Natural Resources for Human Health

visagaa publishing house

Original Research

View Article online

Check for updates

Received 04 February 2025
Revised 12 March 2025
Accepted 15 April 2025
Available Online 30 July 2025

Edited by Kannan RR Rengasamy

KEYWORDS:
Butterbur
Softgel
Capsules
Petasins
Stability
Method of Analysis

Natr Resour Human Health 2025; 5 (4): 596–605
https://doi.org/10.53365/nrfhh/204056
eISSN: 2583-1194

Formulation and Manufacturing Process of Softgel Capsules Containing Common Butterbur Root Extract: A Stability Study and Method of Analysis

Kenza Mansoor[1,*], Khalid Matalka[2], Mathias Schmidt[3]

[1]Department of Pharmaceutical Medicinal Chemistry and Pharmacognosy, University of Petra, Amman, Jordan
[2]Medical Writing, Matalka's Scientific Writing, Burlington, MA, United States
[3]Research and Development, Herbresearch Germany, Mattsies, Germany

ABSTRACT: *Butterbur* root extract offers several health benefits, including anti-inflammatory and antiallergic activities, and developing a standardized, stable finished product of *butterbur* extract is essential to ensure therapeutic efficacy. This study aimed to present the formulation and manufacturing approach for creating a stable, effective, and aesthetically appealing softgel formulation of *butterbur* root extract. Each softgel capsule contained purple *butterbur* root extract (50 mg), lecithin (20 mg), soybean oil (185 mg), and beeswax yellow (10 mg). Stability testing included capsule and fill content weight, capsule rupture time, microbial evaluation, and petasins analysis under both accelerated and real-time conditions for three product lots. Petasins extracted from *butterbur* root extract were analyzed using a validated HPLC method, with percentage recovery ranging from 101.3% to 105.15%. The percent standard deviation for intraday and interday results was 0.29 to 0.31 and 0.39 to 0.79, respectively. This study demonstrated the formulation of softgel capsules containing *butterbur* root extract, effective petasins extraction, a validated method for petasins analysis, and 2-year product stability. It establishes a reliable approach for petasins analysis and the formulation of a *butterbur*-based herbal product.

1. INTRODUCTION

The common *butterbur* (*Petasites hybridus* L.) is a medicinal plant belonging to the Asteraceae family, primarily native to Europe, Southeast Asia, and certain regions of North Africa (Aydın et al., 2013). I It has been traditionally used to treat a wide range of medical conditions, including migraine, hypertension, bronchial asthma, and allergic rhinitis (Blosa et al., 2021; Lipton et al., 2004; Sun-Edelstein and Mauskop, 2009; Thomet and Simon, 2002).

The active constituents of *butterbur* are sesquiterpene compounds, namely petasins, which are predominantly found in the roots. Studies have shown that the anti-inflammatory and antiallergic activities of petasins are based on the inhibition of leukotriene and histamine activity (Blosa et al., 2021; Schapowal, 2005; Thomet et al., 2002; Thomet and Simon, 2002). Although *Petasites hybridus* extracts selectively inhibited COX-2 and prostaglandin expression in microglial cells, this effect was not directly correlated with petasin content (Fiebich et al., 2005). Petasins have also been shown to inhibit calcium channels, making them effective in the treatment of hypertension and migraine (Guo et al., 2020; Sheykhzade et al., 2008; Wang et al., 2010). Furthermore, studies have demonstrated that petasins possess anti-tumor activities (Guo et al., 2020), and that *butterbur* root extract exhibits anti-cancer effects on breast cancer cell line—while sparing non-cancerous cells—primarily through the induction of apoptosis, necrosis, and oxidative stress (Apostolova et al., 2023).

* Corresponding author.
E-mail address: kmansoor@uop.edu.jo (Kenza Mansoor)

A recent review discussed the pharmacology, safety, and clinical efficacy of *butterbur* in the prevention of migraine attacks (Borlak et al., 2022).

Since a dietary supplement containing *common butterbur* root extract may be beneficial for all the conditions mentioned above, developing a standardized and stable finished product of *butterbur* extract is essential to ensure therapeutic efficacy (Kim et al., 2019). Therefore, this study aimed to demonstrate the development process of softgel capsules containing *butterbur* root extract, including the formulation and manufacturing scheme to produce a stable, effective, and aesthetically appealing softgel dosage form. The study also focused on evaluating the stability of the finished product—softgel capsules containing *butterbur*—and establishing an analytical assay method for measuring petasins within these capsules. A high-performance liquid chromatography (HPLC) assay method was selected to analyze petasins, the key reference compounds in the softgel capsules, with stability testing performed under both accelerated and real-time conditions.

2. METHODS

2.1. Purple butterbur root extract

Purple *butterbur* root extract was purchased from RIA International, New Jersey, USA. According to the supplier, the roots were harvested in Germany during late summer to early autumn, when petasin levels were at their peak, then dried at temperatures below 40°C and ground to a 40–60 mesh powder. Extraction was carried out using 80% ethanol at a plant-to-solvent ratio of 1:10 (w/v), employing ultrasonic-assisted extraction at 45°C for 30–60 min, followed by reflux extraction at 65°C for 2–3 h. The extract was subsequently filtered through a 0.45 µm filter and concentrated under vacuum at 40–50°C using a rotary evaporator. The resulting extract underwent charcoal filtration to remove pyrrolizidine alkaloids. After this treatment, the extract was converted into powder form using spray drying at temperatures below 50°C. The *butterbur* extract was then standardized to contain 15% total petasins through fractionation with ethyl acetate and water, followed by quantification of petasins (Table 1).

