

Stability Study of the Active Constituents in the *Centella asiatica* Extract Formulations

P. K. Inamdar,* R. D. Yeole, M. M. Srivastava, and
N. J. de Souza

Research Centre, Hoechst India Limited, Bombay 400 080, India

ABSTRACT

The creams and dragees containing Centella asiatica plant extract were subjected to accelerated storage conditions, and stability of the active constituents, viz. asiatic acid, asiaticoside, madecassic acid, and madecassoside were evaluated, using reverse phase gradient high-performance liquid chromatography.

INTRODUCTION

Asiatic acid (I), asiaticoside (II), madecassic acid (III), and madecassoside (IV) are active principles of the plant *Centella asiatica*. Chemically these compounds are pentacyclic triterpenes, belonging to the β -amyrin ursolic acid group. These compounds are known for various biological activities like chronic venous insufficiency (1), varicose vein and wound healing properties (2). When applied locally on wounds in rats the compounds prompted the proliferation of granulation and increased tensile strength; they decreased wound area of skin necrosis induced by burns (3,4).

The two types of dosage forms of *C. asiatica* extracts were prepared for internal and external applications, i.e., dragees and creams respectively. These formula-

tions were stored for 12 months at different storage conditions of temperature and humidity.

This paper describes stability of the four active constituents (I-IV) of *Centella asiatica* in the formulations. Three batches of creams and four batches of dragees containing *Centella asiatica* extracts of different origins were used for this study.

EXPERIMENT

Chemicals

All the solvents were of HPLC grade. Double distilled, deionized water was used.

The formulations, i.e., dragees and creams, were prepared by our Galenical Department. These formula-

*To whom correspondence should be addressed.

This paper is dedicated to Dr. E. Baltin, Vice Chairman, Hoechst India Ltd., on his 60th birthday.

tions were stored at various accelerated conditions. For the stability study two parameters were ascertained: temperature and humidity. Storage conditions were A/C (air conditioned $25 \pm 1^\circ\text{C}$), R.T. (room temperature $30 \pm 1^\circ\text{C}$), 40°C , and $40^\circ\text{C} + 80\% \text{ R.H.}$

The standard samples of asiatic acid (I), asiaticoside (II), madecassic acid (III), and madecassoside (IV) were supplied by Kilo-scale Lab. Research Centre, Hoechst (I) Ltd.

Sample Preparation

Standard solution: Asiatic acid (I), asiaticoside (II), madecassic acid (III), and madecassoside (IV) were accurately weighed (25 mg each) and dissolved in water:methanol mixture (10:90) (10 ml).

Dragees: Four dragees weighing approximately 100 mg each were powdered and about 100 mg of powder was extracted with water:methanol mixture (10:90) (10 ml). The solution was centrifuged and the supernatant filtered through 0.45μ Millipore filter and used for HPLC analysis.

Creams: About 100 mg of cream was extracted with water:methanol mixture (10:90) (10 ml). The solution was centrifuged and the supernatant was filtered through 0.45μ Millipore filter and used for HPLC analysis.

Placebos of both the formulations were treated in the same manner and investigated on HPLC.

Apparatus

The liquid chromatograph of Waters Associates equipped with constant flow pump model 510; autoinjector WISP 712; 490 programmable multiwavelength detector; 840 data station with digital 350 computer.

Chromatography Conditions (5)

Column: μ -Bondapak, C-18, 10μ , $30 \times 0.39 \text{ cm}$, s.s

Gradient program:

Time (min)	Flow rate (ml/min)	Pump A (% water)	Pump B (% acetonitrile)	Curve No.
0	1.4	80	20	-
30	1.4	45	55	6

Detection: 220 nm

Injection volume: 20 μ l

Calculations

The percentage of active constituents in the samples was determined by external standard quantitation method, comparing areas of the sample peaks with those of the standard.

$$\% \text{ active compound} = \frac{C_{\text{std}} \cdot P_{\text{sample}} \cdot 10}{P_{\text{std}}}$$

where C_{std} = concentration of active constituent in the standard solution, P_{std} = peak area of active constituent in the standard solution, and P_{sample} = peak area of active constituent in the sample.

RESULTS AND DISCUSSION

Figure 1 shows HPLC separation of the active constituents (I-IV) of *Centella asiatica*. Figure 2 shows a chromatogram of HPLC analysis of creams and dragees. The percent content of individual active constituent and total percent content of active constituent of *Centella asiatica* of the various batches of creams are presented in Tables 1-3 and those of dragees are given in Tables 4-7. The residual contents were calculated by comparing the corresponding values with initial percentage. Figure 3a and 3b represents degradation curves for creams and dragees deteriorating according to first-order kinetics.

