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Research Article

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Bioanalytical and Analytical Method Development and HPTLC Method Validation for Pharmaceutical Dosage Formulation

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Abstract

The literature survey determined a need to improve the bioavailability of a very effective molecule viz. Curcumin. Attempts are despite the fact that happening with the utilization of diverse techniques to the molecule are its very low solubility in water. Many marketers like Cyclodextrin, PVP, Gelucire 44/14, Gelucire 50/thirteen, and, Cellulose acetate mannitol has been used to put together stable dispersion for oral dosage paperwork. Smooth and, specific HPTLC methods had been advanced for the HPTLC Bioanalytical approach the prevailing statement is deliberate to prepare the sturdy distribution of Gelucire 44/14 and, Curcumin improves solubility and similarly formulate into Sodium alginate Curcumin Floating Beads (SCFB) for oral use. HPTLC technique became advanced and, showed for the determination of curcumin from serum samples inside the following manner 3albino Wistar rats had been fed with a widespread pellet eating regimen and maintained at a temperature of (25 ± zero. Five° C RH =30%), and 12hrs. Brightness /dim cycle) had been used intended for the have a take a look at.

The technique turns into tailor-made to investigate tablets in their business dosage form (SCFB) without interfering from ingredient. Chromatographic division is completed in excess of TLC that has been coated tableware Merck, Darmstadt, Germany, ("60 F254, 20centimeter 10 cm, 250 m thinness") via a linear-line downhill method the use of the mobile phase constituting toluene: chloroform: methanol: acetic acid (five: four: zero. 5: zero. 5) changed into utilized for the approach improvement. Detection and, quantification have been completed at 426 nm through UV-Spectrophotometer assessment. The investigative ordinary performance of the proposed HPTLC technique become installed consistent by means of the ICH recommendations concerning precision, linearity, quantitation limits, accuracy, detection, quantitation limits, specificity, and robustness. The calibration curve has been linear with the boundaries of two hundred–one thousand ng/spot for Curcumin with relationship coefficients (r2) > 0.9998. The boundaries of detection had been 30 ng/spot for Curcumin. The confirmed HPTLC approach was efficiently applied to the determination of Curcumin within the business polyhedral components.

Keywords: Curcumin, Gelucire forty-four/14, HPTLC Bioanalytical Approach, Sodium Alginate Curcumin Floating Beads (SCFB), etc.

1. Introduction

There have been many researches on the potential of natural products as a result of society's growing interest in traditional medicine, particularly plant-based medicine. Sizeable studies are going on to make use of herbal products to treat illnesses and prevent continual sicknesses. Herbs and Phytopharmaceuticals play crucial function in curing illnesses. Approximately 80% of the world's populace makes use of natural medicines. One of the famous herbal drugs is the rhizomes of Curcuma longa, often known as the roots or rhizomes incorporate a yellow pigment called curcumin.

Curcumin

Advantages and Troubles: Curcumin is one of the maximum essential drugs with a couple of medicinal advantages (Fig. 01) and curcumin is a first-rate lively component of the roots or rhizomes. Curcumin is likewise accountable for the acute yellow shade of the roots or rhizomes.

Curcumin (I) is drastically studied for its chemical, pharmacological and pharmaceutical components. It is a hydrophobic, low molecular weight polyphenol that goes by the chemical names bis-, unsaturated-diketone or Diferuloylmethane. It demonstrates keto-enol (Fig. 02) tautomerism, with a solid "Enol struc-

ture" in an alkaline-middling and a dominating "keto structure" in acidic and neutral environments. Because it's extensively soluble in water, the ability to absorb curcumin is a major issue for formulations incorporating it. Since then, a number of investigations have been conducted that have enhanced Curcumin's bioavailability. Curcumin interaction with polymer has attracted a lot of interest in treating various disease conditions because it increases the solubility and bioavailability of medication in the aqueous media. In the gift study, efforts were undertaken to increase Curcumin's solubility in water.

Due to society's growing interest in traditional medicine, especially plant-based therapy, there has been much research done on the potential of natural products. A lot of research is being done on using natural remedies to cure illnesses and prevent chronic diseases. Herbs and Phytopharmaceuticals are crucial in the treatment of illnesses. Around 80% of people use herbal medications throughout the world. One of the most well-known herbal treatments is curcumin, a yellow pigment originates in the rhizomes or roots of the "Curcuma longa plant."

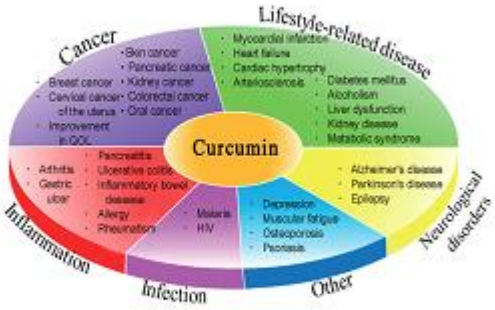


Figure 1: T herap euticApp location of Curcumin

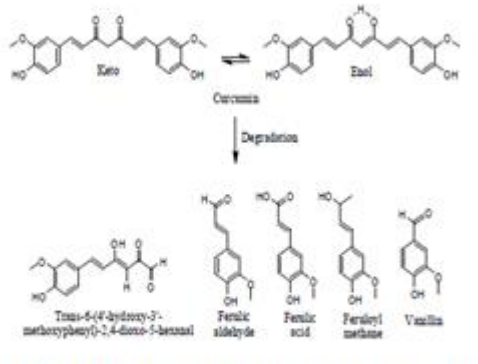


Figure 2: Degradation and various Derivatives of Curcumin

Experimental

Chemicals and reagents: The herbal plant extract dealer for "BAPS life sciences" in Thane, Mumbai, provided a free sample of curcumin. The Gelucire 44/14 current sample was purchased from the Gatteffose Bombay University of Pharmacy in Kalina and Mumbai. The procured samples have been examined to verify their identity, including a UV-visible wavelength test, HPTLC analyses, and a recording of toes-IR spectra. Feet-IR spectra have been discovered inside the [01, 02]. Hiranandani Institute of pharmacy in Ulhasnagar, Kalyan. The sample became organized as KBr pallets for recording the spectra. The UV-seen spectra of curcumin have been recorded using methanol as a solvent while Gelucire forty-four/14 changed into recorded using water as a solvent on SICAN 2301 instrument [01].

