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Stability of erythromycin and some of its esters in methanol and acetonitrile

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Abstract

The stability of erythromycin (EB), erythromycin ethylsuccinate (EES), propionyl erythromycin (PE) and erythromycin estolate (EE) in methanol and acetonitrile was investigated. Methanol is widely used as a solvent for EB and its ester derivatives in compendial identification tests as well as in the sample preparation steps during assay procedures. HPLC with electrochemical detection was used to measure EB concentrations in methanol and acetonitrile solutions – incubated at room temperature (20 ± 0.5°C) over various periods of time. EB was relatively stable in methanol or acetonitrile, remaining intact for over 168 h in acetonitrile and showing less than 5% degradation in methanol over the same period. Approx. 87% of PE remained intact after 168 h in acetonitrile whilst methanol caused rapid hydrolysis to EB (35% remaining after 28 h). Similarly, EES was unstable in methanol, showing rapid and almost complete hydrolysis to EB, with less than 5% remaining after 40 h. Less than 5% degradation, however, occurred over a period of 168 h when EES was dissolved in acetonitrile. EE appeared to be unstable in both acetonitrile and methanol. In acetonitrile, only 13% of EE remained intact after 168 h, whereas in methanol, the degradation was much more rapid with 35% of EE remaining after 28 h.

Keywords: Erythromycin; Erythromycin ethylsuccinate; Propionyl erythromycin; Erythromycin estolate; Stability; Degradation

The British Pharmacopoeia (1988) and the US Pharmacopeia (1990) recommend the use of methanol as a solvent for assay and identification tests of EB and its ester derivatives. Although the instability of erythromycin in aqueous acidic media has been well documented (Clark, 1953; Flynn et al., 1954; Wiley et al., 1957; Kurath et al., 1971; Amer and Takla, 1976; Atkins et al., 1986; Kibwage et al., 1987; Vinckier et al., 1989; Cachet et al., 1989) to our knowledge, data on the stability of erythromycin and its ester derivatives in organic solvents have not previously been reported. A recent report by Fiese and Steffen (1990) indicated a decrease in the degradation rate of EB in acidic aqueous media with increasing acetonitrile concentration when added to an aqueous medium. Systematic studies on the stability of EB, EES, PE and EE in methanol and acetonitrile, solvents of similar polarities (Connors, 1981) and densities

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(Merck Index, 1983) were thus undertaken. Any solvent effects, especially methanol, will obviously have wide ranging implications when such organic solvents are used to make up reference standard solutions and when used in assay procedures as recommended in the British Pharmacopoeia and US Pharmacopeia for erythromycin and its esters.

Solutions of EB, EES, EE (Sigma, St Louis, USA) and PE (Eli Lilly, Indianapolis, USA) in either methanol or acetonitrile were prepared, incubated at room temperature (20 ± 0.5°C) and erythromycin concentrations quantitatively determined by high-performance liquid chromatography (HPLC) over extended periods of time. The HPLC system consisted of a solvent delivery system (Model M112, Beckman Instruments, Inc., CA, USA), an automated sample processor (WISP, Waters Associated, MA, USA), a solvent degasser (Model ERC-3510, Erma Optical Works Ltd, Japan) an electrochemical detector (Coulochem Model 5100 A, E.S.A. Associates Inc., MA, USA) equipped with a Model 5010 analytical cell and a Model 5020 guard cell and a chart recorder (Model 561, Perkin Elmer Corp., IL, USA).

Separation of EES and EE was achieved on an HPLC column custom-packed with Techsil C_{18} (10 μm) packing material (25 cm × 3.9 mm i.d., HPLC Technology, Cheshire, UK) whilst Techsil C_8 (10 μm) packing material was used for the separation of PE and EE. The mobile phase consisted of either acetonitrile/0.05 M phosphate buffer (pH 6.3; 45:55) at a flow rate of 1.4 ml/min (EB and EES), acetonitrile/0.05 M phosphate buffer (pH 4.4; 35:65) at a flow rate of 1.3 ml/min (PE) or acetonitrile/0.05 M phosphate buffer (pH 6.3; 40:60) at a flow rate of 1.5 ml/min (EE). The electrochemical detector was used in the oxidative screen mode at a voltage of 0.85 V set at a gain of 1 × 10 whilst the guard cell was set at 1.00 V. The column temperature was maintained at 30 ± 0.5°C and the mobile phase was degassed through a membrane filter (0.45 μm, Millipore, MA, USA) prior to use. Oleandomycin phosphate (Pfizer Laboratories, Pietermaritzburg, South Africa) was used as the internal standard and the injection volume for all determinations was 20 μl.

