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Research Article

A STUDY OF THE POSSIBLE EFFECT OF POMEGRANATE JUICE ON THE PHARMACOKINATICS OF CANDESARTAN IN RAT PLASMA BY USING A BIOANALYTICALMETHOD- LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY (HPLC/MS/MS)

Ahmed Al-Kawaz[1], Wael Abu Dayyih[1*], Eyad Mallah[1], Mohammed Hamad[2], Tawfiq Arafat[1]

[1]Department of Pharmaceutical Medicinal Chemistry and Pharmacognosy, Faculty of Pharmacy and Medical Sciences University of Petra /Amman- Jordan.

[2]Department of Basic Sciences, Collage of Sciences and Health Professions, King Saud Bin Abdulaziz University for Health Sciences - Jeddah, Saudi Arabia.

Email: wabudayyih@uop.edu.jo

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Abstract

Background: drug interactions with fruit juices (beverages) have received considerable attention from basic scientists, physicians, and industry and drug regulatory agencies. The aim of this study is to develop a new simple, rapid and sensitive method for validation and determination of candesartan in the presence of pomegranate juice. **Methods:** Aspecific LC-MS/MS method, the chromatographic conditions comprised of a reversed-phase RP ACE 5 C18 Column (50 X 2.1 mm), 5µ with a mobile phase consisting of a mixture of Methanol: Formic Acid (50:50 v/v) with flow rate 1 ml/min. The detection was carried out at 269 nm, with retention time of 1.4 min. **Results**, the highest influence on Candesartan concentration was found when it was taken after pomegranate. The standard curve was linear over the range of (10 to 1000) µg/ml with correlation coefficient of the linear calibration graphs for the analytics exceeded (R=0.997). The Candesartan concentrations were successfully estimated at a pH of 2.5. **Conclusions:** Asimple and sensitive method was developed and validated for a qualitative and quantitative analysis of Candesartan and the influence of certain beverages used traditionally in Jordan market.

Key Words: Hypertension, LC-MS/MS, Candesartan, Validation, Pomegranate, Pharmacokinetic.

Introduction

In life Medicines are used to treat health problems. Nevertheless, it must be taken accurately to ensure their efficacy and safety. Diet and beverages can sometimes have a significant impact on drugs. Recently, drug interactions with fruit juices (beverages) have received considerable attention from basic scientists, physicians, and industry and drug regulatory agencies. This interaction can affect the activity of a drug; such effects are either increased or decreased,

or even a newer effect that neither produces on its own. Interaction occur because a lot of juices shown to inhibit cytochrome P-450 enzymes. The interaction can affects the activity of a drug, or modulate intestinal drug absorption via the P-glycoprotien mediated efflux and organic anion-transporting polypeptide (OATP) mediated uptake transport systems in the intestine and liver [1], and so are considered to be responsible for alterations in drug bioavailability, pharmacokinetic and pharmacodynamic changes when drugs are ingested concurrently with juices[2] or beverages [3].Therefore, the combination of different juices with cardiovascular medications may pose a health risk, especially in elderly patients. A number of studies have shown interactions of fruit juice with cardiovascular drugs are likely to cause a change in the pharmacokinetics and pharmacodynamics of these drugs, consequently causing undesirable health effects. Thus, health care professionals and the public need to be advised of the potential risks associated with the concomitant use of fruit juices and interacting medications, especially cardiovascular drugs and agents with a narrow therapeutic index [4].

Candesrtan

Chemically, candesartan is 2-ethoxy-3-[[4-[2-(2H-tetrazol-5 yl) phenyl] phenyl] methyl] benzimidazole-4-carboxylic acid (Figure 1) [5, 6].

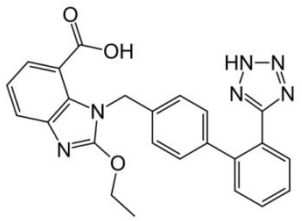

Figure 1: Candesartan chemical structure

Candesartan chemical formula is $C_{24}H_{20}N_6O_3$, with Clearance of 0.37 mL/min/kg, and volume of distribution of 0.13 L/kg and bioavailability of 15% (candesartan cilexetil) and it is highly bonded to the binding proteins. Candesartan is administered as candesartan celextil (CC) which has better availability than candesartan; the prodrug is rapidly and completely hydrolyzed to candesartan during absorption by the gastrointestinal tract.

It belongs to the class of ARBs and binds to angiotensin II receptor type1 selectively and competitively, thus preventing action of angiotensin II and decreasing the blood pressure levels [7]. Candesartan is metabolized by CYP2C9, UDP-glucuronosyl transferase and Prostaglandin G/H synthase [8].

Pomegranate:

Pomegranate (*Punica Granatum*) belongs to the family "Lythraceae"and made its way into the news recently due to its huge reported benefits [9]. Its original native is Persia, and is cultivated in North Africa, Asia and especially in the

Middle East [10]. It can be consumed fresh or transformed into fresh juices, beverages, jellies and as flavoring or coloring agents [11]. Pomegranate considerd as a source of many beneficial compounds; it contains a potent antioxidant of the polyphenolicxavonoid class, which includes tannins and anthocyanins, [12, 13]. Another impressive antioxidant that only founded in pomegranate is punicalagins that can lowers cholesterol level, blood pressure and increase the speed at which heart blockages (atherosclerosis) melt away [14, 15].

