Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Pharmaceutical and Biomedical Analysis 41 (2006) 1116–1123
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Separation and characterization of clindamycin and related impurities in bulk drug by high-performance liquid chromatography-electrospray tandem mass spectrometry

Hui Zhou [a], Zhiguo Zheng [c], Shihua Wu [b], Yuanpo Tai [c], Xiaoji Cao [c], Yuanjiang Pan [c,*]

[a] *Zhejiang University, College of Pharmaceutical Sciences, Hangzhou 310031, PR China*
[b] *Zhejiang University, College of Life Sciences, Hangzhou 310058, PR China*
[c] *Zhejiang University, Department of Chemistry, Hangzhou 310027, PR China*

Received 17 December 2005; received in revised form 5 February 2006; accepted 6 February 2006
Available online 17 April 2006

Abstract

A simple high-performance liquid-electrospray ionization tandem mass spectrometric (HPLC-ESI-MS^n) method has been developed for the rapid identification of clindamycin and its related minor impurities in bulk drug. The ESI-MS^n results obtained allowed us to propose plausible schemes for their fragmentations, which were confirmed further by electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI-FTICR-MS) using collision-induced dissociation (CID) method at high mass resolution. The positive ESI-MS/MS of clindamycin and its derivative compounds showed some diagnostic fragments, such as the neutral losses of H_2O, HCl, methanethiol and 2-methylthio-ethenol, and the residue of 3-propyl-*N*-methylpyrrolidine and 3-ethyl-*N*-methylpyrrolidine, which are specific and useful for the identification of the lincosamide antibiotics and related impurities. According to the fragmentation mechanism of mass spectrometry and HPLC-UV-ESI-MS^n data, six impurities of clindamycin have been identified on-line. Additionally, the positive ion mode extracted ion current (EIC) method has been used to separate and identify these lincosamide compounds.

Keywords: HPLC; Tandem mass spectrometry; Clindamycin; Impurities; Lincosamide

1. Introduction

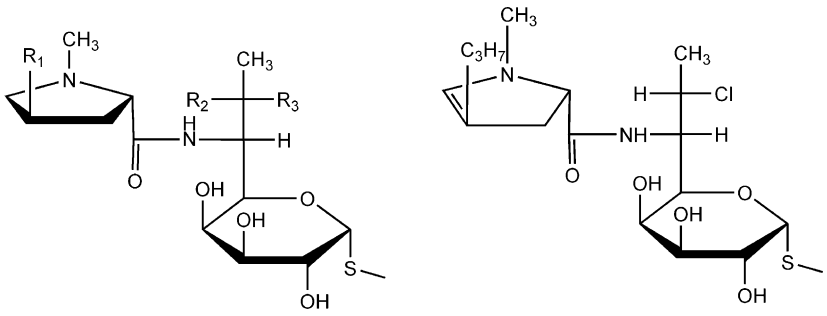
Clindamycin (**1**), a lincosamide antibiotic, is highly efficient against Gram-positive and Gram-negative anaerobic pathogens as well as Gram-positive aerobes by inhibition of bacterial protein synthesis [1]. It was synthesized from lincomycin (**6**) by replacing its hydroxyl group at 7-position with a chlorine atom, resulting in an inversion of the configuration [2]. Clindamycin enjoys widespread use as an antimicrobial in pregnant and non-pregnant patients.

Clindamycin hydrochloride is supplied for administration in capsules. Common impurities (Fig. 1) in clindamycin bulk drug are clindamycin B, 7-epiclindamycin, and a small amount of the lincomycin starting material [20]. Clindamycin B is formed from lincomycin B which is a normal by-product of the fermentation; 7-epiclindamycin is produced during the synthesis of clindamycin [3]. The rapid identification and characterization of minor impurities is an important task.

Several methods have been reported for the determination and analysis of clindamycin in bulk drug, dosage forms and biological fluids, such as microbiological assay [4], spectrophotometry [5], radioimmunoassay [6], gas chromatography [7–9], micellar electrokinetic chromatography (MEKC) [10], and high-performance liquid chromatography (HPLC) with refractive index [11–13] and UV detection [14–20]. Recently, a gradient HPLC method was developed and validated for potency, content uniformity, and impurity determinations for a novel tablet formulation containing clindamycin [21]. However, these methods are not suitable for the identification and characterization of minor impurities. HPLC-MS methods have been described for the determination of clindamycin in biological fluids. An HPLC-atmospheric pressure chemical ionization mass spectrometric (APCI-MS) method was used for human plasma and bone tissue [22] while

* Corresponding author. Tel.: +86 571 87951264; fax: +86 571 87951264.
E-mail address: panyuanjiang@css.zju.edu.cn (Y. Pan).

0731-7085/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2006.02.014



		M.W.	R_1	R_2	R_3
1	Clindamycin	424	CH_3-CH_2-CH_2-	H	Cl
2	7-Epiclindamycin	424	CH_2-CH_2-CH_2-	Cl	H
4	Clindamycin B	410	CH_3-CH_2-	H	Cl
5	7-Epilincomycin	406	CH_3-CH_2-CH_2-	H	OH
6	Lincomycin	406	CH_3-CH_2-CH_2-	OH	H

3 Dehydroclindamycin (M.W. 422)

Fig. 1. Structures of clindamycin and its related impurities.

HPLC-electrospray tandem mass spectrometry (ESI-MS/MS) methods for the determination of clindamycin in human and animal plasma, animal tissues, bovine milk and honey [23–26].

