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Formulation and Analytical Development of Laxative Polyherbal Suspension

Research Article

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Abstract

The aim of study is to formulate and evaluate polyherbal suspension containing aqueous extracts of *Cassia Fistula L, Terminalia Bellerica Gaertn, Terminalia Chebula Retzm, Operculina Turpethum L, and Baliospermum Montanum Muell* for laxative use and develop a validated analytical method by HPLC. The excipients employed in the formulation were Sucrose, Xanthan gum, Tween 80, Sodium methylparaben, Sodium propylparaben and water. The suspension was evaluated for organoleptic properties, Sedimentation volume, Redispersibility, Flow rate, Viscosity, and pH. Optimized formulation was further evaluated to check its stability for 90 days in accelerated conditions. The analytical method was developed for the assay of Rhein in formulated polyherbal suspension and validated by HPLC (Waters instrument with Empower software). The results revealed that an increased concentration of suspending agent increased viscosity, thus reducing the sedimentation and improving the stability of suspension. The validation parameters results indicated that the developed method is simple, reproducible, accurate, robust, precise, and specific. Polyherbal suspension was successfully formulated using varying concentrations of suspending agent and wetting agent by employing a 3^2 factorial design. The validation results obtained for the assay of rhein in polyherbal formulation showed that the developed HPLC method was precise and accurate.

Keywords: Polyherbal suspension, HPLC, Rhein, Method development, Validation, Laxative.

Introduction

Suspension is coarse dispersion of finely divided solid particles of a drug dispersed in a liquid medium, in which the drug is not readily soluble. An aqueous suspension is used to administer insoluble or poorly soluble drugs (1). Suspension is thermodynamically unstable; therefore, it is required to add suspending agent which reduces the rate of settling and allows easy redispersion of settled particles both by protective colloidal action and by increasing the consistency of the suspending medium (2).

Constipation has a very large impact in 20% humans (3), and also largely affects quality of living leading to depletion of mental health and depression. (4, 5). Constipation occurs more in Western countries as about 2% to 27% people suffer to it. Among them ladies, non-whites, and old aged people above 65 years have greater tendency to suffer from constipation (6). Laxatives are the medicines which are used to treat/ cure constipation by increasing the ease and rate of defecation by adding bulk to intestinal contents by retaining the water in bowel or by increasing motility or by stimulating intestinal secretion. Different types of laxative agents are osmotic agents, bulking agents, stimulants and lubricating agents. The major advantage of the plants as laxative over synthetic agents is that they are cheap, available easily and have minimum side effects (7). Many polyherbal formulations were prepared in recent times intended to be administered for laxative purpose as combining multiple herbs gives better systemic effect rather than single herb (8). The oral route for administration of drugs is very convenient method to achieve desirable effects (9).

A polyherbal suspension composed of aqueous extracts of *Cassia fistula* pod, *Terminalia bellerica* fruit, *Terminalia chebula* fruit, *Baliospermum montanum* root and *Operculina turpethum* root was formulated. The laxative activity of *Cassia fistula* pod is due to the presence of anthraquinone aglycones and anthraquinone glycosides in which Rhein is the major ingredient (9). *Terminalia bellerica* fruit is laxative whose pulp is used to treat dropsy, piles and leprosy (10). The *Terminalia chebula* fruit which has laxative activity is also used as homeostatic, antitussive, diuretic and cardiotonic (11). The *Baliospermum montanum* roots are used as laxative to treat dropsy, jaundice, anasarca, rheumatism, anemia, as well as skin diseases, piles, leukoderma and helminthic infection (12). *Operculina turpethum* Root is internally used to treat constipation and other disorders like anorexia, edema, anaemia, ascites, hepatosplenomegaly, hepatitis, abdominal tumors,

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ulcers, wounds, worm infestation, pruritis, and other skin infections (13). Thus, the aim was to formulate a laxative suspension from above extracts and develop an analytical method for the same.

