The history of chromatography dates back to the early 1900s, when it was developed as an analytical tool by Russian-Italian botanist Mikhail Tsvet. Chromatography is so effective that it is still in use today as a means of determining the components or purity of a mixture. The high-performance liquid chromatography (HPLC) technique, developed about 50 years ago, is one of the most commonly used.

More recently (2004) ultra-high-performance liquid chromatography (UHPLC) system has brought more to the HPLC game in terms of increased sample throughout and system efficiency. This has resulted in higher sensitivity and resolution in a shorter amount of time.

While the HPLC technique has been improved upon throughout its history, the past decade has seen some significant advances in the field. The primary goal with improvements to

HPLC/UHPLC have been to increase the efficiency of the method. With this in mind, the following are the major developments in recent history.

HPLC column changes

The conventional size of HPLC columns was 4.6 by 250 mm. Modern columns tend to be shorter now, with a length of 50 to 100 mm. They are also made narrower, with a column ID from 1.0 to 2.1 mm. This results in a faster separation and less solvent consumption.

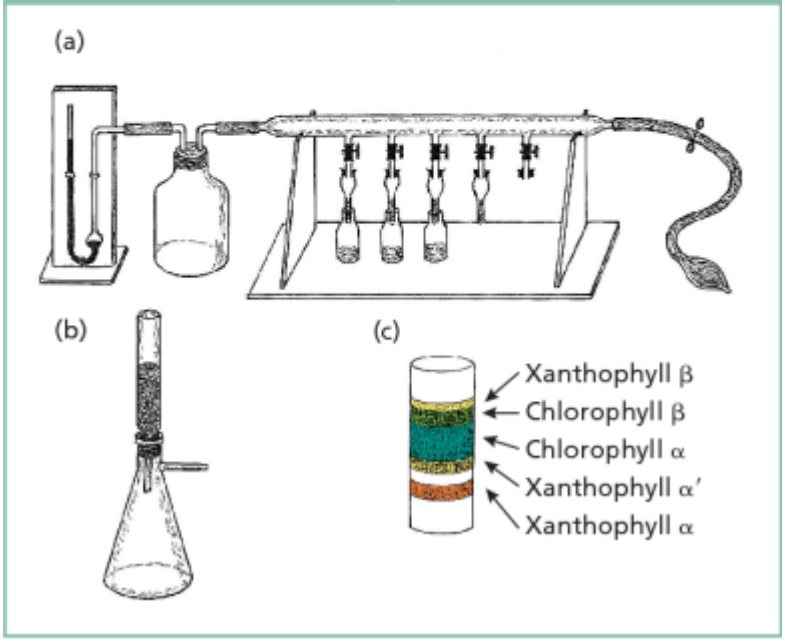
HPLC particle changes

Along with shorter, narrower columns has come the use of smaller particles with different particle morphologies. Traditionally, the particles packed into the column have been 5 µm in diameter and fully porous. These days, particles are trending more toward a diameter of 2 µm or less, and in some cases, with superficially porous particles that have a solid inert core with a thin shell of porous material. As with the change in column length and width, these particle changes help increased the column efficiency and resolution.

Better HPLC pumps

With the changes in the column dimensions and particles used in HPLC, the column backpressure could be very high. This has spurred the development of improved HPLC pumps that can help maintain a desirable column and system pressure. A good pump for modern HPLC system should be able to provide accurate flow with low gradient delay.

Figure 2: Illustrations from Tswett's 1906 paper.[6,10,11] (a) Apparatus for the simultaneous use of as many as five columns. The lower part of the small funnel-like glass pieces (2–3 mm i.d. and 20–30 mm length) served as the packed column. (b) Apparatus for larger samples (1–3 cm i.d., packing length: 5–9 cm). (c) Chromatographic separation of plant pigments as drawn by Tswett. Stationary phase: calcium carbonate; eluent: carbon disulphide.



High-pressure instrumentation

The backpressure for columns with sub-2µm particle size could be more than 600 bar, which exceeds the pressure limit of most traditional HPLC instruments. This has required the development of instrumentation that can operate at pressures as high as 1500 bar.

Optimized stationary phases and mobile phases

The conditions of the stationary phases and mobile phases affect the kinetic performance and selectivity of the column. When it comes to the mobile phase, recent software developments make it easier to optimize the conditions of the mobile phase, such as pH, temperature, and composition. Different type of mobile phase modifier can be used to ensure there are more than one retention mechanism (e.g. reversed-phase LC or ion exchange chromatography) present in a single column.

When it comes to the stationary phase, one of the most recent developments is the introduction of new chiral stationary phases. Mixed-mode stationary phase is also widely used for different selectivity.

Who benefits from modern developments in HPLC?

Every industry that uses HPLC would benefit from these modern developments, including pharmaceutical, biotech, food and environmental, chemical/Polymer, petrochemical/fuel and clinical. After all, everyone wants faster separation with superior resolution and sensitivity. Perhaps one of the greatest beneficiaries is the pharmaceutical industry, because of their need to increase the speed of analyses without compromising accuracy when assessing the purity and potency of the drugs they are manufacturing.

Reach out to our team if you have questions about modern developments in HPLC technology or if you need technical assistance with your work. Phenomenex offers a free, 24/7, online Technical Support service – **Chat Now**.