

Using the LCMS-IT-TOF to identify impurities in pharmaceutical candidates using high mass accuracy and MSⁿanalysis

Overview

Objective.
Structural elucidation for impurities in Erythromycin A oxime

Strategies.
Using accurate mass information and pattern matching with MS/MS spectra of pharmaceutical drug candidate, we have attempted to elucidate the structure of all suspected impurities.

Results
Several by-products were identified by LC/UV/MS.

Component 1
Loss of a methyl group in Area B replaced by a hydrogen

Component 2
Loss of a methyl group in Area A replaced by a hydrogen

Component 3
Mass spectrum inconsistent with erythromycin and not related.

Component 4
2 products.
Product 4.1 relates to an additional methyl group present in Area C.
Product 4.2 corresponds to a loss of oxygen from Area C.

Component X
An additional oxygen in Area B

Introduction

In the development of pharmaceutical candidates it is critical to identify by-products of the reaction mixture. In this paper we describe the application of a novel hybrid instrument platform delivering high mass accuracy data and MSⁿ analysis in identifying impurity products from a relatively impure pharmaceutical candidate.

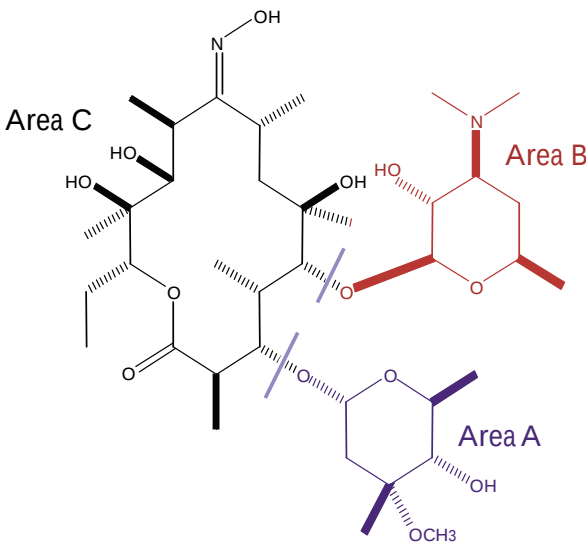
Methods

Instrument configuration	
Liquid Chromatograph Mass Spectrometer	LCMS-IT-TOF
Pump	LC-10AD _{VP}
Auto Injector	SIL-10AD _{VP}
Column Oven	CTO-10A _{VP}
LC conditions	
Column	XterraMS C18 3.5 μm(2.1 mmI.D. × 100 mmL.)
Mobile Phase	0.1% NH ₄ OH/CH ₃ CN(50/50)
Flow Rate	0.2 mL/min
Injection Volume	10μL
Temperature	Ambient
Mass spectrometry conditions	
Ionization mode	Electrospray positive ion mode
Spray Gas flow rate	1.5 L/min
Drying gas pressure	0.1 MPa
Voltage	-3.5 kV
CDL Temperature	200 °C
BH Temperature	200 °C

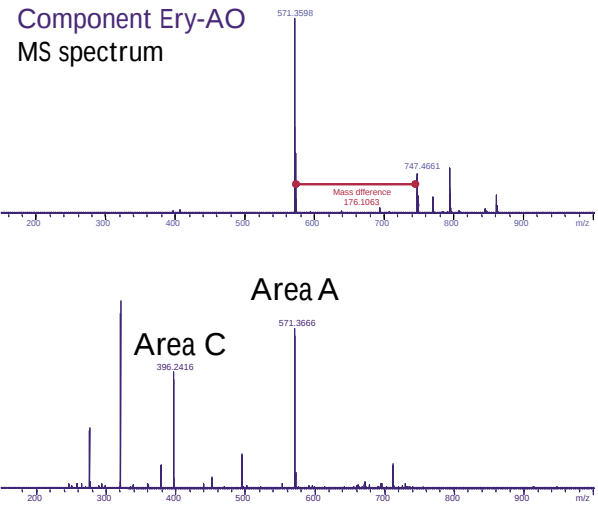
Erythromycin is an important 14 membered macrolide antibiotic active against gram-positive bacteria.

In developing commercial antibiotics it is important to fully characterise impurities (or by-products).

