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Short communication

Xanthine analysis in biological fluids by capillary electrophoresis

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Abstract

Xanthine, a precursor of uric acid, is measured here in serum, urine, and cerebrospinal fluids by capillary electrophoresis (CE) after deproteinization with acetonitrile. The migration time is about 7.5 min with a minimum detection limit of 0.4 mg/l. Different purines and pyrimidines did not interfere with the determination. The method demonstrates the suitability of the CE for determination of small molecules present in a complex matrix at levels of ca. 1 mg/l. It also demonstrates that acetonitrile deproteinization is a simple and effective method for preparing samples for CE, allowing a large volume to be introduced into the capillary.

1. Introduction

Xanthine is a precursor of uric acid. Its concentration in serum increases in several clinical conditions: depressed purine salvage pathways as in the Lesch-Nyhan syndrome [1], decreased xanthine oxidase activity as in hereditary xanthinuria [2,3], or after administration of allopurinol [2]. In addition to that, elevated serum xanthine levels, in general, can indicate tissue depletion of ATP as in prenatal asphyxia, or hydrocephalus [4]. Xanthine is excreted rapidly through the kidney, about ten times faster than uric acid; however, it has low solubility which can lead to stone formation [2,3].

Xanthine levels are very low in serum: about a hundred times less than those of uric acid. The reference intervals for xanthine in different body fluids are listed in Table 2. HPLC [5,6], gas chromatography [7] and enzymatic methods [8,9] have been previously used to measure xanthine levels in serum and other biological fluids. In general, these methods are slow. Here we describe a rapid method based on capillary electrophoresis (CE) for xanthine measurement which is also suitable for measurement of other purines and pyrimidines. The method depends on removing serum proteins by acetonitrile. Previously, we have found that acetonitrile in CE produces a special stacking effect allowing a larger volume of the sample to be introduced into the capillary with an increase in sensitivity such as needed for this assay [10].

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2. Experimental

2.1. Instrument

A Model 2000 CE (Beckman Instruments, Palo Alto, CA, USA) was set at 280 nm, 35°C and 11 kV. The capillary was 500 mm × 50 μm I.D. The running buffer was made of 7 g boric acid mixed with 7 g sodium carbonate/l water (pH 9.2).

2.2. Chemicals

The following compounds were obtained from Sigma Chemicals (St. Louis, MO, USA): hypoxanthine, adenine, inosine, uric acid, guanine, ATP, ADP, AMP, oxypurinol and allopurinol.

2.3. Reagents

Stock standards: 10 mg xanthine, or other purines, were dissolved in 100 ml water with addition of 3 drops of NaOH (1 mol/l). These solutions were stable at room temperature for 30 days. Xanthine was stable enough and did not convert to any other compound detectable on the electropherogram. Working standard: the stock standard was diluted ten-fold in water or serum (prepared daily).

2.4. Procedure

Serum

An aliquot of 100 μl of either serum (or cerebrospinal fluid) was vortex-mixed with 200

