

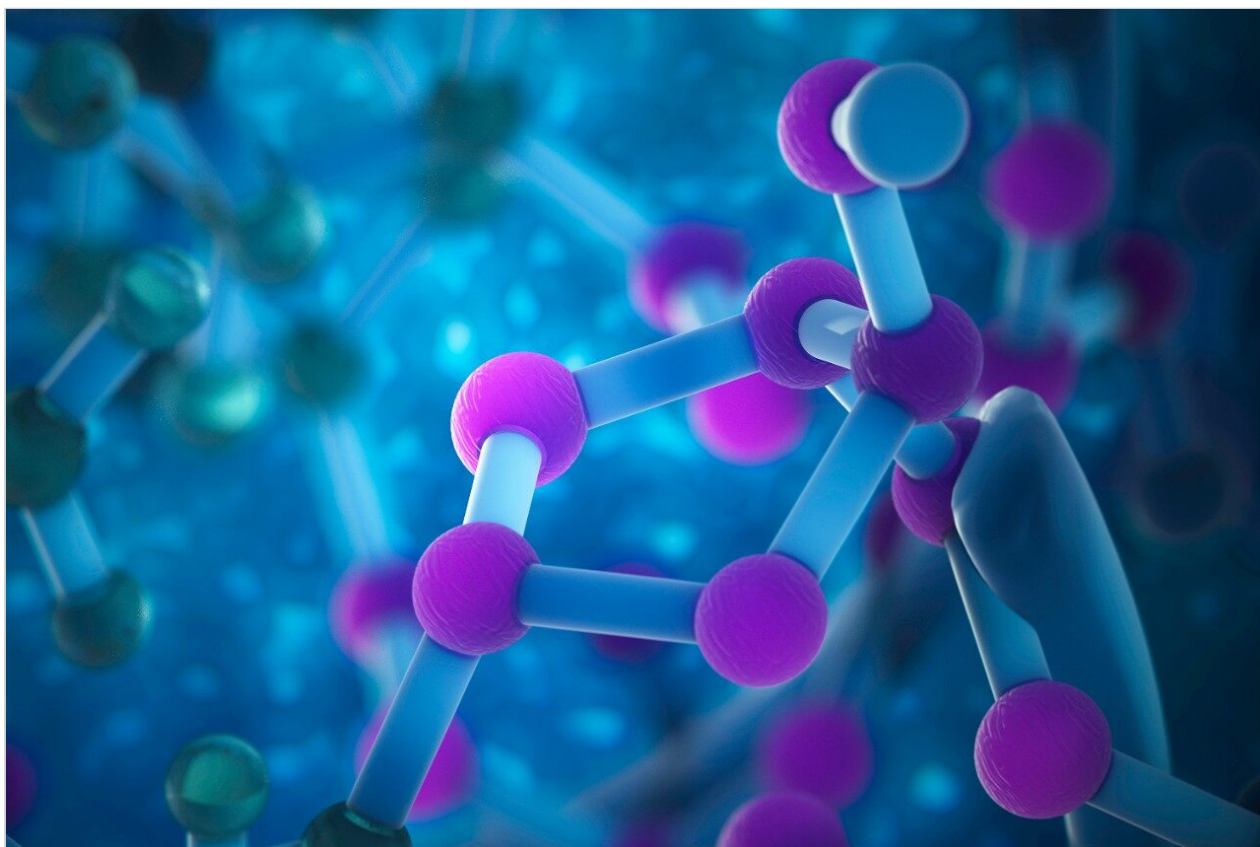
Nota applicativa

# Effect of pH on LC-MS Analysis of Amines

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## Abstract

This application note demonstrates effect of pH on LC-MS analysis of amines.

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## Introduction

Methods development for a LC-MS analysis differs from a traditional HPLC method. The selection of the mobile phase and the buffers must optimize the chromatographic separation, but for mass spectral detection, must promote the ionization of the analytes. In addition, the mobile phase modifiers should produce good peak shape with minimal tailing to maximize sensitivity.

In LC-MS high throughput laboratories need fast gradient separation. Generic methods are desirable to separate as many analytes as possible with the same solvents, buffers and columns. In the past, separations of amines with high pKa's have presented challenges to the chromatographer. On silica based columns, peak tailing has been a problem. Separations in acidic mobile phases reduces the retention of the analytes because they become protonated. The Waters XTerra MS C<sub>18</sub> Columns have unique chemical properties that make them much more stable to extremes of pH. This provides more opportunities to develop suitable methods for optimizing separation and detection.

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## Experimental

### Experimental Conditions

LC-MS:	Waters Alliance HT System:2790 Separations Module, 996 Photodiode Array Detector, ZMD Mass Detector
Compounds:	Diphenhydramine, Oxybutynin, Terfenadine ([M+H] <sup>+</sup> 256, 358, 472)
Column:	Xterra MS C <sub>18</sub> , 2.1 x 30 mm, 3.5 µm, stable at pH 1-12.
Gradients:	30–90% or 50–90% acetonitrile–water or 0.1% formic acid or 5 mM ammonium bicarbonate/carbonate, pH 9.5. Linear gradients

were 1 or 3 minute long at 0.3 or 0.5 mL/min.

