User Guide

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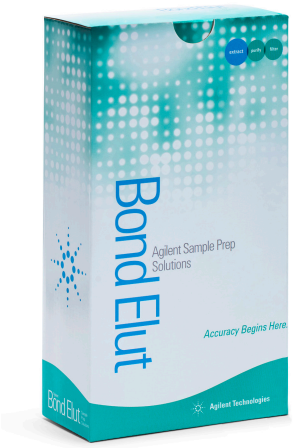
Trusted Answers

Agilent Bond Elut PPL



Introduction

Agilent Bond Elut PPL is a proprietary modified styrene divinylbenzene polymer that is stable at pH extremes and compatible with a wide range of solvents. It retains a broad range of targets from polar to nonpolar analytes. The PPL sorbent provides good retention for relative polar compounds, and the large particle size of 125 µm allows ease of flow for viscous or water samples with particulates. This user guide provides method development and optimization information for using PPL for polar targets or multitarget lists that contain polar targets.

Typical protocol

1. Place a Bond Elut PPL cartridge or plate on the vacuum manifold or positive pressure manifold.
2. Condition with methanol.
3. Equilibrate with water.
4. Load the prepared aqueous sample. Apply low positive pressure or vacuum and gradually increase as needed.
5. Wash with 100% water or buffer for the matrix cleanup.
6. Apply positive pressure or vacuum gently for 30 seconds to completely dry the sorbent bed.
7. Elute with methanol. For incomplete elution with low recovery, perform the parallel elution for method optimization.
8. **Parallel elution:** (A) acidic methanol (MeOH with 0.5% FA) and (B) basic methanol (MeOH with 0.5% NH_4OH) need to be tested separately. For multitarget analysis with a large panel of targets, a sequential elution with acidic and basic methanol may be necessary. If so, apply full vacuum or positive pressure to completely dry the sorbent bed in between.
9. Apply vacuum or pressure for 30 seconds at the end to completely dry the sorbent bed.
10. Evaporate and reconstitute or dilute the sample appropriately before injection to instrument.

Bond Elut PPL protocol charts

It is recommended to start method development based on the generic method. Appropriate adjustment on the loading sample pH can help to improve the retention and the extraction efficiency. This is important not only for Bond Elut PPL but also for other hydrophobic SPE sorbent. Usually, it is recommended to adjust sample pH either two units below targets' pKa for neutral or acidic targets, or two units above targets' pKa for basic targets. This may significantly improve their retention on the SPE polymeric sorbent, especially for polar analytes.

The generic method is viable for most of the polar analytes, but further method optimization to improve the elution is sometimes necessary. Adjusting the pH for an elution solvent (either an acidic or basic organic solvent), could help break the unwanted ionic interaction between the analytes and the sorbent. If the targets panel is relatively small, then either acidic or basic methanol (A or B) elution may be sufficient. If the targets panel is relatively large, especially for multitarget analysis with a broad range of polarity, a sequential elution (A+B) is necessary to achieve good recoveries for all targets. During a sequential elution, it is critical to dry the cartridge completely in between acidic methanol and basic methanol elution. The eluent from both acidic and basic methanol elution is collected into the same collection tube. The combined eluent is then used for further treatment for sample analysis directly.

