# Non-volatile Flavonoid Analysis by HPLC with Evaporative Light Scattering Detection

Application Note


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## Introduction

Flavonoids are a group of water soluble phenolic derivatives found in many vegetables, fruits and drinks. They have been reported as having various potential therapeutic benefits including protection against cancer[1]. The literature also indicates that they play a key role in repairing breaks in DNA strands and base alterations[2]. Additionally, they scavenge reactive oxygen species, promoting vascular relaxation and preventing cardiovascular problems[3]. As non-volatile compounds, characterization and quantification of flavonoids is best assessed using an evaporative light scattering detector, the Agilent ELSD.

The Agilent ELSD also gives even greater sensitivity than UV detection. Solvent peaks are absent and excellent baseline stability is present. The Agilent ELSD is renowned for its rugged design and ability in delivering high performance for demanding HPLC or GPC applications. PLRP-S 100Å columns are ideally suited to the analysis of low molecular weight compounds, such as flavonoids, because the small pore sizes have extremely high surface areas available to the solutes.


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## Instrumentation

| | |
|---|---|
| Column: | PLRP-S 100Å 5 µm, 150 x 4.6 mm (p/n PL1111-3500) |
| Detection: | Agilent ELSD (neb=85 °C, evap=80 °C, gas=1.0 SLM) |

## Materials and Reagents

| | |
|---|---|
| Eluent A: | 5mM Acetic acid |
| Eluent B: | 100% ACN |

## Sample Preparation

Flavonoids, 1 mg/mL Methanol

## Conditions

| | |
|---|---|
| Flow Rate: | 1.0 mL/min |
| Gradient: | 30-80% B in 20 min |

## Results and Discussion

Good baseline stability is apparent in Figure 1 and the very low on-column detection limit, approximately 1.3 µg for each individual flavonoid, is revealed in Figure 2.

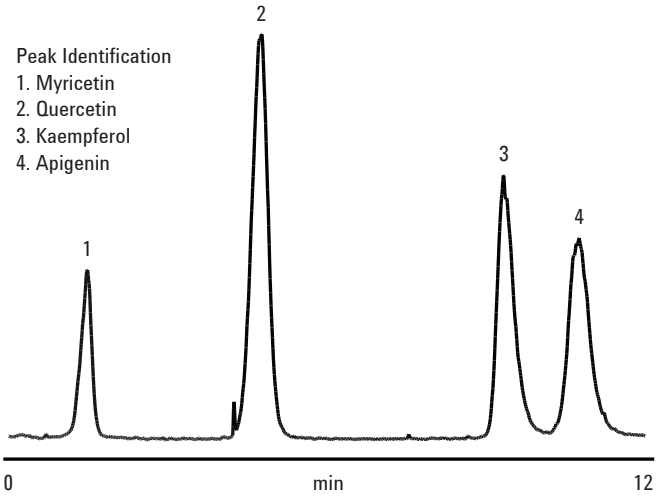


***Figure 1. Separation of flavonoids (40 µL at 1 mg/mL) using the Agilent ELSD evaporative light scattering detector.***

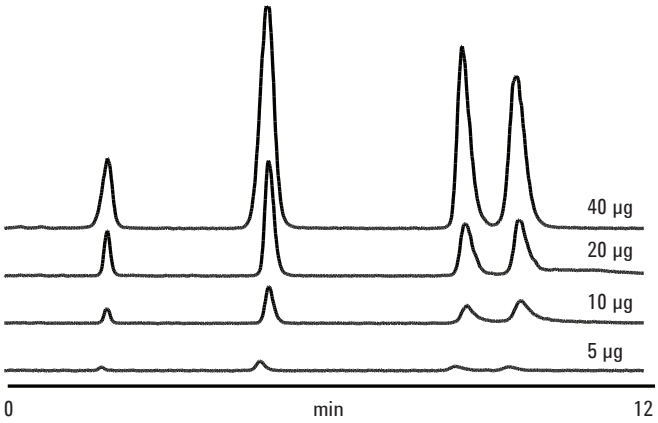


***Figure 2. Low on-column loading of four flavonoids.***

Figure 3 shows the linear responses of the calibration plots.

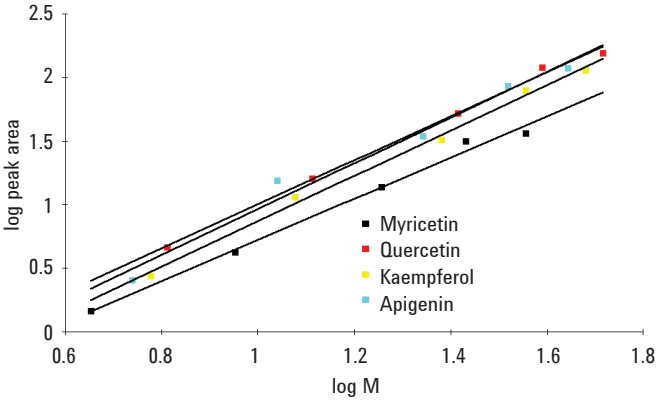


***Figure 3. Calibration curves for four flavonoids.***

## Conclusion

The Agilent ELSD evaporative light scattering detector and a PLRP-S column successfully separated a mixed sample of non-volatile flavonoids.

PLRP-S columns are the preferred choice for the analysis of many small molecules. These columns are more retentive for small molecules than the majority of alkyl bonded silicas. PLRP-S media possess a much greater surface area than alkyl bonded silicas and therefore even polar molecules, such as carboxylic acids, may be retained for much longer resulting in greater resolution.

PLRP-S columns used with the Agilent ELSD is an ideal combination for resolving non-volatile compounds.

## References

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[3] Agati, G., Matteini, P., Goti, A. and Tattini, M. 2007. Chloroplast-located flavonoids can scavenge singlet oxygen. *New Phytol.* 174: 77-89

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