

Polar and Non Polar Metabolite Profiling with a Single Injection - AppNote Poster

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Tandem LC-MS Approach to Metabolite Profiling: Sequential RP + ANP Separation for Comprehensive Coverage

Metabolite profiling often requires analyzing molecules with dramatically different physicochemical properties. Traditional single-mode LC methods struggle to detect both hydrophilic and hydrophobic components in the same run.

The tandem LC-MS approach described here overcomes this limitation by combining Reversed Phase (RP) and Aqueous Normal Phase (ANP) chromatography in a single in-line setup, enabling broad analyte coverage from one injection.

Why Use a Tandem LC Configuration?

A single injection into a tandem LC system allows the sample to pass sequentially through two complementary chromatographic chemistries:

1. Reversed Phase (RP) Column

Ideal for non-polar to moderately polar metabolites.

Approx. 900 molecules detected in urine extract under RP conditions.

2. Aqueous Normal Phase (ANP) Column

Designed for highly polar and ionic metabolites.

Approx. 600 molecules detected on ANP columns in the same extract.

Together, the combined system can detect ~1500 resolved molecules—dramatically improving metabolome coverage compared to conventional, single-mode LC-MS.

How the Tandem LC System Works

The system is designed so that both columns operate in sequence during a single continuous run:

- Hydrophilic compounds are retained primarily on the ANP column.
- Hydrophobic compounds are retained on the RP column.
- After passing through both phases, the eluent enters the mass spectrometer, where data is collected in both positive and negative ionization modes.

This pairing maximizes detection breadth while maintaining robust chromatographic resolution for chemically diverse species.

Key Advantages of Tandem LC-MS for Metabolomics

1. Dual Chemistry = Maximal Coverage

By combining RP and ANP selectivities, the method captures a broader molecular spectrum, including plant metabolites, animal tissue extracts, culture media components, and single-cell organism metabolites.

2. Single Injection Efficiency

Instead of running multiple methods, the system produces all necessary chromatographic data in one file—reducing analyst time and reducing instrument usage significantly.

3. Straightforward Setup

Despite offering multi-mode separations, the system is described as robust and relatively simple to implement, especially when paired with fast polarity-switching MS instruments.

4. Comprehensive Data in One File

Both hydrophilic and hydrophobic chromatographic data, plus positive and negative mode MS scans, are stored in a single output file—streamlining review and processing.

Conclusion

The tandem LC-MS approach enables highly efficient, wide-spectrum metabolite profiling. By coupling RP and ANP in a single in-line configuration, analysts can detect roughly 1500 metabolites from one sample injection—representing a dramatic improvement over single-mode LC methods.

This makes the technique particularly valuable for complex biological samples and high-throughput metabolomics applications.



A new approach to metabolite profiling using tandem liquid chromatography

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Introduction

A tandem liquid chromatography (LC) setup has been developed to analyse a larger subset of molecules in a complex sample from a single injection by coupling multiple chromatographic chemistries. We have coupled reverse phase (RP) chromatography with aqueous normal phase (ANP) chromatography. The LC configuration is shown in the figure below. This tandem LC setup is able to analyse both hydrophilic and complementary stationary and mobile phases. In a metabolite profiling analysis of a urine extract, approximately 900 and 600 molecules can be detected on RP and ANP columns, respectively. The Tandem-LC setup presented here allows detection of approximately 1500 resolved molecules with both hydrophilic and hydrophobic properties. The Tandem-LC setup can be used to resolve many complex samples, including molecules extracted from plant material, animal tissue, culture fluid and single cellular organisms. The Tandem-LC configuration is robust, relatively simple to setup and greatly reduces instrument and analysis times, even more so when coupled with a fast polarity switching MS.



Sample preparation

Mid-stream urine samples were collected from three anonymous volunteers on two consecutive mornings. A second sample was collected 3 hours after the ingestion of both a coffee and a multivitamin tablet (Blackmores, Australia). A 100 μ l aliquot of each urine sample was diluted with 300 μ l of ACN. Aliquots were mixed vigorously and then centrifuged (10min, 14000g, 4°C). 100 μ l of the supernatant was transferred to a microvial, inserted into a vacuum manifold and then resuspended in 100 μ l of 50% v/v methanol with vigorous mixing.

Instrument details

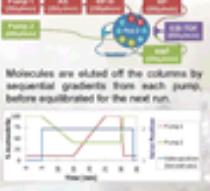
LC: Agilent 1200 series comprising of two degassers, two binary pumps, an auto sampler and a column compartment containing a 10-port 2-position valve (a minimum of 8 ports are required).
APC detector: Cogent Diamond Hydride 2.1 mm \times 100 mm, 2.6 μ m particle size (MicroSolv Technology, Australia).
RP column: Zorbax Eclipse XDB-C18, 2.1 mm \times 100 mm, 1.8 μ m (Agilent) with a corresponding 10mm guard column.
Detector: Agilent 6520 TOF MS detector with fast polarity switching.
Note: Normal operating pressures are less than 250 bar with the described configuration.

LC configuration

Leading step: 2% ACN from Pump 1 flows through the auto sampler (AS) then onto the RP guard column (Pump 2) for 5 minutes to equilibrate the hydrophilic column. Solvent containing unbound molecules is mixed with 100% ACN from Pump 2, to increase ACN to 90.3% in the mixed flow, allowing hydrophilic molecules to bind to the ANP. Remaining unbound molecules are channelled into the MS for detection.



Resolving step: the valve is switched to allow solvent from Pump 1 to flow through the ANP column, whilst solvent from Pump 2 flows onto the ANP. Eluent from both columns is mixed before directed to the MS.

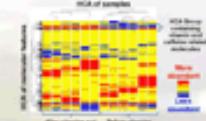


Results

The chromatogram below shows molecules that have no affinity to either the RP or ANP columns eluting before mostly hydrophilic molecules elute then hydrophobic molecules elute.



The HCA below shows 2 major groups from the 12 samples (horizontal tree) which correlate with samples before and after vitamins and caffeine supplements. The sub-groups correlate with replicates from each volunteer. Vitamin and caffeine related molecules are grouped together on the HCA of >1500 molecular features (vertical tree).



Conclusions

We have resolved both hydrophilic and hydrophobic molecules on respective columns from a single in-line injection. A single data file can contain information from both columns as well as data collected in positive and negative mode.



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