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VALIDATED STABILITY INDICATING RP-HPLC METHOD FOR QUANTIFICATION OF BIOACTIVE MARKER IN DRAKSHARISTAM

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Abstract

Draksharistam is an herbal formulation contains main ingredient as dried fruit of *Vitis vinifera*. Vitaceae has been used in the treatment of blood purifier. It contains large amount of polyphenols catechin, epicatechin, gallic acid, quercetin, rutin etc. A present research work was to develop a accurate RP-HPLC method and too validated in terms of ICH guidelines. The analytical method was carried out using column Phenomenex- Luna 5µ C-18(2), solvent phase selected as acetonitrile and water by gradient elution with a flow rate of 1.0ml/min. The detection was effected at 268nm and 280nm. Quercetin and rutin were eluted at 6.8min and 15.6min for respectively. The peak area retort is linear within 5-25µg/ml and correlation coefficient was observed at 0.998. Detection limit and quantitation limit of quercetin and rutin were 1.02µg/ml & 4.18µg/ml and 3.58µg/ml & 10.64µg/ml. The precision studies were satisfactory, and %RSD of sample analysis were in the range. The accuracy showed recoveries are within 98-102%. The developed method can be applied for quantification of biomarker quercetin, rutin and Draksharistam.

Key words : Draksharistam, *Vitis vinifera*, Rutin, Quercetin.

Introduction

In modern drug development single moiety is responsible for therapeutic activity whereas in polyherbalism many component is used, enhances the efficacy of formulation. Biomarkers are therapeutically active component present in herbal drug. Most of the conclusions are drawn in Ayurvedic texts are based on the ancient information and clinical observations; they lack the modern explanation by analytical methods during preparation of a drug. Draksharistam compositions are part of Ayurvedic Formulary of India, standardization and safety parameter are however to be documented. In recent times the medicine is based on experimental data, preclinical data, toxicity, and clinical studies. The present research work is to extend RP-HPLC method and to validate as per ICH guidelines and implement for quality control and routine analysis.

Aristhas have been included in Ayurveda during *CharakSamhita* period as appetizers and stimulants (Tekeshwar K *et al*., 2013), (Kulkani O *et al*., 2015). Aristhas are prepared with decoction of herbs and these possess self-generated alcohols which act as a natural preservative. Draksha is main ingredient of Draksharistam an ayurvedic multi herbal formulation prescribed for digestive impairment, used in respiratory disorder and act as an intestine cleanser. (India, Dept of Health, Ministry of Health and family planning. Ayurveda Formulary of India-I, 2nd Edition. 2003.) It consist of dried fruit of *Vitis vinifra*, Vitaceae (grape vine) is native to southern Europe and Asia, today cultivated globally with 60 different species. They are rich source of flavanoids, sugars, anthocyanidins and revesterol (derivative of stillbene). Grape seed contains high content of polyphenols which includes 25% oligomeric proanthocyanidins and flavanoids-quercetin, rutin, myercetin and kampherol.

A plant pigment quercetin (3, 3'4', 5, 7-pentahydroxyflavanone) is antioxidants bioflavanol present in numerous plant materials. The name quercetin has been derived from *quercetum* (oak forest), after

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quercus-polar naturally act as auxin transport inhibitor. The biological properties form basis of inflammation and immunity in mental /physical performance. Quercetin possesses notable pharmacological benefits as antiobesity, anti inflammatory, anti hypertensive, antiartherosclerotic, and antihypercholesteromic (Mahesh CM *et al.*, 1980) activities. A potential antioxidant rutin (3,3'4', 5, 7-pentahydroxyflavanone-3-rhamnoglucoside) is a bioflavanol - vital nutraceutical. (http://umm.edu/health/medical/altmed/supplement/quercetin.) Rutin name derived from *Ruta graveolens*(known as Vitamin P) chemically it is aglycone quercetin with rhamnoside. Their multi-spectrum pharmacological effect includes cardio/vaso/neuro/cytoprotective anticarcinogenic and hypoglycaemic activity (Colergi *et al.*, 1980), (Hertzog MGL *et al.*, 1993).

Materials and Methods

Chemicals and Solvents

Quercetin and rutin were procured from Natural Remedies, Bangalore, India. All the chemicals and reagents were of analytical grade and purchased from Merck Specialist Pvt, Ltd, Mumbai. The two commercial herbal formulations were purchased from Ayurvedic pharmacy from the local market.

Instrumentation and chromatographic condition

Chromatographic separation be achieved by prepacked column Phenomenex- Luna 5µ C-18(2), equipped with pump, degasser, photodiode array detector and auto sampler. A mobile phase of acetonitrile and water in gradient elution through flow rate1.0ml/min at ambient temperature, quantification was monitored at 268nm and 280nm.

