SWISS PHARMA

Pharmaceutical Quality Assurance
Validation – an important issue of cGMP
Swiss Federal Institute of Technology (ETH), Zürich / University of Basel
Winter Semester 1992/93

6-S/1993

Swiss Review for the Pharmaceutical Industry
Revue suisse pour l'industrie pharmaceutique
Rivista svizzera per l'industria farmaceutica
Revista suiza para la industria farmacéutica
Schweizerische Zeitschrift für die pharmazeutische Industrie

Reinraumtechnik und Kostendruck in der pharmazeutischen Produktion

SWISS PHARMA 3-S/1994

Vorträge der Herbsttagung der Schweizerischen Gesellschaft für Reinraumtechnik (SRRT) vom 29. Oktober 1993 bei Sandoz Pharma AG in Basel

Editorial

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- Dr. rer. nat. Hans-J. Köhler, Ciba-Geigy AG, Stein

Zur Kostenstruktur für den Bau und Betrieb von Reinräumen
- J. Bruderer, Luwa AG, Zürich

Ansätze zur Kostenreduktion für die Bereitstellung von Reinräumen
- Dr. phil. Claude Moirandat, Skan AG, Basel

Répartition en ampoules injectables sous isolateur
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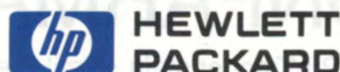

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Impressum 4

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Pharmaceutical Quality Assurance

Validation – an important issue of cGMP

Swiss Federal Institute of Technology (ETH), Zürich

University of Basel

Winter Semester 1992/1993

PD Dr. Hans W. Schmid

Editorial 5

«Pharmaceutical Quality Assurance» and «Validation – an important issue of cGMP»
– St. Marrer, H. W. Schmid, Zürich 5

Papers 7

Pharmaceutical Quality Assurance: Basics of validation
– K. Tomamichel, Cham
– J. Jordan, Basel
– N. Freitag, Schaffhausen
– B. Thurnbauer, Melsungen (D)
– P. Applewhite, Sisseln 7

Production systems for water injection: Validation issues and new technical concepts
– M. J. Moor, Schachen
– H. Buser, Basel
– I. Dentler, Basel
– P. Fürer, Basel
– C. Planzer, Muttenz 16

Pharmaceutical Quality Assurance: Validation of analytical methods in vitamin assays
– E. Frewein, Zürich
– St. Geisshüsler, Schaffhausen
– E. Sibler, Wolhusen 23

Pharmaceutical Quality Assurance: The luciferin-luciferase assay: An example of validating an enzymatic analytical method
– Ch. Goez, Zürich
– T.-D. Luu, Muttenz
– D. J. Meier, Zürich 30

Quality Assurance in the Pharmaceutical Industry: Validation in packaging of pharmaceutical products
– C. Geiger, Basel
– H. Disch, Mitlödi
– P. Jung, Bergdietikon AG
– R. Tengler, Ittigen 33

«Pharmaceutical Quality Assurance» and «Validation – an important issue of cGMP»: Process Validation on Tablets
– F. Bodinka, Liesberg
– St. Merkle, Schaffhausen
– S. Moret, Bern
– F. Naeff, Kirchberg
– R. Naeff, Schaffhausen
– P. Scheidecker, Aesch 39

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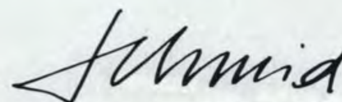
Results of the six project groups of the second postgraduate seminar for Pharmaceutical Quality Assurance, winter semester 1992/93, Department of Pharmacy, Swiss Federal Institute of Technology (ETH), and the University of Basel, Pharmaceutical Faculty.

"Pharmaceutical Quality Assurance" and "Validation – an important issue of cGMP"

Postgraduate Seminar "Pharmaceutical Quality Assurance"
During the past two decades, quality assurance has become a main concept of current good manufacturing practices (cGMP) in the pharmaceutical industry. In view of the complexity of comprehensive quality assurance strategies and the ongoing development in this field, the Pharmaceutical Department of the Swiss Federal Institute of Technology and the Pharmaceutical Faculty of the University of Basel organised the second postgraduate seminar for Pharmaceutical Quality Assurance during the 1992/1993 winter semester. The objective of the seminar was to offer interested representatives from industry, universities and the authorities a forum for a postgraduate education in the rapidly moving and evolving field of quality assurance.

Validation – An Important Issue of cGMP
The subject of the second postgraduate seminar was "Validation – A Key Issue of Good Manufacturing Practices". Validation issues cover the entire spectrum of cGMP concerns, most of which are essentially buildings and equipment, processes, packaging operations, analytical methods and legal aspects. Five project groups of three to six participants selected the topics and defined the objectives. At regular meetings of each project group, data and results were discussed and integrated. Four full-day working meetings with all participants were organised and each project group regularly presented its results. The work of the project groups was challenged and supported by various experts from industry and the authorities. The broad experience and know-how of these experts substantially contributed to the successful development of the seminar. The objective of the seminar was to publish each topic covered by the project groups in a concise form. The papers are published in SWISS PHARMA 6-S/1993. They present a selection of summaries, procedures and approaches which may provide a basis for daily validation work.*



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Reinraum-Monitoring

Warum Reinraum-Monitoring?

Die vielfältigen nationalen wie internationalen Qualitätsnormen sowie die Importanforderungen der Abnehmerländer stellen an eine Pharma-Produktionsanlage sehr hohe Anforderungen. Personal wie Produktionsanlagen und Prozesse müssen diesen Massstäben tagtäglich genügen. Eine interne Qualitätsüberwachung ist daher unumgänglich. Im Reinraumbereich solcher Produktionsanlagen müssen nebst den zu erfüllenden Normen für das Produkt stets auch physikalische Messdaten über den gesamten Produktionszyklus erfasst werden. Nur so können Unregelmässigkeiten erkannt, Pannen vermieden und die Qualität der Produkte gesichert werden. Wichtige Messgrössen hierbei sind: Raumtemperatur und relative Feuchte, Differenzdruck, Luftgeschwindigkeit sowie Partikelbelastung in der Reinluft. Diese Werte können nun über entsprechende Schreiber und sonstige Ablesegeräte angezeigt werden oder aber permanent und jederzeit zu weiteren Datenauswertungen verfügbar, mittels eines Echtzeit-Monitorings erfasst, gespeichert und gesichert werden.

Was ist ein Echtzeit-Monitoring?

Die in den Reinraum-Bereichen installierten Sonden und Fühler erfassen die verschiedenen, momentan herrschenden physikalischen Messgrössen wie Temperatur, Feuchte usw. und übermitteln sie direkt via Schnittstellen (z.B. Analog/Digital-Wandler usw.) an den Computer. Die darauf installierte Echtzeit-Software übernimmt nun diese Werte und zeigt sie zeitgleich in Grafiken, Tabellen, Schaubildern usw. via Farb-Monitor an. Gleichzeitig werden die anfallenden Daten den bereits gespeicherten hinzugefügt, also für spätere Auswertungen konserviert. Ebenfalls gleichzeitig werden aber auch diese Daten in Vergleiche, Berechnungen u.v.m. miteinbezogen. Das Resultat hieraus ist unter anderem:

- Alarmmeldung bei Ueber- oder Unterschreitung eines zuvor gesetzten Limits
- Reaktionen und Steuerbefehle an zugeordnete Elemente der Anlage (Ventil, Pumpen usw.)
- Meldungen an ein übergeordnetes System (Haupt-Computer, Steuerzentrale, Synoptik usw.)

Abb. 1: Komponenten für ein Reinraum-Monitoring

Ein Echtzeitmonitoring, also ein **R**eal **T**ime **D**ata **A**cquisition **S**ystem (RTDAS) erfüllt somit folgende Aufgaben:

- Datenerfassung und deren Darstellung
- Datenspeicherung und deren Sicherung
- Grenzwertüberwachung und bei Ueberschreitung deren Meldung
- Steuerung angegliederter Anlagenkomponenten als Reaktion der gewerteten Messgrössen

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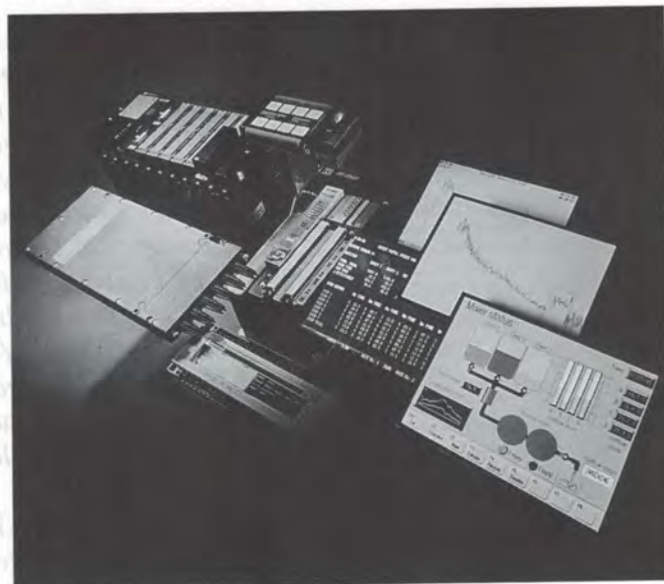

Abb. 2: Optimales Zusammenspiel von Hard- und Software ergeben ein fehlerfreies Reinraum-Monitoring

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Results of the six project groups of the second postgraduate seminar for Pharmaceutical Quality Assurance, winter semester 1992/93, Department of Pharmacy, Swiss Federal Institute of Technology (ETH), and the University of Basel, Pharmaceutical Faculty

Pharmaceutical Quality Assurance

Basics of validation

*K. Tomamichel, J. Jordan, N. Freitag, B. Thurnbauer, P. Applewhite**

The present review discusses the basic official requirements regarding validation outlined in the WHO, EEC/PIC, FDA and Japanese GMP regulations. The first part of the presentation compares the definitions of the three significant terms validation, qualification and calibration presented in the mentioned guidelines. These definitions show a clear tendency toward harmonization. In the second part, the official documents have been studied under the aspects of GMP validation-requirements. The requirements in the various guidelines do not correspond in depth, but they are not contradictory neither. Thus, the basis for an international validation policy meeting all these requirements is definitely given. In a further part, the pros and cons of the various validation approaches for a pharmaceutical manufacturer are discussed. Any validation of a procedure, process, material, or equipment is one crucial part of production and quality control, leading to an increased quality assurance level. The inherent operational costs of validation result in a return on investment if validation is performed in an optimized and consistent way.

Introduction

Validation represents a crucial issue for every producing and researching pharmaceutical company and is a central concern of current Good Manufacturing Practice. Due to the elevated standards, not only of the FDA but also of the WHO, EEC/PIC and Japanese authorities, any person responsible for quality assurance, quality control or production has to be familiar with validation and has to implement "good validation practice" in the workplace, i.e. in process validation, validation of analytical procedures, cleaning validation, laboratory system and computer validation. As a base of a good validation approach, the concepts of qualification and calibration have to be taken into consideration. It is the aim of the following paper to explain current validation-correlated definitions, to give a comparison of different main aspects of validation between selected authorities (EEC/PIC, FDA, Japan, WHO), and to discuss the importance of validation for the pharmaceutical industry.

Terminology

First, a comparison of the definitions for validation, qualification, and calibration (see also *Figure 1*).

Validation

Validation is defined in the EEC-GMP guidelines as *"Action* of proving, in accordance with the principles of Good Manufacturing Practice, that any procedure, process, equipment, material, activity or system actually leads to the expected results" [4]. The FDA defines it as "Establishing *documented* evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its pre-determined specifications and quality characteristics" [7]. Thus, for the FDA, any process, method, etc. can only be regarded as validated if the corresponding complete documentation exists. The "documentation is good science" tendency is also confirmed by the previously adopted WHO guide to good manufacturing practice [1], which defines validation as "The *documented action* of proving that any procedure, process, equipment, material, activity, or system actually leads to the expected results".

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Validation

WHO (1992): The **documented act of proving** that any procedure, process, equipment, material, acivity, or system actually leads to the expected results [1].

Revalidation is generally required [11]:

- if the composition of the pharmaceutical product, the manufacturing procedure or the batch size is changed
- in the event of significant alterations to the processing equipment
- if new equipment is used
- in the event of major changes of processing conditions
- after extensive preventive maintenance work on machines equipment
- in the event of major changes of quality control methods
- if the findings of the in-process and quality-control results indicate the need.

Prospective Validation
Validation conducted prior to the distribution of either a new product, or product made under a revised manufacturing process, where the revisions may affect the product's characteristics [8].

Retrospective Validation
Validation of a process for a product already in distribution based upon accumulated production, testing and control data [8].

Qualification

FIP (1990): Qualification is the formal, systematic, and documented proof that facilities and equipment are suitable for the intended process. Qualification is a **basic requirement for validation and an entire part of this validation.** Qualification of equipment **includes calibration** of measuring equipment [11].

Calibration

FIP (1990): Calibration is the formal, systematic, and documented proof that the used measuring equipment indicates the values within established / defined ranges [11].

Figure 1: Definitions of Significant Validation-Related Terms

Qualification

The concept of qualification is as yet less harmonized than the concept of validation. In the WHO's glossary, the term qualification does not even exist [1]. FIP (Fédération International Pharmaceutique), in contrast, gives a clear and extensive definition, stating that qualification means "the formal, systematic, and documented proof that facilities and equipment are suitable for the intended process. Qualification is a *basic requirement for validation and an entire part of this validation.* Qualification of equipment *includes calibration* of measuring equipment" [11]. The EEC definition falls between these two extreme positions, not adopting a clear standpoint, viz.: "Action of proving that any equipment works correctly and actually leads to the expected results. The term *validation is **sometimes** widened* to incorporate the concept of qualification" [4].

Calibration

The term calibration seems to be the most harmonized of these three terms. In the WHO guide, for example, it is defined as "The set of operations that establish, under specified conditions, the relationship between values indicated by an instrument or system for measuring (especially weighing), recording, and controlling, or the values represented by a material measure, and the corresponding known values of a reference standard. Limits for acceptance of the results of measuring should be established" [1]. This definition corresponds basically to the FIP definition: "Calibration is the formal, systematic, and documented proof that the used measuring equipment indicate the values within established/defined ranges" [11].

We should point out that "calibration" of equipment is an action performed by company personnel. "Proving that the equipment indicates the values within established ranges" when performed by *authorities* is usually called *"official verification"* of the equipment.

Comments

Comparison of current definitions of validation, qualification and calibration in the different official publications shows a tendency toward harmonisation. However, corporations or cultures in different countries often interpret definitions in various ways and build their own specific validation concept. This leads to discrepancies between corporations and authorities. In the current situation, we suggest applying one of the definitions for each validation-related term *(see Figure 1)* and implementing its wording thoroughly in the validation program of development, production and quality control.

Comparison – WHO – EEC/PIC – FDA – Japan

Official Publications

For comparison of the regulations of the above-mentioned areas of validity, the following GMP documents have been considered to be relevant and have therefore been taken into account:

World Health Organisation (WHO)
- Good Manufacturing Practices for Pharmaceutical Products, in: Technical Report Series 823: WHO Expert Committee on Specifications for Pharmaceutical Preparations (1992) [1].

European Community (EEC)
- Guide to Good Manufacturing Practice for Medicinal Products (1992) [4]
- EEC Commission Directive 91/356/EEC (6/1991) laying down the principles and guidelines of good manufacturing practice for medicinal products for human use [3].

Pharmaceutical Inspection Convention (PIC)
- Guide to Good Manufacturing Process of Pharmaceutical Products (1992), PIC-Doc PH 5/89 [10].

United States of America (USA)
- Current Good Manufacturing Practice in Manufacturing, Processing, Packing, or Holding of Drugs; General (4/1992) [7].
- FDA Guideline on General Principles of Process Validation (5/1987) [8].

Japan
- GMP Regulations of Japan, Fourth Edition (1992) [9].

Comparison WHO – EEC/PIC – FDA – Japan

Before discussing the different comparison points mentioned in *Table 1,* let us focus briefly on the relationship between the official publications studied. Since 1992 the *EEC* [4] and the *PIC-GMP* guidelines [10] have been almost identical. Thus, the PIC document will not be further discussed, as it is included in the discussion of the EEC guide [4]. The legal relevance, however, of both documents is different. The EEC guide to Good Manufacturing Practice is only valid for EC members, the PIC documents for PIC members. An overview of the current PIC member and EEC member status is given in *Table 2.* With the EEC guidelines of 1992, the European national requirements, for example the historically relevant "Orange Guide", UK [22], have been superseded.

Furthermore, the revised requirements for GMP presented in Annex 1 of the recently adopted final *WHO document* [1] basically correspond to the 1992 EEC guidelines [4]. In the WHO guide, some sections are more detailed and extensive; others are identical in both documents. However, in the open-ended last section of the WHO guide, the supporting and supplementary guideline section, only two guidelines are included ("Sterile pharmaceutical products"; "GMP for active pharmaceutical ingredients, bulk drug substances"). In the same last, open-ended section of the EEC guide, 12 supplementary guidelines are included. The WHO report has to be considered as the technical basis for the WHO Certification Scheme on the Quality of Pharmaceutical Products Moving in International Commerce.

Whereas the EEC and WHO documents show a considerable development in the sense of new and more harmonized GMP guidelines, in the US additional major validation-related documents have not been issued since 1987 [8]. Nevertheless, the *FDA* applies constantly increasing stringency regarding GMP-requirements. Thus, the "Guideline on General Principles of Process Validation" [8] builds the bases and the secondary literature (i.e. FDA inspection reviews) the actual "update" of the FDA's current GMP interpretation and inspection practice.

Finally, the requirements/recommendations of *Japan's GMP guide* of 1992 [9] are hardly comparable to those previously mentioned (EEC/PIC, WHO, FDA). However, it can not definitely be concluded whether the different way-of-saying stems from translation deficiencies. As an example, the word "validation" is only used two times in the whole guide *("validation* of sterilization of products" and "... quality control, to check the data of *validation* ..."), whereas expressions like "check that" or "show the evidence that" are predominant, indicating an appeal to the company's sense of responsibility rather than of giving firm and detailed GMP recommendations.

