ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF DILTIAZEM HYDROCHLORIDE IN BULK AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

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Abstract: A simple, specific, precise and accurate Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the estimation of Diltiazem HCl (Diltiazem Hydrochloride) in Bulk and Pharmaceutical dosage form. The chromatographic determination was performed on isocratic High performance liquid chromatography system of Agilent model no.1220. The separation was conducted by using column of Zorbax C8 (5µ, 4.6mm×250) with mobile phase consisting of buffer and Acetonitrile in the ratio of (60:40). The mobile phase was delivered at the flow rate of 1.0ml/min. The eluent was monitored at wavelength 240 nm and found a sharp and symmetrical peak with retention time of 4.66min. The method was validated for linearity, accuracy, precision, specificity, robustness. Recovery of Diltiazem HCl was found to be in the range of 98%-102%. The method was found to be linear over the concentration range 50-150 µg/ml with coefficient correlation r² = 0.995. After developing method and studying validation parameter to obtain result complying with USP monograph.

Keywords: Diltiazem HCl, precision, accuracy, Method Validation.

Introduction

Diltiazem HCl is a calcium channel blocker, which has been used in the treatment of various cardiovascular disorders, particularly angina pectoris and systemic hypertension. Chemically it is 1, 5-Benzothiazepin-4(5H)-one, 3-(acetyloxy)-5-[2- (dimethylamino) ethyl]-2, 3-dihydro-2-(4-methoxyphenyl)-, monohydrochloride.

![Fig 1: Structure of Diltiazem HCl](image)

Methods and Material

Diltiazem HCl pure drug generously gifted by local industry (Wockhardt, Aurangabad). The formulation of Diltiazem HCl capsule USP containing 90mg Diltiazem HCl was procured from local market.
**Chemicals and Reagents:**

Potassium monobasic phosphate (Analytical grade), Methanol (HPLC grade), Acetonitrile (HPLC grade), Triethylamine (Analytical grade), and Water (HPLC grade) were purchased from Research –Lab Fine Chem Industries, Mumbai.

**Experimental Conditions:**

Quantitative HPLC was performed on isocratic HPLC of Agilent model no. 1220 with ezchrom elite software G 4286B-1220 infinity isocratic LC manual injector with variable wavelength detector. For method development several trials are carried out. After many trials, the chromatographic conditions are finalised. The separation was conducted by using column of Zorbax [C₈ (5µ, 4.6mm×250)] with mobile phase consisting of buffer and Acetonitrile in the ratio of (60:40). The mobile phase was delivered at the flow rate of 1.0ml/min. The eluent was monitored at wavelength 240 nm and found a sharp and symmetrical peak with retention time of 4.66min. The run time was 10 min.

**Preparation of standard solution:**

Transferred an accurately weighed quantity of about 60mg of Diltiazem hydrochloride into a clean dry 50ml volumetric flask, add methanol to dissolve the content and make up the volume with methanol, Dilute 1ml of resulting solution to 50ml with methanol.(12µg/ml)

**Preparation of sample solution:**

Accurately Weighed 5 Capsules and transferred the powder of capsule 213.28mg,Grind the contents thoroughly, and transfer an accurately weighed portion, equivalent to about 60 mg of Diltiazem hydrochloride, to a 100 ml volumetric flask. Add methanol and make up the volume of volumetric flask, and shake by mechanical means for 30 minutes. Sonicate the resulting solution for 10 minutes to complete the extraction. Diluting 1.12ml of resulting solution to 100 ml methanol. (12µg/ml)

**Diluent:**

The methanol is used as diluent.

**Method Validation**

Validation is an act of proving that any procedure, process, equipment, material, activity or system performs as expected under given set of conditions and also give the required accuracy, precision, sensitivity, etc

**System suitability test:**

Standard solution of Diltiazem HCl injected five times into the HPLC system. The system suitability parameters were evaluated and found to be within the limits.

**Precision:**

The Precision of the method was demonstrated by system precision, method precision studies and intermediate precision. In system precision, the standard solution injected five times as per procedure. In method precision six replicate injections of the standard solution and sample solution prepared as per the proposed method and chromatograms were recorded. In Intermediate precision precision was performed on different day by using different make column of same dimensions.
Accuracy:

Accuracy is calculated the percentage recovery by the assay of the known amount of analyte in the sample or as the difference between the mean and the accepted true value together with confidence intervals. For assay method, spiked samples are prepared in triplicate at three intervals over a range of 50-150% of the target concentration.

Linearity:

It is the ability of an assay to obtain test results, which are directly proportional to the concentration of an analyte in the sample. For the establishment of linearity, a minimum of 5 concentrations required. For assay of a drug substance or a finished product 50-150% of the concentration should be taken.

Specificity:

Specificity study was carried out by injecting blank, standard and sample solution of Diltiazem HCl and it shows no interference of standard and sample in the blank preparation.

