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Analytical Method Development of Process Related Impurities of drug Lacidipine by using RP HPLC Techniques

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ABSTRACT

The synthesis and Retro synthesis of Lacidipine was studied to determine the process-related impurities in Lacidipine formulation. The intermediates of the drug treated as impurities were decided and planned for the synthesis. General Method for 1,4dihyropyridine Synthesis was used. The purity of the intermediate was carried out by column chromatography using dimethyl sulfoxide (DMSO) as mobile phase. The technique was observed to be explicit, direct, delicate, exact and precise. The outcomes showed great middle of the road accuracy. Versatile stage stream rate seemed to have critical impact on power, and thus it was essential to be painstakingly controlled.

Keywords: *Lacidipine, High Performance Liquid Chromatography (HPLC), etc…*

1. INTRODUCTION

Debasements in pharmaceuticals are the undesirable chemicals that stay with the dynamic pharmaceutical fixings (APIs) or create amid detailing or upon maturing of both API and defined API‟s to medications. The nearness of these undesirable chemicals indeed in little sums may impact the viability and security of pharmaceutical items. Debasement profiling (i.e., the personality as well as the amount of pollution within the pharmaceuticals), is presently getting imperative basic consideration from administrative specialists. Debasement profiling is common title of a bunch of explanatory exercises, the point of which is the discovery, identification/structure illustration and quantitative assurance of natural and inorganic debasements as well as remaining solvents in bulk drugs and pharmaceutical formulations.[1,2]

The distinctive pharmacopoeias, such as the British Pharmacopoeia (BP) and the Joined together States Pharmacopoeia (USP) are gradually consolidating limits to passable levels of pollutions display within the API‟s or details. Different administrative specialists like ICH, USFDA, Canadian Medicate and Wellbeing Office are emphasizing on the virtue necessity and the recognizable proof of debasements in Dynamic Pharmaceutical Ingredient's (API‟s). Capability of the debasements is the method of obtaining and assessing information that builds up organic security of person pollution hence, uncovering require and scope of debasement profiling of drugs in pharmaceutical inquire about. Worldwide Conference on Harmonization (ICH) has distributed rules on debasements in unused medicate substances, items and remaining solvents. There's a great noteworthy request for the impurity-reference standards at the side the API reference measures from both administrative specialists and pharmaceutical companies. ICH Q3A covers sedate substances, Q3B covers medicate items and Q3C covers of leftover dissolvable. These rules characterize what examinations and documentation ought to be made in examining debasements and corruption items seen in soundness ponders at prescribed capacity conditions. In common, agreeing to ICH rules on impurities in unused medicates items, distinguishing proof of debasements underneath the 0.1% level isn't considered to be fundamental unless the potential debasements are anticipated to be curiously strong or poisonous. In all cases, pollutions ought to be qualified. In case the information are not accessible to qualify the proposed determination level of a pollution, ponders to get such information may be required (when the normal capability edge limits given underneath are surpassed). Agreeing to ICH, the most extreme every day measurements quality. [3,4]

Objectives

The plethora subscribed in this research paper is directed towards the HPLC study of some intermediates of Lacidipine which may be the part of process related impurities present in Lacidipine. The synthesized intermediates can then be explored as an impurity in Lacidipine formulations.

2. MATERIALS AND METHODS

Acetaldehyde, o-nitrobenzaldehyde, silica gel, and ammonium acetate, Methanol, hexane, ethyl acetate, pyridine, ammonia, benzene, ethylacetoacetate, acetone and the HPLC grade solvents acetonitrile, methanol and water all the chemicals are used of AR grade only and were purchased from Merck Chemicals Pvt. Ltd. Nasik, MS, India. The Lacidipine bulk was obtained as a gift sample for research purpose

Spectroscopic methods

GC-MS Technique

The Q-TOF Micro mass (YA-105) spectrometer capable of recording High Resolution Mass Spectrum (HRMS) both in atomic pressure chemical ionization (APCI) and Electron spray Ionization (ESR) was used for quantitation of synthesized impurities.[5]

