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Chromatographic determination of salivary amoxicillin trihydrate concentration in healthy Iraqi volunteers

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Abstract

Amoxicillin have been widely described by dentists in Iraq, especially in treatment of periodontal and other oral bacterial infections. Amoxicillin is the 4-hydroxy analogue of ampicillin and is used in a similar variety of susceptible infections. Clinical pharmacology of amoxicillin in saliva was not sufficiently studied on normal subjects or patients required amoxicillin treatment. The purpose of this study is to determine the amoxicillin concentration in saliva by using an efficient, low cost and reproducible method. The obtained results from high performance liquid chromatography (HPLC) method revealed that salivary concentration of amoxicillin ranged from 0.22 to 0.44 µg. / ml. The HPLC method was proved to be a successful method for monitoring the amoxicillin in saliva with a detection limit reached to 0.08 µg/ml. It is important to suggest further study to know the therapeutic effect of amoxicillin through saliva against certain oral lesions.

Key word: bio-availability, concentration of amoxicillin in saliva. Chromatography of amoxicillin

Introduction

Amoxicillin is a semi-synthetic antibiotic and analog of ampicillin with a broad spectrum of bactericidal activity against many gram-positive and gram-negative microorganisms. (1) It is widely applied in dental treatments. Amoxicillin is white color, practically odorless crystalline powder, slightly soluble in water and in methyl alcohol; insoluble in carbon tetrachloride, in chloroform, and in benzene. pH of a 0.2% solution in water is between 3.5 and 6.0.(2) . The drug delivery system for this medication is based on the principles of muco-adhesion between gastric mucosa and drug containing polymer particles. The delivery system on reaching the stomach rapidly disintegrates into small particles. (3) Amoxicillin is resistant to inactivation by gastric acid. It is more rapidly and more completely absorbed than ampicillin when given by mouth. (4) Peak plasma-amoxicillin concentrations of about 5 micrograms/ml have been observed 1 to 2 hours after a dose of 250 mg, with detectable amounts present for up to 8 hours. Doubling the dose can double the concentration (5) . The presence of food in the stomach does not appear to diminish the total amount absorbed. Amoxicillin is metabolised to a limited extent to penicilloic acid which is excreted in the urine. About 60% of an oral dose of amoxicillin is excreted unchanged in the urine in 6 hours by

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glomerular filtration and tubular secretion [6] Urinary concentrations above 300 micrograms/ml have been reported after a dose of 250 mg. Amoxicillin is removed by haemodialysis. High concentrations have been reported in bile and some may be excreted in the faeces.

Amoxicillin is the 4-hydroxy analogue of ampicillin and is used in a similar variety of susceptible infections [7]. These include actinomycosis, biliary-tract infections, bronchitis, endocarditis (particularly for prophylaxis), gastro-enteritis (including salmonella enteritis, but not shigellosis), gonorrhoea, Lyme disease, mouth infections, otitis media, pneumonia, spleen disorders (pneumococcal infection prophylaxis), typhoid and paratyphoid fever, and urinary-tract infections[8]. The beta-lactamase inhibitor clavulanic acid widens amoxicillin's antimicrobial spectrum and a combined preparation (co-amoxiclav) can be used when resistance to amoxicillin is prevalent, for example in respiratory-tract infections due to Haemophilus influenzae or Moraxella catarrhalis *(*Branhamella catarrhalis)[9].

High performance liquid chromatography (HPLC) plays a crucial role in the analysis of pharmaceutical compounds. Separation of pure product from manufacturing impurities is necessary before a drug can be sold. [10] In recent years saliva has attracted much attention, in particular among people interested in the determination of drug concentrations, who suggest that saliva might be a substitute for plasma in the areas of pharmacokinetic studies and drug monitoring[11-12]. From this point of view this research was designed to estimate the Amoxicillin concentration in saliva by (HPLC). The HPLC method was applied to capsules to investigate the ability of using this method for evaluation of amoxicillin concentration in saliva. According to our knowledge very few similar researches were conducted to evaluate amoxicillin concentration in saliva till the time of writing this research and no specific method for determination of low salivary amoxicillin concentration has been developed.

