MS-US990609B
Presented at the 47th ASMS Conference on Mass Spectrometry and Allied Topics, Dallas, TX, June 13-17, 1999

High Resolution LC/MS for Analysis of Minor Components in Complex Mixtures: Identification of Impurities and Degradation Products of a Novel Oligosaccharide Antibiotic

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Conventional LC/MS and LC/MS/MS techniques have become an indispensable tool for analysis of minor components in complex mixtures. Such analyses include identification of isomers, drug metabolites, impurities, degradation products, reaction byproducts, individual components in combinatorial chemistry reaction mixtures, etc. However, when there is no prior structural information, such as structures of starting materials for a particular reaction, or a known drug substance that has generated certain metabolites, the complete structural identification becomes a difficult and time consuming task. Often, separation of the compound of interest and further analysis by complementary techniques, such as NMR, are required for full characterization. One way of overcoming these difficulties is the implementation of high resolution LC/MS using a magnetic sector mass spectrometer. Obtaining the elemental composition of parent and most abundant fragment ions provides significant help in the structural identification process. High resolution mass spectrometry has been routinely used for structural confirmation and identification, however, it has been limited to somewhat pure samples, for example, analysis of a typical drug metabolite mixture without the aid of liquid chromatography, has been impractical.

In this work, we report the identification of impurities and degradation products of Ziracin, a member of the Everninomicin class of antibiotics. Everninomicins belong to an important group of oligosaccharides, isolated from the fermentation broth, *Micromonospora Carbonaceae*, and are found to be highly active against Gram-positive bacteria including Methicillin resistant *Staphylococci* and Vancomycin resistant *Enterococci*. With the emergence of drug resistant strains of bacteria, a major effort is being directed towards the reevaluation of the efficacy of existing oligosaccarides and identification of new potential oligosaccaride antibiotics. Currently, the Everninomicin antibiotic Ziracin (SCH27889, Schering-Plough Corporation) is undergoing extensive trials to determine its clinical efficacy. Preliminary results suggest that Ziracin is safe and effective and if it proves successful in the large scale clinical trials, it could become a very important drug for treatment of human infections.

Ten Everninomicin related impurities and degradation products were detected by negative ion HR ESI LC/MS as shown on the reconstructed ion chromatogram below. Exact mass measurements were obtained at a resolution of 5000 and corresponding elemental compositions were proposed with an experimental error of 3 ppm or less (see table).

