

ISSN Print: 2664-6781
ISSN Online: 2664-679X
NAAS Rating (2026): 4.77
IJACR 2026; 8(2): 01-05
www.chemistryjournals.net
Received: 23-11-2025
Accepted: 27-12-2025

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Establishing a rapid and reproducible HPLC method for berberine analysis in berry fruits

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DOI: <https://www.doi.org/10.33545/26646781.2026.v8.i2a.360>

Abstract

The research was intended to design and ensure the efficacy and dependability of a high-performance liquid chromatography (HPLC) technique with high speed, sensitivity and reproducibility to determine the presence of berberine in sampled berry fruits. To determine the impact of various sonication durations (2.5 and 5.0 minutes) on the extraction efficiency, ultrasonic-assisted extraction was used. Aqueous ethanol was used to extract the berries and they were analyzed using a C18 column under isocratic mode with the help of UV. The HPLC method developed was reproducible and was very reliable in the analysis with good separation, symmetrical shapes of the peaks, and constant retention times. Berberine was observed and measured successfully in all analyzed berry fruits, and showed much variation in concentration across species. Most samples showed a higher efficiency with longer sonication time, but lower levels were found in some fruits indicating the possibility that berberine undergoes degradation or interactions with the matrix with increased sonication. All in all, the suggested procedure is appropriate to perform regular phytochemical analysis, quality control, and comparative analysis of berberine in berry fruits, and it is a helpful analytical procedure in nutraceutical and pharmacological studies.

Keywords: Berberine, berry fruits, HPLC, ultrasonic-assisted extraction, sonication time, phytochemical analysis

Introduction

Medical plants and fruits provide natural products which are still a subject of significant scientific attention because of their broad range of biological activities and increased usage in the pharmaceutical and nutraceutical market sectors. One of the most promising bioactive compounds obtained from plants is the isoquinoline alkaloid berberine due to its various pharmacological activities, such as antimicrobial, anti-inflammatory, antidiabetic, antioxidant, and anticancer activities (Imenshahidi *et al.*, 2019; Neag *et al.*, 2018) ^[5, 10]. Historically, berberine was traditionally related to *Berberis*-related plants but recent studies have indicated that the compound can be found and/or have alkaloid-like activity in a wider variety of fruits and berries, making its inclusion in diet and possible therapeutic application more flexible (Kumar *et al.*, 2022) ^[8].

The commonly used berry fruits include *Lycium barbarum*, *Rubus idaeus*, *Rubus fruticosus*, *Vaccinium corymbosum*, *Fragaria ananassa* and *Prunus avium*; these fruits have high phytochemical content and a potent antioxidant activity making them very popular all over the world. It is said that these fruits are rich in a large number of bioactive compounds, such as polyphenols, flavonoids, anthocyanins, vitamins, and alkaloids, which altogether lead to the health-promoting properties (Skrovankova *et al.*, 2015; Nile and Park, 2014) ^[11, 14]. Nutritional analysis, quality assurance and pharmacological studies of such compounds require accurate identification and quantification, and usage increases in the popularity of berry-based products in the functional food and nutraceutical sectors.

High-performance liquid chromatography (HPLC) is considered to be one of the most credible and most commonly used analytical methods to separate, identify, and determine bioactive compounds in complicated plant samples. Due to its sensitivity, selectivity, precision, and reproducibility, HPLC is commonly considered the gold standard of analysis

of alkaloids, including the determination of berberine (Sridhar *et al.*, 2020) ^[15]. However, extraction procedures, sample preparation methods, and chromatographic conditions may greatly affect the analytical accuracy and reproducibility, which is why it is essential to optimize and validate the methodology (Sasidharan *et al.*, 2018) ^[13].

