Journal of Chromatography, 162 (1979) 94—97
Biomedical Applications

CHROMBIO. 221

Note

High-performance liquid chromatographic assay for allopurinol and oxipurinol in human plasma

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(Received March 22nd, 1978)

Allopurinol (4-hydroxy-3,4-*d*-pyrazolopyrimidine) is a potent xanthine oxidase inhibitor used in the treatment of hyperuricemia. As part of an investigation into the pharmacokinetics of allopurinol absorption from a variety of dosage forms, it was necessary to develop an assay capable of detecting the drug and its primary, active metabolite, oxipurinol (3,4-dihydroxy-3,4-*d*-pyrazolopyrimidine), in plasma. Although two high-performance liquid chromatographic (HPLC) methods have been recently published [1,2], we wish to report an HPLC procedure that is equally sensitive but is greatly simplified and requires considerably smaller volumes of plasma.

EXPERIMENTAL

Apparatus

A Glenco System I high-performance liquid chromatograph (Glenco Scientific, Houston, Texas, U.S.A.) equipped with a 254-nm UV detector and a reversed-phase column (Spherisorb ODS 5 μm, 25 cm × 4.1 mm; Laboratory Data Control, Riviera Beach, Fla., U.S.A.) was used for all assays. The detector was connected to an electronic integrator (Autolab Minigrator, Spectra Physics, Santa Clara, Calif., U.S.A.) and chromatograms were recorded on a chart recorder (Linear Instruments, Irvine, Calif., U.S.A.).

Mobile phase

A phosphate buffer (pH 6.0, 0.05 *M*) was prepared by adding 0.508 g of dibasic potassium phosphate and 6.41 g of monobasic potassium phosphate per l of glass-distilled water and adjusting pH to 6.0 with HCl or NaOH. All

solutions were passed through an 0.22-μm filter (Millipore Filter, Bedford, Mass., U.S.A.) and degassed by stirring with a magnetic stirrer before use.

Drug standards

Allopurinol and oxipurinol in pure form were gifts of Dr. George Reddin, Burroughs Wellcome, Research Triangle Park, N.C., U.S.A. Acetaminophen (4′-hydroxyacetanilide), the internal standard, was purchased from Eastman Organic Chemicals, Rochester, N.Y., U.S.A.

Standard solutions

An aqueous solution of allopurinol and oxipurinol, 100 μg/ml of each, was prepared in glass-distilled water. Appropriate volumes were then diluted to 10 ml with human plasma to yield standard solutions of 0.5, 1.0, 2.0, 5.0, 10.0 and 20.0 μg/ml.

The internal standard, acetaminophen, was prepared at a concentration of 100 μg/ml in glass-distilled water.

Assay procedure

To 0.5 ml of plasma were added 0.1 ml internal standard and 0.2 ml 20% trichloroacetic acid. Following mixing (Vortex Genie; Scientific Products, Houston, Texas, U.S.A.) the sample was centrifuged at 8700 *g* (Microfuge B; Beckman, Palo Alto, Calif., U.S.A.) and a 50-μl aliquot injected onto the column. Solvent flow-rate was 2 ml/min (1500 p.s.i.) and the detector was maintained at 0.1 a.u.f.s. The areas under the peaks of interest were calculated by the integrator and standard curves constructed by plotting the ratio of the area under either the allopurinol peak or oxipurinol peak to that of acetaminophen against the concentration of the respective compound.

RESULTS

A chromatogram obtained from blank plasma is shown in Fig. 1A. A chromatogram from standard plasma (allopurinol and oxipurinol, 10 μg/ml of each) in Fig. 1B. Fig. 1C is a chromatogram of a representative sample from a volunteer subject following the oral ingestion of a 300 mg allopurinol tablet (Zyloprim; Burroughs Wellcome).

As can be seen by comparison of Figs. 1A and B, there are no interfering peaks in the areas where allopurinol, oxipurinol and acetaminophen elute. Each of the three peaks is sharp, symmetrical and well defined with respect to the base line. Calibration curves for both compounds were linear from 0 to 20 μg/ml. The lower limit of the assays has been found to be approximately 0.1 μg/ml for both compounds. Recovery over the range 0.5 to 20 μg/ml averaged 98.2 ± 1.75% for oxipurinol and 99.3 ± 0.93% for allopurinol.

The retention times of oxipurinol, allopurinol and acetaminophen were 9.8, 13.0 and 26.0 min, respectively. The following related compounds were found not to interfere in the assay: xanthine, hypoxanthine, 5-fluorouracil, caffeine, theobromine, theophylline, uric acid and 6-thiouric acid. However, 6-mercaptopurine has the same retention time as allopurinol under the conditions used.

