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# A Stability Indicating Assay Method For Verapamil Tablets By High Performance Liquid Chromatography For Stability Studies

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## ABSTRACT

A simple and stability indicating HPLC assay procedure had been developed and validated for verapamil tablets stability samples. The mobile phase consisted of buffer (1.4g $Na_2HPO_4$/1000ml water pH 7.0 by $H_3PO_4$): acetonitrile: in the ratio of (50:50) isocratic elution is carried out under ambient condition at flow rate of 2.0 ml $min^{-1}$ and detector was set at 232 nm. The column selected was thermohypersil, C18, 5µm packing, 4.6 mm x 250 mm and injection volume was 20 µl. The procedure separated verapamil and potential degradation product. The retention time of verapamil is 13.2 min and asymmetry was 1.55. The instrument precision obtained was 0.27 %.The procedure provided a linear response in the range of 50 - 150 % of target concentration (r = 1.000). Forced degradation study shows, response of main drug is reduced in acid, alkali and peroxide degradation. The method was validated for accuracy, robustness and solution stability was obtained up to 25 hrs.


## KEYWORDS

Verapamil;
Cardiovascular drug;
Force degradation;
Validation;
Solution stability;
Stability samples.

## INTRODUCTION

Verapamil is vasodilator, antiarrhythmic and calcium channel blocker agent used in the treatment of angina, hypertension, and for supraventricular tachyarrhythmias[1]. Verapamil is chemically 2-(3,4-dimethoxyphenyl)-5-[2-(3,4-dimethoxyphenyl)ethyl-methyl-amino]-2-(1-methylethyl) pentanenitrile (Figure 1). Literature survey reveals that it is official in U.S.P[2], B.P[3] and several techniques using fluorescence detector[4-5], spectrophotometry[6], voltametry[7], HPLC-gas chromatography[8] and chiral chromatog-

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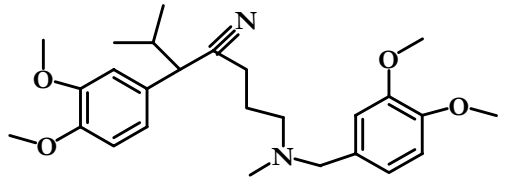

**Figure 1: Chemical structure of verapamil**

raphy[9-10] have been reported for estimation of verapamil in pharmaceutical formulation and in biological samples. In USP titremetry method is suggested for verapamil HCl and HPLC method for tablets using costly reagents, whereas in BP it is by U.V-Vis spectrophotometric method.

Hence to overcome the problem for estimation of verapamil in stability samples where large numbers of sample is to be quantified on regular basis (as HPLC method suggested in USP is not cost effective and spectrophotometric method suggested in B.P is not stability indicating). In the present study we had reported a simple, rapid, accurate and cost effective stability-indicating HPLC assay procedure that can quantify verapamil in stability samples and routine analysis, without concerning about separation of its racemic isomer as both verapamil and norverapamil are active metabolites.

## EXPERIMENTAL

The separation was carried out under isocratic condition with mobile phase prepared by mixing buffer (1.4g $Na_2HPO_4$ /1000ml water pH 7.0 by $H_3PO_4$): acetonitrile in the ratio of (50:50) at a flow rate of 2.0 ml $min^{-1}$ with UV detection at 232 nm. The column temperature was ambient and an injecton volume of 20µl was used. A Thermohypersil C18 column, 5µ, 250 × 4.6 mm was used. A working standard solution containing 200 µg $ml^{-1}$. verapamil was prepared by dissolving verapamil reference standard in mobile phase. A blend of verapamil tablets equivalent to 20 mg of verapamil is transferred to 100 ml volumetric flask. 20 ml of mobile phase was added and sonicated for 5 minutes with immediate shaking and diluted with mobile phase to volume and mix. This solution was centrifuged at about 2000 RPM for 10 minute, and upper clear solution was used for injection. Method validation was performed as per USP 27-NF22[11]. The following validation parameters were addressed: specificity, precision, linearity, accuracy and solution stability of verapamil in mobile phase.

### Specificity

Stress testing of the drug substance can help in identifying the likely degradation products, which in turn help's to establish the degradation pathways and the intrinsic stability of the molecule and validate the stability indicating power of the analytical procedure used[12]. Stress testing is done by exposing the verapamil to following conditions. (TABLE 1).

The result obtained from degradation study shows peak purity of verapamil is 100 % as calculated by PDA detector, proving that no degradation product is interfering with the main peak. (Figure 2A-E).The % residual drug was calculated in comparison with the standard, which is 97.77, 80.87, 69.38, 99.26 and 100.61 for acid, alkali, peroxide, thermal and sunlight degradation respectively.