Method Development by High Performance Liquid Chromatography (HPLC)

High Performance Liquid Chromatography is an analytical technique used for the separation, identification and quantification of each constituent in the mixture. It is a type of column liquid chromatography. The system comprises of pump, injector, column, detector, integrator and display system (14). Rhein (1, 8-dihydroxyanthraquinone-3-carboxylic acid) is a major anthraquinone present in the pod of *Cassia fistula* which is responsible for its laxative activity (9,15). The focus was to develop a HPLC method to perform assay of Rhein in polyherbal formulation.

Method Validation

The analytical method validation is useful for new method so that it is capable of giving reproducible results in same or different laboratories. Method validation can be employed to detect the reliability and quality of analytical results. Use of instrument which complies to the specification, operates correctly and is timely calibrated is necessary for the method validation process (16-18).

Materials and Methods

Cassia Fistula (pod) dry extract was obtained as gift sample from Pukhraj Herbals, Madhya Pradesh and Terminalia Bellerica (fruit) dry extract, Terminalia Chebula (fruit) dry extract, Operculina Turpethum (Root) dry extract, Baliospermum Montanum (Root) dry extract were gifted by Kisalaya Herbals, Indore, Madhya Pradesh.

Rhein reference standard was procured from Yucca enterprises pvt. Ltd., Mumbai.

All other excipients and solvents obtained were of analytical grade.

Determination of λ max of Rhein

1 mg of the accurately weighed Rhein was transferred into a 10 ml of volumetric flask. The standard was then dissolved in 5ml acetonitrile and volume was made up to 10ml with same to get a stock solution.1 ml of above stock solution was pipetted in 10 ml volumetric flask and diluted further with acetonitrile to give concentration of 10µg/mL which was used as standard stock solution. The standard stock solution was then scanned between 800-200nm using acetonitrile as blank solution to determine the maximum absorbance.

Standard calibration curve of Rhein

1mg of rhein was taken in 10mL of volumetric flask and was dissolved in 5 mL of acetonitrile and made upto 10ml with acetonitrile to give primary stock solution to produce concentration 100µg/ml. Dilutions were prepared out of primary stock solution in the concentration range 2 to 10µg/mL by pipetting 0.2, 0.4, 0.6, 0.8, 1ml and diluting each of them to 10ml with diluent. The absorbance was measured for each by UV spectrophotometer at λmax 430nm. The graph was plotted for absorbance vs concentration.

Phytochemical screening

Various tests were performed to determine the presence of phytoconstituents in herbal extracts (19). The tests performed have been summarised in Table No.1.

Table 1: Phytochemical tests

Class	Tests performed
Alkaloids	Mayers test, Wagners test, Hagers test, Dragendorffs test
Carbohydrates	Molisch test, Fehling's test, Benedicts test, Biuret test
Anthraquinone glycosides	Borntrager test
Proteins	Xanthoproteics test, Millons test
Steroids	Lieberman Burchard sterol reaction, Salkowski test
Flavonoids	Shinoda test, Sodium hydroxide test, Lead acetate solution test
Saponins	Froth test
Tannins	10 % $FeCl_3$ test, Dil.HNO_3 test, Acetic acid test
Triterpenes	Salkowski test, Liebermann storch Morawski test

Suspension formulation

Table 2: Formulation table

	Quantity %								
Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Cassia Fistula	2	2	2	2	2	2	2	2	2
Terminalia Bellerica	2	2	2	2	2	2	2	2	2
Terminalia Chebula	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Baliospermum Montanum	3	3	3	3	3	3	3	3	3
Operculina Turpethum	3	3	3	3	3	3	3	3	3
Xanthan Gum	0.15	0.3	0.45	0.15	0.3	0.45	0.15	0.3	0.45
Tween 80	0.25	0.25	0.25	0.5	0.5	0.5	0.75	0.75	0.75
Sucrose	50	50	50	50	50	50	50	50	50
Sodium Methyl Paraben	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Sodium Propyl Paraben	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Flavouring agent	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Purified Water (q.s)	100	100	100	100	100	100	100	100	100

The formulation table (Table 2) was prepared by employing 3^2 factorial design using Xanthan gum (suspending agent) and Tween 80 (wetting agent) as variables.

- Syrup base was prepared by dissolving 50g sucrose (sweetening agent) in 70 ml of water in a 200 ml stainless steel container and heating at 80˚c on a hot plate.

