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Research article

IMPROVED RP-HPLC METHOD FOR QUANTITATION OF SWERTIAMARIN FROM *SWERTIA DENSIFOLIA* LEAVES AND MARKETED HERBAL FORMULATIONS

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ABSTRACT

Objective: Present study aims at developing and validating an improved RP-HPLC method for determination of Swertiamarin from extracts of *Swertia densifolia* (Griseb.) Kashyap leaves and marketed herbal formulations.

Methods: Developed RP-HPLC method is validated and is used for determination of Swertiamarin from hydroalcoholic extracts of test plant material and marketed herbal formulations. The separation is carried out on C_{18} column. The mobile phase used is 10 mM Ammonium acetate: Acetonitrile: Formic acid in ratio 90:10:0.2% (*v/v/v/*). The wavelength selected is 237 nm using UV detector.

Results and Discussion: A seven-point calibration curve over the concentration range of 50.00 –20000.00 ng/mL for Swertiamarin provides an optimum linear detector response (with r^2>0.9970). The % R.S.D. (n = 6) for the low, middle and high quality control samples are within <6.44 and < 9.04 for inter-day and intra-day precision and accuracy respectively.

Conclusion: A simple, sensitive and precise method is developed for quantitation of SWM. Method is validated as per International Conference on Harmonization (ICH) guidelines and its applicability is demonstrated by successful measurement of Swertiamarin from hydroalcoholic extracts of *Swertia densifolia* leaves and selected marketed herbal formulations.

Keywords: RP-HPLC, Swertiamarin, *Swertia densifolia* (Griseb.) Kashyap, Analytical Method Validation, International Conference on Harmonization (ICH).

INTRODUCTION

Swerta chirata (Wall) Clarke is used in various Ayurvedic formulations for its high medicinal values. It is also used as folk medicine in India and Nepal. Owing to its high commercial demands its population in wild are depleted beyond in regeneration capacity (Chhipi Shrestha, J.K., *et.al*, 2013). *Swertia densifolia* (Griscb) Kashyap, the plant selected for current research work, also known as *Swertia decussata* Nimmo.ex Grah., belonging to family *Gentianaceae*, is an annual herb found in Western Ghats at an altitude of 1500-2000 cm. *Swertia densifolia* is well known potential substitute for *Swertia chirata* (Wall) Clarke (Shailajan and Abhishek, 2009). Swertiamarin (SWM) is a secoiridoid glycoside, found in members of Gentianaceae family like *Swertia chirata* (Wall) Clarke, *Swertia ciliata*, *Swertia japonica* Makino, *Swertia angustifolia* Buch.-Ham.ex D. Don and *Swertia densiflora* (Griscb.) Kashyap. Swertiamarin (Fig.1), 4aR,5R,6S)-4a-Hydroxy-1-oxo-5-vinyl-4,4a,5,6-tetrahydro-1H,3H-pyrano[3,4-c] pyran-6-ylβ-D-glucopyranoside, shows antidiabetic, antinociceptive, hepatoprotective, antihyperlipidaemic, antioxidant activities. (Patel, T, *et.al,* 2013, Vaidya, H., *et.al*, 2009a, Jaishree, V, *et.al*, 2009, Jaishree, V. and Badami, S., 2010).

