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Research Article

Pharmacognosy for Efficient Natural Therapy

Marker Based Standardization of Herbal Sunscreen Formulation by Using Validated High-Performance Thin Layer Chromatography Method

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Abstract: Plant based products are worldwide used and trusted for the care and cure of the health. Herbal sunscreen formulations are gaining popularity for their effectiveness and are devoid of side effects. The present investigation is an effort made to develop herbal sunscreen cream containing methanolic extracts of different plants and its standardization for the presence of bioactive compounds. The leaves of *Cymbopogon citratus* (*Stapf),* fruit peel of *Punica granatum (Linn),* flowers of *Butea monosperma* (Lam.) and leaves of *Neolamarckia cadamba* (*Roxb*.) were selected for the preparation of sunscreen cream. The standardization of herbal formulations is very important to determine its quality based on the concentration of their active phytoconstituents. The herbal cream F-5 (2 % w/w) imparted its sun-protective and antioxidant property. It shows free radicals scavenging activity due the presence of flavonoids and phenolic compounds. High performance thin layer chromatography method is used to determine the quality and quantity of the sun protective phytoconstituents present in the product. The method was validated according to ICH guidelines for the estimation butrin (BT), isobutrin (IBT), quercetin (QC), apigenin (API), chlorogenic acid (CA) and gallic acid (GA) using the optimized solvent systems. The estimation of bioactive markers was carried out on silica gel precoated thin layer chromatography plates with $60F_{254}$ as the stationary phase and Camag TC scanner III for densitometric scanning. The average R_f values for the markers were found to be 0.46 for BT, 0.57 for IBT, 0.50 for QC, 0.57 for API, 0.66 for CA and 0.42 for GA. The developed HPTLC method was linear with correlation coefficient 0.999 for BT, 0.998 for IBT, QC, API and 0.9966 for CA and 0.9989 for GA. Limit of detection (LOD) and limit of quantification (LOQ) were recorded. The developed analytical method for quantitative determination of phytoconstituents was found efficient, simple, accurate, and validated.

Keywords: Sunscreen cream, flavonoids, phenolic compounds, bioactive markers, HPTLC

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1. INTRODUCTION

UV radiations are the major cause of harmful effects on human skin when exposed for a longer time to sunlight. UV rays can cause sunburn, premature skin aging, DNA damage, and skin cancer.[1] The UV spectrum is divided into three regions, UVA (320-400 nm) penetrates deep into the skin and its effects are additive to the effects of UVB (280-320 nm) for inducing skin cancer[2] and UVC (100–290 nm), which get filtered through atmospheric ozone layer known as a stratospheric layer and not associated with the harmful effect on the skin.[3] One cannot avoid getting exposed to the sunlight and only the way is to use sunscreens products that can absorb or block UV radiation.[4] Recently, the development of sunscreens with broad-spectrum anti-UV radiation products with a reduced concentration of chemical UV filters emerging in the market.[5] Synthetic agents used in photoprotective have limitations as they cause potential toxicity in humans and their ability to interfere in certain selected pathways of the multistage process of carcinogenesis. When the skin gets more exposure to sunlight, it produces free radicals. They interfere with DNA, protein and fatty acid results in oxidative damage to body system and interfere with the regulation pathways of skin. Whereas phytoconstituents are gaining popularity as ingredients in cosmetic formulations as they can protect the skin from such exogenous and endogenous harmful agents.[6] Herbal sunscreen development provides UV absorption property and skin protection against UVB and UVA radiations with the benefits of the products and compliance of the consumers.[10] Few examples include tea polyphenols, curcumin, silymarin, garlic compounds, apigenin, resveratrol, ginkgo biloba, beta-carotenoids, and ascorbic acid.[7] Important categories of useful phytocompounds are phenolic acids, flavonoids, and high molecular weight polyphenols [8,9]. In recent era crude drugs and herbal products are standardized by using chemical, botanical, spectroscopic and biological methods.[12] This study is an attempt to develop sunscreen cream and its standardization for the purity and quality of bioactive compounds by using HPTLC method. The bioactive compounds selected for High-performance thin layer chromatography are butrin and isobutrin [13], quercetin [17], apigenin [14,17], chlorogenic acid (CGA) [15,17,20], quercetin [16,20] and gallic acid [19]. The proposed High-performance thin layer chromatography (HPTLC) method used is a robust, simplest, rapid, and efficient tool in quantitative analysis of the phytoconstituents [21,22].

2. MATERIALS AND METHODS

2.1 Collection and Authentication of Plant Material

Cymbopogon citratus (Stapf) leaves family *Gramineae* and *Punica granatum* (Linn) fruit peels family *Punicaceae* were collected from medicinal plant garden of Alard College of Pharmacy, Pune. *Butea monosperma* (Lam.) flowers family *Fabacea*e and *Neolamarckia cadamba* (Roxb.) leaves family *Rubiacea*e were collected from Ahmedabad, Gujarat. Plants parts were cleaned, sun dried and authenticated on 25 January 2016 at Botanical Survey of India, Western Regional Centre, Pune with specimen no: BSI/WRC/IDEN.CER. /2016/662-666.

2.2 Preparation of Plant Extracts

Coarse powders of selected parts of plants were passed through a 40-mesh sieve, 100 gm of each powder was refluxed for 2 hrs using 250ml of petroleum ether (60-80^{0}C) to remove non- polar compounds. The marc left after was subjected to continuous hot extraction with 250ml of pure methanol for 36 hrs.[23] The extracts were concentrated at reduced pressure and temperature (40°C) using a rotary evaporator. [24,25] The % yield was found to be 4.54 % w/v, 3.79% w/v, 11.35% w/v, 3.26 % w/v for *Butea monosperma*, *Neolamarckia cadamba*, *Punica granatum* and *Cymbopogon citratus* extract respectively.

2.3 Chemicals and Reagents

Cetomacrogol 1000, Cetostearyl alcohol, Methylparaben, Propylparaben, Light Liquid Paraffin, White Soft Paraffin, Propylene Glycol, Chlorocresol, Sodium Dihydrogen Phosphate Dihydrate purchased from Analab Fine Chemicals, Mumbai. Quercetin, chlorogenic acid, gallic acid, apigenin, were procured from Yucca Enterprises, Mumbai. Butrin and isobutrin from Sigma Aldrich. Ethyl acetate, formic acid, acetic acid was of analytical grade and purchased from Merck, India Ltd, Mumbai, India.

2.4 Formulation of O/W Sunscreen Cream

Aqueous phase prepared by mixing 80% quantity of distilled water, chlorocresol, and sodium dihydrogen phosphate dihydrate and heated at 70°C. Oil-soluble contents like light liquid paraffin and white soft paraffin was mixed together with the addition of cetomacrogol 1000 and cetostearyl alcohol at the temperature 70°C. Then oil phase was mixed with an aqueous phase to get a uniform emulsion. The mixture of extracts (1:1) mixed with propylene glycol and heated at 70°C. Finally, the mixture was added to the mixing phase and quantity was adjusted with the remaining 20% distilled water. 0.5ml of lavender oil was added as perfume to the preparation when it attains a temperature of 35°C, cooled and stored in suitable container. [26]

2.5 HPTLC Conditions

TLC plates (20 × 10 cm) precoated with silica gel 60F254 TLC plates (E. Merck) (0.2 mm thickness) supported with aluminium sheet were used. The spotting was done with CAMAG Linomat V automatic sample spotter (Camag Muttenz, Switzerland); mounted with the 100 µL syringe (Hamilton, Switzerland). Each band of 6mm was spotted with distance 10 mm between each band at the rate of 150 nL/s. The start position along the X-axis was at 15 mm and the application position along the Y-axis was at 8 mm. The plates were developed in a CAMAG glass twin trough chamber of 20 × 10 cm covered with a stainless-steel lid. The densitometer used consisted of a CAMAG TLC Scanner 3 linked to Win CATS Software.

