Validation of Stability Indicating RP-HPLC method for Phloroglucinol in pharmaceutical Oral Solid Dosage Form

[1]Ezhilarasi Azhagesan, [2]Dr. Shantaram Nangude,
[3]Dr. Raja subburayalu, [4]V. Vijay, [5]Dr.K. Devi

[1]PG.Student, [2]Sr. Principal Scientist, [3]Director of R&D, [4]Research officer, [5]Assistant professor [1,5]Annamalai University,
[2,3,4]Archimedis Health care Pvt.Ltd

Abstract: A Simple, accurate and precise reverse phase high performance liquid chromatography (RP-HPLC) method for the Phloroglucinol (PHLO) in marketed formulation was developed. The determination was carried out on Inertsil ODS 3V C18 (250 x 4.6 mm, 5 µm) column using a mobile phase buffer and acetonitrile in the ratio of 90: 10 by isocratic mode. The flow rate was 1.0 ml/min with detection at 265 nm. The retention time for phloroglucinol was 3.8 min. Phloroglucinol showed a linear response in the concentration range of 256 to 384 µg/ml. The correlation co-efficient (' r ' value) for PHLO was 0.999. The percentage recoveries obtained for phloroglucinol ranges from 100.2% to 101.3%. The developed method was validated as per ICH Q2 R1 guidelines and was successfully applied for estimation of phloroglucinol in Synthetic Mixture.

Keywords: RP-HPLC method, Phloroglucinol, ICH.

I. INTRODUCTION:

Phloroglucinol is an organic compound with the formula $C_6H_3(OH)_3$. It is a colorless solid. It is used in the synthesis and explosives. Phloroglucinol is one of three isomeric benzene triols. The other two isomers are hydroxy quinol (1,2,4-benzenetriol) and pyrogallol (1,2,3-benzenetriol). Phloroglucinol, and its benzenetriol isomers, are still defined as "phenols " according to the IUPAC official nomenclature rules of chemical compounds. Phloroglucinol is used to treat the pains in the digestive functional disorders, in the renal colic and in certain pains in gynecology. This drugs fights primarily against the pains due to the spasms, in particular related to some disturb digestive, urinary, with the rules and the contractions of the pregnancy.

The present manuscript describes the degradation behaviour of Phloroglucinol under hydrolysis (acid and alkaline),oxidation, thermal stress conditions, optimization of LC conditions to separate the drug and its degradation products on a reverse phase c18 column and method validation.

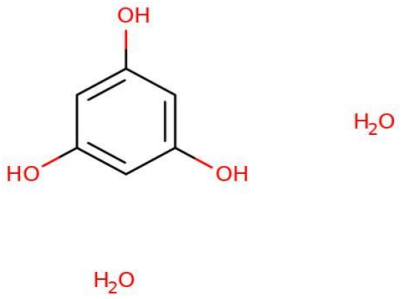

Fig 1 Phloroglucinol dihydrade

II. EXPERIMENTAL:

Materials and Reagents :

Phloroglucinol was obtained as gift sample for research purpose. Commercially available tablet formulation, tablets for the assay studies were purchased from a local pharmacy. HPLC grade acetonitrile, analytical grade orthophosphoric acid, HPLC grade water were purchased from Merck Specialities (Mumbai, India).

Instrumentation :

The development and validation of the method were performed on a HPLC system Alliance ® waters model 2695 with Empower 3 software version was applied for data collection and processing. Detectors 2498 PDA detector .PH meter (lab india pico +), Analytical balance (Radwag) AS82/220.x2.

III. METHOLOGY

Selection of analytical wavelength

The working standards of Phloroglucinol (320µg/mL) were prepared in diluent and inject in HPLC equipped with PDA-detector. The spectra of Phloroglucinol were overlapped and 265 nm were selected as analytical wavelength for quantitative determination of Phloroglucinol .