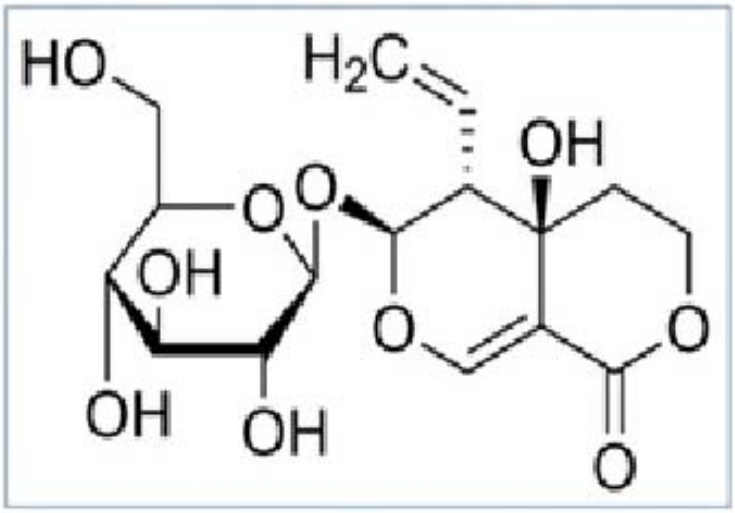

Fig-1: Sewrtiamarine Structure

The current experimental RP-HPLC method is developed for quantification of SWM from plants of *Gentianaceae* family as well as marketed formulations containing plant from *Gentianaceae* family as ingredient. The method is further used to quantitate the contents of the plant material as claimed in the labels of formulations containing plants of *Gentianaceae* family. It ias assumed that the formulations contains the selected experimental plant material as an ingredient. The extraction solvent used is 70% ethanol instead of methanol as reported in earlier methods. Earlier reported methods for quantification of SWM were less sensitive with the LOD up to 4 µg/mL and LOQ up to 6 µg/mL. The linearity range of 4 µg/mL to 80 µg/mL is the lowest range for linearity reported by RP-HPLC method (Ahmad, J., *et.al.*, 2014, Alam, P., *et.al*, 2009, Rana, V.S., *et.al*, 2012,). Current method reports LOD of 25 ng/mL and LOQ of 50 ng/mL. The linearity is from 50 ng/mL to 20000 ng/mL for current improved method.

The solvent ratio of the mobile phase used in current experimental method was 10 mM Ammonium acetate: acetonitrile: formic acid in the ratio of 90:10:0.2% *v/v/v*. To avoid the interfence of excipients from the sample matrix, retention time of 9.0 ± 0.5 min is set with a run length of 15 mins. This time is either almost same or shorter than several previously reported methods, which used runtime from 10 to 30 mins. Sharper peaks are obtained in the current method at wavelength of 237 nm as compared to that of wavelength of 254 as reported by others. (Ahmad, J, *et.al*, 2014, Alam, P., *et.al*, 2009, Rana, V.S, *et.al*, 2012).

MATERIALS AND METHODS

Plant Material: The plant material was collected from Mahabaleshwar, Dist. Satara in the month of March when SWM contents in the plant are reported to be highest (Shailajan, S., *et.al*, 2009). The plant material was authenticated as *Swertia densifolia* (Griseb.) Kashyap, by experts from Blatter Herbarium, Mumbai. The leaves were washed with the water and air dried. Foreign organic matter was removed. The cleaned plant material was shade dried for 4-5 days and then kept in hot air oven at 37 ± 2°C for 48 hours. The dried plant material is powdered sieved through BSS mesh no. 85 to obtain the uniformity in the test plant material. The sieved plant material was stored in polypropylene container till further use.

Chemicals and Reagents: The organic solvents and chemicals used for extraction under study are of analytical grade and procured from Qualigens Fine Chemicals, Mumbai, India. HPLC grade formic acid (88%), acetonitrile, methanol and distilled water were procured from Merck, Mumbai, India. Standard Swertiamarin (98% purity) is procured from Chengdu Biopurify Phytochemicals Ltd., China.

Method Validation

Preparation of standard solution and quality control (QC) samples: Two sets of SWM stock solutions of concentration 1.0 mg/mL prepared by independent weighings in methanol. One set was used to prepare calibrant working solutions while the other set was used to prepare quality control working solutions in diluent. The diluent used is acetonitrile: 10 mM ammonium acetate: formic acid (1:9:0.2 % *v/v/v*). All the stock and working solutions were stored at 2–8°C.

