Reduced Ion-suppression in Bioanalysis by Liquid Chromatography Mass Spectrometry Applying Specially Treated Solid Phase Extraction

Mike Chang, Paul Boguszewski, and Joan Stevens
Agilent Technologies Inc., 25200 Commercentre Drive, Lake Forest, CA 92630

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Agilent Technologies

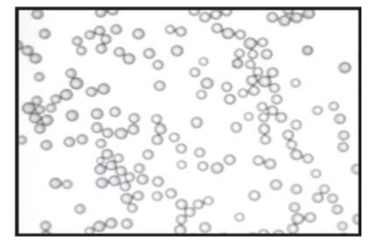
Introduction

Ion-suppression in bioanalysis has been a great challenge to overcome for many scientists handling biological samples with liquid chromatography mass spectrometry. Endogenous materials from biological samples often make a large contribution to ion-suppression leading to poor recovery, unreliable reproducibility, inaccuracy, and increased instrument maintenance time.

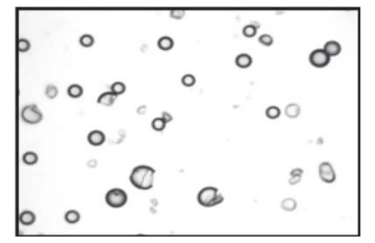
Specially treated surface via hydroxylation on solid phase extraction (SPE) sorbent minimizes attraction of endogenous materials in the biological sample to the sorbent compared to different chemistries such as amide in other SPE sorbents. Amide residues on the surface of the SPE sorbent tend to attract the endogenous materials from the biological samples and bound form of the endogenous interferences are directly responsible for ion-suppression in bioanalysis by liquid chromatography mass spectrometry. Reduction of the interaction between endogenous materials and SPE sorbent resulted in reduced ion-suppression.

The unique chemistry of hydroxylated, spherical, and mono-dispersed polymer based Bond Elut Plexa and Bond Elut Plexa PCX are ideal SPE for non-polar and basic compounds, respectively.

Superior performance in ion-suppression reduction by hydroxylated SPE 96-well plate is demonstrated with good linearity in calibration curves, excellent recovery, reproducibility, and accuracy.



Bond Elut Plexa and Bond Elut Plexa PCX
(Spherical and mono-dispersed polymer)



Competitor SPE polymer image
(Irregular shape and poly-dispersed polymer)

Experimental

Sample Preparation Method

For ion-suppression comparison experiment, blank plasma samples were prepared by the SPE method described below.

	Bond Elut Plexa and its competitors	Bond Elut Plexa PCX and its competitors
Pretreatment	Dilute human plasma 1:3 with 2% aqueous ammonia	Dilute human plasma 1:3 with 2% aqueous H_3PO_4
Condition	500 µL MeOH	500 µL MeOH
	500 µL H_2O	500 µL H_2O
Wash	500 µL 5% MeOH	500 µL 2% formic acid
		500 µL 50:50 MeOH:ACN
Elute	2 X 250 µL 50:50 MeOH:ACN	2 X 250 µL 5% ammonia in 50:50 MeOH:ACN

Table 1. SPE method for Bond Elut Plexa and Bond Elut Plexa PCX plus their corresponding competitor products

Experimental (contd.)

Post-column Infusion Experiment

While injections of blank plasma were done, syringe pump continuously infused solutions containing analytes and the stream of infusion was mixed with injections by a mixer located after Poroshell column.

