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Strategies for structure elucidation using Ultrafast Mass Spectrometry (UFMS): Using nMS^2 as an alternative to MS^3

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1. Introduction

Structure elucidation using tandem mass spectrometry is dominated by approaches that use accurate mass, parent-ion isolation and fragmentation or a combination of both. MS^3 is useful as it allows intermediates in fragmentation pathways to be the assigned but is limited for real-time analysis by lack of speed and consequent loss of peak definition and quantitative reproducibility, particularly for UHPLC (Fig. 1). We have applied UFMS^2 to generate multiple spectra for on-the-fly detection and identification of pharmaceutical compounds and metabolites (Fig. 3).

UFMS with nMS^2	Conventional MS^3
Common parent ion sensitivity at each stage	Generational loss of sensitivity
Signal to noise true for MS^2	Reduced chemical noise
Inferred fragmentation pathways	Certain tracking of fragmentation pathways
Data content with peak definition	Data content sacrifices time (chromatographic) domain
Complimentary spectra with polarity switching or DUIS	Single polarity and source condition prior to fragmentationc

Fig. 1 Comparison of the strengths in favour of nMS^2 and MS^3

Fig. 2 A combination of Nexera LC-30 system with a void volume reducing column oven (CTO-30AS) and a LCMS-8040 Ultrafast Triple Quadrupole Mass Spectrometer

2. Method

LCMS analysis of pharmaceuticals (ciprofloxacin, metoclopramide, oseltamivir and modafinil) on a LC-30-LCMS-8040 (Shimadzu) with water (A) and methanol (B) containing 1% formic acid at 0.5 mL/min in Q3-scan mode identified precursor ions which were then subject to CID with potentials of 10 – 150V in positive and negative ion mode. Spectra were scanned from 50-350 Da at 15,000 µ/sec. The method provided nine simultaneous data channels with a cycle of less than 200 msec including stabilisation time and polarity switching. This was equivalent to each channel delivering 25 points across a UHPLC peak that is 5 sec wide. Channels could be triggered by a MRM cycling at 2 msec per precursor ion. Fragmentation for each spectrum allowed detailed structure assignment.

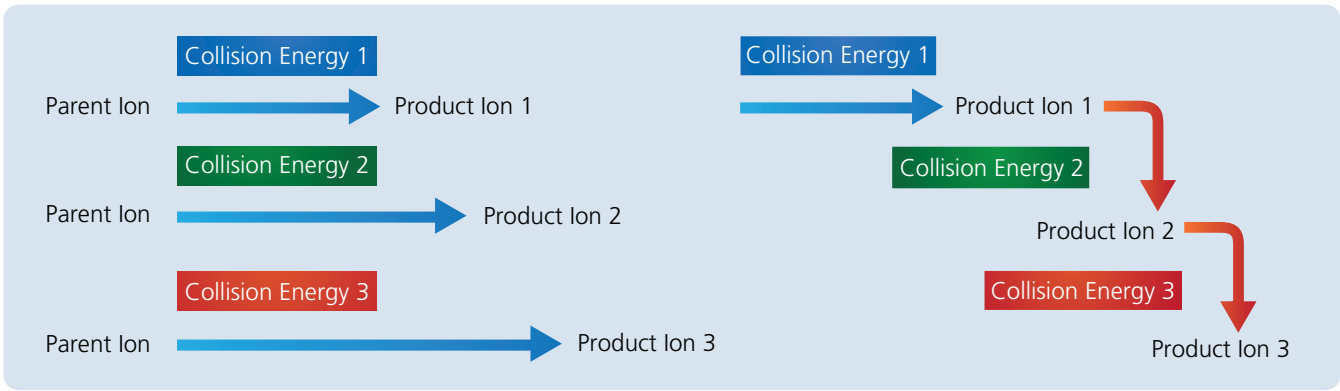

Fig. 3 The strategy of using multiple generations of MS^2 experiment as an alternative to MS^3 is made practical for realtime analysis by UFMS. The nMS^2 method has the advantage of high sensitivity resulting from generating fragments from the same abundant MS parent.