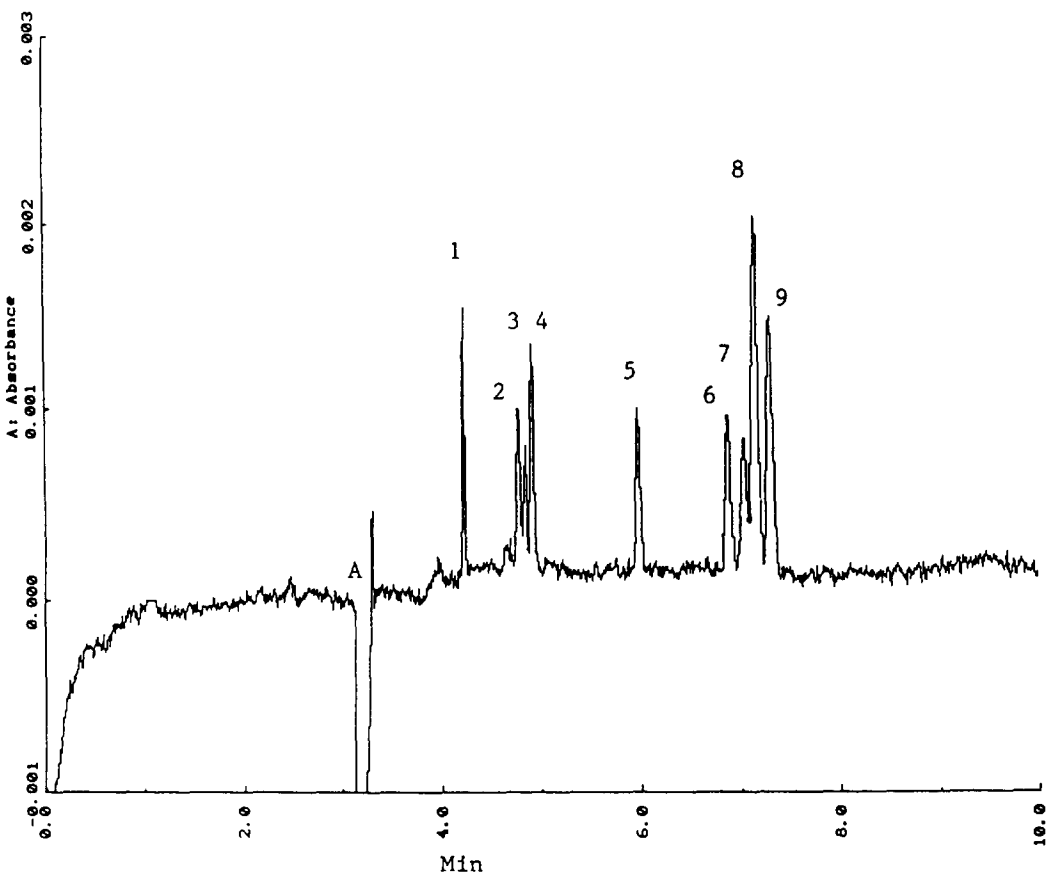

Fig. 1. Electropherogram of different purines and pyrimidines (pH 8.9). A = acetonitrile; 1 = adenine; 2 = guanine; 3 = iothalamic acid; 4 = allopurinol; 5 = hypoxanthine; 6 = inosine; 7 = oxypurinol; 8 = xanthine (5 mg/l); 9 = uric acid (20 mg/l).

