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METHOD DEVELOPMENT AND VALIDATION OF FORCED DEGRADATION STUDIES OF PHLOROGLUCINOL BY USING HPLC

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ABSTRACT

A simple, accurate and reproducible reverse phase isocratic HPLC method has been developed for determination of Phloroglucinol in tablet dosage form. PhG is primarily used to treat the pains in the digestive functional disorders, in the renal colics, and in certain pains in gynecology. The best separation of PhG and its degradation products was achieved on reverse phase CN column. The mobile phase was composed of aqueous solution at 0.5 g/l of H_3PO_4 (85%) at a flow rate of 1.5 ml/min and effluent was monitored at 220 nm. Chromatogram showed a peak of PhG at retention time of 4.9 ± 0.1 min. The linearity range was found to be 100 µg/ml ± 20 µg/ml. Recovery of PhG was found to be in the range of 98.55% ± 0.45%. Degradation products of PhG formed under different forced conditions have been characterized through LC–UV–PDA studies. The drug substance was found to be susceptible to stress condition of oxidation and alkaline but more stable to acid, dry heat and Photolytic condition attempted.

KEYWORDS

Forced degradation, Stress studies, Stability, Hydrolysis and Phloroglucinol.

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INTRODUCTION

Phloroglucinol is an organic compound that 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 [1, 2]. This molecule exists in two forms, or tautomers, 1, 3, 5-

cyclohexanetrione A and 1,3,5-trihydroxybenzene B (Figure No.1).

The present manuscript describes the degradation behaviour of Phloroglucinol (PhG) under hydrolysis (acid and alkaline), oxidation, photolysis and thermal stress conditions[7] , optimization of LC conditions to separate the drug and its degradation products on a reversed phase C18 column and method validation.

MATERIAL AND METHOD

Chemicals and reagents

Pure sample of PhG was kindly provided by Research Laboratory for analyze. The purity range was 99.86% w/w. Tablet formulations containing PhG Actavis 80 mg. Ultra-pure water (HPLC-grade) was obtained from Merck; Hydrogen peroxide (H_20_2), 30 wt. % was purchased from Merck. NaOH and HCl were purchased from Fluka and all the others reagents used were analytical grade.

Instrumentation and chromatographic conditions

The HPLC system used for quantification of PhG consisted of a LaChrom L-7100 Merck Hitachi Pump, LaChrom L-7200 Merck Hitachi Autosampler and a photodiode array detector (L-7450A), was used for the analysis. The chromatogram peaks were quantified by means of PC Multi- System Manager Software (Merck-Hitachi Model D-7000). Chromatography separation for analyte was achieved on Zorbax (SB-CN) column (250 mm x 4.6 mm, 5µm) maintained at ambient temperature. The mobile phases consist of aqueous solution at 0.5 g/l of H_3PO_4 (85%) that was set at a flow rate of 1.5 ml/min. The mobile phase was degassed in an ultrasonic bath prior to use and filtered through 0.45 µm membrane filter before pumping into HPLC system. The injection volume was 20 µl, and a chromatographic peak was detected at 220 nm.

Preparation of mobile phase

Mobile phase was aqueous solution at 0.5 g/l of H_3PO_4 (85%) adjusted to pH 3.0 with ortho phosphoric acid. Mobile phase was filtered through a 0.45 µm nylon filter and degassed for 5 min using an ultrasonicator.

Preparation of standard solution

Accurately weighed about 50 mg of PhG hydrated standard was taken in a 50 ml volumetric flask and was dissolved in 10ml with distilled water. About 30 ml diluent was added and mixture was dissolved by sonication and it was diluted up to mark with water.

Preparation of sample solution

Twenty tablets of PhG were weighed and ground into a fine powder. A quantity of powder equivalent to 50 mg of tablet 80 mg was weighed and transferred into a 50 ml volumetric flask and was dissolved in 10ml with distilled water. After 5 min, 30 ml of mobile phase was added and the mixture was sonicated for 30 min with intermittent shaking and then cooled at room temperature. The resulting solution was diluted with up to the mark with water. After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the sample solution was loaded in the 20 µl fixed – sample loop of the injection port.

