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Sensitive chromogenic method development and validation for the estimation of Vitamin A in the Food and Cosmetics Products

K. Bhavya Sri*[1], G.Manusha [2], Narmada Vallakeerthi[3], Anitha Sadhula[4], Dr. V. Kiran Kumar[5], Dr.M. Sumakanth[6]

[1*]Associate Professor, Department of pharmaceutical analysis,RBVRR women's college of pharmacy, Barkatpura Hyderabad-500027, India.
[2]Research student department of pharmaceutical analysis,RBVRR women's college of pharmacy, Barkatpura Hyderabad-500027, India.
[3,4,5]Assistant professor Department of Pharmacy, University college of Technology, Osmania University, Hyderabad 500007 Telangana, India
[6]Professor and Principal Department of pharmaceutical chemistry,RBVRR women's college of pharmacy, Barkatpura Hyderabad-500027, India

***Corresponding author:**

Dr. K. Bhavya Sri

Associate Professor and Head, Department of pharmaceutical analysis

Email id-bhavya.khagga@gmail.com

ORCID - 0000-0002-9545-937X

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ABSTRACT

Vitamin A which is Retinol Acetate this is simple ,rapid, Quantifying method of vitamin A of different products to make reacting with MBTH reagent at a cold temperature to produce a colored complex which is the basis of an easy-to use and highly sensitive UV-visible spectroscopic approach for the measurement of Vitamin. For colorimetric drug assessment, MBTH Reagent is a chromogenic reagent that contains active methylene groups, phenolic, aromatic amines, and hydroxyl groups. To determine the linearity, precision, robustness, robustness, limit of detection, and limit of quantification, validation was done. A simple, rapid, sensitive methods are used for the determination of Retinol acetate that is added in the food products by using UV-Visible spectroscopy. The method is developed by using chromogenic method (MBTH reagent) according to the ICH Q2(R2) guidelines and With an excellent correlation value (r2) of 0.9988, linear responses were observed in the range of concentrations of 20 µg/ml to 360 µg/ml. The LOD and LOQ were determined at 0.40788 µg/mL & 1.236 µg/ml, respectively.The precision (%RSD ≤ 2) found to be in acceptable limits. The chromogenic method were developed and validated which was extended to food samples that contain high amount of Vitamin A and Quantification of them in food samples

Keywords: *Ferric ammonium sulphate, Retinol Acetate, 3-methyl-2 benzothiazolinonehydrazone, sulphamic Acid, UV visible spectroscopy*

1. INTRODUCTION

The body uses fat-soluble vitamin A for a variety of essential functions. It improves the process of cellular differentiation, which is the regular division of cells. It is essential to have a distinct vision. The first indication of mineral deficiencies.It also affects the functioning of the immune system. rising, bone formation, the act of procreation, and the healing of wounds. One group of compounds, called retinoids, The body converts beta-carotene to vitamin A1. Figure 1 shows the chemical structure of Retinol, a form of vitamin A with a hydroxyl functional group..The optimized method was performed by adding 1 ml of MBTH Reagent and 1 ml of Ferric Ammonium sulphamate and the color appears to be Green Colour. The optimized wavelength was found to be 630nm.2,3,4.

