

A Review on Novel Analytical Techniques Used in Method Development and Validation of Pharmaceuticals

Vilas Shivaji Sawale*¹, Dr.D.Umamaheshwari²

Late Bhagirathi Yashwantrao Pathrikar College of Pharmacy, Phulambri, Aurangabad .¹

Vinayaka Mission College Of Pharmacy, Salem, TN,²

Abstract:

The main objective of this review is to elaborate the novel analytical techniques utilized in method development and validation of varied pharmaceuticals because it is extremely much significant for the steadiness, efficacy and quality of the drug product. Various novel analytical techniques like LC-MS, RP-HPLC Automated development in HPTLC, LC-MS-MS are discussed during this review with suitable samples of drugs concerning ICH Guidelines. Various Validation parameters like Accuracy, Specificity, Precision, Linearity, LOD, LOQ, Ruggedness, and Robustness also are listed concerning ICH Guidelines. Validation is extremely much useful for the standard Control and Quality Assurance of Pharmaceuticals and therefore the safety of patients.

Keywords: Analytical Techniques, ICH Guidelines, Quality, Validation.

INTRODUCTION

The analysis is vital in any product or service, and it is also important in drug because it involves life [1]. The number of drugs introduced in 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. Thus it becomes necessary, to develop newer analytical methods for such drugs. [2]

The amount of medicine introduced within the market is increasing per annum. These drugs could also be either new entities or partial structural modification of the prevailing one. Fairly often there's a delay from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens due to the possible uncertainties within the continuous and wider usage of those drugs, reports of latest toxicities (resulting in their withdrawal from the market), development of patient resistance and introduction of higher drugs by competitors. Under these conditions, standards and analytical procedures for these drugs might not be available within the pharmacopoeias. Thus it becomes necessary, to develop newer analytical methods for such drugs. [2]

Analytical method development

When there are not any definitive techniques are present, new methodologies are being progressed for evaluation of the novel product. to research the presence of either pharmacopoeial or non-pharmacopoeial product novel techniques are developed to scale back the worth besides time for higher precision and strength. These methodologies are optimized and valid through

preliminary runs. Alternate ways are planned and place into practice to exchange this procedure within the comparative laboratory information with all accessible merits and demerits. [7]

The necessity of method development

Drug evaluation exhibits the identity characterization and determination of the drugs together like dosage forms and organic fluids. At some point of manufacturing technique and development of drug, the principal purpose of analytical strategies is to get data regarding efficiency (which could be directly connected with the necessity of a identified dose), impurity (related to safety of the medication), bioavailability (consists of key drug traits like crystal kind, uniformity of drug and release of drug), stability (that shows the degradation product), and effect of producing parameters to verify that the assembly of drug product is steady.

Analyst before the event of latest technologies, don't forget below mention criteria:

- is that this technique possesses the needful sensitivity?
- is that this method sufficiently selective for direct use without interference employing the other element within the sample?
- Are the accuracy and precision doable with this technique?
- Are the reagents and equipment required on this method available or obtained at an inexpensive price?
- is that the time requires to perform this system applicable [5]

System Suitability

System suitability testing originally believed by the industry of pharmaceuticals to make a decision whether a the chromatographic system is being routinely utilized day today in pharmaceutical laboratories where quality of results is most vital which is suitable for a particular analysis.

The parameters utilized in the system suitability tests (SST) report are as follows:

- Several theoretical plates or Efficiency (N).
- Capacity factor (K).
- Separation or Relative retention (α).
- Resolution (Rs).
- Tailing factor (T).
- Relative variance (RSD).

Steps for developing a way

Various steps are involved within the development of an analytical method are as follows:

➤ **Characterization of analyte and standard :**

- ✓ All the known necessary data concerning the analyte and its structure that's to say the physical and chemical properties like solubility, optical isomerism, etc., are collected.
- ✓ the quality analyte is adequate to 100% purity is acquired. the required arrangement is to be created for the right storage (refrigerator, desiccators, and freezer).
- ✓ within the sample matrix, when multiple parts are to be measured the amount of elements is observed duly presenting the knowledge and therefore the accessibility of ordinary are calculated.
- ✓ Techniques like spectroscopy (UV-Visible, FTIR, atomic absorption spectroscopy, etc.), high-performance liquid chromatography and gas chromatography so on and, are however about once coordinated with the steadiness of samples [5].
- ✓ Requirement of the technique:
- ✓ Requirement of the analytical methodology is important to create up the analytical fig. of advantage like linearity, selectivity, specificity, range, accuracy, precision, LOD, LOQ etc. shall be outlined [5].

➤ **Literature survey and prior methods:**

All the info of literature associated with the drug are reviewed for its physical and chemical properties, manufacturing, solubility and applicable analytical ways concerning relevant books, journals, us pharmacopoeia/national formulary(USP/NF), association of official agricultural chemists (AOAC) and American society for testing and materials (ASTM) publications and it's extremely convenient to seem Chemical Abstracts Service automatic computerized literature [5].

➤ **Selecting the tactic**

- ✓ Utilizing the info obtained from the literature, the tactic ology is evolving since the method is being modified wherever needed. Sometimes, it's important to accumulate additional instrumentation to make , alter or replicate and validate existing procedures for analytes and tests.
- If there aren't any past appropriate ways available to research the analyte to be examined [5].

