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Short communication

Determination of the impurity profile of ethyl-2-(4-{(5*R*)-3-[4-(methoxycarboxamidoiminomethyl)-phenyl]-2-oxo-5-oxazolidinylmethyl}-1-piperazinyl) acetate, citrate, a highly active platelet aggregation inhibitor, by liquid chromatography–mass spectrometry

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Abstract

Ethyl-2-(4-{(5*R*)-3-[4-(methoxycarboxamidoiminomethyl)-phenyl]-2-oxo-5-oxazolidinylmethyl}-1-piperazinyl) acetate, a glycoprotein IIb/IIIa antagonist, is a drug substance of the oxazolidinone type from Merck's research to be developed for the chronic oral treatment of thrombotic disorders. For the separation of the byproducts in the bulk drug substance, a reversed-phase HPLC gradient separation was developed. The eluent consisting of a nonvolatile phosphate buffer system had to be changed to a volatile acetate buffer system in order to perform on-line LC–MS coupling.With a triple quadrupole system it was possible to characterize most of the unknown byproducts occuring during synthesis and during scale-up to kg amounts of the bulk drug substance.

Keywords: Pharmaceutical analysis; EMD 122 347; Ethyl-2-(4-{(5*R*)-3-[4-(methoxycarboxamidoiminomethyl)-phenyl]-2-oxo-5-oxazolidinylmethyl}-1-piperazinyl) acetate; Platelet aggregation inhibitors

1. Introduction

The glycoprotein IIb/IIIa antagonist [1] Ethyl-2-(4-{(5*R*)-3-[4-(methoxycarboxamidoiminomethyl)-phenyl]-2-oxo-5-oxazolidinylmethyl}-1-piperazinyl) acetate (EMD 122 347) is a double prodrug in which the N and C-termini of the pharmocologically active drug are blocked by prodrug elements (urethane and ester-type) to achieve good oral bioavailability of the compound. This oxazolidinone-type drug is developed for the chronicc oral treatment of thrombotic disorders (e.g., unstable angina) [2–5]. During process development of the bulk drug material, and especially during scale-up from g to kg scale, numerous side reactions were observed, e.g. partial cleavage of the terminal protecting groups, reesterification, acetylation or amide formation from the amidinium group. To understand the interesting variety of side products, one has to consider the synthetic pathway (Fig. 1).

The analytical task was to elucidate the structure of every single impurity with a concentration of 0.1% or higher [6].

The precursors of EMD 122 347 were available as reference substances and therefore could easily be

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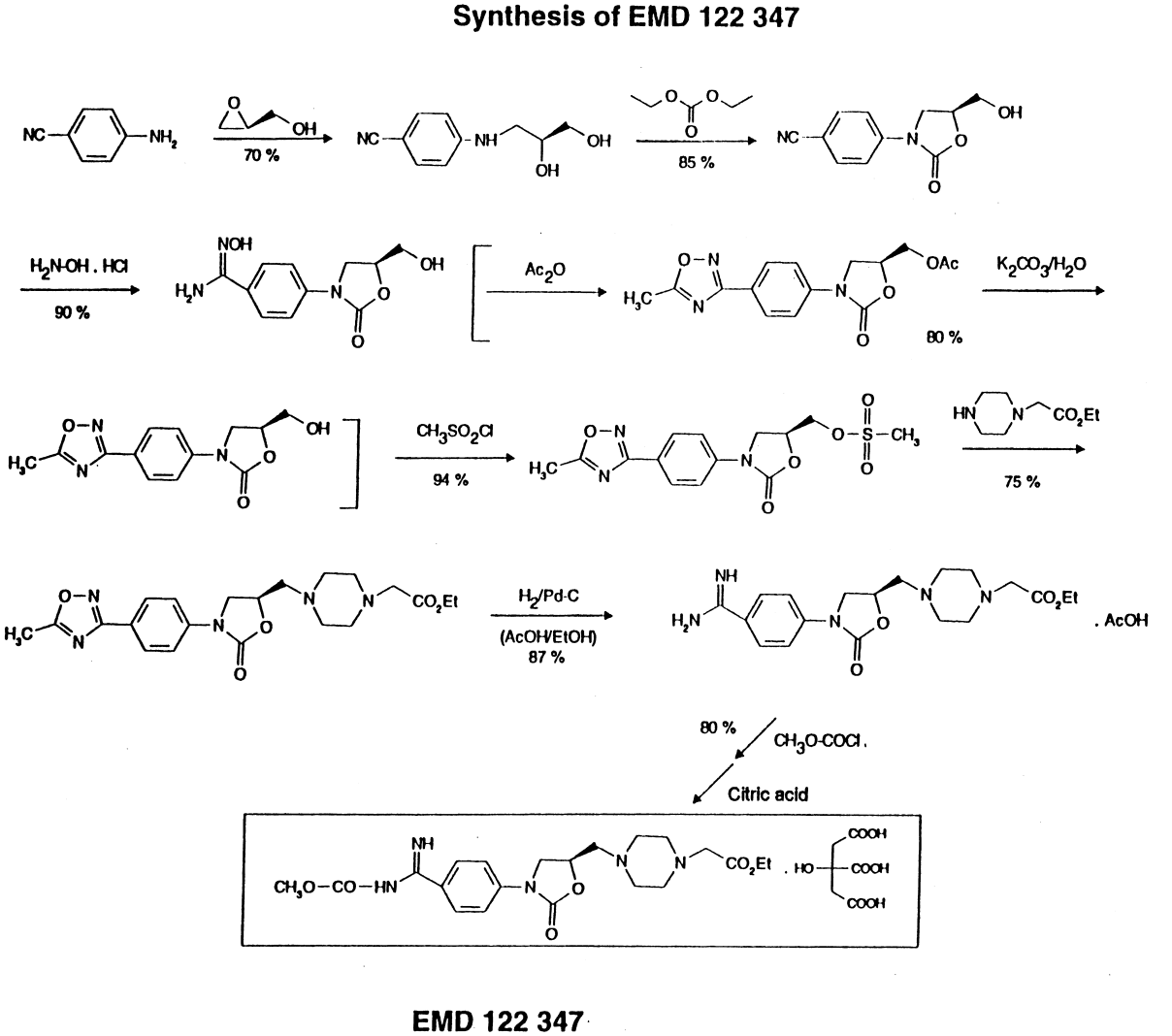