- To the syrup base, Xanthan gum was added in portions with continuous stirring using a mechanical stirrer.
- To the suspending medium, sodium methylparaben and sodium propylparaben (Preservatives) were added (20).
- A weighed amount of dried extracts were added to the suspending medium along with the addition of Tween 80.
- At last sufficient volume of the flavouring agent was added to the suspension with proper mixing and volume was made up to 100ml with purified water (21).

Evaluation of Suspension

Organoleptic properties: Colour, odour, taste and appearance of suspension was evaluated.

pH: The pH of all formulated suspensions was checked using digital pH meter (22).

Determination of Sedimentation Volume: Each suspension was stored in a 100 ml measuring cylinder at room temperature for 1 week in an undisturbed state. The volume of the sediment formed was noted after 24 hrs, 72 hrs and 1 week.

The sedimentation volume was calculated using the formula as follow:

Sedimentation volume (Hs) = Hu/Ho,

Where Hs= sedimentation volume, Hu= Final height of sediment, and Ho= Original height of the suspension before settling (23).

Redispersiblity: Suspension was stored in a stoppered cylinder and moved upside down to redistribute until there was no deposit present at the bottom of the cylinder. Redispersibility was calculated as the number of tilts required for resuspension to take place.

Viscosity: The viscosity of suspensions was measured at 25° C using Brookfield viscometer equipped with spindle RV no.4. Suspensions were taken in a 50ml glass beaker and the spindle was lowered perpendicularly. The spindle was rotated at 100rpm to measure the viscosity in cps (22).

Rheology: Time required for 10 mL suspension to flow through the 10 mL pipette was determined (23) and the flow rate was calculated using the below formula:

$$F = Volume\ of\ pipette\ (ml)/Flow\ time\ (sec)$$

Stability: The stability study was performed at accelerated conditions (Temperature 40 ± 2 ℃ and RH 75 ± 5 %). The study comprised of testing organoleptic properties, viscosity, redispersibility, pH and sedimentation volume which were evaluated at initial, 7, 15 and 30 days (24).

HPLC Method Development and Validation

Chromatographic Conditions: The lambda max of standard solution was detected at 261nm by using PDA detector on Waters Alliance system with injection sample volume 20µl. The column used was YMC Triart C18 of 250mm length, 4.6mm internal diameter and 5µ particle size with mobile phase consisting 2% Glacial acetic acid: Methanol: Acetonitrile in the proportion 30:30:40 which was mixed well and sonicated to degas for about 5 minutes. The flow rate was set to 1.0 ml/min with a run time of 15 minutes.

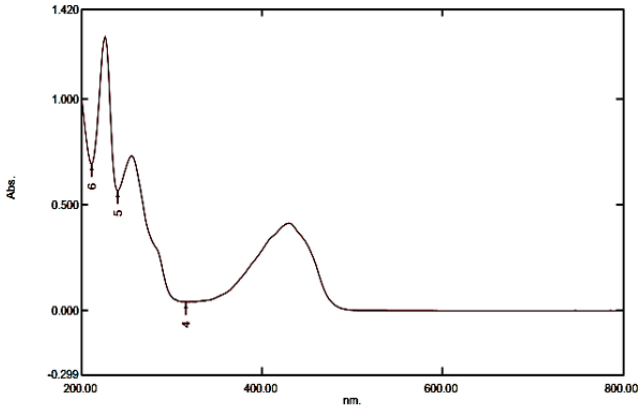
Validation parameters: The developed HPLC method was validated for various parameters including Linearity, Accuracy /Recovery, Precision, Specificity, Robustness, System suitability, Solution stability and Forced degradation studies

Results

Absorbance maxima (λmax)

The λmax of Rhein when scanned in the range 800-200nm was found to be 261 nm (Figure 1) for concentration 10 µg/ml using acetonitrile as diluent.

Figure 1: UV Spectrum of Rhein



Standard calibration curve

The absorbance maximum of Rhein standard solution lying in concentration range 2-10µg/ml (Table 3) was observed at 261nm and accordingly standard calibration curve was plotted. The plotted curve showed linearity with correlation coefficient 0.999 (Figure 2).

The linearity equation was found to be y = 0.0039x – 0.0015.

