International Standard Serial Number (ISSN): 2249-6807

International Journal of Institutional Pharmacy and Life Sciences 6(1): January-February 2016

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Pharmaceutical Sciences

Review Article……!!!

Received: 02-01-2016; Revised: 09-01-2016; Accepted: 10-01-2016

REVIEW: DEVELOPMENT AND VALIDATION OF CHROMATOGRAPHIC METHOD

Sushil D. Patil*, Dr. Sunil V. Amurutkar and Dr. C. D. Upasani

Department of Pharmaceutical Chemistry, SNJB's (Jain Gurukal) Shriman Sureshdada Jain College of Pharmacy, Neminagar, Chandwad-423101.

Keywords:

High performance liquid chromatographic, HPTLC, validation

For Correspondence:

Sushil D. Patil

Department of Pharmaceutical Chemistry, SNJB's (Jain Gurukal) Shriman Sureshdada Jain College of Pharmacy, Neminagar, Chandwad-423101

E-mail:

sushilpharma@rediffmail.com

ABSTRACT

Chromatographic methods development and validation play important roles in the discovery, development and Manufacture of pharmaceuticals drugs &excipient. Method development is the process of proving that a chromatographic method is for used to measure the concentration of drugs, excipient& in biological samples which allow simplified procedures to be employed to verify that an analysis procedure, accurately and consistently will deliver a reliable measurement of an pharmaceutical preparation & bioanalysis. The chromatographic method validation is essential for analytical & bioanalytical method development and tested extensively for specificity, linearity, accuracy, precision, range, detection limit, quantization limit, and robustness.

INTRODUCTION

Chromatographic methods have taken precedence over the conventional methods of analysis. Other than separation of multiple components, the advantage of chromatographic methods is that these possess greater accuracy and sensitivity. HPLC has been very widely employed. It has gained popularity in stability studies due to high-resolution capacity, sensitivity and specificity. Various chromatographic methods that have been used are thin-layer chromatography (TLC), high-performance thin-layer chromatography (HPTLC) and gas chromatography (GC), HPLC and newer technique like capillary electrophoresis [1-2].

The number of drugs introduced into the market is increasing every year. These drugs may be either new entities or partial structural modification of the existing one. Very often there is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, reports of new toxicities (resulting in their withdrawal from the market), development of patient resistance and introduction of better drugs by competitors. Under these conditions, standards and analytical procedures for these drugs may not be available in the pharmacopoeias. It becomes necessary, therefore to develop newer chromatographic &analytical methods for such drugs. [3-7]

General Classification	Specific Method Name	Stationary Phase	Type of Equilibrium
Liquid Chromatography (LC) Mobile Phase = liquid	Liquid-liquid or partition	Liquid adsorbed on a solid	Partition between immiscible liquids
	Liquid-bonded phase	Organic species bonded to a solid surface	Partition between liquid and a bonded surface
	Liquid-solid or adsorption	Solid	Adsorption
	Ion exchange	Ion-exchange resin	Ion exchange
	Size exclusion	Liquid in interstices of a polymeric solid	Partition/sieving
Gas Chromatography (GC) Mobile Phase = gas	Gas-liquid	Liquid adsorbed on a solid	Partition between gas and liquid
	Gas-bonded phase	Organic species bonded to a solid surface	Partition between liquid and bonded surface
	Gas-solid	Solid	Adsorption

General Classification of chromatography Table no.1 [8]

1. CHROMATOGRPHIC (ANALYTICAL & BIOANLYTICAL) METHOD DEVELOPMENT:

Analytical chemistry deals with methods for identification, separation, and quantification of the chemical components of natural and artificial materials. [8] The choice of analytical methodology is based on many considerations, such as: chemical properties of the analyte

and its concentration sample matrix, the speed and cost of the analysis, type of measurements i.e., quantitative or qualitative and the number of samples. A qualitative method yields information of the chemical identity of the species in the sample. A quantitative method provides numerical information regarding the relative amounts of one or more of the analytes in the sample.

