

# Development and validation of stability indicating HPLC method for the simultaneous analysis of amlodipine, hydrochlorothiazide and valsartan in pharmaceutical formulation

## Abstract

A simple, specific, accurate and precise, stability indicating HPLC method for the simultaneous determination of antihypertensive drug combination consisting of amlodipine (AML), hydrochlorothiazide (HCT) and valsartan (VAL) was developed and validated. The optimized conditions for the separation of the three analytes was consisted of ODS C-18 (250mm×4.6mm i.d. 5μm) column, mobile phase: acetonitrile-potassium dihydrogen phosphate pH 3.5- acetonitrile (45:55% v/v). Column temperature was maintained at 40°C; mobile phase flow rate: 1.5mL/min; wave length : 230nm; and injection of 20μL. The precision of the method was measured through adequate repeatability or intraday precision (RSD ≤ 2) and interday precision (RSD ≤ 2). The method demonstrated adequate linearity of detector response over the range of 0.5-250%. The limits of detection for AML, HCT and VAL were 0.011, 0.010 and 0.010μg/mL, while the limits of quantification were 0.032, 0.020 and 0.019μg/mL respectively. The method also showed adequate robustness to variations in mobile phase, pH, and column temperature and acetonitrile concentrations. The full recoveries of each working standard for all compounds were within ICH specifications of 98-101% which showed that the method was accurate. The proposed method proved to be stability indicating by resolution of the analytes from their accelerated storage conditions products. The developed method is rapid (run time 8min), selective, requires simple sample preparation procedures and simple mobile phase combinations. It is also cost effective and represents a good procedure for determination of AML, HCT and VAL in bulk raw materials and pharmaceutical dosage forms.

**Keywords:** column temperature, sample preparation, blood pressure, hypertension, acetonitrile

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**Abbreviations:** AML, amlodipine; HCT, hydrochlorothiazide; VAL, valsartan; FDA, food and drug administration; USP, U.S. pharmacopia; ICH, international conference on harmonization; LOD, limit of detection; LOQ, limit of quantification

## Introduction

Hypertension or high blood pressure, sometimes called arterial hypertension, is a chronic medical condition in which the blood pressure in the arteries is elevated. This requires the heart to work harder than normal to circulate blood through the blood vessels. The main factor that characterizes a rational drug combination is a synergistic action without side effects. In 2009, the US Food and Drug Administration (FDA) and the European Medicines Agency approved a triple fixed-dose combination of AML, HCT and VAL. It was found that the use of this triple combination was generally more effective in reducing blood pressure and providing overall blood pressure control than the dual combination therapies regardless of age, race, gender, ethnicity, or hypertension severity.<sup>1,2</sup>

Amlodipine besylate (AML) is chemically described as 3-Ethyl-5- methyl (±)-2-(2-aminoethoxy) methyl-4-(2-chlorophenyl)-1, 4-dihydro-6-methyl-3, 5- pyridinedicarboxylate, monobenzenesulphonate (Figure 1A). AML tablets are formulated as white tablets equivalent to 2.5, 5, and 10mg of AML for oral administration. AML is formulated as the besylate salt of AML, a long-acting calcium channel blocker.<sup>3</sup>

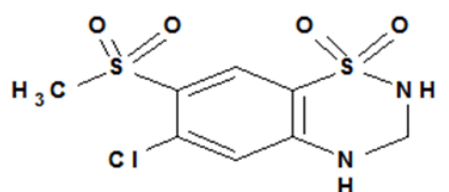
Hydrochlorothiazide (HCT) is a white, practically odorless crystalline powder, slightly soluble in water, sparingly soluble in alcohol and soluble in acetone (pKa 7.0). HCT is chemically described as 6-chloro-1,1-dioxo-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide (Figure 1B).<sup>4-6</sup>

Valsartan (VAL) is a white fine powder. It is soluble in ethanol and methanol but slightly soluble in water. Its melting point is 117°C. VAL is available as tablets for oral administration, containing 40, 80, 160 or 320mg of the drug. VAL is a nonpeptide, orally active and specific angiotensin II receptor blocker acting on the AT1 receptor subtype. VAL is chemically described as N-(1-oxopentyl)-N-[2'-(1H-tetrazol-5-yl) [1, 1'-biphenyl-4-yl] methyl]-L-valine (Figure 1C).

