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Identification of a degradation product in stressed tablets of olmesartan medoxomil by the complementary use of HPLC hyphenated techniques

Tomonori Murakami [a,*], Hidetoshi Konno [a], Naoto Fukutsu [a], Michinobu Onodera [a], Takao Kawasaki [a,1], Fumiyo Kusu [b]

[a] Analytical and Quality Evaluation Research Laboratories, Daiichi-Sankyo Co. Ltd., 1-12-1 Shinomiya, Hiratsuka, Kanagawa 254-0014, Japan
[b] School of Pharmacy, Tokyo University of Pharmacy and Life Science, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan

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ABSTRACT

An unknown degradation product (DP-1) increased in olmesartan medoxomil (OLM) tablets stored at 40 °C/75% r.h., reaching 0.72% after 6 months. The molecular weight and fragment information obtained by LC–MS suggested that DP-1 was a dehydrated dimer of olmesartan (OL) and the presence of ester carbonyl group was indicated by solvent-elimination LC–IR analysis. LC–^{1}H NMR confirmed the structure of DP-1 as an esterified dimer of OL. Rapid and accurate identification of the degradation product was achieved by the complementary use of HPLC hyphenated techniques without complicated isolation or purification processes.

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1. Introduction

The identification and qualification of degradation products in pharmaceuticals are essential during drug development, since such degradation products could affect the efficacy and safety of the pharmaceuticals even in small amounts. The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) has issued workable guidelines regarding stability studies on pharmaceuticals and the identification and quantification of impurities in drug substances and drug products [1–3]. These guidelines provide the reporting, identification and qualification thresholds for impurities based on the total daily intake of the relevant drug. The identification of degradation products is also very effective for estimating the causes and pathways of the degradation of drug substances in pharmaceuticals.

The isolation of degradation products has been used as a general approach for structure elucidation. However, considerable time and the use of several techniques, such as a preparative scale chromatography and multiple extractions, are required to isolate sufficient quantities of impurities for spectroscopic identification. Moreover, special care is required during the isolation processes in cases where the degradation products are labile, since further degradation of the degradants will occur. In fact, hydrolysis and transesterification reactions of the ester group-containing drugs such as candesartan cilexetil have been observed during the solid phase extraction process [4]. HPLC hyphenated techniques are now widely utilized for the structural analysis of trace amounts of the degradation products without complicated isolation processes. LC–MS has been one of the powerful techniques for the identification of small quantities of drug degradation products [5–7]. Recently, LC–NMR has been increasingly applied to obtain detailed structural information on degradation products [8–13]. LC–NMR experiments can be performed in on-flow, stopped-flow and loop-storage modes. However, stopped-flow or loop-storage LC–NMR analysis would be required for detailed structure elucidation due to the relatively low sensitivity of NMR. LC–IR can provide valuable information on the functional groups of the degradation products [14]. Flow-cell and solvent-elimination interfaces have been used in LC–IR analysis and solvent-elimination LC–IR has generally been the tool of choice for structural analysis work because the obtainable spectral information and analytical sensitivity are limited due to intense absorptions of the LC eluent in the flow-cell LC–IR analysis [15,16]. Lately, multiple hyphenated techniques

* Corresponding author. Tel.: +81 463 31 6447; fax: +81 463 31 6479.
E-mail address: murakami.tomonori.a5@daiichisankyo.co.jp (T. Murakami).
[1] Present address: Institute of Applied Medicine, Inc., 32 Kita 2 Nishi 2, Chuo-ku, Sapporo, Hokkaido 060-0002, Japan.

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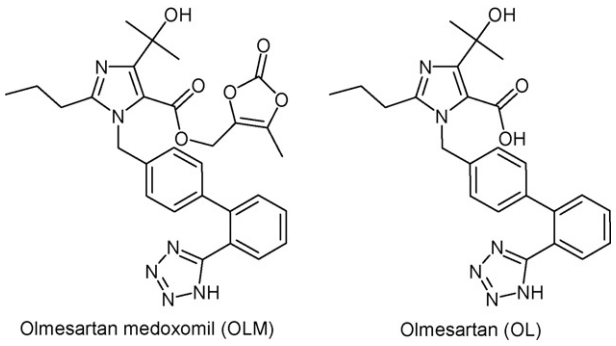

Fig. 1. Chemical structures of OLM and OL.

such as LC–NMR–MS have been reported [17]. However, it is necessary to find a compatible LC method for all the spectrometers in the hyphenated system to perform the analysis. By the complementary use of these HPLC hyphenated techniques, the chemical structures of the degradation products could be elucidated in a significantly shorter time without any isolation or purification processes.