Proper instrumentation and initial studies:

- Installation qualification (IQ), operation qualification (OQ), and performance qualification (PQ) of instrument pertinent to research standard

methodology are examined by an appropriate found out of instruments [5].

Optimization:

- While performing optimization, once a parameter is modified at a time, and a gaggle of conditions are differentiated, before utilizing trial and error approach. This work is required for accomplished supported a scientific organized method plan duly all necessary points and documented with reference to dead ends [5].
- **Proper documentation of analytical fig. of merits:** truth determined analytical fig. of benefit consisting of LOD, LOQ, cost, linearity and evaluation time and planning of samples, etc. also are recorded [5].
- **Evaluation of produced technique with actual specimen:** The specimen solution must prompt specific, complete recognition of the height interest of the medication aside from all different matrix parts [5].
- **Estimation of per cent recovery of real samples and demonstration of quantitative sample analysis:** Percentage recovery of spiked, actual standard medication into a sample grid which incorporates no analyte is evaluated. Optimization to the reproducibility of recuperation from test to check must have appeared. it's not always essential to urge 100% restoration thus far because the outcomes are reproducible to perceive with a high level of assurance [5].

The analysis is significant in any product or service, and it's also important in drug because it involves life [1] the amount of medicine introduced within the market is increasing per annum . These drugs could also be either new entities or partial structural modification of the prevailing one. fairly often there's a delay from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens due to the possible uncertainties within the continuous and wider usage of those drugs, reports of latest toxicities (resulting in their withdrawal from the market), development of patient resistance and introduction of higher drugs by competitors. Under these conditions, standards and analytical procedures for these drugs might not be available within the pharmacopoeias. Thus it becomes necessary, to develop newer analytical methods for such drugs. [2]

Analytical method development:

When there are not any definitive techniques are present, new methodologies are being progressed for evaluation of the novel product. to research the presence of either pharmacopoeial or non-pharmacopoeial product novel techniques are developed to scale back the worth besides time for higher precision and strength. These methodologies are optimized and valid through preliminary runs. Alternate ways are planned and place into practice to exchange this procedure within the

comparative laboratory information with all accessible merits and demerits. [7]

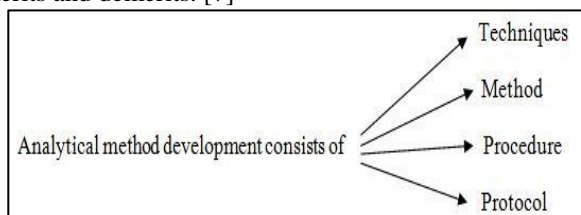


Fig 01: [2]

The necessity of method development:

Drug evaluation exhibits the identity characterization and determination of the drugs together like dosage forms and organic fluids. At some point of manufacturing technique and development of drug, the principal purpose of analytical strategies is to get data regarding efficiency (which could be directly connected with the necessity of a identified dose), impurity (related to safety of the medication), bioavailability (consists of key drug traits like crystal kind, uniformity of drug and release of drug), stability(that shows the degradation product), and effect of producing parameters to verify that the assembly of drug product is steady.

Analyst before the event of latest technologies, don't forget below mention criteria:

- is that this technique possesses the needful sensitivity?
- is that this method sufficiently selective for direct use without interference employing the other element within the sample?
- Are the accuracy and precision doable with this technique?
- Are the reagents and equipment required on this method available or obtained at an inexpensive price?

• is that the time requires to perform this system applicable [5]

System Suitability

System suitability testing originally believed by the industry of pharmaceuticals to make a decision whether a the chromatographic system is being routinely utilized day today in pharmaceutical laboratories where quality of results is most vital which is suitable for a particular analysis.

The parameters utilized in the system suitability tests (SST) report are as follows:

- Several theoretical plates or Efficiency (N).
- Capacity factor (K).
- Separation or Relative retention (α).
- Resolution (Rs).
- Tailing factor (T).
- Relative variance (RSD).

The method validation/evaluation implies the process of documenting or providing that: analytical method provides analytical data for the intended use.

Validation analytical method requires the following

1. Assuring quality
2. Achieving acceptance of products by international agencies.
3. Mandatory requirement purposes for accreditation as per ISO 17025 guidelines.
4. Mandatory requirement for registration of any pharmaceutical product or pesticide formulation.
5. Validation methods are only acceptable for undertaking proficiency testing.
6. Validated/Evaluated method undergoes quality control procedures for further evaluation. [2]

