Application Note 20610

Fast Analysis of Coffee Bean Extracts Using a Solid Core HPLC Column

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Key Words
Accucore RP-MS, fast method, coffee, phenols, polyphenols, chlorogenic acids, caffeic acid, p-coumaric acid, ferulic acid

Abstract
This application note demonstrates the benefits of using Thermo Scientific™ Accucore™ RP-MS HPLC columns for the analysis of coffee extracts. By transferring the established method to the Accucore phase a 75% reduction in analysis time was achieved while maintaining full resolution of all components.

Introduction
It is believed that dietary phenols and polyphenols have the potential to protect the consumer's health and as such there has been a great deal of research into plant products that contain these compounds. One rich source of these antioxidant compounds is coffee, where a range of chlorogenic acids is found. For people who consume coffee beverages regularly, whether prepared from soluble powders or freshly brewed, daily intakes of 500 mg or even more of chlorogenic acids are easily achieved [1]. Chlorogenic acids are a family of esters formed between quinic acid and certain trans-cinnamic acids, most commonly caffeic, p-coumaric and ferulic. As many as 45 of these chlorogenic acids have been reported in coffee [2]. Thus quantification of individual compounds by absorbance detection requires good chromatographic separation.

Accucore HPLC columns use Core Enhanced Technology™ to facilitate fast and highly efficienct separations. The 2.6 µm diameter particles are not totally porous, but instead 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. In this application an Accucore HPLC column was used to speed up the separation of a range of cholorogenic acids from a coffee extract.

Experimental Details

Consumables	Part Number
Fisher Scientific™ LC-MS grade water	W/0112/17
Fisher Scientific Pierce LC-MS grade acetonitrile	TS-511001
Fisher Scientific Pierce LC-MS grade formic acid	PI-28905
9 mm screw thread vial, 300 µL, fused insert	03-FISV
9 mm open top short screw cap, 6 mm hole, blue silicone/PTFE septum	9-SC(B)-ST101

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Separation Conditions			Part Number
Instrumentation:	Thermo Scientific Accela HPLC system		
Column 1:	Fully porous C18, 4 µm, 250 x 2.0 mm		
Column 2:	Accucore RP-MS, 2.6 µm, 150 x 3.0 mm		17626-153030
Mobile phase A:	0.1% formic acid in water		
Mobile phase B:	0.1% formic acid in acetonitrile		
Gradient (column 1)	Time (min)	% B	
	0	5	
	20	10	
	60	35	
Flow rate:	200 µL/min		
Gradient (column 2)	Time (min)	% B	
	0	2	
	8	7	
	15	50	
Flow rate:	700 µL/min		
Backpressure at starting conditions for both columns:	Approx. 200 bar		
Column temperature:	40 °C		
Injection details:	5 µL		
Detection details:	UV absorbance detection at 325 nm		

Sample Preparation
Soluble coffee extract (Futureceuticals Inc. USA) in water/methanol (90:10 v/v)

Data Processing	
Software:	Thermo Scientific Xcalibur Software v1.3

Results

Current methods for the measurement of chlorogenic acid content of commercial coffee bean extracts [3] typically require a gradient method with an analysis time of 60 minutes. Such methods use a 250 x 2.0 mm C18 column packed with conventional 4 µm particles (Figure 1) to achieve adequate separation. Here the first analysis run of a similar coffee bean extract using a 150 x 3.0 mm Accucore RP-MS column is reported. The method was modified to take advantage of the very high performance offered by Accucore columns and reduce the run time. An equivalent separation at a similar backpressure was achieved with an analysis time of only 15 minutes. These excellent results, achieved from the first analysis, meant that no further method development was undertaken (Figure 2).

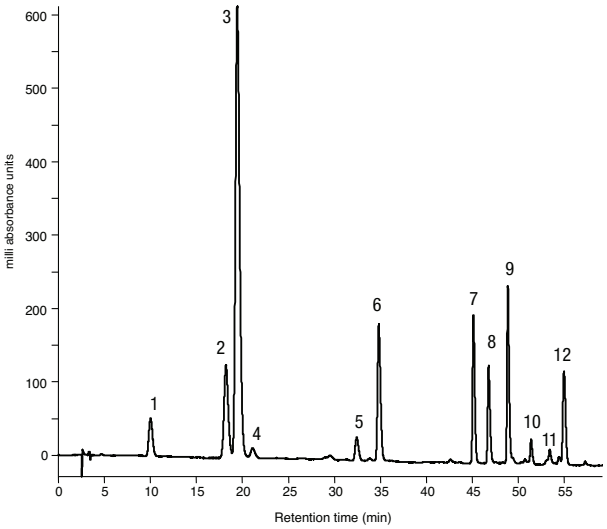

Figure 1: Analysis of a coffee bean extract using a 60 minute gradient on a fully porous C18 HPLC column (4 µm particle size, 250 x 2 mm)

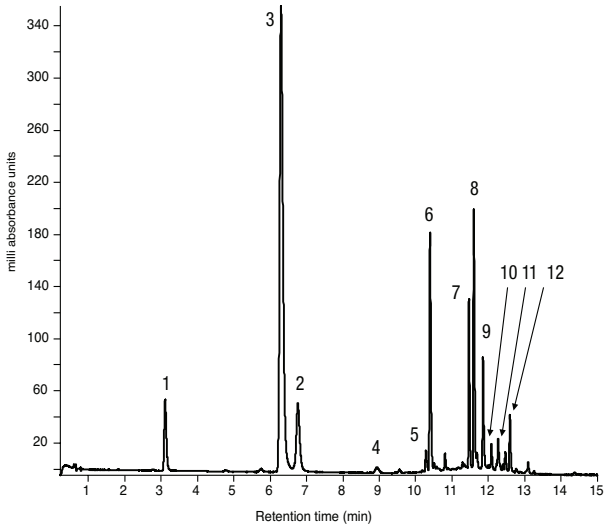

Figure 2: Analysis of a coffee bean extract using a 15 minute gradient on a solid core Accucore RP-MS HPLC column (2.6 µm particle size, 150 x 3.0 mm)

Along with an improvement in the resolution between peaks 2 and 3, the Accucore column also exhibits a change in selectivity, and compound 3 elutes before compound 2.

Peak number	Compound	Peak number	Compound
1	3-O-Caffeoylquinic acid	**7**	3,4-O-Dicaffeoylquinic acid
2	4-O-Caffeoylquinic acid	**8**	3,5-O-Dicaffeoylquinic acid
3	5-O-Caffeoylquinic acid	**9**	4,5-O-Dicaffeoylquinic acid
4	3-O-Feruloylquinic acid	**10**	3-O-Feruloyl-4-O-caffeoylquinic acid
5	4-O-Feruloylquinic acid	**11**	3-O-Caffeoyl-5-O-feruloylquinic acid
6	5-O-Feruloylquinic acid	**12**	4-O-Caffeoyl-5-O-feruloylquinic acid

Table 1: Peak numbers and identities of compounds in coffee extracts

Conclusion

- The Accucore RP-MS HPLC column is suitable for analyzing coffee extracts and quantifying the amount of different chlorogenic acids.
- Transferring the established analysis method to an Accucore RP-MS HPLC column enabled a 75% reduction in analysis time (60 minutes down to 15 minutes).
- Good resolution of all components was observed without additional method development.
- Backpressure was suitable for use on a conventional HPLC systems.

References

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