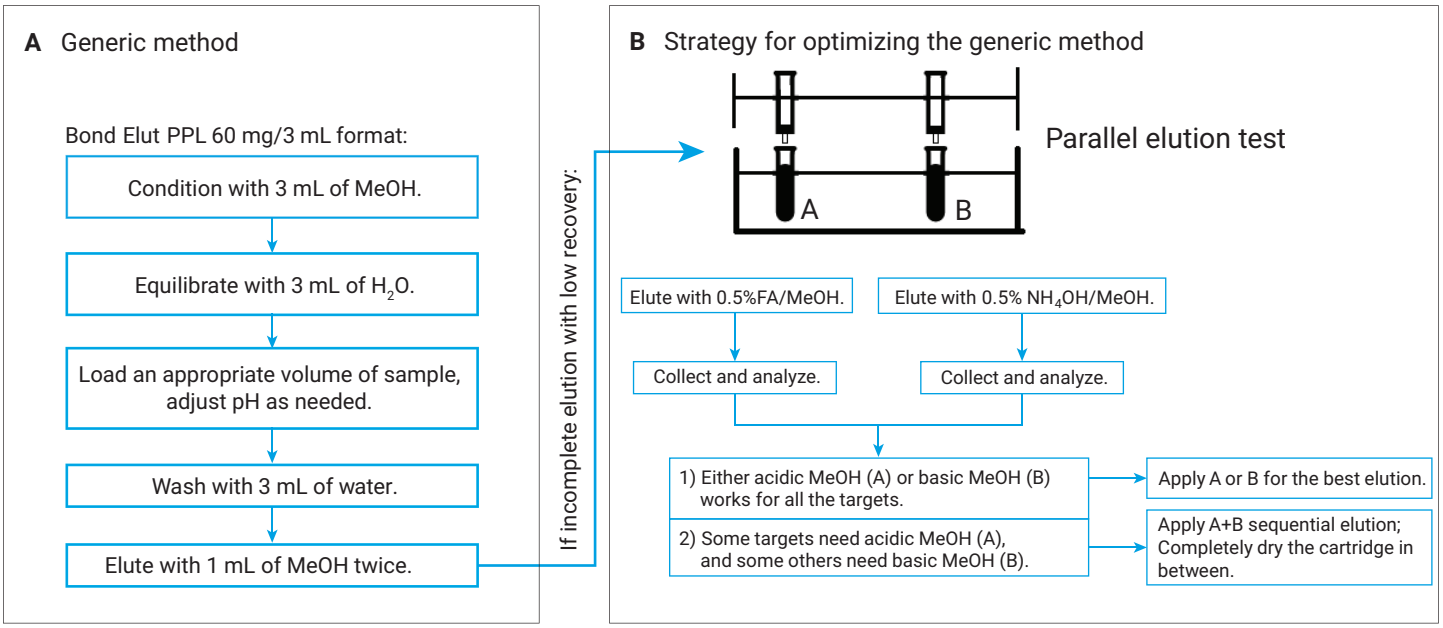


Figure 1. Protocol flow chart.

Method optimization example for polar targets

In this example, a group of polar compounds were selected to demonstrate method optimization, as shown in Table 1. Two sets of experiments were conducted to study: (1) method optimization with Bond Elut PPL on sample loading and elution, and (2) recoveries comparison with typical competitor products.

Table 1. Polar target pool for the Agilent Bond Elut PPL retention capability study.

Analyte	logP	Analyte	logP
Sulfaguanidine	−0.55	HMMNI	−0.4
Methamidophos	−0.9	Ipronidazole-OH	−0.5
Sulfacetamide	−1.0	Acetaminophen	0.5
Oxamyl	−0.5	Cyromazine	−0.2
4-Acetamidoantipyrine	0	Florfenicol	−0.2
Hydroxymetronidazole	−1.3	Amoxicillin	−2.0
Olaquindox	−0.2	Ampicillin	−1.3

As shown in Figure 2, different loading and elution conditions were investigated for the extraction of the polar compounds. The results demonstrated that sample loading pH and elution solvent pH affects the analytes' retention on sorbent, as well as analytes' release from sorbent. In this case, acidic sample loading provided the best overall analytes retention on PPL sorbent, especially for amoxicillin and ampicillin. Neutral MeOH elution provided efficient elution for 9 out of 13 polar targets. The recovery for the remaining four polar compounds were not acceptable. As recommended previously, parallel elution test was conducted using both acidic and basic MeOH for elution. Results showed that acidic methanol elution improved the rest four analytes' recoveries significantly. The acidic elution also provided good recoveries for the previous nine analytes. Therefore, the method was optimized using acidic loading and acidic elution for this group of targets.

