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Therapeutic drug monitoring of digoxin in Jordanian patients: Comparing saliva versus plasma

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ABSTRACT: Therapeutic drug monitoring (TDM) is measuring drug concentrations in plasma, serum or blood, to assure that drug concentrations can be maintained within a target range and to make sure the amount of medicine being administered is both safe and effective. TDM is essential to avoid digoxin toxicity due to its narrow therapeutic window (therapeutic levels of digoxin are 0.8-2.0 ng/mL. The toxic level is >2.4 ng/mL). TDM of digoxin is currently performed by plasma sampling, which is an invasive and painful procedure. The aim of the study is to study the pharmacokinetics of digoxin in plasma and saliva matrices in hospitalized human volunteers; to suggest using non-invasive saliva sampling instead of plasma in monitoring drug's level. Plasma and saliva samples were collected for 20 patients. Plasma and saliva concentrations were determined by validated liquid chromatography mass spectrometry. Excel was used to determine the pharmacokinetics parameters, pbpk modeling was performed using PKSim version 10. Blood and saliva samples were collected from patients prior to taking each dose of digoxin and after; to compare plasma and saliva levels. As a result there was a significant strong correlation between saliva and plasma peak and trough concentrations. There was a significant relationship between gender (p-value 0.01) , weight (p-value 0.013), albumin (p-value 0.008), alanine transaminase (p-value 0.009)and aspartate transaminase (p-value 0.009) with digoxin salivary peak concentration. Creatinine had a strong correlation with saliva AUC values (r =0.5785, p = 0.008).To conclude saliva can be used as an alternative for plasma in monitoring digoxin's levels. This is the first study in digoxin therapeutic drug monitoring using saliva and physiologically based pharmacokinetic modeling.

KEYWORDS: Salivary excretion classification system; therapeutic drug monitoring; digoxin; correlation of concentration with biochemical parameters; pk-sim; saliva/plasma ratio of digoxin.

1. INTRODUCTION

Digoxin is a cardiac glycoside, and a derivative from the foxglove plant is found in digitalis lanata, also reported to be found in the south African plant Digitalis purpurea is traditionally used for treatment of congestive heart failure. Digoxin is digitoxin beta-hydroxylated at C-12. With the chemical formula of $C_{41}H_{64}O_{14}$ and molecular weight equals 780.9 g/mol. Digoxin is used in cases of congestive heart failure (CHF) and atrial fibrillation (AF). In congestive heart failure a lower therapeutic dosage is used due to the inotropic effect seen in digoxin in low doses. Patients of CHF show no benefit from increasing the digoxin dose. Therefore, the therapeutic range falls within 0.5-0.9 µg /L[1-3]. In atrial fibrillation, the purpose of using digoxin is rate control, higher doses are required. Therapeutic doses could range from 0.8-2.0 µg /L. When serum digoxin concentrations are higher than 2 µg /L, there are more negative effects of digoxin including those with the gastrointestinal system(anorexia, vomiting, nausea, diarrhea, constipation, abdominal pain), the central nervous system (headache, fatigue, insomnia, confusion, vertigo), visual disturbances symptoms, the cardiovascular system (atrioventricular block or dissociation, bradycardia, premature ventricular contractions, ventricular tachycardia), will show signs of toxicity if the serum concentration is 2.5 µg /L or above [1]. Digoxin poisoning can cause severe bradycardia, heart block, vomiting, and shock in patients. Hyperkalemia occurs in situations of acute severe poisoning.