The steps of method development and method validation the steps of method development and method validation

- Method development plan definition
- Background information gathering
- Laboratory method development, it includes various stages namely sample preparation, specific analytical method, detection and data processing
- Generation of test procedure
- A well-developed method should be easy to validate. A method should be developed with the goal to rapidly test preclinical samples, formulation prototypes, and commercial samples. There are five common types of analytical methods, each with its own set of validation requirements
- Identification tests
- Potency assays
- Quantitative tests for impurities
    - Limit test for the control of impurities
- Specific tests

The most widely used methods for quantitative determination of drugs and metabolites in biological matrices such as blood, serum, plasma, or urine includes Gas chromatography, (GC) High-performance liquid chromatography, (HPLC) [9-10] Thin layer chromatography, (TLC) combined GC and LC mass spectrometric (MS) procedures such as LC-MS [11-12] LC-MS-MS, [13-14] GC-MS, [15-16] and GC-MS-MS, techniques like NMR is used for structure identification. Chromatography in different forms is the leading analytical method for separation of components in a mixture. The chromatographic procedure for the separation of substances is based on differences in rates of migration through the column arising from different partition of the compounds between a stationary phase (column packing) and a mobile phase transported through the system. [17]

Chromatographic methods can be classified according to the physical state of the mobile phase into the following basic categories:

- Gas chromatography, (GC)
- Supercritical fluid chromatography (SFC) and
- Liquid chromatography (LC). [18-19]

Today TLC is rapidly becoming a routine analytical technique due to its advantages of low operating costs, high sample throughput and the need for minimum sample preparation. The

major advantage of TLC is that several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC thus reducing the analysis time and cost per analysis. [20-21]. An enhanced form of thin layer chromatography (TLC) is called as High performance thin layer chromatography (HPTLC). [22-23]

A number of enhancements can be made to the basic method of thin layer chromatography to automate the different steps, to increase the resolution achieved and to allow more accurate quantitative measurements Liquid chromatography can be categorized on the basis of the mechanism of interaction of the solute with the stationary phase as:

- Adsorption chromatography (liquid-solid chromatography)
- Partition chromatography(liquid-liquid chromatography)
- Ion-exchange chromatography (IEC)
- Size exclusion chromatography (SEC) and
- Affinity chromatography

Early work in liquid chromatography was based on highly polar stationary phases, and nonpolar solvents served as mobile phases, this type of chromatography is now referred to normal-phase liquid chromatography (NPLC).[24] Chromatography on bare silica is an example of normal-phase chromatography. In reversed-phase high performance liquid chromatography, (RP-HPLC) the stationary phase is nonpolar, [8, 26] often a hydrocarbon, and the mobile phase is relatively polar. [27]

- **Sample preparation**.
- **Detection**

Table No.2: Use of analytical methods - generics

CLINICAL	PHARMACEUTICAL	METHODS
At Initial Phase of pharmaceutical Development		
To determine bioavailability in healthy volunteers	To develop a stable and reproducible formulation for the manufacture of bioequivalence, dissolution, stability and pilot-scale validation batches	To understand the profile of related substances and to study stability, To start measuring the impact of Key product and manufacturing process parameters on consistent FPP quality
At advanced phase of pharmaceutical development		
To prove bioequivalence after critical variations to the prequalified dossier	To optimize, scale-up and transfer a stable and controlled manufacturing process for the prequalification product	To be robust, transferable, accurate and precise for specification setting, stability assessment and QC release of prequalified product batches

2. Analytical Method validation

Successful acceptance of the validation parameters and performance criteria, by all parties involved, requires the cooperative efforts of several departments, including analytical development, QC, regulatory affairs and the individuals requiring the analytical data. The operating procedure or the Validation Master Plan (VMP) should clearly define the roles and responsibilities of each department involved in the validation of analytical methods. [28]

2.1 Method Validation:

A method should be validated when it is necessary to verify that its performance parameters are adequate for use for a particular analytical problem. For example:

- Method just developed
- Revised method or established method adapted to a new problem;
- When a review of quality control indicates an established method is changing with time;
- When an established method is used in a different laboratory, with different analysts or with different equipment;
- Demonstration of the equivalence between two methods, e.g. a new method and a Standard. Certain areas of analytical practices, such as in clinical chemistry will specify Validation requirements relevant to the method. This ensures that particular validation Terminology together with the statistics used is interpreted in a manner consistent within the relevant sector. Official recognition of a method may require characterization using a collaborative study.