From the percent residual content, stability of creams and dragees can be expressed as:

Creams

Batch no. 1: Stable at A/C and R.T. for 6 months and at 40°C for 3 months

Batch no. 2: Stable at A/C for 12 months

Batch no. 3: Not stable at 40°C and $40^\circ\text{C} + 80\% \text{ R.H.}$

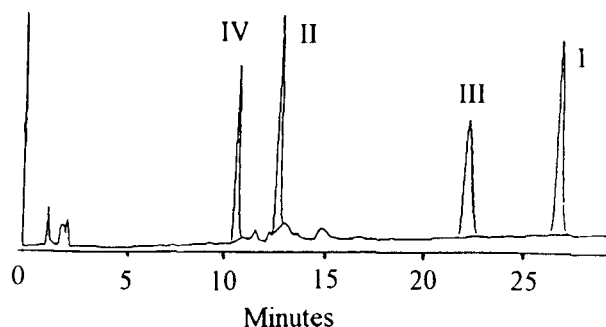


Figure 1. HPLC separation of active constituents (I-IV) of *Centella asiatica*.

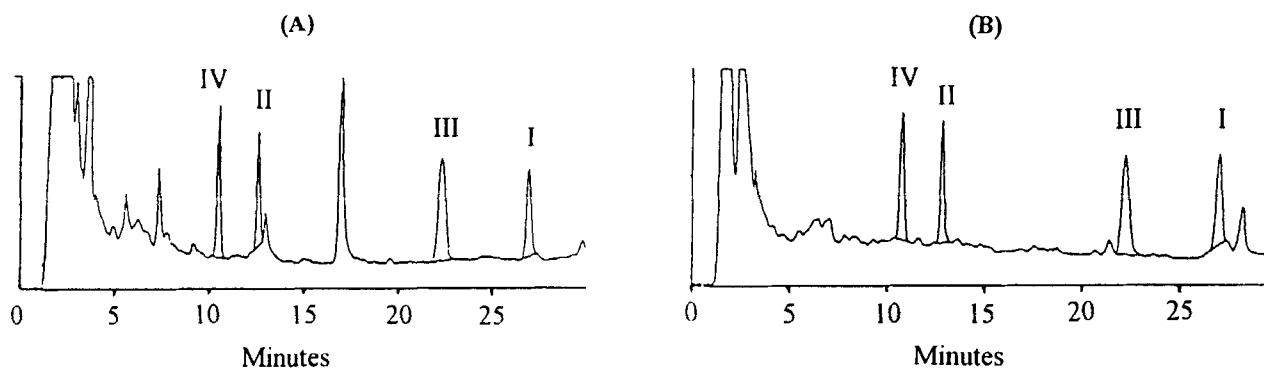


Figure 2. HPLC separation of active constituents (I-IV) in the creams (A) and dragees (B) containing *Centella asiatica* extract.

Table 1

Stability Study Results of C. Asiatica Cream (Batch no. 1)

Storage Period	Storage Condition	% Made-cassoside	% Asiaticoside	% Madecassic Acid	% Asiatic Acid	% Total Content	% Residual Content
Initial		0.18	0.11	0.41	0.27	0.97	100
3 months	R.T.	0.18	0.10	0.41	0.27	0.96	98.96
	40°C	0.18	0.09	0.39	0.23	0.89	91.70
6 months	A/C	0.17	0.11	0.40	0.20	0.88	90.72
	R.T.	0.17	0.10	0.40	0.20	0.88	90.72
	40°C	0.17	0.09	0.39	0.20	0.85	87.60
12 months	A/C	0.17	0.11	0.39	0.16	0.83	85.50
	R.T.	0.16	0.10	0.40	0.16	0.82	84.50
	40°C	0.16	0.09	0.39	0.15	0.79	81.40

Table 2

Stability Study Results of C. Asiatica Cream (Batch no. 2)

Storage Period	Storage Condition	% Made-cassoside	% Asiaticoside	% Madecassic Acid	% Asiatic Acid	% Total Content	% Residual Content
Initial		0.26	0.26	0.56	0.45	1.53	100
3 months	R.T.	0.19	0.22	0.55	0.35	1.31	85.60
	40°C	0.17	0.19	0.42	0.35	1.13	73.80
6 months	A/C	0.30	0.26	0.54	0.43	1.53	100
	R.T.	0.18	0.22	0.52	0.32	1.24	81.04
	40°C	0.18	0.19	0.40	0.33	1.10	71.89
12 months	A/C	0.31	0.26	0.54	0.41	1.53	100
	R.T.	0.16	0.20	0.49	0.30	1.15	75.16
	40°C	0.17	0.18	0.40	0.29	1.04	67.97

