Journal of Advances and Scholarly Researches in Allied Education
Vol. 16, Issue No. 6, May-2019, ISSN 2230-7540

A Review Study of Reverse Phase HPLC & UV-Visible Methods for Active Pharmaceutical Ingredients [API] In Pure and Their Formulations Perindopril–Amlodipine Dihydropyridine

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Abstract – An accurate and precise HPLC method was developed for the simultaneous determination of perindopril and amlodipine. The UV range used remains in between 190-380 nm. The instrument used in the genre is known as ultraviolet - Visible spectrophotometer. A molecule absorbs ultra-violet radiation when the energy of such radiation becomes equal to the energy necessary to performing an electronic transaction of the molecule.

Keywords: Reverse Phase Hplc, UV-Visible Methods, Perindopril, Amlodipine Dihydropyridine etc.

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I. INTRODUCTION

Perindopril erbumine is a non-sulfhydryl prodrug that belongs to the angiotensin converting enzyme (ACE) inhibitor class of medications. Chemically, it is (2S, 3aS, 7aS)-1- [(2S)-2-[[(2S)-1-ethoxy-1-oxopentan-2-l]amino]propanoyl] 2,3,3a,4,5,6,7,7a-octahydroindole2-carboxylic acid. (Fig.1). Amlodipine is a Dihydropyridine calcium antagonist (calcium ion antagonist or slow-channel blocker) that inhibits the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle. Amlodipine besylate is chemically described as 3-ethyl-5-methyl (±)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1,4- dihydro-6-methyl-3,5-pyridinedicarboxylate, monobenzenesulphonate1,2 (Fig. 2). A few spectroscopic, GC7, HPLC, LC-MS14 and TLC15 methods were reported earlier for the individual determination of perindopril and amlodipine in pharmaceutical dosage forms. Till now no HPLC method has been developed for the estimation of these drugs simultaneously [1]. In this communication, we proposed a rapid, sensitive, accurate and precise HPLC method for the simultaneous estimation of perindopril and amlodipine in bulk samples and in tablet dosage forms [2].

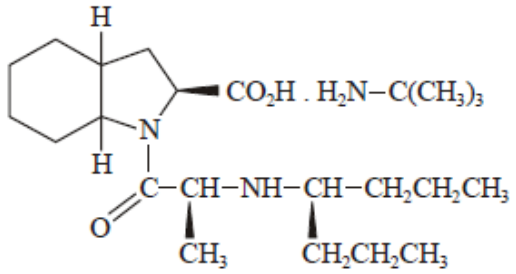


Fig. 1: Chemical structure of perindopril

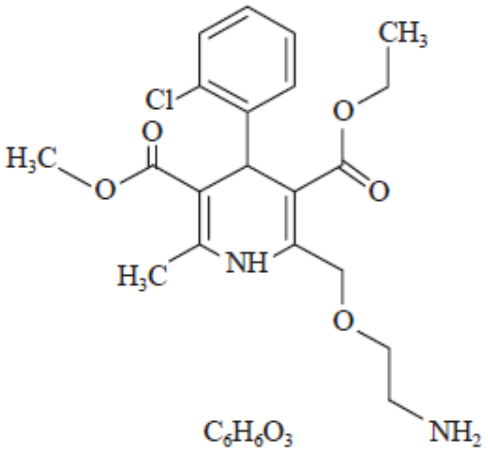


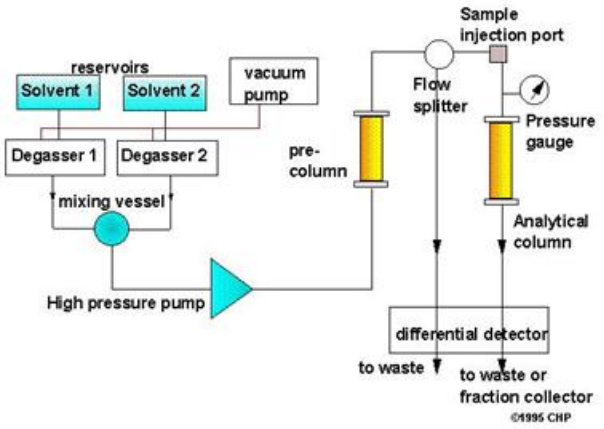
Fig. 2: Chemical structure of amlodipine

Analytical chemistry is to deal with different methods of chemical analysis for determining properties of different matters available naturally and artificially [3]. This stream of chemistry is based on the analysis of functional groups and various rings present in a molecule. There are two forms of analytical chemistry: qualitative analysis method and quantitative analysis method.

