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Short communication

Identification, isolation and characterization of impurities of clindamycin palmitate hydrochloride

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ABSTRACT

Clindamycin palmitate hydrochloride is a water soluble hydrochloride salt of the ester of clindamycin and palmitic acid. It is inactive in vitro, rapid in vivo hydrolysis converts this compound to the antibacterially active clindamycin. Total 12 impurities at levels ranging from 0.05% to 0.5% were detected by isocratic reverse-phase high performance liquid chromatography (HPLC) using RI detector. The molecular weights of impurities were determined by LC–MS analysis. Two impurities were starting materials and the remaining impurities were isolated from crude samples/enriched mother liquors using reverse-phase preparative HPLC. Based on the spectral data the structures of these impurities were characterized as, clindamycin palmitate sulphoxides α-/β-isomers (impurity I); clindamycin laurate (impurity II); lincomycin palmitate (impurity III); clindamycin myristate (impurity IV); epiclindamycin palmitate (impurity V); clindamycin palmitate 3-isomer (impurity VI); clindamycin pentadecanoate (impurity VII); clindamycin B-palmitate (impurity VIII); clindamycin heptadecanoate (impurity IX) and clindamycin stearate (impurity X). Structural elucidation of all impurities by spectral data (^{1}H NMR, ^{13}C NMR, MS and IR) and formation of these impurities have been discussed in detail.

1. Introduction

Clindamycin palmitate hydrochloride is an antibacterial drug. It is chemically designated as methyl 7-chloro-6,7,8-trideoxy-6-(1-methyl-*trans*-4-propyl-L-2-pyrrolidinecarboxamido)-1-thio-L-threo-α-D-galacto-octopyranoside-2-palmitate monohydrochloride. Its molecular formula is $C_{34}H_{63}ClN_2O_6S{\cdot}HCl$ and molecular weight is 699.85 amu.

The scheme used for the synthesis of clindamycin palmitate hydrochloride was reported in the literature [1]. Analytical HPLC methods were reported in the literature for the separation of clindamycin hydrochloride and related impurities [2], separation and characterization of clindamycin and related impurities by LC–MS [3], development and validation of a gradient HPLC method for determination of clindamycin and related compounds in tablet formulation [4], HPLC method for clindamycin hydrochloride related compounds was reported in US pharmacopeia [5] and in European pharmacopoeia [6], but only few methods were reported for the determination of clindamycin palmitate hydrochloride, GC method was reported for the assay of clindamycin palmitate hydrochloride in United States pharmacopeia [7]. Analytical methods for identification, separation and characterization of impurities in clindamycin palmitate hydrochloride were not reported in the literature till date to the best of our knowledge. HPLC method described in this paper is aimed for separation of all impurities with isocratic mode using RI detector. Another HPLC method was also described in this paper for determination of clindamycin hydrochloride content in the drug substance using UV detector.

The HPLC analysis of clindamycin palmitate hydrochloride bulk drug revealed the presence of 12 impurities, which were up to 0.5%. As per the regulatory requirements, the impurity profile study has to be carried out for any final product [8]. This paper describes the identification of impurities present in clindamycin palmitate hydrochloride, detection of masses by LC–MS, isolation by preparative HPLC and characterization of impurities using spectral data.

2. Experimental

2.1. Samples

The investigated sample, clindamycin palmitate hydrochloride was synthesized in APL Research Centre (a unit of Aurobindo

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Pharma Limited, Hyderabad, India). The scheme for synthesis of clindamycin palmitate hydrochloride is shown in Fig. 1. The gas chromatographic purity of palmitoyl chloride used in synthesis is >99.0%. Five impurities lauroyl chloride, myristyl chloride, pentadecanoyl chloride, heptadecanoyl chloride and stearoyl chloride ranging from 0.05% to 0.2% were present in palmitoyl chloride. Reagents used for analysis, i.e., ammonium acetate (AR grade), methanol (HPLC grade) were procured from Merck (India) Limited. Water used was Milli-Q grade.

2.2. High performance liquid chromatography

For identification of impurities: A Waters 2695 separation module equipped with Waters 2414 refractive index detector with Empower pro data handling system [Waters Corporation, Milford, MA, USA] was used. The analysis was carried out on Sunfire C18, 250 mm long, 4.6 mm i.d., 5 μm particle diameter column. Mobile phase was prepared by dissolving 1.5 g of ammonium acetate in 50 ml of water, and made up the volume to 1000 ml with methanol. RI detection was carried out at sensitivity 64 and flow rate was kept at 1.0 ml/min. Column oven temperature and RI detector oven temperature were maintained at 30 °C. Data acquisition time was 50 min.

For determination of clindamycin hydrochloride content: A Shimadzu HPLC system equipped with LC10AT VP pumps with gradient mixer assembly, CTO-10ASVP column oven, SPD-M10A VP UV diode array detector with class VP, version 6.12 SP2 data handling system [Shimadzu Corporation, Analytical Instruments Division, Kyoto, Japan] was used. The analysis was carried out on Sunfire C18, 250 mm long, 4.6 mm i.d., 5 μm particle diameter column. Buffer was prepared by dissolving 1.5 g of ammonium acetate in 1000 ml of water. Mobile phase was prepared by mixing buffer and methanol in the ratio of 15:85 (v/v) and degassed. Diluent was a degassed mixture of water and methanol in the ratio of 5:95 (v/v). Pump mode was isocratic and flow rate was kept at 1.0 ml/min. Column oven temperature was maintained at 30 °C. UV detection was carried out at 226 nm and data acquisition time was 15 min.

2.3. Preparative liquid chromatography

A Shimadzu LC-8A preparative liquid chromatograph equipped with SPD-10A VP, UV–vis detector [Shimadzu Corporation, Analytical Instruments Division, Kyoto, Japan] was used. Hyperprep HS C18 (250 mm long × 21.2 mm i.d.) preparative column packed with 10 μm particle size was employed for isolation of impurities. The mobile phase consisted of (A) 0.1 M ammonium acetate solution and (B) methanol. Flow rate was kept at 30 ml/min and UV detection was carried out at 210 nm. The gradient program was as follows: time (min)/A (v/v):B (v/v); $T_{0.01}$/50:50, $T_{10.0}$/30:70, $T_{25.0}$/20:80, $T_{40.0}$/15:85, $T_{60.0}$/10:90, $T_{80.0}$/5:95, $T_{100.0}$/0:100.

