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A REVIEW: METHOD DEVELOPMENT AND VALIDATION OF AMLODIPINE AND VALSARTAN.

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Abstract:

The key role of the amlodipine drug is, it is the calcium channel blocker. Amlodipine is the drug molecule used to treat the high blood pressure; also, this medication is used for the coronary artery disease. In 1990 amlodipine was approved for its medical use and it was patented in 1982. In comparison with the other class of medications used to lower the blood pressure, amlodipine as a calcium channel blocker is having the greater protection against the stroke. With the variety of the other medication, amlodipine can be used as the combination for treatment. Valsartan is one of the combinations that can be taken along with amlodipine to treat the hypertension where, valsartan is the angiotensin II receptor blocker. Along with the high pressure and heart failure, valsartan is the medication that can be used to treat diabetic kidney disease. For analyzing this combination qualitatively and quantitatively, analytical techniques were found to be effective. Various research articles, literature papers were collected and reviewed reporting the analytical techniques mostly by HPLC and spectrophotometric for amlodipine and losartan and their combination. Review articles will find to be advantageous for developing an accurate and precise analytical method and for their validation studies.

Keywords: *QBD approach, Amlodipine, Valsartan, RP-HPLC, method development and validation.*

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INTRODUCTION:

For the treatment of high blood pressure, Amlodipine is a medication that can be used as a calcium channel blocker. Also it can be used for coronary artery disease. Amlodipine can also be used for the other medications. The effect of this drug can lasts for at least a day and can be taken orally by mouth. Amlodipine is considered as one of the essentials medicines, which is listed on the World Health Organization's. Amlodipine is also present in the form of generic medication. In 1990, amlodipine was approved for its medicinal use and also it was patented in 1982. The dose for the medication may vary as per requirements, for the elderly individuals or for the patients with the liver problems doses should be reduced. It is still not clear that, whether the amlodipine medication is safe during pregnancy or breastfeeding. Amlodipine works partly by increasing the size of arteries. Amlodipine works as calcium channel blocker of dihydropyridine type, as long acting chain. There are side effects also of this medication in some cases, common side effects include dizziness, nausea, swelling, stomach ache, feeling tired, serious side effects should be heart attack, low blood pressure. While Valsartan is also used for the treatment of blood pressure and also helps to prevents kidney which may affect due to diabetes. Losartan is the drug of class angiotensin II receptor blocker, which works by relaxing the blood vessels so as to flow the blood easily. It is also one of the essential drugs listed in the world health organization and approved by united states in 1996 as medicinal use and was patented in 1990. It is taken in the combination with other drugs. Valsartan can also be used to treat the type II diabetes, hypertension. Common side effects may arise due to this medication like, back pain, respiratory infections, dizziness mostly in adults, while those with diabetes and kidney issues may come across diarrhea, fatigue, low blood pressure, allergic reactions, chest pain, increase in potassium level.

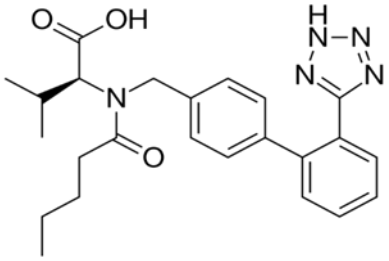


Fig. Amlodipine

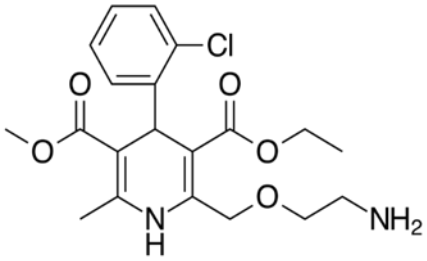


Fig. Valsartan

Pharmacology:

Amlodipine is a long-acting calcium channel antagonist that selectively inhibits calcium ion influx across cell membranes.[32] It targets L-type calcium channels in muscle cells and N-type calcium channels in the central nervous system which are involved in nociceptive signalling and pain perception.[33][34] Amlodipine has an inhibitory effect on calcium influx in smooth muscle cells to inhibit contraction. Amlodipine ends up significantly reducing total vascular resistance without decreasing cardiac output expressed by pressure-rate product and cardiac contractability in comparison with verapamil, a non-dihydropyridine.[35] In turn, following treatment lasting a month, with amlodipine, cardiac output was significantly enhanced.[35] Unlike verapamil which has efficacy in moderation of emotional arousal and reduces cardiac load without lowering cardiac output demands, amlodipine increases the cardiac output response concomitantly with increased functional cardiac load.

