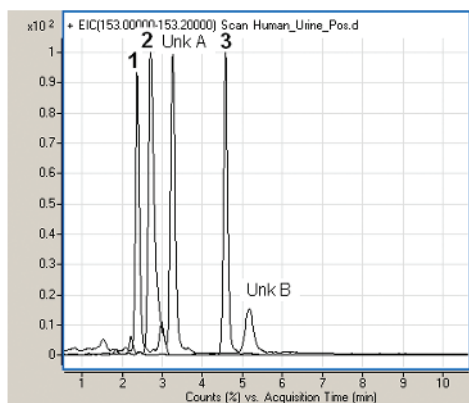


Determination of Xanthine, Uric acid & Hypoxanthine From Human Fluids



Sample Peak:

1. Xanthine (X) 153.04070 m/z
2. Uric acid (UA) 169.03560 m/z
Unknown A 153.06080 m/z
3. Hypoxanthine (HX) 137.04580 m/z
Unknown B 153.06606 m/z

Notes:

Xanthine oxidase, an enzyme which catalyzes the oxidation of hypoxanthine (HX) to xanthine (X) to uric acid (UA) can be inhibited by allopurinol and other drugs. Uric acid lowering drugs are used in the treatment of gout and the prevention of tumor lysis syndrome. High concentrations of UA in blood (hyperuricemia) cause deposition of urate crystals, which could ultimately result in chronic joint inflammation and renal impairment. The determination of UA has been one of the tests in the clinical chemistry laboratory performed for patient diagnosis of gout.

Method Conditions

Column: Cogent Diamond Hydride™ 4µm, 100Å.

Catalog No.: 70000-10P-2

Dimensions: 2.1 x100 mm

Solvents: A: DI Water + 0.1% formic acid

B: Acetonitrile + 0.1% formic acid

Gradient:	Time	%B	Time	%B
	0.0	95	9.0	80
	0.2	95	10.0	50
	8.0	80	12.0	50

Post Time: 5 min

Flow Rate: 0.4 mL/min

Sample: Human Urine

Sample Prep: 400 microL of acetonitrile was added to 100 microL of human urine and sample was centrifuged (3000 g). Next 20 microL of the supernatant was mixed with 10 microL of the 50% acetonitrile/50%DI water + 0.1% formic acid.

Detection: ESI – pos - Agilent 6210 MSD TOF mass spectrometer.

Discussion

A simple ANP method was developed for the determination of xanthine (X), uric acid (UA), and hypoxanthine (HX) at concentrations in human urine (can be used for human serum) to support pharmacodynamic (PD) studies of a novel xanthine oxidase inhibitor during its clinical development. PD biomarkers (UA, X, and HX) were well separated from each other. In addition xanthine was separated from two isobaric unknowns (unknown A and B) present in this particular urine sample. Current HPLC methods for UA/X/HX measurements suffer from low sensitivity, poor selectivity, and/or inefficient sample throughput. The developed ANP method is fast and sensitive and it will allow high sample throughput.

For more information visit www.MTC-USA.com

Cat. No.	Description
----------	-------------

70000-10P-2	Cogent Diamond Hydride™ HPLC Column, 100Å, 4µm, 2.1x100mm
-------------	---