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Rapid identification and confirmation of unknown substances using Direct Insertion Probe – Exactive GC Orbitrap mass spectrometry

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Keywords

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Goal

The purpose of the experiments described here was to evaluate a quick and simple procedure for the identification of unknown suspicious substances using direct sample introduction and high resolution Orbitrap™ mass spectrometry.

Introduction

The detection and identification of illicit or suspicious substances of unknown origin can be a challenging task, often involving complex analytical processes that will delay the end result. In addition, it is essential that the final identification is made with a high degree of confidence and with numerous points of confirmation. Generally, all suspicious related seizures by law enforcement agencies must be sent to the forensic science providers for examination. While drug-testing kits can be used for ad-hoc tests of certain classes of drugs, it is often difficult to identify the exact active substance in an unknown powder or liquid, especially emerging substances in drugs

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such as “legal highs”.[1] When using analytical techniques such as mass spectrometry-based detectors, obtaining high mass accuracy data is essential to provide the required selectivity in complex matrices and to increase the confidence in compound identification. Determining the elemental mass of a chemical with sufficient accuracy allows the chemist to determine the elemental composition and use isotopic ratios and fragmentation patterns to identify the chemical structure of the substance.[2]

In this study a near-instant method for confident identification of unknown substances is described. The method uses the Direct Insertion Probe (DIP) coupled to an Exactive GC Orbitrap mass analyzer with both electron ionization (EI) and chemical ionization (CI) to quickly confirm compound identity.

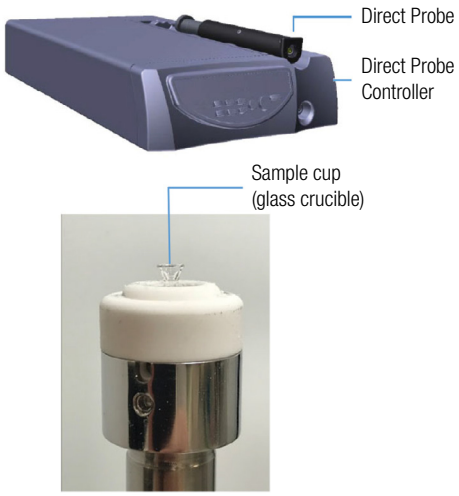
Experimental

In the experiments described here, a Thermo Scientific™ Exactive GC™ Orbitrap™ mass spectrometer was used. Direct analyses of samples were performed with the Thermo Scientific™ Direct Insertion Probe.[2] The DIP is a tool available on all Thermo Scientific GC-MS products equipped with the vacuum probe interlock (VPI) option. It provides a quick, simple method for sample introduction directly into the mass spectrometer ion source, allowing for accurate analysis of highly polar, thermally labile compounds, polymers, composites, solid or liquid samples, or organisms. The Exactive GC mass spectrometer was operated in full scan using 60,000 mass resolution (measured as FWHM at *m/z* 200). Data was acquired and processed with Thermo Scientific™ TraceFinder™ software. Additional details regarding the DIP and MS conditions are given in Table 1.

Table 1. DIP and mass spectrometer analytical parameters

DIP parameters	
Initial temperature:	80 °C
Hold:	20 s
Rate:	100 °C/min
Final temperature:	350 °C
Hold:	40 s

Exactive GC mass spectrometer parameters	
Ionization type (gas type):	EI PCI (methane) NCI (methane)
Ion source temperature:	230 °C (EI) 180 °C (PCI and NCI)
Electron energy:	70 eV
Acquistion mode:	Full scan
Mass range:	50–700 *m/z*
Mass resolution (FWHM at *m/z* 200):	60,000



Samples

Morphine (CAS 57-27-2) and methadone (CAS 76-99-3) solid standards were diluted in methanol to a final concentration of 1 mg/mL, and 0.5 µL of sample was loaded into the DIP glass cup (P/N 119329-0001) (Figure 1).