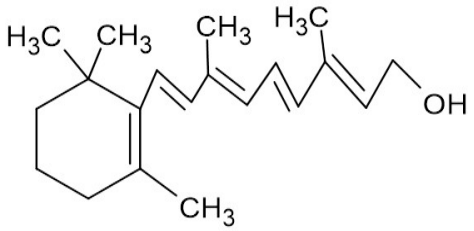
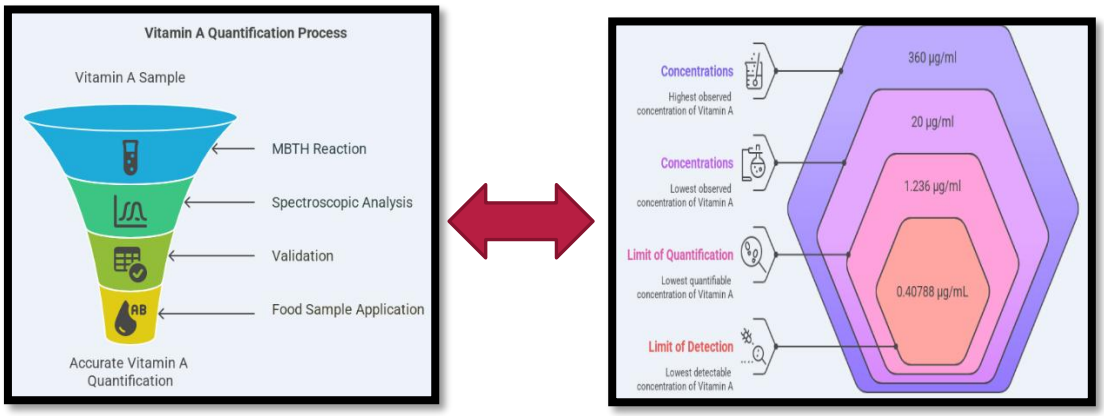


Figure 1: Structure of Retinol Acetate

Graphical Abstract



During oxidation, MBTH loses one proton and two electrons, creating an electrophilic intermediate. The intermediate, in the combination of MBTH (Figure 2shows the chemical structure of Retinol, a form of vitamin A with a hydroxyl functional group.) and ferric ammonium sulphate at a cold temperature of 5°C, combines with its hydroxyl group by an electrophilic attack on the primary nucleophilic as site (i.e., para or ortho position) to produce the green color[5,6,7.]

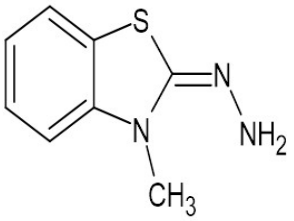


Figure 2 : Structure of MBTH

2. Materials and Methods

2.1 Chemicals and reagents:

All of the chemicals and reagents used were freshly made in distilled water and were of analytical purity.

2.2 2% Preparation of ferric ammonium sulphate:

Taken 600mg ferric ammonium sulphate and 400mg of sulphamicacid and make up with 40ml distilled water and keep at 5°freeze for 10 min.

2.3 1% Preparation of3-methyl-2 benzothiazolinonehydrazone(MBTH)reagent :

Taken 200mg of MBTH (3-methyl-2 benzothiazolinonehydrazone) reagent and 400mg of sulphamic acid and make up with 40ml distilled water and keep at 5°freeze for 10 min

2.4 Instrumentation:

The absorbance of the resultant solution was measured using a double-beam ELICO SL 210 UV-Visible Spectrophotometer 1100, which has a spectrum the bandwidth of 1.8 nm, frequency accuracy of ± 0.5 nm, and two quartz cells that are matched in terms of path length, measuring 1 cm.[8,9,10.]

2.5 StandardPreparation :

The standard solution was prepared by weighing 10mg of Retinol Acetate and dissolving a few milliliters (mL) of Dimethyl Sulfoxide that had been distilled in a 10 mL volumetric flask.The following dilutions are made further.

trial 1 : Retinol Acetate with MBTH

1.6 ml of RA a standard solution was taken and 0.5 ml MBTH reagent was added, and it was shaken for 10 minutes. After 10 minutes, add 0.5 ml of FeCl3 and top it off with diluent. λ max peak was not detected, when scan was done in UV Visible spectrophotometer of ELICO -210.

Trial 2: Retinol Acetate with MBTH

1.6 ml of the standard solution was taken and added 0.5 ml MBTH reagent and shaken for 10 minutes. After 10 minutes, add 0.5 ml of the ferric ammonium sulphate along with top it off with diluent. Absorbance was not to be found and λ max peak was not highlighted.