Olmesartan medoxomil (OLM), (5-methyl-2-oxo-1,3-dioxolen-4-yl)methoxy-4-(1-hydroxy-1-methylethyl)-2-propyl-1-{4-[2-(tetrazol-5-yl)-phenyl]phenyl}methylimidazol-5-carboxylate is an angiotensin II receptor blocker [18,19]. This drug is an ester prodrug of the pharmacologically active metabolite olmesartan (OL) [20]. The chemical structures of OLM and OL are shown in Fig. 1. In the stability study of OLM tablets, two major degradation products were observed during 6 months of open storage at 40 °C/75% r.h. One of the degradation products was OL, an ester hydrolysate of OLM, and the other was an unknown degradation product (DP-1) which reached a level of 0.5%. Although a significant increase of DP-1 was observed only in OLM tablets stored at a high temperature and humidity, from a safety standpoint it was important to identify this degradation product.

In this study, the structure of DP-1 was elucidated by the hyphenated techniques of LC–MS, solvent-elimination LC–IR and LC–NMR using LC conditions compatible with each technique. The complementary use of HPLC hyphenated techniques and the adjustment of the HPLC separation conditions for each technique proved to be very effective in the rapid and accurate identification of degradation products without isolation or purification processes.

2. Experimental

2.1. Chemicals and reagents

OLM 5 mg tablets were manufactured by Daiichi-Sankyo (Tokyo, Japan) and these tablets were stored at 40 °C/75% r.h. for 6 months. Separately, OLM tablets were stored in a water-saturated atmosphere at 70 °C for 5 days in order to generate a sufficient amount of DP-1 for the LC–IR and LC–NMR analyses (hereinafter referred to as DP-1-enriched tablets). OL was synthesized by Daiichi-Sankyo. Acetic acid, phosphoric acid, potassium dihydrogen phosphate and triethylamine (Et_3N) of guaranteed grade, and acetonitrile and methanol of HPLC grade were purchased from Wako Pure Chemical Industries (Osaka, Japan), deuterium oxide (D_2O, 99.9% D), deuterated acetonitrile (CD_3CN, 99.8% D) and deuterated methanol (CD_3OD, 99.8% D) were purchased from Cambridge Isotope Laboratories (Andover, MA, USA). Water was purified with a Milli-Q Gradient A10 system (Millipore, Bedford, MA, USA).

2.2. Sample preparation

One OLM tablet was dissolved in 5 ml of methanol using an ultrasonic bath (1 mg OLM/ml). The homogenous suspension was centrifuged at 3000 rpm (rotor radius 21.4 cm) for 10 min with a Hitachi CF8DL centrifuge (Hitachi, Tokyo, Japan) and the clear supernatant was subjected to LC–UV and LC–MS analyses.

Separately, two DP-1-enriched tablets were dissolved in 1 ml of methanol (10 mg OLM/ml) and filtered through a 0.45-μm filter (13 mm GD/X disposable filter, Whatman, Springfield Mill, UK) and the filtrate was subjected to LC–IR and LC–NMR analyses.

For the acquisition of reference LC–IR and LC–NMR spectra of OL, an OL solution of 1 mg/ml concentration in methanol was prepared.

2.3. LC–UV analysis of OLM tablets

LC–UV analyses were performed on an Agilent 1100 LC system (Agilent Technologies, Santa Clara, CA, USA) consisting of an online degasser G1379A, a binary pump G1312A, an autosampler G1329A, a temperature regulated column compartment G1316A, a UV–vis variable-wavelength detector G1314A and a diode array detector G1315B. The chromatographic conditions were as follows: column, TSKgel ODS-100V (150 mm × 4.6 mm, 3 μm particle size, Tosoh, Tokyo, Japan); column temperature, 40 °C; mobile phase, phosphate buffer (15 mM, pH 3.9)–acetonitrile; gradient elution; flow rate, 0.8 ml/min. The acetonitrile content was increased linearly from 35% to 80% in 25 min and held for 15 min at 80%. The analytes were detected by UV detection at 250 nm and the online UV spectra were collected between 200 and 400 nm. The injection volume was 10 μl and the run time was 40 min. Agilent Chemstation version A.09.03 software was used for the data acquisition.

2.4. LC–MS and MS/MS analyses

The liquid chromatograph was the Agilent 1100 LC system described previously. The HPLC separation was conducted under the same conditions as those used for the LC–UV analysis, except for the use of volatile mobile phase modifiers and the use of a 2.0 mm i.d. column at a mobile phase flow rate of 0.2 ml/min in order to get efficient solvent evaporation and ionization. The mobile phases consisted of acetonitrile and 0.1% Et_3N in water adjusted to pH 3.9 with glacial acetic acid. The injection volume was 1 μl. A hybrid quadrupole time-of-flight mass spectrometer Q-Tof2 (Waters, Milford, MA, USA) equipped with an electrospray ionization (ESI) source was used and operated in positive ESI mode. Nitrogen was used with flow rates of 20 l/h for nebulization, 50 l/h for cone gas, and 400 l/h for desolvation. The source and desolvation temperatures were 120 and 200 °C, respectively. The capillary voltage was 3.0 kV and the cone voltage was 35 V. The collision energy was set to 10 eV for MS measurements, 25 eV for the MS/MS measurement. Mass spectra were acquired over an m/z range of 50–2000 with a resolution of approximately 10,000 at full-width half-maximum. For the MS/MS operation, argon was used as a collision gas and the isolation widths of the parent ions were set to 1.7 m/z units. Accurate masses were measured by comparison to a reference compound, leucine enkephalin (m/z of the protonated molecule 556.2771) infused into the lock spray reference channel. MassLynx v 3.5 software (Waters) was used to calculate the molecular formulas of the protonated molecules according to the accurate mass data.

