Nota de aplicación

Improved Transmission of Labile Species on the Xevo™ G3 QTof Mass Spectrometer with the StepWave™ XS

Lisa Reid, David Pickles

Waters Corporation

This is an Application Brief and does not contain a detailed Experimental section.

For *in vitro* diagnostic use. Not available in all countries.

Abstract

The unwanted fragmentation of unstable ions within the ion optics of a mass spectrometer can lead to reduced analyte ion transmission and misleading MS spectra. This can detrimentally impact assay limits of detection, database searching, and structural elucidation. To address this challenge the Xevo G3 QTof is equipped with the novel StepWave XS ion guide, which improves signal transmission up to 22-fold. Use of a “soft-transmission” mode can further improve this increase up to 57.8-fold for specific, fragile analytes from the compounds demonstrated in this assessment. The increase in ion transmission results in a significant improvement in sensitivity and intact analyte to fragment ion ratio, improving both analyte ion detection and structural confirmation.

Benefits

Demonstrate the increase in transmission of labile species and resulting improvement in sensitivity and intact analyte to fragment ion ratio as a result of the new StepWave XS ion guide. Validate optimised parameters for labile compound transmission on the StepWave XS to accommodate simple and rapid transition from default parameters.

Introduction

Accurate mass spectrometry is extensively employed for the analysis labile compounds in many disciplines ***e.g.,*** pharmaceutical QC, proteomics, metabolomics, natural products, drug metabolism, environmental monitoring, forensic toxicology, and food analysis, to both profile constituents and identify individual analytes. In all these application areas sensitivity and specificity are critical for the detection and structural analysis of analytes that can exhibit significant unintended fragmentation such as: small molecule pharmaceuticals, drug metabolites, pesticides, and endogenous biomolecules.

Unintended analyte fragmentation typically occurs due to dissociation of unstable molecular bonds caused by the energy imparted during ion transfer from the ion source to the mass analyzser. This fragmentation can lead to reduction, or even complete loss of intact analyte ion signal and potentially result in misidentification of key analytes. Using other commercially available QTof mass spectrometers to verify or quantify these labile compounds may require the integration of product ions rather than the analyte (precursor) ion of interest and prevent confident identification or quantification of the analyte.

The Waters Xevo G3 mass spectrometer (Figure 1) is equipped with the StepWave XS ion guide, a new design shown to facilitate the intact transmission of labile ions. Here we demonstrate that even with default StepWave XS settings, the fragmentation of labile analytes is significantly reduced on the Xevo G3 QTof compared to QTof instruments equipped with a previous ion transfer optics design. We also illustrate the ability to tune the StepWave XS settings to facilitate a “soft transmission” mode, which further reduces unintended fragmentation effects and improves detection of the analyte ion.