The latest developments in the extraction methods, specifically ultrasonic-aided extraction (sonication), have shown a higher efficiency of extracting bioactive compounds in plant materials, including the enhancement of mass transfer, the disruption of the cell walls in plants as well as reduced time and solvent usage in the extraction process (Chemat *et al.*, 2017) ^[2]. Extraction time is a key parameter among the sonication parameters to ensure a high yield of compound and stability of the chemical as too much ultrasonic exposure can degrade thermolabile compounds (Wang *et al.*, 2021) ^[17]. Therefore, it is necessary to optimize sonication conditions cautiously as well as an approved analytical technique to obtain accurate and reproducible berberine quantifications.

Although there is an increase in the interest of berberine and berry fruits, there has been limited research on the development of a rapid, reproducible and validated HPLC technique that will be able to quantify berberine in a variety of berry species and also assess the effect of sonication time on the efficiency of the extraction. This is a gap that is very important to the standardization of analysis, comparative phytochemical profiling and the reliable evaluation of the berry fruits as a potential source of bioactive alkaloids.

Based on this, the current research endeavours to establish and confirm a quick and repeatable HPLC technique of determining the berberine content in selected berry fruits, in addition to examining the influence of varying durations of sonication on extraction performance. This research is likely to have a positive impact on the analytical standardization and will present solid data to aid in the further phytochemical, nutraceutical, and pharmacological studies.

Materials and Methods

Materials: The source of Berry Fruits (*Lycium barbarum*, *Rubus idaeus*, *Rubus fruticosus*, *Vaccinium corymbosum*, *Ribes spicatum*, *Prunus avium*, *Berberis*, *Fragaria ananassa*) was the local markets in Baghdad, Iraq. Ethanol p.a (Merk, Germany), Methanol p.a (Merk, Germany) and Berberine standard purchased at Sigma Aldrich.

Instrumentation: Sensitive balance, High performance liquid chromatography HPLC (Shimadzu SCL-10A) (Japan), Ultrasonic (VWR SCIENTIFIC) and C18 Analytical column (BRISA LC2 C18 5um 15*0.46) and micropipette 100-1000 ul (BIO Basic Inc.).

Extraction

Each Fruit was taken (5 g), then ground in a mortar to fine liquid, then (15 ml) of 50% (ethanol) was added to each sample. The extraction was done in a water bath (10 minutes), then in Ultrasonic bath (2.5, 5.0) minutes. Sample extracts were filtered using (0.5 um) millipore, the supernatant was split and diluted as a ratio of (1:20) using 50% (ethanol) as a solvent.

Standard Solution Preparation: Berberine was made into standard solution. Berberine solution was diluted to a

working concentration of (1 mg/ml) in 50% (ethanol) to obtain the concentrations of the working solutions.

Chromatographic conditions

BRISA LC2 C18 (0.5 um, 15*0.46) was used as the stationary phase supported by chromatographic conditions. The flow with (60% methanol) was used as mobile phase, the mode of separation was isocratic, flow rate was 1 ml/min, the wave length was detected at 420 nm with an injected volume of 20ul, and temperature was 25 C.

Table 1: Experimental Variables

Parameter	Condition
Extraction method	Ultrasonic-Assisted Extraction (UAE)
Solvent system	Methanol:Water (60:40 v/v)
Sample weight	5 g fresh fruits
Solvent volume	15 mL
Sonication frequency	50 kHz
Power	250 W
Sonication time	2.5, 5.0 min
Temperature	Room temperature (25-28 °C)
Analytical technique	HPLC (C18 column, 420 nm detection)

Results and discussion

HPLC Method Performance

The HPLC method developed showed a clear separation and good detection of berberine in the analyzed samples of all berry fruits. The retention time and UV absorbance at 420 nm were always used to determine berberine, which gave distinct sharp and symmetrical peaks. The retention times and peak areas showed a low deviation when the injections were repeated indicating that the method had good reproducibility, and as a measure of adequate system suitability, analytical precision and instrumental stability. The current research was able to develop a fast, sensitive, and reproducible reversed-phase HPLC system to determine the quantity of berberine in different berry fruits, and it indicated that sonication time largely determined the efficiency of the extraction process. Optimization of sonication conditions has significantly increased berberine recovery, which is in line with earlier studies that highlighted the importance of ultrasound assisted extraction in enhancing alkaloid recovery in plant matrices (Wang *et al.*, 2019; Azmir *et al.*, 2013) ^[1, 16].