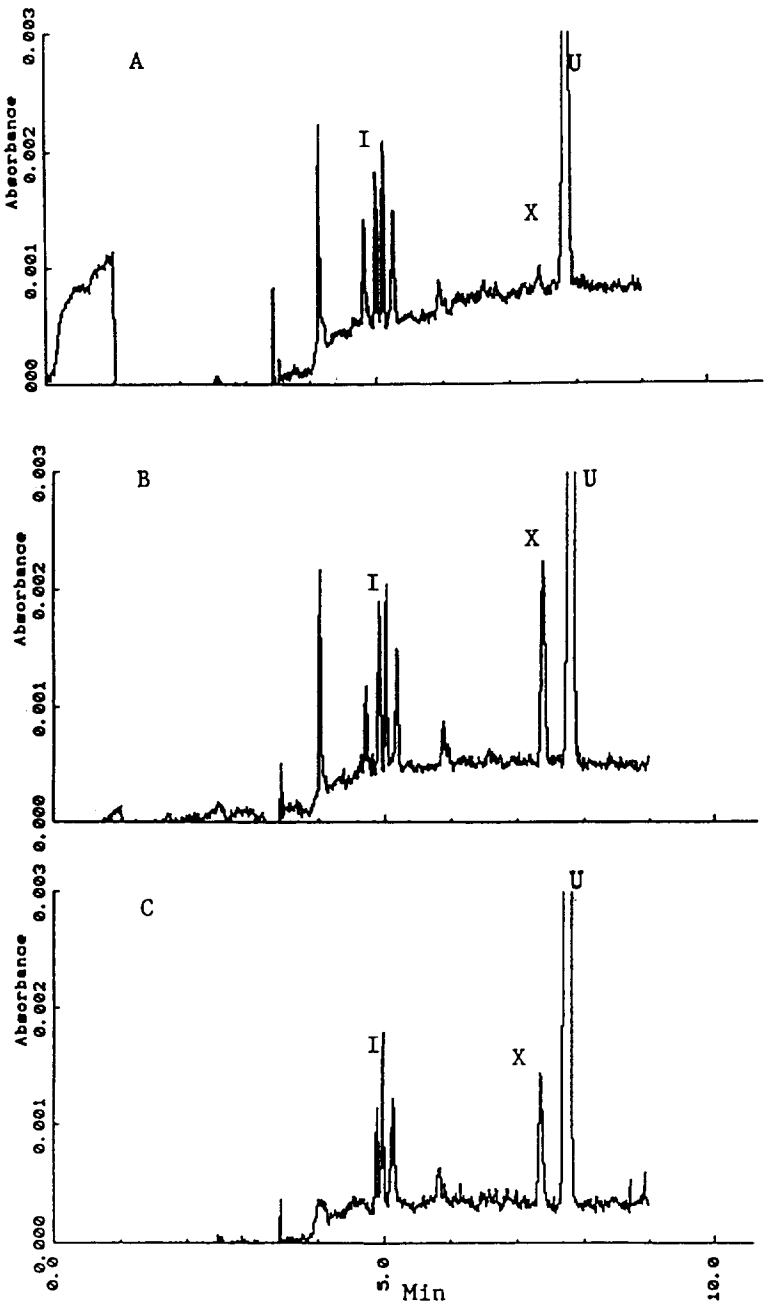

Fig. 2. Electropherogram of xanthine in serum (buffer pH 9.2) from: (A) a patient with normal level, 0.45 mg/l; (B) same patient spiked with 4.5 mg/l xanthine; and (C) patient with elevated xanthine, 3.1 mg/l. (X = xanthine; U = uric acid; I = internal standard.)

μl of acetonitrile containing iothalamic acid (100 mg/l) as an internal standard. The mixture was centrifuged at 14 000 *g* for 1 min and the supernatant was injected into the capillary by pressure injection for 20 s.

Urine

Urine was diluted five-fold with water and a 100 μl aliquot was added to 200 μl acetonitrile as for serum.

Tissues

Guinea pig liver (100 mg) was homogenized in 1.0 ml water and a 100 μl aliquot was added to 200 μl acetonitrile containing the internal standard as for serum.

2.5. Calculation

Peak height was used for calculation. The internal standard, iothalamic acid, was not used for calculation.

3. Results and discussion

The separation of xanthine from the common purines and pyrimidines is illustrated in Fig. 1. It

