Research Article

# Development and Validation of Stability Indicating RP-HPLC Method for Estimation of Manidipine Hydrochloride in Pharmaceutical Dosage Form

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## ABSTRACT

A novel, precise, accurate and rapid isocratic reversed-phase high performance liquid chromatographic (RP-HPLC) method was developed, optimized and validated for determination of Manidipine HCL. novel stability-indicating RP-HPLC method has been develop and validated for quantitative analysis of Manidipine HCL in in its pharmaceutical dosage forms using Column -Inertsil ODS 3v column (150 mm x 4.6 mm i.d., 5 μm)  with Phosphate buffer (pH-2.2) : Acetonitrile (60:40) as isocratic mobile phase at a flow rate of 1.4 ml/min and wavelength of 228 nm. The calibration curves were linear over the concentration ranges of 20-150 μg/ml for Manidipine HCL. The limit of detection (LOD) and limit of quantification (LOQ) for Manidipine HCL were 0.48 and 1.47 μg/ml. Recovery of Manidipine HCL the pharmaceutical dosage form ranged from 99.89-100.71%.
Manidipine HCL was subjected to stress conditions (Hydrolysis (acid, base), oxidation, thermal and photo degradation) and the stressed samples were analysed by use of the method. Degradation was observed in acid, base, and 30% $H_2O_2$.

**Keywords:** Manidipine HCL, RP-HPLC, Validation, Force Degradation

## INTRODUCTION

Manidipine HCL(MND)is chemically 2-[4-(diphenylmethyl)piperazin-1-yl]ethyl methyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate(Figure 1).is a well-known Antihypertensive agent[1]. Manidipine is a dihydropyridine calcium-channel blocker. It causes vasodilation by blocking the entry of calcium into the arteriolar muscle cells. Manidipine also appears to have some renally protective effects [2-3].Manidipine is not official in IP, USP and BP but official in JP.JP describe liquid chromatography method for its estimation [4]. Literature survey reveals UV [5], HPLC [6-8]methods for estimation of MND. Manidipine HCL(MND) is official in Japanese Pharmacopeia (JP). JP liquid chromatography method for its estimation.

Development of stability indicating assays, using the approach of stress testing as determined by the International Conference on Harmonization (ICH) guidelines [9], is highly recommended for the QC of pharmaceutical formulations [10-12]. Manidipine HCL(MND) is commercially available but at the moment is not available in any pharmacopoeia the aim of the present research was to develop and validate a simple stability-indicating high performance liquid chromatographic method for the quantitative analysis of MND in tablet dosage form[12-16]

## EXPERIMENTS

### Apparatus

· RP-HPLC instrument equipped with a UV-Visible detector and a photodiode array detector, (Shimadzu, LC-2010C$_{HT}$, Japan,) and agilent (EZ chrome software) auto sampler, (Inertsil ODS 3v column (150 mm x 4.6 mm i.d., 5 µm))and Shimanzu CLASS VP software were used.

· Analytical balance (Sartorius CP224S, Germany)

· Triple distillation unit consisting of borosilicate glass

· Digital pH meter (LI 712 pH analyzer, Elico Ltd., Ahemedabad)

· Ultra sonic cleaner (Frontline FS 4, Mumbai, India)

### Reagents and materials

Manidipine HCL was purchased from trader. mili-Q (HPLC grade) water, Acetonitrile (AR Grade, S. D. Fine Chemicals Ltd., Mumbai, India) and Whatman filter paper no. 41 (Millipore, USA) were used in the study.

### Preparation of mobile phase:

Phosphate buffer (pH-2.2)*: ACN (60:40) was taken in glass bottle, mixed it properly and sonicated for 15 minutes.

3.4 gm of KH$_2$PO$_4$ was weighed and dissolved in to 1000 ml mili-Q (HPLC grade) water; pH was adjusted to 2.2with Orto-phosphoric acid to give a phosphate buffer (pH-2.2).

### Preparation of standard stock solutions

A 50 mg of standard Manidipine was accurately weighed and transferred to a 50ml volumetric flask and dissolved in 15 ml diluent (ACN:Water,50 : 50). The flask was sonicated for 10 min. The flask was shaken and volume was made up to the mark with diluent to give a solution containing 1000 µg/ml of Manidipine.

### Preparation of Sample Solution

Twenty tablets were weighed. Powder equivalent to 50 mg of Manidipine was transferred to 50 ml of volumetric flask containing 20 ml diluent (ACN : Water, 50: 50), sonicated for 20 min. The flask was shaken and volume was made up to the mark with diluent (ACN: Water, 50:50).The above solution was filtered through Milipore filter (0.45 µ). 5 ml of aliquot was taken and transferred to volumetric flask of 50 ml capacity and volume was made up to the mark with the diluent to give a solution containing 100 µg/ml Manidipine. This solution was used for the estimation of manidipine in tablet dosage form.**(Table-1)**

## FORCED DEGRADATION STUDY [17-22]

### Acid Degradation:

50 mg of Manidipine HCL API was accurately weighed and transfer it to a 50ml volumetric flask. Add 5ml of diluent (ACN: Water, 50:50)in to this flask and 5ml of 5N HCL and kept this flask at 80$^0$C for 3 hrs. After that kept this flask at room temperature for cooling. After cooling add 4.5 ml of 5M NaOH to neutrilize the solution and made up the volume up to the mark with diuent. From this solution 5ml aliquot was taken into another 50ml volumetric flask and made volume upto the mark with diluent (100 µg/ml) and chromatographed to check the interference from the degradation products. Similarly, tablet powder equivalent to 50mg of Manidipine was accurately weighed and transfer it to a 50ml volumetric flask and degradation was carried out similarly as that with API.

### Alkali Degradation:

It was carried out as per the procedure described for the acid degradation using 0.1 M NaOH instead of 5 N HCl.