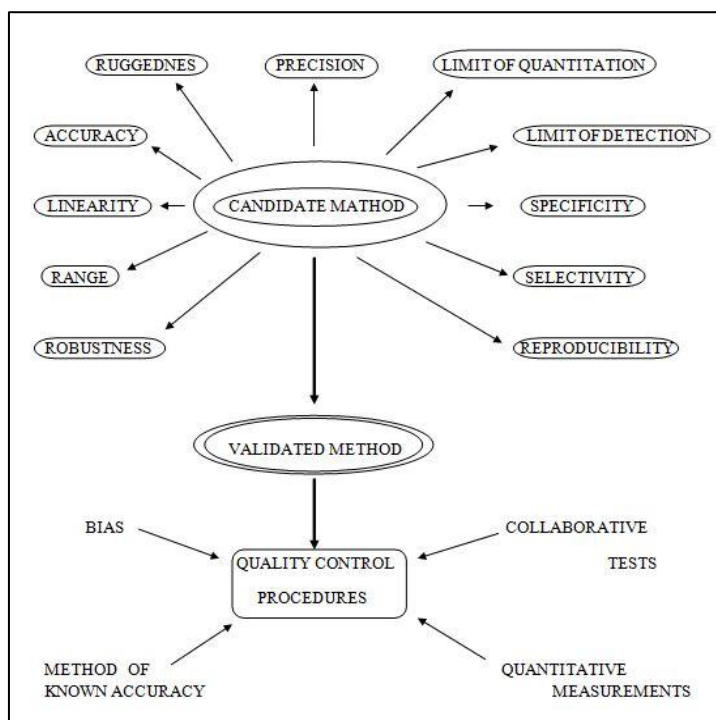


Fig 2: Flow chart showing different steps in analytical method development [2]

Validation

Validation is an idea that has developed in the U. S. in 1978. The idea of validation has extended during that point to understand an in depth sort of activities from analytical approaches utilized for the standard control of medication to computerized systems for clinical trials, marking or process control, validation is established on, however not endorsed by regulatory specifications and is best seen as a critical and necessary part of current good manufacturing practice (cGMP).

The phrase validation implies for evaluation of validity or activity of demonstrating viability. Validation may be a workforce effort where it entails humans from various departments of the plant. Validation is required for any new or amended technique to verify that it's capable of giving consistent and reliable results, once utilized by different operators using similar instrumentation within the same or completely different laboratories [7]. Validation is an important component of quality assurance; it includes the efficient investigation of systems, facilities, and procedures aimed toward deciding if they execute their planned capacities sufficiently and reliably as determined.

➤ Analytical methods have been validated in pursuance of ICH guidelines of Q2 (R1) Validation parameters are:

1. System suitability
1. Specificity
2. Linearity
3. Precision
4. Accuracy
5. LOD
7. LOQ
8. Robustness

➤ Validation should during this way be considered within the accompanying circumstances:

- Completely new procedure.
- Latest equipment.
- Procedure and equipment which are adjusted to suit altered needs and,
- Procedure where the finished result test is a poor and undependable marker of product quality [6].

➤ Importance of validation

- Assured high quality.
- Time foundation.
- Optimization of the method.
- Minimum batch product failure, enhanced efficiency, manufacturing, and productivity.
- Quality cost decreased.
- Rejection decreased.
- Yield increases.
- Fewer complaints about process-related issues.
- Fast and realistic start-up of new equipment's.

→ Increased worker consciousness of the process [8].

HPLC

High-Performance Liquid Chromatography (HPLC) is one mode Of chromatography, one among the foremost used analytical techniques. The chromatographic process can be defined as a separation technique Involving mass-transfer between stationary and mobile phase. HPLC utilises a liquid mobile phase to separate the components of a mix . The stationary phase are often a liquid or a solid phase. These Components are first dissolved during a solvent then forced to flow through a chromatographic column under high . In the Column, the mixture separates into its components. The amount Of resolution is vital and depends upon the extent of Interaction between the solute components and the stationary phase. The stationary phase is defined because the immobile packing within the column. The interaction of the solute with mobile and stationary Phases are often manipulated through different choices of both solvents And stationary phases has developed an lc method For the determination of amiodarone hydrochloride in tablet And injectable formulations [10]. An HPLC method was also developed And validated for the determination of amiodarone hydrochloride and its related compounds in amiodarone hydrochloride injections By Christopherson et al. [11]. As a result, HPLC acquires a high degree of versatility not found in other chromatographic systems And it can easily separate a wide variety of Chemical Mixtures. High-performance liquid chromatography may be a Highly improved sort of chromatography . A simple, Precise, Accurate, and stability-indicating liquid chromatographic method Was validated for the determination of amiodarone hydrochloride Impurities (amiodarone impurity D and impurity E) as well as for the Determination of amiodarone hydrochloride in tablet formulations. Liquid chromatography with a UV detector at a wavelength of 240 Nm using a C18 column was employed in this study. Isocratic elution Was employed employing a mixture of solution ph 5.0, methanol, And acetonitrile (30:30:40, v/v/v). This method was validated for the Determination of amiodarone hydrochloride following USP requirements for assay determination, which include accuracy, precision, selectivity, linearity and range [12]. High-performance liquid Chromatographic (HPLC) separation on a BDS Hypersil C18 column Using disodium hydrogen phosphate buffer (0.02 M): Methanol (25: 75, v/v) ph adjusted to 7 with ortho-phosphoric acid because the mobile phase. The proposed methods were validated as per ICH guidelines And successfully applied for the determination of investigated drugs in tablets [13]. High-performance liquid chromatography [14] and LC/ESI-MS/MS [15] methods have been reported for the estimation of Gatifloxacin in dosage forms and from human plasma. This study aimed to develop and validate for determination of piroxicam in Human plasma by new rapid HPLC method and to compare the relative Bioavailability of two branded formulations of piroxicam in healthy

Korean volunteers [16]. Bioanalytical, HPLC and stability-indicating HPLC methods are reported for its determination and in combination with other drugs [17-20].

