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HPLC-PDA Method Development and Validation of *Lagenaria siceraria* (Molina) Standl. Fruit Extracts Supported by Multianalytical Techniques

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The fruit of *Lagenaria siceraria* belonging to Cucurbitaceae family commonly recognized as bottle gourd is rich in bioactive compounds exhibited an extensive range of therapeutic and pharmacological properties that include antioxidant, lipid lowering, cardioprotective, diuretic, central nervous system stimulant and immunosuppressive, *etc.* Due to the broad spectrum of bioactivities, researchers have been consistently working on identification, qualitative and quantitative analyses of a new series of bioactive compounds. Therefore, the current investigation was performed to develop and subsequently validation of a rapid, simple, reliable, sensitive and multianalytical methods to standardize constituents of *L. siceraria* fruits. The simultaneous identification and quantitation of phenolic and flavonoid glycosides, including (*E*)-4 hydroxymethyl phenyl-6-O-caffeoyl-B-D-glucopyranoside (**LS1**) and kaempferol 3-O-rutinoside (**LS2**) were done by ultra-high-performance liquid chromatography (UHPLC) method for the *L. siceraria* fruits extract. A high-performance thin-layer chromatography-based (HPTLC) approach for phenolic and flavonoid glycosides has been developed as a tool for quality assessment for chemical fingerprinting and quick screening. Additionally, mass spectrometry was used to characterize compounds, **LS1** and **LS2** in sample matrices. The aforementioned compounds **LS1** and **LS2** were successfully identified using the qualitative HPTLC-based approach. The results of the method's performance and data validation showed that the UHPLC method was robust (RSD < 5.0%), linear (r^2 > 0.99), precise (RSD < 5.0%) and accurate (recoveries 80-110%). It could also be used for quantification. The standardization, quality evaluation, quantification, authenticity and foundation for future research in the field would be benefited from this multianalytical method for identifying the compounds in *L. siceraria* fruit extract.

Keywords: *Lageraria siceraria*, Dietary supplement, UHPLC, HPTLC, Flavonoids, Glycosides.

INTRODUCTION

The family Cucurbitaceae, which includes a variety of species like *Lagenaria siceraria, Momordica charantia* and *Cucurbita pepo*, is abundant in phytochemicals with important pharmacological characteristics. Not only are these plants essential to conventional medicine, but they also contain a variety of bioactive chemicals that enhance their potential as therapeutics. For instance, cucurbitosides, triterpenoids, carotenoids and cucurbita glycosides are found in *Cucurbita pepo* and have been associated with several health advantages [1]. Cucurbitacins are well known for their anticancer effects, especially cucurbitacin B, which prevents microtubule polymerization in cancer cells [2]. *M. charantia* and *C. maxima* extracts have shown antimalarial efficacy, dramatically lowering parasitaemia in infected mice [3]. The findings also demonstrated that cucurbits have anti-inflammatory and anticancer properties, as seen by decreased inflammatory cytokine release and decreased colon cancer cell viability [4]. Even with their advantages, several cucurbit species can be toxic if consumed improperly, emphasizing the necessity to use caution. Overall, the research on the Cucurbitaceae family looks very promising.

Commonly referred as bottle gourd, *L. siceraria* holds noteworthy phytochemical and pharmacological characteristics, rendering it an invaluable asset in conventional medicine. It's numerous bioactive constituents support a range of health advantages such as antihyperglycemic, antihyperlipidemic and antioxidant properties. The composition of phytochemistry includes flavones, C-glycosides, cucurbitacins and triterpenoids

and consist of pectin and vitamins B and C in high levels [5]. The fruit juice extracts indicate flavonoids, tannins, saponins and cardiac glycosides [6]. Ethanol extracts demonstrate high DPPH radical scavenging capacity, indicating robust antioxidant properties [7]. Because of their flavonoid concentration, methanol extracts decrease the blood glucose levels of diabetic rats while fasting [8]. In atherogenic diet rats, fresh fruit juice reduces triglyceride and cholesterol levels [6]. Although *L. siceraria* exhibits promising health advantages, more studies are required to understand its mechanisms and possible adverse consequences. The therapeutic and nutritional qualities of *L. siceraria*, are mostly attributed to the phenolic and flavonoid glycosides that are found in it. These substances are well-known for their antioxidant properties as well as possible medical advantages. Numerous phenolic compounds found in *L. siceraria*, such as hydroxycinnamate conjugates and flavonol glycosides, are essential for both human health and plant defense [9,10]. Studies have demonstrated that ethanol extracts from the fruit have excellent DPPH radical scavenging activity, indicating a high phenolic content, which indicates that the fruit is rich in antioxidants [7]. The phenolic compounds found in *L. siceraria* have been associated with a number of health advantages including hepatoprotective, cardioprotective and anti-inflammatory actions [5,11]. These compounds possessing antioxidant properties may reduce the effects of oxidative stress-related illnesses, increasing their potential in the treatments and further applications [11]. Although the advantages of phenolic and flavonoid glycosides are often highlighted, it is important to consider the broader spectrum of plant-based compounds, as *L. siceraria* also contains other phytochemicals that contribute to its overall health promoting properties.

