[TECHNICAL NOTE]

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SYNAPT G2 HIGH DEFINITION MASS SPECTROMETRY: ION MOBILITY SEPARATION AND STRUCTURAL ELUCIDATION OF NATURAL PRODUCT STRUCTURAL ISOMERS

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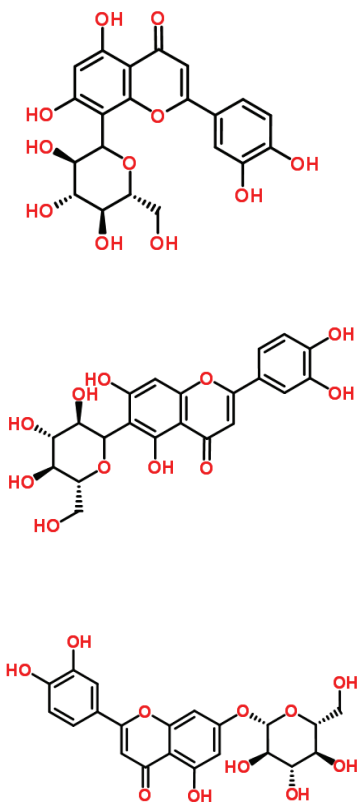
INTRODUCTION

We demonstrate the ability of the SYNAPT™ G2 High Definition Mass Spectromerty™ (HDMS™) System to separate and identify three natural product structural isomers, shown in Figure 1, which differ in collision cross-section by less than 8 Å^2 without LC separation.

We also demonstrate the use of collision-induced dissociation (CID) after T-Wave™ ion mobility separation in order to elucidate the structure of the three small molecule isomers, in combination with peak detection and interpretation with DriftScope™ Software.



Luteolin 8 C-glucoside
MW 448.3769
$C_{21}H_{20}O_{11}$

Luteolin 6 C-glucoside
MW 448.3769
$C_{21}H_{20}O_{11}$

Luteolin 7 O-glucoside
MW 448.3769
$C_{21}H_{20}O_{11}$

Figure 1. Chemical structures of three luteolin gylcoside isomers.

EXPERIMENTAL

SYNAPT G2 HDMS is an innovative hybrid quadrupole IMS orthogonal acceleration time-of-flight (oa-Tof) mass spectrometer, featuring second-generation Triwave™ Technology[1], shown in Figure 2. This region provides enhanced ion mobility (IM) resolution by up to a factor of four through the increased length and gas pressure of the IMS T-Wave. A novel helium-filled entry cell is employed to ensure the enhanced IM resolution (afforded by the IMS T-Wave) can be provided while maintaining high transmission efficiency of ions from the low pressure TRAP T-Wave into the elevated pressure of the IMS T-Wave.

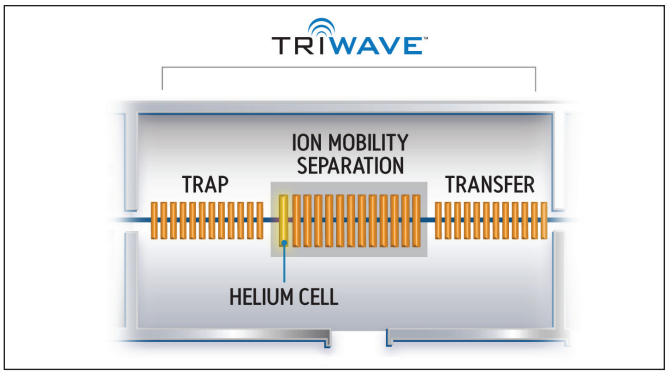


Figure 2. Schematic of the second-generation Triwave Technology in the SYNAPT G2 HDMS System.