Depending on the dosage form the bioavailability of digoxin is 100% for IV injection, 80% for IM, 90-100% for oral capsules, 63-75% for oral tablets and 75-80% for oral solutions [1,2]. Digoxin follows a two-

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compartment model starting in the plasma (small Vd) and then distributing into a larger Vd (tissues). The Vd is around 7.3L/Kg. However, it is affected by diseases and concomitant drugs used. Protein binding of digoxin is 20-30%, while it increases in patients with uremia and hypoalbuminemia [2]. Digoxin has renal clearance that almost equals CrCl. While the metabolic clearance is approximately 0.57 to 0.86 mL/kg/min. digoxin Cl is affected by diseases and concomitant drugs used [3]. 50 – 70% of Digoxin is excreted unchanged renally, via glomerular filtration with some tubular secretion. 30 – 50% of Digoxin is excreted non-renally, through biliary and intestinal tracts. The half-life ($t_{1/2}$) depends on age and renal function, it averagely ranges from 38-48 hrs for adults.

1.1. Therapeutic drug monitoring of digoxin

Digoxin is currently monitored through sampling plasma. Samples are obtained 12-24 hours after dose initiation a loading dose administration, 6 hours after the dose is given to make sure distribution is complete. And 3-5 days (3 to 5 days for digoxin to reach the steady state and to make sure the distribution is complete) without a loading dose. For monitoring of maintenance dose, a sample is collected after 5 to 7 days to ensure the drug has reached steady state. In oral doses samples are collected 30 minutes before next dose or 6 hours after administration, in IV doses right before next dose or after 4 hours. In patients with end stage renal disease, it may take 15-20 days for the steady state to be reached [4,5].

1.2. Salivary drug monitoring of digoxin

Based on the salivary excretion classification system (SECS) drugs with low permeability and high fraction unbound are classified as class II drugs. Class II drugs are excreted in saliva. Unbound digoxin fraction is 0.71 and effective intestinal permeability is in the range of ($0.24x10^{-4}$cm/sec _ $0.32x10^{-4}$ cm/sec) and based on the data above digoxin is classified as SECS class II drug. In this study, the feasibility of using saliva for digoxin therapeutic drug monitoring and the pharmacokinetics of digoxin in plasma and saliva matrixes in hospitalized human volunteers were investigated, to evaluate using non-invasive saliva sampling instead of plasma in monitoring drug's level [6-8]. This is the first study in digoxin therapeutic drug monitoring using saliva and physiologically based pharmacokinetic modeling.

2. RESULTS AND DISCUSSION

The clinical part was done at AlBashir hospitals with Institutional Review (IRB) from the Ministry of Health (MOH). A total of 20 patients (13 male and 7 female) were involved in this study. The mean age of the participants was (61.5). The weight range was (50-90) kg. Total weight was used in calculations of creatinine clearance. All patients were receiving digoxin therapy and hospitalized at the time of data collection. Informed consent was taken from all patients, IRB number Moh/REC/2022/72 and date of approval 24/03/2022. All sociodemographic and clinical data are demonstrated in Table 1.

The study entails gathering plasma and saliva samples from patients to measure the digoxinconcentration in both matrices after reaching steady state prior to each dose for trough concentration and 6 hours after dose administration for peak concentration. Several laboratory tests were also acquired such as creatinine, albumin and both alanine aminotransferase (ALT) and aspartate aminotransferase (AST) [9-20].

2.1 Digoxin's plasma concentrations

Plasma concentration of digoxin were measured in 20 patients at trough and peak concentrations, the trough concentrations were in the range of 1.12-2.3 ng/mL, the mean value was 1.40, standard deviation (SD) equals 0.50- and the coefficient of variation (CV-) equals 0.36. for the peak concentrations the range was from 1.2 to 2.15 ng/mL, the mean value was 1.5, standard deviation (SD) equals 0.5 and the coefficient of variation (CV) equals 32 %.

