Industry Perspective on the Validation of Column-Based Separation Processes for the Purification of Proteins

蛋白质净化柱层分离工艺验证的工业观点

I. Foreword 前言

The purpose of this document is to outline some of the significant issues related to the validation of column-based separation processes used in the purification of proteins produced by recombinant DNA (rDNA), hybri-doma technology, or peptide synthesis. While validation of these processes has been identified as a first priority by the PDA Biotechnology Task Force, the issues raised in certain sections of this document may have broader applications, including processes for non-protein phar-maceutical products purified by HPLC, as well as protein pharmaceuticals which are not produced by rDNA, hybridoma, or peptide synthesis technologies. 此文件目的是列出与DNA重组 (rDNA)，杂交瘤技术或肽合成产生的蛋白质净化所使用的柱层分离工艺验证相关的一些重要问题。这些工艺的验证是PDA生物技术特别小组最重要的任务，此文件某些章节提到的问题可能有更广泛的应用，包括由HPLC净化的非蛋白质药品工艺，非DNA重组 (rDNA)，杂交瘤技术或肽合成技术产生的蛋白质产品。

While column-based separations are key purification techniques in the production of recombinant proteins and monoclonal antibodies, little has been written regarding the validation of these processes. In general, process validation is the assurance that product quality is derived from careful attention to a number of factors, including process design, selection and use of quality parts and materials, and control of the process through appropriate in-process and end-product testing. In May 1987, the Center for Drugs and Biologies and the Center for Devices and Radiological Health of the Food and Drug Administration published the "Guideline on General Principles of Process Validation." While this guideline is useful, it does not include all of the specific elements required in the validation of a manufacturing process. Therefore, the PDA established the Biotechnology Task Force on Purification and Scale-up to develop a practical guide for the validation of column-based separations used in the manufacture of proteins intended for use as therapeutic or diagnostic agents. 柱层分离是重组蛋白质和单克隆抗体类生产的关键净化技术，这些工艺验证文件很少。一般来说，工艺验证是为确保产品质量经过谨慎处理，包括工艺设计，选择，质量部分和材料的使用，适当的过程中测试及制成品测试的工艺控制等因素。1987年5月，药品和生物中心，设备中心和食品药品管理局放射卫生组出版了“工艺验证一般原则指南”。虽然这个指南有其用途，但不包括生产工艺验证要求的所有指定部件。因此，PDA成立了净化和工艺放大生物技术特别小组，以建立用于治疗剂或诊断剂中蛋白质生产使用的柱层分离验证的实用指南。

The goal of validation is to demonstrate that a process, when operated within established limits, produces a product of appropriate and consistent quality. While it may seem that successful validation involves an inordinate amount of work over a long period of time and consumes scarce resources, a strategy which provides for the validation of critical process parameters is vital from both a quality and business perspective. Because no two processes are identical, it is impossible to define exactly what work should be done to accomplish a satisfactory validation. During validation, the critical process parameters should be identified, and, based on sound scientific principles, appropriate studies should be performed to demonstrate that the parameters can be met on a consistent basis. 验证目的是证明工艺在规定范围内操作会产生合适且质量相同的产品。成功的验证似乎需要长时间的过量工作，且消耗稀少资源，但关键工艺参数验证的方法从质量和商业角度都是至关重要的。因为没有两个工艺是相同的，所以不可能准确定义如何完成满意的验证。验证过程中，应确定关键工艺参数，根据合理的科学原理进行相关研究，以证明可以持续达到参数要求。

Validation should be considered as early in the development of a process as is practical. In this way, data required for validation can be collected during development studies and the production of batches for clinical studies. The most appropriate validation data are collected during the production of products to be used in Phase III clinical trials. In addition to the in-process testing, the evaluation of product in humans under carefully monitored clinical trials provides the ultimate test of the safety and efficacy of the product. 工艺前期应考虑验证是否实际。从而验证需要的数据可以在开发研究和临床研究生产过程中进行收集。最重要的验证数据可在临床试验第3阶段的产品生产过程中收集。除了过程中测试，临床试验人员仔细监测的产品评估将提供产品安全性和效力的最终测试。

In preparing this document, we have assumed that the reader is familiar with protein purification in general and column-based separations in particular. For background reading on this subject, the reader is referred to references (1-5). 文件编制时，我们假设读者都熟悉蛋白质净化，特别是柱层分离。关于此主题的背景材料，见参考文件（1-5）。

II. Introduction 介绍

Biotechnology in the pharmaceutical industry combines advances in genetic engineering, cell fusion technology, classical biochemistry and microbiology, traditional pharmaceutical technology, and biochemical engineering to produce once scarce proteins for testing as potential therapeutic or diagnostic agents. These proteins are produced in a suitable host cell by fermentation or cell culture processes. The protein may be either retained intracellularly or secreted into the culture medium. If the protein remains inside the cell, then the cells must be harvested, disrupted, and the debris that is created removed to yield a particulate-free extract for further purification. If the protein is secreted, the cells must be separated from the conditioned culture medium prior to purification. These steps, shown schematically in Figure 1, are often referred to as upstream processing, isolation, or primary separation steps. These upstream steps

usually provide limited purification of the product. 制药行业的生物技术结合了基因工程，细胞融合，经典生物学和微生物学，传统制药技术和生物化学工程的发展，以产生治疗剂或诊断剂测试的一次性稀有蛋白质。这些蛋白质通过发酵或细胞培养基产生在合适的寄主细胞上。这种蛋白质可保留在细胞内或隐藏在培养液中。如果这种蛋白保存在细胞内，那么细胞必须被收集并进行干扰。除去碎片以产生无微粒提取液，用于进一步净化。如果这种蛋白质被隐藏，细胞必须在净化前与培养基进行分离。这些步骤，如图1，通常被称为上游工艺，隔离或主要分离步骤。这些上游步骤通常会提供产品的有限净化。

The downstream processing of biotechnology products usually includes several column-based purification steps. Typically, several column-based separations are necessary to achieve the level of purity required for a protein to be used as a therapeutic agent. These purification processes are based on different properties of the biomolecule, such as shape, size, net ionic charge, hydrophobicity, or specific affinity to another biomolecule. Consequently, there are four main types of column-based separations: gel filtration or size exclusion, ion exchange, reverse-phase or hydrophobic, and affinity. Normally these operations are carried out in a packed column connected to a pump to move fluids through the column and a detector to monitor the effluent stream of the column. These processes are generally batch processes with the packed columns intended for repeated use. 生物技术产品的下游加工通常包括几个柱层净化步骤。通常情况下，一些柱层分离是必要的，以实现作为治疗剂使用的蛋白质纯度。这些净化工艺根据生物分子的不同属性，如形状，大小，净离子电荷，疏水性或与其他生物分子的亲和性。因此，有四个主要类型的柱层分离：凝胶过滤或体积排阻，离子交换，反相或疏水性，亲和性。通常这些活动在连接到泵的填充柱进行，以通过柱排出液体，检测器用于监测柱体流出的气体。这些过程通常为批工艺，填充柱可重复使用。

Many, if not most production scale column separations are adsorption/desorption operations. In adsorption or on-off chromatography, the process stream is fed onto a column until the desired amount of product or impurities are bound to the media. The product or impurity moves rapidly through the column until an unoccupied binding site is found where they adsorb and remain until the elution conditions are changed. Weakly bound species are displaced by more tightly binding ones. After loading, unabsorbed or weakly bound material is washed from the column and the desired species eluted by changing the elution conditions either gradually in a gradient or in a single step. In gel filtration, or size exclusion chromatography, molecules are continuously separated by their differential migration through a packed bed. 许多，即使不是大多数的生产规模柱层分离为吸附/解吸附操作。在吸附或通断色谱法过程中，工艺蒸汽通入柱层直到到达介质所需产品量和杂质量。产品或杂质迅速通过柱层，直到发现未使用的结合位置，在此位置进行吸附并保存直至洗脱条件发生变化。结合力弱的物种由紧密结合的物种代替。载入后，未吸附或结合力弱的材料从柱层冲洗出，通过逐步或单独改变排出条件来排出期望物种。在凝胶过滤或分子排除色谱法中，分子通过填充床的移位持续进行分离。

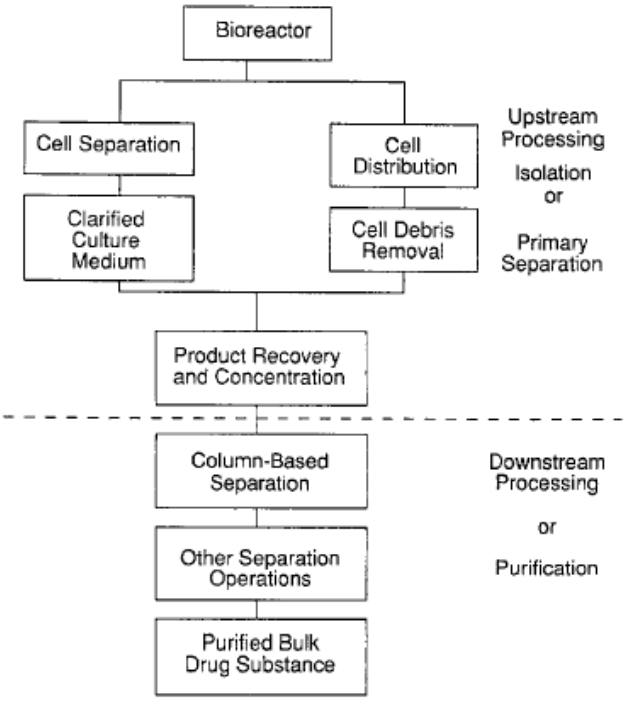

Figure I—Recovery and Purification of rDNA Proteins and Monoclonal Antibodies

Validation is a scientifically rigorous and well-documented study which demonstrates that a process or piece of equipment consistently does what it is intended to do. Due to the complex nature of proteins and the relatively short histories of some cell lines in pharmaceutical manufacturing, it is difficult, if not impossible, to fully characterize a protein product. Thus, final product testing alone is insufficient to ensure consistent manufacture of these products. Therefore, the processes used for the purification of proteins must be designed and validated to remove potential contaminants. The contaminants, which may arise from source material equipment or purification reagents, may include endotoxins, viruses, nucleic acids, and proteins, as well as media constituents, process chemicals, ligands leached from chromatography media, and modifications or inactive forms of the protein itself. 验证是一个科学严谨而详实的研究，证明工艺或设备持续执行既定安排。由于蛋白质的复杂性和一些药品生产细胞系相对较短的历史，完全描述蛋白质产品的特性比较困难，但并非不可能。因此，仅进行最终产品测试不足以确保这些产品的持续生产。因此，必须设计并验证蛋白质净化使用的工艺，以清除潜在污染物。可能产生于原料设备或净化剂的污染物包括内毒素，病毒，核酸和蛋白质，以及介质构成，工艺化学品，从色谱介质析出的配体，蛋白质本身的修改或非活性形态。

