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High Performance liquid chromatographic (HPLC) Selection of an Important Medicinal Plant *Stevia rebaudiana* Bertoni. Based on the Percentage of Sweet Diterpene Glycosides

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Abstract

Diabetes is the fastest spreading disease that occurs when the blood glucose, is very much high, and being main source of energy, we need to regularly monitor blood glucose. *Stevia rebaudiana* as a natural sweetener, with its all-medicinal properties, had great potential to keep an eye on blood glucose. Therefore, the aim of this research is to investigate the most efficient *S. rebaudiana* plants via HPLC among the 84 test plants in their high rebaudioside and stevioside content. A lot of variation was observed among the individuals, as the percentage of rebaudioside-A exceeded up to 9.37% followed by 8.65, 8.62%, and 8.24%. Similarly, in the case of stevioside among 84 individuals the percentage of stevioside varied from 0.23- 8.64%. It would be concluded that HPLC is a fantastic method that offers an accurate analysis that can facilitate the knowledge of more diverse plants that can be used for creating hybrid vigor among the Stevia plants that in turn would help the pharmaceutical industry for making more efficient stevia artificial sweeteners from Stevia.

Keywords: *Stevia rebaudiana*, HPLC, Rebaudioside, Stevioside, Medicinal plant

Introduction

Stevia rebaudiana Bertoni, an incredible member of the family Asteraceae, is one of the most valuable tropical and subtropical perennial medicinal plant [1]. It is at first originated as a South American wild plant [2] and in Paraguay, it had been used as a natural sweetener for centuries [3]. After its Introduction in India at Bangalore, the Institute of Himalayan Bioresource Technology (IHBT-CSIR), Palampur, introduced two accessions of stevia for domestication and cultivation in Himachal Pradesh [4]. Stevia is anti-bacterial, anti-fungal [5], anti-inflammatory, anti-viral [6,7], anticancerous [8] cardiotonic, antihypertensive [9] diuretic and hypoglycemic [10]. It is used as a tabletop sweetener, in soft drinks, baked food items, pickles, fruit juices, jams, jellies, candies and chewing gums. It is recommended for diabetics and has been extensively tested on animals and used by humans with no side effects [11,12].

Stevia is highly distinguished by the presence of sweet diterpene glycosides: rebaudioside-A, rebaudioside-C, stevioside and dulcoside in its leaf tissue. Wide variation in the percentage of these sweet diterpenoid glycosides has been reported in the leaves and other parts of *S. rebaudiana* and this has been ascribed to both genetic and environmental factors. The stevioside is the major component but it has an unpleasant bitter after-taste. However, rebaudioside-A, normally present in lower amount (25% to 45% of stevioside content) in leaves, does not have bitter after-taste and has a sweetening power 1.2 to 1.6 times higher than stevioside [13]. Usually, stevioside is the dominant glycoside, but types rich in rebaudioside-A have also been reported [14]. The presence of these high potency sweeteners has attracted a huge interest in stevia production. Nearly three decades of breeding and selection have increased glycoside concentration in stevia leaves up to levels of 20 per cent [14]. However, this improvement

is based on biochemical selection and measurements are based on High Performance Liquid Chromatography (HPLC) which lead to the selection for plants producing high amounts of glycosides. The objective of this research is to investigate the most efficient plants via HPLC among the 84 test plants in their high rebaudioside and stevioside content in *S. rebaudiana*. Therefore, HPLC is a fantastic method that offers rapid analysis that can facilitate the knowledge of more diverse plants that can be used for future breeding of Stevia that in turn would help pharmaceutical industry for making artificial sweeteners from Stevia. This study would directly enhance the quality of raw materials in artificial sweetener industry.

Materials and Methods

The estimates of Rebaudioside-A and Stevioside were made based on High Performance Liquid Chromatography (HPLC) grade chemicals procured from standard firms such as Sisco Research Laboratories Pvt. Ltd., Maharashtra, India, Sigma (Sigma-Aldrich, Bangalore, India) and Chromadex, Irvine, USA. Plant material i.e. leaves were collected from the field grown 84 individual plants and their fresh weight was taken individually on the day of collection. The collected leaves kept at room temperature for drying for few days till they left around 70% of their moisture. They were then oven dried (60°C) and weighed thrice at regular intervals till their dry weight became constant. The dried leaf samples were then powdered by grinding them in pestle and mortar. The powdered samples were weighed (300 mg), transferred to thimbles and refluxed with dichloromethane on a boiling water bath to remove colour by using soxhlet apparatus individually. Non-polar compounds and colours were removed through extraction with dichloromethane. The boiling point of dichloromethane is 39.75°C so during dichloromethane reflux, the water bath was continuously switched on and off and the process was continued till the color was completely removed.

The residual samples in thimbles were then refluxed with methanol in soxhlet apparatus to extract its sweetness. The process is continued till the samples in thimbles become tasteless. Methanol was used for final sweet compounds extraction. The extract was then distilled off to recover methanol. The concentrated extract was dissolved in 15 ml acetonitrile: water (78:22) mixture and these plant extracts were then filtered through Millipore filter paper using Millipore filter assembly and filtered samples were kept in volumetric flasks and the samples were then analysed by HPLC. Before injecting the solutions of reference compounds and samples, HPLC column was run in HPLC grade methanol for few hours and then with acetonitrile: water (78: 22) solvent mixture.

HPLC Conditions

Instrument: Waters HPLC unit with Waters HPLC pump 515 and dual-absorbance detector 2487

Mobile phase: Acetonitrile: water (78:22)

Flow Rate: 1.0 ml/min.

Column: 5 µm spherisorb NH2 column (4.6 mm x 250 mm).

Volume injected: 20µl

Detection: 210 nm

Percentages of rebaudioside-A and stevioside were calculated using the following formula:

$$Sweet\ Compound\ (\%) = \frac{Test\ Area}{Standard\ Area} \times \frac{Weight\ of\ Standard\ Compound}{Standard\ Compound\ dilution} \times \frac{Test\ sample\ dilution}{Test\ sample\ weight} \times 100$$

Using the above formula rebaudioside-A and stevioside content of all the 84 test plants were estimated.

Result and Discussion

The percentage of rebaudioside-A and stevioside was obtained on high performance liquid chromatography (HPLC) for all the 84 test plants. Standard curve for rebaudioside-A and stevioside were prepared using standard rebaudioside-A and stevioside (Table 1, Figure 1a and b). All the 84 individuals were scored for their percentage of rebaudioside-A and stevioside (Table 2) which is based on the peaks obtained. Percentage of rebaudioside-A and stevioside was calculated on the basis of the formula given in section of Material and Methods. Rebaudioside-A ranged from 0.00 to 9.37% whereas stevioside ranged from 0.23% to 8.64% in the test plants (Table 2) (Figure 2).

Table 1: Standard curve area for rebaudioside-A and stevioside.

Concentration (ppm) Rebaudioside-A/ Stevioside	Area under curve (AUC) Rebaudioside-A	Area under curve (AUC) Stevioside
500	273853	339038
750	416613	537588
1000	553534	710642

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Table 2: Percentage of rebaudioside-A and stevioside among 84 test individuals.

S.No.	Plant Code	Rebaudioside-A (%)	Log of % age content of Rebaudioside-A	Stevioside (%)	Log of % age content of stevioside content
1.	1	0.67	-0.17	8.46	0.93
2.	2	7.3	0.86	4.62	0.66
3.	3	4.09	0.61	3.59	0.55
4.	4	4.12	0.61	4.69	0.67
5.	5	6.16	0.8	5.07	0.7
6.	6	4.55	0.66	4.74	0.68
7.	7	1.21	0.08	3.4	0.53
8.	8	1.55	0.18	3.5	0.54
9.	9	5.86	0.77	2.68	0.43
10.	10	7.54	0.88	2.99	0.48
11.	11	4.37	0.64	2.73	0.43
12.	12	0.93	-0.03	3.81	0.58
13.	13	9.37	0.97	3.28	0.51
14.	14	4.35	0.64	6.45	0.81
15.	15	0.81	-0.09	4.93	0.69
16.	16	0.55	-0.26	5.26	0.72
17.	17	0.75	-0.12	3.38	0.52
18.	18	3.42	0.53	3.7	0.57
19.	19	2.89	0.46	1.22	0.09
20.	20	0.4	-0.4	7.83	0.89
21.	21	1.45	0.16	7.46	0.87
22.	22	0.44	-0.35	6.89	0.84
23.	23	4.86	0.69	2.5	0.4
24.	24	5.96	0.78	4.92	0.69
25.	25	5.95	0.77	2.34	0.37
26.	26	2.99	0.48	3.3	0.52
27.	27	3.98	0.6	3.8	0.58
28.	28	3.43	0.54	3.8	0.58
29.	29	3.74	0.57	2.32	0.37
30.	30	3.19	0.5	2.2	0.34
31.	31	0	0	8.6	0.93
32.	32	2.38	0.38	5.05	0.7
33.	33	2.85	0.45	4.59	0.66
34.	34	3.36	0.53	5.59	0.74
35.	35	4.5	0.65	6.71	0.83
36.	36	3.62	0.56	4.79	0.68
37.	37	3.28	0.51	2.89	0.46
38.	38	0.97	-0.01	5.45	0.74
39.	39	4.06	0.6	4.71	0.67
40.	40	2.98	0.47	3.38	0.53
41.	41	3.08	0.49	3.34	0.52

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42.	42	2.82	0.45	2.2	0.34
43.	43	3.23	0.51	8.18	0.91
44.	44	0.82	-0.09	7.33	0.87
45.	45	8.24	0.92	3.75	0.57
46.	46	6.14	0.79	4.21	0.62
47.	47	4.42	0.65	6.88	0.84
48.	48	6.85	0.84	5.69	0.75
49.	49	5.42	0.73	5.05	0.7
50.	50	7.51	0.88	5.83	0.77
51.	51	4.66	0.67	6.13	0.79
52.	52	6.73	0.83	4.9	0.69
53.	53	1.77	0.25	3.67	0.56
54.	54	2.45	0.39	2.31	0.36
55.	55	2.23	0.35	3.93	0.59
56.	56	2.38	0.38	6.47	0.81
57.	57	3.08	0.49	6.21	0.79
58.	58	2.95	0.47	2.09	0.32
59.	59	0.72	-0.14	4.85	0.69
60.	60	0.34	-0.47	6.62	0.82
61.	61	3.35	0.53	7.37	0.87
62.	62	3.69	0.57	5.63	0.75
63.	63	4.11	0.61	6.76	0.83
64.	64	4.51	0.65	5.63	0.75
65.	65	2.37	0.37	8.63	0.94
66.	66	2.92	0.47	3.14	0.5
67.	67	2.52	0.4	2.87	0.46
68.	68	4.45	0.65	5.21	0.72
69.	69	8.62	0.94	6.58	0.82
70.	70	1.99	0.3	2.06	0.31
71.	71	0	0	8.64	0.94
72.	72	2.37	0.37	3.14	0.5
73.	73	3.07	0.49	2.51	0.4
74.	74	4.98	0.7	5.51	0.74
75.	75	3.12	0.49	5.13	0.71
76.	76	2.31	0.36	5.8	0.76
77.	77	8.65	0.94	2.19	0.34
78.	78	0.85	-0.07	2.73	0.44
79.	79	2.04	0.31	1.65	0.22
80.	80	4.55	0.66	3.53	0.55
81.	81	0.17	0	0.23	-0.64
82.	82	4.43	0.65	3.72	0.57
83.	83	0	0	3.76	0.58
84.	84	5.09	0.71	3.35	0.53

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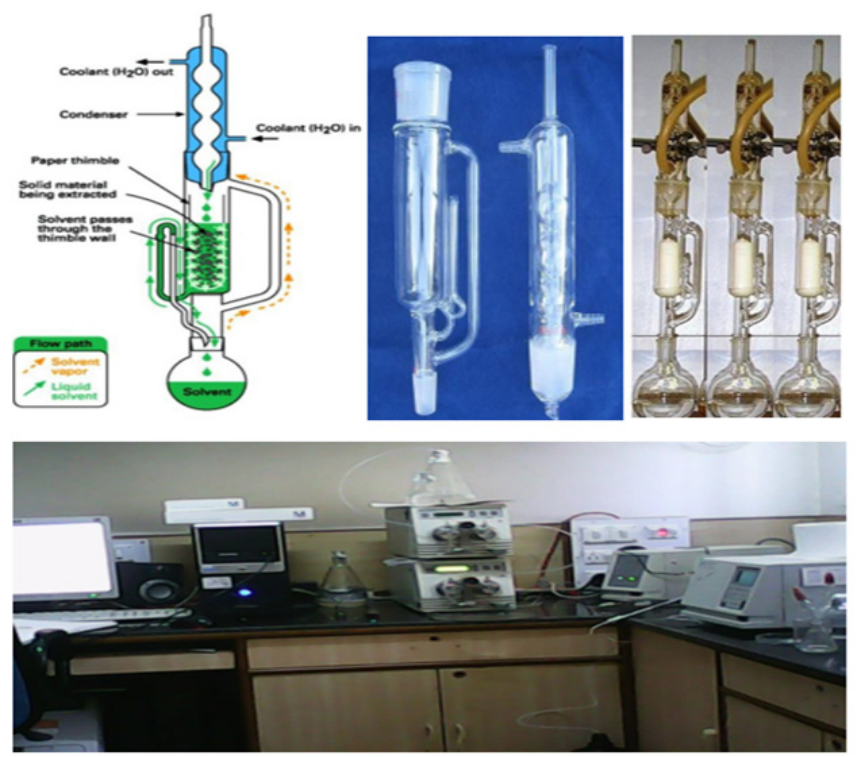

Figure 1: Soxhlet apparatus and other equipment's used to carry out HPLC.

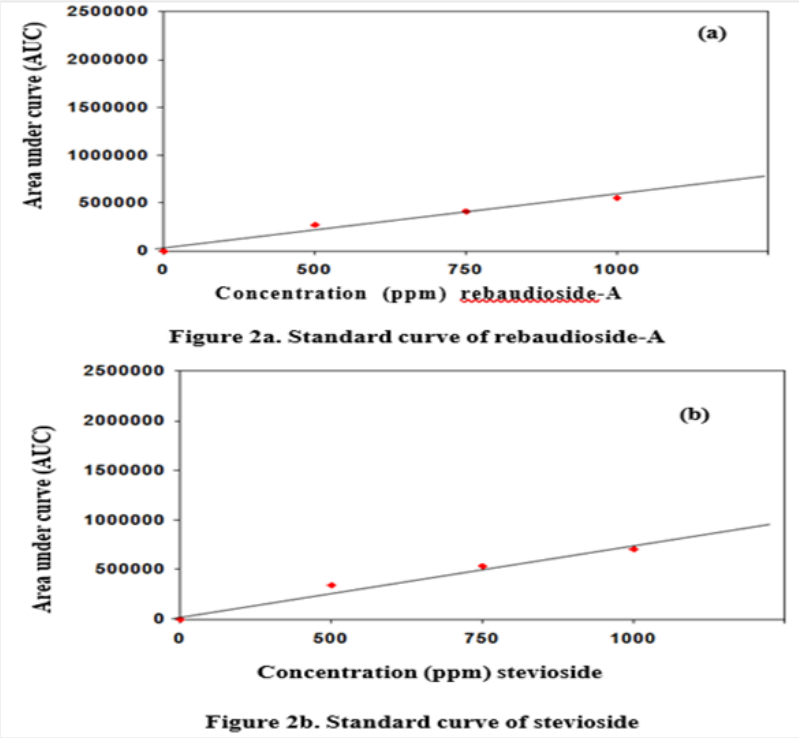

Figure 2: In Figure 2(a) and (b) X-axis depicts concentration of reference standard compound in ppm while on Y axis Area under curve values are presented.

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On observing the data, it was clearly seen that there was lots of variation among the individuals, as the percentage of rebaudioside-A exceeded upto 9.37% (13) followed by 8.65 (77), 8.62% (69), 8.24% (45). Similarly, in case of stevioside among 84 individuals the percentage of stevioside varied from 0.23 (81) -8.64 (71). Depending on the analysis the scientists can use the most divergent stevia individuals and looking at their variation hybrid vigour can be created.

Conclusion

The chromatographic results obtained in this study have clearly demonstrated that HPLC is a promising technique to discover the best divergent *Stevia rebaudiana* with high rebaudioside content and low stevioside content (because of mild bitterness) for the material selection process to create hybrid vigour. Additionally, the study also added to our knowledge that leaf parts of *Stevia rebaudiana* can produce the finest possible products due to its highest content of bioactive compounds. Therefore, HPLC is a fabulous method that offers accurate analysis that can facilitate the quality herbal products in pharmaceutical industry and hence would help the health-conscious people or people suffering from diabetes. This study would definitely boom in pharmaceutical industry for making artificial sweeteners from Stevia. This study would directly enhance the quality of raw materials in artificial sweetener industry.

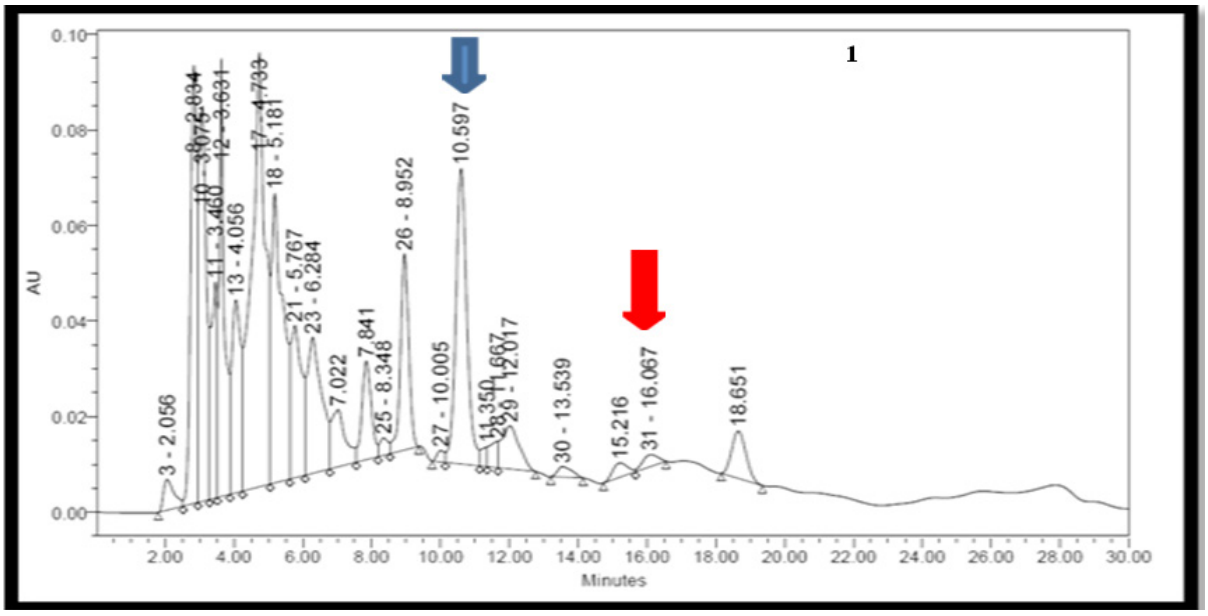
Declarations

Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

S. No.	Plant Code	Stevioside Retension Time	Stevioside Area	Stevios-ide (%)	Rebaudioside-A Retention Time	Rebaudioside-A Area	Rebaudioside-A (%)
1	1	10.597	1202278	8.46	16.067	74557	0.67
2	2	10.569	657113	4.62	15.985	808216	7.3
3	3	10.557	509779	3.59	15.972	453185	4.09
4	4	10.541	667326	4.69	15.92	456134	4.12
5	5	10.537	720219	5.07	15.858	682319	6.16
6	6	10.559	674731	4.74	15.904	504419	4.55

Plate 1: Percentage of Stevioside and Rebaudioside-A based on retention time and Area under curve.



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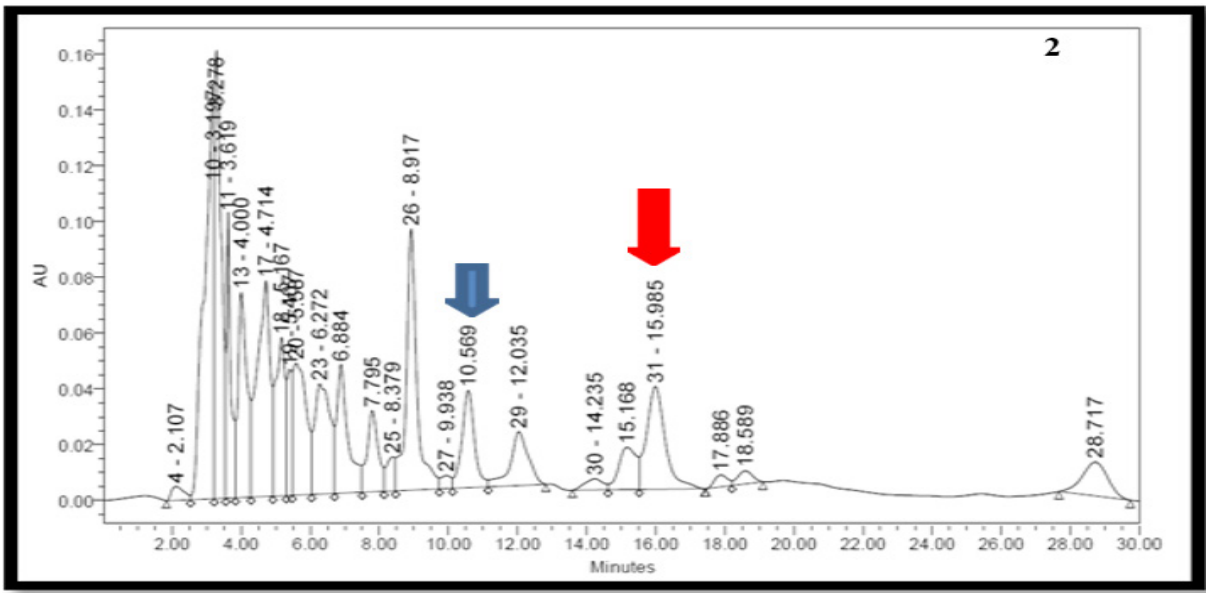

