Automated Sample Preparation by Protein Precipitation for High Throughput Bioanalysis

Application Note

BioPharma

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Abstract

Protein removal is an essential step in sample preparation for LC/MS/MS analysis of compounds in biological matrices. Protein precipitation followed by centrifugation is one of the most popular sample preparation techniques for removing proteins. However, it is not well-suited for high throughput environments due to the multiple manual steps involved. Fully automatable in-well precipitation in a 96-well plate format would reduce the time, cost, and manpower required for sample preparation in high throughput labs.

Agilent introduces a new protein precipitation filtration plate, Captiva ND, with non-drip technology for simple operation. Specially designed filtration materials effectively hold organic solvents used for precipitation with no dripping. This prevents sample loss and enables trouble-free automated methods. Simply add organic solvent to precipitate followed by biological samples, mix them in the well, and apply vacuum to filter out precipitated proteins. The result is particulate-free, protein-free samples in just a few minutes–five times faster than centrifugation methods.

Agilent Technologies

Materials and Methods

Reagents and solutions

Add 10 µL formic acid to 10 mL ACN to produce a 0.1% formic acid in ACN crash solvent.

Sample preparation using Captiva ND

SPE: Agilent Captiva ND 96-well plate, 0.45 µm, 10 mg (p/n A5969045)

Figure 1 shows the sample preparation method using Captiva ND.

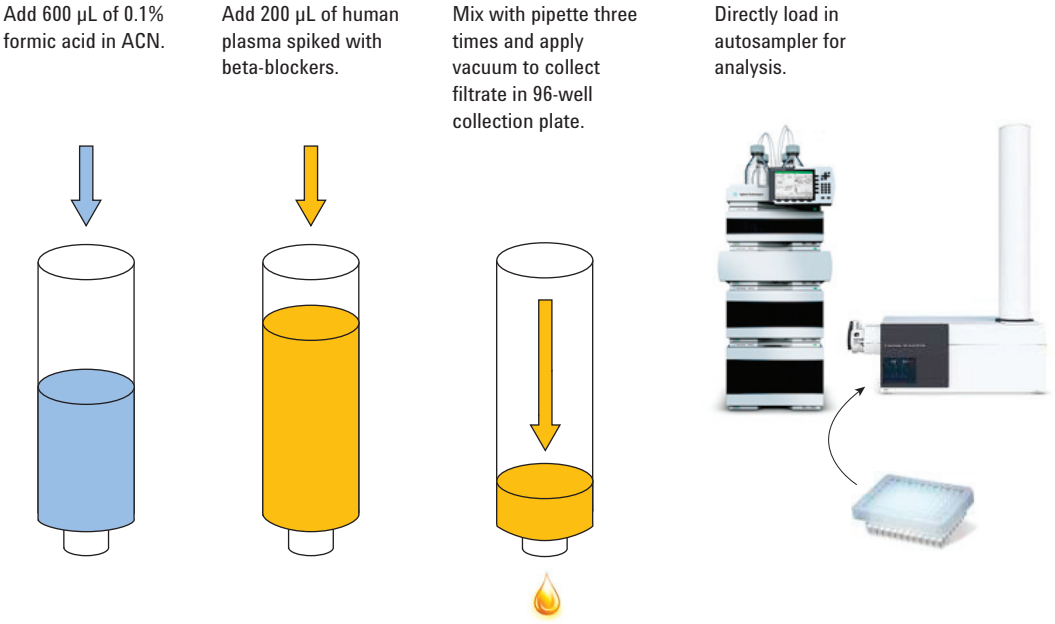

Figure 1. Schematic of sample preparation method for Captiva ND.

Sample preparation by centrifugation of protein precipitation

Centrifuge: Eppendorf centrifuge 5424 with 24 centrifuge tube holders.

1. Add 600 µL of 0.1% formic acid in ACN and 200 µL of human plasma spiked with beta-blockers.
2. Centrifuge at 10,000 RPM for 10 min or more.
3. Filter supernatant if needed.
4. Carefully transfer (filtered) supernatant to injection vials manually for analysis.

LC conditions

Column	Agilent ZORBAX Eclipse Plus RRHD C18, 2.1 × 5.0 mm, 1.8 µm (p/n 959757-902)
LC/MS/MS	Agilent 1290 Infinity UHPLC coupled with a generic MS system
Eluent A	0.1% formic acid in H_2O
Eluent B	0.1% formic acid in ACN
Flow rate	0.5 mL/min
Injection volume	1 µL
Gradient	Time (min) %B 0 30 1.0 90 1.1 30 1.5 30
Temperature	Ambient
Ion-source	ESI+
Drying gas temperature	300 °C
Drying gas pressure	18 psi
Nebulizer	55 psi
Vortex gas temperature	300 °C
Vortex gas pressure	25 psi
Needle voltage	4,000 V
CID gas pressure	1.5 mTorr

Table 1. Samples

	pKa	log P	MS/MS transition	Collision energy	Capillary
Nadolol	9.67	0.81	310.4 → 254.0	15.5	120
Propranolol	9.42	3.48	260.3 → 115.9	15.0	120
Pindolol	9.25	1.75	249.3 → 115.9	17.5	120
Metoprolol	9.70	1.90	268.4 → 115.9	18.0	120

Results and Discussion

A longevity experiment with continuous injections over an extended period of time is the easiest and most effective way to demonstrate the cleanliness of biological samples. Samples prepared by Captiva ND were tested in a 5,000-injection longevity experiment while monitoring retention times, MS area counts, and backpressure (see Figures 2 to 4).

