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Short communication

# Comparison of capillary electrophoretic and liquid chromatographic determination of hypoxanthine and xanthine for the diagnosis of xanthinuria

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**Abstract**

A capillary electrophoretic (CE) method for the determination of hypoxanthine and xanthine in urine was developed to diagnose xanthinuria. The linearity was excellent up to 200 $\mu$mol $l^{-1}$ for the two compounds and the limit of quantitation was 2 $\mu$mol $l^{-1}$. A comparison o the results obtained using CE was made with those obtained by the high-performance liquid chromatographic (HPLC) technique described previously. With regard to specificity, sensitivity and reproducibility, the results are similar but CE is more rapid than HPLC.

*Keywords:* Capillary electrophoresis; Hypoxanthine; Xanthine; Purines; Uric acid

## 1. Introduction

Hypoxanthine and xanthine are two important purine bases which result from purine catabolism. A block of purine nucleotide degradation due to a deficiency of xanthine oxidase occurs in xanthinuria. This enzyme defect causes increased urinary concentrations of hypoxanthine and xanthine. Because of its high efficiency and rapidity [1], capillary electrophoresis (CE) was chosen to develop a method for the determination of hypoxanthine and xanthine in urine to diagnose xanthinuria. This determination can also be a measure of foetal hypoxia [2] and a tool in the diagnosis of ischaemic heart disease [3]. This paper reports a comparison of the results obtained using the CE method and those obtained by the high-performance liquid chromatographic (HPLC) technique described previously [4].

## 2. Experimental

### 2.1. Chemicals and reagents

Xanthine and hypoxanthine were purchased from Sigma (St. Quentin Fallavier, France). Potassium dihydrogenphosphate, orthophosphoric acid, diammonium hydrogenphosphate and sodium hydroxide were obtained from Merck (Nogent-sur-Marne, France) and xanthine

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oxidase in suspension (activity 20 U/ml) from Boehringer (Meylan, France).

### 2.2. Chromatographic conditions

The chromatographic system consisted of Hypersil ODS (3 $\mu$m) as the stationary phase and 0.02 *M* $KH_2PO_4$ buffer adjusted at pH 3.65 with orthophosphoric acid as the mobile phase. The flow-rate was 1.5 ml/min and detection was performed at 254 nm.

### 2.3. Electrophoretic conditions

CE was performed with an automated P/ACE 2000 system (Beckman Instruments, Gagny, France) controlled by Beckman System Gold software. Separations were achieved within a fused-silica capillary (37 cm × 50 $\mu$m I.D., 30 cm from sample-end electrode to the detector) and using 0.04 *M* $(NH_4)_2HPO_4$ (pH 8.1) as the running buffer.

Injections were made hydrodynamically by pressure at the anodic end for 5 s. The applied voltage was 20 kV, giving a current of 80 $\mu$A. The temperature was kept at 25°C. Detection was performed at 254 nm. The daily initial preparation of the capillary involved a 10-min rinse with 1 *M* NaOH followed by a 10-min rinse with running buffer. Before each run, the capillary was rinsed with 0.05 *M* NaOH for 2 min and equilibrated with running buffer for 5 min.

### 2.4. Urine collection

Urine was collected during 24 h and stored at −20°C. The urine samples were diluted fivefold before analysis.

### 2.5. Peak identification

The identification of hypoxanthine and xanthine peaks was confirmed by the enzymatic peak shift technique using xanthine oxidase as enzyme.

## 3. Results and discussion

Fig. 1a shows the electropherogram of a standard solution including hypoxanthine, xanthine and uric acid and Fig. 1b shows a representative electropherogram of a urine sample from a patient with xanthinuria. It can be seen that the compounds of interest can be separated with high resolution in only 3.5 min. The concentration of hypoxanthine and especially the concentration of xanthine are increased in the urine sample from the patient with xanthinuria. The increase is associated with very low uric acid concentration. Concentrations of the compounds were calculated from the respective calibration graphs. The calibration graphs were linear up to 200 $\mu$mol $l^{-1}$. For quantification, correction of the peak area by the migration time is not necessary, the migration time being constant from run to run.

