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Research Article

HPLC METHOD DEVELOPMENT FOR EMBELIN ESTERS DERIVATIVES

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ABSTRACT
A simple reverse phase liquid chromatographic method has been developed and subsequently validated for the derivative of the Embelin Esters substituted derivatives. The separation was carried out using a mobile phase consisting of acetic acid buffer in HPLC grade water (as mobile phase A) and methanol (as mobile phase B). An Inertsil ODS, 3.0 V (250 X 4.6 mm and 5.0 µ), HPLC column was used with flow rate of 1.0 ml / min using UV-Vis detection at 260 nm of Shimadzu HPLC (model: LC-2010). The analytical method, data integrations, processes and calculations were processed by LC solution soft ware. There are ten Embelin substituted ester derivatives are taken for analysis viz., RLS 33, 34, 36, 37, 38, 40, 41, 42, 44 and 45. 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 ten derivatives interims of validation parameters viz., separation, system suitability, System Precision and linearity in a single simple HPLC run.
Keywords: Embelin ester derivatives, High Performance Liquid Chromatography, Separation and Linearity

INTRODUCTION

Embelin (2, 5-dihydroxy-3-undecyl-1, 4-benzoquinone) is a naturally occurring alkyl substituted hydroxy benzoquinone and a major constituent of *Embelia ribes* Burm. (Family: Myrsinaceae). The plant is indicated in traditional medicine for the treatment of various diseases. The fruit is bitter in taste, good appetizer, cures tumors, ascites, bronchitis, jaundice, brain tonic, mental disorders, dyspnoea, diseases of the heart, urinary discharges, scorpion-sting, snake-bite and tooth ache[1]. It also exhibits antioxidant properties in diabetic animals and anti-inflammatory to relieve rheumatism and fever[2,3]. Embelin showed antifertility[4], antiimplantation[5], antitumor, analgesic[6], antioxidant, hepatoprotective, wound healing, antibacterial and anticonvulsant activities[7]. In this study, ten novel esters of Embelin derivatives were synthesized and analyzed for separations and linearity[8,9] in a single HPLC run.

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 10 µl and the analysis was performed at ambient temperature. The HPLC column was used Inertsil ODS, 3.0 V, 5µ, 250 X 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.

- 2,5-Di-O-(3-fluorophenylcarbonyl)-3-undecyl-1,4-enzoquinone (RLS 38)
- 2,5-Di-O-(4-chlorophenylcarbonyl)-3-undecyl-1,4-benzoquinone (RLS 33)
- 2,5-Di-O-(2-bromophenylcarbonyl)-3-undecyl-1,4-benzoquinone (RLS 34)
- 2,5-Di-O-(4-bromophenylcarbonyl)-3-undecyl-1,4-benzoquinone (RLS 36)
- 2,5-Di-O-(3-nitrophenylcarbonyl)-3-undecyl-1,4-benzoquinone (RLS 40)
- 2,5-Di-O-(2-fluorophenylcarbonyl)-3-undecyl-1,4-benzoquinone (RLS 41)
- 2,5-Di-O-(4-Nitrophenylcarbonyl)-3-undecyl-1,4-benzoquinone (RLS 42)
- 2,5-Di-O-(3-methoxyphenylcarbonyl)-3-undecyl-1,4-benzoquinone (RLS 44)
- 2,5-Di-O-(4-methoxyphenylcarbonyl)-3-undecyl-1,4-benzoquinone (RLS 45)
- 2,5-Di-O-(2-iodophenylcarbonyl)-3-undecyl-1,4-benzoquinone (RLS 37)

Preparation of Test Solution

10 mg of the each standard samples were weighed in to the 10 ml standard volumetric flask, dissolved and make up to the mark separately with diluents Methanol : Water (60:40) ratio. The concentration of the sample is 1000 ppm. From this 1000 ppm concentrations, by dilutions 20, 100, 200, 500 and 1000 ppm sample concentrations were prepared.