Prior to beginning validation, the final protein product should be defined in terms of its physical and biological characteristics. Specifications should be established to ensure uniformity, and the required level of purity should be established based upon the indicated use of the product. Assays used to determine product purity should be validated. The sensitivity of the analytical test methods should permit accurate detection and quantitation of the product as well as impurities. Once these criteria are met, validation of the purification process and equipment can begin. 开始验证之前，最终蛋白质产品应根据其物理和生物特性进行定义。应建立规范以确保一致性，并根据产品的使用建立纯度要求的等级。应验证确定产品纯度的分析方法。分析测试方法的灵敏度应允许对产品和杂质进行精确检测和定量。一旦满足这些标准，可以开始净化工艺和设备的验证。

While validation at full production scale is preferred, cost, practical issues, and safety often make it impractical to generate all the necessary data. For these reasons, laboratory studies utilizing scaled-down columns and "spiking" experiments can yield acceptable validation data. These studies must be designed to model the production process, and product yields and purity must be comparable to those obtained in typical full production runs. Studies characterizing the physicochemical and biochemical interactions in the purification process may be performed at smaller scale. Also, clearance studies performed by challenge or spiking studies with radiolabeled chemicals, toxic chemicals, or infectious biological agents should be done at small scale for reasons of worker safety and to avoid contamination of production equipment. Each process and each validation study should be examined on a case-by-case basis to assess the legitimacy of scaled-down experiments. 虽然在整体生产规模下进行验证是首选，但成本，实际问题和安全性往往导致不能生成所有必要数据。由于这些原因，实验室利用按比例缩小的柱形和“滴定”实验产生可以接受的数据。这些研究必须设计生产工艺模型，产品产量和纯度必须与典型全面投产运行得到的数据类似。净化工艺中关于理化特征及生化特征之间的关系可能以较小规模进行。此外，考虑到工人安全并避免生产设备受污染，用放射性化学品，有毒化学品，传染性生物制剂进行的挑战测试或峰值进行的余隙研究应小规模进行。应逐个检查每个工艺和验证，以评估规模缩小实验的合法性。

Studies involving performance characteristics that are intrinsically dependent on equipment design cannot be modeled on scaled-down equipment and should be performed using the actual production equipment. The effectiveness of washing and cleaning cycles, in the control of endotoxin and bioburden levels are examples of such performance characteristics. The equipment features affecting these characteristics involve the nature of fluid flow and the presence of void spaces in pumps, valves, flow adapters, the column bed itself and, therefore, cannot be modeled in scaled-down experiments. 涉及性能特点且本质上依赖于设备设计的研究无法按比例缩小的设备建模，应该用实际生产设备进行。在控制内毒素和生物负荷等级条件下，性能特征指洗涤和清洁周期的有效性等。该设备影响包括流体的性质及泵、阀、流量适配器、柱床本身余隙的特征，因此，不能按比例缩小的实验建模。

As in all scientific testing, it is important that validation tests and challenges be repeated enough times to assure reliable and meaningful results. Demonstration at production scale that the manufacturing process consistently removes known and potential contaminants may eliminate the need for testing every production batch for certain impurities. Revalidation may be required in the event a process step is removed, added or modified, or when raw materials are obtained from new sources. 正如所有科学试验中提到的，应重复足够次数的验证测试和挑战试验以确保结果可靠并有意义。生产规模中生产工艺持续除去已知和潜在污染物，可能不需要为除去某些杂质而测试每个生产批。验证步骤删去，增加或修改时或从新来源得到原始材料时可能需要再验证。

Process validation of column-based separations will usually cover four major areas: process chemicals, column packing materials, equipment qualifications, and performance qualification of the process itself. Equipment qualifications are normally broken down into installation qualifications (IQ) and operational qualifications (OQ). These qualifications ensure that the equipment is properly installed, calibrated, and functioning according to specification. Performance qualification (PQ) of the process will establish that the process is effective and reproducible, and that the final product meets all established release specifications. In performing process validation, it is important that protocols are prepared which clearly specify the procedures and tests to be conducted, the data to be collected, and the acceptance criteria. The purpose for which data are collected must be clear, the data must reflect facts, and the data must be collected carefully and accurately. The protocol should also specify a sufficient number of replicate process runs to demonstrate reproducibility and to provide an accurate measure of variability among successive runs. All important process variables should be identified, monitored, and documented. Analysis of the data collected will establish the variability of process parameters for individual runs and will establish whether or not the equipment and process controls are adequate to assure that product specifications will be met. 柱层分离的工艺验证通常包括四个主要领域：工艺化学品，柱层包装材料，设备确认和工艺本身的性能确认。设备确认通常分为安装确认（IQ）和运行确认（OQ）。这些确认确保设备已正确安装，校准并按照规范运作。工艺性能确认（PQ）证明工艺是有效，可重复的，且最终产品符合所有既定放行规范。执行工艺验证时，方案应明确规定程序，将进行的测试，将收集的数据和验收标准。数据的收集目的必须明确，数据必须反映事实，且必须认真准确地收集。方案还应该指定足够数量的重复工艺运行以证明其重复性，并在连续运行期间提供准确的测量差。所有重要的工艺变量应查明，监测并记录。对收集的数据进行分析将确定独立运行的工艺参数变量，并确定设备和工艺控制是否足以保证满足产品规格。

While we have divided the validation of column-based separations into several sections which outline some approaches to their validation, it should be noted that the separation of topics is arbitrary. Depending upon the company structure, different organizational approaches are possible. In the biopharmaceutical industry today, process validation studies are typically performed by multidisciplinary groups with representatives from Manufacturing, Engineering, Process Development, Quality Control, and even from Research. 虽然我们将柱层分离验证分为几个部分，并列出验

证的一些方法，但应该指出的是主题的分配是任意的。根据公司结构，可能出现不同的组织方式。在当今生物制药行业，工艺验证研究通常由来自生产部门，工程设计，工艺开发，质量控制，甚至是研究部门的代表执行。

III. Process Chemicals 工艺化学品

Chemical reagents, such as buffer salts, used to prepare solutions for column-based separations should be handled and controlled in the same manner as other raw materials used in pharmaceutical production. For example, appropriate raw material sampling plans and specifications should be developed and approved by Quality Control. Test procedures and stability data should be developed and validated. Procedures should be developed to ensure that only approved materials which have been adequately sampled and tested are released for production use. In addition to chemical identity and purity specifications, raw material specifications should also include limits for bacterial endotoxin and bioburden levels. 化学试剂，如用于制备柱层分离溶液的缓冲盐，应与药品生产使用的其他原料以相同的方式处理和控制。举例来说，适当的原料取样计划和规范应由质量控制部门制定和批准。应开发并验证测试程序和稳定数据。应制定有关程序以确保只有经批准且充分采样和测试的材料可以用于生产。除了化学特性和纯度规范，原料规范还应包括细菌内毒素和生物负荷水平的极限。

The water used in column-based purification steps should have consistent quality appropriate for the process as the type of water may influence the quality of the final product. The bacterial endotoxin and bioburden levels of the water used in column-based separations are important as they will affect the level of these contaminants in the final product. In addition, the metal ion content of process water may be critical in some column-based separations. Water for Injection is generally used for buffer preparation in column-based separations, particularly for the final steps of a purification process. In any case, the water used in column-based separations should meet pre-defined specifications and the water producing system itself should be properly validated. 柱层净化步骤中使用的水应持续有工艺要求的质量，因为水的类型可能影响最终产品的质量。柱层分离用水中细菌内毒素和生物负荷水平非常重要，因为它们会影响最终产品中污染物的含量。此外，工艺用水的金属离子含量在某些柱层分离中可能是至关重要的。注射用水一般用于柱层分离的缓冲液制备，尤其是净化工艺的最后步骤。在任何情况下，柱层分离使用的水应符合预先定义的规格，水生产系统本身应适当验证。

IV. Column Packing Materials 柱层包装材料

Chromatography media for column-based separations are selected to accomplish well-defined functions in the purification process and may have a significant effect on the purity, uniformity, and other characteristics of the product. The packing material should be treated like any other process raw material, i.e., quarantined upon receipt and released for use only after meeting specified criteria. If the media supplier adheres to the PDA's vendor certification plan (6) then a certificate of analysis specifying the results of physical, chemical, and functional tests of the media, along with appropriate

