RP-HPLC method development and validation by ICH Guidelines for Pharmaceutical Dosage Forms.

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INTRODUCTION

- **Nowadays HPLC and RP-HPLC methods are most commonly used for separation and analysis of single and multicomponent dosage forms in QA-QC department of PHARMA-INDUSTRY.**
- **High speed and more accurate, easy handling,**
- **Greater sensitivity and selectivity.**
- **Ideal, for costly drugs which can be analyzed in low concentration,**
- **Improved separation and easy Instrumentation.**
- **Precise and reproducible, applicable for biological estimation by using internal standard.**

PLAN OF WORK

- **STAGE I : Literature Survey**
- **STAGE II: Development of HPLC and Spectrophotometric methods for reference standard drugs.**
- **STAGE III: Development of HPLC for drugs in multicomponent Pharmaceutical formulations.**
- **STAGE IV: The developed methods were validated by using validation parameters as per ICH guidelines**

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Chromatography

- High Performance Liquid Chromatography (HPLC) is one of the most widely used techniques for Separation, Identification, Quantification as well as Purification of mixtures of Pharmaceutical compounds as well as Formulations.
- In HPLC, as in all chromatographic methods, components of a mixture are partitioned (separated) between an adsorbent (the stationary phase) and a solvent (the mobile phase) under high pressure.

Aims and Objectives

- To develop and validate HPLC methods for simultaneous estimation of drugs from multicomponent all Pharmaceutical dosage forms as Tablets, Capsules, Syrup Injectables as well.
- To develop analytical methods for the estimation of each drug simultaneously in combined dosage form, which is not available in official books is the task of QA-QC Lab.
- To estimate individual drugs present in multicomponent dosage forms is difficult due to cumbersome (tedious) extraction or isolation and separation.

PARAMETERS USED

- Solubility of drug components
- Stationary Phase
- Mobile Phase and its ratio(Composition of Mobile Phase)Polar and Non polar
- Detection wavelength of each component
- Selection and fixing of wavelength
- Absorbance of drugs at different concentration obeying Beer's law
- Sensitivity of instrument
- Stability of solutions at room temperature
- Selection of Internal Standard

EXPERIMENTAL

Chemical and Reagents used

Chemicals, buffers & reagents are of AR grade, solvents like Methanol, Acetonitrile were HPLC grade and Millipore water.

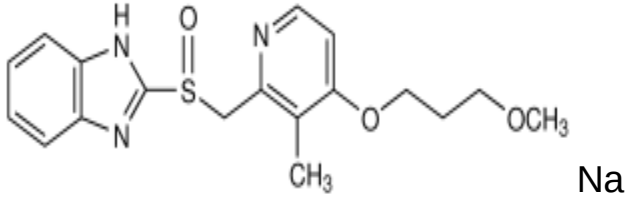
RABLET-D Capsules

Instruments used

- **Sartorius digital balance (BSR223S)**
- **Systronic pH meter, µpH system 361,**
- **Shimadzu UV -1700 Pharmaspec,**
- **Waters HPLC system with Breeze 3.3 data processor and UV-Detector,**
- **Shimadzu® liquid chromatographic system with Class-VP 6.01 data station SPD M-10AVP photo diode array detector,**
- **Stationary phase: Phenomenox C_{18} column (25cm X 4.6 mm i.d., 5µm particle size) Princeton C_{18} (15cm x 4.6mm i.d., 5µm particle size).**

RABEPRAZOLE SODIUM[1]

- **Chemical name** : **2-({[4-(3-Methoxypropoxy)-3-methyl-2-pyridyl]methyl}sulfinyl)-1*H*-benzimidazole sodium**
- **Molecular Formula** : **$C_{18}H_{20}N_3NaO_3S$**
- **Molecular Weight** : 381.4
- **Therapeutic use** : It is used in treatment of active peptic ulcer disease
- **Chemical Structure** :

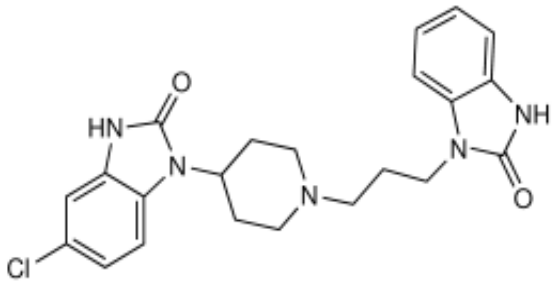


Internal Standared used

Pantaprozole Hcl 10 mg/ml

DOMPERIDONE[1]

- **Chemical name** : **(5-chloro-1-{1-[3-(2,3-dihydro-2-oxo-1H-benzimidazol-1-yl)propyl]-4-piperidinyl}benzimidazolin-2-one)**
- **Molecular Formula** : **$C_{22}H_{24}ClN_5O_2$**
- **Molecular Weight** : 425.9
- **Therapeutic use** : It is used as an antiemetic for the short term treatment of nausea and vomiting
- **Chemical Structure** :



METHOD DEVELOPMENT FOR REFERENCESTANDARD DRUGS

- **By using High performance chromatography**
- **Optimization of chromatographic conditions**
- **Estimation of drugs in Multicomponent dosage forms by reversed phase HPLC.**
- **Validation of developed Chromatographic Method.**

OPTIMIZATION OF CHROMATOGRAPHIC CONDITIONS

- Selection of solvent and reagents,
- Selection of wavelength,
- Selection of initial separation conditions,
- Nature of the stationary phase,
- Nature of mobile phase, (pH, peak modifier, solvent strength, ratio & flow rate)
- Sensitivity,
- Selection of internal standard

Chromatographic conditions for seperation

Stationary Phase ; Phenomenex C_{18} (250 x 4.6 mm, i.d., 5µ)