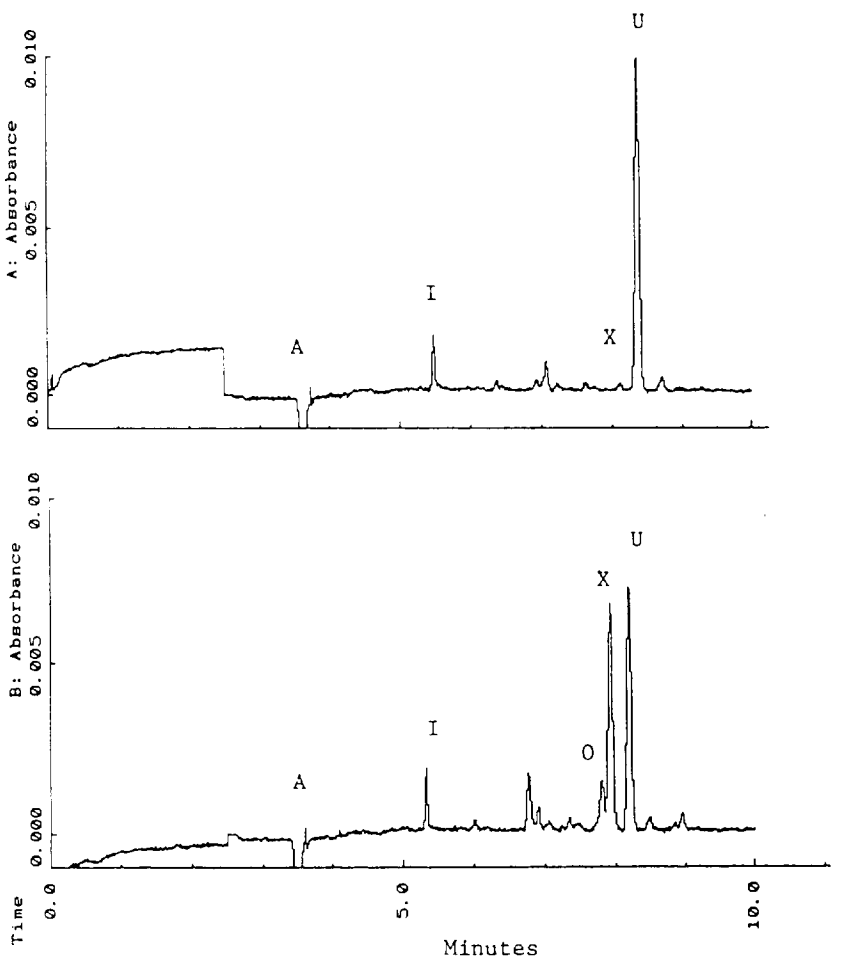

Fig. 3. Electropherogram of urine from: (top) a patient with normal level of xanthine (1.2 mg/l), and (bottom) a patient with elevated xanthine (279 mg/l). (X = xanthine; U = uric acid; I = internal standard; A = acetonitrile; O = oxypurinol.)

is pH-dependent with a better separation for the majority of the compounds at pH 8.9. However, the separation between xanthine and uric acid deteriorates at a pH lower than 8.9 and improves at a pH above that (Fig. 2). The migration time for xanthine was ca. 7.5 min. It is slightly faster than that of uric acid. AMP, ADP and ATP are more acidic and migrate at about 15 min and thus they too do not interfere with the test.

Figs. 2 and 3 illustrate the separation of xanthine in serum and urine samples. The different peaks in these figures were identified based on the electrophoretic mobility and with sample spiking with standards. Based on standards prepared in serum, the test was linear between 0.4 and 20 mg/l [Conc. = 0.16 × peak height (mm) − 0.17; $r = 0.999$], with 0.4 mg/l as the lower limit of detection (3S.D. of baseline noise). From a practical point of view, values less than 1 mg/l do not have any diagnostic significance. For instruments which utilize diode array detection, the spectrum of xanthine is very helpful for peak confirmation (Fig. 4). The relative standard deviation of the assay is illustrated in Table 1. The recovery of 10 mg/l of xanthine added to serum was 101% relative to that in water and 82% to that in 1% NaCl ($n = 3$). We prefer to prepare the standard in the same type of fluid being measured (serum, urine, or cerebrospinal fluid) to avoid differences in sample matrix.

We measured the levels of xanthine in serum

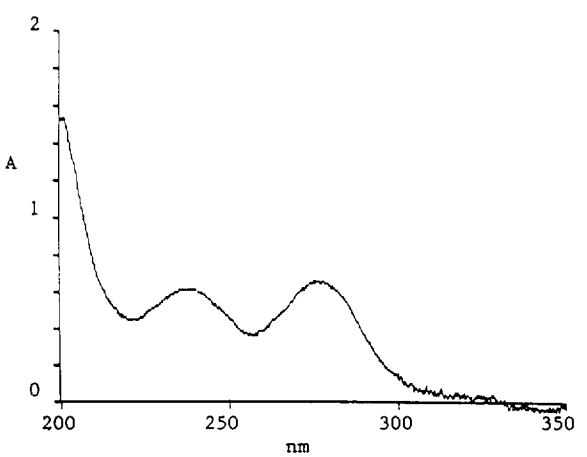

Fig. 4. Spectrum of xanthine.