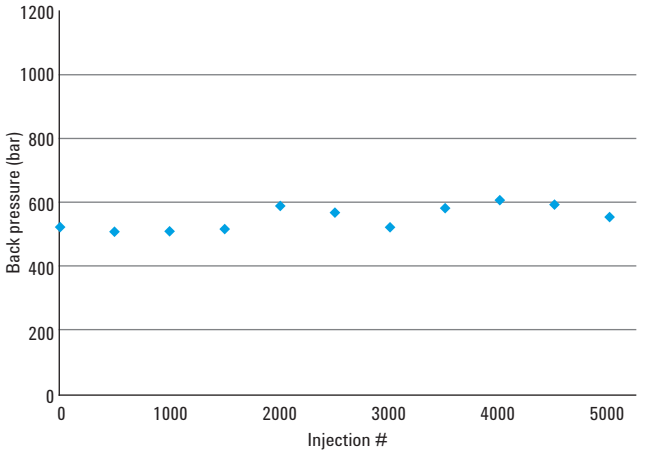

Figure 2. System backpressure data for longevity experiment using Agilent Captiva ND.

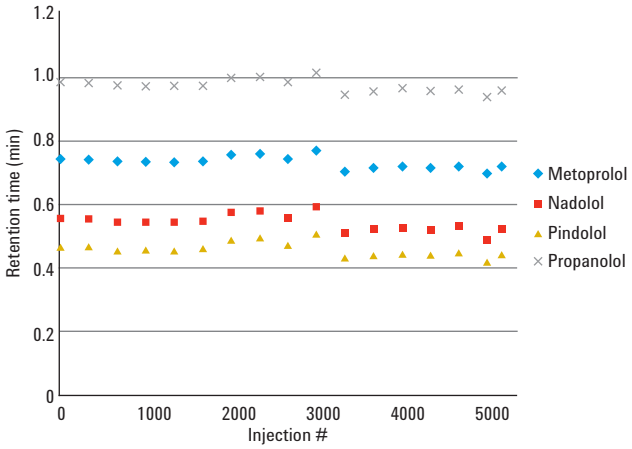

Figure 3. Retention time data for 5,000 continuous injections of Agilent Captiva ND samples.

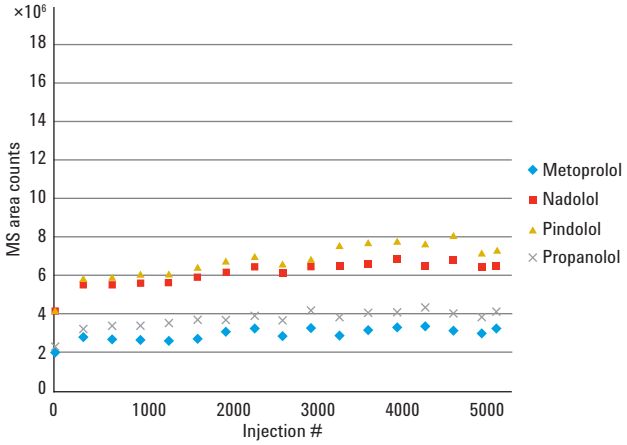

Figure 4. MS area count data for plasma samples spiked beta-blockers prepared by Agilent Captiva ND.

As implied by stable monitoring parameters in the longevity experiment such as backpressure, retention times, and MS area counts (see Figures 2 to 4), Captiva ND showed superb capability in protein removal, and delivered ultra cleanliness in biological samples. The longevity experiment, using over 5,000 injections, proved Captiva ND's ideal applicability in a high throughput environment especially using sub-2-µm columns and UHPLC systems. Comparison of high throughput applicability between Captiva ND and centrifugation protein precipitation is summarized in Table 2.

Table 2. Sample Preparation Time Comparison Between Agilent Captiva ND and Centrifugation Protein Precipitation Methods

Centrifugation protein precipitation	Time (min)	Captiva ND*	Time (min)
Add 0.2 mL of spiked plasma sample and 0.6 mL of ACN + 0.1% formic acid to centrifugation tubes or an empty 96-well plate.	5	Add 0.2 mL of spiked plasma sample and 0.6 mL of ACN + 0.1% formic acid to Captiva ND 96-well plate.	5
Centrifuge at 10,000 RPM for 10 min.	11	Mix each well with a pipette 5 times and apply vacuum.	
Transfer supernatant to 2 mL injection vials (if tubes were used) or a new empty 96-well plate for analysis (if plate format was used).	10	Directly transfer injection plate for analysis.	0
Total time required for sample preparation.	26	Total time required for sample preparation.	5

* Based on automation with robotic system such as Tomtec or Hamilton.

Approximately 80% reduction in cycle time is anticipated by switching from centrifugation protein precipitation to Captiva ND. Switching from centrifugation protein precipitation to Captiva ND saves time, money, and training.

Conclusion

Captiva ND plate is an optimal solution for the high throughput analytical industry. It enables the production of a large volume of samples in just a few minutes, with an extremely easy to use method. The three major monitoring parameters in the longevity experiment, backpressure, retention times, and MS signals, were all stable assuring the performance of Captiva ND.

For More Information

These data represent typical results. For more information on our products and services, visit our Web site at www.agilent.com/chem.

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