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Determination of Total Gossypol of Bt Cottonseed and Non-Bt Cottonseed using HPLC and New (UV)-Spectrophotometer Methods

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ABSTRACT: *Gossypol exits in two different forms, free and total in cottonseeds. However, both the forms of gossypol can be toxic to animals and humans, which limit the usefulness of cottonseed as animal feed. In this study the total gossypol content in Bt cottonseed and non-Bt cottonseed were investigated using HPLC and UV-Spectrophotometer, and comparison studies on total gossypol of various genetic types of Bt and non-Bt cottonseed (Barakat90, Mixed and Abdeen) was done. Among the all Non-Bt cottonseed varieties Barakat (90) had shown higher amounts of total gossypol 1320 and 1243 ppm by using HPLC and UV-Spectrophotometer respectively. Moreover, Bt cottonseed variety (Sini 1) had shown less amount 960 and 890 ppm by both methods HPLC and UV-Spectrophotometer respectively. Spectrophotometric method is the more available analytical method as quick, cheap and simple; hence, the method can be applied for determination of total gossypol in cottonseed and cottonseed product samples. A mathematical formula was formed, in which a corrtion factor was calculation for the relation between UV and HPLC result. The factor may be used for future analysis using UV Spectrophotometer instead of HPLC.*

Key Words: **gossypol, Bt cottonseed, Non-Bt cottonseed, HPLC, UV Spectrophotometer**

1. Introduction

Cottonseed is a good source of high quality meal and edible oil. But a toxic phenolic compound called gossypol [1]. Which is an anti-nutrition factor that limits the use of cottonseed and its products due to it is toxicity associated to its reactions with amino acid and minerals [2].

Gossypol exists in cottonseed products in two forms (free and bound). Free gossypol (FG), as defined by AOCS official methods, are those gossypol and gossypol derivatives that are soluble in aqueous acetone and are physiologically active. Bound gossypol (BG) forms during conventional cooking and processing of cottonseed by the reaction of gossypol with free amino groups of proteins and peptides. It is insoluble in ether, chloroform, or aqueous acetone. BG form the most part is physiologically inactive[3]. It is not measured directly but is calculated as the difference between total and free gossypol. Total gossypol (TG) is briefly defined as the amount of gossypol, and gossypol derivatives, both free and bound, and gossypol-like pigments extracted during hydrolysis[4, 5].

2. Material and methods

2.1 Material

The cottonseeds used as sample for the analysis were collected from the seed unit, at ARC, Al-Gazira State, Sudan. The variety of cottonseed used is Normal cotton (Barakat 90, Abdeen, and Mixed) and Modified cotton (Sini1 (Bt)). The collected seeds were properly cleaned so to remove farm residues and other impurities. All the cottonseed samples were manually crushed followed by grinding samples into power form. Then oil was extracted by soxhlet with n-hexane.

2.2 Methods

Spectroscopy methods is the branch of science dealing with the study of interaction between Electromagnetic radiation and matter. It is a most powerful tool available for the study of atomic and molecular structure/s and is used in the analysis of wide range of samples. Optical spectroscopy includes the region on electromagnetic spectrum between 100 Å and 400 µm.

UV-Visible spectrophotometry is one of the most frequently employed technique in pharmaceutical analysis. It involves measuring the amount of ultraviolet or visible radiation absorbed by a substance in solution. Instrument which measure the ratio, or function of ratio, of the intensity of two beams of light in the U.V-Visible region are called Ultraviolet-Visible spectrophotometers.

In qualitative analysis, organic compounds can be identified by use of spectrophotometer, if any recorded data is available, and quantitative spectrophotometric analysis is used to ascertain the quantity of molecular species absorbing the radiation. Spectrophotometric technique is simple, rapid, moderately specific and applicable to small quantities of compounds.

In this study, Ultra visible spectrophotometer and high performance liquid chromatography were used.

2.3 Experimental Section

2.3.1 Determination of Total Gossypol by Ultra Visible (UV) Spectrophotometer Procedure

2.3.1.1 **Apparatus:** UV-VIS spectrophotometer equipped with a pair of 1-cm path length quartz cuvettes was used for absorbance measurements.

