ThP578

Development of a Data Processing Approach to Support Ultra High-Throughput MS Acquisition

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1. Introduction

Ultra-High-throughput analytical systems (UHTS) are needed in various field of the industry to assay large quantities of samples in a short time or to improve response time.

UHT-LC-MS systems have gained popularity thanks to the versatility of LC separation modes, high automation, sensitivity and selectivity of MS. In addition, other introduction modes such as Laser Diode Thermal Desorption are used for extremely short analysis. Hardware became extremely fast. However, still some time is needed for software to upload settings and receive the ready state between two samples. This extra amount of time can ruin the throughput of the system (see figure 1). A solution is to record all data in a single data file, but a software able to process it was needed.

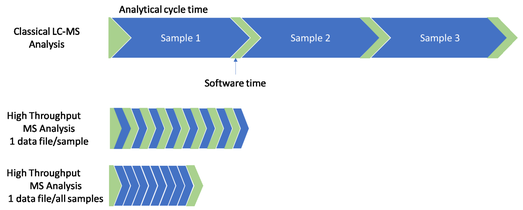

Figure 1 Impact of software time on analytical throughput

2. Material and Reagents

2-1. LDTD-MS/MS

Laser Diode Thermal Desorption (Luxon, Phytronix, Québec, Canada) coupled to triple quadrupole mass spectrometer LCMS-8060 (Shimadzu Corp., Kyoto, Japan) was used to assay testosterone, 17-hydroxyprogesterone, androstenedione and cortisol in human plasma. Double charcoal-stripped human plasma (BioIVT) was spiked with standard solution to reach concentrations from 0.4 to 100 ng/mL.^{13}C3-labelled analogues (D_4 for cortisol) were used at internal standards at a concentration of 25 ng/mL in plasma. Samples were prepared by supported-liquid extraction and assayed by Luxon-MS/MS. 3 µL of extract were transferred in LazWell plate wells and allow to dry. Analysis time was of 3.5 seconds per sample. Acquisition was performed using APCI positive ionization and MRM mode. 2 transitions per target compound and 1 transition for ISTD were recorded.

2-2. LC-MS/MS

Analytical standards of Clozapine, Nor-Clozapine, and Clozapine-D_8 , Nor-Clozapine-D_8 (as internal standards) were purchased from Wako Chemicals. Individual stock solutions at 100 mg/mL were prepared in Methanol and further diluted to make calibration standards (8 levels). The calibration range was from 0.5 to 75 µg/L.

The instrumental configuration includes a Nexera™ XR LC system with LCMS-8050 (Shimadzu Corp.). The newly designed autosampler SIL-40C XR, which provides an ultrafast injection cycle time (<7 sec) was used in single channel configuration. SIL-40C XR Autosampler can be programmed to provides multiple injections at specific interval time (30 seconds) and multiple rinsing sequences.
Detailed analytical conditions can be provided upon request.

2-3. Software

A software was developed (Reifycs Inc, Tokyo, Japan) in order to read the original data files generated by the LC/MS software (LabSolutions, Shimadzu Corp, Kyoto, Japan). In both analysis cases, one full 96-well plate was assayed within one data file. For UHPLC-MS/MS, samples were split by applying a constant time interval. For LDTD-MS/MS, as the analysis time is shorter, it was necessary to take into account the little time shift generated by the movement of the plate from sample to sample. Therefore, the laser shooting log was recorded (Start and end time) and automatically embedded in the data file. The software was then using this information in order to split the data accurately.

3. Results

3-1. LDTD-MS/MS

A 96-well plate containing the plasma samples was assayed first in single-file acquisition mode. The total time to record was less than 7 minutes. The complete profile is shown in figure 2.
Then the file was processed by the new software to separate peaks and assign results to each sample. The processed data is shown in figure 3.

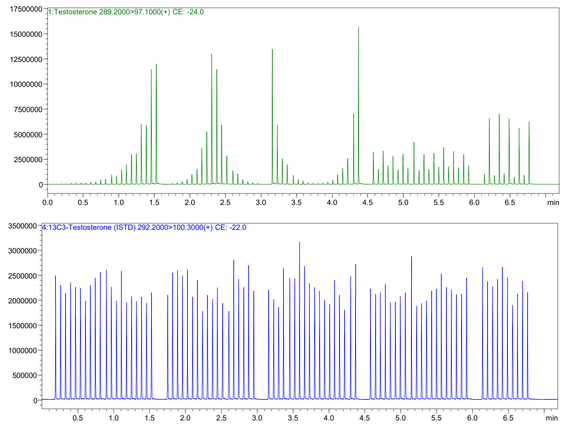

Figure 2 MRM profile of single file acquisition for Testosterone (green) and $^{13}C_3$-Testosterone (blue).

