

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.

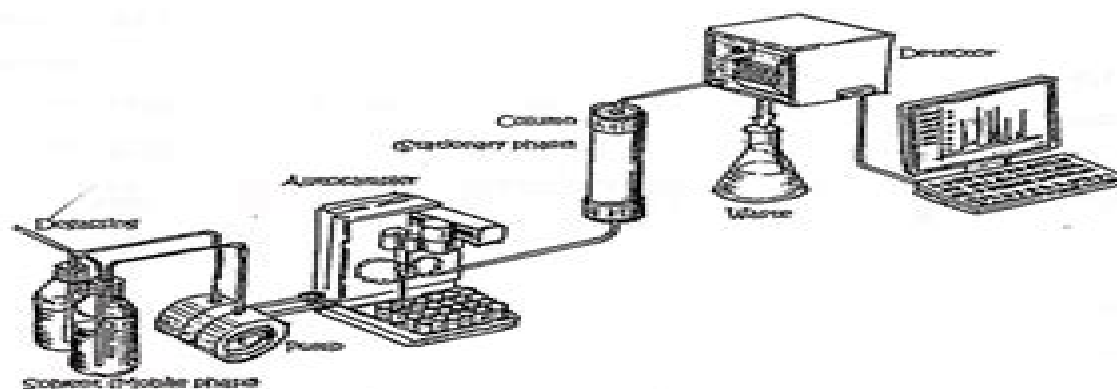


Figure 6.1: Schematic Diagram of a High Performance/ Pressure Liquid Chromatography.

HPLC

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.

Hplc Lab Manual

J Elliott



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Biochemistry Laboratory Manual Mr. Rohit Manglik, 2024-07-30 Hands on manual with detailed protocols and experiments for conducting fundamental and advanced biochemistry lab work *Food Analysis Laboratory Manual* S. Suzanne Nielsen, 2010-03-20 This second edition laboratory manual was written to accompany Food Analysis Fourth Edition ISBN 978 1 4419 1477 4 by the same author The 21 laboratory exercises in the manual cover 20 of the 32 chapters in the textbook Many of the laboratory exercises have multiple sections to cover several methods of analysis for a particular food component of characteristic Most of the laboratory exercises include the following introduction 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

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also discusses pollutants produced from ethanol and mixtures containing ethanol the status of ways to control these pollutants and what can be done to minimize the harm to the earth's ecosystems due to ethanol and gasoline reactions

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