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A Recent Review On Analytical Method Development and Validation

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ABSTRACT

In this review articles, the development, formulation, and manufacture of drugs, analytical method development & validation play a critical role. Methods are developed for ensuring purity, identity, potency, and performance of pharmaceutical products. Methods should be applied to the extent that they are sufficient for their intended purpose. Throughout the life cycle of a drug product and substance, a range of activities are associated with developing and validating methods. An objective of method validation is to prove that the procedure can be used as intended. Once the method is developed, validation is performed. Different national and international committees have defined the parameters for method validation. The International Conference on Harmonization attempted to harmonize pharmaceutical applications. In accordance with the ICH, other organizations define Linearity, Selectivity/Specificity, Range, Accuracy, Precision (repeatability, intermediate precision, and reproducibility), Limit of quantitation, Limit of detection, Ruggedness, and Robustness.

Keywords: Validation, HPLC, Stationary Phase, LOD, LOQ, ICH.

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INTRODUCTION

It is a process of creation a test to measure a parameter of a sample. It is done for new products and existing products. Analyzing methodology is determined by many factors, such as chemical properties of analytes, their sample concentrations, analysis speed, analysis cost, number of samples and type of measurements likewise quantitative or qualitative. It is important to develop analytical methods for herbal products, new reactions and processes, residues (micro analysis active ingredients (macro analysis), new molecules, analysis of impurities, and components of interest in different media. As part of method development, it is necessary to begin documenting the development process at the very beginning, and then establish a system for documenting the entire development process. Laboratory notebooks are electronic databases for all data pertaining to these studies.[1]

The steps involved in method development are

a)Analyte standard concentration,

b) Literature search and prior methodology.

c) Choosing a method. d) Instrumentation setup initial studies. e) Optimization.

f) Documentation of analytical figures of merits. g) Actual samples are used to evaluate the method development.

Strategy for Method Development

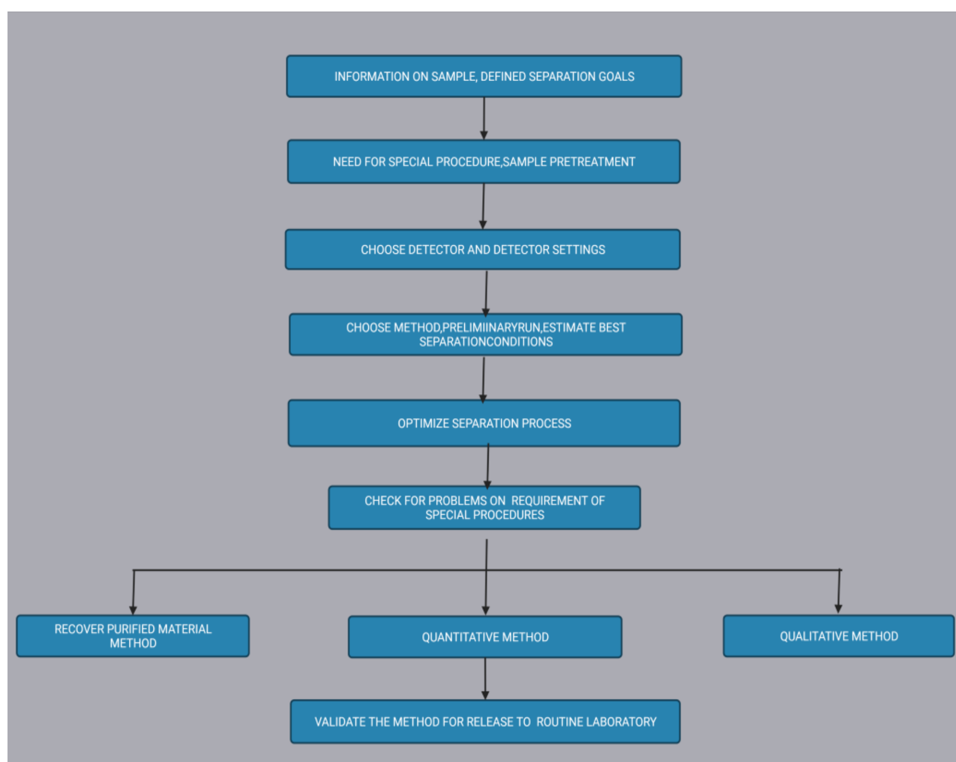

Figure 1: Strategy for method development (created by Biorender.com)

Selection of spectroscopic mode or chromatographic are Electrophoresis, RP-HPLC, Chiral HPLC, HPLC, TLC, GC, Spectroscopy, UV-Vis.[1]

Analytical Method Validation

DEFINITION: The process by which the method performance characterization is determined by laboratory studies response to the need of the analytical application.