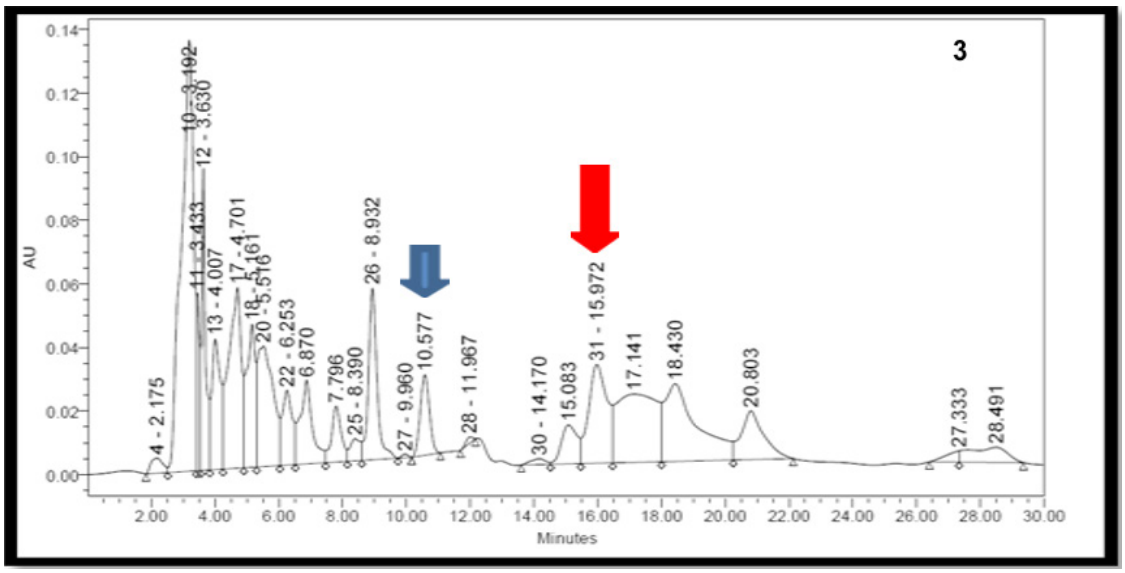

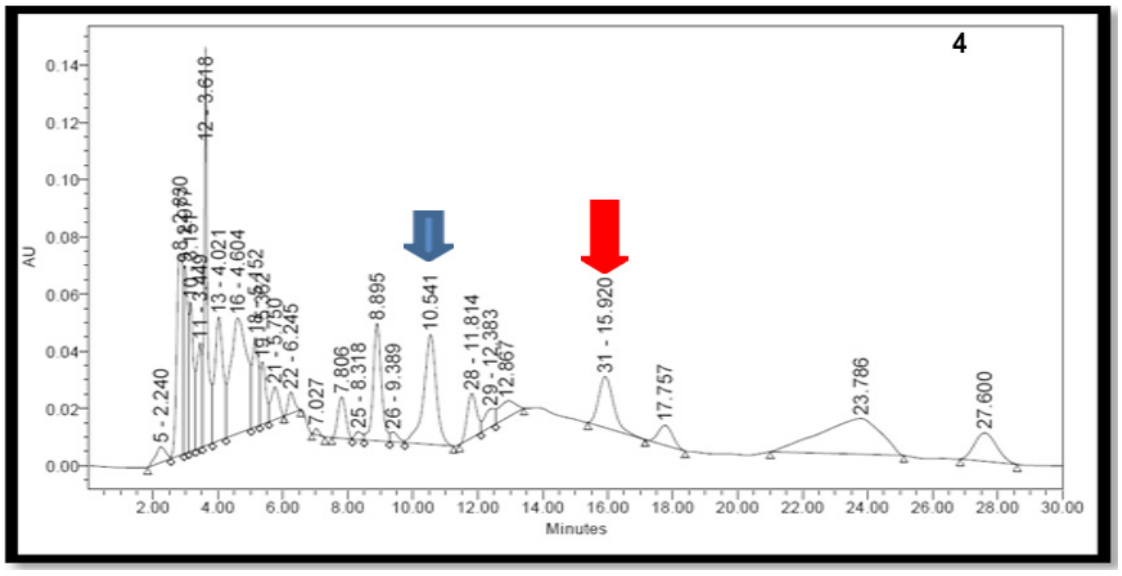

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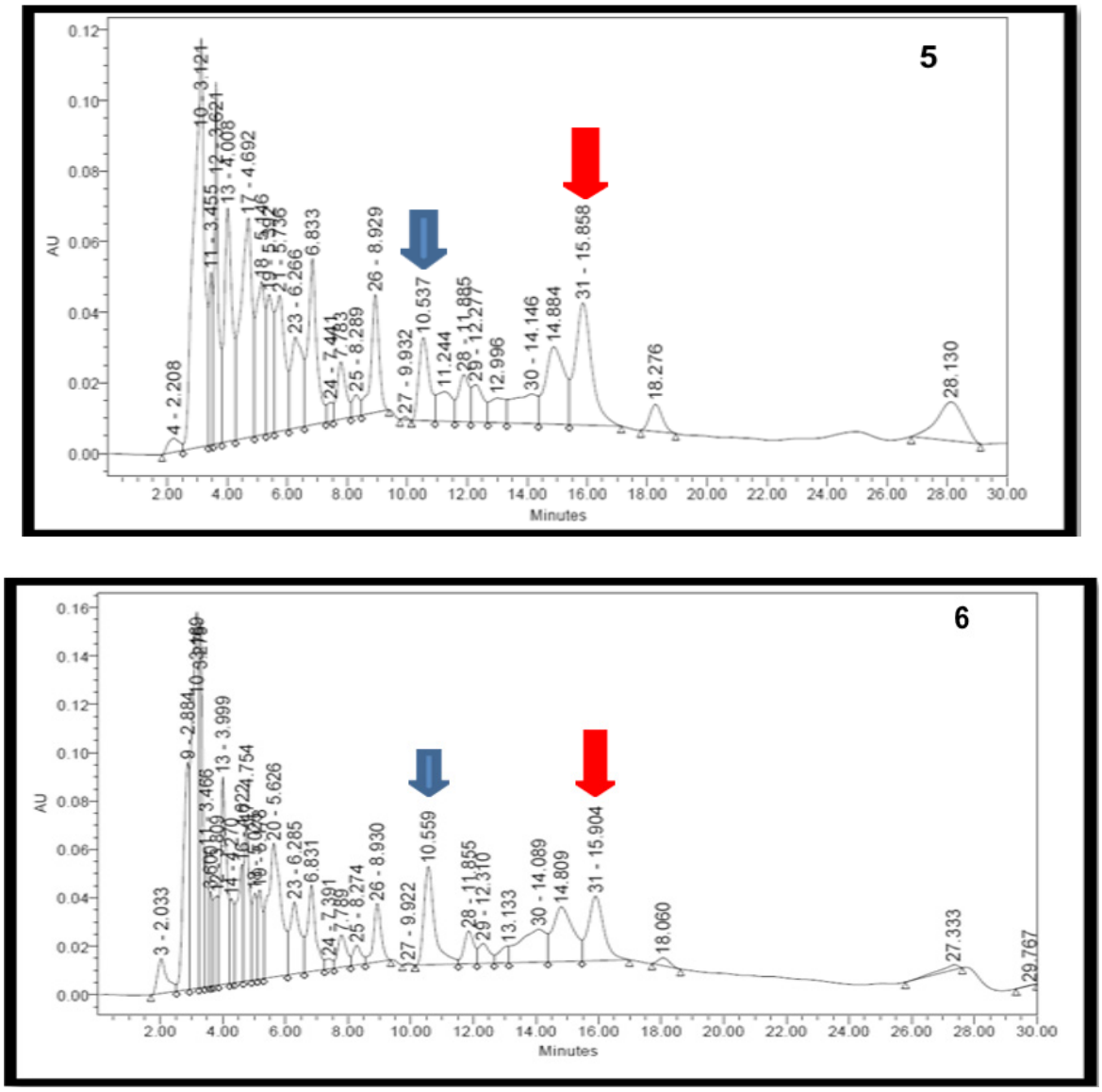

Plate 1: Blue arrow depicts Stevioside peak and Red arrow depicts Rebaudioside A peak in all the plates.

S. No	Plant Code	Stevioside Retension Time	Stevioside Area	Stevioside (%)	Rebaudioside-A Retention Time	Rebaudioside-A Area	Rebaudioside-A (%)
7	7	10.542	483388	3.4	15.803	133517	1.21
8	8	10.516	498424	3.5	15.772	171627	1.55
9	9	10.472	381222	2.68	15.755	648722	5.86
10	10	10.462	425194	2.99	15.702	834351	7.54
11	11	10.438	388260	2.73	15.695	483506	4.37
12	12	10.439	542462	3.81	15.669	103081	0.93

Plate 2: Percentage of Stevioside and Rebaudioside-A based on retention time and Area under curve.

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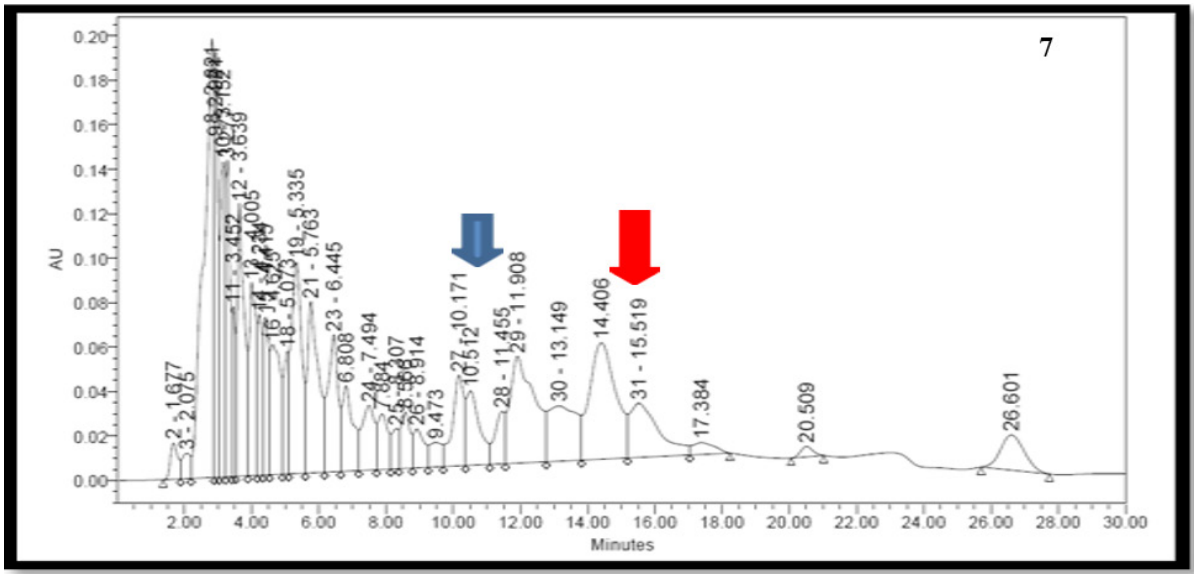

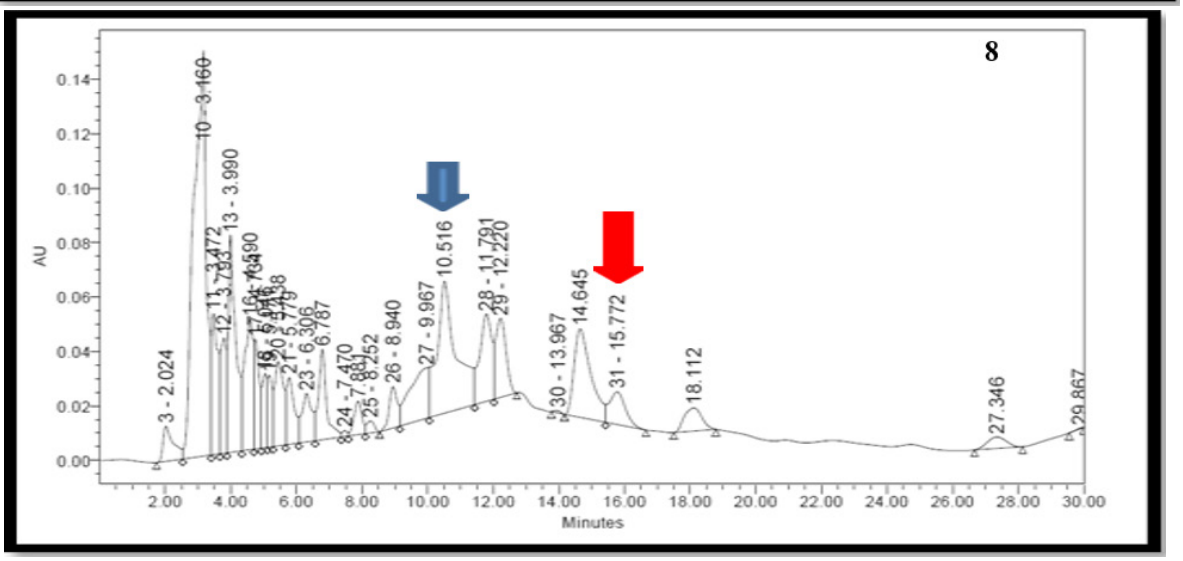

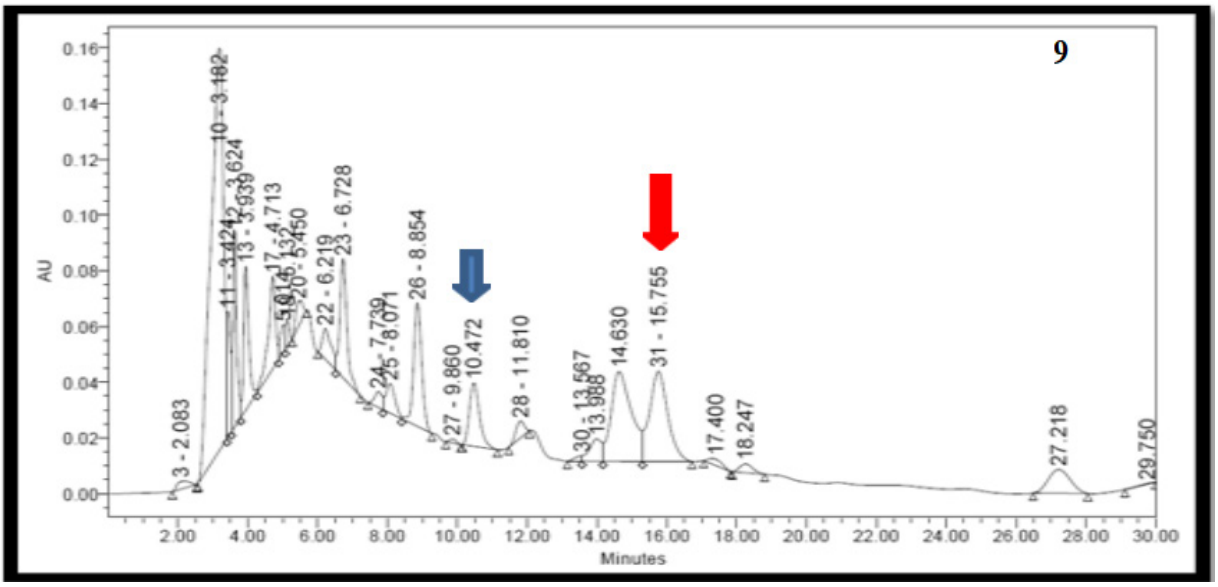

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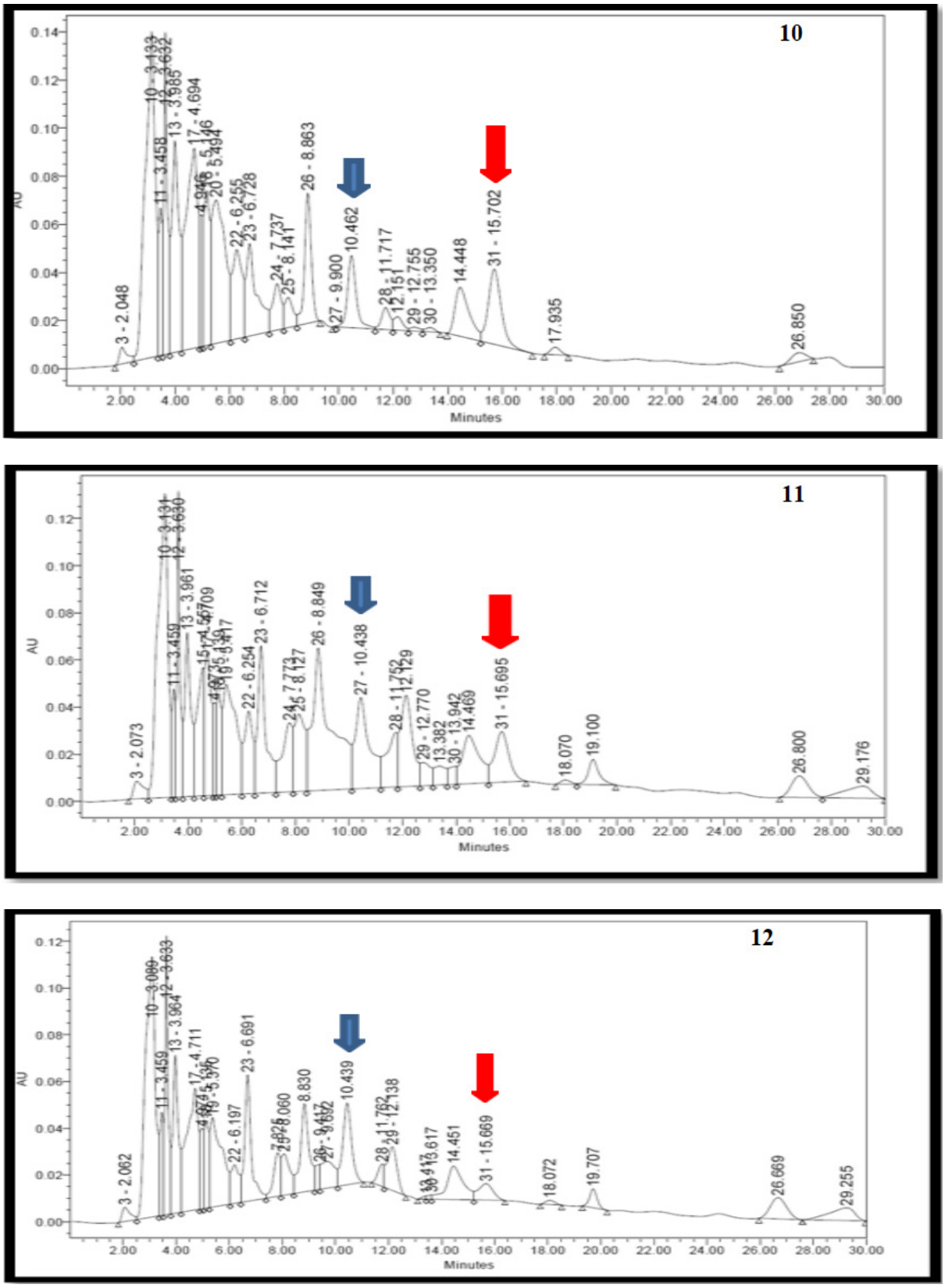

Plate 2: Blue arrow depicts Stevioside peak and Red arrow depicts Rebaudioside A peak in all the plates.

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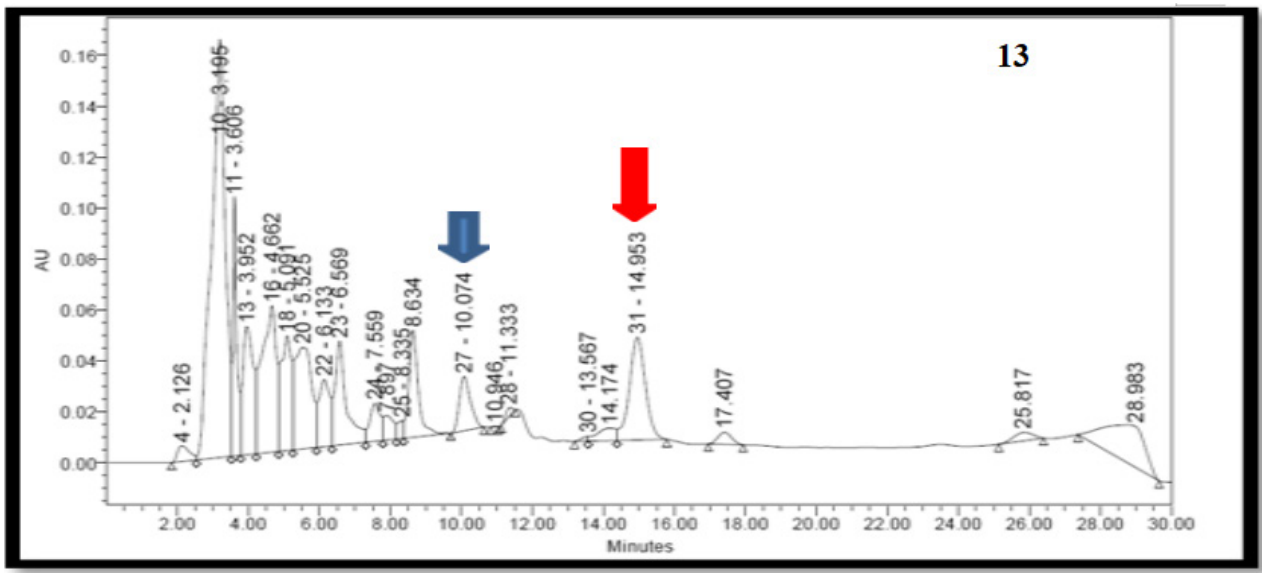
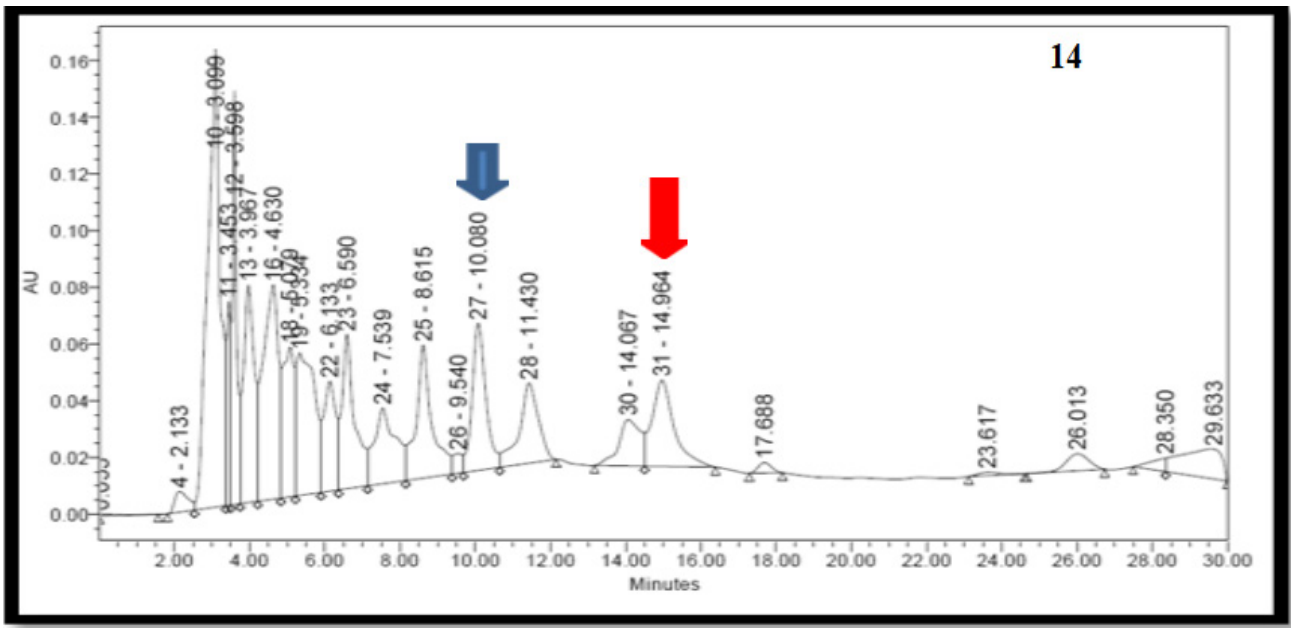
S. No.	Plant Code	Stevioside Retention Time	Stevioside Area	Stevioside (%)	Rebaudioside-A Retention Time	Rebaudioside-A Area	Rebaudioside-A (%)
13	13	10.074	466721	3.28	14.953	1037359	9.37
14	14	10.08	916930	6.45	14.964	482305	4.35
15	15	10.081	700904	4.93	14.76	89694	0.81
16	16	11.064	748254	5.26	17.208	60873	0.55
17	17	11.02	481214	3.38	17.19	83772	0.75
18	18	11.026	527037	3.7	17.12	378620	3.42

Plate 3: Percentage of Stevioside and Rebaudioside-A based on retention time and Area under curve.