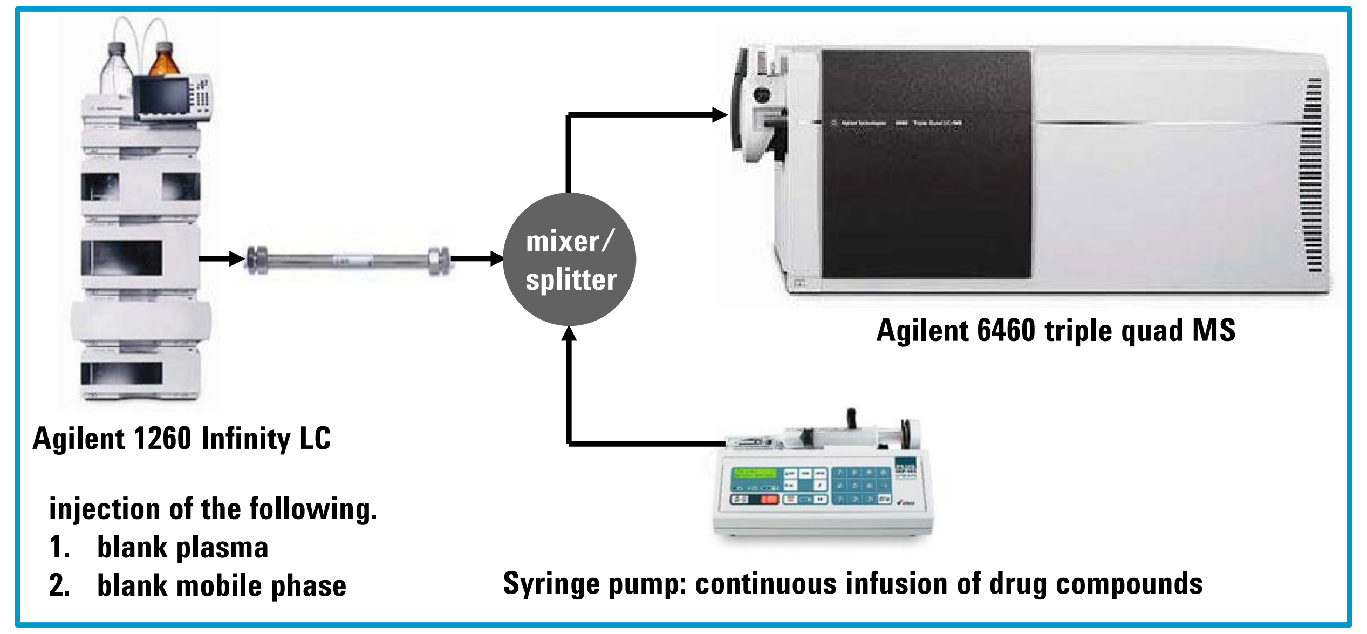


Fig 1. Schematic of post-column infusion experiment

	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
Pindolol	9.25	1.75	249.2 → 116.1	12	92
Metoprolol (ISTD)	9.70	1.90	268.2 → 116.2	16	92

Table 2. Compound list for analysis

LC/MS Conditions

Column: Agilent Poroshell 120 EC-C18, 2.1 mm X 5.0 mm, 2.7 µm (p/n 699775-902)
LC/MS system: Agilent 1260 Infinity LC coupled with 6460 triple quad MS
A: 0.1% formic acid in H_2O
B: 0.1% formic acid in MeOH
Injection volume: 10 µL
Gradient: Ramp 10 – 90% B in 4 min, back to 10% B in 0.1 min, hold at 10% B for 2.4 min
Temperature: sample (25 °C), column (ambient)
Ion-source: ESI+ with JetStream
Gas temp.: 350 °C
Nebulizer: 35 psi
Sheath gas temp.: 400 °C
Capillary: 4000 V

Results and Discussion

Bond Elut Plexa PCX (cation exchange mechanism) – LC/MS chromatograms, detection limits, recovery data with % RSD (n=6), and correlation coefficients, R^2

