Application Note 20583

Determination of Catechins and Phenolic Acids in Red Wine by Solid Phase Extraction and HPLC

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Key Words

Catechins, phenols, polar compounds, food analysis, HyperSep Retain PEP, perfluoroalkyl phase, Accucore PFP

Abstract

This application note demonstrates a simple and rapid HPLC method for the analysis of nine catechins and phenolic acids in red wine. Extraction of these polar analytes was achieved on Thermo Scientific™ HyperSep™ Retain PEP material. The polyphenols in the extracts were quantified by a matrix-standard calibration, with extracts from a wine sample spiked with increasing amounts of analytes. Determination of the polyphenols was performed by HPLC, using a Thermo Scientific Accucore™ PFP HPLC column under gradient mobile phase conditions.

Introduction

Dietary polyphenols comprise a wide range of aromatic compounds that are responsible for numerous organoleptic characteristics of plant-derived food and beverages. In addition to color and taste properties, polyphenols are reported to have antioxidant characteristics, making them responsible for the healthy features of fruit, vegetables and plant-derived beverages.

The polyphenols that are present in foods can be divided into two main groups: non-flavonoids and flavonoids. Non-flavonoids are mostly monocyclic acids and can be further divided into two main sub-classes: phenolic acids and stilbenes (e.g. resveratrol). Phenolic acids are subdivided into benzoic acids and hydroxycinnamic acids.

Flavonoids share a common nucleus consisting of two phenolic rings and an oxygenated heterocycle. They form a diverse range of compounds and can be categorized into many classes, such as anthocyanins, flavonols (e.g. quercetin), flavanols (e.g. catechins), flavones, and chalcones.[1]

The catechin group of flavanols are major components in wine and are reported to have antioxidant, antimicrobial, antimutagenic and anticarcinogenic activities. Some of the main catechins present in red wine are shown in Figure 1.

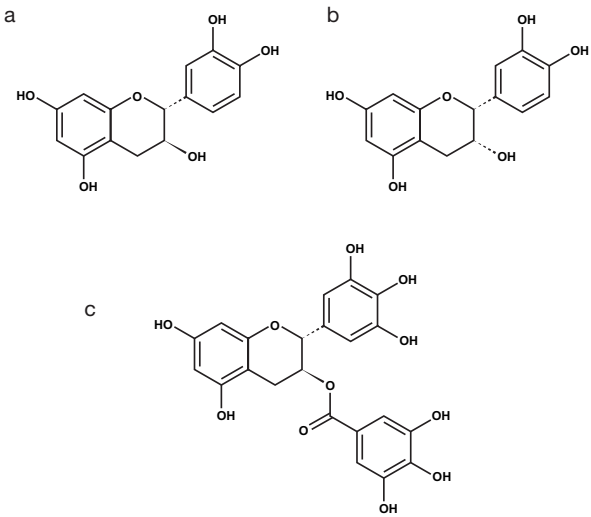

Figure 1: Catechins: a. catechin, b. epicatechin, c. gallocatechin gallate

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The presence of polyphenols in plant matter is highly variable. Some compounds are ubiquitous; whereas others are restricted to specific species. Large variations may also occur because of environmental conditions, ripening stages, genetic variations, and part of the fruit considered (e.g. peel or pulp). Polyphenols are also highly unstable species. For these reasons, assaying polyphenols can be very difficult. However, since polyphenols contribute to the taste, appearance, and formation of unappetizing flavors in foods and drinks, compositional studies have gathered momentum in recent years.[2]

Most phenolic substances are water-soluble and aromatic; therefore, reversed-phase HPLC with UV detection is the technique of choice. However, since polyphenols are structurally similar, their analysis requires high chromatographic selectivity and resolution.

The method described in this application note uses the Accucore PFP (pentafluorophenyl) HPLC column for the fast and efficient chromatographic determination of several catechins and other polyphenols in red wine under gradient HPLC conditions.

Accucore columns use Core Enhanced Technology™ to facilitate fast and highly efficient separations. The 2.6 µm diameter particles are not totally porous, but have a solid core and a porous outer layer. The optimized phase bonding creates a series of high coverage, robust phases. The tightly controlled 2.6 µm diameter of the Accucore particles results in much lower backpressures than typically seen with sub-2 µm materials. Introduction of fluorine groups into the Accucore PFP stationary phase leads to significant changes in the analyte-stationary phase interactions. This chemistry is well suited to the analysis of polar compounds containing hydroxyl, carboxyl, nitro, or other polar groups. Furthermore, its selectivity is more evident when the functional groups are located on an aromatic ring, making the Accucore PFP HPLC column the ideal candidate for the analysis of polyphenols and catechins.

