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 cluents 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.

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Hplc Lab Manual

Jiyuan Zhang

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HPLC Marvin C. McMaster, 2006-12-18 This Second Edition of the classic handbook details how to set up an HPLC system that capitalizes on the latest innovations It covers new techniques in high temperature micro flow and ultra fast chromatography the linking of an HPLC to a mass spectrometer and more Complete appendices and supplementary material online this guide has everything chromatographers need to know to confidently separate identify purify and quantify Quality Control Training Manual Syed Imtiaz Haider, Syed Erfan Asif, 2016-04-19 Written to help companies comply with GMP GLP and validation requirements imposed by the FDA and regulatory bodies worldwide Quality Control Training Manual Comprehensive Training Guide for API Finished Pharmaceutical and Biotechnologies Laboratories presents cost effective training courses that cover how to apply advances in the life sciences to produce commercially viable biotech products and services in terms of quality safety and efficacy This book and its accompanying downloadable resources comprise detailed text summaries test papers and answers to test papers providing an administrative solution for management Provides the FDA Health Canada WHO and EMEA guidelines directly applicable to pharmaceutical laboratory related issues Offers generic formats and styles that can be customized to any organization and help management build quality into routine operations to comply with regulatory requirements Contains ready to use training courses that supply a good source of training material for experienced and inexperienced practitioners in the biotechnology biopharmaceutical industries Includes downloadable resources with downloadable training courses that can be adopted and directly customized to a particular organization Supplies ready to use test papers that allow end users to record all raw data up to the issuance of the attached certificate The biotechnology bioscience industries are regulated worldwide to be in compliance with cGMP and GLP principles with particular focus on safety issues Each company must create a definite training matrix of its employees The training procedures in this book enable end users to understand the principles and elements of manufacturing techniques and provide documentation language ranging from the generic to the specific The training courses on the downloadable resources supply valuable tools for developing training matrices to achieve FDA Health Canada EMEA MHRA UK WHO and GLP compliance The Organic Chem Lab Survival Manual James W. Zubrick, 2020-02-05 Teaches students the basic techniques and equipment of the organic chemistry lab the updated new edition of the popular hands on guide The Organic Chem Lab Survival Manual helps students understand the basic techniques essential safety protocols and the standard instrumentation necessary for success in the laboratory Author James W Zubrick has been assisting students navigate organic chemistry labs for more than three decades explaining how to set up the laboratory make accurate measurements and perform safe and meaningful experiments This practical guide covers every essential area of lab knowledge from keeping detailed notes and interpreting handbooks to using equipment for chromatography and infrared

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