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# Investigation of unknown related substances in commercial erythromycin samples with liquid chromatography/mass spectrometry

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**A selective reversed phase liquid chromatography/mass spectrometry (LC/MS$^n$) method is described for the identification of erythromycin impurities and related substances in commercial erythromycin samples. Mass spectral data are acquired on a LCQ ion trap mass spectrometer equipped with an electrospray interface operated in positive ion mode. The LCQ is ideally suited for identification of impurities and related substances because it provides on-line LC/MS$^n$ capability. Compared with UV detection, this hyphenated LC/MS$^n$ technique provides as a main advantage efficient identification of novel substances without time-consuming isolation and purification procedures. Using this method four novel related substances were identified in commercial samples. Copyright © 2000 John Wiley & Sons, Ltd.**

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Erythromycin is a mixture of macrolide antibiotics produced by fermentation of strains of *Saccharopolyspora erythrea*.[1] Treatment with this antibiotic drug in human and veterinary practice is still very common, because of the high activity against gram-positive and a few gram-negative strains.[2] Erythromycin A (EA) is the main component, but during fermentation several related substances such as erythromycins B (EB), C (EC), D (ED), E (EE), F (EF), A *N*-oxide (EANO) and *N*-demethylerythromycin A (NdMeEA) are formed in small amounts. Figure 1 shows the chemical structures. A characteristic feature is the neutral sugar glycosidically linked to the 3 position (cladinose for EA, EB, EE, EF, NdMeEA and EANO and mycarose for EC and ED) and the dimethylamino sugar desosamine linked to the 5 position of the 14-membered lactone ring. In addition, degradation products such as pseudoerythromycin A enol ether (PsEAEN), formed in alkaline medium, and anhydroerythromycin A (AEA) and erythromycin A enol ether (EAEN), which are formed under mild acidic conditions, may also be present.[3]

Ample work has been carried out on the optimization of the separation of EA and its related substances by LC.[4–16] These methods have been applied over the years to qualitative and quantitative analysis in fermentation media, purity assessment of raw materials, assay of pharmaceutical dosage forms and measurement in biological samples such as blood, plasma, serum, urine and tissue.[17] With the development of more performant methods, new unidentified peaks have been separated. In the past the structure of these unidentified compounds was elucidated after time-consuming isolation and purification procedures and subsequent off-line mass spectrometric analysis and NMR investigation. To prevent laborious preparative LC using large amounts of sample and solvents, we considered developing a selective LC/MS$^n$ method for the identification of the erythromycin impurities or related substances in commercial samples. Recent literature about mass spectrometric investigation of erythromycin is limited to characterization of decomposition products,[18] quantitative determination of EA in salmon tissue[19] and human plasma,[20] and a partial structural elucidation of EA and two analogues.[21] No suitable method for the separation of the components of commercial erythromycin samples compatible with on-line MS detection has been described.

This study describes the development of a reversed phase LC method, suitable for UV detection and compatible with MS. UV detection of erythromycin needs low wavelengths and high concentrations. MS detection is more sensitive. In a previous study it has been shown that an electrospray interface operated in positive ion mode is suitable for analysis of EA[22] by mass spectrometry. For this paper, an LCQ ion trap mass spectrometer, providing on-line LC/MS$^n$ capability, was used. Because of the complexity of the 14-membered lactone structures some understanding of the fragmentation reactions of erythromycin derivatives was required. We acquired MS and MS$^n$ spectra of known related substances available in our laboratory. This resulted in the creation of a library of spectra, which was useful for identification of related substances in commercial samples by comparison of fragmentation patterns. The final objective of this study was to screen commercial samples from various sources for related substances and impurities with the developed LC/MS$^n$ method, not only to confirm the presence of the related substances depicted in Fig. 1, but also to identify unknown compounds, present in amounts above 0.1%.

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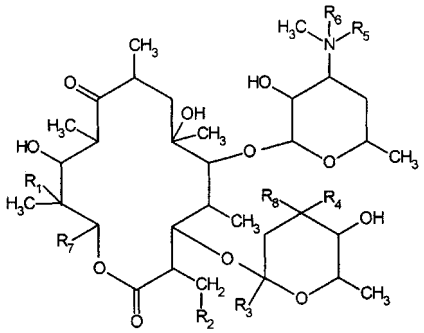

