Agilent Bond Elut Plexa PCX – Cation Exchange SPE

A Destination to a Better Sensitivity in LC/MS Bioanalysis Resulting from Minimized Ion-Suppression

Application Note

Small Molecule Pharmaceuticals & Generics

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Introduction

Throughout the drug development process in pharmaceutical industry, it is of essence to develop and validate fast methods for bioanalysis without losing sensitivity. Ion-suppression often can be the most commonly encountered issue in achieving that goal, which causes low recovery, inaccuracy, as well as increased instrument maintenance cost and time. While ion-suppression cannot be fully avoided when biological samples are handled, it should be avoided as much as possible.

The nature of hydroxylated surface on Agilent Bond Elut Plexa PCX makes it stand out among other cation exchange SPE products with amide residue on the surface of the sorbent. The presence of amide residue causes increased interaction between the SPE sorbent and the endogenous material in biological sample, which can be directly responsible for ion-suppression during bioanalysis. Due to hydroxylation of the sorbent's surface, Bond Elut Plexa PCX reduces the interaction between the sorbent and the endogenous material in the biological matrices, hence, they achieve improved sensitivity. The following experiment shows clear evidence of ion-suppression reduction and improved sensitivity with Bond Elut Plexa PCX, mono-dispersed polymeric SPE.

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Materials and Methods

SPE reagents and solutions

2% H_3PO_4	Add 20 µL H_3PO_4 to 1 mL H_2O
2% formic acid	Add 20 µL formic acid to 1 mL H_2O
MeOH	Reagent grade or better
50:50 MeOH:ACN	Add 1 mL MeOH to 1 mL ACN
5% ammonia in 50:50 MeOH:ACN	Add 50 µL diluted NH_4OH to 1 mL 50:50 MeOH:ACN

SPE Method

All samples were processed by the same SPE method.

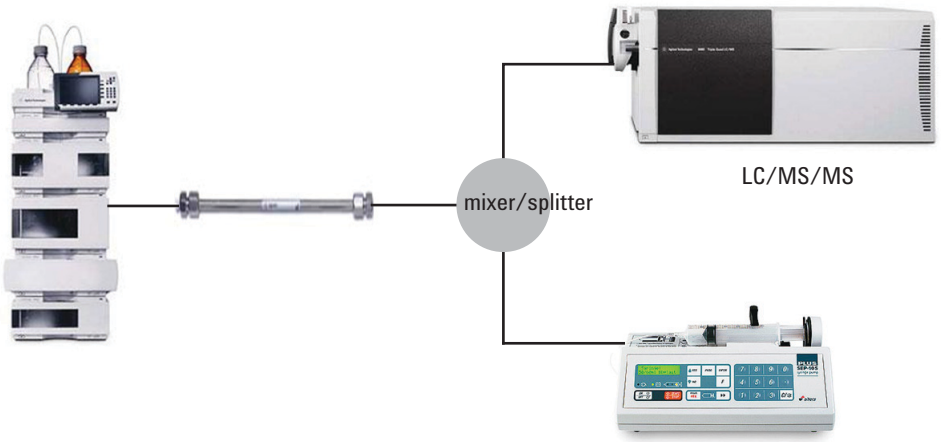
SPE products	Agilent Bond Elut Plexa PCX 96-well plate (10 mg) (p/n A4968010)
	Competitor W 96-well plate (10 mg)
	Competitor P 96-well plate (10 mg)
Sample	100 µL human plasma[1]
Pretreatment	Dilute with 300 µL 2% H_3PO_4
Conditions	1. 500 µL MeOH
	2. 500 µL H_2O
Load	400 µL diluted sample from pretreatment (actual plasma amount 100 µL)
Wash	1. 500 µL 2% formic acid
	2. 500 µL 50:50 ACN:MeOH
Elute	2 × 250 µL 5% ammonia in 50:50 ACN:MeOH

Experiment Design

For ion-suppression comparison, drug compound mixture (50 ng/mL) was continuously infused by a syringe pump at 20 µL/min while a blank plasma sample was injected. Blank plasma samples were prepared by Agilent Bond Elut Plexa PCX and two competitor's products based on the SPE methods specified in the previous section. MS transition 184 → 184 was selected for lipid contents monitoring during the analysis.

