IJRAR.ORG E-ISSN: 2348-1269, P-ISSN: 2349-5138

INTERNATIONAL JOURNAL OF RESEARCH AND ANALYTICAL REVIEWS (IJRAR) | IJRAR.ORG

An International Open Access, Peer-reviewed, Refereed Journal

Analytical Techniques In Pharmaceutical Analysis: A Review

Deep Sharma*, Rekha Rana, Lokender Singh and Neha Sharma

Department of Pharmaceutical Sciences,DDM College of Pharmacy,Una(HP),India-174303

Shanti Niketan College of Pharmacy,Mandi(HP),India

Abstract

Pharmaceutical analysis plays a very prominent role in quality assurance as well as quality control of bulk drugs and pharmaceutical formulations. These pharmaceuticals would serve their intent only if they are free from impurities and are administered in an appropriate amount. To make drugs serve their purpose various chemical and instrumental methods were developed at regular intervals. Recent development in analytical methods has been resulted from the advancement of analytical instruments, which are involved in the estimation of drugs, reduced the time of analysis, increased precision and accuracy. Analytical instrumentation and methods play an important role in detecting impurities at various stages of pharmaceutical development, transportation and storage which makes it risky to be administered. Thus they must be detected and estimated. This review spotlights the role of the analytical instrumentation and methods in estimating the quality of the drugs and also development of new techniques.

Keywords: Analytical method development, Quality control, Validation.

1. Introduction

Pharmaceutical research in the past has played a significant role in the progress of development of analytical techniques and drug development. The contribution of chemistry, pharmacology and biochemistry has set a standard in the drug discovery where new drugs are no longer generated only by the imagination of chemists but these new drugs are the outcome of exchange of ideas between chemist and biologists.

The process of drug development starts with the innovation of a drug molecule that has showed therapeutic value to fight, control and treat diseases. The synthesis and characterization of such molecules which are also called active pharmaceutical ingredients (APIs) and their analysis to produce preliminary safety and therapeutic efficacy data are necessary for identification of drug candidates for more detailed exploration[1].

The investigations on the pre drug discovery are based on knowing the basic cause of the disease to be treated, the information on how the genes are altered that cause the disease, the interaction of proteins and the affected cells and changes brought by these affected cells and how they affect these cells. Based on these facts a compound is developed which interacts with the affected cells and finally could become the drug molecule or active pharmaceutical ingredient (A.P.I).

The ‘‘compound’’ which is set to become the drug molecule undergoes safety tests and a series of experiments to prove that it is absorbed in the blood stream, distributed to proper site of action in the body, metabolized sufficiently and demonstrates its non-toxicity thus, can be considered safe and successful. Once the compound is finalized the preclinical research i.e. In vitro studies followed by the animal testing to check kinetics, toxicity and carcinogenicity tests are performed. After passing the pre-clinical tests the regulatory authorities grant permission for the clinical trials. The clinical trials check whether the drug is working in the proposed mechanism or not, its optimum dose and schedule while the last two stages generate statistically important data about efficacy, safety and overall benefit risk association of the drug. In this phase the potential interaction of the drug with other medicines is determined and monitors drug’s long term effectiveness. After a successful completion of the clinical trials, the drugs are launched in the market for patients. The summary of various stages of clinical trials are listed in Table 1.

Development of single-enantiomer drugs was also made possible by asymmetric synthesis and chiral separation techniques. Several guidelines dealing with chiral drugs have been published in FDA’s European Medicines which encouraged the development of single enantiomer drugs for pharmaceutical manufacturers. The quality of chiral drugs was stipulated by the guideline of the International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH). The guideline recommends applicants to consider other enantiomer as an impurity and to set the identity tests capable of distinguishing both enantiomers and the racemic mixture. It is required to provide tools for efficient quality systems ensuring safe and proper manufacturing processes[2]. However, insufficient in-process control may result in the products suffering from surface irregularities[3]. In addition, the finished products may contain unidentified foreign matter particles. The foreign matter has to be identified and its source should be defined in order to prevent further contamination. Hence it is required to provide an efficient detection and identification procedure of foreign matter from the dosage forms by utilization of analytical techniques[4].