Automated development in HPTLC:

High performance thin layer chromatography (HPTLC) is an enhanced form of a thin layer Chromatography (TLC). Several enhancements can be made to the essential method of thin-layer chromatography to automate the different steps, to extend the resolution achieved and to permit more accurate quantitative measurements. Automation is useful to overcome the uncertainty in droplet size and position when the sample is applied to the TLC plate by hand. Nowadays, HPTLC has become a routine analytical technique thanks to its advantages of reliability in quantitation of analytes at the micro and even in nanogram levels and price effectiveness [21]. Recently an HPLC and HPTLC method has been reported for simultaneous estimation of levocetirizine dihydrochloride and Montelukast sodium in pharmaceutical dosage forms which are either tedious or expensive methods [21,22]. This chromatographic process relies on the property of biologically active substances to make stable, specific, and reversible complexes.

Development of RP-HPLC:

An easy and rapid method for the determination of ATP, ADP, AMP, NADP⁺, NAD⁺, NADPH, and NADH in human erythrocytes. The analysis is performed by reverse phase high-performance liquid chromatography on a 5- μ m Supelcosil LC-18 column and UV detection. Reversed-phase HPLC (RP-HPLC or RPC) features a non-polar stationary phase and an aqueous, moderately polar mobile phase. A simple, fast and precise reversed-phase high performance liquid chromatographic method has been developed for the simultaneous determination of Camylofin dihydrochloride and Diclofenac Potassium using Methylparaben as an indoor standard. Efficient chromatographic separation was achieved on Inertsil C18 column (250mm x 4.6 mm, 5 μ m) as a stationary phase with a mobile phase comprising of 0.05 M KH₂PO₄ in water: Methanol (35:65,v/v) at a flow of 1.5mL min⁻¹, column temperature of 27°C and UV detection at 220 nm. The proposed method was validated for linearity, accuracy, precision, sensitivity, robustness and solution stability. Linearity, accuracy and precision were found to be acceptable over the ranges of 250 -750 μ g mL⁻¹ for both camylofin dihydrochloride and Cataflam . The test solution was found to be stable for 48 hours. It are often conveniently adopted for routine internal control analysis [23]. The literature revealed no method was available for simultaneous determination of this drug in such pharmaceutical preparation by HPLC [24-28]. a replacement simple, rapid and precise reverse -phase high-pressure liquid chromatography (RP-HPLC) method was developed for the simultaneous estimation of amoxicillin trihydrate and bromhexine hydrochloride from oily suspension. An ODS C18 (250 X 4.5mm ID), 5 μ particle size with mobile phase methanol and glacial acetic acid

(50:50 v/v) were used [29]. An improved derivatives RP-HPLC method with PDA detection has been developed and validated for the simultaneous estimation of tranexamic acid and mefenamic acid in combined tablet dosage form [30]. A rapid, sensitive and specific RP-HPLC method involving UV detection was developed and validated for determination and quantification of Moxifloxacin HCl in a tablet dosage form. The tactic does require only 10 min as a run time for analysis which proves the adaptability of the tactic for the routine quality control of the drug [31,32].

LC-MS Method

LC/MS methods apply to a good range of compounds of pharmaceutical interest, sensitivity, selectivity, speed of study, and cost-effectiveness. These analytical features have continually improved, resulting in easier to use and more reliable instruments. These improvements were timely and coincided with the aforementioned developments within the pharmaceutical industry. Analytic technique by which chemical substances are identified by sorting gaseous ions by Mass using electric and magnetic fields. A spectrometer uses electrical means to detect the sorted ions, while a mass spectrometer uses photographic or other non-electrical means either device is mass spectroscopy. The method is widely wont to measure masses and relative abundances of various isotopes, to research products of separation by liquid or gas chromatography, to check vacuum integrity in high-vacuum equipment, and to live the geological age of minerals.

Applications of this method in clinical samples

- ✓ LC-MS/MS method used for Simultaneous Quantification of Seven Anti-HIV Medicines in Plasma of HIV-infected Patients [32].
- ✓ A liquid chromatography-tandem mass spectrometry (LC-MSMS) method for the screening and confirmation of mescaline inhuman urine samples and to use this method to routine testing inpatient samples.
- ✓ A sensitive, rapid assay method has been developed and validated for the simultaneous estimation of tolmetin (TMT) and MED5 in human plasma with liquid chromatography coupled to tandem mass spectrometry with electrospray ionization within the positive-ion mode. an easy solid-phase extraction process was used to extract TMT and MED5 alongside mycophenolic acid (internal standard, IS) from human plasma.
- ✓ LC-MS/MS method for determination of the Paclitaxel in human plasma was developed and validated [33].
- ✓ Quantitative Estimation of Amlodipine Desolate, Olmesartan Medoxomil and Hydrochlorothiazide in Tablet Dosage Form [34].
- ✓ Epigenetic regulators have quickly become one among the foremost widely studied therapeutic agents for a huge array of diseases, making histone deacetylase inhibitors (HDIs) and DNA methyltransferase(DNMT) inhibitors commonly used

molecules in pre-clinical and clinical anti-cancer studies [35].

