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Note

New, simple and rapid method to determine sodium oestrone sulphate in vagitories by reversed-phase paired-ion liquid chromatography

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Sodium oestrone sulphate is a steroidal drug that possesses, *inter alia*, proliferative activity in the endometrium. Combined with a bacterio- and mycostatic agent (*e.g.* broxychinoline), it has been indicated for the topical treatment of atrophic colpitis.

The *United States Pharmacopeia* describes[1] a method for the determination of piperazine oestrone sulphate in creams and tablets. This procedure involves a multi-step extraction and hydrolysis of the drug substance and subsequent measurement of free oestrone spectrophotometrically. This method is also applicable to the assay of sodium oestrone sulphate as oestrone after hydrolysis, but it is tedious and time-consuming to perform.

Other analytical methods for assaying conjugated steroids have been described, including high-performance liquid chromatography (HPLC). Most HPLC methods have been developed for biological fluids[2–5] and only a few for pharmaceutical preparations[6]. This paper describes a new reversed-phase paired-ion liquid chromatographic method for the determination of sodium oestrone sulphate in pharmaceuticals.

EXPERIMENTAL

Chemicals

The sodium oestrone sulphate was a bulk drug, and the content was assayed UV-spectrophotometrically after hydrolysis by comparison with the USP oestrone reference standard (H. Lando International, NY, U.S.A.). All chemicals were either analytical or liquid chromatographic grade. Methanol and acetonitrile were obtained from Orion Pharmaceuticals (Orion, Espoo, Finland). Waters PIC A ion-pairing reagent (tetrabutylammonium phosphate in phosphate buffer, pH 7.5) was purchased from Millipore Finland (Waters Assoc., Milford, MA, U.S.A.) and it was used according to instructions. HPLC solutions were filtered through 0.45-μm membrane filters (Sartorius Filters, Hayward, CA, U.S.A.) before use.

Chromatographic system

The liquid chromatograph used was Hewlett-Packard Model 1084B equipped

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with a variable-wavelength UV–VIS detector Model 79875A and an autosampler, Model 79842A (Hewlett-Packard, Palo Alto, CA, U.S.A.). A stainless-steel column (100 mm × 4.6 mm I.D.) packed with 5 μm octadecylsilane reversed-phase material (Hypersil ODS, Hewlett-Packard) was used. Water–methanol (43:57, v/v) containing PIC A was used as the mobile phase at a flow-rate of 1.0 ml/min. The absorbance of the effluent was monitored at 268 nm against a reference wavelength of 600 nm. The absorption maximum of sodium oestrone sulphate was determined by scanning the UV spectrum using stop-flow technique. The column was kept at a constant temperature of 40°C, and the injection volume was 50 μl.

Standard solution preparation

Approximately 25 mg of sodium oestrone sulphate, accurately weighed, were dissolved in 100 ml of methanol, and 4 ml of this solution were diluted to 50 ml with water–methanol (50:50, v/v) containing PIC A reagent.

Sample preparation

Five vagitories were crushed to a homogenous mass, from which two parallel determinations were performed. A portion of this mass, corresponding to *ca.* 1 mg of sodium oestrone sulphate, was accurately weighed and transferred into a 100-ml volumetric flask. Then 25 ml of acetonitrile–methanol (15:10, v/v) were added, in which the sample was dissolved by shaking and using an ultrasonic bath with gentle heat. Upon complete dissolution, 25 ml of water containing PIC A were added with shaking. The solution was cooled to room temperature with occasional shaking. The sample solution was then filtered through a filter paper before HPLC, because the vagitory base wax precipitates upon cooling.

Chromatographic procedure

Replicate injections of the standard solution were made to check the column equilibration. The coefficient of variation of detector response should agree with the reproducibility of the chromatographic system (coefficient of variation not more than 1.0%). Single injections of the sample solutions were made. The quantification of sodium oestrone sulphate in the samples was made according to the external standard calculation method used by the integrator.

RESULTS AND DISCUSSION

Sample preparation

Although the official USP assay[1] for piperazine oestrone sulphate in tablets is adequate for sodium oestrone sulphate in vagitories, the vagitory assay procedure is tedious and time-consuming. The method involves a lengthy shake-out technique by separatory funnel (five steps including seventeen shakings), leading to a hydrolysis step followed by UV spectrophotometric measurement of the free steroid. This process is especially inefficient for large numbers of samples, dosage uniformity tests and dissolution tests. With the HPLC procedure described here, the simplified sample preparation avoids laborious extraction and eliminates the use of harmful chloroform.

In this HPLC procedure, sodium oestrone sulphate as well as the vagitory wax

base dissolves in the warm acetonitrile–methanol mixture. When the aqueous–PIC A solution is added, the wax precipitates and filtration is necessary. Small fluoropolymer HPLC filters (Acro LC13, Gelman Sciences, MA, U.S.A.) were observed to adsorb sodium oestrone sulphate, so conventional paper filtration was chosen. The addition of aqueous PIC A to the sample solution was essential to achieve good peak symmetry in HPLC.

Chromatography

The use of tetrabutylammonium phosphate in the mobile phase prevents tailing of the sodium oestrone sulphate peak and makes possible good peak symmetry and precise quantification. The improvement of the peak shape is possibly due to the ion-pairing effect of tetrabutylammonium phosphate in the mobile phase. The retention time of sodium oestrone sulphate was *ca.* 2.4 min. Typical chromatograms of sodium oestrone sulphate standard and sample solutions are shown in Fig. 1.

