Biological Forum – An International Journal **15(6): 957-965(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Method Development for Identification of Manidipine HCl using High Performance Liquid Chromatography

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(Received: 04 May 2023; Revised: 20 May 2023; Accepted: 27 May 2023; Published: 15 June 2023)
(Published by Research Trend)

ABSTRACT: Manidipine HCl (MND) is a third-generation dihydropyridine calcium (Ca) channel antagonist that is lipophilic and highly selective for the vasculature, leading to significant peripheral vasodilation and minimal cardio depression. MND appears to increase insulin sensitivity without altering metabolic function and helpful in hypertensive individuals with comorbidities such as type 2 diabetes mellitus and/or renal impairment. MND is a first-line medication for people with essential mild-to-moderate hypertension as a result.

The discovery, development, and production of pharmaceuticals depend heavily on the development and validation method, among all HPLC is one of them due to its very effective separations and often high detection sensitivity, HPLC is the most widely used separation method in contemporary pharmaceutical and biomedical analysis. Its numerous benefits, includes its speed, specificity, accuracy, precision, and ease of automation, the majority of medications in multi-component dosage forms can be examined using this technique. The development and validation of HPLC procedures are crucial to novel discoveries, the creation of pharmaceutical medications, and numerous other human and animal investigations.

This study provides details on the various steps that go into developing and validating a HPLC technique for MND. According to ICH Guidelines, its include testing for system appropriateness as well as accuracy, precision, specificity, linearity, range and limit of detection, limit of quantification, robustness, and other performance characteristics. The developed HPLC method effectively identified and quantified Manidipine HCl, including impurities, with reliable results.

Keywords: Manidipine HCL (MND), Identification, Validation, High Performance Liquid Chromatography (HPLC), Method development.

INTRODUCTION

A well-known antihypertensive medication, Manidipine HCL (MND) is chemically 2-[4-(diphenyl methyl) piperazin-1-yl] ethyl methyl 2, 6-dimethyl-4-(3-nitrophenyl)-1, 4 dihydropyridine-3, 5-dicarboxylate. (Cheer and McClellan 2001) Manidipine is the third-generation antihypertensive drug effective in depressing BP (Blood Pressure) among those who have mild to moderate essential hypertension (HTN). It is useful since long duration without signs of tolerance (Bellinghieri *et al.,* 2003). It has a moderate start & a lengthy duration of action, successfully sustaining lower BP levels over the one-day dosing period (Casiglia *et al.,* 2004). Its ability to reduce blood pressure is comparable with other well-known DHPs (Dihydropyridines) and angiotensin-converting enzyme inhibitors (Cavalieri and Cremonesi 2009). The therapy is beneficial for patients with mild to severe HTN who are diabetic and very old (Fogari *et al.,* 2011). It is often well tolerated and has no effects on glucose and lipids metabolism. Thus, a first choice in decreasing BP in those with mild-to-moderate.

MND prevents the passage of calcium into the arteriolar muscle cells, it dilates the blood vessels. Additionally, MND appears to have some renal protective properties. (McKeage and Scott 2004; Richy and Laurent 2011; Martínez Martín 2009). MND determination is officially recognized by the Japanese Pharmacopoeia (JP). In JP liquid chromatography was used to estimate MND. It is strongly advised for the quality control (QC) of pharmaceutical formulations to develop stability indicating assays utilising the method of stress testing as outlined by the International Conference on Harmonization (ICH) recommendation. Although MND is commercially accessible, it is not yet included in any other pharmacopoeia. The current study's goal was to create and test a straightforward HPLC approach for the

quantitative analysis of MND (Patel and Prajapati 2014)

MATERIAL AND METHODS

A standard stock solution of MND was prepared by diluting 10 mg of the MND in 10 ml of a diluent consisting of a 50-45 mixture of 0.1% Trifluoroacetic acid (TFA) and Methanol. The analytical wavelength range was set between 200-400 nm. The mobile phase composition comprised Methanol in Mobile Phase A and 0.1% TFA in Mobile Phase B. Key parameters such as linearity, accuracy, precision, LOD, and LOQ were assessed to validate the method.

