HALO®

Small Molecule Selectivity Sampler

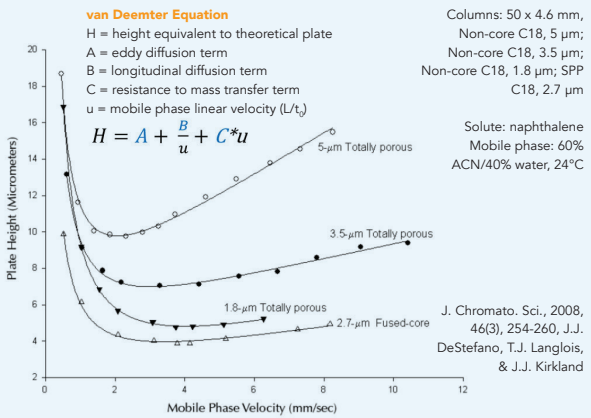
HALO 90 Å C18, AQ-C18, BIPHENYL

Fused-Core® Particle Technology

Why Choose Fused-Core® Particle Technology?

Fused-Core® Out-Performs Totally Porous Particles.
HALO® particles deliver higher efficiencies without the back pressure penalty when compared to sub-2 µm totally porous particles.

The van Deemter plot for 2.7 µm Fused-Core® to the right demonstrates a reduction in both the A term (eddy diffusion) and B term (longitudinal diffusion) which provide a significant reduction in theoretical plate height, which corresponds to higher efficiency. This ultimately means more resolution and more separation power!



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Achieving Selectivity in Reversed Phase Liquid Chromatography

Of the three variables in the general resolution equation, including efficiency (N) and retention (k), selectivity (α) is the most powerful parameter for adjusting and improving resolution (R_s) between peaks in a chromatographic separation.

Stationary phase selectivity is one of the most useful parameters for adjusting HPLC separation selectivity. Methods are easier to reproduce, especially between labs, if the selectivity comes from the stationary phase while using simple mobile phases.

EFFICIENCY SELECTIVITY RETENTION

$$R_S = \left(\frac{\sqrt{N}}{4}\right) x \left[\frac{(\alpha - 1)}{\alpha}\right] x \left[\frac{k_2}{(1+\bar{k})}\right]$$

where

$$\bar{k} = \frac{(k_1 + k_2)}{2}, \quad \alpha = \frac{k_2}{k_1} \text{ and } N = \frac{L}{H} = \frac{L}{h \times d_p}$$

Stationary Phase Selection

The gold standard C18 has reached its acclaimed status as being a very versatile, retentive, and stable phase for reversed phase liquid chromatography with simple binary mobile phases and gradients.

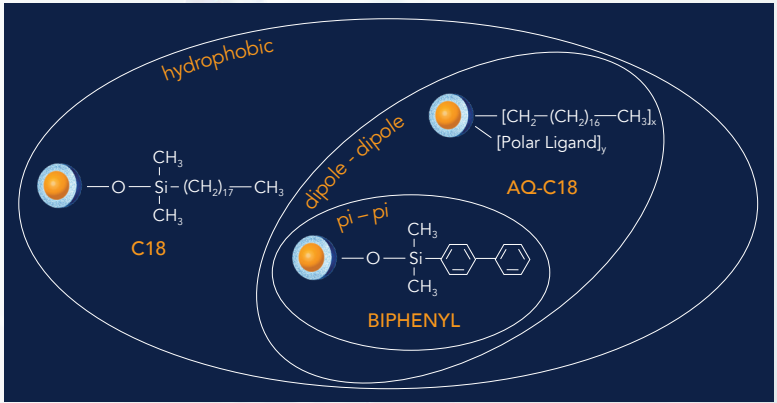
When to try a C18 alternative:

- Complex mixtures and isomers or very similar analytes cannot be resolved.
- When analytes are strongly retained or when hydrophilic and hydrophobic analytes are both present.
- Polar analytes not well resolved or well retained.
- Polar analytes not well retained with low or no organic modifier.

HELP WANTED: Suitable Alternatives to C18. Please Apply!

Consider the interactions between stationary phase and compounds of interest. What forces can be utilized for chromatographic resolution with challenging separations?