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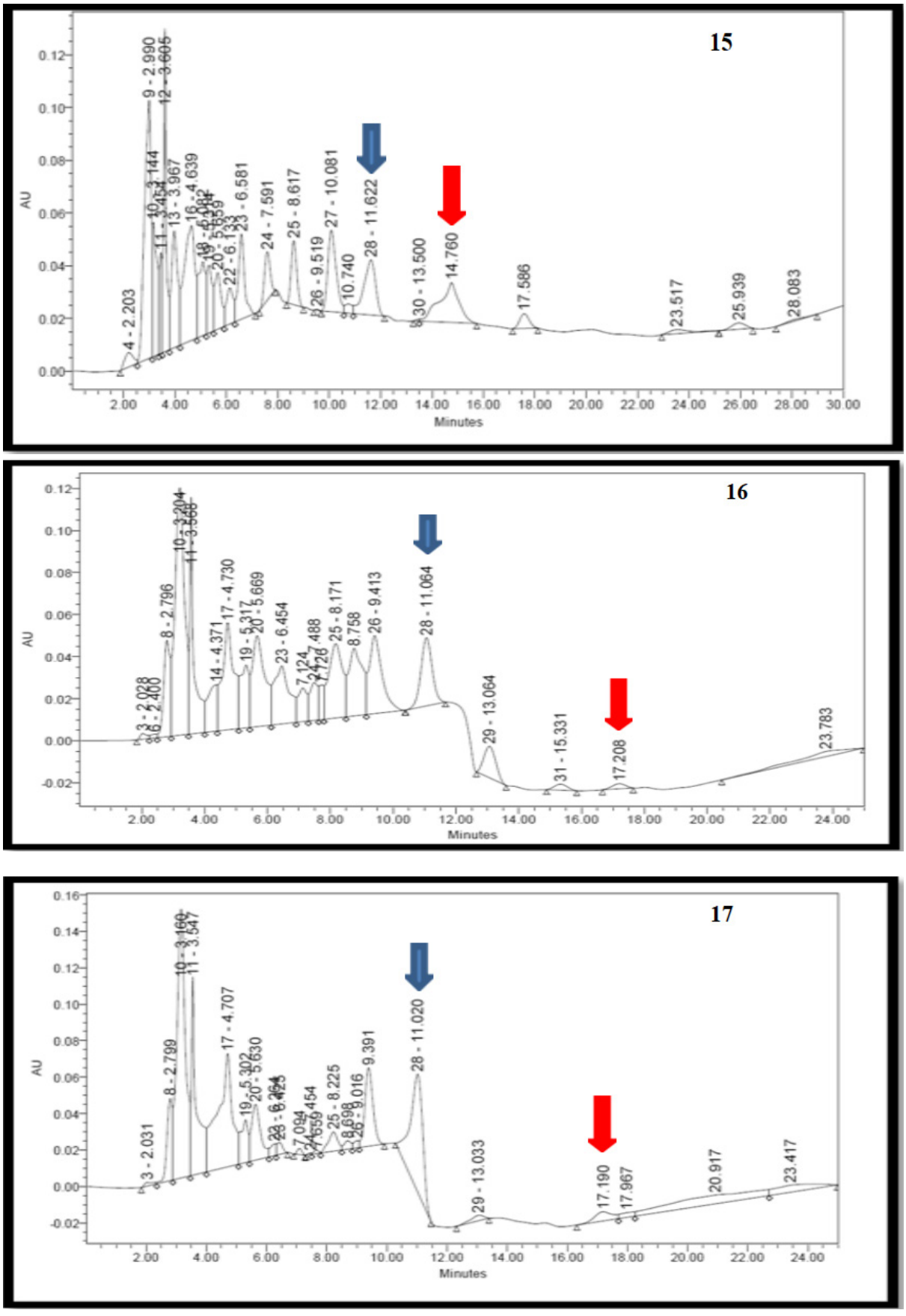

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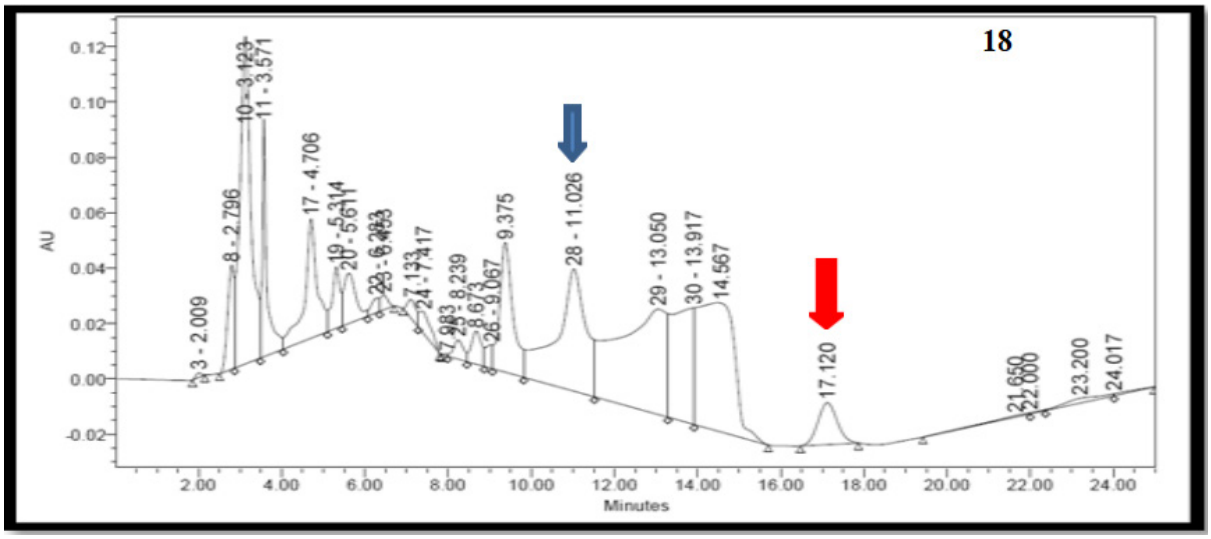
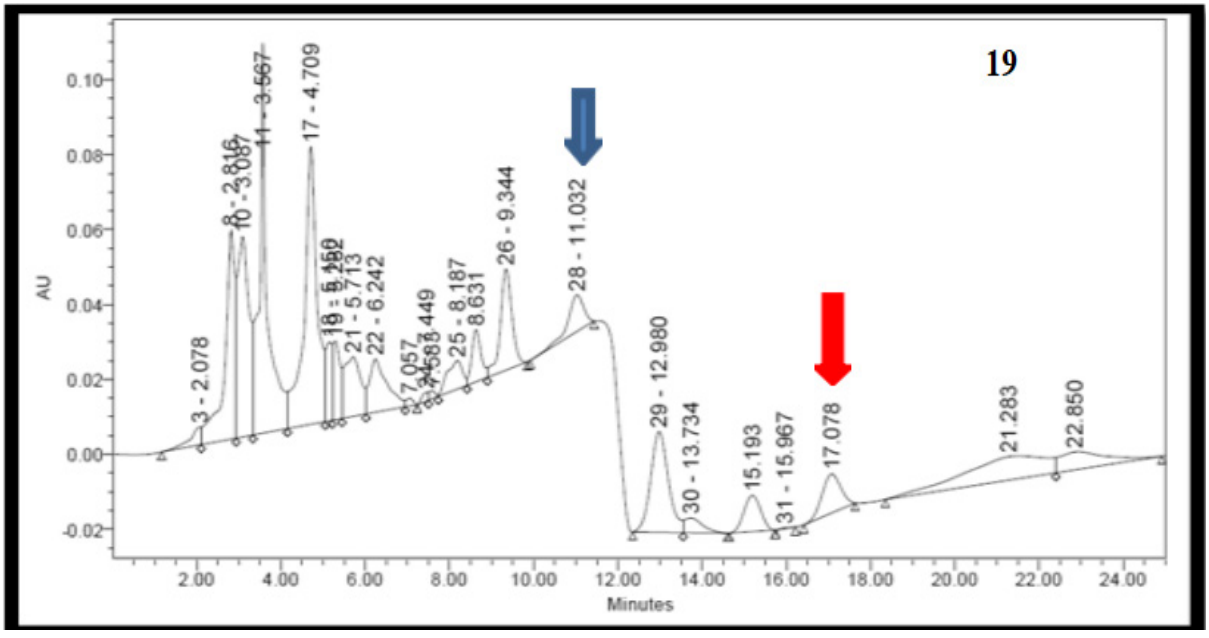


Plate 3: Blue arrow depicts Stevioside peak and Red arrow depicts Rebaudioside A peak in all the plates.

S. No.	Plant Code	Stevioside Retension Time	Stevioside Area	Stevioside (%)	Rebaudioside-A Retention Time	Rebaudioside-A Area	Rebaudioside-A (%)
19	19	11.032	174452	1.22	17.078	319780	2.89
20	20	11.016	1113792	7.83	17.05	44739	0.4
21	21	10.981	1060927	7.46	17.054	161119	1.45
22	22	11.462	979336	6.89	17.882	48843	0.44
23	23	11.415	356617	2.5	17.846	538370	4.86
24	24	11.415	699733	4.92	17.797	659823	5.96

Plate 4: Percentage of Stevioside and Rebaudioside-A based on retention time and Area under curve.



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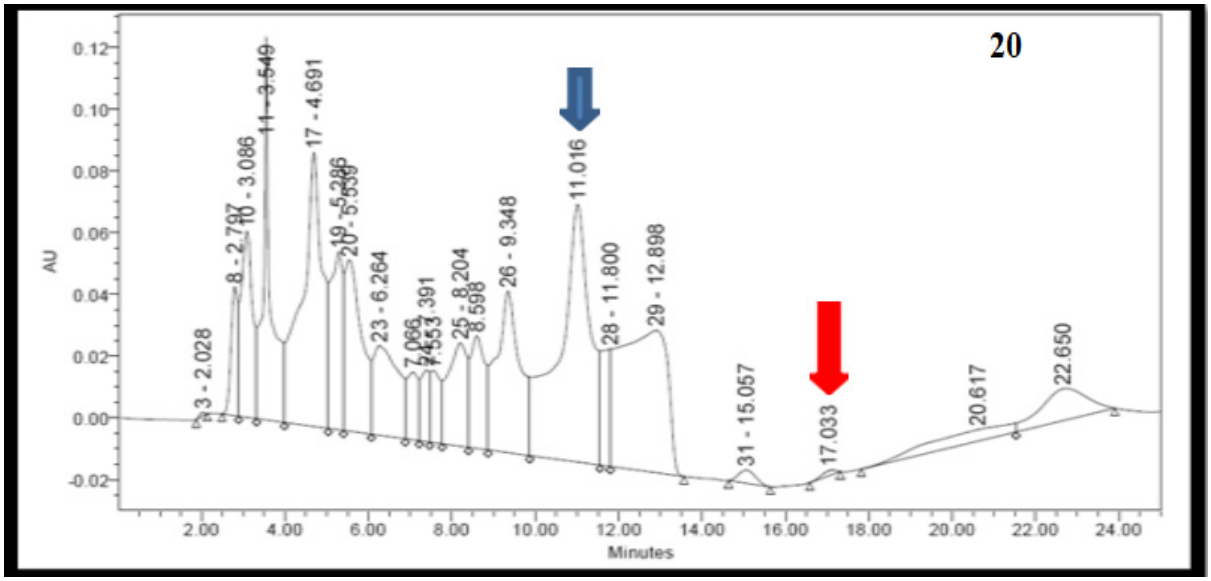

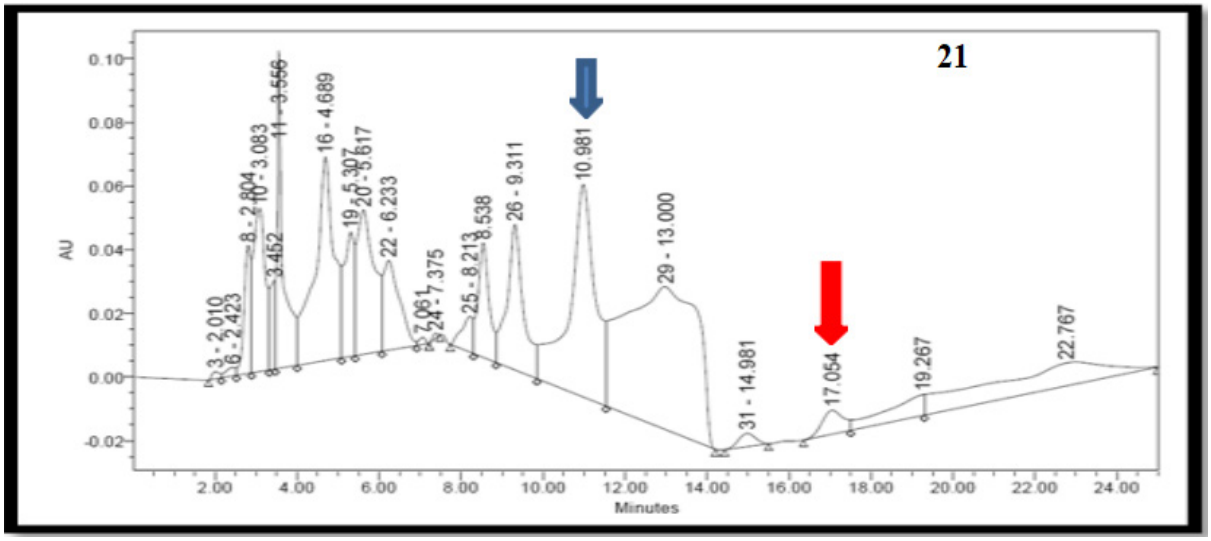

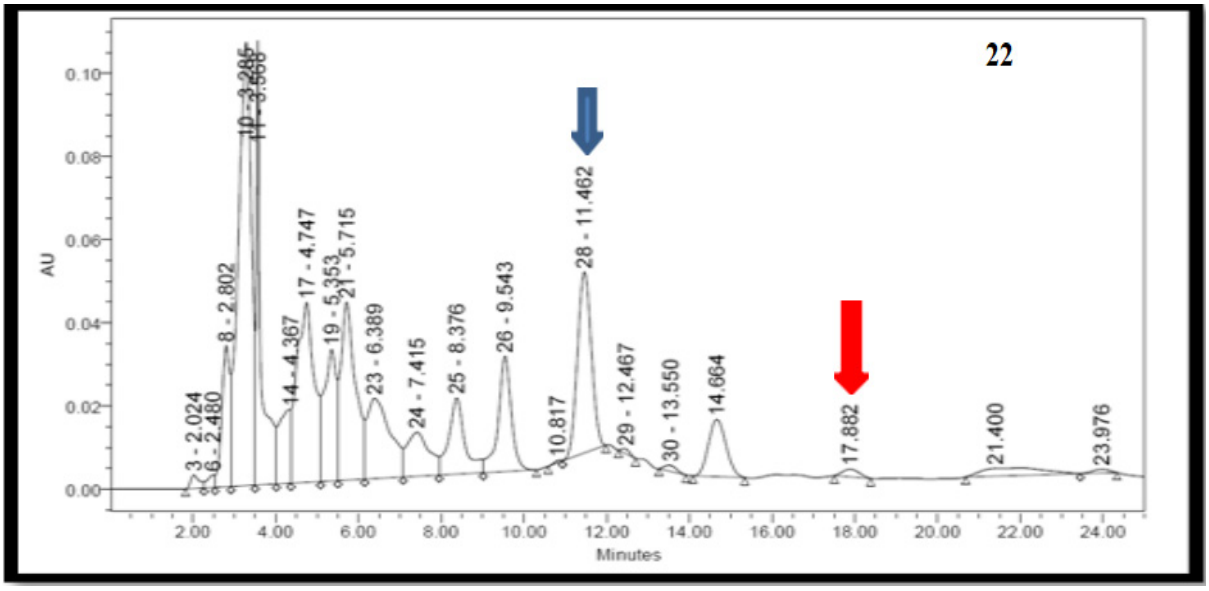

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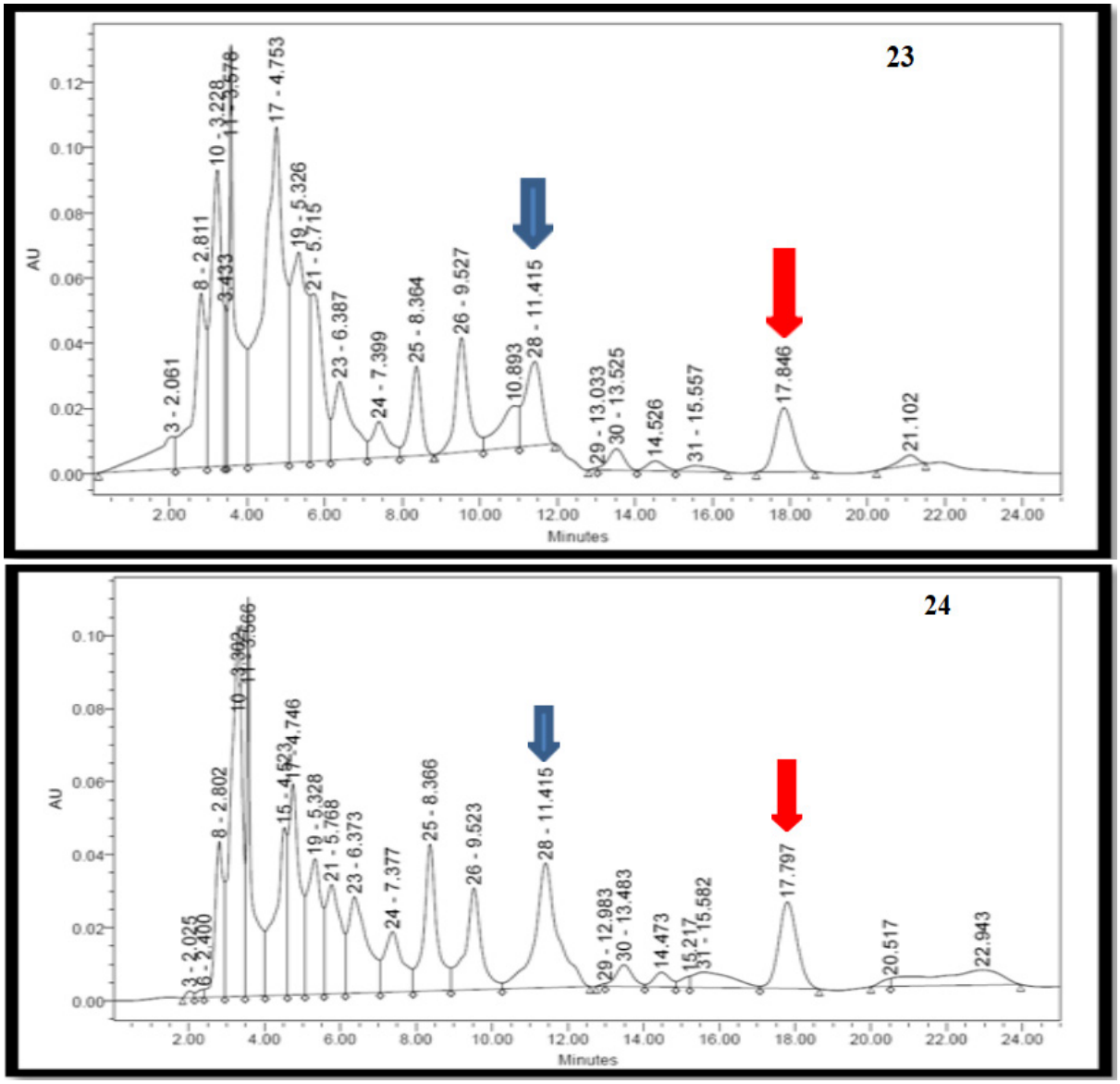

Plate 4: Blue arrow depicts Stevioside peak and Red arrow depicts Rebaudioside A peak in all the plates.

S. No.	Plant Code	Stevioside Retension Time	Stevioside Area	Stevioside (%)	Rebaudioside-A Retention Time	Rebaudioside-A Area	Rebaudioside-A (%)
25	25	11.416	333085	2.34	17.774	658638	5.95
26	26	11.376	469922	3.3	17.744	330579	2.99
27	27	11.365	539681	3.8	17.738	441050	3.98
28	28	11.344	540080	3.8	17.689	379850	3.43
29	29	11.315	329893	2.32	17.623	414067	3.74
30	30	11.253	312084	2.2	17.579	354043	3.19

Plate 5: Percentage of Stevioside and Rebaudioside-A based on retention time and Area under curve.

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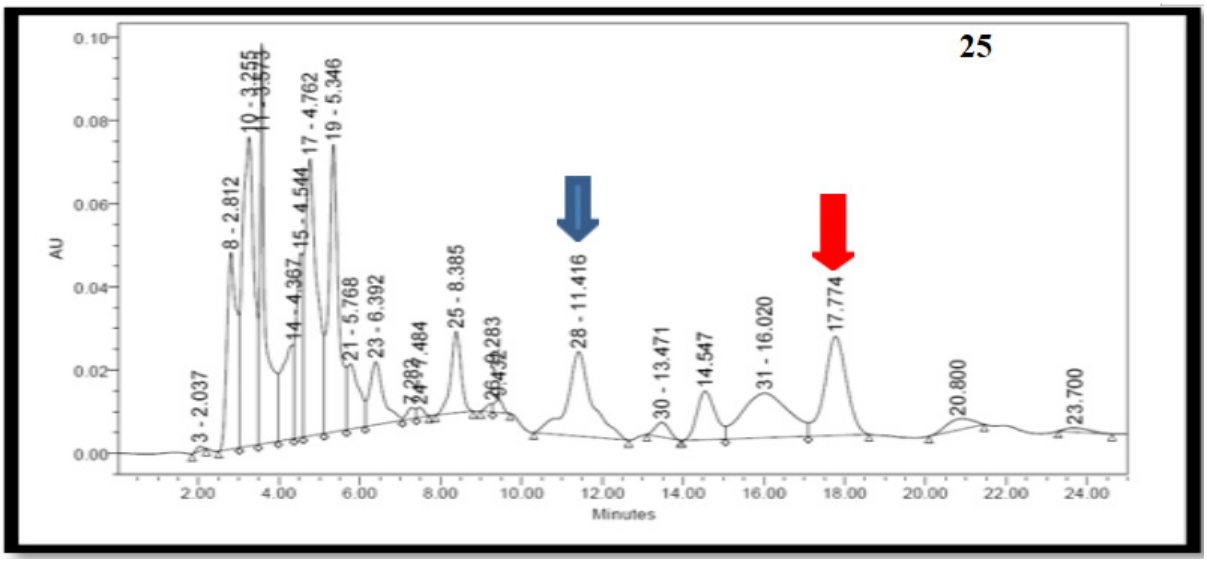