- ✓ A sensitive HPLC method was established and evaluated for determining the concentrations of phenol in mice plasma. 180 KM male mice of 22~28g weight were divided into three groups randomly and purified phenol, Moutan Cortex decoction and Rhubarbmoutan decoction were orally administered at equivalent doses of 10 mg•kg⁻¹ phenol [36].
- ✓ to research the bioequivalence of the ultimate tablet formulation of eslicarbazepine acetate (ESL) and therefore the tablet formulation used in pivotal clinical studies [37].
- ✓ The bioavailability of nimesulide was 89.42% after i.m administration. These pharmacokinetic data suggest that nimesulide given intramuscularly could also be useful within the treatment of disease conditions in bovines [38]
- ✓ LC-MS/MS assay was developed for the determination of bupropion and its metabolite hydroxybupropion in human plasma using lidocaine. Supported the 90% confidence interval of the individual ratios it had been concluded that the test formulation is bioequivalent to the reference formulation with respect to the speed and extent of absorption of both bupropion and hydroxybupropion which food intake before the drug the administration didn't affect the relative pharmacokinetic parameters. However, the hyperlipidemic meal significantly increased bupropion absorption [39].
- ✓ The foremost important feature in transbuccal drug delivery is that the low drug passage through the buccal mucosa [40].
- ✓ The concept of sustained delivery of rapamycin using ReGel as a promising strategy to inhibit SMC proliferation for the prevention of hemodialysis arteriovenous graft stenosis [41]. Simultaneous determination of multiple components by the flow injection technique (MC FIA) of varied configurations with a single detector (single or multichannel), or with several detectors in series or parallel.
- ✓ An easy, rapid and precise reversed-phase liquid chromatographic method is developed for simultaneous determination of Atorvastatin, Ezetimibe and Fenofibrate in their a ternary mixture of economic pharmaceutical preparations. This HPLC method is successfully applied to the simultaneous quantitative analysis of the drugs in tablets [42].
- ✓ Within the present study, comprehensive stress testing of tenatoprazole was administered consistent with ICH guide-line Q1A (R2). Tenatoprazole was subjected to worry conditions of hydrolysis, oxidation, photolysis and neutral decomposition. Extensive degradation was found to occur in acidic, neutral and oxidative conditions. Mild degradation was observed in basic conditions. The drug is comparatively stable within the solid-state.

- ✓ Tablet formulation of eslicarbazepine acetate (ESL) and therefore the tablet formulation utilized in pivotal clinical studies.

Application of UV-Spectroscopy and First Order Derivative

- ✓ Method for Determination of Tamsulosin Hydrochloride in Bulk and Tablets [43].
- ✓ DNA-based tetravalent dengue vaccine can induce balanced neutralizing antibody responses against all four sorts of dengue virus (DENV1–4), using naive mice. The dengue tetravalent DNA vaccine could provide a balanced induction of dengue antibody responses even in mice preimmunized with any of the three flaviviruses, a minimum of after the second vaccination [44].
- ✓ V-5 Immunitor (V5) has been evaluated in patients with chronic hepatitis C with concomitant HIV and Mycobacterium Tuberculosis infections [45].
- ✓ The aim of this study was to develop a single-laboratory validated (SLV) method using high-performance liquid chromatography with different detectors. the tactic does not require any sample cleanup/preconcentration steps except centrifugation and filtration [46].
- ✓ Some liquid chromatographic (LC) methods for determination of milnacipran combined with other antidepressants in human plasma have already been published [47-51].

Automated injection technique

Automation may be a critical demand in modern pharmaceutical analysis and internal control, since strict legislation regarding Good Laboratory (GLP) and Manufacturing Practice (GMP) require extensive analyses of huge amounts of samples during all stages of the manufacturing process of a pharmaceutical formulation. An automatic flow injection

Determination of some phenothiazine derivatives, supported their oxidation with iron (III) during a strongly acidic medium. A flow injection the spectrophotometric procedure is proposed for determining adrenaline in pharmaceutical formulations. A simple, rapid and precise reversed-phase the liquid chromatographic method is developed for simultaneous

Determination of Atorvastatin, Ezetimibe and Fenofibrate in their ternary mixture of economic pharmaceutical preparations. Extensive analyses of giant amounts of samples during all stages of the Manufacturing process of a pharmaceutical formulation [54].

Automation may be a critical demand in modern pharmaceutical analysis and internal control, since strict legislation regarding Good Laboratory (GLP) and Manufacturing Practice (GMP) require extensive analyses of huge amounts of samples during all stages of the manufacturing process of a pharmaceutical formulation.