	Loading Conditions	Elution Conditions
Neutral	Sample prepared in 100% water	100% MeOH
Acidic	Sample prepared in 0.1% TFA/water	0.5% FA/MeOH
Basic	Sample prepared in 0.5% NH_4OH/water	0.5% NH_4OH/MeOH

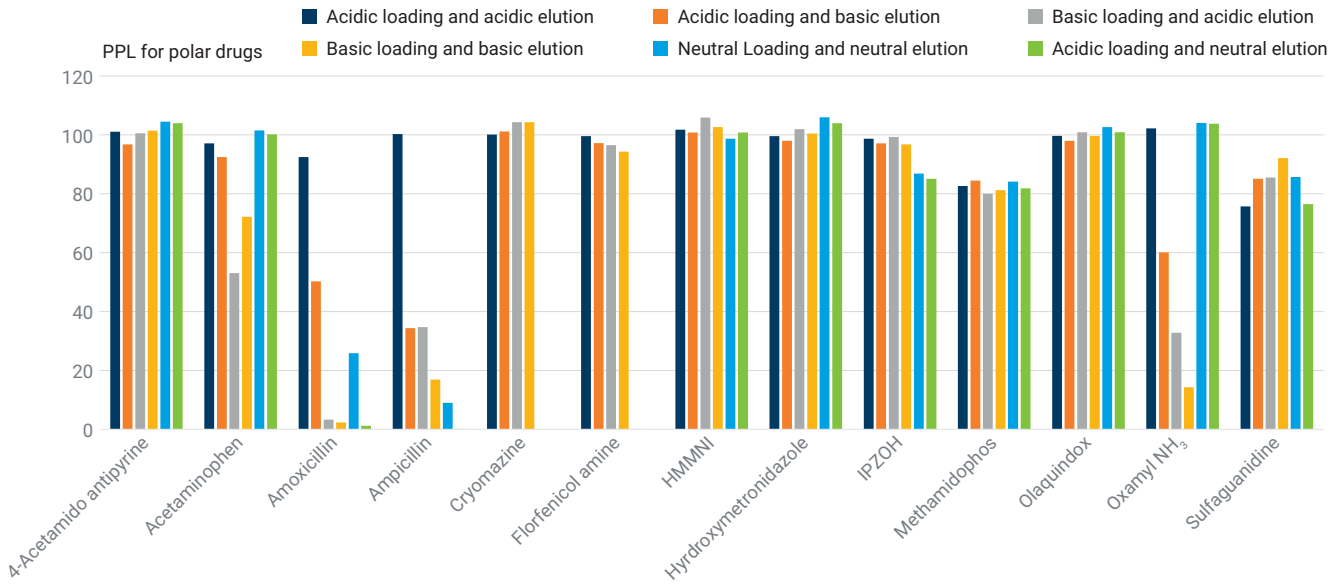


Figure 2. Method optimization on sample loading and elution for polar group of analytes.

The retention of Bond Elut PPL for polar targets was compared with two different commercial HLB cartridges using the same group of polar analytes. The acidified samples were loaded to SPE cartridges and washed with water. For HLB cartridges, MeOH was used for elution; while for Bond Elut PPL cartridges, acidified MeOH was applied for elution. From the results shown in Figure 3, Bond Elut PPL provides higher recovery and better reproducibility than HLB cartridges for amoxicillin and methamidophos. Furthermore, Bond Elut PPL demonstrates faster flow rate and more consistent elution than corresponding commercial HLB cartridges, with either MeOH or water elution under gravity (Figure 4). This certainly can be advantageous for the product ease of use.

