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Development of Validated Spectrophotometric and Chromatographic Methods for Simultaneous Estimation Taxifolin and Bergapten in Marketed Formulation

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Keywords
HPTLC, UV-Vis Spectrophotometry, Taxifolin, Bergapten

Abstract
Background: Bergapten (BER) and taxifolin (TXN), a biomarkers which has many therapeutic activities such as anti-oxidant, anti-inflammatory, anti-microbial, and other pharmacological actions. This shows a new, simple, precise, and accurate High Performance Thin Layer Chromatography (HPTLC) and UV methods for estimating Taxifolin (TXN) and Bergapten (BER) as biomarkers in marketed formulations. Aim: The study determines simultaneous estimation and Q-analysis of UV-Vis spectrophotometry as well as to develop and validate method parameter in High Performance Thin Layer Chromatography (HPTLC). Objective: Application of developed method to quantify these biomarkers in commercial formulation. Materials and methods: The chemicals and reagents were purchased from Merck in India and were of analytical grade. The biomarkers were used of Tokyo Chemical Industry Co., Ltd. bergapten (98.0% w/w) and (+)-taxifolin (95.0%). The biomarkers were estimated and analysed with UV Probe 2.0 software and winCATS software relatively. Methods performed in UV-Vis spectrophotometry (i) Simultaneous estimation and (ii) Q-analysis whereas for HPTLC the biomarkers were simultaneously estimated on parameters of method development. Results: The method for UV-Vis spectroscopy were analysed with a LOD and LOQ of 0.5 and 1 respectively where the %RSD does not exceed more than 2%. The biomarkers were separated by chromatography on precoated aluminium plates as the stationary phase and where optimized by using mobile phase such as toluene:ethyl acetate:formic acid (4:6:0.1 v/v/v) at 306 nm densitometry, and where validated at separate zones. Taxifolin and Bergapten were resolved satisfactorily, with Rf values of 0.64 and 0.82, respectively, in accordance with ICH guidelines. Conclusion: The newly designed UV-Vis Spectroscopy and HPTLC technique has improved specificity, precision and accuracy. The quality of biomarkers containing a flavonoid and furanocoumarins were being validated and quantify these biomarkers in commercial formulation.

1. Introduction

Dihydroquercetin is another name for taxifolin. It is well known for being a bioactive chemical and an antioxidant. Synonyms for Larix dahurica Turcz, ex Trautv, Larix gmelinii (Rupr.) Kuzen, and Larix sibirica Ledeb[1], milk thistle[2], onions[3], Douglas fir[4], and French maritime pine bark[5] can be found in taxifolin. It is also present in numerous other plants. It has hardly ever been used as an individual element. When larch wood is processed industrially, taxifolin is created in crystalline form as an active medicinal component. By preventing cancer cell lipogenesis, it also exhibits anti-proliferative effects on a variety of cancer cell types.

Bergapten (C12H8O4), also known as 5-methoxy psoralen, is a psoralen chemical that is present in grapefruit juice, various citrus essential oils, and bergamot essential oil. Additionally, a large number of therapeutic plants from the Rutaceae and Umbelliferaceae families contain these furanocoumarins derivatives. Bergapten belongs to the chemical class of furanocoumarins. Additionally, they have a wide range of biological effects, such as antibacterial, antioxidant, immunomodulator, apoptotic, and anticancer agents. The biomarkers were tested and validated on Shimadzu 1900 and Camag Linomat V. The method was developed to quantify these biomarkers bergapten (98.0% w/w) and (+)-taxifolin (95.0%). In this research, the biomarkers were

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studied for method analysis in UV-Vis spectroscopy and validating method parameters in high-performance thin layer chromatography as per ICH guidelines.

2. Material and methods

Material:

The biomarkers used by Tokyo Chemical Industry Co., Ltd. are bergapten (98.0% w/w) and (+)-taxifolin (95.0%). The chemicals and reagents were purchased from Merck in India and were of analytical grade.

Instrumentation:

UV-Vis Spectrophotometry

UV-Vis Spectrophotometry was operated on UV-1900 Shimadzu with UV Probe 2.0 software which was used in the validation of biomarkers. The equipment was manufactured in 2019 and was assembled by Shimadzu Manufacturing Asia Sdn, Bhd, Sendayan Techvalley, NS, Malaysia. The instrumentation of single and double-beam spectrophotometers is substantially identical. The incident light beam travels through both the sample and reference cells at the same time, which distinguishes it from a single-beam UV-Vis spectrophotometer. The incident light is separated and directed to the reference and sample cuvettes. The detectors pick up the transmitted or refracted beam. A twin-beam UV-Vis spectrophotometer requires two detectors that detect electron ratio to measure or calculate absorbance in a test sample. It also demands a stable voltage supply [28].

