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RP-HPLC ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR NEWLY SYNTHESIZED ISOEUGENOLINDOLE-3-ACETIC ACID

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ABSTRACT
Analytical method development and validation on RP-HPLC for isoeugenolindole-3-aceticacid (IEIAA). The HYPERSIL column used for determination was carried out on the acetonitrile:water mobile phase with a ratio 80:20v/v with 0.5ml/min flow rate (UV detection at 340nm). The retention time for isoeugenolindole-3-aceticacid was 7.538 min. isoeugenolindole-3-aceticacid in the concentration range of 4-24 ppm showed a linear response and the correlation co-efficient ('r ' value) of isoeugenolindole-3-aceticacid was 0.999. To be validated the developed method with regard to precision, accuracy, linearity, selectivity, range, and force degradation study and found the method to be specific, accurate, linear, and precise. The RSD for injector repeatability and inter-assay precision have been observed to be> 2 %. The percentage recoveries obtained for isoeugenolindole-3-aceticacid range from 98.28 – 1to 01.65 % and with an overall % mean recovery of 100.27%.
Keywords: Isoeugenolindole-3-aceticacid, Linearity, Accuracy, Precision, Inter-assays Precision
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INTRODUCTION

The science branch of analytical chemistry determining the composition uses advanced technologies by analytical techniques.[1-5] Play a major role in the analytical instruments to achieve reliable and high-quality analytical data.[6] The process of selecting an analytical method for development is an accurate assay procedure to determine the formulation composition. The analytical method validation process is proving that it is acceptable to use measuring the concentration in the laboratory to subsequent samples.[7]Instrumental RP-HPLC analytical methods should be used within GLP and GMP environments and must be developed[8-11].The existing work describes the improvement, and validation of the reverse section excessive overall performance liquid chromatographic (RP-HPLC) method, which can quantify these aspects simultaneously. Confirmation of the applicability of the developed approach was once validated in accordance with the International Conference on Harmonization (ICH) for the willpower of isoeugenolindole-3-aceticacid.

EXPERIMENTAL

HPLC/AR grade solvents were used and a standard sample of isoeugenolindole-3-aceticacid is used by in-house double purified and isoeugenolindole-3-aceticacid technical used by in-house preparation.[12]

Chromatographic Conditions

HPLC system, (HPLC, Shimadzu, LC-20AD with PDA detector) with HYPERSIL (C18, 5.0 µ x 250 x 4.6mm) column were used for the study. Data processing software was used as an Empower for the HPLC system and the mobile phase usedwas of isocratic elusion performed acetonitrile: water (80:20v/v) with 0.5ml/min flow rate and 340nm detection was carried out.

Stock Solution of Standard

A stock solution of a standard of isoeugenolindole-3-aceticacid was prepared by weighing 100mg accurately of the isoeugenolindole-3-aceticacid standard; adding 5ml of acetonitrile diluting up to 20mL

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with acetonitrile (stock solution-I). From the above solution transfer 5 ml solutions to a 50ml volumetric flask and diluted up to the mark with acetonitrile as inventory solution-II labeled.

Stock Solution of Sample

The isoeugenolindole-3-aceticacid sampleof 100 mg, was diluted to 10mLwithacetonitrile.[13]From the above stock solution, 5ml solution was transferred into a 50ml volumetric flask and diluted up to the mark with acetonitrile.

Calibration Curve

For isoeugenolindole-3-aceticacid, standard solution pipette into a 100 ml volumetric flask. The concentration range 4, 8, 12, 16, 20, and 24 ppm of volume was marked with acetonitrile for isoeugenolindole-3-aceticacid. Each concentration of duplicate dilutions was prepared separately for each drug. Each concentration of isoeugenolindole-3-aceticacid was injected 20µl injections from these duplicate solutions into the RP-HPLC system separately and chromatograph underthe above conditions. Evaluation of isoeugenolindole-3-aceticacid was performed with a 340nm UV detector.[14-15]

Method Validation

The validated of a method for the various parameters such as; robustness, force degradation, system suitability studies, precision, selectivity, accuracy, range, and linearity.

Specificity

Specificity was determined by scanning of diluent solution and standard solution of isoeugenolindole-3-aceticacid having concentrations 20µg/ml. Derivatized[16] solutions of isoeugenolindole-3-aceticacid into the chromatographic system sample and standard were injected after injecting solvent blank, reagent blank, and sample blank to demonstrate that there is no interference at the retention time of 2 isoeugenolindole-3-aceticacid from any reagent or solvent (mobile phase) blank.

Linearity

The assay method for linearity test solutions was concentrations of different levels such as; 4, 8, 12, 16, 20, and 24ppm were prepared from a stock solution. 20µl of everysolutionwas injected into the system of HPLC and the noted peak area from the chromatogram was obtained. The toplocationtowardsawarenessstatisticsturned intoanalysis with least-squares linear regression. The y-intercept and slopewere reportedforthe calibration curve.