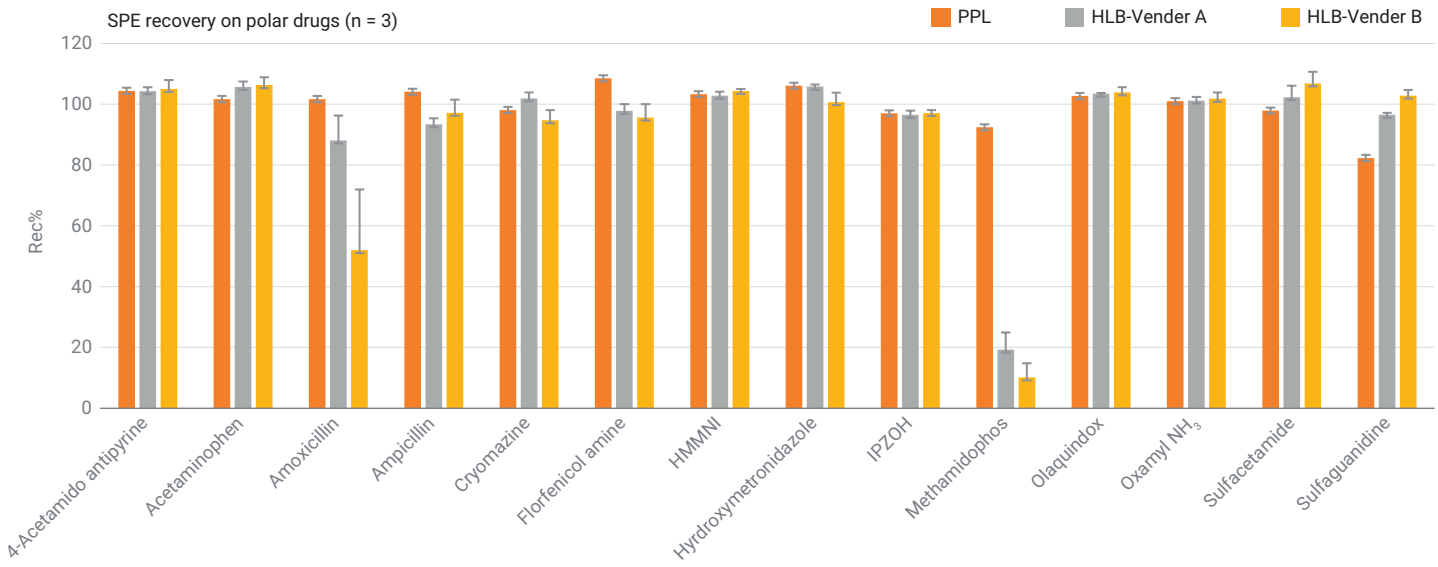


Figure 3. Comparison of Agilent Bond Elut PPL with HLB from two commercial brands on the recoveries and reproducibility of polar targets.

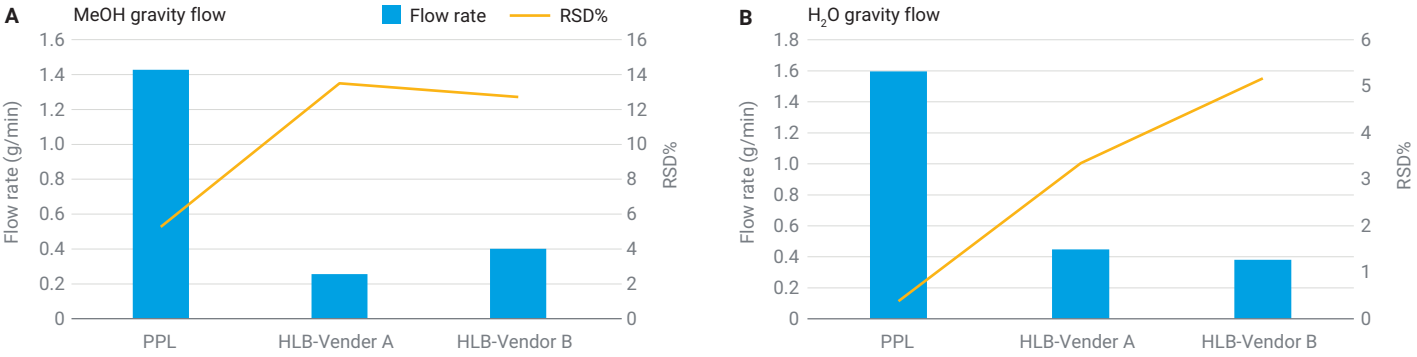


Figure 4. Flow rate comparison of Agilent Bond Elut PPL with HLB from two commercial brands with 60 mg/3 mL format under gravity flow with both MeOH and Water (n = 3).

Method optimization example for a broad range of multitarget analysis

Bond Elut PPL can also be applied for multiple targets with a broad range of polarities. The results shown in Figure 5 demonstrate the recovery heat map of multitarget analysis in water using Bond Elut PPL. The water sample was loaded in neutral, then eluted using different elution conditions including (A) MeOH, (B) MeOH with 0.5% formic acid, (C) MeOH with 0.5% NH_4OH, and (D) sequential elution with acidic MeOH and basic MeOH. With condition A, neutral MeOH worked well for some of the targets but was not efficient for others. With condition B or C, either acidic or basic MeOH elution improved certain analytes' recoveries dramatically. Neither B nor C provided good results for all of the targets. The sequential elution D provided better results than elution B and C. However, the recoveries for several relatively nonpolar compounds (fenbendazole, nicarbazine, coumaphos, and febantel) were still low. To achieve good recoveries for all the targets, sequential elution with a stronger organic solvent, 1:1 ACN/MeOH with pH adjustment instead of elution D, could be applied to better improve the nonpolar targets elution as shown in Figure 6. The recoveries of the four nonpolar targets were improved, and the recoveries of the polar targets were not affected. This demonstrates that both the strength and the pH of the solvent affect the interaction between the analytes and the PPL sorbent.

