the paper chromatogram. The conditions specified in the method give reproducible results. Other tests demonstrated that the aqueous dimethylformamide extraction and the paper chromatography are quantitative. Since the recoveries are consistent and very reproducible, a factor of 2.0 is used to calculate the amount present.

Recovery of Gossypol. Known amounts of gossypol were added at various levels to representative samples, such as fat and fatty acid samples, containing no free gossypol and a fatty acid sample containing free gossypol. The samples were analyzed by the proposed method. To eliminate prejudice and to increase the precision, the visual judgments were done separately by three to four persons. Each value is the average of three to four determinations. Data on the recovery of the method are shown in Table II. The average recovery was 97%.

TABLE II

Recovery of Gossypol

Sample	Number analyzed	Gossypol			Recovery
		Present	Added	Found	
		(p.p.m.)	(p.p.m.)	(p.p.m.)	(%)
Fat	3	0	100	95	94
Fat	4	0	50	49	98
Fat	2	0	10	11.6	116
Fatty acid	1	0	100	80	80
Fatty acid	1	0	50	44	88
Fatty acid	1	0	10	10	100
Fatty acid	1	20	50	63	86

Reproducibility of the Method. Samples of gossypol alone, tallow with added gossypol, and an acidulated cottonseed foots, containing an unknown amount of free gossypol, were analyzed by this method. Each value represents the average of three to four determinations. The standard deviation from the mean for all samples examined was ± 3 p.p.m. Data on the reproducibility of the method are presented in Table III.

TABLE III

Reproducibility of the Method

Sample	Number analyzed	Gossypol		Standard deviation
		Added	Found	
		(p.p.m.)	(p.p.m.)	(p.p.m.)
Gossypol solution	4	40	42.5	3.1
Fat	8	100	97.5	1.6
Fat	4	50	50	0
Unknown	6	0	87	2.0

Discussion

The method, with slight modifications, was also applied to meals. One gram of cottonseed meal was extracted with 50 ml. of 7:3 acetone-water solution (6, 11) and filtered. The filtrate was diluted with 200 ml. of distilled water, acidified with 2 ml. of concentrated hydrochloric acid, and extracted twice with 50-ml. portions of chloroform. The chloroform layer was dried with anhydrous sodium sulfate, concentrated to a suitable volume, and chromatographed for free gossypol. The recovery is quantitative, and therefore no calibration factor is required.

Total gossypol (7, 8) can also be determined by this method. The sample is hydrolyzed, then analyzed by the given procedure.

Gossypol derivatives, such as dianilino- and 2,3-dimethyl-butadiene-gossypol, were chromatographed in the heptane-chloroform-acetic acid solvent. The derivatives have different R_f values, and they give different color reactions with phloroglucinol. Therefore this solvent can be used for the differentiation between gossypol and its derivatives.

Summary

A quantitative method for the determination of traces of free gossypol in oils and fatty acids was developed. The method is based on the concentration of gossypol by extraction and quantitative paper chromatography of the extract. The method is specific for free gossypol and is not subject to interferences. The new method is both accurate and reproducible. The lower limit of detection is 10 p.p.m. The method is intended primarily for p.p.m. levels but is suitable for all concentrations. With slight modifications the method is applicable to meals.

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Erratum

THROUGH AN OVERSIGHT the References were omitted for the paper entitled "Solubility of Cottonseed Proteins in Hydrochloric Acid," by Mann, Rubins, Carney, and Frampton, which was published in the May 1958 issue of the Journal of the American Oil Chemists' Society (*35*, 244–246). The bibliography follows.

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