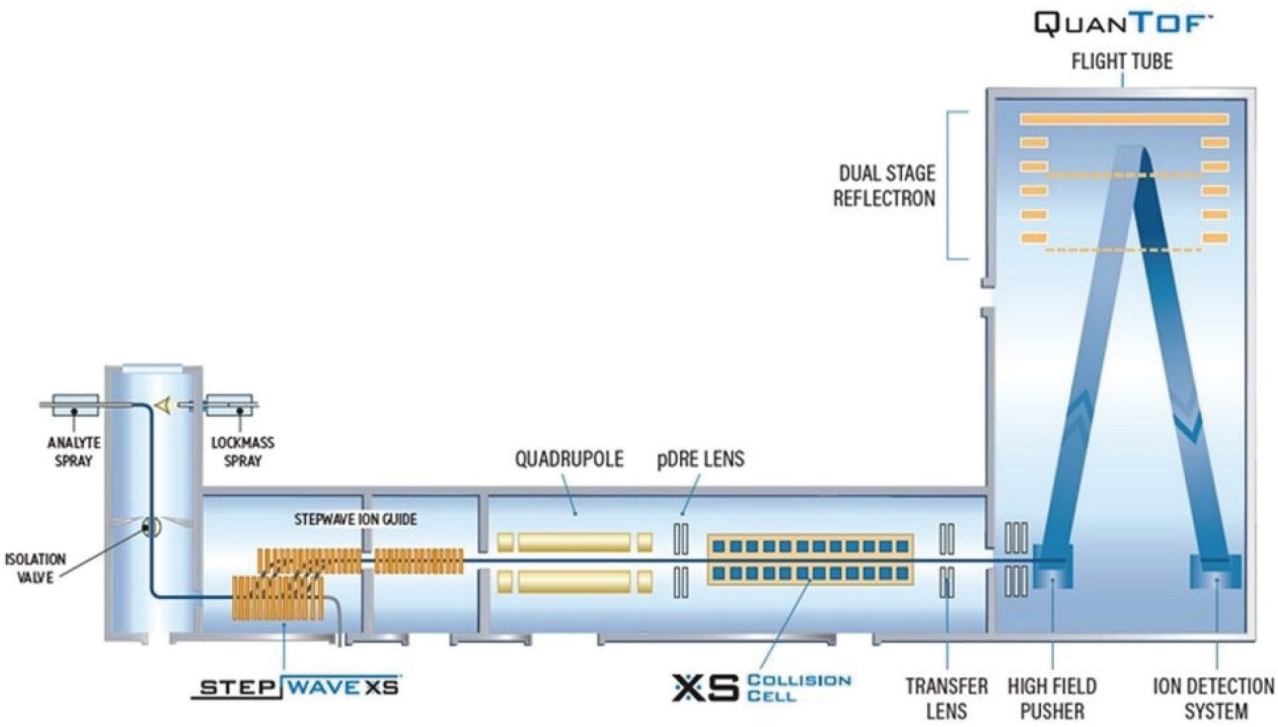

Figure 1. The Xevo G3 schematic depicting the StepWave XS design.

Results and Discussion

To assess the benefit of the StepWave XS ion guide during LC/MS analysis, a mixture of small molecule compounds known to be suspectable to fragmentation were analysed on both the Xevo G3 QTof and previous generation Xevo G2-XS QTof. The analytes selected are listed below (Table 1):

- Small molecule pharmaceuticals: Amphetamine, Norsetraline, Ibuprofen, Aspirin.
- Pesticide: Chloropropham.
- Endogenous compound: 25-Hydroxyvitamin D3.

These test compounds were analysed using a reversed-phase chromatographic gradient and detected in both positive and negative ionization MS mode. Assessment was performed on a QTof instrument containing the

previous StepWave design (Xevo G2-XS QTof) using default StepWave tune parameters, and on the Xevo G3 QTof using the default StepWave XS tune parameters, as well as the optimized "soft transmission" tune parameters (Table 2). Six injections of the test solution were performed on each instrument in each mode of operation and the average peak area values for the precursor ion measured for each mode of analysis.

Compound	Formula	Precursor ion *m/z*	Fragment ion *m/z*
Amphetamine	$C_9H_{13}N$	136.113	91.05
Chlorpropham	$C_{10}H_{12}ClNO_2$	214.064	172.02
Norsertraline	$C_{16}H_{15}Cl_2N$	292.066	158.98
25-Hydroxyvitamin D3	$C_{27}H_{44}O_2$	401.342	365.32
Ibuprofen	$C_{13}H_{18}O_2$	205.123	161.13
Aspirin	$C_9H_8O_4$	179.034	93.03

Table 1. Compound precursor and fragment ion details.

Tune settings	Default parameters	Soft transmission
StepWave RF (*Vp-p*)	150	50
Body gradient (*V*)	20	5
Transfer collision energy (*V*)	2	2

Table 2. Tune parameters for the StepWave XS for both default settings and optimized "soft transition" mode settings.

When default tune parameters were employed, a significant increase in intact analyte ion response was observed on the Xevo G3 QTof when compared to the Xevo G2-XS QTof for all analytes tested. The improvement was compound dependent and ranged from 3.9-fold increase in precursor peak area for Ibuprofen to a 22.7-fold increase in precursor peak area for Amphetamine. Additionally, a further increase in the intact analyte ion response was seen for all analytes tested when the Xevo G3 QTof StepWave XS tune settings were optimized for "soft transition", compared to those obtained when the Xevo G3 QTof was operated with the default StepWave XS tune settings. This further improvement in intact analyte ion response resulted in an overall increase in precursor peak area of between 5.6 and 57.8-fold increase for the Xevo G3 QTof compared to that obtained with the Xevo G2-XS QTof. (Figure 2).