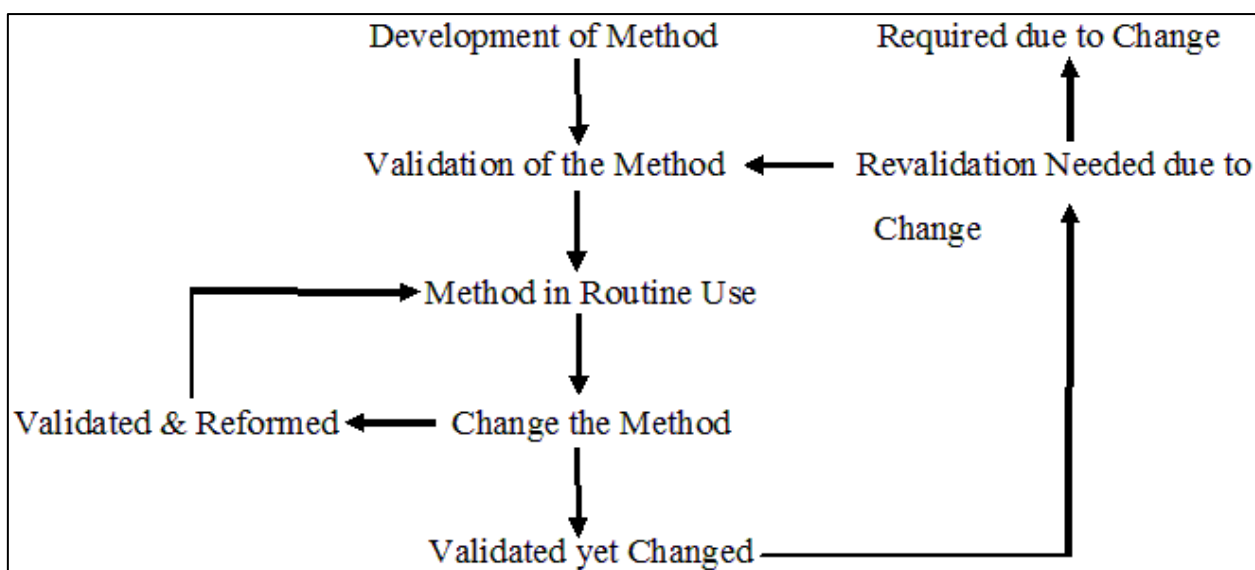


Fig. 03: Life cycle of the analytical method [7]

Future trends in automated injections:

The auto-injectors are having a rapid climb within the fields of Pharmacy. This text discusses the benefits of driving this growth, the present state of the technology. An auto-injector could also be described as a tool which completely or partially replaces the activities involved in parenteral drug delivery from a typical syringe. There'll likely be continued growth in the next few years within the sort of auto-injectors, with competition to meet requirements spurring innovation in new therapy areas. The deserved success is underpinned by the principles of quality, safety and efficacy and can certainly still be a crucial part of patients' lives, particularly those that enjoy the advantages of taking a lively role in their treatment.

CONCLUSION:

The main purpose of development of analytical methods are for identification, purification and eventually to quantification any required drug etc., the most activities involved within the analytical development of a way are separation and characterization of impurities also as degraded products, analytical investigations, studies for identification and eventually fixing of parameters optimization to specific requirements. Therefore the salient points enumerated within the above review are immense use to an analyst while estimating the pharmaceutical formulations also as bulk drugs. The results demonstrated that this analytical Technique are accurate, precise, specific, linear, reliable, sensitive, and fast.

REFERENCES:

- [1] Hema, Swati Reddy G. A Review On New Analytical Method Development And Validation By Rp-HPLC. *Int Res J Pharm Biosci* 2017;4:41-50.
- [2] Ravisankar Et.Al A Review On Analytical Method Development Indian Journal Of Research In Pharmacy And Biotechnology Issn: 2321-5674(Print) Issn: 2320 – 3471(Online) Ijrbp.
- [3] Chauhan A, Mittu B, Chauhan P. Analytical method development and validation: a concise review. *J Anal Bioanal Tech* 2015;6:1.
- [4] Patel A, Dwivedi N, Kaurav N, Bashani S, Patel S, Sharma HS, et al. Chemical analysis of pharmaceuticals: a review. *J Med Pharm Innov* 2016;3:4-7.
- [5] Ravisankar P, Navya CN, Pravalika D, Sri DN. A review of step-by-step analytical method validation. *IOSR J Pharm* 2015;5:7-19.
- [6] Jatto E, Okhamafe AO. An overview of pharmaceutical validation and process controls in drug development. *Trop J Pharm Res* 2002; 1:115-22.
- [7] Mahar P, Verma A. Pharmaceutical process validation: an overview. *Int J Pharm Res Biosci* 2014;3:243-62.
- [8] Lavanya G, Sunil M, Eswarudu MM, Eswaraiah MC, Harisudha K, Spandana BN, et al. Analytical method validation: an updated review. *Int J Pharm Sci Res* 2013;4:1280
- [9] Lacroix PM, Curran NM, Wing-Wah SY, Gorecki DKJ, Thibault P, et al. (1994) Liquid chromatographic determination of amiodarone hydrochloride and related compounds in raw materials and tablets. *J AOAC Int* 77: 1447-1453
- [10] Thyagarajapuram N, Alexander KS (2003) A simplified method for the estimation of amiodarone hydrochloride by reverse-phase high-performance liquid chromatography. *J Liq Chrom & Rel Technol* 26: 1315-1326.
- [11] Christopherson MJ, Yoder KJ, Miller RB (2004) Validation of a stability-indicating HPLC method for the determination of amiodarone HCl and its related substances in amiodarone HCl injection. *J Liq Chrom & Rel Technol* 27: 95-111.
- [12] Al-Rimawi F (2010) Validation of an HPLC-UV method for the Determination of Amiodarone Impurities in Tablet Formulations. *Pharm Anal Acta* 1:105.
- [13] Rathore AS, Sathiyarayanan L, Mahadik KR (2010) Development of Validated HPLC and HPTLC Methods for Simultaneous Determination of Levocetirizine Dihydrochloride and Montelukast Sodium in Bulk Drug and Pharmaceutical Dosage Form. *Pharm Anal Acta* 1:106
- [14] Liang H, Kays MB, Sowinski KM (2002) Separation of levofloxacin, ciprofloxacin, gatifloxacin, moxifloxacin, trovafloxacin and cinoxacin by HPLC: application to levofloxacin determination in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 772: 53-63.
- [15] . Sistla R, Tata VS, Kashyap YV, Chandrasekar D, Diwan PV (2005) Development and validation of a reversed-phase HPLC method for the determination of ezetimibe in pharmaceutical dosage forms. *J Pharm Biomed Anal* 39: 517-522.
- [16] Sonawane SS, Shirkhedkar AA, Fursule RA, Surana SJ (2006) Application of UV-Spectrophotometry and RP-HPLC for