The sample preparation for wine requires a solid phase extraction. In this application note we demonstrate the efficacy of the versatile HyperSep Retain PEP (polar enhanced polymer) material. HyperSep Retain PEP products consist of high-purity, highly porous polystyrene divinylbenzene material, modified with urea groups. This material provides exceptional recoveries for polar and non-polar analytes. Additionally, pH stability across the 0–14 range, fast sample preparation, fast method development and consistent recoveries are key attributes exhibited by HyperSep Retain PEP products and demonstrated here.

Experimental Details

Consumables	Part Number
Fisher Scientific™ formic acid (AR grade)	F/1900/PB08
Fisher Scientific methanol (HPLC grade)	M/4056/17
Fisher Scientific acetonitrile (HPLC grade)	A/0626/17
Fisher Scientific water (HPLC grade)	W/0106/17

Sample Handling Equipment	Part Number
Thermo Scientific Finnpipette™ F2 pipettor kit 10 µL–100 µL, 100 µL–1000 µL, 1 mL–10 mL	PMP-020-220F
Thermo Scientific Finntip™ pipette tips, 10 µL	PMP-107-110W
Thermo Scientific Finntip pipette tips, 200 µL	PMP-107-600F
Thermo Scientific Finntip pipette tips, 1000 µL	PMP-103-206K
Thermo Scientific Finntip pipette tips, 10 mL	PMP-107-040R
Thermo Scientific HyperSep Retain PEP (200 mg/3 mL)	60107-204
Thermo Scientific SPE 16-port vacuum manifold	60104-232
Thermo Scientific borosilicate glass vials (2 mL, 12 mm x 32 mm) with 8 mm black screw cap fitted with a silicone/PTFE seal	60180-600

Sample Preparation

Analytical Standards:

Primary analytical standards of catechin, epicatechin, gallocatechin gallate, syringic acid, hydroxybenzaldehyde, p-vanillin, myricetin, resveratrol, and quercetin were prepared separately. Catechin, epicatechin, gallocatechin gallate, syringic acid, hydroxybenzaldehyde, and p-vanillin standards were prepared in water. Myricetin, resveratrol, and quercetin were prepared in water / methanol (50:50 v/v). A mixed working standard was prepared by combining 1000 µL of each primary standard.

Solid Phase Extraction Method Development:

The extraction procedure was optimized by performing an elution profile to determine the best wash and elution conditions for the SPE. This was achieved by aliquoting 2 mL of working standard mixture (prepared in water) onto the HyperSep Retain PEP cartridges (following conditioning and equilibration with 2 mL of methanol and water, respectively). Washes with increasing elutropic strengths of solvent were applied, starting with 0:100:0.1 (v/v/v) methanol / water / 0.1% formic acid and increasing stepwise by 10% to 100:0:0.1 (v/v/v) methanol / water / 0.1% formic acid.

Four wash steps (using 1 mL of 100% methanol for each step) were then performed. Each wash stage was collected and analyzed by HPLC. The data obtained are presented in Figure 2, which shows an optimal wash condition of 20% methanol before compounds start to elute from the cartridge. Figure 2 shows that 90% elution solvent is strong enough to elute all of the components. However, a 100% methanolic solution was used to reduce the time taken on the solvent evaporation stage.

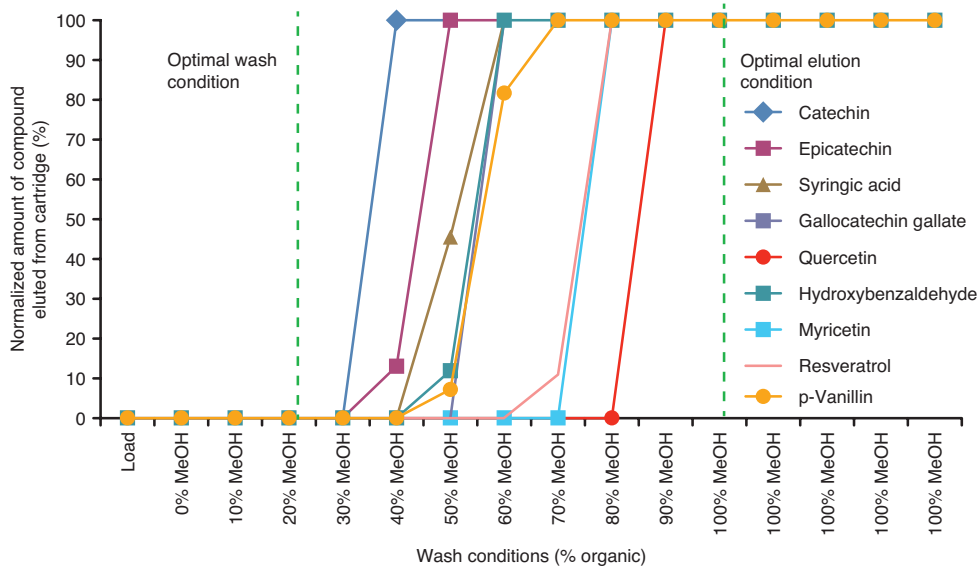

Figure 2: HyperSep Retain PEP column elution profile for catechins and phenolic compounds

