RP-HPLC METHOD DEVELOPMENT FOR THE SUBSTITUTED BENZENE DERIVATIVE OF EUGENOL

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ABSTRACT

A simple Reverse phase liquid chromatographic method has been developed and subsequently validated for the derivative of the Phenyl substituted derivatives on the hydroxyl proton of Eugenol. The separation was carried out using a mobile phase consisting of 0.5 %v/v Acetic Acid buffer in HPLC grade water (Mobile phase A) and Methanol (Mobile phase B). The column Inertsil ODS, 3V (C18, 5 μ , 25 cm $\times$ 4.6 mm id with flow rate of 1.0 ml / minute using PDA detection at 260 nm. The described method was linear over a concentration range of 10-100 μ g/ml (ppm). All the five mixtures are well separated and the resolution complies as per the pharmacopeia method development requirements. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the identification and quantifications of the said derivatives of 4-allyl-2-methoxyphenyl 3,4-dimethoxybenzoate (RLS-228), 4-allyl-2-methoxyphenyl 3,4,5-trimethoxybenzoate (RLS-222), (Z)-4-allyl-2-methoxyphenyl 3-(4-methoxyphenyl) acrylate (RLS-205), 4-allyl-2-methoxyphenyl 4-ethoxy-3-methoxybenzoate (RLS-193) and 4-allyl-2-methoxyphenyl benzoate (RLS-218)

Keywords: Eugenol, phenyl substituted derivatives, High Performance Liquid Chromatography, Method Validation

INTRODUCTION

The Eugenol molecule (2-methoxy-4-(2-propenyl) phenol)^{1,2} is derived from Clove oil. This is the main content of the clove oil (72 – 90 %). It is a clear to pale yellow oily liquid, it is slightly soluble in water and soluble in organic solvents, the characteristic clove smell is due to the presence of this in the clove. It is one of the ancient medicines used as a local antiseptic and anesthetic³, in the recent trend is used in the manufacturing of perfumeries also. This formulated with metal oxides and the resulting Metal oxide, which has been used in the restorative and prosthodontic application⁴ in dentistry as healing agents. The Eugenol is highly significant to study the substituted derivative of this drug molecule also important because of its medical properties. In this paper will illustrate the HPLC (RP) phase method validation⁵ for separating the five phenyl substituted derivatives of Eugenol^{6,7}

MATERIAL AND METHOD

Reagents and chemicals used

Methanol HPLC grade, E. Merck (India) Ltd, Water HPLC grade was obtained from a Milli-Q Reverse Osmosis (RO) water purification system. A working standard internally qualified used; Analytical reagent grade Glacial Acetic Acid and Volumetric flask and glassware of class A used for the analysis.

Instrument

Chromatographic separation was performed on a Shimadzu® liquid chromatographic system HPLC (Shimadzu LC-2010), equipped with a LC-10AT-vp solvent delivery system (pump), SPD M-10AVP photo diode array detector, Rheodyne 7725i injector with 50 ml loop volume. Class-VP 6.01 data station was applied for data collection and processes. The injection volume was 5 μ l and the analysis was performed at ambient temperature. The HPLC column was used Inertsil ODS, C18, 5 μ , 25 cm $\times$ 4.6 mm id.

Preparation of standard solutions

Weigh accurately about 5.0 mg of each of the following internal qualified working standard in to a 5 ml volumetric flask respectively. Dissolved and dilute up to mark with diluents and further diluted to get 100 ppm standard solution. This Solution is injected for the RT confirmations.

- 4-allyl-2-methoxyphenyl 3,4-dimethoxybenzoate (RLS-228)
- 4-allyl-2-methoxyphenyl 3,4,5-trimethoxybenzoate (RLS-222)
- (Z)-4-allyl-2-methoxyphenyl 3-(4-methoxyphenyl) acrylate (RLS-205)
- 4-allyl-2-methoxyphenyl 4-ethoxy-3-methoxybenzoate (RLS-193)
- 4-allyl-2-methoxyphenyl benzoate (RLS-218)

Preparation of Test Solution

Weighed 5.0 mg of each of the above five sample in to a 5 ml volumetric flask. Dissolved and dilute up to mark with diluents (1000 ppm). This Solution is further diluted to get 100,200,400 and 800 ppm of the solutions for analysis.

HPLC method

The Mobile phase selected for this method contained 0.5 %v/v Acetic acid buffer (5 ml /1000 ml) and Methanol; this was filtered through 0.45-micron membrane filter. Flow rate employed was 1.0 ml/min (Gradient). The injection volume was 10 μ l and the Auto sampler and column compartment was maintained at ambient temperature (25°C). Detection of eluent was carried out at 260 nm using PDA detector. With the optimized chromatographic conditions, a steady baseline was recorded, the mixed standard solution was injected, chromatogram were recorded. The retention time of the said five Eugenol derivatives (working standard) were eluted at 15.6, 17.4, 20.8 and 21.7 minutes, respectively for RLS 228, 222, 193, 218 and 205. Inject the Test preparation once separately for entire five solutions for RT confirmations and six replicate of the five mixture sample.