### Peroxide Degradation:

It was carried out as per the procedure described for the acid degradation using 30% H$_2$O$_2$ instead of 5 N HCl.

**Thermal Degradation:**
API and Tablet of Manidipine HCL were kept in the Hot air oven at 100˚C for 3 days and from this 100 µg/ml solution containing Manidipine was prepared. This solution was chromatographed to check the specificity.

**Photo Degradation:**
API and Tablet of Manidipine were kept in the UV chamber at 254-366nm (energy: 1.2 lux/hr) for 48 hrs and from this 100 µg/ml solution containing Manidipine was prepared. This solution was chromatographed to check the specificity.

For each degradation, blank solutions were also prepared without taking API or tablet powder as per the same procedure described above. Each blank was injected separately and chromatoghraphed.

Chromatograms of blank solutions were compared with respective sample chromatograms to check the interference of HCl, NaOH and $H_2O_2$, Thermal and Photolytic.

**Note**: Minimum 10 to 30 % degradation should be achieved in applied stress conditions. Final concentration of all the sample preparations should be approximately equal to the test concentration specified in method and final dilution shall be in diluent.

**Chromatographic Condition**
- Column : Inertsil ODS 3v column (150 mm x 4.6 mm i.d., 5 µm)
- Mobile phase: Phosphate buffer(pH-2.2) : Acetonitrile (60:40)
- Flow rate: 1.4 ml/min
- Injection volume: 10 µl
- Wave length: 228 nm
- Column temperature: 40°C
- Run time: 12 min

## METHOD DEVELOPMENT [23-26]
Developed RP-HPLC method was validated by different parameters as per ICH guidelines.

**Specificity**
Specificity of an analytical method is ability to measure specifically the analyte of interest without interferences from blank and placebo.

**Check for interference from blank and forced degradation product**
The API and drug samples were prepared as described in 5.1.4. After degradation, samples were analyzed and separation of the degradant product peaks from main peaks was checked.

**Linearity and range**
Appropriate volume of aliquots (1, 2, 3, 4, 5, 6,7.5 ) from standard stock of Manidipine containing (1000 µg/ml) were transferred to volumetric flasks of 50 ml capacity. The volume was adjusted to the mark with diluent (ACN: Water, 50:50) give a solution containing 20, 40, 60, 80, 100, 120 and 150 µg/ml Manidipine. The standard solutions was chromatographed for 12 min using mobile phase at a flow rate of 1.4 ml/min. Graph was plotted for peak area vs. concentration for the drug.

**Precision**
a)**Repeatability:** Method precision for assay was established by determining the assay of six sample preparations of Manidipine(100 µg/ml) under same conditions. Six replicates of sample were prepared at sample concentration and analyzed on same day, and % RSD was reported.
b) **Intermediate precision (Reproducibility)**
The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day (intraday) and 3 on different days(interday) for 3 different concentrations of standard solutions Manidipine (50, 100 and150 µg/ml). The results were reported in terms of relative standard deviation (% RSD).

**Accuracy (% Recovery)**
Accuracy was determined over the range 50 % to 150 % of the sample concentration.

Calculated amount Manidipine API was added in placebo to attain 50 %, 100 % and 150 % of sample concentration.

Each sample was prepared in triplicate at each level and injected. The chromatograms were recorded and from the peak area of drug, % recovery was calculated from regression equation of the calibration curve.

### Robustness

The following parameters were changed one by one and their effects were observed on area of peak, when chromatographed, every condition is repeated for the three times.

a) Mobile phase ratio (± 2% absolute) as buffer: ACN (58: 42), buffer: ACN (62: 38).

b) pH of the mobile phase (± 2% absolute) as 5.2 pH and 5.7 pH of the mobile phase.

### Limit of detection & Limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by using the following equations as per International Conference on Harmonization (ICH) guidelines.

LOD = 3.3 × σ /S

LOQ = 10 × σ /S

Where σ = the standard deviation of the response

S = Slope of calibration curve.

## RESULTS AND DISCUSSION

Selection of mobile phase:

Various mobile phase systems were tried such as,

1. Methanol : water at concentration of 50:50

2. Acetonitrile : water at concentration of 50:50

3. Acetonitrile : phosphate buffer (pH 4.6) at concentration of 50:50.

4. Acetonitrile:, phosphate buffer (pH 3) at concentration of 60:40,

5. Acetonitrile:, phosphate buffer (pH 2.2) at concentration of 60 : 40, at a flow rate of 1.4 ml/min and the chromatograms were recorded.

### Specificity

Placebo and standard drugs were used to determine the specificity of the developed method and chromatogram was recorded **(fig.2,3& 4)**

### Linearity

Solutions of different concentrations were prepared (20-150 µg/ml of Manidipine) and chromatographed and area was recorded **(Table 2).** From the linearity graph **(fig.5&6),** correlation coefficient, slope and intercept were calculated and shown in **(Table-3).** Correlation coefficient is in accordance with ICH guidelines

### Accuracy

Recovery was checked at three concentration level (50%, 100% and 150%). % Recovery for individual and mean value (n=3) at each level was recorded in **(Table -4)**

### Method Precision (% Repeatability)

Six sample preparations containing 100 µg/ml of Manidipine was chromatographed and % RSD of area was calculated **(Table-5)**

### Intermediate Precision (Reproducibility)

Three replicate of three concentration were chromatographed in traday and inerday and % RSD were calculated .**(Table-6)**

### LOD and LOQ

LOD and LOQ for Manidipine were found to be 0.48 µg/ml and 1.47 µg/ml.

