density at the wavelength of each particular TC for a solution containing 1 mcg. of TC per milliliter, using a 1-cm. cell.

A standard calibration curve of ATC HCl was prepared in chloroform solutions. In the analysis of ATC HCl, a linear relationship over the range of 0.025–1.5 mg./50 ml. is obtained. The absorptivity for TC and ETC was determined in alkaline methanol at 366 mμ and found to be 3.29×10^{-2} as previously described (2). The determination of absorbances of the column chromatography eluates provided a quantitative spectrophotometric method for assaying ATC, EATC, TC, and ETC in solvent solutions when compared with standards treated in a like manner.

To test the accuracy of this column chromatographic procedure for TC mixtures, a known mixture was made of standards of ATC, EATC, TC, and ETC, which was analyzed by the column procedure with the recoveries shown in Table I.

In the two experiments performed, recoveries of 98.0–102.0% were obtained on TC mixtures. Quantitative analysis of synthetic mixtures of TC was achieved with high degree of accuracy (Table I) on the column of acid-washed diatomaceous earth treated with buffer consisting of 0.1 *M* EDTA, glycerin, and PEG 400 at pH 7.0. Therefore, this method has the advantage of determining the entire content of a TC mixture on a single chromatogram.

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Spectrophotometric Assay of Potassium Permanganate Tablets (USP XVII)

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Abstract □ Assay of potassium permanganate tablets (USP XVII) was accomplished by a spectrophotometric procedure. This method is comparable in accuracy to the USP XVII titrimetric procedure.

Keyphrases □ Potassium permanganate tablets—analysis □ Colorimetric analysis—spectrophotometer

The quantitative determination of potassium permanganate by a spectrophotometric method has been reported by Bastian *et al.* (1). The application of this method to the assay of USP XVII potassium permanganate tablets, as compared to the standard USP XVII titrimetric assay for potassium permanganate tablets (2), is reported in this article.

The spectrophotometric method reported here is comparable in accuracy to the USP XVII assay, but it is considerably simpler to do and results in an appreciable saving of time, labor, and materials.

EXPERIMENTAL

Apparatus—Spectra and absorbance measurements were made with a spectrophotometer[1] (slit width 5 Å). Matched cells with a 1-cm. optical path were used.

Reagents and Chemicals—Potassium permanganate solution (0.1 *N*)[2] was standardized against 0.1 *N* oxalic acid solution.[3] Potassium permanganate tablets USP XVII (300 mg.)[4] were assayed. All other reagents used where of the highest commercial grade available.

[1] Bausch and Lomb, model 505.
[2] Fisher Certified reagent.
[3] Fisher Certified reagent.
[4] Eli Lilly and Co., Indianapolis, Ind.

Table I—Absorbance of Known Potassium Permanganate Solutions

Concentration of $KMnO_4$, mg./l.	Absorbance
44.354	0.693
47.515	0.743
50.676	0.792
53.737	0.841
56.898	0.891
60.059	0.939
63.220	0.988

Procedure—Standardized solutions of potassium permanganate were prepared and their absorbances measured. Twenty 300-mg. potassium permanganate tablets USP XVII were weighed and finely powdered. An accurately weighed portion of the powder, 50–55 mg., was dissolved in water and diluted to 1 l. The absorbance of the solution was measured at 526 mμ and compared with the standards to determine its concentration. The same samples were also assayed according to the USP XVII titrimetric procedure.

RESULTS AND DISCUSSION

Absorbance readings for the standardized potassium permanganate solutions in the concentration range of 44–64 mg./l. were obtained (Table I). A graph of absorbance *versus* concentration was linear with a slope of 0.0156.

The percent potassium permanganate in the sample used is found from:

$$\% KMnO_4 = \frac{A}{0.0156 \times W} \times 100 \qquad \text{(Eq. 1)}$$

where A = absorbance, and W = weight in milligrams of the $KMnO_4$ sample.

USP XVII standards for potassium permanganate tablets contain not less than 95% and not more than 105% of the labeled amount of $KMnO_4$ for tablets of 300 mg. or more, and not less

than 93% and not more than 107% for tablets of less than 300 mg.

Determinations of five potassium permanganate 300-mg. tablet samples were performed. The mean percent of the labeled amount was 99.65 with a *SD* of 0.43 when assayed spectrophotometrically and 99.89 with a *SD* of 0.59 when assayed titrimetrically.