Material required for the Method development for identification of MND is given as per the below table

Table 1: list of materials and instruments.

Materials / Instruments Used	Specifications
HPLC	Agilent 1260 Infinity II
Software	Open lab Ezchrom
Quat.Pump with Degasser	G7111A
Auto-Injector	G7129A
DAD Detector	G7115A
Column	Agilent Technologies
Nylon membrane 0.45µm 15mm Syringe Filters	Qualsil
Nylon 6,6 membrane 0.45µm 47mm Filters	Pall India Pvt. Ltd., Mumbai
All Glass Filter Holder- 47mm	Borosil Glass works Ltd., Mumbai
Melting Point Apparatus	Vigo

HPLC method validation protocol (Horacio, 2022)

1. System Suitability:

Chromatographic Conditions

— Analytical column: Agilent Zorbax Bonus RP (250 x 4.6 mm, 5µ)

— Mobile phase: Methanol: 0.1% (TFA) trifluoroacetic acid at ratio (55-45)

— Flow rate: 1ml/min

— Injection volume: 10 µl

— Detection wavelength: 240 nm.

— Runtime: 10 min

Preparation of MND standard stock solution (SSS-1). (Validation of analytical procedures Q2 (R2) 2022) MND 10 mg was taken in a volumetric flask with 10 ml of diluent and vortexes for 1 min. then it was sonicated for 5 min. stock solution has a concentration of 1000 µg/ml.

Preparation of working standard (WS). 1 ml of SSS-1 Solution was transferred in a volumetric flask (10 ml) and with diluent solution final volume was make up which was further vortexes in the flask for 1 minute, which has a concentration 100µg/ml.

Selection of analytical wavelength. During the initial injection, the MND and diluent mixture was scanned between 200 and 400 nm. The Wavelength was selected was done on the basis of highest intensity of main peak.

Selection of mobile phase and its strength. The column was saturated with mobile phase and continuous back pressure, Different ratios of Methanol: 0.1% TFA was used to evaluate the solution for 10 minutes at a flow rate of 1ml/min. Methanol in Mobile Phase A and 0.1% TFA in Mobile Phase B. A solution of MND at a concentration 100 µg/ml was prepared in diluent and filtered using a syringe filter before being introduced into the HPLC system.

2. Specificity: (Guideline, 1994; Borman & Elder 2017).

A. Specificity by identification (ID):

Working standards were prepared according to the analytical method.

ID solution for diluent: For ID diluent solution filtered diluent 0.1% TFA: Methanol (50-50) as diluent was used. Major peak was diluent peak.

ID solution for working Standard: For ID working standard solution, it was prepared using MND. The major peak other than diluent peak was MND peak.

Procedure: System suitability was performed as per analytical method. Performing single injection of each ID solution. Perform single injection of placebo, FP sample.

Acceptance criteria:

(a) The peaks due to impurities, MND should be well separated from any peaks due to diluent and mobile phase.

(b) There should be no significant interference due to diluent and MP at the RT of the specified MND peak.

B. Specificity by forced degradation

This study was performed by applying the following forced degradation parameters (Table 2) to sample preparation. All the preparations were analysed on an HPLC system with photo diode array detector, array detector. During forced degradation study, observation such as solubility and phase separation were documented.

Table 2: Forced Degradation pathways and conditions.

Degradation pathways	Condition
Acid	0.1 N Hydrochloric Acid, 1.0 ml, 60 minutes
Base	0.1 N Sodium Hydroxide, 1.0 ml, 60 minutes
Peroxide	30% H_2O_2, 1.0 ml, for 30 minutes
UV light	254 nm for 8 hours.
Dry heat	Thermal (110°C for 8 hours in oven)

Controls:

Control Working Standard: (WS)

Prepare WS (without MND) according to the analytical method by using Diluent instead of API

Acid degradation:

Acid blank: Add 1 ml of 0.1 N HCl into a 10 ml volumetric flask. Use diluent to adjust the volume. Mix thoroughly.

