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Analytical Method Development of Process Related Impurities of Lacidipine by UV Spectroscopy and Chromatographic Techniques

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A B S T R A C T

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. Isolation of the both the intermediate was done by column chromatography. The separation was carried out by Thin Layer Chromatography (TLC) and column chromatography. 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.

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 a person pollution hence, uncovering the 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 synthesis and characterization 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, ethyl acetoacetate, 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

Methodology

The synthesis and Retro synthesis of Lacidipine was studied to determine the process-related impurities in Lacidipine formulation.

Synthesis of Impurities

The intermediates of the drug treated as impurities were decided and planned for the synthesis. The synthon had been decided using disconnection approach.[5]

General Method for 1,4dihyropyridine Synthesis

Hantzsch in 1882 synthesized the 1,4-DHPs using aldehyde, α or β ketoester with ammonia or with ammonium acetate in presence of alcohol to reflux for 3-5 hr respectively. Thus, the yield was obtained was not in higher percentage so further can be modified by using some other materials and solvents to increase the yield.[6]

Isolation Method

Isolation of the both the intermediate was done by column chromatography using silica gel as stationary phase and dimethyl sulfoxide as mobile phase.[7]

Separation Methods

The separation was carried out by Thin Layer Chromatography (TLC) and column chromatography.

Purity determination

The purity of the intermediate was carried out by column chromatography using dimethyl sulfoxide (DMSO) as mobile phase.

Characterization of Impurities
The structure of the synthesized intermediate was established by using various analytical technique viz., FT-IR, NMR, elemental analysis and hyphenated techniques viz., LC-MS, UPLC and GC-MS. Hence, these impurities will be further identified as intermediates in Lacidipine formulation.[8, 9]

The characterization of synthesized impurities by using following parameters;

Preliminary Evaluations
Molecular structure and mass can be distinguished using elemental analysis and spectral data. The color, odor and the nature of the synthesized impurity was physically evaluated.

Physicochemical Properties
Melting Point Determination: The melting point of the organic compounds was carried out by an open capillary in a heavy liquid paraffin bath. Melting point is a valuable criterion of purity for an organic compound, as a pure crystal is having definite and sharp melting point.

Percentage yields: The percentage yield of the synthesized intermediate was determined by mathematical expressions.

Elemental analysis: The C, H, O elemental analyses for the synthesized intermediate were calculated in percentage and were compared to standards reference values.[10]

Solubility: The solubility of the synthesized intermediate was carried out for various polar and non-polar solvents. The tests were performed using 1mg/ml of compound and solvent. The solubility of the both intermediate was determined by official (IP. 2010) standard.

Chromatographic methods
Column chromatography
The column chromatography was performed using the column of 50cm height and 1cm to diameter and thus the purity for the synthesized impurity was performed using dimethyl sulfoxide (DMSO) as mobile phase using alumina for elution. The compound was dried at oven temperature 125° C approximately. The quantity for the synthesized intermediates was taken around 200mg.[11]

Thin Layer Chromatography
Thin layer Chromatography (TLC) is an important technique to identify the formulation of new compounds and also to determine the purity of the compounds. The Rf value is the characteristic for each of the compound. Depending upon the polarity and non-polarity of solvents the separation of the synthesized intermediates can be evaluated and the Rf value can be determined. The TLC plates was prepared using silica gel for TLC as stationary phase and the iodine chamber was used for identification of spot at UV cabinet, thus the Rf value of the synthesized impurity was determined using the following expression;
Rf = distance travelled by solute/distance travelled by solvent

Spectroscopic methods
FT-IR technique

FT-IR can be routinely used to identify the functional groups and quality control of raw material and finished products. The Fourier transform Infrared Spectrophotometer Model No 8400s Shimadzu Inc. was used for the determination of the functional group of the synthesized intermediate. The pure and dried KBr were used for identification of spectra using potassium bromide (KBr) disc method for the identification of probable structure of synthesized intermediate.[12]

UV-Visible spectrophotometer

The UV detection can be selected by using UV Spectrophotometer (UV-1650 PC) SHIMADZU INC. The range was selected up to 200-800 nm. The 10ppm dilution was prepared using methanol as a solvent for calibration and the wavelength was selected for further estimation.

3. Results and Discussion

The Experimental Work Comprises of Scheme

Preparation of diethyl 1,4-dihydro-2,6-dimethyl-4-(o-nitro phenyl) pyridine -3, 5-dicarboxylate

0.01 mole of o-nitrobenzaldehyde, 0.02 mole of ethyl acetoacetate were added in round bottom flask. To it add 5ml of ammonia and 10ml of methanol stir vigoursly. Refluxed for 4 hours and pour the solution in cold water and the solution was kept overnight in freezer. Filter at the vacuum and recrystallized from methanol. Purity was checked by TLC with hexane: ethyl acetate: formic acid (6:3:1) as mobile phase Rf-0.73.

