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Note

Simultaneous determination of allopurinol and oxipurinol in biological fluids by mass fragmentography

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The mass fragmentographic analysis of xanthine and hypoxanthine as described previously [1] was applied to the determination of allopurinol and its major metabolite, oxipurinol. We present, in this paper, a specific and sensitive method for the simultaneous determination of xanthine, hypoxanthine, allopurinol and oxipurinol (Fig. 1) in serum and urine, and its application to studies involving patients treated daily with 200 mg of allopurinol (Zyloric®, Wellcome) for a week.

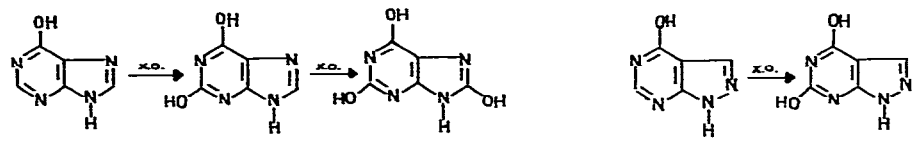

Fig. 1. Oxidative transformation of hypoxanthine to uric acid and allopurinol to oxipurinol by xanthine oxidase (X.O.).

EXPERIMENTAL

Analytical procedure

The determinations were carried out on a quadrupolar gas chromatography—mass spectrometry system (Hewlett-Packard 5985 B) in the electron-impact mode by the method of selected ion monitoring.

Each serum and urine sample was analyzed according to the procedure

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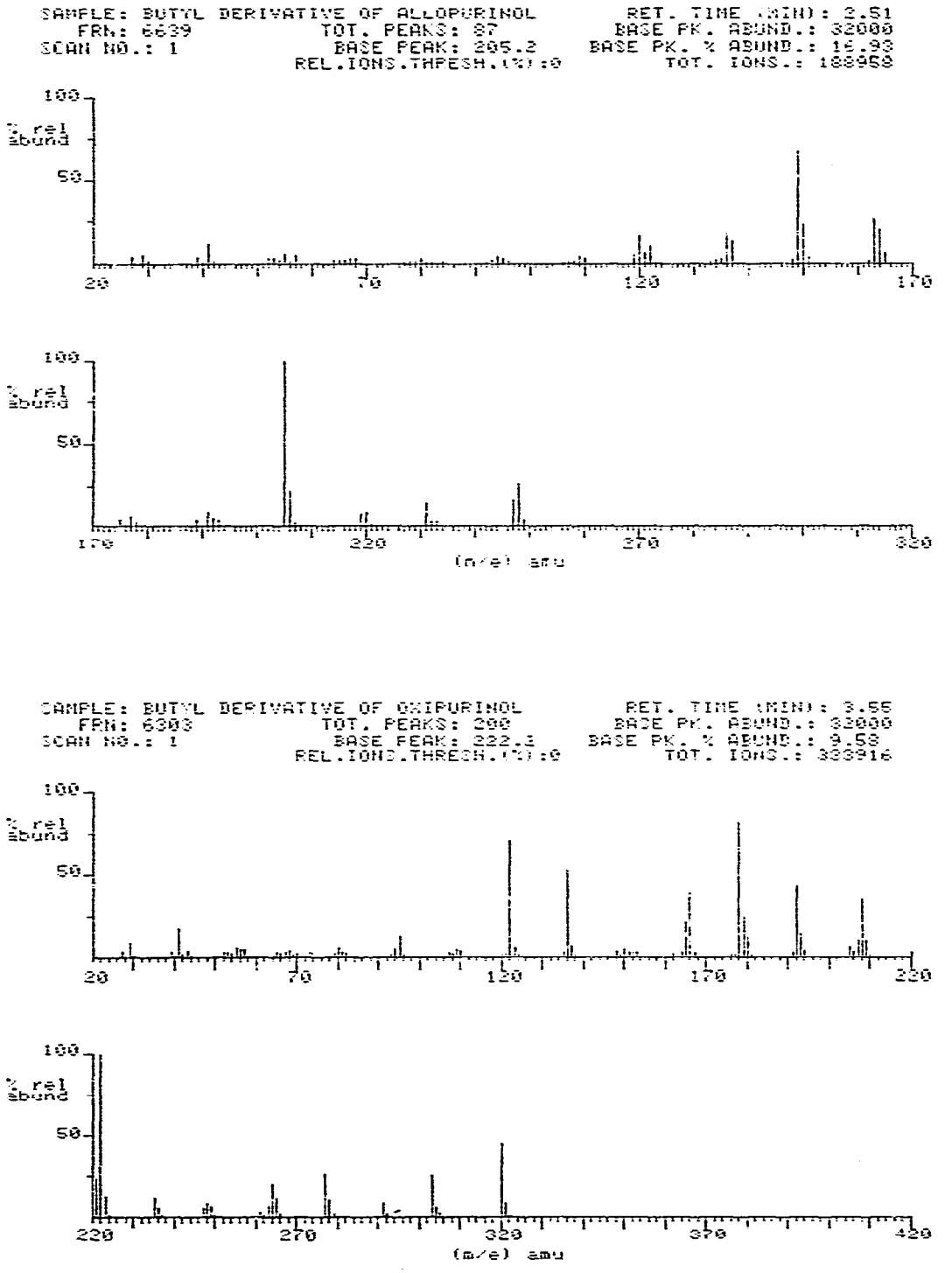

