To Develop a Simple (UV-VIS Spectrometric) Method for the Estimation of Multivitamin with Special Reference to Capsules & Tablets

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Abstract: Fat-soluble vitamin regulation is of particular significance in cystic fibrosis. A vitamin is an organic compound required as a nutrient in tiny amounts by an organism. In the present study, we had developed a simple method to estimate the fat soluble vitamin by Spectrophotometer. The vitamin A, D, E and K were estimated by spectrophotometer at 325 nm, 264 nm, 525 nm and 635 nm respectively. After calibration of the curves for the absorbance, the data were validated against label claimed on the packaging material. The precision, repeatability, intermediate precision and accuracy were tested. The accuracy of the method was determined by spiking working standards of the four vitamins into the placebo at different concentration levels: 80, 100 and 120% of target concentration of each of the vitamins. The mean recoveries (%) of vitamins A, D, E and K were found to be 99.32, 98.68, 99.10 and 99.28 respectively which are within the acceptance limit. The spectrophotometer estimation is easier than other existing techniques in the pharmaceutical laboratories.

Keywords: Fat soluble vitamins, UV-visual spectroscopy, Estimation, Tablets, Capsule.

Introduction

Vitamins are non-energy producing organic compound, essential for normal human metabolism that must be supplied in small quantities in the diet. Fat-soluble vitamins are absorbed through the intestinal tract with the help of lipids (fats). Because they are more likely to accumulate in the body, they are more likely to lead to hypervitaminosis than are water-soluble vitamins. Fat-soluble vitamin regulation is of particular significance in cystic fibrosis. A vitamin is an organic compound required as a nutrient in tiny amounts by an organism. A compound is called a vitamin when it cannot be synthesized in sufficient quantities by an organism, and must be obtained from the diet. Thus, the term is conditional both on the circumstances and the particular organism. For example vitamins D and K are required in the human diet only in certain circumstances. The term vitamin does not include other essential nutrients such as dietary minerals, essential fatty acids, or essential amino acids, nor does it encompass the large number of other nutrients that promote health but are otherwise required less often. Vitamins are classified by their biological and chemical activity, not their structure. Thus, each vitamin may refer to several vitamer compounds that all show the biological activity associated with a particular vitamin. Such a set of chemicals are grouped under an alphabetized vitamin generic descriptor title, such as vitamin A, which includes the compounds retinal, retinol, and many carotenoids. Vitamins have diverse biochemical functions, including function as hormones (vitamin D), antioxidants (vitamin E), and mediators of cell signaling and regulators of cell and tissue growth and differentiation (vitamin A).

Vitamin A acetate chemically, 15-apo-β-caroten-15-ol,3,7-Dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,4,6,8-tetraen-1-
It is a yellowish powder. Vitamin A occurs in nature in several forms\textsuperscript{9}. Retinal (Vitamin A\textsubscript{2}) is an unsaturated alcohol containing an ‘ionone’ ring. It is present in marine fish (cod, shark and halibut) liver oil, egg yolk, milk and butter. Dehydroretinol (Vitamin A\textsubscript{3}) is present in fresh water fishes. Carotenoids (\(\alpha\), \(\beta\) and \(\gamma\)) and cryptoxanthin, which are fat soluble lipochromes found in plants are converted into vitamin A (active form) in the intestinal mucosa or liver. Retinal generated by reversible oxidation of retinol is a component of the light sensitive pigment Rhodopsin which is synthesized by rods during dark adaptation. This pigment gets bleached and split into its components by dim light and in the process generates a nerve impulse through a G-protein called Transducin. Retinal so released is synthesized in the cones- responsible for vision in bright light, colour vision and primary dark adaptation. It maintains the functional and structural integrity of epithelial cells throughout the body by preventing its metaplasia to stratified squamous type. This vitamin A probably does by stabilizing the \(-\text{SH}\) groups in proteins, maintaining the normal amounts of mucoprotein, glycoprotein and keratoprotein in epithelial structures. Vitamin A is also required for bone growth. A deficiency is night blindness (nyctalopia) i.e. inability to see in poor light. Increased tendency to urinary stone formation may be due to shedding of ureteric epithelial lining which acts as a nidus\textsuperscript{10}.