Chromatographic Conditions: Linomat 5 was used to detect and develop the cell segment's On HPTLC plates covered with Silica gel (0.2mm covering thicknesses), "toluene, chloroform, methanol, as well as acetic acid" were used in a 5:4:0.5:0.5 ratio. The mobile market with the best resolution is chosen for the method's development. For the development of the method, a mobile component including "toluene, chloroform, methanol, and acetic acid ("5:4:0.5:0.5")" was used. The factors used to enhance the method are listed under.

Table: 01- Chromatographic parameters:[4-9]

Application mode	Camag Linomat V, Hamilton Syringe
Development mode	Ascending technique
Stationary Phase	Precoated silica gel 60 GF_{254} plates (thickness 0.2mm)
Mobile Phase	Toluene: Chloroform: Methanol: Acetic Acid (5: 4: 0.5: 0.5 v/v/v/v)
Chamber saturation	30 minutes
Development distance	18mm
Scanner	Camag TLC Scanner 3
Integrator	Win CATS Planar Chromatography Manager Software version1.4.4.6337
Detection	426 nm
Sample	Standard Curcumin and SCFB Formulation

Preparation of Solution-Standard Solutions: Blood was extracted in the amount of 1 mL by cutting a hole in the end's retro-orbital plexus. For around 20 to 30 minutes, set aside. The clot blood was centrifuged at 5000 rpm to get serum 30 minutes later. 20 to 100 µg of curcumin (10 mg/10 ml) in 30 µl of serum. The serum sample was deproteinised by the addition of chloroform and methanol in the ratio of (1:1) followed by centrifugation. The supernatant containing the extracted curcumin 10µl was applied on an HPTLC plate. The sample was utilized for the development of the technique intended or designed for the purpose of "curcumin or turmeric active constituents" beginning serum.

The Bioanalytical HPTLC Method's Validation

A Proposed of analytical technique was approved in accordance with the Q2 (R1) of the "International Conference on Harmonization (ICH) requirements."10 The HPTLC technique become verified -for parameters viz. Specificity, linearity, LOD, LOQ, Precision, recovery, accuracy, Robustness, and so on [10,11].

A. Specificity: A Methanolic solution of the reference well known of the Curcumin sample changed into carried out 20µl, (Baps existence Sciences) and the methanolic extract of the serum pattern spiked with standard curcumin (20 µl) changed into applied to a silica gel GF254 HPTLC platter. The platter was evolved using the cellular segment and condition as described above, underneath all the situations mentioned above, the spot with Rf of 0.56 turned into observed to be corresponding to the Curcumin widespread, and the wavelength test of the pattern spot and the spot of general curcumin have been co-inclusive of and the 426 nm ʎ max value was determined to be 426 nm. A height purity takes a look at became additionally done for the spectra of the spot. A plain serum sample processed as consistent with the process accompanied for the pattern (20µl) become additionally spotted in parallel as a manipulate. The result is offered in fig: 01.

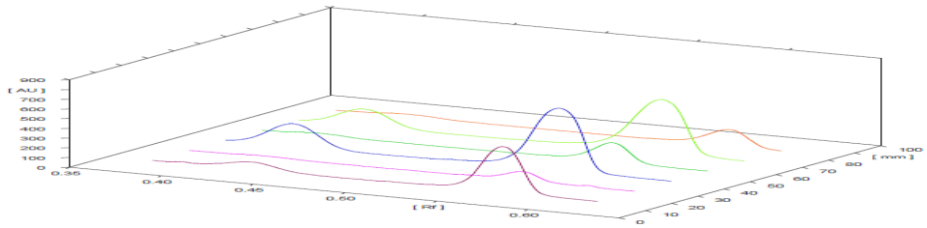


Figure 3: Data Obtained from Specificity Data Obtained from Specificity

B. Linearity: For you to establish linearity Curcumin 10 µl corresponding to 10 mcg became spiked inside the 20 µg and five special concentrations viz 20µg, forty µg, 60 µg, 80 µg and, a hundred µg were carried out on the TLC plate. The plates have been advanced and the response of the detector for the exclusive concentrations of the reference substance was measured. The experiment changed into finished in triplicate and the mean became calculated. A graph was plotted of the drug peak vicinity against the concentration of Curcumin calculating the linear variety and regression of the coefficient.

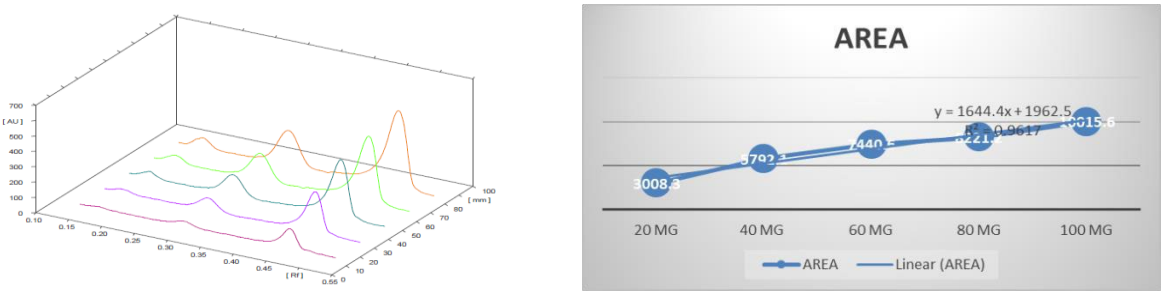


Figure 4: data obtained from linearity (Mean ± S.D, N = 3 from the results it was observed that the linearity range is 20 µg to 100 µg.)

Conc.	Area of peak (Mean ± S.D.)	Rf value
20µg	3008.3 ± 1.53	0.44
40µg	5792.1 ± 2.70	0.45
60µg	7440.6 ± 1.8	0.44
80µg	8221.2 ± 1.83	0.45
100µg	10015.6 ± 3.2	0.45

Table 2: data obtained from linearity

C. Limit of Detection and Quantifications: Reference standard solution of Curcumin at a concentration in the lower part of the linear range of the calibration plot was used to determine the Limit of detection (LOD) and Limit of quantification (LOQ). These were determined from the slope of the calibration plot and the standard deviation (S.D.) of the blank sample by use of equations - S.D = 166.92, S = 139.45.

L.O.D.

= 3.3 × S.D./S

= 3.3 × 166.92/ 139.45

= 3.95

L.O.Q.

= 10 × S.D./S

= 10 × 166.92/139.45

= 11.96

Where SD – ("Standard Deviation") of the empty reply in addition to S is the incline of "calibration intrigue".

