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METABOLOMIC APPLICATIONS USING EXACT NEUTRAL LOSS ACQUISITIONS ON A Q-TOF

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SUMMARY

- Flavonoids play essential role in plant physiology
- Beneficial to human health
- Tomatoes contain only small amounts
- Aim to upregulate the flavonoid biosynthesis in the fruit by genetic engineering
- Many variations of the flavonoid aglycon exist as additions of various sugar moieties to free hydroxyl groups
- Exact neutral loss acquisitions used to detect accumulation of new flavonoid-glycosides in transgenic fruit

INTRODUCTION

Flavonoids are a large and diverse group of phenolic compounds ubiquitous in plants that play essential roles in plant physiology. They are normal constituents of the human diet that are beneficial to human health due their antioxidative and other health promoting effects. Since some major crops, e.g. tomato, contain only small amounts of flavonoids in their edible parts the aim is to upregulate the flavonoid biosynthesis by means of genetic engineering. Apart from the aglycon, many variations exist on this basic structure by the addition of various sugar moieties to free hydroxyl groups, resulting in a large group of related molecules. In addition, since many of these compounds are unstable, spontaneous loss of one or more glycosidic side chains during MS-analysis, even in a low energy mode, is a commonly observed feature. This problem can be resolved by the use of precursor and neutral loss acquisitions.

These techniques prove to be a very valuable tool as a selective way to search for common losses or common ions, which will be diagnostic of a particular compound group like flavonoid glycosides. Until recently this work has been carried out with triple quadrupole technology using nominal masses. The advantage of the hybrid time of flight mass spectrometer is the selectivity that can be achieved using the accurate mass capabilities for both precursor ion and neutral loss acquisitions. This enables survey functions to be carried out with a very high level of specificity and at the same time eliminating matrix related interference, which may occur when analysing complex biological matrices.

Exact neutral loss detection is achieved via sequential high and low energy MS acquisitions. When the loss of a sugar or combination of sugars is detected from a predefined list within a mass tolerance window of typically +/- 20 mDa, data dependent MS/MS is automatically performed on the precursor. The continual acquisition of data using the high and low energy mode allows further interrogation of the data for full scan MS information.

Here we present flavonoid compositions of control and transgenic tomato fruits as determined by LC/MS/MS analysis, using exact neutral loss acquisitions for the loss of glycosides on a Q-Tof Ultima fitted with LockSpray using a tolerance window of +/- 20mDa. The MS/MS spectra were generated with mass accuracies of <5ppm RMS. The transgenic plant was transformed with a gene construct that enhances flavonoid biosynthesis. Expression of the two genes in the fruit appeared to result in the accumulation of several new flavonoid-glycosides, which were easily resolved by exact neutral loss acquisitions.

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These results underline the usefulness of exact neutral loss acquisitions for the analysis of complex plant extracts.

EXPERIMENTAL CONDITIONS

- Sample Preparation:
  - Plants were grown simultaneously in a greenhouse
  - Tomato fruits harvested in liquid nitrogen and ground
  - 0.5 g of the material was extracted in 2mL of 62.5% methanol: 37.5% water +0.125% formic acid and sonicated for 15 min then filtered
  - Injection volume 30 µL
- Solvent Delivery System: Waters 2795
  - Column : Waters Xterra MS C18 3.5µ 2.1x150 mm
  - Mobile Phase A: Water +0.1% formic acid
  - Mobile Phase B: MeCN +0.1% formic acid
  - Gradient: 0 min 95%A, 25 min 75%A, 35 min 65%A, 37min 50%, 41min 50%A, 42 min 95%A
  - Flow rate: 0.25 mL/min
- Mass-Spectrometer: QTof Ultima
  - Ionisation mode: Positive ion Electrospray
  - Exact Neutral Loss Acquisition : Low Energy 5eV, High Energy 25eV
  - MS/MS: CE1= 10eV , CE2= 30eV, argon collision gas

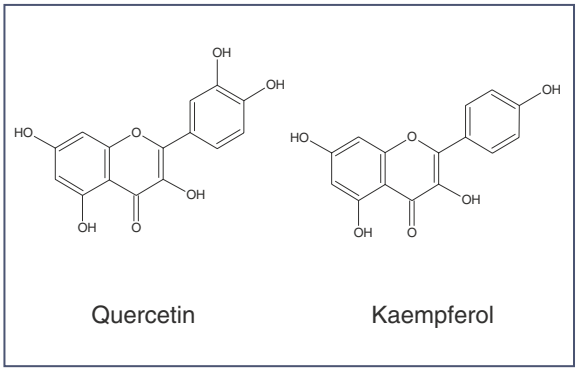

Figure 1. Examples of Flavonoid Structures

POTENTIAL GLYCOSYLATIONS

- Pentoses $C_5H_{10}O_5$ - monoisotopic neutral loss 132.0422
- Deoxyhexose $C_6H_{12}O_5$ - monoisotopic neutral loss 146.0579
- Hexosamine $C_6H_{13}NO_5$ - monoisotopic neutral loss 161.0688
- Hexose $C_6H_{12}O_6$ - monoisotopic neutral loss 162.0528
- Hexuronic acid $C_6H_{10}O_7$ - monoisotopic neutral loss 176.0321

* Different combinations of all of these were also monitored for neutral losses. 19 individual masses and/or combined were analysed

