



Original Research Article

Determination of amount of retinyl palmitate and ascorbic acid of extract gel of sweet corn fibre by UV and HPTLC method

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ABSTRACT

Sweet corn fibres of about 15 gm were extracted with methanol for 5 hours in heating mantle at 40°C and filtered and allowed to dry. The dried gel was further analyzed for estimation of Retinyl palmitate by spectrophotometrically by laboratory method and found to be 140 mg/kg. The dried extract gel was further estimated for ascorbic acid both by UV and HPTLC and found to be linear in the range of 1-5 ug/ml and 5-10ug/ml, correlation coefficient was found to be 0.997 and 0.998 and the amount of ascorbic acid was found to be 338 ng/ml and 9.9 ng/ml by UV and HPTLC respectively. The method was found to be linear.

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1. Introduction

1.1. The Corn Fiber

Corn is an agricultural product with large quantities of starch. Manufacturers extract this from the plant fibers, break it down into sugars, ferment them and separate them into polymers.

1.2. The advantages in the use of corn fiber

The fabric made from corn fiber hardly requires maintenance, is fairly cheap and comfortable to wear. There are also the advantages of the fiber being stain-resistant and does not fade easily. So applications like readymade apparel, diapers, bedding, carpets and upholstery come out well

Retinol, also known as vitamin A₁-alcohol, is a vitamin in the vitamin A family¹ found in food and used as a dietary supplement.² As a supplement it is ingested to



Fig. 1: The fibre of sweet corn

treat and prevent vitamin A deficiency, especially that which results in xerophthalmia.¹ In regions where deficiency is common, a single large dose is recommended to those at high risk a couple of times a year.³ It is also used to reduce the risk of complications in those who have measles.³ It is used by mouth or injection into a muscle.¹

Retinol at normal doses is well tolerated.¹ High doses may result in an enlarged liver, dry skin, or hypervitaminosis

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A.¹⁴ High doses during pregnancy may result in harm to the baby.¹ It is converted in the body to retinal and retinoic acid through which it acts.² Dietary sources include fish, dairy products, and meat.²

Retinol was discovered in 1909, isolated in 1931, and first made in 1947.^{5,6} It is on the World Health Organization's List of Essential Medicines.⁷

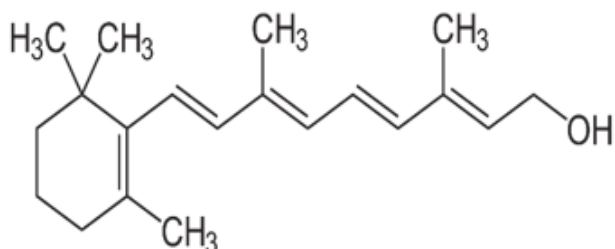


Fig. 2: Structure of retinol

1.3. Retinol is used to treat vitamin A deficiency.

Vitamin C (also known as ascorbic acid and ascorbate) is a vitamin found in various foods and sold as a dietary supplement.⁸ It is used to prevent and treat scurvy.⁸ Vitamin C is an essential nutrient involved in the repair of tissue and the enzymatic production of certain neurotransmitters.^{8,9} It is required for the functioning of several enzymes and is important for immune system function.^{9,10} It also functions as an antioxidant.¹¹

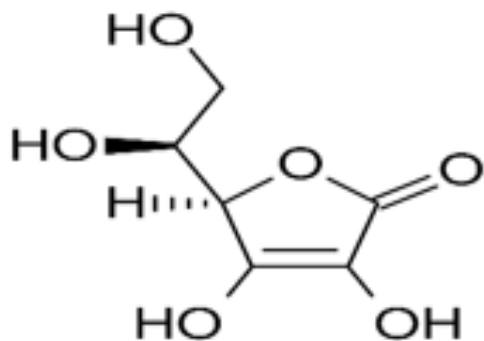


Fig. 3: Structure of ascorbic acid

There is some evidence that regular use of supplements may reduce the duration of the common cold, but it does not appear to prevent infection.^{11,12} Vitamin C is generally well tolerated.⁸ Large doses may cause gastrointestinal discomfort, headache, trouble sleeping, and flushing of the skin.^{8,11} Normal doses are safe during pregnancy.⁷ The United States Institute of Medicine recommends against taking large doses.⁹