Liquid chromatographic separations of conjugated oestrones have been described elsewhere. Reversed-phase columns with buffered mobile phases, normal-phase columns and anion-exchanger columns have been used mainly in biological applications[2–6]. Different modifiers such as ammonium sulphate, alkylamines, alkylsulphonic acids and cetyltrimethylammonium bromide have been used with reversed-phase columns showing an apparent ion-pairing effect[4,5]. In one study, conjugated oestrogens in tablet form have been separated by normal-phase partition chromatography[6].

Assay characteristics

The specificity of the HPLC method was tested by chromatographing vagitory base wax and broxychinoline (the other drug in the preparation) under the same conditions. No peaks were detected in the sodium oestrone sulphate area in the

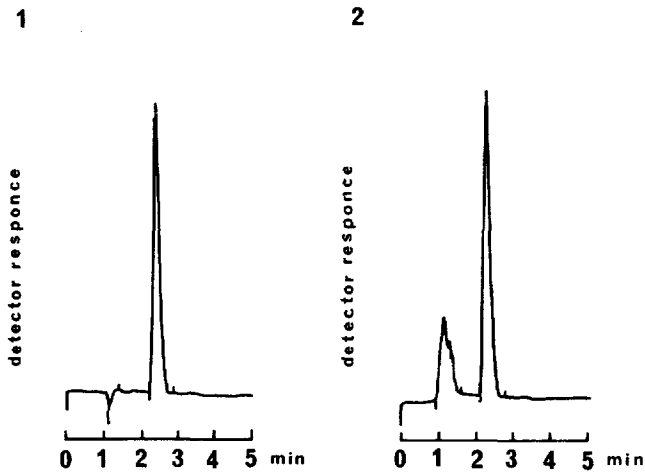

Fig. 1. A typical chromatogram of sodium oestrone sulphate standard (1) and sample (2) solution at a concentration of 20 μg/ml. Chromatographic system: Hypersil ODS 5 μm, 100 mm × 4.6 mm I.D. reversed-phase column; mobile phase, water–methanol (43:57, v/v) containing Waters PIC A ion-pairing reagent in phosphate buffer (pH 7.5); flow-rate, 1.0 ml/min; UV detection at 268 nm against 600 nm.

TABLE I

RECOVERY OF SODIUM OESTRONE SULPHATE IN SYNTHETIC VAGITORY PREPARATIONS

Batch	*Theoretical content*	*Found content (HPLC)*	*Recovery (%)*
1	1.81 mg/vagitory	1.80 mg/vagitory	99.0
2	1.83 mg/vagitory	1.77 mg/vagitory	96.9
3	1.87 mg/vagitory	1.81 mg/vagitory	96.7
4	0.76 mg/g of vagitory mass	0.77 mg/g of vagitory mass	101.2 (average 98.5)

chromatogram. The sodium oestrone sulphate peak diminished or disappeared when the standard solution was exposed to acidic, basic or oxygenating conditions.

The linearity of the HPLC method was shown in the range 0–30 μg/ml of sodium oestrone sulphate. The coefficient of correlation was 0.9999.

The precision of the method was demonstrated by the coefficient of variation of replicate standard and sample injections. The coefficient of variation for the standard solution was 0.96% (n = 11) and for separate sample solutions 1.48% (n = 7). Test concentrations of sodium oestrone sulphate were *ca.* 20 μg/ml.

The accuracy was demonstrated by assaying synthetic sodium oestrone sulphate vagitory masses and vagitories, and expressed as the recovery of sodium oestrone sulphate (see Table I). The detection limit of sodium oestrone sulphate was 20 ng per peak.

A comparison with the USP method[1] (piperazine oestrone sulphate in tablets) was made by assaying the same synthetic batches of sodium oestrone sulphate vagitories using both methods (see Table II).

Applications

The HPLC method reported here was applied to dissolution tests described in pharmacopeias. Depending on the dissolution medium, the solution to be injected into the chromatographic system must be changed to be as near as possible to the HPLC mobile phase composition to achieve good peak symmetry. Furthermore, a suitable standard concentration for the sodium oestrone sulphate solutions derived from the dissolution apparatus must be checked.

TABLE II

COMPARISON OF UV SPECTROPHOTOMETRIC AND HPLC ASSAY OF SODIUM OESTRONE SULPHATE IN SYNTHETIC VAGITORY PREPARATIONS

Batch	*UV spectrophotometry*	*HPLC*	*Percentage UV/HPLC*
1	1.83 mg/vagitory	1.80 mg/vagitory	101.7
2	1.74 mg/vagitory	1.79 mg/vagitory	97.2
3	1.70 mg/vagitory	1.80 mg/vagitory	94.4
4	1.79 mg/vagitory	1.77 mg/vagitory	101.1 (average 98.6)

This HPLC method is also applicable to dosage uniformity tests described in pharmacopeias, and it can also be used for other drug formulations by modifying the sample preparation step.

CONCLUSION

The reversed-phase paired-ion liquid chromatographic procedure reported here offers a specific, precise, accurate and rapid method for the determination of sodium oestrone sulphate in vagitories, and it is also applicable to other drug formulations. The sample preparation is easy and the rapid chromatographic analysis allows for determination of a large number of samples in a day.

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