- The slit dimensions were kept as 5× 0.45 mm with the scanning speed of 20 mm/s. The mobile phase saturated for 25 min at room temperature and developed up to 80 mm. and after plates were dried for 15 min. The optical densitometric scanning was done at λ_{max} 330nm for Butrin (BT) and isobutrin (IBT) using ethyl acetate: methanol: formic acid: water (4:0.8:0.2:0.2 v/v/v/v) as solvent system. Quercetin, chlorogenic acid and apigenin scanning at λ_{max} 252nm using ethyl acetate: formic acid: acetic acid: water (10:1.1:1.1:0.6 v/v/v/v) as a solvent system. Gallic acid scanned

at λ_{max} 275nm using Toluene: ethyl acetate: formic acid: methanol (3:3:0.8:0.2 v/v/v/v) as a solvent system.

2.6 Preparation of Stock Solution[27]

The stock solutions of standard markers i.e. BT, IBT, QC, APG, CA and GA were prepared by dissolving 10 mg /10ml pure methanol. Different dilutions were prepared in 10 ml of volumetric flask from stock solution and the calibration curve was plotted for each standard marker.

2.7 Sample Preparation of Sunscreen cream

5gm of the formulation was weighed accurately and extracted with 20 ml of pure methanol and 20 ml of hexane by means of separating funnel. The mixture was shaken vigorously and kept it for 5 min for separating the two layers. The methanolic layer was separated and evaporated to dryness. 25 mg of dry methanolic extract was dissolved in 25 ml of pure methanol by ultrasonication for 10 min and filtered through a membrane filter. Further diluted for the analysis of marker compounds.

2.8 Quantitative Analysis of Markers

10 µl of sample solutions were spotted on a TLC plate and the peak areas were recorded. The calibration curve was plotted to determine the amount of each marker present in sample. The analysis was repeated in triplicate and % content was determined.

2.9 Method Validation [22,23]

Validation of the method was carried as per the International Conference on Harmonization (ICH. Q2A, ICH. Q2B) guidelines for linearity, precision, the limit of detection (LOD), the limit of quantification (LOQ), accuracy, repeatability, selectivity, and robustness.

2.9.1 Linearity

The calibration curve was plotted for each standard solution with the application of concentration in the range of 200 - 1000ng. The plates were developed in respective solvent systems, dried and scanned in 200-400 nm of range. After densitometric scanning, the peak area was recorded for each concentration. Peak areas vs concentrations were plotted, and the slope & correlation coefficient was noted. The procedure was repeated in triplicates.

2.9.2 Precision

Intraday precision was carried out with an analysis of six replicate applications of the three different concentration 100, 120 and 140 ng/band of freshly prepared solutions of the standard and 10 µl, 20 µl and 40 µl of cream F-5 solution on the same day. Interday precision was evaluated on two different days and analysis was done in six replicate applications as previous. Instrumental precision was measured with application of same marker band for ten times. The % RSD of peak areas was calculated.

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2.9.3 Limit of Detection and Limit of Quantification

The limit of detection (LOD) and Limit of quantitation (LOQ) was determined using formulae LOD = 3.3 X (σ /s) and LOQ = 10 X (σ /s) respectively. Where σ is the SD of the response (y-intercept) and S is the slope of the linearity[28].

2.9.4 Accuracy

Accuracy is measured by the recovery study of standard BT, IBT, QC, APG, CA and GA by standard addition method (80, 100 and 120 %) on F-5 cream sample solutions. Their response was measured in terms of peak area and % recovery was determined. The accuracy was performed in triplicate.

2.9.5 Repeatability

The repeatability was done by analysing 200 ng/spot of BT, IBT, QC, API, CA and GA individually spotted on TLC plate at the same conditions. The method was repeated 6 times and % RSD was determined.

2.9.6 Selectivity

The selectivity of an analytical method has the ability to measure the analyte accurately and specifically that may be present in the sample like impurities, degradation products, and matrix components. It was determined from the sample peak area or R_f value. The bands of BT, IBT, QC, API, CA and GA of cream samples were compared with the R_f and spectra of standards. The purity of peak of each sample was analysed at the starting, middle and end position of the bands.

2.9.7 Robustness

Change in the mobile phase composition, its volume, and chamber pre-saturation time used to study robustness. Robustness was done in six replicates at a concentration level of 200ng/band of BT, IBT, QC, CA, APG, and GA. The % Relative Standard Deviation of peak areas was calculated.

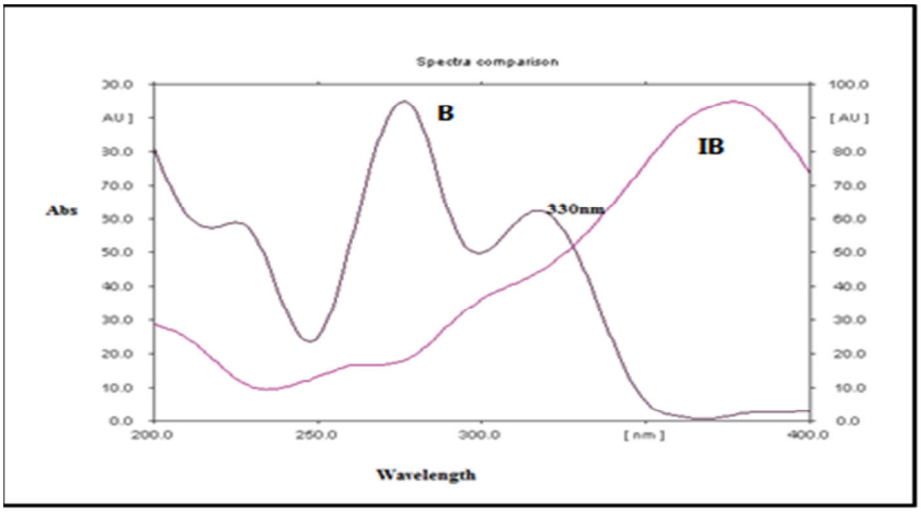
3. STATISTICAL ANALYSIS

The data obtained were analysed using Microsoft Excel 2016. The data were represented as mean±SD and % RSD of the values obtained for different parameters.

