Application Note 10494

Impurity Profiling of Pharmaceutical Starting Materials Using Gas Chromatography Coupled with High-Resolution Accurate Mass Spectrometry

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Key Words
Pharmaceutical active ingredients, impurities, accurate mass, high resolution, Q Exactive GC

Introduction

Pharmaceutical impurities are unwanted chemicals present in starting and intermediate materials used in the manufacturing of active pharmaceutical ingredients (API) which, even in small amounts, can affect the efficiency of the pharmaceutical product and ultimately can pose health risks to patients.[1] In general, most of these impurities are small molecules formed during the manufacturing process of the API or originated from the contact of the active ingredient with the packaging materials.

The impurity profiling process (defined as compound detection, identification and quantitation) is currently a mandatory step in the manufacturing of pharmaceutical products and is receiving rigorous attention from regulatory authorities, such as the International Conference Harmonization (ICH), United States Food and Drug Administration (FDA).

The large number and diversity of impurity compounds that can be present in starting and intermediate materials poses a significant analytical challenge for detection, quantitation and chemical characterization of these chemicals. Amongst the various analytical tools used to detect and characterize impurities in API, gas chromatography coupled with mass spectrometry (GC-MS), is commonly used to detect volatile and semi-volatile chemicals throughout the active pharmaceutical ingredients manufacturing process, as well as in the final product.

Until recently, GC-MS analysis of impurities has traditionally been performed by Electron Ionization (EI) or Chemical Ionization (CI) on single-quadrupole systems. However, developments of Time-of-flight mass spectrometry (TOFMS) technology have allowed high resolution accurate mass measurement to be utilized in this application.[2] In addition to EI and CI, soft Ionization techniques, such as Atmospheric Pressure Chemical Ionization (APCI) coupled with ToF mass spectrometers have been applied to GC-HRMS analysis. However, these systems have limited linear dynamic range and higher chemical background noise compared to vacuum GC-MS systems.[3] Moreover, the incompatibility of the mass spectra obtained with the APCI-GC-MS with existing commercial mass spectral libraries makes compound identification difficult.[3]

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This study describes the capability of the Thermo Scientific™ Q Exactive™ GC Orbitrap™ GC-MS/MS system for the detection and structural characterization of a number of small organic molecules (and their associated impurities) used in synthesis of pharmaceutical active ingredients. Here it is demonstrated that the Q Exactive GC mass spectrometer delivers high resolving power and routine accurate mass measurements over a large range of concentrations and that reliable quantitative results are easily obtained. The outstanding analytical performance of this instrument was combined with an automatic compound detection, identification and quantification workflow to deliver an ideal tool for impurity profiling of the starting and intermediate materials used in active pharmaceutical ingredients. This includes data acquisition using high resolution full-scan accurate mass using electron ionization, confirmation of the molecular ions of putatively identified compounds with chemical ionization, as well as compound quantification over a large concentration range. The data presented in this work was published in collaboration with a pharmaceutical partner.[5]

Experimental Conditions

Sample Preparation

The samples analyzed for their impurity content were both solid and liquid chemicals that represent a range of typical starting materials routinely used during the manufacturing of pharmaceutical products. Details of the samples, suppliers and solvent used to dilute them are given in Table 1. The liquid samples, such as (3S)-3-methylmorpholine (SAFC®, Buchs, Switzerland), N,N,N'-trimethylethylenediamine and 1,2-A imidazo pyridine were diluted in methanol to a working solution of 1% v/v. The solid samples, such as 4-fluorobenzonitrile, 3,5-difluorophenol, and 2,6-difluorobenzyl bromide were diluted to 100 µg/mL (w/v) in methanol Optima™ LC/MS grade (Fisher Scientific, UK). The 2,6-diflurobenzyl bromine sample was further diluted to 2.5 µg/mL (w/v) in methanol. Also, N,N,N'-trimethylethylenediamine was diluted in DMSO (Fisher BioReagents, Fisher Scientific, Loughborough, UK) (to 1% and serial dilution to 0.0001% v/v).

Table 1. Samples analyzed.

