



Quantitative analysis of genotoxic impurities by high resolution mass spectrometer

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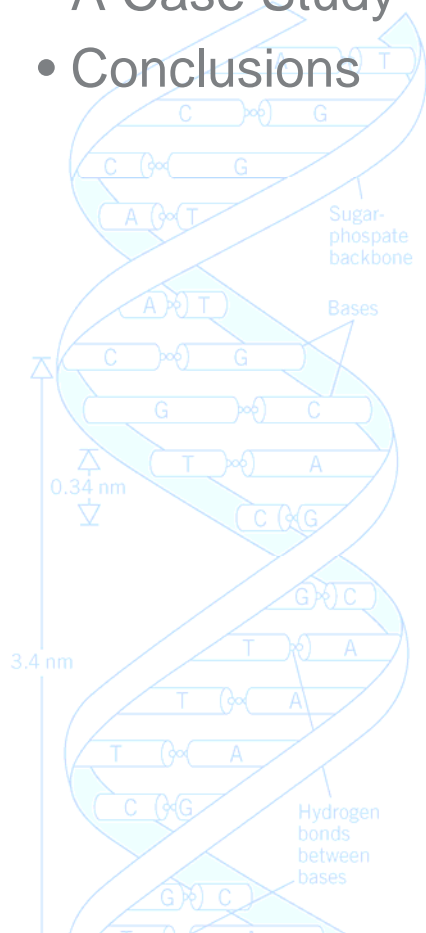
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Thermo Scientific Annual Mass Spectrometry Users Meeting

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Outlines

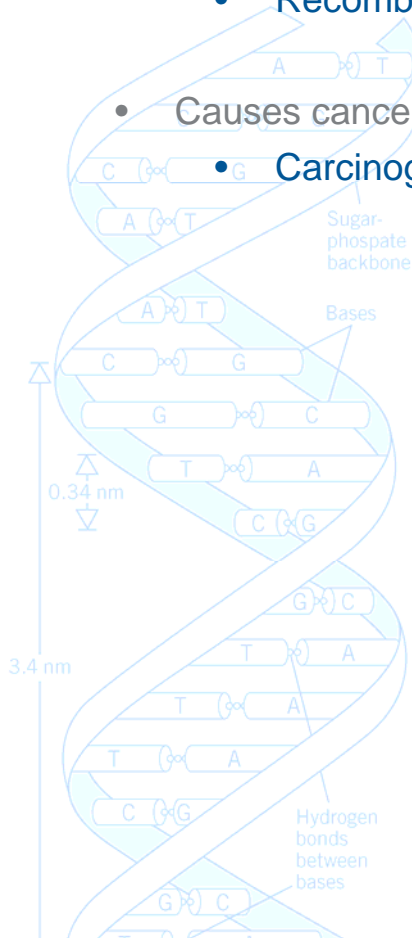
- Background of Genotoxic Impurities
- New Instrumentation
- A Case Study – Oncology Small Molecule
- Conclusions



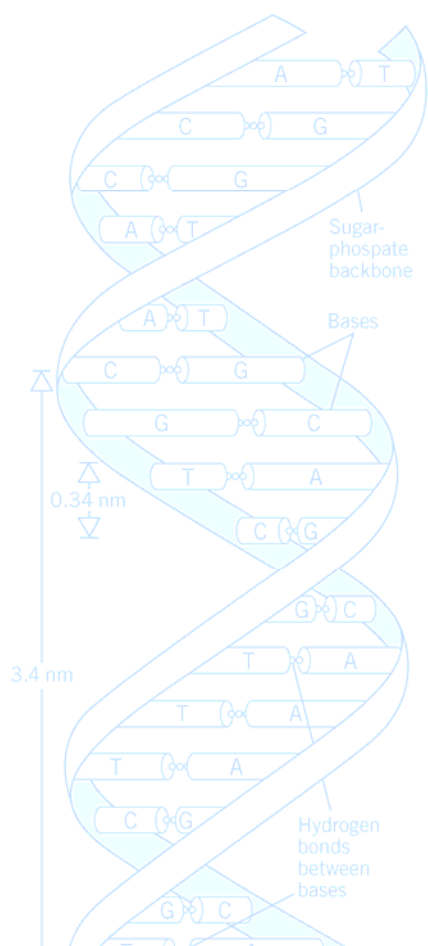
Genotoxicity

- Induces genetic damage and fixation
 - Gene mutation
 - Larger scale chromosomal damage
 - Recombination and numerical chromosome changes

