Journal of Chromatography, 193 (1980) 160–162

CHROM. 12,650

Note

Analysis of dexamethasone sodium phosphate formulations by high-performance liquid chromatography

DENISE GAGNÉ and BRUCE A. LODGE

Drug Research Laboratories, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario K1A 0L2 (Canada)

(Received January 3rd, 1980)

Dexamethasone disodium phosphate has the same actions as dexamethasone, and is one of the most soluble of the adrenocorticosteroidal agents[1]. It is therefore very suitable for intravenous use, as an inhalent and in topical preparations, particularly ophthalmic formulations. In the *United States Pharmacopeia* (USP)[2], monographs for a cream, injection, ophthalmic ointment and ophthalmic solution are found. The analytical procedure for the ophthalmic preparations and the cream[2] is based on a modified Oorter–Silber reagent (0.065 g of phenylhydrazine dissolved in 40 ml of water, 60 ml of sulfuric acid and 50 ml of 2-propanol), and this method has presented considerable difficulties in our organization in the case of the ophthalmic solution, because of very low absorbance readings and poor reproducibility. There was therefore a need to develop an improved analytical procedure for this formulation and a study was made of high-performance liquid chromatographic (HPLC) methods. A recent publication[3] described the quantitative determination of dexamethasone sodium phosphate by HPLC without the use of an internal standard. The method described herein, on the other hand, uses an internal standard, a procedure which experience has shown to give better accuracy and precision.

EXPERIMENTAL

Apparatus

A Waters Assoc. liquid chromatograph was used. This consisted of a 440 absorption detector operating at 254 nm, a 6000A pump and a U6K septumless injector. The column was a μBondapak C_{18} (Waters; 30 cm × 3.9 mm I.D.) and peak areas were determined by a Hewlett-Packard Model 3370B integrator. The solvent filtering system was from Millipore.

Materials

The mobile phase was 0.07 *M* potassium dihydrogen phosphate–tetrahydrofuran–methanol (300:18:182) with a flow-rate of 1.5 ml/min. All solvents were HPLC grade (Fisher Scientific, Pittsburgh, Pa., U.S.A.), with the water being double glass distilled and deionized (house system).

The internal standard was diethyl phthalate (Aldrich, Milwaukee, Wisc., U.S.A.) —used as 1 mg/ml in mobile phase— and the reference standard was USP

reference standard dexamethasone phosphate (30 mg, accurately weighed, dissolved with the aid of 5 ml of 0.1 *M* sodium hydroxide and made up to 50 ml with mobile phase).

Procedure

Linearity of internal standard. The linearity of the internal standard was checked over the range 0.5–2.5 μg, and was found to be linear, with a correlation coefficient of 0.996.

Standard curve. The linearity of dexamethasone phosphate in this sytem was checked against the internal standard over the range 0.15–1.5 μg, and was found to be linear, with a correlation coefficient of 0.99992.

Reference standard solution for assays. To 2 ml of stock solution of USP dexamethasone phosphate was added 4 ml of internal standard solution. The mixture was made up to 10 ml with mobile phase.

Formulations. Formulations were prepared for chromatography as follows:

Ophthalmic solution. To a 1-ml aliquot was added 4 ml of internal standard solution, and the mixture was made up to 10 ml with mobile phase. Aliquots of 5 μl were injected into the HPLC, and likewise 5-μl aliquots of reference standard solution. The peak area ratio of drug to internal standard in the sample preparation was compared to the same ratio in the reference standard preparation.

Injection. A 1-ml aliquot of injection was diluted to 10 ml with mobile phase. From this solution, a 2.5-ml aliquot was taken, mixed with 4 ml of internal standard solution, and the mixture was diluted to 10 ml with mobile phase. Aliquots of 5 μl were injected into the HPLC, and comparison was made with the reference standard solution as for the ophthalmic solution (see above).

Inhalent. With an adapter attached, the cartridge was sampled 10 times into a closed container containing 4 ml of internal standard and 6 ml of mobile phase. A needle was inverted in the container seal to allow the propellant to escape. The cartridge was shaken between each sampling. Aliquots of 5 μl of this solution were injected into the HPLC, and comparison made with the reference standard as for the ophthalmic solution (see above).

RESULTS AND DISCUSSION

The retention times for the principal steroidal degradation products and other materials of interest such as excipients and commonly co-formulated drugs are shown in Table I, and the results of assays on commercial formulations are shown in Table II. Although the method was originally developed for the ophthalmic solution, its suitability for use in assaying the injectable preparation since such preparations are also aqueous solutions, and its applicability to the inhalent formulation, for which there is no compendial procedure, we also evaluated. In spite of a recent report[4] concerning the presence of excessive amounts (≈ 50%) of 17-ketone degradation products in commercial injections, there was no evidence of any such decomposition in the products examined by us, nor did they interfere with the HPLC method when deliberately added to the samples.

Comparison with compendial analytical procedures was impossible in the case of the ophthalmic solutions, since the obtained absorbances were so low (≈ 0.1) and

TABLE I

RETENTION TIMES OF DEXAMETHASONE DISODIUM PHOSPHATE, DIETHYL PHTHALATE AND OTHER COMPOUNDS OF INTEREST

Compound	*Retention time (min)*
Dexamethasone sodium phosphate	10.6
Diethyl phthalate	13.6
Xylocaine*	3.2
Benzyl alcohol**	3.8
Methyl paraben**	5.6
Ethyl paraben**	8.4
Propyl paraben**	18.2
Dexamethasone***	18.9
Neomycin sulphate* (not detectable at 254 nm)	
17-Ketone decomposition product***	19.7

* Active ingredient occasionally formulated with dexamethasone.
** Excipient used in formulations examined.
*** Impurity.

TABLE II

ANALYSES OF SOME COMMERCIAL DEXAMETHASONE PHOSPHATE FORMULATIONS

All relative standard deviations (RSD) are based on at least four replicate assays.

Formulation	*Content of dexamethasone phosphate*	
	Label claim (%)	*RSD (%)*
Ophthalmic solution	107.6	0.51
	110.7*	0.54
Injection	97.3	0.39
	100.3**	0.47
	97.0	0.83
Inhalation	99.9	0.51

* Formulations also contained neomycin sulphate.
** Formulation also contained xylocaine.

so erratic that no confidence could be placed in them. USP analyses[2] on the three injectable formulations gave results in the range 102–106%, but with a relative standard deviation of 3%, seemingly inferior to the HPLC procedure. The inhalation was included in the study to see if the technique could be applied to its analysis, and the preliminary results are certainly encouraging.

HPLC is therefore a suitable procedure for the analysis of dexamethasone sodium phosphate injection and ophthalmic solution, and also appears to be applicable to analysis of the inhalation.

REFERENCES

1 *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, Pa., 15th ed., 1975, p. 891.
2 *United States Pharmacopeia, XIXth revision*, Mack Publishing Co., Easton, Pa., 1975.
3 V. Das Gupta, *J. Pharm. Sci.*, 68 (1979) 926.
4 E. C. Juenge and J. F. Brower, *J. Pharm. Sci.*, 68 (1979) 551.