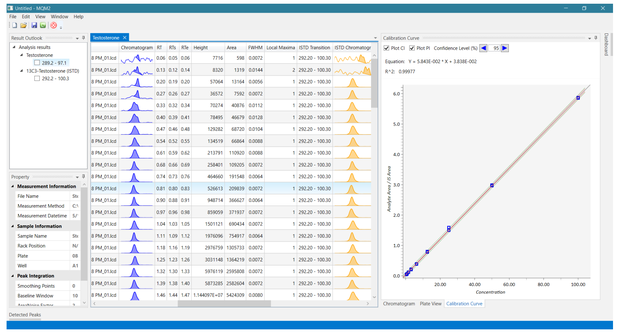

Figure 3 LDTD-MS/MS single file data processed

Another plate was assayed using 1 data file acquisition per sample. The total time to assay the 96 samples was of 27 minutes.

3-2. LC-MS/MS

The Clozapine and Nor-Clozapine quantitation was performed using a single datafile which containing all the analytical samples (a system blank followed by 9 calibration points and 2 blank samples). The total run time was 6 minutes for 12 samples , and is compatible with ultra – high throughput analysis (48 minutes per plate). The chromatogram of single file acquisition is shown in figure 4.

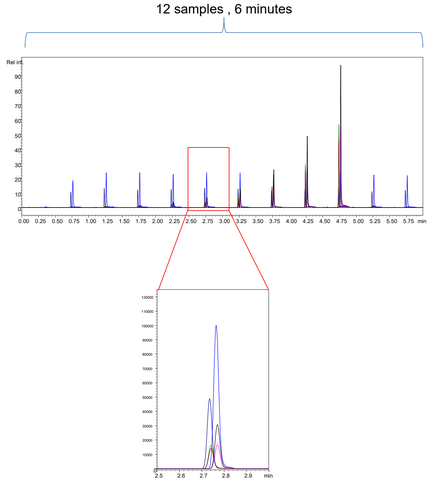

Figure 4 MRM profile of single file acquisition in LC-MS/MS

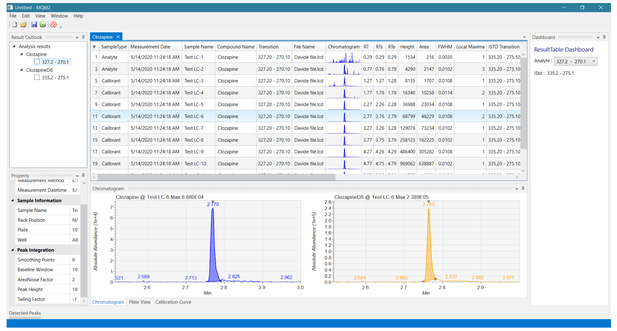

Figure 5 LC-MS/MS single file data processed

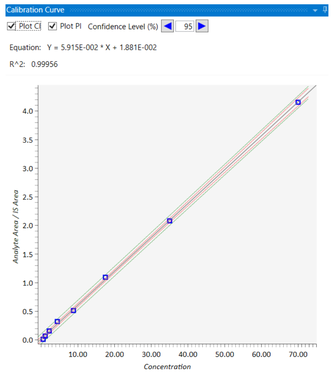

Figure 6 LC-MS/MS single file data calibration curve of Clozapine

Data processed from the single file by the new software and a calibration curve are shown in figure 5 and 6.

The same samples were injected in the classical one data per sample mode. The time necessary to acquire all data was doubled.

4. Conclusion

- Acquisition in single datafile mode demonstrated to be suitable for ultra-high throughput analysis by strongly reducing the overall acquisition time compared to the traditional approach (one file – one sample).
- The use of a novel software for retrieving the quantitation information from the single datafiles allowed to streamline the post – processing workflow without modifying the analytical parameters traditionally used for method validation (retention time , Internal standard ratio etc..)