Fig. 1. Synthesis of EMD 122 347.

spiked to a LC run. LC–MS and LC–MS–MS were used to identify the structure of every new impurity peak generated during variations of the synthesis or variations of the cleanup procedures.

2. Experimental

2.1. Chemicals

All chemicals (buffer salts, acids, solvents, HPLC-water) and the LC columns used in this work were from Merck (Darmstadt, Germany).

2.2. HPLC separation

For the routine quality control of the different batches from our central process development department, a LC-gradient method was developed. LC separations were performed with a Merck–Hitachi system consisting of an L-6200 intelligent pump and an L-4200 UV–Vis detector. The A/D converter was

a D-7000 interface for data aquisition. Results were calculated by a Siemens/Nixdorf SCENIC Pro C5 computer. The column was a 250×4 mm LiChrospher 60 RP-select B (5 μm) operated at a flow-rate of 1.0 ml min^{-1}. For gradient separations, eluent A was a mixture of 11.04 g NaH_2PO_4, 800 ml water and 200 ml acetonitrile. Eluent B consisted of 6.90 g NaH_2PO_4, 500 ml water and 500 ml acetonitrile. Both eluents were adjusted to pH 6.5 with 1 *M* NaOH. Starting with an isocratic run of eluent A for 17 min, the composition was changed from 100% A to 100% B in 8 min. From 25 to 35 min, 100% B was run, followed by a 20 min reequilibration with 100% A. The detection wavelength was set at 285 nm.

2.3. LC–MS analyses

The liquid chromatography system used for the LC–MS experiments consisted of a Merck–Hitachi L-6200 pump and a L-4200 UV–Vis detector. A Finnigan MAT TSQ-700 (San Jose, CA, USA) triple quadrupole instrument equipped with a Finnigan API-1 electrospray ionization (ESI) interface was used for MS measurements, data aquisition and processing.

Only volatile buffer systems can be used for LC–MS therefore we had to change from a phosphate to an acetate buffer system. The column used was a 125-4 LiChrospher 60 RP-select B (5 μm) at a flow-rate of 0.5 ml min^{-1}. Eluent A for the gradient separation consisted of 3.08 g ammonium acetate, 800 ml water and 200 ml acetonitrile, eluent B consisted of 1.93 g ammonium acetate, 500 ml water and 500 ml acetonitrile. Both eluents were adjusted to pH 6.5 with acetic acid. Starting with an isocratic run of 100% A for 25min., the elution power was increased by a linear gradient changing from 100%

