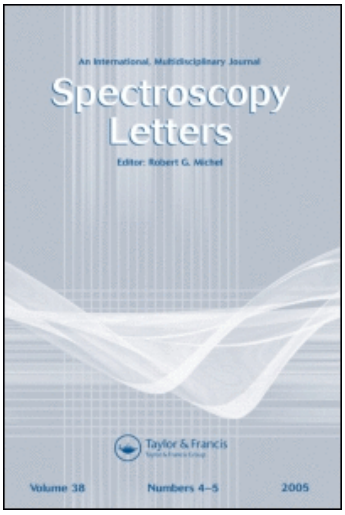

Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

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M. S. Jovanović[a]; M. Gutalj[b]; D. Ivanović[c]
[a] Laboratory for Radioisotopes, Vinca Institute of Nuclear Sciences, Belgrade, Yugoslavia [b] ICN Jugoslavia, Belgrade, Yugoslavia [c] Department of Pharmaceutical Chemistry and Drug Analysis, Faculty of Pharmacy, Belgrade, Yugoslavia

To cite this Article Jovanović, M. S. , Gutalj, M. and Ivanović, D.(2000) 'Influence of Lanolin and White Soft Paraffin on the Determination of Vitamin a in Fats', Spectroscopy Letters, 33: 2, 227 — 234
To link to this Article: DOI: 10.1080/00387010009350072
URL: http://dx.doi.org/10.1080/00387010009350072

SPECTROSCOPY LETTERS, 33(2), 227–234 (2000)

INFLUENCE OF LANOLIN AND WHITE SOFT PARAFFIN ON THE DETERMINATION OF VITAMIN A IN FATS

Key words: UV spectrophotometry, vitamin A palmitate, anhydrous lanolin, white soft paraffin, pharmaceutical interaction

M. S. Jovanović
Laboratory for Radioisotopes, Vinca Institute of Nuclear Sciences, P. O. Box 522, 11000 Belgrade, Yugoslavia

M. Gutalj
ICN Jugoslavija, Quality Control, 29 Novembar 111, 11000 Belgrade, Yugoslavia

D. Ivanović*
Department of Pharmaceutical Chemistry and Drug Analysis, Faculty of Pharmacy, Vojvode Stepe 450, 11000 Belgrade, Yugoslavia

ABSTRACT

Pharmaceutical interaction of the most frequently used substances with fats: lanolin, white soft paraffin and vitamin A palmitate was studied. The mixtures were examined by TLC with different ratios of lanolin and vitamin A palmitate, and white soft paraffin and vitamin A palmitate. Vitamin A in the mixtures was determined by the UV spectrophotometric method according to BP 93. It was established that the rise of lanolin concentration resulted in an increased apparent content of vitamin A in fats as a consequence of lanolin absorption at the wave length of maximum absorption of vitamin A. By an increase of white soft paraffin concentration, an apparent decreased in the content of vitamin A in fats was obtained. Therefore, the suggested UV spectrophotometric method for vitamin A determination has a limited application on fats with high content of lanolin and white soft paraffin.

* To whom correspondence should be addressed

INTRODUCTION

A series of methods have been described in the literature that are used to determine vitamin A and its esters in pharmaceutical preparations, food, biological fluids, and tissues. To determine vitamin A acetate and vitamin A palmitate in a mixture, an IR - spectrophotometric method[1] was used. In order to differentiate and determine vitamin A and its isomers in different biological fluids, the HPLC method was used with electrochemical, fluorescent, and UV detection[2-6]. For the determination of vitamin A in serum, the fluorimetric method[7] was also applied.

Other described methods for the determination of vitamin A, directly or after saponification, are the UV spectrophotometric and colorimetric methods such as the Carr-Price reaction, Budowski and Bondi method, a method with glycerol dichlorhydrin and others[8]. The UV spectrophotometric method for vitamin A determination in pharmaceutical preparations has been recommended in national[7-10]. Pharmacopoeias, like USP XXIII and BP 93.

The degradation products of vitamin A - isomers of vitamin A as well as other carotenoids and sterols may interfere with vitamin A or inhibit the color influencing the specific and accurate spectrophotometric and colorimetric determination of vitamin A. Therefore it is necessary in some cases to have previous chromatographic cleansing of the sample, which complicates the procedure even more.

By the suggested UV method - spectrophotometric method according to BP 93 for vitamin A determination in different medicinal fats, it was established that in fats with different content of fatty basis an unrealistic low or high percentage of vitamin A content might be observed. It was presumed that probably a component of the base influenced the determination of vitamin A. The largest amount and most frequently used additional substances in fats have been lanolin and white soft paraffin. The purpose of this study was to investigate the presence of a pharmaceutical interaction of lanolin and white soft paraffin, and vitamin A.

EXPERIMENTAL

Apparatus

The VARIAN CARY 3 UV - VIS Spectrophotometer with a 1 cm i.d. quartz cell was used.

