ISOTOPE DILUTION ASSAY FOR DEXAMETHASONE IN PHARMACEUTICAL PREPARATIONS

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The determination of 9-α-fluoro-16-α-methylprednisolone (dexamethasone) in drugs using isotope dilution method is described. Dexamethasone is tritiated with tritium-labelled acetic acid and in the course of the isotopic dilution the activity of tritium is measured with a liquid scintillator.

Isotope dilution methods, while not always practicable in routine analysis, merit consideration as a referee procedure in critical situations. This method has been applied to the analysis for the steroid dexamethasone (9-α-fluoro-16-α-methylprednisolone) in an ointment and an oil injection form. These applications are described below. The ointment assays were part of a stability study. The analysis of the oil injection form was performed to check the potency of a preparation of foreign origin.

Experimental

Labelled dexamethasone

The steroid was labelled with tritium by catalytic exchange between dexamethasone-21-acetate and 70% tritiated acetic acid for five days at 85°C in the presence of platinum catalyst.

The acetate was removed and purified by partition column chromatography. Details concerning the exchange condition and the chromatographic purification were described in the “Proceedings of the Second Euratom Conference on the Labelling and Storage of Marked Molecules”.\(^1\) The purified acetate was converted to the 21-alcohol by treatment with sodium methoxide in an atmosphere of nitrogen. The crystallized product had a specific activity of \(\sim 7 \mu\text{Ci/mg}\). Its absorption spectrum in methanol exhibited a \(\lambda_{\text{max}} = 238 \text{ m\mu}\) with an absorbance in 1% solution at 1 cm of 392 vs. a value of 390 which was found for pure material in these Laboratories. Chromatography on Whatman \#1 paper saturated with formamide and developed with benzene indicated identical mobilities for the tritiated and for authentic non-radioactive dexamethasone.

The stock tracer solution of radioactive dexamethasone was prepared in methanol at a concentration of \(\sim 0.1-0.2 \text{ mg per ml}\). One ml of tracer solution was added for isotope dilution assays.
Preparations analyzed

One sample was 0.1% dexamethasone alcohol in a gelled isopropyl myristate base which contained neomycin sulfate, citric acid (anhydrous), dibasic sodium phosphate (anhydrous), lanolin alcohols, white wax and isopropyl myristate in addition to the steroid. This ointment was measured after storage at room temperature and at 37°C to estimate stability of the dexamethasone contained therein.

The second preparation, labelled “Decadron” oil injection* was of foreign origin and of unknown base composition, and contained (label claim) 1 mg of dexamethasone phosphate disodium per ml. This is equivalent to 0.761 mg of dexamethasone alcohol per ml. This injectable preparation was also found to contain \( \approx 8\% \) benzyl alcohol.

Procedures

Approximately one gram of ointment containing \( \approx 1 \) mg of steroid alcohol \( (X) \) was equilibrated with 1 ml of tracer \( (Y \text{ mg}; A-\text{cpm}) \) solution and 25 ml of isooctane in a separatory funnel. The funnel was shaken until the ointment was completely dispersed. This dispersion was extracted with 25 ml of 1/1 methanol/water, discarding the isooctane phase, and the aqueous methanol washed twice with 25 ml isooctane. The methanolic solution was then extracted twice with 25 ml of methylene chloride. The latter extracts were combined and evaporated to dryness. The residue was dissolved in 10.0 ml of methanol and the radioactivity \( (B \text{ cpm}) \) and quantity of dexamethasone \( (Z) \) in this solution were determined as described below.

The oil injection was treated somewhat differently because the phosphate (disodium salt) was present, instead of the free alcohol and the solution contained considerable benzyl alcohol. The dexamethasone phosphate salt had first to be hydrolyzed to the alcohol. For this purpose, 1 ml of sample \( (X \text{ mg}) \) to which 0.2 ml of tracer solution had been added \( (A-\text{cpm}, Y-0.098 \text{ \mu g}) \) was incubated for 2 hours at room temperature with 3.4 mg of the enzyme, alkaline phosphatase (Worthington Biochemical Corp.), in 4 ml pH 9 borate buffer containing Mg\(^{++}\). This vehicle was only faintly colored and a simpler extraction procedure appeared feasible. The enzymatically treated sample was extracted with methylene chloride to remove dexamethasone, and this solution was washed four times with 5 ml water. The solvent was removed by evaporation and heating overnight at 52°C under vacuum to remove the benzyl alcohol.

The faintly greyish residue was dissolved in methanol, and purified further by chromatography on paper wet with propylene glycol as stationary phase and developed with toluene saturated with propylene glycol—methanol \( (1:1) \). Dexamethasone was eluted from the paper in methanol, and the UV absorption spectrum and radioactivity \( (B \text{ cpm}) \) of the eluate were determined. The amount of pure steroid present in the eluate \( (Z \text{ mg}) \) was calculated from the known extinction coefficient.

* Decadron\(^{\text{R}} \) is the trade name for the steroid with the generic name dexamethasone.

\(^{\text{R}}\) J. Radioanal. Chem. 1 (1968) 163—167