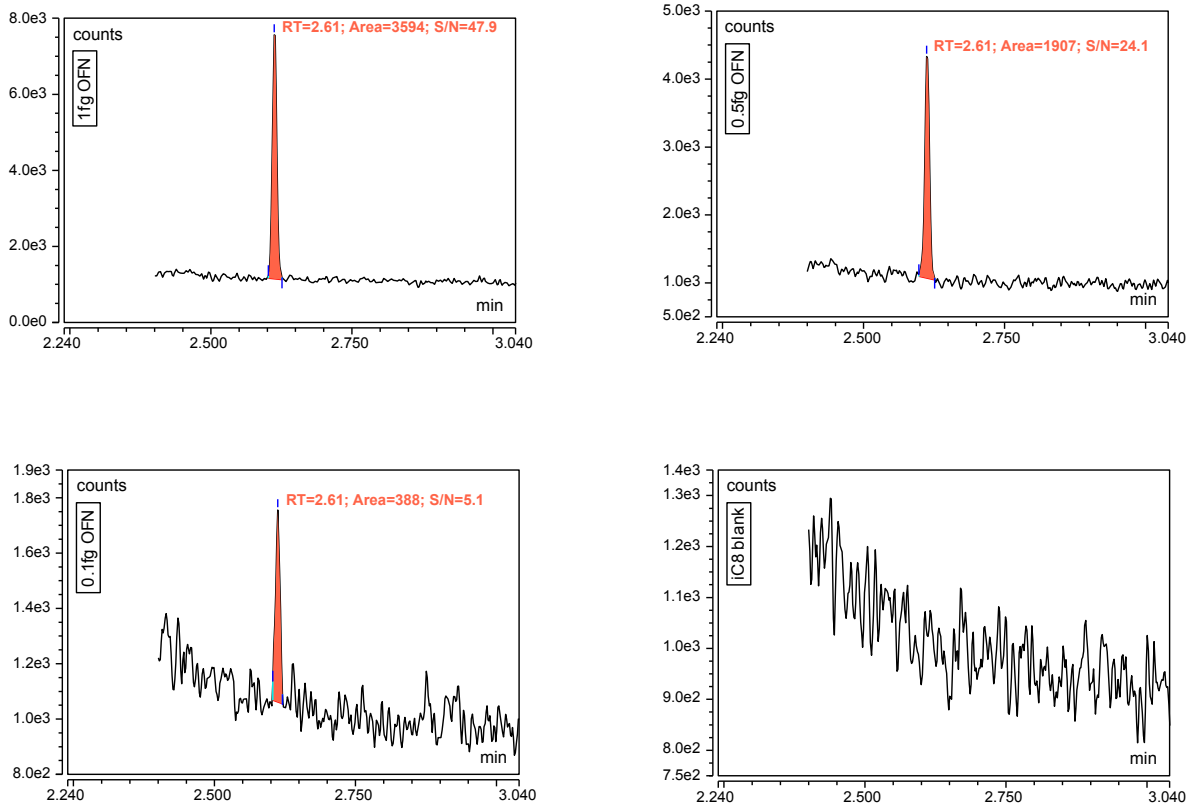

Figure 7. Comparison of various low level OFN injections showing the ability of the ISQ 7000 to easily detect levels as low as 100 ag on-column. Also shown in the isooctane blank chromatogram.

Effect of ion source temperature and reagent methane flow rates

The mass spectrum in NCI mode is sensitive to mass spectrometer run conditions such as ion source temperature and reagent gas flow rates. Figure 8 shows the effect of ion source temperature on the mass spectrum of 4TMS-BaPT at 1.5 mL/min methane flow. Decreasing the ion source temperature results in lower abundance of low mass ions such as *m/z* 284 versus higher mass ones such as *m/z* 446. A similar trend can be seen for derivatized 11-nor-9-Carboxy-Δ^9-THC in Figure 9 where relative abundance of *m/z* 620 (M-HF) versus *m/z* 492 increases with lower ion source temperatures. Generally, high mass ions are preferred since they give higher selectivity to compounds of interest which in turn improves the detection limits for such compounds due to higher signal to noise ratios. An adverse effect of decreasing the ion source temperature for 4TMS-BaPT is an increase in asymmetry of the peak as shown in Figure 10 while the effect of ion source temperature on peak shape for derivatized 11-nor-9-Carboxy-Δ^9-THC (Figure 11) is minimal — even when at much lower source temperatures. This shows that peak shapes are dependent on the analyte properties and that silylated molecules are more prone to adsorption effects within the ion source leading to increased tailing effects at low temperatures versus non-silylated molecules (fluorinated in this instance). Very low ion source temperatures (< 150 °C) are not recommended with methane-CI as it decreases the robustness of the system due to matrix adsorption and fouling of the ion volume by methane reagent ions. It is therefore important to find a balance amongst the ion source temperature, peak tailing factors and the desired robustness for the desired application.