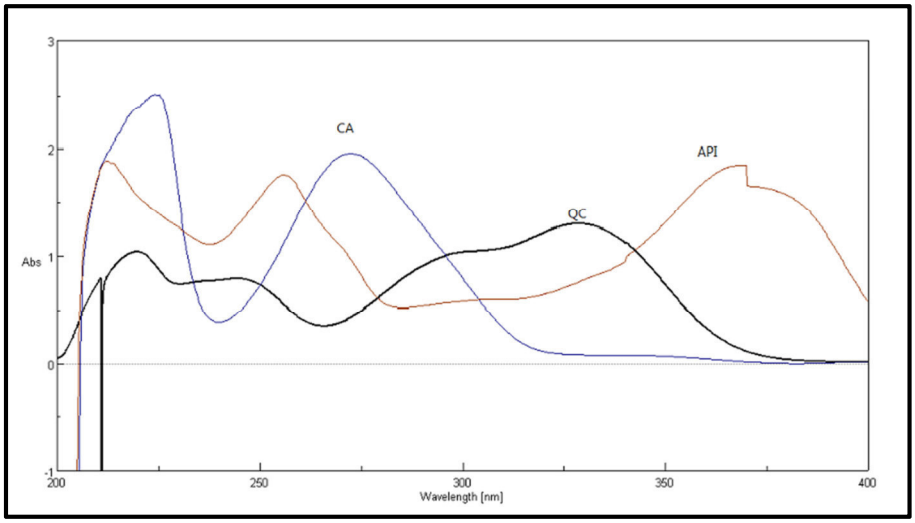
4. RESULTS AND DISCUSSION

4.1 Selection of Wavelength

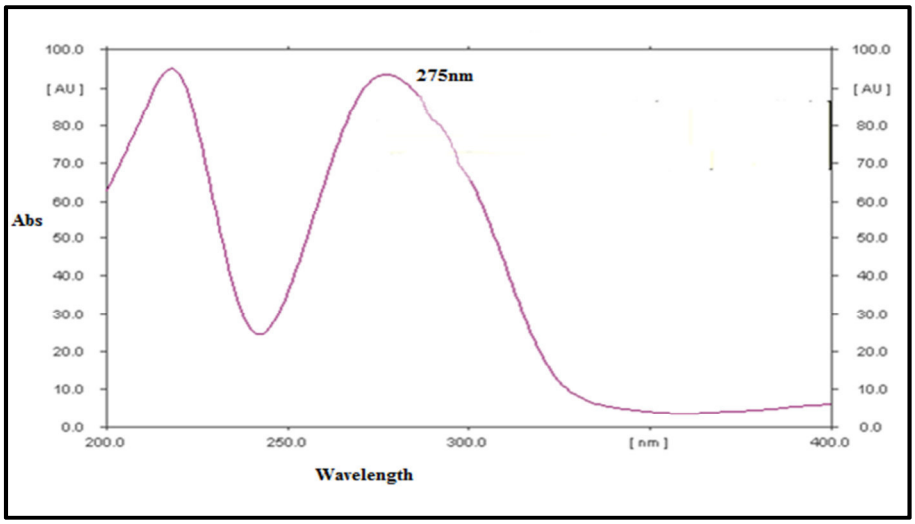
The spectra were scanned for each marker and overlain spectra were taken to find the iso absorptive point. The isoabsorptive point for butirn and isobutrin was λ_{max} 330 nm. The quercetin, apigenin, and chlorogenic acid were at λ_{max} 252nm and gallic acid maximum absorbance at λ_{max} 275nm. The scanning wavelength for marker compounds are shown in Fig 1



(a) Scanning Wavelength for Butrin and Isobutrin



(b) Scanning Wavelength for Quercetin, Apigenin and Chlorogenic Acid



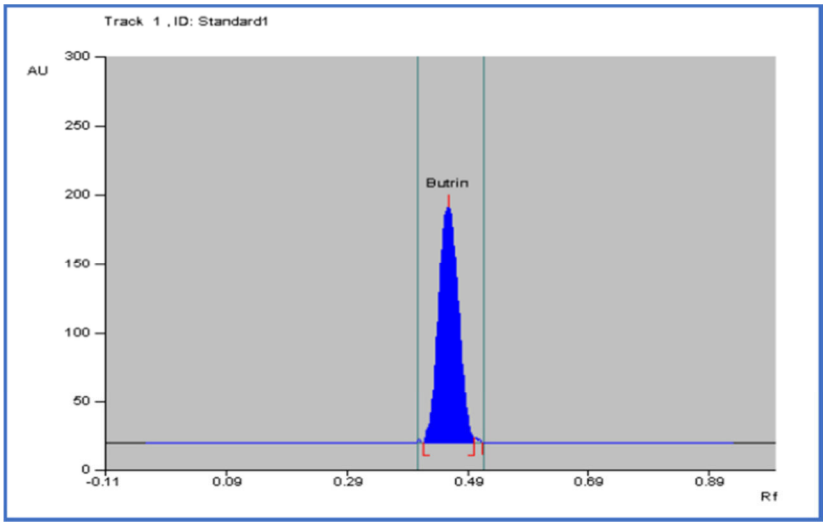
(C) Scanning Wavelength for Gallic acid

Fig 1. Scanning Wavelength and spectra of each marker compound (a) *Butrin and Isobutrin (b) Quercetin, Apigenin and Chlorogenic Acid and (c) Gallic acid*

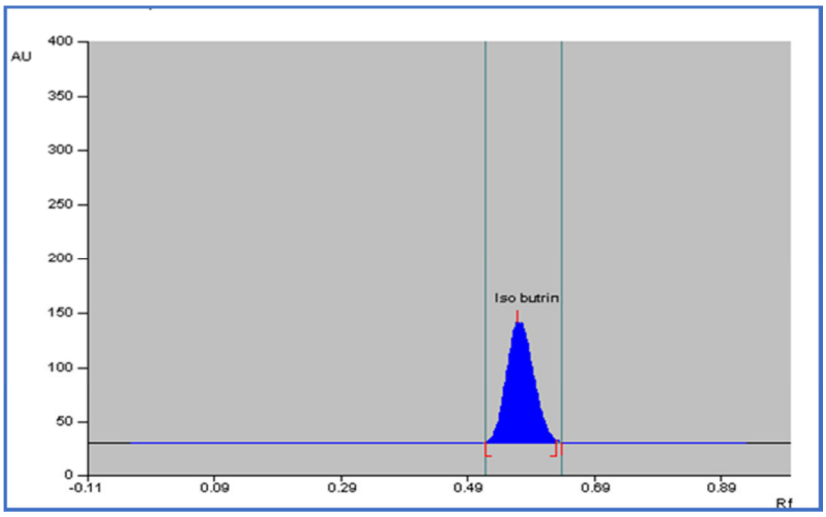
4.2 Optimization of HPTLC Method

Different polarity solvents such as ethyl acetate, methanol, toluene, water, formic acid, and acetic acid were tired in different proportions for separation of the pure compounds. The effect of changing the proportion of mobile phase on the retention factor, resolution of the tested analytes and peak shapes were studied. The optimised mobile phase shown well

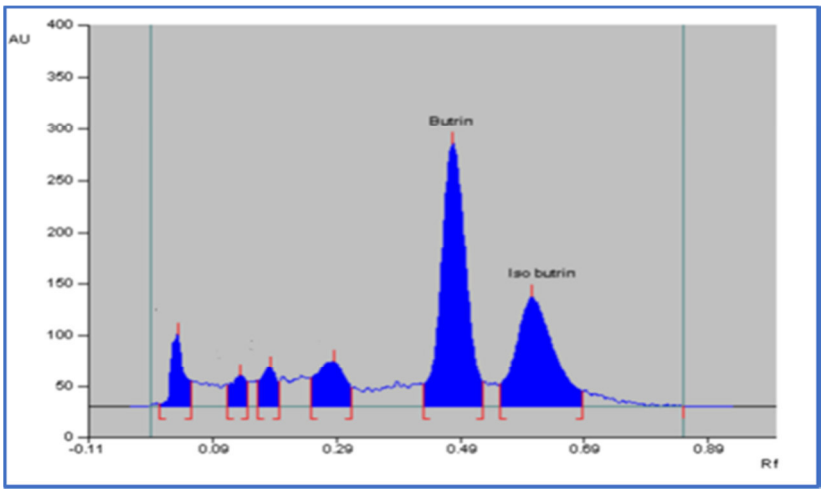
resolved peak shape was selected for further analysis. The retention factor in respective mobile phase for BT, IBT, QC, API, CA and GA were found to be 0.46 ± 0.04, 0.57±0.004, 0.50±0.004, 0.57 ±0.006, 0.66 ±0.004 and 0.42±0.006 respectively. The Densitogram for each marker in sample are shown in Figure 2,3 and 4. Selected phytoconstituent were standardized for herbal sunscreen cream formulation (F-5) and the % content was determined as reported in Table 1.



(a) *Densitogram for Butrin (BT)*

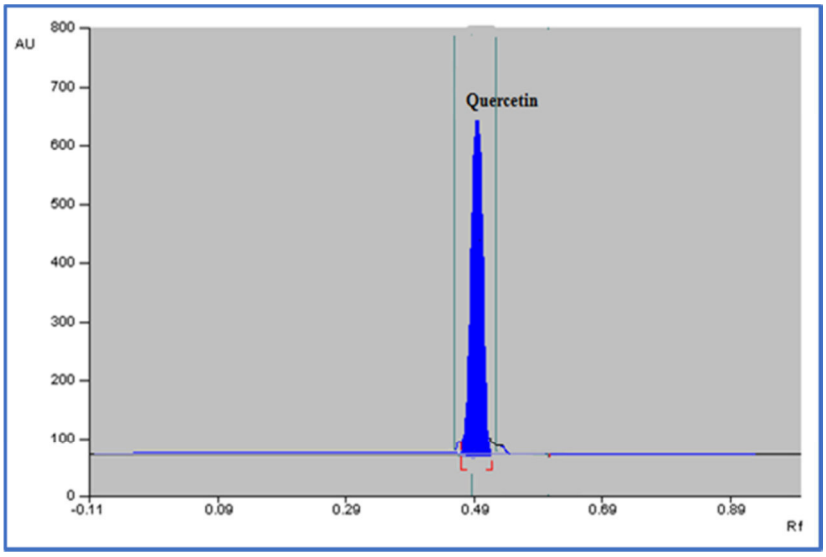


(b)Densitogram for Isobutrin (IBT)