Vitamin D\textsubscript{1} is chemically, (5\(Z\), 7\(E\))-(3\(S\))-9, 10-secoholesta-5, 7, 10(19)-triene-3-ol\textsuperscript{11}. It is a white crystalline compound\textsuperscript{a}. Vitamin D is the collective name given to antirachitic substances synthesized in the body and found in foods activated by ultraviolet radiation. Vitamin D\textsubscript{1} which mixture of antirachitic substances found in food-only of historic interest. Vitamin D\textsubscript{2} (calciferol), present in irradiated food-yeasts, fungi, bread and milk. Vitamin D\textsubscript{3} (cholecalciferol) is synthesized in the skin under the influence of UV rays. Vitamin D in food requires bile and acids for its proper absorption from the small intestines. Vitamin D itself is inactive. In the body it is converted by hydroxylation to calcifediol and 1, 2, 5-dihydroxycholecalciferol (calcitriol). Alfacalcidol is a precursor of calcitriol (one of the two active metabolites of vitamin D). The primary action of vitamin D is to increase the absorption of calcium and phosphorus from the intestines, and also to reciprocally increase the renal excretion of phosphorus. Such an action is needed for proper mineralization of bone, and for the regulated of normal plasma calcium levels. It is known that large of vitamin D cause decalcification of bone. A deficiency is rickets (in children) and osteomalasia (adult rickets)\textsuperscript{10}.

Vitamin E acetate chemically, (2\textsuperscript{RS}, 4\textsuperscript{RS}, 8\textsuperscript{RS})-6-acetoxy-2, 5, 7, 8-tetramethyl-2-(4\textsuperscript{RS},8\textsuperscript{RS},12\textsuperscript{RS}-trimethyltridecyl)chroman(all-rec\(-\alpha\)-tocopherol acetate)\textsuperscript{12}. It is a clear, slightly greenish yellow viscous oily liquid\textsuperscript{9}. Vitamin E is absorbed from intestine through lymph with the help of bile; it circulates in plasma in association with â-lipoprotein, is stored in tissue and excreted slowly in bile and urine as metabolites. Vitamin E acts as antioxidant, protecting unsaturated lipids in cell membranes, coenzyme Q, etc. from free radical oxidation damage and curbing generation of toxic peroxidation products. No clear-cut vitamin E deficiency syndrome has been described in humans, but vitamin E deficiency has been implicated in certain in hepatobiliary disease and some cases hemolytic anaemia\textsuperscript{10}.

Vitamin K\textsubscript{3} chemically, 2-methyl-1,4-naphthaquinone\textsuperscript{13}. It is a pale yellow crystalline powder\textsuperscript{9}. It is a fat-soluble dietary principle required for the synthesis of clotting factors. Vitamin K has a basic naphthaquinone structure, with or without aside chain (R) at position 3. The side chain in K\textsubscript{1} is phytol, in K\textsubscript{2} prenyl, while in K\textsubscript{3} there is no side chain. Daily requirement is uncertain, because a variable amount os menaquinone (vitamin K\textsubscript{2}) produced by colonic bacteria becomes available. Vitamin K acts a cofactor at late stage in the synthesis by liver of coagulation protein-prothrombin, factors VII, IX and X. The vitamin K dependent change (beta carboxylation of glutamate residues of these zymogen proteins) confers on them capacity to bind Ca\textsuperscript{2+} and to get bound to phospholipid surfaces-properties essential for partition in the coagulation cascade. Vitamin K deficiency is an increased bleeding tendency. Epistaxis, haematuria, ecchymoses, gastrointestinal bleeding, postoperative haemorrhage and intracranial haemorrhage are common\textsuperscript{14}.
Material and Methods

Instrumentation

Shimadzu-1700 UV-Visible double beam spectrophotometer with 1 cm matched quartz cell.

Vitamin A Acetate (Retinol)

Weigh equivalent to 500 IU of sample was taken into round bottom flask. In bottom flask, 2 ml of potassium hydroxide solution (50% w/v), 10 ml glycerol and 50 ml methanol added and mixed well, then reflex for 45 minutes on boiling water bath and cool. Wash the flask with distil water and taken washing in separator then extract with 4×25 ml diethyl ether, combined ether extract washed it with water. Discard the water layer then taken ether layer in dry 100 ml volumetric flask by passed through anhydrous sodium sulphate and make up to 100 ml with diethyl ether, mixed well. It was absorbance recorded at 325 nm against blank.

Vitamin D<sub>3</sub> (Cholecalciferol)

Standard preparation: Weigh accurately 25 mg vitamin D<sub>3</sub> working standard was taken 25 ml volumetric flask with solution mixture (chloroform and methanol in ratio 1:9) dissolved and dilute with solution mixture and make up to the mark mix well.

Sample preparation: Weigh accurately equivalent to 40, 00000 IU vitamin D<sub>3</sub> of sample was taken 25 ml volumetric flask with solution mixture (chloroform and methanol in ratio 1:9) dissolved and dilute with solution mixture and make up to the mark mix well.

