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Assessment of Quercetin Content in Selected Vegetables and Fruits by Conventional Extraction and High Performance Liquid Chromatography

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ABSTRACT

One of the dietary flavonoids which can be found in a variety of vegetables and fruits is Quercetin (3,3′,4,5,7-pentahydroxyflavone).Quercetin reduce infection risk and also has unique biological property which improves the physical performance. The current research work describes the extraction and characteristic of quercetin present in carrot (Daucus carota sp. sativus) and grapes (genus vitis). A liquid-solid extraction method of quercetin contained in carrot and grapes was developed, in which Quercetin is extracted from a solid mixture using a liquid solvent (methanol). Determination of Quercetin is studied by using High performance liquid chromatography. The separation study was performed on Zodiac C18, 250mmx 4.6mm, 5μm column, detection at 280nm and flow rate applied 1mL/minute. The limits of detection(LOD) and quantification(LOQ) parameters were in the ranges of 0.1–0.3 and 0.3–1.0 μg/mL respectively. The results of carrot and grape meet the specified specification limit. The detection of the active substance in carrot and grapes using the HPLC method has the advantage of being simple, fast, and accurate and the reported method was validated.

Keywords: Quercetin, Extraction, High performance liquid chromatography, Carrot (*Daucus carota sp. sativus*), Grapes (*Genus vitis*), Method validation.

INTRODUCTION

Quercetin (QRN) is a flavonol (plant polyphenol) found in fruits, vegetables, seeds and edible parts. It is also present in medicinal botanicals, plays a key role in developing antioxidants. Phenolic compound play a crucial role in maintaining nutritious substances which help to improve human health from chronic diseases. Fig. 1 shows the chemical structure of QRN. Whose name is derived from the Latin word "quercetum,"which signifies"oak forest," it is a category in the class of flavonoids, and a sub class of flavonol[1]. Flavonoids are phenolic compounds with a three-ring system, composed of 15 carbon atoms in the form of C_6,C_3,C_6. This compound cannot be synthesised by the human body[2]. QRN a yellow crystalline substance which is soluble in alcohol is anticipated that persons with balanced nutrition consume 25–50 mg per day[3]. The optimal effective dose of QRN for decreasing blood

pressure and inflammation has been determined[4]. QRN is not a carcinogen, and may protect against Genotoxicants[5]. In the food sector, antioxidant supplementation may help to avoid mycotoxin toxicity[6]. The bioctivity and solubility of QRN in the body increase when combined with metal ions to build a complex[7]. Green tea infusions and bitterness are due to poly phenols were investigated8. Deep Eutectic Solvents (DESs) gives high yield for extraction of QRN and its glycosylated form from onion peels[9]. Phenolic compounds have antioxidant, antimutagenic, anticarcinogenic, anti-inflammatory, and antimicrobial activities[10]. QRN play a vital role in the treatment of rheumatoid arthritis[13] and it has been demonstrated to lessen the risk of death in COVID-19 patients due to presence of anti-inflammatory and antioxidant activities.[11]

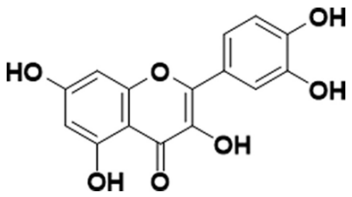

Fig. 1. Structure of QRN

The carrot and grape contains the most important phenolic compound which act as anti-oxidant activities and non–carcenogenic along with other nutritional compounds. The author reported QRN content present in vegetable (carrot) and fruit (grape) extraction and characteristics by using HPLC-UV method.

MATERIALS AND METHODS

Solvents and chemicals

Reference standard of QRN was purchased from Sigma-Aldrich. Methanol, acetonitrile and HPLC-grade water, orthophosphoric acid, acquired from Merck, India

Plant materials

The fresh and healthy vegetable carrot (*Daucus carota sp. sativus*) and fruit grape (*Genus vitis*) were procured by cultivators from local market, Visakhapatnam, Andhra Pradesh, India. The selected vegetable and fruit samples were stored in glass containers and kept at room temperature.

