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Note

Simultaneous determination of allopurinol, oxipurinol and uric acid in human plasma by high-performance liquid chromatography

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Allopurinol (4-hydroxy[3,4-*d*]pyrazolopyrimidine) and its major active metabolite oxipurinol (3,4-dihydroxy[3,4-*d*]pyrazolopyrimidine) are potent xanthine-oxidase inhibitors used in the treatment of hyperuricemia. The mode of action is inhibition of the conversion of hypoxanthine into xanthine and further into uric acid in the purine metabolism.

In the present study a high-performance liquid chromatographic (HPLC) procedure for use in bioavailability studies has been developed. The method makes it possible to determine allopurinol (parent compound), oxipurinol (active metabolite) and uric acid (effect) simultaneously by direct injection of plasma onto the column.

EXPERIMENTAL

Chromatography

A Waters liquid chromatography system consisting of a solvent delivery system 6000A, a universal injector U6K and a UV-absorbance detector 440 was used. The chromatograms were recorded on a Servogor 210 chart recorder. The column was μBondapak C_{18}, particle size 10 μm (300 mm × 3.9 mm I.D.) connected to a pre-column (23 mm × 3.9 mm I.D.) dry packed with Bondapak C_{18}/Corasil of particle size 37—50 μm. The mobile phase was 0.05 *M* phosphate buffer, pH 6.0 (as used by Kramer and Feldman [1] at a flow-rate of 2.5 ml/min. The detector wavelength was 254 nm, at a setting of 0.01 a.u.f.s. for allopurinol and oxipurinol, and 0.2 a.u.f.s. for uric acid.

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Sample preparation

A 75-μl volume of internal standard solution (acetaminophen in distilled water, 10 μg/ml) was added to 0.5 ml of plasma and mixed. A 20-μl aliquot of this sample was injected directly onto the column system.

RESULTS AND DISCUSSION

Fig. 1 shows a typical chromatogram of a blank plasma sample. Fig. 2 shows a chromatogram of a sample taken from a volunteer 2 h after the oral administration of 200 mg of allopurinol. In Fig. 2, peak 1 corresponds to a concentration of 35.0 μg of uric acid, peak 2 to 1.9 μg of oxipurinol and peak 3 to 0.7 μg of allopurinol per ml of plasma.

The retention times for uric acid, oxipurinol, allopurinol and the internal standard (acetaminophen) were 2.2, 6.7, 7.2 and 18.0 min, respectively.

Calibration curves for allopurinol and oxipurinol (0.5—10.0 μg/ml) and uric acid (7.5—120.0 μg/ml) were constructed using spiked plasma and water with six determinations at each of five levels. The curves were linear (r = 1.00) and identical for plasma and water for all three compounds in the ranges mentioned above.

Accuracy and precision of the method are shown in Table I. Detection limits for allopurinol and oxipurinol were 0.15 μg/ml and for uric acid, with 0.2 a.u.f.s., about 5 μg/ml.

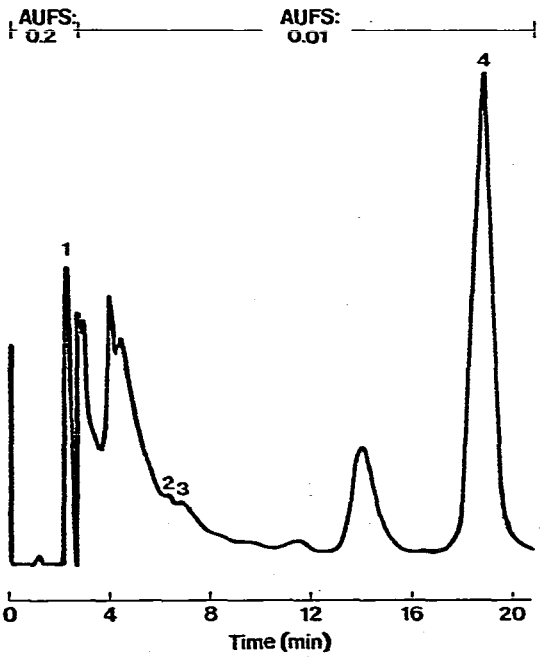

Fig. 1. HPLC chromatogram of blank plasma obtained according to the procedure. Peaks: 1 = uric acid; 2 = oxipurinol (blank value); 3 = allopurinol (blank value); 4 = acetaminophen (internal standard).