API	Phase	Solvent	Working Concentration
(3S)-3-methylmorpholine	liquid	Methanol	1% v/v
N,N,N'-trimethylethylenediamine	liquid	DMSO	1% v/v
1,2-A imidazo pyridine	liquid	Methanol	1% v/v
4-fluorobenzonitrile	solid	Methanol	100 µg/mL (w/v)
3,5-diflurophenol	solid	Methanol	100 µg/mL (w/v)
2,6 diflurobenzyl bromide	solid	Methanol	100 µg/mL (w/v)

GC-MS Analysis

The impurities in the samples were analyzed using the Q Exactive GC Orbitrap GC-MS/MS mass spectrometer. Sample introduction was performed using a Thermo Scientific™ TriPlus™ RSH™ autosampler, and chromatographic separation was obtained with a Thermo Scientific™ TRACE™ 1310 GC system. All samples, except N,N,N'-trimethylethylenediamine, were analyzed on a Thermo Scientific™ TraceGOLD™. 30 m × 0.25 mm I.D. × 0.25 µm film capillary column (P/N 26096-1420), using a flow rate of 1.0 mL/min of helium with an inlet temperature of 250 °C, oven temperature program from 50 to 320 °C at 20 °C/min. For the N,N,N'-trimethylethylenediamine impurity analysis the column used was a Stabilwax®-DB 30 m × 0.32 mm × 0.5 µm (Restek®) (equivalent TraceGOLD TG-WaxMS B, Thermo Scientific can also be used) with a flow rate of 1.0 mL/min of helium, an injector temperature of 250 °C and a GC oven gradient from 50 °C held for ten minutes to 200 °C at 45 °C/min. A 1µL injection was used with a split ratio of 40:1. The EI source was operated at 70 eV at a temperature of 250 °C with a GC transfer line temperature of 250 °C. Positive chemical ionization (PCI) experiments were performed when methane was used as reagent gas at 1.0 mL/min. Internal mass correction was achieved using a background ion as a lock mass (*m/z* 207.03235) (Table 2).

Table 2. Mass spectrometer parameters.

Q Exactive GC Mass Spectrometer Parameters	
Transfer Line (°C):	250
Ionization:	EI & PCI
Ion Source:	250 (EI) & 185 (PCI)
Electron Energy (eV):	70
Acquisition Mode):	full scan
Mass Range (Da):	50–750
Mass Resolution (FWHM at *m/z* 200):	60 k
Lock Mass, Column Bleed (*m/z*):	207.03235

Data Acquisition and Processing

Data was acquired in full-scan high resolution and processed using the Thermo Scientific™ TraceFinder™ software for automatic peak detection, spectral deconvolution, elemental composition elucidation and quantitation assessment.

Results and Discussion

Automatic Compounds Detection and Identification Workflow

Each sample was acquired using full-scan with a default instrumental resolving power set to 60,000 (FWHM at *m/z* 200). Electron ionization data was used for tentative compound identification using existing commercial libraries (NIST), whereas positive chemical ionization with methane was used to confirm the compound molecular ions. In addition to this, MS/MS experiments were performed using PCI in order to confirm the chemical structure of the impurities detected. For all experiments, a solvent blank injection was used as reference background for binary data comparison (sample versus solvent blank). An example of the total ion current chromatograms (TIC) obtained is shown in Figure 1 for the (3S)-3-methylmorpholine sample.

