VALIDATION

OF

ANALYTICAL METHODS

GERT BEUVING

INTERNATIONAL PHARMACEUTICAL OPERATIONS
INTERNATIONAL QUALITY SYSTEMS

TASKS:

- Internal auditing
- Auditing of suppliers and contract manufacturers
- Preparing for and guiding of external inspections
- Review of and advice on procedures & validations

Validation

FDA-guidelines:

Validation is 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**

EU-guidelines

Action of **proving**, in accordance with **GMP**-principles that any procedure, process, equipment, material, activity or system **actually** leads to the **expected results**

Conclusion:

- Need for pre-determined operational & performance user requirements (**URS**) of process or system
- Provide evidence of **meeting pre-defined operational & perfomance requirements**
- Provide evidence on **consistency** of meeting these requirements

More specific:

“Methods validation is the process of demonstrating that analytical procedures are suitable for their intended use”

(ICH Topic Q2B, March 1995)

Why validation?

1. GMP-legislation

2. Good economics

3. Good science practices

Validation guidelines

Guidelines

1. ICH Q2A
   Text on validation of analytical procedures: Definitions and terminology (March 1995)

2. ICH Q2B
   Validation of analytical procedures: Methodology (June 1997)

3. FDA
   (Draft) Guidance for Industry: Analytical procedures and methods validation

4. Pharmacopoeias
   USP and European Pharmacopoeia

What methods to be validated?

Defined for:

- identification
- quantitative tests for content of impurities
- limit tests for control of impurities
- quantitative tests for active moiety in drug substances and drug products

Referred to:

- dissolution testing
- particle size determination (drug substance)

When should methods be validated?

Development and tox:

No validation required

Phase 1

No validation data required

Phase 2

For both drug substance and drug product supporting validation data on analytical methods should be available on request

When should methods be validated?

Phase 3 (Pivotal studies):

- Appropriate validation information should be provided.
- Assay validation should cover accuracy, precision, specificity (including stress testing), quantitation & detection limits, linearity and range (where appropriate)
- Degradation should be identified, qualified and quantified

NDA submission

- Full validation reports of relevant methods must be included

What aspects to cover?

Specificity:

Definition:

Ability to assess unequivocally the analyte in the presence of of components which may be expected to be present (impurities, degradants, matrix)

Aspects:

- Identification
- Purity tests
- Assay (Content/potency)

Linearity:

Definition:

Ability (within a specified range) to obtain test results which are directly proportional to the concentration of analyte in the sample

Aspects:

- Test across the range (at least 5 concentrations)
- Evaluate linearity by visual inspection of the plot and by statistical techniques
- Calculate corr. coefficient, y-intercept, slope and res. sum of squares

Range:

Definition:

Interval between upper and lower concentration of the analyte in the sample for which it has been demonstrated that the procedure has a suitable level of precision, accuracy and linearity

Aspects:

- Defined from linearity study
- Depends on the application of the method (assay, dissolution test, content uniformity)

Accuracy

Definition:

Expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

Methods:

Drug substance

- use of reference standard with known purity
- comparison with independent, well-characterised procedure
- may be inferred once precision, linearity and specificity are established

Accuracy

Drug product

- spiking of placebo mixture
- addition of analyte to ‘active’ material
- comparison of results obtained with independent, well-characterised procedure
- may be inferred once precision, linearity and specificity are established

Impurities

- spiking of product samples
- use of independent, well-characterised procedure

Accuracy

Recommended data

- Assessed by 9 determinations over a minimum of 3 concentration levels covering the specified range
- To be reported as percent recovery

Precision

Definition

Closeness of agreement ('scatter') between a series of measurements obtained from multiple sampling of the same homogeneous sample.

Aspects

- Repeatability
- Intermediate precision
- Reproducibilty

Precision - Repeatability

Definition

Precision under the same operating conditions over a short interval of time.

Method

- 9 determinations covering the specified range
- or: 6 determinations at 100% of the test concentration

Precision - Intermediate precision

Definition

Expresses within laboratory variations.

Method

- Depends on circumstances of usage of the methods
- Should include variations in days, analists, columns

Precision - Reproducibility

Definition

Precision between laboratories

Method

- Dependent on usage of method
- Should include interlaboratory study

Detection limit

Definition

Lowest amount of an analyte in a sample which can be detected but not necessarily quantitated.