### Robustness

The following parameters were changed one by one and their effects were observed on area of peak, when chromatographed, every condition is repeated for the three times. Recorded in **(Table-7 & 8)**

### Force Degradation Study-

In order to establish whether the analytical method for the assay was specific or not, both

the tablet and pure active pharmaceutical ingredient (API) of Manidipine was subjected to various stress conditions. Stress studies were carried out under the various conditions like acid/base hydrolysis, oxidation, and thermal degradation**(Table-9)**

**Acidic Degradation:** 4.97 % degradation of API and 3.55 % degradation of tablet of Manidipine was observed while refluxed in 5 N HCl at 80°C for 3hrs. The major degradation products formed in API and Tablet of Manidipine were eluted at retention time (RT) of 1.547, 5.8, 6.5 & 10.8 min respectively. **(fig.7)**

**Alkali Degradation:**
25.67 % degradation of API and 11.77 % degradation of tablet of Manidipine was observed while refluxed in 0.1 M NaOH at room temperature for 15 min.The major degradation products formed in API and Tablet of Manidipinewere eluted at retention time (RT) of 2.481 and 20.4 min respectively.**(fig.8)**

**Peroxide Degradation:**
The oxidative degradation was carried out in 30 % v/v H2O2 for 3 hrs at 80 °C.3.23% of API and 1.28% of tablet degradation was observed. Major degradation product of Manidipine was eluted at RT 8.3 & 10.8 min. **(fig. 9).**

**Thermal Degradation:**
No degradation was found in API and tablet of Manidipine upon placing them in hot air oven at $100^0$C for 3 days**. (fig. 10)**

**Photo Degradation**
No degradation was found in API and tablet of Manidipine upon placing them in hot air oven at UV-chamber at 254-366nm (energy: 1.2 lux/hr) for 48 hrs.**(Fig. 11)**

## CONCLUSION

A simple, selective, precise RP-HPLC method has been established and validated for analysis of Manidipine in bulk and tablet dosage form. The wave length having maximum absorbance 228 nm was selected as detection wavelength. Forced degradation study shows major degradation in alkaline condition. Regression analysis data for the calibration plot indicated good linear relationships between response and concentration over the range of 20-150 μg/ml. The correlation coefficient, $R^2$ was 0.9993. The method was validated for precision, recovery and robustness. The limits of detection and quantification were 0.48 μg/ml and 1.47 μg/ml, respectively.
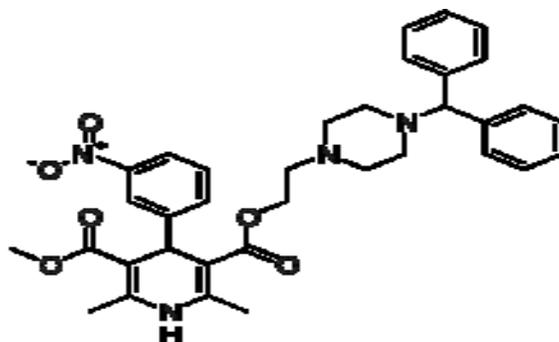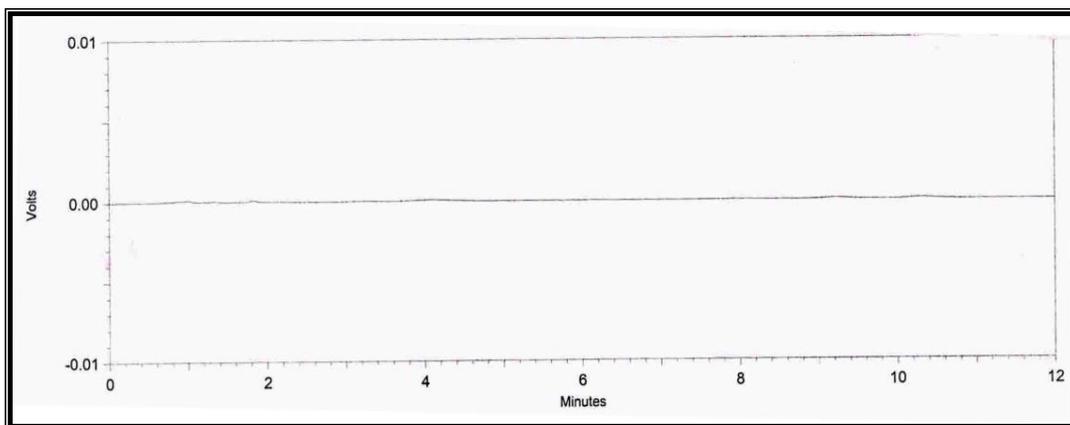

Fig. 1: Chemical structure of Manidipine HCL

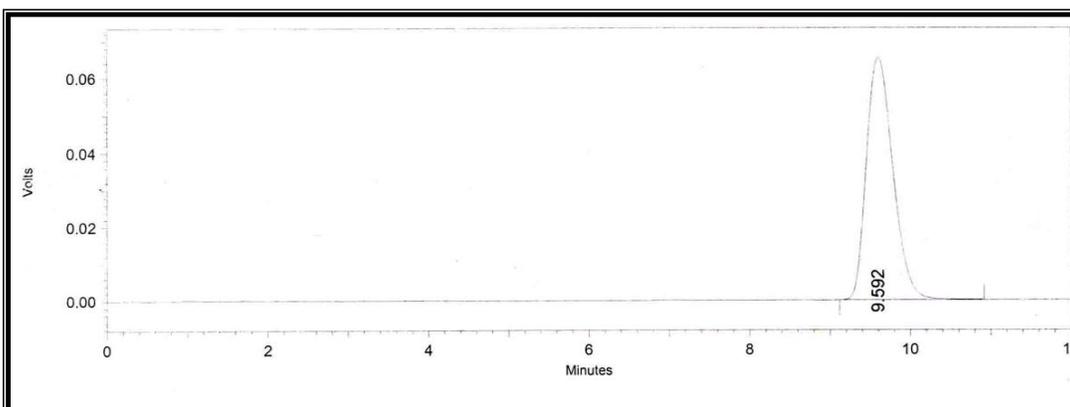Figure 2: Chromatogram of the placebo of tablet of Manidipine



