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Microwave Mediated Synthesis and Analytical Method Development for the Estimation of Novel 1,4-Dihydropyridines in Bulk by RP-HPLC

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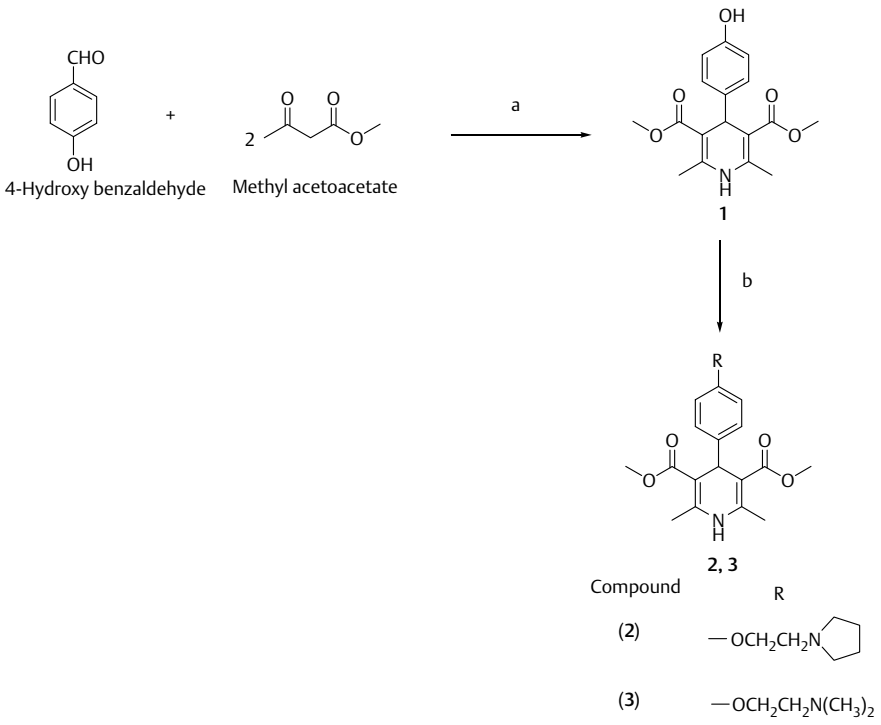
ABSTRACT

The present work describes a rapid and green microwave mediated method for the synthesis and a simple and precise isocratic reverse phase HPLC method for the estimation of the biologically significant dihydropyridines. The conventional synthesis of these dihydropyridines has been previously reported from our lab. The analysis of a standard solution (1 mg/ml) was accomplished on a symmetry (4.6 mm I.D x 250 mm) C-18 column using mobile phase acetonitrile:water:triethylamine (TEA) (70:30:0.1 v/v/v) at a flow rate of 0.7 ml/min. Detection was monitored at 354 nm. The retention time for all the compounds was accomplished as less than 10 min. The compounds showed the linear response over the concentration range 10–100 µg/ml. The study is aimed to develop a rapid method for the quantification of these potent molecules. Various parameters like linearity (10–100 µg/ml), USP tailing and plate count were found to be satisfactory. The investigated parameters were studied with the freshly prepared solutions.

Introduction

Antihypertensive drugs are prescribed worldwide to cure the common cardiovascular diseases and related complications [1]. Calcium channel blockers (CCBs) have emerged as the most favourable choice for treating hypertension as they have been proven to control the various coronary and stroke related risks [2, 3]. CCBs of the type 4-aryl-1,4-dihydropyridine are of interest to medicinal chemists for the treatment of cardiovascular disorders because of their high potency, selectivity of action and heterogeneity [4, 5]. CCBs control the calcium influx through voltage-dependant calcium channels in the smooth muscles and dilate coronary and peripheral arteries and reduce heart after-load [6–8]. Dihydropyridines (DHPs) have been reported to be the most frequently prescribed CCBs for the treatment of hypertension in many regions of eastern Asian countries including China [9, 10]. The fascinating biological spectra of the calcium channel antagonists have led to the development of newer technologies to improve conventional methods of preparation using various catalysts such as copper (II) triflate [11], molecular iodine [12], organocatalyst [13], trifluoroacetic acid [14]. Further to overcome inherited drawbacks of longer reaction times and poor yields, microwave assisted organic synthesis (MAOS) [15, 16] has been adopted. Syntheses of DHP and pyridines have been demonstrated using microwave in batch [17] and flow reactor in the presence of a catalyst to replace the traditional two-step synthesis [18].

