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Simultaneous Determination of *trans*-Resveratrol and Emodin in *Polygonum cuspidatum* Root Extracts and Pharmaceutical Preparations by High Performance Liquid Chromatography†

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A simultaneous determination of *trans*-resveratrol and emodin by high performance liquid chromatography (HPLC) in *Polygonum cuspidatum* root, extracts and dosage forms was developed and validated. HPLC analysis was performed on a reversed phase C_{18} column using the gradient formed by 0.1% (v/v) phosphoric acid in water and acetonitrile at the flow rate of 1 mL/min with photodiode array detection at 280 nm. The linear regression analysis data for the calibration plots for *trans*-resveratrol and emodin showed good linear relationship with correlation coefficients (r^2) 0.9995 ± 0.0002 and 0.9995 ± 0.00025, in the concentration range 0.5–2.0 and 0.25–1.00 μg per injection, respectively. The method was validated for precision and recovery. The limit of detection (LOD) and limit of quantification (LOQ) were 3.06, 9.27 and 1.03, 3.13 ng per injection, respectively, for *trans*-resveratrol and emodin. Statistical analysis confirmed that the method is accurate, precise and selective and can be applied for the identification and quantification of *trans*-resveratrol and emodin, simultaneously, in herbal extracts and pharmaceutical preparations.

Key Words: *trans*-Resveratrol, Emodin, *Polygonum cuspidatum*, High performance liquid chromatography,

INTRODUCTION

The dried roots of *Polygonum cuspidatum* (Family: Polygonaceae) are a well known traditional medicine in China as well as in Japan. In China, *Polygonum cuspidatum* is commonly known as Hu Zhang or Hu Chang and in Japan as Kojo-kon[1]. In addition to Asia, *Polygonum cuspidatum* was also distributed throughout North America and it is known as Mexican bamboo and Japanese knotweed.

Polygonum cuspidatum was used as a laxative in folk medicine. It is found to be useful for the treatment of atherosclerosis, cough, asthma, hypertension, cancer

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and hyperlipidimia[1–3]. Pharmacological properties of *Polygonum cuspidatum* were studied extensively over the last two decades and the activity was mainly attributed to anthraquinones, stilbenes, flavonoids and other phenolics, present in the product[4–7].

Polygonum cuspidatum roots are a rich source of *trans*-resveratrol, a stilbene and a potent antioxidant. The roots also contain considerable amounts of emodin, an anthraquinone, which affects the immune system, vasomotor systems and metabolic processes[8] and found to be toxic in rats at higher concentrations[9].

Because of beneficial effects of *trans*-resveratrol and the adverse effects of high concentrations of emodin on human health, several researchers have focussed their attention on the development of analytical methods to determine *trans*-resveratrol in wines, foods, human plasma and other natural products. The methods for *trans*-resveratrol include gas chromatography-mass spectrometry (GC-MS)[10–12], which involves the extraction and derivatization procedures requiring a significant amount of time, liquid chromatography-mass spectrometry (LC-MS)[13, 14], capillary electrophoresis (CE)[15], high performance thin layer chromatography (HPTLC)[16] and spectrophotometric[17] techniques.

High performance liquid chromatography (HPLC), presently the method of choice for quantification of *trans*-resveratrol[18–22] and emodin[23, 24], is based upon this application.

Capillary electrophoresis (CE)[25] and HPTLC[26] methods are reported for simultaneous estimation of *trans*-resveratrol and emodin in *Polygonum cuspidatum* and no HPLC methods have been reported for the simultaneous estimation of *trans*-resveratrol and emodin. In the present paper, a high performance liquid chromatographic method for the simultaneous estimation of *trans*-resveratrol and emodin in *Polygonum cuspidatum* roots, extracts and pharmaceutical preparations has been described. The proposed method was validated as per guidelines given by United States Pharmacopoeia[27].