Sample and standard solution preparation

The fruit and vegetable material procured from cultivators were dried and washed with plenty of water after washing/cleaning, the materials were properly cut into small slices or pieces and placed on a clean filter paper for further work. Then the plant material is finely grounded by using a potable grinding machine, Maceration technique was adopted for the extraction procedure in which 100 g of each sample was soaked in small portions with (1:20) methanol and 1:1 aqueous Hydrochloric acid solution in a conical flask with occasional shaking for two hours and using orbital shaking incubator for 60 minutes. The contents are occasionally heated for 1 h at 60°C on water bath and the contents were cooled and subjected to filtration. The process was repeated for 3 times and filtrate was mixed. The filtrate obtained was dried by using a rotary vaccum evaporator at 40°C to get viscous concentrate sample and stored for analysis.

Mobile phase preparation

Prepare a mixture of Methanol and Water in a ratio of 700:300 v/v with 1.0 mL formic acid.

Preparation of Standard Solution

10 mg of QRN standard was taken into a clean and dry 50.0 mL volumetric flask. 5 mL of diluent was added, then dilute to the desired volume with diluent. Transfered 1.0 mL into 10.0 mL volumetric flask and dilute to the volume with diluent. Further 1.0 mL of this solution take into 20.0 mL volumetric flask and dilute to the volume with diluent.

Preparation of test solution

Weighed accurately and transfer about 1000 mg of test sample into a clean and dry 10.0 mL volumetric flask. Add about 5 mL of diluent, sonicate to dissolve the content and dilute to the volume with diluent.

Sample preparation optimization

Various methods have been reported for the extraction and quantification of QRN[12-16]. The procedure was optimized with regard to mobile phase Acetonitrile-Water, Methanol and Water in a ratio of 700:300 v/v with 1.0 mL formic acid.

Chromatographic conditions

Agilent Technologies, 1260 was used for method development, quantification and method

validation. The various chromatographic conditions were given in Table 1.

Table 1: Chromatographic conditions

Instrument	High Performance Liquid Chromatograph, Agilent 1260
Detector	UV
Diluent	Mobile phase
Column	Zodiac C18, 250mmx 4.6mm, 5µm
Wavelength of detection	280nm
Injection volume	10µL
Chromatogram run time	30 min
Column temperature	35ºC
Sampler cooler temperature	10ºC
Flow rate	1.0 mL/min
Pump mode	Isocratic

RESULTS AND DISCUSSION

The most abundant dietary flavonoid is QRN and is used to reduce the blood pressure, inflammation, blood sugar etc. The QRN content in fruits and vegetables is very imperative. QRN maximum wavelength was obtained at 285nm in Methanol. The optimum mobile phase was a mixture of acetonitrile-water and 700:300v/v with 1.0 mL formic acid. The chromatograms show a very good baseline resolution of analytes. Chromatograms of QRN were presented in Fig. 2-5. The QRN content in selected carrot and grape samples were presented in Table 2.

Method validation

Method validation was performed as per AOAC (Association of Official Analytical Chemists) and ICH guidelines. Limit of Detection (LOD), Limit of Quantitation (LOQ), Precision at LOQ level, System suitability, Specificity, Linearity, Accuracy etc were studied.

Table 2: QRN content in grape and carrot

Fruit/vegetable	Sample-1	Sample-2	Sample-3	Sample-4	Sample-5
Grape(mg/100g) white	1.43	1.57	1.29	1.59	1.31
Carrot(mg/100g)	0.84	0.37	0.65	0.49	0.92