Synthesis of some new analogues of 4-aryl-1,4-dihydropyridines (1,4-DHPs) possessing significant calcium channel blocking activity along with good vasodilatory profile has been reported [14] from our lab. With chemical synthesis, achieving high yields in reasonable times is a great challenge, therefore there is a need to develop an efficient method to synthesize these compounds with minimum hazards and less time. We have tried to develop an efficient and greener method for the preparation of novel 1,4-DHPs exhibiting excellent vasodilation activity as an environment friendly approach. As the regulatory authorities have made the complete as-



▶ **Fig. 1** Synthetic route to the synthesis of the dihydropyridines. Reagents and reaction conditions: **a** Microwave 75 °C, 25-30 min, Liq. NH_3 **b** Microwave 75 °C, 50 min, EMK, anhyd. K_2CO_3, respective amine.

sessment even more rigorous for new drug candidates and understanding that the detection limits are completely varied for a vast range of applications, consistent increase in the method development for commercial calcium channel blockers such as amlodipine [19–21] felodipine [22] and nimodipine is obvious [23].

Analytical methods based on HPLC [24, 25] have long been the methods of choice for the quantification of dihydropyridines as the technique is sensitive and rapid. High therapeutic index of dihydropyridines has promoted the analytical method development for their assessment in bulk, dosage and in biological fluids. Literature study reveals that several analytical methods for the quantitative determination of dihydropyridines such as spectrophotometry [26, 27], spectrofluorimetry [28], electrochemical methods [29] and HPLC-MS [30, 31] have been reported. The inherent simplicity, reproducibility and highly selective nature of analysis make the liquid chromatography based estimations as the most suitable for quality control laboratories. The first phase of the research work is aimed to develop a greener and rapid synthetic method for 1,4-DHP via microwave irradiation and the second phase involves development of a simple and efficient RP-HPLC method for the quantitative estimation of these potent dihydropyridines.

Materials and Methods

General Information

All reactions were carried out under an inert atmosphere, unless otherwise stated using microwave synthesizer (Initiator Exp. Biotage). Solvents were dried and purified by standard methods prior to use. The progress of all reactions was monitored by TLC using glass plates pre-coated with silica gel 60 F254 with a thickness of 0.5 mm. The Veego melting point apparatus (VMP-D) was used to measure melting points of the synthesized compounds. The melting points reported are uncorrected.

Microwave mediated synthesis

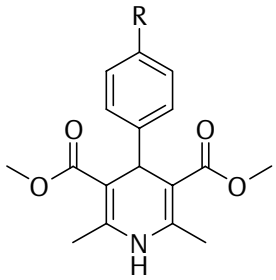
Synthesis of the parent 1,4-dihydropyridine scaffold **1** was carried out by reacting 4-hydroxybenzaldehyde, methyl acetoacetate and liquor ammonia under stirring at 75°C in a microwave vial for 25 min (▶ **Fig. 1**). The residue obtained was washed with cold ethanol (25 %) and crystallised in acetone. Further alkylation of the parent dihydropyridine with hydrochlorides of 1-(2-chloroethyl)pyrrolidine and 2-(diethylamino)ethyl chloride in ethyl methyl ketone (EMK) using anhydrous potassium carbonate afforded compounds **2** and **3**, respectively. Reaction progress was monitored with the help of the TLC. The comparative data for the formation of dihydropyridines using conventional and microwave methods has been provided in ▶ **Table 1**.

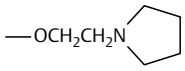
Analytical method development

Chromatographic conditions

The chromatographic conditions for analysis of the molecules throughout the experimental work were maintained as detailed in ▶ **Table 2**. All analyses were performed in an air-conditioned lab.

▶ **Table 1** Physical, chemical and comparative data for the formation of investigated compounds.