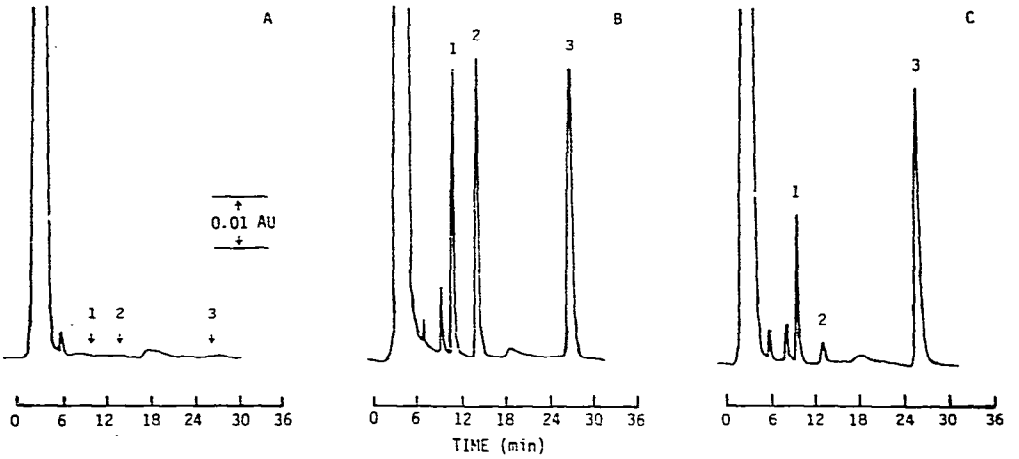

Fig. 1. Chromatograms of (A) blank plasma, (B) standard plasma containing allopurinol and oxipurinol, 10 μg/ml of each and (C) plasma sample from a volunteer subject 4 h after the ingestion of a 300-mg tablet of allopurinol. 1=Oxipurinol, 2=allopurinol; 3=acetaminophen (internal standard).

DISCUSSION

The assay method described in this paper is rapid, sensitive and specific. A representative plot of plasma allopurinol and oxipurinol concentrations vs. time for a volunteer subject following a 300-mg oral dose is shown in Fig. 2 and demonstrates the utility of the method.

Endele and Lettenbauer [1] and Brown and Bye [2] have reported HPLC assays for allopurinol and oxipurinol utilizing anion exchange columns. In each case, 1 ml of plasma was required and samples were subjected either

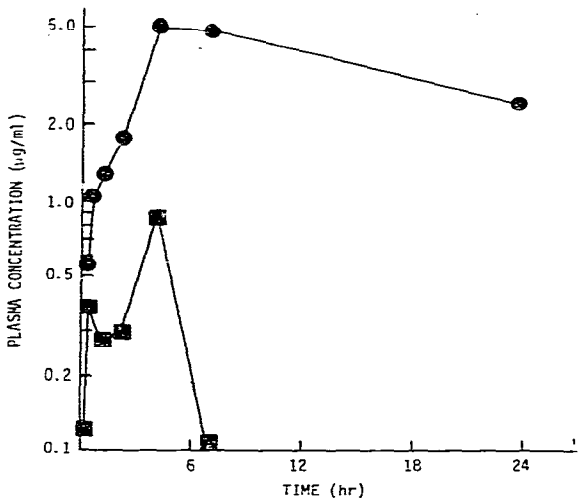

Fig. 2. Plasma allopurinol (■) and oxipurinol (●) concentration—time curves obtained in a volunteer subject following the ingestion of a 300-mg tablet of allopurinol.

to solvent extraction [1] or column [2] clean-up procedures before chromatography. Our procedure requires no extraction and therefore has fewer steps and requires less time. Further, by use of the appropriate volumes of internal standard and trichloroacetic acid, the assay may be run using as little as 100 μl of plasma, making it appropriate for clinical use even in pediatric patients.

Although the injection of the supernate obtained from trichloroacetic acid-precipitated plasma can potentially clog or contaminate the column, we have found that the use of a 2-μm inline filter (Model 7302, Column Inlet Filter, Rheodyne, Berkeley, Calif., U.S.A.) prevents column damage as evidenced by consistent peak shapes and retention times. Filters are replaced periodically when back pressure increases above normal. In addition, due to the force generated by centrifugation at 8700 g, essentially no particulate matter may be detected in the supernate.

A reversed-phase HPLC assay for allopurinol and its metabolite, oxipurinol, in human plasma, has been described. The method may be applied to samples as small as 0.1 ml, has a lower limit of sensitivity of 0.1 μg/ml of either compound, and is suitable for pharmacokinetic and clinical studies.

REFERENCES

1 R. Endele and G. Lettenbauer, J. Chromatogr., 115 (1975) 228.
2 M. Brown and A. Bye, J. Chromatogr., 143 (1977) 195.