Stress decomposition studies

The international conference on harmonization (ICH) [8] guideline entitled stability testing of new drug substances and products require that stress testing be carried out to elucidate the inherent stability characteristics of the active substance.

Our objective of this work was to perform the stress degradation studies on the PhG using the method developed.

Acid induced hydrolysis

Preparation of standard solution

Accurately weighed about 50 mg of PhG hydrated standard was taken in a 50 ml volumetric flask and was dissolved in 10 ml with 0.1 N HCl. About 30 ml of distilled water was added and mixture was dissolved by sonication and it was diluted up to mark with distilled water. The resulting solution was kept in the dark place to exclude the possible degradation effect of light and was carried out in thermostatically controlled water bath.

Preparation of sample solution

A quantity of powder equivalent to 50 mg of tablet 80 mg was weighed and transferred into a 50 ml volumetric flask and was dissolved in 10 ml with 0.1 N HCl. After 5 min, 30 ml of distilled water was added and the mixture was sonicated for 30 min with intermittent shaking and then cooled at room temperature. The resulting solution was diluted with water up to the mark and was kept in the dark place to exclude the possible degradation effect of light. Then, the solution was carried out in thermostatically controlled water bath.

Alkaline induced hydrolysis

Preparation of standard solution

Accurately weighed about 50 mg of PhG hydrated standard was taken in a 50 ml volumetric flask and was dissolved in 10 ml with 0.1 NaOH. About 30 ml of the water was added and mixture was dissolved by sonication and it was diluted up to mark with water. The resulting solution was kept in the dark place to exclude the possible degradation effect of light and was carried out in thermostatically controlled water bath.

Preparation of sample solution

A quantity of powder equivalent to 50 mg of tablet 80 mg was weighed and transferred into a 50 ml volumetric flask and was dissolved in 10 ml with 0.1 NaOH. After 5 min, 30 ml of water was added and the mixture was sonicated for 30 min with intermittent shaking and then cooled at room temperature. The resulting solution was diluted with water up to the mark. The resulting solution was kept in the dark place to exclude the possible degradation effect of light and was carried out in thermostatically controlled water bath.

Dry heat induced degradation

Tablet of PhG 80 mg were taken in petriplate and exposed to a temperature of 90ºC for 48 hours in an oven. After 48 hours, 50 mg of the sample and of the standard were diluted with water in order to make the volume up to 50 ml.

Oxidative degradation

Preparation of standard solution

Accurately weighed about 50 mg of PhG hydrated standard was taken in a 50 ml volumetric flask 1ml of 30% H_2O_2 was added then the volume made up by water. The flask was kept in dark lace to exclude the possible derivative effect of light, and analysed.

Preparation of sample solution

Accurately weighed about 50 mg of tablet 80 mg and transferred into a 50 ml volumetric flask and 1ml of 30% H_2O_2 was added then the volume made up by water. The flask was kept in dark lace to exclude the possible derivative effect of light, and analysed.

Photolytic degradation

Preparation of standard solution

Accurately weighed about 50 mg of PhG hydrated standard and transferred into a 50 ml volumetric flask and it was diluted up to mark with water. The solution was exposed to light from UV lamps for 24 hours.

Preparation of sample solution

Accurately weighed about 50 mg of tablet 80 mg and transferred into a 50 ml volumetric flask and it was diluted up to mark with water. The solution was exposed to light from UV lamps for 24 hours.

Validation

Linearity and Range

Linearity of the method was evaluated by using 5 linearity solutions of different concentrations. Accurately measured aliquots of solution standard were taken in five different 50 ml volumetric Brown flask and diluted up to the mark with the mixture solvent such that the final concentrations of Phloroglucinol were 80 $\mu g\ ml^{-1}$, 90 $\mu g\ ml^{-1}$, 100 $\mu g\ ml^{-1}$, 110 $\mu g\ ml^{-1}$ and 120 $\mu g\ ml^{-1}$. A 10 µl aliquot of each linearity solution was injected in triplicate. The peak area values were plotted against the corresponding analyses concentrations to obtain the linear calibration. The coefficient of this dependence was calculated to be 0.9998. The standard solutions were prepared by diluting an appropriate volume of stock solution with water. Each solution was analyzed in triplicate [9, 10].

