



Research Article

Exploring the Pharmacokinetics and Bioavailability of Naturally Occurring Picroside-II Through Ultra-High-Performance Liquid Chromatography (UHPLC) and Mass Spectrometry Analysis

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Abstract

Picroside II (PK-II), the primary bioactive iridoid glycoside derived from *Picrorhiza kurroa*, has attracted significant interest for its diverse range of biological activity. The aim is to develop a UHPLC-MS/MS method that is efficient, accurate, and responsive in measuring the concentration of PK-II in rat plasma. Sixty rats were administered a dose of PK-II (10 mg/kg intravenously and 20 mg/kg orally), and the results were determined using ultra-high-performance liquid chromatography–mass spectrometry (UHPLC–MS/MS) in negative-ion mode. Blood samples were collected at time intervals of 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 hours. The newly developed method demonstrated significant selectivity, accuracy, and rapidity (10 min) for the quantitative measurement of PK-II in biological samples. These results have implications for a better understanding of the pharmacokinetic effect of PK-II, which will be beneficial in its research and development.

Keywords: Picroside-II, Pharmacokinetics, Bioavailability, UHPLC-MS/MS, Stability, Natural Products

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Introduction

Natural compounds derived from medicinal plants have long been a source of diverse and potent therapeutic agents, captivating the attention of researchers for their potential pharmaceutical applications [1]. Among these, Picroside-II, a glycoside found in various plant species such as *Picrorhiza kurroa*, holds promise due to its reported pharmacological activities, including anti-inflammatory, hepatoprotective, and antioxidant properties. Despite its potential, the pharmacokinetic behavior and bioavailability of Picroside-II remain relatively unexplored [2].

Using cutting-edge analytical methods including Ultra-High-Performance Liquid Chromatography (UHPLC) and Mass Spectrometry (MS), this investigation seeks to delve into the specifics of Picroside-pharmacokinetics II's and bioavailability. Because of their superior sensitivity and precision, these analytical techniques are invaluable for deciphering the intricate pharmacokinetic profile of bioactive substances [3].

A comprehensive understanding of the pharmacokinetics of Picroside-II is important in order to clarify its mechanisms of absorption, distribution, metabolism, and excretion within the biological system. Further analysis of its bioavailability—the proportion of the compound that enters the systemic circulation

after administration—offers valuable information regarding its therapeutic capacity and assists in the development of dose plans that maximize effectiveness.

In light of ongoing investigations into natural chemicals as prospective therapeutic candidates, it is crucial for the pharmaceutical industry to possess a thorough comprehension of the pharmacokinetics and bioavailability of Picroside-II. Acquiring this knowledge not only facilitates the advancement of innovative treatment procedures but also illuminates the complexities and possibilities that arise when attempting to use the medicinal properties of natural items.

Mostly with goal of elucidating Picroside-pharmacokinetic II's behavior and bioavailability, this study adopts a rigorous technique that includes sample collection, preparation, and analysis utilizing UHPLC-MS. Research like this adds to our developing understanding of natural product pharmacology and has the potential to inform future studies and drug development including Picroside-II [4].

Material and Methods

Chemicals, Standards, and Instrumentations

Mass spectrometric grade (99.2%) pure formic acid was acquired from Merck Chemicals, Mumbai, India. LC-grade methanol and acetonitrile (ACN) were obtained from JT Baker, Mumbai, India. Milli-Q the in-house LabLink Extrapure system was used to get water for making the movable phase, seal washes, and rinsing solvent. Picroside-II (Purity 99.2% by HPLC) in house isolated from *Picrorhiza kurroa rhizomes*.

Instrument

The LC-MS/MS analysis was performed utilizing a cutting-edge Sciex system, which integrated sophisticated components to enable precise and dependable readings. A Sciex UHPLC system featuring binary AD pumps, a Degasser, AD Autosampler, AC Column oven, PDA Detector, Controller, and a QTRAP-4500 triple quadrupole-linear ion trap mass spectrometer formed this experimental configuration. Chromatographic separation was performed by employing a Phe-Hex column (100 x 4.6 mm, 2.7 μ m Poroshell 120 Agilent) and Analyst 1.7.3 software to handle the complete system. The meticulously selected and all-encompassing amalgamation of chemicals, standards, and apparatus serves as the fundamental basis for the rigorous analytical methodology implemented in this research [5].

Bioanalytical method development

Utilizing a sophisticated and sensitive analytical methodology, the Bioanalytical LC-MS/MS (Liquid Chromatography-Tandem Mass Spectrometry) method is utilized to quantify and identify a wide range of chemicals, including Picroside II. By integrating the substantial separation efficiency of liquid chromatography with the extraordinary sensitivity and specificity of tandem mass spectrometry, this approach achieves an unprecedented result. Within the framework of our research concerning the hepatoprotective properties of Picroside II, the Bioanalytical LC-MS/MS technique functions as a potent instrument for

precisely quantifying the Picroside II concentration in biological samples. This facilitates the evaluation of the substance's pharmacokinetics, distribution, metabolism, and excretion from the organism [6].

Optimization of Compound and Source Parameters of Mass Spectrometer

To enhance the analytical accuracy of the mass spectrometer, a tuning solution containing 200 ng/ml Picroside II in acetonitrile was carefully created. The tuning solution was systematically administered using the built-in syringe pump to maintain a regulated flow rate of 10 μ l/min and guarantee consistent delivery. A number of compound factors, including de-clustering potential (DP), entrance potential (EP), collision energy (CE), and collision cell exit potential, were the focus of the optimization procedure (CXP). The tuning solution of Picroside II was employed to methodically adjust these parameters with the objective of augmenting the mass spectrometer's sensitivity and specificity with respect to the molecule being studied [7].

