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Identification of volatile degradants in formulations containing sesame oil using SPME/GC/MS

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Abstract

Solid-phase microextraction (SPME), in combination with gas chromatography/mass spectrometry (GC/MS), was used to identify an unknown degradant observed during stability studies of a pharmaceutical formulation containing sesame oil. SPME is a solvent-less, rapid, sensitive, and inexpensive extraction method that minimizes sample preparation. SPME combined with GC is a widely used technique in certain fields, such as food, environmental analysis, forensics, and consumer products, but has only rarely been used for the analysis of pharmaceutical formulations. Hexanal, octanal, 2-octenal, 2-decenal, 2-undecenal, and 2,4-decadienal can be detected and identified by GC/MS, but they cannot be detected by LC/MS due to their volatility and low ionization efficiency under atmospheric pressure ionization conditions. Combining the MS data from the GC/MS with LC/DAD data resulted in the identification of the unknown degradant in the formulation as 2,4-decadienal. The presence of this and other aldehydes was attributed to the oxidative degradation of the unsaturated fatty-acid component in vegetable oils.

Keywords: Solid-phase microextraction; SPME/GC/MS; 2,4-Decadienal; Volatile degradants; Unknown identification

1. Introduction

For drug products, any degradation product observed in stability studies conducted at the recommended storage condition should be identified when present at a level greater than the identification thresholds given in the ICH Guidance for Industry Q3B(R2) [1]. It is necessary to identify the degradation products observed in early drug formulation development so that they can be qualified, reduced, or eliminated during later development stages. In addition, the identification can shed light on the degradation pathways and the origins of the degradants, and thus to possible prevention of the formation of the degradation products.

A stability study was conducted on exploratory capsule formulations containing sesame oil as an excipient. An unknown degradant was observed as an incompletely resolved doublet peak in the LC/UV chromatogram. The unknown degradant was not detected at the initial time point, but increased significantly upon storage at 40 °C/75% RH. At the 3-month time point, the peak reached a level of 0.50% relative to the active pharmaceutical ingredient (API) in the LC/UV chromatogram, but could not be detected in an LC/MS chromatogram.

This study describes the identification of the unknown degradant using solid-phase microextraction (SPME) and gas chromatography (GC)/mass spectrometry (MS) combined with the information given by LC/DAD/MS. Liquid chromatography combined with photodiode array detector and mass spectrometry (LC/DAD/MS) is a standard technique to obtain information on chromophores and mass spectra for observed degradation products. This technique is limited to compounds that are chromophoric, non-volatile, and ionizable. Gas chromatography (GC) complements this technique, but often requires a simple and clean sample matrix due to the potential thermal degradations at high temperatures in the injector.

SPME can separate the analytes from their complex matrix without using a solvent. It is a rapid, sensitive, and inexpensive extraction method that minimizes sample preparation. SPME/GC is a widely used technique in certain fields, such as food [2], environmental analysis [3], forensics [4], and consumer products [5]. There are a few reports where SPME/GC was used to determine organic volatile impurities and residual solvents in the pharmaceutical field [6]. However, to our knowledge, this

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is the first time that SPME/GC/MS has been utilized to identify an unknown degradant in a pharmaceutical formulation. This is also the first time that 2,4-decadienal has been identified as a degradant in formulations containing vegetable oil excipients.

2. Experimental

2.1. Reagents and chemicals

All solvents for mobile phases and diluents were high purity grade from Burdick and Jackson. The prototype capsules containing the active pharmaceutical ingredient suspended in oil were prepared at Amgen, and stored in plastic vials (1.8 mL CryoTubes, Nunc A/S, Denmark). (*E*,*E*)-2,4-Decadienal (90%) was purchased from Alfa Aesar (Ward Hill, MA 01835, USA).

2.2. Sample preparation for HPLC/DAD analysis

Binary mixtures of the API with each of the excipients in the oil suspension formulation were prepared in a 1:10 ratio (API: excipient, w/w). The binary mixture samples and sesame oil itself were stored at 50 °C for 2 weeks. Both the compatibility and capsules samples were dissolved in 60/40 (v/v), acetonitrile (ACN) and water to give a concentration of 0.1 mg/mL of API, 25 μL injections were analyzed using HPLC/DAD gradient method.