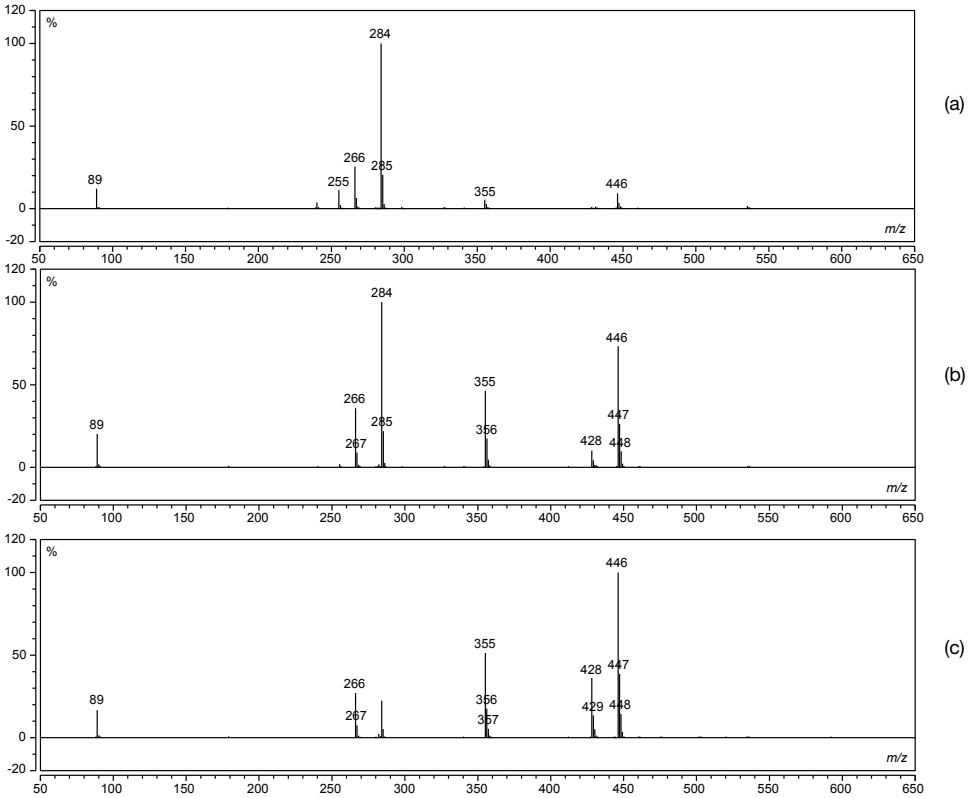

Figure 8. Comparison of mass spectra for 4TMS-BaPT at varying ion source temperatures (a) 300 °C, (b) 250 °C and (c) 200 °C