By determining the range, linearity with a limit of detection (LOD), the limit of quantification (LOQ), precision, accuracy, recovery and stability, the suggested methodology is developed, refined and then considered for the validation protocol for the simultaneous analysis of the two bioactive constituents *L. siceraria* fruits extract (LSFE). Furthermore, a quick and easy HPTLC method was also developed. This can be utilized as a visual quality assessment for the quick fingerprint analysis and identification of (*E*)-4 hydroxymethyl phenyl-6-O-caffeoyl-B-D-glucopyranoside (**LS1**) and kaempferol 3-O-rutinoside (**LS2**) in LSFE. A sensitive, robust and reproducible HPLC coupled with MS/MS method was developed for the analysis of targeted compounds of LSFE.

Existing research on the flavonoid glycosides in *L. siceraria* highlights gaps in the standardization and quantification of these important phytochemicals. While various studies have explored the phytochemical composition and specific flavonoids, comprehensive methodologies for the quantification and characterization of flavonoid glycosides remain unexplored. Similarly, total phenolic and flavonoid contents have been estimated using spectrophotometric techniques, but these methods lack specificity for the individual glycosides. High-performance thin-layer chromatography (HPTLC) reports majorly deals with quercetin quantification, yet it does not address the understanding on complete fingerprinting of flavonoid glycosides and other bioactively potent analogues present in the extracts. Hence, further exploration through mutlianalytical approaches will partially address this gap. Considering the vast pool of bioactive compounds specifically polyphenols and flavonoid glycosides in LSFE, it is imperative to explore them through further phytochemical and pharmacological investigations. As per our understanding, there are limited reports available on marker based standardization for *L. siceraria* fruit extracts. Hence, two compounds in the current study, **LS1** and **LS2** (Fig. 1) were selected for isolation based on the initial response and resolution of the respective peaks observed in the extract. The HPLC profile of the extract was developed to improve the resolution. The isolation and characterization of the data revealed the structures. Both compounds are previously well known for their bioactivities. The **LS1**, (*E*)-4-hydroxymethyl phenyl-6-O-caffeoyl-B-D-glucopyranoside is shown the significant antioxidant potential [12], whereas, **LS2**, kaempferol-3-O-rutinoside, earlier reported for significant anti-inflammatory activity and antidiabetic potential [13].

The standardization against these two compounds possibly be an important starting point and crucial in the authentication of ingredient, controlling any possible adultration and subsequent bioefficacy and formulation work. The work could be important step towards the industrial scale up, once coupled with safety and efficacy studies.

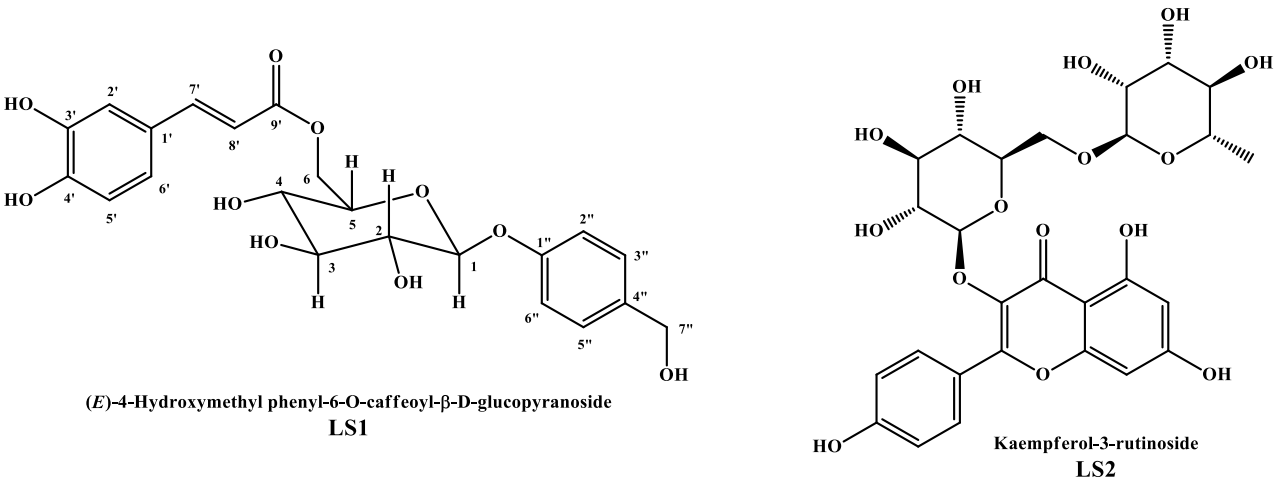

Fig. 1. Chemical structures of isolated compounds from LSFE

EXPERIMENTAL

The markers which used as reference are, (*E*)-4 hydroxymethyl phenyl-6-O-caffeoyl-B-D-glucopyranoside (**LS1**) and kaempferol 3-O-rutinoside (**LS2**) were extracted and further taken for isolation from LSFE. Water, methanol, acetonitrile and formic acid were procured from Rankem and JT Baker chemical companies. *L. siceraria* fruits (LSF) were collected from Gujarat state, India and the specimen identified with an authentication code no. PHPL/ADIC/HBS/0622/010 was deposited in the herbarium of Pharmacognosy and Botany center of the Pharmanza Herbal Pvt. Ltd., Anand, India.

