Electrospray mass spectrometry for assay of erythromycin A extracted from fermentation liquor

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Electrospray Mass Spectrometry (ESI-MS) can accurately distinguish and quantify erythromycins by their mass:charge ratios. This procedure is suitable for assay in complex fermentation media, and is compared with previous techniques which depend for detection of erythromycin by the low UV absorbance of erythromycin, after separation of the 5 analogues by HPLC, TLC, GLC, and with biological assays which employ erythromycin-sensitive bacterial strains e.g. of Arthrobacter citreus.

Introduction

Erythromycin is the oldest and most widely used macrolide antibiotic. It exists in five forms; erythromycin A, B, C, D and E. Erythromycin A is the most active form and predominates in pharmaceutical preparations. This drug is used primarily against Gram positive organisms and, for instance, where individuals are allergic to penicillin. Erythromycin A operates by selectively inhibiting bacterial protein synthesis, binding to the 50S RNA subunit inhibiting the translocation process (Hugo & Russell, 1992.). All five analogues are produced by Saccharopolyspora erythraea and contain a 14-membered macrocyclic lactone ring with two attached glycosidal groups (Fig.1).

Due to the similarity of these erythromycin analogues in terms of size and structure, their separation is difficult, but has been satisfactorily carried out especially by using HPLC (Tsuji and Goeze, 1978). However, due to the low UV absorbance of the erythromycins (Grigurinovich and Matthews, 1988), this technique requires a large amount and hence large samples of a particular analogue for its detection. Thus the drug must be present at high concentrations (>1mg/ml), or the sample must be laboriously extracted and concentrated before detection and assay become possible. Even the most recently upgraded extraction procedure uses HPLC separation with UV detection (Heydarian et al., 1997).

Figure 1 Chemical structures of erythromycin analogues.

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Figure 2  ESI Chromatogram of the Erythromycin Analogues.

Figure 3  Erythromycin A calibration curve.

Here we present a novel approach to erythromycin A quantification using Electrospray Mass Spectrometry (ESI-MS); this allows samples containing as little as 1 µg erythromycin A/ml to be detected. ESI MS depends on generating ionised molecules and separating them on the basis of their mass/charge ratios. Molecules with a molecular weight of up to 3000 amu can be analysed. Erythromycin A with a molecular weight of 733.93 is well within the ESI-MS range. In ESI-MS a solvent is pumped through a stainless steel capillary which carries a high potential (here: 3.5 kV). The strong electric field generated by this potential causes the solvent to be sprayed from the end of the capillary thus producing highly charged droplets. This fine spray desolvates in a stream of warm gas to yield singly charged ions, such as [M + H]+, which are separated in the analyser and then detected (Smith et al., 1990).

Materials and methods

Strain and culture media

A high yielding mutant of *Saccharopolyspora erythraea* (designated as EA-4) was used throughout. The culture storage medium consisted of 10% (v/v) glycerol and 5% (w/v) lactose in distilled water. Preculture medium contained the following major nutrients (all w/v): 1.0% dextrin, 1.0% dextrose, 2.0% soybean meal, 1.0% Pharmamedia, 0.2% CaCO₃ in distilled water. The pH was adjusted to 6.85 with NaOH prior to autoclaving.

Fermentation medium contained (all w/v): 6% soybean oil, 1.8% soybean meal, 1.0% Pharmamedia, 2.0% corn starch, 0.75% corn steep powder, 0.75% CaCO₃, 0.032% (NH₄)₂SO₄, 0.0035% CuCl₂·2H₂O, 0.05% (v/v) polypropylene glycol (Mn 2000) in distilled water. The pH was adjusted to 6.85 with NaOH prior to autoclaving. n-Propanol 1.0% (v/v) was added after autoclaving just before inoculation of *S. erythraea* into media.

Frozen stocks of *S. erythraea* spores were stored at −84°C of which 0.5 ml was inoculated into a 250 ml Erlenmeyer flask containing 20 ml (~10⁶/ml) of the preculture medium, incubated at 32°C on a rotary shaker at 220 rev/min⁻¹. After 48 h agitation, 0.25 ml was removed and inoculated into a 250 ml Erlenmeyer flask containing 25 ml of fermentation medium, incubated at 32°C on a rotary shaker at 220 rev/min⁻¹ for 6–8 days.

Procedure for erythromycin extraction

A 0.4 ml sample of fermentation broth was mixed by vortexing with 1.6 ml methanol for 15 min. It was then