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Review Article

SIGNIFICANCE OF VARIOUS CHROMATOGRAPHIC TECHNIQUES IN DRUG DISCOVERY AND DEVELOPMENT

Manisha Chavan[1*], Manisha Sutar[2] and Swati Deshmukh[2]

[1]Department of Pharmaceutical Chemistry, Alard College of Pharmacy, Hinjewadi, Pune, Maharashtra, India.
[2]Department of Pharmacognosy, Alard College of Pharmacy, Hinjewadi, Pune, Maharashtra, India.

ABSTRACT
This review deals with discussion of the conventional as well as sophisticated chromatographic techniques from the standpoint of its working principle and applicability for diverse samples. Depending upon a wide range of test samplesof different physical and chemical properties to be separated, the specific chromatographic method is selected which justifies the separation, identification and analysis with optimum outcomes for that particular sample. The study signifies the appliance of chromatography at various stages of drug discovery and development. The aptness of various chromatographic methods for different types of components to be separated was reviewed.

Keywords: Drug discovery, HPLC, Affinity Chromatography, Chiral Chromatography.

INTRODUCTION

There are very few, if any, methods for chemical analysis that are exclusive for a single chemical species. Generally analytical methods are selective for a few species or a class of species. Consequently, the separation of the analyte from potential interference is quite often a rate limiting step in the research. Chromatography is the separation and identification technique. Structurally or chemically similar components of homogeneous mixture can be separated using this technique. Separation is based upon component's relative ability to adsorb and/or partition between mobile phase and the stationary phase. Depending upon separation principle, geometry of method, mode of chromatography, the technique is classified in various types.

At the early stage of drug discovery when a large no. of closely related compounds is synthesized, these are required to be separated. Its identification and purity testing is very essential. Revealing the purity and/or impurity of the synthetic compounds is inadequate without the chromatography. During the various preclinical and clinical studies, chromatography plays essential role. The development stage is the part where analytical methods are developed and validated[1]. These are promoted to manufacturing department during the subsequent manufacturing stages.

While selecting the chromatographic method for analysis of any complex mixture, one must think on the chemical composition, nature, physical and chemical properties, and physical state of that test substance. The chemical composition of the test substance may comprise different type of functional group present, Chemical properties constitutes chemical nature, pH, its acidic or basic behavior. Physical property may cover polarity, molecular mass, refractive index, density, viscosity, whether it is solid, liquid or gas. Definitely, after studying and considering these entire parameters one can select the specific and selective method to achieve foolproof results. Depending upon the chromatographic method's applicability on test candidate, the technique is selected for analysis. Chromatography encompasses a diverse and important group of methods that allow the separation, identification, and determination of

closely related components of complex mixtures; many of this separation are impossible by other means. Chromatography is not restricted to analytical separations. It may be used in the preparation of pure substances, the study of kinetics of reactions, structural investigations on the molecular scale, and the determination of physicochemical constants, including stability constants of complexes, enthalpy, entropy, and free energy[2].

Chromatographic techniques

In all the chromatographic techniques, the sample is dissolved in a mobile phase which may be a liquid, a gas, or a supercritical fluid. This mobile phase is allowed to run over the stationary phase which is fixed in place on a solid surface. The two phases are selected so that the components of the sample distribute themselves between the mobile and stationary phase to varying degrees. Those components strongly retained by the stationary phase move slowly with the flow of mobile phase and elutes later on. In contrast, components that are weakly held by the stationary phase travel rapidly down the column and elutes first. This difference in migration rates make the sample components to separated into discrete zones which can be analyzed qualitatively or quantitatively[3, 4]. There are various parameters, based upon it the chromatographic methods can be categorized which is elaborated in “table 1”.

Paper chromatography

This is probably the simplest, type of chromatography, where a drop of a test mixture is placed on a piece of chromatography paper and allowed to dry. The mixture separates as the solvent front advances past the mixture. Separation is the most efficient if the atmosphere is saturated in the solvent vapor. Paper chromatography works by the partition of solutes between water in the paper fibers (stationary phase) and the solvent (mobile phase) [5].