C18 – hydrophobic
AQ-C18 – hydrophobic, dipole – dipole
Biphenyl – hydrophobic, pi – pi, dipole – dipole



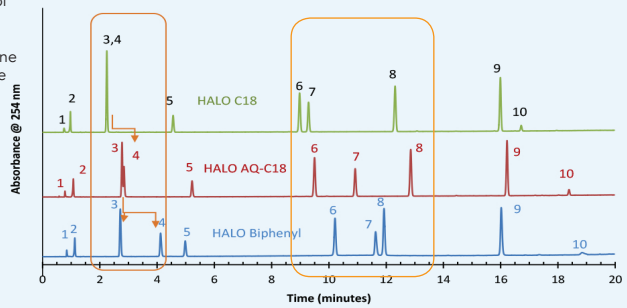
Selectivity Differences

In this example of substituted aromatic compounds with different functional groups including hydroxyl, nitro, ether, ketone, and benzene rings, the compound polarities and pi-pi interactions demonstrate the selectivity differences of the three phases. Note the separation and resolution achieved from the earlier eluting 4-nitrophenol and benzonitrile on the HALO® Biphenyl which is the most orthogonal with these three columns.

Columns: HALO 90 Å C18, 2.7 µm, 4.6 x 100 mm; HALO 90 Å AQ-C18, 2.7 µm, 4.6 x 100 mm; HALO 90 Å Biphenyl, 2.7 µm, 4.6 x 100 mm; Flow rate: 1.2 mL/min; Mobile Phase A: water; Mobile Phase B: methanol; Gradient: A/B 55/45 to 5/95 in 20 min; Injection Volume: 2 µL; Temp: 30 °C; Detection: 254 nm, VWD

PEAK IDENTITIES

1. uracil
2. resorcinol
3. 4-nitrophenol
4. benzonitrile
5. anisole
6. valerophenone
7. trans-stilbene
8. biphenyl
9. o-terphenyl
10. impurity



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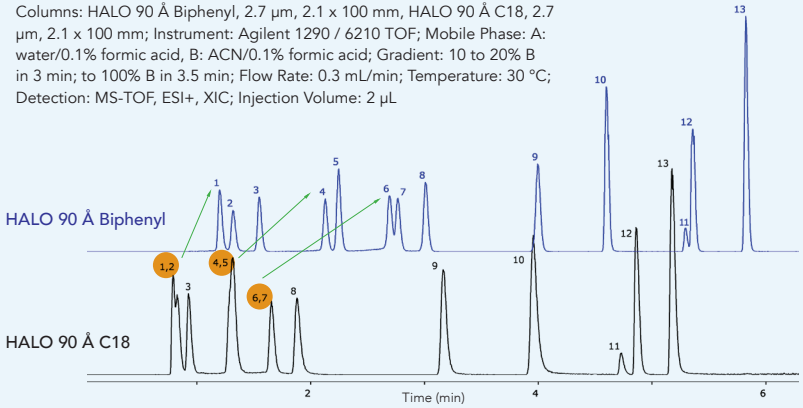
Pain Reliever

In this common 13 opiate pain panel screen on the next page used by clinical laboratories, the hydrophobic interactions of a C18 are not sufficient to separate six of the analytes of interest. The HALO® Biphenyl demonstrates the benefits of leveraging additional selectivity interactions.

Columns: HALO 90 Å Biphenyl, 2.7 µm, 2.1 x 100 mm, HALO 90 Å C18, 2.7 µm, 2.1 x 100 mm; Instrument: Agilent 1290 / 6210 TOF; Mobile Phase: A: water/0.1% formic acid, B: ACN/0.1% formic acid; Gradient: 10 to 20% B in 3 min; to 100% B in 3.5 min; Flow Rate: 0.3 mL/min; Temperature: 30 °C; Detection: MS-TOF, ESI+, XIC; Injection Volume: 2 µL



PEAK IDENTITIES

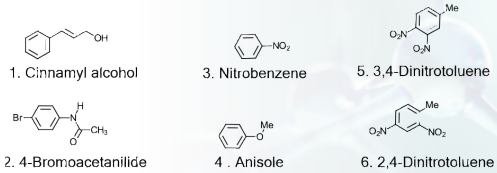
1. Morphine
2. Oxymorphone
3. Hydromorphone
4. Naloxone
5. Codeine
6. Naltrexone
7. Oxycodone
8. Hydrocodone
9. cis-Tramadol HCl
10. Meperidine
11. Fentanyl
12. Buprenorphine
13. (±)-Methadone