Discussion of Table 1

In the following, the different comparison points, listed in Table 1, are discussed:

Responsibility for Validation

In 1980, the FIP stated that the manufacturer of a pharmaceutical product is responsible for the complete validation work to be done [11]. The EEC and WHO guides narrow this by saying that the head of quality control and the head of production are the persons responsible for validation [1, 4]. The Japanese guide places validation responsibility in a stricter quality assurance system, assigning it to the production security manager, who is superior to the production control manager and the quality control manager. Our working group's opinion is that it is important that

Table 1: Summary of Important/Major Aspects in the WHO, EEC/PIC, FDA and Japanese Regulations

Responsibility for validations:	WHO/ EEC:	Head of quality control resp. head of production [1, 4]
	FDA:	
	Japan:	Product security manager, production control manager, quality control manager [9]
Documentation of Validation Data:		
- general prescriptions	WHO/ EEC	**SOPs and associated records** of actions taken or,..., conclusions reached should be available for...equipment assembly and validation... analytical apparatus and calibration... [1] *) [4]
	FDA	The manufacturer must prepare a **written validation protocol** which specifies the procedure to be conducted and the data to be collected... [8] The **protocol** should specify a specific number of replicate number of runs to demonstrate reproducibility and provide an accurate measure of the variability amongst the runs [8] **Validation documentation** should include evidence of the suitability of the materials and the performace of equipment and systems... [8]
- for equipment	WHO/ EEC	**Logbooks** should be kept for major or critical equipment recording any validations, calibrations [1] *) [4]
	FDA	**A written record** of major equipment cleaning, maintenance (except routine...) [7]
	Japan	–
Validation of Production Processes:		
- general remarks	WHO/ EEC 1)	Processes and procedures should be established on the basis of validation study and undergo periodic revalidation... [1] *) [4] **Critical processes** should be validated, prospectively or retrospectively [1] When any **new master formula** or **method of preparation** is adopted, steps should be taken to demonstrate its suitability for routine processing. [1]*) [4] / [3] **Significant amendments to the manufacturing process**, including any change in equipment or materials that may affect product quality and/or the reproducibility of the process, should be validated. [1] *) [4]
	EEC	Experimental studies validating the manufacturing process, where a **non-standard method** of manufacture is used or where it is **critical**... [2]
	FDA	Process validation is a requirement of cGMP [8] Process validation is a key element in assuring that quality assurance goals are met [8]
	Japan	...where a **method different from that prescribed in the letter of manufactring approval** is given...to check if it is based on scientific grounds... [9] ...where a **change in manufacturing method** of a starting material is evident, supplemental specifications and test methods shall be established, if necessary [9]

Validation of Production Processes (con't):		
- sterile products:		
- sterilization processes in general	WHO/ EEC	**All sterilization processes** should be **validated** [1] [4] Care should be taken that validations do not hazard the processes [1] [4] Sterilization process... its suitability for the product and its efficacy in achieving the desired sterilizing conditions in all parts of each type of load should be demonstrated. ...**repeated at scheduled intervals**, at least anually, and whenever significant modifications have been made to the equipment.
	FDA	Appropriate procedures, designated to prevent microbiological contamination of the drug products, purporting to be sterile, shall be established and followed. Such procedures shall include **validation of any sterilization process** [7]
	Japan	...should implement **validation of sterilization** of products to be sterilized [9]
- aseptic processes	WHO/ EEC	The use of nutrient media...is a valuable part of **overall validation of an aseptic process.** *) [4]...at least 3000 units of production... The target should be zero growth and anything above 0.1% contamination of units... unacceptable [1]
	FDA	-
	Japan	...should implement **validation** of sterilization of products to be sterilized and **aseptic procedure** as well [9]
- equipment used for processing of sterile products	WHO/ EEC	All **equipment**, including sterilizers, air-filtration systems, and water-treatment systems including stills, should be subject to **planned maintenance, validation, and monitoring**; its approved use following maintenance work should be documented [1] *) [4].
	FDA3)	-
	Japan	...**sterilization apparatus** shall be used after thorough **examination of its performance**. The performance shall be examined regularly. A suitable method for prevention of bacterial contamination shall be devised. The contamination shall be examined regularly [9] **Sterilization apparatus**... should be used on **checking them thoroughly for efficiency** and such efficiency checked regularly [9]
- Cleaning Validation	WHO	Particular attention... to the **validation of** processing, testing, and **cleaning** procedures [1]
	EEC	(Annex 2: biological medicinal products:) The adequacy of **cleaning and decontamination procedures** should be **validated** [4]. (Annex 9: Liquids, creams and ointments:) After any chemical sanitization of the water systems, a **validated flushing procedure** should be followed... [4] (Annex 10: Pressurized metered dose aerosol preparations for inhalations:) Containers and valves should be **cleaned using a validated procedure** [4]
	FDA	–
	Japan	...**hygienic control of the buildings and facilities**..., Hygienic control of personnel......(a) Method of **confirmation of the result of cleaning**... (b) Method of confirmation of health conditions.

Validation in Quality Control:	WHO	**Test methods** must be **validated** [1] **Testing procedures** described in documents should be **validated** in the context of available facilities and equipment... [1]
	EEC 2)	The standard error of the **method,** its reliability and the acceptability limits of the results shall be specified, and, if necessary, explained [2] Laboratory documentation... available to the Quality Control Department: **Validation records** of **test methods** [4]
	FDA	The accuracy, sensitivity and reproducibility of the **test methods** employed by the firm shall be established and documented [7]
	Japan	...**test methods** and the method of evaluating test results right... [9] ...a test varying from that of approval is conducted for the sake of quality control, to check the **data of validation showing the propriety of such tests** [9].
Validation of Processing Systems:		
- general remarks	WHO	Data may be recorded by electronic data-processing systems or by photographic or reliable means... **accuracy of the records should be checked**... [1] *) [4]
	EEC	When electronic, photographic or other data processing systems are used ... the manufacturer shall have **validated the systems** [3]
	FDA	**Equipment** (**automatic**, mechanical or **electronic**) shall be routinely **calibrated, inspected or checked**. Written records shall be maintained. [7]
	Japan	–
- computerized systems	WHO	–
	EEC	(Annex 12: computerized systems:)...appropriate expertise is available and used to provide advice on aspects of design, **validation**, installation and operation of computerized systems [4] ...The **extent of validation** necessary will depend on a number of factors... Validation should be considered as part of the complete life cycle of a computer system. This cycle includes the stages of planning, specification, programming, testing, commissioning, documentation, operation, monitoring and modifying [4].
	FDA	–
	Japan	–
Calibration:	WHO/ EEC	Measuring, weighing, recording, and control equipment and instruments should be serviced and **calibrated** at prespecified intervals and records maintained [1] *) [4]
	FDA	Equipment (automatic, mechanical or electronic) shall be routinely **calibrated, inspected or checked**. Written records shall be maintained. [7]
	Japan	...manufacturing facilities and apparatuses ... checked regularly... [9] ...checks the manufacturing facilities and apparatuses as to **calibration**...to keep them in order, and to ascertain if such checks for keeping them in order are implemented regularly [9] ...**system to prevent errors** in weighing is established [9]

Table 2: PIC/EC Member Status (4/93)

PIC Member Only	EC Member Only	EC and PIC Member
Australia Austria Finland Hungary Iceland Liechtenstein Norway Romania Sweden Switzerland	Greece Netherlands*) Spain	Belgium Denmark France Germany Ireland Italy Portugal United Kingdom

*) and South Africa, Czechoslovakia, Turkey, Poland and Slowenia have initiated the PIC-accession procedure.

the person(s) responsible for validation have the competence to interrupt processing by a non-validated production process or analytical method. However, the necessary operational steps which have to be taken into consideration, usually planned by working groups, can be delegated [17].

Documentation of Validation Data
As already stated in discussing the definitions, the FDA is mainly concerned to have an entire and complete documentation of all validation work (including qualification, calibration, revalidation programs...) relating to a product and/or production line at disposal [7, 8]. The "validation protocol, a written plan stating how validation will be conducted, including test parameters, product characteristics, production equipment, and decision points on what constitutes acceptable test results" [8], the "validation documentation including the evidence of the suitability of the materials and the performance of equipment and systems" [8], as well as all development work and further maintenance actions (excluding routine testing), have to be consistently documented in a so-called "Master Validation Plan". This document may also be called differently. Its scientific quality is an accurate gauge of a company's adequacy as regards actual validation testing [19] and is therefore thoroughly assessed by the inspection authorities. Furthermore, SOPs (Standard Operating Procedures) for all cleaning and maintenance actions such as recalibrations, for self-inspection etc. must be available [12, 13, 14, 15]. The SOPs including logbooks for major and critical equipment, are also required by the EEC and WHO guides [1, 4].

Validation of Production Processes
As is well known, critical, non-standard or new methods of preparation as well as, in the case of adoption of a new master formula, *methods of preparation*, have to be validated. The WHO officially indicates that validation may be performed either "prospectively" or "retrospectively" [1]. However, it is reported that for the FDA retrospective validation has been losing in importance. The FDA no longer accepts retrospective validation without additional prospective validation data. Thus, data obtained from the annual product review ("retrospective validation" [16]) will have less importance in future.
Detailed validation instructions are given for sterile pharmaceutical products. Explicitly, all sterilisation processes have to be validated and validation repeated at scheduled intervals, annually at least [1, 4, 7, 9]. For aseptic production processes, in contrast to the EEC guide the new WHO guide gives clear instructions concerning the validation requirements ("at least 3000 units"; "target ... anything above 0.1% contamination ... unacceptable" [1] which are in accordance with the FDA Guideline on Sterile Products Produced by Aseptic Processing already adopted in 1987 [21]. Instead of following these strict instructions, any responsible person should fix the number of units to be tested according to the batch size, in a way that allows a statistically significant evaluation of the results obtained. It should also reflect the significance of the nature and the dosage of a contamination agent in the field of application of the drug.
Furthermore, it has to be mentioned that "examination of performance" resp. "checking of efficiency of sterilisation apparatus", i.e. the qualification concept, plays a major role in the complete validation concept, especially for sterile products, in the Japanese GMP regulations [9]. Generally, there is a marked similarity of the Japanese to the FDA's prospective validation concept. It outlines the FDA concept in the following three steps: "equipment installation qualification" – "process performance qualification" – "product performance qualification" [8], defined in Table 3. The FDA regards this concept as a proposal for the validation/qualification issue. It is only important that a complete qualification be performed on any equipment and its result documented [25].
In the broader context of process validations, the publications dealing with *cleaning validation* have also been studied. In the EEC guide, only cleaning procedures relating specifically to special products (i.e. "biological medicinal products", "liquids, creams and ointments" and "pressurized metered dose aerosol preparations for inhalations" (in: annexes 2., 9., and 10. of the GMP guide [4]) are to be found. However, the previously adopted WHO guide contains the following general instruction: "Particular attention should be accorded to the validation of processing, testing, and cleaning procedures" [1]. Similarly, the Japanese guide, in accordance with its appeal to comprehensive quality assurance, says that "hygienic control of the buildings and personnel" has to be established by a *"method of confirmation of the result of cleaning..."*. In conclusion, in the WHO and Japanese guides, the cleaning procedure seems to be part of the complete production process of a medicinal product and has, consequently, to be treated as such.

Validation in Quality Control
The concept of validation of analytical test methods is well established and also thoroughly adopted in the different official documents studied and in selected pharmacopoeial monographs (e.g. USP). We shall therefore not discuss this item further.

Validation of Processing Systems
This comparison point was assessed with particular regard to computer systems. The EEC GMP guide, annex 10, is dedicated to computerized systems, describing the "extent of validation (as) depending on a number of factors" and as "being a part of the complete life cycle of a computer system. This cycle includes the stages of planning, specification, programming,

Table 3: General Concept of Process Validation of the FDA [8]

Installation Qualification – Establishing confidence that process equipment and ancillary systems are capable of consistently operating within established limits and tolerances. *Process Performance Qualification* – Establishing confidence that the process is effective and reproducible. *Product Performance Qualification* – Establishing confidence through appropriate testing that the finished product produced by a specified process meets all release requirements for functionality and safety.

testing, commissioning, documentation, operation, monitoring and modifying" [4]. The requirements concerning any laboratory system in the Current Good Manufacturing Practice of the FDA [7] pertain also to *computerized* laboratory systems [19], i.e. the requirement that "the calibration of instruments, apparatus, gauges, and recording devices at suitable intervals in accordance with a written program containing specific directions, schedules, limits for accuracy and precision" must be carried out [7]. More detailed instructions regarding computer validation can be found in the secondary literature [19, 20].
In conclusion, the documents studied do not contain contradictory GMP/validation recommendations. Thus, it should be a possible and desirable aim of a corporation to meet all current validation requirements in the EEC/PIC, WHO, FDA and Japanese guides. This calls for a high standard of organisation and planning.

Significance of Validation in the Pharmaceutical Company

The significance, pros and cons, of validation of any process, procedure, equipment etc. is outlined in *Table 4*.
The only con found – the costs – is worth discussing further. In a presentation given by Naumann [23], the transfer of validation and the synergistic relationship between the 3 quality assurance means, namely in-process control, finished product controls and validation, have been discussed.
Validation transfer is the transfer of validation work done on an existing system to a new system. It is, for example, necessary to include the validation costs in the investment calculation for a new processing unit. In case a validated or a not validated processing unit have both been evaluated as suitable, the validation costs could be decisive when purchasing the already validated unit, since a great part of these costs would no longer have to be taken into consideration.
Concerning the three quality assurance tools (in-process control, finished product controls and validation), Anisfeld [24] also points out their synergistic effect, saying "Let us have the courage to come to the logical conclusion, namely to validation – and relegate pharmacopoeial testing methods to the archives of time, and close down our in-process and finished product control and our on-going stability testing laboratories". In other words, if validation has been conducted carefully and shows that every step of the production process is under complete control, then additional quality control measures can be significantly reduced. In this sense, a positive economic effect of validation is obvious.
Validation performed in an exaggerated or inconsistent way causes high costs and brings no additional benefit to the patient. However, optimally conducted validation leads to a higher level of quality assurance and, by avoiding mistakes, optimizes the system and reduces other quality assurance expenses. Thus it is also economically justified.
The pros of adequate validation, listed in *Table 4*, are evident. Particularly in the pharmaceutical industry, where a quality deficiency may result in serious consequences, validation has to be an integral factor of quality assurance. The "validation fever" ascendant in Europe, however, is only justified within reasonable limits. Validation should be rationally organized and concentrated on the essential critical parameters.

Conclusions

The definitions of validation, qualification, and calibration given in the different GMP regulations (WHO, EEC/PIC, FDA, Japan) studied are fundamentally similar. However, details are weighted slightly differently. Furthermore, the validation-related GMP requirements given in the same regulations do not contradict each other.
In our opinion, the basis for a validation policy meeting all these requirements exists.
Nevertheless, for a number of reasons the practical validation work done by different companies in various countries will never be uniform. For example, nations and companies follow their own philosophies; this leads to varying interpretations and/or practical implementation of the same GMP requirements. Moreover, the assessment of parameters judged to be critical and therefore needing to be validated is a subjective procedure. For the particular case, a generally valid pattern cannot be defined.
Generally applicable, however, is the objective of validation as such, namely to prove that a particular system, process or procedure is fully under control.
Finally, we suggest this to any person who is practically confronted with validation: Validation should not be considered to be more difficult than it is in fact. Only critical parameters should be validated. Validation should not be uncritically employed as an additional means of quality assurance. It should be integrated into the quality assurance system, assessing whether the extent of the quality assurance means actually applied, e.g. in-process controls or quality control measures, might be reduced if validation has been performed carefully.
It is the duty of the manufacturer of proprietary medicinal products to strive for an optimized extent of quality assurance, as additional costs have finally to be borne by the patient.

Table 4: Pros and Cons of Validation

	Pro	Con
1	In contrast to in-process and finished product controls, it is possible, by validation data, to predict in which range system parameters have to be maintained. Through validation of a system, the system is controlled, deficiencies are detected which otherwise may not have been noticed and, most importantly, an intensive scrutiny of the complete system is conducted.	additional costs
2	drug safety and thus safety for the patient is improved	
3	possibly, the system is optimized	
4	the probability of a product recall is reduced	
5	lately occurring system deficiencies are reduced	
	the validation documentation can be used:	
6	– for presentation in case of an inspection	
7	– as legal proof of safety in a product liability case	
8	– as a document for a marketing authorisation application and a certification	

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Production systems for water for injection

Validation issues and new technical concepts

*M. J. Moor, H. Buser, I. Dentler, P. Fürer, C. Planzer**

GMP standards have evolved very rapidly over recent years. As part of this process, qualification and validation concepts have been optimized by the pharmaceutical industry in order to keep pace and increase the safety of medicinal products. This article presents a general discussion of regulatory requirements for equipment qualification and process validation. A new concept for a Water for injection (WFI) production system is reviewed. In contrast to the traditional distillation procedures, it is proposed to use a 2-phase reverse osmosis system in combination with ultra-filtration. Lower operational costs are one of the main advantages of this particular design. For energy reasons as well, WFI is stored and distributed at room temperature. To prevent microbial growth, the recirculated WFI is continuously treated with ozone. Before the WFI reaches the points-of-use, the ozone is eliminated by UV-radiation. With a regard to GMP-required qualification and validation procedures, we propose that even the preliminary concepts should be qualified and validated. However, new technologies will only be successful if they offer significant advantages in respect of production costs and quality. Increasing costs of validation programs make it necessary to review current validation policies critically without losing control of quality standards. Today's challenge is to concentrate on the essentials and to set priorities while ceasing from validating for validation's sake.

Introduction

Commonly accepted GMP standards have evolved in recent years and repeatedly challenged the pharmaceutical industry.

As a central part of pharmaceutical quality management and a basic requirement of governmental regulations, equipment qualification and process validation have become essential concepts for the design, installation and operation of pharmaceutical facilities [1]. Qualification and validation policies have also been established for pharmaceutical production and, most recently, for manufacturing of bulk pharmaceutical chemicals [2]. Although regulatory validation policies have been completely accepted by the pharmaceutical industry, critical discussions of the extent of validation requirements and the cost-efficiency of validation programs have recently been published [3, 4].

Water for injection (WFI) is a central ingredient for pharmaceutical products and is necessary for many processes and operations in the pharmaceutical industry. Quality standards and production methods for WFI are defined by national and international pharmacopoeias. Since standard specifications and standard product lines are not available for WFI production systems, validation procedures have to be redefined on a project basis.

The present article discusses basic aspects of equipment qualification and process

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validation. We will present a concept for the planning, qualification and validation of a WFI system. Using a rather unconventional concept for a WFI facility, we will demonstrate that the consequent qualification and validation of a plant design is one of the most important tools for the design and optimization of new cost-effective facilities.
Our discussion does not include the important issue of qualification of buildings for pharmaceutical production. An excellent compendium covering this field has recently been published [5].

