WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 8, Issue 13, 123-133. Research Article ISSN 2277– 7105

STUDY OF FACTORS AFFECTING ON SOLVENT EFFECT AND PEAK SHAPE IN REVERSED PHASE CHROMATOGRAPHY

Xiaobing Zhan[1] and Dr. Vishal Shinde[2]*

Wanbang Biopharmaceuticals Rand D Center, Xuzhou, China.

Article Received on
06 Oct. 2019,

Revised on 27 Oct. 2019,
Accepted on 17 Nov. 2019

DOI: 10.20959/wjpr201913-15937

***Corresponding Author**
Dr. Vishal Shinde
Wanbang
Biopharmaceuticals Rand D
Center, Xuzhou, China.

ABSTRACT

In the application of chromatography, the Peak-shape problem is the most common trouble. It causes integration problems and poor resolutions. The goal of the present work is to, study the factors affecting on solvent effect and the peak shape of the chromatogram such as of different pH in the diluent (pH 3.0- pH6.8), addition of 0.5% SDS the solution, Different column temperature (30^0C-50^0C), Different column type (Column 1: 100-5-C18 (250×4.6mm, 5µm), Column 2: Zorbax SB-C18 (150×4.6mm,5µm) and mobile phase with different composition, In this study Aniline used as analyte and set the Chromatographic conditions as Column: 100-5-C18 (250×4.6mm, 5µm) Detection wavelength: 254mn, Flow rate: 1ml/min, Injection volume: 10µl. It explained the reason for the solvent effect, and it redefined the concept of the solvent effect. The solvent effect caused due to the above difference is because of the large difference between the distribution coefficient of the stationary phase-diluent and the distribution coefficient of the stationary phase-mobile phase. It results from the three-media effect i.e. stationary phase, diluent and mobile phase. Therefore, when assessing the risk of solvent effects, it may combine with the specific situation of these three media and to assess the strength of the interaction between the three media. In the distribution coefficient of the analyte in the stationary phase-diluent and the stationary phase-mobile phase, the partition coefficient may affect the different pH in the diluent, addition of SDS solution in the diluent, Different column temperature, Different column type and different mobile phase with different compositions and it can also be adjusted to optimize the effect of the solvent effect.

KEYWORDS: Liquid chromatography, Solvent effect, Reversed-Phase chromatography, Peak shape, Distribution coefficient, Aniline.

1. INTRODUCTION

In the application of chromatography, the Peak-shape problem is the most common trouble. It causes a distorted peak for integration problems and often for poor resolutions. When simple organic anions were injected in ion-exchange HPLC, the peak height and shape depended on the relative strengths of the injection solvent and the mobile phase. Injection solvent strength was varied by changing the solvent's eluent ion strength or by changing the solvent's organic composition.[2] The injection of a pulse of different viscosity from that of the mobile phase is underlying causes of distortion in early eluting bands strong eluting solvents (of exactly equal viscosity coefficients) are caused by broadening, not of peak distortions.[3] The effect of sample and mobile phase viscosities on peak shape, and detected that instabilities occur at the front boundary of a sample plug when this is less viscous than the mobile phase, in accordance proposed several years before in no chromatographic context.[4,5] Peak distortions generated in an aminopropyl silica column using arbutin as probe solute.[6] It can predict differences between sample solvent and car mobile phase viscosities but also by the solute and the sample solvent migration velocities shall affect the distortions of the solute band. It detected several studies of peak distortion in RPLC that may explain in terms of viscosity mismatch in a no exhaustive survey of the literature.[7-12,14] Hoffman, Pan, and Rustum observed leading peaks or peak splitting when a strong injection solvent was used and that when the elute was injected in a large volume of strong solvent, peak height was significantly reduced in RPLC.[13] However, there is little research on the relation between solvent effect and peak distortion. The goal of the present work is to, study the different factors affecting on solvent effect and the peak shape of the chromatogram, such as of different pH in the diluent, addition of solution in the diluent, Different column temperatures, Different column type and different mobile phases with different compositions. Set the Chromatographic conditions as Column: 100-5-C18 (250×4.6mm, 5µm) Detection wavelength: 254mn, Flow rate: 1ml/min, Injection volume: 10µl.[15-21]

2. MATERIALS AND METHODS

Study the factors affecting on solvent effect and the peak shape of the chromatogram such as of different pH in the diluent(pH 3.0- pH6.8), addition of 0.5% SDS solution, Different column temperature(30^0C-50^0C), Different column type (Column 1: 100-5-C18 (250×4.6mm, 5µm), Column 2: Zorbax SB-C18 (150×4.6mm,5µm) and mobile phase with different composition, In this study Aniline was used as a analyte and set the Chromatographic conditions as Column: 100-5-C18 (250×4.6mm, 5µm) Detection wavelength: 254mn, Flow

rate: 1ml / min, Injection volume: 10µl.