2.5. Solvent-elimination LC–IR analysis

The HPLC separation was conducted under the same conditions as those used for the LC–MS analysis. The injection volume was 10 μl. The HPLC eluent was evaporated by nebulizing with compressed air at a pressure of 30 psi at a temperature of 150 °C using an LC-Transform Series 600 interface (Lab Connections, Northborough, MA, USA). The resultant eluate was deposited on a 60 mm o.d. × 2 mm thickness rear-surface-aluminized germanium disk with a rotation rate of 10°/min. The FT-IR spectra of the deposited eluate were recorded on a Spectrum 100 FT-IR Spectrometer (PerkinElmer, Norwalk, CT, USA) equipped with a triglycine sulfate detector from 400 to 4000 cm^{-1} with 4 cm^{-1} resolution. The transient data of 10 scans was accumulated. As a check on the stability of the samples during the LC–IR analysis, the deposited samples were re-subjected to LC–UV analysis after the IR measurements.

2.6. Stopped-flow LC–NMR analysis

The LC–NMR experiments were performed in the stopped-flow mode on an Avance 600 spectrometer (Bruker BioSpin, Rheinstetten, Germany) working at 600.13 MHz for ^{1}H, coupled to the Agilent 1100 LC system and a Bruker peak sampling unit (BPSU-12) interface. Two HPLC methods were used for the LC–NMR analyses. The first method was the same as that used for the LC–UV analysis, except for the use of a 2.0 mm i.d. column at a mobile phase flow rate of 0.2 ml/min in order to reduce the elution volume of the component of interest and make sure that the maximum amount of the component was inside the flow cell for NMR detection. In the second method, methanol was used in place of acetonitrile as an organic component of the mobile phase and the methanol content was increased linearly from 55% to 85% in 30 min and held for 20 min at 85%. All the mobile phases were prepared using deuterated solvents (D_2O, CD_3CN and CD_3OD) in order to minimize the solvent background. The injection volume was 2 μl. For the NMR experiments, a ^{1}H–^{13}C/^{15}N triple-resonance inverse (TCI) 5 mm cryoprobe (active volume 60 μl) with cold ^{1}H- and ^{13}C-coils and preamplifiers and a *z*-gradient accessory was used. The hyphenated system was controlled by Bruker Hystar software (version 2.3). One-dimensional ^{1}H NMR spectra were recorded using the double presaturation nuclear Overhauser effect spectroscopy pulse

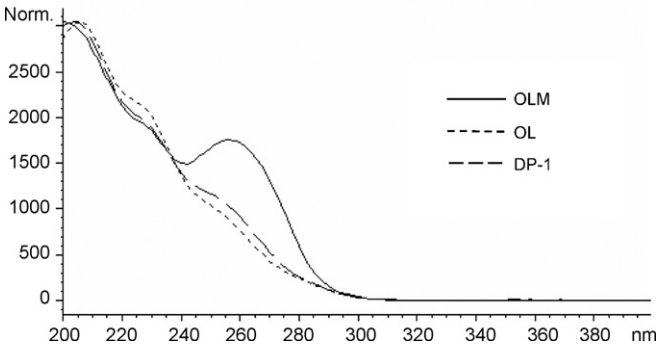

Fig. 3. On-line UV spectra of OLM, OL and DP-1.