2.2. Formulation, preparation of softgel capsules, and manufacturing process

The manufacturing and quality control processes of the *butterbur* softgel capsules are outlined in Figure 1 and Table 1. Briefly, the shell material is prepared using gelatin, glycerin, purified water, titanium dioxide, FD&C Red No. 40, and FD&C Yellow No. 6. The mixture is heated and stirred until fully dissolved, forming the gelatin solution (Figure 1). Purple *butterbur* root extract (50 mg) is combined with soybean oil, beeswax yellow, and lecithin to create a uniform fill mixture (Figure 1). The fill material and the gelatin solution are then combined and stirred thoroughly to ensure even distribution of the extract and excipients. Next, the gelatin solution is fed into a rotary die encapsulation machine to form the softgel shells, while the fill material is simultaneously encapsulated within the gelatin shells. The filled softgel capsules are sealed and cut to the desired size and shape. Quality control steps are conducted on the gelatin mix, during mixing, and after softgel encapsulation using specified criteria and test methods, as outlined in Table 2.

Table 1
Specifications and testing methods for the butterbur root extract.

Test Parameter	Method	Specification
Petasin Content	HPLC	15% ± 0.5%
Pyrrolizidine Alkaloids	HPLC-MS	<0.1 ppm
Moisture Content	Karl Fischer	≤5%
Microbial Limits	*Total Plate Count*	$\leq 10^4$ *CFU/g*
Heavy Metals (Pb, Cd, Hg, As)	ICP-MS	Below regulatory limits
Shelf-Life Stability	40°C, 75% RH	No major degradation over 6 months

CFU = colony forming unit; g = gram; HPLC = high-performance liquid chromatography; HPLC-MS= high-performance liquid chromatography-mass spectrometry; ICP-MS= inductively coupled plasma mass spectrometry; RH = relative humidity; ppm = part per million.

2.3. Drying and conditioning

The softgel capsules are dried under controlled temperature and humidity conditions to achieve the desired moisture content and hardness. After drying, the capsules are conditioned to ensure uniformity and stability (Figure 1, Table 2).

2.4. Identification of Purple Butterbur Root Extract

The purple *butterbur* root extract in the finished product was identified from the content of no less than 10 capsules using thin layer chromatography (TLC) with a 0.50-mm layer of chromatographic silica gel mixture. Following extraction by heating under reflux for 20 minutes with about 40 ml of light petroleum on a water bath, the filtrate was concentrated to approximately 1 ml. A volume of 20 µL of both