Figure 1. DIP sample loading procedure

Results and discussion

After the methanol evaporated (passively at room temperature) the DIP was directly inserted into the mass spectrometer via the VPI, and the probe containing the sample was subjected to the DIP temperature program (Table 1). As the quartz crucible was heated at a programmed temperature rate, the sample components volatilized and were ionized using EI. The EI generated spectra can be used for putative compound identification using spectral libraries such as NIST. An example of the total ion chromatogram (TIC) obtained in EI is shown in Figure 2.

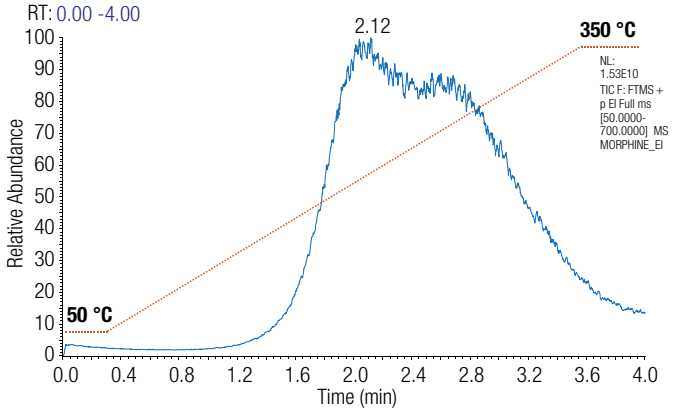
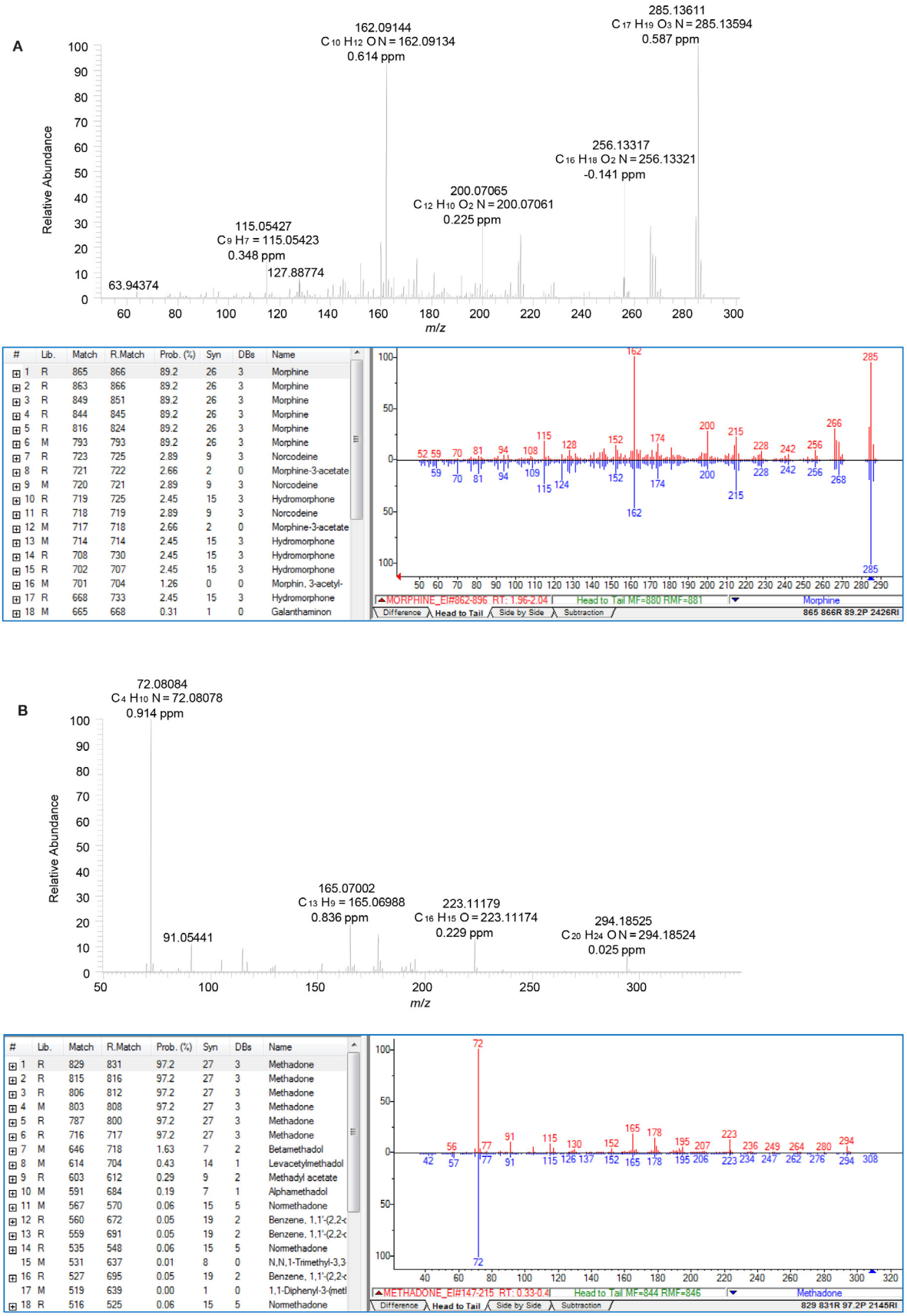


