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Degradation of vitamin D_3 in a stressed formulation: The identification of esters of vitamin D_3 formed by a transesterification with triglycerides

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Abstract

Four unknown degradants in the LC-UV profile of a stressed experimental tablet formulation that contains vitamin D_3 have been identified by a combination of Ag^+-cationization electrospray ionization (ESI) LC/MS and atmospheric pressure chemical ionization (APCI) LC/MS/MS. The peaks elute in the method chromatography in two pairs of two peaks. The first pair of peaks has *m/z* 511 while the second pair has *m/z* 539. The major, first peak of each set of peaks corresponds to the octanoate and decanoate ester of vitamin D_3, respectively. These are formed by a transesterification with the two major fatty acid components (octanoate and decanoate) of the triglycerides present in the formulation. The formation of two degradation products with each fatty acid is due to the presence of both vitamin D_3 (major component) and the isomeric pre-vitamin D_3 (minor component) in the stressed formulation.

Keywords: Vitamin D_3; Triglycerides; Transesterification; APCI; Ag^+-cationization ESI; LC/MS

1. Introduction

Vitamin D_3 (**1**; trivial name: cholecalciferol) has been described as "... a vitamin imposed on man by civilization." [1]. This is because, although necessary for good health, vitamin D_3 can be synthesized in the skin of mammals by the action of sunlight on the immediate precursor, 7-dehydrocholesterol (Fig. 1), and therefore does not meet the standard definition of a vitamin. It is a steroid hormone related to cholesterol and is the naturally occurring anti-rachitic component of fish liver oils. Dietary intervention is necessary only in the absence of sufficient natural irradiation, but because of the involvement of vitamin D_3 in the intestinal absorption of calcium and bone calcium metabolism, it is routinely added to infant formula and milk for the prevention of rickets in children and osteomalacia in adults [2]. It is also widely used as an additive to animal feedstuffs, multivitamin preparations and other food products.

One of the major hurdles on the road from active pharmaceutical ingredient to marketable drug formulation is to demonstrate the chemical stability of the proposed commercial product [3]. The definitive demonstration of chemical stability is embodied in long-term formal stability studies on the marketed formulation in its intended package. As an aid in the interpretation of stability study results, forced degradation studies are conducted on drug substance and drug product to elucidate the potential degradation mechanisms and gain information on the primary degradation products. Experimental formulations will often be subjected to thermal, oxidative, acidic, basic, humidity, and light (both UV and visible) stress.

The chemical reactivity of vitamin D_3 has been studied extensively, because of its biological importance. Particular attention in the literature has been paid to chemically, photochemically and thermally induced isomerizations, and to the oxidation chemistry. The most facile thermal isomerization of vitamin D_3 is to pre-vitamin D_3 (**2**), an intermediate in the production of vitamin D_3 from 7-dehydrocholesterol. The dynamic equilib-

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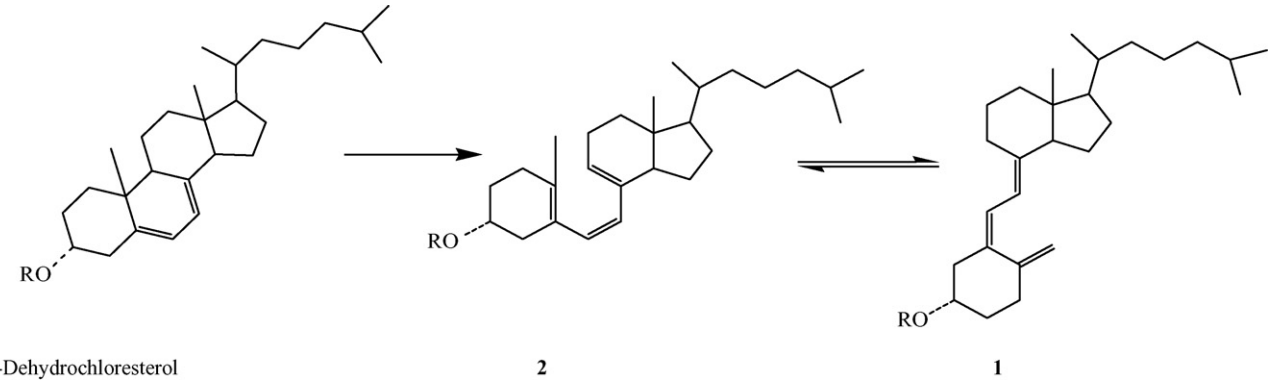


Fig. 1. The production of vitamin D_3 (**1**) and pre-vitamin D_3 (**2**) from 7-dehydrocholesterol.

rium between vitamin D_3 and pre-vitamin D_3 favors vitamin D_3, and pre-vitamin D_3 can thus be treated as an active form of vitamin D_3. A number of older reviews provide a good foundation in the isomerization chemistry of vitamin D_3 [1,4]. Among oxidation studies are those that have addressed the selective epoxidation of the triene system [5,6], reaction with singlet oxygen [7–9], the biomimetic oxidation by an iron–sulfur protein model cluster and molecular oxygen [10] and the oxidation in an ethanol solution or adsorbed on a finely powdered solid support, such as silica gel [11]. The free radical autoxidation (induced by 2,2′-azobis-isobutyronitrile) of vitamin D_2, which differs from vitamin D_3 only in the side-chain, has also been studied and several degradants were identified [12].

Despite its widespread use in vitamin supplements for humans and animals, the authors are aware of only a few prior reports on the stability of vitamin D_3 in formulations [13]. However, none of these studies identified any degradation products. This paper describes the detection and identification of several new degradants from a thermal stability study with an experimental formulation of tablets that contained vitamin D_3.