Solid Phase Extraction Analysis:

The optimized extraction procedure was performed by using mixtures of the phenolic compounds and catechins dissolved in a wine matrix (Table 1). The standard mixtures were prepared at six concentration points (Std 1- 11, Table 2), by carrying out serial dilutions from the working standard solution. The wine chosen for this study was a red Bonarda Shiraz from Argentina (year 2010). The same extraction procedure used for the standard mixtures was then applied to the wine sample. The wine sample was diluted in water by a factor of three prior to SPE and prior to the standard mixtures spiking to ensure retention of the analytes.

Condition	2 mL methanol (MeOH)
Equilibration	2 mL water
Load	2 mL sample
Wash	2 mL water + 0.1% formic acid (FA)
Wash	2 mL 20% MeOH + 0.1% FA
Elute	4 x 1 mL MeOH + 0.1% FA

Table 1: SPE procedure

Chromatographic Conditions			Part Number
Instrumentation:	Thermo Scientific Accela™ UHPLC system		
Column:	Thermo Scientific Accucore PFP 2.6 µm, 100 mm x 2.1 mm		17426-102130
Mobile phase:	A: Water + 0.1% formic acid; B: acetonitrile + 0.1% formic acid		
Gradient:	Time (min)	% B	
	0	2	
	0.1	2	
	7.1	65	
	7.2	95	
	7.9	95	
	8.0	2	
	10.0	2	
Flow rate:	0.4 mL/min		
Column temperature:	30 °C		
Autosampler temperature:	Ambient		
Detection:	UV at 280 nm		
Injection volume:	1 µL		
Run time:	10 minutes		
Syringe flush:	Mobile phase		

Results

Under the conditions adopted for this analysis, good retention and baseline separation of nine polar molecules can be accomplished in approximately five minutes. The chromatography is presented in Figure 3. The total run time is ten minutes due to the column equilibration necessary at the end of the gradient.

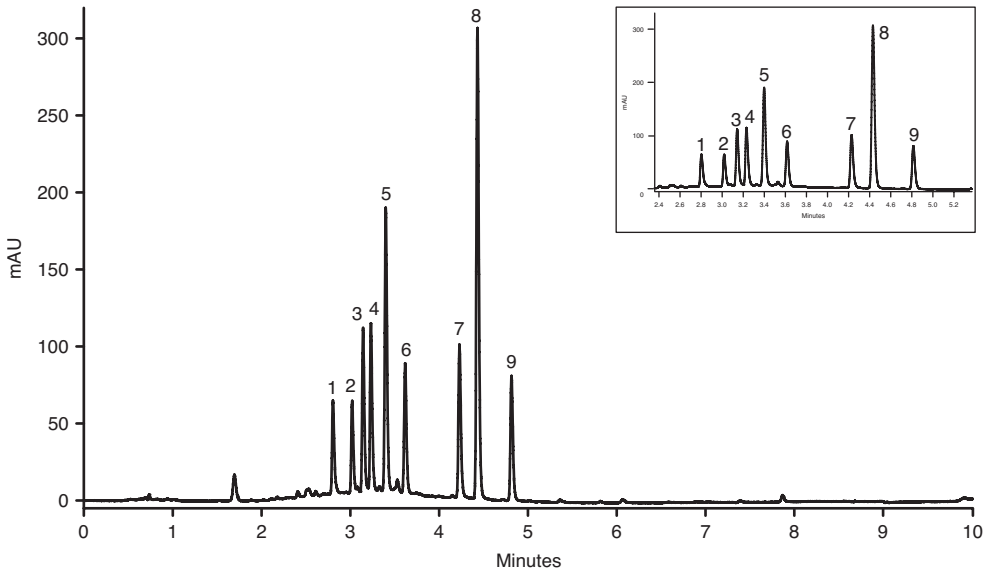

Figure 3: Chromatogram for standard mixture 1, containing nine polyphenol standards prepared in a red wine matrix and extracted by SPE. Order of elution: 1. catechin; 2. epicatechin; 3. syringic acid; 4. gallocatechin gallate; 5. hydroxybenzaldehyde; 6. p-vanillin; 7. myricetin; 8. resveratrol; 9. quercetin.