Chromatographic conditions: The mobile phase used is 10 mM Ammonium acetate: Acetonitrile: Formic acid in the ratio 90:10:0.2% *v/v/v*. Flushing solvent used for the experiment was mobile phase. UV detector, Jasco UV-970, was used at a wavelength of 237 nm. Maximum pump pressure of 500 kg/cm^2 is set at Jasco PU-980 pump. Autosampler (Jasco AS-1527), temperature is set at 4°C ± 2°C. Other chromatographic conditions like flow rate of 1.0 mL/min along with the injection volume as 50 µL are set. Cosmosil C_{18} (4.6 mm i.d. x 150 mm, 5 µ particle size) is used as column.

System Suitability: System suitability test is performed to verify that whether system is adequate for the intended analysis. Replicate injections of the standard solution of 1 µg/mL (n = 6), are compared to ascertain whether requirements for precision meet. The data generated by these injections is used to calculate % relative standard deviation.

Linearity and Quality control samples: Linearity (calibration curve) is obtained by analyzing working standard solutions of SWM at 7 different concentration levels (50, 100, 500, 1000, 5000, 10000 and 20000 in ng/mL). Quality control (QC) samples at three different concentration levels (150, 2500 and 15000 ng/mL as low, medium and high) are prepared independent of the calibration standards.

The peak areas and their respective concentration of SWM obtained are subjected to regression analysis for evaluating the correlation coefficient (r^2) and regression equation (y = mx + c) by the least square method (Niazi, S.K., 2000).

Specificity: The specificity of the method developed is ascertained by analyzing the standard and samples. The peak of SWM in samples were confirmed by comparing the retention time and the spectra of standard. The peak purity of SWM is assessed by comparing the spectra at three different levels *i.e.* peak start, peak apex and peak end position of the chromatogram (Ahamad, *et.al.*, 2014).

Precision and Accuracy: The precision and accuracy of the system is determined by measuring repeatability of sample application and measurement of concentration for six replicates at three different concentrations of 150, 2500, 15000 ng/mL. Intra and inter-day variation for the determination of SWM were also carried out. The intraday precision is carried out on the same day while inter-day precision (intermediate precision) was studied by comparing assays performed on three different days. The precision of the system and method are expressed as percent Relative Standard Deviation (% R.S.D.) and % Relative error (% R.E.) (Nair, S.N., *et al*, 2012).

Robustness and Ruggedness: Robustness of the method is determined by changing the flow rate (0.95 to 1.05 mL/min), mobile phase composition (10 mM Ammonium acetate: Acetonitrile: Formic acid, 91:09:0.1% to 89:11:0.3 *% v/v*) whereas, Ruggedness is determined by change in instrument, column and analyst of assay procedure (Ahamad, *et.,al.*, 2014).

Limit of Detection (LOD) and Limit of quantification (LOQ): The limit of detection (LOD) and limit of quantitation (LOQ) for the developed method is determined by injecting progressively low concentrations of the standard solution of SWM. Limit of detection (LOD = 25 ng/mL) and limit of quantitation (LOQ = 50 ng/mL) are established at a signal to noise ratio of 3:1 and 10:1 respectively (Shailajan, S., *et.al*, 2010).

Assay: 1 µg/mL pure standard (Swertiamrin), leaf extracts of *S. densifolia* and extracts of four marketed herbal formulations (Formulation 1, Formulation 2, Formulation 3 and Formulation 4) were injected into HPLC system and their respective areas under curve for SWM were recorded. 70% ethanol is used as extraction solvent. 1 gm of test plant material and marketed herbal formulation 1 and 2 (powders) and 2 mL of formulation 3 and 4 (extracts) were extracted with 10 mL of extraction solvent for 18 hours. This extract was centrifuged and filtered and is used for determination of SWM using developed RP-HPLC method. Peaks of SWM were assigned according to their retention times and by peak start time, peak apex and end point of peak with reference standard. The UV spectra for both the reference standard and samples under same chromatographic condition are also compared for specificity. The amount of SWM present per gram or per mL of sample is calculated using the regression equation (Shailajan, S., *et.al*, 2010).