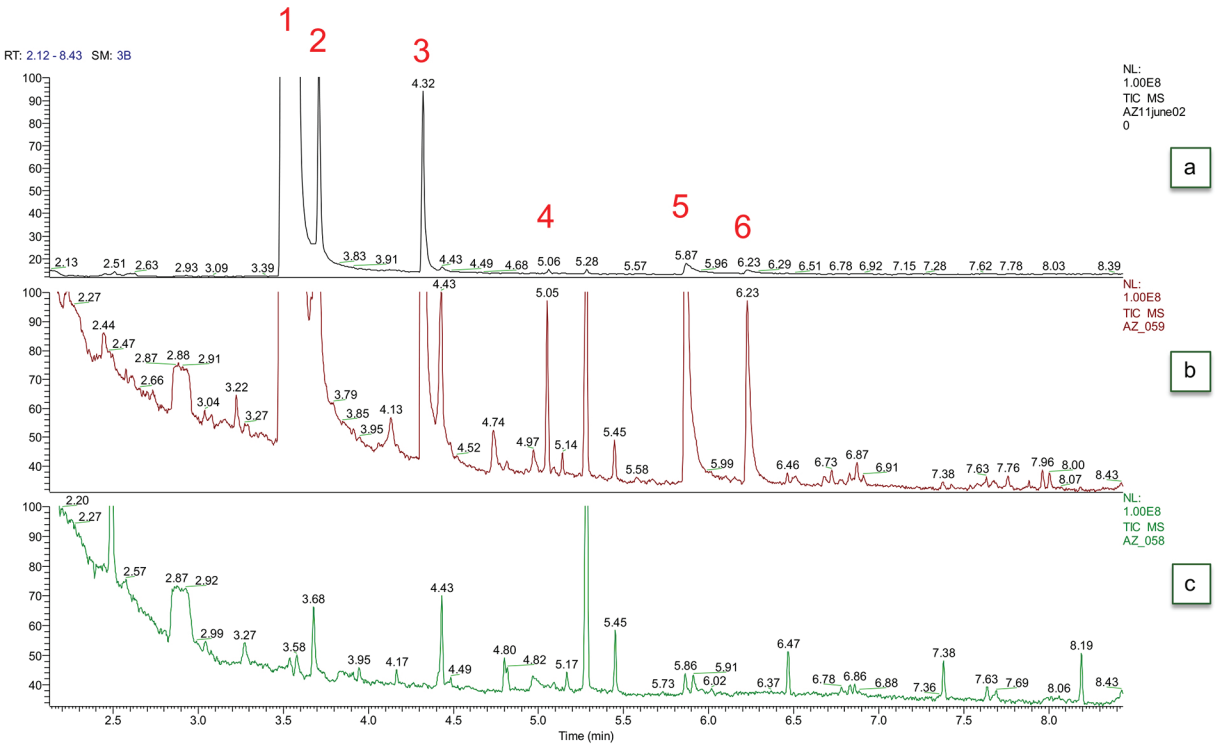
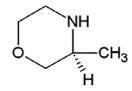
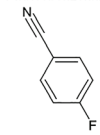
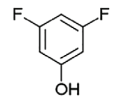
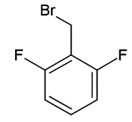
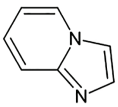
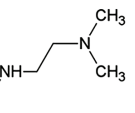


Figure 1. TICs showing the (3S)-3-methylmorpholine peak at 10 µg/mL (w/v) on column (1) and several major impurities (2-6) at >0.1% level. Data acquired in full-scan EI (a) and PCI (b) using 60,000 resolution (FWHM at *m/z* 200). A solvent (methanol) blank injection (c) was used as a reference background.

All molecular ions of the starting and intermediate materials ingredients were confidently confirmed with mass accuracies of <1ppm using both EI and PCI (Table 3).

The TICs obtained were refined using the spectral deconvolution function in TraceFinder to detect impurity at levels greater than 0.1% peaks (according to ICH guidelines) and generate clean mass spectra. TraceFinder uses a unique high-resolution filtering (HRF) score to refine low library searches using accurate mass. For each compound with a library match, an HRF score is derived and it represents the relative number of explainable ions in the measured spectra as compared to the proposed elemental composition of the library match.[4] An example of compound detection and identification is shown in Figure 2 where 4-fluorobenzonitrile was detected only in the sample (AZ15june001) and was absent in the solvent blank (AZ15june000). Deconvoluted mass spectra (e) was searched against the NIST library (d) for compound identification and a validation of the library hits (b) was made using the accurate mass data and elemental composition (f).

Table 3. Accurate mass confirmation of the molecular ions for the samples analyzed. Data obtained from the EI was confirmed using PCI experiments. The compounds' chemical structures as well as the mass difference from the theoretical (Δppm) are annotated.

Active Pharmaceutical Ingredient	Exact Mass (EI)	Measured Mass (EI)	Δppm	Exact Mass (PCI)	Measured Mass (PCI)	Δppm
(3*S*)-3-methylmorpholine  Molecular Formula = $C_5H_{11}NO$	101.08352	101.08358	0.6	102.09134	102.09136	0.2
4-fluorobenzonitrile  Molecular Formula = C_7H_4FN	121.03223	121.03223	0.0	122.04005	122.04012	0.5
3,5-difluorophenol  Molecular Formula = $C_6H_4F_2O$	130.02247	130.02256	0.7	131.03030	131.03032	0.2
2-(bromomethyl)-1,3-difluorobenzene  Molecular Formula = $C_7H_5BrF_2$	204.94625	204.94608	-0.9	206.96155	206.96162	0.3
imidazo[1,2-*a*]pyridine  Molecular Formula = $C_7H_6N_2$	118.05255	118.05256	0.1	119.06037	119.06038	0.0
N,*N*,*N*'-trimethylethane-1,2-diamine  Molecular Formula = $C_5H_{14}N_2$	102.11515	102.11520	0.5	103.1073	103.12300	0.3