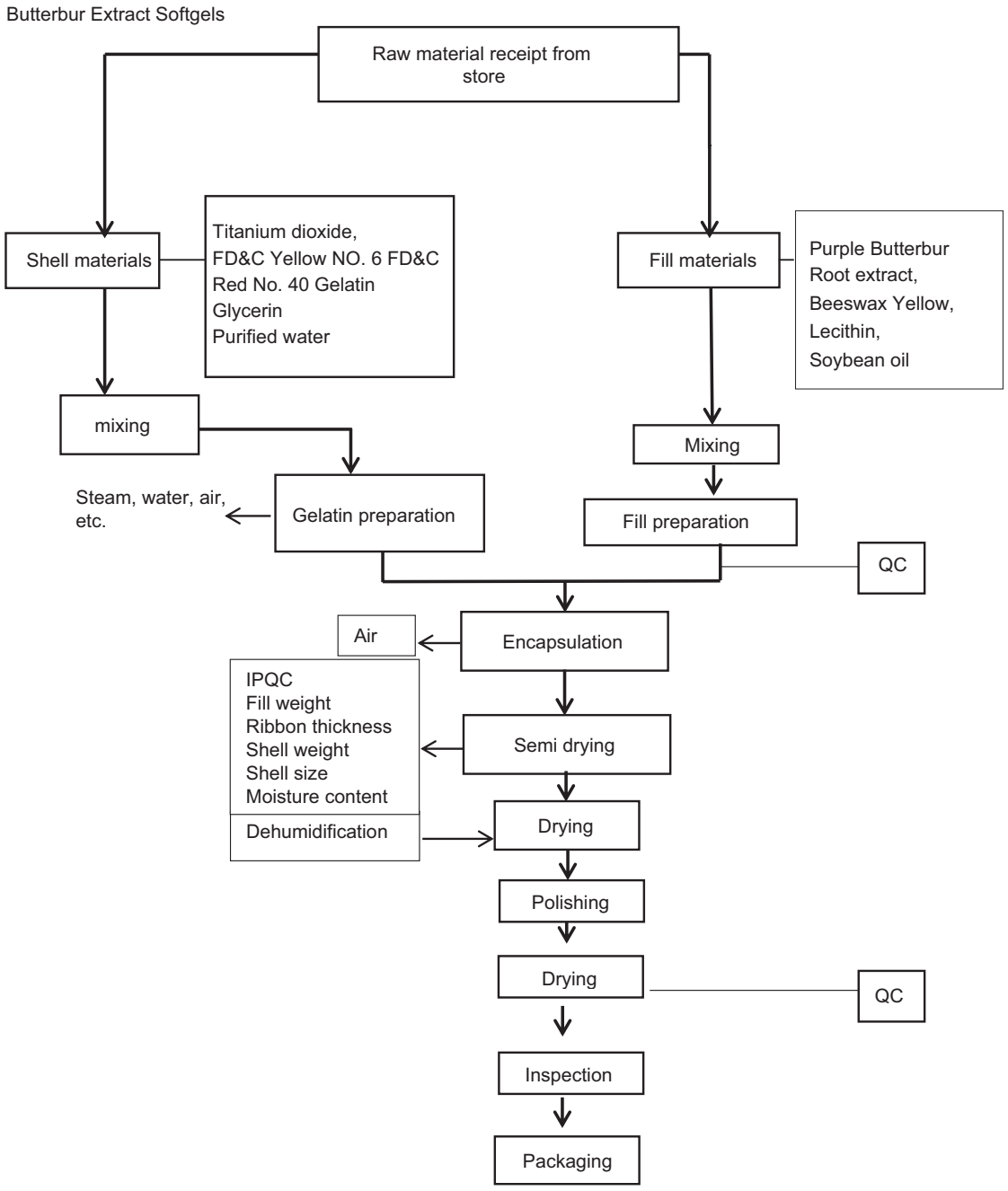

Figure 1. Manufacturing process of *butterbur* softgel capsules. Quality control (QC) steps were performed as outlined in Table 1.

the standard and sample solutions was applied to the TLC plate, with chloroform used as the mobile phase. The plates were sprayed with a mixture of 0.5 ml anisaldehyde and 10 ml glacial acetic acid, then heated at 120°C for 7–10 minutes. The plates were examined under UV light at 230 nm. The sesquiterpenes petasin and isopetasin were detected in the Rf range of 0.4–0.45 as green-blue fluorescent zones.

2.5. Capsule Weight and fill content weight

Twenty softgel capsules were randomly selected and weighed, and the average weight was recorded. Weight variation was calculated according to USP-NF <2091> Weight Variation of Dietary Supplements. The average fill content was measured by weighing 10 intact capsules selected at

Table 2
Quality control parameters for *butterbur* softgel capsules.

Manufacturing Stage	*Tests*	Specifications	Test Method
Before starting	i) Environment control	i) Temperature: NMT 30°C, RH: NMT 50%	i) Hygrometer
	ii) Cleanliness of the equipment & utensils	ii) Should be clean	ii) Visual inspection
Making of the Gelatin Mix	i) Gel mass moisture	i) NMT 5.0%	Analytical Method of Finished Product
	ii) Gel mass Viscosity	ii) 2.8 – 4.5 mPa s	
	iii) Gel Strength	iii) 150 – 200 bloom	
Making of the Mix	Appearance	Light yellow colored paste.	
	Moisture Content	NMT 2.0%	
Softgel Encapsulation	Displacement pump temperature	35°C ± 2°C	
	Sealing Temperature	37°C – 40°C	
	Humidity	20% RH	
Finished product (Coated Softgels)	Appearance	5 minims oval shaped opaque orange colored softgels containing yellowish green colored paste.	
	Average weight	390.00 mg ± 10%	
	Average Fill Weight	270.00 mg ± 10%	
	Weight Variation	± 10% of average weight	
	Rupture Time	NMT 15 min.	
	Assay Each Softgel Contains Claim		
	Purple Butterbur Root Extract Providing: 50 mg *Petasins*	NLT 90% & NMT 110% NLT 15.0% of the labeled amount of the *Purple Butterbur root extract* (NLT 7.5mg)	
Setting batch number & expiry date	Batch no. & Exp. date	According to running batch	Visual inspection
Packaging	Leakage test	Should be leak proof	Leak Test
	Correctness & legibility of Batch no., Mfg. date & Exp. date on carton	According to running batch	Visual inspection

NLT = Not Less Than; NMT = Not More Than

random; each capsule was then opened, and its contents were completely recovered using a solvent. The empty shells were kept at room temperature for 30 minutes to allow the solvent to evaporate, after which each shell was weighed. The average shell weight was calculated, and the average fill weight content was determined by subtracting the average shell weight from the average capsule weight.

2.6. Rupture time of capsules

The rupture time of softgel capsules was determined according to USP-NF <2040> Disintegration of Dietary Supplements. Briefly, each capsule was placed in a dissolution vessel containing 500 ml of water, and the dissolution apparatus was run for 15 minutes. Rupture time was recorded within this 15-minute period.

2.7. Microbial testing

The microbial testing applied was according to USP-38 protocol.

2.8. Analytical method of petasins

2.8.1. Analysis

Dry and chopped rhizomes of *P. hybridus* (Freising, Germany) or samples from the softgel capsules were collected and processed as follows. Extraction was performed using a stoichiometric plant-to-solvent ratio of 1:10 (w/v). The material was steeped in ethanol at 38°C for 1 hour, and the resulting extract was filtered through filter paper and a 0.2 µm RC membrane filter at room temperature. The filtrate exhibited a yellowish color with a characteristic *butterbur* scent.

