Quick, easy, and fully automatable lipid removing sample preparation for antioxidants from Traditional Chinese Medicine (TCM) in plasma by LC-MS/MS

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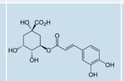
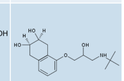
Introduction

Bergenin and chlorogenic acid are both polyphenolic compounds commonly found in plants and used for Traditional Chinese Medicine (TCM) and in many other herb medicines.

Chlorogenic acid can easily be found in plant food including vegetables, fruits, and coffee beans and exhibits diverse biological and pharmacological effects such as antioxidant, anti-carcinogenic, an antihypertensive activities.

Bergenin can be extracted from Bergenia and is reported to have multiple properties including antihepatotoxic, antiulcerogenic, antiarrhythmic,, anti-inflammatory, immunomodulatory characteristics and burn wound healing effects.

	Chlorogenic acid	Bergenin	Nadolol (internal std)
log P	-1.01	-1.06	0.81
pKa	2.66	5.46	9.67
			

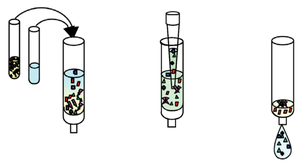
Experimental

Sample Preparation Method

Captiva ND*Lipids* 96-well plate is used for this study due to its simplicity in its operation during the sample preparation especially with biological samples. Captiva ND*Lipids* 96-well plates are extremely useful for high-throughput laboratories since they can virtually be employed in biological sample preparation without any method development process. Its main characteristics include non-drip feature for complete in-well protein precipitation and lipid/protein removal process during filtration when vacuum is applied. Filtered elution can directly be used for LC/MS analysis.

Captiva ND*Lipids* 96-well plate is analyte independent and easy-to-use lipid removing sample preparation plate.

Experimental (contd)



Step1
Add crash solvent (750 µL MeOH) followed by spiked biological sample (250 µL).

Step2
Pipette five times for protein precipitation. Complete automation can be achieved by utilizing a liquid handler.

Step3
Filter through by applying vacuum. Proteins and lipids will be removed and elution can be directly introduced to LC/MS for analysis.

- Salt
- Proteins
- Lipids
- Analyte

Note: In Step1, optimal crashing ratio is 3:1 with MeOH or acidified MeOH.

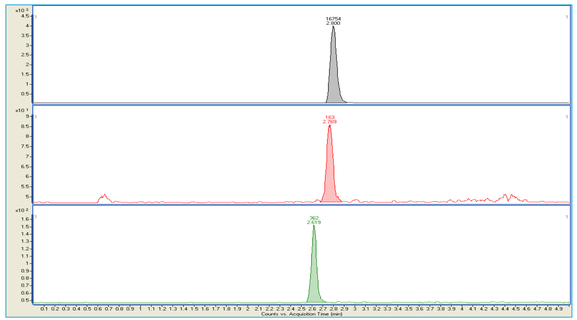
Fig 1. Sample preparation method using Captiva ND*Lipids*.

LC-MS/MS Conditions

Column:	Poroshell 120 EC-C18, 2.7 µm, 2.1 X 50 mm (pn: 699975-302)
Sample prep:	Captiva ND*Lipids* 96-well plate (pn: A59640002I)
LCMS:	Agilent Infinity 1290 UHPLC 6460 triple quad with JetStream
A:	0.1% formic acid in water
B:	MeOH + 0.1% formic acid
Flow rate:	0.4 mL/min
Gradient:	10 – 90%B in 3 min, back to 10% B in 0.1 min, equilibrate at 10% B for 1.9 min
Inj. Vol.:	5 µL
Gas temp.:	350 °C
Gas flow:	10 L/min
Nebulizer:	35 psi
Sheath gas:	350 °C, 12 L/min
Capillary:	3500 V (pos), 3500 V (neg)
MRM:	Chlorogenic acid (353.1 → 191.1, fragmentor = 92, CE = 8) Bergenin (327.1 → 192.0, fragmentor = 123, CE = 16)

Results and Discussion

LC/MS Chromatogram



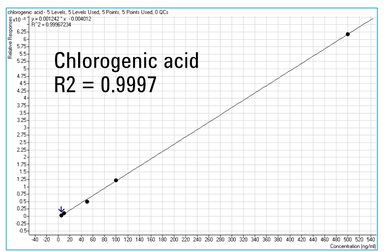
Nadolol (internal std)

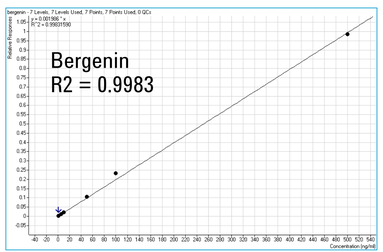
Chlorogenic acid

Bergenin

Fig 2. LC/MS chromatogram of spiked plasma sample at 5 ng/mL processed by Captiva ND*Lipids*.

Calibration Curves





Chlorogenic acid
R^2 = 0.9997
Range = 5 – 500 ng/mL
(5, 10, 50, 100, 500 ng/mL, 5 levels)

Bergenin
R^2 = 0.9983
Range = 0.5 – 500 ng/mL
(0.5, 1, 5, 10, 50, 100, 500 ng/mL, 7 levels)

For calibration standards, 50 µL of standard mixture and 50 µL of internal standard were spiked in 1 mL of human plasma.
750 µL of MeOH was added to Captiva NDLipids 96-well plate.
250 µL of spiked human plasma was added.
Pipette mixing or liquid handler mixing for five times.
Apply vacuum to filter.
Filtered sample was collected in a 96-well collection plate and directly loaded to LCMS autosampler.

Results and Discussion (contd)

Recovery and LOQ

	LOQ	Low (10 ng/mL)		Mid (100 ng/mL)		High (500 ng/mL)	
		Ave (ng/mL)	%RSD	Ave (ng/mL)	%RSD	Ave (ng/mL)	%RSD
Chlorogenic acid	5 ng/mL	11.7	8.8%	99.54	6.6%	522.0	4.5%
Bergenin	0.5 ng/mL	11.06	5.8%	103.34	3.7%	493.3	8.8%

Recovery study was performed at three different concentration levels in human plasma. Spiked concentrations of 10, 100 and 500 ng/mL were chosen as low, mid, and high concentrations, respectively.

Recovery study sample preparation method was the same as calibration standard preparation.

All recovery data are based on 8 replicate experimental values.

At all low, mid, and high concentration levels, %RSD values were all single digit numbers.

Conclusions

- Captiva ND*Lipids* 96-well lipid depletion plate can be used for high-throughput biological sample analysis.
- Analyte independent result was obtained with a single digit %RSD for both chlorogenic acid and bergenin.
- Superb calibration curves were achieved for chlorogenic acid and bergenin with R^2 values of 0.9997 and 0.9983, respectively.
- LOQ's of chlorogenic acid and bergenin in human plasma were 5, and 0.5 ng/mL, respectively.
- Complete automation can be implemented with liquid handler.
- No time-consuming evaporation and reconstitution were needed.

References

- Yu, W., Wang, Y., Zhang, Y., Zhang, D., Lan, J., Liu, Z., Gu, J., Fawcett, J. P., J Chrom B, 877 (2009) 33 – 36
- Matsui, Y., Nakamura, S., Kondou, N., Takasu, Y., Ochiai, R., Masukawa, Y., J Chrom B, 858 (2007) 96 – 105