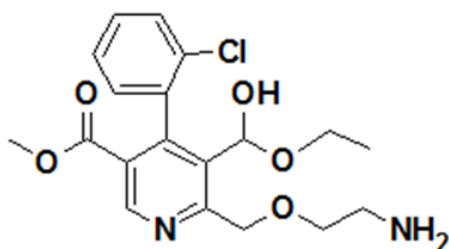
Literature survey revealed that a number of methods have been reported for estimation of AML, HCT and VAL individually or in combination with other drugs. However, there is very few analytical methods reported for the simultaneous analysis of these drugs in a combined dosage formulation. The reported methods are UV spectrophotometric,<sup>7-12</sup> spectrofluorimetric<sup>13</sup> HPLC and HPTLC,<sup>14-25</sup> capillary electrophoresis<sup>26-29</sup> and electrochemical<sup>30-32</sup> methods.

Stability testing is an important issue in the process of drug product development. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors, such as temperature, humidity, and light, and enables recommendation of

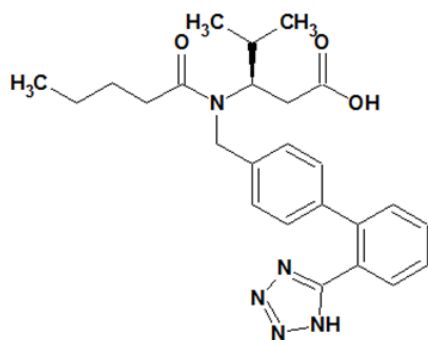
storage conditions, retest periods, and shelf lives to be established. The two main aspects of a drug product that play an important role in shelf life determination are assay of the active drug and degradation products generated during the stability study. The drug product in a stability test sample needs to be determined using a stability indicating method, as recommended by the International Conference on Harmonization (ICH) guidelines<sup>33</sup> and U.S. Pharmacopia (USP).<sup>34</sup> Although stability indicating methods have been reported for assay of various drugs in drug products, most of them describe assay procedures for drug products containing only one active drug substance. To best of our knowledge no HPLC stability indicating method for the simultaneous determination of AML, HCT and VAL was reported. Therefore the aim of this work is to develop stability indicating HPLC method for the simultaneous determination of AML, HCT and VAL in mixtures without the need of prior separation step.



IA: HCT.



IB: AML.



IC: VAL

**Figure 1** Chemical structures of amlodipine (AML), hydrochlorothiazide (HCT) and valsartan (VAL).

## Experimental

### Materials

AML, HCT and VAL working standards were obtained from Azal pharmaceutical industries company Ltd, (Khartoum, Sudan). Acetonitrile HPLC grade, Methanol HPLC grade, potassium dihydrogen orthophosphate AR was purchased from Scharlau chemical (Spain). High purity deionised water was obtained from Azal pharmaceutical (Khartoum, Sudan) purification system.

## Methods

### Instrumentation

Chromatographic separation was performed with Shimadzu high performance liquid chromatography having C18, ODS (250mm×4.6mm), 5.0µm analytical column with photodiode array detector provided by Auto sampler and the Chromatographic data were recorded by LC Solution software. Absorbance was carried out by using UV-visible spectrophotometer model Shimadzu 1800 with quartz cells of 1cm optical path length, pH meter was used for pH measurements, analytical balance and ultrasonic bath.

### Chromatographic conditions

For HPLC a number of preliminary trials were conducted with combinations of different organic solvents, compositions, and flow rate to check the retention time, shape, resolution, and other chromatographic parameters. Among all tried experiments, the mobile phase combination is potassium dihydrogen orthophosphate AR (0.05M) in water:acetonitrile (45:55v/v) adjust to pH 3.5. The solution was degassed in an ultrasonic water bath for 5minutes and filtered through 0.45µm nylon filter. The instrumental settings flow rate of 1.5mL/min; the column temperature is 40°C, and detector wavelength is 230nm found to be most suitable.