2.3 Parameters for method validation:

The parameters for method validation have been defined in different working groups of National and international committees and are described in the literature. Unfortunately, some of the definitions vary between the different organizations. An attempt at harmonization was made for pharmaceutical applications through the ICH where representatives from the Industry and regulatory agencies from the United States, Europe and Japan defined Parameters, requirements and, to some extent, methodology for analytical methods validation [29-30]

The various parameters are:

- Selectivity/Specificity
- Precision and Reproducibility
- Accuracy and Recovery
- Stability
- Range
- Limit of Detection
- Limit of Quantization
- Repeatability
- Reproducibility
- Measurement Uncertainty Sensitivity
- Ruggedness

3. BIOANALYTICAL METHOD DEVELOPMENT AND VALIDATION

Bioanalytical methods employed for the quantitative determination of drugs and their metabolites in biological matrix (plasma, urine, saliva, serum etc) play a significant role in evaluation and interpretation of bioavailability, bioequivalence and pharmacokinetic data [31]. Both HPLC and LCMS-MS can be used for the bioanalysis of drugs in plasma. Each of the instruments has its own merits. HPLC coupled with UV, PDA or fluorescence detector can be used for estimation of many compounds. The main advantages of LCMS/MS include low detection limits, the ability to generate structural information, the requirement of minimal sample treatment and the possibility to cover a wide range of analytes differing in their polarities [32]. Bioanalytical method validation includes all of the procedures that demonstrate that a particular method used for quantitative measurement of analytes in a given biological matrix, such as blood, plasma, serum, or urine, is reliable and reproducible for the intended use [33-34] .The fundamental parameters for this validation include selectivity, accuracy, precision, linearity and range, limit of detection, limit of quantification, recovery, robustness and stability. This guideline provides assistance to sponsors of investigational new drug applications (INDs), new drug applications (NDAs), abbreviated new drug applications (ANDAs) and supplements in developing bioanalytical method validation information used in human clinical pharmacology, bioavailability (BA) and bioequivalence (BE) studies requiring pharmacokinetic (PK) evaluation. This guideline also applies to bioanalytical methods used for non-human pharmacology/toxicology studies and preclinical studies. For studies related to the veterinary drug approval process, this guidance applies only to blood and urine BA, BE and PK studies.

3.1 Steps for bioanalytical method:

- Sample collection and preparation
- Liquid – Liquid extraction
- Solid Phase Extraction (SPE)
- Protein Precipitation

3.2 Need of Bioanalytical Method Validation:

1. It is essential to used well-characterized and fully validated bioanalytical methods to yield reliable results that can be satisfactory interpreted.

2. It is recognized that bioanalytical methods and techniques are constantly undergoing changes and improvements; they are at the cutting edge of the technology.

3. It is also important to emphasize that each bioanalytical technique has its own characteristics, which will vary from analyte to analyte, specific validation criteria ma need to be developed for each analyte.

4. Moreover, the appropriateness of the technique may also be influenced by the ultimate objective of the study. When samples analysis for a given study is conducted at more than one site, it is necessary to validate the bioanalytical methods at each site and provide appropriate validation information for different sites to establish inter-laboratory reliability [35]

Typical parameters to validate are included; selectivity, accuracy, precision, linearity and range, limit of detection, limit of quantification, recovery, robustness and stability.

3.3 Specific Recommendation for Bioanalytical Method Validation:

1. For validation of the bioanalytical method, accuracy and precision should be determined using a minimum of five determinations per concentration level. The mean value should be within 15% of the theoretical value. Other methods of assessing accuracy and precision that meet these limits may be equally acceptable.

2. The accuracy and precision with which known concentrations of analyte in biological matrix can be determined should be demonstrated. This can be accomplished by analysis of replicate sets of analyte samples of known concentrations QC samples from an equivalent biological matrix.

3. The stability of the analyte in biological matrix at intended storage temperature should be established.

4. The stability of the analyte in matrix at ambient temperature should be evaluated over a time period equal to the typical sample preparation, sample handling and analytical run times.

5. Reinjection reproducibility should be evaluated to determine if an analytical run could be reanalyzed in the case of instrument failure.