The first kind of analysis provides information on different functional groups and rings present in the molecules of the elements and compounds. On the other hand, the quantitative analysis method provides data on the quantity of analyte present in the targeted compound or sample. Ultra-Violet (UV) spectroscopy is one of the most-used instrumental techniques of analysis in the pharmaceutical industry. The UV range used remains in between 190-380 nm. The instrument used in the genre is known as ultraviolet - Visible spectrophotometer. A molecule absorbs ultra-violet radiation when the energy of such radiation becomes equal to the energy necessary to performing an electronic transaction of the molecule [4] [5].

II. HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

In the technique of high-performance liquid chromatography, a separation is made whereby a static phase is obtained in a column with one end is attached to the source of pressurized liquid eluent or the mobile state [6]. In HPLC, the right selection of the mobile phase is important. The polarity of the static phase determines that eluting capacity of the mobile phase.



A. Reversed-Phase Chromatography (RPC)

This is the primary choice of most of the regular samples. In comparison to other forms of chromatography, RPC is better as far as the utilities of all chromatography are concerned. High performing RPC columns are stable, reproducible, and efficient. Due to the efficiency of the solvents, the detection process in RPC is easier and faster. Moreover, users have more exposure to RPC than HPCL.

Several organic compounds are there possessing limited solubility in the mobile phase but that does not create any practical restriction since these compounds are injected in minute quantities. Even then, if the solubility of a compound in RPC mobile phases is found to be really weak, normal-phase chromatography is applied.

B. Normal-Phase Chromatography (NPC)

In the case, the static phase shows high polarity than the mobile phase. This is just opposite to RPC. In the mobile phase, three or more organic solvents are mixed together and inorganic absorbent like silica or alumina is used for column packing. Sometimes, in mobile phase, amino, cyano, and dilo on a silica base are used to form a polar bonded phase that is used instead of polar bonded phase. Regardless of the static or mobile phase, the retention of the sample in NPC improves with the decreasing mobile phase polarity that is just opposite of RPC. Both ionic and non-ionic compounds could be separated with the help of NPC. In the mobile phase of NPC for separating ionic samples, water may be used but the retention process is a bit complicated. If NPC is applied for separating ionic samples, experts advise using triethylamine to the mobile phase for separating the basic compounds and formic acid for separating acidic compounds.

Non-ionic or neutral samples could be separated perfectly by NPC and RPC. In NPC, feeble polar compounds elute first and the strong polar compounds elute at the end. In RPC just the opposite happens.

C. Ion-Pair Chromatography (IPC)

Several features of IPC and HPCL are common. The mobile phase and column of both are almost similar. One visible difference is in the use of ion-pair reagent used in the mobile phase. It is advisable to use RPC first for ionic samples before trying IPC. IPC is more complicated than RPC and requires additional experimental segregation due to weaker band spacing. IPC is considered as a follow-up for RPC separation techniques that need more improvement.

D. Ion-Exchange Chromatography (IEC)

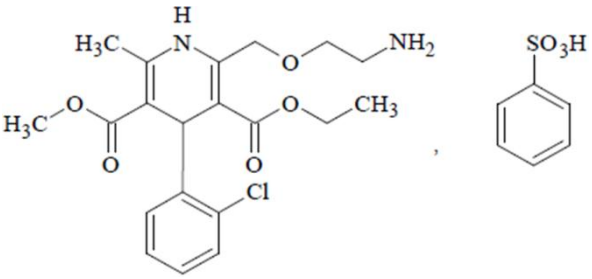
There was a time when IEC was an important HPLC technique. Today, it is applied in some special samples only such as mixtures of organo-metallics, biological origin, and inorganic salts. Because of several similarities, IEC is comparable to IPC. But, the later one has some advantages

such as more stable columns and higher column efficiency.