Thin layer chromatography (TLC)

In thin layer chromatography, the stationary phase is a thin layer of a solid such as alumina or silica supported on an inert base such as glass, aluminum foil or insoluble plastic. The mixture is ‘spotted’ at the bottom of the TLC plate and allowed to dry. The plate is placed in a closed vessel containing solvent (the mobile phase) so that the liquid level is below the spot. The solvent ascends the plate by capillary action, the liquid filling the spacesbetween the solid particles. Solutes adsorb on the stationary phase depending on its affinity towards the stationary phases. Those which adsorbs more retained and which adsorbs less, eluted first. TLC has advantages over paper chromatography in that its results are more reproducible, and that separations are very efficient because of the much smaller particle size of the stationary phase[5].

Ion exchange Chromatography

Here stationary phase is ion exchange resin and ions of the opposite charge are electrostatically bound to the surface of the resin (insoluble, high molecular mass solid). When the mobile phase (always a liquid) is passed through the resin, the electrostatically bound ions are released as other ions are bonded preferentially. This technique involves the exchange equilibria between ions in solution and ions of like sign on the resin surface[6].

Molecular exclusion

The separation of high molecular mass molecules, mostly in biochemical situations, can be accomplished in a column which works on the basis of molecular exclusion. Molecular exclusion differs from other types of chromatography in that no equilibrium state is established between the solute and the stationary phase. Instead, the mixture passes through a porous gel (stationary phase). The pore size is designed to allow the large solute particles to pass through uninhibited. The small particles, however, permeate the gel and are slowed down. Thus smaller the particles, the longer it takes for them to get through the column. Thus separation is according to particle size. molecules larger than the largest pores of the swollen gel particles elute out first and molecules small enough to penetrate gel particles, eluted laterSEC can now be used for determining absolute molecular weight, size, and branching, as well as polymer solution properties, and polymer chain conformation, The technique is mainly used to separate high molecular mass, natural product from low-molecular-mass species and from salts. e.g. separation of proteins from amino acids and low-molecular-mass peptides. It is also applicable to separation of a series of fatty acids[7].

Gas chromatography (GC)

In this technique a gas is used as the mobile phase, and the stationary phase can either be a solid or a non-volatile liquid coated on inert support particles. If a solid stationary phase is used the technique is described as gas-solid adsorption chromatography, and if the

stationary phase is liquid it is referred as gas-liquid partition chromatography. The latter is mostly used technique. The stationary phase is held in a narrow column in an oven. While gaseous mobile phase travels through column. In Gas chromatography, the solutes of a vaporized sample are separated as consequence of being partitioned between a mobile gaseous phase and a liquid stationary phase held in a column. Unlike most other type of chromatography, the mobile phase does not interact with molecules of analyte but only transport the analyte[5] through the column.The technique is applicable to complex organic, metal-organic biochemical entities made of volatile species and generally thermostable compounds[7].

High performance liquid chromatography (HPLC)

Liquid chromatography is similar to gas chromatography but uses a liquid mobile phase. The stationary phase is usually an inert solid or a liquid held on the inert solid. Mobile phase travels through the column forcibly with the aid of the high pressure pump. Solutes of the sample separated on column and eluted with mobile phase[5].
The technique is applicable to thermally fragile samples, e.g. amino acids, proteins, nucleic acids, hydrocarbons, antibiotics, steroids, drugs, inorganic and may organic substances[7].

Affinity chromatography

Affinity chromatography is based on selective non-covalent interaction between an analyte and affinity ligand. These affinity ligands are antibodies, enzyme inhibitors which selectively and reversibly bind to analyte molecules in the sample. These affinity ligands are covalently bonded to the solid support. When sample passes through the column, these ligands bind selectively to some of the analyte molecule and other molecules may elute out. Once these eluted out retained molecule may be made to elute by changing the elution parameters.Affinity chromatography often utilized to analyze biomolecules[7].

