Reduced Ion Suppression and Improved LC/MS Sensitivity with Agilent Bond Elut Plexa

Application Note

Small Molecule Pharmaceuticals & Generics

Author

Mike Chang
Agilent Technologies, Inc.
25200 Commercentre Drive
Lake Forest, CA 92630
USA

Abstract

In a comparison of SPE products, Agilent Bond Elut Plexa polymeric SPE reduces ion suppression for better sensitivity and low LOD and LOQ. Beta blockers are extracted with excellent correlation coefficients, recoveries, and % RSD.

Introduction

Ion suppression is often an issue in bioanalysis by LC/MS, resulting in poor recovery and inaccuracy, as well as increased instrument maintenance cost and time. Ion suppression cannot be avoided completely when biological samples are handled. However, it should be avoided wherever possible.

Agilent Bond Elut Plexa has a hydroxylated surface whereas other competitor's products have amide residues on the surface of the sorbent. The presence of amide residue can cause increased interaction between the SPE sorbent and the endogenous materials in biological samples, which can be directly responsible for ion suppression during bioanalysis. Due to hydroxylation of the sorbent's surface, Bond Elut Plexa reduces the interaction between the sorbent and endogenous materials in biological matrices to improve sensitivity. In this example, we present clear evidence of ion-suppression reduction and improved sensitivity with Bond Elut Plexa mono-dispersed polymeric SPE. The sample was human plasma spiked with beta blockers.

Agilent Technologies

Materials and Methods

SPE reagents and solutions

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

SPE method

All samples were processed by the same SPE method.

SPE products	Agilent Bond Elut Plexa 96-well plate (10 mg) (p/n A4969010)
	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% aqueous ammonia
Condition	1. 500 µL MeOH
	2. 500 µL H_2O
Load	400 µL diluted sample from pretreatment (actual plasma 100 µL)
Wash	500 µL 5% MeOH
Elute	Twice with 250 µL 50:50 ACN:MeOH

Experiment set up

For ion-suppression comparison, a drug compound mixture (50 ng/mL) was continuously infused by syringe pump at 20 µL/min. The blank plasma sample was injected. Blank plasma samples were prepared by Agilent Bond Elut Plexa and two other competitor's products based on the SPE methods specified above. MS transition 184 → 184 *m/z* was selected for lipid content monitoring during the analysis. Figure 1 shows the experimental set up.

Injections of the following
1. Blank plasma
2. Mobile phase (or MeOH)

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

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

LC Conditions

Column	Agilent Poroshell 120 EC-C18, 2.1 mm × 5.0 mm, 2.7 µm (p/n 699775-902)
Instrument	Agilent 1260 Infinity LC/MS
Mobile phase A	0.1% formic acid in H_2O
Mobile phase 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 temperature	350 °C
Gas flow	10 L/min
Nebulizer	35 psi
Sheath gas temperature	400 °C
Sheath gas flow	12 L/min
Capillary	4000 V

Samples

Beta blocker	pKa	log P	MS/MS transition	Collision energy	Fragmentor
Acebutolol	9.40	1.71	337.2 → 116.1	20	128
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
Pindolol	9.25	1.75	249.2 → 116.1	12	92
Metoprolol (ISTD)	9.70	1.90	268.2 → 116.2	16	92

Results and Discussion

Good separation and retention between all analytes were obtained, as shown in Figure 2. Reduced ion-suppression delivered higher sensitivity resulted, as demonstrated in Figure 3. The figure shows an overlay of extracted MS chromatograms for nadolol from all three SPE products when blank plasma sample was injected and continuous infusion of drug mixture were executed simultaneously. The signal from Bond Elut Plexa was higher than from the other products during most of the analysis. Figure 4 shows an overlay of extracted MS/MS chromatogram 184 → 184 *m/z* for all three SPE products. The data show clearly that Bond Elut Plexa has reduced ion-suppression when compared to the others.

[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.