### Preparation of standard and sample solutions

**Stock standard solutions of AML, HCT and VAL (200, 200 and 250µg/mL) respectively:** An accurately 20,20 and 25mg of AML, HCT and VAL standards were dissolved in mobile phase, transferred into 100mL volumetric flask, diluted with same solvent and mixed well.

**Standard solutions of AML, HCT and VAL (40, 40 and 50µg/mL):** The standard solutions were prepared by dilution of the stock standard solution with mobile phase to reach a concentration range 0.20-80µg/mL for AML, 0.2-80µg/mL for HCT and 0.25-100µg/mL for VAL. Triplicate 20µL injections were made for each concentration and chromatographed under the condition described above. The peak area of each concentration was plotted against the corresponding concentration to obtain the calibration graph and regression equation was computed.

### Assay of commercial tablets

Ten Exforge HCT tablets were weighed and their mean was determined. After grinding the tablets into a fine powder in glass mortar, an accurately weighed quantity of the tablet powder equivalent 5mg AML, 12.5mg HCT and 160mg VAL the solution was stirred for 10min then diluted with mobile phase into a 100-mL volumetric flask. The sample solution was then filtered using 0.45µm filters (Millipore, Milford, MA).

### Stability study

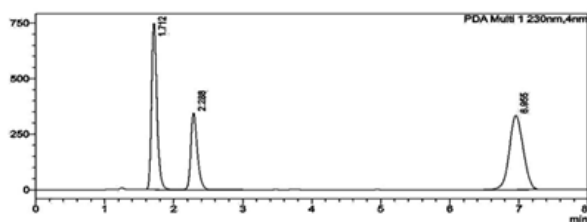
The samples were kept in the stability chamber (Thermaolab) set at accelerated storage conditions<sup>38</sup> of 40±2°C and 75±5% relative humidity and in real storage conditions of 25±2°C and 60% relative humidity. The samples were periodically monitored by the HPLC analysis. Analyses were carried out at time 0 and after storage of 1, 2, and 3months in the stability chamber.

## Results

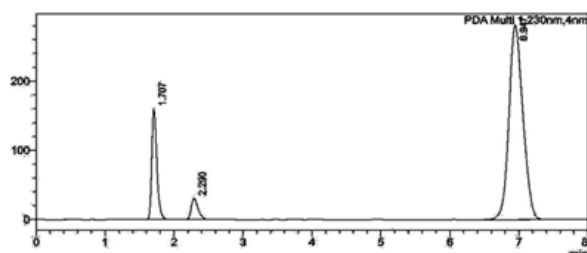
### Chromatography optimization

The chromatographic conditions were optimized to achieve the best resolution and peak shape for AML, HCT, and VAL. Different

mobile phases containing acetonitrile in phosphate buffer were examined. With acetonitrile content 45% or less, the retention time for VAL was far (retention time >10min) and the resolution between the AML and HCT less than 2.5, with decreasing of acetonitrile more than 40% the retention time of VAL too far up to 15min eluted. On the other hand to give short time increased the acetonitrile to 60%, the retention time for valsartan was short (retention time < 5.5min) but the resolution between the AML and HCT less than 2.0, moreover phosphate buffers of pH 3.0-7.0 were tried but the peak shapes for the drugs were sufficiently symmetrical only for pH value below 4 and the resolution warmed over. Therefore, the mobile phase containing acetonitrile and phosphate buffer at pH 3.5 (55:45, v/v) was selected as optimal for obtaining well defined and resolved peaks with mean retention times of 1.7, 2.2 and 6.0 min, for standard solution HCT, AML and VAL, respectively, as shown in Figure 2.



**Figure 2** Chromatogram of the standard solutions of HCT, AML and VAL respectively at optimized HPLC conditions.



**Figure 3** Chromatograms of HCT, AML and VAL respectively in tablets formulation at optimized conditions HPLC.

## Validation of the method

Method validation is the process of determining, through laboratory studies, that the performance characteristics of the method meet the requirements for its intended analytical applications. The characteristics that are studied during method validation include sensitivity, accuracy, precision, specificity, linearity of detector response, range of analyte concentration and robustness.

