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Note

High-pressure liquid chromatographic analysis for triamcinolone acetonide in rice starch

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In some European countries, triamcinolone acetonide (10% by wt.) is mixed with rice starch and, in turn, is used by pharmacists for individual formulations. The problem for the manufacturer is to ensure that the pharmacist receives a well-blended mixture of correct concentration, thus requiring the analysis of numerous representative samples of the blended powder. This has stimulated the development of a ready and rapid method that has not previously appeared in the literature. An adaption of the method in the United States Pharmacopeia (USP)[1] for triamcinolone acetonide analysis is too slow for a reasonable uniformity test.

EXPERIMENTAL

Sample preparation

A quantity of powder equivalent to *ca.* 4 mg of triamcinolone acetonide (TACA) was weighed into a 10-ml centrifuge tube. 5-ml of dichloromethane (analytical grade, E. Merck, Darmstadt, G.F.R.) were added and the stoppered tube was shaken on a Vortex mixer for 1 min, then centrifuged at 2000 *g* for 3 min.

Standard preparation

Approximately 8 mg of TACA standard were accurately weighed, then dissolved and brought to a volume of 10 ml with dichloromethane.

Chromatography

The chromatography was carried out using a constant-flow metering pump (Chromatronix, Model CMP-1) and a spectrophotometer (Beckman, Model DB-G) equipped with a 8-μl dual-beam flow-cell (designed and constructed by the author) and a strip chart recorder (Beckman, Model 1005). A 1 m × 2 mm I.D. glass column (Chromatronix) was packed with Zipax (DuPont, Wilmington, Del., U.S.A.) using the tap-and-fill dry-packing technique[2]. The mobile phase consisted of dichloromethane (reagent grade; Merck) that had been freshly distilled and washed with water. The flow-rate of the water-saturated mobile phase was 20 ml/h, resulting in a column pressure drop of 18 atm. Injections of 5-μl amounts of sample were made using an automatic valve with a pneumatic actuator (Chromatronix, Model CSVA).

The quantity of TACA in the rice starch was calculated from the following formula:

$$\frac{SPL}{STD} \cdot \frac{\text{Standard weight (mg)}}{\text{Sample weight (mg)}} \cdot 200 = \%\ \text{TACA in rice starch}$$

where SPL and STD are the peak heights of the sample and standard, respectively.

RESULTS AND DISCUSSION

Table I indicates that there is good agreement between the high-pressure liquid chromatographic (HPLC) analyses and the theoretical values calculated from the production records of three lots of blended TACA (10% by wt.) in rice starch. It also shows that the individual lots are homogeneous after blending, since in each case the four samples were taken from different areas of the batch. The same three lots of blended rice starch powder were analyzed by methanol extraction of samples of the powder followed by the assay in the USP[1] for TACA. Table II illustrates that the values thus obtained correlate with the HPLC method, although there does appear to be a slight negative bias in the extraction followed by the USP procedure.

Accuracy and precision

Precision data for the HPLC assay were obtained by repeatedly injecting a single sample over the period of a normal working day, and had a 95% confidence

TABLE I

HPLC ANALYSIS OF TACA (10% by wt.) IN RICE STARCH AFTER BLENDING

Values are expressed as a percentage of the theoretical value.

Sample	*Lot A*	*Lot B*	*Lot C*
1	107.1	106.5	102.1
2	106.3	104.7	103.7
3	105.7	105.5	102.0
4	102.4	104.9	101.0
Average	105.4	105.4	102.2
Theoretical	106.9	107.0	102.0

TABLE II

ANALYSIS OF TACA (10% by wt.) IN RICE STARCH POWDER BY METHANOL EXTRACTION FOLLOWED BY THE USP ASSAY

Values are expressed as a percentage of the theoretical value.

Sample	*Lot A*	*Lot B*	*Lot C*
1	103.4	105.0	101.2
2	102.9	105.0	103.0
3	101.6	101.7	99.0
Average	102.6	103.9	101.0
Theoretical	106.9	107.0	102.0