The scope for application of validation is manufacturing process control [raw material], pre-formulation evaluation of dosage forms, stability studies, environmental control, cleaning controls. [1]

In accordance with ICHQ2, the following test procedures need to be validated.

a) Quantitative tests to detect impurities
b) Identification tests for analytes
c) Limit tests to identify impurities;
d) Quantitative tests for active ingredients in drug sample or final product or other preferred component(s) in the drug product.

The steps for validations are

- Establish validation of proposed protocols.
- Perform experimental studies.
- Evaluate the analytical results.
- Carry out statistical evaluation.
- Prepare the report documenting all the results.

Validation features include range, linearity, accuracy, specificity, precision (intermediate accuracy, repeatability), quantification limit, detection limit and robustness. [2]

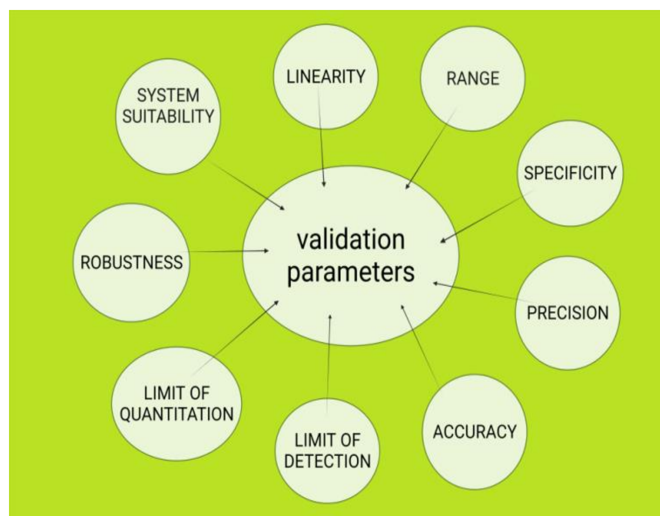

Figure 2: Validation Parameters (created by Biorender.com)

Accuracy

The analytical procedure is to show that the test results are close to the true value. This is calculated in % bias.

$$\%Bias = \frac{\text{observed value} - \text{true value}}{\text{true value}} \times 100$$

It can be estimated by analyzing a sample at a known concentration and comparing the calculated value with the actual value supplied with the material.[2]

Table 1: Accuracy for different Drugs (drug substance and drug products)

Accuracy for drug substances	Accuracy for drug products
To analyze the purity (e.g. reference materials) is the prime application of a analytical procedure.	By applying analytical procedure to the components of a drug product, the amount of the drug can be tested.
The results of the proposed analytical procedure is compared with a second well-characterized procedure, to defined the accuracy.	If it is not possible to obtain all drug product components of a sample, it may be followed by adding known quantities of the analyte to the drug product or comparing the results found from a second procedure, where the accuracy is defined.
Accuracy can be inferred after accuracy; linearity and specificity have been established.	Accuracy can be deduced when accuracy, linearity and specificity have been determined.

Precision

It is the ability of a method to generate repeatability. It is indicated as SD/RSD. The procedure involves Reproducibility, Repeatability and Intermediate precision.

Repeatability: It is the ability of a method to generate alike results for multiple composition of the same sample by the analyzing same instrument in short time duration.

Intermediate precision/ Ruggedness: - The variability of method results are measured where samples are tested and compared with different analyst, days and equipment.

Reproducibility: it is a precision when the prepared samples are compared with different testing sites. [2]

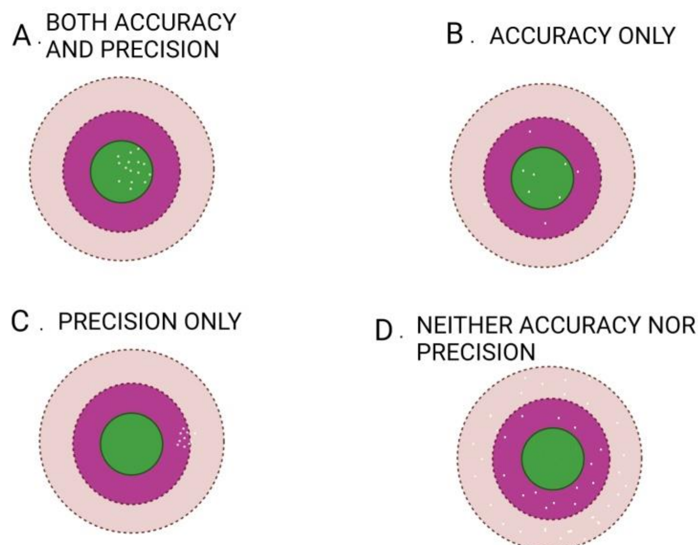

Figure 3: Accuracy and precision (created by Biorender.com)

