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Introduction

Flavonoids are naturally occurring antioxidants that are thought to have therapeutic importance in cardiovascular disease and as cancer preventive agents [1, 2]. Resveratrol, quercetin, and other polyphenolic flavonoids are not only found in wine, but also green tea, chocolate, cocoa and several other food products.

A method is presented for the analysis of epicatechin (EC), epigallocatechin (EGC), epigallocatechingallate (EGCG), myricetin, quercetin, keampferol and cis and trans resveratrol. An ALEXYS gradient LC system with electrochemical detection has been applied.



Fig. 1. ALEXYS[®] system for Polyphenols.

Method

HPLC ALEXYS Phenol analyser (p/n 180.0094A)
Flow cell VT-03 with 2 mm GC WE, HyREF[™]
Temperature 35 °C
Flow rate 200 µL/min

Gradient analysis

Because of the difference in retention behaviour between the catechins and polyphenols a gradient has been applied.

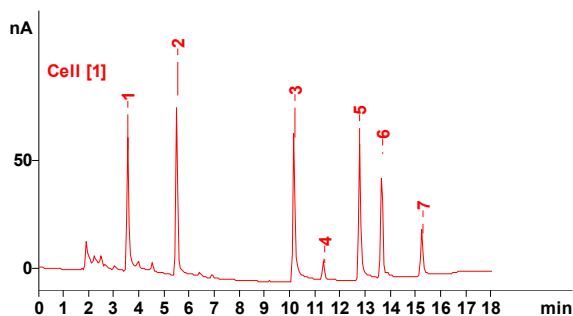


Fig. 2. Resolving power of the gradient LC system. Analysis of 2 µM standards: epicatechin (1), epigallocatechin (2), myricetin (3), trans-resveratrol (4), quercetin (5), keampferol (7) and cis-resveratrol (6). Linear gradient from 5% (t=0) to 40% B (t=8) to 100%B (t=14).

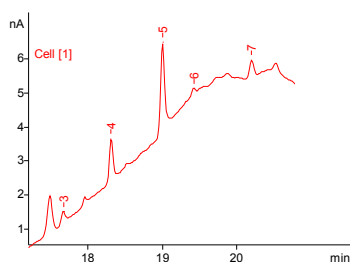


Fig. 3: Analysis of 100 µl, 1 nM myricetin (3), trans-resveratrol (4), quercetin (5), cis-resveratrol (6), and keampferol (7). This large volume injection enhances the LOD.

Linearity and reproducibility

The linearity has been studied in the range 10 - 100 nM using a HPLC gradient. Correlation coefficient *r* is better than 0.998 in all cases. A detection limit of 2-5 nM was found for all 7 polyphenols for 5 µL injections. Detection limits can easily be improved by increasing the sample load on column (fig. 3). At 20 nM an RSD of 2% for peak heights and areas was found.

Analysis of food and beverages

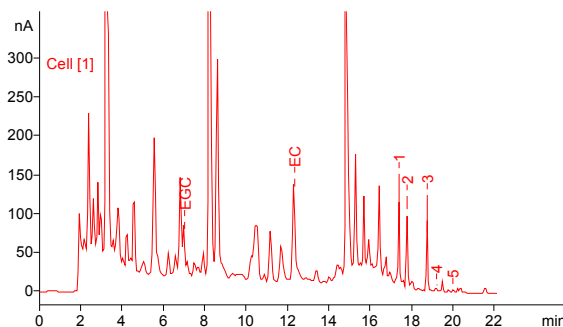


Fig. 4: Chromatogram of 10X diluted Santa Digna cabernet sauvignon (1), 2003, Chile; 1= Myricetin, 2= trans-Resveratrol, 3= Quercetin, 4= cis-Resveratrol and 5= Keampferol

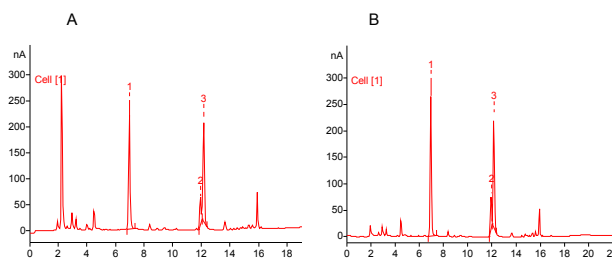


Fig. 5A: Analysis of 50X diluted Lipton green ice tea, Unilever, 1= EGC, 2= EC, 3=EGCG and (5B) 50X diluted green tea, Pickwick; 1= EGC, 2= EC and 3= EGCG.

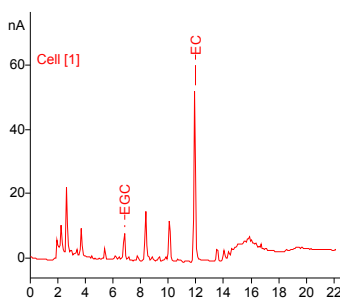


Fig. 6: Chromatogram of Milk chocolate (Trésor, the Convenience company), extracted and diluted 50X; 1= EGC, 2= EC

Conclusion

An ALEXYS Phenol Analyser has been used for the analysis of polyphenols in wine and food products. The system has excellent performance as to detection reproducibility, linearity and detection limits.

References

- Otto Daniel, Matthias Samuel Meier, Josef Schlatter, and Peter Frischknecht; Selected Phenolic Compounds in Cultivated Plants: *Ecologic Functions, Health Implications, and Modulation by Pesticides*; Environmental Health Perspectives 107 (1999) 109-114
- E. de Rijke, P. Out, W.M. Niesse, F. Ariese, C. Gooijer, U.A. Brinkman; *Analytical separation and detection methods for flavonoids*; J Chromatogr A. 1112(1-2) (2006) 31-63