ACCELERATED SEMIMICRO METHOD OF DETERMINING THE AMOUNT OF FREE GOSSYPOL IN COTTON SEEDS AND THE PRODUCTS OF THEIR PROCESSING

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UDC 665.3/35:667.71

A semimicro method is proposed for determining the amount of free gossypol in liquid and solid materials using 70% aqueous acetone as the gossypol extractant.

Several methods have been suggested for the quantitative determination of gossypol in seeds, oil, press-cake, and meal [1]. Nevertheless, an accurate, simple, and rapid determination of gossypol remains a problem requiring solution.

It has been shown in the literature that chloroform and ethanol solutions of gossypol absorb light intensively in the UV region of the spectrum [2]. The absorption maximum at 366 nm (chloroform solutions) is due to the presence of aldehyde groups. We have studied the absorption spectra of gossypol in ethanol. It has been found that a direct relationship exists between the absorption index at the position of the maximum (376-378 nm) and the concentration of gossypol in solution. Figure 1 gives a calibration curve for ethanolic solutions of gossypol (curve 1) with the aid of which it is possible to rapidly determine the amount of gossypol in a sample being analyzed. However, it is known that the most complete extraction of gossypol from seeds without preliminary defatting is achieved by using a mixture of acetone and water (70 + 30) which extracts a minimum amount of oil but readily dissolves gossypol [3, 4]. At the same time, acetone is used in the complex processing of cotton seeds. Consequently, we have dwelt on a study of the UV spectra of aqueous acetone solutions of gossypol.

It was found that the relationship between the absorption at the point of the maximum (380 nm) and the amount of gossypol in 70% aqueous acetone was linear, as in the case of ethanolic solutions. A calibration curve for aqueous acetone solutions of gossypol in the range of concentrations of from 0.002 to 0.03 mg/ml is given in Fig. 1 (curve 2).

For analysis we took the following samples: 1) seeds before defoliation; 2) after defoliation; 3) oil cake; 4, 5) oil cake after treatment in a cyclone. To evaluate the method the amounts of free gossypol in the samples were determined by the p-anisidine method; the results obtained are the means of three parallel determinations (%):

Sample	Proposed method	p-Anisidine method
1	0,77	0,75
2	0,43	0,46
3	1,09	1,06
4	2,00	2,05
5	2,70	2,95

As we see, the values obtained by the two methods are fairly close.

The accuracy and reproducibility of the method was checked by the spectrophotometric measurement of a large number of model samples of pure gossypol in 70% aqueous acetone. After the absorption coefficient at the point of the maximum at 380 nm had been measured, the amounts of gossypol were determined from a calibration curve:

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 608-612, September-October, 1979. Original article submitted February 22, 1979.

 0009-3130/79/1505-0530$07.50

Amount of gossypol used, mg	Determined from the calibration curve, mg	Error, %
0,110	0,114	+3,6
0,144	0,140	−2,8
0,160	0,158	−1,2
0,176	0,178	+1,1
0,220	0,220	0
0,270	0,260	−3,7
0,288	0,300	+4,2
0,480	0,484	+0,9
0,576	0,600	+4,2

The facts given above show that the proposed method permits the determination of semimicro amounts of gossypol with an error not usually exceeding ±5%.

A. L. Markman has shown that "gossypol itself gives a spectrum which changes greatly with time" [1]. Consequently, colorimetric and spectrophotometric methods are usually used not with gossypol itself but with various stable derivatives of it [5]. In view of this, to investigate the influence of the length of storage on absorption we periodically recorded the spectra of the same solutions of gossypol in aqueous acetone during their keeping in the refrigerator for 10 days. The nature of their spectrum in the 340-400-nm determined region remained unchanged. Results on the amount of gossypol in the stored solutions obtained with the aid of the calibration curve are given below (mg):

Gossypol taken, mg	Storage for 1 day	3 days	10 days
0,110	0,114	0,110	0,106
0,176	0,178	0,180	0,184
0,220	0,220	0,226	0,218
0,270	0,260	0,260	0,260