Chromatographic fractionation of the filtrate was carried out using an HPLC-UV system (Merck-Hitachi HPLC system, Tokyo, Japan) equipped with an auto-sampler, binary pump L-7100, and Diode-Array Detector L-7450, controlled by LaChrom D-7000 HPLC-System-Manager. Separation was performed on an Agilent Zorbax Eclipse XDB-C18 reversed-phase column (100 mm × 3.0 mm i.d., 3.5 µm particle size) using elution solvents of methanol–ammonium acetate (10 mM aq.) in a 90:10 (v/v) ratio and ammonium acetate (10 mM aq.)–methanol in a 90:10 (v/v) ratio at pH 7.4 as the organic and aqueous phases, respectively, at a flow rate of 0.4 mL/min. Chromatograms were recorded at a wavelength of 230 nm. Fractions were collected using a Pharmacia LKB Frac-100 auto-fraction sampler (Uppsala, Sweden) at 1-minute intervals. From each fraction, a 15 µL volume was injected and repeated four times to increase the total amount of collected drug constituents. Fractions corresponding to a chromatographic mobile phase volume of 1.6 mL (i.e., 4 × 0.4 mL) were evaporated at room temperature under continuous nitrogen flow. The mobile phase consisted of methanol–ammonium acetate (10 mM aq.) in a 90:10 (v/v) ratio and ammonium acetate (10 mM aq.)–methanol in a 90:10 (v/v) ratio at pH 7.4 as the organic and aqueous phases, respectively. The standard solution was prepared from pure petasin (98.0%) purchased from TLC Pharmaceutical Standards.

The percentage of the labeled amount of petasin in the capsule was calculated using the following equation:

$$\text{Concentration of Petasins} = \frac{rU}{rS} \times \frac{CS}{CU} \times 100$$

where

rU peak area of petasins from the *Sample solution*
rS peak area of petasins RS from the *Standard solution*
CS concentration of petasins RS in the *Standard solution* (mg/mL)
CU nominal concentration of petasins in the *Sample solution* (mg/mL)

The acceptance criterium was not less than (NLT) 15.0% of petasin of the labeled amount of purple butterbur root extract.

2.8.2. Validation

Accuracy was determined using five different levels of the working concentration, with two replicates for each concentration level. The calculated petasin content was compared to the percent recovery for each preparation against the standard solution. The percent recovery at each level was required to be between 90% and 110%.

Precision was determined by repeatability (intraday) precision and intermediate (interday) precision. Repeatability

Table 3
Description and composition of each softgel capsule.

Color	Opaque orange	**Average fill weight (mg)**			270.00
Size	5 minims	**Average weight (mg)**			390.00
Shape	Oval	**Average weight of 10 softgels (g)**			3.9
Raw Material Source		**% OV***	**Amount/Cap. (mg)**	**Function**	**Protocol**
Active Ingredients	**Per Cap. (mg)**				
Purple butterbur root extract	50.00	10	55.0	Active	In House
In-Active Ingredients					
Beeswax Yellow		–	10.00	Stiffening agent	USP-38
Lecithin		–	20.00	Emulsifying agent	USP-38
Soybean Oil		–	185.00	Vehicle	USP-38
In-Active Shell Ingredients					
Titanium Dioxide		–	0.24	Opacifying agent	USP-38
FD&C Red No. 40		–	0.36	Coloring agent	FCC
FD&C Yellow No. 6			0.60	Coloring agent	FCC
Gelatin		–	76.8	Gelling Agent	USP-38
Glycerin		–	34.8	Plasticizer	USP-38
Purified Water		–	7.20	Vehicle	USP-38
Total Weight			390.00		

* %OV = Percent Overages added.

was evaluated by assaying six determinations at 100% of the working concentration, using samples from the same batch of the finished product, and calculating the relative standard deviation (RSD) for the six results. Intermediate precision was assessed by comparing the results of repeated assays performed on different days. The % RSD should not exceed 2%.

Linearity was determined using five different solutions covering a concentration range of 80% to 120%. Each solution was measured individually, and the results were plotted against the actual concentrations. The method should exhibit linearity within the desired range, with a correlation coefficient (R) of 0.99 or higher.

2.9. Stability

Stability studies on three lots of the finished product (11908S) were conducted over a 24-month period under real-time conditions of 30°C ± 2 and 65% ± 5% RH, and over a 6-month period under accelerated conditions of 40°C ± 2 and 75% ± 5% RH. The stability assessments included average fill weight, weight variation, rupture time, assay of petasins, and microbial testing.

2.10. Data analysis

Data were entered into Microsoft Excel 365 and presented as mean values. The percent recovery, percent relative standard deviation, and correlation coefficient with regression equation were calculated as described in each legend.