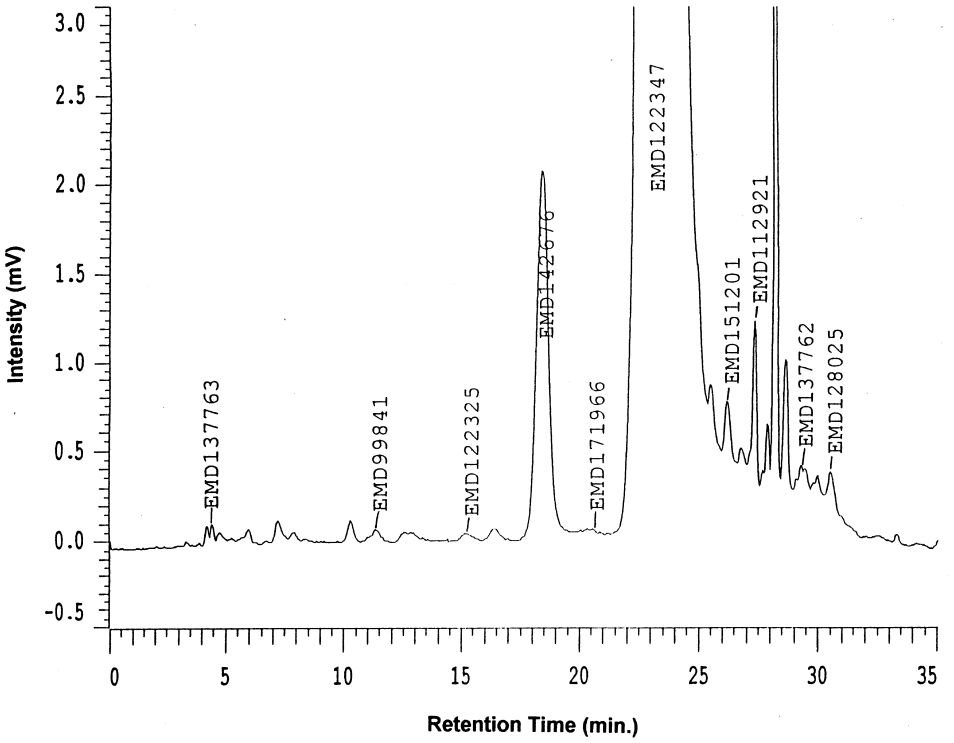


Fig. 2. HPLC run phosphate system. HPLC conditions: column: 250×4 mm LiChrospher 60 RP-select B (5 μm); detection wavelength: 285 nm; flow: 1.0 ml $min;^{-1}$ eluent A: 11.04 g NaH_2PO_4+800 ml water+200 ml acetonitrile (adjusted to pH 6.5 with 1 *M* NaOH); eluent B: 6.90 g NaH_2PO_4+500 ml water+500 ml acetonitrile (adjusted to pH 6.5 with 1 *M* NaOH); gradient: from 0–17.0 min: 100% A, from 17.0 to 25.0 min: from 100% A to 100% B, from 25.0 to 35.0 min: 100% B, from 35.1 min to 55.0 min: 100% A.

A to 100% B in 10 min. Then 100% B was run for additional 10min., followed by a 10 min. reequilibration with 100% A.

The detection wavelength was also set to 285 nm.

3. Results

As pointed out in the introduction, some of the precursors were available as reference substances for spiking experiments. After structure elucidation by LC–MS of the frequently occuring byproducts, these new impurities were synthesized and used as reference materials. Fig. 2 shows an example of one production batch and Fig. 3 shows the same batch spiked with eleven impurities.

Our results are summarized in Fig. 4.

It was most interesting and surprising to find that beneath the other trace contaminants there was another byproduct which could be identified via LC–MS: EMD 151 201 with m/z 574, which is {4-[(4-{(5*R*)-3-[4-iminomethoxycarbonylaminomethyl)-phenyl]-2-oxo-oxazolidin-5-ylmethyl}-piperazin-1-yl)-acetyl-piperazin-1-yl}-acetic acid ethyl ester. This molecule could have been formed when a contaminated batch of ethyl-1-piperazinylacetate was used. (Compare Fig. 1 from stages 6 to 7). Probably, partial aminolysis of the ethyl ester happened with the basic piperazine nitrogen of another molecule (Fig. 5).

The third panel in Fig. 6, the User track shows the peak of interest marked with an arrow in the LC acetate system.

One additional problem was the structure elucidation of another impurity with m/z 462. Two different species of this mass can be generated during synthesis, namely the ethylurethaneethylester and the methylurethaneisopropyl ester analogs. This question was answered by MS–MS experiments which clearly show different fragmentation patterns.