2. Experimental

2.1. Chemicals

Tablets of an experimental formulation that contained pharmaceutical grade granules of vitamin D_3 (0.14 mg vitamin D_3/tablet) were obtained from Formulation Design, Merck Research Laboratories, West Point. 1,3-Dicyclohexylcarbodiimide, octanoic acid, silver tetrafluoroborate, 4-dimethylaminopyridine, trifluoroacetic acid and crystalline vitamin D_3 were purchased from Aldrich Chemical Company Inc. (Milwaukee, WI) and the mixture of medium chain triglycerides (Captex 300 EP) was from Abitec Corp. (Columbus, OH). Phosphoric acid, tetrahydrofuran, dichloromethane, hexane and HPLC grade acetonitrile, methanol and water were obtained from Fisher Scientific (Fair Lawn, NJ).

2.2. Sample preparation

Tablets were stored at 60 °C/ambient RH for 7 months. Ten tablets were combined and extracted by sonication with 100 mL of water–methanol (5:95, v/v) for 0.5 h, followed by stirring for 3 h. A portion of the solution was clarified by centrifugation prior to analysis by LC-UV and LC/MS.

2.3. LC-UV analysis

Aliquots (100 μL) of the solution (sample tray at 5 °C) were injected onto an HPLC. Gradient chromatography was performed on a Platinum EPS C18 column (150 mm × 4.6 mm; 3 μm particles) held at 17 °C, with UV detection at 265 nm. The organic component of the mobile phase was acetonitrile, while the aqueous component was 0.1% phosphoric acid. Gradient program, %-organic (time, min): 4 (0), 4 (7), 80 (20), 85 (48), 95 (50), 95 (60), 100 (61), 100 (68), 4 (75).

2.4. Transesterification of vitamin D_3 with triglycerides

Crystalline vitamin D_3 (~2 g) was dissolved in a mixture of medium chain triglycerides (100 mL; Captex 300 EP) by sonication. Trifluoroacetic acid (700 μL) was added with shaking, and cooling in an ice-bath. After 10 days at ambient temperature, an aliquot (1 mL) was diluted to 50 mL with methanol–water (95:5, v/v) for analysis.

2.5. Synthesis and purification of octanoate ester of vitamin D_3

1,3-Dicyclohexylcarbodiimide (605 mg) was added slowly to a stirred solution of crystalline vitamin D_3 (about 1 g), octanoic acid (465 mg) and 4-dimethylaminopyridine (40 mg) in dichloromethane (10 mL). After 2 h, hexane (50 mL) was added and the mixture was filtered (Millipore, 22 μm) under vacuum to remove the dicyclohexylurea. The filtrate was washed with 0.2 N NaOH, 1 N acetic acid and water, then dried over anhydrous sodium sulfate. Hexanes were removed on a rotary evaporator to give the product as a light yellow oil. The oil was re-dissolved in tetrahydrofuran for isolation by preparative HPLC using a Platinum EPS C18 column (250 mm × 22 mm; 5 μm particles) with an acetonitrile–water (90:10, v/v) mobile phase at a flow rate of 10 mL/min.

2.6. Partial conversion of octanoate ester of vitamin D_3 to octanoate ester of pre-vitamin D_3

A solution of the isolated octanoate ester of vitamin D_3 was heated in a methanol–water mixture (95:5, v/v) containing a trace of tetrahydrofuran at 65–70 °C for 45 min.

2.7. LC/MS analysis

All samples were analyzed on an HPLC system interfaced to a ThermoFinnigan LCQ mass spectrometer. The following chromatographic methods were used:

Method A: Alltech Platinum EPS C-18 column (250 mm × 4.6 mm; 5 μm particles) at ambient temperature. The mobile phase was water–acetonitrile at a flow rate of 1.0 mL/min, with the following gradient program, %-organic (time, min): 80 (0), 100 (30), 100 (45), 80 (46), 80 (56).
Method B: Alltech Platinum EPS C-18 column (250 mm × 4.6 mm; 5 μm particles) at ambient temperature. The isocratic mobile phase was water–acetonitrile (7:93, v/v) at a flow rate of 1.0 mL/min.
Method C: Alltech Platinum EPS C-18 column (250 mm × 4.6 mm; 5 μm particles) at ambient temperature. The mobile phase was water–methanol at a flow rate of 0.9 mL/min, with the gradient program described for Method A. This was used for Ag^+-cationization LC-ESI-MS.

Chromatographic peaks were tracked and labeled in terms of relative response time (RRT). The shifts in retention time between the three methods were determined by characterizing selected samples by all three chromatographic methods, and are presented below in terms of Method A, unless specified otherwise.

For LC-APCI-MS, chromatography was by Method A or B, and the corona discharge was set at 4.5 kV. The APCI probe was operated with the vaporizer at 450 °C and the heated capillary at 250 °C (standard conditions) or with the vaporizer at 250 °C and the heated capillary at 150 °C (reduced thermal load).

Ag^+-Cationization LC-ESI-MS was performed at a spray voltage of 4.5 kV with post-column addition of a solution of $AgBF_4$ in water (115 mg/L) at 0.1 mL/min via a high-pressure mixing tee (Upchurch) placed after the UV detector. The concentration of Ag^+ entering the ESI source was about 6.3 μg/mL. A Spiral Link™ reaction coil (0.030 in. i.d. × 200 cm; Upchurch) was in-line between the mixing tee and the ESI probe; transit time through the reaction coil was approximately 60 s. The heated capillary was held at 250 °C. The sheath gas (N_2) and auxiliary gas (N_2) settings were 70 and 30 arbitrary units, respectively, for all experiments. MS/MS experiments were performed with an isolation width of 5 amu centered on the ^{13}C isotope signal. Dissociation was achieved by applying an energy equivalent to 35% of the maximum amplitude.