## Specificity

The specificity of the HPLC method was ascertained by analyzing standard drug and sample solution. The retention time of AML, HCT and VAL were confirmed by comparing the retention time with that of standard.

## Linearity

The linearity of the proposed HPLC procedure was evaluated by analyzing a series of different concentrations for each of the three analytes. The linear regression equations were generated by least squares treatment of the calibration data. Under the optimized conditions described above, the measured peak areas were found to be proportional to concentrations of the analytes. Table 1 presents the performance data and statistical parameters including linear regression equations, concentration ranges, and correlation coefficients. The data obtained from the linearity determination experiments was

subjected to linear regression analysis. The linearity equations were  $y = 23,447.2x + 3,996.1$ ,  $R^2 = 0.9996$  for Amlodipine

$y = 37,204.5x + 66,638.8$ ,  $R^2 = 0.9993$  for hydrochlorothiazide and

$y = 40,039.99x + 22,825$ ,  $R^2 = 0.9993$  for valsartan. Correlation coefficient  $R^2$  values were in all cases greater than 0.999 indicating a strong correlation between the concentrations of the analytes and the peak areas and therefore the method could be applied in the assay of all or any of the three analyte compounds. A summary of the linearity analysis results obtained is shown in Table 1. The ICH guidelines recommend that for the establishment of linearity a minimum of five concentrations be utilized over the range of 80 to 120%.<sup>40, 41</sup> This method was found to be linear over the range tested for all the three compounds.

## Accuracy

The accuracy of an analytical method is the closeness of the test results obtained by that procedure to a true known value. The accuracy for the developed method was determined by spiking the finished commercial products with working standards of compounds under study. The difference between spiked sample result and the unspiked sample resulted was calculated as percentage of the known added spike concentration. For this purpose one commercial compound that contained all the 3 compounds was used. The standards were added to the samples at 3 concentrations, 50, 100 and 150% of the assay concentrations and injected in triplicate. The standards were added at amounts that would increase the concentration by 20%. The % recovery of each added working standard was regarded as the accuracy (Table 2). The recoveries of all compounds were within the specified guidelines (ICH) of 98-103%.<sup>33, 34</sup>

## Precision

The precision of an analytical procedure as the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. The ICH5 requires repeatability to be tested from at least six replications measured at 100 percent of the test target concentration or from at least nine replications covering the complete specified range and calculation the relative standard deviation for various assays done on same day or different days. The results obtained are summarized in Table 3.<sup>34,35</sup> The relative standard deviation in both cases was below 2% indicating that the method was precise.

## Sensitivity

**Limit of detection:** The limit of detection (LOD) is the lowest amount of analyte in a sample that can be detected but not necessarily quantified using an analytical technique under specified experimental conditions. The LOD was established by signal over noise (S/N) method where 6 injection of blank (mobile phase) were injected and the mean peak height was determined. Determination of LOD is indicated for method developed for determination of restricted substances and degradation products that are usually present in small quantities in the sample<sup>34</sup> while the LOQ was determined for this method to give an idea of the amount of each compound which can be quantified with adequate precision and accuracy to enable the profiling of related substances and degradation products during stability studies. The low limit of detection indicates that the developed method can be used for detection of low concentration of active constituents extracted from samples (Table 1).

**Limit of quantification:** The limit of quantification (LOQ) is the lowest amount of analyte in a sample that can be determined with acceptable precision under specified experimental conditions. The degree of precision considered to be acceptable for purposes of LOQ determination from peak areas of six injections, the low limit of quantification indicates that the developed method can be used for quantification of low concentration of active constituents extracted from samples (Table 1).

## Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in

method parameters and provides an indication of its reliability during normal usage.<sup>34</sup> In liquid chromatography variations of pH in mobile phase, mobile phase composition, column, temperature and flow rate are considered. Robustness of the method was determined from the degree of variation observed in peak areas and retention times from the same working standard solution analyzed while adjusting each of the liquid chromatographic factors indicated. Six replicate injections of the same working standard solution were run after having adjusted a single chromatographic parameter and coefficient of variation of peak areas of component peaks calculated. The degree of variation observed was then used to infer the method's robustness Table 4.