| | m/z $[M+H]^+$ | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | $R_6$ | $R_7$ | $R_8$ |
|---|---|---|---|---|---|---|---|---|---|
| Erythromycin A | 734 | OH | H | H | $OCH_3$ | $CH_3$ | - | $C_2H_5$ | $CH_3$ |
| *Related substances* | | | | | | | | | |
| Erythromycin B | 718 | H | H | H | $OCH_3$ | $CH_3$ | - | $C_2H_5$ | $CH_3$ |
| Erythromycin C | 720 | OH | H | H | OH | $CH_3$ | - | $C_2H_5$ | $CH_3$ |
| Erythromycin D | 704 | H | H | H | OH | $CH_3$ | - | $C_2H_5$ | $CH_3$ |
| Erythromycin E | 748 | OH | -O- | | $OCH_3$ | $CH_3$ | - | $C_2H_5$ | $CH_3$ |
| Erythromycin F | 750 | OH | OH | H | $OCH_3$ | $CH_3$ | - | $C_2H_5$ | $CH_3$ |
| N-demethylerythromycin A | 720 | OH | H | H | $OCH_3$ | H | - | $C_2H_5$ | $CH_3$ |
| Erythromycin A N-oxide | 750 | OH | H | H | $OCH_3$ | $CH_3$ | O | $C_2H_5$ | $CH_3$ |
| *Novel related substances* | | | | | | | | | |
| IM1 | 706 | OH | H | H | OH | $CH_3$ | - | $CH_3$ | $CH_3$ |
| IM2 (2 postulations) | 706 | OH | H | H | OH/H | $CH_3$ | - | $C_2H_5$ | H/OH |
| IM3 | 720 | OH | H | H | $OCH_3$ | $CH_3$ | - | $CH_3$ | $CH_3$ |
| IM4 | 748 | OH | H | H | $OCH_3$ | $CH_3$ | - | $C_3H_7$ | $CH_3$ |

**Figure 1.** Chemical structures of erythromycin A and related substances and proposed structures for the novel related substances IM1, IM2, IM3 and IM4.

## EXPERIMENTAL

### Chemicals

Acetonitrile (HPLC grade S) was purchased from Rathburn (Walkerburn, UK). 2-Propanol (Chromasolv®) was obtained from Riedel-de Haën (Seelze, Germany). 2-Methyl-2-propanol p.a. (Merck Eurolab, Leuven, Belgium) was distilled before use. Ammonium carbonate p.a. was purchased from Acros (Geel, Belgium) and glacial acetic acid p.a. was obtained from Merck Eurolab. A Milli-Q water purification system (Millipore, Bedford, MA, USA) was used to further purify glass-distilled water.

### Samples and sample preparation

A pure standard of EA was obtained by open column chromatography and subsequent crystallization in our laboratory. Secondary standards of EE,[23] EF,[24] EANO,[25] NdMeEA,[26] PsEAEN,[27,28] AEA,[29] and EAEN[30] were prepared according to procedures found in literature. Reference substances EB and EC were obtained from the European Pharmacopoeia Laboratory. A commercial sample, known to contain ED, as verified by thin-layer chromatography, was also available.[31]. All these compounds will be called reference substances in this paper.

Four commercial samples were investigated: two (production 1980) were from Proter (Milan, Italy), one (production 1984) was from Chemielux (Capellen, Luxemburg) and one (production 1995) was from Alpha Pharma (Zwevegem, Belgium).

All samples were dissolved in a 30:70 mixture of acetonitrile/water. Reference substances EA, EB, EC, EE, EF, EANO, NdMeEA, PsEAEN, AEA and EAEN were dissolved separately at a concentration of 0.01 μg/μL. The commercial sample containing ED was dissolved at a concentration of 2 μg/μL. Commercial samples investigated were dissolved at concentrations of 0.1, 2 and 10 μg/μL.

### LC instrumentation and chromatographic conditions

The LC apparatus consisted of a SpectraSYSTEM P1000XR quaternary pump, a SpectraSERIES AS100 autosampler equipped with a 20 μL loop, a variable wavelength Spectra 100 UV-VIS detector set at 215 nm, all from Thermo Separation Products (Fremont, CA, USA), and an integrator Model 3390A (Hewlett-Packard, Avondale, PA, USA). The XTerra™ RP $C_{18}$ column (3.2 μm, 100 × 2.1 mm i.d.) (Waters, Milford, MA, USA) was immersed in a waterbath at 30 °C. The following mobile phases were used for separation: (A) 2-methyl-2-propanol/2-propanol/0.2 M ammonium carbonate buffer pH 7.5/water (7.5:7.5:5:80); (B) 2-methyl-2-propanol/acetonitrile/0.2 M ammonium carbonate buffer pH 7.5/water (15:3:5:77). The ammonium carbonate buffer was adjusted to the required pH by adding glacial acetic acid. The organic solvents were first mixed and the buffer/water mixture added. Fresh mobile phases were prepared every two days. The one step gradient elution was performed as follows: 0–30 min, 100% A; 30–50 min, 100% B; 50–60 min, 100% A. The mobile phase was degassed by sparging helium. The LC pump was operated at a flow rate of 200 μL/min.

### Electrospray ionization tandem mass spectrometry

The mass spectral data shown in this paper were acquired on a LCQ ion trap mass spectrometer (Finnigan MAT, San

 

