Generic method recommendations

	Acids	Neutrals		Bases
Analyte	LogP>1.0 pK_a < 5	LogP > 1.5 pK_a 3-5	 pK_a 6-10	LogP > 0.8 pK_a 6-10
	Plexa PAX	**Plexa** (Acid load method)	**Plexa** (Base load method)	**Plexa PCX**
Sample Treatment	2% NH_4OH	1% HCO_2H	2% NH_4OH	2% H_3PO_4
Sorbent Condition	100% MeOH	100% MeOH		100% MeOH
Equilibration	100% H_2O	100% H_2O		100% H_2O
Wash	100% H_2O	5% MeOH in H_2O		2% HCO_2H in H_2O
Elution 1	100% MeOH *Neutrals*	100% MeOH *Neutrals*		1:1 MeOH/ACN *Acids, Neutrals*
Elution 2	5% HCO_2H in MeOH *Acids*			5% NH_3 in 1:1 MeOH/ACN *Bases*

Note: This user guide is a convenient starting point for any SPE method development. Further optimization may be required to adjust the method to your application needs.

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Bond Elut Plexa SPE method guide

Accuracy Begins Here

The Bond Elut Plexa Family is a new generation of polymeric SPE products, designed for simplicity, improved analytical performance and ease-of-use.These advanced SPE sorbents offer excellent flow characteristics due to their monodisperse particle size distribution, affording superior ease-of-use, with minimal clogging of the packed bed.

Optimized surface chemistries and extraction protocols deliver ultra clean extracts with minimized ion suppression.

The Measure of Confidence

Agilent Technologies

Method development and troubleshooting for plasma samples

Bond Elut Plexa PAX

Bond Elut Plexa PAX contains a strong anion exchange functionality. Simple generic methodology and excellent batch to batch reproducibility offer robust anion exchange SPE workflow.

Strong Anion Exchange SPE for Acidic Analytes	
SPE device Pre-conditioning	1. 500 µL MeOH 2. 500 µL H_2O
Sample	100 µL Plasma
Pretreatment	Dilute 1:3 with: 2% NH_4OH in H_2O
Washes	1. 500 µL H_2O 2. 500 µL MeOH
Elution	2 x 250 µL 5% HCO_2H in MeOH

Volumes stated for all methods are for a 30 mg, 1 mL SPE format device.

pH adjustment – To improve ion exchange interactions on Plexa PAX, ionize analytes prior to loading. For acidic analytes the pH should be at least 2 pH units above the pK_a.

Bond Elut Plexa

Bond Elut Plexa is a non-polar divinylbenzene-based neutral polymeric sorbent. This sorbent is the best choice for non-ionic extraction of a wide range of acidic, neutral and basic analytes from different matrices.

Non-Polar SPE for neutrals and moderately acidic or basic analytes	
SPE device Pre-conditioning	1. 500 µL MeOH 2. 500 µL H_2O
Sample	100 µL Plasma
Pretreatment	Dilute 1:3 with: 2% NH_4OH *(neutrals and bases)* 1% HCO_2H in H_2O *(acids)*
Washes	500 µL 5 % MeOH in H_2O
Elution	2 x 250 µL MeOH

pH adjustment – To improve hydrophobic interaction on Plexa, neutralize analytes prior to loading. Basic analytes should be at least 2 pH units above the pK_a. Acidic analytes should be 2 pH units below the pK_a.

Bond Elut Plexa PCX

Bond Elut Plexa PCX is a cation exchanger with mixed mode sorbent characteristics and is therefore suitable for the extraction and clean-up of polar and non-polar bases from biofluids.

Strong Cation Exchange SPE for Basic Analytes	
SPE device Pre-conditioning	1. 500 µL MeOH 2. 500 µL H_2O
Sample	100 µL Plasma
Pretreatment	Dilute 1:3 with: 2% H_3PO_4 in H_2O
Washes	1. 500 µL 2% HCO_2H in H_2O 2. 500 µL MeOH:ACN (1:1, v/v)
Elution	2 x 250 µL 5% NH_3 (28-30%) in MeOH: ACN (1:1, v/v)

pH adjustment – To improve ion exchange interactions on Plexa PCX, ionize analytes prior to loading. Basic analytes should be at least 2 pH units below the pK_a. Acidification is also necessary to disrupt analyte-protein interaction.

Troubleshooting	Bond Elut Plexa	Bond Elut Plexa PCX	Plexa PAX
Analyte(s) eluting in the wash step(s)	• Reduce volume of washing step • Reduce concentration of organics in the wash step		
	• Rinse with either 2% NH_3 for basic analytes or 1% formic acid for acids to ensure hydrophobic interactions • Increase sorbent bed mass	• Increase sorbent bed mass for increased ion exchange capacity	
Inadequate Elution (Eluent does not contain >90% of the analyte.)	• Decrease flow rate, (1 mL/min is recommended) • Check solubility of analyte in the eluent • Increase strength of elution solvent • Increase the eluent volume or use multiple aliquots of eluent		
	• Add modifier (depending on analyte type) to the elution solvent, thereby promoting ionization	• Use up to 10% ammonia (28-30%) in solvents such as MeOH and ACN	• Use up to 10% formic acid in MeOH for anion exchange elution