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Quantitative Separation of Free Estrogens by Liquid Partition Chromatography

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Abstract □ A mixture of structurally related estrogens containing estrone, equilin, equilenin, 17α-estradiol, 17α-dihydroequilin, and 17α-dihydroequilenin was separated by a partition column chromatographic system based on a lipophilic polydextran stationary support and a composite organic solvent. The chromatographic column yielded 25 theoretical plates per centimeter of column height at 0.6 ml./min. flow rate; column efficiency was studied as a function of solvent flow rate, and an inverse relationship between the two parameters was observed. Since the complete separation of the six estrogens required 10 hr., the column was shortened to yield a 3-hr. elution, which separated completely four of the estrogens with only partial overlap between estrone and equilin. The overlap between estrone and equilin was resolved quantitatively by specific colorimetric and fluorometric determinations. The method may be scaled up for preparative purposes, it is applicable to other steroids that are too labile for gas chromatography, and the same chromatographic column can be used repeatedly.

Keyphrases □ Estrogens, free—quantitative separation □ Chromatography, liquid partition—separation □ Fluorometry—analysis □ Colorimetric analysis—spectrophotometer □ UV spectrophotometry—analysis

The separation and quantitative determination of estrogens were studied by a number of investigators. The problem was already approached by gas chromatography (1, 2), TLC (3), and liquid chromatography (4–6). However, no quantitative chromatographic method applicable to an estrogen mixture containing closely related structures such as estrone, equilin, equilenin, estradiol, dihydroequilin, and dihydroequilenin was reported in the literature. Although this study is based only on the analysis of an arbitrary mixture of these synthetic free estrogens, the sulfate esters of these free estrogens are the principal ingredients of the naturally occurring estrogenic hormones (7, 8).

Another consideration was the need for a chromatographic procedure that would be applicable to the separation of structurally related free estrogens for preparative purposes. Liquid column chromatography is ideally suited for this purpose. Furthermore, such a system may also be applicable to the analysis of other steroid structures that are too labile for gas chromatography. For example, Vandenheuvel and Horning (9) observed that gas chromatography of C-21 steroids containing an α-ketol side chain led to side-chain cleavage. Other internal rearrangements of C-21 steroids were observed by Brooks (10). Such rearrangements could lead to complications in the quantitation and the

Table I—Elution Volumes of Free Estrogens as a Function of the Solvent System

Solvent System[a]	Estrone N[e]	Estrone El. Vol.[f]	Estrone Fr. Vol.[g]	Estrone Ovl. Vol.[h]	Equilin El. Vol.	Equilin Rel. Vol.[i]	Equilin Fr. Vol.	Equilenin El. Vol.	Equilenin Rel. Vol.	Equilenin Fr. Vol.	Estradiol[b] El. Vol.	Estradiol[b] Fr. Vol.	DHEQ[c] El. Vol.	DHEQ[c] Fr. Vol.	DHEQN[d] El. Vol.	DHEQN[d] Fr. Vol.
A	1770	88	12	4.5	97	1.10	14	116	1.32	24						
B	1090	80	13	5.5	88	1.10	14	104	1.30	22						
C	910	75	17	8.5	83	1.11	16	96	1.28	20	161	24	185	26	230	32
D	1500	112	18	3.0	127	1.12	18	164	1.45	28	226	32	269	36		
E	700	71	18	9.0	80	1.13	18	99	1.40	19	138	31	159	33	211	46
F	950	62	16	6.0	70	1.13	12	84	1.36	20	111	22	132	26	160	40

[a] Solvent system: A = cyclohexane–xylene–methanol–triethylamine (400:400:75:5); B = cyclohexane–toluene–methanol–trimethylamine (400:400:75:5); C = cyclohexane–benzene–methanol–trimethylamine (400:400:75:5); D = cyclohexane–benzene–methanol (500:150:75); E = cyclohexane–benzene–methanol (500:150:75); and F = cyclohexane–benzene–methanol–trimethylamine (500:150:75:5). Column height for Systems A, B, C, and D = 65 ± 5 cm.; and column height for Systems E and F = 45 ± 2 cm. All elutions were carried out at 0.6 ± 0.1 ml./min. flow rate. All volumes except the relative volume are expressed in milliliters. [b] 17α- or 17β-Estradiol. [c] 17α- or 17β-Dihydroequilin. [d] 17α- or 17β-Dihydroequilenin. [e] Theoretical plate number; based on elution volume and peak width at half peak height. [f] The elution volume of a given estrogen at its maximum concentration. [g] The total volume of eluent containing a given estrogen fraction. [h] The overlap volume between estrone and equilin. [i] The elution volume of a given estrogen relative to the elution volume of estrone (estrone relative volume = 1).