In this paper, we describe the identification of clindamycin and its related impurities in bulk drug using HPLC-ESI-MS^n. At first ESI-MS^n in the positive ion mode was employed to obtain the protonated $[M+H]^+$ ions of the molecular species and the MS^n spectra. As a result, the useful and characteristic fragment ions have been found to propose plausible fragmentation pathways. Moreover, the proposed fragmentation mechanisms were further supported by electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI-FTICR-MS) using collision-induced dissociation (CID) method at high mass resolution, which can demonstrate the unambiguous elemental composition of fragment ion. Then, the bulk drug has been analyzed using HPLC-UV-ESI-MS^n. By comparing the retention time (t_R) and product ion spectra of the compounds with those of authentic standards or literature data, the valid identifications of clindamycin and six related impurities in bulk drug have been provided. To our knowledge, this is the first attempt to characterize clindamycin together with dehydroclindamycin, 7-epiclindamycin, clindamycin B, 7-epilincomycin, lincomycin, and clindamycin diastereoisomer by both ESI-MS^n and HPLC-ESI-MS^n.

2. Experimental

2.1. Samples and chemicals

The investigated clindamycin bulk drug and standards of clindamycin (**1**), 7-epiclindamycin (**2**), dehydroclindamycin (**3**), clindamycin B (**4**), 7-epilincomycin (**5**), and lincomycin (**6**) were kindly provided by Hihang Pharm. Inc., Hangzhou, China. Acetonitrile and methanol used for analytical and preparative HPLC were of chromatographic grade and purchased from Merck, Darmstadt, Germany. Deionized water (18 MΩ) was obtained from a Milli-Q purification system, Millipore, Bedford, MA, USA.

2.2. High-performance liquid chromatography (HPLC)

The analytical HPLC was performed on an Agilent 1100 chromatography system including a G1312 BinPump, G1314A variable-wavelength detector (VWD), model 7725 injector fitted with 20 μl sample loop, a PT100 column oven and an Agilent ChemStation for LC. The column used was a reversed-phase C18 column (YMC-PACK ODS-A, 150 mm × 4.6 mm i.d., 5 μm, 120 Å) at 25 °C. The mobile phase was acetonitrile and pH 7.5 phosphate buffer (dissolve 6.8 g of monobasic potassium phosphate in 1 l of water, and adjust with 8 M potassium hydroxide to pH 7.5) (50:50, v/v). The flow-rate was 0.8 ml/min, and the effluent was monitored at 210 nm.

The semi-preparative HPLC separation for validating the HPLC-ESI-MS^n method was performed using a Waters Delta 600 semi-preparative chromatography system equipped with Waters 600 controller, Waters 2996 photodiode array detector (PAD), Rheodyne Injector Model 7725i with 5.0 ml loop and Millennium^{32} Chromatography Manage. The column employed was a reversed-phase column (Symmetry® C18, 100 mm × 7.8 mm i.d., 5 μm, 100 Å) at 25 °C, and the mobile phase used was methanol and pH 7.5 phosphate buffer (60:40, v/v). The flow-rate was 4 ml/min, and the effluent was monitored at 210 nm. Peak fractions were collected according to the elution profile and analytical HPLC detection.

2.3. HPLC-ESI-MS^n analysis

The HPLC-ESI-MS^n was simultaneously performed with HPLC-UV by coupling an Agilent 1100 series with Bruker Esquire 3000^{plus} mass spectrometer equipped with an ESI interface, ion trap analyzer system and acquired data in software Esquire 5.0. The same column as described in Section 2.2 was used at 25 °C. The mobile phase was acetonitrile and 0.01 M ammonium acetate (50:50, v/v). The flow-rate was 0.8 ml/min, the effluent was monitored at 210 nm. For the HPLC-ESI-MS analysis, the flow was subject to a split of 1:5 before being introduced into the ion source. There was a short delay of about 0.2 min between the UV and MS detectors because of the connecting tubing between HPLC and MS.

Table 1
ESI-MS^2 product ions obtained from the $[M+H]^+$ ions of standards **1–6**

Production	1	2	3	4	5	6
$[M+H]^+$	425 (76.9)	425 (86.7)	423 (25.9)	411 (39.8)	407 (1.7)	407 (1.5)
$[M+H\text{-}H_2O]^+$	407 (28.8)	407 (47.3)	405 (15.6)	393 (23.2)	389 (10.0)	389 (7.6)
$[M+H\text{-}HCl]^+$	389 (23.1)	389 (10.4)	387 (12.9)	375 (17.0)	–	–
$[M+H\text{-}HSCH_3]^+$	377 (100)	377 (100)	375 (10.0)	363 (100)	359 (24.5)	359 (25.8)
$[M+H\text{-}HSCH_3\text{-}HCl]^+$	341 (7.7)	341 (4.7)	339 (6.9)	327 (6.3)	–	–
$[M+H\text{-}CHOHCHSCH_3]^+$	335 (9.6)	335 (6.6)	333 (5.2)	321 (8.0)	317 (6.0)	317 (4.3)
$[M+H\text{-}HCl\text{-}CHOHCHSCH_3]^+$	299 (2.9)	299 (1.9)	297 (1.7)	303 (1.5)	–	–
A^+	126 (61.5)	126 (77.4)	124 (31.9)	112 (1.3)	126 (100)	126 (100)

The ESI-MS^n ion source temperature was 250 °C, and the source voltage was set at 3.5 kV. Nitrogen was used as the sheath and nebulization gas set at 6 l/min and 20 psi, respectively. Helium was introduced into the system to an estimated pressure of 6×10^{-6} mbar to improve trapping efficiency, and also provided as the collision gas during the MS^n experiment. For MS/MS spectra, the mass spectrometer was tuned optimizing the specific collision energy between 0.5 and 1.1 V to maximize the ion current of sequential MS^n of clindamycin and its related impurities. The mass spectra were acquired with a scan rate of 13,000 μm/s.