- Causes cancer or heritable changes
 - Carcinogenicity more easily detected



Definition of impurity classifications



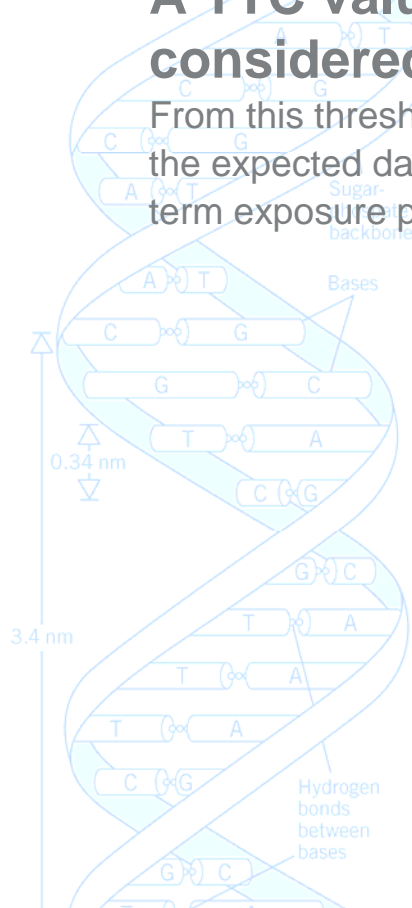
Impurity classification	Definition	Guidance on control of human exposure ^b
Category 1	Precedent for mutagenicity and carcinogenicity	<div style="text-align: center;"> <div>PDE or TTC</div> <div style="font-size: 2em;">↓</div> <div>ICH Q3</div> </div>
Category 2	Mutagens with unknown carcinogenic potential or a “close-in” structural analog	
Category 3	Alerting structure—unique and unknown mutagenic potential	
Category 4	Alerting structure—non-unique and qualified in comparison to API	
Category 5	No structural alerting features	

Reg Tox and Pharm, 2006

Threshold of Toxicological Concern (TTC)

A TTC value of 1.5 µg/day intake of a genotoxic impurity is considered to be associated with an acceptable risk.

From this threshold value, a permitted level in the active substance can be calculated based on the expected daily dose. Higher limits may be justified under certain conditions such as short-term exposure periods.



Case study – An oncology small molecule

- 6 Compounds with Structural Alerts (Category 3)

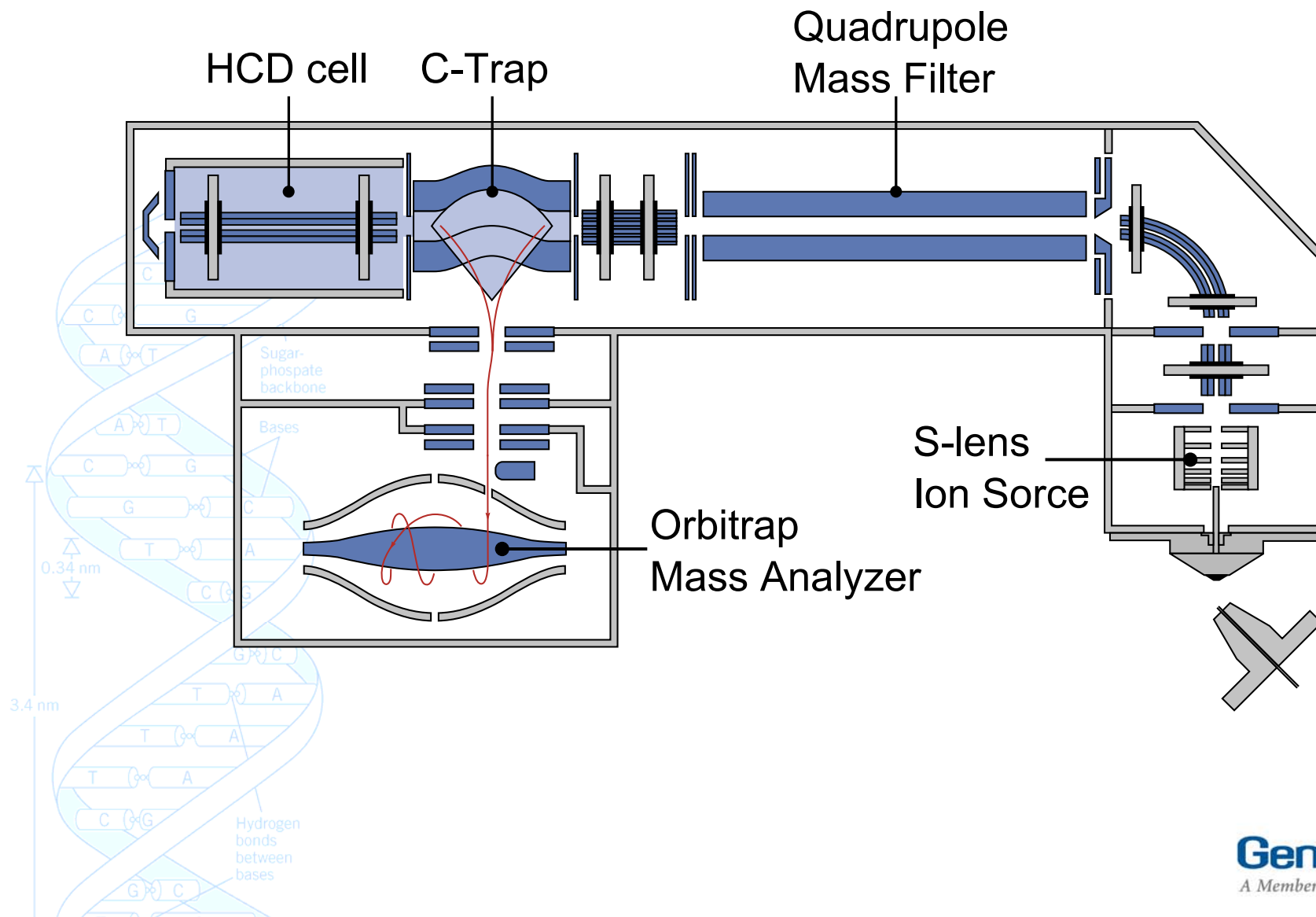
- Aromatic Nitro
- Aromatic Azo
- Aromatic Azoxy
- Aromatic Hydroxylamine
- Aromatic Amine/Amide