Material and methods

Subjects, Reagents and Solutions

All chemical reagents used in this study were either from Fluka or BDH companies suitable for HPLC analysis. Amoxicillin 500 mg caps were obtained from Sinapharm weight variation, content uniformity, assay and dissolution studies were all carried out according to united state pharmacopeia (USP) procedures.[13]

Fifteen clinically healthy Iraqi male volunteers were participating in this study. Their age ranged from 24-26 years. Subjects were given orally a single dose of one 500mg capsule of amoxicillin in a randomized fashion with 200 ml of water. Food and drinks (other than water), were not allowed for 4 hours after dosing to all volunteers. Approximately 0.5 ml saliva samples were collected into centrifuge tubes at (0 hr) and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, 36 and 48 hrs after initial dosing. The samples were centrifuged at 3000 rpm for 15 min; samples were separated and kept frozen at -4 ◦C in coded glass tubes.[14]

Chromatographic Conditions

A reversed phase HPLC method was developed to estimate salivary concentration of amoxicillin. The apparatus was a Waters HPLC system (Waters, Ireland), consisting of a model intelligent solvent delivering pump, a computerized system controller, and a SPD-6AV UV

detector. Chromatographic separation was performed using L1 (4.0 mm ×25 mm, Waters, Ireland) column: Diluent: dissolved 13.6 gm. of monobasic potassium phosphate in 1000 ml of water and adjust with 45 % (W/W) solution of potassium hydroxide to a pH of 5.0±0.1. Mobile phase is prepared by filtration of suitable amount of mixture of diluent and acetonitrile (96:4) V/V. The liquid chromatograph is equipped with a 280-nm detector, a 4.0-mm × 25-cm L1 column that contains 5-µm packing L1, The flow rate is about 1.5 mL per minute.[11]

Sample Preparation

For protein precipitation 100 µl of 10 % zinc sulfate was added to 300 µl of saliva in a 5 ml test tube. Samples were vortexes, placed in refrigerator for 15 min and centrifuged at 3000 rpm for 15 min. Supernatant layer was separated of which 20 µL was injected into the column and peak areas were recorded [12] .

Results

The assays, dissolution and other USP parameters are listed in (Table 1). in which the retention time of amoxicillin was 10.42 min. This allows for the analysis of about 3 samples per hours. The obtained chromatogram recorded abroad peak of amoxicillin. The retention times as well as the area under the curves are listed in (Table 2). The method is highly sensitive, with the lower limit of quantization of amoxicillin at 0.08 µg/ml. The calibration curve was linear in saliva with a regressions of r=0.9997. The concentrations of amoxicillin in saliva were ranged from 0.22-0.44 µg/ml (Table-3). Despite the fact that many modern HPLC methods have been described for the assay of amoxicillin is very effective efficient, low coast and reproducible. This method is very effective to be applied to both research and therapeutic drug monitoring in biological fluids. The maximum concentrations as well as the minimum concentration in saliva are listed in table4. The dissolution profile is plotted in (Figure 1), while the separation chromatogram of amoxicillin is plotted in (Figure 2),

Discussion

In this study, however the determination of amoxicillin concentration in saliva was done, but its therapeutic effect in oral cavity against certain lesions is unknown, so further study is needed to evaluate the effect of amoxicillin against certain oral lesions through saliva [15]. Systemic amoxicillin can eliminate bacteria that cannot be removed mechanically by scaling, polishing and root planning, therefore it has been shown that amoxicillin can arrest bone loss and suppress Actinobacillus actinomycintemcommitance level in conjunction with scaling, polishing and root Planning [16].

Saliva is also considered as one of the components of the periodontal pocket so that saliva may enrich the pocket with additional amount of Amoxicillin and this may be beneficial in reduction of pathogenic microorganisms which are sensitive to Amoxicillin. [17]

Amoxicillin was well detected in saliva of all volunteers. This method is very simple for monitoring of amoxicillin concentration in saliva. The short time of analysis, simplicity, [18], and sufficient sensitivity makes the method particularly useful for pharmacokinetic and bioequivalent studies of Amoxicillin even following oral single dose (1 caps/6 hrs.). All products met the Pharmacopeia specifications for weight variation,

assay, and. dissolution profiles. The dissolution test revealed that 90 % of stated Amoxicillin was released effectively. Therefore, either formulation met the USP dissolution specifications stating that not less than 80% of drug content should be released at dissolution time [11].