2.4. LC–MS/MS analysis

LC–MS/MS analysis was carried out using PerkinElmer triple quadrupole mass spectrometer (API 2000, PE SCIEX) coupled with a shimadzu HPLC equipped with Shimadzu refractive index detector (RID-10A) and LC 10 AT VP pumps. Analyst software was used for data acquisition and data processing. Turbo ion spray voltage was maintained at 5.5 kV and temperature was set at 375 °C. The auxillary gas and curtain gas used was high pure nitrogen. Zero air was used as nebulizer gas. LC–MS spectra were acquired from *m*/*z* 100 to 1000 in 0.1 amu steps with 2.0 s dwell time. Clindamycin palmitate hydrochloride laboratory batch sample was subjected to LC–MS/MS analysis using ESI source in both +ve and −ve ion modes. The analysis was carried out using RI detector and the remaining conditions were followed as given in Section 2.2. Data acquisition time was

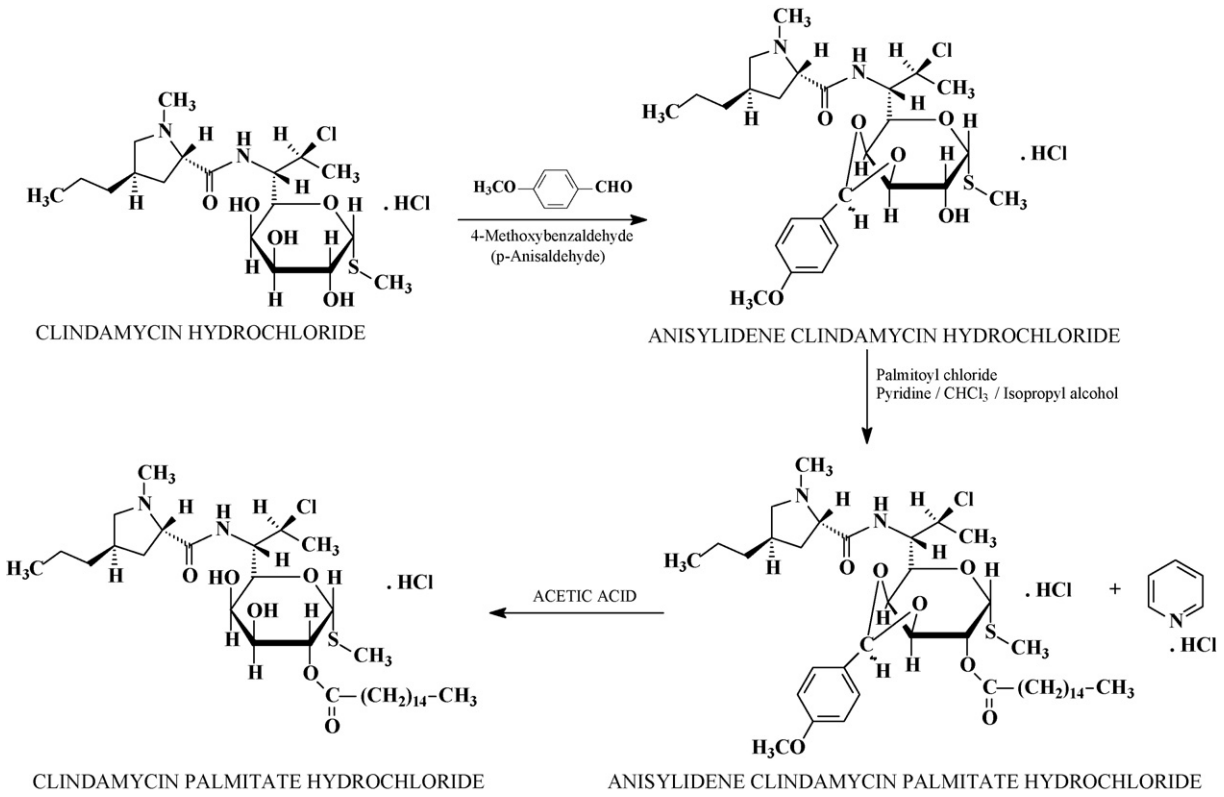

Fig. 1. Scheme for the synthesis of clindamycin palmitate hydrochloride.

50 min. Twelve impurity peaks were detected in this sample. The masses of detected peaks were 425.2 [(MH)$^+$], 427.1 [(MH+2)$^+$] clindamycin; 255.4 [(M–H)$^-$] palmitic acid; 679.8 [(MH)$^+$], 681.5 [(MH+2)$^+$]; 607.4 [(MH)$^+$], 609.4 [(MH+2)$^+$]; 645.5 [(MH)$^+$]; 635.4 [(MH)$^+$], 637.5 [(MH+2)$^+$]; 663.8 [(MH)$^+$], 665.7 [(MH+2)$^+$]; 663.8 [(MH)$^+$], 665.7 [(MH+2)$^+$]; 649.6 [(MH)$^+$], 651.5 [(MH+2)$^+$]; 649.6 [(MH)$^+$], 651.5 [(MH+2)$^+$]; 677.4 [(MH)$^+$], 679.3 [(MH+2)$^+$]; 691.4 [(MH)$^+$], 693.5 [(MH+2)$^+$] respectively. From mass values the structures given in Fig. 3 were suggested.

2.5. NMR spectroscopy

The ^{1}H NMR, ^{13}C NMR (proton decoupled) and DEPT spectra were recorded on Bruker 300 MHz spectrometer using DMSO-d_6 as solvent and tetramethylsilane (TMS) as internal standard.

2.6. Mass spectrometry

Mass spectra were recorded on PerkinElmer PE SCIEX-API 2000 mass spectrometer equipped with a Turboionspray interface at 375 °C. Detection of ions was performed in electrospray ionization, positive ion mode.

2.7. FT-IR spectroscopy

FT-IR spectra of impurities were recorded as KBr pellet on PerkinElmer instrument model—spectrum one.

2.8. Isolation of impurities by preparative HPLC

Impurities were present in the crude samples/enriched samples at percentage levels of 8.0% (impurity I), 0.5% (impurity IV), 0.5% (impurity V), 25.0% (impurity VI), 0.6% (impurity VIII) and 70.0% (impurity X) by area normalization. These impurities were isolated by preparative HPLC by using the conditions described in Section 2.3. Based on *m*/*z* values from LC–MS analysis, impurities II, III, VII and IX were prepared by synthetic procedures and these were spiked with clindamycin palmitate hydrochloride batch to confirm the retention times. Even though clindamycin palmitate hydrochloride has low UV response, UV detector (210 nm) was used for isolation by preparative HPLC for collecting the fractions. RI detector was not used for isolation due to the baseline disturbances caused by gradual increase of methanol and over ranges observed during loading. Impurities were eluted at >90% organic phase. Collected fractions were analyzed by analytical HPLC using RI detector and as per the conditions mentioned in Section 2.2. Fractions of >90% chromatographic purity were pooled together, concentrated on rotavapour to remove methanol. The aqueous solutions were lyophilized using freeze dryer (Virtis advantage 2XL). Impurities II, III, IV, VII and IX were prepared as hydrochloride salts and obtained as white powders with chromatographic purities of 97.3%, 82.3%, 98.9%, 96.4% and 97.8% respectively. Impurities I, V, VI, VIII and X were obtained as gummy solids and they were not prepared as salts. Their chromatographic purities are 93.4%, 90.1%, 87.7%, 95.3% and 95.6% respectively.

