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Marker Based Standardisation of Saptang Guggul Using HPTLC Validated Method for Guggulsterone, Gingerol, Gallic Acid, Piperine Quantification

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ABSTRACT

For the purpose of standardizing Saptang Guggul, a straightforward, accurate, and precise High-Performance Thin-Layer Chromatography (HPTLC) method was created and approved, a classical Ayurvedic polyherbal formulation. The method involved estimation of [insert key marker compounds, e.g., guggulsterone, gallic acid, etc.] using a suitable mobile phase and detection wavelength. Validation was done in compliance with ICH rules, assessing linearity, accuracy, precision, specificity, & robustness. The developed method provides an effective tool for quality control and standardization of *Saptang Guggul*.

1. Introduction

Saptang Guggul (Saptanga Guggulu) is a classical Ayurvedic polyherbal formulation comprising Commiphora mukul (guggul) and six other ingredients: *Piper longum* (Pippali), *Piper nigrum* (Maricha), *Zingiber officinale* (Shunthi), *Terminalia chebula* (Haritaki), *Terminalia bellirica* (Bibhitaki), and *Emblica officinalis* (Amalaki). Traditionally, Saptang Guggul is prescribed for ailments of the anal and abdominal region (e.g. hemorrhoids, fistula, chronic ulcers), especially in post-operative care, owing to its wound-healing, anti-infective and analgesic properties. The formulation combines *Triphala* (the three Terminalia fruits) and *Trikatu* (the three pungent herbs), which are believed to kindle digestive fire (Agni) and balance Vata and Kapha doshas; guggul itself is credited with anti-inflammatory and detoxifying effects that address tissue discharge and inflammation. These diverse ingredients act synergistically in traditional practice to alleviate inflammation, clear obstructions, and promote tissue regeneration in anorectal disorders.

Ensuring consistent quality of complex polyherbal medicines like Saptang Guggul requires modern analytical standardization. Marker-based standardization – the selection and quantification of characteristic bioactive compounds as reference markers – is a key strategy for quality control of herbal formulations. This approach is advocated in pharmacopeial and regulatory guidelines, which emphasize chromatographic fingerprinting and marker assays to confirm identity and potency of each constituent herb. Identification of "major and unique" phytochemicals from the component herbs, followed by validated analytical monitoring, ensures reproducible pharmacological activity and detects adulteration or batch variation. In practice, this means selecting marker compounds that reflect the pharmacologically relevant ingredients and developing robust methods to measure them.

High Performance Thin Layer Chromatography (HPTLC) is a preferred analytical technique for such standardisation. It enables simultaneous qualitative and quantitative analysis of multiple markers in a single run with high precision, sensitivity, and cost-effectiveness. For Saptang Guggul, HPTLC can effectively standardise compounds such as guggulsterones (from Commiphora mukul), gingerol-6 (from Zingiber officinale), gallic acid and epigallocatechin (from Triphala components), and piperine (from Piper species). These markers represent the major pharmacologically active components of the formulation and are critical to its therapeutic profile.

Using validated HPTLC methods ensures batch-to-batch consistency, aids in detecting adulteration or substandard raw materials, and supports regulatory compliance and clinical acceptance of Ayurvedic formulations. The goal of this project is to create a marker-based standardization approach using HPTLC. This method will be important for quality assurance and for incorporating traditional formulations like Saptang Guggul into evidence-based healthcare systems.

2. Materials and Methods

2.1 Materials

- Formulation: Marketed and in-house prepared Saptang Guggul
- Standards: Guggulsterone Z , gallic acid, epigallocatechin, gingerol-6 and piperine
- Solvents and chemicals: Analytical reagent (AR) grade solvents including methanol, toluene, ethyl acetate, and formic acid were procured from SD fine chemicals.
- Stationary phase: Precoated silica gel 60 F254 TLC plates (E. Merck, Germany)
- Instrumentation: CAMAG HPTLC system equipped with Linomat 5 sample applicator, twin-trough development chamber, derivatization chamber, and TLC Scanner with winCATS software.

2.2 Sample & standard Preparation

Using sonication for 30 minutes, 10 mL of methanol was used to extract an accurately weighed amount (around 1 g) of Saptang Guggul powder. Whatman No. 1 filter paper was

used to filter the extract, and the filtrate was then concentrated under low pressure. The final volume was brought up to 10 mL with methanol and then utilized for HPTLC analysis.

Respective reference standards were prepared with concentration of 1mg/ml in chloroform except gallic acid and epigallocatechin in ethanol.

2.3 Chromatographic Conditions

The stationary phase was the precoated silica gel aluminum plate 60F254 (20 cm × 10 cm with a thickness of 250 µm; E. Merck, Darmstadt, Germany, supplied by Anchrom Technologists, Mumbai). The test solutions were spotted in the shape of bands of width 6 mm with a CAMAG microlitre syringe. Before chromatography, the plates were rinsed with methanol and heated to 60°C for 5 minutes. The slit size was 5 mm × 0.45 mm, the bandwidth was 7 mm, and the scanning speed was 10 mm/s. The mobile phase was made up of varying amounts of toluene, ethyl acetate, and formic acid v/v/v to get good resolution of each stand. The linear rising development took place in a twin trough glass chamber that was full of the mobile phase. The best period for the mobile phase to saturate the compartment was 30 minutes at room temperature (25 ± 2°C). The chromatogram run lasted 80 mm. The plate was then left to cure at room temperature. The bands that were separated on the HPTLC plates were scanned between 200 and 400 nm. The source of radiation used was a tungsten lamp, a deuterium lamp, or a mercury lamp. The quantification of each standard was executed at their respective maximum absorbance wavelength.

2.4 Method Validation

The High-Performance Thin-Layer Chromatography (HPTLC) method created to measure marker chemicals in Saptang Guggul was tested and found to be legitimate according to the International Conference on Harmonisation (ICH) guidelines Q2(R1). The validation parameters encompassed linearity, accuracy, precision, specificity, limit of detection (LOD), limit of quantitation (LOQ), and robustness. These criteria were evaluated to confirm the method's reliability, repeatability, and suitability for routine analysis.

1. Linearity

By examining standard solutions of the chosen marker chemicals at five distinct concentrations, linearity was assessed. Three applications of each concentration were made. Plotting the peak area against the corresponding concentration allowed for the construction of the calibration curve. To establish linearity, the correlation coefficient (r2) was computed. For all marker chemicals, good linearity was demonstrated by a r2 value greater than 0.999.

2. Accuracy

Recovery studies utilizing the conventional addition approach were used to determine accuracy. The pre-analyzed sample was spiked with known quantities of standards at three concentration levels: 80%, 100%, and 120%. Every level was examined three times. The following formula was used to get the percentage recovery:

% Recovery = [(Amount found − Original amount) / Amount added] × 100

3. Precision

Two levels of precision evaluation were conducted: intraday and interday. While interday precision was evaluated across three consecutive days, intraday precision was evaluated by examining the sample three times on the same day under the same experimental settings. Relative standard deviation (RSD%) was used to express the findings.

4. Specificity

The standard, sample, and blank chromatograms were compared to ascertain specificity.

5. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Using the response's standard deviation and the calibration curve's slope, LOD and LOQ were computed using the following formulas:

LOD = 3.3 × (σ/S)

LOQ = 10 × (σ/S)

where S is the slope and σ is the response's standard deviation.

6. Robustness

Small intentional adjustments to the chromatographic settings, such as slight changes in the detection wavelength and mobile phase saturation time, were used to assess robustness.

Results and Discussion

3.1 Chromatographic Separation and Rf Values

The developed HPTLC method successfully provided a distinctive chromatographic fingerprint for the Saptang Guggul formulation. Multiple marker compounds, including guggulsterone Z, gingerol-6, piperine, gallic acid, and epigallocatechin, were identified and quantified based on their retention factor (Rf) values & peak characteristics. Guggulsterone Z, gingerol-6, piperine standard compounds showed good separation and resolution under the optimized mobile phase conditions of Toluene: Ethyl acetate: Formic acid (7:3:0.1 v/v/v). The Rf values for each of the markers guggulsterone Z, gingerol-6, piperine were 0.52, 0.43, 0.36 respectively. **(Figure no.1)**

The other two marker compounds namely, gallic acid and epigallocatechin are polar compound thus, these two compounds were separated and well resolved under the optimized mobile phase comprising same solvents with different ratio, Toluene: Ethyl acetate: Formic acid (4:4.5:0.5 v/v/v). **(Figure no.1)**

Each marker band was well separated from other constituents. Specificity was confirmed by spectral overlay: the UV spectrum of each band in the sample matched that of the corresponding standard at start, middle& end of the band, with no co-eluting peaks. The method's selectivity and successful chromatographic separation of the selected markers in the polyherbal matrix are demonstrated by these results.**(Figure no.2)**

3.2 Method Validation

Linearity: Calibration curves for each marker were linear over the chosen range. Guggulsterone Z showed an excellent linear response (r^2=0.9992) across 8–128 mcg/spot, piperine was linear (r^2=0.9998) over7 – 112 mcg/spot and 6-gingerol was linear (r^2=0.9996) over 5-80 mcg/spot. Epigallocatechin and gallic acids also yielded linear plots (r^2= 0.9997 and 0.9998) in conc. range of 4-64 mcg/spot & 2-32 mcg/spot respectively. These high correlation coefficients (>0.98) indicate robust quantitation capability. **(Figure no.3)**

Accuracy (Recovery): The method proved highly accurate. Standard-addition recovery studies gave mean recoveries near 100%. For instance, guggulsterone Z recovery was 99.77 to 101.58%, 6-gingerol recovery was 100.64– 102.59%, piperine showed recovery in the range of 99.92 to 101.58% whereas epigallocatechin and gallic acid recoveries were in the range of 100.71 to 101.49% and 100.70 to 103.54%, respectively. Such near-complete recovery confirms that the extraction and analysis recover the true marker amounts. **(Table no.1)**

The mean recovery values for the markers ranged between 99.77% and 102.59%, indicating accuracy of method.

Precision: The assay was precise with very low variability. Intra-day and inter-day repeatability produced %RSD values typically <1.97%. RSDs (well below 2%) demonstrate excellent precision for both the marker standards and sample determinations. **(Table no.2)**

Sensitivity (LOD/LOQ): The limits of detection & quantitation for each marker were in low ng to mcg range, reflecting high sensitivity. The LODs of markers were about 8.8 ng–2.79 µg/spot and LOQs 26.59 ng/spot– 9.08µg/spot. These low detection limits are suitable for detecting the markers even at minor levels in the herbal extract. **(Figure no.1 and Table no.3)**

Specificity: As noted, each marker produced a single, well-resolved band with no interfering peaks at the same Rf. The identity and purity of marker zones were confirmed via Rf comparison and UV spectral overlay with standards. This confirms that excipients or other botanicals in Saptang Guggul do not co-elute with the marker analytes. **(Figure no.1 and 2)**

Robustness: Deliberate small changes in analytical parameters; slight chamber saturation time or detection wavelength variations did not significantly affect Rf or quantification. The marker spot characteristics (area and Rf) remained essentially unchanged, indicating the method is robust. **(Table no.4)**

Overall, the validation data meet ICH criteria: linear calibration (r^2 ≥0.9998), recoveries = 99.77–102.59 %, precision RSD<2%, and sensitive LOD/LOQ levels. This affirms that the HPTLC method is accurate, precise, specific and reliable for five markers in Saptang Guggul.

Marker Content in In-House Samples

In our study, the in-house Saptang Guggul showed approximately 1.68% of guggulsterone Z, 1.48% of piperine, 0.32% of gingerol-6 and respectively triphala group gallic acid and epigallocatechin was found to be 4.28 % and 1.64 % respectively **(Figure no.1 and table no. 5).**

The findings highlight the critical role of marker-based HPTLC standardization in ensuring Saptang Guggul quality. By defining characteristic fingerprints and quantifying active markers, the method provides a scientific basis for product specification. High HPTLC selectivity and sensitivity (with rapid analysis) make it ideal for routine quality control. In line with regulatory expectations, our marker profiling confirms that authenticated formulations deliver consistent levels of key bioactives, whereas marked variability could compromise efficacy and safety. The identification of unique marker compounds and their quantification are key steps in herbal standardization. Indeed, developing standardized herbal products is considered a "valuable approach" to assure chemical consistency and therapeutic activity.

