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CHROMATOGRAPHY: A REVIEW

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ABSTRACT

Chromatography is the term given to the collection of procedures that are used to detect, quantify, and separate the chemical components that may be present in complicated mixtures. Comparable to spectroscopy in many respects, this method is widely used and achieves excellent results in both preparative and analytical techniques. This process enables the production of compounds of an extremely high purity level. Chromatography may be described as "the technique in which the components of a mixture are separated based upon the rates at which they are carried or moved by a gaseous or liquid mobile phase through a stationary phase (column)". Chromatograms are visual representations of signal intensity in relation to volume or time. They exhibit peaks, or sample components, with an area corresponding to each component's amount, at various points in time, or retention durations.

Keywords: - *chromatography, chemical components, analytical techniques.*

INTRODUCTION

Chromatography is a valuable technology that may be used to identify, cleanse, and separate the components that make up a mixture. Mikhail Tswett, a botanist working in Warsaw in 1906, was the first person to come up with the technique for dissecting colored compounds into their individual constituents. Since that time, a number of alterations have been made to this approach. As a result, it is now possible to separate almost any given combination of compounds utilizing a number of different chromatographic modalities. The term chromatography derives from the Ancient Greek terms khromatos (meaning "color") and graphos (meaning "writing"), both of which mean "to write." Chromatography is an umbrella term that refers to a wide range of techniques for the identification, separation, and purification of substances that are only present in mixtures in very trace amounts.

Chromatography is the name given to a series of techniques that are used in the process of extracting individual components from a mixture. High-Pressure Liquid Chromatography (HPLC), also known as High-Performance Liquid Chromatography (HPLC), is a specialized approach that use liquid chromatography and columns to separate, characterize, and examine the active moieties that are present in the mixture. HPLC is also known as High-Performance Liquid Chromatography (HPLC). The high-performance liquid chromatography (HPLC) technique is the focus of this particular study, in which its basics, sorts, instruments, applications, advances, and patents are discussed. The high-performance liquid chromatography (HPLC) technique is crucial for quantitative as well as qualitative analysis; it is used to examine biological and pharmaceutical components. For the purpose of ensuring that medication components meet quality standards, this chromatographic analytical approach is the fastest, most versatile, safest, and most adaptable option

available. The authors of this piece have also made an effort to present a summary of a selection of more current inventions and patents that make use of the HPLC technology for the purpose of analysis. This piece of writing will help readers better understand the role and relevance of this analytical approach in the context of quality control for pharmaceutical and biological products.

Chromatography is comprised of chromatography's two phases: the stationary phase and the mobile phase. The first is the stationary support, which keeps the components in place to variable degrees, and the second is the mobility phase, which makes it easier for the components to move about. When using TLC, the stationary phase is in the form of thin layers; whereas, when using HPLC, the stationary phase is in the form of a packed column. The mobile phases may be either solvent mixes or pure solvents; this holds true across all modalities. It is possible that they be normal phase modes or reversed phase modes, however this will depend on the properties of both the stationary and mobile phases. In normal phase chromatography, the stationary phase is more polar than the mobile phase, but in reversed phase chromatography, the mobile phase is more polar than the stationary phase.

There are several different ways in which one might define chromatography. The explanation that "chromatography is a separation technique applicable to essentially molecular mixtures that relies on distribution of the mixture between stationary and mobile phases" is the one that seems to be the most appropriate, "Chromatography is a separation technique applicable to basically molecular mixtures." "Chromatography is meant those processes which allow the resolution of mixtures by effecting separation of some or all of their components in concentration-zones on or in phases different from those in which they are originally present, irrespective of nature of force or forces causing the substances to move from one phase to another" is an additional definition of chromatography offered by Williams and Weil respectively. The two basic levels at which the various forms of chromatography differ from one another are as follows:

- variances in the kinds of distribution systems that are put into use, particularly with respect to the steps that are included.

- variations in the contraction or manipulation of the phases. Four main categories of chromatography exist.

TSWETT Chromatography Or Adsorption Chromatography

Variations in the adsorption coefficients of the solutes are what form the foundation of this idea. In this scenario, the solutes are adsorbed at different sites along the adsorbent column, and the stationary solid phase is made up of materials such as silica gel, alumina, or magnesium oxides. After that, the components that have adsorbed to the column are eluted by passing the column through the mobile phase, which is comprised of the suitable solvents. Conventional techniques for liquid chromatography, such as column chromatography, are in great demand due to the fact that they are simple to implement and have a brief history in the scientific community. It was put to extensive use for a broad variety of functions, including the harvesting and processing of ecological and natural resources, among other things. In chromatography, sorbents of many different kinds, including silica, alumina, florisil, and a great many others, are used. It was an innovation known as high-pressure liquid chromatography (HPLC) that was credited for reviving this technology. It is considered to be an improvement that is both more sophisticated and better.