### Precision

The system precision was determined by performing six replicate injections of standard solutions, the % RSD of 0.27 is obtained .The method precision was determined by performing six consecutive assays of verapamil by preparing six independent samples, the % label claim of 103.28 was obtained. (TABLE 2).

### Accuracy

The accuracy was evaluated by the recovery of

**TABLE 1: Summary of stress testing conditions for verapamil**

| S.No. | Degradation | Conditions | Figure No. |
|---|---|---|---|
| 1 | Acid | 2 ml, 2 M HCl and heated at 70°C for 1 hr. | 2A |
| 2 | Alkali | 2 ml, 2 M NaOH and heated at 70°C for 1 hr. | 2B |
| 3 | Peroxide | 1 ml, 30% $H_2O_2$ and heated at 70°C for 1hr. | 2C |
| 4 | Thermal | Heated at 70°C for 1hr. | 2D |
| 5 | Sunlight | Exposed for 2 hrs. | 2E |

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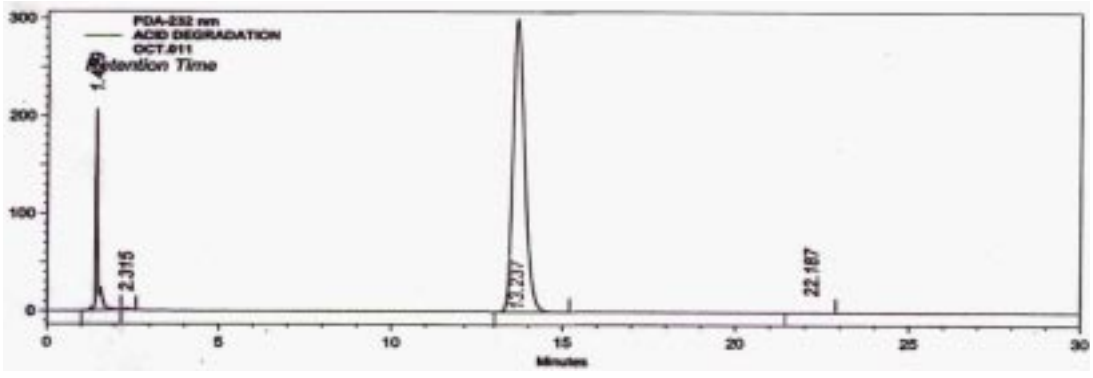