Ultimate 3000 HPLC (Thermo Scientific) was used for this study. HPLC Grade Methanol, acetonitrile were purchased from Merck. All other reagents and chemicals were of analytical grade hydrochloric acid, phosphoric acid, potassium dihydrogen phosphate, sodium hydroxide, aniline (the AR, Sinopharm Chemical Reagent Co., Ltd.), sodium lauryl sulfate (the CP, from Sinopharm Chemical Reagent Co., Ltd.).

3. RESULTS AND DISCUSSION

3.1 Effect of different pH values of diluent

Sample Preparation

Weighed and transferred about 20mg of Aniline in 100ml flask and diluted up to the mark with respectively 0.1mol / L hydrochloric acid, pH 3.0 phosphate buffer, pH 6.8 phosphate buffer.

Chromatographic conditions

Mobile phase: water (adjusted to pH 3.0 with phosphoric acid) - acetonitrile (80:20)
Column: 100-5-C18 (250 × 4.6mm, 5µm)
Detection wavelength: 254mn, flow rate: 1ml / min, column temperature: 30 ℃,
Injection volume: 10µl.

DISCUSSION AND ANALYSIS

For the ionizable components of weak electrolytes, the part of the ionization equilibrium is the ion state and the other part is the molecular state. The pH value of the diluent has a very large influence on the ionization equilibrium. The distribution of the two ionization states in the stationary phase and the mobile phase has a difference. In a reversed-phase system, the molecular state is more tightly bound to the stationary phase and the retention is stronger. The ionic state is more inclined to the mobile phase, and the retention is weaker. If the component to be tested has differs greatly in ionization state between the diluent and the mobile phase, it is likely to cause an abnormal peak shape. The results shown in Figure no. 1.

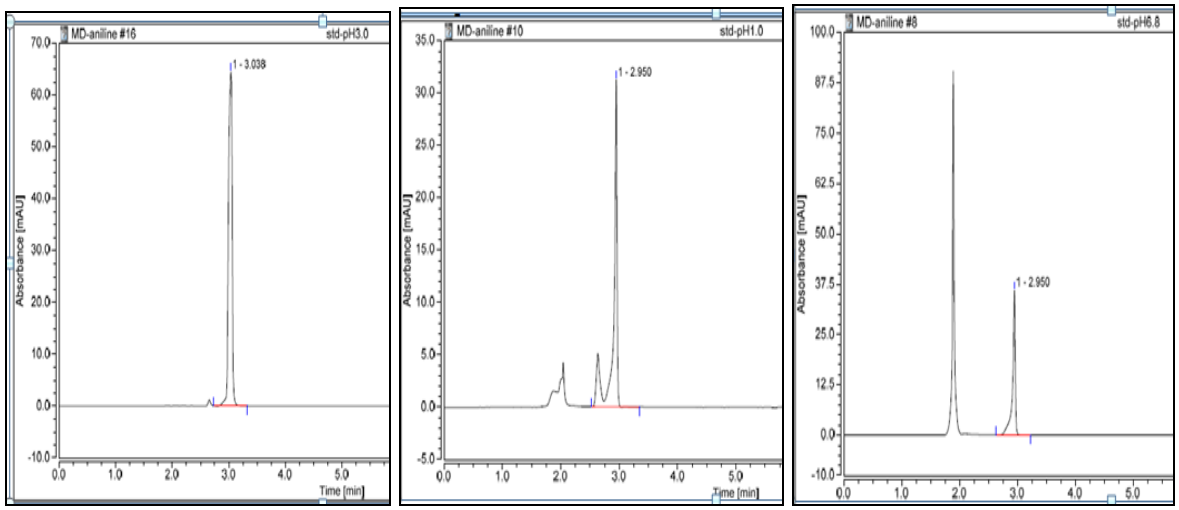

Fig no.1: Chromatograms of Effects of Different Ph (3.0, 1.0, and 6.8 Respectively) Values of Diluent in Solvent Effect and Chromatographic Peak Shape.