Fig. 2. Mass spectra of butylated derivatives of allopurinol and oxipurinol.

described in detail previously [1]. [7,9-^{15}N]Xanthine was added to each sample as internal standard.

Serum (0.5 ml) was deproteinized by ultrafiltration in Amicon CF 25 Centriflo cones, and was then assayed in the same way as the urine samples (0.1 ml).

After extraction by shaking with 2 ml of *n*-butanol at pH 4.2, followed by butylation according to Greeley's method [2], the chromatographic separation was carried out on a glass column (150 cm × 2 mm I.D.) packed with 3% OV-17 on Gas-Chrom Q, programmed from 190 to 260°C at 10°C/min.

Mass spectra

The electron-impact mass spectrum of the dibutylated derivative of allopurinol (Fig. 2) exhibits a slight molecular ion at *m/e* 248; so, for quantitative analysis, we selected the base peak *m/e* 205. Oxipurinol shows a fragmentation pattern (Fig. 2) similar to that of xanthine when derivatized by butylation. The molecular ion (*m/e* 320, 48%) was selected for the quantitation of oxipurinol. The ions found to be suitable for the mass fragmentographic analysis of xanthine, [7,9-^{15}N]xanthine and hypoxanthine were *m/e* 320, *m/e* 322 and *m/e* 231, respectively, as reported previously [1].

A typical mass fragmentogram from a serum extract is shown in Fig. 3.