The drugs which are marketed may have different dosage forms. Formulation can be categorized according to the route of administration[5]. Pharmaceutical development information provides the scientific rationale for formulation development and justification for a suitable dosage form. Regulatory guidance provides only limited details of the requirements for the data sets associated with the pharmaceutical development[6] but more detailed information are available for the toxicological assessment of excipients[7] . Excipients are the major fraction of the solid dosage forms which serve as diluents to allow the formulation of appropriately sized tablets and coatings to protect the tablet from undesirable organoleptic qualities of the drug substance. Solid state reactions in the dosage form can occur when the drug substance is reactive and may be accelerated by physical and chemical interaction with excipients. In some cases excipients do not interact chemically but promotes the degradation of drug substance[8].

In the field of pharmaceutical research, the analytical investigation of bulk drug materials, intermediates, drug products, drug formulations, impurities and degradation products, and biological samples containing the drugs and their metabolites is very important. From the commencement of official pharmaceutical analysis, analytical assay methods were included in the compendia monographs with the aim to characterize the quality of bulk drug materials by setting limits of their active ingredient content. In recent years, the assay methods in the monographs include titrimetry, spectrometry, chromatography, and capillary electrophoresis can be seen in the literature.

From the stages of drug development to marketing and post marketing, analytical techniques play a great role, be it understanding the physical and chemical stability of the drug, impact on the selection and design of the dosage form, assessing the stability of the drug molecules, quantitation of the impurities and identification of those impurities which are above the established threshold essential to evaluate the toxicity profiles of these impurities to distinguish these from that of the API, when applicable and assessing the content of drug in the marketed products. The analysis of drug and its metabolite which may be either quantitative or qualitative is extensively applied in the pharmacokinetic studies. This review highlights the role and criteria of development of

various analytical techniques and their corresponding analytical methods in the analysis of pharmaceuticals.

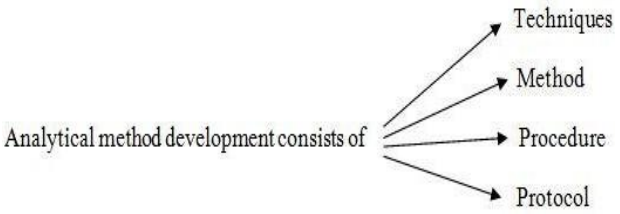
Table 1 Summary of phase wise clinical trial

Phase of clinical trial	Number and type of subjects	Investigation
Phase 1	50–200 healthy subjects (usually) or patients who are not expected to benefit from the IMP	Is the IMP safe in humans? What does the IMP do to the body? (pharmacodynamics)
Phase 2	100–400 patients with the	Will the IMP work in patients? Is the IMP* safe in patients?
Phase 3	1000–5000 patients with the	
Phase 4	Many thousands or millions of patients	Does the IMP seem to work in patients? Is the IMP really safe in patients?

Source: guidelines in clinical trials: 2007 edition. The Association of the British Pharmaceutical Industry, 12 Whitehall London.

1.1 Need to develop analytical methods

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 which resulting in their withdrawal from the market, development of patient resistance and introduction of better drugs by competitors[9]. 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.



The method development provides the following requirements to the analyst so as to enable him to estimate the drug:

1. The required data for a given analytical problem.
2. The required sensitivity.
3. The required accuracy.
4. The required range of analysis.
5. The required precision.