Specificity

The specificity of the RP-HPLC method was determined by elution of PhG (Fig 3). The tailing factor for peak obtained was satisfactory because it was less than 2%. The retention time for PhG was

found to be 4.9±0.1 min for six replicates. The peak obtained for PhG was sharp with clear baseline result of the method validation experiments are given in Table No.1.

Precision

A standard and sample (tablet) solution containing PhG of 100 µg/ml were prepared. The standard and sample solution (n = 6) were injected and the Peak of PhG present in pure and formulation were determined. Statistical evaluations of tablet analysis were showed in Table No.1. The intraday and interday precisions were determined and results are given in Table No.2.

Accuracy

Accuracy of method was evaluated as a percentage of recovery obtained from analysis of sample spiked with known amount of PhG (80.0, 100.0 and 120.0 µg/ml). The accuracy was carried out three times at each level of recovery. The results of study along with its evaluation are given in Table No.3.

Detection limit and quantification limit

The LOD and LOQ were separately determined (Table No.1) based on the standard calibration curve. The residual standard deviation of y-intercepts of regression lines LOD = 3.3x D/S and LOQ = 10x D/S of regression lines and S is the slope of calibration curve[11].

Robustness

It was observed that by making changes in chromatographic parameters, absolute difference between percent assay under altered condition and mean percent assay obtained during repeatability was not more than 2.0%. %RSD of area response and retention time was below 2%. The results of Robustness evaluation are shown in Table No.4.

RESULTS AND DISCUSSION

In this method to optimize chromatographic parameters several mobile phase compositions were tried. A satisfactory separation, good peak symmetry and to achieve good retention time was obtained with economic mobile phase consisting an aqueous solution at 0.5 g/l of H_3PO_4 (85%) adjusted to pH 3.0 with ortho phosphoric acid. The flow rate was 1.5 ml/min with UV detection at 220 nm. The calibration curve was found to be linear in the range of 90-120 µg/ml for PhG with correlation coefficient of 0.9998. The validation parameters are presented in Table No.1. The LOD and LOQ for PhG were determined in the basis of peak response and slope of the regression equation. The LOD and LOQ of the drug were found to be 0.04 µg/ml and 0.14 µg/ml respectively. The low % RSD value for intraday and interday precisions revealed that the proposed method is reproductible and robust. No interfering peaks were found in the chromatogram indicating that the excipients used in tablet formulations did not interfere with the estimation of drug by the proposed HPLC method.

Forced degradation study was carried out by subjecting the drug to acid and alkaline hydrolysis, chemical oxidation and photolytic conditions and its chromatograms were showed in Figure.4.

The forced degradation studies of PhG tablet formulation was done on stress degradation by hydrolysis under alkaline conditions by using 0.1 N NaOH was found to be 15.16% for 60 min, stress degradation by using 0.1N HCl and product degradation was found to be 0.3% for 60 min. Dry heat induced degradation was done by using 90 ºC temperature was found to be 0.25% for 48 hours. Oxidative degradation was done by using hydrogen peroxide 30% and product degradation was found to be 5% for 15 min. Photolytic degradation was found to be 0.2% for 24 hours. The PhG was found to be stable of the condition like photolytic stress degradation, acid degradation and thermal degradation but instable to rest of the conditions degradation

Table No.1: Validation and System Suitability Parameters

Parameters	**Phloroglucinol**
Linearity	80-120 µg/ml
Slope	23709
Intercept	18006
Coefficient of correlation	0,9998
T_r	4.9±0.1 min
Tailing Factor	1,08
LOD µg/ml	0,04 µg/ml
LOQ µg/ml	0,14 µg/ml
Theoretical plates Ph Eup	10225

Table No.2: Intraday and Interday Precisions

Intraday precision	**Interday precision**	**%RSD**	**%RSD**
Mean %±SD	Mean %±SD	Intraday	Interday
97.7 ± 1.1	97.2 ± 0.3	0.77	1.15

