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Note

Analysis of piperazine estrone sulfate in tablets by ion-pair high-performance liquid chromatography

G. CARIGNAN*, B. A. LODGE and W. SKAKUM
Bureau of Drug Research, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario K1A 0L2 (Canada)
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Piperazine estrone sulfate is a synthetic water-soluble estrogen conjugate which is more effectively absorbed than the other estrogens. It is used in the treatment of menopausal and postmenopausal symptoms.

The U.S.P. XX[1] introduced a reversed-phase high-performance liquid chromatography (HPLC) procedure for free estrone and the assay of piperazine estrone sulfate in raw material only. As piperazine estrone sulfate is a salt of the strongly acidic sulfate ester of estrone, it is an ideal candidate for ion-pair chromatography[2,3]. The addition of quaternary alkylammonium ions to the mobile phase increased the affinity of the estrone sulfate salt for the lipophilic stationary phase. Moreover, this offered an additional way to improved resolution by careful selection of the length of the alkyl chain of the counter ion as well as its concentration[4].

EXPERIMENTAL

Apparatus

A modular HPLC system was used, consisting of a pump (Constametric II, Laboratory Data Control, Riviera Beach, FL, U.S.A.) operated at 2.0 ml/min., a variable wavelength UV detector (Schoeffel Model SF770, Westwood, NJ, U.S.A.), set at 225 nm and loop injector adjusted for 7000 p.s.i. (equipped with a 10 μl loop) (Rheodyne Model 7120, Berkeley, CA, U.S.A.). The column (250 × 4.6 mm I.D.) was packed with totally porous irregularly shaped micro-silica particles to which an octadecyl group had been chemically bonded (RP-18; Brownlee Labs., Santa Clara, CA, U.S.A.).

Peak retention times and areas were obtained by the use of a reporting integrator (Automation System 3385A, Hewlett-Packard, Avondale, PA, U.S.A.).

Reagents

Piperazine estrone sulfate, estrone and 17β-estradiol were USP reference standards.

17α-Estradiol, methylparaben, ethylparaben, *n*-propylparaben, *n*-butylparaben (Sigma, St. Louis, MO, U.S.A.) and biphenyl (Aldrich, Milwaukee, WI, U.S.A.) were reagent-grade and used without further purification.

Sodium salts of equilin sulfate, 17α-estradiol sulfate and 17β-estradiol sulfate were buffered mixtures (Ayerst Labs, Montreal, Canada).

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Cetyltrimethyl ammonium bromide (CTMABr; Aldrich) was recrystalized from methanol–diethyl ether. Acetonitrile (Fisher Scientific, Fair Lawn, NJ, U.S.A.) was HPLC grade. Water was double-distilled in glass.

Mobile phase

CTMABr (0.003 *M*) in a 55% acetonitrile in phosphate buffer solution (pH 5.0, 0.02 *M*) filtered through a 0.2 μm membrane (F.H.O.2 μ; Millipore, Bedford, MA, U.S.A.) and degassed, was used.

Internal standard solution

A solution of biphenyl in mobile phase was prepared at a concentration of 0.1 mg per ml.

Standard preparations

Piperazine estrone sulfate standard solution was prepared in internal standard solution at a concentration of 0.3 mg per ml.

Estrone standard solution was prepared in internal standard solution at a concentration of 6 μg per ml.

Sample preparation

Not less than 20 tablets were weighed and finely powdered. An amount of powder equivalent to one tablet (3 mg of piperazine estrone sulfate) was accurately weighed into a 15-ml centrifuge tube equipped with a PTFE-lined cap. Internal standard solution (10.0 ml) was added. The tube was capped and vigorously shaken for 30 min. The tube was then centrifuged to obtain a clear solution.

Procedure

A 10 μl aliquot of piperazine estrone sulfate standard preparation, estrone standard preparation, and sample preparation were successively injected into the chromatograph. The peak area ratios of piperazine estrone sulfate and of estrone to the internal standard were calculated. The quantity of piperazine estrone sulfate and of free estrone per tablet were calculated using their respective formulas:

Piperazine estrone sulfate:

$$C_{u_2} = 10\, C_{s_2} \cdot \frac{R_{u_2}}{R_{s_2}} \cdot \frac{W_t}{W_u}$$

where C_{s_2} = concentration of piperazine estrone sulfate in standard preparation in mg/ml; R_{u_2} = area ratio of piperazine estrone sulfate in sample preparation; R_{s_2} = area ratio of piperazine estrone sulfate in standard preparation; W_t = average weight per tablet; W_u = weight of sample taken.

Free estrone:

$$C_{u_1} = 333\, C_{s_1} \cdot \frac{R_{u_1}}{R_{s_1}} \cdot \frac{W_t}{W_u}$$

where C_{u_1} = percent of free estrone; C_{s_1} = concentration of estrone in standard

preparation in mcg/ml; R_{u_1} = area ratio of estrone in sample preparation; R_{s_1} = area ratio of estrone in standard preparation; W_t = average weight per tablet; W_u = weight of sample taken.

RESULTS AND DISCUSSION

Complete resolution was achieved between free estrone, piperazine estrone sulfate, internal standard, preservatives (methylparaben and *n*-propylparaben) and coloring agent used in tablet formulation (Table I).

TABLE I

CAPACITY FACTORS OF PIPERAZINE ESTRONE SULFATE AND OF OTHER COMPOUNDS OF INTEREST

$k' = (t - t_0)/t_0$, where t = retention time of peak and t_0 = time for elution of a non-retained peak.

Compound	k'
Piperazine estrone sulfate	8.06
Sodium equilin sulfate	7.63
Sodium α-estradiol sulfate	6.45
Sodium β-estradiol sulfate	5.30
Estrone	2.78
α-Estradiol	2.16
β-Estradiol	1.95
Biphenyl	11.04
Methylparaben	0.85
Propylparaben	1.86
Coloring agent	4.54

Retention of the piperazine estrone sulfate ion-pair increased with ion-pair concentration while that of neutral compounds remained unchanged. Resolution of these latter was affected by acetonitrile concentration only. So, for a particular column, resolution could be adjusted by varying these two factors.

Although there was significant mobile phase light absorption (maximum at 212 nm, 1.4 a.u., against water) at 225 nm, the sensitivity was still better than at other wavelengths (269 nm and 280 nm for piperazine estrone sulfate and estrone, for UV maximum absorption, respectively). Amounts of estrone as low as 0.01 μg were easily detected and quantified.

Sample preparations were relatively stable, no significant changes being detected when solutions were re-assayed 24 h later.

Linear response *versus* concentration was determined for piperazine estrone sulfate (0–5 μg) and estrone (0–0.06 μg). Within the ranges studied, the standard curves passed close to the origin and their correlation coefficients were 0.9998 and 0.986, respectively.

Quantitative analysis of a commercial formulation gave results comparable to the U.S.P. procedure for piperazine estrone sulfate (98.2%, coefficient of variation

(C.V.) 0.46%, and 98.5%, C.V. 0.52%, respectively). The free estrone content (2.7%, C.V. 3.8%) was found to be slightly above compendial limit for raw material (2.0%); however, no limit exists for tablets. Good reproducibility was obtained as shown by the C.V. Single tablet analysis showed there was no content uniformity problem with the product.

The use of separate estrone standard solutions allowed the direct estimation of free estrone in the sample without any correction for the contribution of free estrone from piperazine estrone sulfate reference standard.

CONCLUSION

This HPLC procedure is fast and accurate. It has been specially designed for single dosage form analysis as required in U.S.P. content uniformity test.

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