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Table 1. Sociodemographic and clinical characteristics of participants (n = 20)

	Age(years)	Weight (kg)	Gender	Creatinine (mg/dL)	ALT(U/L)	AST(U/L)	Albumin(g/l)	Creatinine clearance (mL/min)
1	69	81	M	0.791	9.8	34.7	3	100.9798
2	57	70	M	0.92	7.2	15.7	.	87.71135
3	69	80	F	1.07	10.1	31.3	37.02	62.66874
4	67	75	F	1.29	.	.	.	50.10497
5	61	80	M	0.305	13	.	23.1	287.796
6	70	90	M	1.02	16.9	11	.	85.78431
7	76	73	F	1.53	88.6	49.6	30.8	36.04938
8	54	60	M	0.57	24.6	45.7	42.6	125.731
9	75	70	F	1.59	6.6	14.3	30.1	33.78319
10	68	88	F	1.29	.	.	.	57.9845
11	17	50	M	11.26	13	14.3	.	7.58585
12	56	79	M	0.86	33.8	23.9	32.12	107.1705
13	62	65	F	0.75	18	10	.	79.80556
14	66	75	M	0.74	24.3	32.6	35.95	104.1667
15	35	55	M	0.76	14.5	19	34.8	105.5373
16	70	62	F	0.78	12.7	18.3	34.9	65.68732
17	57	90	M	0.76	5.7	.	23.2	136.5132
18	84	82	M	0.76	3.7	17.8	30.03	83.91813
19	59	90	M	0.59	11.8	12.1	37.3	171.6102
20	58	77	M	1.4	18.6	33.4	32.7	62.63889
SD	14.27	11.33		2.27	18.52	12.03	9.16	58.16

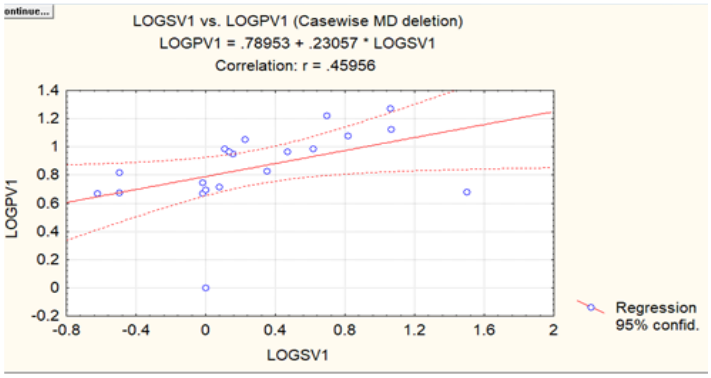

Figure 1. Correlation between trough plasma and saliva concentrations

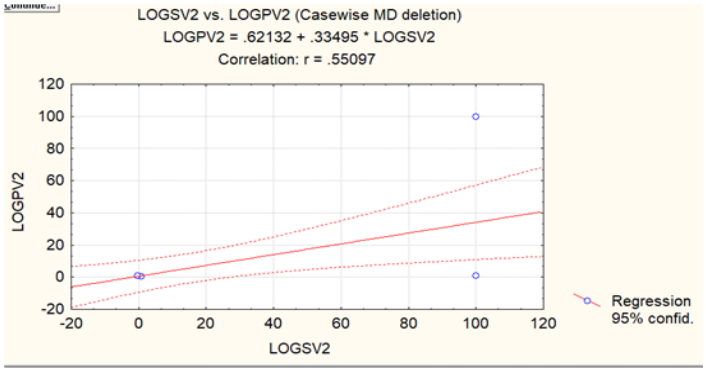

Figure 2. Correlation between peak plasma and saliva concentrations.

Table 2. AUC values for plasma and saliva and their log values

Patient Number	AUC Saliva	AUC Plasma	log AUC Saliva	log AUC Plasma
1	17.4	33	1.24	1.51
2	19.92	37.08	1.29	1.56
3	.	44.52	.	1.64
4	.	.	.	.
5	67.8	31.32	1.83	1.49
6	.	38.4	.	1.58
7	4.68	32.52	0.67	1.51
8	5.04	35.76	0.70	1.55
9	5.16	41.28	0.71	1.61
10	14.64	34.8	1.16	1.54
11	34.44	50.76	1.53	1.70
12	119.64	28.8	2.07	1.45
13	10.8	31.32	1.03	1.49
14	6.48	34.08	0.81	1.53
15	.	0.00	.	.
16	4.8	37.68	0.687	1.57
17	.	.	.	.
18	.	.	.	.
19	6.00	30	0.77	1.47
20	2.76	26.88	0.44	1.42
SD	31.64	10.15	1.5	1.00

. no data

The AUC for plasma and saliva samples was calculated and transformed to logarithmic values as seen in Table 2, then the correlation was measured with the r and p values to also give a significant, strong correlation (r =0.5785, p = 0.008) shown in Figure 3.