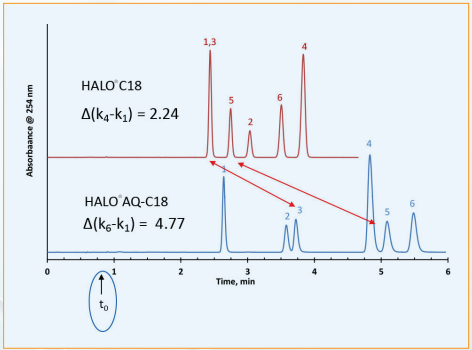
Aqueous Advantage

In the case of the separation of these six aromatic compounds the C18 phase proves inadequate to resolve Cinnamyl alcohol and Nitrobenzene.

The use of an alternative phase, in this case the HALO® AQ-C18, provides the necessary resolution as well as demonstrates selectivity differences.

Columns: 4.6 x 100 mm, HALO 90 Å C18, 2.7 µm (top), HALO 90 Å AQ-C18, 2.7 µm (bottom); Mobile Phase A: water; Mobile Phase B: methanol; Isocratic: 48/52 A/B instrument mixed; Flow Rate: 1.4 mL/min; Temperature: 30 °C; Detection: 254 nm; Injection: 0.5 µL





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Problematic Polar Compounds?

The use of low organic or totally aqueous mobile phases can be a useful tool for adequate retention and resolution of polar compounds. Due to the dewetting of hydrophobic phases, these types of mobile phases cannot be reliably used with C18, especially under isocratic conditions.

What is dewetting?

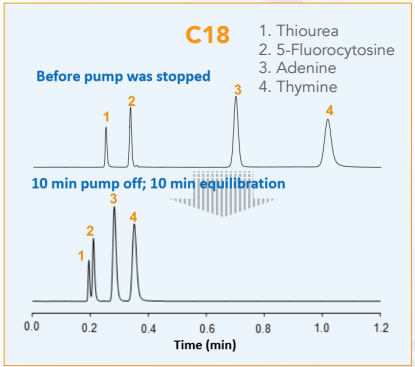
Dewetting occurs when the stationary phase is highly hydrophobic and the mobile phase is changed from one with a high amount of organic solvent component (>40% ACN or MeOH) to one that is entirely or mostly aqueous. When the column is under pressure, the aqueous mobile phase is forced into the porous structure where most of the retention occurs. When the pump is stopped, the aqueous mobile phase is no longer forced into the pore and is expelled from the interior of the particles. Restarting the pump will not force the aqueous mobile phase back into the pores since the phase is hydrophobic. When the stationary phase is dewetted, analyte retention is lost, especially for the most polar components.

Defeating Dewetting

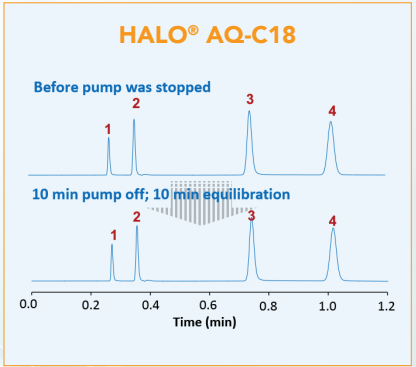
The HALO® AQ-C18 and Biphenyl are polar phases, which are wettable with low or no organic modifier. Their wettability and unique selectivities allow them to perform reproducibly in highly aqueous conditions.

The following figure demonstrates what happens to retention and resolution in a separation when dewetting occurs on a standard C18 compared to the HALO® AQ-C18.

The C18 shows a dramatic loss of retention after the pump is shut off for 10 minutes in 100% aqueous mobile phases.



Alternatively, the HALO® AQ-C18 maintains identical retention.



