Product Information

YMC EUROPE GMBH

UltraHT Hydrosphere
Polar Analytes
Method transfer

UltraHT Hydrosphere

Analytical LC - 110 - 17
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In addition to diabetes and hypertension, gout has become one of the diseases of civilisation which are probably due to dietary habits. The symptoms have been known for many hundreds years and most of the mechanisms have been elucidated.
Gout is caused by an increase of uric acid (hyperuricemia) in the blood stream. This results in the crystallisation of uric acid and monosodium urate in the joints, tendons and surrounding tissue, which causes severe pain. The reason for the presence of uric acid in our metabolism is connected to the purine degradation process, which ends at uric acid (please see fig. 1).

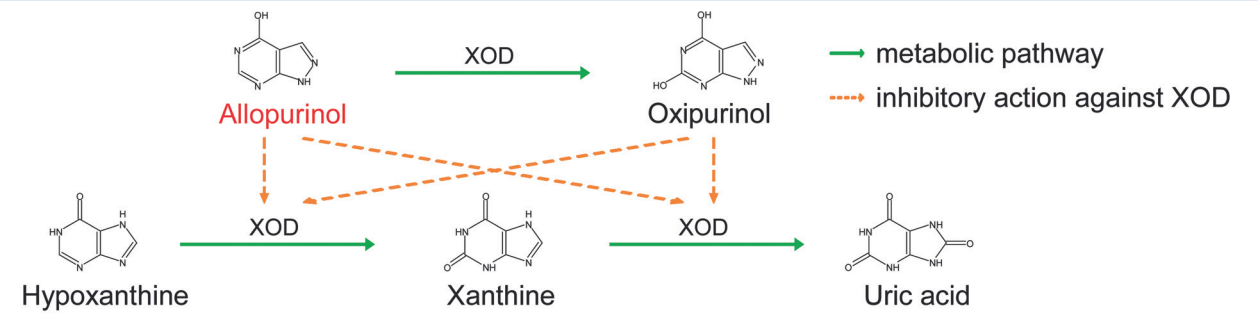

Figure 1: Xanthine oxidase inhibitor and related metabolites

One of the degradation mechanisms is carried out by the enzyme xanthine oxidase, which converts hypoxanthine via xanthine to uric acid. Allopurinol, a structural isomer of hypoxanthine which is also a xanthine oxidase inhibitor is often administered to avoid an uric acid excess or pain in passing urine (see fig. 1).

Therefore, it is most important to have an analytical method, which monitors all of these related metabolites.

Experiment

Eluent:	10 mM CH3COOH-CH3COONH4 (pH 4.5)
Temperature:	37 °C
Detection:	UV at 254 nm
Sample:	standard (100 µg/ml)

1. Uric acid
2. Hypoxanthine
3. Xanthine
4. Oxipurinol
5. Allopurinol

Results
The above isocratic method has been successfully developed using Hydrosphere C18. The application enables a baseline separation of all five metabolites (see fig. 2).

1.0 ml/min

7.5 µl injection

1250 psi

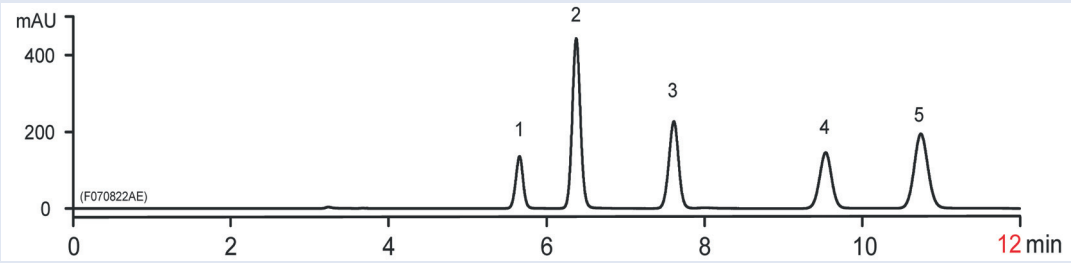

Figure 2: Isocratic method for polar metabolites

This method is useful for analysing xanthine oxidase metabolites and monitoring xanthine oxidase inhibitors and their related metabolites.

Method Transfer

In order to transfer the above application developed for conventional LC systems onto ultra-fast LC systems, YMC-UltraHT Hydrosphere C18 (2 µm particle) is the column of choice. As this is the same sorbent, the same degree of selectivity is provided, so that method transfer is very easy. The separation remains exactly the same but takes only 4 minutes instead of 12 minutes (see fig. 3).