HPLC method

The Mobile phase selected for this method contained 0.25 %v/v Acetic acid buffer (2.5 ml /1000 ml of HPLC grade water) 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 µl and the Auto sampler and column compartment was maintained at ambient temperature (25°C). Detection of eluent was carried out at 260 nm using UV-Vis detector. With the optimized chromatographic conditions, a steady baseline was recorded, the mixed standard solution was injected, chromatogram ware recorded. The retention time of the said five Embelin ester derivatives (working standard) were eluted at 24.4, 48.6, 30.2, 60.8, 25.8, 15.1, 28.6, 21.4, 18.7 and 40.3 minutes, respectively for samples RLS 38, 33, 34, 36, 40, 41, 42, 44, 45 and 37 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, system precision and system suitability

Resolution

One of the system suitability parameters is resolution; the acceptance criteria of the resolution is Not Less Than (NLT) 1 and for the tailing factors is Not More Than (NMT) 1.5, the percentage RSD of replicate of injections is NMT 2.0 %

Linearity

The ten derivatives were found to be in a linear in a concentrations range of 20 – 1000 ppm. 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 1.

Resolution and Linearity

The said 10 molecules are separated with resolution NLT 1.5 minutes with the linearity correlation coefficient NLT 0.999. Refer the Annexure 4 respective chromatograms and linearity calculations.

System Precision and System suitability

One of the system suitability concentration solution was injected and the % RDS for 6 replicates injection was achieved NLT 15. Refer the Annexure 5 respective chromatograms and calculations.

Table 1: Precision (Repeatability)

Precision (RLS 41)

Title	RT	Area	Theoretical Plates	Tailing Factor	Resolution
500 ppm-R1.lcd	15.034	77923	10860	1.128	0
500 ppm-R2.lcd	15.058	77757	10889	1.125	0
500 ppm-R3.lcd	15.077	77590	10915	1.126	0
500 ppm-R4.lcd	15.105	77542	10896	1.122	0
500 ppm-R5.lcd	15.132	77693	10877	1.123	0
500 ppm-R6.lcd	15.141	77734	10884	1.124	0
Average	15.09	77706.50	10886.83	1.12	0
STDV	0.04	134.90	18.48	0.00	0
% RSD	0.28	0.17	0.17	0.19	0

Precision (RLS 45)

Title	RT	Area	Theoretical Plates	Tailing Factor	Resolution
500 ppm-R1.lcd	18.603	219100	10843	1.07	5.54
500 ppm-R2.lcd	18.642	219009	10823	1.069	5.54
500 ppm-R3.lcd	18.671	219101	10823	1.07	5.55
500 ppm-R4.lcd	18.721	218597	10759	1.069	5.56
500 ppm-R5.lcd	18.769	218555	10800	1.069	5.58
500 ppm-R6.lcd	18.785	218798	10773	1.07	5.59
Average	18.70	218860.00	10803.50	1.07	5.56
STDV	0.07	246.54	32.38	0.00	0.02
% RSD	0.39	0.11	0.30	0.05	0.38

Precision (RLS 44)

Title	RT	Area	Theoretical Plates	Tailing Factor	Resolution
500 ppm-R1.lcd	21.285	30289	11335	1.074	3.55
500 ppm-R2.lcd	21.333	37982	11416	1.078	3.55
500 ppm-R3.lcd	21.362	38499	11445	1.084	3.55
500 ppm-R4.lcd	21.416	37610	11477	1.082	3.54
500 ppm-R5.lcd	21.476	38499	11366	1.081	3.54
500 ppm-R6.lcd	21.495	38612	11346	1.066	3.54
Average	21.39	36915.17	11397.50	1.08	3.545
STDV	0.08	3268.74	57.39	0.01	0.01
% RSD	0.39	8.85	0.50	0.61	0.15

Precision (RLS 38)