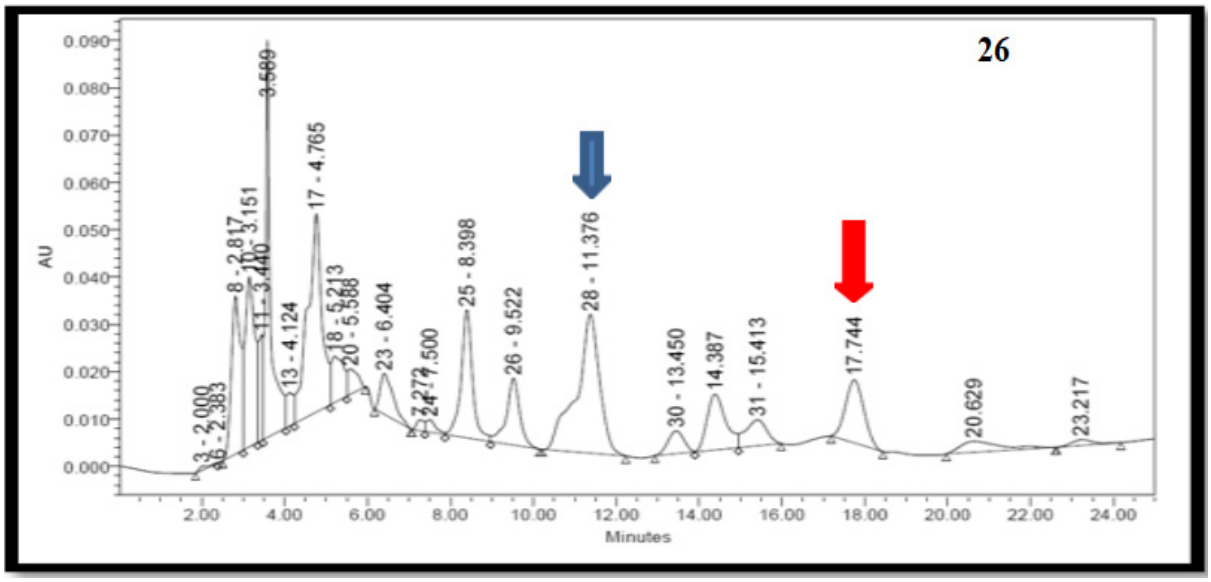

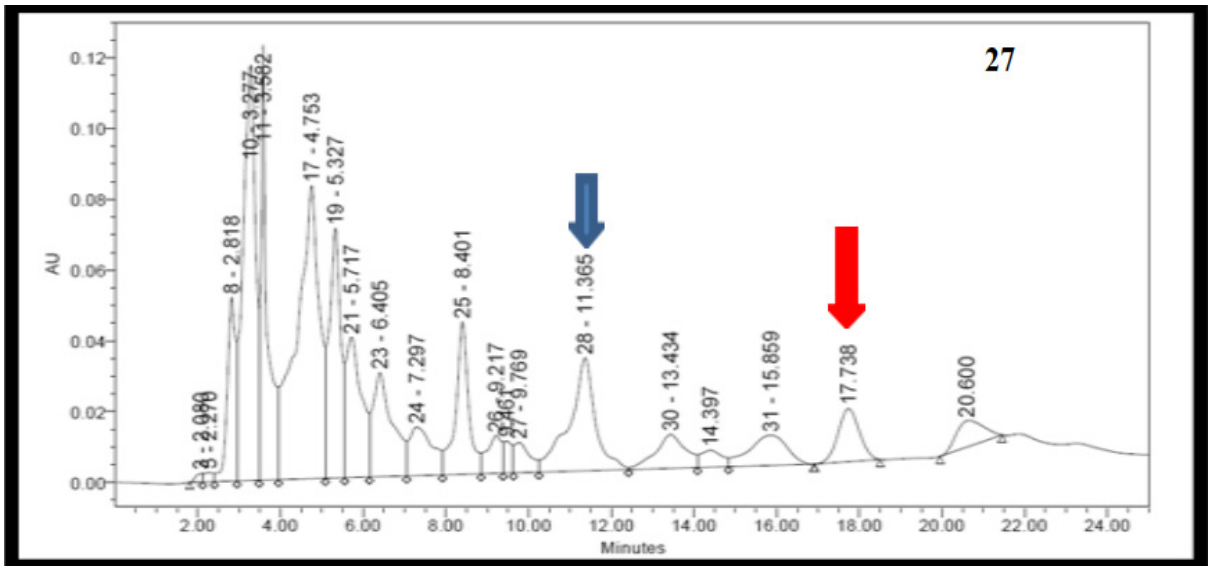

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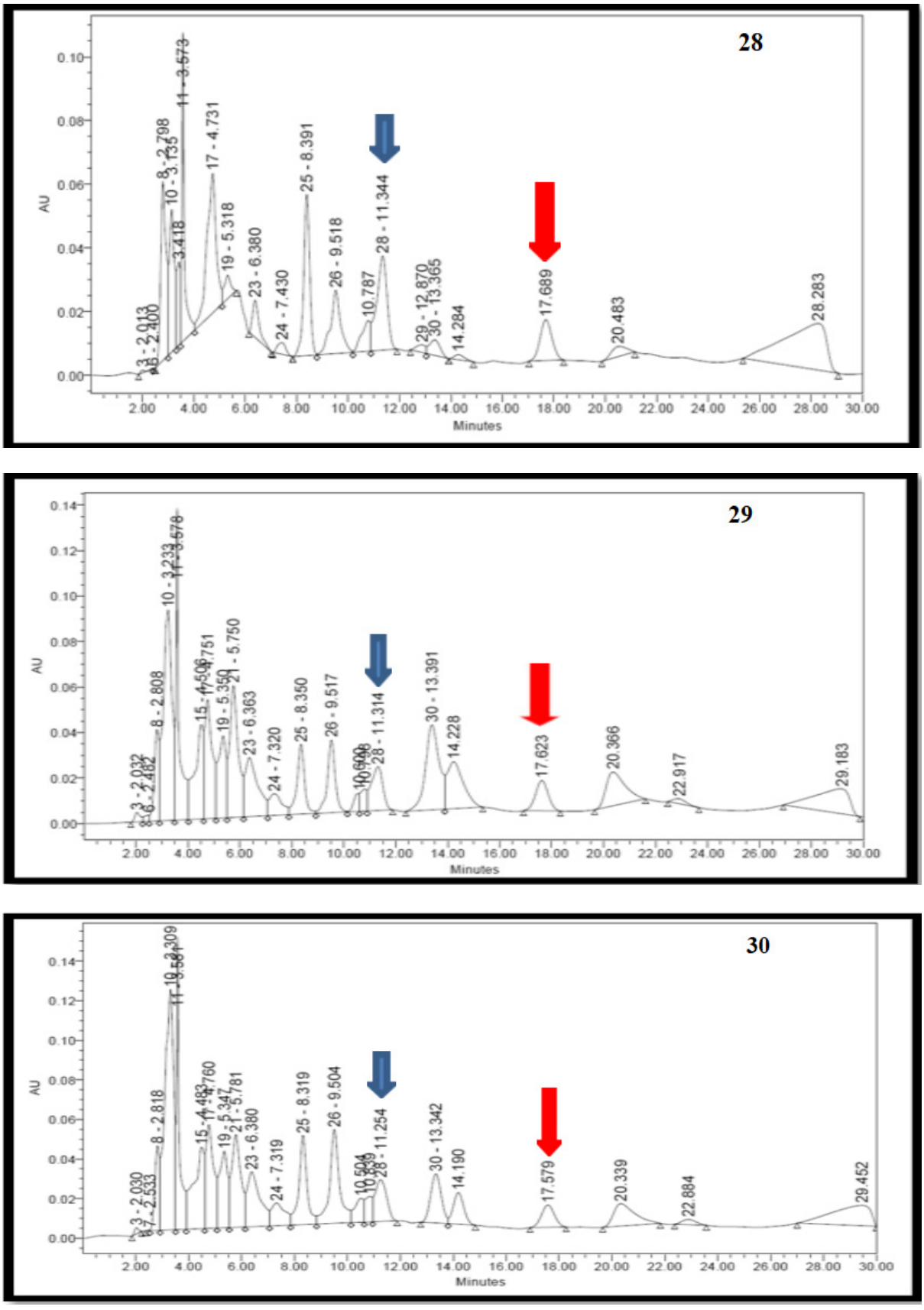

Plate 5: Blue arrow depicts Stevioside peak and Red arrow depicts Rebaudioside A peak in all the plates.

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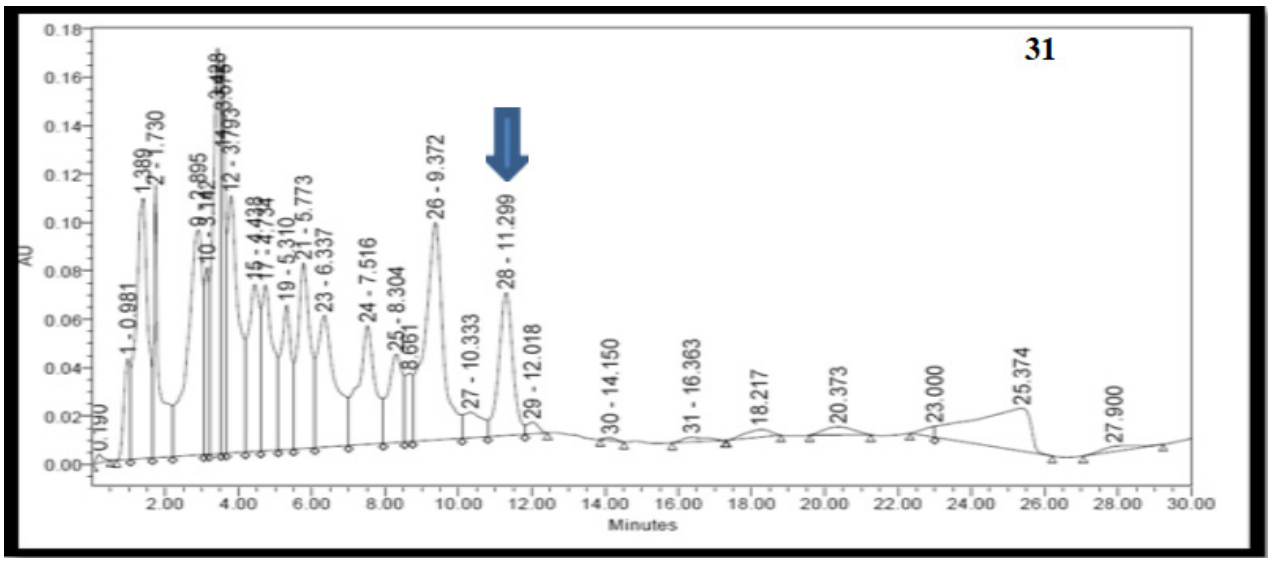
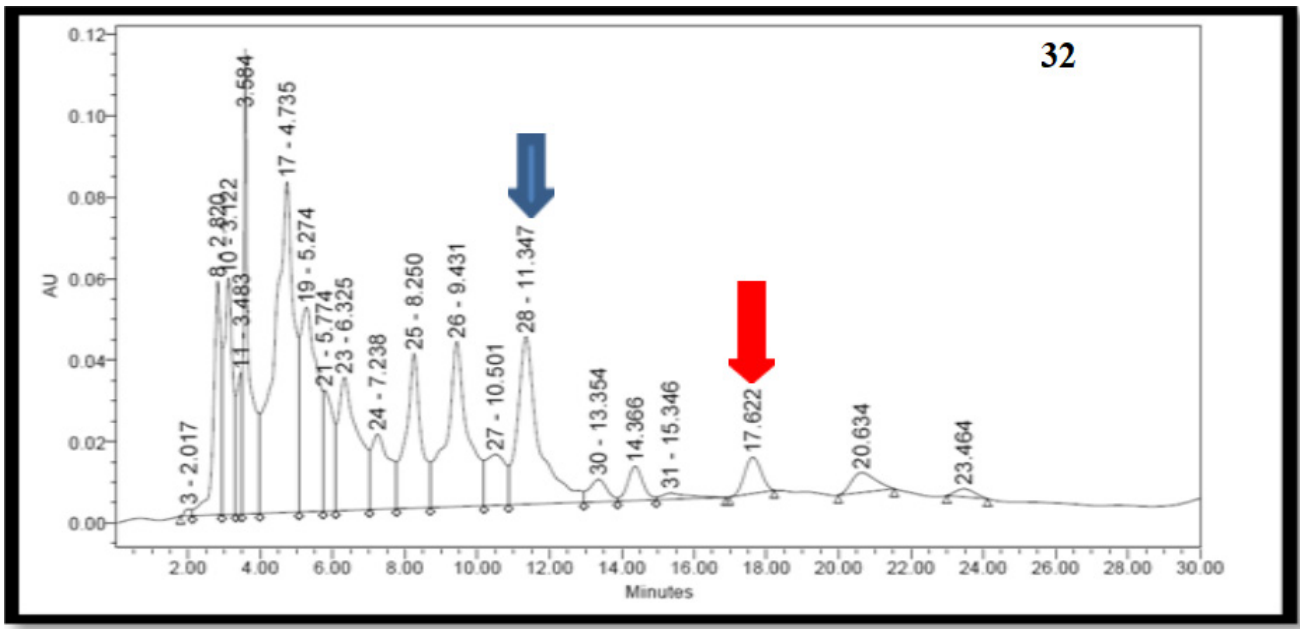
S. No.	Plant Code	Stevioside Retension Time	Stevioside Area	Stevioside (%)	Rebaudioside-A Retention Time	Rebaudioside-A Area	Rebaudioside-A (%)
31	31	11.299	1223348	8.6	No peak	-	-
32	32	11.347	718592	5.05	17.622	264014	2.38
33	33	11.247	652331	4.59	17.504	315755	2.85
34	34	11.271	794697	5.59	17.49	372048	3.36
35	35	11.252	954039	6.71	17.449	498532	4.5
36	36	11.494	680419	4.79	18.005	401238	3.62

Plate 6: Percentage of Stevioside and Rebaudioside-A based on retention time and Area under curve.





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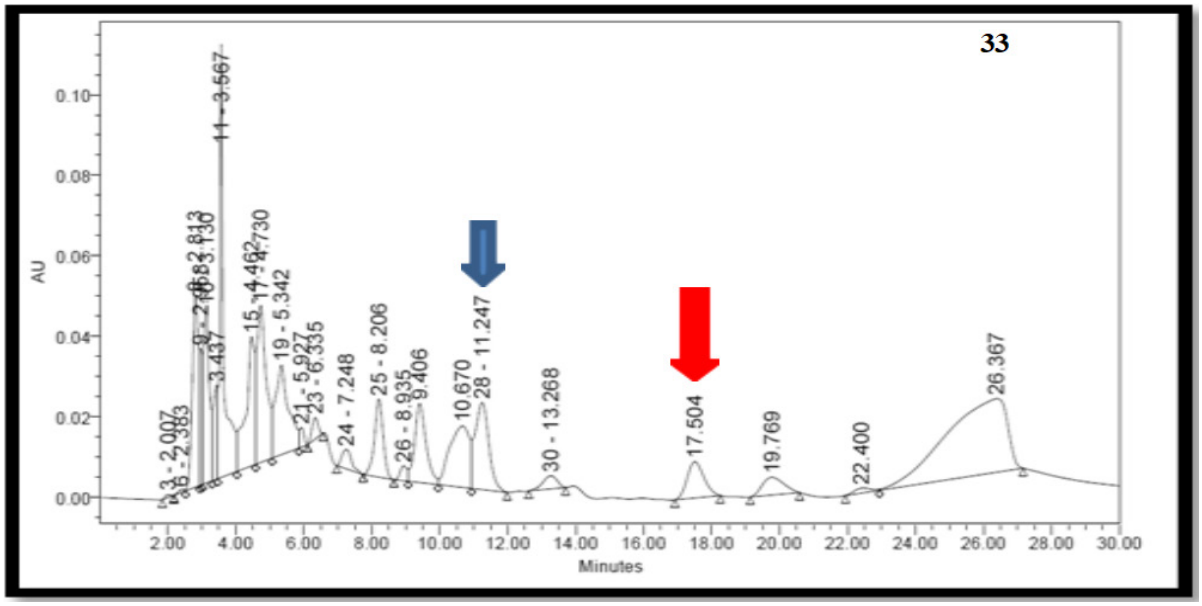

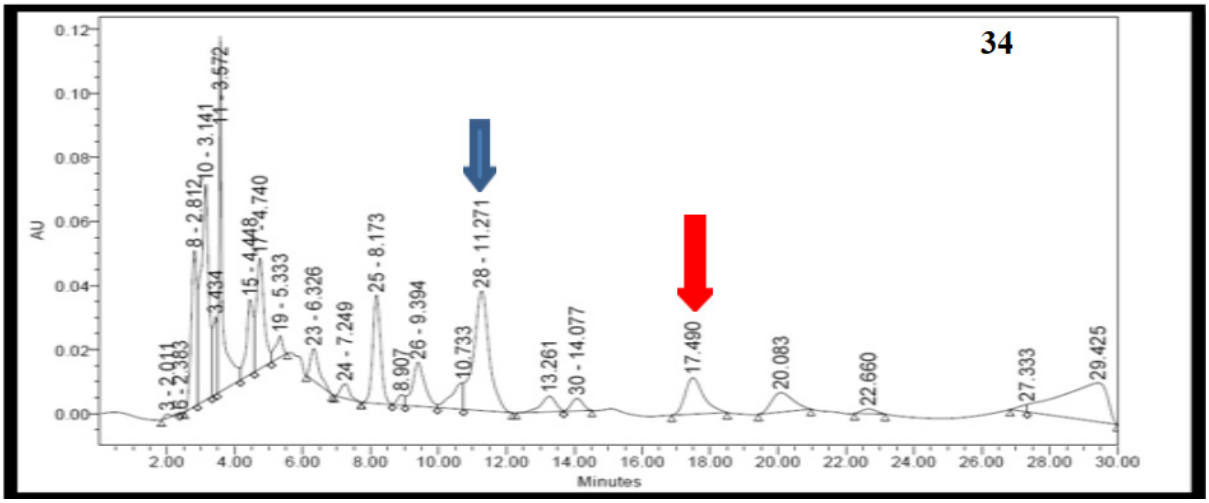

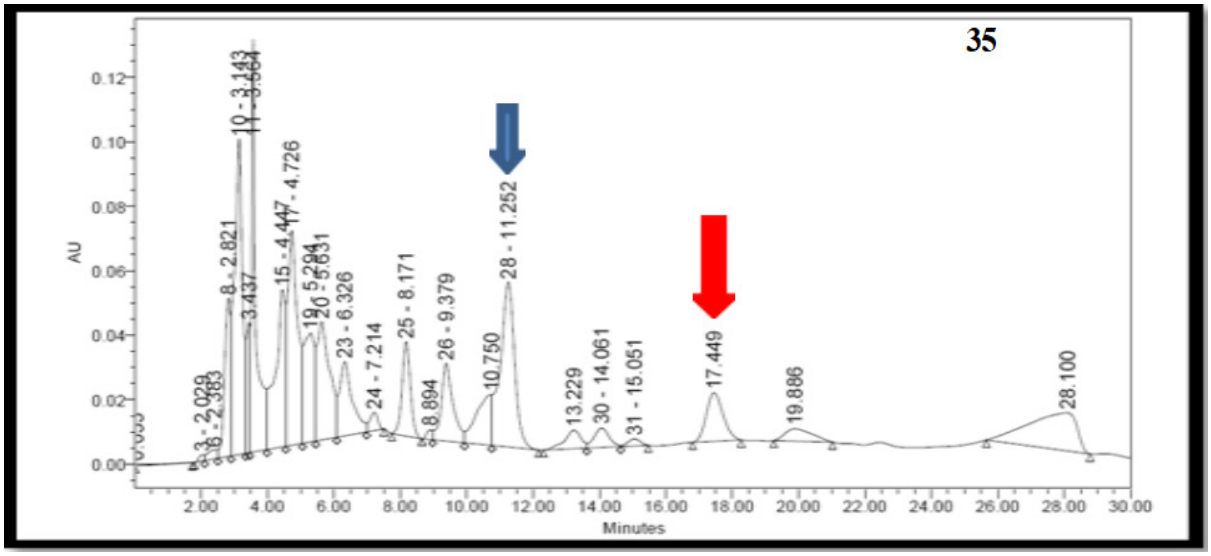

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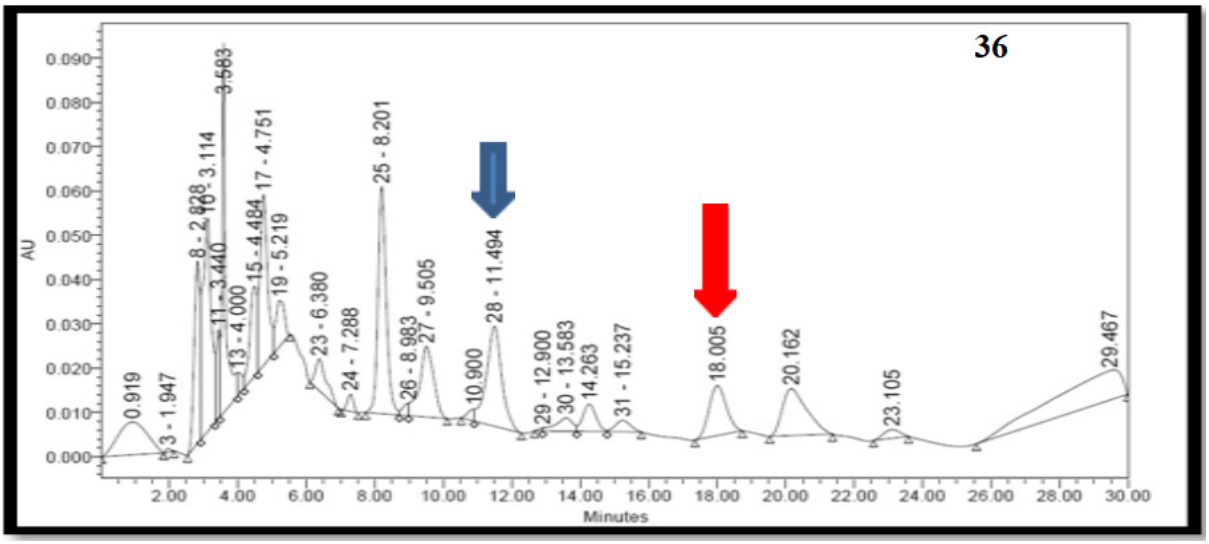
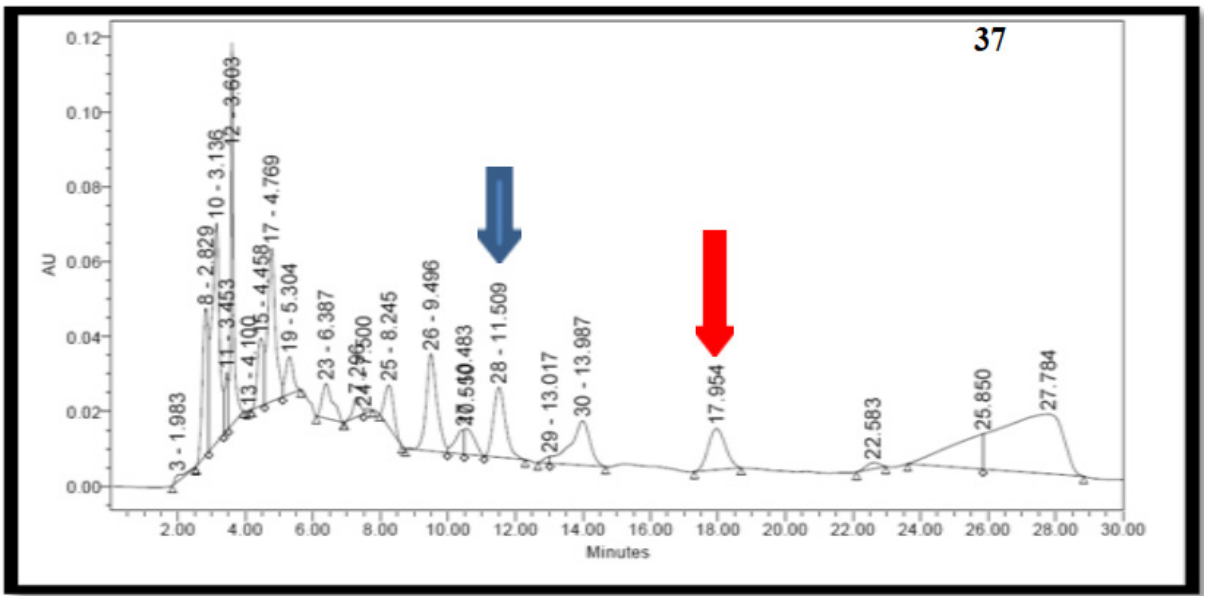


Plate 6: Blue arrow depicts Stevioside peak and Red arrow depicts Rebaudioside A peak in all the plates.

S. No.	Plant Code	Stevioside Retension Time	Stevioside Area	Stevioside (%)	Rebaudioside-A Retention Time	Rebaudioside-A Area	Rebaudioside-A (%)
37	37	11.509	410853	2.89	17.954	364166	3.28
38	38	11.487	774227	5.45	18.003	107740	0.97
39	39	11.302	836760	4.71	17.501	561711	4.06
40	40	11.294	481040	3.38	17.52	330343	2.98
41	41	11.242	475102	3.34	17.486	341406	3.08
42	42	11.262	312410	2.2	17.452	312452	2.82

Plate 7: Percentage of Stevioside and Rebaudioside-A based on retention time and Area under curve.