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

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

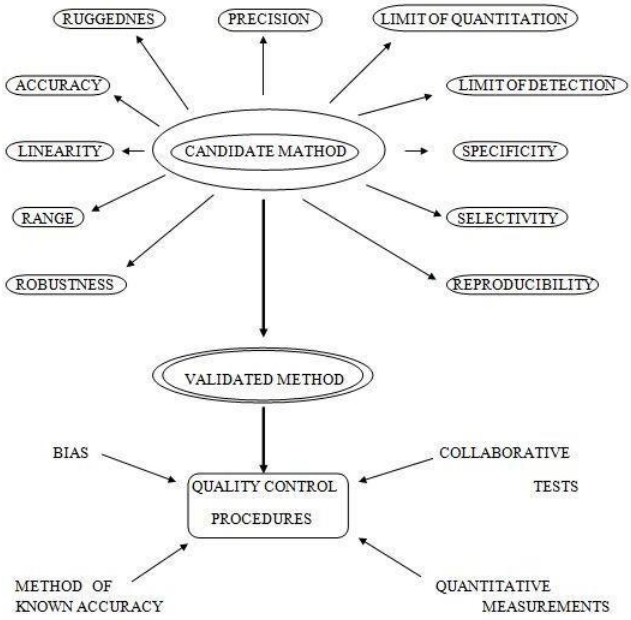


Figure.1. Flow chart showing different steps in analytical method development

1.2 Steps involved in method development

Documentation starts at the very beginning of the development process. A system for full documentation of development studies must be established. All data relating to these studies must be recorded in laboratory notebook or an electronic database.

1.2.1 Analyte standard characterization

a) All known information about the analyte and its structure is collected i.e., physical and chemical properties.

b) The standard analyte (100 % purity) is obtained. Necessary arrangement is made for the proper storage (refrigerator, desiccators and freezer).

c) When multiple components are to be analyzed in the sample matrix, the number of components is noted, data is assembled and the availability of standards for each one is determined.

d) Only those methods (spectroscopic, MS, GC, HPLC etc.,) that are compatible with sample stability are considered.

1. **Method requirements:** The goals or requirements of the analytical method that need to be developed are considered and the analytical figures of merit are defined. The required detection limits, selectivity, linearity, range, accuracy and precision are defined.
2. **Literature search and prior methodology:** The literature for all types of information related to the analyte is surveyed. For synthesis, physical and chemical properties, solubility and relevant analytical methods, books, periodicals, chemical manufacturers and regulatory agency compendia such as USP / NF, are reviewed. Chemical abstracts service (CAS) automated computerized literature searches are convenient.

4. Choosing a method: Using the information in the literatures and prints, methodology is adapted. The methods are modified wherever necessary. Sometimes it is necessary to acquire additional instrumentation to reproduce, modify, improve or validate existing methods for in-house analyte and samples.

5. Instrumental setup and initial studies: The required instrumentation is to be setup. Installation, operational and performance qualification of instrumentation using laboratory standard operating procedures (SOP's) are verified. Always new consumables (e.g. solvents, filters and gases) are used. For example, method development is never started on a HPLC column that has been used earlier. The analyte standard in a suitable injection / introduction solution and in known concentrations and solvents are prepared. It is important to start with an authentic, known standard rather than with a complex sample

matrix. If the sample is extremely close to the standard (e.g., bulk drug), then it is possible to start work with the actual sample.

6. **Optimization:** During optimization one parameter is changed at a time and set of conditions are isolated, rather than using a trial and error approach. Work has been done from an organized methodical plan, and every step is documented in caseof dead ends.
7. **Documentation of analytical figures of merit:** The originally determined analytical figures of merit are limit of quantitation (LOQ), limit of detection (LOD), linearity, time per analysis, cost, sample preparation etc., are documented.
8. **Evaluation of method development with actual samples:** The sample solution should lead to unequivocal, absolute identification of the analyte peak of interest apart from all other matrix components.
9. **Determination of percent recovery of actual sample and demonstration of quantitative sample analysis:** Percent recovery of spiked, authentic standard analyte into a sample matrix that is shown to contain no analyte is determined. Reproducibility of recovery (average + / - standard deviation) from sample to sample and whether recovery has been optimized or not has been shown. It is not necessary to obtain 100 % recovery as long as the results are reproducible and known with a high degree of certainty. The validity of analytical method can be verified only by laboratory studies. Therefore documentation of the successful completion of such studies is a basic requirement for determining whether a method is suitable for its intended applications.