3. Results and discussion

3.1. Detection of impurities

A typical analytical LC-chromatogram of a laboratory batch of clindamycin palmitate hydrochloride bulk drug is recorded using the LC method as described in Section 2.2. This sample was subjected to LC–MS/MS analysis using the method described in Section 2.4. The prepared and isolated impurities were co-injected with clindamycin palmitate hydrochloride to confirm the retention times. All the impurities were well resolved from clindamycin palmitate peak. The resolution mixture chromatogram is shown in Fig. 2. Isopropyl palmitate (RRT—0.78) was present in the drug substance at a level of 0.02%. Chemical structures of all impurities are shown in Fig. 3.

3.2. Structural elucidation of impurities

3.2.1. Impurity I (clindamycin palmitate sulphoxides α-/β-isomers)

ESI mass spectrum of impurity I (RRT—0.44 and 0.46) exhibited a molecular ion at *m*/*z*, 679.5 [(MH)$^+$], 681.5 [(MH+2)$^+$] with chloro pattern in positive ion mode, indicating that the molecular weight of this impurity was 16 amu more than that of clindamycin palmitate. Two closely eluted peaks corresponding to this mass were observed in LC–MS, have suggested that the impurity may be existing as two isomers *m*/*z*, 425 is the major fragment of clindamycin palmitate and this is from clindamycin moiety and *m*/*z* 441.5 is the fragment observed in this impurity and suggested that the change in the molecule is on clindamycin moiety. Being more polar than that of clindamycin palmitate, it was suggested that the oxygen addition might have happened on sulphur atom. In ^{1}H NMR spectrum, it was observed that the methyl group, C(7)H3 attached to sulphur atom had shifted from 2.06 ppm to 2.30 and 2.31 ppm and in ^{13}C spectrum, C(7) methyl carbon attached to sulphur atom had shifted from 13.3 ppm to 33.5 ppm. Most of the remaining protons and carbons were observed at two chemical shift values due to the epimers at sulphur atom. Based on the

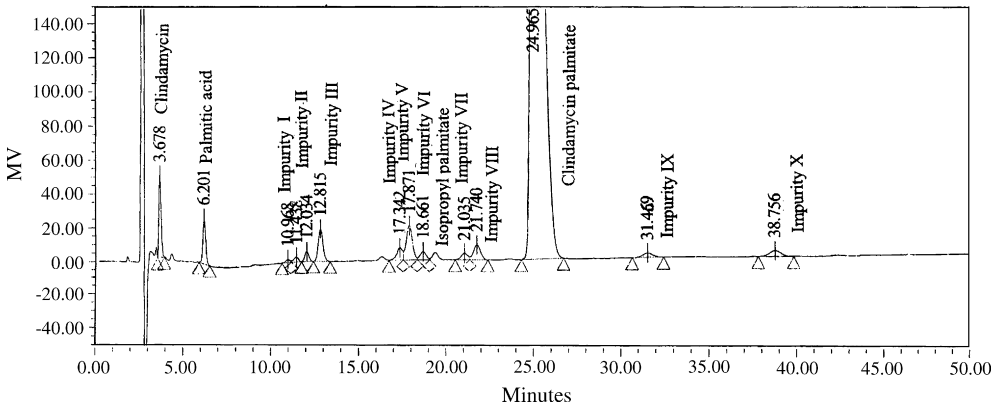

Fig. 2. Typical LC-chromatogram of clindamycin palmitate hydrochloride sample spiked with impurities.

