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Research Article

REVERSED-PHASE-HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF RUTIN AND QUERCETIN AS WELL AS PHARMACOKINETIC EVALUATION IN RAT PLASMA

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ABSTRACT

Objectives: Rutin and quercetin are widely present flavonoids in many herbal extracts, with potential therapeutic benefits in various diseases. Their pharmacokinetic interactions and combined effects on bioavailability have not been extensively studied. This research aims to develop and validate a bioanalytical method for studying the pharmacokinetic interactions between rutin and quercetin in rats and to estimate their plasma concentration when administered alone and in combination.

Methods: In this study, a bioanalytical method was developed and validated for interflavonoid combination. Pharmacokinetic interactions of rutin and quercetin were estimated using six rats, and divided into three groups for a pharmacokinetic interaction study between R (100 mg/kg) and Q (40 mg/ kg), administered alone as well as in combination. The total plasma concentration of rutin and quercetin was determined by the high-performance liquid chromatography method. The estimation method was developed and validated using international council for harmonization guidelines.

Results: The outcomes of these studies show characteristic differences in the pharmacokinetics of rutin and quercetin. The bioavailability of quercetin increased compared to the bioavailability of rutin. The maximum plasma concentration of Q in combination was 736.3058621 µg/mL×h, whereas it was 675.0596359 µg/mL×h when given alone. In the case of rutin, the maximum plasma concentration was 323.585 µg/mL×h in combination, and 319.1925 µg/mL×h when administered alone. From the outcomes, it was proven and estimated that there is an interflavonoid interaction between rutin and quercetin. The possible interaction between the two flavone constituents with hydrolyzing enzymes in their combination enhances bioavailability.

Conclusion: It is concluded that if both drugs are used in combination, they may produce a beneficial effect in the treatment of neuropathic pain

Keywords: Rutin, Quercetin, Bioanalytical method, Reversed-phase-high-performance liquid chromatography, Fourier transform infrared, Rat plasma.

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INTRODUCTION

Flavonoids are a type of polyphenolic substance found in numerous plants and vegetables, especially onions, apples, and tea. Several plants with flavonoids have been frequently utilized in conventional treatments for a variety of diseases [1,2]. On a typical diet, people consume 1–2 g of flavonoids per day. Humans commonly ingest the secondary metabolites produced by plants known as flavones and flavonols [3,4]. The average daily intake, which is primarily quercetin (60–75%), has been estimated to range between 3 and 70 mg, depending on the country or eating habits. The primary sources, which vary widely between nations, are not just berries, tea, wine, onions, or apples but also fruits and green vegetables generally contribute significantly as well [5,6]. Plants get protection from flavonoids from herbivores such as insects and mammals, as well as ultraviolet radiation, pathogens, and bacterial infections. Numerous human ailments, such as cancer and cardiovascular disorders, are helped by these substances. In addition, its anti-oxidant, anti-inflammatory, anti-tumoral, and antiviral capabilities have also been highlighted [7,8]. Rutin and quercetin are secondary metabolic byproducts of plants termed flavonoids. Both of those composites are the most naturally distributed flavonoids in herbs and plant-based diets, and these are chemically identical due to rutin is a very-very prevalent quercetin glycoside. Flavones are ingested by humans since they are found in food and medicinal plants. Their primary sources include fruits, vegetables, tea, and wine [9,10]. To comprehend the mechanism of action of natural products as well as conventional drugs, it is crucial to understand the pharmacokinetics of the active components. With the growing use of extracts of plants for use as medication as an alternative, there are increasing numbers of clinical cases involving herb-drug interactions that are being researched [11,12]. The natural action of flavonoids is attributed to their antioxidant properties, but they can also influence cellular activity by binding to a large variety of protein adenosine triphosphate-binding sites or by specifically interfering with different protein kinase and lipid kinase signaling pathways [13]. Previous research has focused on the physical combinations of two or more pure substances or individual pure compounds. Subsequent investigations have demonstrated that quercetin appears to be eliminated at a relatively low rate, but rutin absorption occurs more slowly than quercetin [14]. Quercetin (3, 3', 4', 5, 7-pentahydroxyflavone) (Q) is one of the most frequently studied polyphenolic group constituents due to its eventuality to reduce inflammation, oxidative damage, platelet aggregation, and capillary permeability. Moreover, quercetin demonstrates protective activity in cardiovascular, hepatoprotective, neuroprotective, antiviral, anti-inflammatory, anticancer, and anti-obesity exertion with antidepressant effects. Another well-understood flavonoid is rutin (R), also commonly called quercetin-3-O-rutinoside or 3',

4', 5, 7-tetrahydroxy-flavone-3-rutinoside. It has been exhibited to possess anti-inflammatory, antiplatelet, vasoactive, antihypertensive, antiallergic, antispasmodic, hypolipidemic, cytoprotective, antitumor, antiprotozoal, antibacterial, and antiviral properties. It may similarly be able to inhibit the vital proteins severe acute respiratory syndrome coronavirus 2 [15,16].