Figure 2. TIC of morphine analyzed using DIP-Orbitrap MS. The red dotted line represents the DIP probe programmed temperature rate. The components of the sample loaded are separated according to their boiling points up to highest masses selected in the full scan acquisition method. Data was acquired in full scan using EI and 60,000 resolution.

The EI data obtained can be used for candidate compound identification against existing commercial libraries. Automatic comparison of the EI spectra against the NIST 2017 library resulted in putative identification of morphine and methadone with an SI score of >830 and probability >87% (Figure 3).

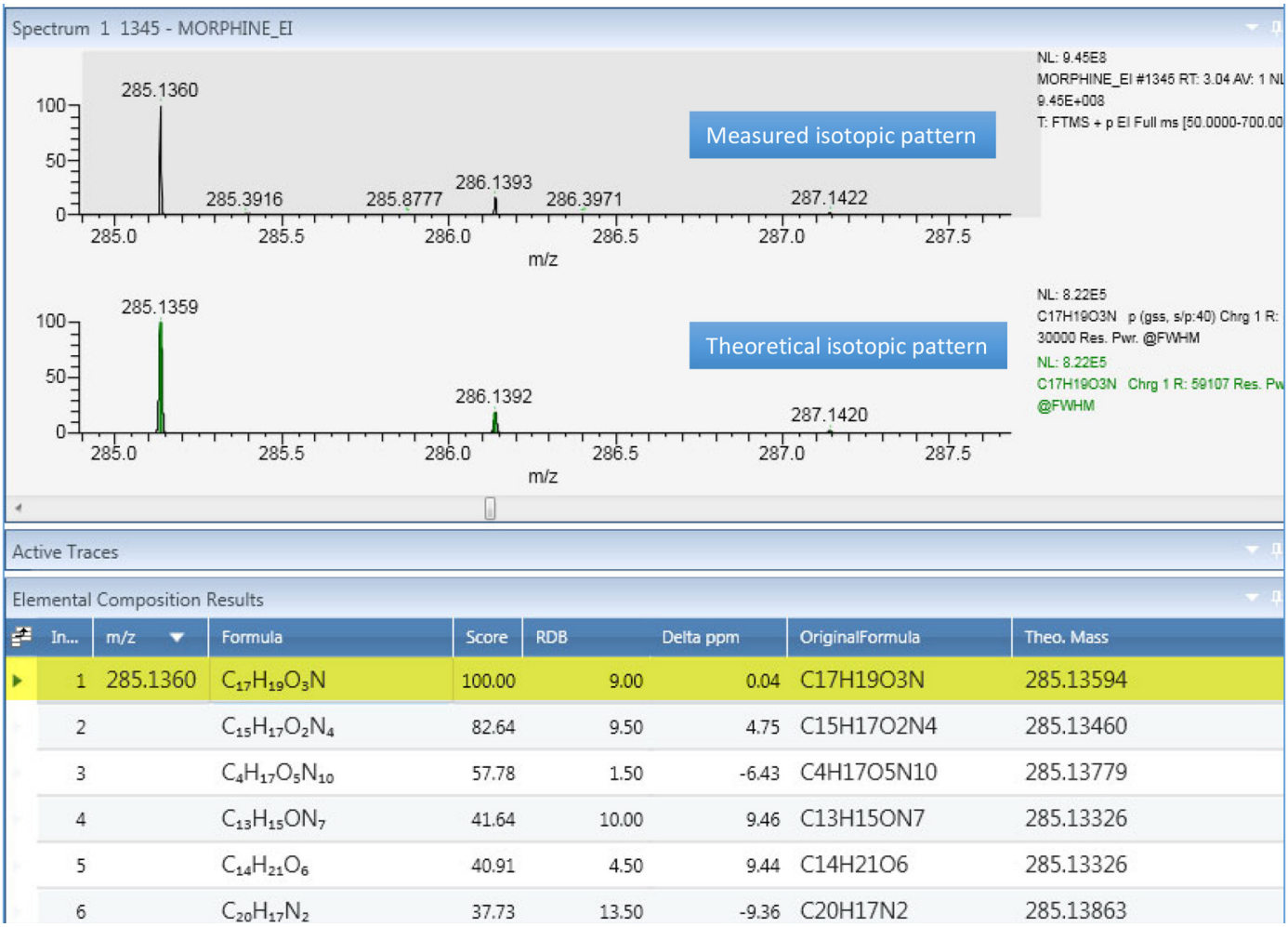
The compounds proposed from the NIST search were then further assessed using the fragmentation pattern, mass accuracy, and isotopic ratios to confirm the proposed elemental composition for morphine and methadone. As shown in Figure 4, a number of elemental compositions are proposed based on the accurate mass. In addition to mass accuracy, the isotopic fidelity on the Orbitrap mass analyzer allows for further confirmation based on the isotopic pattern of the proposed formula with the measured pattern. These additional identification points allow the correct formula ($C_{17}H_{19}O_3N$, highlighted in yellow in Figure 4) to be the top hit with a mass accuracy of 0.04 ppm and a 100% match of the measure isotope pattern with the theoretical. The other hits, although viable based on accurate mass, do not have the same isotope pattern agreement as the correct answer. These features allow for a fast and confident identification of unknown compounds.