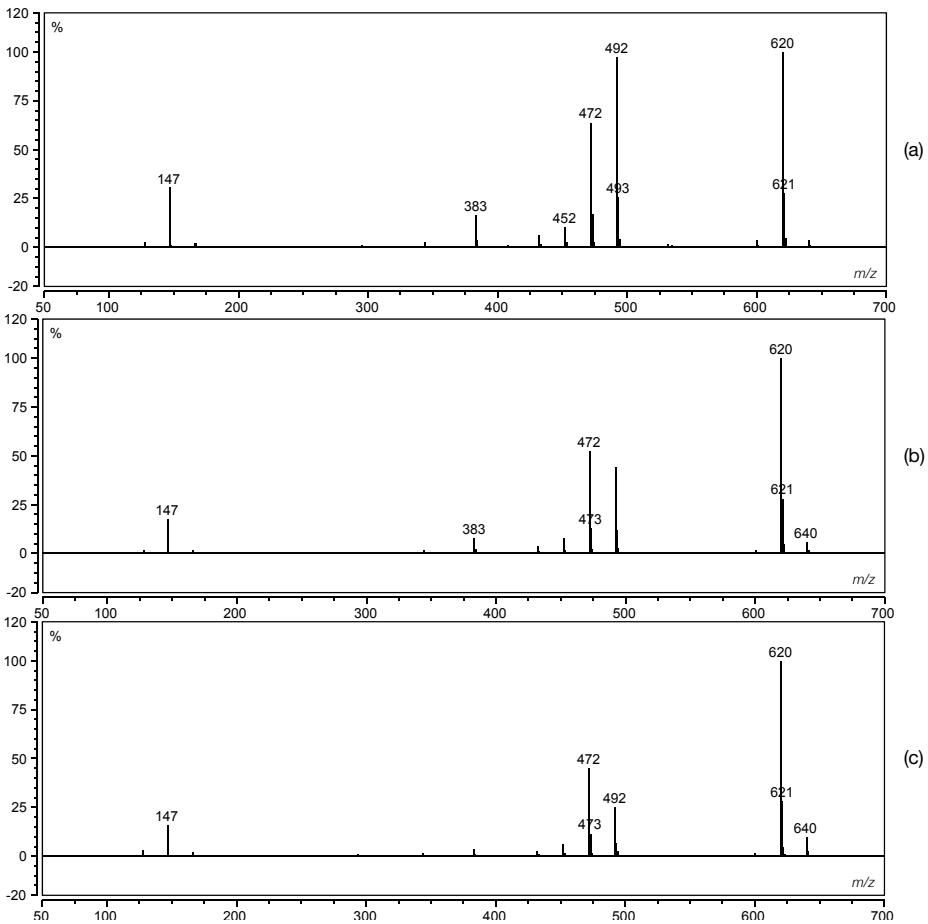

Figure 9. Comparison of mass spectra for PFPA-HFIP-derivatized 11-nor-9-Carboxy-Δ^9-THC at varying ion source temperatures (a) 200 °C, (b) 175 °C and (c) 150 °C

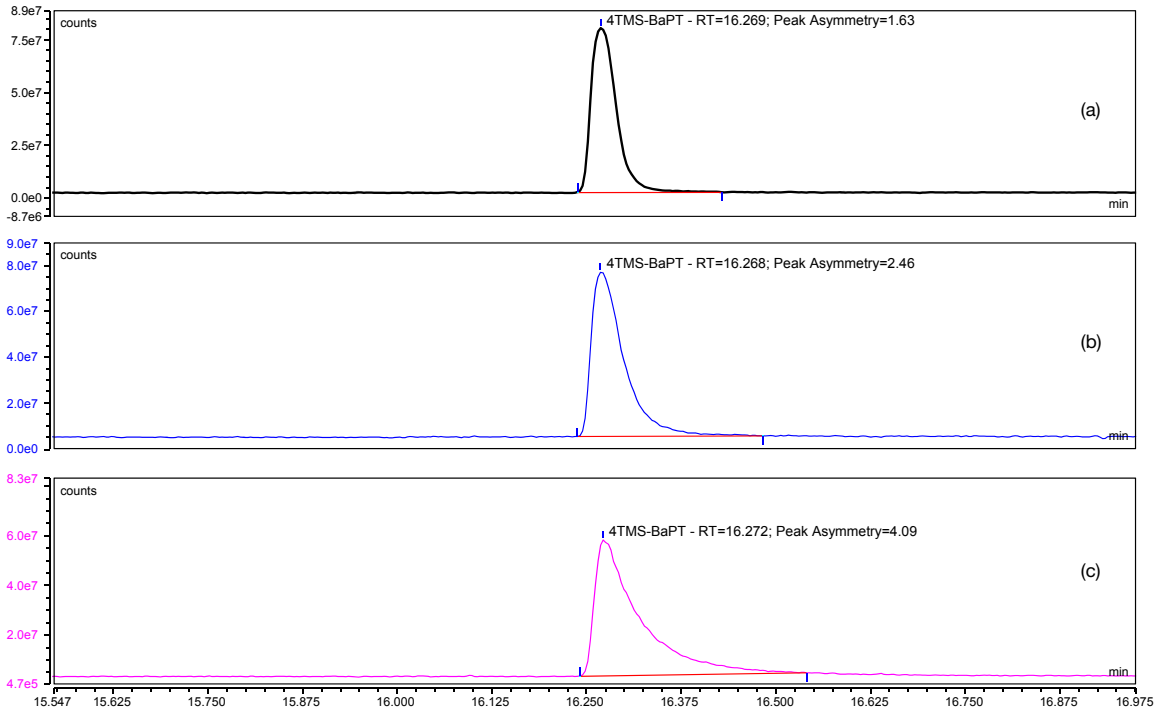

Figure 10. Chromatographic peak shape comparisons for TMS derivatized BaPT at varying ion source temperatures (a) 300 °C, (b) 250 °C and (c) 200 °C