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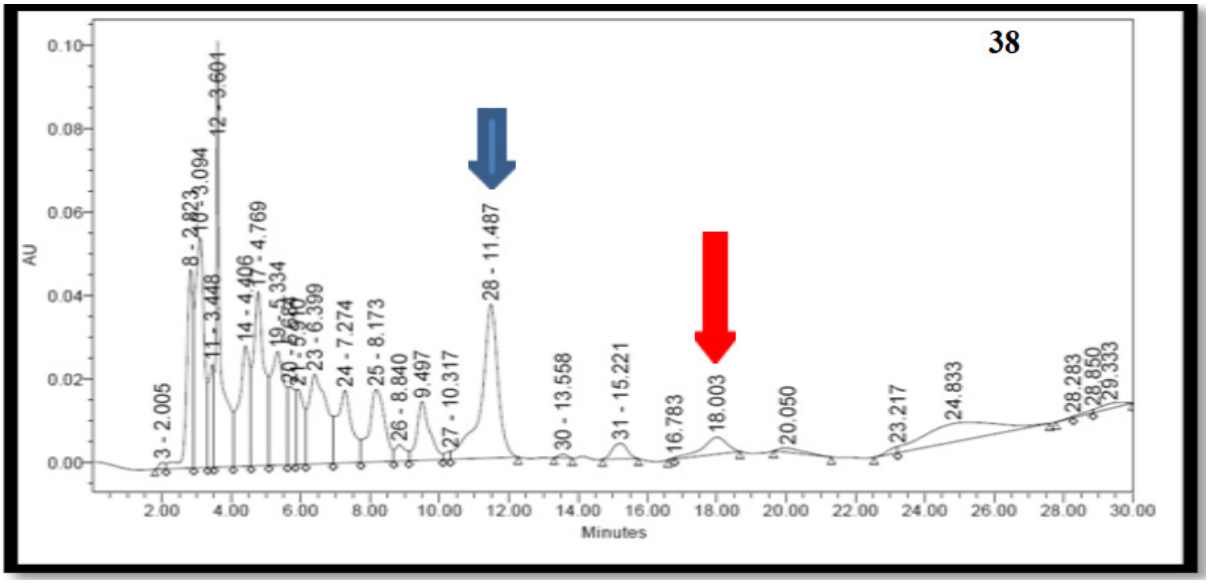

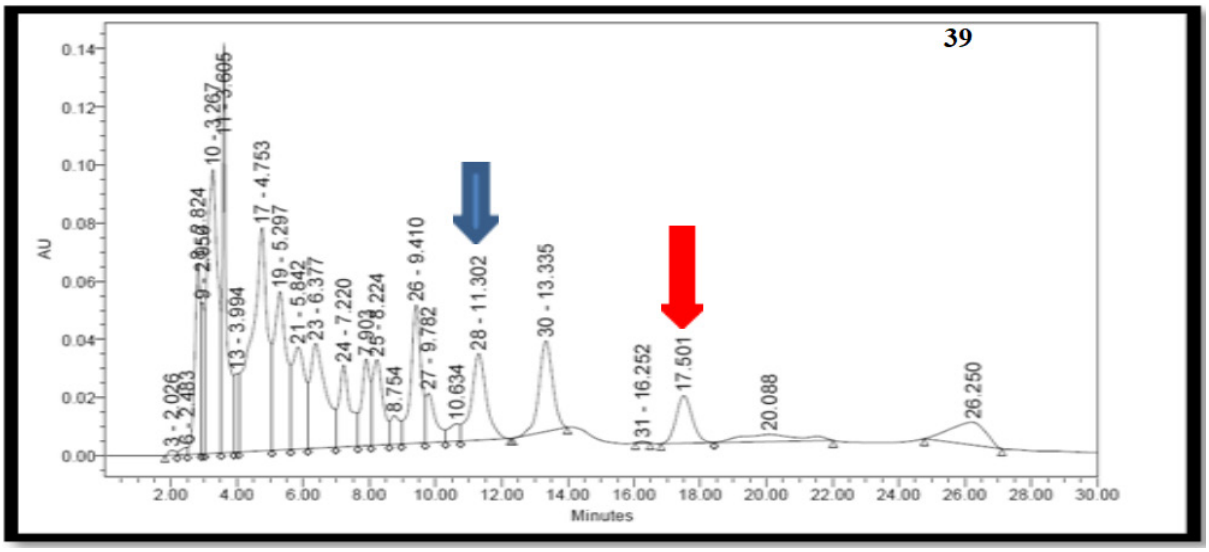

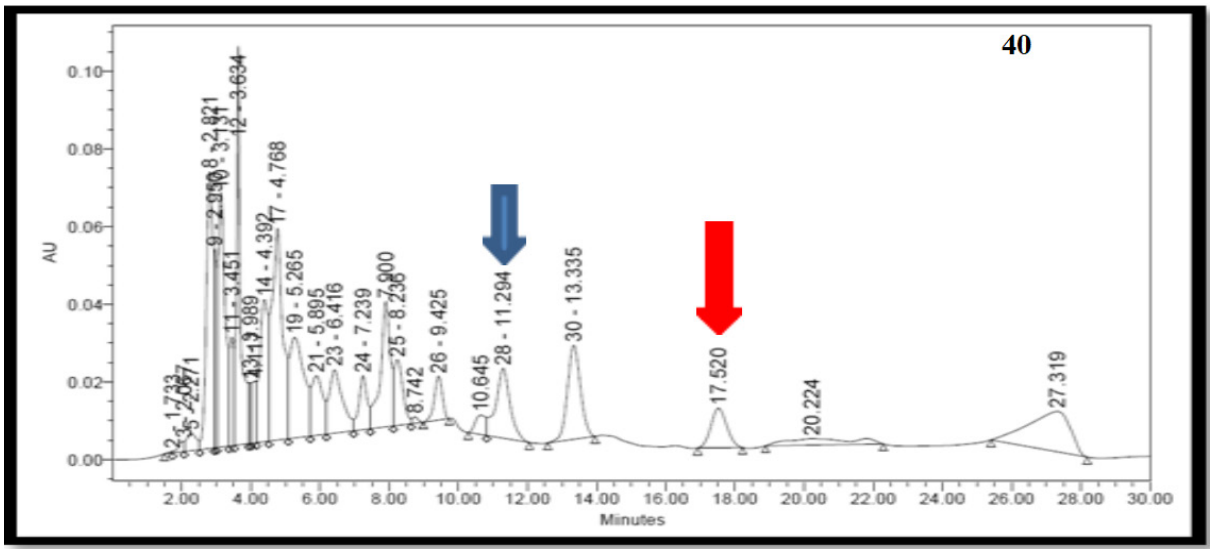

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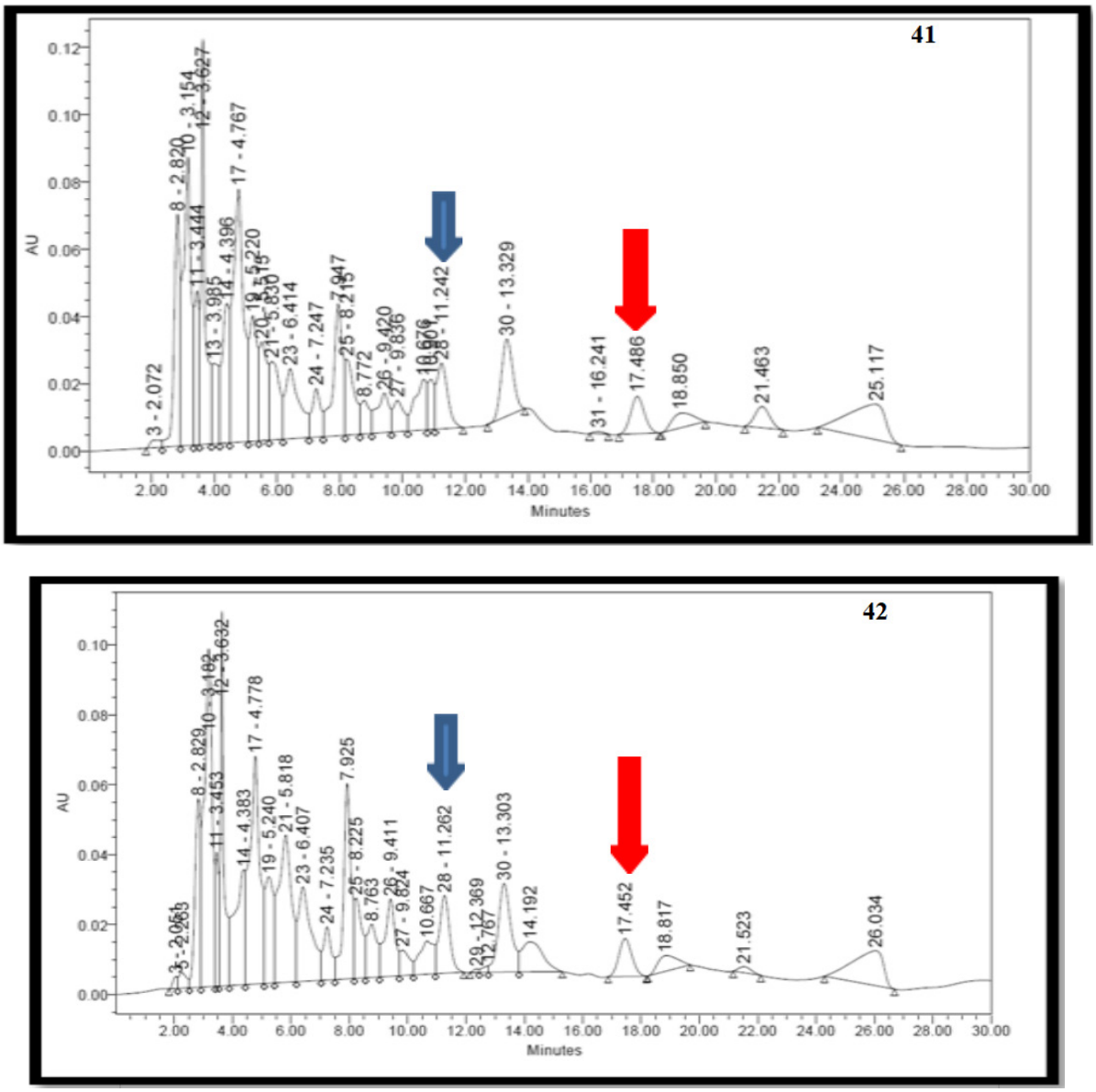

Plate 7: Blue arrow depicts Stevioside peak and Red arrow depicts Rebaudioside A peak in all the plates.

S. No.	Plant Code	Stevioside Retension Time	Stevioside Area	Stevioside (%)	Rebaudioside-A Retention Time	Rebaudioside-A Area	Rebaudioside-A (%)
43	43	11.282	1163192	8.18	17.555	358134	3.23
44	44	11.265	1042402	7.33	17.217	90583	0.82
45	45	11.243	533044	3.75	17.408	911954	8.24
46	46	11.245	598905	4.21	17.414	680259	6.14
47	47	11.212	977668	6.88	17.321	489505	4.42
48	48	11.211	808622	5.69	17.319	758573	6.85

Plate 8: Percentage of Stevioside and Rebaudioside-A based on retention time and Area under curve.

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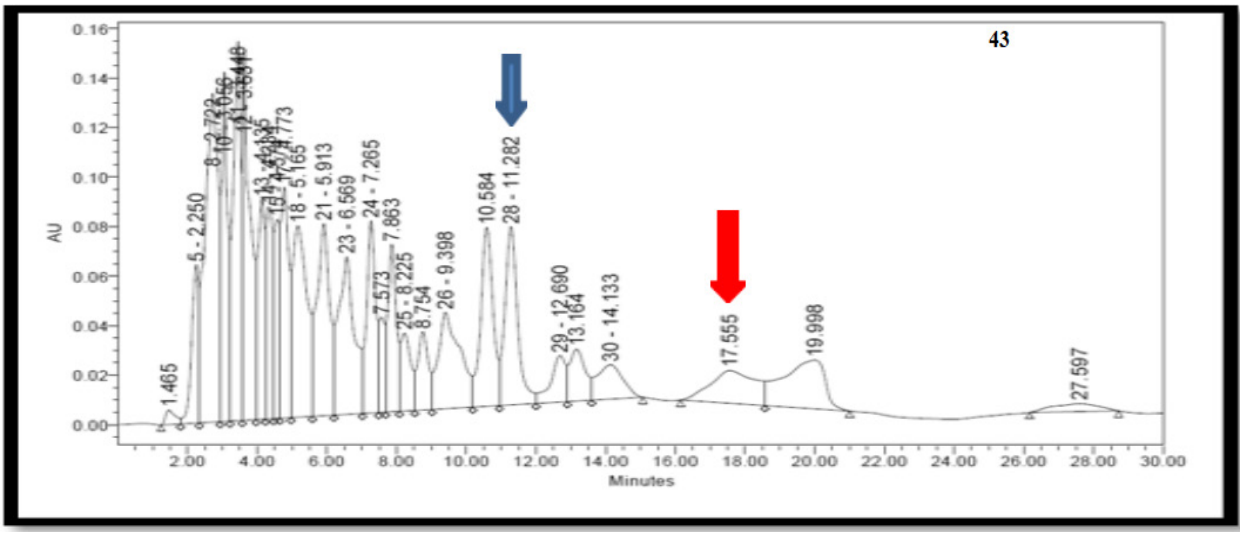

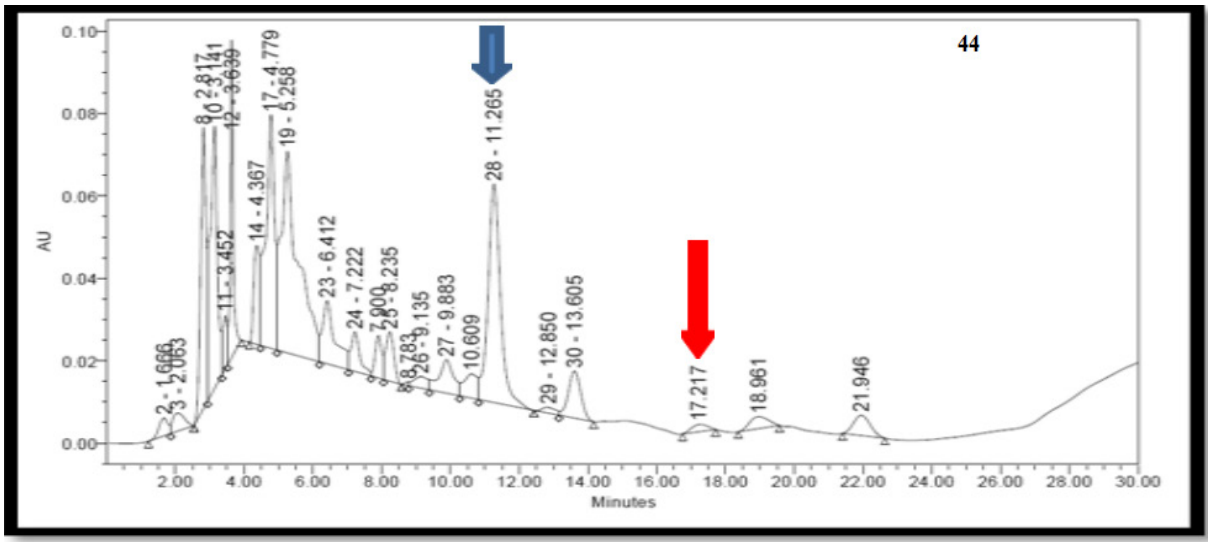

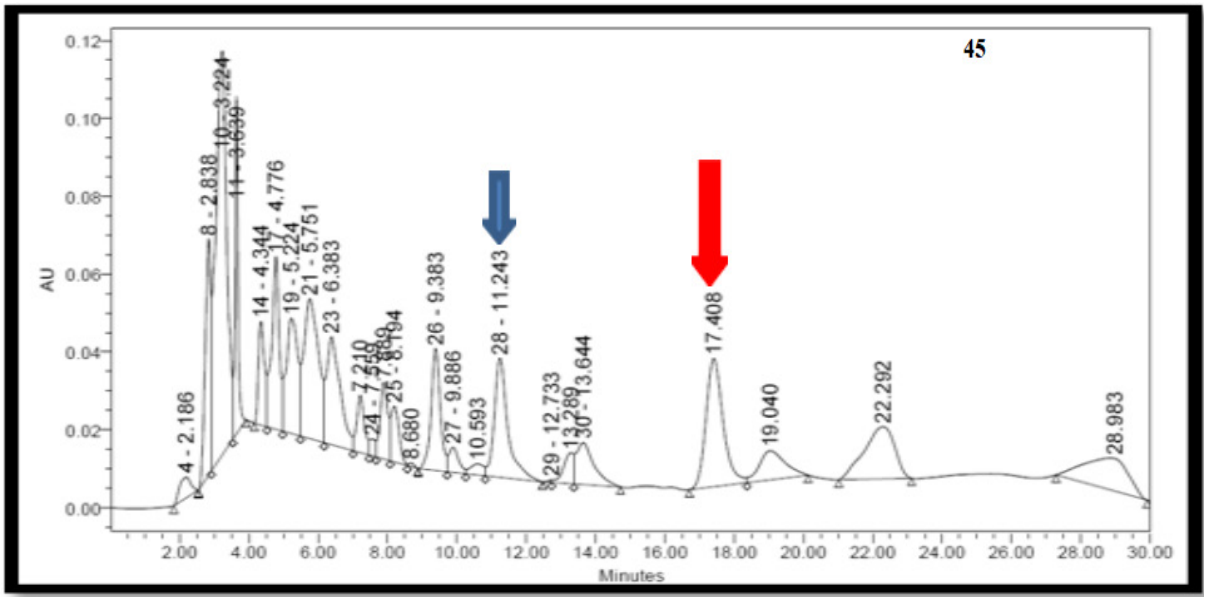

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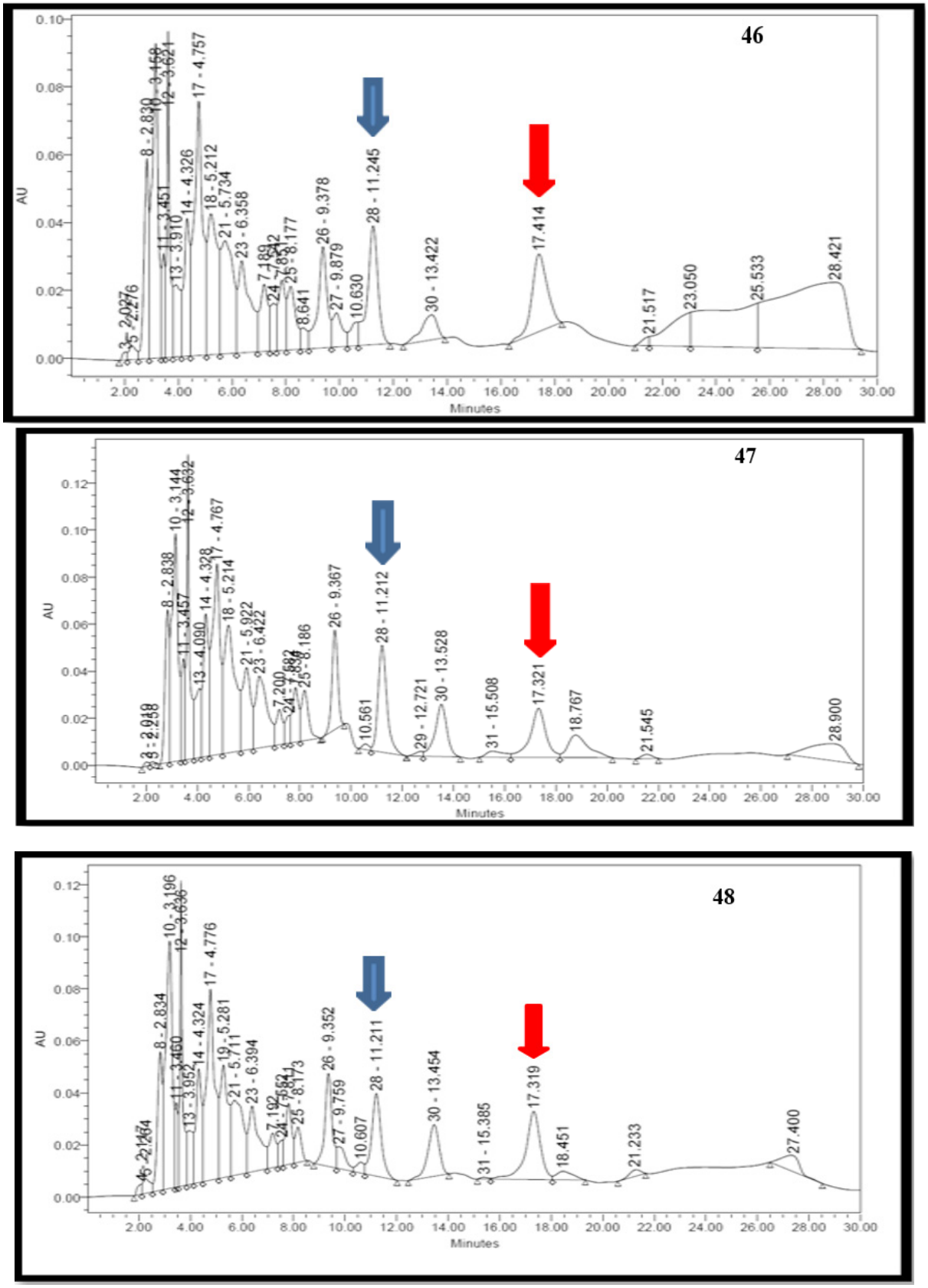

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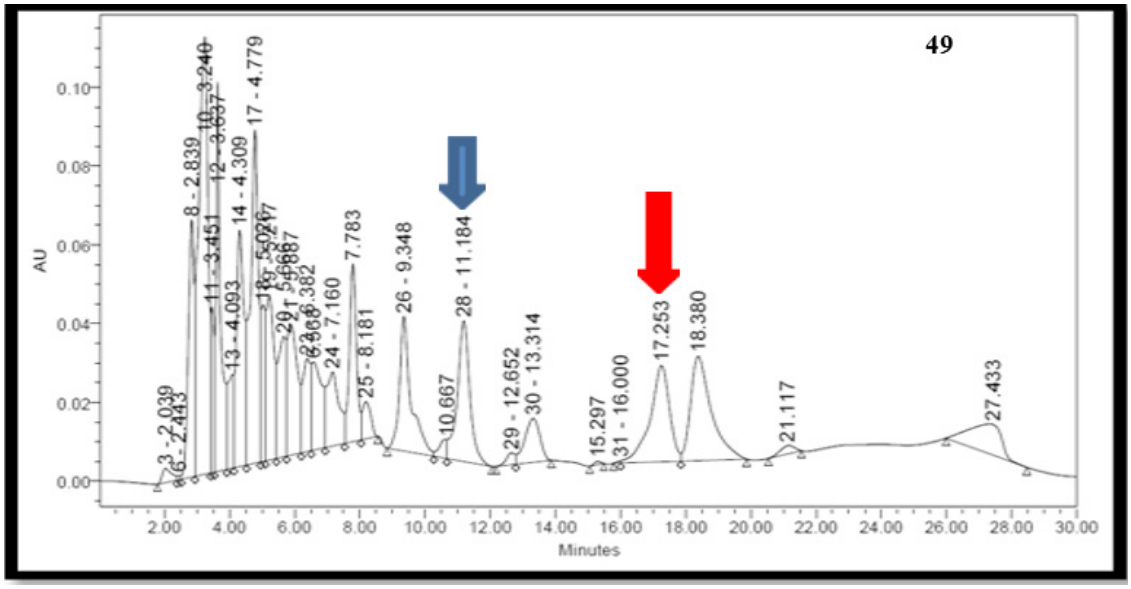
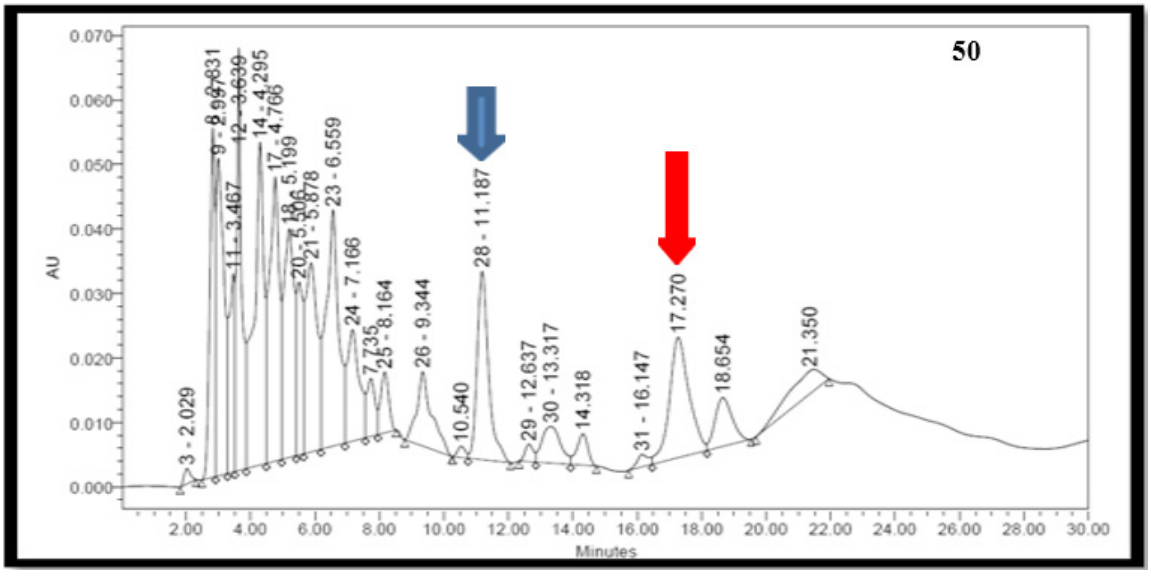
S. No.	Plant Code	Stevioside Retension Time	Stevioside Area	Stevioside (%)	Rebaudioside-A Retention Time	Rebaudioside-A Area	Rebaudioside-A (%)
49	49	11.184	717690	5.05	17.253	599819	5.42
50	50	11.187	552918	5.83	17.27	554392	7.51
51	51	11.154	871938	6.13	17.176	516309	4.66
52	52	11.141	695820	4.9	17.162	745513	6.73
53	53	11.114	521900	3.67	17.13	196244	1.77
54	54	11.199	328745	2.31	17.242	271267	2.45

Plate 9: Percentage of Stevioside and Rebaudioside-A based on retention time and Area under curve.