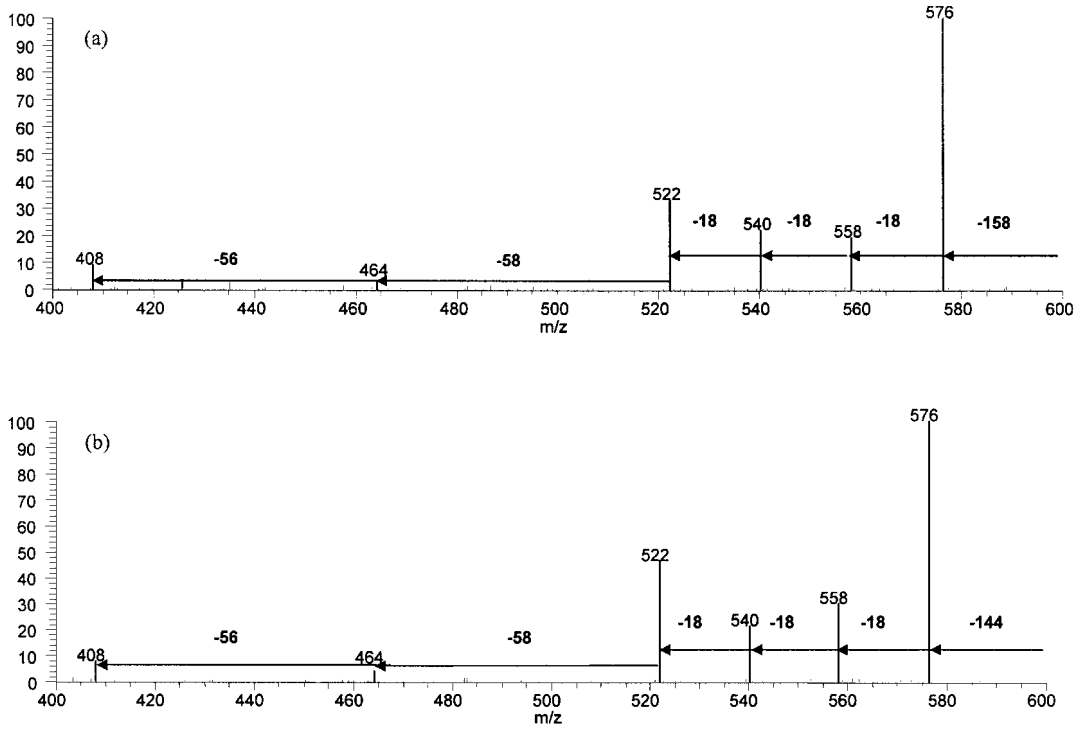


**Figure 2.** Product ion spectra (MS/MS) of erythromycin A (a) and erythromycin C (b), the result of monoisotopical isolation and collisional activation in the ion trap of the parent ions *m/z* 734 (EA) and *m/z* 720 (EC). The masses of the fragments produced and mass losses are indicated in the figure.

Jose, CA, USA) equipped with an electrospray interface operated in positive ion mode.

### Mass spectrometric investigation of the reference substances

The solution of EA reference substance was used to tune on. Infusion into the mass spectrometer was performed as follows: the flow of EA solution coming from the built-in syringe pump at a flow rate of 10 μL/min was mixed with mobile phase A (200 μL/min) through a T-piece. The ESI source and MS parameters were automatically optimized and saved in a tune file. This tune file was used during the subsequent investigation of commercial samples. Nitrogen supplied by a Nitroprime TM membrane unit, type SNIFF (AGA, Lidingö, Sweden) was used as auxiliary and sheath gas. Helium was used as collision gas in the ion trap. A Pentium II (Gateway 2000, North Sioux City, SD, USA) equipped with the standard LCQ software package was used for instrument control, data acquisition and processing.

Solutions of EA, EB, EC, EE, EF, NdMeEA, EANO and EAEN reference substances were introduced into the LCQ by direct injection with the syringe pump, similar to the tuning procedure. Full mass spectra were acquired over the mass range *m/z* 250–800. For MS/MS investigation the protonated erythromycin molecules were isolated monoisotopically in the ion trap and collisionally activated with different collision energies (CE) to find the optimal CE for a distinct fragmentation. The CE which generated the highest intensity of the daughter ion needed for further $MS^3$ experiments was chosen as the final value. $LC/MS^3$ spectral data were obtained for the compounds relevant to the identification of the unknowns (EA, EB, EC, EF and NdMeEA). CE was optimized as described above for MS/MS. The reference substances ED, PsEAEN and AEA were chromatographed before MS/MS and $MS^n$ investigation, because they were not sufficiently pure.

### Mass spectrometric investigation of the commercial samples

Solutions of commercial samples were injected onto the XTerra™ column. Full MS acquisition over the mass range *m/z* 250–800 was performed on the 0.1 μg/μL solution to gain some idea of the commercial sample composition. 2 μg/μL solutions were injected for additional full mass spectral information and MS/MS investigation of each peak in the chromatogram. CE was optimized as described above. $LC/MS^3$ experiments on new related substances required 10 μg/μL solutions.

## RESULTS AND DISCUSSION

### Development of the liquid chromatographic method

Important features in combining LC and MS are the incompatibility with MS of non-volatile mobile phase additives and of high flow rate. Until now LC of EA and related substances has been performed with mobile phases containing non-volatile additives and using column dimensions of 250 × 4.6 mm i.d. Based on the method recently developed by Chepkwony and co-workers, a mobile phase with a volatile ammonium carbonate buffer, instead of the original non-volatile potassium phosphate buffer, was developed using a 100 × 2.1 mm i.d. XTerra RP $C_{18}$ column™, allowing a flow rate of 200 μL/min.[16] The resulting method was less selective than the original one.