3.2 Effect of Sodium Dodecyl Sulfate (SDS) was Added Diluent

Sample Preparation

Weighed and transferred about 10mg of Aniline in 100ml flask and diluted up to the mark with diluted with 0.5% SDS solution, shake.

Chromatographic Conditions

Mobile phase: 0.005mol / L potassium dihydrogen phosphate solution (adjusted to pH 3.0 with phosphoric acid) - acetonitrile (80:20)

Column: 100-5-C18 (250 × 4.6mm, 5μm)

Detection wavelength: 254mn,

Flow rate: 1ml / min,

Column temperature: 30 ℃,

Injection volume: 10μl.

Discussion and Analysis

The chromatographic peak shape of the test substance in which the diluent is 0.5% SDS solution has a bifurcation phenomenon. The reason may be that the analyte is a basic compound, and the cation of the analyte forms an ion association with the dodecyl sulfate anion. The substance is retained in the reversed phase chromatography to be greatly enhanced relative to the analyte, and the mobile phase cannot form the association with the analyte. The mobile phase requires a more efforts to eliminate the influence of the association, thereby

causes an abnormal shape of the peak. This phenomenon of solvent effect will be effect on the peak shape of the analyte. The results shown in Figure no. 2.

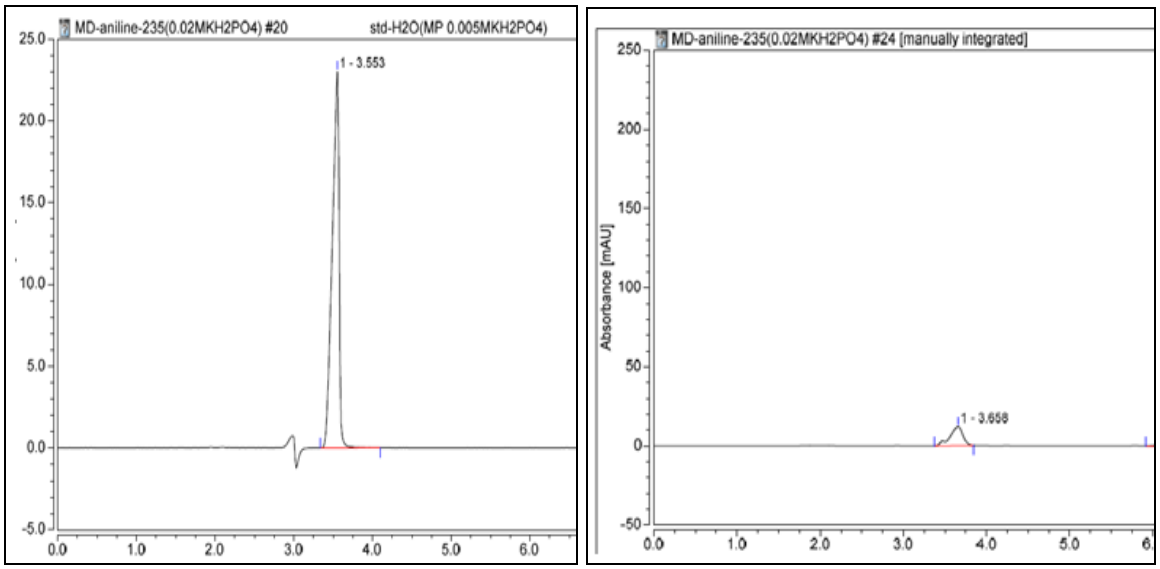

Fig. No 2: Two chromatograms shows effects of SDS addition in diluent on solvent effect and peak shape. First chromatogram shows normal peak shape when standard prepared in water and second chromatogram shows the solvent effect and peak distortion due to the standard prepared in 0.5 % SDS.

3.3 Effect of Column Oven Temperature

Sample Preparation

Prepared 0.1 mol/L aniline sample solution in hydrochloric acid, and the column temperature was changed. The other chromatographic conditions were the same as first study. The test results were as follows:

Discussion and Analysis

Generally temperature produces the smallest changes in selectivity; however, it can be usefully used to optimize separations, especially when the analyte involved in ionization.