Hence, there is a need and importance to investigate when both are given simultaneously, whether there is any interaction between them, and the effect on their bioavailability. In the current work, using *in vivo* pharmacokinetics in rats, we estimated the pharmacokinetic behavior of both quercetin as well as rutin with their co-administration. In the present examination, we simultaneously determined the two active constituents, quercetin and rutin, in rat plasma following oral treatment utilizing a safe reversed-phase high-performance liquid chromatography (RP-HPLC) technology. Rutin and quercetin's plasma pharmacokinetics were calculated [17].

Table 1: Chromatographic conditions

S. No.	Parameters	Conditions
1	Column	4.6 mm×250 mm, 5-µm Shim-Pack solar (C18)
2	Column temperature	37°C
3	Detector	Photo-diode array
4	Flow rate	1.0 mL/min
5	Pump mode	Isocratic
6	Injection volume	20 µL
7	Run time	20 min
8	Mobile phase	Methanol: Water: Formic acid (65:33:02)

Table 2: Peaks for functional groups

Standard wavelength	Functional group	Sample A (rutin)	Sample B (quercetin)	Sample C (rutin+ quercetin)
3400–3600	-OH	3660	3689	3650
3000–3100	-C=C-H	2881,2828	3014,2883	3014,2883
1600–1662	-C=C-	1649	1665	1662

MATERIALS AND METHODS

Materials

Drugs and chemicals

From Yucca Enterprises, WADALA (E), MUMBAI 400 037, INDIA, received Quercetin (QU, 99) and Rutin (RU, 99). For HPLC analysis, methanol (HPLC grade - Merck Co., India) and formic acid (Merck Co., India) were utilized.

Experimental animals

Wistar rats (180–270 g) were used and their housing took place in the central animal house facility, Ashokrao Mane College of Pharmacy, Peth-Vadgoan, Maharashtra, India, accompanied for 12-h light/dark cycle and a constant temperature of 25–2°C. The committee for the control and supervision of experiments on animals (CCSEA) granted its approval for this study under protocol number IAEC/AMCP/01/2022–2023, and every step of the process was followed exactly as per CCSEA.

Methods

Spectrophotometric analyses using the Fourier transform infrared (FTIR)

The FTIR spectra of extracts for rutin, quercetin, and combination were recorded by ATR FTIR technology of the BRUKER spectrometer and analyzed within the range 400–4000 cm.