3. Results and discussion

The LC-UV profile at 265 nm of a solution of an experimental formulation of vitamin D_3 containing-tablets that had been stressed at 60 °C under ambient relative humidity for 7 months is shown in Fig. 2. The four unknown degradant peaks labeled A (RRT 1.37; 5.57% claim), B (RRT 1.40; 0.63% claim), C (RRT 1.50; 3.05% claim) and D (RRT 1.54; 0.42% claim), respectively, were of immediate interest. From their chromatographic retention relative to vitamin D_3 under the reversed phase conditions, they were less polar than vitamin D_3. Initial LC/MS analysis of the solution (HPLC Method A) was performed under atmospheric pressure chemical ionization (APCI) conditions, since compounds such as vitamin D_3 (non-ionic and lacking

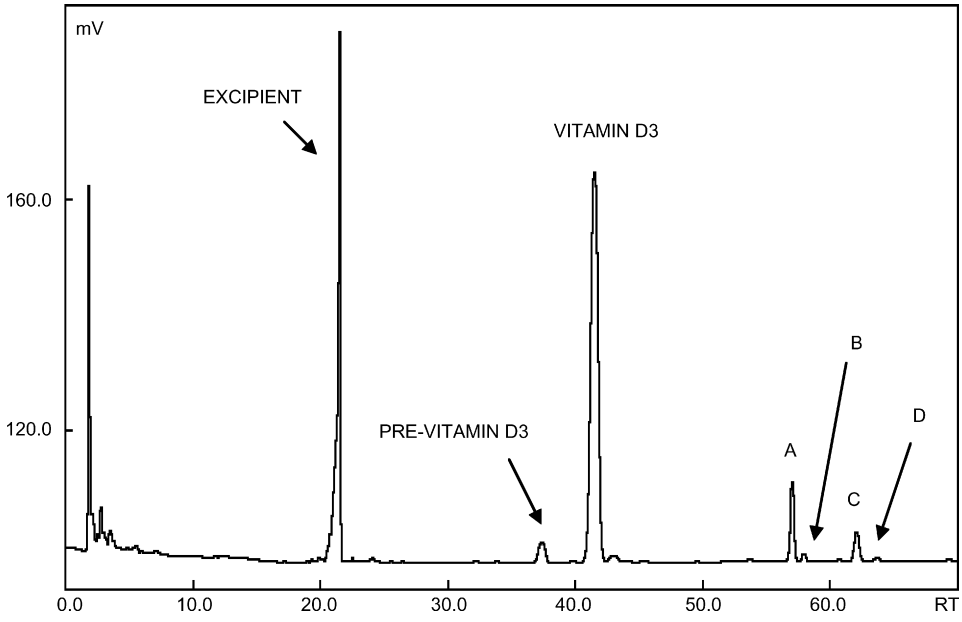


Fig. 2. HPLC-UV profile of solution of experimental formulation tablets stressed at 60 °C/ambient RH for 7 months.

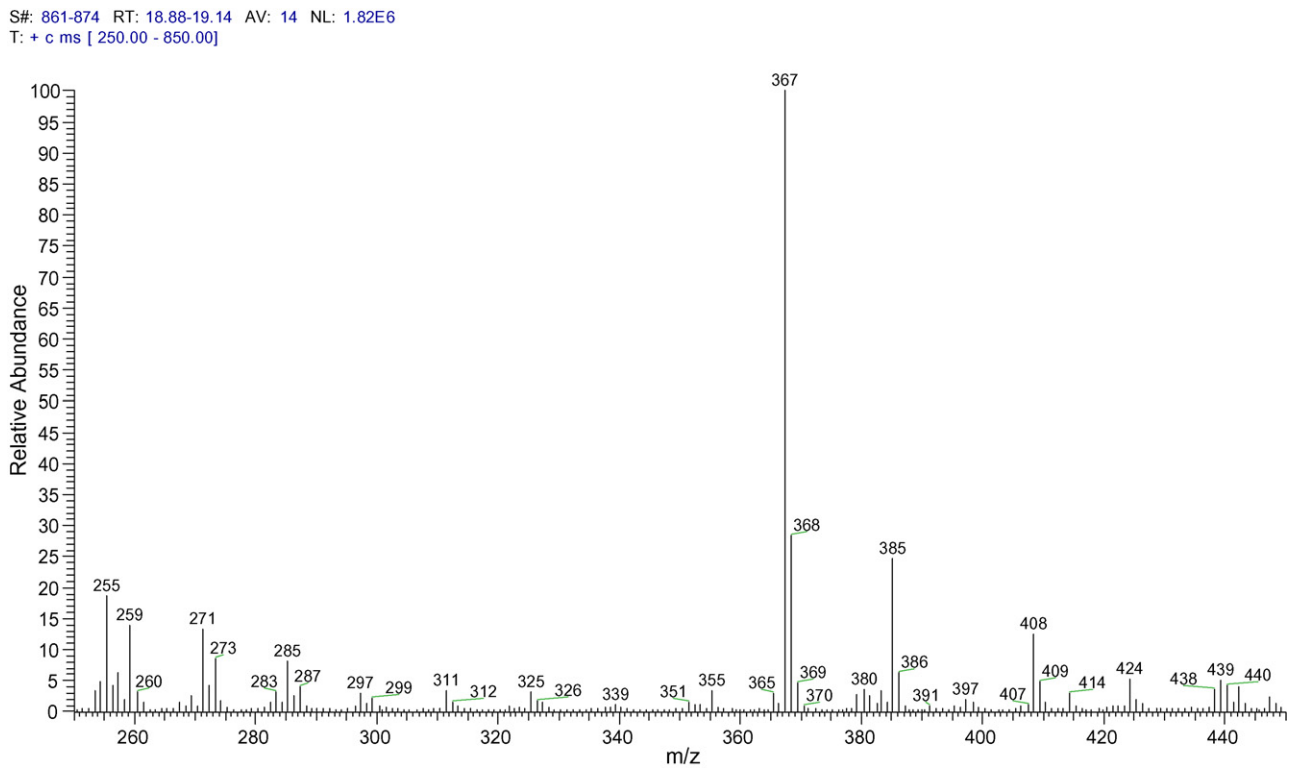


Fig. 3. APCI-MS spectrum of vitamin D_3.

