# LC-MS based metabolomics and accurate mass measurements in complex extracts

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## Introduction

Metabolomics represents the in-depth profiling of metabolites intrinsic to several organisms. An important feature of metabolomics is identifying differences in metabolite patterns, thus demanding accurate measurements in analytical devices. Here we discuss accurate mass measurement on a Q-TOF micro (Waters) equipped with a Lockspray device.

## Aim

This part of our research investigates the possibility of accurate mass measurements in a metabolomic context. Our goal was to establish the mass accuracy of a Q-TOF micro (Waters), using the features available in the Masslynx software. A comparison was made between leucine-enkephalin as lockmass, and a mixture of compounds that covers the mass range 50-800.

## Materials

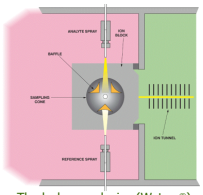
*HPLC Conditions:*
- Alliance 2695 (Waters®)
- Column:
  - Atlantis™ dC18, 2.1 x 150mm (Waters®)
- Mobile Phase:
  - Eluent A: 100% water + 0.1% formic acid
  - Eluent B: 90/10 acetonitrile/water + 0.1% formic acid
- Flow Rate: 200µl/min
- Injection Volume: 50µl

*MS Conditions:*
- Mass Spectrometer: Q-TOF micro (Waters®)
- Ion Source: Lockspray™ in positive ion mode
- Software: Masslynx 4.0
- *m/z*-range: 50-800
- Capillary Voltage: 3000V
- Cone Voltage: 30V

*Metabolites:*
Amino acids, carbohydrates, nucleotides, plant hormones, steroids, fatty acids, polyamines, carotenoids and others were used from stock solutions at 1mg/ml

*Lockspray solution:*
- Leucine-enkephalin 1ng/µl in 50/50 AcN/$H_2O$
  *m/z*-value: 556.2771
- Lockmix:
  - Acetanilide (*m/z* 136.0762)
  - Pentazocine (*m/z* 286.2171)
  - Trazodone (*m/z* 374.1747)
  - Dipyridamole (*m/z* 505.3251)
  - Aconitine (*m/z* 646.3227)

*Extraction procedure:*
*Arabidopsis thaliana* leaves were homogenized with an Ultra-Turrax mixer (Ika) in 2:6:2 $H_2O$:MeOH:$CHCl_3$. Samples were centrifuged for 10 minutes and the supernatant (after spiking) was evaporated under nitrogen. Before injection, samples were dissolved in 75/25 $H_2O$/AcN + 0.1% FA.



The lockspray device (Waters®)

## Methods

❖A mixture of 5 compounds with lockmasses covering the typical mass range in metabolomic research from 50-800, is considered to give better accurate mass measurements than a single compound. Leucine-enkephalin is the standard product in accurate mass measurement using lockspray devices. Both lockspray compositions were tested to evaluate the best accurate mass measurement.

❖Accurate mass measurement is an important feature in identifying metabolites in metabolomics. Subtle differences from multivariate data analysis can thus be attributed to specific metabolites, revealing complex biochemical regulations in e.g. plants.

## Results

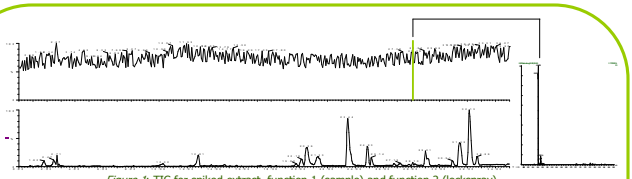


*Figure 1*: TIC for spiked extract, function 1 (sample) and function 2 (lockspray), using leucine-enkephalin as a lockmass; spectrum of leu-enk.