*Mean of six determination (n=6)

Table No.3: Recovery Studies of PhG in Tablet Dosage Form

% Taken	**%Recovered**	**%Recovery ±SD**	**%RSD**
80%	98.1	98.1±0.4	0.24
100%	98.2	98.2±0.9	0.54
120%	99.0	99.0±1.4	0.80

Table No.4: Result of Robustness Studies

Method parameter	**Altered condition**	**%Assay**	**%RSD**
Flow rate	1.425 ml min^{-1}	98.48	1.18
	1.50 ml min^{-1}	98.20	0.54
	1.575 ml min^{-1}	99.12	0.76
Temperature	23 °C	98.82	0.92
	25 °C	98,20	0.54
	27 °C	98.55	0.46
Wavelength (nm)	215 nm	98.92	0.95
	220 nm	98.20	0.54
	225 nm	100.34	0.72
Column	Lot-1	98.20	0.54
	Lot-2	99.05	1.09

Table No.5: Summary of Results of Stress Degradation Study

Stress condition	Test	%Assay	% degradation	Remarks
Photolytic degradation	Standard Simple	98.85% 99.4%	<0.5% <0.5%	No degradation No degradation
Alkaline induced hydrolysis (NaOH 0.1N)	Standard Simple	97.9% 63.9%	<0.5% 15.2%	No degradation Degradation observed
Oxidative hydrolysis (H_2O_2 at 30%).	Standard Simple	90.9% 87.6%	1.43% ≈ 4.99%	Degradation observed Degradation observed
Acid induced hydrolysis (HCl 0.1N)	Standard Simple	96.8% 98.3%	<0.5% <0.5%	No degradation No degradation
Dry heat induced degradation	Standard Simple	99.0% 99.9%	<0.5% <0.5%	No degradation No degradation