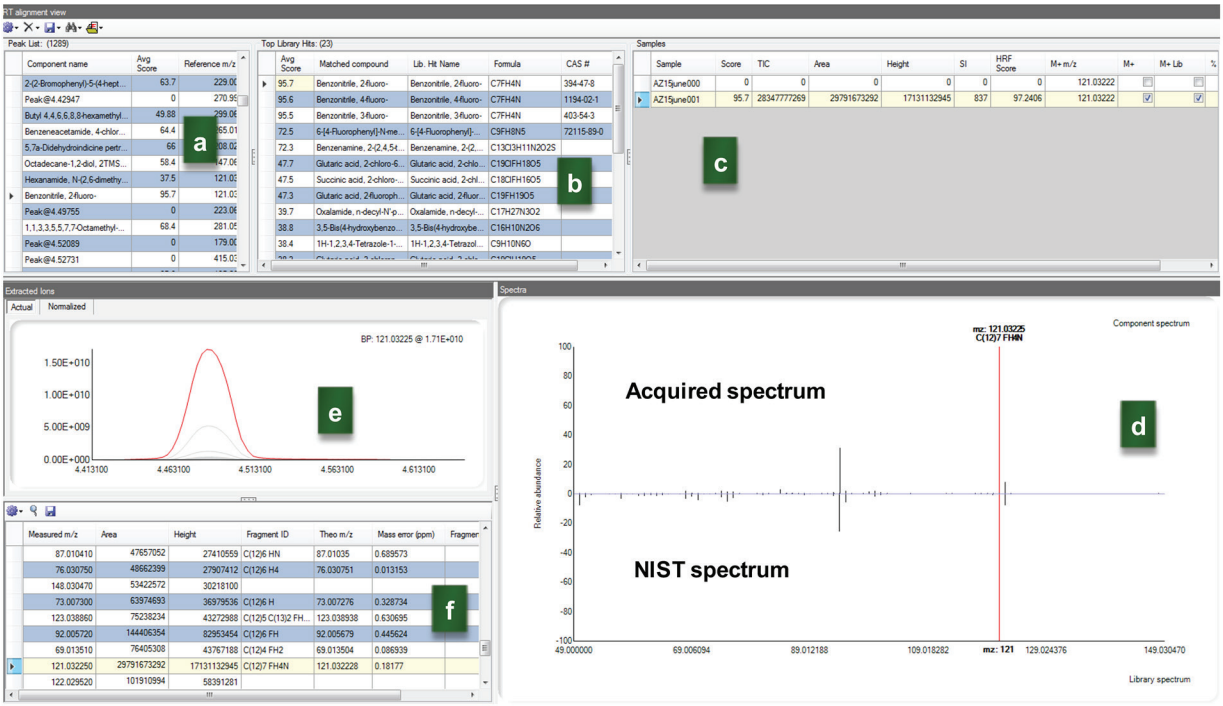


Figure 2. Impurities detection and identification in a 4-fluorobenzonitrile sample using TraceFinder. Spectral deconvolution of the TIC, library search index and the HRF score together with additional criteria are used for intelligent compound identification.

Several impurities were detected in the (3S)-3-methylmorpholine sample. Proposed tentative impurities identification from the deconvolution software were validated using PCI data. The molecular ions observed in EI and PCI for the compounds detected are shown with the mass measurement error from theoretical in Table 4. All measurements made were within 1 ppm mass accuracy which resulted in a significant reduction of the number of possibilities in assigning molecular formula taking into account the most common chemical elements (C, H, O, N, S and P). All determined chemical formulae of the (3S)-3-methylmorpholine impurities (including impurities without baseline resolution) were assigned to structures that could be justified as being impurities generated in the manufacturing process.

Table 4. Accurate mass measurements of the molecular ions for the main impurities observed in (3S)-3-methylmorpholine. Data obtained by EI and PCI at 60,000 resolution (FWHM at *m/z* 200).

