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Note

Gas chromatographic analysis of pharmaceuticals based on pyrimidine and purine substances

V. MILLER, V. PACÁKOVÁ and E. SMOLKOVÁ

Department of Analytical Chemistry, Charles University, Albertov 2030, 128 40 Prague 2 (Czechoslovakia)

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For the analysis of pyrimidine and purine bases, the application of microbiological, titrimetric, spectrophotometric or fluorimetric methods[1–5] is limited by their poor sensitivity and poorer specificity. In analyses for these substances, which are the active components in a number of pharmaceuticals, their separation is also important; consequently, chromatographic methods, especially paper chromatography[6] and thin-layer chromatography (TLC)[3], have been used. Gas–liquid chromatography (GLC) can also be utilized, after conversion of the substances into volatile derivatives; this method satisfies the demands of good sensitivity, versatility and rapidity, especially in applications to biological materials.

A study of the course of silylation of some pyrimidine and purine bases and nucleosides[7] has permitted the application of GLC in the analysis of some therapeutic compounds; four such substances have been studied, *viz.*, 5-fluorouracil, ftorafur (1-[2-tetrahydrofuryl]-5-fluorouracil), 6-azauridine and allopurinol (1 *H*-pyrazole-[3,4-*d*]-pyrimidin-4-ol).

5-Fluorouracil and ftorafur are chiefly used in the treatment of carcinoma of the stomach, large intestine or rectum, the antineoplastic effect involving inhibition of thymidylate synthetase and hence blocking of the methylation of deoxyuridylic acid to form thymidylic acid[8]. However, ftorafur is much less toxic, and has virtually the same neoplastic efficiency as 5-fluorouracil[9]. 6-Azauridine is used particularly in the treatment of leukaemia; it blocks decarboxylation of orotidylic acid in the organism, thus inhibiting the synthesis of pyrimidine nucleotides[10]. Allopurinol inhibits conversion of xanthine and hypoxanthine into uric acid[11].

GLC has not to our knowledge been used for determining any of these substances except 5-fluorouracil[12–15]; the methods so far employed involve iodometric determination[2], spectrophotometry[16] and TLC[3]. In view of the similarity between these substances and the nucleic acid components studied earlier[7], it can be assumed that ftorafur, allopurinol and 6-azauridine will also be determinable by GLC as their trimethylsilyl (TMS) derivatives.

EXPERIMENTAL

Materials

5-Fluorouracil. This substance, both in the pure form (white crystals, readily

soluble in dilute ammonia) and as an injection solution of its sodium salt (50 mg/ml) was obtained from Hoffman-LaRoche (Basle, Switzerland).

Ftorafur. The pure form of this substance (as colourless crystals, readily soluble in hot water, alcohol and dimethylformamide) was obtained from Dr. H. Tománková (State Institute for Drug Control, Prague, Czechoslovakia); injection solution of the sodium salt of ftorafur (concentration 4%) was obtained from Medexport (Moscow, U.S.S.R.).

6-Azauridine. This substance, both in the pure form (white crystals, very easily dissolved in water) and as a powdered solid-dosage drug was obtained from Spofa (Prague, Czechoslovakia).

Allopurinol. The pure form of this substance (a white powder, slightly soluble in water or ethanol and readily soluble in ammonia) was obtained from Dr. H. Tománková; the pharmaceutical form (Zyloric tablets, each containing 100 mg of allopurinol) was obtained from the Burroughs Wellcome Co. (Dartford, Great Britain).

Silylation reagent. BSTFA (Regis Chemical Co., Morton Grove, Ill., U.S.A.).

Solvent. Pyridine p.a. (Lachema, Brno, Czechoslovakia).

Internal standard. Phenanthrene (The British Drug Houses Ltd., Poole, Great Britain).

Gas chromatographic apparatus

All measurements were carried out by using a Packard Type 7409 instrument, with a dual-column system, flame ionisation detectors and programmed-temperature control. Sample injection was made directly into the column. A two-channel Goerz recorder was used.

Preparation of TMS derivatives

A portion (1.0 ml) of a standard solution of the pure substance in dilute ammonia, corresponding to 0.50 mg of the test compound, was evaporated to dryness at 90° in a stream of argon. The dried residue was heated with 250 μl of BSTFA and 200 μl of pyridine at 150° for 20 min, with vigorous stirring; for 6-azauridine, heating was for 30 min. TMS derivatives of the compounds in injection solutions (corresponding to 0.50 mg of the active substance) were prepared in the same way. For Zyloric tablets, half of a tablet was stirred for 30 min with 50 ml of 1.5 *N* ammonia solution at 60°, then the insoluble material (excipients) was filtered off; 0.5 ml of the filtrate was evaporated and silylated as described above.