The experimental results show that the column temperature has a significant influence on the peak shape of the analyte. The reason may be that the change of the column temperature changes the ionization equilibrium constant of the analyte. The ratio of the ion state of the analyte to the molecular state is changed, thus changing the chromatographic behavior of the analyte. The results shown in Figure no. 3.

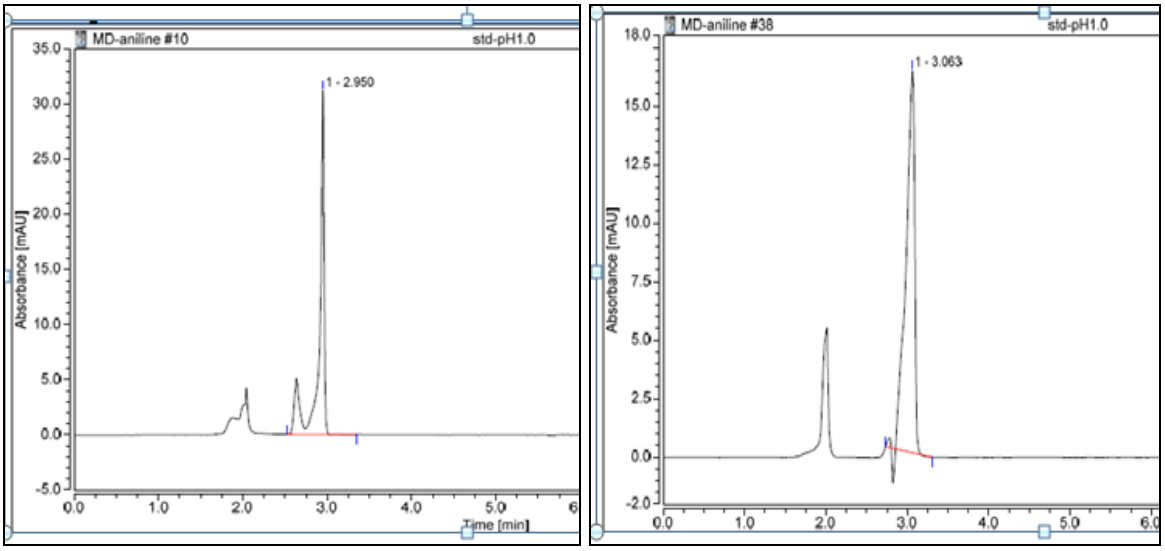

Fig No. 3: Chromatograms of Effect of Column Temperature (Column Oven Temperature 30^0c And 50^0c Respectively) on Peak Shape Chromatograms Shows Change In Chromatographic Behavior Of The Analyte.

3.4 The Effect of The Mobile Phase

Sample Preparation

Prepared 0.1 mol/L aniline sample solution in hydrochloric acid, and study was done with different mobile phase. The other chromatographic conditions were the same as first study. The test results were as follows:

Chromatographic Conditions

Mobile phase 1: Water (pH adjusted to 3.0 with phosphoric acid) - acetonitrile (80:20)

Mobile phase **2**: water - methanol (1: 1)

Mobile phase 3: 0.02mol / L potassium dihydrogen phosphate solution (adjusted to pH 3.0 with phosphoric acid) - acetonitrile (80:20)

Column: 100-5-C18 (250 × 4.6mm, 5μm)

Detection wavelength: 254mn,

Flow rate: 1ml / min,

Column temperature: 30 ℃,

Injection volume: 10μl.

Discussion and Analysis

Generally the mobile phase pH can have a drastic effect on the selectivity of a separation, especially when acidic or basic analyte molecules are involved, for acidic analyte the pH

range 2.5 to 6.5 is particularly important. Small changes in pH can bring about large changes in retention behavior of certain peaks within the chromatogram. For this particular separation, it would be difficult to identify a single pH value at which all peaks are satisfactorily

The pH of the mobile phase is usually a key parameter for selectivity optimization when dealing with analyte molecules that have ionizable groups. Changes in mobile phase pH should be undertaken carefully as not all silica based HPLC columns are resistant to extremes of pH.