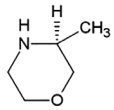
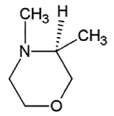
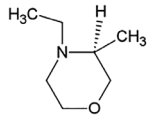
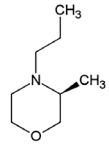
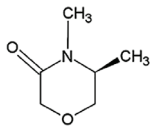
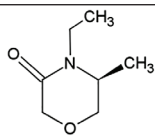
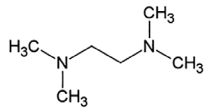
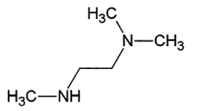
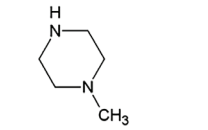
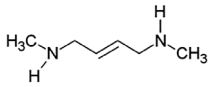
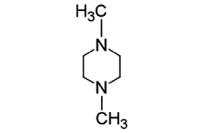
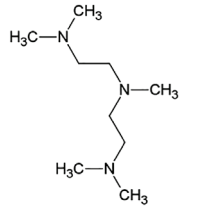
Retention Time (min)	Compound ID	Exact Mass (EI)	Measured Mass (EI)	Δppm	Exact Mass (PCI)	Measured Mass (PCI)	Δppm
3.55	 (3*S*)-3-methylmorpholine Molecular Formula = $C_5H_{11}NO$	101.08352	101.08358	0.6	102.09134	102.09136	0.2
3.75	 Molecular Formula = $C_6H_{13}NO$	115.09917	115.09925	0.7	116.10699	116.10703	0.4
4.32	 Molecular Formula = $C_7H_{15}NO$	129.11482	129.11486	0.4	130.12264	130.12268	0.3
5.06	 Molecular Formula = $C_8H_{17}NO$	155.13047	155.13048	0.1	156.13829	156.13825	0.3
5.87	 Molecular Formula = $C_6H_{11}NO_2$	129.07843	129.07843	0.0	130.0862	130.08634	0.3
6.23	 Molecular Formula = $C_7H_{13}NO_2$	143.09408	143.09414	0.4	144.10193	144.10193	0.2

Table 5. Accurate mass measurements obtained by EI and PCI at 60,000 resolution (FWHM at *m/z* 200) for impurities observed in N,N,N'-trimethylethylenediamine.

Retention Time (min)	Compound ID	Exact Mass (EI)	Measured Mass (EI)	Δppm	Exact Mass (PCI)	Measured Mass (PCI)	Δppm
2.79	 Molecular Formula = $C_6H_{16}N_2$	116.13080	116.13085	0.5	n.d.	n.d.	n/a
3.20	 Molecular Formula = $C_5H_{14}N_2$	102.11515	102.11520	0.5	103.1230	103.12300	0.3
3.67	 Molecular Formula = $C_5H_{12}N_2$	99.09167	99.09171	0.4	101.10730	101.10733	0.1
4.52	 Molecular Formula = $C_6H_{14}N_2$	113.10730	113.10736	0.3	115.1230	115.12311	1.2
5.00	 Molecular Formula = $C_6H_{14}N_2$	114.1151	114.11524	0.8	115.12298	115.12304	0.5
12.4	 Molecular Formula = $C_9H_{23}N_3$	173.18865	n.d.	n.a.	174.19647	174.19658	0.6

Furthermore, confirmation of the chemical structures of the impurities tentatively identified was made using PCI-MS/MS experiments. For this, the protonated molecular ions of the impurities were selected and fragmented in the higher energy collisionally activated dissociation HCD cell using 15V energy. By assessing the resulting fragment ions it was possible to assign a chemical structure to each of the measured mass in addition to the accurate mass information, all these allowing for unambiguous compound structural elucidation. An example of PCI-MS/MS spectrum is shown in Figure 3 for two impurities detected in the (3S)-3-methylmorpholine sample.