EXPERIMENTAL

Standard *trans*-resveratrol and emodin were purchased from Sigma Chemical Co. (USA). Methanol and acetonitrile were of HPLC grade and phosphoric acid was purchased from Qualigens (Mumbai, India). Ultra pure water generated by the Barnstead nanopure system (model D3750, USA) was used. Methanol was used as a solvent for preparation of standards and samples. 0.1% (v/v) phosphoric acid in water and acetonitrile were used as mobile phase. All solutions were filtered through 0.45 μm pore size membrane filter using a Millipore-Swinnex type filtration unit. *Polygonum cuspidatum* roots and extracts were provided by M/s Laila Impex, Vijayawada, India. Dosage form capsules and tablets were purchased from the United States of America.

Sample Preparation

***Polygonum cuspidatum* root:** Weighed about 1 g *Polygonum cuspidatum* root powder into a round bottom flask, added about 30 mL of methanol and refluxed on a water bath for about 30 min. Filtered and repeated the same

operation (2 × 30 mL) with methanol; combined all the alcoholic fractions and made up to 100 mL with methanol. Filtered on 0.45 μm membrane filter.

***Polygonum cuspidatum* root extracts:** 10 mg of sample was dissolved in 50 mL of methanol, sonicated for 10 min. diluted to 100 mL with methanol and filtered through 0.45 μm membrane filter.

Pharmaceutical preparations

The average weights of the capsule and tablets were determined by weighing 20 capsules or tablets. Hard gelatin shells were removed in case of capsules and the contents were finely powdered. Weighed an amount of powder equivalent to 10 mg *trans*-resveratrol, added 50 mL of methanol, sonicated for 10 min. and made up to 100 mL with methanol and filtered through 0.45 μm membrane filter.

Calibration: 1 mg/mL *trans*-resveratrol and emodin solutions were prepared in methanol. Standard working solutions were prepared by diluting stock solution with methanol in the concentration range 25.0–100.0 ng/μL for *trans*-resveratrol and 12.5–50.0 ng/μL for emodin. 20 μL from each working standard solution was injected in six replicates. Calibration curve was generated by linear regression based on the peak areas.

HPLC Instrumentation: *trans*-Resveratrol and emodin were analyzed in a Shimadzu HPLC system with Alltima C_{18}, 5 μ (4.6 × 250 mm) column, LC-10AT VP pumps, SCL-10AVP system controller, SIL-10AD VP auto injector, SPD-M10 AVP photodiode array detector set at a wavelength 280 nm for detection and class VP software was used.

Analytical method: The column was kept at ambient temperature; the mobile phase consisted of a binary solvent systems using 0.1% (v/v) phosphoric acid in water (solvent A) and 100% acetonitrile (solvent B), kept at a flow rate 1 mL/min. The gradient program started with 70% solvent A and 30% solvent B which ramped linearly for 0.0–15.0 min, 10% solvent A and 90% solvent B for 15.0–17.5 min, 100% solvent B for 17.5–28.0 min and 70% solvent A and 30% solvent B for 28.0–35.0 min. The total run time was 35 min. Detection and quantification were performed at 280 nm using class VP software. Peaks were initially assigned by spiking the samples with standard compounds and comparison of their retention times.

RESULTS AND DISCUSSION

The composition of the HPLC mobile phase was optimized to achieve a good resolution between peaks. The best resolution and peak shapes were obtained by a gradient 0.1% (v/v) phosphoric acid in water as solvent A and acetonitrile as solvent B (Fig. 1). The compound with a retention time 11.1 ± 0.3 min was identified as *trans*-resveratrol and 23.6 ± 0.3 min was identified as emodin. Peak purity of each component was determined by PDA detector, which was completely in agreement with the standards.

To assess the validity of the method, validation tests were run. All test parameters were carefully chosen to cover the range of samples and concentrations involved. The linearity of standard curves were expressed in terms of the