2.3. HPLC/DAD/MS conditions

HPLC analysis was performed on an Agilent LC 1100 with DAD detector and a mass spectrometer, either an Agilent quadruple MSD SL (Agilent Technologies, Inc., Santa Clara, CA, USA) or a Finnigan LCQ DECA (Thermo Scientific, Waltham, MA, USA). A Waters SymmetryShield^{TM} RP18, 5 μm particle size, 150 mm × 4.6 mm i.d. HPLC column (Waters Corporation, Milford, MA, USA) was used. The detection wavelength was 274 nm, and a UV scan from 200 to 400 nm was collected for identification. The flow rate was 1.0 mL/min with a sample injection volume of 25 μL. Mobile phase A was a mixture of 20/80/0.1, v/v/v ACN, water, and trifluoroacetic acid (TFA). Mobile phase B was a mixture of 95/5/0.1, v/v/v ACN, water, and TFA. A gradient method was used in which mobile phase B was set to 10% at time zero, subsequently increased linearly to 25% at 5 min, 60% at 20 min, and 85% at 27.5 min, held at 85% to 32 min, decreased linearly to 10% at 32.1 min, and held at 10% until 35 min.

2.4. Isolation of unknown degradants

An analytical HPLC column was used in attempt to separate and isolate a small quantity of the unknown degradant from the stressed capsules. The HPLC column and detection wavelength were the same as in Section 2.3. Mobile phase A was a mixture of 20/80 (v/v) acetonitrile (ACN) and water. Mobile phase B was a mixture of 95/5 (v/v) ACN and water. TFA was not included in the mobile phases to prevent potential acidic degradation of the fractions. The sample injection volume was increased to 100 μL. A gradient method was used in which the mobile phase B was held at 40% for 16 min, subsequently increased linearly to 100% at 16.1 min, held at 100% to 26 min, changed to 40% at 26.1 min, and held at 40% until 30 min. The peak corresponding to the unknown degradant was collected from multiple injections and pooled together. The pooled fraction was directly injected (1 μL) for GC analysis prior to the concentration step. After the pooled fraction was concentrated under a nitrogen stream, the concentrate was also directly injected (1 μL) for GC analysis.

2.5. Sample preparation for GC/MS analysis—SPME procedure

An open capsule was sealed in a headspace vial at room temperature with an SPME device (Supelco Polydimethylsiloxane, 100 μm film, Cat# 57300-U). The fiber was exposed to the interior headspace for 2 h. The absorbed analytes were then desorbed in a GC injector port at 250 °C, and injected into the GC column without being diluted.

2.6. GC/MS conditions

The drug capsule was analyzed using headspace GC/MS (Agilent Headspace 7694, GC 6890, MS 5973). The sample vials were heated in a headspace oven at 95 °C for 5 min before injection.

In addition, collected sample fractions from procedure 2.4 were analyzed by GC/MS via direct injection. The injection volume for all measurements was 1 μL with splitless mode. A Phenomenex Zebron ZB-WAX, 30 m × 0.32 mm i.d. column was used, with a 0.5 μm film thickness. The oven temperature program was set at 40 °C for initial, held at 40 °C for 1 min, increased to 230 °C at the rate of 20 °C/min, and held at 230 °C for 5 min. Helium was the carrier gas with a flow rate of 2.5 mL/min. The detector mass scan was from 25 to 200 Da.

3. Results and discussion

An unknown degradant (not detected at the initial timepoint) was observed as a doublet peak in the capsule formulation containing sesame oil excipient after storage for 1 month under accelerated stability conditions of 40 °C/75% RH (Fig. 1). For the sake of simplicity, the unknown degradant will be referred

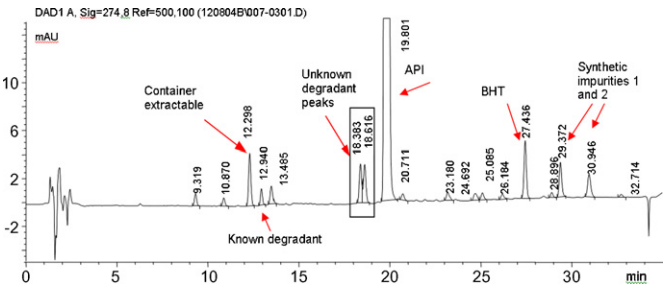

Fig. 1. HPLC chromatogram of capsule sample stored at 40 °C/75% RH showing unknown doublet peaks.