MS and IMS conditions:

MS system:	SYNAPT G2 HDMS System
Ionization mode:	nanoESI Positive
Capillary voltage:	1000 V
Cone voltage:	35 V
TRAP CE:	7 V
TRANSFER CE:	4 V
TRAP/TRANSFER gas:	Ar
IMS gas:	N_2 (~2.5 mbar)
IMS T-Wave speed:	600 m/sec
IMS T-Wave height:	40 V
Acquisition range:	*m/z* 50 to 500

Collision cross-section (Ω) calculation

The first stage in the analysis was to separate the isomers using ion mobility and derive the collision cross-section values (average rotational cross-sectional). To do this accurately, the IMS T-Wave was calibrated using a mixture of haemoglobin tryptic peptides and polyglycine with known collisional cross sections (http://www.indiana.edu/~clemmer). The position of the glucoside moiety in the luteolin 8 *C*-, 6 *C*-, and 7 *O*-structures has a dramatic effect on the T-Wave-derived collision cross sections (Ω) as demonstrated in Figure 3. The 8 *C*-glu being the most compact, and 7 *O*-glu was the most extended.

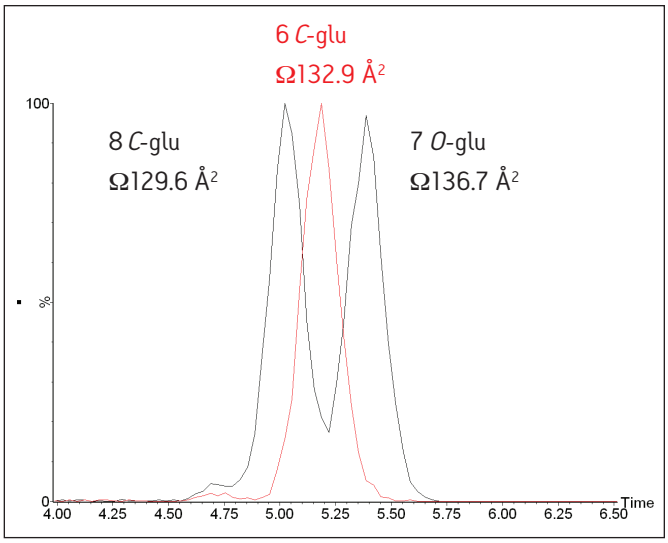


Figure 3. T-Wave ion mobility arrival time (msec) chromatograms and T-Wave derived collision cross-sections for the three luteolin glycoside structural isomers.

Characterization of the separated isomers, in parallel

In the second stage of the analysis, collision-induced dissociation (CID) was performed in the TRANSFER T-Wave to enable structural elucidation of each separated isomer. This approach allowed mobility separation of structural isomers based on differences in collision cross-section, followed by arrival time-resolved fragmentation. Since the CID generated fragment ions correlate with the drift times of the precursor ions, distinct fragmentation spectra can be produced for each isomer, as shown in Figure 4B. DriftScope Software provides the ability to quickly recover the Exact Mass fragment ion information for structural elucidation via automated peak detection.

In Figures 4A, 4B, 4C, and 4D, it can be seen that luteolin 8 *C*- and 7 *O*-glucoside differ in their mobility arrival times and fragmentation patterns. The parallel fragmentation (collision energy 60 V applied to Transfer T-Wave) of luteolin 8 *C*-glucoside (Figure 4A), results in many dehydration events and cross-ring cleavages, whereas the dominant fragmentation pathway of the luteolin 7 *O*-glucoside (Figure 4D) shows the neutral loss of the glucoside moiety due to the