Mechanism of action:

Valsartan blocks the actions of angiotensin II, which include constricting blood vessels and activating aldosterone, to reduce blood pressure.[31] The drug binds to angiotensin type I receptors (AT1), working as an antagonist.[32] This mechanism of action is different than that of the ACE inhibitor drugs, which block the conversion of angiotensin I to angiotensin II. As valsartan acts at the receptor, it can provide more complete angiotensin II antagonism since angiotensin II is generated by other enzymes as well as ACE. Also, valsartan does not affect the metabolism of bradykinin like ACE inhibitors do.

Pharmacokinetics:

Amlodipine has been studied in healthy volunteers following oral administration of 14C-labelled

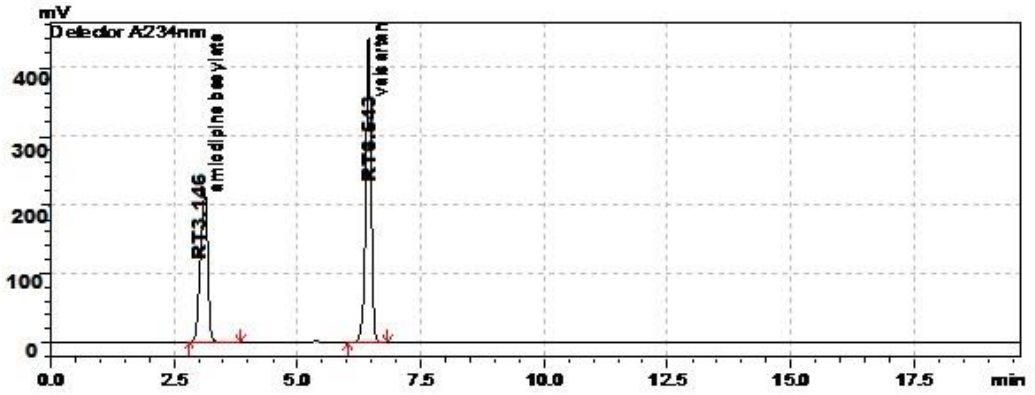
drug.[43] Amlodipine is well absorbed by the oral route with a mean oral bioavailability around 60%; the half-life of amlodipine is about 30 h to 50 h, and steady-state plasma concentrations are achieved after 7 to 8 days of daily dosing. In the blood it has high plasma protein binding of 97.5%.[33] Its long half-life and high bioavailability are largely in part of its high pKa (8.6); it is ionized at physiological pH, and thus can strongly attract proteins.[5] It is slowly metabolized in the liver by CYP3A4, with its amine group being oxidized and its side ester chain being hydrolyzed, resulting in an inactive pyridine metabolite.[44] Renal elimination is the major route of excretion with about 60% of an administered dose recovered in urine, largely as inactive pyridine metabolites. However, renal impairment does not significantly influence amlodipine elimination.[45] 20-25% of the drug is excreted in the faeces.

Medicinal Benefits:

Combination of these two drugs i.e., Amlodipine and losartan is used as a medication for the treatment of hypertension, that is used as an anti-hypertensive drug for the treatment of high blood pressure. It works by widening and relaxing blood vessels to reduce the high blood pressure. Losartan comes under the class of angiotensin II receptor antagonist that prevents the narrowing of blood vessels by lowering the blood pressure and increase the blood flow. Losartan also works in case of type II diabetes by slowing down the kidney damage and also treat the heart stroke. Amlodipine is used to treat the conditions or an effect that arises due to coronary artery disease. Amlodipine is used to increase the blood flow by widening the blood vessels, also helps to lower high pressure or chest pain as it works as a calcium channel blocker.

