MASS SPECTRA OF VITAMIN A ALCOHOL AND RELATED COMPOUNDS IN DEHYDRATED FOOD SYSTEM

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SUMMARY : The mass spectra of vitamin A alcohol and related compounds were investigated in a dehydrated food system using a Karto mass spectrometer. Upon electron impact, each compound gave its molecular ion, m/e=M^+ and peaks showing the loss of its functional group, thereby providing a quick and unambiguous method of identification. The molecular ions, m/e 328 and m/e 284 were the base peaks for retinyl acetate and vitamin A aldehyde respectively. The molecular ion (m/e 286) was the base peak for vitamin A alcohol and its isomers, which were found more complex and similar to each other. The occurrence of m/e=M^+-18 or fragment derived from this ion, predicted the presence of the hydroxyl group. Approximately 1 ug of pure compound was needed for measurable identification.

Key Words : Vitamin A, alcohol.

INTRODUCTION

Vitamin A contents in dehydrated foods is greatly affected by the moisture contents, oxygen, uv rays or sunlight, the presence of mineral elements, and chlorinated solvents used during preparation and extraction (1-4). The major degradative products of vitamin A during photolysis in chlorinated solvents (chloroform and dichloromethane) were 13-cis, 9-cis, 9,13-dicis, 11-cis and 11,13-dicis (1) which have lower biological activities i.e., 75%, 21%, 24%, 24%, 15%, respectively except all-trans vitamin A, a biological substance 100% active in human body (5).

Literature regarding the mass spectra of the degradative products of vitamin A could not be traced, although some reports have been published on the mass spectra of retinyl acetate and some vitamin A compounds (6-8) in foods. Mass spectra have also reported on the isolation of metabolites of retinoic acid particularly the 13-cis retinoic acid (9). Keeping in view the nutritional significance of dietary vitamin A and its isomers, it required further experimental investigation. The isomers were detected by TLC and HPLC in the present study. However, it was felt necessary to confirm these results by mass spectrometry.

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MATERIALS AND METHODS

Materials

Standard vitamin A alcohol (retinol), retinyl acetate and vitamin A aldehyde were purchased from Fluka, AG (Buchs, Switzerland). The 9-cis, 13-cis vitamin A aldehyde and 13-cis vitamin A alcohol vitamin A alcohol were supplied by Sigma Chemical Co (UK). Since 9-cis vitamin A alcohol was not available in the market, it was prepared by reduction of 9-cis vitamin A aldehyde to 9-cis vitamin A alcohol with sodium borohydride. The identification of 9,13-dicis, 11-cis and 11, 13-dicis were based on the previous work of Manan (2). HPLC grade methanol, hexane and propan-2-ol were supplied by Rathburn Chemical Co (UK). All other chemicals used were of AnalaR grade.

Analytical method

Mass spectra of the degradative products of vitamin A alcohol in a dehydrated food system were obtained on approximately 1 μg of each of the isomers using the direct inlet probe of the sample into a Kartos mass spectrometry (MS 80 RFA). The purity of these compounds were established by TLC and HPLC just prior to mass spectral analysis. Each vitamin A alcohol isomer appeared as a single spot on TLC plate and one peak on HPLC chromatogram.

From TLC plate, the individual isomers were scrapped and dissolved in methanol. The solutions was filtered through Millipore filter paper and transferred to 10 μl micro pipette, which were covered with aluminum foil. The solution was transferred to mass spectrometry tube and the solvent was evaporated under vacuum at 40°C. The dried sample was directly inserted into a mass spectrometry probe for scanning. Each sample was scanned from mass 500 to 17 at 1 second per decade, electron input (El) 70v and the source temperature used was 150°C.

Table 1: The most prominent peaks of vitamin A alcohol and related compounds.