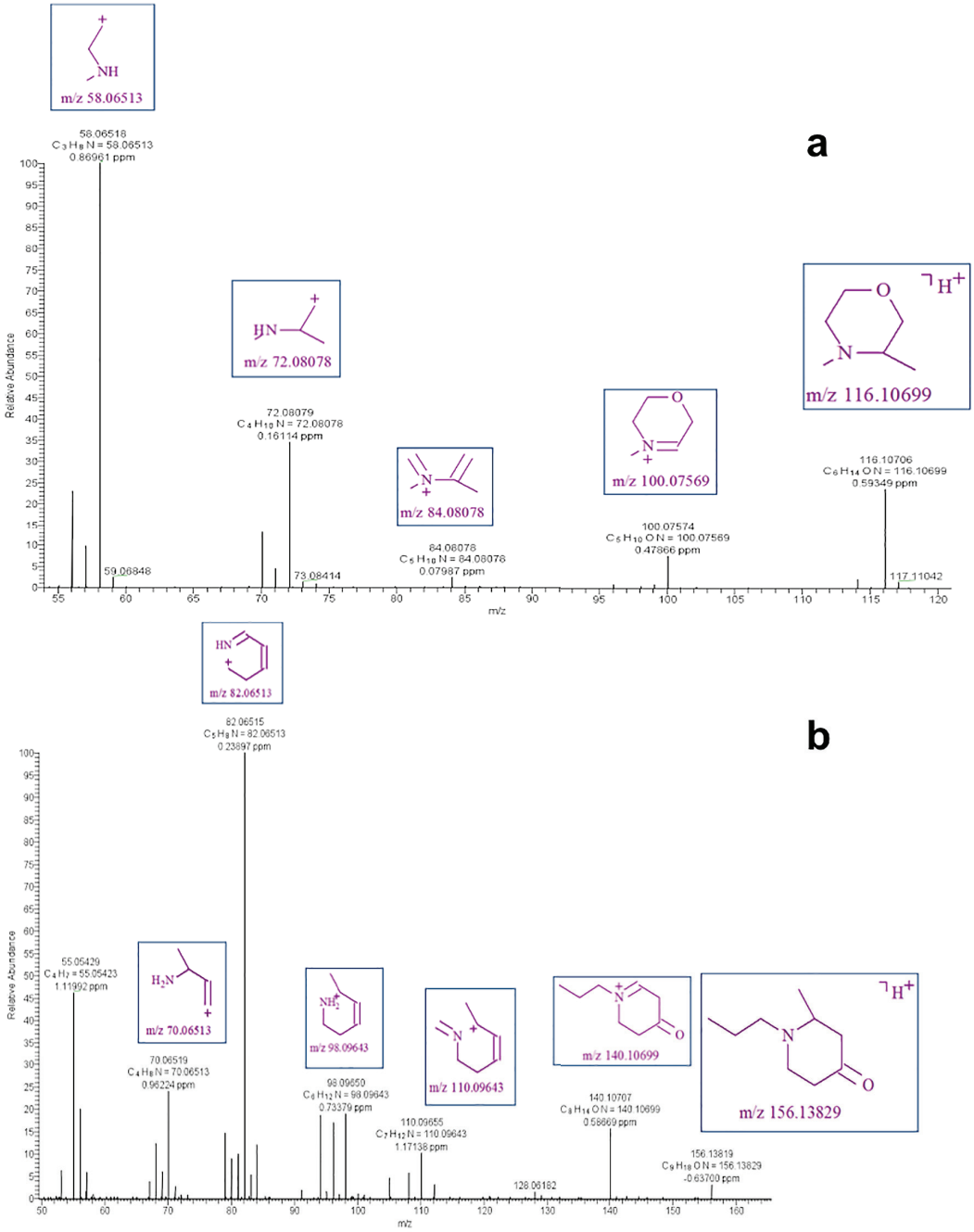
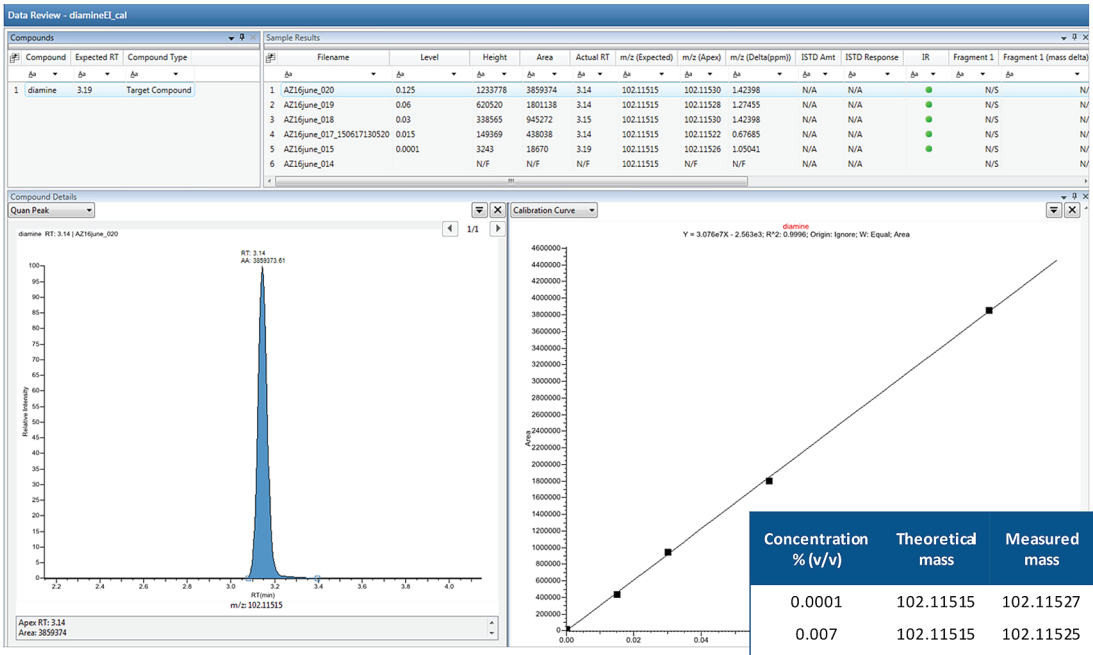