LC Conditions

Column	Agilent Poroshell 120 EC-C18, 2.1 × 5.0 mm, 2.7 µm (p/n 699775-902)	
LC/MS	Agilent 1260 Infinity LC/MS	
A	0.1% formic acid in H_2O	
B	0.1% formic acid in MeOH	
Flow rate	0.4 mL/min	
Injection volume	10 µL	
Gradient	Time (min)	%B
	0	10
	4.0	90
	4.1	10
	6.5	10
Temperature	sample (25 °C), column (ambient)	
Ion-source	ESI+ with JetStream	
Gas temperatue	350 °C	
Gas flow	10 L/min	
Nebulizer	35 psi	
Sheath gas temperature	400 °C	
Sheath gas flow	12 L/min	
Capillary	4000 V	



Injection of blank plasma.

Syringe pump: continuous infusion of drug mixture (50 ng/mL at 20 µL/min)

Figure 1. Schematic of ion-suppression comparison experiment setup.

1. For calibration and recovery, plasma was spiked with drug compounds of corresponding concentrations. For ion-suppression comparison, blank plasma samples were processed with SPE.

Table 1. Samples

	pKa	log P	MS/MS transition	Collision energy	Fragmentor
Acebutolol	9.40	1.71	337.2 → 116.1	20	128
Ranitidine	8.20	0.27	315.2 → 176.1	12	92
Nadolol	9.67	0.81	310.2 → 254.1	12	92
Atenolol	9.60	0.16	267.2 → 190.1	12	92
Propranolol	9.42	3.48	260.2 → 116.2	16	92
Procainamide	9.32	0.88	236.2 → 120.1	16	92
Metoprolol (ISTD)	9.70	1.90	268.2 → 116.2	16	92

Results and Discussion

Good separation and retention among all analytes were achieved and shown in Figure 2. Chromatograms shown in Figure 3 were obtained during continuous infusion of drug mixture with blank plasma sample injections processed by each SPE product. The data show clearly that Agilent Bond Elut Plexa PCX has reduced ion-suppression when compared to its competitive SPE products.

Excellent limit of detection (LOD) and limit of quantitation (LOQ) were achieved with Bond Elut Plexa PCX. A recovery experiment was performed at three different concentration levels (low, mid, and high, n = 6) and the data are shown in Table 1 with excellent recovery and % RSD. All compounds showed good linearity with correlation coefficients $R^2 \geq 0.995$ (Figure 4).