Title	RT	Area	Theoretical Plates	Tailing Factor	Resolution
500 ppm-R1.lcd	24.327	30587	12941	1.054	3.68
500 ppm-R2.lcd	24.383	30351	12914	1.058	3.68
500 ppm-R3.lcd	24.411	30283	12943	1.065	3.68
500 ppm-R4.lcd	24.475	30450	12888	1.049	3.68
500 ppm-R5.lcd	24.526	30097	12716	1.066	3.64
500 ppm-R6.lcd	24.545	30055	13071	1.068	3.66
Average	24.44	30303.83	12912.17	1.06	3.67
STDV	0.09	204.40	114.96	0.01	0.02
% RSD	0.35	0.67	0.89	0.71	0.46

Precision (RLS 40)

Title	RT	Area	Theoretical Plates	Tailing Factor	Resolution
500 ppm-R1.lcd	25.711	935781	11942	1.293	1.52
500 ppm-R2.lcd	25.751	934123	12008	1.292	1.52
500 ppm-R3.lcd	25..775	934211	11976	1.293	1.52
500 ppm-R4.lcd	25.817	932579	11907	1.288	1.48
500 ppm-R5.lcd	25.855	932201	11830	1.287	1.46
500 ppm-R6.lcd	25.873	931413	11854	1.287	1.47
Average	25.80	933384.67	11919.50	1.29	1.50
STDV	0.07	1606.74	69.26	0.00	0.03
% RSD	0.27	0.17	0.58	0.23	1.88

Precision (RLS 42)

Title	RT	Area	Theoretical Plates	Tailing Factor	Resolution
500 ppm-R1.lcd	28.514	172027	14099	1.067	2.95
500 ppm-R2.lcd	28.557	173084	14064	1.081	2.95
500 ppm-R3.lcd	28.583	171029	14077	1.069	2.95
500 ppm-R4.lcd	28.628	170490	14108	1.06	2.94
500 ppm-R5.lcd	28.668	170340	14028	1.063	2.93
500 ppm-R6.lcd	28.667	170407	13938	1.064	2.93
Average	28.60	171229.50	14052.33	1.07	2.94
STDV	0.06	1107.24	62.75	0.01	0.01
% RSD	0.22	0.65	0.45	0.69	0.33

Precision (RLS 34)

Title	RT	Area	Theoretical Plates	Tailing Factor	Resolution
500 ppm-R1.lcd	30.095	174738	8481	1.856	1.38
500 ppm-R2.lcd	30.144	190383	8048	1.895	1.38
500 ppm-R3.lcd	30.175	184137	7868	1.941	1.37
500 ppm-R4.lcd	30.223	180577	8334	1.923	1.39
500 ppm-R5.lcd	30.266	175995	8465	1.877	1.4
500 ppm-R6.lcd	30.29	175258	8491	1.865	1.4
Average	30.20	180181.33	8281.17	1.89	1.39
STDV	0.07	6185.02	262.92	0.03	0.01
% RSD	0.25	3.43	3.17	1.77	0.87

Precision (RLS 37)

Title	RT	Area	Theoretical Plates	Tailing Factor	Resolution
500 ppm-R1.lcd	40.097	48214	13479	1.15	7.3
500 ppm-R2.lcd	40.193	47871	13314	1.104	7.34
500 ppm-R3.lcd	40.238	47781	13268	1.119	7.3
500 ppm-R4.lcd	40.238	47781	13268	1.119	7.3
500 ppm-R5.lcd	40.393	48238	13047	1.134	7.42
500 ppm-R6.lcd	40.447	47799	13122	1.117	7.45
Average	40.27	47947.33	13249.67	1.12	7.35
STDV	0.13	218.52	151.51	0.02	0.07
% RSD	0.32	0.46	1.14	1.42	0.91

Precision (RLS 33)