Mobile phase ; 30 mM ammonium sulphate(pH 5.5): Acetonitrile

Mobile phase ratio : 60:40 % v/v

Detection wavelength : 220 nm

Flow rate : 1 ml / min

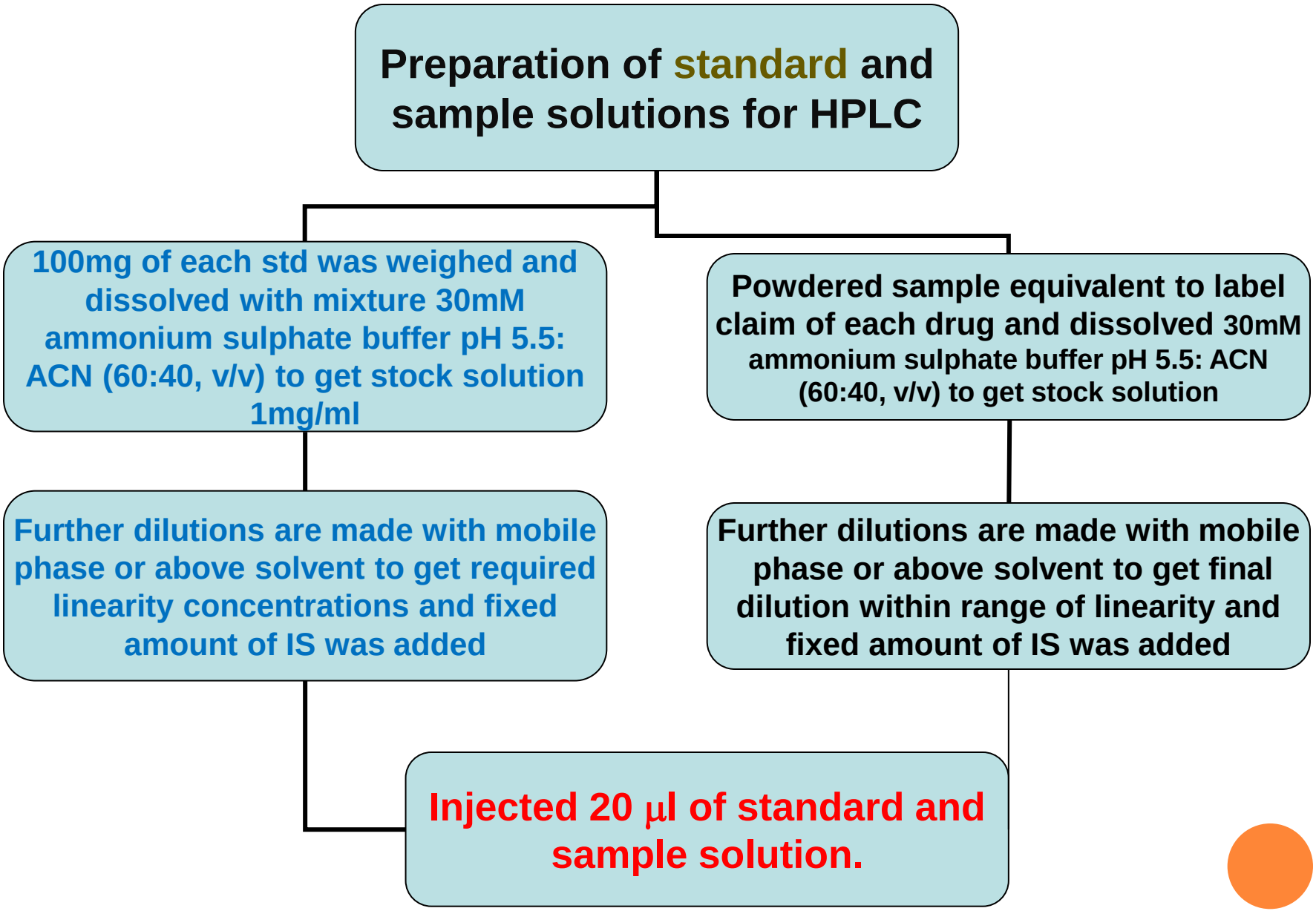
Sample size : 20 µl

Temperature : Room temperature

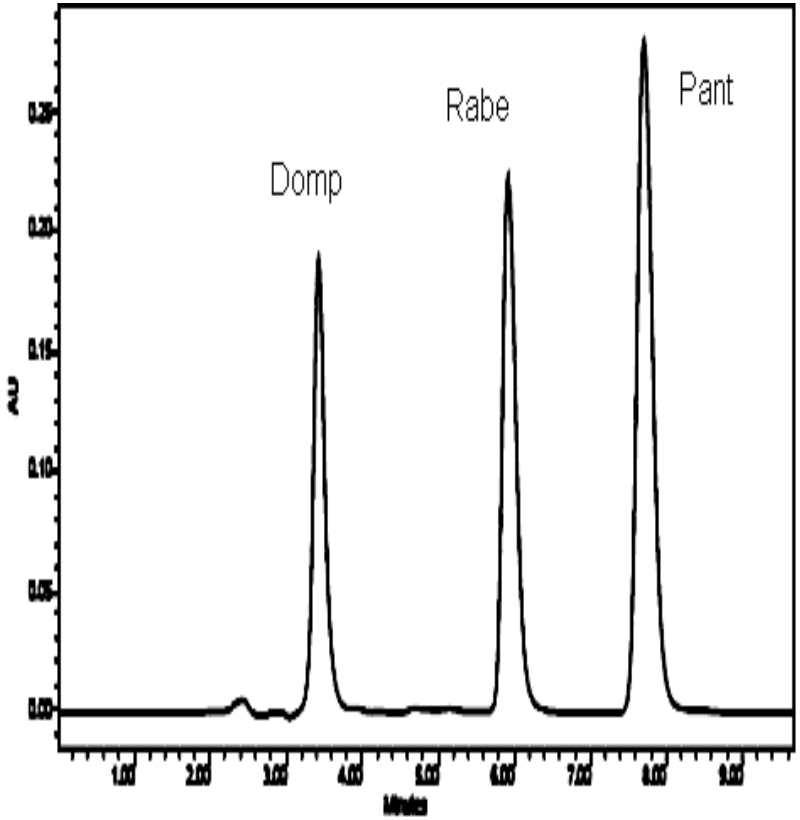
ESTIMATION OF DRUGS IN THEIR PHARMACEUTICAL FORMULATIONS

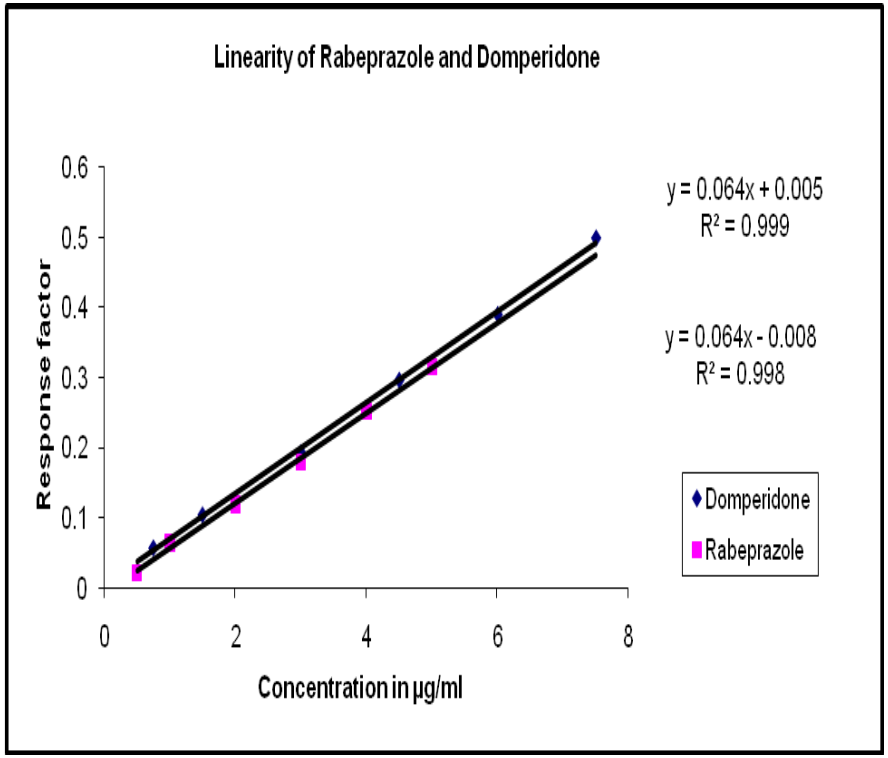
- Preparation of standard solutions and further diluted to different concentrations to obtain linearity and range by reversed phase HPLC