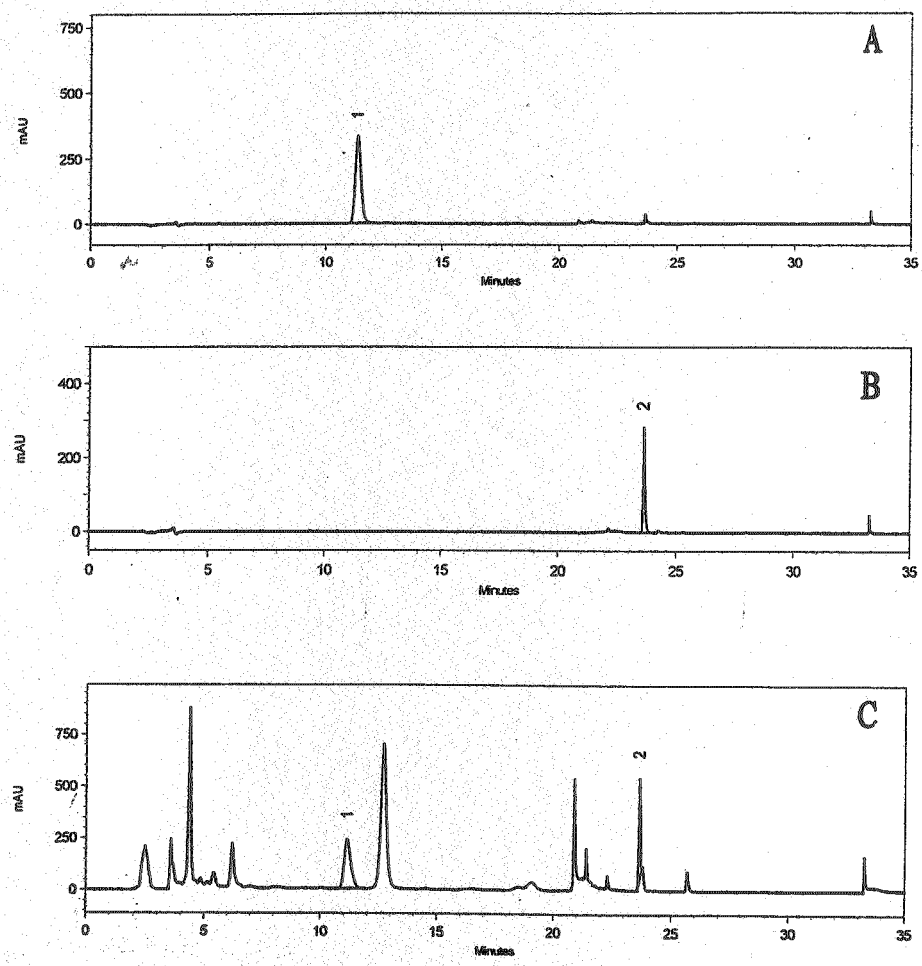

Fig. 1. HPLC Chromatograms: A. *trans*-Resveratrol standard (1), B. Emodin standard (2), C. *Polygonum cuspidatum* root extract

determination of correlation coefficient from plots of the integrated peak area *vs.* concentration of the same standard (μg per injection). Calibration curves were constructed in the concentration range 25.0–100.0 ng/μL for *trans*-resveratrol and 12.5–50.0 ng/μL for emodin. Linearity was found over the concentration range 0.5–2.0 μg per injection with a correlation coefficient 0.9995 ± 0.0002 for *trans*-resveratrol and 0.25–1.00 μg per injection with a correlation coefficient 0.9995 ± 0.00025 for emodin.

The precision of the method was validated by both intra- and inter-day variation. Six determinations of three concentrations of standard *trans*-resveratrol and emodin, on the same day (intra-day), and on different days (inter-day), were carried out and expressed as per cent relative standard deviation (% RSD). The results depicted in Table-1 showed the RSD % values of intra-day between 0.39–0.94% and 0.24–1.30% and inter-day between 0.28–1.27% and 0.36–1.33% for *trans*-resveratrol and emodin respectively. The precision of the method was satisfactory with acceptable values.

TABLE-1
INTRA- AND INTER-DAY PRECISION OF HPLC METHOD (n = 6)

Amount (ng)	Intra-day precision		Inter-day precision	
	SD	RSD (%)	SD	RSD (%)
(a) *trans*-Resveratrol				
490.0	2.17	0.44	1.37	0.28
1030.0	4.05	0.39	12.93	1.27
1640.0	15.45	0.94	9.04	0.56
(b) Emodin				
275.0	0.91	0.33	3.64	1.33
560.0	1.34	0.24	4.13	0.73
840.0	10.87	1.30	3.03	0.36

The accuracy of the method was determined from recovery studies. The pre-analyzed samples were spiked with three concentrations of standard *trans*-resveratrol and emodin and the mixtures were analyzed by proposed method.

