STUDIES ON THE DETERMINATION OF VITAMIN A IN THE PRESENCE OF NEOVITAMIN A

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The methods of vitamin A determination described in the Pharmacopeia of several countries are mostly based on the ultraviolet spectrophotometric studies in which three points correction suggested by Morton and Stubbs (1) is employed. The methods of the United States Pharmacopeia (USP) XVI (2), British Pharmacopeia (BP) (3), Japan Pharmacopeia (JP) VII (4) and Deutsches Arzneibuch (DAB) (5) are quite similar in the points that the absorbancies of the sample in isopropyl alcohol are estimated at 310, 325 and 334 mµ to obtain the correction factor, f, and that the I.U. value per g of the sample is calculated from the following formula where F is the conversion factor.

\[ E_1^{\lambda} cm^{-1} (325 m\mu) \times f \times F \]

However, they are different in the values of F and f as shown in Table I and hence the I.U. values of vitamin A determined by each method are different.

<table>
<thead>
<tr>
<th>Pharmacopeia</th>
<th>F</th>
<th>Calculation of f</th>
</tr>
</thead>
<tbody>
<tr>
<td>USP XVI, BP</td>
<td>1830</td>
<td>[ 6,815 - 2,555 \times E_{313}/E_{325} - 4,260 \times E_{334}/E_{325} ]</td>
</tr>
<tr>
<td>JP VII</td>
<td>1900</td>
<td>[ 7 - 2,625 \times E_{313}/E_{325} - 4,375 \times E_{334}/E_{325} ]</td>
</tr>
<tr>
<td>DAB</td>
<td>1830</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

Note: (a) In USP XVI and BP, it is defined that \( 0.970 \leq f \leq 1.030 \).
(b) According to BP, alumina chromatography is to be employed when the ultraviolet absorption maximum of the sample solution does not lie at 323 to 327 mµ or \( E_{300}/E_{325} > 0.73 \).

Morton-Stubs method was originally employed by USP XIV (1950) and it is still being used in the present JP VII. The USP XIV method was based on the World Health Organization’s (WHO) decision of 1948 in which 0.344 µg of crystalline all-trans vitamin A acetate was defined as 1 I.U. and on the recommendations of WHO (6) in which \( E_1^{\lambda} cm^{-1} 325 m\mu (E_1^{\lambda} cm^{-1} 325) \) of all-trans vitamin A alcohol

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³ BP involved another method in which the sample is dissolved in cyclobexane without saponification.
was 1750 and the ratio $E_{310}/E_{325} = E_{334}/E_{325}$ was 6/7.

Afterwards, however, Bolding et al. (7) studied on the more purified vitamin A and found that $E_{1\%}^{1\text{cm}^2}$ of all-trans vitamin A alcohol was not 1750 but 1820 and the ratio $E_{310}/E_{325} = E_{334}/E_{325}$ was not 6/7 but 5.815/6.815. Because of these facts, they suggested the revision of the USP XIV method. In 1958 the International Union of Pure and Applied Chemistry (IUPAC) accepted their suggestion and employed a new method based on the values reported by them (8, 9).

Accordingly, USP also revised in the second addendum of XV in 1958 and finally fixed the present method in XVI (1960). BP revised following USP in its addendum in 1960. On the other hand, JP VII employed the USP XIV method, though this was fixed in 1961, a year after the publication of USP XVI. This is due to the following reasons: (a) it was expected that when the USP XVI method was employed instead of USP XIV, the determined values of vitamin A would be 6% lower and hence this might disturb markets; (b) it was assumed that since the USP XVI method was based on the constants of all-trans vitamin A, the determination values would be correct for the sample containing only all-trans vitamin A, while the determined values would be lower than the biological potencies for the sample containing about 10 to 40% of neovitamin A such as cod-liver oil (10) or pharmaceutical preparations (11). However, the latter, being only a speculation, was not confirmed by experiments at the publication of JP VII.

The methods of DAB (1959) is the moderate one and is situated between USP XVI and JP VII. But the reason why such method was employed is not clear. One can only assume that this would be due to the same reason as that encountered in the publication of JP VII.

It seems to be obvious that the application of Morton-Stubbs method to the samples containing both all-trans vitamin A and neovitamin A is not appropriate and that the determination of each component after the separation is more suitable. But no adequate separate method has been reported.

Therefore, the present studies were undertaken on the mixed sample of pure all-trans vitamin A and neovitamin A according to the USP XVI, JP VII and DAB methods in an attempt to determine whether there were any suitable method to give the approximate values for biological potencies without separate treatment or not.

**EXPERIMENTAL**

**Materials**

1. **Crystalline All-trans Vitamin A Acetate**

The material of Riken Vitamin Oil Co., Ltd. was used. The properties of this crystalline all-trans vitamin A acetate and its unsaponifiable matters (all-trans vitamin A alcohol) are given in Tables II and III. They are in good agreement with those previously reported.