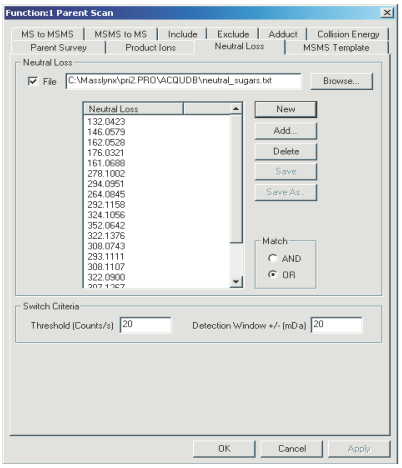

Figure 2. Neutral Losses Monitored

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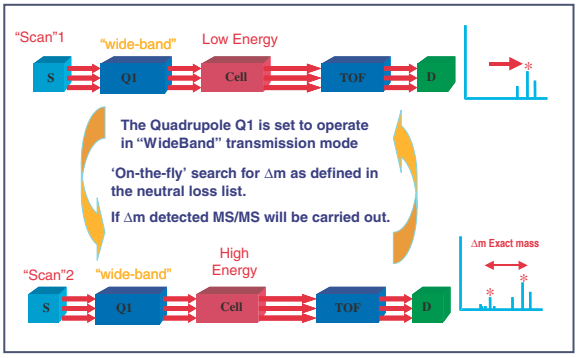

Figure 3. Neutral Loss Acquisitions on a QTof

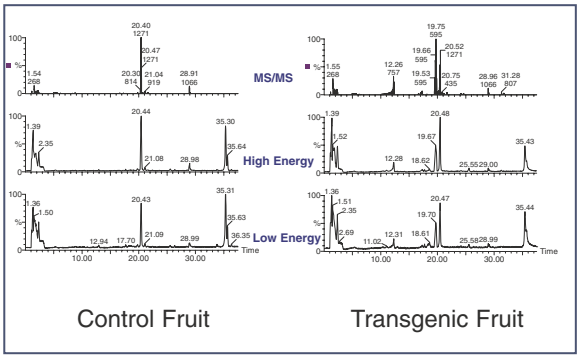

Figure 4. Neutral loss chromatograms from control and transgenic tomatoes

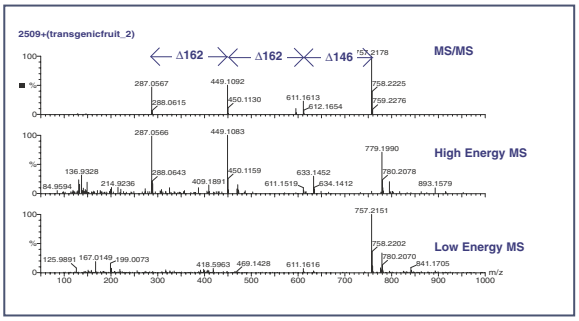

Figure 5. Spectra from peak at 12.3 min showing loss of 3 sugars (deoxyhexose + 2 hexose)

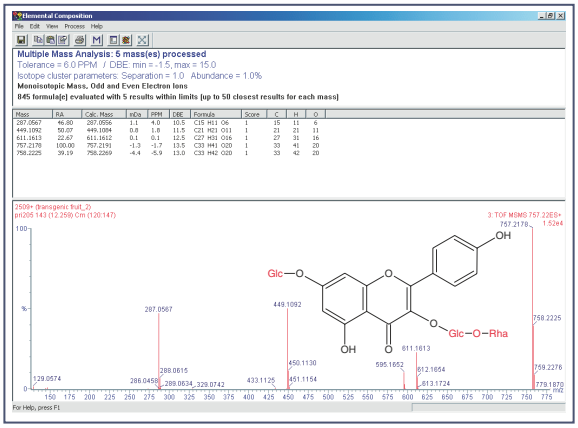

Figure 6. Elemental composition report and postulated structure for peak at 12.3 min

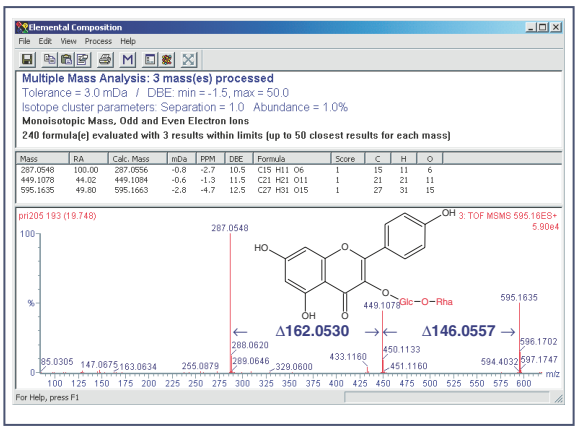

Figure 7. MS/MS spectrum,elemental composition report and postulated structure for peak at 19.7 min

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CONCLUSIONS

- Selective and sensitive (+/- 20mDa window) tool for screening for glycosylated metabolites in complex extracts (19 putative sugar losses monitored)
- Fast screening method for differentially produced glycosides in control and transgenic mutant plants, containing MS and MS/MS information from one single chromatographic run
- Exact mass measurements (<5ppm RMS) and elemental compositions reports provide information on the putative metabolites and their aglycon fragments
- The flavonoid content of the tomatoes were increased by a factor of >50 with no difference in taste being discernible

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