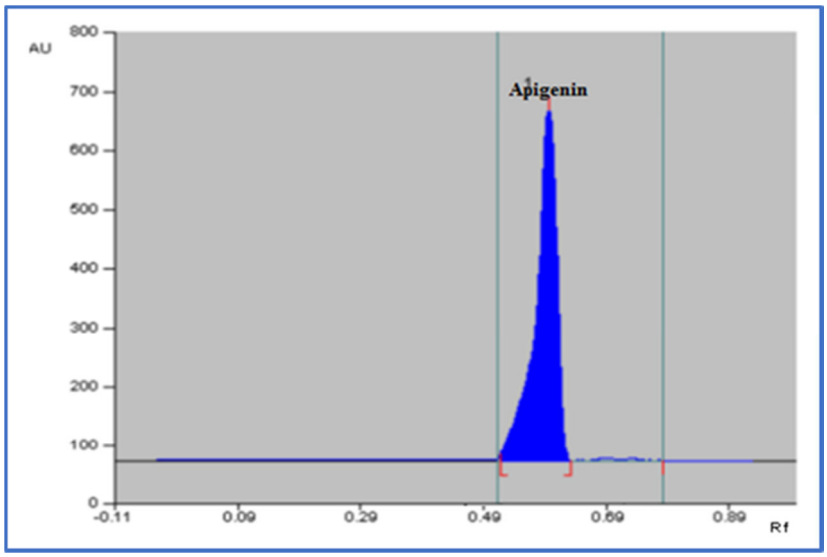


(c) Densitogram for BT and IBT in Formulation

Fig 2. Densitogram for (a) Butrin (BT), (b) Isobutrin (IBT) marker and (c) BT and IBT in Formulation

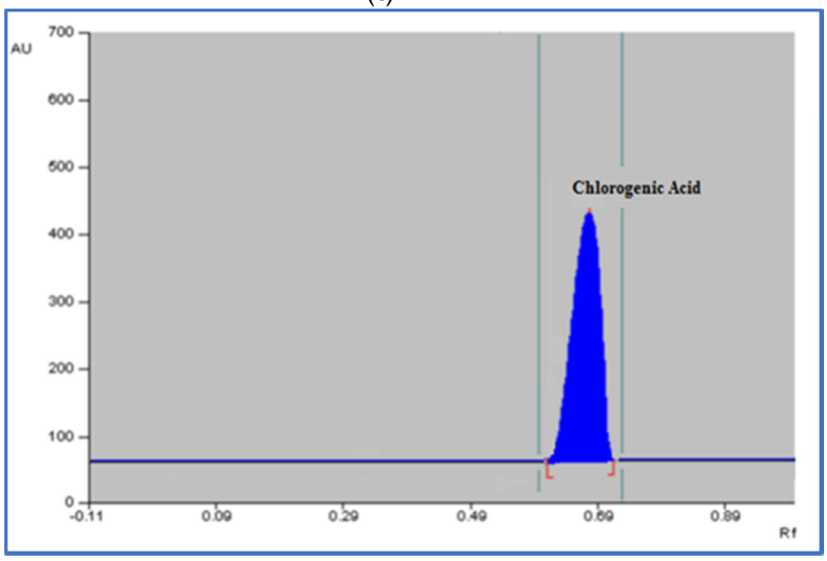


(a) Densitogram for Quercetin (QC)

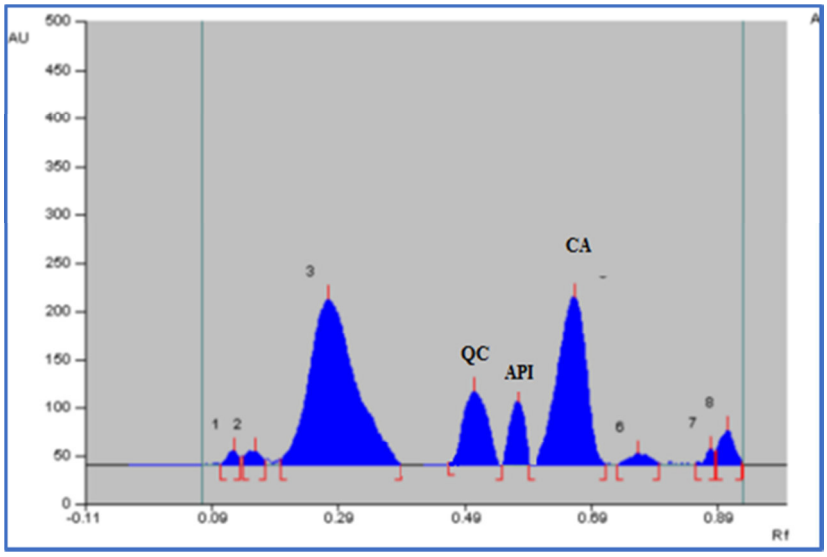


(b) Densitogram for Apigenin (API)

(c)



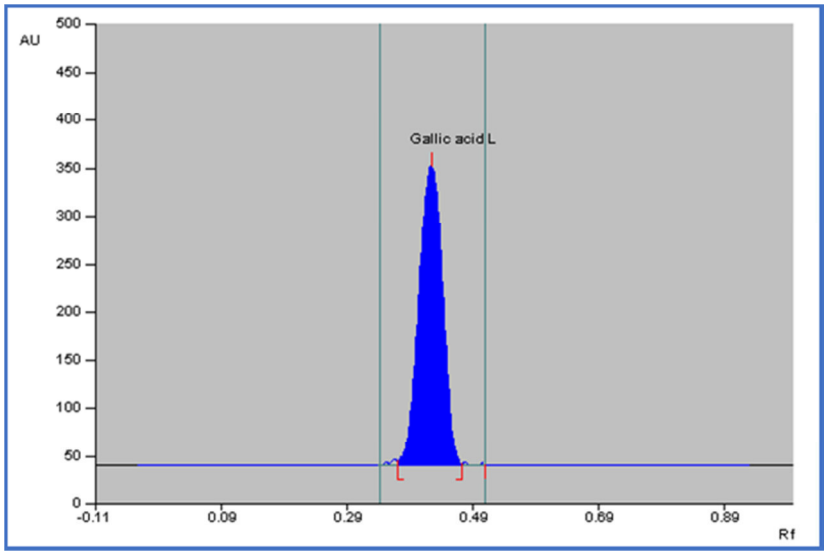
(C) Densitogram for Chlorogenic Acid (CA)



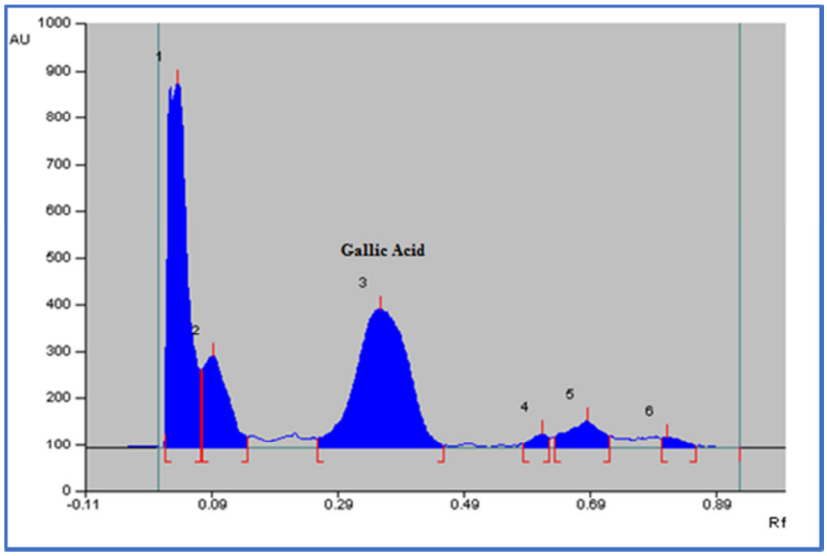
(d) Densitogram for QC, API and CA in Formulation

(e)