- Estimation of drugs in multicomponent dosage forms by reversed phase HPLC

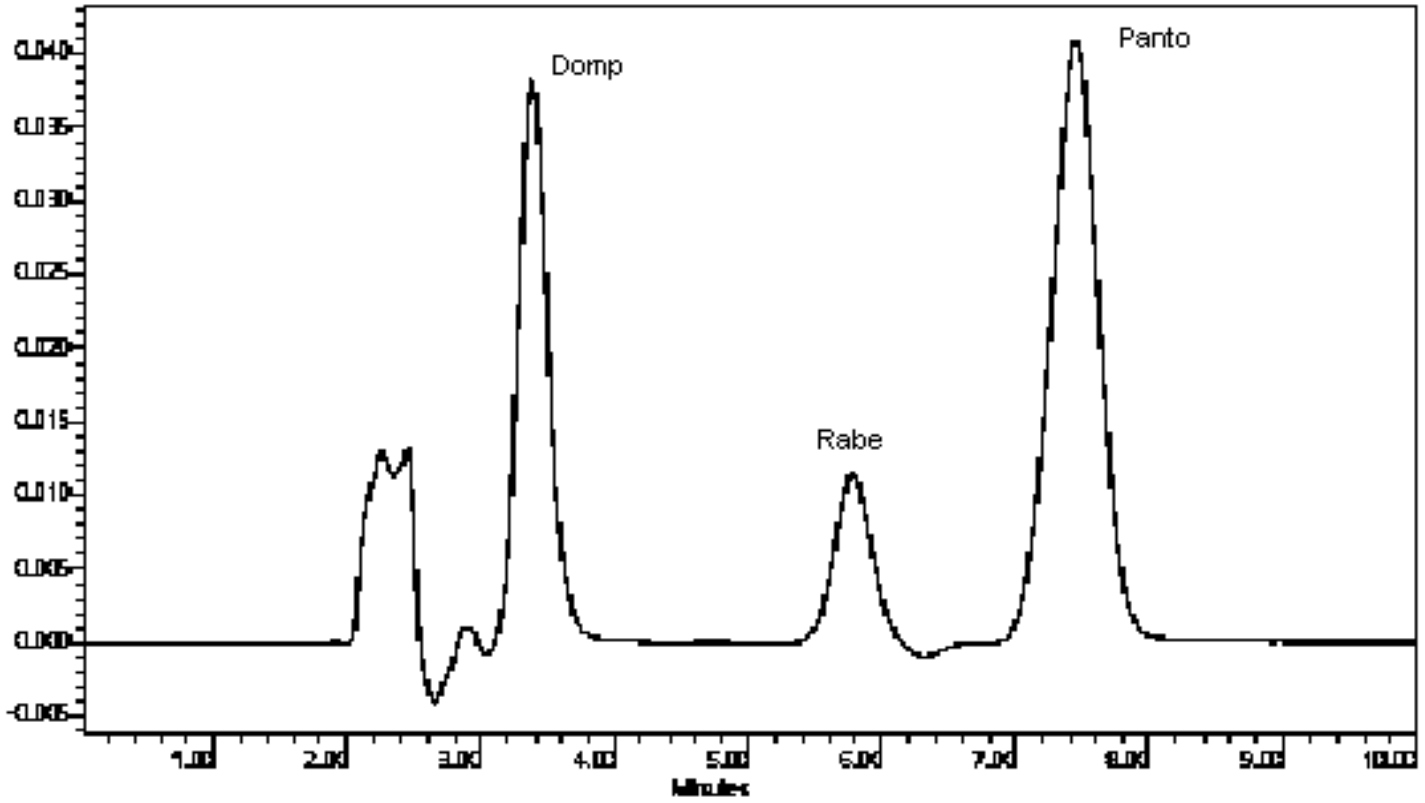

Preparation of standard and sample solutions for HPLC
100mg of each std was weighed and dissolved with mixture 30mM ammonium sulphate buffer pH 5.5: ACN (60:40, v/v) to get stock solution 1mg/ml
Powdered sample equivalent to label claim of each drug and dissolved 30mM ammonium sulphate buffer pH 5.5: ACN (60:40, v/v) to get stock solution
Further dilutions are made with mobile phase or above solvent to get required linearity concentrations and fixed amount of IS was added
Further dilutions are made with mobile phase or above solvent to get final dilution within range of linearity and fixed amount of IS was added
Injected 20 µl of standard and sample solution.

Chromatogram of Standard Domperidone, Rabeprazole and IS