- Goal of the study

To develop a reproducible and sensitive LC-MS method to quantify whether the amounts of PGIs are above or below the controlled level in final API lot. (< 1.5 ug/day)

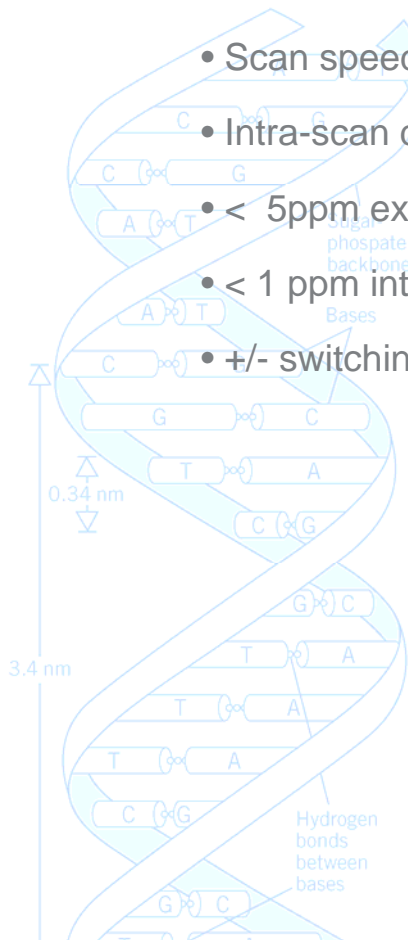


Schematic of Q Exactive™ Benchtop LC-MS/MS

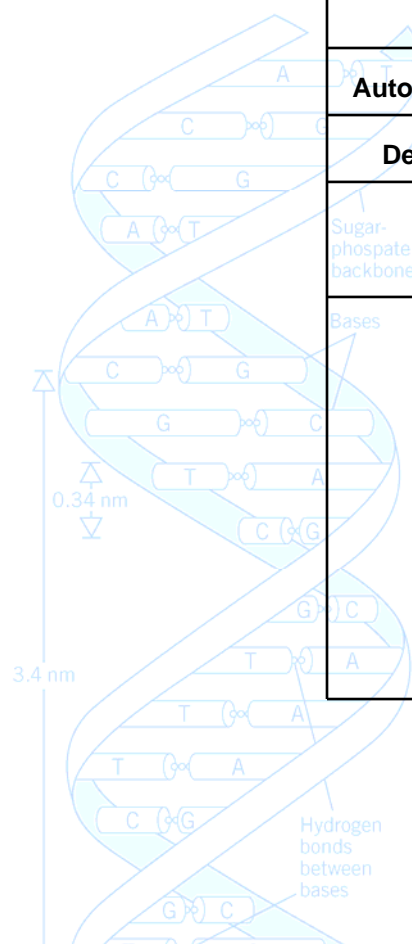


Q Exactive™ Specifications

- Mass range for full scans: $50 < m/z < 4000$
- Scan range: first mass $< m/z < 15 \times$ first mass
- Max resolution: 140,000
- Scan speed: up to 12 HZ
- Intra-scan dynamic range $> 5000:1$
- $< 5\text{ppm}$ external
- $< 1\text{ ppm}$ internal
- \pm switching within 1 sec



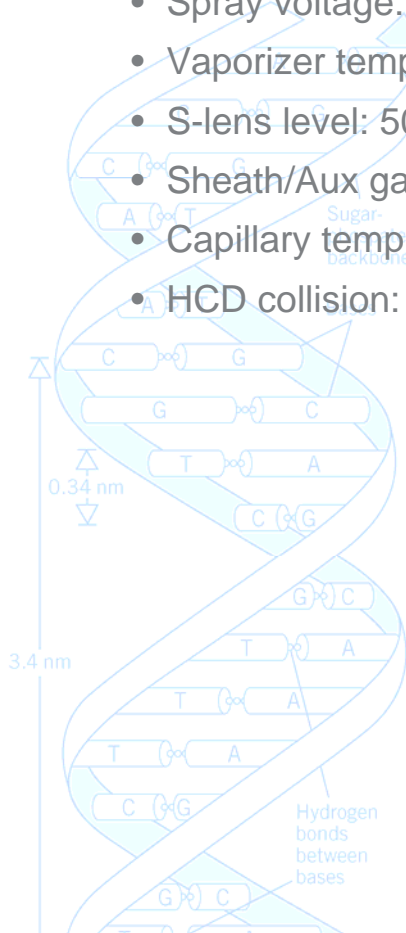
Chromatograph



Column	ACE 3 C18, 100x4.6mm		
Column temperature	25 °C		
Flow rate	1 mL/min		
Injection volume	5 ul		
Auto-sampler temperature	4 °C		
Detection wavelength	UV at 265 nm		
Mobile phase	A: 0.1% Acetic acid in water B: ACN		
Gradient	Time (min)	%A	%B
	0	90	10
	7	10	90
	9.5	10	90
	9.6	90	10
	14	90	10

