A trace enrichment high performance liquid chromatography technique for determining the dissolution rate of adrenocortical tablets

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Dissolution rate is a useful quality control test for many tablets and the concentration of active ingredient in the dissolution medium is often determined by ultraviolet spectroscopy; this technique is used in the U.S.P. XIX for adrenocortical tablets. The sensitivity of the procedure is however barely adequate for low content tablets. Thus with a 1 mg prednisolone tablet the maximum absorbance observed is about 0·05 when the 1 cm cell prescribed in the U.S.P. test is used. Interference can also be encountered from irrelevant absorption either derived from the tablet excipients or introduced during filtration of the dissolution medium.

When investigating dissolution methods for tablets containing 0·5 or 0·25 mg of betamethasone using water as the dissolution medium, it became clear to us that procedures based on ultraviolet spectroscopy lacked the necessary sensitivity and specificity. The steroid concentration in the dissolution medium was also too low for conventional high performance liquid chromatography techniques to be applied. When testing tablets containing fludrocortisone acetate Abdou et al (1978) overcame the problem by designing a miniaturized apparatus in which the volume of the dissolution medium was reduced from 900 ml to 30 ml.

It has been found, however, that adequate sensitivity can be achieved using the pharmacopoeial volume of dissolution medium by adopting the trace enrichment technique recently described for reversed-phase systems (Frei 1978; Krummen & Frei 1977; Kummert et al 1978; Little & Fallick 1975; Schauwecker et al 1977). In this procedure a large volume of a solution of the compound in a solvent that is distinctly more polar than the mobile phase used for chromatography is injected onto a reversed-phase column. The compound is fully retained during sample injection, but it is subsequently eluted as a compact peak when mobile phase is passed through the column.

The technique was found to be applicable to solutions of betamethasone in water when a 20 × 0·45 cm internal diameter column packed with 10 μm Spherisorb ODS was used at a temperature of 60 °C with aqueous methanol as the mobile phase and a flow rate of approximately 2 ml min^{-1}. The optimum concentration of methanol in the mobile phase varied between 40 and 55% v/v depending on the characteristics of the column used.

The chromatograph was constructed from a Haskel model number 28646 air driven pump (Olin Energy Systems Ltd., Sunderland), a Cecil 212 ultraviolet monitor (Cecil Instruments Ltd., Cambridge) and a Servoscribe 1s recorder (Smiths Industries Ltd., London). The temperature of the column was controlled with a water jacket and the solution was injected onto the column with a Rheodyne 7120 syringe loading injector (Phase Separations Ltd., Clwyd).

For a tablet containing 0·25 mg of betamethasone dissolved in 1000 ml of water (B.P. 1973, Addendum 1977), the injection of 1500 μl of the filtered solution gave a satisfactory chromatogram. The effect of injection volume on the peak height obtained was assessed by injecting approximately 10 to 1500 μl volumes of aqueous solutions of betamethasone using calibrated loops of different sizes. The betamethasone concentrations of the solutions used were adjusted so that approximately the same amount of betamethasone was injected onto the column for each size of loop. Peak heights per μg of betamethasone injected were calculated from the measured peak heights for each injection volume and the reduction in peak height per μg of betamethasone was found to be less than 10% when the injection volume was increased from 10 μl to 1500 μl.

Aqueous solutions containing betamethasone in the ranges 0·1 to 1 μg per ml (500 μl injection volume) and 0·05 to 0·5 μg per ml (1500 μl injection volume) gave a rectilinear relationship between concentration and detector response. When preparing the standard solution of betamethasone it was necessary to use methanol (0·1% v/v in the final solution) to dissolve the steroid and then dilute to volume with water. Incorporation of methanol at up to 2% of the total volume of the betamethasone solution did not affect the peak heights obtained significantly when a 1500 μl injection volume was used.

Injection of up to 1500 μl of water disturbed the base line of the chromatogram but this did not interfere with the steroid peak. Chromatograms of water filtered through a 25 mm diameter MF-Millipore 1·2 μm white plain filter showed several small peaks. The size of these peaks was reduced by rejecting the first 10 ml of filtrate and the next 10 ml of filtrate showed no peaks that interfered with the steroid peak. This technique provided a suitable means of clarifying the dissolution medium before chromatography. No interfering peaks were observed when placebo tablets were examined at ten times the test concentration for a tablet containing 0·25 mg of betamethasone.