As previously mention pomegranate juice has reduce blood pressure, by reducing systolic blood pressure and inhibits serum angiotensin converting enzyme [14]. Also it was found that pomegranate can inhibit CYP3A in the body, thus it will alter the pharmacokinatics of any drug metabolized by this enzyme [16]. Recently, we illustrate that there was a significant increase in the metronidazole pharmacokinetic parameters (Cmax and AUC, $P<0.05$) after pretreatment with multiple dose of pomegranate juice [17].

Experimental Part

Chemicals and Reagents

The pure drug CC was obtained from Jordanian Pharmaceutical Manufacturing Co., Amman, Jordan. Irbesartan (rawmaterial) (Nanopure (Fisher Scientific). Methanol advanced gradient grade (Fisher scientific, B# 1155904). Acetonitrile advanced gradient grade (Fisher scientific, B# 1156250). Formic acid advanced gradient grade (GPR Rectapur, B# 07L210512). Dimethyl Sulfoxide DMSO (Tedia, B# DR0469-001), Sodium hydroxide powder (GPR , B# B0057050) were purchased from the local agent and were of analytical grade, Rats, Plasma, (harvested from Rats). Freshly prepared liquorice juice, freshly squeezed pomegranate juice, and freshly squeezed orange juice

Instrumentation

An API Mass spectrometer was used and composed of the following: On-line vacuum Degasser (Agilent 1200), Solvent delivery systems pump (Agilent 1200). Autosampler (Agilent 1200). Thermostat column compartment (Agilent 1200). API 3000 Mass Spectrometer, ACE 5, C18 (50 x 2.1 mm), 5µm. Computer System, Windows XP, SP3, Data Management Software 1.5.2 Other instruments include: Bath Sonicator Crest model-175T (Ultra Sonics CORP.), Sartorius balance BP 2215, Sartorius pH meter (Professional meter PP-25), Centrifuge (eppendorf 5417C).

Experimental:

All animal experiments were performed in compliance with FELASA guidelines (Federation of European Laboratory Animal Science Association) and the study protocol was approved by the Research Committee (No. 5, January 30/2013) at the Faculty of Pharmacy, University of Petra, Amman, Jordan. Adult male Sprague Dawley laboratory

rats were supplied by the animal house of Petra University. The Average weight of rats was approximately 220.0g, and they were in healthy condition. They were placed in air-conditioned environment (20-25°C) and exposed to a photoperiod cycle (12hours light/ 12 hours dark) daily.

Methods

Preclinical study design

After preparation of candesartan solution, the rats received a certain amount of candesartan solution; this amount is to be calculated according to their weights, the solution was given by oral cavage. The rats were divided into 20 groups, every group contain an average of six rats, eight groups received candesartan only, six groups received candesartan with liquorice, other three groups received candesartan with orange while the last three groups received candesartan with pomegranate. Pomegranate juice was given to the rats as multiple doses, before the administration of the drug, by oral cavage. Each rat had been weighed then the tip of the tail has been cut and a few drops of blood were taken in eppendorff tube, then the rat received the drug solution orally, and after giving the dose of drug to the rat a few drops of blood were taken after 30 min and 1, 2, 3, 4 and 6 hours of administration.

Blood samples were centrifuged for 10 minutes to get the plasma required for the analytical process.

Preparation of Stock Solutions

Preparation of Candesartan Solution to be given to the rats

22 mg of candesartan raw material dissolved in 3 ml of DMSO then the volume were completed to 100ml with distilled water.

Preparation of stock solution of Candesartan:

Weight equivalent to 10.00 mg of candesartan working standard was dissolved it in 10 ml of Methanol to get concentration 1000 µg/ml stock solution of candesartan.

Preparation of stock solution of Irbesartan Internal Standard:

Weight equivalent to 10.0 mg of Irbesartan working standard was dissolved it in 10 ml of ACN to get concentration of 1000.0 µg/ml stock solution of Irbesartan.

Preparation of working solutions:

Preparation of working solution of Irbesartan I.S:

200 µl from Irbesartan stock solution (1000.0µg/ml) were taken and diluted it to 100 ml of ACN as I.S working solution (B-IS) of concentration 2.0 µg/ml Irbesartan.

Preparation of working solution for candesartan:

200.0 µl from 1.0 mg/ml stock solution was added to 10.0 ml of 1:1 water/methanol in volumetric flask to obtain 20.0 µg/ml working solution.

Preparation of Candesartan serial spiking samples in plasma:

Samples of standard curve in plasma were prepared by spiking 100.0 µl from serial solution into 10.0 ml of plasma, using seven concentrations, not including zero to obtain standard concentrations of: 10, 25, 75, 250, 400, 600, and 1000 ng /ml for Candesartan in plasma (Table 1). Each concentration of the plasma sample was divided to 25 µl in 1.5 ml eppendorf tube and kept at (-30˚C), standard samples were given daily together with the quality control samples.

Table-1: Serial Spiked Plasma Samples.