Figure 2: Calibration curve of Rhein

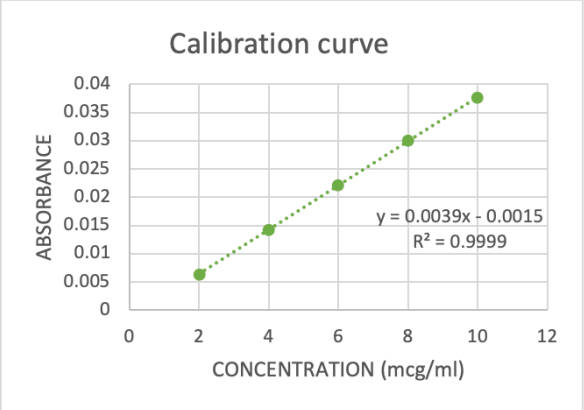


Table 3: Linearity

Concentration [mcg/ml]	Absorbance
2	0.0063
4	0.0143
6	0.022
8	0.03
10	0.0376

Phytochemical screening

Results of phytochemical tests (Table No.4) showed the presence of Alkaloids, Carbohydrates, Proteins, Flavonoids, Tannins, Steroids, Saponins, Triterpenes and Anthraquinone Glycosides in the herbal extracts.

Table 4: Phytochemical test results (25)

	Cassia Fistula	*Terminalia Bellerica*	*Terminalia Chebula*	*Operculina Turpethum*	*Baliospermum Montanum*
Alkaloids	+	+	+	+	+
Carbohydrates	+	+	+	+	-
Proteins	+	+	+	-	-
Flavonoids	+	+	+	+	+
Tannins	+	+	+	+	+
Steroids	+	+	-	-	-
Saponins	+	-	-	-	-
Triterpenes	+	+	+	+	+
Anthraquinone glycosides	-	+	+	-	-

Evaluation of suspension

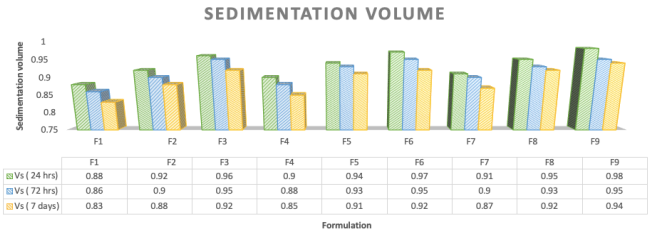
Organoleptic properties of suspension

Organoleptic properties showed that all suspensions were brown coloured with characteristic odour, smooth appearance and palatable taste.

Sedimentation volume: The sedimentation volume of formulations containing 0.45% Xanthan gum was found to be highest at the end of every phase i.e., 24 hrs, 72 hrs and 1 week compared to 0.3% and 0.15% Xanthan gum. A slight decrease in sedimentation volume in each formulation was observed after 72 hrs and 7 days. Increase in the concentration of wetting agent also showed marginal increase in sedimentation volume of each formulation. Formulations F5 [0.3% Xanthan gum and 0.5 % Tween 80] and F8 [0.3% Xanthan gum, 0.75 % Tween 80] were found to be most stable throughout 7 days compared to other formulations. The results are shown in Fig.3

Flow rate: The flow rate was found to be inversely proportional to the concentration of suspending agent added. The formulation containing highest concentration of Xanthan gum showed lowest flow rate whereas varying the concentrations of Tween 80 had no significant impact on flow rate. Results are given in Table 5.

Fig 3: Bar graph of sedimentation volume results of all formulations at different time-points



	F1	F2	F3	F4	F5	F6	F7	F8	F9
Vs (24 hrs)	0.88	0.92	0.96	0.9	0.94	0.97	0.91	0.95	0.98
Vs (72 hrs)	0.86	0.9	0.95	0.88	0.93	0.95	0.9	0.93	0.95
Vs (7 days)	0.83	0.88	0.92	0.85	0.91	0.92	0.87	0.92	0.94