Method

- Based on visual evaluation
- Based on signal-to-noise ratio (3:1)
- Based on st.dev. (SD) of response and slope (DL=3.3xSD/S)
- Report results and method of choice

Quantitation limit

Definition

Lowest amount of an analyte in a sample which can be quantitatively determined with a suitable precision and accuracy

Method

- Based on visual evaluation
- Based on signal-to-noise ratio (10:1)
- Based on st.dev. (SD) of response and slope (DL=10xSD/S)
- Report results and method of choice

Robustness

Definition

Measure of the capacity of a method to remain unaffected by small variations in method parameters.

Aspects

- To be considered during development
- To be used for establishment of system suitability criteria
- Include testing of stability of solutions
- To be tested by introducing small variations in method parameters

System Suitability Test

Definition

Set of parameters and criteria thereoff to ensure the system is working properly.

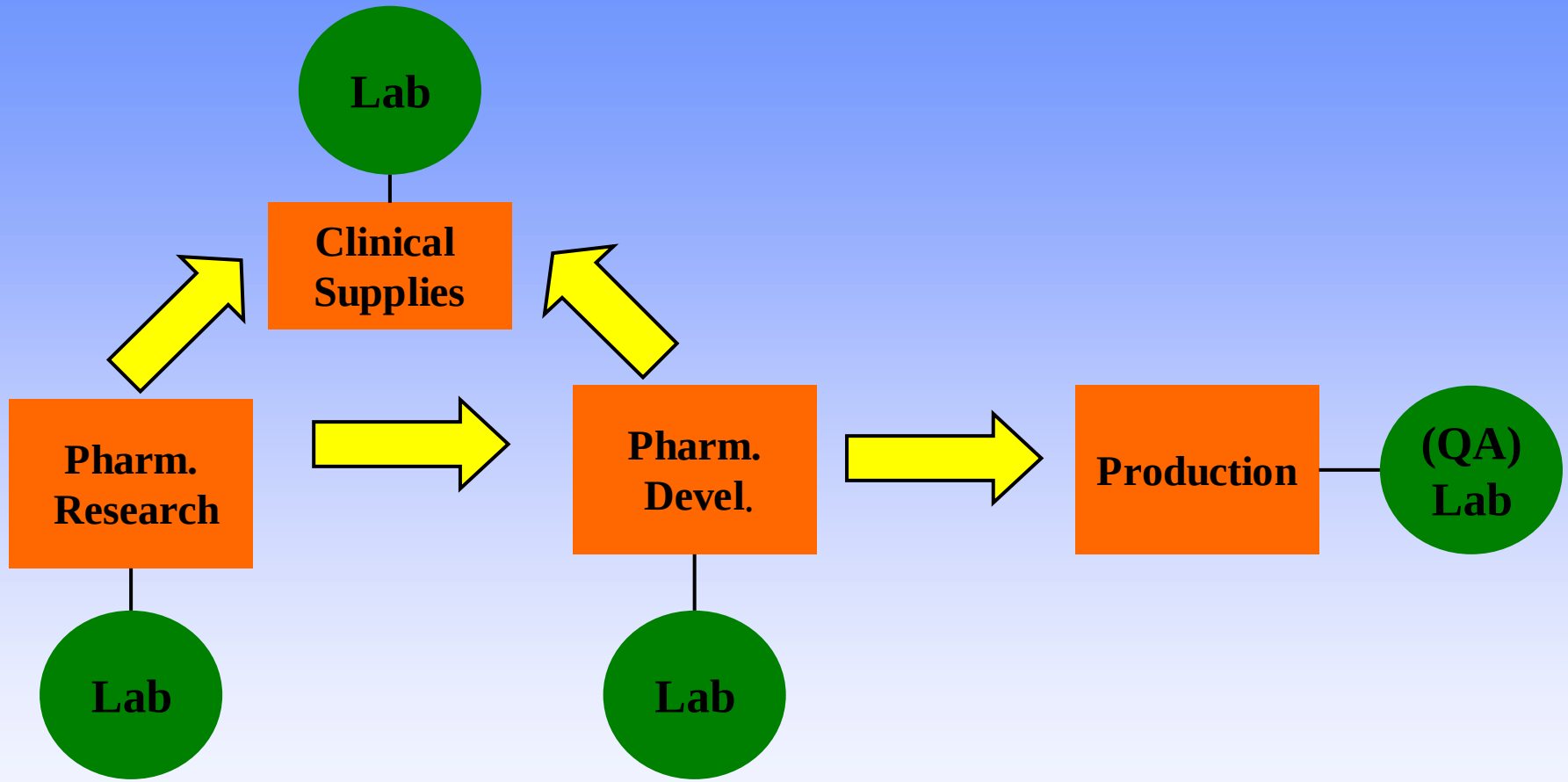
Aspects

- Dependent on type of test
- For chromatographic methods: tailing factor, rel. retention times, resolution factor, rel. st. deviation, number of theoretical plates
- To be checked before start of run and to be verified afterwards
- Described in Pharmacopoeias

Recommended Validation characteristics of various Types of Tests

Type of tests/ Characteristics	Identification	Testing for impurities		Assay/ Dissolution	Specific Tests
		Quantitative	Limits		
Accuracy	-	+	-	+	+
Precision-repeatability	-	+	-	+	+
Precision- Intermediate precision	-	+	-	+	+
Specificity	+	-	+	+	+
Detection limit	-	+	+	-	-
Quantitation Limit	-	+	-	-	-
Linearity	-	+	-	+	-
Range	-	+	-	+	-
Robustness	-	+	-	+	+

Analytical method development



Implementation of Guidelines

- Standard protocols
- Set up as procedures
- Mutual agreement on tests
- Mutual agreement on criteria
- Mutual agreement on documentation

==> MUTUAL DEVELOPMENT PROCEDURES (MDP)

MDP 6-01

"Validation of the assay method of active compounds by HPLC, capillary electrophoresis or gas chromatography in drug products"