Preparation of mobile phase :

0.136 g of potassium dihydrogen phosphate in 1000ml of water adjust with PH 3.0 of ortho phosphoric acid

Preparation of Standard Solution (Con about 320ppm):

Weigh accurately 82mg of Phloroglucinol working standard and transfer into 200 mL volumetric flask. Add 150ml of diluent and sonicate to dissolve. Make up the volume with diluent up to the mark and mix well.

Preparation of Sample Solution

Weigh and transfer 10 tablets into 250ml volumetric flask. Add 150ml diluent and sonicate for 20minutes with intermediate shaking. Make up the volume with diluent and mix well. Centrifuge the solution 3000RPM for 10 min.

5ml above clear solution to 50ml with diluent mix and filter through 0.45 µ PVDF filter.

Procedure

Inject 20µl of blank solution, standard solution, and sample solution record the chromatogram and calculate percentage of assay.

Optimized Chromatographic Condition

Table-1 Optimized Chromatographic Condition

Stationary Phase	Inertsil ODS 3V C18 (250 x 4.6 mm, 5 µm)
Elution Mode	Isocratic
Mobile Phase	Buffer and ACN(90:10)
Diluent	Mobile phase
Flow Rate	1.0ml/min
Detection wavelength	265nm
Injection Volume	20 µL
Column temperature	30° c
Run Time	20 mins

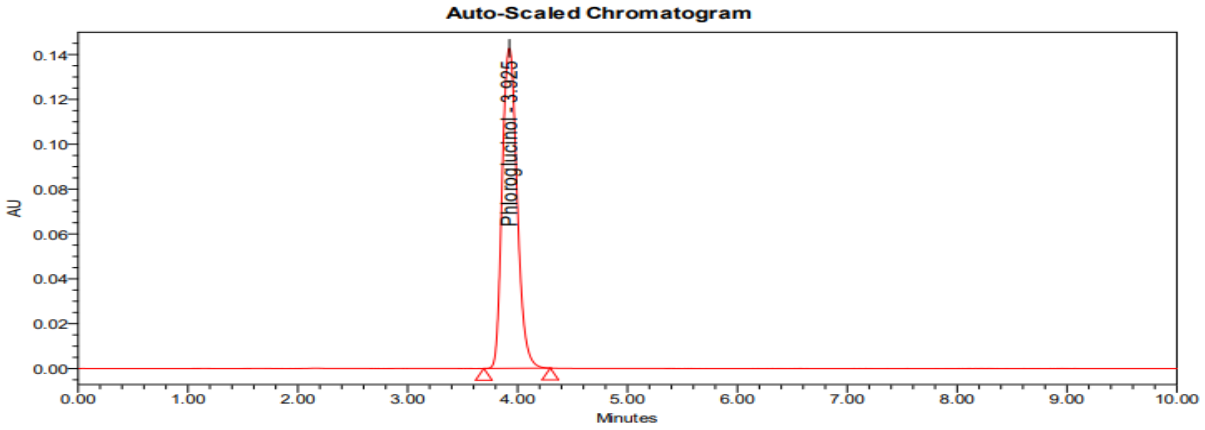

Fig 2. Standard Chromatograph

Selection of Mobile Phase for Method Optimization and experimental condition:

Several trials have been taken for the proper optimization of RP-HPLC Method by changing Different Mobile Phase with different ratio. And finally the mobile phase for optimized condition Was selected and given follows . And Optimized parameters for phloroglucinol was given Table (1).

Assay:

Assay of tablet formulation containing 80mg of phloroglucinol and Six injections of above prepared sample and standard solutions were injected . The assay of sample was calculated by comparing the areas of standard and Sample peaks. The assay formulation found within limit.

IV.METHOD VALIDATION:

The validation of RP-HPLC Method for the determination of phloroglucinol as per the protocol and to demonstrate that the method is appropriate for its intended use was studied for the following parameters. All the validation parameters were carried out according to ICH.

The parameters assessed were Specificity (Blank, placebo interference, forced degradation studies, Linearity, Method Precision (Repeatability), Intermediate precision (Ruggedness), Accuracy and Robustness.

Specificity :

Blank/placebo interference :

Examine the Blank and placebo interference at the retension time of main peak retention time.