Fig 3. Densitogram for(a) Quercetin (QC), (b) Apigenin (API), (C) Chlorogenic Acid (CA) marker and (d) for QC, API and CA in Formulation



(a) Densitogram for Gallic Acid (GA)



(b) Densitogram for Gallic Acid (GA) in Formulation

Fig 4 Densitogram for (a) Gallic Acid (GA) marker and (b) Gallic Acid (GA) in Formulation

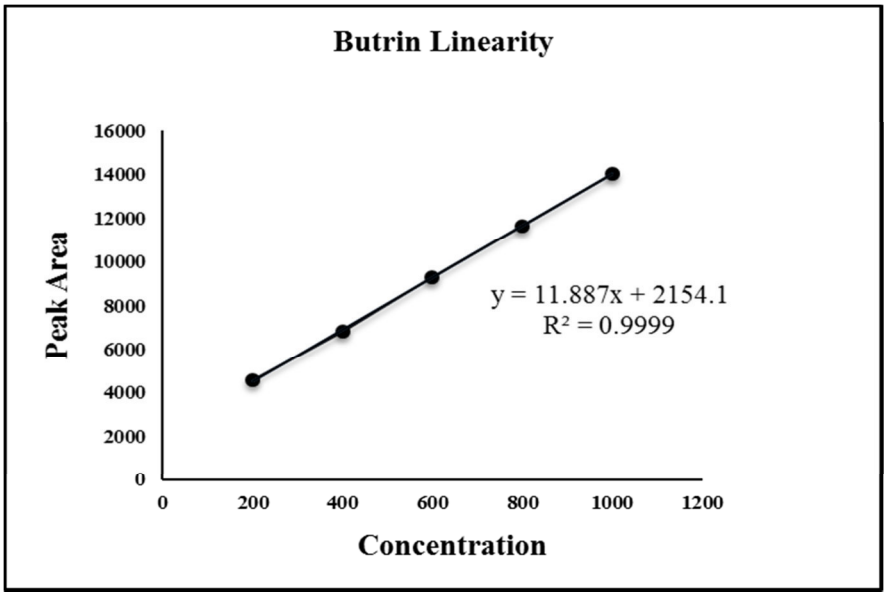
Table 1. *Content of BT, IBT, QC, API, CA and GA in Herbal Sunscreen Cream*

S. No	Marker	% Content
1	BT	0.92
2	IBT	0.38
3	QC	2.25
4	CA	1.08
5	API	0.52
6	GA	2.08

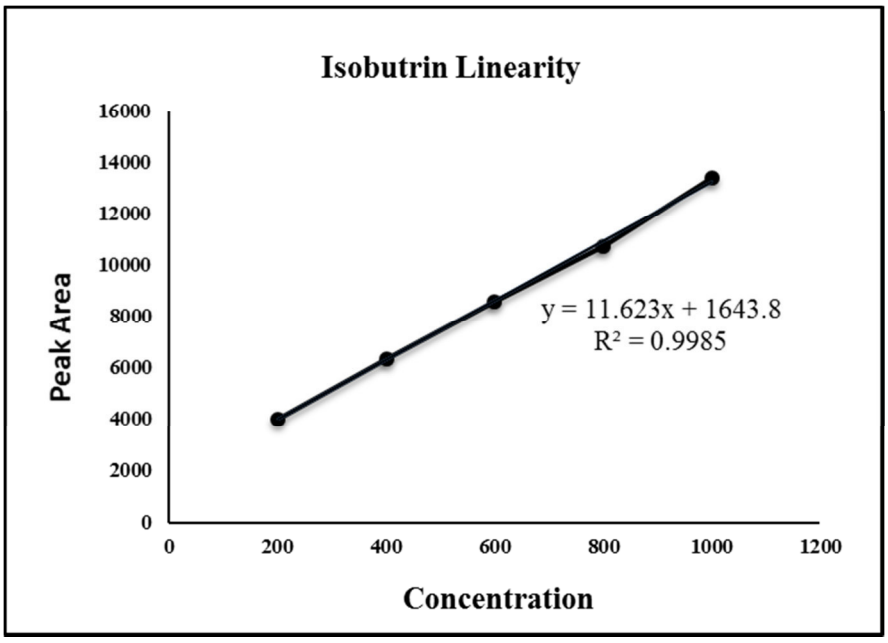
4.3 HPTLC Method Validation

4.4.1 Linearity, Limit of Detection and Limit of Quantification

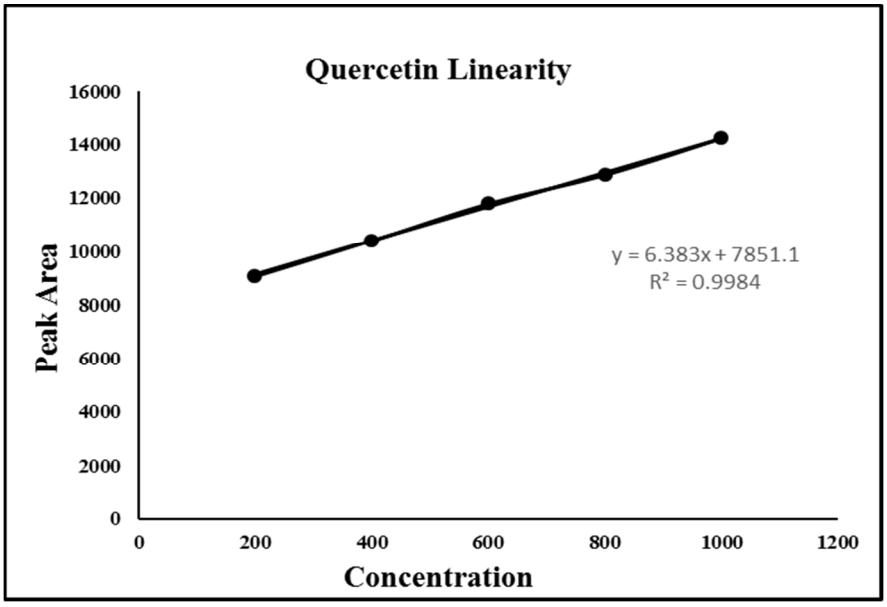
The calibration curves for BT, IBT, QC, API, CA and GA at 200 -1000ng/band were found in a linear range. The calibration curve with the correlation coefficient (r^2) and regression equations were noted in Graph 1 -6 for each marker compound [29]. The LOD and LOQ was calculated [30] and reported in Table 2.



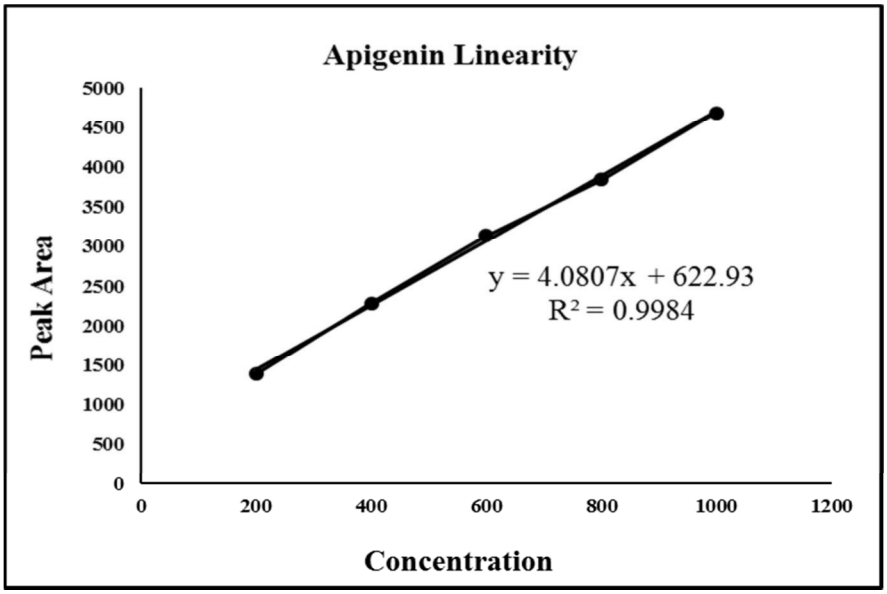
Graph 1. *Calibration Curve for Butrin (BT)*