The results obtained by CE for hypoxanthine and xanthine were similar to those obtained by HPLC. The correlation between the concentrations was tested by linear regression analysis ($A = 1.03$, $B = 0.3$, $r = 0.98$ for hypoxanthine and $A = 0.99$, $B = 0.5$, $r = 0.97$ for xanthine).

The run-to-run and the day-to-day reproducibilities were tested at 20 $\mu$mol $l^{-1}$ ($n = 10$). The relative standard deviations (R.S.D.s) for the migration times were 0.4% and 1.7% for hypoxanthine and 0.6% and 2.1% for xanthine. The R.S.D.s for the concentration were less than 3% for both compounds. The minimum detectable concentration based on a signal-to-noise ratio of 3:1 was about 2 $\mu$mol $l^{-1}$. These values were similar to those obtained by HPLC.

Under the conditions described, the Hypersil ODS column has a longer lifetime than the capillary; ca. 500 samples were injected on to the column without any deterioration compared with 200 samples in the capillary. Further, there was no interference with the compounds of interest from related endogenous compounds such as uric acid, guanine and guanosine or from drugs such as allopurinol, oxypurinol, 6-mercaptopurine, caffeine and theophylline.

The analysis times of the two methods were then compared. Using HPLC, the separation of

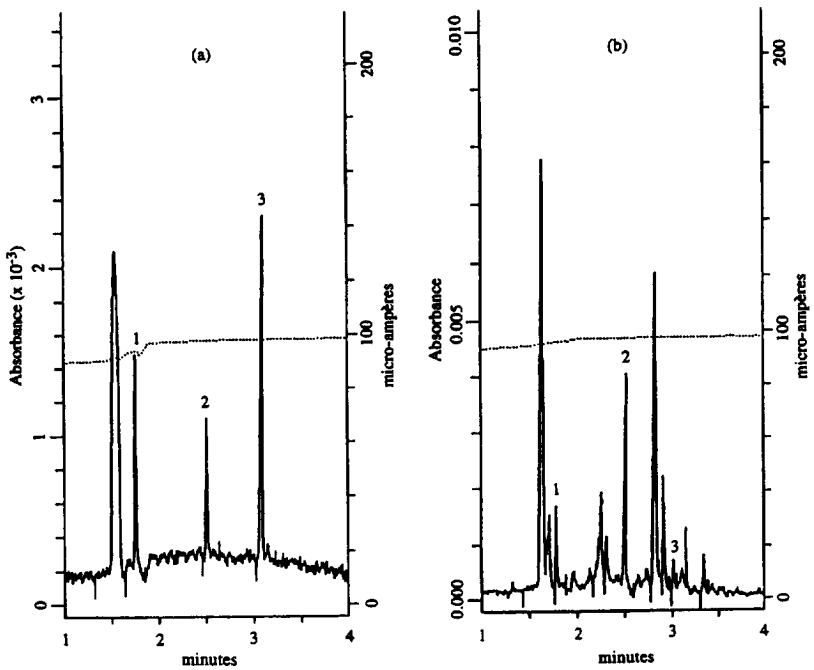


Fig. 1. Electropherograms of (a) a standard solution and (b) a fivefold diluted urine sample from a patient with xanthinuria. The analyses were carried out as outlined under Experimental. Peaks: 1 = hypoxanthine [(a) 20 $\mu$mol $l^{-1}$, (b) 113 $\mu$mol $l^{-1}$ (136 $\mu$mol per 24 h)]; 2 = xanthine [(a) = 20 $\mu$mol $l^{-1}$, (b) 450 $\mu$mol $l^{-1}$ (540 $\mu$mol per 24 h)]; 3 = uric acid [(a) = 70 $\mu$mol $l^{-1}$, (b) traces].

the compounds of interest lasts 6 min but the elution of all the compounds present in normal urines needs at least 30 min. Using CE, the separation lasts 4 min but the analysis includes preconditioning of the capillary for 7 min. Therefore, the analysis time in CE is three times shorter than that in HPLC.

## 4. Conclusion

The results obtained concerning specificity, sensitivity and reproducibility are similar for CE and HPLC. Both of these techniques represent suitable and useful tools for the diagnosis of xanthinuria, but CE is simpler and more rapid than HPLC.

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