Supercritical fluid chromatography (SFC)

Supercritical fluid chromatography is a separation technique in which the mobile phase is a fluid above and relatively close to its critical temperature and pressure. SFC is a hybrid of a gas chromatography and liquid chromatography that combines some of the best features of each. For certain applications it is superior to GC and LC. SFC permits the separation and determination of group of compounds not conveniently handled by either GC or LC. These compound (1) are either nonvolatile or thermally unstable so the that GC procedures are inapplicable and (2) contain no functional groups that make possible detection by the spectroscopic or electrochemical techniques used in HPLC [7].

Chiral chromatography

Chiral chromatography involves the separation of stereoisomers. In the case of enantiomers, these have no chemical or physical differences apart from being three-dimensional mirror images. Conventional chromatography or other separation processes are incapable of separating them. To enable chiral separations to take place, either the mobile phase or the stationary phase must themselves be made chiral, giving differing affinities between the analytes. Chiral chromatography HPLC columns (with a chiral stationary phase) in both normal and reversed phase[8] are commerciallyavailable.
Many of the drugs are available in its racemic mixture form after synthesis. Only one of the enantiomer is having therapeutic activity and other enantiomer is either inactive or having adverse reactions. It is very important to separate them, Separation of the enantiomers comprising the racemate, i.e., the resolution of the racemate, is a common problem in stereochemical research as well as in the preparation of biologically active compounds, in particular, drugs. Chiral chromatograph made feasible to separate these racemic mixtures.

High Performance Thin Layer Chromatography (HPTLC)

High-performance thin-layer chromatography (HPTLC) is a form of thin-layer chromatography (TLC) that provides superior separation power using optimized coating material, automated procedures for mobile-phase feeding, layer preconditioning, précised sample application, chromatogram development scanning, and photo-documentation. It promotes for higher separation efficiencies, shorter analysis time, lower amounts of mobile phase, and efficient data acquisition and processing.
HPTLC has strong potentials as a surrogate chromatographic model[9]for estimating partitioning properties in support of combinatorial chemistry, environmental fate, and health effect studies[9]. The method can be used to validate the simultaneous estimation of two or more drug combinations in a dosage form[10].
One of the available chromatographic techniques is HPTLC, which is used for the

identification of constituents, identification and determination of impurities, and quantitative determination of active substances. The use of modern apparatus such as video scanners, densitometers, and new chromatographic chambers, and more effective elution techniques, high-resolution sorbents with selected particle size or chemically modified surface, the possibility of combining with other instrumental methods, and development of computer programs for method optimization all make HPTLC an important alternative method to HPLC or gas chromatography. Specifically, HPTLC is one of the ideal TLC techniques for the analytical purposes because of its increased accuracy, reproducibility, and ability to document the results, compared with standard TLC. Because of this, HPTLC technologies are also the most appropriate TLC technique for conformity with GMPs[11].

Ultra performance liquid chromatography (UPLC)

The ultramodern technology Ultra performance liquid chromatography (UPLC) has taken an advantage of technological march made in particle chemistry performance, pressure optimization, detector design, and data processing and control. Using particles of 2.5µ or less size[12,13] and mobile phases at high linear velocities, and instrumentation that operates at higher pressures than those used in HPLC, dramatic increases in resolution, sensitivity, and speed of analysis can be obtained. This new category of analytical separation science retains the practicality and principles of HPLC while creating a step function improvement in chromatographic performance[14].

According to the Van Deemter equation, as the particle size decreases to less than 2.5 µ, not only is there a significant gain in efficiency, but the efficiency does not diminish at increased flow rates or linear velocities. By using smaller particles, speed and peak capacity (number of peaks resolved per unit time in gradient separations) can be extended to new limits, termed Ultra Performance Liquid Chromatography, or UPLC. The technology takes full advantage of chromatographic principles to run separations using columns packed with smaller particles and/or higher flow rates for increased speed, with superior resolution and sensitivity[15].