1.3 Chromatographic techniques

1.3.1 Thin layer chromatography

Although an old technique yet it finds a lot of application in the field of pharmaceutical analysis. In thin layer chromatography, a solid phase, the adsorbent, is coated onto a solid support as thin layer usually on a glass, plastic, or aluminum support. Several factors determine the efficiency of this type of chromatographic separation. First the adsorbent should show extreme selectivity toward the substances being separated so as to the dissimilarities in the rate of elution be large. For the separation of any given mixture, some adsorbents may be too strongly adsorbing or too weakly adsorbing. Thin layer chromatography is a popular technique for the analysis of a wide variety of organic and inorganic materials, because of its distinctive advantages such as minimal sample clean-up, wide choice of mobile phases, flexibility in sample distinction, high sample loading capacity and low cost. TLC is a powerful tool for screening unknown materials in bulk drugs[10]. It provides a relatively high degree of assertion that all probable components of the drug are separated. The high specificity of TLC has been exploited to quantitative analytical purpose using spot elution followed by spectrophotometric measurement. TLC plays a crucial role in the early stage of drug development when information about the impurities and degradation products in drug substance and drug product is inadequate. Various impurities of pharmaceuticals have been identified and determined using TLC[11].

1.3.2 High performance thin layer chromatography

With the advancement of the technique, high performance thin layer chromatography (HPTLC) emerged as an important instrument in drug analysis. HPTLC is a fast separation technique and flexible enough to analyze a wide variety of samples. This technique is advantageous in many means as it is simple to handle and requires a short analysis time to analyze the complex or the crude sample cleanup. HPTLC evaluates the entire chromatogram with a variety of parameters without time limits. Moreover, there is simultaneous but independent development of multiple samples and standards on each plate, leading to an increased reliability of results. HPTLC has been used to quantitate drugs as ethinyl estradiol and cyproterone[12], alfuzosin and tramadol and pentazocine[13].

1.3.3 High-performance liquid chromatography (HPLC)

HPLC is an advanced form of liquid chromatography used in resolution of complex mixture of molecules encountered in chemical and biological systems, in order to identify better the role of individual molecules. It was in the year1980, HPLC methods appeared for the first time for the assay of bulk drug materials and has become the principal method in USP and European Pharmacopeia. The specificity of the HPLC method is excellent and simultaneously sufficient precision is also attainable. However, it has to be stated that the astonishing specificity, precision and accuracy are attainable only if wide-ranging system suitability tests are carried out before the HPLC analysis. For the reason the expense to be paid for high specificity, precision and accuracy is also high.

During the survey of the literature it was observed that among the chromatographic techniques HPLC has been the most widely used system. In liquid chromatography the choice of detection approach is critical to guarantee that all the components are detected. One of the widely used detectors in HPLC is UV detector which is capable of monitoring several wavelengths concurrently; this is possible only by applying a multiple wavelength scanning program. If present in adequate quantity, UV detector assures all the UV-absorbing components are detected. A photodiode array (PDA) is a lined array of discrete photodiodes on an integrated circuit (IC) chip for spectroscopy. It is placed at the image plane of a spectrometer to allow a range of wavelengths to be sensed concurrently. When a variable wavelength detector (VWD) is used a sample must be injected numerous times, with changing wavelength, to be sure that all the peaks are detected. In the case of PDA, when it is used a wavelength range can be programed and all the compounds that absorb within this range can be identified in a single analysis. PDA detector can also analyze peak purity by matching spectra within a peak. PDA detector finds its application in the method development of Iloperidone in pharmaceuticals[14]. The refractive index detector is the detector of choice when one needs to detect analytes with restricted or no UV absorption such as alcohols, sugars, carbohydrates, fatty acids, and polymers. Decent trace detection performance is secured through a low noise. This detector is having the lowest sensitivity among all detectors but suitable at high analyte concentrations. Lakshmi and Rajesh utilized the refractive index detector to analyze the content of volgibose in pharmaceutical formulations[15]. The electrochemical detector responds to the substances that are either oxidizable or reducible and the electrical output results from an electron flow triggered by the chemical reaction that take place at the surface of the electrode. This detector was applied recently to analyze the content of glutathione in human prostate cancer cells and lung adenocarcinoma cells.