Definitions and Legal Requirements

Definitions
Definitions of basic concepts are available from numerous sources, such as the American Food and Drug Administration (FDA), the Pharmaceutical Inspection Convention (PIC) guidelines, the GMP regulations of the European Community and the World Health Organization (WHO), etc. Representative definitions are summarized in *Table 1.* A more detailed discussion has recently been published [6].
In general, "qualification" should be used for system components or independent modules of a complete system and "validation" whenever referring to processes or a complete facility. However, in publications and regulation alike, "qualification" and "validation" are used rather interchangeably.

Legal requirements
Equipment qualification as part of process validation is required implicitly or expressly in most national and international drug laws and GMP's, e.g.

Table 1: Basic Definitions

Concepts	Definitions
Qualification [1]	Establishing confidence that process equipment and ancillary systems are capable of consistently operating within established limits and tolerances.
Validation [1]	Establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its pre-determined specifications and quality attributes.
Calibration [10]	The set of operations that establish, under specified conditions, the relationship between values indicated by an instrument or system for measuring, recording, and controlling, or the values represented by a material measure, and the corresponding known values of a reference standard. Limits for acceptance of the results of measuring should be established. Details about setting measuring limits can be found in DIN 1319, Part 3, Pt. 8.3.2. [16].

- GMP requirements according to the Code of Federal Regulations (CFR 21) Parts 210 and 211 [7]
- PIC Guidelines [8]
- GMP Guidelines of the European Community [9]
- WHO GMPs [10]

Specifications for WFI
The quality requirements for WFI are specified in pharmacopoeia monographs. Specifications of USP XXII, Ph. Eur. 2nd Ed. and an industry standard are outlined in *Table 2.*

General Agenda of Plant Qualification

All planned qualification and validation activities have to be summarized in a central documentation. This is extremely important in the case of production systems for WFI, since such facilities need to be designed according to individual needs and specifications. The validation master plan should contain the complete validation program, including the conceptual design, plant specifications, required SOPs, qualification and validation protocols, test reports, etc. Overall validation activities will cover

- Production processes;
- Analytical methods;
- Operational procedures and monitoring programs;
- Training of personnel.

After the approval of basic concepts and specifications, the following general qualification steps are required:
- Installation Qualification (IQ);
- Operational Qualification (OQ);
- Performance Qualification (PQ).

Table 2: WFI Specifications

Specifications for WFI			
Test	USP XXII	Ph. Eur. 2nd. Ed.	Industry [19]
Bacterial endotoxins	< 0.25 EU/ml	free from pyrogens	< 0.25 EU/ml
Micro-organisms	–	–	10/100 ml
Pseudomonas aeruginosa	–	–	not detectable in 100 ml
Pseudomonas cepacia	–	–	not detectable in 100 ml
pH	5.0–7.0	complies with test	5.0–7.0
Chlorides	not detectable	not detectable	100 ng/ml
Sulphates	not detectable	not detectable	1000 ng/ml
Ammonium	< 0.3 ppm	< 0.2 ppm	200 ng/ml
Calcium/Magnesium	not detectable	not detectable	2000 ng/ml
Carbonate	not detectable	–	
Heavy metals	not detectable	< 0.1 ppm (Pb)	100 ng/ml (Pb, Ag, Hg, Cu, Co)
Oxidisable substances	not detectable	not detectable	640 ng/ml
Residue on evaporation	< 10 ppm	< 10 ppm	10 μg/ml
Conductivity [13]	(proposed 1.25 μS/cm)	–	5.0 μS/cm
Preparation method	Distillation Reverse Osmosis	Distillation	

This schedule is in accordance with FDA guidelines [1] and the Pharmaceutical Manufacturers' Association (PMA) concept for the process validation of bulk pharmaceutical chemicals (BPCs)[2]. A detailed general program for IQ, OQ and PQ is outlined in *Figure 1.*

Installation Qualification (IQ)
The PMA [2] definition of the Installation Qualification is;
"IQ is the documented verification that all key aspects of the equipment and ancillary system installation adhere to the approved design intentions and that the recommendations of the manufacturer are suitably considered."
In most companies the engineering department is responsible for the preparation of the documents as well as the proper execution and record-keeping of the IQ tests, which include the following items:
(i) Design qualification;
(ii) Pre-qualification or module-qualification;
(iii) Equipment and installation acceptance;
(iv) Equipment start-up.

(i) Design Qualification
The following project documents make up the basis of the IQ:
- Project performance specifications;
- Calculation- and design-documentation;
- Process and instrument flow diagrams;
- Apparatus and instrument specifications;
- Automatic control performance specifications.

The project performance specifications will preferably be prepared and approved by the project manager in cooperation with the quality assurance, the engineering and the production departments. Other specialists may be involved where necessary for approval of required technical documents.

(ii) Pre-Qualification or Module Qualification
All equipment elements must be checked to see if they meet the specifications. In the case of devices and instruments this should occur as follows:
- Release of the manufacturer's production drawings;
- Acceptance test by buyer *or* equipment acquisition including qualification certificate from the manufacturer;
- General inspection of incoming goods;
- Calibration and functional control of instruments;
- Documentation check such as apparatus descriptions, spare part lists, operating and service, inspection and maintenance procedures.

(iii) Equipment and Installation Acceptance
The completely installed equipment will be tested for
- Mechanical completion
  - Equipment control for completeness;
  - Equipment control in respect to working safety.

- Functional control
  - Check of motor's rotation direction;
  - Tightness high/low pressure;
  - Line test as well as functional control of instruments;
  - Functional control and acceptance of electrical installations.

(iv) Equipment Start-up
After equipment acceptance, technical start-up is initiated by a test run with water. Pharmaceutical aspects are of minor importance during this phase.
- Tests:
  - Functional control of equipment control systems;
  - Instrument calibrations;
  - System tests by simulation of the production, cleaning and sterilization processes;
  - Functional control of safety programs;
  - Preparation of the equipment log book;
  - Preparation of the operation and maintenance manuals for users and the maintenance department.

- For the documentation of IQ activities the following format can be used:
  - Introduction;
  - Protocol and SOPs for test programs;
  - Approved test reports;
  - Final IQ reports.

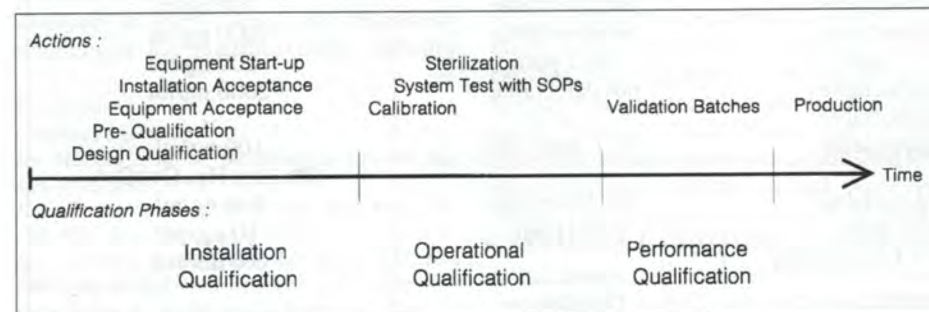

Fig. 1: General Agenda for Process Validation

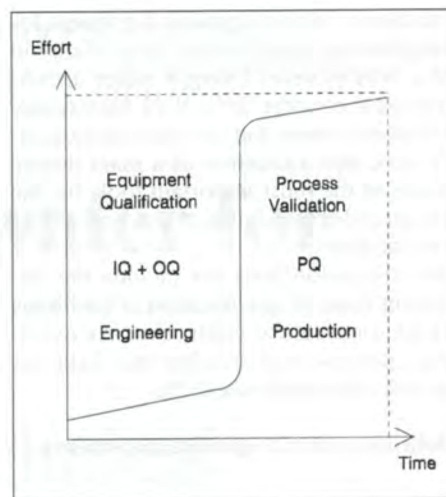

Fig. 2: Complementary Responsibilities of Engineering and Production Departments

Operational Qualification (OQ)
Definition according to the PMA BPC concept [2]:
"OQ is the documented verification that the equipment and ancillary systems perform as intended throughout anticipated operating ranges."
The project manager is generally responsible for proper execution and record-keeping of the OQ (see *Fig. 2)*
The following documentation and SOPs must be available for the OQ:
- System description;
- Maintenance procedures;
- Instrument calibration procedures;
- Sanitation procedures;
- Sampling procedures;
- Analytical methods for product testing.

Using these documents, the equipment will be checked according to approved test protocols. Besides physical, chemical and microbial parameters, system performance and capacity issues will be investigated.
These tests belong to the primary equipment qualification of the production system and will be documented. Calibrations and fine-tuning will be carried out by the responsible engineering group. Test protocols and reports will be part of the OQ documentation.

Performance Qualification (PQ)
In this phase of the validation program, project responsibility is transferred from engineering to production. The overall scope is to evaluate the robustness and reproducibility of the process. Process parameters will be varied to identify allowable limits. All critical parameters will be monitored during this phase and product

specifications verified according to existing procedures (SOPs).
All qualification activities have to be properly documented and summarized in a final report specifying the process parameters. Whenever the production system is changed or production parameters are altered, performance qualification needs to be repeated.

Specifications and Production Methods

Water for injection is mainly produced by distillation techniques but membrane separation processes are also of importance. Three possible procedures are summarized in *Figure 3*. Specifications for the final product are outlined in *Table 2*.

Concept Study

Basis of Design
For our concept study we have chosen a membrane process instead of a conventional distillation technique. Lower investment and operational costs are the main advantages of reversed osmosis based systems. To guarantee highest safety standards for the final product, the 2-step reversed osmosis was combined with an ultra-filtration unit to remove pyrogens from potential microbial system contaminations. The produced WFI is collected in a stainless steal storage tank to cover peak consumption. WFI is stored at room temperature and distributed to the points-of-use by a loop system. The detailed design of the proposed production and distribution facility is outlined in *Figure 4*.
To control microbial growth WFI should be stored and distributed to the points-of-use via a hot, continuously recirculating loop. Temperature recommendations of various authorities are summarized in *Table 3*. WFI at ambient conditions should not be stored for longer than 24 hours [20]. Long-term storage at room temperature has become practicable by ozonization of the water in circulation.

Critical plant components
As part of the plant validation, all critical components such as the reversed osmosis, the ultra filtration and the storage and distribution system have to be carefully qualified and continuously controlled during

Table 3: Temperature Recommendations for WFI Storage and Distribution

Guidelines	Storage and Distribution Temperature for WFI
FDA [20]	> 80ºC
EC/PIC [8, 9]	> 70ºC
WHO [10]	< 4˚C or > 80˚C
Orange Guide[18]	> 65˚C

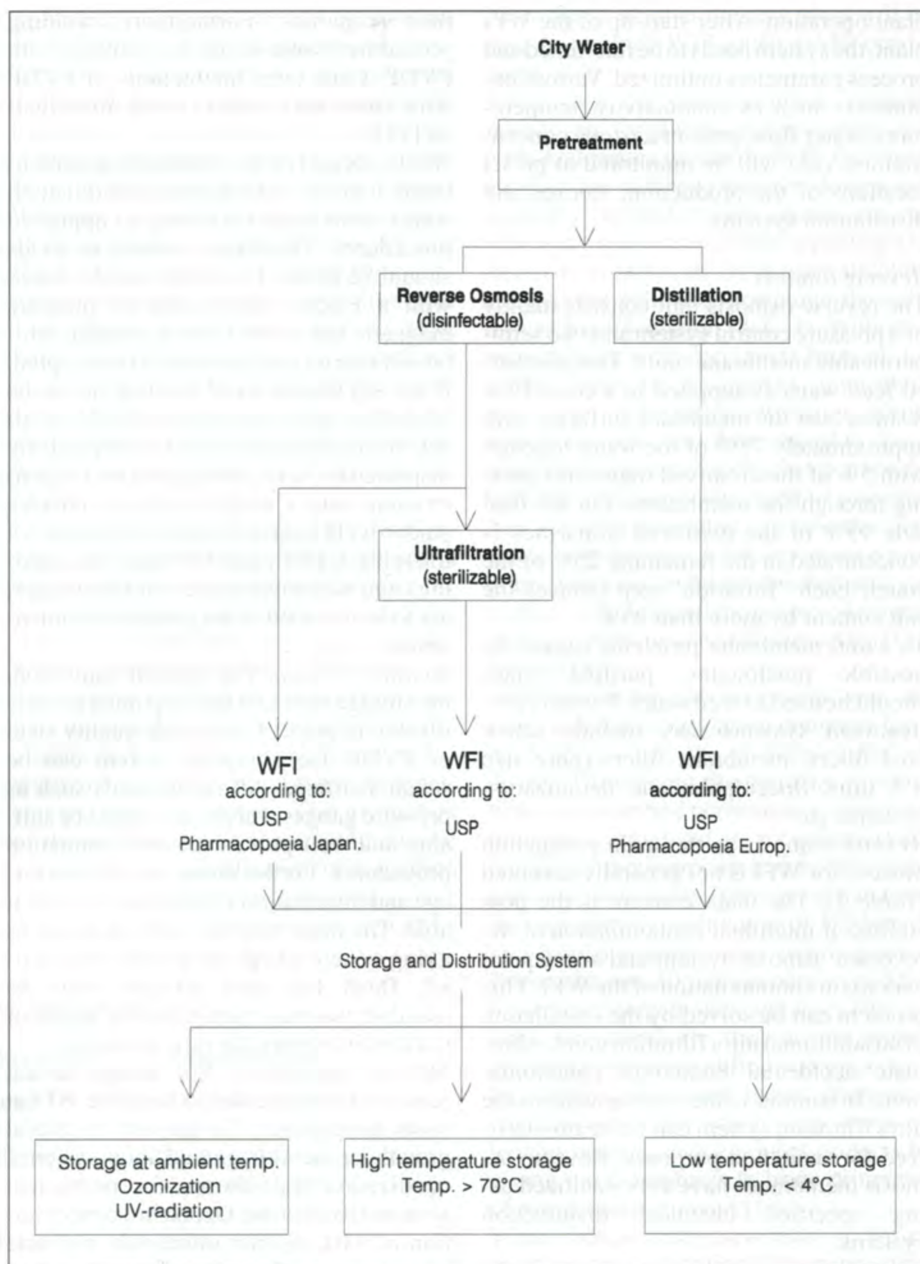

Fig. 3: Main Procedures for the Production of WFI

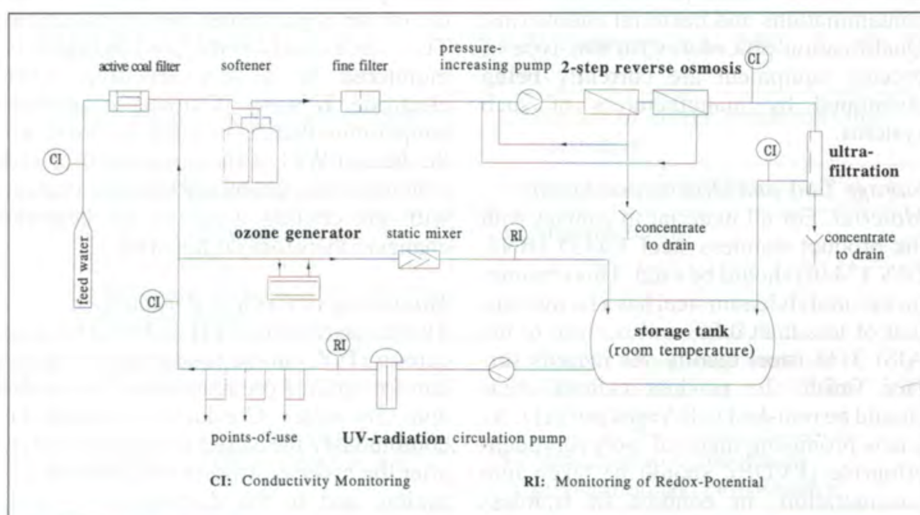

Fig. 4: Design of WFI Production and Distribution Facility

plant operation. After start-up of the WFI plant, the system needs to be fine-tuned and process parameters optimized. Various parameters such as conductivity, temperatures, water flow, pressure, ozone concentrations, etc., will be monitored at preset locations of the production, storage and distribution systems.

Reverse osmosis
The reverse osmosis unit consists mainly of a pressure control system and two semi-permeable membrane units. The pretreated feed water is supplied in a cross-flow fashion over the membrane surfaces, with approximately 75% of the water together with 5% of the dissolved impurities passing through the membranes. On the feed side 95% of the dissolved impurities is concentrated in the remaining 25% of the water. Each "filtration" step reduces the salt content by more than 95%.
To avoid membrane problems caused by possible precipitates, purified water should be used as feed water. Possible pretreatment systems may include active coal filters, membrane filters (pore size < 5 µm), flocculation and deionization systems, etc.
Reverse osmosis as the main production process for WFI is not generally accepted *(Table 2)*. The main concern is the possibility of microbial contamination of the reversed osmosis system and subsequent endotoxin contamination of the WFI. This problem can be solved by the installation of an additional ultra filtration unit to eliminate accidental endotoxin contaminations. In contrast to the reverse osmosis the ultra filtration system can be steam-sterilized for sanitation purposes. Reverse osmosis membranes have to be sanitized using specified chemical disinfection systems.

Ultra filtration
The ultra filtration unit acts as a fine filter and removes minute particles, microbial contaminations and bacterial endotoxins. Qualification procedures for this type of process equipment are currently being elaborated by manufacturers of such systems.