Limit of Detection

The limit of detection is the point of an analytical procedure, it is the lowest amount of analyte in the sample perhaps it can be detected but can't be quantized under experimental conditions.

LOD =3σa\b

Where, a = standard deviation of response

b = slope of the calibration curve. 2

Specificity/Selectivity

Specificity is the ability to absolutely assess the analyte in the presence of other compounds such as degradants, matrix, impurities, etc.

OR

It is the ability of a method to measure the analyte specifically and accurately in presence of matrix and other components like sample matrix and impurities, degradation products, Lack of specificity of any analytical procedure may be make amends by other analytical procedure(s). [2]

Linearity

The linearity of an analytical procedure is its capability (within a given range) to obtain test results directly proportional to the amount (concentration) of analyte in the given sample. Test results should be computed by appropriate statistical methods, such as, by calculating a regression line by the least squares method.

The Y interception, the slope of the regression line, the correlation coefficient and the residual sum of the squares should be presented. At least five concentrations are recommended. [2]

Limit of Quantitation

The limit of quantification for an analytical procedure is the smallest quantity of analyte in a sample that can be quantitatively obtained with appropriate precision and precision. It is a parameter of quantitative assays for low levels of analytes in sample matrices. It is mainly used for determining degradation products and/or impurities.

Quantitation limit (QL) may be expressed as: $QL=10\sigma /S$

Whereas,

σ= the standard deviation of the response.

S=the slope of the calibration curve.[2]

Robustness

The robustness is a measure of its capability to remain unaffected by small voluntary variations in method parameters and it provides an indication of its reliability during normal usage.

The method validation report provides the results from the validation efforts. Explain the choice of acceptance criteria and how method was developed. The report of method validation is to be submitted to the regulators. [2]

S. Suresh Kumar et al developed a specific, simple, precise and accurate HPLC method for aspirin with its degradation product. The developed method was validated with ICH guidelines. They used /Hypersil BDS C18 (100X4.6mm 5µ) in the column as stationary phase and mobile phase taken were isopropyl alcohol: acetonitrile: sodium perchlorate buffer having (pH 2.5) (1:14:85 % v/v) with UV detection limit 275nm. They found a best resolution of both aspirin and its degraded products.[3]

Abbas khan et al, developed an isocratic UV/RP-HPLC method to determine the cefdinir and cefixime drug simultaneously in human plasma with good experimental parameters and chromatographic condition. First, they did the separation of analytes by Supelco Discovery HS C18 (150 mm × 4.6 mm, 5 µm) column with a guard column named Perkin Elmer C18.The mobile phase is the combination of three solvents i.e., acetonitrile: methanol (50/50, *v*/*v*): trifluoroacetic acid 0.05% (19:81, *v*/*v*), the elusions are monitored in 285 nm. This method has been used for pharmacokinetic study for healthy volunteer.[4]

Purvi Shah et al has developed an HPTLC method to determine clonazepam and paroxetine HCL simultaneously by CCD design with some independent variables like the content of butanol in the mobile phase, distance travel and saturation time. They have used a pre-coated silica gel 60 F254in an aluminum plate as a stationary phase and water: butanol: acetic acid in a ratio of (0.5:9:2 % v/v/v) as a mobile phase. The method parameters were validated with ICH guidelines. Methods has been used to test the quality of marketed tablet dosage forms.[5]

Mahua Sarkar et al, had developed the UV and HPLC method for the simultaneous determination of the anti-retroviral drugs. The analytical performance parameters were validated as per ICHQ2B guidelines. That method was an isocratic method. The UV range was 270,265, and 313 for lamivudine, stabudine and nevirapine respectively. This method was reliable, cheap, sensitive, accurate and shorter duration of time. Its recovery was good. It has been used to routine analysis of anti-retroviral drug dosage forms.[6]

