the meclizine peak area to the dinonyl phthalate peak area in the standard solution before and after sample injections.

RESULTS AND DISCUSSION

A typical chromatogram of meclizine and dinonyl phthalate is shown in Fig. 1. Figure 2 shows the linear relationship of various amounts of meclizine to a constant amount of dinonyl phthalate.

The accuracy and precision of the method were tested by the following experiment. Two weights of a placebo blend in which known quantities of meclizine hydrochloride had been added were assayed per day for 3 consecutive days. The average recovery (Table I) was 100.4%. The estimate of precision (Table II) for injection to injection within a weight on a day, excluding variability due to days and weights, was ±1.7%. The standard error for the average of three injections is ±2.4%.

Meclizine hydrochloride yields a symmetrical peak, identical with meclizine base. A possible explanation for this phenomenon is that two HCl molecules are thermally removed from the meclizine molecule inside the injection port (16). By taking advantage of this effect, sample preparation is simplified since a two-phase liquid extraction step is eliminated.

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Spectrophotometric Determination of Thyroxine Iodine in Thyroid Preparations

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Abstract □ An improved quantitative determination of thyroxine iodine in USP thyroid preparations is described. The iodine-containing proteins are dissolved in 8 *M* urea and separated from cell debris and excipient materials by centrifugation and filtration. One aliquot of the clear yellow filtrate is adjusted to pH 9.0, and a second aliquot is adjusted to pH 5.0. The difference in absorbance between the two is observed at 325, 350, and 360 nm. These values are inserted into an equation utilizing the appropriate absorptivities of the iodoamino acids and dilution factors, and the amount of thyroxine iodine is calculated. The relative standard deviation of the method is ±6.0%. Data are presented to demonstrate the application of this method to several thyroid formulations, thyroid powder USP, tablets, and enteric-coated tablets. Correlation with biological data is shown.

Keyphrases □ Thyroxine iodine—UV titrimetric analysis in thyroid preparations □ Thyroid preparations—UV titrimetric analysis of thyroxine iodine □ UV spectrophotometric titration—analysis, thyroxine iodine in thyroid preparations

Thyroxine contained in pure proteins can be determined by spectrophotometric titration, taking advantage of the unique ionization and absorption properties of the iodoamino acids (1, 2). Various other methods to measure chemically both thyroxine and triiodothyronine (liothyronine) in pharmaceutical thyroid preparations have been described (3–13). These methods involve: chemical or enzymic hydrolysis of the thyroproteins; extraction of the released iodoamino acids; separation of these acids by paper chromatography, TLC, or gel permeation chromatography; and quantitation by iodometry. The methods are time consuming and the stability of the iodoamino acids in solution also has been a problem (14–16). The USP

Table I—Precision of Measurement of Thyroxine in Thyroid Preparations

Preparation	Average Value	Range	*RSD*, %
Powder USP, mcg./g.	207.0	191.0–222.0	±6
120-mg. tablets USP, mcg./tablet	24.2	22.7–25.3	±5
200-mg. enteric-coated tablets USP, mcg./tablet	32.8	30.2–33.9	±6

Table II—Thyroxine Iodine and Total Organic Iodine Contents of Thyroid Preparations from the Same Starting Material

Preparation	Thyroxine Iodine, mcg./Tablet Spectrophotometric	Bioassay[a]	Total Organic Iodine, mcg./Tablet
120-mg. enteric-coated tablets USP	22.7	30.8	253.0
60-mg. enteric-coated tablets USP	12.0	14.9	120.0
120-mg. tablets USP	22.5	29.5	242.0
60-mg. tablets USP	11.5	14.9	120.0
30-mg. tablets	6.1	7.2	61.5
Precursors to above preparations:			
Powder USP	209.0 mcg./g.	258.0 mcg./g.	2120.0 mcg./g.
Desiccated crude material	NA[b]	914.0 mcg./g.	7500.0 mcg./g.

[a] Bioassay values were calculated by reducing the value obtained for the desiccated crude material in proportion to the total organic iodine. [b] NA = not available.

XVIII method measures the total iodine content (17), which is not truly reflective of the physiological activity of the preparation (10, 18, 19).

This report presents the application of spectrophotometric titration to pharmaceutical thyroid preparations. The data are compared with data obtained by bioassay. The rapidity of the method and the parallel data to bioassay should make this a useful method for control of thyroid preparations in conjunction with biological or chemical evaluation of the raw material.