The experimental results show that different mobile phase have significant effects on chromatographic behavior. The mobile phase 1 and the mobile phase 3 have the same pH value, and the concentration of the salt is different, and the shape of the chromatographic peak of the analyte is significantly different. It is speculated that the mobile phase 3 can improve its buffering capacity and can change the analyte more strongly. The ionization state is consistent with the mobile phase, reducing the adverse effects of the difference in ionization state. The mobile phase 2 has no buffering capacity, so the peak of the analyte can hardly be detected. The results shown in Figure no.4.

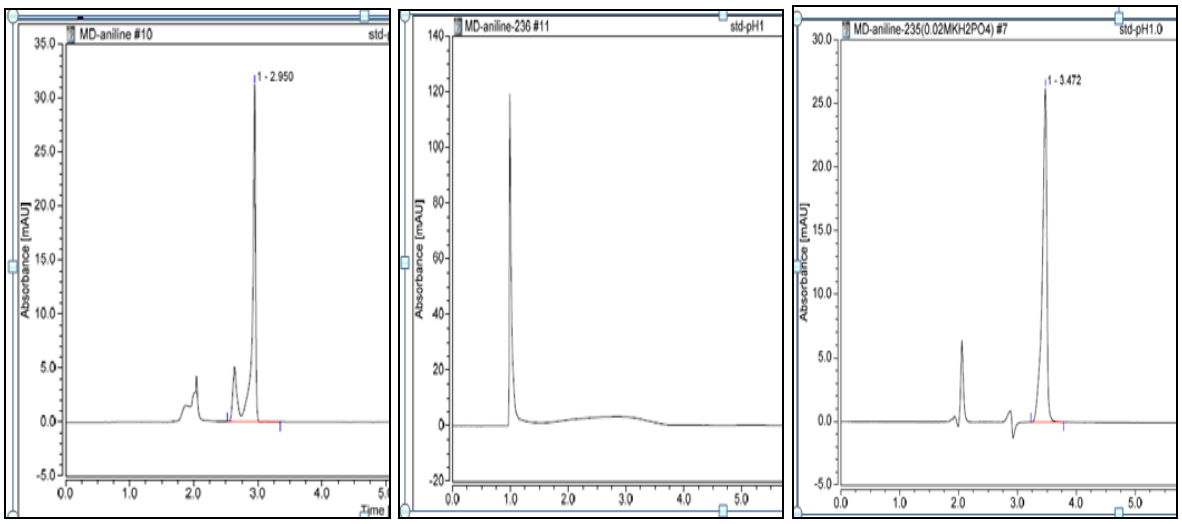

Fig. No 4: Chromatograms of Effect of Mobile Phase (Mobile Phase 1, 2 And 3 Respectively) Shows Different Solvent Effect And Peak Shape.

3.5 Effect of different Column

Sample Preparation

Weighed and transferred about 10mg of Aniline in 100ml flask and diluted up to the mark with acetonitrile: water (4: 1).

Chromatographic conditions

Mobile phase: water - methanol (1: 1)

Column 1: 100-5-C18 (250 × 4.6mm, 5μm)

Column 2: Zorbax SB-C18 (150 × 4.6mm,5μm)

Detection wavelength: 254mn, flow rate: 1ml / min, column temperature: 30 ℃, injection volume: 10μl.

Discussion and Analysis

Significant differences in both the reduced and non-reduced axial diffusion behaviour of solutes in the RP and HILIC modes were observed on columns made from the same silica[22]**.** Here we can see how changing the column stationary phase can affect the selectivity of a separation. The hydrophobicity, polarity, and nature of the base silica all play a critical role in the physicochemical interaction with the analyte. As a chromatographer one of the most powerful options to change a separation will come from changing the stationary phase. Picking the correct stationary phase is amongst the most important choices to be made when developing methods.

The experimental results show that different types of column also have significant effects on chromatographic behavior. Generally the peak shape of analyte is depends on the below factors.

1. Silica type/acidity: Fully hydroxylated silica and silica metal content (Silica silanol ionization, Hydrogen bonding with silica silanols and Column bonding). 2. Endcapping and 3. Type of bonded phase (Packing Pore Size/Structure).