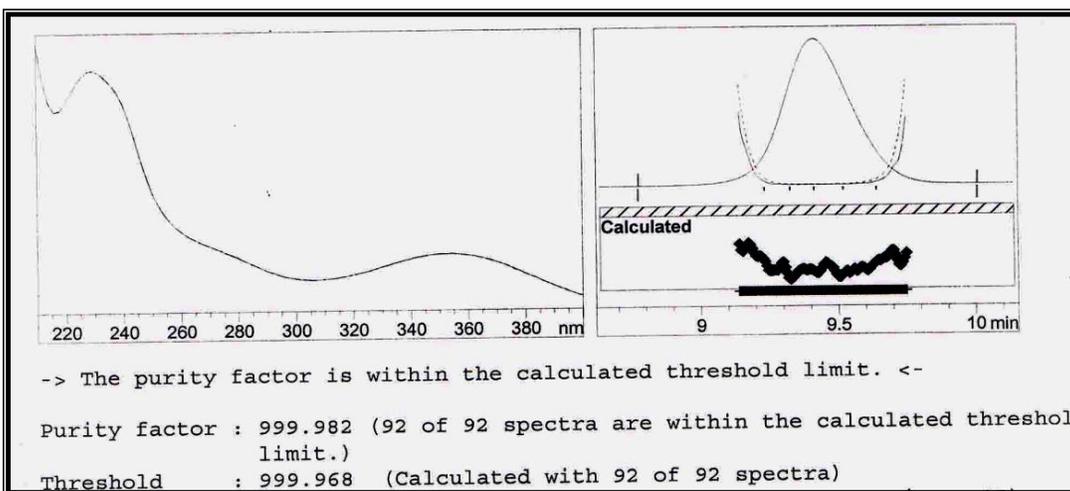Figure 3: Chromatogram of the Standard of Manidipine
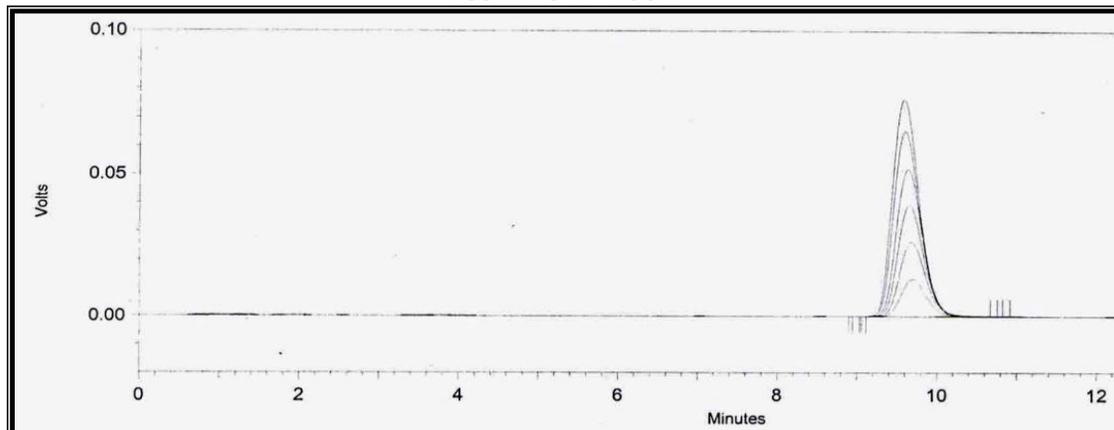


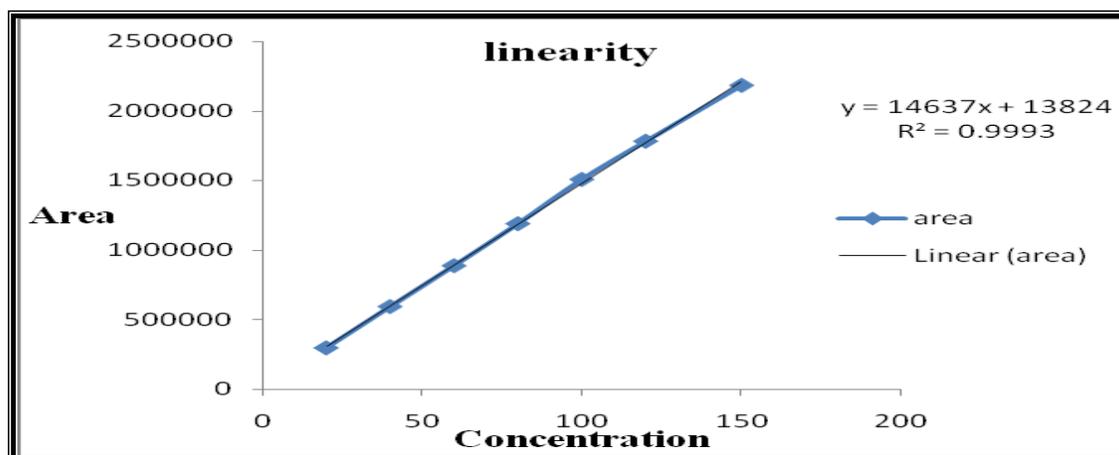Figure 4:Purity spectrum of the Standard of Manidipine

