The Application of MS/MS Directed Purification to the Identification of Drug Metabolites in Biological Fluids

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Introduction

The identification of drug metabolites following animal or human volunteer studies is essential to the drug discovery & development process and regulatory submissions. Traditionally this has been achieved by the use of liquid chromatography or gas chromatography coupled to mass spectrometry[1,2]. More recently the use of hyphenated techniques such as LC/NMR and LC/NMR/MS have become more common place in the drug metabolism laboratory allowing a more precise identification of the site of metabolism[3-,4]. While LC/NMR and LC/NMR/MS are extremely powerful tools they are low throughput and sensitivity limited. The loading capacity of analytical columns restricts the amount of material that can be loaded on to the column before the column exhibits either volume or mass overloading effects and the chromatographic resolution is lost. Thus making it less attractive for the analysis of highly potent compounds dosed at low levels or those compound that undergo extensive metabolism. In such cases it is often necessary to perform a pre-concentration step such as SPE or L/L extraction, these are both time consuming and risk loosing valuable information.

The use of MS directed auto-purification, using semi preparative scale columns (typically 20mm i.d), is now common place within the pharmaceutical industry, especially to support lead candidate purification. This approach has also been applied to the isolation of drug metabolites with some success[5]. The extra sensitivity and selectivity of MS/MS mass spectrometry should allow for the more precise selection of drug metabolites, and the use of neutral loss and precursor ion scanning detection modes will facilitate the collection of drug metabolites without the need for prior knowledge of compound metabolism.

This paper shows how tandem quadrupole mass spectrometry has been employed with both analytical and semi-preparative scale chromatography for the isolation of the metabolites of common pharmaceuticals, from urine. The application of MRM, neutral loss and precursor ion scanning is demonstrated. We also demonstrate how the use of MS/MS directed purification facilitates the combination of samples from several chromatographic runs.

System

Waters Alliance—Quattro Micro LC/MS

Waters Alliance® HT 2795 with a SunFire™ C_{18} 5µm 4.6 x 100 mm column at 40 °C. Flow is split 1:20 with a Valco Tee. 95% of the flow passes the 2996 Detector to the Waters Fraction Collection III. The other 5% is to the Quattro micro Mass Spectrometer equipped with an ESCi™ Multi-Mode Ionisation Source.

Methods

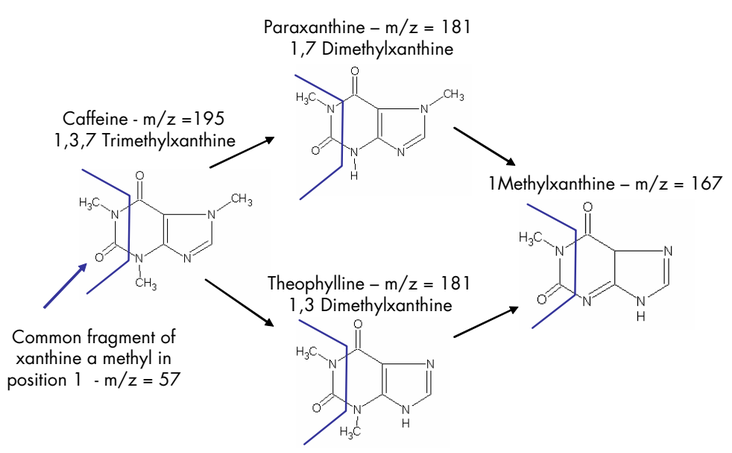
Caffeine Metabolites

Separation

Water: Acetonitrile: 0.1%Formic acid, 1.25 ml/min total flow gradient. 0 – 5 minutes: 0%, 5 – 35 minutes: 0 – 10% B, 35 – 35.5 minutes: 10 – 95% B, 35.5 – 39.5 minutes: 95% B, 39.5 – 40 minutes: 95 – 5% B, 45 minutes end

MS detection

Electrospray positive, 3 kV capillary voltage, 30 V cone voltage, 20 V collision energy (for MS/MS experiments)



Metabolism of caffeine by demethylation. Metabolites which maintain the methyl group in the 1 position have a common fragment of 57.