an acidic or basic group) cannot readily be detected by conventional electrospray ionization (ESI) mass spectrometry. As shown in Fig. 3, vitamin D_3 was detected by the $[M+H]^+$ signal at *m/z* 385 and the intense signal at *m/z* 367 $[M+H-H_2O]^+$. The mass spectra of peaks A, B, C and D were also all characterized by the presence of *m/z* 367 as the base signal. The mass spectrum of peak A also exhibited many signals at higher mass, for example, *m/z* 408 ($[M+H-H_2O+MeCN]^+$; assumed Mw 366 amu), 461, 511 and 605 with 5–15% relative abundance (RA) (Fig. 4); the spectrum of peak C similarly showed low-level signals at *m/z* 408, 461, 539, 568, 593 and 633. The initial interpretation of these data was that, based upon the *m/z* 367 and 408 signals, all four peaks were due to isomeric, dehydrated forms of vitamin D_3. However, diode array UV data revealed that none of these four degradants showed the shift of the absorbance maximum from 265 nm to 305 nm, that has been reported for a 3,4-unsaturated, dehydrated vitamin D_3 (9,10-secocholesta-3,5,7,10 (19)-tetraene) [14]. Further, all of these degradants are significantly more retained than vitamin D_3 in the reversed phase HPLC method, hinting at a larger, more hydrophobic structure. Pure vitamin D_3 is known to be highly susceptible to oxidation [15] and it was therefore speculated whether these four degradants were actually of higher molecular weight, such as peroxy-linked "dimers", but were decomposing under the high temperatures of the APCI interface. A more gentle alternative method of LC/MS analysis was then sought. The 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) adduct [16], formed by a Diels-Alder cycloaddition, has been used in LC/MS studies to enhance sensitivity for the quantitation of trace levels of 1,25-dihydroxy-vitamin D_3 [17,18]. This approach was not used here because (a) sensitivity was not an issue and (b) formation of the adduct requires that the C-10-19:C-5-6 diene structure of vitamin D_3 is intact, which was not certain for these unknown degradants.

The use of silver cationization to generate mass spectra of non-polar, hydrophobic compounds (e.g. olefins, aromatics, steroids, terpenes, unsaturated fatty acids, etc.) under ESI conditions has been reported previously [19–21]. This approach takes advantage of the stable π complexes that are formed between Ag^+ and unsaturated compounds. Since ^{107}Ag and ^{109}Ag isotopes occur with 51.8% and 48.2% RA, the detection of $[M+Ag]^+$ ions is facilitated by the appearance of a doublet signal with ~1:1 ratio.

Some initial feasibility experiments were performed to investigate the behavior of vitamin D_3 under conditions of Ag^+-cationization ESI LC/MS. To minimize the potential deposition of silver salts in the ESI interface, a sample prepared from a methanol solution of vitamin D_3 (8 mL; 150 ppm) that had been doped with an aqueous solution of silver tetrafluoroborate (1 mL; 11 ppm) was used. Isocratic chromatography with water–acetonitrile (10:90, v/v) resulted in $[M+Ag+MeCN]^+$ as the base signal, with $[2M+Ag]^+$ at about 20% RA and $[M+Ag]^+$ at about 2% RA. In contrast, chromatography with water–methanol (10:90, v/v) gave $[2M+Ag]^+$ as the base signal and the $[M+Ag]^+$ signal at about 55% RA, while the $[M+Ag+MeOH]^+$ signal was only detectable by inspection of the appropriate mass chromatograms. Furthermore, the total ion chromatogram signal for vitamin D_3 was about 70 times greater for the water–methanol chromatography than for the water–acetonitrile chromatography. This enhanced sensitivity