QC testing by the user may be all that is required on a lot-to-lot basis. User tests might include, but are not limited to, pyrogen or bacterial endotoxin testing, particle size and distribution measurements, determination of the swelling factor for dry packing material in buffer, titration curves for ion exchange media, the separation of a standard protein mixture for size exclusion media and binding capacity for all adsorption media. 柱层分离的色谱介质用于完成净化工艺的良好功能，可能对纯度，均匀性及产品的其他特征有显著影响。包装材料应作为任何其他工艺原料处理，即接收后进行隔离，只有在符合特定标准后才能放行使用。如果介质供应商遵循PDA的供应商认证计划（6），指定物理、化学结果、介质功能测试的分析证书，以及用户进行的相关质量控制测试结果可能是批对批的所有要求。用户测试可能包括，但不仅限于，热源或细菌内毒素检测，颗粒大小及分布测量，缓冲区干包装材料膨胀系数的确定，离子交换介质的滴定曲线，根据介质尺寸排除法和所有吸附介质的结合能力进行的标准蛋白质混合物分离。

However, these tests may not always be sufficient to qualify the media for use in the production process. Therefore, it is essential that the user carefully analyze the process to determine if extra testing is required. Additional tests would then be applied to each incoming lot of media. The most important criterion is that the packing material gives a specified purity and yield of the desired product. Other factors, such as selectivity or available capacity for the substance of interest should be defined within set limits. 然而，这些试验可能并不总是足以确认生产过程中使用的介质。因此，用户应仔细分析工艺以确定是否需要额外测试。额外测试将应用于介质的每个输入批。最重要的标准是，包装材料有规定的纯度和所需产品量。其他因素，如物质选择性或有效功率应在设定极限内。

Once media specifications are established it should be possible to reach an agreement with the manufacturer that allows only a sample of the media to be purchased for user testing while the required quantity of the same batch is quarantined pending acceptance. If this course of action is taken, it is advisable to discuss the in-process and quality control procedures that define the acceptance criteria with the media manufacturer, and to include these specifications in the purchase agreement. This will increase the probability that the media is suitable since the manufacturer can verify it meets the defined specifications before purchase. In addition, the user should audit the media supplier's manufacturing facilities for compliance with Good Manufacturing Practices. 一旦建立了介质规范，应与生产商达成协议，即用户测试仅允许使用一个介质样品，而同批所需介质应在接收前进行隔离。如果采用此做法，建议讨论工艺中程序和质量控制程序，与介质生产商确定验收标准，并在收购协议中包括这些规范。这将增加介质适用的概率，因为生产商可确认其符合购买前确定的规范。此外，用户应审计介质生产商的生产设施是否符合良好生产规范。

In systems where the process batch size is small, or in highly automated and recycled systems, prepacked columns may be obtained from the vendor in a ready-to-use format. Prepacked columns present a special case since the contents and the product contact surfaces cannot be inspected by the user. In these cases, the user must have assurance in the form of a certificate of analysis from the

manufacturer that the column meets previously agreed upon specifications with respect to materials of construction, media properties, column packing procedures, and performance specifications. 工艺批尺寸小的系统或高度自动化及再循环系统，可能会从供应商获得现成格式的预包装柱体。预包装柱体代表特殊情况，因为用户无法检测到含量和产品接触表面。在这种情况下，用户必须获得生产商的分析证明，说明柱体在构成材料，介质属性，柱体包装程序和性能指标方面满足先前规定的规格。

V. Equipment Qualification 设备确认

A. Installation Qualifications 安装确认

The *Installation Qualification* (IQ) of process equipment for column systems is documented verification that all aspects of the installation of the equipment adhere to manufacturer's recommendations, appropriate federal, state and local safety, fire and plumbing codes, approved company specifications and design intentions. The IQ demonstrates that the user of the equipment has purchased and installed the right equipment for the specific task. This document demonstrates that the user has considered aspects of compatibility of the equipment with the process and that the user has standard operating procedures (SOPs) for keeping the equipment calibrated and in good operating condition through a preventative maintenance program and spare parts inventory. This document also demonstrates that the user has analyzed the operation of the equipment and determined the level of operator training required by preparing written SOPs covering these activities. The information contained in the IQ document should be verified and the document signed off by representatives of Quality Assurance, Manufacturing, and Engineering. The column system IQ is similar to IQs for other process equipment and might contain some or all of the following information. 柱层系统的工艺设备安装确认（IQ）为文件确认，即设备安装的所有方面符合生产商建议，相关联邦、州和地方安全，防火和管道规范，批准的公司规范和设计意图。IQ证明设备用户已采购并安装特定任务所需的设备。这份文件表明，用户已经考虑设备与工艺的兼容性，而用户的标准操作规程（SOP）可保持设备的校准，并通过预防性维护计划和库存备件保证设备处于良好的运行状态。该文件还表明，用户通过编写SOP已分析了设备的操作，并确定了操作人员的培训水平。应确认IQ文件中包含的信息，该文件需由质量保证部门，生产部门和工程部门签署。柱层系统的IQ与其他工业设备的IQ类似，可能包含以下部分或全部信息。

System Application: This section should briefly describe what processes are to be performed and where the equipment is located. As an overview of the system, a schematic may be included to facilitate review of the IQ document. 系统应用性：本节应简要描述工艺要执行什么及设备置于何处。作为系统的概述，可能包含示意图以方便IQ文件的审查。

Equipment Information Summary: A detailed description of the column system including an equipment summary (manufacturer, model number, serial number) and a description of the components should be provided. The information should be specific enough to clearly define the system. For example, in a typical column system, each

component will be listed and described separately. This may include feed tanks, tubing or piping, pumps, filters, pressure gauges, valves, detectors and the column itself. 设备信息摘要：应提供柱层系统的详细说明，包括设备摘要（制造商，型号，序列号）和部件说明。信息应能明确界定系统。例如，在一个典型的柱层系统中，将分别列出并描述每个部件。这可能包括供给罐，管或管道，泵，过滤器，压力计，阀门，探测器和柱本身。

Utility Description: All utilities supporting the column system should be described and checked to ensure proper installation. For example, the electrical source (voltage, amperage, etc.) should be listed and checked against local codes and the electrical specifications of the system. If the system requires compressed gases or steam, their quality and source should be described and verified. 公用工程说明：所有支持柱系统的公用工程应进行说明和检查，以确保正确安装。例如，电源（电压，安培等）应列出并按照地方法规和系统的电气规范进行检查。如果系统需要压缩气体或蒸汽，应说明并确认其质量和来源。

Standard Operating Procedures (SOP) and Manual Listing: The title and location of all appropriate manuals should be listed and a checklist prepared to ensure that the manuals exist and have been referenced in the installation of a piece of equipment. All SOPs relating to the installation, operation, and maintenance of the equipment should be listed. These documents should contain the drawings and schematics necessary for installing, maintaining, and repairing the column system. 标准操作规程（SOP）以及手动列表：应列出所有相关手册的标题和位置，制成清单，以确保存在手册并在设备安装过程中进行了参考。应列出所有与设备安装，运行和维护相关的SOP。这些文件应包含柱层系统安装，维护和修理必要的图纸和图表。

Spare Plans: A detailed list of recommended spare parts and their location is usually included in the IQ. This spare parts list may either be a separate list or included in the manuals. 备件计划：一份建议备件的详细清单和他们的位置通常包含在IQ中。此备件列表可以作为单独列表或包含在手册中。

Operating Logs: A listing of the name and location of log books which document the use of process equipment is usually included in the IQ document. 操作日志：记录工艺设备的日志名称及位置列表通常包含在IQ文件中。

Process Instrumentation: The type, manufacturer, range, use, and calibration schedule of all process instrumentation should be listed. This list should be divided into critical and non-critical instruments. A *critical instrument* is one whose failure would adversely affect the product's quality or safety. Depending on the system design and complexity, not all instruments are critical instruments. For example, if a system is equipped with a flow meter but process performance is not a strong function of flow rate, then the flow meter may be considered a convenience or non-critical instrument in this system. The distinction is important because critical instruments will be calibrated and maintained on a more rigorous schedule than non-critical instruments. Also,

change orders for a critical instrument will undergo more extensive examination, and failure of a critical instrument during the process will be reviewed more carefully than failure of a non-critical instrument. 工艺仪表：应列出所有工艺仪表的类型，制造商，范围，使用和校准时间表。这个列表应分为关键和非关键仪表。关键仪表指其故障会对产品质量和安全产生不利影响。根据系统设计和复杂性，并不是所有的仪表都是关键仪表。例如，如果一个系统配有流量计，但工艺性能并不是流量的重要功能，那么流量计可以被看作是便捷工具或系统的非关键仪表。这种区别很重要，因为关键仪表将比非关键仪表具有更严格的校准和维护时间表。此外，改变关键仪表的订单需进行大量审查，工艺生产过程中关键仪表的故障将比非关键仪表需要更仔细的审核。

Materials of Construction: Those items which come, or may come in contact with the product, should be described and verified to be compatible with the product and/or process. All components of the system, including lubricants, with the potential for contacting the product, filters, valves, and the column itself, should be included. Table I lists some materials of construction that have been satisfactorily used in column systems to purify proteins. Equipment vendors can often provide appropriate compatibility data; however, the user may have to confirm such data with actual process fluids. If materials leach from the system into the product stream then it should be demonstrated that subsequent process steps remove them. 构成材料：接触或可能会接触产品的部分应予以说明，并核实其与产品和/或工艺的兼容性。系统所有部件应包括可能接触产品的润滑油，过滤器，阀门柱体本身。表1列出了已成功用于蛋白质净化系统的一些材料。设备供应商通常可以提供适当兼容性的数据，但是，用户可能要与实际工艺流体确定这些数据。如果材料从系统渗入产品蒸汽，应证明随后的处理步骤可除去材料。