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As far as the treatment in the present studies is concerned, both the USP XVI and BP are essentially the same and they are denoted as USP XVI for convenience in the following description.
TABLE II
Properties of All-trans Vitamin A Acetate

<table>
<thead>
<tr>
<th>Properties</th>
<th>Values found</th>
<th>Values in literatures or the theoretical</th>
</tr>
</thead>
<tbody>
<tr>
<td>mp (°C)</td>
<td>57 to 58</td>
<td>57.5 to 58.0 (12)</td>
</tr>
<tr>
<td>$E_{1%}^1$ cm$^{-1}$ mL$^{-1}$</td>
<td>1,545</td>
<td>1,535 (13)</td>
</tr>
<tr>
<td>t.c./g (USP XVI)</td>
<td>2,833,000</td>
<td>2,907,000</td>
</tr>
<tr>
<td>t.c./g (JP VII)</td>
<td>3,021,000</td>
<td>2,907,000</td>
</tr>
<tr>
<td>t.c./g (DAB)</td>
<td>2,910,000</td>
<td>2,907,000</td>
</tr>
</tbody>
</table>

TABLE III
Properties of Unsaponifiable Matters of All-trans Vitamin A Acetate

<table>
<thead>
<tr>
<th>Properties</th>
<th>Values found</th>
<th>Values in the literatures or the theoretical</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_{314}/E_{265}$</td>
<td>0.853</td>
<td>0.853</td>
</tr>
<tr>
<td>$E_{314}/E_{265}$</td>
<td>0.853</td>
<td>0.853</td>
</tr>
<tr>
<td>$f_{[USP XVI]}$</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>$f_{[JP VII]}$</td>
<td>1.027</td>
<td>1.029a</td>
</tr>
<tr>
<td>$f_{[DAB]}$</td>
<td>1.027</td>
<td>1.029a</td>
</tr>
</tbody>
</table>

a Calculated for $f$ of JP VII and DAB, using the theoretical value of $E_{314}/E_{265} = E_{334}/E_{325} = 0.853$.

2. Crystalline Neovitamin A Alcohol

The material of Sumitomo Chem. Ind. Co., Ltd. was used. The properties of this crystalline neovitamin A alcohol are given in Table IV. They are in good agreement with those previously reported. Because of its unstable property the sample was sealed in a N$_2$-filled ampoule and preserved at $-30^\circ$. Weighing was carried out rapidly in a weighing bottle with a ground glass stopper. When the crystalline neovitamin A alcohol was dissolved in isopropyl alcohol, it was stable for 3 to 4 hours.

On other reagents JP VII is to be refered.

Methods

Isopropyl alcohol solution of the unsaponifiable matters of crystalline all-trans vitamin A acetate was prepared by the conventional method. The vitamin A concentration of the solution was determined by USP XVI and adjusted to
2 μg/ml (6.67 I.U./ml). This was denoted as the all-trans vitamin A solution.

On the other hand, crystalline neovitamin A alcohol was weighed and dissolved in isopropyl alcohol to make 2 μg/ml concentration. This was denoted as neovitamin A solution.

The mixed sample solutions containing the weight ratio of all-trans vitamin A to neovitamin A of 10:0 to 5:5 were prepared by mixing the all-trans vitamin A and neovitamin A solutions. Determinations of the vitamin A values of these mixed sample solutions were carried out without chromatographic separation by three different methods, USP XVI, JP VII and DAB.

**RESULTS**

As shown in Table V, the values of the mixed sample solutions and of the neovitamin A solution were compared with the calculated biological potencies by setting the biological potency of all-trans vitamin A as 100 and that of neovitamin A as 75.3 according to Ames et al. (15). The differences between the two are listed in Table VI.

**DISCUSSION**

$E_{318}/E_{325} = E_{334}/E_{325}$ of all-trans vitamin A alcohol was found to agree well with 0.853 (=5.815/6.815), as was pointed out by Bolding et al. (7). The $f$ value of all-trans vitamin A alcohol estimated by the USP XVI method was 1.000, whereas that estimated from the JP VII and DAB methods were greater than 1.000.

5 It is obvious that the USP XVI method gives a proper value for all-trans vitamin A.
Because of these observations and the fact that the vitamin A values of crystalline all-trans vitamin A acetate determined by the latter two methods exceeded the theoretical value, it might be concluded that the USP‡Y method was most suitable for the determination of all-trans vitamin A rather than other two methods, as mentioned previously.

It can also be concluded that the USP‡Y method is the most desirable one because it gives the closest values to the calculated biological potencies of the mixtures of all-trans vitamin A and neovitamin A and separate determination of all-trans vitamin A and neovitamin A is not necessary.

**SUMMARY**

It has been confirmed that the USP XVI method is the most desirable one for the determination of all-trans vitamin A and of a mixture of all-trans vitamin A and neovitamin A.

**ACKNOWLEDGEMENT**

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**REFERENCES**