Concurrently, optimization was performed on source parameters, including capillary voltage, source temperature, GS-1 flow, GS-2 flow, and CAD gas flow, by flow injection analysis (FIA). By employing this methodology, the source conditions could be further optimized, resulting in enhanced ionization and transfer of Picroside II from the sample matrix to the mass spectrometer. By meticulously calibrating the mass spectrometer through the iterative process of tweaking and optimizing both compound and source parameters, subsequent studies can be certain to get an optimal performance level for the exact measurement of Picroside II [8].

Selection of Columns (Stationary Phases) and Mobile Phases

Based on the physicochemical features of the drug, suitable columns (stationary phases) and mobile phases were chosen for the LC-MS/MS analysis of Picroside II (PK-II). For optimal resolution, numerous mobile phases were rigorously evaluated on lipophilic stationary phases. In this examination, columns of various packing materials and particle sizes from various manufacturers were utilized. Efficient separation of components required an examination of the mobile phase composition, which encompassed the combination of organic solvents (methanol (MeOH), acetonitrile (ACN), and isopropyl alcohol (IPA) with modifiers (acetic acid, formic acid, and ammonium formate) [9-10].

Preparation of Reference Solutions

A volumetric flask containing 50 ml of PK-II was utilized to sonicate the 50 milligrams of the compound, which had been accurately weighed with a pre-calibrated analytical balance. A concentration of 1 mg/ml was attained through dilutions using acetonitrile (Stock-I). In order to mitigate evaporation-induced loss and protect the reference solution contained within the flask, it was enveloped in foil and sealed with paraffin film.

Preparation of Calibration Standards (CAL STD)

Stock-I solutions were subsequently diluted with acetonitrile of MS grade to produce various working stock solutions. In order to satisfy the following seven calibration standards: 25, 50, 100,

200, 400, 800, and 1600 ng/ml, appropriate amounts of Stock-I were diluted.

Preparation of Quality Control Standards (QC STD)

To make stock-II solutions with a concentration of 1 mg/ml, PK-II was weighed, dissolved, and diluted to the necessary volume. Every day of the experiment, these Stock-II solutions were diluted to a new concentration, and three quality control standards were generated using PK-II at concentrations of 30, 800, and 1500 ng/ml.

Preparation of System Suitability Standards (SS STD)

Solution of Stock-III (1 mg/ml) was made using the same method as that which was outlined for Stock-II. Stock-III solutions were diluted on the day of the experiment, and 30.00 ng/ml system suitability standards (SS STD) were established.

Validation of LC-MS/MS method

Method validation was conducted in compliance with International Conference on Harmonization norms (ICH). Linearity, range, accuracy, precision, specificity, limit of quantification, and robustness were the parameters evaluated [13].

Specificity

This study validated its LC-MS/MS methodology to accurately quantify Picroside II in complicated biological matrices. Picroside II was distinguished from probable interferences by chromatographic separation of blank and spiked samples. Injecting a Picroside II solution and evaluating its unique ion signals proved mass spectrometric specificity. Picroside II and co-eluting chemicals were injected to test selectivity and ensure peak distinctness. Analysing samples containing matrix components like biological samples analysed matrix effects. Injecting Picroside II solutions with probable degradation products confirmed the method's resistance against degradation and distinguished Picroside II from its deteriorated variants. Picroside II measurement in complicated biological matrices is reliable and precise with this full method validation [14-16].

System suitability (SS)

For the system suitability test, the System Suitability Standard (SS STD) at a concentration of 30 ng/ml was employed. Prior to sample analysis, nine replicate injections of the SS STD were performed. Acceptance criteria were set at less than 0.5% relative standard deviation (R.S.D.) for retention time and less than 1.5% R.S.D. for peak area, accompanied by a requirement of more than 3500 plates for each peak.

Linearity and range

In order to evaluate the linearity of Picroside II (PK-II) and determine an appropriate concentration range, seven-point calibration curves were painstakingly generated. These curves encompassed a predetermined range of PK-II concentrations. The correlation between concentrations and

peak areas was examined by applying linear least square regression analysis to the plots that were obtained. To evaluate the dependability and uniformity of the calibration curves, daily and intra-day linearity were exhaustively examined. The thorough examination of linearity and range in this research establishes a fundamental basis for precise quantification of Picroside II throughout a range of concentrations in following investigations [17-18].

Accuracy and precision

The accuracy and precision of the LC-MS/MS approach were methodically assessed in order to ascertain its reliability in quantifying Picroside II. In order to ascertain recovery, pre-determined concentrations of Picroside II standards were created and examined by comparing the concentrations obtained with the expected values. This procedure, which was performed in duplicate at different concentrations, confirmed the correctness of the approach. In order to evaluate the reliability-critical precision, the relative standard deviation (R.S.D.) for retention time and peak area was computed both intra-day and inter-day. The acceptance criteria, which stipulate an accuracy range of 90 to 110 percent and a root mean square deviation of less than 2 percent for precision, validate the method's capacity to consistently produce accurate outcomes. This establishes a strong basis for subsequent analyses involving the quantification of Picroside II [19-20].

Lower limit of quantitation (LLOQ)

In order to establish the lower limit of the LC-MS/MS method's quantification range for Picroside II, the LLOQ was calculated. A sequence of Picroside II standards was created, varying in concentration, in order to cover the expected lower limit of quantification. The standards underwent examination with the LC-MS/MS technique. The LLOQ, which stands for the minimum concentration of Picroside II that could be quantified with acceptable precision and accuracy (between 80-120 percent) and range deviation (R.S.D. < 20 percent), was established. The LLOQ examination comprised numerous injections of the lowest standard concentration, and a comprehensive evaluation of the signal-to-noise ratio and precision metrics confirmed the concentration at the LLOQ. The method's capacity to withstand fluctuations in concentration below this threshold guarantees its appropriateness for precisely measuring minute quantities of Picroside II in intricate biological matrices, hence enhancing the overall dependability of the analytical methodology [21].