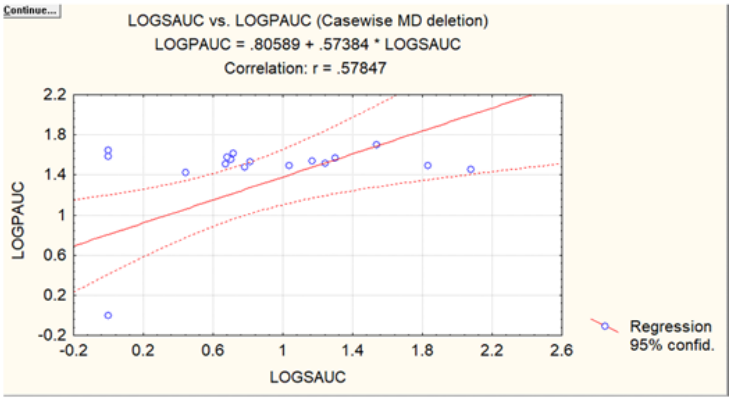

Figure 3. Correlation between AUC plasma and saliva concentration.

The ratios for both peak and trough concentrations for saliva and plasma were calculated as seen in Table 3. The theoretical ratio for digoxin was 0.705. As seen in Table 3 the actual normal saliva range was 0.304-1.446 µg /L [33-38].

2.2. Digoxin's saliva concentrations

Saliva concentration of digoxin were measured in 20 patients at trough and peak concentrations, the trough concentrations were in the range of 0.06-7.9 ng/mL, the mean value was 0.83, standard deviation (SD) equals 1.75 and the coefficient of variation (CV) equals 2.11. For the peak concentrations the range was from 0.12 to 5.13 ng/mL, the mean value was 1.07, standard deviation (SD) equals 1.36 and the coefficient of variation (CV-) equals 1.26.

Table 3. Peak and trough plasma and saliva concentrations and their ratios.

Patient no.	Saliva min	Saliva max	Plasma min	Plasma max	S/Pmin	S/Pmax
1	0.24	1.21	1.17	1.58	0.20	0.76
2	0.56	1.1	1.68	1.41	0.33	0.78
3	1.46	.	1.66	2.05	0.87	.
4	1.44	.	2.33	.	0.61	.
5	0.52	5.13	1.21	1.4	0.42	3.66
6	0.82	.	1.5	1.7	0.54	.
7	0.18	0.21	1.12	1.59	0.16	0.13
8	0.3	0.12	1.29	1.69	0.23	0.07
9	0.21	0.22	1.41	2.03	0.14	0.10
10	0.06	1.16	1.17	1.73	0.05	0.67
11	0.62	2.25	2.08	2.15	0.29	1.04
12	7.9	2.07	1.2	1.2	6.58	1.72
13	0.16	0.74	1.21	1.4	0.13	0.52
14	0.25	0.29	1.24	1.6	0.20	0.18
15	.	.	0	0	.	.
16	0.08	0.32	1.64	1.5	0.04	0.21
17	0.24	.	1.39	.	0.17	.
18	0.34	.	2.3	.	0.14	.
19	0.37	0.13	1.15	1.35	0.32	0.09
20	0.08	0.15	1.18	1.06	0.06	0.14
Mean	0.83	1.07	1.39	1.49	Average S/Pmin: 0.60	Average S/Pmax: 0.72
SD	1.71	1.33	0.48	0.46	1.42	0.93
	Normal plasma range: 0.5-2 µg /L				Normal saliva range: 0.304-1.446 µg /L	

. no data, data below 1 ng/ml were shown for presentation and calculation purposes.