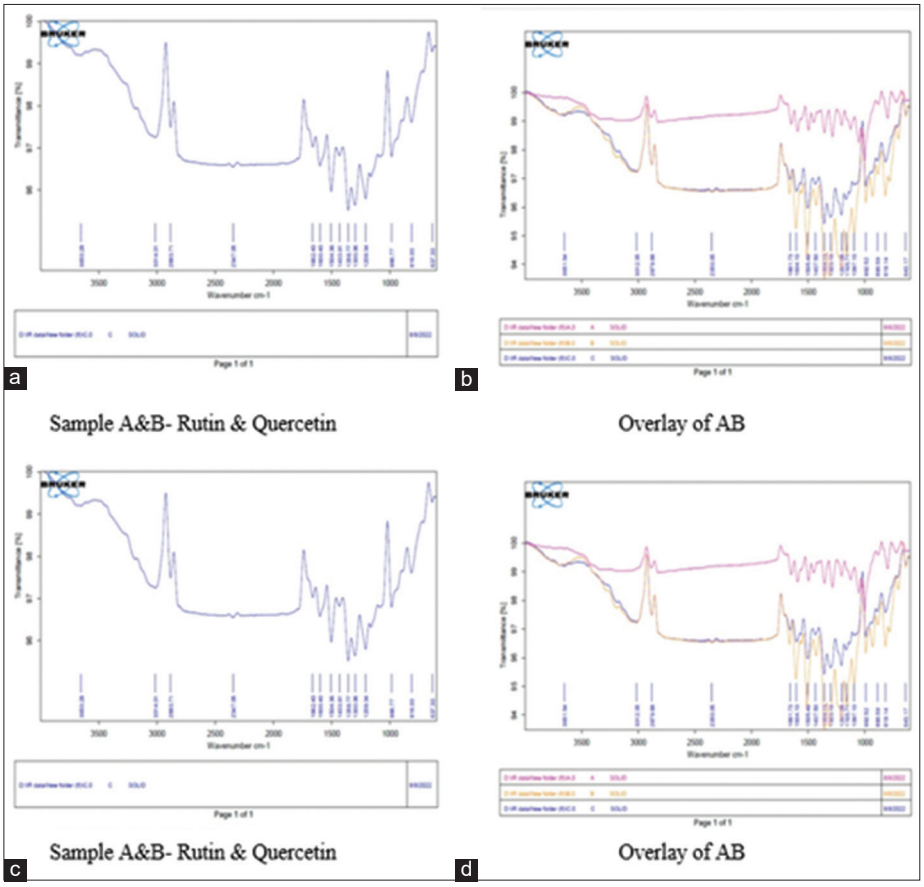

Fig. 1: (a) Spectra of rutin, (b) quercetin, (c) combination of both, and (d) overlay of both

Method development and chromatographic conditions

For the determination, rutin and quercetin powder modified HPLC method was applied, Shimadzu, Prominence modular (Japan), an Inertsil C18 column (150 mm×4.6 mm, 5 μm) with an auto-injector was utilized. Methanol water formic acid 65 33 2(v/v/v) was determined as the mobile phase [18]. ICH guidelines were followed, and validation studies were conducted [19]. (ICH1995)([R1] and 2005) [20,21]. Chromatographic conditions are illustrated in Table 1.

Preparation of mobile phase

Methanol, water, and formic acid were combined for the production of the mobile phase in the proportion of 65:33:02, respectively, which is then ultrasonically reprocessed as long as for up to 10 min.

Table 3: Results of the linearity study, rutin and quercetin

Calibration curve		
Conc.	**Rutin**	**Quercetin**
0	0	0
20	1716789	4812942
40	2868056	9705562
60	5343140	13518087
80	7532792	20660383
120	11305706	29060437
Equation	y=99307×-602375	y=246482×-223386
	R^2=0.9932	R^2=0.9929

Preparation of standard stock solution (pure extract rutin and quercetin)

To produce a stock solution, 10 mg of dry powder, or precisely weighed quantities of quercetin and rutin (5 mg each), were dissolved in 100 mL of a methanol-filled volumetric flask. The solution was carried out at 100 μg/mL or 0.1 mg/mL of quercetin and rutin. The final concentration was kept at 10 μg/mL by pipetting off 1 mL of the stock solution and dissolving it once again in 10 mL of methanol.

Preparation of sample (stock) solution

The final concentration of rutin and quercetin in the methanol solvent was maintained at 10 μg/mL.

Pharmacokinetic study

For the pharmacokinetic study, 18 rats were used and divided into three groups of six rats each. Quercetin 40 g/kg, 100 mg/kg of rutin, and a combination of the two medicines were employed in the oral dose. Group 1 rats were given 100 mg/kg of rutin, Group 2 rats were given 40 mg/kg of quercetin, and Group 3 rats were given a combination of both medications. Blood samples (0.2 mL) were taken from the first four rats in each group for 0 h, 0.5 h, 1 h, and 2 h, and from the following four rats in each group at 5 h, 10 h, 18 h, and 24 h. The vials containing potassium oxalate were used for anticoagulant actions at these times. Then, samples of blood were carefully combined and centrifuged for 15 min at 35,000 g. After that, the plasma was removed into 5-mL vials, which were then safely closed and processed following the guidelines provided. The PK solver

Table 4: Results of the accuracy study of rutin and quercetin

Calculation parameters	Rutin			Quercetin		
	LQC	**MQC**	**HQC**	**LQC**	**MQC**	**HQC**
	80	100	120	80	100	120
	7532792	10742075	11632747	20264856	23490592	34400437
	7588322	10762066	11647224	20397564	23405259	34402080
	7537066	10791067	11632747	20344209	23522059	34295600
	7508687	10804067	11604079	20309996	23554040	34251578
	7545460	10712025	11605706	20280580	23594229	34403868
	7519726	10722067	11734553	20280490	23415259	34184650
Average	7538676	10755561	11642843	20312949	23496906	34323036
STDEV	27591.32	37026.82	47990.79	50119.62	75463.91	93576.01
% RSD	0.365997	0.344257	0.412191	0.246737	0.321165	0.272633
Actual concentration	80	100	120	80	100	120
Percentage recovery	100	100	100	100	100	100

CON: Concentration, SD: Standard deviation, RSD: Relative standard deviation, LQC: Lower quality control, MQC: Middle quality control, HQC: Higher quality control, STDEV: Standard deviation