High Performance Thin Layer Chromatography:

A Camag Hamilton (100 µl) syringe was used to apply the sample to Merck's precoated silica plate 60F254. The sample was applied to the TLC plates using the automatic sample applicator Camag Linomat V, which was linked to a nitrogen gas unit. The sample application made use of 6 mm-long bands. The TLC plates were cleaned in a Camag twin trough chamber 20 cm x 20 cm. In a Camag twin trough chamber (20 cm 10 cm), the chromatographic development was carried out in a linear increasing fashion. Densitometric analysis was performed using a Camag TLC scanner III (Muttenz, Switzerland). Migration distance was 14 mm, band separation was 10.0 mm, sample application location was 8.0 mm, solvent front location was 80.0 mm, and scanning speed was 20 mm/s. [6]

Reagents and chemicals:

Preparation of standard stock solution

Taxifolin and bergapten standard stock solutions were made by dissolving precisely weighed volumes of the biomarkers in methanol at 700 and 1000 µg/ml concentrations as per formulation, respectively.

Preparation of standard working solution

The standard stock solution was diluted with methanol to a concentration of 70 µg/ml for taxifolin and 100 µg/ml for bergapten as per the marketed formulation. The marketed formulation was estimated simultaneously with the same concentration as per standard. The commercial formulation, which is utilized as a system and metabolic corrective, has been shown to be antistress, adaptogenic, and comprehensive tablets. The tablets were used in validation parameters using High-Performance Thin Layer Chromatography and in analysis for UV-Vis spectrophotometry. Tablets being validated and acquired by using a working solution.

Preparation of standard stock solution for UV-Vis Spectrophotometry

The standard stock solution was diluted with methanol to a concentration, using biomarkers such as taxifolin and bergapten individually with a concentration of 100 µg/ml, respectively. The working solution was validated, and scanned; for bergapten at 309 nm and taxifolin at 289 nm respectively. Six working standard solutions were made (n=6) with methanol from a stock solution. The resulting solutions were measured at respective λmax and the isobestic point (299 nm) was plotted at the calibration curve to get the linearity and regression equation.

Determination of λmax by UV-Vis Spectrophotometry and HPTLC

The detection of the compounds was done using UV-Vis spectrophotometry since both the biomarkers; TXN and BER, the calibration curve was plotted at a concentration of 2-12 µg/ml. Both were determined using a working solution (n=6) prepared in two separate series of 10 ml volumetric flasks with methanol as the solvent. All the solutions were screened for absorbance at respective λmax. By graphing the concentration versus absorbance, where

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each value represented the average of three determinations, the calibration curves were created. The detection of the compound in High-Performance Thin Layer Chromatography a Camag TLC scanner III was used for multi-wavelength detection mode (both short and long wavelength), the densitometric analysis of produced, air-dried HPTLC plates was carried out to determine the maximum concentration between short-wave UV light 254 nm and long-wave 366nm. They were scanned individually on Camag TLC scanner III with the trace of spots on an automatic sample applicator connected to a nitrogen gas unit using equipment called Camag Linomat V. The spots were accurate, and scanned at a visible range to acquire densitogram. [7, 8]

Methods:

Simultaneous equation method

The absorption maxima of the biomarkers bergapten and taxifolin are used in the simultaneous equation method of analysis. Two wavelengths selected for the development of the simultaneous equations are 289 nm and 309 nm. The absorptivity values determined for taxifolin are 0.213 (ax1), 0.13 (ax2), and for bergapten 0.317 (ay1), 0.185(ay2) at 289 and 309 nm, respectively. These are the averages of six (n=6) calculations. To calculate drug concentrations, the absorbance and absorptivity at various wavelengths were replaced into equations (1) and (2). [7, 8]

$$Cx = A2\ (0.317) - A1\ (0.185)/0.01 \quad (1)$$

$$Cy = A1\ (0.13) - A2\ (0.213)/0.01 \quad (2)$$

Where A1 and A2 represent mixture absorbance at 289 nm and 309 nm, respectively, ax1 and ax2 represent TXN absorptivity at 1 and 2, respectively, ay1 and ay2 represent BER absorptivity at 1 and 2, respectively, and Cx and Cy represent TXN and BER concentrations, respectively. [9, 10]

Q-analysis

The absorbance ratio analysis technique is based on absorbance at two different wavelengths, one of which is an isobestic point and the other is the wavelength of maximum absorption of one of the two components. For the construction of Q absorbance equations (3) and (4), the wavelengths 299 nm isobestic and 289 nm (max of taxifolin) are chosen from the spectra. The absorptivity values determined for taxifolin are 1.494 (ax1), and 1.338 (ax2), and for bergapten are 2.219 (ay1), and 1.915 (ay2) at 289 nm and 309 nm, respectively. These values are the average of estimations. To calculate medication concentrations, the absorbance, and absorptivity at various wavelengths were replaced into equations (3) and (4). [9, 10]

$$Qx=ax2/ax1,\ Qy=ax2/ax1,\ Qm=A2/A1$$

$$Cx= Qm – Qy/Qx- Qy \times A1/ax1 \quad (3)$$

$$Cy= Qm – Qx/Qy- Qx \times A2/ay1 \quad (4)$$

Where,

Cx, Cy - Concentrations of TXN and BER respectively (g/100ml).