3. RESULTS AND DISCUSSION

3.1. Chemical composition of the finished product softgel capsules

Each softgel capsule contains 50 mg of purple *butterbur* root extract, lecithin (20 mg), soybean oil (185 mg), and beeswax yellow (10 mg) as inactive ingredients (Table 3). The

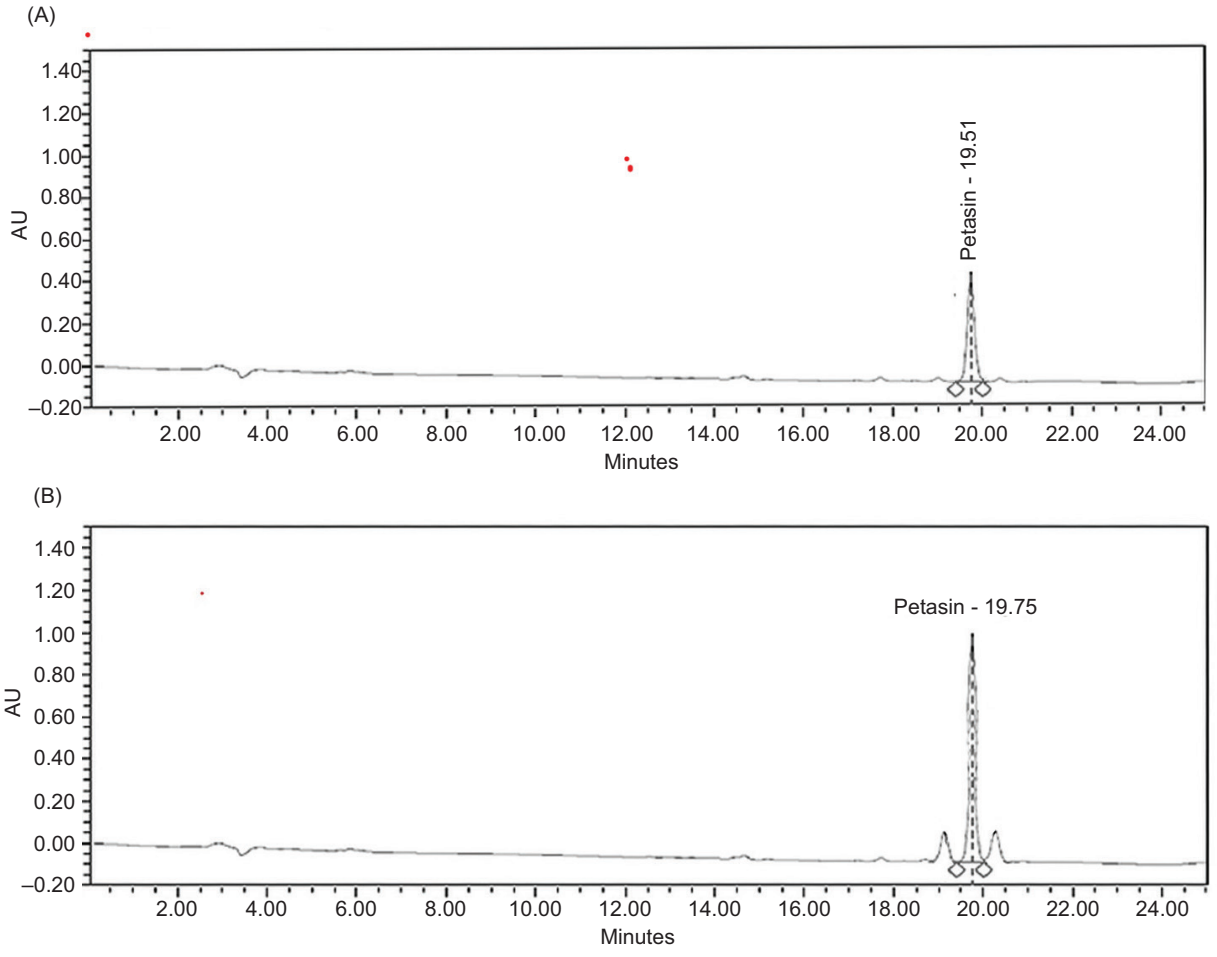

Figure 2. Chromatogram of petasin in the standard solution (A) and extracted from *butterbur* (B), both showing a retention times of 19.75 min.

Table 4
Percentage recovery of petasins extracted from the softgel capsules.

Sample No.	Con. Level	No. of Preparation	Added Petasins (mg/ml)	Recovered Petasins (mg/ml)	% Recovery*
1.	Level 1 (80.0%)	1	6.00	6.20	103.0
		2	6.00	6.16	
2.	Level 2 (90.0%)	1	6.75	6.89	101.30
		2	6.75	6.78	
3.	Level 3 (100.0%)	1	7.50	7.78	102.27
		2	7.50	7.56	
4.	Level 4 (110.0%)	1	8.25	8.87	105.15
		2	8.25	8.48	
5.	Level 5 (120.0%)	1	9.00	9.34	104.83
		2	9.00	9.53	

*%Recovery was calculated from the mean recovered petasins divided by the added petasins multiplied by 100%.

Table 5
Precision analysis of petasin extracted from the softgel capsules.