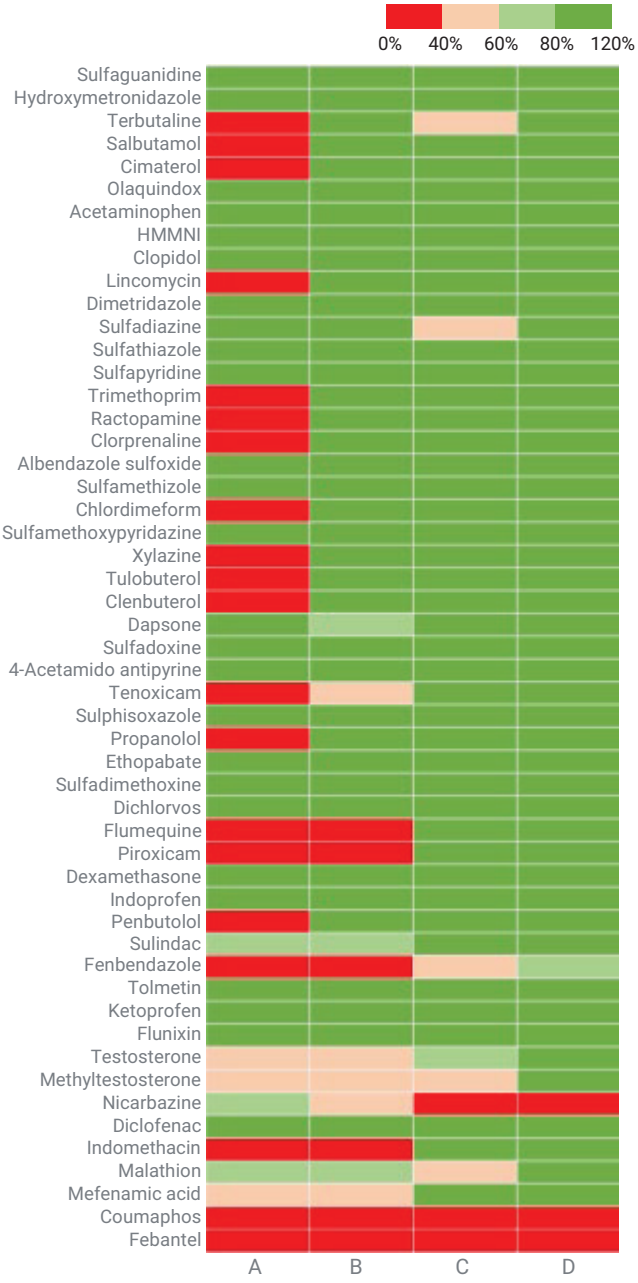


Figure 5. A heat map of the recoveries for multitarget analysis with Bond Elut PPL under the elution conditions of (A) MeOH, (B) MeOH with 0.5% formic acid, (C) MeOH with 0.5% NH_4OH, and (D) Sequential elution with B and C. Red represents the recoveries are in 0 to ~40%, pink represents the recoveries are in 40 to ~60%, light green represents the recoveries are in 60 to ~80%, green represents the recoveries are in 80 to ~120% (n = 3).

