

TROUBLESHOOTING GUIDE – HPLC

Before starting any troubleshooting, whether it is related to instruments or columns, it is essential that safe laboratory practices be observed. The chemical and physical properties of any solvents used should be known and the material safety data sheet (MSDS) for these solvents should be readily available.

All electrically powered instruments should be powered down and unplugged from the main supply before the removal of their covers, etc...

Wear eye protection when troubleshooting the detector (with the cover removed) as ultraviolet light is emitted during UV and fluorescence detector operation and this will damage the eye's cornea irreversibly.

There are many areas in a HPLC instrument that give rise to system and chromatographic problems. This guide will deal with each one in the following sections:

- visual inspection
- pressure
- baseline irregularities
- changes in chromatography
- qualitative results
- quantitative results

I- Visual inspection

When a problem occurs, it is advisable to perform a quick visual check of the instrument and column. This will pick up leaks, loose or disconnected tubing, changes in instrument settings, etc...

II- Pressure

System pressure is affected by a number of variables including the viscosity of the solvent used, column variables, flow rate and temperature. It is important to have a reference point when high or low pressures to the norm. This reference point should be the pressure generated in the system when everything is functioning correctly.

Pressure problems fall into one of three categories: high, low or fluctuating pressure. They can occur suddenly or be a gradual process. Sudden pressure rises tend to be due to particles from the sample, blocked or damaged tubing or column packed bed collapse. Gradual pressure rises can also be due to particles in the sample, but they can also arise from particles generated in the instrument, for example, debris from vial septa or degrading seals.

Before releasing any high pressure build-up in a system, be aware that the solvent may form an aerosol or spray when loosening connections. Eye protection should be worn and ideally the connection to be positioned above and adsorbent material to soak up all released solvents.

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Toshihiko Hanai



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High-Temperature Liquid Chromatography Thorsten Teutenberg, 2010-06-03 High temperature liquid chromatography has attracted much interest in recent years but has not yet recognized its full potential in the chromatographic community There is a widespread reluctance in industry to use temperature to speed up the separation process influence the selectivity of a separation or implement novel detection techniques However the technology has now matured and could revolutionize chromatography as we see it today Better equipment such as heating systems able to generate faster heating rates is becoming more readily available Also columns based on silica gel which can withstand higher temperatures for an extended period are now being introduced Nevertheless further technological and methodical efforts are needed to establish the method in a regulated environment like the pharmaceutical industry This is the only text to cover all the practical aspects as well as the underlying theoretical principles of setting up an HPLC system for high temperature operation It is not intended solely for academics but will also benefit the researcher interested in more practical considerations The author is a recognized expert and has conducted several studies with partners from industry to validate the method Many real examples from these studies have been included in the book The aim is to support practitioners in the creation of their own protocols without the need to rely solely on trial and error The book starts with a brief definition of high temperature liquid chromatography before going on to cover system set up the heating system mobile phase considerations suitable stationary phases method development using temperature programming analyte stability and special hyphenation techniques using superheated water as a mobile phase In each chapter experimental data is used to illustrate the main statements and the advantages over conventional HPLC are evaluated The book concludes with a critical outlook on further developments and applications underlining the necessary advances needed to make high temperature HPLC more robust

HPLC Toshihiko Hanai, 1999 How can these compounds be separated Why was that method used These are the two basic questions often asked by students of chromatography HPLC A Practical Guide provides the answers enabling the reader to grasp the concepts of the technique using simple representative chromatograms Divided into six chapters this practical guide covers basic concepts of HPLC instrumentation stationary phase materials eluents column efficiency and the influence of physical chemistry on separations Focusing on the basic considerations such as selection of stationary phase and eluent rather than specific applications sections on troubleshooting are also included Uniquely the descriptions of chromatographic separations are based on solubility using molecular properties and solubility parameters are used to analyse the selections of chromatographic mode and column Presenting the chemistry of liquid chromatography for undergraduate students this valuable practical guide will also be useful for laboratory staff in industry and academia *HPLC* Marvin C.