Acid API: Put a precise 10 mg weight of API Sample into a volumetric flask (10 ml). Transfer 1.0 ml of 0.1 N HCl. Make up to volume using diluent. Keep standing for 60 minutes and following working standard procedure. (Note: solution was filtered using 0.45µm Nylon syringe filter)

Base degradation:

Base blank: Transfer 1 ml of 0.1 N NaOH to a 10 ml clean and dried volumetric flask. Dilute to volume with diluent. Mix well

Base - API:

(Note: inject within 1 hour of composition)

Put a precise 10 mg weight of API Sample into a 10 ml volumetric flask. Transfer 1.0 ml of 0.1 N NaOH, and place the volumetric flask at room temperature for about 30 minutes. Make up to volume using diluent and resultant solution was filtered using 0.45µm Nylon syringe filter

Peroxide degradation:

Peroxide blank: Add 1.0 ml of 30% H_2O_2 in a 10ml clean and dried volumetric flask. Make up to volume using diluent. Mix well.

Peroxide - API: Put a precise 10 mg weighed API Sample in 10ml water in volumetric flask. Transfer 1.0 ml of 30% H_2O_2 for 30 minutes and let it reduce the temperature to room temperature. Volume made up with diluent and resultant solution was filtered using 0.45µm Nylon syringe filter.

Dry heat degradation:

Heat blank: Keep 10 ml of diluent in volumetric flask and keep in hot air oven at 110°C for 8 hours and analyze immediately thereafter.

Heat - API: 5 mg of API Sample should be precisely weighed and added to a 10ml volumetric flask. For around eight hours, place the flask in an oven set to 110°C. The flask should be taken out of the oven and let cool to room temperature. Using diluent, dilute to desired volume (Note: filter the solution using a 0.45 µm Nylon syringe filter).

UV degradation:

UV blank: Keep 10 ml of diluent in volumetric flask and keep in UV Cabinet for 8 hours at 254 nm and analyze immediately thereafter.

UV - API: Put 5 mg of API Sample should be precisely weighed and added to a 10ml volumetric flask. Expose the placebo to UV light at 254 nm for about 8 hours. Finally make up to volume using diluent (Note: Filter the solution using 0.45µm Nylon syringe filter)

Procedure: Perform System suitability as per analytical method.

Inject the control sample, blank preparations, exposed blank and sample preparations, single injection each.

Acceptance criteria:

(a) MND peak should be well separated from any peaks due to diluent, placebo and other degradation peaks generated during stress conditions.

(b) Peak purity should be greater than 0.998 for the MND.

(c) There should be no significant interference due to diluent and placebo at the RT of the specified impurity peak or MNDpeak.

(d) MNDpeak should give between 5% - 25% degradation in any one forced degradation conditions. If required, forced degradation condition shall be altered with new sample preparation to achieve these criteria.

3. Stability of analytical solutions (Solution stability): (Pingale & Topiwala 2013).

Working standard and finished product sample solution stability was determined during validation and was included in the final method. All solutions were stored in the original container.The solution stability demonstrated by periodic analysis of the same working standard and FP sample solution shall be evaluated as per the procedure below.

Procedure:

(a) Inject blank each time to identify peaks due to diluent.

(b) Working Standard Stability:

— Perform System suitability as per analytical method at 0 hours (the area of working standard injection from the system suitability is considered as stability T0).

— Keep the working standard solution in the original volumetric flask while parafilmingit and storing it at room temperature to test the stability of the solution at various time intervals.

— Inject the stored solution as a part of system suitability in a new sequence.