In order to determine the concentrations of possible degradation products when present, anhydroerythromycin and erythromycin enol ether (Abbott Laboratories, North Chicago, USA) were incorporated into all the test solutions. Five replicate samples at four different concentrations of the calibration standards in acetonitrile were assayed by injecting 20 μl of each standard solution into the HPLC column and calibration lines established by plotting peak height ratios of the relevant compounds to internal standard vs concentration. Preliminary studies indicated EE to be unstable in acetonitrile (showing approx. 10% degradation over 12 h), hence these standards were frozen immediately after preparation and stored at −80°C until required. The calibration standards were allowed to reach room temperature prior to analysis. Freezing of the reference standards decreased degradation to less than 6% over 28 h.

The initial concentration of each test solution was analyzed within approx. 3 min of preparation. Samples were prepared in triplicate in each of the solvents, incubated at room temperature and samples withdrawn for analysis at appropriate intervals of time. The internal standard (oleandomycin phosphate in acetonitrile, 1 mg/ml) was added to each sample immediately prior to analysis. EB samples in either methanol or acetonitrile were withdrawn every 12 h over a period of 28 h and EES and PE samples in methanol at 90-min intervals over the same period of time. EES and PE samples in acetonitrile were monitored at 12-h intervals. EE samples were prepared and monitored in the same way as described for EES and PE.

Linearity of EB, EES, PE and EE was established over the range of concentrations investigated, typically 0.2–2.5 × 10^{-4} and 0.2–3.5 × 10^{-4} mol/l in methanol and acetonitrile, respectively.

System precision (%RSD) was established for each compound in methanol and acetonitrile following analysis of five replicates of each solution. Mean %RSD values for EB, EES, PE and EE in methanol were found to be 0.82, 4.34, 6.57 and 5.03, respectively, whilst the corresponding values for determinations of each compound in acetonitrile were 2.80, 0.91, 4.84 and 3.13, respectively.