表1

Column tube construction 柱形管构成 borosilicate glass, stainless steel1, PTFE2-coated stainless steel, E-CTFE3-coated stainless steel, polysulfone, TPX4, epoxy coated fiberglass, high density polypropylene, plexiglass 硼硅玻璃，不锈钢1，PTFE2涂层不锈钢，E-CTFE3涂层不锈钢，聚砜，TPX4，环氧玻璃纤维，高密度聚丙烯，有机玻璃
Column end pieces 柱端片 stainless steel, PTFE-coated stainless steel, E-CTFE-coated stainless steel, polysulfone, ox- irane glass/polycarbonate, polyamide-covered aluminum, polyvinyl chloride 不锈钢，聚四氟乙烯涂层不锈钢，E-CTFE-涂层不锈钢，聚砜，环氧乙烷玻璃/聚碳酸酯，聚酰胺涂层铝，聚氯乙烯
Column O-rings 柱体O形环 EPDM5, FPM6, NBR7, PTFE, silicone rubber (NOTE: The presence of carbon black in O-rings may present a problem. Most nitrile rubber rings contain carbon black) EPDM5，FPM6，NBR7，聚四氟乙烯，硅橡胶（注意：O形环为碳黑色可能存在问题。多数丁腈橡胶圈含有碳黑色）

Netting *网片* polypropylene, stainless steel, polyethylene, polyester, polyamide, silicone rubber, polyvinyl, chloride, PTFE 聚丙烯，不锈钢，聚乙烯，聚酯，聚酰胺，硅橡胶，聚氯乙烯，氯，聚四氟乙烯 *Valves* 阀门 stainless steel, polypropylene, polyvinyl chloride, PTFE 不锈钢，聚丙烯，聚氯乙烯，聚四氟乙烯
1. Stainless steel should always be 316,316L or better quality 不锈钢应为316，316L或更高质量 2. Polytetrafluoroethylene 聚四氟乙烯 3. CTFE: Halor 三氟氯乙烯 4. Polymethyl pentene 聚戊烯 6. fluorocarbon rubber 氟橡胶 7. nitrile rubber 丁腈橡胶

B. Operational Qualification *运行确认*

The *Operational Qualification* (OQ) is documented verification that the equipment, when assembled and used according to the standard operating procedures, does in fact perform its intended function. As with the IQ, the OQ is concerned with the equipment, and not with the product or process per se. The OQ demonstrates that the user has tested the equipment and has found it to be free from mechanical defects or design defects before use in the production process. 运行确认（OQ）书面证明设备按照标准操作规程装配和使用时，能执行预期功能。如同IQ，OQ与设备相关，与产品或工艺本身不相关。OQ表明用户已测试设备，并证明设备在生产使用前无机械故障或设计缺陷。

Before starting the OQ for a column system, the IQ on that system should be completed. Any required calibration for the system should also be completed. Calibration may be part of the IQ or OQ depending on the format chosen by the organization. The IQ and OQ for supporting utilities such as water systems, lighting, heating/cooling and electrical should also be completed prior to starting the OQ for the column system. The OQ document may include the following: 开始柱系统的OQ前，应完成该系统的IQ。系统要求的任何校准也应完成。取决于组织选择的格式，校准可能是IQ或OQ的一部分。 支持公用设备的IQ和OQ，如水系统，照明，加热/冷却和电力也应在开始柱系统的OQ之前完成。该OQ文件可能包括以下内容：

SOP Audits: It should be verified that operators have received the proper training and are able to operate the equipment as intended by following the appropriate operating SOPs. If the equipment is automated, the tests should verify that the equipment responds to the controller as designed. SOP审计：应确认操作者已接受适当培训，并能够根据相关操作SOP来运行设备。如果设备为自动，测试应确认该设备按照设计响应控制器。

System Integrity: The column system should be tested at operating pressures to establish that it is capable of the out leaks. The simplest means of detecting leaks is by visual inspection of the fluid path. Leaks may also be detected in complex systems by demonstrating that the fluid output equals fluid input (fluid mass balance). 系统完整性：该柱系统应以运行压力进行测试，以证明系统可能会泄漏。最简单的检漏方法为目检流径。 通过证明流量输出与输入相等（流体质量平衡）可检测出复杂系统的泄露。

Flows/Pressures: Pumps should be tested to show that they deliver the required flow under normal operating conditions. Tolerances may be established for variations in flow rates. 流量/压力：应测试泵，证明其在正常运行条件下输出要求的流量。可能确定流量的偏差允许范围。

Gradient Formation: For systems operating in a non-isocratic mode, the ability of the system to deliver reproducible gradients of the desired shape and slope should be demonstrated. The effect of variations in the formulation of individual solutions in the final gradient should also be determined. 梯度构成：对于在非恒溶剂模式下运行的系统，应证明系统具有传递所需形状和坡度的可再生梯度的能力。应确定最终梯度中每个溶液配制偏差的影响。

Fraction Collector: If a fraction collector is used, the correct functioning of the fraction collector should be established. The accuracy of the timer/volume counter and correct positioning of the delivery arm should be demonstrated. 馏分收集器：如果使用馏分收集器，应确立馏分收集器的正确功能。应证明计时器/流量计的精确度和传递装置定位正确。

Detectors/Recorders: If the data generated by detectors is to be used in process control, then the acceptable operating range, the limits of linearity response, the reaction time, and the response of the detectors and recorders with operating flow rates should be established. 检测器/记录器：如果检测器生成的数据将用于工艺控制，应确定可接受的运行范围，线性响应极限，反应时间，检测器及记录器与运行流量的响应。

Filters: Filters and filter housings should be examined to verify that they are appropriate for use with the flow rates and pressures likely to be encountered in the system. They should be suitable for their intended purpose whether that be sterilization or particle removal. If filters are used for sterilization, then they should be validated as such. 过滤器：应检查过滤器和滤壳，确认其适用于系统流量和压力。应适用于既定用途，灭菌或颗粒移除。如果过滤器用于灭菌，则需验证。

Computer Control: If computer control is to be used in the operation or cleaning of the column system, validation of the control software and hardware in the system must be addressed (7). It should be shown that the software functions correctly and is protected from unauthorized alteration. The ability of the system hardware to perform its assigned task should also be shown. A schematic of the control logic, including "if-then loop paths," should be included. The validation of computer-controlled systems should be treated in a similar manner to the validation of other computer-controlled processes. 计算机控制：如果柱系统的操作或清洗将使用计算机控制，必须说明系统

软件和硬件控制的验证（7）。应该表明软件功能正常，并防止未授权修改。应说明该系统执行分配任务的能力。控制逻辑图，“如果-然后循环路径”等应包括在内。计算机控制系统验证应该与其他计算机控制的工艺以同样的方式处理。

Alarms: All alarms should be tested by simulation of "alarm conditions" either by actually challenging the system or by electronic simulation. For example, a pressure alarm may be tested by increasing the pressure in the system using pumps and valves; alternately the high pressure may be simulated by sending the appropriate voltage to the alarm mechanism. 报警：所有警报系统都应该以“报警条件”进行模拟，无论是实际挑战系统或电子模拟。例如，压力报警可通过泵和阀门增加系统压力；或者可通过发送适当电压到报警机制来模拟高压。

Other Features / Components: Finally, each system may have unique features or components not found in conventional column systems or in systems used for other applications. Appropriate tests to demonstrate the correct functioning of these features or components should be included in the OQ. 其他功能/组件：最后，每一个系统可能有独特的特征，传统柱层系统或其它应用系统中没有的特征。能证明这些特征或部件运行正确的相关测试应包括在OQ中。

VI. Process Validation of Column-Based Separations 柱层分离工艺验证

A. Performance Qualifications 性能确认

Validation of a column-based separation process involves *Performance Qualifications* which require rigorous testing to demonstrate the effectiveness and reproducibility of the process. The goal of performance qualification is to establish confidence in the performance of a column system under normal as well as "worst case" production conditions. The FDA's guideline on validation discusses the concept of "worst-case" conditions as the extreme of normal operating conditions. The worst case challenge in process validation should simulate those conditions that may *actually* be encountered during the production process. Unrealistic extremes do not constitute a worst-case challenge. Clearance studies, described below, may be used to approximate worst-case challenges with regard to certain contaminants. Validation protocols for performance qualification should list approved test procedures which provide detailed instructions for carrying out testing, formats for records to be developed for the validation data package, and necessary analysis of data to determine acceptability of test results. Performance testing should involve replicate production runs or simulated production runs. Normally, three runs are the minimum required to establish confidence in the consistency of performance. 柱层分离工艺验证包括性能确认，需要严格的测试以证明工艺的有效性和可重复性。性能确认的目标是建立正常条件下柱层系统的性能以及“最坏情况”的生产条件。FDA的验证指南讨论作为正常运行条件的极限的“最坏情况”的概念。工艺验证的最坏情况应模拟实际生产工艺可能遇到的条件。不实际的极限不能构成最坏情况挑战。以下描述的余隙研究可用于某些污染物的最坏情况挑战。性能确认的验证方案列出经批准的程序，提供执行测试，

验证数据包记录格式及确定可接受测试结果的必要分析数据的详细说明。性能确认应包括重复生产运行或模拟生产运行。通常情况下，建立性能持续性至少需要3次运行。

Performance qualification is often carried out using a *concurrent validation* approach in which the process is sampled during actual production runs. However, because it may be impractical and uneconomical to perform reproducibility studies on the actual process, a scaled-down version of the process can be used to conduct *prospective* validation studies. During these prospective studies, radiolabeled reagents, viruses, and other reagents may be employed. When designing these experiments, the scaled down process should be a linear extrapolation of the equipment and process parameters used in the manufacturing process. An important factor to consider when scaling-down any process is the flow rate. It should be scaled-down by the ratio between the cross-sectional area of the production column and the scaled-down column so that the linear velocity remains constant. The column bed height should remain the same as that used in production so the contact time of the feed solution with the media is not altered. For adsorption separations, gradient slope and volume, which are scaled by the ratio between the total volume of the production column and the scaled-down column. The ratio of product loaded to column volume should be kept constant and the product should be present during the tests at the same relative concentrations that it is present during the actual manufacturing process. Finally, to be valid, the yield and purity of the product recovered from the scaled-down column should be consistent with that of the production column. The extent to which a given column-based separation is scaled down for validation will depend upon the actual production scale and the smallest scale that can reliably reproduce the production process. 性能确认通常使用并行验证方法，工艺在实际生产运行中取样。然而，因为在实际工艺进行可重复性研究可能不实际，也不经济。工艺按比例缩小方法可用于进行前瞻性验证研究。在这些前瞻性研究中，可能使用放射性试剂，病毒和其他试剂。设计这些实验时，按比例缩小的工艺应是生产工艺的设备和工艺参数的线性推断。缩小任何工艺需考虑的重要因素是流量。应当按生产柱和缩小柱截面积的比率进行缩小，从而使线速度保持不变。柱床应保持生产时的高度，因此供给溶液与介质的接触时间不变。对于吸附分离，梯度和容量都是由生产柱的总量和比例缩小柱的比率得到的。加载到柱体积的产品比率应保持不变，测试过程中，产品的相对浓度应与实际生产工艺相同。最后，为保证有效，比例缩小柱的产量和产品纯度应与生产柱一致。指定柱层分离按什么程序进行缩小验证取决于实际生产规模和能可靠再生生产工艺的最小规模。

