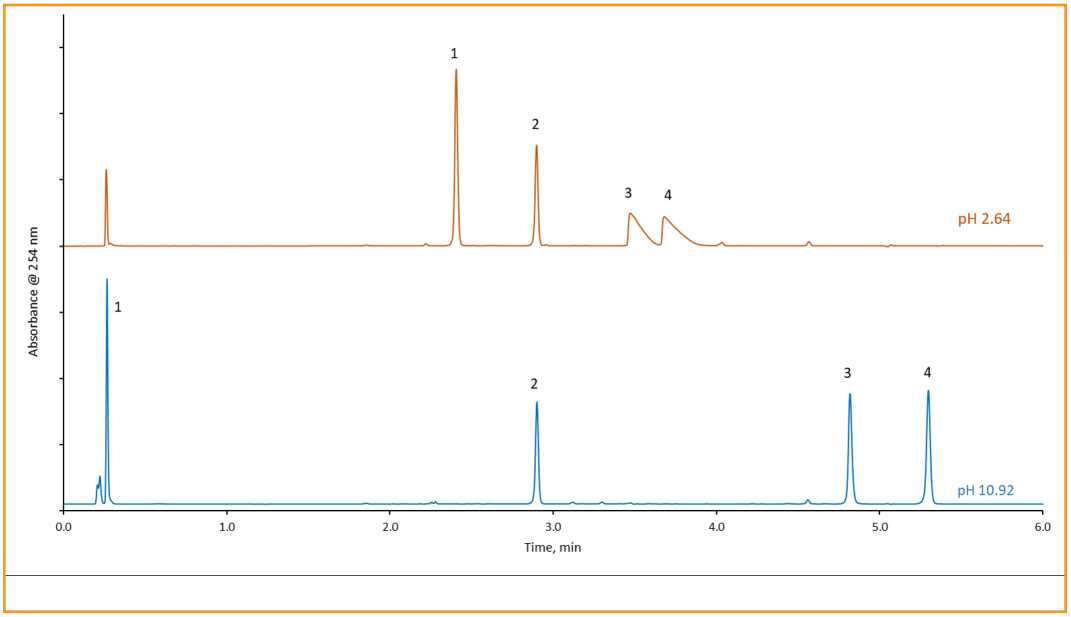
HALO®

PHARMACEUTICALS

Impact of pH on Retention for HALO® Elevate

379



PEAK IDENTITIES:

1. 2-Chlorobenzoic Acid, pKa 2.94
2. Dimethyl Phthalate
3. Imipramine, pKa 9.2
4. Trimipramine, pKa 9.4

TEST CONDITIONS:

Column: HALO 120 Å ELV, 2.7 µm, 2.1 x 50 mm
Part Number: 92272-402
Mobile Phase A:
Water + 0.1% Formic Acid, pH 2.64
Water + 0.1% Ammonium Hydroxide, pH 10.92
Mobile Phase B: Acetonitrile

Gradient:	**Time**	**%B**
	0.0	5
	5.5	85
	6.0	85
	6.1	5

Flow Rate: 0.4 mL/min
Back Pressure: 185 bar
Temperature: 30 °C
Injection Volume: 1.0 µL
Sample Solvent: 1:1 Water:Acetonitrile
Detection: UV/PDA, 254 nm
Flow Cell: 1 µL
Data Rate: 100 Hz
Response Time: 0.025 sec.
LC System: Shimadzu Nexera X2

A mixture containing a neutral, acid, and bases is separated on the HALO® Elevate column. A range of pH's was used to demonstrate how different compounds will shift in retention based on their pKa's. Under acidic conditions with no salt, bases will tail on C18 phases. If the pH is increased, the bases may have reduced tailing, but will still be partially ionized.

At the high pH the bases have great peak shape and are retained even more while the acidic compound has lost all retention. With the stable Elevate C18 phase, any conditions ranging from acidic to basic can be used in order to obtain the optimal separation for your sample set.

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