4. Discussion

For Saptang Guggul, the selected markers – guggulsterones, gingerol, gallic acid and piperine – represent the principal bioactive types in the formulation's herbs. Guggulsterones (E- and Z-isomers) are steroidal constituents of *C. mukul* that exhibit potent biological activities; they antagonize the farnesoid X receptor and upregulate bile acid export, leading to hypolipidemic and antiobesity effects. In addition, guggulsterones have documented anti-inflammatory and cardioprotective properties, consistent with the traditional use of guggul resin in inflammatory & metabolic disorders. [6]-Gingerol, the main pungent phenol from *Z. officinale*, has been displayed in numerous studies to have strong anti-inflammatory and analgesic activity. It inhibits inflammatory mediator synthesis and modulates pain pathways, which aligns with the use of ginger (Shunthi) in digestive and pain-related indications in Ayurveda. Gallic acid, a polyphenolic antioxidant abundant in *Terminalia* fruits and *Emblica*, has broad pharmacological effects – notably antioxidant, anti-inflammatory and wound-healing actions. This marker reflects the presence of the Triphala ingredients (Haritaki, Bibhitaki, Amalaki) and their contribution to tissue repair and infection control. Finally, piperine – the principal alkaloid of *Piper longum* and *Piper nigrum* – not only exhibits anti-inflammatory and analgesic effects, even acts as a bioavailability enhancer for other phytoconstituents. Piperine's ability to inhibit drug-metabolizing enzymes increases systemic exposure of co-administered compounds, potentially potentiating the efficacy of Saptang Guggul. By quantifying these four markers, the HPTLC-based method will thus provide a comprehensive standardization profile that correlates with the formulation's therapeutic attributes.

Marker-based standardisation offers a modern scientific approach to address these challenges. By identifying and quantifying specific bioactive compounds (markers) that are pharmacologically relevant and characteristic of the formulation, one can establish reproducible quality standards. This method helps validate the authenticity, purity, and concentration of key therapeutic constituents in Saptang Guggul.

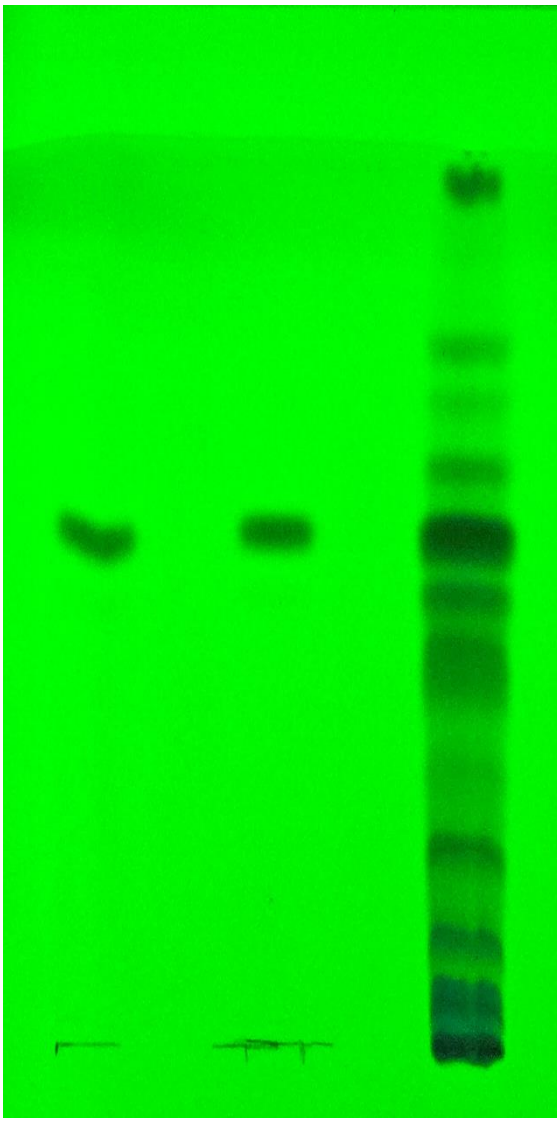
High-performance thin-layer chromatography (HPTLC) is especially well-suited for this purpose. HPTLC fingerprinting enables simultaneous analysis of multiple samples and markers with high resolution and throughput. The technique can be rigorously validated (for linearity, precision, accuracy, etc.) and generates characteristic chromatographic profiles for complex mixtures. Indeed, HPTLC is recommended by official guidelines for herbal drug authentication, as it allows visualization of distinct bands corresponding to individual markers and co-developed compounds. In summary, HPTLC provides a rapid, economical and sensitive platform for both qualitative fingerprinting and quantitative estimation of markers in polyherbal drugs, thereby serving as an integral tool in modern standardization of Ayurvedic formulations.

5. Conclusion

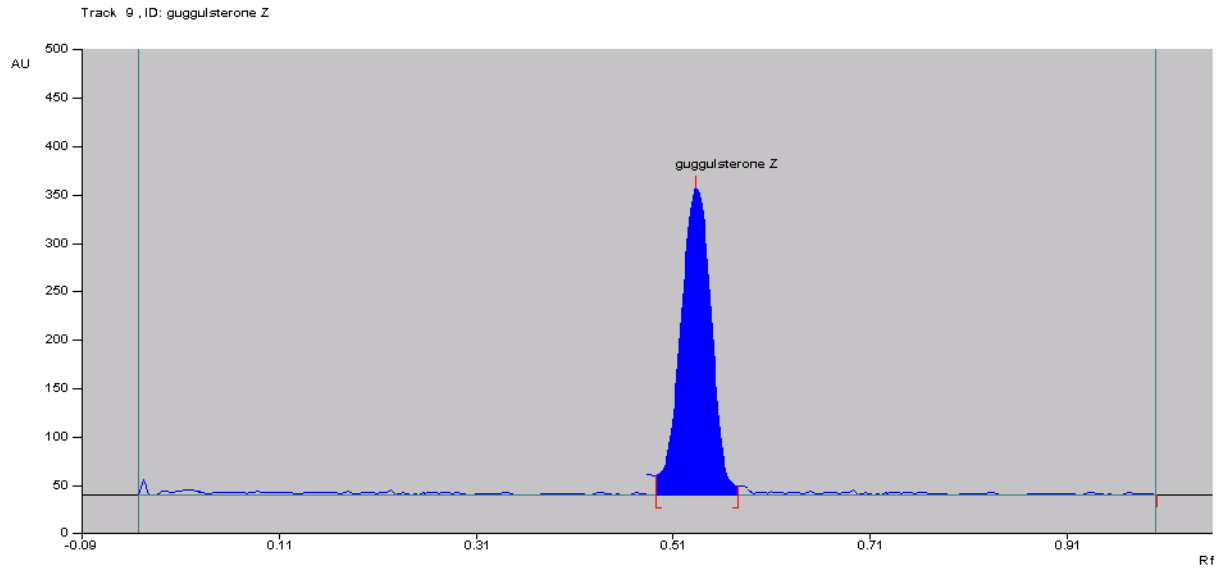
In conclusion, the validated HPTLC method efficiently separates and quantifies the five selected markers with high accuracy and precision. The chromatographic profile (fingerprint) and quantitation results serve as an authentication toolpmc.ncbi.nlm.nih.gov. Implementation of this marker-based standardization strategy will help guarantee that every batch of Saptang Guggul meets defined quality criteria, thereby assuring reproducible potency and supporting safe, effective use.

FIGURE NO.1

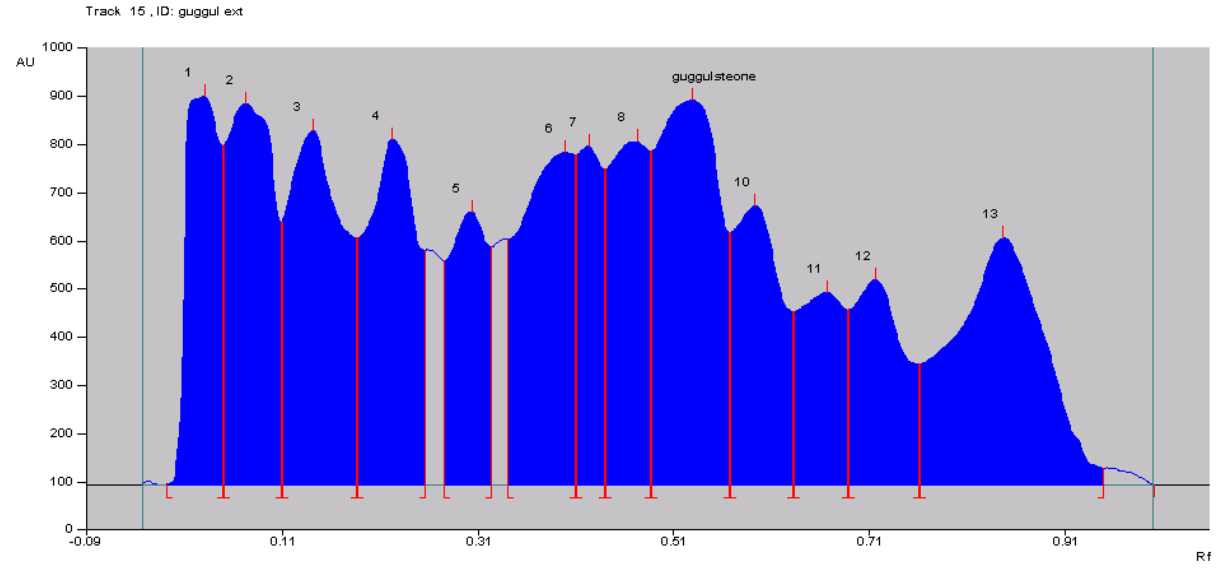
Guggulsterone Z



Track 1: Reference Standard Guggulsterone Z
Track 2: Isolated Guggulsterone Z
Track 3 : Guggul extract

