A Simple and Cheap Methods for Measuring Serum Vitamin A in Cattle Using Only a Spectrophotometer

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Vitamin A is an essential nutrient and has crucial roles in diverse biological functions including vision, reproduction, immunity and cell growth, and its deficiency is one of the most important nutritional disorders in mammals [3, 5, 6]. Vitamin A can be classified into three related compounds, retinol (vitamin A alcohol), retinal (vitamin A aldehyde) and retinoic acid (vitamin A acid). In serum, most vitamin A is present in the form of retinol. Measurement of retinol has been recently achieved by using high-performance liquid chromatography (HPLC), and the values obtained by the HPLC method are believed to be more reliable than those obtained by other measurements such as the colorimetric method [1]. However, the HPLC apparatus is very expensive and its operation requires skillful techniques. We therefore developed a cheap and simple method for the measurement of serum retinol only using a spectrophotometer. The values obtained by this method were comparable to those using HPLC.

Cattle serum was extracted by hexane, and the extract was developed by thin-layer chromatography according to the method of Fung et al. [4]. The chromatogram (Fig. 1), photographed under ultraviolet light, showed that the hexane extract consisted of two main ultraviolet absorbable materials, retinol and β-carotene, which were identified by the simultaneous run of the two compounds. Figure 2 shows the absorption spectra of retinol and β-carotene together with the hexane extract of the serum. Retinol had its absorption maximum at 325 nm, and had no absorbance at 453 nm. β-carotene showed its maximum at 453 nm, but still had absorbance at 325 nm. The spectra of the hexane extract near 453 nm was similar to that of β-carotene, suggesting that absorbance at 453 nm in the extract was largely derived from β-carotene. Indeed, β-carotene concentrations obtained by measuring \( A_{453} \) were in good accordance with those obtained by HPLC (data not shown).

Considering all the results in Figs. 1 and 2 together, we can assume that the net concentration of retinol can be calculated by the subtraction of \( A_{325} \) due to β-carotene from total \( A_{325} \), the absorbance of which is mainly the sum of

![Fig. 1. Thin-layer chromatogram of a hexane extract of cattle serum. Serum (1 ml) was mixed with 1 ml of ethanol containing 20 μg of butylated hydroxytoluene (an anti-oxidant), then with 2 ml of hexane, and shaken mechanically for 10 min. After centrifugation at 800 x g for 10 min, the hexane layer was evaporated under N₂ gas, and redissolved in 50 μl of chloroform-methanol (1:1). An aliquot (20 μl) was spotted on a plate (Kiesel gel 60, Merck Sharp & Dohme, Darmstadt, FRG) and developed by acetone-light petroleum (18:82) in the dark for 40 min. Two μg of retinol (Sigma Chemicals, St. Louis, MO, U.S.A.) and 1 μg of β-carotene (Nacalai Tesque, Kyoto, Japan) were also run for the standards. Spots were detected by UV light.](image-url)
Table 1. Comparison of retinol concentrations determined by the spectrophotometric and HPLC methods

<table>
<thead>
<tr>
<th>Serum #</th>
<th>A_{453}</th>
<th>β-carotene (µg/dl)</th>
<th>A_{325} due to β-carotene</th>
<th>A_{325}</th>
<th>D-C</th>
<th>Retinol* (µg/dl)</th>
<th>Retinol (HPLC) (µg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.026</td>
<td>10.1</td>
<td>0.002</td>
<td>0.071</td>
<td>0.069</td>
<td>37.9</td>
<td>41.3</td>
</tr>
<tr>
<td>2</td>
<td>0.707</td>
<td>274.0</td>
<td>0.047</td>
<td>0.085</td>
<td>0.038</td>
<td>20.9</td>
<td>15.0</td>
</tr>
<tr>
<td>3</td>
<td>0.272</td>
<td>105.4</td>
<td>0.018</td>
<td>0.116</td>
<td>0.098</td>
<td>53.8</td>
<td>57.9</td>
</tr>
<tr>
<td>4</td>
<td>0.759</td>
<td>294.2</td>
<td>0.050</td>
<td>0.165</td>
<td>0.115</td>
<td>63.2</td>
<td>54.8</td>
</tr>
<tr>
<td>5</td>
<td>2.048</td>
<td>793.8</td>
<td>0.135</td>
<td>0.243</td>
<td>0.108</td>
<td>59.3</td>
<td>53.7</td>
</tr>
<tr>
<td>6</td>
<td>1.093</td>
<td>423.6</td>
<td>0.072</td>
<td>0.202</td>
<td>0.130</td>
<td>71.4</td>
<td>65.6</td>
</tr>
<tr>
<td>7</td>
<td>2.753</td>
<td>1,067.1</td>
<td>0.181</td>
<td>0.289</td>
<td>0.108</td>
<td>59.3</td>
<td>57.4</td>
</tr>
<tr>
<td>8</td>
<td>0.627</td>
<td>243.0</td>
<td>0.041</td>
<td>0.112</td>
<td>0.071</td>
<td>39.0</td>
<td>44.4</td>
</tr>
<tr>
<td>9</td>
<td>0.144</td>
<td>55.8</td>
<td>0.009</td>
<td>0.093</td>
<td>0.084</td>
<td>46.2</td>
<td>40.8</td>
</tr>
<tr>
<td>10</td>
<td>0.433</td>
<td>167.8</td>
<td>0.029</td>
<td>0.128</td>
<td>0.099</td>
<td>54.4</td>
<td>54.8</td>
</tr>
</tbody>
</table>

