Sensitive and Selective Detection and Characterization of Drug Metabolites in Human Plasma by LC-HRMS: Application of Matrix Removal-Based Data Processing Strategy

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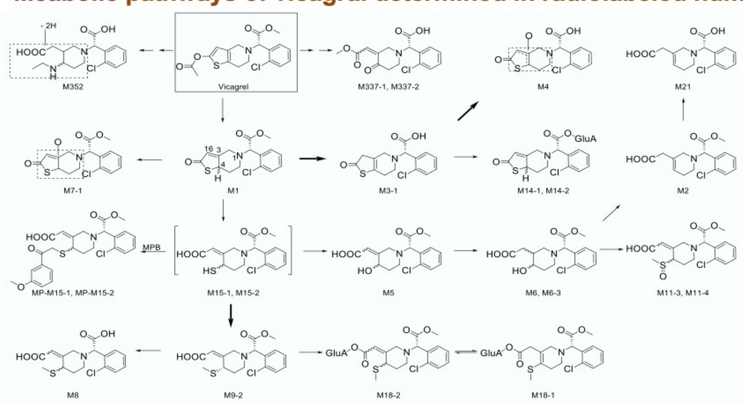
INTRODUCTION

- Metabolite profiling and identification of a testing drug in human plasma by LC-HRMS are routinely carried out in clinical phase I trial. Results provide crucial information for metabolites in safety testing (MIST) and guide pharmacological activity and DDI evaluation of plasma metabolites.
- However, due to low levels of metabolites in plasma and interferences of biological and formulation components, detection and identification of plasma metabolites using common LC-HRMS methods are time-consuming and often failed.
- The main objective of the study was to develop and evaluate a matrix removal-based data processing (MRDP) workflow for sensitive and comprehensive profiling and structural characterization of human plasma metabolites by LC-HRMS.

Meabolic pathways of vicagral determined in radiolabeled human ADME study[1]



GluA: glucuronic acid. MPB: derivation reagent (2-bromo-3'-methoxyacetophenone).

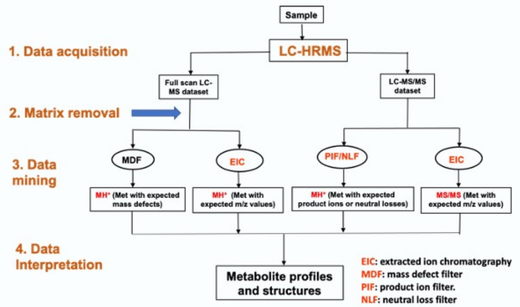
[1] Y Zheng et al. Mass balance, and metabolism of [14C]vicagrel, a novel irreversible P2Y12 inhibitor in humans. Acta Pharmacologica Sinica (2021) 42:1535 – 1546.

EXPERIMENTAL APPRAOCH

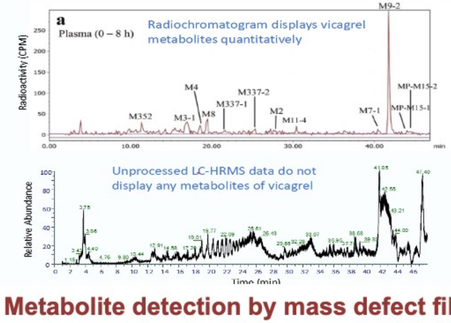
- Human plasma samples (0–168 h) were collected from six healthy male subjects after a single oral dose of 20 mg (100 μCi) [14C]vicagrel[1].
- Accurate mass LC-MS and LC-MS/MS datasets of predose and AUC-pooled (0-8 h) plasma samples were acquried by LC-HRMS (QE plus) and processed by the MRDP workflow using in-house developed software. Structures of detected metabolites were characterized based on spectral interpretation and biotransformation knowledge.
- Metabolite profiles of the pooled plasma sample determined using the MRDP workflow in this study were compared with those previously determined by LC-radiodetection/HRMS[1] .

RESULTS

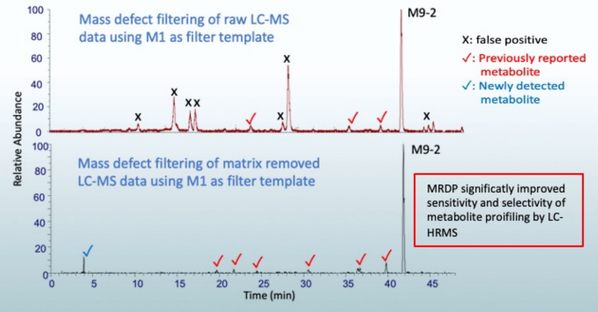
Matrix removal-based data process (MRDP)



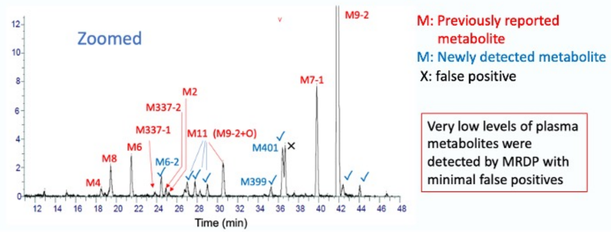
Detection of vicagrel metabolite in human plasma (0 – 8 h) by LC-radiodetection/HRMS



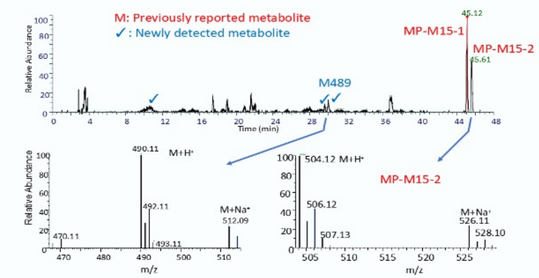
Metabolite detection by mass defect filtering of unprocessed and matrix-removed LC-HRMS data



Metabolites detected by mass defect filtering of matrix-removed LC-HRMS data using M1 as template



Metabolites detected by mass defect filtering of materix-removed LC-HRMS data using M3+GluA as template



CONCLUSIONS

- A new matrix removal-based data processing (MRDP) workflow has been developed and applied for detection and characterization of drug metabolites in human plasma by LC-HRMS
- Results from metabolite profiling of plasma samples from human ADME study of [14C]vicagrel demonstrate that the combination of LC-HRMS and MRDP is a useful tool for comprehensive detection and structural characterization of human plasma metabolites
- MRDP may have broad applications in fast and sensitive metabolite profiling of various drug modalities in complex biological samples