The MS–MS studies started with the bulk drug substance EMD 122 347 (m/z 448) using direct

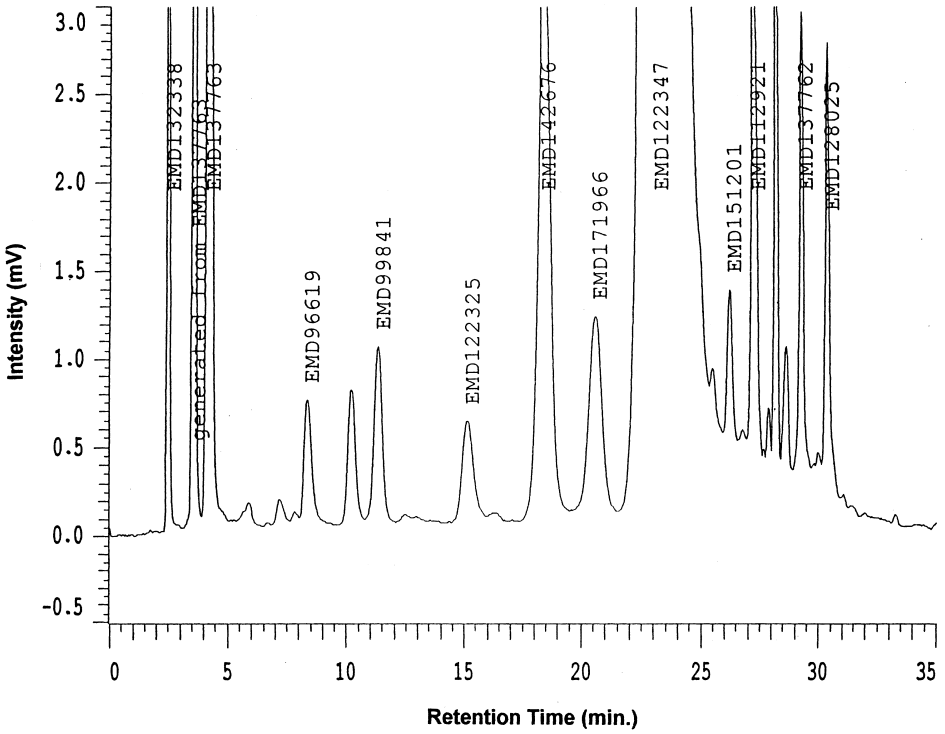


Fig. 3. HPLC run phosphate system + spiked substances. Conditions as in Fig. 2.

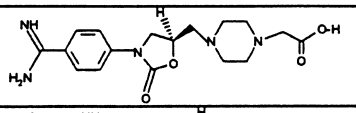
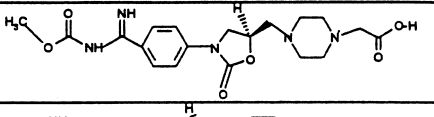
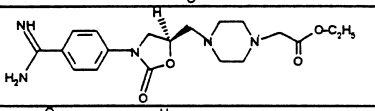
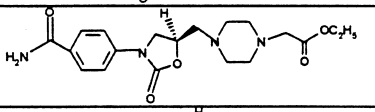
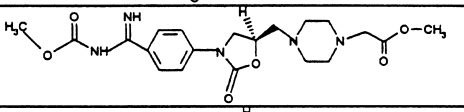
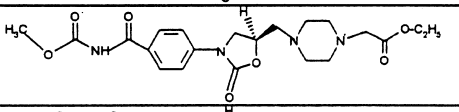
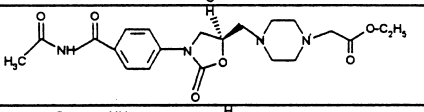
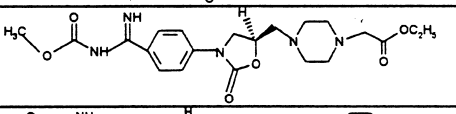
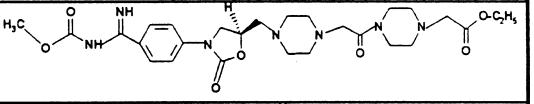
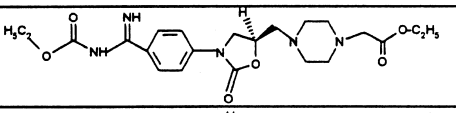
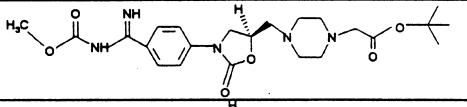
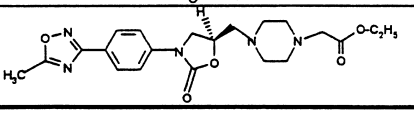
Retention time (min)	Name	Formula
2.49	EMD 132 338	
4.25	EMD 137 763	
8.35	EMD 96 619	
11.33	EMD 99 841	
15.23	EMD 122 325	
18.37	EMD 142 676	
20.32	EMD 171 966	
23.05	EMD 122 347 main component	
26.17	EMD 151 201	
27.31	EMD 112 921	
29.25	EMD 137 762	
30.48	EMD 128 025	

Fig. 4. Results: retention time, name and formula.

syringe introduction into the triple quadrupole MS instrument. As one can see in Fig. 7, the most intensitive fragment can be correlated with the loss of the methoxy group of the urethane. The next fragment can be associated with the loss of the complete methoxycarboxamido residue.