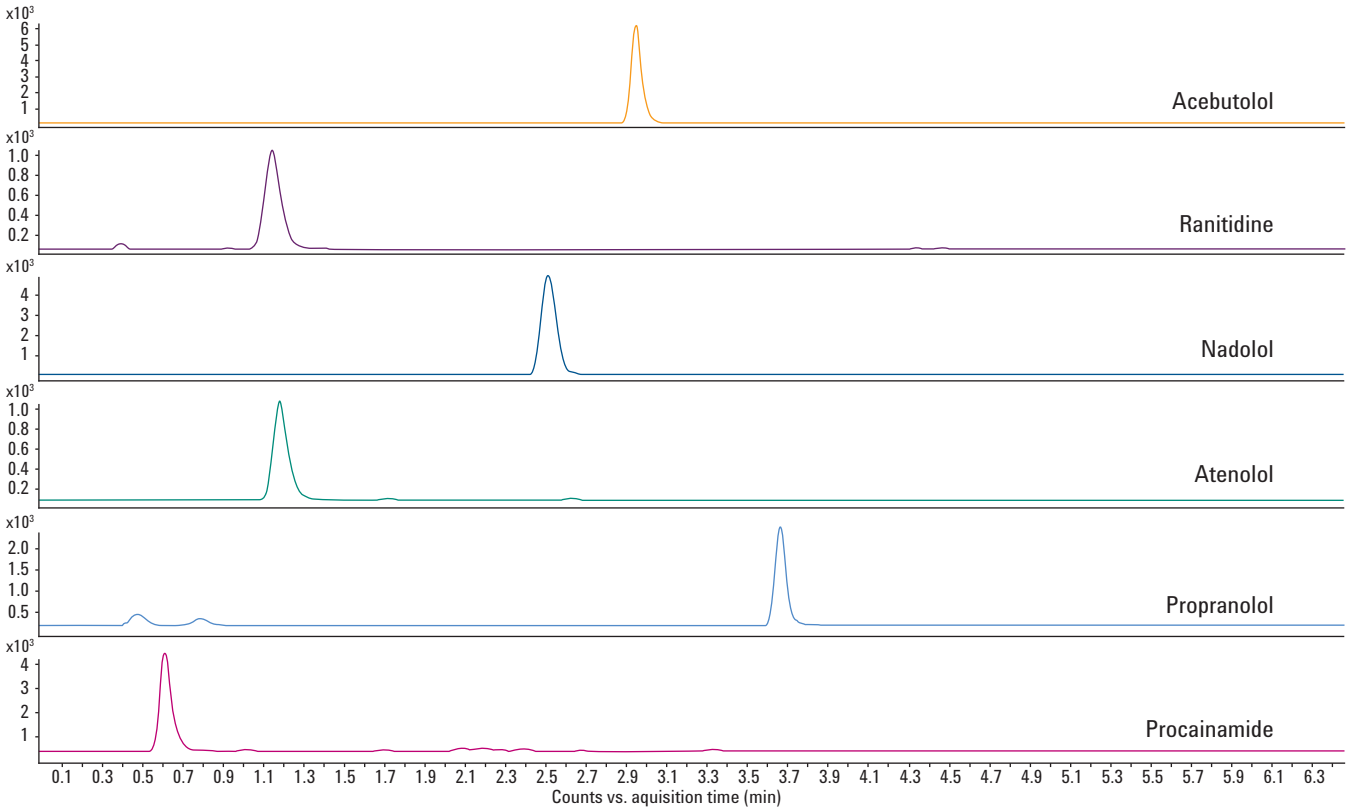


Figure 2. MS chromatogram of spiked plasma sample processed by Agilent Bond Elut Plexa PCX (5 ng/mL each).