Table 1
Reproducibility (R.S.D.) of xanthine analysis in serum samples

Concentration (mg/l)	R.S.D. (%)	
	Peak height	Migration time
5	2.8 ($n = 9$)	1.5 ($n = 9$)
2.2	4.2 ($n = 18$	2.1 ($n = 18$)
0.4	9.3 ($n = 9$)	1.2 ($n = 9$)

from individuals with different disorders as shown in Table 2. Normal individuals have a mean of 0.6 mg/l ± 0.3 mg/l, which is very close to that reported using HPLC [6] or the enzymatic method [9]. Patients treated with allopurinol have elevated serum and urine xanthine (Table 2). Oxypurinol, the metabolite of allopurinol, migrates immediately ahead of xanthine (Fig. 3). Some patients with slightly elevated uric acid also had slightly elevated xanthine levels. In general, urine levels of xanthine are higher than those of serum. They can be

Table 2
Xanthine levels in normal individuals and patients with various disorders

	n	Concentration (mean ± S.D.) (mg/l)
Serum		
Normal	22	0.6 ± 0.3
Allopurinol-treated	9	3.4 ± 3.9
Elevated uric acid	12	2.2 ± 1.1
Leukemia	10	0.6 ± 0.8
Reference interval[a]		(1–3) (0.03–0.9 mg/l)
Urine		
Normal	12	2.6[b] ± 2.4[c]
Allopurinol-treated	5	160 ± 165
Reference interval[a]		(5–12 mg/l)
CSF		
Different disorders	12	0.9 ± 0.4
Reference interval[a]		(none)

[a] Based on Refs. [6,9].
[b] 4.6 mg/g creatinine.
[c] 6.9 mg/g creatinine.