McMaster, 2007-01-29 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 with a CD ROM and appendices this guide has everything chromatographers need to know to confidently separate identify purify and quantify compounds Note CD ROM DVD and other supplementary materials are not included as part of eBook file **The HPLC Solvent Guide** Paul C. Sadek, 1996-09-06 An indispensable working resource for anyone who performs liquid chromatography The HPLC Solvent Guide provides an opportunity for analytical chemists to expand their experimental toolkits It features extensive and detailed coverage of HPLC solvents currently used in most laboratory settings Over the course of seven chapters each one focusing on a specific solvent class the author provides all the practical information needed to develop separations using these solvent classes A valuable working reference for experimentalists in a broad array of industrial and research fields The HPLC Solvent Guide Divides solvents according to chemical class then arranges the methods by field of application and finally by groups of analytes Provides complete descriptions of separations including sample matrices lists of analytes separated and quantified chromatographic parameters and results Features tables listing individual solvents and their physical chemical and chromatographic properties safety and health parameters and availability both in terms of supplier and performance specifications Includes figures that show the chemical structure of each solvent discussed Solvent selection is perhaps the most commonly overlooked of experimental parameters in high performance liquid chromatography When performing separations even the most experienced analytical chemist tends to select one of three solvents acetonitrile methanol or water overlooking the fact that many different classes of solvent can be used very effectively in HPLC By providing extensive detailed coverage of HPLC solvents currently used in a wide range of separations The HPLC Solvent Guide offers chemists an opportunity to expand their experimental repertoires The author begins with an in depth review of the role of solvents in HPLC This is followed by a series of chapters devoted to the different classes of solvent To facilitate easy reference chapters are first grouped by solvent classes including alcohols alkanes ethers ketones and nitriles They are then further divided by field of application e g environmental pharmaceutical and by specific analyte class e g priority pollutants anticancer drugs Throughout each separation is described as fully as possible listing the sample matrix analytes separated and quantified chromatographic parameters used and abbreviated results As a consequence even novice chromatographers have sufficient information to begin to develop a separation on the basis of a citation alone Appealing to anyone doing HPLC are the four tables appearing in each of the seven chapters dealing with individual solvent classes These tables list physical chemical and chromatographic properties safety and health parameters and availability both in terms of supplier and performance specifications A separate table provides the chemical structures of the solvents presented in each chapter In writing this book the author has drawn heavily from contemporary references in which the chromatograms for described separations were included thus providing readers with clear reference points from which to conduct a more extensive historical literature review if needed Providing the most detailed treatment of HPLC solvents to date The HPLC Solvent Guide is a valuable

working resource for anyone who performs liquid chromatography *HPLC* Joel K. Swadesh, 2000-12-21 Product specifications regulatory constraints and tight production schedules impose considerable pressures on separation scientists in industry The first edition of *HPLC Practical and Industrial Applications* helped eliminate the need for extensive library or laboratory research when confronting a problem an unfamiliar technique or work in a n **The HPLC Solvent Guide** Paul Charles Sadek, 2002-02-27 High performance liquid chromatography HPLC is a procedure for separating components from a mixture of chemical substances a combination of separation identification and quantitative measurements Solvent selection is perhaps the most commonly overlooked parameter in HPLC Even the most experienced analytical chemist tends to select one of three familiar solvents The *HPLC Solvent Guide* provides detailed coverage of all commonly used HPLC solvents used in a wide range of separations HPLC is a mature but substantial market and one that Wiley reaches successfully and well The HPLC list is established and this second edition of a successful title will build upon the success of the first This is a revised and expanded edition in a field that is still growing into areas of analysis and methods *A Practical Guide to HPLC Detection* Donald Parriott, 1993 *HPLC Columns* Uwe D. Neue, 1997-07-28 An in depth guide to HPLC column technology High performance liquid chromatography and its derivative techniques have become the dominant analytical separation tools in the pharmaceutical chemical and food industries environmental laboratories and therapeutic drug monitoring Although the column is the heart of the HPLC instrument and essential to its success until now no book has focused on the theory and practice of column technology *HPLC Columns* provides thorough state of the art coverage of HPLC column technology for the practicing technician and academician alike Along with a comprehensive discussion of the chemical and physical processes of the HPLC column it includes fundamental principles separation mechanisms and available technologies column selection criteria and special techniques Special features include Comprehensive overview of state of the art HPLC column technology Explanation of the underlying principles of HPLC columns Methods for selecting columns Practical advice on using and applying columns including examples Section by M Zoubair El Fallah on methods development Special techniques including preparative chromatography continuous chromatography and the simulated moving bed Troubleshooting section *HPLC Columns* helps laboratory practitioners make better choices in column selection methods development and troubleshooting it is also an excellent textbook for graduate level courses and HPLC short courses *A Guide to the HPLC Literature* Henri Colin, 1984 HPLC has largely contributed to the development of pharmacology biology food research and the biomedical sciences as demonstrated by the growing number of meetings dedicated to this topic and by the proliferation of companies offering equipment products or services for HPLC users It is becoming highly difficult to follow the current literature particularly in the area of applications *Modern HPLC for Practicing Scientists* Michael W. Dong, 2016-04-06 A comprehensive yet concise guide to Modern HPLC Written for practitioners by a practitioner *Modern HPLC for Practicing Scientists* is a concise text which presents the most important High Performance Liquid Chromatography HPLC fundamentals