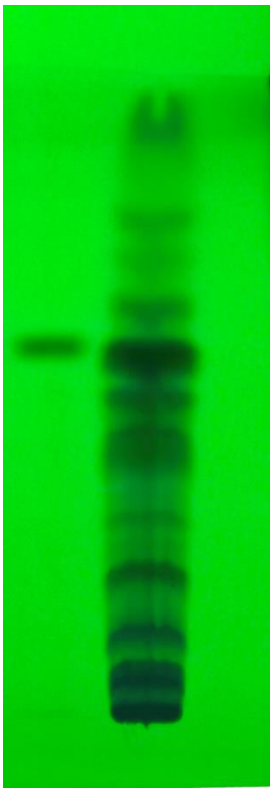


Reference Standard Guggulsterone Z

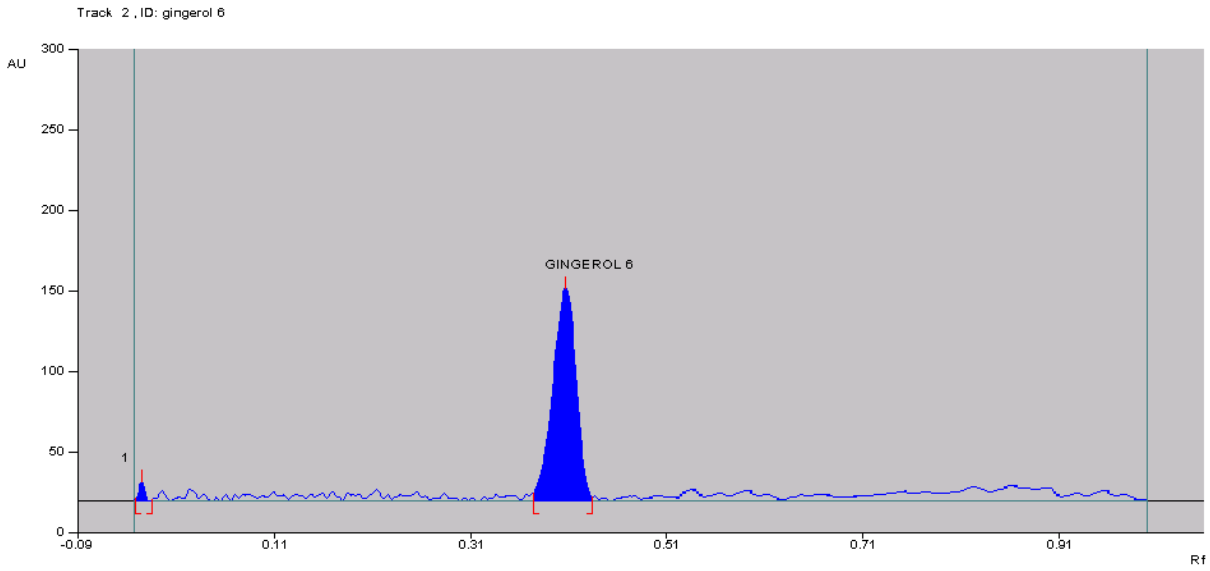


Guggul extract

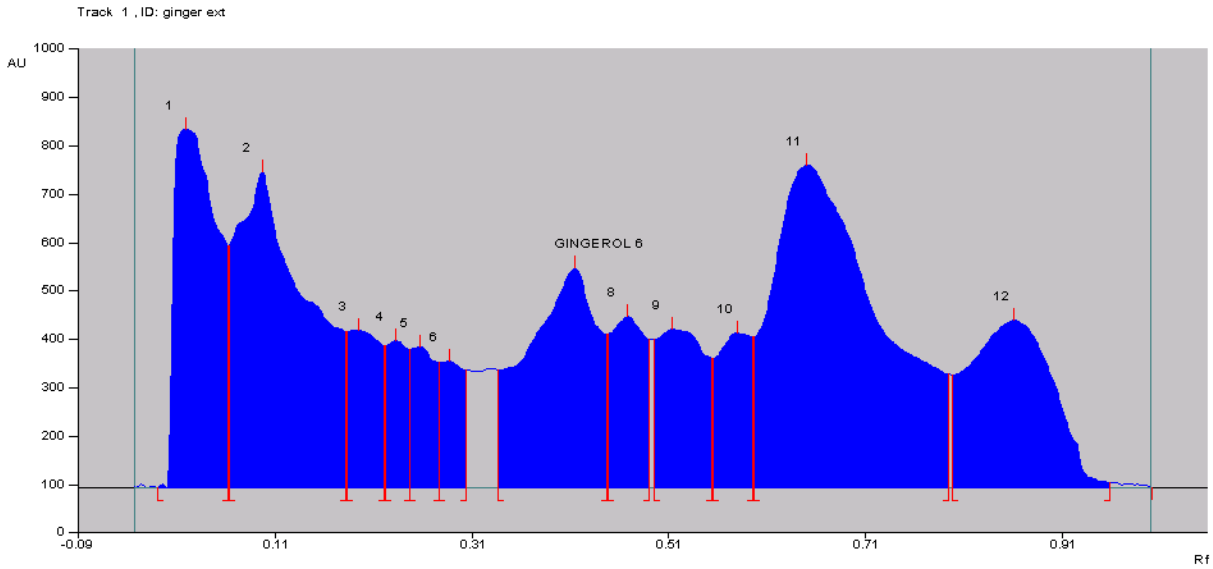
Gingerol 6



Track 1: Reference Standard Gingerol 6
Track 2: Ginger Extract

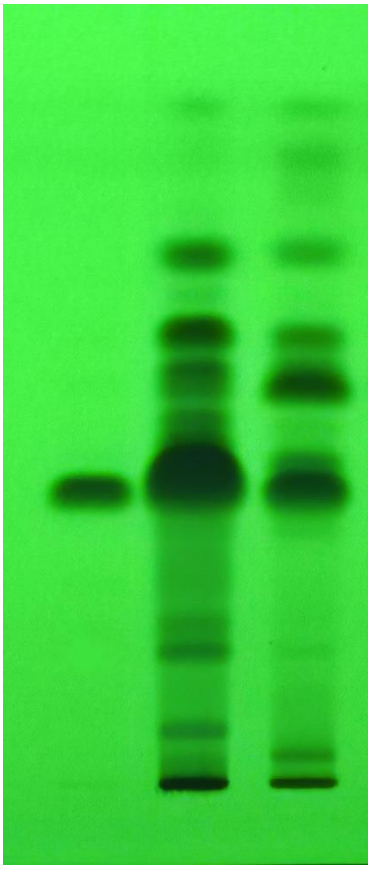


Reference Standard Gingerol 6

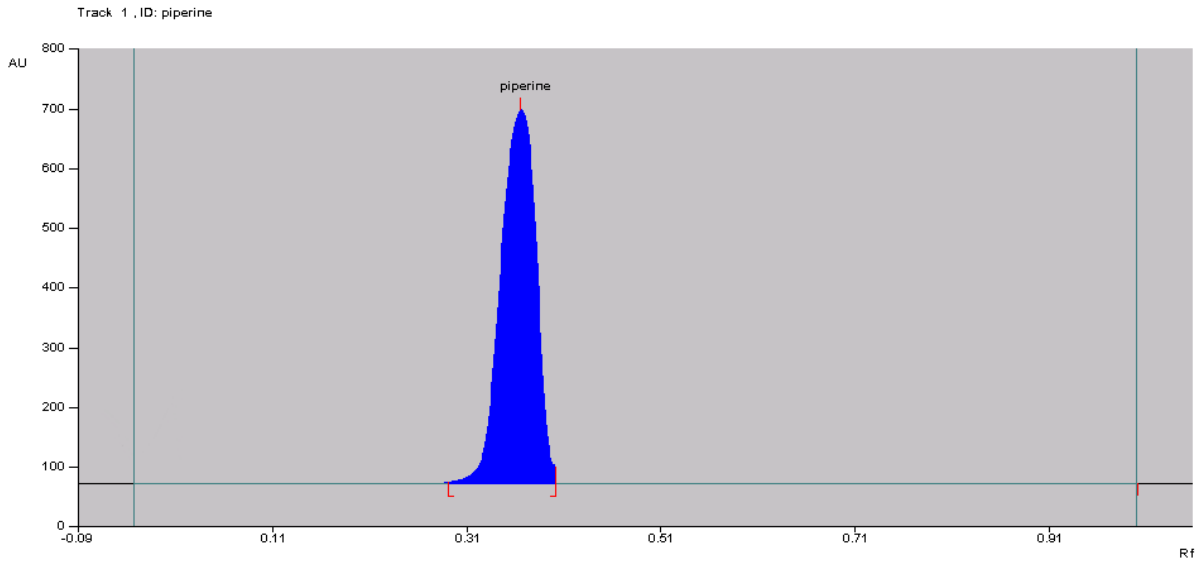


Ginger extract

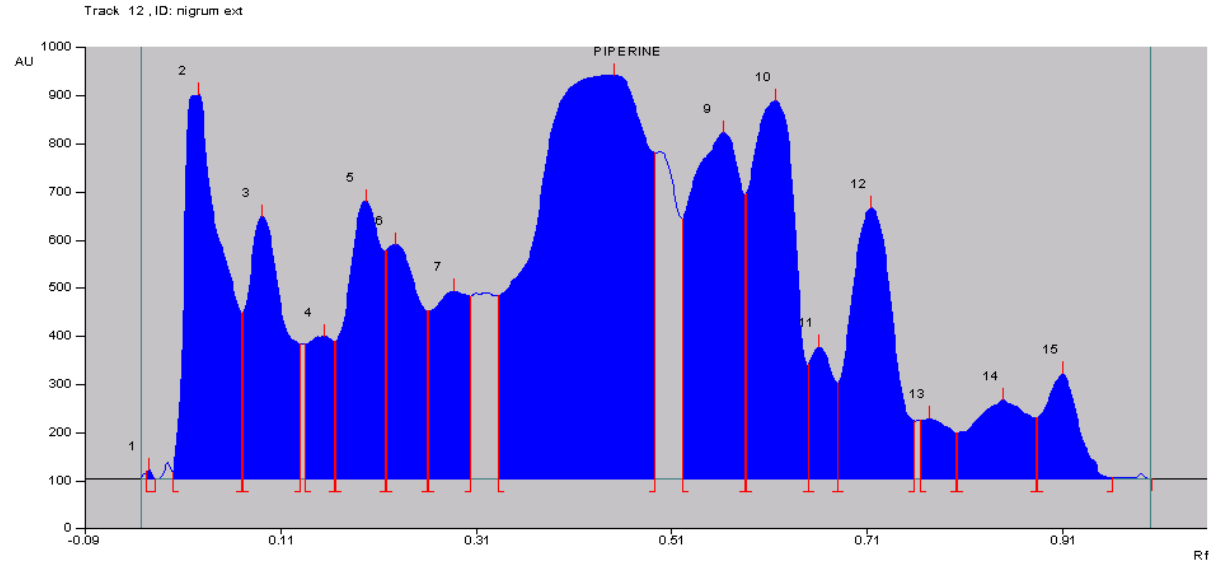
Piperine



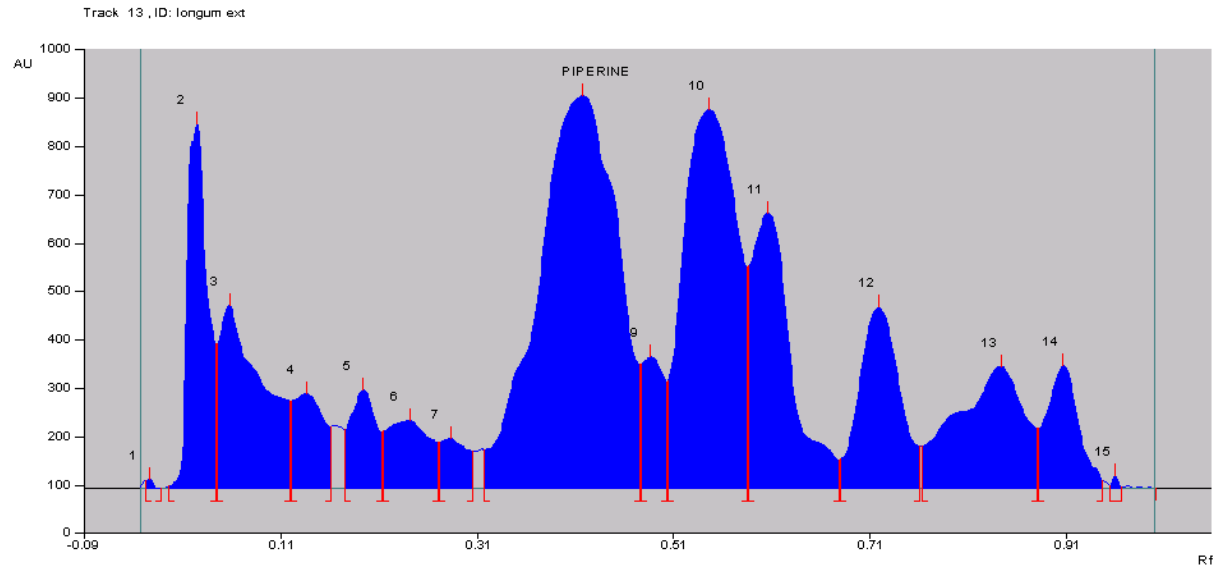
Track 1: Reference Standard Piperine
Track 2: Piper nirgum Extract
Track 3: Piper longum Extract