2.4. Mass spectrometry (ESI-FTICR-MS^2)

All accurate mass measurements were carried out on an Apex III (7.0 T) FTICR-MS (Bruker, Billerica, MA, USA). Solutions were infused from the ESI source at 3 μl/min with parameters: capillary, −4305 V; end plate, −3265 V; skimmer 1, 12.44 V; skimmer 2, 6.61 V; offset, 0.9 V; RF amplitude, 580 Hz; dry gas temperature, 150 °C. Nitrogen was used as the nebulizer gas and drying gas, and argon was used as the collision gas. MS/MS parameters: correlation sweep pulse length, 1000 μs; correlation sweep attenuation, 15 dB; ejection safety belt, 100 Hz; ion activation pulse length, 250,000 μs; ion activation attenuation, 47.5 dB; frequency offset from activation mass, 600 Hz; user delay length, 3 s.

3. Results and discussion

3.1. ESI-MS^n analyses of the reference substances

Standards of clindamycin (**1**), 7-epiclindamyin (**2**), dehydroclindamycin (**3**), clindamycin B (**4**), 7-epilincomycin (**5**), lincomycin (**6**) (Fig. 1) were first studied. Some characteristic fragment ions, such as the losses of neutral molecules H_2O, HCl, methanethiol and 2-methylthio-ethenol, and the residue of 3-propyl-*N*-methylpyrrolidine or 3-ethyl-*N*-methylpyrrolidine, are summarized in Fig. 2 and Table 1, which are specific and useful for the identification of clindamycin and its impurities.

ESI-MS of clindamycin (**1**) (Fig. 3a), shows a characteristic protonated isotope pattern at *m/z* 425 and 427 with about 3:1 ratio of relative abundance corresponding to the presence of one chlorine atom in the molecule. After scanning the fragment at *m/z* 425 with collision-induced dissociation (CID), the product ion spectrum (Fig. 3b) showed the prominent fragment ions at *m/z* 407, 389, 377, 341, 335, 299, and 126. The fragment ion at *m/z* 126 corresponds to the 3-propyl-*N*-methylpyrrolidine ion after loss of the rest of the molecule, and the ion at *m/z* 407 is due to loss of one H_2O from unknown elimination location while the ion at *m/z* 377 corresponds to the loss of $HSCH_3$ from

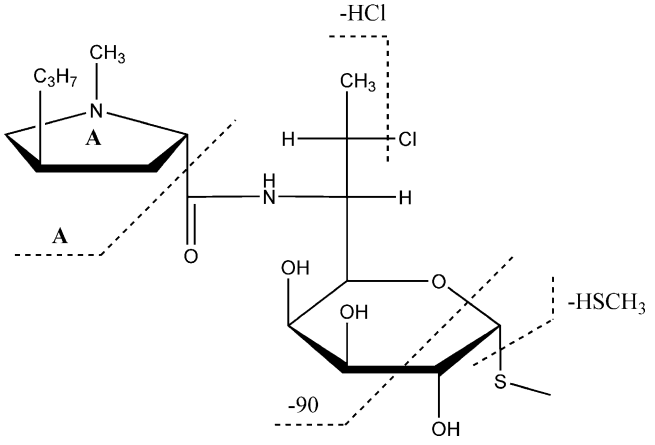


Fig. 2. Observed major fragmentation of clindamycin and its related impurities.

Table 2
Observed and calculated mass of proposed neutral loss of the ion at *m/z* 425.1871 of clindamycin in ESI-FT-ICR-MS^2

Production	Proposed formula	Proposed neutral loss	Calculated mass	Error (ppm)
425.1871	$C_{18}H_{34}O_5N_2SCl$	–	425.1871	0
407.1766	$C_{18}H_{32}O_4N_2SCl$	H_2O	407.1766	0
389.2106	$C_{18}H_{33}O_5N_2S_1$	HCl	389.2105	0.2
377.1838	$C_{17}H_{30}O_5N_2Cl$	$HSCH_3$	377.1838	0
341.2070	$C_{17}H_{29}O_5N_2$	$HSCH_3 + HCl$	341.2071	−0.3
335.1733	$C_{15}H_{28}O_4N_2Cl$	$CHOHCHSCH_3$	335.1732	0.3
299.1969	$C_{15}H_{27}O_4N_2$	$HCl + CHOHCHSCH_3$	299.1965	1.3
126.1277	$C_8H_{16}N_1$		126.1277	0