2.3.1.2 **Reagents and Standards:** all the chemical used were of analytical reagent grade.

a) A 1.79 ⍰10 $^{-2}$ M iron(III) solution was prepared by dissolving hydrated ferric nitrate ($Fe(NO_3).9H_2O$, BDH AnalaR) in 40:60 (v/v) hexane-isopropyl alcohol containing a few drops of concentrated hydrochloric acid. The complexing agent solution was prepared by mixing 2 ml of 3-amio-1-propanol with 10 ml of glacial acetic acid. The solution was cooled to room temperature and diluted to 100 ml with di-methylformamide in a volumetric flask.

b) A4.821 ⍰10 $^{-3}$ M standard solution of gossypol was prepared by dissolving gossypol acetic acid in complexing agent solution. A working solution of gossypol was prepared by diluting a 10 ml aliquot of standard solution to 100 ml with 40:60 (v/v) hexane-isopropyl alcohol in a volumetric flask.

2.3.1.3 **Procedure for extraction of Gossypol from Cottonseeds and Cottonseed press cakes:** samples of four varieties of Bt-cottonseed and non-Bt-cottonseed, a weighed quantity of samples containing 2-20 mg of total gossypol was transferred into a 100 ml Erlenmeyer flask and 10 ml of complexing agent solution was added to it. The mixture was heated in a boiling water bath for 30 min, cooled, and diluted to about 30 ml with 40/60 (v/v) hexane-isopropyl alcohol. The solution was filtered and diluted to 50 ml with hexane-isopropyl alcohol in a volumetric flask.

2.3.1.4 **Procedure for Determination of Gossypol:** an aliquot of the solution containing 0.2-2.0 mg of gossypol was transferred into a 25 ml volumetric flask and 2 to 4 drops of 5M hydrochloric acid were added to it. A 5 ml aliquot of iron(III) solution was added to the flask, the solution were mixed well an then allowed to stand for 5 min. 1 ml of distilled water was added to the flask, and the solution was immediately diluted to volume with 40:60 (v/v) hexane-isopropyl alcohol. The absorbance of the colored solution was measured at 620nm against the hexane-isopropyl alcohol mixture as a reference. Calibration curves are prepared by measuring the absorbance of the solutions containing known amount of the gossypol by the same procedure.

2.3.2 Determination of Total Gossypol by High Performance Liquid Chromatography (HPLC)

Extraction of total gossypol is carried out by N,N-dimethyl formamide, during the process of reaction evaporation of N,N-di Methyl formamide is performed at high temperature till an oily material containing gossypol was obtained.

2.3.2.1 **Complex Reagent Preparation:** 2 ml of 3-amino proponl was added to 10 ml of glacial acetic acid and it is this solution was then made up to 100 ml using N,N-di Methyl formamid. Solvent-based method is the technique used for extraction of gossypol from cottonseed samples. Presence of gossypol in the solvent can be confirmed by yellow color reaction.

2.3.2.2 **Estimation of Total Gossypol:** for 1 gm of each sampl, 25 ml of complex reagent was added and then placed in water bath at 100 ^{0}C for 2 hr. This process results in the formation of yellow color indicating the presence of gossypol. After color reaction was observed, samples were allowed to cool to room temperature upon which filtration was preformed. Dilutions were preformed by taking 1 ml of filtrate to which 4 ml N,N-di Methyl formamide was added. A 20 µl of this sample was injected for HPLC analysis. Gossypol standard has

been shown peak at Retention time 6 min with Pressure of 90-95 Kgf and Flow rate 1 ml/min at Wavelength of 254 nm.

2.3.2.3 Analysis of Total Gossypol Content: total from of gossypol is analyzed for it is presence in both Bt and non-Bt samples, all these samples were allowed to run in two different batche under same optimized conditions of temperature and percentage of solvents used for extraction of total gossypol.

3. Result and Discussion

From table (1,2) the range of total gossypol determination by UV-Spectrophotometer and HPLC are (890 – 1260) ppm and (960 – 1320) ppm respectively , Barakat (90) it has the highest total gossypol, And Sini (1) has lowest total gossypol.

Table (1): Total Gossypol Content determination by UV-spectrophotometer in Different varieties

	Total	
	ppm	**%**
Sini (1)	890	0.082
Barakat (90)	1243	0.124
Abdeen	914	0.091
Mixed	1017	0.102

Gossypol is a phenolic compound that is why spectrophotometric method was applied for its detection. The reason for this is that all phenolic compounds are aromatic and they show intense absorption in the UV region (200-350 nm) of the spectrum. Spectral methods are therefore especially important for identification and quantitative analysis of phenolic compounds[6].