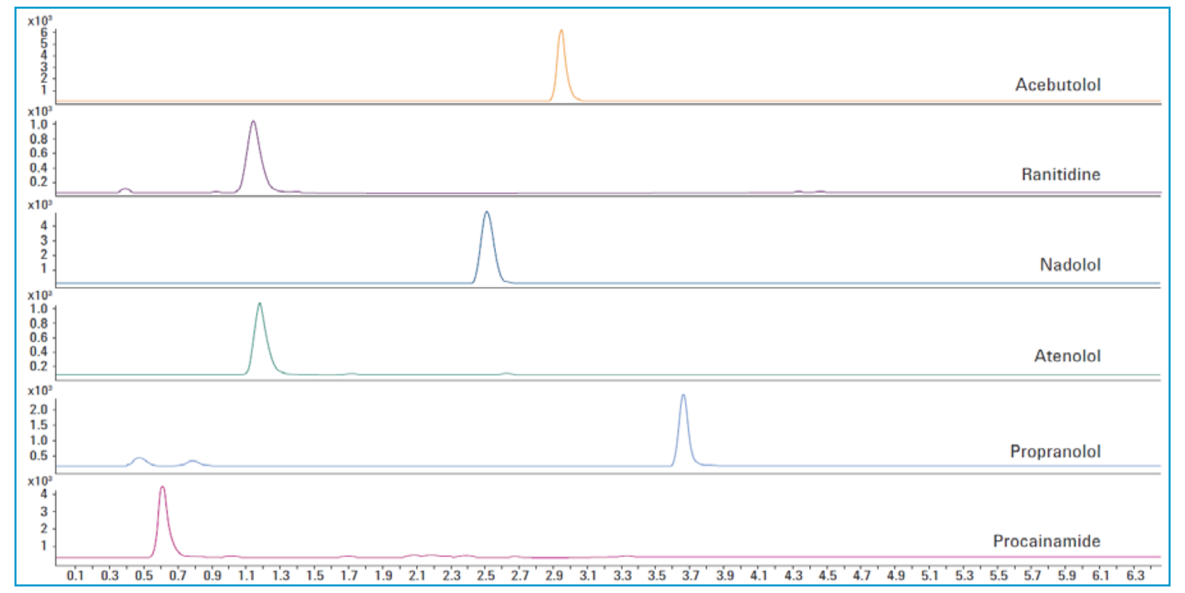


Fig 2. LC/MS chromatogram of spiked human plasma sample processed by Agilent Bond Elut Plexa PCX

	LOD (ng/mL)	LOQ (ng/mL)	5 ng/mL		50 ng/mL		100 ng/mL		R^2
			Recovery	% RSD	Recovery	% RSD	Recovery	% RSD	
Atenolol	0.05	0.1	109.0	1.2	95.6	2.3	95.5	3.3	0.997
Nadolol	0.01	0.05	110.8	1.4	120.7	1.5	95.4	1.6	0.998
Acebutolol	0.01	0.1	113.9	0.9	108.6	2.0	98.7	2.4	0.999
Propranolol	0.05	0.1	120.2	1.1	103.5	2.7	93.6	2.5	0.999
Procainamide	0.05	0.1	93.0	2.1	104.5	1.8	96.9	3.9	1
Ranitidine	0.05	0.1	90.7	1.9	96.4	2.7	91.1	3.9	1

Table 3. Summary of detection limits, recovery with % RSD (n=6), and correlation coefficient data for Bond Elut Plexa PCX (All samples were spiked in human plasma.)

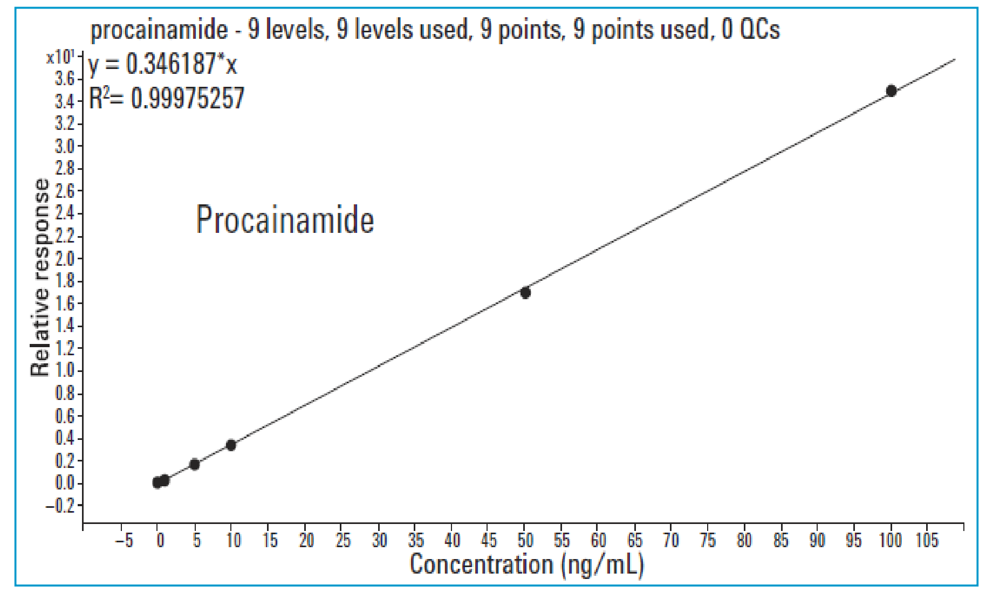


Fig 4. Calibration curve of procainamide in human plasma by Bond Elut Plexa PCX (Other analytes also showed superb calibration curves. See R^2 values in Table 3.)