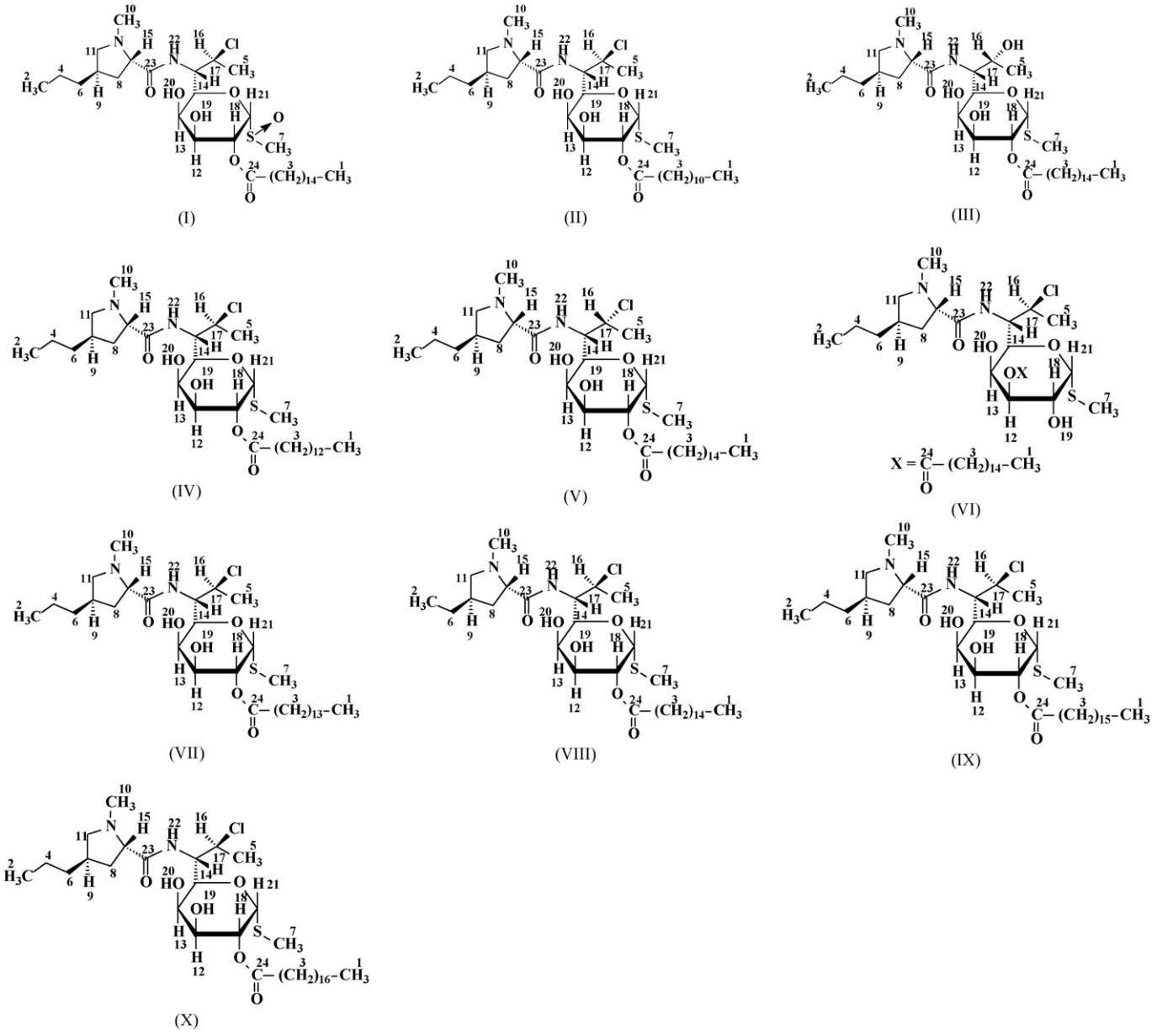

Fig. 3. Chemical structures of impurities.

above spectral data the molecular formula of this impurity was confirmed as $C_{34}H_{63}ClN_2O_7S$ and the corresponding structure was characterized as methyl 7-chloro-6,7,8-trideoxy-6-(1-methyl-*trans*-4-propyl-L-2-pyrrolidinecarboxamido)-1-thio-L-threo-D-galacto-octopyranoside-2-palmitate sulphoxide (clindamycin palmitate sulphoxides α-/β-isomers).

3.2.2. Impurity II (clindamycin laurate)

The electrospray ionization mass spectrum of impurity II (RRT—0.48) exhibited molecular ion peak at *m*/*z* 607.4 [$(MH)^+$], 609.4 [$(MH+2)^+$] with chloro pattern in positive ion mode. The molecular weight of this impurity is 56 amu ($4 \times CH_2$) less than that of clindamycin palmitate. Based on *m*/*z* value, this impurity was synthetically prepared using lauroyl chloride as starting material. Retention time of the prepared impurity matched with impurity present in clindamycin palmitate hydrochloride drug substance. Chemical shift values of proton and carbon signals observed in ^1H NMR and ^{13}C NMR spectra of impurity were unchanged, when compared to spectra of clindamycin palmitate hydrochloride, but 18 protons were observed at 1.24 ppm as broad singlet instead of 26 protons in clindamycin palmitate. Based on this data the molecular formula of this impurity was confirmed as $C_{30}H_{55}ClN_2O_6S$ and the structure was characterized as methyl 7-chloro-6,7,8-trideoxy-6-(1-methyl-*trans*-4-propyl-L-2-pyrrolidinecarboxamido)-1-thio-L-threo-D-galacto-octopyranoside-2-laurate (clindamycin laurate).

3.2.3. Impurity III (lincomycin palmitate)

ESI mass spectrum of impurity III (RRT—0.51) exhibited a molecular ion peak at *m*/*z*; 645.5 [$(MH)^+$] in positive ion mode. Molecular weight of this impurity is 18 amu less than that of clindamycin palmitate. Interesting observation is that chloro pattern was not observed in mass spectrum. This pattern has immediately suggested that the hydroxy group is present in place of chloro group and it had formed due to the presence of lincomycin in clindamycin hydrochloride, which further reacts with palmitic

acid to form lincomycin palmitate. Retention time of synthetically prepared lincomycin palmitate was matched with the impurity present in the drug. In ^{1}H NMR spectrum there was a shift in C(5)H3 methyl group from 1.36 ppm to 1.03 ppm and in ^{13}C NMR spectrum C(5) methyl carbon was shifted from 23.5 ppm to 19.0 ppm. C(16)H group was shifted from 4.47 ppm to 4.10 ppm. The molecular formula of impurity III was confirmed as $C_{34}H_{64}N_2O_7S$ and the corresponding structure was characterized as methyl 6,8-dideoxy-6-(1-methyl-*trans*-4-propyl-L-2-pyrrolidinecarboxamido)-1-thio-D-erythro-D-galacto-octopyranoside-2-palmitate (lincomycin palmitate).

3.2.4. Impurity IV (clindamycin myristate)

This impurity (RRT ~ 0.69) exhibited a molecular ion peak at *m*/*z* 635.4 [(MH)$^+$] and 637.5 [(MH+2)$^+$] with chloro pattern in positive ion mode. The molecular mass of this impurity was 28 amu less than that of clindamycin palmitate. Fragment peak was observed at *m*/*z* 425, which corresponds to clindamycin moiety. All the chemical shift values observed in the impurity spectra were similar to the values of clindamycin palmitate hydrochloride but the total number of protons observed in ^{1}H NMR spectrum were 59 instead of 63 protons in clindamycin palmitate. Only 22 protons were observed at 1.24 ppm as broad singlet instead of 26 protons in clindamycin palmitate. This has confirmed that this impurity is having 2 × CH_2 groups (28 amu) less in the palmitic acid chain. Based on this spectral data the molecular formula of this impurity was confirmed as $C_{32}H_{59}ClN_2O_6S$ and the corresponding structure was characterized as methyl 7-chloro-6,7,8-trideoxy-6-(1-methyl-*trans*-4-propyl-L-2-pyrrolidinecarboxamido)-1-thio-L-threo-D-galacto-octopyranoside-2-myristate (clindamycin myristate). The structure of this impurity was also confirmed authentically by synthetic preparation using myristoyl chloride as starting material and matching the retention time.

3.2.5. Impurity V (7-epiclindamycin palmitate)

ESI mass spectrum of this impurity (RRT—0.72) showed a molecular ion peak at *m*/*z* 663.8 [(MH)$^+$] and 665.7 [(MH+2)$^+$] with chloro pattern in positive ion mode and is of same molecular weight as that of clindamycin palmitate. Fragmentation pattern was similar to clindamycin palmitate. Structurally there is a possibility of epimerization at 7th position of clindamycin. To confirm this, ^{1}H and ^{13}C NMR spectra of isolated impurity were studied. C(5)H3 methyl was slightly shifted from 1.36 ppm to 1.43 ppm and C(5) methyl carbon was shifted from 23.5 ppm to 20.7 ppm in ^{13}C NMR spectrum. There was no shift observed for the remaining signals. Based on the spectral data the molecular formula of this impurity was confirmed as $C_{34}H_{63}ClN_2O_6S$ and the corresponding structure was characterized as methyl 7-chloro-6,7,8-trideoxy-6-(1-methyl-*trans*-4-propyl-L-2-pyrrolidinecarboxamido)-1-thio-D-erythro-D-galacto-octopyranoside-2-palmitate (7-epiclindamycin palmitate). Later the structure of this impurity was also confirmed by synthetic preparation of epiclindamycin palmitate to a level of 60.0% in the lab which was subjected to preparative HPLC to get pure material. The data of synthetically prepared sample was identical with the data of sample isolated from crude material.