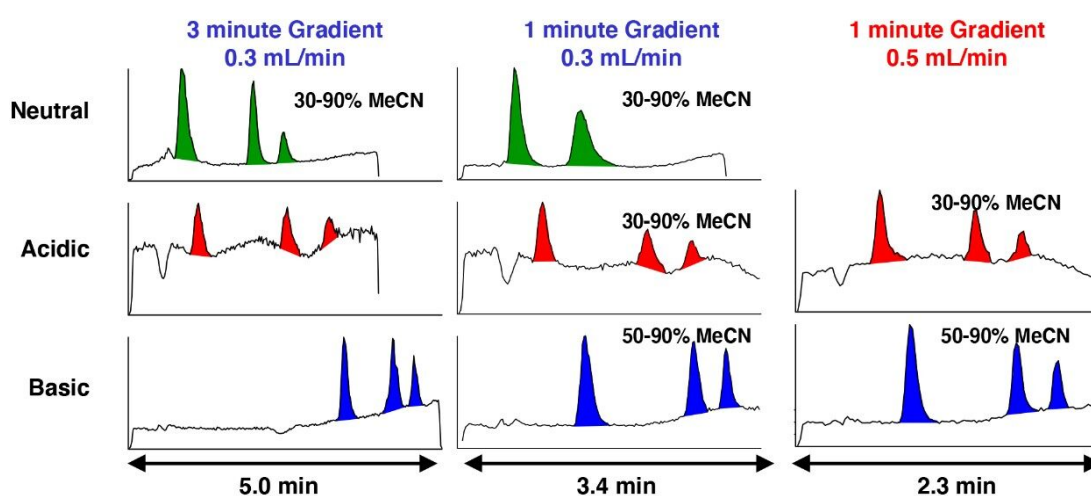
Detection:

ESI<sup>+</sup>, scan 100-600 *m/z*. ZMD was tuned for with the mobile phase containing formic acid.

## Results and Discussion

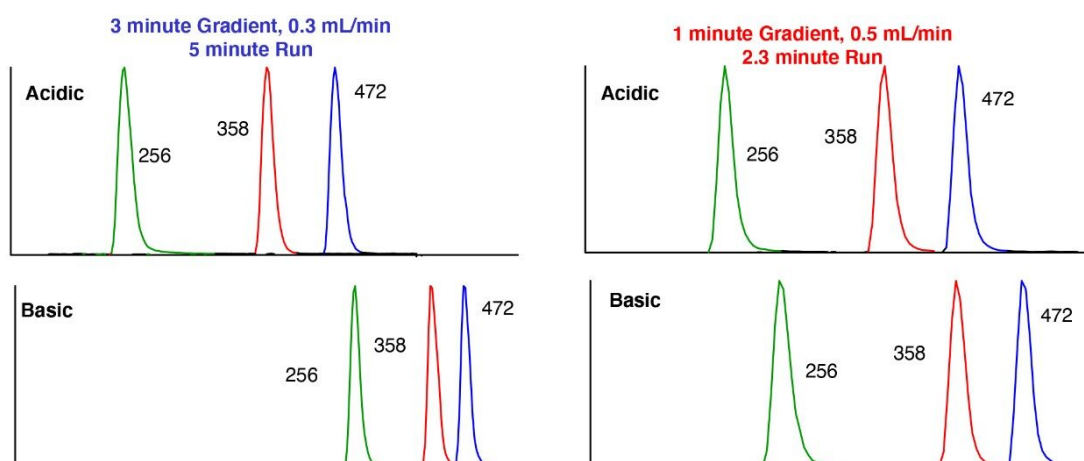
### Effect of pH and Gradient Time on the Separation of Amines

1. Effect of pH on amine separation. Diphenhydramine, oxybutynin, terfenadine were separated with a neutral acetonitrile-water gradient (upper row), with the same gradient in acid mobile phase (middle row), or in basic (pH 9.5) mobile phase (bottom row). Retention times were longest at pH 9.5 as the amines are forced to the unionized state. Compare the retention times in the left-hand column. Electrospray positive ionization mode was used in all cases to obtain the TIC (total ion current) mass chromatograms.
2. To obtain similar retention time between the acid and basic mobile phases, the initial %B was increased in the basic conditions. Reducing the gradient time from 3 to 1 minutes decreased the run time by 32% (middle column). However, at neutral pH resolution between peaks 2 and 3 was lost.
3. For a further reduction in run time, the flow rate can be increased from 0.3 mL/min to 0.5 mL/min (right column).



## Effect of pH and Gradient on Peak Shape - overlaid extracted masses $[M+H]^+$

In the left panel, the peaks eluted at basic pH have peak widths at 5% that are 17% narrower, with an average increase in peak height of 18%. However, when the gradients are optimized for speed, the peak widths in acidic and basic mobile phases are similar. The peak heights are also similar.



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## Conclusion

Before developing an LC-MS method, objectives must be stated (e.g. speed or sensitivity, ruggedness, etc.). New types of columns like the XTerra MS  $C_{18}$  are stable at extremes of pH and at higher temperatures. Therefore during methods development, different chromatographic parameters should be tried. Also do not assume basic compounds will ionize better in electrospray negative mode at high pH. In this example, the amines ionized equally well in the electrospray positive mode at pH 9.5. Through experimentation a rapid gradient method can be developed with good peak resolution and peak shape.

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