**Table 1** Parameters of Linearity data for the Amlodipine (AML), Hydrochlorothiazide (HCT), and Valsartan (VAL)

Parameter	AML	HCT	VAL
Measurement wavelength (nm)	230	230	230
Linear range (µg/mL)	0.2 - 80	0.2 - 80	0.25 - 100
Standard deviation of the Blank	74.9	74.9	74.9
Intercept	3,996.10	66,639	22,824.90
Slope	23,447.10	37,204.50	40,040
Correlation coefficient (r)	0.99996	0.9993	0.9993
Limit of detection, LOD (µg/mL)	0.011	0.01	0.01
Limit of quant., LOQ (µg/mL)	0.032	0.02	0.019

**Table 2** Recovery studies for the determination of Amlodipine (AML), Hydrochlorothiazide (HCT), and Valsartan (VAL) by the proposed method

Sample No	Sample Content (µg/ mL)	Average of Amount Found (µg/ mL)	Assay %	Recovery %	RSD %	Average of Recovery and RSD
(Relative to 100% Concentration )						
Amlodipine						
AML	20	19.73	49.325	98.65	0.41	99.55%
	40	39.88	99.7	99.7	0.17	0.23%
	60	60.18	150.45	100.3	0.12	
HCT						
HCT	20	19.74	49.35	98.7	0.21	99.52%
	40	39.88	99.7	99.7	0.22	0.18%
	60	60.09	150.23	100.15	0.12	
Valsartan						
VAL	25	24.88	49.76	99.52	0.15	100.01%
	50	49.99	99.98	99.98	0.17	0.15%
	75	75.41	150.82	100.55	0.13	

**Table 3** Influence of small variation in the assay condition on the analytical performance of the Proposed HPLC method for determination of Amlodipine (AML), Hydrochlorothiazide (HCT) and Valsartan (VAL)

Change In	Before Change	Flow Rate	Flow Rate	Mobile Phase	Mobile Phase	λ nm	λ nm
Parameter		1.4ml/min	1.6 ml/min	+10% acetonitrile	-10% acetonitrile	-5nm (225)	+5nm (235)
Amlodipine							
Retention Time	2.133	2.374	2.095	2.005	3.096	2.234	2.233
Tailing Factor	1.277	1.288	1.272	1.276	1.288	1.275	1.277
Hydrochlorothiazide							
Retention Time	1.694	1.809	1.588	1.322	1.83	2.234	1.69
Tailing Factor	1.222	1.247	1.244	1.212	1.234	1.223	1.244
Valsartan							
Retention Time	6.332	6.939	6.129	6.122	10.526	6.534	6.521
Tailing Factor	1.16	1.252	1.121	1.172	1.234	1.18	1.19

**Table 4** Precision studies for the determination of Amlodipine (AML), Hydrochlorothiazide (HCT), and Valsartan (VAL) by the proposed method

Sample No	Intraday Precision		Interday Precision	
	Assay %	RSD %	Recovery Mean	RSD %
Amlodipine				
1	99.96	0.27	99.94	0.36
2	99.4	0.23	98.1	0.32
3	99.81	0.34	99.34	0.29



Table Continued...

Sample No	Intraday Precision		Interday Precision	
Average	99.72	0.28	99.13	0.32
All assay		99.43	RSD %	0.42
Hydrochlorothiazide				
1	98.76	0.31	98.3	0.12
2	98.24	0.22	99.87	0.18
3	98.55	0.27	99.23	0.31
Average	98.52	0.27	99.13	0.2
All assay		98.83	RSD %	0.44
Valsartan				
1	98.86	0.33	99.84	0.18
2	101.76	0.24	99.05	0.13
3	99.45	0.45	99.43	0.34
Average	100.02	0.34	99.44	0.22
All assay		99.73	RSD %	0.41

**Table 5** Stability studies for the determination of Amlodipine (AML), Hydrochlorothiazide (HCT), and Valsartan (VAL) by the proposed method