M. Saeedarayne et al have developed RP-HPLC for metformin in API form and all dosage forms. They have used C18 column as a stationary phase and, mobile phase are water: methanol in a ratio of (70:30 v/v). They also used diazepam as an internal standard. The result of this method was good related to statistical and pharmacopoeial content. [7]

Amandine Dispas et al had performed a comparative study from method development, validation and application between UHPSFC and UHPLC. The method was developed in a design space strategy for quality control of pharmaceuticals to optimize robust method. The design space was optimized and gave a guarantee that no need of robust optimization prior to the validation. By using accurate and based on the total error both UHPLC and UHPSFC method were validated. Finally, the UHPSFC method was validated and it was use for analysis of real sample and the results were similar to that of UHPLC method (reference method).[8]

A. Soman et al, have developed an isocratic RP-HPLC method to determine the presence of formaldehyde in drug substance. The analytical column was C8, particle size (3-µm) analytical column (150 mm × 4.6 mm), the detector was diode – array, absorbance was at 360 nm. The results are linear, accurate, robust based and specific range. They also used to derivatize the solvent but there was no absorption of drug substance.[9]

D.H. Shewiyo, et al has developed an HPTLC method for pharmaceutical substance to achieve a good precision and trueness as comparable to other method like HPLC. They have developed and also validated the method for quantitative assay of different pharmaceuticals.[10]

S. Azhagvuel, et al have developed a capillary zone electrophoresis to determine the citrizine dihydrochloride, paracetamol and phenylpropanolamine HCL simultaneously by using uncoated fused silica capillary and the detection was at 195 nm. Here internal standard was ibuprofen for quantitative analysis. [11]

Emirhan Nemutlu et al developed a capillary electrophoresis for analysis of meloxicam by the use of fused silica capillary and borate buffer containing 5% methanol as mobile phase and detection was at 205 nm. The internal standard was diflunisal. The method showed linearity, precision, accuracy& other parameters are to be validated accordingly. [12]

S.H. Gan, et al developed and validated the liquid chromatography method to determine tramadol by HPLC liq – liq extraction and UV detection. Linearity, accuracy, precision, selectivity and recovery were done with the help of internal standard phenacetin. This was claimed for determination of tramadol in human plasma.[13]

T.A. Phazna Devi et al has developed RP-HPLC method to determine the paracetamol by using stationary phase i.e.C18 column and orthophosphoric buffer as a mobile phase. It was detected at 207nm. The statistical validation parameters like accuracy, precision, linearity was checked and from the obtained result it was decided that this method may be adoptable for routine qualitative analysis of paracetamol.[14]

Abu Bakar Ruzilawati et al emerged a HPLC method for determination of concentration of repaglinide in plasma of human. In this method indomethacin was used as an internal standard. Here C18 column is a stationary phase, and ammonium formate & acetonitrile (40:60) are mobile phase. The method was registered for pharmacokinetics study of repaglinide in human plasma. Method was passed all validation parameters like Linearity, precision, accuracy, LOD, LOQ, and recovery.[15]

B. dhandapani et al and his colleagues has developed a RP-HPLC method and validated simultaneously to determine ofloxacin & ornidazole by using phosphate buffer with acetonitrile as a mobile phase. The column was phenomenex C18 having rate of flow 1ml/min having PDA detection at 293nm. Here internal standard was gatiflixacin. From the above results it has been concluded that the method was passed all validation parameters and it is to be used in routine analysis in a bulk drug, and its dosage form.[16]

J.J. Berzas Nevado et al developed a capillary zone electrophoresis for analysis of omeprazole and its enantiomers. The mobile phase was phosphate buffer (pH 2.2 with cyclodextrin and sodium diphosphate). This method was applied to five pharmaceutical preparations with good recovery and other validation parameters. This electrophoresis method was another method to HPLC It was suitable for routine use, simple to operation, low cost and flexible to handle. [17]

Silvana e. Vignaduzzo et al developed an RP-HPLC method to determine meloxicam, pridinolmesylate tablet in combining form. The column C18 and mobile mixture was isopropanol and methanol and potassium phosphate buffer. The detection was at 225nm. This method was followed the validation parameters with good robustness. This Method may be demonstrated to determine the drug content of two commercial formulations.[18]

Ceren Yardımc et al have developed a capillary zone electrophoresis for rosiglitazone and metformin simultaneously. They have used a fused silica capillary column with buffer containing

acetate. It was detected at 203 nm. Here internal standard was Verapamil. This method was validated all the analytical parameters like LOD, LOQ, specificity, accuracy, precision, etc. The result of developed method was compared with different liquid chromatography and found that no significance difference was found.[19]