Title	RT	Area	Theoretical Plates	Tailing Factor	Resolution
500 ppm-R1.lcd	48.338	46039	16874	0.964	5.77
500 ppm-R2.lcd	48.48	46150	17153	0.97	5.77
500 ppm-R3.lcd	48.576	46129	16969	0.974	5.77
500 ppm-R4.lcd	48.704	46691	16751	0.981	5.69
500 ppm-R5.lcd	48.842	45022	16973	0.959	5.8
500 ppm-R6.lcd	48.915	46942	16576	0.977	5.78
Average	48.64	46162.17	16882.67	0.97	5.76
STDV	0.22	663.96	200.07	0.01	0.04
% RSD	0.45	1.44	1.19	0.85	0.66

Precision (RLS 36)

Title	RT	Area	Theoretical Plates	Tailing Factor	Resolution
500 ppm-R1.lcd	60.431	65870	14026	1.091	6.9
500 ppm-R2.lcd	60.619	60104	14611	1.078	6.96
500 ppm-R3.lcd	60.724	61433	14215	1.1	6.89
500 ppm-R4.lcd	60.954	60956	14316	1.08	6.91
500 ppm-R5.lcd	61.07	66171	13750	1.075	6.83
500 ppm-R6.lcd	61.189	67811	13560	1.042	6.78
Average	60.83	63724.17	14079.67	1.08	6.88
STDV	0.29	3265.32	384.21	0.02	0.06
% RSD	0.47	5.12	2.73	1.84	0.93

Table 2: Linearity

Sample/ Con (ppm)	20	100	200	500	1000
RLS - 41	3052	15599	25843	77898	153870
RLS - 45	7856	44127	73041	219164	433320
RLS - 44	1387	7313	12086	37952	75921
RLS - 38	1045	5255	9711	30673	60682
RLS - 40	37480	187773	311826	936094	1850347
RLS - 42	6407	34072	56343	173339	340600
RLS - 34	6760	33734	57562	175249	333316
RLS - 37	1854	8253	14828	48222	97020
RLS - 33	1942	9932	15383	46637	92700
RLS - 36	2558	13418	21122	63018	126689