New validated stability indicating rp-hplc method for simultaneous estimation of amlodipine besylate and valsartan in pharmaceutical formulation:

Stability indicating RP-HPLC method was developed and validated for the simultaneous estimation of Amlodipine Besylate and Valsartan in pharmaceutical formulation which is found to be a simple, specific, accurate and precise. Sodium acetate buffer (pH 5.0) and methanol (35:65% v/v) with a flow rate of 1 mL/min was considered as a mobile phase. The method was developed using Enable C 18G column with specification of (250 × 4.6 mm, 0.5 µm). The wavelength for the detection was carried out at 234 nm. The retention time for Amlodipine Besylate and Valsartan were found to be 3.146 and 6.543 min respectively. The proposed method was validated for linearity, range, accuracy, precision, robustness, LOD and LOQ. Linearity was observed over a concentration range 0.5-250 µg/ml for amlodipine besylate (r2 = 0.9996) and 1-90 µg/ml for Valsartan (r2 = 0.9984). The % RSD for Intraday and Interday precision was found to be 0.37 and 0.57 for Amlodipine Besylate and 0.48 and 0.75 for Valsartan. The LOD was found to be 0.01µg/ml, and LOQ were 0.04µg/ml for Amlodipine Besylate and LOD and LOQ were found to be 0.04 and 0.14 µg/ml for Valsartan respectively. Amlodipine Besylate and Valsartan were subjected to stress conditions of degradation including acidic, alkaline, oxidative, and thermal and photolysis. Below are the chromatograms that are observed during the analysis



Chromatogram of amlodipine besylate and valsartan

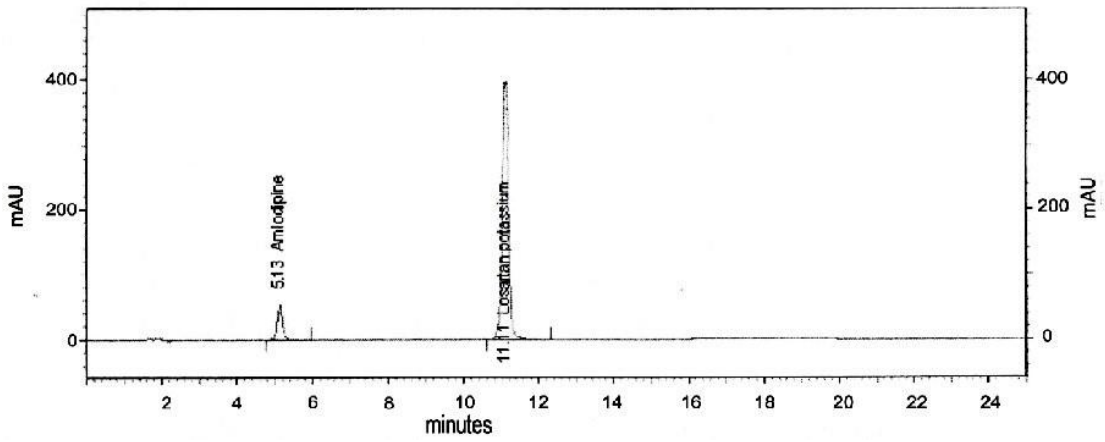
Simultaneous analysis of losartan potassium, amlodipine besylate, and hydrochlorothiazide in bulk and in tablets by High-Performance Thin Layer Chromatography with UV-absorption densitometry:

A Simple high-performance thin layer chromatography (HPTLC) method for separation and

quantitative analysis of losartan potassium, amlodipine, and hydrochlorothiazide in bulk and in pharmaceutical formulations has been established and validated. After extraction with methanol, sample and standard solutions were applied to silica gel plates and developed with chloroform: methanol: acetone: formic acid 7.5: 1.3: 0.5: 0.03 (v/v/v/v) as mobile phase. Zones were scanned densitometrically at 254 nm. The Rf values of amlodipine besylate, hydrochlorothiazide, and losartan potassium were 0.35, 0.57, and 0.74, respectively. Calibration plots were linear in the ranges 500–3000ng per spot for losartan potassium, amlodipine and hydrochlorothiazide, the correlation coefficients, *r*, were 0.998, 0.998, and 0.999, respectively. The suitability of this method for quantitative determination of these compounds was by validation in accordance with the requirements of pharmaceutical regulatory standards. The method can be used for routine analysis of these drugs in bulk and in formulation.

RP-HPLC method development and validation of amlodipine and losartan in binary mixture:

A Novel, selective and rapid Gradient Reversed Phase High Performance Liquid Chromatographic (RPHPLC) method for the analysis of Amlodipine and Losartan in binary mixture has been developed and validated. The chromatographic system consisted of a LC-20AT VP series model chromatograph equipped Spinchrome software. The separation was achieved from a Inertsil ODS 3V C18 (150 X 4.6 mm, 5µm) at ambient temperature with a mobile phase containing a mixture mobile Phase-A with 70% v/v of buffer pH-3.7 and 30%v/v of acetonitrile and mobile phase–B containing 70% v/v of acetonitrile and 30% v/v of buffer pH-3.7. The samples were monitored at 237 nm for detection at a flow rate of 1.0 mL/min and the retention time was about 5.13 and 11.11min for Amlodipine and Losartan respectively. The calibration curve was linear over the concentration range 1.25-7.5 µg/mL and 12.5-75 µg/mL for Amlodipine and Losartan respectively. The proposed method is accurate in the range of 99.95% - 100.133% recovery and precise (%RSD of intraday variation and %RSD of inter day variation were found to be within the acceptance criteria). Therefore, this method can be used as a more convenient and efficient option for the analysis of Amlodipine and Losartan in Quality control laboratory.



