A New Kind of Amide-free Polymer for Simplified, Improved SPE

Rich Motyka, Arnie Aistars, William C Hudson, David Jones

[1]Agilent Technologies, 25200 Commercentre Drive, Lake Forest, CA 92630, USA

Agilent Technologies

Abstract

A new polymer for SPE, Bond Elut Plexa, based on a water-wettable amide-free surface, allows improved bioanalysis due to a unique approach to matrix removal. Plexa technology offers a highly hydrophilic surface which excludes proteins and lipophilic interferences from binding. A unique phase transfer system based on a polarity gradient within the polymer bead allows analyte binding at the core of the particle. This provides improved clean up directly at the load step of SPE, improving overall sensitivity and simplifying method development. A broad range of drugs was screened with a general method to prove efficacy over a large range of analyte pKa and log P. Results are compared to traditional polymeric SPE with respect to recovery and cleanliness.

Additionally, analytical methods were developed for extracting Propranolol and Metoprolol out of 100 µL of human plasma. A working calibration range of 1.0 – 1000 ng/mL was established with good linearity over the entire working range. Precision was determined to be 4% at the mid level concentration range. Method accuracy was measured to the calibration curve with deviations less than 10%. Absolute recoveries of the analytes were measured against a mobile phase standard to compare any loss in sensitivity and recoveries greater than 90% were achieved. Ion suppression data is also presented to confirm the removal of proteins and lipids which interfere with sample ionization.

Method

Normally recoveries are measured against an extracted calibration curve when performing analysis. As long as the linearity is good recoveries will always be nominal. However when the extracted sample response was compared to a spiked mobile phase the recoveries were much lower. Sample loss is thought to be due to poor retention or irreversible binding, but extract cleanliness can also cause loss of signal. Proteins and other indigenous matrix interferences will compete with the sample for ionization in the Electro spray source. In order to determine the actual response loss the signal loss due to ion suppression was calculated by analyzing a mobile phase spike compared to a non-extracted standard (NEX).

Any loss in signal is attributed to ion suppression effects from protein or fats in the sample extract and not problems with the sorbent extraction mechanism since none of the drug passed through the sorbent bed.

Mobile phase std – 10 µL of 500 ng/mL compound mix is added to 80 µL of 80:20 0.1% formic acid. (Final volume = 100 µL)

NEX – 100 µL of Human Plasma is extracted per SPE method. Post extraction it is spiked with 10 µL of 500 ng/mL compound mix.

MS Parameters

- ➔ Argon was used as the collision gas
- ➔ Compound structures, collision energies and capillary inlet voltage are shown in Figure 1.

Compound	Transition	Collision Energy (eV)	LogP
Metoprolol	268.2 → 116.0	-15.0	2.5
Propranolol	260.0 → 116.0	-13.5	3.6

Figure 1. *Compounds*

Method

Sample:	100 µL Human plasma
Pretreatment:	Dilute 1:3 w/ 200 mM NH_4Ac pH 10
Conditioning:	1. 150 µL MeOH 2. 150 µL H_2O
Washes:	1. 150 µL 2 x 95:5 H_2O:MeOH
Elution:	2 x 100 µL MeOH

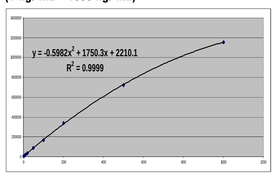
Liquid Chromatography

Column:	Agilent Pursuit C18 3 µm, 2.0 x 50 mm
Mobile Phase:	A: 0.1% Formic acid B: 100% Methanol mixed through a high pressure static mixer with a 10 µL internal volume
LC Gradient:	t_0 to 0.5 min 80% A, 20% B t = 1.5 to 3.0 min 20% A, 80% B t = 3.5 to 5.0 min 80% A, 20% B
Injection Volume:	10 µL
Total Run Time:	4.5 min

Figure 2. *Compounds*

All samples were evaporated to dryness and reconstituted with 100 µL of mobile phase.