Chromatography By Segments Of A Whole

In partition chromatography, the separation of solutes occurs as a result of the phenomena of solutes being able to partition between stationary (liquid) and mobile phases. In paper chromatography, fixed phases may be composed of a liquid that has been strongly adsorbed on a solid that acts as a support. This support may be a piece of paper. In this particular scenario, the solute is spread out throughout both the stationary phase, which is represented by the fixed liquid, and the mobile phase, which is represented by the solvent.

The Chromatography Of Ions By Exchange

Ion exchange chromatography uses the ion exchange concept to separate chemicals. In general, it helps with ionic compound resolution. Either cation or anion exchange might be involved. However, sometimes, additional adsorptive forces play a role in the process of separation.

Chromatography By Means Of Emulsions And Foams

The chemicals that produce foam or micelles are used, and the solutes are partitioned between the foam and the mobile phase. Micellar chromatography is another name for this technique. The surfactant used in miceller chromatography contains a charged top and an extended series hydrocarbon end. When the concentration of counter ions in the water mobile phase is elevated to a level that is higher than a critical micelle concentration, micelle organization will take place. A total of forty to one hundred ions come together to create particles that are about spherical in shape. The hydrophilic head of the particle is oriented so that it faces the outside of the micellar particle, and the hydrophobic end is tilted such that it faces the center of the particle. As a consequence of this, an extra step is produced, which makes it possible for uncharged species to disintegrate inside the micelles.

As was said earlier, every chromatographic technique requires the movement of a mobile phase over a stationary phase in order to get the desired results. As a direct consequence of this, the solute is separated between the two phases, and the central component of the chromatographic system explains in further detail how this separation took place.

Chromatography By Phase Separation (Psc)

In 1938, Izmailov and Sharaiber were the first people to describe thin layer chromatography, often known as TLC. Chromatography may also be referred to as drop, strip, spread layer, or surface chromatography. Other names for thin layer chromatography include these. Because of its low cost, ease of use, sharpness of separation, high sensitivity, speed of separation, and simplicity in recovering separated compounds, thin layer chromatography is finding more and more applications in analytical chemical research across all subfields. It has been shown that this approach is effective for doing analytical research on compounds that are either not accessible or are only present in very minute or trace concentrations. The utilization of any and all chromatographic principles that are applicable to liquid-liquid and solid-liquid systems is possible in thin-layer chromatography. The TLC technique may also make advantage of ion exchange and partitioning, although the adsorption phenomenon is the one that has seen the most use. The choice of chromatographic principle is determined by the chemical composition of the molecules (that need to be resolved) as well as the pattern of fractionation that is desired. Chromatography on a thin layer is regarded to be more successful than chromatography on a column or on paper for a number of major reasons, including the following:

1. It requires a lesser amount of material overall.
2. The development time ranges from 15 to 45 minutes, which is a more manageable amount of time.
3. It is possible to spray chromatoplates with strong acids without risking their integrity for the purpose of identification.
4. Thin-layer chromatography has a greater capability for adsorption of compounds as compared to paper chromatography.
5. It is not impossible to maintain standards while simultaneously working with caustic combination

REVIEW LITERATURE

Oliveira, D. C. et. al. (2012), has developed Reverse phase liquid chromatography method using the PDA detector for the analysis and dissolution studies of fexofenadine hydrochloride in dosage forms. This method also validated for its suitability and adequacy. Mobile phase used is composition of pH 3.2 1% triethylamine phosphates, ACN, CH3OH in the proportion of 50:30:20. The detection wavelength is 210 nm. Phenomenex C18 column was used for analysis. The method validation parameter are recovery, precision (RSD < 1%), linearity (r2 = 0.9999), and robustness.

Nirogi, R et. al. (2018), has develop a HPLC/ MS/ ESI-Mass spectroscopyprocedure for simultaneous estimation fexofenadine and pseudoephedrine combination. This method is based on an internal standard method where mosapride is used in plasma sample. The fexofenadine and pseudoephedrine were resolved in simple isocratic reverse phase chromatography. It is attached to mass spectroscopy where sample mass are monitored at specific [M+H]+ ions. For fexofenadine m/z is 502/466, 166/148 for pseudoephedrine and the internal standard mass to charge ratio 422/198. Developed procedure is found precise and sensitive.