NMR Spectra

The ^{1}H and ^{13}C NMR was recorded by using NM achieved Varian NMR Mercury 300 MHz spectrometer. The DMSO was used as a solvent and TMS were used as an internal reference standard for the proton experiment. All experiments were conducted at 25°C, and no shift relaxation agents were employed. The 1H and 13C NMR chemical shift values were reported on the δ scale in ppm, relative to TMS (δ=0.00) respectively. [6]

HPLC Method Development

The HPLC method was developed by using LC20AD Prominence Liquid Chromatography SPD20-A Schimadzu, Japan. The UV-Vis detector and C18 column with dimens ion on 25 x 0.6 cm was used for the method development with flow rate 1.0ml/min at wavelength 275 nm. The acetonitrile: buffer in proportion of (60:40) as a mobile phase was selected for development of method validation for synthesized impurities and various parameters according to ICH guidelines (Q2B) were studied. [7]

Analytical Method validation

A suitable analytical method was developed and validated for identification. New drug development requires meaningful and reliable analytical data to be produced at various stages of development. [8]

1) Selection of analytical method for method development.

2) Screening of chromatographic conditions and phases.

3) Optimization of method to set the parameters related to ruggedness and robustness.

Preparation of Mobile phase

The selection of mobile phase was according to polarity and non-polarity of solvents. The acetonitrile: buffer (60:40) was selected as mobile phase in ratio of 60:40 and was filtered on membrane filter (0.45µ) to remove degassing and was stirred for 10-15 min. [9]

Preparation of Stock solution (Impurity)

The stock solution was prepared according to the standard procedure viz., 10mg of intermediates I was accurately weighed on analytical balance, and using mobile phase it was dissolved to make volume up to 100ml stock solution.

The sample was prepared in the ppm in the range of 2-12 ppm in concentrations respectively for the method validation by HPLC. [9]

Preparation of standard solution (Formulation)

The Lacidipine Hydrochloride solution was prepared in 100ml stock solution for quantification of intermediates in Lacidipine formulation. The 4ml of standard solution was measured and volume up to 100ml with mobile phase was diluted and thus in the range of 20-120 ppm in concentration of standard solution were prepared for quantification of intermediate in the Lacidipine formulation. The peak was observed thus the retention time and the peak area were noted. Hence, these peaks were quantified and comparative study to sample was identified. The method was validated with respect to the parameter. [9]

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly

proportional to the concentration (amount) of analyte in the sample. The sample solution for linearity was prepared in the range of 20-120 ppm in concentrations from the stock solution in 10ml each. The sample solutions were analyzed individually to calculate regression coefficient and the slope for quantification of intermediate. [9]

Precision

The precision is the measure of the degree of repeatability of the analytical method under normal operation and is normally expressed in percentage relative standard deviations. The sample solution were prepared in the same concentration (50 ppm) repeatedly in 10ml each and identified to calculate average, standard deviation (SD) and percentage relative standard deviation (%RSD) for quantification of intermediate. [10]

Accuracy

The accuracy is the measure of exactness of an analytical method, or the closeness of agreement between the measured value and the value accepted as conventional, true value. The sample solution was prepared in the different concentration in percentage related to 50%, 100% and 150% individually. The sample and test solution were prepared in the range of 60, 80 and 100ppm each in triplicate to calculate the percentage recovery for quantification of intermediate. [10]

Limit of Detection (LOD)

The LOD is the lowest concentration of an analyte in a sample that can be detected, though not necessarily quantitated in the stated experimental conditions. The LOD for the sample was calculated by slope and intercept by using following formula;

$LOD = 3.3 \times \sigma / S$

Where, Slope

σ = the standard deviation of the response.

S = the slope of the calibration curve.

Limit of Quantification (LOQ)

The LOQ of an individual analytical method is the lowest concentration of an analyte in a sample, which can be quantitatively determined with suitable precision and accuracy under stated experimental conditions. The LOQ of the sample was calculated by slope and intercept by using following formula. [10]

$LOQ = 10 \times \sigma / S$

Slope

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve

Robustness

It is the capacity of a method to remain unaffected by small deliberate variations in the method parameters. The robustness of a method is evaluated by varying method parameters. The change in analyst and the concentration of 4 ppm were used to determines robustness of intermediates I. Thus, area and height of chromatogram were reported to calculate standard deviation (SD), relative standard deviation (RSD) and percentage relative standard deviation (RSD).