"The limit of detection and Limit of Quantification for" a bioanalytical method of curcumin from serum samples were found to be 3.95 and, 11.96 µg.

D. Precision: Precision was determined at two levels, "repeatability" (intra-day) and "intermediate" (between-days), in accordance with ICH recommendations. While intermediate accuracy was assessed by measuring the intraday day variation for triplicates determination of the reference standard, repeatability of the sample application was determined as intraday day variation Curcumin (40 µg, 60 µg, 80µg, and, 100 µg)

Normal Concentration	Concentration Found	Standard deviation	Precision C.V.%	Accuracy	R.S. D
40 µg	37.01	0.943	94.35 %	92.52 %	0.0515
60 µg	67.10	74.09	110.42%	111.83 %	1.850
80 µg	76.0	82.00	105.50%	95.00%	2.00
100 µg	86.49	91.18	105.52%	86.49 %	1.82

Table 3: Data of Inter- day precision

Normal Concentration	Concentration Found	Standard deviation	Precision C.V.%	Accuracy	R.S. D
40 µg	37.64	0.934	93.44 %	94.10 %	0.050
60 µg	69.45	72.54	104.4%	110.0 %	0.710
80 µg	84.66	89.18	105.33%	105.82%	0.730
100 µg	105.73	101.02	95.54%	105.73%	0.601

Table 4: Data of Intra- day precision

E. "Accuracy and Recovery": The accurateness of the technique at low, medium, and high concentrations was determined by six-fold replicate application and chromatography of 40µl, 40µl (1), 40µl (2), and, 40µl (3). HPTLC analysis was performed for both the spikes of the sample's solution. Recovery was calculated by use of the equation:

Accuracy = [C/ N] × 100

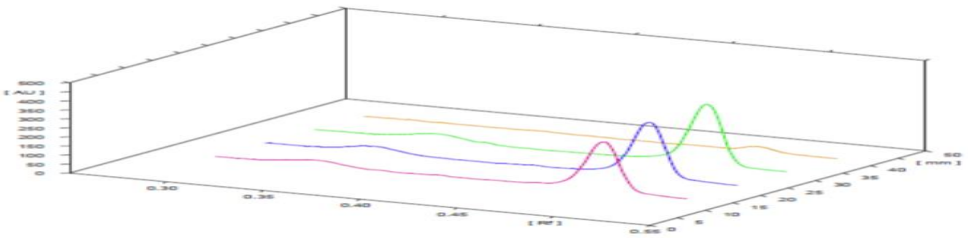


Figure 5: data obtained from the accuracy of standard curcumin in serum

µg	Area	Rf
40 µg	4684.8	0.48
40 µg	5302.2	0.48
40 µg	5397.8	0.48
40 µg	5568.4	0.49

Table 5: data obtained from the accuracy of standard curcumin in serum

Conc µg	Normal Conc µg	Conc Found µg	% accuracy
40 (0) µg	40 µg	35.19 µg	87.97 %
40 (1) µg	40 µg	39.45µg	98.62 %
40 (2)µg	40 µg	41.87 µg	104.67 %
40 (3)µg	40 µg	29.29 µg	73.22 %

Table 6: Data of accuracy of standard Curcumin

F. Recovery: Recovery = [(A – B)/C] × 100
Where A is a quantity of Curcumin standard, B is a quantity of SCFB; C is a quantity of added

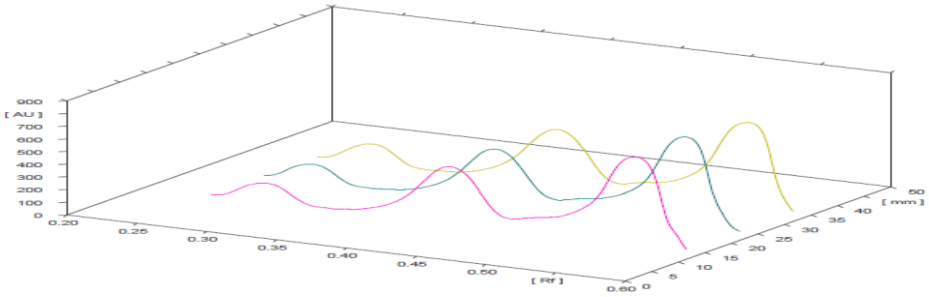


Figure 6: graph Recovery of standard Curcumin

Concentration(µg)	Area of peak	Rf value
40 + 100 µg	28529.0	0.55
60 + 100 µg	29778.6	0.55
80 + 100 µg	30291.7	0.55

Table 7: data obtained from recovery:

Normal Concentration µg	Concentration Found µg	Recovered Standard µg	% Recovery	RSD
100 µg	108 µg	--	--	0.12
40 + 100 µg	137 µg	33.58 µg	91.66 %	0.22
60 + 100 µg	147 µg	47.77 µg	85.84 %	0.11
80 + 100 µg	166 µg	65.38 µg	84.78 %	0.10

Table 8: recovery of standard Curcumin sample

G. Robustness of the technique: Each parameter's standard deviation of peak areas was computed, and RSD was found to be within acceptable bounds. The proposed HPTLC technique's robustness was demonstrated by the near to the ground standards of S.D. (3.0) and % R.S.D. (1.2) that were obtain by making tiny intentional adjustments to the approach.

Concentration	Area	Rf	Height	% Area
60 µg	10983.1	0.46	318.9	69.03
80 µg	16466.5	0.46	444.6	69.18
100 µg	14200.2	0.46	384.2	68.03

Table 09: Data from the mobile phase (5: 4: 1) v/v/v toluene, chloroform, and methanol

Concentration	Area	Rf	Height	% Area
60 µg	10785.6	0.52	386.2	65.78
80 µg	11711.2	0.52	417.2	63.49
100 µg	13933.7	0.53	445.4	62.32

Table 10: Data for the mobile phase for "toluene, chloroform, methanol, and acetic acid (5: 4: 0.5: 0.5 v/v/v/v)"

Concentration	Mobile phase "T:C:M: A (5:4:0.5:0.5 v/v/v/v) % RSD"	Mobile Phase "T: C: M (5: 4: 1 v/v/v) % RSD"
60 µg	0.24	0.30
80 µg	0.42	0.30
100 µg	0.22	0.25

Table 11: data of robustness of validation method:

The results (desk eleven) of Robustness for the analytical approach of dedication of curcumin from organic pattern indicated that the % RSD is less than the most allowed RSD this is 2%. Therefore, it may be concluded that the technique qualifies for the take a look at for Robustness. The method become then utilized for the estimation of curcumin from serum samples in the bioavailability studies.