#	Lib.	Match	R.Match	Prob. (%)	Syn	DBs	Name
1	R	865	866	89.2	26	3	Morphine
2	R	863	866	89.2	26	3	Morphine
3	R	849	851	89.2	26	3	Morphine
4	R	844	845	89.2	26	3	Morphine
5	R	816	824	89.2	26	3	Morphine
6	M	793	793	89.2	26	3	Morphine
7	R	723	725	2.89	9	3	Norcodeine
8	R	721	722	2.66	2	0	Morphine-3-acetate
9	M	720	721	2.89	9	3	Norcodeine
10	R	719	725	2.45	15	3	Hydromorphone
11	R	718	719	2.89	9	3	Norcodeine
12	M	717	718	2.66	2	0	Morphine-3-acetate
13	M	714	714	2.45	15	3	Hydromorphone
14	R	708	730	2.45	15	3	Hydromorphone
15	R	702	707	2.45	15	3	Hydromorphone
16	M	701	704	1.26	0	0	Morphin, 3-acetyl-
17	R	668	733	2.45	15	3	Hydromorphone
18	M	665	668	0.31	1	0	Galanthaminon

#	Lib.	Match	R.Match	Prob. (%)	Syn	DBs	Name
1	R	829	831	97.2	27	3	Methadone
2	R	815	816	97.2	27	3	Methadone
3	R	806	812	97.2	27	3	Methadone
4	M	803	808	97.2	27	3	Methadone
5	R	787	800	97.2	27	3	Methadone
6	R	716	717	97.2	27	3	Methadone
7	M	646	718	1.63	7	2	Betamethadol
8	M	614	704	0.43	14	1	Levacetylmethadol
9	R	603	612	0.29	9	2	Methadyl acetate
10	M	591	684	0.19	7	1	Alphamethadol
11	M	567	570	0.06	15	5	Normethadone
12	R	560	672	0.05	19	2	Benzene, 1,1'-(2,2-c
13	R	559	691	0.05	19	2	Benzene, 1,1'-(2,2-c
14	R	535	548	0.06	15	5	Normethadone
15	M	531	637	0.01	8	0	N,N,1-Trimethyl-3,3-
16	R	527	695	0.05	19	2	Benzene, 1,1'-(2,2-c
17	M	519	639	0.00	1	0	1,1-Diphenyl-3-(metl
18	R	516	525	0.06	15	5	Normethadone

