

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.

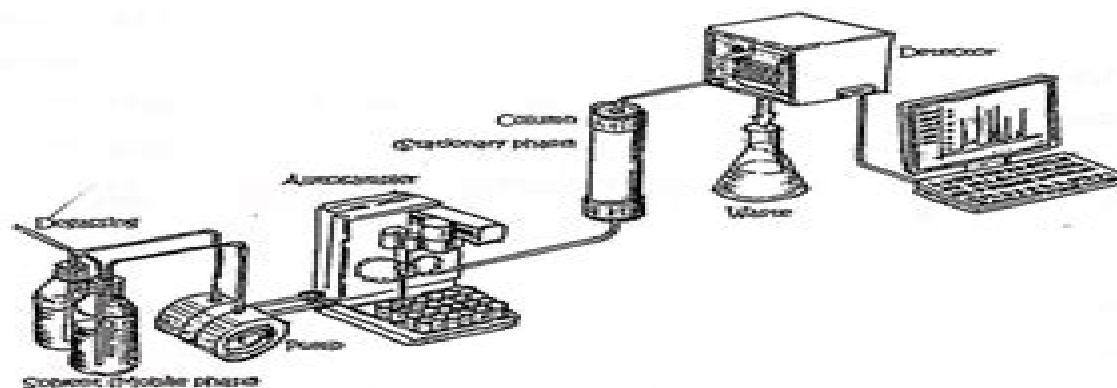


Figure 6.1: Schematic Diagram of a High Performance/ Pressure Liquid Chromatography.

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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Yicheng Fang



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Analysis Laboratory Manual B. Pam Ismail, S. Suzanne Nielsen, 2024-06-20 This fourth edition laboratory manual was written to accompany Nielsen's Food Analysis Sixth Edition by the same authors New to this fourth edition of the laboratory manual are three new chapters that complement both the textbook chapters and the laboratory exercises The book again contains four introductory chapters that help prepare students for doing food analysis laboratory exercises The 26 laboratory exercises in the manual cover 24 of the 35 chapters in the textbook Many of the laboratory exercises have multiple sections to cover several methods of analysis for a particular food component or characteristic Most of the laboratory exercises include the following background reading assignment objective principle of method chemicals reagents precautions and waste disposal supplies equipment procedure data and calculations questions and references This laboratory manual is ideal for the laboratory portion of undergraduate courses in food analysis **The Science of Ethanol** Walter E.

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