CHROMATOGRAPHY OF SAMPLE FORMULATION



REGULATORY GUIDELINES

- ICH-Q2R1/Q2A "Text on Validation of Analytical Procedure (1994)
- ICH-Q2R1/Q2B "Validation of Analytical Procedures Methodology (1995)
- CDER "Reviewer Guidance: Validation of Chromatographic Method" (1994)

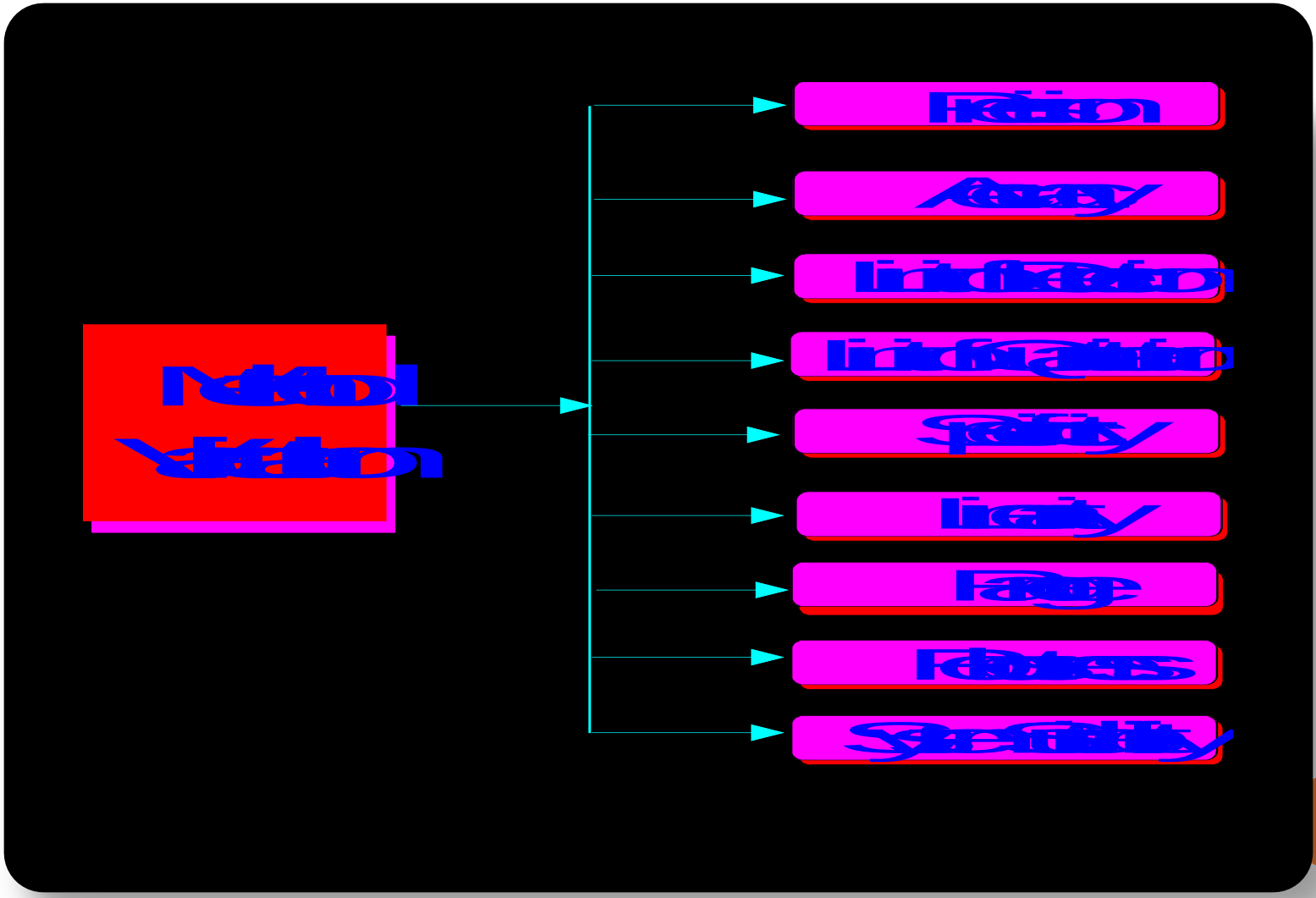
Goal of ICH

- To promote international harmonization by bringing together representatives from the three ICH regions (EU, Japan and USA)
- To discuss and establish common guidelines.
- To make information available on ICH, ICH activities and ICH guidelines to any country or company that requests the information
- To promote a mutual understanding of regional initiatives in order to facilitate harmonization processes related to ICH guidelines regionally and globally
- To strengthen the capacity of drug regulatory authorities and industry to utilize them.

