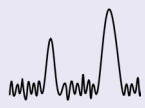
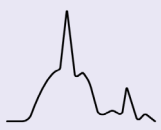
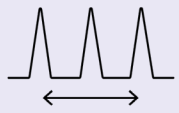
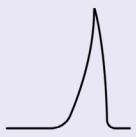
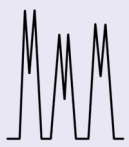
LC Purification Troubleshooting Guide

12 Prep Separation Issues & Actions for Resolving Them

Isolation success is achieving the desired target purity and yield and completing the purification task within the desired timeframe. To stay productive and avoid isolation failures, it is important to recognize issues early and address them quickly. This first-responder's guide may help you minimize risk of sample loss or target purity.

	Issue	Potential cause(s)	Action to Fix
1	Weak peak detection in trace 	■ Metal-sensitive targets interacting with column surfaces ■ Injection volume too low	■ Employ column with inert surface treatment ■ Inject more sample volume/ higher concentration
2	Broad, poorly shaped peaks 	■ Metal-sensitive targets interacting with column surfaces ■ Bad choice of mobile phase pH/column chemistry	■ Employ column with inert surface treatment ■ Choose pH at which target is unionized or alternate column
3	Target peak not fully resolved 	■ Selectivity of current stationary phase not sufficient	■ Employ column with alternate selectivity (different base particle or ligand bonding) ■ Employ method improvements
4	Target peak unretained (or minimally) 	■ Stationary phase not ideal	■ Increase aqueous mobile phase at initial conditions (up to 95%) ■ Employ column packed with polar stationary phase (aqueous compatible)
5	Ghost peaks appear in trace 	■ Sample not fully resolved, solids remain in crude	■ Clean-up crude sample by filtering with Celite or syringe filter ■ Extend high organic wash at end of gradient/reequilibrate/employ ACD
6	Peaks all broadened (smeared) 	■ Injection volume overload ■ Poor diluent choice ■ Basic target interacting with silica particle of stationary phase ■ Column degraded (or voided)	■ Concentrate sample into less diluent before injection ■ Select mobile phase as diluent ■ Avoid (or use less) strong solvents to dissolve sample ■ Test column performance with standard mix or replace with new
7	Target eluting late/drifting 	■ Insufficient flow	■ Measure system flow to ensure match to method
8	Column pressure building up (or excessive) 	■ Sample precipitation/build up on column inlet	■ Clean column with organic solvent (repeat injections or flush)
9	Regular spiking in trace 	■ Leak or bubble in the LC system	■ Check for leaks, tighten fittings ■ Prime HPLC pumps ■ Test flow to ensure check valves function
10	Excessive peak fronting 	■ Sample mass overload	■ Inject less in next run or employ larger column
11	Peak(s) appear flat across top 	■ Detector response being maximized by large sample load	■ Continue with separation and fraction collection ■ Assess purity of fraction prior to combining for evaporation ■ Install a shorter pathlength flow cell
12	Peaks that are splitting 	■ Inlet frit blocked in column (or guard)	■ Replace guard column ■ Flush column to clear blockage

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Column selection and method help

Waters OBD Preparative Columns Ordering Guide

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