— To determine the working standard solution stability at different time interval, use peak area of working standard injected from initial time to the end of stability study and determine cumulative % RSD of working standard peak area.

Acceptance Criteria:

— The working standard solution will be considered stable if the cumulative % RSD of area of MND is NMT 2.0%.

Precision: Borman & Elder (2017); Sundararajan & Kumar (2012).

A. Instrument precision:

System suitability performed as a part of any analysis may be considered as instrument precision.

Mobile phase and working standard were prepared according to analytical method.

Acceptance Criteria:

(a) For the MND peak from six duplicate injections of the working standard, the %RSD for the peak area should be NMT 2.0%.

(b) Asymmetry for MND peak in all working standard injections should be NMT 2.0.

(c) All operational standard injections of the MND peak should include NLT 2000 theoretical plates.

For MND, the total% RSD for the peak region from all injections of working standard should be NMT 2.0%.

Method Precision & Accuracy (Assay)

Unspiked control sample: Six control samples were prepared according to the analytical method for Assay and relatedcompounds.

Blank: Blank prepared according to the analytical method by using diluent instead of API.

Spiking stock sample solution-1 (SSSS-1)

Quantitatively transfer 10 mg of MND that has been accurately weighed in a volumetric flask (10 ml). Fill it upto volume using diluent after filling it to two-thirds full, vortexes it with the cap, and sonicates it to dissolve it. When the solution has reached room temperature, add diluent until the desired volume is reached. Mix thoroughly. (MND Conc. approx. = 1000 µg/ml).

Spiked Samples: Make duplicate preparations for every stage. Weigh accurately about 'X' ml SSS-1 directly into a 10ml volumetric flask. Make up to volume using diluents. (Note: Filter the solution using 0.45µm Nylon syringe filter, if necessary)

Control Working Standard for Accuracy:

In a 10 ml volumetric flask, add 1 ml of SSSS-1 and 1 ml of SSS-2. With a diluent, dilute to volume.

Procedure: Perform System suitability as per Analytical method.

Inject working standard for accuracy in 3 replicates.

Acceptance criteria:

(a) MND should meet % Assay as per the specification in the unspiked samples.

(b) The relative standard deviation of % Assay of six sample preparations should be NMT 2.0 % for MND.

(c) Average % Recovery at each spiked level should be between 95.0% to 103.0% for MND.

(d) For MND, the % RSD of% recovery at each spiking level should be NMT 2.0%.

LOD and LOQ of MND: The calculation shown below, together with results from the Precision and Linearity of MND technique, should be used to establish the Limit of Detection (LOD) and Limit of Quantitation (LOQ).

Calculations

LOD

DL = 3.3 x σ/ S

Where σ = the response's standard deviation

S = the calibration curve's slope

LOQ

DL = 10 x σ/ S

Where σ = the response's standard deviation

S = the calibration curve's slope

Acceptance Criteria: For LOD: Peak should be detected in all six replicate injections. (Note that a LOQ number that is also a LOD is one where the % RSD of the region of six duplicate injections is 2%).

4.3 Intermediate precision (ruggedness study): In order to demonstrate ruggedness, the second analyst will use different mobile phase preparation, different column lot number (if possible) and different HPLC system (If Possible)

Prepare the mobile phase and working standard according to analytical method.

WS preparation for intermediate precision: Prepare six WS samples according to the analytical method.

Blank: Prepare blank according to the analytical method by using diluent instead of API.

Procedure: Perform system suitability as per analytical method.

Inject intermediate precision samples in 6 replicates.

Acceptance criteria:

(a) % Assay of MND should meet the specification in the unspiked samples.

(b) The relative standard deviation of % Assay from six unspiked sample preparations should be NMT 3.0%.

For MND, NMT 3.0 % should be the gap between the mean assay achieved in method precision and intermediate precision.

Linearity: Linearity of MND (120 to 0.01% of assay concentration) in order to establish the linearity for assay standard response of MND to calculate related compounds.