Before a column is used in production, during routine production, and after prolonged storage, it is important that the quality of the packing is checked. This may be performed by measuring the height equivalent to a theoretical plate value (HETP). In practice, a sample solution which represents 2-3% of the packed column volume is run on the column at a chosen flow rate, and the eluted sample peak is recorded by measuring pH, conductivity, ultraviolet light absorbance or other parameters. The test results are assessed from both the HETP value and the peak shape. The peak should be smooth in profile and have a high degree of symmetry with little or no tailing. It is difficult to give examples of acceptable HETP values since they depend upon the test

conditions and column packing, but in general the lower the HETP value the better. The choice of sample used for HETP determinations depends upon the packing material. For example, size exclusion and affinity media can be tested with a 1% sodium chloride solution using water as the eluent. Ion exchange media may be tested with a sample of buffer that is ten times more concentrated than the equilibration buffer. For example, if a cation exchange column is equilibrated in 0.025 M sodium acetate buffer, a 0.25 M sodium acetate solution is a suitable sample. With these samples, the eluted peak is easy to measure with a flow-through conductivity meter connected to a recorder. Other innocuous reagents, such as benzyl alcohol, can be used to collect similar column performance data. Benzyl alcohol is a strong UV-absorbing compound and thus may provide an easy on-line measurement to assess changes in the physical state of the packed bed. The HETP of reverse phase columns can be checked by using compounds generally considered to be safe such as the parabens. 柱用于生产前，日常生产时及长期储存后，对包装质量的检查非常重要。这可能通过测定高度来确定，相当于一个理论板值（板高度）。在实践中，含2-3%填充柱容量的样品溶液以选定的流量在柱层运动，通过测量pH值，电导率，紫外线吸收或其他参数来记录洗脱后的样品峰值。测试结果通过板高度值和峰形进行评估。峰值应为光滑的曲线，并有很少或没有拖尾的高度对称性。很难给出可接受的板高度的例子，因为它们取决于测试条件和柱包装，但一般板高度值越低越好。确定板高度的样品取决于包装材料。例如，尺寸排除和亲和介质可用1%氯化钠溶液进行测试，水作为洗脱液。离子交换介质可用缓冲液样品进行测试，它比平衡缓冲液的浓度至少高10倍。例如，如果一个阳离子交换柱在0.025 M醋酸钠缓冲液中可稳定存在，0.25M醋酸钠溶液则为合适的样本。有了这些样本，洗脱峰值可轻易地通过连接到记录仪的流量电导率仪进行测量。其他无害的试剂，如苯甲醇，可用于收集类似的柱性能数据。苯甲醇是一种强烈的紫外线吸收化合物，可以提供一个简单的在线测量，以评估填充床物理状态的变化。反相柱的板高度通常可以用安全的化合物进行检查，如对羟基苯甲酸酯类。

Column packings may also be assessed by analyzing the peak shape and column performance of a freshly packed column using actual production conditions. In this case, the elution profile of the first manufacturing run on a new column is compared to that obtained during production on a previously used column. Assuming that there are no process changes associated with the new column, the elution profile should be similar to previous profiles on the old column or to a control, thus building a historical or retrospective column record. 柱包装也可通过分析峰形和实际生产条件下的新填充柱的性能进行评估。在这种情况下，新柱第一次生产运行的洗脱曲线与先前柱的曲线进行比较。假设不存在新柱的工艺变更，洗脱形状应与原先柱曲线或控制类似，从而建立柱的历史或可追溯记录。

The most important criterion for validation of a column-based separation is the demonstration that when operated in a specified manner, the overall process, or process step, yields a product of consistent quality which conforms to specifications. Validation should demonstrate that the process will not fail when carried out within the normal operational range of critical process parameters such as buffer pH and ionic strength, gradient shape, amount of material applied per unit volume of packing

material, temperature, flow rate, and system back pressure. Validation of some of the operational ranges of process variables may be conducted either at scale or in scaled-down experiments. The studies may be conducted by changing one parameter at a time with all other parameters fixed, or by using a multifactorial design in which many parameters are changed in a systematic way. 柱层分离验证最重要的标准是证明在制定方式下运行，整个工艺或工艺步骤能产出质量稳定且符合规范的产品。验证应证明工艺在关键参数的正常运行范围内执行不会导致失败，如缓冲液pH值，离子强度，梯度形状，单位包装材料的数量，温度，流量和系统背压。工艺变量的运行范围验证可能在规模试验或规模缩小试验中进行。这些研究可能通过改变一个参数而所有其他参数固定，或通过使用多因素设计，许多参数有规则地改变来执行。

As part of the overall validation effort, the column input feed stream should be characterized in terms of general composition, product concentration, and levels of specific contaminants. The eluate from the column similarly should be characterized and the yield of product measured. Product yield and quality data will serve as indicators of process step reproducibility, especially when analyzed in relation to variations in the column operating parameters. Quality data include biological or immunological activity, protein concentration, as well as product purity. 作为全面验证工作的一部分，柱输入蒸汽应按一般构成，产品浓度和指定污染物的等级进行描述。柱的洗脱液应同样进行描述，产量需测量。产品产量和质量数据将作为工艺步骤可重复性指标器，特别是分析柱运行参数的变化。质量数据包括生物或免疫活动，蛋白质浓度以及产品纯度。

Since production columns are frequently used for a multiple of batches, their cleaning, regeneration, and useful life should be validated. The most common method for validating the cleaning, regeneration, and useful life of packed columns is to manufacture a number of batches of product and analyze these batches using validated analytical and biological assays. Since the lifetime of a packed column may be hundreds or even thousands of cycles, it is usually impractical to validate column lifetimes except concurrently during normal production. One approach would be to establish a set of working criteria for evaluation of column performance and monitor this performance during production over an extended period of time. In this manner, the column's performance is evaluated with each production run against previous successful lots. With time, a database would be generated correlating column life with product quality and yield. Such a data base on each column used in purifying a protein will serve as an aid to determining column life. 由于生产柱通常用于多个批，应验证填充柱的清洗，再生和使用寿命。验证填充柱的清洗，再生和使用寿命的最常用方法是生产一定数量的产品批，并用已验证的分析和生物检定法分析批次。由于填料柱的寿命可能是成百或上千个周期，通常验证柱的寿命不切实际，除非在正常生产的同时进行。一种方法是建立一组工作标准，用于评估柱性能并在生产延长时间内监测此性能。这种方式下，柱性能通过每次运行与先前成功批的对比进行评估。随着时间的推移，将生成关于柱使用周期，产品质量和产量的数据库。这种用于净化蛋白质的每个柱的数据库将作为确定柱使用周期的辅助工具。

During production, careful consideration should be given to avoid irreversible fouling of the column with contaminants which may then leach into subsequent batches. Clearance studies are helpful in determining whether a regeneration process fully removes specified impurities from the column. If the regeneration process does not completely remove these impurities, then it is important to demonstrate that they do not leach off the column during subsequent production runs. 生产过程中，应认真考虑以避免不可挽回的污染物可能会渗入随后的批次。余隙研究有助于确定再生工艺是否能完全除去柱中的指定杂质。如果再生工艺不能完全去除杂质，则要证明其在随后的生产运行期间不会溶于柱中。

Specific criteria by which production columns may be evaluated before and after each use include physical, performance, and microbiological parameters. For smaller columns constructed of acrylic or glass, the column may be physically inspected for cracks or channels in the packed bed and discoloration of the bed or deposits on the top of the bed. Any abnormalities observed should be recorded in a column use log and correlated to product yield and purity. Since it is common for media to become discolored with repeated use, such discoloration may be acceptable if it does not adversely affect product quality. Also, during column equilibration and production, the column back pressure should be monitored. If the column back pressure rises above a predetermined level, appropriate actions can be taken. These actions may include repacking or replacing the media. 生产柱的具体标准可能在每次使用前后进行评估，包括物理，性能及微生物参数。对于较小的由丙烯酸或玻璃构成的柱，可物理检测柱的填充床是否有裂缝或缺口，填充床是否变色或床上方是否有沉积物。发现的任何异常应记录在柱日志上并联系到产量和纯度。既然介质重复使用后通常会变色，不影响产品质量的变色是可以接受的。另外，在柱平衡和生产中，应监测柱的背压。如果柱的背压超过预定的水平，应采取适当措施。这些行动可能包括重新包装或更换介质。

Packed columns may be further evaluated by monitoring the bacterial endotoxin level of the column eluate prior to sample loading and in the purified product. For example, if the bacterial endotoxin level of the column eluate is less than some predetermined value (e.g., < 1 EU/mL by the Limulus Amoebocyte Lysate [LAL] assay) then the column would be acceptable for use. Such a measurement would supplement the rabbit pyrogen test indicating no bacterial endotoxin contamination in either the columns or the final product. 样品装载前，填充柱可通过监测柱洗脱液和纯化产品中细菌内毒素水平来进一步评估。例如，如果柱洗脱液中细菌内毒素水平低于一些预先确定的值，那么该柱可以使用。这种测量将补充家兔热原试验，表明柱或最终产品中无细菌内毒素污染。