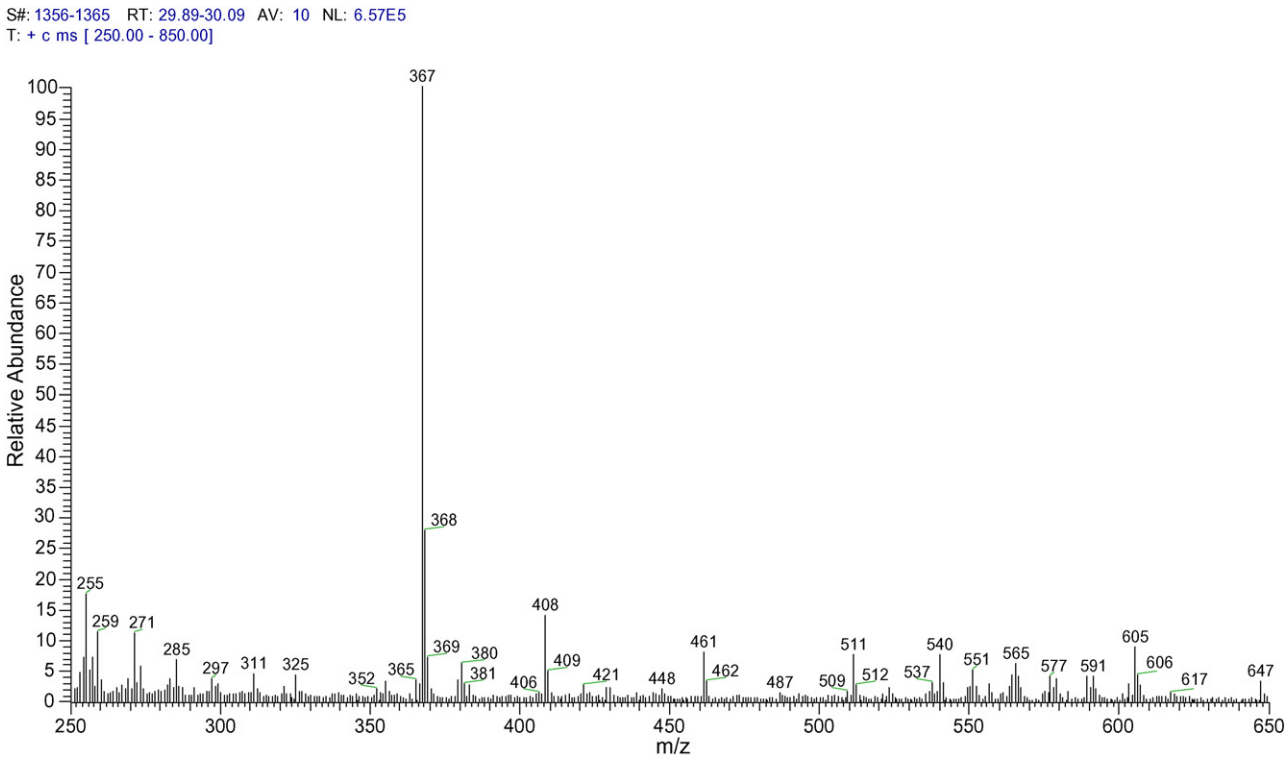


Fig. 4. APCI-MS spectrum of peak A.

with methanol as the mobile phase component is similar to some results with other compounds that were reported during early investigations of the thermospray ionization LC/MS interface [22,23]. Direct infusion experiments (5 μL/ min) were then conducted to optimize the ESI interface parameters. Initial use of a mixture of the 140 ppm vitamin D_3 solution in methanol with an 11 ppm aqueous $AgBF_4$ solution (10:1, v/v) resulted in detection of vitamin D_3 by the $[M+Na]^+$ signal as base signal; interestingly, moderate intensity signals for $[M+H]^+$ and $[M+H-H_2O]^+$ were also observed, while the $[M+Ag]^+$ signal was very weak. When the mixture ratio was reversed to 1:10, only $[M+Ag]^+$ was present in the molecular ion region and the signal was readily optimized. Subsequent chromatography of the tablet solution was therefore performed with a water–methanol mobile phase (HPLC Method C) and the concentration of the aqueous $AgBF_4$ solution for post-column addition was increased 10-fold, to 115 ppm.

When the LC/MS analysis of the stressed tablet solution was repeated under ESI conditions with post-column addition of an aqueous solution of silver tetrafluoroborate [21], peaks A and B both gave $[M+Ag]^+$ signals at *m/z* 617, 619 (Fig. 5; peak A) while peaks C and D both gave $[M+Ag]^+$ signals at *m/z* 645, 647 (Fig. 6; peak C). This determined a molecular weight of 510 amu for the A, B peaks and a molecular weight of 538 amu for the C, D peaks, respectively, and confirmed the suspicion that higher molecular weight species had decomposed in the APCI interface. It was noted that the vitamin D_3 granules used in the experimental tablet formulation contain triglycerides. The fatty acid composition of these medium chain triglycerides is mainly caprylic acid ($C_7H_{15}COOH$; 54.9%) and capric acid ($C_9H_{19}COOH$; 42.6%) [24]. This suggested that peaks A and C are due to the octanoate and decanoate esters, respectively, of vitamin D_3 (**1**, Fig. 7) formed by a transesterification with the triglycerides.

Peaks B and D are then due to the octanoyl and decanoyl esters, respectively, of pre-vitamin D_3 (**2**, Fig. 7; see discussion of UV spectra below). The identification of the degradant peaks as esters accounts for several observations, i.e. (1) the high hydrophobicity (relative to vitamin D_3) as manifested by reversed-phase HPLC retention times, (2) the presence of apparent dehydration products due to the facile elimination of RCOOH in the APCI interface, and (3) why none of the standard chemical, oxidative stress procedures applied to either crystalline vitamin D_3 or the granules or tablets was able to generate these degradants.

The ready elimination of a fatty acid group under APCI LC/MS conditions to give *m/z* 367 as the base signal and the $[M+H]^+$ ion at about 10–12% RA has been reported for the palmitoyl and stearoyl esters of vitamin D_3 [25]. The same authors also derivatized these esters with PTAD, which resulted in 53% RA and 93% RA for the $[M+H]^+$ ions of the two esters, respectively. However, as shown below, such derivatization is not necessary to obtain a high RA for $[M+H]^+$ under APCI-MS conditions.

As described above, LC/MS analysis of vitamin D_3 under standard APCI conditions (vaporizer: 450 °C; heated capillary: 250 °C) results in a weak $[M+H]^+$ signal at *m/z* 385 and a strong $[M+H-H_2O]^+$ signal at *m/z* 367. Some experiments were then performed with a vitamin D_3 solution to determine better conditions (i.e., producing less thermal degradation) for APCI-MS