MND linearity stock solution (MLSS-1): Put 10 milligrams of MND into a volumetric flask with a capacity of 10 ml. Add diluent up to volume, and then combine. With the cap on, fill the diluent vortex halfway. Next, add diluent up to volume, and then combine. Allow the solution to settle to room temperature for 30 minutes. (Approx. MND Conc. = 1000 µg/ml)

MND linearity stock solution (MLSS-2): Transfer accurately 0.1ml of LSS-1 into 10ml volumetric flask. Add diluent up to volume, and then combine. With the cap on, fill the diluent vortex halfway. Next, add diluent up to volume, and then combine. Allow the solution to settle to room temperature for 30 minutes. (Approx. MND Conc. = 10 µg/ml)

Linearity solutions:

Transfer X.0 ml of the ML**SS-1 or MLSS-2,** in a 10ml volumetric flask. Add diluent up to volume, then combine.

Procedure: Perform System suitability according to analytical method.

Inject the linearity samples single injection each.

Acceptance criteria:

(a) r^2 (coefficient of determination) should be NLT 0.998 for MND. Report y-intercept, slope and residual sum of squares.

Robustness. Robustness will be studied by slightly changing the chromatographic conditions and solution preparation, as shown below. Change only one condition at a time.

Table 3: Robustness conditions.

Condition / Change	Mobile Phase A	Column Oven Temperature (°C)
Decrease	53%	28°C
Normal	55%	30°C
Increase	57%	32°C

WS preparation: Prepare one WS according to analytical method.

Blank preparation: Prepare Blank according to the analytical method by using Diluent instead of API.

***Procedure for each robustness condition*:**

Perform System suitability as per Analytical method.

Inject Blank, Working Standard, and single injection each.

Acceptance criteria:

(a) The system suitability should meet the acceptance criteria.

(b) % Assay of MND should meet the specification in FP sample.

Note: If the above criteria are not met for any of the robustness conditions, a moderate variation may be attempted until it achieves the above acceptance criteria.

Results (HPLC Method validation Report)

Method development

From the literature, it was incurred that MND was very slightly soluble in water but able to solubilize in organic solvents like methanol and ethanol. An initial test was performed by adding 10 mg MND to 10 ml methanol. The solution was vortexed, sonicated. All the solids dissolved quickly. Therefore, methanol was the choice of diluent for initial development. As the diluent was methanol, one of the Mobile phases for elucidation of MND was methanol.

To decide the second mobile phase, acid buffer of 0.1% Trifloroacetic acid was used. As MND is a non-polar molecule water-based column was to be used. Agilent's Zorbax Bonus RP column with dimensions' length of 250 mm, column diameter 4.6mm, and particle size 5 micron was selected.

Method validation

Specificity by identification: The identification solutions were prepared as specified in the protocol and injected. The retention time for MND was 4.29 mins.

Specificity by forced degradation

This study was performed by applying the following forced degradation parameters as specified in the protocol to sample preparation. All preparations were analyzed on an HPLC system with diode array (DAD) detector. The results of forced degradation study are shown in Table 4.

Table 4: Specificity by Forced Degradation% Degradation of MND.

Sample name	Degradation condition	% Assay	Peak purity	% Degradation
Control	NA	100.00	1.000	NA
Acid	0.1 N, HCl 1.0 ml, at RT for 10 min	91.22	0.998	8.78
Base	0.1 N, NaOH, 1.0 ml, at RT for 30 min.	87.14	0.996	12.86
Peroxide	30% H_2O_2, 1.0 ml, at RT for 60 min.	94.99	0.992	5.01
UV	254 nm for 8 hours	95.85	0.990	4.15
Dry heat	Thermal (80°C for 8 hours in an oven)	95.46	1.000	4.54

Table 5: Working standard (Solution stability at room temperature).