As these facts show, the storage of solutions of gossypol for 10 days does not lead to appreciable quantitative changes. This fact opens up the possibility for the spectrophotometric determination of gossypol as such and not in the form of its derivatives. However, one cannot completely exclude the possibility of a change in the gossypol during the preparation of its derivatives because of the length of the process. The possibility is not excluded of the reaction of the staining reagents with substances accompanying the gossypol, which must affect the absorption index. The majority of the existing colorimetric and spectrophotometric methods were designed for determining the amounts of gossypol in seeds, oil cake, and meal (i.e., in solid materials), and considerably fewer for determining it in oil and miscella. However, the extraction of gossypol with 70% aqueous acetone permits the proposed method to be applied to the analysis of liquids, as well. In samples of factory unfractionated cottonseed oil (1-4) and oil obtained by extracting the kernel with binary solvents (sample 5) we determined the amounts of free gossypol by the proposed method and by the p-anisidine method (%):

Sample of oil	Proposed method	p-Anisidine method
1	0,24	0,22
2	0,10	0,10
3	0,20	0,22
4	0,14	0,12
5	1,18	1,22

Thus, the results are fairly close, but the proposed method has undoubted advantages in simplicity and time.

Since the method of determining gossypol adopted by a GOST [State Standard] is the gravimetric aniline—pyridine method, we have compared the proposed method with this by determining the amounts of free gossypol in a number of samples of seeds and oils (%):

Sample	Aniline–pyridine method	Proposed method
	Seeds	
1	0,62	0,65
2	1,12	1,04
3	0,96	0,95
4	0,59	0,57
	Oil	
1	0,81	0,79
2	0,61	0,57
3	0,94	0,99

The discrepancy of the results obtained by the two methods is within acceptable limits.

To determine the sensitivity of the method, known amounts of pure gossypol were introduced into several samples of refined cottonseed oil. The results of the determination of gossypol in these models are as follows (%):

Gossypol added, % on the oil	Proposed method	Aniline–pyridine method
0,0024	0,0022	Not det.
0,027	0,024	Not det.
0,105	0,100	0,095
0,76	0,700	0,700

Thus, the proposed method permits the detection of thousandths of a percentage part of gossypol in a sample, which cannot be achieved by using the aniline–pyridine method.

EXPERIMENTAL

Preparation of Pure Gossypol. Gossypol was obtained from previously hexane-defatted cottonseed pulp by its extraction with diethyl ether containing water (3-5%). The product was recrystallized from a mixture of diethyl ether and petroleum ether (1:2) and it was repurified via gossypol acetate.

The purity of the product obtained, as determined by the reduction of Fehling's solution [6], was 98.5%, mp 180-181°C.

A standard solution of gossypol was prepared by dissolving 20 mg of pure gossypol in 100 ml of ethanol or 70% aqueous acetone, so that 1 ml of solution contained 20 µg of gossypol.

To plot the calibration curves, solutions of pure gossypol in ethanol and in 70% acetone were diluted with a standard solution to the corresponding concentrations. The optical densities were determined on a Hitachi spectrophotometer.

Preparation of Aqueous Acetone Solutions of Gossypol from Cotton Seeds and Oils. Ground cottonseed kernels, oil cake, or meal (25-30 mg) or a weighed amount of oil was covered with

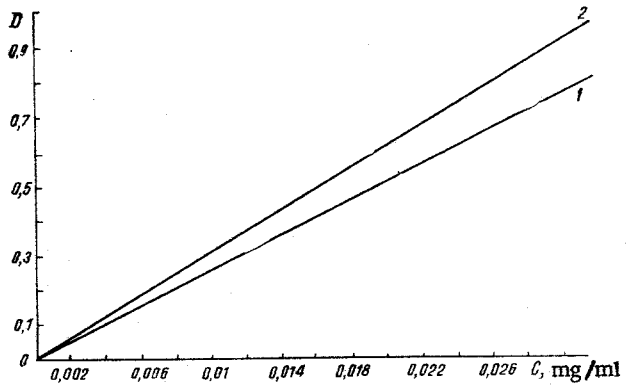

Fig. 1. Calibration curves of gossypol solutions: 1) in ethanol; 2) in 70% aqueous acetone; D) optical density; and C) concentration of gossypol in the solution.