Extraction of *L. siceraria* fruits: At 55 ± 5 ºC, about 1 kg of dried and powdered fruits of *L. siceraria* was refluxed in ethanol and water in a ratio of 9:1. Three iterations of this extraction procedure were carried out and combined. After filtering and condensing the combined extract, the semi-solid mass was then triturated with ethanol at ambient temperature, filtered, concentrated to dryness to get 104 g of dried powder. Compounds **LS1** and **LS2** were further isolated using the extract.

Isolation of LSFE compounds: In current investigation, we have carefully isolated phenolic and flavonoid glycosides (**LS1** and **LS2**) from the fruit extract of *L. siceraria* using enrichment and column chromatography procedures as per literature [12].

Standard solution: The reference standards, **LS1** and **LS2** were taken up for preparation of stock solution in methanol with a concentration of (1000 µg/mL). The calibration curve was constructed keeping the range between 25-450 µg/mL at 5 different concentrations for the developed HPLC-PDA method.

Sample solution: The LSFE samples were finely powdered and thoroughly homogenized, after which approximately 25,000 µg/mL were dissolved in methanol. The solution was filtered using a 0.22 µm PTFE membrane filter before injecting in to the system.

Chromatographic analyses

HPTLC: The method was developed on a Camag HPTLC system (Switzerland) equipped with a Camag TLC Scanner III Camag (Muttenz, Switzerland) and a Linomat V, an automatic sample applicator. The compounds were separated using a pre-coated silica gel aluminium plate 60 F_{254} (10 cm × 10 cm; E. Merck, Germany). Before being used, the HPTLC plates were pre-washed using methanol. Afterwards, drying was carried out in an oven set to 105 ºC for 30 min. The sample solution was applied on an HPTLC plate using a Linomat V automatic applicator keeping 2 µL injection volume.

Plate images under white light were captured with a Camag TLC visualiser. Prior to derivatization, spots were visualized and scanned at 254 nm and 366 nm with Camag winCATS software. Using a 100 µL syringe (Hamilton) and an automated application apparatus, the standards, **LS1** and **LS2** were detected on precoated plates within an 8 mm bandwidth. A 10 cm × 10 cm twin trough glass chamber that was kept for saturation consisting of the optimized mobile phase for 20 min was used to conduct the linear ascending development. After being taken out of the chamber, the plate was dried in an air flow. The device control and image processing were done using VisionCATS software (V3.1).

Quantitative determination of LS1 and LS2 in LSFE by HPLC-PDA: An Agilent 1260 infinity II HPLC-PDA system was used for the analysis. The chromatographic separation was achieved on a Agilent Infinity Lab Poroshell 120 EC-C18 (100 mm × 4.6 mm, 4.0 µm ID) column. The mobile phase consisted of a mixture of 0.1% formic acid in water (A) and acetonitrile (B) in the gradient elution mode: 0.01-3.0 min, 15-30% B; 3.0-6.0 min, 30% B; 6.0-7.0 min, 30-15% B; 7.0-10 min, 15% B. Both a strong (95/05; acetonitrile/water) and a weak (10/90; acetonitrile/water) needle wash solution was used. The analysis run time is 10 min, with a 0.8 mL/min flow rate, a 5.0 µL injection volume and a 330 nm of detection wavelength. In order to identify the peaks, the samples were spiked using reference chemicals, UV spectra and retention time comparisons.

Confirmation of LS1 and LS2 in LSFE by HPLC-TQ-MS: The peaks observed through HPLC-ESI-MS/MS (LC-8045, Shimadzu) system, matches with that of compound for identification and confirmation in the LSFE. A precursor scan was used to confirm the product ions in the analysis, which was conducted under scan mode in the optimized MS/MS conditions in positive and negative ion modes. At 300 ºC, the ESI interface was configured. The optimized temperatures were kept at 300 and 250 ºC for the heat block and desolvation line, respectively. The flow rate for the nebulizing gas was kept 3.0 L min^{-1} and 10.00 L min^{-1} for the heating gas and for the drying gas. The remaining LC conditions were the same as per the HPLC-PDA analysis. Identification and confirmation were performed by comparing the retention times, UV spectra, and mass spectra of **LS1** and **LS2** with those reported in the literature.

HPLC-PDA method validation: The validation was performed according to the guidelines of International Conference on Harmonization (ICH) [14,15]. The novel developed HPLC-PDA method was validated for system suitability, precision (intra and inter-day), recovery, specificity, linearity, LOD and LOQ. The LOD and LOQ were calculated as per linearity. It was calculated as [LOD = 3.3 × Standard deviation ÷ Slope] and the LOQ is calculated as [LOQ = 10 × Standard deviation ÷ Slope]. The recovery (accuracy) was determined using 80%, 100% and 120% concentration levels in triplicate. The precision (intra and inter-day) was assessed for two consecutive days in assay. The assay was conducted three times on six concentrations of sample solution in intermediate moderate precision. The stability of the autosampler solution was assessed up to 60 h with a 12 h injection interval.

Chromatographic determination of bioactive compounds in LSFE samples: For the purpose of assessing the effectiveness of the method, the samples were subjected for qualitative and quantitative analysis of the targeted compounds. To this end, six batches of LSFE were subjected to a qualitative and quantitative investigation of certain bioactive components. The external standard approach is used to quantify the targeted chemicals.