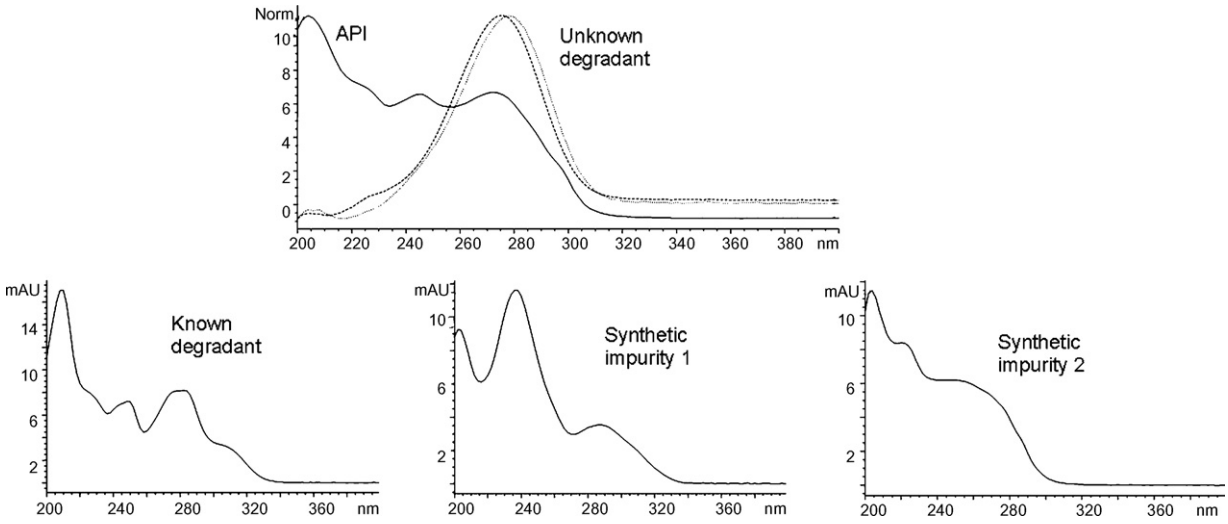

Fig. 2. UV spectra of API, unknown peak, known API degradant and synthetic impurities.

to as a single entity in the present work, although it was detected as two peaks in the HPLC chromatogram. The total level of the unknown degradant increased over time and varied from batch to batch, reaching a level of up to 0.50% (w/w versus API) at 40 °C/75% RH after 3 months.

The UV spectrum of the unknown degradant did not show any similarity to the API or its known degradants or synthetic impurities (Fig. 2). This indicated that the degradation was probably not from the API, as would normally be expected, but was likely to be from other sources such as excipients, excipient impurities, or container leachables.

The absence of the unknown degradant peak in the LC/UV chromatogram of the container extractions ruled out the possibility that the degradant was leached from the container. Interestingly, the unknown degradant was neither detected in a forced degradation study of the API under acidic, basic, oxidative, heat, and light stress conditions, nor in an excipient compatibility study. The failure to detect the unknown degradant in these studies was probably due to the batch to batch variations of oil excipients and/or shorter storage conditions as compared to the original stability studies.

The unknown degradant in the capsules could not be detected by LC/MS with either ESI or APCI methods. These experiments indicated that the degradant might be volatile, difficult to be ionized by ESI or APCI, and/or not sufficiently concentrated for LC/MS detection. The loss of the degradant upon evaporation of the isolated unknown fractions from the capsule samples also indicated that the degradant might be volatile. GC/MS analysis by direct injection of the isolated fractions prior to the evaporation did not give any useful information, probably due to the low levels of the degradant.