Precision

The proposed method precision (intra-day precision and injector repeatability) fixed concentration of six replicates was ascertained by determinationof the linearity range of the mixture of the isoeugenolindole-3-aceticacid on the differentday and on same day, different analyst, different column, etc.

Accuracy (Recovery Studies)

By comparing the area before and after the addition of the working standard, the percent recovery was calculated. Recovery was carried out in the same manner for both drugs. This common addition method was used at 20, 60, 80, 100 and 120% levels,and the recovery percentage was computed.

RESULTS AND DISCUSSION

A developed and validation technique of RP-HPLC, isoeugenolindole-3-aceticacid compound and the isoeugenolindole-3-aceticacid were resolved on HYPERSIL RP C18 column using acetonitrile: water (80:20v/v) and the 0.5ml/min flow rate were wavelength detection at 340 nm. Overall, did not interfere with isoeugenolindole-3-aceticacid peaks from the data demonstrated that the excipients, indicated that the method is selective. Analytes were complete separated from the accomplished in < 10 min.[17]

HPLC Method Optimization and Development

The samples initially analyzed were mobile phase using water:acetonitrile (20:80, v/v) at a 0.5mL/min flow rate. Under these conditions, observed well-resolved peaks with good sharpness and symmetry. Therefore, the acetonitrile:water mobile phase with the ratio 80:20v/v was chosen for the entstudy'study best chromatographic response.[18]

System Suitability Studies of Method Validation

The system suitability test performed is a complete testing system to ensure that it is suitable for the intended application. Measured the various parameters and in all measurements were observed peak at 7.538 min, the average retention time was less than 2.0. This peak area varied, and the tailing factor was less than 2, and more than 2000 observed theoretical plates for the isoeugenolindole-3-aceticacid peak. The high sensitivity offers the proposed method and can be detected for the peak accurately.[19] The isoeugenolindole-3-aceticacid in all the cases, separated from the peak was well,excipients.

Specificity

The retention time of isoeugenolindole-3-aceticacid was 7.538min.There was no peak interfering from the blank at the retention time of isoeugenolindole-3-acetic acid, and hence the determination of isoeugenolindole-3-aceticacid proposed method is specific.[20-22]

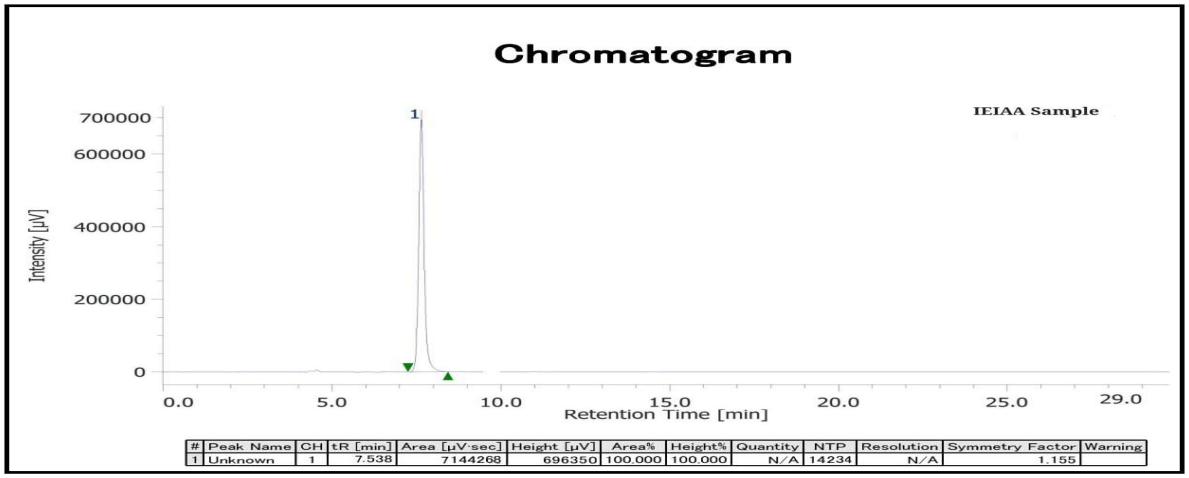

Fig.-1: Specificity Peak Purity Chromatogram of Isoeugenolindole-3-Acetic acid

Linearity

Observed the linear calibration curve for isoeugenolindole-3-aceticacid over the 4-24ppm concentration range. By linear regression analysis, the isoeugenolindole-3-aceticacid data for the peak area in the treated was concentration corresponds (Table-1) and calibration curves of the equations of regression (Figure-2) were found to be Y = 329,556.503891x + 628,290.300000(Figure- 2)with a coefficient of correlation of 0.99962, which equals to unity.[23]