Method Validation

The developed method was validated in terms of the parameters viz., Resolution, linearity, precision (inter and intraday), LOD, LOQ and correlation co-efficient.

Resolution

One of the system suitability parameters is resolution; the acceptance criteria of the resolution is Not Less Than (NLT) 1.0 and for the tailing factors is Not More Than (NMT) 1.5, The percentage of replicate of injections is NMT 2.0 %

Linearity

The five derivatives were found to be in a linear in a concentrations range of 0.488 – 500 µg/ml. The detection of the samples was measured at 260 nm (Uv – Vis) detector and the linear graph was plotted using concentration on X-axis and area on Y-axis. The linearity of the five samples was given in the Figure 1 – 5 and the determined area counts were given in the Table 4.

Precision (Repeatability)**Inter day precision**

This analysis was carried out by same analyst and HPLC instrument. The % of RSD in terms of Retention Time (RT), area, height, theoretical plates, tailing factor and resolution were given in the Table 1.

Intraday precision

This analysis was carried out by same day of sample preparation. The percentage RSD in terms of Retention Time (RT), area, height, theoretical plates, tailing factor and resolution were given in the Table 2.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD is determined based on the Standard Deviation of the Response and the Slope and the LOQ is determined based on the Based on the Standard Deviation of the Response and the Slope

The LOD is calculated from the formula = $3.3 \sigma / S$

Where: σ = the standard deviation of the response,

S = the slope of the calibration curve

The LOQ is calculated from the formula = $10 \sigma / S$

Where: σ = the standard deviation of the response,

S = the slope of the calibration curve

The calculated LOD and LOQ values are given in the Table 3; Table 1: Precision – Inter Day

Precision – Inter day (RLS 228)

Title	RT	Area	Height	Theoretical Plates	Tailing Factor	Resolution
230513 03.lcd	15.473	773352	27391	6771.143	1.07	0.000
230513 04.lcd	15.508	746708	26961	6929.802	1.063	0.000
230513 05.lcd	15.491	739517	26813	6943.979	1.069	0.000
230513 06.lcd	15.51	745984	27107	7007.304	1.05	0.000
230513 07.lcd	15.396	770623	27363	6714.191	1.041	0.000
230513 08.lcd	15.447	773142	27258	6684.008	1.039	0.000
Average	15.47	758221	27149	6841.74	1.06	0.000
% RSD	0.282	2.075	0.850	1.980	1.314	0.000
STDV	0.04	15732.30	230.76	135.46	0.014	0.000

Precision – Inter day (RLS 222)

Title	RT	Area	Height	Theoretical Plates	Tailing Factor	Resolution
230513 03.lcd	17.498	854841	30919	9039.446	1.033	2.721
230513 04.lcd	17.535	855629	30812	8995.598	1.036	2.731
230513 05.lcd	17.518	856113	30783	8958.942	1.037	2.733
230513 06.lcd	17.515	855621	31087	9132.419	1.036	2.719
230513 07.lcd	17.415	855457	30909	8925.148	1.034	2.713
230513 08.lcd	17.472	854815	30822	8950.396	1.036	2.711
Average	17.49217	855412.7	30889	9000.32	1.04	2.721
% RSD	0.25	0.06	0.36	0.84	0.15	0.33
STDV	0.04	503.32	111.50	75.97	0.00	0.009

Precision – Inter day (RLS 193)

Title	RT	Area	Height	Theoretical Plates	Tailing Factor	Resolution
230513 03.lcd	20.675	606656	25128	15914.824	0.983	4.566
230513 04.lcd	20.727	607756	25031	15822.39	0.98	4.563
230513 05.lcd	20.711	609736	25570	16390.12	0.982	4.601
230513 06.lcd	20.674	608277	25192	15931.139	0.983	4.551
230513 07.lcd	20.587	609524	25119	15628.119	0.983	4.544
230513 08.lcd	20.656	605829	24927	15671.188	0.985	4.552
Average	20.67167	607963	25161	15892.96	0.98	4.563
% RSD	0.24	0.25	0.88	1.72	0.17	0.45
STDV	0.05	1548.15	220.34	273.28	0.00	0.020

Precision – Inter day (RLS 218)