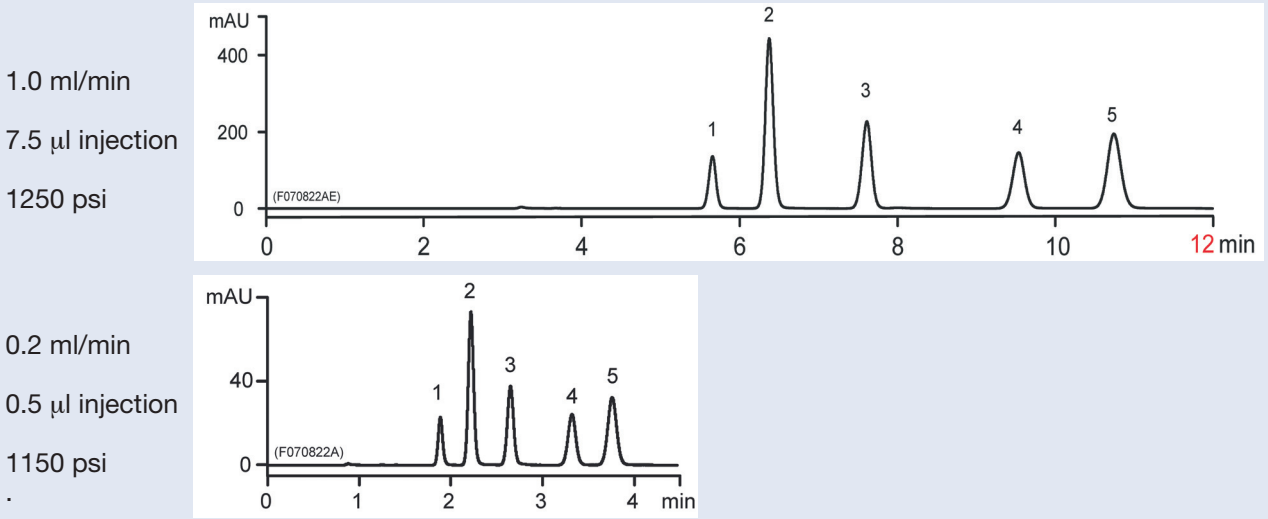

Figure 3: Method transfer from conventional LC to ultra-fast LC

In order to optimise the application further, the flow rate can be increased by the factor 3. A further retention time reduction can be achieved without compromising the resolution (see fig. 4).

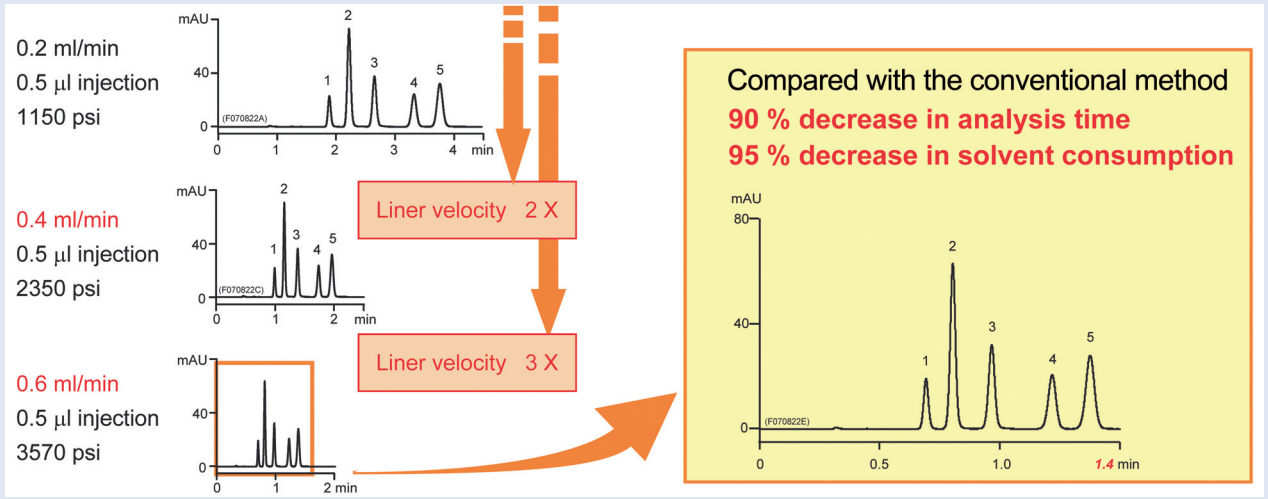

Figure 4: Ultra-fast LC optimisation