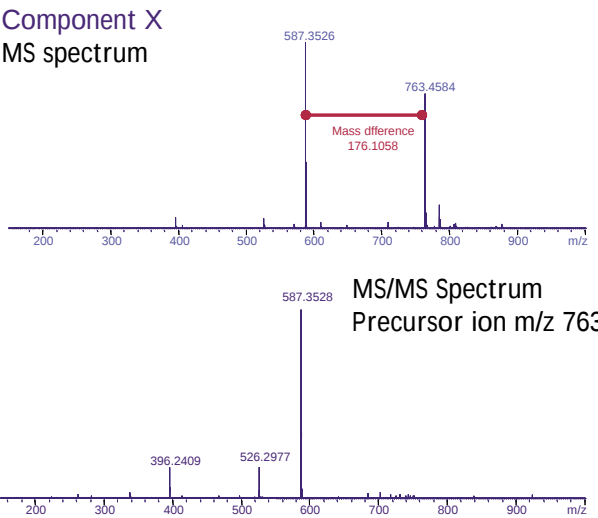
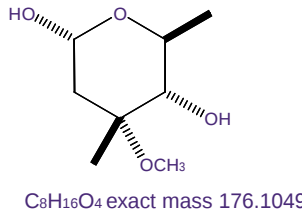
The chemical structure can be simply represented as 3 domains or areas. In this regard we have nominated area A to correspond to the desosamine sugar whilst area B relates to cladinose.



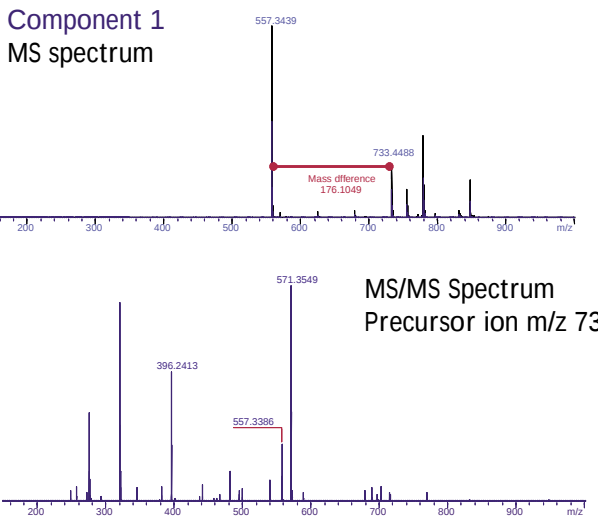
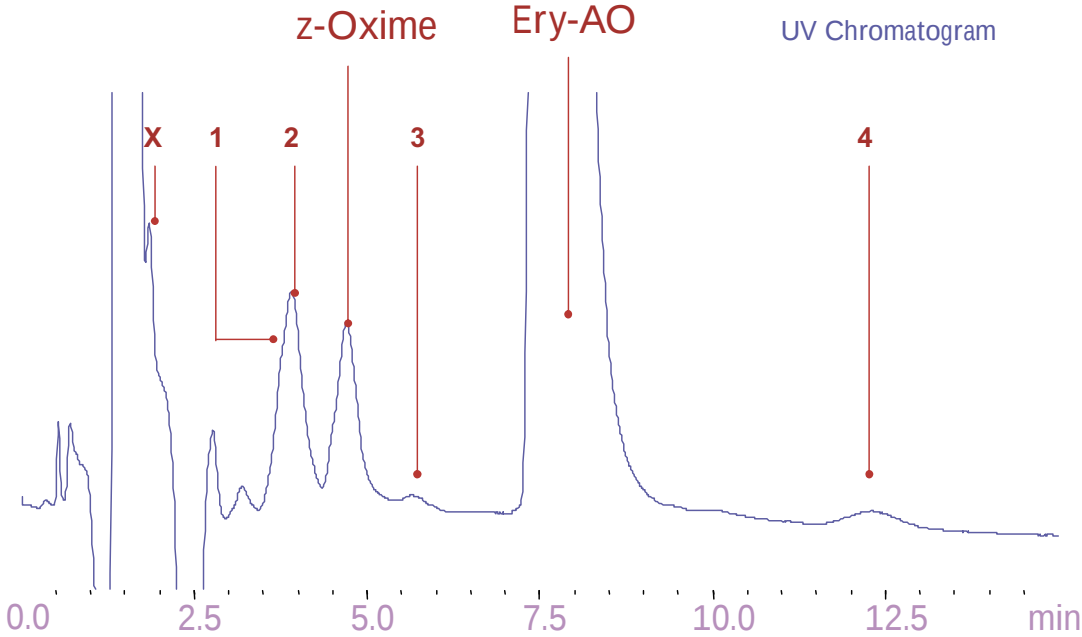
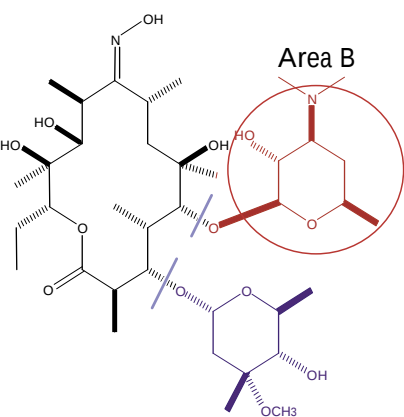
Results



Component Ery-AO
Generally, cleavage of the glycosidic linkages attaching the sugars to the macroliding and water losses constitute the major fragmentation pathways for most of the protonated compounds. A diagnostic fragment ion assigned as the desosamine ion m/z176.1049 is referred to as **Structure A**).