Radhakrishna, T et. al.(2014), has developed a Reverse Phase Liquid Chromatographic method which is useful to estimate fexofenadine HCl and fexofenadine related substance. Fexofenadine related compound B which is meta-isomer related substance is separated and estimated by C8 High performance liquid chromatographic column. The chromatographic column was used as an Eclipse C8, 150 x 4.6 millimeter, 5 micron, for related substance separation from fexofenadine. 60% 3.70 pH phosphate with 1% TEA, 20% methanol and 20% acetonitrile mixture was used as mobile phase. As an internal standard for determination of fexofenadine, 5-Methyl 2-nitrophenol was used.The optimized analytical method is linier form 0.7 ppm to 18.7 ppm for related substance and for fexofenadine potency estimation linear range was 60 ppm to 750 ppm.

Sharaf El-Din M. K et. al.(2015), has developed RP-LC method for estimation of fexofenadine with its degradants. The developed reversed phase liquid chromatographic is stability indicating. In present of potential degradant which is sensitive to acid, base and peroxide was separated from fexofenadine peak in this method. A mobile phase has 35% KH2PO4 buffer and 65% acetonitrile mixture. The mobile phase pH was adjusted to 5.5 with OPA. To separate the degradant from fexofenadine the octadecylsilane C18 HPLC column with dimension of 150 x 4.6 millimeter internal diameter was used. 25°C temperature was kept for Column oven. The mobile phase FR was 1.0 milliliter per minute. The output signals were recorded in detector at 225 nm. The developed test procedure can be successfully applied for routine analysis of FEX finished product.

S.M. Chen et. al.(2016), studied sample of nicardipine photo stability chamber for 3 hours. This sample was made in methanol & applied photo irradiated. In this sample chromatogram 4 potential photodegradent observed by using HPLC technique. Further the same sample is subjected for LC MS analysis. Outcome of this study was potential degradation is because of dihydro pyridine in nicardipine.

RESEARCH METHODOLOGY

Method

Metaxalone (MTX), a 2-oxazolidinone derivative (5-(3, 5- dimethyl phenoxy methyl)-2-oxazolidinone) with skeletal muscle relaxant activity. Analytical methods using RP-HPLC [3, 4]; liquid chromatography mass spectrometry (LC–MS/MS) and ultra-violet (UV) spectroscopy has been successfully reported for MTX quantification. However, none of them adopt systematic statistical optimization; rather those methods pursued traditional approach i.e. varying one factor at a time, while keeping the others constant. Since, the RP-HPLC method optimization is a complex process, variety of parameters and chemical factors such as mobile phase pH, buffer concentration, flow rate, column temperature, detector wave length, etc., are to be simultaneously monitored to achieve separation selectivity and other performance criteria. Moreover, the traditional approach based on trials and error methodologies are inefficient and time-consuming and may not be able to identify the optimal condition.

DATA ANALYSIS

Some of the most important components of chromatographs are columns, which are apparatuses designed expressly for molecular separation, as well as high-performance pumps that deliver solvent at a constant flow rate. These pumps are among the numerous technologies that have been developed for chromatography. As the technologies that are associated to it progressed, the method that is often referred to as High Performance Liquid Chromatography started to be known simply as "LC." In today's world, there has been a rise in the use of ultra-high performance liquid chromatography (UHPLC), a technique that enables rapid data analysis.

Materials and reagents used

Metaxalone (99% purity) was procured from a local manufacturing unit in Hyderabad, India. Glass-distilled and de-ionized water (Nanopure, Bransted, USA) was used throughout the experiments. Acetonitrile (HPLC grade) was purchased from Merck (Mumbai, India). Potassium dihydrogen phosphate, disodium hydrogen phosphate, glacial acetic acid, sodium hydroxide, potassium hydroxide etc. (all of analytical grade) purchased from S.D. Fine Chemicals (Mumbai, India) were used.

Preparation of standard and working solutions

Stock standard solution (1mg/ml) of MTX was freshly prepared in acetonitrile. For use in development and optimization of the RP-HPLC method, working standard solution (50µg/ml) was made by diluting the stock standard solution with acetonitrile.