Serial solution of Candesartan from working solution of 1000 µg/ml					**Plasma spiking solution**			
Solution No:	Working Solution Conc. (µg/ml)	Stock Conc. (µg/ml)	Volume taken from stock (µl)	Total Volume (ml)	Cal ID	Volume taken from w.s (µl)	Total Volume (ml)	Final concentration (ng/ml)
1	0.4	1000	4	10	S1	25	1	10
2	1.0	1000	10	10	S2	25	1	25
3	3.0	1000	30	10	S3	25	1	75
4	10.0	1000	100	10	S4	25	1	250
5	16.0	1000	160	10	S5	25	1	400
6	24.0	1000	240	10	S6	25	1	600
7	40.0	1000	400	10	S7	25	1	1000

Preparation of Candesartan Quality Control Samples in plasma:

Samples of QC in plasma were prepared by spiking 100.0 µl from serial solution into 10.0 ml of plasma to obtain QC concentrations of: 30, 50 and 800 ng /ml for Candesartan in plasma (Table 2).

Table-2: QC Spiked plasma samples.

Serial solution of Candesartan from working solution of 1000 µg/ml					**Plasma spiking solution**			
Solution No:	Working Solution Conc. (µg/ml)	Stock Conc. (µg/ml)	Volume taken from stock	Total Volume (ml)	Cal ID	Volume taken from w.s (µl)	Total Volume (ml)	Final concentration (ng/ml)

			(µl)					
8	1.2	1000	12	10	QcL	25	1	30
9	20.0	1000	200	10	QcM	25	1	500
10	32.0	1000	320	10	QcH	25	1	800

Each concentration of the plasma sample was divided to 25 µl in 1.5 ml eppendorf tube and kept at (-30˚C), standard samples were given daily together with the quality control samples.

Preparation of fresh juices: All of juices were freshly prepared at the day of experiment.

Sample preparation:

The procedures described were applied for subject samples, calibrator and quality control samples. In order to perform the sample extraction, the following experimental procedure was performed:

Briefly, appropriate numbers of disposable eppendorf tubes were placed in a rack and properly labeled. Then, pipette 50.0 µl aliquots of each test sample (blank, zero, standards, QCL, QCM, QCH or Rat samples) into the appropriate tubes. Then, 150.0 µl of internal standard (2.0µg/ml Irbesartan) was added. Vortex each sample vigorously for 1.0 min and centrifuge at 14000 rpm for 15 minutes.

Results and Discussion

Results

Validation

A full method validation was performed according to ICH and EMA guidelines for any analytical method to demonstrate the reliability of a particular method for the determination of candesartan concentration in the rat plasma. Method validation was performed in three separate days. In each day, seven standard calibration levels (not including zero) was prepared. Serum samples of method validation represented blank, zero, standard calibration curve, six replicates of QC samples (Q.C. Low, Q.C. Mid, and Q.C. High). The validation parameters (sensitivity, intra and inter day accuracy and precision, linearity) should not exceed the limits by the Food and Drug Administration (FDA) Guidance for Industry and United state pharmacopeia (USP), All of the chromatographic and mass detector conditions are mentioned in (Table-3).

Table-3: Summery Table of Chromatographic Conditions and Mass Spectrometric Conditions.

HPLC Conditions	Pump Flow Rate		Autosampler Injection Volume		Autosampler Temp		Column Oven Temp	
	1.0 ml/min		5 µl		4˚C		30˚C	
Chromato	Mobile phase Gradient Elution	Step	Total Time(min)	Flow Rate	A (%) Methanol	B (%) 0.2%		

graphy						
			(µl/min)		F.A
		0	0.00	1000	50.0	50.0
		1	0.01	1000	50.0	50.0
		2	0.02	1000	100.0	0.0
		3	0.70	1000	100.0	0.0
		4	0.71	1000	50.0	50.0
		5	2.00	1000	50.0	50.0
	Column type	ACE 5 C18 Column (50 X 2.1 mm), 5µl				
	Expected Retention times(minutes)	Candesartan		Irbesartan (I.S)		
		1.4		1.0		

MRM Detection Conditions	Analytes	Q1 Mass	Q3 Mass	Dwell	FP	DP	EP	CE	CXP
	Candesartan	441.200	263.200	150	70	81	10	19	22
	Irbesartan (IS)	429.453	207.300	150	70	26	10	8	22

MS Conditions	CUR	CAD	IS	TEM	NEB
	10	6	5500	400	5

In addition, representative chromatograms of candesartan zero, candesartan LLOQ and candesartan QC Mid are shown in figures 2, 3, and 4