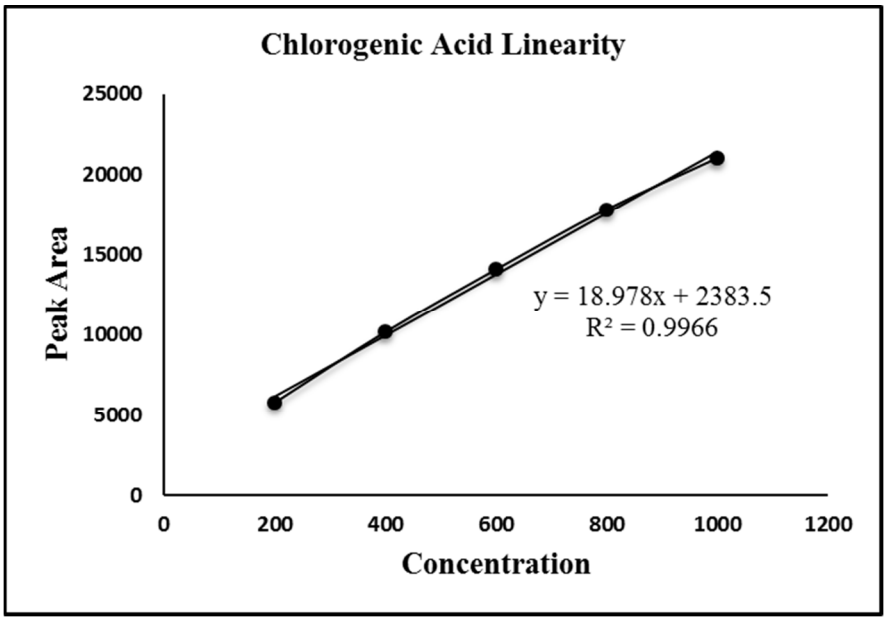
Graph 2. *Calibration Curve for Isobutrin (IBT)*



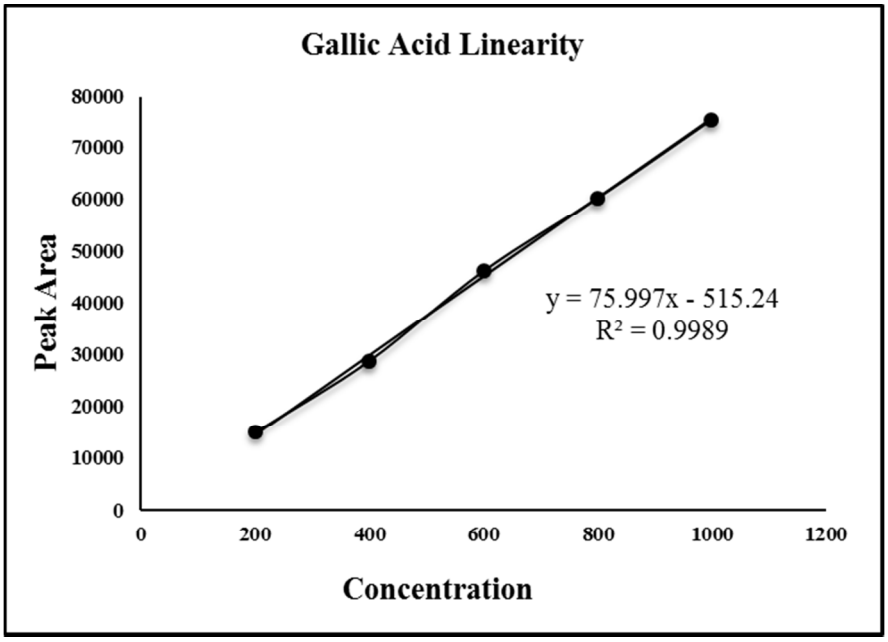
Graph 3. *Calibration Curve for Quercetin (QC)*



Graph 4. *Calibration Curve for Apigenin (API)*



Graph 5. *Calibration Curve for Chlorogenic Acid (CA)*



Graph 6. *Calibration Curve for Gallic Acid (GA)*

Table 2. *HPTLC Validation for Linearity, LOD and LOQ*

S.No	Parameter	BT	IBT	QC	API	CA	GA
1	Linearity range (ng)	200-1000	200-1000	200-1000	200-1000	200-1000	200-1000
2	Correlation coefficient (r^2)	0.9999	0.9985	0.9984	0.9984	0.9966	0.9989
3	Regression equation	y=11.887 x+2154.1	y=11.623 x+1643.8	y=6.383 x+7851.1	y=4.080 x+622.93	y=18.978 x+2383.5	y=75.997 x+515.24
4	LOD	13.75	46.13	48.41	48.26	70.2	39.63
5	LOQ	41.68	139.81	146.69	146.263	212.74	120.09

4.4.2 Precision

The intra-day and inter-day precision expressed as the % RSD for peak area were determined for standards BT, IBT, QC, API, CA and GA by repeated analysis (n = 6). Intra-day relative standard deviation of BT, IBT, QC, API, CA and GA were found between 1.72 to 1.57 %, 0.55 to 0.65 %, 1.21 to 0.93 %,1.13 to 0.52 %,1.06 to 0.63 and 0.97 to 0.66 % respectively for standard marker compound and 1.80 to 1.69 % , 1.08 to 1.15 % ,1.09 to 1.24% ,1.57 to 1.43 %, 1.28 to 0.98 % and 1.18 to 0.65 % respectively for cream formulation. The Inter-day relative standard deviation BT, IBT, QC, API, CA and GA were found between 1.05-1.56 %, 0.61- 0.52% ,1.15 to 0.77%, 0.60 to 0.57 %, 0.68 to 0.77 % and 1.18 to 0.65 % respectively for standard marker compound 1.75 to 1.84 %, 1.34 to 1.64, 1.23 to 1.12%, 1.37 to 1.58 %, 1.31 to 0.79 % and 1.31 to 0.77 % respectively for cream formulation whereas instrumental precision showed relative standard deviation of 1.08% for peak area. The precision for standard and sample are shown in Table No.3

Table 3. *Intraday and Interday Precision for HPTLC*

S. No	Markers	Concentration (ng/band)	Standard		Cream	
			Intra-day % RSD	Inter-day % RSD	Intra-day % RSD	Inter-day % RSD
1	BT	100	1.72	1.05	1.80	1.75
		120	1.81	1.54	1.85	1.82
		140	1.57	1.56	1.69	1.84
2	IBT	100	0.55	0.61	1.08	1.34
		120	0.69	0.44	1.21	1.68
		140	0.65	0.52	1.15	1.64
3	QC	100	1.21	1.15	1.09	1.23
		120	0.92	1.49	1.44	1.42
		140	0.93	0.77	1.24	1.12
4	API	100	1.13	0.60	1.57	1.37
		120	0.81	0.87	1.26	1.28
		140	0.52	0.57	1.43	1.58
5	CA	100	1.06	0.68	1.28	1.31
		120	0.94	0.59	1.11	1.20

		140	0.63	0.77	0.98	0.79
6	GA	100	0.97	1.06	1.18	1.31
		120	0.78	0.88	0.97	0.98
		140	0.66	0.81	0.65	0.77

**** Analysis was done in 6 replicates (n=6) and % RSD is relative standard Deviation.***

4.4.3 Accuracy by Recovery

The recovery studies were performed by spiking standards at a known amount of analyte in a cream formulation. The recovery studies performed in triplicate and % recovery was found to be in the range 99.18% - 99.42%, 100.21 % to 100.33 %, 99.95 % to 99.86 %, 100.18 % to 99.90 %, 99.85-100.54 % and 99.82-100.53% for BT, IBT, QC, API, CA and GA respectively as shown in Table 4.