Preparation of buffer solutions

Since, pH was opted as one of the independent factor during method of optimization phase; phosphate buffers of three different pH values (4.0, 5.0 and 6.0) were used as a part of mobile phase composition. (i) In order to prepare buffer pH 4.0, 5.04 g of disodium hydrogen phosphate and 3.01 g of potassium dihydrogen phosphate were dissolved in 1000 ml water and final pH was adjusted to 4.0 with glacial acetic acid. (ii) For buffer pH 5.0, 6.8 g of potassium dihydrogen phosphate was suspended in 1000 ml of water and pH adjusted with 10M potassium hydroxide. (iii) For preparing buffer pH 6.0,ml of the 0.2M potassium dihydrogen phosphate and 5.6 ml of 0.2M sodium hydroxide were placed in a 200-ml volumetric flask and the volume was made up with water.

Optimization Method

Response surface methodology (RSM) was used to recognize the significant factors influencing the elution of MTX in the proposed RP-HPLC method. Factors for instance buffer pH, % organic phase (acetonitrile), and flow rate, each at three levels (low, medium & high) were monitored for the observed responses such as retention time (min.), theoretical plates, and tailing factor. The three responses were optimized all together by a multiple response algorithm using a statistical programme Design Expert Version-8.0.4 (Stat-Ease Inc.). Optimal condition was established along with the robustness of the method using a BBD.

RESULTS

The response data for all the 17 experimental trials by BBD were performed in accordance with Table to analyze the effect of three independent factors on the responses. The values for the investigated responses, i.e. theoretical plates (N), retention time (tR) and tailing factor because of the combined factorial effects are denoted.

Table 1: Box-Behnken design of three variables and the experimental observed responses.

Runs	Independent Variables			Responses		
	pH of the Buffer	% Organic phase	Flow rate	Retention time(tR)	Tailing factor	Theoretical plates (N)
1	6.00	60	1.00	4.963	1.991	6264.75
2	6.00	40	1.00	12.746	0.980	4443.73
3	5.00	50	1.00	7.105	0.989	1959.95
4	5.00	50	1.00	7.367	0.983	2105.35
5	5.00	40	1.20	10.103	1.094	4013.32
6	5.00	40	0.80	15.786	1.107	2598.12

7	5.00	50	1.00	7.369	0.932	2059.13
8	5.00	50	1.00	7.361	0.971	2095.56
9	6.00	50	1.20	6.200	1.485	4982.7
10	5.00	60	1.20	4.131	1.568	6142.87
11	5.00	50	1.00	7.367	0.973	2115.84
12	4.00	40	1.00	12.486	1.292	3678.44
13	4.00	50	1.20	5.927	1.485	4777.99
14	4.00	60	1.00	4.907	1.548	5466.29
15	5.00	60	0.80	6.2	1.603	6901.59
16	6.00	50	0.80	8.817	1.342	5190.93
17	4.00	50	0.80	8.477	1.366	6368.18

A chemometric approach was adapted to develop an improved isocratic RP-HPLC method for the determination of metaxalone in bulk and tablet dosage form. A response surface methodology with the aid of Box–Behnken design was efficiently employed to identify buffer pH, % organic phase and flow rate as significant factors influencing the chromatographic elution of the drug.

CONCLUSION

The outcome demonstrates that, at least in terms of specifications, the procedure is linear. Recovery values of pure medication ranged from 98 to 102%, as indicated in the accuracy table, proving the method's reliability. Therefore, the proposed procedure worked. System suitability characteristics were found to be within the limit when the flow rate, wave number, and mobile phase composition were varied, proving the method's resilience. The approach is linear, accurate, exact, robust, and sensitive; it may be utilized for the measurement of both amlodipine and telmisartan in their dose forms. The chromatographic technique for the examination of film-coated tablets of Triolmezest and Inderal passed the robustness test, yielding accurate findings for all four substances. Defining system suitability limits based on the worst-case results for which the circumstances were anticipated from the robustness test, permits to prevent an unpleasant situation when a technique is judged to be resilient for its quantitative aspect but certain externally set system suitability requirements are violated.

REFERENCES

1. Oliveira, VítorTodeschini& Clarice MadalenaBueno Rolim.2011.Rapid simultaneous determination of aliskiren and hydrochlorothiazide from their pharmaceutical formulations by monolithic silica hplc column employing experimental designs. Journal of LiquidChromatography&Related Technologies,vol.34(17), pages 1976-1996.

2. Nirogi R.V., M Shukla.,MudigondaV.N Kandikere. K.,Komarneni& P Mayurya S..,(2006), Simultaneous quantification of fexofenadine and pseudoephedrine in human plasma by liquid chromatography/tandem mass spectrometry with electrospray ionization: method development, validation and application to a clinical study, Rapid. Comm. Mass. Spectrom.,20, 3030-3038.

