Acetaminophen Toxicity: QUAL/QUAN High Resolution MS Approaches for Drug Metabolism and Metabolomics Investigations

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Introduction

Acetaminophen (APAP) overdose has been described as the major cause for acute liver failure in developed countries before viral hepatitis. A reactive metabolite of APAP, N-acetyl-p-benzoquinone imine (NAQUI), produced under certain conditions (*e.g.* overdoses) has been considered as the prime event that leads to acute liver failure [1]. Modification of APAP metabolism is then the first critical parameter to investigate when looking at possible effects of APAP on different pathologies. APAP metabolism was then often investigated to ensure its safety on patients with chronic liver diseases, mainly on cirrhosis resulting from hepatitis and/or alcohol abuse [2]. However, despite its large use as post surgical pain reliever, the metabolism of the potential hepatotoxic APAP on patients that underwent major hepatic resections has been poorly described. The further exploration of this exogenous metabolism is a definite first step to distinguish post surgical problems and to determine a potential further intoxication due to therapeutic ingestion of APAP for these patients. Metabolomics exploration is also of great interest to understand mechanism of post surgical metabolic changes that can be due to APAP ingestion.

The approach we present herein combines on a single analytical platform and with a single sequence the quantitative and qualitative measurements of samples of interest. The quantification of APAP and two of its metabolites with HR-SRM (High Resolution-Selected Reaction Monitoring) was first performed. The screening and identification of exogenous metabolites using principal component analysis (PCA) and principal component variable grouping (PCVG) [3] tools was then performed subsequently on subset of patients that were treated with APAP and those that were not. A list of potential candidates was then obtained and screened with the list of expected APAP metabolites. A confirmation step was then made using mass accuracy of TOFMS scan and HR-MS/MS (FragAll). Finally, after removal of peaks related to APAP metabolites, PCVG was performed to determine potential biomarkers. The step to remove exogenous metabolites was crucial as they, due to their large variation as a function of time and across groups, tend to pollute PCA outcome. Potential biomarkers lists were then generated and compared to metabolomics databases. The confirmation of these candidates was then performed using structural identification and MS/MS similarities with MS/MS databases using FragAll approach.

Materials and Methods

Samples from 3 different groups were considered for this study. A first group where patients received a 2 g APAP dose after a major hepatic surgery, a second group with the same APAP dose administered after a general surgery and a third control group representing patients with general surgery that did not receive APAP. Plasma samples were collected from 16 different individuals at different time points (total of 34 samples). Plasma samples (50 µL) underwent a protein precipitation in MeOH/EtOH (4:1; v/v) with acetaminophen-d4 and L-phenyl-S-Cysteine as internal standards. Analyses were performed using UHPLC (UltiMate 3000 Dual RSLC, Dionex) coupled to quadrupole-TOFMS (TripleTOF 5600, AB Sciex). The column was a YMC Ultra-HT Hydrosphere C18, S-2µm, 150x2.0 mm i.d. The MS acquisition strategy is shown in Figure 1.

