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VALIDATION AND DETERMINATION OF PIRACETAM IN RAT PLASMA BY USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY/UV/VIS SPECTROMETRY (HPLC/UV/VIS) IN PRESENCE OF POMEGRANATE AND LIQUORICE JUICES FOR PHARMACOKINETIC STUDY

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ABSTRACT

The study aimed to develop a simple and rapid chromatographic method for quantifying piracetam in rat plasma and study the pharmacokinetic parameters with and without pomegranate and liquorice juices.

A new validated, simple and rapid method for determination of piracetam in presence of pomegranate and liquorice juices was developed by using High Performance Liquid

Chromatography–Ultra Violet- Visible Spectroscopy. The mobile phase was composed of 98 % of Water and 2 % of Acetonitrile. BDS C18 Column (150mm x 4.6 mm, 5µm), and a flow rate of 1.0 ml/min were used, the autosampler injection volume was 5 microliters. Cefadroxil was used as an internal standard.

Rats were divided into 3 groups, A (n=6) received a single dose of piracetam only (50mg/Kg), B (n=6) received piracetam with pomegranate juice and C (n=6) received piracetam with liquorice juice. The precision of the predicted measurements for piracetam was high (mean CV% <10%). The accuracy for piracetam over all the three days of validation and all the four-tested target concentration was within the accepted criteria. The standard curves for piracetam matched the requirements, linear relation (R^2) ranged between (0.9992 to 1).

Pharmacokinetic data showed that the Cmax for piracetam alone was (61.254µg/ml), with juices of pomegranate (62.038µg/ml) and liquorice (78.238µg/ml). The total body clearance of piracetam significantly increased ($p < 0.05$) when combined with either pomegranate or licorice. However, in spite this significant increment, this does not seem to affect the whole pharmacokinetic profile of piracetam when given with pomegranate or licorice juices.

Keywords: Piracetam, Pomegranates, Liquorice, HPLC, Pharmacokinetics

INTRODUCTION

Piracetam

Piracetam ($C_6H_{10}N_2O_2$) is one of the racetam drug family. It is a (2-(2-oxopyrrolidin-1-yl) acetamide) and shares the characteristic 5-carbon oxopyrrolidone ring (figure 1). Structurally it is similar to the neurotransmitter GABA [1, 2].

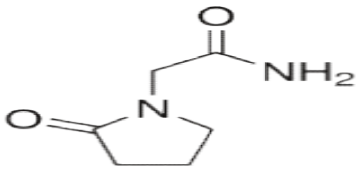


Figure 1: Chemical structure of piracetam

It is a nootropic agent used to improve cognition and to enhance memory [3, 4]. It is a non sedative non-stimulant agent that influences neuronal and vascular functions and influences cognitive function and it works as a positive allosteric modulator of α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptors [5]. In addition, piracetam improves the function of the neurotransmitter acetylcholine via muscarinic cholinergic (ACh) receptors, which are implicated in memory processes and, it is involved in the learning and memory processes by affecting N-Methyl-D-aspartate (NMDA) glutamate receptor [6]. Piracetam side effects are rare and of short

period, it may be limited to anxiety, insomnia, drowsiness and agitation [1].

Methods for analysis of piracetam were applied; an HPLC method was developed to determine and validate of piracetam in film coated tablets [7], also RP-HPLC ultraviolet method was used for quantification of piracetam and levetiracetam [8]. A simple and rapid HPLC method for the separation and determination of piracetam and its four impurities, was developed [9]. In addition, piracetam determination in human palsma was done using LC–tandem MS method [10] and by HPLC [11].

Pomegranate juice

The pomegranate is considered as a popular 'superfood' since it has high antioxidant content and disease prevention properties [12]. It was used for treating many diseases in folk medicine such as acidosis, dysentery, hemorrhage, microbial infections, and respiratory pathologies [13, 14, 15]. The potential therapeutic properties of pomegranate are wide-ranging and include treatment and prevention of cancer, cardiovascular disease, Alzheimer's disease, and male infertility [16, 17, 18, 19]. Also, pomegranate – drug interaction was studied for metabolism of some drugs such as carbamazepine [20], and midazolam [21]. The effect of pomegranate fresh juice on the Metronidazole pharmacokinetics using HPLC /MS method was applied and found to be good for intestinal but not hepatic enzyme inhibitor for Metronidazole metabolism [22]. Recently, the effect of pomegranate juice on sildenafil citrate was evaluated in rats plasma [23] and on Candesartan [24].