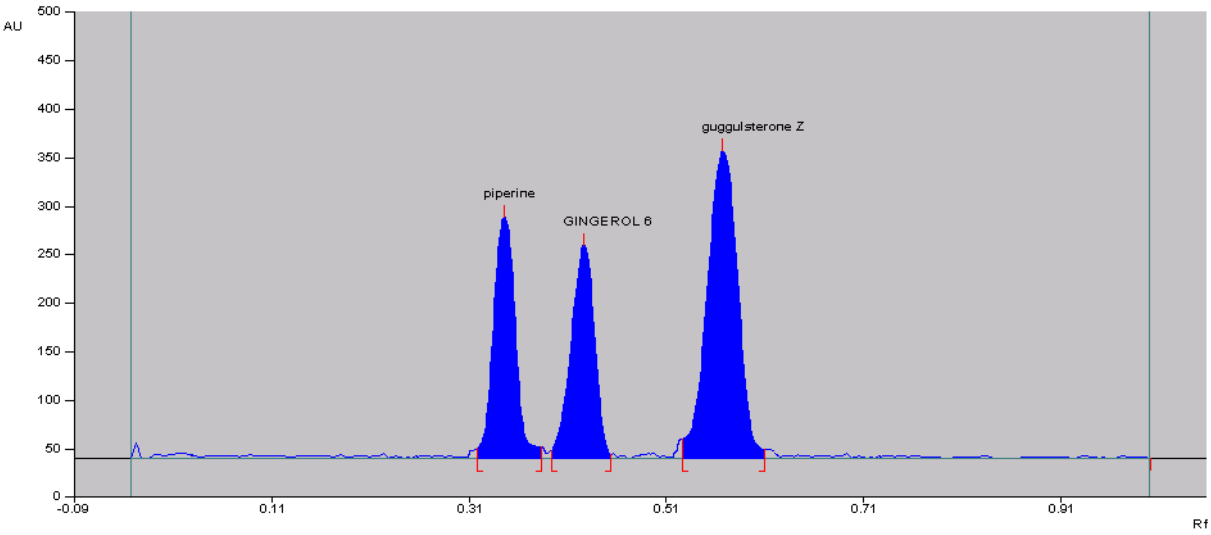
Reference Standard Piperine



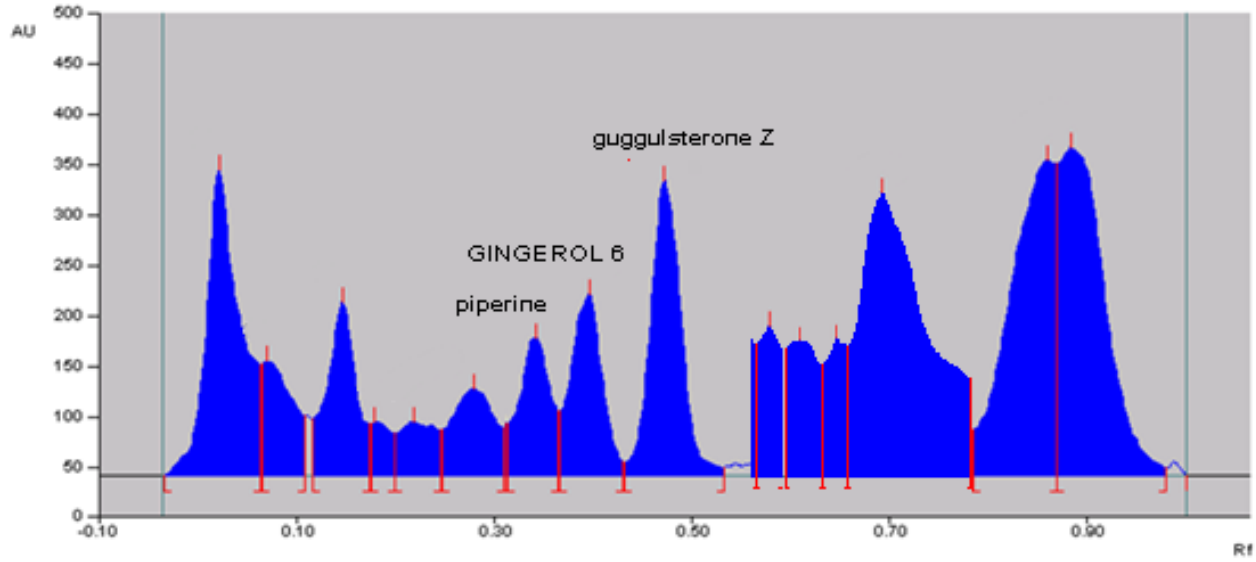
Piper nigrum extract



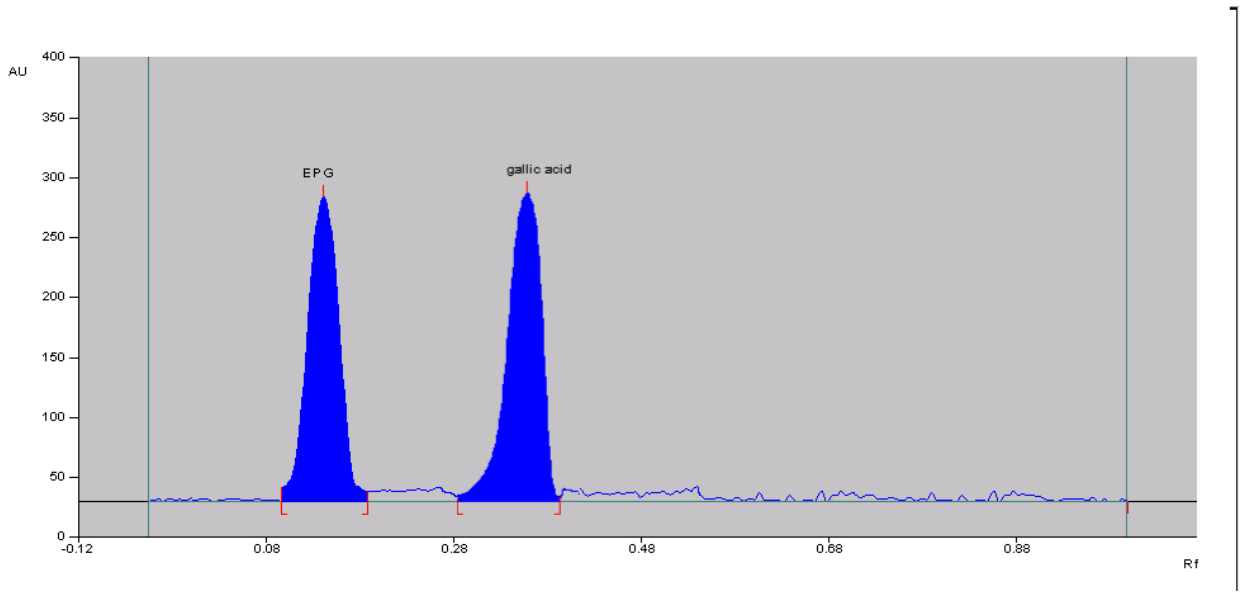
Piper longrum extract

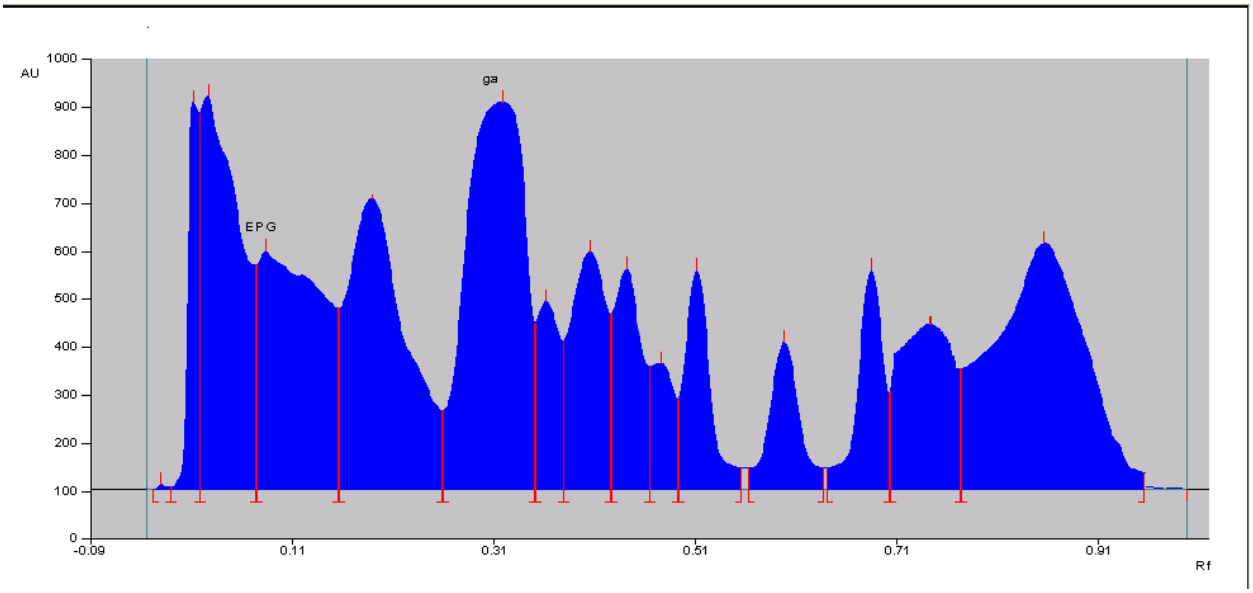


Standard chromatogram



Spatang guggul sample

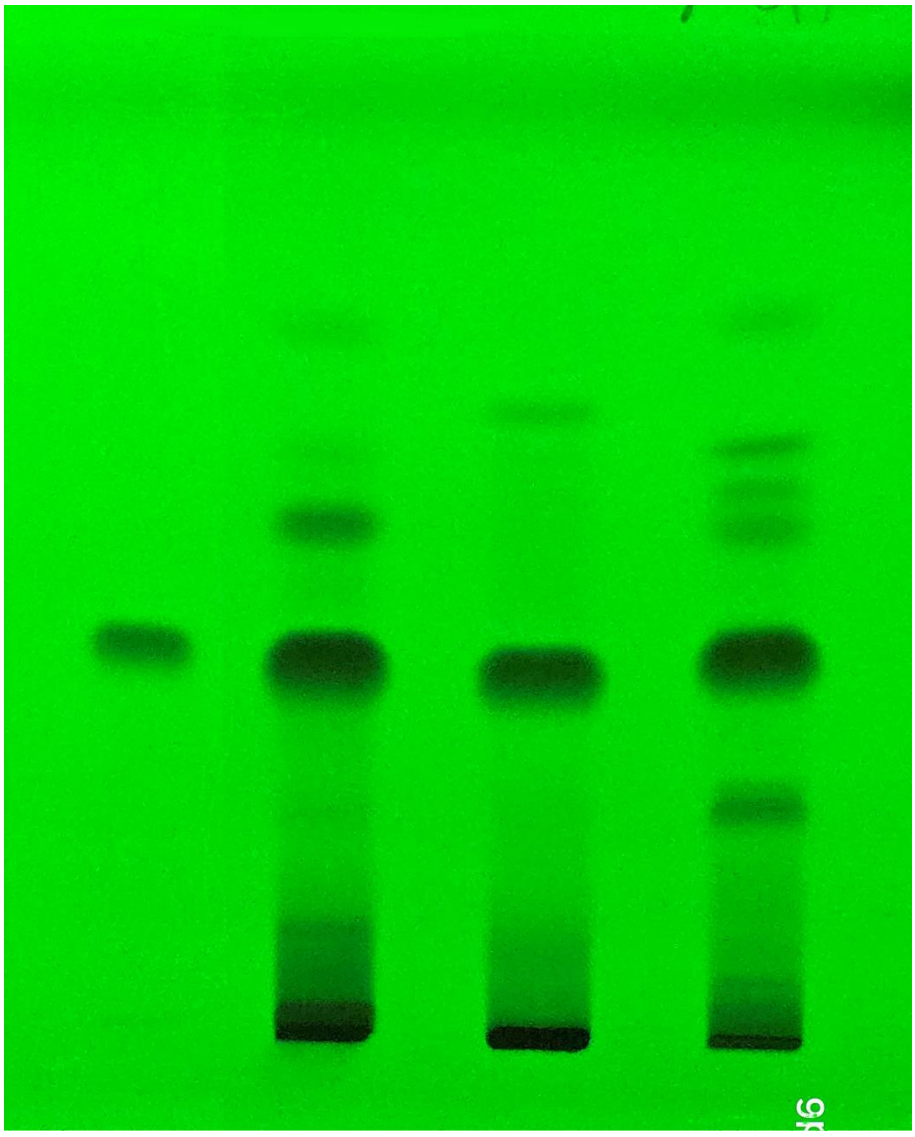




Spatang guggul sample