3.2.6. Impurity VI (clindamycin palmitate 3-isomer)

ESI mass spectrum of this impurity (RRT—0.75) exhibited a molecular ion peak at *m*/*z* 663.8 [(MH)$^+$] and 665.7 [(MH+2)$^+$] with chloro pattern in positive ion mode. This molecular weight is same as that of clindamycin palmitate and suggested that this impurity may be an isomer. By thorough study of ^{1}H and ^{13}C NMR spectra, it was observed that the protons and carbons present at 2 and 3 positions of D-galacto-octopyranoside ring were disturbed and remaining were at the same chemical shift positions. Proton at 3-position C(12)H was shifted to down field from 3.62 ppm to 4.33 ppm and the proton situated at 2-position C(18)H shifted to upfield from 5.0 ppm to 4.2 ppm. Based on these shifts, it was confirmed that the palmitic acid chain was attached at 3-position instead of 2-position. Based on the spectral data, the molecular formula of this impurity was confirmed as $C_{34}H_{63}ClN_2O_6S$ and the corresponding structure was characterized as methyl 7-chloro-6,7,8-trideoxy-6-(1-methyl-*trans*-4-propyl-L-2-pyrrolidinecarboxamido)-1-thio-L-threo-D-galacto-octopyranoside-3-palmitate (clindamycin palmitate 3-isomer).

3.2.7. Impurity VII (clindamycin pentadecanoate)

The ESI mass spectrum of this impurity [RRT—0.84] exhibited a molecular ion peak at *m*/*z* 649.6 [(MH)$^+$], 651.5 [(MH+2)$^+$] with chloro pattern in positive ion mode. The molecular weight of this impurity was 14 amu less than that of clindamycin palmitate. Twenty-four protons were observed at 1.24 ppm as broad singlet instead of 26 protons in clindamycin palmitate and all the remaining signals were present at the same chemical shift positions and with same number of protons. Based on this data, it was confirmed that one —CH_2 group (14 amu) was less in the palmitic acid chain. Based on the spectral data, the molecular formula of this impurity was confirmed as $C_{33}H_{61}ClN_2O_6S$ and the corresponding structure was characterized as methyl 7-chloro-6,7,8-trideoxy-6-(1-methyl-*trans*-4-propyl-L-2-pyrrolidinecarboxamido)-1-thio-L-threo-D-galacto-octopyranoside-2-pentadecanoate (clindamycin pentadecanoate).The structure of this was further confirmed by synthetic preparation using pentadecanoyl chloride as starting material. Spectral data and retention times of synthetically prepared material were similar to the data of isolated sample.

3.2.8. Impurity VIII (clindamycin B-palmitate)

ESI mass spectrum of this impurity [RRT ~ 0.87] exhibited a molecular ion peak at *m*/*z* 649.6 [(MH)$^+$], 651.5 [(MH+2)$^+$] corresponding to chloro pattern in positive ion mode. The molecular weight of this impurity was 14 amu less than that of clindamycin palmitate. Mass fragmentation pattern, *m*/*z* 411.2 indicated that the change in the molecule had happened in the clindamycin moiety and it was not in the palmitate chain. ^{1}H NMR and ^{13}C NMR spectra showed that all the signals were present except C(4)H2 signal. Twenty-four protons were observed at 1.23 ppm as broad singlet instead of 26 protons in clindamycin palmitate. In ^{13}C NMR spectrum, C(2) signal of propyl side chain was shifted from 14.8 ppm to 13.5 ppm. Signal at 21.3 ppm corresponding to –C(4) methylene of propyl group was absent and methylene C(6) signal of propyl group was shifted from 34.4 ppm to 27.5 ppm. Based on NMR and fragmentation data, it was concluded that ethyl group was present in the molecule instead of propyl chain and the molecular formula of this impurity was confirmed as $C_{33}H_{61}ClN_2O_6S$ and the corresponding structure was characterized as methyl 7-chloro-6,7,8-trideoxy-6-(1-methyl-*trans*-4-ethyl-L-2-pyrrolidinecarboxamido)-1-thio-L-threo-D-galacto-octopyranoside-2-palmitate (clindamycin B-palmitate).

3.2.9. Impurity IX (clindamycin heptadecanoate)

ESI mass spectrum of this impurity [RRT ~ 1.26] exhibited a molecular ion peak at *m*/*z* 677.4 [(MH)$^+$], 679.3 [(MH+2)$^+$] with chloro pattern in positive ion mode. The molecular weight of this impurity was 14 amu more than that of clindamycin palmitate. All the chemical shift values observed in ^{1}H and ^{13}C NMR spectra of impurity were similar to clindamycin palmitate spectra, but total number of protons observed in ^{1}H NMR spectrum were 65 instead of 63 protons. Twenty-eight protons were observed at 1.24 ppm

Table 1
Comparative ^{1}H NMR assignments for clindamycin palmitate hydrochloride and its impurities