Liquorice juice

Liquorice is an esteemed crude drug that originates from the dried roots of several Glycyrrhiza species [25, 26]. Liquorice has been employed as a flavoring and sweetening agent as well as a demulcent and expectorant [27]. In addition, Licorice has been also reported to possess anti-allergic [28], neuroprotective [29], antioxidative [30], and anti-inflammatory activities [31]. The efficacy and safety profiles of a drug may be affected when administered concomitantly with liquorice, since its components induced the hepatic CYP450 isozymes in mice [32] and rats [33]. These enzymes are responsible for oxidative, peroxidative, and reductive metabolic transformations of drugs, environmental chemicals, and natural compounds [34]. Recently, the effect of liquorice juice on pharmacokinetics of atorvastatin, simvastatin, and lovastatin in rat's plasma using LC/MS spectrometry was studied by [35].

This study aimed to develop a simple and rapid chromatographic method for quantifying piracetam in rat plasma and study the pharmacokinetic parameters for the piracetam in rats pre-fed with liquorice and pomegranate juices to examine the possible effect of these juices on piracetam pharmacokinetic parameters.

MATERIALS AND METHODS

Chemicals

Methanol and Acetonitrile of HPLC grade (Chromanorm), Phosphoric Acid, (GRP RECTAPUR), Deionized Water (Chromanorm), Triethylamine (TEDIA), Perchloric acid 60% (GCC ANALYT)

Rats Plasma, (harvested from Animals of UOP animal house). Piracetam raw material Freshely prepared liquorice juice (Local market) Freshly squeezed pomegranate juice (Local market).

Instrumentation

An HPLC (FINNIGAN SURVEYOR) was used and composed of: ChromQuest software 4.2.34, Solvent delivery systems pump (LC Pump Plus). Autosampler Plus, UV-VIS Plus Detector, Hypersil Thermo Electron Corporation, BDS C-18 Column (150 mm x 4.6 mm, 5µm) and Computer System, Windows XP, SP3.

Animals

All animal experiments were performed in compliance with Federation of European Laboratory Animal Science Association (FELASA) guidelines. The study protocol was approved by the Research Committee 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 250.0g, and they were in healthy condition. They were placed in air-conditioned environment (20-25°C) and exposed to a photoperiod cycle (12 hours light/ 12 hours dark) daily.

The rats were divided into 3 groups; A (n=6) received piracetam (50mg/kg) only, B (n=6) received piracetam with liquorice, and C (n=6) received piracetam with pomegranate. Fruit juices were given to the rats (liquorice and pomegranate) as single dose (12ml/kg), before the administration of the drug, by oral cavage. Each rat was weighed then the tip of the tail was cut and a few drops of blood collected in eppendorf tube, then the rat received the drug solution orally. A blood samples were collected from each rate in Eppendorf tubes after 30 min and 1, 1.5, 2, 3, 4, 6, 8 and 24 hours of administration. Eppendorf tubes were centrifuged for 10 minutes to get the plasma required. Plasma

samples were labeled and stored at (-30 °C) until HPLC analysis.

Preparation of Solutions

Stock solution of Piracetam

80 mg of piracetam working standard were weighed accurately and dissolved in 10 ml of Methanol to get a concentration of 8.0 mg/ml stock solution of piracetam.

Stock solution of the Internal Standard (IS) Cefadroxil

20.0 mg of Cefadroxil working standard were weighed accurately and dissolved in 10 ml of Methanol to get a concentration of 2.0 mg/ml stock solution of Cefadroxil.

Working solution of Cefadroxil

3.0 ml from Cefadroxil stock solution (2.0 mg/ml) were taken and diluted to 100 ml of 5% Perchloric acid to get working solution contains 60 µg/ml of Cefadroxil as IS.

Piracetam serial solutions and plasma spiking samples

A serial solutions and plasma spiking samples were prepared using the stock solution of Piracetam (8.0 mg/ml) as shown in table 1.

Piracetam Quality Control Samples in plasma

Samples of QC were prepared by spiking plasma with 100.0 µl from serial solution into 10.0 ml of plasma to obtain QC concentrations of: 6, 50 and 80 µg/ml as shown in 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.