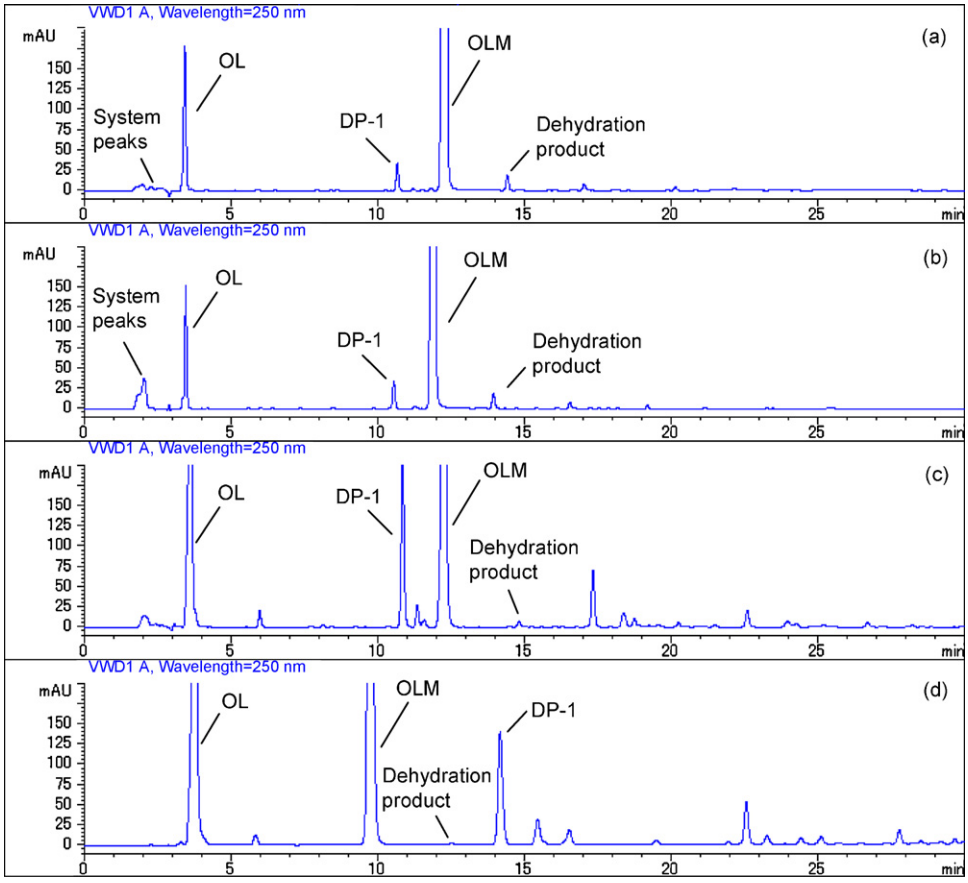

Fig. 2. Typical HPLC chromatograms: (a) stressed OLM tablets (6 months at 40 °C/75% r.h.) analyzed by the LC-UV method (phosphate buffer pH 3.9-acetonitrile eluent), (b) stressed OLM tablets (6 months at 40 °C/75% r.h.) analyzed by the LC-MS method (triethylamine/acetic acid solution pH 3.9-acetonitrile eluent), DP-1 enriched tablets analyzed by LC-NMR methods using (c) acetonitrile eluent and (d) methanol eluent.

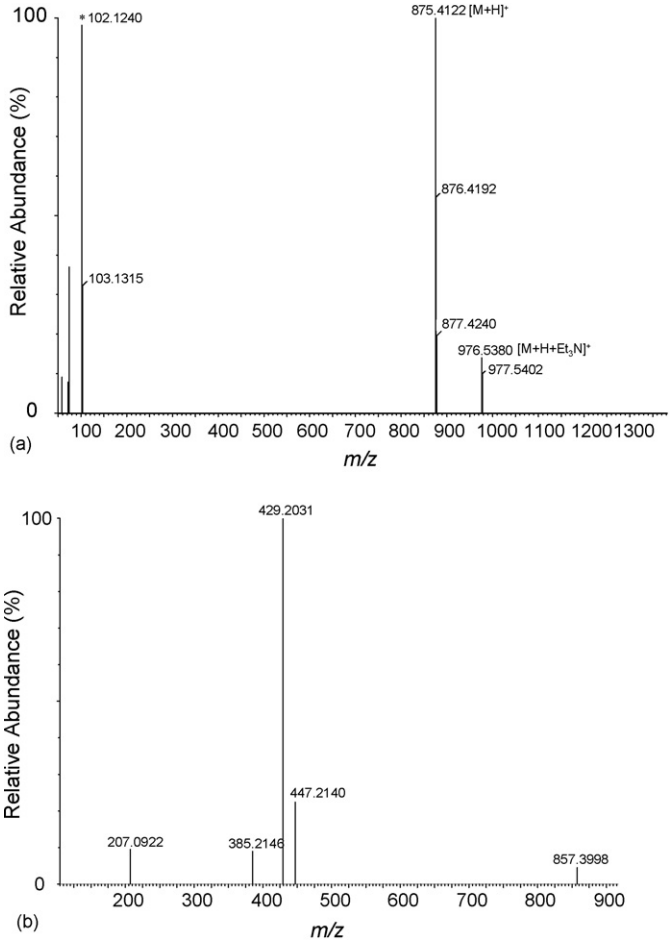

Fig. 4. (a) LC-MS and (b) LC-MS/MS spectra of DP-1. *Signals of the protonated molecule of triethylamine in the mobile phase.

sequence with shaped pulses for suppression of the acetonitrile and the water signals. Spectra were acquired with a 12 019 Hz spectral width and 16K data points, giving digital resolution of 0.73 Hz per point. A total of 256–512 scans were accumulated. All the spectra were processed with an exponential function, a line broadening of 1 Hz and a zero filling factor of 2.