Fig. 5:Linearity Overlain for Manidipine


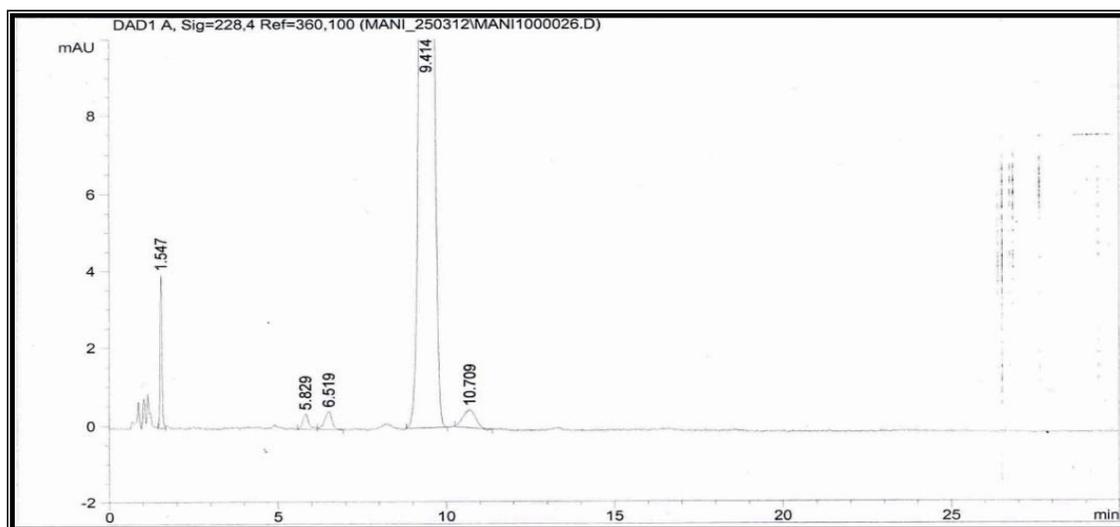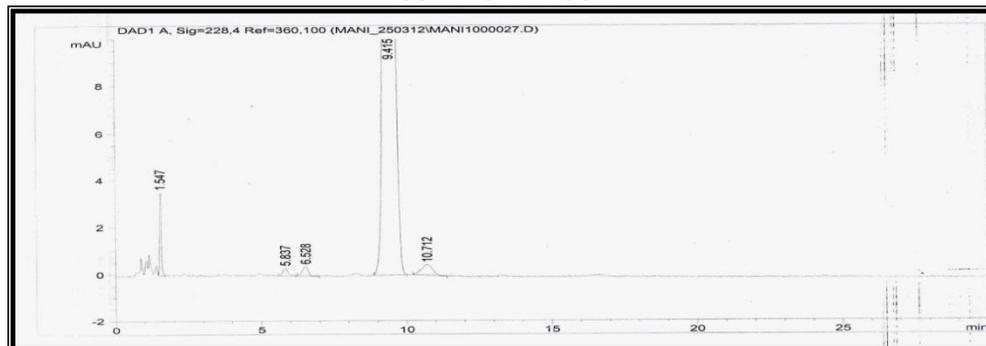
Fig. 6: Calibration curve for linearity
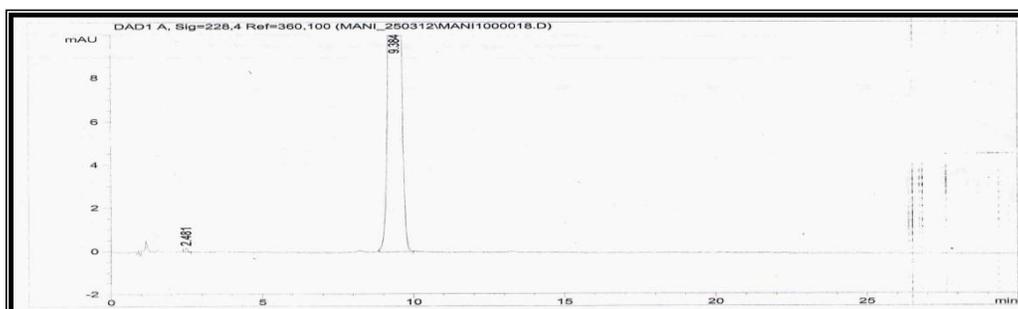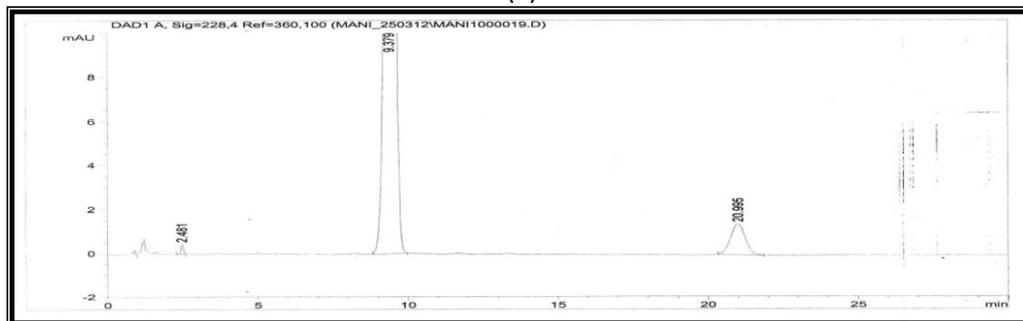


(a)

(b)