Preparation of juices

Pomegranate fruits (*Punica granatum*) were purchased from local market in Jordan. Fruits were manually cut up and arils were collected manually in a stainless container. Then juice mechanically extracted by using hand operated fruit juicer.

Licorice roots from *Glycyrrhiza glabra* (Fabaceae) cultivated in Jordan, which were used to prepare the licorice juice. The roots were soaked in distilled water for 1 hour. The wet licorice paste then was taken in a clean white tissue and hold on a holder. Finally, the cold water was allowed to drizzle over the tied licorice cushion in a period of 2 hours. The prepared licorice juice has no additives and it was stored at 4°C and used within 48 hours.

Table 1: Preparation of Piracetam serial dilution and spiked plasma samples

	Serial solutions				Plasma spiking			
	Solution No:	Volume taken from stock (µl)	Total volume (µl)	Working solution concentration (µg/ml)	Cal ID	Volume taken from working solution (µl)	Total Volume (µl)	Final concentration (µg/ml)
Calibration points	1	10	1000	80	C1	25	1000	2
	2	40	1000	320	C2	25	1000	8
	3	80	1000	640	C3	25	1000	16
	4	150	1000	1200	C4	25	1000	30
	5	240	1000	1920	C5	25	1000	48
	6	350	1000	2800	C6	25	1000	70
	7	500	1000	4000	C7	25	1000	100
QC points	8	30	1000	240	QCL	25	1000	6
	9	250	1000	2000	QCM	25	1000	50
	10	400	1000	3200	QCH	25	1000	80

Sample collection and processing

Few drops blood samples were collected in Eppendorf tubes containing Ethylenediaminetetraacetic acid (EDTA) by cutting the rats tail. Blood samples were collected from each group at time points: 0.0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0 and 24.0 hours of administration. Blood samples then centrifugated at 5,000 RPM for 10 minutes and plasma samples were taken in labeled eppendorf tubes and stored at -30^{o}C till the analysis.

Sample extraction were done by taking 100 µl of each sample (blank, zero, standards, QCL, QCM, QCH or Rat samples) and 100 µl of internal standard working solution (60 µg/ml of Cefadroxil in 5% perchloric acid) were taken in a tube and vigorously vortexed for 1.0 min; after centrifugation for 10 min at 14000 rpm the supernatant was transferred into auto-sampler tubes then 5 µl was injected for analysis. This procedure was used for all samples; rat, calibration and quality control samples.

Method validation

Accuracy and Precision

Within-batch accuracy and precision, evaluations were determined by analysis of 6 replicates quality control samples from each level. The between-batch precision and accuracy was determined by analyzing three sets of within-batch quality control sequence in three separate batches. The quality control samples were randomized daily, processes and analyzed in position either; a) immediately following the standard curve, b) in the middle of batch or c) at the end of the batch. The acceptance criteria for within and between –batch precision and accuracy were

20% for LLOQ and 15 % for the other concentrations.

Specificity

Specificity is the ability of an analytical method to differentiate and quantify the analytes in presence of other components in the sample. The specificity of the method was evaluated by screening six different lots of blank plasma. These lots were analyzed as blank and zero samples then compared with LLOQ to confirm lack of endogenous peaks.

Linearity

Rats plasma samples were spiked with Piracetam to prepare calibrators, these samples were extracted and assayed. Each calibration curve was completed by plotting the ratio versus nominal concentration values.

Chromatographic conditions

Chromatographic conditions of piracetam analysis were summarized in Table 2.

Table 2: Summery Table of Chromatographic Conditions.

HPLC conditions	**Pump flow rate ml/ min**	**Auto sampler injection volume (µl)**	**Auto-sampler temp(˚C)**	**Column oven temp(˚C)**
	1.0 ml/min	**5 µl l**	**4 ˚C**	**40 ˚C**
Chromatography	**Mobile phase**	**98 % of Water Contains (1 ml Triethylamine per 1 liter) 2 % of Acetonitrile pH= 3.50, adjust with H_3PO_4**		
	Column type	**Hypersil Thermo Electron Corporation, BDS C18(150mm x 4.6 mm, 5µm)**		
	Expected retention times (minutes)	**Cefadroxil (I.S)**	**4.9**	
		Piracetam	**2.6**	
Detection condition	**Wave length**	**205 nm**		

RESULTS AND DISCUSSION

Method validation

A full method validation according to ICH [36], and EMA [37] guidelines was performed in this analytical method to demonstrate the reliability of the method for the determination of piracetam concentration in rat plasma. The HPLC/UV developed method used to separate, identify and determine the concentration of piracetam in plasma was validated to meet the requirements for a global regulatory filing.