To overcome these problems, SPME/GC/MS was used to investigate the unknown degradant. There are several types of SPME fibers available with different coatings, such as polydimethylsiloxane (PDMS), polyacryrate, PDMS/divinylbenzene (DVB), Carbowax/DVB, DVB/carboxen/PDMS. We chose PDMS based on its recommended use for volatile and relatively non-polar compounds, since the LC/MS results indicated that the unknown degradant is most likely volatile and non-polar. However, the identification of the unknown degradant was complicated by the large number of peaks observed in the SPME/GC/MS chromatogram (Fig. 3). Comparing the mass spectra of these peaks with the NIST MS library, five of them were identified as hexanal, octanal, 2-octenal, 2-decenal, and 2-undecenal. In addition, three peaks in Fig. 3 were identified as 2,4-decadienal. The multiple peaks of 2,4-decadienal are due to four possible isomers, namely (*E*,*E*), (*Z*,*Z*), (*E*,*Z*), and (*Z*,*E*)-2,4-decadienals.

Most of the aldehydes were commercially available except for the (*Z*,*Z*), (*E*,*Z*), and (*Z*,*E*)-2,4-decadienals. The available aldehydes were purchased as authentic samples and analyzed by LC/DAD to compare with the unknown doublet peaks in Fig. 1. Fig. 4(a) shows the LC/UV chromatogram of the authentic (*E*,*E*)-2,4-decadienal. The small front shoulder peak is probably due to other stereoisomeric forms that were not completely separated by the reverse phase HPLC method. Comparison of the HPLC retention times (Fig. 4(a and b)) and UV spectra (Fig. 4(d)) indicated that the later-eluting peak of the unknown doublet was (*E*,*E*)-2,4-decadienal. The earlier-eluting peak of the doublet was most likely due to another stereoisomer of 2,4-decadienal because its retention time (Fig. 4(a and b)) and UV spectrum (Fig. 4(c)) matched with the front shoulder of the authentic (*E*,*E*)-2,4-decadienal peak.

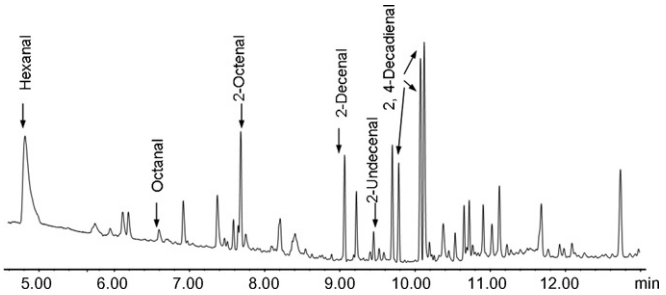

Fig. 3. Total ion chromatogram using SPME/GC/MS of the volatiles from the capsule formulation.