Robustness

Analyzing quality control standards under a variety of conditions specifically, by varying the intensity of the volatile buffer and the composition of the mobile phase the robustness of both analytical procedures was carefully assessed. Modified chromatographic conditions for the assessment of robustness were $\pm 1^\circ\text{C}$ deviation in column temperature, ± 0.05 ml/min deviation in flow rate of mobile phase and ± 1 unit deviation in volume of mobile phase. For the robustness study, a solution (800 ng/mL) was repeatedly ($n=5$) analyzed for retention time and amount of PK-II using above-mentioned modified chromatographic conditions. Acceptance was determined based on a variation in final results of less than 2 percent subsequent to alterations in mobile phase

composition and buffer strength; this criterion was established in order to determine the method's resistance to change. By subjecting the procedures to various experimental settings, this robustness testing guarantees their dependability and reproducibility

Stability

A sequence of stability studies was conducted to ascertain the stability of PK-II in plasma. These tests involved the use of spiked plasma samples at varying doses. The experiments comprised the following: (a) a one-month deep freeze storage period at -80 °C, (b) three consecutive freeze-thaw cycles, (c) a twenty-four-hour storage period at ambient temperature, and (d) a short-term stability assessment at 10 °C within the autosampler. For the stability study, a solution (800 ng/mL) was repeatedly (n=5) analyzed for amount of PK-II using above-mentioned modified chromatographic conditions. Strictly adhering to each designated storage condition, the materials underwent rigorous processing and analysis. The stability testing conducted offers vital insights regarding the LC-MS/MS method's capacity to withstand diverse storage conditions and probable fluctuations in sample handling, specifically in regards to PK-II analysis.

In-vivo pharmacokinetic studies

The purpose of this research was to compare a pharmacokinetics of Naturally Occurring PK-II. Wistar rats and Swiss albino mice were procured from Crystal Biological Solutions Pune, and the handling protocols adhered to the rigorous standards established by the Good Laboratory Practice (GLP) as defined in the CPCSEA guidelines. Every experimental procedure received careful scrutiny and authorization from the Institutional Animal Ethics Committee, substantiated by the allocation of registration number 2030/ PO/ RcBiBt/ S/18/ CPCSEA, and project proposal number CRY/2223/084.

Absorption Study of PK-II

A randomized study was done to assess the pharmacokinetics of PK-II. The studies were conducted using male Wistar rats weighing between 130 and 150 g. Animals were acclimatized for a week in natural light and dark, with free access to water and a temperature of 25°C and 50–60% relative humidity. The animals were fasted overnight before to the experiment. PK-II was given intravenously to one group of six rats at a dose of 10 mg/kg of body weight, and orally to the other group of six rats at a dose of 20 mg/kg of body weight. The oral gavage and syringe were used to administrate the PK-II formulations. Approximately 200 µL of blood samples were collected at specific time intervals of 00, 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 hours after administering the dosage [22-23].

Tissue Distribution Study of PK-II

The Wistar rats were randomly divided into six groups. PK-II solutions were orally administrated at a dose of 20 mg/kg to rats of each group. At determined time points (0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24), six animals at each time point from each group were given anesthesia and blood was

collected from the postorbital vein into heparinized tubes and was centrifuged to get the plasma samples. Tissues of interest liver, heart, spleen, lung, kidney, brain, testicle, uterine, and ovary were collected immediately after cervical dislocation at different time points and weighed rapidly. They were placed into normal saline solution to remove the blood or content and blotted dry with tissue paper. The plasma and tissue samples were frozen at -20°C until analysis [24-25].

Plasma and tissue sample analysis

To analyze plasma samples, an optimized liquid-liquid extraction method was applied. 600 µL ml LC-MS grade ACN was added to the 200 µl spiked plasma samples (PK-II added externally to achieve 30 ng/ml, 800 ng/ml, and 1500 ng/ml conc. in plasma). The supernatant was collected in a new microcentrifuge tube after the plasma sample was vortexed for two minutes and centrifuged for ten minutes at 10,000 rpm. The supernatant was evaporated using a centrifugal vacuum concentrator and reconstituted in 500 µl of LC-MS grade ACN. The sample was filtered using a 0.45 µ nylon filter and analyzed using LC-MS.

Results and discussion

Optimized Compound and Source Parameters of Mass Spectrometer

Before optimizing the UHPLC conditions, a thorough tuning process was conducted on the mass spectrometer utilizing two distinct tuning solutions—one composed entirely of organic solvent and the other of an organic solvent with an aqueous component—in order to evaluate their influence on the PK-II mass spectrum. At first, the operation of the syringe pump was conducted at a rate of 30 µl/min; however, it was subsequently refined to 10 µl/min. The optimization procedure centered on the conditions of the parent mass.

Scanning Mode

In order to ascertain the parent mass's optimal intensity, the Mass Spectrometer functioned in both positive and negative modes. Following meticulous experimentation, it was discovered that the negative mode is best appropriate for the identification of the target parent mass of PK-II.

Q1MS Scan

The PK-II solution was scanned at a rate of 200 Da/s within a range of 100 to 900 Da during the Q1MS analysis. The desired parent mass was determined to be 510.88 Da in positive mode. Using the Q1MI scan mode, many ions of the parent mass of interest were detected subsequent to the Q1MS scan. The entry potential and de-clustering potential for Q1 were optimized throughout the experiment.

MS2 Scan

The MS2 scan was utilized to detect potential constituents of the parent target mass, which weighed 510.88 Da. The parent target mass remained constant throughout the experiment, but the collision energy and exit potential of the collision cell were increased in order to determine the most likely pieces. The 234.80 Da peak was identified as the most intense daughter ion peak.

Multiple Reaction Monitoring (MRM)

Q1 was established at 510.88 in the MRM mode, while Q3 was placed at 234.80. All parameters were held constant,

DP, EP, CE, and CXP. The TIC and XIC modes were utilized to monitor the intensity of the MRM transition from 510.88 to 234.80, as shown in tables 1, 2, and the figure 1 & 2.