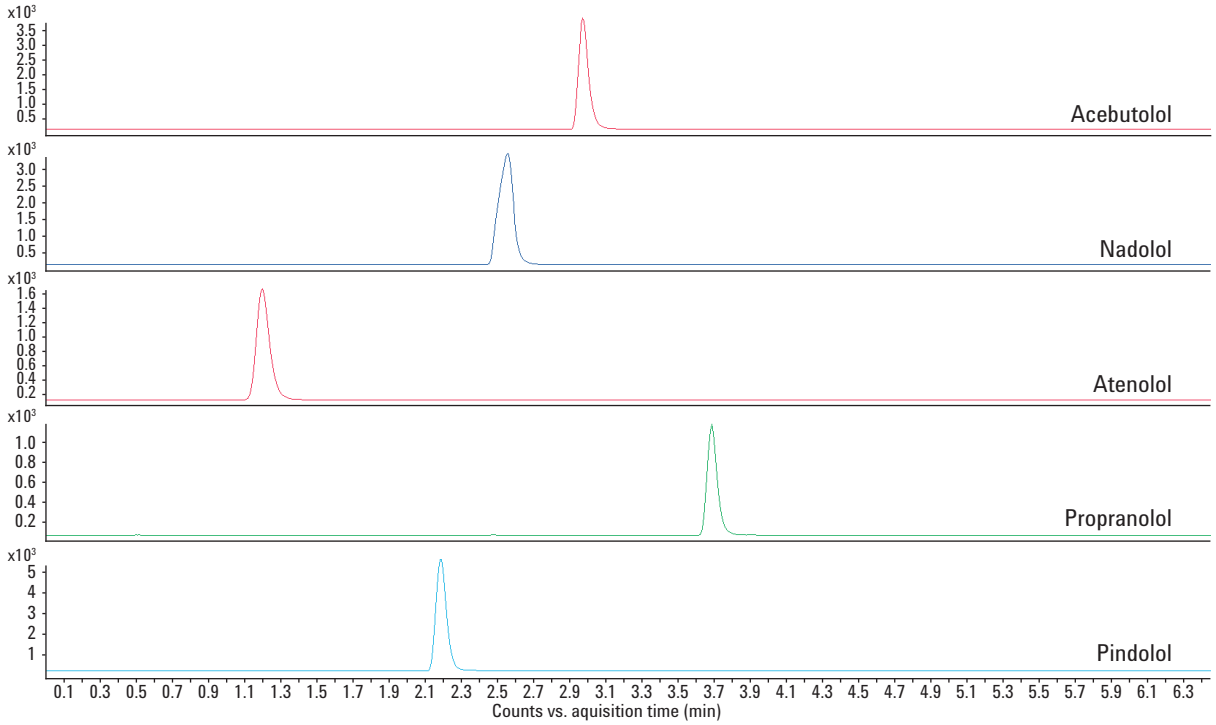

Figure 2. MS chromatogram of spiked plasma sample processed by Agilent Bond Elut Plexa (5 ng/mL of each analyte).

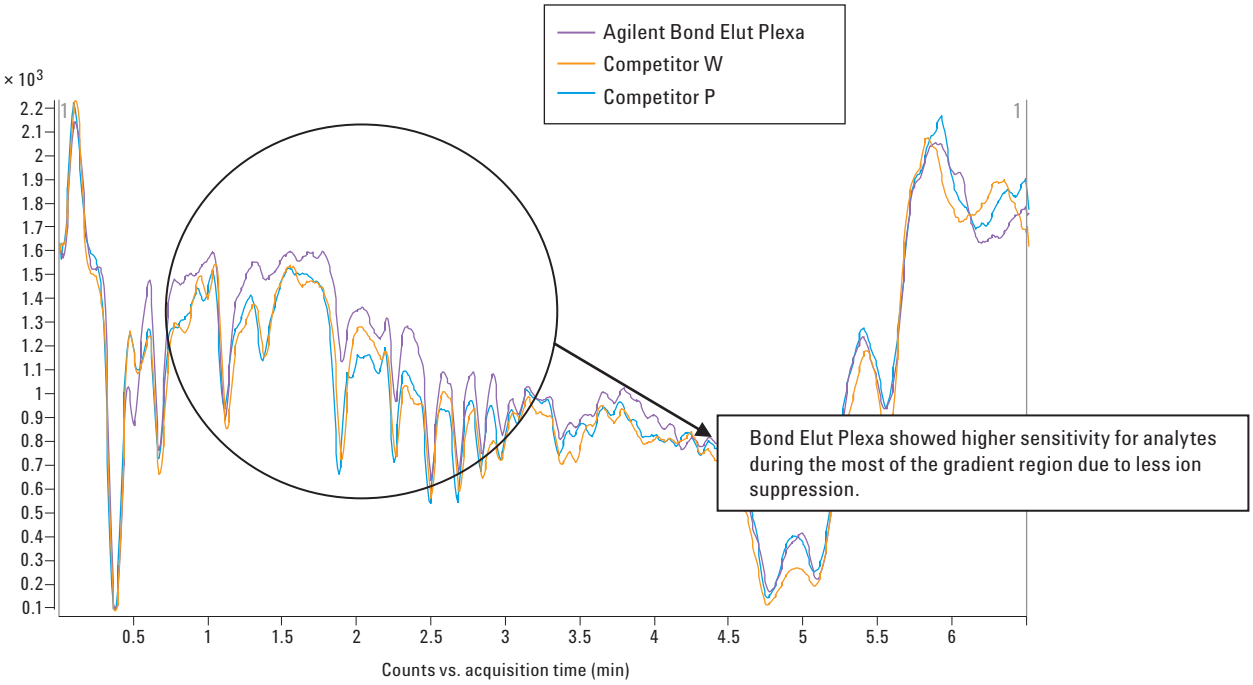

Figure 3. Overlay of the nadolol signal from simultaneous injection of blank plasma sample and continuous infusion of drug mixture, showing the superiority of Agilent Bond Elut Plexa.