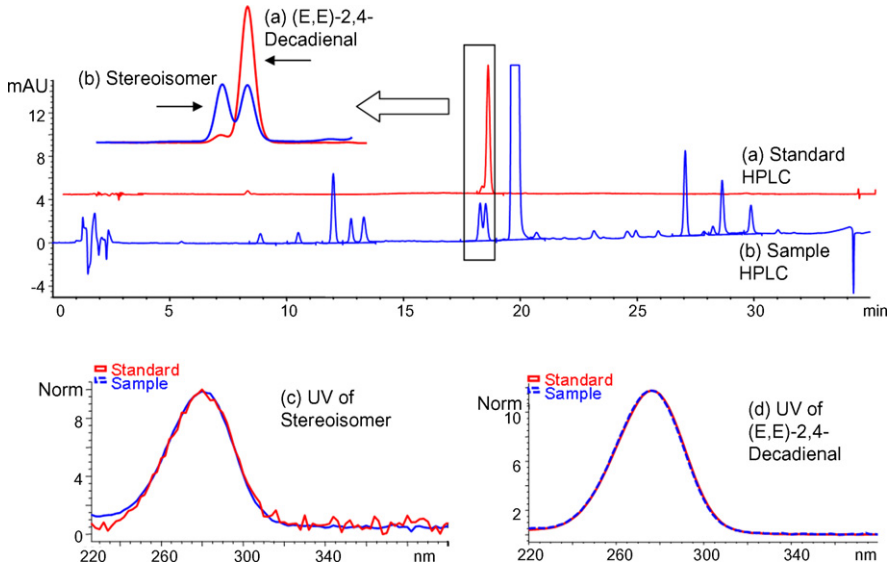

Fig. 4. (a) HPLC chromatogram of the (*E*,*E*)-2,4-decadienal standard (shown in red). (b) HPLC chromatogram of the capsule sample (shown in blue). (c) UV spectra of the early-eluting stereoisomer peak indicated in (b) show a match between the sample and standard. (d) UV spectra of the (*E*,*E*)-2,4-decadienal standard peak indicated in (a) show a match between the sample and standard. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

After the identification of 2,4-decadienal by SPME/GC and LC/DAD, an ion chromatogram (Fig. 5(b)) was retrospectively obtained by extraction of the 2,4-decadienal molecular ion ($MH^+ = 153$) from the LC/MS total ion chromatogram acquired previously for the capsule sample. Although the unknown peak was not observed in the total ion chromatogram due to the poor signal to noise ratio, the extraction showed a peak that matched the retention time of the unknown peak observed in the LC/UV chromatogram (Fig. 5(a)). The presence of the 2,4-decadienal molecular ion of $MH^+ = 153$ (Fig. 5(c)) further confirmed the identification.

The present work shows the first identification of 2,4-decadienal as a degradant in a drug product. 2,4-Decadienal is known to be formed by the free radical autoxidation of oils containing linoleic acid under specific conditions [7,8]. Sesame oil is a mixture of fatty acid glycerides. A typical analysis of refined sesame oil indicates the composition of the fatty acid glycerides as: linoleic acid 40.4%; oleic acid 45.4%; palmitic acid 9.1%;

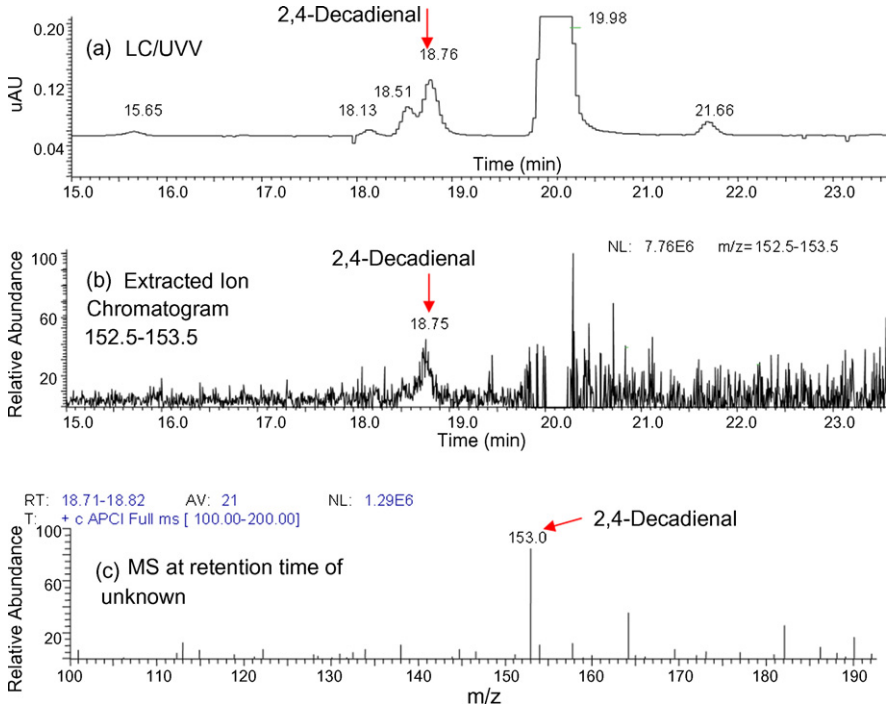

Fig. 5. LC/MS (APCI) of the capsule sample. The arrows indicate peaks from the unknown which correspond to 2,4-decadienal.