MDP 6-01 - Selectivity

Tests

- Inject solutions of standard, product, impurities, known degradation products, excipients;
- Inject solutions of degraded/stressed products and placebo
    - 2 hours art. daylight (70-90 klux)
    - 1 week at 75°C/amb. humidity and 75°C/100% RH
    - 24 hrs 3% H_2O_2, 1 mol/L HCl, 1 mol/L NaOH
- Demonstrate separation
- Demonstrate peak purity

MDP 6-01 - Selectivity

Criteria

- Separation between relevant peaks of at least Rs > 2.0
- Peak of analyte should be pure

Documentation

- Chromatograms of all solutions
- retention times
- peak purity results
- data of contents of active substance and degradation products in stress samples

MDP 6-01 - Linearity

Tests

- Inject solutions of 25%, 50%, 75%, 100%, 125% and 150% of expected concentration in duplicate;
- Calculate by statistical techniques the order of function (first or second), significance of intercept and correlation coefficient
- In case of second order and/or significant deviation of intercept from zero: determine the degree of linearity in the range of 70-130%.

MDP 6-01 - Linearity

Criteria

- Use of one reference concentration is acceptable when:
  - regression line is linear (lack of fit test)
  - true zero is within 95% conf. interval of calculated intercept

or in case of second order curve:

  - if experimental rel. response at 70% and 130% does not deviate by more than 1% from the calculated values

- Linear when corr. coefficient > 0.9990

MDP 6-01 - Linearity

Documentation

- Plots of peak height and peak areas
- Statistical results (equations, significance of intercept, lack-of-fit test, rel. responses, corr. coefficient)
- Plots of peak area and peak heights residuals

MDP 6-01 - Accuracy

Test

- Prepare placebo sample
- Prepare spiked placebo samples: 3 replicates over 3 concentration levels (e.g. 70%, 100%, 130% of theoretical strength)
- Carry out the method
- Calculate mean percent recoveries and rel. standard deviation (RSD) from both peak area and peak height responses.