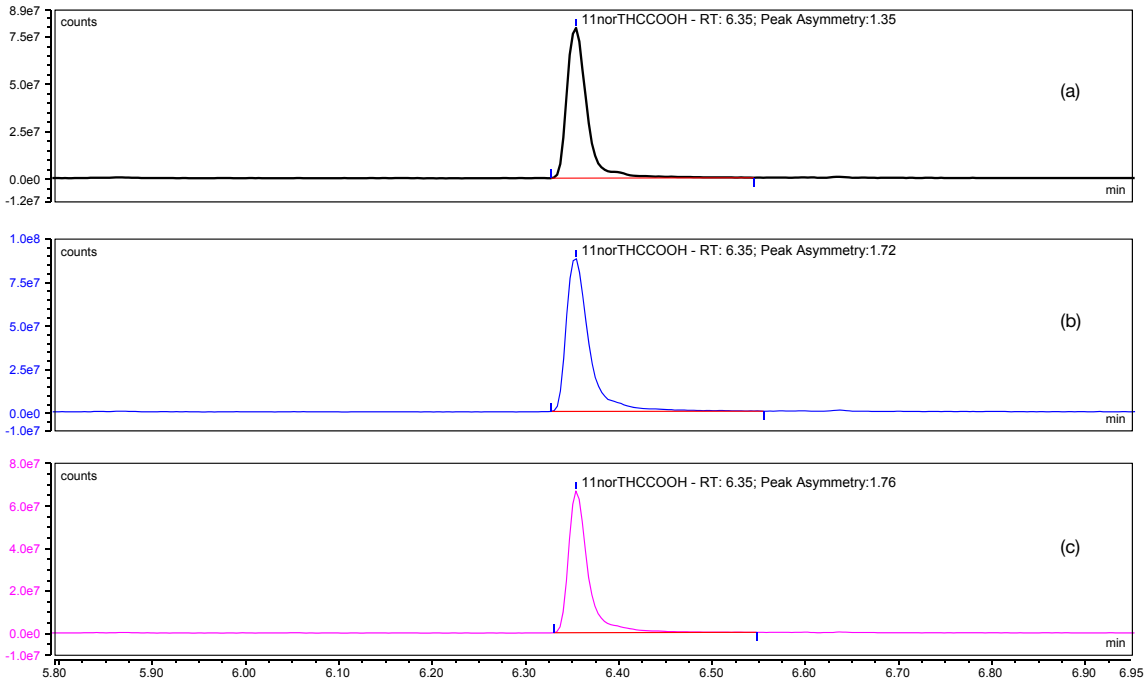

Figure 11. Chromatographic peak shape comparisons for PFPA-HFIP-derivatized 11-nor-9-Carboxy-Δ^9-THC at varying ion source temperatures (a) 200 °C, (b) 175 °C and (c) 150 °C

The effect of methane reagent gas flow rate on mass ratios for 4TMS-BaPT at ion source temperature of 300 °C is shown in Figure 12. Increasing the methane flow rate results in a higher degree of undesired fragmentation. This could be attributed to the greater abundance of thermal electrons resulting from the higher flow rate of methane, which in turn results in greater dissociative electron capture reactions. An extreme case of high ion source temperature and high methane flow rate is shown in Figure 13. Ion source temperature of 350 °C and a methane flow rate of 3 mL/min results in almost complete disappearance of mass 446; the peak asymmetry for this example was 1.0.

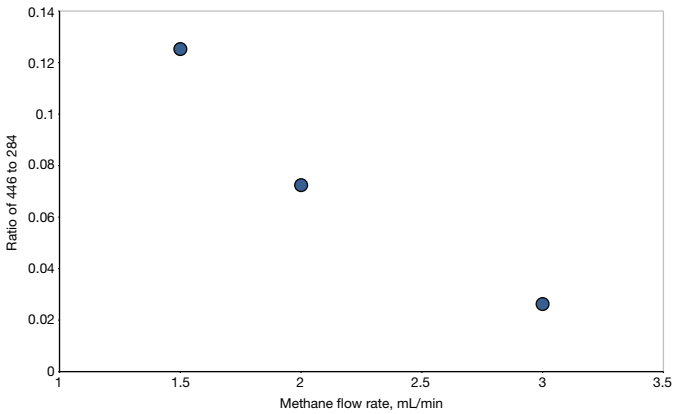

Figure 12. Effect of reagent methane gas flow rate on ratio of *m/z* 446 to *m/z* 284