The same fragmentation types could also be observed during the investigation of the two peaks

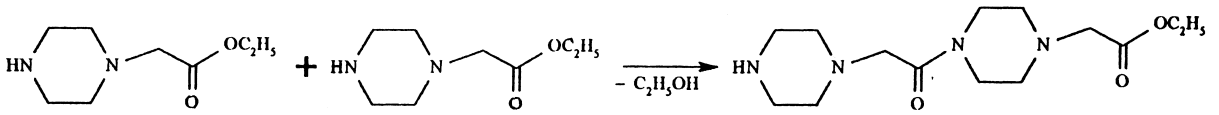


Fig. 5. Possible reaction pathway leading to the formation of EMD 151 202.

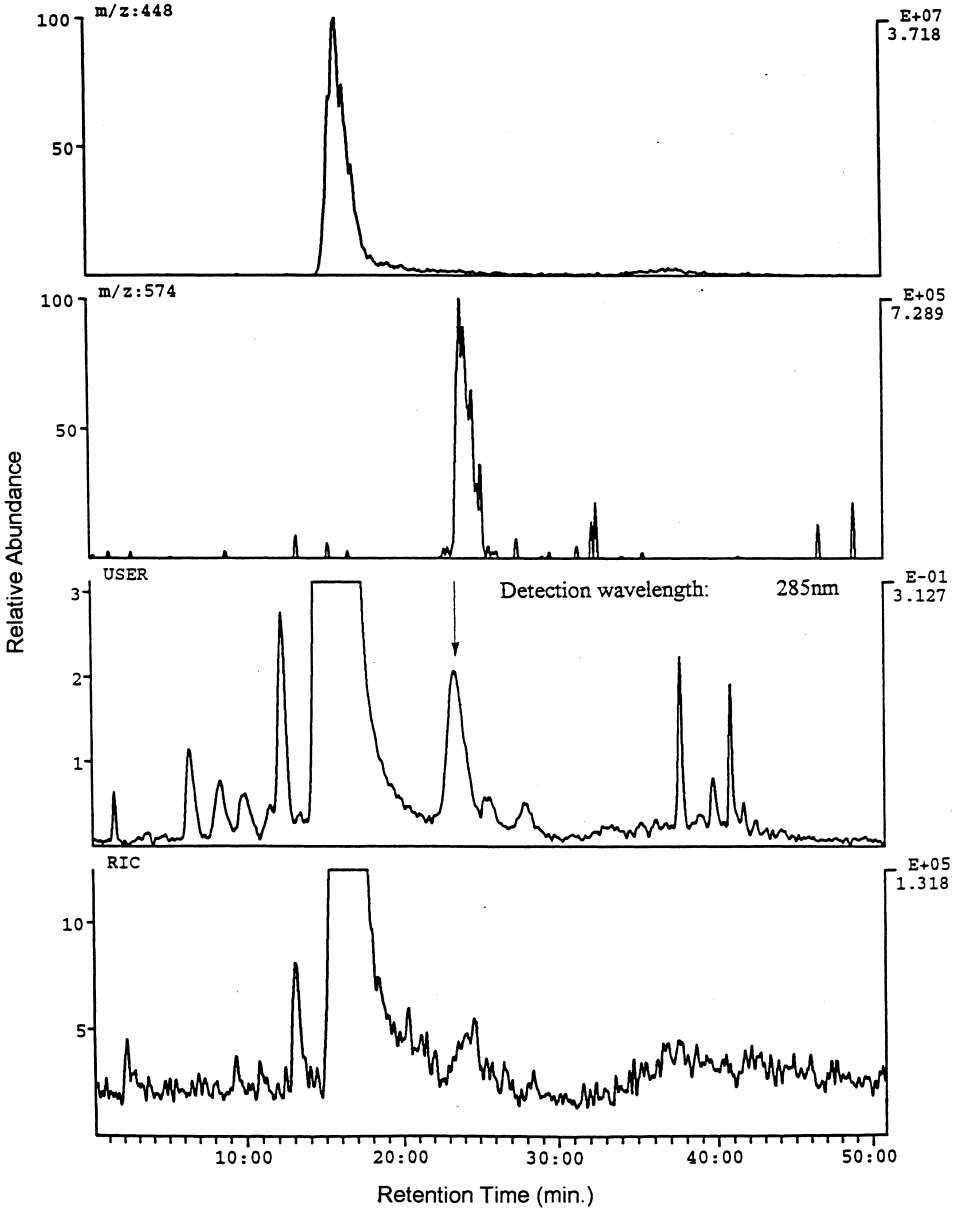


Fig. 6. ESI–HPLC–MS of the main component EMD 122 347 (m/z) 448 and one of the main side products EMD 151 201 (m/z 574).

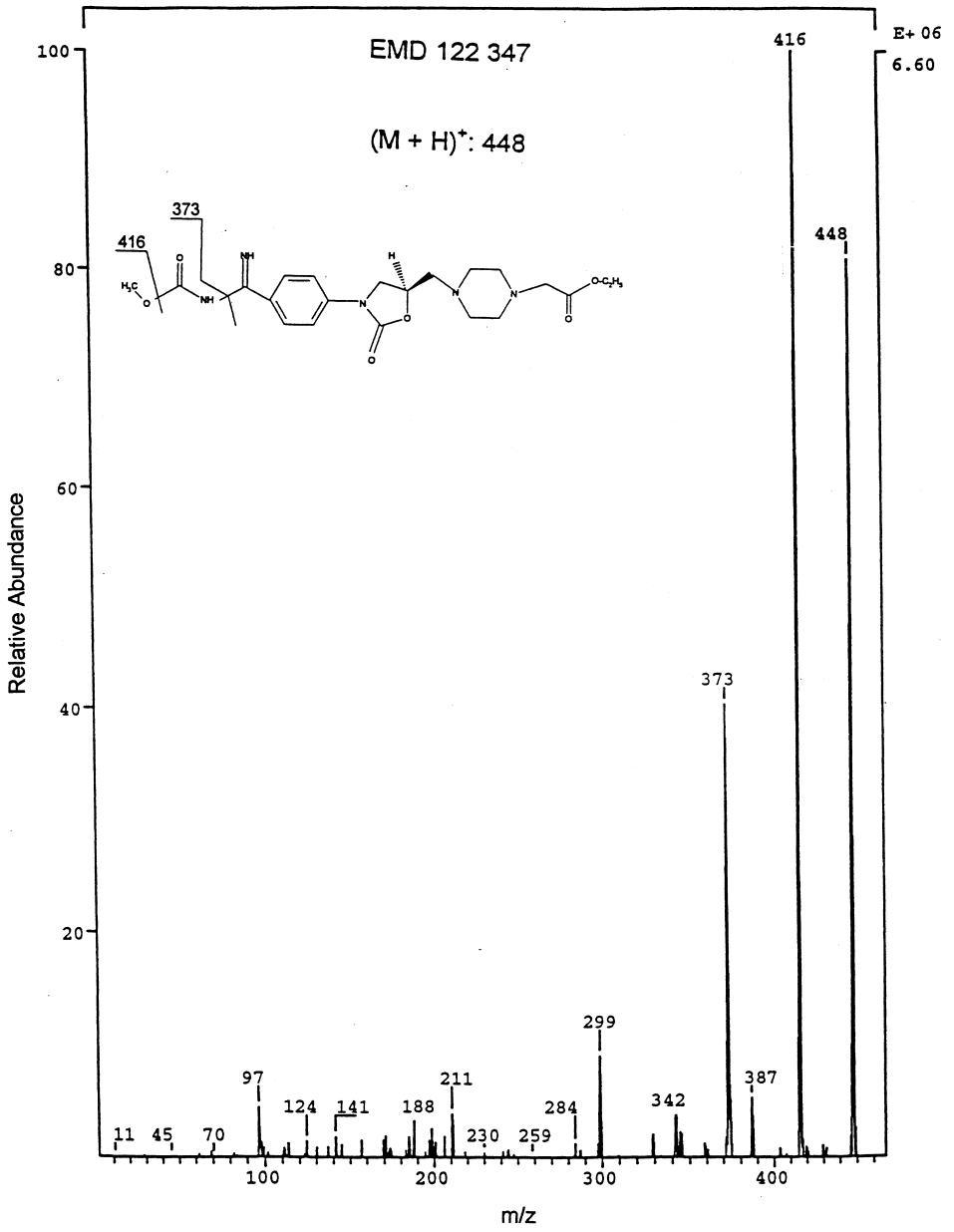


Fig. 7. MS–MS of EMD 122 347.