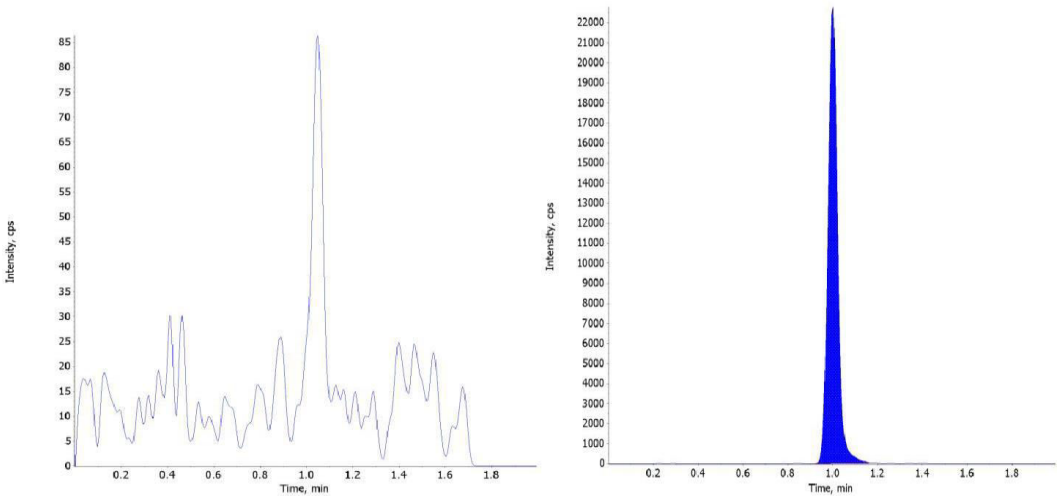

Figure 2: Candesartan zero chromatogram.

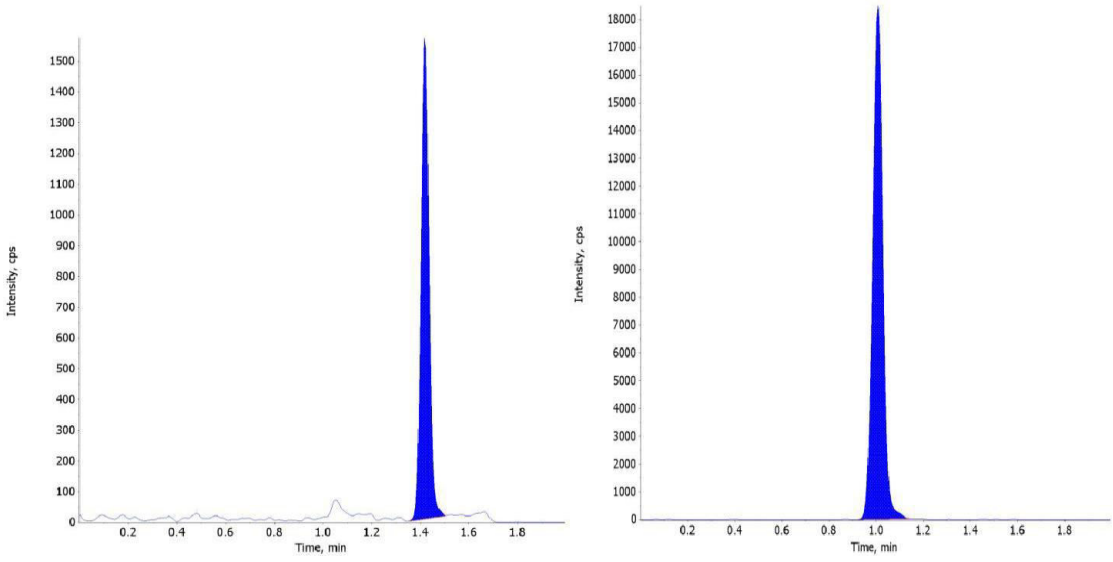

Figure 3: Candesartan LLOQ chromatogram.

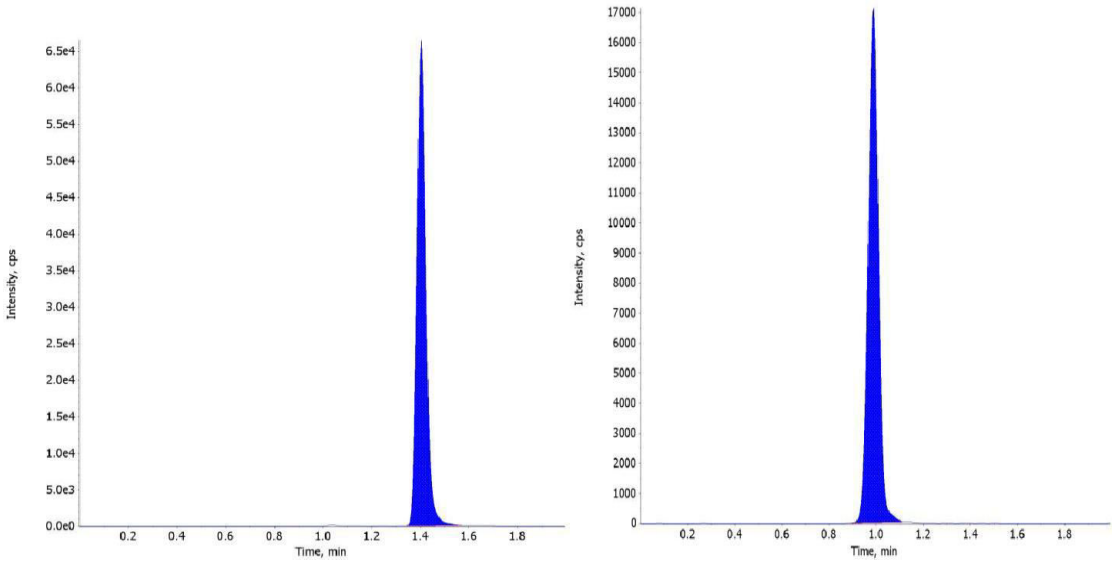

Figure 4: Candesartan QC Mid chromatogram.