Standard preparation of Quercetin and Rutin

The common stock solutions were prepared by dissolving 10mg of both drugs in methanol and transferred to a 100 volumetric flask to obtain 100µg/ml. The volumes were prepare with the methanol to get concentration series from 5-25 µg/ml and were measured in triplicate. (Fig. 1 & 2)

Sample preparation

The two marketed formulation of each 0.5ml of Draksharistam was pipette out into 10ml volumetric flask, and 5ml of methanol was added and sonicated for 10mins. The volume was prepared with methanol and filtered throughout 0.2µ.

HPLC method development

Optimization a selective and sensitive method for analysis of Quercetin and rutin was done on HPLC with optimized parameter. (Table 1)

HPLC method validation

The developed methods were compliance with ICH guidelines (ICH-Q2A, Q2B, 1995). (Table 2)

System suitability: Six replicates were injected of each marker. HETP was more than 2000 and tailing factor less than 2.

Linearity: Graph was plotted by peak area versus concentration and least square regression was determined (Fig. 3). A 10µl of each concentration range 5-25 µg/ml of standard quercetin and rutin was injected in column. The scanning was done for standard and two marketed formulation were quantified by way of calibration curve.

Detection Limit (DL) and Quantitation Limit (QL)

For determination of DL and QL, standard deviation response and the slope were followed. The DL expressed as: $3.3\sigma/S$ and quantitation limit as $10\sigma/S$ where; σ= standard deviation of the response and S is slope of the calibration curve. From the intercept, slope and residual standard deviation, DL and QL were calculated.

Accuracy

Standard addition method was used to determine accuracy by calculating recovery of quercetin and rutin. A known amount of standard solutions were injected at 50%, 100%, 150% of a prequalified sample solution and peak response was measured and %RSD calculates. Percentage recovery was within 98-102%.

Precision

Six replicates of each concentration containing quercetin and rutin were used for analysis of instrumental precision was performed within a day and different days at three different concentration levels of 5, 15, 25 µg/ml for quercetin and rutin. %RSD of peak area was calculated.

Robustness

The robustness was carried by slightly changing in

Table 1: HPLC Parameters.

Parameter	Description
Column	Phenomenex- Luna 5ì C-18(2),
Column size	4.6mm*250mm*5µ
Detector	Photodiode array detector
Mobile phase	Acetonitrile : Water (70: 30 v/v)
Flow rate	1.0 ml/min
Injection volume	20µl
Elution	Gradient elution
Temperature	Ambient

Table 2: Retention time, Regression coefficient, Limit of Detection and Quantitation.

Compound	Time (min)	R^2	Concentration (µg/ml)	LOD (µg/ml)	LOQ (µg/ml)	Regression Equation
Quercetin	6.8 min	0.982	5-25(µg/ml)	1.02µg/ml	4.18µg/ml	Y=55878x+72365
Rutin	15.6 min	0.997	5-25(µg/ml)	3.58µg/ml	10.64µg/ml	Y=41334x+1177055

Table 3: Interday Method Precision Data of Quercetin and Rutin.

Replicates	Peak Area					
	Quercetin			Rutin		
	5µg	15µg	25µg	5µg	15µg	25µg
1	972876	1544790	2056096	1393715	1554770	2156096
2	943409	1541256	2056823	1367531	1531266	2146923
3	978546	1549743	2054676	1378523	1538743	2154666
4	978368	1578986	2053458	1398643	1578986	2133458
5	951788	1519987	2056990	1420756	1590977	2166990
6	979578	1508120	2051986	1379697	1598120	2159869
Mean	967427.5	1540480.333	2055004.833	1389811	1565477	2153000
Standard Deviation	14388.182	22564.0796	1828.088293	18851.78	27921.4887	10599.4
% RSD	1.487262043	1.464743114	0.088957859	1.356428	1.78357706	0.49231

Table 4: Intraday Method Precision Data of Quercetin and Rutin.

Replicates	Peak Area					
	Quercetin			Rutin		
	5µg	15µg	25µg	5µg	15µg	25µg
1	972323	1546789	2056096	1303115	1531970	2165096
2	972836	1542178	2056823	1321531	1511056	2196423
3	972452	1542135	2054676	1320523	1528743	2144666
4	974839	1544657	2053458	1300643	1518986	2123458
5	974928	1543565	2056990	1312756	1501977	2156990
6	972088	1546789	2051986	1310697	1566120	2185869
Mean	973244.3333	1544352.167	2055004.833	1311544	1526475.33	2162084
Standard Deviation	1180.267296	1924.911116	1828.088293	8630.513	22365.3641	24410.6
% RSD	0.121271427	0.12464198	0.088957859	0.658042	1.46516381	1.12903

Table 5: Quantification.