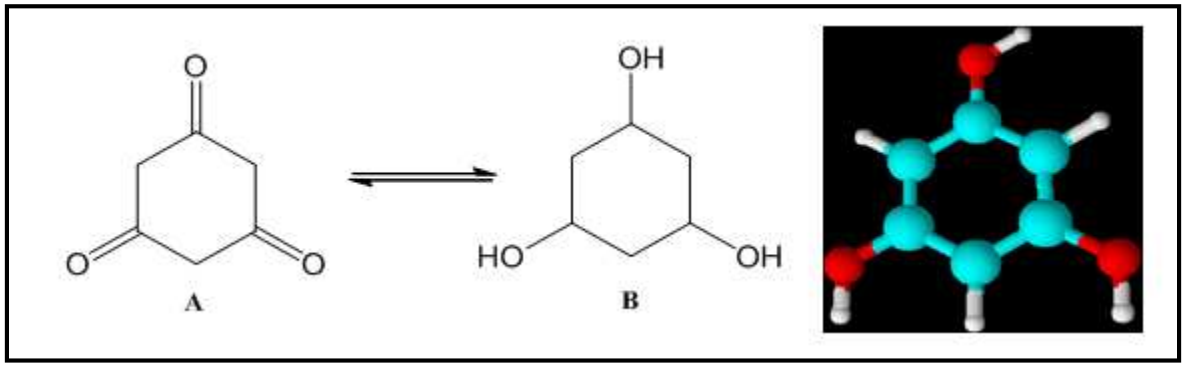


Figure No.1: Chemical Structure of Phloroglucinol

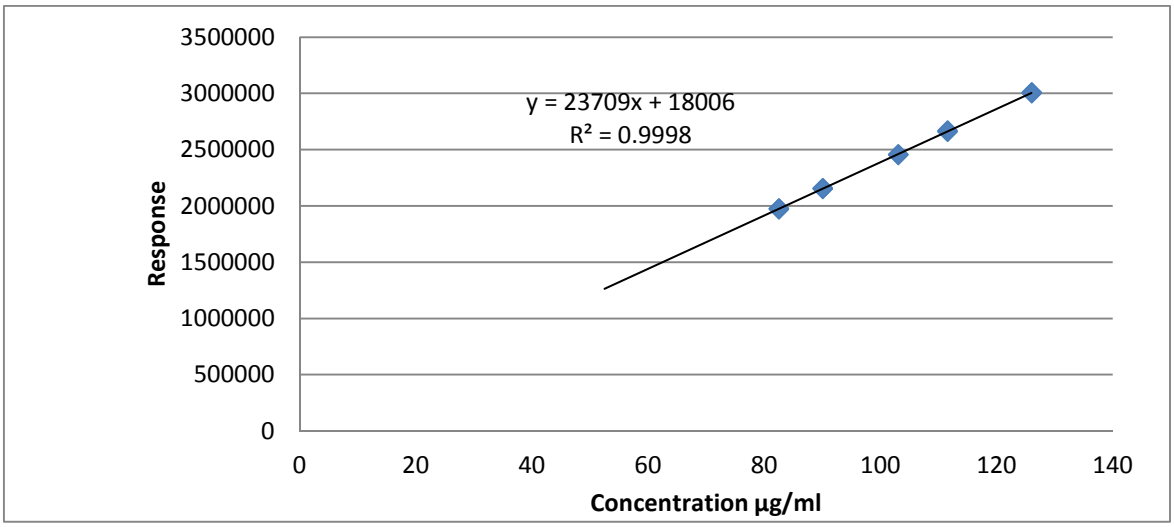


Figure No.2: Linearity of Phloroglucinol

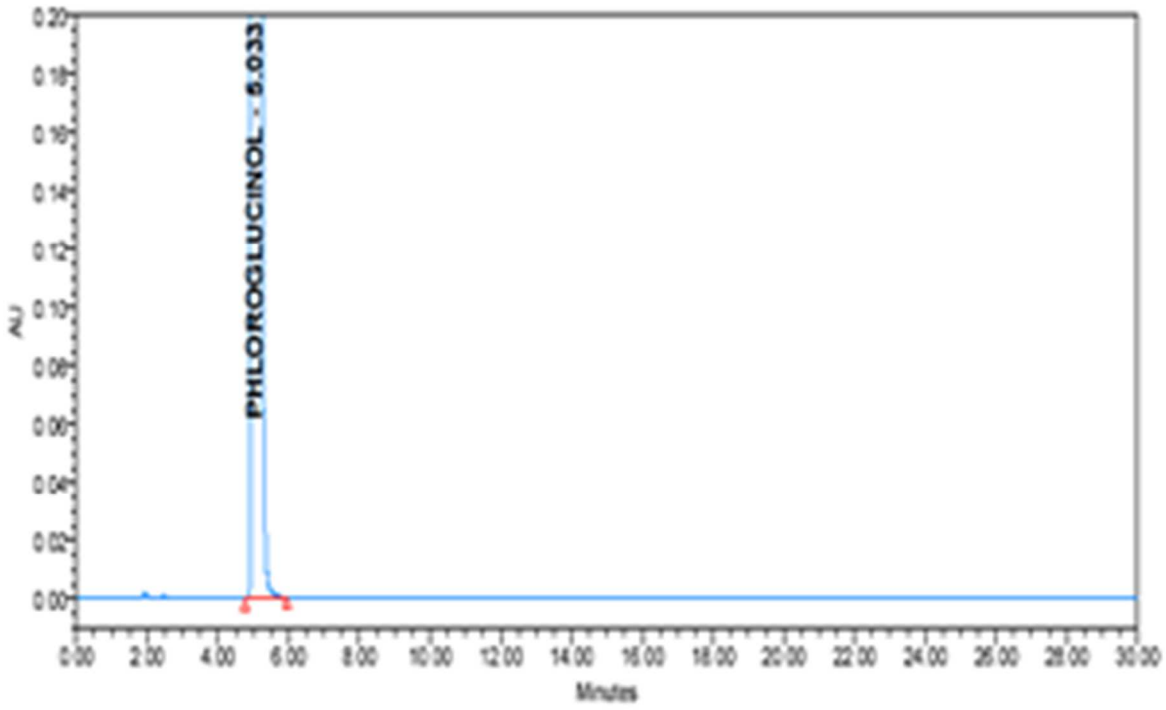
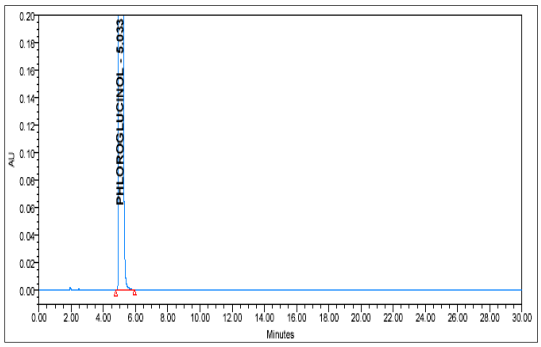