 

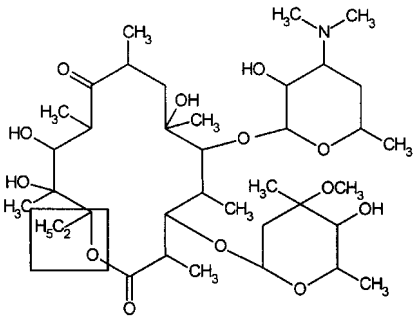

**Figure 3.** The aglycone of erythromycin is formed by incorporation of seven propionate units, according to described biosynthetic routes, one of which is used as the starter acid. The starter acid of erythromycin A is boxed.

## Mass spectrometric results of the reference substances

MS/MS analysis of EA confirmed the results of Gates and co-workers.[21] The initial losses are due to the cladinose sugar and three water molecules. Further losses of masses 58 and 56 Da to 464 and 408, respectively, are presented in the MS/MS spectrum in Fig. 2(a). The deuterated experiments of Gates and co-workers with a Fourier transform ion cyclotron resonance (FTICR) apparatus showed that the first loss of 58 Da is due to the starter acid unit of the polyketide ring (Fig. 3) and the second loss of 56 Da is assumed to arise from the neighbouring oxygen-containing unit of the ring.[21] Further $MS^3$ experiments performed in our laboratory on EA proved that, after isolation and collisional activation, the daughter ion at *m/z* 522 dissociates predominantly to the fragment ions with *m/z* 464 and 408 (data not shown).

Mass spectra for EB, EC, ED, EE, EF, NdMeEA, EANO, PsEAEN, AEA and EAEN were similarly recorded. The MS/MS spectrum for erythromycin C is presented in Fig. 2(b), because the structural elucidation of two unknown compounds was established by comparison with these data. After isolation and collisional activation the protonated erythromycin C molecule with *m/z* 720 yields predominantly the daughter ion at *m/z* 576, by loss of the mycarose moiety, and the daughter ions 558, 540 and 522 by subsequent water losses. Further losses of masses 58 and 56 Da respectively to give 464 and 408 are present in the MS/MS spectrum. Collisional activation of the ion with *m/z* 522 leads to formation of the fragment ions 464 and 408 ($MS^3$ data not shown).

Mass spectrometric data were used to compose a complete library with as the main objective the confirmation of their presence in a commercial sample and the structural elucidation of unknowns by comparison. The fragmentation patterns are not displayed here, because they were not applicable in the structural elucidation of the novel related substances.

## Mass spectrometric results of the commercial samples

EA, EB and ED were identified by their *m/z* ratio. For the related substances with a similar *m/z*, i.e. EC, NdMeEA and an unknown related substance with *m/z* 720, EANO and EF with *m/z* 750, EE and an unknown related substance with *m/z* 748, AEA and PsEAEN with *m/z* 716, we identified the corresponding peaks by comparison of the MS/MS data with the fragmentation patterns of the reference substances collected in the library. Due to the high retention time of EAEN under our chromatographic conditions and hence its very good separation, we did not analyze for this compound in the commercial samples. Four peaks with *m/z* 706, 706, 720 and 748 were related substances, of which the fragmentation pattern did not match the fragmentation pattern of any of the standards in the library. They were noted respectively as IM1, 2, 3 and 4. A typical total ion