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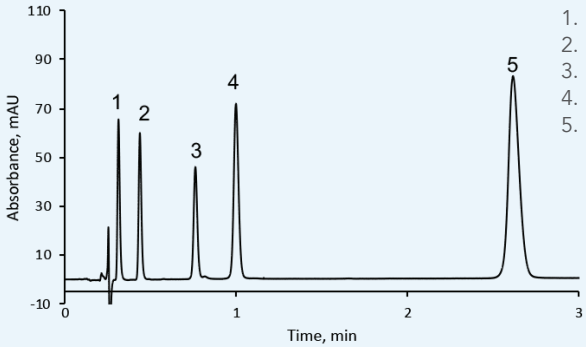
Brain Neurotransmitters Using 100% Aqueous Mobile Phase

This separation of biogenic amines demonstrates the benefits of utilizing a 100% aqueous mobile phase with isocractic conditions. The HALO® AQ-C18 was designed for this!

Column: HALO 90 Å AQ-C18, 2.7 µm, 2.1 x 50 mm;
Mobile phase: 0.1% TFA in water; Flow rate: 0.5 mL/min;
Temperature: 30 °C; Detection: UV 210 nm, PDA;
Injection volume: 1 µL

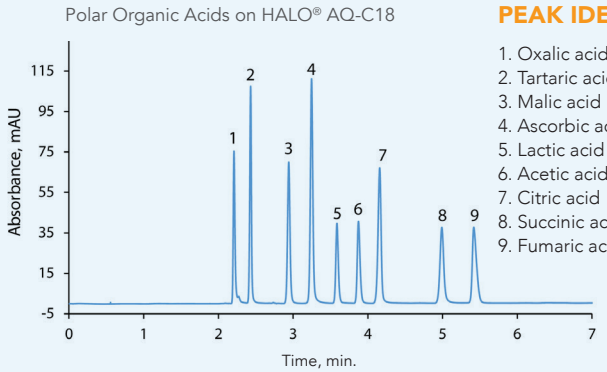


1. Norepinephrine
2. Epinephrine
3. Dopamine
4. L-DOPA
5. Serotonin

Polar Organic Acids

This application of polar organic acids highlights the advantages of using an aqueous C18 (HALO® AQ-C18) for excellent retention and resolution in a 100% buffered mobile phase.

Column: HALO 90 Å, AQ-C18, 2.7 µm, 4.6 x 250 mm; Isocratic: 20 mM Potassium Phosphate Monobasic buffer pH: 2.7; Flow Rate: 1.0 mL/min; Pressure: 307 bar; Temperature: 40 °C; Detection: UV 214 nm, PDA; Injection Volume: 20 µL; Flow Cell: 1 µL; LC System: Shimadzu Nexera X2



PEAK IDENTITIES

1. Oxalic acid
2. Tartaric acid
3. Malic acid
4. Ascorbic acid
5. Lactic acid
6. Acetic acid
7. Citric acid
8. Succinic acid
9. Fumaric acid

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What's the Goal?

Is the C18 in use not doing the job?
Like the C18, the HALO® AQ-C18 has a USP L1 designation. When slightly different selectivity is needed for polar analytes, the HALO® AQ-C18 should be evaluated to determine if it can provide the desired retention or resolution changes.

The HALO® Biphenyl carries a USP L11 designation and offers more orthogonality from the C18.The HALO® Biphenyl should also be considered as part of a column and condition screening protocol for method development. It demonstrates an alternate selectivity when C18 columns do not provide sufficient retention or resolution, especially for analytes with aromatic or heterocyclic rings.

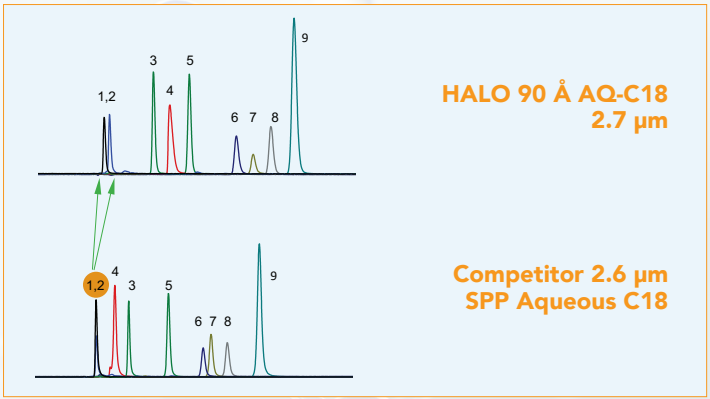
Competitive Advantage

The HALO 90 Å AQ-C18 column stands out in its ability to resolve this set of purines. These molecules are highly polar and are not retained under reversed-phase conditions that include an organic modifier.