1.3.4 Gas chromatography

Moving ahead with another chromatographic technique, gas chromatography is a powerful separation technique for detection of volatile organic compounds. Combining separation and on-line detection allows accurate quantitative determination of complex mixtures, including traces of compounds down to parts per trillions in some specific cases. Gas liquid chromatography commands a substantial role in the analysis of pharmaceutical product[16]. The creation of high-molecular mass products such as polypeptides or thermally unstable antibiotics confines the scope of this technique. Its main constraint rests in the comparative non-volatility of the drug substances therefore, derivatization is virtually compulsory. Recently, gas chromatography has been used for assay of drugs such as isotretinion, cocaine and employed in the determination of residual solvents in betamethasone valerate[17]. Gas chromatography is also an important tool for analysis of impurities of pharmaceuticals. In recent years GC has been applied to estimate the process related impurities of the pharmaceuticals, residual solvents listed as impurity by the International Conference of Harmonization are analyzed by the GC using a variety of detectors.

1.4 Spectroscopic techniques

1.4.1 Spectrophotometry

Another important group of methods which find an important place in pharmacopoeias are spectrophotometric methods based on natural UV absorption and chemical reactions. Spectrophotometry is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength.

The advantages of these methods are low time and labor consumption. The precision of these methods is also excellent. The use of UV–Vis spectrophotometry especially applied in the analysis of pharmaceutical dosage form has increased rapidly over the last few years[18]. The colorimetric methods are usually based on the following aspects:

- Complex-formation reaction.
- Oxidation-reduction process.
- A catalytic effect.

It is important to mention that colorimetric methods are regularly used for the assay of bulk materials. For example, the blue tetrazolium assay is used for the determination of corticosteroid drug formulations. The colorimetric method is also exploited for the determination of cardiac glycosides and is presented in European Pharmacopoeia. Several approaches using spectrophotometry for determination of active pharmaceutical ingredients in bulk drug and formulations have been reported.

Derivative spectroscopy uses first or upper derivatives of absorbance with respect to wavelength for qualitative investigation and estimation. The concept of derivatizing spectral data was first offered in the 1950s, when it was shown to have many advantages. However, the technique received little consideration primarily due to the complexity of generating derivative spectra using early UV–Visible spectrophotometers. The introduction of microcomputers in the late 1970s made it generally convincing to use mathematical methods to generate derivative spectra quickly, easily and reproducibly. This significantly increased the use of the derivative technique. The derivative method has found its applications not only in UV-spectrophotometry but also in infrared, atomic absorption, fluorescence spectrometry and fluorimetry[19]. The use of derivative spectrometry is not restricted to special cases, but may be of advantage whenever quantitative study of normal spectra is problematic. Disadvantage is also associated with derivative methods; the differential degrades the signal-to-noise ratio, so that some form of smoothing is required in conjunction with differentiation.

1.4.2 Near infrared spectroscopy (NIRS)

Near infrared spectroscopy (NIRS) is a rapid and non-destructive procedure that provides multi component analysis of al- most any matrix. In recent years, NIR spectroscopy has gained a wide appreciation within the pharmaceutical industry for raw material testing, product quality control and process monitoring. The growing pharmaceutical interest in NIR spectroscopy is probably a direct consequence of its major advantages over other analytical techniques, namely, an easy sample preparation without any pretreatments, the probability of separating the sample measurement position by use of fiber optic probes, and the expectation of chemical and physical sample parameters from one single spectrum. The major pharmacopoeias have generally adopted NIR techniques. NIR spectroscopy in combination with multivariate data analysis opens many interesting perceptions in pharmaceutical analysis, both qualitatively and quantitatively.

