

## Introduction

### The Combined Effects of pH and Percent Methanol on the HPLC Separation of Benzoic Acid and Phenol:

Many mobile-phase variables can affect an HPLC (High Performance Liquid Chromatograph) separation. Among these are pH and the percent and type of organic modifier. The pKa of a weak acid is the pH at which the acid is equally distributed between its protonated (uncharged) and unprotonated (charged) forms. This is illustrated by the Henderson–Hasselbalch equation:

$$\text{pH} = \text{pKa} + \log ([A_-]/[HA])$$

where  $[A_-]$  is the concentration of the weak acid in its unprotonated form and  $[HA]$  is the concentration of the weak acid in its protonated form.

If the weak acid is equally distributed between its two forms,  $([A_-]/[HA]) = 1$ ,  $\log ([A_-]/[HA]) = 0$ , and  $\text{pH} = \text{pKa}$ . If the weak acid is not equally distributed between its two forms, then the pH will be either less or greater than the pKa of the weak acid.

For example, if  $[A_-] < [HA]$ ,  $([A_-]/[HA]) < 1$ ,  $\log ([A_-]/[HA]) < 0$ , and  $\text{pH} < \text{pKa}$ . Thus, a weak acid exists primarily in its protonated form at a pH below the pKa and therefore has a greater affinity for the nonpolar stationary phase. If  $[A_-] > [HA]$ ,  $([A_-]/[HA]) > 1$ ,  $\log ([A_-]/[HA]) > 0$ , and  $\text{pH} > \text{pKa}$ . Thus, a weak acid exists primarily in its unprotonated form at a pH above the pKa and therefore has a greater affinity for the polar mobile phase.

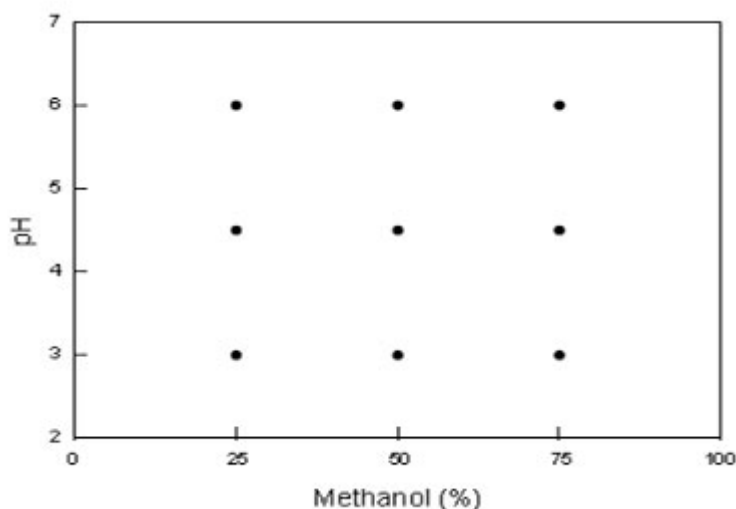


Fig. 1 - A three-level, two-factor full-factorial experimental design

# pH in Methanol

Organic modifiers also have an effect on the retention of solutes in HPLC. In the reversed-phase mode (polar mobile phase, nonpolar stationary phase), the most polar solute component will elute first. This is because the most polar component interacts least with the nonpolar stationary phase.

As the polarity of the mobile phase is increased, those solute components that were previously highly retained (nonpolar components) will be retained even more.

Two species that are of public interest because of their classification as moderate environmental and health hazards are benzoic acid ( $pK_a = 4.202$ ) and phenol ( $pK_a = 9.98$ ). The purpose of this study is to investigate the combined effects of pH and percent methanol on the reversed-phase HPLC separation of these compounds.

A three-level, two-factor fullfactorial experimental design will be used to specify nine mobile phases for consideration in this study. The levels of pH were chosen to bracket the  $pK_a$  value of benzoic acid (below, near, and above 4.202). It was not possible to study a mobile phase with a  $pH > 7.5$  owing to the pH range limit of the column. A methanol/water mobile phase was selected for this study because methanol is readily available in most undergraduate labs and relatively inexpensive. In addition, both solutes elute in a relatively short time, making completion of this lab during one or two lab periods possible.

**Table 1. Mobile Phases Specified by the Experimental Design**

Phase No.	Methanol %	pH
1	25	3.0
2	25	4.5
3	25	6.0
4	50	3.0
5	50	4.5
6	50	6.0
7	75	3.0
8	75	4.5
9	75	6.0

## Major Observation

At low mobile-phase methanol concentration (25%), as pH increases, the retention time of phenol appears to be unaffected, whereas the retention time of benzoic acid decreases significantly. Over the pH range investigated, the mobile-phase pH is below the pKa of phenol. Thus, phenol will remain in its protonated form and should be unaffected by these mobile-phase changes. However, as pH increases, benzoic acid shifts from its protonated to its unprotonated form, decreasing its affinity for the nonpolar stationary phase and decreasing its retention time.

At intermediate (50%) and high (75%) mobile-phase methanol concentrations, as pH increases, the retention time of phenol remains unaffected by increases in pH while the retention time of benzoic acid decreases. This is consistent with the behaviour at low methanol concentration.

At pH 3.0, as percent methanol increases, the retention times of both phenol and benzoic acid decrease significantly. Because both solutes are polar, increasing mobile-phase polarity causes both to be retained less tightly. At pH 4.5 (slightly above the pKa of benzoic acid) and pH 6.0 (well above the pKa of benzoic acid) as percent methanol increases, the retention times of phenol and benzoic acid decrease. This is consistent with the retention behaviour at pH 3.0.

## Typical Process Details

- Customer plant: Bulk drug plant
- Application: This is 4 cycle application. There will be a pipe connected to inlet which allows process to flow through the column and the same will be sent out from another pipe at outlet.
- pH measurement is typically required at both the inlet and outlet. Temp: 30-40° C. pH range shall be 7 to 7.5. Between this range the customer can take necessary action to control his process.
- Conductivity max. 300 micro siemens/cm.
- Cycle 1: Process contains 95% liquid methanol, 2% liquid ammonia, 3% water.
- Cycle 2: Process contains 30% liquid methanol, 70% water.
- Cycle 3: Process contains 90% liquid methanol, 5% liquid ammonia, 3% water, 2% sugar content.
- Cycle 4: The column will be cleaned by flushing with DM water.

## Tangible benefit

More reliable and accurate analysis of pH which helps to improve end product quality.

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### YOKOGAWA ELECTRIC CORPORATION

#### World Headquarters

9-32, Nakacho 2-chome, Musashino-shi, Tokyo 180-8750, JAPAN

<http://www.yokogawa.com/an/>



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