Part – [B]

Introduction

Ultra Performance Liquid Chromatography
1. INTRODUCTION OF UPLC

History of chromatography can give an idea about improvement in technology from conventional column chromatography to high performance liquid chromatography and finally at this stage an ultra performance liquid chromatography or in other way a combination of pressurized chromatographic technology and sub 2 (two) micron particle size of stationary phase technology leads to advance Ultra Performance Liquid Chromatography (UPLC) or Rapid Resolution Liquid Chromatography (RRLC) technology.

Technology of sub 2 (two) micron particle size leads many modifications in hardware part of the system like reduction of system volume, higher pump pressure capacity, injector and needle part, and cell volume of detector as well as in software area, data acquisition rate or capacity was increased for sufficient data collection.

In brief detail, small particle size columns leads to increase in pump pressure so that area was improved and for accurate and precise injection volume needle in needle technology with teflon material was came into the picture. Detector cell volume was reduced for better signals and resolution.

Smaller particle size of 2 micron technology altered the machine and its application for faster way of analysis in current scenario of separation science. Requirement of this technology can be explained by van deemter equation\(^1,4\) and plot as shown in fig 1. From this plot it reveals that there is minimum HETP against the linear velocity with the almost constant relation or maximum the theoretical plates can be achieved with particle size less than 2 micron. Finally as a known fact increasing in \(N\) leads to increase in Resolution as shown in formula;

\[
Rs = \sqrt{\frac{N}{4}} \left( \frac{\alpha - 1}{\alpha} \right) \left( \frac{k}{k+1} \right)
\]

\[\begin{array}{ccc}
\text{System Efficiency} & \text{Selectivity} & \text{Retentivity}
\end{array}\]
Now, for method conversion from HPLC to UPLC or for comparison of both the technology following aspects needs to take in consideration\[2-3\].

- Ratio of column length to particle size (L/dp) needs to keep constant.
  
i.e. 150 mm/5 µm = 30,000 is closest to 50mm/1.7 µm = 29,500

- Column selection should be based on same basic column chemistry
  
i.e. C\textsubscript{18} column should be replaced by C\textsubscript{18} column

- 5 µm to 1.7 µm particle size leads to increase in speed of 9X along with 9X pressure
- 3 µm to 1.7 µm particle size leads to increase in speed of 3X along with 3X pressure
- 5 µm to 1.7 µm particle size leads to increase in peak height of 1.7X
- 3 µm to 1.7 µm particle size leads to increase in peak height of 1.3X
- 5 µm to 1.7 µm particle size leads to decrease in peak width of 0.6X
- 3 µm to 1.7 µm particle size leads to decrease in peak width of 0.8X
Column efficiency (N) is inversely proportional to dp

\[ N \propto \frac{1}{dp} \]

i.e. 5 µm to 1.7 µm particle size leads to increase in column efficiency (N) 3X but resolution also increase by 1.7X

Based on above fact practically an example for chromatogram comparison against column dimension for run time and resolution is shown in fig 2.

Remark
Here, X is used to express the mathematical relation in multi fold.
e.g. pressure increased by 3X i.e. pressure increase by three times

Method Development

Method development in UPLC remains same as of HPLC but few areas of chromatographic conditions are different e.g. for gradient elution column equilibration time is very less as compare to HPLC due to lower column volume.

Advance technology in column filled material for HPLC as well as UPLC allows higher pH and temperature stability for column for wider choice of mobile phase for different applications. e.g. pH of mobile phase or its buffer can selected based on compound chemical nature and that can be explain by fig.3 for reversed phase retention plot.
From the above plot and fundamental theory of solvent gives following information for development consideration.
Facts for basic compound

1. Alkaline pH increases retention of basic analytes
2. Methanol increases retention of all components compared to acetonitrile
3. Similar basic analytes differ little in selectivity, respective to one another, when they are either fully charged or uncharged
4. Largest selectivity differences between bonded phases occur with methanol and analytes in their unionized state

Facts for acid compound

1. Acidic pH increases retention of acidic analytes
2. Methanol increases retention of all components compared to acetonitrile
3. Large differences in selectivity are observed when change in pH alters charge state
4. Largest selectivity differences between bonded phases occur with methanol and analytes in their unionized state

Column chemistry for known columns UPLC are shown in fig 4.

Figure 4: Column chemistry of UPLC column

By many recent research and development, UPLC presents the ability to extend and expand the utility of separation science at a time when many scientists have reached separation barriers, pushing the limits of conventional HPLC. New chemistry and instrumentation technology can provide more information per unit of work as UPLC begins to fulfil the promise of increased speed, resolution, and sensitivity predicted for liquid chromatography. As this is the concept for the scientist many of industries may
take time to use in routine but it can be the future of the liquid chromatography. Hence present research work includes the extended area of HPLC to UPLC as a part of technology updating or a balance form of present HPLC and improved LC or UPLC for future scope for separation science.

2. References

2. UPLC waters seminar presentation at Singapore (2006).