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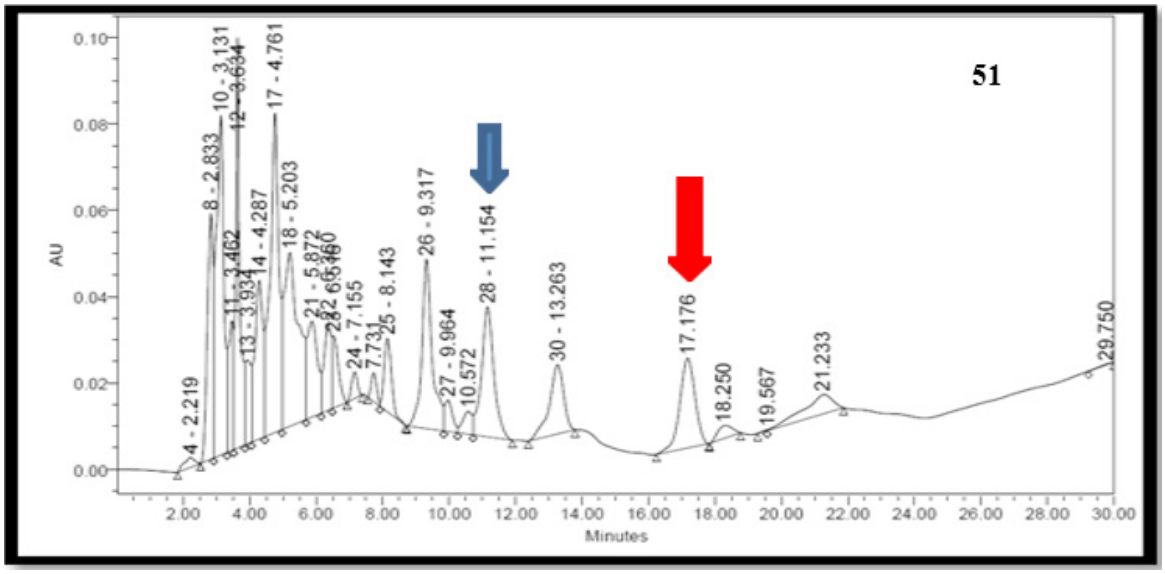

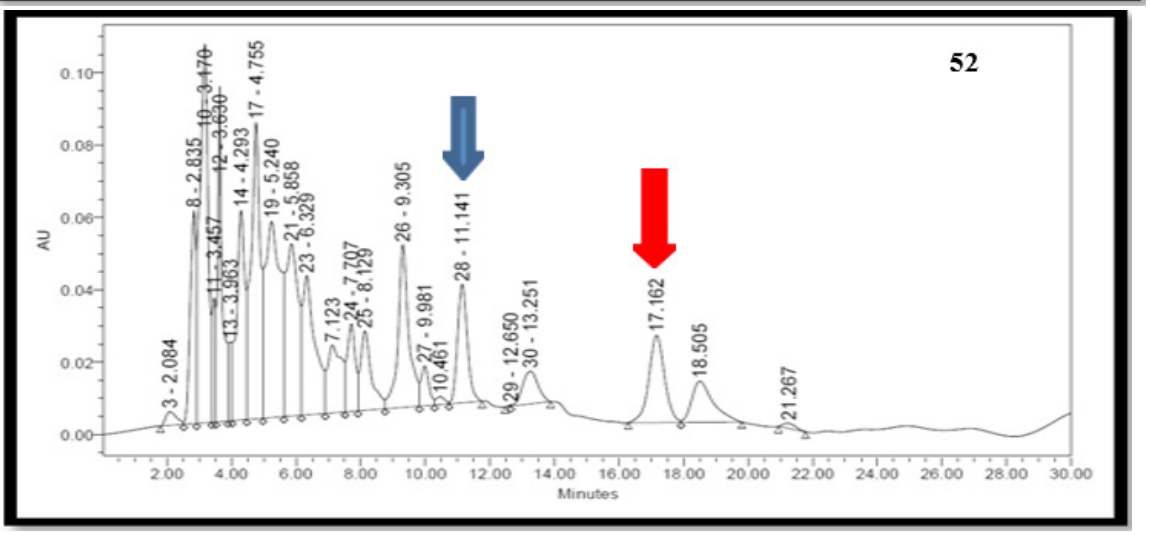

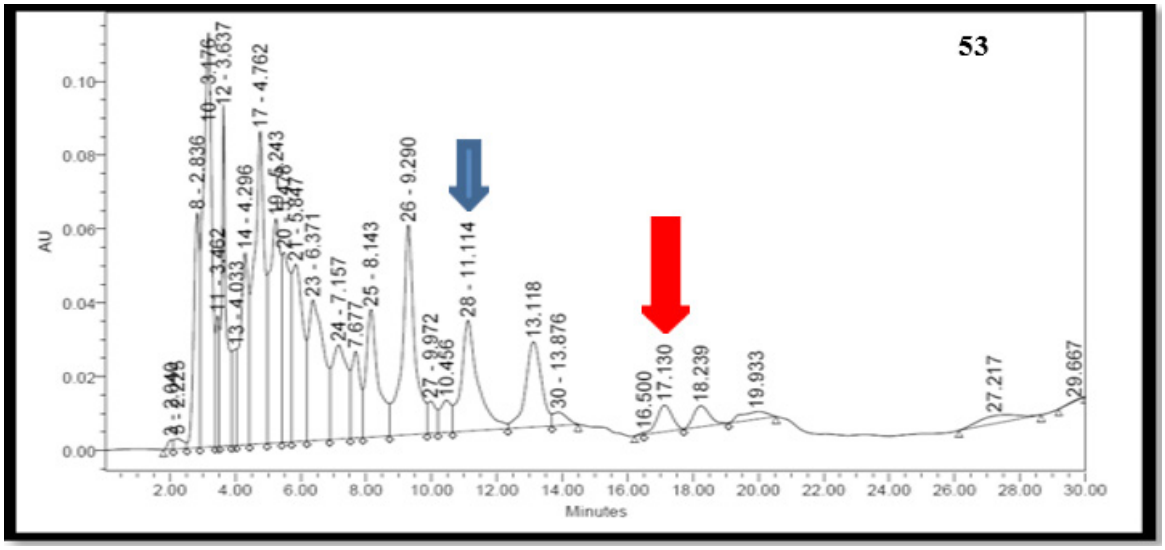

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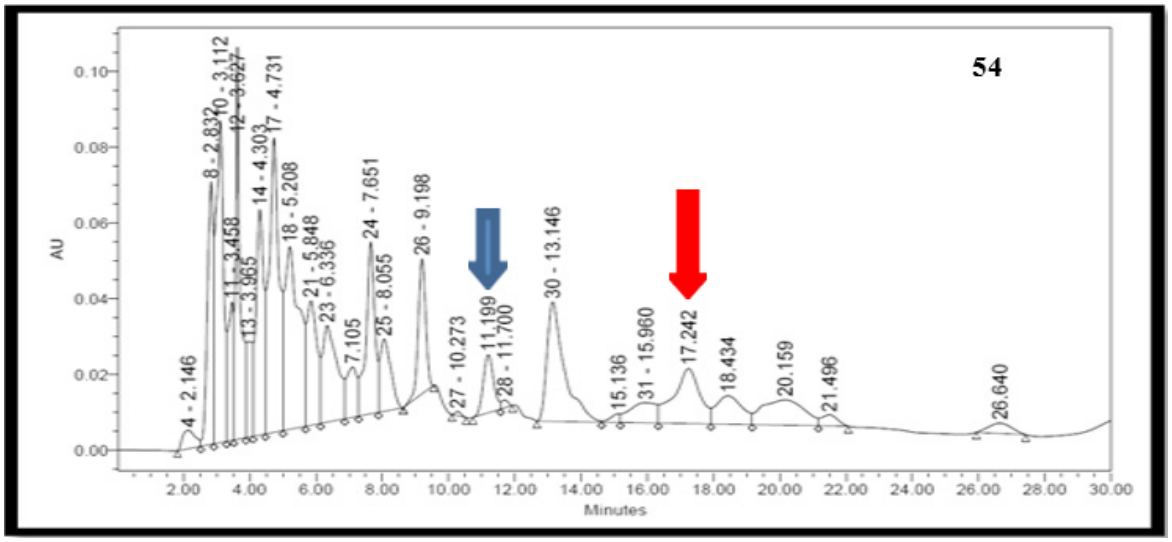
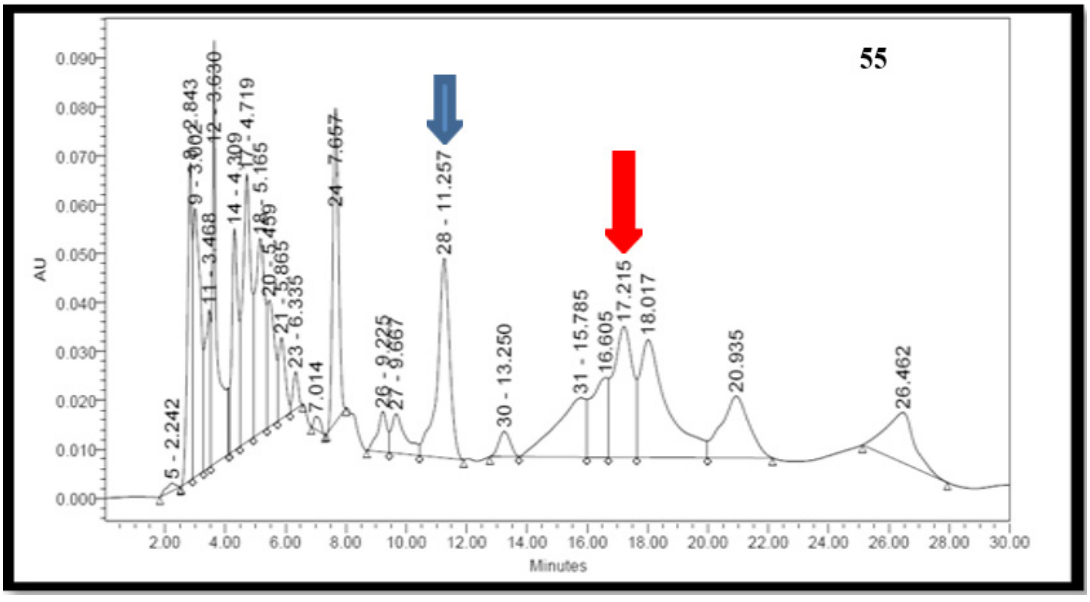


Plate 9: Blue arrow depicts Stevioside peak and Red arrow depicts Rebaudioside A peak in all the plates.

S. No.	Plant Code	Stevioside Retension Time	Stevioside Area	Stevioside (%)	Rebaudioside-A Retention Time	Rebaudioside-A Area	Rebaudioside-A (%)
55	55	11.257	559416	3.93	17.215	247208	2.23
56	56	11.079	919662	6.47	17.56	263634	2.38
57	57	11.043	882598	6.21	17.753	340943	3.08
58	58	10.894	297320	2.09	16.645	326836	2.95
59	59	10.89	689037	4.85	16.621	79834	0.72
60	60	10.902	941265	6.62	16.556	38162	0.34

Plate 10: Percentage of Stevioside and Rebaudioside-A based on retention time and Area under curve.



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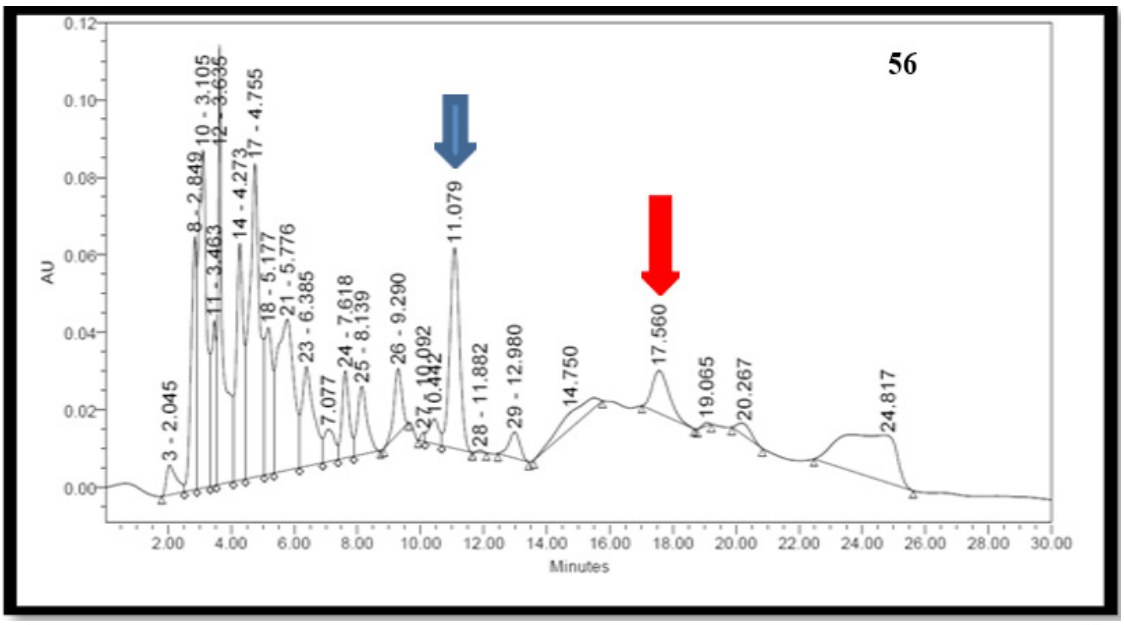

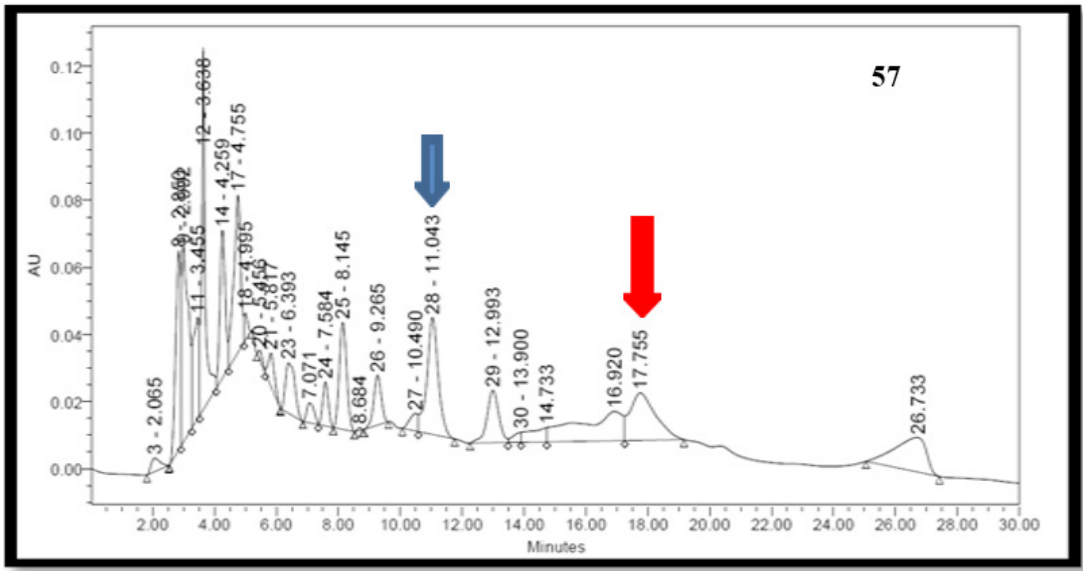

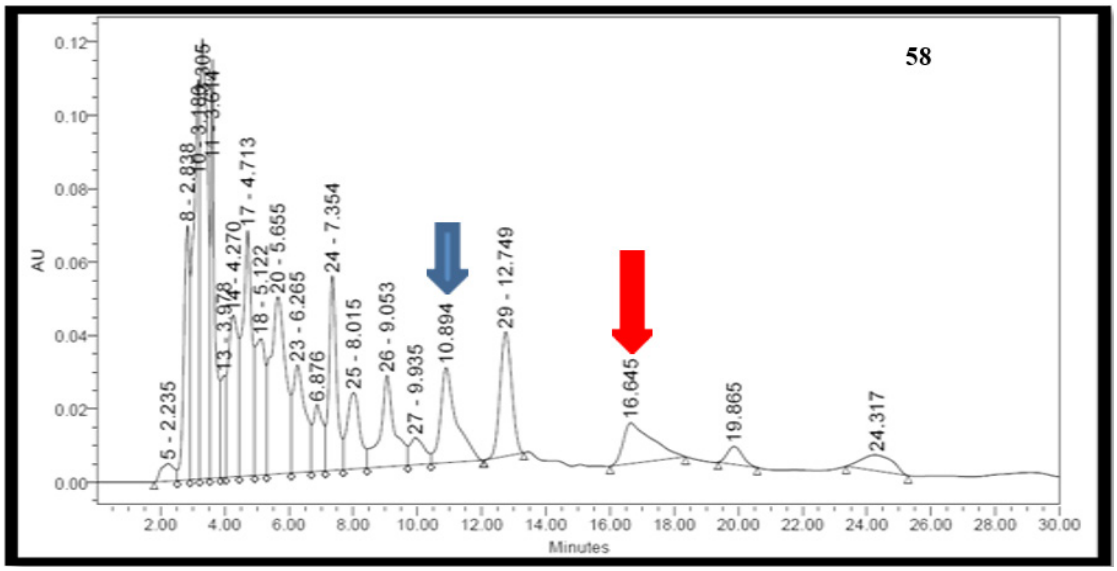

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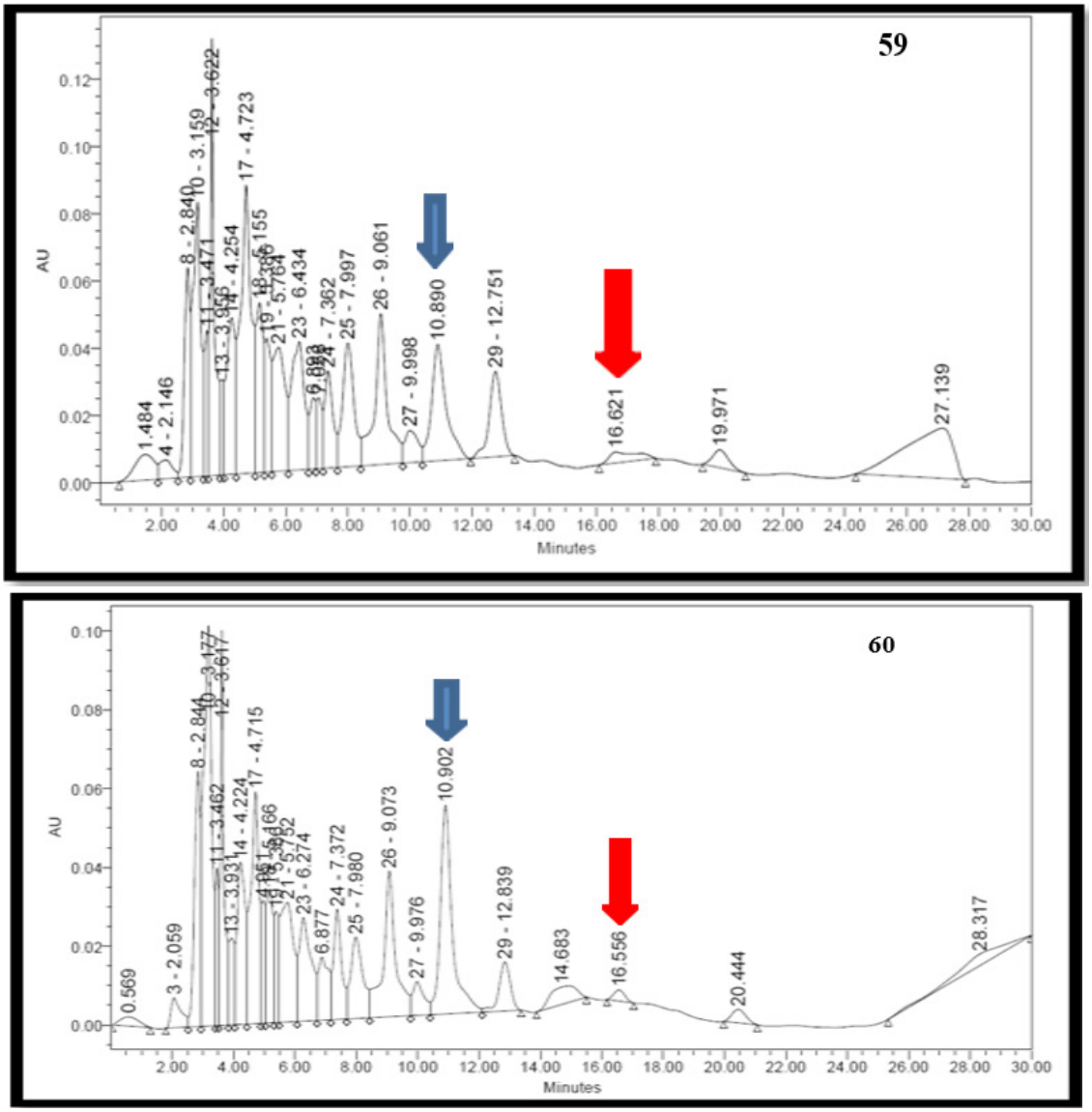

Plate 10: Blue arrow depicts Stevioside peak and Red arrow depicts Rebaudioside A peak in all the plates.

S. No.	Plant Code	Stevioside Retension Time	Stevioside Area	Stevioside (%)	Rebaudioside-A Retention Time	Rebaudioside-A Area	Rebaudioside-A (%)
61	61	10.904	1047692	7.37	17.02	370593	3.35
62	62	10.859	801176	5.63	16.565	408964	3.69
63	63	10.041	960406	6.76	14.917	455480	4.11
64	64	10.821	800783	5.63	16.52	499666	4.51
65	65	10.816	1226181	8.63	16.533	263171	2.37
66	66	10.802	447566	3.14	16.47	324290	2.92

Plate 11: Percentage of Stevioside and Rebaudioside-A based on retention time and Area under curve.