Ruggedness

Ruggedness is the degree of responsibility of the results obtained under variety of conditions. The sample solution was prepared in the range of 20-100 ppm respectively and with change in flow rate up to 0.8ml/min to determine the area and height of chromatogram of the sample to calculate standard deviation and percentage relative standard deviation.

Interday

The Interday of the method was developed for the intermediate I. The same sample after six hour with concentration 20-100 ppm was used for the quantification of peak area and height of chromatogram for the sample. Thus the standard deviation and relative standard deviation were calculated.

Intraday

The intraday of method was developed for the intermediate I and the sample after 24hrs were used for the quantification and the area of chromatogram was reported. Hence the standard deviation and relative standard deviation were calculated.

Quantitation of Impurity

The total amount of impurity present in Lacidipine formulation was calculated for the intermediate and the result was

compared to ICH limit for impurities in new drug substances is 0.1%.

3. RESULTS AND DISCUSSION

Gas Chromatography-Mass Spectroscopy (GC-MS)

The Q-TOF Micro mass (YA-105) spectrometer capable of recording High Resolution Mass Spectrum (HRMS) both in atomic pressure chemical ionization (APCI) and Electron spray Ionization (ESR) were used for quantitation of synthesized impurities. [11, 12]

The m/e ratio was calculated for intermediate I and was uncorrected.