Compound	Molecular ion	Interpretive Peaks	The ten most intense peaks
Standard vitamin A alcohol	286	268 $[M\text{-}H_2O\ (18)]^+$ 255 $[M\text{-}CH_2\ OH\ (31)]^+$	41, 91, 286, 105, 255, 55, 79, 268, 197, 225
Standard vitamin A aldehyde	284	269 $[M\text{-}CH_3\ (15)]^+$ 255 $[M\text{-}CHO\ (29)]^+$ 251 $[M\text{-}H_20\ (18)\text{-}CH_3\ (15)]^+$	41, 91, 105, 266 119, 145, 55, 284, 77, 255
Standard retinyl acetate	328	268 $[M\text{-}CH_3COOH\ (60)]^+$ 253 $[M\text{-}CH_3COOH\ (60)\text{-}CH_3\ (15)]^+$	43, 328, 41, 253, 91, 69, 105, 55, 119, 145
Vitamin A alcohol extracted from MS	286	268 $[M\text{-}H_2O\ (18)]^+$ 255 $[M\text{-}CH_2OH\ (31)]^+$	268, 41, 91, 105, 119, 55, 145, 77, 131, 255
13-cis Vitamin A alcohol extracted from MS	286	268 $[M\text{-}H_2O\ (18)]^+$ 255 $[M\text{-}CH_2OH\ (31)]^+$	43, 69, 286, 255, 91, 105, 55, 123, 145, 159
9-13-cis Vitamin A alcohol alcohol extracted from MS	286	268 $[M\text{-}H_2O\ (18)]^+$ 255 $[M\text{-}CH_2OH\ (31)]^+$	43, 57, 268, 91, 69, 105, 119, 145, 286, 255
9-cis Vitamin A alcohol extracted from MS	286	268 $[M\text{-}H_2O\ (18)]^+$ 255 $[M\text{-}CH_2OH\ (31)]^+$	43, 286, 69, 255, 123, 91, 55, 105, 268, 145
11-cis vitamin A alcohol extracted from MS	286	268 $[M\text{-}H_2O\ (18)]^+$ 255 $[M\text{-}CH_2OH\ (31)]^+$	43, 57, 69, 91, 268, 109, 255, 145, 79, 197
11,13-cis vitamin A alcohol extracted from MS	286	268 $[M\text{-}H_2O\ (18)]^+$ 255 $[M\text{-}CH_2OH\ (31)]^+$	43, 123, 69, 109, 55, 95, 286, 268, 81, 145

Figure 1: Mass spectra of standard vitamin A alcohol.

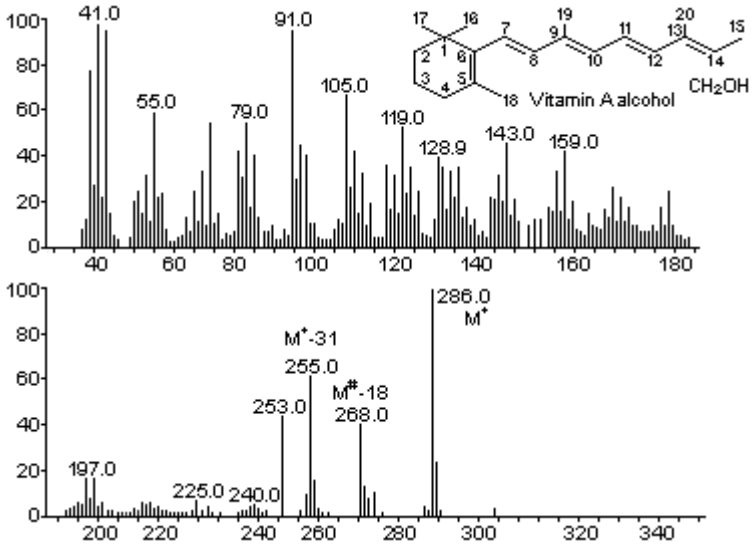
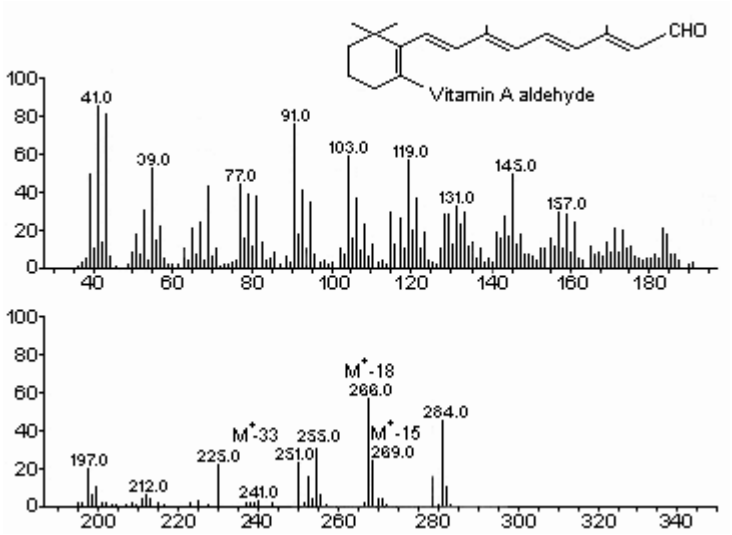


Figure 2: Mass spectra of standard vitamin A aldehyde.