Swathi Vaka et al developed a RP-HPLC to estimate the sacubitril & valsartan in pharmaceutical dosage forms simultaneously. In this method for separation, C18 column was used. Methanol &water are used as a mobile phase in a ratio of 80:20, the detection point was 241nm. This also validated all parameters and gave good studies of recovery at three different levels. This method was suitable for routine analysis in institute for research, and also helpful for quality control department of different pharmaceutical industries.[20]

ParvinZakeri-Milani et al have developed a method HPLC to estimate ketoprofen and naproxen sodium along with a marker called phenol red simultaneously. The mobile phase consisted of methanol, acetonitrile, water and triethylamine. It was detected at 270nm. This method was passed the validation parameters like precision, accuracy, linearity etc.[21]

Siladitya Behera et al have developed a simple UV spectrometric method to determine paracetamol. Here they have used methanol and water as diluents and the method has been validated and passed all parameters according to ICH guidelines. This also used in institution for routine analysis.[22]

Jignesh Prajapati et al has developed and validated RP-HPLC method for the determination of amlodipine besylate& perindopril Erbumine in combined dosage form. Here the stationary phase was Eclipse XDB C-8 column and the mobile phase was buffer and acetonitrile. The detection was at 210 nm. This method was simple and do not involve in time consuming sample preparation. [23]

Robter Heineet al have developed a simple method for assay of atazanavir, amprenavir, indinavir, darunavir, lopinavir, nelfinavir, nelfinavir, aquinavir, ritonavir, tipranavir and nevirapine and efavirenz in human plasma. In this method, stationary phase was C18 column and acetonitrile and methanol was as mobile phase. The method was validated with current FDA guidelines for bio-analytical method validation due to small calibration range and signal to noise ratio was higher at the LLQ level. So, the method was use to routine analysis for drug monitoring and pharmacokinetic studies in HIV infected patients.[24]

Gadapa Nirupa et al have expanded a HPLC method and validated to determine glimepride, pioglitazone, metformin and their dosage forms simultaneously. This method was carried out by using Intertsil ODS-3V column and acetonitrile, tetrahydrofuran and buffer as a mobile phase. It was detected at 228nm. All validation parameters like precision, linearity, accuracy, ruggedness

and robustness were validated. Due to successful validation, it was concerned to dosage form available in the market, and their result was good and reproducible.[25]

Junge Zhanga et al developed an optimized and qualified gel electrophoresis method for impurity and purity analysis of monoclonal antibody under the no reduced and reduced conditions. Investigation of sample preparation parameters with alkylation condition and reduction conditions has been done. It was noticed that the slightly acidic sample buffer was good for analysis, so it was used for sample preparation. This method was shown that no interference with sample buffer matrix. It was found that it shows linearity, accuracy, precision, and LOQ. So, it was used in quality control department.[26]

Vander Heyden et al developed HPLC method to determine the ketoconazole and formaldehyde at 250 and 345nm respectively. They have taken inter chromnucleosil column and acetonitrile-phosphate buffer as a mobile phase. They checked that there was no interference between drugs and other excipients of the shampoo. The formaldehyde has an advantage because of different selectivity between two drugs which was found on different column.[27]

E. Castellanos Gil et al produced a method of capillary electrophoresis for the analysis of doxycycline. A central composite design was developed to optimize the method. This method showed good repeatability, selectivity, sensitivity and linearity. Lastly this method results were compared with other liquid chromatographic method from European pharmacopoeia.[28]

H.R. Liang et al have developed a LC/MS method to determine of methadone enantiomers quantitatively in the plasma of human. The chiral –AGP column have applied for quantification of drugs without other effect of the matrix. This method showed good sensitivity, linearity, ruggedness and successfully applied for clinical analysis in industries.[29]

R. Sistla et al have developed a novel RP-HPLC for assaying ezetimibe in dosage forms by using a Kromasil 100 C18 column, and water – acetonitrile is mobile phase. The drug was detected at 232 nm. The validation parameters were under the acceptance range. The method was applied for routine drug analysis in the institute. [30]

Catalina Ortega et al developed and validated a method for determination of major and minor volatile compound from wine by using carbowax capillary column. The wine was diluted with water, ammonium sulphate and extracted with dichloromethane. The detection method was flame ionization detection. Totally around 30 volatile compounds are detected with all analytical characteristics like linearity, precision and accuracy. From all parameters, it was decided that the method was helpful in quality control and give information related to compounds for wine making processes. [31]