1.4.3 Nuclear magnetic resonance spectroscopy (NMR)

Since the first report appeared in 1996[20] describing the use of NMR spectroscopy to screen for the drug molecules, the field of NMR based screening has proceeded promptly. Over the last few years, a variety of state of the art approaches have been presented and found a widespread application in both pharmaceutical and academic research. Recently NMR finds its application in quantitative analysis in order to determine the impurity of the drug, characterization of the composition of the drug products and in quantitation of drugs in pharmaceutical formulations and biological

fluids. Many reviews on the application of NMR in pharmaceuticals have been published[21].

1.4.4 Fluorimetry and phosphorimetry

The pharmaceutical industries continuously look for the sensitive analytical techniques using the micro samples. Fluorescence spectrometry is one of the techniques that serve the purpose of high sensitivity without the loss of specificity or precision. A gradual increase in the number of articles on the application of fluorimetry[22] and phosphorimetry in quantitative analysis of various drugs in dosage forms and biological fluids has been noticed in the recent past.

1.4.5 Electrophoretic methods

Another important instrument essential for the analysis of pharmaceuticals is capillary electrophoresis (CE). CE is a relatively new analytical technique based on the separation of charged analytes through a small capillary under the impact of an electric field. In this technique solutes are perceived as peaks as they pass through the detector and the area of individual peak is proportional to their concentration, which allows quantitative estimations. In addition to pharmaceutical studies it finds an application in the analysis of biopolymer analysis and inorganic ions. CE analysis is generally more effective, can be performed on a quicker time scale, requires only a small amount, lesser up to Nano liter injection volumes and in most cases, takes place under aqueous conditions. These four characteristics of CE have proven to be beneficial to many pharmaceutical applications. Several reports have appeared on the application of this technique in the routine drug analysis[23] and affinity capillary electrophoresis have been developed and applied to pharmaceutical purity testing and in bio analysis of drugs.

1.4.6 Kinetic method of analysis

Kinetic method of analysis has been developing since 1950s and yet in modern days it is taking a major resurgence in activity. The repetitive interest in the kinetic methods can be credited to the advancements made in principles, in automated instrumentation, in understanding the chemical and instrumentation, in data analysis methods and in the analytical application.

From the literature it is evident that the kinetic approach to analytical chemistry is rather general with several advantages over traditional equilibrium approach[24]. Essentially, kinetic methods trust the measurements of concentration changes in a reactant with time after the sample and reagents have been mixed manually or mechanically.

The usage of catalysts to accelerate analytical reactions is feasible with both reaction rate and equilibrium estimations. The use of micellar media in kinetic method is recently encouraged to enhance the rate of reaction, through micellar catalysis and may additionally improve the sensitivity and the selectivity which in turn lessen the analysis time for the analyte.

Two new approaches i.e. kinetic wavelength pair method and H-point standard addition method[25] have been proposed for dealing with overlapping spectra of components in the binary mixtures.

2. Conclusion

The main aim of the pharmaceutical drugs is to serve the human to make them free from potential illness or prevention of the disease. For the medicine to serve its intended purpose they should be free from impurity or other interference which might harm humans. This review is aimed at focusing the role of various analytical instruments in the assay of pharmaceuticals and giving a thorough literature survey of the instrumentation involved in pharmaceutical analysis and development of analytical methods are for identification, purification and finally to quantification any required drug etc., The main activities involved in the analytical development of a method are separation and characterization of impurities as well as degraded products, analytical investigations, studies for identification and finally setting up of parameters optimization to specific requirements. Therefore the salient points enumerated in the above review article are immense use to ananalyst while estimating the pharmaceutical formulations as well as bulk drugs.