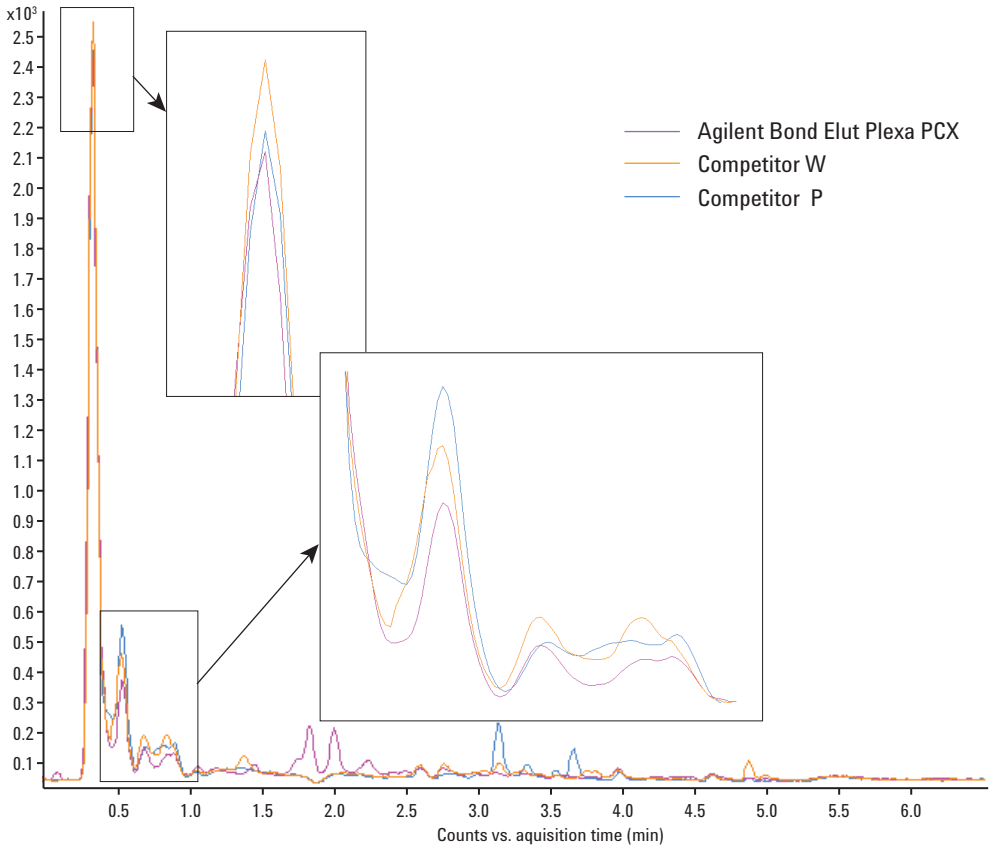
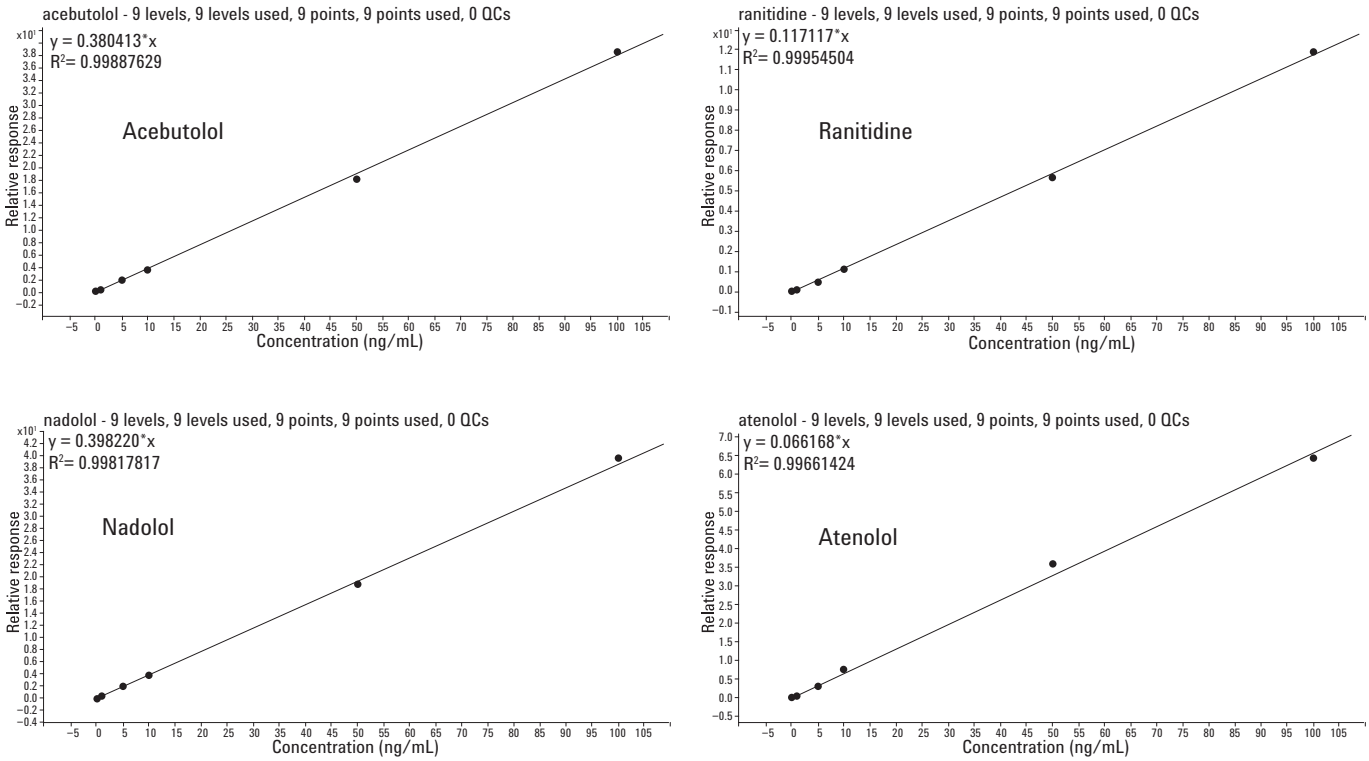