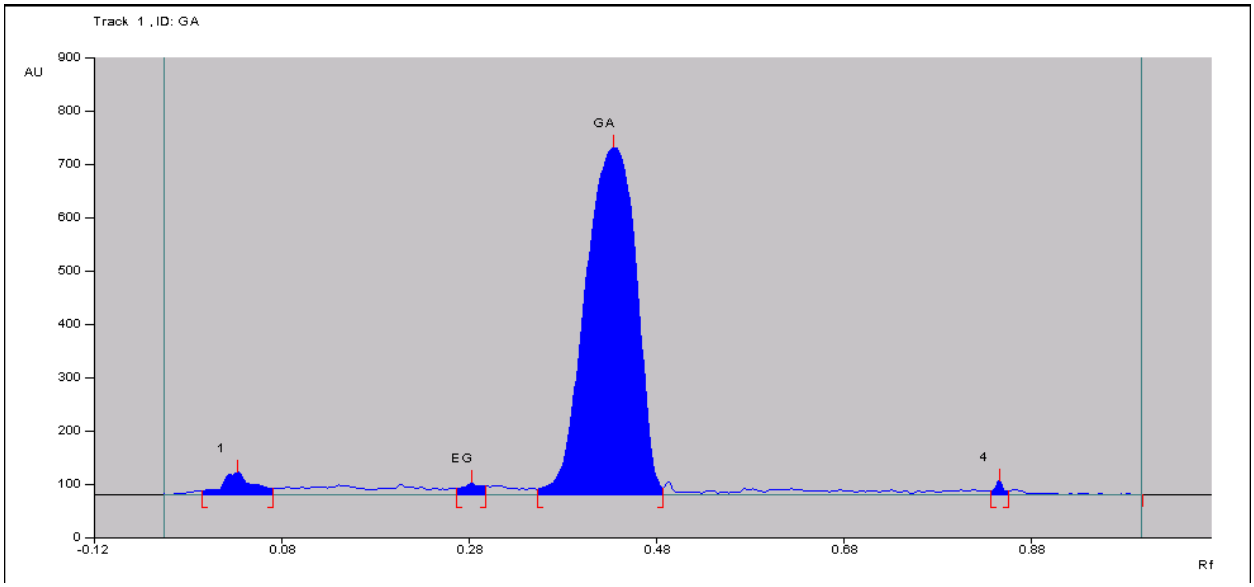
FIGURE NO. 2

Gallic acid

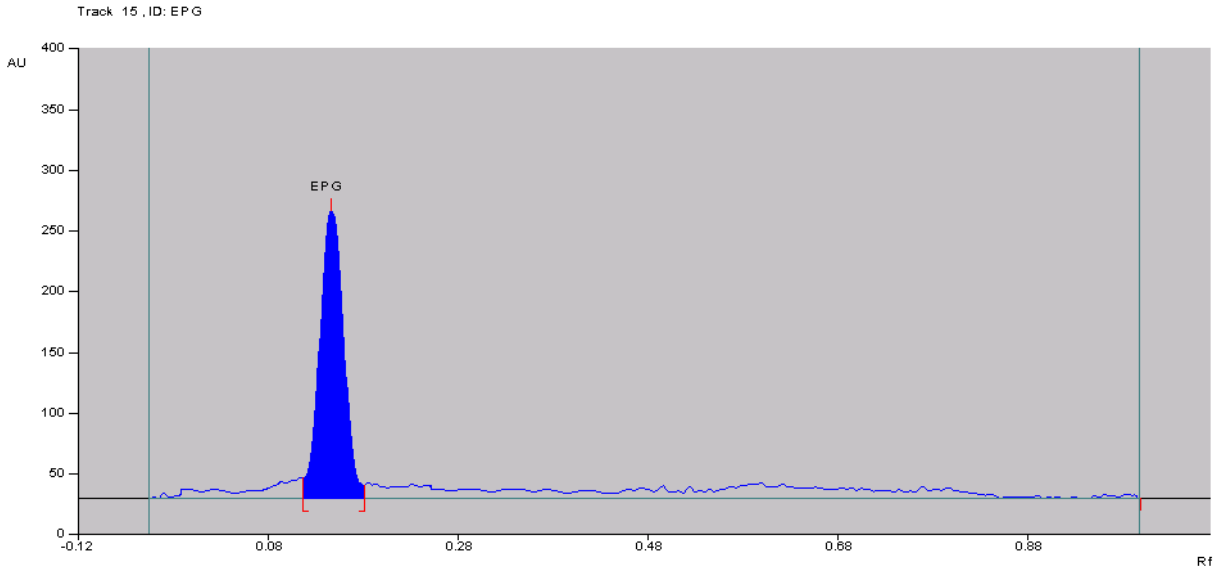


Track 1: Reference Standard gallic acid
Track 2: hareda Extract
Track 3 : Baheda Extract
Track 4 : Amla Extract

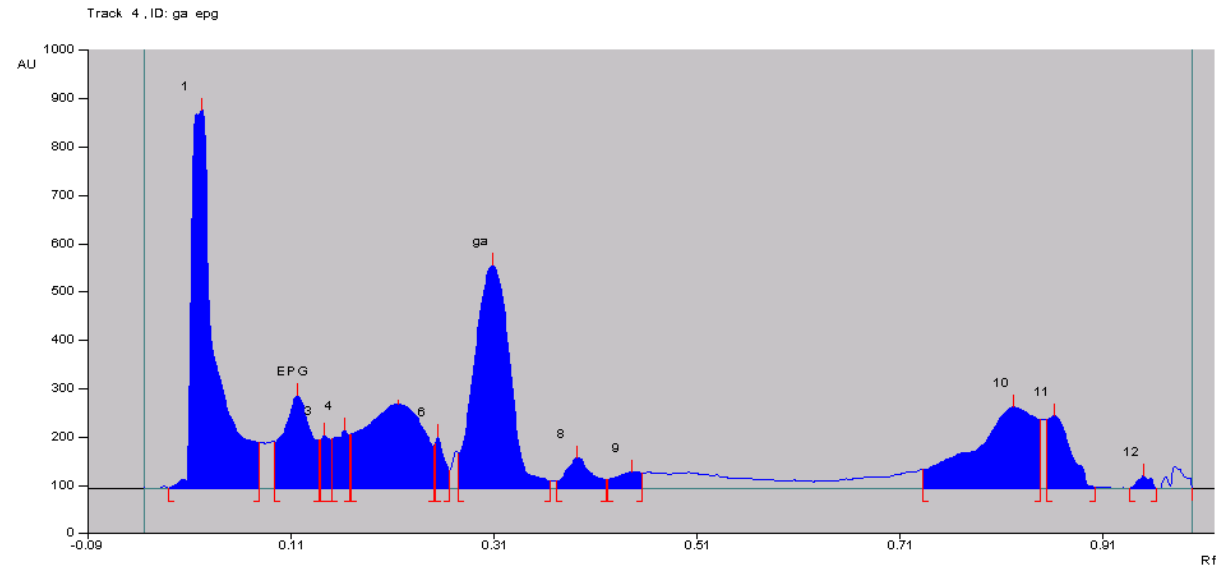
Track 1: Reference Standard epigallocatechin
Track 2: hareda Extract
Track 3 : Baheda Extract
Track 4 : Amla Extract