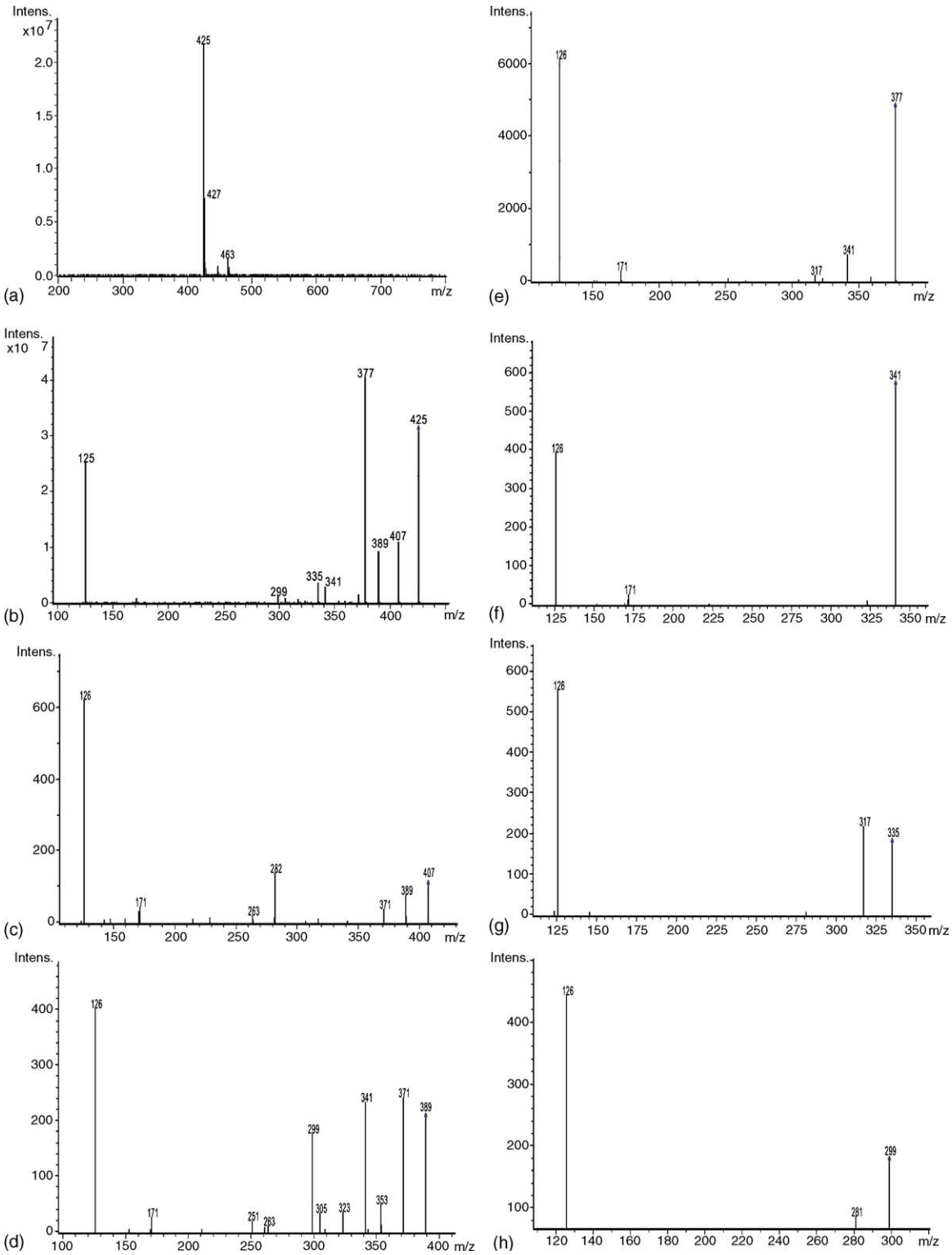


Fig. 3. ESI-MS^n of clindamycin standard. (a) MS; (b) MS^2 of the ion at *m/z* 425 from MS; (c) MS^3 of the ion at *m/z* 407 from MS^2; (d) MS^3 of the ion at *m/z* 389 from MS^2; (e) MS^3 of the ion at *m/z* 377 from MS^2; (f) MS^3 of the ion at *m/z* 341 from MS^2; (g) MS^3 of the ion at *m/z* 335 from MS^2; (h) MS^3 of the ion at *m/z* 299 from MS^2.

the precursor. The fragment at *m/z* 389 corresponding to the loss of molecular mass 36 Da from clindamycin, however, can be considered to be due either to the loss of one HCl, or to elimination of two H_2O from the precursor ion. Although the loss of 36 Da was attributed to two H_2O in previously reported ESI-MS^2 [25], it seemed to lack direct evidence. ESI-MS^2 gives two kinds of possible mechanisms, and it is necessary to establish which one is correct. Moreover, two minor product ions at *m/z* 341 and 335 are present in ESI-MS^2, which also need to be elucidated.

Then ESI-FTICR-MS experiment using CID method was performed, which can demonstrate the unambiguous elemental composition of fragment ions at high resolution. As shown in Table 2, ESI-FTICR-MS^2 results closely corresponded to the above elucidation and clearly exhibited that the fragment at *m/z* 389 corresponding to the loss of molecular mass 36 Da from clindamycin is attributed to the loss of neutral molecule HCl not to the reported elimination of two H_2O. The result implies that the loss of HCl from the molecule of clindamycin is easier than the elimination of two water molecules, which is also supported by the fact that HCl has the lower proton affinity than water (5.8 eV versus 7.2 eV) [27]. Thus, according to Fields rule, elimination of HCl is favoured over elimination of water.

ESI-MS^3 spectra (Fig. 3c–h) from precursor ions at *m/z* 407, 389, 377, 341, 335, and 299, clearly indicate the presence of the prominent fragment ion at *m/z* 126 corresponding to the 3-propyl-*N*-methylpyrrolidine ion after loss of the rest of the molecule from the precursor ions, respectively. From the precursor ion at *m/z* 407 (Fig. 3c), two interesting fragment ions were observed, one is the ion at *m/z* 389 corresponding to the loss of one H_2O which implied it is possible to eliminate two H_2O from clindamycin molecule with the increase of collision energy, the other is the ion at *m/z* 371 corresponding to loss of 36 Da, which can be attributed to the loss of HCl. Furthermore, the ESI-MS^3 from the precursor ions at *m/z* 389 (Fig. 3d), 377 (Fig. 3e) and 335 (Fig. 3g) also showed the characteristic elimination of one and/or two H_2O which is further to indicate that the elimination of H_2O is possible by increasing of collision energy. The product ion at *m/z* 341, found in the ESI-MS^3 from the precursor ions at *m/z* 389 (Fig. 3d) and 377 (Fig. 3e), was formed by the loss of $HSCH_3$ and the elimination of HCl from the two precursor ions, respectively, which was supported by ESI-FTICR-MS^2 data (Table 2); the molecular formula of the ion at *m/z* 341 is $C_{17}H_{29}O_5N_2$. However, the ion at *m/z* 335 was not observed in the ESI-MS^3 from the precursor ions at *m/z* 407, 389, 377, 371 and 341. This implied that the ion at *m/z* 335 in ESI-MS^2 (Fig. 3b) was formed directly by the elimination of 2-methylthio-ethenol with mass weight loss of 90 Da. In addition, ESI-MS^3 indicated the ion at *m/z* 299 in ESI-MS^2 was also produced by the elimination of 2-methylthio-ethenol with mass weight loss of 90 Da from the precursor ion at *m/z* 389. Therefore, the plausible fragmentation of clindamycin may be proposed as in Fig. 4.