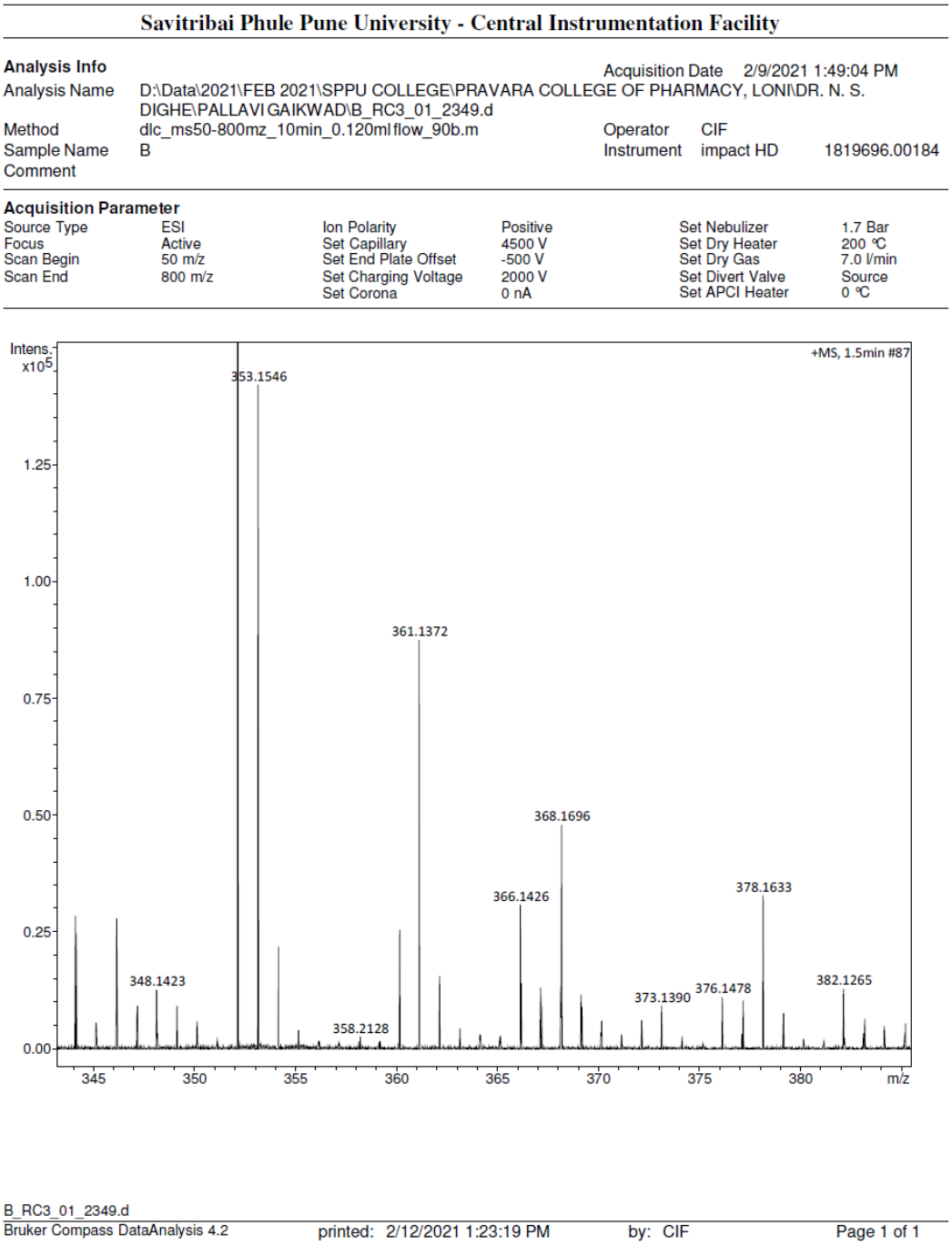

Figure 1: Mass spectra for intermediate

NMR Spectra

NMR Spectroscopy enables us to record differences in magnetic properties of the various magnetic nuclei present and to deduce in the large measure about the position of these nuclei within the molecule. We can trace how many different kinds of environment are there in the molecules and also which atoms are present in neighboring groups. The proton NMR spectra, enables us to know different chemical and magnetic environments corresponding to protons in molecules. The 1H and 13C NMR was recorded by using NM achieved Varian NMR Mercury 300 MHz spectrometer using DMSO as solvent and TMS as an internal reference standard. The chemical shift data were expressed as δ-values related to TMS. [11, 12]