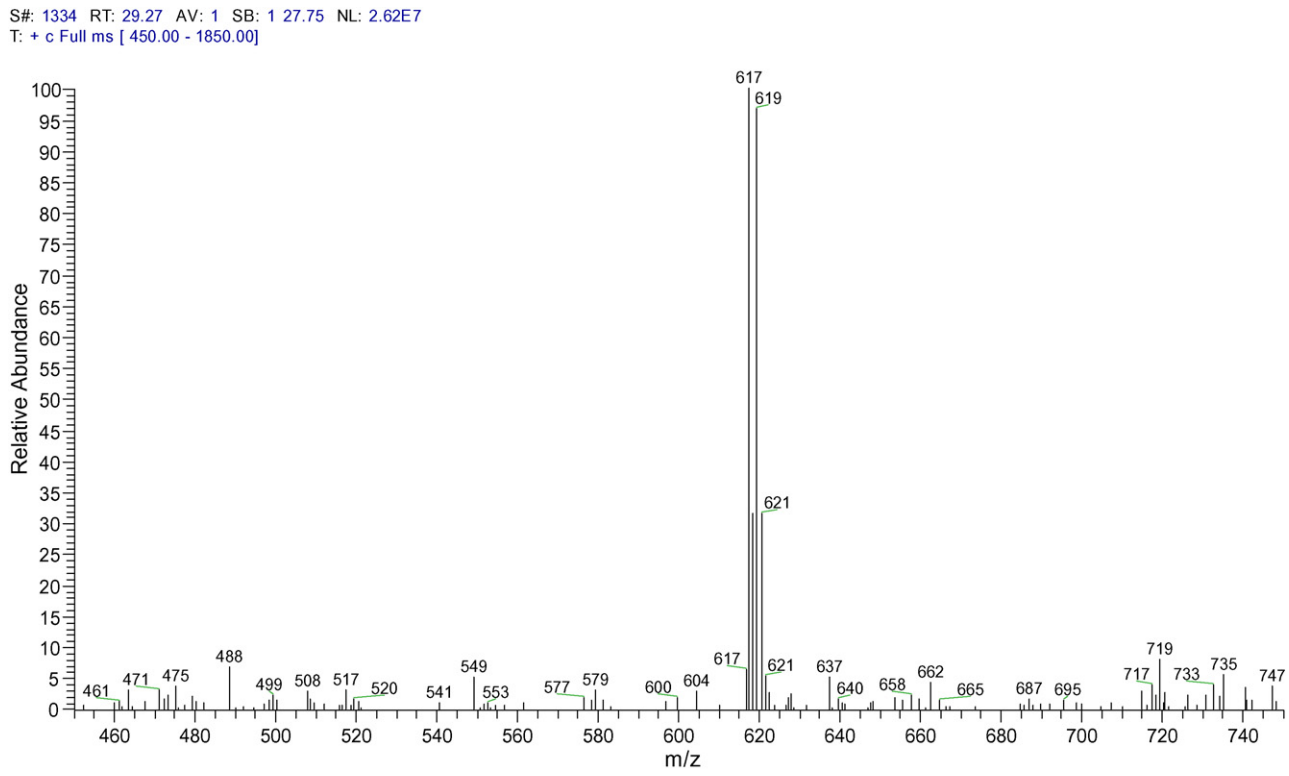


Fig. 5. Ag^+-cationization ESI-MS spectrum of peak A.

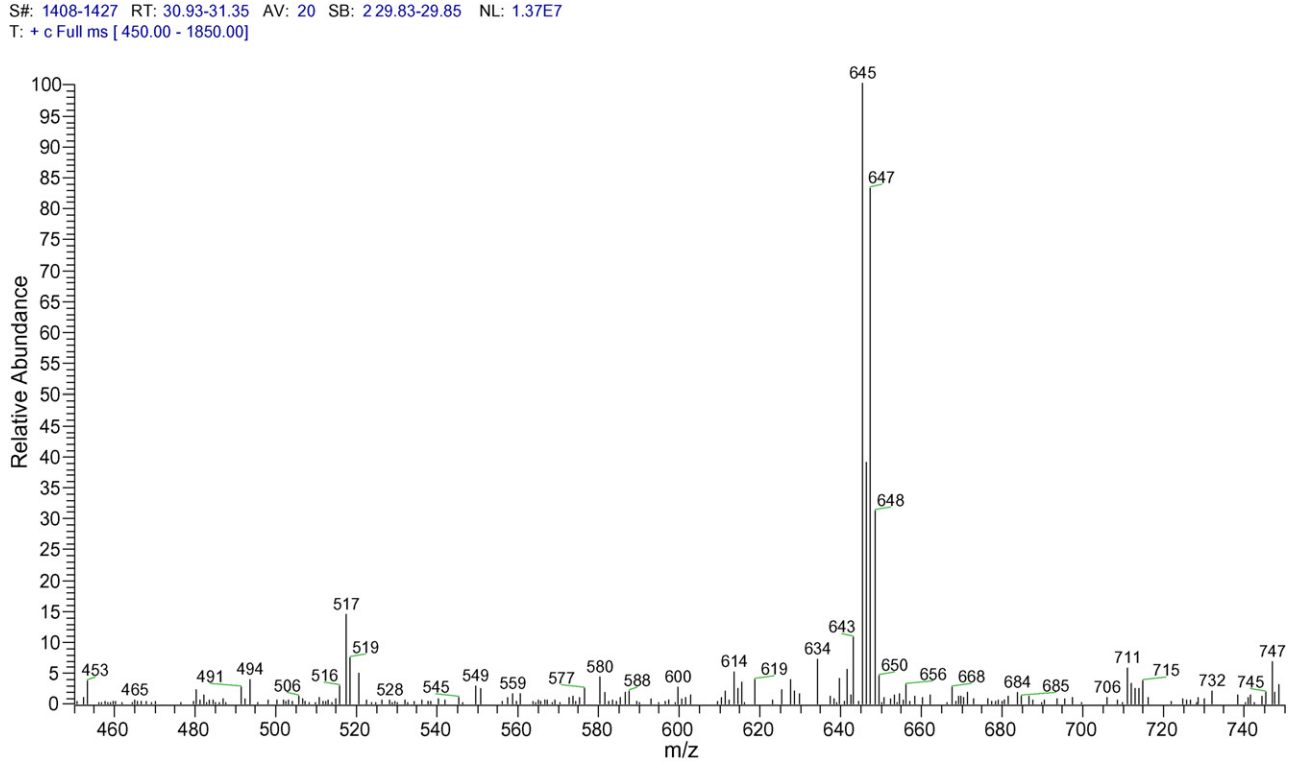
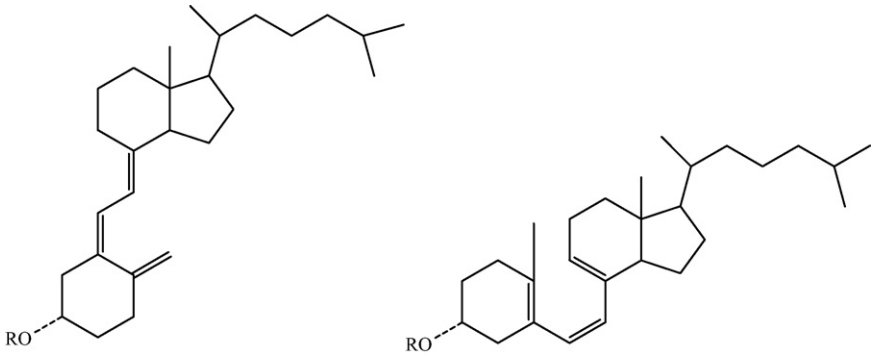


Fig. 6. Ag^+-cationization ESI-MS spectrum of peak C.