Chromatogram of Amlodipine and Losartan

Stability Indicating RP-HPLC Method for Simultaneous Estimation of Valsartan and Amlodipine in Capsule Formulation:

Present work describes a precise, accurate and reproducible Reverse phase High Performance Liquid Chromatographic (RP-HPLC) method for simultaneous estimation of Amlodipine besylate (AMLB) and Valsartan (VAT) on RP-HPLC. Column that was used for validation was C-18 Column (Kromasil, 250 x 4.6 mm). Optimized condition was acetonitrile: phosphate buffer (0.02M, pH 3.0), (56:44 v/v) as mobile phase at a flow rate of 1.0 ml/min and the detection wavelength was 234 nm. The retention time for amlodipine and valsartan was found to be 3.07 and 6.20 min, respectively. The method was also applied for the determination of AMLB and VAT in the presence of their degradation products formed under variety of stress conditions. Proposed method was validated for precision, accuracy, linearity range, robustness and ruggedness.

QbD based method development for simultaneous quantification for Amlodipine besilate, Hydrochlorothiazide and olmesartan medoxomil film coated tablet dissolutions in different dissolution media by RP-HPLC:

The scientific way to develop a simple and robust analytical HPLC method for the critical separations is QbD approach. Quality-by-design (QbD) is a systematic approach to product or process development, which begins with predefined objectives, and uses science and risk management approaches to gain product and process understanding and ultimately process control. The concept of QbD can be extended to analytical methods. A simple Analytical method was developed and used to identify and quantify simultaneously the three active pharmaceutical ingredients Amlodipine (AML), Hydrochlorothiazide (HCTZ) and Olmesartan medoxomil (OLM)in presence of major degradants, sample matrix and other extraneous peaks from different dissolution medias, namely pH1.2 Hydrochloric acid, pH 4.5 Acetate buffer and pH 6.8 Phosphate buffer solutions by reverse phase HPLC method. The identified CQA (Critical quality attributes) are resolution between acetate peak from HCTZ peak, resolution between HCTZ and Olmesartan (Metabolite of Olmesartan medoxomil) and the resolution between OLM and AML which will affects the quality of the product and Analytical method performance**.** The CPP (critical process parameters) were identified in initial phase of method development and design space developed for the robust method. The optimized methodology was achieved on C18 (typically 75mm length, 4.6mm ID and 3.5µm) column with optimized conditions of Mobile phase 0.1% Ortho phosphoric acid (pH-2.1): Acetonitrile: Methanol (67:28:5 v/v/v).35°C Column temperature, sampling rate 5pts/sec at 230nm. All the validation parameters were performed for the assessment of quality of drug product in development and stability samples of Amlodipine, Hydrochlorothiazide and Olmesartan medoxomil film-coated tablets.

A new RP-HPLC method development and validation for simultaneous estimation of amlodipine and valsartan in tablet dosage form:

A simple, accurate, rapid and precise isocratic reversed-phase high-performance liquid chromatographic method has been developed and validated for simultaneous determination of Amlodipine and Valsartan in tablets. The chromatographic separation was carried out on an cosmosil packed column 5c-18 ms II (250×4.6 i.d) with a mixture of acetonitrile:methanol: phosphate buffer pH 3 adjusted with Ortho phosphoric acid (20:50:30, v/v) as mobile phase; at a flow rate of 1.0 ml/min. UV detection was performed at 239 nm. The retention times were 4.915 and 8.056 min. for Amlodipine and Valsartan respectively. Calibration plots were linear (r2>0.998) over the concentration range 10-60 µg/ml for Amlodipine and 10-60 µg/ml Valsartan. The method was validated for accuracy, precision, specificity, linearity, and sensitivity. The proposed method was successfully used for quantitative analysis of tablets. No interference from any component of pharmaceutical dosage form was observed. Validation studies revealed that method is specific, rapid, reliable, and reproducible. The high recovery and low relative standard deviation confirm the suitability of the method for routine determination of Amlodipine and Valsartan in bulk drug and tablet dosage form.