2. Results

The dried extract gel was further estimated for ascorbic acid both by UV and HPTLC and found to be linear in the range of 1-5 ug/ml and 5-10ug/ml, correlation coefficient was found to be 0.997 and 0.998. The amount of ascorbic acid was found to be 338 ng/ml and 9.9 ng/ml by UV and HPTLC respectively. The method was found to be linear.

3. Materials and Methods

Corn fibres of about 15 gm were extracted with methanol for 5 hours in heating mantle at 40 °C and filtered and allowed to dry and diluted with hot water for further analysis.

The extract sample was dissolved in warm water to dissolve the matrix of vitamin A fortificant compound. The extract solution is diluted 1:2 with sodium hydroxide and then is extracted into hexane. Retinyl palmitate concentration is determined by recording the absorbance of this solution at 326 nm. This method does not usually require irradiation with UV light, because the absorbance of the extract at 326 nm is mainly due to the retinyl palmitate.

3.1. Reagents

Absolute ethanol, Phenolphthalein solution-1%, Hexane (C₆H₁₄), Sodium hydroxide solution-0.1 N.

3.2. Procedure

1. Weigh approximately 1 g of corn fibre extract and add about 100 mL hot water at 85°C. Use a glass rod to completely dissolve the sample.
2. Cover the beakers with a watch glass or aluminum foil
3. Cool them to room temperature in a dark place. An ice bath can be used for this purpose.
4. Measure 5 mL of the solution prepared in steps (4 or 5) into a 50 mL test tube.
5. Prepare triplicates for each sample
6. Add 5 mL of 0.1 N-sodium hydroxide to each tube and mix in a Vortex for 30 seconds.
7. Add 2-3 drops phenolphthalein-1% m/v to the same tubes. Then, add 5 mL absolute ethanol to each tube. Mix in the vortex mixer for 5 seconds.
8. Measure 5 mL of hexane and add it to each tube from step (8). Immediately close with a cap each tube and mix vigorously with the vortex mixer for 30 seconds to ensure complete extraction of the retinyl palmitate. Open the tubes briefly to release the vapor pressure. Allow separation of phases. The aqueous phase has a fuchsia color, and the top organic solvent phase is colorless.
9. Recording absorbance of the extracted vitamin A
10. As soon as possible, transfer the organic phase, using a Pasteur pipette to a 1 cm light path spectrophotometer

cuvette and read the absorbance at 326 nm. Adjust the zero of spectrophotometer with hexane before each reading.

11. Calculations The retinyl palmitate concentration of the sugar sample is calculated using the following equation:

$$\text{Retinyl palmitate(mg/kg)} = \frac{\text{Abs}_{corrected} \times Q}{V_{org} / V_{sam} \times V_i / w \times CF / R \times D}$$

Where: $\text{Abs}_{corrected} = \text{Abs}_{sample} - \text{Abs}_{blank}$ And Abs_{blank} is the average for the three readings, which should be less than 0.050

The equation parameters are: To express the results as unesterified retinol, the ratio of the molecular weights of retinol/retinyl palmitate ($286.46/524.84 = 0.546$), must be taken into consideration.