Table 5: Results of precision study rutin and quercetin

Precision	Rutin				Quercetin			
	Interday 1	**Interday 2**	**Analyst 1**	**Analyst 2**	**Interday 1**	**Interday 2**	**Analyst 1**	**Analyst 2**
	MQC	**100 Microgram/mL**	**MQC**	**100 Microgram/mL**	**MQC**	**100 Microgram/mL**	**MQC**	**100 Microgram/mL**
	100	100	100	100	100	100	100	100
	12185261	10899565	12185261	10899565	19201058	17998946	19201058	17998946
	12089512	10807085	12089512	10807085	19201060	17859383	19201060	17859383
	12164148	10829622	12164148	10829622	19206598	17729712	19206598	17729712
	12147044	10850182	12147044	10850182	19201012	17859390	19201012	17859390
	12111235	10823010	12111235	10823010	19045919	17871539	19045919	17871539
	12217794	10868277	12666666	10868277	19201112	17859987	19201112	17859987
Average	12152499	10846290.17	12152499	10846290.17	19176127	17863159.5	19176126.5	17863159.5
STDEV	47270.12113	33744.96735	47270.12113	33744.96735	63826.84	85270.11282	63826.84044	85270.11282
Percentage RSD	0.388974491	0.311119902	0.388974491	0.311119902	0.332845	0.477351797	0.332845324	0.477351797

STDEV: Standard deviation, RSD: Relative standard deviation, STDEV: Standard deviation, MQC: Middle quality control

Table 6: Results of robustness study rutin and quercetin

Rutin						Quercetin						
Robustness	Flow rate	100		Robustness	Wavelength			Robustness	Flow rate	AREA		Robustness	Wavelength		
					LW	MW	HW						LW	MW	HW
Flow rate	0.8	1	1.2	Flow rate	249	254	259	Flow rate	0.8	1	1.2	wavelength	249	254	259
	6967042	7032490	7137060		7030490	7032490	7032490		25060776	17998946	24824559		17991215	17998946	17981259
	6917670	7034680	7164038		7032680	7034680	7034680		24998211	17859383	24810091		17991516	17859383	17951287
	6917657	7044492	7191641		7012492	7044492	7012492		25021937	17729712	24772778		17992025	17729712	17971230
	6987107	7062412	7173333		7010412	7062412	7032414		24967196	17859390	24793213		17991512	17859390	17985412
	6923793	7045410	7134193		7008410	7045410	7032416		24866046	17871539	24767869		17974512	17871539	17975641
	6935145	7025010	7134189		7025125	7025010	7035019		24734647	17859987	24742043		17951230	17859987	17986451
Average	6941402	7040749	7155742	Average	7019935	7040749	7029919	Average	24941469	17863160	24785092	Average	17982002	17863160	17975213
STDEV	29060.68	13097.94	24268.97	STDEV	10767.46	13097.94	8619.136	STDEV	120875.8	85270.11	30166.3	STDEV	16548.79	85270.11	13079.51
% RSD	0.418657	0.186031	0.339154	% RSD	0.153384	0.186031	0.122606	% RSD	0.484638	0.477352	0.121711	% RSD	0.09203	0.477352	0.072764

STDEV: Standard deviation, RSD: Relative standard deviation

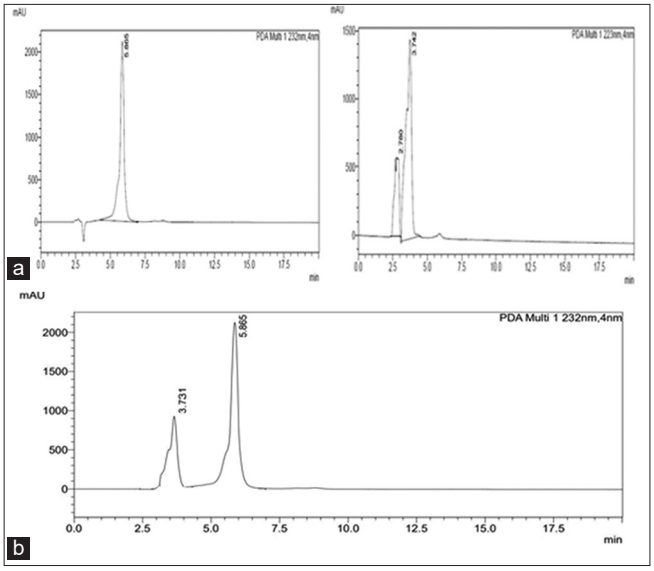

Fig. 2: (a) Chromatogram of standard (I) rutin and (II) quercetin (alone) and (b) chromatogram of rutin and quercetin combination

