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NOTE

Identification of Related Substances in Erythromycin Drug by Liquid Chromatography/Mass Spectrometry

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A new LC-MS method has been developed for the determination of related substances in erythromycin. For method development, various solvent systems were used. An Ashaipak column was used at with a mobile phase consisting of 0.023 M ammonium formate (pH 10.3): water:acetonitrile (35:25:40) v/v/v. Using mass spectra as interpretative templates, seven known related substances in erythromycin samples were identified *viz.*, erythromycin F, N-demethylerythromycin A, erythromycin E, anhydroerythromycin A, erythromycin enol ether, erythromycin B and erythromycin C along with their fragmentation pattern.

Key Words: Erythromycin, LC-MS, Related substances.

Erythromycin is macrolide antibiotic that has an antimicrobial spectrum similar to or slightly wider than that of penicillin[1] and is often used for the people who have an allergy to penicillins. Erythromycin is isolated from the strain of the actinomycete *Saccharapolyspora erythraea*, formerly known as *Streptomyces erythraeus*[2]. Erythromycin is used for respiratory tract infections and has better coverage of typical organisms including mycoplasma and legionellosis[3].

The investigated samples of erythromycin drug substance were obtained from Generic Research Laboratory of Alembic Research Centre, Alembic Limited, Vadodara, India.

Method of analysis (LC-MS): Several experiments were carried out to develop the LC-MS method using different buffers, solvents, column and column temperature. An agilent separation module equipped with binary pump, degasser, auto sampler and UV detector (200 series) was linked with applied bioscience mass spectrometer (API-2000) for the purpose. Ashaipak ODP-50 HPLC column having dimensions 250 mm × 4.6 mm i.d. and 5 µm particle size was used for analysis. Injection volume is 70 µL and the run time is 70 min. The flow rate was adjusted to 0.8 mL/min and column was maintained at 50 °C using column oven. The eluent was monitored at 215 nm. The data was recorded using software analyst 1.3.1.

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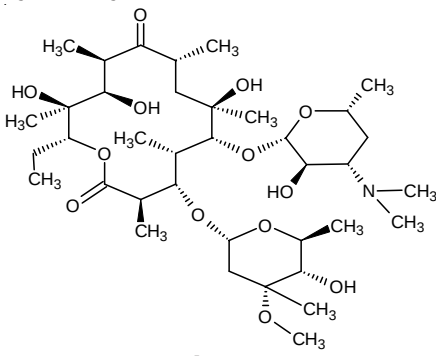
Mass spectrometer parameters: Curtain gas: 10 mL/min; ion spray voltage: 3500 V; temperature: 450 °C; ion spray gas 1: 20 mL/min; ion spray gas 2: 30 mL/min; declustering potential: 60 V; entrance potential: 10 V.

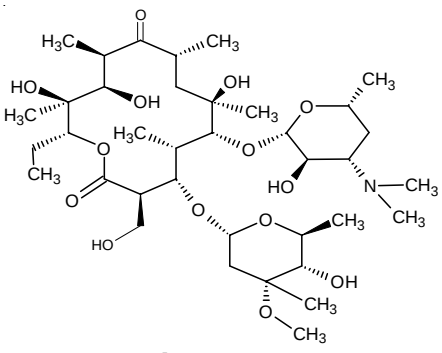
Sample preparation: Weighed and transferred *ca.* 70 mg of test sample and 2 mg each of N-demethyl erythromycin, anhydroerythromycin A and erythromycin enolether into a 25 mL volumetric flask, added 10 mL of acetonitrile and sonicated to mix and make up the volume with mobile phase.