Storage Tank and Distribution System
Material. For all material in contact with the product stainless steel 1.4435 (BN2, DIN 17440) should be used. This chrome-nickel-molybdenum steel has a ferrite content of less than 0.5% and is close to the AISI 316L steel quality. As regards surface finish, the product contact areas should be polished to RA=0.4 µm [11]. As a new promising material, polyvinylideneflouride (PVDF)* should be taken into consideration. In contrast to stainless steel, PVDF requires neither electropolishing nor passivation due to its ideal surface properties. Furthermore, welding procedures seem to be less critical with PVDF. Long-term interactions of PVDF with ozone are currently being investigated [12].
Welds. As part of the validation documentation it needs to be demonstrated that all welds were done according to approved procedures. The ferrite content in welds should be below 1%, which can be tested with a Fischer Ferritscope to measure magnetic induction. Only automatic orbital welding techniques should be accepted. If for any reason hand welding has to be applied on parts in contact with the product, such connections have to be carefully inspected by X-ray procedures or dye penetration tests. Careful attention must be paid to weld seam elevations which are not tolerable. Certificates for materials, weldings and welding licenses for the welders are to be included in the general documentation.
Sanitary Design. For system sanitation, the storage tank and the loop must be sterilizable in place. Using high-quality steel or PVDF, the complete system can be steam-sterilized. All components such as pressure gauges, valves, etc., must be suitable and well proven for such sanitation procedures. Furthermore, the entire storage and distribution system must be drainable. The main loop has to be designed as a ring system where the WFI is recirculated. Dead legs and pockets must be avoided; the maximum tolerable length of pockets is 6 times the pipe diameter.
Storage Conditions. For energy-saving reasons it was decided to keep the WFI at room temperature. To prevent microbial growth in the storage and loop systems, ozonization was chosen for permanent system disinfection. Ozone in a concentration of 0.04 mg/liter effectively prevents bacterial growth and pyrogen build-up [11]. The ozone is generated on line in an electrolytic cell. Before the ozone-containing water reaches the points-of-use, the ozone is eliminated by UV radiation. The effectiveness of the UV treatments is monitored by a very sensitive redox electrode. If water is stored at ambient temperature there is no need to cool down the heated WFI at the points-of-use, and contamination problems through contact with the cooling water of the heat-exchangers therefore do not arise.

Monitoring of Critical Parameters
The measurement of pH and total organic carbon (TOC) in the feed water is important for optimal pretreatment of the available city water. Conductivity should be continuously measured in the feed water, after the reverse osmosis and the ultra filtration, and in the distribution system. Ozone concentrations are determined after the static mixer, in the storage tank and following the UV radiation by monitoring of the redox potential. This allows the control of the ozone concentration within the validated concentration range. Additionally, low and overfill levels in the storage tank and differential pressures in the WFI distribution loop should be monitored and linked to a central alarm system. The most critical parameters need to be registered on a recorder.
The number of viable particles in the feed water and after the reverse osmosis should be checked at pre-defined intervals. The ultra filtrate and the WFI at various locations in the loop must be checked daily. Pyrogen levels must also be investigated on a daily basis. In contrast to physical parameters, microbial and pyrogen data are not immediately available. Trend analysis and system maintenance procedures are thus of critical importance.
Complete chemical and microbial analysis of the feed water and the WFI should be carried out at pre-defined intervals according to pharmacopoeia specifications *(Tab. 2)*. All test results need to be documented and the monitoring and control devices calibrated at appropriate intervals.

Discussion and Conclusions
According to regulatory guidelines, water for injection (WFI) has to be used for a wide variety of pharmaceutical and biotechnical applications. Specifications for WFI are defined in national and international pharmacopoeias such as the USP XXII or the Ph. Eur. 2nd Ed. Besides low conductivity values and the absence of heavy metals, the most critical requirements are microbial and endotoxin limits *(Tab. 2)*. Traditionally, WFI is produced by distillation. The water is collected in a storage tank and then distributed to the various points-of-use by a loop-system. To avoid microbial growth, hot loop designs are standard, with temperatures kept at 70°C or higher *(Tab. 3)*. All metal components are of special stainless steel quality and finish.
Welds of stainless steel tubings are considered to be very critical parts of the distribution system. Technical details are specified in national and international standards [11]. Improper welds can lead to intolerable ferrite ion concentrations in the WFI and to microbial growth on weld seam elevations. As concerns cost, certified welds including the required documentation are very expensive. In this regard PVDF appears to have some significant advantages, although its long-term resistance to ozone is still not fully known. The necessary investigations have recently been initiated by several pharmaceutical companies, which shows the overall

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interest in this material for pharmaceutical applications [12].
Distillation systems as described are still standard plants for the production of WFI. However, in the meantime alternative production technologies, new control mechanisms for microbial growth limitation, and new materials with very promising properties have become available. Therefore, alternative systems are currently being discussed, but in many cases their use is as yet limited by regulatory specifications.
The discussed WFI plant *(Fig. 4)* takes advantage of recent technical developments relating to energy-saving production elements. Reverse osmosis systems are especially advantageous in respect of operating cost, which is up to 10 times lower than with traditional distillation techniques [19]. Energy consumption has been further reduced by running the storage and distribution system at ambient temperature. This became possible thanks to progress in disinfection technology based on ozonization/de-ozonization of the recirculated water. For maximal safety, the 2-step reverse osmosis has been combined with an ultra-filtration unit.
According to Ph. Eur., WFI has to be produced by a distillation process *(Tab. 2)*. The development of new technologies for the production of WFI is therefore limited by such requirements, even if the product itself would meet the pharmacopoeia specifications. This problem has recently been addressed and it will be proposed to the European Pharmacopoeia Commission to eliminate production system requirements from the WFI monograph [12]. As regards WFI specifications *(Tab. 2)*, replacement of the traditional chemical tests by one physical parameter is currently being discussed. Recently, conductivity limits of 1.25 µSi/cm have been proposed [13]. Such low conductivity requirements might have a significant impact on the design of WFI production systems, since not all currently available techniques can deliver a product to meet these new specifications.
Looking at the overall agenda for the planning and installation of WFI facilities, a very critical point is approached after the proposal of the preliminary basis of design. At this point the most important decision of the project has to be made: can the proposed design be accepted or must it be modified or even replaced? Such decisions have to be based on critical evaluations of technical, regulatory, commercial and financial aspects. This evaluation of the preliminary concept could also be considered as a qualification or validation process of the initial planning, since through the reviewing process it will be determined whether the new plant will be able to produce WFI under GMP conditions.
Up to now our discussion was focused mainly on regulatory requirements and technical issues related to WFI facilities. Besides the validated basis of design and a clearly defined overall agenda for the validation project, two other aspects are of decisive importance in every validation project: the validation team and the validation documentation *(Tab. 4)*. Without a project team with specialists from all involved areas of a company, competent evaluation of the project at various points will not be possible. With regard to documentation, a general policy needs to be established, defining what kind of information has to be generated and how this data has to be presented. A documentation program will have to be established and coordinated throughout the project. An interesting concept has been discussed by D.H. Artiss [15].
GMP guidelines have evolved over the years and basic validation concepts have been accepted by the pharmaceutical industry. Throughout the industry, installation, operation and performance qualifications are carried out in accordance with regulatory requirements and have become standard procedures. Various publications dealing with the validation of WFI facilities have discussed such overall agendas. During the past 10 years general schedules have not changed substantially *(Tab. 5)*. Only the names for the various project phases have been modified to some extent. In addition, the most recent discussion contains very interesting suggestions on how to manage such complex projects by giving more emphasis to basic principles of project management [14].
What, then, is really new today in the area of process validation? The procedures have not changed substantially. What has

Table 4: Organization and Documentation of Validation Projects

Validation Team	Organizational Structure	Documentation
– Engineering – Research & Development – Production – Maintenance – QC – Regulatory Affairs	Management ↓ Manager of Validation Project ↓ Validation Team	– Validation Masterplan – Approved Specifications – Technical Documentation – Approved SOPs – Approved Validation Protocols – Validation Report

Table 5: Validation Programs for WFI Systems

PDA 1983 [17]:	D.H. Artiss, 1986 [15]:	W.V. Collentro et al. 1992 [14]:
1. System description, construction and operating considerations	1. Prevalidation of the total system	1. Review of design parameters and alternatives
2. Installation qualification	2. Construction validation	2. Design and engineering
3. Operational checks of equipment and instruments	3. Start-up validation: a. Functional operation b. Procedures verification c. Quality limits	3. Manufacturing
4. Initial WFI qualification testing	4. System qualification	4. Completion of equipment manufacturing, documentation and testing
5. Documentation and monitoring program	5. Approval of the system for use	5. Validation documentation preparation and performance
		6. Review and ongoing support

changed are regulatory standards and validation requirements, which have become more extensive and more sophisticated. As a consequence for the industry, validation efforts and costs have increased substantially. Although it is never questioned that as a general concept validation is undoubtedly important, current tendencies must also be critically reviewed and discussed with a view to the impact of validation activities on production costs. On the basis of recent investigations it was concluded that overall validation cost may amount to as much as 20% of the production cost [4]. It is thus conceivable that a point might be reached where the cost of validation programs outweighed their safety benefits. It therefore follows that current validation policies should be kept under critical review, but without losing control of drug safety. Today's challenge is to concentrate on the ciritical issues, set priorities – and stop validating for validation's sake.

To revert to our proposed system for the production of WFI: As a departure from the standard technology of the last ten years, is it just a technical fiction? In the perspective of the current regulatory environment this might appear to be the case. From the engineer's point of view, however, it is real and is already on its way to becoming more than merely another new concept.

Acknowledgments

Various experts and colleagues have contributed to our seminar publication with their valuable comments. We are especially indebted to Mr. W. Setz of CIBA-GEIGY AG for his remarks concerning the design of modern WFI facilities and Drs. W. Hecker, B. Vogel and M. Zimmer of Sandoz Pharma AG for information on quality attributes and specifications for WFI.

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Results of the six project groups of the second postgraduate seminar for Pharmaceutical Quality Assurance, winter semester 1992/93, Department of Pharmacy, Swiss Federal Institute of Technology (ETH), and the University of Basel, Pharmaceutical Faculty

Pharmaceutical Quality Assurance

Validation of analytical methods in vitamin assays

*E. Frewein, St. Geisshüsler, E. Sibler**

Keywords:
Validation, vitamin assays, microbiology, analytical methods, HPLC

Validation of *analytical methods is not only indispensable from a scientific point of view, but also a requirement from the regulatory and health authorities. This ensures that assay methods meet proper standards, are suitable for their intended use, and are adequately documented. Validation commonly refers to* physico-chemical *methods, although this requirement applies to* microbiological *test procedures as well. However, the complexity of microbiological assay methods makes their validation a difficult task.*

This papier discusses the validation parameters that are known from physico-chemical methods and attempts to show how they are applicable to microbiological assay methods. For this purpose* vitamins *are chosen as an exemplary category of compounds where physico-chemical (mainly chromatographic) as well as microbiological methods can be used for quantitation.

Depending on the objective and the target criteria envisioned, i.e. high sensitivity, high selectivity, pronounced ruggedness for routine work, speed, etc., one method principle may be superior to another. However, it cannot be denied that the trend points clearly in the direction of physico-chemical, i.e. chromatographic methods, although the microbiological alternative is still well established for the quantitation of* folic acid, vitamin B12, and biotin. *Moreover, microbiological determinations may even be required by the authorities in some cases.

Introduction

Today's assay methods in analytical chemistry of pharmaceuticals, food supplements, etc. are highly sophisticated, use complex extraction procedures, advanced instrumentation technology and often a high degree of automation, especially in routine labs. However, data and appropriate documentation must demonstrate that a method is scientifically sound and that it has been systematically evaluated. As a rule the following parameters are applied to show evidence of the performance, effectiveness and suitability of an analytical method:

- precision;
- accuracy;
- selectivity;
- linearity;
- limit of quantitation;
- ruggedness

These requirements are commonly summarized by the term *validation*. It is the procedure used to prove that a test method consistently yields what it is expected and required to do. Current Good Manu-

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facturing Practice guidelines require that test methods, which are employed for assessing compliance of pharmaceutical products with established specifications, meet proper standards of accuracy and reliability, and sufficient laboratory data must be available to document the validity of procedures. Validation of analytical methods therefore is an indispensable part of a modern assay method development. The pharmaceutical industry is especially interested in method validation because of the steadily increasing list of new products associated with clinical and environmental concerns and because each new product requires a set of appropriate assay methods. Nowadays, an increasing amount of effort, time and money is absorbed by the validation work of an analytical method. Furthermore, most regulatory and health authorities have declared validation mandatory [1–5].
Usually analysts are familiar with the validation requirements for physico-chemical, chromatographic or spectroscopic methods. This field is well covered by a number of authors [e.g. 6–14] and official publications [15–18]. Validation is not only required for physico-chemical methods, but is also mandatory for microbiological and enzymatic assay methods. Seyfarth [19] states that "even if microbiological test procedures present certain problems in validation due to the large deviation of the results, one should demonstrate that the methods are suitable for the intended use". Also Shah et al. [20] emphasize that validation criteria of microbiological assays should be based on the requirements of the study and should match those of chromatographic methods. In this domain of validation, however, the available knowledge, the number of specialists and the amount of published work are very limited. Even compendia give only a few hints on the subject.
The aim of this papier is to discuss validation criteria for microbiological assay methods and to show how they compare with those known from validation of physico-chemical (e.g. chromatographic) assay methods. Vitamins will be used as an example, since several compounds in this class can be quantitated by physico-chemical as well as by microbiological methods.
Aspects of validation criteria in analytical methods using enzymes will be discussed in another publication [21].

Vitamin Assays:
The Choice of Methods

Vitamins are an important group of organic compounds required for normal growth and maintenance of life by man and animals. Furthermore, they are widespread in pharmaceutical products and nutritional supplements for human and animal use *(added vitamins)*. Vitamins are also (main) ingredients in a variety of natural food such as fruit, vegetables, etc. *(endogenous vitamins)*. The ubiquity of this chemically heterogeneous group of compounds explains the need for analytical methods that allow for their quantitation. Vitamin activity or potency can be measured by one of the following principles:
In *biological methods,* animals are used where physiological changes serve as a quantitative indicator of the amount of a vitamin (e.g. the curative rat test for *vitamin D)*. Despite the reliability and specificity of biological methods, for several reasons they have become less popular and have been replaced by alternative, mostly physico-chemical methods. Therefore, they will not be covered in this paper.
Most vitamins can now be quantitated by *physico-chemical methods*. Chromatography, especially high-performance liquid chromatography (HPLC), is predominant, but titrimetry (e.g. for *vitamin C)* or photometry (e.g. for *vitamin B_6)* are also employed. In these methods vitamins are determined by using the reducing property (redox titration of *vitamin C)*, by measuring the spectrophotometrical response of a reaction product/derivative (e.g. *vitamin B_6)*, or by integrating the signal of an HPLC-UV system (e.g. *vitamin PP)*.
Microbiological methods are a third way of quantitating vitamins. At the end of the 19th century scientists observed that growth of some fungi could depend on "essential factors". But it was only in 1937 that Snell [22] found that microorganisms could be used for quantitative measurement of vitamins. The principle: strains of microorganisms are selected which need a certain vitamin for growth or metabolism. Quantitation then is done titrimetrically (titration of the lactic acid produced by Lactobacillus in the presence of *vitamin B_2)*, gravimetrically (weighing of the mycel built from Neurospora in the presence of *vitamin B_6)*, or turbidimetrically (measurement of the turbidness due to cell propagation in the presence of *vitamin B_{12})*.
Finally some vitamins can be determined by radioimmunological assay (RIA) methods (e.g. *vitamin B_{12})* or by mass spectrometry. An overview is given by Friedrich [23].
For some vitamins the choice of two (or more) methods is given. *Table 1* gives an overview of the microbiological and physico-chemical methods officially used by the USP XXII, USP XXII Nutritional Supplements [24] and the Schweizerisches

Table 1: Overview of compendial methods for vitamin quantitation

Vitamin	Microbiological Methods				Physico-chemical Methods			
	Turbid.	*Titration*	*Gravim.*	*Fluorim.*	*Photom.*	*Titration*	*HPLC*	*GC*
A (Retinol)					SLB/USP		SLB	
D (Cholecalciferol)					USP		SLB/USP	
E (α-Tocopherol)					SLB/USP		SLB	USP
K (Phyllochinon)					SLB		SLB	
B_1 (Thiamine)				SLB/USP			USP	
B_2 (Riboflavin)		SLB		SLB/USP			SLB/USP	
B_6 (Pyridoxine)	SLB		SLB		SLB		SLB/USP	
B_{12} (Cyanocobalam.)	SLB/USP						USP	
Biotin	SLB/USP	SLB					USP	
Folic acid	SLB	SLB					USP	
PP (Niacin)	USP	SLB			USP		SLB/USP	
Panthenol	USP	SLB			SLB		SLB	
C (Ascorbic acid)					SLB	SLB/USP	SLB	
Rutin					SLB			
Inosit			SLB					SLB

SLB = Schweizerisches Lebensmittelbuch 1989
USP = United States Pharmacopeia XXII, incl. Nutritional Supplements

Lebensmittelbuch (SLB) [25]. Other compendia such as the British, German or European Pharmacopeia do not include microbiological methods for the quantitation of vitamins.

Method Validation

In the following the validation criteria commonly applied to physico-chemical methods (especially HPLC methods) are cited and attempts are made to explain how they compare with microbiological methods. Depending on the objective, the target criteria envisioned and the nature of the compound in question (reaction intermediate, bulk drug substance, formulated dosage form, endogenous food ingredient, biological sample, etc.), the appropriate validation parameters have to be selected and adapted, and a procedure must be individually outlined.

Precision

Precision usually is divided into system precision and method precision. *System precision* is the repeatability or reproducibility of replicate injections in the case of e.g. HPLC. It is therefore applicable whenever an instrumentation with a dosing device such as an automatic injector is involved in the quantitation. System precision in instrumental chromatography should not exceed 2% [26–27], but modern instrumentation such as HPLC with autoinjectors give precision values well below 1% RSD (relative standard deviation). *Method precision,* on the other hand, can be determined by carrying independent analyses – at least six – of a homogeneous sample through the complete analytical procedure from sample preparation to the final test result. The precision is usually expressed as the RSD and is a degree of the reproducibility under normal operating circumstances. It is customary to assume that microbiological assays may be subjected to an inherent error of 10–15% and to accept as identical, replicates that do not differ by more than that amount [28].

Accuracy

The *accuracy* expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value (e.g. international standard) and the (mean) value obtained by applying the test procedure a number of times. The accuracy provides therefore an indication of *systematic errors.* It is, however, important to realize that there is no method of determining the true value of the concentration of a component in a natural product; thus there is no method of measuring the systematic error. The use of an internal standard or of recovery tests can give at most a hint of whether an appreciable systematic error exists. For microbiological determinations, recoveries of 90 to 110% are considered acceptable [29].

