

## **Solid-phase microextraction for untargeted LC-MS metabolomics studies using benchtop Orbitrap instrument**

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### **Novel Aspect**

This is the first successful use of in vitro and in vivo SPME for untargeted LC-MS metabolomics studies.

### **Introduction**

Metabolomics applications require sample preparation methods that are fast, reproducible and able to extract a wide range of analytes of differing polarities. The efficiency of metabolism quenching and stability of analytes in selected biofluid dictate how accurately the analytical results represent true metabolome composition at the time of sampling. However, complete quenching of metabolism is not easily accomplished and/or changes due to poorly stable compounds can occur, so the processes of sampling and sample preparation can significantly affect metabolome's composition. We propose the use of in vitro and in vivo solid-phase microextraction (SPME) as an effective sample preparation method for untargeted LC-MS metabolomics studies for the first time.

### **Methods**

SPME coatings suitable for untargeted metabolomics studies of biofluids using direct extraction mode were developed because the existing commercial coatings have very poor extraction efficiency towards polar compounds. The performance of 40 types of commercial sorbents was compared using 30-component metabolite standard spanning a wide polarity and molecular weight range. LC-MS analysis was performed using two complementary methods to achieve broad metabolite coverage: reverse-phase (using pentafluorophenyl column) and HILIC (using unmodified silica column). Final SPME extract was directly compatible for the analysis by both methods. The performance of optimized SPME method was compared to plasma protein precipitation and ultrafiltration in terms of metabolite coverage, matrix effects and method precision for untargeted metabolomics study of human plasma.

### **Preliminary Data**

The best coatings for simultaneous extraction of both hydrophilic and hydrophobic metabolites were mixed-mode, polar-modified polystyrene divinylbenzene and phenylboronic acid. The use of biocompatible SPME coating enables the extraction of small molecules while large macromolecules are excluded, thus ensuring an accurate representation of the metabolome at the time of sampling. The use of short extraction times (2-5 min) provided good metabolite coverage with ~ 1500-2000 features detected for mixed-mode coating. Metabolite coverage could be further enhanced by use of complementary coatings or increasing extraction time. In comparison to traditional methods, SPME reduced ionization suppression effects throughout the entire chromatographic space due to its non-exhaustive nature. In positive ESI mode, the best metabolite coverage was found for plasma protein precipitation with methanol/ethanol (3245 features), whereas in negative ESI mode, the best metabolite coverage was achieved by SPME (3320 features). Method precision achieved by SPME was similar or better than traditional methods with median RSD of 11 and 17% in positive and negative ESI mode respectively. To obtain free concentration information, SPME provided significantly better performance than ultrafiltration in terms of method precision and more balanced coverage of hydrophilic and hydrophobic species. Plasma precipitation methods were found to perform poorly for the analysis of many polar species due to very significant matrix effects and/or solubility issues. In addition to excellent method precision, metabolite coverage and reduction of ionization suppression effects, additional benefits of SPME for metabolomics studies include (i) high sample throughput (ii) reduction in overall number of sample preparation steps which can help minimize inadvertent sample losses (iii) suitability for in vivo sampling as shown in a proof-of concept study on mice (iv) ability to perform simultaneous in vivo sampling of biofluids and tissues and/or longitudinal sampling (v) elimination of need to quench metabolism and (vi) good metabolite stability in coating.