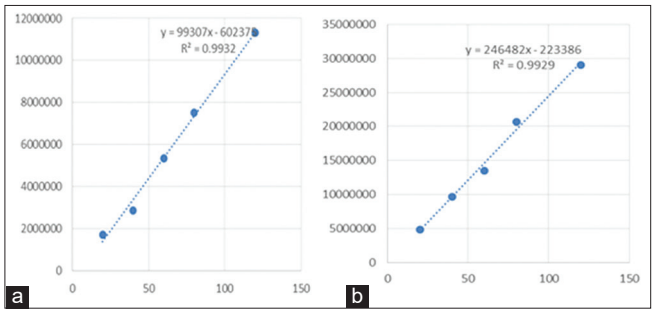

Fig. 3: (a) Calibration curve of rutin and, (b) quercetin

Table 7: Result of LOD and LOQ

Rutin		Quercetin
Analytical Parameters	**Concentration**	**Concentration**
LOD	1 µg/mL	1 µg/mL
LOQ	10.51523/µg/mL	1.565902 µg/mL

LOD: Limit of detection, LOQ: Limit of quantification

2.0 software was used to assist with the area under the curve (AUC) (AUC0- t and AUC0-) calculations, [22,23].

FTIR spectrophotometry

FTIR analyses of rutin, quercetin, and the combination were carried out to look at the changes in the distinguishing signals of spectra by ATR FTIR technology of the BRUKER spectrometer and interpreted.

Method validation

Linearity

A linearity study was performed by preparing six different dilution concentrations of rutin and quercetin, namely, 0, 20, 40, 60, 80, and 120 µg/mL. Dilutions are further diluted and injected into the HPLC column with an injection volume of 20 µL. From the linearity study, it was found that the regression coefficient of rutin and quercetin was 0.9929 and 0.9956, respectively [24].

Table 8: Pharmacokinetic parameters of rutin and quercetin alone and in combination

Parameter	Unit	Quercetin	Rutin	Quercetin in combination	Rutin in combination
$t_{1/2}$	h	4.82831389	5.982220791	5.440383735	6.351784074
T_{max}	h	5	5	2	5
C_{max}	µg/mL	49.6	26.67	57	28.78
AUC_{0-t}	µg/mL×h	675.0596359	319.1925	736.3058621	323.585
$AUC_{0-\infty}$	µg/mL×h	691.3595716	343.0990411	717.9396359	348.9684141

AUC: Area under the curve

Accuracy

Accuracy is a measurement of how closely test findings that were achieved using a procedure matched the actual value. By conducting the recovery studies at three different amounts of standard stock solution applied to the samples, the method's accuracy was evaluated. Accuracy is the proximity of the result. The percentage recovery for rutin and quercetin was calculated and reported [25,26].

Precision

Studying repeatability and intermediate accuracy allowed for the determination of precision, which is a metric for the reproducibility of the entire analytical procedure. The results obtained for precision for interday 1, analyst 1, intraday 1, and intraday 2 [27,28].

Robustness

Robustness is the analytical process that remains unaffected by small, deliberate changes in system parameters. The robustness was carried out at different flow rates, that is, 0.8 mL/min, 1m L/ min, and 1.2 mL/ min. 249 and 254 nm was selected for detection of rutin and quercetin respectively[24,27].

Ruggedness

The capability to reproduce an analytical method in various laboratories or environments without the emergence of unanticipated variations in the acquired results is typically referred to ruggedness of the analytical method [29].

RESULTS AND DISCUSSION

Results of FTIR

Studies showed that sample A rutin got a peak at 3660 nm for the functional group -OH,28281,2828 for the functional group -C=C-H and 1649 for the functional group -C=C-. Sample B quercetin got a peak at 3689 nm for the functional group -OH,3014,2883 for the functional group -C=C-H and 1665 for the functional group -C=C- (Fig. 1). When both drugs were analyzed at a time, there were no extra peaks in combination, so it indicates no interaction between Samples A and B (Table 2) [30,31].

Method development

The method of RP-HPLC was developed and validated for the qualitative estimation of rutin and quercetin from the pure drug, that is, dry powder of rutin and quercetin, and the method was found to be the most accurate, specific, and stable, indicating at different validation parameters. The different combinations of mobile phase of methanol, water, and formic acid at different ratios such as in blank 65:35:05, rutin (65:35, 70:30, 75:25,65:30:05,65:33:02), quercetin (65:35, 70:30, 75:25, 65:30:05, and 65:33:02), and rutin and quercetin in combination 65:33:02, these different ratios were tried, and finally, it was optimized at 65:33:02. Results of these trials given idea about shape and retention time of rutin and quercetin based on these data; we finalized the mobile phase and wavelength in which both drugs eluted with good resolution, that is, distance between two phases is more than 2 min as it shows satisfactory separation and well resolved peak at 3.737 min and 5.858 min for standard or pure rutin and quercetin; similarly, it shows 3.731 min and 5.865 min of retention time for formulation of rutin and quercetin, respectively (Fig. 2).