The only practical method suitable for estimating the accuracy of a result is the *comparison* of various methods. It is important, however, that the methods are truly independent. Unfortunately, agreement does not ensure accuracy; both methods could be wrong. Only in case of high selectivity can it be assumed that the accuracy of an analytical method is constant, otherwise the systematic error is influenced by other components (active substances and excipients) in the sample (e.g. multivitamin preparations, food). In official analysis the accuracy is of secondary importance because it is rarely recognized that by definition the result of an ideal analysis is identical with the true value. The method has therefore to be standardized and defined by law *(official methods).*

In pharmaceutical preparations, vitamins are usually regarded as well-defined chemical compounds like most conventional active substances; therefore methods of determining the *recovery* are accepted to be a measure for accuracy. Recovery studies must be made in the sample matrix with care taken to design realistic spiked samples. If the sample matrix cannot be adequately duplicated because it is not free of analyte, the standard addition method can be applied [30]. Reliable statistical evaluation of this approach can be performed on any personal computer using a spread sheet program (e.g. LOTUS 123 or EXCEL) or programmable pocket calculator.

Selectivity

The *selectivity or specificity* of an analytical method is its ability to determine accurately and specifically the analyte in the presence of components that may be expected to be present in the sample, whether from the matrix or from other (unknown) compounds (e.g. vitamers in natural products). Modern HPLC methods used for the determination of vitamins in pharmaceutical preparations are considered to be highly selective. With the availability of high-efficiency columns, appropriate mobile phases, and function-selective detectors (e.g. UV/VIS, fluorescence, electro-chemical), this is generally not a problem for routine assay work. The peak purity can easily be demonstrated by numerous techniques such as peak ratioing, continuous UV scanning, TLC of the detector eluent or sample spiking. Chemometric methods, e.g. Principle Component Analysis (PCA), in conjunction with powerful microcomputers facilitate the integration of these validation tools into modern chromatographic systems.

Microbiological methods of vitamin determination are based on the nutritional requirement of a microorganism for a certain vitamin. Although it is essential that a test organism needs the vitamin being assayed, this does not ensure that it is appropriate for use. Many microorganisms can synthesize a vitamin from precursors, derivatives, or breakdown products that would not occur in the metabolism of animals or humans. Simple and reliable procedures require the test organism to possess the following characteristics:

- specific requirement for the vitamin forms that are biologically active in higher animals;
- genetic constancy during prolonged response;
- rapid growth cycle;
- growth response that is not easily influenced by neutralization salts or other substances which may be present in an extract of a sample (e.g. antibiotics and preservatives in pharmaceutical and cosmetic preparations);
- non-pathogenic property.

The extraction solution might contain impurities which exert a *stimulating* (upward drift) or *inhibiting* effect (downward drift) on the growth of the microorganisms. A possible means for eliminating the upward drift is the enrichment of the basal medium with one or more nutrient factors which are suspected to be the cause of the drift. The growth-inhibiting substances responsible for the downward shift consist most frequently of extractable materials. In seeking a method for the optimum freeing of the vitamin from a particular material, this consideration must be taken into account. The best test for the reliability of a microbiological determination is its comparison with a physico-chemical and/or biological method, or measurement with other test strains. A literature search for the selection of a suitable test strain may be necessary for vitamin determination in food.

Folic acid can be cited as an example where the length of the glutamate chain markedly influences the growth response of the test microorganism. The number of naturally occurring folates (approx. 140) and the chemical lability and variability of these compounds make their quantitation difficult [23]. For this reason a survey of an improved microbiological assay method for *folic acid* is at present being carried out by the Commission of the European Communities, Bureau of Reference, Brussels [31].

Linearity

The *linearity* of an analytical method is its ability to elicit test results that are directly, or by well-defined mathematical transformation, proportional to the concentration of analyte in samples. With most physico-chemical methods, the response is,

within a given working range, directly proportional to the concentration (amount) of analyte in the sample. For microbiological assays, it is necessary to measure with a sufficient number of standards to adequately define the relationship between concentration and response. This relationship must be demonstrated to be continuous and reproducible. The simplest relationship for response versus concentration should be determined and the fit should be statistically tested (ANOVA).

In general, experimental data can be analyzed using two types of models: *Mechanistic models* are based on a specific formula derived from theoretical considerations of the process which connects a dependent (or response variable) with one or more independent variables. The Michaelis-Menten equation is a well-known example of a mechanistic model. An *empirical model* is used when the exact form of a response variable with respect to the independent variable(s) is unknown; essentially it is an approximation of the real but unknown model, e.g. the logit-log transformation in RIA. Most of these functional relationships cannot be written in linear form. A popular graphical approach was to transform mathematically the model equation into a form resulting in a straight-line plot (e.g. Lineweaver-Burk plot, Scatchard plot). The linear transformation may distort (usually magnify) the experimental variability, leading to biased estimates for the values of the parameters. A correctly weighted linear regression can give satisfactory results, but non-linear regression is often better. Statistical computer programs (e.g. SPSS, BMDP, SAS) are now widely available and provide powerful procedures for linear and non-linear regression suitable for many kinds of bioassays. A word of caution is appropriate: an elegant mathematical model or a sophisticated computer program can never substitute for poor and unreliable experiments. In most microbiological vitamin assays that use turbidimetry or acidimetry, a standard curve is prepared by plotting growth (turbidity, titratable acid) versus µg or ng of vitamin per test tube. From the standard curve the amount of vitamin in the various levels of test solutions is determined by interpolation. Unfortunately, the working range is restricted to less than one order of magnitude (decade). A relatively exact initial estimation of the vitamin level in the specimen is essential for a proper extraction and dilution scheme. The design and analysis (cf. USP XXII <111>) of a microbiological vitamin assay is therefore an important step for reliable results, especially in comparison with modern HPLC methods.

Limit of Quantitation

The *limit of quantitation* (LOQ) is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. The LOQ is expressed as the concentration of analyte in the sample. The LOQ is also called *limit of reliable measurement* [13]. The lowest amount of analyte in a sample which can be detected but not quantitated as an exact value is called *limit of detection* (LOD). The LOD is mostly a parameter of limit tests and therefore of less interest in this context. The *sensitivity* is the capacity of the test procedure to record small variations in concentration; the slope of the calibration curve is therefore a measure of sensitivity.

The determination of the LOQ of an analytical method may vary, depending on whether it is an instrumental or an non-instrumental method. For *instrumental* procedures (most physico-chemical methods), the LOQ is determined by statistical analysis of the calibration curve [32–34]. A word of caution is needed at this point: all these computations are based on assumptions (standards known with no error, population variability constant on all levels, normal distribution, calibration and measurement environments essentially similar), and it is advisable to validate this limit by the analysis of a suitable number of samples known to be near or prepared at the LOQ. For *non-instrumental* procedures (most biological methods, including microbiological vitamin assays), the LOQ is determined empirically by analysis of samples with known concentrations of analyte.

When comparing microbiological and physico-chemical methods for vitamin analysis, it is evident that the LOQ for some microbiological methods is of several magnitudes lower than for HPLC methods *(vitamin B_{12}, biotin, vitamin B_6)*. *Vitamin B_6* concentrations as low as 50 ng/mL are determinable with microbiological methods whereas 10 µg/mL are needed for HPLC determination according to the SLB. As of now there are no reliable physico-chemical alternatives for the quantitation of *vitamin B_{12}* and *biotin* in common pharmaceutical preparations.

Ruggedness

For practical purposes it is very important that a proposed method has a high *ruggedness or robustness*. This means that small changes in the conditions of the analytical procedure (pH, temperature, purity of analytical reagents, storage time of the samples, wavelength of measurement, etc.) lead to insignificant changes in the results. A measure of the robustness of a method is its reliability, as this implies that the variation of the systematic and random errors with time and the number of the extreme values which have to be excluded for statistical treatment of the results are low, and that there is a good match between the results of different laboratories.

Depending on the purpose of the investigation, the various analytical procedures have to satisfy different requirements. Where routine work is involved, a high robustness is important.

Robustness is a critical point in the *validation* of a microbiological method [35]. The conditions (incubation, preparation of inoculum, buffer solutions, etc.) have to be well defined and closely followed. Berg et al. [36] report that the complex nature of a microbiological vitamin assay also implies that many differences will remain between various laboratories. These differences are due to the fact that each microbiological laboratory has its own "traditions" with respect to work procedures, glassware, instruments, etc. Additionally, the work routine at each laboratory varies with the room, the number of assays to be performed, etc. For this reason an intra- and interlaboratory survey *(cross-validation)* with two or more analysts/laboratories should be conducted in order to ensure ruggedness. In some cases the comparison with an official (or at least different) method may be indicated. Whenever discrepancies arise they should be subject to discussion with the issuing analyst/laboratory [37].

Discussion

Niacin (vitamin PP) is an example for which the USP XXII [38] describes both a physico-chemical (photometric) and a microbiological method. In addition the USP XXII Nutritional Supplements contain a HPLC method. Quantitation of *niacin* has also been covered by several authors [39–43]. Van Niekerk et al. [44] demonstrated that the *niacin* assay results obtained by a microbiological procedure were in close agreement with those from a HPLC method. The microbiological assay gave a comparable recovery and even a slightly better precision. Furthermore, the HPLC method was found to be susceptible to changes in the extraction conditions.

When comparing the *niacin* determination procedures of the three compendial methods it is obvious that physico-chemical methods are more straightforward, faster and probably less error-prone than the microbiological method. A short extraction is followed by either injection onto a HPLC column or by a derivatization step leading to the product that subsequently is determined photometrically in the visible range. Microbiological assays, on the other hand, make the preparation of a number of reagents, buffer solutions and the inoculum from the Lactobacillus cell suspension necessary, followed by a photometric determination. Sterilizing the material and incubating the test organisms with the sub-

strates are quite time-consuming. The cell suspension has to be recultivated periodically (usually monthly) and the approximate number of reproductive microorganisms has to be determined by turbidimetry before using for microbiological quantitation. Also the pH of the medium, the incubation conditions (time, temperature), the microbiological purity of the glassware and the water, etc. are factors of importance. Bell [45] reports on a number of such issues when dealing with microbiological assays for the quantitation of *vitamins of the B group*.

Voss et al. [46–47] also report on the *B group vitamins* and compared photometric with microbiological methods. They found that the latter are distinguished by greater sensitivity (LOD in the *pg*-range) and selectivity. Both methods showed comparable results for precision and accuracy.

Gregory [48] compared a HPLC method with microbiological methods for *vitamin* B_6 in cereals and found that the HPLC method was more satisfactory because of its simplicity, accuracy, and high precision.

Although Shah et al. [20] claim that the criteria for precision and accuracy of microbiological assays should match those of chromatographic methods, most microbiological methods described in the literature were found to give a much wider precision range than physico-chemical methods. The assay results of Voigt et al. [49] show a pronounced variation; also the SLB sets ± 10% from the mean and a RSD of 5% as limit for the precision. According to Bognar [50], "available analytical methods for vitamins are usually complicated, labor-intensive and of low precision. Errors of more than 10% are not infrequent. Up to 50% fluctuation in the results for *vitamins* B_1, B_2 *and* B_6 has been found in a collaborative study on vitamin analysis in food ... in which 18 laboratories of various European countries participated". Precision and accuracy are obviously two issues of concern. Subsequently, the author presents HPLC methods for several vitamins, including such valuable information as precision, recovery, LOD, and sample throughput.

Barna [51] demonstrated in his studies with *vitamin* B_2 in baby food samples that a good correlation between results determined by the microbiological and the HPLC method can be found. In another paragraph, however, he expresses his concerns by saying that "the microbiological riboflavin assay ... is very sensitive and selective but needs too much time and manual work and in the presence of antimicrobiological substances (antibiotics, preservatives) it cannot be used".

Finally, the SLB gives some information on the methods described therein. For *vitamin* B_6, for instance, the microbiological procedure is more sensitive than the photometric method and has to be used whenever *endogenous* B_6 in food has to be measured: it accounts for all three B_6 *vitamers*. The subsequent HPLC method is recommended for quantitation of *added vitamins* in food and cosmetic products only.

Conclusion

Table 2 shows the advantages and disadvantages of microbiological assay methods of vitamins.

Physico-chemical as well as microbiological methods have their strengths and weaknesses. Although microbiological testing procedures for vitamins and amino acids are being widely displaced by chromatographic methods, microbiological procedures can still be superior to chemical methods for the analysis of substances that have a growth-regulating influence on microorganisms. Microbiological assays, however, cannot be applied to *fat soluble vitamins and vitamin C*, since bacteria do not need them for growth [52].

Whereas microbiological methods are generally more time-consuming, demand well-trained people, and are usually less precise in comparison with physico-chemical methods, they are able to make up in selectivity or in sensitivity *(vitamin* B_6, *vitamin* B_{12}*, biotin)*. In these cases they still have a widespread use in quality control laboratories of pharmaceutical companies and state/federal institutions. To obtain reliable and reproducible results the operating procedures have to be well-defined for each assay and strictly followed.

However, the trends clearly go towards chemical, i.e. chromatographic methods where – thanks to modern instrumentation – a high degree of precision, accuracy and selectivity as well as automation can be achieved. The HPLC method has an advantage when a small number of samples is to be analyzed. But for a large number of samples HPLC is only competitive when an automatic sampler enables the chromatograph to run continuously. Yet costs have to be taken into consideration, too. The HPLC equipment necessary for doing reliable (and unattended) work can hurt the budget effectively.

The *validation* of a microbiological vitamin assay is a very complex process, since numerous steps are involved in the assay procedure. Kavanagh et al. [35] state that "microbiological assaying is one of the more difficult analytical fields" and that "it encompasses basic operations of analytical chemistry, use of a living organism as the indicator of response, non-linear calibration lines, and multitudinous unknown interferences". So, the number of critical steps to be controlled and validated is considerably larger than in physico-chemical assays, where validation is more straightforward and the time and effort needed not any different from that for other chemical compounds. Inaccurate data can be expected from analysis employing organisms that do not reach a stabilized response, since the presence of interfering agents (especially inhibitors) in sample extracts could not be overcome by an extended incubation period [53]. The materials used, such as buffer solutions, and diluents, test organisms, culture media, etc., as well as the utensils (e.g. autoclave, laminar flow bench, incubators), have to be qualified and validated, since the results depend strongly on their reliability [54].

Proper validation of a microbiological method often goes beyond the possibilities, capacities and financial resources of a laboratory that is not specialised in microbiological methods and is lacking the expertise for running such assays on a routine basis. And since validation of assay methods is a requirement of increasing importance, laboratories tend to hand their samples for microbiological vitamin quantitation to official laboratories or institutions (like the *Swiss Vitamin Institute*

Table 2: Advantages and disadvantages of microbiological assay methods of vitamins

Microbiological Assay Methods	
Advantages	*Disadvantages*
fast and simple (routine laboratory)	not suitable for occasional use
suitable for semi-automatization	time-consuming preparation of reagents etc.
low cost (instrumentation)	periodical recultivation of test organisms
preferred for assaying *endogenous* vitamins and vitamers	danger of contamination with other microorganisms
unmatched sensitivity for *vitamin* B_6, *vitamin* B_{12} and *biotin*	validation difficult and not clearly defined
highly selective	interferences (preservatives etc.)

Basel) that have the knowledge and expertise to perform such assay work.

As an abbreviated validation procedure for a microbiological assay method we suggest focussing on the following criteria:

- *Precision study*
  Five to ten complete determinations of a vitamin should be carried out under the same conditions, according to the assay method procedure. Results should be within ±10% of the mean and exhibit a RSD <5%.
- *Accuracy study*
  The method should be compared with another, independent method – preferably HPLC – described in a compendium or in the literature. The results should be in close agreement. To get an estimation of the recovery standard, additions of 50 and 100% to a sample can be performed.
- *Selectivity*
  If available a placebo should be run to ensure selective quantitation of the compound (i.e. vitamin) of interest.

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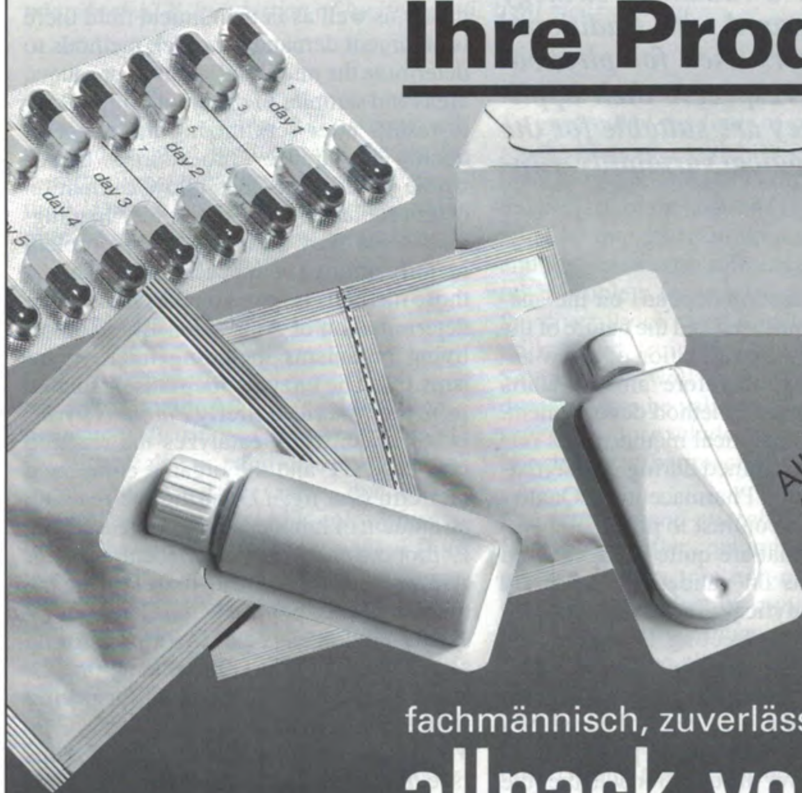

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Pharmaceutical Quality Assurance

The luciferin-luciferase assay: An example of validating an enzymatic analytical method

*Ch. Goez, T.-D. Luu, D. Meier**

The luciferin-luciferase assay for ATP determination is scrutinized for critical control points. Hints are given for a validation strategy of single reaction steps. The method is compared with traditional microbial procedures. Performance parameters used for physico-chemical analytical methods are discussed in respect to their applicability to this method. It is concluded that they are suitable for the luciferin-luciferase assay due to the small biological variability within the system.