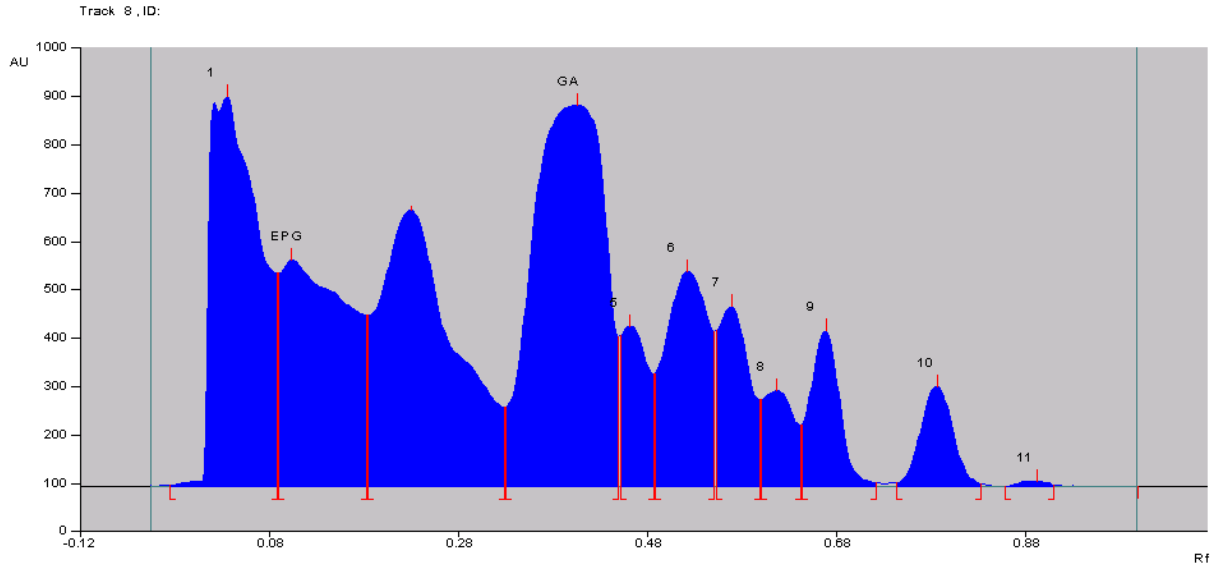
Reference Standard Gallic acid



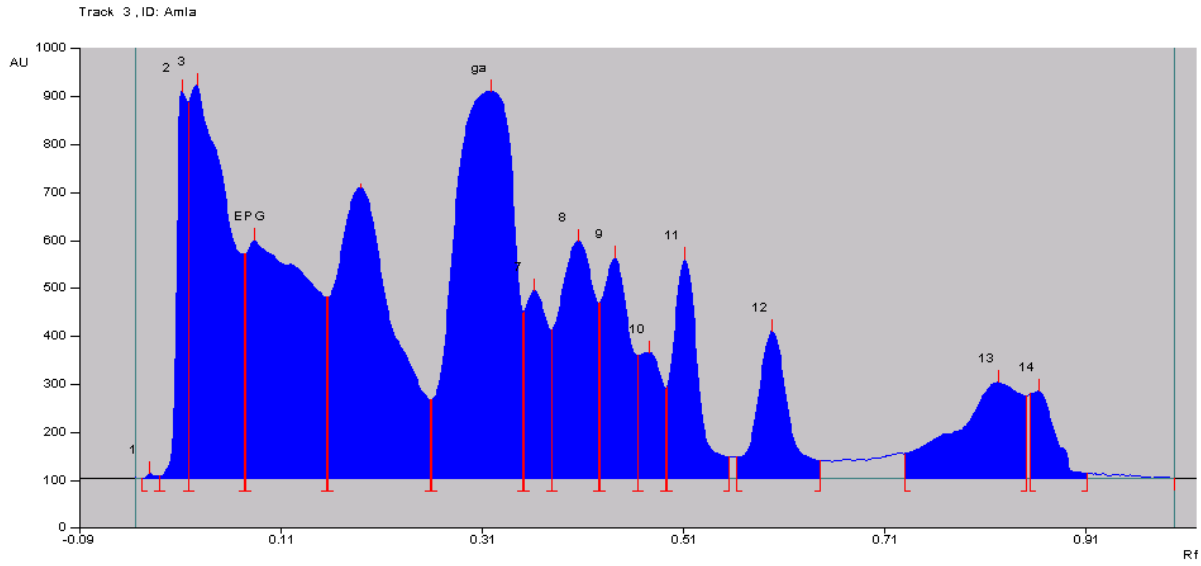
Reference Standard



Hareda extract

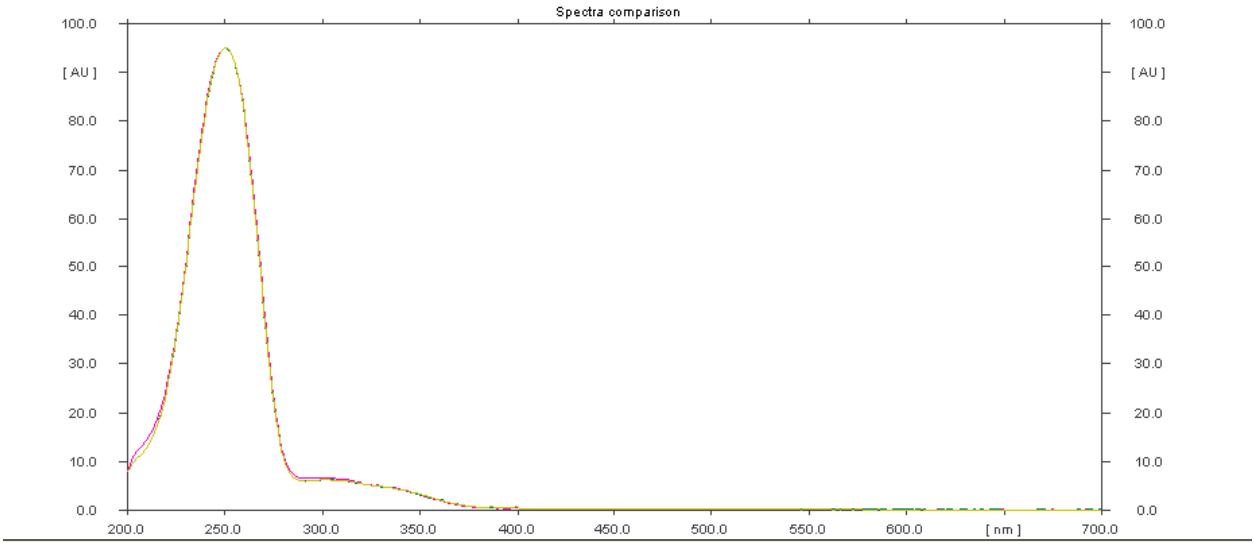


Baheda extract

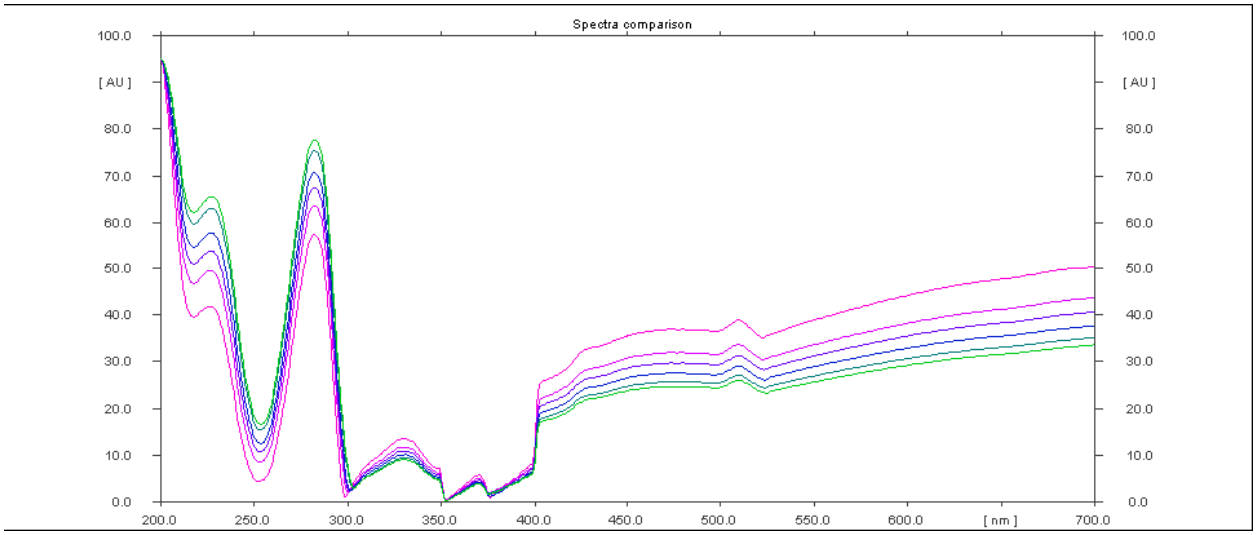


Amla extract

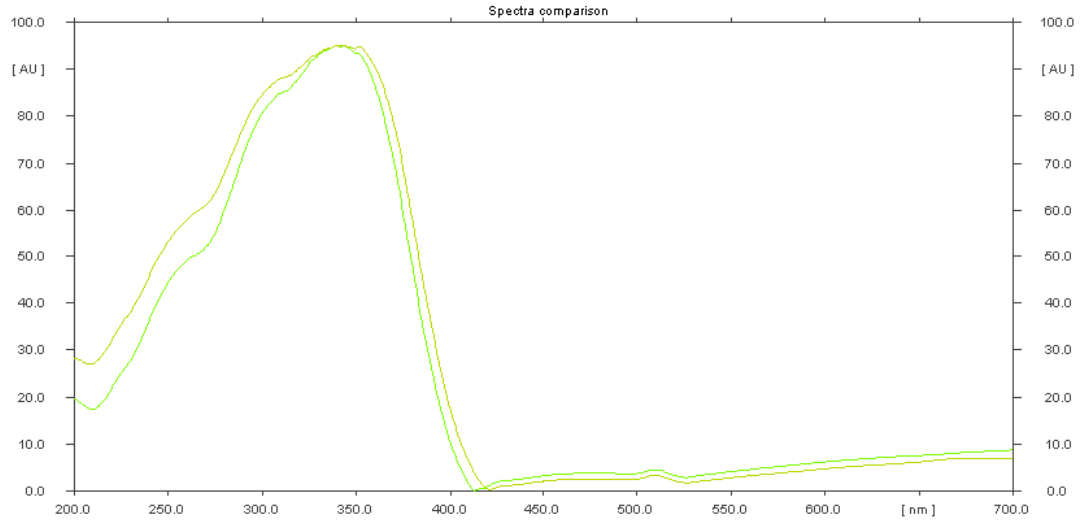
FIGURE NO. 2



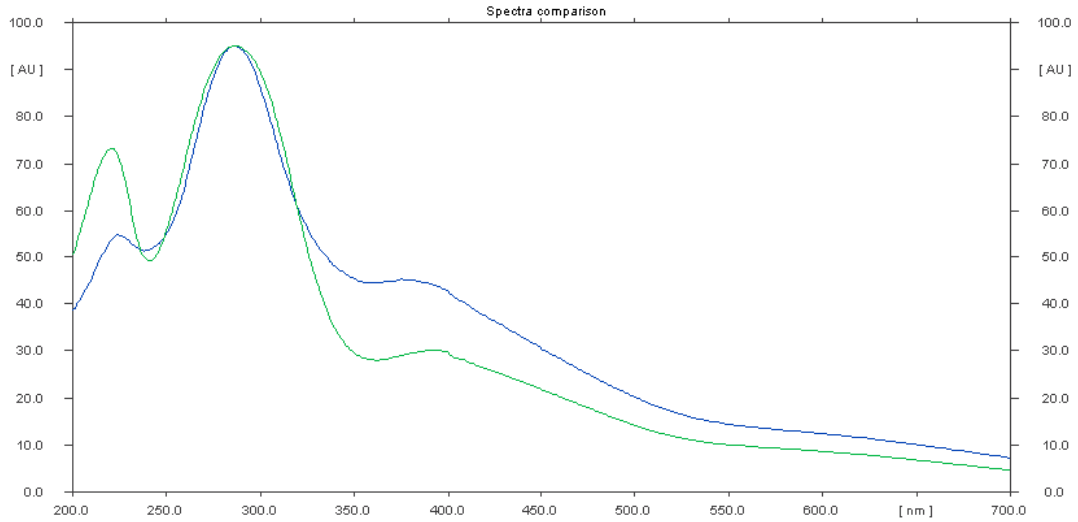
UV spectrum overlay of guggulsterone Z standard and sample



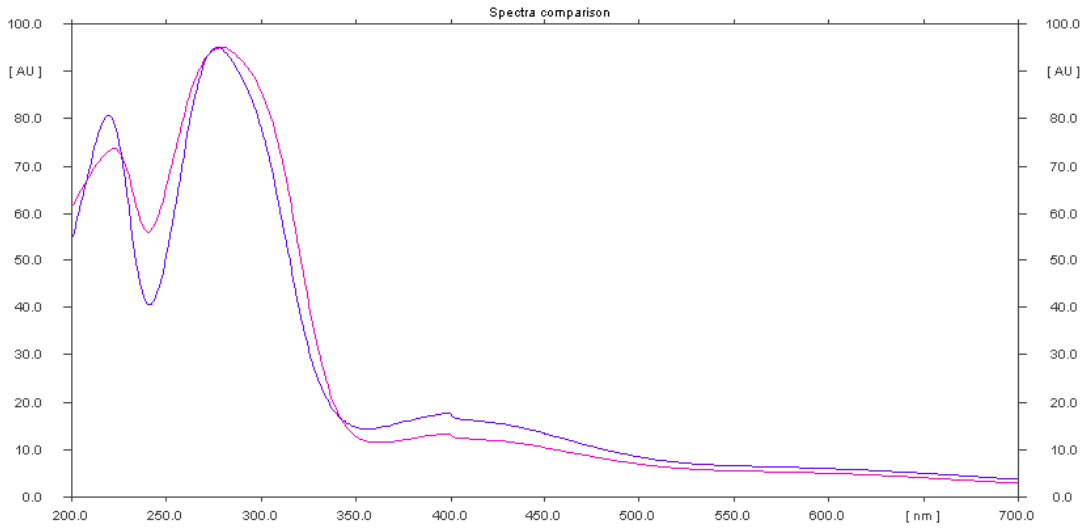
UV spectrum overlay of gingerol-6 standard and sample