RESULTS AND DISCUSSION

Optimization of chromatographic conditions: HPLC method for quantification of SWM in plants and marketed formulation has been reported earlier (Alam, *et.al*, 2009, Ahamad, *et.al*, 2014) with a total run length of 30 min. Another method for quantification of SWM in plants and marketed formulations with run length 10 min using methanol: distilled water in ratios ranging from 50:50 to 90:10 *v/v* (Ahamad, *et.al*, 2014, Alam, *et.al*. 2009, Rana, V.S., *et.al*, 2012) is also reported.

A comparison of chromatographic results using C18 and C8 columns with different specifications were performed. It is observed that good separation, better resolution, sensitivity and selectivity were accomplished on a C18 column to which the binding of analyte is stronger. Further, it is seen that the type and concentration of organic modifier (acetonitrile or methanol), the formic acid concentration in the mobile phase and the buffer concentration intensed the chromatographic separation. Acetonitrile on comparison with methanol gave a significant increase in signal intensity of SWM. A wide range of concentrations of formic acid (0.1 % to 1.0 %) was investigated.

It is observed that peak response of SWM increased considerably when the concentration of formic acid in the phase is 0.2 %. Several different concentrations of ammonium acetate and ammonium formate (2–10 mM) buffer solution were tested. It is found that 10 mM ammonium acetate resulted in a good response and improved the peak shape for the analyte. Based on above observations, isocratic mobile phases of different combinations were tested. However, irrespective of the phase composition tried, it is seen that there are co-eluting peaks from the hydroalcoholic extracts of selected test plant material and marketed formulations at the retention time of the analyte. The ratio of the mobile phase is therefore finalized at 90:10:0.2% *v/v/v* that of 10 mM Ammonium acetate: acetonitrile: formic acid. The run length of the method is also increased upto 15 mins resulting in good resolution of the analyte from endogenous peaks from the test samples. Under the optimized chromatographic conditions, the retention time for SWM is at 9.0 ± 0.5 mins.

System Suitability: The system suitability was carried out by injecting (n = 6) freshly prepared 1 µg/mL solution of SWM. The system suitability is evaluated by calculating % R.S.D. for areas and retention times obtained for SWM in experimental setup. On calculation % R.S.D. is found to be 3.63 and 0.02 for area values and retention times respectively (Table no.1).

Limit of Detection (LOD) and Limit of Quantification (LOQ): The least LOD and LOQ values in reported methods are 4 µg/mL and 6 µg/mL respectively (Ahmad, J., *et.al*, 2014, Alam, P., *et.al*, 2009, Rana, V.S., *et.al*, 2012,). As compared to these reported methods, current method is found to be more sensitive with LOD and LOQ of 25 ng/mL and 50 ng/mL respectively. This indicates that proposed method is more sensitive to effectively determine lower concentrations of SWM from samples.

Specificity: The specificity of proposed method was determined by comparing the sample and standard peak for its retention time and UV spectra. Three point peak purity *i.e.* peak start, peak apex and peak end, was compared and is found to be superimposing. This indicates that the standard and sample peaks were not merging with any other components or impurities from the sample matrix.

Linearity: A wide linear range for SWM have been reported in earlier methods like 4 µg/mL to 80 µg/mL, 17.25 µg/mL to 56.92 µg/mL and 10 µg/mL to 1000 µg/mL, to obtain calibration curve (Ahmad, J., *et.al.*, 2014, Alam, P., *et.al.*, 2009, Rana, V.S., *et.al.*, 2012,). In the current method calibration curve area versus concentration (ng/mL) was found linear in the range of 50-20000 ng/mL. The linear regression data for the calibration curve shows good linear relationship over the concentration ranges of 50-20000 ng/mL with respect to peak area. The typical regression equation for the seven-point calibration curve (50-20000 ng/mL) obtained by the least squares regression for SWM is y = 67.38 (± 0.7347) x – 2959.52 (± 158.7023), r2= 0.9989 ± 0.0016.