6. The specificity of the assay methodology should be established using a minimum of six independent source of the same matrix. [36]

3.4 Method development for Nonclinical and clinical

Nonclinical and clinical bioanalytical method development (MD) activities are not required to be performed in compliance with GLPs but should be adequately documented to support a reproducible method document for validation. Method development is intended to define the method and provide sound scientific evidence for method design and suitability for its intended purpose. If the MD experiments outlined in Table no. 3 are incorporated into the

MD plan and properly executed, then the resultant method design should be deemed suitable to proceed with validation.

The following aspects should also be considered during MD:

1. Carryover should be addressed and minimized during method development. If carryover is inevitable or unavoidable, it should be noted in the method and a non-randomized sequence should be used with extra blanks inserted after the high calibration standard and high quality control (QC) injections.

2. Precautions should be taken to eliminate any possibility of contamination of the mobile phases, diluents, wash buffers, stocks, and working stocks. Use of disposable glassware, pipet tips with filters, and automation, whenever possible, is highly recommended.

3. Analyte stability (freeze/thaw and use of stabilizer agents) should be assessed during MD to help avoid delays in validation activities due to QC (analyte in matrix) stability issues.

4. Selection of the anticoagulant should be made by the MD scientist and should remain consistent throughout the entire toxicology program (nonclinical and clinical). If the anticoagulant or counter ion is changed for a PK/TK study, at a minimum an equivalency experiment must be performed to show that the existing method can be used for the new anticoagulant. Additional proof of stability may also be necessary.

4. Whole blood stability and collection process stability are not often performed as a method development activity; however, assessment may provide valuable information for both nonclinical and clinical studies. The analyte may adsorb to cellular or proteinaceous components during the time period between collection and sample processing.

5. An impact assessment on quantitation of analyte interference in matrix containing lipids and hemolysis is not required by the FDA Bioanalytical Guidance; however, considerations should be made for this assessment if the desired submission will target Brazil or an EMA country.

6. Effect of the dosing vehicle on the matrix should also be evaluated during MD, particularly for nonclinical studies, depending on the dose route. The dosing vehicle can cause potential interference or matrix ionization effects, as the vehicle may be present at relatively high levels for nonclinical samples. This may not be an issue for the clinical method, as the dosing vehicle is typically present at low levels in clinical study samples. Depending on the excipient, this is a requirement within the EMA draft guidance.

7. If metabolites are known and standards are available, then an evaluation of metabolite impact on quantitation of analyte is required. In nonclinical studies, these are not often known, and if they are, there may be no reference standards available or characterized.

8. During MD, specific instrument parameters must be clearly defined to ensure a consistent parameter approach can be set and remain fixed during validation: mobile phase composition, gradient profile,ionization mode, HPLC column identity. Minor parameters may be optimized to account for instrument to instrument variability and improve response (i.e., voltage and gas settings, precursor and product ion mass adjustment to optimize mass center).

9. Once the sample preparation parameters are refined, they must remain fixed during validation. Changes in aliquot volume or sample preparation parameters necessitate at least partial revalidation. [39]

3.5 General recommendation for bioanalytical method validation

- *Accuracy*
- *Precision*
- *Repeatability*
- *Reproducibility*
- *Linearity*
- *Selectivity and specificity*
- *Limit of Detection (LOD)*
- *Limit of Quantitation*
- *Quantification Range*
- *Recovery*
- *Standard Curve (calibration curve)*
- *Stability*
    - *a. Short - term stability*
    - *b.Long - term stability*
    - *c.Freeze and Thaw Stability*
    - *d.Bench – Top stability*
    - *e.Stock Solution Stability*
- *Range*
- *Robustness*
- *Ruggedness*

Overview of Validation Exercises and Criteria for Nonclinical and clinical Table no.3