Mass spectrometry

- Mass spectrometer: Thermo Scientific Q-Exactive benchtop high resolution, accurate mass system
- Resolution: $R=70,000$ FWHM at full scan; $R=35,000$ FWHM at MSMS
- Ion source: HESI-II, positive
- Spray voltage: 3.8 kV
- Vaporizer temp: 500 °C
- S-lens level: 50%
- Sheath/Aux gas: 50/15 units with N₂
- Capillary temp: 320 °C
- HCD collision: NCE 40



Experiments

Full MS Experiment

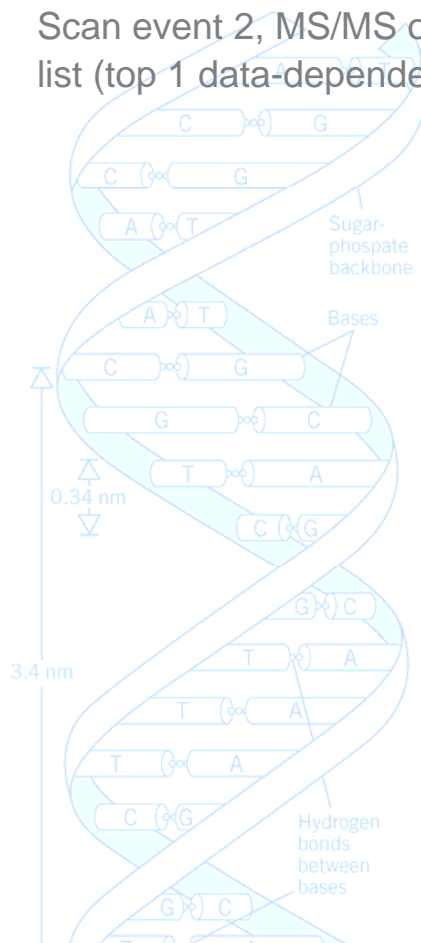
Scan event 1, 150-500 m/z (Full mass)

Scan event 2, MS/MS of top 1 from the inclusion list (top 1 data-dependent scan)

t-SIM Experiment

Single ion monitoring of masses of interest

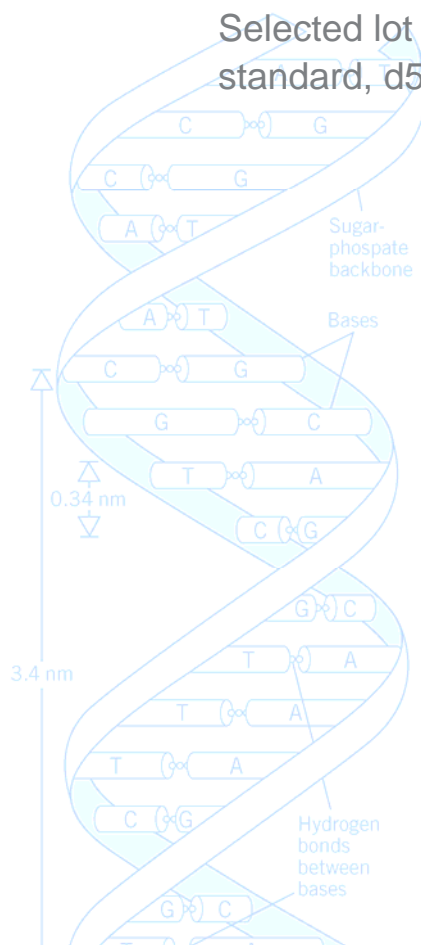
SIM time ranges were adjusted in response to changes in analyte retention