Analyte	Analyst	Number of samples analyzed of petasins (7.5 mg/ml)						Standard deviation	%RSD*
		1	2	3	4	5	6		
Petasin (mg/ml) (Day 1) (Intraday)	Analyst 1	7.56	7.58	7.61	7.60	7.58	7.62	0.02228	0.29
	Analyst 2	7.62	7.63	7.59	7.64	7.58	7.62	0.02338	0.31
Petasin (mg/ml) (Day 2) (Inter day)	Analyst 1	7.55	7.58	7.70	7.67	7.68	7.66	0.06033	0.79
	Analyst 2	7.67	7.68	7.65	7.64	7.62	7.69	0.02639	0.34

RSD = relative standard deviation.
*%RSD was calculated by dividing the standard deviation by the mean of petasins measured by each analyst, multiplied by 100%.

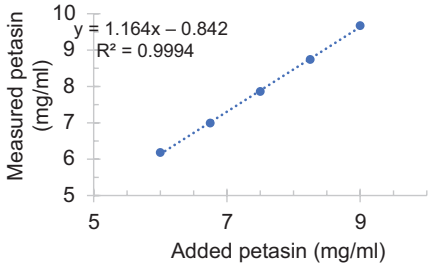

Figure 3. Correlation between the measured and added petasin extracted from the softgel capsules. The relationship linear, with a correlation coefficient (R^2) > 0.99 and a regression equation of y = 1.164x + 0.842.

shell ingredients consist of gelatin, glycerin, titanium dioxide, coloring agents, and purified water.

The composition of each capsule was designed to ensure that the *butterbur* extract is emulsified in an oil vehicle and that the product remains stable for a long period of time (Table 3) (Szuhaj, 2003). Lecithin is known to reduce viscosity and prevent adhesion of sticky products, whereas titanium dioxide in the shell protects the capsule ingredients from light-induced degradation and heat (Blundell et al., 2022; Szuhaj, 2003). Since sesquiterpenes are the active ingredients in *butterbur* extracts and have poor solubility (Disch et al., 2018), the gelatin shell content with lecithin and a lipophilic vehicle were used as a drug-dosage form to increase the bioavailability of sesquiterpenes, ultimately resulting in high dosage accuracy and uniformity (Damian et al., 2021). Furthermore, adding glycerin as a plasticizer forms stable thermoreversible gel networks and lowers moisture resistance, especially in oil-based fill formulations (Naharros-Molinero et al., 2023).

3.2. Petasin Assay validation

An HPLC method was developed and validated according to the definitions of ICH guideline Q2 (R2) (ICH, 2005). The petasin chromatogram extracted from *butterbur* was equivalent to the petasin standard solution with regard to the retention time of 19.75 min (Figure 2A and B).

The accuracy of the analytical method for petasin extracted from the capsules was validated using five different working concentrations ranging from 6 to 9 mg/mL, corresponding to 80% to 120% of the target concentration of 7.5 mg/mL. The results showed percentage recovery values between 101.3% and 105.15% (Table 4). Precision was validated by two different analysts on two separate days, each performing six runs per day. The intraday and interday %RSD values ranged from 0.29 to 0.31 and 0.39 to 0.79, respectively (Table 5). Linearity, based on measurements of five petasin-containing solutions, yielded a correlation coefficient (R^2) of 0.9994 (Figure 3). These results were all within acceptable limits.

3.3. Realtime and accelerated stability of the softgel capsules

Stability studies for three lots of the finished product (11908S) were initiated under real-time conditions of 30°C ± 2 and 65% ± 5% relative humidity, and under accelerated conditions of 40°C ± 2 and 75% ± 5% relative humidity (ICH, 2003). The stability data demonstrated that the softgel capsules (11908S) remained within specifications throughout the study under both conditions (Tables 6 and 7). For instance, the concentration of the active ingredient, petasins, remained above the NLT required percentage and within 90% of the initial reported petasin concentration at the end of both testing periods. Although petasin is known to spontaneously transform into isopetasin (Kleeberg-Hartmann et al., 2021), the HPLC method used in this study measures both petasins and isopetasin. Notably, both forms exhibit therapeutic effects (Kleeberg-Hartmann et al., 2021; Borlak et al., 2022). While petasins are prone to degradation by oxidation, hydrolysis, light, and heat, the formulation developed in this study effectively stabilized the compound, preventing degradation for up to 24 months. Additionally, average fill weight, capsule weight variation, identification tests, and rupture time all met the acceptance criteria. Microbial testing for aerobic bacteria, yeasts, and molds also remained within acceptable limits. Based on the results of these stability studies, the shelf life of the product is concluded to be 24 months.

4. CONCLUSION

The present study demonstrated the formulation of softgel capsules containing *butterbur*, a validated method for the analysis of petasins—the active sesquiterpene ingredients in *butterbur*—and the 2-year stability of the finished product. This study represents a method of choice for the analysis and formulation of a *butterbur*-containing herbal product.

Table 6
Real-time stability data for a single batch of the softgel capsules.