UV spectrum overlay of piperine standard and sample

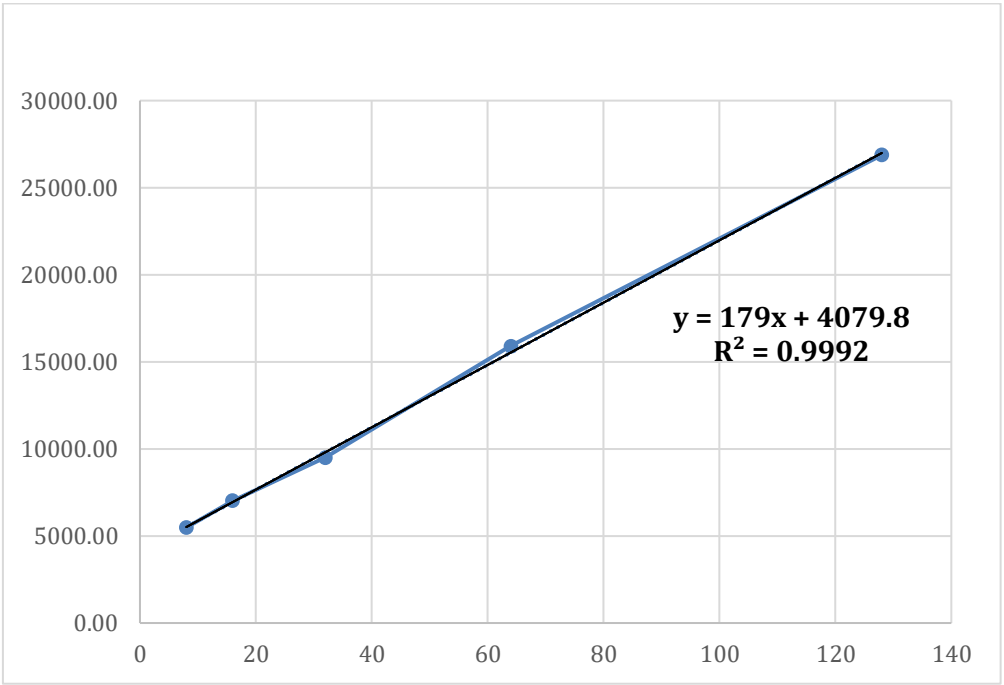


UV spectrum overlay of epigallocatechin standard and sample

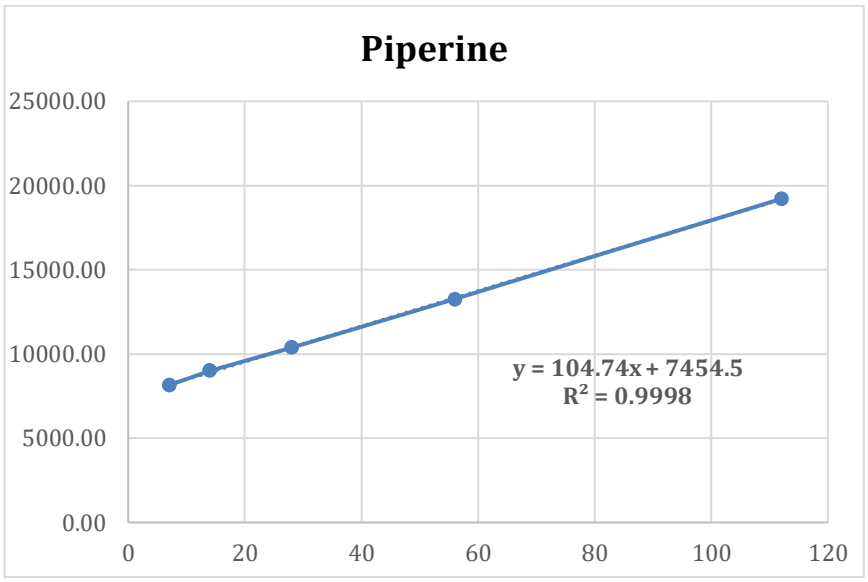


UV spectrum overlay of epigallocatechin standard and sample

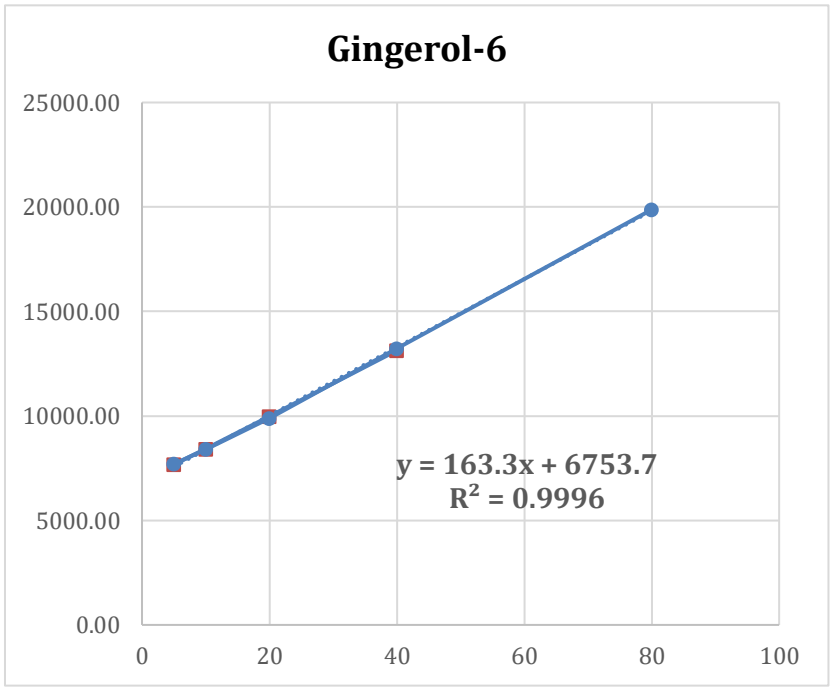
FIGURE NO. 3



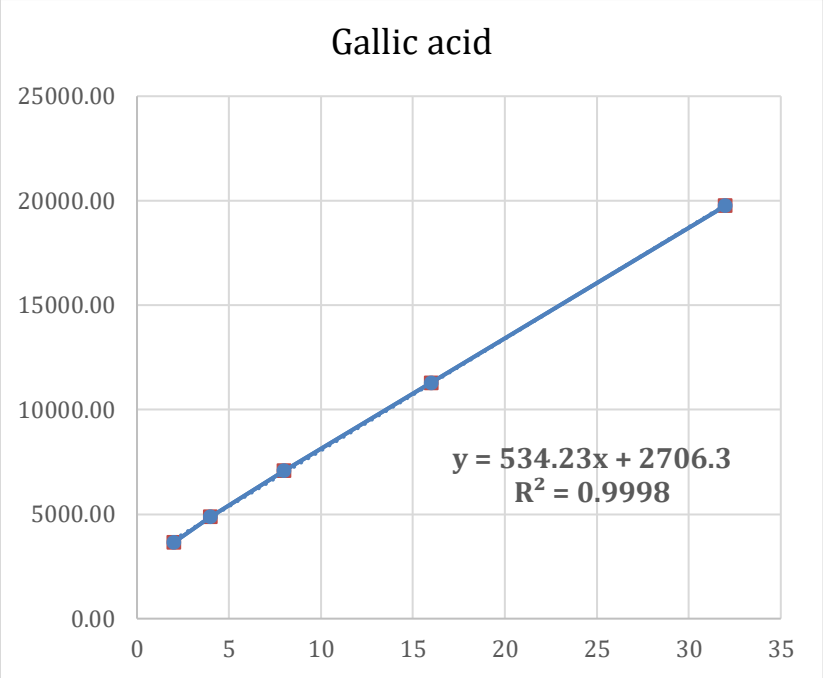
Linearity graph of standard Guggulsterone Z



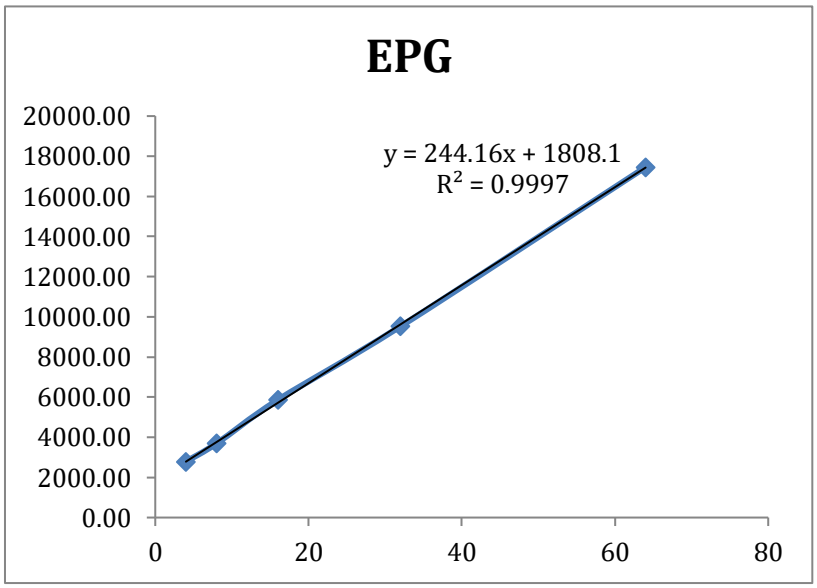
Linearity graph of standard Piperine



Linearity graph of standard gingerol-6



Linearity graph of standard Gallic acid



Linearity graph of standard EPG

TABLE NO. 1

Accuracy data of Guggulsterone Z

			80%			
Sr no.	**µgm/ml**	**Amt added**	**Area**	**Amt found**	**Amt recovered**	**% recovered**
1	36	28.8	15765.48	65.28	29.28	101.68
2	36	28.8	15536.9	64.01	28.01	97.24
3	36	28.8	15699.26	64.91	28.91	100.39
			Mean	64.73	28.73	99.77
			SD	0.66	0.66	2.28
			%RSD	1.01	2.29	2.29
			100%			
Sr no.	**µgm/ml**	**Amt added**	**Area**	**Amt found**	**Amt recovered**	**% recovered**
1	36	36	17198.14	73.29	37.29	103.57
2	36	36	17003.26	72.20	36.20	100.55
3	36	36	16953.25	71.92	35.92	99.77
			Mean	72.47	20.58	101.30
			SD	0.72	0.72	2.01
			%RSD	1.00	3.51	1.98
			120%			
Sr no.	**µgm/ml**	**Amt added**	**Area**	**Amt found**	**Amt recovered**	**% recovered**
1	36	43.2	18458.29	80.3268	44.33	102.61
2	36	43.2	18201.46	78.8920	42.89	99.29
3	36	43.2	18325.03	79.5823	43.58	100.88
			Mean	79.60	43.60	101.58
			SD	0.72	0.72	1.66
			%RSD	0.90	1.65	1.64

*N= 3

Accuracy data of Piperine

			80%			
Sr no.	µgm/ml	Amt added	Area	Amt found	Amt recovered	% recovered
1	28	22.4	12756.01	50.62	22.62	100.96
2	28	22.4	12725.63	50.33	22.33	99.67
3	28	22.4	12713.11	50.21	22.21	99.14
			Mean	50.38	22.38	99.92
			SD	0.21	0.21	0.94
			%RSD	0.42	0.94	0.94

			100%			
Sr no.	µgm/ml	Amt added	Area	Amt found	Amt recovered	% recovered
1	28	28	13381.68	56.59	28.59	102.11
2	28	28	13341.3	56.20	28.20	100.73
3	28	28	13352.68	56.31	28.31	101.12
			Mean	56.37	20.58	101.32
			SD	0.20	0.20	0.71
			%RSD	0.35	0.97	0.70

			120%			
Sr no.	µgm/ml	Amt added	Area	Amt found	Amt recovered	% recovered
1	28	33.6	13932.12	61.8448	33.84	100.73
2	28	33.6	13895.52	61.4953	33.50	99.69
3	28	33.6	14002.68	62.5184	34.52	102.73
			Mean	61.95	33.95	101.58
			SD	0.52	0.52	1.55
			%RSD	0.84	1.53	1.52

*N= 3

Accuracy data of Gingerol-6

			80%			
Sr no.	µgm/ml	Amt added	Area	Amt found	Amt recovered	% recovered
1	20	16	12675.25	36.27	16.27	101.66
2	20	16	12638.28	36.04	16.04	100.25
3	20	16	12704.16	36.44	16.44	102.77
			Mean	36.25	16.25	101.56
			SD	0.20	0.20	1.26
			%RSD	0.56	1.24	1.24

			100%			
Sr no.	µgm/ml	Amt added	Area	Amt found	Amt recovered	% recovered
1	20	20	13285.26	40.00	20.00	100.01
2	20	20	13305.16	40.12	20.12	100.62
3	20	20	13327.46	40.26	20.26	101.30
			Mean	40.13	20.58	100.64
			SD	0.13	0.13	0.65
			%RSD	0.32	0.63	0.64

			120%			
Sr no.	µgm/ml	Amt added	Area	Amt found	Amt recovered	% recovered
1	20	24	14007.17	44.4224	24.42	101.76
2	20	24	14042.14	44.6365	24.64	102.65
3	20	24	14069.47	44.8039	24.80	103.35
			Mean	44.62	24.62	102.59
			SD	0.19	0.19	0.80
			%RSD	0.43	0.78	0.78

*N= 3

Accuracy data of Gallic acid

			80%			
Sr no.	µgm/ml	Amt added	Area	Amt found	Amt recovered	% recovered
1	8	6.4	10456.36	14.51	6.51	101.68
2	8	6.4	10399.36	14.40	6.40	100.02
3	8	6.4	10412.25	14.43	6.43	100.39
			Mean	14.44	6.44	100.70
			SD	0.06	0.02	0.87
			%RSD	0.39	0.34	0.87

			100%			
Sr no.	µgm/ml	Amt added	Area	Amt found	Amt recovered	% recovered
1	8	8	11414.28	16.30	8.30	103.76
2	8	8	11341.28	16.16	8.16	102.05
3	8	8	11458.96	16.38	8.38	104.81
			Mean	16.28	20.58	103.54
			SD	0.11	0.11	1.39
			%RSD	0.68	0.54	1.34

			120%			
Sr no.	µgm/ml	Amt added	Area	Amt found	Amt recovered	% recovered
1	8	9.6	12125.28	17.6319	9.63	100.33
2	8	9.6	12142.26	17.6637	9.66	100.66
3	8	9.6	12141.09	17.6615	9.66	100.64
			Mean	17.65	9.65	101.58
			SD	0.02	0.02	0.18
			%RSD	0.10	0.18	0.18

*N= 3

Accuracy data (Intraday) of Epigallocatechin

			80%			
Sr no.	µgm/ml	Amt added	Area	Amt found	Amt recovered	% recovered
1	16	12.8	8869.36	28.92	12.92	100.94
2	16	12.8	8852.17	28.85	12.85	100.39
3	16	12.8	8875.22	28.94	12.94	101.13
			Mean	28.91	12.91	100.82
			SD	0.05	0.05	0.38
			%RSD	0.17	0.37	0.38

			100%			
Sr no.	µgm/ml	Amt added	Area	Amt found	Amt recovered	% recovered
1	16	16	9658.63	32.15	16.15	100.96
2	16	16	9678.46	32.23	16.23	101.47
3	16	16	9700.76	32.33	16.33	102.04
			Mean	32.24	20.58	101.49
			SD	0.09	0.09	0.54
			%RSD	0.27	0.42	0.53

			120%			
Sr no.	µgm/ml	Amt added	Area	Amt found	Amt recovered	% recovered
1	16	19.2	10452.36	35.4041	19.40	101.06
2	16	19.2	10396.69	35.1761	19.18	99.88
3	16	19.2	10458.25	35.4282	19.43	101.19
			Mean	35.34	19.34	100.71
			SD	0.14	0.14	0.72
			%RSD	0.39	0.72	0.72

*N= 3

TABLE NO.2

Precision data of Guggulsterone Z

Intraday Precision			Area							
Sr No.	Conc	I	II	III	Mean	Amt Found	% Amt Fnd	SD	%RSD
1	6	5847.17	5786.24	5968.29	5867.23	6.08	101.38	92.67	1.58
2	12	8662.55	8774.19	8639.22	8691.99	11.97	99.74	72.14	0.83
3	24	14458.2	14568.55	14752.28	14593.01	24.27	101.11	148.56	1.02