TABLE I

ACCURACY AND PRECISION OF SIX DETERMINATIONS AT EACH LEVEL OF ALLOPURINOL, OXIPURINOL AND URIC ACID IN SPIKED HUMAN PLASMA

Allopurinol				Oxipurinol				Uric acid			
Added (μg/ml)	Found (μg/ml)	Accuracy (found, %)	Precision (S.D., %)	Added (μg/ml)	Found (μg/ml)	Accuracy (found, %)	Precision (S.D., %)	Added (μg/ml)	Found (μg/ml)	Accuracy (found, %)	Precision (S.D., %)
0	0	—	—	0	0	—	—	0	32.4	—	2.1
0.45	0.50	111	6.1	0.45	0.48	107	5.7	7.5	6.7*	89	12.1
0.90	0.91	101	3.5	0.90	0.95	106	3.2	15.0	13.9*	93	14.0
1.75	1.75	100	4.1	1.75	1.69	97	1.8	30.0	28.5*	95	5.6
3.40	3.33	98	2.1	3.40	3.23	95	1.5	60.0	60.0*	100	1.0
6.35	6.40	101	2.0	6.35	6.43	101	1.0	80.0	81.0*	101	1.0
Mean		102	3.6			101	2.8			96	6.7

*Corrected for blank value.

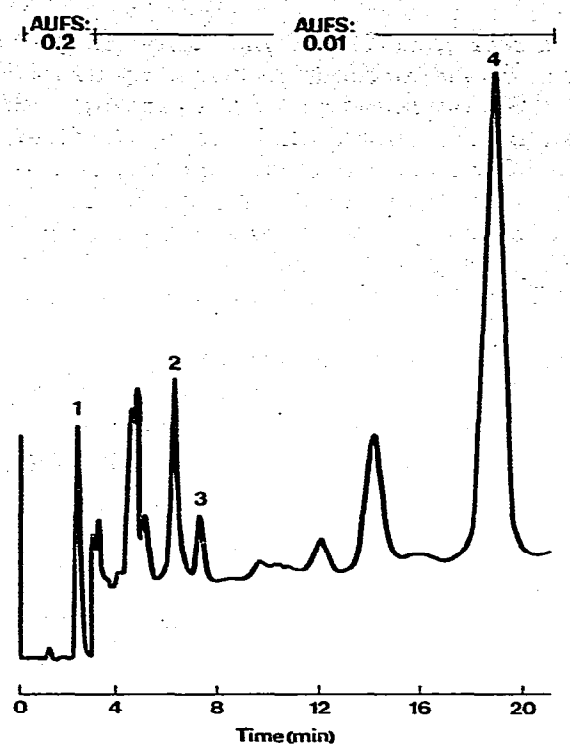

Fig. 2. HPLC chromatogram obtained according to the procedure 2 h after oral ingestion of 200 mg of allopurinol. Peaks: 1 = uric acid; 2 = oxipurinol; 3 = allopurinol; 4 = acetaminophen (internal standard).

In a bioavailability study of allopurinol including eleven subjects [2], blank values corresponding to allopurinol and/or oxipurinol in the range 0.15—0.25 μg/ml were found in six of the blank plasma samples. The blank values were not caused by the intake of any medicine and xanthine, hypoxanthine, caffeine, theobromine and theophylline were found not to interfere with the assay. The interfering substances have not been identified but they are found to be constant throughout the day.

Since uric acid is found naturally in human plasma (normally 30—75 μg/ml) the specificity was confirmed by co-elution with a known standard and by absorbance ratios at 220, 254 and 290 nm as described by Yost et al. [3]. Furthermore, the concentration of uric acid obtained with the method showed good correlation (± 6%) with results obtained by a uricase method described by Praetorius and Poulsen [4].

Compared with other methods described in the literature for the detection of uric acid, allopurinol and oxipurinol [5], for allopurinol and oxipurinol [1,6—9] or for uric acid separately [10], the sample preparation in this method is simple and rapid seeing that the only pre-treatment prior to injection is the addition of the internal standard to the plasma. This means that it is only necessary to use very small blood samples (ca. 50—100 μl), which do not require a venous puncture (they can be taken from an ear or finger, for example). Furthermore, it is possible to achieve increased sensitivity by this method.

It has often been mentioned that direct injection of plasma/serum onto the column causes column deterioration. In the methods described by Kramer and Feldman [1] and Sved and Wilson [9], precipitation of the proteins with 20% trichloroacetic acid and injection of the supernatant are used. Chang and Kramer [11] report that, because of the low pH in the injected sample, column deterioration occurs. Instead, they recommend ammonium sulfate for deproteinization. Wung and Howell [5] use perchloric acid for deproteinization and neutralize before injection and they do not use any internal standard. The recovery of allopurinol is significantly lower than 100% (89%—95%) with this procedure.

In this study, the same main column was used for about 500 applications without showing any signs of deterioration. The pre-column was renewed after 50—70 applications because of clogging. Generally, the pressure was about 140 bars and the indicator for renewing the pre-column was a pressure exceeding 210 bars.

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