2.6 Optimized Trial :

The optimized method trial was performed with 10µg/ml Pipetted out 1.0 ml of the standard solution that worked into a 10 ml volumetric flask, added 1 ml of the MBTH reagent, and allowed to sit at room temperature for 20 minutes. Then 2% ferric ammonium sulphate(1ml) was added and left for 15mins at cool temperature. After that make up with distilled water. The resulting solution was observed in UV-Visible spectroscopy and scanned ranging from 400-800nm. the color appears to be a Green Colour The optimized wavelength was found to be 630nm the absorbance 0.5758 Figure 3 displays the absorbance spectrum indicating a peak at 630 nm, which is identified as the optimized wavelength for analysis with a corresponding absorbance of 0.5758, confirming the formation of a green-colored complex.

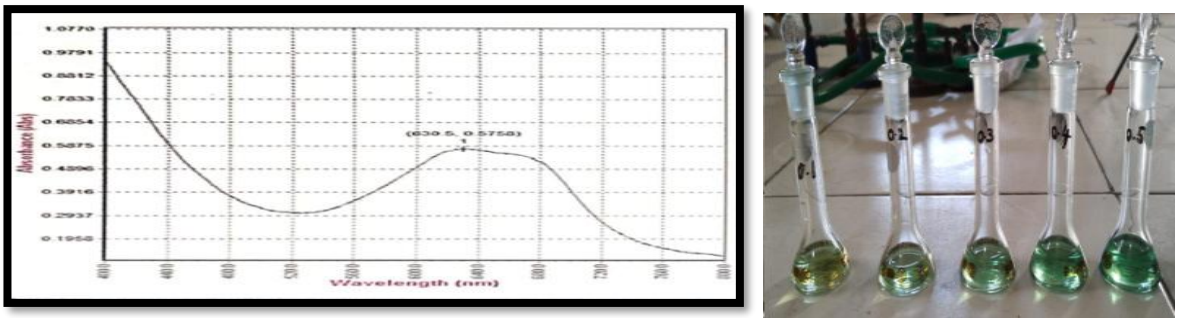


Figure 3 : the absorbance graph indicating the peak used to determine the optimized wavelength for analysis.

2.7 Chemical Reaction : 1 ml of MBTH Reagent and 1 ml of Ferric Ammonium sulphamate and the color appears to be a complicated Green Colour Figure 4 illustrates the derivatization reaction of Retinol Acetate with MBTH reagent in the presence of ferric ammonium sulphate at 5°C, forming a green-colored complex. The reaction scheme highlights the structural transformation resulting from the coupling process.

