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CHIETI
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Venue: New Rectorate Auditorium

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Fast one-step liquid chromatography determination of purine compounds in blood with photodiode array detector

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Uric acid (UA), a polyedric functional compound, is a strong natural antioxidant in humans where it is the end product of purine metabolism. The rational process for this study was the clinical interest of the metabolites as markers for energy disturbance in ischemia/hypoxia, antioxidant capacity, and disease activity in vivo. Unfortunately, reports about changes in UA levels in the neurodegenerative disorders have been conflicting.

We propose a pratical one-step high performance liquid chromatography (HPLC) method with photodiode array detector (PDA) for the simultaneous determination of hypoxanthine (HX), UA, and xanthine (X) in human plasma.

Blood samples were obtained from the healthy adult volunteers (University workers/30). For stability study, 200 µL of plasma was deproteinized through a Millipore-Amicon Ultracel.

Waters-HPLC system consisted of a 600E Pump; 2998 PDA; Empower TM2 Data Software; Atlantis T3 analitycal column, 3µm; a 10µl injection volume. The m.f. was a 40 mmol/L potassium phosphate buffer, pH 2.2 at a flow rate of 1.0 mL/min. The spectral range of the PDA was 200-400 nm. The optimal wavelength for detecting a mixture of standards was 254 nm.

We have obtained an execellent base-line separation of HX/UA/X from the interfering compounds of plasma samples, in a short elution time of less than 10min. The RT for HX/UA/X were 5.5; 6.6; 8.7 min, respectively. The chosen wavelength of 254 nm provides a higher sensitivity with a clean chromatogram. The reported reference ranges from 30 healthy normal subject were 1.2-17.9 µM, 151-442 µM, and 0.2-5.8 µM, for HX/UA/X respectively. Peaks were identified by matching the RT against those of authentic standards, and by spiking standard solutions to ultrafiltrated plasma. This HPLC-PDA system is very useful tool for the assay of UA and purine derivatives both in research assays and in clinical laboratories.