Table 4. *Recovery Studies of Markers*

S. No	Marker	Amount added (ng)	Mean Recovery (%) ± S. D
1	BT	184	99.18 ± 0.71
		230	99.22 ± 1.49
		276	99.42 ± 1.80
2	IBT	152	100.21 ± 0.45
		190	99.96 ± 1.06
		228	100.33 ± 1.24
3	QC	160	99.95 ± 1.59
		200	100.15± 1.82
		240	99.86 ± 1.11
4	API	140	100.18 ± 1.53
		130	99.68 ± 1.42
		156	99.90 ± 1.35
5	CA	108	99.85 ± 1.59
		135	99.96 ± 1.06
		162	100.54 ± 1.82
6	GA	208	99.82 ± 1.80
		260	99.22 ± 1.49
		312	100.53 ± 1.24

****Values represented with mean recovery (n=3) ± S.D (Standard Deviation)***

4.4.4 Repeatability

The repeatability was done by analysing 200 ng/spot of BT, IBT, QC, API, CA, and GA individually spotted on TLC plate at the same conditions. % RSD for repeatability were 0.47, 0.55, 0.98, 0.86, 0.78 % and 0.65 % respectively as shown in Table 5.

Table 5. *Repeatability of Marker Compounds*

S. No	Markers	Concentration (ng/band)	(%) RSD
1	BT	200ng/band	0.47
2	IBT	200ng/band	0.55
3	QC	200ng/band	0.98
4	API	200ng/band	0.86
5	CA	200ng/band	0.78
6	GA	200ng/band	0.65

4.4.5 Specificity

The bands for BT, IBT, QC, API, CA and GA from sample solutions were confirmed by comparing the retention factor and the spectra of the bands to those of the standard markers. Peak purity data confirmed that the proposed method was specific by comparing the spectra at three different levels, i.e., peak start (S), peak apex (M), and peak end (E) positions of each compound[31].

4.4.6 Robustness

% RSD for all the standards viz. BT, IBT, QC, API, CA and GA after changing the mobile phase composition, mobile phase volume, time from spotting to chromatography, time from chromatography to scanning, % RSD for peak area was calculated [32] and found to be less than 2% as shown in Table 6.

Table 6. ***Robustness Study for HPTLC Method (% RSD)***

S. No	Parameters	BT	IBT	QC	API	CA	GA
1	The proportion of mobile phase (Ethyl acetate) ± 0.2 mL	0.80	1.08	0.80	0.62	1.25	0.72
2	The volume of mobile phase (± 2mL)	1.05	0.63	1.05	0.73	0.87	1.15
3	Time from spotting to chromatography	1.21	0.87	1.21	0.64	1.37	1.23
4	Time from chromatography to scanning	0.83	0.59	0.83	1.46	1.07	0.79

Analysis done n = 6, Concentration- 200ng/band.

The formulated cream contains more than one plant extract and various phytochemicals. The cream formulation was previously studied for its antioxidant potential. It has shown considerable amount of phenolic and flavonoid content which could be responsible for its sun protective effect.[33] The selected phytoconstituents are phenolic and flavonoid in nature. Validation of HPTLC methods for different parameters were studied according to the ICH guidelines. Ethyl acetate: methanol: formic acid: water (4:0.8:0.2:0.2 v/v/v/v) was used as solvent system for Butrin (BT) and isobutrin (IBT)[34] and both were previously studied for antioxidant activity.[35] Ethyl acetate: formic acid: acetic acid: water (10:1.1:1.1:0.6 v/v/v/v) was used as solvent system estimation of quercetin [36] ,chlorogenic acid and apigenin [37] in extract and formulation. Gallic acid was estimated using Toluene: ethyl acetate: formic acid: methanol (3:3:0.8:0.2 v/v/v/v) as solvent system.[38] All solvent systems were saturated for 25 min at room temperature and developed up to 80 mm. The peaks obtained were shown well resolved and compact retention factors. Methods were found to be in linear in a concentration range of 2µg-10 µg/band (n=5) with respect to peak area. Table 2 revealed a good linear relationship over the concentration range studied and demonstrated its suitability for the analysis. The lowest amounts of phytoconstituent were detected and quantified during LOD and LOQ studies. The precision found was less than 2 % conforming the method was precised. The recovery values obtained during our studies were in acceptable limits which demonstrated the accuracy of the method. Specificity of the method was confirmed by peak purity data by comparing the spectra at three different levels, i.e., peak start (S), peak apex (M), and peak end (E) positions of each compound. Spotting and analysing the same amount of drug several times (n=6) ensued the repeatability of our developed method as well as proper functioning of the HPTLC system. The low % RSD values obtained after introducing small changes in the developed HPTLC method confirmed the robustness of the method. All the parameters studied were helpful for the accurate identification and assurance of the quality of phytoconstituents present in herbal sunscreen cream.

5. CONCLUSION

The HPTLC method used for qualitative and quantitative analysis of markers i.e BT, IBT, QC, API, CA and GA in prepared herbal sunscreen formulation of 2% w/w and validated for the different analytical parameters. The mobile phases shown well resolve peaks of respective compounds separation. The markers selected are flavonoid and phenolic in nature. It would be supportive to study the sunscreen property of the formulation with antioxidant activity. The method was found to be simple; cost effective, sensitive, specific, robust and repeatable. This is a useful tool for routine quality control analysis of phytoconstituents in herbal industry. Further the formulation is required to study for its in vitro and in vivo studies.

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7. AUTHORS CONTRIBUTION STATEMENT

Ms. Manisha P Sutar designed and collected the data required to conduct the study. Dr. Sanjay R Chaudhari analysed these data and suggested necessary inputs. Authors discussed the methodology, results and contributed to the final manuscript. Authors ensuring the descriptions are accurate and agreed by all authors for publication.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

9. REFERENCES

1. Xu Y, Shao Y, Voorhees JJ, Fisher GJ. Oxidative inhibition of receptor-type protein-tyrosine phosphatase kappa by ultraviolet irradiation activates epidermal growth factor receptor in human keratinocytes. J Biol Chem. 2006 Sep 15;281(37):27389-97. doi: 10.1074/jbc.M602355200, PMID 16849327.
2. Lucas R, McMichael T, Smith W, Armstrong B. Solar ultraviolet radiation: global burden of disease from solar ultraviolet radiation. Vol. 13 Geneva: Switzerland; 006). World Health Organization. Environmental burden of disease series. Available from: https://apps.who.int/iris/handle/10665/43505.
3. Manikrao Donglikar M, Laxman Deore S. Sunscreens: A review. Phcogj;8(3):171-9. doi: 10.5530/pj.2016.3.1.
4. Ferrero L, Pissavini M, Marguerie S, Zastrow L. Sunscreen in vitro spectroscopy: application to UVA protection assessment and correlation with in vivo persistent pigment darkening. Int J Cosmet Sci. 2002;24(2):63-70. doi: 10.1046/j.1467-2494.2002.00130.x. PMID 18498497.
5. Velasco MV, Sarruf FD, Salgado-Santos IM, Haroutiounian-Filho CA, Kaneko TM, Baby AR. Broad spectrum bioactive sunscreens. Int J Pharm. 2008 Nov 3;363(1-2):50-7.