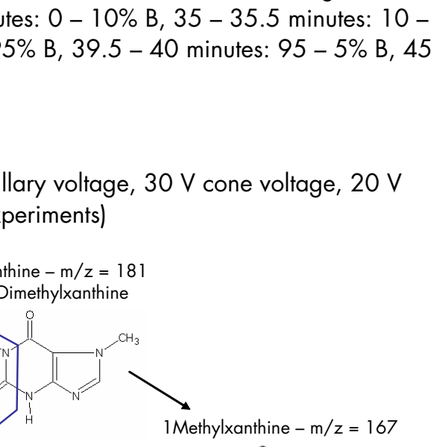
Ibuprofen Metabolites

Separation

Water: Acetonitrile: 10 mM ammonium formate, 1.25 ml/min total flow gradient. 0 – 5 minutes: 5%, 5 – 35 minutes: 5 – 60% B, 35 – 35.5 minutes: 60 – 95% B, 35.5 – 39.5 minutes: 95% B, 39.5 – 40 minutes: 95 – 5% B, 45 minutes end

MS detection

Electrospray negative, 3 kV capillary voltage, 30 V cone voltage, 20 V collision energy

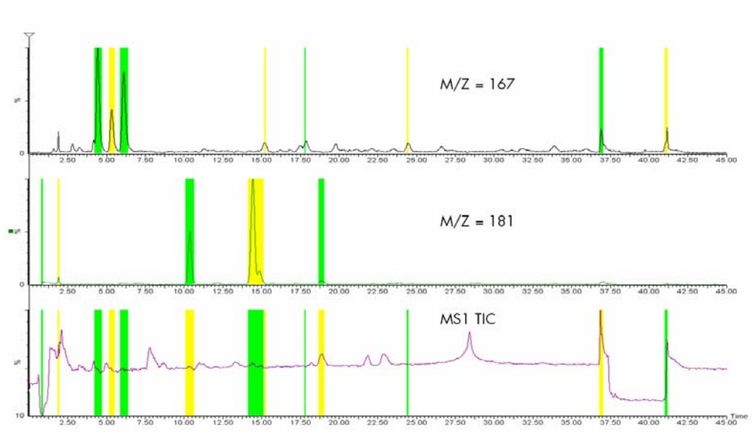


Ibuprofen gluceronide metabolite with a common daughter ion on 193[7].

Single Quadrupole Purification

With single quadrupole directed purification, all ions generated in the source are passed through the quadrupole and detected. This mode is possible on the Quattro micro by using the "Scan" mode of acquisition. Only MS1 is scanned and there is no collision energy or scanning of Q3. Because all the ions generated are detected, complex mixtures can contain numerous isobaric interferences. Consequently, multiple fractions can be generated from a single m/z value. Additional analysis is then required to identify fraction of interest.

Example Data - Caffeine metabolites m/z = 167 and 181



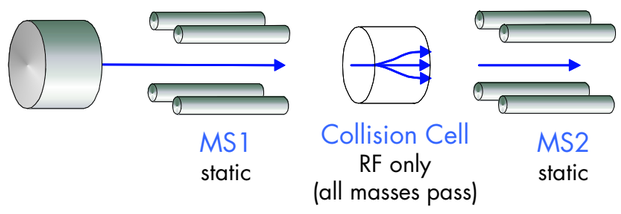
Fractionation based only on scanning the 1st quadrupole.

- 8 fractions collected for m/z 167 and 5 fractions collected for m/z 181
- Additional analysis required to determine fraction of interest

Tandem Quadrupole Purification

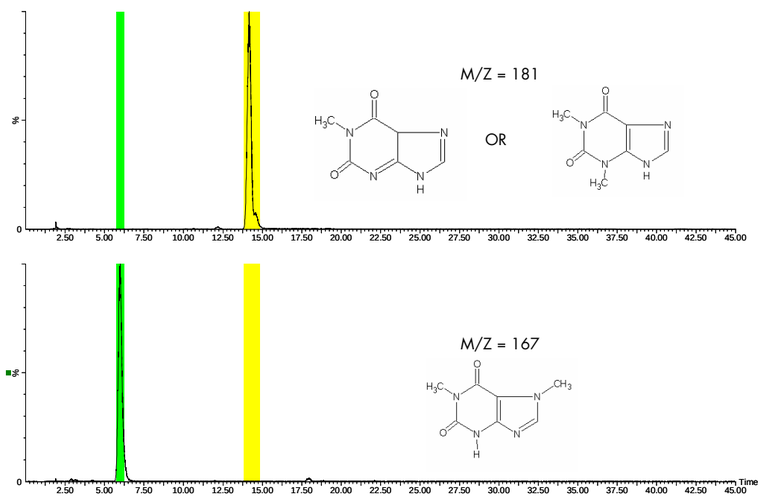
MRM Collection

With MRM (Multiple Reaction Monitoring) data acquisition, MS1 is static on the parent mass and MS2 is static on a specific daughter ion.