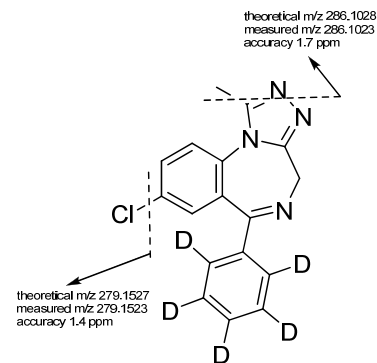
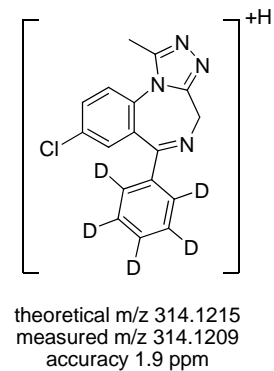
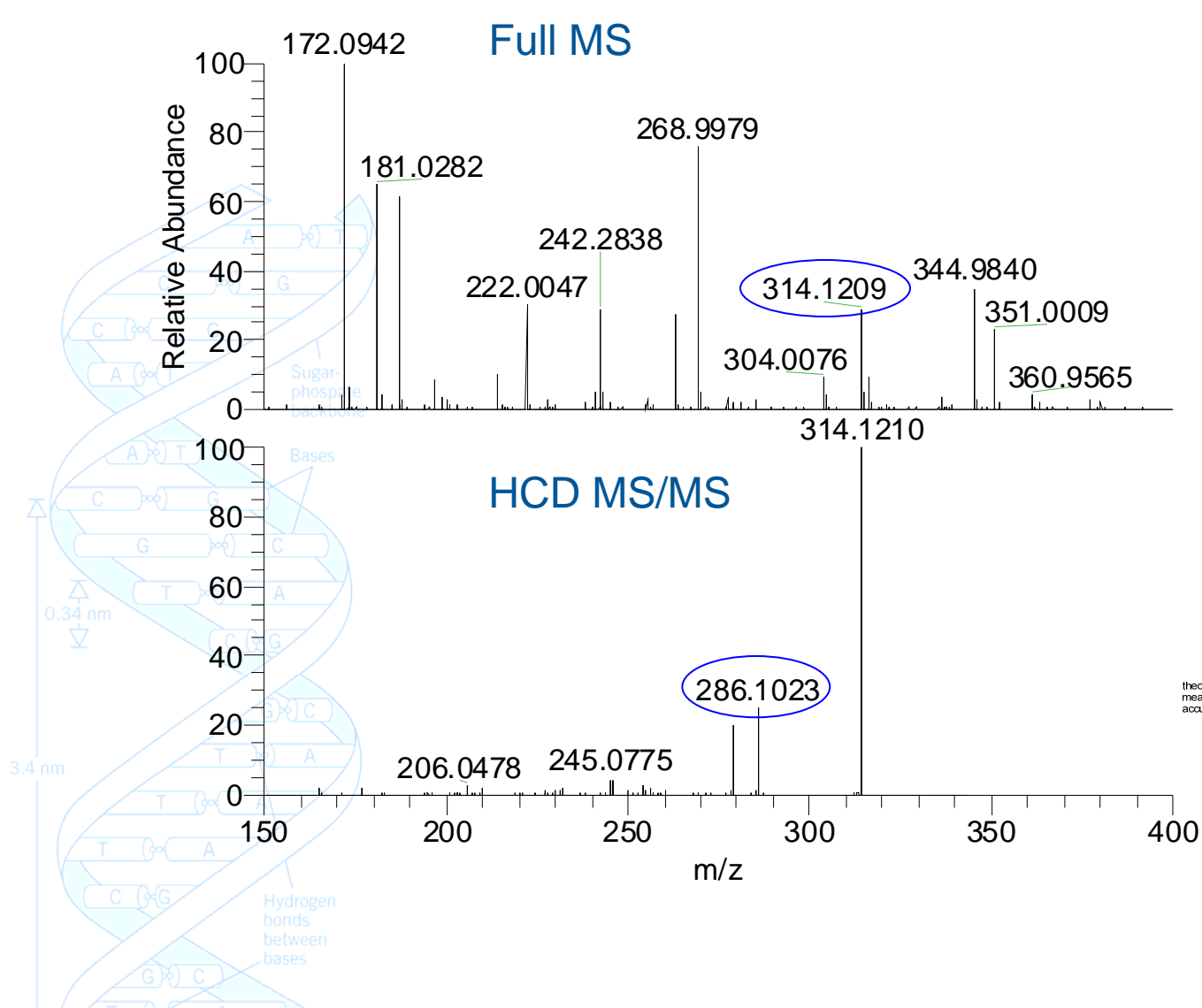
Sample preparation

Calibration solutions, with concentrations of 0.1, 0.5, 1, 5, 10, 50 ppm, were prepared by serial dilution of the stock solution in MeOH with 5 ng/mL of internal standard, d5-alprazolam, and 4 mg/mL of API.

Selected lot samples, were prepared at 4 mg/mL in MeOH with 5 ng/mL of internal standard, d5-alprazolam.