Qx - Ratio of absorptivity of TXN at 289 nm and 309 nm.

Qy - Ratio of absorptivity of BER at 289 nm and 309 nm.

Qm - Ratio of absorbance of Mixture at 289 nm and 309 nm.

A - Isobestic point absorbance of a mixture.

Ax - Absorptivity of TXN at Isobestic point.

Ay - Absorptivity of BER at Isobestic point.

High Performance Thin Layer Chromatography

Precision was checked by repeated scanning of spots (i) system precision 4, 5, 6 µl for standard and (ii) method precision 4, 5, and 6 µl for tablet preparation. The reading was taken six times and was expressed in terms of relative standard deviation (%RSD). The repeatability of the method was done by analysing the spots of standard solutions of TXN and BER after application on the HPTLC plate (n = 6) and expressed in terms of % RSD. Variability of the method was studied by analysing aliquots of standard solutions of TXN and BER (1, 2, 3, 4, 5, 6 μlspot^{-1}) on the same day (intraday precision) and on different days (interday precision) and the results were expressed in terms of %

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RSD. The accuracy of the method was determined by three levels of recovery studies (100%, 80%, and 120%). The biomarkers were analysed and the percent recovery was calculated[11]. Different dilutions of standard solutions of TXN and BER in methanol were applied. The limit of detection (LOD) and limit of quantification (LOQ) were determined on the basis of-signal to-noise ratios of 3: 1 and 10: 1.

Application of developed biomarker in marketed formulation:

a) Simultaneous equation method

Two wavelengths were selected for the method (289 nm and 309 nm) as the absorbance maxima of TXN and BER respectively in methanol. Standard stock solutions (100µg/ml for both biomarkers) were prepared separately in methanol. The stock solutions of both biomarkers were further diluted separately with methanol to get a series of standard solutions of 2-12 µg/ml for TXN and BER, respectively. The absorbance was measured at the selected wavelengths and absorptivity for both the biomarkers at both wavelengths was determined. Equations (1) and (2) were used to compute the concentrations in the samples. [7, 9, 10]

b) Q-analysis

Two wavelengths were selected for the method (289nm and 299nm) as one is the maximum wavelength for TXN and the other is the isobestic point respectively in methanol. Standard stock solutions (100µg/ml for both biomarkers) were prepared separately in methanol. The stock solutions of both biomarkers were further diluted separately with methanol to get a series of standard solutions of 2-12 µg/ml for TXN and BER. The absorbance at the relevant wavelengths was measured, and the absorptivity for both drugs was determined for both wavelengths. Equations (3) and (4) were used to compute the concentrations in the samples. [7, 9, 10]

c) High Performance Thin Layer Chromatography

HPTLC is an inexpensive method of analysis in the laboratory as well as in the field. It is dependable, sensitive, and appropriate for both qualitative and quantitative analysis when HPTLC techniques are combined with the automated sample application and densitogram scanning[12]. The ability to visualize and save chromatographic fingerprints as digital images make it a useful tool for fingerprint identification. An overview of HPTLC's modern analytical technique;

- Selection of chromatographic layer.
- Layer pre-washing.
- Layer pre-conditioning.
- Application of sample and standard.
- Samples and standard preparation.
- Optimization of the mobile phase.
- Chromatographic development.
- Scanning and documentation of chromatoplate.
- Detection of spots.

Validation of method:

a) Specificity

UV-spectrum of blank solvent (Methanol) and a solution containing taxifolin and bergapten individual was scanned between the range of 400-200 nm and observed for the interference of any absorbance at 289 nm and 309 nm respectively with an isobestic of 299 nm. The specificity in HPTLC was determined using a TLC plate and the standard drug solution, sample solution, diluent, and mobile phase. The plate was then developed and scanned for possible interference. [13, 14]

b) Precision

The precision study of UV-Vis spectroscopy was carried out within (2-12 µg/ml) between both biomarkers. The readings were taken on an interval time period on the same day (intraday precision) and on different days (interday precision). The precision system of HPTLC was determined by applying a standard solution of bergapten, 4, 5, 6 µlspot^{-1}, to TLC plates six times. The intra-day and inter-day precision were validated, where 4, 5, and 6 µlspot^{-1} were applied in triplicate. Calculating Conc ± SD and % RSD on both days. The system precision, intraday precision, and interday precision were validated precisely. [13, 14]

c) Accuracy

In studying the method validation parameter for biomarker recovery, three levels of analysis were