Bond Elut Plexa (non-polar interaction mechanism) – LC/MS chromatograms, detection limits, recovery data with % RSD (n=6), and correlation coefficients, R^2

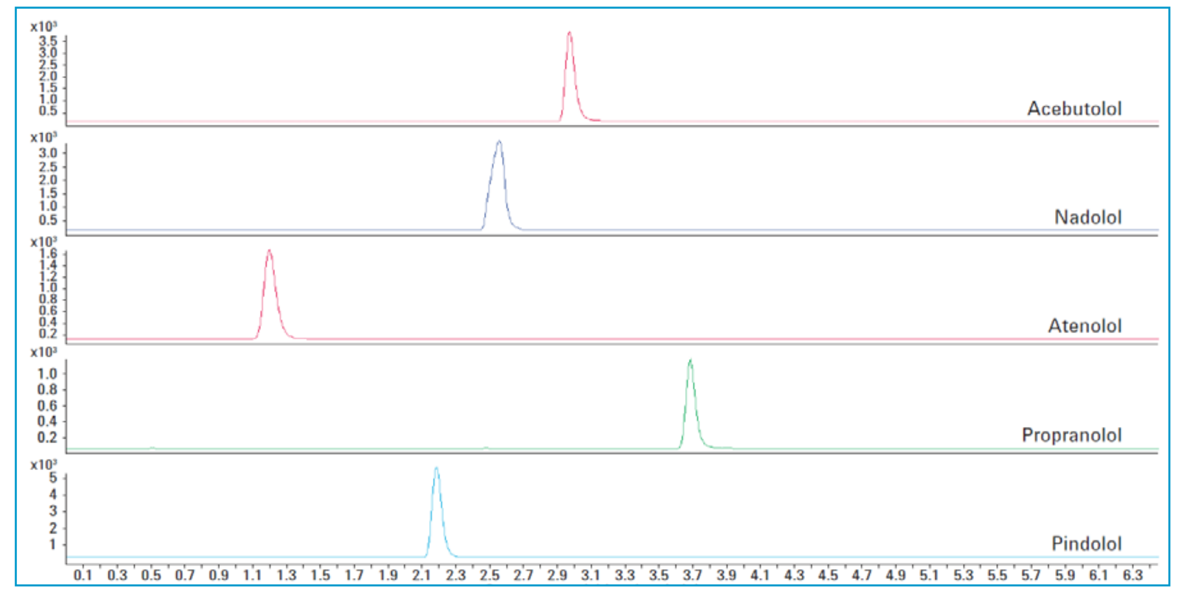


Fig 3. LC/MS chromatogram of spiked human plasma sample processed by Agilent Bond Elut Plexa

	LOD (ng/mL)	LOQ (ng/mL)	5 ng/mL		50 ng/mL		100 ng/mL		R^2
			Recovery	% RSD	Recovery	% RSD	Recovery	% RSD	
Acebutolol	0.01	0.05	79.3	0.5	84.9	0.7	97.0	0.4	0.996
Nadolol	0.01	0.05	98.5	0.8	94.7	1.4	108.1	0.8	0.997
Atenolol	0.05	0.5	119.7	2.9	104.0	2.5	109.0	4.5	1
Propranolol	0.05	0.5	106.2	3.7	109.9	7.3	126.9	9.7	0.995
Pindolol	0.01	0.05	111.6	1.3	106.0	3.0	115.1	2.8	0.998

Table 4. Summary of detection limits, recovery with % RSD (n=6), and correlation coefficient data for Bond Elut Plexa (All samples were spiked in human plasma.)