5-10 ml of aqueous acetone and the mixture was shaken for 10-15 min, filtered into measuring flasks, and made up to the mark with aqueous acetone.

SUMMARY

A rapid spectrophotometric semimicro method of determining free gossypol in cotton seeds and the products of their processing is proposed which permits thousandths of a percentage part of gossypol in a sample to be detected.

Calibration curves are given for determining the amount of gossypol in ethanolic and aqueous acetone solutions.

LITERATURE CITED

1. A. L. Markman and V. P. Rzhekhin, Gossypol and Its Derivatives [in Russian], Moscow (1965), p. 194.
2. V. P. Rzhekhin and A. B. Belova, New Methods of Isolating Gossypol from Cotton Seeds, Oil, and Meal [in Russian], Moscow (1961), p. 7.
3. W. A. Pens and J. D. Guthrie, J. Am. Oil Chemists' Soc., 26, 671 (1949).
4. A. L. Markman and Z. Sabirov, Maslob-Zhir. Prom., No. 2, 14 (1962).
5. J. M. S. Mathur, N. D. Sharma, and Munshi Singh, J. Food Sci. Technol., 9, 138 (1972).
6. M. Z. Podol'skaya, Zh. Prikl. Khim., 17, No. 11-12, 657 (1944).

NONGLYCERIDE COMPLEX OF THE SEED OIL OF *Onopordum acanthium*

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UDC 547.915+21.29+922

Extraction of the comminuted seeds has yielded an oil from which have been isolated: C_{33}-C_{25}, C_{18} and C_{17} paraffinic hydrocarbons, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$, $C_{17:1}$, $C_{17:2}$ and $C_{17:3}$ olefinic hydrocarbons, ethyl esters of $C_{32:0}$, $C_{31:0}$, $C_{30:0}$, $C_{29:0}$, and $C_{28:0}$ fatty acids, sterols with molecular weights of 414, 412, and 400, and the alcohols α-amyrin and lupeol with their natural acetates. Extraction of the uncomminuted seeds has shown that the paraffinic hydrocarbons, ethyl esters, and alcohol acetates pass into the oil from the husks of the seeds. This is the first time that the $C_{31:0}$ and $C_{29:0}$ fatty acids have been detected as natural compounds, and it is the first time that the ethyl esters of C_{34}, C_{33}, C_{32}, C_{31}, and C_{30} fatty acids have been isolated from seed oils of higher plants.

In a study of the seed oils of *Onopordum acanthium* (Scotch thistle), family Compositae, growing in Central Asia, attention is attracted by the considerable variation in the amount of "unsaponifiables" in it (0.5-1.5% on the oil). To explain this fact, we have investigated the nonglyceride complex of the oil. The oil was subjected to column chromatography (CC) with a mixture of hexane and ether. Three fractions of oil were collected. The migration of the substances of these fractions on Silufol plates (systems 1, 2, and 3) corresponded to the migration of model samples of hydrocarbons, pentacyclic alcohols, and sterols (results of one of the experiments) (Table 1).

The total substances of fraction I were rechromatographed by the CC method (systems 1 and 4). When system 1 was used, as was shown by IR and NMR spectra, only the hydrocarbons passed into the first portions of the eluate. The mass spectra of the readily volatile substances when a sample of hydrocarbons was present in the glass tube for direct introduction and the reaction with iodine on Silufol showed the presence in the oil of two groups of hydrocarbons, C_{17} and C_{18}. The hydrocarbons within each group differed from one another by their degree of unsaturation, i.e., they contained from 0 to 3 ethylenic bonds. On complete introduction, saturated paraffins were detected in the mass spectrum with molecular weights

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent.

Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 612-615, September-October, 1979. Original article submitted February 22, 1979.

0009-3130/79/1505-0533$07.50