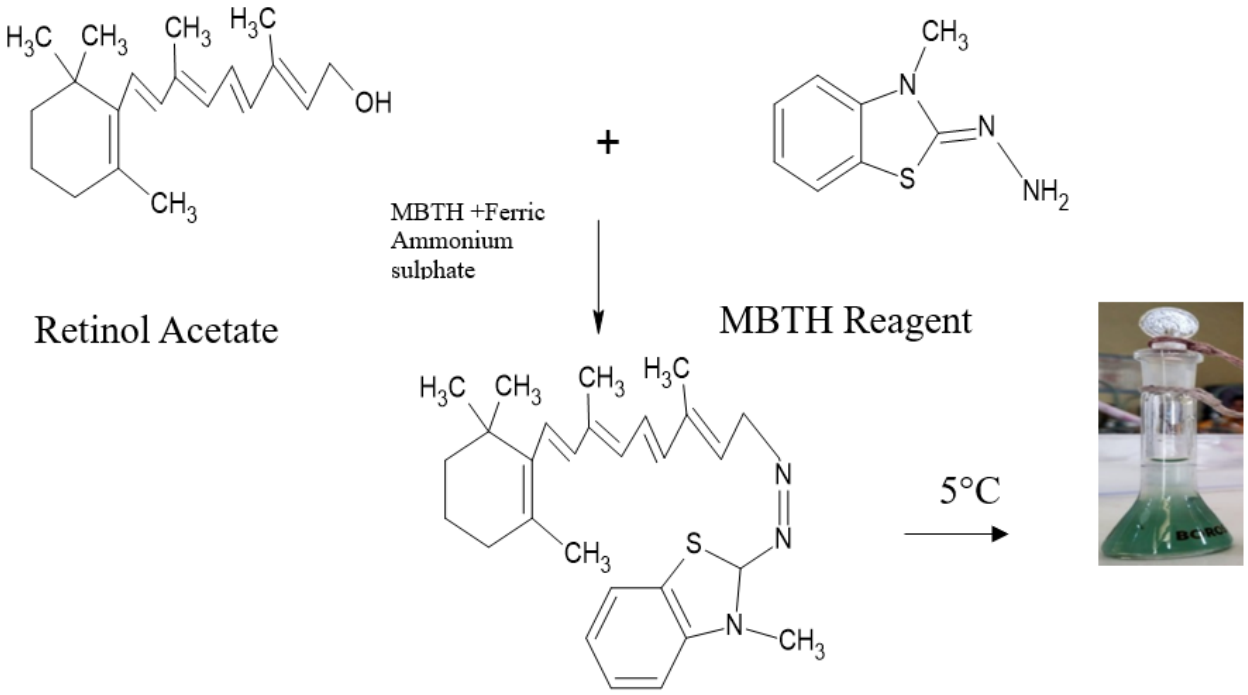


Figure 4 : Reaction scheme showing Retinol Acetate derivatization using MBTH at 5°C

2.8 Method Optimization with Temperature and colour stability :

At ambient temperature R^2 value was found to be 0.0168 was very low compared to cool temperature. Absorbance was measured for every 30 mins and Stability of drug colour reduces due to changes in duration of time for longer period. The absorbance was stable for 2 hours Figure 5 presents two graphs: the left graph shows method optimization at different

temperatures, indicating a strong correlation (R^2 = 0.9993) for one data trend, suggesting increased absorbance with temperature. The right graph illustrates color stability over time, showing that absorbance remains stable up to 120 minutes before significantly decreasing. [11,12.]

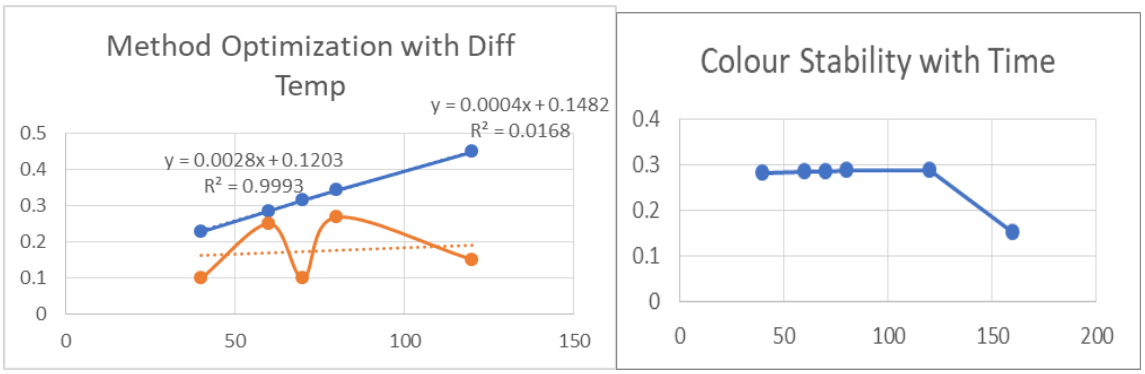


Figure 5 : Method Optimization with Diff Temperature and colour stability

3. Result and Discussion :

3.1 Validation Parameters :

Linearity: The correlation coefficient (r2) has been determined to be 0.9988, being within the acceptable range according to ICH criteria Figure 6 shows a linearity graph for Retinol Acetate with a strong correlation (R^2 = 0.9988), confirming excellent linearity of absorbance versus concentration. The accompanying spectral overlay further supports consistent absorbance patterns across samples.[13,14,15,16]