III. DRUG PROFILE

A. Amlodipine besylate

Molecular Structure of Amlodipine Besylate



Chemical Name

3-ethyl 5-methyl2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1, 4-dihydropyridine-3, 5-dicarboxylate benzene sulphonate.

Molecular Formula

C26H31ClN2O8S

Molecular Weight

567.1g/mol

Description

Physically it looks white or almost white and it is available in the powdered form.

Solubility

It is weakly soluble in water and 2-proponole, freely soluble in methanol, and moderately soluble in ethanol.

Storage

Should be stored away from intense light in an airtight container.

PKA Value

8.6

Purity

98.0 to 102.00

Amlodipine Besylate acts as a calcium channel blocker, vasodilator, and anti-hypertensive agent.

Indicators and Dosage

Elderly: Initially 2.5 mg once daily that can be increased as required later

Maximum dose: 10mg/day only for adults

Mechanism of action

Amlodipine Besylate is capable of controlling the movement of calcium ions in the body. Calcium ions are positive ions that move across the cell membranes of the myocytes (muscular cells). The action of the drug is even higher in the arterial vessels causing vasodilatation and after load drop. However, its action in the myocardium is quite low. With the application of Amlodipine Besylate it clinically proven that the blood pressure is reduced considerably without disturbing the heart rate. In the patients with angina pectoris, it helps in reducing the after load that again reduces the requirement of myocardial oxygen. Thus the risk of angina reduces and exercise tolerance increases.

Pharmacokinetics

Absorption

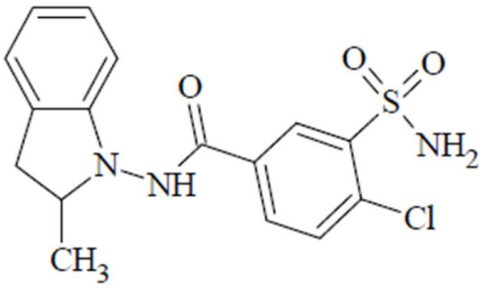
The drug is absorbed promptly and rapidly if the bio-availability is systematic with 60-65% presence of Amlodipine in the orally applicable drug [7].

Adverse Effect

Following effects noticed effects of which vary from patient to patient: a headache, fatigue, peripheral edema, mild hypokalaemia, hyperglycemia, insomnia, and gingival enlargement.

B. Indapamide

The molecular structure of Indapamide is as follows:



Chemical Name

The chemical name of Indapamide is as follows:

4-chloro-N-[(2RS)-2-methyl-2, 3-dihydro-1H-indol-1-yl]-3-sulphamoylbenzamide

Molecular Weight

365.84 g/mol

Molecular Formula

C16H16ClN3O3S

Description

Physically it is a White or almost white powdery substance

Solubility

Mostly insoluble in water, easily soluble in alcohol, and weakly soluble in ether

Storage

Store protected from light.

Indications and dosage

At lower dosage shows antihypertensive effects. At higher doses shows diuretic actions also. It relaxes the vascular muscles by inhibiting net Ca2+ inflow.

Adult: Primarily 5 mg once daily which can be increased after 7-14 day

Elderly: Primarily 2.5 mg/day that can be increased later as per the requirement

Maximum Dose: 10mg/day

Mechanism of Action

Indapamide obstructs the sluggish section of the delayed rectifier potassium current (IKs) without varying the speedy section (IKr) or the inward rectifier current. It specifically blocks or antagonizes the actions of two proteins: KCNQ1 and KCNE1. Indapamide is also thought to stimulate the separation of the vasodilator hypertensive prostaglandin PGE2.

IV. HYPERTENSIVE PATIENTS

Hypertension is a major risk factor for the onset of cardiovascular and renal disease and is a triggering factor for entry of individuals into the cardiovascular continuum. In 2000, hypertension was estimated to be present in 26% of the world's population and, by 2025, this is predicted to increase by 60% and so to affect 1.56 billion people. Although awareness and management of hypertension have improved during the last two decades, the treatment of many hypertensive patients remains suboptimal, and as many as 30% do not achieve adequate BP control. Furthermore, 30% of the hypertensive patients remain unaware of their condition, leaving diagnosis to be made at a late stage when accelerated decline down the cardiovascular. lar continuum has already occurred, resulting in end-organ damage. The main target of management in hypertension should be to arrest the pathophysiological continuum and prevent entry or decline down the cardiovascular continuum [8].