Method Development → Method Validation → Method Transfer

VALIDATION OF DEVELOPED METHODS

THE DEVELOPED METHODS WERE VALIDATED BY USING FOLLOWING VALIDATION PARAMETERS AS PER Q2B ICH GUIDELINES,



Specificity/Selectivity

- Identification,Assay and Test for Impurities
- Identification
  - Discrimination between compounds of closely related structures
- Assay and impurities/degradants
  - Discrimination of analytes where impurities/degradants are available
- Peak purity
- Overlapping peaks in HPLC

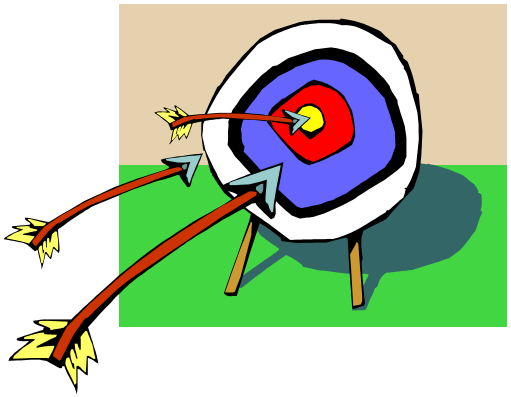
ACCURACY

- 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 i.e. individual observation or mean of measurements
- Assessment of samples spiked with known amounts of pure or impurities, In case certain impurities or change in purity of products are unavailable.
- Min 9 determinations on the different 3 concentrations in triplicate.

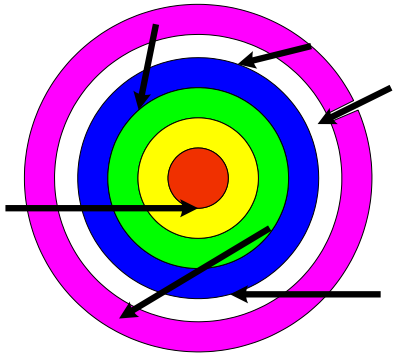
PRECISION

- The measure of the degree of agreement (degree of scatter) among test results when the method is applied repeatedly to multiple samplings of a homogeneous sample
- Expressed as %RSD for a statistically significant number of samples
  - At three IMP Levels gives information of
    - Repeatability
      - 9 determinations (3 x 3) covering the specified range
        or
      - 6 determinations at 100% of the test concentration
    - Intermediate precision
      - Effects of random events on the precision of the procedure, e.g.
        - Days
        - Analysts
        - Equipment
    - Reperoduciblity

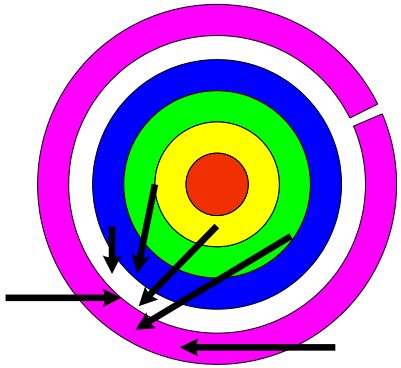
To be performed with a test solution prepared from the drug product