Figure 3. EI mass spectra, corresponding accurate masses (ppm) and elemental composition. NIST library spectral match showing methadone (A) and morphine (B) as top hits with SI scores >830. Data acquired in EI at 60,000 resolution (FWHM, at *m/z* 200).



In...	m/z	Formula	Score	RDB	Delta ppm	OriginalFormula	Theo. Mass
1	285.1360	$C_{17}H_{19}O_3N$	100.00	9.00	0.04	C17H19O3N	285.13594
2		$C_{15}H_{17}O_2N_4$	82.64	9.50	4.75	C15H17O2N4	285.13460
3		$C_4H_{17}O_5N_{10}$	57.78	1.50	-6.43	C4H17O5N10	285.13779
4		$C_{13}H_{15}ON_7$	41.64	10.00	9.46	C13H15ON7	285.13326
5		$C_{14}H_{21}O_6$	40.91	4.50	9.44	C14H21O6	285.13326
6		$C_{20}H_{17}N_2$	37.73	13.50	-9.36	C20H17N2	285.13863

Figure 4. Elemental composition and isotopic pattern comparison for morphine indicating clearly $C_{17}H_{19}O_3N$ as the most likely elemental composition with a mass accuracy of 0.04 ppm and an isotopic pattern score of 100% (direct match between measured versus theoretical isotopic match)

To further increase the confidence in compound identification, methadone and morphine samples were analyzed using positive chemical ionization (PCI) and negative chemical ionization (NCI) with methane as reagent gas. The source exchange from EI to CI is performed without breaking vacuum and the system is operational within minutes.

Chemical ionization is essential to be able to identify the molecular ion in a mass spectrum. Chemical ionization is key for molecular ion confirmation of a compound molecular mass as it is readily identified from the *m/z* value of the molecular ion adducts. Chemical ionization is critically important when chromatography is not used and pure chemical standards to check compound identity are not available. Low eV (or soft EI) alone would not be sufficient to identify the molecular ion, as without the mass tag of the ion adduct (ex: M^+H, $M^+C_2H_5$), it is possible that the largest mass in the spectrum could simply be a high mass fragment. This would lead to incorrect identification, a time-consuming process and low confidence in the compound characterization.