Introduction

Application of well characterized and fully validated analytical methods in routine analysis is of crucial importance. Only methods producing reliable and reproducible results are appropriate for quality assessment of products and processes [1]. Performance of analytical methods is judged by parameters such as selectivity, accuracy, precision, limit of detection, linearity and ruggedness [2, 3, 4]. Which of these parameters is to be applied to the problem in question depends on the analytical technique used and the nature of the sample [1, 3]. Any validation of a new analytical method therefore already starts during the phase of method development. Validation of analytical methods was one of the topics examined during a postgraduate seminar on "Pharmaceutical Quality Assurance". In contrast to physico-chemical methods that are quite often covered in publications on validation, the rarely treated bioanalytical procedures were focused upon at this meeting. Discussing the example of ATP determination by the luciferin/luciferase reaction, part of the team tried to analyse critical control points of a defined enzymatic analytic procedure as well as to answer the question whether the performance parameters mentioned above are sufficient criteria for the assessment of this method.

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Principle of the luciferin method

In pharmaceutical, cosmetic and food industry as well as in the clinical field there is an urgent demand for quick methods to determine the microbial status of products, areas and samples of different origin. Rapid results after scrutinizing the effect of quality assurance procedures and/or whole production steps allow estimation of microbial contamination and loading; vice versa they facilitate rapid problem identification or better patient care. One of those methods is based on the quantitative determination of ATP, a constituent of all living organisms including microorganisms [5]. The enzyme luciferase, isolated from the American firefly *Photinus pyralis* [6], specifically catalyzes the reaction between ATP and the organic compound luciferin (see *fig. 1*). During the reaction an amount of luminescence is released that is proportionate to the concentration of ATP in the sample and therefore to the number of cells present [7, 8]. Consequently this method can be used for monitoring a great number of ATP producing or consuming reactions [6] or as an unspecific indicator of biomass.

The procedure consists of several steps (see *fig. 2*) [8]: First a representative sample has to be drawn and worked up in an appropriate way to allow measurement. Following extraction and removal of

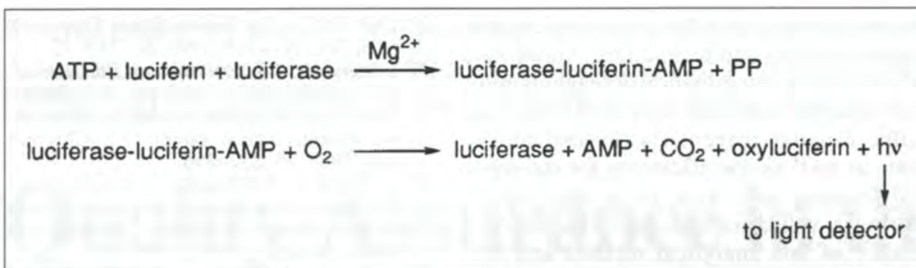

Fig. 1: luciferase light reaction

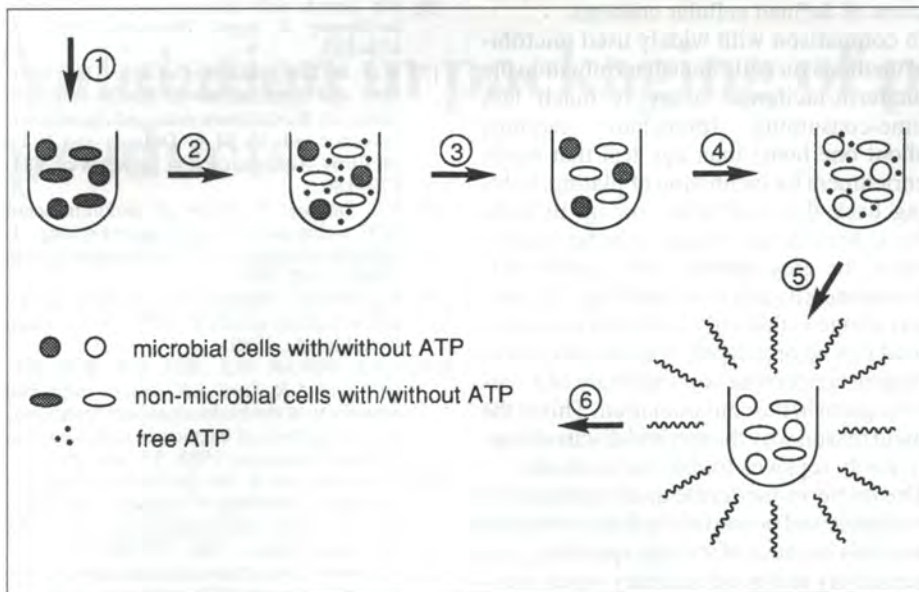

Fig. 2: reaction steps of the luciferin/luciferase assay. 1: sampling/sample work-up, 2: extraction of non-microbial ATP, 3: removal of non-microbial ATP, 4: extraction of microbial ATP, 5: addition of luciferase, 6: light measurement

somatic ATP, microbial ATP is liberated; after addition of luciferin and the luciferase the light signal emitted can be measured. During validation all these steps must be checked for critical control points and validated separately.

Critical control points

Sample preparation
Microbial contamination is seldom homogeneously distributed in a given sample [9]. Especially in solid or semisolid matrices and on surface areas, but also in homogeneous liquid media, colony formation at the surface, container wall or floor is observed. Thorough sample homogenisation can eliminate this problem. As further reaction steps require a liquid or at least a suspension, homogenisation of solid and semisolid matrices is indispensable. On the other hand, loss of microbial cells by lysis has to be prevented, and homogenisation must be done in a moderate way [8]. Apt conditions should be validated using test organisms embedded in the matrix in question.

To ensure that the limit of quantitation is reached, samples with little contamination have to be enriched by centrifugation, filtration [10] or incubation [11]. These steps must be integrated in the overall method and validated with reference probes. Another problem for validation is caused by the diversity of sample matrices [8] (e.g. milk, blood, fruit juice, waste water etc.). Sampling as well as working-up procedures have to be validated for each matrix separately to exclude unforeseen matrix effects on further reaction steps.

Extraction of non-microbial ATP
By nature or after contamination the sample can contain free non-microbial ATP, deriving from plant or somatic cells. Before actual measurement these sources of ATP have to be eliminated with suitable chemicals or surfactants, otherwise the amount of ATP detected would indicate a false high level of microbial contamination. During the validation process it has to be assured that reagents added at this stage exclusively dissolve the cell walls of somatic cells, without affecting microbial cells [8]. In this case experiments with defined microbial solutions could help verification. The fact that a bacterium like *Pseudomonas aeruginosa* is partially lysed by the widely used triton X-100 shows that this is no trivial problem [12]. The addition of detergents is also problematic with regard to a further aspect: any influence on further reaction steps has to be strictly avoided. Main interferences which might occur are either quenching of the light signal emitted or a change in the kinetics of the luciferase reaction itself [8].

Removal of non-microbial ATP
With the help of an ATPase the released non-microbial ATP is degraded. The more enzyme is added, the faster this reaction step proceeds. However, the amount of ATPase used is critical as it means an increasing danger of carry-over of ATP-cleaving activity to the following steps unless the ATP degrading enzyme is removed, inhibited or denatured [8]. Residual ATPase would diminish the level of microbial ATP and consequently lead to an underestimation of microbial contamination. So far no methods are known that completely inhibit the ATPase added and do not disturb luciferase activity. Nevertheless, if trichloroacetic acid is used to extract microbial ATP (see next step), followed by pH readjustment, operation with excess ATPase is regarded as possible and guarantees complete degradation of non-microbial ATP [8]. As in the preceding step it has to be shown that none of the reagents added influences the results obtained.

Extraction of microbial ATP
Extraction of microbial ATP can be achieved by addition of organic solvents, detergents or acids [8, 13], with the main requirement being quantitative release. Different response of microbes to the procedure used has to be investigated with experiments using suspensions containing defined type and number of microbial cells. Cells sticking together and forming aggregates can rather hinder the effectiveness of extraction.
As mentioned above, the reagents used in this step must also be tested for interference with the terminating luciferase reaction.

Addition of luciferase
The activity of enzymes like luciferase can only be validated if enzyme preparations of specified quality are used. For example, ATP-producing activity was detected in partially purified luciferase preparations, which certainly can destroy the specifity of this method [5, 7]. Consequently, before a new series of analyses is started, it is necessary to check the activity of the enzyme preparation used. Undercover activ-

ity can be detected by measuring the same sample several times over a period.

Like other enzymes, luciferase only works properly in a very narrow range of optimum conditions [5, 6, 14]. The method's design has to guarantee that these defined outer conditions, which were examined during the method evaluation period, are also maintained in routine application.

Measurement

The light signal of the luciferin/luciferase reaction reaches a maximum level 0.4 seconds after mixing of the reagents [7]. The actual time of measuring is thus of great importance. It is also noteworthy that the result of the measurement is given in RLU (relative light units) and cannot be correlated to any SI unit. Because of this calibration has to be carried out using defined ATP standard solutions [5, 15, 16].

Several matrix compounds as well as added reagents cause quenching of the light signal [7, 8]: in comparison with pure ATP solutions the intensity of luminescence is markedly reduced, which results in false cell number correlations. Dilution of the sample [7, 14] or addition of an internal ATP standard help to solve this problem. The identical sample is measured twice, once without and the second time after addition of a known amount of ATP; thus the influence of either the matrix or the reagent on the result is eliminated.

The impact of the luminometer used is a problem of qualification [17].

Conclusions

The luciferin-luciferase assay determines ATP levels and correlates ATP contents in the sample to the number of microbial cells present. This approach causes some general problems for validating this method. The calculation of the cell number relies on average ATP content in one microbial cell, which depends not only on the kind of organism (yeast or bacteria), but also on the developmental stage of the cells. In addition, procedures such as centrifugation, filtration or addition of reagents can lead to cellular stress, which again decreases the ATP content to a large extent. A period of reconditioning has to be introduced for processed samples before actual work-up starts.

As experiments by Simpson and co-workers indicate [16], the sensitivity of this method can be significantly improved when ATP-free media and reagents are used. Equipment and instruments are also often contaminated by ATP. Thus it becomes essential to work not only in a sterile, but also in an ATP-free manner during sample workup and analysis. Blind experiments can prove the effectiveness of cleaning procedures and techniques. Additional microbial contamination of reagents and instruments is another source of false results. Regular reagent checks and blind tests as well as specifications for the signal-to-noise ratio have to be developed during a validation process. The performance of this analytical method and its suitability for a specific task can be concisely examined by comparing it with already validated procedures for the cell count determination or with cell suspensions of defined cellular contents.

In comparison with widely used microbial methods for cell count determination the luciferin-luciferase assay is much less time-consuming (procedure requires about one hour) than any test that needs enrichment by incubation or plating, holding back the final result for up to three days. Another advantage is the favourable price, the great number of available self-contained kits and easy handling. The disadvantage is that only a general microbial load can be registered, without any information concerning sort and share of a specific germ in the contamination. This is the main reason why the method discussed only partly replaces traditional methods.

The luciferin-luciferase assay seems to be a suitable and powerful method for routine analysis because of its high specifity, high sensitivity and good accuracy when introducing an internal ATP standard. Compared with conventional microbiological methods the precision of this method is much greater and can even be improved by complete automation. Automation additionally increases the possibility of controlling reaction conditions and therefore the ruggedness of the method.

In summary, it can be concluded that the performance parameters mentioned above [2] are also suitable for the validation of enzymatic analytical methods. The influence of biological variability among bioanalytical methods is increasing from enzymatic and microbiological procedures through cell line assays to animal experiments and clinical studies. Enzymatic methods stand at the very beginning of this sequence. In terms of their properties and the performance parameters applicable, they are still close to physico-chemical methods.

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Quality Assurance in the Pharmaceutical Industry

Validation in packaging of pharmaceutical products

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Key Words:
packaging, validation, GMP, terminology, validation guideline, risk factors.

The packaging process is part of the production process and should be validated. Since the various stages in a packaging process can be quite complex, a pharmaceutical manufacturer's own validation guideline should exist in order to meet the legal and company specific validation requirements best. It has proven useful to subdivide packaging processes into two phases: filling and packaging. In each phase risks exist in connection with machinery, product and personnel, as well as materials flow and external suppliers. These risks should be analyzed and ranked, and appropriate validation measures should then be taken, based fundamentally on the results of calibrations and qualifications. Provided that calibration and qualification have been adequately performed, the quality of a packaging process can thus be safely assessed by in-process-control-results and control data of the finished product; they are, so to speak, the equivalent of validation.

Introduction

The key function of pharmaceutical packaging is to guarantee the correct presentation of a qualitatively unobjectionable product [1]. To accomplish this goal various requirements applying to the material [2] and printing [3] of the packaging components must be fulfilled:

- The packaging material must be physically, chemically and microbiologically suitable.
- The individual packaging components must be in conformity with the product's name and specification, the type of application and dosage, the quantity of content (number, volume, weight), the production and expiry date, the product's batch number, and even the article number of the packaging material itself.
- The integrity of the packaging components must be ensured with respect to both primary packaging material (containers, covers and all aids which are in direct contact with the product) and the secondary packaging material (labels, folders, boxes, applicators).

On the face of it there is nothing exciting about packaging. Quite often simple equipment lacking "high-tech" glamour is used. In practice, however, we are confronted with various problems. A crucial factor is the risk of cross-contamination. To appreciate the importance of cross-contamination it must be realized that various products are frequently packed on numerous lines, often in the same room.

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Furthermore, the configuration of the packaging lines can vary according to the different types of products being handled on each line. For example, a device such as a bundling machine is transferred from line to line in order to use capacity to the optimum.
The tendency to rationalize processes entails standardization of the packaging components and increases the rate of the goods being passed through. Thus visual controls tend to become more and more ineffective.
The importance of the staff in packaging must not be underestimated. Packaging is a complex process in which various persons are involved at different levels. As the packaging process still consists of a lot of manual work typically characterized by the repetition of multiple monotonous steps, mistakes can be overlooked easily. To reduce the risk of packaging errors, effective quality management is obviously needed.
To date the calibration of instruments, qualification of personnel and machinery devices, end- and in-process-controls and, last but not least, the proper description of the critical stages of the packaging process in the form of SOPs have been the classical measures for better control of packaging procedures.
The coordination of end-controls and in-process-controls gives a much higher rate of process reliability than do exclusive end-controls. But even the most intensive in-process- and end-controls do not enable us to ascertain how precisely the given process parameters have to be adhered to or which deviations from the rated values of the procedure are acceptable without adversely influencing product quality. For this we need validation [4].
In general, validation comprises "the documented act of proving that any procedure, process, equipment, material, activity, or system actually leads to the expected results" [5, p. 22].
The following article tries to illustrate and comment on this relatively new subject of GMP, referring to the legal, organizational, and practical aspects of process validation in packaging.

Legal Requirements

When considering the current GMP situation it has to be realized that demands for the validation of pharmaceutical manufacturing processes are not new [6]. As the final stage of production, packaging plays a role as important as the planning, development, formulation and production of pharmaceuticals. But how about legal requirements? In *Table 1* several guidelines have been listed and evaluated with respect to process validation and packaging validation. The results reveal the following:

Tab. 1: Summary of the current guidelines with special respect to the requirement of packaging validation

process validation	*packaging validation*
WHO	
5.1. …Processes and procedures should be established on the basis of a validation study and undergo periodic revalidation to ensure that they remain capable of achieving the intended results… 5.2. Critical processes should be validated, prospectively or retrospectively	not explicitly
EEC	
EEC-Note on development pharmaceutics and process validation (April 1988). "The proposed manufacturing process is a suitable one"	not explicitly
"Orange Guide"	
5. Manufacture validation	not explicitly 5.38: "all possible steps to avoid labelling and packaging errors"… "appropriate measures"
FDA	
"General principles of process validation" (21 CFR 10.90)	Packaging is obviously an integral part of process validation
DIN ISO 9000-9004 (EN 29000-29004)	
"Process and manufacturing guidelines"	not explicitly Packaging is listed under "handling and post-production functions"

- The PIC/EEC GMP Rules [7; 8; 9] and the "Orange Guide" [10] do not explicitly show a relation between process validation and the field of packaging.
- Due to the intensive interplay between the pharmaceutical manufacturer and his suppliers (e.g. just-in-time-deliveries), the ISO-9000-standards [11] are a factor of increasing importance (reduction of time-consuming controls for incoming goods). However, on the one hand the standards describe procedures for "Quality in Production (Process Controll" in ISO 9001 (clause 4.9) and 9002 (clause 4.8), but on the other hand packaging itself is listed under "handling and post-production functions".
- Up to now the FDA has taken the most advanced approach; the paper "General Principals of Process Validation" [12] includes packaging aspects explicitly.
- The latest WHO GMP-guideline [5] takes into account the aspect of validation and in particular the validation of production processes. According to the glossary the packaging procedures are included analogously. In order to express the legal requirement applicable to packaging processes more clearly, we recommend specifying the relevant text in [5, p. 27] by adding the term "in production":

"[...]
5. Validation
5.1.... Processes and procedures *in production* should be established on the basis of a validation study and undergo periodic revalidation to ensure that they remain capable of achieving the intended results...
5.2. Critical processes *in production* should be validated, prospectively or retrospectively. [...]"

This would give us a system of well-structured definitions, as depicted in *Figure 1*.

Organization

Specific procedures for carrying out and documenting validation work in packag-

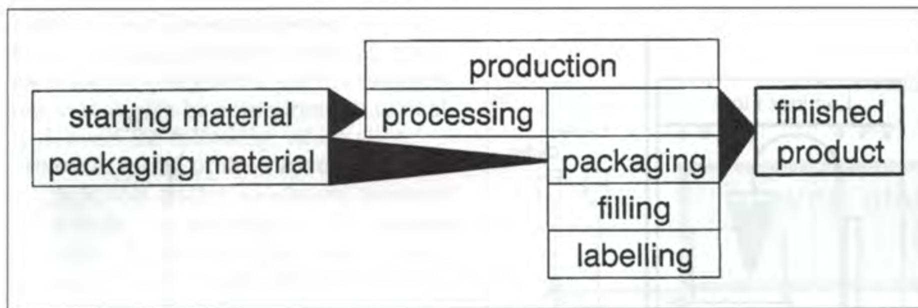

Fig. 1: A systematic approach to the terminology in pharmaceutical production according to the WHO/GMP-guideline 1992

ing are mentioned neither by the various GMP rules nor by any authority. For example, *P. Vogel*, a FDA official, states that it is virtually impossible for him or anyone at FDA to give a succinct, all-encompassing answer or formula to follow [13]. Not all steps of a packaging process need to be validated, but only the critical functions [4; 5; 14]. From experience it has proved useful to start the validation work with a company-specific concept laying down the goals and priorities. In any case the highest priority should be assigned to new packaging facilities. The concept should also take into consideration specific conditions such as organizational structures, responsibilities and "business philosophy" in the workplace. A uniform standardized method of solving validation problems is not only rational and effective but above all cost-saving [4]. Especially for packaging it appears necessary to develop a "validation action plan", since a company's packaging department

- has extensive contact with company internal departments as well as external suppliers;
- consists of complex processes (interaction of different machines, e.g. filling, coding, boxing, wrapping often regulated by EDP systems);
- shows a strong interaction between man and machine;
- has to fulfill numerous company-specific requirements with respect to the packaging lines (e.g. one product on each line or many different products on one line), flow of material of the packaging components (EDP controlling, structure of suppliers, just-in-time-deliveries), process conditions (continuous or discontinuous, and sort of packaging (final packaging by hand, on-line filling of sterile products in combination with final packaging, risk packaging).

