Application Note 00926

Data Acquisition and Analysis of 7-ethoxycoumarin and its Metabolites Using TurboDDS™ Software in the 500-MS Ion Trap

Fran Lai
Varian, Inc.

Introduction

The ion trap is a powerful tool for metabolism studies, due to the MS^n capability for the identification of metabolites and structure elucidation of unknown peaks. The TurboDDS™ software on the Varian 500-MS LC Ion Trap eliminates the need to do multiple runs in order to identify metabolites. Once the expected *m/z* of a metabolite is entered into the method, the 500-MS will automatically look for this *m/z* and when found, will immediately trigger an MS^n (where n can be 2 or higher) experiment.

7-ethoxycoumarin (Figure 1A) is commonly used for metabolism studies, including hepatocyte studies (1), liver perfusion techniques (2) and cytochrome P450 studies (3). This application note describes an MS^n data acquisition method using Varian's TurboDDS for the analysis of 7-ethoxycoumarin and two of its metabolites, 7-hydroxycoumarin (Figure1B) and 7-hydroxycoumarin ß-D-glucuronide (Figure 1C).

Instrumentation

- Varian 500-MS LC/MS equipped with ESI source
- Varian ProStar™ 420 AutoSampler
- Varian 212-LC Binary Solvent Delivery Modules equipped with 150 µL Static Mixer

Materials and Reagents

7-ethoxycoumarin (CAS number 31005-02-4), 7-hydroxycoumarin (CAS number 93-35-6) and 7-hydroxycoumarin ß-D-glucuronide sodium salt (CAS number 168286-98-4) from Sigma Aldrich, St. Louis, MO.

All other chemicals were reagent grade or HPLC grade.

Sample Preparation

To simulate a typical mixture of a drug and its metabolites from metabolic study samples, a mixture of 7-ethoxycoumarin (7-EC), 7-hydroxycoumarin (7-HC) and 7-hydroxycoumarin ß-D-glucuronide sodium salt (7-HC glu) in 50:50 methanol:water was prepared, with concentrations of 0.12, 5.5 and 15 ng/µL respectively. (These concentration levels were used in order to show comparative peak sizes in the resultant chromatograms.)

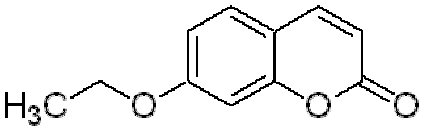

Figure 1A Structure of 7-ethoxycoumarin (7-EC).

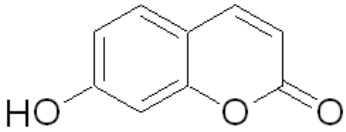

Figure 1B Structure of 7-hydroxycoumarin (7-HC).

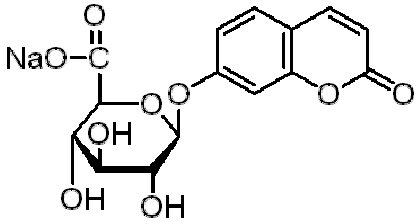

Figure 1C Structure of 7-hydroxycoumarin glucuronide (7-HC glu).

HPLC Conditions

Column: Pursuit™ XRs C18 5 µm, 150 x 2 mm ID (Varian Part No. A6000150X020)

Solvent A: 0.1% formic acid in water

Solvent B: 0.1% formic acid in acetonitrile

LC Program:

Time (min:sec)	%A	%B	Flow (µL/min)
0:00	60	40	200
5:00	15	85	200
5:30	15	85	200
5:42	60	40	200
7:00	60	40	200

Injection Volume: 20 µL

Injection Solvent: 50:50 methanol:water

MS and API Parameters

API Drying Gas:	25 psi at 400 ^{o}C
API Nebulizing Gas:	25 psi
Needle:	5000 V
Capillary:	74 V
Shield:	500 V
Enhanced Scan Mode:	5000 Da/sec
RF loading:	72%

TurboDDS™ Scan Parameters

Survey scan: *m/z* 100-400

"Include List" for MS^n triggers:

- *m/z* 191 (for 7-EC)
- *m/z* 163 (O-deethylation [-28] to 7-HC)
- *m/z* 339 (glucuronidation [+176] to 7-HC glu)

Data Dependent Scans (DDS) with MS^2 and MS^3 were included in the method.

