Probing Drug and Metabolite-Covalent Binding with a Non-Radiolabeled Workflow: Osimertinib as an Example

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Abstract

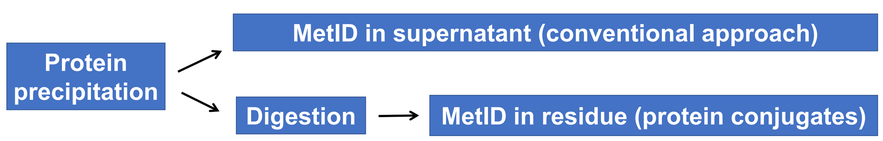
Protein precipitation using organic solvents is a standard technique in metabolite identification (MetID) studies. However, protein binding, especially covalent binding, often results in low recovery rates of parent drugs and their metabolites in both *in vivo* and *in vitro* MetID studies. This suggests that organic solvents may not effectively extract all protein-bound compounds. With the rising interest in covalent drugs, the likelihood of covalent protein binding has significantly increased. We established a workflow for MetID in protein residues, including sample preparation, LC-MS analysis, and data processing. Using Osimertinib as a model, we found that low extraction recovery rates in plasma, liver microsomes, and hepatocytes were due to covalent binding with matrix proteins. Protein-bound products of Osimertinib were detected in these matrices. This method supports the study of drug covalent binding, offering a more comprehensive metabolite profile without the need for radiolabeled compounds.

Objective

To develop an LC-MS based workflow to identify protein-conjugated drug-related compounds, addressing low recovery rates in MetID studies for potential covalent binding without using radiolabeled compounds.

Method

◆Workflow overview



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◆MetID in supernatant after protein precipitation

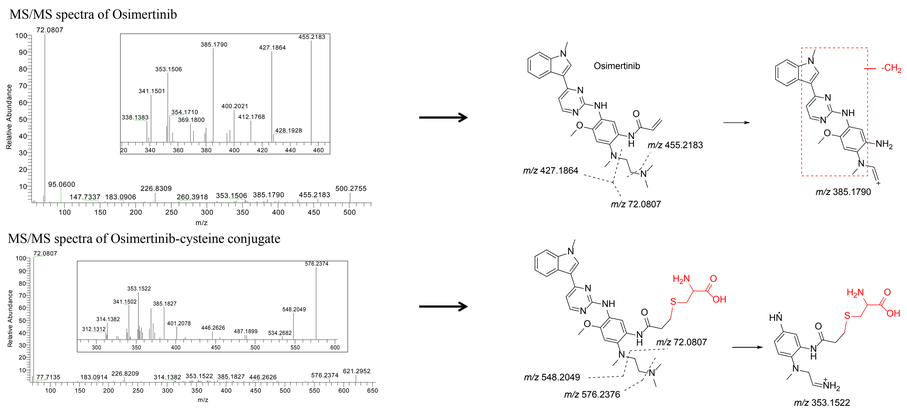
The compound was incubated in mouse plasma for 18 hours, and in liver microsomes (1 mg/mL protein) for 120 min and in hepatocytes (1 × 10^6 cells) for 240 minutes at 37°C with 5% CO_2 and 95% humidity. The incubation was terminated by adding ice-cold acetonitrile containing 0.1% formic acid, followed by centrifugation. The supernatant was collected, and the residue was washed twice with water. The combined supernatant was dried under nitrogen and reconstituted in 30% acetonitrile/water containing 0.1% formic acid. The samples were then analyzed using LC-UV-HRMS/MS.

◆MetID in residue after protein precipitation

The residue from extraction above was subjected to proteolytic digestion by adding PBS and pronase, after twice-wash with PBS. The digestion was terminated by adding acetonitrile containing 0.1% formic acid, followed by drying under nitrogen. The extracted digested samples were then analyzed using LC-UV-HRMS/MS.