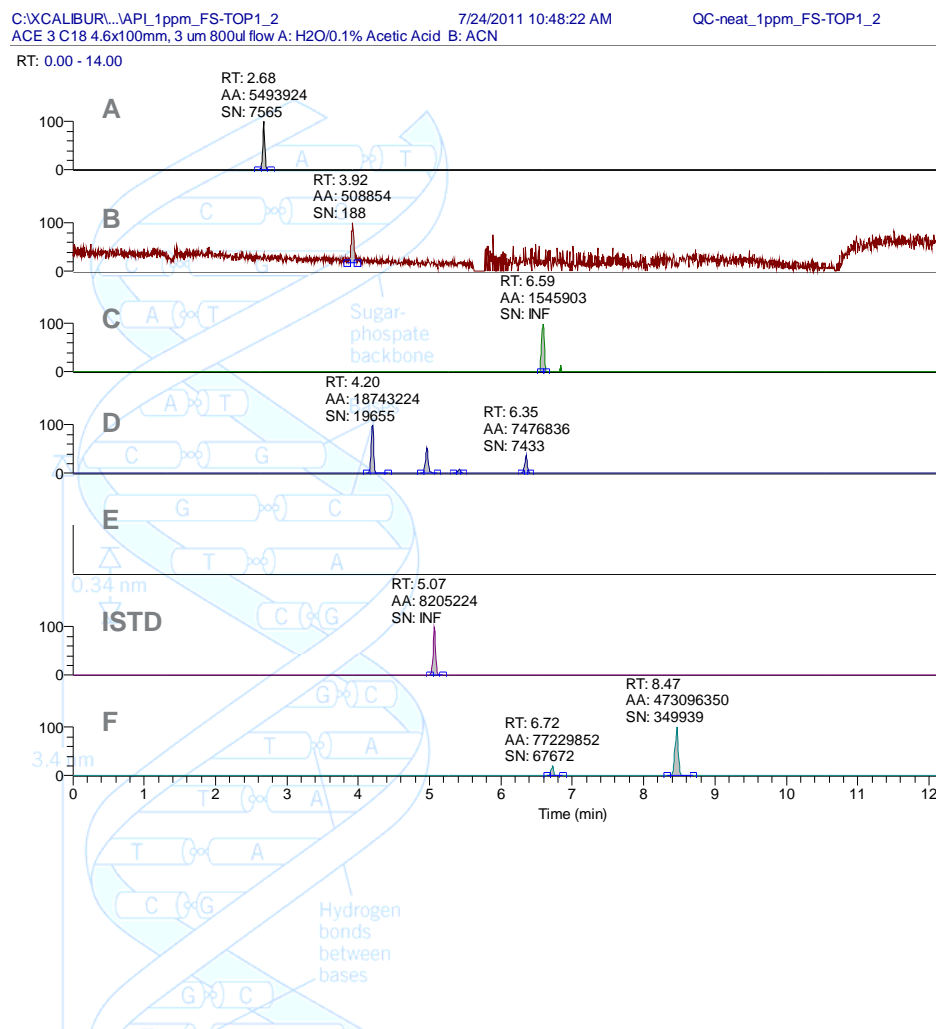


Mass accuracy of Alprazolam-D5, ISTD

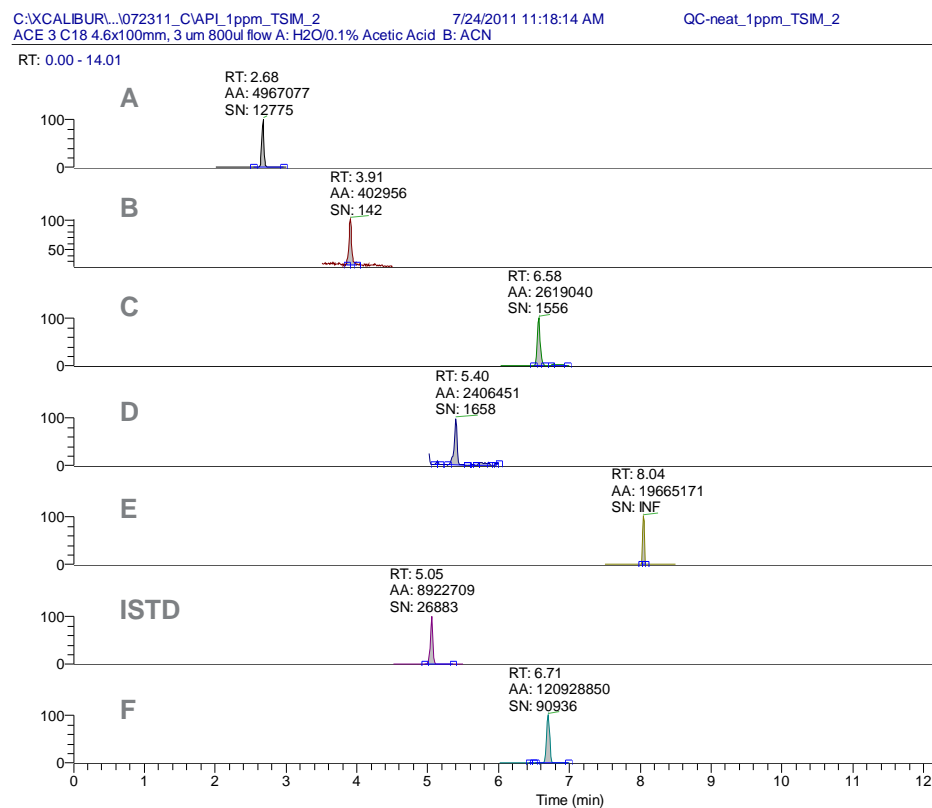


Typical SIC chromatogram at 1 ppm level in API

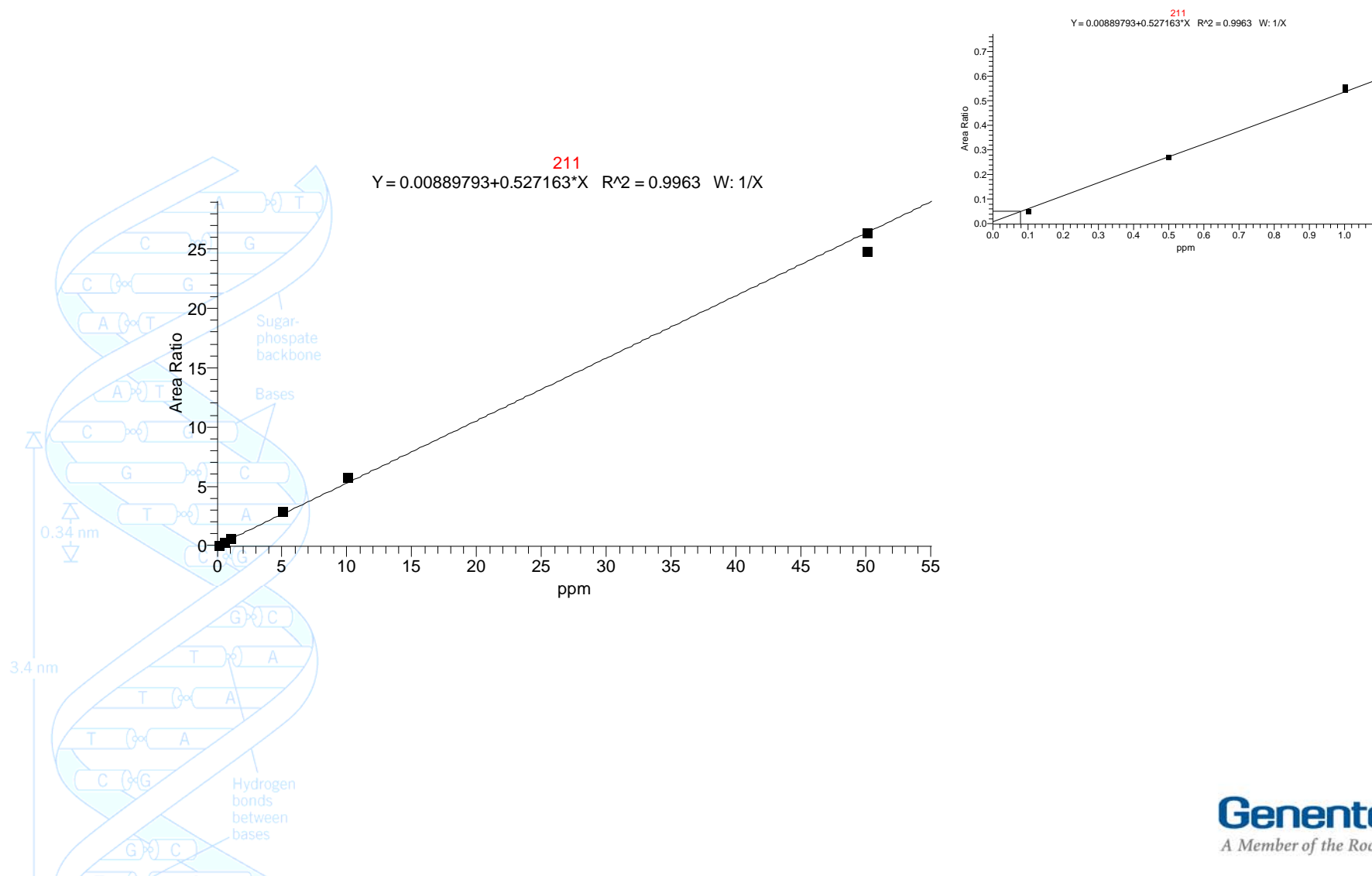