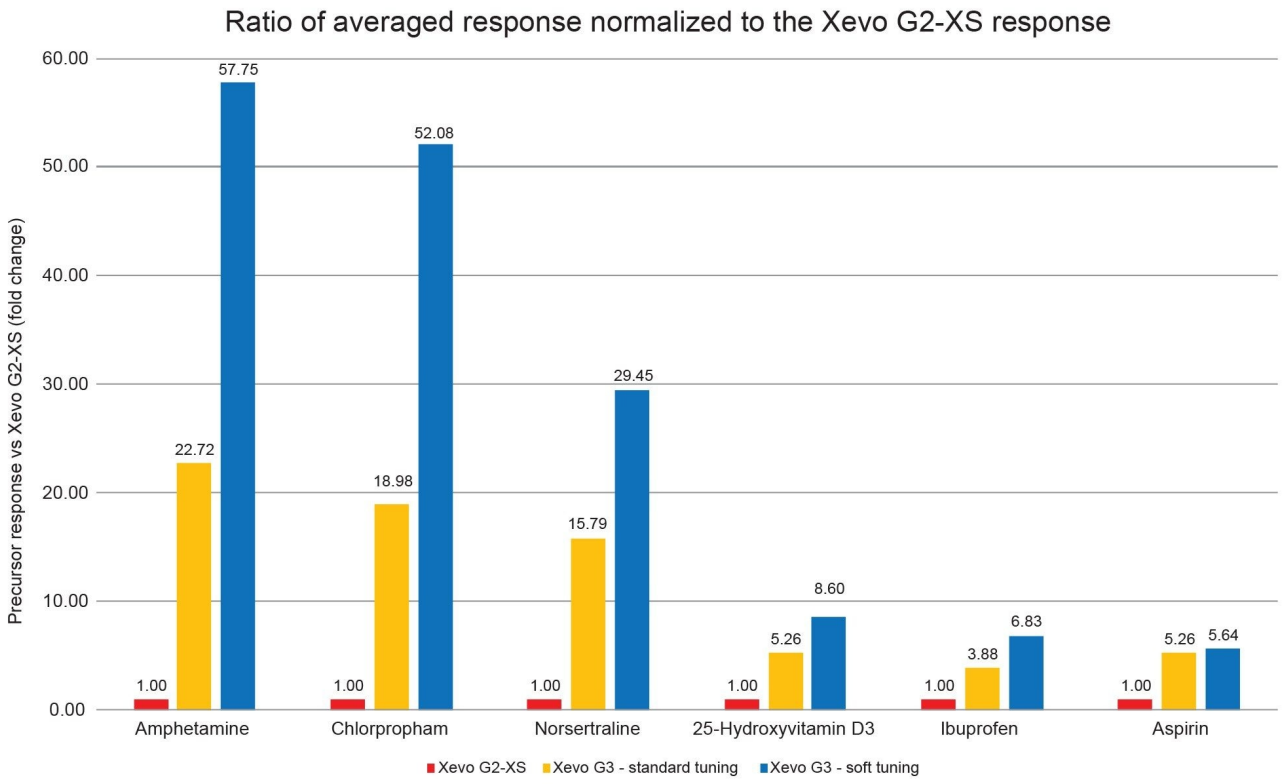

Figure 2. Summary of the improvement in sensitivity for each labile analyte, the response has been normalized against the Xevo G2-XS data. Showing the fold change improvement over the precursor response observed on the Xevo G2-XS.

As well as monitoring the increase in peak area response for the labile analytes tested it was possible to evaluate the unintended fragmentation as a percentage of the analyte ion. This fragmentation value was calculated as a percentage of the intact analyte precursor ion response to the total ion response of the precursor and fragment ions (precursor/(precursor+fragment)*100). Using this calculation, it can be seen that the Xevo G3 QTof produced significantly less fragmentation of the analyte precursor ions compared to the Xevo G2-XS QTof for five of the six analytes tested. Ibuprofen, which already demonstrates a relatively high precursor ion signal compared to its fragment ion (>50%) across both instruments, is the only analyte that does not show an improvement when analysed on the Xevo G3 QTof.

The compounds analysed all showed an improvement in intact analyte ion intensity compared to combined total ion signal, when analysed using the “soft transmission” tune parameters on the Xevo G3 QTof. Norsertraline

showed >20-fold increase (0.7% to 20.3% equivalent) using the Xevo G3 QTof with “soft transmission” parameters compared to the conventional ion optics in the Xevo G2-XS QTof. Two compounds, Chloropropham and Aspirin both exhibited >99% fragmentation when analysed. Though the fragmentation is still significant, both these analytes did show a slight improvement in intact precursor ion signal when implementing the “soft transmission” tune parameters on the Xevo G3 QTof (Figure 3).