Comp.	R	m.p. (°C)	Crystallizing Solvent	Time/Yield	
				Conventional Method	Microwave Method
1	OH	231-233	Acetone	3 h / 87.43 %	25 min /51.1 %
2		155-156	Ether/ethyl acetate	8 h /41.48 %	50 min /40.21 %
3	$-OCH_2CH_2N(CH_3)_2$	169-171	Ether/ethyl acetate	8 h/45.78 %	50 min /41.32 %

Standard Solution

An accurately weighed pure sample (10 mg) was dissolved in methanol (10 ml) to make a stock solution. The solution was sonicated for 5 min. Dilutions (10–100 µg/ml) were further made using this stock solution. The solutions were filtered through 0.22 µm PTFE membrane filter (Millipore) and were used for analysis.

Results and Discussion

Microwave mediated synthesis

As observed from ▶ **Table 1**, the current synthetic method has a clear advantage of shorter reaction time and higher yields in the absence of any catalyst and solvent for the synthesis of dihydropyridine analogues.

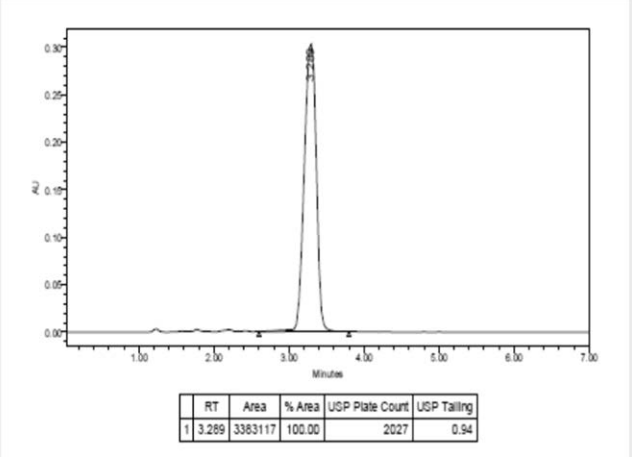
HPLC method development

To achieve the best chromatographic conditions, the detection wavelength (200–400 nm) and the mobile phase composition were adequately selected. Pre-conditioning of the RP-phase columns before runs was always performed by gradually changing the flow rates and mobile phase composition in the gradient mode. The main objective was to develop a reverse phase liquid chromatographic method allowing the determination of dihydropyridine analogues in the shortest time. At 354 nm the dihydropyridines showed enough absorption and the calibration has a good linearity and the best S/N ratio. Because of less polar nature of dihydropyridines they are readily soluble in organic solvents such as ethanol, methanol and DMSO and hence strongly retained on RP-HPLC columns. The column selection has been done based on certain parameters including back-pressure, peak shape, theoretical plates and reproducibility of retention times on these columns.

Different types of C-18 columns (4.6 × 250 mm) (BEH, SUN, Symmetry and Spherisorb from Waters) with various mobile phase combinations were investigated. This included methanol + water; acetonitrile + water; methanol + water + TEA and acetonitrile + water + TEA systems in gradient mode. Though all the columns performed satisfactorily for one or the other compound but the aim was to develop one column for all the compounds under investigation. The best choice came as symmetry column, which could resolve all the compounds in less than 10 min of run time (▶ **Figs. 2–4**). Selected stationary phase has shown better repeatability with low back pressure. Acetonitrile was preferred as it has shown lesser UV cut-off response compared to methanol. This was

▶ **Table 2** Details of the various parameters used for RP-HPLC method.

System: Waters HPLC Model 2996
Column: Symmetry (4.6 mm I.D x 250 mm) C-18
Detector: Variable wavelength programmable UV/VIS detector PDA
Mobile phase: Acetonitrile + Water + Triethylamine (TEA) (70:30:0.1)
Detection wavelength: 354 nm
Mode: Isocratic
Syringe: Hamilton syringe. (7202, 25 µL)
Flow rate: 0.7 ml/min.
Injector: Rheodyne-7725i
Type of Injector: Manual
Temperature: Room temperature



▶ **Fig. 2** Optimised chromatogram of compound **1** (100 µg/ml).