Elution profiles can also be monitored for consistency from run to run. Qualitative changes in a profile, such as changes in peak width or symmetry may indicate that the packing material is approaching the end of its useful life. More quantitative changes, such as peak retention times, resolution between peaks, and loss of capacity, may also be used as indicators. Conversely, process deviations, such as feed stream inconsistency, or changes in other control parameters such as flow rate, temperature

or elution conditions, may produce altered profiles. 洗脱曲线图也可以监测运行间的一致性。形状的质变，如峰宽或对称的变化可能表明包装材料已接近其使用寿命。更多量变，如峰值保留时间，峰之间的转换，能量损失也可作为指标。相反，工艺偏差，如供给蒸汽不一致或其他控制参数的变化，如流量，温度或洗脱条件，可能会改变曲线。

Each column-based separation step in the downstream process must have a list of SOPs and appropriate batch records with provisions for recording process deviations. These SOPs and batch records will contain instructions on column packing, column equilibration, column loading and subsequent product recovery. The purification step must be defined and described in terms of intended use and expected characteristics of the eluate. 每个下游工艺的柱层分离步骤必须有SOP列表和相关批记录，包括记录工艺偏差。这些SOP和批记录包括柱包装，柱平衡，柱装载和随后产品复原的说明。净化步骤必须按预期用途和预期的洗脱液的特征加以确定和说明。

To ensure that changes in elution profile do not result from changes in the packed column bed, visual inspection and HETP determination can be used. Over a period of time, changes in HETP values may be correlated with column performance and an acceptance limit set. If used, HETP measurements should be repeated when unexpected results are obtained from a column. 为确保洗脱曲线不受填充柱床的变更影响，可目检和确定板高度值。经过一段时期，板高度值的变化可能与柱的性能和可接受极限相关。如果使用，得到柱的意外结果时应重复板的高度测量。

Finally, validation studies in which scaled down laboratory columns are cycled repetitively may be useful in determining approximate column lifetimes. These studies can support ongoing studies of the production columns and data generated from these small scale studies can be included in the overall validation database. 最后，重复比例缩小实验柱的验证研究，有助于确定柱的大概寿命。这些研究可以支持生产柱的研究，这些缩小研究得到的数据可包括在整个验证数据库中。

VII. Clearance Studies 余隙研究

Purification processes may contain a number of column separations designed to inactivate or remove viral, nucleic acid, immunogenic, and pyrogenic contaminants without effecting the biological activity of the desired product. Successful measurement of elimination or inactivation of contaminants can be determined by specific assays such as radioimmunoassays, enzyme immunoassays and protein blotting directed toward the contaminants. However, the development of such assays can often lag behind the development of the purification process or may not be technically feasible. It is therefore prudent to employ both end-point testing and clearance studies as part of process validation to provide assurance that the process will effectively eliminate or inactivate specified contaminants from the product. 净化过程可能包含大量柱分离，用于灭活或去除病毒，核酸，免疫原和热原污染物，而不影响所需产品的生物活性。污染物去除或灭活的成功测量可通过具体分析确定，如放射免疫分析，酶免疫测定和蛋白质印迹法。然而，这些分析方法的发展往往落后于净化工艺的发展或在技术上并不可行。

因此，使用端点检测和余隙研究作为工艺验证的一部分需谨慎，保证工艺将有效减少产品指定污染物或对其进行灭活。

If noxious or infectious agents are to be used during clearance studies, it is unwise to allow these materials to be applied to actual production columns. These agents may contaminate clinical or commercial product or place manufacturing workers at unnecessary risk. Instead, these studies should be conducted on smaller-scale columns where the process is accurately reproduced to assure that the data generated can be extrapolated to production-scale columns. To accomplish this scale-down in an effective manner, the column media under test should be of the same type and preferably, the same production lot as that used in the process-scale column. Furthermore, all significant process parameters such as protein load to column volume ratios, column bed height, linear velocities, temperature, buffer compositions, and ratios of mobile phase volume to column volume should be maintained. 如果有毒剂或传染剂将用于余隙研究，则不允许将这些材料应用于实际生产柱。这些试剂可能污染临床或商业产品或使生产工人遭遇不必要的风险。相反，这些研究应用于小规模柱，工艺准确再生，确保生成的数据可推断出生产规模的柱。为有效完成比例缩小研究，测试中的柱介质应为相同的类型，最好与工艺规模柱是同一产品批。此外，应维持所有重要工艺参数，如蛋白质与柱容量的比，柱床高度，线性速度，温度，缓冲液成分，流动相体积与柱体积的比例。

In clearance studies, a particular contaminant is added to the input feed stream on a small scale and the recovery of the contaminant is measured at each stage of the process step such as the column flow-through, product pool and regeneration fractions using scaled-down columns. The addition of the contaminant should be kept to a minimum so the concentration of the feed stream is not significantly changed. The addition of the contaminant should not significantly alter the behavior of product recovery. Measurements for mass balance calculations should be performed on column flow-through (non-binding materials), eluted fractions, regeneration, and cleaning steps. Mass balance during regeneration and cleaning steps is critical in assessing whether or not the column packing material can be reused. 在余隙研究中，特殊污染物添加到小规模的输入供给蒸汽，在每个工艺步骤阶段测量污染物的复原情况，如比例缩小的柱流，产品库和再生馏分。污染物的增加应保持最低限度，从而供给蒸汽浓度不会有明显改变。污染物的增加应该不会明显改变产品复原。质量平衡计算的测量应包括柱流（非结合性材料），洗脱馏分，再生和清洗步骤。再生和清洗步骤的质量平衡在评估柱包装材料是否可再使用方面非常关键。

A clearance factor can be calculated as shown in equation 1 by dividing the number of units introduced by the number of units recovered in the product after that step: 余隙系数可以根据公式1计算出，产品单元数除以复原的单元数：

$$CFi = I/O \qquad (1)$$

where CF, is the clearance factor for the ith step in the process, I represents the number of units introduced at the start of the process step and *O* represents the number of units recovered after the process step. CF代表工艺中第i步的余隙系数，I代表工艺步骤开始时的单元数，O代表工艺结束后复原的单元数。

Each step in a purification process should be challenged separately. The clearance of a particular contaminant by each step of the process can then be calculated. In general, the overall clearance factor for the process (CF_t) is the product of the clearance factors for each step: 净化工艺中的每一步都应分别进行挑战。工艺每个步骤的特殊污染物余隙可以计算出来。一般而言，工艺的整体余隙系数是每步余隙系数的乘积：

$$CF, = (CF, \bullet CF_2 \bullet CF_y \ldots CF_{„}) \quad (2)$$

When radiolabeled tracers are used in clearance studies, the interpretation of clearance factors may be more complex. If the tracer is a homogeneous species, or if the tracer behaves as if it were a homogenous species in the process under study, then the clearance of the tracer in each step is independent of the sequence in which the steps are performed. In this case, the clearance factors measured at each step in the process may be multiplied together with the resulting product representing the clearance factor for the entire process. 余隙研究中使用放射性示踪剂时，余隙系数的说明可能更加复杂。如果示踪剂是同质种类，或者如果示踪剂作为工艺中的同质种类发挥作用，每个步骤中的示踪剂余隙则独立于步骤中的顺序。在这种情况下，工艺中每步测量的余隙系数可能会成倍增加，导致其乘积代表整个工艺的余隙系数。

If the tracer used is not homogeneous, then the interpretation of clearance factors may be more difficult. [P^{32}]DNA commonly used in clearance studies is an example of a heterogeneous tracer. It is a chemically diverse population; i.e. the population consists of molecules of different nucleotide sequences of various lengths with a distribution of molecular weights. Some of the separation methods used in the purification of recombinant proteins are insensitive to either the nucleotide sequence or the molecular weight distribution. For these processes, the assumption that [P^{32}]DNA behaves as a single homogeneous species is a valid one, and the clearance factors obtained at each step may be multiplied together to give an overall clearance factor for the process. 如果使用的示踪剂不是同质的，那么余隙系数的说明可能更加困难。 余隙研究常用的DNA是异类示踪剂。这是化学多样化群种；即群种由不同核苷酸序列的分子构成，长度不同，按分子量分布。重组蛋白质净化使用的一些分离方法不受核苷酸序列或分子量分布影响。对于这些工艺，假设DNA作为单一同质种类工作是有效的，每步得到的余隙系数可能会大量增加，形成工艺的整体余隙系数。

Radiolabeled host cell protein is another example of a chemically diverse population of molecules which behaves as an heterogeneous population in protein purification processes. The population may consist of several hundred labeled proteins which are heterogeneous with respect to charge, hydrophobicity, thiol content, and molecular weight. Therefore, the product of the clearance factors obtained from each

step may have no practical meaning. 放射性宿主细胞蛋白则是分子化学多样性群体的另一例子，作为蛋白质净化工艺的异质群体。群体可能由数百个标记的蛋白质构成，电荷，疏水性，硫醇含量和分子量都不同。因此，每一步得到的余隙系数乘积可能没有实际意义。

The principle contaminants which may require clearance studies are media components, host cell proteins, nucleic acids, viruses, pyrogens, and materials leached from bio-affinity media. 可能需要余隙研究的主要污染物为介质成分，宿主细胞蛋白质，核酸，病毒，热原，从生物亲和介质浸出的材料。