Interday Precision			Area						
Sr No.	Conc	I	II	III	Mean	Amt Found	% Amt Fnd	SD	%RSD
1	6	5876.49	5874.21	5841.27	5875.35	6.10	101.67	19.71	0.34
1	12	8744.29	8758.26	8746.39	8751.28	12.09	100.77	7.53	0.09
3	24	14691.28	14674.26	14852.29	14682.77	24.45	101.89	98.24	0.67

Repeatability			Area						
Sr No.	Conc	I	II	III	Mean	Amt Found	% Amt Fnd	SD	%RSD
1	12	8769.28	8724.13	8755.18	8746.71	12.08	100.69	23.10	0.26

*N= 3

Precision data of Piperine

Intraday Precision			Area						
Sr No.	Conc	I	II	III	Mean	Amt Found	% Amt Fnd	SD	%RSD
1	14	8936.07	8958.54	8985.21	8959.94	14.37	102.67	24.60	0.27
2	28	10333.89	10287.41	10664.51	10428.60	28.40	101.41	205.62	1.97
3	56	13236.01	13234.81	13294.81	13255.21	55.38	98.90	34.30	0.26

Interday Precision			Area						
Sr No.	Conc	I	II	III	Mean	Amt Found	% Amt Fnd	SD	%RSD

1	14	8912.04	8949.4	8955.14	8930.72	14.09	100.67	23.40	0.26
1	28	10487.73	10371.34	10439.14	10429.54	28.40	101.44	58.46	0.56
3	56	13451.81	13288.78	13461.74	13370.30	56.48	100.86	97.12	0.73

Repeatability			Area						
Sr No.	Conc	I	II	III	Mean	Amt Found	% Amt Fnd	SD	%RSD
1	28	10388.43	10326.88	10623.89	10357.66	27.72	98.99	156.76	1.51

*N= 3

Precision data of Gingerol-6

Intraday Precision			Area						
Sr No.	Conc	I	II	III	Mean	Amt Found	% Amt Fnd	SD	%RSD
1	10	8409.46	8409.85	8415.46	8411.59	10.16	101.57	3.36	0.04
2	20	9988.26	9994.21	10184.14	10055.54	20.22	101.12	111.41	1.11
3	40	13477.11	13442.26	13112.14	13343.84	40.36	100.90	201.41	1.51

Interday Precision			Area						
Sr No.	Conc	I	II	III	Mean	Amt Found	% Amt Fnd	SD	%RSD
1	10	8422.58	8424.26	8417.96	8423.42	10.23	102.29	3.26	0.04
1	20	9955.28	10185.49	9967.28	10070.39	20.31	101.57	129.59	1.29
3	40	13292.09	13279.46	13329.28	13285.78	40.00	100.01	25.90	0.19

Repeatability			Area						
Sr No.	Conc	I	II	III	Mean	Amt Found	% Amt Fnd	SD	%RSD
1	20	10007.28	10082.28	9987.26	10044.78	20.16	100.79	50.09	0.50

*N= 3

Precision data of Gallic acid

Intraday Precision			Area						
Sr No.	Conc	I	II	III	Mean	Amt Found	% Amt Fnd	SD	%RSD
1	4	4852.29	4871.39	4857.19	4860.29	4.03	100.80	9.92	0.20
2	8	7031.36	7085.94	7044.28	7053.86	8.14	101.73	28.52	0.40

3	16	11205.28	11188.46	11324.28	11239.34	15.97	99.83	74.04	0.66

Interday Precision			Area						
Sr No.	Conc	I	II	III	Mean	Amt Found	% Amt Fnd	SD	%RSD
1	4	4871.33	4844.97	4852.27	4858.15	4.03	100.70	13.61	0.28
1	8	7022.64	7124.25	7112.26	7073.45	8.18	102.19	55.53	0.79
3	16	11252.28	11246.23	11925.25	11249.26	15.99	99.95	390.30	3.47

Repeatability			Area						
Sr No.	Conc	I	II	III	Mean	Amt Found	% Amt Fnd	SD	%RSD
1	8	6982.85	7144.47	7035.16	7063.66	8.16	101.96	82.47	1.17

*N= 3

Precision data (Intraday) of Epigallocatechin

Intraday Precision			Area						
Sr No.	Conc	I	II	III	Mean	Amt Found	% Amt Fnd	SD	%RSD
1	8	3785.29	3791.25	3714.85	3763.80	8.01	100.12	42.49	1.13
2	16	5764.82	5786.94	5811.46	5787.74	16.30	101.87	23.33	0.40
3	32	9694.98	9601.28	9611.25	9635.84	32.06	100.19	51.46	0.53

Interday Precision			Area						
Sr No.	Conc	I	II	III	Mean	Amt Found	% Amt Fnd	SD	%RSD
1	8	3812.25	3798.66	3795.46	3805.46	8.18	102.26	8.91	0.23
1	16	5784.19	5782.22	5796.36	5783.21	16.28	101.75	7.66	0.13
3	32	9701.24	9711.34	9758.64	9706.29	32.35	101.09	30.64	0.32

Repeatability			Area						
Sr No.	Conc	I	II	III	Mean	Amt Found	% Amt Fnd	SD	%RSD
1	16	5784.12	5758.25	5712.26	5771.19	16.23	101.45	36.40	0.63

*N= 3

TABLE NO. 3

LOD and LOQ of compounds

Sr. No.	Compounds	Parameters	
		LOD (mcg/spot)	LOQ (mcg/spot)
1.	Guggulsterone Z	2.99	9.08
2.	Piperine	2.79	8.45
3.	Gingerol-6	0.92	2.77
4	Gallic acid	0.19	0.58
5	Epigallocatechin	0.008	0.026

TABLE NO. 4

Robustness study of Guggulsterone Z

mp saturation time changed from 15 min to 17min

Sr No.	µgm/ml	Area	Amt found	%
1	36	10524.71	36.0051	100.01
2	36	10625.82	36.5699	101.58
3	36	10658.29	36.7513	102.09
	Mean	10602.94	36.44	101.23
	SD	71.50	0.40	1.11
	%RSD	0.67	1.10	1.10

Mobile Phase

mp saturation time changed from 15 min to 12min

Sr No.	µgm/ml	Area	Amt found	%
1	36	10613.24	36.4997	101.39
2	36	10580.71	36.3179	100.88
3	36	10490.36	35.8132	99.48
	Mean	10561.4	36.21	100.58
	SD	23.00	0.13	0.36
	%RSD	0.22	0.35	0.35

Wavelength= 252

Sr No.	µgm/ml	Area	Amt found	%
1	36	10552.37	36.1596	100.44
2	36	10591.58	36.3787	101.05
3	36	10479.49	35.7525	99.31
	Mean	10541.1	36.10	100.27

	SD	27.73	0.15	0.43
	%RSD	0.26	0.43	0.43

Wavelength= 248

Sr No.	µgm/ml	Area	Amt found	%
1	36	10641.5	36.6575	101.83
2	36	10611.71	36.4911	101.36
3	36	10668.62	36.8091	102.25
	Mean	10640.6	36.65	101.81
	SD	21.06	0.12	0.33
	%RSD	0.20	0.32	0.32

*N= 3

Robustness study of Piperine

mp saturation time changed from 15 min to 17min

Sr No.	µgm/ml	Area	Amt found	%
1	28	9085.29	27.9636	99.87
2	28	9178.14	28.4823	101.72
3	28	9045.12	27.7392	99.07
	Mean	9102.85	28.06	100.22
	SD	65.65	0.37	1.31
	%RSD	0.72	1.31	1.31

Mobile Phase

mp saturation time changed from 15 min to 12min

Sr No.	µgm/ml	Area	Amt found	%
1	28	9061.75	27.8321	99.40
2	28	9154.6	28.3508	101.25
3	28	9021.58	27.6077	98.60
	Mean	9079.3	27.93	99.75
	SD	65.65	0.37	1.31
	%RSD	0.72	1.31	1.31

Wavelength= 252

Sr No.	µgm/ml	Area	Amt found	%
1	28	9158.1	28.3704	101.32
2	28	9119.68	28.1558	100.56
3	28	9056.46	27.8026	99.29
	Mean	9111.4	28.11	100.39
	SD	27.17	0.15	0.54
	%RSD	0.30	0.54	0.54

Wavelength= 256

Sr No.	µgm/ml	Area	Amt found	%
1	28	8997.27	27.4719	98.11
2	28	9200.68	28.6083	102.17
3	28	9127.86	28.2015	100.72
	Mean	9108.6	28.09	100.34
	SD	143.83	0.80	2.87
	%RSD	1.58	2.86	2.86

*N= 3

Robustness study of Gingerol-6

mp saturation time changed from 15 min to 17min

Sr No.	µgm/ml	Area	Amt found
1	20	10018.78	19.9987
2	20	10079.12	20.3682
3	20	10085.28	20.4059
	Mean	10061.06	20.26
	SD	42.67	0.23
	%RSD	0.42	1.11

mp saturation time changed from 15 min to 12min

Sr No.	µgm/ml	Area	Amt found
1	20	10074.19	20.3380
2	20	10112.28	20.5712
3	20	10176.29	20.9632
	Mean	10120.92	20.62
	SD	26.93	0.32
	%RSD	0.27	1.53

Wavelength= 252

Sr No.	µgm/ml	Area	Amt found
1	20	10182.46	21.0010
2	20	10162.09	20.8762
3	20	10047.29	20.1732
	Mean	10130.61	20.68
	SD	14.40	0.45
	%RSD	0.14	2.16

Wavelength= 256

Sr No.	µgm/ml	Area	Amt found
1	20	10042.98	20.1468
2	20	10143.17	20.7604
3	20	10175.33	20.9573
	Mean	10120.49	20.62
	SD	70.85	0.42
	%RSD	0.70	2.05

*N= 3

Robustness study of Gallic acid

mp saturation time changed from 15 min to 17min

Sr No.	µgm/ml	Area	Amt found
1	8	7084.66	8.1961
2	8	7054	8.1387
3	8	7042.19	8.1166
	Mean	7060.28	8.15
	SD	21.68	0.04
	%RSD	0.31	0.50

mp saturation time changed from 15 min to 12min

Sr No.	µgm/ml	Area	Amt found
1	8	7122.02	8.2660
2	8	6998.24	8.0343
3	8	6989.33	8.0177
	Mean	7036.53	8.11
	SD	87.53	0.14
	%RSD	1.24	1.71

Wavelength= 252

Sr No.	µgm/ml	Area	Amt found
1	8	6985.39	8.0103
2	8	6988.77	8.0166
3	8	6996.36	8.0308
	Mean	6990.17	8.02
	SD	2.39	0.01
	%RSD	0.03	0.13

Wavelength= 256

Sr No.	µgm/ml	Area	Amt found
1	8	6996.88	8.0318
2	8	6984.19	8.0080
3	8	6985.55	8.0106
	Mean	6988.87	8.02
	SD	8.97	0.01
	%RSD	0.13	0.16

*N= 3

Robustness study of Epigallocatechin

mp saturation time changed from 15 min to 17min

Sr No.	µgm/ml	Area	Amt found
1	16	5785.28	16.2892
2	16	5774.46	16.2449
3	16	5762.11	16.1943
	Mean	5773.95	16.24
	SD	7.65	0.05
	%RSD	0.13	0.29

mp saturation time changed from 15 min to 12min

Sr No.	µgm/ml	Area	Amt found
1	16	5801.21	16.3545
2	16	5712.21	15.9900
3	16	5742.69	16.1148
	Mean	5752.04	16.15
	SD	62.93	0.19
	%RSD	1.09	1.15

Wavelength= 252

Sr No.	µgm/ml	Area	Amt found
1	16	5722.81	16.0334
2	16	5734.95	16.0831
3	16	5748.52	16.1387
	Mean	5735.43	16.09
	SD	8.58	0.05
	%RSD	0.15	0.33

Wavelength= 256

Sr No.	µgm/ml	Area	Amt found
1	16	5714.28	15.9984
2	16	5728.33	16.0560
3	16	5742.84	16.1154
	Mean	5728.48	16.06
	SD	9.93	0.06
	%RSD	0.17	0.36

*N= 3

TABLE NO. 5

Analysis of Formulation for Determination of % Content of Bioactives

Sr. No	Markers	% Content in Formulations
1.	Guggulsterone Z	1.68
2.	Gallic acid	2.19
3.	Epigallocatechin	0.84
4	Piperine	1.48
5	Gingerol-6	0.32

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