Reference

1. Burvenich, C., Duchateau, L., Jansen, F.H., De Spiegeleer, B. (2011). Malaria J. 10, 51. http://dx.doi.org/10.1186/1475-2875-10-51. Valagaleti, R., Burns, P.K., Michael Gill, (2003). Drug Inform. J. 37, p.407–438
2. ICH (2009). International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. Pharmaceutical Development Q8(R2).
3. Akseli, I., Mani, G.N., Cetinkaya, C. (2008). Int. J. Pharm. 360, p.65– 76.
4. Pajander, J., Haugshoj, K.B., Bjorneboe, K., Wahlberg, P., Rantanen, J. (2013). J. Pharma. Biomed. Anal. 80, p.116–125.
5 Pifferi, G., Santoro, P., Pedrani, M. (1999). IL Farmaco, 541. Pospisilova, M., Kavalirova, A., Polasek, M. (2005). J. Chromatogr. A 1081, p.72–76.
6 U.S. Department Health and Human Services (2003). Food and Drug Administration, Centre for drug evaluation and Research,Centre for Biologics Evaluation and Research. Guidance for Industry, Drug Product, Chemistry Manufacturing and Control Information, Draft Guidance, Rockville, Maryland, p.1–62.
7. U.S. Department Health and Human Services, 2002. Food and Drug Administration, Centre for drug evaluation and Research, Centre for Biologics Evaluation and Research. Draft Guidance for Industries, ‘‘Nonclinical studies for Development of Pharmaceutical Excipients’’ (2002), p.1–7.
8. Bryn, S.R.,Xu, W., Newman, A. (2001). Adv. Drug Del. Rev. 48, p.115– 136
9. Conner’s K A, A Text Book of Pharmaceutical Analysis, CBS Publishers and Distributors Pvt. Ltd., New Delhi, 3rd (2001), p.3-6.
10. Szepesi, G., Nyiredy, S. (1996). Pharmaceutical and drugs. In: Sharma, J., Fried, B. (Eds.), Handbook of Thin-Layer Chromatography, 2nd ed. Marcel Dekker, New York, p. 208–235.
11. White, D., Varlashkin, P., Rusch, D.N. (1992). J. Pharm. Sci. 81, p.1204–1209.
12. Pavic, K., Cudina, O., Agbaba, D., Valdimirov, S.(2003). J. Planar Chromatgr.-Mod. TLC 16,p.45–47.
13. Fayed, A.S., Shehata, M.A-A., Hassan, N.Y., ElWeshahy, S.A. (2006). J. Sep. Sci. (2006), p.2716–2724.
14. Devi Manjula, A.S., Ravi, T.K. (2012). Int. J. Pharm. Tech. Res. 4, p.576–581.
15. Lakshmi, K., Rajesh, T. (2010). Eur. J. Chem. 1, p.262–265.
16 Watson, D.G. (1999). Pharmaceutical Analysis. Churchill Livingstone, Edinburg, p. 208.
17 Somuramasami, J., Wei, Y.-C., Soliman, E.F., Rustum, A.M., (2011).
18 Tella, A.C., Olabemiwo, O.M., Salawu, M.O., Obiyenwa, G.K.(2010). Int. J. Phy. Sci. 5, p.379–382.
19 O’Haver, T.C. (1976). In: E.L., Wehry (Ed.), In: Modern Florescence Spectroscopy, Vol. 1. Plenum Press, New York, p. 65.
20 Shuker, S.B., Hajduk, P.J., Meadows, P.P., Fesik, S.W. (1996). Science 274, p.1531–1534.
21 Holzgrabe, U., Deubner, R., Schollmayer, C., Waibel, B.(2005). J. Pharm. Biomed Anal. 38, p.806–812.
22 Rahman, N., Khatoon, A., Rahman, H. (2012). Quim Nova 35, p.392– 397.
23 Neméth, K., Tárkányi, G., Varga, E., Imre, T., Mizsei, R., Iványi, R., Visy, J., Szemán, J., Jicsinszky, L., Szente, L., Simonyi, M. (2011). J. Pharm. Biomed. Anal. 54, p.475–481.
24 Pardue, H.L. (1989). Anal. Pifferi Chim. Acta 216, p.69–107.
25 Pena, J.M., Rubio, S., Perez-Bendito, D. (1991). Anal. Chim. Acta 244, p.81–88.