RESULTS AND DISCUSSION

Characterization of LS1 and LS2 using spectroscopic data: The isolations were carried out and two bioactive com-

pounds (**LS1** and **LS2**) were subsequently isolated. The compounds were identified and confirmed using NMR and mass data by comparing the obtained values with that of reported in the literature [12,13].

HPTLC method development for chemical fingerprinting: The chemical fingerprinting for LSFE were conducted using a novel HPTLC analysis. One of the objectives of the current study is to optimise simultaneous HPTLC fingerprint profiles for **LS1** and **LS2** in LSFE [16-18]. An excellent separation was achieved after trying different mobile phase combinations, *viz.* ethyl acetate:toluene (40:60, 45:55, 65:35 v/v), ethyl acetate:formic acid:methanol (7:0.2:3, 8:0.2:2% v/v/v) and ethyl acetate:toluene:glacial acetic acid:formic acid (5:4:1:0.1, 6:2.5:1.5:0.5, 8:3:2.2:1.0% v/v/v/v). From the multiple assessed mobile phase combinations, optimum resolution observed with the ethyl acetate: toluene: glacial acetic acid: formic acid (4.5:5.5:1.1:1.1 % v/v/v/v) for **LS1** and **LS2**. The R_f values were observed as per the respective chromatographic bands for **LS1** and **LS2**, at the R_f equivalent to 0.18 and 0.23, respectively (Fig. 2).

Optimization of HPLC-PDA method for two compounds in LSFE: In chromatographic analysis for *L. siceraria* compounds, the separation depends on the various parameters including polarity of the matrix, resolution of neighbouring peaks, chemistry of targeted compounds and run time, in the simultaneous analysis. To achieve separation, the chromatographic parameters such as selection of column, temperature, mobile phase composition, flow rate and sample extraction technique were optimized.

In sample extraction, the effects of temperature and extraction time along with polar and mid-polar solvents were examined. In comparison to methanol, acetonitrile, water and methanol:water (65:35, 80:20) experiments, methanol (100%) diluent provided the highest extraction and reproducibility. The optimization of column was achieved with trials of Agilent infinity lab poroshell 120 EC- C_{18} (50 mm × 4.6 mm, 2.7 µm ID), Acquity UPLC® HSS T3 (150 mm × 4.6 mm, 5 µm ID), for the purpose of chromatographic optimizations.

For separation in LSFE targeted analytes, Shimadzu shimpack Velox C_{18} (150 mm × 4.6 mm, 5.0 µm ID) and Agilent infinity lab poroshell 120 EC- C_{18} (100 mm × 4.6mm, 4.0 µm ID) were used. The Agilent Infinity Lab Poroshell 120 EC-C_{18} (100 mm × 4.6 mm, 4.0 µm ID) used in the development exhibited more distinct and better resolved peaks (**LS1** and **LS2**). For the simultaneous detection of *L. siceraria* compounds, the wavelength was set at 330 nm. For improved resolution, the mobile phase was optimized using a gradient flow of water-acetonitrile as compared to different water-methanol combinations. Furthermore, formic acid improved the resolution peak shape of the targeted analytes at a flow rate of 0.8 mL/min.

HPLC-PDA method validation: The HPLC-PDA method was verified by assessing its specificity, accuracy, linearity, precision, range, solution stability, limit of quantification and detection. Following the ICH guidelines, the validation was carried out [14,15].

Linearity, range, LOD and LOQ: The calibration curve was constructed by plotting peak area against five different concentrations. The calibration data showed good linearity with regression coefficient $r^2 > 0.99$ between concentration ranging from 25-450 µg/mL. The LOD and LOQ values were 4.151 and 0.512 µg/mL and 12.582 and 1.541 µg/mL, respectively (Table-1). The experiment was carried out in three replicates.

Specificity: By injecting individual standard solutions, the specificity of method was assessed and no analyte interference was found (Fig. 3).

The chromatograms were examined to check additional peaks. By examining UV-spectrum data at different timepoints, such as the peak apex and peak start-end; peak purity

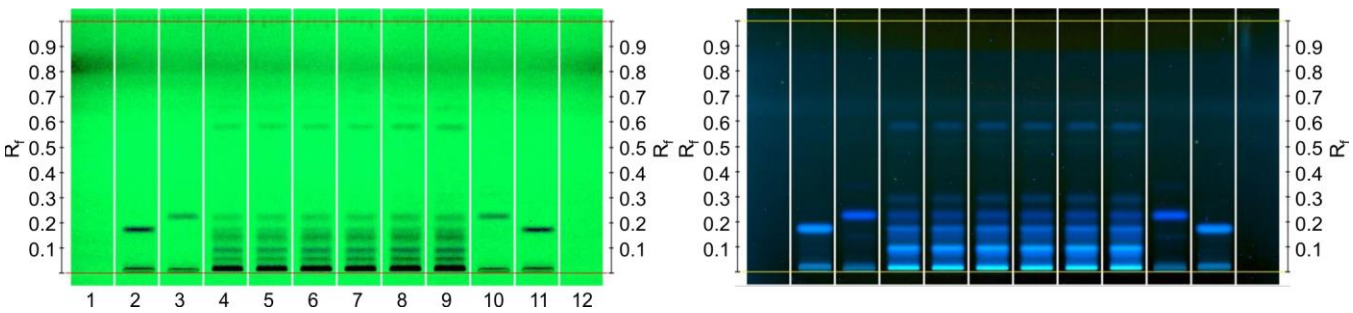

Fig. 2. HPTLC fingerprint of *Lagenaria siceraria* fruit extract (LSFE). Blank (T1 and 12), LS1 (T2 and T11), LS2 (T3 and T-10), LSFE (T4-T9), before derivatization at 254 nm and 366 nm