Test	Specifications	Initial Time Report	3 Months	6 Months	9 Months	12 Months	18 Months	24 Months
Description	5 minims oval shaped opaque orange colored softgels containing yellowish green colored paste	Complies	Complies	Complies	Complies	Complies	Complies	Complies
Average Fill Weight (mg)	270.00 ± 10%	274.87	274.89	274.98	275.01	275.22	275.54	275.98
Weight Variation	± 10 % of Average Weight	Complies	Complies	Complies	Complies	Complies	Complies	Complies
Identification test	To Comply	Complies	Complies	Complies	Complies	Complies	Complies	Complies
Rupture Time (min)	NMT 15 minutes	2	2	3	3	3	3	3
Assay: Each softgel contains								
Content of Petasins (mg)	NLT 7.5 mg (NLT 15.0%)	12.06	12.0	11.85	11.72	11.54	11.27	11.04
Microbiological Test								
Total Aerobic Microbe Count (CFU/g)	NMT 3,000	<10	<10	<10	<10	< 20	<20	<20
Total Yeast and Molds Count (CFU/g)	NMT 300	<10	<10	<10	<10	< 20	<20	<20
Salmonella	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
E. coli	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Staphylococcus aureus	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Pseudomonas aeruginosa	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

NLT = not less than; NMT = not more than; CFU = colony forming unit.

Table 7
Accelerated stability data for a single batch of the finished softgel capsules.

Test	Specifications	Initial Time Report	1 Months	2 Months	3 Months	6 Months
Description	5 minims oval shaped opaque orange colored softgels containing yellowish green colored paste	Complies	Complies	Complies	Complies	Complies
Average Fill Weight (mg)	270.00 ± 10%	274.87	274.90	274.96	274.97	275.60
Weight Variation	± 10% of Average Weight	Complies	Complies	Complies	Complies	Complies
Identification test	To Comply	Complies	Complies	Complies	Complies	Complies
Rupture Time (min)	NMT 15 minutes	2	3	3	3	3
Assay: Each softgel contains						
Content of Petasins (mg)	NLT 7.5 mg (NLT 15.0%)	12.06	11.50	11.44	11.20	11.05
Microbiological Test						
Total Aerobic Microbe Count (CFU/g)	NMT 3,000 CFU/g	<10	< 10	< 20	< 20	<20
Total Yeast and Molds Count (CFU/g)	NMT 300 CFU/g	< 10	< 10	< 10	< 10	<10
Salmonella	Absent	Absent	Absent	Absent	Absent	Absent
E. coli	Absent	Absent	Absent	Absent	Absent	Absent
Staphylococcus aureus	Absent	Absent	Absent	Absent	Absent	Absent
Pseudomonas aeruginosa	Absent	Absent	Absent	Absent	Absent	Absent

NLT = not less than; NMT = not more than; CFU = colony forming unit.

DATA AVAILABILITY

The raw data is available from K.A.M and M.S.

AUTHOR CONTRIBUTIONS

K.A.M. and M.S. contributed to the study conception, analysis, and data curation. K.Z.M. performed part of the statistical analysis and drafted the original manuscript. K.A.M. reviewed the manuscript. All authors contributed to the manuscript revision and read and approved the submitted version.

CONFLICT OF INTEREST

The authors declare no conflict of interest with respect to research, authorship and/or publication of this article.

FUNDING

No funding.

ORCID

Kenza Mansoor 0000-0002-8171-2055
Khalid Matalka 0000-0002-7350-1186
Mathias Schmidt 0000-0002-4488-4026

REFERENCES

Apostolova, S., Oreshkova, T., Uzunova, V., Georgieva, I., Maslenkova, L., and Tzoneva, R. (2023). A Standardized Extract of Petasites hybridus L., Containing the Active Ingredients Petasins, Acts as a Pro-Oxidant and Triggers Apoptosis through Elevating of NF-κB in a Highly Invasive Human Breast Cancer Cell Line. Frontiers in bioscience (Landmark edition) 28, 111. https://doi.org/10.31083/j.fbl2806111

Aydın, A.A., Zerbes, V., Parlar, H. and Letzel, T., 2013. The medical plant butterbur (*Petasites*): Analytical and physiological (re)view. *Journal of Pharmaceutical and Biomedical Analysis*, 75, pp.220–229. https://doi.org/10.1016/j.jpba.2012.11.028

Blosa, M., Uricher, J., Nebel, S., Zahner, C., Butterweck, V. and Drewe, J., 2021. Treatment of early allergic and late inflammatory symptoms of allergic rhinitis with *Petasites hybridus* leaf extract (Ze 339): results of a noninterventional observational study in Switzerland. *Pharmaceuticals (Basel, Switzerland)*, 14. https://doi.org/10.3390/ph14030180

Blundell, R., Butterworth, P., Charlier, A., Daurio, D., Degenhardt, M., Harris, D., Hancock, B., Johnston, M., Kasina, R., Kaye, J. et al., 2022. The role of titanium dioxide (E171) and

the requirements for replacement materials in oral solid dosage forms: an IQ Consortium working group review. *Journal of Pharmaceutical Sciences*, 111, pp.2943–2954. https://doi.org/10.1016/j.xphs.2022.08.011

Borlak, J., Diener, H.C., Kleeberg-Hartmann, J., Messlinger, K. and Silberstein, S., 2022. *Petasites* for migraine prevention: new data on mode of action, pharmacology and safety. A narrative review. *Frontiers in Neurology*, 13, p.864689. https://doi.org/10.3389/fneur.2022.864689