Results & Discussion

Table 1A TurboDDS results with MS^2:

Analyte	Precursor Ion (*m/z*)	Product ion (*m/z*)
7-EC	191	163
7-HC	163	119
7-HC glu	339	163

Table 1B TurboDDS results with MS^3:

Analyte	Precursor Ion (*m/z*)	Product ion (*m/z*)
7-EC	163	119
7-HC	119	91
7-HC glu	163	119

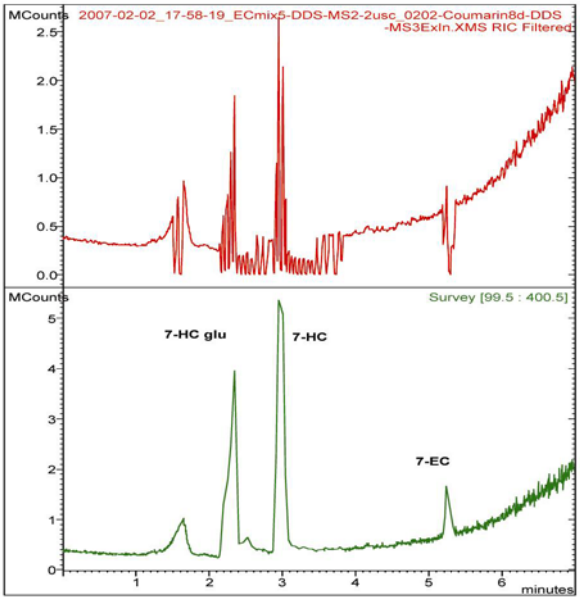

Figure 2 Chromatogram of 7-EC, 7-HC and 7-HC glu (top). All scan descriptors, (bottom) Survey scan.

Figures 3-5 (tree reports) show MS^2 and MS^3 data tabulated in Tables 1A and 1B.

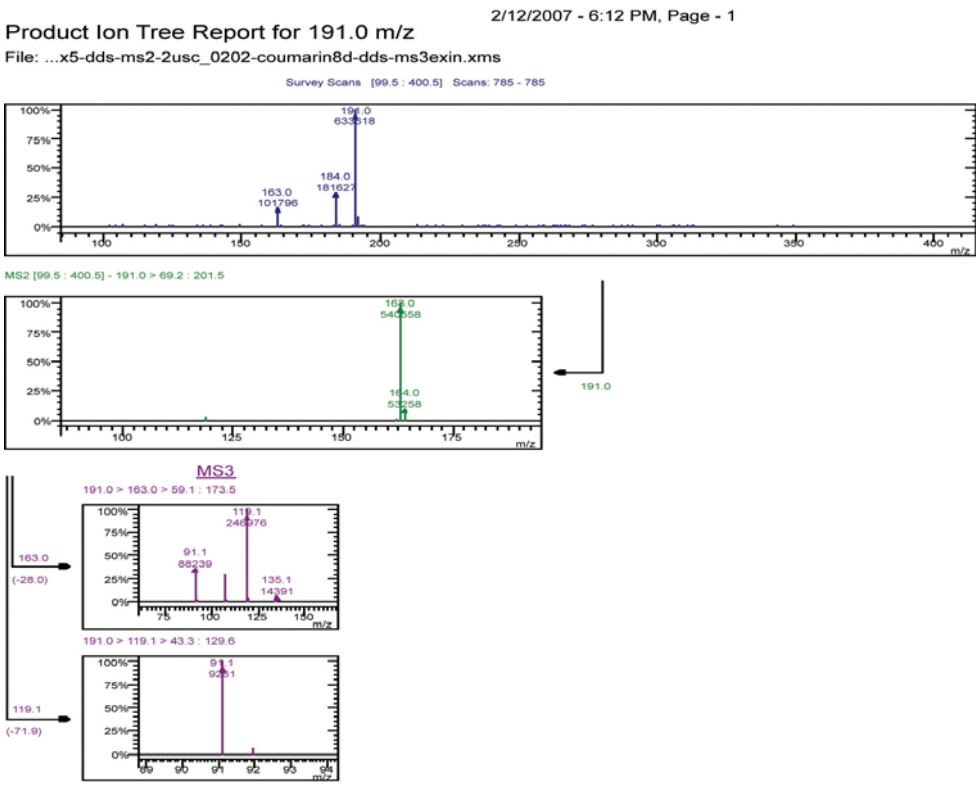

Figure 3 Survey, MS^2 and MS^3 spectra of 7-EC (peak #3) from TurboDDS.