Table 1: Optimized Values for MRM transition

Q1 Mass	Q3 Mass	DP	EP	CE	CXP
510.88	234.800	-95 V	-10 V	-30 V	-11 V

Table 2: Optimized Source Parameters

Curtain Gas Flow	CAD Gas	Ion-spray Voltage	Source Temperature	GS-1 Flow	GS-2 Flow
30	Medium	-4500 mV	5500C	20	20

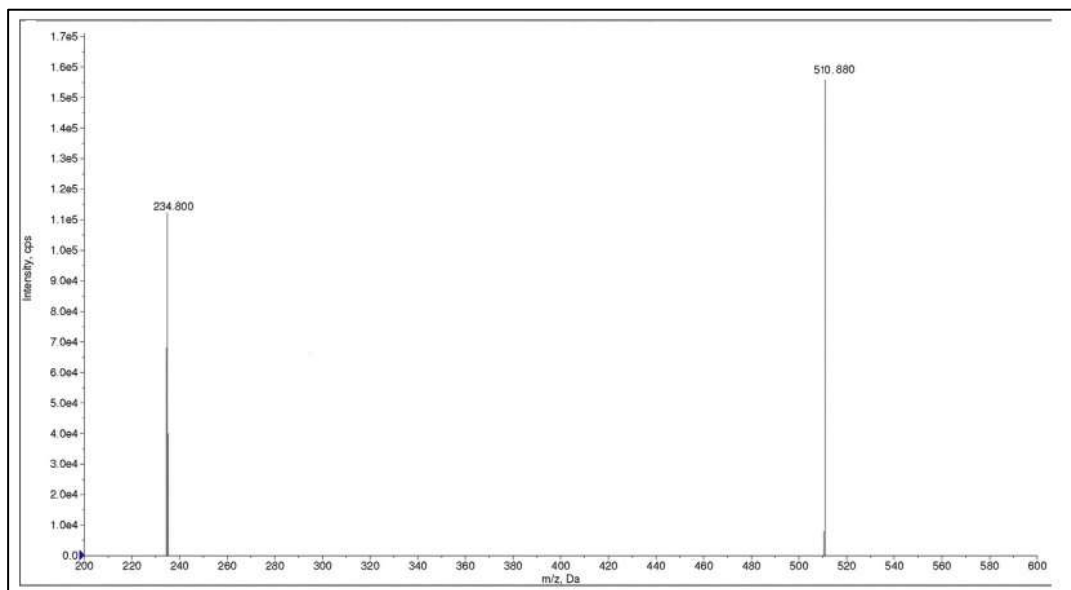


Fig. 1. Mass spectrum of isolated PK-II

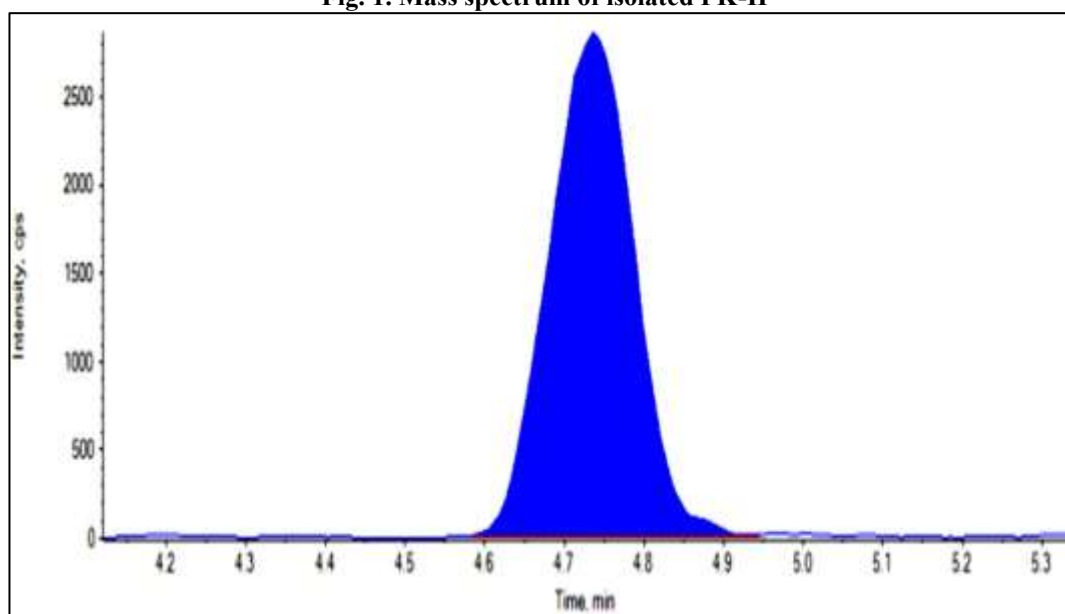


Fig.2: MRM Transition of Picroside II, m/z 510.88 to 234.80 Da.

Optimized UHPLC Conditions for PK-II

To achieve accurate and dependable characterization of Picroside II (PK-II), a comprehensive investigation was

undertaken into its chemical composition and physical characteristics. The systematically recorded data comprised crucial characteristics such as the pH, solubility, and lipophilicity

of a 1% solution of PK-II. Following an extensive assessment, a C-18 stationary phase including particles of diverse sizes was chosen. After consulting relevant scholarly works, a variety of volatile buffers were selected for further experimentation. The retention time and peak characteristics that were observed were utilized to determine the subsequent UHPLC conditions, which consistently yielded the anticipated outcomes.

By customizing UHPLC conditions to the specific attributes of PK-II, these conditions not only guarantee reliable

separation but also satisfy rigorous standards for precision and accuracy. The settings that have been provided establish a strong basis for the following quantitative examination of PK-II in intricate biological matrices. The findings validate the appropriateness of the selected UHPLC parameters for the dependable quantification of PK-II, providing a foundation for subsequent deliberations regarding the pharmacokinetics and bioavailability of the chemical (Table 3).