Table-1: System suitability

Sample code	Injection Number	Area Values	Retention Time
SYS01	1	63834	8.917
	2	60183	8.920
	3	61181	8.918
	4	59133	8.915
	5	57236	8.916
	6	60399	8.917
Mean		**60328**	**8.917**
S.D.		**2192.59**	**0.0017**
% R.S.D.		**3.63**	**0.02**

Acceptance criteria: The % R.S.D of the area ratio and retention time for respective drug should be ≤ 5.

Precision and Accuracy: Results of Precision and Accuracy for inter-day and intra-day (three consecutive days) are shown in **Table no.2**. The low values of % R.S.D. (<6.44 for intra-day assay and < 9.04 for inter-day assay) and % R.E. (< 0.76 for intra-day assay and < 1.40 for inter-day assay) indicate the high degree of repeatability and intermediate precision of the current method.

Robustness and Ruggedness: Robustness and Ruggedness was determined to evaluate the influence of small but deliberate variation in the chromatographic conditions in the assay procedure. To determine Robustness the changes in area values for 1 ppm standard were recorded and % R.S.D. was calculated. Low value of the % R.S.D. (< 0.87 for change in flow rate and < 0.93 for change in mobile phase composition) indicates the robustness of the method as shown in Table no. 3.1 and 3.2. To determine Ruggedness, % R.S.D. and % difference for 1 ppm standard which was processed and analyzed by same analyst, instrument and column was compared with another instrument, analyst and column. The value obtained for % R.S.D. is found to be <1.03, whereas value found for % difference was 0.53. The obtained values showed no significant variation between both the sets of experiments (Table no. 3.3).

Quantification of Swertiamarin in leaf extracts of *S. densifolia* and marketed herbal formulations: The peaks of SWM from sample solution were identified by comparing the Rt obtained from the peaks with those of standard. HPLC profile of the 70 % ethanolic leaf extracts of the *S. densifolia* is developed through the same condition as estimation of standard SWM (Rt value 9.0 ± 0.5 min). The peak purity of extract is confirmed by superimposing the peaks of standard and extract. (Fig. 2, 3, 4, 6, 7).

Table-2: Summary of inter-day and intra-day precision and accuracy for quality control samples of Swertiamarin

Concentration of QC sample(ng/mL)	Back calculated conc. ± SD	% R.S.D.	% R.E.
Inter-day assay (n=6 replicates of each concentration, three days)			
150	151.14 ± 2.55	1.69	0.76
2500	2391.24 ± 142.63	5.96	-4.35
15000	15046.64 ± 969.35	6.44	0.31
Intra-day assay (n=6 replicates of each concentration, same day)			
150	152.09 ± 0.95	0.62	1.40
2500	2425.11 ± 219.14	9.04	-3.00
15000	15062.19 ± 845.45	5.61	0.41
Acceptance criteria: The % R.S.D and % R.E. of intra-day and inter-day precision and accuracy for quality control samples of respective drug should be ≤ 15 %.			

Table-3.1: Robustness - Change in Flow rate (n=3)

Flow rate	Mean peak area values	(±) S.D.	% R.S.D.
0.95 mL/min	75767.39	638.50	0.84
1.0 mL/min	75968.02	659.26	0.87
1.05 mL/min	76006.88	526.89	0.69

Table-3.2: Robustness - Change in Mobile phase composition (n=3)

Ratio (10 mM Ammo. Acet.: ACN: F.A.)	Mean peak area values	(±) S.D.	% R.S.D.
91:09:0.1%	74602.32	405.04	0.54
90:10:02%	76866.23	211.83	0.28
89:11:0.3%	73655.99	687.10	0.93

Table-3.3: Ruggedness - Change in analyst, instrument and column (n=3)

Set of experiment	Mean Area values	(±) S.D.	% R.S.D.	% Difference
Set 1	74822.66	387.28	0.52	---
Set 2	75217.99	771.49	1.03	0.53
Acceptance criteria: The % R.S.D samples of respective drug should be ≤ 15 %.				