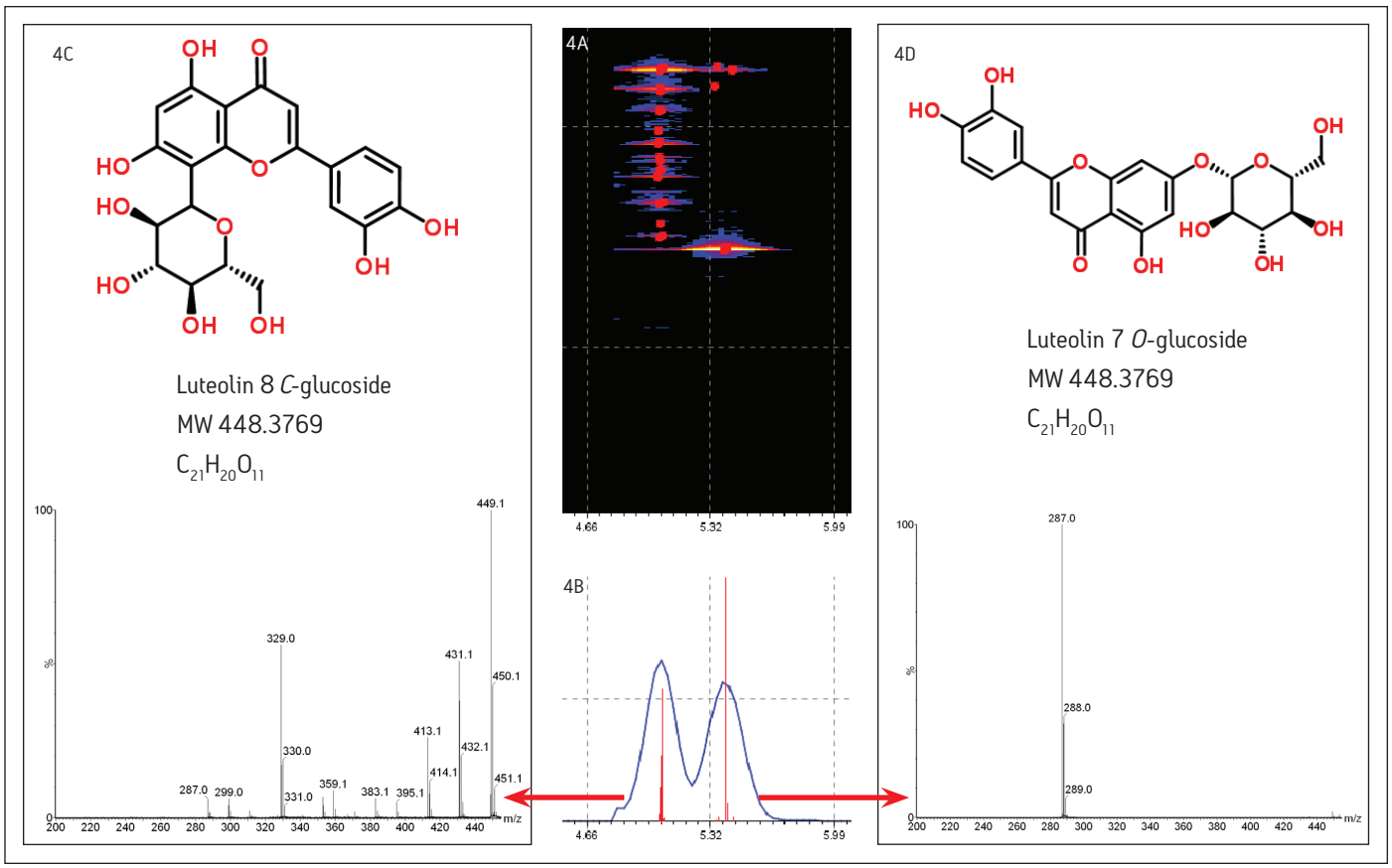


Figure 4. Fragment ion analysis of structural isomers.
A) Visualization (drift time vs. m/z, with intensity from blue to yellow) of the parallel fragmentation data (detected peaks as red spots, 60 V) from the luteolin structural isomeric compounds in DriftScope Software, v. 2.1;
B) Visualization of the separated structural isomeric luteolin compounds in drift time;
C) Fragment ion spectrum of luteolin 8 C-glucoside;
D) Fragment ion spectrum of luteolin 7 O-glucoside

very labile 7 *O*-glucoside bond. Without prior ion mobility separation, the fragmentation spectra of luteolin 8 *C*- and 7 *O*-glucoside would overlap, making structural interpretation very challenging.

CONCLUSIONS

- The enhanced ion mobility resolution of SYNAPT G2 HDMS enables clear separation of three natural product luteolin glycoside structural iosomers.
- The T-Wave derived collision cross-section measurements of three isomeric species showed they differed by less than 8 $Å^2$.
- No LC separation was required to identify the luteolin glycoside structural iosomers; separation was achieved by ion mobility separation alone.
- Combining parallel fragmentation of isomers with Triwave and data interpretation with DriftScope Software, v. 2.1, which incorporates a novel peak detection routine, enables rapid compound identification and structural elucidation.

Reference

1. The travelling wave device described here is similar to that described by Kirchner in U.S. Patent 5,206,506 (1993).

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May 2009 720003041EN AG-PDF

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