Title	RT	Area	Height	Theoretical Plates	Tailing Factor	Resolution
230513_03.lcd	21.476	232603	10913	21718.47	1.139	1.294
230513_04.lcd	21.528	234102	10895	21472.372	1.132	1.286
230513_05.lcd	21.502	234803	11001	21742.483	1.136	1.286
230513_06.lcd	21.472	234103	10982	21717.533	1.137	1.289
230513_07.lcd	21.931	237559	10998	21043.068	1.127	1.288
230513_08.lcd	21.46	232614	11037	21852.884	1.111	1.295
Average	21.5615	234297.3	10971	21591.14	1.13	1.290
% RSD	0.85	0.78	0.50	1.37	0.92	0.30
STDV	0.18	1826.17	55.22	296.00	0.01	0.004

Precision – Inter day (RLS 205)

Title	RT	Area	Height	Theoretical Plates	Tailing Factor	Resolution
230513_03.lcd	26.609	308754	12029	24013.913	1.055	8.085
230513_04.lcd	26.661	286524	11914	24296.828	1.053	8.071
230513_05.lcd	26.591	309584	11906	23528.519	1.061	7.973
230513_06.lcd	26.559	308456	11890	23483.094	1.064	7.973
230513_07.lcd	26.47	309159	11946	23426.948	1.059	7.927
230513_08.lcd	26.524	309606	11953	23551.287	1.06	7.963
Average	26.569	305347.2	11940	23716.76483	1.059	7.9986667
% RSD	0.25	3.02	0.42	1.49	0.38	0.80
STDV	0.07	9232.57	49.91	353.89	0.00	0.064

Table 2: Precision – Intraday
Precision – Intraday (RLS 228)

Title	RT	Area	Height	Theoretical Plates	Tailing Factor	Resolution
220513_11.lcd	15.927	768520	27764	7459.514	1.035	0.000
220513_12.lcd	15.864	771976	27567	7264.052	1.04	0.000
220513_13.lcd	15.856	771585	27441	7195.638	1.042	0.000
220513_14.lcd	15.838	772190	27632	7265.819	1.04	0.000
220513_15.lcd	15.842	771737	27449	7187.954	1.041	0.000
220513_16.lcd	15.889	772841	27477	7236.024	1.043	0.000
Average	15.87	771474.8	27555	7268.17	1.04	0.000
% RSD	0.212	0.196	0.459	1.367	0.268	0.000
STDV	0.03	1512.68	126.55	99.36	0.003	0.000

Precision – Intraday (RLS 222)

Title	RT	Area	Height	Theoretical Plates	Tailing Factor	Resolution
220513_11.lcd	17.955	850841	31283	9842.924	1.036	2.775
220513_12.lcd	17.905	852115	31027	9603.16	1.035	2.767
220513_13.lcd	17.901	850552	30966	9592.529	1.036	2.767
220513_14.lcd	17.87	851852	31140	9644.003	1.037	2.763
220513_15.lcd	17.888	851557	30889	9506.48	1.036	2.763
220513_16.lcd	17.937	851577	30862	9537.649	1.035	2.764
Average	17.90933	851415.7	31028	9621.124	1.036	2.7665
% RSD	0.18	0.07	0.52	1.24	0.07	0.16
STDV	0.03	600.27	160.18	119.14	0.00	0.005

Precision – Intraday (RLS 193)

Title	RT	Area	Height	Theoretical Plates	Tailing Factor	Resolution
220513_11.lcd	21.117	600758	25290	17154.873	0.98	4.620
220513_12.lcd	21.086	601317	25124	16833.143	0.981	4.607
220513_13.lcd	21.091	600494	25094	16863.779	0.98	4.620
220513_14.lcd	21.041	600993	25171	16854.071	0.982	4.609
220513_15.lcd	21.083	601311	24971	16666.319	0.981	4.607
220513_16.lcd	21.136	600627	24938	16717.337	0.982	4.608
Average	21.09233	600916.7	25098	16848.254	0.981	4.612
% RSD	0.15	0.06	0.52	1.01	0.09	0.14
STDV	0.03	349.09	130.09	170.15	0.00	0.006

Precision – Intraday (RLS 218)

Title	RT	Area	Height	Theoretical Plates	Tailing Factor	Resolution
220513_11.lcd	21.898	232697	10994	22962.189	1.131	1.277
220513_12.lcd	21.874	231013	10882	22961.038	1.134	1.280
220513_13.lcd	21.878	229991	10840	22766.534	1.141	1.280
220513_14.lcd	21.828	232582	10926	22557.127	1.134	1.280
220513_15.lcd	21.874	232413	10851	22363.24	1.141	1.278
220513_16.lcd	21.926	231360	10834	22573.793	1.138	1.276
Average	21.87967	231676	10888	22697.320	1.1365	1.279
% RSD	0.15	0.46	0.57	1.06	0.36	0.14
STDV	0.03	1075.65	62.14	241.25	0.00	0.002

Precision – Intraday (RLS 205)