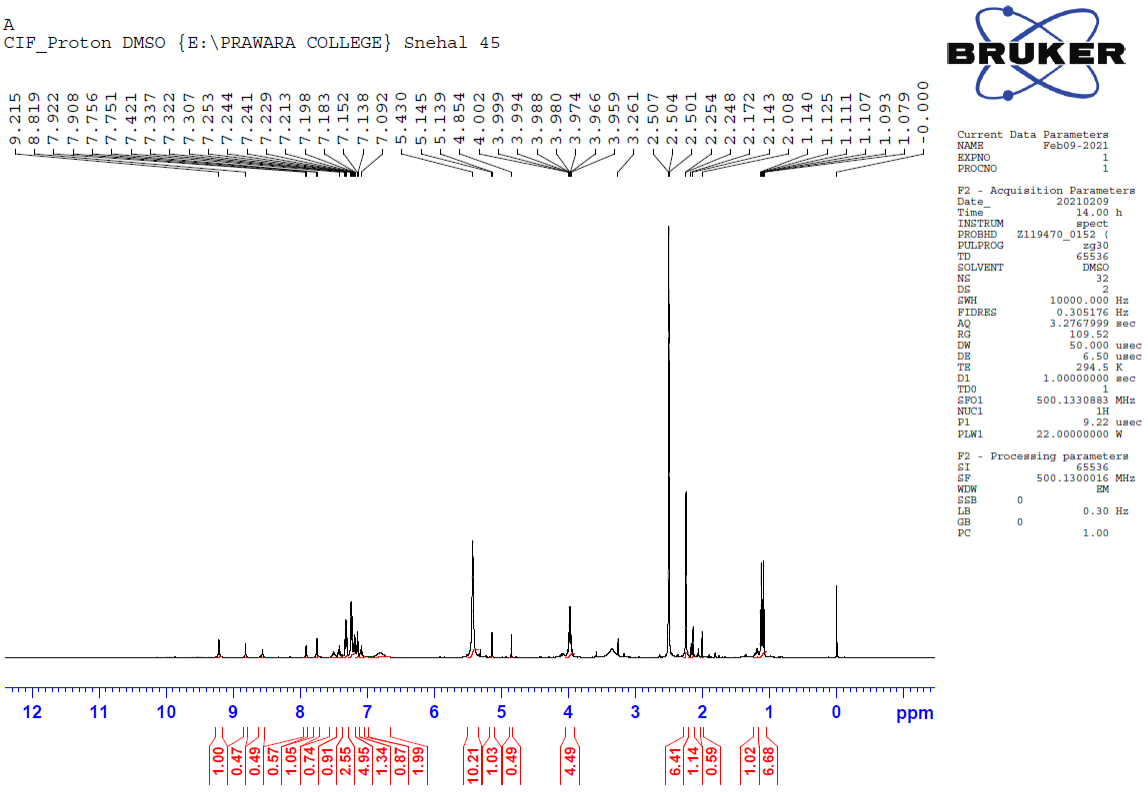

Figure 2: 1H NMR spectra for intermediate

Spectral data

Intermediate I

IR (KBr) cm-1: 3344 (N-H stretching), 2931,2870 (C-H stretching),1705 (C=O stretching), 1647 (C=C stretching), 1523, 1473 (N-O stretching), 1373 (C-H deformat ion), 1300 (CH3 deformation), 1261 (C=O stretching), 1118 (C-O-C stretching), 744, 750, 766 (ring bending). **1H NMR (DMSO):** δ=1.2 (OCH2CH3), 2-2.4 (CH3), 2.5 (N-H), 4 (CH2CH3), 5 (CH2), 7.4-(benzeneproton), 9 (deshieldedproton). **13CNMR** δ= 14, 18, 39, 59, 102, 120, 121, 122, 129, 134, 146, 148, 150, 160, 166. **GC-MS (m/e) :**374.15 (100%), 375.15, 376.15.

HPLC Method Development

Analytical Method

The ICH Q2B guidelines discuss the analytical method validation on HPLC. Currently the vast majority of process-related impurity determinations are performed by HPLC. It offered the desired sensitivity for trace level determinations with a high degree of automation. A wide variety of stationary phases and operation modes make HPLC applicable to all drug classes. The typical detection limits for process-related impurities by HPLC are 0.1% or lower and this can be routinely met in the majority of circumstances using conventional UV detectors. These methods involved the prediction of likely impurities within the synthetic process, their isolation and identification by suitable analytical techniques.

The last step of the present study was to check method's validation for linearity, precision, accuracy, intra/inter-day precision, ruggedness. The optimized HPLC method was specific in relation to the placebo used in this study. All placebo chromatograms showed no interference peaks. [9]

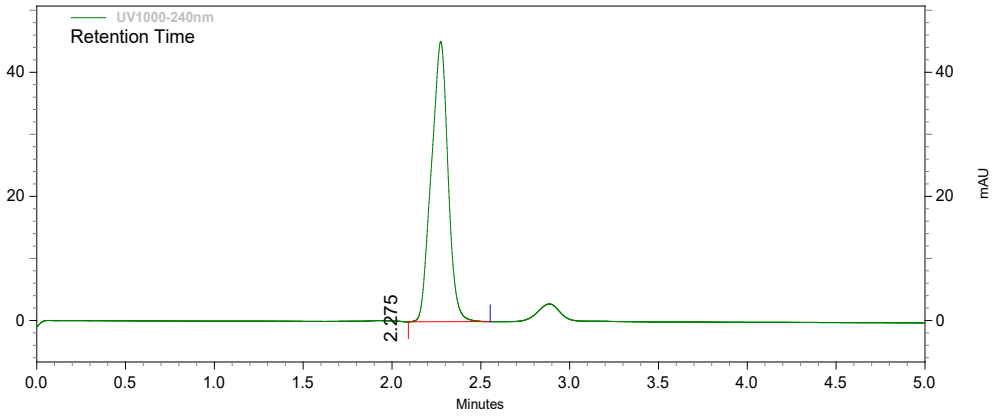

Figure 3: HPLC chromatogram for Intermediates I.