S. no.[a]	Clindamycin palmitate HCl	Impurity I	Impurity II	Impurity III	Impurity IV	Impurity V
1	0.83–0.90 (m, 6H)	0.83–0.85 (m, 6H)	0.83–0.90 (m, 6H)	0.83–0.90 (m, 6H)	0.83–0.90 (m, 6H)	0.83–0.87 (m, 6H)
2						
3	1.24 (brs, 26H), 1.53 (m,	1.24 (brs, 28H), 1.54 (m,	1.24 (brs, 18H), 1.52 (m,	1.24 (brs, 26H), 1.53 (m,	1.24 (brs, 22H), 1.52 (m,	1.23 (brs, 26H), 1.51 (t,
4	2H) and 2.29 (t, 2H)	2H) and 2.38 (t, 2H)	2H) and 2.29 (t, 2H)	2H) and 2.27 (t, 2H)	2H) and 2.29 (t, 2H)	2H) and 2.29 (m, 2H)
5	1.36 (m, 5H)	1.37 and 1.42 (2d, 3H)	1.35 (m, 5H)	1.03 (d, 3H)	1.39 (m, 5H)	1.43 (d, 3H)
6		1.24 (m, 2H)		1.39 (m, 2H)		1.23 (m, 2H)
7	2.06 (s, 3H)	2.30 and 2.31 (2s, 3H)	2.06 (s, 3H)	2.01 (s, 3H)	2.06 (s, 3H)	2.01 (s, 3H)
8	2.06 and 2.29 (2m, 2H)	1.70 and 1.95 (2m, 2H)	2.06 and 2.24 (2m, 2H)	2.17 (m, 3H)	2.06 and 2.27 (m, 2H)	1.60 and 1.75 (2m, 2H)
9	2.20 (m, 1H)	2.05 (m, 1H)	2.21 (m, 1H)		2.24 (m, 1H)	2.20 (m, 1H)
10	2.84 (s, 3H)	2.67 and 2.72 (2s, 3H)	2.84 (s, 3H)	2.81 (s, 3H)	2.84 (s, 3H)	2.29 (s, 3H)
11	2.85 and 3.61 (2m, 2H)	1.98 and 3.17 (2m, 2H)	2.85 and 3.62 (2m, 2H)	2.82 and 3.55 (2m, 2H)	2.85 and 3.62 (2m, 2H)	1.93 and 3.13 (2m, 2H)
12	3.62 (m, 1H)	3.71 (brs, 1H)	3.62 (m, 1H)	3.60 (m, 1H)	3.62 (m, 1H)	2.20 (m, 1H)
13	3.85 (brs, 1H)	4.05–4.45 (m, 2H)	3.84 (brs, 1H)	3.79 (m, 1H)	3.84 (brs, 1H)	3.75 (m, 1H)
14	4.13 (m, 2H)		4.13 (m, 2H)	3.82 (m, 1H)	4.14 (m, 2H)	4.18 (m, 1H)
15		2.89 (m, 1H)		4.20 (m, 1H)		2.78 (m, 1H)
16	4.47 (m, 2H)	4.54–4.80 (m, 2H)	4.46 (m, 2H)	4.14 (m, 1H)	4.48 (m, 2H)	4.37 (m, 2H)
17				4.00 (m, 1H)		
18	4.99 (dd, 1H)	5.34 (dd, 1H)	5.0 (m, 1H)	5.01 (dd, 1H)	5.0 (dd, 1H)	4.99 (dd, 1H)
19	5.05 and 5.25 (2brs, 2H)	4.88 (2d, 1H) and 5.25	5.10 and 5.25 (2brs, 2H)	–	5.10 and 5.25 (2brs, 2H)	4.92 and 5.11 (2d, 2H)
20		(2m, 1H)		–		
21	5.42 (d, 1H)	5.76 (s, 1H)	5.42 (d, 1H)	5.38 (d, 1H)	5.42 (d, 1H)	5.37 (d, 1H)
22	8.77 (d, 1H)	7.62 (2d, 1H)	8.74 (d, 1H)	8.50 (d, 1H)	8.74(d, 1H)	7.97(d, 1H)
25	9.81 (brs, 1H NH^+)	–	9.80 (brs, 1H, NH^+)	9.73 (brs, 1H, NH^+)	9.80 (brs, 1H, NH^+)	–

S. no.[a]	Impurity VI	Impurity VII	Impurity VIII	Impurity IX	Impurity X
1	0.83–0.87 (m, 6H)	0.83–0.90 (m, 6H)	0.83–0.88 (m, 6H)	0.83–0.90 (m, 6H)	0.83–0.87 (m, 6H)
2					
3	1.23 (brs, 26H), 1.48 (m,	1.24 (brs, 24H), 1.52 (m,	1.23 (brs, 24H), 1.53 (t, 2H) 2.28 (t, 2H)	1.24 (brs, 28H), 1.53 (t,	1.24 (brs, 30H), 1.52 (t,
4	2H) and 2.28 (m, 2H)	2H) and 2.29 (t, 2H)	–	2H) and 2.29 (t, 2H)	2H) and 2.26 (t, 2H)
5	1.38 (d, 3H)	1.35 (d, 3H)	1.37 (d, 3H)	1.36 (m, 5H)	1.38 (d, 3H)
6	1.23 (m, 2H)	1.42 (m, 2H)	1.32 (m, 2H)		1.24 (m, 2H)
7	2.07 (s, 3H)	2.06 (s, 3H)	2.07 (s, 3H)	2.07 (s, 3H)	2.05 (s, 3H)
8	1.75 (m, 2H)	2.06 and 2.20 (2m, 2H)	1.75 and 1.86 (2m, 2H)	2.07 and 2.30 (2m, 2H)	1.70 and 1.85 (2m, 2H)
9	2.00 (m, 1H)	2.18 (m, 1H)	1.90 (m, 1H)	2.15 (m, 1H)	2.15 (m, 1H)
10	2.32 (s, 3H)	2.83 (s, 3H)	2.31 (s, 3H)	2.73 (s, 3H)	2.31 (s, 3H)
11	1.99 and 3.14 (2m, 2H)	2.83 and 3.62 (2m, 2H)	2.0 and 3.16 (2m, 2H)	2.73 and 3.57 (2m, 2H)	1.97 and 3.16 (2m, 2H)
12	4.33 (m, 1H)	3.62 (m, 1H)	3.66 (m, 2H)	3.61 (m, 1H)	2.87 (m, 1H)
13	3.75 (m, 1H)	3.82 (brs, 1H)		3.77 (brs, 1H)	3.66 (m, 2H)
14	4.01 (m, 1H)	4.12 (m, 2H)	4.05 (m, 1H)		
15	2.89 (m, 1H)		2.90 (m, 1H)	4.10 (m, 2H)	4.04 (m, 1H)
16	4.57 (m, 2H)	4.47 (m, 2H)	4.55 (m, 1H)	4.44 and 4.52 (2m, 2H)	4.35 (m, 1H)
17			4.35 (m, 1H)		4.56 (m, 1H)
18	4.20 (m, 1H)	5.01 (dd, 1H)	4.99 (dd, 1H)	5.0 (m, 1H)	4.98 (dd, 1H)
19	4.95 and 5.34 (2d, 2H)	5.01 and 5.25 (2brs, 2H)	4.75 and 5.05 (m, 2H)	5.01 and 5.25 (2m, 2H)	4.77 and 5.04 (2d, 2H)
20					
21	5.29 (d, 1H)	5.42 (d, 1H)	5.41 (d, 1H)	5.43 (d, 1H)	5.41 (d, 1H)
22	7.71 (d, 1H)	8.68 (d, 1H)	7.64 (d, 1H)	8.50 (brs, 1H)	7.64 (d, 1H)
25	–	9.76 (brs, 1H, NH^+)	–	9.75 (brs, 1H, NH^+)	–

[a] Refer Fig. 3 for numbering.

as broad singlet instead of 26 protons in clindamycin palmitate. Based on the spectral data, the molecular formula of this impurity was confirmed as $C_{35}H_{65}ClN_2O_6S$ and the corresponding structure was characterized as methyl 7-chloro-6,7,8-trideoxy-6-(1-methyl-*trans*-4-propyl-L-2-pyrrolidinecarboxamido)-1-thio-L-threo-α-D-galacto-octopyranoside-2-heptadecanoate (clindamycin heptadecanoate). The structure of this impurity was further confirmed by synthetic preparation using heptadecanoyl chloride as starting material instead of palmitoyl chloride.