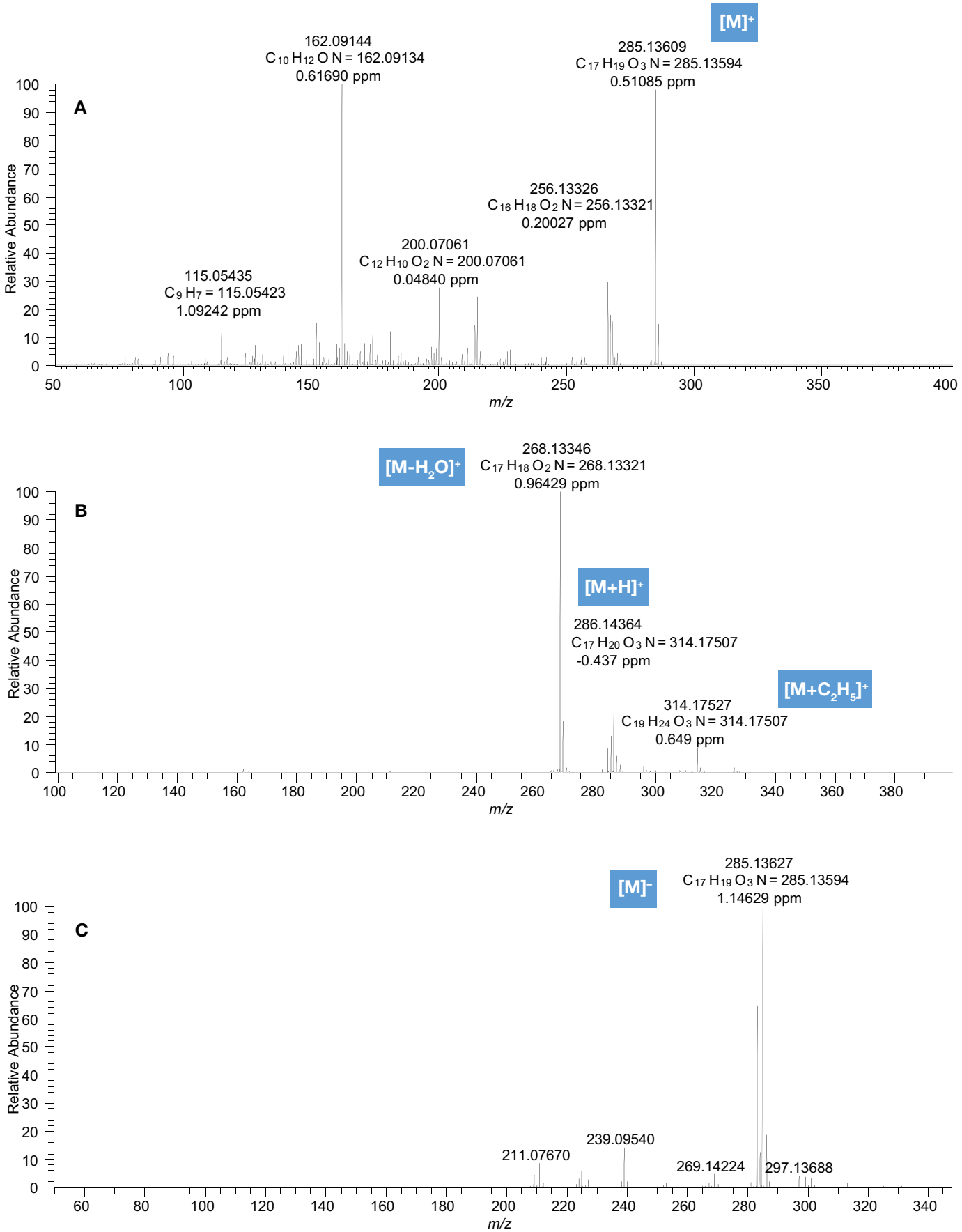


Figure 5. Confirmation of morphine-based mass accuracy measurements: (A) electron ionization mass spectrum, (B) positive chemical ionization mass spectrum, and (C) negative chemical ionization mass spectrum, annotated with mass (*m/z*), elemental composition, theoretical mass, and delta (ppm). Morphine molecular ion confirmed based on adducts formed ($[M+H]^+$, $[M+C_2H_5]^+$, $[M]^-$) as well as excellent mass accuracy.

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Conclusions

These results demonstrate that the direct insertion probe in combination with the high-resolution mass spectrometry on the Exactive GC platform offers a powerful solution for the analysis of unknowns in suspicious samples. Using this approach, routine analysis of thermally labile or polar chemicals can be performed with ease and without the need of chromatographic separation.

Spectra obtained using electron ionization can provide immediate clues on the possible identity of unknown chemicals though spectral library matching.

Additionally, the power of high resolution and sub ppm mass accuracy together with isotopic pattern comparison can further increase the confidence in compound identification.

Importantly, availability of soft ionization such as PCI and/or NCI allows for molecular ion confirmation of putatively identified compounds with timely, vent-free exchange from electron ionization via the vacuum probe interlock option.

References

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2. Thermo Fisher Scientific WP10456: The Power of High Resolution Accurate Mass Using Orbitrap Based GC-MS (https://assets.thermofisher.com/TFS-Assets/CMD/Reference-Materials/WP-10456-GC-MS-Orbitrap-High-Resolution-Accurate-Mass-WP10456-EN.pdf)
3. Thermo Fisher Scientific DEP/DIP User Guide: https://assets.thermofisher.com/TFS-Assets/CMD/manuals/ISQ-TSQ8000-Direct-Probe-User-Guide.pdf

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