Forced degradation studies :

Forced degradation studies were performed on phloroglucinol to prove the stability- indicating property of the Method. The stress conditions employed for degradation study of phloroglucinol include Acid hydrolysis (0.1 M HCl), Base hydrolysis (0.1M NaoH), Thermal (105°C) and Humidity (90%RH) degradation. For Acid, Base the monitoring period was 1hr whereas for Thermal and Humidity it was 24 hrs. Peak purity of the principal peak in the chromatogram of stressed samples of phloroglucinol tablets was checked using PDA detector.

Linearity

Weigh accurately 82 mg Phloroglucinol working standard and transfer into 100 mL volumetric flask. Add 150ml of diluent and sonicate to dissolve. Make up the volume with diluent up to the mark and mix well. From this solution prepare different working standards in the concentration range 256 to 384 µg/ml for Phloroglucinol in a suitable volumetric flask.

Method Precision (Repeatability)

Method precision was studied by injecting six sample preparation of the assay on the single batch of the drug product as per the methodology. Calculating the Percentage of relative standard deviation (%RSD) for six determinations of peak areas of phloroglucinol.

Intermediate Precision (Ruggedness)

Ruggedness according to the USP, is the degree of reproducibility of the results obtained under a Variety of conditions, expressed as % relative standard deviation (RSD). These conditions include differences in analyst, instruments, days and experimental periods.

Accuracy :

Accuracy of analytical method was performed by spiking of sample with standard solution. Target concentration of 320 µg/mL for Phloroglucinol respectively was selected and spiked at 50, 100 and 150%. Each spiked concentration was analyzed for three times and overall mean % recovery was determined.

Robustness Study

Robustness of the method was demonstrated by deliberately changing the chromatographic conditions. The flow rate Of the mobile phase was changed from rate (1.0 ml/min of set value i.e. 0.9 ml/min and 1.1 ml/min) and variation of Temperature (30°C set value i.e. 25°C and 35°C) and changed the wavelength (260nm and 270nm)

V. RESULTS AND DISCUSSION

Specificity

Blank Placebo interference

No interference at retention time for main peaks . It demonstrates that the absence of interference from the other materials and therefore confirm the Specificity of the proposed method.

Table 2: Specificity Blank/Placebo Interference Result

SAMPLE ID	RETENTION TIME	INTERFERENCE AT SITA/ MET
Blank	No peaks observed at retention time of principle peak	Nil
Placebo	No peaks observed at retention time of principle peak	Nil

Table 3: Specificity – Retention Time

Component Name	Retention Time(Standard)	Retention Time(Sample)
Phloroglucinol	3.925 mins	3.924 mins

Table 4: Specificity – Peak Purity Results

Component Name	Peak Purity			
	Standard		Sample	
	Peak purity Index	Single point Threshold	Peak purity Index	Single point Threshold
Phloroglucinol	0.999923	0.999796	0.999991	0.999980

Table 5 : Forced degradation study data

Degradation conditions	% Degradation of Phloroglucinol
Acid 0.1M Hcl	0.5
Base 0.1M NaOH	8.1
Thermal (105°c)	0

Humidity (90%RH)	0.1
Peroxide	6.6

Linearity

To determine linearity a calibration graph was obtained by plotting concentration against peak area. Linearity was good in the concentration range 80-120 µg/ml for component. The correlation coefficient was found 0.999 for Phloroglucinol.

Table 3 Linearity

Sr.No.	**Phloroglucinol**		
	Cons.in ppm	**Area**	**Correlation coefficient**
1	80	1342683	0.999
2	90	1344327	
3	100	1342965	
4	110	1342635	
5	120	1342563	