1 **2**

VITAMIN D_3	R = H	PRE-VITAMIN D_3
VITAMIN D_3 OCTANOATE	R = COC_7H_{15}	PRE-VITAMIN D_3 OCTANOATE
VITAMIN D_3 DECANOATE	R = COC_9H_{19}	PRE-VITAMIN D_3 DECANOATE

Fig. 7. Structures of vitamin D_3, pre-vitamin D_3 and their octanoate and decanoate esters.

analysis. It was found that a reduced thermal load (vaporizer: 250 °C; heated capillary: 150 °C) gave an intense signal at $[M+H]^+$ and a weak signal at $[M+H-H_2O]^+$. The use of even lower temperatures was viable, but resulted in significant memory effects, i.e., deposition of variable amounts of vitamin D_3 in the APCI interface that were released into the mass spectrometer when temperatures were subsequently increased. A large reduction of the temperatures of the vaporizer and the heated capillary was possible because of the very high organic content (93% acetonitrile) of the chromatographic mobile phase. These modified APCI conditions were then applied to enable MS/MS spectra to be generated from the $[M+H]^+$ ions of the components in the solution of the stressed formulation. As expected for isomers, the MS/MS spectra of the *m/z* 385 pair (vitamin D_3 and pre-vitamin D_3), the *m/z* 511 pair (peaks A, B) and the *m/z* 539 pair (peaks C, D) were identical within each pair. Each showed *m/z* 367 (loss of H_2O or $C_7H_{15}COOH/C_9H_{19}COOH$ from $[M+H]^+$, as appropriate) as the most intense signal, together with an envelope of low level signals (*m/z* 150–350); see Fig. 8 for MS/MS spectrum of $[M+H]^+$ for peak A.

To confirm the identity of the proposed esters of vitamin D_3, a solution of vitamin D_3 in Captex 300EP (tricaprylin/tricaprin mixture; fatty acid composition: caprylic acid, 54.9%, capric acid, 42.6%) acidified with trifluoroacetic acid, was stored at ambient temperatures for 10 days. LC-UV analysis (Method B) of the reaction mixture showed the formation of four degradant peaks eluting in the HPLC method as two pairs of peaks with characteristic relative abundances (RRT 1.67, large; 1.92, small; 2.48, large; 2.94, small). Diode array UV data acquired from the stressed tablet solution had shown that the spectra of the vitamin D_3 peak and the pre-vitamin D_3 peak are quite different. Furthermore, the spectra of peaks A and C were identical to that of vitamin D_3, while the spectra of peaks B and D were identical to that of pre-vitamin D_3. This strengthened the presumption that the first-eluting peak in each pair was derived from vitamin D_3 and the second-eluting peak was derived from pre-vitamin D_3. LC-APCI-MS analysis (reduced thermal load) determined molecular weights of 510 amu and 538 amu for the first and second degradant pairs, respectively. MS/MS spectra generated from the $[M+H]^+$ signals at *m/z* 511 and 539 were identical with those generated from the stressed tablet sample. These data confirmed the identification of the major degradants in the stressed experimental formulation as the octanoate and decanoate esters of vitamin D_3 and pre-vitamin D_3.

Preparation of a larger amount of the octanoate ester of vitamin D_3 was sought for determination of the response factor relative to vitamin D_3. Esterification of vitamin D_3 with octanoic acid and isolation of the ester by preparative HPLC gave a soft, off-white gel. Analytical LC-UV of the isolated material showed that it was contaminated with about 5% of a later-eluting impurity. The impurity peak was increased to about 10% by warming the analytical solution at 65–70 °C for 45 min. LC/MS analysis confirmed that the octanoate ester of vitamin D_3 had partially converted into the octanoate ester of pre-vitamin D_3. The thermal equilibrium between the two ester isomers, which mirrors that between vitamin D_3 and pre-vitamin D_3 [26], explains why the impurity cannot be fully eliminated from the vitamin D_3 ester under ambient conditions.

The vitamin D_3 granules used in the tablet formulation also contained sucrose (MW 342 amu) in about 100-fold excess over the vitamin D_3. It was therefore of interest to see whether any sucrose esters had been formed. Because such esters are not detectable at the wavelength (265 nm) of the LC-UV analysis, this was investigated by plotting mass chromatograms of *m/z* 575–577 and 603–605, $[M+Ag]^+$ for the mono-octanoate and mono-decanoate esters of sucrose, respectively, from the LC/MS analysis of the stressed tablets. These mass chromatograms showed peaks at RRT 0.64 and 0.77 for the two $[M+Ag]^+$