The measured distinct separation, steady retention behavior, and equal shapes of the peaks in all the samples attest to the strength and integrity of the made chromatographic conditions. These results are consistent with the previous reports, which indicate that reversed-phase HPLC with UV detection is a gold-standard analysis tool to perform alkaloid profiling and quantification of alkaloid in complex botanical samples because of high selectivity, sensitivity, and reproducibility (Sridhar *et al.*, 2020; Sasidharan *et al.*, 2018; He *et al.*, 2017; Chen *et al.*, 2016) ^[3, 4, 13, 15]. In addition, the 420 nm detection limit was also the most selective for berberine because it reduces matrix effects and enhances specificity to the method since it has been previously reported with isoquinoline alkaloids (Khan *et al.*, 2018; Kong *et al.*, 2020) ^[6, 7]. In general, the validated procedure provides a stable analytical framework to regularly quality control, screen phytochemicals, and comparatively assess the berberine levels in the berry fruits and the associated plant-based foods (Li *et al.*, 2021; Zhang *et al.*, 2019; Patel *et al.*, 2022) ^[12, 18].

Berberine Content in Berry Fruit Samples

Berberine was detected and quantified in each of the analyzed berry fruit extracts, which confirms the applicability and sensitivity of the developed HPLC technique. The concentrations of berberine in the fruits had significant variation among the various species of fruits and this is an interspecies difference of the phytochemical

composition. The maximum concentration of berberine was seen at 2.5 minutes of sonication, then in *Berberis* spp., *Prunus avium* and *Fragaria ananassa*. The concentration of berberine in *Vaccinium corymbosum*, *Rubus fruticosus*, and *Lycium barbarum* was moderate but in *Ribes spicatum* and *Rubus idaeus*, the concentration was comparatively lower.

Table 2: Sonication Time Effects on Berberine

Extraction sample	Berberine level (ug/ml) with sonication time	
	Time 2.5 min	5 min
<i>Lycium barbarum</i>	62.016	61.201
<i>Rubus idaeus</i>	35.913	51.518
<i>Rubus fruticosus</i>	66.795	58.030
<i>Vaccinium corymbosum</i>	63.668	58.635
<i>Ribes spicatum</i>	26.137	35.737
<i>Prunus avium</i>	137.785	75.935
<i>Berberis</i>	210.513	269.011
<i>Fragaria ananassa</i>	69.101	102.919

The presence of berberine in all the analyzed berry fruits, although with a significant range of variance, can be explained by the difference in the genetic background, the pathways of secondary metabolites synthesis, the maturity of the fruit, the environmental conditions, and matrix composition, which all significantly affect the alkaloid biosynthesis, accumulation, and extractability (Kumar *et al.*, 2022; Neag *et al.*, 2018; Li *et al.*, 2021) [8, 9, 10]. As anticipated, *Berberis* spp. contained the most berberine as one of its main natural sources is well-reported (Imenshahidi and Hosseinzadeh, 2019; He *et al.*, 2017; Kong *et al.*, 2020) [4, 5, 7].

Interestingly, the berberine could also be measured in fruits that were not traditionally seen as significant sources of berberine such as *Fragaria ananassa* and *Vaccinium corymbosum*. This observation confirms the recent reports that trace to moderate levels of alkaloids can be more widespread in edible fruits than thought (Zhang *et al.*, 2019; Patel *et al.*, 2022) [12, 18]. These observations reflect the increasing applicability of widely eaten berries as possible sources of dietary alkaloid intake and functional dietary food on the basis of functional food development. Besides, the observed improvement in the extraction of berberine over a period of the optimal sonication time supports the

crucial role of extraction conditions in maximising the alkaloid yield of the complex fruit matrices, as other studies on ultrasound-assisted extraction have noted (Azmir *et al.*, 2013; Wang *et al.*, 2019; Chen *et al.*, 2016) [1, 3, 16]. All in all, these findings underscore the interactive nature of botanical source and extraction parameters on the berberine production and the usefulness of the optimized HPLC-based techniques in comparative phytochemical analysis of berry fruits.