TABLE-1
LINEARITY (n = 5 × 3), RANGE, LOD AND LOQ (n = 6), % RECOVERY (n = 3), INTER AND INTRA-DAY PRECISION (%, RSD) (n = 6 × 3), IN THE HPLC-PDA METHOD FOR **LS1** AND **LS2**

Analyte	RT (min)	RRT	Range (µg/mL)	Linear regression equation	r^2	LOD (µg/mL)	LOQ (µg/mL)	Average Recovery (± SD)	Method precision		Intermediate precision (%, RSD)
									Day-1 (%, RSD)	Day-2 (%, RSD)	
LS1	4.277	1.000	150-450	3.3095x - 39.584	0.999	4.151	12.582	98.636 ± 2.218	1.236	1.568	1.619
LS2	4.948	1.157	25-75	9.1106x - 40.965	0.999	0.512	1.541	95.171 ± 3.172	0.872	1.842	1.427

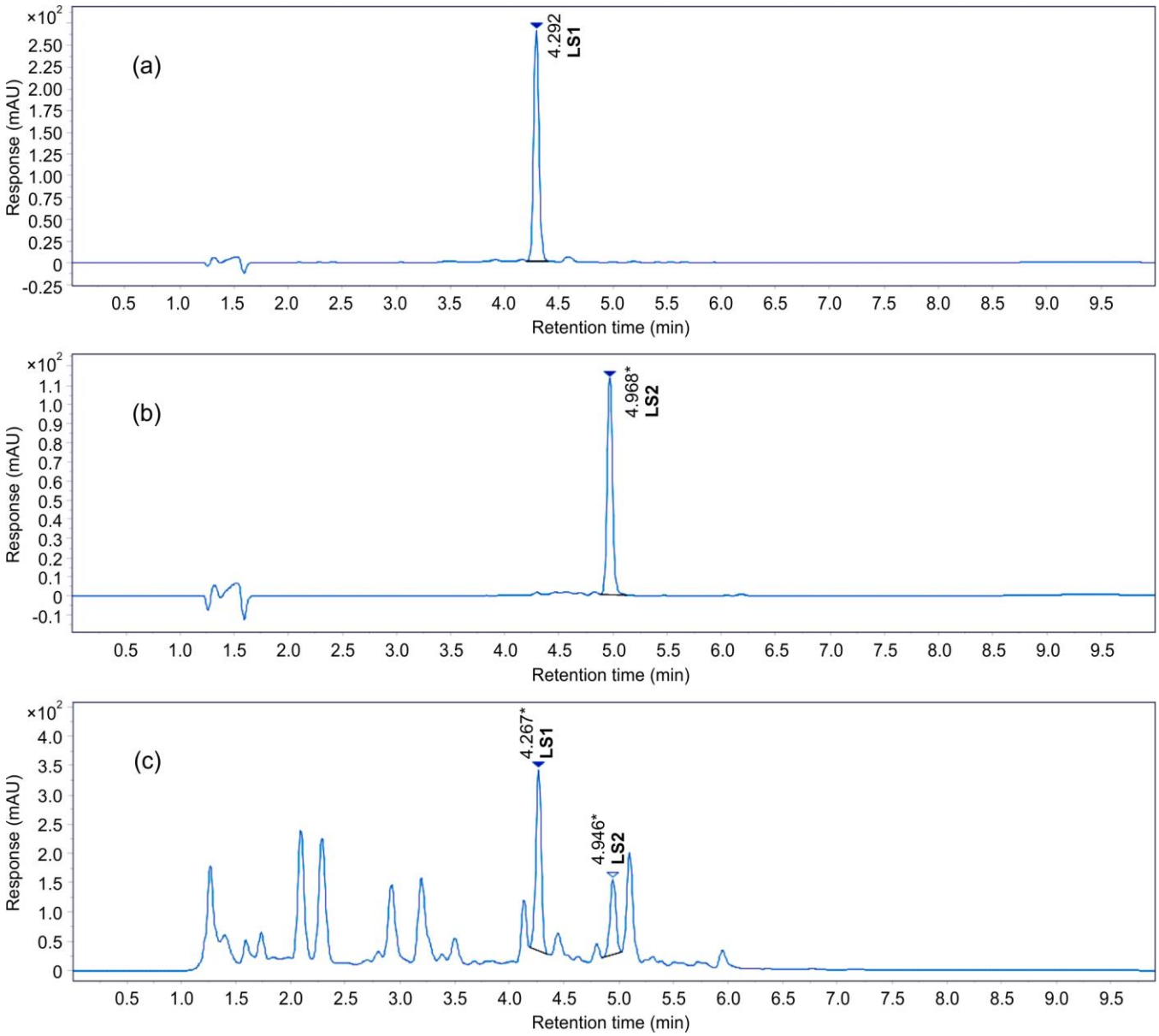

Fig. 3. (a & b) HPLC-PDA chromatograms of the LS compounds (**LS1** & **LS2**) at 330 nm & (c) HPLC-PDA chromatogram of LS fruits extract at 330 nm

and identification were confirmed. The UV spectra at all time-points were the same. It was determined that the chromatographic peak purity was acceptable.

