Separation of Quercetin and its Organic Impurities per USP Monograph

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Overview

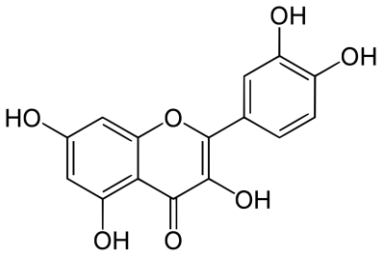
Quercetin is a potent antioxidant flavonoid found in fruits and vegetables. The development of a quick and efficient analysis of Quercetin and its related organic impurities is of interest for generic drug manufacturers. In this application note, we report the separation of Quercetin and its related organic impurities using a Luna Omega 5 µm C18 compared with a Inertsil 5 µm ODS-3 column according to the USP monograph for Quercetin, which specifies a column containing L1 (C18) packing and dimensions of 250 x 4.6 mm.

System suitability per USP Monograph for Quercetin is the same for both Assay and Organic Impurities: Resolution no less than (NLT) 1.5 between Kaempferol and Isorhamnetin, a column efficiency NLT 2000 theoretical plates, and a percent relative standard deviation (%RSD) of no more than (NMT) 2.0 % for Quercetin. All system suitability requirements for Quercetin Assay and Organic Impurities were met by both the Luna Omega and Inertsil columns. Other columns, including a Luna 5 µm C18(2) column, a InertClone™ 5 µm ODS-3 column, and a Prodigy™ 5 µm ODS-3 column were also tested but did not meet acceptance criteria for impurity content according to the USP monograph for Quercetin.

It is important to note that Retention Time %RSD is included because there was variability between injections across all columns tested. The column temperature was not specified in the USP monograph, so it was assumed to be ambient. To maintain a reasonably constant column temperature during sample analysis, the HPLC column was placed inside the column oven with the oven door closed and the oven turned off.

All solutions were prepared as indicated in the USP Monograph for Quercetin. USP Quercetin RS (Catalog No. 1592409), USP Kaempferol RS (Catalog No. 1594900), and USP Isorhamnetin RS (Catalog No. 1591800) were purchased from USP.

Figure 1. Quercetin



LC-UV Conditions

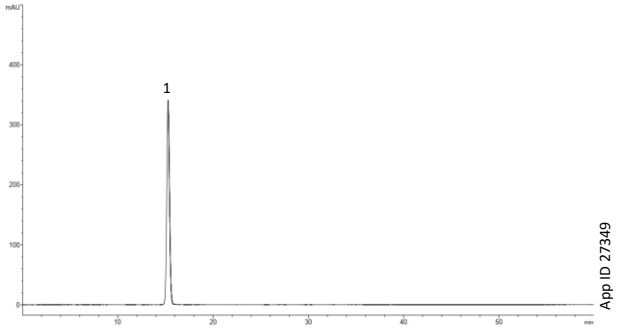
Column: Luna™ Omega 5 µm C18 (00G-4785-E0)
Inertsil® 5 µm ODS-3
Dimensions: 250 x 4.6 mm
Mobile Phase: Methanol / Water / Phosphoric Acid (100:100:1, v/v/v)
Flow Rate: 1.0 mL/min (Isocratic)
Injection Volume: 20 µL
Temperature: Ambient
Detector: UV @ 370 nm (Assay) or 270 (Organic Impurities)
LC System: Agilent® 1260

Table 1. Preparation of Solutions

Solution	Composition
System Suitability Solution – Assay and Organic Impurities	0.02 mg/mL each of USP Quercetin RS, USP Kaempferol RS, and USP Isorhamnetin RS in Methanol.
Standard Solution – Assay and Organic Impurities	Transfer 5 mg of USP Quercetin RS to a 25-mL volumetric flask and add 10 mL of Methanol to dissolve. Add 10 mL of water, mix, and dilute with Methanol to volume.

Figure 2. Standard Solution – Assay and Organic Impurities

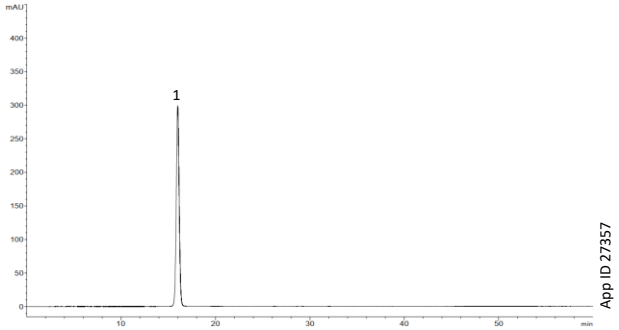
Luna™ Omega 5 µm C18



Peak No.	Analyte	RT (min)	RT %RSD	Area	Area %RSD	Theoretical Plates
1	Quercetin	15.20	1.84	6767.8	0.58	13603.17

N = 6 Injections

Inertsil® 5 µm ODS-3

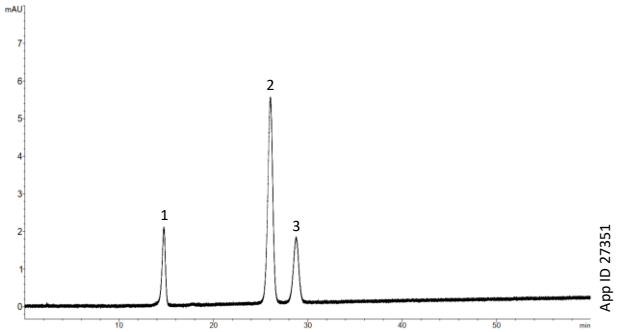


Peak No.	Analyte	RT (min)	RT %RSD	Area	Area %RSD	Theoretical Plates
1	Quercetin	16.45	2.39	6357.62	0.06	13077

N = 6 Injections

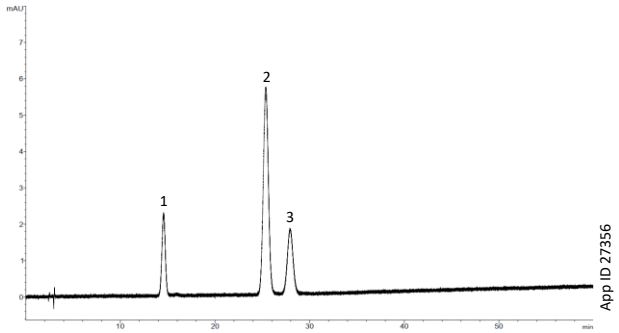
Figure 3. System Suitability Solution – Assay and Organic Impurities

Luna Omega 5 µm C18



Peak No.	Analyte	RT (min)	RT %RSD	Area	Width	Resolution
1	Quercetin	14.79	0.67	46.47	0.33	-
2	Kaempferol	26.11	0.70	184.87	0.49	2.82
3	Isorhamnetin	28.86	0.68	63.4	0.65	

Inertsil 5 µm ODS-3



Peak No.	Analyte	RT (min)	RT %RSD	Area	Width	Resolution
1	Quercetin	14.60	0.96	56.8	0.42	-
2	Kaempferol	25.43	1.03	215.4	0.63	2.45
3	Isorhamnetin	37.989	0.97	75.3	0.69	

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