UV1000-240nm Results					
Name	**Retention Time**	**Area**	**Area Percent**	**Theoretical Plates**	**Asymmetry**
Intermediate	2.275	293043	100.000	2572	0.97

Linearity

The linearity of the proposed method was estimated by regression analysis at six concentration levels in the range of 20-120ug/ml for intermediate and was successfully quantitated shown in **Table 1**. The slope and intercept of calibration curves is fig no 16. The correlation coefficients (R2) was found to be 0.9988 and intercept y=8094.1 was linear.

Table 1: Linearity data of Intermediate

Sr. No.	**Conc. in ppm**	**Area (mv-sec)**
1	20	151005
2	40	286771
3	60	456431
4	80	629274
5	100	776194
6	120	955951

Figure 4: Graph of peak area vs. conc. in ppm.

Regression coefficient of the linearity was R2 – 0.9988; indicates linear relationship. Equation of regression y = 8094.1 x – 23980.

Precision

The precision of the synthesized intermediates I was quantified for repeated concentration 50 µg/ml in the range and was reliable with their area of chromatogram as shown in the table no 4. The standard deviation (SD) and relative standard deviation (RSD) was found to be 8.987 and 0.7564 precise.

Table 2: Standard and % relative standard deviation data for precision

Sr. No.	Concentration in ppm	Area in mv sec	SD	%RSD
1	50	427347	8.987	0.7564
2	50	427035		
3	50	426199		

Accuracy

The accuracy with known concentration of each intermediate in formulation was determined. The recovery assessment was performed by the analysis of Lacidipine formulation spiked with known amounts of each impurity at three concentration levels in triplicate of 50%, 100% and 150% and was found to be accurate shown in the **Table 3**. A very good percentage recovery was obtained for 50% but increase in concentration recovery is low.

Table 3: Percentage recovery data for intermediate

Sr. No.	Sample	Amount found in mg (n=3) in %			% Amount Recovered			SEM	SD	% RSD
		50	100	150	50	100	150			
1	A	172.45	254.32	309.76	101.76	96.78	92.65	1.27	2.76	1.7
2	B	168.76	238.65	308.76	97.98	93.26	90.86	1.76	3.10	1.5

Limit of Quantitation and detection

The LOD and LOQ values were calculated for intermediates I based on the noise level and the values are shown in **Table 4**.

Table 4: Method validation summary for HPLC

Sr. No.	Parameter	Standard
1	Linearity range	20-120 µg/ml
2	Slope	8094.1
3	Intercept	23980
4	Correlation coefficient	0.9988
5	LOD	0.903ug/ml
6	LOQ	2..721 ug/ml

Robustness

The robustness of the intermediate I was carried out for three changes in analyst and method was found to be robust at standard deviation to 2.64 and percentage relative standard deviation to 2.23 as shown in the **Table 5**.

Ruggedness

The ruggedness of method were performed for change in flow rate up to 0.8ml/min and method was rugged with high standard deviation to 6.86 and % relative standard deviation to 4.30 as shown in **Table 5**.

Table 5: Robustness, ruggedness and precision of the method

Sr. No.	Parameter	SD	% RSD
1	Precision (n = 3)	8.987	0.7564
2	Intraday precision (n = 3)	0.45	1.879
3	Inter day precision (n = 5)	103.67	1.764
4	Ruggedness (change in flow rate; n =5)	6.86	1.621
5	Robustness (change in analyst; n = 3)	2.64	1.524

System Suitability Parameter

To know reproducibility of the method, system suitability test was carried out to determine the chromatographic parameter such theoretical plates, tailing factor, % RSD by analyzing sample solution shown in the **Table 6**. The values obtained demonstrated the suitability of the system for the analysis of intermediate I.

Table 6: Robustness and ruggedness of the method (System suitability parameters)

Sr. No.	Parameter	Theoretical Plate	Peak area	Tailing Factor	Retention time
1	Flow rate (0.8 ml/min)	7375	167.64	3.2	4.21
2	Flow rate (1 ml/min)	6354	102.10	3.2	3.34
3	Analyst 1	7660	118.70	1.81	3.34

Formulation

As below in the **Table 7** shows better peak and area for impurity present in the Lacidipine formulation at the retention time of 2.860 minutes was reported accurately. The linearity for Lacidipine formulation was determined and the graph conc. vs. area was linear and the regression coefficient was found to be 0.9988 and the intercept was 4877.7 thus, we can determine the synthesized impurity is present in the Lacidipine formulation.