Accuracy, Precision and Measurement error

Tables 3 and 4 shows the precision and accuracy data resulted from the HPLC/UV. These data presented that the used method is precis, accurate, and reproducible as per the requirements of the ICH and EMA guidelines for the quantification piracetam with and without fruit juices in rat's plasma.

The inter and intra-day accuracy results (table 3-4) over the targeted concentrations range (97 and 108%) in each day. 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. Further, results of intra-day accuracy lied in the same accepted rang.

Inter and Intra-day precision (CV%) found to be less than 20% for LLOQ and less than 15% for the other targeted concentrations. The CV% evaluation over the three days was less than 6.51% for LLOQ, which indicates the closeness of the measurements (Table 3-4).

The overall all mean measurement error were an underestimate of 0.61 ug/ml, 0.789 ug/ml and 0.572 ug/ml for day 1,2 and 3 respectively and looking at all the 3 days of validation one would conclude an overall mean measurement error of 0.657 ug/ml (overestimate on average) for the validation experiments of Piracetam (table 3-4).

Linearity

Equation of Linear regression was used to calculate 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. In Validation process all R^2 were above 0.99. Correlation, slope, R^2 and intercept of the standard curve are found in table 5 and figure 2. The three validation days data were within the accepted ranges in terms of linearity and all the linear regression models were statistically significant. Also, the data confirmed a good linearity in the range 2–100 µg/ml for piracetam in rat's plasma and showed a good correlation coefficient (R^2) of more than 0.999 (Table 5, Fig. 2).

Sensitivity

The protein direct precipitation procedure was specified and sensitive for Piracetam, where both blank and zero samples that examined from six deferent lots of plasma were attained the required clean chromatogram for specific method (figures 3 and 4).

Stability

The autosampler stability test is agreed according to the ICH accepted range, where the accuracy % is less than 15% (table 6). Short term stability data indicated by two QC concentrations (low, high) for Piracetam after preparation procedure (auto-sampler stability), T=4 C °.

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 is within the accepted range which is 85-115%, table 6.

The short-term stability at room temperature or processing temperature was tested using freshly prepared two QC's concentrations (0 hour) were taken as a reference upon calculating stability of Piracetam at room temperature. All the results are within the accepted criteria which are in the range 85%-115%, as shown in table 4.

Recovery of Piracetam and Cefadroxil

Table 7 shows the data of absolute recovery of piracetam and cefadroxil in both spiked plasma and mobile phase using the QC samples.

Absolute recoveries for Piracetam and IS were calculated by dividing average peak area of triplicate from each QC level of plasma samples over the same set of QC samples that were prepared in mobile phase multiplied by 100%. The recovery % of Piracetam and IS show a high and acceptable recovery % at the studied concentrations.

Effect of fruit juices on Piracetam levels in plasma

The plasma concentration of piracetam with and without fruit juices was measured in rats on a sample size of 6 using three types of samples; piracetam only, piracetam combined with liquorice juice and piracetam combined with pomegranate juice. The measurements were repeated at 9 time intervals following drug administration to a maximum time of 24 hours.

Data in table 8 showed that the maximum level of piracetam in the first type of samples was (61.254 µg/ml) after 2 hours and decline gradually to reach the minimum level (4.031µg/ml) at 24 hours. While in the samples of piracetam with pomegranate the maximum concentration was 62.038µg/ml after 2 hours of administration and minimum concentration of 4.031µg/ml at 24 hours. The samples of piracetam with liquorice reached maximum concentration (78.238µg/ml) after 1 hour and decline to (5.628µg/ml) after 24 hours.

The data from this study showed a nonsignificant difference between the pharmacokinetic parameters; Cmax, Tmax and AUC between the samples of piracetam alone, piracetam with pomegranate and piracetam with liquorice juices. Also, the overall elimination pattern for the three drug samples showed no big changes. Tables 8 and 9 show the kinetic parameters of

compartmental and non-compartmental analysis.