Appliance of the chromatographic techniques in drug discovery and development

After the review of these chromatographic techniques the discussion is now focused on the significance of these techniques in the drug research. The aim of the drug researcher is a discovery of a new, safe and active chemical entity (NCE)[16] which has theoretic value for any disease or disorder. While studying the target and drug interactions the Affinity chromatography has proven to be key-method in the study of drug or hormone interactions with binding proteins[17].There are several ways to discover the potential lead compounds i.e. screening natural products from microbes, plants, or animals[18]. Natural Products are usually tested as crude extracts first, followed by isolation and identification of active compounds. Other approaches are (random) collections of discreetly synthesized compounds, computer assisted drug design, compounds synthesized with combinatorial chemistry.

Isolation and identification of the crude extracts is very challenging, one can confidently and effectively satisfy the 100% identity rule of the cGMP to isolate and identify crude raw materials and their powdered or liquid extracts as well as identify a majority of such ingredients in finished products with diverse matrixes. HPTLC Technique can be used for Fingerprint analysis and the determination of identity and quality of Herbal drugs effectively[19].Applications of HPTLC for phytochemical analysis, biomedical analysis, herbal drug quantification, finger print analysis are in the routine practice. HPTLC-MS, HPTLC-FTIR and HPTLC-Scanning Diode Laser mode are powerful analytical tools in the field of analysis. It is noteworthy that utilization of instrumental HPTLC toward the analysis of drug formulations, Bulk drugs, natural products, clinical samples food stuffs, environmental, and other relevant samples will increase in the future.

Thin-layer chromatography (TLC) is a very commonly used technique in synthetic chemistry for identifying compounds, determining their purity and following the progress of a reaction by studying the components present; and in separating reaction intermediates. The TLC based chemical screening approach has been developed for the investigation of metabolites from the microbial culture[20]. The large numbers of products created by the combinatorial chemistry are then identified by fast LC-MS methods.

Almost half of the drugs in use are chiral. Molecules which are revealed from combinatorial chemistry may be mixture of two enantiomers. It is well known that the

pharmacological effect is restricted in most of the cases to one of the enantiomers. Only about 25% of drugs are administered as pure enantiomers. There can be qualitative and quantitative differences in the activity of the enantiomers. The pharmacologically inactive enantiomers can show unwanted side effects; in some cases antagonistic and even toxic effects are observed. The enantiomers can differ in absorption, distribution, protein binding and affinity to the receptor. Furthermore, the metabolic pathways can differ. Therefore, the separation of racemic mixtures of intermediate or final products is often required. For enantiomers' separation on analytical scale agreat variety of methods based on chiral chromatographic techniques such as HPLC, GC, SFC, TLChave been developed where chiral reagent is added in mobile phase or chiral stationary phase is used[21].

Capillary column gas chromatography (GC)/mass spectrometry (MS) have been used to achieve more powerful separation and to perform structural analysis of molecules. The development of high-performance liquid chromatography (HPLC) has proven a milestone in the drug discovery and allowed faster separations of fragile biological macromolecules. Liquid chromatography LC/MS is more suitable for biomedical applications than GC/MS because almost all biomolecules are heat sensitive. Furthermore, a combination of various mass spectrometers has been used even for proteins directly. Improving the sensitivity of nuclear magnetic resonance spectrometry (NMR) has permitted a direct connection with LC. Fast LC analysis using a column switching technique was introduced for aromatic amino acid metabolites and guanidino compounds[22].

After the lead compounds are selected, the next steps involve pharmacological, metabolic and pharmacokinetic studies for lead optimization. For these studies high sensitivity and selectivity is required. HPLC coupled with tandem mass spectrometry i.e. HPLC-MS/MS. As well LC coupled with nuclear magnetic resonance methods LC-NMR, LC coupled with Infrared methods LC-IR methods has proved its application in the drug discovery.

Novel development in this field has been the evolution of ultra performance liquid chromatography (UPLC) which enables use of short columns to be used (5 cm or less) and rapid analyses (e.g., 5 min or even less than 1 min). The subsequent development is then UPLC-MS/MS, UPLC-DAD, UPLC-NMR which has made DMPK (1) analysis possible in hardly any time, which has fasten the lead optimization step and trimmed down the time and cost of analysis. The novel generic UPLC/MS/MSmethodology was successfully introduced within early Drug Discovery and resulted in a four-fold increase of throughput as well as a significant increase in sensitivity compared to other in-house generic LC/MS methods[23].