Schemes for the synthesis of Lacidipine

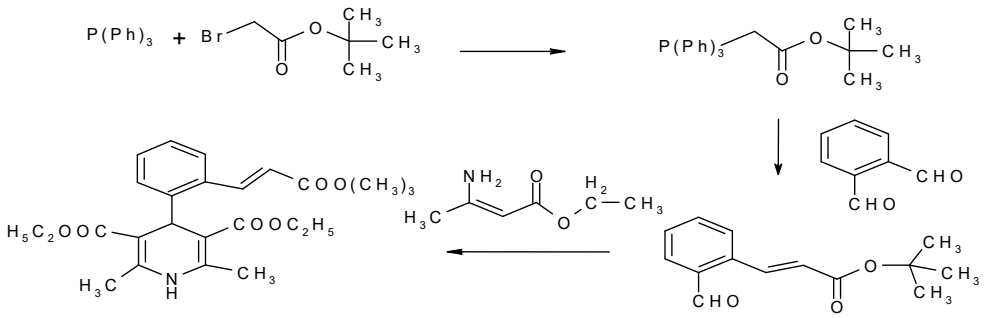


Fig No. 1: Process for synthesis of Lacidipine.

Retrosynthesis of Lacidipine

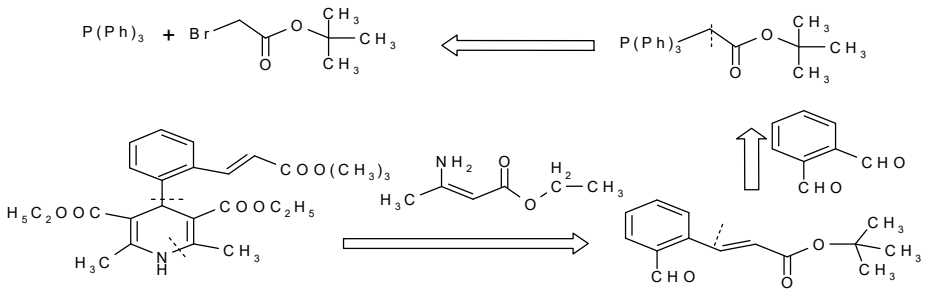
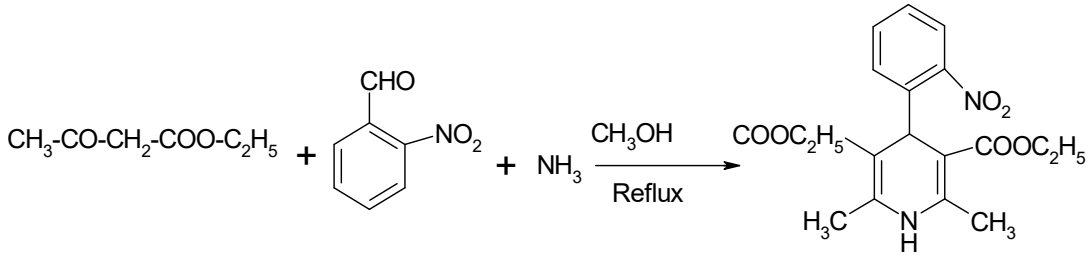


Fig No. 2: Retrosynthesis of Lacidipine.

Synthesis of Lacidipine Intermediate (I)
Synthesis of Intermediates



Characterization of Synthesized Intermediates

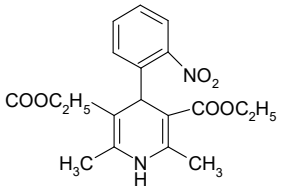
Once an impurity is detected, it is necessary to estimate or quantify it. Initial estimation of impurities present in the new drug molecule involves use of reference standard API. If estimation indicates that impurity present in sample is more than 0.1 % then it must be characterized as per FDA guidelines. Even authentic samples of expected impurities like raw material related impurities, degradation products, process intermediates, byproducts and excipient can be used for characterization of impurities.

Generally, both chromatographic and non-chromatographic techniques are used for isolation of impurities prior to its characterization.

Highly sophisticated instrumentation, such as HPLC, GCMS, LCMS, and UPLC are inevitable tools in the identification of minor components in various matrices. The popularity of LC-MSMS systems for complex mixture analysis of thermally labile and biologically relevant molecules is largely attributed to the "soft" nature of Atmospheric Pressure Chemical Ionization (APCI) and Atmospheric Pressure Ionization (APPI). HPLCDAD- MS (HPLC coupled with a Diode Array UV Detector and a Mass Spectrometer), and such other techniques are almost routinely used. NMR has now been added to this combination to provide HPLCDAD-NMR-MS capabilities in instruments.

Characterizations of the synthesized impurities includes following data;

Intermediate
Preliminary evaluations
Molecular structure



IUPAC Name - Diethyl, 1, 4-dihyro-2, 6-dimethyl-4-(2-nitrophenyl) pyridine- 3, 5-dicarboxylate
Molecular mass – 374.39 gm
Molecular formula - $C_{19}H_{22}N_2O_6$
Color - it appears as light yellows solid crystalline powder
Odor- is about to characteristics

Nature – appear as a solid state

Physicochemical properties

The physicochemical property of the synthesized intermediate was evaluated by considering the elemental analysis, percentage yields, melting point and Rf value. All the data were precise and accurate with comparisons to official standards. The following table describe about physicochemical properties of synthesized intermediate.