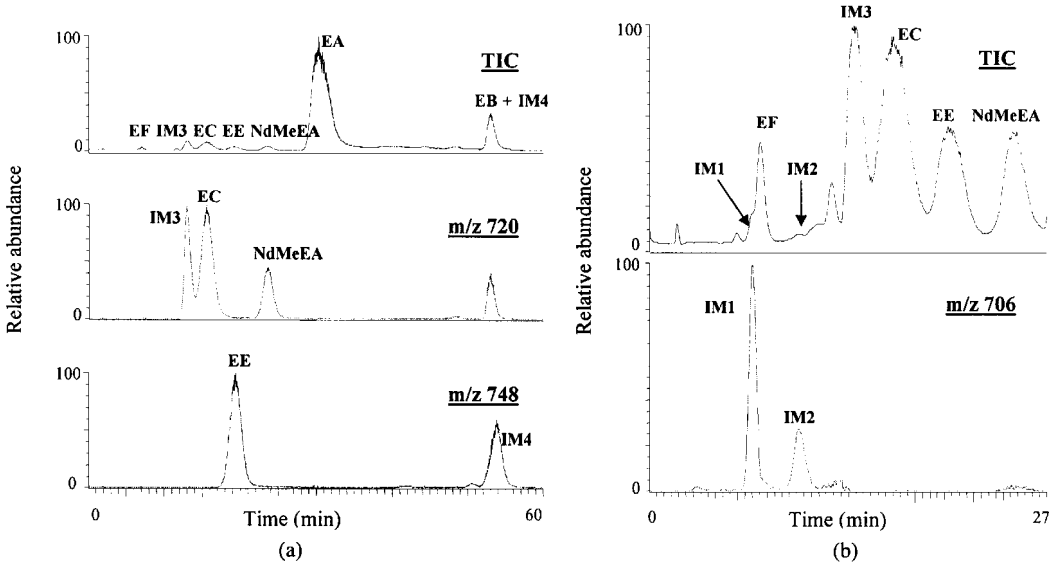


**Figure 4.** Typical chromatograms of a commercial erythromycin sample with sample concentrations (a) 0.1 μg/μL; (b) 2 μg/μL. Stationary phase, Waters XTerra® $RP_{18}$, 3.5 μm (100 × 2.1 mm i.d.); column temperature, 30 °C; mobile phase, (A) 2-methyl-2-propanol/2-propanol/0.2 M $[NH_4]_2CO_3$ buffer pH 7.5/water (7.5:7.5:5:80) (B) 2-methyl-2-propanol/acetonitrile/0.2 M $[NH_4]_2CO_3$ buffer pH 7.5/water (15:3:5:77); gradient elution 0–30 min (100% A), 30–50 min (100% B) and 50–60 min (100% A); flow rate, 200 μL/min; injection volume, 20μL Top: Total ion current (TIC) chromatograms. Bottom: Extracted pseudomolecular ion current chromatograms are shown in (a) for IM3 (*m/z* 720) and IM4 (*m/z* 748) and in (b) for IM1 (*m/z* 706) and IM2 (*m/z* 706).

 

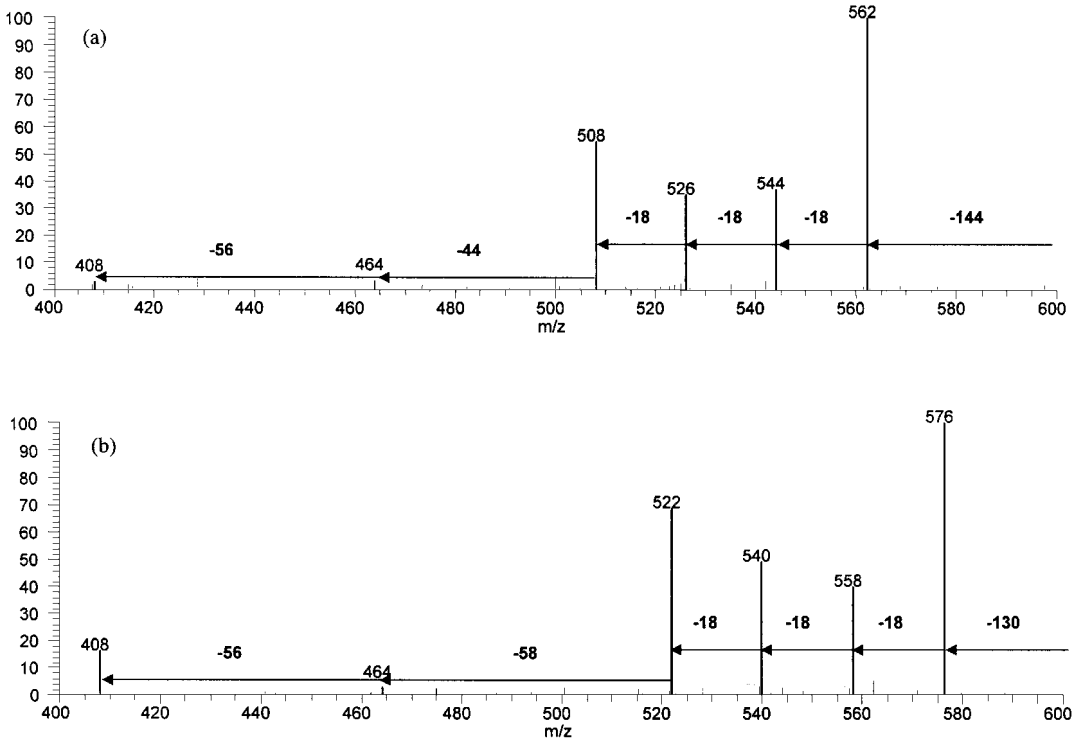


**Figure 5.** Product ion spectra (MS/MS) of IM1 (a) and IM2 (b), the monoisotopic species is selected for collisional activation in the ion trap for the parent ions *m/z* 706 for IM1 and IM2. The masses of the fragments produced and mass losses are indicated in the figure.

current (TIC) chromatogram of a 0.1 μg/μL solution of a commercial sample (Fig. 4 (a), top) shows IM3 and IM4, which is coeluted with EB. Screening of the 2 μg/μL solution revealed two additional related substances IM1 and IM2 (Fig. 4(b), top). These compounds are not fully separated from EF and an additional unidentified compound, which elutes before IM3. Extracted pseudomolecular ion current chromatograms are shown in Fig. 4(a) (bottom) for IM3 (*m/z* 720) and IM4 (*m/z* 748) and in Fig. 4(b) (bottom) for IM1 (*m/z* 706) and IM2 (*m/z* 706). The peak in the extracted pseudomolecular ion chromatogram (*m/z* 720) which eluted at the same retention time as EB is due to an isotopic peak of EB (*m/z* 718).