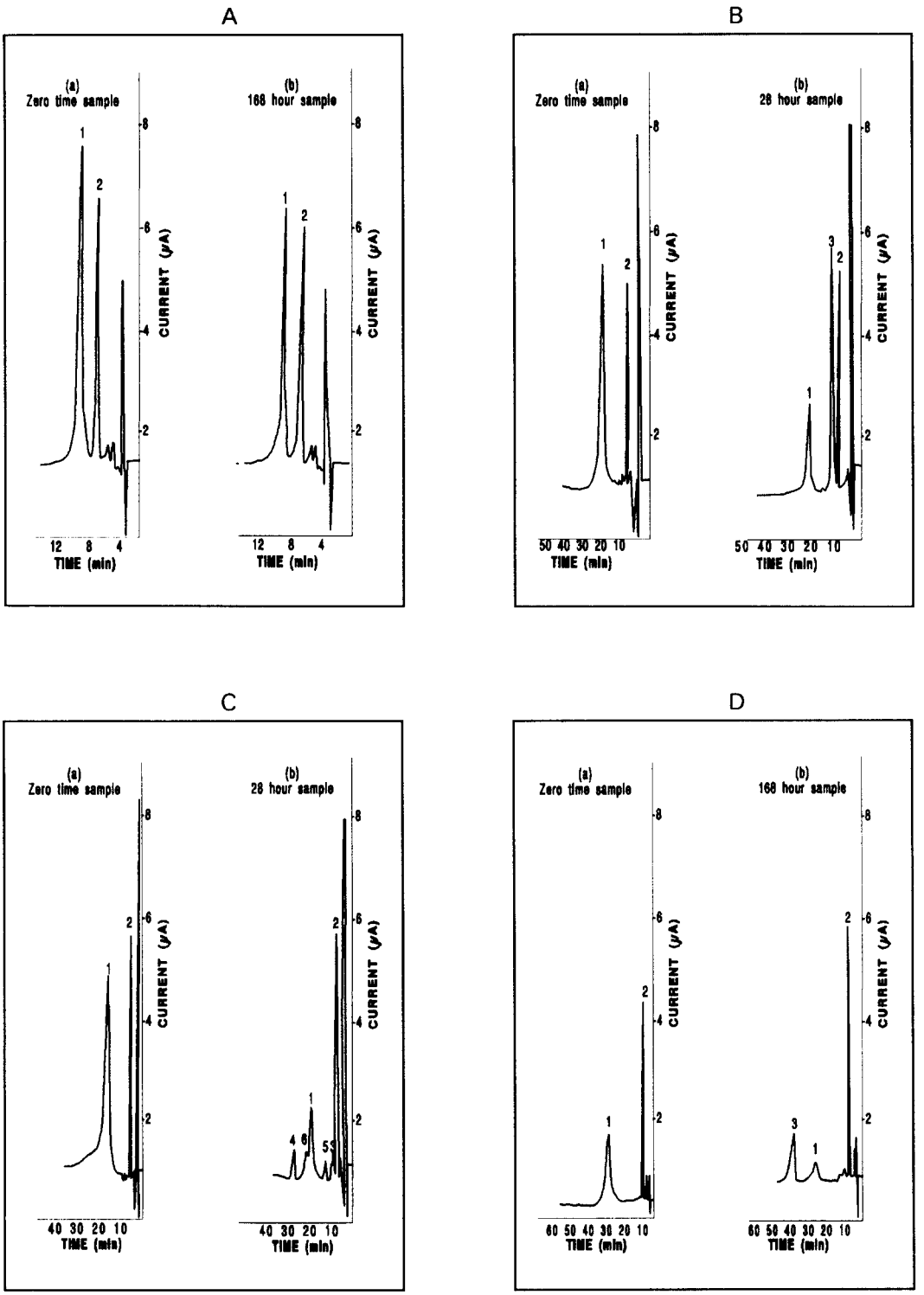

Fig. 1. HPLC chromatograms showing the separation of the various erythromycins and associated degradation products. (A) Peak 1, erythromycin base (EB); peak 2, internal standard; solvent, methanol. (B) Peak 1, propionyl erythromycin (PE); peak 2, internal standard; peak 3, erythromycin base (EB); solvent, methanol. (C) Peak 1, erythromycin estolate (EE); peak 2, internal standard; peak 3, erythromycin base (EB); peak 4, unknown compound; peak 5, anhydroerythromycin; peak 6, erythromycin enol ether; solvent, methanol. (D) Peak 1, erythromycin estolate (EE); peak 2, internal standard; peak 3, unknown compound; solvent, acetonitrile.

Erythromycin was stable in methanol showing no evidence of any degradation products up to 168 h following solution (Fig. 1A). Similar results were obtained for the stability of EB in acetonitrile (Fig. 2).

In methanol, PE undergoes solvolysis to form EB (Fig. 1B and 3) whilst remaining relatively stable in acetonitrile (Fig. 2). Approx. 65% solvolysis to EB occurred within 28 h whilst in acetonitrile more than 85% PE remained intact over 168 h. The rate of solvolysis calculated from the integral method (Taylor et al., 1985; Taylor and Shivji, 1987) was approx. 6.22×10^{-4} min^{-1}. Similarly, EES undergoes solvolysis to form EB whilst remaining stable in acetonitrile. EES showed rapid degradation in methanol resulting in almost complete solvolysis to EB, with less than 5% remaining after 40 h (Fig. 3). The rate constant calculated for EES in methanol was approx. 1.57×10^{-3} min^{-1}.

Erythromycin estolate was unstable in both methanol and acetonitrile (Fig. 1C and D). In acetonitrile, approx. 87% of EE degraded to an unknown compound over 168 h (Fig. 2). This reaction did not appear to be due to solvolysis, since no EB formation was observed (Fig. 1D). The degradation in methanol is more complex with 65% EE degrading in 28 h. Initially EB was formed and after 12 h the EB concentration decreased and an unknown compound was formed (Fig. 1C). This unknown compound differed from that formed in acetonitrile (Fig. 1D). After 12 h the concentration of the unknown compound increased at the expense of EE whilst the concentration of EB also increased. At approx. 20 h after initiation of the reaction, the concentration of EB once again decreased and at this time anhydroerythromycin was formed (Fig. 1C). Shortly thereafter, when most of EE had disappeared, erythromycin enol ether started to appear. From the above it seems that EB is an intermediate from which one or all of the degradation products are formed. The rates of EE