The pharmacokinetic profile of piracetam is linear and time-independent with low inter subject variability over a large range of doses. This is consistent with the high permeability, high solubility and minimal metabolism of piracetam. It is rapidly and almost completely absorbed. The extent of oral bioavailability, assessed from the AUC, is close to 100% [38].

Clearance of piracetam is dependent on the renal creatinine clearance, and it is excreted almost completely in urine and the fraction of the dose excreted in urine is independent of the dose given [38, 39]. The data showed that the total body clearance (Cl) of piracetam significantly increased ($p<0.05$) when combined with either pomegranate or licorice. Assuming that Vd is constant the increment in Cl might be attributed to a kind of interaction on reabsorption from kidney. Both juices might inhibit partially some of the transporters that are responsible for this partial reabsorption.

While, the overall elimination pattern for the drug with or without these the two juices showed no notable changes, since piracetam is excreted almost completely in urine and not metabolized while the major effect of pomegranate and licorice is on the liver. In addition, the volume of distribution (Vd) and MRT of piracetam in the body from time zero up to 24 hrs in water (control) or with pomegranate or with licorice was almost the same (non-significant $p>0.05$).

Table 3: Results of intra- day precision and accuracy and measurement error for the quality control samples of Piracetam in the three days of validation.

Day 1	LLOQ	QC_{Low}	QC_{Mid}	QC_{High}
Target conc.	2.0 ug/ml	6.0 ug/ml	50.0 ug/ml	80.0 ug/ml
Calculated conc. ±SD	2.162±0.14 1	5.98±0.278	48.929±0.632	78.81±1.254
SE	0.058	0.113	0.258	0.512
Accuracy ± SD	108.10±7.04	99.71±4.64	97.86±1.26	98.51±1.57
Precision (CV%)	6.51	4.655	1.291	1.591
Range	2.004-2.348	5.624-6.360	48.304-49.684	77.248-80.832
Day 2				
Target conc.	2.0 ug/ml	6.0 ug/ml	50.0 ug/ml	80.0 ug/ml
Calculated conc. ±SD	2.076±0.0.111	6.151±0.236	50.566±1.344	82.364±1.879
SE	0.045	0.096	0.549	0.767
Accuracy ± SD	103.80±5.57	102.51±3.93	101.13±2.69	102.96±2.35
Precision (CV%)	5.369	3.835	2.659	2.281
Range	1.884-2.204	5.888-6.536	49.276-52.884	80.11 - 84.54
Day 3				
Target conc.	2.0 ug/ml	6.0 ug/ml	50.0 ug/ml	80.0 ug/ml
Calculated conc. ±SD	1.997±0.052	6.163±0.162	50.82±1.625	81.302±3.060
SE	0.021	0.066	0.45	0.48
Accuracy ± SD	99.85±2.69	102.72±2.69	101.64±3.25	101.63±3.82
Precision (CV%)	2.624	2.622	3.179	3.764
Range	1.914-2.048	5.938-6.391	49.023-50.123	77.39-84.63

Table 4: Inter day accuracy, precision and measurement error for the quality control samples of Piracetam in the three days of validation.

Target conc.	2.0 ug/ml (LLOQ)	6.0 ug/ml (QC_{Low})	50.0 ug/ml (QC_{Mid})	80.0 ug/ml (QC_{High})
Mean ± STD	2.078±0.123	6.099±0.232	50.105±1.473	80.825±2.570
CV%	5.913	3.811	2.939	3.179
Accuracy %	103.92	101.65	100.21	101.03

Table 5: Linearity and linear working range of six calibration curves of piracetam data based on the measured concentration

Calibration Curve #	Measured concentration for each standard point (ug/ml)						
	2	8	16	30	48	70	100
1	2.001	8.023	15.916	29.83	46.86	70.646	102.193
2	1.893	8.472	16.133	29.749	48.266	68.681	100.807
3	2.019	7.785	15.46	29.739	48.656	72.368	101.206
4	2.296	7.035	14.978	30.04	49.005	71.823	98.823
5	2.269	7.496	14.987	29.266	47.281	70.939	101.762
6	2.004	7.961	16.006	29.621	47.306	70.315	102.509
Mean ± SD	2.08±0.163	7.795±0.491	15058±0.515	29.708±0.257	47.896±0.866	70.795±1.286	101.217±1.328
CV%	7.847	6.297	3.308	0.866	1.807	1.817	1.312
Accuracy %	104.02	97.44	97.37	99.03	99.78	101.14	101.22

