

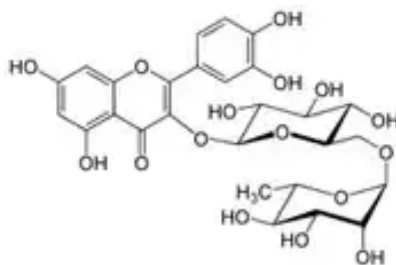
# Determination of Rutin in Anti-Aging Skin Cream Formulations Using Chromolith<sup>®</sup> HighResolution RP-18 Endcapped Column and UV Detection

## INTRODUCTION

Rutin, also known as rutoside, quercetin-3-O-rutinoside, or sophorin, is a quercetin glycoside found in various plants, including buckwheat and asparagus. The chemical structure contains units of Rutoside and the disaccharide rutinose ( $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 6)- $\beta$ -D-glucopyranose).

Rutin is believed to possess antioxidant properties, and/or posses anti-aging effects on human dermal fibroblasts and human skin. Like other bioflavonoids, Rutin is investigated for its biological activity and often formulated as skin cream, i.e. semi-solid emulsions of oil and water, products.

HPLC analysis of skin cream formulations can profit from use of monolithic rods over particle based columns, due to higher tolerance against matrix heavy samples. This application illustrate how Rutin can be determined in skin cream samples using a monolithic Chromolith<sup>®</sup> HighResolution RP-18 endcapped column with UV detection. Cream samples would only require ethanol-solubilization and filtration prior to analysis, lowering both the cost and time of analysis.

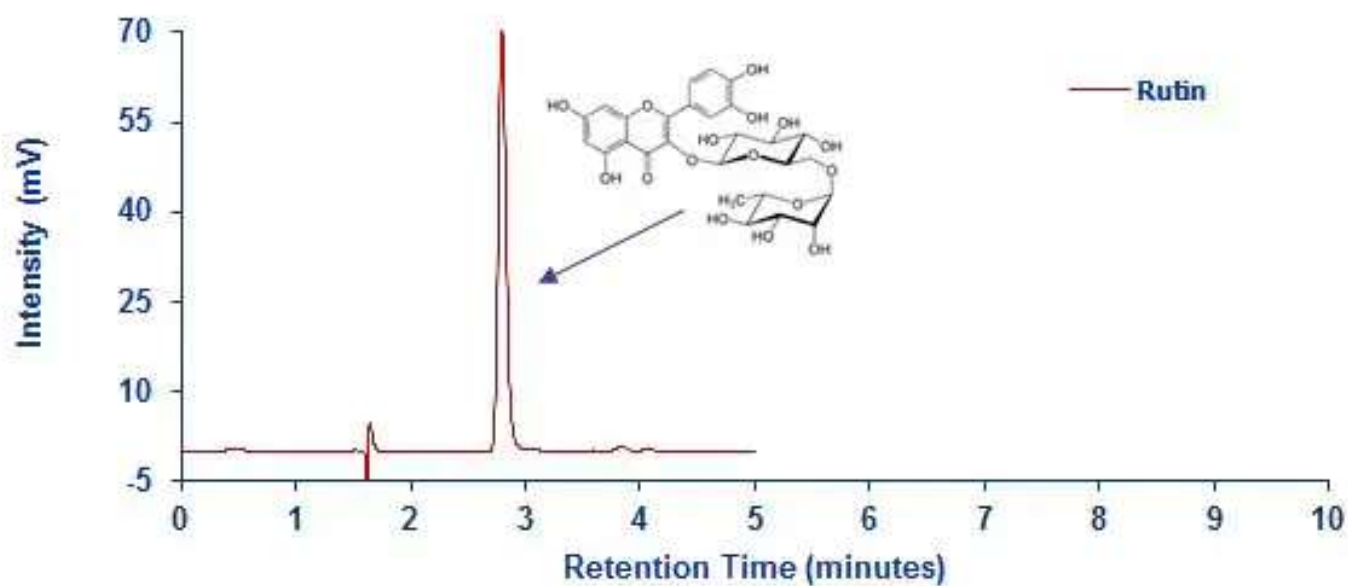


Column	Chromolith® HighResolution RP-18e 100x4.6 mm		
Injection volume	1 µL		
Flow Rate	1mL/min		
Temperature	25 °C		
Pressure Drop	56 - 85 Bar (812-1233 psi)		
Detection	Dionex Ultimate 3000 VWD-3400, 5Hz, Response Time 0.1s, UV=220nm		
Mobile phase A	Phosphate buffer (10mM, pH 3)		
Mobile Phase B	Methanol		
Gradient	Time (min)	A (%)	B (%)
	0	55	45
	5	55	45
	10	10	90
	10.1	55	45
	15	45	45

Diluent	Ethanol
Standard solution (282 µg/mL)	Rutin was accurately weighed and dilute to volume with diluent.
Sample preparation (cream)	50mg Day-Cream were weight into aq 25mL volumetric flask and filled up with ethanol. The cream suspension was placed into an ultra sonic bath for 20 minutes. The sample solution was filtered through a 0.45 PTFE µm filter directly into the HPLC vial prior to analysis and placed in the autosampler.