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performed. Standard biomarkers were spiked into the pre-analyzed sample solution at levels of 80%, 100%, and 120%, and triple analyses were performed on these solutions. By carrying out the amount in tablet, % recovery and average % recovery ±SD. [13, 14]

d) Robustness of method

The method varied in the duration of mobile phase saturation (10 and 20 minutes) and change in the composition of the mobile phase (6:4:0.1 and 5:5:0.1 v/v/v of toluene, ethyl acetate, and formic acid). The robustness of the UV parameter was extended by stock solution with the solvent methanol. Using the same solvent replicating in working solution containing biomarkers individually and where at all different wavelengths measured. [13, 14]

e) The limits of detection (LOD) and quantification (LOQ)

LOD and LOQ were calculated using the standard deviation of the regression line's y-intercept. Calculating the signal-to-noise ratio (S/N); 3.3 for LOD and 10 for LOQ using the following equation as defined by the ICH guidelines[7, 11].

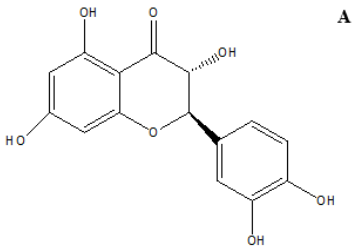
$$LOD = 3.3\sigma/S$$

$$LOQ = 10\sigma/S$$

Where, σ= standard deviation response and S= slope of the calibration curve

3. Result:

Structure of compounds



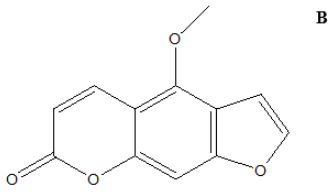
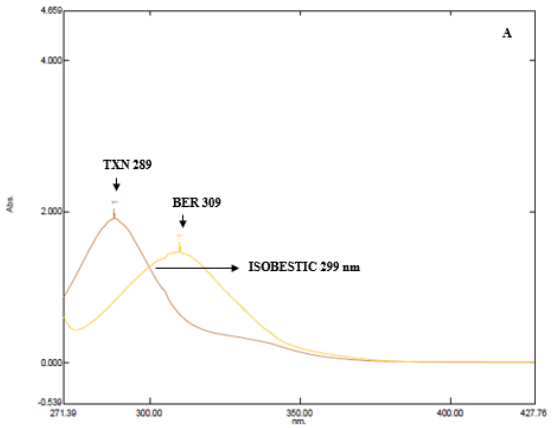


Fig. 1. Structure (A) Taxifolin and (B) Bergapten as both biomarkers.

Determination of wavelength of maximum absorbance (λ_{max}) and Isobestic Point



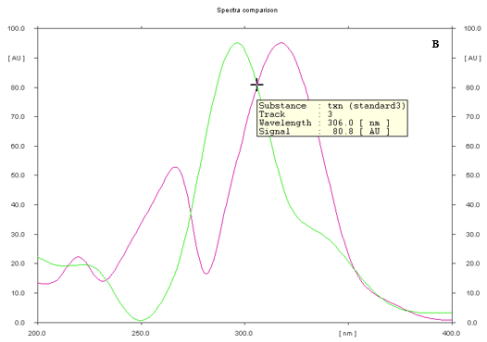


Fig. 2 Determining λ_{max} of isobestic point TXN and BER in both (A) UV spectroscopy and (B) in HPTLC.

Spectral overlay of taxifolin and bergapten in HPTLC

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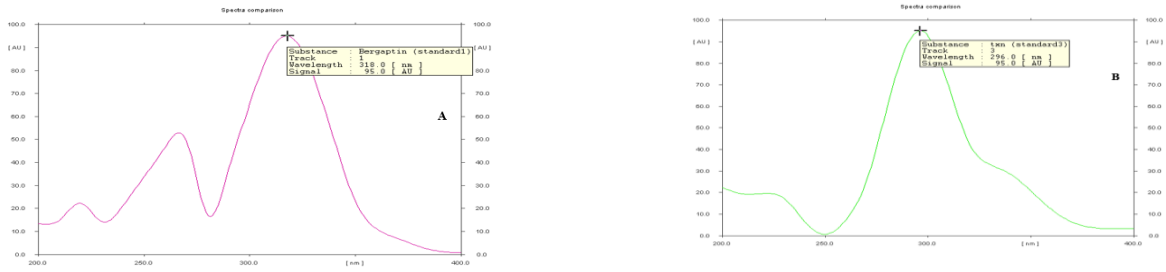

Fig. 3. Spectral scanning of both the biomarkers (A) bergapten and (B) taxifolin.

Analysis of biomarker in formulation:

Table 1. Analysis of biomarker in marketed formulation.