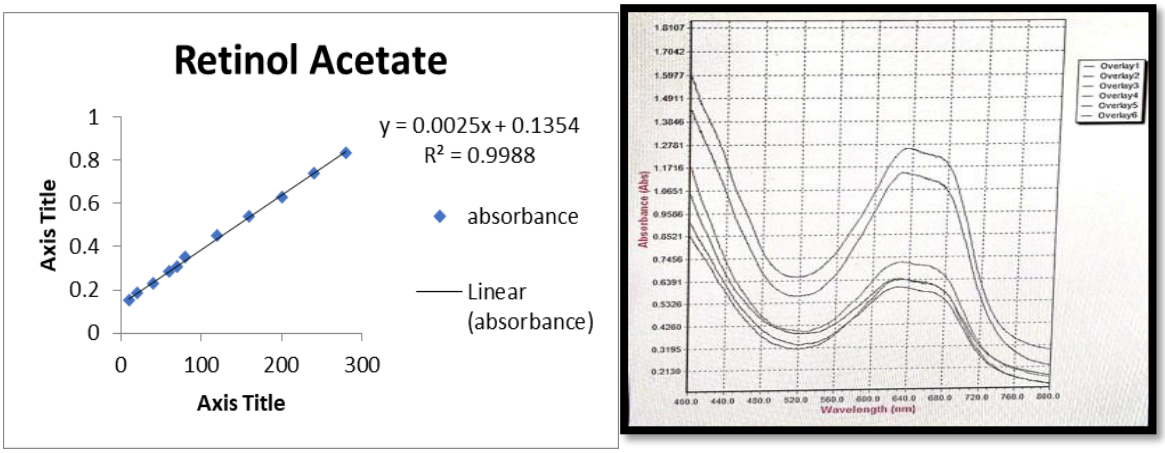


Figure 6 :Linearity

Precision:

Results of the preparation of six times 6 solutions are listed below. It was discovered that the Retinol Acetate %RSD of repeatability was 0.0536.RSD for intra-day precision was determined to be 0.3127 in the morning and 0.152279 in the evening.Similarly, the percentage RSD for inter-day precision was determined to be 0.24644 on day 1 and 0.15764 on day 2.All of those results, as per ICH criteria, were within the acceptable range.[17,18]

Accuracy: Three degrees of additions of chemicals and reagents (50%, 100%, and 150%) were made. 1 ml of MBTH Reagent and 1 ml of Ferric Ammonium sulphamate and the color appears to be a complicated Green Colour Table 1show tabulated data evaluating analytical method accuracy using spiked recovery at different concentration levels (50%, 100%, and 150%), with corresponding absorbance readings and calculated percentage recoveries.[19,20]

The Results: The accuracy recovery range was determined to be 85–115%,

PERCENTAGE LEVEL	SAMPLE ABSORBANCE	SPIKING ABSORBANCE	TOTAL ABSORBANCE (sample Abs +Spiking Abs)	% RECOVERY	MEAN % RECOVERY
50% (70ppm +20ppm	0.2281	0.1861	0.4121	98.87%	98.5%
			0.4113	98.44%	
			0.4112	98.38%	
100% (70ppm +40ppm)	0.2281	0.2281	0.4561	99.95%	99.8%
			0.4559	99.86%	

			0.4555	99.69%	
150% (70ppm + 60ppm)	0.2281	0.2861	0.5145	100%	100%
			0.5149	100.24%	
			0.5150	100.27%	

Table 1 : Accuracy

The Limits of Quantification (LOQ) and Limits of Detection (LOD):
It was determined that the limits of detection (LOD) and limits of quantification (LOQ) were, respectively, 0.40788µg/ml and 1.236µg/ml Table 2 shows the Limit of Detection (LOD) and Limit of Quantification (LOQ) for Retinol Acetate.[21,22]