By selectively detecting a product ion, the signal-to-noise ratio is optimised, thus reducing the isobaric interference and allow only the target to be collected. This mode of acquisition requires previous knowledge of the exact parent and the exact daughter ions before purification.

This example data shows the MRM acquisition and collection of the caffeine metabolites. The metabolites of interest for isolation have the transitions 181 to 134 and 167 to 110.

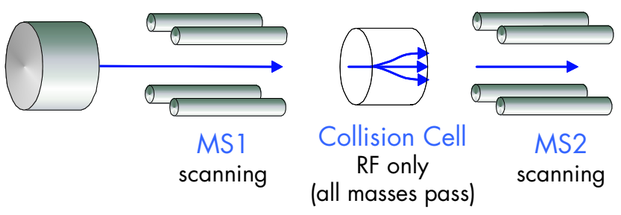


Fractionation based on MRM acquisition.

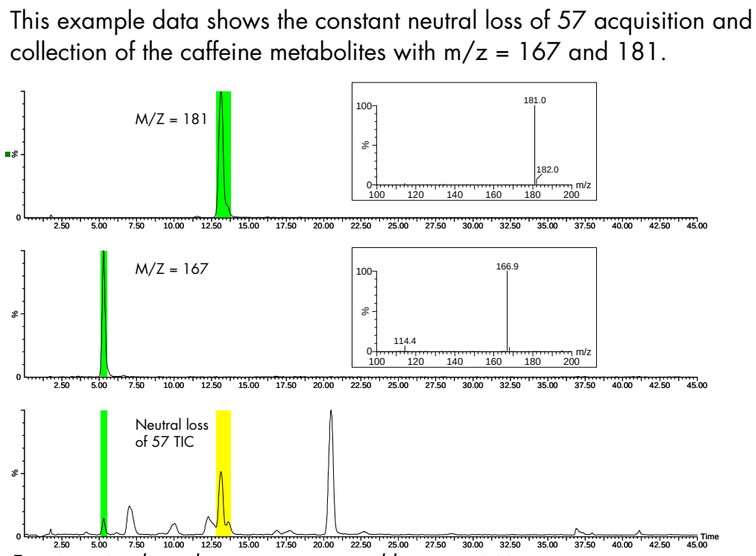
- For the peak present in the chromatogram, both the specific parent and the specific daughter ion.
- The chromatogram shows that only one fraction for each mass was collected. Note: Only 1 of the 2 possible dimethyl metabolites was present in this sample. Further analysis is necessary to determine its identity.

Constant Neutral Loss Collection

With constant neutral loss acquisition, MS1 and MS2 are scanned in synchronisation. When MS1 transmits a specific parent ion, MS2 "looks" for a daughter which is the parent minus the neutral loss value. If the correct daughter is present, it registers at the detector. The constant neutral loss "spectrum" shows the only the masses of all the parents that lost the specific mass.



This example data shows the constant neutral loss of 57 acquisition and collection of the caffeine metabolites with m/z = 167 and 181.



Fractionation based on constant neutral loss acquisition

- 2 Fractions are collected, 1 for each mass.
- Fractions contain the target mass and have the specific neutral loss.

Applications for Fraction Collection from Constant Neutral Loss Acquisition

Mass Triggered Collection

With constant neutral loss acquisition, the only peaks detected are the ones with the loss of the specific mass, in this case 57. Depending on the specificity of the loss, numerous ions can be detected. This leads to complex total ion chromatograms. Therefore, when triggering by a specific mass, the collected target must contain the parent of interest and have a specific neutral loss.

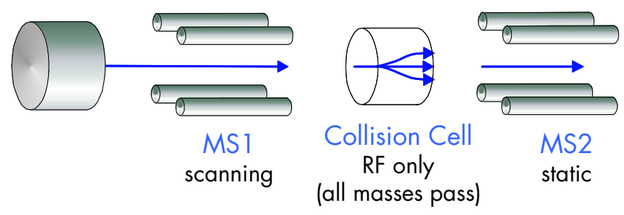
Collection Triggered on TIC

When using this mode of acquisition and collection, all the peaks with a specific neutral loss are collected. This is valuable when the metabolites have a specific loss related to the drug's structure. It could also be used for isolating a class of metabolites with a generic loss, for example sulfates (- 80) or glucuronides (- 176). The parent mass for each fraction can then be extracted and used to aid in the identification of the metabolites.