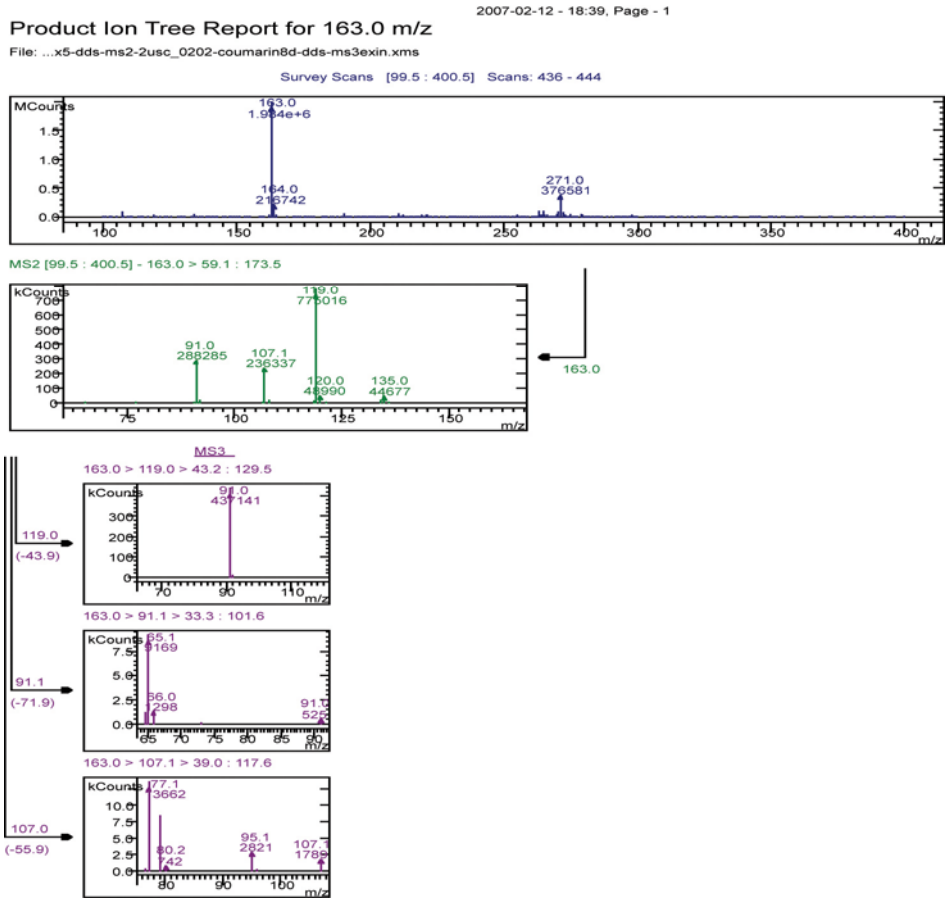

Figure 4 Survey, MS^2 and MS^3 spectra of 7-HC (peak #2) from TurboDDS™.

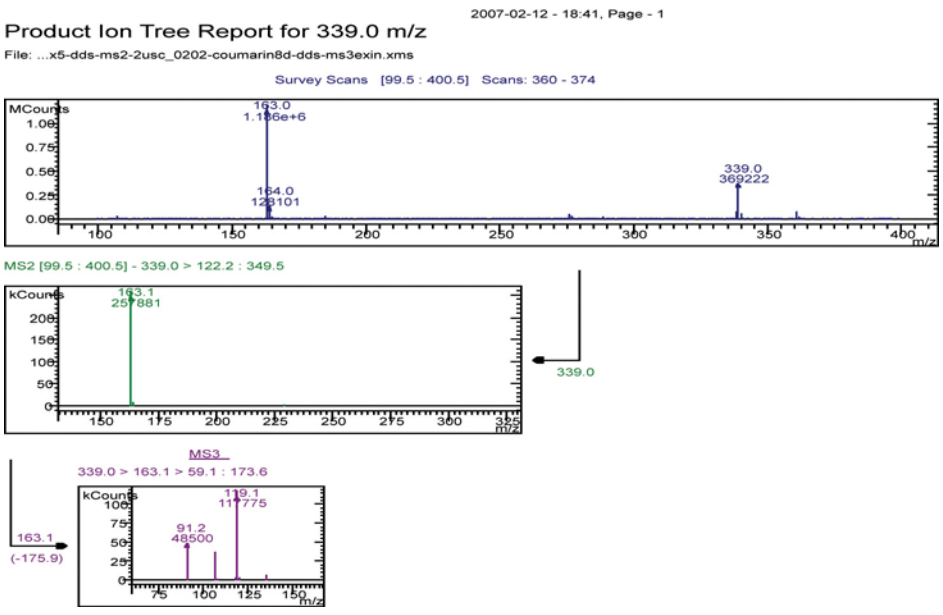

Figure 5 Survey, MS^2 and MS^3 spectra of 7-HC glu (peak #1) from TurboDDS.

These tree reports (Figures 3-5) make it very easy for an operator to view product ions and trace them back to the original precursor ions. Also, mass differences are displayed at each MS/MS step, a clear benefit for structural elucidation.

Conclusion

The Varian 500-MS LC/MS TurboDDS™ software demonstrates excellent performance for data dependent scans of 7-ethoxycoumarin and two of its metabolites.

MS^n was performed up to MS^3 in this application note. However, further MS/MS experiments could be performed in the same run as long as there is enough sample. Without TurboDDS, the same data would have required multiple runs, but with it, the time saving is tremendous and the data reporting for the multiple MS/MS stages is streamlined.

References

1. Carlile et. al., *Drug Metabolism and Disposition* 26 (1998) 216.
2. Andersson et. al., *Drug Metabolism and Disposition* 11 (1983) 494.
3. Yamazaki et. al., *Biochemical Pharmacology* 51 (1996) 313.

These data represent typical results.
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