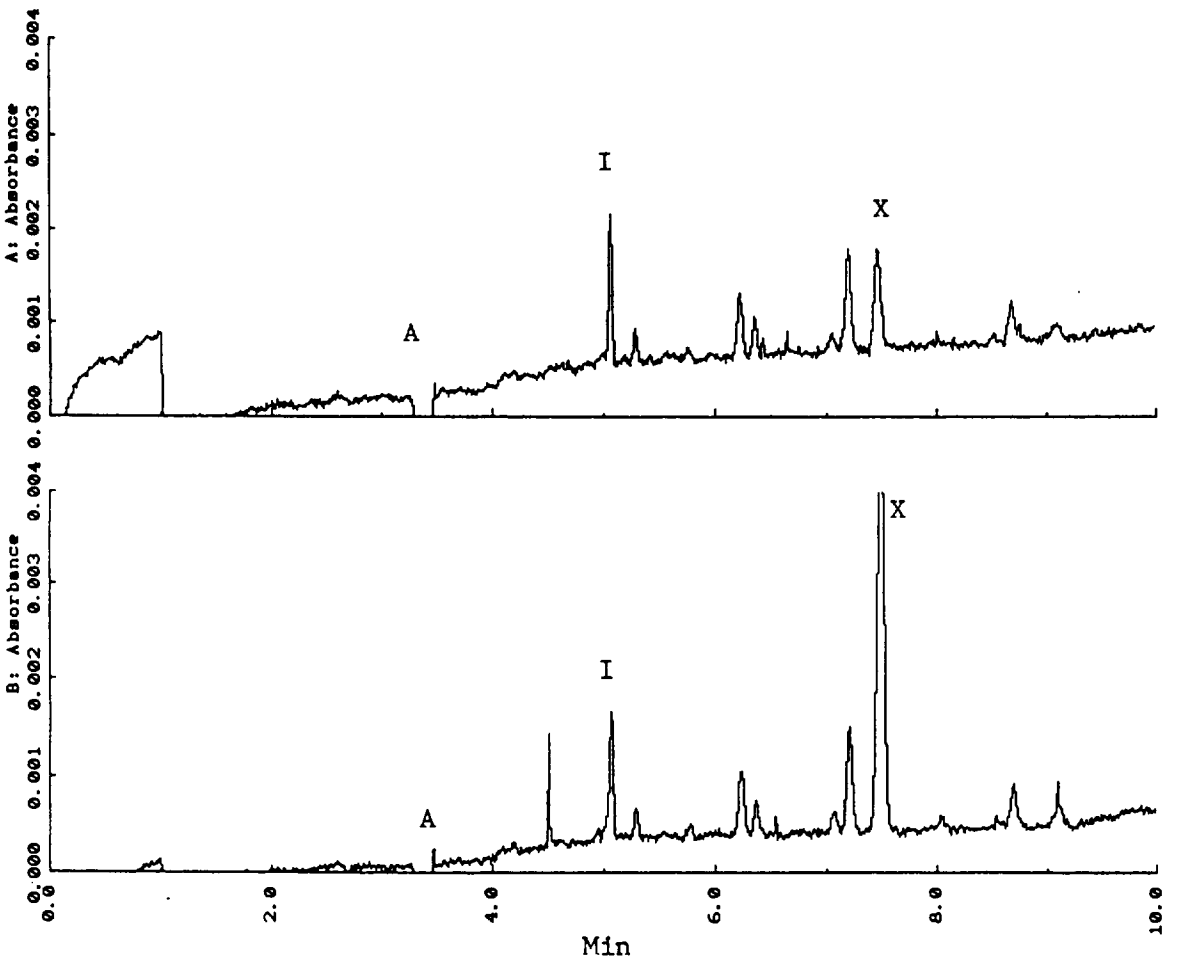

Fig. 5. Xanthine: (top) from guinea pig liver homogenates (28 μg/g wet tissue); (bottom) the same, but after spiking with xanthine. (X = xanthine; I = internal standard; A = acetonitrile.)

determined by this method after diluting the urine five-fold with water.

Iothalamic acid was chosen as an internal standard because it had a migration time which was different from other compounds present in serum or urine. However, because it is structurally different from xanthine, it is not suitable for calculation based on a ratio. Its peak height can change slightly independent of that of xanthine. In general, uric acid, together with the neutral peak of acetonitrile and the iothalamic acid all serve as internal standards to detect and correct for slight changes in the migration time.

Uric acid migrated ca. 30 s after xanthine and can be measured using this method also. Since no other compounds were found to interfere with the uric acid peak, this procedure may serve as a candidate reference method for uric acid determination, especially if it is coupled to the enzyme uricase.

Acetonitrile deproteinization in HPLC leads to sample dilution; however, in CE it leads to a special type of stacking which has been described before [10,11], allowing a large volume of the sample to be introduced into the capillary with little band spreading. In this case the sample is filled with about 3% of the capillary volume leading to the sensitivity needed for this analysis.

The CE method is much more rapid than the HPLC methods [5,6] without the usual problems of the HPLC column such as high pressure, the need for solvent gradients, or expensive columns. It is applicable to many body fluids. We used the method for measuring xanthine in whole blood and tissue homogenates too (Fig. 5). Furthermore, the enzymes involved in xanthine metabolism such as xanthine oxidase, can also be determined by a modification of this method.

The importance of this work is that it demonstrates that CE can be used successfully to measure low levels of small molecules (ca. 1 mg/l) present in complex matrices such as serum or urine. It also demonstrates that acetonitrile deproteinization is a simple and effective method for preparing samples for CE.

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