Bioavailability of SCFB System

Bioavailability studies on Sodium alginates Curcumin floating beads had been finished in albino rats by way of oral administration of the formulation (equivalent to curcumin 60 mg/kg frame weight in line with oral.) and simple Curcumin, beads in the equivalent dose (60 mg/kg B.W.P.O.). The method containing Gelucire 44/14, sodium bicarbonate, and calcium carbonate become selected for the observed because the formula indicated an excellent release profile in in-vitro dissolution testing. The blood samples had been withdrawn at30-minute periods and a serum sample changed into obtained after proper clotting of the blood. The serum samples had been analyzed for Curcumin content the use of the tested HPTLC technique. The effects are provided in desk and, Fig.

A). HPTLC Bioavailability Results in the 30 Minutes- Different Animals

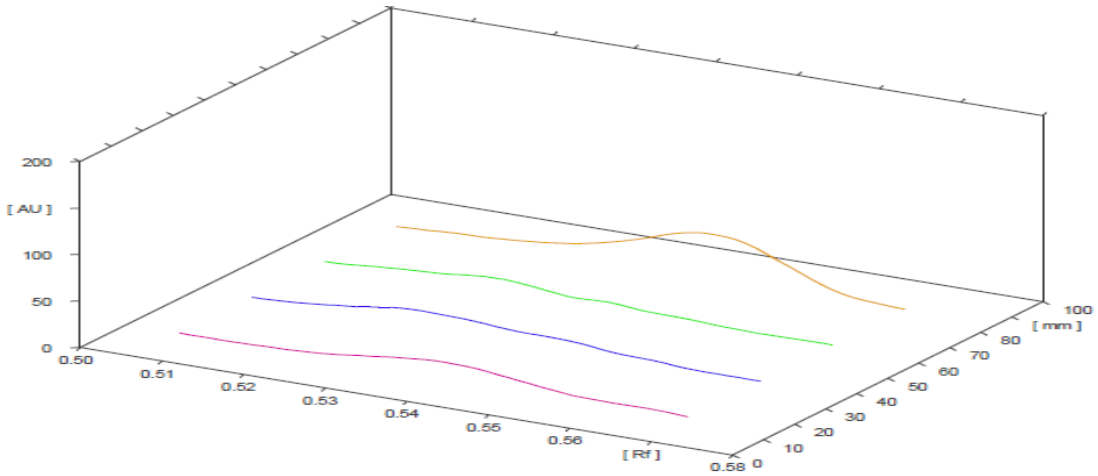


Figure 7: Serum Sample after the 30 minutes

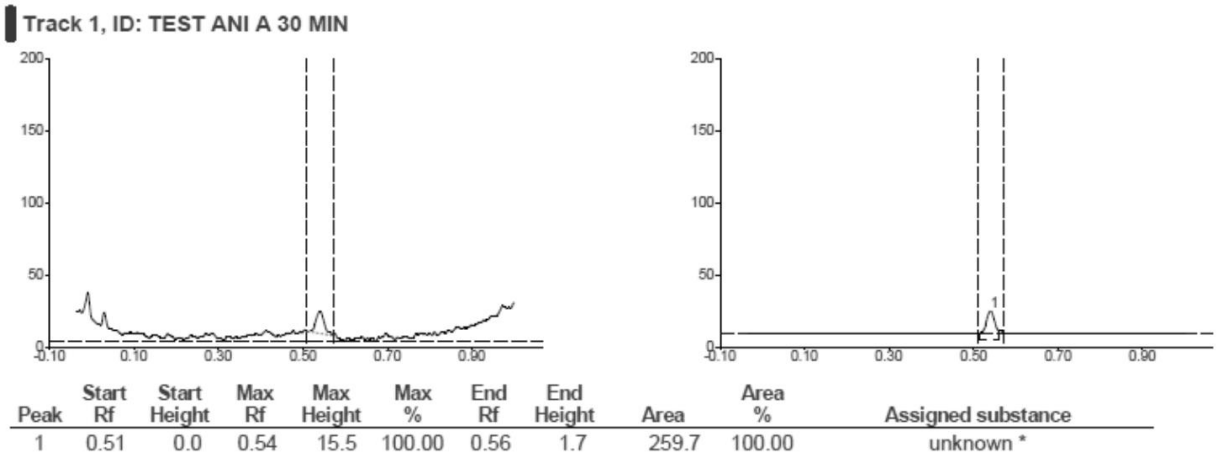
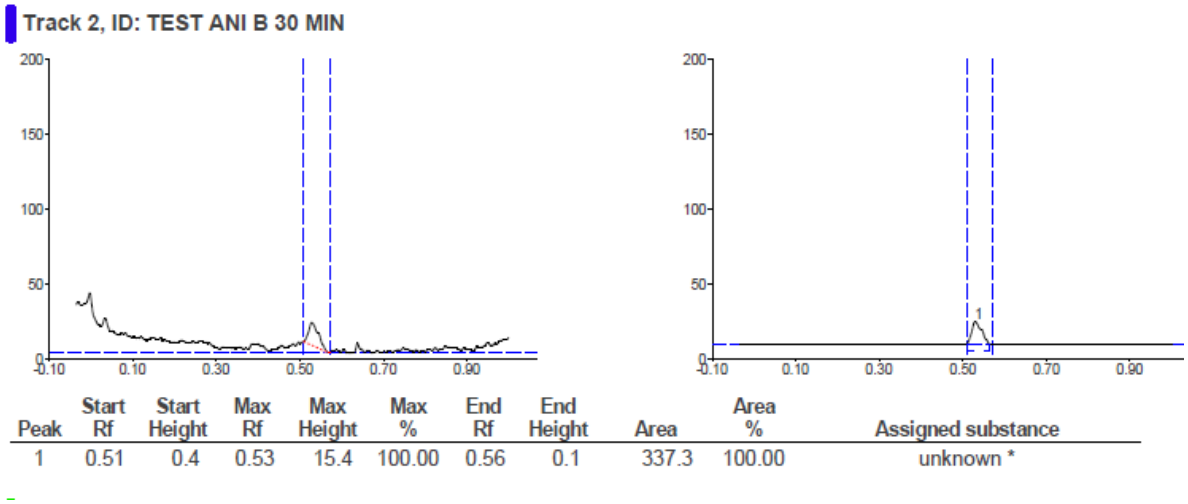


Figure 8: First Animal Serum Sample After 30 minutes



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.51	0.4	0.53	15.4	100.00	0.56	0.1	337.3	100.00	unknown *

Figure 9: Second Animal Serum Sample after 30 minutes.