2.3. Relationship between plasma and saliva concentrations of digoxin

All the data obtained for saliva and plasma were converted into logarithmic values to normalize the original data. Using the program Statistica (v.4.5) the correlation (r) was measured along with the p-value, giving a significant, strong correlation for trough plasma and saliva concentrations, r and p values were 0.4596 and 0.041, respectively. Figure 1 below shows the correlation mentioned. For peak plasma and saliva concentrations there was also a significant, strong correlation with r and p values of 0.5510 and 0.012, respectively, as seen in Figure 2.

2.4. Digoxin saliva concentration and weight and gender

Several parameters were studied for correlation to observe the effect they have on digoxin concentration in either saliva or plasma or both. It was noticed that both weight and gender showed a significant correlation with digoxin's maximum saliva concentration with p-value 0.01 and 0.013, respectively .

2.5. Digoxin saliva concentration and liver function tests

When studying the correlation between maximum salivary concentrations of digoxin and alanine transaminase (ALT), a strong correlation was found(P value 0.009). The correlation with aspartate transaminase (AST) was also strong (P value 0.009).

2.6. Digoxin saliva concentration and albumin

Albumin was also correlated with salivary concentrations of digoxin and was found to have a strong correlation with the maximum salivary concentrations (P value 0.008).

2.7. Digoxin saliva and plasma concentration and creatinine

There was a significant but weak correlation between creatinine and digoxin in both plasma and saliva minimum and maximum concentrations; for saliva, the p values were 0.002 regarding the minimum concentration and 0.028 regarding maximum concentration, and for plasma, p values were 0.026 and 0.047 regarding the minimum and maximum concentrations, respectively.

2.8. Digoxin concentration and other parameters

Several other parameters were correlated with digoxin concentration at trough and peak concentrations for saliva and plasma to find any relationship with digoxin concentration. However, no significant relationships were found with the other parameters studied such as age and creatinine (Table 4) [25-43].

Table 4. Digoxin peak and trough plasma and saliva concentration p-value.

	Saliva $_{min}$	**Saliva $_{max}$**	**Plasma $_{min}$**	**Plasma $_{max}$**
Age	0.682	0.080	0.092	0.409
Weight	0.564	0.01 (significant)	0.820	0.910
Gender	0.885	0.013(significant)	0.700	0.648
Cr	0.002(significant)	0.028(significant)	0.026(significant)	0.047(significant)
ALT	0.892	0.009(significant)	0.207	0.306
AST	0.934	0.009(significant)	0.763	0.344
ALB	0.898	0.008(significant)	0.216	0.671

3. CONCLUSION

Based on this study, the following conclusions were made: There was a significant strong correlation between saliva and plasma peak and trough concentrations. Saliva can be used as an alternative for plasma in monitoring digoxin's levels. Gender and weight has an effect on maximum concentration of digoxin in saliva.. There was a significant relationship between gender, weight, albumin, alanine transaminase (ALT) and aspartate transaminase (AST) with digoxin salivary peak concentration. Creatinine had a strong correlation with saliva and plasma minimum and maximum concentrations. Digoxin's concentration is not affected by other parameters. Other clinical and sociodemographic parameters had no significant effect on salivary trough concentration of digoxin and both peak and trough plasma concentrations. This is the first study in digoxin therapeutic drug monitoring using saliva and physiologically based pharmacokinetic modeling.