Time (Month)	Accelerated Storage Conditions			Real Storage Conditions		
	40°C and 75%			25°C and 60%		
	AML %	HCT %	VAL %	AML %	HCT %	VAL %
0 Time	99.72	99.13	100.02	99.72	99.13	100.02
1 month	99.2	100.02	98.8	101	98.6	99.47
2 month	99.4	100.2	99.55	98.74	100.22	99.31
3 month	99.14	99.57	99.93	98.9	99.34	99.56

### Stability tests

The stability test results in terms of the average content of AML, HCT, and VAL per tablet, in percentage with respect to the labeled amount, are presented in Table 5. The quantity of AML, HCT, and VAL remained fairly constant in accelerated and real storage until the end of the stability study period (3months). The stability results suggest that No significant degradation was observed and the relative standard deviation between the result of time initial and after 1, 2, and 3months in the stability chamber.

### Analysis of pharmaceutical dosage form

The optimized HPLC procedure was applied for the assay of this drug combination in the pharmaceutical formulation (Exforge HCT tablets). The active ingredients were extracted with the same solvent used for the preparation of the standard stock solutions (HPLC-grade acetonitrile and phosphate buffer 55%:45%) then dilution was made with same solvent to reach concentration levels within the specified ranges. The active ingredients eluted at their specific retention times as shown in Figure 3. No interfering peaks were observed from any of the inactive ingredients or the colored coat of the analyzed tablets. The diode-array detection enables peak purity verification where no signs of co-elution from any of the inactive adjuncts were detected. Recoveries were calculated using both external standard and standard addition methods. The assay results revealed satisfactory accuracy and precision as indicated from % recovery, SD and RSD% values Table 2 & 4. Although no monograph for the sample combinations are present in official pharmacopoeia, the assay limits specified in the British Pharmacopoeia and United States Pharmacopoeia for single component containing any of the three ingredients were used as a basis for determining whether the products met quality specifications. In all the three cases, the pharmacopoeia specified assay limits of 90-110% for each drug component<sup>3,36</sup> (Table 4). It is evident from these results that the proposed method is applicable to the assay of this drug combination with a satisfactory level of selectivity, accuracy and precision.

### Discussion

In this study a method for the simultaneous determination of Amlodipine (AML), hydrochlorothiazide (HCT) and valsartan (VAL) an antihypertensive fixed dose combination in the market was developed. The optimum conditions for the separation of the AML, HCT and VAL was determined using isocratic reversed phase HPLC method by using ODS C-18 (250mm×4.6mm i.d. 5µm) column because these types of columns are the ones routinely and cheaply used in liquid chromatography analysis of pharmaceuticals. And a mobile phase consisting of acetonitrile-0.05 M KH<sub>2</sub>PO<sub>4</sub> pH 3.0 (55:45 % v/v). Column temperature was maintained at 40°C and the flow rate was 1.5ml/min. Detection was at 230nm and injection of 20µL. All components eluting within 6min. The order of elution of the component was HCT, AML and VAL respectively. The % recovery was found to be within limits of the acceptance criteria with recovery range 98.6 to 100.3% for AML, 98.7 to 100.1% for HCT and 99.5 to 100.5% for VAL. The high percentage of recovery indicates that the proposed method is highly accurate. The detection limit of the proposed method was 0.011µg/mL, 0.10µg/mL and 0.10µg/mL and the quantification limit was 0.032µg/mL, 0.019µg/mL and 0.020µg/mL for AML, VAL and HCT respectively, which indicate the sensitivity of the method. The assay procedures were repeated more times and the results were found to give 99.43%, 98.83% and 99.73% for AML, HCT and VAL respectively. The number of theoretical plates calculated was 3019, 2404 and 6094 for AML, VAL and HCT respectively, which indicates efficient performance of the column. No interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in tablet formulations did not interfere with the simultaneous estimation of the drugs HCT, VAL and AML by the proposed HPLC method. The study focused on optimization of the conditions for simple, rapid and cost effective analysis including selection of routinely used columns and mobile phases to obtain satisfactory results.

### Conclusion

The present paper described the development of simple, sensitive

and accurate analytical methods for the simultaneous determination of AML, HCT and VAL in mixtures without the need of prior separation step. The procedure presented here does not need any expensive apparatus; therefore the proposed method can be used advantageously as a routine method for the determination of AML, HCT and VAL in quality control and industry, our method may be applied to the determination the content of the cited drugs in commercial tablets.