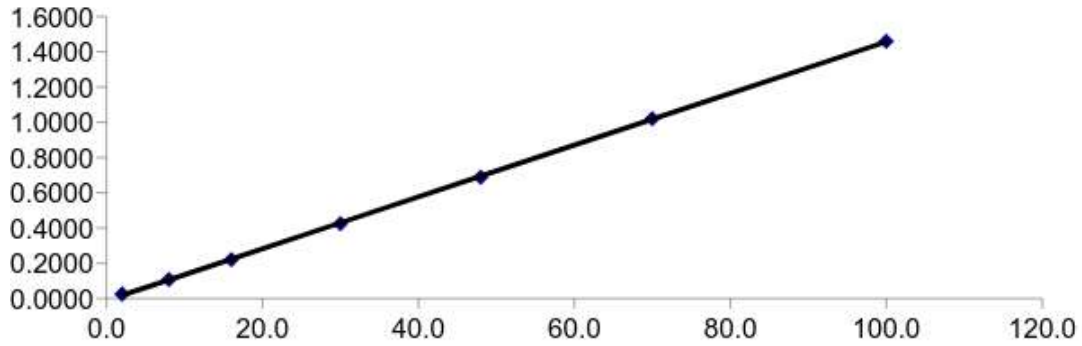
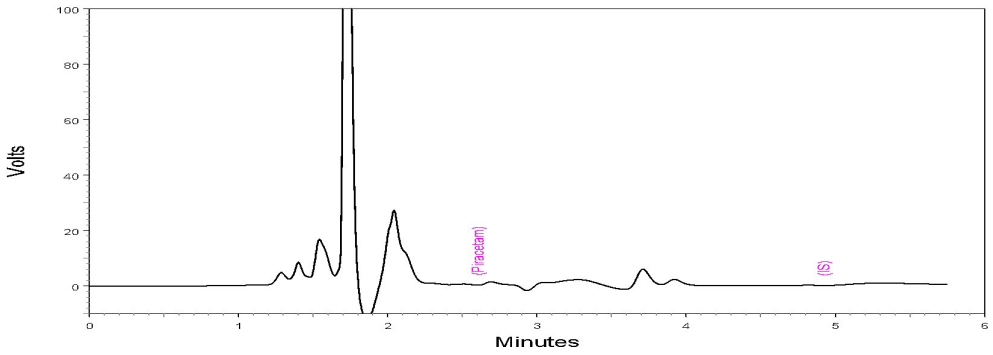


Figure 2: The plot of linearity of mean six calibration curve levels for Piracetam quantification against their analytical response and regression linear equation



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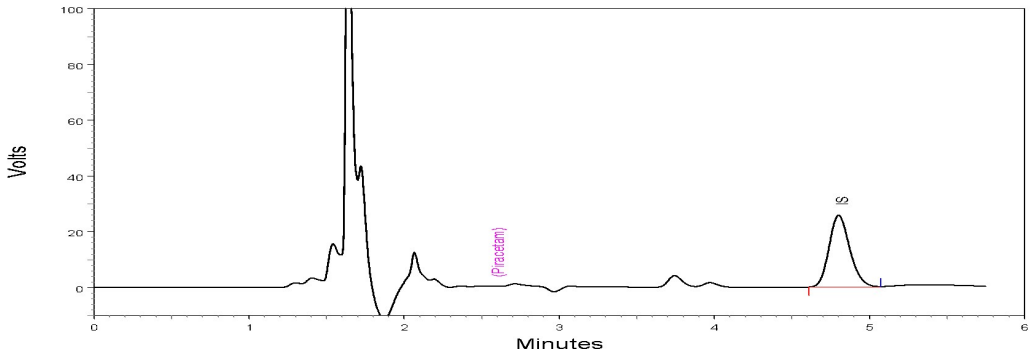