4. MATERIALS AND METHODS

4.1. Chemicals and reagents

The method required digoxin as the standard analyte. Reagents used were purified water, HPLC/SPECTRO grade methanol and acetonitrile, HPLC grade tertiary butyl ether and analytical reagent grade dimethyl sulfoxide. Apparatus used included micropipettes of different volumes (10-100µL, 20-200µL, and 100-1000 µL), a vortex mixer (IKA) for 36 samples, an Eppendorf centrifuge 5810 R, RAD WAG (4Y) and Mettler Toledo analytical balances, a freezer at -20^{o}C, a refrigerator at 2-8^{o}C, a synchronized clock timer, a dual-timer clock, an Eppendorf dispenser (1-5), and a stable temperature water bath. The LC-MS/MS components used in the analysis include an Agilent 1260 series pump and auto-sampler, an API 5500 Applied Biosystems, MDS SCIEX detector, USA. The chromatographic separation was achieved by an ACE C_{18} 5µmx 4.6 x 100 mm analytical column.

4.2. Chromatographic Conditions

The chromatographic parameters were set at a flow rate of 0.60 mL/min, a column temperature of 25^{o}C, an auto-sampler temperature of 5^{o}C (0.2), an injection volume of 15.0 µL, and a total run time of 3.0 min. the Digoxin was detected by MRM transition 779.50> 649.40. The retention time was 1.9 minutes.

4.3. Preparation of stock, intermediate and calibrator solutions

For the preparation of the stock solution 25.0 mg of digoxin are accurately weighed and dissolved into 2 mL of dimethyl sulfoxide and the volume is completed to 25 ml with methanol. The solution is mixed using the vortex. The resulting concentration is 1.0 mg/mL of digoxin. For the working solution, From the standard solution prepared 2500.0 µg is added into 50 mL of acetonitrile (ACN): water (H_2O), v:v 50:50%. Mixed with vortex, resulting in the concentration of 10.0 µg/mL of digoxin. To prepare the calibration standards from the working solution (10.0 µg/mL) (diluent:(H_2O): ACN v/v, 50%:50%) resulting in 8 calibration standards with the concentrations (10.0, 20.0, 100.0, 200.0, 400.0, 600.0, 800.0, 1000.0) ng/mL. The

mobile phase is then prepared by mixing of acetonitrile with ammonium formate buffer in the ratio of 70:30 %) and shaken well.

4.4. Preparation of quality control samples

To prepare the QC solutions from the 10.0 µg/mL working solution 50:50 (v/v) mixture of H_2O: ACN was used resulting in low, mid, high QC standards with concentrations of 30, 450, 750 ng/mL (, respectively. Both the calibration standards and the quality control samples are prepared by spiking blank plasma/saliva with working solutions containing the analytes. Spiked plasma/saliva QC samples should be stored with the samples to be analyzed at the same storage conditions.

4.5. Extraction of digoxin from plasma and saliva samples

Extraction of digoxin is done for both plasma and saliva by pipetting 270 µL of blank plasma/saliva /300µL of spiked plasma into pre-labelled tube, adding 30.0 µL of calibration curve solution into blank plasma/saliva, vortex the samples for about 10 sec, dispensing 5.0 mL of tertiary methyl ether, then vortex the samples for 2.0 min and centrifuge the samples at 4000 rpm for 5.0 minutes, at 25°C. Decant the organic layer into another labeled clean test tube, evaporate the solvent under a stream of compressed air in water bath at 35°C; this step should be conducted in fume hood. Reconstitute the samples with 200 µL mobile phase and vortex about 1.0 min, transfer the supernatants to the auto sampler vial insert, to analyze in LC-MS/MS.

4.6. Limits of quantitation (LLOQ)

According to the US FDA bioanalytical method validation guidance; the analytical method was developed and validated to measure the lowest concentration that can be identified with appropriate precision and accuracy. The precision of these limits was less than 20%, with the LLOQ in both plasma and saliva being 1.0 ng/mL

4.7. Selectivity and specificity

There were no interferences at the retention time of both digoxin and the internal standard. The peaks were in good shape, completely resolved from the plasma and saliva components. The matrix peak was less than 5% of the peak area of the internal standard, which is acceptable per the US FDA guidance.

