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Estimation of Erythromycin concentration In saliva of healthy volunteers

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Abstract

Erythromycin is an extensively used group of antibiotic medicines and dentistry. Clinical pharmacology for erythromycin in saliva was not clear until the time of this research. The evaluation was achieved by using suitable method (efficient, low cost, and reproducible). The purpose of this study is the determination of the erythromycin concentration in saliva.

Methods: Ten Subjects were given orally a single dose of 500 mg erythromycin very 6 hours. Samples 0.5 ml saliva was collected into centrifuge tubes at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, and 24 hrs after dosing. Samples were centrifuged and the superannuated were injected to HPLC using USP-27 method of assays (2).

Results: The results of this study indicated that erythromycin concentration in saliva ranged from 0.11-0.29 µg. / mL. The other pharmacokinetic parameters are: the mean concentration of erythromycin C max 0.27±0.08 µg /ml, AUC 0-∞ 217.25±9.25 µg. h/ml, and Tmax. 7.28 ± 0.14 hr. and T *1/2* 8.33±2.68 hr.

Conclusion: There was possibility to detection of erythromycin in saliva The HPLC method provided successful methods for monitoring the erythromycin with a detection limit reach 0.06 ng / ml. The analysis method is sensitive, reproducible, low cost and efficient for low concentration. The detection of erythromycin in saliva represents the distribution of the drug in saliva and indicated the suitability for erythromycin in treatments of dental and oral infections.

Key word: bio-bioavailability of erythromycin – concentration of erythromycin in saliva

Introduction

Erythromycin is a broad-spectrum macrolide antibiotic which possesses antimicrobial activity against gram-positive and a few gram negative micro organisms. [(1)] Its widely used in dentistry for treatment of oral infections such as pericoronitis, gingivitis, and chromatic conditions associated with secondary infections, especially for patients who are allergic

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to penicillin's. [2] Erythromycin is one of the drugs recommended by the American heart association for prevention of bacterial endocarditic in susceptible patients. [3] Erythromycin and its salts and esters are generally well tolerated and serious adverse effects are rare. Gastrointestinal disturbances such as abdominal discomfort and cramp, nausea, vomiting, and diarrhoea are fairly common after both oral and parenteral use, probably because of the stimulant activity of erythromycin on the gut. [4] Gastrointestinal effects are dose-related and appear to be more common in young than in older subjects. Supra-infection with resistant organisms may occur and pseudomembranous colitis has been reported. [5] All forms of erythromycin should be used with care in patients with existing liver disease or hepatic impairment, and the estolate is best avoided in such patients. [6] Repeated courses of the estolate or use for longer than 10 days increase the risk of hepatotoxicity. Erythromycin may aggravate muscle weakness in patients with myasthenia gravis. [7,8] Erythromycin and other macrolides have the potential to interact with a large number of drugs through their action on hepatic cytochrome P-450 isoenzymes, particularly CYP1A2 and CYP3A4. [9,10] Such interactions can result in severe adverse effects, including ventricular. [11]

Materials and methods

Reagents and Solutions:

All chemical reagents used in this study were either from Fluka or BDH companies suitable for HPLC analysis. Erythromycin 250 mg caps were obtained from SDI Company [1]

Weight variation, content uniformity, assay and dissolution studies were all carried out according to USP procedures [12].

Ten healthy Iraqi males were used, without any pathologic conditions. Their age ranged from 25-28 years. Each person was given 500 mg. of erythromycin caps every 6 hours for one day. Subjects were given orally a single dose of 500-mg tablets in a randomized fashion with 200 ml of water. Foods and drinks (other than water, which was allowed after 2 hours) were not allowed for 4 hours after dosing to all volunteers. Samples of approximately 0.5 ml saliva samples were collected into centrifuge tubes at (0 hr) and 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, 36 and 48 hr. after dosing. Participants were reminded for sample collection by cell phones calls. The centrifuge samples were centrifuged at 3000 rpm for 15 min; plasma samples were separated and kept frozen at -4 ◦C in coded glass tubes (12,13).

Chromatographic Conditions:

A reversed phase HPLC method was developed to quantities salivary levels of erythromycin. The chromatographic procedure may be carried out using a column (25 cm × 4.6 mm) packed with styrene-divinylbenzene copolymer (8 µm) with a pore size of 100 nm (PLRP-S is suitable), as mobile phase at a flow rate of 2.0 ml per minute a solution prepared in the following manner: to 50 ml of a 3.5% w/v solution of dipotassium hydrogen orthophosphate adjusted to pH 9.0 with 1M orthophosphoric acid add 400 ml of water, 165 ml of 2-methylpropan-2-ol and 30 ml of acetonitrile and dilute to 1000 ml with water and (c) a detection wavelength of 215 nm. Maintain the temperature of the column at 70° using a water bath for the column and at least one third of the tubing preceding the column. The flow rate is about 2 mL per minute [12].

Sample Preparation

300 µL of saliva from each sample was injected into a 5 ml test tube by using micropipette. For protein precipitation 100 µl of 10 % zinc sulfate was added, samples were vortexed, placed in refrigerator for 15 min and centrifuged at 3000 rpm for 15 min. Supernatant layer was separated of which 20 µL was injected onto the column and peak areas were recorded [(14)].

