

EXPERIMENT 6

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY/HIGH PRESSURE LIQUID CHROMATOGRAPHY (HPLC)

THEORY/ BACKGROUND

In recent years the technology used in chromatographic analysis has greatly improved. Advances in this area have led to the common use of High Performance Liquid Chromatography, a laboratory technique that allows for the very efficient separation of small amounts of the components of a mixture. The technique has essentially the same operational basis as liquid chromatography, but HPLC allows for far better separation of the components of a mixture. The column in an HPLC instrument contains tiny particles of only about 5 μm diameter, ensuring a very large surface area to which molecules may adsorb. These particles comprise the *stationary phase* of the chromatographic system. Because these tiny particles are so tightly packed, the *mobile phase* solvent must be forced through the column under very high pressure. A detector and a computer are connected to the HPLC instrument in order to signal when eluents are coming off the column and fractions should be collected. The diagram in Figure 6 illustrates the main components of an HPLC instrument.

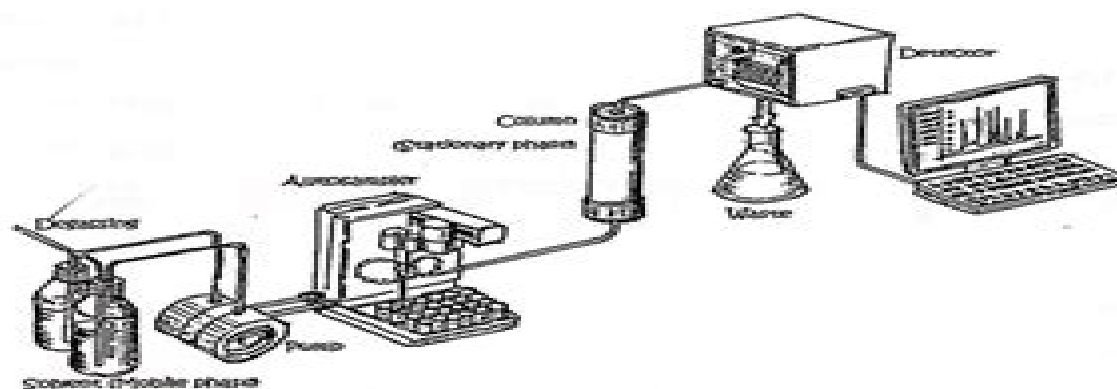


Figure 6.1: Schematic Diagram of a High Performance/ Pressure Liquid Chromatography.

Identification of samples is carried out by comparison of their retention times. A known amount of a known compound (a standard) is injected, the retention time is recorded and the area under the peak can be calculated. A sample containing the same analyte is then injected under the same experimental parameters. If the analyte is the same compound as the standard, then they should give identical retention times. The ratio of peak areas should give the amounts of the components present.

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1. Understanding the eBook Hplc Lab Manual
 - The Rise of Digital Reading Hplc Lab Manual
 - Advantages of eBooks Over Traditional Books
2. Identifying Hplc Lab Manual
 - Exploring Different Genres
 - Considering Fiction vs. Non-Fiction
 - Determining Your Reading Goals
3. Choosing the Right eBook Platform
 - Popular eBook Platforms
 - Features to Look for in an Hplc Lab Manual
 - User-Friendly Interface
4. Exploring eBook Recommendations from Hplc Lab Manual
 - Personalized Recommendations
 - Hplc Lab Manual User Reviews and Ratings
 - Hplc Lab Manual and Bestseller Lists
5. Accessing Hplc Lab Manual Free and Paid eBooks
 - Hplc Lab Manual Public Domain eBooks
 - Hplc Lab Manual eBook Subscription Services
 - Hplc Lab Manual Budget-Friendly Options

6. Navigating Hplc Lab Manual eBook Formats
 - ePub, PDF, MOBI, and More
 - Hplc Lab Manual Compatibility with Devices
 - Hplc Lab Manual Enhanced eBook Features
7. Enhancing Your Reading Experience
 - Adjustable Fonts and Text Sizes of Hplc Lab Manual
 - Highlighting and Note-Taking Hplc Lab Manual
 - Interactive Elements Hplc Lab Manual
8. Staying Engaged with Hplc Lab Manual
 - Joining Online Reading Communities
 - Participating in Virtual Book Clubs
 - Following Authors and Publishers Hplc Lab Manual
9. Balancing eBooks and Physical Books Hplc Lab Manual
 - Benefits of a Digital Library
 - Creating a Diverse Reading Collection Hplc Lab Manual
10. Overcoming Reading Challenges
 - Dealing with Digital Eye Strain
 - Minimizing Distractions
 - Managing Screen Time
11. Cultivating a Reading Routine Hplc Lab Manual
 - Setting Reading Goals Hplc Lab Manual
 - Carving Out Dedicated Reading Time
12. Sourcing Reliable Information of Hplc Lab Manual
 - Fact-Checking eBook Content of Hplc Lab Manual
 - Distinguishing Credible Sources
13. Promoting Lifelong Learning
 - Utilizing eBooks for Skill Development
 - Exploring Educational eBooks
14. Embracing eBook Trends
 - Integration of Multimedia Elements

- Interactive and Gamified eBooks

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