7-Epiclindamyin (**2**) is the epimer of clindamycin (**1**) with the chlorine at different configuration. Its ESI-MS showed the same characteristic protonated isotope pattern at *m/z* 423 and 425 with about 3:1 ratio of relative abundance while ESI-MS^2 spectra both showed the very similar characteristic fragment ions as shown in Table 1.

The ESI-MS of dehydroclindamycin (**3**) shows the characteristic protonated isotope pattern at *m/z* 423 and 425 with about 3:1 ratio of relative abundance corresponding to the presence of one chlorine atom in the molecule. Its ESI-MS^2 (Fig. 5) clearly shows the diagnostic ions at *m/z* 405, 387, 375, 339, 333 and 124 corresponding to the losses of neutral molecules H_2O, HCl, methanethiol and 2-methylthio-ethenol, and the residue of 2,3-dihydro-1-methyl-4-propyl-1H-pyrrole, respectively.

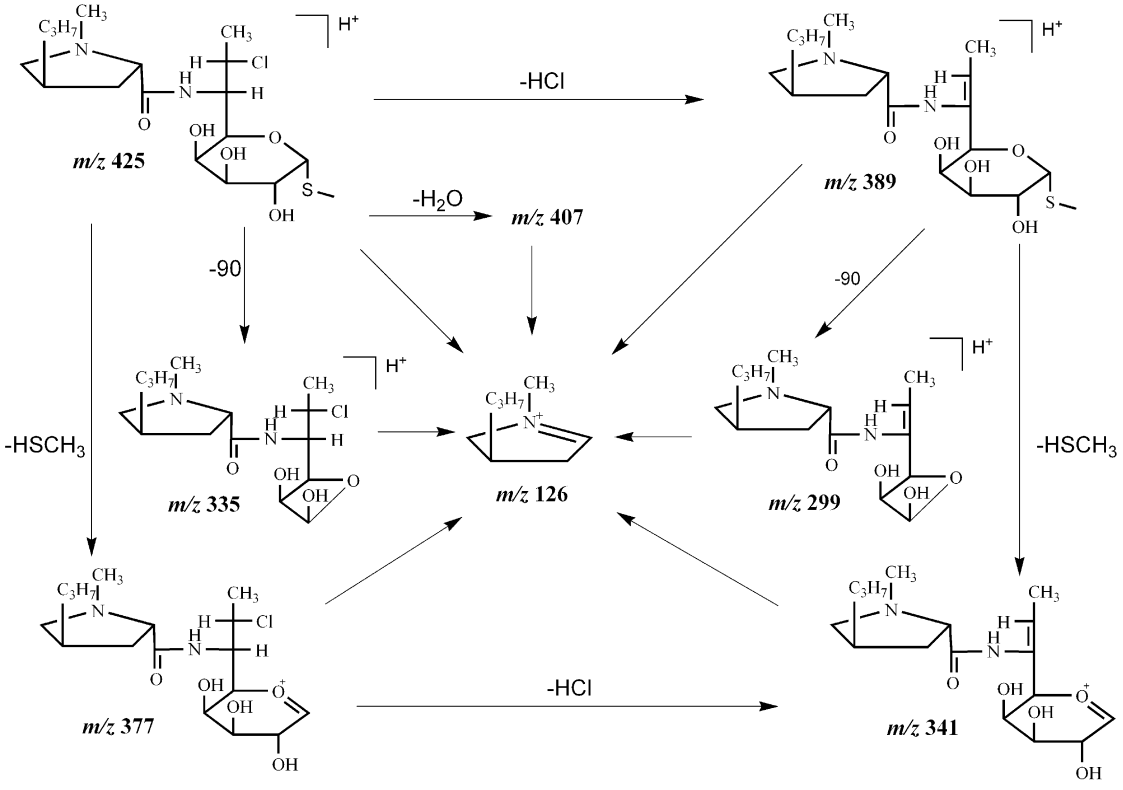


Fig. 4. Proposed fragmentation pathway of clindamycin.

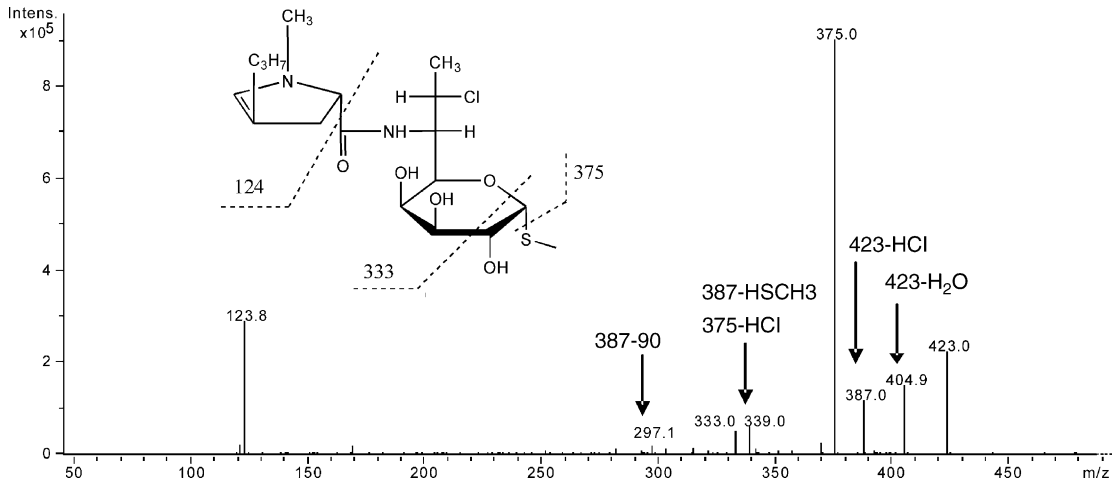


Fig. 5. ESI-MS^2 of dehydroclindamycin.