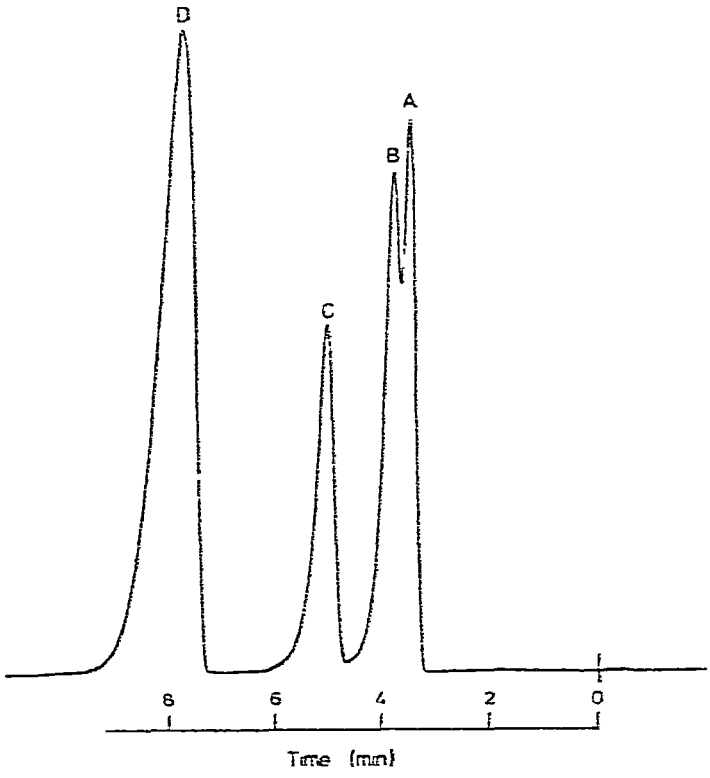

Fig. 1. HPLC chromatogram of TACA and related steroids on Zipax with dichloromethane as the mobile phase. A = Triamcinolone acetonide 11,21-diacetate and 11-acetate; B = triamcinolone acetonide 21-acetate; C = triamcinolone 16,21-diacetate; and D = TACA.

interval of $\pm$ 0.90%. A study of the accuracy was made on six rice starch samples which had been treated with TACA. The data obtained on analyzing these samples had a 95% confidence interval of $\pm$ 1.02%. The average percentage of TACA observed (100.2% of the theoretical value) indicates that the assay had no positive or negative bias.

The calibration graph was linear throughout the TACA concentration range of interest (0–1.4 mg/ml), and it had a zero intercept when monitoring at wavelengths between 240 and 280 nm.

Separation of related steroids

In Fig. 1 it can be seen that TACA is well separated from the 11β- and 21-monoacetates and 11β,21-diacetate, these being residual compounds that could remain after the steroid synthesis[3]. Also resolved, although not directly related to the analysis being considered, was triamcinolone 16,21-diacetate, another related compound commonly used in pharmaceutical formulations[4]. The most likely side-chain degradation product, the 21-aldehyde[5], was not eluted from the Zipax column under the conditions described in the Experimental section. This was demonstrated by oxidizing TACA with copper diacetate as described by Lewbart and Mattox[5] and

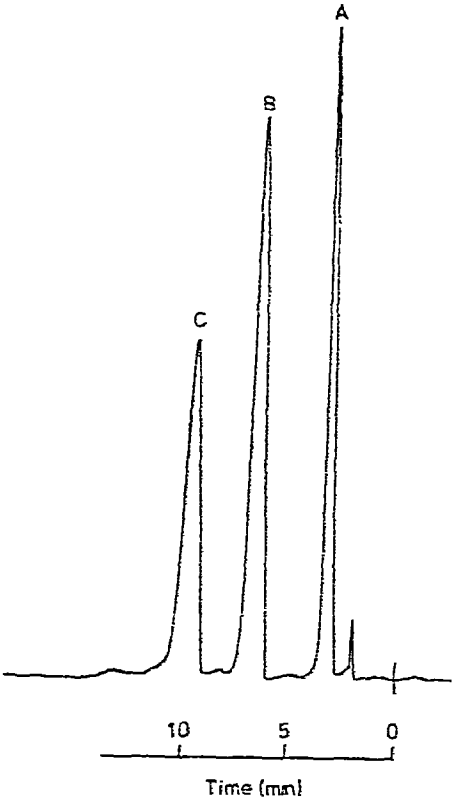

Fig. 2. HPLC chromatogram of mixed acetates on Zipax with 2-chloropropane as the mobile phase. A = triamcinolone acetonide 11,21-diacetate; B = triamcinolone acetonide 11-acetate; and C = triamcinolone acetonide 21-acetate.

following the reaction by HPLC. The 16α,17α-acetonide degradation to the *cis*-diol and eventual *d-homo* derivatives[6] resulted in products which were so polar that they were not eluted from the column under the conditions used in the analysis.

In Fig. 1 triamcinolone acetonide 21-acetate is poorly resolved and the 11-acetate isomer is not separated at all from the diacetate. These compounds can be separated on the Zipax column using water-saturated 2-chloropropane as eluent (Fig. 2); however, with such a non-polar solvent, the elution time of TACA was too long to be practical in routine analysis.

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