3. Radhakrishna T. and G.O Reddy.,(2002), Simultaneous determination of fexofenadine and its related compounds by HPLC, J. Pharm. Biomed. Anal., 29, 681-690.

4. Sharaf El-Din M.K., M.E.K.Wahba, F Ibrahim., &M.I Eid.,(2011), Validated stability indicating liquid chromatographic method for the determination of fexofenadine hydrochloride in presence of its degradation products. Application to tablets and content uniformity testing, Journal of Pharmacy Research,4, 2377-80

5. Chen S.M.,(2008), Separation and structure determination of nicardipine photoproducts by LC-ESI-MS. Biomed Chromatogr., 22 ,1008-1012.

6. Nalwade S, Ranga Reddy V, DurgaRao D, KoteswaraRao I.2011.Rapid simultaneous determination of telmisartan, amlodipine besylate and hydrochlorothiazide in a combined poly pill dosage form by stability- ndicating ultra performance liquid chromatography.Sci Pharm., vol. 79(1),69-84.

7. Jabir Aboobacker et al., 2012. Method Development and Validation of Hydrochlorothiazide, Amlodipine Besylate and Telmisartan in Tablet Dosage Form by RP-HPLC Method, Research Journal of Pharmaceutical, Biological and Chemical Sciences, 3(3), 509-517.

8. Dragica Zendelovska, Trajce Stafilov.2004.Development of solid-phase extraction method and its application for determination of hydrochlorothiazide in human plasma using HPLC.BiomedicalChromatography.vol.18(2), pages 71–76.

9. N. V. S. Ramakrishna, K. N. Vishwottam, S. Manoj, M. Koteshwara, S. Wishu and D. P. Varma.2005.Sensitive liquidchromatography–tandem mass spectrometry method for quantification of hydrochlorothiazide in human plasma.Biomedical Chromatography,vol.19(10), pages 751–760.

10. T. Hemke, M. V. Bhure, K. S. Chouhan, K. R. Gupta, and S. G. Wadodkar.2010.UV Spectrophotometric Determination of Hydrochlorothiazide and OlmesartanMedoxomil in Pharmaceutical Formulation. E-Journal of Chemistry, vol.7(4), Pages 1156-1161.

11. Vidhya et al., 2011. Validated HPLC method for simultaneous estimation of atenolol, hydrochlorothiazide and amlodipine besylate in bulk drug and formulation, International Journal of Analytical and Bio Analytical Chemistry, 1(3), 70-76.

12. Panchal et al., 2012. Development and Validation of Reversed-Phase LC Method for Simultaneous Determination Telmisartan, Amlodipine and Their Degradation Products in Fixed Dose Combination Tablets. Eurasain Journal of Analytical Chemistry3 (1), 28-42.

13. Rajitha et al., 2013. Method Development and Validation of Telmisartan and Amlodipine Besylate by RP-HPLC in Tablet Dosage Form. International Journal of Pharma Sciences, 3(5), 365-369.

14. Ashraf et al., Method development and validation of telmisartan and amlodipine besylate by RP-HPLC in Tablet Dosage form. Pharma Research Library.

15. Kayal et al., 2011. Method development and validation of telmisartan and amlodipine besylate by RP-HPLC in Tablet Dosage form. International Journal of Pharmaceutical Research and Development, 3(5).

16. Paul Kumar et al., 2011. Method development and validation of telmisartan and amlodipine besylate by RP-HPLC in Tablet Dosage form. International Journal of Pharmacy, 1(2).

17. Rajeswari et al., 2013. RP-HPLC method development and validation for Simultaneous estimation of amlodipine besylate and Telmisartan in tablet dosage form. International Journal of Pharmaceutical Research and Analysis, 3(1), 13-17.

18. Suresh Kumar GV et al., Development and validation of reversed-phase HPLC method for simultaneous estimation of telmisartan and amlodipine in tablet dosage form. International Journal of Pharma and Pharmaceutical Sciences, 1(3),128-131.

19. Hamed M.EL-Fatatry et al., 2013. Method Development and Validation of Telmisartan and Amlodipine Besylate by RP-HPLC in Tablet Dosage Form. International Journal of Biological and pharmaceutical Research,4(10),697- 701.

20. Paul et al., 2011. Method Development and Validation of Telmisartan and Amlodipine Besylate by RP-HPLC in Tablet Dosage Form. International Journal of Pharmaceuticals, s1(2),105-109.