Figure 3. Lipid contents monitoring of blank plasma sample injection by 184 → 184 m/z transition.



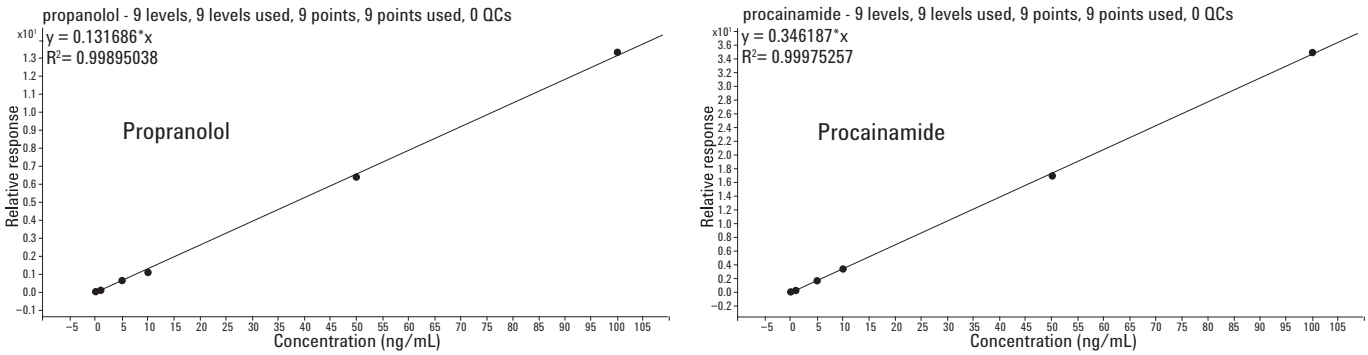


Figure 4. Calibration curves of six beta blockers at nine concentration levels (0.01, 0.05, 0.1, 0.5, 1, 5, 10, 50, and 100 ng/mL).

Table 2. Agilent Bond Elut Plexa PCX Data Summary

					5 ng/mL		50 ng/mL		100 ng/mL		
	pKa	log P	LOD (ng/mL)	LOQ (ng/mL)	Recovery	% RSD	Recovery	% RSD	Recovery	% RSD	Correlation coefficient, R^2
Atenolol	9.60	0.16	0.05	0.1	109.0	1.2	95.6	2.3	95.5	3.3	0.997
Nadolol	9.67	0.81	0.01	0.05	110.8	1.4	120.7	1.5	95.4	1.6	0.998
Acebutolol	9.40	1.71	0.01	0.1	113.9	0.9	108.6	2.0	98.7	2.4	0.999
Propranolol	9.42	3.48	0.05	0.1	120.2	1.1	103.5	2.7	93.6	2.5	0.999
Procainamide	9.32	0.88	0.05	0.1	93.0	2.1	104.5	1.8	96.9	3.9	1
Ranitidine	8.20	0.27	0.05	0.1	90.7	1.9	96.4	2.7	91.1	3.9	1

Conclusion

Agilent Bond Elut Plexa PCX showed reduced ion-suppression when compared to their competitive SPE products. Low LOD (0.01 – 0.05 ng/mL) and LOQ (0.05 – 0.5 ng/mL) were obtained resulted from minimized ion-suppression. Excellent correlation coefficients ($R^2 \geq 0.995$) and good recovery data were obtained with very good % RSD as well.

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