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Simultaneous Analysis of Quercitrin, Isoquercitrin, and Hyperoside on the Hitachi LaChrom Elite® Liquid Chromatography System

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Quercetins modified with various carbohydrate glycosides via an ether linkage are often extracted from the hawthorn plant (Hypericum japonicum) for use as antioxidants[1]. These flavinoid compounds, collectively known as quercitrins, might show health benefits in humans when dosed as supplements. Quercetin, itself, is a widely occurring compound, found in most produce, and the corresponding quercetin/saccharide ethers are more prevalent than free quercetin. Three common quercitrins—quercetin-3-O-galactoside (hyperoside, or HYP), quercetin-3-O-glucoside (isoquercitrin, or ISO), and quercetin-3-O-rhamnoside (quercitrin, or QUE)—were investigated for this application note. The goal of this study was to effectively resolve a mixture of the three components and show linearity and system suitability for each. Using the Hitachi LaChrom Elite® liquid chromatography system, these three compounds were easily resolved using a gradient pumping method and UV detection.

Experimental Conditions

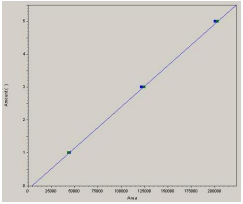
Module	Conditions and Other
Pump (L-2130)	Mobile Phase A: 0.5% Acetic Acid Mobile Phase B: Acetonitrile See Gradient Profile Below
Autosampler (L-2200)	Injection Volume: 5, 3, and 1 µL of a 16.7 µg/mL solution of each component
Oven (L-2300)	30°C
UV Detector (L-2420)	UV λ: 355 nm
Column	Phenomenex® Luna ODS(2) 5 µm 4.6X250 mm
Standards	QUE: Sigma-Aldrich® – Q3001, 90% pure ISO: BioChemika – 17793, >90% pure HYP: BioChemika – 83388, >97% pure Prepared at 50 µg/mL each 50/50 MP A and B

Gradient Table for Quercitrins:

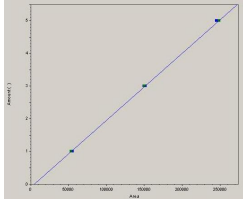
Time (min)	%A	%B	Flow Rate (mL/min)
0.0	84	16	1.5
15.0	80	20	1.5
15.1	84	16	1.5
20.0	84	16	1.5

Results - Linearity of Standards (HYP, ISO, and QUE, n=3):

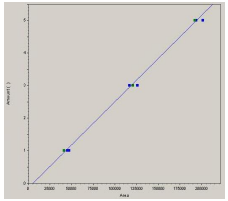
Hyperoside



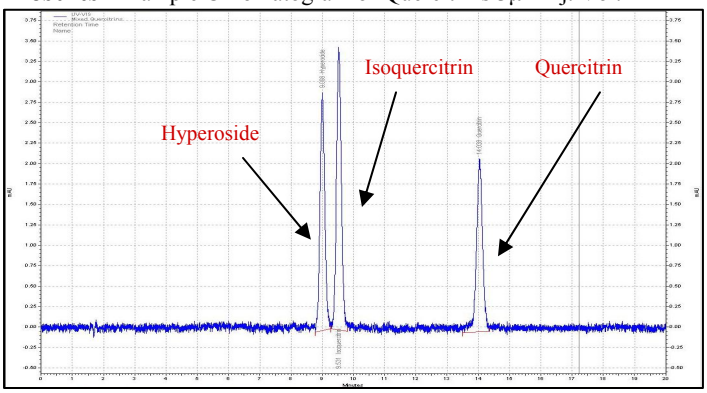
Isoquercitrin



Quercitrin



Results–Example Chromatogram of Quercitrins 3µL inj. Vol.



Reproducibility and Linearity of Standards

Compound	%RSD	Linearity
HYP 5µL inj.	0.6	$r^2 = 0.9999$
HYP 3µL inj.	1.2	-
HYP 1µL inj.	1.4	-
ISO 5µL inj.	0.7	$r^2 = 0.9999$
ISO 3µL inj.	0.6	-
ISO 1µL inj.	1.8	-
QUE 5µL inj.	2.5	$r^2 = 0.9991$
QUE 3µL inj.	2.8	-
QUE 1µL inj.	2.6	-

Discussion

Hitachi's LaChrom Elite® Liquid Chromatography System, equipped with a 5-µm particle size Phenomenex® column, resolves the quercetin flavinoids hyperoside, isoquercitrin, and quercitrin. The system is suitable, and reproducibility of the standards is shown. Reproducibility (<3 %RSD) and system suitability (theoretical plates: N > 1000 and tailing factor: T < 2.0) are shown at the low quercitrins (HYP, ISO, and QUE) concentrations.

References:
1 – Chang, Q. et al. "HPLC Method for Simultaneous Determination of Hawthorn Active Components in Rat Plasma." *J Chromatogr B Biomed Sci Appl*, 688 (1) 1997.

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