Method validation

Linearity

A linearity study was performed by preparing six different dilution concentrations of rutin and quercetin, namely, 0, 20, 40, 60, 80, and 120 µg/mL. Dilutions are further diluted and injected into an HPLC column with an injection volume of 20 µL. From the linearity study, it was found that the regression coefficient of rutin and quercetin was 0.9929 and 0.9956, respectively, as shown in Fig. 3 and Table 3.

Accuracy

Accuracy is a measurement of how closely test findings that were achieved using a procedure match the actual value. By conducting the recovery studies at three different amounts of standard stock solution applied to the samples, the method's accuracy was evaluated. Accuracy is the proximity of the result. Rutin and quercetin's percentage recovery has been calculated and reported in Table 4.

Precision

Studying repeatability and intermediate accuracy allowed for the determination of precision, which is a metric for the reproducibility of the entire analytical procedure. The following results were obtained for precision, as shown in Table 5.

Robustness

The use of robustness is the analytical procedure that is not impacted by slight, deliberate alterations in robustness results parameters. The robustness was completed at distinct flow rates, that is, 0.8 mL/min, 1 mL/min, and 1.2 mL/min for rutin as well as quercetin, and at different wavelengths 249 nm, 254 nm, and 259 nm (Table 6).

Limit of detection (LOD) and limit of quantification (LOQ) (LOD and LOQ studies)

In HPLC analyses, the linearity range of R and Q was 10–120 µg mL^{-1}, and the regression equation was y=602375×–99.307 for R, and y=246482×–223386 for Q. Correlation coefficient (r^2) of R was 0.9932±0.0009 and for Q r^2=0.9929±0.0001. LOD and lower LOQ (LLOQ) were calculated based on the "Standard Deviation of the Response and the Slope" approach. LOD and LLOQ values for R and Q were found to be 1 µg.mL^{-}, 10.51 µg.mL^{-1}, and 1 µg mL^{-1}, 1.56 µg.mL^{-1}, respectively. Recovery of the method was 98–100%. With RSD values of <5%, the method for R and Q was regarded as precise due to repeatability and intermediate precision (Table 7).

Pharmacokinetic parameters

Bioavailability studies were performed using a method developed and using the PK2Solver computer software; the pharmacokinetic parameters derived from the plasma concentration-time- time curve are shown in Table 8.

Since quercetin must be digested by the cecal microbiota, but the small intestine absorbs rutin. It was absorbed moreover slowly than quercetin. Quercetin can be absorbed and digested in both the large and small intestines. As a conclusion, it seems that there are more opportunities for this quercetin to be absorbed than there are for rutin. It is very important to note that rutin increases the concentarion of

quercetin. Similar impact we have also observed in the treatment of neuropathic pain when these two drugs are given in combination to the rats against alcohol induced neuropathy.

Validation studies were performed according to the international council for harmonisation (ICH) guidelines, and the analysis results fulfill the ICH requirements.

CONCLUSION

The current investigation demonstrated that when rutin and quercetin are administered orally, their combination accelerated the oral bioavailability of quercetin and rutin compared to single administration. Thus, it may be concluded that the oral dosage of combined extracts may have a remarkable biopharmaceutical advantage over the single drug. As a result, the interaction between flavonoids of quercetin and rutin was clarified in plasma. There are no extra peaks in the combination study of IR, so it indicates no interaction between rutin and quercetin. Hence, the established technique can be applied to various pharmaceutical studies to investigate the drug's pharmacokinetics and biodistribution, and if these two drugs are used in combination my produce a synergistic effect.

AUTHORS' CONTRIBUTIONS

Prashant S. Kumbhar: Literature review, methodology, data curation, writing-original draft, and evaluation; Safina. I. Mulla: Literature review; Bimlesh Kumar: Writing original draft, review and editing, supervision, evaluation, and visualization; Ganesh Mote: Writing original draft; and Dileep Singh Baghel: Writing original draft. All authors have read and approved the final version of the manuscript and agree to be accountable for all aspects of the research.

CONFLICTS OF INTEREST

The authors express no conflicts of interest.

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Nil.