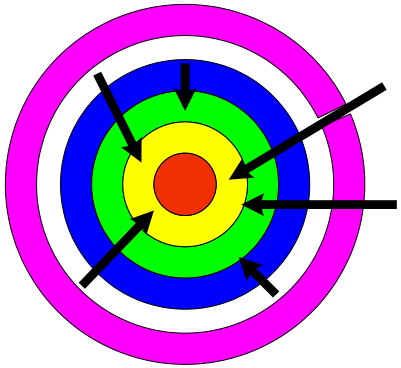
ACCURACY & PRECISION



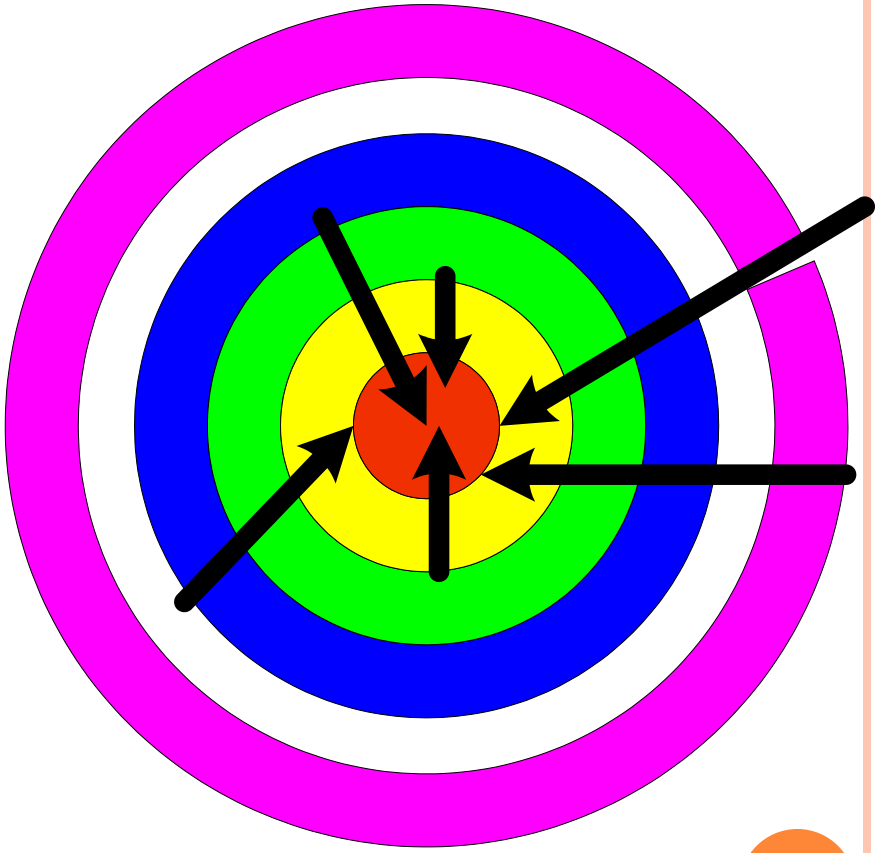
Inaccurate & imprecise



Inaccurate but precise



Accurate but imprecise



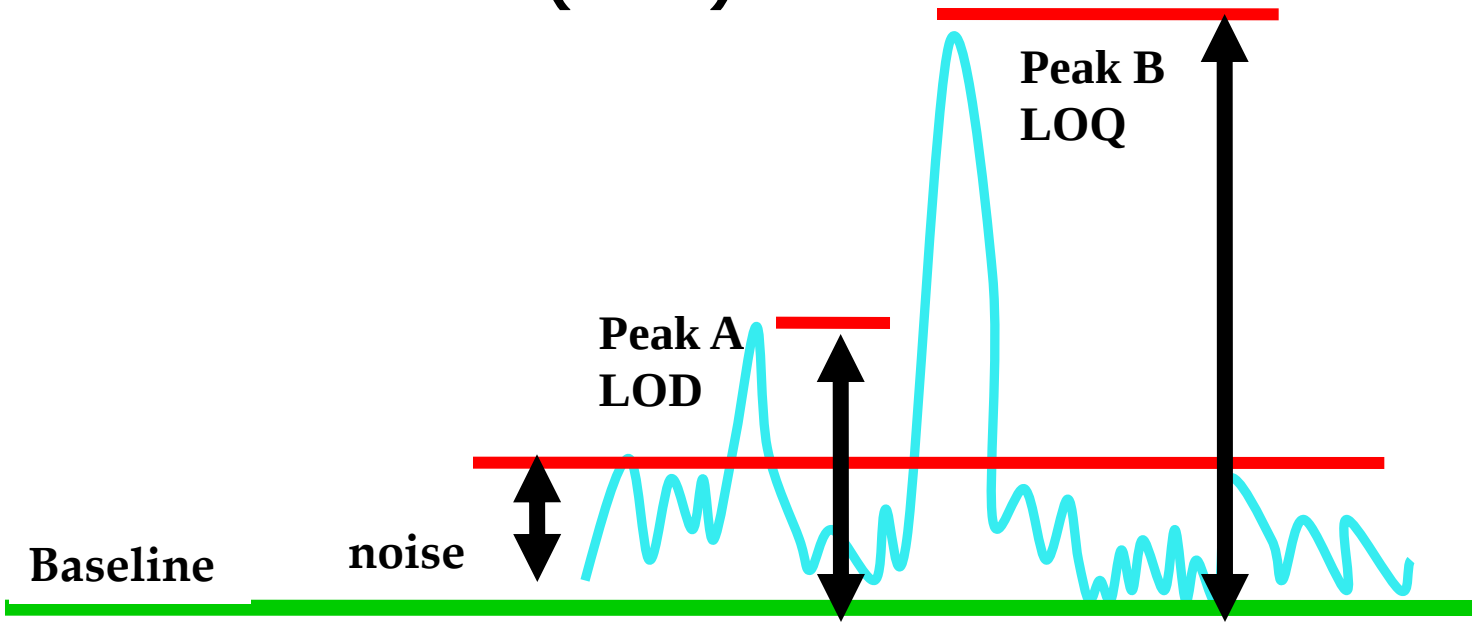
Accurate and precise

LOD, LOQ AND SNR

Limit of Quantitation (LOQ)

Limit of Detection (LOD)

Signal to Noise Ratio (SNR)



LOD The lowest concentration of an analyte in a sample that can be detected, not quantified

Expressed as a concentration at a specified signal:noise ratio

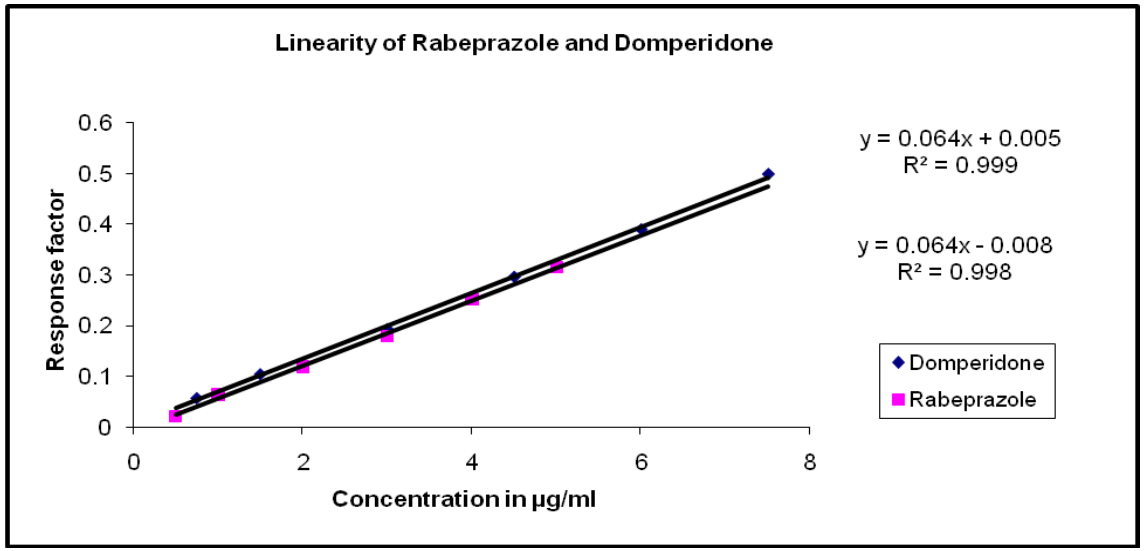
- Determination based on
  - Visual evaluation (non-instrumental and instrumental methods)
  - Signal to Noise (baseline noise)
  - Standard deviation of response (σ) and slope (S)
    - $DL=3.3\sigma/S$
      - Estimation of S
        - from the calibration curve of the analyte
      - Estimation of σ
        - from the standard deviation of the blank
        - from the standard deviation (regression line or y-intercept) of a calibration curve in the range of the DL