Here Even though both columns are packed with base deactivated C18 stationary phases, the band spacing (selectivity) between peaks is very different on the two columns which caused different solvent effect. The results shown in Figure no. 5.

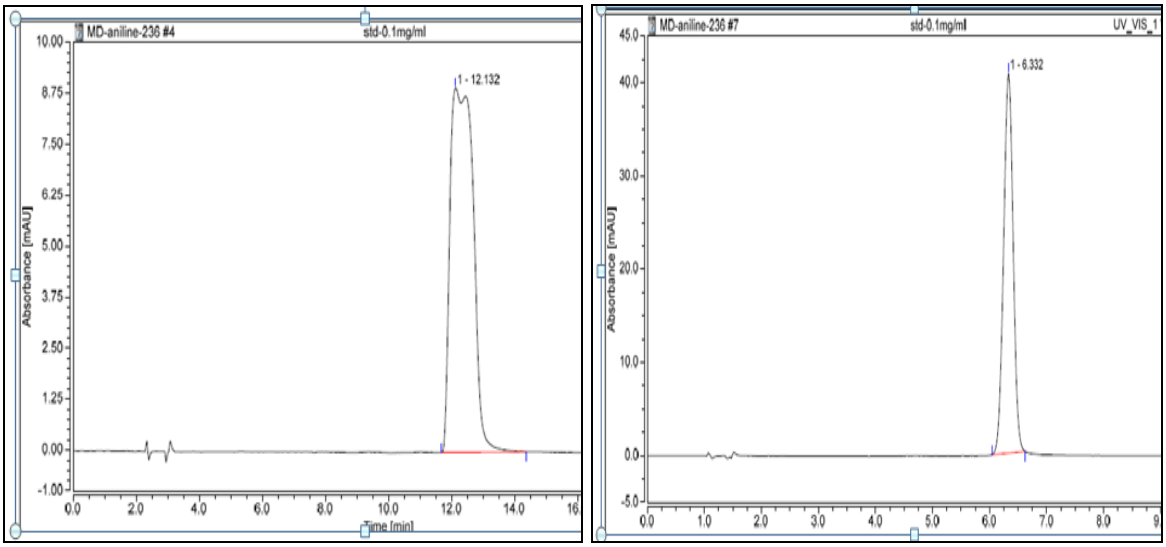

Fig. no 5: Chromatograms of effect Of Different Column (Column 1 And Column 2 Respectively) On Peak Shape.

The currently accepted understanding of the solvent effect is the solvent strength of the diluent is greater than that of the mobile phase. If the sample is dissolved in pure acetonitrile and injected into an acetonitrile-water (18:82) reversed phase system, the result of peak bifurcation or tailing will result. However, this concept does not explain other chromatographic behavioral anomalies caused by diluents in practice, such as the above-mentioned factors different pH values in the diluent and the addition of SDS in the diluent caused abnormal peak shape of the chromatogram. In these cases, the solvent strength of the diluent is not necessary, it is larger than the mobile phase, but the result in abnormal chromatographic behavior has occurred.

Since many chromatographic behavioral anomalies caused by diluents cannot be explained by the current solvent effects[14], I believe it is necessary to re-explain the concept of solvent effects: solvent effects are due to the specific components in the test sample in the diluent. The adverse effects of differences in the state of the mobile phase on chromatographic behavior[15]. If this adverse effect is more serious, it may cause obvious chromatographic behavior abnormalities, such as peak bifurcation, tailing, and the like.

The solvent effect caused by the above differences is caused by the large difference between the distribution coefficient of the stationary phase-diluent and the distribution coefficient of the stationary phase-mobile phase. It is the result all three media stationary phase, diluent and mobile phase play important role. Therefore, when assessing the risk of solvent effects, it

should be combined with the specific situation of the three media to assess the strength of the interaction between the three media.

Hence, the effect of eliminating or reducing the solvent effect can be started from the three aspects of diluent, mobile phase and stationary phase. If the diluent is close to the mobile phase, the amount of diluent can be reduced, or prepare the mobile phase is close to the nature of the diluent. Enhance the ability of the mobile phase to change the state of the analyte; or change the type and model of the stationary phase to reduce the solvent effect of the difference in the state of the component to be tested on the chromatographic behavior.