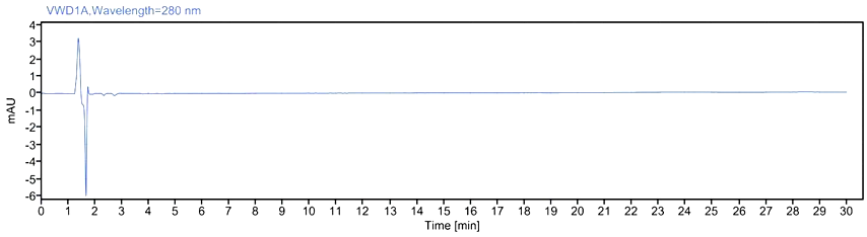

Fig. 2. Blank chromatogram

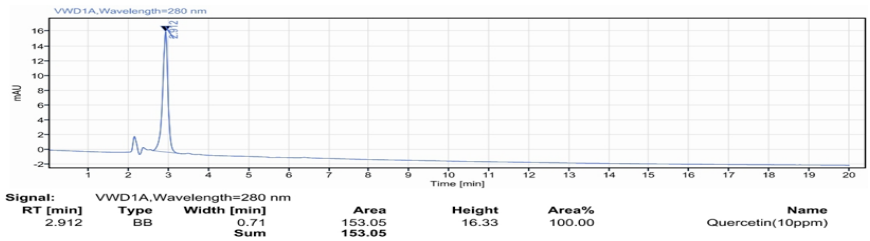

Fig. 3. QRN-HPLC Standard chromatogram

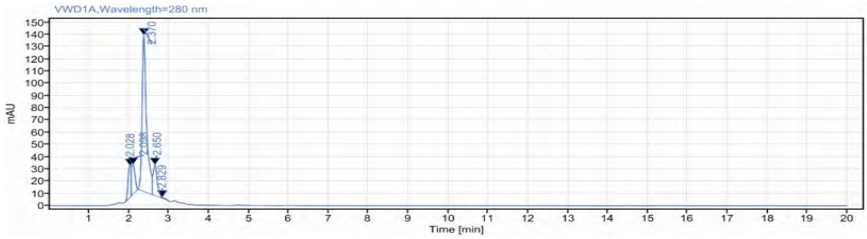

Fig. 4. QRN HPLC chromatogram-Carrot sample

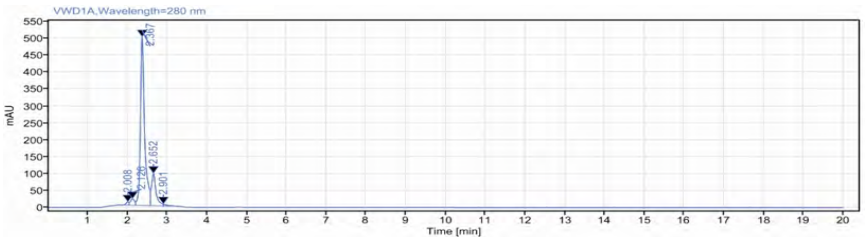

Fig. 5. QRN HPLC chromatogram-Grape sample

Limit of detection and Limit of Quantification

LOQ : Transfer 2.5 mL of standard solution in to 10 mL volumetric flask and make up to mark with diluent. **LOD**: Transfer 3.3 mL of LOQ Solution into 10 mL volumetric flask and dilute to the volume with diluent. Limit of Quantification is considered for this validation is 25% of the specification and Limit of Detection is considered for this validation is 33% of the LOQ Solution. Injected blank followed by six injections of LOQ solution and inject LOD solution in triplicate. The areas of standard, LOQ and LOD were presented in Table 3.

Table 3: Area of Standard LOQ and LOD

					Area of Standard solution					
Sr. No	Name	I-1	I-2	1-3	I-4	1-5	I-6	Average	SD	RSD%
1	Quercetin	88.29	89.06	88.86	90.19	90.38	94.81	90.27	2.37	2.62
					Area of LOQ solution					
		I-1	I-2	1-3	I-4	1-5	I-6	Average	SD	RSD%
2	Quercetin	19.52	19.3	19.35	19.21	19.4	19.35	19.355	0.10	0.53
					Area of LOD solutions					
		I-1	I-2	Injection-3	Average	SD	RSD%			
3	Quercetin	5.94	6.14	6.15	6.08	0.12	1.95			

I:Injection SD: Standard deviation RSD

System suitability

Injected system suitability solution in six replicates. The %RSD values (given in Table 4) of the peak area and Retention time of all analytes were less than 2.0% which satisfy the acceptance criteria.

Table 4: Percentage of RSD

					Area of Standard solutions					
Sr. No	Name	I-1	I-2	1-3	I-4	1-5	I-6	Average	SD	RSD%
1	Quercetin	91.95	90.89	91.16	90.67	92.34	91.12	91.36	0.65	0.71

Specificity

Specificity reveals that the method is proficient of resolving the analyte(s). Accurately weighed and transfer about 10 mg of QRN standard into a clean and dry 50.0 mL volumetric flask. Further dilutions were done and dilute to the volume with diluent.