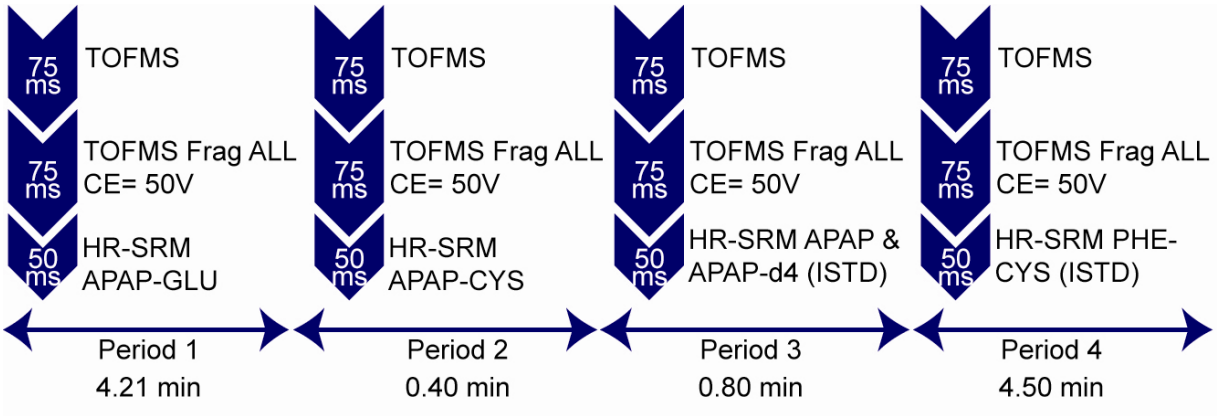

Fig 1.: Acquisition mode for QUAL/QUAN MS experiments: Each MS acquisition period contains two TOFMS scans at low and high collision energy followed by an HR-SRM scan for the quantification of APAP and its metabolites.

Data acquisition was performed on Analyst TF 1.5.1. PeakView (v. 1.1, AB Sciex) and its add-in LCMS Peak Statistics and Formula Finder were used for general spectra and data quality evaluation. MultiQuant (v. 1.2, AB Sciex) was used for quantification purposes. MarkerView software (v. 1.2.1, AB Sciex) was used for peak picking, PCA and PCVG.

Results

Validation of the analytical method for the quantification of APAP and its two metabolites of interest *i.e.* acetaminophen-glucuronide (APAP-GLU), one of the two major circulating metabolite metabolites and acetaminophen-cysteine (APAP-CYS), a degradation product of the detoxifying glutathione linked acetaminophen, was performed in HR-SRM mode (TripleTOF 5600) and compared to SRM mode (4000 QTRAP). Performances in terms of Limit of Quantification (LOQ), precision and accuracies were comparable for the two instruments: 20 ng/mL for APAP and 50 ng/mL for APAP-CYS and APAP-GLU; accuracies and precision within

15%, 20 % for LOQ. Results for the quantification of APAP on patients revealed a delay and reduction in APAP metabolism for patients that underwent hepatic surgery compared to those that underwent general surgery. Levels of APAP-CYS were also found to be more important in the first group after 24 hours indicating also a reduced metabolism or an increased production of toxic metabolite.

The exploration of exogenous metabolites was then performed with PCA and PCVG (Fig. 2a-2b). The obtained features that had the same variation profiles between the general surgery group with or without APAP determined peaks of interest regarding potential exogenous metabolites (fig. 2c). Peaks of interests were then extracted and MS/MS spectra obtained with the FragAll approach. A companion software of PeakView was used to deconvolute and assign MS/MS ions coming from the same precursor to ease identification (Fig. 2d).