Solution stability: The solution stability was carried out to determine the stability in the autosampler (at set temperature) for 60 h at a fixed intervals. The standard and sample solution were examined both initially and at various points for upto 60 h. Both the sample solution and the standard solution showed no significant variations in area with time in the components.

Precision: The variations in intra- and inter-day analysis were determined for the samples, by repeating in triplicate. The samples were processed six times on two separate days as per the protocol. For **LS1**, the intraday RSD was 1.236% and for **LS2** it was 0.872% (Table-1). For inter-day, it was 1.568% for **LS1** and 1.842% for **LS2** (Table-1). The samples were prepared six times, each in triplicate, for determining the intermediate precision. The RSD was 1.619% for **LS1** and 1.427% for **LS2** (Table-1).

System suitability: A mixture of standard solutions was injected atleast five times to perform the test. An acceptable limit of no more than 2% was observed for the RSD, which was between 0.072 to 0.598. The theoretical plate observed in the range of 35126.291-46493.751, which was within the acceptable limit of not less than 2000. The achieved resolution for these two compounds was 7.32, which was also within the acceptable limit.

Accuracy: The method accuracy was determined by spiking sample with a known concentration of standard mixtures consisting of LSFE. It was performed in triplicate at a three-concentration level of 80-120%, with results of sample recovery as 98.636% and 95.171% (Table-1).

Identification and confirmation of LS1 and LS2 using HPLC-ESI-MS/MS: The pool of phytochemicals observed

in HPLC-MS/MS chromatograms of the LSFE as observed through the peaks (Fig. 4). In the positive ion mode, the ion intensity was tend to be higher as compared to the other one. The compounds were analyzed in ESI mode using a full scan order to optimize mass spectrometer conditions [19]. In the $[M + Na]^+$ mode, the most abundant *m/z* was 471.12 for **LS1** and the adduct $[M + 2ACN + H]^+$ mode, the abundant *m/z* was 677.30 for **LS2** (Table-2).

Applicability of the developed HPTLC and HPLC methods: Two bioactive compounds in six different LSFE samples were effectively identified qualitatively using the HPTLC technique (Fig. 1). There are no significant differences observed among the samples according to the HPTLC fingerprint profile. Different collection sources, such as source, harvesting period or other environmental conditions, may result in variations in the fingerprint profile and chemical composition. Although the fingerprints showed the similarities to the authorized samples, additional research is required to validate them using different analytical methods. This method will work for quick visual comparisons of the variations between different *L. siceraria* species and for rapid authentication. It offers a quick and affordable tool for preliminary qualitative screening. Two targeted compounds were quantified in six batches of LSFE using this validated method. The average content value (n = 3) for the target compounds in the samples were determined. The slight content variation among sample set, indicates the possible impact of processing and storage conditions that may have differed (Table-3, Fig. 5).

Conclusion

For the qualitative and quantitative studies of the two bioactive compounds of *L. siceraria* fruits extract (LSFE), an effective, quick and sensitive, robust and reproducible HPTLC, HPLC-PDA and HPLC-ESI-MS/MS methods were developed. Through validation, the sensitivity and specificity were established. This was further utilized to analyze the LSFE samples. It confirmed that the HPTLC, HPLC-PDA and HPLC-MS/MS methods were suitable for the quality control purposes. This could also be the reliable quality assessment tool for the LSFE analysis. The suggested techniques were observed accurate, precise, rapid and cost-effective. Analysis of botanical-based leads and supplements will benefit from a combination of HPLC, HPTLC and HPLC-MS/MS techniques. Precisely for *L. siceraria* fruit extracts, the combinatorial strategy for standardization based on HPTLC, HPLC-PDA and HPLC-MS/MS has not been sufficiently explored in the past. The current study will help in the design of efficacy studies and scale-up model for large-scale applications of this important botanical ingredient.