Preparation of test solution

Weigh accurately and transfer about 1000 mg of test sample into a clean and dry 10.0 mL volumetric flask. Add about 5 mL of diluent, sonicate to dissolve the content and dilute to the volume with diluent.

Preparation of Spiked solution

Weighed accurately and transfer about 1000 mg of test sample into a clean and dry 10.0 mL volumetric flask. Add about 5 mL of diluent, sonicate to dissolve the content and added 0.5 mL of Stock Standard solution and dilute to the volume with diluent.

No significant interference of blank and Impurity peak with analyte peak was observed.

Linearity

The linearity test reveals that the method

of detection has a linear retort to concentration over the range of concentrations of the fastidious product. Linearity to be performed separately by preparing in the range 25%-200% of Impurities concentration. Correlation coefficient of each impurity should be more than 0.99. Areas of standard and linearity were given Table 5 and 6, the linearity graph was shown in Fig. 6. Based on final result the Correlation Coefficient was found with in acceptance criteria.

Table 5: Area of standard solution

	Area of standard solution								
Name	I-1	I-2	I-3	I-4	I-5	I-6	Average	SD	RSD%
Quercetin	91.95	90.89	91.16	90.67	92.34	91.12	91.36	0.65	0.71

I -Injection

Table 6: Linearity

Sr. No	Injection Id	Areas of linearity	
		Percentage	Quercetin
1	Solution-1	25	19.91
2	Solution-2	50	44.35
3	Solution-3	100	93.02
4	Solution-4	150	138.3
5	Solution-5	200	193.36
Correlation Coefficient			0.9985

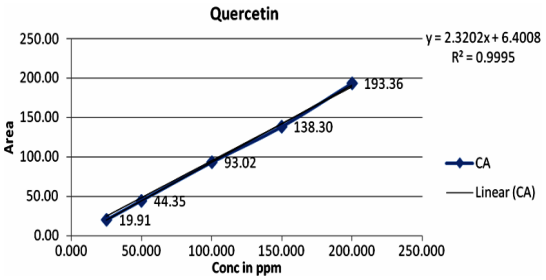

Fig. 6. Linearity graph of QRN

Accuracy

Generally accuracy reveals the potential of the method to recuperate a identified quantity of active or degradant etc. from the placebo matrix. To demonstrate accuracy for Quercetin impurities, revival test was performed using solutions containing 50%, 100% and 200% of the theoretical active concentration in the end product. Every level was performed in triplicate and the mean value for QRN level was calculated and reported. Percentage of RSD, LOQ level not more than 15.0%. The acceptance criteria for impurities in this parameter are that recovery for each of the concentration levels is within the limits 80.0%–120.0%. The accuracy results were given in Table 7 and recovery in Table 8.

Table 7: Accuracy

		Accuracy at LOQ			
I- 1	I-2	I-3	Average	SD	%RSD
9.04	9.10	9.19	9.11	0.08	0.83
		Accuracy at 50%			
22.39	21.84	22.11	22.11	0.28	1.24
		Accuracy at 100%			
61.99	62.3	62.04	62.11	0.17	0.27
		Accuracy at 200%			
163.17	164.00	162.95	163.37	0.55	0.34

I- Injection

Table 8: Percentage recovery

Name	Accuracy at LOQ Result	Accuracy at 50% Result	Accuracy at 100% Result	Accuracy at 200% Result
Quercetin	101.15	104.04	102.14	100.51

CONCLUSION

The QRN content varies between geographical areas, cultivators and plant parts. Methanol was used for the extraction of grape and carrot and the high total phenolic content shows strong antioxidant activities. Determination of quercetin is studied by using High performance liquid chromatography. The chromatographic separation was performed on Zodiac C18, 250mmx 4.6mm, 5μm column, detection at 255nm and flow rate 1mL/minute. The limits of detection(LOD) and quantification(LOQ) parameters were in the ranges of 0.1–0.3 and 0.3–1.0 μg/mL. The detection of the active substance in carrot and grapes using the HPLC method has the advantage of being simple, fast, accurate and the reported method was validated.

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