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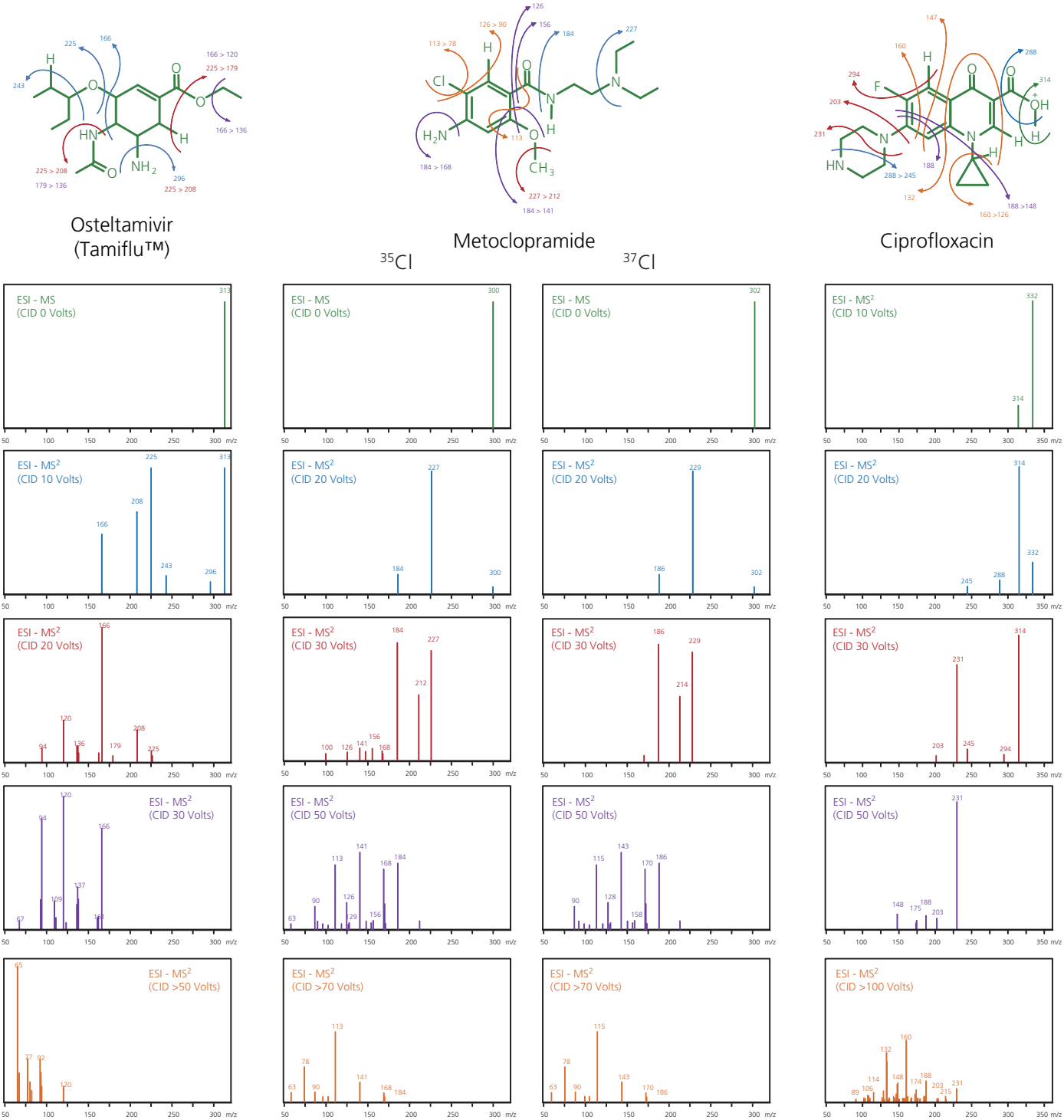

Fig. 4 The analysis of osteltamivir, metoclopramide and ciprofloxacin using UFMS^2 at various collision energies simultaneously generates complimentary fragmentation data that is useful for structure elucidation. Five spectra could be triggered from a MRM event in 150 msec and preserve UHPLC peak definition