B). HPTLC Bioavailability Results in Data Obtained in the 1hr.- Different Animals.

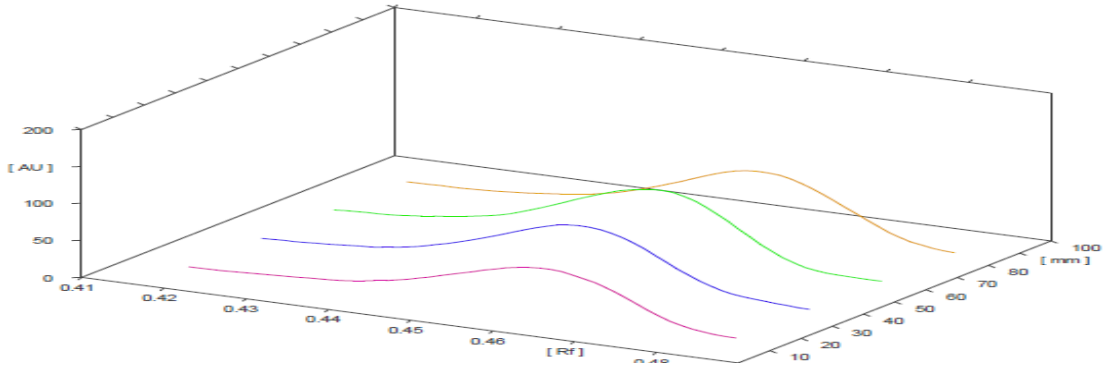
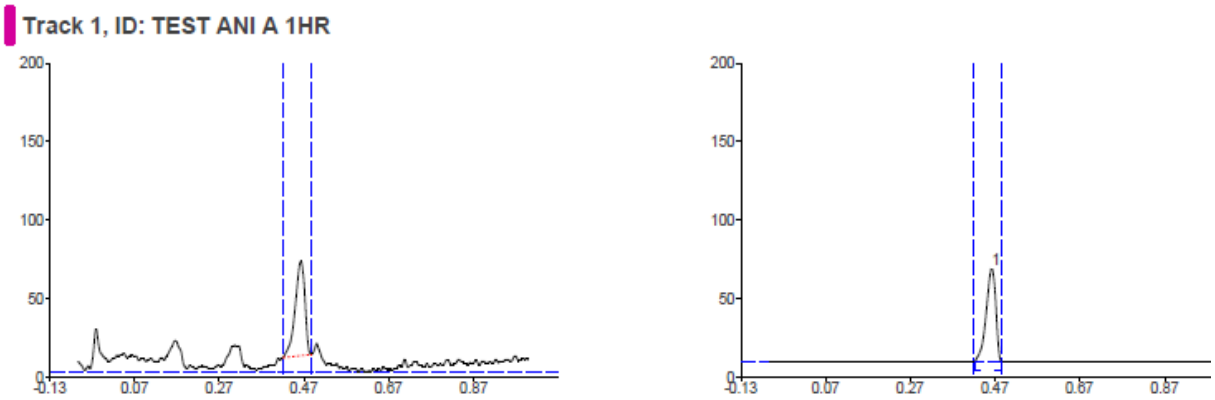
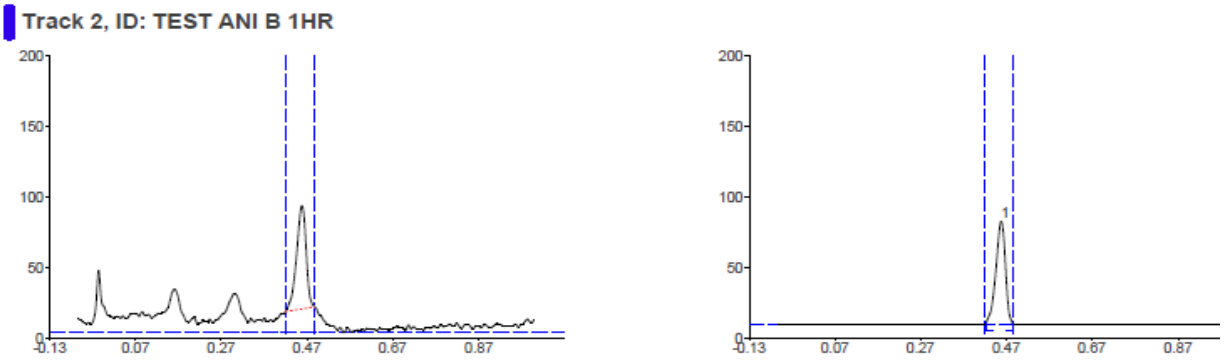


Figure 10: Serum Sample after 1hr.



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.42	0.1	0.46	59.9	100.00	0.48	0.4	1175.0	100.00	unknown *

Figure 11: First Animal Serum Sample after 1hr.



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.42	1.3	0.46	73.3	100.00	0.48	0.4	1410.2	100.00	unknown *

Figure 12: Second animal Serum Sample after 1hr.

C). HPTLC Bioavailability result in data obtained in the 2hr.- different animals.