MDP 6-01 - Accuracy

Criteria

- The average result of the mean for each level should be 98.0 - 102.0%
- Range for response of placebo within -1% and +1%
- RSD of pooled results should be < 2%

Documentation

- Details on sample preparations
- Individual results (peak areas and peak heights)
- Calculated % recovery and pooled RSD

MDP 6-01 - Repeatability of system

Test

- Inject in six-fold one of the 100% solutions from the accuracy experiment
- Calculate RSD for both peak height and peak area

Criterion

RSD < 1.5%

Documentation

Results and statistical calculation

MDP 6-01 - Repeatability of method

Test

- Analyse within one day by one operator with one column 6 times a homogeneous sample of the product
- Calculate the RSD for results of both peak height and peak area

Criterion

RSD < 2%

Documentation

Results and statistical calculation

MDP 6-01 - Intermediate precision

Test

- Same as for repeatability of the method but by at least 2 analists, more days, different labs, different (batches of) columns
- Calculate the RSD on overall results

Criterion

RSD < 2.5%

MDP 6-01 - Intermediate precision

Documentation

- Description of preparation of homogeneous sample
- Description of experimental conditions
- Results and statistical evaluation

MDP 6-01 - Detection and quantitation limit

Determination not necessary

Only applicable for impurities and degradation products

MDP 6-01 - Range

No specific test:

Normally a range of 70-130% is acceptable, unless a wider range is required based upon the nature of the dosage form (e.g. metered dose inhalers)

MDP 6-01 - Robustness (1)

Test on stability of solutions

- Prepare 2 sample and 2 reference standard solutions
- Store in refrigerator and at room temperature
- Analyse at zero time and after at least 24 and 72 hours storage
- Calculate differences between samples

Criterion

Storage period is defined by period with no more than 1% difference between room temperature and refrigerator

MDP 6-01 - Robustness

Documentation

- Individual results
- Calculations, difference between room and refrigerator samples

MDP 6-01 - Robustness (2)

Implementation

Test on variations

- Vary relevant analytical parameters e.g.
    - composition and/or pH of mobile phase
    - column temperature
    - different column (other batch or brand/supplier)
    - stability of chromatographic system

Criteria

- Chromatographic results meet system suitability criteria
- Typically plate count should not decrease by more than 50%

MDP 6-01 - Robustness (2)

Documentation

- Relevant chromatograms
- Calculations and results of system suitability parameters

Use results from method development experiments!!

MDP 6-01 - System Suitability Testing

Test

- Collect all data from previous experiments with regard to
    - number of theoretical plates
    - tailing factor
    - relative retention
    - resolution factor
    - precision of the system
- Include information on minimum resolution between analyte and most-difficult-to-resolve impurity/degradation product

MDP 6-01 - System Suitability Testing

Criteria

- Criteria dependent on development and validation results.
- Evaluate and optimise defined criteria when more experience is gained with the method.

Documentation

- Summary of data on individual parameters
- Calculations and relevant chromatograms

MDP 6-04

"Validation of the determination of an impurity in a drug product by HPLC, capillary electrophoresis or gas chromatography"

MDP 6-04 - Selectivity

Tests and documentation

Same as for determination of active substance.

Criteria

- Assay of impurity should not be influenced by any other peak originating from other components in the sample solution.
Resolution factor between 2 peaks should be at least > 1.5.
Resolution between active substance and impurity should be > 2.

MDP 6-04 - Linearity

Tests

- Inject solutions of 10%, 50%, 100%, 150%, and 200% of expected concentration in duplicate (concentration based upon registered limit; if not defined then 1%);
- Calculate by statistical techniques the order of function (first or second), significance of intercept and correlation coefficient
- In case of second order and/or significant deviation of intercept from zero: determine the deviation in the relative response of the 10% and 200% points.

MDP 6-04 - Linearity

Criteria

- Use of one reference concentration is acceptable when:
  - regression line is linear (lack of fit test)
  - true zero is within 95% conf. interval of calculated intercept

or in case of second order curve:

  - if deviation of the rel. response of 10% point isd less than 20% and of the 200% point is less than 5%values

- Linear when corr. coefficient > 0.995

MDP 6-04 - Linearity

Documentation (same as for DS)

- Plots of peak height and peak areas
- Statistical results (equations, significance of intercept, lack-of-fit test, rel. responses, corr. coefficient)
- Plots of peak area and peak heights residuals

MDP 6-04 - Range

No specific test:

Normally a range of 10-200% is acceptable. In most cases 100% is 1% relative to the drug substance.