Table 3*Stability Study Results of C. Asiatica Cream (Batch no. 3)*

Storage Period	Storage Condition	% Made-cassoside	% Asiaticoside	% Madecassic Acid	% Asiatic Acid	% Total Content	% Residual Content
Initial		0.31	0.24	0.41	0.35	1.31	100
3 months	40°C	0.27	0.21	0.30	0.31	1.09	83.20
	40°C, 80% RH	0.28	0.19	0.30	0.26	1.03	78.60
6 months	R.T.	0.25	0.17	0.31	0.27	1.00	76.33
	40°C	0.23	0.15	0.27	0.19	0.84	64.10
	40°C, 80% RH	0.23	0.15	0.30	0.18	0.86	56.20
12 months	R.T.	0.27	0.18	0.25	0.23	0.93	70.90
	40°C	0.18	0.18	0.22	0.16	1.74	56.50
	40°C, 80% RH	0.17	0.13	0.16	0.10	0.56	42.80

Table 4*Stability Study Results of C. Asiatica Dragee (Batch no. 1)*

Storage Period	Storage Condition	% Made-cassoside	% Asiaticoside	% Madecassic Acid	% Asiatic Acid	% Total Content	% Residual Content
Initial		1.11	0.92	1.52	0.66	4.21	100
1 month	40°C, 80% RH	1.14	0.91	1.51	0.65	4.22	99.28
3 months	A/C	1.13	0.91	1.49	0.64	4.17	99.04
	40°C	1.14	0.88	1.45	0.63	4.10	97.38
	40°C, 80% RH	1.14	0.87	1.45	0.63	4.09	97.15
6 months	R.T.	1.09	0.86	1.38	0.62	3.95	93.82
	40°C, 80% RH	1.00	0.82	1.30	0.60	3.72	90.73
12 months	R.T.	0.83	0.65	1.10	0.56	3.14	74.58
	40°C	0.81	0.62	0.86	0.51	2.80	66.50
	40°C, 80% RH	0.76	0.57	0.87	0.55	2.75	65.32

Table 5*Stability Study Results of C. Asiatica Dragee (Batch no. 2)*

Storage Period	Storage Condition	% Made-cassoside	% Asiaticoside	% Madecassic Acid	% Asiatic Acid	% Total Content	% Residual Content
Initial		1.30	1.40	1.42	0.93	5.06	100
1 month	40°C, 80% RH	1.13	0.90	1.32	0.91	4.26	84.18
3 months	A/C	1.12	0.86	1.31	0.87	4.16	82.21
	40°C	1.09	0.81	1.25	0.79	3.94	77.86
	40°C, 80% RH	1.06	0.76	1.23	0.76	3.81	75.29
6 months	R.T.	1.01	0.82	1.23	0.62	3.68	72.72
	40°C, 80% RH	1.02	0.76	1.20	0.61	3.59	70.94
12 months	R.T.	0.81	0.62	0.79	0.59	2.81	55.53
	40°C	0.73	0.56	0.88	0.40	2.57	50.79
	40°C, 80% RH	0.71	0.51	0.87	0.57	2.66	52.56

Table 6
Stability Study Results of C. Asiatica Dragee (Batch no. 3)

Storage Period	Storage Condition	% Made-cassoside	% Asiaticoside	% Madecassic Acid	% Asiatic Acid	% Total Content	% Residual Content
Initial		1.20	0.94	1.48	1.18	4.80	100
1 month	40°C, 80% RH	1.16	0.93	1.47	1.06	4.62	96.25
3 months	A/C	1.13	0.92	1.45	0.97	4.47	93.12
	40°C	1.11	0.90	1.43	0.96	4.40	91.66
6 months	40°C, 80% RH	1.11	0.90	1.43	0.92	4.36	90.80
	R.T.	1.10	0.86	1.32	0.59	3.87	80.60
12 months	40°C, 80% RH	1.06	0.87	1.25	0.60	3.78	78.75
	R.T.	0.78	0.65	0.77	0.52	2.72	56.66
	40°C	0.72	0.60	0.91	0.57	2.80	58.30
	40°C, 80% RH	0.71	0.52	0.84	0.58	2.65	55.21