Nilesh Jain, et al have developed a sensitive spectrophotometric method to determine the amlodipine besylate at 240 nm. Due to less solubility of the drug, use of a solubilizing agent named sodium acetate solution. This method was validated in accordance with ICH guidelines and was used for routine analysis. [32]

F. Al-Rimawi et al has developed a HPLC and validated to analyze metformin HCL & 1-cyanoguanidine in tablet form. It was developed by using methanol and ammonium dihydrogen phosphate buffer as a mobile phase and Nova –Pak silica column is a stationary phase. Light detection at 232 nm. Validated method was related to all USP requirement for quantitative detection.[33]

Deanne L. Hertzog et al have developed and validated RP-HPLC method to simultaneously determine the losartan potassium, hydrochloro thiazide with their degradation products. They used waters symmetry C8 column and acetate buffer- acetonitrile as a mobile phase. This method passed the validation parameters like accuracy, linearity, precise etc. [34]

Vaijanath G. Dongre et al have developed & validate a RP-UPLC method for determination of primaquine phosphate drug by using a waters acquity BEH C18 column. This method was validated in accordance with regulatory guidelines, such as precision, accuracy, specificity, linearity and robustness. Again, the forced degradation method has been carried out to check the stability of this method. From this, it was concluded that it was a better method as compared to HPLC related to time, performing good resolution than other.[35]

Yuan Xiong et al and his friends performed a stability indicating RP-HPLC method for separation and quantitation of betamethasone and dexamethasone and other compound with having ACXE phenyl column and mobile phase were water-acetonitrile, and acetonitrile- isopropanol at a wavelength 240 nm. The method was qualified all the validation parameters such as precision, reproducibility, high sensitivity, linearity, specificity and robustness. Therefore, this method was used for quality control labs for routine analysis of drug molecules in dosage form. [36]

Ghada M. Hadad et al has developed, validated the HPLC method with all parameters to determine paracetamol, dantrolene, cetirizine pseudoephedrine. Main purpose of this method was for stability studies of all drugs in quality control laboratories. Here column was RP HS C18 analytical column and the mobile phase was sodium dihydrogen phosphate, haptaneosulphonic acid sodium salt and acetonitrile. The detect point was 214 nm. The method was passed the parameters such as sensitivity, accuracy, precision, linearity, repeatability, reproducibility, robustness, specificity. This method was used for routine analysis of drugs for quality checking.[37]

Faud Al-Rimawi et al have developed and also validated a HPLC method determination of oleuropein by using C18 column and acetonitrile- phosphate buffer as a mobile phase and it was detected at 280nm. This method was validated according to the requirement includes accuracy, precision, selectivity, robustness, LOQ, LOD, range and linearity. [38]

Roberto Romero González et al have developed a UHPLC-MS method for the determination of (6 neurotransmitters and its 3 metabolites) aminobutyric acid, choline, glutamate, dopamine,5-hydroxyindole-3- acetic, acetylcholine, 3,4-dihydroxyphenylacetic acid, serotonin, and homo vanillicacidin a rat brain. The separation of analytes was done by the use of ion paired reagent and the mobile phase was heptafluorobutyric acid and the detectors was electrospray ionization. It showed good linearity, intraday–interday, precision and low LOD and LOQ, specificity. This method was performed for the routine analysis of neutral diseases.[39]

A. k. moharan et al have developed a spectrophotometric method to determine mesalamine with obeying beer's law. This method was followed the statistical evaluation like accuracy, precision, linearity, sensitivity, LOD, LOQ. It was applied for routine analysis in lab and research institution.[40]

P. Pérez-Lozano and his co-researcher had developed a HPLC method to determine the alprazolam tablets. It was carried out in ODS C18 column and phosphate buffer solution with acetonitrile a mobile phase. The UV detector was detected at 254nm. All the validation parameters were fulfilled as per ICH guidelines. This method was applied in routine analysis for quality control department to quantify the drug, its degradation product and to check the content uniformity.[41]

CONCLUSION

Method developments are the method very much helpful for the industries for saving of time and expenditure before sending the newer drugs to the markets. There are many methods for identifying the active pharmaceutical ingredient or new medicines. It is a one type of test or analysis of the medicaments present in the formulations. By choosing the desired method and validate the parameters at the time of manufacturing processes. The methods must be convenient for routine use in institutions and economic for the purpose of quality control in the manufacturing unit. Methods must be easy, convenient to use, specific, accurate and easy recovery.

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