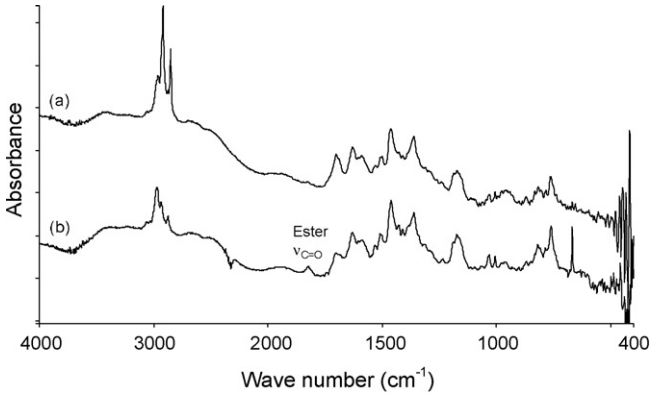

Fig. 6. Solvent-elimination LC-IR spectra of (a) OL and (b) DP-1.

All NMR spectra were recorded at a constant temperature of 25 °C, and the chemical shifts were referenced to the methyl signal of the residual acetonitrile at 1.93 ppm or that of the residual methanol at 3.30 ppm.

3. Results and discussion

3.1. LC–UV analysis of stressed OLM tablets

In the LC–UV analysis, an unknown degradation product DP-1 was detected at 0.72% by the peak area percent at 250 nm in OLM 5 mg tablets stored at 40 °C/75% r.h. for 6 months. OL was also detected as another major degradation product formed by the ester hydrolysis of OLM. There was no loss of mass balance in the stressed tablets. A typical HPLC chromatogram of the stressed tablets and the online UV spectra of OLM, OL and DP-1 are shown in Figs. 2a and 3, respectively. DP-1, as well as OL, did not show the intensive UV absorption maximum at 260 nm characteristic of the 5-methyl-2-oxo-1,3-dioxolen-4-yl-methyl group (ester moiety) in OLM, suggesting that DP-1 lacks the ester moiety.

3.2. Identification of DP-1

In the LC–MS and LC–IR analyses, a separation condition using volatile mobile phases was developed and the resulting separa-

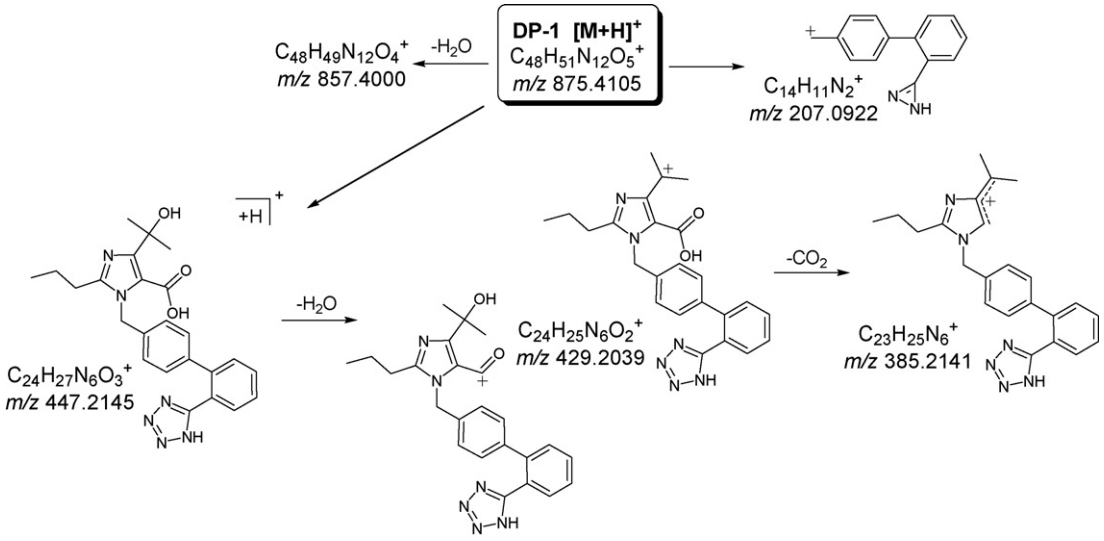

Fig. 5. Proposed fragmentation scheme for DP-1 in the LC-MS/MS analysis.