Drug Name	LOD	LOQ
Retinol Acetate	0.40788 µg/mL	1.236 µg/mL

Table 2: LOQ and LOD

Robustness and Ruggedness:
Two separate analysts conducted the ruggedness analysis; the results were Analyst1%RSD 0.21203 and Analyst2%RSD 0.086261 We examined the robustness using two wavelengths, +1(%RSD 0.14748) along with -1(%0.131958) and the findings were within the acceptable ranges according to ICH guidance.[23,24,25,26]

3.2 Marketed Formulations of Vitamin A :

6.1 Sample Solutions Preparation : Take off the capsule shells from 10 vitamin A capsules. Pour 75 ml of distilled water into the measuring cylinder, then add the MBTH and ferric ammonium sulfate. Take out 0.7ml of it and dilute with purified water. The absorbance at 630nm Figure 12This figure shows a container labeled "Vitamin A Capsules," which is collected from local market representing the formulation used in the analysis. It illustrates the sample source for the quantitative determination of Vitamin A content. [27,28,29]

Figure 7 :Vitamin A Formulation

y=mx+Cc

***X*= 70.88 µg/mL**

=70.88 * 100/ 70

Recovery % =101.25%

3.3 Extraction Method for Juices,Raw Vegetables and Cosmetics :

3.3.1 Samples Used for GROUP - I

Weigh 5mlof fruit juice. After weighing the sample in a beaker, 5 ml of methanol was added, and the layer has been separated by hexane using a separating funnel and vortexing for 10 minutes. The cleared solution was then centrifuged for 10 minutes before being filtered.Gather sample, dilute supernatant in a 100 ml volumetric flask with water that has been supernated using Whatman filter paper.Absorbance of extract was determined at 630 nm.Figure 8shows Different samples were collected from local market which contains Vitamin A for determination.[30,31,32]

Figure 8 : Samples collected from local market

3.3.2 Samples Used for GROUP-II

Weighed 1g of the food sample was weighed in the Beaker to that Solution added 5ml of cooled acetone and vortex for ten minutes. Spin for ten minutes.The cleared solution was then centrifuged for 10 minutes before being filtered Gather sample, dilute supernatant in a 100 ml volumetric flask with water that has been supernated using Whatman filter paper.Absorbance of extract was determined at 630 nm.Figure9 shows Fruits and Vegetables were collected from local market which contains Vitamin A for determination.[33,34,35]