Table no. 4: Determination of Swertiamarin content in test plant material and marketed formulations.

Sample	Label claim for *Gentianaceae* family contents	Expected Swertiamarin contents	Contents of Swertiamarin Observed	Back calculated *Gentianaceae* family contents
S. densifolia	---	----	1.350 mg /g	-----
Formulation 1	3.375 g / 10 g	0.465 mg /g	0.51 mg /g	3.77 g/ 10 g
Formulation 2	3.35 g / 10 g	0.452 mg /g	1.1 mg /g	8.15 g / 10 g
Formulation 3	25.00 g / 100 mL	0.338 mg /mL	0.27 mg /mL	20.01 g / 100 mL
Formulation 4	0.115 g / 100 mL	0.002mg /mL	0.005 mg /mL	0.371 g / 100 mL

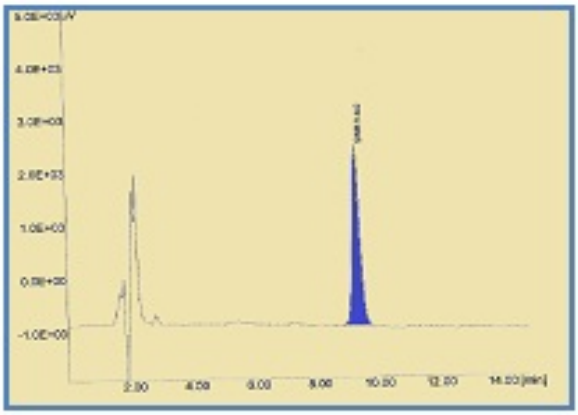

Fig.2: Swertiamarin standard (1 ppm)