Stability time interval	Cumulative % RSD of MND
Intraday	
Working Standard – Intraday Morning	NA
Working Standard – Intraday Evening	0.81
Interday	
Working Standard – Interday 1 days	NA
Working Standard – Interday 2 days	0.74

(a) The working standard is found stable for 1 day when stored at room temperature, in parafilmed, original volumetric flask.

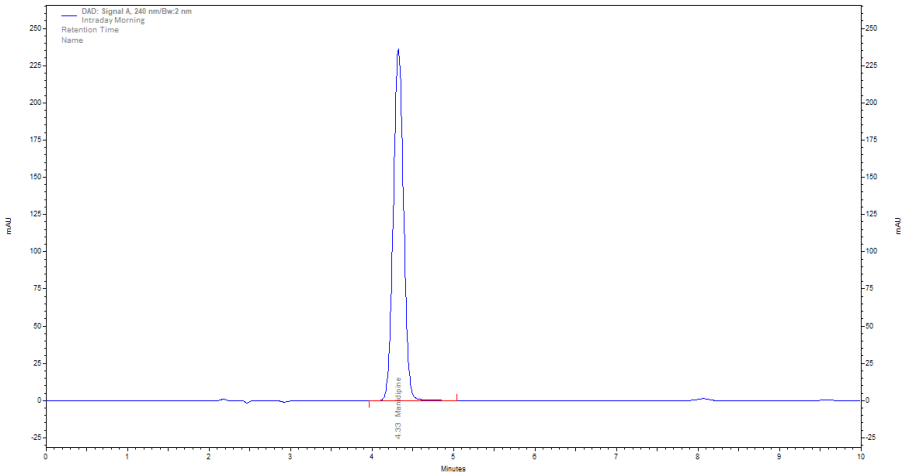

Fig. 1. Intraday stability – morning.

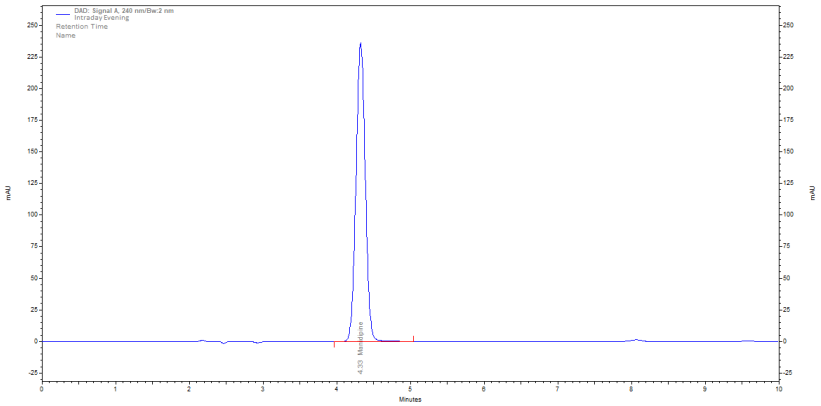

Fig. 2. Intraday stability – evening.

The method system suitability requirements were met for each analysis performed. Following Table 6 shows instrument precision data.

Accuracy (assay): The Accuracy (assay) study was performed at each level in triplicate and injected. The results of analysis are shown in Table 7.

LOD and LOQ. The limit of detection (LOD) and limit of quantitation (LOQ) were determined. The results of analysis are shown in Table 8.

Table 6: Instrument precision results.

Repeatability		System Suitability			
Sample ID	**Area**	**RT**	**TP**	**Asymmetry**	**Peak Purity**
100% Rep 1	4346061	4.33	5429	1.01	1.00
100% Rep 2	4328745	4.33	5581	1.03	1.00
100% Rep 3	4368941	4.33	5297	1.04	1.00
100% Rep 4	4317854	4.33	5347	1.01	1.00
100% Rep 5	4325847	4.33	5322	1.05	1.00
100% Rep 6	4314756	4.33	5378	1.02	1.00
AVG	4333701	4.33			
STDEV	20449.52266	9.7295207			
RSD	0.47	0.00			

Table 7: Accuracy results (MND).