4.8. Data analysis

Physiologically based PK-SIM®, modelling of digoxin: PK-sim®, a software that can simulate physiologic based pharmacokinetics of the body, PK-sim has been showed out to be of significant help in preclinical and clinical research for research and development companies. PK-sim® has been used to simulate a model regarding digoxin in cases of a single oral dose and the chemical and physical properties used are mentioned in table 5. Based on the previous data input in the program (PK-sim) the following results were obtained. Figure 4 shows both the Pk-sim® simulation along with the observed data, the results show very little differences**.**

Table 5. Physical and chemical properties of digoxin [1,3,9,33].

Parameter	Literature	Unit
Molecular weight	780.9	g/mol
Lipophilicity	1.26	Log Units
Solubility	64.8	mg/L
Fraction unbound	.71	%
Intestinal permeability	2.67E-07	dm/min
Partition coefficients	Rodgers and Rowlands	
Cellular permeabilities	1.01E-05	dm/min

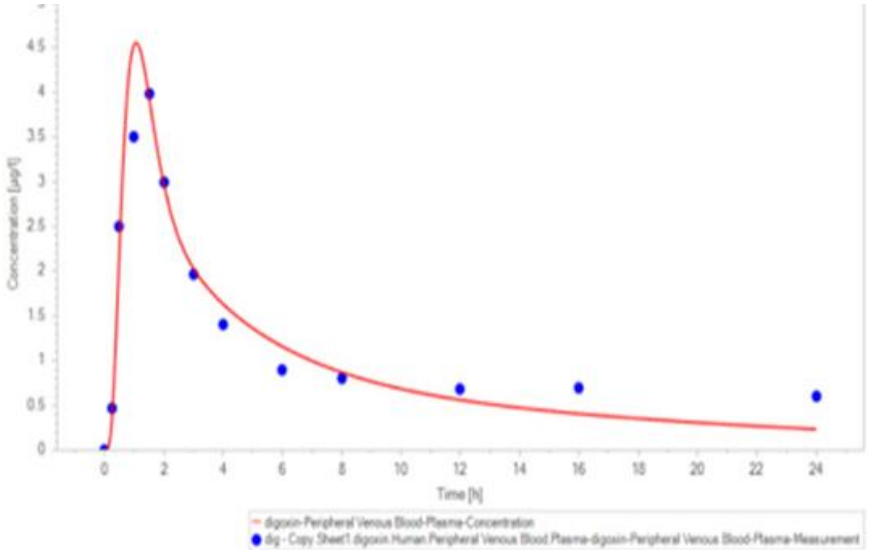

Figure 4. PK-sim simulation and observed data of digoxin concentration in plasma vs time.

4.9. Pharmacokinetic calculation

The equations used for calculating the average concentration (Cp) and area under the curve (AUC) are [1]:

$$Cp = \frac{C_{min} + C_{max}}{2}$$

$$Cp = \frac{F*D}{Cl} = \frac{AUC}{*\ tau}$$

$$AUC = Cp* tau$$

The theoretical ratio between saliva and plasma concentrations are calculated using the following formula

$$S/P \text{ (basic drug)} = \frac{((1+10(pKa-pHs))*Fup}{((1+10(pKa-pHs))*Fus}$$

Where pH for the plasma=7.4, for saliva=6.9, fraction unbound for plasma 0.71, for saliva=1
The pKa used was 13.5 and -3 then the average for both was taken giving the final answer 0.705. The creatinine clearance was calculated using the Cockcroft-Gault equation without adjustment for ideal body weight.
((140 – age (yr)) * weight (kg)) / ((72* serum creatinine (mg/ dL)) * (0.85 female)).

4.10. Statistical calculation method

The results of this study were achieved by using excel for the calculation of the mean, the standard deviation, coefficient of variance, distributive statistics, and ratio statistics. Systat version 5 was used for the ANOVA and Statistica version 4.5 was used for the correlation analysis and the calculation of the r and p values[21-26].

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