Factorial design was used to develop and validate the HPLC method for Valsartan nanoparticle determination.

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Abstract:
According to the ICH guidelines, a high performance liquid chromatographic technique was devised, optimised, and validated. In this investigation, the mobile phase for the measurement of valsartan was composed of 20 mM ammonium formate andacetonitrile in a 57:43 ratio. The influence of variable components was optimised using a full factorial design. Peak area, tailing factor, and number of theoretical plates were the answers. On peak area, the quadratic effects of flow rate and wavelength both separately and in combination were most significant (p 0.0001 and p 0.0086, respectively); the

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1 **Introduction**:
These days, cardiovascular diseases are the leading cause of death worldwide, not only in underdeveloped nations. Coronary heart disease, hypertension, ischemic heart disease, cerebrovascular illness, rheumatic heart disease, heart failure, elevated cholesterol levels, etc. are all linked to cardiovascular problems. Considering the patterns of mortality in emerging nations By 2015, around 1.5 billion fatalities are anticipated as a result of this illness (Grundy et al., 2004; Kearney et al., 2005; Gonzalez et al., 2009; Solanki et al., 2014). A major contributing factor to cardiovascular disease is hypertension. For the treatment of hypertension, a variety of antihypertensive medications are available. Blockers of the angiotensin II receptor (subtype AT1) are the principal medications used to treat hypertension. It is Valsartan.

2. **Instrumentation**:
In this investigation, a Shimadzu Corporation, Kyoto, Japan, LC-2010CHT model HPLC system with a dual wavelength UV detector, column oven, and auto sampler was utilised. This system was linked to a PDA (Photodiode Array, model no. SPD-M20A PDA with 220-230 V). Computerized equipment was used to record and process chromatograms. use the LC solution 5.57 release. The separation was carried out using a C18 Phenomenex, security Guard column with a HyperClone (Phenomenex) C18 column (250 mm 4.6 mm id, 5 lm, BDS 130 A) as the separation column. The mobile phase was filtered using a vacuum pump-attached Millipore glass filter assembly with a cellulose nitrate grid that has a 0.22 l capacity and a 47 mm diameter.

2.2 Chemicals and solvents:
Valsartan was received as a gift Sample from Lupin Ltd. in Goa, India. The company Sisco Research Laboratories Pvt. Ltd. sold ammonium formate (Mumbai, India). Acetic acid bought it from RFCL Limited (New Delhi, India). We bought Acetonitrile HPLC Grade from Finar Limited (Ahmadabad, India). I bought Methanol HPLC Grade from Labort Fine Chem Pvt. Ltd. (Surat, India). A Direct-Q 3 water purification system from Millipore Corporation generated high purity water (18.2 MX cm resistivity) (Billerica, MA, USA).

2.3. Buffer preparation for the mobility phase:
For the mobile phase, an ammonium formate buffer was produced.The buffer solution containing 20 mM ammonium formate was made. Ammonium formate was dissolved in 1000 mL of Milli Q water, and formic acid was used to adjust the pH to 3.0.

2.4. Setting up the mobile phase.
The ratio of buffer to acetonitrile in the mobile phase was 57:43. Because it is volatile and does not precipitate in a column or HPLC system, ammonium formate was employed to prepare the mobile phase (Dolan, 2014). So there are less risks of developing excessive back pressure. Before being pumped into the HPLC system for degassing, the mobile phase was passed through a filter with a 0.22 l pore size and sonicated for 15 min.

2.5. HPLC technique development and optimization.
C18 silica packed HPLC column was employed in the current investigation for improved separation. The separation was performed with a C18 Phenomenex, 4 3.0 mm id, security guard column and a HyperClone (Phenomenex) C18 Column (250 mm 4.6 mm id, 5 lm, BDS 130 A). Because acetonitrile has a low viscosity, it was utilised as an organic solvent to reduce the high internal pressure of the column. In the HPLC process, the flow rate, buffer solution pH, and wavelength all matter a lot. Therefore, based on DOE, these three parameters were optimised in the current study. Valsartan has a 4.9 pKa value and is a moderately acidic medication (Cagigal et al., 2001). Ammonium formate comes in three pH ranges, including 2.8,

2.6. HPLC method validation:
According to the ICH Q2 (R1) guidelines for the following parameters, including linearity, accuracy, precision, limit of detection (LOD), and limit of quantitation (LOQ) (Solanki et al., 2014; ICH Harmonized Tripartite Guideline and Methodology Q2 (R1), 2005), the developed and optimised method was validated.

2.6.1. System suitability:
Valsartan stock solution of 6 lg/mL was used to determine the appropriateness of the system. The HPLC analysis of six duplicate injections of this standard solution. The acceptability criteria for peak area, tailing factor (5%), tailing factor (10%), and number of theoretical plates were studied from these repeat injections.