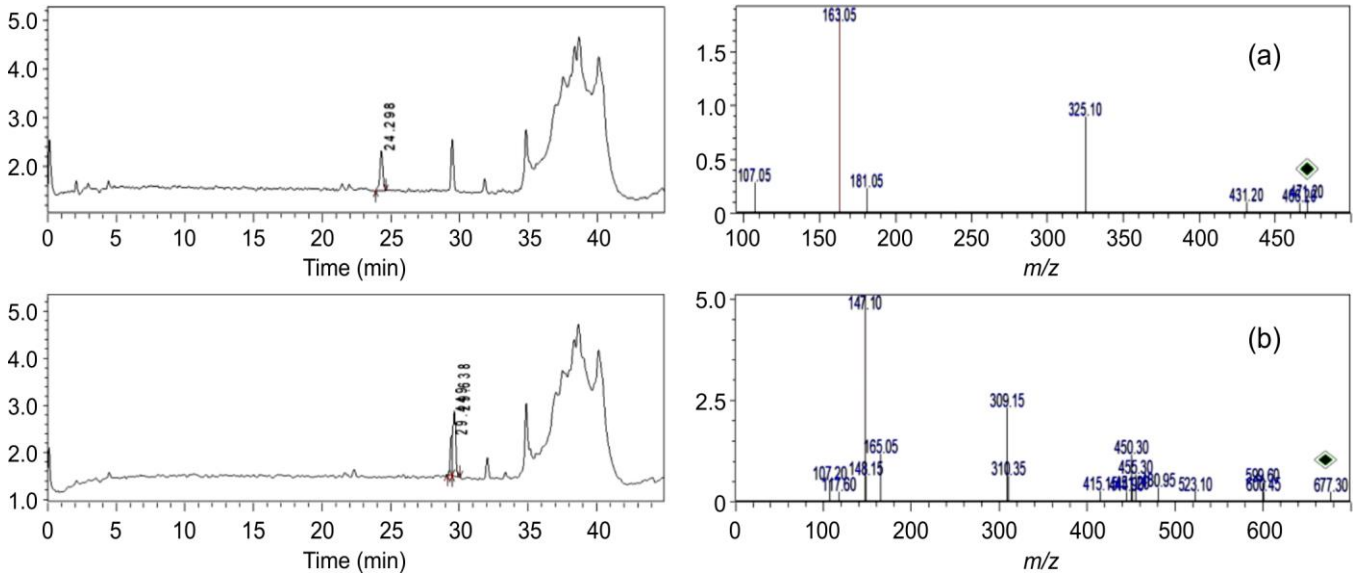

Fig. 3. The confirmation of two compounds in LSFE in full scan (SRM) mode with extracted ion chromatogram (EIC) in the developed HPLC-ESI-MS/MS method with mass spectra at respective *m/z* values in the positive ion mode

TABLE-2
LC-BASED ESI-MS/MS IDENTIFICATION AND MASS SCAN OF LS COMPOUNDS (**LS1** AND **LS2**) IN LSFE

RT (min)	λ_{max} (nm)	Analyte	m.f.	m.w.	Polarity	Mass ion	Precursor ion
4.277	330	LS1	$C_{22}H_{24}O_{10}$	448.1	+	$[M + Na]^+$	471.12
4.748	330	LS2	$C_{27}H_{30}O_{15}$	594.5	+	$[M + 2ACN + H]^+$	677.30

TABLE-3
CONTENT (%) OF TWO COMPOUNDS (**LS1** AND **LS2**) IN LSFE DIFFERENT SIX BATCHES (*n* = 3 ± SD)

Analyte	**LSFE-1**	**LSFE-2**	**LSFE-3**	**LSFE-4**	**LSFE-5**	**LSFE-6**
LS1	1.423 ± 0.181	1.369 ± 0.143	1.381 ± 0.231	1.459 ± 0.451	1.418 ± 0.302	1.437 ± 0.317
LS2	0.231 ± 0.051	0.246 ± 0.043	0.254 ± 0.038	0.226 ± 0.042	0.238 ± 0.037	0.251 ± 0.035