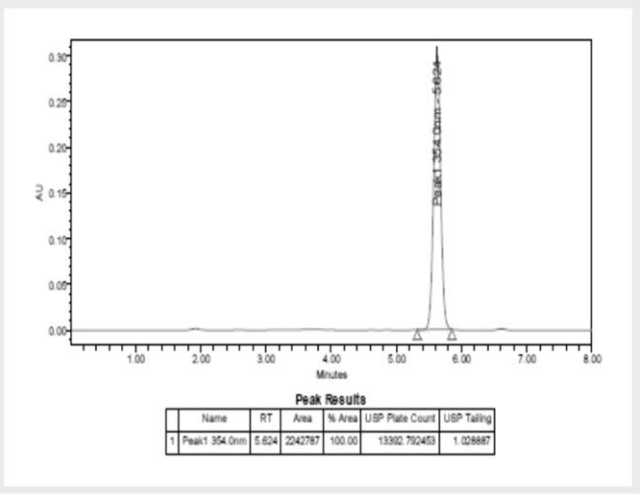
further improved for the individual compounds and the run time was brought down to less than 6 min. Addition of TEA led to improved baseline and peak quality.

Validation of system suitability parameters

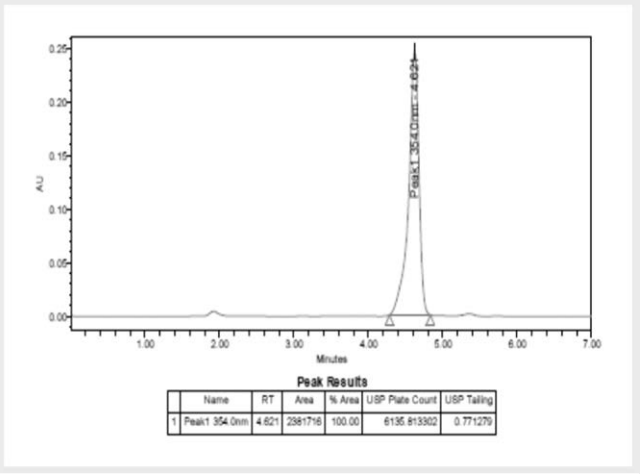
According to USP, system suitability tests were carried out on standard stock solution of compounds. 20 µL of the solution was injected into the chromatographic conditions. Parameters studied to evaluate the suitability of system were retention time, area under curve, USP tailing and number of theoretical plates, statistical parameters and LOD/LOQ values (▶ **Table 3**).

The straight line graph (with zero intercept value) has been obtained after plotting the peak area values as a function of the concentration values (in triplicates). This clearly confirms the linearity through this measured range as well as lack of bias. The statistical parameters of the calibration have been described in ▶ **Table 3**.

The mobile phase acetonitrile: water: TEA (70:30:0.1) (v/v/v %) was selected as it gave sharp peaks of the compounds with retention time of less than 6 min. Wavelength was selected by scanning standard solutions of drugs over 200 nm to 400 nm. The compounds showed good response at 354 nm. The low value of standard deviation indicates that system suitability parameters are stable over the given chromatographic conditions. The value of coefficient of correlation reflects the method is linear over the concentration range of 10-100 µg/ml. The low relative standard deviation values indicate a high accuracy of the method.



▶ **Fig. 3** Optimised chromatogram of compound **2** (100 µg/ml).



▶ **Fig. 4** Optimised chromatogram of compound **3** (100 µg/ml).

Conclusion

A rapid, green and efficient microwave irradiated synthesis methodology has been developed for the preparation of novel biologically active dihydropyridines in this study. Furthermore, for the estimations of these dihydropyridines a simple, sensitive and precise RP-HPLC method has been developed. Since the analysis is completed within 6 min, it clearly indicates that the method is rapid and thus it could be used for routine studies of the potent dihydropyridine based drug candidates.

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Ethical approval: This article does not contain any studies with human participants performed by any of the authors.

Conflict of Interest

Authors declare no conflict of interest.

▶ **Table 3** Various chromatographic parameters obtained with symmetry column.

Parameters	(1)	(2)	(3)
Retention Time	3.28 min	5.6 min	4.6 min
Peak Area (mean)	1192170	1109994	1134391
Theoretical Plates(mean)	2083	13277	6647
Tailing Factor(mean)	0.94	1.02	0.77
LOD;LOQ	0.628 µg/ml; 2.09 µg/ml	0.45 µg/ml; 1.53 µg/ml	0.58 µg/ml; 1.19 µg/ml
Statistical parameters of the calibration	1st order linear curve; $r^2 = 0.9989$	1st order linear curve; $r^2 = 0.9984$	1st order linear curve; $r^2 = 0.9970$

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