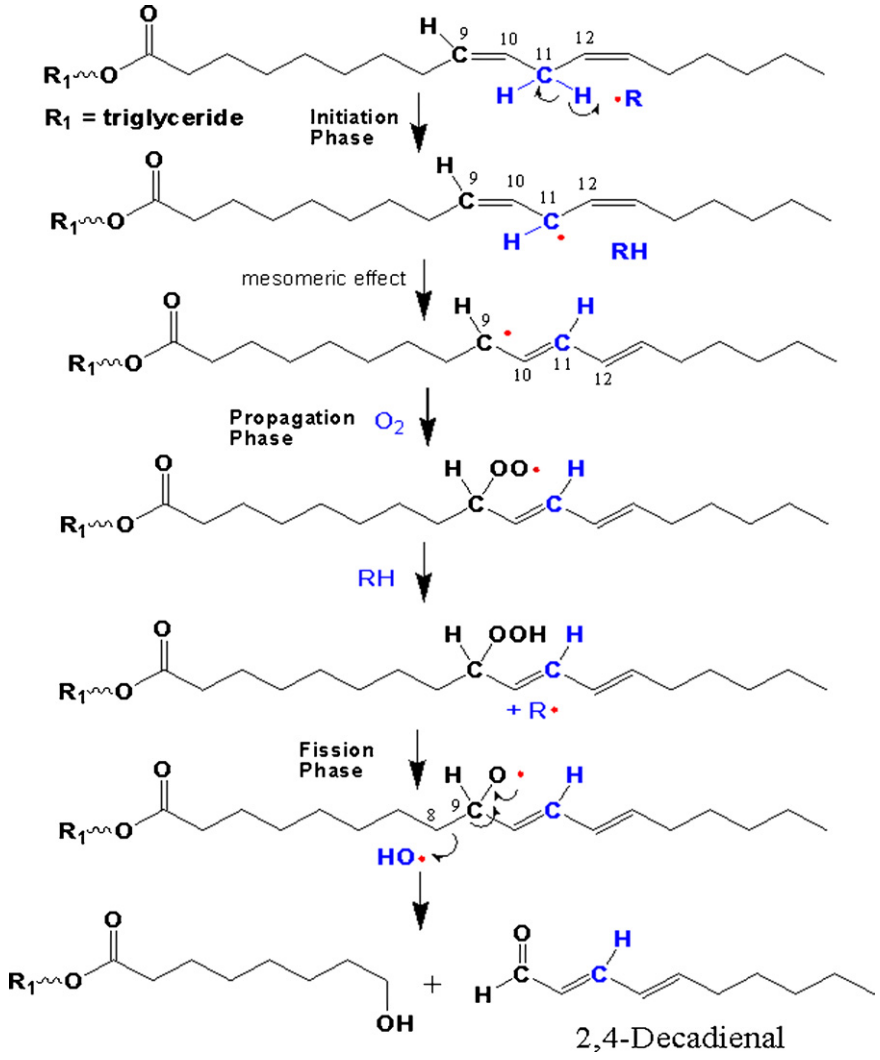

Fig. 6. Mechanism proposed for autoxidation of oils containing linoleic acid groups to form 2,4-decadienal [7].

arachidic acid 0.8%; and stearic acid 4.3% [9]. In the food and agriculture literature, 2,4-decadienal has been observed as one of the numerous aldehydic degradants from oils that contain linoleic acid [10–14]. However, in the pharmaceutical literature, there have not been any reports of the detection of 2,4-decadienal as a degradant, despite the common use of oils, such as soybean, sesame, canola, corn, cotton seed, olive, and peanut oils in drug products.

Since 2,4-decadienal was formed from the linoleic acid groups in sesame oil, it could also form in any oil containing linoleic acid. As expected, 2,4-decadienal was found in soybean oil that had been stressed at 90 °C for 7 days. Therefore, the identification of 2,4-decadienal is valuable for stability investigations of pharmaceutical formulations using any oils that contain linoleic acid groups.