Columns: HALO 90 Å AQ-C18, 2.7 µm, 2.1 x 100 mm; Mobile phase: 0.1% Formic Acid; Flow rate: 0.5 mL/min; Temperature: 35 °C; Detection: UV 254 nm; Injection: 1 µL

Samples: 1. 5-Amino-imidazole-4-carboxamide 2. Azepinomycin 3. Guanine - substrate 4. 2,6-Diaminopurine 5. Uric Acid 6. Xanthine – product 7. 8-Azaxanthine 8. 8-Azaguanine 9. Allopurinol

Separation of Highly Polar Purines in 100% Aqueous Mobile Phase



Comparative results presented here may not be representative for all applications.

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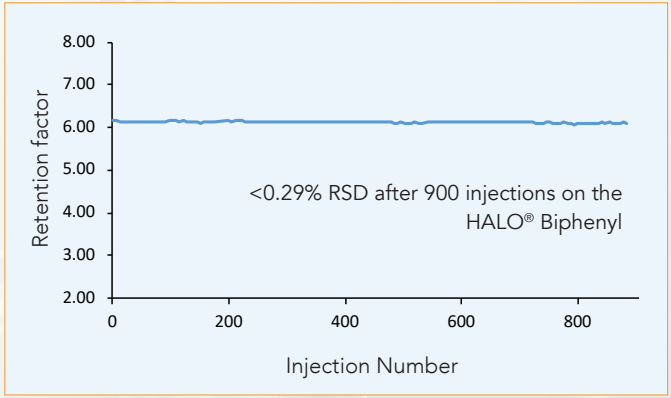
Quality You Can Count On

How rugged is your column?

In addition to the competitive advantage of HALO®, AMT understands that ruggedness of your method, AND your column are equally important. HALO® provides quality you can count on. Every time.

Column: HALO 90 Å Biphenyl, 2.7 µm 2.1 x 100 mm; Mobile Phase: 60/40 Methanol/ H_2O, 0.1% Formic Acid, 10mM Ammonium Formate; Temperature: 35°C; Flow Rate: 0.6 mL/min; Detection: 254 nm; Injection Volume: 0.5 µL; Sample: Naphthalene

HALO® Biphenyl Stability

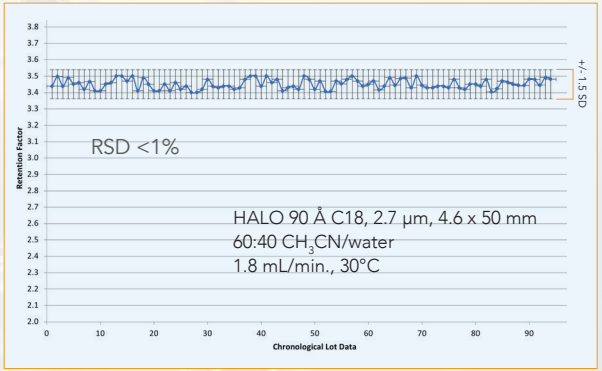


Performance You Can Rely On

Advanced Materials Technology led the revolution in Fused-Core® particle technology with the development of the first commercially available sub-3 µm particle with the original HALO® 2.7 µm HPLC column. Our innovation and manufacturing expertise carry forward into being the leader in SPP development. From our beginning in 2006, quality and performance always have been our focus and remain our focus.

HALO® - Innovation you can trust - Performance you can rely on.