Chromatographic Conditions:

Precision

At the first day of validation, the variability of errors (precision) in predicted concentration ranged between as low as 2.645% observed with the High target concentration of 800 ng/ml to a maximum coefficient of variation of (CV%) of 5.963% at the mid target concentration. The precision for low and mid concentrations of target was 5.778%, 5.963% respectively.

At the second day of validation, the variability of errors (precision) in predicted concentration ranged between as low as 1.839% observed with the mid target concentration of 500 ng/ml to a maximum coefficient of variation of (CV%) of 5.677% at the low QC target concentration of 30 ng/ml. The precision for low and high concentrations of target was 5.677%, 1.916% respectively.

At the third day of validation, the precision of predicted concentration ranged between as low as 2.833% observed with the High QC concentration of target of 800ng/ml to a maximum coefficient of variation of (CV %) of 5.030% at the QC low target concentration of 30ng/ml. The precision for LLOQ and mid concentration of target was 4.816%, 3.681% respectively. The precision (CV %) is not exceed 20% for LLOQ, and 15% for the other concentrations which prove the closeness of the measurements.

Accuracy

At the first day validation, the accuracy of mean predicted value compared to target concentration ranged between a minimum of 96.103% at the QC Low concentration of target 30 ng/ml to a maximum accuracy of 103.319% at the QC high concentration for target 800ng/ml. The overall all average accuracy at the first day was 100.55 %. Accuracy

range for six replicates of LLOQ, QC low, QC mid, QC high samples was (97.01%-107.13%), (90.89%-102.56%), (106.42%-93.34%), (107.14%-99.37%) respectively.

At the second day of validation, the accuracy of mean predicted value compared to target concentration ranged between a minimum of 94.482% at the high concentration of target 800ng/ml to a maximum accuracy of 105.302% at the LLOQ target concentration of 10ng/ml. The overall all average accuracy at the second day was 98.78%. Accuracy range for six replicates of LLOQ, QC low, QC mid, QC high samples was (98.70%-109.52%), (93.90%-109.07%), (92.90%-97.80%), (91.87%-96.91%) respectively.

At the third day validation, the accuracy of mean predicted value compared to target concentration ranged between a minimum of 95.691% at the mid concentration of target 500ng/ml to a maximum accuracy of 101.529% at the LLOQ target concentration of 10ng/ml. The overall all average accuracy at the third day was 99.78%. Accuracy range for six replicates of LLOQ, QC low, QC mid, QC high samples was (93.68%-108.88%), (94.46%-109.59%), (90.30%-99.81%), (96.38%-105.07%) respectively.

Comparing with the accepted criteria which is 85-115% for all concentration except for LLOQ which is 80-120%, the accuracy obtained is within the required criteria in terms of accuracy.

Measurement error

The mean measurement error at the first day assessment ranged between 1.169 ng/ml lower than the target concentration at the QC low concentration 30ng/ml of target to 26.549ng/ml higher than the target concentration) at the high concentration of target 500 ng/ml. The overall all mean measurement error at the first day was an overestimate of 7.74ng/ml. The mean measurement error at the second day of assessment ranged between 44.145ng/ml (lower than the target concentration) at the high concentration 800ng/ml of target to 0.530ng/ml higher than the target concentration at the LLOQ concentration of target of 10ng/ml. The overall all mean measurement error at the second day was an underestimate of 17.91ng/ml.

The mean measurement error at the third day of assessment ranged between 21.545ng/ml lower than the target concentration at the mid concentration 500ng/ml of target to 2.033ng/ml higher than the target concentration at the high concentration of target of 800ng/ml. The overall all mean measurement error at the third day was an underestimate of 6.07ng/ml.

Looking at all the 3 days of validation one would conclude an overall mean measurement error of 10.3ng/ml (underestimate on average) for the validation experiments of candesartan.

Linearity

Linearity is determined by calculating the regression line using a mathematical treatment of the results (i.e. least mean squares) vs. analyte concentration [18]. The determination coefficient (R^2) measures the amount of variation in the response (dependent) variable explained by changes in the explanatory (independent variable). A value of 1 for R^2 indicates a perfect linear relation between target concentration and predicted concentration. The closer the value of R^2 to 1 the stronger is the linear relation. A strong regression indicates a strong dose-response relationship between predictor and outcome, which in turn supports a stronger validity for predicted concentration of the drug.

The linear regression equation was used for calculating the predicted drug concentration at the start of each validation experiment, using one unique target concentration for getting the "D area/ IS area" at each of the 3 days of validation for each drug. The R^2 was a perfect dose-response relationship for candesartan at the 3 days of validation. All the linear regression models were statistically significant, (table 4) and (figure 5, 6). The accuracy range for 3 days of validation (table 5) was ranged between 90.98% and 109.05%.