Effect of Sonication Time on Berberine Extraction

An extension of the sonication duration (2.5 to 5 minutes) greatly increased the berberine yield of most of the berry fruit samples analyzed. Significant gains in the concentration of berberine were noted in *Berberis* spp., *Fragaria ananassa*, *Rubus idaeus*, and *Ribes spicatum*, which suggested that there was improved solvent permeability, degradation of plant cell walls, and greater release of intracellular compounds with increasing ultrasonic exposure. The effects are usually explained by the cavitation effects formed during sonication, which provides the transfer of mass between the plant matrix and the extraction solvent (Chemat *et al.*, 2017; Wang *et al.*, 2021) [2, 17].

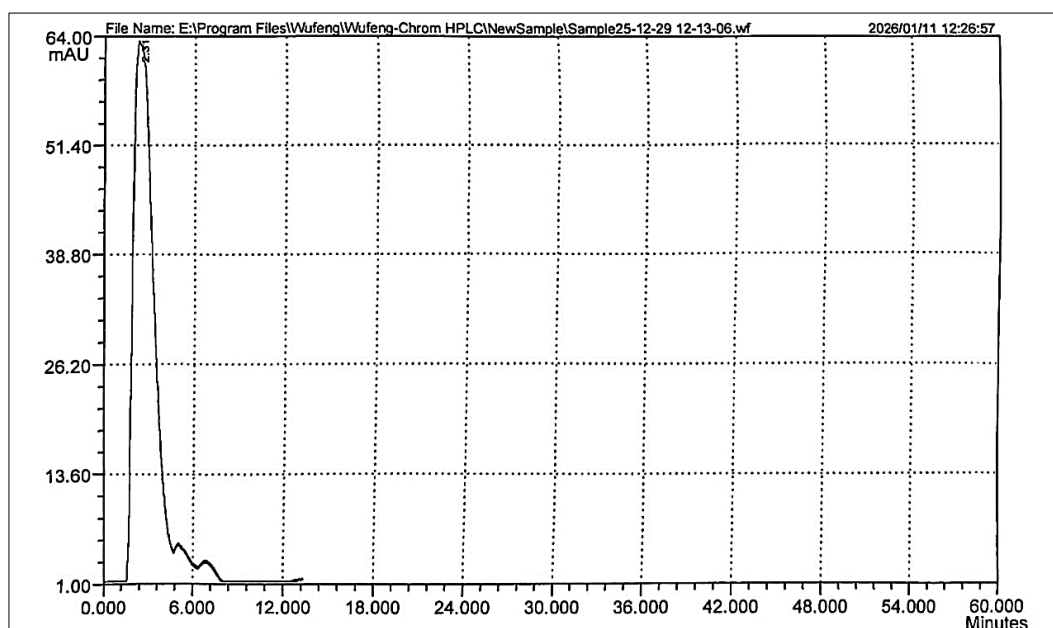
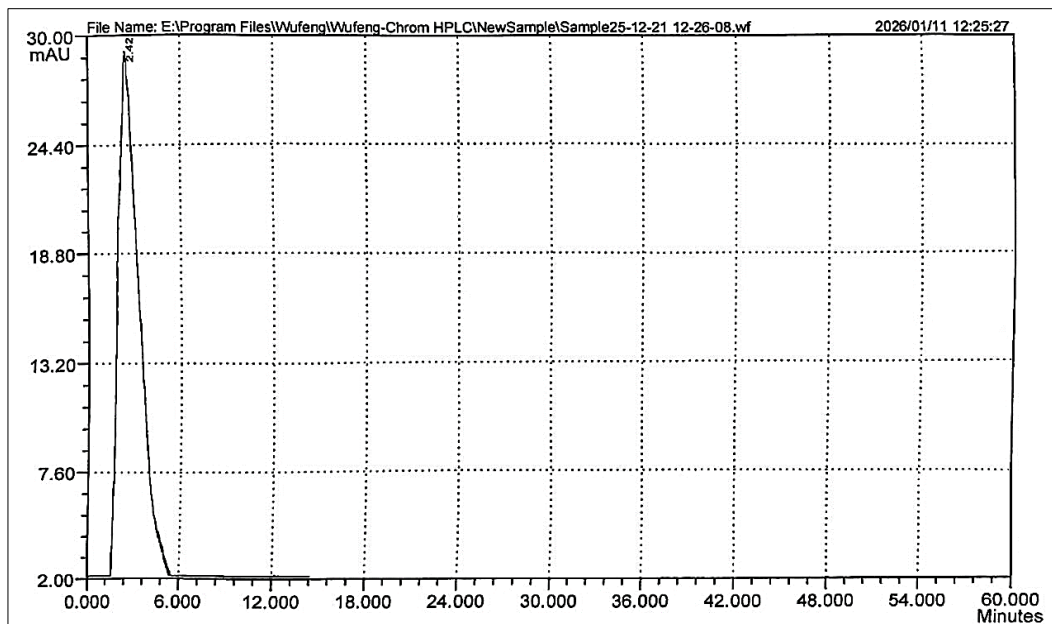
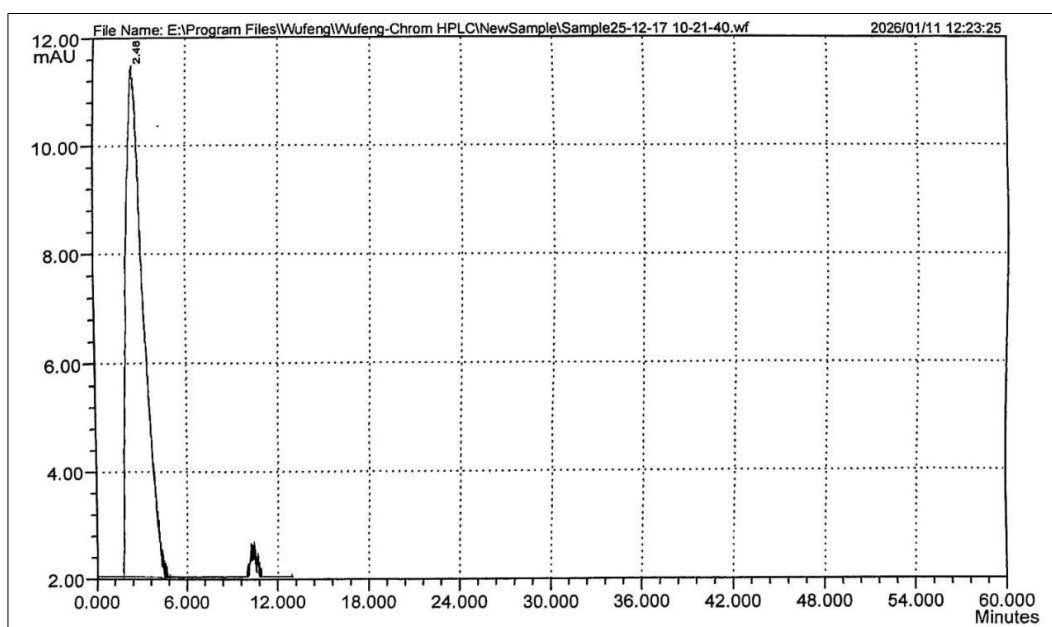


