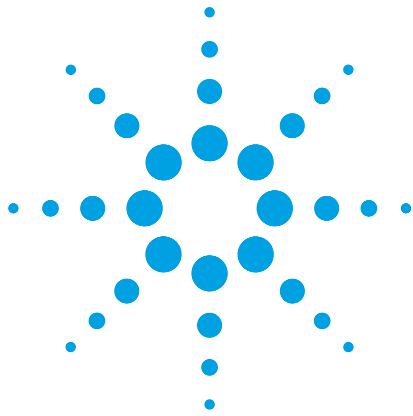

Streamlined Sample Preparation of Bergenin and Chlorogenic Acid in Human Plasma

Application Note

Author

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Abstract

Bergenin and chlorogenic acid are polyphenolic compounds commonly found in plants that are used in traditional Chinese medicine and in many other herbal medicines. Quick and easy sample preparation for simultaneous measurement of bergenin and chlorogenic acid in human plasma is an excellent tool for meeting the needs of high throughput bioanalysis.

Introduction

Faster and robust bioanalysis methods using LC/MS/MS have always been pursued by scientists. All components in the entire bioanalysis process need to work in harmony to achieve this goal, from quicker biological sample preparation techniques, to excellent chromatographic performance by employing a nonporous column, to UHPLC technology for fast and reproducible biological sample analysis.

This application note features the fast and reproducible sample preparation capability of Agilent Captiva ND Lipids plates in bioanalysis. Its performance is analyte-independent, and the entire sample preparation process can be streamlined.

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Materials and Methods

Figure 1 shows the arrangement for using the Agilent Captiva ND Lipids 96-well plate. The sample preparation procedure for the isolation of bergenin and chlorogenic acid is as follows.

1. Add 0.75 mL of MeOH to Captiva ND Lipids 96-well plate.
2. Add 0.25 mL of spiked human plasma to the plate.
3. Mix loaded samples five times by aspirating and releasing the loaded sample.
4. Apply vacuum to filter samples; leave the vacuum on until filtration is complete.
5. Place the 96-well collection plate in autosampler for LC/MS/MS bioanalysis.

The methanol was LC/MS grade. MeOH containing 0.1% formic acid was prepared by adding 1 mL of formic acid to 1 L of MeOH, and 0.1% formic acid was prepared by adding 1 mL of formic acid to 1 L of water.

Sample preparation by Agilent Captiva NDL plate can be automated depending on the robotic systems available in the laboratory, which can vary from fully automatable systems, such as Agilent Bravo or Encore liquid handling systems (or equivalent), to semiautomatic hand-operated liquid handling robots, or by employing a multichannel pipette for multiple sample process simultaneously. Individual sample preparation is also possible by using a single-channel pipette for a small number of samples.

Conditions

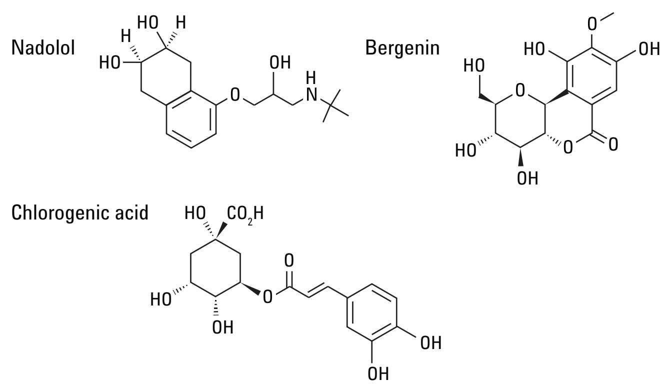
Column:	Agilent Poroshell 120 EC-C18, 3.0 × 50 mm, 2.7 µm (p/n 699975-302)
Sample prep:	Agilent Captiva ND Lipids (p/n A59640002I)
Samples:	See Table 1
Eluent:	A, 0.1% formic acid B, MeOH + 0.1% formic acid
Injection volume:	5 µL
Flow rate:	0.4 mL/min
Gradient:	Time (min) % B 0 10 3 90 3.1 10 5 10
LC/MS/MS:	Agilent 1290 Infinity LC with Agilent 6460 Triple Quadrupole LC/MS
Drying gas:	350 °C, 10 L/min
Sheath gas:	350 °C, 12 L/min
Nebulizer:	35 psi
Capillary:	3,500 V (positive), 3,500 (negative)
Nozzle voltage:	0 V

Figure 1. Stacked Agilent Captiva ND Lipids with a CaptiVac collar and 96-well collection plate (above). The bottom image shows the CaptiVac collar alone.

Table 1. Bergenin and chlorogenic acid.

	Chlorogenic acid	Bergenin	Nadolol (internal standard)
log P	-1.01	-1.06	0.81
pKa	2.66	5.46	9.67
MRM	353.1 → 191.1	327.1 → 192.0	310.2 → 56.1
Polarity	-	-	+

Structures



Results and Discussion

Biological sample preparation by Captiva ND Lipids was streamlined by eliminating the common steps typically required for solid phase extraction (SPE), such as conditioning, equilibration, washing, evaporation, and reconstitution. Figures 2 and 3, and Table 2 summarize the performance of Captiva ND Lipids with excellent recovery and linearity data regardless of the different pKa values of the analytes.