REFERENCES

1. Sharma P, Dhiman T, Negi RS, Oc A, Gupta K, Bhatti JS, *et al*. A comprehensive review of the molecular mechanisms driving skin photoaging and the recent advances in therapeutic interventions involving natural polyphenols. S Afr J Bot. 2024;166:466-82. doi: 10.1016/j.sajb.2024.01.035
2. Kammalla AK, Ramasamy MK, Chintala J, Dubey GP, Agrawal A, Kaliappan I. Comparative pharmacokinetic interactions of quercetin and Rutin in rats after oral administration of European patented formulation containing *Hippophae rhamnoides* and Co-administration of quercetin and Rutin. Eur J Drug Metab Pharmacokinet. 2015;40(3):277-84. doi: 10.1007/s13318-014-0206-9, PMID 24888486
3. Chand P, Kumar H, Jain R, Jain A, Jain V. Flavonoids as omnipotent candidates for cancer management. S Afr J Bot. 2023;158:334-46. doi: 10.1016/j.sajb.2023.05.025
4. Havsteen BH. The biochemistry and medical significance of the flavonoids. Pharmacol Ther. 2002;96(2-3):67-202. doi: 10.1016/s0163-7258(02)00298-x, PMID 12453566
5. Manach C, Morand C, Demigné C, Texier O, Régérat F, Rémésy C. Bioavailability of rutin and quercetin in rats. FEBS Lett. 1997;409(1):12-6. doi: 10.1016/s0014-5793(97)00467-5, PMID 9199494
6. Ramadhan F, Mukarramah L, Risma FA, Yulian R, Annisyah NH, Asyiah IN. Flavonoids from endophytic bacteria of *Cosmos Caudatus* Kunth. Leaf as anticancer and antimicrobial. Asian J Pharm Clin Res. 2018;11(1):200-4. doi: 10.22159/ajpcr.2018.v11i1.21987
7. Fernández SP, Wasowski C, Loscalzo LM, Granger RE, Johnston GA, Paladini AC, *et al*. Central nervous system depressant action of flavonoid glycosides. Eur J Pharmacol. 2006;539(3):168-76. doi: 10.1016/j.ejphar.2006.04.004, PMID 16698011
8. Mustarichie R, Runadi D, Ramdhani D. The antioxidant activity and phytochemical screening of ethanol extract, fractions of water, ethyl acetate and n-hexane from mistletoe tea (*Scurrula atropurpurea* BL. DANS). Asian J Pharm Clin Res. 2017;10(2):343-7. doi: 10.22159/ajpcr.2017.v10i2.15724
9. Nieoczym D, Socała K, Raszewski G, Wlaź P. Effect of quercetin and rutin in some acute seizure models in mice. Prog Neuropsychopharmacol Biol Psychiatry. 2014;54:50-8. doi: 10.1016/j.pnpbp.2014.05.007, PMID 24857758
10. Lotha R, Sivasubramanian A. Flavonoids nutraceuticals in prevention and treatment of cancer: A review. Asian J Pharm Clin Res. 2018;11(1):42-7. doi: 10.22159/ajpcr.2017.v11i1.23410
11. Gouws C, Steyn D, Du Plessis L, Steenekamp J, Hamman JH. Combination therapy of Western drugs and herbal medicines: Recent advances in understanding interactions involving metabolism and efflux. Expert Opin Drug Metab Toxicol. 2012;8(8):973-84. doi: 10.1517/17425255.2012.691966, PMID 22612723
12. Joshi B, Bhandari NL, Shrestha S, Gyawali R, Thapa P. Comparative study of polyphenol, flavonoid, and antioxidant activity of various medicinal plants collected from different altitudes. Asian J Pharm Clin Res. 2021;9:87-93.
13. Dajas-Bailador F, Bantounas I, Jones EV, Whitmarsh AJ. Regulation of axon growth by the JIP1-AKT axis. J Cell Sci. 2014;127(1):230-9. doi: 10.1242/jcs.137208, PMID 24198394
14. Li G, Zeng X, Xie Y, Cai Z, Moore JC, Yuan X, *et al*. Pharmacokinetic properties of isorhamnetin, kaempferol and quercetin after oral gavage of total flavones of *Hippophae rhamnoides* L. in rats using a UPLC-MS method. Fitoterapia. 2012;83(1):182-91. doi: 10.1016/j.fitote.2011.10.012, PMID 22056665
15. Ghate NB, Chaudhuri D, Das A, Panja S, Mandal N. An antioxidant extract of the insectivorous plant *Drosera burmannii* Vahl. Alleviates iron-induced oxidative stress and hepatic injury in mice. PLoS One. 2015;10(5):e0128221. doi: 10.1371/journal.pone.0128221,PMID 26010614
16. Thenmozhi M, Jayanthi M. Phytochemical screening and antioxidant activity of *Eclipta alba* L. Asian J Pharm Clin Res. 2019;12:215-8.
17. Pandey NK, Kumar B, Singh SK, Baghel DS, Sudhakar K, Singh S, *et al*. Original Article validated reversed-phase high-performance liquid chromatography method for the estimation of tetrabenazine in self-nano emulsifying drug delivery systems. Int J Appl Pharm. 2024;16:388-94.
18. Zhao Z, Dong L, Wu Y, Lin F. Preliminary separation and purification of rutin and quercetin from *Euonymus alatus* (Thunb.) Siebold extracts by macroporous resins. Food Bioprod Process. 2011;89(4):266-72. doi: 10.1016/j.fbp.2010.11.001
19. ICH Harmonised Tripartite Guideline. Validation of Analytical Procedures: Text and Methodology. Vol. 5. European: European Medicines Agency; 2005. p. 1-20.
20. Mahanur VB, Rajge RR, Pal RS, Chaitanya MV, Vishwas S, Gupta S, *et al*. Harnessing unexplored medicinal values of the red listed South African weed *Erigeron bonariensis*: From ethnobotany to biomedicine. S Afr J Bot. 2023;160:535-46. doi: 10.1016/j.sajb.2023.07.031
21. Das S, Sultana KW, Chandra I. Characterization of polyphenols by RP-HPLC in *Basilicum polystachyon* (L.) Moench with their antioxidant and antimicrobial properties. S Afr J Bot. 2022;151:926-40. doi: 10.1016/j.sajb.2022.11.016
22. Kumar B, Singh SK, Prakash T, Bhatia A, Gulati M, Garg V, *et al*. Pharmacokinetic and pharmacodynamic evaluation of Solid self-nanoemulsifying delivery system (SSNEDDS) loaded with curcumin and duloxetine in attenuation of neuropathic pain in rats. Neurol Sci. 2021;42(5):1785-97. doi: 10.1007/s10072-020-04628-7, PMID 32885394
23. Dilek E, Dener M. Computer vision applications in intelligent transportation systems: A survey. Sensors (Basel). 2023;23(6):2938. doi: 10.3390/s23062938, PMID 36991649
24. Awasthi A, Kumar A, Kumar R, Vishwas S, Khursheed R, Kaur J, et al. RP-HPLC method development and validation for simultaneous estimation of mesalamine and curcumin in bulk form as well as nanostructured lipid carriers. S Afr J Bot. 2022;151:529-37. doi: 10.1016/j.sajb.2022.05.044
25. Scholz J, Mannion RJ, Hord DE, Griffin RS, Rawal B, Zheng H, *et al*. A novel tool for the assessment of pain: Validation in low back pain. PLoS Med. 2009;6(4):e1000047. doi: 10.1371/journal.pmed.1000047, PMID 19360087
26. Marcelín-Jiménez G, Angeles-Moreno AP, Contreras-Zavala L, García-González A, Ramírez-San Juan E. Comparison of fasting bioavailability among 100-mg commercial, 100-mg generic, and 50-mg chewable generic sildenafil tablets in healthy male Mexican volunteers: A single-dose, 3-period, crossover study. Clin Ther. 2012;34(3):689-98. doi: 10.1016/j.clinthera.2012.01.021, PMID 22386826