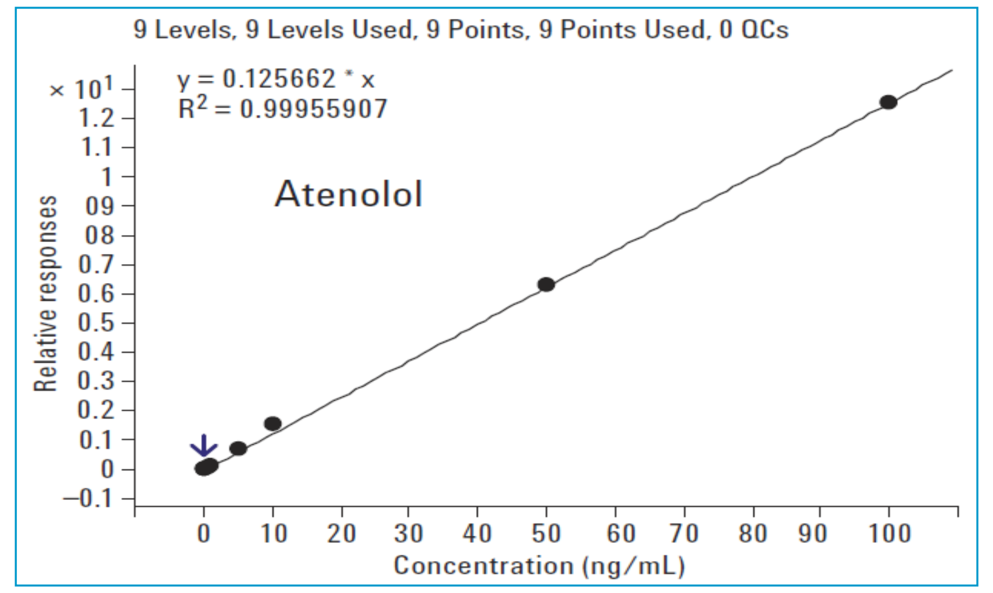


Fig 5. Calibration curve of atenolol in human plasma by Bond Elut Plexa PCX (Other analytes also showed superb calibration curves. See R^2 values in Table 4.)

Direct lipid trace monitoring data during LC/MS analysis

These are lipid content MS intensity during analysis. **Lower lipid signal = less interference = better analyte signal !!!**

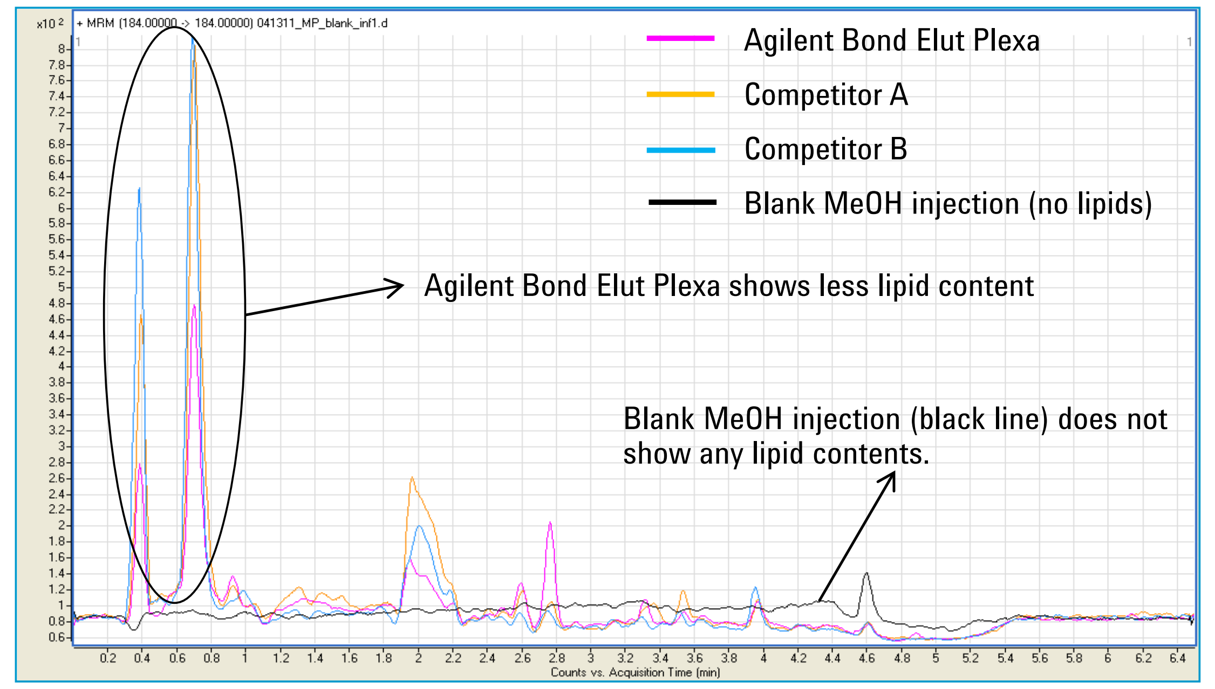


Fig 6. Lipid traces monitored by 184 → 184 m/z MS transition in blank plasma samples process by Bond Elut Plexa and its competitors