Sr. no.	**Biomarker**	**Concentration (µg/ml)**	**% found**	**Relative Standard Deviation (%RSD)**
1.	Taxifolin	700	99.17±0.295	0.042
2.	Bergapten	515	99.85±0.367	0.071

Estimation of Taxifolin and Bergapten

Table 2. Characteristics of biomarkers in UV spectrophotometry

Parameters	**Method I**		**Method II**	
	Taxifolin	**Bergapten**	**Taxifolin**	**Bergapten**
Wavelength λ_{max} (nm)	289 nm	309 nm	Isobestic point at 299 nm	
Correlation coefficient R^2	0.981	0.9411	0.9966	0.9567
Slope	0.2165	0.1796	0.1753	0.1549
Intercept	0.4515	0.2299	0.2669	0.2266

B

Densitogram of standard biomarkers

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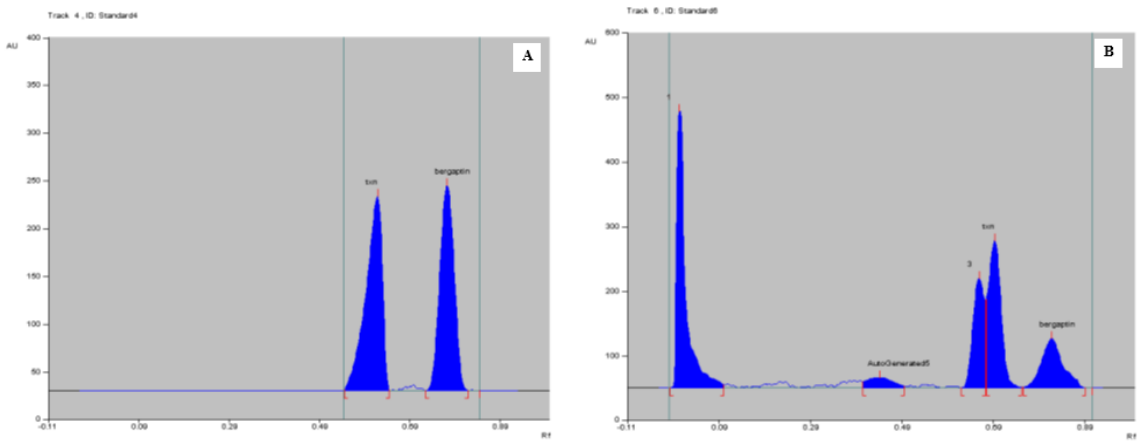

Fig. 4. Densitogram scanning of standard biomarkers (A) and polyherbal scanning (B) TXN and BER.

Validation parameter of method parameter:

a) Linear regression data for calibration curve

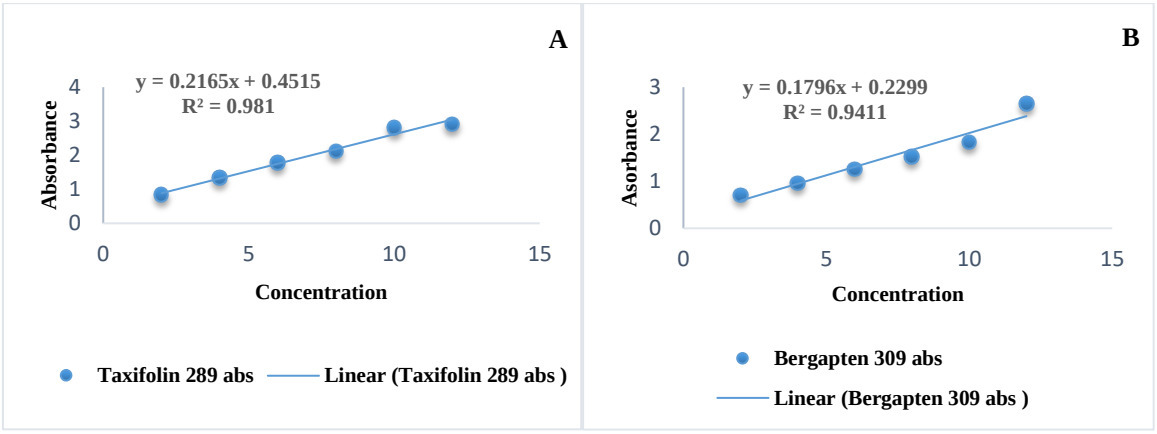

Fig. 5 Calibration curves of (A) TXN in methanol at wavelength (λ_{max}) 289 nm, (B) BER in methanol at wavelength (λ_{max}) 309 nm by UV-Vis spectroscopy.