doi: 10.1016/j.ijpharm.2008.06.031, PMID 18662760.
6. Aburjai T, Natsheh FM. Plants used in cosmetics. Phytother Res. 2003 Nov;17(9):987-1000. doi: 10.1002/ptr.1363, PMID 14595575.
7. F'guyer S, Afaq F, Mukhtar H. Photochemoprevention of skin cancer by botanical agents. Photodermatol Photoimmunol Photomed. 2003;19(2):56-72. doi: 10.1034/j.1600-0781.2003.00019.x. PMID 12945805.
8. Robbins RJ. Phenolic acids in foods: an overview of analytical methodology. J Agric Food Chem. 2003 May 7;51(10):2866-87. doi: 10.1021/jf026182t, PMID 12720366.
9. King A, Young G. Characteristics and occurrence of phenolic phytochemicals. J Am Diet Assoc. 1999 Feb;99(2):213-8. doi: 10.1016/S0002-8223(99)00051-6, PMID 9972191.
10. Tabrizi H, Mortazavi SA, Kamalinejad M. An in vitro evaluation of various Rosa damascena flower extracts as a natural antisolar agent. Int J Cosmet Sci. 2003 Dec;25(6):259-65. doi: 10.1111/j.1467-2494.2003.00189.x.
11. Bijauliya RK, Alok S, Chanchal DK and M. Kumar, A Comprehensive Review on Standardization of Herbal Drugs, International journal of pharmaceutical sciences and research. 2017 Sep;8(9): 3663-3677. Doi:10.13040/IJPSR.0975-8232.8 (9).3663-77.
12. Ekka NR, Namdeo KP, Samal PK. Standardization strategies for herbal drugs-an overview. Res J Pharm Technol. 2008 Oct–Dec;1(4):310-2. http://www.indianjournals.com/ijor.aspx?target=ijor:rjpt&volume=1&issue=4&article=003
13. Sharma AK, Deshwal N. An overview: on phytochemical and pharmacological studies of Butea monosperma. Int J PharmTech Res. 2011 Jan;3(2): 864-71.http://sphinxsai.com/vol3.no2/pharm/pharmpdf/PT=38(864-871)AJ11.pdf.
14. Bachhav RS, Buchake VV, Saudagar RB. Analgesic and anti-inflammatory activities of Anthocephalus Cadamba Roxb. leaves in Wistar rats. Res J Pharm Technol; 2009 Jan-March;2(1):164-167. http://rjptonline.org/HTMLPaper.aspx?Journal=Research+Journal+of+Pharmacy+and+Technology%3bPID%3d2009-2-1-80
15. Kapil A, Koul IB, Suri OP. Antihepatotoxic Effects of chlorogenic acid from Anthocephalus Cadamba. Phytother Res. 1995 May;9(3):189-93. doi: 10.1002/ptr.2650090307.
16. Xaio P. Recent developments on medicinal plants in China. J Ethnopharmacol. 1983 Jan;7(1):95-109. doi: 10.1016/0378-8741(83)90083-1.
17. Tchoumbougnang F, Zollo PH, Dagne E, Mekonnen Y. In vivo antimalarial activity of essential oils from Cymbopogon citratus and Ocimum gratissimum on mice infected with Plasmodium berghei. Planta Med. 2005 Jan;71(1):20-3. doi: 10.1055/s-2005-837745, PMID 15678368.
18. Negrelle RR, Gomes EC. Cymbopogon citratus (DC) Stapf: chemical Composition and Biological Activities. Braz J Med Plants. 2007;9(1):80-92. https://pdfs.semanticscholar.org/0af7/c90c0ec2b89f32c5104aec34147bcdae0898.pdf?_ga=2.13794100.201754 29.1587996588-481671444.1586637515
19. Saxena R, Sharma R, Nandy BC. Chromatographic determination of phenolic profile from Punica granatum Fruit Peels. Int Res J Pharm. 2017 Feb;8(1):61-5. doi: 10.7897/2230-8407.080112.
20. Hmid I, Elothmani D, Hanine H, Oukabli A, Mehinagic E. Comparative study of phenolic compounds and their antioxidant attributes of eighteen pomegranate (Punica granatum L.) cultivars grown in Morocco. Arab J Chem. 2017 May;10(2): S2675-84. doi: 10.1016/j.arabjc.2013.10.011.
21. ICH Q2B. Validation of analytical procedure: methodology. Available from: https://www.fda.gov/media/71725/download. In: International Conference on Harmonization, Geneva, 1996.
22. ICH Q2A. Validation of analytical procedures: text and methodology. Available from: https://www.fda.gov/media/71724/download. In: International Conference on Harmonization, Geneva, 2005.
23. Deore LP, Bachhav DG, Nikam VK, Heda AJ. Study of anthelmintic and antimicrobial activity of peel extract of Punica granatum Linn. Eur J Pharm Med Res. 2016;3(4):292-7.https://storage.googleapis.com/journal-uploads/ejpmr/article_issue/1459413604.pdf
24. Sapkale AP, Thorat MS, Dighe DA, Munot NM, Singh MC. Investigation of anti-inflammatory activity of whole flower extract of Butea monosperma by ex-vivo and in-vitro techniques and their correlation. Int J Pharm Pharm Sci. 2013 May;5(2):84-6. https://innovareacademics.in/journal/ijpps/Vol5Suppl2/6131.pdf
25. Kaur K, Shetye SS, Valsamma W. In-vitro evaluation of two polyherbal formulations containing Neolamarckia Cadamba for their antioxidant activity. World J Pharm Res. 2016: 5(9); 6987:1336-48. doi: 10.20959/wjpr20169.
26. Panchal H, Amin A, Shah M. Development of Validated High-performance Thin-layer Chromatography Method for Simultaneous Determination of Quercetin and Kaempferol in Thespesia populnea. Pharmacogn Res. 2017 Jul–Sep;9(3):277-81. doi: 10.4103/0974-8490.210326, PMID 28827970.
27. Rowe RC, Sheskey PJ, Quinn ME. Handbook of Pharmaceutical Excipients.6thEdition. Available from: https://adiyugatama.files.wordpress.com/2012/03/handbook-of-pharmaceutical-excipients-6th-ed.pdf. Vol. 659(536). Pharmaceutical Press; 2009. p. 596, 170, 447,474.
28. Tuzimski T, Bartosiewicz A. Correlation of Retention Parameters of pesticides in Normal and RP Systems and their Utilization for the Separation of a Mixture of ten urea Herbicides and Fungicides by two-Dimensional TLC on Cyanopropyl-Bonded Polar Stationary Phase and two-Adsorbent-Layer Multi-K Plate. Chromatographia. 2003 Dec;58(11):781-8. doi: 10.1365/s10337-003-0116-5.
29. Linearity E J, In: Ermer J, Miller Mc.B. (eds.). Method validation in Pharmaceutical Analysis, published by Wiley-VCH, Weinheim. 2005
30. Yuwono M and Indrayanto G. Validation of Chromatographic Methods of Analysis. In: Brittain G. (ed.) Profiles of Drugs Substances, Excipients and Related Methodology, Vol. 32, published by Academic Press Elsevier Inc, San Diego 2005.

31. Srivastava M.M. High-Performance Thin- Layer Chromatography (HPTLC). Springer Verlag Berlin Heidelberg 2011: 32-60.
32. Vander Hyden Y, Nijhuis A, Smayers-Verbeke J, Vandeginste B.M.G, Massart D.L. Guidance for robustness/ruggedness test in method validation. J Pharm. Biomed. Anal. 2001 Mar; 24(5-6): 723–3. DOI: 10.1016/s0731-7085(00)00529-x.
33. Sutar MP and Chaudhari SR. Formulation And *In Vitro* Evaluation of Antioxidant Activity of Herbal Sunscreen Formulation, International Research Journal of Pharmacy, 2020 M*arch;* 11 (3): 58-65. DOI: 10.7897/2230-8407.110332.
34. Shirole RL, Kshatriya AA , Sutariya BK, Saraf MN. Mechanistic evaluation of butea monosperma using *in vitro* and *in vivo* murine models of bronchial asthma, International Journal of Research in Ayurveda and Pharmacy. 2013 June;4(3):322-331. http://www.ijrap.net/.../1008_pdf.pdf
35. Sutariya B, Badgujar L, Somani S, Saraf M. Anti-Nephritic Potential of N-Butanolic Fraction of *Butea Monosperma* (Lam.) Flowers on Doxorubicin Induced Nephrotic Syndrome in Rats, International Journal of Research in Ayurveda and Pharmacy. 2015 July- Aug; 6(4): 478-488. DOI: 10.7897/2277-4343.06492
36. Pawar NP and Salunkhe VR. Development and Validation of HPTLC Method for Simultaneous Estimation of Rutin and Quercetin in Hydroalcoholic Extract of Triphala Churna. International Journal of Pharm Tech Research. 2012 Oct-Dec; 4(4):1457-1463.
37. Claudia C. Analysis of Some Natural Antioxidants by Thin-Layer Chromatography and High Performance Thin-Layer Chromatography, Journal of Liquid Chromatography & Related Technologies.2006; 29(7-8): 1125–1142. doi.org/10.1080/10826070600574911.
38. Sawant L, Pandita N, Prabhakar B. Determination of gallic acid in *Phyllanthus emblica* Linn. dried fruit powder by HPTLC. Journal of pharmacy & bioallied sciences, 2012 Apr- June; 2(2):05–108. DOI:org/10.4103/0975-7406.67012.