Materials and reagents

All substances and solvents used were of analytical reagent grade. The substances were obtained from commercial sources:
White soft paraffin - CHEMAG GMBH, Wien, Austria
Lanolin - CHEMAG GMBH, Wien, Austria

Vitamin A palmitate 1 : 1.500000 - Merck, Darmstadt, Germany
Diethylether - Lek, Ljubljana, Slovenia
Chloroform - Zorka, Sabac, YU
Cyclohexane - Kemika, Zagreb, Croacia
Anhydride of vinegar acid - Merck, Darmstadt, Germany
Absolute ethanol - Zorka, Sabac, YU
Isopropanol - Reanal, Budapest, Hunagary
Diethylacetate - Merck, Darmstadt, Germany
Potassium-hydroxide - Zorka, Sabac, YU
Hydrohinone - Reanal, Budapest, Hungary
Natrium-sulphate, anhydrous - Zorka, Sabac, YU
Antimony (III)-chloride - Kemika, Zagreb, Croacia
Silica-gel G plates 0.25mm, 20x20cm - Merck, Darmstadt, Germany

Procedure

Mixtures were prepared with a different mass ratio of lanolin and vitamin A palmitate (lanolin/vitamin A palmitate = 10-1400) and white soft paraffin and vitamin A palmitate (white soft paraffin/vitamin A palmitate = 10-1400).

TLC method

Chloroform solutions (0.1 ml) of the working standard of vitamin A palmitate were spread on the silica-gel G plates, samples of mixtures with different content of white soft paraffin and lanolin, and white soft paraffin and lanolin of the same concentration as in the mixtures. Vitamin A palmitate concentration in all solutions was 100 μg/ml. As a mobile phase, a cyclohexane: diethylether mixture (4 : 1) was used. After drying, the plates were sprinkled with the solution of antimony(III) chloride with anhydride of vinegar acid and heated at 100° C 5-10 minutes.

UV spectrophotometric method

The UV spectrophotometric method was used for the determination of vitamin A in mixtures, with a prior saponification, according to BP 93. The concentration of vitamin A in ether extract was 2-4 μg/ml.

RESULTS AND DISCUSSION

Pharmaceutical interaction of lanolin, white soft paraffin, and vitamin A in mixtures with different mass ratios of lanolin, white soft paraffin, and vitamin A palmitate was studied using the TLC method (Table 1). It was determined that the chromatograms of the standard of vitamin A palmitate of all samples of the mixtures with different content of lanolin, (A, B, C) and the mixture with a lower content of white soft paraffin (D) had the same intensity, shape and Rf value (0.90) as the smears which had originated from vitamin A palmitate. On the

TABLE 1
Examined mixtures with a different ratio of vitamin A palmitate and lanolin/white soft paraffin

Mixture	lanolin/white soft paraffin vitamin A palmitate
A	10
B	200
C	1400
D	10
E	900
F	1400

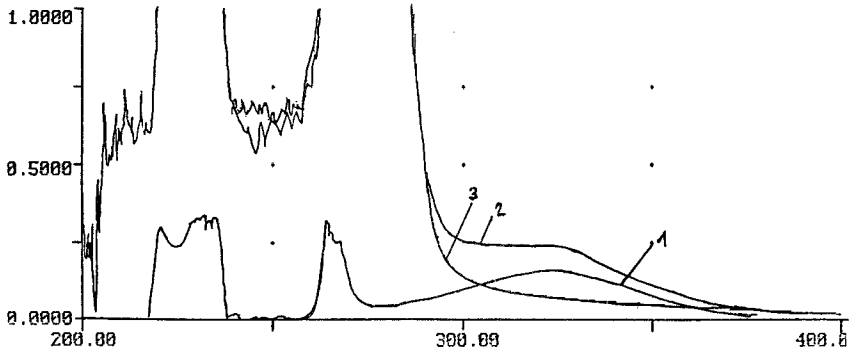

Figure 1.
Absorption spectra of ether extracts of the standard of vitamin A (curve 1), mixture C (curve 2) and lanolin (curve 3). $C_{vit.A}$ = 2.3 μg/ml

chromatograms of the samples of the mixtures with a high content of white soft paraffin (E and F), the smear originating from vitamin A palmitate had a lower Rf value (0.77) compared with the smear of the standard. White soft paraffin alone showed no smears on the chromatogram. It suggests a possible existence of pharmaceutical interaction of white soft paraffin and vitamin A.

Vitamin A was determined, in the mentioned mixtures, by the UV spectrophotometric method according to BP 93. After saponification, vitamin A was extracted by diethylether. Absorption spectra of etheric extracts of the