It was absorbance recorded at 264 nm against blank.

Vitamin E Acetate (Tocopherol)

Standard preparation: Weigh accurately 25 mg vitamin E acetate working standard was taken into 100 ml volumetric flask and 50 ml of methanol was added to dissolve. The volume was made up to the mark with methanol.

Sample preparation: Weigh accurately equivalent to 25 IU sample of vitamin E acetate was taken in round bottom flask. In round bottom flask, 2 ml of potassium hydroxide solution (50% w/v), 10 ml glycerol and 25 ml methanol was added and mixed well, then reflex for 45 minutes on boiling water bath, cool transfer the solution into separating funnel and extract with 50 ml ether for 5 minutes. Discard the water layer and ether layer filter through anhydrous sodium sulphate. After that evaporate the ether layer to dryness and dissolved with methanol in 100 ml volumetric flask.

Procedure: 5 ml of the standard, sample and blank solution was taken in 25 ml volumetric flask. In each volumetric flask, 2 ml of 0.1% 2, 2 bilyridil solution (in methanol) and 1 ml of 0.1 % ferric chloride solution (in water) added and mixed well. It was diluted 25 ml with methanol and absorbance recorded at 525 nm against blank.

Vitamin K<sub>3</sub> (Menadione)

Standard preparation: Weigh accurately 25 mg of menadione working standard was taken into 100 ml volumetric flask and 50 ml of chloroform was added to dissolve. The volume was made up to the mark with chloroform. After that filter and further 1 ml was taken into 50 ml volumetric flask made up to the mark with chloroform.

Sample preparation: Weigh equivalent 250 mcg of sample was taken into separator. In separator, 5 ml of water was added, mixed well and extract with 4×10 ml chloroform. Discard the water layer then taken chloroform in dry 50 ml volumetric flask by passed through anhydrous sodium sulphate and made up to 50 ml with chloroform.

Procedure: 5 ml of the standard, sample and blank solution was taken into test tube. In each test tube, added 2 ml of 0.2% solution of 2, 4-dinitrophenyl hydrazine (in hydrochloric acid and alcohol in ratio of 1:5 v/v) and mixed well. After that heat on water bath until to almost dryness and cool at room temperature. 15 ml solution mixture (ammonia and alcohol in ratio of 1:1) was added in each test tube. It was absorbance recorded at 635 nm against blank.<sup>[15]</sup>

Statistical Analysis

The proposed methods were successfully applied to the analysis of vitamins (vitamin A, vitamin D<sub>3</sub>, vitamin E and vitamin K<sub>3</sub>) in tablets and capsule. These data were subjected to ANOVA.
test to see any significant difference between the data sets as calculated for p = 0.05.

**Calculation**

\[
\text{Mean} = \frac{\sum(x_1 + x_2 + \cdots + x_n)}{\text{Number of item}(N)}
\]

\[
\text{Standard Division} = \sqrt{\frac{\sum(x - \text{mean})^2}{(n-1)}}
\]

\[
\text{RSD} = \frac{\text{Standard Deviation}}{\text{Mean}} \times 100
\]

The amount of vitamin A was calculated by the following formula:

\[
\text{Amount of vit IU} = \frac{\text{Sample Absorbance}}{\text{Standard Absorbance} \times \text{Factor(1800)} \times \text{Sample Dilution} \times \text{Standard Weight \times Average Weight}}
\]

The amount of vitamins (D_3, E and K_3) was calculated by the following:

**Results and Discussion**

**Calibration Curves**

The linearity for the four vitamins were determined solutions with concentrations of 5-20 µg/ml of vitamin A, 3-20 µg/ml of vitamin E, 5-25 µg/ml of vitamin D_3 and 5-20 µg/ml of vitamin K_3 prepared using working standards of each of the four vitamins. The linear regression data for the calibration curves indicate that the response is linear over the concentration range studies with coefficient of correlation (r) value as 0.9993 of vitamin A, 0.9998 of vitamin E, 0.9981 of vitamin D_3 and 0.9989 of vitamin K_3. Results are summarized in Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>Vitamin D_3</td>
</tr>
<tr>
<td>λ max (nm)</td>
<td>325</td>
</tr>
<tr>
<td>Beer’s law limits (µg/mL)</td>
<td>5-35</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9993</td>
</tr>
<tr>
<td>Regression equation (Y)*</td>
<td></td>
</tr>
<tr>
<td>Slope, b</td>
<td>0.0694</td>
</tr>
<tr>
<td>Intercept, c</td>
<td>0.0943</td>
</tr>
</tbody>
</table>

*Y = bX + c, where X is the concentration of drug in µg/ml.