Table 5: Results of redispersibility, flow rate, viscosity and pH

Formulation	Re-dispersibility (strokes)	Flow rate (ml/sec)	Viscosity [cps]	pH
F1	1	1.25	181.5	4.51
F2	3	0.26	265.4	4.56
F3	4	0.071	338.2	4.5
F4	1	1	193.4	4.59
F5	2	0.24	274.3	4.72
F6	4	0.064	341	4.81
F7	1	0.67	187.6	4.5
F8 (optimized)	3	0.29	276.5	4.67
F9	5	0.067	332.7	4.867

Viscosity: From the results (Table 5) it was observed that viscosity of suspensions containing 0.3% Xanthan gum [F2, F5 and F8] were viscous enough to cause reduction in sedimentation thereby contributing to its stability. Whereas formulations containing 0.45 % Xanthan gum [F3, F6 and F9] were highly viscous because of intermolecular interaction and greater entanglement density of Xanthan Gum. The suspensions containing 0.15% Xanthan gum [F1, F4 and F7] had low viscosity thus unable to prevent particles from aggregating to keep them in flocculated state. Tween 80 didn't have any observable effect on viscosity. Hence F2, F5 and F8 were satisfactory formulations (27). Results are given in Table 5.

pH: The pH of all the formulations were in the range 4.5 – 4.9. Results are given in Table 5.

Stability testing [F8 formulation] (26)

On exposing to accelerated conditions (temperature 40 ± 2° C and RH 75 ± 5 %) it was found that no significant changes were observed in organoleptic properties, sedimentation volume, viscosity, pH and redispersibility in the formulation. Sedimentation volume was observed to decrease at the end of 3 month. The viscosity of suspension also decreased, the change in both the cases was less suggesting a stable formulation. pH value was almost same therefore indicating no chemical change when kept for long duration. Redispersibility results were also satisfactory (28). Results are given in Table 6.

Table 6: Stability results

Optimised Formulation F8 - 40° C /75% RH					
Evaluation Parameters	**Initial**	**7 days**	**15 days**	**1 month**	**3 month**
Appearance	Smooth	Smooth	Smooth	Smooth	Smooth
Odour	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic
Taste	Palatable	Palatable	Palatable	Palatable	Palatable
Colour	Brown	Brown	Brown	Brown	Brown
pH	4.67	4.57	4.74	4.73	4.86
Viscosity [cps]	276.5	273.1	271.8	267.9	261.3
Redispersibility (strokes)	3	3	3	3	3
Sedimentation volume	0.95	0.92	0.92	0.90	0.87

Analytical Method Development (29,30)

Selection of diluent and mobile phase: Since rhein is insoluble in water and methanol, acetonitrile (40%) was used to dissolve the drug and further volume was made up to 100% with equal volumes of methanol (30%) and 2% Glacial acetic acid (30%) as combination of organic solvents and buffer has been found to elute peaks faster and give sharper peaks. Acetonitrile was used within limits (enough to dissolve drug) as it is an expensive organic solvent and methanol was used instead as it is quite economic comparatively. Optimised chromatographic conditions for developed method are summarised in Table No.7 with standard and sample chromatograms in Fig.4 and 5 respectively.