The recoveries are in the range 99.97–100.61% and 99.57–100.06% for *trans*-resveratrol and emodin respectively (Table-2). Limit of detection (LOD) and limit of quantification (LOQ) were studied to check the sensitivity of the method under the working conditions proposed. Both followed USP criteria[25]. These limits referring to the concentrations in *Polygonum cuspidatum* roots, extracts and dosage forms were detected and quantified. The LOD and LOQ was 3.06 ng and 9.27 ng for *trans*-resveratrol and 1.03 ng and 3.13 ng for emodin, respectively.

TABLE-2
RECOVERY STUDY (n = 6)

Amount added (μg)	Amount recovered (μg)	Recovery (%)	RSD (%)
(a) *trans*-Resveratrol			
245.3	246.8	100.61	0.44
488.9	490.5	100.32	0.69
733.4	733.2	99.97	0.66
(b) Emodin			
233.6	232.6	99.57	0.32
467.1	466.6	99.89	0.58
700.7	701.1	100.06	0.70

Application of the method

The method was applied for determination of *trans*-resveratrol and emodin in two different *Polygonum cuspidatum* roots samples, four different *Polygonum cuspidatum* root extracts, three different tablets and two different capsules available in the market. Extracts from *Polygonum cuspidatum* roots are used as a therapeutic agent in several different ways. The contents of *trans*-resveratrol and emodin are evaluated. Table-3 shows that the *Polygonum cuspidatum* roots contain higher

amounts of emodin, whereas extracts and dosage forms contain relatively lesser amounts of emodin than *trans*-resveratrol.

TABLE-3
ESTIMATION OF *TRANS*-RESVERATROL AND EMODIN BY PROPOSED HPLC METHOD (n = 6)

Sample	Labelled amount (mg)		Estimated amount (mg)	%RSD			RSD (%)	SE
	trans-Resveratrol	Emodin	*trans*-Resveratrol		S.E.	Emodin		
Polygonum cuspidatum root[a]								
Sample 1	—	—	*0.56	1.34	0.01	*0.23	1.38	0.01
Sample 2	—	—	*0.70	1.19	0.01	*0.40	1.39	0.01
Polygonum cuspidatum root extracts[b]								
Sample 1	—	—	*58.47	1.33	0.31	*1.30	1.18	0.01
Sample 2	—	—	*56.81	1.00	0.23	*1.63	1.68	0.01
Sample 3	—	—	*25.10	0.49	0.05	*3.03	1.44	0.02
Sample 4	—	—	*30.74	1.30	0.16	*2.88	1.17	0.01
Pharmaceutical preparations[c]								
Tablet 1	13.00	—	12.36	0.40	0.02	4.46	0.53	0.01
Tablet 2	—	—	1.98	0.97	0.01	2.54	1.93	0.02
Tablet 3	20.00	—	19.14	1.08	0.04	6.07	0.90	0.02
Capsule 1	10.00	5.00	8.73	0.76	0.03	4.89	0.82	0.02
Capsule 2	15.00	—	19.77	0.36	0.03	1.32	1.76	0.01

*Expressed as mg/100 mg.
a: *Polygonum cuspidatum* root samples supplied by M/s Laila Impex, India.
b: *Polygonum cuspidatum* root extract samples supplied by M/s Laila Impex, India.
c: Tablet 1: KAL-Resveratrol.
Tablet 2: Jarrows formulas Resveratrol Synergy .
Tablet 3: Source Naturals Resveratrol.
Capsule 1: Natrol Protykin® Resveratrol.
Capsule 2: Solaray Resveratrol.

Conclusion

It is evident from a number of reports that *trans*-resveratrol is mainly responsible for the biological activity of *Polygonum cuspidatum* and appropriate concentration of emodin is desirable. Therefore, techniques for their estimation in plant materials and commercial products are of great importance. The HPLC method described is useful for accurate quantification of *trans*-resveratrol and emodin, simultaneously, in the range 25.0–100.0 ng/μL and 12.5–50.0 ng/μL in the plant materials, extracts and pharmaceutical dosage forms. The HPLC method developed is statistically validated for linearity, accuracy and precision; thus it also complies with international standards required for analysis of pharmaceuticals for human use.

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