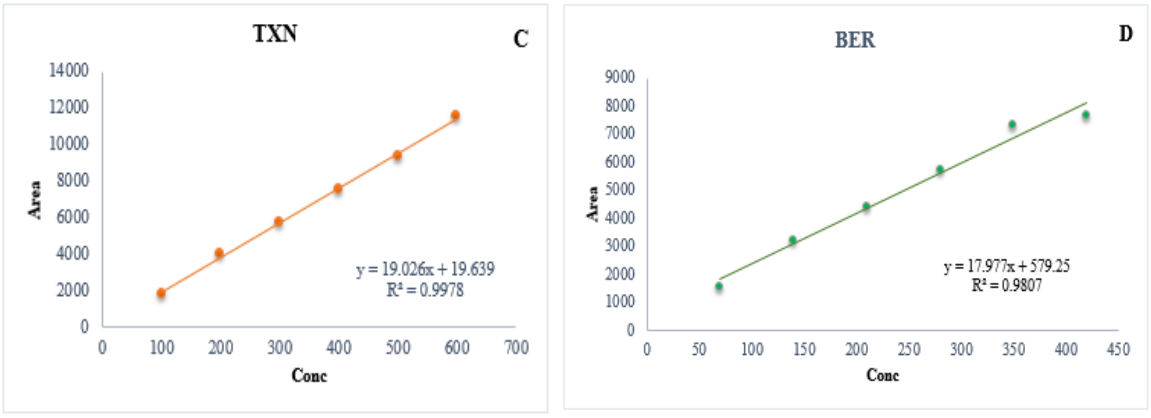

Fig. 6 Calibration curves of (C) TXN in methanol at wavelength (λ_{max}) 296 nm, (D) BER in methanol at wavelength (λ_{max}) 319 nm by determining in HPTLC.

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Table 3. Linear regression data of following method validation.

Parameters	**Results**			
	HPTLC		**UV**	
	Taxifolin	**Bergapten**	**Taxifolin**	**Bergapten**
Linearity range	1-6µl	1-6µl	2-12 µg/ml	2-12 µg/ml
Wavelength	296 nm	318 nm	289 nm	309 nm
Determination of λ_{max}	306 nm	306 nm	299 nm	299 nm
Correlation coefficient (r^2)	0.9978	0.9807	0.981	0.9411
Slope	19.026	17.977	0.2165	0.1796
Intercept	19.639	579.25	0.4515	0.2299
Standard Deviation	1.870	1.870	3.741	3.741
Limit of Detection (µg/ml)	0.1	0.2	0.5	0.5
Limit of Quantification (µg/ml)	0.5	0.5	1	1

b) Precision of method

Table 4. Precision study of intraday and interday to validate method parameter.

Drug	**Conc (µg/ml)**	**Intraday found Conc ± SD**	**Relative Standard Deviation (%RSD)**	**Interday found Conc ± SD**	**Relative Standard Deviation (%RSD)**
Taxifolin	4	388.81±0.455	0.117	387.10±0.490	0.127

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		382.97±0.427	0.112	387.09±0.461	0.119
		372.27±0.230	0.062	368.81±0.508	0.138
	5	484.09±0.115	0.024	466.3±0.560	0.120
		475.27±0.230	0.049	402.25±0.288	0.072
		460.18±0.173	0.038	375.14±0.346	0.092
	6	582.17±0.670	0.116	546.06±0.479	0.088
		572.13±0.086	0.015	439±0.057	0.013
		555.13±0.334	0.060	399.54±0.404	0.101
Bergapten	4	395.06±0.363	0.092	436.69±0.461	0.106
		396.61±0.479	0.121	400±0.115	0.029
		391.72±0.540	0.140	366.38±0.461	0.126
	5	500±0.034	0.071	529.55±0.461	0.087
		499.94±0.533	0.107	500±0.173	0.035
		499.94±0.533	0.107	472.09±0.288	0.061
	6	598.64±0.461	0.077	574.86±0.513	0.089
		596.44±0.277	0.046	626.23±0.230	0.037
		595.05±0.473	0.080	600±0.381	0.063

c) Accuracy of method

Table 5. Recovery Study

Biomarker	**% Level**	**Amount in tablet (µg/ml)**				**% recovery ±SD**	**%RSD**
		TXN*	**Concentration added**	**Total**	**Concentration obtained**		
Taxifolin	80	700	560	1260	1249	99.12 ±0.450	0.032%
	100	700	700	1400	1387	99.07±0.305	0.022%
	120	700	840	1540	1529	99.28±0.350	0.023%
Bergapten	80	515	412	927	919	99.13±0.285	0.031%
	100	515	515	1030	1023	99.32±0.308	0.030%
	120	515	618	1133	1129	99.64±0.478	0.042%

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d) Robustness of method

Table 6. Robustness of method

Concentration (µl)	Parameters		Rf	Assigned Substances
4	Optimised mobile phase v/v/v	Toluene : ethyl acetate : formic acid (4:6:0.1) v/v/v	0.64	TXN
			0.82	BER
4	Composed mobile phase v/v/v	Toluene : ethyl acetate : formic acid (6:4:0.1) v/v/v	0.26	TXN
			0.68	BER
		Toluene : ethyl acetate : formic acid (5:5:0.1) v/v/v	0.53	TXN
			0.75	BER
4	Saturation time	10 minutes	0.64	TXN
			0.79	BER
		20 minutes	0.33	TXN
			0.69	BER
4	Temperature		0.60	TXN
			0.79	BER

