drug or dosage formulation (even upto 1.0 μg/ml) is required for analysis. The proposed methods can be used for the routine determination of CPD in the pure form and in pharmaceutical formulations depending upon the availability of chemicals.

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Colorimetric Determination of Vitamin-A with 4- Hydroxy-3-Methoxy-Benzaldehyde

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A simple Spectrophotometric method in the visible region is described for the estimation of vitamin A or its esters. The method is based on the formation of a coloured condensation product with 4-hydroxy-3-methoxy benzaldehyde which shows maximum absorption at 610 nm.

A few colorimetric methods of assay have been reported for a analysis of vitamin A or its ester in bulk samples and pharmaceutical formulations[1-7] H.P.L.C. procedures[8-9] were also developed for the estimation of vitamin A. The present work describes a new, simple, rapid, selective and sensitive method which is based on the reaction with 4-hydroxy-3-methoxy benzaldehyde which gives an intense blue colour with maximum absorption at 610 nm.

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Ethanolic solution of 4 hydroxy-3-methoxy benzaldehyde (0.5% W/V) and ethanolic solution of potassium hydroxide (9% w/v) were prepared. Analytical grade ethanol, sodium sulphate, solvent ether, isopropanol and sulphuric acid were used in the investigation.

Chemito 2500 u.v. visible scanning spectrophotometer was used for all absorbance measurements.

The standard solution of vitamin A was prepared by transfering an accurately weighed portion of vi-

Table - 1: Optical Characteristics, Precision and Accuracy of Vitamin A

Beer's law limits i.u/ml.	10 iu - 50 iu
Molar extinction coefficient (1 mol^{-1} cm^{-1})	2.24×10^4
Sandel's sensitivity (mcg/cm^2/0.001 absorbance Unit)	0.01312
Recoveries (%)	99.1
Relative Standard deviation (%)	1.86

Table - 2: Results of Analysis of Vitamin A in Pharmaceutical Formulations

Sample tested		Lable Amount (I.U.)	Reported[7] method (I.U.)	Prepared method (I.U.)
1.	MULTI-VITAMIN DROPS: Vitamin A 10,000 IU, plus other vitamins	10,000	13,150	13,165
2.	SYRUP : Vitamin A 2000 IU Vitamin C 150 mg. Vitamin D2 150 IU.	2,000	2,015	2,035
3.	TABLETS: Vitamin A 10,000 IU	10,000	11,515	11,545
4.	CAPSULES: Vitamin A 10,000 IU plus other vitamins	10,000	12,115	12,140

High values obtained reflect the overages of Vitamins added.

tamin A (5 mg 15,000 I.U. approx.) to a round bottom flask and refluxed with alcoholic KOH 40 ml for 30 min. The solution was cooled and 30 ml of water was added and the resulting solution was transferred to a conical separator. After adding 2 g of sodium sulphate, the solution was extracted with 50 ml of solvent ether by shaking for 3 min. Extraction was repeated with three additional portions of ether, 20 ml each time. The ether extracts were combined and washed with 20 ml portions of water by swirling gently. The washings were repeated until it was neutral to phenophthalein. The ether extract was evaporated to dryness and the residue was dissolved in ethanol and diluted to a concentration of vitamin A 100 mcg (300 IU. approx)/ml with ethanol.

An accurately weighed portion of the sample (tablets or capsules or syrups or drops) equivalent to 5 mg of vitamin A was treated in the same manner as described under the standard drug solution preparation. In formulations containing vitamin A and D together, vitamin A was separated from vitamin D by passing the solution obtained after saponification, through a column packed with alumina. The vitamin

A that was retained in the column was eluted with ethyl ether. Ether was evoporated and the residue was dissolved in ethanol before development of colour.

Aliquots of ethanolic solution containing 100 IU-500 IU of vitamin A (standard solution) were transferred into a series 10 ml volumetric flasks. To each flask, appropriate volumes of ethanol was added to bring the total volume to 2 ml. In one flask 2 ml of ethanol was added which serve the purpose of the blank. To each flask added 1 ml of 0.5% w/v 4-hydroxy-3-methoxy benzaldehyde solution and 1ml of Conc. sulphuric acid was added cautiously. After 5 min the volume was made upto the mark with isopropanol and the absorbance of the blue coloured solution was measured at 610 nm against the reagent blank. A standard graph was then plotted of absorbance versus concentration. The amount of vitamin A in the sample solution was computed from the standard graph. The coloured product was stable for 6 h.

Beers law limits, molar absorptivity, Sandel's sensitivity, % recovery and % standard deviations are summerised in Table-1. Several commercial formulations containing vitamin A (tablets, capsules, syrups or drops) were determined by the proposed as well as reported methods and data is presented in Table-2.

To evaluate the validity and reproducibility of methods known amount of pure drug was added to the previously analysed samples and mixtures were again analysed by the present method and the results are presented in Table-1 and 2.

The proposed procedure could be useful for the determination of vitamin A with reasonable precision and accuray.

No interference either from other active ingredients or from the excipients used in formulations was noticed in the current estimation and the results obtained were reproducible. The additional advantage of the proposed method is that the coloured product formed is very stable (3 h) unlike many of the reported methods (less than 1 min). The colour of the product formed during the reaction of vitamin A with antimony trichloride[1] or phosphotungstic acid[1] or aluminium chloride[1] or trifluoroacetic acid[2,3] persists up to 5-20 sec. only.

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