The concept of "company guidelines" has already been successfully implemented in the field of pharmaceutical bulk product validation. The basic principles can be extended to the packaging of pharmaceuticals as well. The concept should include the following aspects [15]:

Responsibilies
A body of experts, e.g. the heads of Quality Control/Quality Assurance, Production, Development, Materials Management and Engineering, should set up a steering committee "validation" and a master plan. The steering committee, which consists of delegates of the above-mentioned departments, develops standards for the validation projects (e.g. action plans for technical inspections, calibration of instruments, qualification of rooms and machinery, process validation). Furthermore, the committee coordinates the various validation activities and supervises the timetables of the projects and the validation policy in general. The actual validation is performed by the validation teams, which are headed by area-responsible experts and specialists in Quality Control, Production, Development, Materials Management, Engineering and others *(Figure 2)*.

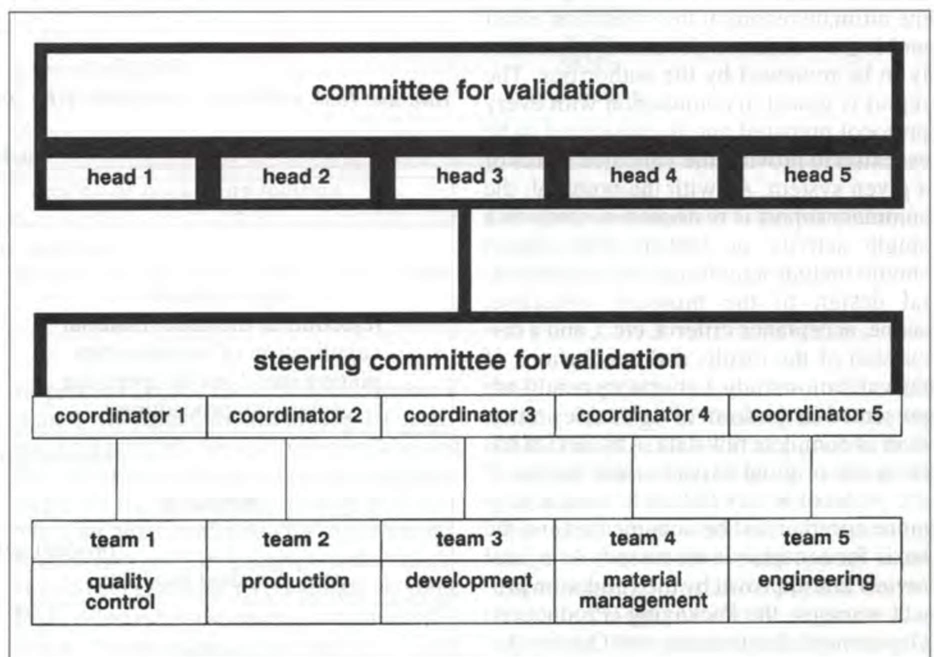

Fig. 2: A possible way to organize validation

Prerequisites
First and foremost, the quality profiles of the new (finished) product components must be established under optimized production procedures, along with the appropriate manufacturing and test instructions (operational qualification). The personnel should be qualified, i.e. skilled, instructed and trained.
New rooms and equipment should have passed the necessary technical inspections and the instruments should have been calibrated in order to undergo further qualification (installation qualification). Special areas (e.g. clean room zones) should also be qualified.

Documentation
The most essential element of an effective validation effort is complete and accurate documentation. Much of what is now considered to be effective validation was formerly classified as simply good engineering or development practice. Although these practices were carried out, they were sometimes incompletely documented, resulting in the loss of valuable information. A validation project file should be established in which all specifications, drawings, vendor literature and other documentation supporting the validation test are stored. At project completion, this file is maintained by and remains available to the operating department, serving as a reference guide to correct operation and maintenance of the system.
The major elements of validation documentation are as described below:

Master Plan
The master plan is the first formal document to be prepared in conjunction with a

validation project. Although not essential, it is a valuable planning tool for large projects, serving to lay out a preliminary plan for protocol preparation. The primary aim of the master plan is to provide a broad, general overview of the proposed validation effort and to serve as a source of information for management, peripherally involved company internal areas, and possibly for the authorities. It may also be useful for determining approximate budgetary requirements for the validation project and in obtaining management support for these expenditures.

Protocol
The validation protocol is a systematic, written plan, prepared in advance of validation start-up, which states specifically how a validation will be conducted. It describes the process, the critical parameters to be examined, and when and how samples are to be drawn. It also defines the expected product characteristics, stipulates appropriate acceptance criteria and assigns personnel responsibilities. The protocol contains basically the same elements as does the master plan. The chief differences between the protocol and the master plan lie in the areas of scope and degree of detail. Whereas the master plan provides a generalized overview of the proposed validation of an entire process, the protocol focuses on a specific step in the process or on an individual piece of equipment, describing in detail exactly how the validation will be performed.

Summary Report
The summary report comprises an abstract of the actual protocol results. It represents the ultimate record of the validation effort and is the validation document most likely to be reviewed by the authorities. The report is issued in conjunction with every protocol prepared and is considered to be essential to proving the validated status of a given system. As with the protocol, the summary report is restricted in scope to a single activity or system. This report should include a review of the experimental design of the protocol (objective, scope, acceptance criteria, etc.), and a discussion of the results and conclusions of the validation study. Laboratory results are presented in the form of data tables rather than as complete raw data. Any deviations from the original experimental design of the protocol or any failure to meet acceptance criteria must be documented and the basis for acceptance discussed. As a final review and approval by the validation project manager, the Packaging (Production) Department, Engineering and Quality Assurance management are required for acceptance.

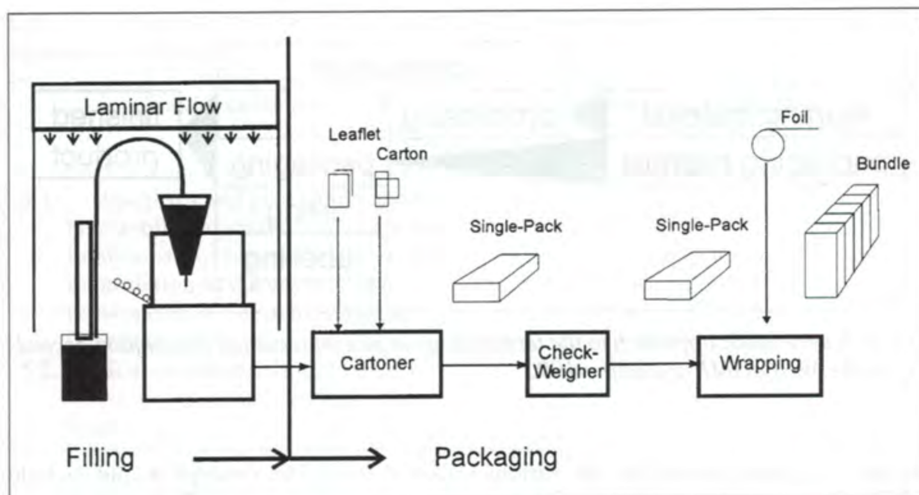

Fig. 3: Scheme of a filling Line

Tab. 2a: Risk analysis of the filling process

filling	
critical parameters	*measures*
machine-related aspects	
chemical / physical parameters contamination uniformity of dosage putting into / out of operation (cleaning procedures)	calibration / qualification / IPC IPC / monitoring / SOPs IPC SOPs / monitoring
personnel-related aspects	
hygiene compliance	instruction / SOPs / monitoring instruction / SOPs / monitoring
product-related aspects bulk products	primary packaging material
identity	specification (release-)certification IPC = visual control

Tab. 2b: Risk analysis of the packaging process

packaging	
critical parameters	*measures*
machine-related aspects	
error control rejection of incorrect material application of variable data putting into / out of operation (cleaning procedures)	code-reader / IPC (test run) IPC (test run) IPC (visual control) SOPs / monitoring
personnel-related aspects	
compliance	instruction / SOPs / monitoring
product-related aspects semi-finished products	secondary packaging material
identity	specification (release-)certification IPC = visual control

Final Project Summary Report
For a validation project involving multiple protocols, an overall project summary report may also be appropriate. It typically delivers the following information:
- a title listing of the various validation protocols and/or validation summary reports
- a short synopsis of the results
- number and titles of new directions, instructions, and standards
- protocol of staff information (e.g. sort of information, names of participants, dates of meetings).

Practical Approach
As already mentioned above, only critical functions and parameters need to be validated. To fulfill this requirement in an efficient way it has been proven that subdividing a complex process into smaller units is quite helpful in order to estimate the various individual risks more easily [4]. Based on the judgements of the experts and specialists in the validation teams, all possible risks of a process stage should be listed and weighed. Then the reasons for the risks which are ranked high and middle are further investigated. Finally, validation measures are stipulated and testing plans are set up.
This kind of procedure is transferred to a filling line as illustrated in *Figure 3.* The process can be subdivided into the stages filling and packaging. The specific risk analysis could then be performed for the following criteria:
- machinery related aspects;
- product related aspects;
- personnel related aspects.

The critical parameters and the suggested appropriate measures are listed in *Tables 2a and 2b.*

– Machinery and Product Related Aspects
With the critical parameters identified, operating limits must be developed for each variable. This is typically done in a multilevel fashion as shown in *Figure 4.* The edge of failure level, the extremes of which constitute the worst case situation, represents the broadest possible limits. Operation of the equipment or process outside these limits will result in a product which does not meet acceptance criteria. The edge of failure limits may be developed at the developmental stage especially for packaging materials; this, however, is not always possible.
The next level, the proven acceptable range (PAR), is the most ciritical. The extremes of the PAR represents the outermost limits within which satisfactory products can *safely* be produced (widest possible operating range). These must be defined through experimentation or can be obtained by using (validated) results of the in-process and/or end-controls. Approximate PAR values should ideally be established during the development phase and are communicated to the packaging area as part of the technology transfer package. They are then confirmed by experimentation during the commercial scale validation. For a retrospective validation, some PAR value information may be obtainable from accumulated packaging batch records. In order to give an added degree of safety in the event of process upsets, the PAR is typically set at a level somewhat within the edge of failure; however, the PAR should be as wide as possible to allow maximum flexibility in running the process.
The final level, the normal operating range, is that which would appear in the manufacturing procedure. Again, normal operating ranges would be set somewhat within the PAR values as a safety measure. The degree of setback would depend on the level of confidence in the PAR values

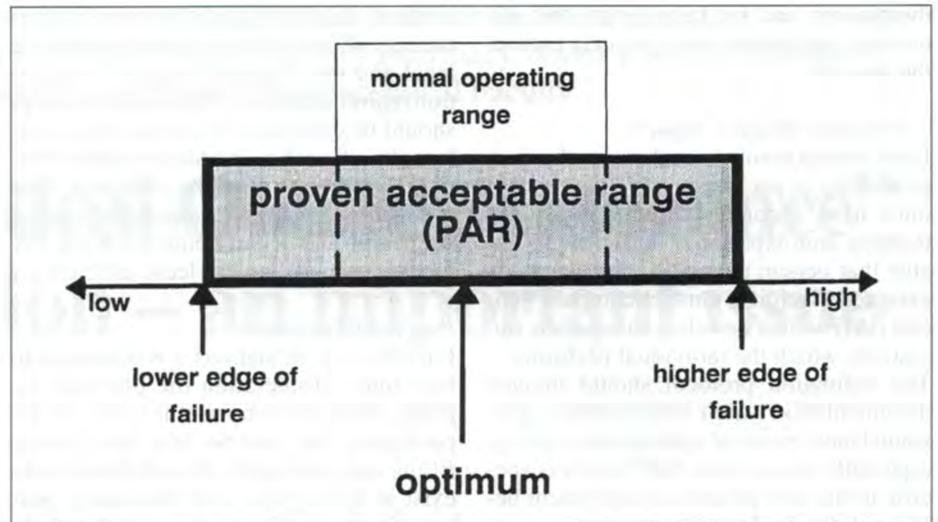

Fig. 4: PAR approach to process validation

Tab. 3: Summary of the validation in packaging processes

critical parameters	measures	
	specific	as a whole
machines		
instrumentation EDP-systems tests cleaning procedures	calibration validation validation, SOPs validation	qualification
staff		
knowledge hygiene compliance	professional training instruction instruction, SOPs	qualification
internal flow of material		
incoming material control procedures storage returned goods EDP systems	specification validation SOPs SOPs validation	
external suppliers		
quality of packaging material	certification DIN/ISO standards quality audits	qualification

themselves and on confidence that the existing equipment can accurately control the process.

– Personnel Related Aspects
Each person involved in the manufacture, processing or packaging of a drug product must have a combination of education, training and experience sufficient to enable that person to perform the functions assigned. Training must encompass general GMP issues as well as the specific operations which the individual performs. The validation protocol should include documentation which verifies that all personnel have received appropriate training, especially on any new SOP which is specific to the new process or equipment being validated. Training should be conducted as defined in a separate guideline. Documentation should minimally include the name of the trainer, a brief description of the training given, the date on which training or instruction was conducted, and the signatures of those having received training.

Conclusions

Legal Requirements
A clear terminology is required: packaging is part of the production (process) and should therefore be validated. This commitment should become an integral part of all relevant GMP guidelines and rules.

Organization
Since the various stages in a packaging process can be quite complex, a pharmaceutical manufacturer's own validation concept should exist in order to meet the legal and the company's specific validation reqirements best. This kind of concept should be oriented also on the firm's philosophy of quality. It enables transparency with respect to planning, expenses, time schedules, resources, responsibilities of the people and departments involved, risk factors and, last but not least, efficiency.

Practical Approach
For effective validations it is important to take into consideration the practical aspects. Thus it can be useful to divide the packaging line process into two phases: filling and packaging. In each phase risks exist in connection with machinery, personnel, internal flows of material and external suppliers. Therefore a risk analysis should preferably take into account these four areas. The risks can then be regulated by applying various measures, as listed in *Table 3*, leading to process validation.

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Results of the six project groups of the second postgraduate seminar for Pharmaceutical Quality Assurance, winter semester 1992/93, Department of Pharmacy, Swiss Federal Institute of Technology (ETH), and the University of Basel, Pharmaceutical Faculty

"Pharmaceutical Quality Assurance" and "Validation – an important issue of cGMP"

Process Validation on Tablets

F. Bodinka[1], St. Merkle[2], S. Moret[3], F. Naeff[4], R. Naeff[5], P. Scheidecker[6]

Based on the requirements of the 1987 FDA-Guideline on validation and of similar publications of the EC and WHO, the following paper tries to show critical items in validation of a tabletting process with special regard to fluid-bed granulation and to compression. The principles of a validation dossier are presented. The main aim of this presentation is to show the method of initiating process validation.

Introduction
FDA requirements appertaining to validation as an essential part of Good Manufacturing Practices have existed since 1978. The definitions were modified and worked out more precisely and finally presented in the FDA-Guideline on process validation in 1987[1)]. It defines process validation as follows:
"Validation – Establishing documented evidence that provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes."
This definition requires specifications on a process to be fixed before validation starts and written documentation to certify its consistency. In the same guideline[1)] the validation protocol is defined as follows:
Validation protocol – A written plan stating how validation will be conducted, including test parameters, product characteristics, production equipment, and decision points on what constitutes acceptable test results. The FDA validation requirements have been widely accepted and were picked up in the EC GMP-guideline in 1989[2)] and in the WHO document[3)].
These guidelines have not brought an end to discussions on validation. For some aspects there may be a lack of common sense on what is really needed for validation. For others, as for example Analytical Methods-Validation, consensus on the necessary volume of validation work has already been reached. For the process validation segment numerous catalogues of requirements are available[4a,b)], but there is a lack of complete and detailed documentation on process steps that may be of interest as examples for similar problems. We would like to present such a documentation for the manufacturing of tablets (*Fig. 1*).
During the manufacturing of tablets there are several process steps that more or less influence the quality of the final product. If fluid-bed granulation is involved this step may be critical, but the compression of tablets may also cause difficulties. This paper concentrates on these two steps and in addition presents an example for a validation protocol.

Manufacturing of Tablets – an Example

Fluid-Bed Granulation
The manufacturing of a granulation using Fluid Bed Technology can be subdivided into several process steps:
- Preparation of the granulation liquid
- Preblending
- Wet Granulation
- Drying
- Dry Granulation / Sifting
- Final Blending

Of special interest for the Validation Manager will be the Fluid Bed Process itself with its several coherent process parameters. Due to different standards in instru-

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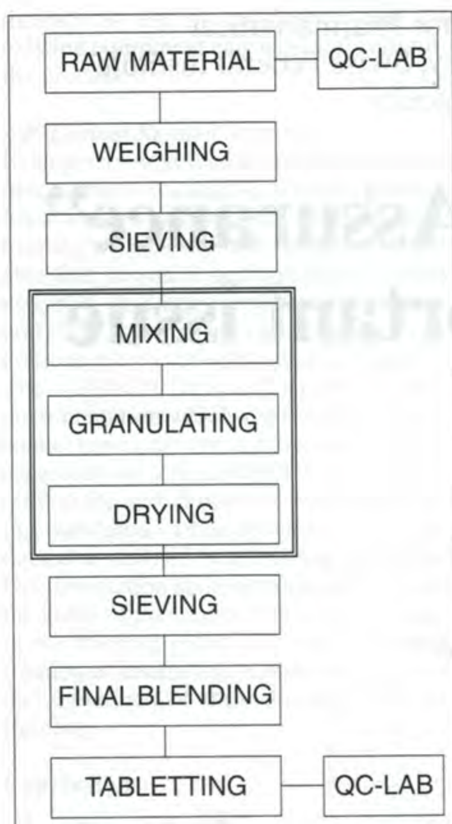

Fig. 1: Flow Chart of a Tablet Manufacturing Process

mentation of the fluid bed units it may be difficult to control every relevant parameter continuously. This special situation needs significant in-process controls on the granulation to guarantee a proper documentation of the process.
A prerequisite for effective validation of this process, as well as for every other process, is an exact and well regulated calibration of all measuring and regulating instrumentation.