Std Wt. (mg)	**% Purity**	**Stock Conc. (ug/ml)**
10	99.7	997.00
Std Area	4333700.667	

Sample ID	**Reps**	**Spiked Conc (ug/ml)**	**Area**	**Amount Recovered (ug/ml)**	**% Recovery**	**AVG**	**STDEV**	**% RSD**
80%	Rep 1	79.76	3474602	79.94	100.22	100.37	0.16	0.16
	Rep 2		3485711	80.19	100.54			
	Rep 3		3478542	80.03	100.33			
100%	Rep 1	99.70	4346061	99.98	100.29	100.33	0.46	0.46
	Rep 2		4328745	99.59	99.89			
	Rep 3		4368941	100.51	100.81			
120%	Rep 1	119.64	5209618	119.85	100.18	100.40	0.24	0.24
	Rep 2		5234789	120.43	100.66			
	Rep 3		5219745	120.08	100.37			

Table 8: Summary of LOD/LOQ results for MND.

LOD	0.89 ug/ml
LOQ	2.69 ug/ml

The data demonstrate that the accuracy, linearity, LOD and LOQ are established for MND. The method is linear and accurate in above ranges.

Intermediate Precision (Ruggedness): The ruggedness study for MND carried out by two different analysts using same HPLC systems and same HPLC columns. The detail of analysis is shown in Table 9:

Table 9: Ruggedness analysis details.

	Analyst-1 - YH	Analyst-2 - AT
HPLC	LC-01	LC-02
Date of analysis	22-02-2021	23-02-2021
HPLC column	Zorbax SB-Aq	Zorbax SB-Aq

Six working standard samples were prepared as ruggedness samples by analyst-2 according to the analytical method.

Table 10: Ruggedness results (area).

Sample ID	Area
IP-1	4278542
IP-2	4268972
IP-3	4278541
IP-4	4252357
IP-5	4278985
IP-6	4256745
AVG	4269023.667
STDEV	11907.58218
RSD	0.28

The absolute difference of % RSD obtained in method precision and intermediate precision is 0.17 % for MND.

Linearity

(a) [Linearity of Manidipine HCL (˜120% to ˜0.05% of test concentration) in order to establish the linearity for assay standard response of Manidipine HCL to calculate related Compounds.]

(b) linearity solutions were prepared as per the protocol and injected. The results of Manidipine HCL linearity study are shown in the following Table 11.

Table 11: Linearity Results (MND).

% Level	Conc (ug/ml)	Retention Time	Peak Area	Theoretical Plates	Asymmetry
0.01	0.01	4.28	1408	0	0.65
0.05	0.05	4.28	4819	4330	0.99
0.1	0.1	4.29	10016	3902	0.83
0.5	0.5	4.29	27745	6203	1.05
1	1	4.30	61194	5879	1.02
5	5	4.31	273022	6176	1.02
10	10	4.30	544690	6145	1.05
20	20	4.29	870831	6119	1.05
40	40	4.31	1729988	6133	1.01
60	60	4.31	2604382	6067	1.01
80	80	4.33	3474602	5702	1.01
100	100	4.33	4346061	5429	1.01
120	120	4.33	5209618	5091	1

Acceptance criteria:

(a) r^2 (coefficient of determination) should not be NLT 0.998 for Manidipine HCL

(b) Report y-intercept, slope and residual sum of squares.

Linearity Results:

(a) r^2 (coefficient of determination) is = 0.9997.

(b) Slope: 43185

(c) y-intercept: 23183

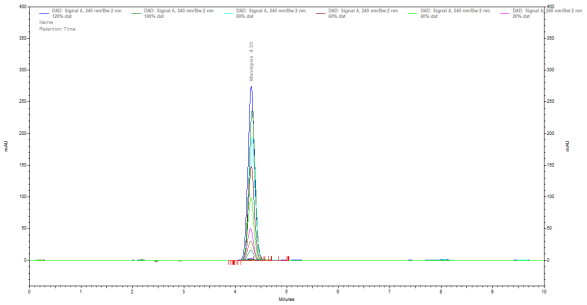

Fig. 3. linearity overlay graph MND.