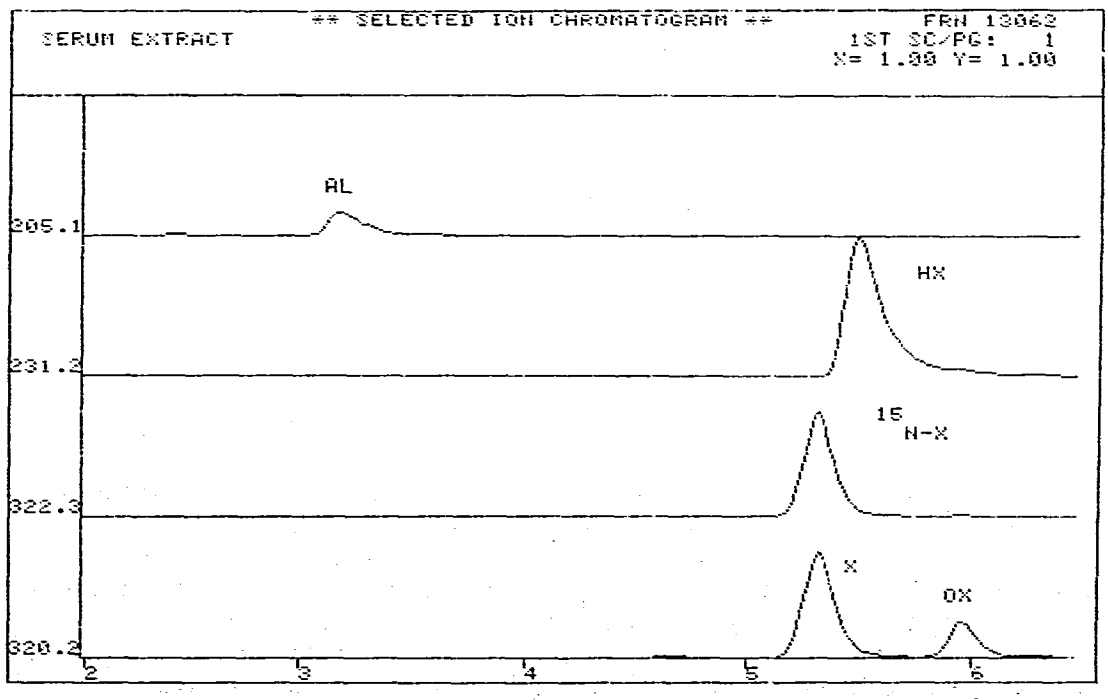

Fig. 3. Mass fragmentogram of butylated derivatives of allopurinol (AL), oxipurinol (OX), xanthine (X), [7,9-^{15}N]xanthine (^{15}N-X) and hypoxanthine (HX) in a serum extract.

Human studies

The study was conducted on 31 subjects receiving allopurinol (200 mg) orally once daily for a week, before lunch. Blood was withdrawn on the eighth day by venipuncture (20 ml in dry tubes) after overnight fasting, and urine was collected over the previous 24 h.

RESULTS AND DISCUSSION

Evaluation of the method

The limit of sensitivity was approximatively 50 ng of xanthine, hypoxanthine and oxipurinol per ml of serum and 25 ng/ml for allopurinol.

The inter-assay reproducibility of the method was determined by quadruplicate analysis of high and low standards in both serum (Table I) and urine (Table II).

TABLE I

PRECISION OF THE SIMULTANEOUS DETERMINATION OF XANTHINE, HYPOXANTHINE, ALLOPURINOL AND OXIPURINOL IN HUMAN SERUM

	Theoretical (μg/ml)	Calculated (μg/ml ± S.D.)	Coefficient of variation (%)	Mean error (%)
Hypoxanthine	5.0	4.97 ± 0.07	1.4	0.6
	2.0	2.09 ± 0.19	9.1	4.5
	0.5	0.46 ± 0.05	10.8	8.0
Xanthine	5.0	4.99 ± 0.01	0.2	0.2
	2.0	2.03 ± 0.05	2.5	1.5
	0.5	0.46 ± 0.02	4.3	8.0
Allopurinol	5.0	5.05 ± 0.06	1.2	1.0
	2.0	2.02 ± 0.21	10.4	1.0
	0.5	0.52 ± 0.06	11.5	4.0
Oxipurinol	5.0	5.00 ± 0.03	0.6	0.0
	2.0	2.07 ± 0.11	5.3	3.5
	0.5	0.50 ± 0.05	10.0	0.0

The accuracy of the method is indicated by the coefficient of variation and the mean error between the detected and the theoretical values.

The extraction recovery was found to be 65% for allopurinol at a 0.5 μg/ml level, and 62% for oxipurinol at a 5 μg/ml level, both in serum. The extraction recovery appears to be better than the xanthine recovery (55%).

Standard curves for the various compounds were linear over the concentration range investigated in both serum (Fig. 4) and urine. Correlation coefficients for serum standard curves (0.5—5.0 μg/ml) were better than 0.99. Correlation coefficients were equally good (> 0.99) for urine calibration curves for xanthine, hypoxanthine, allopurinol (2.5—25 μg/ml) and oxipurinol (10—100 μg/ml). Each sample was analyzed in duplicate and a third analysis was performed if the results differed by more than 10%.