- Simultaneous Determination of Atorvastatin Calcium and Ezetimibe in Pharmaceutical Dosage Form. *Eurasian Journal of Analytical Chemistry* 1: 31-41.
- [17] Kumar DA, Sujun DP, Vijayasree V, E Rao JVLNS (2009) Simultaneous Determination of Simvastatin and Ezetimibe in Tablets by HPLC. *Journal of Chemistry* 6: 541-54
- [18] Singh S, Singh B, Bahuguna R, Wadhwa L, Saxena R (2006) Stress degradation studies on ezetimibe and development of a validated stability-indicating HPLC assay. *J Pharm Biomed Anal* 41: 1037-1040a.
- [19] Doshi AS, Kachhadia PK, Joshi HS (2008) Validation of a Stability-Indicating LC Method for Assay of Ezetimibe in Tablets and Determination of Content Uniformity. *Chromatographia* 67: 137-142.
- [20] Oswald S, Scheuch E, Cascorbid I, Siegmund W (2006) *J Chromatogr B* 830: 143-150a.
- [21] Shuijun L, Gangyi L, Jingying J, Xiaochuan L, Chen Y (2006) *J Pharm Biomed Anal* 40: 987-992.
- [22] Vishwanathan K, Bartlett MG, Steward JT (2001) Determination of gatifloxacin in human plasma by liquid chromatography/electrospray tandem mass spectrometry. *Rapid Commun Mass Spectrom* 15: 915-919.
- [23] Song HH, Choi KS, Kim CW, Kwon YE (2009) Pharmacokinetic Profiles of Two Branded Formulations of Piroxicam 20mg in Healthy Korean Volunteers by a Rapid Isocratic HPLC Method. *J Bioequiv Availab* 1: 074-081.
- [24] Ni Y, Liu Y, Kokot S (2011) Two-dimensional fingerprinting approach for comparison of complex substances analysed by HPLC-UV and fluorescence detection. *Analyst*. 136:550-559.
- [25] Elbarbry FA, Mabrouk MM, El-Dway MA (2007) Determination of the analgesic components of Spasmomigraine tablet by liquid chromatography with ultraviolet detection. *J AOAC Int* 90: 94-101a.
- [26] Hinz B, Chevts J, Renner B, Wuttke H, Rau T, et al. (2007) Bioavailability of diclofenac potassium at low doses. *Br J Clin Pharmacol* 59: 80-84.
- [27] G Subramanian, P Musmade, S Agarwal, N Udupa (2004) Simultaneous RP HPLC estimation of tinidazole, diclofenac potassium and paracetamol in tablets. *Indian journal of pharmaceutical sciences* 66: 694-696a.
- [28] Barde PS, Desai AY, Roy MNS, Vaidya VV (2008) Simultaneous RP HPLC determination of Camylofin dihydrochloride in Pharmaceutical preparation. *TSI Journal* 7(10)a.
- [29] Singh RR, Rathnam MV, Vegesna R (2008) Simultaneous RP HPLC determination of Camylofin dihydrochloride and Paracetamol in Pharmaceutical preparations. *TSI Journal* 7(11).
- [30] Sethi PD (1992) Identification of Drugs in Pharmaceutical Formulations by Thin Layer Chromatography. CBS Publishers, New Delhi...
- [31] Ashok Kumar S, Senthil Raja M, Perumal P (2009) RP-HPLC Method Development and Validation for Simultaneous Estimation of Montelukast Sodium and levocetirizine Dihydrochloride. *International journal of pharmaceutical research* 1:8-12.
- [32] Smita Sharma MC, Sharma DV, Kohlib AD, Sharma C (2010) Development and validation of TLC-densitometry method for simultaneous quantification of montelukast sodium and levocetirizine dihydrochloride pharmaceutical solid dosage form. *Der Pharmacia Lettre* 2: 489-494.
- [33] Tournel G, Houdret N, Hédouin V, Deveaux M, Gosset D, et al. (2001) High-performance liquid chromatographic method to screen and quantitate seven selective serotonin reuptake inhibitors in human serum. *Journal of Chromatography B* 761:147-158aF
- [34] Duverneuil C, de Grandmaison GL, de Mazancourt P, Alvarez JC (2003) A High- Performance Liquid Chromatography Method with Photodiode-Array UV Detection for Therapeutic Drug Monitoring of the Nontricyclic Antidepressant Drugs. *Therapeutic Drug Monitoring* 25: 565-573a