Using full MS method



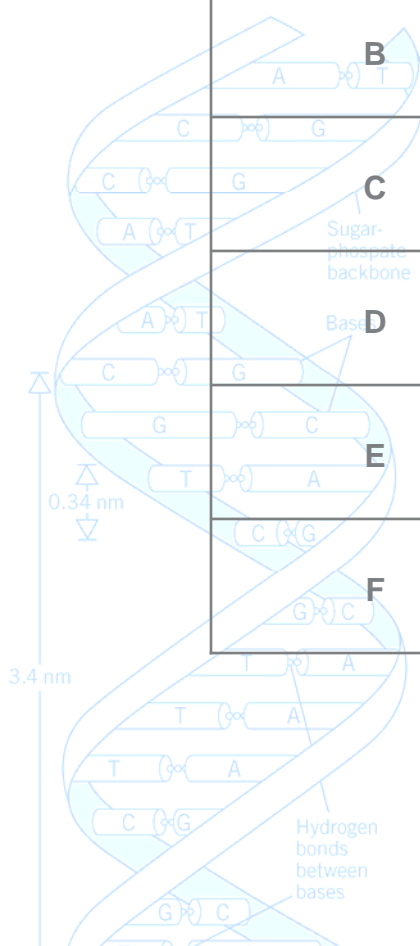
Using tSIM method



Representative calibration curve of compd A over the range of 0.1-50 ppm, linear regression and 1/x weighting