with the same m/z of 462. Fig. 8 shows the fragmentation of EMD 112 921, which is ethyl-2-(4 - {(5*R*)- 3 -[4-(ethoxycarboxamidoiminomethyl)-phenyl]-2-oxo- 5 -oxazolidinylmethyl}-1-piperazinyl) acetate, the 'ethyl–ethyl' derivative. Fig. 9 shows the fragmentation of one distinct peak which was a heartcut from a production batch called Ans. 97/Mü/006-4, and was also introduced via syringe into the MS instrument. This clearly points out that the peak of interest is the isopropyl ester analog of EMD 122

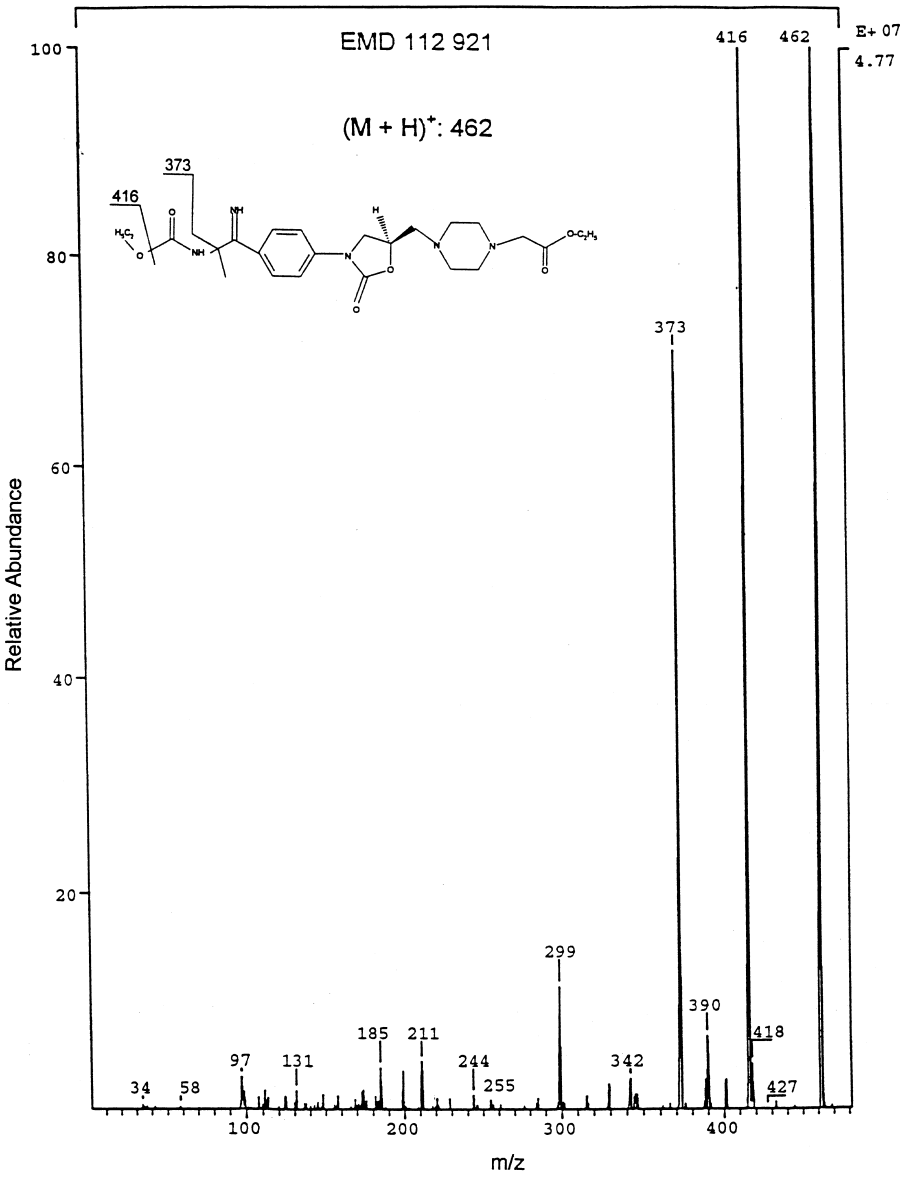


Fig. 8. MS–MS of EMD 112 921.

347. Loss of the isopropoxyacetyl residue may lead to fragment m/z 360, probably followed by an additional loss of a methyl group leading to m/z 345.

The reason for this side product was an isopropanol-contaminated ethanol batch used for catalytic hydrogenation in stage 8.