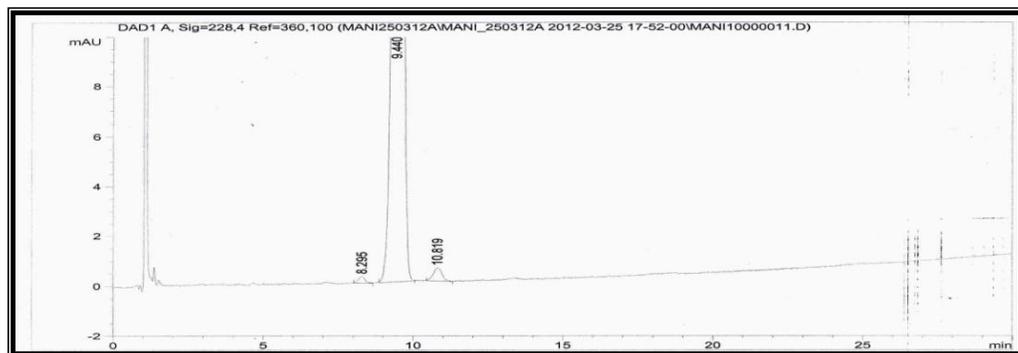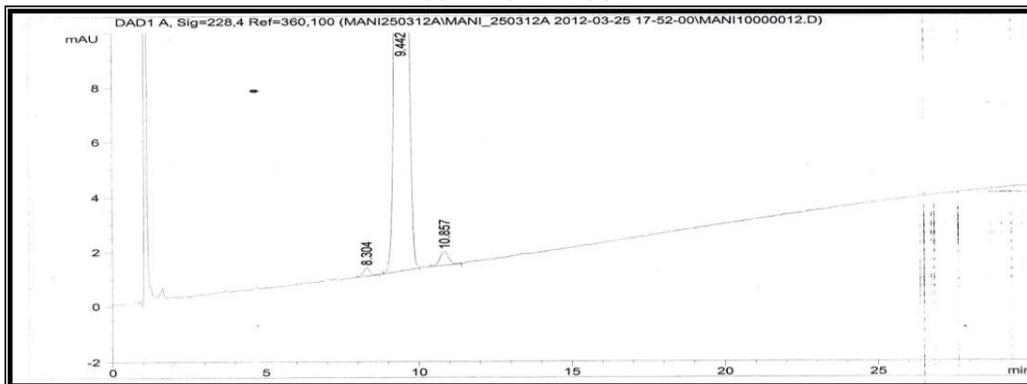Fig. 7:Chromatograms of acid degraded (a) API and (b) tablet preparation of Manidipine
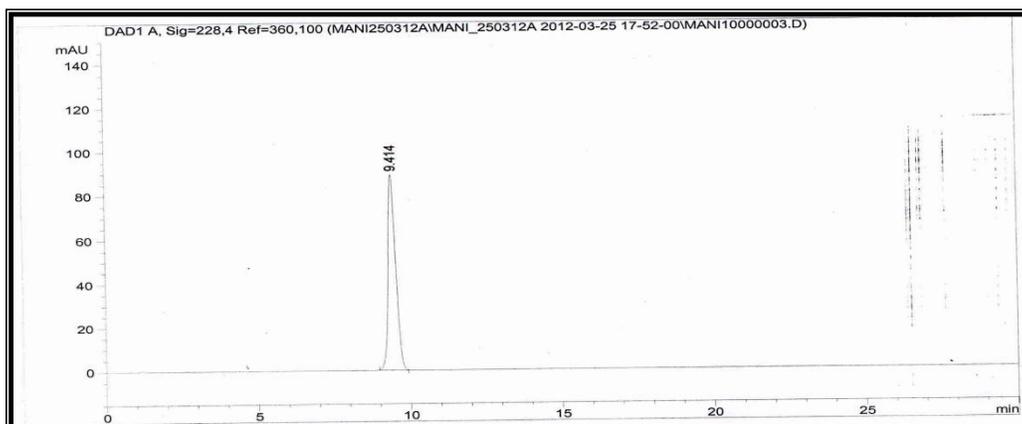


(a)



(b)

Fig. 8:Chromatograms of alkali degraded (a) API and (b) tablet preparation of Manidipine
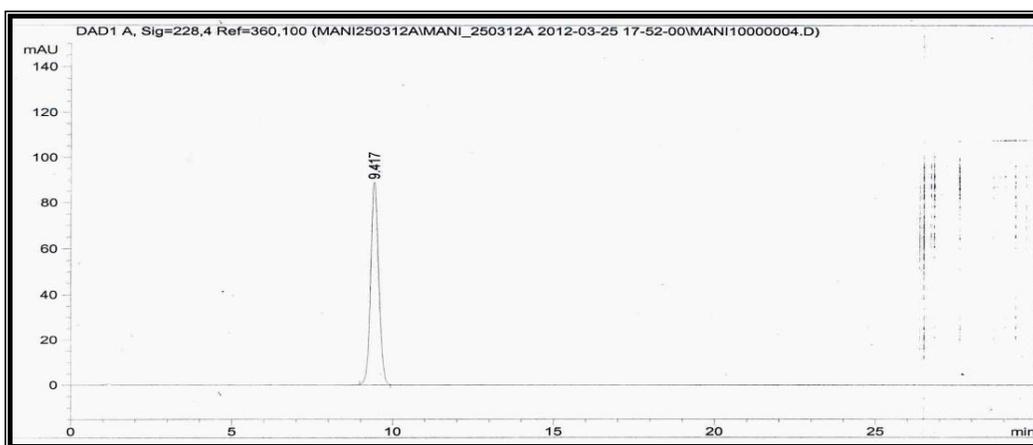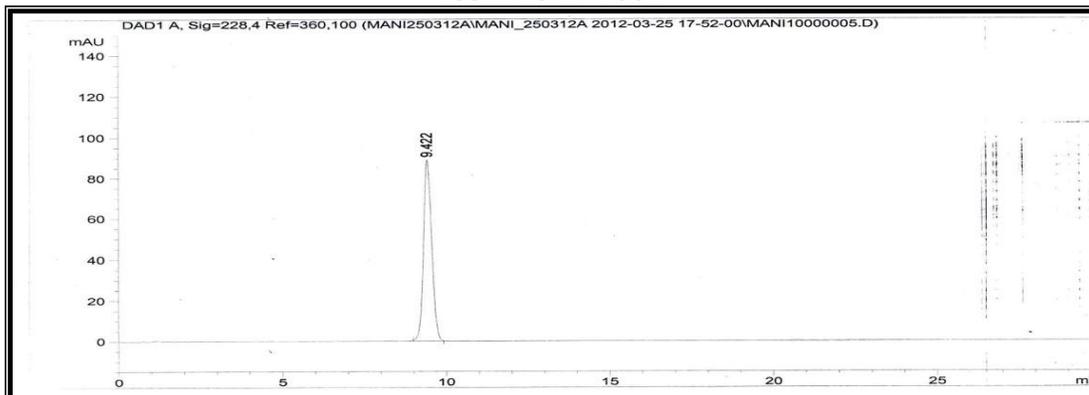