Results

The assays, dissolution as well as other USP investigations are listed in (table 1). The separation chromatogram of erythromycin is plotted in figure 1. The retention time of erythromycin was 5.88 min (Figure 1). This allows for the analysis of about 3 samples per h. the obtained chromatogram recorded broad peak of erythromycin. The retention time as well as the area under the curves is listed in (table 2). The method is highly sensitive, with the lower limit of quantitation of erythromycin at 0.05 µg/ml. The calibration curve was linear in saliva with a regression of r=0.99990. The concentrations of erythromycin in saliva were ranged from 0.11-0.29 µg/ml (table 3). Despite the fact that many modern HPLC methods have been described for the assay of erythromycin there is justification for investigating newer methods, especially where they are more cost-effective and there is an appreciable reduction in the procedural time compared to older methods. [(12)] It may be applied to both research and therapeutic drug monitoring as demonstrated by the concentration time curves (pharmacokinetic curve in Figure 2). While the pharmaco-kinetic parameters are shown in (table 4). In conclusion, the HPLC assay method developed in this study using a reversed-phase system was found to be simple, rapid, not expensive and sensitive for assaying erythromycin concentration as low as 0.06 µg/ml in saliva.

Discussion

All products met the pharmacopoeia specifications for, weight variation, assay, and. dissolution profiles (table 1). The dissolution test revealed that 88.5 ± 2.54 of stated erythromycin was released from SDI Iraq (n = 6). Therefore, either formulations met the USP dissolution specifications stating that not less than 80% of drug content should be released at dissolution time [(12)].

HPLC assay:

High performance liquid chromatography represent erythromycin can easily be detected after 5.88 minutes of capsules ingestion.

All chromatograms were free from any interference at the retention times of erythromycin, and both compounds were eluted completely and appeared as one big separate resolved peak with peak tailing followed with small one which could be related to the erythromycin isomers, it was possible to calculate peak height or peak area of standard curves. The retention times for erythromycin were 5.88 min. Linear relationships were found when the peak area ratios of erythromycin to the standard of erythromycin were plotted versus the erythromycin saliva concentration ranging from 0.11-0.29 (r2 = 0.99990). The use of external erythromycin standard to increase the accuracy of the assay whose availability is an important issue in HPLC assays and avoid interferences

of other internal standard such as capsules additive.

Erythromycin was well detected in saliva of all volunteers. This method was very simple for monitoring of erythromycin in saliva. The short time of analysis, simplicity and sufficient sensitivity makes the method particularly useful for pharmacokinetic and bioequivalent studies of erythromycin even following oral single dose (1 caps/6 hrs). Based on estimated pharmacokinetic parameters and statistical analyses [16]. These finding support our recommendations for using erythromycin in treatments of dental infections and oral soft tissues [17,19], practically , traumatic ulcers end even aphus ulcers associates with secondary infections [20-22]. In summary, a rapid, sophisticated and sensitive HPLC method is described for determination of erythromycin in human saliva.

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No.	Test	No of tabs	result
1	Wt. variation	20	3.35 %
2.	Disintegration time	6	4.12 min.
3.	Specification	6	Comply with USP
4.	Dissolution	6	88%
5.	assay	6	104 %

Table 1: The USP parameters of erythromycin capsules

Table 2: The chromatogram of erythromycin separation

Replicate	Peak area	Retention time (minutes)
1	26670420	5.92
2	26770554	5.93
3	25982575	5.89
Av.	26474516.333	

Table 3. The erythromycin concentration in saliva

Patients number	Concentration (µg/ml)
1	0.19
2	0.22
3	0.17
4	0.11
5	0.14
6	0.23
7	0.29
8	0.25
9	0.27
10	0.16

Table 4. The pharmaco-kinetic parameters for erythromycin .

Parameters	Test (mean ± SD)	P- values
C *max*. µg. /ml	0.27. ±0.08	0.187
AUC *0*-∞ (µg.h /ml)	217.25±9.25	0.782
T *max*.(hr.)	7.28±0.14	0.630
T *1/2* (hr.)	8.33±2.68	0.937

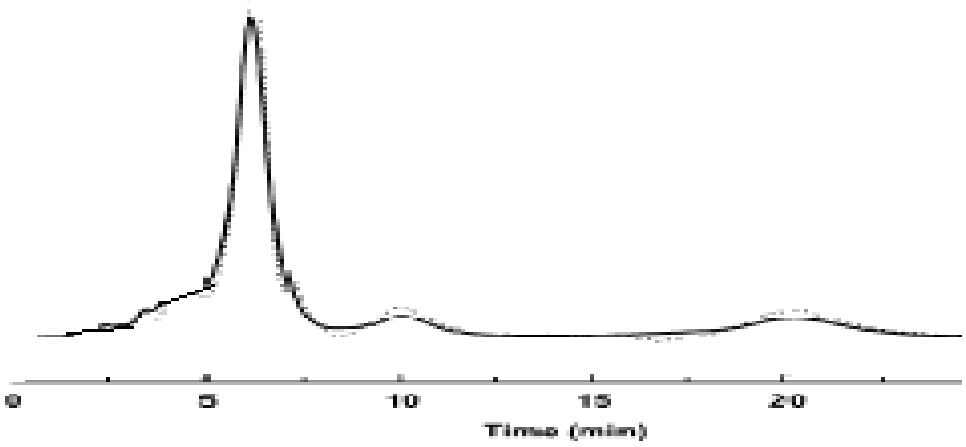

Figure 1 the erythromycin separation chromatogram in saliva.

Figure 2. Changes of erythromycin concentration with time.