MS/MS spectra of the two impurities IM1 and IM2 with *m/z* 706 are shown in Fig. 5. The protonated IM1 molecule fragments in Fig. 5(a) to the daughter ion *m/z* 562 by means of a loss of 144 mass units, identified as mycarose. Three subsequent water losses lead to formation of the daughter ions *m/z* 544, 526 and 508. Further losses of masses 44 and 56 Da are probably due to the starter acid unit and the neighbouring unit. From experiments with EA and analogues it was concluded that the identification of the starter acid and the neighbouring unit is possible by generating the daughter ions of the fragment ion that is yielded from the protonated target molecule obtained after loss of the neutral sugar moiety and three water molecules. Fragmentation of the daughter ion *m/z* 508 resulted in a loss of 44 Da and a subsequent loss of 56 Da, which confirmed our postulation. One of the fragment ions of *m/z* 508 is also the desosamine sugar ($MS^3$ data not shown). It was concluded that the losses in the MS/MS spectrum of IM1 (Fig. 5 (a)) are identical to those observed for EC (Fig. 2 (b)), except for the loss of 44 instead of 58 Da. The change in the first ring fragment loss indicates that the propionate starter acid is replaced by an acetate starter acid. The presence of the desosamine moiety and the loss of the mycarose moiety indicate that there is no change in the sugar moieties. The identity and structure of IM1 was thus established as 13-methyl-13-desethyl-erythromycin C. This compound, of which a reference substance was no longer available in our laboratory, has previously been described by Kibwage and co-workers after isolation from a mother liquor concentrate of erythromycin.[32] The existence of 13-methyl-13-desethyl-erythromycin C can be explained in the context of previously proposed biosynthetic routes, if acetate rather than propionate is incorporated into the aglycone as the starter unit.[33]

The mass spectrum of IM2 exhibited a protonated molecule at *m/z* 706. The mass losses in the MS/MS spectrum in Fig. 5 (b) are similar to EC (Fig. 2 (b)), except for the loss of 130 instead of 144 Da. Collisional activation of the fragment ion *m/z* 522 revealed a fragment ion at *m/z* 158 (data not shown), confirming the presence of the desosamine sugar. The loss of mass 130 instead of 144 could indicate a defective sugar. Earlier biogenetic studies by Column *et al.*[34] reported the existence of two erythromycin metabolites, which resulted from aberrant mycarose biosynthesis. It is interesting to note that the sugar moiety attached here in the defective glycosides 3-*O*-(2″,6″-dideoxy-α-L-ribo-hexopyranosyl)erythronolide B and 3-*O*-(2″,6″-dideoxy-α-L-arabino-hexopyranosyl)erythronolide B had a mass of 130. The authors failed though to obtain intact erythromycin analogues starting from the aberrant monoglycosides.[34] It is proposed that IM2 is EA with a defective sugar, probably 2,6-dideoxy-α-L-ribo-hexopyranose or 2,6-dideoxy-α-L-arabino-hexopyranose. Further investigation should be carried out using NMR after isolation of this compound.

Compound IM3 is clearly detectable both in the MS

 