2.6.2. Linearity:
The 0.1 to 12 lg/mL concentration range was used to produce the standard calibration curve. For this investigation, three duplicate injections of each concentration were examined. The graph between peak area and concentration revealed the linear regression and correlation coefficient.

2.6.3. Accuracy (Precision):
A research called "precision" is based on both intra-day and inter-day precision. By using repeatability, the new method's precision was assessed (six times of each concentration)

2.6.4. Reliability (Accuracy):
By calculating Recovery values, the precision of the devised approach was investigated. The value that is close to the value of the reference is what is meant by the word accuracy. The standard medication in the percentage range of 75%, 100%, and 125% is used to determine accuracy. The sample solution was combined with the sample's concentration (2 lg/mL), yielding final concentrations of 3, 4, and 5 lg/mL. The identical procedure has been carried out three times. The following formula was used to determine the % recovery (Sagirli et al., 2007) of the standard medication administered to the test samples:
% recovery is equal to [(Cs-Cf)/Cs] 100.

Where Cf is the concentration of analyte in the nano-formulation, Cs is the standard concentration, and Cc is the concentration of analyte present in the combination of standard and test.

2.6.5. Limits of quantification and detection:
For the limit of detection (LOD), the sigma approach was employed (Awotwe-Otoo et al., 2012). The slope and least standard deviation found in the answer serve as the foundation for this methodology. LOD was calculated using the formula LOD = 3.3r/SP, where r represents the response's tiniest standard deviation number and SP represents the calibration curve's slope. Similar to this, valsartan's reaction was used to compute the limit of quantitation (LOQ). LOQ was calculated using the following formula: LOQ = 10r/SP, where r represents the response's least significant standard deviation and SP represents the response's slope.

3. Findings and commentary (Results and Discussion):

3.1. Development and improvement of the HPLC technique:
The peak area, tailing factor (5%), and number of theoretical plates were determined by doing the 27 runs in this investigation. To calculate the impact of variables on peak area, tailing factor, and number of theoretical plates, perturbation plots were made using Design Expert version 9.0.3.1 software. Perturbation plots show how self-governing variables affect dependent factors and how independent factors affect peak area. It demonstrates that the peak area is unaffected by wavelength (B) or buffer pH (C), but is greatly impacted by flow rate (A). The peak area gets smaller as the flow rate goes up. Grants the Peak area (R1) is calculated as follows: +(3.261E + 005) -68744.94A -2848.56B + 325.83C 91.58AB -1326.42AC - 269.92BC +13321.83 A2 + 1468.33B2 +27.17c2.

R2's tailing coefficient is 1.14. (1.111E-003) A + (1.111E-004) (1.111E-004) B + 0.039C + (2.500E-004) (2.500E-004) AB – (2.917E-003) (2.917E-003) AC –(1.167E-003) (1.167E-003) BC – (2.222E-004) (2.222E-004) A2 + (1.111E-004) (1.111E-004) B2 +0.011C2.
R3 = +12240.15 -1937.06A – 57.33B – 1124.67C + 52.75AB – 748.33AC + 3.33BC – 528.94A2 + 1108.89B2 – 578.11C2.

Where, correspondingly, Peak Area, Tailing Factor, and Number of Theoretical Plate are the Response Factors R1, R2, and R3. The buffer's flow rate, wavelength, and pH are denoted by letters A, B, and C, respectively.

The independent quadratic effects of flow rate and wavelength The quadratic effect of buffer pH was also most significant effect (p 0.0001) on tailing factor (5%) while the quadratic effect of flow rate and wavelength individually was significant (with p = 0.0006 and p = 0.0265, respectively). Additionally, interaction was most significant (p 0.0001 and P 0.0086, respectively) on peak area.

3.2. Validation of optimised factors, Section:
The outcomes of independent variables that were optimised, such as flow rate, wavelength, and buffer pH, were confirmed by evaluating the differences between expected and actual results. As indicated in table 3, there was only an 11% discrepancy between the actual and projected outcomes. The percent residual value is calculated using the formula: Percent residual = Predicted outcomes - Observed results. Results predicted / Predicted result 100. The optimization factor's attractiveness is demonstrated by the desirability values, which are typically in the range of 0 to 1. A score close to zero indicates that the method's solution is weak, while a value closer to one side indicates that the method's solution is extremely robust. The approach is quite powerful, as evidenced by the highest (i.e. 1) desirability value that was found.

3.3. **Method validation:**
3.3.1. Selectivity:
According to ICH Q2 (R1) criteria (ICH Harmonized Tripartite Guideline and Methodology), the study was carried out.Q2 (R1), 2005). The chromatogram shows 6 lg/mL and a blank. Valsartan concentration is depicted in the drug's retention time was discovered to be 10.177 minutes.