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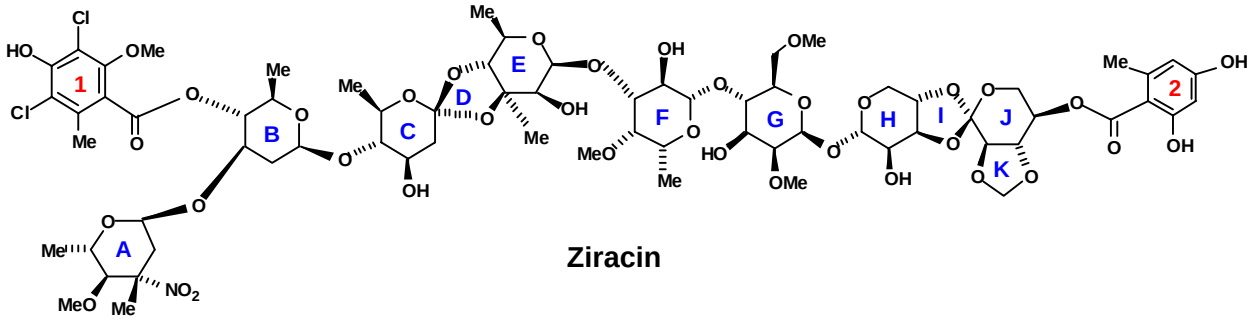
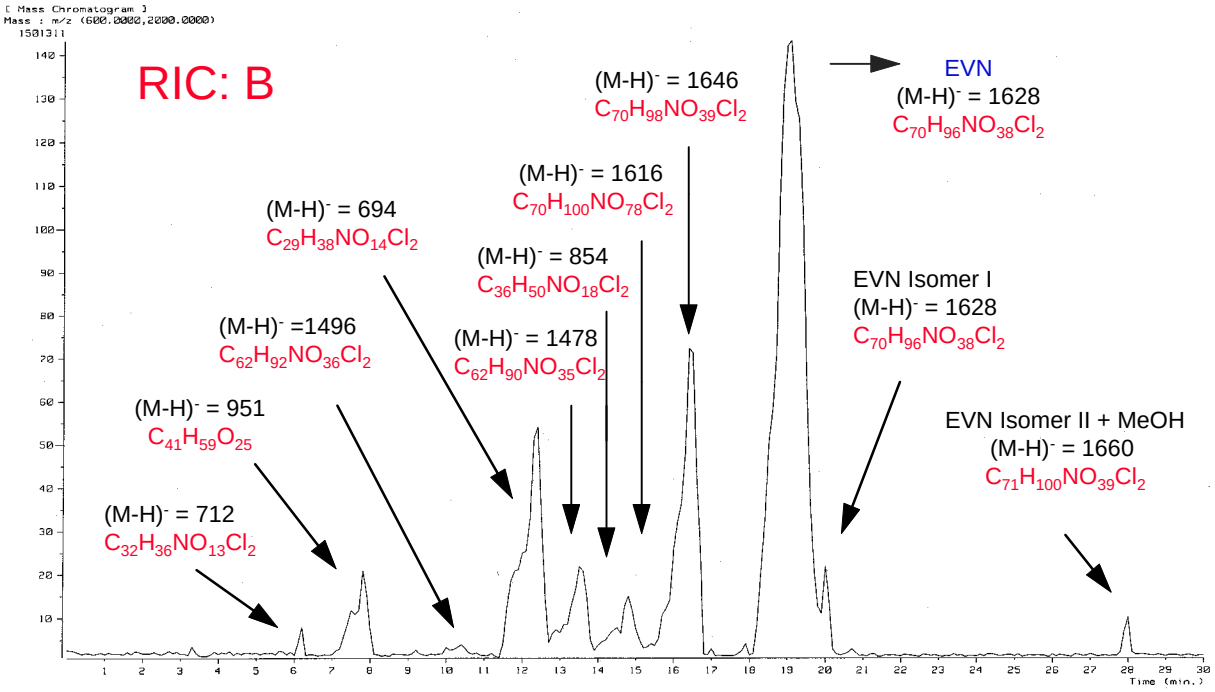


Ziracin



t_R (min)	(M-H)$^-$	elemental composition	theoretical value	experimental value	Δ (ppm)
6.1	712	$C_{32}H_{36}O_{13}N_1Cl_2$	712.1564	712.1583	2.7
7.5	951	$C_{40}H_{55}O_{26}$	951.3345	951.3353	1.1
10.2	1496	$C_{62}H_{92}O_{36}NCl_2$	1496.4776	1496.4781	0.4
12.1	694	$C_{29}H_{38}O_{14}NCl_2$	694.1669	694.1678	1.3
13.3	1478	$C_{62}H_{90}O_{35}NCl_2$	1478.4670	1478.4689	1.2
13.5	854	$C_{36}H_{50}O_{18}NCl_2$	854.2405	854.2414	1.1
14.3	1616	$C_{70}H_{100}O_{37}NCl_2$	1616.5351	1616.5369	1.1
15.7	1646	$C_{70}H_{98}O_{39}NCl_2$	1646.5093	1646.5082	0.7
18.1	1628	$C_{70}H_{96}NO_{38}Cl_2$	1628.4987	1628.4999	0.7
27.4	1660	$C_{71}H_{100}O_{39}NCl_2$	1660.5250	1660.5262	0.8