Title	RT	Area	Height	Theoretical Plates	Tailing Factor	Resolution
220513 11.lcd	27.111	306682	11497	23207.663	1.062	8.082
220513 12.lcd	27.117	303028	11350	23007.739	1.061	8.090
220513 13.lcd	27.152	307891	11637	23575.446	1.074	8.193
220513 14.lcd	27.035	307325	11548	23198.436	1.059	8.064
220513 15.lcd	27.129	307884	11529	23228.471	1.062	8.102
220513 16.lcd	27.153	307147	11498	23239.714	1.063	8.066
Average	27.11617	306659.5	11510	23242.912	1.0635	8.100
% RSD	0.16	0.60	0.81	0.79	0.50	0.59
STDV	0.04	1837.65	93.64	184.02	0.01	0.048

Table 3: Calculation of LOD and LOQ

Sample/ Con (ppm)	0.488	1.953	7.812	31.25	125	RSD	Slope	LOD	LOQ
RLS - 228	11525	47106	194736	774015	3044114	6644.128	24342.57	0.90	2.73
RLS - 222	12564	53662	214149	852357	3356073	6553.835	26834.15	0.81	2.44
RLS - 193	8782	36906	160878	657921	2612974	3596.531	20923.71	0.57	1.72
RLS - 218	3123	13911	67307	281073	1122888	1580.679	9003.574	0.58	1.76
RLS - 205	4049	21491	76120	306612	1214159	1881.122	9707.089	0.64	1.94

Table 4: Linearity

Sample/ Con (ppm)	0.488	1.953	7.812	31.25	125	500
RLS - 228	11525	47106	194736	774015	3044114	12665390
RLS - 222	12564	53662	214149	852357	3356073	13932059
RLS - 193	8782	36906	160878	657921	2612974	10845523
RLS - 218	3123	13911	67307	281073	1122888	4684712
RLS - 205	4049	21491	76120	306612	1214159	5017317

Figure 1: Linearity of RLS-228

Figure 2: Linearity RLS-222

Figure 3: Linearity RLS-193

Figure 4: Linearity RLS-218

Figure 5: RLS-205

CONCLUSION

On this basis of this study that the proposed HPLC method for separation of all the said five derivatives were separated and validated on the parameters viz., Resolution between any two peaks: NLT 1.0, Peak tailing factor of any peak in the mixture solution: NMT 1.5, RSD for six replicate injections for mixture preparation: NMT 2.0 % was achieved as per the Pharmacopeias requirements.

REFERENCES

1. Shu Juan Liu, Zong Tao Lin, Hong Wang and Shi Zhong Chen. Quantification of eugenol and bancroftone in Caryophylli Fructus using high performance liquid chromatography. Journal of Chinese Pharmaceutical Sciences, R927.2, A, 1003-1057(2010)-459-05. [http://www.jcps.ac.cn/qikan/manage/wenzhang/1003-1057\(2010\)6-459-05.pdf](http://www.jcps.ac.cn/qikan/manage/wenzhang/1003-1057(2010)6-459-05.pdf)
2. Yong Hong Li, Zhi Hao Sun and Pu Zheng. Determination of Vanillin, Eugenol and Isoeugenol by RP-HPLC; Chromatographia; 2004. p. 709-713. <http://rd.springer.com/article/10.1365%2Fs10337-004-0440-4>. <http://dx.doi.org/10.1365/s10337-004-0440-4>
3. Jadhav BK, Khandelwal KR, Ketkar AR, Pisal SS. Formulation and evaluation of muco adhesive tablets containing eugenol for the treatment

of periodontal diseases. Drug Dev Ind Pharm 2004; 30(2): 195–203. <http://www.ncbi.nlm.nih.gov/pubmed/15089054>. <http://dx.doi.org/10.1081/DDC-120028715> PMID:15089054

4. Jack L Ferracane. Materials in Dentistry; Principles and Applications, 2001, 2nd Edition, Lippincott Williams and Wilkins; 2013.
5. ICH Harmonised Tripartite Guideline Validation Of Analytical Procedures: Methodology (Q2R1) International Conference on Harmonization; 1997.
6. Hyekyung Ha, Chang Seob Seo, Seong Sil Kim and Jung Hoon Kim. Simultaneous Determination of Gallic Acid, Ellagic Acid, and Eugenol in *Syzygium aromaticum* and Verification of Chemical Antagonistic Effect by the Combination with *Curcuma aromatica* Using Regression Analysis. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3708417>.
7. Sagar Saran, Sasikumar Menon, Sunita Shailajan and Priyanka Pokharna. Validated RP-HPLC method to estimate eugenol from commercial formulations like Caturjata Churna, Lavangadi Vati, Jatiphaladi Churna, Sitopaladi Churna and clove oil. Journal of Pharmacy Research 2013; 6: 53E60.

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