Fig 2: Chromatogram for diluent

Fig 3: Chromatogram for blank

Fig 4: Specificity Standard chromatogram of Diltiazem HCl
Robustness:

The robustness of the methods was determined by performing the assay of the triplicate by deliberately alternating parameters and that the results are not influenced by different changes in the above parameters.
Change in wavelength: ± 2nm
Change in flow rate: ± 0.2ml/min.
Change in pH: ± 0.2

Stability of analytical solution:

Evaluate the stability in analytical solution by injecting the standard preparation and sample preparation of Diltiazem HCl at regular interval. The stability of solution is carried out by 0, 3, 6, 12, 24, hrs.

Results and Discussion

The aim is to develop accurate and precise method for the quantitative estimation of Diltiazem HCl in bulk and pharmaceutical dosage form. For method development several trials are carried out. After many trials, the chromatographic conditions are finalized. The separation was conducted by using column of Zorbax [C₈ (5µ, 4.6mm×250)] with mobile phase consisting of buffer and Acetonitrile in the ratio of (60:40). The mobile phase was delivered at the flow rate of 1.0ml/min. The eluent was monitored at wavelength 240 nm and found a sharp and symmetrical peak with retention time of 4.66min. The run time was 10 min.

| Sample  | Amount taken | Area   | 98.44% |
|---------|--------------|--------|--------|
| Standard| 60 mg        | 836126 |
| Sample  | 213mg        | 864991 |

| Sr. No | Sample | Area | R.T. | Theoretical plates | Tailing factor |
|--------|--------|------|------|--------------------|----------------|
| 1.     | Blank  | -    | -    | -                  | -              |
| 2.     | Std. 1 | 857171 | 4.643 | 12643              | 1.08           |
| 3.     | Std. 2 | 874529 | 4.653 | 15479              | 0.73           |
| 4.     | Std. 3 | 860542 | 4.650 | 12840              | 1.08           |
| 5.     | Std. 4 | 884768 | 4.653 | 8631               | 0.82           |
| 6.     | Std. 5 | 897971 | 4.660 | 8701               | 0.84           |
| Average|        | 87496.2 | 4.651 | 11658.8            | 0.91           |
| %RSD   |        | 1.93   |      |                    |                |

Table 01:
Result of Assay

Table 02:
Results of System suitability Test
The blank, standard solution and sample solution are injected. There should be no interferences of standard and sample in blank preparation.

**Table 03:**
Results of System Precision

| Sr. No | Sample | Area   | R.T  | Theoretical plates | Tailing factor |
|--------|--------|--------|------|--------------------|----------------|
| 1      | Std. 1 | 934671 | 4.627| 7740               | 0.80           |
| 2      | Std. 2 | 950554 | 4.627| 8328               | 0.86           |
| 3      | Std. 3 | 934881 | 4.630| 8432               | 0.82           |
| 4      | Std. 4 | 956217 | 4.627| 7653               | 0.81           |
| 5      | Std. 5 | 908143 | 4.627| 7823               | 0.82           |
| 6      | Std. 6 | 932430 | 4.630| 7574               | 0.82           |
| **Average** |       | 936149 | 4.628| 7925               | 0.82           |
| **% RSD** |       |        |      |                    | 1.79           |

The precision is done to check for the consistent results and which are in the limits. The method and intermediate precisions are showing the results within the limits.

**Table 04:**
Results of Method Precision and Intermediate Precision

| Sr.No | Standard (% assay) | Sample (% assay) |
|-------|--------------------|------------------|
| 1     | 99.23              | 101.69           |
| 2     | 99.65              | 98.66            |
| 3     | 98.56              | 99.72            |
| 4     | 98.89              | 99.05            |
| 5     | 99.12              | 98.78            |
| 6     | 99.63              | 100.66           |
| **Average** | 99.18          | 99.76            |
| **%RSD**   | 0.42              | 1.26             |

**Table 05:**
Results of specificity

| Sr. No | Sample | Area   | R.T  | Theoretical plates | Tailing factor |
|--------|--------|--------|------|--------------------|----------------|
| 1      | Blank  | 897971 | 4.660| 8701               | 0.84           |
| 2      | Std. 1 | 900018 | 4.673| 9011               | 0.87           |
| 3      | Std. 2 | 890884 | 4.680| 8074               | 0.83           |
| 4      | Std. 3 | 896700 | 4.687| 8005               | 0.82           |
| 5      | Std. 4 | 894887 | 4.627| 8421               | 0.82           |
| **Average** | 896092   | 4.665 | 8442 | 0.836              |
| **%RSD**   | 0.38              |

**Table 06:**
Results of Linearity

| Concentration [µg/ml] | Peak Area |
|-----------------------|-----------|
| 6                     | 3119245   |
| 9                     | 5438299   |
| 12                    | 6925899   |
| 15                    | 8684575   |
| 18                    | 9735335   |

The proposed method is linear and the range is 6µg/mL to 18µg/mL and correlation coefficient is 0.998.
Fig 6: Graph of linearity