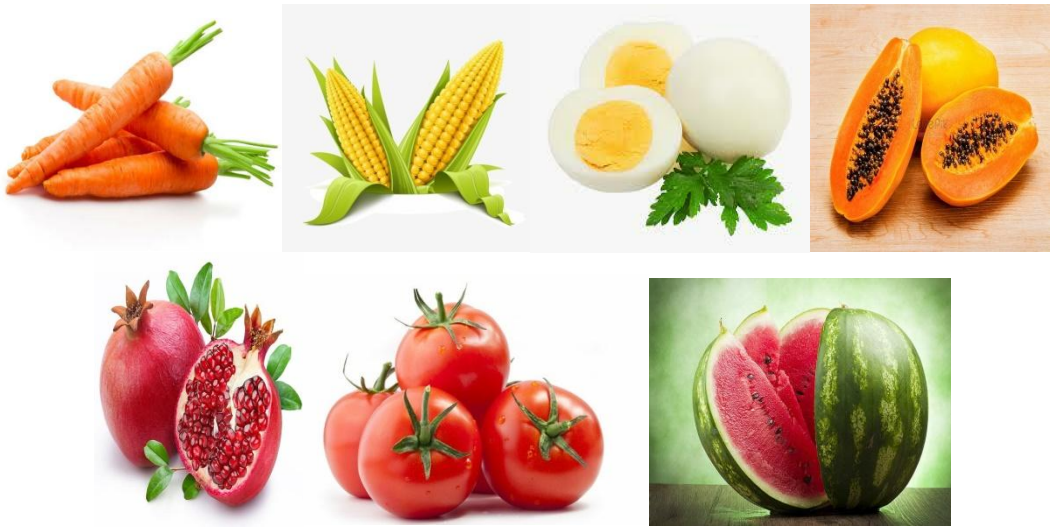


Figure 9 : Raw Vegetables collected from local market

3.3.3 Samples Used for GROUP-III

Weighed 0.5g of food Sample weighed in a Volumetric Flask Diluted up by Ethanol Solution Stirred by Vortex / Sonication for 5min. The cleared solution was then centrifuged for 10 minutes before being filtered.[20, 21,22]Gather the sample, dilute supernatant in a 100 ml volumetric flask with water that has been supernated using Whatman filter paper. Absorbance of extract was determined at 630 nm. Figure 10 shows various cosmetics were collected from local market for determination.[36,37]

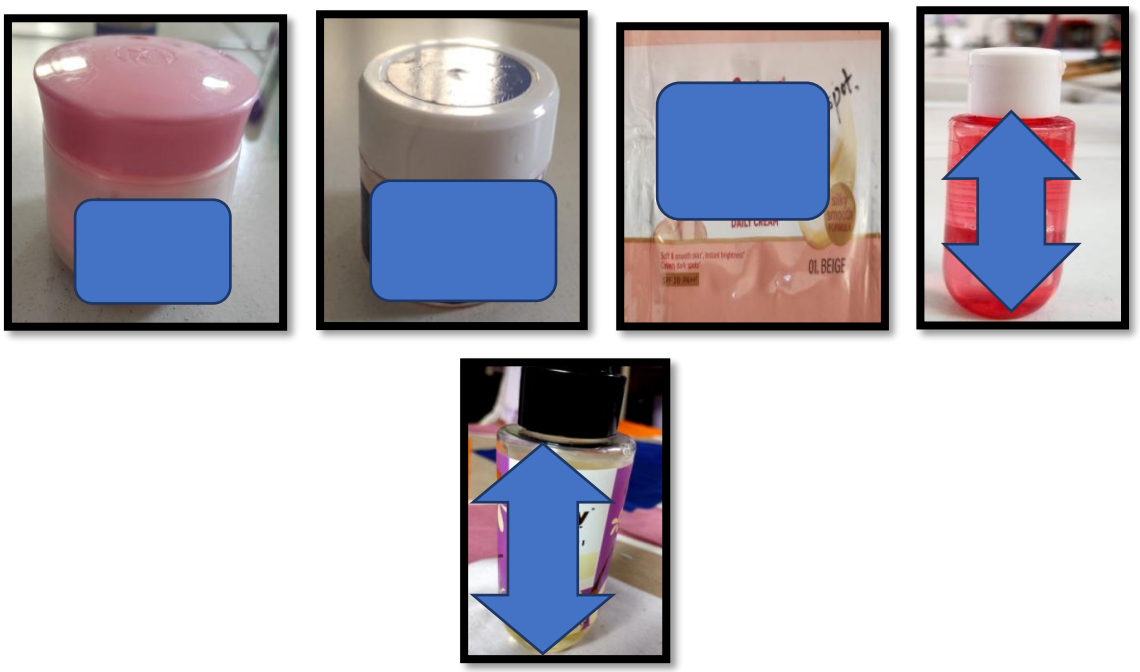


Figure 10 : Cosmetics collected from local malls

3.3.4 Vitamin A Content in Various Samples :

Samples which is procured from locally available Market which contains Vitamin A Content in various SamplesafterExtraction Figure 11is a Extraction of vitamin A from different samples by and proceeding with the MBTH to get green complex.[38]