Affinity-based chiral separations and the use of affinity chromatography for the study of drug or hormone interactions with binding proteins. Some areas of possible future developments are then considered, such as tandem affinity methods and the use of synthetic dyes, immobilized metal ions, molecular imprints, as affinity ligands for clinical analytes[17].

Ion exchange chromatography has been applied to a variety of organic and biochemical systems, drugs, their metabolites serum, food preservative, vitamin mixtures, and pharmaceutical preparations. Ion exchange is probably the most frequently used chromatographic technique for the separation and purification of proteins, polypeptides, nucleic acids, polynucleotides and other charged biomolecules[24].

As quoted earlier in this review, Gel permeation/Molecular exclusion chromatography can be effectively applied to biological samples of proteins, fatty acids where the differing decisive factor is the molecular mass of the components of the mixture to be analyzed. Gel permeation chromatography with differential refractometry[25] is used to obtain molecular weight distributions (MWD) of poly-(ε-caprolactams).

Supercritical Fluid chromatography has choice over LC And GC as quoted above because of the much lower mobile phase viscosity, higher diffusivity and lower column pressure drop, the packed column supercritical fluid chromatography(pSFC) has made pSFC-MS[26], a very powerful tool for high-throughput, qualitative, and quantitative analyses in drug discovery. The SFC has wide range of applicability to rapid method development, structure characterization and achiral/chiral purification. PrepSFC has been used to purify fatty acid esters, synthesis intermediates, steroids, most importantly, chiral compounds. The technique has become more attractive because it offers high speed, better efficiency, unique selectivity and it has aqueous-free purification capabilities.

Once the lead has optimized for it therapeutic potential then the analytical methods are developed which will be utilized during manufacturing, of drug or dosage form. The methods are validated and approved for using

in the production department. These methods are identification tests, impurity testing, assay, stability, the dosage form content uniformity, dissolution, etc. As per requirement and specificity of the test component, the chromatographic techniques are opted and implemented to accomplish one of the step of drug discovery.
Assessment of identify of the active ingredient in the test sample or drug formulation is utmost important. It can be affected using LC-PDA or LC-MS [27]. It is mandatory to characterize the impurity profile to establish the therapeutic safety of the drug candidate in pharmaceutical research. Many of the governing regulations like ICH, USFDA has emphasized on identification of impurities in Active Pharmaceutical ingredients. It will no be exaggerate if one says that impurity profiling is impossible without chromatographic methods. Chromatographic methods along with spectroscopic detection technique in identification of the impurities has brought milestone in the drug discovery and development of pharmaceutical industry. Various hyphenated techniques LC-MS LC-NMR, LC-NMR-MS, GC-MS, and LC-MS/MS[28] are conducted for these purpose depending upon the chemical and physical status of the sample. Obviously assay, content uniformity and dissolution testing are important from the standpoint of consumers' safety and therapeutic effect of dosage form. One has to follow the pharmacopoeial norms for the methods to be used. IP, BP, USP and many more pharmacopoeias has described the chromatographic methods and conditions while performing the tests.
The stability testing [29, 30] is conducted to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of temperature, humidity, and light, and to establish a re-test period for the drug substance or a shelf life for the drug product and recommended storage conditions. The stability program[31] also includes the study of product-related factors that influence its quality, for example, interaction of API with excipients, container closure systems and packaging materials.
After conducting these studies, the drug product or formulation is tested for its quality, purity, strength, presence of any degradation products. Chromatographic techniques (HPLC, HPTLC) have played a crucial role in stability studies for evaluation of the decomposition of the drug, presence of possible degradation products, strength of active drug.
The objective of the cleaning validation[32] is to verify the effectiveness of the cleaning procedure for removal of product residues, degradation products, preservatives, excipients, and/or cleaning agents as well as the control of potential microbial contaminants. In addition one need to ensure there is no risk associated with cross-contamination[33]of active ingredients. The analytical method involved in here should be sensitive, specific, fast and accurate to establish the assurance that the equipments used in manufacturing are free of the above unwanted impurity, presence of which may alter the safety and efficacy of the drug product. HPLC, UPLC techniques have established their role in pharmaceutical cleaning validation.
IPQC testing are integral part during manufacturing process, which give assurance that any process in manufacturing is running as per the laid standards and will produce the products with predetermined specifications. HPLC, GC, UPLC studies are commonly utilized to check drug release, dissolution testing, content uniformity etc.
Identification of leachable is utmost important in pharmaceutical manufacturing. Chromatographic techniques have justified its role in detection of leachable.