Table 7: Linearity of Lacidipine formulation

Sr. No.	Conc. in ppm	Area in mv/s
1	20	385105
2	40	742803
3	60	1120592
4	80	1516931
5	100	1886593

Figure 5: HPLC chromatogram for Lacidipine Formulation

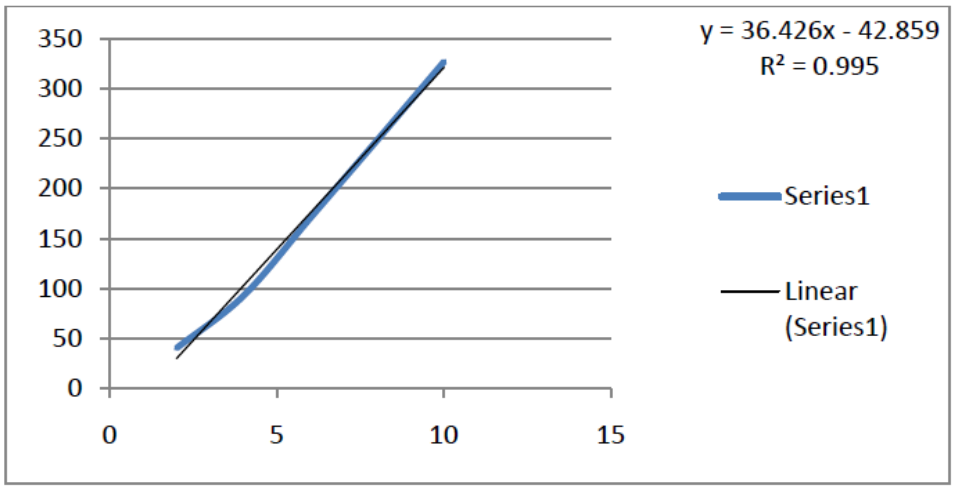

Figure 6: Linearity graph for Lacidipine formulation.

Quantitation of Impurity

The total amount of impurity present in the formulation of Lacidipine is quantified. The 40 µg/ml and 20 µg/ml of both standard as well as sample solution were injected and the retention time was 2.860 minutes and shows the better area of chromatogram. Hence, the following data express the total amount of impurity present in formulation;

Table 8: Data for Lacidipine formulation

Conc.(ppm)	Retention time in min.	Area in mv/s
20	2.860	385105

The 40 µg/ml area represents 742803 mv/s

The 20 µg/ml area represents 385105 mv/s

Then,

Sample/standard

385105/742803 = 0.652 µg/ml

40 µg/ml of Lacidipine---------- 0.5184

100 µg/ml ----------?

= 1.296 µg/ml

Convert into percentage

= 0.01296 %.

4. CONCLUSION

The cycle related debasement of Lacidipine diethyl 1, 4-dihydro-2, 6-dimethylpyridin e 3, 5 dicarboxylate in definition was integrated, portrayed and the RP HPLC technique was created by ICH Q2B rules. The combination of transitional was done by Hantzsch measure utilizing aldehyde, ketoester, alkali and methanol as dissolvable for impetuses. The yield was (84%), by the expressed technique. The item was recrystallized and filtered. The starter assessment was done on lab scale viz., dissolving point, dainty layer chromatography and natural examination. At last, the RP-HPLC technique was created to distinguish the pollution in Lacidipine plan according to ICHQ2B rules. The technique was read for the linearity, exactness, precision, LOD, LOQ and the heartiness and toughness. The technique was observed to be straight, exact, precise, vigorous and tough. Lacidipine definition was additionally investigated for quantitation of diethyl 1, 4-dihydro-2, 6-dimethyl pyridine 3, 5 dicarboxylate as a contamination. The measure of this pollutant was observed to be 0.016% and the formally adequate restriction of the expressed debasement diethyl 1, 4-dihydro-2, 6-dimethyl pyridine 3, 5 dicarboxylate is 0.1%.

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Conflict of interest: None

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