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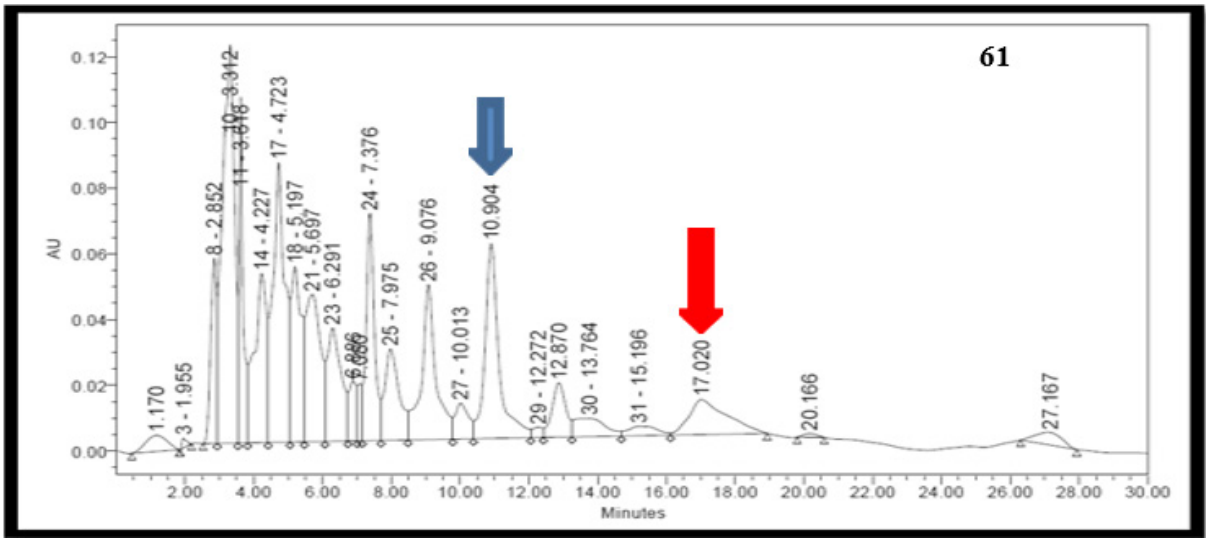

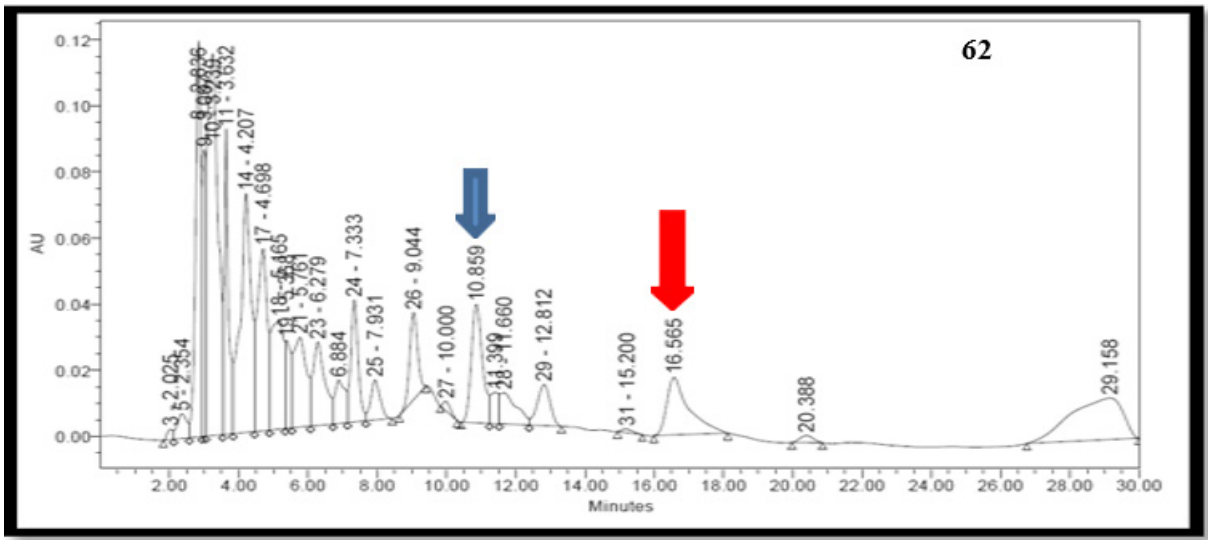

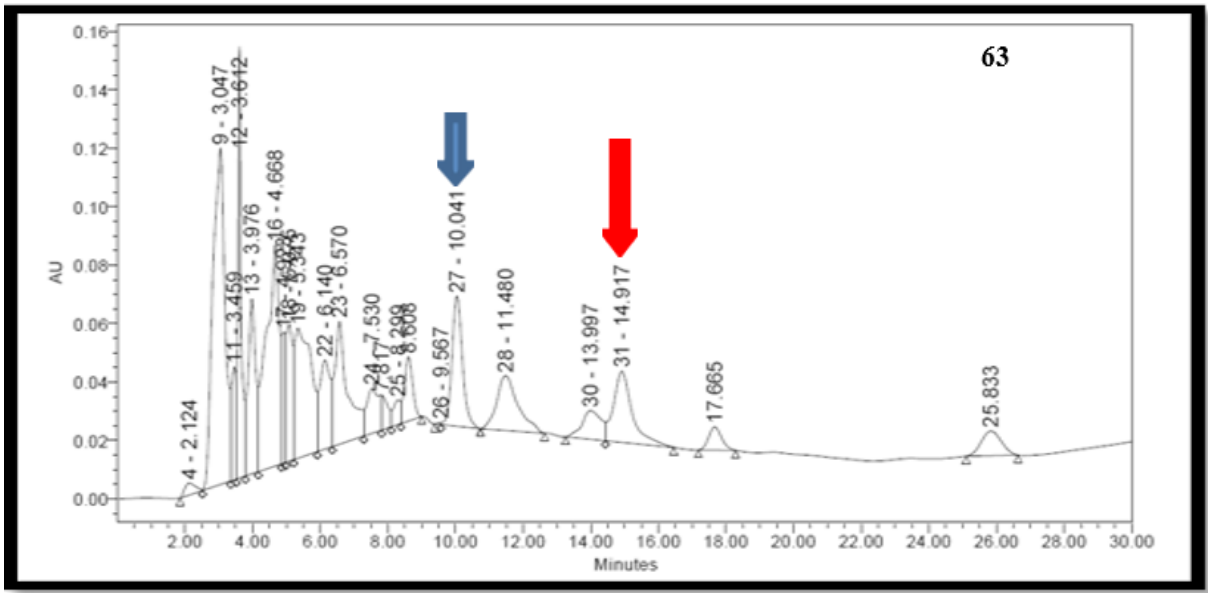

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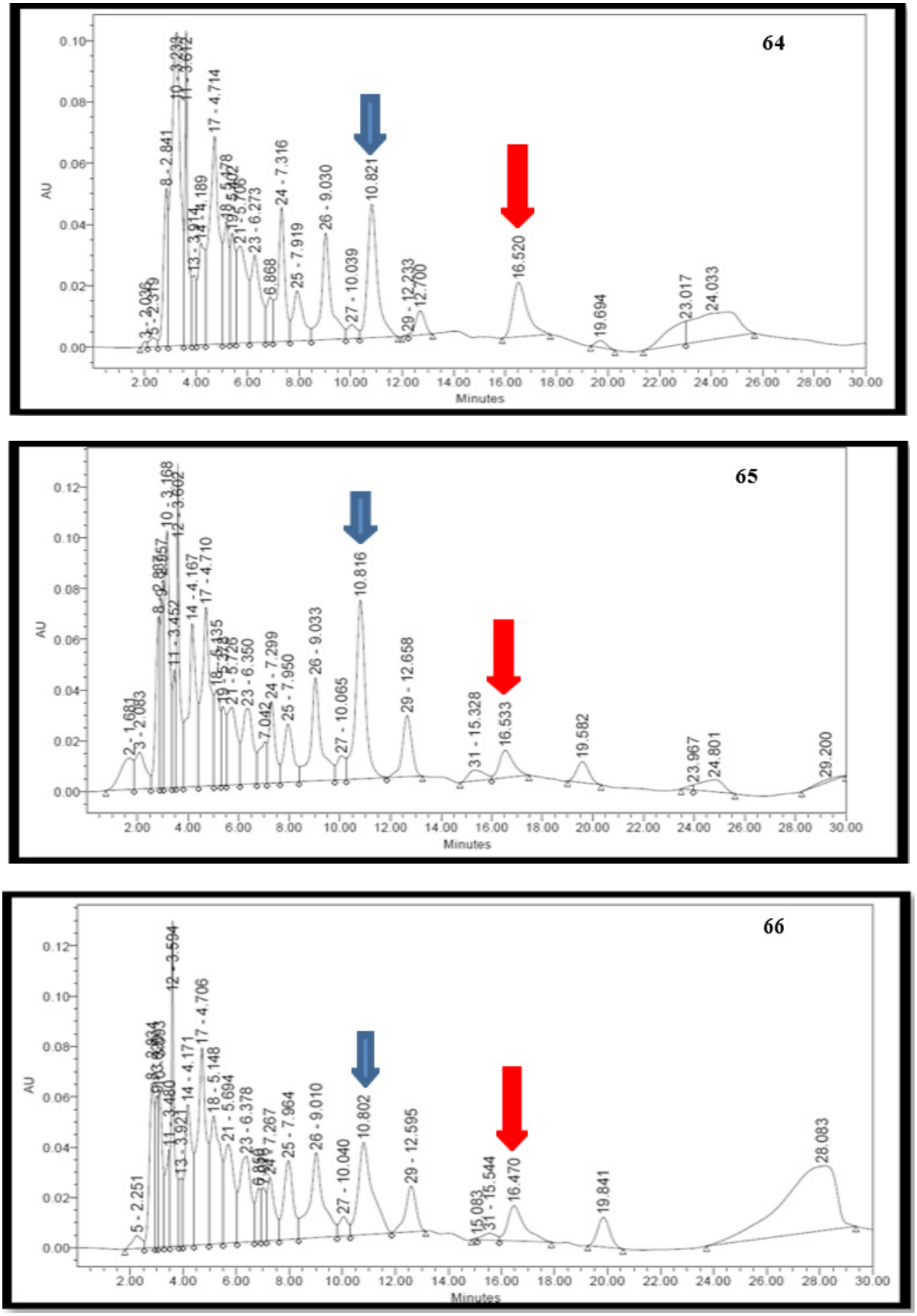

Plate 11: Blue arrow depicts Stevioside peak and Red arrow depicts Rebaudioside A peak in all the plates.

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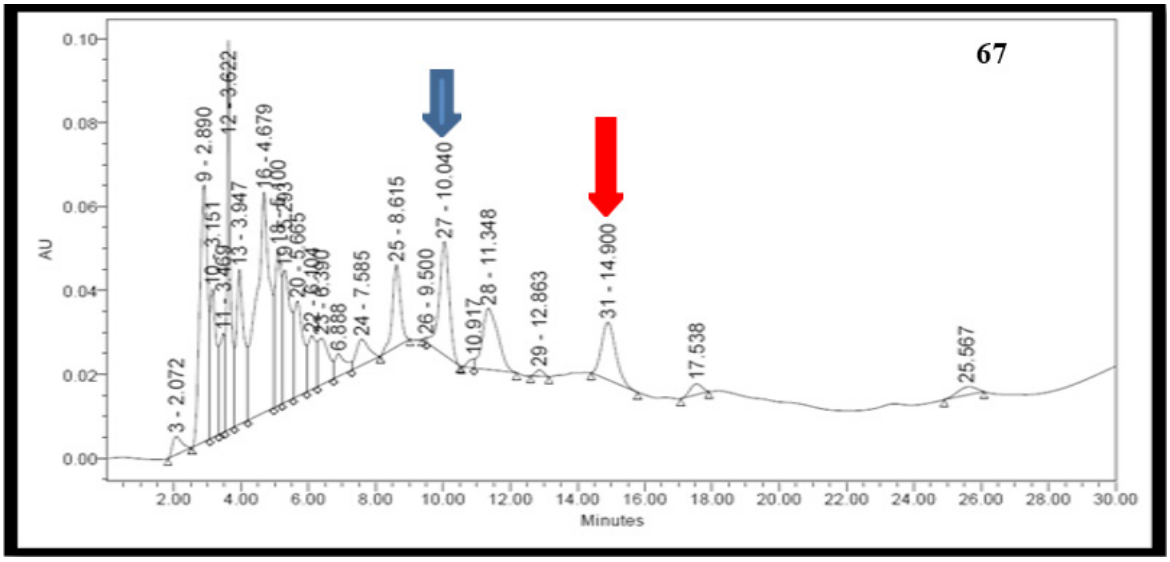
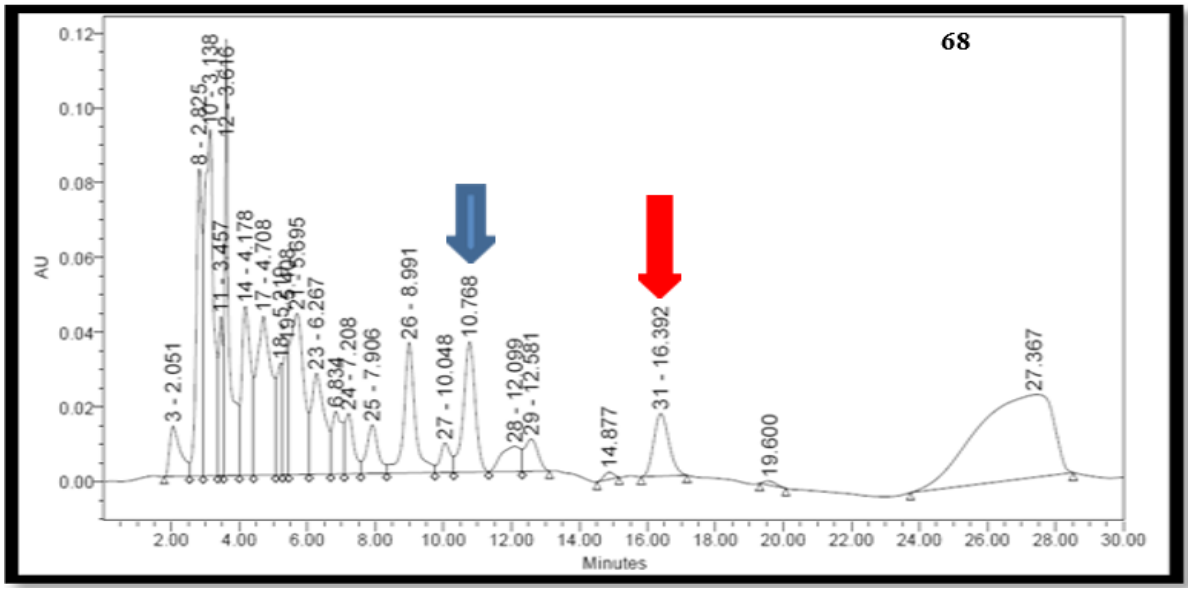
S. No.	Plant Code	Stevioside Retension Time	Stevioside Area	Stevioside (%)	Rebaudioside-A Retention Time	Rebaudioside-A Area	Rebaudioside-A (%)
67	67	10.04	407958	2.87	14.9	279525	2.52
68	68	10.768	694708	5.21	16.392	462696	4.45
69	69	10.751	935374	6.58	16.449	954673	8.62
70	70	10.036	292999	2.06	14.854	221207	1.99
71	71	10.027	1228826	8.64	No peak	-	-
72	72	10.713	419091	3.14	16.297	245845	2.37

Plate 12: Percentage of Stevioside and Rebaudioside-A based on retention time and Area under curve.





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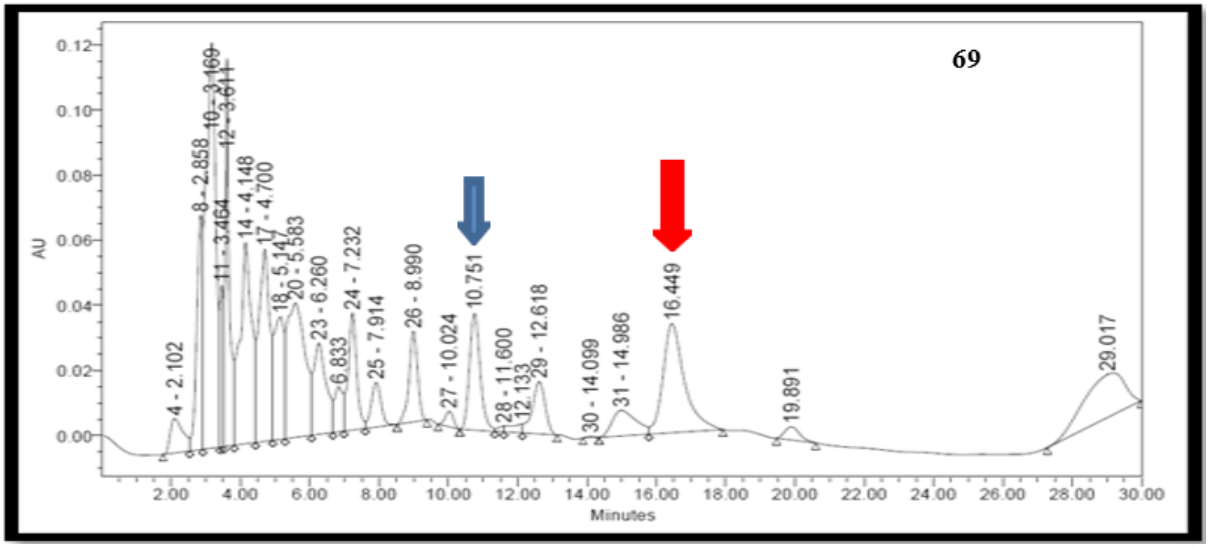

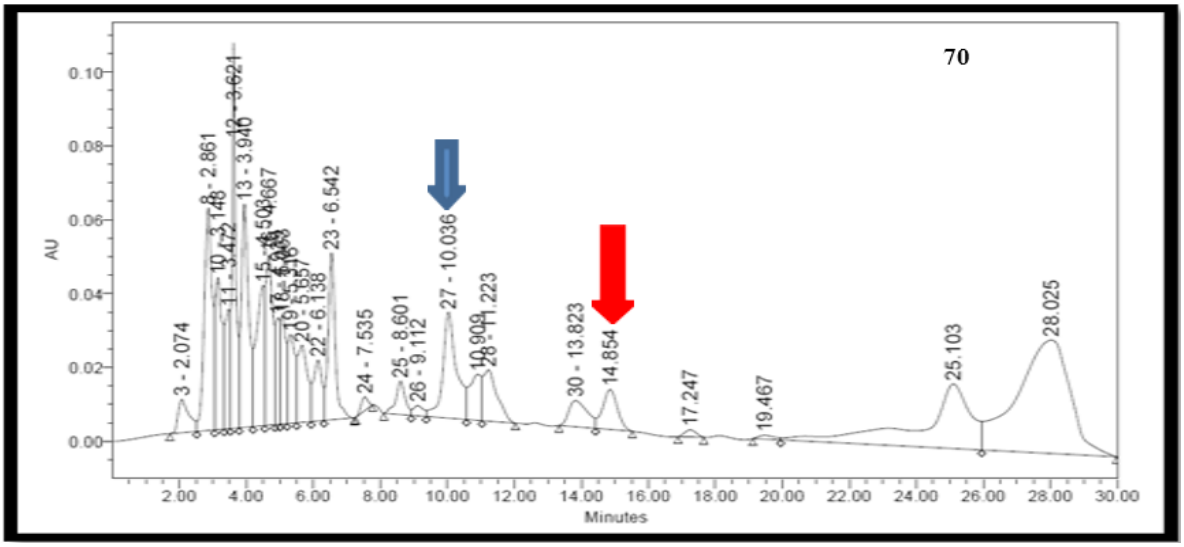

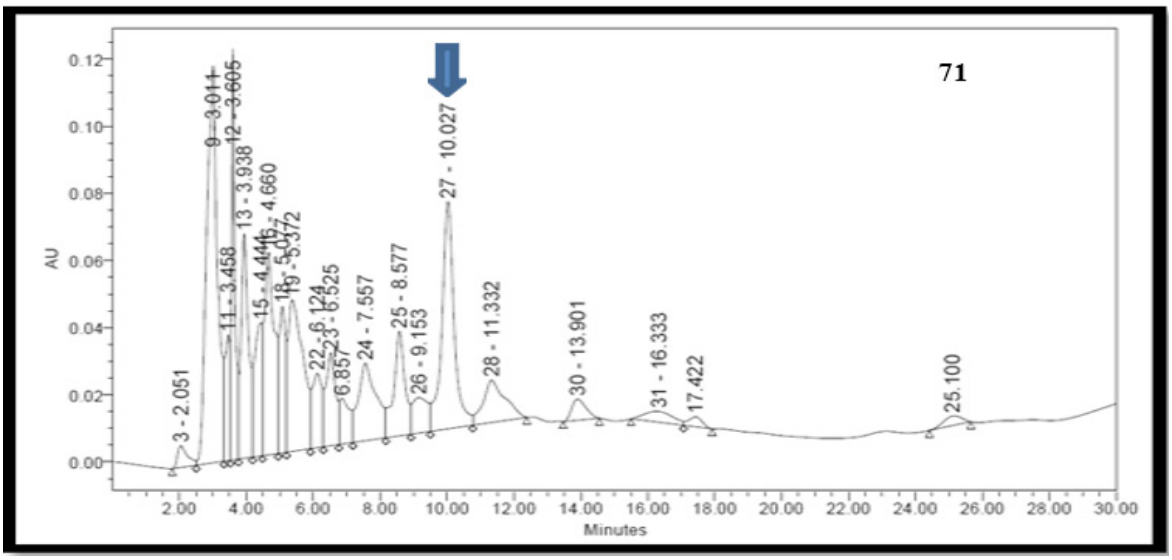

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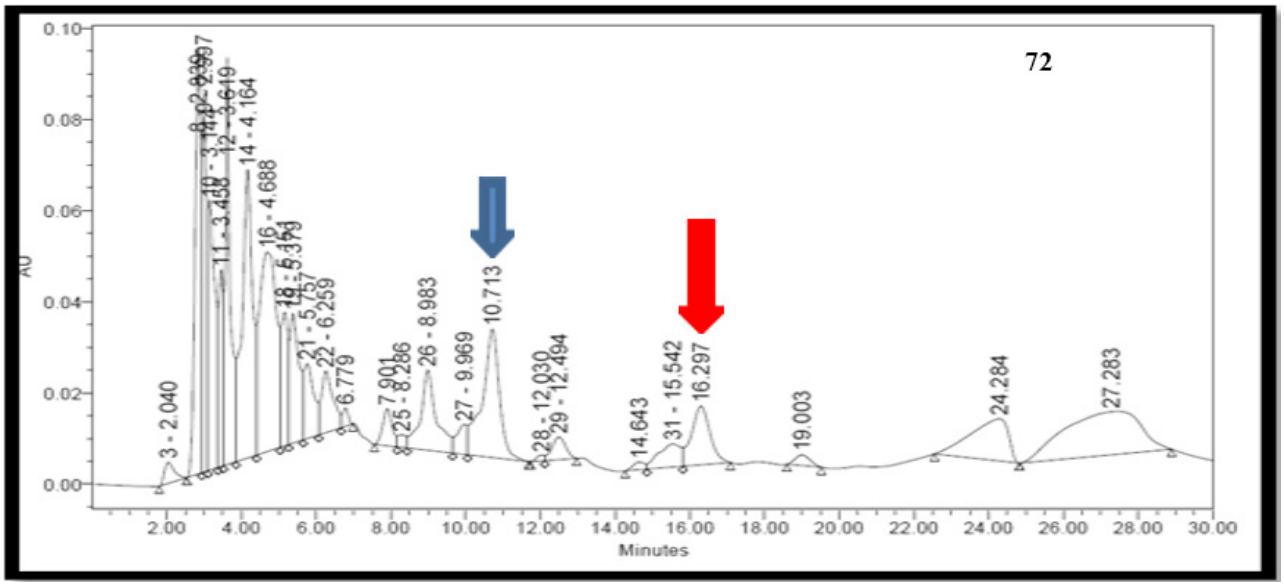
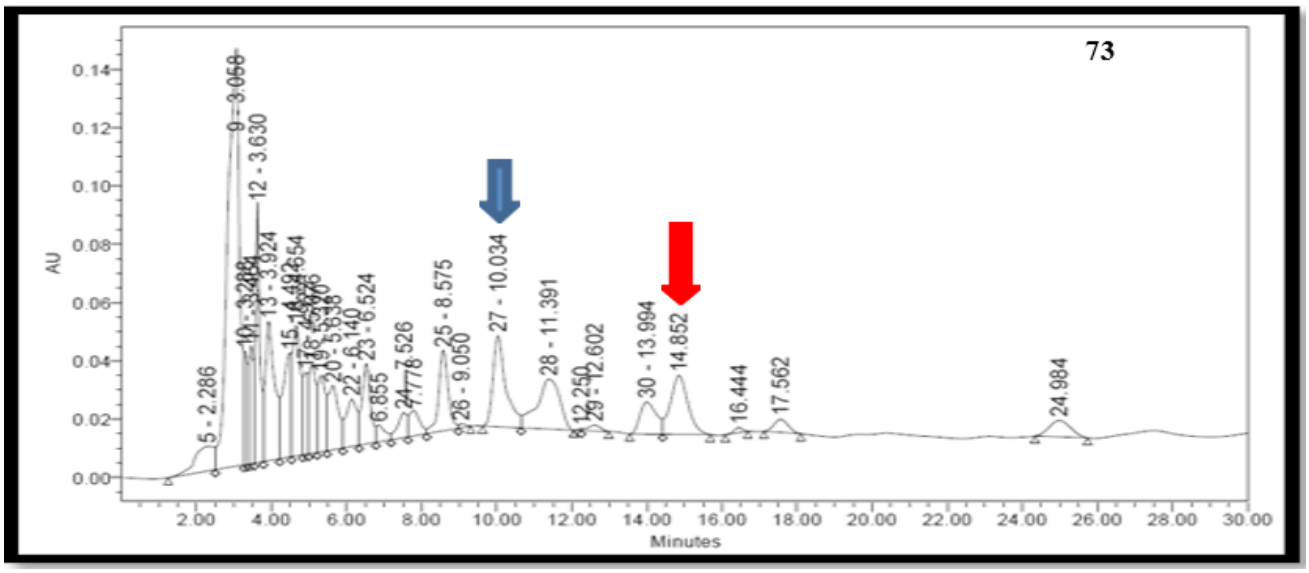


Plate 12: Blue arrow depicts Stevioside peak and Red arrow depicts Rebaudioside A peak in all the plates.

