SUPPLEMENTARY MATERIAL

RP-HPLC analysis of seco-iridoid glycoside swertiamarin from different *Swertia* species

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Abstract

Genus *Swertia* is valued for its great medicinal potential, mainly *Swertia chirayita* (Roxb. ex Fleming) H. Karst. is used in traditional medicine for a wide range of diseases. Seco-iridoid glycosides like swertiamarin referred with enormous pharmacological potentials. Aim of the study was to identify suitable substitute to *S. chirayita* by quantifying seco-iridoid swertiamarin from five different *Swertia* species endemic to Western Ghats. The reverse phase high performance liquid chromatography diode array detector analyses was performed and chromatographic separation was achieved on a Lichrospher 100, C18e (5 µm) column (250-4.6 mm). Mobile phase consisting of acetonitrile and water (25:75) was used for separation. Results indicated that concentration of marker compound has been found to vary largely between and within the species from different localities. Content of swertiamarin was highest in *S. chirayita* compared to other species studied herein. Advocating use of *S. minor* as alternate source to *S. chirayita*.

Keywords: *Swertia*; Western Ghats; Swertiamarin; RP-HPLC.

1. Experimental

1.1 Collection of plant materials and extract preparation

Plant material of all the species were obtained from different localities of Western Ghats. The whole plant material was air-dried at room temperature and ground to fine powder in laboratory grinder. Extracts were prepared by adding 250 mg plants powder in 25 ml methanol for 24 hrs. The filtrates were re-volumised and subjected to RP-HPLC analysis after passing through 0.2µm nylon filters.

1.2 Chemicals and standard

All solvents and chemicals used during the study were of HPLC grade. A HPLC grade swertiamarin (99.3% pure) was procured from ChromaDex, USA. A accurately weighed standard swertiamarin (CID:442435) was dissolved in known amount of methanol to obtain mg/ml concentration stock. The stock solution was then diluted to obtain desired working concentrations (10, 20, 40, 80, 100, 250, 500 ppm).

1.3 Reverse phase-high performance liquid chromatographic analysis

The RP-HPLC analysis was performed on Shimadzu chromatographic system consisting of a quaternary pump, manual injector and dual λ UV absorbance diode array detector. The built in LC-Solution software system was used for data processing. Chromatographic separation was achieved on a Lichrospher 100, C18e (5 μ m) column (250-4.6 mm). Mobile phase consisting of acetonitrile and water (25:75) was used for separation with injection volume 20 μ L. The flow rate was 1 ml/min and the detection wavelength of the dual λ absorbance detector beam was set at 238 nm. The analysis time was 5 min for both standard and sample. The system suitability test was assessed by three replicate injections of the standard solutions at a particular concentration.

Figure Captions

Fig 1. RP-HPLC profile chromatograms of Swertia species and Standard swertiamarin a) *S. minor* b) *S. densifolia* c) *S. lawii* d) *S. corymbosa* e) *S. angustifolia* var. *pulchella* f) *S. chirayita* g) 100 ppm standard swertiamarin h) Seven point calibration curve for swertiamarin.

Abbreviations

RP-HPLC: Reverse phase high-performance liquid chromatography; **UV:** Ultraviolet; λ_{max} : Maximum absorption wavelength; **LOD:** Limit of detection; **LOQ:** Limit of quantification; **SD:** Standard deviation; **RSD:** Relative standard deviation; **PDA:** Photodiode array; **N:** Theoretical plate number (#); **T:** Tailing factor; **ppm:** Parts per million; **RT:** Retention time.

Table S1. HPLC validation parameters for Swertiamarin

Parameters	Swertiamarin
RT (min)	3.611
Linearity (10-500 ppm)	$y=18601.5.x + 111259.8$
R^2	1.000
LOD (ppm)	0.0594 ± 0.003
LOQ (ppm)	0.1799 ± 0.009
RT RSD (%)	0.125
Theoretical plate # (N)	12923.736 ± 4568.947
Tailing Factor (T)	1.271 ± 0.068

Figure S1. RP-HPLC profile chromatograms of *Swertia* species and Standard swertiamarin a) *S. minor* b) *S. densifolia* c) *S. lawii* d) *S. corymbosa* e) *S. angustifolia* var. *pulchella* f) *S. chirayita* g) 100 ppm standard swertiamarin h) Seven point calibration curve for swertiamarin.