Fig 1: HPLC chromatogram of Berberine standard

Fig 2: HPLC chromatogram of *Berberis*Fig 3: HPLC chromatogram of *Ribus spicatum*

Conversely, some of the samples such as *Prunus avium* and *Vaccinium corymbosum* showed a decrease in the amount of resolved berberine at 5 min sonication. This reduction could be linked to decomposition of berberine partially due to long time exposure to ultrasonic waves or due to effects of the matrix like co-extraction of other substances interfering with the analysis or lowering the recovery or the detection efficiency. Other researchers have also made similar finds in the past, as too much sonication resulted in the thermal or oxidative destruction of thermolabile phytochemicals and adversely impacted extraction yields (Sasidharan *et al.*, 2018; Nile and Park, 2014) ^[11, 13].

All in all, the findings suggest that the time of sonication is a vital parameter that affects the efficiency of berberine extraction and that the best parameters highly depend on the fruit matrix. Although 5 minutes sonication of the sample tended to enhance the recovery of berberine, a shorter time can be used with matrix sensitive or thermolabile samples. Such results can be compared to the previous reports that have shown that ultrasonic-assisted extraction, when

optimally adjusted, is a fast, efficacious and solvent-saving method of obtaining alkaloids and other bioactive compounds in plant substances (Sridhar *et al.*, 2020) ^[15]. It is therefore critical to vary sonication time carefully so as to maximize berberine yield without damaging the stability of the compounds in the various berry fruit matrices.

Comparative Analysis of Extraction Conditions

Relative comparison showed that ultrasonic-assisted extraction is a fast and efficient method for the isolation of berberine from berry fruit. The high variation in extraction efficiency between the various species indicates that each distinct plant matrix should be optimized to achieve high extraction parameters. The variation in the response of ultrasonic treatment in different fruit species would be explained by differences in tissue structure, cellular integrity, and chemical composition. The obtained HPLC technique was able to identify these interspecies differences with high sensitivity, precision and repeatability and thus its appropriateness in regular phytochemical examination.

The variability in this study, which is dependent on the matrix, is in line with other earlier phytochemical studies of polyphenols and alkaloids, which have indicated that the efficiency of extraction is highly affected by plant morphology, localization of metabolites, and co-extractive compounds (Skrovankova *et al.*, 2015; Kumar *et al.*, 2022)^[8, 14]. The sensitivity of the developed HPLC technique in capturing these variations also provides further evidence of its use in comparative phytochemical profiling, in quality control and in standardization of plant-derived products. Comprehensively, the results of the current work can be used to analyze the data analytically by proving that an

isocratic, short-run, RP-HPLC approach with ultrasonic-assisted extraction is a dependable and reproducible method of quantifying berberine in various berry fruit samples. Such a combined method approach has a practical benefit of providing clear practical benefits to everyday analysis, assurance of quality of nutraceuticals and pharmacological research in the future. Furthermore, the finding of berberine in the typically used berry fruits confirms the new research that dietary fruits can be used as a supplement of bioactive alkaloids with possible health-promoting effects (Neag *et al.*, 2018; Imenshahidi and Hosseinzadeh, 2019)^[5, 10].

Table 3: Key of Analytical Indicators

Parameter	Observed Value	Interpretation
Retention Time (Rt)	2.42min	Rapid elution of berberine under polar conditions
Peak Height	28.9 mAU	Strong signal intensity
Area	298,483.1 mAU·s	High concentration and purity
Concentration	100% (standardized)	Calibration accuracy
Tailing Factor	3.44	Acceptable peak symmetry
Theoretical Plates	60	Adequate column efficiency

Conclusions: A high-performance liquid chromatographic technique was reliably established to determine berberine in various berry fruits. The ultrasonic-assisted extraction was found to be effective and the extraction efficiency depended heavily on sonication time. All samples were positive for berberine with significant diversification across fruit species. The strategy is appropriate to regular examination, quality management, and future nutraceutical and phytochemical research.

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