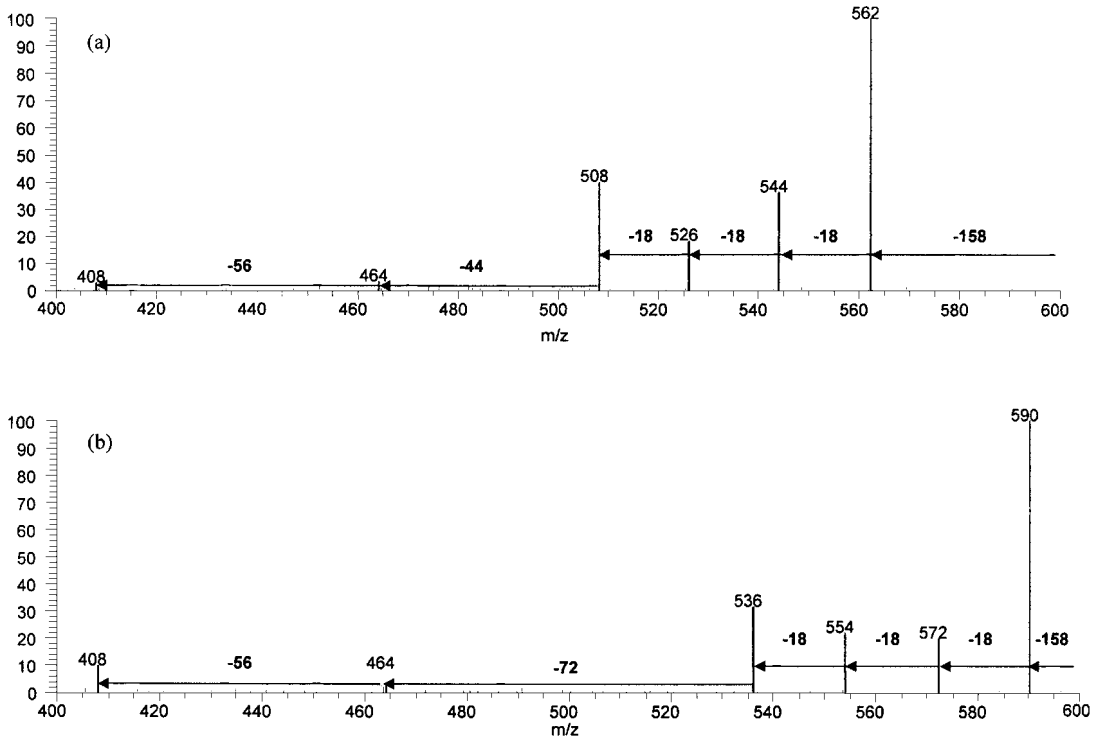


**Figure 6.** Product ion spectra (MS/MS) of IM3 (a) and IM4 (b), the monoisotopic species is selected for collisional activation in the ion trap for the parent ions *m/z* 720 (IM3) and *m/z* 748 (IM4). The masses of the fragments produced and mass losses are indicated in the figure.

profile and the UV chromatogram. LC/MS$^n$ experiments resulted in identification of this structure as 13-methyl-13-desethyl-erythromycin A. This compound was also previously obtained by Gates and co-workers from relatively impure biological synthetic sources.[21] It was not described though in a liquid chromatographic study of commercial samples probably due to the coelution of this compound with EC. Comparison of the MS/MS spectrum of EA (Fig. 2 (a)) and IM3 (Fig. 6 (a)) showed that several fragment ions of EA (576, 558, 540, 522) were shifted down by 14 mass units in the MS/MS spectrum of IM3 (*m/z* 562, 544, 526, 508). MS/MS spectra of this compound showed the same losses as observed in the MS/MS data of EA, except for the loss of 44 instead of 58 Da. The MS$^3$ spectrum (data not shown) shows the loss of 44 Da and the subsequent loss of 56 Da, respectively, due to the starter acid and the neighbouring unit, after isolation and collisional activation of the ion with *m/z* 508. Fragmentation of *m/z* 508 generated in addition a fragment ion at *m/z* 158, confirming the presence of the desosamine, while the presence of the cladinose sugar was clearly detectable by loss of mass 158 in the MS/MS spectrum. The change in the first ring fragment loss indicates that the propionate starter acid is replaced by the acetate starter acid analogous to 13-methyl-13-desethyl-erythromycin C (IM1), which can be explained in the context of previously proposed biosynthetic routes.[33] The lower reversed phase LC retention time of 13-methyl-13-desethyl-erythromycin C and 13-methyl-13-desethyl-erythromycin A compared with the retention time of EC and EA, respectively, is consistent with the decreasing lipophilicity as the chain length of the side chain at position 13 of the lactone ring decreases.

The identity of IM4 was established as 13-propyl-13-desethyl-erythromycin A on the basis of spectral data, from the following observations. MS$^n$ investigation resulted in a MS/MS spectrum which shows the loss of 158 Da, which is confirmation for the presence of the cladinose sugar (Fig. 6 (b)) and an MS$^3$ spectrum (data not shown) which shows a fragment ion at *m/z* 158, attributable to desosamine. The difference with EA is the loss of 72 instead of 58 Da in the fragmentation of the fragment ion yielded after loss of the cladinose sugar and three subsequent water losses. It was concluded that the propionate starter acid is replaced in the biosynthesis by a butyrate starter acid. The higher reversed phase LC retention time is consistent with the increasing lipophilicity as the chain length of the side chain at position 13 of the lactone ring increases.

These compounds were present in different amounts in all the samples examined. The amount present of the two related substances with *m/z* 706 could not be defined with UV detection due to partial coelution with EF and an additional unidentified compound. The UV signal of IM3 was in the range of 0.5 to 1%, expressed as EA with respect to the EA content. IM4 could be resolved from EB with a mobile phase containing phosphate buffer. Based on UV absorbance, its content, expressed as EA with respect to the EA content, corresponded to approximately 0.5%. Other peaks visible in the TIC chromatogram in Fig. 4 (b) have not yet been identified and are not discussed in this paper.

## CONCLUSIONS

This study clearly illustrates the suitability of the developed LC/MS$^n$ method with electrospray ionization in positive ion mode for the analysis of erythromycin A and related substances in commercial samples.

An LC method, suitable for UV detection and compatible with MS detection, was developed based on reversed phase

 

liquid chromatography. Three impurities, of which the fragmentation spectra did not match the fragmentation spectra of the reference substances in the library, were identified. The structures were elucidated by identifying the sugars attached to the lactone ring, the starter acid and the neighbouring unit of the lactone ring, and by comparison of the MS$^n$ data of the standards with the spectra of the unknowns. The compounds 13-methyl-13-desethyl-erythromycin C and 13-methyl-13-desethyl-erythromycin A have previously been described by Kibwage and Gates, respectively, but their presence in commercial samples has never been reported. The novel related substance 13-propyl-13-desethyl-erythromycin A has not been described previously as being present in commercial samples. The fourth compound, which could only be partially identified as having an aberrant sugar, will need other techniques such as NMR spectroscopy for further characterization.

Compared with UV detection, this hyphenated LC/MS technique provides enhanced confirmation of identity and its main advantage is the on-line identification of novel related substances without recourse to time-consuming isolation and purification procedures.