Table-1:Linearity Data of Isoeugenolindole-3-Acetic acid Standard[24]

Linearity Sol Level	Conc ppm	Replications	Peak Area Counts	Means Area
L1	4.002	R1	1827272	1848070
		R2	1868868	
L2	8.004	R1	3398969	3348594.5
		R2	3298220	
L3	12.006	R1	4630008	4648431.5
		R2	4666855	
L4	16.008	R1	5923290	5914624.5
		R2	5905959	
L5	20.01	R1	7176955	7176062.5
		R2	7175170	
L6	24.012	R1	8530736	8530546.5
		R2	8530357	

Precision

The precision (intra-assay and injector repeatability) of the method was determined by the isoeugenolindole-3-acetic acid standard solutions. The % RSD for repeatability and intra-assay precisions were less than 2%, indicating a high degree of precision.[25]

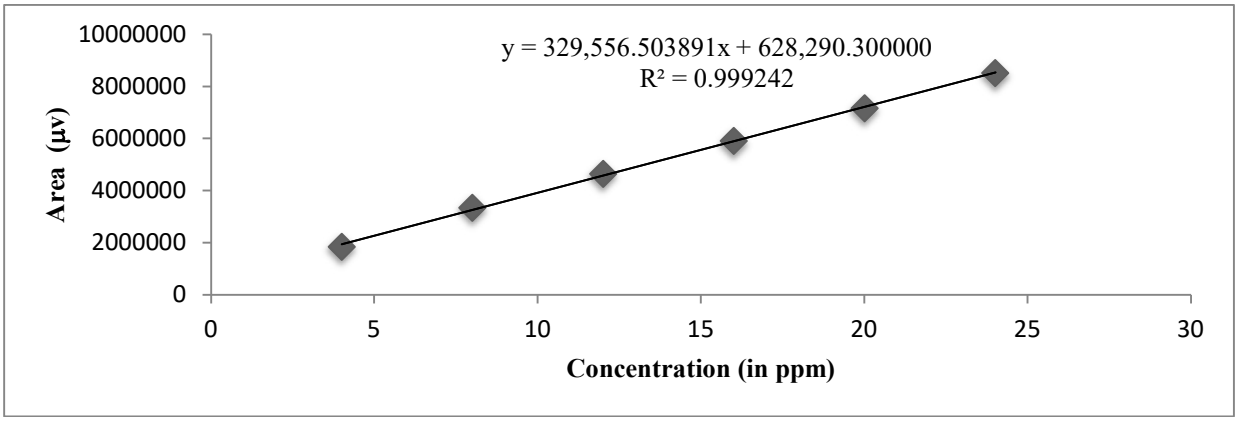

Fig.-2: Linearity Graph of Isoeugenolindole-3-acetic acid Standard

Table-2: Injection Repeatability (IR) for Isoeugenolindole-3-Acetic acid

Sample no.	Conc in ppm	Area (mv)	% Content
IR Sample-1	20.02	7144269	99.72
IR Sample-2	20.06	7144538	99.53
IR Sample-3	20.02	7132033	99.55
IR Sample-4	20.09	7143383	99.36
IR Sample-5	20.03	7103493	99.10
IR Sample-6	20.07	7134097	99.33
Average	NA	NA	99.43
STDEV	NA	NA	0.22
% RSD	NA	NA	0.22

Table-3: Intra-Assay (IA) Data of Isoeugenolindole-3-Acetic acid Technical

Sample No.	Conc. in ppm	Area (mv)	% Content
IASample-1	20.02	7144636	99.63
IASample-2	20.10	7144277	99.23
IASample-3	20.20	7214520	99.71
IASample-4	20.03	7143585	99.57
IASample-5	20.01	7134696	99.55
IASample-6	20.05	7134097	99.34
Average	NA	NA	99.51
STDEV	NA	NA	0.18
% RSD	NA	NA	0.18

Table-4: Comparison between Analyst-1 and 2

	Mean % Content	Absolute Difference
Analyst 1	99.43	0.07
Analyst 2	99.51	

Accuracy

Less than 2.0 with a recovery resultant % RSD was found to be 98.28 -101.65% and with the overall % mean recovery of 100.27 for isoeugenolindole-3-aceticacid. This indicates the process of removal of both positive and negative blank interferences. According to the above results, the analyte recovery data are within the limit, and thus the proposed method is accurate.[26]

Table-5: Accuracy Data for Isoeugenolindole-3-Acetic acid Technical

Level (%)	Sample Wt (in mg)	Conc. (in ppm)	Area (mv)	% Recovery	% Mean Recovery	STDEV	% RSD
20_1	4.09	4.09	1468068	100.35	100.27	0.10	0.10
20_2	4.10	4.10	1468868	100.16			