RESULTS AND DISCUSSION

The mass spectra and structures of vitamin A alcohol and related compounds are presented in Figures 1-9. The molecular ion was the base peak for vitamin A alcohol (m/e 286), vitamin A aldehyde (m/e 284) and retinyl acetate (m/e 328). The relatively abundant

Figure 3: Mass spectra of standard retinyl acetate.

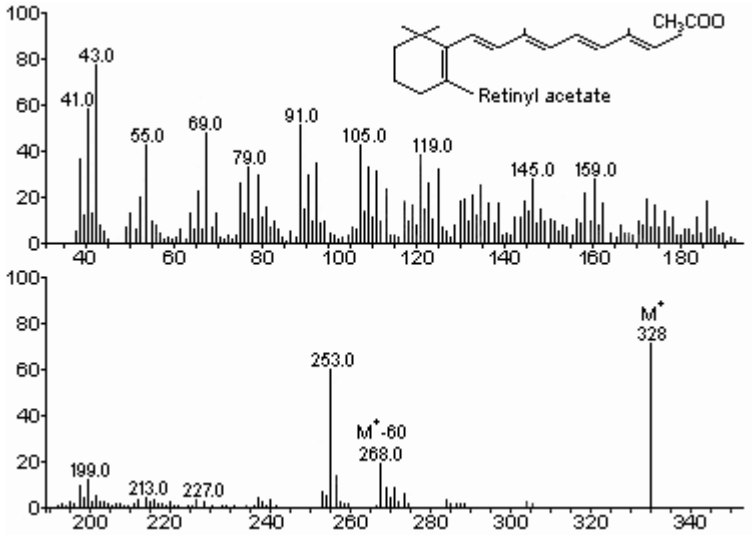
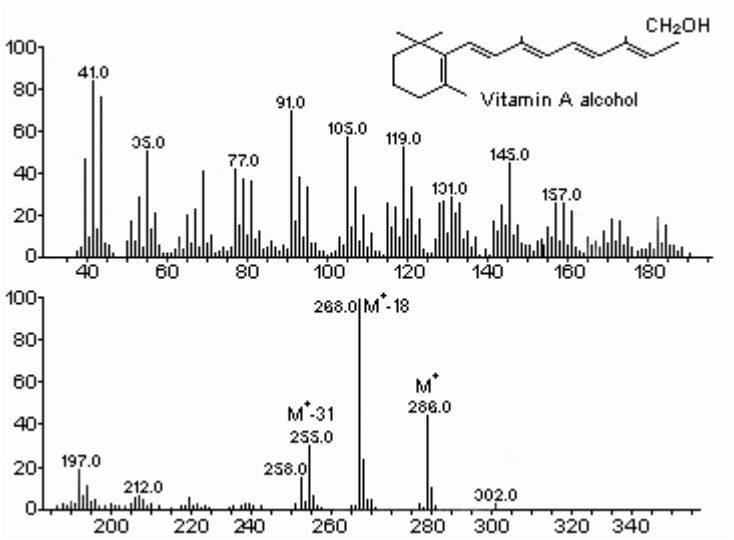


Figure 4: Mass spectra of vitamin A alcohol extracted from model dehydrated food system.



molecular ion observed for oxidized form of vitamin A may be stabilized by conjugation of the carbonyl group with the double bond in the acyclic chain (10,11). In comparison, vitamin A alcohol [M^+-18 (H_2O)] and retinyl acetate [M^+-60 (CH_3COOH)] dehydrate rather easily. The presence of ions from vitamin A aldehyde, m/e 255 [M^+-29 (CHO)], resulting from the loss of the functional groups rendered additional help in identifica-

Figure 5: Mass spectra of 13-cis vitamin A alcohol extracted from model dehydrated food system.