(a)

(b)

Fig. 9:Chromatograms of $H_2O_2$ degraded (a) API and (b) tablet preparation of Manidipine



(a)



(b)

Fig. 10:Chromatograms of Thermal degraded (a) API and (b) tablet preparation of Manidipine

(a)
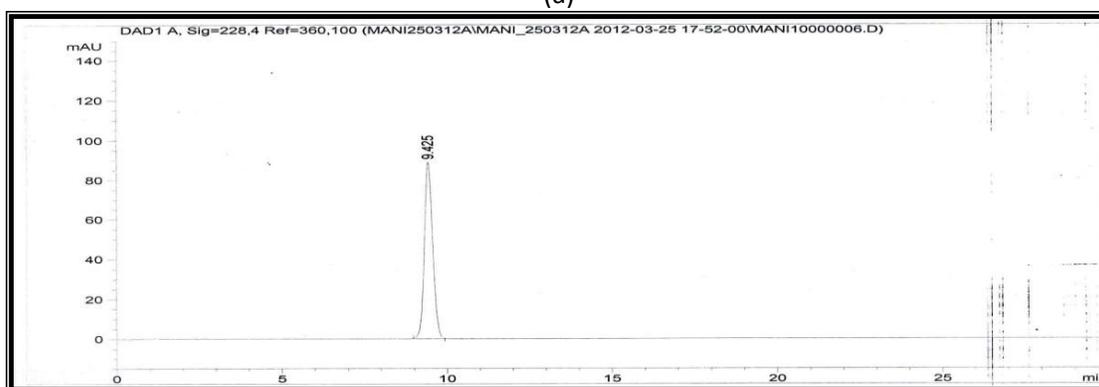


(b)

Fig. 11:Chromatograms of Photo-degraded (a) API and (b) tablet preparation of Manidipine

Table 1: Analysis of Formulation (n = 6)

| Sample No. | Label Claim (mg) | Amount Found (mg) | % Label Claim |
|---|---|---|---|
| 1 | 50 | 49.84 | 99.68 |
| 2 | 50 | 49.94 | 99.87 |
| 3 | 50 | 49.03 | 98.05 |
| 4 | 50 | 49.25 | 98.49 |
| 5 | 50 | 49.06 | 98.12 |
| 6 | 50 | 49.46 | 98.92 |
| Mean | | 49.43 | 98.84 |
| S.D. | | 0.36 | 0.77 |

Table 2: Linearity of Manidipine  by RP-HPLC method

| Concentration(µg/ml) | Mean area±S.D. | %RSD |
|---|---|---|
| 20 | 300546.3±3624.14 | 1.21 |
| 40 | 592104±2367.44 | 0.39 |
| 60 | 889920.3±6192.45 | 0.70 |
| 80 | 1186793±10258.64 | 0.87 |
| 100 | 1511226±15048.74 | 0.99 |
| 120 | 1774339±25681.2 | 1.45 |
| 150 | 2185589±11719.7 | 0.54 |

Table 3: Regression Analysis Data of MND for proposed Method

| Parameters | HPLC method |
|---|---|
| | **MND** |
| Detection wavelength(nm) | 228 |
| Concentration range (µg/ml) | 20-150 µg/ml |
| Regression equation Y= mX + c | y = 14637x + 13824.0 |
| Correlation coefficient | 0.9993 |
| Slope | 14637 |
| Intercept | 13824 |
| LOD(µg/ml) | 0.48µg/ml |
| LOQ(µg/ml) | 1.47µg/ml |

Table4:Recovery Data for the proposed Method

| Level | Amt. of drug added (mg) | Amt. of drug recovered(mg) | % Recovery | Mean | R.S.D. |
|---|---|---|---|---|---|
| 50% | 25 | 24.9 | 99.6 | 100.52 | 1.15 |
| 50% | 25 | 25.03 | 100.13 | | |
| 50% | 25 | 25.45 | 101.82 | | |
| 100% | 50 | 50.86 | 101.71 | 100.713 | 0.95 |
| 100% | 50 | 50.32 | 100.63 | | |
| 100% | 50 | 49.9 | 99.8 | | |
| 150% | 75 | 74.25 | 99 | 99.89 | 1.14 |
| 150% | 75 | 75.8 | 101.18 | | |
| 150% | 75 | 74.62 | 99.5 | | |

Table 5: Intra-day and Inter-day precision (n = 3)

| Drug | Conc. (µg/ml) | Intra-day precision | | Inter-day precision | |
|---|---|---|---|---|---|
| | | Mean ± SD | % RSD | Mean ± SD | % RSD |
| SIL | 50 | 742049±3219.37 | 0.43 | 742894±4914.03 | 0.66 |
| | 100 | 1502783±11661.26 | 0.78 | 1512507±22984.76 | 1.52 |
| | 150 | 2178827±18768.66 | 0.86 | 2178086±30237.12 | 1.39 |

Table 6: Repeatability of Sample Application (n = 6)

| Sr. No | Peak Area |
|---|---|
| 1 | 1507282 |
| 2 | 1507564 |
| 3 | 1506998 |
| 4 | 1508112 |
| 5 | 1509965 |
| 6 | 1512563 |
| Mean | 1508747.33 |
| SD | 2147.25 |
| % RSD | 0.14 |