Phytoconstituent	DM-I	DM-II
Quercetin	40.174µg/ml	38.32602µg/ml
Rutin	10.32602µg/ml	11.82602µg/ml

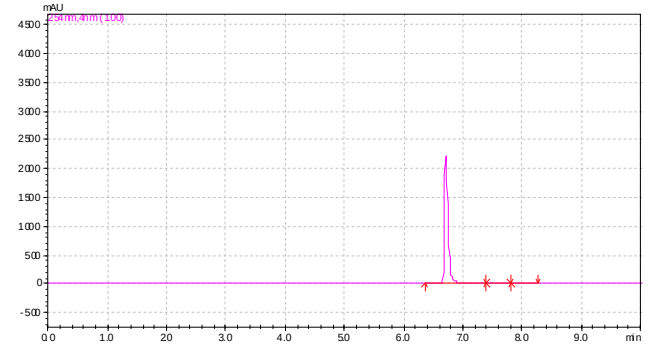

Fig. 1: Chromatogram for Retention time of Sample Quercetin.

flow rate and effects on the results were examined. The column temperature and wavelength were varied at range of 5%. The same three determinations were checked as mentioned in precision for robustness.

Results and Discussion

A reverse phase column C18 was utilized to develop the chromatograms with elution gradient.

Acetonitrile and water (70:30v/v) as solvent system in isocratic mode with flow rate of 1.0ml/min and run time was observed at 6.8min, 268nm for quercetin and 15.6 min at 280nm for rutin. The system suitability reports were within the criteria limits. The calibration curve was resolute by injecting replicates of 5-25µg/ml and showed good linear correlation coefficients within the desired

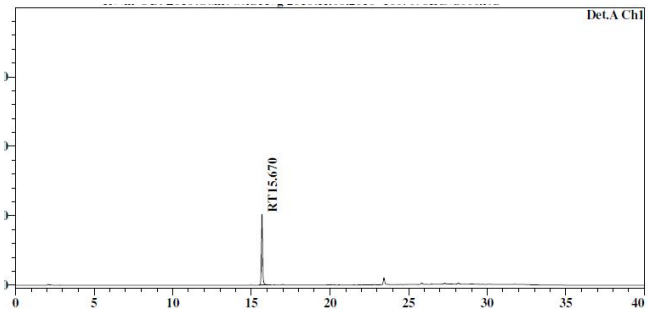

Fig. 2: Chromatogram for Retention time of Sample Rutin.

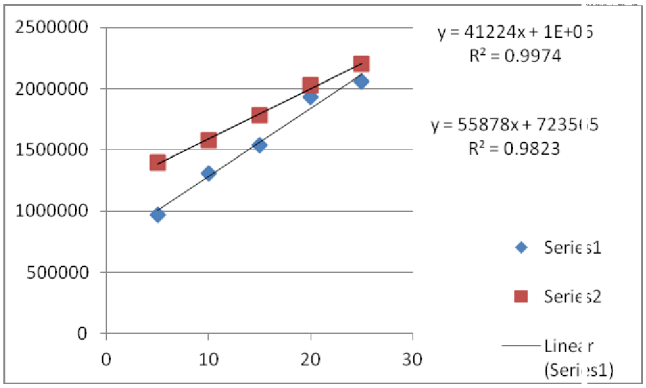

Fig. 3: Linearity of Quercetin and Rutin.

ranges. All the low values of %RSD for precision study obtained was within the acceptance of less than 2%. The accuracy was done during recovery studies of quercetin and rutin by spiking the quercetin at three different levels of 50%, 100% and 150% with pre-analyzed samples of known pre-determined concentration. The average recovery was within 98-102% and in total agreement with acceptance criteria. The DL and QL was performed by standard deviation method and quercetin was found to be1.02µg/ml and 4.18µg/ml for 3.58µg/ml and10.64µg/ml for rutin respectively. Robustness was studied by small changes but deliberate in mobile phase, flow rate, wave length and temperature and it exhibit the developed method wasn't affected by changes. The assay results obtained for analysis of quercetin in two marketed formulation D-I and D-II were found to be 40.174µg/ml and 38.32602µg/ml and for rutin 10.32602µg/ml and 11.82602µg/ml respectively. The above proposed method can be conveniently executed for the quality of quercetin and rutin in herbal/ayurvedic formulation.

Conclusion

The new design developed RP-HPLC methods were successfully validated and can be applied for routine analysis of Ayurvedic formulation.

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