

THE DETERMINATION OF OESTRADIOL DIPROPIONATE IN OILS

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A method of assay of oestradiol dipropionate in pharmaceutical preparations is described in which interfering substances are removed from the solvent oils by partition chromatography. Oestradiol dipropionate is determined as oestradiol after acid hydrolysis of the oil solution using the iron phenol reagent.

OESTRADIOL dipropionate although not one of the most widely used oestrogens, nevertheless is manufactured and administered in large enough quantities to make a precise chemical determination a useful tool in both control and analytical work.

Difficulties have been experienced in the determination of oestradiol dipropionate in pharmaceutical preparations by the method described in U.S.P. XVI in that it has not been possible to duplicate results. The problem seems to lie in the final step of the method when an extraction of the coloured solution is made with ethyl ether. It appears that varying amounts of ether dissolve in the reagent causing dilution so that different values are obtained.

Because of this it appeared necessary to develop an alternate procedure. Since oestradiol dipropionate is administered parenterally as an oil solution the problem seemed to be to separate the oestrogen from the oil without carrying along substances which might subsequently interfere. The difficulty in this was to find a solvent partition system which would effectively separate the oestrogen from the oil.

A second approach was then tried in which the oestradiol dipropionate was hydrolysed and the free oestradiol was determined. This appeared to be the preferable procedure and was therefore used in the development of a method for this steroid.

METHODS

Reagents

Chloroform—reagent grade; ethanol—absolute; isooctane—reagent grade; methanol—reagent grade; *n*-hexane—practical grade; light petroleum—ligroine, B.P. 30–60°, reagent grade; polyethyleneglycol 600—Carbowax 600, Carbide and Carbon Chemicals Co.; hydrochloric acid, concentrated—reagent grade; sulphuric acid—10 N; sulphuric acid—35 per cent; sodium hydroxide—N; Celite No. 545—Johns Manville; iron-phenol reagent—U.S.P. XVI, page 277.

Celite Column

The column used to remove interfering substances from the oil solvent is a modification of one used by Theivagt and Campbell (1959) to separate vitamin D from vitamin A in multivitamin mixtures.

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Five g. of Celite No. 545 is wetted with 25 ml. of isooctane and 2 ml. of polyethyleneglycol 600. The whole is vigorously stirred until the mixture is thick in consistency. It is then transferred to a column, 10 mm. in diameter, fitted with a coarse porosity fritted disc at one end, and firmly packed down. The column is then ready for use.

Preparation of Extract

A sample of oestradiol dipropionate in oil equivalent to 1.0 mg. of the steroid (1-2 ml.) is diluted with 5 ml. of *n*-hexane and quantitatively

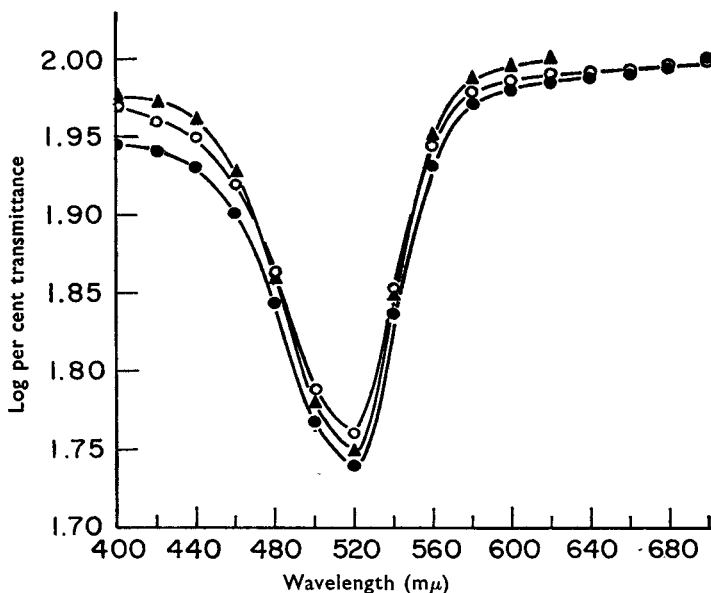


FIG. 1. Absorption curves for extract of hydrolysate of oestradiol dipropionate in sesame oil before and after column treatment.

- ▲ 15 mg. oestradiol.
- Oestradiol dipropionate in sesame oil 14.16 mg. oestradiol eq. before column.
- Oestradiol dipropionate in sesame oil 14.16 mg. oestradiol eq. after column.

transferred to a celite column prepared as described. The oily solution is washed into and through the column with small portions of *n*-hexane until a total of 75 ml. has been used. The eluate is collected 25 ml. at a time and evaporated in a 50 ml. round bottomed flask with a standard taper ground joint on the neck. The *n*-hexane is evaporated from the oil on a steam bath under a current of air. Ten ml. of methanol, 0.5 ml. of concentrated hydrochloric acid and two glass beads are added to the flask and the mixture is refluxed on a steam bath for 15 min. The condenser is removed and while the flask is still on the steam bath the methanol and a large part of the acid is driven off under a stream of air. When the oily residue stops bubbling and looks dry it is transferred to a 125 ml. separatory funnel with four 15 ml. portions of light petroleum.