HPLC included many pharmaceutical applications include analgesics, antibiotics, steroids, and experimental drug metabolites. The methods used were specific to each separation,. Several solvents and additives were used. Acetonitrile, buffer was the most commonly used components in the mobile phase. HPLC represent Amoxicillin which can easily detect after 6-8 hours of ingestion of its capsules. [19-21]

In conclusion all chromatograms were free from any interference at the retention times of Amoxicillin, and both compounds were eluted as completely and appeared as one separate resolved peak with peak tailing in such a way that it was possible to calculate peak height or peak area of standard curves. The retention times for Amoxicillin (10.42 min). A linear relationship was achieved between peak area and Amoxicillin concentration. The retention time, Amoxicillin standard and Amoxicillin concentration in saliva was similar .The Amoxicillin concentration in saliva was ranged from 0.20-0.44bµg/ml. The use of external Amoxicillin standard to increase the accuracy of the assay whose availability is an important issue in HPLC assays and avoid interferences with other internal standard

In summary, a rapid, non sophisticated and sensitive HPLC method is described for determination of Amoxicillin in human saliva. Addition of zinc sulfate followed by cooling allowed efficient detection of Amoxicillin in saliva by HPLC. The amoxicillin concentration ranged from 0.22-0.44 µg/ml.

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Table 1. The USP parameters of amoxicillin capsules

No.	Test	No of caps	result
1	Wt. variation	20	1.16 %
2.	Disintegration time	6	3.16 min.
3.	Specification	6	Comply with USP
4.	Dissolution	6	90%
5.	Assay	6	106 %

Table 2. The chromatogramic peaks area and retention time of amoxicillin trihydrate

Replicate	Peak area	Retention time(minutes)
1	233455	10.40
2	233460	10.44
3	233470	10.39
Av.	233490	10.42

Table 3: The amoxicillin concentration in saliva for each patient

Patients number	Concentration (µg/ml)
1	0.22
2	0.24
3	0.30
4	0.44
5	0.36
6	0.40
7	0.41
8	0.33
9	0.39
10	0.25
11	0.33
12	0.35
13	0.42
14	0.27
15	0.29

Table 4. The concentration range of amoxicillin in saliva

Parameters	Test (mean ± SD)
C *max*. µg. /ml	0.44 ±0.004
C *min*. µg. /ml	0.22±0.006

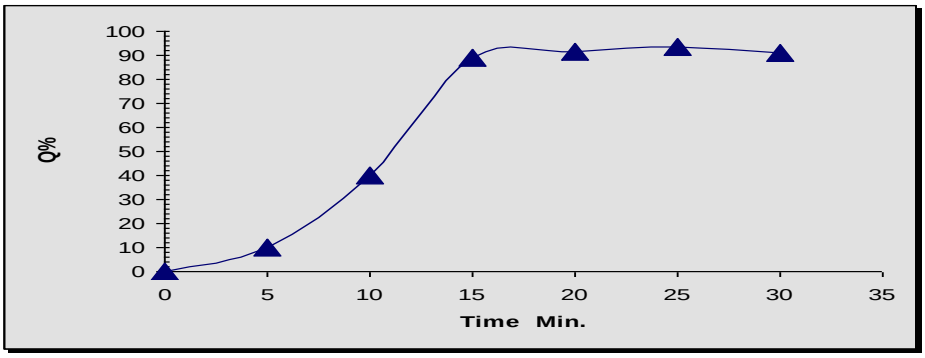

Figure-1: Dissolution profile of amoxicillin capsules

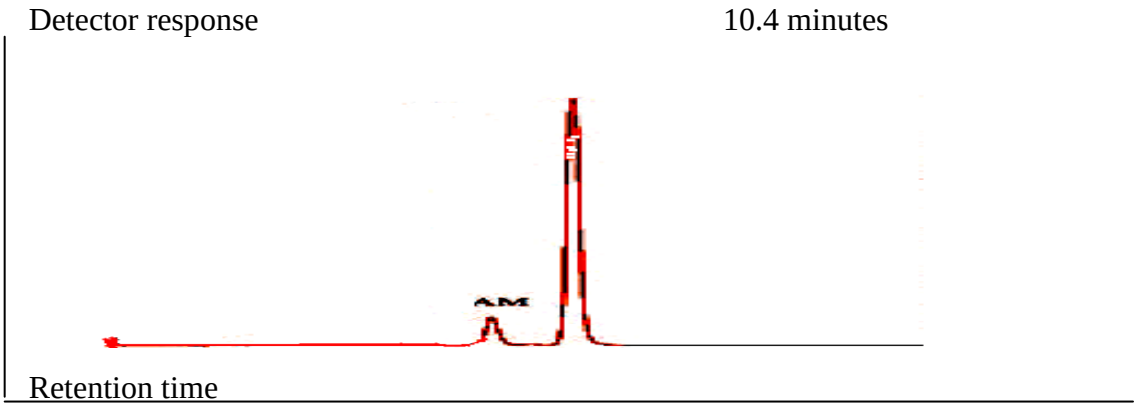

Figure 2 : The amoxicillin separation chromatogram in saliva.