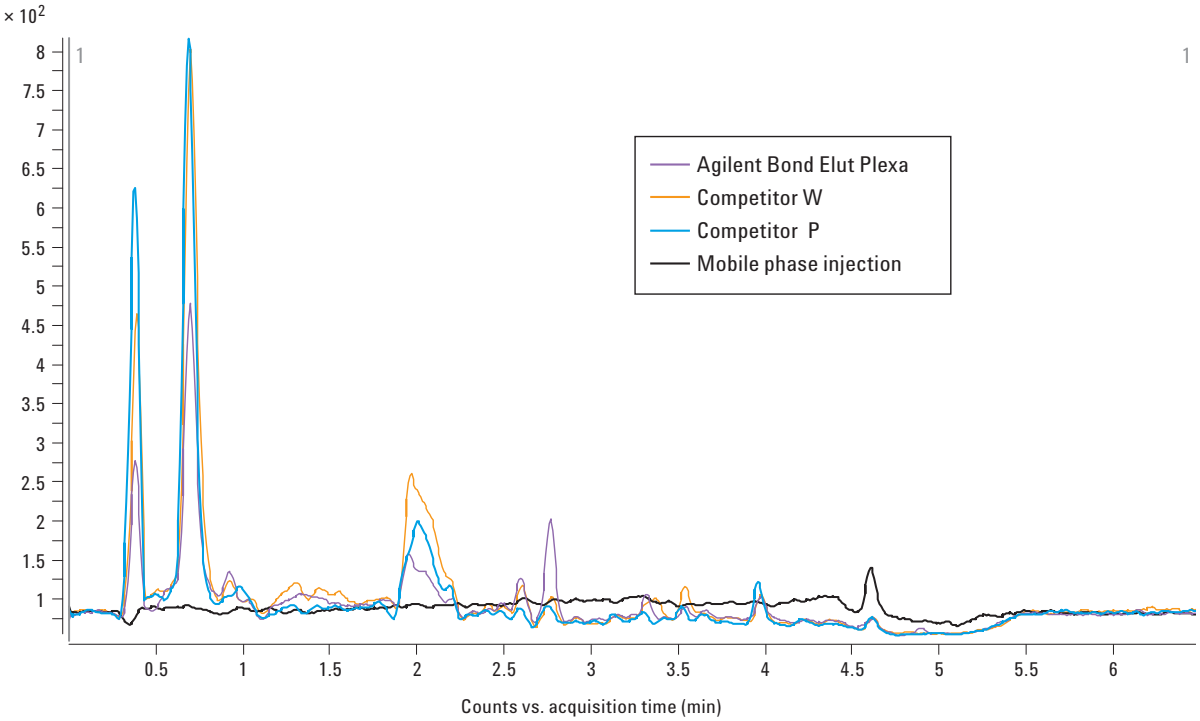

Figure 4. Lipid content monitoring of blank plasma sample injection by 184 → 184 m/z transition, with reduced ion suppression using Agilent Bond Elut Plexa.

Bond Elut Plexa achieved excellent limit of detection (LOD ≤ 0.05 ng/mL) and limit of quantitation (LOQ ≤ 0.5). A recovery experiment was performed at three different concentration levels (low, mid, and high, n = 6) and the data are summarized in Table 1, with excellent recovery and % RSD. Calibration curves were created with nine concentration levels (0.01 – 100 ng/mL) and all compounds showed good linearity with correlation coefficients $R^2 \geq 0.995$ (Figure 5).