LOQ The lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under stated operational conditions
Expressed as concentration of analyte

- Determination based on
  - Visual evaluation (non-instrumental and instrumental methods)
  - Signal to Noise (baseline noise)
  - Standard deviation of response (σ) and slope (S)
    - $QL=10\sigma/S$
      - Estimation of S
        - from the calibration curve of the analyte
      - Estimation of σ
        - from the standard deviation of the blank
        - from the standard deviation (regression line or y-intercept) of a calibration curve in the range of the QL

LINEARITY AND RANGE

- The Ability of the method to obtain test results that are directly proportional to concentration within a given range
- Method: dilution of stock solution/separate weighing
- Expressed as the variance of the slope of the regression line
- Correlation coefficient, y-intercept, slope of regression line and residual sum of squares should be presented together with plot of the data



Linearity expresses differences in precision at different points of a given range.

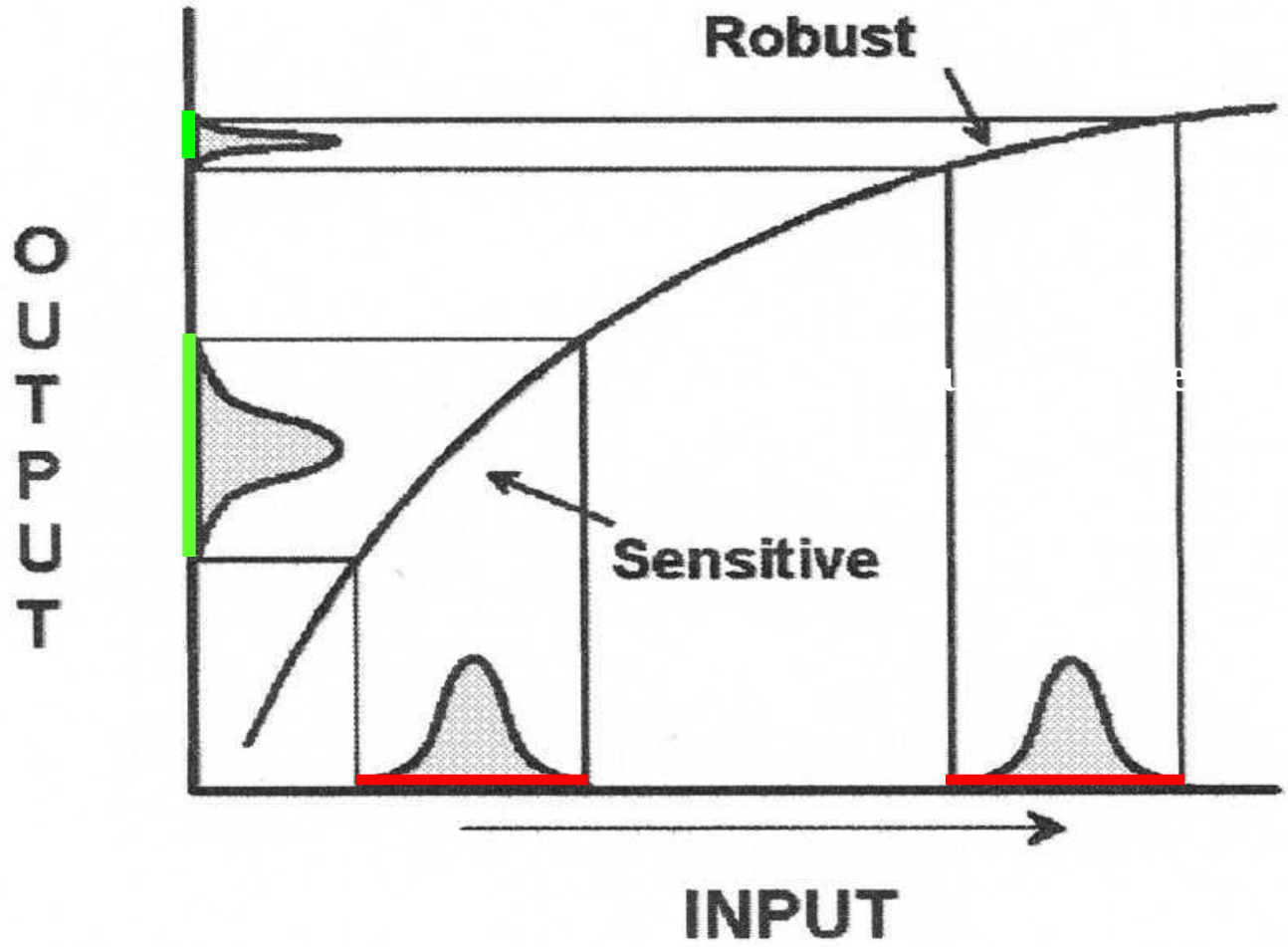
The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration (amount) of analyte in the sample

- Interval between upper and lower levels of analyte demonstrated by the method
- Confirms that the analytical procedure provides acceptable degree of linearity, accuracy and precision when applied to samples containing amounts of analyte within or at the extremes of the specified range
- Minimum 5 concentrations

ROBUSTNESS

- Measure of the capacity to remain unaffected by small (deliberate) variations in method parameters
  - Indication of reliability during normal use
- Reliability of an analysis with respect to deliberate variations in method parameters
  - Susceptibility to variations in analytical conditions?
- In the case of liquid chromatography
  - Influence of variations of pH in a mobile phase
  - Influence of variations in mobile phase composition
  - Influence of columns (different lots and/or suppliers)
  - Influence of temperature
  - Influence of flow rate

