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Note

Analytical separation of bleomycin

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Bleomycin is an antitumour antibiotic used mainly in the treatment of well differentiated squamous cell carcinomas. The preparation used clinically consists of a mixture of several bleomycins, it is controlled by the specification set down by either the U.S. Food and Drug Administration (FDA) or in the Japanese Pharmacopoeia[2]. One of these specifications consists of the chromatographic separation of the bleomycin complex. Both methods, which are based upon the original paper of Umezawa[3], use several milligrams of bleomycin and take three to four days to complete the analysis.

The method presented in this paper uses about 300 μg of bleomycin and the analysis can be completed in about 15 h. The results obtained compare favourably with those from the existing methods.

EXPERIMENTAL

Materials

The bleomycin used was part of the proposed first International Reference preparation supplied by Nippon Kayaku (Tokyo, Japan). For each analysis 300 μg were dissolved in 200 μl of 0.025 *M* ammonium formate just before the analysis CM52 ion-exchange cellulose was obtained from Whatman (Clifton, N.J., U.S.A.) and equilibrated in 0.025 *M* ammonium formate. All other chemicals were analytical reagent grade.

Equipment

A Pharmacia K9/30, 30 × 0.9 cm, column and end pieces were used with an LKB 12000 Varioperpex peristaltic pump. A Cecil CE272 spectrophotometer with a simple flow cell was used as the detector coupled to a Servoscribe recorder. An LKB gradient mixer was used to produce a linear gradient of ammonium formate.

RESULTS

The sample of bleomycin was eluted with a linear gradient consisting of 75 ml each of 0.025 and 0.6 *M* ammonium formate. The flow-rate was 7 ml/h. In order to

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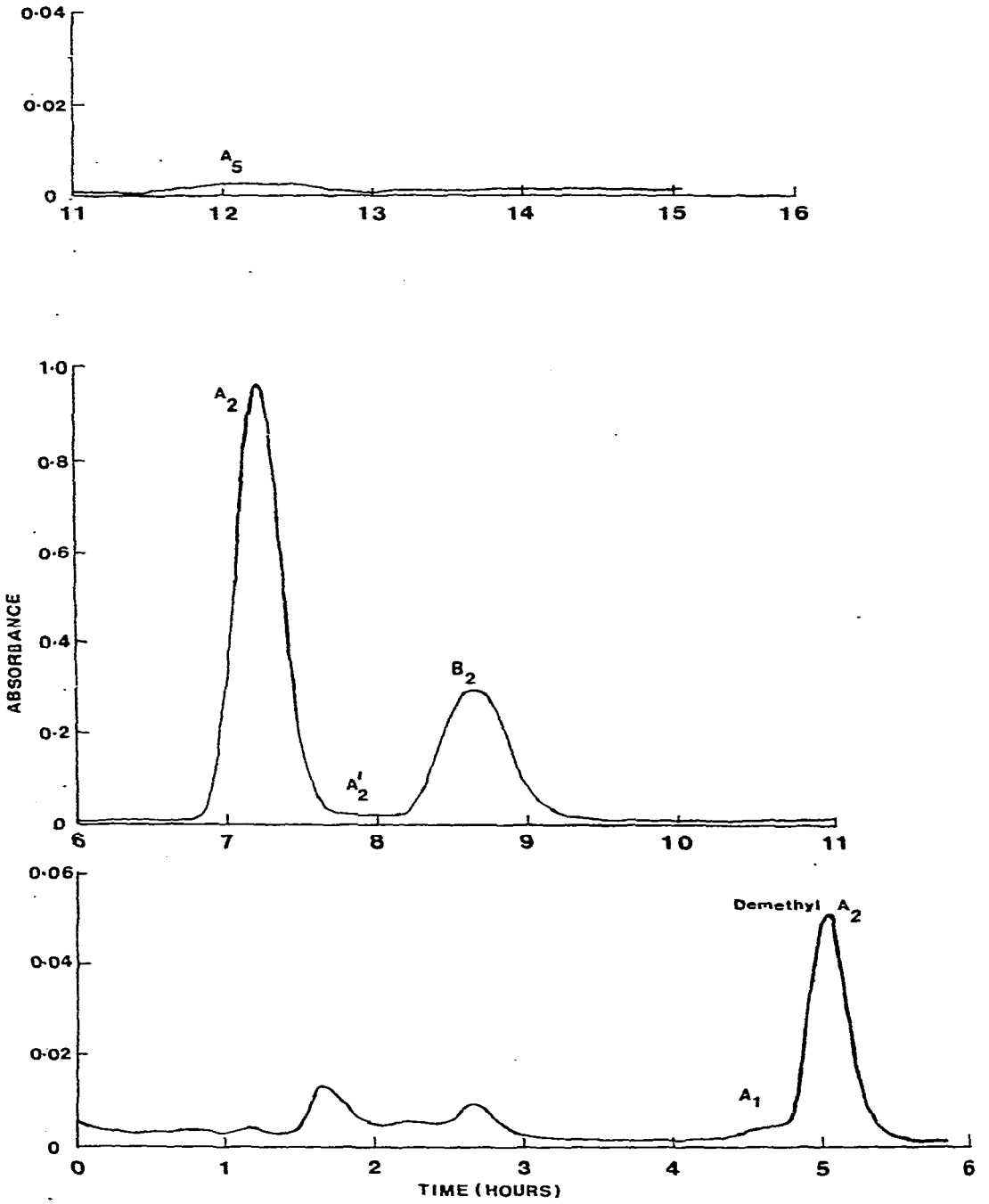

Fig. 1. Chromatography of bleomycin. Changes in absorbance scale correspond to changes made in spectrophotometer sensitivity by the automatic switching device.

cope with the widely differing amounts of the various bleomycins a simple switching devise has been incorporated that changes the spectrophotometer sensitivity from scale 2 to 0.2 at preset times during the run. A typical separation of a proposed International Reference preparation, after distribution into ampoules is shown in Fig. 1. The proportional content of each component derived from it agreed closely with the values reported by the manufacturer (Table I).

TABLE I

CONTENT OF BLEOMYCINS (%)

	A_1	*Demethyl* A_2	B_1	A_2	A'_2	B_2	A_5	B_4
Manufacturer's analysis	0.1	1.2	—	69.0	0.2	28.5	0.1	—
NIBSC analysis	*	1.9	—	69.3	*	28.9	*	—

* Component detected.

A significant amount of demethyl A_2 is present in the preparation, this component is the immediate degradation product of A_2 and its formation has been attributed to the effects of freeze drying and oxidation[4]. However, in the ampoulling of International Reference preparations oxygen is almost completely excluded[5,6]. After sealing in glass ampoules under nitrogen the proposed International Reference preparation of bleomycin contained less than 0.3% (v/v) of oxygen. The results of an accelerated degradation study of these sealed ampoules is shown in Table II. The proportion of demethyl A_2 increased in six weeks, even at room temperature the recommended storage temperature for the sample used clinically.

TABLE II

CONTENT OF BLEOMYCINS (%)

Storage for 6 weeks at	A_1	*Demethyl* A_2	B_1	A_2	A'_2	B_2	A_5	B_4
−20°	*	1.9	—	69.3	—	28.9	*	—
20°	0.2	3.5	—	67.7	0.6	27.8	0.3	—
37°	0.3	5.5	—	64.6	0.7	28.4	0.3	0.3
58°	*	14.7	—	54.2	0.8	30.3	*	—

* Component detected.

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