Table 1. Results of a Recovery Experiment using AgilentBond Elut Plexa

					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
Acebutolol	9.40	1.71	0.01	0.05	79.3	0.5	84.9	0.7	97.0	0.4	0.996
Nadolol	9.67	0.81	0.01	0.05	98.5	0.8	94.7	1.4	108.1	0.8	0.997
Atenolol	9.60	0.16	0.05	0.5	119.7	2.9	104.0	2.5	109.0	4.5	1.000
Propranolol	9.42	3.48	0.05	0.5	106.2	3.7	109.9	7.3	126.9	9.7	0.995
Pindolol	9.25	1.75	0.01	0.05	111.6	1.3	106.0	3.0	115.1	2.8	0.998

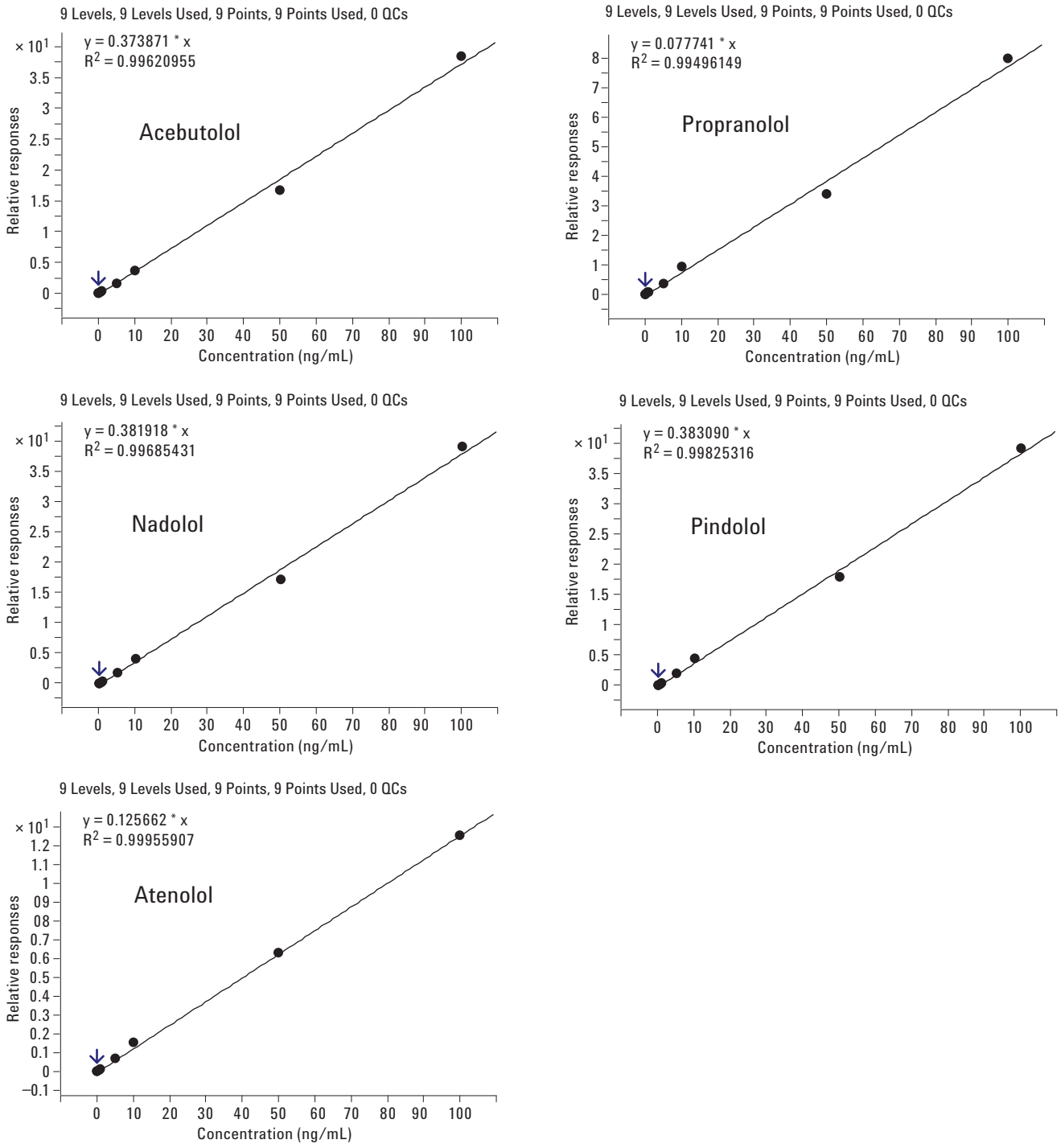

Figure 5. Calibration curves of five beta blockers at nine concentration levels (0.01, 0.05, 0.1, 0.5, 1, 5, 10, 50, and 100 ng/mL).

Conclusion

Agilent Bond Elut Plexa reduced ion suppression when compared to other SPE products, resulting in better sensitivity. The improved sensitivity delivered low LOD (0.01 – 0.05 ng/mL) and LOQ (0.05 – 0.5 ng/mL). In addition, excellent correlation coefficients ($R^2 \geq 0.995$) and good recovery data were obtained with very good % RSD.

For More Information

For more information on our products and services, visit our Web site at www.agilent.com/chem.

www.agilent.com/chem

Printed in the USA
December 1, 2013
5990-8388EN

Agilent Technologies