CONCLUSION

In the drug discovery and development process the chromatography has proven a crucial role. It may be concluded that drug discovery phenomenon is incomplete with out chromatographic techniques. Depending on the nature of analyte if proper chromatographic method is supported with suitable detection technique, the analysis is no longer a challenge. Appliance of selective and specific chromatographic technique in the various steps of the drug discovery has declined the time and cost of drug research from discovery to manufacturing stage.

Table 1: Classification of Chromatographic Technique based on various parameters

Parameters	Principle of Separation	Mobile Phase Used	Geometry of Technique	Scale of Operation	Mode of technique	Elution method
Types	Partition	GC	Planar -Paper, TLC, HPTLC	Preparative	Normal phase	Gradient
	Adsorption	LC				
	Size Exclusion	Super-critical Fluid C	Column- GC, HPLC, SFC, UPLC	Analytical	Reverse Phase	Isocratic
	Affinity					
	Ion Exchange					

REFERENCES

1. Shulamit Levin, High Performance Liquid Chromatography (HPLC) in the pharmaceutical analysis, Medtechnica, Feb 2010.
2. Robert L. Grob Ph, Eugene F. Barry, Modern Practice of Gas Chromatography, Fourth Edition, John Wiley & Sons, IncJuly 2004, 605-641.
3. Chromatography: James M. Miller.Concepts and Contrasts, 2nd edition, John Wiley & Sons, Inc, 2005, 37-44
4. J Cazes, R.P.W. Scott, Chromatography Theory, New York Dekker, 2002 John Wiley & Sons, 2005, 520.
5. THE ROYAL SOCIETY OF CHEMISTRY, Modern Chemical techniques, Unilever 5. Chromatography, 116-159
6. Corrado Sarzanini, Recent developments in ion chromatography, Elsevier Journal of Chromatography A, 956 (2002) 3–13
7. D.A. Skoog, F.J. Holler, S.R. Crouch, Instrumental Analysis, Cengage Learning, 2007.836, 858, 865, 885, 893, 981-921, 923-925, 927.
8. Analytical Chiral Separation Methods, (IUPAC Recommendations 1997) Pure &App/. Chem., Vol. 69, No. 7, pp. 1997.
9. MM. Srivastava , An Overview of HPTLC: A Modern Analytical Technique with Excellent Potential for Automation, Optimization, Hyphenation, and Multidimensional Applications, High-performance thin-layer chromatography (HPTLC) 2011, pp 3-24
10. 2 Kaul N, Dhaneshwar SR, Agrawal H, Kakad A, Patil B, Application of HPLC and HPTLC for the simultaneous determination of tizanidine and rofecoxib in pharmaceutical dosage form, Journal of Pharmaceutical and biomedical analysis, 2005 Feb 7;37(1):27-38.
11. Mahesh Attimarad, KK Mueen Ahmed, Bandar E Aldhubaib, Sree Harsha, High-performance thin layer chromatography: A powerful analytical technique in pharmaceutical drug discovery, Pharmaceutical Methods, , an addendum to Journal of Young Pharmacist, Year : 2011, Volume : 2 ,Issue : 2,Page : 71-75
12. A B Roge, S N Firke, R M Dhane, V J Gunjkar, S M Vadvalkar, Novel Achievement of HPLC: UPLC, International Journal of PharmTech Research, Vol.3, No.3, , July-Sept 2011,pp1423-1429
13. UPLC: A Permanent Technique in Pharmaceuticla Analysis, Ashok Kumar, Gautam Saini, Anroop Nair and Rishabh Sharma, Acta Poloniae Pharmaceutica ñ Drug Research, 2012,Vol. 69 No. 3 pp. 371-380,
14. Michael E. Swartz UPLC : An Introduction and Review, Journal of Liquid Chromatography & Related Technologiesw, 28:, 2005, 1253–1263.
15. Michael E. SwartzUltra Performance Liquid Chromatography, (UPLC): An Introduction, Sepration Science Refined, May, 2005, 9-14.
16. Shulamit Levin, High Performance Liquid Chromatography (HPLC) in the pharmaceutical analysis, Medtechnica, Feb 2010
17. Hage DS.,Affinity chromatography: a review of clinical applications. Cliical Chemistry, 1999 May;45(5):593-615.
18. http://www.alzforum.org/drg/tut/tutorial .asp
19. Sudberg S, Sudberg EM, Terrazas J, Sudberg S, Patel K, Pineda J, Fine B, Fingerprint analysis and the application of HPTLC to the determination of identity and quality of botanicals, from an industry perspective, Journal of AOAC International, 2010 Sep-Oct;93(5):1367-75.