## Acknowledgements

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## REFERENCES

1. McGuire JM, Bunch RL, Anderson RC, Boaz HE, Flynn EH, Powell HM, Smith JW. *Antibiot. Chemother.* 1952; **2**: 281.
2. Kibwage IO, Hoogmartens J, Roets E, Vanderhaeghe H, Verbist L, Dubost M, Pascal C, Petitjean P, Levol G. *Antimicrob. Agents Chemother.* 1985; **28**: 630.
3. Bryskier A, Agouridas C, Gasc J-C. in Bryskier AJ, Butzler J-P, Neu HC, Tulkens PM (eds). *Macrolides: Chemistry, Pharmacology and Clinical Uses*, Arnette Blackwell: Paris, 1993; 10–12.
4. Omura S, Suzuki Y, Nakagawa A, Hata T. *J. Antibiot.* 1973; **26**: 794.
5. Tsuji K, Goetz JF. *J. Chromatogr.* 1978; **147**: 359.
6. Tsuji K, Kane MP. *J. Pharm. Sci.* 1982; **71**: 160.
7. Kibwage IO, Roets E, Hoogmartens J, Vanderhaeghe H. *J. Liquid Chromatogr.* 1985; **330**: 275.
8. Cachet Th, Kibwage IO, Roets E, Hoogmartens J, Vanderhaeghe H. *J. Liquid Chromatogr.* 1987; **409**: 91.
9. Cachet Th, De Turck K, Roets E, Hoogmartens J. *J. Pharm. Biomed. Anal.* 1991; **9**: 547.
10. Cachet Th, Quintens I, Paesen J, Roets E, Hoogmartens J. *J. Liquid Chromatogr.* 1991; **14**: 1203.
11. Paesen J, Roets E, Hoogmartens J. *Chromatographia* 1991; **32**: 162.
12. Paesen J, Claeys P, Roets E, Hoogmartens J. *J. Liquid Chromatogr.* 1993; **630**: 117.
13. Paesen J, Calam DH. Miller JHMcB, Raiola G, Rozanski A, Silver B, Hoogmartens J. *J. Liquid Chromatogr.* 1993; **16**: 1529.
14. Khan K, Paesen J, Roets E, Hoogmartens J. *J. Liquid Chromatogr.* 1994; **17**: 4195.
15. Nasr MM, Tschappler TJ. *J. Liquid Chromatogr. Relat. Technol.* 1996; **19**: 2329.
16. Chepkwony HK, Vanderriest I, Nguyo JM, Roets E, Hoogmartens J. *J. Chromatogr.* 2000; **870**: 227.
17. Kanfer I, Skinner MF, Walker RB. *J. Chromatogr.* 1998; **812**: 255.
18. Volmer DA, Hui JPM. *Rapid Commun. Mass Spectrom.* 1998; **12**: 123.
19. Pleasance S, Kelly J, LeBlanc MD, Quilliam MA, Boyd RK, Kitts DD, McErlane K, Bailey MR, North DH. *Biol. Mass Spec.* 1992; **21**: 675.
20. Li Y-X, Neufeld K, Chastain J, Curtis A, Velagaleti P. *J. Pharm. Biomed. Anal.* 1998; **16**: 961.
21. Gates PJ, Kearney GC, Jones R, Leadlay PF, Staunton J. *Rapid Commun. Mass Spectrom.* 1999; **13**: 242.
22. Morgan J, Rowlands RT, Joyce-Meneksçe ME, Gilbert IH, Lloyd D. *J. Biotechnol. Tech.* 1998; **12**: 435.
23. Martin JR, Egan RS, Goldstein AW, Collum P. *Tetrahedron* 1975; **31**: 1985.
24. Martin JR, De Vault L, Sinclair AC, Stanaszek RS, Johnson P. *J. Antibiot.* 1982; **35**: 426.
25. Flynn EH, Sigal MV, Wiley PF, Gerzon K. *J. Am. Chem. Soc.* 1954; **76**: 3121.
26. Freiberg LA. US Patent 1972; **3**: 725 385; *Chem. Abstr.* 1996; 5c.
27. Kibwage IO, Busson R, Janssen G, Hoogmartens J, Vanderhaeghe H. *J. Org. Chem.* 1987; **52**: 991.
28. Kirst HA, Wind JA, Paschal JW. *J. Org. Chem.* 1987; **52**: 4359.
29. Wiley PF, Gerzon K, Flynn EH, Sigal MV, Weaver O, Quarck UC, Chauvette RR, Monahan R. *J. Am. Chem. Soc.* 1957; **79**: 6062.
30. Kurath P, Jones PH, Egan RS, Perun TJ. *Experientia* 1971; **27**: 362.
31. Kibwage IO, Roets E, Hoogmartens J. *J. Chromatogr.* 1983; **256**: 164.
32. Kibwage IO, Janssen G, Busson R, Hoogmartens J, Vanderhaeghe H. *J. Antibiot.* 1987; **40**: 1.
33. Corcoran JW, Kaneda T, Butte JC. *J. Biol. Chem.* 1960; **235**: 29.
34. Column P, Egan RS, Goldstein AW, Martin JR. *Tetrahedron* 1976; **32**: 2375.

 