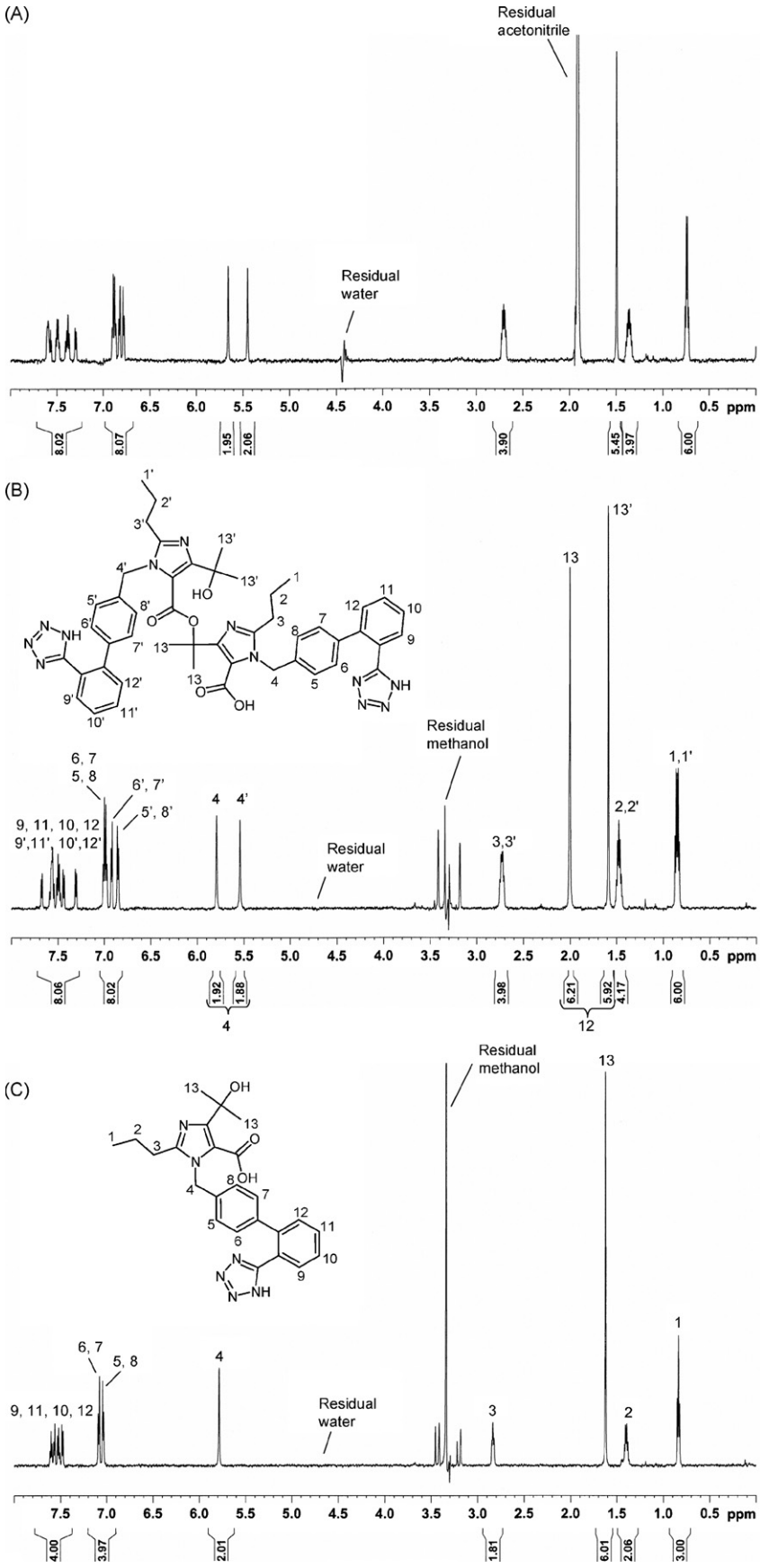

Fig. 7. LC-^{1}H-NMR spectra of (A) DP-1 obtained using CD_3CN eluent, (B) DP-1 obtained using CD_3OD eluent and (C) OL obtained using CD_3OD eluent. The relative integral intensities for the resonances are indicated below the axis.

tion (Fig. 2b) was equivalent to that obtained in the LC–UV analysis (Fig. 2a). Comparative chromatograms of the DP-1-enriched tablets obtained by the LC–NMR methods using acetonitrile eluent (Fig. 2c) and methanol eluent (Fig. 2d) showed that the use of methanol changed the retention order and caused little peak broadening, however, a sufficient separation of main impurities in the DP-1 enriched tablets was achieved.