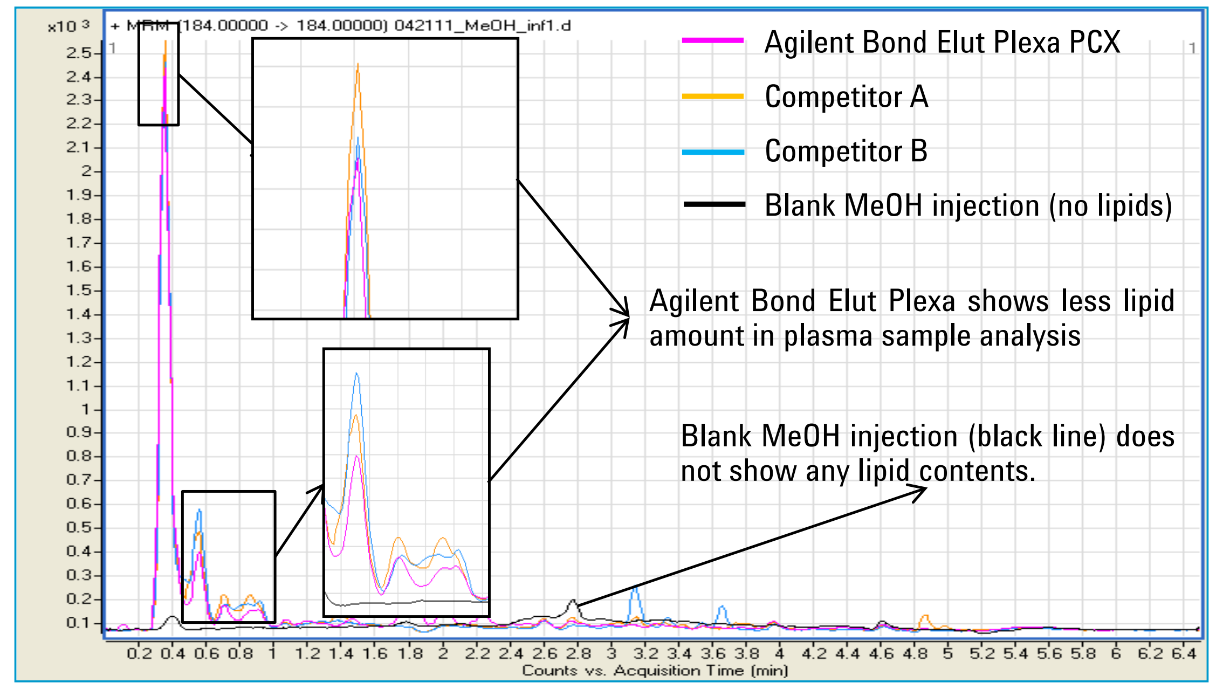


Fig 7. Lipid traces monitored by 184 → 184 m/z MS transition in blank plasma samples process by Bond Elut Plexa PCX and its competitors

Non-polar mechanism SPE	MS area count of Nadolol (5ng/mL spiked in plasma)
Bond Elut Plexa	**2010**
Competitor A	1548
Competitor B	1554

Cation exchange mechanism SPE	MS area count of Propranolol (5ng/mL spiked in plasma)
Bond Elut Plexa PCX	**9708**
Competitor A	8112
Competitor B	6974

Table 5. Some examples of MS area count comparison Bond Elut Plexa, Bond Elut Plexa PCX, and their corresponding competitor products

Conclusions

- Both Bond Elut Plexa and Bond Elut Plexa PCX showed excellent detection limits, recovery with great % RSD (n=6), and correlation coefficient, R^2 as summarized in Table 3 and 4.
- Being amide-free on the surface of the SPE sorbent led to minimum interference between the sorbent and the endogenous materials, hence, less ion-suppression was experienced during LC/MS analysis.
- Better LC/MS sensitivity was achieved with reduced ion-suppression.

References

- Agilent application note: 5990-8388EN
- Agilent application note: 5990-8400EN