20. Alexander Hillisch, Rolf Hilgenfeld, Modern Methods of Drug Discovery, Springers, 2003, 98.
21. Gerald Gu¨ bitz, and Martin G. Schmid, Chiral Separation by Chromatographic and Electromigration Techniques. A Review : Biopharmaceutics and drug Disposition, (2001), 22: 291–336.
22. Hanai T. Chromatography and computational chemical analysis for drug discovery, Current Medicinal Chemistry, 2005;12 (5):501-25.
23. Mensch J, Noppe M, Adriaensen J, Melis A, Mackie C, Augustijns P, Brewster ME Novel generic UPLC/MS/MS method for high throughput analysis applied to permeability assessment in early Drug Discovery, Journal of Chromatography B – Analytical technologies in the biomedical and Life Sciences, 2007 Mar 1;847(2):182-7. Epub 2006 Nov 13.
24. Bonnerjera, J., Oh, S., Hoare, M., Dunhill, P., The right step at the right time. Bio/Technology, 4, 954-958 (1986),
25. Elena Mateos1, Vicente L. Cebolla1, Luis Membrado1, Elena Piera, and Miguel A. Caballero, Journal of Chromatogr Science (2007) 45 (8): 524-530.
26. Yining Zhao, Pat Sandra, Gregory Woo, Samuel Thomas, Kyung Gahm and David SeminPacked-Column Supercritical Fluid Chromatography–Mass Spectrometry for Drug Discovery Applications Pharmaceutical Discovery, November/December, 2004, 1-8.
27. Gilliard J.A, Ritter, C., Use of simulated liquid chromatography--diode array detection data for the definition of a guide curve in peak purity assessment by spectral comparison, Journal of Chromatography A, Volume 786, issue 1 (October 24, 1997), p. 1-11.
28. Kavita Pilaniya, Harish K. Chandrawanshi, Urmila Pilaniya, Pooja Manchandani, Pratishtha Jain, and Nitin Singh Recent trends in the impurity profile of pharmaceuticals, J Adv Pharm Technol Res. 2010 Jul-Sep; 1(3): 302–310.
29. Stability Testing of new Drug Substances and Products, ICH Topic Q 1 A (R2),(CPMP/ICH/2736/99), August 2003, 4.
30. Kim Huynh-Ba, Handbook of Stability Testing in Pharmaceutical Development, Springer Science & Business Media, 2009, 1.
31. World Health Organization, Annex 2, Stability testing of active pharmaceutical ingredients and finished pharmaceutical products WHO Technical Report Series, No. 953, 2009, 88.
32. Q7A Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients, Cleaning Validation , August 2001, ICH, 34.
33. GUIDE-0028, Cleaning Validation Guidelines, Drugs and health products, Health Canada.