Table 3: MS & UHPLC Conditions for PK-II

S.No	Parameter	LC-MS/MS
1	Instrument	Sciex QTRAP 4500
2	Column	Phe-Hex, 100 X 4.6 mm, 2.7 µm Poroshell 120 Agilent Column Oven Temp: 40°C
2	Mobile Phase	0.1 Formic acid in water: ACN (80:20 v/v)
3	Flow rate	0.6 ml/min
4	R.T.	4.75 min
5	Plasma Recovery	ACN :89.64 to 91.42 % MeoH: 73.48 to 75.18 %
7	Linearity & Range	> 0.997 & 25 to 1600 ng/ml
7	Accuracy	-1.09and + 4.89
8	Precision	0.5780 and 1.6474

Validation of LC-MS/MS method

Specificity

The specificity of the method was confirmed by comparing extracted blank plasma with plasma samples spiked with PK-II. No interference was observed at the respective retention time of PK-II. This outcome substantiates the method's ability to selectively detect and quantify PK-II without being influenced by potential interferences.

System Suitability

The objective of the system suitability evaluation for the LC-MS/MS method that was developed was to verify its resilience and efficacy in the examination of Picroside II (PK-II). The

critical parameters, which comprised the number of theoretical plates, retention duration, and peak area, were evaluated in accordance with the predetermined acceptance criteria specified in Table 4.

The results of the system suitability assessment validate the method's capacity to consistently satisfy the predetermined acceptance criteria for critical parameters. The low percent RSD values for retention time, peak area, and the number of theoretical plates were well within the set limits, showing that the approach is precise and suitable for the accurate analysis of PK-II. The aforementioned positive results verify the LC-MS/MS technique's applicability for subsequent investigations and prove its dependability.

Table 4: System Suitability Parameters for PK-II

Parameters	Acceptance Criteria	Average	% RSD	Status
Retention Time	% RSD < 0.5	4.73 min	0.1846	Passed
Peak Area	% RSD <1.5	6155	0.3254	Passed
Theoretical Plates	> 3500	4586	0.2784	Passed

Linearity & Range:

Over a variety of concentrations, the linearity of the LC-MS/MS technique for Picroside II (PK-II) was evaluated, and the peak areas corresponding to this was calculated. The findings are succinctly presented in Table 5 and illustrated in Figure 2.

The significant correlation between the concentration of PK-II and the associated peak regions, as indicated by the high coefficient of determination ($R^2 = 0.9964$), provides evidence for the linearity of the approach. The y-intercept and slope data provide additional evidence that the calibration curve is linear.

The broad range of concentrations analysed by the method (25 to concentrations. The results of this study demonstrate that the 1600 ng/mL) validates its capacity to deliver precise and reliable technique is appropriate for the subsequent measurement of PK-II quantitative outcomes for PK-II across a variety of in intricate biological matrices.

Table 5: Linearity of Picroside II

Sr. No	Conc. (ng/mL)	Peak Area
1	25	4897.6
2	50	44541.6
3	100	116676
4	200	186988
5	400	455329.6
6	800	1137737
7	1600	2439175

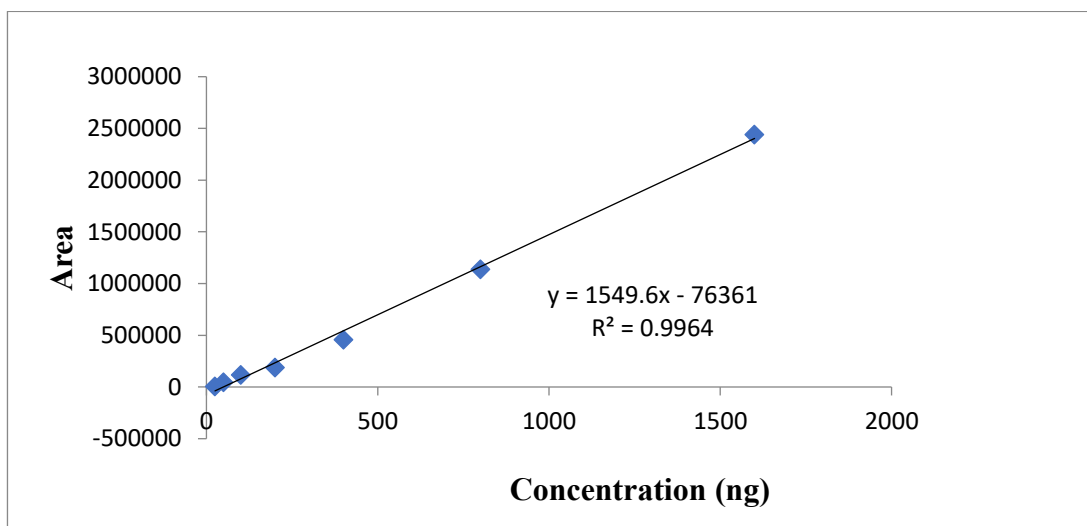


Fig.3: Calibration curve of Picroside II

Accuracy & Precision

Systematic evaluations of the LC-MS/MS method's accuracy and precision were conducted via recovery studies and intra-day and inter-day precision assessments. The accuracy of the procedure is illustrated in Table 6 for control samples of low, medium, and high quality. The results are consistent and fall within acceptable variation percentages. The precision data

presented in Table 7 illustrates the reproducibility of the procedure both within and between sets. The results of this study provide consistent evidence that the approach accurately measures Picroside II concentrations in intricate biological matrices; hence, it establishes a strong basis for its utilisation in research pertaining to pharmacokinetics and bioavailability.