EXPERIMENTAL

Thyroxine Iodine—An amount of the thyroid preparation, equivalent to 500–1000 mg. of USP material, is transferred to a centrifuge tube. The material is suspended in 10 ml. 8 *M* urea containing 1 *M* guanidine hydrochloride, as described by Covelli *et al.* (2), and is mixed intermittently for 1 hr. The tubes are then centrifuged for 15 min. at 2000 r.p.m. The yellow, slightly turbid supernate is filtered with gentle suction through a Whatman glass fiber pad on a Gooch crucible with the aid of a bell jar microfiltration assembly. The clear yellow liquid is collected in a small conical flask. Equal amounts (2 ml.) of the filtrate are pipeted into each of two test tubes. To one tube an equal volume of 8 *M* urea–1 *M* guanidine hydrochloride, buffered to pH 5.0 with 0.2 *M* acetate, is pipeted. To the other tube an equal volume of 8 *M* urea–1 *M* guanidine hydrochloride, buffered to pH 9.0 with 0.2 *M* tromethamine, is similarly added. After mixing, the solutions are transferred to matched silica cells and the difference in absorbance (ΔA) between them is measured at 325, 350, and 360 nm.

The concentration of thyroxine iodine in the cell is given by the following equation[1]:

$$\text{mcg. thyroxine iodine/ml.} = \frac{(\Delta A_{350} - \Delta A_{360})5960 - \Delta A_{325} \times 280}{10.6 \times 10^3} \times 507.6 \frac{\text{mg. iodine}}{\text{mmole thyroxine}} \quad \text{(Eq. 1)}$$

The amount of thyroxine iodine per unit of the thyroid preparation is obtained by applying the appropriate dilution factors.

The thyroid preparations tested were: desiccated crude thyroid glands, thyroid powder USP, ground tablets, and enteric-coated tablets (decoated with dilute acid and then cracked to expose the interior).

[1] For the derivation of this expression, see *Reference 2.*

Table III—Thyroxine Iodine and Total Organic Iodine of Thyroid Preparations at Various Stages of Manufacture

Preparation	Thyroxine Iodine—Spectrophotometric	Thyroxine Iodine—Bioassay	Total Organic Iodine
A:			
200-mg. enteric-coated tablets USP, mcg./tablet	33.0	44.0	380.0
Powder USP, mcg./g.	178.0	232.0	2000.0
Desiccated crude material, mg./g.	680.0	927.0	8000.0
B:			
200-mg. enteric-coated tablets USP, mcg./tablet	35.0	51.5	380.0
Powder USP, mcg./g.	213.0	292.0	2200.0
Desiccated crude material, mcg./g.	NA[a]	983.0	7400.0
C:			
Powder USP, mcg./g.	209.0	333.0	2100.0
Powder USP, mcg./g.	198.0	333.0	2100.0
Desiccated crude material, mcg./g.	NA	1250.0	7900.0

[a] NA = not available.

Bioassay—Bioassay was performed by either a mouse anoxia method (20) or a rat goiterogenic method (21).

Total Iodine—Total iodine was determined by the USP XVIII method (17).

RESULTS AND DISCUSSION

To obtain the precision of the method, five replicate analyses were run on composite samples of the various thyroid formulations. The results are shown in Table I.

Tablets and enteric-coated tablets of various dose sizes, prepared from the same lot of desiccated crude thyroid, were assayed (Table II). As expected, the spectrophotometric results are consistently lower than biological activity calculated from the value measured for the desiccated crude material in proportion to the total iodine content of the tablet or enteric-coated tablets.

Table III also displays the relation between thyroxine iodine and bioassay values. The first line in each section shows the values obtained or calculated for the preparation. The material represented on the first line was made from the powder represented on the second line which, in turn, was made from the desiccated crude material shown on the third line.

The presence of triiodothyronine is ignored by this method, since it is not measured spectrophotometrically (1, 2). This compound is reported to be at least five times as active as thyroxine (22). The deviation between the spectrophotometric and bioassay values in Tables II and III can thus be accounted for by an amount of triiodothyronine representing 5–10% of the thyroxine level.

This method may prove useful as a control assay in the manufacture of thyroid formulations. It is probably best used in conjunction with biological or chemical assay of the desiccated crude thyroid glands entering the process as raw material.