Host Cell Proteins: The clearance of host cell proteins may be measured by using a direct immunoassay of these proteins in the actual process stream. Production source proteins are prepared from the host organism or cell which contains a plasmid constructed to have all the DNA sequences except those for the gene encoding the protein product, or from the parent myeloma cell of a hybridoma. These proteins are isolated and polyclonal antibodies prepared against them. As an alternative, clearance of host cell proteins can be determined by radiolabelling these production source proteins and then adding or "spiking" them into the appropriate crude feed stream or intermediate stream to the column under scaled down test conditions. The total radioactivity of all the fractions collected is determined and compared to the total loaded radioactivity for mass balance determinations. Fractions containing product are then pooled and the clearance factor calculated. The clearance factor is the ratio of total loaded radioactivity to radioactivity contained in the product fraction. The overall clearance factor for the purification process will vary according to specificity, selectivity, and the number of process steps. 宿主细胞蛋白：宿主细胞蛋白质的余隙可通过对实际工艺蒸汽中蛋白质的直接免疫测定来测量。生产源蛋白是从宿主有机物或含质粒的细胞中制备，除了基因编码的蛋白质产品外都有全部的DNA序列，或来自杂种细胞的母体骨髓瘤细胞。这些蛋白质相互隔离并有克隆抗体。或者，宿主细胞蛋白的余隙可通过对产品源蛋白进行射线标记来确定，接着在比例缩小测试条件下将其添加或“滴定”到相关粗供给蒸汽或中间蒸汽中。确定所有收集的馏分放射性并与质量平衡确定值的总装载放射性进行比较。储存含产品的馏分并计算余隙系数。余隙系数是总装载放射性与产品馏分中放射性的比率。净化工艺的整体余隙系数将因特异性，选择性以及工艺步骤数量而异。

Nucleic Acids: Production source DNA, extracted from the host strain or cell line, may be radiolabeled by nick-translation. After separating free radioactivity from the labeled DNA, the labeled DNA is spiked into the feed stream and applied to the column under test. Radioactivity is measured in every fraction, similar to the host cell protein example above. As with host cell protein, it is important that the labeled DNA be essentially free of low molecular weight radioactivity which will bias the results. The size of the labeled DNA should be similar to that found in the initial crude extracts or conditioned media, since the ability of different chromatographic steps to clear DNA can be a function of the molecular weight of the nucleic acid. Clearance factors are determined by calculating the ratio of radioactivity loaded on the column to the

radioactivity contained in the product fraction(s). 核酸：从宿主菌株或细胞系提取的生产源DNA，可通过缺口平移进行放射标记。从标记的DNA分离出自由放射物后，该标记的DNA添加到供给蒸汽中并应用于测试柱。需测量每个分馏物的放射性，类似于以上宿主细胞蛋白范例。关于宿主细胞蛋白质，标记的DNA应没有能影响结果的低分子量放射性。标记的DNA大小应类似于初始粗提物或条件培养液中的尺寸，因为DNA不同色谱步骤的能力可作为核酸分子量的功能团。余隙系数通过计算柱放射性与产品馏分放射性的比率确定。

In addition to scaled down studies using radiolabeled nucleic acids, DNA clearance can also be determined by directly measuring the DNA levels in the actual production stream using non-radioactive assay methods to directly measure the DNA in column fractions (16). 除了比例缩小研究使用放射标记核酸，DNA余隙也能通过直接测量实际生产蒸汽中的DNA等级来确定，即用非放射性分析法直接测量柱馏分中的DNA（16）。

Viruses: When mammalian cells are used as substrates to produce a protein product, there is concern that the cell lines may harbor viruses. Endogenous retroviruses are widespread in animal populations and have been described in species as diverse as reptiles, birds, and many mammals. For example, murine hybridomas used in the production of monoclonal antibodies are known to express retroviruses which may have the potential to transform cells. Other rodent cell lines such as Chinese Hamster Ovary (CHO) and Baby Hamster Kidney (BHK) may also contain these endogenous retroviruses. In the absence of a specifically identified viral contaminant in the product cell line, the potential presence of retroviral particles is of greatest concern. In addition, concerns regarding bovine viruses and prions are also increasing among regulatory agencies. 病毒：当哺乳动物细胞用于培养基产出蛋白质产品时，令人担心的是细胞系可能带有病毒。内源性反转录病毒普遍存在于动物种群中，如爬行动物，鸟类和许多哺乳动物。例如，用于单克隆抗体生产的小鼠杂交瘤细胞会有反转录病毒，可能会改变细胞。其他啮齿动物细胞系，如中国仓鼠卵巢（CHO）和幼仓鼠肾（BHK）也可能包含这些内源性反转录病毒。产品细胞系无确定的病毒性污染物时，潜在的病毒粒子是最令人关注的。此外，监管机构也越来越关注牛病毒和朊病毒。

The most appropriate way to assure that viruses do not co-purify with product is to test and select production cells and media components that are free from known adventitious viral contamination. Since most cell lines currently used in production are derived from sources that cannot be certified as free of endogenous viruses, and since adventitious agents may enter the production process and propagate in cells, viral clearance studies for products derived from cell culture are essential. Virus clearance is most readily measured by small scale spiking experiments. Viral clearance should include both virus removal and inactivation. Although there are no FDA or industry written guidelines, it is desirable to have clearance factors several logs greater than the theoretical titer of infectious virus per dose of product. A theoretical worst case titer may be estimated from electron microscope (EM) pictures of the cell culture fluids from which the product is purified. This information, combined with the process yields and

the expected dose size, is used to compute the number of viruses which would be carried into the dosage unit if there were no clearance by the purification process. 确保病毒不会影响产品的最恰当方式是测试并选择不含已知病毒污染的生产细胞和介质组件。由于目前生产中使用的大多数细胞系来源不能被视为无内源性病毒，由于不定制剂可能进入生产工艺并在细胞中繁殖，对细胞培养得到的产品进行病毒余隙研究则非常重要。通过小规模滴定实验测量病毒余隙是最容易的。病毒余隙应包括病毒清除和失活。虽然没有书面FDA或行业准则，最好每剂产品的余隙系数比理论传染性病毒滴度大几个对数。滴度的理论最坏情况可能从产品纯化细胞培养液的电子显微镜图片中估算出。此信息与工艺产量和预期剂量大小相结合，可用于计算如果净化工艺不产生余隙的情况下，将进入剂量单位的病毒数。

In addition to characterizing the viruses contained in the cell line, it is important to demonstrate that the purification process can remove and/or inactivate those viruses which may be indigenous to the cell line but remain undetected. It is, therefore, desirable to perform spiking experiments with viruses that can be cultivated to a high titer, which have well established detection assays, and which do not present health hazards. 除了描述细胞系中病毒的特征，重要的是证明净化工艺可除去和/或灭活细胞系中可能固有但仍未检测到的病毒。因此，最好用可以培养到高滴度的病毒进行滴定实验，其有完备的检测分析方法且不会对健康形成危害。

For proteins produced by recombinant DNA technology or naturally by human cell lines, virus removal or inactivation validation should include a collection of model viruses possessing a range of biophysical and structural features (15). Table II lists several viruses which have been used in virus validation studies. The viruses used should include enveloped and non-enveloped DNA and RNA viruses which have different diameters and geometries. DNA viruses such as Herpes Simplex 1 (enveloped) and SV-40 (non-enveloped) and the RNA viruses, Sabin Type I Polio (non-enveloped) and Influenza Type A (enveloped) represent typical challenge viruses. When rodent cells such as CHO, BHK, C127, and murine hybridomas are used for production, then Moloney murine leukemia virus may be used as a model retrovirus. When choosing an appropriate challenge virus, preference should be given to those viruses which display a significant resistance to physical and/or chemical agents. 对于由重组DNA技术或人类细胞系产生的蛋白质，病毒去除或灭活验证应包括有生物物理和结构特点（15）的病毒模型。表2列出了已用于病毒验证研究的几种病毒。使用的病毒应包括包膜和非包膜DNA和RNA病毒，有不同的直径和几何形状。DNA病毒，如单纯性疱疹（包膜）和SV - 40（非包膜），RNA病毒，萨宾I型脊髓灰质炎（非包膜）和A型流行性感冒（包膜）代表典型的挑战病毒。啮齿目动物的细胞，例如CHO，BHK，C127和小鼠杂交瘤细胞用于生产时，莫洛尼鼠类白血病病毒可用于反转录病毒模型。选择一个适当的挑战病毒时，应优先考虑那些对物理/或化学剂有抗药性的病毒。

TABLE II

Examples of Viruses Which Have been Used in Virus Validation Studies 病毒验证研究用到的病毒样例

Virus 病毒	Family 系	Natural Host 天然宿主	Genome 基因组	Enveloped 包膜	Size (nm) 大小	Shape 形状	Resistance to Physiochemical Reagents 对物理化学剂的抗药性
Poliovirus 脊髓灰质炎病毒	Picorna 细小核糖核酸	Man 人	RNA	No	25-30	Icosahedral二十面体	Medium 中
Sabin Type 1 萨宾							
Reovirus 3 呼肠孤病毒	Reo氧化物	Various 多种	RNA	No	60-80	Spherical 球体	High 高
SV40	Papova 组病毒	Monkey 猴子	DNA	No	45	Icosahedral 二十面体	High 高
Murine鼠科病毒	Retro	Mouse 鼠	RNA	Yes	80-110	Spherical 球体	Low 低
Leukemia 白血病							
Virus 病毒 (MuLV)							
HIV	Retro	Man 人	RNA	Yes	80-100	Spherical 球体	Low 低
Vesicular水泡	Rhabdo 棒状病毒	Bovine 牛	RNA	Yes	80-90	Bullet 锥形体	Low 低
Stomatitis 口腔炎							
Virus 病毒							
Parainfluenza 副流感病毒	Paramyx o副粘液病毒	Various 多种	RNA	Yes	150-300	Pleo-Sphere 多面球体	Low 低
Virus 病毒							
Pseudorablies 假性狂犬病	Herpes 疱疹	Swine 猪	DNA	Yes	120-200	Spherical 球体	Medium 中
Virus 病毒							