Currently recommended targets for the management of hypertension are, 140/90 mmHg. ACE inhibitors are recommended as first-line therapy in the management of hypertension in patients aged, 55 years and in those with compelling indications such as HF, left ventricular (LV) dysfunction, MI, diabetes, or recurrent stroke. Perindopril has proven anti-hypertensive efficacy and is well tolerated with low rates of cough and first-dose hypotension. With one of the highest trough-to-peak ratios of the ACE inhibitor class, perindopril provides 24-h anti-hypertensive coverage in a single daily intake. A primary healthcare trial in over 13 000 patients demonstrated that mono therapy with perindopril reduces BP by219.7/10.5 mmHg in the general hypertensive population and by 214.9/8.4 mmHg in patients unresponsive to other anti-hypertensive agents [9].

A. Combination Therapy in Hypertensive Patients

The use of more than one anti-hypertensive agent is often necessary to achieve target BP and prevent target-organ damage. In this context, perindopril has been confirmed as a suitable choice for fixed-combination therapies in hypertension, notably with the thiazide-like diuretic, indapamide. The potential advantages of combining perindopril with the calcium channel blocker amlodipine were highlighted in the large-scale, randomized, double-blind Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT). ASCOT included hypertensive patients (n¼19 257) at moderate cardiovascular risk who were randomly allocated to BP lowering with amlodipine, with the addition of perindopril as required, or beta-blocker, or with the addition of diuretic as required. After a median of 5.5 years, ASCOT was stopped prematurely because the amlodipine/perindopril group showed an 11% reduction in all-cause mortality (P¼0.0247), compared with beta-blocker/diuretic. The early termination of ASCO Treduced the power of the study, and the relative risk reduction (RRR) of 210% in the primary end point (non-fatal MI including silent MI and fatal coronary heart disease) with amlodipine/perindopril did not reach statistical significance. Nevertheless, treatment with amlodipine/perindopril significantly reduced a range of other endpoints, including cardiovascular mortality, total coronary events, and total cardiovascular events and procedures [10].

A review of the ASCOT data highlighted that cardiovascular mortality was slightly higher in the amlodipine/perindopril treatment arm until 2 years into the study, when the arms started to diverge. At

this time point, the majority of amlodipine-treated patients had also been receiving perindopril for at least 1 year. When the trial was stopped, 68.4% of the patients in the amlodipine/perindopril group were receiving perindopril, and cardiovascular mortality was reduced by 14% (P, 0.05).

The mean BP in the amlodipine/perindopril group was 2.7/1.9 mmHg lower than in the beta-blocker/diuretic group. However, this difference could not entirely account for the differences in outcome. The Conduit Artery Functional Evaluation sub study of 2199 patients from ASCOT showed that despite similar reductions in brachial artery BP, both central aortic BP and central aortic PP were significantly reduced with amlodipine/perindopril vs. beta-blocker/diuretic (24.3 and 23.0 mmHg, respectively, both P, 0.0001). Moreover, central aortic PP was significantly associated with an increased risk of cardiovascular events or procedures and the development of renal impairment. These findings suggest that central arterial PP lowering by perindopril and amlodipine combination therapy disrupts the pathophysiological continuum, there by slowing progression onto the cardiovascular continuum in hypertensive patients [11].

The ASCOT trial results overturned established thinking in the treatment of hypertension and prompted an early revision of the treatment and management guidelines for hypertension by the British Hypertension Society. The findings of ASCOT also form the rationale behind the new fixed combination of perindopril and amlodipine for the treatment of hypertension. The anti-hypertensive efficacy of this combination is being investigated in ongoing trials.

B. Patients with Type 2 Diabetes

Type 2 diabetes mellitus is a major risk factor for the onset of cardiovascular disease. Moreover, over 70% of diabetics also suffer from hypertension, and the primary cause of death in diabetics is cardiovascular disease. For this reason, patients with both hypertension and diabetes need to be aggressively managed, and BP targets are, 130/80 mmHg.