Validation Exercise	Minimum Experiments	Performance Criteria
Selectivity	Matrix blanks: 6 lots, n=1 for each lot Matrix blank fortified with IS: 6 lots, n=1 for each lot LLOQ Selectivity Sample: 6 lots, fortified with analyte at LLOQ level and IS. n=3 for each lot	At least 5 out of the 6 lots must meet the following criteria: Response for the analyte in matrix blanks or matrix blank fortified with IS must be ≤20% of the mean analyte response in the acceptable LLOQ calibration standards Response for IS in matrix blanks must be ≤5% of the mean IS response in the acceptable LLOQ calibration standards At least two-thirds of the selectivity LLOQ replicates for each lot must meet accuracy acceptance limit, and the mean accuracy must be within ±20.0% of the nominal concentration
Cross-analyte Interference	Each analyte at ULOQ evaluated separately. IS at the level of use evaluated separately	Interference must be ≤ 20% of the mean analyte peak response or ≤ 5% of the mean IS response of the acceptable LLOQ calibration standards

Carryover	Minimum of 2 blank samples after the injection of each ULOQ standard	Analyte response ≤20% of the mean analyte response in the acceptable LLOQ standards, IS response ≤5% of the mean IS response in the acceptable LLOQ standards
Linearity	Minimum of 6 non-zero calibration standard (CS) levels	(R2) ≥0.985
Calibration Standards: Accuracy	Injected at the beginning and end of the analytical run	Minimum 6 non-zero (or 75% of total) CS must be within ±15.0% RE of nominal (exception: LLOQ within ±20.0 %RE)
QC Samples – Core Validation	Three concentration levels: Low, Mid, High; n=6 at each level	Minimum 50% of the QC replicates at each level and 66.7% of all QCs must be within 15.0% RE of nominal Mean inter- and intra-assay accuracy within ±15.0% RE of nominal; precision ≤15.0% RSD
QC Samples – Ancillary Runs	Three concentration levels: Low, Mid, High; n ≥ 2 at each level	≥66.7% of all QCs and at least 50% at each level must be within 15.0% RE of nominal
LLOQ Samples (Sensitivity)	n=6, ≥ 1 run	Mean accuracy within ±20.0%RE of nominal; precision ≤20.0% RSD

Recovery	Analyte at low, medium and high levels, and IS at the level of use: preextraction spiked samples (n=6) are compared with mean response of post-extraction spiked matrix samples (n=6)	Recovery for analyte and IS must be relatively consistent across all QC levels
Dilution (Parallelism)	One level (minimum 10 fold dilution); n=6	Mean accuracy within ±15.0% RE of nominal; precision ≤15.0% RSD
Matrix Effect	Post-extraction spiked samples (n=6, at each QC low, mid and high level) are compared with mean response of 6 injections of analyte or IS in solvent	MF will be calculated and reported for the analyte and for the IS
Ruggedness	Minimum of two variables over the course of validation (e.g. different column, instrument and/or analyst)	Mean inter- & intra-assay accuracy within ±15.0% RE of nominal; precision ≤15.0% RSD

Overview of Validation Exercises and Criteria for Nonclinical and clinical Table no.4

Validation Exercise	**Minimum Experiments**	**Performance Criteria**
Stock Solution	n ≥6; long term at typical storage conditions; bench top at conditions representing typical processing conditions for ≥6 hours	Precision of area response or relative response must be ≤15.0% RSD; RD within 7.0% for analytes, 20.0% for internal standards

Bench top	≥ 4 hours n ≥6 at QC Low and High levels	Mean accuracy within ±15.0% RE of nominal; precision ≤15.0% RSD
Freeze/Thaw	3 freeze/thaw cycles n ≥6 at QC Low and High levels	Mean accuracy within ±15.0% RE of nominal; precision ≤15.0% RSD
Long term	n ≥ 6 at QC Low and High levels at -10 to -30°C or -50 to -90°C for at least 1 and 4 months.	Mean accuracy within ±15.0% RE of nominal; precision ≤15.0% RSD
Reinjection Reproducibility	Calibrations standards (CS) and QCs (n=6 at each level) reinjected from an acceptable validation batch run, maintained at autosampler temperature for ≥72 hours.	Mean accuracy within ±15.0% RE of nominal; precision ≤15.0% RSD; calculated using calibration standards from re-injected run
Extract Stability	Stored extracts at QC Low, Mid, and High levels (n=6) maintained at autosampler temperature for ≥72 hours.	Mean accuracy within ±15.0% RE of nominal; precision ≤15.0% RSD; calculated using freshly extracted curves or back calculated using the original curves from the batch the aged extracts were originally extracted and injected

