A new approach to metabolite profiling using tandem liquid chromatography

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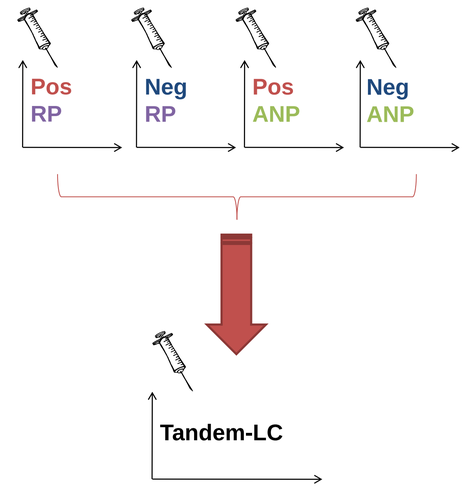
Introduction

A tandem liquid chromatography (LC) setup has been developed to analyze 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 allows sequential separation of hydrophilic and hydrophobic compounds on respective, 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 analyst time, 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 later, after the ingestion of both a coffee and a multivitamin tablet (Blackmores, Australia). A 100µL aliquot of each urine sample was diluted with 300 µL of ACN. Aliquots were mixed vigorously and then centrifuged (10min, 14000g, 4°C). 100µL of the supernatant was transferred to a micro-vial insert, speedvac evaporated then resuspended in 100µL 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)

ANP column: Cogent Diamond Hydride 2.1 mm × 100 mm, 4µm particle size (MicroSolv Technology, Australia).

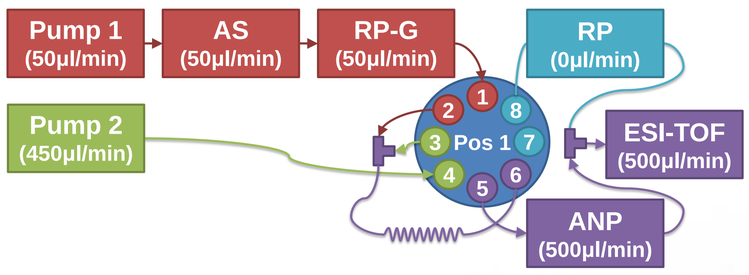
RP column: Zorbax Eclipse XDB-C18, 2.1 mm × 100 mm, 1.8µm (Agilent) with a corresponding 10mm guard column.

Detector: Agilent 6520 TOF MS detector with fast polarity switching.

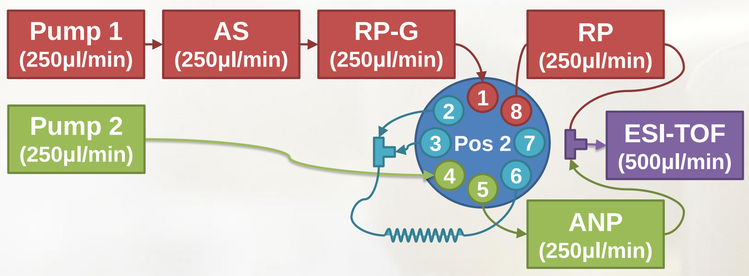
Note: Normal operating pressures are less than 250 bar with the described configuration.

LC configuration

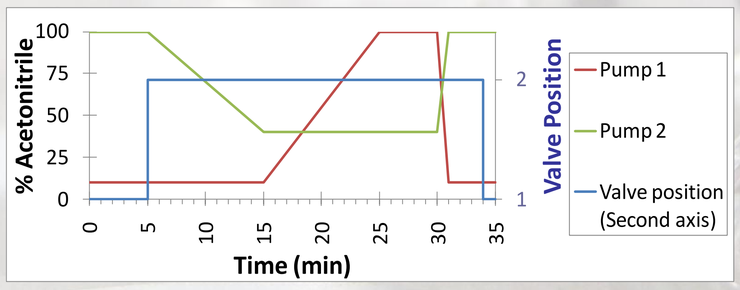
Loading step: 2% ACN from Pump 1 flows through the auto sampler (AS) then onto the RP guard column (RP-G) for 5 minutes to retain hydrophobic molecules. Solvent containing unbound molecules is mixed with 100% ACN from Pump 2, to increase ACN to 90.3% in the mixed flow, allowing hydrophilic molecules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



Resolving steps: the valve is switched to allow solvent from Pump 1 to flow through the RP-G then the RP analytical column, whilst solvent from Pump 2 flows onto the ANP. Eluant from both columns is mixed before directed to the MS.

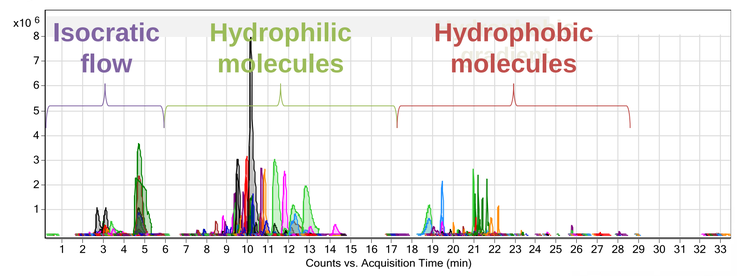


Molecules are eluted off the columns by sequential gradients from each pump, before equilibrated for the next run.

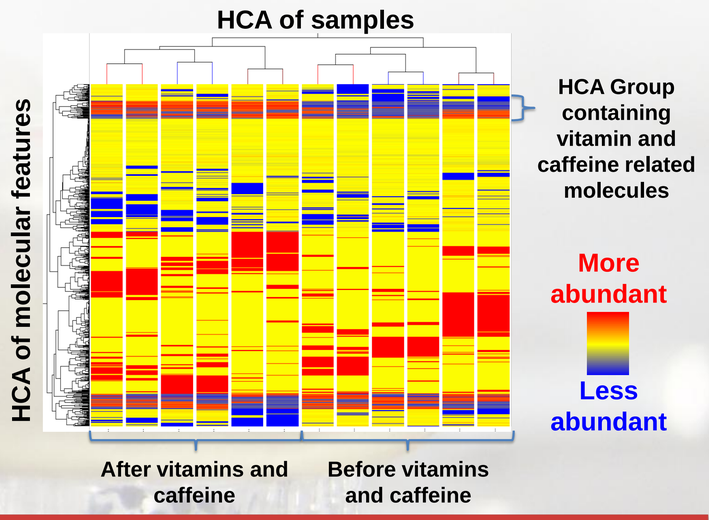


Results

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 vitamin 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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