Figure No 3: Atypical chromatogram of standard Phloroglucinol

PHLOROGLUCINOL I

a



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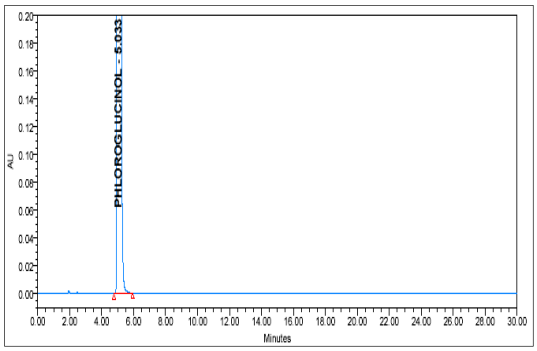
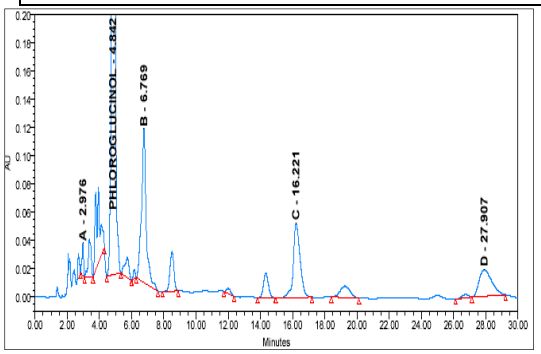


PHLOROGLUCINOL II

c



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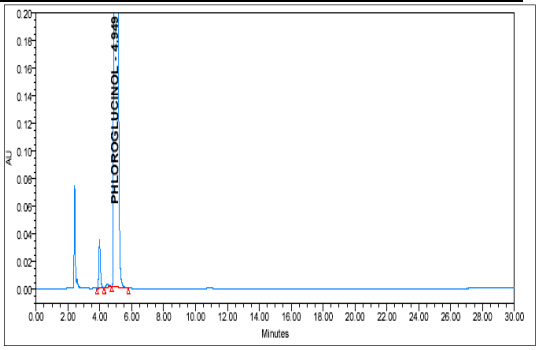
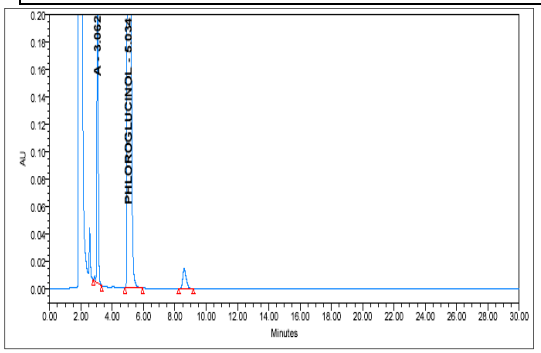


PHLOROGLUCINOL III

e



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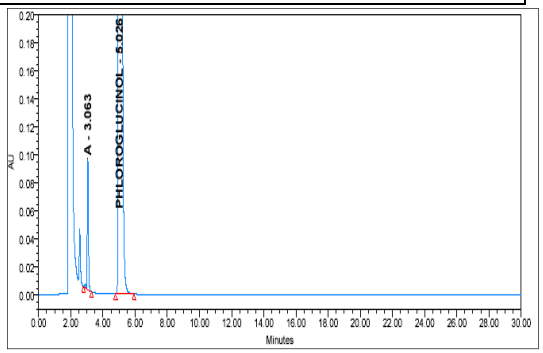