[39]

4. General Methodology for HPTLC Method Development

Method development demands primary knowledge about the physicochemical characteristics of sample, nature of the sample, such as structure, polarity, volatility, stability and solubility. It involves considerable trial and error procedures. Steps involved in HPTLC method

development are selection of stationary and mobile phase, application of sample, development, derivatization, documentation of plate, labeling, quantitative evaluation of chromatogram, and documentation of work performed. Instrument installation and operation qualification (IQ/OQ) and good laboratory practice/ good manufacturing practice (GLP/GMP) requirements in regulated laboratories are met by many of the latest TLC instruments, such as sample applicators, developing chambers and densitometers.

4.1 Method Development for HPTLC

I. *Sample Preparation*

II. *Selection of Stationary Phase*

III. *Layer Prewashing*

IV. *Selection and Optimization of Mobile Phase*

V. *Sample Application*

VI. Chromatogram Development

VII. Plate Labeling

VIII. Derivatization

IX. Documentation

X. Detection

XI. Quantitation

4.2 Validation Parameters:

- *Linearity*
- *Accuracy*
- *Precision*
- *Repeatability*
- *Intermediate precision*
- *Reproducibility*
- *Limit of Detection*
- *Limit of Quantification[37]*

5. Stability Indicating By LC-MS Method

It was shown that liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) is a suitable tool for the identification of organic impurities in the pharmaceutical substance of interest. Using these techniques, the methods for separation and identification were developed which allow determining both, process impurities and degradation impurities

which were formed during the stability study of the tested substance. The mass spectra, fragmentation spectra and chromatographic data were used to identify the impurities1.

5.1 Need of stability study

- To develop a stability test methods.
- To develop quantitative analytical procedure.
- To study degradation pathways of drugs.
- To determine storage condition.
- To detect drug stability in different biological fluids.
- To relevant legal requirements concerned with the identity, strength, purity and quality.
- To minimize financial loss.

5.2 Application

- LC-MS in drug metabolism studies
- Analysis of drug & metabolites
- Analysis of chiral impurities in pharmaceuticals
- Molecular weight determination
- Structural determination e.g. Ginsenoside
- Biochemical application e.g. rapid protein identification using capillary LC/MS/MS

5.3 Analytical Method Development

- Method development usually requires the choice of columns, mobile phase, detectors, and method of
- Quantization etc.New drug development requires meaningful and reliable analytical data to be produced at various stages of the development.
- a) Sample set selection for analytical method development
- b) Screening of Chromatographic conditions and Phases,typically using the linear solvent- strength model of gradient elution
- c) Optimization of method to fine-tune parameters related to ruggedness and robustness

5.4 Validation Methods

1. Limit of Detection
2. Limit of Quantification
3. Linearity
4. Range
5. Robustness
6. Ruggedness [38]

CONCLUSION

The efficient development and validation of chromatographic (analytical& bioanalytical) methods are critical elements in the development of pharmaceuticals. Success in these areas can be attributed to several important factors, which, in turn, will throw in to regulatory compliance. Recent development in pharmaceutical and biotechnological field generates a cumulative demand for analytical methods. Rapid and accurate quantification of the substrate and drug product is important in the process development. Improvements in analytical instrumentation leads to development of new techniques like isocratic and gradient RP-HPLC,LC-MS, which evolved as the primary techniques for the analysis of nonvolatile drugs and impurities. These analytical methods are critical elements of pharmaceutical development so it is very important to develop efficient and accurately validated analytical methods to develop safe and effective drugs.