Table No. 1: Physicochemical and analytical data for synthesized intermediate

Comp.	Molecular Formula	Mol. Wt.	M.P.	Rf Value	%	Elemental analysis cal.		
I	$C_{19}H_{22}N_2O_6$	374.3	158	0.73	96	60.95 (60.69)	5.92 (5.68)	7.48 (7.28)

Solubility

The solubility profile of synthesized intermediates was determined with reference to (I.P 2010) as official standard and was studied for various polar and non-polar solvents respectively. The following table shows solubility data for synthesized intermediate

Table No. 2: Solubility parameter for the synthesized intermediate

Sr. No.	**Test Solvent**	**Observation**
1	Methanol	Sparingly soluble
2	Benzene	Slightly soluble
3	DMF	Freely soluble
4	DMSO	Freely soluble
5	Acetone	Freely soluble
6	Pyridine	Freely soluble
7	Pet ether	Completely soluble
8	Toluene	Soluble
9	Hexane	Slightly soluble

Chromatographic methods

Column chromatography

Column chromatography was studied to obtain the intermediates in pure form for further analysis. The dimethyl sulfoxide (DMSO) was selected as mobile phase and the column was 50 cm in height and 1cm in diameter. The very good yield was obtained after drying the intermediates at oven. Further this intermediate was used for analytical method validation by HPLC.

Thin layer chromatography

The Rf value was for the both the synthesized intermediates was determined. The benzene: pyridine: methanol in the ratio of 5:3:2 was selected as mobile phase for quantification of intermediate 0.80.

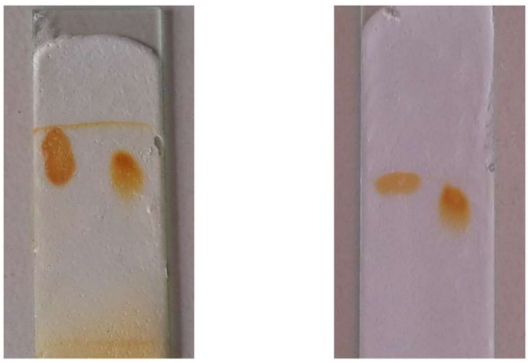


Fig No. 3: Identification of Intermediate by TLC method.

Spectral Study

U V Spectroscopy

The UV analysis of the synthesized intermediates was calibrated in the range of 400-800 nm appropriately. The well calibration peak was obtained using methanol as solvent and the wavelength 275 nm with absorbance 0.621 was reported.

Infra-Red spectra

The IR spectra for the synthesized intermediate were recorded by using Fourier Transform Infrared Spectrophotometer Model No. 8400S SHIMADZU. The peaks in the IR Spectrum gave an idea about the probable structure of the compound. A very good result was obtained by using potassium bromide (KBr) press pellet technique, which showed different vibration levels of molecules. The following graph shows IR spectral data with their types of vibration respective to their IR bands in cm-1 for both intermediates.

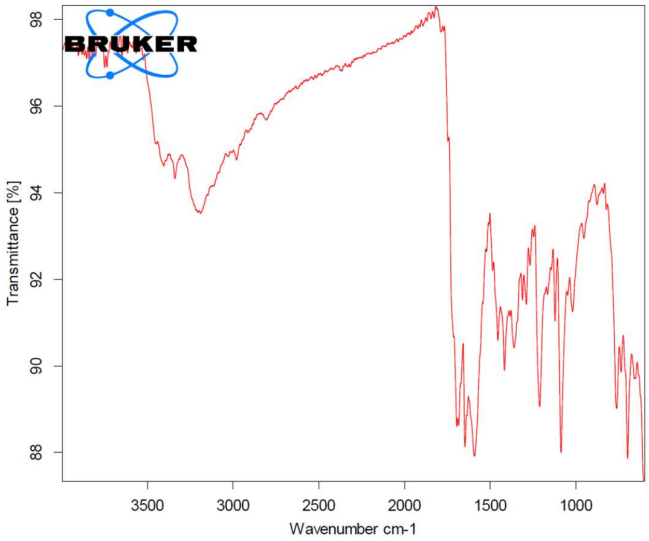


Fig No. 4: IR spectra for intermediate

4. Conclusion

An effective isocratic turned around stage elite fluid chromatography strategy was created, advanced and approved to examine the cycle related debasement of Lacidipine in details.

Season of investigation, chromatographic enhancement capacity, goal and nature of the pinnacles were all the while upgraded. 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. It is presumed that the cycle related debasement in medications ought to be limited to adequate levels to further develop therapeutics viability and wellbeing of the medication.

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Source of support: Nil.

Conflict of interest: None

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