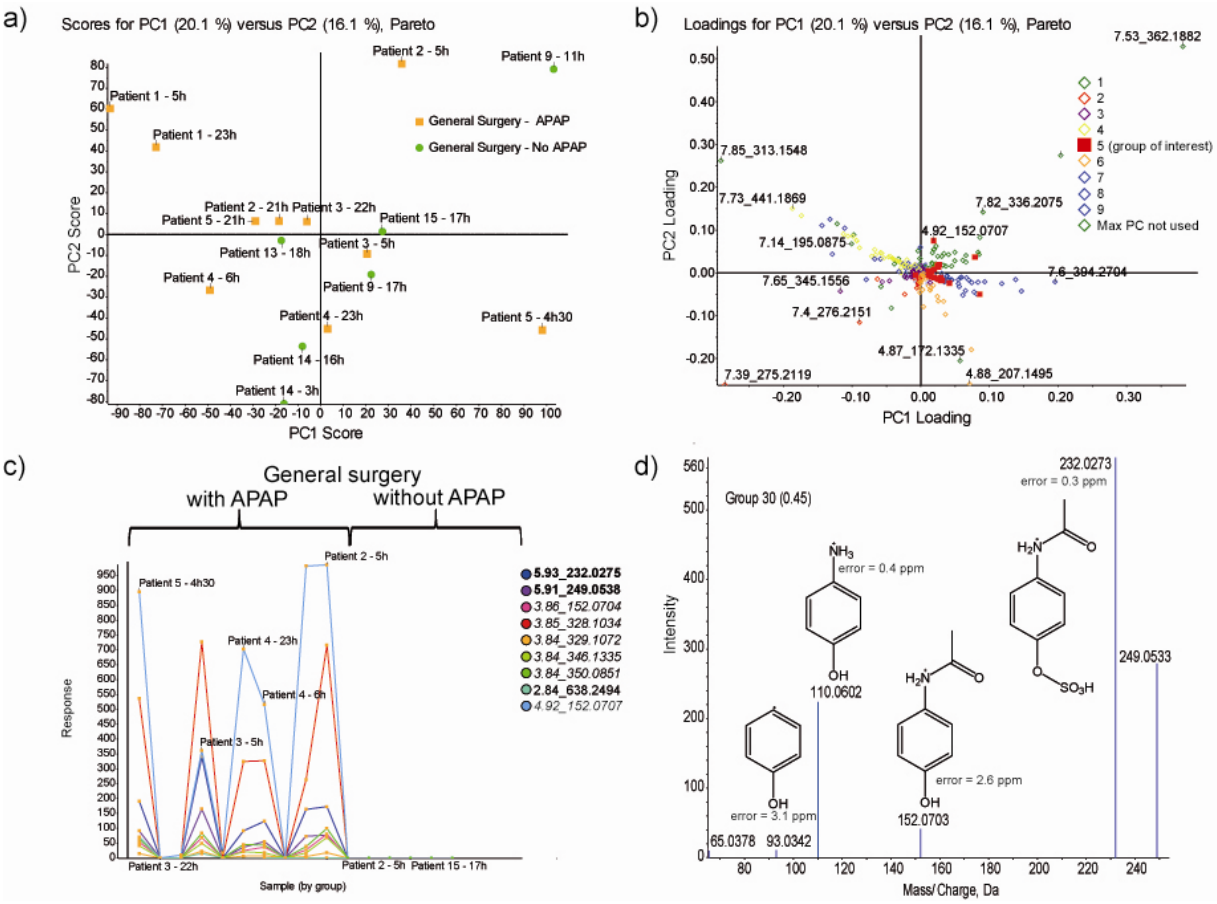

Fig 2: a) Scores plot obtained with PCA for the patients groups with/without APAP administration; b) Loadings plot after PCVG treatment; c) Extracted list of potential exogenous metabolites from PCVG; d) Deconvoluted MS/MS spectrum obtained with FragAll for precursor at *m/z* 232.0275: identification of acetaminophen-sulfate metabolite.

APAP exogenous metabolites levels are variables that changes dramatically during time; hence they change the PCA outcome. Once these features removed, PCA and PCVG were performed to determine potential biomarkers that differentiate hepatic surgery with general surgery patients. A candidate list was then submitted to available web databases for identification and completed as above with the high resolution MS/MS spectra as above. One identified potential biomarker was uric acid.

Conclusion

An analytical platform using a single high resolution MS has been presented for the simultaneous quantification of exogenous metabolites along with exogenous and endogenous metabolites screening and identification. Quantification performances (LOQ, precision, accuracy) for targeted metabolites analysis on TripleTOF 5600 (HR-SRM) were comparable to 4000 QTRAP (SRM). The TripleTOF allows short duty cycles (50 msec in PIS) compatible with UHPLC and is more generic; in particular, the fragment ion can be selected post-acquisition. Investigation of exogenous metabolism and endogenous metabolites allowing substance identification in one single run using multiple experiments (FragAll, PIS) was performed. High resolution was essential for selectivity and elemental composition determination of precursors and fragments.

References

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