REFERENCES

1. Syed G Musharraf., Urooj Fatima., Rahat sultanan. Stress degradation studies anddevelopment of stability indicating –TLC densitometry method for determination of Predensolone and chloramphenicol in their individual and combined pharmaceutical formulations reversed phase. Chem.Central.J 2012; 6(7): 134 – 137.
2. Nahla Nour El-Din Ahmed Salama., Afaf Osman Mohamed. Stability-Indicating Methodsfor the Determination of Sertaconazole Nitrate by Micelle-Enhanced Spectrofluorimetryand TLC-Densitometry. Bull. Chem. Soc. Ethiop. 2009; 23(2):167- 174.
3. Swarbrick James., and Boylan James., Encyclopedia of pharmaceutical technology, Volume I, Marcel Dekker Inc., New York, 1998, 217 - 224.
4. Beckett,A.H., Stenlake J.B., Practical Pharmaceutical Chemistry, 4th edition, Part 2, CBS Publishers and distributors, 1997, 275-337.
5. Glenn A.L., Journal of pharmacy and pharmacology,1960, 12,598-608.
6. Jain H.K., Agrawal R.K., Indian Journal of pharmaceutical sciences, 2002, 64(1), 88-71.
7. Shankar M.B., Mehta F.A., Bhatt K.K., Mehta R.S., and Geetha M., Indian Journal of pharmaceutical sciences, 65(2), 167-170.
8. Skoog DA, West DM, Holler FJ Fundamentals of analytical chemistry. 8thEdn, Fort Worth: Saunders College Pub. 1996.
9. Yue PF, Yuan HL, Yang M, Zhu WF Preparation, Char-acterization and Pharmacokinetics in Vivo of Oxymatrine-Phospholipid Complex. J Bioequiv Availab 2009, 1: 099-102.
10. Maithani M, Singh R Development and Validation of a Stability-Indicating HPLC Method for the Simultaneous Determination of Salbutamol Sulphate and Theophylline in Pharmaceutical Dosage Forms. J Anal Bioanal Techniques 2011, 1:116.
11. Chitlange SS, Chaturvedi KK, Wankhede SB Development and Validation of Spectrophotometric and HPLC Method for the Simultaneous Estimation of Salbutamol Sulphate and Prednisolone in Tablet Dosage Form. J Anal Bioanal Techniques 2011, 2:117.
12. Saber AL, Amin AS Utility of Ion-Pair and Charge Transfer Complexation for Spectrophotometric Determination of Domperidone and Doxycycline in Bulk and Pharmaceutical Formulations. J Anal Bioanal Techniques 2011, 1:113.
13. Bai L, Ma Z, Yang G, Yang J, Cheng J A Simple HPLC Method for the Separation of Colistimethate Sodium and Colistin Sulphate. J Chromatograph Separat Techniq 2011, 1:105.
14. Babu ARS, Thippeswamy B, Vinod AB Determination of Tacrolimus in Rat Whole Blood Utilizing Triple Quadrupole LC/MS. J Anal Bioanal Techniques 2011 2:118.
15. Junior EA, Duarte LF, Pereira R, Pozzebon JM, Tosetti D, et al. Gabapentin Bioequivalence Study: Quantification by Liquid Chromatography Coupled to Mass Spectrometry. J Bioequiv Availab (2011) 3: 187-190.

