Chloroform quantitatively elutes ergocalciferol from the column. The trifluoroacetic acid colorimetric method recommended by Clements *et al.* (15) was chosen due to its simplicity and obvious advantages over the antimony trichloride method.

Procedure B was also applied to commercially available tablets containing milligram quantities of ergocalciferol. The results obtained (Table II) were compared with those obtained for samples from the rat bioassay (17) and the USP XVIII chemical method (7). Recoveries of reference standard ergocalciferol added to the stabilized powder in quantities of 0.3 and 3.0 mg for each gram of powder were 99 and 97%, respectively, which are within experimental error of the assay.

The results of Procedure B (Table II) are mostly slightly higher than those of the USP chemical method. A loss through the long procedure of saponification, solvent–solvent extraction, and column chromatography in the latter method is a possibility. The biological assay gave higher results than Procedure B for most samples. Procedure B (average deviation ≤2.6%) takes only 1.5 hr for completion; for quality control purposes, it is a suitable assay for high potency ergocalciferol preparations.

SUMMARY

Ergocalciferol could be assayed when present in microgram quantities in tablets by refluxing followed by column chromatography and differential spectroscopy using trifluoroacetic acid and hydrogen peroxide. The simplicity and accuracy of Procedure A make it adequate for quality control analysis. Preparations containing milligram quantities of ergocalciferol could be assayed for their ergocalciferol content (Procedure B) by partition/extraction chromatography followed by spectrophotometry using trifluoroacetic acid.

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Quantitative Determination of Phenol in Phenolated Calamine Lotion USP

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Abstract □ A method for the quantitative analysis of phenol in phenolated calamine lotion USP is described. The method is based on spectrophotometrically measuring the color produced by reacting phenol with either ferric chloride or ferric nitrate. Beer's law is followed. The effect of ferric-ion concentration on the sensitivity of the assay method is reported.

Keyphrases □ Calamine lotion, phenolated—colorimetric analysis of phenol □ Phenolated calamine lotion—colorimetric analysis of phenol

Phenolated calamine lotion is an official USP (1) product. Although phenol is a potent therapeutic agent, the USP does not require its quantitative analysis in phenolated calamine lotion.

Kiposki and Allen (2) reported a bromometric method for the analysis of phenol. Stanko and DeKay (3) recommended a complicated colorimetric method for the quantitative analysis of phenol in phenolated calamine lotion based on reaction with copper sulfate. This report describes a simple colorimetric assay method for the analysis of phenol in phenolated calamine lotion USP. The method is based on an identification test with ferric chloride as described for phenol USP (4).

EXPERIMENTAL

Reagents and Chemicals—All chemicals and reagents used were USP, NF, or ACS grade. $FeCl_3 \cdot 6H_2O$[1], $Fe(NO_3)_3 \cdot 9H_2O$[2], and phenol loose crystals[3] were purchased and used without further purification.

[1] Mallinckrodt Chemical Works.
[2] J. T. Baker Chemical Co.
[3] Matheson, Coleman and Bell.

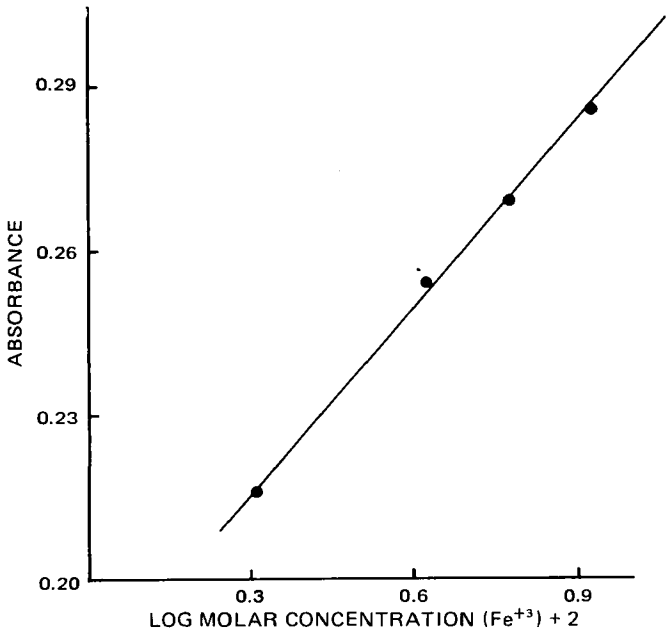

Figure 1—*Plot of log ferric-ion concentration* versus *absorbance.*

Table I—Assay Results on Phenolated Calamine Lotion and Calamine Lotion USP

Assay Number	Results, %	
	Phenolated Calamine Lotion	Calamine Lotion
1	100.2	0.0
2	100.5	0.0
3	100.2	0.0
Average deviation ± 0.13%		

Preparation of Solutions—All solutions of phenol, ferric chloride, and ferric nitrate were prepared (w/v) in distilled water using a simple solution method.