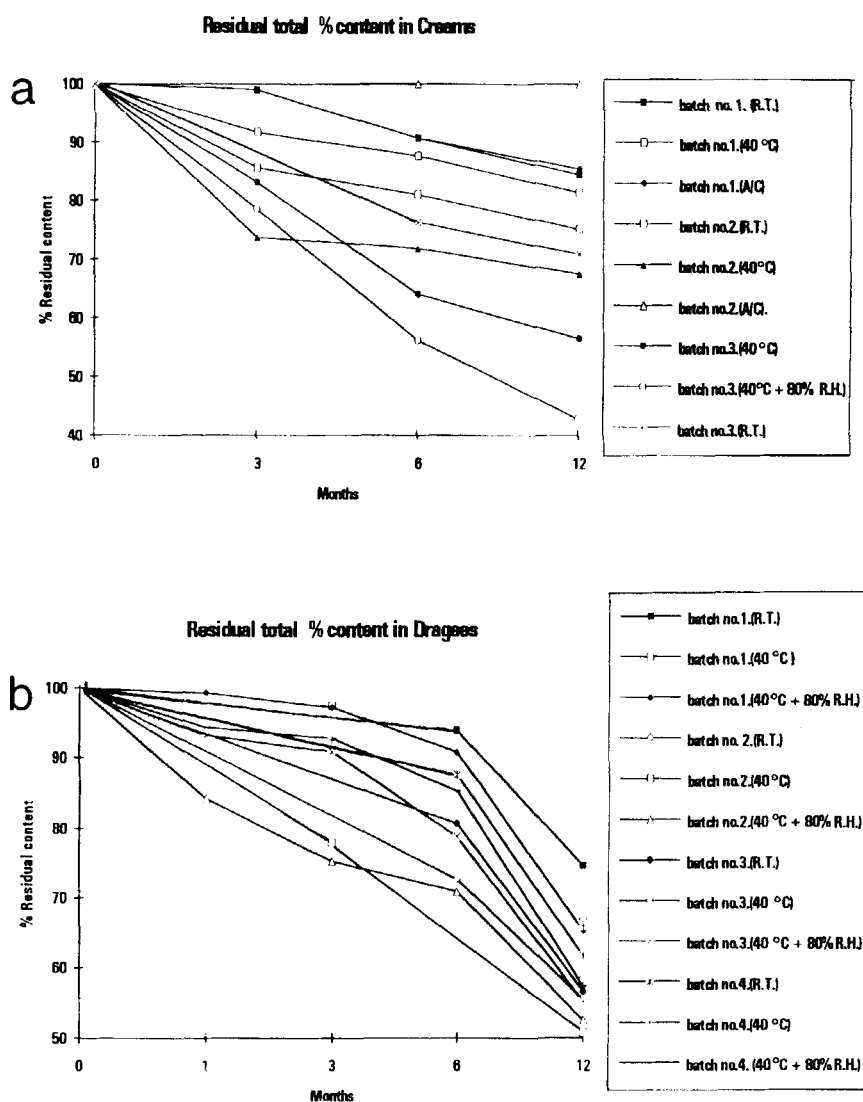


Table 7
Stability Study Results of *C. Asiatica* Dragee (Batch no. 4)

Storage Period	Storage Condition	% Made-cassoside	% Asiaticoside	% Madecassic Acid	% Asiatic Acid	% Total Content	% Residual Content
Initial		1.23	0.88	1.36	0.94	4.41	100
1 month	40°C, 80% RH	1.20	0.87	1.36	0.73	4.16	94.30
3 months	A/C	1.20	0.84	1.34	0.71	4.09	92.70
	40°C	1.19	0.85	1.34	0.71	4.08	92.52
	40°C, 80% RH	1.18	0.85	1.34	0.72	4.09	92.70
6 months	R.T.	1.09	0.85	1.33	0.59	3.86	87.53
	40°C, 80% RH	1.02	0.80	1.34	0.60	3.76	85.26
12 months	R.T.	0.78	0.63	0.80	0.51	2.72	61.68
	40°C	0.80	0.56	1.01	0.54	2.91	65.98
	40°C, 80% RH	0.72	0.52	0.83	0.46	2.53	57.37

Dragees

Batch no. 1: Stable at A/C, 40°C, and 40°C + 80% R.H. for 6 months

Batch no. 2: Not stable at A/C, 40°C, and 40°C + 80% R.H.

Batch no. 3: Stable at A/C, 40°C, and 40°C + 80% R.H. for 3 months

Batch no. 4: Stable at A/C, 40°C, and 40°C + 80% R.H. for 3 months

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