16. Hsieh CL, Wang HE, Ker YB, Peng CC, Chen KC, et al. GC/MS Determination of N-butyl-N-(3-carboxypropyl) Nitrosamine (BCPN) in Bladder Cancers - The Skewed Molecular Interaction Caused Retention Time Shift. J Anal Bioanal Techniques 2011 1:115.
17. Ekeberg D, Norli HR, Stene C, Devle H, Bergaust L Identification of Brominated Flame Retardants in Sediment and Soil by Cyclohexane Extraction and Gas Chromatography Mass Spectrometry. J Chromatograph Separat Techniq 2010 1:102.
18. Ravali R, Phaneendra M, Bhanu Jyothi K, Ramya Santhoshi L, Sushma K Recent Trends in Analytical Techniques for the Development of Pharmaceutical Drugs. J Bioanal Biomed 2011 R1: 002.
19. M.S. Tswett, TR Protok, Otd. Biol., 1905, 14, 20.
20. Verzele M, Dewaele C Preparative High Performance Liquid Chromatography, A Practical Guidline. TEC Dent, Belgium. 1985.
21. Dhaneshwar SR, Salunkhe JV, Bhusari VK Validated HPTLC Method for Simultaneous Estimation of Metformin Hydrochloride, Atorvastatin and Glimepiride in Bulk Drug and Formulation. J Anal Bioanal Techniques 2010,1:109.
22. Abdelkawy M, Metwaly F, El Raghy N, Hegazy M, Fayek N Simultaneous determination of Ambroxol Hydrochloride and Guaifenesin by HPLC, TLCSpectro densitometric and multivariate calibration methods in pure form and in Cough Cold Formulations. J Chromatograph Separat Techniq 2011, 2:112.
23. Bari SB, Bakhshi AR, Jain PS, Surana SJ Development and Validation of Stability-Indicating Tlc Densitometric Determination of Ropinirole Hydrochloride in Bulk and Pharmaceutical Dosage Form. Pharm Anal Acta 2011 2:125.
24. Jain PS, Khatal RN, Jivani HN, Surana SJ Development and Validation of TLC-densitometry Method for Simultaneous Estimation of Brimonidine tartrate and Timolol maleate in Bulk and Pharmaceutical Dosage Form. J Chromatograph Separat Techniq 2011, 2:113.
25. Subbaiah PR, Kumudhavalli MV, Saravanan C, Kumar M, Chandira RM Method Development and Validation for estimation of Moxifloxacin HCl in tablet dosage form by RP-HPLC method. Pharm Anal Acta 2010, 1:109.
26. Sultana N, Arayne MS, Naveed S RP-HPLC Method for Simultaneous Determination of Captopril and Diuretics: Application in Pharmaceutical Dosage Forms and Human Serum. J Chromatograph Separat Techniq 2011,2:109.
27. Liu L, Cheng Y, Zhang H Phytochemical analysis of anti-atherogenic constituents of Xue-Fu-Zhu-Yu-Tang using HPLC-DAD-ESI-MS. Chem Pharm Bull2004, 52: 1295-1301.
28. Nakashima Kenichiro, High-Performance Liquid Chromatography of drug of abuse in biological samples, Journal of Health Science,2005,51(3) 272-277.
29. Boulanger B., Chiap P. Dewe W., Crommen J., Hubert Ph., An analysis of the SFSTP guide on validation of chromatographic bioanalytical methods: progresses and limitations Journal of Pharmaceutical and Biomedical Analysis, 2003,32, 753-765.
30. Bressolle F, Bromet P, Audran M. Validation of liquid chromatography and gas chromatographic methods application to pharmacokinetics. J. Chromatogr. B.1996, 686, 3-10.
31. Rao R, kalakuntla K, Kumar S. BioanalyticalMethod Validation: A Quality Assurance Auditor View. Journal of Pharmaceutical Sciences and Research.2009, 3, 2-3.
32. Eric Reid, Ian D. Wilson. Methodological Survey in Biochemistry and Analysis: Analysis for Drug and Metabolites, Including Anti-infective Agents1990, 20, 1-57.
33. Food and Drug Administration Guidance for Industry Bioanalytical Method Validation, 2001.
34. Tiwari G. Tiwari R. Bioanalytical MethodValidation: An updated review: Pharm Methods (2010) 1: 25-38.
35. A. Kirthi1,R. Shanmugam,M. Shanti Prathyusha,D. Jamal Basha a review on bioanalytical method development and validation by RP – HPLC Journal of Global Trends in Pharmaceutical Sciences 5(4) - (2014) 2265 – 2271.
36. Patel Rashmin, Patel Mrunali, Dubey Nitin, Dubey Nidhi Patel Bharat HPTLC Method Development and Validation: Strategy to Minimize Methodological Failures Journal of Food and Drug Analysis, Vol. 20, No. 4, 2012, Pages 794-804.
37. N. M. Gaikwad, S. H. Gatkal, K. Pratyusha, P. M. Patil, P.D. Chaudhari Stability Indicating By Lc-Ms Method International Journal of Pharmaceutical Sciences Review and Research Volume 12, Issue 2, 2012; 141-147.
38. Monica Whitmire, Jennifer Ammerman, Patricia de Lisio, Jacqueline Killmer, Devon Kyle, Emily Mainstone, Lynann Porter and Tianyi Zhang LC-MS/MS Bioanalysis Method Development, Validation, and Sample Analysis: Points to Consider When Conducting Nonclinical and Clinical Studies in Accordance with Current Regulatory Guidances J Anal Bioanal Techniques 2011, 1-10.