The light petroleum solution is extracted five times with 10 ml. portions of N sodium hydroxide. The flask which contained the hydrolysate is rinsed with the portions of sodium hydroxide before adding to the separatory funnel. The combined alkaline extracts are acidified carefully with 10N sulphuric acid to pH 2-3 and allowed to stand for 1hr. After

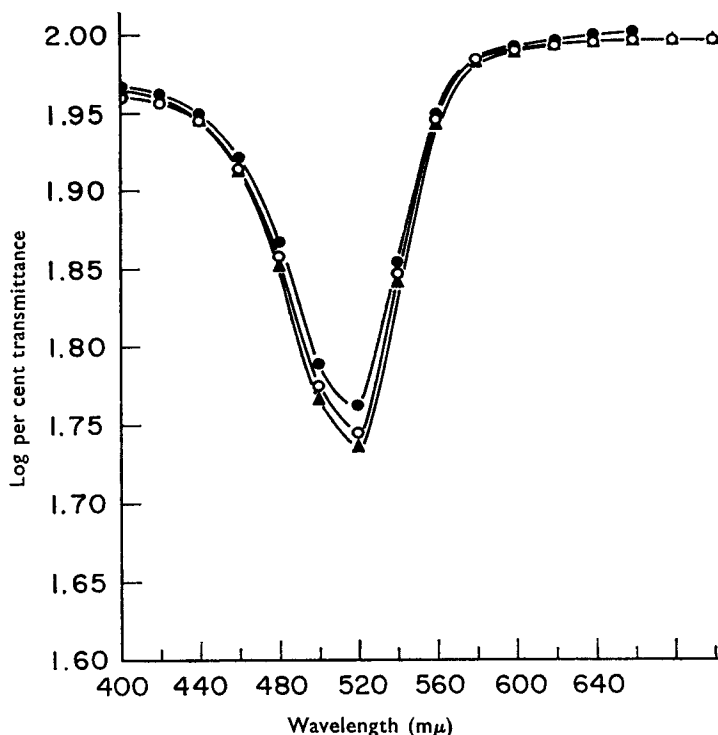


FIG. 2. Absorption curve for extract of hydrolysate of oestradiol dipropionate in corn oil before and after column treatment.

- ▲ 15 mg. oestradiol.
- Oestradiol dipropionate in corn oil to 14.16 mg. oestradiol eq. before column.
- Oestradiol dipropionate in corn oil to 14.16 mg. oestradiol eq. after column.

checking to make sure the mixture is still acid it is extracted with four 15 ml. portions of chloroform. The chloroform extracts are run into a 100 ml. volumetric flask and taken to dryness on a steam bath. The residue is then dissolved in ethanol and the flask is made up to volume with the same solvent. Aliquots of this solution are taken for the determination of oestradiol.

Determination of Oestradiol

Oestradiol is determined by the U.S.P. XIV method, parts of which have been modified to suit conditions in this laboratory.

An aliquot of the extract (prepared above) equivalent to 10-20 μg .

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oestradiol dipropionate is transferred to a 10 ml. volumetric flask and taken to dryness on a boiling water bath. Aliquots of a standard solution of oestradiol also in ethanol are taken so that 5, 10, 15 and 20 μg . of the steroid are placed in four separate flasks. These aliquots are also taken to dryness.

A blank is prepared by evaporating 1 ml. of ethanol in a 10 ml. volumetric flask and then carrying it through the same procedure as for the sample or standard.

To each flask is added 1.5 ml. of the iron-phenol reagent. The flasks are allowed to stand for 30 min. with frequent shaking and are then placed