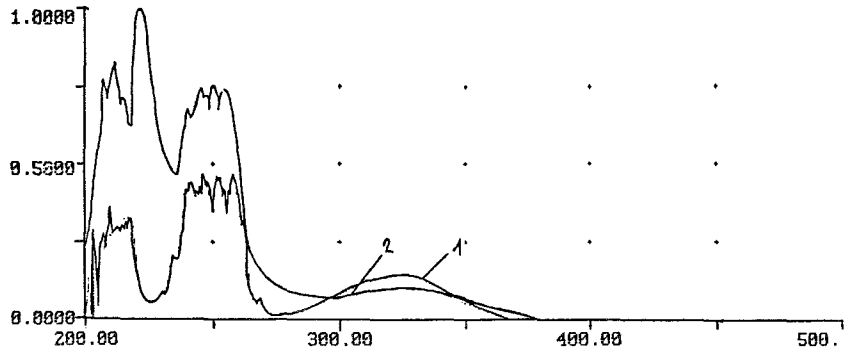

Figure 2.
Absorption spectra of ether extracts of the standard of vitamin A (curve 1) and mixture F (curve 2). $C_{vit.A}$ = 2.3 μg/ml

standard of vitamin A and the examined mixtures ($C_{vit.A}$ = 4 μg/ml) within the range of 200-400 nm, with diethylether as a blind test, were recorded. The absorption spectra of the standard vitamin A, mixture C and mixture F are presented in **Figs. 1 and 2.** The maximum absorption of vitamin A is at λ = 325 nm. The readings of absorbency are 300, 310, 325 and 334 nm.

The applied spectrophotometric method was not specific enough because, in the presence of compounds that absorb light at the same wave length (300-350 nm) like vitamin A, unrealistic results of the vitamin A content were obtained. Therefore a correction was made of the value of absorbency of the solution at 325 nm, according to BP 93:

$$A_{325\,(cor)} = 6.815 \times A_{325} - 2.555 \times A_{310} - 4.260 \times A_{334}$$

When calculating the content of vitamin A, the value of the corrected absorbency of 325 nm may be used only if the maximum absorbency of the solution is between 323 and 325, as well as when the ratio of absorbencies measured at 300 and 325 is lower than 0.73.

In Table 2 the results are shown for the UV spectrophotometric determination of vitamin A content in mixtures with different lanolin content. In mixtures A and B, in which there is 10 to 100 times more lanolin than vitamin A, the real content of vitamin A was obtained (105.76% and 107.31%). Lanolin in the concentrations in the mixtures A and B scarcely absorbed at the wavelength of the maximum of the absorption of vitamin A at 325 nm (Table 2). The Table shows that $A_{300}/A_{325} > 0.73$ for the mixture C with a high content of lanolin. In this

TABLE 2

Vitamin A content in mixtures with different lanolin content

Sample	$A_{325\ (non\ cor)}$	A_{300}/A_{325}	Vitamin A content (%) n = 5
Standard vitamin A for A and B	0.3306	0.71	–
A	0.3360	0.72	105.76
B	0.3583	0.65	107.31
Standard vitamin A for C	0.1514	0.60	–
C	0.2313	1.10	160.26
Lanolin A	0.0000	–	–
Lanolin B	0.0090	–	–
Lanolin C	0.0698	–	–

TABLE 3

Vitamin A content in the mixture C after a decrease of absorbency for the value of the lanolin absorbency

Sample	$A_{325(non\ cor)}$ $(A_c - A_{lanolin})$	A_{300}/A_{325}	Vitamin A content (%) n = 5
Standard vitamin A	0.1514	0.60	–
C	0.1615	0.72	99.31

TABLE 4

Vitamin A content in mixtures with a different content of white soft paraffin

Sample	A_{300}/A_{325}	Vitamin A content (%) n = 5
Standard vitamin A for E	0.65	–
E	0.63	89.64
Standard vitamin A for F	0.56	–
F	0.79	65.93

case the total signal, counted as an uncorrected value of absorbency, resulted in a high content of vitamin A (i.e., 160.26%). The lanolin absorbency value at 325 nm, as in the fat C mixture, are significant. The absorbency of ether extracts of mixture C were small and were corrected according to the given equation. Table 3 shows that the absorbency of ether extracts of mixture C were small relative to the ether extracts of lanolin at the same concentration as in the mixture C, yielding a more realistic vitamin A content of 99.31%.

In Table 4 the results are shown of the vitamin A content determination in the mixtures with a high content of white soft paraffin (E and F). For the mixture with a higher content of white soft paraffin the result was $A_{300}/A_{325} > 0.73$, and that was the reason why the correction was not made, but it was calculated with an uncorrected value of absorbency. For both mixtures a lower content of vitamin A was obtained (89.64% and 65.93%). The absorption spectrum of the ether extract of white soft paraffin showed that white soft paraffin did not absorb at the examined wavelengths and did not influence the determination of the vitamin A content.

CONCLUSIONS

These examinations showed that with an increase of lanolin quantities a higher vitamin A content was obtained, above the allowed limits (±10%) in mixtures as a consequence of lanolin absorption at the wave length of the maximum of absorption of vitamin A. The problem may be solved by correcting absorbency of the mixture for the value of absorbency of lanolin of the same concentration or by measuring, with an ether extract as a blind test. An increase of concentration of white soft paraffin resulted in a decrease of vitamin A content in the mixtures with white soft paraffin (10 - 35%) which, along with the chromatographic investigations, speaks in favor of the existence of a pharmaceutical interaction of white soft paraffin and vitamin A. The influence of white soft paraffin on the determination of the vitamin A content by the UV spectrophotometric method cannot be avoided and it should be kept in mind when formulating fats and selecting an analytical method of determination of vitamin A content in them.

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Date Received: January 30, 1999
Date Accepted: September 18, 1999