A proposed degradation pathway is schematically illustrated in Fig. 6. The proposed degradation mechanism is based on autoxidation, i.e., the reaction between molecular oxygen (O_2) and organic molecules. Autoxidation is often initiated by trace amounts of impurities, such as metal ions or hydroperoxides. Those impurities may not be identifiable in the reaction mixture. Fig. 6 shows three phases of the reaction: initiation, propagation, and fission. We believe that the free radical can be initiated at *C*11 of linoleic acid since the free radical formed at this carbon can be stabilized by both double bonds at *C*9 and *C*12. Due to the mesomeric or resonance effect, the free radical can also be shifted to the *C*9 position with extended double-bond conjugation at *C*10 and *C*12. The free radical at *C*9 may react with O_2 to give peroxide in the propagation phase. Assuming the decomposition of peroxide and fission between *C*8 and *C*9, 2,4-decadienal would be produced in the fission phase. The formation of 2,4-decadienal from the linoleic acid moiety in the oils was also confirmed by the presence of 2,4-decadienal in linoleic acid.

In addition to 2,4-decadienal, other aldehydic degradants were observed in the SPME/GC/MS chromatogram (Fig. 3). These additional aldehydes can be formed by the oxidation pathways illustrated in Fig. 7. Similar to the degradation pathway described in Fig. 6, these aldehydes can also be formed by autoxidation, which may involve three phases: initiation, propagation, and fission. Fig. 7 shows only the position of free radicals and fissions in the fatty acid groups that lead to the identified aldehydes.

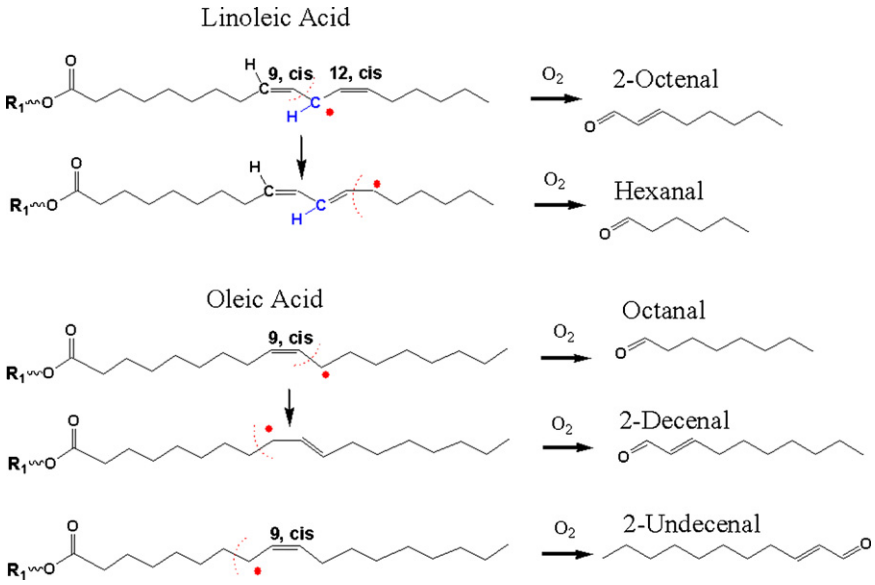

Fig. 7. Oxidation pathways for the formation of other aldehydes.

4. Conclusion

An unknown degradant in a capsule formulation containing an API suspended in a vegetable oil excipient was identified as 2,4-decadienal. Multiple analytical techniques were used in the separation and identification efforts. SPME coupled with GC/MS was used in the identification of this low level degradant, which is the first time that this technique has been reported for pharmaceutical drug product analysis. SPME/GC/MS was thus shown to be an effective way of identifying trace amounts of volatile compounds in complex sample matrices, such as drug products.

In addition, this is the first time that 2,4-decadienal was identified as an unknown degradant in a pharmaceutical formulation. The formation of 2,4-decadienal in the capsule formulation was attributed to the oxidation of linoleic acid groups in sesame oil, and could potentially occur in other vegetable oils that are used as excipients. One of the structural analogues of 2,4-decadienal, namely, 2,4-hexadienal, was reported to be carcinogenic in rats and mice [15]. As a result, there is an interest to identify the presence of 2,4-decadienal and its analogues in pharmaceutical formulations. This study demonstrated that SPME/GC/MS is a simple and fast method to identify those volatile degradants in pharmaceutical formulations.

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