Figure 3. PCI-MS/MS of impurity eluting at RT=3.70 min (a) and RT=5.06 min (b).

Linearity

Determining the concentration of impurities in the starting and intermediate materials used for API manufacturing is often required and for this reliable quantification should be obtained. For this reason, the performance of the Q Exactive GC system for compound quantification was tested in terms of the linearity of the method as well as the number of scans/peak at various resolution settings.

An example of compound linearity, is shown below for N,N,N'-trimethylethylenediamine. The linear dynamic range of N,N,N'-trimethylethylenediamine extended from 0.0001 to 0.1250 % (v/v in DMSO) with a correlation coefficient r^2 >0.9996 (at 60,000 RP at *m/z* 200) using EI and the peak area of *m/z* 102.11515 (molecular ion). Noticeable, the mass accuracy was <1.1ppm at each concentration level (Figure 4).



Concentration % (v/v)	Theoretical mass	Measured mass	Mass difference [ppm]
0.0001	102.11515	102.11527	1.1
0.007	102.11515	102.11525	0.9
0.015	102.11515	102.11525	0.9
0.03	102.11515	102.11526	1.0
0.06	102.11515	102.11525	0.9
0.125	102.11515	102.11524	0.9

Figure 4. Linearity of N,N,N'-trimethylethylenediamine across a concentration range of 0.0001 to 0.1250% (v/v in DMSO). Extraction ion chromatogram of the quantification ion (*m/z* 102.11515) with the corresponding peak area, the linear regression with the coefficient of determination (r^2), as well as the mass accuracy measured at each concentration level are shown. Data is acquired at a resolution of 60,000 FWHM (at *m/z* 200) using EI.

Fast Scan Speed and Consistent Mass Accuracy

Reliable impurities quantitation requires accurate peak integration and this, in turn, is directly related to the number of scans (points) across a chromatographic peak. To demonstrate that the Q Exactive GC system is able to scan fast enough, even at the highest resolution,[2,6] difluorobenzyl bromide was used. A standard solution of 2.5 µg/mL (w/v) was acquired in full-scan PCI using various resolution settings. The main component peak has a width of three seconds under the chromatographic conditions used. Figure 5 shows the effect of altering the resolution setting has on the number of scans across a chromatographic peak, and that even at the highest resolution (120,000 FWHM at *m/z* 200) sufficient scans are obtained for accurate peak integration. Additionally, Figure 6 shows the mass accuracy for each scan across the peak acquired at 60,000 resolution, all measurements are <0.3 ppm showing excellent scan-to-scan mass accuracy.