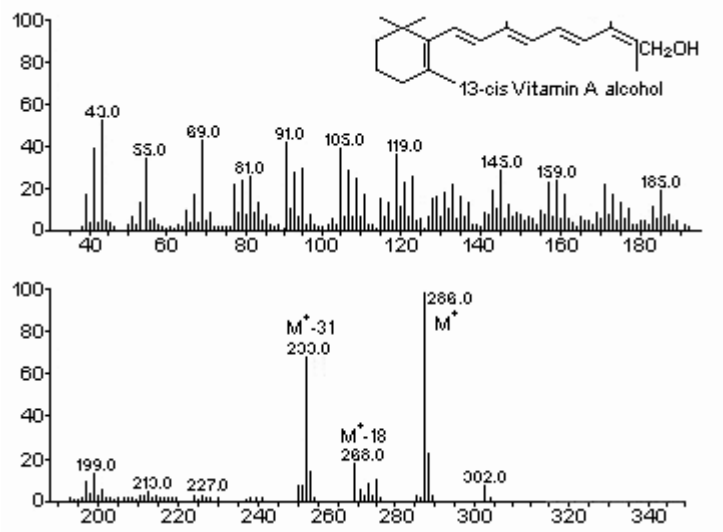
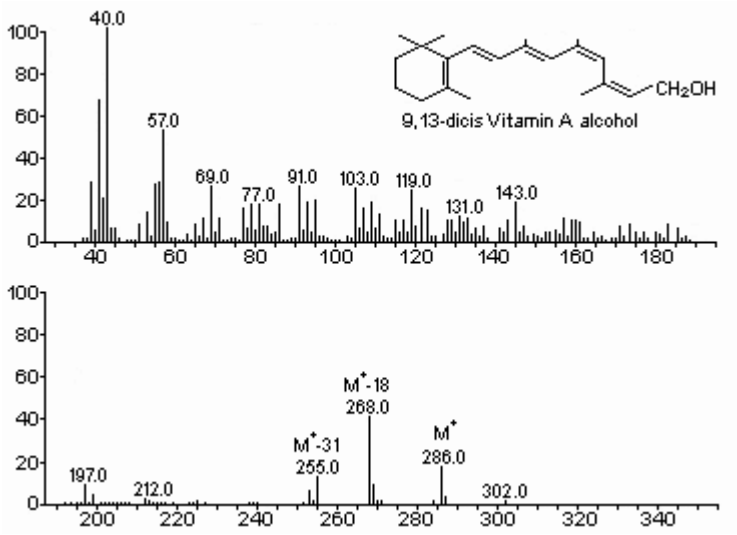


Figure 6: Mass spectra of 9, 13-dicis vitamin A alcohol extracted from model dehydrated food system.



tion. Moreover, vitamin A aldehyde lost a methyl group [M^+-15] prior to the loss of their functional groups i.e., m/e 269, while vitamin A alcohol and retinyl acetate did not behave the same. The elimination of a methyl group may also have occurred following the loss of the functional group, i.e., vitamin A alcohol, m/e 253 [M^+-(H_2O+CH_3)], vitamin A aldehyde, m/e 241 [M^+-(CO+CH_3)] and retinyl acetate, m/e 253

Figure 7: Mass spectra of 9-cis vitamin A alcohol extracted from model dehydrated food system.

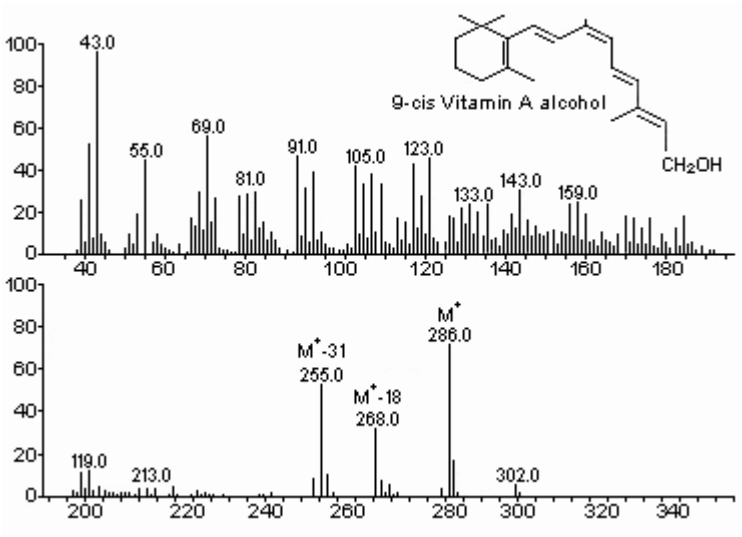
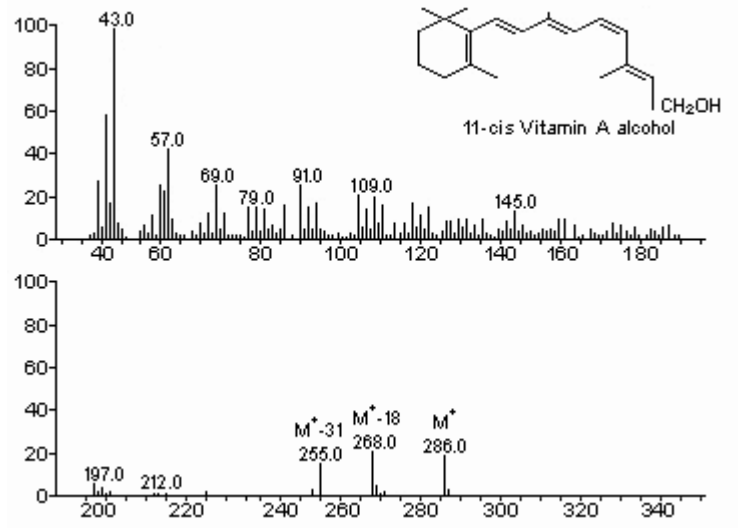