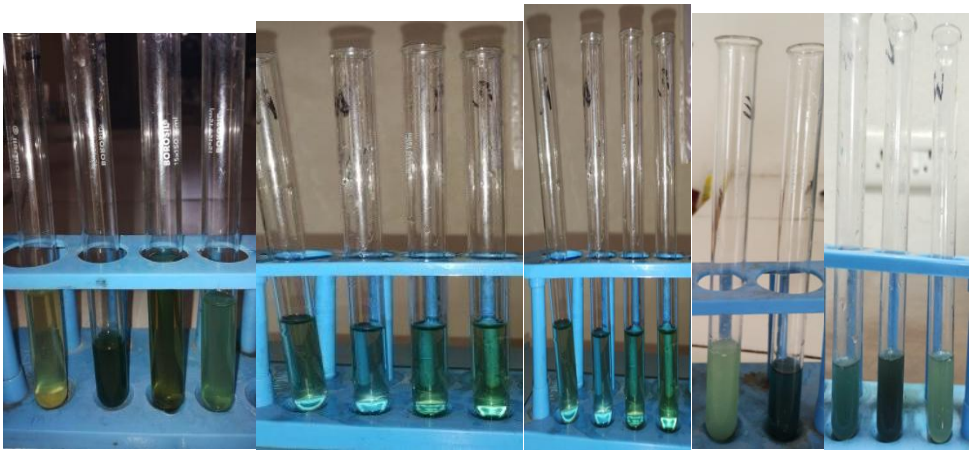


Figure 11 : Represent the results of extraction

Here are the various food items which contains vitamin A where concentration was absorbed and sample absorbance was determined by using the formula (y= mx+c) Table 3(GROUP–I: Fruits & Fruit juices) shows the absorbance and corresponding vitamin A concentration in various fruit samples.[27, 28] Table 4 (GROUP–II: Vegetables) presents vitamin A concentration in vegetable samples based on absorbance values. Table 5 (GROUP–III: Mixed samples) combines results from diverse food items, highlighting high vitamin A content in certain mixed or processed samples.[39,40]

GROUP – I	Sample Absorbance	Concentration
G I - 1	0.2929	63.32 µg/mL
G I - 2	1.9084	709.52 µg/mL
G I - 3	0.5171	152.68 µg/mL
G I - 4	0.4890	141.44 µg/mL
G I - 5	0.0396	38.32 µg/mL
G I - 6	0.3584	89.2 µg/mL
G I - 7	0.3249	75.8 µg/mL
G I - 8	1.336	480.24 µg/mL
G I - 9	0.665	211.84 µg/mL
G I - 10	1.356	488.24 µg/mL

Table 3: GROUP – I Fruits& Fruit juices

GROUP-II	Sample Absorbance	Concentration
G II - 1	0.4879	141 µg/mL
G II - 2	0.1596	9.68 µg/mL
G II - 3	0.355	87.84 µg/mL
G II - 4	1.068	373.04 µg/mL
G II - 5	0.977	336.64 µg/mL

G II - 6	0.543	159.44 µg/mL
G II - 7	1.093	383.04 µg/mL

Table 4 :GROUP-II Vegetables

GROUP-III	Sample Absorbance	Concentration
G III - 1	0.453	127.04µg/mL
G III - 2	1.068	373.04µg/mL
G III - 3	0.880	297.84 µg/mL
G III - 4	1.053	367.04 µg/mL
G III - 5	1.095	383.84 µg/mL

Table 5 : GROUP-III Cosmetics

2. 4. CONCLUSION:

This study set out to create and test a straight forward technique for measuring Retinol Acetate in a variety of food products. Analytical validation was made simple, cost-effective, and precise with the above-mentioned method. The developed method is helpful for routine analysis because the results are within the ranges.

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6. Funding/Conflicts Of Interest

The authors declare no conflicts of interest related to this publication and confirm that all data and methods are accurately represented, ensuring transparency and reproducibility.

7. Author Contribution In this Research Paper

Conceptualization & Methodology: K. Bhavya Sri
Writing - original draft preparation: G. Manusha
Investigation: Narmada Vallakeerthi
Review and editing: Anitha Sadhula
Validation: Dr. V. Kiran Kumar
Formal Analysis: Dr. M. Sumanth

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