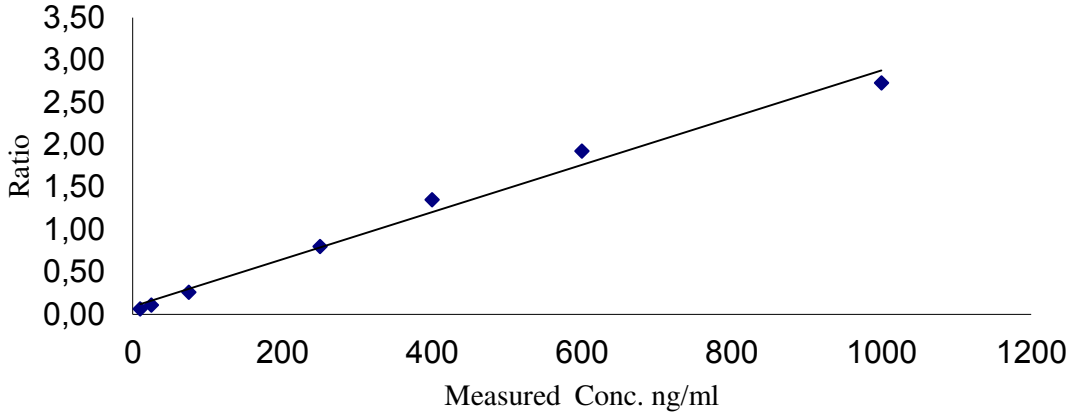

Figure 5: The plot of calibration curve levels against their analytical response, in day one validation for candesartan (Y = 0.00296X +0.0326 (R= 0.9962))

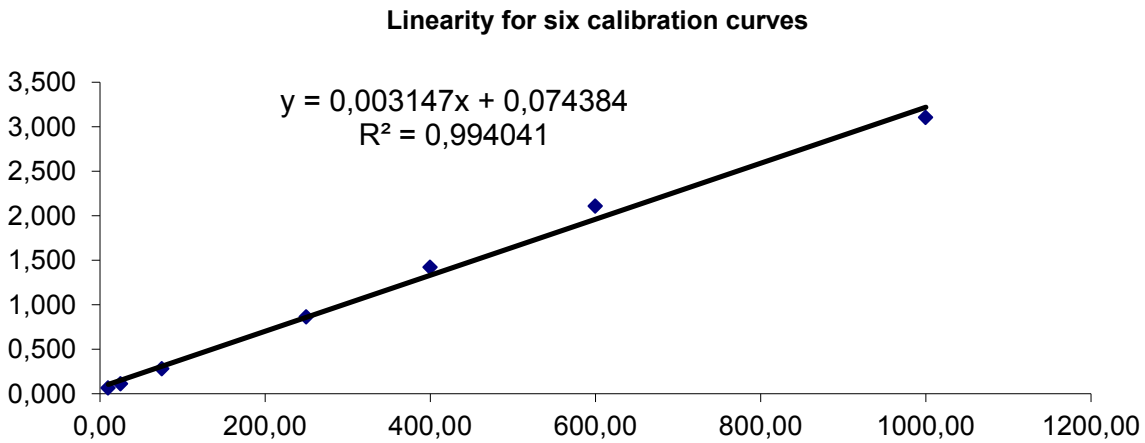

Figure 6: The plot of linearity of calibration curve levels for candesartan quantification against their analytical response and regression linear equation.

Table-4: Equations and R of the 3 days of validation.

Days	Equations	R
1	Y = 0.00296X +0.0326	0.9962
2	Y = 0.0036X + 0.0268	0.9978
3	Y = 0.00366X + 0.0355	0.9987

Table-5: Accuracy % of the standard calibration curves of 3 days for candesartan.

Theoretical conc.ng/ml	Accuracy % Day 1	Accuracy % Day 2	Accuracy % Day 3
10.00	90.98	96.82	97.76
25.00	96.87	90.20	94.95
75.00	100.36	100.71	106.08
250.00	103.13	109.05	99.19
400.00	111.23	106.56	98.52
600.00	106.41	103.31	107.56
1000.00	91.02	93.35	95.95

Since the accepted criteria according to USFDA are 85%-115% except for the LLOQ is 80%-120%, the results of three days of validation passed the required criteria in terms of accuracy.

Stability

Auto sampler stability test is passed according to the ICH accepted range where the accuracy % doesn't exceed 15%. The short term stability indicated by two QC concentrations (low, high) for candesartan after preparation procedure (auto-sampler stability), T=4 C°. The short term stability at room temperature or processing temperature, freshly prepared 0 hour two QC's concentrations were taken as a reference upon calculating stability of candesartan at room temperature. After 24 hours incubation of the samples at room temperature all the results are within the accepted criteria (LQC= 99.56% and HQC=97.79%) which are in the range 85%-115%. The accuracy % was 98.93- 103.44% for LQC and 98.21 – 100.26% for HQC. Regarding the freeze and thaw stability: the QC samples are stored and frozen in the freezer at the intended temperature and thereafter thawed at room or processing temperature. After complete thawing, samples are refrozen again applying the same conditions. At each cycle, samples should be frozen for at least 12 hours before they are thawed. The accuracy for QC low and high after 3 cycles were (97.60 – 104.92% for LQC and 97.67 – 101.64% for HQC and all are within the accepted range which is 85-115%. The stability was 99.78% for LQC and 98.16% for HQC.

Sensitivity: The protein direct precipitation procedure was specified and sensitive for candesartan, where both blank and zero samples that examined from six deferent lots of plasma were attained the required clean chromatogram for specific method.

The modifying effect of combining fruit juices with candesartan

The serum concentration of candesartan with or without pomegranate juice was evaluated on rats on a sample size of 8 for drug alone and another sample size of 3 when candesartan is combined with pomegranate. The measurements were repeated at 6 time intervals following drug administration to a maximum of 6 hours.