Preparation of the Granulation Liquid
The following parameters may be of relevance for the preparation of the granulation liquid:
- Temperature
- Stirrer / Homogenizer (Type)
- Mixing Time
- Viscosity
- Absence of Particles
- Microbial Contamination

The decision as to whether one of these parameters is more or less critical depends very much on the specific formulation and the manufacturing conditions. For many products it may be sufficient to adequately control all manufacturing conditions (equipment, temperature, mixing time) to guarantee reproducibility of this process step.

Pre-blending, Granulation, Drying
The following parameters may influence the fluid bed process:
- Spray Rate
- Inlet-Air Volume
- Inlet-Air Temperature
- Inlet-Air Humidity
- Filling Grade of Fluid Bed Bowl
- Number of Spray Guns
- Position of Spray Guns
- Setting of Spray Guns
- Nozzle Tip Diameter
- Outlet-Air Filter (Materials, Mesh Size)

The following variables can be observed during the process as results of the above parameters:
- Product Temperature
- Product Humidity
- Outlet-Air Temperature
- Differential Pressure (Bottom Sieve, Outlet-Air Filter)
- Overall Process Time (Mixing-, Spraying-, Drying-Time)
- Delta T

Because of the way a fluid bed unit is constructed, it is necessary to clean the outlet-air filter during the process. Suppliers offer several solutions for this purpose. It should be possible to guarantee the reproducibility of the filter cleaning settings.
The results of a fluid bed process can be observed in the granulation properties:
- Particle Size Distribution
- Loss on Drying
- Visual Aspect
- Loose/Tap Density
- Flowing Properties
- Content Uniformity

The control of all the process parameters cannot be a validation objective. This work has to be done during the development of a new formulation (or is not necessary at all in the validation of an established process). Some variables are normally fixed by the limitations of the equipment used.
The recording of the process parameters depends to a great extent on the equipment. The measurement of the actual inlet-air volume is often not possible, even though technically feasible. In this case, the air volume may be documented by the capacity of the ventilator and by the air gap setting. If these units are calibrated properly this weakness can be considered as non-critical.
For the control of the inlet-air humidity a fine-tuned air conditioning system would be the optimum solution to eliminate influences on process variables, as for example an extension of the drying time. The minimum that should be done here is to record the outside air humidity.
The differential pressure that is measured at the bottom sieve as well as at the outlet-air filter of most standard-equipped fluid bed units to indicate the air flow through the system varies on account of the unequal motion of the granulation in the fluid bed. A recording of these measurements therefore serves no purpose.
Spray rate, inlet-air temperature and volume should be constant throughout the whole wet granulation process in order to simplify reproducibility and automatization of the process.
The end-point for the drying of a granulation is given by the optimum moisture content for compression. The end-point can be indicated by a rapid increase in the product temperature during drying. The Delta T method is also used for end-point detection. The moisture content of the granulation can only be ascertained with certainty by determining the loss on drying. Because of a tendency toward process automatization, methods for in-process determination of the granulation moisture content are required. Currently the IR method looks most promising[5a,b].
As regards granulation properties, the particle size distribution shows most distinctively the successful course of granulation. Neither high fines nor large amounts of agglomerates should occur in a good granulation.
Some authors require determination of content uniformity in the granulation. It may be sufficient to do a representative sampling throughout the granulation (proper sampling procedure is very important to avoid surprises), store the samples, and assay only if problems in connection with the content uniformity of the tablets are observed.

Sieving
In the sifting procedure the process parameters have to be documented and the result has to be analyzed by a sieve analysis.
- Equipment
- Mesh Size
- Speed

Final Blending
The following variables have to be documented:
- Equipment (Type, Size)
- Blending Time
- Blending Speed

It is feasible to rely on experience to adjust the mixing time. It is obviously not feasible to try to document the distribution of the glidant in the granulation by analytical methods. The measurement of the ejection force can be taken as an indicator of an optimum distribution of the glidant.

Sampling of the granulation after discharging from the blender for determination of the content uniformity may be useful.

Tabletting
Provided that a properly qualified rotary tablet press has been set up with appropriate tooling, tablet quality should be a function of granulation properties and of uniform filling of the dies. Variations of granulation can be influenced to some extent by varying compaction force and filling speed.

Tooling
Initial qualification and periodical recalibration of the tabletting tools are necessary to avoid machine problems such as wedging or tabletting problems such as sticking and picking that may be caused by worn-out punches and dies.

Granulation
Granulation properties are the main influence on tabletting. Criteria that influence tabletting directly are well documented in the literature[6a,b].

Compression
Validation of this process means to control the compression of an equal amount of granulates into tablets of constant volume. The influence of granulation parameters is obvious, due to the volumetric controlled filling of the dies. The compaction force is closely correlated to the filling volume of the die, and small changes in filling volume can be indicated by recording the compaction force even before weight variation is observed.
Rotary tablet presses of the latest generation are equipped with strain gauges on the compaction rollers linked to a computer system that varies the position of the lower punch. The position of the lower punch at the filling station determines the die filling volume. Decreases in compaction force indicate insufficient amount of granulation inside the die. Decrease of compaction force under a defined value will lead to a correction of the filling volume by lowering the position of the lower punch and vice versa.
It is nevertheless dangerous to rely on measurement of the compaction force only for a validation of the compression process. Sticking of granulation to the punch surface, for example, may increase the compaction force without any change in the filling volume of the dies.
To ensure validation of the filling volume of the die we propose recording the tablet weight simultanously with the compaction force measurement. There are already systems available that automatically measure the tablet weight after ejection and correlate the results to the compaction force for every station of the tablet press. Validation work on tablet presses without sophisticated instrumentation relies on the recording of tablet properties such as weight, band height, disintegration time, dissolution, hardness and friability.
Standardized and qualified methods should be used for the measurement of all these parameters. For hardness testing special regard should be paid to constant time span between compression and measurement to guarantee reproducible results.

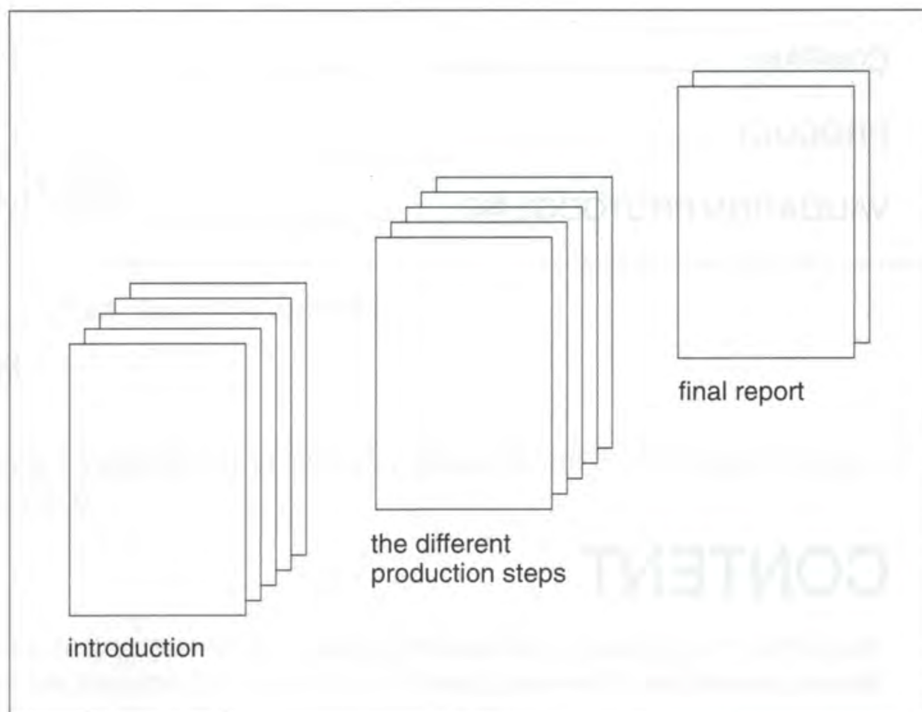

Fig. 2: Validation Protocol

Validation Protocol

As pointed out in the introduction, the validation protocol, the formal documentation of raw data, the statistical interpretation and the review of the results are the main components of validation work. It is also very time-consuming to get proper hard copies of all validation results.
For this purpose we propose the following set of protocol sheets: *Remarks and examples are correlated to the special purpose of a tabletting process (Fig. 1)*
Figure 2 shows a set of Protocol Sheets[7] that can be subdivided into 3 parts:
- Introduction and Organization
- Process Steps
- Data Interpretation and Results

The protocol sheets should serve as a concept to simplify the generation of a validation dossier for a distinct project by analyzing different process steps with the same screen.
In the light of our experience special regard should be paid to the following aspects of documentation:
- Clear Definition of the Validation Project
- List of Responsible and Involved Persons
- List of Equipment Used
- Time Schedule
- Flow Chart of the Process
- Critical Steps and Special Control Methods
- Documentation of Process Data
- (Statistical) Data Interpretation
- Validation Result

Literature

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■

COMPANY ______________________ PROCESS VALIDATION FOR:

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page 1 of

CONTENT

page

1. INTRODUCTION 2

2. OVERVIEW OF THE PROCESS 3

3. CRITICAL PROCESS STEPS 4

4. REQUIRED CONTROLS 5

5. THE DIFFERENT PRODUCTION STEPS 6 ff

5.1. control of raw material

5.2. weighing

5.3. granulation

5.4. compression of tablets

5.5. control of finished product

6. ANALYSIS OF THE RESULTS X

7. FINAL REPORT last page

prepared by: ______________ approved by: ______________ date: ______________

1. INTRODUCTION

Under this section the following should clearly be outlined:

1.1. Purpose of Validation
Validation required externally / selected for validation / new production / change in manufacture / complaint / revalidation.

1.2. Organisation
Members of the validation team composed of at least: production, quality control and quality assurance managers (if necessary: technical department, research and development, general management). Define clearly: addressee, responsibilities, reporting relationships.

1.3. Subject of Validation
Precise description according to section 2.

1.4. Time Schedule
Deadlines for the formation of the validation team, training period, intermediate reports / briefings, production control, final report.

1.5. Required Results
Detailed final report, summary, conclusion and proposals to the addressee.

1.6. Actions to solve unexpected problems
Treat immediately problems that can be eliminated within time schedule. Formulate proposals for urgent measures, short-term improvements or long-term changes. Define period of revalidation. Formulate provisos concerning running qualifications that are required for this validation.

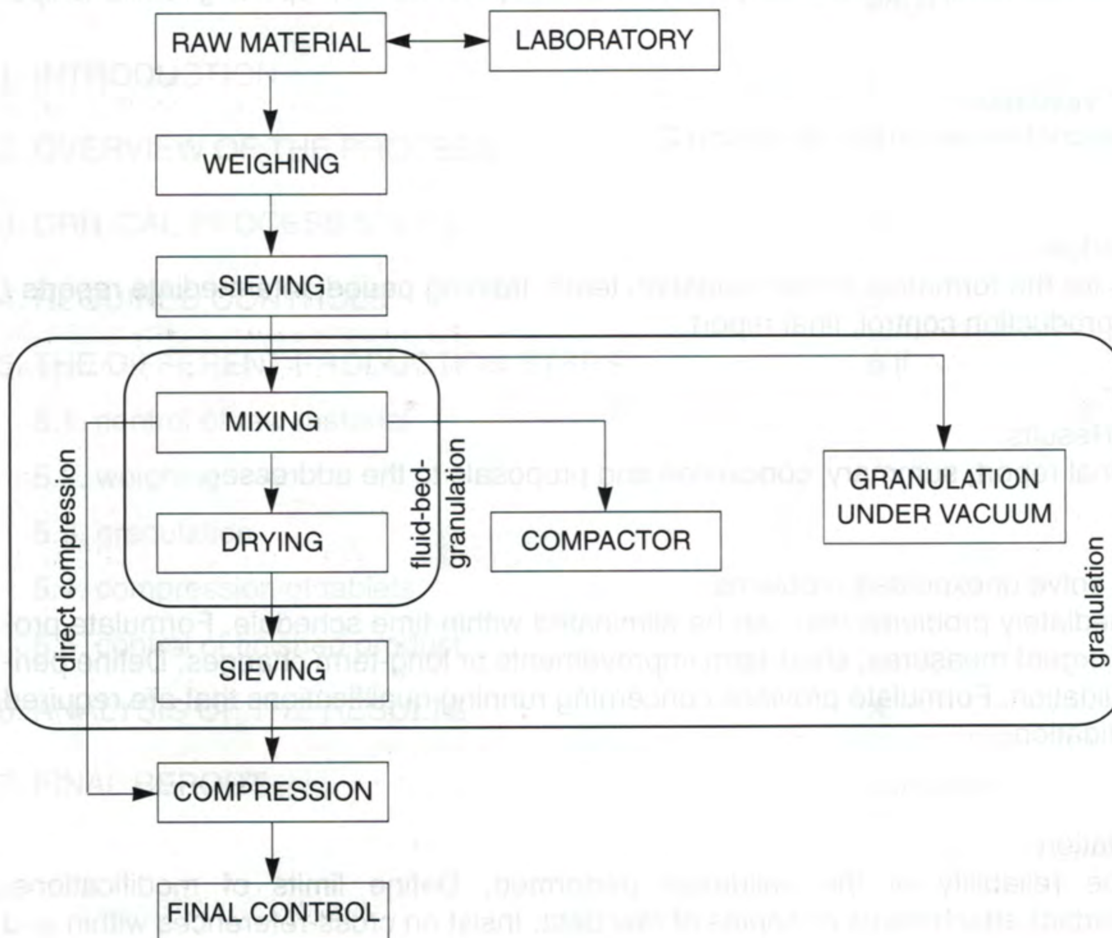
1.7. Documentation
Outline the reliability of the validation performed. Define limits of modifications. Allow (or forbid) attachments or copies of raw data. Insist on cross-references within and outside this dossier. Define a distribution list (cf. 1.2.).

COMPANY ______	PROCESS VALIDATION FOR:
PRODUCT ______	CODE ______
VALIDATION PROTOCOL NO. ______	YEAR ______

page 3 of ______

2. OVERVIEW OF THE PROCESS

The following flow-sheet provides an overview of the whole process to be validated.



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3. CRITICAL PROCESS STEPS

In accordance with the flow sheet in section 2, all critical steps have to be listed and an explanation given of why they influence the quality of the final product (in case of revalidation it might also be helpful to list steps indicated as less critical).

3.1. Raw Materials
Their specification influences the processing and also the quality of the end product considerably.

3.2. Weighing
The precision of weighing determines the concentration of the active ingredients. Cleanliness in the handling of raw materials, tools and containers influences the purity of the final product. This applies to all steps from opening of the first container through packaging of the tablets.

3.3. Conditions of Granulation
Homogeneity, particle size, humidity and flow characteristics influence subsequent compression and the dissolution of active ingredients.

3.4. Conditions of Compression
These determine dimensions, stability and disintegration of the tablet.

3.5. Control of finished Product
The only criterion is compliance with the specification within the defined limits.

prepared by: ______________ approved by: ______________ date: ______________

4. REQUIRED CONTROLS

In line with the above-listed critical steps the following controls are relevant for process validation. For each single manufacturing step the required test procedure has to be prepared and carried out. Tables have to be prepared for recording the measured values.
During validation supplementary additional data may be added. If anything might be omitted, a justification must be added and approved.

4.1. Raw Materials
All raw materials have to be controlled and released according to their specification. If not specially mentioned under 1.3. a reference to special raw material validation or supplier audits may be given.

4.2. Weighing
All relevant SOPs and weighing procedures have to be checked on site. Training of personnel has to be verified. Cleaning procedures and their application are to be controlled by residual analysis and microbial tests to establish that cleaning instructions and weighing procedures are sufficient and are being followed and controlled by the personnel.

4.3. Granulation
Suitable and measurable parameters have to be proposed that influence the critical steps according to 3. (and subsequently, in section 5, have to be imposed). Several of these values can only be judged retrospectively at the end of granulation or compression.

4.4. Compressing
Suitable and measurable parameters have to be proposed that control the critical steps specified in 3.4. and can be monitored throughout the compression stage, in order to document that the whole batch was compressed under the same conditions. These parameters have to be imposed subsequently in section 5.

4.5. Control of finished product
The methods of final control analysis normally are not a subject of process validation unless specially mentioned under 1.3. A reference to separate validation reports and SOPs may be given.

COMPANY ____________________ PROCESS VALIDATION FOR:

PRODUCT ____________________ CODE ____________

VALIDATION PROTOCOL NO. ____________ YEAR ____________

page 6 ff of ________

5. THE DIFFERENT PRODUCTION STEPS

In this main part of the validation protocol each single production step (cf. section 2) should be dealt with according to the following schema:

5.1. ____ ____ 1. INTRODUCTION

____ 2. OVERVIEW OF THE PRODUCTION STEP

____ 3. CRITICAL POINTS IN THIS STEP

____ 4. IN-PROCESS CONTROLS

____ 5. EXECUTION OF THE STEP IN DETAIL

____ 6. RESULTS

____ 7. CONCLUSION: Is this step consistent with the required specifications? Are there inconsistencies that need to be examined further?

5.2. ____

prepared by: ____________ approved by: ____________ date: ____________

COMPANY ______________________ PROCESS VALIDATION FOR:

PRODUCT ______________________ CODE ______________

VALIDATION PROTOCOL NO. ______________ YEAR ______________

page X of ______________

6. ANALYSIS OF THE RESULTS

Compilation of all relevant data from the different production steps according to section 5. Analysis of tables and diagrams. Detailed comments on limits, deviations and reproducibility. Compare with complete end-control values.

last page of ______________

7. FINAL REPORT

Has the aim of this validation been achieved? Which points need further clarification? When should a revalidation be envisaged?
Recommendations and signatures of the members of the validation team listed in 1.2.

prepared by: ______________ approved by: ______________ date: ______________

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LEBENSQUALITÄT

Ob in der Dermatologie, Gynäkologie, Immunologie oder Nephrologie – unsere Arzneimittel haben weltweit unzähligen Menschen ein gutes Stück ihrer verlorenen Lebensqualität zurückgegeben.

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