Damian, F., Harati, M., Schwartzenhauer, J., Van Cauwenberghe, O. and Wettig, S.D., 2021. Challenges of dissolution methods development for soft gelatin capsules. *Pharmaceutics*, 13. https://doi.org/10.3390/pharmaceutics13020214

Disch, L., Forsch, K., Siewert, B., Drewe, J. and Fricker, G., 2018. In vitro and in situ absorption and metabolism of sesquiterpenes from *Petasites hybridus* extracts. *Planta Medica*, 84, 795–805. https://doi.org/10.1055/s-0044-100401

Fiebich, B.L., Grozdeva, M., Hess, S., Hüll, M., Danesch, U., Bodensieck, A. and Bauer, R., 2005. *Petasites hybridus* extracts in vitro inhibit COX-2 and PGE2 release by direct interaction with the enzyme and by preventing p42/44 MAP kinase activation in rat primary microglial cells. *Planta Medica*, 71, 12–19. https://doi.org/10.1055/s-2005-837744

Guo, L., Kang, J.S., Kang, N.J. and Choi, Y.W., 2020. S-petasin induces apoptosis and inhibits cell migration through activation of p53 pathway signaling in melanoma B16F10 cells and A375 cells. *Archives of Biochemistry and Biophysics*, 692, 108519. https://doi.org/10.1016/j.abb.2020.108519

ICH, 2003. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. Stability testing of new drug substances and products Q1A (R2). In: ICH Harmonised Tripartite Guideline. Current Step 4 version.

ICH, 2005. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. Validation of Analytical Procedures: Text and Methodology Q2 (R1). In: ICH Harmonised Tripartite Guideline.

Kim, J.H., Lee, K., Jerng, U.M., and Choi, G., 2019. Global comparison of stability testing parameters and testing methods for finished herbal products. Evidence-based complementary and alternative medicine: eCAM, 2019, 7348929. https://doi.org/10.1155/2019/7348929

Kleeberg-Hartmann, J., Vogler, B. and Messlinger, K., 2021. Petasin and isopetasin reduce CGRP release from trigeminal afferents indicating an inhibitory effect on TRPA1 and TRPV1 receptor channels. Journal of Headache Pain, 22, 23. https://doi.org/10.1186/s10194-021-01235-5

Lipton, R.B., Göbel, H., Einhäupl, K. M., Wilks, K. and Mauskop, A., 2004. Petasites hybridus root (butterbur) is an effective preventive treatment for migraine. Neurology, 63, pp.2240–2244. https://doi.org/10.1212/01.WNL.0000147290.68260.11

Naharros-Molinero, A., Caballo-González, M., de la Mata, F.J. and García-Gallego, S., 2023. Shell formulation in soft gelatin capsules: design and characterization. Advanced Healthcare Materials, e2302250. https://doi.org/10.1002/adhm.202302250

Schapowal, A., 2005. Treating intermittent allergic rhinitis: a prospective, randomized, placebo and antihistamine-controlled study of Butterbur extract Ze 339. Phytotherapy Research, 19, 530–537. https://doi.org/10.1002/ptr.1705

Sheykhzade, M., Smajilovic, S., Issa, A., Haunso, S., Christensen, S.B., and Tfelt-Hansen, J., 2008. S-petasin and butterbur lactones dilate vessels through blockage of voltage gated calcium channels and block DNA synthesis. European Journal of Pharmacology, 593, 79–86. https://doi.org/10.1016/j.ejphar.2008.07.004

Sun-Edelstein, C., and Mauskop, A., 2009. Foods and supplements in the management of migraine headaches. The Clinical Journal of Pain, 25, 446–452. https://doi.org/10.1097/AJP.0b013e31819a6f65

Szuhaj, B. F., 2003. PHOSPHOLIPIDS | Properties and Occurrence. In: B. Caballero, ed. Encyclopedia of Food Sciences and Nutrition. 2nd ed. Oxford: Academic Press, 4514–4519.

Thomet, O.A., Schapowal, A., Heinisch, I.V., Wiesmann, U.N., and Simon, H.U., 2002. Anti-inflammatory activity of an extract of Petasites hybridus in allergic rhinitis. International Immunopharmacology, 2, 997–1006. https://doi.org/10.1016/s1567-5769(02)00046-2

Thomet, O. A., and Simon, H. U., 2002. Petasins in the treatment of allergic diseases: results of preclinical and clinical studies. International Archives of Allergy and Immunology, 129, 108–112. https://doi.org/10.1159/000065884

USP-NF <2040>. The United States Pharmacopeial Convention, 2009. *Disintegration and dissolution of dietary supplements. In: USP 32*. Rockville, MD: United States Pharmacopeial Convention.

USP-NF <2091>. The United States Pharmacopeial Convention, 2009. *Weight variation of dietary supplements*. In: USP 32: Rockville, MD: United States Pharmacopeia Convention.

Wang, G.J., Lin, Y.L., Chen, C.H., Wu, X.C., Liao, J.F., and Ren, J., 2010. Cellular calcium regulatory machinery of vasorelaxation elicited by petasin. Clinical and Experimental Pharmacology & Physiology, 37, 309–315. https://doi.org/10.1111/j.1440-1681.2009.05283.x