**Table 1.** Experimental Conditions

## STANDARD SOLUTION

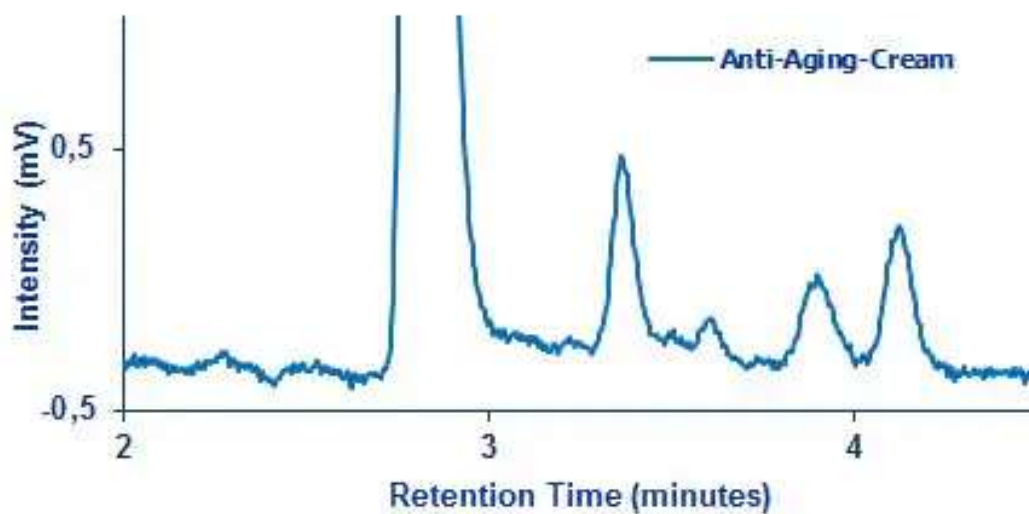
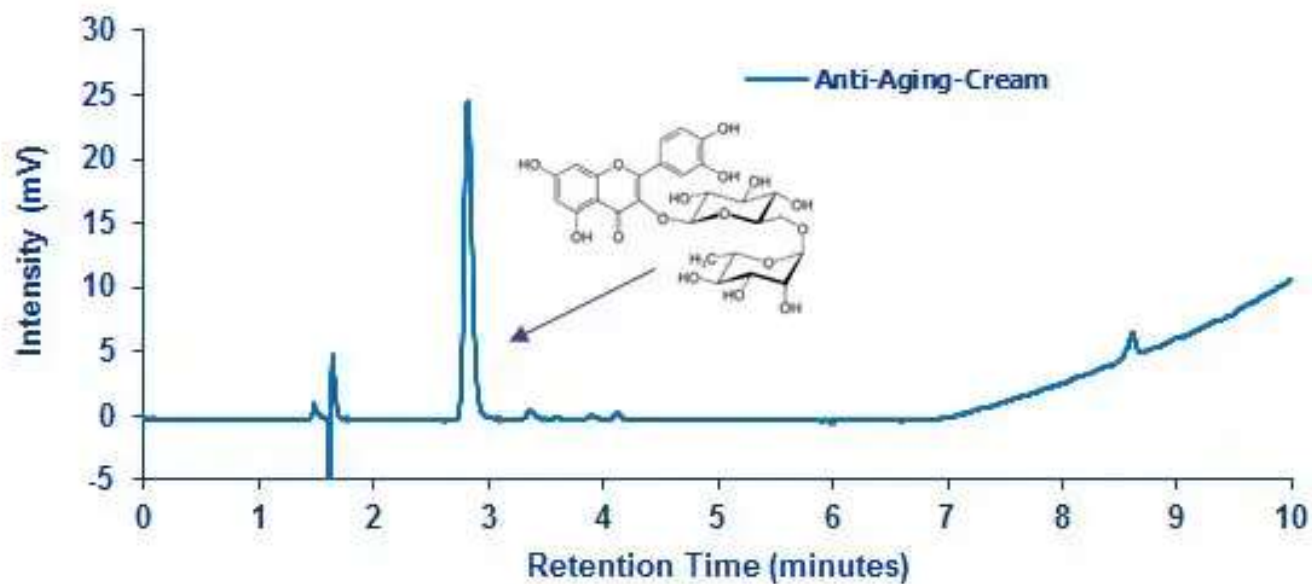


**Figure 1.** Determination of Rutin - Standard Solution

No.	Compound	Retention Time (min)	Area (mAU*min)	Tailing Factor
1	$t^0$ void volume	2.0		
2	Rutin	2.8	2,266	1.2

Table 2. Chromatographic Data [Standard Solution (282 µg/mL)]

## CREAM SAMPLES



Figures 2 and 3. Cream samples

1. Specificity: Inject standard solution of Rutin and determine the retention time, peak purity, tailing factor and column efficiency