3.2.1. LC–MS and MS/MS analyses

The accurate mass spectrum of DP-1 exhibited a protonated molecule $[M+H]^+$ and an Et_3N adduct ion $[M+H+Et_3N]^+$ at m/z 875.4122 and 976.5380, respectively (Fig. 4a). In the MS/MS spectrum (Fig. 4b), the product ions equivalent to the protonated molecule of the OL substructure and the dehydrated ion of the protonated molecule of DP-1 was detected at m/z 447.2140 and 857.3998, respectively. All the other product ions detected in the MS/MS spectrum of DP-1 at m/z 429.2031, 385.2146 and 207.0922 were also detected in the MS/MS spectrum of OL. From these results, DP-1 was estimated to be a dehydrated condensation product ($C_{48}H_{50}N_{12}O_5$, calculated mass for $[M+H]^+$ 875.4105, error 1.9 ppm) of two OL molecules and the product ions of DP-1 were proposed as shown in Fig. 5. However, there were 6 possible dimer structures formed as reaction products of the carboxylic group of one of the OL molecules with the alcoholic group on the imidazole moiety, with the secondary amino group of the tetrazole moiety, or with the carboxyl group of the others (ester, amide, or acid anhydride formation) and reaction products of the alcoholic group on the imidazole moiety of one of the OL molecules with the secondary amino group of the tetrazole moiety of the other.

3.2.2. Solvent-elimination LC–IR analysis

In the LC–IR spectrum, DP-1 showed a similar absorption pattern to OL except for the band corresponding to the ester carbonyl stretching ($\nu_{C=O}$) (Fig. 6a and b). From these results, in conjunction with the LC–MS results, DP-1 was estimated to be an esterified dimer of OL.

3.2.3. Stopped-flow LC–NMR analysis

Further confirmation was performed by LC–NMR. The LC–^{1}H NMR spectrum of DP-1 obtained using CD_3CN as an organic component of the mobile phase is shown in Fig. 7a. The total number of protons which appeared in the ^{1}H spectrum obtained from the relative integral intensities of the proton signals (40) was 6 less than that expected from the estimated dimeric structure (46, total 50, H-D exchangeable 4). One possible explanation for this is that the methyl signal of the residual acetonitrile overlapped with the signals of DP-1. Further LC–NMR analyses were therefore performed using CD_3OD in place of CD_3CN as an organic component of the mobile phase. The LC–^{1}H NMR spectrum of DP-1 using CD_3OD eluent is shown in Fig. 7b. A signal of 6 protons at 2.0 ppm not observed in the analysis using CD_3CN eluent was noted, indicating that this signal was overlapped with the residual acetonitrile signal. The LC–^{1}H NMR spectrum of DP-1 was similar to that of OL (Fig. 7c) as well as the UV and IR spectra, except for the presence of two more singlet signals at 2.0 and 5.6 ppm due to the methyl groups of the isopropanol moiety and the methylene group, respectively. The peak integrals from DP-1 spectrum indicated that the number of protons observed in each resonance region (8, 8, 4, 4, 12, 4, 6 from the lower field to the higher field) is double that of OL (4, 4, 2, 2, 6, 2, 3). These results confirmed that DP-1 was a dimer of OL and that the newly formed ester bond between the two OL molecules shifted the resonance of H13 to a lower field than that of H13′ and the resonance of H4′ to a higher field than that of H4.

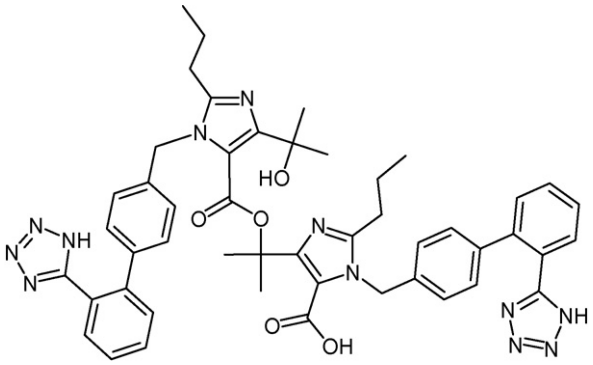

Fig. 8. Chemical structure of DP-1.

Thus, DP-1 was successfully identified as an esterified dimer of OL by the complementary use of HPLC hyphenated techniques, as shown in Fig. 8, and all the results of the on-line and at-line spectroscopic and spectrometric analyses (UV, MS, IR and NMR) were quite consistent with this structure. As things turned out, the rapid identification using HPLC hyphenated techniques without isolation was presumed to be preferable, since it is well known that compounds containing ester groups are susceptible to degradation, such as hydrolysis.

Two dimeric degradation products were observed in the stressed tablets (3 years at 40 °C/75% r.h.) of losartan, the first angiotensin II receptor blocker to be marketed. However, the two dehydrated dimers of losartan were formed by the reaction of the alcoholic group on the imidazole moiety and the amine functionality at the tetrazole [21]. It was assumed that DP-1, the esterified dimer of OL, was predominantly formed in the OLM tablets because the DP-1 formation reaction involving the carboxylic group which losartan lacks occurred more easily during the storage at 40 °C/75% r.h. On the other hand, the formation of similar esterified dimeric degradation products, acetylsalicylsalicylic acid and salicylsalicylic acid, has been reported in the case of aspirin tablets and those studies have suggested that basic lubricants such as magnesium stearate in tablets accelerate the degradations [22–24]. Basic lubricants are suggested to have a similar effect on the formation of DP-1 in OLM tablets.