S. No.	Plant Code	Stevioside Retension Time	Stevioside Area	Stevioside (%)	Rebaudioside-A Retention Time	Rebaudioside-A Area	Rebaudioside-A (%)
73	73	10.034	356545	2.51	14.852	340312	3.07
74	74	10.713	419091	3.14	16.282	245845	2.37
75	75	10.684	729963	5.13	16.107	345588	3.12
76	76	10.687	823840	5.8	16.173	256446	2.31
77	77	9.951	311588	2.19	14.711	957834	8.65
78	78	10.661	388437	2.73	16.061	94118	0.85

Plate 13: Percentage of Stevioside and Rebaudioside-A based on retention time and Area under curve.



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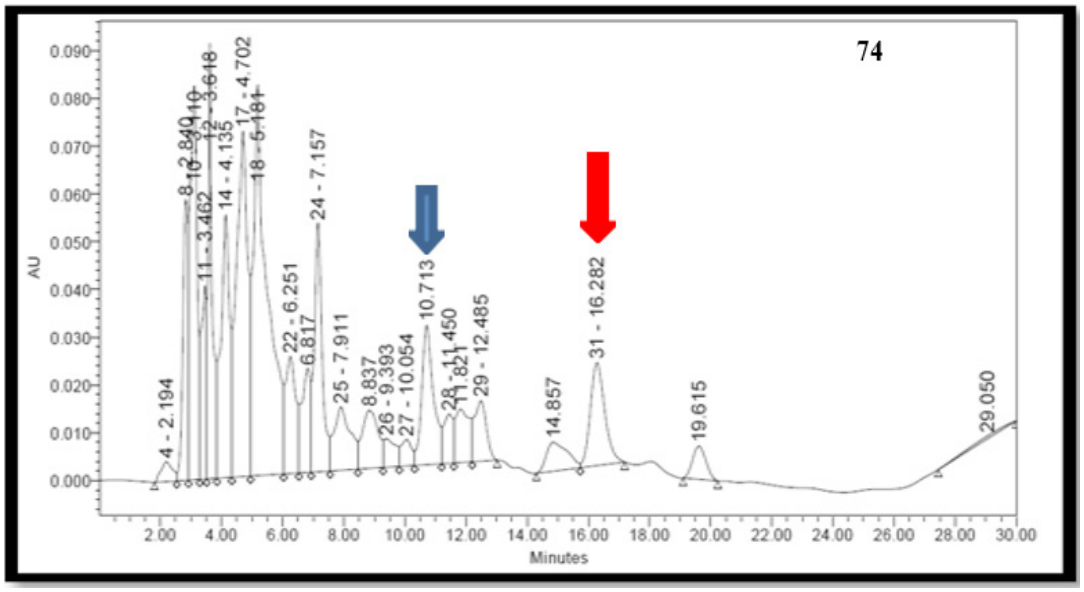

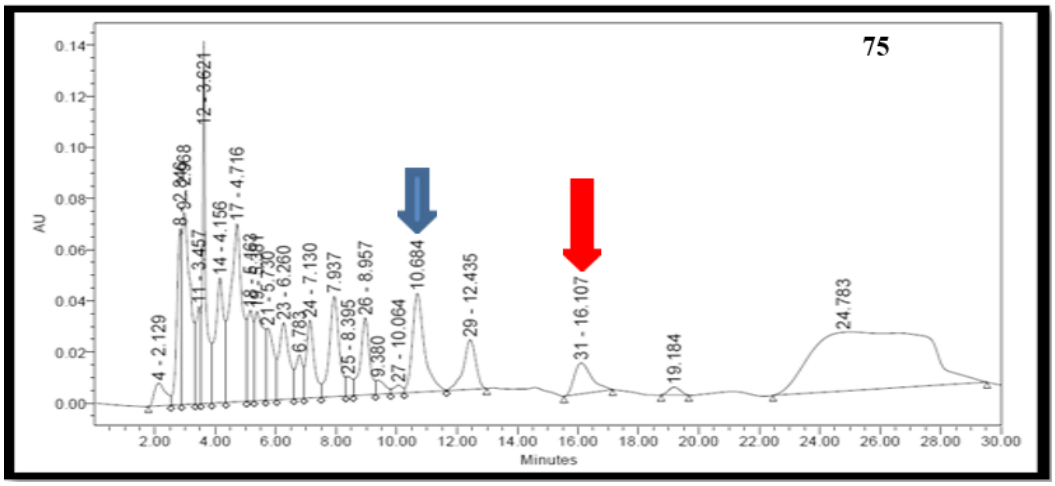

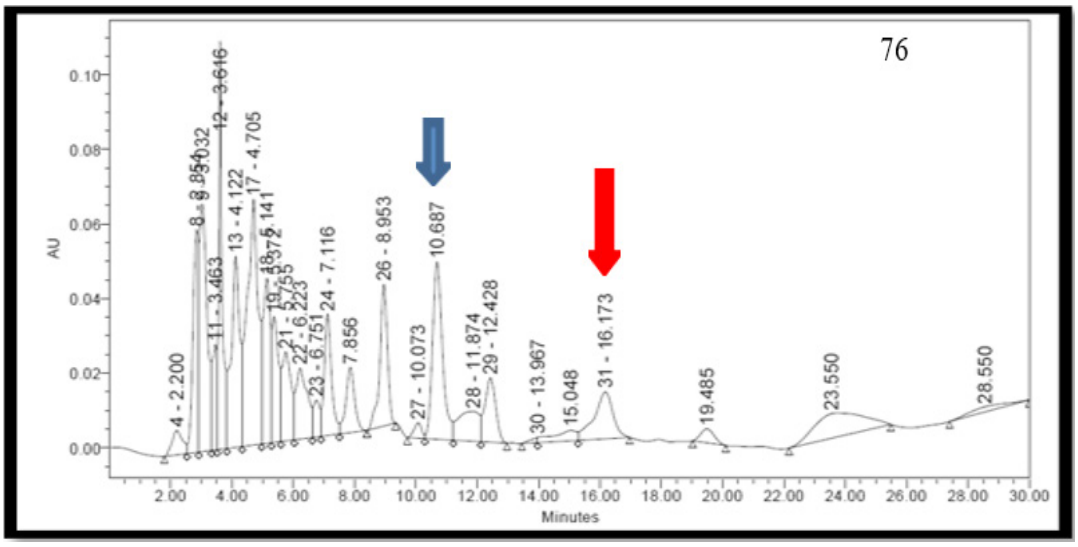

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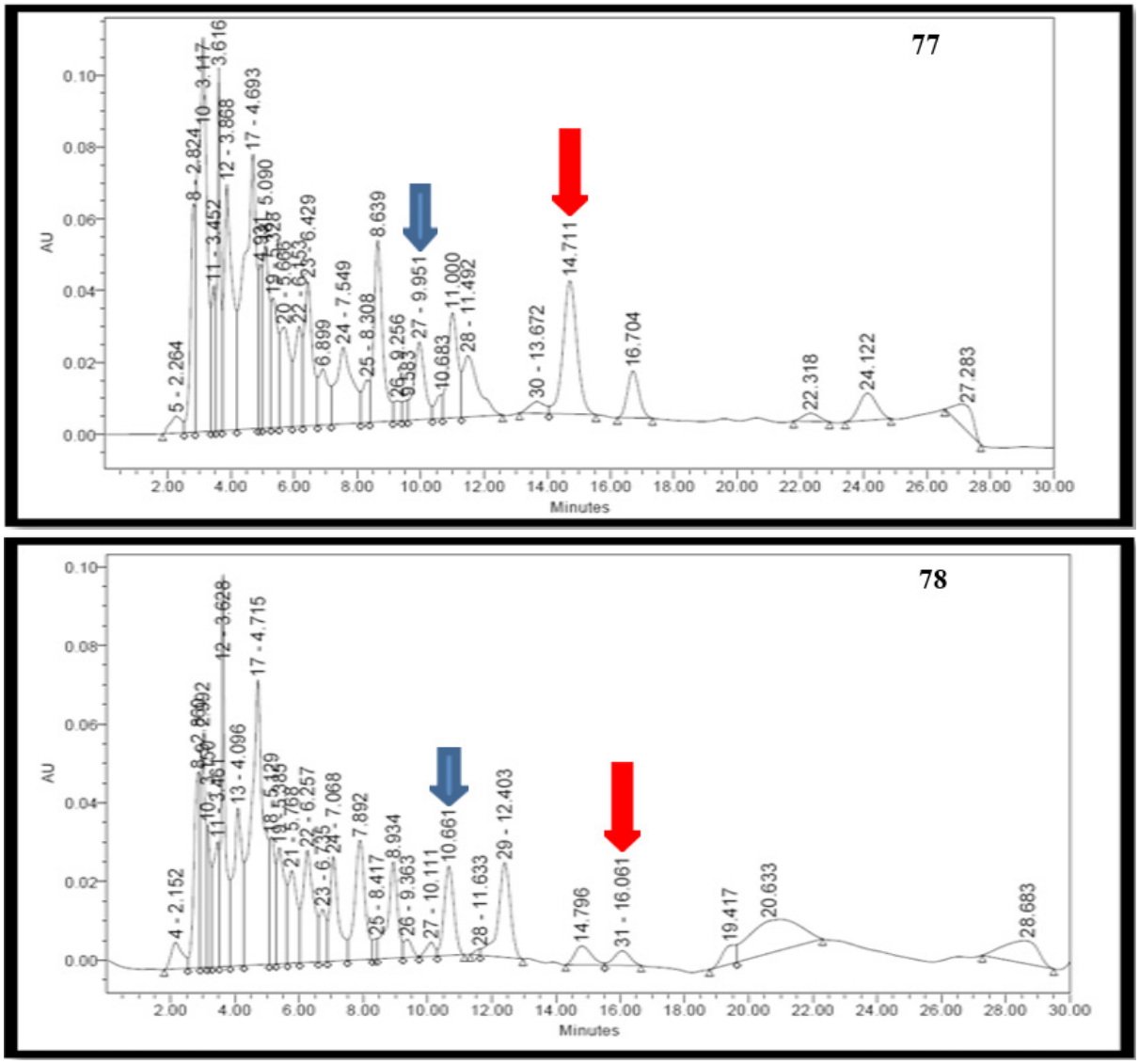

Plate 13: Blue arrow depicts Stevioside peak and Red arrow depicts Rebaudioside A peak in all the plates.

S. No.	Plant Code	Stevioside Retension Time	Stevioside Area	Stevioside (%)	Rebaudioside-A Retention Time	Rebaudioside-A Area	Rebaudioside-A (%)
79	79	10.008	234660	1.65	14.8	225411	2.04
80	80	9.997	502426	3.53	14.795	503743	4.55
81	81	10.723	32695	0.23	16.22	19042	0.17
82	82	10.622	528993	3.72	16.097	491106	4.43
83	83	10.65	535203	3.76	No peak	-	-
84	84	10.611	476256	3.35	16.035	563486	5.09

Plate 14: Percentage of Stevioside and Rebaudioside-A based on retention time and Area under curve.

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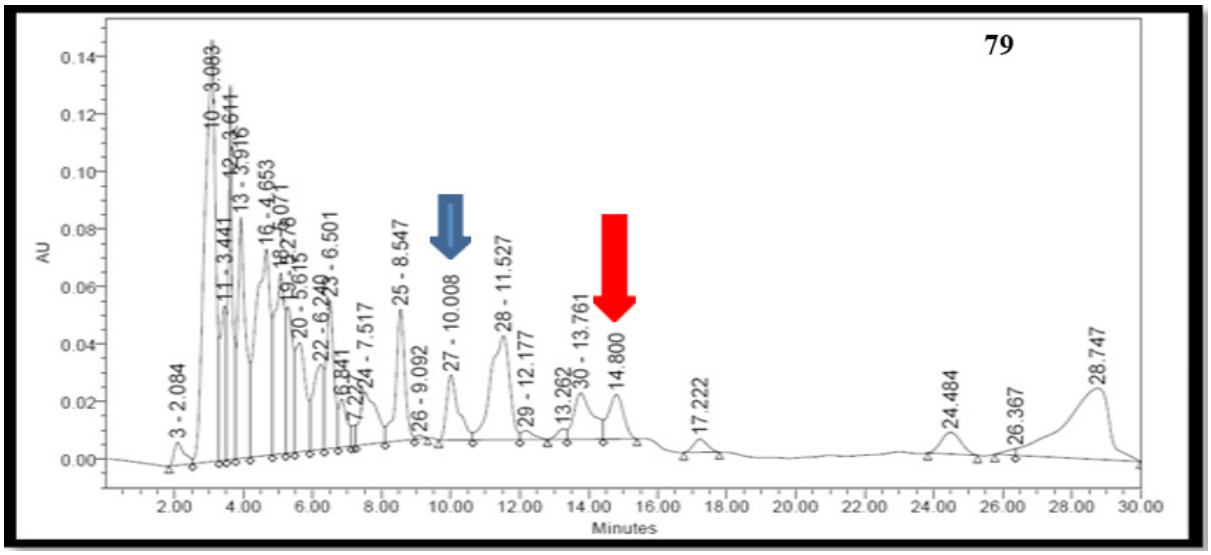

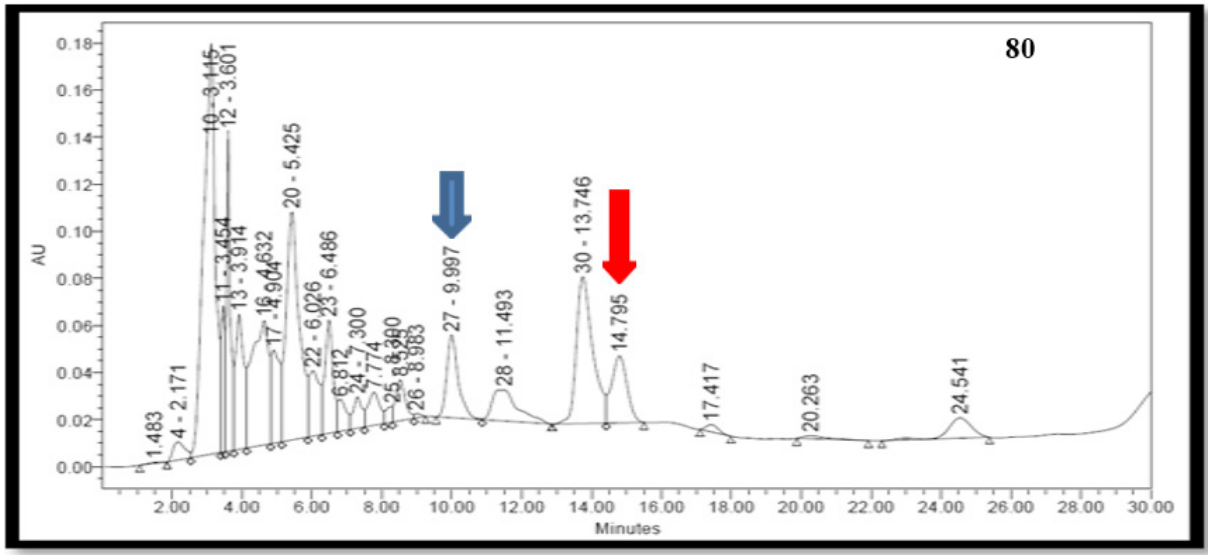

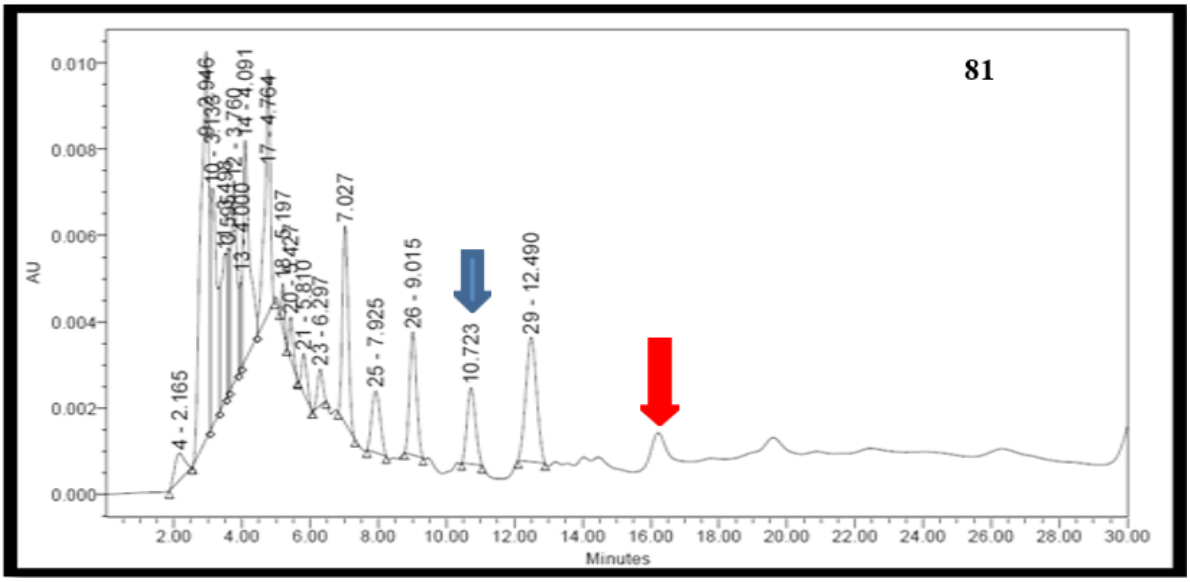

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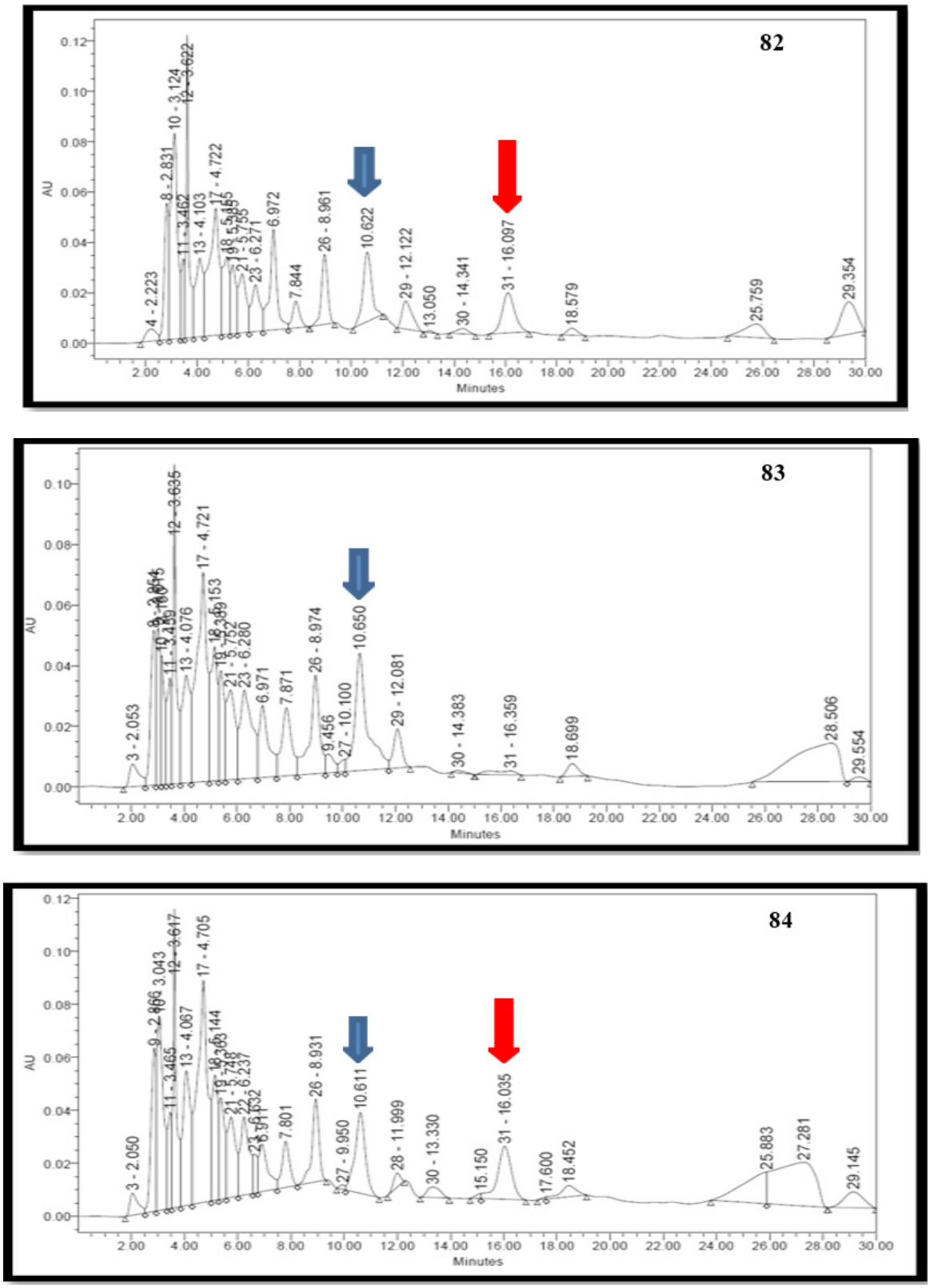

Plate 14: Blue arrow depicts Stevioside peak and Red arrow depicts Rebaudioside A peak in all the plates.

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