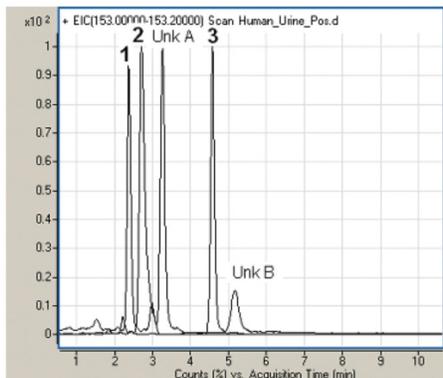


Determination of Xanthine, Uric acid & Hypoxanthine From Human Fluids



Method Conditions

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

Catalog No.: 70000-10P-2

Dimensions: 2.1 x 100 mm

Solvents: A: DI H₂O + 0.1% formic acid

B: Acetonitrile + 0.1% formic acid

| Gradient: | time (min.) | %B |
|-----------|-------------|----|
| | 0 | 95 |
| | 0.2 | 95 |
| | 8 | 80 |
| | 9 | 80 |
| | 10 | 50 |
| | 12 | 50 |

Post Time: 5 min

Flow rate: 0.4 mL/min

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

Sample: 400µL of acetonitrile was added to 100µL of human urine and sample was centrifuged (3000 g). Next, 20µL of the supernatant was mixed with 10µL of the 50% acetonitrile/ 50% DI H₂O + 0.1% formic acid

Peaks: 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

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.

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.