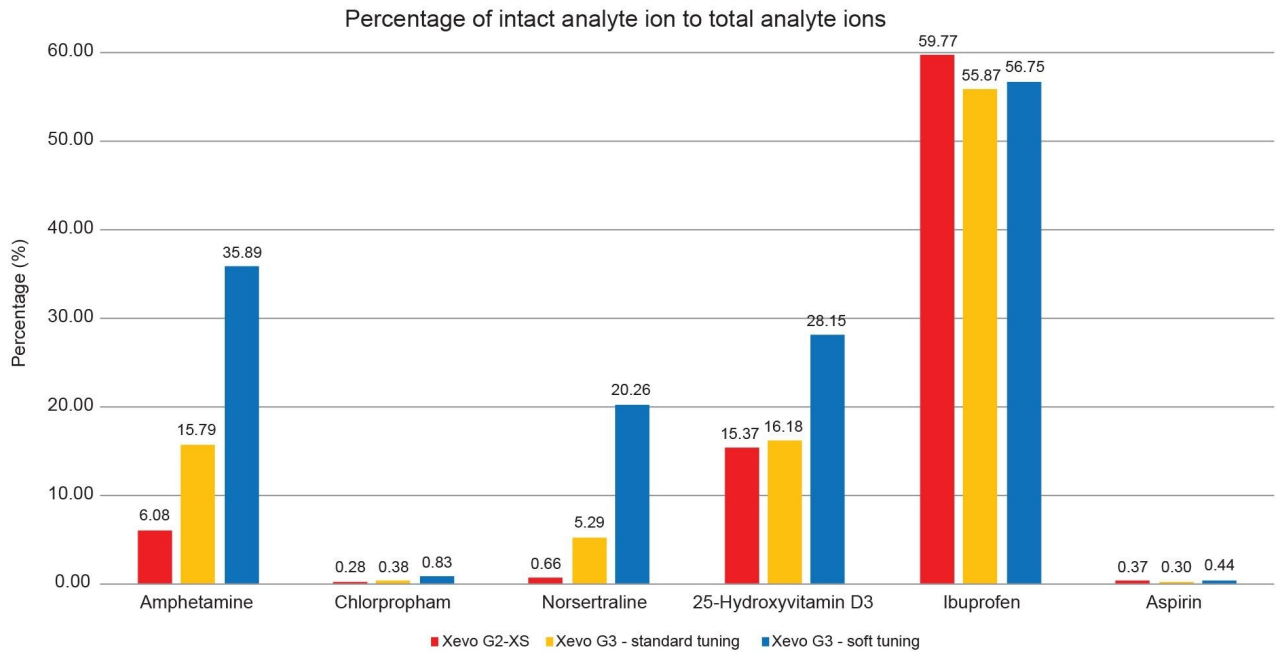

Figure 3. Summary of the improvement in percentage precursor ions to total analyte ions for each labile analyte.

Conclusion

Labile compounds can undergo fragmentation in the source and ion optics of a mass spectrometer, this can detrimentally impact, peak detection, assay limits of sensitivity, database searching, and structural elucidation. The Waters StepWave XS design has been shown to mitigate unwanted fragmentation of labile ions within the ion optics of the mass spectrometer. This study demonstrated that the Waters StepWave XS ion guide can significantly improve the transmission, for intact analyte ions of labile compounds, particularly when the

parameters are optimized using the “soft transmission” mode. For more information regarding the StepWave technology please see the document: StepWave - Enhancing MS Sensitivity and Robustness (720004175 < https://www.waters.com/webassets/cms/library/docs/720004175en.pdf>).

Of the six labile compounds tested: four ionized most efficiently in positive ionization mode and two ionized most efficiently in negative ionization mode. The majority of compounds analysed in this dataset demonstrated an improved intact analyte ion signal compared to the “total” combined ion intensity. All analytes injected showed a significant improvement in response extracted ion chromatogram ((XIC) peak area of the precursor ion) using the StepWave XS compared to the previous StepWave design. All analytes also showed improvement when analysed on the Xevo G3 QTof using optimized “soft transmission” StepWave parameters compared to the default StepWave settings.

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StepWave <https://www.waters.com/waters/nav.htm?cid=134673601>

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