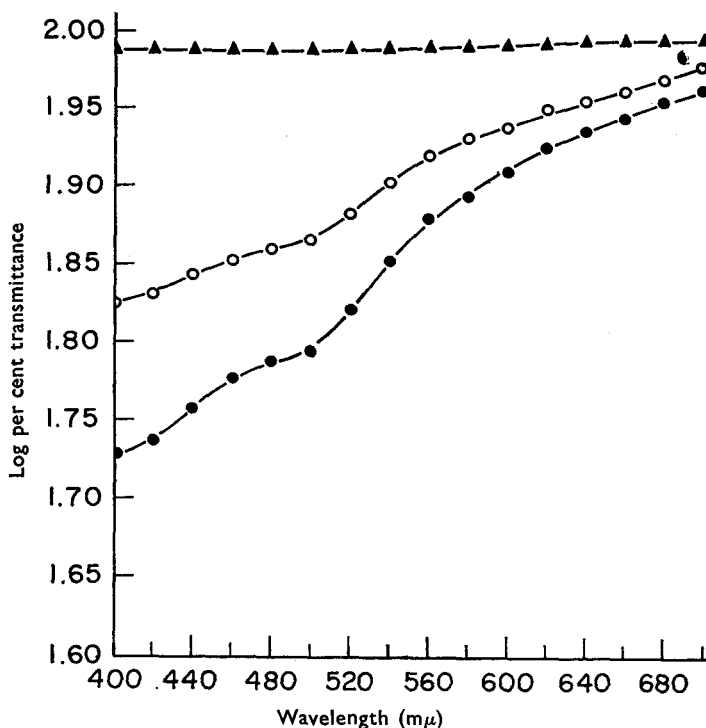


FIG. 3. Absorption curves for extract of hydrolysate of sesame oil before and after column treatment.

- 1 ml. sesame oil before column treatment.
- 2 ml. sesame oil before column treatment.
- ▲ 1 ml. sesame oil after column treatment.

in a boiling bath. After heating for 2-3 min. they are stoppered and after 5 min. are again well shaken. The heating is continued for 1 hr. At the end of this time the flasks are thoroughly cooled and 6 ml. of 35 per cent sulphuric acid is added to each. They are well shaken and after transferring to appropriate cuvettes are read against the blank in a Coleman Junior spectrophotometer at 520 $m\mu$.

The amount of oestradiol in the aliquot of the extract is found by comparison with the standard curve. It is then calculated in terms of oestradiol dipropionate by multiplying by a factor of 1.4117.

RESULTS AND DISCUSSION

Hydrolysis

In preliminary work it was found that alkaline hydrolysis caused saponification of the oil and troublesome emulsions occurred during the subsequent separation of oestradiol from the hydrolysate. With a change to acid hydrolysis employing hydrochloric acid this difficulty was overcome and the oestradiol was easily separated. However, when the amount of oestradiol present in hydrolysates of standard solutions was determined, variable recoveries in terms of oestradiol dipropionate were

TABLE I
RECOVERY OF OESTRADIOL DIPROPIONATE FROM OIL SOLUTIONS BEFORE AND AFTER COLUMN TREATMENT

Oil	Typical recovery per cent	
	Before column	After column
Sesame	115.1	102.0
	113.5	99.6
Corn	93.7	100.7
	94.3	101.3
Cottonseed	73.3	99.0
	97.0	100.6
Peanut	97.2	100.8
	99.0	102.4

found. These recoveries were higher than 100 per cent when sesame oil was used and usually lower with corn, cottonseed and peanut oils.

Removal of Interfering Substances

With an extract of the hydrolysate of oestradiol dipropionate in sesame oil an interfering colour developed which gave an absorption curve slightly different than that for standard oestradiol. However, if the sesame oil sample containing the oestradiol dipropionate is first put through the Celite column described above this interfering colour does not develop. The removal of this interference can be clearly seen in Fig. 1. This interference has previously been noted by Dracass and Foster (1943) when determining stilboestrol dipropionate in oily solution.

With corn, cottonseed and peanut oils a lower recovery than 100 per cent is generally found with an absorption curve that is identical in all respects with that of oestradiol. The curves shown for corn oil in Fig. 2, both before and after column treatment are typical of cottonseed and peanut oils also.

Apparently in these instances there is something in the oil which inhibits full colour development of the oestradiol with the iron-phenol reagent. When these oil solutions are put through the same column as that used with sesame oil the inhibitors are removed and it can be seen in Table I that a good recovery of oestradiol dipropionate is achieved.

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Typical recoveries of oestradiol dipropionate from commercial preparations are shown in Table II.

When aliquots of 1 and 2 ml. of sesame oil alone were put through the same procedure as for oestradiol dipropionate in oil there was definite colour formation with the iron-phenol reagent. If, however, the samples of oil were first put through the column it is clearly shown in Fig. 3 that the substances causing interfering colour formation were removed.

TABLE II
RECOVERY OF OESTRADIOL DIPROPIONATE IN MULTIPLE ASSAYS OF
COMMERCIAL PREPARATIONS

Preparation	Labelled	Found
A	1 mg./ml.	0.97 mg./ml.
		0.99 "
		0.98 "
		0.98 "
B	1 mg./ml.	1.06 "
		1.04 "
		1.00 "
		1.02 "

When the same procedure was carried out with corn, cottonseed and peanut oils there was no interfering colour formation either before or after column treatment.

These interfering substances are not artifacts produced by the acid hydrolysis of an oil solution since they are present to the same extent in aliquots of oil which have been extracted without being put through the hydrolysis procedure. Neither are they oxidation products from oil constituents since rancid oils do not contain any more of these interfering components than fresh oils.

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