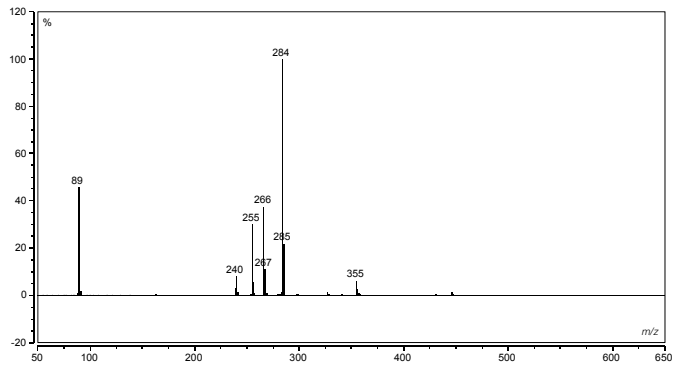

Figure 13. Mass spectrum of 4TMS-BaPT at 350 °C and 3 mL/min methane gas flow rate

Conclusion

In conclusion, this technical note illustrates the NCI capabilities of the ISQ 7000 single quadrupole GC-MS system, with detection limits as low as 100 attograms OFN on-column. An appropriate balance of ion source temperature and reagent gas flow rate is required to have a suitable abundance of high mass ions as well as satisfactory peak asymmetry. For applications involving silylated compounds careful consideration needs to be given to optimization of source temperature, in order to avoid excessive fragmentation of higher mass ions and excessive peak tailing. For most applications however, ion source temperatures between 200 and 250 °C, and methane flow rates between 1 and 2 mL/min provide a good balance between sensitivity and robustness.

References

1. Rosenfelder, N.; Vetter, W. Gas chromatography coupled to electron capture negative ion mass spectrometry with nitrogen as the reagent gas – an alternative method for determination of polybrominated compounds. Rapid Commun. Mass Spectrom. 2009, 23, 3807-3812.
2. Tagami, T.; Kajimura, K.; Takagi, S.; Satsuki, Y.; Nakamura, A.; Okihashi, M.; Akutsu, M.; Obana, H.; Kitagawa, M. Simultaneous analysis of 17 organochlorine pesticides in natural medicines by GC/MS with negative chemical ionization. Yakugaku Zasshi: Journal of the Pharmaceutical society of Japan 2007, 127(7), 1167-1171.
3. Xiao, X-Y.; McCalley, D.; McEvoy, J. Analysis of estrogens in river water and effluents using solid-phase extraction and gas chromatography-negative chemical ionization mass spectrometry of the pentafluorobenzoyl derivatives. J. Chromatogr. A 2001, 923, 195-204.
4. Sparkman, O. D.; Penton, Z. E.; Kitson, F. G. Gas Chromatography and Mass Spectrometry – A Practical Guide; Academic Press, 2011.
5. Day, B.; Naylor, S.; Gan, L-S; Sahali, Y.; Nguyen, T.; Skipper, P.; Wishnok, J.; Tannenbaum, S. Molecular Dosimetry of Polycyclic Aromatic Hydrocarbon Epoxides and Diol Epoxides via Hemoglobin Adducts Cancer Research 1990, 50, 4611-4618.
6. Huestis, M., A.; Barnes, A.; Smith, M. L. Estimating the Time of Last Cannabis Use from Plasma Δ9 -Tetrahydrocannabinol and 11-nor-9- Carboxy-Δ9 -Tetrahydrocannabinol Concentrations. Clinical Chemistry 2005, 51-12, 2289-2295.
7. Ciceri, E.; Albertini, T; Caruso, A. Sensitive determination of THCCOOH in hair to regulatory requirements using Triple Quadrupole GC-MS/MS. AN 10493, Thermo Fisher Scientific.
8. Harrison, A. G. Chemical Ionization Mass Spectrometry; CRC press, 1992.
9. McLafferty, F. W.; Tureček, F. Interpretation of Mass Spectra; University Science Books, 1993.
10. Gujar, A.; Anderson, T.; Cavagnino, D.; Patel, A. Practical determination and validation of instrument detection limit for the Thermo Scientific ISQ 7000 Single Quadrupole GC-MS system with Advanced Electron Ionization source. TN 10597, Thermo Fisher Scientific.

Find out more at **thermofisher.com/unstoppableGCMS**

TN10654-EN 1018S

ThermoFisher SCIENTIFIC