3.2.10. Impurity X (clindamycin stearate)

ESI mass spectrum of this impurity exhibited a molecular ion peak at *m/z* 691.4 [$(MH)^+$], 693.5 [$(MH+2)^+$] with chloro pattern in positive ion mode. The molecular weight of this impurity was 28 amu more than that of clindamycin palmitate suggested that $2 \times CH_2$ groups were probably more in palmitic acid side chain. This impurity was synthetically prepared to a level of 70% using stearoyl chloride as starting material to give clindamycin stearate. Further this was purified by preparative HPLC. The retention time and *m/z* values of synthetically prepared material were correlated to the impurity present in clindamycin palmitate drug substance at RRT—1.55. Based on the spectral data, the molecular formula of this impurity was confirmed as $C_{36}H_{67}ClN_2O_6S$ and the corresponding structure was characterized as methyl 7-chloro-6,7,8-trideoxy-6-(1-methyl-*trans*-4-propyl-L-2-pyrrolidinecarboxamido)-1-thio-L-threo-α-D-galacto-octopyranoside-2-stearate (clindamycin stearate).

The ^{1}H and ^{13}C NMR chemical shift values of clindamycin palmitate hydrochloride and all impurities are given in Tables 1 and 2 respectively. The FT-IR spectral data is given in Table 3.

3.3. Formation of impurities

3.3.1. Impurity I

Clindamycin palmitate hydrochloride may get oxidized at sulphur atom resulting in this impurity as α-/β-isomers.

Table 2
Comparative ^{13}C NMR and DEPT assignments for clindamycin palmitate hydrochloride and its impurities

S. No.[a]	Clindamycin palmitate HCl ^{13}C (δ, ppm)/DEPT	Impurity I ^{13}C (δ, ppm)/DEPT	Impurity II ^{13}C (δ, ppm)/DEPT	Impurity III ^{13}C (δ, ppm)/DEPT	Impurity IV ^{13}C (δ, ppm)/DEPT	Impurity V ^{13}C (δ, ppm)/DEPT	Impurity VI ^{13}C (δ, ppm)/DEPT	Impurity VII ^{13}C (δ, ppm)/DEPT	Impurity VIII ^{13}C (δ, ppm)/DEPT	Impurity IX ^{13}C (δ, ppm)/DEPT	Impurity X ^{13}C (δ, ppm)/DEPT
1	14.7 CH_3	14.8 CH_3	14.7 CH_3	14.7 CH_3	14.7 CH_3	14.8 CH_3	14.8 CH_3	14.7 CH_3	14.8 CH_3	14.8 CH_3	14.8 CH_3
2	14.8 CH_3	15.0 CH_3	14.8 CH_3	14.8 CH_3	14.8 CH_3	15.0 CH_3	15.0 CH_3	14.8 CH_3	13.5 CH_3	14.9 CH_3	15.0 CH_3
3	23.0, 25.2, 29.3, 29.6, 29.8, 29.9, 32.2, 35.2 CH_2	22.9, 24.7, 25.1, 29.3, 29.6, 29.7, 29.8, 29.9, 32.1, 37.7 CH_2	23.0, 25.2, 29.3, 29.6, 29.8, 29.9, 32.2, 35.2 CH_2	23.0, 25.2, 29.3, 29.6, 29.8, 29.9, 32.2, 35.3 CH_2	23.0, 25.2, 29.3, 29.6, 29.8, 29.9, 32.2, 35.3 CH_2	23.0, 25.2, 29.2, 29.6, 29.8, 29.9, 32.2, 36.6 CH_2	23.0, 25.1, 29.3 29.6, 29.7, 29.9, 32.2, 36.2 CH_2	23.0, 25.2, 29.3, 29.4, 29.6, 29.8, 29.9, 32.2, 35.2 CH_2	23.0, 25.2, 29.2, 29.5, 29.8, 32.1, 34.3 CH_2	23.0, 25.3, 29.4, 29.6, 29.9, 30.0, 32.2, 35.3 CH_2	23.0, 25.2, 29.2, 29.6, 29.8, 29.9, 32.2, 36.6 CH_2
4	21.3 CH_2	21.9, 21.94 CH_2	21.3 CH_2	21.3 CH_2	21.3 CH_2	21.9 CH_2	21.8 CH_2	21.3 CH_2	–	21.4 CH_2	21.4 CH_2
5	23.5 CH_3	23.6 CH_3	23.5 CH_3	19.0 CH_3	23.5 CH_3	20.7 CH_3	23.4 CH_3	23.5 CH_3	23.5 CH_3	23.6 CH_3	23.5 CH_3
6	34.4 CH_2	34.1, 34.3 CH_2	34.2 CH_2	34.4 CH_2	34.4 CH_2	34.4 CH_2	34.3 CH_2	34.4 CH_2	27.5 CH_2	34.5 CH_2	34.4 CH_2
7	13.3 CH_3	33.5 CH_3	13.3 CH_3	13.6 CH_3	13.3 CH_3	13.8 CH_3	13.0 CH_3	13.3 CH_3	13.3 CH_3	13.4 CH_3	13.3 CH_3
8	36.1 CH_2	36.5 CH_2	36.1 CH_2	35.7 CH_2	36.1 CH_2	37.7 CH_2	37.9 CH_2	36.1 CH_2	37.5 CH_2	36.2 CH_2	37.8 CH_2
9	36.5 CH	36.8 CH	36.5 CH	36.5 CH	36.5 CH	37.9 CH	38.2 CH	36.5 CH	36.0 CH_2	36.6 CH	37.8 CH
10	41.0 CH_3	42.0, 42.1 CH_3	40.9 CH_3	40.9 CH_3	40.9 CH_3	42.0 CH_3	42.1 CH_3	40.7 CH_3	42.1 CH_3	41.0 CH_3	42.1 CH_3
11	61.0 CH_2	63.1, 63.2 CH_2	61.0 CH_2	61.1 CH_2	61.0 CH_2	63.2 CH_2	63.0 CH_2	61.0 CH_2	62.8 CH_2	61.1 CH_2	63.2 CH_2
12	68.5 CH	68.9, 69.1 CH	68.5 CH	69.1 CH	68.5 CH	69.5 CH	74.5 CH	68.5 CH	69.3 CH	68.6 CH	69.2 CH
13	68.2 CH	68.3, 68.5 CH	68.2 CH	68.5 CH	68.2 CH	69.4 CH	70.2 CH	68.2 CH	68.8 CH	68.3 CH	68.9 CH
14	71.3 CH	70.9, 71.1 CH	71.4 CH	71.3 CH	71.4 CH	71.1 CH	65.3 CH	71.3 CH	71.3 CH	71.4 CH	71.3 CH
15	67.8 CH	67.2 CH	67.8 CH	68.1 CH	67.8 CH	68.4 CH	68.9 CH	67.8 CH	68.4 CH	67.9 CH	68.4 CH
16	60.2 CH	60.2, 60.3 CH	60.1 CH	66.7 CH	60.1 CH	59.2 CH	60.2 CH	60.1 CH	60.5 CH	60.3 CH	60.5 CH
17	53.4 CH	52.3, 52.8 CH	53.4 CH	54.6 CH	53.4 CH	53.7 CH	51.9 CH	53.4 CH	52.0 CH	53.5 CH	52.1 CH
18	69.8 CH	75.7, 77.0 CH	69.8 CH	69.9 CH	69.8 CH	70.0 CH	66.0 CH	69.8 CH	70.2 CH	69.9 CH	70.3 CH
21	84.8 CH	87.6, 92.1 CH	84.7 CH	84.9 CH	84.7 CH	85.1 CH	88.7 CH	84.7 CH	84.7 CH	84.8 CH	84.7 CH
23	168.5 –	173.5, 173.8 –	168.5 –	167.8 –	168.4 –	173.5 –	173.5 –	168.4 –	173.5 –	168.5 –	173.6 –
24	173.6 –	174.6, 174.7 –	173.5 –	173.6 –	173.5 –	174.7 –	174.7 –	173.5 –	174.7 –	173.6 –	174.7 –