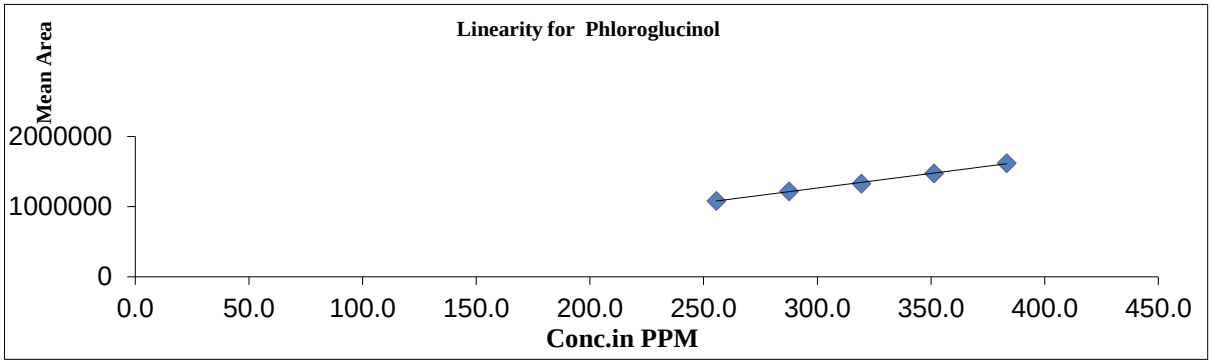

Fig 3 Linearity plot for Phloroglucinol

Method Precision (Repeatability)

Method Precision was established by determining the %RSD for six determination of Assay.

Table 6 Method Precision (Repeatability)

Sr.No.	**Sample Number**	**%Assay Phloroglucinol**
1	Sample-1	99.9
2	Sample-2	99.9
3	Sample-3	99.9
4	Sample-4	99.9
5	Sample-5	100.0
6	Sample-6	99.9
Mean		**99.9**
Standard Deviation(SD)		**0.041**
%RSD		**0.0**
95% Confidence interval		**0.0**

Intermediate Precision (Ruggedness)

Intermediate precision was established by determining the overall %RSD for (intra-day and inter-day) method precision and Intermediate Precision for assay

Table 7 Intermediate Precision :(Ruggedness)

Sr.No.	**Sample Number**	**% Assay(Repeatability)**	**% Assay (Ruggedness)**

1	Sample-1	99.9	99.2
2	Sample-2	99.9	99.5
3	Sample-3	99.9	99.5
4	Sample-4	99.9	99.5
5	Sample-5	100.0	99.4
6	Sample-6	99.9	100.2
Mean		**99.9**	**99.6**
Standard Deviation(SD)		**0.041**	**0.339**
%RSD		**0.0**	**0.3**
95% Confidence interval		**0.0**	**0.3**
Overall %RSD(n=12)for Phloroglucinol		**. 0.300**	

Accuracy study

The accuracy of the method was assessed by determination of recovery for three concentrations covering the range of the method. The mean recovery of Phloroglucinol was between 100.2 to 101.3% which is satisfactory

Table 8 Accuracy Study

Accuracy Level	**Amount Recovered (in PPM)**	**% Recovery**
50% Level Sample - 1	158.12	101.3
50% Level Sample - 2	158.26	101.0
50% Level Sample - 3	158.09	101.1
100% Level Sample - 1	312.46	100.4
100% Level Sample - 2	312.56	100.6
100% Level Sample - 3	312.43	100.6
150% Level Sample - 1	468.63	100.2
150% Level Sample - 2	468.61	100.3
150% Level Sample - 3	468.62	100.2
	Over All Mean	100.6
	SD	0.4
	% RSD.	0.4

Robustness Study

The robustness of the method was assessed by assaying test solutions under different analytical conditions deliberately changed from the original conditions. For each different analytical condition the standard solution and test solution were prepared separately. The result obtained from assay of the test solution was not affected by varying the conditions and was in accordance with the true value.

Conclusion

Thus proposed method was found to be simple, accurate, precise selective, stability indicating and economical for routine analysis of Phloroglucinol in solid oral dosage form. The proposed method described a new RP-HPLC were developed and validated asper ICH guidelines, the standard deviation and %RSD calculated for the proposed method are good, indicating high degree of precision of the method. The recovery study performed show the high degree of accuracy and has the use of inexpensive solvent were it has the ability to separate these drugs from their degradation products.

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