4. Discussion

The described mobile phase and LC–MS conditions were sufficient to give detailed information about the impurity profile of a new drug substance in the early stages of process development. LC–ESI–

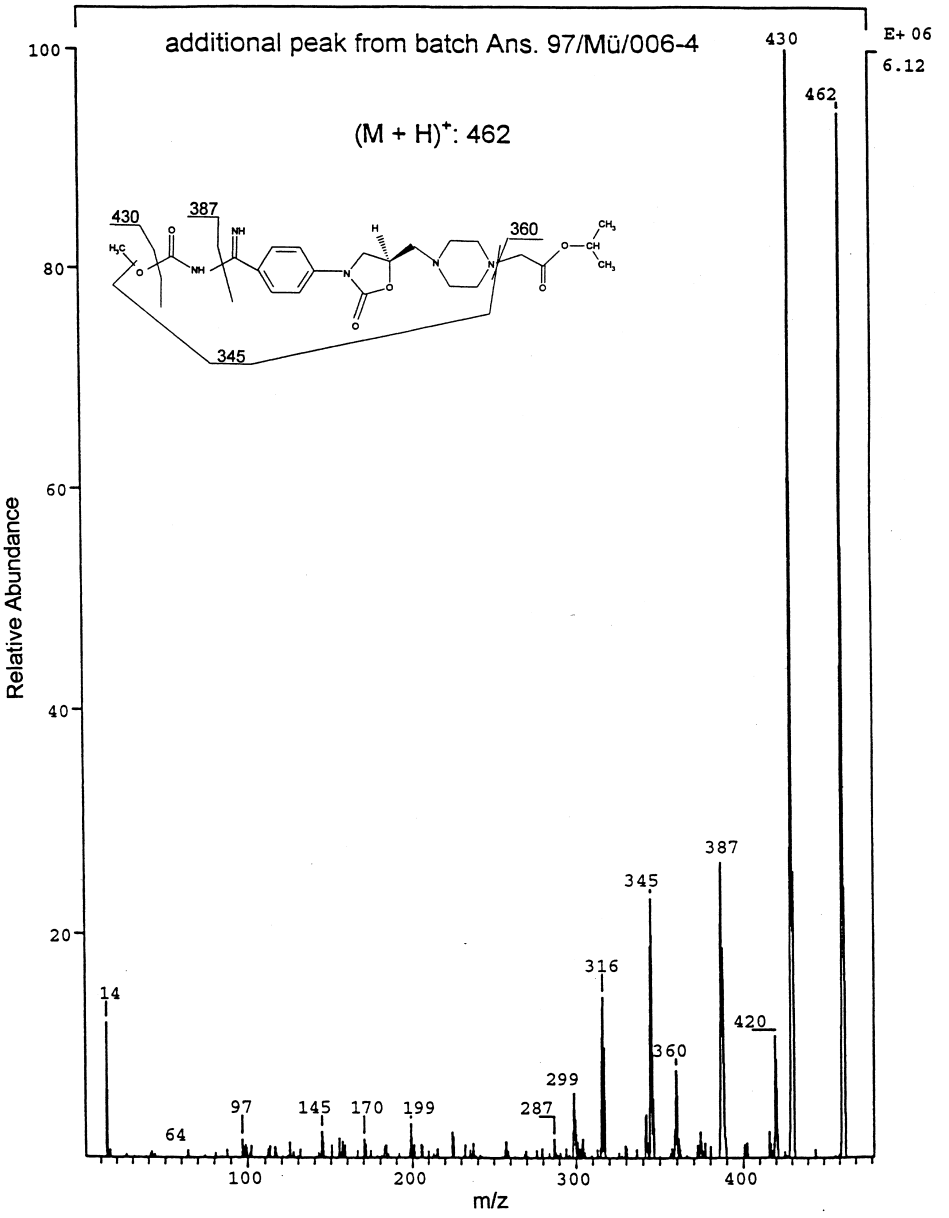


Fig. 9. MS–MS of the additional peak in batch Ans. 97/Mü/006-4.

MS and LC–ESI–MS–MS are suitable for the investigation of side reactions in organic chemistry. The MS or MS–MS information combined with the retention characteristics (polarity, lipophilicy, molecular size) from a LC run quickly gives an idea of the molecular mass of the interesting species. ESI–MS in the positive mode is a powerful tool to get quick information, especially in the field of nitrogen-containing heterocyclic components [7].

The use of these hyphenated techniques enables the early recognition of reaction paths leading to failures, such as contaminated drug substances or

decreased product yields. This can help to save time and money during process development in chemical companies working in this field.

References

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