MDP 6-04 - Accuracy

Test

- Prepare placebo sample
- Prepare spiked placebo samples: 3 replicates over 3 concentration levels (e.g. 2 x QL, 100% and 200% of 1% of the drug substance concentration)
- Perform analysis
- Calculate mean percent recoveries and rel. standard deviation (RSD) from both peak area and peak height responses.

MDP 6-04 - Accuracy

Criteria

- The average result of the mean for 100% and 200% level should be 90-110% and for 2xQL 70-130%
- RSD of 100 and 200%: < 5% and 2xQL level: < 15%

Documentation

- Details on sample preparations
- Individual results (peak areas and peak heights)
- Calculated % recovery and pooled RSD

MDP 6-04 - Repeatability of system

Test

- Inject in six-fold one of each of the strengths of the reference solutions from the accuracy experiment
- Calculate RSD for both peak height and peak area

Criterion

RSD (2xQL) < 15%; RSD (100% and 200%) < 5%

Documentation

Results and statistical calculation

MDP 6-04 - Repeatability of method

Test

- Inject in six-fold one of each of the strengths of the samples used in the accuracy experiment
- Calculate the RSD for results of both peak height and peak area

Criteria

RSD (2xQL) < 15%; RSD (100% and 200%) < 5%

Documentation

Results and statistical calculation

MDP 6-04 - Intermediate precision

Test and documentation

Same as for assay but tested on spiked samples at 1% level

Criterion

RSD < 10%

MDP 6-04 - Detection and quantitation limit

Test

- Determine peak-to-peak distance of baseline at the position of the analyte in a blank sample. Calculate noise as 0.5 times this distance
- Calculate the detection limit as 3 times noise and quantitation limit as 10 times noise
- Verify the calculated DL and QL by injecting at least one solution with a concentration at or near the DL and QL.

MDP 6-04 - Detection and quantitation limit

Criterion

The Quantitation Limit is, by preference, less than 0.1% relative to the drug substance.

Documentation

- Chromatograms used for calculations
- Chromatogram of sample at a concentration near DL and QL

MDP 6-04 - Robustness (1)

Test and documentation on stability of solutions

Same as for assay

Criterion

Storage period is defined by period with no more than 5% difference between samples stored at room temperature and in the refrigerator

MDP 6-04 - Robustness (2)

Test, Criteria and Documentation on variations

Same as for assay

MDP 6-04 - System Suitability Testing

Test, Criteria and Documentation

Same as for Assay

Implementation - A practical example

Livial capsules 1.25 mg

Product is developed for post-menopausal complaints and also prevents osteoporoses

An analytical method was developed to determine drug substance and main degradation products simultaneously

Implementation- Example

Analytical procedure

Extraction:

Sonification and mixing with ethanol (conc. OD 14: 0.156 mg/ml)

HPLC:

- column: Nova-pak 18, 150x3.9 mm, dp = 4 mcm
- mobile phase: Tetrahydrofuran+water (28+72)
- column temperature: 40°C
- Detection: UV 210 (OD 14) + UV 240 (degradation products)

Analytical procedure - Specificity

Results of stress-testing

Condition	Content OD 14	Pur. factor OD 14	Content OM 08	Content OM 06	Content OM 38	Total others
Non stressed	100%	0.9985	<0.1	0.1	0.2	nd
2 hrs art. daylight	80.3 %	0.9985	<0.1	0.3	0.2	0.3
1 wk 75°C/amb. RH	83.6%	0.9993	2.1	2.0	10.9	0.1
1 wk 75°C/100% RH	4.6%	-	1.5	3.8	12.3	nd
2 hrs 1M HCl	nd	nd	nd	<0.1	90	0.7
2 hrs 1M NaOH	nd	nd	0.4	0.1	53.4	1.2
2 hrs 3% H2O2	91.4%	0.9981	<0.1	0.2	0.2	<0.1

Implementation- Example

Legend

1. Org OD 14 RT: 14.4 min

2. Org 30205 RT 15.8 min

3. OM 38 RT: 7.3 min

4. OM 08 RT: 3.5 min

5. OM 06 RT: 5.1 min

6. OH 45 RT: 33.6 min

Implementation- Example

Analytical procedure - Linearity

OD 14

Concentrations of 1, 25, 50, 75, 100, 125 and 150% of 0.15 mg/mL

Degradation products

Concentrations of 0.1, 0.5, 1.0, 1.5, 2.0, and 2.5% with respect to concentration of OD 14 (0.15 mg/mL)

Solutions prepared and injected in duplicate.