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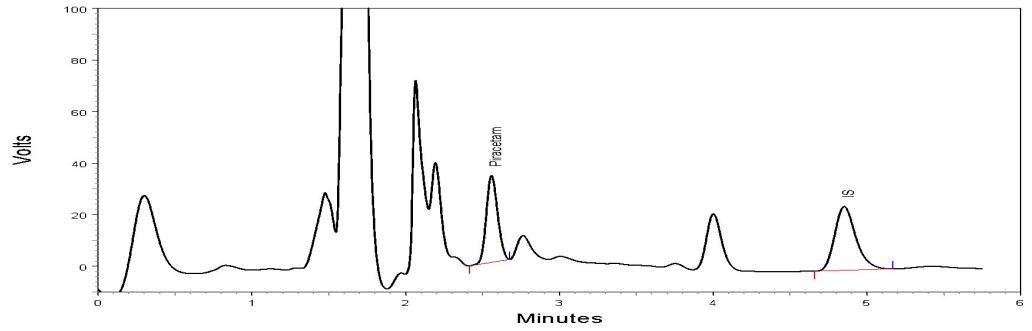


C

Figure 3: (A) Blank plasma, (B) Zero plasma with I.S and (C) Piracetam QC Mid (50µg/ml) with I.S chromatograms.

Table 6: Stability Piracetam under different storage conditions (n = 6).

Storage Condition	QC (ug/ml)	Measured Mean± SD at 0.0 h (ug/ml)	Measured Mean± SD at 24.0 h (ug/ml)	Accuracy (%)	Stability (%)
Autosampler stability 24 h at 4°C	6	6.013 ± 0.137	6.031 ± 0.068	100.52	98.82
	80	81.117 ± 1.181	80.735 ± 1.065	100.92	99.53
Freeze and thaw stability at -20°C (3 cycles)	6	6.013 ± 0.137	6.017 ± 0.153 (3 cycles)	102.22	96.45
	80	81.117 ± 1.181	79.766 ± 2.099 (3 cycles)	107.96	98.33
Room Temperature stability for 24h	6	6.103 ± 0.317	6.022 ± 0.091	97.38	99.67
	80	81.117 ± 1.181	79.726 ± 3.356	101.32	98.29

Table 7: Absolute recovery of Piracetam and Cefadroxil from plasma and mobile phase (n=3)

	QC Concentration (ug/ml)	Spiked Plasma Mean AUC	Mobile Phase Mean AUC	Absolute Recovery (%)
Piracetam	6.0	19642	20104	97.70
	50.0	169668	171517	98.92
	80.0	270149	274533	98.40
Cefadroxil	6.0	241300	245530	98.28
	50.0	239130	245306	97.48
	80.0	238159	243988	97.61

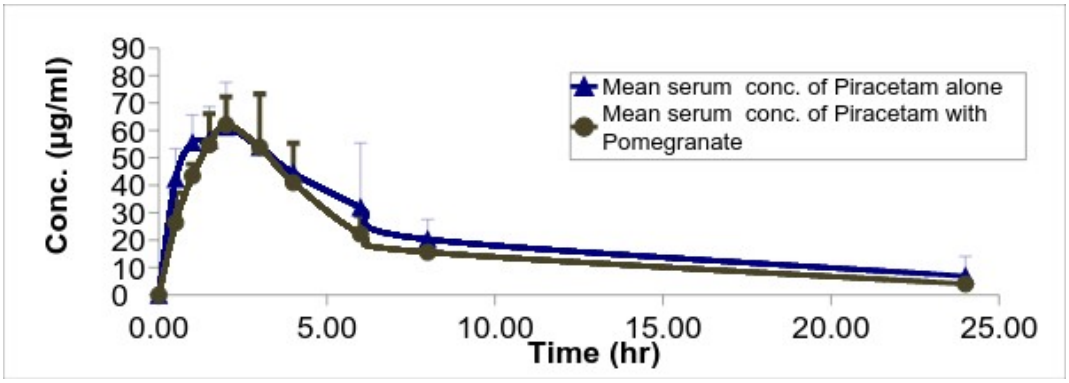
Table 8: Mean plasma concentration of piracetam using for all types of samples; piracetam only, piracetam combined with liquorice juice and piracetam combined with pomegranate juice. (n=6).