4. Conclusions

This investigation has demonstrated that the unambiguous identification of the degradation product formed in stressed tablets of OLM was achieved by the hyphenated techniques of LC–MS, solvent-elimination LC–IR and LC–NMR using LC conditions compatible with each technique. LC–MS and MS/MS spectra provided the molecular formula and substructural information of the degradation product and LC–IR analysis determined the presence of the ester functional group. The structure of the degradation product was definitely confirmed by LC–NMR. Complementary use of these hyphenated techniques facilitated the rapid structure elucidation of the degradation product without complicated purification or isolation processes. In the LC–NMR analysis, a change of the organic modifier of the mobile phase, e.g. from CD_3CN to CD_3OD, would be efficient for resolving the overlapping signals of the sample and solvents. These approaches can be very effective in the pharmaceutical development process for the rapid and accurate identification of degradation products.

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References

[1] International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), ICH Harmonised Tripartite Guideline Q1A(R2): Stability Testing of New Drug Substances and Products, Step 4, February 6, 2003. Available at: http://www.ich.org/.
[2] International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), ICH Harmonised Tripartite Guideline Q3A(R2): Impurities in New Drug Substances, Step 4, October 25, 2006. Available at: http://www.ich.org/.
[3] International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), ICH Harmonised Tripartite Guideline Q3B(R2): Impurities in New Drug Products, Step 4, June 2, 2006. Available at: http://www.ich.org/.
[4] N. Ferreiros, S. Dresen, R.M. Alonso, W. Weinmann, J. Chromatogr. B 855 (2007) 134–138.
[5] J. Ermer, M. Vogel, Biomed. Chromatogr. 14 (2000) 373–383.
[6] Y. Wu, Biomed. Chromatogr. 14 (2000) 384–396.
[7] L. Tollsten, in: S. Gorog (Ed.), Identification and Determination of Impurities in Drugs, Elsevier, Amsterdam, 2000, pp. 266–298.
[8] S.X. Peng, B. Borah, R.L.M. Dobson, Y.D. Liu, S. Pikul, J. Pharm. Biomed. Anal. 20 (1999) 75–89.
[9] W. Feng, H. Liu, G. Chen, R. Malchow, F. Bennett, E. Lin, B. Pramanik, T.M. Chan, J. Pharm. Biomed. Anal. 25 (2001) 545–557.
[10] S.X. Peng, Biomed. Chromatogr. 14 (2000) 430–441.
[11] I.D. Wilson, L. Griffiths, J.C. Lindon, J.K. Nicholson, in: S. Gorog (Ed.), Identification and Determination of Impurities in Drugs, Elsevier, Amsterdam, 2000, pp. 299–322.
[12] J.C. Lindon, J.K. Nicholson, I.D. Wilson, in: K. Albert (Ed.), On-line LC–NMR and Related Techniques, Wiley, Chichester, 2002, pp. 45–87.
[13] T. Murakami, N. Fukutsu, J. Kondo, T. Kawasaki, F. Kusu, J. Chromatogr. A 1181 (2008) 67–76.
[14] N. Fukutsu, T. Kawasaki, K. Saito, H. Nakazawa, J. Chromatogr. A 1129 (2006) 153–159.
[15] G.W. Somsen, C. Gooijer, N.H. Velthorst, U.A.Th. Brinkman, J. Chromatogr. A 811 (1998) 1–34.
[16] G.W. Somsen, C. Gooijer, U.A.Th. Brinkman, J. Chromatogr. A 856 (1999) 213–242.
[17] I.D. Wilson, U.A.Th. Brinkman, Trends Anal. Chem. 26 (2007) 847–854.
[18] M. Mizuno, T. Sada, M. Ikeda, N. Fukuda, M. Miyamoto, H. Yanagisawa, H. Koike, Eur. J. Pharmacol. 285 (1995) 181–188.
[19] M. Takemoto, K. Egashira, H. Tomita, M. Usui, H. Okamoto, A. Kitabatake, H. Shimokawa, K. Sueishi, A. Takeshita, Hypertension 30 (1997) 1621–1627.
[20] K. Puchler, J. Nussberger, P. Laeis, P.U. Witte, H.R. Brunner, J. Hypertens. 15 (1997) 1809–1812.
[21] Z. Zhao, Q. Wang, E.W. Tsai, X.Z. Qin, D. Ip, J. Pharm. Biomed. Anal. 20 (1999) 129–136.
[22] J.C. Reepmeyer, R.D. Kirchhoefer, J. Pharm. Sci. 68 (1979) 1167–1169.
[23] P.V. Mroso, A. Li-Wan-Po, W.J. Irwin, J. Pharm. Sci. 71 (1982) 1096–1101.
[24] G.C. Ceschel, R. Badiello, C. Ronchi, P. Maffei, J. Pharm. Biomed. Anal. 32 (2003) 1067–1072.