Triple Quad vs. Q-Exactive (at Limit of Quantitation, 1 ppm)

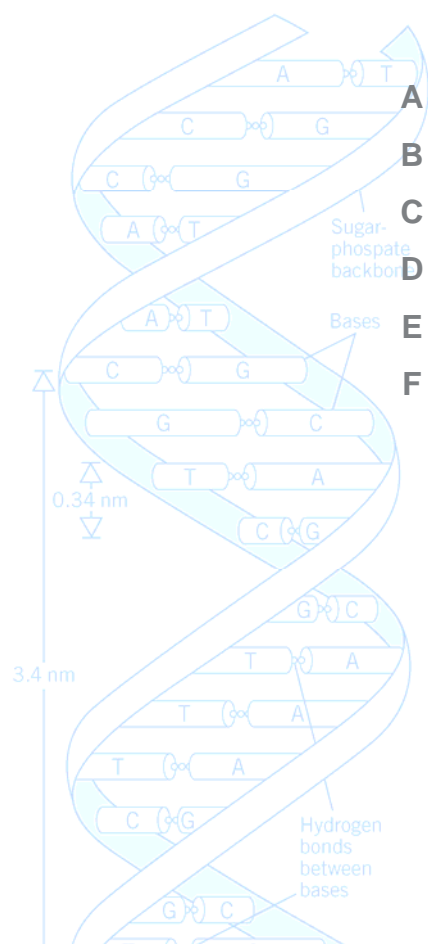


The diagram illustrates a DNA double helix with the following labels and dimensions:

- Base Pairs:** A-T, C-G, G-C, T-A, A-T, C-G, G-C, T-A, C-G, A-T, G-C, T-A, C-G, A-T, G-C, T-A.
- Dimensions:**
 - 0.34 nm: Distance between adjacent base pairs.
 - 3.4 nm: Length of one full DNA helical turn.
- Structural Labels:**
 - Sugar-phosphate backbone:** The outer structure of the DNA molecule.
 - Base:** The nitrogenous bases (A, T, C, G) that form the rungs of the helix.
 - Hydrogen bonds between bases:** The forces that hold the base pairs together.

PGI		Triple Quad	HR-MS
A	S/N	553	21122
	%RSD	14.4	1.5
B	S/N	2755	297
	%RSD	3.5	1.8
C	S/N	882	1450
	%RSD	1.7	3.9
D	S/N	448	1721
	%RSD	4.9	3.1
E	S/N	1127	108265
	%RSD	0	1.1
F	S/N	1047	INF
	%RSD	14.7	3.8

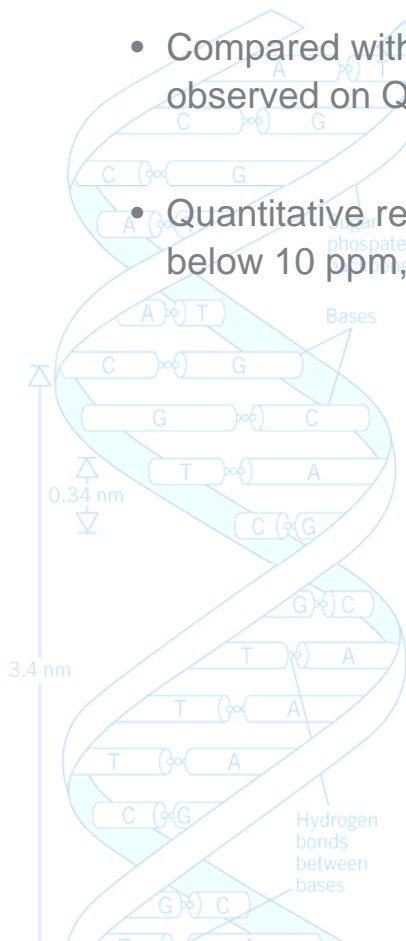
PGIs concentrations in selected lot samples



	Lot1		Lot2		Lot3	
	Mean measured conc. (ppm)	% RSD	Mean measured conc. (ppm)	% RSD	Mean measured conc. (ppm)	% RSD
A	<0.1	NA	<0.1	NA	<0.1	NA
B	0.442	3.0	0.457	0.5	0.446	2.7
C	<0.1	NA	<0.1	NA	<0.1	NA
D	0.419	0	0.404	0	0.440	0.5
E	<0.1	NA	<0.1	NA	<0.1	NA
F	<0.1	NA	<0.1	NA	<0.1	NA

Conclusion

- High resolution mass spectrometer, Q-Exactive, coupled with HPLC provides a sensitive and quantitative method to detect trace-level genotoxic compounds in the presence of large amount of APIs.
- Compared with traditional triple-quad method, there is no sensitivity and selectivity compromise observed on Q-Exactive.
- Quantitative results showed that the levels of all 6 potential genotoxic impurities in final APIs are below 10 ppm, which is below the controlled level.



Acknowledgement

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