4. Discussion

The linearity range for Taxifolin and Bergapten is 2–12 µg/ml at respective selected wavelengths. The coefficient correlation for taxifolin at 289 nm and for bergapten at 309 nm is 0.981 and 0.9411, respectively. Both biomarkers showed good regression values at their respective wavelengths, and the results of a recovery study revealed that any small change in the biomarker concentration in the solution could be accurately determined by the proposed methods[9]. Whereas, High-performance thin layer chromatography (HPTLC) is a sophisticated instrumental technique based on the full capabilities of thin-layer chromatography. The benefits of automation, scanning, full optimization, the selective detection concept, minimal sample preparation, hyphenation, and so on. Enable it to be a powerful analytical tool for chromatographic information of complex mixtures of inorganic, organic, and biomolecules [12]. HPTLC fingerprinting has been demonstrated to be a linear, exact, and accurate approach for herbal identification, as well as a tool for authenticating and characterizing medicinally significant plants. The developed HPTLC fingerprints will help the manufacturer with quality control and standardization of herbal formulations[15]. Precision was determined by studying intraday and interday precision. The % RSD was calculated for all the

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biomarkers as the % RSD should not exceed more than ≤ 2%.

5. Conclusion:

The analysis on HPTLC and UV-vis Spectrophotometry method was developed for bergapten and taxifolin analysis which was found to be simple, precise, accurate, and specific. The developed method was extremely sensitive. This method would be useful in the analysis and formulation of taxifolin and bergapten. As the authors are using these biomarkers for further development and validation of biomarkers using chromatography and spectrophotometry in the comparison of biomarkers in traditional medication vs herbal medication.

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References

[1] Pew JC. A flavonone from Douglas-fir heartwood. J Am Chem Soc.1948;70(9):3031- Sawant RL, Hadawale SD, Dhikale GK, Bansode CA, Tajane PS. Spectrophotometric methods for simultaneous estimation of rabeprazole sodium and aceclofenac from the combined capsule dosage form. Pharm Methods. 2011;2(3):193–7.doi: 10.4103/2229-4708.903624. doi: 10.1021/ja01189a059, PMID 18882535.

[2] Wallace SN, Carrier DJ, Clausen EC. Batch solvent extraction of flavanolignans from milk thistle (Silybum marianum L. Gaertner). Phytochem Anal. 2005;16(1):7-16. doi: 10.1002/pca.803. PMID 15688950.

[3] Slimestad R, Fossen T, Vågen IM. Onions: A source of unique dietary flavonoids.J Agric Food Chem. 2007;55(25):10067-80. doi: 10.1021/jf0712503, PMID 17997520.

[4] Kiehlmann E, Li EPM. Isomerization of dihydroquercetin. J Nat Prod.1995;58(3):450-5. doi: 10.1021/np50117a018 R. Slimestad R, Fossen T, Vågen IM. Onions: A source of unique dietary flavonoids. J Agric Food Chem.2007;55(25):10067-80, doi: 10.1021/jf0712503, PMID 17997520.

[5] Rohdewald P. A review of the French maritime pine bark extract (Pycnogenol),a herbal medication with a diverse clinical pharmacology. Int J Clin Pharmacol Ther. 2002;40(4):158-68. doi: 10.5414/cpp40158, PMID 11996210.

[6] M. C. Chavan, Navratne AR, Patil RB, Vanjari SS. Development and Validation of Stability Indicating High Performance Thin Layer Chromatography Method for Analysis of Bergapten. Tathawade; Maharashtra, India, J. Pharm. Sci. & Res. Vol. 11(9), 2019, 3237-3242

[7] Srinivas B, Ashraf S, Vivek N, Rajashree H. Development and validation of bivariate UV-visible spectroscopic method for simultaneous estimation of curcumin and piperine in their combined nanoparticulate system. J Appl Pharm Sci. 2021;doi: 10.7324/japs.2021.110509

[8] Gomathi D, Kalaiselvi M, Ravikumar G, Sophia D, Gopalakrishnan VK, Uma C. Secondary metabolite credentials of Evolvulus alsinoides by high performance thin layer chromatography (HPTLC). J Biomed Res. 2012;26(4):295–302.doi: 10.7555/JBR.26.20110128

[9] Sawant RL, Hadawale SD, Dhikale GK, Bansode CA, Tajane PS. Spectrophotometric methods for simultaneous estimation of rabeprazole sodium and aceclofenac from the combined capsule dosage form. Pharm Methods. 2011;2(3):193–7.doi: 10.4103/2229-4708.90362

