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Note

High-performance liquid chromatographic determination of phosphate esters of dexamethasone and prednisolone and their sulphite adducts

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Corticosteroids are widely used in therapeutics in different formulations. During the preparation of a formulation containing corticosteroids and during its storage decomposition may occur. To prevent oxidative decomposition of corticosteroid esters in aqueous solution sodium metabisulphite is used as an antioxidant.

Decomposition of corticosteroids due to this added bisulphite has been reported for prednisolone[1,2] and dexamethasone[2]. Smith *et al.*[2] reported that addition of bisulphite took place at the A-ring system at C-1. When prednisolone was used both the 1α- and 1β-sulphonates were detected[2], but with dexamethasone only the 1β-sulphonate was detected[2]. This addition is illustrated for prednisolone in Fig. 1.

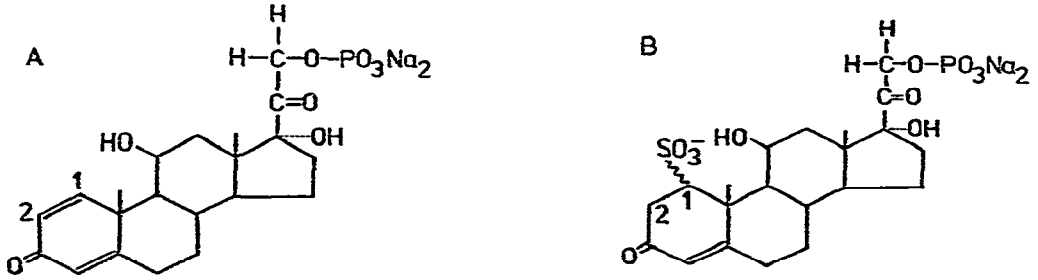

Fig. 1. Prednisolone sodium phosphate (A) and its bisulphite adduct (B).

Specific and sensitive methods are required to study the addition of bisulphite to corticosteroids in aqueous solutions and to determine the adducts formed and the remaining undecomposed corticosteroid phosphates in aqueous formulations. Therefore we developed an ion-pair reversed-phase high-performance liquid chromatographic procedure for the determination of the phosphate esters of dexamethasone and prednisolone and their respective sulphite adducts.

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MATERIALS AND METHODS

The chemicals used were of European Pharmacopoeia quality unless mentioned otherwise. Prednisolone sodium phosphate and dexamethasone sodium phosphate were of B.P. quality. PIC-A reagent (tetrabutylammonium phosphate in phosphate buffer) was purchased from Waters Assoc. (Milford, MA, U.S.A.).

A liquid chromatograph (Waters Assoc.), a stainless-steel column (30 cm × 3.9 mm I.D.) and a UV detector Model 440 (Waters Assoc.) were used. Detection was performed at 254 nm. The column packing comprised porous silica particles, permanently bonded to a monomolecular layer of organosilane (μBondapak C_{18}; Waters Assoc.). The mobile phase was methanol (analytical reagent grade)–water (1:1 v/v), containing 0.01 or 0.02 *M* PIC-A.

Peak areas were measured and computed with an Hewlett-Packard Type 3390 A integrator. The flow-rate was 1.5 ml/min and the sensitivity was 1 a.u.f.s. The temperature was 24°C. After dilution of aqueous solutions containing the corticosteroids and adducts formed, volumes of 5 μl and 20 μl respectively were injected.

Dilution appeared to have no influence on the equilibrium between the corticosteroid and the adducts.

RESULTS AND DISCUSSION

A chromatogram of an aqueous solution of dexamethasone sodium phosphate (peak 1) and the adduct formed (peak 2) is illustrated in Fig. 2A. A chromatogram of

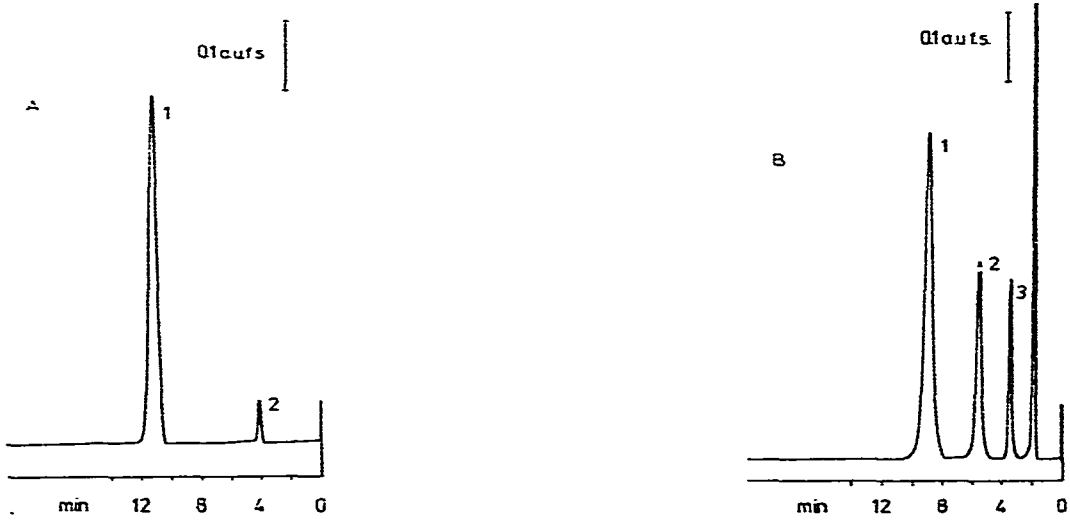

Fig. 2. A. HPLC of an aqueous solution of dexamethasone sodium phosphate (peak 1) (11 μg) and the adduct formed (peak 2) (0.6 μg). Solvent: 0.01 *M* PIC-A in methanol–water (1:1, v/v). Injection volume, 20 μl. Column: μBondapak C_{18} (30 cm × 3.9 mm I.D.). Temperature: 24°C. Flow-rate; 1.5 ml/min. Wavelength 254 nm. Sensitivity: 1 a.u.f.s. B, HPLC of an aqueous solution of prednisolone sodium phosphate (peak 1) (4.1 μg) and the adducts formed (peaks 2 and 3) (1.4 μg each). Solvent: 0.02 *M* PIC-A in methanol–water (1:1, v/v). Injection volume: 5 μl. Other parameters as in A.

prednisolone sodium phosphate (peak 1) and the two adducts formed (peaks 2 and 3) is shown in Fig. 2B.

Detailed studies on the addition of bisulphite to corticosteroids revealed that the molar absorption of the adducts at 254 nm was about 10% less than the molar absorption of the intact A-ring system. Consequently, peak area measurements should be corrected for these absorption differences.

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