Colorimetric Determination of Sterols in Vegetable Oils with Chloramine T and Sulfuric Acid Reagent

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ABSTRACT

A new colorimetric method for determination of sterols in vegetable oils using chloramine T and conc sulfuric acid reagent is described. After saponification of oils, the sterols present in the unsaponifiable matter are separated by thin layer chromatography (TLC) on Silica Gel G plates in the solvent system ethyl ether/petroleum ether (40-60 C) (1:1). The sterols are treated with 1% chloramine T in conc H_2SO_4 at 100 C for 12 min to develop the yellowish-brown color which is read at 400 nm. Sterol content is calculated against a cholesterol standard. The method is at least 5 times more sensitive than that which uses Liebermann-Burchard reagent; color formed is stable for 48 hr.

INTRODUCTION

A colorimetric method using Liebermann-Burchard reagent (cold acetic anhydride and sulfuric acid) by the modified Sperry-Webb procedure (1) is used for the determination of sterols in vegetable oils (2-4). Norcia et al. (3) reported the presence of some fast-reacting sterols with this reagent in some vegetable oils which may be the source of error (5). The other main drawback in the colorimetric determination of sterols using this reagent is the instability of the color which reaches its maximal intensity after a certain period depending on conditions such as light, temperature and composition of the reagent (6). In this communication, a new colorimetric method for the determination of sterols using chloramine T and conc H_2SO_4 is described. Although this reagent has broad specificity and reacts with other sterols (7) as does the Liebermann-Burchard reagent (6), the method is at least 5 times more sensitive than the Liebermann-Burchard reaction and the color formed is stable for 48 hr. The chromogenic reagent not only is quite stable, but easy to use, as well.

MATERIALS AND METHODS

Reagents

(a) 1% Chloramine T (Rhodia, France) solution in cold conc sulfuric acid was used (analytical reagent). The reagent can be kept for a period of 1 mo in amber-colored bottles stored in a refrigerator.

(b) The cholesterol standard solution was prepared by dissolving 10 mg cholesterol (mp 149 C) (May and Baker, London) in chloroform and adjusting the vol to 50 ml.

(c) The chloroform (analytical grade reagent) and petroleum ether (40-60 C) used should be free of aldehydes and ketones.

(d) Ethyl ether should be peroxide-free.

(e) Silica Gel G (B.D.H. India) was used; thin layer chromatography (TLC) plates were activated at 105 C for 1 hr prior to use.

Apparatus

(a) We used Spectronic-20-Bausch and Lomb colorimeter with a cell vol of 10 ml and path length 1 cm.

(b) Glass columns (0.8 x 21 cm) were plugged with cotton before use.

Preparation of Calibration Curve

Standard cholesterol solution was pipetted out in increments of 0.05, 0.1, 0.2, 0.3, 0.4, 0.5 . . . up to 1 ml into small glass crucibles and the solvent evaporated to dryness on a water bath. To each we added 1 ml chloramine T reagent and mixed thoroughly. The colored solution was quantitatively transferred into test tubes using 5 ml of conc H_2SO_4 (4 ml conc H_2SO_4 along with the colored solution may be added first into the test tube, and 1 ml conc H_2SO_4 may be used for rinsing the crucibles). A blank without cholesterol was prepared similarly. The test tubes were incubated in a boiling water bath for 12 min, cooled and the color was read at its absorption maxima at 400 nm.

Procedure for Determination of Sterol Content in Vegetable Oils

(a) We extracted oil from various plant seeds by solvent extraction procedure using hexane.

(b) About 2 g oil were weighed accurately and the unsaponifiable extract was prepared by AOAC (8) method. The vol of the unsaponifiable extract was raised to 50 ml using ethyl ether.

(c) TLC was performed on activated Silica Gel G plates and 0.5 ml unsaponifiable extract was applied as a band with a guide spot of cholesterol. The plate was developed in a glass chamber well saturated with solvent ethyl ether/petroleum ether (1:1) (8). After ca. 16-cm run the developed plate was removed and air-dried. The main band was covered with a glass plate and the identification spot of cholesterol was located using 1% chloramine T in H_2SO_4 as a spray reagent (7). The band corresponding to the identification spot was scraped off and put into a glass column with a cotton plug and eluted with 10 ml chloroform. The corresponding blank was prepared by evaporating 10 ml chloroform and passing through a similar column. The color was developed in the experimental sample after evaporation of the solvent, as well as with the blank as just described.