27. Kumar B, Malik AH, Sharma P, Rathee H, Prakash T, Bhatia A, *et al*. Validated reversed-phase high-performance liquid chromatography method for simultaneous estimation of curcumin and duloxetine hydrochloride in tablet and self-nanoemulsifying drug delivery systems. J Pharm Res. 2017;11:1166-78.
28. Patil SR, Kumar L, Kohli G, Bansal AK. Validated HPLC method for concurrent determination of Antipyrine, carbamazepine, furosemide and phenytoin and its application in assessment of drug permeability through Caco-2 cell monolayers. Sci Pharm. 2012;80(1):89-100. doi: 10.3797/scipharm.1109-03, PMID 22396906
29. Dejaegher B, Vander Heyden YV. Ruggedness and robustness testing. J Chromatogr A. 2007;1158(1-2):138-57. doi: 10.1016/j.chroma.2007.02.086, PMID 17379230
30. Gao L, Zheng Y, Zhao C, Teng H. Investigation on effect of basalin coated silver nanoparticles as antioxidant for alleviating peripheral neuropathy in mice treated with oxaliplatin. J Photochem Photobiol B. 2017;177:56-61. doi: 10.1016/j.jphotobiol.2017.10.003, PMID 29069632
31. Kuthati Y, Busa P, Goutham Davuluri VN, Wong CS. Manganese oxide nanozymes ameliorate mechanical allodynia in a rat model of partial sciatic nerve-transection induced neuropathic pain. Int J Nanomedicine. 2019;14:10105-17. doi: 10.2147/IJN.S225594, PMID 31920306