Figure 4: Standard chromatogram

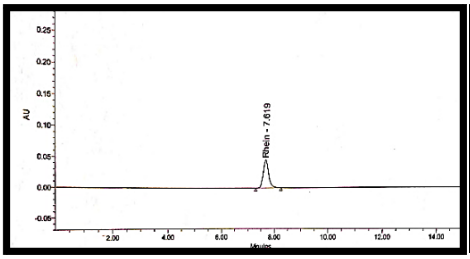


Figure 5: Sample chromatogram

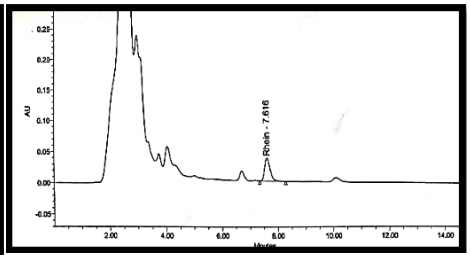


Figure 6: Placebo chromatogram

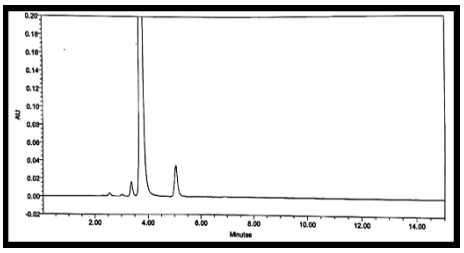


Table 7: Optimised chromatographic conditions

Chromatograph	HPLC (Waters with Empower software)
Column	YMC Triart C18 (250mm x 4.6mm, 5 microns)
Mobile phase	2%GAA: Methanol: ACN (30: 30: 40)
Flow rate	1ml / min
Wavelength	261 nm
Injection volume	20 µl
Column temperature	30 ̊C
Sample temperature	8 ̊C
Run time	15 minutes
Retention time	7.6 minutes

Analytical method validation

Method was validated according to ICH guidelines

System suitability: All the system suitability criteria were met as the USP plate count was greater than 2000, USP tailing was less than 2 and the % RSD for the standard peak of Rhein (5 replicate injections) was less than 2. Results are depicted in Table 8.

Specificity: From the figure (Fig.6) which shows chromatogram of placebo preparation, it can be concluded that there is no interference of the excipients at the retention time (7.67 minutes) of drug, and therefore the peak of Rhein is pure. Hence, the developed method is specific.

Table 8: System suitability of Rhein

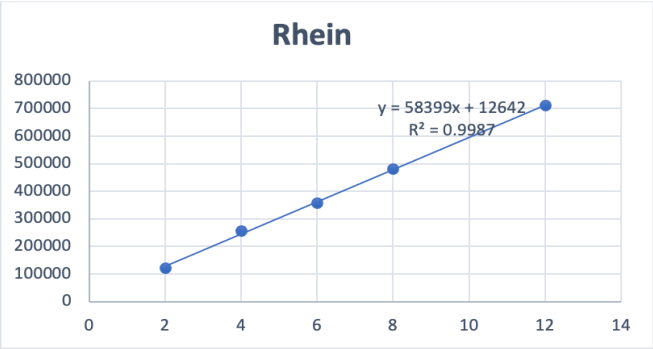
Inj. No.	Peak area of Rhein	Tailing factor	USP Plate count
1	553308	1.31	8765
2	556627	1.29	8856
3	557405	1.29	8810
4	557441	1.28	8705
5	557852	1.28	8822
Mean	556527	-	-
SD	1852.961	-	-
% RSD	0.33	-	-

Linearity: The mean linear regression equation obtained. Was $y=58399x + 12642$, with y as peak area and x as standard solution concentration in mcg/ml. The correlation coefficient (r^2) value was found to be 0.9987 thereby indicating that developed method is linear and will provide correct results by using UV – detector. The results are given in Table 10 and linearity plot in figure 7.

Table 10: Linearity data for Rhein

Sr.No.	Concentration (mcg/ml)	Mean Area	%RSD
1	2	121511	0.35
2	4	258750	0.08
3	6	357563	0.06
4	8	482854	0.06
5	12	711313	0.12

Figure 7: Standard calibration curve



Precision: The % RSD for assay of sample was found to be 0.41 thus concluding the method to be highly precise. From the intermediate precision results, it can be concluded that there were no significant changes in the results after analysing the sample set on different instrument (Shimadzu Prominence) and using different column (Epic C18) as %RSD was 0.34 even after comparison with method precision results. Retention time shifted to 8.95 minutes (from 7.6) which was acceptable as these variations are liable to occur due to change in column and instrument. Results are depicted in Table 11.

Table 11: Precision result

	%RSD
System precison	0.33
Method precison	0.41
Intermediate precison	0.34

Accuracy: Accuracy results (Table 12) were between 98% to 102% which was satisfactory for developed method.

Table 12: Accuracy data

%Spike level	Pre analysed Standard conc (mcg/ml)	Amount Added (mcg/ml)	Mean % Recovery
50%	8	4	99.67
100%	8	8	100.68
150%	8	12	100.59
		Mean	100.34
		% RSD	0.47

Analytical solution stability: Standard solution and sample solution both were stable for 48 hours as calculated %RSD was below 2, whereas at 72 hrs standard solution was found to be unstable and sample solution was found to be stable. Results are given in Table 13.