Parameters	Amlodipine	Valsartan
lmax (nm)	239	239
Beers law limit (µg/ml)	10-60	10-60
Correlation coefficient (r)	0.9994	0.9993
Regression equation (y=mx+c)	y= 230808.9x + 366397	y=249058 x + 2721008
Slope (m)	230808.9	249058
Intercept (c)	366397	2721008
LOD (µg/ml)	0.1379	0.0677
LOQ (µg/ml)	0.4180	0.2051
Standard Error	134339.4	162471.7

Table: the parameter for the linearity of simultaneous estimation of Amlodipine and valsartan

Qbd based RP-HPLC method development and validation for simultaneous estimation of amlodipine besylate and lisinopril dihydrate in bulk and pharmaceutical dosage form:

The objective of this experiment was to develop and validate a simple, robust, and accurate QbD based Reverse-Phase High-Performance Liquid Chromatography method for Simultaneous estimation of Amlodipine besylate and Lisinopril dihydrate in bulk and Pharmaceutical Dosage form. A box-Behnken design was employed for optimizing the mobile phase, flow rate and pH of buffer, the optimized chromatographic conditions were Phosphate buffer: Methanol (25: 75 v/v), pH of buffer: 6.5 and flow rate: 1mL/min. Furthermore, formulation injected and observed that the additives do not interfere with the peak of Amlodipine besylate and Lisinopril dehydrate. Both drugs are well resolved and Retention times were found to be 2.332 min and 3.584 min respectively. Linearity was observed in the concentration range of 10µg to 50µg/mL (r2=0.999). The accuracy range was 99.75 to 100.04%. Intra-day and Inter-day precision was found to be less than 2% RSD. The proposed method was useful for the best analysis of Amlodipine besylate and Lisinopril dihydrate in Bulk, pharmaceutical dosage forms and was successfully applied to routine analysis.

Spectrophotometric and HPLC methods for simultaneous estimation of amlodipine besylate, losartan potassium and hydrochlorothiazide in tablets:

Two UV-spectrophotometric and one reverse phase high performance liquid chromatography methods have been developed for the simultaneous estimation of amlodipine besilate, losartan potassium and hydrochlorothiazide in tablet dosage form. The first UV spectrophotometric method was a determination using the simultaneous equation method at 236.5, 254 and 271 nm over the concentration range 5-25, 10-50 and 5-25 µg/ml for amlodipine besilate, losartan potassium and hydrochlorothiazide, respectively. The second UV method was a determination using the area under curve method at 231.5-241.5, 249-259 and 266-276 nm over the concentration range of 5-25, 5-25 and 10-50 µg/ml for amlodipine besilate, hydrochlorothiazide and losartan potassium, respectively. In reverse phase high performance liquid chromatography analysis is carried out using 0.025 M phosphate buffer (pH 3.7): acetonitrile (57:43 v/v) as the mobile phase and Kromasil C18 (4.6 mm i.d×250 mm) column as stationery phase with detection wavelength of 232 nm linearity was obtained in the concentration range of 2-14, 20-140 and 5-40 µg/ml for amlodipine besilate, losartan potassium and hydrochlorothiazide, respectively. Both UV-spectrophotometric and reverse phase high performance liquid chromatography methods were statistically validated and can be used for analysis of combined dose tablet formulation containing amlodipine besilate, losartan potassium and hydrochlorothiazide.

Assay method development and validation for valsartan using high performance liquid chromatography:

Our main objective is to develop a simple, accurate, precise and stability indicating HPLC method for the determination of valsartan and its impurities. An inertsil ODS-3v; (150 × 4.6) mm; 5 µm column is used for the separation of drugs. The flow rate was maintained at 1.0 mL/min and the wave length used for detection was 230 nm. The linearity was observed in the range of 0.025-50µg/ml of spiked impurities in valsartan, impurity B and impurity C with a correlation coefficient of 0.990, 0.996 and 0.997 respectively. The drug undergoes degradation under acid, base, H2O2, thermal and humidity conditions. Linearity, accuracy, precision and robustness parameters for the suggested method were estimated for validation as per ICH guidelines. The developed method can be utilized in the analysis of valsartan tablets**.**

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