[10] Bhaskar KL, Lakshmi DS, Sumalatha G, Suji G, Kumar KAT. Q-analysis and simultaneous equation method for estimation of domperidone and naproxen by UV spectrophotometry in bulk and tablet dosage form. Res J Pharm Technol. 2020;13(12):6050–4.doi: 10.5958/0974-360x.2020.01054.9

[11] A. K. S. Rawat, A. P. Singh, D. P. Singh, M. M. Pandey, R. Govindarajan, and Sharad Srivastava. Separation and Identification of Furocoumarin in Fruits of Heracleum candicans DC. Hindawi Publishing Corporation; Volume 2013, Article ID 915762, 4 pages, 2013.doi: 10.1155/2013/915762

[12] Srivastava, M. (2010). An Overview of HPTLC: A Modern Analytical Technique with Excellent Potential for Automation, Optimization,

ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4
Journal of Coastal Life Medicine

Hyphenation, and Multidimensional Applications. High-Performance Thin-Layer Chromatography (HPTLC), 3–24. doi:10.1007/978-3-642-14025-9_1

[13] ICH guidelines, Q1A(R2): Stability Testing of New Drug Substances and Products. (revision 2). International Conference on Harmonization. 2003.

[14] Urška Jug, Irena Vovk, Vesna Glavnik, Damjan Makuc, Katerina Naumoska. Off-line multidimensional high performance thin-layer chromatography for fractionation of Japanese knotweed rhizome bark extract and isolation of flavan-3-ols, proanthocyanidins and anthraquinones. Journal of Chromatography A 1637 (2021) 461802; 2020.

[15] Sampathkumar S, Ramakrishnan N. Chromatographic fingerprint analysis of Naringi crenulata by HPTLC Technique. Asian Pac J Trop Biomed 2011(1S); 195-8.

[16] Yogesh P Pancham, Girish B and Shailendra Suryawanshi Sanjay, UV-Spectrophotometric method for quantification of ascorbic acid in bulk powder, The Pharma Innovation Journal 2020; 9(5): 05-08

[17] Glassco J. Analytical Method Development and Validation. LLS Health CDMO. Lubrizol CDMO.2019; [cited 2023 May 12]. Available from: https://lubrizolcdmo.com/technical-briefs/analytical-method-development-and-validation/

[18] Le Borgne E, Cicchetti E, Bertrand T. HPTLC methods for qualitative and quantitative analysis of selected furocoumarins in essential oils. Flavour Fragr J. 2017;32(5):330–9. http://dx.doi.org/10.1002/ffj.3394

[19] Mignot B, Guillaume Y, Makki S, Murret E, Cavalli E, Truong TT, et al. High-performance thin-layer chromatographic determination of 5-methoxypsoralen in serum from patients. J Chromatogr. 1997;700(1–2):283–5.doi: 10.1016/s0378-4347(97)00229-6

[20] Sawant RL, Hadawale SD, Dhikale GK, Bansode CA, Tajane PS. Spectrophotometric methods for simultaneous estimation of rabeprazole sodium and aceclofenac from the combined capsule dosage form. Pharm Methods 2011;2(3):193–7.doi: 10.4103/2229-4708.90362

[21] Pancham YP, Sanjay SS. UV-Spectrophotometric method for quantification of ascorbic acid in bulk powder [Internet]. Thepharmajournal.com. [cited 2023 May 12]. Available from: https://www.thepharmajournal.com/archives/2020/vol9issue5/PartA/8-6-15-737.pdf

[22] Jinxiu Hou, Mingyang Hu, et al, Dietary Taxifolin Protects Against dextran Sulfate Sodium-Induced Colitis via NF-κB Signaling, Enhancing Intestinal Barrier and Modulating Gut Microbiota, Frontiers in Immunology, 2021, Volume 11. doi: 10.3389/fimmu.2020.631809

[23] Sunil C, Xu B. An insight into the health-promoting effects of taxifolin(dihydoquercetin).Phytochemistry.2019; 166:112066.doi10.1016/j.phytochem.2019.112066

[24] Melough, M. M., Cho, E., Chun, O. K., Food Chem. Toxicol. 2018, 113, 99-107

[25] Hung, W. L., Suh, J. H., Wang, Y., Food Drug Anal. 2017, 25, 71-83

[26] Kubrak, T., Podgorski, R., Stompor, M., Eur. J. Clin. Exp. Med. 2017, 15, 169-175

[27] Moura FCS. Taxifolin stability: In silico prediction and in vitro degradation with HPLC-UV/UPLCeESI-MS monitoring. Journal of Pharmaceutical Analysis. 2020 Jun 29;11:232–40.

[28] UV-Vis spectroscopy: Principle, instrumentation, and applications [Internet]. PSIBERG. 2021 [cited 2023 May 12]. Available from: https://psiberg.com/uv-vis-spectroscopy/