Development of in-SPE derivatization method for GC-MS metabolome analysis

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【Introduction】
Due to complex and long-time sample preparations, including solvent extraction, centrifugal concentration, freeze-drying and derivatization, technical skills have been required in a conventional metabolome analysis for GC-MS. It causes discrepancies in the acquired data from multiple experiments, especially between analysis batches. Furthermore, it causes a critical problem when the statistical analysis is done because a lot of samples should be handled in the metabolome analysis. The purpose of this study was to develop a rapid and robust method of sample preparation for metabolomics using solid phase extraction (SPE).

【Methods】
Extracted samples with acetonitrile were loaded on an ion-exchange cartridge SPE. Ion-exchange SPEs were used depending on what kind of chemical functional group the compounds have. The target compounds were retained in the SPE, then the cartridge was washed by acetonitrile for dehydratation. Derivatization was done by methoxime/pyridine and MSTFA which were directly added sequentially on the SPE. After derivatization, the object substances were eluted by acetonitrile/hexane. The 10µl of derivatized compounds were injected in the GC-MS with a Large Volume Injection system equipped with a splitless liner.

【Results】
A cation-exchange column, C18, was used for amino acids which have cationic amino group, and an anion-exchange column, AX1, was applied for the compounds which have carboxyl and hydroxyl group, such as organic acids and sugars. Washing with acetonitrile after loading the target compounds enabled not only to eliminate other interferences but also to dehydrate at the same time. It took several hours to dry the samples in the conventional method, while dehydration was done in a few minutes using SPE. In this method, target metabolites which were retained in the SPEs by ion-exchange interaction were methoxymethylated by methoxymethyl hydroxide in pyridine followed by trimethylsilylation effectively. Derivatized metabolites were changed into less-polar compounds and easily eluted using organic solvent, acetonitrile/hexane. The total preparation time from sample loading on the column to derivatization was within 10 minutes, and good reproducibility was obtained.

【Discussion】
The derivatization processing time is greatly reduced compared to the conventional method. Since SPE derivatization method for GC-MS metabolome analysis has been established, it is possible to handle in a shorter time and less labor in the metabolome analysis. The purpose of this study was to develop a rapid and robust method of sample preparation for metabolomics using solid phase extraction (SPE).

【Conclusion】
By means of development of in-SPE derivatization method, we can achieve rapid and robust method of sample preparation for GC-MS metabolome analysis. By using this solid phase derivatization, 1. With holding targeted compounds into solid phase and washing with ACN, we could achieve dehydration process become quite easy and quickly. 2. As Methoxime and MSTFA directly added into the strong ion-exchange solid phase which hold the targeted compounds, the derivatization reaction was proceeding promptly on the solid phase. 3. The operating time of dehydration and derivatization was greatly reduced from 19 hours (conventional method) to 10 minutes.