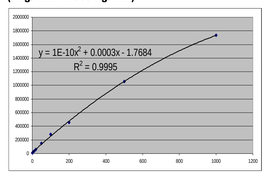
Calibration curve for metoprolol on Plexa (1 ng/mL – 1000 ng/mL)



Recoveries

Conc	% Rec	RSD(n=6)
0.5 µg/mL	100%	5.5%
1.0 µg/mL	100%	5.4%

Calibration curve for propranolol on Plexa (1ng/mL – 1000 ng/mL)



Recoveries

Conc	% Rec	RSD(n=6)
0.5 µg/mL	102%	6.2%
1.0 µg/mL	101%	6.2%

Figure 3. *Calibration curves and recoveries*

Good linearity was achieved on both analytes over a wide linear range (1.0 ng/mL to 1000 ng/mL). Recoveries were calculated versus the standard curve.

Abs recoveries vs mobile phase std, metoprolol

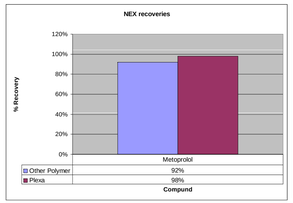
	Plexa (n=6)		Other polymer	
Conc	rec (%)	RSD (%)	rec (%)	RSD (%)
5	89	9	83	5
10	81	7	69	5
20	77	18	87	4
50	95	15	79	3
100	83	6	57	5
200	92	5	74	3
500	97	5	80	4

Abs recoveries vs mobile phase std, propranolol

	Plexa (n=6)		Other polymer	
Conc	rec (%)	RSD (%)	rec (%)	RSD (%)
5	77	9	27	11
10	62	7	24	31
20	56	7	25	12
50	59	8	28	14
100	55	4	32	14
200	55	3	35	10
500	58	4	32	12

Figure 4. *Absolute recovery at different concentrations*

Analyte recoveries were calculated using a spiked mobile phase for the standard. But when sample response is measured against a spiked mobile phase the recoveries are much lower.



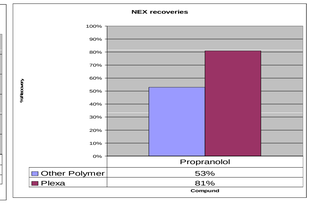
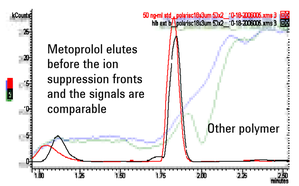


Figure 5. *Recoveries of NEX samples versus the mobile phase standards*

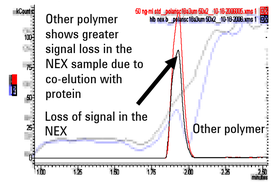
Metoprolol experienced little sample loss.

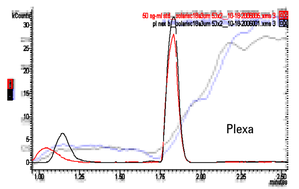
Propranolol experienced significant sample loss in the competitive polymer.

Metoprolol



Propranolol





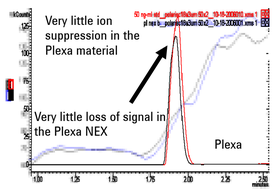


Figure 6. *Ion suppression chromatograms and signal loss*

Overlapped chromatograms

Ion suppression - mobile phase blank is overlapped with a blank extract. A 10 µg/mL drug standard is infused directly into the mass spec simultaneously with sample elution

Analyte chromatograms - mobile phase spike is overlapped with NEX spike to demonstrate loss of response and co-elution with ion suppression front.

Conclusions

1) Good recoveries were easily achieved with standard calibration curves but true signal loss and sensitivity were not demonstrated. Any reduced signal due to native matrix interferences were normalized out the analyses (Figure 3).

2) When extracted samples were compared to spiked mobile phase standards a significant loss in response was seen (Figure 4).

3) The loss of response due to poor sample cleanliness can be seen in the NEX recoveries (Figure 5). All analytes were spiked post extraction of 100 µL human plasma. Drug standards were not extracted through the sorbent bed. Poor recoveries could only be due to matrix interferences in the ESI source and not extraction efficiency.

4) Signal loss was further correlated with ion suppression chromatograms overlaid with NEX chromatograms (Figure 6). Metoprolol experiences little signal loss and clearly elutes before the ion suppression caused by plasma proteins. Propranolol, which suffers much greater signal loss directly co-elutes with the plasma proteins.

5) Bond Elut Plexa did a much better job of removing these sample proteins and generating cleaner extracts. The unique hydrophilic pore gradient allowed Plexa to extract the drugs of interest without extracting unwanted proteins.

6) Better propranolol response was achieved through cleanliness of the extract.

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