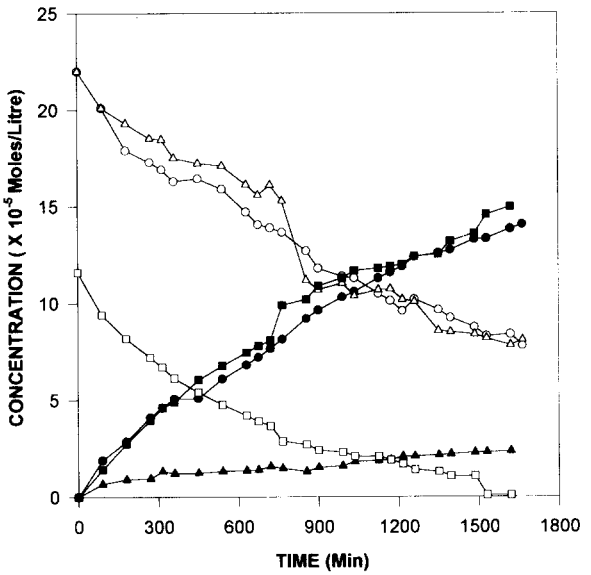

Fig. 3. Plot showing the stability of propionyl erythromycin, erythromycin estolate and erythromycin ethyl succinate in methanol. (○) Degradation of propionyl erythromycin (PE); (●) formation of erythromycin base from propionyl erythromycin; (△) degradation of erythromycin estolate (EE); (▲) formation of erythromycin base from erythromycin estolate; (□) degradation of erythromycin ethyl succinate (EES); (■) formation of erythromycin base from erythromycin ethyl succinate.

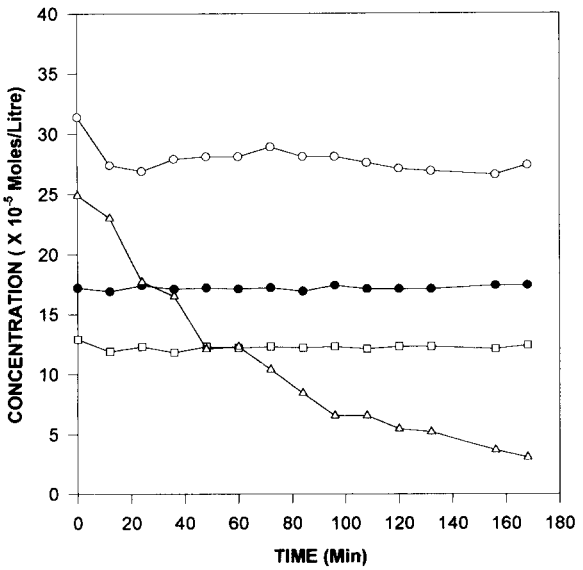

Fig. 2. Plot showing the stability of erythromycin base, propionyl erythromycin, erythromycin estolate and erythromycin ethyl succinate in acetonitrile. (●) Erythromycin base (EB); (○) propionyl erythromycin (PE); (△) erythromycin estolate (EE); (□) erythromycin ethyl succinate (EES).

degradation in acetonitrile and methanol were calculated and found to be 1.24×10^{-2} h^{-1} and 6.70×10^{-4} min^{-1}, respectively (Fig. 2 and 3).

It is thus evident that whilst erythromycin is stable in methanol, the esters are not, all of which showed extensive degradation in this organic solvent. Furthermore, the degradation appeared to follow different pathways to those proposed for the aqueous acidic degradation of these compounds (Atkins et al., 1986; Kibwage et al., 1987; Cachet et al., 1989; Vinckier et al., 1989). For both the ethylsuccinate and propionyl esters, the reactions appeared to be simple solvolysis with base formation. This is in agreement with the findings of Steffansen and Bundgaard (1989). However, erythromycin estolate was not studied by these authors. The estolate exhibited extensive and complex degradation and the reaction appeared to follow a reverse mechanism to that found in aqueous acidic solutions, with the anhydroerythromycin forming before the enol ether. Thus, degradation in methanol does not appear to be acid mediated.

These findings suggest that methanol should either not be used or used with caution as a solvent in assay and identification tests for EES, PE and EE, since the presence of this organic solvent causes degradation of the drug, which will result in erroneous conclusions. The US Pharmacopeia (1990) and British Pharmacopoeia (1988) recommend methanol as a solvent in their assay and identification tests for erythromycin and its esters and their salts. The use of methanol is thus contraindicated for making up standards of erythromycin esters for determination of their concentrations in bioavailability and pharmacokinetic studies. Since the analysis period for these studies usually involves a lengthy time period (up to and sometimes > 24 h), any standards made up in methanol would degrade over the analysis period resulting in inaccurate results.

Whilst only erythromycin was stable in methanol, the base, ethylsuccinate and propionate are stable in acetonitrile. However, the estolate exhibited instability in acetonitrile, although the rate of degradation was much slower than that found for this compound in methanol. Furthermore, the degradation pathway followed a different route to that found in either aqueous acidic media or in methanol, with the single unknown compound (differing to the unknown compound formed when this compound was placed in methanol) being formed.

We thus conclude that methanol should not be considered as the solvent of choice for PE, EES and EE, all of which showed instability in this organic solvent. It would appear that for the ethylsuccinate, propionate and the estolate, acetonitrile is a more suitable solvent. However, despite the preferred use of acetonitrile as a solvent for the estolate derivative, samples should be analyzed within 2 h in order to minimize degradation ($< 2\%$).

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