Table (2): Total Gossypol Content Determination by HPLC in Different varieties

Cottonseed Varity	**Total gossypol**	
	ppm	**%**
Sini (1)	960	0.096
Barakat (90)	1320	0.132
Abdeen	988	0.099
Mixed	1200	0.120

One of the studies aimed at determining gossypol content among different cotton variations in gossypol in gossypol content emphasizing differences in genetic makeup pigmented glands or dot glands. Investigation found high amount of total from the seeds non Bt varieties of, while Bt variety showed low amount of total. Chandrashaker et al research paved a conclusion that Bt varieties show low gossypol content compared with non-Bt varieties. Therefore, these results do not compatible with Chandrashaker et al research.

Several method have been reported for the determination of gossypol in a variety of samples in a variety samples[7] include spectrophotometric, an NMR method[8], gas-liquid chromatographic[9] method, High Performance Liquid Chromatography[10]. Most of these methods are tedious, time-consuming and suffer. Therefore, it is necessary to look for the development of a simple and precise method for the routine analysis of gossypol in cottonseed materials.

From the results of total gossypol were determined by (UV Spectrophotometer) and (HPLC) we derive this relationships:

a, (corrected factor) =

Average of results determined by (HPLC) – Average of results determined by (Uv Spectrophotometer)

% gossypol (UV Spectrophotometer) = % gossypol (HPLC) ± a

where: a = 0.009%.

Table (3): Total Gossypol Content Determination by HPLC and UV-Spectrophotometer in Different varieties

	UV		HPLC		UV with equ.	
	ppm	%	ppm	%	ppm	%
Sini (1)	890	0.082	960	0.096	980	0.091
Barakat (90)	1243	0.124	1320	0.132	1350	0.135
Abdeen	914	0.091	988	0.099	1000	0.100
Mixed	1017	0.102	1200	0.120	1110	0.111

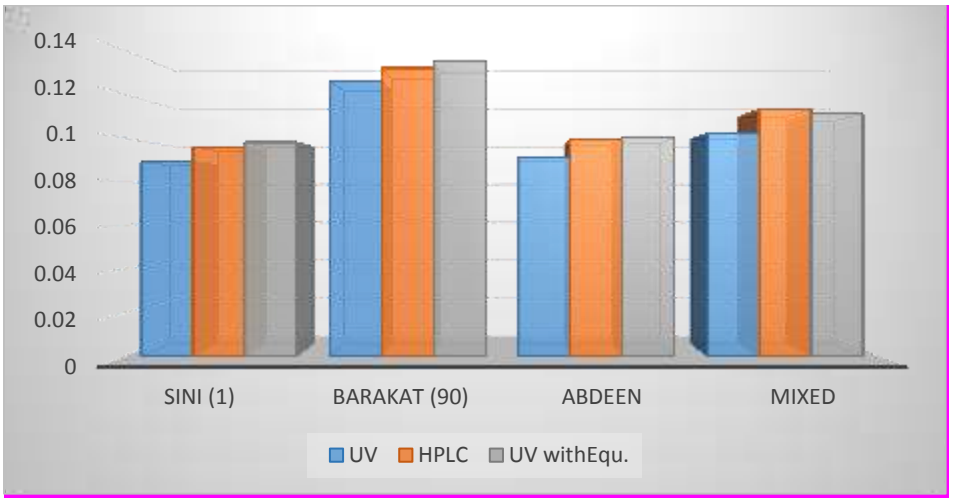

Figure (1): comparison of Total Gossypol Content in Seed by Different Methods (percentage)

4. Conclusions

- Among the all non-Bt cottonseed samples, Barakat (90) had shown higher amount of total gossypol. Bt cottonseed sample (Sini (1)) had shown less gossypol amount than non-Bt cottonseed samples.
- HPLC method is more accurate, effective and specialized but UV-Spectrophotometer is simple, rapid. When gossypol is a phenolic compound this lead to easy of using the most available analytical method UV-Spectrophotometer as quick, cheap and available. The result that get from UV-Spectrophotometer can be modalized mathematically, (equation 1) using the more equate HPLC and then have reasonable result without using HPLC method.

5. Recommendation

This study recommend that: the mathematical equation (% gossypol (UV Spectrophotometer) = % gossypol (HPLC) ± a) results can be corrected to give the same results as HPLC.

6. Accknowledgement:

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