Effect of combination on candesartan

As shown in figure7 and table 6, when candesartan was administered alone its serum level reached its maximum (Cmax=964.692 ng/ml) after half an hour (Tmax) and then gradually declines to reach a minimum concentration of (272.679 ng/ml) after 6 hours from the administration of candesartan. The combination of candesartan and pomegranate shows a decrease in serum concentration approximately the half the concentrations when candesartan is given alone. At the first half an hour (Tmax) of administration it reaches its maximum serum concentration (Cmax=475.9673ng/ml) and then gradually declines to reach a minimum concentration of (188.1737ng/ml) at the end of follow up period (6 hours).

Table-6: Pharmacokinetic data of candesartan.

Drug	Cmax (ng/ml)	Tmax (hr)	AUC (ng/ml*hr)
Candesartan	964.692±374.2553	0.5	2864.291±409.3088
Candesartan with pomegranate juice	475.6973±139.5053	0.5	1561.537±259.2953

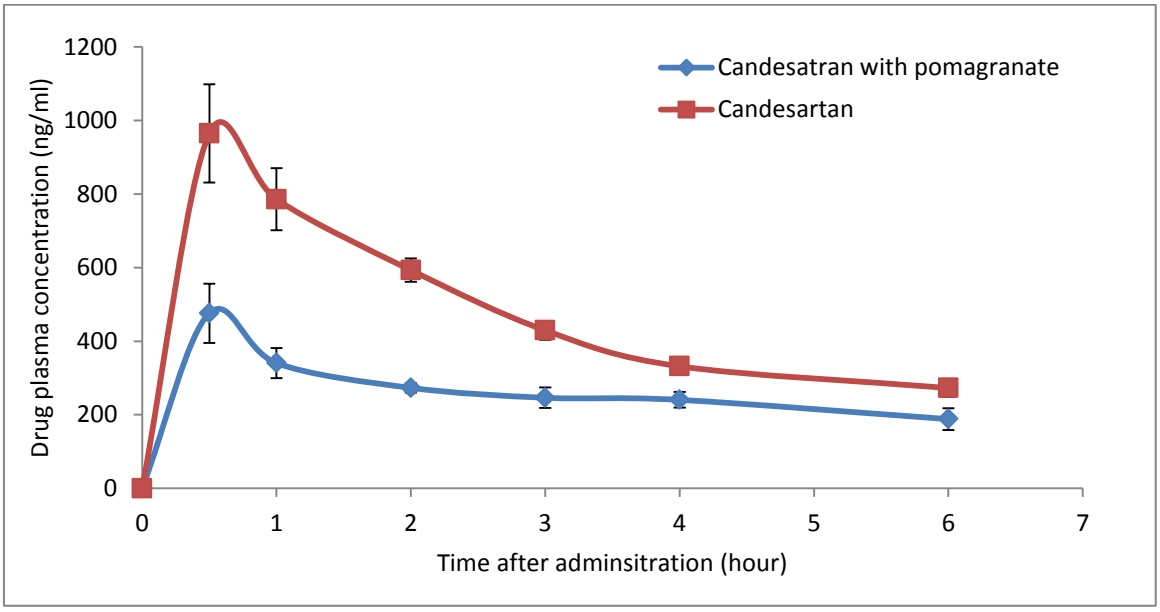

Figure 7: Rat plasma profile showing the changes in mean serum candesartan concentration with time after drug administration, comparing candesartan with pomegranate juice and solitary drug use, each data point represents the mean ± SEM (n=3).

No significant difference was detected between Cmax of Candesartan alone and candesartan with pomegranate (P>0.05).While for the AUC, the difference between candesartan alone and candesartan with pomegranate is significant (P<0.05).

As shown in table 7 the mean serum candesartan concentration when used in combination with pomegranate was lowered by a percentage ranged between 25 and 57% at different time intervals compared to its isolated administration. The effect of this combination on serum candesartan level compared to its single drug use was evaluated as a large effect (Cohen's ranged from d=1.398 to d=4.877). This decrease in serum candesartan concentration is significant (P<0.05) for most of the time intervals.

Table-7: Comparing the mean serum candesartan drug concentration at selected time intervals after administration between single and combined drug use.

	Plasma concentration (ng/ml)					
Drug assessed candesartan	30 min	1 hour	2 hours	3 hours	4 hours	6 hours
candesartan (n=8)						
Mean	964.692	785.724	593.097	429.240	331.759	272.679
SD	377.7636	238.8949	90.2467	71.11296	45.06003	68.71602
SD.Error	133.5596	84.46212	31.90703	25.14223	15.93113	24.29478
Range	(581.313-1586.134)	(545.327-1087.437)	(445.422-701.449)	(350.796-560.130)	(279.878-404.249)	(175.348-386.792)
candesartan with pomegranate (n=3)						
Mean	475.97	340.53	273.12	246.20	240.45	188.17
SD	139.51	71.38	21.50	47.99	36.87	50.76
SE	80.54	41.21	12.42	27.71	21.29	29.31
Range	315.02-562.21)	(284.49-420.89)	(248.31-284.72)	(190.96-277.60)	(200.21-272.62)	(134.50-235.41)
Effect of combination of candesartan with pomegranate compared to solitary drug effect						
Difference between 2 means	-488.722	-445.194	-319.977	-183.04	-91.309	-84.509
Cohen's d	1.7163	2.5251	4.8777	3.0173	2.2178	1.3989
Percent change compared to solitary	-0.50.66%	-56.66%	-53.95%	-42.64%	-27.52%	-30.99%
P (t-test)	0.0424	0.0131	0.0002	0.0029	0.0125	0.0876