In the constant neutral loss example shown, collection could have also been triggered from the TIC. All peaks in the –57 TIC would be collected and additional analysis or data review would be required to find the desired fractions.

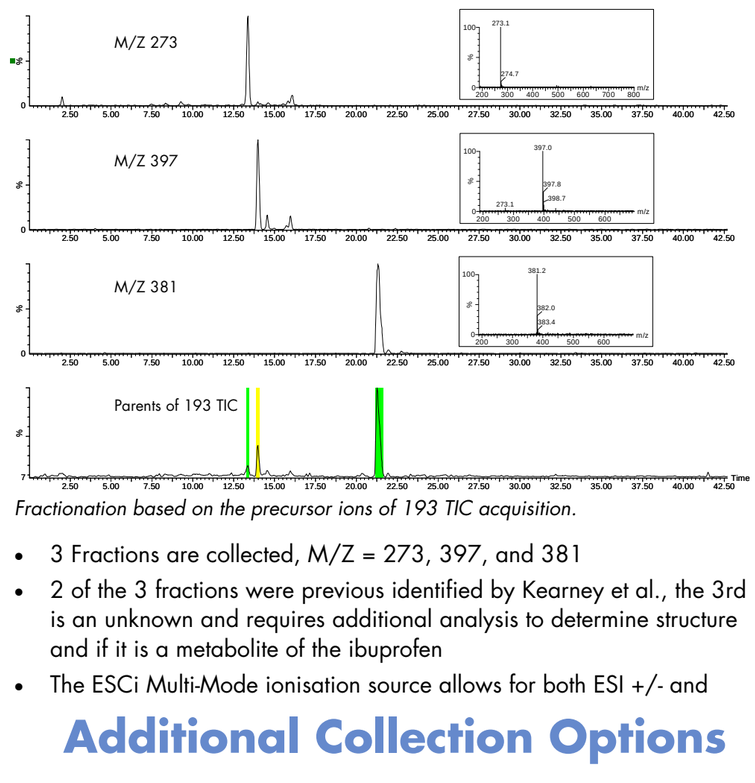
Precursor Ion Collection

With precursor ion acquisition, MS1 is scanning and MS2 is fixed on a specific daughter ion. If the specific daughter ion is observed, it is registered and the detector. The "spectrum" only shows the masses which have that specific daughter.



Fraction collection from a precursor ion acquisition has to be from the TIC, since the parent mass is unknown. This mode of fraction collection is valuable when the metabolites are unknown, but there is a common fragment of the core compound that can be detected.

This example data shows the collection of ibuprofen metabolites from the precursor ion of 193 acquisition.



Fractionation based on the precursor ions of 193 TIC acquisition.

- 3 Fractions are collected, M/Z = 273, 397, and 381
- 2 of the 3 fractions were previous identified by Kearney et al., the 3rd is an unknown and requires additional analysis to determine structure and if it is a metabolite of the ibuprofen
- The ESCi Multi-Mode ionisation source allows for both ESI +/- and APCI +/- acquisition to occur within the same run. This allows for fraction collection to be triggered form any of the acquisition channels, thus proving useful if the metabolites require different ionisation. With these type of samples, the only options for collection would be to split the sample and run in different modes or time base fractionate and the analysed all the fractions by both modes to determine the targets.
- Mixed trigger fractionation allows for boolean logic strings to trigger collection from multiple data traces. For example, collection can occur only when Mass A is present and Mass B is not or when a peak is present in 2 different traces.

Additional Collection Options

Conclusions

- Fraction collection with a tandem quadrupole mass spectrometer is now possible using 4 different modes of data acquisition, scan, MRM, constant neutral loss and precursor ion, allowing for improved versatility for fraction collection options.
- Scan mode uses only a single quadrupole so all ions formed are detected. This has the potential to increase the number of isobaric inferences detected and collected.
- MRM is the most selective by only monitoring a specific parent and a specific daughter. This greatly reduces the isobaric interferences but requires previous knowledge of the parent / daughter transition.
- Constant neutral loss can be used for a collecting a class of compounds with a target specific loss or a mode generic loss for a broader study. It can also be used as a second filter, where the target has to have a specific mass and the neutral loss.
- Collection from a precursor ion TIC scan allows for all the parents with a specific daughter ion to be collected. This mode of collection can be valuable when the metabolites are unknown, but there is a common fragment of the core compound that can be detected.
- The different modes of collection have different applications based on 1) the requirements of the study, 2) the level of previous knowledge about the sample and 3) the characteristics of the sample like ionisation and fragmentation.

References

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