Of 40 sera used, the results for 10 sera are shown in the Table. *Calculated spectrophotometrically from the values of E. For HPLC measurement, hexane extract of serum was prepared using 5 ml of hexane and, after shaking, centrifugation and evaporation as in Fig. 1, the extract was dissolved in 100 µl of ethanol-water (95:5), then an aliquot of 20 µl was applied to a TSK gel ODS-120A column (4.6 x 25 cm) at a flow rate of 1 ml/min.

![Absorption spectra of retinol (A), β-carotene (B) and hexane extract of serum (C). Retinol and β-carotene were dissolved in hexane at a concentration of 1 µg/ml. Serum (1 ml) was mixed with 1 ml of ethanol and 3 ml of hexane, then shaken and centrifuged as described in Fig. 1. The hexane layer was used without evaporation. The samples were scanned using a Hitachi 220A spectrophotometer.](image)

![Correlation between serum retinol concentrations determined from the spectrophotometric (SPM) and HPLC methods. A regression line obtained was y=0.92x+1.59 (n=40).](image)

retinol and β-carotene. The following equations were used:

(1) β-carotene (µg/dl) = A_{453}/0.00258;
(2) retinol (µg/dl) = (A_{325} - β-carotene conc. x 0.00017)/0.00182,

where A_{453} at 1 µg/dl of β-carotene=0.00258, A_{325} at 1 µg/dl of β-carotene=0.00017, and A_{325} at 1 µg/dl of retinol = 0.00182. These absorption coefficients obtained using freshly prepared retinol and β-carotene were similar to the reported
values [2]. At first, we measured $A_{453}$ of the hexane extract to determine the $\beta$-carotene concentration (Table 1). Next, $A_{325}$ of the extract was measured. From the $A_{325}$, the absorbance derived from $\beta$-carotene was subtracted, and finally the subtracted value was converted to the retinol concentration.

Using 40 cattle sera, we compared the values determined from the spectrophotometric and HPLC methods, and obtained a good correlation coefficient ($r=0.740$) in the range of 15–70 $\mu$g/dl concentrations (Fig. 3). The spectrophotometric values sometimes showed higher (up to 1.4-fold) than the HPLC values. The reason for the higher values can be explained, at least partly, by the presence of minor unidentified substances in the hexane extract. By HPLC, minor peaks near the retinol peak were detected (figure not shown). There is a tendency, when the minor peaks are relatively large, for the spectrophotometric method to yield higher values. However, the spectrophotometric method is practically available to determine the gross concentration of serum retinol. This method is simple and the cost required is very low as compared to the HPLC method. We herein propose that this method is useful for routine serum vitamin A assay in a large majority of local veterinary facilities which have no HPLC apparatus.

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REFERENCES


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分光光度計を使用するだけの牛血清中ビタミンAの簡易かつ安価な測定法 (短報)：鈴本淳一・加藤憲夫1)（山形県家畜保健衛生所, 1)家畜衛生試験場北海道支場）——分光光度計を使用するだけで、牛血清中のビタミンAを測定する簡易かつ安価な方法を開発した。本法で得られた測定値は高速液体クロマトグラフィーで得られた値と良く相関した。