applications and developments It describes basic theory and terminology for the novice and reviews relevant concepts best practices and modern trends for the experienced practitioner Moreover the book serves well as an updated reference guide for busy laboratory analysts and researchers Topics covered include HPLC operation Method development Maintenance and troubleshooting Modern trends in HPLC such as quick turnaround and greener methods Regulatory aspects While broad in scope this book focuses particularly on reversed phase HPLC the most common separation mode and on applications for the pharmaceutical industry the largest user segment Accessible to both novice and intermediate HPLC users information is delivered in a straightforward manner illustrated with an abundance of diagrams chromatograms tables and case studies and supported with selected key references and Web resources With intuitive explanations and clear figures Modern HPLC for Practicing Scientists is an essential resource for practitioners of all levels who need to understand and utilize this versatile analytical technology

Handbook of Pharmaceutical Analysis by HPLC Satinder Ahuja, Michael Dong, 2005-02-09 High pressure liquid chromatography frequently called high performance liquid chromatography HPLC or LC is the premier analytical technique in pharmaceutical analysis and is predominantly used in the pharmaceutical industry Written by selected experts in their respective fields the Handbook of Pharmaceutical Analysis by HPLC Volume 6 provides a complete yet concise reference guide for utilizing the versatility of HPLC in drug development and quality control Highlighting novel approaches in HPLC and the latest developments in hyphenated techniques the book captures the essence of major pharmaceutical applications assays stability testing impurity testing dissolution testing cleaning validation high throughput screening A complete reference guide to HPLC Describes best practices in HPLC and offers tricks of the trade in HPLC operation and method development Reviews key HPLC pharmaceutical applications and highlights current trends in HPLC ancillary techniques sample preparations and data handling

Practical HPLC Methodology and Applications Brian A. Bidlingmeyer, 1993-05-06 Of related interest Trace and Ultratrace Analysis by HPLC Satinder Ahuja Written by a leading scientist in the field this monograph provides the first definitive and technically up to date treatment of the theory equipment and applications of chemistry's most powerful reliable analytical technique Coverage includes an encyclopedic compendium of common substances that require trace and ultratrace analysis and features clear discussion of such important topics as considerations for HPLC equipment sensitive detectors sample preparation method development selectivity and computer based optimizations optimizing detectability and much more 1991 0 471 51419 5 432 pp High Performance Liquid Chromatography in Biotechnology Edited by William S Hancock Analytical chemists biochemists and chemical engineers will find this up to date guide to HPLC's recent developments essential for enhancing on the job technical expertise Extensive coverage includes the broad applications of HPLC ranging from major chromatographic techniques including reversed phase ion exchange affinity and hydrophobic interaction chromatography to specific separations such as those in monoclonal antibody and nucleic acid purification Techniques for quality control programs and advanced technology are also discussed

1990 0 471 82584 0 564 pp Unified Separation Science J Calvin Giddings This advanced text monograph brings together for the first time the variety of techniques used for chemical separations by outlining their common underlying mechanisms The mass transport phenomena underlying all separation processes are developed in a simple physical mathematical form facilitating analysis of alternative separation techniques and the factors integral to separation power The first six chapters provide background material applicable to a wide range of separation methods while the final five chapters illustrate specific techniques and methods 1991 0 471 52089 6 320 pp **An Introduction to HPLC for Pharmaceutical Analysis** Oona