m, multiplet; s, singlet; d, doublet; dd, double doublet; brs, broad singlet.

[a] Refer Fig. 3 (structures) for numbering.

Table 3
FT-IR spectral data for clindamycin palmitate hydrochloride and its impurities

S. no.	Compound	IR (KBr) absorption bands (Cm^{-1})
1	Clindamycin palmitate hydrochloride	3293 (br and m) OH stretch, 2946, 2930, 2912, 2860 (br and s) aliphatic CH stretch, 1742 (m) C=O stretch (ester), 1694 (m) amide C=O stretching, 1464, 1377 (s) aliphatic CH bend, 721(m) CH_2 rock
2	Impurity I	3317 (br and s) OH stretch, 2922, 2852 (br and s) aliphatic CH stretch, 1742 (s) C=O stretch (ester), 1662 (s) amide C=O stretching, 1456, 1378 (s) aliphatic CH bend, 719 (m) CH_2 rock
3	Impurity II	3223 (m) OH stretch, 2939, 2909, 2856 (br and s) aliphatic CH stretch, 1740 (s) C=O stretch (ester), 1693 (m) amide C=O stretching, 1460, 1377 (s) aliphatic CH bend, 721(m) CH_2 rock
4	Impurity III	3218 (m) OH stretch, 2932, 2910, 2852 (br and s) aliphatic CH stretch, 1732 (m) C=O stretch (ester), 1682 (m) amide C=O stretching, 1463, 1377 (s) aliphatic CH bend, 721 (m) CH_2 rock
5	Impurity IV	3307 (m) OH stretch, 2958, 2927, 2856 (br and s) aliphatic CH stretch, 1742 (m) C=O stretch (ester), 1693 (m) amide C=O stretching, 1458, 1377 (s) aliphatic CH bend, 721 (m) CH_2 rock
6	Impurity V	3321 (m) OH stretch, 2924, 2854 (brand s) aliphatic CH stretch, 1741 (s) C=O stretch (ester), 1661 (m) amide C=O stretching, 1457, 1379 (s) aliphatic CH bend, 720 (m) CH_2 rock
7	Impurity VI	3329 (m) OH stretch, 2924, 2853 (br and s) aliphatic CH stretch, 1735 (s) C=O stretch (ester), 1662 (m) amide C=O stretching, 1457, 1379 (s) aliphatic CH bend, 721(m) CH_2 rock
8	Impurity VII	3218 (br and m) OH stretch, 2913, 2849 (br and s) aliphatic CH stretch, 1741 (s) C=O stretch (ester), 1694 (m) amide C=O stretching, 1455, 1377 (s) aliphatic CH bend, 721(m) CH_2 rock
9	Impurity IX	3218 (br and m) OH stretch, 2944, 2895, 2864, 2847 (br and s) aliphatic CH stretch, 1744 (m) C=O stretch (ester), 1693 (s) amide C=O stretching, 1463, 1377 (s) aliphatic CH bend, 721(m) CH_2 rock
10	Impurity X	3360 (br and s) OH stretch, 2962, 2945, 2912, 2849 (br and s) aliphatic CH stretch, 1739 (m) C=O stretch (ester), 1667 (s) amide C=O stretching, 1470, 1377 (m) aliphatic CH bend, 718 (m) CH_2 rock

w, weak; s, strong; m, medium; br, broad.

3.3.2. Impurity II

Starting material, palmitoyl chloride may contain lauroyl chloride impurity, which participates throughout the synthesis to yield this impurity.

3.3.3. Impurity III

Lincomycin is the starting material for the preparation of clindamycin. Clindamycin hydrochloride may contain lincomycin impurity, which condenses with palmitic acid results in this impurity.

3.3.4. Impurity IV

Starting material, palmitoyl chloride may contain myristoyl chloride impurity, which participates in the synthesis to yield this impurity.

3.3.5. Impurity V

During the preparation of clindamycin hydrochloride, a small quantity of material may get epimerized at 7th position to form 7-epiclindamycin, which further reacts with palmitic acid to yield 7-epiclindamycin palmitate. Clindamycin epimer (7-epiclindamycin) is a known impurity in clindamycin hydrochloride.

3.3.6. Impurity VI

During the preparation of clindamycin palmitate, a small quantity of palmitoyl chloride may condense with 3-hydroxy group of galacto-octopyranoside ring instead of 2-position leading to this impurity.

3.3.7. Impurity VII

Starting material, palmitoyl chloride may contain pentadecanoyl chloride impurity, which condenses with clindamycin leading to this impurity.

3.3.8. Impurity VIII

Starting material clindamycin hydrochloride may contain clindamycin B-palmitate impurity, which condenses with palmitoyl chloride results in this impurity.

3.3.9. Impurity IX

Starting material, palmitoyl chloride may contain heptadecanoyl chloride impurity, which condenses with clindamycin hydrochloride results in this impurity.

3.3.10. Impurity X

Starting material, palmitoyl chloride may contain stearoyl chloride impurity, which condenses with clindamycin hydrochloride results in this impurity.

4. Conclusion

The process related impurities in clindamycin palmitate hydrochloride bulk drug were identified, isolated and characterized by HPLC (analytical and preparative), LC–MS, FT-IR, NMR (^{1}H, ^{13}C and DEPT) techniques.

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