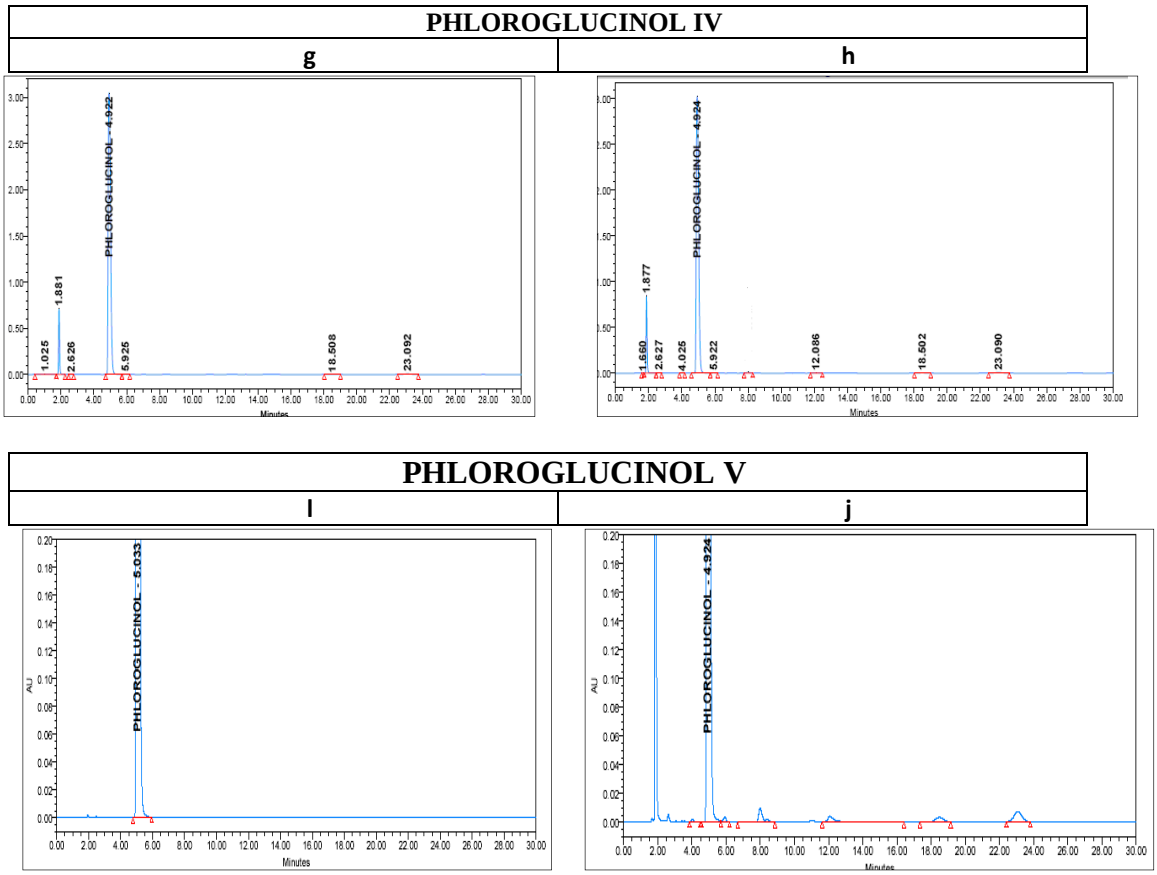

Figure No.4: The chromatograms sample (a,c,e,g,i) and chromatograms standard (b,d,f,h,j) showing the decomposition of Phloroglucinol in : I (photolytic degradation), II (alkaline induced hydrolysis), III (oxidative hydrolysis), IV (Dry heat induced hydrolysis) and V (acid induced hydrolysis).

CONCLUSION

The proposed method described a new RP-HPLC were developed and validated as per ICH guidelines, the standard deviation and % RSD calculated for the proposed method are low, 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 where it has the ability to separate these drugs from their degradation products, related substance; excipients found in tablet dosage forms and can be applied, for the routine analysis in quality control laboratory.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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