Results evaluated for peak heights and peak areas

Analytical procedure - Linearity

Summary of Results for OD 14

	Curve	Corr. coefficient	p-value intercept	p-value LoF-test
Area	**Linear, 1st order**	**0.9999**	**0.37**	**0.11**
Height	**Linear, 1st order**	**0.9997**	**0.41**	**0.62**
Criteria	**Linear**	**>0.9990**	**>0.05**	**>0.05**

Implementation- Example

Implementation- Example

Analytical procedure - Accuracy

Summary of Results for OD 14

	Area Response			Height Response		
Replicate	**70%**	**100%**	**130%**	**70%**	**100%**	**130%**
1	**100.3**	**100.0**	**99.7**	**97.9**	**99.7**	**100.9**
2	**98.2**	**99.7**	**100.3**	**98.2**	**99.1**	**102.1**
3	**100.4**	**99.1**	**97.5**	**99.5**	**99.0**	**99.0**
Mean	**99.6**	**99.6**	**99.2**	**98.5**	**99.3**	**100.7**
RSD	**1.25**	**0.46**	**1.49**	**0.86**	**0.38**	**1.55**

Criterion for mean recovery: 98-102%
Criterion RSD: <2.0%

Analytical procedure - Accuracy

Summary of Results for OM 08

	Area Response			Height Response		
Replicate	0.1%	1.0%	2.0%	0.1%	1.0%	2.0%
1	80.9	104.4	106.8	96.1	103.9	103.5
2	80.9	104.4	109.0	94.1	103.8	104.8
3	79.9	103.5	107.1	95.4	104.0	104.0
4	83.8	103.9	107.8	98.3	104.2	104.2
Mean	81.4	104.0	107.7	96.0	104.0	104.1
RSD	2.09	0.42	0.91	1.81	0.15	0.52

Criterion for mean recovery at 0.1%: 70-130%
Criterion for mean recovery at 1.0 and 2.0%: 90-110%
Criterion RSD at 0.1%: <15%
Criterion RSD at 1.0 and 2.0%: <5.0%

Analytical procedure - Repeatability

OD 14:

Calculated from pooled standard deviation of the accuracy results covering the range from 70 to 130%

Peak area: RSD = 1.15%

Peak height: RSD = 1.06 %

Criterion: < 2.0%

Degradation products:

Calculated from accuracy results per concentration level

Analytical procedure - Intermediate Precision

Scheme for testing of intermediate precision of OD 14

Day	Number of analyses	Analyst	HPLC apparatus	HPLC column
1	3	A	I	c1
2	3	A	II	c2
3	3	B	I	c2