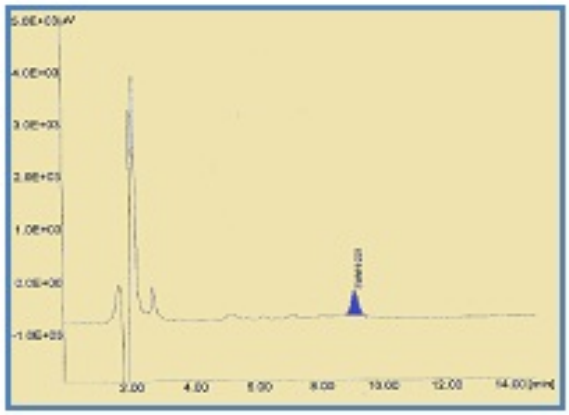

Fig.3: Leaf extract of *S. desnsifolia* – 1.35 mg/g

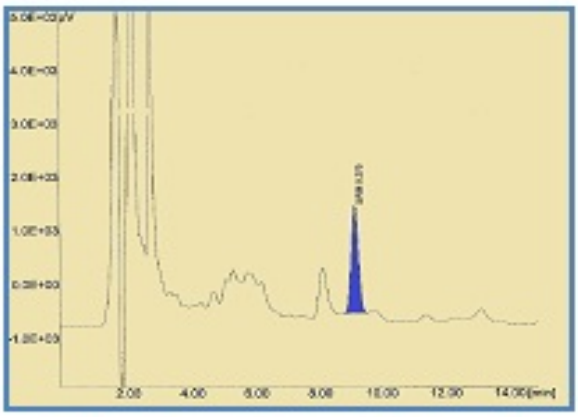

Fig.4: Formulation 1- 0.51 mg/g

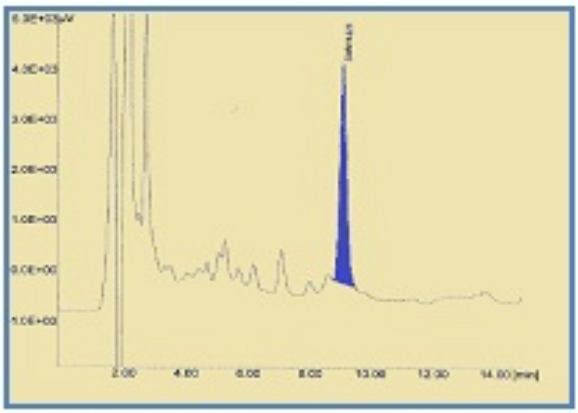

Fig.5: Formulation 2- 1.1 mg/g

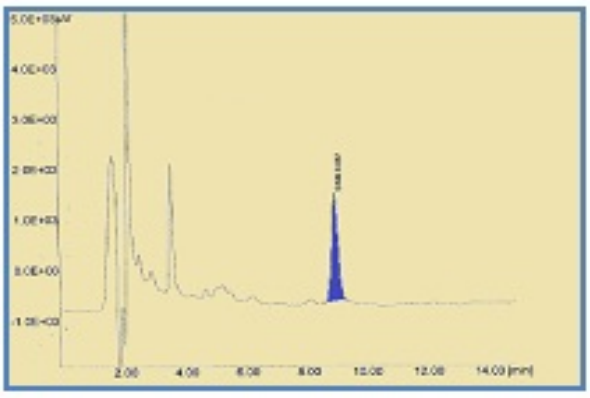

Fig.6: Formulation 3- 0.27 mg/mL

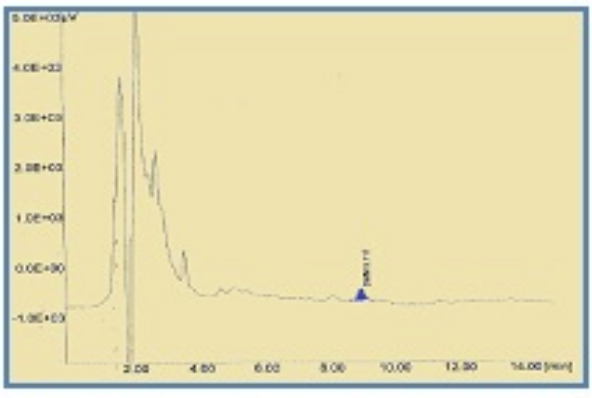

Fig.7: Formulation 4- 0.005 mg/mL

A single peak was observed at the same Rt in the samples of marketed formulations 1, 2, 3 and 4. There was no interaction between the SWM and other excipients present in the marketed formulations. The SWM content as per label claim mentioning the contents of plant members of *Gentianaceae* family present per gram or mL, was back calculated. The SWM contents obtained for various marketed formulations are tabulated in Table no. 4.

According to label claim Formulation 1 contains 3.375g/10g, Formulation 2 contains 3.35g/10g, Formulation 3 contains 25g/100 mL and Formulation 4 contains 0.115 g/ 100 mL of the plant contents from *Gentianaceae* family. The results obtained for Formulation 1 are satisfactory as it meets the label claim criteria for the plant members from *Gentianaceae* family. Formulation 2 and 4 gave the higher back calculated contents of plant members of *Gentianaceae* family as compared to label claim. Formulation 3 shows less concentration of SWM as compared to label claim.

CONCLUSION

An improved, simple, sensitive, rapid and precise RP-HPLC method for quantitation of SWM has been developed. The analytical method is validated as per ICH guidelines and statistical evaluation has proved the repeatability and reproducibility of the results. The RP-HPLC method is found to be suitable for the quantification of Swertiamarin from ethanolic extracts of *S. densifolia* leaves as well as marketed formulations containing plant members from *Gentianaceae* family as an ingredient. The method can be successfully used for the routine analysis of Swertiamarin in both crude drugs and prepared formulations, for standardization and quality control of raw materials and marketed herbal products of traditional system of medicine.