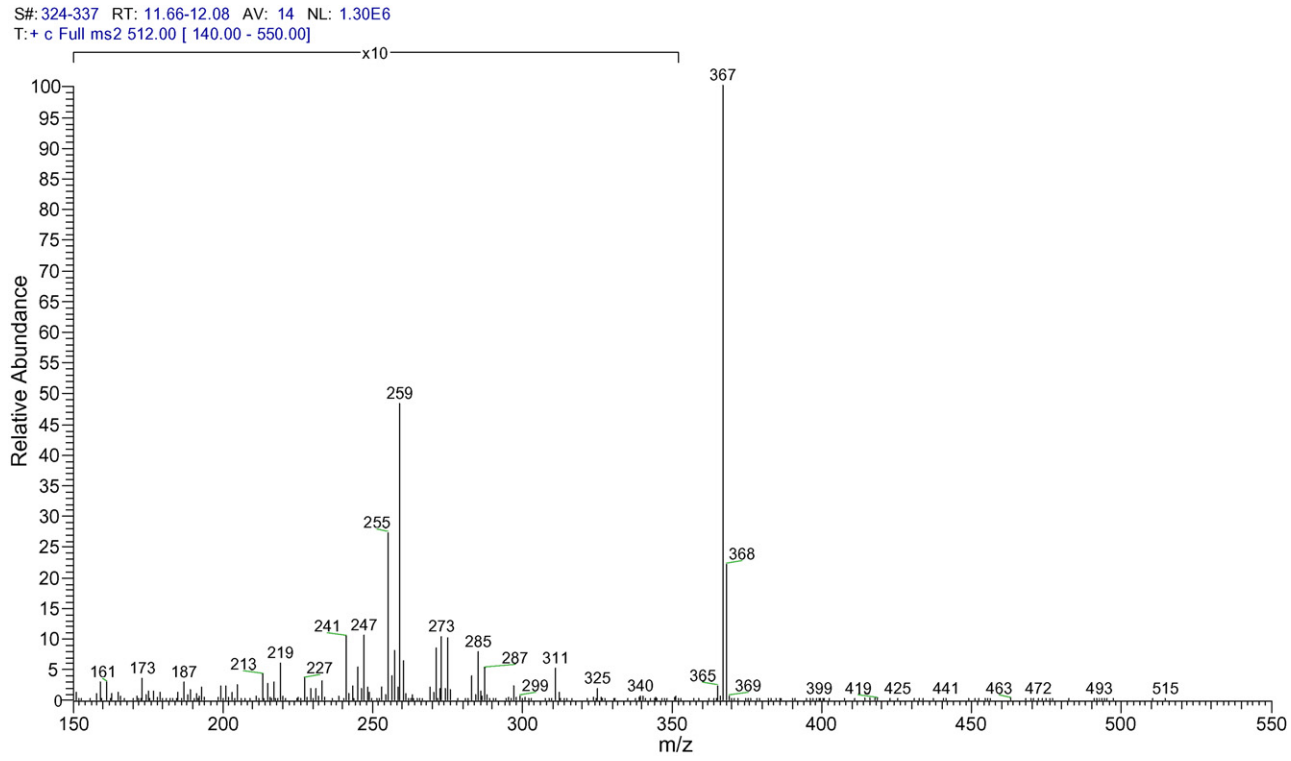


Fig. 8. APCI-MS/MS spectrum of *m/z* 511 for peak A with reduced source temperatures (vaporizer: 250 °C; heated capillary: 150 °C).

values, respectively. Although the formation of sucrose mono-esters is presumptive rather than definitive, the RRT values noted above are appropriate for these relatively more polar compounds. No higher degree of esterification was detected. The detection of these mono-esters is not surprising, since sucrose fatty acid esters (widely used as additives in pharmaceuticals, cosmetics and foods) have been prepared industrially by transesterification of sucrose with fatty acid methyl esters and triglycerides in a melt or solvent-free state [27].

Reports of acylation or esterification of drug substances by excipients in formulations are relatively rare in the pharmaceutical literature. The solid-state acetylation of codeine phosphate [28,29], phenylephrine hydrochloride [30], acetaminophen [31] and homatropine [32] by aspirin have been reported. Codeine phosphate in the solid state has also been reported to be esterified by the acid component (citric or tartaric acid) in an effervescent tablet formulation [33]. Another example, more closely related to the present case, is the esterification of fatty acids and transesterification of triglycerides that occurred during storage of cockle tinctures [34]. The formation of ethyl-2-mercapto-acetate from residual ethanol present in an excipient (sodium starch glycolate) and compounds related to a thermal stabilizer present in PVC packaging material has been reported recently [35]. The acylation of peptides by lactic acid and glycolic acid inside degrading microspheres of poly(lactic acid) and poly(lactic-*co*-glycolic acid) has also been observed [36]. Methylparaben (methyl *p*-hydroxybenzoate), used widely as an anti-microbial preservative, has been shown to undergo transesterification reactions with aldoses and alditols which are common excipients in pharmaceuticals [37].

Esters of vitamin D_3 are of considerable interest because of their implication in the storage and transport of the vitamin in the body. This interest stems from the observations that vitamin D_3 in salt-water fish is esterified [38,39], and that esters (mainly palmitate, stearate, linoleate and oleate) are synthesized in rats that are fed the vitamin [40]. Human pancreatic carboxyl esterase has been shown to catalyze the esterification of vitamin D_3 with oleic acid [41], and the gastro-intestinal absorption of the palmitate ester has been studied in human neonates [42]. The antirachitic activity of various esters in rats and poultry has also been investigated [43,44]. Liquid multi-vitamin formulations that contain esters of vitamin D_3, e.g. palmitate, and that improve stability upon storage and taste are the subject of patent claims [45,46].

4. Conclusions

Four major degradants formed in a thermally stressed, experimental tablet formulation that contained vitamin D_3 granules are identified as the octanoate and decanoate esters of vitamin D_3 and pre-vitamin D_3, formed by a transesterification with triglycerides present in the formulation. This observation reinforces the need to be aware of potential interactions when designing formulations of apparently inert excipients and active pharmaceutical ingredients. The degree of trans-esterification reactivity that was responsible for the degradants identified in the present work is relatively minor. However, even minor drug-excipient reactivity can be significant in the long-term stability of pharmaceutical products due to the stringent quality standards to which pharmaceutical formulations are held.

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