Table 1: Parameters to calculate the retinyl palmitate

Parameter	Explanation	Value
Q	Retinyl palmitate absorption coefficient in hexane($\text{mg}^{-1}\text{cm}^{-1}\text{L}$)	0.092
V_{org}	Volume of organic phase (ml)	5
V_{sample}	Volume of sample phase analysed(ml)	5
V_i	Initial volume (ml)	100
w	Weight of the sample(g)	1
R	Recovery	0.906
CF sample	Correction factor of the ideal spectrophotometer	1
D	Dilution factor	1

$$\text{Retinyl palmitate(mg/kg)} = \frac{\text{Abs}_{corrected} \times 1639.4}{w \times CF_{spec}} = 140.98$$

3.3. Spectrophotometric determination of ascorbic acid in the extract

Preparation of Standard solution: Different concentrations of ascorbic acid were prepared by diluting the ascorbic acid with water to make 1000 ug/ml. And it is further diluted to 1-5 ug/ml concentrations and absorbance value were noted at 260 nm. And the calibration curve was drawn.

Table 2: Linearity values for the calibration curve

Concentration(ug/ml)	Absorbance(nm)
0	0
1	0.099
2	0.198
3	0.324
4	0.445
5	0.570
Unknown concentration of corn fibre	0.338

And the absorbance value of corn powder gel was also determined and found to be 0.338 nm and from the calibration graph the amount of ascorbic acid was found to be 2.89 ug/ml.

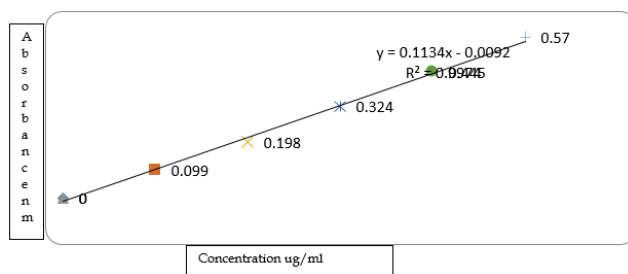


Fig. 4: Calibration curve of ascorbic acid

3.4. HPTLC determination of corn fibre extract gel

The Chromatographic estimation was performed by spotting standards and extracted samples on pre-coated silica gel aluminium plate. Plates were developed using a mobile phase consisting of Methanol and water (8:2v/v). Linearity of the method was studied by spotting various concentrations and found to be linear in the range of 5-10 ug/ml

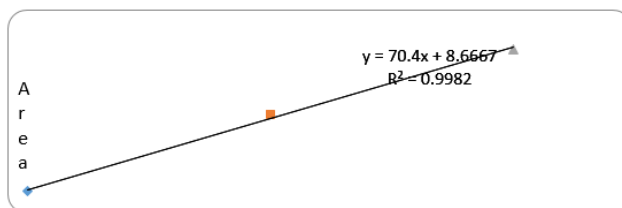


Fig. 5: Linearity curve of Ascorbic acid by HPTLC

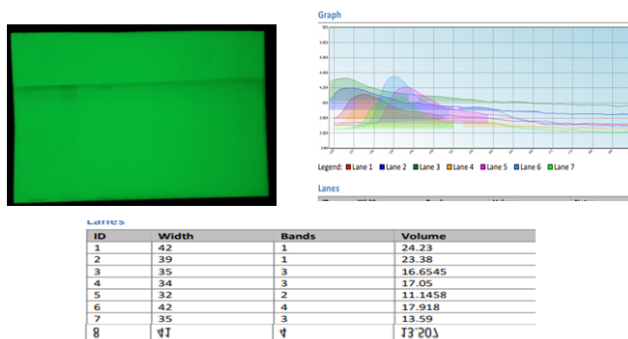


Fig. 6: Densitogram of the corn fibre extract and ascorbic acid

4. Discussion

The dried gel was further analyzed for estimation of retinol by spectrophotometric ally by laboratory method and found to be 140 mg/kg. The linearity for ascorbic acid both by UV and HPTLC found to be 1-5 ug/ml and 5-10ug/ml, correlation coefficient was 0.997 and 0.998. The amount of ascorbic acid was found to be 338 ng/ml and 9.9 ng/ml by UV and HPTLC respectively.

5. Conclusion

The method was found to be linear and very economical was researched for the first time.

6. Acknowledgement

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7. Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

8. Source of Funding

None.

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