4. CONCLUSION

Since the distribution coefficient of the analyte in the stationary phase-diluent and the stationary phase-mobile phase, the partition coefficient are also affected by the pH of diluent, addition of SDS in diluent, column temperature, column type and mobile phase with different compositions and can also be adjusted to optimize the effect of the solvent effect and peak shape distortion.

5. ACKNOWLEDGEMENTS

This study was supported by Wanbang Biopharmaceutical Biopharmaceuticals R& D center, Pantaoshan south road, Xuzhou city, China.

6. AUTHORS CONTRIBUTION STATEMENT

Mr. Xiaobing Zhan conceptualized and Dr. Vishal Shinde gathered the data with regard to this work. Mr. Xiaobing Zhan and Dr. Vishal Shinde analyzed these data and necessary inputs were given towards the designing of the manuscript. All authors discussed the methodology and results and contributed to the final manuscript.

REFERENCES

1. Xiaobing Zhan, Dr. Vishal Shinde, International Journal of Science and Research (IJSR), 2019, 21275/ART20197459.
2. Lee HK, Hoffman N E. INJECTION SOLVENT EFFECT ON PEAK HEIGHT IN ION-EXCHANGE HPLC[J]. Journal of Chromatographic Science, 1992; 30(10): 415-421.
3. Hoffman N E, Chang J H Y. Injection Solvent Enhancement of Peak Height in Reversed-Phase Liquid Chromatography[J]. Journal of Liquid Chromatography, 1991; 14(4): 651-658.

4. S. Hill, Chem. Eng. Sci., 1952; 1: 247.
5. P.G. Saffman, G. Taylor, Proc. R. Soc. London, Ser. A, 1958; 245: 312.
6. R.C. Castells, C.B. Castells, M.A. Castillo, J. Chromatogr. A, 1997; 775: 73.
7. F. Khachik, G.R. Beecher, J.T. Vanderslice, G. Furrow, Anal. Chem., 1988; 60: 807.
8. P.K. Tseng, L.B. Rogers, J. Chromatogr. Sci., 1978; 16: 436.
9. M. Zapata, J.L. Garrido, Chromatographia, 1991; 31: 589.
10. N.E. Hoffman, S. Pan, A.M. Rustum, J. Chromatogr, 1989; 465: 189.
11. J. Kirschbaum, S. Perlman, R.B. Poet, J. Chromatogr. Sci., 1982; 20: 336.
12. D. Vukmanic, M. Chiba, J. Chromatogr, 1989; 483: 189.
13. N.E. Hoffman, S-L. Pan, and A.M. Rustum. Injection of eluites in solvents stronger than the mobile phase in reversed-phase liquid chromatography. *J. Chromatogr*, 1989; 465: 189-200.
14. Castells C B, Castells R C. Peak distortion in reversed-phase liquid chromatography as a consequence of viscosity differences between sample solvent and mobile phase[J]. Journal of Chromatography A, 1998; 805(1–2): 55-61.
15. Poole CF The essence of chromatography. Elsevier, Amsterdam, 2003.
16. Anali S, Haddad PR, Poole CF, Riekkola M-L (eds) Liquid chromatography, 2nd edn. Elsevier, Amsterdam, 2017; 1(2).
17. Snyder LR, Kirkland JJ, Glajch Practical HPLC method development, 2nd edn. Wiley-Interscience, New York, (1997).
18. Roses M, Subirats X, Bosch E Retention models for ionizable compounds in reversed-phase liquid chromatography. Effect of variation of mobile phase composition and temperature. J Chromatogr A, 2008; 1216: 1756–1775.
19. Vailaya A, Horvath C Retention in reversed-phase liquid chromatography: partition or adsorption. J Chromatogr A, 1998; 829: 1–37.
20. Tan LC, Carr PW Revisionist look at solvophobic driving forces in reversed-phase liquid chromatography—2. Partitioning vs. adsorption mechanism in monomeric alkyl bonded phase supports. J Chromatogr A., 1997; 775: 1–12.
21. Wang M, Mallette J, Parcher JF Fundamental mathematical relationships for retention volumes in liquid chromatography. J Chromatogr A., 2008; 1213: 105–109.
22. James C. Heaton, Xiaoli Wang, William E. Barber, Stephan M.C. Buckenmaier, David V. McCalley, Journal of Chromatography A., 2014; 1328: 7–15.