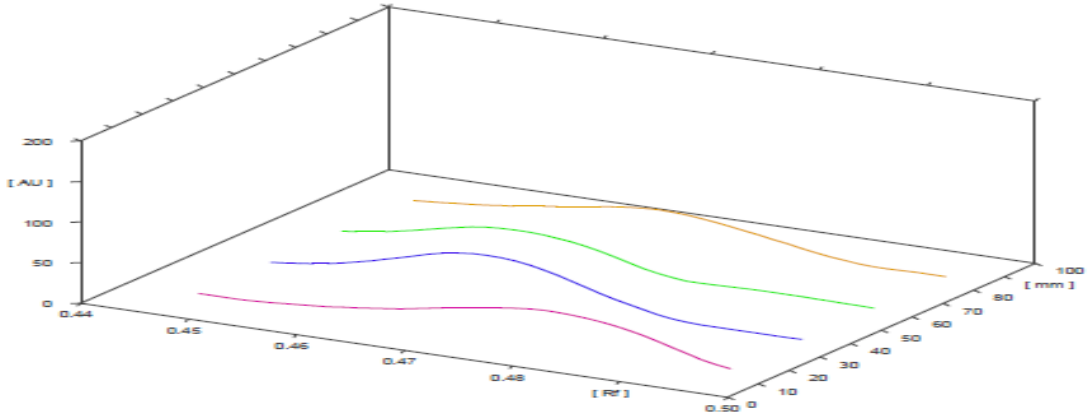
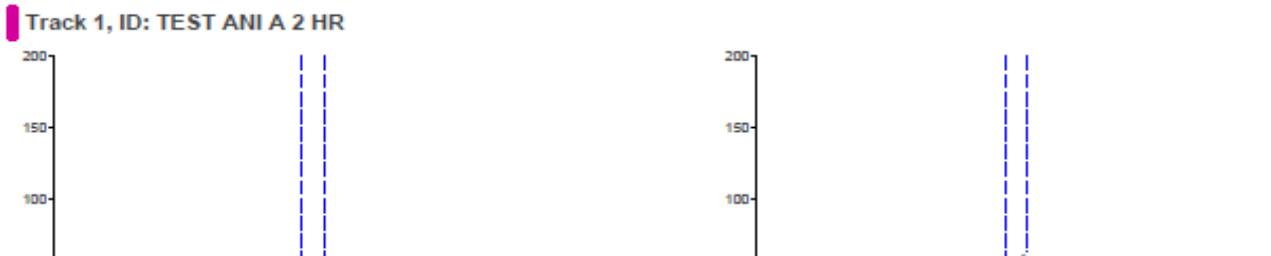
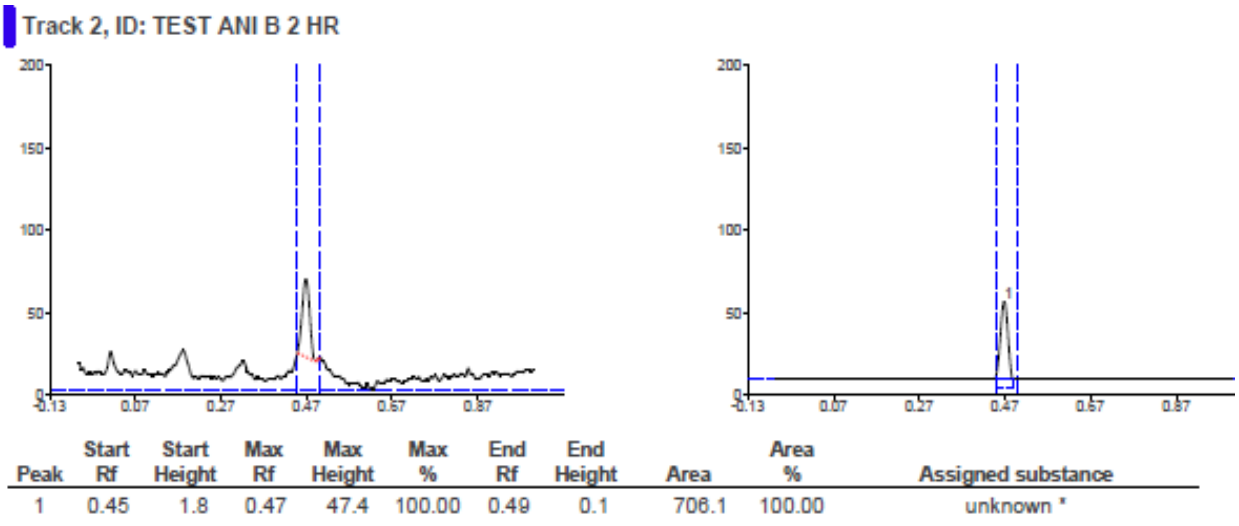


Figure 13: Serum Sample after 2hr.



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.45	0.1	0.48	41.2	100.00	0.50	2.3	727.0	100.00	unknown *

Figure 14: First Animal Serum Sample after 2hr.



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.45	1.8	0.47	47.4	100.00	0.49	0.1	706.1	100.00	unknown *

Figure 15: Second Animal Serum Sample after 2hr.

Bioavailability of standard Curcumin [12-19]: Dosing the 80mg of standard Curcumin through orally on rat's estimation of Curcumin from serum after the dosing of Curcumin in different time intervals (30min, 1hr. and 2hr.)

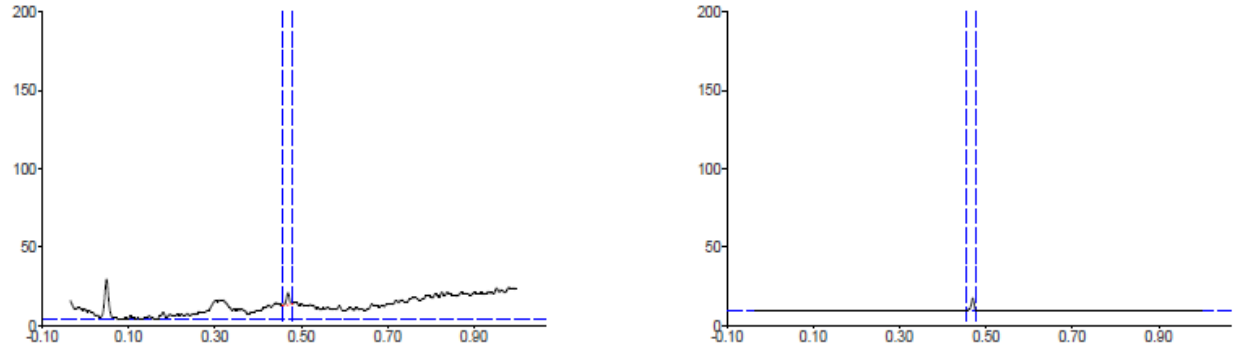
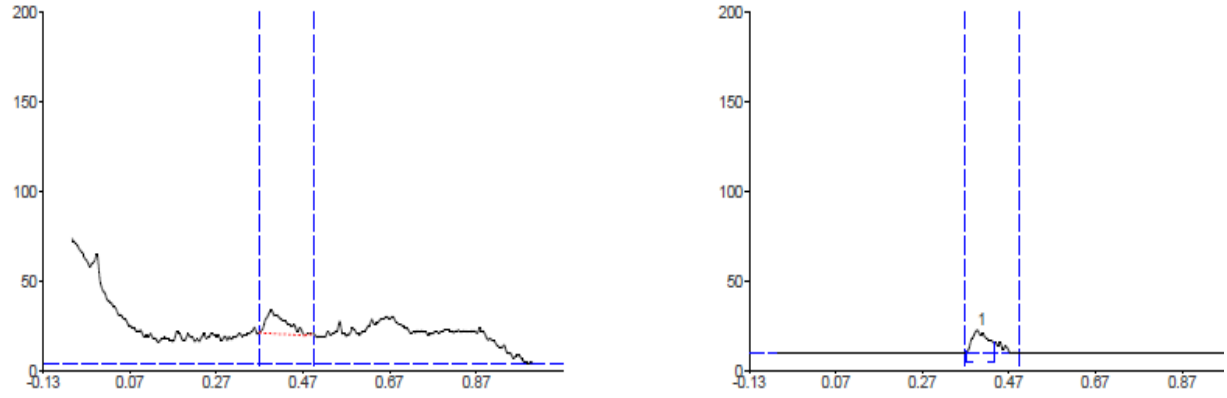