		Retention Time (min)	Plates (USP))	Tailing Factor	Peak Purity
1	Rutin	2.8	8004	1.2	1.0000

**2. LOD & LOQ**

Concentration (ppm)	Mean Area (mAU*min)
LOD (ppm)	5,5
LOQ (ppm)	16,7

**3. Linearity (area mAU\*min)**

Concentration (ppm)	Mean Area
2,820	0,06
7,050	0,13
14,100	0,26
28,200	0,50
56,400	1,17
141,000	2,86
282,000	5,77

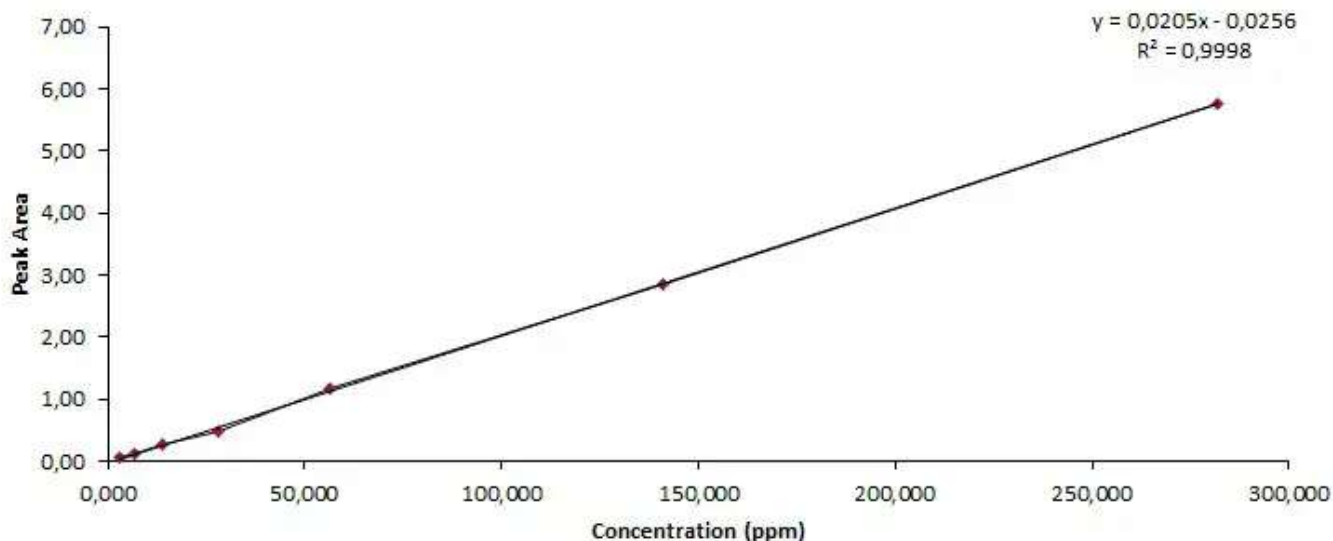




Figure 4. Determination of Rutin - Validation Data

## Materials

Product No.	Description	SDS	Pricing
<a href="#">1.52022</a>	<b>Chromolith® HighResolution RP-18 endcapped</b> L x I.D. 100 mm x 4.6 mm HPLC column		<a href="#">Expand</a> ▾
<a href="#">1.06007</a>	<b>Methanol</b> gradient grade for liquid chromatography LiChrosolv® Reag. Ph Eur	<a href="#">↓</a>	<a href="#">Expand</a> ▾
<a href="#">1.15333</a>	<b>Water</b> for chromatography (LC-MS Grade) LiChrosolv®	<a href="#">↓</a>	<a href="#">Expand</a> ▾
<a href="#">1.00983</a>	<b>Ethanol</b> absolute for analysis EMSURE® ACS,ISO,Reag. Ph Eur	 <a href="#">↓</a>	<a href="#">Expand</a> ▾
<a href="#">78095</a>	<b>Rutin trihydrate</b> analytical standard	<a href="#">↓</a>	<a href="#">Expand</a> ▾
<a href="#">R5143</a>	<b>Rutin hydrate</b> ≥94% (HPLC), powder	<a href="#">↓</a>	<a href="#">Expand</a> ▾
<a href="#">438081</a>	<b>Phosphoric acid</b> ACS reagent, ≥85 wt. % in H <sub>2</sub> O	 <a href="#">↓</a>	<a href="#">Expand</a> ▾
<a href="#">71640</a>	<b>Sodium phosphate dibasic</b> puriss. p.a., ACS reagent, anhydrous, ≥99.0% (T)	<a href="#">↓</a>	<a href="#">Expand</a> ▾
<a href="#">SLHVX13NK</a>	<b>Millex Syringe Filter, Durapore® ( PVDF ), Non-sterile</b> 0.45 µm pore size, 13 mm diameter, Millex-HV Durapore® ( PVDF ) membrane, hydrophilic		<a href="#">Expand</a> ▾