RESULTS AND DISCUSSION

The yellowish-brown colored reaction product of chloramine T with cholesterol obeys Lambert-Beer's Law in the concentration range 1.6 μg-32.8 μg/ml at its absorption maximum which appears at 400 nm. The color was stable up to 48 hr after which a slight increase in absorbance was observed. The color was stabilized when the sterols were heated for a minimal period of 12 min in a boiling water bath. Heating for longer periods did not affect the absorbance. As with the Liebermann-Burchard reaction, this reaction also has a broad specificity, giving color reactions with various steroids as reported earlier (7); other interfering substances are aldehydes, ketones, phospholipids and epoxides. Triglycerides and free fatty acids did not interfere. The reaction of chloramine T/conc H_2SO_4 reagent

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TABLE I

Percentage of Unsaponifiable Matter and Sterols in Vegetable Oils

Botanical name	Common name	Unsaponifiable matter (%)	Sterol content (%)	
			Modified Sperry-Webb method[a]	Our method
Linum usitatissium L.	Flax/Linseed	1.10	0.40	0.42
Helianthus annuus L.	Sunflower	1.01	0.58	0.62
Arachis hypogaea L.	Peanut/ Groundnut	0.84	0.26	0.24
Eruca sativa Mill	Taramira[b]/ Roquette (Fr.)	0.42	0.31	0.28
Brassica campestris L. Var. Toria (Duthie Fuller) Watt	Toria[b] Indian rapeseed	0.71	0.33	0.31
Brassica campestris L var. Glauca (Rexb) Watt	Yellow sarson/ Indian cotza	0.68	0.51	0.50
Sesamum indicum L.	Sesame	1.26	0.37	0.35
Brassica campestris L. Var. Dichotma (Roxb) Watt	Brown sarson[b]	0.78	0.53	0.53
Brassica juncea L. Cess	Raya[b]	0.80	0.52	0.51
Gossypium arboreum L.	Cottonseed	1.09	0.28	0.30
Ricinus communis L.	Castor bean	0.70	0.46	0.44
Cocos nucifera L.	Coconut	0.24	0.21	0.18
Prunus amgydalus L. Batsch	Almond	0.60	0.32	0.30
Glycine max L. Merr	Soybean	0.61	0.27	0.28
Carthamus tinctorius L.	Safflower	0.78	0.41	0.36

[a]Corrections for fast-reacting sterols have not been made. A correction factor of 100/65.5 was applied to the sterol content as reported by Norcia and Rosenthal (1966).

[b]Common name in India; other names are universal.

with sterols is more sensitive than the other colorimetric methods reported earlier for determination of sterols (1,9). The sensitivity of the method as determined for cholesterol was 0.028 μg/ml, $\log \frac{I_0}{I}$ = .001. The method is reproducible as shown by replicate experimental data. (Six determinations of 16.6 μg/ml of the solution gave a mean value of 16.0 μg/ml, standard deviation 0.25 μg/ml, relative standard deviation as 1.56%, showing that this method is reasonably accurate and reproducible. Higher concentrations of chloramine T, i.e., up to 5% did not affect the absorbance. However, 1% chloramine T solution in conc H_2SO_4 was considered desirable because of the low blank reading. Repeat experiments (TLC followed by elution through glass column) with standard cholesterol solution (0.5 ml standard solution) showed that recovery was 97.6 ± 0.3% (6 determinations).

Determination of Sterol Content in Vegetable Oils

Data given in Table I show the unsaponifiable matter and total sterol content as determined by the modified Sperry-Webb method (1) and by our method. These results show a close agreement of sterol content as determined by both methods. Because of the greater sensitivity of the method, it is possible to determine sterol content in quantities as low as 100 mg oil. Furthermore, since the absorbance of various phytosterols such as β-sitosterol, stigmasterol and that of cholesterol with this reagent is the same, it is possible to use cholesterol as a standard and there is no need to apply a correction factor for a difference in the absorbance of cholesterol and phytosterols as with the Liebermann-Burchard reagent (4).

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