Peak Area: RSD = 1.71%
Peak Height: RSD = 1.65%
Criterion: RSD < 2.5%

Implementation- Example

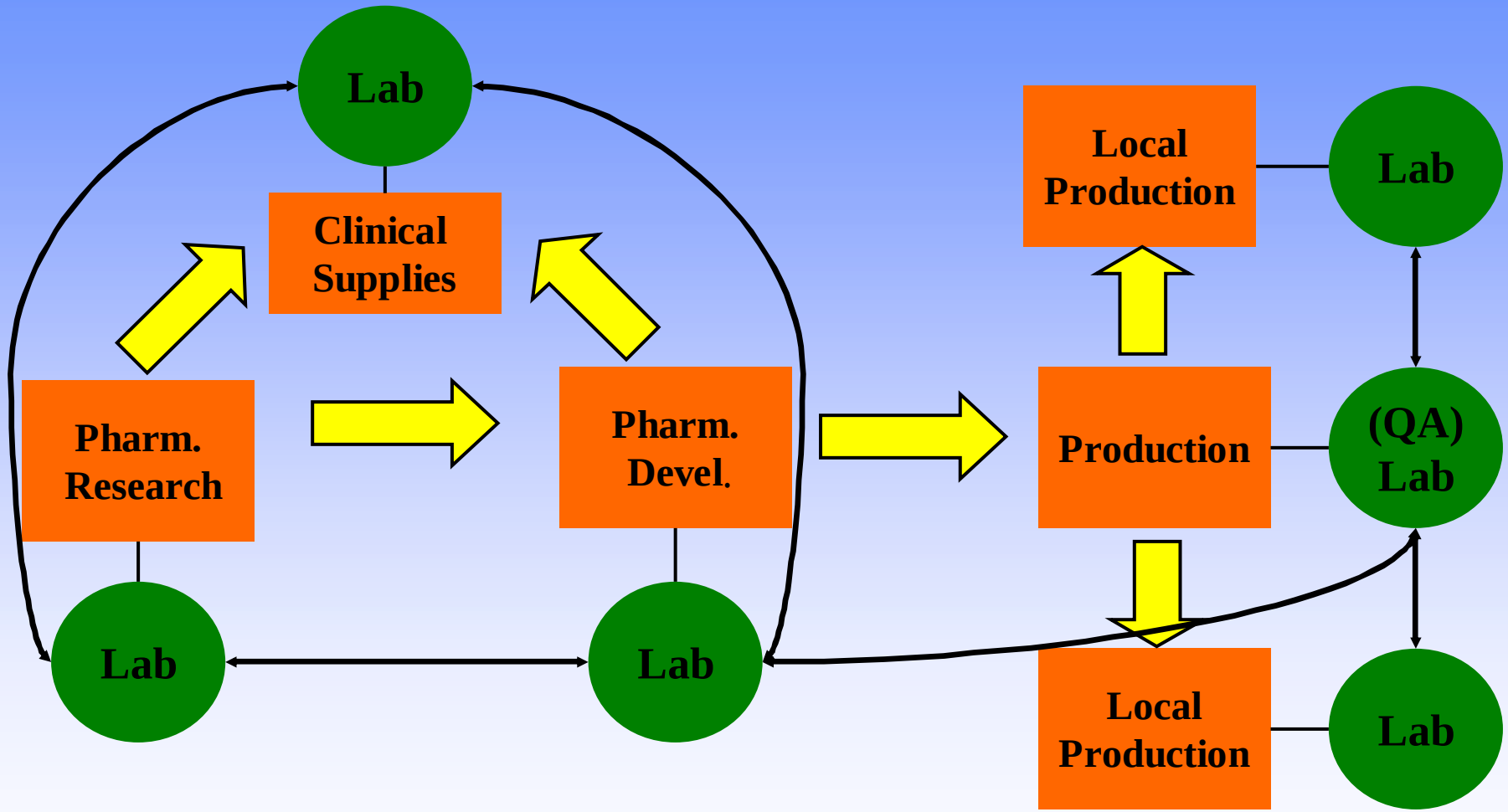
Implementation- Example

Analytical procedure - System Suitability Testing

Criteria obtained from validation data

Retention time (tR) of OD 14 (min)	12.0 < tR < 16.0
Number of theoretical plates (N)	N > 3000
Tailing Factor (T)	0.9 < T < 2.0
Rel. St. deviation (RSD) of reference solution	RSD < 1.5%
Ratio of mean response factors of standards	0.985 < Q < 1.015

Analytical transfers



MDP 6-02

"Analytical Method Transfer Procedure"

MDP 6-02

- Select labs
- Prepare protocol including:
  - detailed description of analytical method
  - samples to be tested
  - items to be checked: assay, precision (reproducibilty & intermediate precision), SST values
  - calculation formulas
  - way of reporting
- Carry out analyses and report results
- Perform statistical analysis on results and report on conclusions

Transfer - Example

Test:

OD 14 and degradation products in 1.25 mg tablets

Labs involved:

Organon, Oss (NL)

Organon, Swords (IRL)

Tested on:

Non-stressed and stressed (1 month 60°C/Amb. RH) tablets

Transfer - Example

Test schedule and criteria for transfer

Day	Number of analyses	Analyst	HPLC apparatus	HPLC column
1	3	A	I	c1
2	3	A	II	c2
3	3	B	I	c1

Total: 9 results for each type of sample

Criteria for method transfer

	Assay	Degradation products
Method repeatability	< 2.0%	< 5%
Intermediate precision	< 2.5%	< 10%
Reproducibility	< 3.0%	< 15%
Max. difference of mean between labs	No stat. signif. difference or < 2%	

THANK YOU FOR YOUR ATTENTION!!!!

QUESTIONS??

REMARKS??