Table 13: Analytical solution stability data

Time interval(hrs)	%RSD of standard	%RSD of sample
4	0.47	0.69
8	0.68	0.68
12	1.07	0.69
24	1.68	1
48	1.88	1.46
72	3.56	1.9

Robustness: Robustness results (Table No.14) for all the parameters in every condition were good as RSD values were below 2%.

Table 14: Robustness results

Parameter	Condition	Retention time of standard	Retention time of sample	% Assay	% RSD
	Optimised	7.627	7.616	96.08	
Flow rate	0.9 ml/min	8.190	8.174	93.48	1.31
	1.1 ml/min	6.673	6.504	97.71	0.87
Wave length	259 nm	7.120	7.204	98.30	1.12
	263 nm	7.354	7.553	95.49	0.9
Mobile phase	-5% Methanol	8.022	7.988	93.74	1.34
Composition	+5% Methanol	7.188	7.153	99.62	1.73

Forced degradation studies

From the below results (Table 15) it can be seen that, oxidative stress had maximum impact on sample degradation as 18.36% of it degraded. Whereas exposure to water hydrolysis, acidic stress and alkali stress didn't have effect on the sample. % Degradation in all conditions was observed to be within limit of 20%.

Table 15: Forced degradation studies data

Stress condition	Sample taken (mg)	Area of peak	% Assay	% Degradation
Untreated	1000.2	491979	101.75	-
Water hydrolysis	1000.3	490078	101.34	0.41
Acidic stress	1000.3	467788	96.73	5.02
Alkali stress	1000.2	486032	100.52	1.23
Oxidative stress	999.8	403075	83.39	18.36

Discussion

Herbal suspension was prepared and stability parameters were evaluated. Xanthan gum (suspending agent) and Tween 80 (wetting agent) was used for preparation of herbal suspension. The suspensions containing 0.15% Xanthan gum had low viscosity thus unable to prevent particles from aggregating to keep them in flocculated state. Tween 80 didn't have any observable effect on viscosity. The prepared suspension formulation was found to have redisperbility with sedimentation studies shows F8 formulation was acceptable. Forced degradation study shows that acidic and alkali stress didn't have more effect on sample.

Conclusion

Polyherbal suspension was successfully formulated using varying concentration of suspending agent and wetting agent by employing 3^2 factorial design. Further it was evaluated for various parameters

including pH, viscosity, sedimentation volume, flow rate, redispersibility and organoleptic properties. Among the nine formulations, F8 gave best results and was selected as optimum formulation from the overlay plot for stability studies. The results of stability studies revealed that the formulation was stable throughout the 3-month period when kept at 40° C and 75% RH as no major changes were seen in all evaluation parameters. The validation results obtained for assay of rhein in polyherbal formulation showed that developed HPLC method was precise, accurate, linear, robust, specific and is applicable to study stress testing of Rhein in different conditions.

Abbreviations

HPLC: High Performance Liquid Chromatography, **λ max:** Absorbance maximum, **mg**: Milligram, **ml**: Millilitre, **µg/mL**: Microgram /Millilitre, **nm**: Nanometres, UV:Ultraviolet, **No.**: Number, **%$FeCl_3$**: Percentage Ferric Chloride, **Dil.HNO_3**: Dilute Nitric acid, **g**: Grams, **°C :**Degree Celsius, **q.s.**: Quantity sufficient, **hrs :**Hours, **Hs**: Sedimentation volume, **Hu**: Final height of sediment**, Ho**: Original height of the suspension, **rpm** :Rotations per minute, **RH**: Relative humidity, **µl** : Microlitre, **µ** : Microns, **ml/min** : Millilitres / Minute, **%RSD** : Percentage Relative standard deviation, **ACN** : Acetonitrile, **Conc** : Concentration, **cps** : Centipoise seconds, **RH** : Relative humidity, **mm** : Millimetres, **GAA** : Glacial acetic acid, **min** : Minutes, **r^2** : Correlation coefficient, **mcg/ml** : Micrograms /Millilitre, **% RSD** : Percentage Relative standard deviation, **ml/sec** : Millilitres / Second

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