It has been observed that all the related impurities are well separated and properly ionized in above mentioned conditions. From UV chromatographic pattern of erythromycin, it has been concluded that erythromycin eluted at retention time about 13.9 min and showed mass 734.5 ($M+H^+$). Erythromycin F eluted at relative retention time 0.3 with respect to erythromycin showed mass 750.8 ($M+H^+$), while N-demethylerythromycin A eluted at relative retention time 0. 42 and showed mass 750.5 ($M+H^+$). Erythromycin C eluted at relative retention time 0.5 and showed mass 719.3 ($M+H^+$), erythromycin E eluted at relative retention time 0.9, showed mass 748.5 ($M+H^+$). Anhydroerythromycin eluted at relative retention time 1.43 and showed mass 716.5 ($M+H^+$), erthromycin B eluted at relative retention time 1.89 showed mass 717.3 ($M+H^+$) and finally erythromycin enolether eluted at relative retention time 3.95, showed mass 716.7 ($M+H^+$).

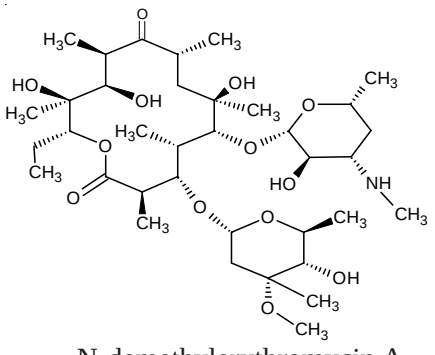
Erythromycin related substances:



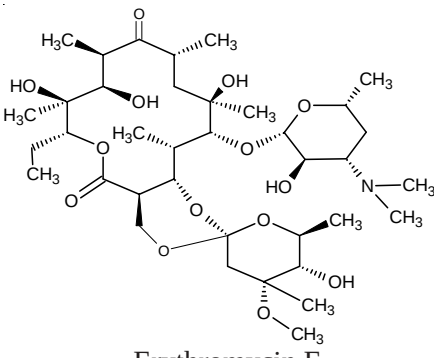
Erythromycin



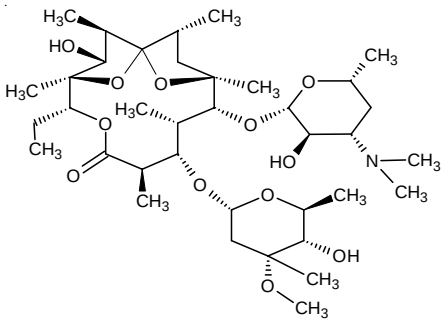
Erythromycin F



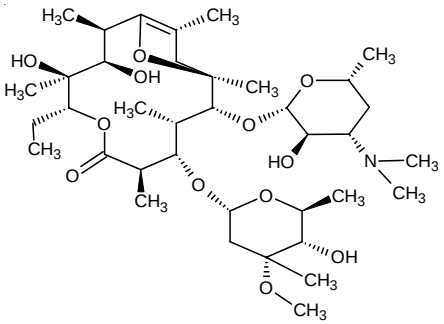
N-demethylerythromycin A



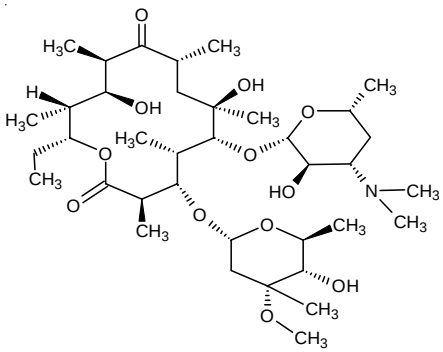
Erythromycin E



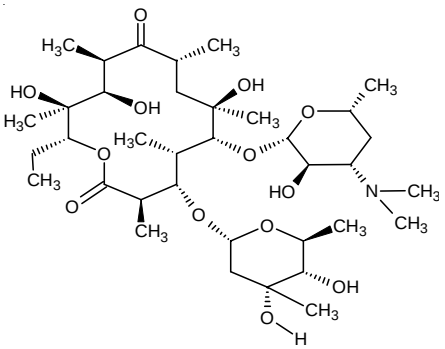
Anhydroerythromycin A



Erythromycin enol ether



Erythromycin B



Erythromycin C

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