Figure 16: Data obtained from the First animal serum sample after 30 minutes.



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.37	1.1	0.39	12.9	100.00	0.44	5.8	409.8	100.00	unknown *

Figure 17: Data Obtained from the First Animal Serum Sample after 1hr.

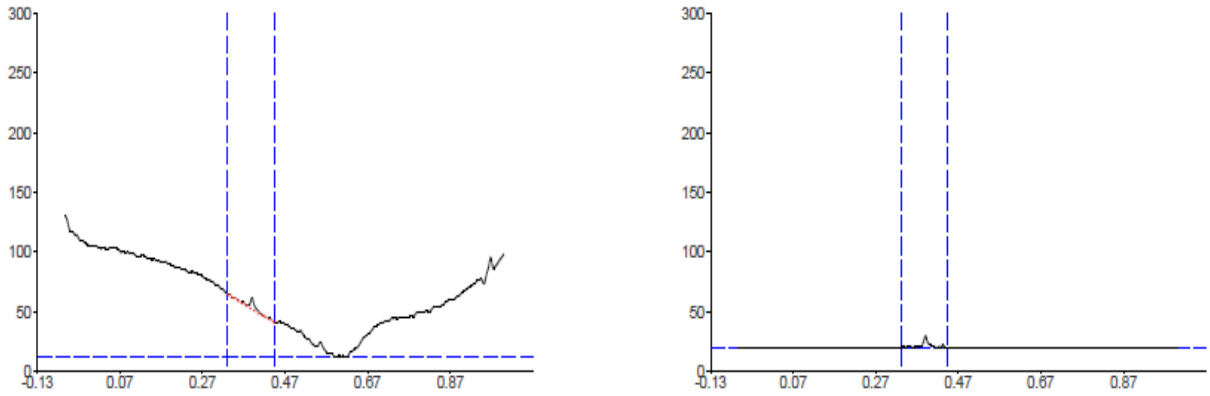
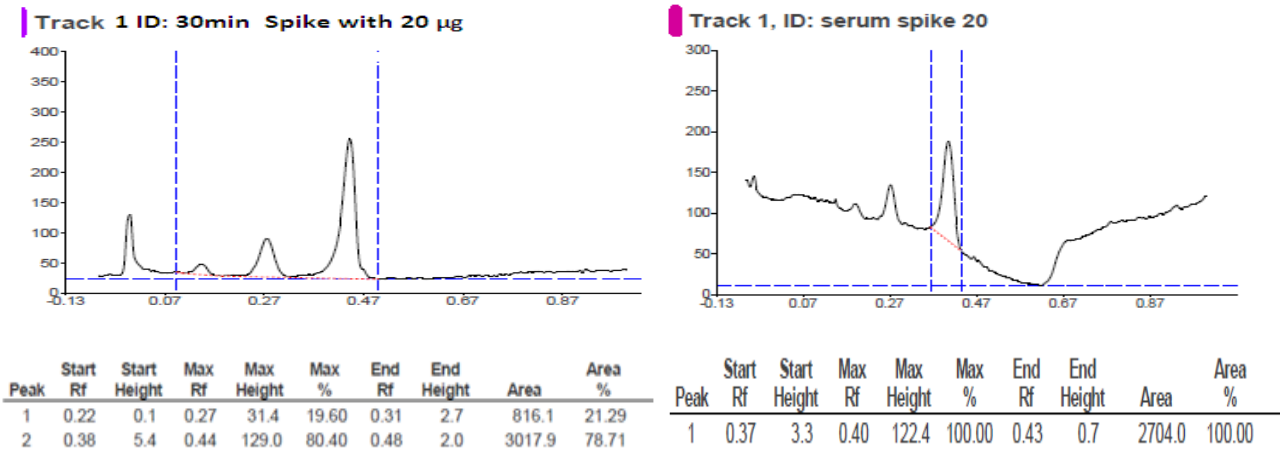


Figure 18: Data Obtained from the First Animal Serum Sample After 2hr.

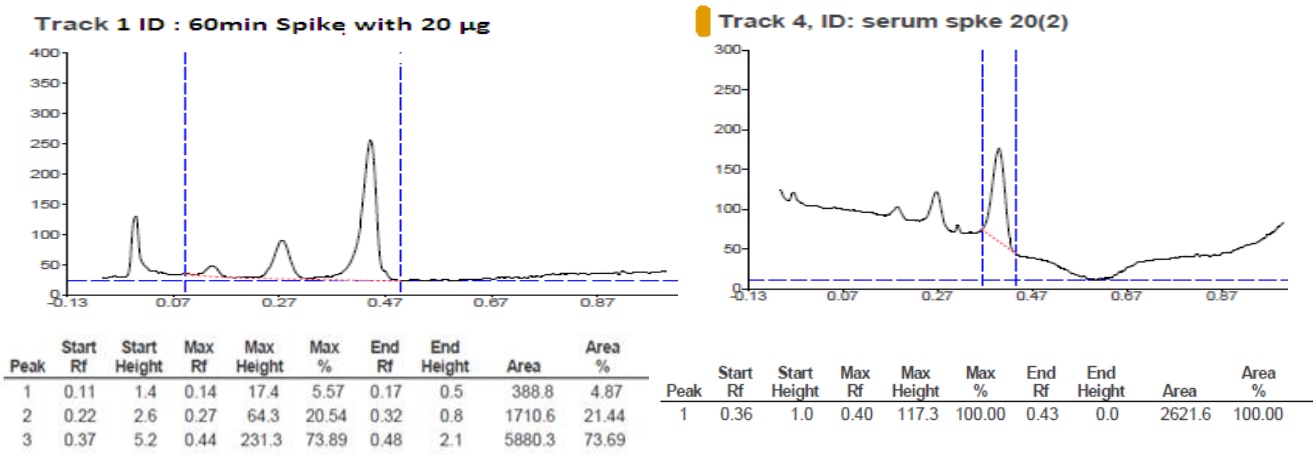
Spiking Method for Bioavailability Studies