The evaluation of label claims of four marketed formulations indicate significant variations in the content of SWM with respect to the amount of plant material claimed in the label. This highlights the need for better quality control especially for formulations containing plants from *Gentianaceae* family.

REFERENCES

Ahmad, J, Amin, S, Mir, S.R, (2014). Development and validation of HPLC-UV method for estimation of swertiamarin in *Enicostemma littorale*. Journal of pharmaceuticals and biosciences. 2(1), 9-16.

Alam, P, Ali, M, Singh, R, Shakeel, F, (2009). Estimation of swertiamarin in *Enicostemma littorale* and marketed formulations using HPLC-UV Method. Journal of Bioanalysis and Biomedicine. 1 (1), 22-27.

Bhave, R. and Barve, S, S, (2014). HTLC method validation for detection and quantification of Betulinic acid in *Ancistrocladus heyneanus* Wall Ex. J. Graham. International Journal of Pharmacy and Pharmaceutical Sciences. 6 (8): 328-331.

ICH. (2005). Validation of analytical procedures: Text and Methodology. ICH-Q2 (R1), International Conference on Harmonization: Geneva.

Jaishree, V., Badami, S, Rupesh Kumar, M, Tamizhamani, T, (2009). Antinociceptive activity of swertiamarin isolated from *Enicostemma axillare.* Phytomedicine. 16, (2-3): 227-232.

Jaishree, V, and Badami, S, July 2010. Antioxidant and Hepatoprotective effect of Swertiamarin from *Enicostemma axillare* against D-galactosamine induced acute liver damage in rats. Journal of Ethanopharmacology. 130 (1): 103-106.

Nair, S, N, Menon, S, Shailajan, S, (2012). A liquid chromatography/electrospray ionization tandem mass spectrometric method for quantification of Asiatic acid from plasma: application to pharmacokinetic study in rats. Rapid Comminucations in Mass Spectrometry. 26: 1899-1908.

Niazi SK. (2000). Handbook of Bioequivalence Testing, Informa Health care. U.S.A., pp: 250-253.

Patel, T., Soni, S., Parikh, P., Gosai, J., Chruvattil, R., Gupta, S., (2013). Swertiamarin: An Active Lead from *Enicostemma littorale,* Regulates Hepatic and Adipose Tissue Gene Expression by Targeting PPAR- γ and Improves Insulin Sensitivity in Experimental NIDDM rat Model. Hindawi Publishing Corporation, Evidence-Based Complementary and Alternative Medicine, Article no. 358673.

Rana, V, S, Dhanani, T, Kumar, S, (2012). Improved And Rapid HPLC-PDA Method For Identification And Quantification Of Swertiamarin In The Aerial Parts Of Enicostemma axillare. Malaysian Journal of Pharmaceutical Sciences. 10 (1): 1-10.

Chhipi Shrestha, J, K, Bhattarai, T, Sijapati, J, Rana,N, Maharjan, J, Rawal, D, S, (2013). Assessment of Genetic Diversity in Nepalese Populations of Swertia chirayita (Roxb. Ex Fleming) H. Karst Using RAPD-PCR Technique. American Journal of Plant Sciences. 4, 1617-1628.

Shailajan, S, Singh, A, Tiwari, B, (2010). Quality Control and Standardization of an Ayurvedic Taila formulation. International Journal of Biomedical Research and Analysis. 1 (2): ISSN 2229-3353.

The Wealth of India, (2003). A Dictionary of India Raw Material, Council of Scientific and Industrial Research, 99 Volume- 10,

Vaidya, H. Rajani, M, Sudarsanam, V, Padh, H, Goyal, R, (2009a). Antihyperlipidaemic activity of Swertiamarin, a secoiridoid glycoside in poloxamer-407-induced hyperlipidaemic rats. Journal of Natural Medicine. 63, 437-442.

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