Time (Hrs)	Piracetam Mean ± STD plasma conc. (ug/ml)	Piracetam + Pomegranate Mean ± STD plasma conc. (ug/ml)	Piracetam + Liquorice Mean ± STD plasma conc. (ug/ml)
0.50	42.283±11.08	26.351±10.90	63.456±8.35
1.00	55.452±10.20	43.421±1.745	78.238±15.45
1.50	56.410±12.21	54.764±11.37	71.352±11.36
2.00	61.254±16.28	62.038±10.22	61.351±13.22
3.00	53.841±18.46	53.874±19.51	49.025±11.97
4.00	44.435±10.02	40.962±14.44	36.986±6.47
6.00	31.922±23.48	22.245±6.75	27.191±15.77
8.00	20.248±7.29	15.581±2.31	15.150±6.23
24.00	6.860±7.22	4.031±0.71	5.628±2.5

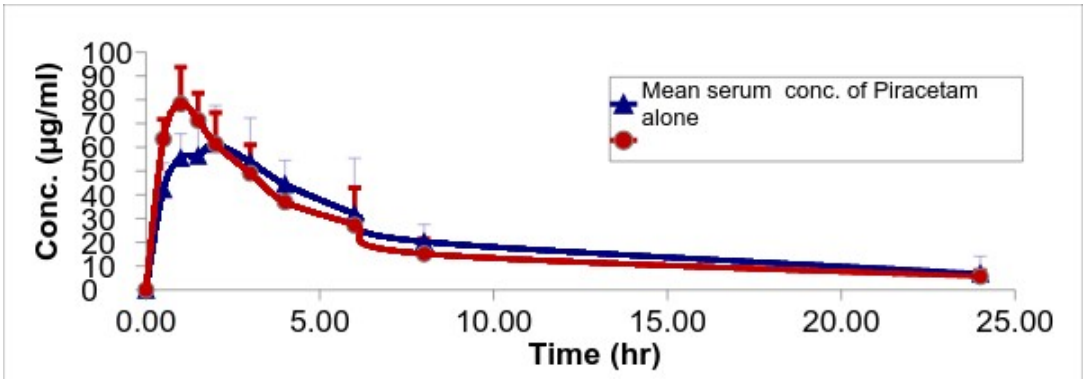
Table 9: Results of compartmental analysis of piracetam with and without pomegranate and liquorice

Parameter	Piracetam	Piracetam + pomegranate	Piracetam+ liquorice
C_{max} (ng/ml)	61.254	62.038	78.238
T_{max} (h)	2.00	2.00	1.00
Kel (/hr)	0.099	0.11	0.112
t ½ (Hr)	7.0	6.3	6.2
Vd (ml)	231.0	231.0	231.0
Cl (ml/hr)	22.869	25.41*	25.872*

C_{max}: Maximum plasma concentration, t_{max}: time require to achieve maximum concentration, Kel: elimination rate constant, t1/2: elimination half- life, Vd: Volum of Distribution, Cl: Clearance
***Significant p<0.05, non-significant p>0.05, 95% CI**



A



B

Figure 4: Rat plasma profile showing the changes in mean serum piracetam concentration with time after drug administration, (A) piracetam with pomegranate juice and (CB) piracetam with liquorice juice, each data point represents the mean ± SEM (n=6).

CONCLUSION

A new simple, rapid and sensitive (HPLC / UV method is established for validation and determination of piracetam in the presence of each juices; pomegranate and liquorice.

The effect of single dose of fresh pomegranate and licorice juices on the pharmacokinetics profile for Piracetam was investigated. In comparison with distilled water-fed rats, non-compartmental analysis showed a nonsignificant effect of single dose pre-treatment on the pharmacokinetic parameters of piracetam.

An interesting observation was that the total body clearance of piracetam was significantly increased ($p<0.05$) when combined with either pomegranate or licorice, this increment in Cl might be attributed to a kind of interaction on reabsorption from kidney. Juices might inhibit partially some of the transporters that are responsible for this partial reabsorption. However, in spite that the " Cl" showed significant increment, this does not seem to affect the whole PK profile of piracetam when given with pomegranate or licorice juices.

The results of non-compartmental analysis showed a nonsignificant effect of pomegranate and licorice on piracetam pharmacokinetic (PK) parameters; maximum plasma level (Cmax) and the area under plasma level-time curves (AUC) ($p>0.05$).

Further *in vitro* and *in vivo* investigations are suggested. This study can lead to many possible future studies such as the administration of different quantities of pomegranate and liquorice juices to detect any dose-dependent changes in PK parameters.

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