Discussion

Candesartan is an angiotensin receptor blocker (ARBs) drug that binds to angiotensin II receptor type1 selectively and competitively and results in an overall decrease in blood pressure, it is a drug used chronically. In the recent years, an increase in beverage intake was noticed; drug interactions with beverages have received considerable attention. The interaction can affect the activity of a drug, because a lot of juices shown to inhibit or induce liver

enzymes or modulate intestinal drug absorption via the P-gp mediated efflux and OATP-mediated uptake transport systems the intestine and liver. It has been well documented that the components of grapefruit, such as bergamottin and (R)-6′,7′-dihydroxybergamottin, demonstrate potent inhibition of CYP 450 system[19, 20].

Pomegranate is a rich source of several chemicals such as pectin, tannins, flavonoids, and anthocyanins. However, debated data are available on whether the component(s) of pomegranate inhibits or induce the metabolism and/or absorption of drugs. Pomegranate has been widely consumed in many countries including the Middle East region.

The aim of the current study was to develop a new validated simple chromatographic method for quantifying candesartan and to study the effect of pomegranate on candesartan serum concentration in a pre fed rats.

According to the data presented in this study, the manner in which candesartan concentration level was altered is caused by the component(s) of pomegranate. Therefore, it is of interest to determine the identity of the chemical(s) in pomegranate juice that exhibits the stated result to enable health care professionals to avoid beverage-drug interactions. Furthermore, identifying situations in which the inhibition or induction of the liver and/or intestinal enzymes and/or transporters may be of therapeutic benefit.

In this study, we demonstrated the influence of pomegranate juice on the pharmacokinetics of candesartan in rats, in comparison with water. The AUC of candesartan decreased, (approximately 1 fold) upon administration to pomegranate juice (Table 7).

As shown in figure 4, when candesartan was administered alone its serum level reached its maximum (964.692 ng/ml) after half an hour and then gradually declines to reach a minimum concentration of (272.679ng/ml) after 6 hours from the administration of candesartan. and when candesartan was administered to a pomegranate pre-fed rat groups, a significant decrease in serum concentration were resulted, at the first half an hour of administration candesartan reaches its maximum serum concentration (475.967 ng/ml) and then gradually declines to reach a minimum concentration of (188.174 ng/ml) at the end of follow up period (6 hours). The metabolic effect resulted from up taken pomegranate juice is not necessary to be only via the hepatic enzymes, and since candesartan is mainly excreted unchanged, then results of this study revealed that pomegranate juice might affect the intestinal metabolic system which resulted in beverage-drug interactions. The mechanism of beverage-drug interactions in the intestine consists of several systems that include metabolism and absorption [21]. The P-glycoprotein is believed to play an important role in the efflux of drugs, which results in poor absorption of these drugs. Therefore, pomegranate juice might be an inducer of P-glycoprotein in the intestine and reduce the absorption of candesartan. Also it has been

reported that pomegranate juice affects intestinal uptake transporters as well as P-glycoprotein [21, 22]. Thus, we conclude that the decrease in the AUC of candesartan by pomegranate juice could be due to the induction of enteric p-glycoprotien activity and/or due alteration in the intestinal uptake transporters system. However, further studies required to explore the molecular exact effect of pomegranate on drugs.

In addition, the difference in T max between rats and humans, candesartan maximum concentration reached after 30min (½ hr) in rats compared to 3-4 hr's in human. The overall rate of biotransformation of candesartan in rats is markedly different from that in humans; however, the major metabolic pathways of the drug are almost similar in both rats and humans. Nevertheless, it is difficult to extrapolate our results, which were obtained in rats, to humans. Quantitative evaluation of pomegranate-drug interaction in humans needs to be verified by studies in humans. Therefore, further investigations in humans are necessary.

Conclusion

A new simple, rapid and sensitive method for validation and determination of candesartan in the presence of each juice (pomegranate, liquorice and orange) has been done by using High Performance Liquid Chromatography–Mass Spectrometry (HPLC-MS/MS). Plasma candesartan level was affected by the administration of pomegranate. The reduction in plasma candesartan level in pomegranate pre-fed group was reduced to the half comparing to candesartan alone drug use.

The difference between Cmax (single administration vs. combination with pomegranate) is insignificant, where the difference in AUC is significant ($P<0.05$).

According to these results pomegranate juice might affect the intestinal metabolic system which resulted in beverage-drug interactions. Thus, the decrease in the AUC of candesartan by pomegranate juice could be due to the induction of enteric p-glycoprotien activity and/or due alteration in the intestinal uptake transporters system. Also, it is difficult to extrapolate our results, which were obtained in rats, to humans and in this time we recommend not to take candesartan with pomegranate juice due to its potential interaction.

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Corresponding Author:

Dr. Wael Abu Dayyih (PhD, Bpharm).

Email: *wabudayyih@uop.edu.jo*