**Figure-2A**

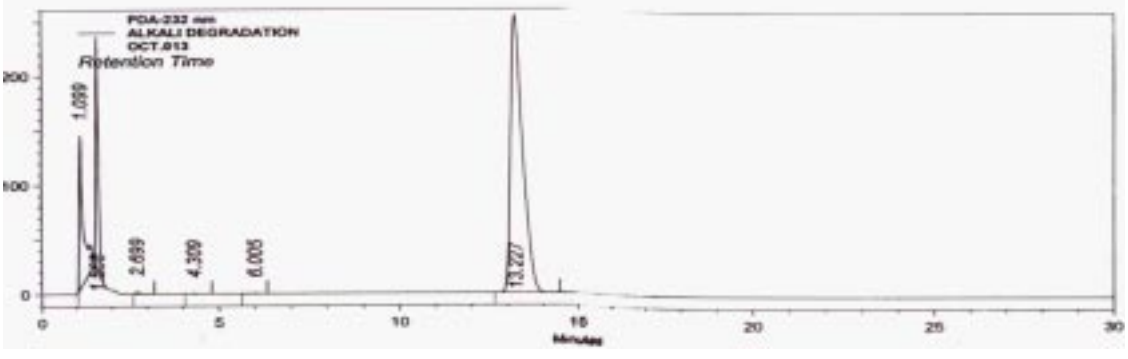


**Figure-2B**

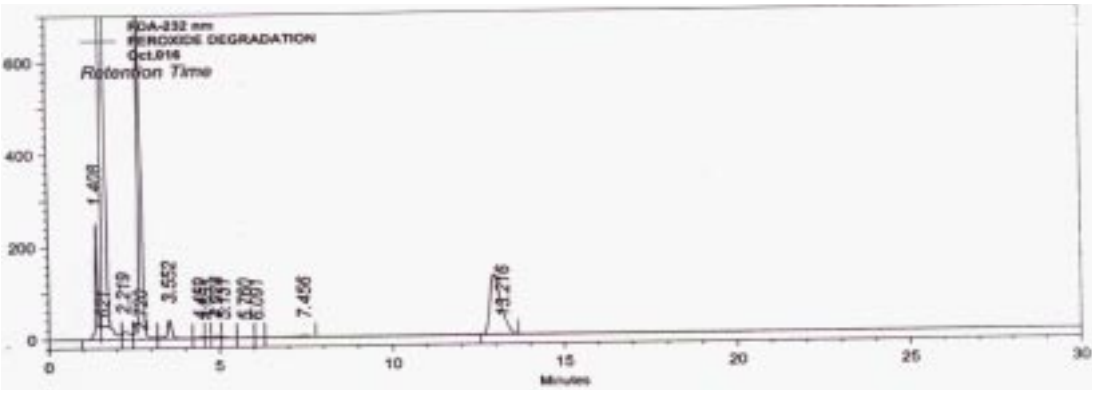


**Figure-2C**

verapamil at three different levels (80,100 and 120%) using three preparations for each level tested three times. The mean recovery data for each level is within accepted values (100.97, 101.29 and 98.61 respectively). Therefore, these results indicated a good accuracy of the method for verapamil. The mean recovery is 100.29% (TABLE 2) and % RSD is 1.46.

**Linearity**

The linearity of detector response for verapamil standard was determined by preparing and injecting solutions in the concentration range of 100-300 µg/ml (50-150% of assay conc.) of verapamil standard. A calibration curve was constructed using characteristic parameters for regression equation (y = a + bx) of the HPLC method obtained by least squares treatment of the results confirmed the good linearity of the method developed. (TABLE 2).

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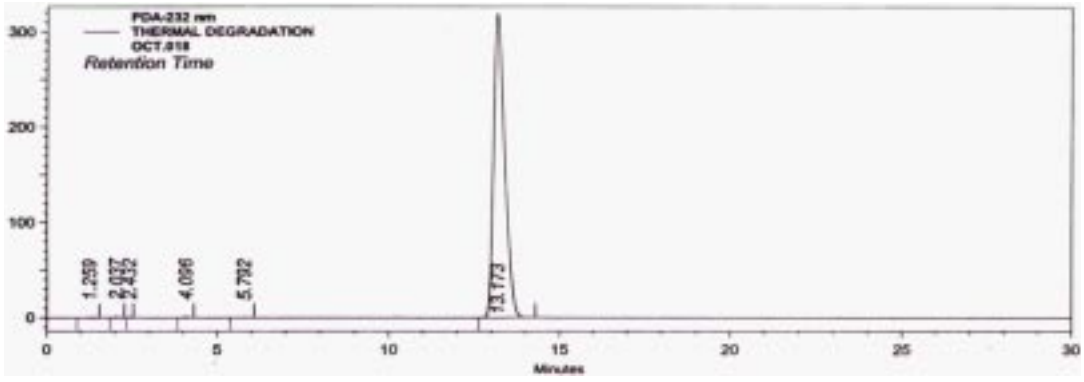


**Figure-2D**

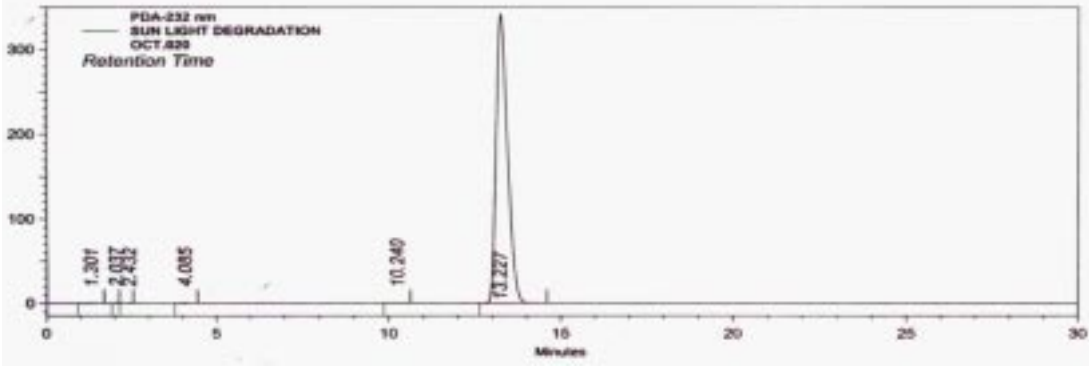


**Figure-2E**

**TABLE 2: Summary of the performance parameters of the HPLC procedure for verapamil tablets.**

| S.no | Parameters | Observed value |
|---|---|---|
| 1 | System suitability<br>a. Theoretical plates<br>b. Tailing Factor | -<br>8155<br>1.55 |
| 2 | Instrument Precision | RSD 0.27% |
| 3 | Method Precision | Label Claim 103.28% |
| 4 | Linearity and range | Correlation coefficient(r) = 1.0000 |
| 5 | Accuracy | Mean recovery 100.29% |
| 6 | Specificity | Peak Purity of verapamil peak after degradation is 100%. |
| 7 | Robustness | Difference from original condition 0.15% |
| 8 | Solution stability | 25 hrs |

### Robustness

The robustness study helps us in demonstrating that transferring the methodology can be done successfully or not. In this study we had compared the results between normal operating conditions and by deliberately changing certain parameters like changing analyst, instrument, column (Inertsil, C 18, 250 *4.6 mm, 5μ). The result obtained shows that by changing deliberately some internal and external parameters of the method does not influence the results obtained. (TABLE 2).