Preparation of Phenolated Calamine Lotion USP—A phenolated calamine lotion was prepared according to the USP (1).

Selection of Wavelength of Maximum Absorption—A 2.0-ml quantity of phenol solution (0.5%) was mixed with 2.0 ml of ferric chloride solution (4%), and the mixture was brought to volume (10.0 ml) with distilled water. The solution was scanned in the visible range using a spectrophotometer[4], and a broad peak was recorded between 528 and 556 nm. For further investigations, a wavelength of 550 nm was selected.

Effect of Ferric-Ion Concentration on Sensitivity of Assay Method—A 2.0-ml quantity of phenol solution (0.05 *M*) was mixed with 2.0, 4.0, 6.0, or 8.0 ml of ferric chloride solution (0.1 *M*), and the mixture was brought to volume (10.0 ml) with distilled water. The absorbance of the solution was measured at 550 nm against a reagent blank, prepared by substituting distilled water for phenol solution (Fig. 1).

Preparation of Calibration Curves—A 2.0-ml quantity of ferric chloride[5] solution (either 2 or 4%) was mixed with 2.0, 2.5, 3.0, 3.5, or 4.0 ml of phenol solution (0.5%), and the mixture was brought to volume (10.0 ml) with distilled water. The absorbance of each solution was measured as already described (Fig. 2).

Assay Procedure for Phenolated Calamine Lotion USP—A 10.0-ml quantity of phenolated calamine lotion was transferred on a filter paper. The filtrate was collected in a suitable container, and the precipitate on the filter paper was washed with 3 × 10-ml portions of distilled water. The precipitate was then rinsed with small portions of distilled water to bring the filtrate to volume (50.0 ml). It was mixed and refiltered when necessary. Then 8.0 ml of the clear filtrate was mixed with 2.0 ml of ferric chloride solution (4%), and the absorbance was measured at 550 nm as already described. The results, calculated by using the standard curve, are presented in Table I.

[4] Beckman model DB.

[5] Ferric nitrate may be substituted for ferric chloride on a mole per mole basis.

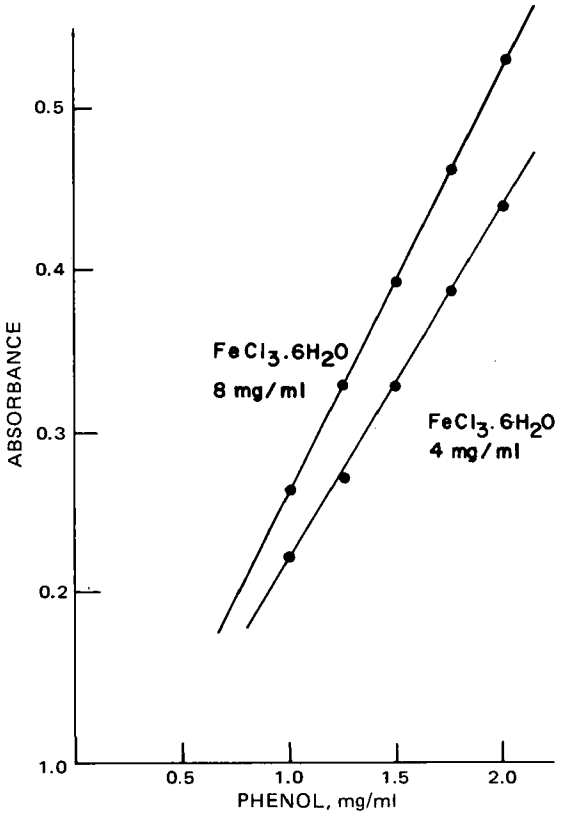

Figure 2—*Standard curves for phenol.*

Interference from Other Ingredients—The described procedure was repeated using calamine lotion USP. The results are presented in Table I.

DISCUSSION AND CONCLUSIONS

The results (Table I) show that phenol can be assayed in phenolated calamine lotion USP using a simple method (reaction with ferric chloride or ferric nitrate). Beer's law is followed (Fig. 2) at different concentrations of ferric ion. The concentration of ferric ion may be increased for more sensitivity in the assay technique, and the blank value is increased also. The log of the ferric-ion concentration is linearly related to absorbance (Fig. 1).

The reaction between phenol and ferric ion may be an oxidation–reduction or the formation of a complex. There is no interference from the other ingredients (Table I). It is important to keep the ferric-ion concentration constant since the sensitivity is dependent on this factor (Fig. 1). A 4.00% solution of ferric chloride is recommended for routine use for the quantitative analysis of phenol in phenolated calamine lotion USP.

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