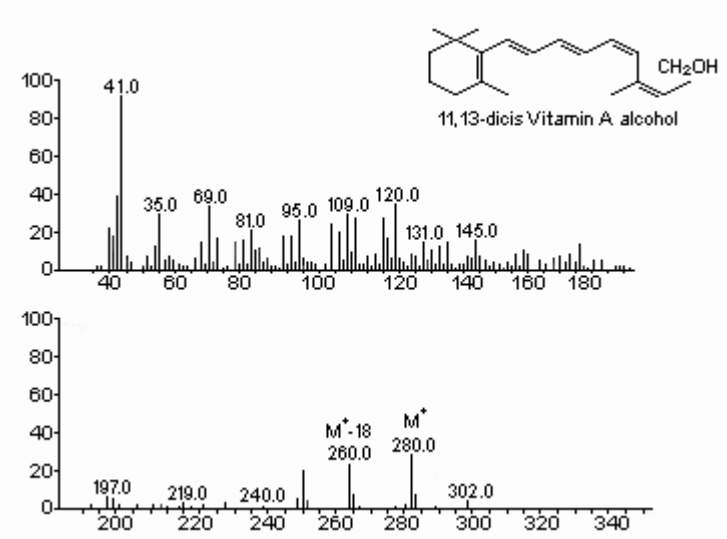
Figure 8: Mass spectra of 11-cis vitamin A alcohol extracted from model dehydrated food system.



$[M^{+}-(CH_3COOH+CH_3)]$. The labile methyl group was probably attached to the double bond in the ring as explained by the mass spectrum of hexadenterated beta-ionone (12). However, it may arise from other methyl groups in the vitamin A molecule as well.

The ten prominent peaks and those characteristic of vitamin A alcohol and related compounds are shown in Table 1. Although the ion resulting from the loss of a

Figure 9: Mass spectra of 11, 13-dicis vitamin A alcohol extracted from model dehydrated food system.



functional group is not always one of the ten most intense peaks, it proved very crucial in establishing the identity of the compound. Hence, based on a rather limited number of interpretive peaks, it is possible to identify each of these compounds by the apparatus of its molecular ion and the fragment ions corresponding to the loss of functional and methyl groups.

The isomers of vitamin A alcohol (13-cis, 9,13-dicis, 9-cis, 11-cis, 11,13-dicis) formed during sunlight photolysis gave spectra which were similar to that obtained from standard vitamin A alcohol. The spectra of these isomers are shown in Figures 5-9. The molecular ion, m/e 286 was the base peak for all the vitamin A alcohol isomers in this study. Reid *et. al.* (9) reported that the major difference in the fragmentation of vitamin A isomers was the very small M^+-18 peak in the cis-isomers which may be useful in identifying the cis-trans vitamin isomers. But in our study, it is somewhat true only in case of 13-cis isomer, which has a smaller M+-18 peak than all-trans vitamin A alcohol. It is reported that the differences between the mass spectra of the trimethyl silyl derivatives of cis-trans isomers of retinol were very small (9). Other workers (6-8) support our findings mass spectra can differentiate between different retinoid compounds conveniently but its fragmentation patterns for vitamin A isomers resemble each other to a great extent.

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