### Solution stability

The solution stability study was performed by injecting a standard solution in duplicate at different time intervals, the peak areas were compared with the initial areas, it was found that there were no significant changes in the peak areas up to 25 hrs. Hence the solution is not needed to be injected immediately. (TABLE 2).

## RESULT AND DISCUSSION

There was no cost effective method reported as per our knowledge which can be used on regular ba-

sis for quantification of verapmil specially when many samples are to be analysed, during long term and short term stability studies. We used previously reported data such as pka value of verapamil is 8.9[13], so a neutral pH of 7.0 is selected for which buffer choice of $Na_2HPO_4$ is taken and various trials of organic phase acetonitrile is tried to get desired retention time, so that all degradation products are well separated and peak shape of verapamil has good asymmetry. The previous procedures mention uses of short C18 columns as 4.6 mm × 12.5-15 cm, where it is observed that it creates back pressure and resolution problem as in stability samples lot of impurities are generated and large number of samples are to be analysed, hence a long column of 4.6 mm × 25.0 cm is selected. detector wavelength is selected at 232 nm as verapamil has dual lambda max in u.v. region, at 232 and 278 nm, as the mobile phase selected has cutoff value below 200 nm, hence lower wavelength is selected to detect large number of impurities.

## CONCLUSION

A stability-indicating, rapid, cost effective and reliable HPLC assay method was developed for the assay of Verapamil tablets useful for long term and short term stability samples. This chromatographic assay fulfilled all the requirements such as specificity, precision, accuracy, linearity, robustness and solution stability up to 25 hrs. The peak shape obtained though out the study shows asymmetry of 1.55 as well as the retention time of the verapamil is 13.2 (±0.1) min, showing a good column life and fast analysis of large numbers of samples in short time period, therefore this method is suitable for routine sample analysis and preferably for samples with short term stability and long term stability studies.

### Supplementary informations

The HPLC system used for this study is shimadzu LC-10ATvp solvent delivery pump with SIL -10Avp autoinjector, shimadzu SPD-M10Avp detector (photo diode array), Pentium 4 computer with class VP data integrating software. HPLC grade acetonitrile and analytical grade disodium hydrogen orthophosphate was purchased from Merck India and National chemicals Ltd respectively. High quality pure water was prepared by using Millipore milli Q Plus purification system.

## REFERENCES


[1] Drug Bank, APRD # 00335.
[2] United states pharmacopia 27- NF22, 1934 **(2005)**
[3] British pharmacopia CD-ROM., **(2003)**.
[4] PA.Hynning, P.Anderson, U.Bondesson, LO. Boreus; Clinical Chemistry, **34**, 2502-2503 **(1988)**.
[5] P.C.Ho, D.J.Saville, S.Wanwimolruk; Journal of Liquid Chromatography and Related Technologies, **23**, 1711-1723 **(2000)**.
[6] Nafisur Rahman, Syed Najmul Hejaz Azmi; Il Farmaco, **59**, Issue -7, July, 529-536 **(2004)**.
[7] Ensaf Aboul Kasim, M.A.Ghandourb, M.T.El-Hatyc, M.Mahasen, J.Ahmedr.Journal; of pharmaceutical and Biomedical Analysis, **20**, Issue 4, November, 921-929 **(2002)**.
[8] PA.Hynning, P.Anderson, U.Bondesson, LO. Boreus; Clinical Chemistry, **34**, 2502-2503 **(1988)**.
[9] Pham Thi Thanh Haa, Inge Sluytsa, Sigrid Van Dycka, Jie Zhanga, Ron A.H.J.Gilissenb, Jos Hoogmartensa, Ann Van Schepdaela; J.of Chromatogr.A., **1120**, Issue 1-2 , 7 July, 94-101 **(2006)**.
[10] E.Brandsteterova, I.W.Wainer; J.of Chromatogr.B, **732**, 395-405 **(1999)**.
[11] ICH, Draft Guidelines on validation procedures: definition and terminology, Fedral Register, **60**, March 1, 11260 **(1995)**.
[12] ICH, stability testing of new drug substance and products (Q1AR)., International conference on Harmonisation, IFPMA, Geneva, **(2000)**.
[13] J.Hasegawa, T.Fujita, Y.Hayashi, K.Iwamoto, J.Watanabe; J.pharma.Sci., **73(4)**, 442-5 **(1984)**.