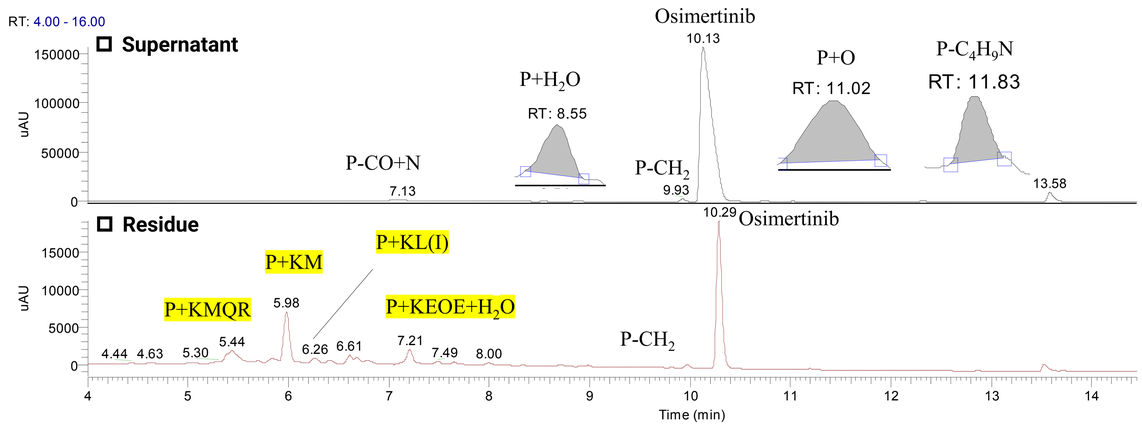
Results

1. Structure elucidation of Osimertinib and Osimertinib-cysteine conjugate

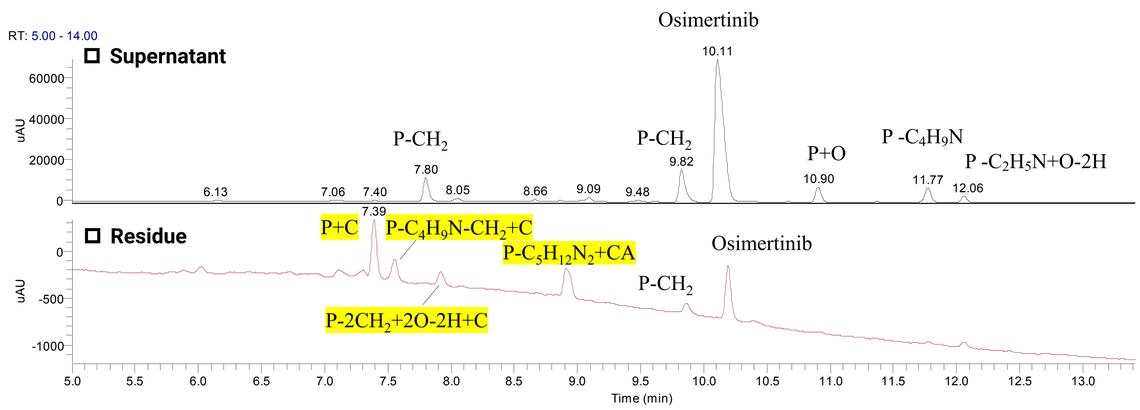


Results

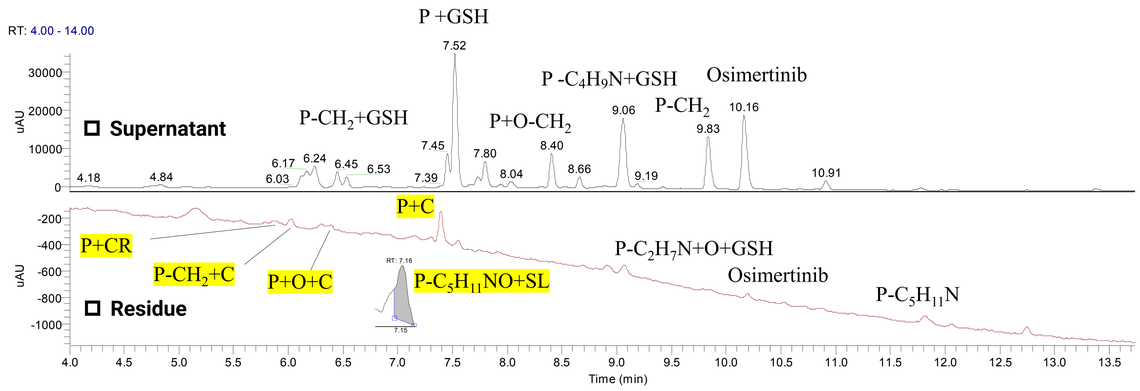
2. Major metabolites in *in vitro* rat plasma



3. Major metabolites in rat microsome



4. Major metabolites in rat hepatocytes



Conclusion

In addition to the conventional metabolite identification, we established a workflow for MetID in protein residues, encompassing sample preparation, LC-MS analysis, and data processing. Protein-bound products of Osimertinib were detected in plasma, liver microsomes, and hepatocytes. This method provides a supportive tool for studying drug covalent binding, offering a comprehensive metabolite profile.

References

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