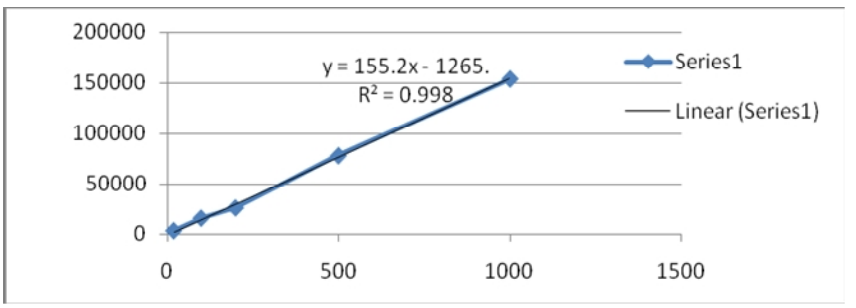

Figure 1: Linearity of RLS-41

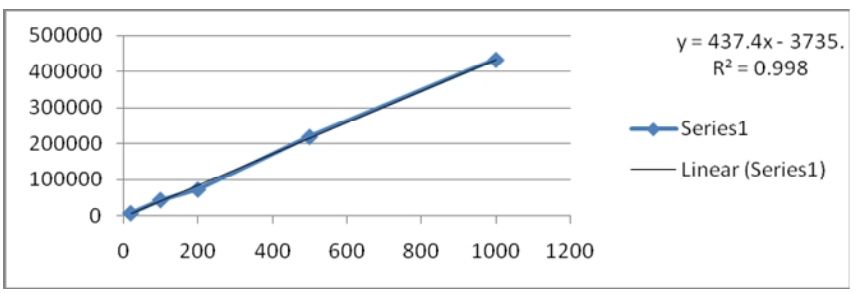

Figure 2: Linearity of RLS-45

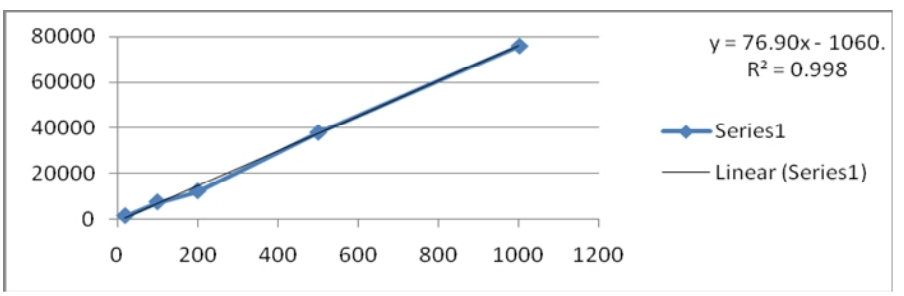

Figure 3: Linearity of RLS-44

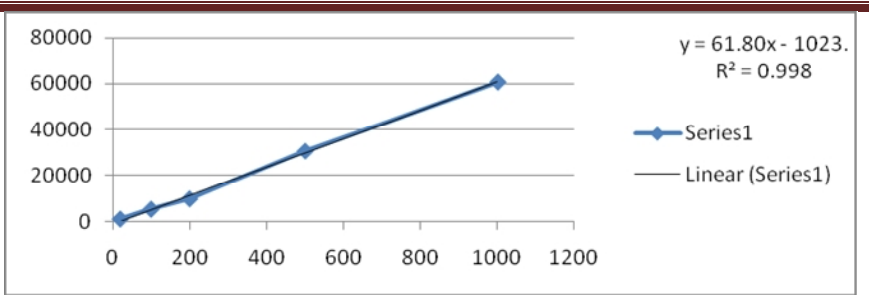

Figure 4: Linearity of RLS-38

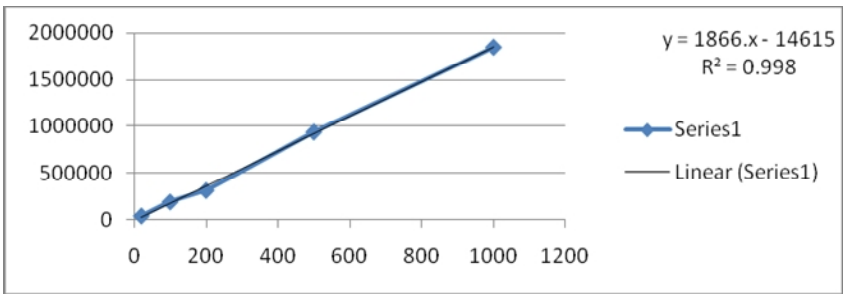

Figure 5: Linearity of RLS-40

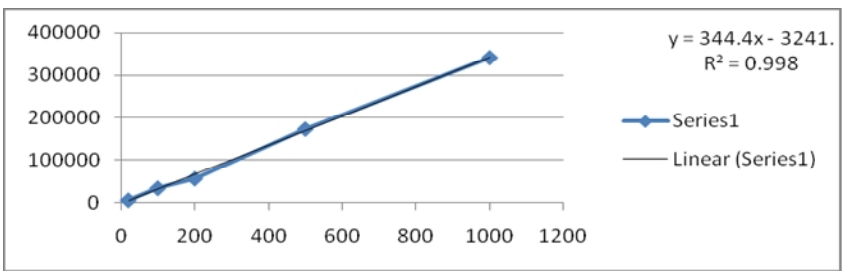

Figure 6: Linearity of RLS-42

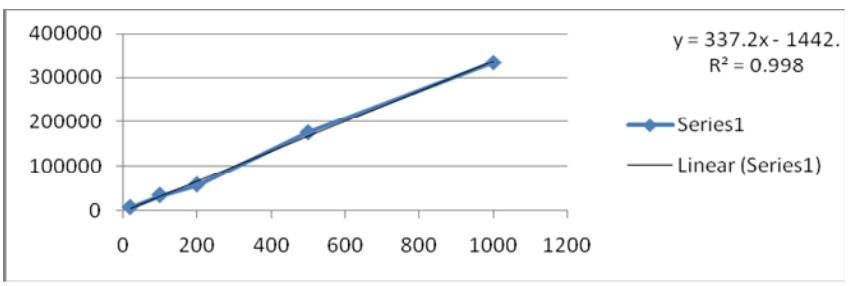

Figure 7: Linearity of RLS-34

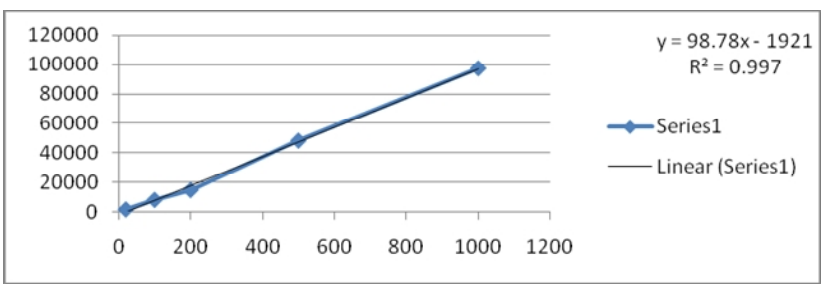

Figure 8: Linearity of RLS-37

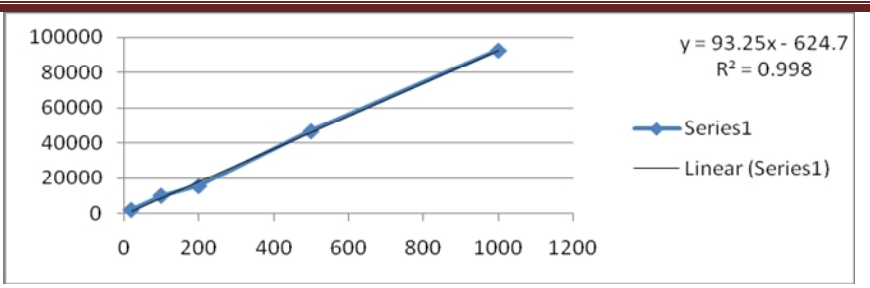

Figure 9: Linearity of RLS-33

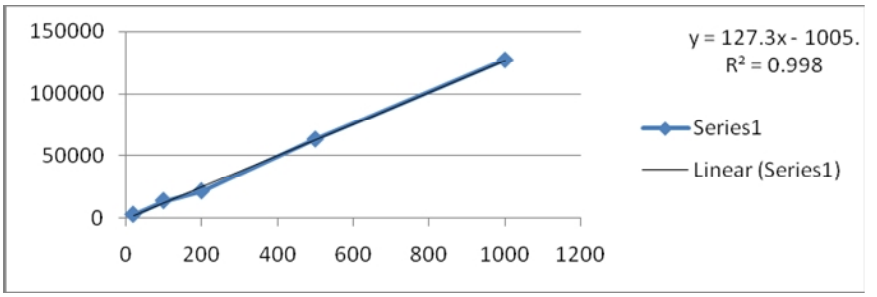

Figure 10: Linearity of RLS-36

CONCLUSION

Separation

The separation between the two peaks is achieved as NLT 1.5 minutes. The minimum requirement of the Pharmacobial limits is achieved

Linearity

The Linearity correlation co-efficient is achieved NLT 0.999. The minimum requirement of the Pharmacobial limits is achieved

System Precision

The system precision is achieved as the % RDS for 6 replicates observed as 5.125 for the RLS 36 sample, hence the minimum requirement of the Pharmacobial limits NMT 15 % RSD is achieved

System suitability

The system suitability parameters like theoretical plates, asymmetric and % RSD (in system precision) are achieved as per the minimum requirement of the Pharmacobial limits.

Details of the Laboratory work were carried out

Quality Control Lab, Dhirubhai Ambani Life Sciences Centre.R-282, TTC industrial area, M.I.D.C., Thane Belapur Road, Opposite to Rabale Station, Navi Mumbai, India.

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