Clindamycin B (**4**) is the ethyl derivative of clindamycin (**1**). Its ESI-MS and ESI-MS^2 also shows the common fragment ions (Table 1), such as the losses of neutral molecules H_2O, HCl, methanethiol, and 2-methylthio-ethenol, and the residue of 3-ethyl-*N*-methylpyrrolidine.

7-Epilincomycin (**5**) and lincomycin (**6**) is a pair of epimers which contain hydroxyl groups at different configurations. ESI-MS shows the characteristic protonated ion $[M+H]^+$ at *m/z* 407 corresponding to their molecular formula of $C_{18}H_{34}N_2O_6S$. As described above for clindamycin and its epimer, the ESI-MS^2 of lincomycin and 7-epilincomycin also showed very similar characteristic ions such as the loss of neutral molecules H_2O, $HSCH_3$ and 2-methylthio-ethenol, and the residue of 3-propyl-*N*-methylpyrrolidine as shown in Table 1.

The specific ESI-MS^n fragmentation mechanisms of $[M+H]^+$ discussed above can be used for the identification of the lincosamide compounds in bulk drug of clindamycin.

3.2. Determination of clindamycin and its related impurities in crude bulk drug by HPLC-UV

The HPLC method used for quantitative analysis of clindamycin and its related compound was based on the USP [19] and Ph. Eur. [20] methods. Fig. 6 shows the separation of the main component and its impurities. The quantities of the impurities calculated from their peak areas are presented in the legend of Fig. 6.

3.3. The analyses of bulk clindamycin by HPLC-UV-ESI-MS^n

The above HPLC method (Fig. 6) using nonvolatile phosphate buffer can achieve high resolution for the minor impurities; however, it is not compatible with HPLC-ESI-MS^n. For this reason we used an ESI-MS-compatible buffered mobile phase (0.01 M ammonium acetate) avoiding the use of an ion-pairing reagent such as TFA which may cause non-reproducible retention times. Fig. 7 shows the HPLC-UV and HPLC-ESI-MS scans of the bulk drug. The assignments of the HPLC peaks were confirmed by comparison of retention times and ESI-MS^2 fragmentation patterns with authentic standards. Furthermore, the peak assignments of above HPLC analysis (Fig. 6) was also confirmed by preparative separation of the impurities and comparison of retention times. Additionally, positive ion mode extracted ion current (EIC) analyses were performed in order to confirm these assignments.

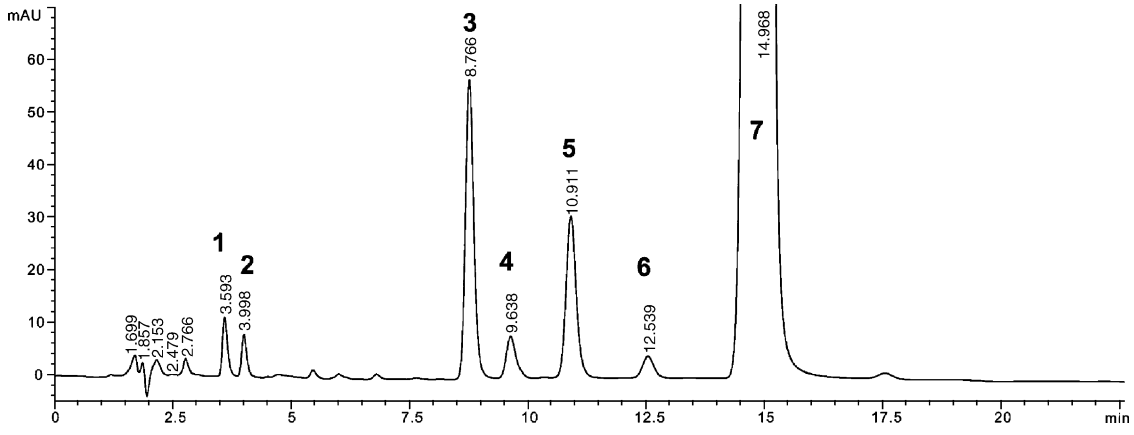


Fig. 6. HPLC analysis of crude clindamycin. Key: 1. lincomycin; 2. 7-epilincomycin; 3. clindamycin B; 4. dehydroclindamycin; 5. 7-epiclindamycin; 6. clindamycin diastereoisomer; 7. clindamycin. For the chromatographic conditions see Section 2.2. Contents (%): **1**, 0.25; **2**, 0.18; **3**, 2.17; **4**, 0.44; **5**, 1.41; **6**, 0.20; **7**, 94.92.

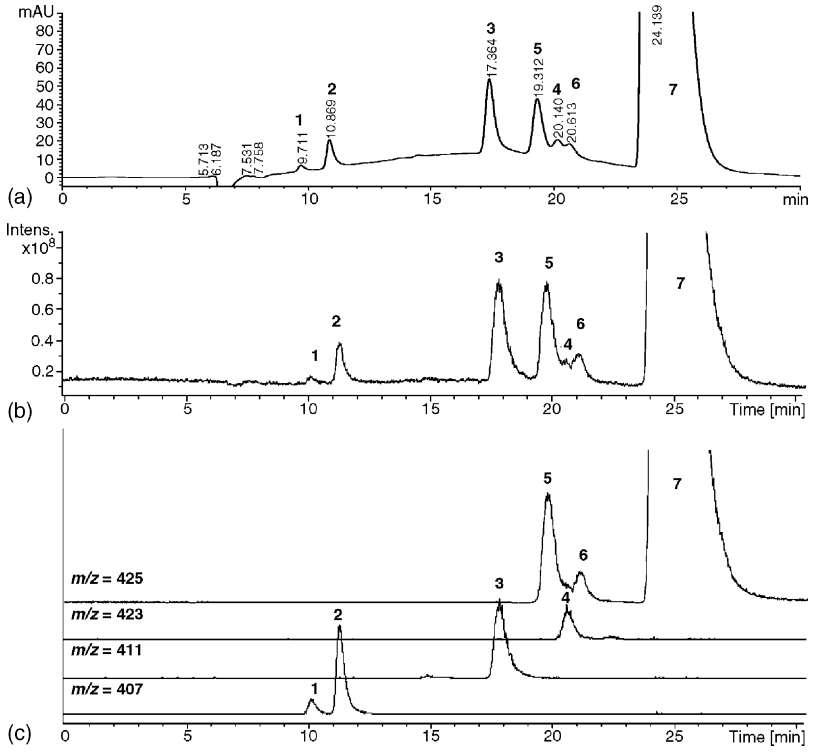


Fig. 7. HPLC-UV-ESI-MS analyses of the clindamycin bulk drug: (a) UV detection at 210 nm; (b) positive ion mode ESI-MS total ion current monitoring; (c) the corresponding EIC chromatograms with SIM at *m/z* 425, 423, 411 and 407. Key: as in Fig. 6.