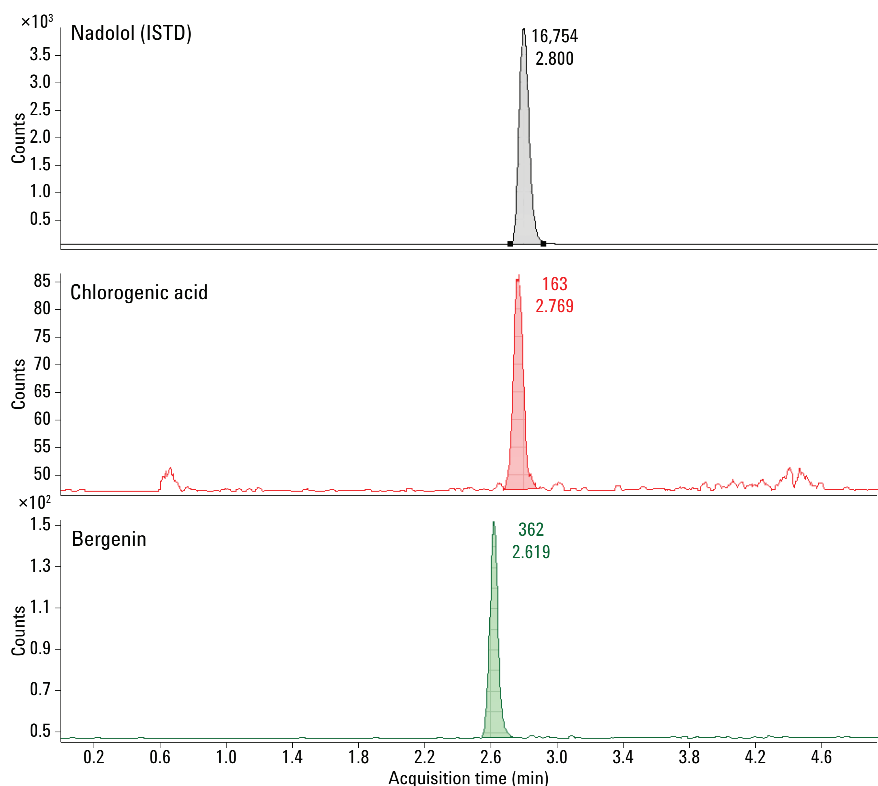


Figure 2. MS chromatograms of a human plasma sample spiked with chlorogenic acid and bergenin at 5 ng/mL.

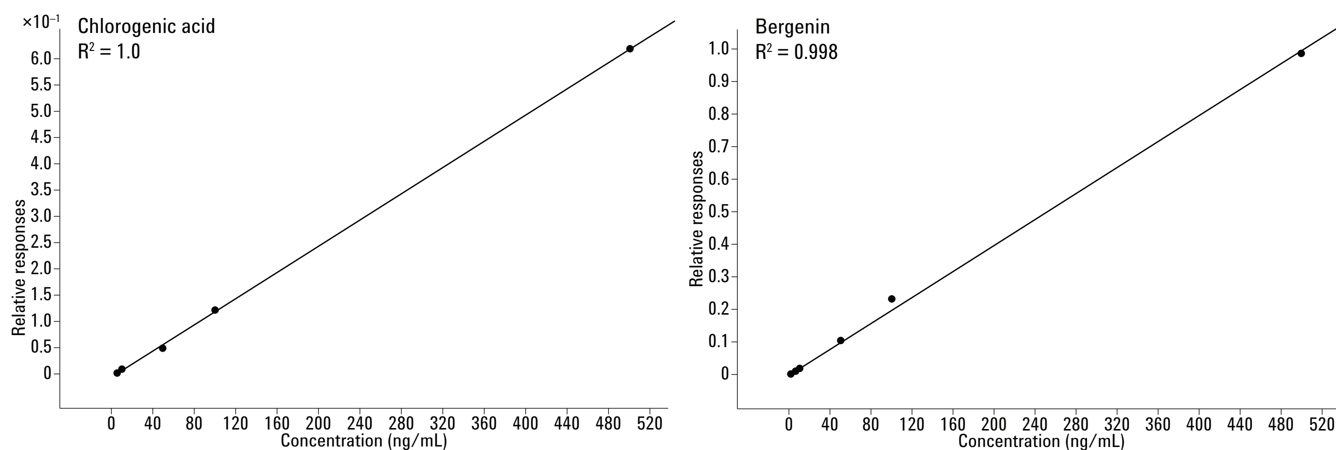


Figure 3. Calibration curves for chlorogenic acid and bergenin in human plasma.

Excellent linearity was achieved in the calibration curves with $R^2 \geq 0.998$ (Figure 3). Nadolol was used as an internal standard and showed similar retention characteristics as the two analytes. Nadolol had MRM transition under positive polarity while the other two compounds were measured under negative polarity. With the superior polarity switching capability of the Agilent 6460 Triple Quadrupole LC/MS between positive and negative, excellent quantitative results were obtained with good recoveries at three different plasma concentration levels (10, 100, and 500 ng/mL), ranging from 98.7 to 117.0 %. The low %RSD data shown in Table 2 indicate the precision of Captiva ND Lipids.

Conclusions

Streamlined and fully automatable biological sample preparation was demonstrated by Agilent Captiva ND Lipids. By eliminating the multiple steps conventionally required for SPE techniques, such as conditioning, equilibration, washing, evaporation, and reconstitution, high throughput biological samples preparation was achieved with good accuracy and precision.

Table 2. Summary of data with limits of quantitation (LOQ), calibration curve linearity (R^2), recovery, and %RSD (n=8 for each concentration).

			10 ng/mL (low)		100 ng/mL (mid)		500 ng/mL (high)	
	LOQ (ng/mL)	Linearity, R^2	% Recovery	%RSD	% Recovery	%RSD	% Recovery	%RSD
Bergenin	0.5	0.9983	110.6	5.8	103.3	3.7	98.7	8.8
Chlorogenic acid	5	0.9997	117.0	8.8	99.5	6.6	104.4	4.5

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