Small-scale studies in diabetic hypertensive show that perindopril reverses arterial remodeling and stiffening, which are important steps on the pathophysiological continuum. In ASCOT, 27% of the study population (n¼5137) had diabetes at baseline, but no history of cardiovascular disease. In this subpopulation, amlodipine/perindopril reduced total cardiovascular events and procedures by 23% when compared with beta-blocker/diuretic (P, 0.05). Further analyses of the diabetic subpopulation showed that amlodipine/perindopril reduced a variety of cardiovascular endpoints vs. beta-blocker/diuretic.28Importantly, the composite end point of fatal and non-fatal stroke was reduced by 25% (P, 0.05), whereas that of peripheral artery disease, fatal coronary heart disease, and non-fatal MI was reduced by 48% (P,0.001). The endpoint of requirement for revascularization was reduced by 57% (P, 0.001).

More recently, the Action in Diabetes and Vascular disease: Preter Ax and Diamicro N-MR Controlled Evaluation (ADVANCE) study evaluated the clinical benefits of background BP lowering with a fixed combination of perindopril and Indapamide on top of standard management in a cohort of 11 140 diabetic patients [12]. Over a mean of 4.3 years, treatment with perindopril/indapamide fixed combination reduced the incidence of the compo-site primary endpoint of macro vascular (non-fatal stroke, non-fatal MI, and cardiovascular death) and micro vascular (new or worsening nephropathy and retinopathy) events by 9% vs. placebo (P, 0.05). Cardiovascular and all-cause mortalities were also reduced by 18 and 14%, respectively (both P, 0.05). The beneficial effects of fixed combination of perindopril and indapamide on the primary endpoint were similar in the hypertensive and non-hypertensive patients in ADVANCE.

Hypertension is often associated with impaired glucose tolerance, insulin resistance, and obesity, and many hypertensive patients develop diabetes. A further complication to this problem is that some classes of anti-hypertensive agents, such as beta-blockers, increase the risk of new-onset diabetes, whereas others, notably ACE inhibitors, reduce the risk. In this context, perindopril has been shown to improve insulin sensitivity, glycemic control, and glucose metabolism in hypertensive patients [13]. These effects are likely to account for the 30% reduction in the risk of new-onset diabetes with amlodipine/perindopril vs. beta-blocker/diuretic in ASCOT (P, 0.0001).

V. CONCLUSION

Basic, quick, exact and precise UV Spectrophotometric strategy as well as RP-HPLC technique were produced and approved for evaluation of Amlodipine besylate as well as Indapamide in the tablet measurement structure. From dissolvability profile and soundness ponders, methanol pursued by Borate cushion pH 8.0 has been picked as a typical dissolvable for evaluation of Amlodipine besylate as well as Indapamide. Example arrangements of 10 µg/ml Amlodipine besylate as well as Indapamide in the methanol pursued by Borate cradle - pH 8.0 arranged exclusively and the arrangements were filtered in wavelength extend from 200-400 nm by utilizing methanol as well as 0.2M Borate cushion - pH 8.0 like clear. Overlaid spectra related to blend of Amlodipine besylate as well as Indapamide has been documented. From spectra, 339.0 nm Amlodipine besylate as well as 293.0 nm Indapamide has been chosen as wavelength. Straightforward, fast and precise UV Spectroscopic

(the First request subordinate technique) as well as isocratic RP – HPLC strategies indicated astounding affectability, reproducibility, exactness, along with repeatability, that is confirmed by low rate relative quality deviation. The outcomes acquired in recuperation ponders were showing that there lies no impedance from excipients utilized in the detailing. By looking at two techniques, UV Spectroscopic strategies were observed to be financial when contrasted with RP-HPLC. Consequently it is recommended suggested UV Spectroscopic as well as isocratic RP-HPLC strategies can be successfully connected for the standard investigation of Amlodipine besylate as well as Indapamide in mass as well as in the tablet plan. The outcomes drawn are introduced as in the Figures or Tables at a proper spot for example as grouping viz. recognizable proof of tests to recuperation Studies including framework appropriateness test parameters.

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