Gas chromatographic analysis

An aliquot (1–3 μl) of the silylation-reaction mixture was chromatographed on two glass columns (2 m × 0.4 cm I.D. each), with stationary phases of 3% OV-101 and 3% OV-17 on Chromosorb W HP AW DMCS (100–120 mesh). The column temperature was 150° for analysis of the derivatives of 5-fluorouracil, ftorafur and allopurinol, and 225° for that of 6-azauridine.

Quantitative analysis for the active constituents of the pharmaceuticals was carried out on the OV-17 column by the standard-addition method, the concentration of each substances being calculated from the relationship:

$$m_t = \frac{V_s}{V_t} \cdot \frac{m_s}{\frac{A_{is}}{A_t} \cdot \frac{v_t}{v_{ts}} \cdot \left(1 + \frac{V_s}{V_t}\right) - 1}$$

where v_t is the injected volume of the silylated reaction mixture from the pharmaceutical with unknown concentration m_t,

v_{ts} is the injected volume of the mixture from the sample with the standard addition,

A_t is the peak area for injected volume v_t,

A_{ts} is the peak area for injected sample volume v_{ts},

V_t is the volume of the sample (of concentration m_t) mixed with the standard addition, and

V_s is the standard-addition volume of known concentration m_s.

RESULTS

Qualititative analysis

The TMS derivatives of 5-fluorouracil, ftorafur, allopurinol and 6-azauridine prepared from the standard ammoniacal solutions were chromatographed on both columns with OV-101 and OV-17 stationary phases; all the substances yielded single symmetrical elution peaks on both columns. For identification purposes, the retention indices were measured; the values are shown in Table I.

TABLE I

RETENTION INDICES OF TMS DERIVATIVES OF THE TEST COMPOUNDS ON COLUMNS WITH OV-101 AND OV-17 PHASES OPERATED AT 150°

Substances	$I_{OV\text{-}101}$	$I_{OV\text{-}17}$
5-Fluorouracil	1317.6	1419.8
Ftorafur	1318.7	1420.0
6-Azauridine*	—	2531
Allopurinol	1612.6	1723.6

* Column temperature 225°.

Quantitative analysis

The good chromatographic properties of the substances tested, as found during qualitative measurements, have made possible their determination in medicinal preparations by GLC.

Quantitative analysis of 5-fluorouracil and ftorafur in injection solutions and of allopurinol in tablets was carried out by the standard-addition method on a column with OV-17 as stationary phase at 150°. The average concentration of these substances in pharmaceuticals (c) was calculated from five measurements (c_i) and the average measuring error (s_i) was determined.

The average concentration of 5-fluorouracil in the injection solution was 48.96 $\pm$ 0.54 mg/ml (see Table II), which was 97.9 $\pm$ 1.1% of the concentration specified by the manufacturer.

Also shown in Table II are the concentrations of ftorafur found in the injection preparation; the calculated average value of was 98.3 $\pm$ 0.9% of that specified by the manufacturer. In the manufacturer's literature[2], the permitted tolerance for ftorafur content in injections is given as 98.5–100.5% of the amount specified (determined by

TABLE II

MEASURED CONCENTRATIONS OF THE TEST COMPOUNDS IN PHARMACEUTICALS

Measured values (c_i), mg/ml	*Mean value (c), mg/ml*	*Mean error (s_c), mg/ml*
For 5-fluorouracil in an injection solution		
49.1, 50.4, 47.3, 49.7, 48.3	48.96	0.54
For ftorafur in an injection solution		
38.9, 39.1, 39.3, 40.7, 38.7	39.34	0.39
For allopurinol extracted from a Zyloric tablet		
97.0, 98.1, 94.5, 99.5, 98.1	97.44	0.83

iodometric titration). The value found by GLC in this work is at the lower limit of the permitted tolerance.

The amount of allopurinol in a single Zyloric tablet was 97.44 $\pm$ 0.83 mg (see Table II); the theoretical amount specified by the manufacturer is 100 mg. The British Pharmacopoeia[16] permits a range 92.5–107.5% of the prescribed amount of allopurinol in one tablet (as determined by UV spectrophotometry at 250 nm). The GLC result corresponded to 97.4% of the amount specified by the manufacturer, *i.e.*, in the middle of the permitted range.

6-Azauridine was supplied as the pure substance. The relative molar response of its TMS derivative with respect to phenanthrene was measured on an OV-17 column operated at 225°; the average value was 0.67 unit.

Easy silylation and the good chromatographic properties of the resulting derivatives indicate that GLC analysis will be applicable to the determination of the substances studied in biological materials.

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