HALO® Reproducibility Data for Ten Years
(QA Retention Factor - Naphthalene)



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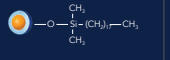
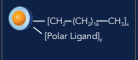
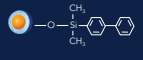
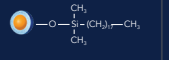
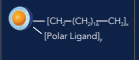
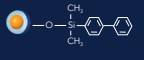
HALO 90 Å, 2.7 µm Specifications

			
	C18	**AQ-C18**	**Biphenyl**
Functional Group	dimethyloctadecyl	proprietary	dimethylbiphenyl
USP Designation	L1	L1	L11
Particle Size (µm)	2.7	2.7	2.7
Pore Size (Ångstroms)	90	90	90
Carbon Load (%)	7.7	6.7	7.0
Surface Area (m2/g)	135	135	135

			
	C18	**AQ-C18**	**Biphenyl**
Endcapped	Yes	Yes	Yes
pH Range	2-9	2-9	2-9
100% Aqueous Compatibility	No	Yes	Yes
Max. Temp. °C (pH 2)	60	60	60
Max. Temp. °C (pH 9)	40	40	40
Maximum Pressure	600 bar	600 bar	600 bar

HALO 90 Å 2.7 µm Capillary and Microbore Columns

Dimensions (ID x Length, mm)	C18	AQ-C18	Biphenyl
0.075 x 50	98219-402	98219-422	98219-411
0.075 x 100	98219-602	98219-622	98219-611
0.075 x 150	98219-702	98219-722	98219-711
0.1 x 50	98218-402	98218-422	98218-411
0.1 x 100	98218-602	98218-622	98218-611
0.1 x 150	98218-702	98218-722	98218-711
0.2 x 50	98217-402	98217-422	98217-411
0.2 x 100	98217-602	98217-622	98217-611
0.2 x 150	98217-702	98217-722	98217-711
0.3 x 50	98216-402	98216-422	98216-411

Dimensions (ID x Length, mm)	C18	AQ-C18	Biphenyl
0.3 x 100	98216-602	98216-622	98216-611
0.3 x 150	98216-702	98216-722	98216-711
0.5 x 50	98215-402	98215-422	98215-411
0.5 x 100	98215-602	98215-622	98215-611
0.5 x 150	98215-702	98215-722	98215-711
1.0 x 30	92811-302	92811-322	92811-311
1.0 x 50	92811-402	92811-422	92811-411
1.0 x 75	92811-502	92811-522	92811-511
1.0 x 100	92811-602	92811-622	92811-611
1.0 x 150	92811-702	92811-722	92811-711

HALO 90 Å 2.7 µm Analytical Columns

Dimensions (ID x Length, mm)	C18	AQ-C18	Biphenyl
2.1 x 20	92812-202	92812-222	92812-211
2.1 x 30	92812-302	92812-322	92812-311
2.1 x 50	92812-402	92812-422	92812-411
2.1 x 75	92812-502	92812-522	92812-511
2.1 x 100	92812-602	92812-622	92812-611
2.1 x 150	92812-702	92812-722	92812-711
2.1 x 250	92812-902	92812-922	92812-911
3.0 x 20	92813-202	92813-222	92813-211
3.0 x 30	92813-302	92813-322	92813-311
3.0 x 50	92813-402	92813-422	92813-411
3.0 x 75	92813-502	92813-522	92813-511
3.0 x 100	92813-602	92813-622	92813-611
3.0 x 150	92813-702	92813-722	92813-711
3.0 x 250	92813-902	92813-922	92813-911
4.6 x 20	92814-202	92814-222	92814-211
4.6 x 30	92814-302	92814-322	92814-311
4.6 x 50	92814-402	92814-422	92814-411
4.6 x 75	92814-502	92814-522	92814-511
4.6 x 100	92814-602	92814-622	92814-611
4.6 x 150	92814-702	92814-722	92814-711
4.6 x 250	92814-902	92814-922	92814-911
10.0 x 50	92810-402	92810-422	92810-411
10.0 x 75	92810-502	92810-522	92810-511
10.0 x 100	92810-602	92810-622	92810-611
10.0 x 150	92810-702	92810-722	92810-711

HALO 90 Å 2.7 µm Guard Columns, 3-pack

Dimensions (ID x Length, mm)	C18	AQ-C18	Biphenyl
2.1 x 5	92812-102	92812-122	92812-111
3.0 x 5	92813-102	92813-122	92813-111
4.6 x 5	92814-102	92814-122	92814-111

Guard Column Holder	94900-001

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