McPolin,2009-03-01 If you are new to HPLC this book provides an invaluable guide to how HPLC is actually used when analysing pharmaceuticals It is full of practical advice on the operation of HPLC systems combined with the necessary theoretical knowledge to ensure understanding of the technique Key features include A thorough discussion of the stationary phase enabling the reader to make sense of the many parameters used to describe a HPLC column Practical advice and helpful hints for the preparation and use of mobile phase A complete overview of each of the different components which together make up a HPLC system A description of the contents of a typical HPLC analytical method and how to interpret these A step by step guide on how to follow a method and set up a HPLC analysis A discussion of system suitability criteria and how to interpret the values obtained during an analysis Explanation of the common methods of calibration and quantification used for pharmaceutical analysis **Manual for HPLC** Ramu BANDAMEEDI,Saibaba SV,Chittela

BABU,2018-02 This text is intended to be a guide for both the novice to preparative HPLC and as an aid to the chemical engineer planning to introduce this black art into the industrial environment The first question to ask is What is preparative To many the isolation of a few grams of an extremely potent molecule may be considered as largescale In some instances 50 g of a vaccine will supply the annual market for a particular disease state In more traditional drug therapies a few tonne may be more typical The second question to be answered is What is HPLC This abbreviation is often derived from the term High Performance Liquid Chromatography though the term High Pressure Liquid Chromatography is often preferred since high performance can also be achieved at low pressure Just to confuse the issue is this the pressure created by the resistance to liquid flow through the column or the pressure at which the column is packed **HPLC Analysis of Biological**

Compounds William S. Hancock,James T. Sparrow,1984 *Practical HPLC Method Development* Lloyd R. Snyder,Joseph J. Kirkland,Joseph L. Glajch,2012-12-03 This revision brings the reader completely up to date on the evolving methods associated with increasingly more complex sample types analyzed using high performance liquid chromatography or HPLC The book also incorporates updated discussions of many of the fundamental components of HPLC systems and practical issues associated with the use of this analytical method This edition includes new or expanded treatments of sample preparation computer assisted method development as well as biochemical samples and chiral separations **Liquid**

Chromatography ,1996 **Optimization in HPLC** Stavros Kromidas,2021-11-08 Learn to maximize the performance of

your HPLC or UHPLC system with this resource from leading experts in the field Optimization in HPLC Concepts and Strategies delivers tried and tested strategies for optimizing the performance of HPLC and UHPLC systems for a wide variety of analytical tasks The book explains how to optimize the different HPLC operation modes for a range of analyses including small molecules chiral substances and biomolecules It also shows readers when and how computational tools may be used to optimize performance The practice oriented text describes common challenges faced by users and developers of HPLC and UHPLC systems as well as how those challenges can be overcome Written for first time and experienced users of HPLC technology and keeping pace with recent developments in HPLC instrumentation and operation modes this comprehensive guide leaves few questions unanswered Readers will also benefit from the inclusion of A thorough introduction to optimization strategies for different modes and uses of HPLC including working under regulatory constraints An exploration of computer aided HPLC optimization including ChromSwordAuto and Fusion QbD A treatment of current challenges for HPLC users in industry as well as large and small analytical service providers Discussions of current challenges for HPLC equipment suppliers Tailor made for analytical chemists chromatographers pharmacologists toxicologists and lab technicians Optimization in HPLC Concepts and Strategies will also earn a place on the shelves of analytical laboratories in academia and industry who seek a one stop reference for optimizing the performance of HPLC systems Reversed Phase HPLC ,

Beginners Guide to Liquid Chromatography Waters Corporation, 2014-04-21 The Beginners Guide to Liquid Chromatography a 56 page paperback book is a useful tool for those learning about the technology of liquid chromatography LC with a focus on high performance liquid chromatography HPLC The Beginners Guide offers an uncomplicated look at LC HPLC and includes clear and colorful diagrams to acquaint the reader with basic concepts and terminology No previous scientific training is required to obtain a better working knowledge of this powerful technology Looking for something else Learn a new technique or technology with the Waters Primers Series view other titles available here <http://www.wiley.com/go/waters>

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