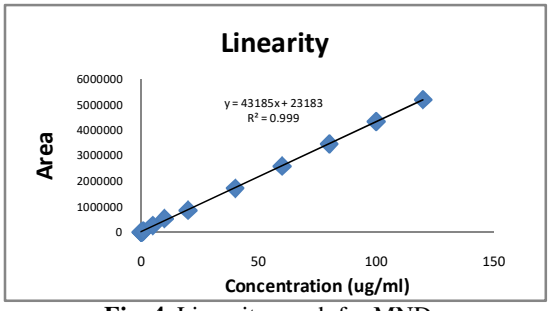

Fig. 4. Linearity graph for MND.

The data demonstrate that the method is linear over the range of 120% to 0.01% for MND

Robustness

Robustness was studied by changing the chromatographic conditions mobile phase composition and Column oven temperature one at a time. The results of analysis are shown in Table 12 and 13.

Table 12: Change in column oven temperature.

Condition	Sample ID	Manidipine HCL				
		RT	TP	Asymmetry	Area	Peak Purity
28˚C	Blank	-	-	-	-	-
	WS	4.33	5321	1.02	4289871	1.00
30˚C	Blank	-	-	-	-	-
	WS	4.33	5429	1.01	4346061	1.00
32˚C	Blank	-	-	-	-	-
	WS	4.33	5412	1.04	4310127	1.00

Table 13: Change in mobile phase combination.

Condition	Sample ID	MND				
		RT	TP	Asymmetry	Area	Peak Purity
MP A Increase (57M-43TFA)	Blank	-	-	-	-	-
	WS	4.42	5378	0.99	4297886	1.00
Normal (55M-45TFA)	Blank	-	-	-	-	-
	WS	4.33	5429	1.01	4346061	1.00
MP A decrease (53M-47TFA)	Blank	-	-	-	-	-
	WS	4.25	5456	1.02	4217415	1.00

All robustness conditions met the system suitability criteria

RESULTS AND DISCUSSION

The validated HPLC method followed ICH guidelines. MND showed limited solubility in water but higher solubility in organic solvents like methanol and ethanol. The calibration curve showed excellent linearity with a regression coefficient (R2) exceeding 0.997. The LOD and LOQ were determined to be 0.021µg/ml and 0.063 µg/ml, respectively. The method showed satisfactory linearity and accuracy in the tested concentration ranges. A water-based column was used for the analysis of MND, considering its non-polar nature. The absolute % RSD difference between method precision and intermediate precision for Manidipine HCl was 0.17%. The proposed method demonstrated accurate results with % RSD for recovery studies below 2%. The data confirmed the method's linearity from 0.01% to 120% concentration range for MND.

CONCLUSIONS

HPLC method development presented in this study shows that this method is suitable for the identification of Manidipine HCL. An HPLC method for the identity, assay, and purity evaluation of Manidipine HCL and its impurities has been successfully developed. Study has demonstrated that the method is rugged. It has also been extensively validated.

Acknowledgement. The author would like to extendtheir sincere regard and gratitude to the university for giving permission to research conduct and guide for his valuable support

Conflict of Interest. None.

FUTURE SCOPE

To compare a defined characteristic of the drug substance or drug product to predetermined acceptance criteria for that characteristic, an analytical technique is designed which can be used for identification, assay and purity determination of MND and other drugs.

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How to cite this article: Jadhav A.B., Nawale R.B. and Thorat S.T. (2023). Method Development for Identification of Manidipine HCl using High Performance Liquid Chromatography. *Biological Forum – An International Journal, 15*(6): 957-965.

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