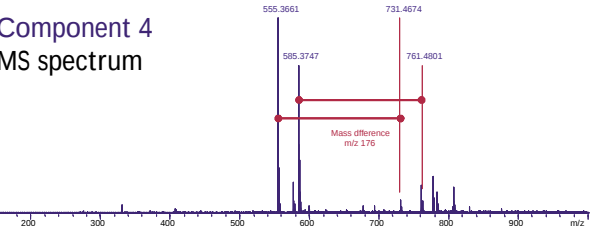
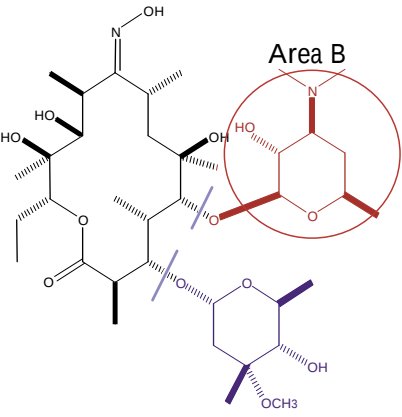


Component X.
The mass difference between the main product Ery-AO (m/z747.4661) and a previously unknown Impurity labeled 'X' (m/z763.4584) is 15.9923 (u). This mass difference is equal to Oxygen, O (15.9949(u)) and provides strong evidence that this impurity has the structure with a single Oxygen atom in 'Area B' of the molecule.

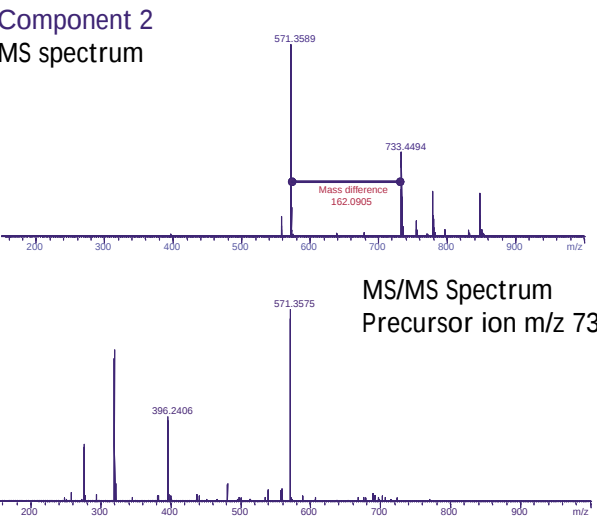
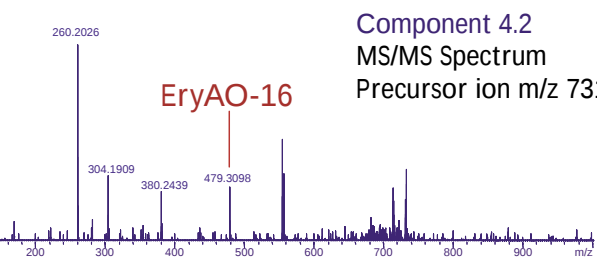
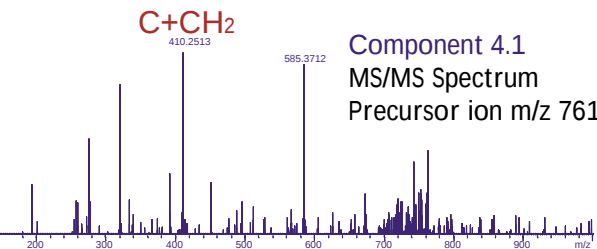


Component 1.
The mass difference between Ery-AO (m/z747.4661) and Impurity 1 (m/z733.4488) is 14.0173 (u) corresponding to CH₂ (14.0156(u)). In the MS/MS spectrum the ion at m/z396.2413 corresponds to Area C (theoretical mass value is m/z396.2386) and the ion at m/z571.3549 relates to Area A.

As a result, the difference in structure can be assigned to a loss of a methyl group in Area B.



Component 4.
2 products co-elute resulting in product 4.1 and product 4.2. As the spectrum of product 4.1 includes an ion at m/z410.2513 this would strongly suggest a methyl group is present in Area C (m/z396.2386) + CH₂(14.0156(u))=410.2542). Product 4.2 corresponds to the loss of oxygen from Area C (the ion at m/z 380.2439 supports the loss of oxygen from the diagnostic ion at m/z396.2386 for Area C).



Component 2.
In the MS spectrum the mass difference between m/z733.4494 and m/z571.3589 is 162.0905 (u). This mass difference is 14.0144 (u) and corresponds to CH₂ (14.0156 (u)). As the m/z396.2406 ion is present (Area A) and the ion at m/z571.3575 is also present (relating to the loss of Area A and a CH₂group) the data is consistent with the loss of a methyl group in Area A.

Component 2 is a loss of a methyl group in Area A.