The concentration technique was also found to be applicable to dilute aqueous solutions of dexamethasone, prednisone and prednisolone. Under the chosen conditions all four steroids had retention times between 3 and 4 min. Prednisone is suitable as an internal standard in the determination of betamethasone and

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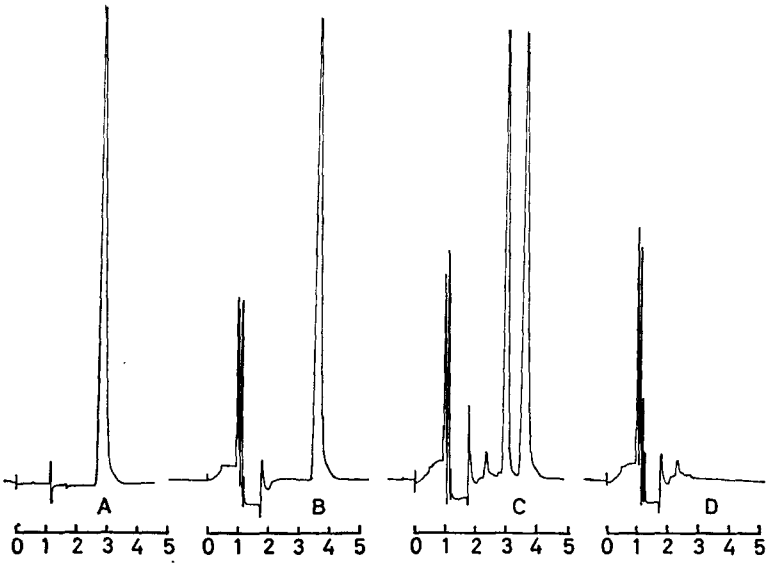

FIG. 1. Chromatograms of (A) betamethasone, 38 μg ml^{-1} in water; injection: loop, nominal 10 μl (B) betamethasone, 0·25 μg ml^{-1} in water; injection: loop, nominal 1500 μl (C) betamethasone, 0·25 μg ml^{-1} and prednisone, 0·15 μg ml^{-1} in water, filtered through MF-Millipore 1·2 μm as described in text; injection: loop, nominal 1500 μl (D) water, filtered through MF-Millipore 1·2 μm as described in text; injection: loop, nominal 1500 μl. Abscissa: time (min). Chromatographic conditions: column, 20 cm × 0·45 cm i.d. packed with 10 μm Spherisorb ODS, temperature 60 °C; mobile phase, 40% v/v methanol in water; flow rate, 2 ml min^{-1}; detection, u.v. at 254 nm, 0·05 absorbance unit full-scale.

dexamethasone; betamethasone is suitable as an internal standard in the determination of prednisone and prednisolone. However the reproducibility of injection was sufficiently precise to render the use of an internal standard unnecessary. Injection of (a) ten 200 μl volumes of water containing 1·1 μg of prednisone ml^{-1}, (b) ten 500 μl volumes of water containing 0·5 μg of betamethasone ml^{-1} and (c) ten 1500 μl volumes of water containing 0·25 μg of betamethasone ml^{-1} gave coefficients of variation of peak height of 0·6, 0·6 and 0·8% respectively.

In aqueous solution betamethasone shows an absorption maximum at 240 nm and initially the detector was set at this wavelength to achieve maximum sensitivity. Adoption of 254 nm as the detector wavelength enables a fixed wavelength detector to be used and also has the advantage that chromatograms show a smoother base line with a reduction in the size of the peaks due to the injection of water. The loss in sensitivity on changing from 240 to 254 nm was less than 10%with the detector used. Typical chromatograms are shown in Fig. 1.

The technique described provides a quick, sensitive and precise method for determining the steroid present in the dissolution medium when testing low content adrenocortical tablets for dissolution. The procedure has been found to be particularly useful when examining tablets where ultraviolet spectroscopic measurements are not sufficiently sensitive and specific.

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