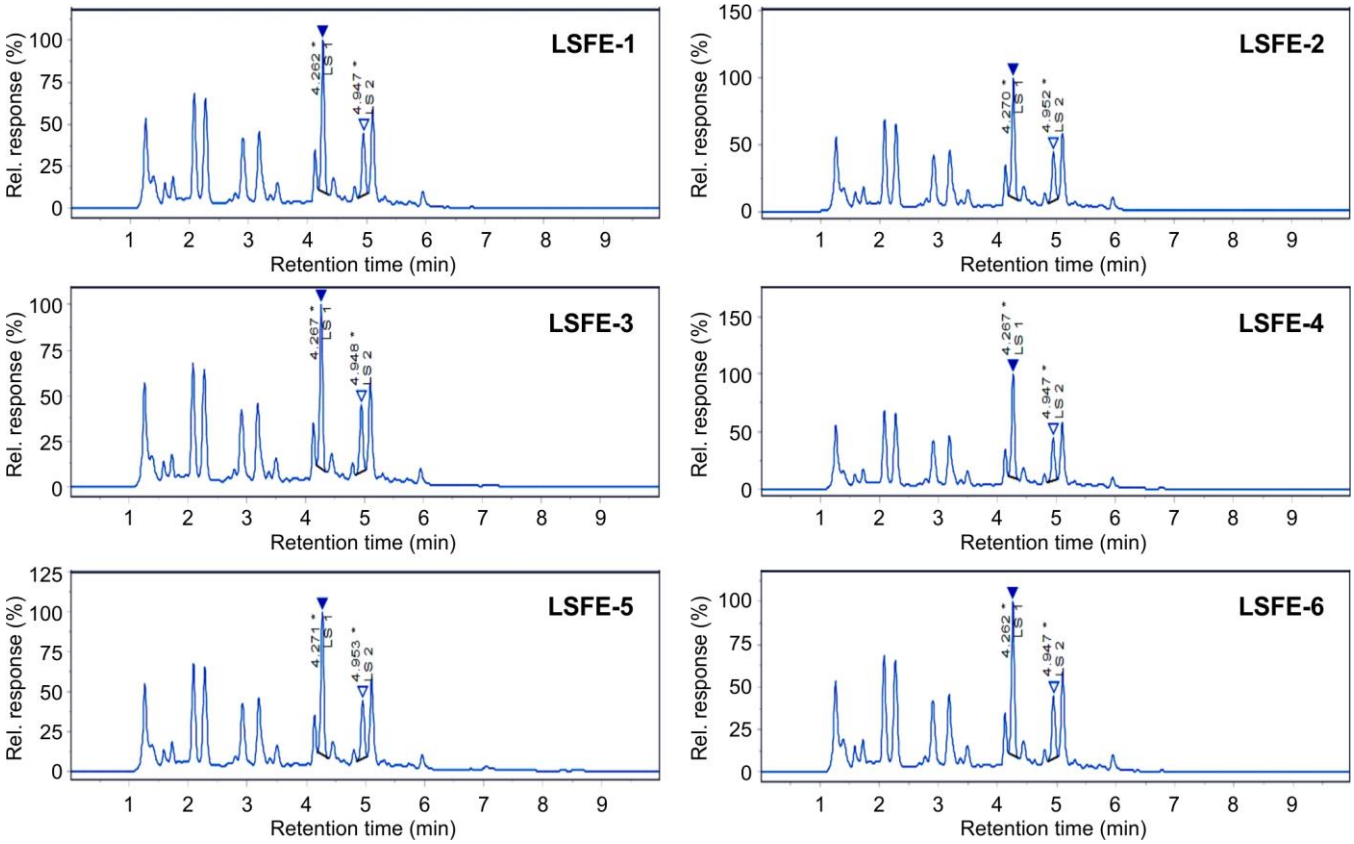

Fig. 5. HPLC-PDA comparative profile of LSFE batches (n = 6)

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. S. Kaur, A. Panghal, M.K. Garg, S. Mann, S.K. Khatkar, P. Sharma, and N. Chhikara, *Nutrition Food Sci.*, **50**, 384 (2019); https://doi.org/10.1108/NFS-05-2019-0143
2. S. Duangmano, P. Sae-Lim, A. Suksamrarn, F.E. Domann and P. Patmasiriwat, *BMC Complement. Altern. Med.*, **12**, 185 (2012); https://doi.org/10.1186/1472-6882-12-185
3. C.Z. Amorim, A.D. Marques and R.S.B. Cordeiro, *Mem. Inst. Oswaldo Cruz*, **86(suppl 2)**, 177 (1991); https://doi.org/10.1590/S0074-02761991000600040
4. B. Zieniuk and M. Pawełkowicz, *Metabolites*, 13, 1081 (2023); https://doi.org/10.3390/metabo13101081
5. A.J. Modi, S.S. Khadabadi, U.A. Deokate, I.A. Farooqui, S.L. Deore and M.R. Gangwani, *J. Pharmacogn. Phytother.*, **2**, 34 (2010).
6. P. Nainwal, K. Dhamija and S. Tripathi, *Int. J. Pharm. Pharm. Sci.*, **3**, 88 (2011).
7. S.B. Patel, U.A. Attar and S.G. Ghane, *Thai J. Pharm. Sci.*, **42**, 90 (2018); https://doi.org/10.56808/3027-7922.2385
8. P. Saha, U.K. Mazumder, P.K. Haldar, S.K. Sen and S. Naskar, *Diabetol. Croat.*, **40**, (2011).
9. M. Saeed, M.S. Khan, K. Amir, J. B. Bi, M. Asif, A. Madni, A.A. Kamboh, Z. Manzoor, U. Younas and S. Chao, *Front. Nutr.*, **9**, 927361 (2022); https://doi.org/10.3389/fnut.2022.927361.
10. A.M. Boudet, *Phytochemistry*, **68**, 2722 (2007); https://doi.org/10.1016/j.phytochem.2007.06.012
11. P. Erasto and Z.H. Mbwambo, *Tanzan. Health Res. Bull.*, **11**, (2009); https://doi.org/10.4314/thrb.v11i2.45206
12. R. Mohan, R. Birari, A. Karmase, S. Jagtap and K.K. Bhutani, *Food Chem.*, **132**, 244 (2012); https://doi.org/10.1016/j.foodchem.2011.10.063
13. B. Kashyap, K. Saikia, S.K. Samanta, D. Thakur, S.K. Banerjee, J.C. Borah and N.C. Talukdar, *J. Ethnopharmacol.*, **301**, 115788 (2023); https://doi.org/10.1016/j.jep.2022.115788
14. International Conference on Harmonization (ICH). Validation of Analytical procedure, text, and methodology, Q2(R1). Geneva, Switzerland: ICH (2005).
15. USP, Validation of Compendial Procedures. United States Pharmacopoeia XXXVII Natl. Formul. XXXII (2007).
16. B. Kumbhalkar, S. Tamhankar and A. Upadhye, *J. Planar Chromatogr. Mod. TLC*, **28**, 294 (2015); https://doi.org/10.1556/1006.2015.28.4.5
17. S. Parmar, R. Prajapati and M. Kalarias, *Folia Med.*, **64**, 84 (2022); https://doi.org/10.3897/folmed.64.e59492
18. M. Irshad, I. Ahmad, H.C. Goel and M.M.A. Rizvi, *Res. J. Phytochem.*, **4**, 242 (2010); https://doi.org/10.3923/rjphyto.2010.242.247
19. A. Girme, P. Bhoj, G. Saste, S. Pawar, A. Mirgal, D. Raut, M. Chavan and L. Hingorani, *J. AOAC Int.*, **104**, 1634 (2021); https://doi.org/10.1093/jaoacint/qsab067