**Validation of the Method:** The amount found in the claiming the label and their precision for repeatability (intraday-assay precision) and intermediate precision are listed in the table 2.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Label claim</th>
<th>Amount Found*</th>
<th>Percentage*</th>
<th>Standard Deviation*</th>
<th>Precision** Repeatability (intra-assay precision)</th>
<th>Intermediate Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinol (Vitamin A Acetate)</td>
<td>5000 IU</td>
<td>6352.7 IU</td>
<td>127.05</td>
<td>0.3631</td>
<td>0.2858</td>
<td>0.4063</td>
</tr>
<tr>
<td>Cholecalciferol (Vitamin D3)</td>
<td>200 IU</td>
<td>344.53 IU</td>
<td>172.26</td>
<td>0.5326</td>
<td>0.3092</td>
<td>0.3115</td>
</tr>
<tr>
<td>Tocopherol (Vitamin E Acetate)</td>
<td>25 IU</td>
<td>27.09 IU</td>
<td>108.36</td>
<td>0.3268</td>
<td>0.3016</td>
<td>0.7375</td>
</tr>
<tr>
<td>Menadione (Vitamin K3)</td>
<td>70 mcg</td>
<td>74.89 mcg</td>
<td>106.98</td>
<td>0.8533</td>
<td>0.7976</td>
<td>0.9723</td>
</tr>
</tbody>
</table>

*Mean of six determinations. **%RSD of six determination Precision
The validity of the methods for the assay of vitamin was examined by determining precision and accuracy [1]. In this study, we had validated the protocol for UV-visual spectrophotometer and four vitamins were estimated by the same instrument. These were determined by analyzing six replicates of the drug within the Beer’s law limits. The results of analysis of dosage forms are given in Table 2. Precision was measured in terms of repeatability of application and measurement date. Repetability of a standard sample was carried out six sample of the same batch. Precision is usually expressed as the relative standard deviation (co-efficient of variation). Precision should be considered at different levels as follows:

Repeatability (Intra-assay Precision)
Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision. Repeatability of the sample preparation from same homogenous of marketed sample (10, 1000, 250 and 5 µg/ml of vitamin A, vitamin D₃, vitamin E and vitamin K₃ respectively). The low values of relative standard deviation (R.S.D.) indicate good precision of the methods.

The RSD for repeatability of standard preparation is 0.4431, 0.3438, 0.5915 and 0.3216 for vitamin A, vitamin D₃, vitamin E and vitamin K₃ respectively, whereas the RSD for repeatability of sample preparation is 0.2858, 0.3092, 0.3016 and 0.7976 vitamin A, vitamin D₃, vitamin E and vitamin K₃ respectively. This show that the method is precise as RSD is below 2.0% (RSD is not more than 2.0% as a suggested in IP 2010) [17]. Table 2 represents the precision data obtained for the method.

Intermediate precision is usually demonstrated by repeated measurements of the sample used in the repeatability experiment within the same laboratory. Usually the repeatability experiment is repeated on the same sample by a different analyst, on a different day, using different equipment if possible.

Accuracy
The accuracy of the method was determined by spiking working standards of the nine vitamins into the placebo at different concentration levels: 80, 100 and 120% of target concentration of each of the vitamins. The resulting solutions were assayed in triplicate and results obtained were compared with the expected results and expressed as percentage [18, 19]. The mean recoveries (%) of vitamins A, D₃, E and K₃ were found to be 99.32, 98.68, 99.10 and 99.28 respectively which are within the acceptance limit.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Accuracy of Vitamins in Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. No.</td>
<td>Vitamin A</td>
</tr>
<tr>
<td>1</td>
<td>99.12</td>
</tr>
<tr>
<td>2</td>
<td>100.02</td>
</tr>
<tr>
<td>3</td>
<td>98.82</td>
</tr>
<tr>
<td>Mean</td>
<td>99.32</td>
</tr>
<tr>
<td>±SD</td>
<td>0.6244</td>
</tr>
<tr>
<td>RSD</td>
<td>0.6286</td>
</tr>
</tbody>
</table>

Conclusion
A simple, sensitive, highly accurate UV-visual spectrophotometric method for determination of vitamins (vitamin A, vitamin D₃, vitamin E and vitamin K₃) in pharmaceutical capsule and tablet dosage form was developed and validated. The study proved that the UV-VIS Spectrophotometer was the best instrument for estimation of fat soluble vitamin in easier way.

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References