A matrix-matched calibration line was obtained from the standard mixtures prepared in wine. The standard mixtures were prepared at six concentration points (Std 1- 11), by carrying out serial dilutions from the working standard solution.

Table 2 summarizes the concentrations of catechins and polyphenols in the standard mixtures spiked into a sample of red Bonarda Shiraz.

Linearity in detector response was observed over the concentration ranges investigated (as reported in Table 2), with correlation coefficients greater than 0.995 for all nine analytes. An example of linearity in detector response for catechin (over the concentrations reported in Table 2) is presented in Figure 4. Catechin was chosen as a representative for the linear responses of the phenolic compounds investigated in this application note.

Recoveries were calculated by comparing the detector response of the extracted top standard mixture against an unextracted standard at the same concentration (Table 2). Accuracy values (calculated by comparing the backcalculated values with the actual values) are shown in Table 2.

	Accuracy (%)											Std 1 Recovery (%)
	Std 1 (55 µg/mL)	Std 2 (40 µg/mL)	Std 3 (25 µg/mL)	Std 4 (20 µg/mL)	Std 5 (15 µg/mL)	Std 6 (10 µg/mL)	Std 7 (8 µg/mL)	Std 8 (6 µg/mL)	Std 9 (5 µg/mL)	Std 10 (4 µg/mL)	Std 11 (3 µg/mL)	
Catechin	100.0	101.0	84.0	94.6	111.5	93.7	N/A	N/A	N/A	N/A	N/A	93.7
Epicatechin	97.5	104.3	100.4	98.2	101.4	98.2	N/A	N/A	N/A	N/A	N/A	95.4
Gallocatechin gallate	100.3	99.5	99.2	99.5	102.9	98.2	N/A	N/A	N/A	N/A	N/A	93.3
Myricetin	100.7	98.5	99.6	100.4	102.9	98.2	N/A	N/A	N/A	N/A	N/A	89.3
Resveratrol	99.8	100.2	99.6	98.6	102.9	98.2	N/A	N/A	N/A	N/A	N/A	95.7
Quercetin	100.0	100.2	98.2	99.5	104.3	97.3	N/A	N/A	N/A	N/A	N/A	94.8
Syringic acid	N/A	N/A	N/A	100.4	103.8	92.7	100.0	N/A	105.2	100.0	N/A	95.1
Hydroxybenzaldehyde	N/A	N/A	N/A	98.9	N/A	105.0	96.3	98.6	N/A	102.3	100.0	97.5
***p*-Vanillin**	N/A	N/A	N/A	N/A	97.1	105.4	101.7	N/A	96.4	97.1	100.0	95.9

Table 2: Concentration levels for the mixtures of catechins and phenolic compounds spiked into the wine sample, with accuracies and recoveries. N/A indicates that this concentration level was not used for this compound.

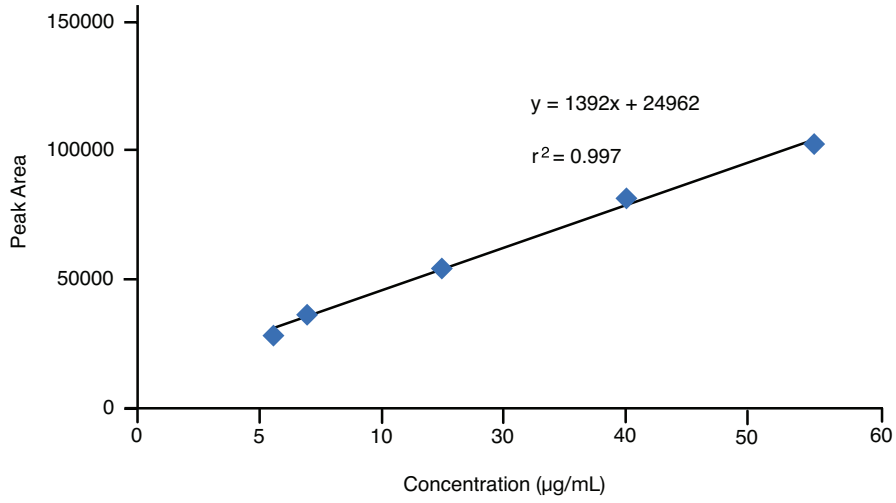

Figure 4: Calibration graph for catechin

Conclusion

In this application note an HPLC method for the analysis and quantitation of nine catechins and phenolic acids from red wine was developed. Extraction of these polar analytes was achieved on HyperSep Retain PEP material, and shows excellent recovery. The polyphenols in the extracts were quantified by a matrix-standard calibration, with extracts from a wine sample spiked with increasing amounts of the analytes. The unique selectivity offered by the Accucore PFP HPLC column provides exceptional separation performance to resolve these very structurally similar compounds.

References

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