| Name compound | Accurate mass (Da) | Mass accuracy (ppm; n=3) |
|---|---|---|
| *leucine* | 132.1025 | 8.83 ± 1.58 |
| *phenylalanine* | 166.0868 | 11.24 ± 2.28 |
| *s-adenosylmethionine* | 399.1451 | -2.92 ± 4.00 |
| *lactose* | 365.106 | -4.93 ± 8.55 |
| *maltotriose (Na+)* | 527.1588 | 2.85 ± 1.71 |
| *uracil* | 113.0351 | 3.24 ± 5.03 |
| *cytidine* | 244.0933 | 2.59 ± 12.31 |
| *IMP* | 349.0594 | 6.11 ± 5.45 |
| *zeatin* | 220.1198 | -3.03 ± 2.15 |
| *jasmonic acid* | 211.1334 | -3.16 ± 7.28 |
| *abscisic acid* | 265.144 | 0.00 ± 5.07 |
| *epibrassinolide* | 481.3529 | 1.94 ± 3.72 |
| *spermidine* | 146.1657 | -0.23 ± 3.77 |

*Table 1:* Several spiked metabolites with accurate mass (Da) and mass accuracy in ppm, using leucine-enkephalin as a lockmass (n=3)

Leucine-enkephalin (*Figure 1*) is presented as lockmass in accurate mass measurement. By means of the accurate mass measure tool in Masslynx, *m/z* correction throughout the entire chromatogram is possible. When analysing complex LC-MS matrices, data processing software bunches mass with retention time. In this perspective, masses are taken at most intense peak signal. Mass deviations up to 40ppm are obtained (results not shown). Such deviations are too large for identification purposes but adequate for multivariate data analysis where binning of masses is the rule. When mass correction is performed at a signal intensity (e.g. at the foothill of a peak) close to the intensity of the lockmass, however, mass differences lay within 10ppm (*Table 1*).

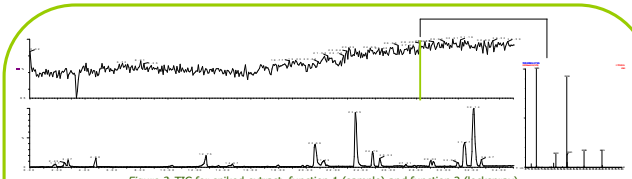


*Figure 2*: TIC for spiked extract, function 1 (sample) and function 2 (lockspray), using the lockmix for accurate mass measurement; spectrum of lockmix.

| Name compound | Accurate mass (Da) | Mass accuracy (ppm; n=3) |
|---|---|---|
| *leucine* | 132.1025 | 42.39 ± 9.66 |
| *phenylalanine* | 166.0868 | 45.96 ± 27.76 |
| *s-adenosylmethionine* | 399.1451 | 38.00 ± 16.20 |
| *lactose* | 365.106 | 37.07 ± 6.17 |
| *maltotriose (Na+)* | 527.1588 | -1.83 ± 2.08 |
| *uracil* | 113.0351 | 49.24 ± 12.89 |
| *cytidine* | 244.0933 | -36.87 ± 26.31 |
| *IMP* | 349.0594 | 11.27 ± 7.04 |
| *zeatin* | 220.1198 | 9.24 ± 3.22 |
| *jasmonic acid* | 211.1334 | 13.74 ± 5.76 |
| *abscisic acid* | 265.144 | -2.39 ± 10.05 |
| *epibrassinolide* | 481.3529 | 16.20 ± 2.40 |
| *spermidine* | 146.1657 | 41.28 ± 9.29 |

*Table 2:* Several spiked metabolites with accurate mass (Da) and mass accuracy in ppm, using the lockmix for accurate mass measurements (n=3)

Via a tool in Masslynx, called secondary reference correction, a possibility is provided to correct masses using a mixture of compounds. Here we present a lockmix of 5 compounds covering the *m/z* range 50-800 (*Figure 2*). The secondary reference tool corrects masses with the lockmass closest to the *m/z* of the sample metabolite. Theoretically, better lockmass correction could be envisaged. Nevertheless, larger deviations are observed as can be seen in *Table 2*.

## Conclusion

❖We tried to obtain a better lockmass-correction in the *m/z* range 50-800 using a mixture of 5 compounds. Nevertheless, leucine-enkephalin seems the best solution for *m/z*-correction in a lockspray setting.

❖Accurate mass measurements with a Q-TOF micro are feasible within a mass range of 10ppm, if the signal intensity of compound and lockspray are matched.