Table 6: Recovery studies of PK-II

Sr. No.	Sample	Nominal Conc. (ng/ml)	Accuracy (Diff. %)					
			Intra-run (n=9)			Inter-run (n=9)		
			Set 1	Set 2	Set 3	Set 1	Set 2	Set 3
1	LQC	30	3.98	4.82	4.89	4.19	4.69	4.18
2	MQC	800	2.82	3.65	3.96	3.54	3.47	3.42
3	HQC	1500	-1.09	-0.88	-0.25	-0.88	-0.70	-0.64

Table 7: Intra & Inter-day precision data for PK-II

Sr. No.	Sample	Nominal Conc. (ng/ml)	Precision (% CV)					
			Intra-run (n=9)			Inter-run (n=9)		
			Set 1	Set 2	Set 3	Set 1	Set 2	Set 3
1	LQC	30	0.9745	1.6474	0.8674	1.3023	1.0268	1.3943
2	MQC	800	0.6726	0.6726	0.6240	0.6389	0.8267	0.9166
3	HQC	1500	0.7594	0.7888	0.5865	0.9819	0.5780	0.7734

*The validation was performed separately in terms of sets.

Lower Limit of Quantitation (LLOQ)

The Lower Limit of Quantitation (LLOQ) for PK-II was established at 20 ng/ml, signifying the lowest concentration at which reliable quantification can be achieved.

Robustness

Robustness Intentionally, slight deviations in the composition of the mobile phase and the strength of the buffer were incorporated in order to evaluate the resilience of the LC-MS/MS technique. The implemented modifications did not yield substantial changes in the performance of the method, as

indicated by the consistent values of retention time, resolution, peak characteristics, and theoretical plates. The % RSD values were found to be less than 2 (table 8). The robustness of the approach implies that it can withstand little modifications in experimental circumstances, hence enhancing its dependability for the accurate and precise measurement of PK-II. The resilience of this method is crucial in order to guarantee consistent and reliable outcomes under a wide range of analytical settings, hence expanding its practicality in several experimental contexts.

Table 8. Robustness study for PK-II

Sr.	Parameter	Setting	PK-II			
			RT	% RSD	Amount (ng)	% RSD
1	Column Temperature (°C)	39	4.821	0.1231	798.67	0.1287
		40	4.751	0.0264	800.12	0.1057
		41	4.971	0.0763	801.92	0.1043
2	Mobile phase flow rate (ml/min)	0.5	4.801	0.0458	799.32	0.1384
		0.6	4.755	0.0627	799.98	0.7880
		0.7	4.698	0.0575	800.83	0.3016
3	Mobile phase composition (% v/v)	80.8:19.2	4.759	0.0864	800.01	0.3077
		80:20	4.752	0.0421	798.12	0.8721
		79.8:20.2	4.750	0.0348	799.95	0.1737

Stability study

A detailed examination of the stability of the LC-MS/MS technique was conducted across a range of circumstances, as outlined in Table 9. Systematically, the stability of the autosampler, storage stability in plasma at -80°C, freeze-thaw stability, and short-term stability in plasma at room temperature were evaluated. The theoretical concentrations and

percent RSD values (Table 9) offer valuable insights regarding the robustness and dependability of the approach when subjected to various storage and handling conditions. The stability assessments play a significant role in enhancing the method's applicability for precise PK-II measurement in practical experimental situations.

Table 9. Stability data of PK-II

Sr.No.	Condition	Theoretical conc. (ng/ml)	% RSD (Acceptable limits (% RSD < 15).
1	Storage stability in plasma (-80oC)		
	0 month	801.71	3.7812
	1 month	798.98	7.4261
2	Freeze-thaw stability		
	0 cycles	799.37	4.3712
	3 cycles	798.92	5.7523
3	Short-term stability in plasma at room temperature		
	0	801.23	2.2716
	24 h	800.32	6.8392
4	Autosampler stability		
	0	799.03	2.9230
	24 h	800.67	7.8932

Pharmacokinetic studies of Picroside-II

Oral Absorption and Bioavailability

A comprehensive investigation was conducted on the pharmacokinetic parameters of Picroside II subsequent to both oral and intravenous (IV) administration. This analysis yielded valuable insights into the behavior of the compound within the organism, as summarized in Table 10 and illustrated in Figures 4, 5.

The pharmacokinetic profile of Picroside II, which was assessed subsequent to oral and intravenous (IV)

administration, unveils discernible patterns in multiple parameters, providing significant knowledge regarding its activity inside the biological system. An IV administration results in a considerably greater C_{max}, which signifies an immediate systemic exposure, as opposed to the oral route which requires absorption and gastrointestinal processing. IV treatment results in a practically immediate time to achieve maximum concentration (T_{max}), but oral administration causes a little delayed absorption process. It is worth noting that oral administration of Picroside II results in a more

pronounced accumulation of exposure over time, as indicated by larger AUC 0-∞ values. The shorter half-life ($t_{1/2}$) that is observed following oral administration indicates a potentially accelerated clearance, which may be ascribed to metabolic and excretion mechanisms. The volume of distribution (Vd) values for both pathways are comparable, suggesting that they are widely distributed throughout the body. Nevertheless, the increased clearance (CL) subsequent to oral ingestion suggests a more streamlined

mechanism of elimination. The orally delivered Picroside II has a systemic availability that is approximately 69.73 percent, as indicated by its calculated bioavailability, in contrast to the IV administration. The combined results of this study make a substantial contribution to the understanding of the pharmacokinetic profile of Picroside II. This knowledge is vital for future research endeavors and the development of possible therapeutic applications.