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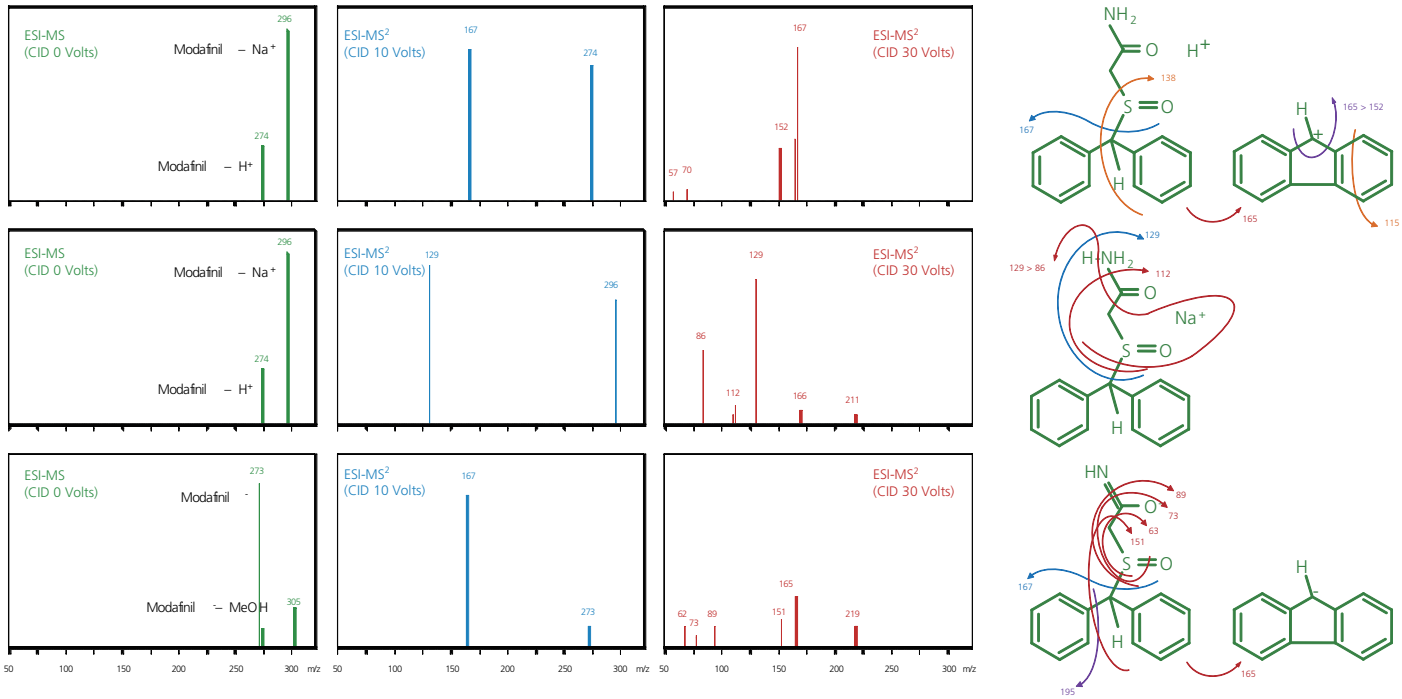

Fig. 5 The simultaneous analysis of modafinil, its sodium adduct and negative ion form using UFMS^2 at various collision energies. The spectral array could be triggered from a MRM event and the data set collected in approximately 100 msec including UF-polarity switching in 15 msec

3. Discussion and Conclusion

UFMS requires quadrupole mass filtering (15000 μ/sec at 0.1 μ mass definition (10 points per 1 μ rather than interpolated masses), Ultrafast polarity switching (15 msec), negligible mass axis displacement or loss of sensitivity for constant dwell time and negligible cross-talk.

We have applied UFMS to generate multiple product ion spectra (PIS) across a UHPLC peak with CID at varying energies. The nMS^2 approach yields an array of MSMS spectra derived from the same parent ion but exhibiting mechanistically distinct fragment ions (Fig. 4 and 5). Typically, we have driven simple neutral losses at low collision energy (10 V potential difference across the collision cell), fission of pendant moieties (30-50 V) and more significant aromatic rearrangements and formation of fused cyclic structures at higher collision energies. We have also tracked halogenated fragments of metoclopramide with parallel PIS from the MH+2 across the collision energy range. UFMS has enabled on-the-fly detection and identification of novel compounds (e.g. metabolites) with arrayed MS^2 data.

Unlike MS^3, the n MS^2 approach is not subject to the transfer loss limitations on sensitivity because the same parent ion is selected from the first mass filter rather than from collisional debris. Ultrafast polarity switching allows one or more of the elements in the PIS array to be based on either negative or positive ions and so increases the informing power of the data set. Similarly, nMS^2 allows the selection of multiple parents (isotopic contributors or co-resident adducts) as well as the fragmentation of products from source induced fragmentation or neutral loss (pseudo-MS^3.)

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