Table 07: 
Results of Accuracy

| Spike Level in % | Area        | Amt. Added | Amt. Found | %recovery | Mean   | SD  | %RSD |
|------------------|-------------|------------|------------|-----------|--------|-----|------|
| 50%              | 4142188     | 0.006      | 0.0060     | 100.66    | 98.89  | 1.55| 1.57 |
|                  | 3938906     | 0.006      | 0.00585    | 97.70     |        |     |      |
|                  | 4048460     | 0.006      | 0.00597    | 98.33     |        |     |      |
| 100%             | 5110526     | 0.0012     | 0.00122    | 101.66    | 101.3  | 0.47| 0.472|
|                  | 5285208     | 0.0012     | 0.00121    | 100.83    |        |     |      |
|                  | 5314775     | 0.0012     | 0.00122    | 101.66    |        |     |      |
| 150%             | 8651678     | 0.0018     | 0.00177    | 98.33     | 98.51  | 0.32| 0.32 |
|                  | 8722465     | 0.0018     | 0.00178    | 98.89     |        |     |      |
|                  | 8519110     | 0.0018     | 0.00177    | 98.33     |        |     |      |

The accuracy of the method was determined by the recovery studies, carried out at different levels 50%, 100% and 150%.

Results of stability solution

Evaluate the stability in analytical solution by injecting the standard preparation and sample preparation at regular interval.

Table 08: 
Results of stability solution

| Stability in hours | % Assay |
|--------------------|---------|
| 0                  | 98.50   |
| 3                  | 99.90   |
| 6                  | 99.93   |
| 12                 | 101.25  |
| 24                 | 101.75  |
| Average            | 100.26  |
| %RSD               | 1.27    |
Conclusion

The developed method was found to be simple, accurate, precise, specific and robust and this method can be applied for routine quantitative analysis of Diltiazem HCl in bulk and pharmaceutical formulations like capsule.

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References

1. United States pharmacopoeia, XXIV, United States Pharmacopoeial convention, Inc. Rockville, M.D., 2000; 573-576
2. Vivekanand A.Chatpalliwar, Pawan.K.Porwal, Neeraj Upmanyu. Validated gradient stability indicating HPLC method for determining Diltiazem hydrochloride and related substances in bulk drug and novel tablet formulation. Journal of Pharmaceutical Analysis; 2(3): 2012, 226–237
3. R. Pravin Cumar, Dr. M. Vasudevan, Dr. Deeca Raman. RP-HPLC Method development and validation for the estimation of Diltiazem in bulk and tablet dosage forms; Asian Journal of Pharmaceutical and Clinical Research, Vol 5, Issue 3, 2012, 62-64
4. H. Rowe, S. Wahab, B. Voigt. Method Development and Validation of a Stability-Indicating HPLC Assay Method for the Quantitation of Diltiazem Hydrochloride in Compounded Oral Preparations, United States Pharmacopeia
5. Shabana Naz, Najma Sultana, M. Saeed Arayne,Nighat Shafi. Simultaneous determination of Prazosin and Calcium channel blockers in raw materials, pharmaceutical formulations and human serum by RP-HPLC; International Journal of Pharmaceutical Research and Development, VOV-2/ISSUE-9, 2010, 6-12.
6. Vidyav V. Gawande and Dr. A. V. Chandewar. Development and validation of spectroscopic method for estimation of diltiazem hydrochloride in solid dosage form; Journal of Pharmacy Research 3(12), 2010 3032-3033
7. Jean Paul Remon, Geert J. Vergote, Chris Vervaet, Tony Haemers, Francis Verpoort. Near-infrared FT-Raman spectroscopy as a rapid analytical tool for the determination of Diltiazem hydrochloride in tablets; European Journal of Pharmaceutical Sciences 16 (2002) 63–67
8. M.S.Bhatia, S.Kulkarni, S. D. Jadhav, S.S.Khetmar Development of Chromatographic Technique for Simultaneous Estimation of Lovastatin and Diltiazem Hydrochloride; Mahidol University Journal of Pharmaceutical Sciences; 39 (3-4), 2012, 17-23
9. Farhan Ahmed Siddiqui, Najma Sultana, M. Saeed Arayne, Nighat Shafi, and Azhar Hussain. Development of a RP-HPLC method for the simultaneous analysis of Diltiazem and statin: Application in pharmaceuticals and human serum; Analytical Methods, 2010, 2, 1571-1576 | 1571
10. Farhan Ahmed Siddiqui Najma Sultana, M. Saeed Arayne, Nighat Shafi, and Azhar Hussain. Development and Validation of New Assay Method for the Simultaneous Analysis of Diltiazem, Metformin, Pioglitazone and Rosiglitazone by RP-HPLC and applications in pharmaceuticals and human Serum; Journal of Chromatographic Science, Vol. 49, 2011, 774-779.
11. Chaudhari B. G, Patel J. Development and validation of dual wavelength spectrophotometric method for simultaneous estimation of Rosuvastatin and Diltiazem in combined dosage form; International Journal for Pharmaceutical Research Scholars. V-1, I-3, 2012, 146-153
12. Carsten coors, Hans-George schulz, Frank Stache. Development and validation of a bioanalytical method for the quantification of Diltiazem and Desacetyl Diltiazem in plasma by capillary zone electrophoresis, Journal of Chromatography A, 717 (1995) 235-243.