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GLC of Cycloserine

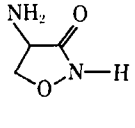
DAVID L. SONDACK▲, FRANK E. GAINER, and HAROLD J. WESSELMAN

Abstract □ A GLC assay is described for cycloserine (D-4-amino-3-isoxazolidinone). The sample is dissolved in pyridine–water (2:1), an aliquot is evaporated, and the residue is treated with *N,O*-bis(trimethylsilyl)acetamide and trimethylchlorosilane. After heating for a few minutes, chromatography is performed using a column containing methyl vinyl silicone, a relatively nonpolar liquid phase. Favorable quantitation is achieved by using hexamethylbenzene as the internal standard. The observed peak is the *N,N'*-bistrimethylsilyl derivative of cyloserine. The procedure eliminates interference from the cycloserine dimer, which does not chromatograph under these conditions.

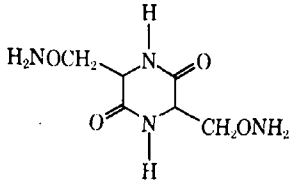
Keyphrases □ Cycloserine—GLC analysis using trimethylsilyl derivatives □ GLC—analysis of cycloserine trimethylsilyl derivatives □ Trimethylsilyl derivatization—GLC analysis of cycloserine

Cycloserine (D-4-amino-3-isoxazolidinone, I) equilibrates with its dimer, (+)-*cis*-3,6-bis(aminoxymethyl)-2,5-piperazinedione (II) even in the solid state (1, 2). In solution, pH extremes cause degradation of the dimer to 3,6-dimethylene-2,5-piperazinedione and to β-aminoxyalanine (3, 4). At or near neutral pH, dimerization predominates (4).

Quantitative determination of cycloserine in pharmaceutical preparations has been done by the nitroprusside method (5), titrimetrically and by UV spectrophotometry (6), or microbiologically against suitably susceptible microorganisms. The chemical treatment and solvents required for these assays cause formation of degradation products (1–4), which may interfere with the assay or give inaccurate results. This report presents a more specific GLC method for the quantitative analysis of cycloserine.



I



II

EXPERIMENTAL

Equipment—A gas chromatograph[1], equipped with a flame-ionization detector, was used. The detector signal was fed to a computer[2] for peak integration and to a 1-mv. recorder[3] with a chart speed of 15 in./hr. and a 1-sec. fullscale response. Samples were injected with a 10-μl. syringe[4]. Helium was used as the carrier gas, and electrolytic hydrogen and oxygen were used in the detector. The stationary phase was 3.8% UC W98 on Diatoport S[5] (80–100 mesh) packed in borosilicate glass columns 0.91-m. × 0.64-cm. o.d. All chemicals used were reagent grade or the best quality available.

Operating Conditions—The column was operated isothermally at 115° with the detector block and injection port at 150°. The helium flow rate was 55 ml./min. with an inlet pressure of 40 p.s.i. The electrometer range was 100 with an attenuation of 32. Sample injections of between 1 and 5 μl. were made.

Internal Standard—Hexamethylbenzene, 1 mg./ml., in chloroform was used.

Cycloserine Analysis—Solutions of cycloserine (I) reference standard or samples were prepared with pyridine–water (2:1) mixed prior to use and cooled to room temperature. Enough material was dissolved to provide a 1-mg./ml. solution. After mixing, excipient material was allowed to settle for 15 min. A 1-ml. aliquot of each was transferred by pipet to small screw-capped vials with Teflon septa. A stream of filtered dry air was used to evaporate the solvent. A 1-ml. portion of the internal standard solution was pipeted into each vial. The caps were screwed on firmly, and 100 μl. of *N,O*-bis(trimethylsilyl)acetamide and 10 μl. of trimethylchlorosilane[6] were added with a tuberculin syringe. After heating the vials for 10 min. on a heating block at 80°, the samples were ready for chromatography.

UV Spectra—Solutions of cycloserine reference standard and of 3,6-bis(aminoxymethyl)-2,5-piperazinedione (II) were prepared

[1] Hewlett-Packard model 402.
[2] IBM 1800.
[3] Honeywell Electronik 16.
[4] Hamilton No. 701.
[5] Hewlett-Packard, Avondale, Pa.
[6] Pierce Chemical Co.