- [35] . Puozzo C, Filaquier C, Zorza G (2004) Determination of milnacipran, a serotonin and noradrenaline reuptake inhibitor, in human plasma using liquid chromatography with spectrofluorimetric detection. *Journal of Chromatography B* 806: 221-228a. Shinozuka T, Terada M, Tanaka E (2006) Solid-phase extraction and analysis of 20 antidepressant drugs in human plasma by LC/MS with SSI method. *Forensic Science International* 162: 108-112.
- [36] Labat L, Deveaux M, Dallet P, Dubost JP (2002) Separation of new antidepressants and their metabolites by micellar electrokinetic capillary chromatography. *Journal of Chromatography B* 773: 17-23.
- [37] Patti A, Pedotti S, Sanfilippo C (2007) Chiral HPLC analysis of milnacipran and its FMOC-derivative on cellulose-based stationary phases. *Chirality* 20: 63-68.
- [38] Choudhari VP, Nikalje AP (2010) Simultaneous Estimation of Atorvastatin, Ezetimibe and Fenofibrate in Pharmaceutical Formulation by RP-LC-PDA. *Pharm Anal Acta* 1:111.
- [39] Manassra A, Khamis M, el-Dakiky M, Abdel-Qader Z, Al-Rimawi F (2010) Simultaneous HPLC Analysis of Betamethasone and Clotrimazole in Cream Formulations. *Pharm Anal Acta* 1:113.
- [40] Zhang LJ, Yao YM, Sun JJ, Chen J, Jia XF, et al. (2010) An LC-MS/MS Method for Simultaneous Quantification of Seven Anti-HIV Medicines in Plasma of HIV infected patients. *Pharm Anal Acta* 1:102.
- [41] Rajender G, Narayana NGB (2010) Liquid Chromatography-tandem Mass Spectrometry Method for Determination of Paclitaxel in Human Plasma. *Pharm Anal Acta* 1:101.
- [42] Sharma HK, Jain N, Jain SK (2011) Development of Spectrophotometric Method for Quantitative Estimation of Amlodipine Besylate, Olmesartan Medoxomil and Hydrochlorothiazide in Tablet Dosage Form. *Pharm Anal Acta* 2:126.
- [43] de Almeida VR, Brunetto AL, Schwartzmann G, Roesler R, Abujamra AL (2011) De-mystifying the Epigenetic Free for All: Pharmacophore Modeling for Epigenetic Cancer Therapy. *Pharm Anal Acta* 2:102e.
- [44] Jing W, Rui Z, Gui-yan Y, Rui-chen G (2011) Pharmacokinetics of Purified Paeonol and Paeonol in Moutan Cortex Decoction and Rhubarbmoutan Decoction. *Pharm Anal Acta* 2:124.
- [45] Mahapatra L, Sahoo GR, Panda MK, Parija S (2009) Pharmacokinetic Profile of Nimesulide in Bovine Calves. *J Bioequiv Availab* 1: 121-026.
- [46] Moreira RF, Rigato HM, Borges BC, Sverdlhoff CE, Oliveira RA, et al. (2009) Effect of Hyperlipemic Food on the Comparative Bioavailability of Two Bupropion Formulations after Administration of a Single Oral Dose of 150 mg in Healthy Human Volunteers. *J Bioequiv Availab* 1: 103-111.
- [47] De Caro V, Giandalia G, Siragusa MG, Campisi G, Giannola LI (2009) Galantamine Delivery on Buccal Mucosa: Permeation Enhancement and Design of Matrix Tablets. *J Bioequiv Availab* 1: 127-134
- [48] Zhu W, Masaki T, Cheung AK, Kern SE (2009) In-vitro Release of Rapamycin from a Thermosensitive Polymer for the Inhibition of Vascular Smooth Muscle Cell Proliferation. *J Bioequiv Availab* 1: 3-12.
- [49] Lima R, Vasconcelos T, Cerdeira R, Lefebvre M, Sicard E, et al. (2009) Bioequivalence of Final Tablet Formulation and Research Tablet Formulation of Eslicarbazepine Acetate in Healthy Volunteers. *J Bioequiv Availab* 1: 093- 098.
- [50] Bari SB, Bakshi AR, Jain PS, Surana SJ (2011) Application of UV Spectroscopy and First Order Derivative Method for Determination of Tamsulosin Hydrochloride in Bulk and Tablets. *Pharm Anal Acta* 2:120.
- [51] Konishi E, Takizawa Y (2010) Effect of Pre-Existing Immunity to Flaviviruses on Balanced Induction of Neutralizing Antibodies by a Dengue Tetravalent DNA Vaccine in Mice. *J Vaccines Vaccin* 1: 102
- [52] Sharma Et Al. A Review On Analytical Method Development And Validation *Int J App Pharm, Vol* 10, Issue 6, 2018, 8-15
- [53] Ravali R, Phaneendra M, Bhanu Jyothi K, Ramya Santhoshi L, Sushma K (2011) Recent Trends in Analytical Techniques for the Development of Pharmaceutical Drugs. *J Bioanal Biomed* S11. DOI:10.4172/1948-593X.S11-002