20_3	4.09	4.09	1467545	100.31			
60_1	12.04	12.04	4301694	99.88	99.28	0.70	0.71
60_2	12.01	12.01	4272451	99.45			
60_3	12.00	12.00	4228422	98.51			
80_1	16.27	16.27	5914180	101.62	101.65	0.22	0.22
80_2	16.28	16.28	5907159	101.44			
80_3	16.21	16.21	5907142	101.88			
100_1	20.13	20.13	7183655	99.77	99.85	0.08	0.08
100_2	20.09	20.09	7176255	99.86			
100_3	20.10	20.10	7183676	99.92			
120_1	24.31	24.31	8697461	100.02	100.28	0.32	0.32
120_2	24.27	24.27	8697461	100.19			
120_3	24.16	24.16	8697515	100.64			
Overall % Recovery				100.27			
Overall STDEV				0.86			
Overall % RSD				0.86			

Range

The range for the isoeugenolindole-3-acetic acid is evaluated[26] from 20% i.e. 4 ppm to 120% i.e. 24 ppm.

Table-6: Range for Isoeugenolindole-3-Aceticacid

Solution	20% (4 ppm)	120% (24 ppm)
1	1768272	8697500
2	1767868	8697751
3	1767121	8697676
4	1742242	8697240
5	1768968	8697232
6	1767376	8697254
Average	1763641.17	8697442.17
STDEV	10503.98	234.03
% RSD	0.60	0.00

Forced Degradation Studies

Overall, isoeugenolindole-3-acetic acid proved to be a stable drug substance in metallic condition (0.05M $FeCl_3$) at room temperature, basic condition (1N NaOH) at room temperature, acidic condition (1N HCl) at room temperature, oxidation condition (3% H_2O_2) photolytic at room temperature, reduction condition (1% Na_2S), photolytic condition (Exposure to 1.2 million lux/hour) and thermal degradation conditions of 105°C.In all the above degradation conditions, each degradant peak is well separated from the blank and main peak. The correlation of an increase in degradant impurities with a decrease in assay value for isoeugenolindole-3-aceticacid was found satisfactory. On the basis of the above validation data, it is concluded that the above method for isoeugenolindole-3-aceticacid by HPLC is a specific and stability-indicating method.[27]

Table-7: Forced Degradation Calculation of Isoeugenolindole-3-Aceticacid Technical

Condition	Sample Wt. (in mg)	Conc. (in ppm)	Area (mv)	% Assay	% Total Imp.	Mass Balance
As such	20.19	20.19	7113863	100.49	0.000	NA
0.05M_FeCl3_24 Hrs	20.06	20.06	7003930	99.58	0.131	99.2
1N_NaOH_24 Hrs	20.19	20.19	7090221	100.16	0.539	100.2
1N_HCl_24 Hrs	20.09	20.09	7015517	99.60	0.143	99.3
3% H2O2_24 Hrs	20.11	20.11	7039130	99.83	1.476	100.8
1% Na2S_24 Hrs	20.03	20.03	7115517	101.32	1.044	101.9
Photo @ 1.2 million lux/Hr	19.96	19.96	7009788	100.16	0.899	100.6
Thermal @ 105°C_24 Hrs	20.03	20.03	7113580	101.29	0.000	100.8

Table-8: Force Degradation of Isoeugenolindole-3-Acetic acid of Impurity Profile

% impurity (by Area normalization)								
RT about -->	Unk @ 2.90	Unk @ 3.15	Unk @ 3.26	Unk @ 3.45	Unk @ 3.99	Unk @ 6.82	Unk @ 8.32	Total Imp
As such	-	-	-	-	-	-	-	0.000
0.05M $FeCl_3$ 24 Hrs	-	-	-	-	-	0.131	-	0.131
1N NaOH 24 Hrs	0.0812	-	0.204	0.254	-	-	-	0.539
1N HCl 24 Hrs	-	0.078	-	-	0.065	-	-	0.143
3% H_2O_2 24 Hrs	-	-	1.476	-	-	-	-	1.476
1% Na2S 24 Hrs	-	-	1.044	-	-	-	-	1.044
Photo @ 1.2 million lux/Hr	-	-	-	-	-	-	0.899	0.899
Thermal @ 105°C_24 Hrs	-	-	-	-	-	-	-	0.000

CONCLUSION

The isoeugenolindole-3-aceticacid compound was validated and developed and applied for the determination of isoeugenolindole-3-aceticacid and was found the method to be specific, accurate, precise, and robust. 2 isoeugenolindole-3-acetic acid elutes a short time (<8min) and pharmaceutical dosage form any components no interference was seen. In conclusion, the high reproducibility, accuracy, good selectivity, and sensitivity of isoeugenolindole-3-aceticacid for simultaneous determination are suitable for the proposed method.

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