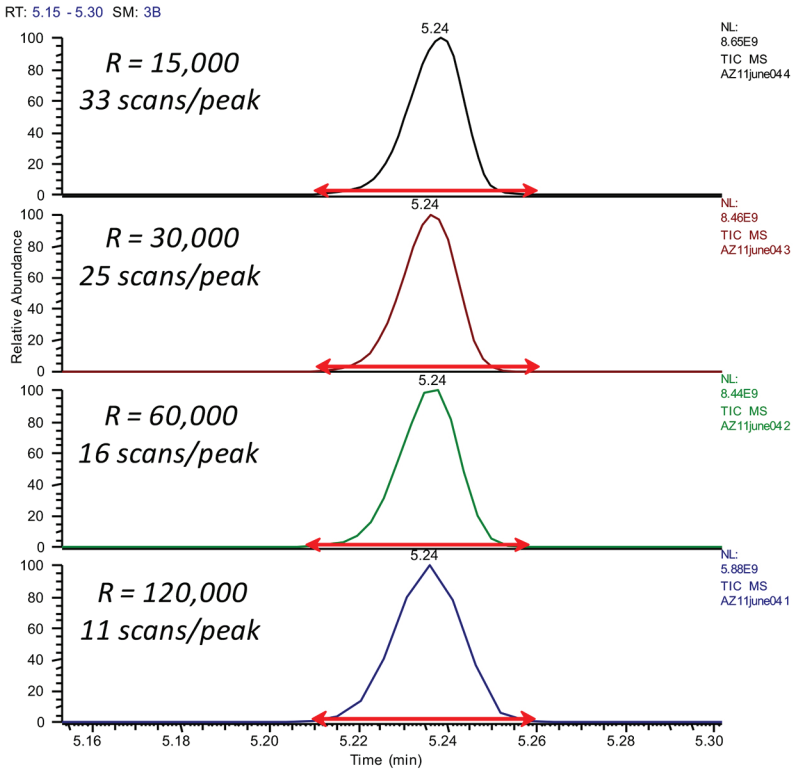


Figure 5. Achieving enough scans/peak at high resolving power. Accurate peak integration is ensured by obtaining enough scans even at high resolving powers of 120,000 FWHM for a 3 second wide peak (2,6-difluorobenzyl bromide, XIC of base peak *m/z* 127.03583). Data acquired in PCI using methane as a reagent gas.

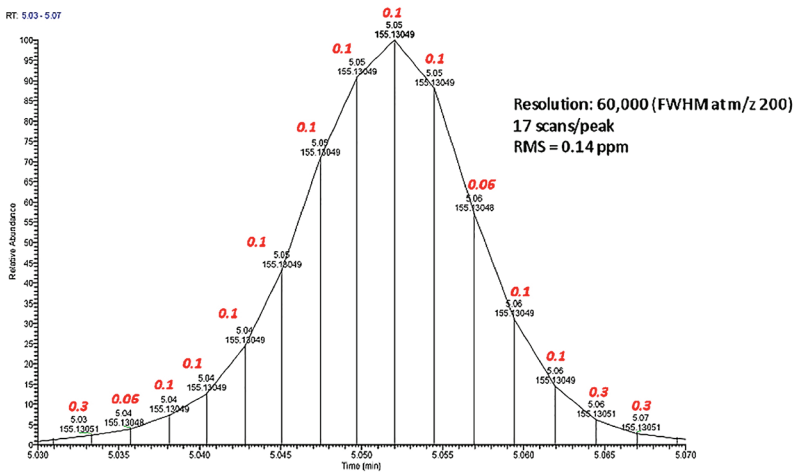


Figure 6. An example of the number of scans/peak obtained in EI at high resolving powers of 60,000 FWHM for a ~3 sec wide peak (impurity in 3,S-methyl morpholine eluting at RT= 5.06 min). Annotated in red is scan-to-scan mass error (in ppm). Calculated ppm RMS as well as the number of scans/peak obtained is indicated.

Conclusions

- The Thermo Scientific Q Exactive GC Orbitrap mass spectrometer has been evaluated for both qualitative and quantitative analysis of impurities present in starting and intermediate materials used for the manufacturing of active pharmaceutical ingredients.
- Automatic peak detection, spectral deconvolution and putative impurities identification were performed using TraceFinder software. Importantly, compound identification was taking into account the NIST library match score in addition to fragments rationalization using the accurate mass information and elemental composition of the proposed chemical.
- Excellent system sensitivity together with a wide dynamic range enabled the detection of both low and high level impurities with routinely achieved sub-ppm mass accuracy measurements allowing for clear assignment of chemical elemental composition of unknown compounds. Coupling these performance features with MS/MS experiments allowed for confident structural elucidation of the impurities detected in the samples.
- Scan speeds compatible with GC peaks, even at the highest resolution mode of 120,000, allowed for clear peak definition against a high background of chemical noise and reliable compound quantification.
- The Q Exactive GC system is a versatile tool allowing for both EI and PCI experiments to be rapidly performed and making this analytical platform a powerful tool with significant advantages to pharmaceutical industries for research and development.

References

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