Sensitivity and Robustness



SYSTEM SUITABILITY

- The checking of a system, before or during analysis of unknowns, to ensure system performance.
  - *“No sample analysis is acceptable unless the requirements for system suitability have been met.” (USP Chapter 621)*
  - Plate Count, Tailing, Resolution
  - Determination of reproducibility (%RSD)
    - For %RSD < 2.0%, Five replicates
    - For %RSD > 2.0%, Six replicates
- System Suitability "Sample“ - A mixture of main components and expected by-products utilized to determine system suitability
- *“Whenever There is a Significant change in Equipment or Reagents System Suitability Testing Should be Performed”* (USP Chapter 621)

Stability of analytical solution

Solutes may readily decompose prior to chromatographic investigations e.g. during sample preparation , extraction, cleanup, phase transfer or storage of prepared vials (refrigerators or automatic sampler). Method development should investigate the stability of the analytes standards.

System stability

- Stability of the samples being analyzed in a sample solution.
- Measure of the bias in assay results generated during a preselected time interval e.g. 1 – 48 hours using a single solution
- Should be determined by replicate analysis of the sample solution.
- Considered appropriate when the RSD, calculated on the assay results obtained at different time intervals, Less than 20 percent of the corresponding value of the system precision

Range (minimum requirements)

- Assay of an API or a FPP: ± 20% of the test concentration.
- Content uniformity: ± 30% of the test concentration (unless a wider more appropriate range, based on the nature of the dosage form (e.g., metered dose inhalers), is justified).
- Dissolution testing: ± 20 % over the specified range.
- Impurity: from the reporting level of an impurity to 120% of the specification. (Unusually potent or toxic impurities, LOD and LOQ should be commensurate with ICH requirement.)
- If assay and purity are performed together as one test and only a 100% standard is used, linearity should cover the range from the reporting level of the impurities to 120% of the assay specification

Main Points Again

- Validation of analytical procedures is a critical requirement in risk assessment and management:
  - establishment of product-specific acceptance criteria, and
  - stability of APIs and FPPs.
- Validation should demonstrate that the analytical procedure is suitable for its intented purpose.
- HPLC systems and method validation deserves special attention during the inspection of QC laboratories.

RESULTS AND DISCUSSION

The following steps were carried out to obtain the results

- **Optimized chromatographic conditions,**
- **The percentage label claim of the formulations analyzed by the developed HPLC.**
- **The accuracy of the method,**
- **The precision data,**
- **Linearity and range,**
- **The limit of detection (LOD) and limit of quantification (LOQ) of the developed HPLC method.**
- **The system suitability and**
- **The comparative analytical statement of developed HPLC method.**

Table No 1 HPLC Parameters (Rabeprazole and Domperidone)

Sr. No	Drug Combinations	Column	Mobile phase composition	Internal standard (µg/ml)	Detection wavelength (nm)	Flow rate (ml/min)	Runtime in (minutes) #
1	Rabeprazole +Domperidone capsules	C_{18}	30mM ammonium sulphate buffer pH 5.5: ACN (60:40, v/v)	Pantaprazole 10 µg/mL	220	1	13

Table No 2 System suitability (Rabeprazole and Domperidone)

Sr.No.	Parameter	Rabeprazole	Domperidone
1	Retention time (minutes)	6.63± 0.03	9.01± 0.05
2	Theoretical plates*	4967	10654
3	Resolution	4.76	
4	Asymmetry factor	0.98	1.01
5	Calibration range(µg/ml)	0.5-5	0.75-7.5
6	Correlation coefficient(r)	0.998	0.999
7	LOD (ng/ml)	55	58
8	LOQ (ng/ml)	170	175

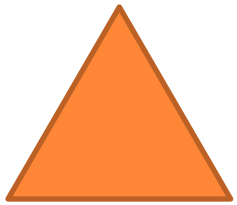
Table III Analysis of RABLET-D Capsules formulation, recovery and precision studies by HPLC

Drug	Amount (mg/capsule)		% Label claim*	% Recovery*	Precision (% RSD)*	
	Labeled	Found*			Interday	Intraday
Rabeprazole sodium	20	19.85	98.72±1.560	99.99±0.426	0.546	0.746
Domperidone	30	29.88	100.04±0.990	101.02±0.97	0.212	0.684

* (n=6)

Formulation ;- Domperidone and Rabeprazole (Capsules)

RABLET-D Capsules



Conclusion

- The proposed HPLC method developed is
  - simple,
  - accurate,
  - precise,
  - linear and rapid,
  - reproducible,
  - rugged and rebourst,
  - stable at room temperature for 24 hours

They are thus suitable for

- ✓quality control of raw materials,
- ✓formulations and in dissolutions studies.

The newly developed analytical methods may be used in various fields like

- ✓research institutions quality control department in industries,
- ✓approved testing laboratories,
- ✓bioequivalence studies and clinical pharmacokinetic studies.

REFERENCES

1. The Martindale The complete drug reference. 35thed. published pharmaceutical press, lambeta high street, London SEI 75M, UK, 2006; 92, 114, 1211, 1277, 1417, 2846.
2. Snyder LR, Kirkland J J, Glejch J L. Practical HPLC Method Development 2nd ed. Wiley-Interscience, New york ,1997; 1-20, 21 - 57, 234 - 57.
3. Miller JM, Chromatography Concepts and contrasts.2nd ed. Jhon wiley & sons, Inc., Publication, New Jersey. 2005; 35-182.
4. Sethi PD, High performance liquid chromatography, 1st edition, CBS publisher and distributors, New Delhi, 2001; 5-21,59-65, 117 - 20, 151 - 95.

5. ICH, Q2A: Text on validation of analytical procedures, International Conference on Harmonization. October 1994.
6. ICH, Q3B: Validation of analytical procedures: Methodology, International Conference on Harmonization. November1996.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