Table 10: Pharmacokinetic Parameters of Picroside II

Parameters	Unit	PK-II (IV)	PK-II (ORAL)
Cmax	µg/ml	2087.8000	354.1481
Tmax	Hr	0.0833	1.0000
AUC 0-t	µg.hr/ml	977.4456	1363.2952
AUC 0-∞	µg.hr/ml	1028.6617	1414.5607
$t_{1/2}$	Hr	11.3759	7.8949
Vd	ml/kg	159.5805	161.0724
CL	ml/hr/kg	9.7214	14.1387
Bioavailability	%	-	69.73

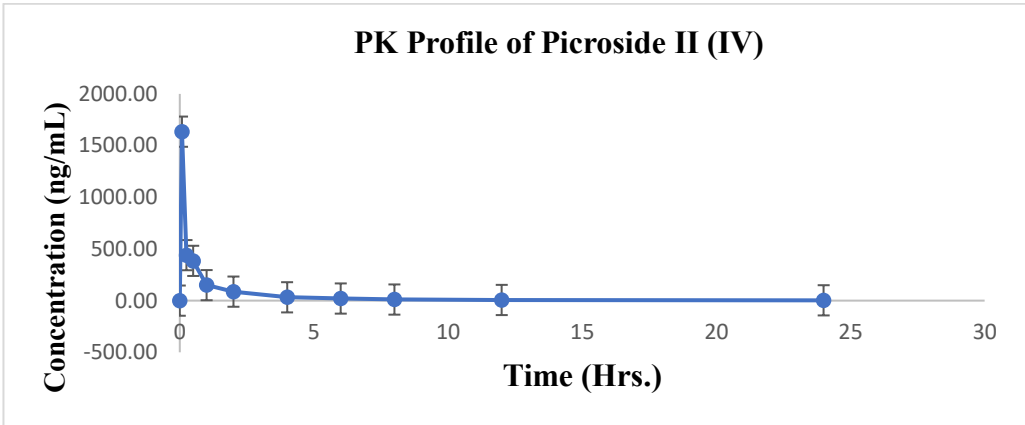


Fig.4: PK Profile of Picroside II (IV)

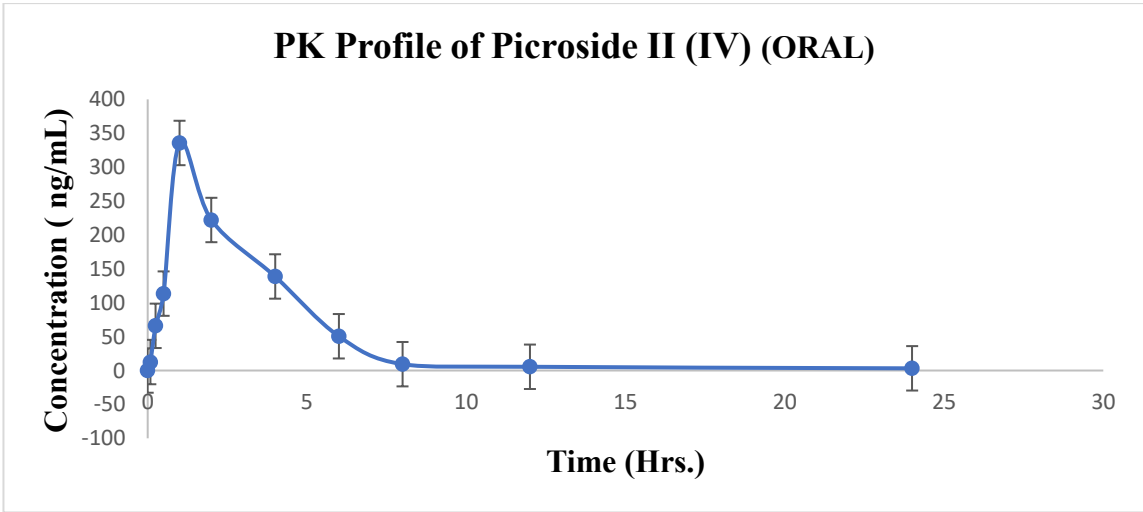


Fig.5: PK Profile of Picroside II (IV) (oral)

Tissue Distribution Study

The tissue distribution study provides insights into the concentration dynamics of Picroside II by depicting its trajectory through different physiological tissues at different

time intervals. The tissue distribution study illustrates temporal variations in the distribution of Picroside II among different bodily tissues, with particular emphasis on tissue preferences during several stages of its progression, as detailed in Table 11

and illustrated in Figure 5. Significantly, discernible accumulation patterns are identified in gonadal and brain tissues, indicating possible therapeutic ramifications that extend beyond the systemic circulation outlined in Table 12

and illustrated in Figure 7. It is probable that the chemical influences reproductive and neurological processes, as evidenced by its affinity for these particular organs.

Table 11: Tissue Distribution at Different Time Points

Time (Hrs)	Liver	Kidney	Spleen	Heart	Llung	Stomach	Intestine	Brain
0.0833	5.168	6.089	1.05	2.16	4.16	1.65	3.59	1.25
0.25	20.15	25.168	3.68	5.69	9.49	2.61	5.26	4.1658
0.5	60.15	72.155	8.94	12.46	18.64	3.49	10.95	6.42
1	98	102.15	12.6	15.16	25.16	6.68	14.15	10.05
2	70.19	60.15	7.69	12.35	19.48	4.63	9.63	7.59
4	10	15.158	4.165	5.64	6.48	1.2	5.12	4.16
6	5.16	3.45	1.28	2.16	1.528	0.79	1.84	1.28