TABLE II

PRECISION OF THE SIMULTANEOUS DETERMINATION OF XANTHINE, HYPO XANTHINE, ALLOPURINOL AND OXIPURINOL IN URINE

	Theoretical (μg/ml)	Calculated (μg/ml ± S.D.)	Coefficient of variation (%)	Mean error (%)
Hypoxanthine	25.0	25.0 ± 0.1	0.4	0.0
	10.0	10.5 ± 0.4	3.8	5.0
	2.5	2.5 ± 0.2	8.0	0.0
Xanthine	25.0	25.1 ± 0.2	0.8	0.4
	10.0	10.3 ± 0.6	5.8	3.0
	2.5	2.5 ± 0.2	8.0	0.0
Allopurinol	25.0	24.9 ± 0.1	0.4	0.4
	10.0	10.4 ± 0.5	4.8	4.0
	2.5	2.4 ± 0.2	8.3	4.0
Oxipurinol	100	100.4 ± 0.6	0.6	0.4
	50	51.2 ± 1.8	3.5	2.4
	10	10.0 ± 0.7	7.0	0.0

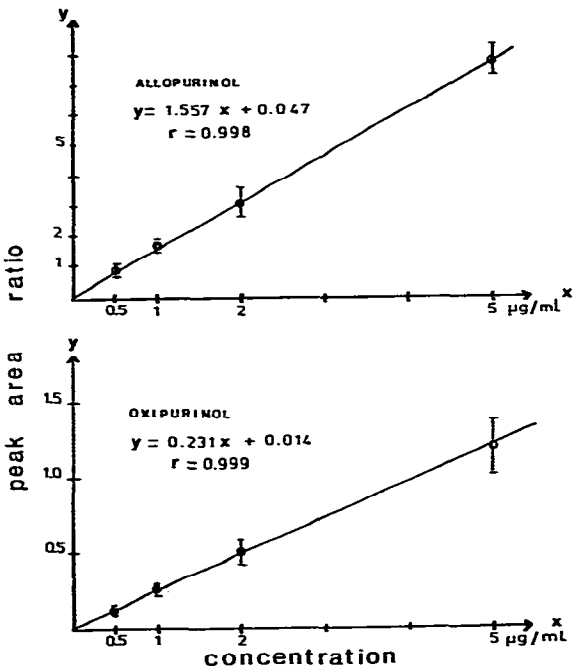

Fig. 4. Calibration curves for allopurinol and oxipurinol in serum. Each point represents the mean and standard deviation of seven determinations carried out on different days. The length of the bar corresponds to the estimated S.D.

Application of assay

Means and standard deviations for the serum concentrations and the urinary eliminations of xanthine, hypoxanthine, allopurinol and oxipurinol are listed in Table III. It can be seen that there is practically no allopurinol in the blood

TABLE III

SERUM CONCENTRATION AND URINARY ELIMINATION OF XANTHINE, HYPOXANTHINE, ALLOPURINOL AND OXIPURINOL IN 31 SUBJECTS TREATED WITH ALLOPURINOL

	Serum (μg/ml; n = 31)		Urine (mg per 24 h; n = 31)	
	Mean	S.D.	Mean	S.D.
Hypoxanthine	3.2	3.4	16.4	13.7
Xanthine	0.9	0.3	25.0	15.0
Allopurinol	0.1	0.1	7.8	5.3
Oxipurinol	3.7	1.9	122.9	48.6

samples; this conforms to our knowledge about the rapid transformation of allopurinol into oxipurinol, and is due to the important time delay between administration of the drug and the blood sampling.

The values obtained for xanthine and hypoxanthine in serum and especially in urine are higher than those observed in our first study on normal subjects, which is in agreement with the expected mechanism of action of allopurinol.

In conclusion, the method presented here is sensitive and accurate enough to allow the monitoring, in parallel to a pharmacokinetic study of allopurinol, of the influence of this drug on purine metabolism.

REFERENCES

1 J.L. Chabard, C. Lartigue-Mattei, F. Vedrine, J. Petit and J.A. Berger, J. Chromatogr., 221 (1980) 9.
2 R.H. Greeley, J. Chromatogr., 88 (1974) 229.