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.22	0.1	0.27	31.4	19.60	0.31	2.7	816.1	21.29
2	0.38	5.4	0.44	129.0	80.40	0.48	2.0	3017.9	78.71

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.37	3.3	0.40	122.4	100.00	0.43	0.7	2704.0	100.00

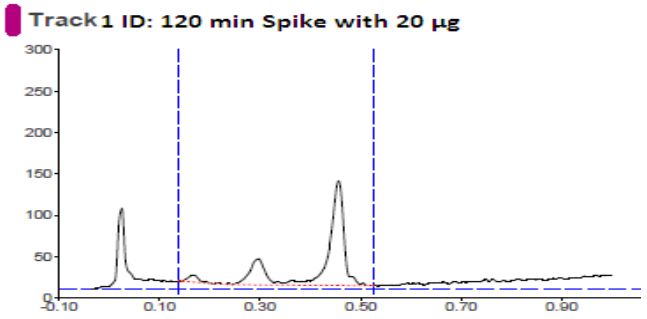
Figure 19: Data Obtained from the Spiking Method for the Study of Bioavailability Studies after 30 min.



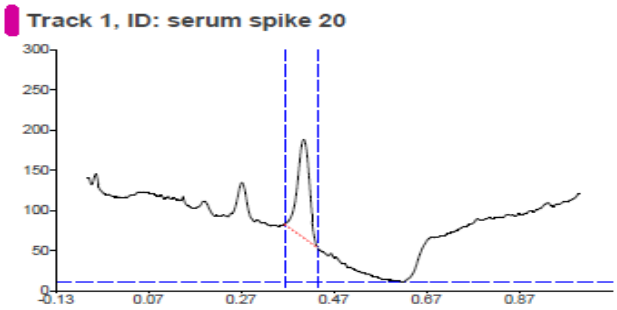
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.11	1.4	0.14	17.4	5.57	0.17	0.5	388.8	4.87
2	0.22	2.6	0.27	64.3	20.54	0.32	0.8	1710.6	21.44
3	0.37	5.2	0.44	231.3	73.89	0.48	2.1	5880.3	73.69

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.36	1.0	0.40	117.3	100.00	0.43	0.0	2621.6	100.00

Figure 20: Data Obtained from the Spiking Method for the Study of Bioavailability Studies after 60 min.



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.25	1.1	0.29	31.7	19.98	0.33	2.7	847.6	22.08	unknown *
2	0.40	5.9	0.45	126.8	80.02	0.50	2.1	2990.6	77.92	unknown *



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.37	3.3	0.40	122.4	100.00	0.43	0.7	2704.0	100.00

Figure 21: Data Obtained from the Spiking Method for the Study of Bioavailability Studies after 120 min.

Animal	"Amount of curcumin in serum sample (µg) Mean ± SEM"		
	30 minute	60 minute	120 minute
Group1	12.62 µg ± 0.35	30.43 µg ± 0.32	10.30µg ± 0.34
Group 2	0.000 µg ± 0.000	0.020 µg ± 0.000	0.000 µg ± 0.000

Table 12: Effect of Curcumin formulation on bioavailability after dosing in different time intervals

Group3	Spike of serum sample with 20 µg of standard curcumin	Amount of curcumin in serum sample (µg) mean ± SEM		
		30 min	60 min	120 min
Animal A	20µg	30.94 µg ± 0.23	48.34 µg ± 0.41	33.76µg ± 0.51

Mean ± SEM N = 3

"Validation of the planned technique the recommended approaches were approved in peace with the ICH's (International Conference on Harmonization) 2005 guidelines."

Table 13: Effect of STD. Curcumin on Bioavailability after Dosing in Different time Intervals.

2. Results and Discussion

"Curcumin is one of the main sources of energy that comes from the underground stems or sprouts of Curcuma longa". It has been discovered that the roots are medicinal. "(1E, 6E)-1, 7-bis (4-hydroxy- 3-methoxyphenyl) -1, 6- heptadiene-3, 5-Dione" is the compound name for turmeric active ingredient. and it has a low bioavailability. The goal of the current work was to create a complex between Gelucire 44/14 and curcumin, which is a great way to make curcumin more water-soluble and, consequently, boost its bioavailability. The complex was then transformed into sodium alginate floating beads for oral administration of curcumin. Through oral administration to rats and the execution of the creation and validation of the Bioanalytical technique, the bioavailability of the beads was evaluated. The approach was then specifically designed to investigate pharmaceuticals in their industrial dose form (SCFB) without interference from substances.

Utilizing a linear ascending approach, over precoated TLC plates, chromatographic separation is carried out. ("60 F254, 20 cm 10 cm, 250 m thickness, Merck, Darmstadt, Germany"). The technique is created with a combination of "toluene, chloroform, methanol, and acetic acid (5:4:0.5:0.5)". Utilizing UV-Spectrophotometer analysis, finding and quantification were achieved at 426 nano meter. In accordance with the ICH guidelines for "linearity, accuracy, precision, detection, quantitation limits, robustness, and specificity", the analytical general efficacy of the proposed HPTLC technique was linked. The calibration curves for curcumin have been linear with limits of 200–1000 ng/spot and 'correlation coefficients (r2) >0.9998'. The finding levels for curcumin are 30 ng/spot. The commercial polyhedral components containing curcumin were effectively identified using the tried-and-true HPTLC approach.

3. Conclusion

The scientifically advanced and verified bioanalytical method using HPTLC was sufficient to quantify the samples from the SCFB formulation since it was selective, sensitive, accurate, and correct. According to what we know, this is the main HPTLC method used to treat plasma samples following SCFB management; as a result, it is a great alternative to UV-Vis and MS/MS in bioavailability study.

Conflict of Interest Statement: The authors claim that the work covered in this book is not at odds with any known financial or personal interests."

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Adherence to Moral Principles

Prior to the in-vivo investigation, we made a moral statement by approving protocol “SVBCP/IAEC/PG/13-14/53” for the use of animals. The task was completed under the guidance of research mentor Dr. Shailesh B.

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