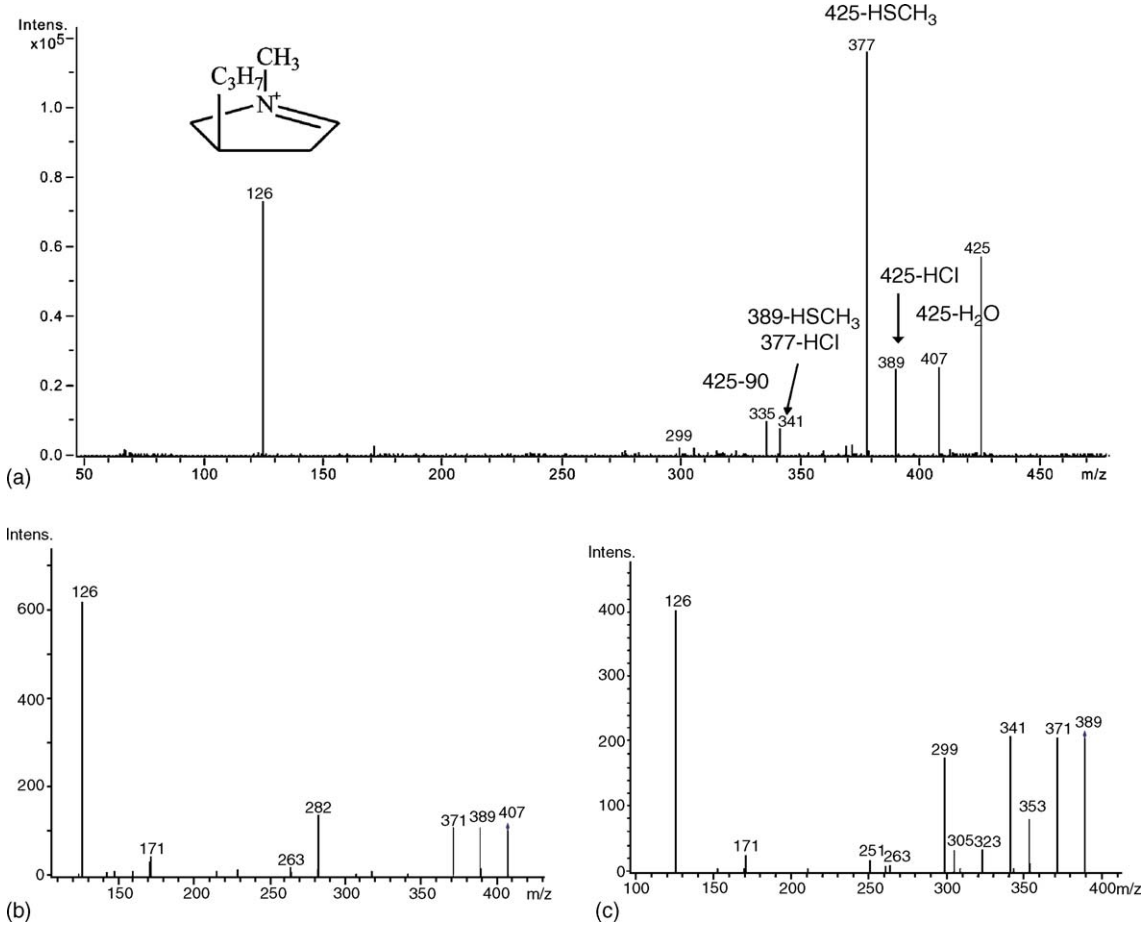


Fig. 8. ESI-MS^2 and MS^3 of peak 6.

As shown in Fig. 7c, positive ion mode EIC chromatogram at *m/z* 407 exhibited two peaks 1 and 2 at retention times of 9.71 and 10.89 min, respectively, which were matched with the t_R of standard lincomycin and 7-epilincomycin. Furthermore, ESI-MS^2 showed the diagnostic fragments corresponding to the neutral loss of H_2O, $HSCH_3$ and 2-methylthio-ethenol, and residue of 3-propyl-*N*-methylpyrrolidine, which are in close agreement with the characteristic fragments of standards. Thus, peaks 1 and 2 are identified as lincomycin and 7-epilincomycin, respectively.

The EIC chromatogram at *m/z* 411 and 423 (Fig. 7c) showed single peak 3 at 17.36 min and peak 4 at 20.14 min, which were matched with the t_R of standard clindamycin B and dehydroclindamycin, respectively. In addition, ESI-MS and ESI-MS^2 of peak 3 are in close agreement with the characteristic fragments of clindamycin B standard, whereas ESI-MS and ESI-MS^2 of peak 4 are also consistent with the characteristic fragments of dehydroclindamycin standard. Thus, peaks 3 and 4 are identified as clindamycin B and dehydroclindamycin, respectively.