3.3.2. System suitability:
The percent relative standard deviations of the peak area, the tailing factor (5%), the tailing factor (10%), and the number of theoretical plates were used to indicate the system appropriateness. Six duplicates of the 6 lg/mL test were run to see whether the system was suitable. Reports the percentage RSD of peak area, tailing factor (5%), tailing factor (10%), and number of theoretical plate data. Peak size, tailing factor (10%), and number of theoretical plates all had percent RSD values that were less than 0.5%, while the percent RSD value of the tailing factor (5%) was determined to be less than 1%.

3.3.3. Linearity:
The accepted value for correlation coefficients is r2 = 0.9976, which is shown on the standard calibration curve for the valsartan concentration range of 0.1–12 lg/mL. The concentration range determined by the aforementioned linear regression is y = 54061x + 10210.

3.3.4. Accuracy (precision):
The intra-day and inter-day precision of the newly devised approach were assessed, and the precision was reported as a percentage relative to peak area standard deviations. Six repetitions of 1, 6, and 12 lg/mL were performed to test intra-day and inter-day precision. Table 5 lists the percentage RSD of the peak region of the intra-day and inter-day precision findings. All three concentrations'% RSD values for intra-day precision and inter-day precision, respectively, were determined to be less than 1%.

3.3.5. Reliability. (Accuracy):
Three different concentration levels of the standard drug—75%, 100%, and 125%—were added to samples to conduct the accuracy study. The percent recovery ranged from 98.57 to 100.27% for these three distinct concentration levels. This paper recommends a practical approach for doing routine experimental drug formulation analysis.

3.3.6 Limits of Quantitation and Detection:
The sigma technique was used to determine the limit of detection and limit of quantitation. The thresholds for quantitation and detection were discovered to be 14.833 ng/mL and 44.95 ng/mL, respectively.

4. **Finality (Conclusion):**
DOE was used to effectively create and optimise the approach, while Design Expert 9.0.3.1 software was used to evaluate the results. ANOVA was used to examine the significant effects of independent variables, and the results were also shown as perturbation plots. Effective tools for the optimization of variable parameters for HPLC method development are provided by experiment design. Additionally, the approach was verified, and according to the findings, it is brand-new, straightforward, accurate, exact, affordable, and resilient for the study of valsartan in Nano formulations.

References:

1. Awotwe-Otoo, D., and C. Agarabi Lee, S., Khan, M.A., and Shan, R.B. (2012). Faustin, P.J., Habib, M.J. Application of quality by design principles in the creation and improvement of a protamine sulphate analytical technique J. Pharmaceutics boomed from 1962 to 1967.
2. Bozkir, A., and Saka, O.M. creation and study of 5-Fu Nano-Particles using experiments based on factorial designs. Farmaco II, 60, 840, 846.
3. Cagigal, E., Gonzalez, L., Alonso, R. M., and Jimenez, R. M. Angiotensin II receptor antagonist (ARA-II) Pka determination by spectroscopy. Drug industry boomed, 26,477-486.
4. B.K. Sharma, Instrumental Method of Chemical Analysis: HPLC, 24th Edition, Goel Publisher, 2005, pp. 286-300.
5. Design optimization, manufacture, and evaluation of valsartan dispersion granules and formulation into tablets. Kapadia, J., Shrivastava, R., and Ursekar, B. 2009. Delivery of Current Drugs 6:28–37.
6. Smith P.'s (1996) in vitro approach for assessing intestinal permeability and metabolism. Bio-Tech 13-34 Pharama.

7. HAN, J., MA, B, and LI, J. Valsartan dispersible tablet preparation and content analysis. 145–146, Shizhen Guoyi Guoyao, V.18 (2007).
8. High-performance liquid chromatographic analysis of the angiotensin-II receptor by Daneshta lab, Lewanczuk, and Jammalik. Valsartan is an antagonist that is extracted using liquid. 2002; J Chromatogr B; 766; 345-9. (Pub. Med.)
9. Okafo, N., Ye, C., Liu, J., and 2000. JMP- (SAS Institute) designed the experiment and analysed the data in the validation of the analytical procedure. 23, 581-589 J. Pharmaceut. Biomed.
10. Yang, Y.J., Song, D.M., Jiang, W.M., and Xiang, B.R. Simultaneous detection of sildenafil, vardenafil, and tadalafil in pharmaceutical preparations and fake medications using the Rapid Resolution RP-HPLC-DAD technique. 43, 373–380, Anal. Lett.
11. Pathak, K., Philip, A.K., and Singh, J., 2008. Design and assessment of orodispersible indomethacin formulation optimization studies. 9, 60–66, AAPS PharmSciTech.