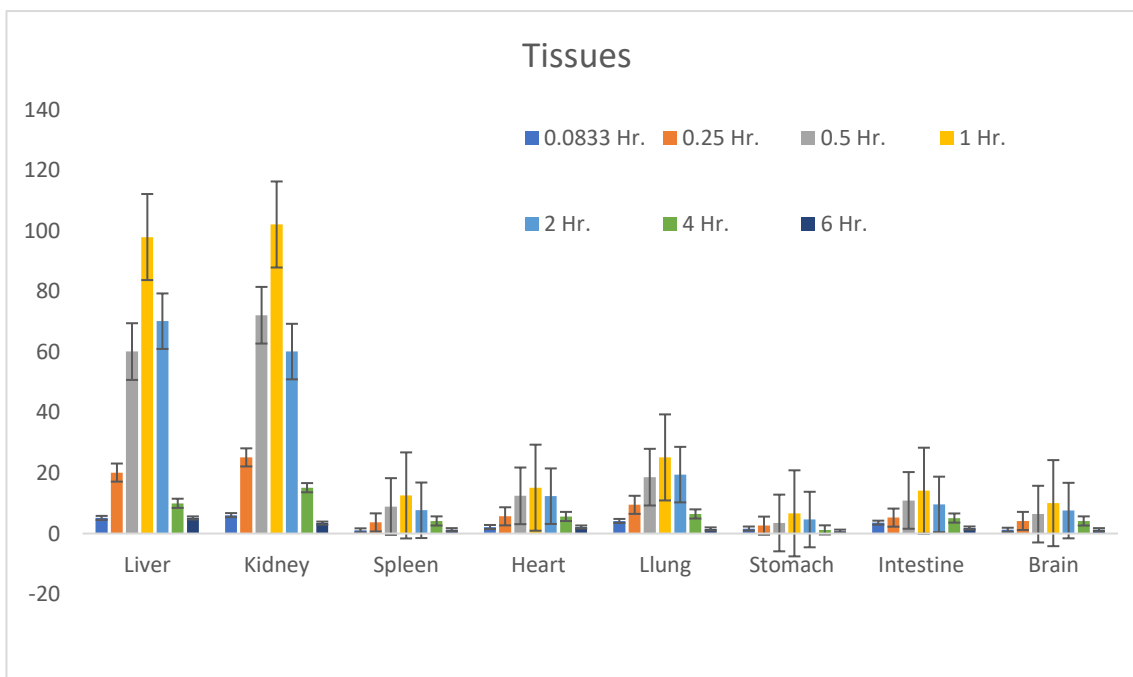


Fig.6: Tissue Distribution at Different Time Points

Table 12: Brain and gonadal tissue distribution studies

Time (Hrs)	Brain	Uterus	Ovary	Testis
0.0833	1.25	5.03	4.35	4.68
0.25	4.1658	20.15	18.15	19.254
0.5	6.42	55.36	32.15	35.15
1	10.05	70.18	40.18	50.164
2	7.59	40.15	29.32	31.258
4	4.16	20.15	18.42	20.19
6	1.28	2.18	1.28	1.25

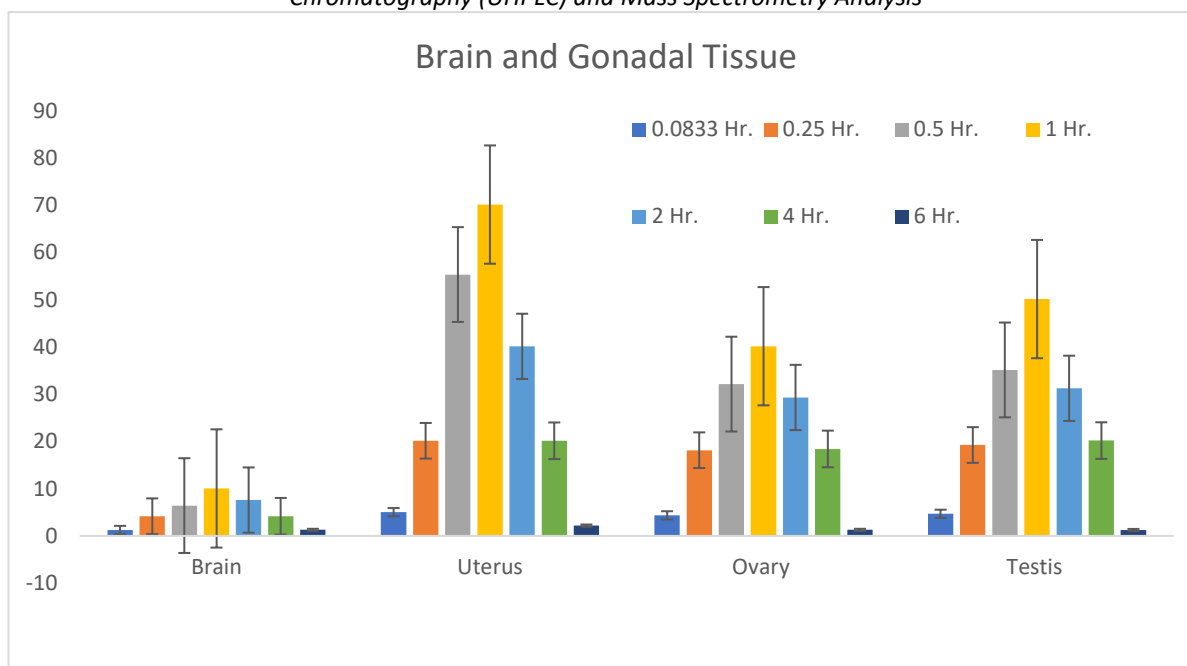


Fig.7: Brain and gonadal tissue distribution studies

In conclusion, our investigation utilizing Mass Spectrometry and Ultra-High-Performance Liquid Chromatography has yielded exhaustive knowledge regarding the pharmacokinetics and bioavailability of Picoside-II. The stringent methodologies utilized exhibited accuracy and consistency over a range of concentrations. The results make a substantial contribution to the comprehension of Picoside-activity II's in biological systems, encompassing absorption and elimination. This study highlights the significance of employing sophisticated analytical methods when investigating the pharmacokinetics of natural products.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Abbreviations

LC-MS/MS: Liquid Chromatography-Mass Spectrometry
 PK-II: Picoside-II
 UHPLC: Ultra-High-Performance Liquid Chromatography
 R.S.D.: Relative Standard Deviation
 ACN: Acetonitrile
 PDA: Photodiode Array

DP: De-clustering Potential

EP: Entrance Potential

FIA: Flow Injection Analysis

LLOQ: Lower Limit of Quantitation

LOQ: Limit of Quantitation

% CV: Percentage Coefficient of Variation

R2: Coefficient of Determination

CAD: Curtain Air Device

GS: Gas Source

TIC: Total Ion Chromatogram

XIC: Extracted Ion Chromatogram

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