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Table 7: Change the ratio of mobile phase

| Standard repetitions(n=3) | 60 : 40 | 58 :42 | 62 : 38 |
|---|---|---|---|
| Mean Area ± %RSD | 1505198±0.38 | 1512211±1.03 | 1508183±1.04 |

Table 8: Change the pH of mobile phase

| Standard repetitions(n=3) | 2.2 | 2 | 2.4 |
|---|---|---|---|
| Mean Area ± %RSD | 1512115±0.88 | 1525744±1.1.09 | 1503554±0.97 |

Table 9: Results of forced degradation study of Manidipine

| Stress condition/duration | API | | SAMPLE | |
|---|---|---|---|---|
| | %Assay | %Degradation | %Assay | %Degradation |
| Acidic-heating on water bath at $80^0$C for 3hrs with 5ml 5N Hcl | 95.03 | 4.97 | 96.45 | 3.55 |
| Basic-kept at room temperature for 15 min with 5ml 0.1 M NaOH | 74.33 | 25.67 | 88.23 | 11.77 |
| Peroxide-heating on water bath at $80^0$c for 3 hrs with 30%$H_2O_2$ | 96.84 | 3.16 | 98.72 | 1.28 |
| Thermal-kept in Oven for 3 days at $100^oC$ | 99.92 | - | 99.35 | - |
| UV light-exposed to UV light at 254-366nm (energy:1.2 lux/hr) for 48 hrs under UV light in photo stability chamber | 99.46 | - | 99.67 | - |

↓ REFERENCES

1. Japan Pharmacopoeia, society of japanese pharmacopiea ;15th Edn; 1901-1902
2. drugs.com/international/manidipine.htm
3. en.wikipedia.org/wiki/Manidipine-wikipedia.
4. Japan Pharmacopoeia, society of japanese pharmacopiea; 15th Edn; 1901-1902.
5. Sivasubramanian L, Manikandan K, Venkatesan M, Lakshmi K.S,Quantitative analysis of manidipine dihydrochloride in bulk and synthetic mixtures by visible spectrophotometry; J Young Pharmacists ;2009; 1 ;175-182
6. Jing J, Ren W and He H, Determination and pharmacokinetics of manidipine in human plasma by HPLC/ESIMS; Biomed Chromatogr;2007; 21(8) ; 836-40.
7. Ohkubo T, Uno T, Sugawara K. , Liquid chromatographic determination of manidipine in serum with

electrochemical detection, J Chromatogr B,Dec 1996; 2 ; 413-421

8. Tsukasa U, Tadashi O and Kazunobu S., Enantioselective high performance liquid chromatographic determination of manidipine in human plasm; J of Liquid Chrom. & Related Tech; 1999 ;22 (8) ; 1117-1128.

9. ICH Q2B Text on validation of analytical procedures: Methodology International Conference on Harmonization Nov. 1996. Harmonization tripartite guidelines, adapted 27 Oct 1994.

10. Mendham J, Denney RC, Baraes JD, Thomas MJK, Vogel's Textbook of Quantitative Chemical Analysis, 5th Edn, Pears Edjucation, 1991, 5-11.

11. Shrma PP, How to Practice GMPs, Good Manufacturing Practices, 3rd Edn, Vandana Publication Pvt Ltd, 2001, 214.

12. Meyer VR, Practical High Performance Liquid Chromatography, 2nd Edn, John Wiley and Sons, 1993 , 26-7, 40, 222, 246 and 256.

13. Ahuja S, Handbook of Modern Phamaceutical Analysis, United States of America: Academic Press, 2001, 349.

14. Mendham J, Denney RC, Baraes JD, Thomas MJK, Vogel's Textbook of Quantitative Chemical Analysis, 5th Edn, Pears Edjucation, 1991 ; 217-35.

15. Szepesi Gabor, HPLC in Pharmaceutical Analysis, CRC Press, Vol-I, 1990, 101-73.

16. Gurdeep R. Chatwal,Sham K. Anand,"Instrumental Methods of chemical analysis,1st Edition ,Himalaya Publishing House, 1979, 2.168-2.169.

17. Singh SS, Bakshi M., Development of Validated Stability Indicating Assay Methods;Critical Review; J Pharm Bio Anal 2002 ; (28); 1011-1040.

18. Singh SS, Bakshi M, "Guidance on Conduct of Stress Test to Determine Inherent Stability of Drugs", Pharm Tech 2000; 1-14.

19. Heyrman AN, Henry RA, Importance of Controlling Mobile Phase pH in RP- HPLC, Technical Bull, 1999 ; 1-7.

20. O' Neil MJ, Smith A, Heckelman PE, Kinneary JF, The Merk Index,An Encyclopedia of Chemicals, Drugs and Biologicals, 12th Edn, Merck Research Laboratories, 1996 ; 146.

21. Sweetman SC, Martindale, The complete Drug Reference, 33rd Edn, London: Pharmaceutical Press, 2002; 842, 863, 966.

22. Touchstone J C, Practice of thin layer chromatography, 3rd Ed., Published by John wiley and sons, Inc., USA, 1992.

23. Sethi P D and Charegaonkar D, Identification of drugs in pharmaceutical formulations by thin layer chromatography, 2nd Ed., CBS Publishers and distributors, USA, 1999.

24. Validation of Analytical Procedure: Methodology, ICH Harmonized Tripartite Guidelines, 1996; 1-8.

25. Green JM, "A Guide to Analytical Method Validation", J Amer Chem, 1996(68) ; 305-309.

26. Validation of Analytical Procedure: Text and Methodology ICH Q2 (R1). 2005.