The EIC chromatogram at *m/z* 425 (Fig. 7c) shows three peaks: 5 at 19.31 min, 6 at 20.81 min and 7 at 24.34 min. The retention times of peaks 5 and 7 were well matched with the t_R of authentic 7-epiclindamycin and clindamycin, respectively. ESI-MS of the peaks exhibited the same characteristic protonated isotope pattern at *m/z* 425 and 427 with about 3:1 ratio of relative abundance. ESI-MS^2 of the three peaks showed the characteristic fragment ion at *m/z* 425, 407, 389, 377, 341, 335, 299 and 126, which are in close agreement with that of 7-epiclindamycin and clindamycin. All of these data indicate the three peaks are three isomers of clindamycin. By comparison of retention times and ESI-MS^2 fragmentation patterns with authentic standards, peak 5 and peak 7 were identified as 7-epiclindamycin and clindamycin, respectively. However, peak 6 is neither clindamycin nor 7-epiclindamycin. ESI-MS^2 (Fig. 8a) of peak 6 is very similar to clindamycin, and further ESI-MS^3 from precursor ions at *m/z* 407 (Fig. 8b) and 389 (Fig. 8c) were almost matched the spectra of clindamycin. Thus, peak 6 may be a clindamycin diastereoisomer. Although under the present HPLC-UV-ESI-MS condition (Fig. 7) excellent resolution was not obtained especially for HPLC peaks 2–4 contrasting with good separation using non-volatile phosphate buffer (Fig. 6), it is very practical and useful for the rapid identification of the clindamycin and its impurities in bulk drug.

4. Conclusions

In this study, clindamycin and six related lincosamide compounds have been characterized by ESI-MS^n and losses of neutral molecules supported by FT-ICR-MS^2 have been proposed, which are useful for the identification of the lincosamide compounds. An HPLC-UV-ESI-MS^n method was developed to separate and identify clindamycin and six impurities in bulk drug. The experimental results demonstrate that both ESI-MS^n and HPLC-UV-ESI-MS^n are powerful analytical tools for the rapid identification of the lincosamide compounds.

Acknowledgements

Financial support for this work was provided by the Natural Scientific Foundation of China (20375036), Scientific Research Fund of Zhejiang Provincial Education Department (G20050356) and China Postdoctoral Science Foundation.

References

[1] H.A. Stevens, Prim. Care Update Ob/Gyns 4 (1997) 251–253.
[2] R.D. Birkenmeyer, F. Kagan, J. Med. Chem. 13 (1970) 616–619.
[3] J.B. Landis, M.E. Grant, S.A. Nelson, J. Chromatogr. 202 (1980) 99–106.
[4] T.F. Brodasky, C. Lewis, J. Antibiot. 25 (1972) 230–238.
[5] F.A. El-Yazbi, S.M. Blaih, Analyst 118 (1993) 577–579.
[6] C. Duckworth, J.F. Fisher, S.A. Carter, C.L. Newman, C. Cogburn, R.R. Nesbit, C.H. Wray, J. Antimicrob. Agents Chemother. 31 (1993) 581.
[7] G. Gatti, J. Flaherty, J. Bubp, J. White, M. Borin, J. Gambertoglio, J. Antimicrob. Agents Chemother. 37 (1993) 1137.
[8] L.W. Brown, J. Pharm. Sci. 63 (1974) 1597–1600.
[9] T.O. Oesterling, J. Pharm. Sci. 59 (1970) 63–67.
[10] P. Dehouck, A. Van Schepdael, E. Roets, J. Hoogmartens, J. Chromatogr. A 9322001 1245–152.
[11] L.W. Brown, J. Pharm. Sci. 67 (1978) 1254–1257.
[12] G. La Follette, J. Gambertoglio, J.A. White, D.W. Knuth, E.T. Lin, J. Chromatogr. 431 (1988) 379–388.
[13] A. Hornedo-Nuñez, T.A. Getek, W.A. Korfmacher, F. Simenthal, J. Chromatogr. 503 (1990) 217–225.
[14] J.A. Orwa, K. Vandenbempt, S. Depuydt, E. Roets, J. Hoogmartens, J. Pharm. Biomed. Anal. 20 (1999) 745–752.
[15] J.A. Orwa, K. Vandenbempt, S. Depuydt, E. Roets, J. Hoogmartens, J. Pharm. Biomed. Anal. 23 (2000) 771.
[16] H. Fieger-Büschges, G. Schüβler, V. Larsimont, H. Blume, J. Chromatogr. B 724 (1999) 281–286.
[17] S. Cho, H. Im, W. Park, Y. Ha, Y. Choi, K. Lee, Biomed. Chromatogr. 19 (2005) 783–787.
[18] G.C. Batzias, G.A. Delis, M. Koutsoviti-Papadopoulou, J. Pharm. Biomed. Anal. 35 (2004) 545–554.
[19] United States Pharmacopeia, 29, United States Pharmacopeial Convention, Inc., Rockville, Maryland, 2006, p. 536.
[20] European Pharmacopoeia, Supplement 4.2 to fourth ed., Council of Europe, Strasbourg, France, 2002.
[21] D.J. Platzer, B.A. White, J. Pharm. Biomed. Anal. 41 (2006) 84–88.
[22] J. Martens-Lobenhoffer, P. Banditt, J. Chromatogr. B 755 (2001) 143–149.
[23] L. Yu, C. Chao, W. Liao, T. Twu, C. Liu, T. Yang, E.T. Lin, J. Chromatogr. B 724 (1999) 287–294.
[24] G.N. Rechberger, G. Fauler, W. Windischhofer, H. Köfeler, W. Erwa, H. Leis, Rapid Commun. Mass Spectrom. 17 (2003) 135–139.
[25] M. Cherlet, S. Croubels, P. De Backer, J. Mass Spectrom. 37 (2002) 848–853.
[26] D.W.M. Sin, C. Ho, Y.C. Wong, S.K. Ho, A.C.B. Ip, Anal. Chim. Acta 517 (2004) 39–45.
[27] The National Institute of Standards and Technology (NIST), Standard Reference Database Number 69, NIST Chemistry WebBook, available on http://webbook.nist.gov/chemistry/.