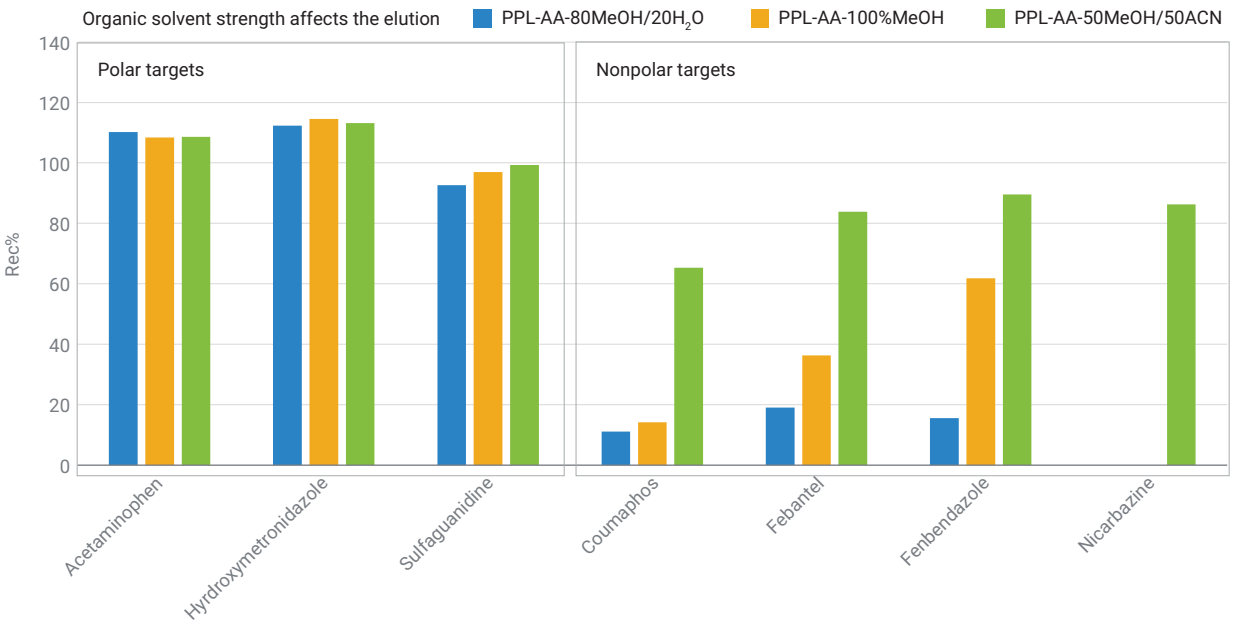


Figure 6. The impact of organic solvent strength on the elution of nonpolar targets.

Conclusion

Agilent Bond Elut PPL has been proven to work well for the extraction of polar compounds with a logP of –2 to 0.5, with efficient retention. The pH-adjusted parallel elution test demonstrates the opportunity to deliver good recoveries, especially for multitarget analysis. This SPE product is suitable for the application of both polar compounds and multiple targets analyses, with a broad range of polarity when combined with careful method optimization.

Typical applications

- Determination of Organophosphates in Lake Water Application Note (SI-02094)
- Determination of Drug Residues in Surface and Municipal Wastewaters by SPE and LVI-LC/MS/MS (SI-02188)
- Analysis of Olaquindox in Fodder Using SPE with LC/MS/MS (5994-1942EN)
- Determination of Phenols in Drinking Water by Solid Phase Extraction and Capillary Column Gas Chromatography/Mass Spectrometry (GC/MS) (EPA Method 528)

Ordering information

Bond Elut PPL

Part Number	Description
12105001	Bond Elut PPL cartridge, 25 mg, 1 mL, 100/pk
12105002	Bond Elut PPL cartridge, 50 mg, 1 mL, 100/pk
12105003	Bond Elut PPL cartridge, 100 mg, 1 mL, 100/pk
12105004	Bond Elut PPL cartridge, 100 mg, 3 mL, 50/pk
12105005	Bond Elut PPL cartridge, 200 mg, 3 mL, 50/pk
12105006	Bond Elut PPL cartridge, 500 mg, 3 mL, 50/pk
12255001	Bond Elut PPL cartridge, 500 mg, 6 mL, 30/pk
12102148	Bond Elut PPL cartridge, 1 g, 3 mL, 50/pk
12255002	Bond Elut PPL cartridge, 1 g, 6 mL, 30/pk
12256087	Bond Elut PPL cartridge, 5 g, 60 mL, 16/pk
7531901C*	Bond Elut PPL VersaPlate tubes, 100 mg, 24/pk
7551901C*	Bond Elut PPL VersaPlate tubes, 100 mg, 96/pk
7541901C	Bond Elut PPL VersaPlate preassembled 96-well plate, 100 mg, 1/pk

* Tubes need to be inserted into a VersaPlate base plate (p/n 75400000).

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