Clearance studies similar to those described above for host cell proteins and DNA, may be performed by spiking model viruses into the production stream and measuring their removal on scaled-down columns. The clearance of virus particles may also be measured using radiolabeled virus. Radiolabeled virus can be prepared in a similar manner to the preparation of labeled host cell proteins using [H^3], [C^{14}] or [S^{35}]-labeled amino acids. As mentioned above, care should be taken to prepare labeled virus which

is free from low molecular weight labeled contaminants. Each stage of the purification process should be individually assessed for its ability to remove or inactivate virus. The overall clearance factor can be determined from individual clearance factors. Care should be taken in calculating the overall clearance factor however. The assumption that clearance factors of different steps may be multiplied to give the overall clearance factor may not always be valid. Clearance factors may only be additive, for example, if the mechanism of virus removal in two different steps is the same. 余隙研究类似于上述的宿主细胞蛋白质和DNA，可将模型病毒滴加到生产蒸汽中，并在按比例缩小的柱上进行测量。病毒颗粒的余隙也可以通过放射标记病毒进行测量。放射标记病毒可以与标记的宿主细胞蛋白一样用[H^3], [C^{14}] or [S^{35}]-标记氨基酸制备。如上所述，应小心制备无低分子量标记的污染物病毒。应分别评估每个工艺阶段去除或灭活病毒的能力。整个余隙系数可通过单个余隙系数确定。应小心计算总余隙系数。假设每步的余隙系数都成倍增加，那么总余隙系数可能并不总是有效的。余隙系数只能累积，例如，两个不同步骤的病毒去除机制是相同的。

Since membrane-enveloped virus may shed surface proteins, the assay for virus particles should include steps to distinguish viral particles from shed proteins. Alternatively, since retroviruses contain a specific enzyme marker, reverse transcriptase, it may be possible to demonstrate their clearance through the use of an enzymatic or immunologic assay for reverse transcriptase. However, the reverse transcriptase assay is inaccurate at low concentrations and in crude samples care should be taken to avoid interferences from cellular DNA polymerases. 由于包膜病毒可能脱落表面蛋白质，病毒颗粒的分析应包括能区别病毒颗粒与脱落蛋白质的步骤。另外，由于反转录病毒含有一种特殊的酶标记，反转录酶，分析它的酶和免疫可能会证明余隙。然而，在低浓度和原样品中，反转录分析法是不准确的，应注意避免细胞DNA聚合酶的干扰。

In addition to demonstrating removal of viral particles, virus inactivation should also be measured. Retroviruses are labile species and a well-designed process may include steps which can be validated as virus inactivation steps. A column-based separation may provide viral inactivation as well as removal, especially if non-neutral pH, denaturing reagents, or organic solvents (as used for HPLC) are used. To demonstrate virus inactivation, the virus may be spiked into a process solution and incubated under time and temperature conditions which model the normal production process. When conducting these inactivation studies, it is desirable to determine the kinetics of inactivation as well as the extent of inactivation because virus inactivation has been demonstrated in some cases to be a complex reaction with a "fast phase" and a "slow phase" (15). The inactivation study should be performed in such a way that samples are taken at different times and an inactivation curve constructed. To do these studies, a high titer virus stock is needed, as well as the appropriate infectivity assay. 除了证明能除去病毒颗粒，也应测量病毒灭活。反转录病毒是不稳定物种，精心设计的工艺可能包括可作为病毒灭活验证的步骤。柱层分离可提供病毒灭活及去除，特别是使用非中性pH，变性试剂或有机溶剂（如用于HPLC）。为了证明病毒灭活，病毒可滴定到工艺溶液中，在正常生产工艺时间和温度条件下培养。进行这些失活研究时，最好确

定失活的动力学以及失活程度，因为在某些情况下病毒失活与“快速阶段”和“慢速阶段”有复杂的作用。进行失活研究时，应在不同的时间进行取样，形成失活曲线。进行这些研究时，需要高滴度病毒库以及相关的感染检测。

Pyrogens: The LAL assay for gram-negative bacterial endotoxins is sensitive enough for detection at concentrations at least an order of magnitude below levels which will produce a pyrogenic reaction in the rabbit pyrogen test. The possibility of inhibition of the LAL assay by the protein product, however, must be ascertained through validation of the LAL test. Since a sensitive assay is available and protein inhibition may be overcome, clearance studies may not be necessary. Good process control and hygiene, i.e., LAL testing of all raw materials, microfiltration or ultrafiltration of process buffers, and cleaning and sanitization of columns after each use, will minimize the potential for endotoxin contamination and, hence, the need for clearance studies. If it is desired to perform clearance studies, they may be carried out similarly to the methods described above. Clearance studies for bacterial endotoxin removal may also be valuable in identifying the step(s) useful in reprocessing batches that fail the final pyrogen test. Many column-based separation methods are useful in reducing bacterial endotoxin levels, provided that the selectivity of the media is such that co-purification of the bacterial endotoxin and product is avoided. 热原：革兰氏阴性细菌内毒素的LAL对低于等级的浓度有足够的敏感度，从而产生兔热原试验的热原反应。蛋白质产品的LAL分析可能有抑制作用，但必须通过LAL试验验证。由于存在敏感性检测，蛋白质抑制作用可以克服，可能没有必要进行余隙研究。良好的工艺控制和卫生，即对所有原材料，工艺缓冲液的微滤和超滤，使用后柱的清洗和灭菌进行LAL测试将减少内毒素污染的可能性，因此需要进行余隙研究。如果想要执行余隙研究，方法可能与上述类似。细菌内毒素去除的余隙研究也可用于确定最终热原试验失败的再处理批的步骤。许多柱层分离方法可减少细菌内毒素水平，只要选择的介质能避免细菌内毒素和产品的相互净化。

Ligand Leakage From Affinity Media: Affinity media are prepared by covalently coupling a biospecific ligand to the support antibodies (mono- and polyclonal) or proteins, such as Protein A, have specific binding affinity for the protein product. There are many chemistries available for coupling the ligand to the chromatographic support. The choice of chemistry is determined on a case-by-case basis. Affinity columns are known to release or leak ligand (protein) from the support during purification processes. This ligand leakage may result from either chemical cleavage of the covalent bond or physical sloughing of media fragments containing the ligand. Ligand leakage can change with time, and leakage may be higher when the media are new. In either case, the released ligand becomes a contaminant in the product. Since these ligands may themselves be derived from rDNA or hybridoma technology, it is important that the ligand be of high purity, well characterized, and produced by validated processes before coupling to the chromatographic support. 亲和介质配体泄漏：亲和介质由生物特异性配体共价耦合到载体抗体（单和多细胞系）或蛋白质，如蛋白A对蛋白产品具有特定的结合亲和。有多种化学物质可将配体耦合到色谱载体。化学品是逐个确定的。亲和柱通常在净化工艺中从载体中释放或泄漏配体（蛋白质）。这种配体泄漏可能由共价键

的化学分裂或包含配体的介质碎片的物理分离造成。配体泄漏可随着时间而改变，当介质是新的时，渗漏率可能会更高。在两种情况下，释放的配体都成为产品污染。由于这些配体可能自行从rDNA或杂交技术产生，配体需有高纯度，特点描述良好，耦合到色谱载体前由已验证的工艺产出。

In order to detect ligand leakage, appropriate assays with high sensitivity may need be developed to measure the level of ligand in the product. In addition to monitoring ligand leakage in the actual production process stream, radiolabeled ligand can be coupled to a support on a small scale and leakage rates monitored under continuous exposure to binding and elution conditions. 为了检测配体渗漏，可能需要开发高灵敏度的相关分析法，以测量产品的配位水平。除了监测实际生产工艺蒸汽中的配体渗漏，放射标记的配体可以耦合到小规模载体，渗漏率在持续暴露于黏合和洗脱的条件下监测。

Ligand leakage in the product can be minimized by choosing the appropriate support and coupling chemistry and conditioning the column by repeated washing with binding and elution buffers prior to use in production. Also, inclusion of additional processing steps after the affinity step that specifically remove leaked ligand from the product may provide assurance that the final product will be free of the affinity ligand. If such affinity processing steps are included, clearance studies demonstrating the ability of the process to remove leaked ligand from the product should be performed. 产品配体渗漏可通过选择适当的载体和耦合化学剂来减少，使用前，用黏合和洗脱缓冲液重复清洗处理。此外，亲和性步骤之后增加的处理步骤可专门从产品中除去渗漏配体，保证最终产品中无亲和性配体。如果包括这些亲和性处理步骤，应进行能证明工艺除去产品中渗漏配体能力的余隙研究。

VIII. Summary 总结

Validation of column-based separations is necessary to ensure the quality and safety of protein and peptide products produced by rDNA, peptide synthesis, and hybridoma technologies. Process validation for column-based separations includes qualification of raw materials, equipment, and the purification process. Combined with in-process control and quality control of the final product, column validation ensures that a uniform product is produced consistently from batch to batch. 柱层分离验证可确保rDNA，肽合成物和杂交瘤技术产生的蛋白质和肽产品的质量和安全性。柱层分离的工艺验证包括原材料，设备及净化工艺的确认。结合最终产品的过程中控制和质量控制，柱验证确保每批生产出统一的产品。

In the best case, validation is designed into the process. During process design, techniques are selected which can remove impurities and contaminants. Equipment and chromatographic media which can perform reproducibly are selected. Column performance standards, cleaning and regeneration routines, and column life should be considered as early as possible. Clearance studies should be planned and implemented to ensure that a product is produced with the requisite purity. 在最好的情况下，工艺包括验证。工艺设计过程中，选择的技术可以去除杂质和污染物。选择可

再生产的设备和色谱介质。应尽早考虑柱性能标准，清洁，再生程序和柱的使用寿命。应规划并执行余隙研究，以确保生产出所需产品纯度。

There are no explicit rules for process validation of column-based separation processes. This document is intended to serve as a starting point for those needing to validate column-based separation processes. 柱层分离的工艺验证没有明确的规定。本文件仅作为柱层分离工艺验证的起始点。