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## Conflicts of interest

None.

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None.

## References

- Calhoun DA, Lacourciere Y, Chiang YT, et al. Triple antihypertensive therapy with amlodipine, valsartan, and Hydrochlorothiazide. *Hypertension*. 2009;54(1):32–39.
- Chrysant SG. Single-pill triple-combination therapy: an alternative to multiple-drug treatment of hypertension. *Postgrad Med*. 2011;123(6):21–31.
- Reynolds JEF. MARTINDALE. The Extra Pharmacopoeia. (6<sup>th</sup> edn), A publication of Pharmaceutical Press, UK. 2009;1:1214–1420.
- Nikam MB, Dhamane H, Aligave A, et al. Simultaneous estimation of valsartan, amlodipine besylate and hydrochlorothiazide by first order derivative UV spectrophotometric method. *Int J Pharmacy and Technology*. 2010;2:642–650.
- United States Pharmacopoeia United States Pharmacopoeial Convention, Twin Brook Parkway, Rockville, USA. 2007. p.2288–3447.
- British pharmacopoeia 2001. A publication of stationary office Ltd, UK. 2001. p.116–860.
- Anandakumar K, Jayamariappan M. Absorption Correction Method For The Simultaneous Estimation of Amlodipine Besylate, Valsartan and Hydrochlorothiazide in Bulk and in Combined Tablet Dosage Form. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2011;3(1):23–27.
- Lakshmi KS, Lakshmi S. Simultaneous Spectrophotometric Determination of Valsartan and Hydrochlorothiazide by H-Point Standard Addition Method and Partial Least Squares Regression. *Acta Pharmaceutica*. 2011;61(1):37–50.
- Stolarczyk M, Maslanka A, Krzek J, et al. Application of Derivative Spectrophotometry for Determination of Enalapril, Hydrochlorothiazide and Valsartan in Complex Pharmaceutical Preparations. *Acta Poloniae Pharmaceutica*. 2008;65(3):275–281.
- Gupta KR, Mahapatra AD, Wadodkar AR, et al. Simultaneous UV Spectrophotometric Determination of Valsartan and Amlodipine in Tablet. *International Journal of ChemTech Research*. 2010;2(1):551–556.
- Tatar S, Saglik S. Comparison of UV- and Second Derivative-Spectrophotometric and LC Methods for the Determination of Valsartan in Pharmaceutical Formulation. *Journal of Pharmaceutical and Biomedical Analysis*. 2002;30(2):371–375.
- Satana E, Altinay S, Goger NG, et al. Simultaneous Determination of Valsartan and Hydrochlorothiazide in Tablets by First-Derivative Ultra-violet Spectrophotometry and LC. *Journal of Pharmaceutical and Biomedical Analysis*. 2001;25(5–6):1009–1013.
- Shaalán R, Belal TS. Simultaneous Spectrofluorimetric Determination of Amlodipine Besylate and Valsartan in Their Combined Tablets, *Drug Test Anal*. 2010;2(10):489–493.
- Kul D, Dogan-Topal B, Kutucu T, et al. High-Performance Liquid Chromatographic and First Derivative of the Ratio Spectrophotometric Determination of Amlodipine and Valsartan in Their Binary Mixtures. *J AOAC Int*. 2010;93(3):882–890.
- Wei X, Yang G, Qi L, et al. Determination of Nicardipine and Amlodipine in Human Plasma Using On-Line Solid-Phase Extraction with a Monolithic Weak Cation-Exchange Column. *Talanta*. 2009;77(3):1197–1202.
- Ramani AV, Sengupta P, Mullangi R. Development and Validation of a Highly Sensitive and Robust LC-ESI-MS/MS Method for Simultaneous Quantitation of Simvastatin Acid, Amlodipine and Valsartan in Human Plasma: Application to a Clinical Pharmacokinetic Study. *Biomed Chromatogr*. 2009;23(6):615–622.
- Alsarra IA. High-Performance Liquid Chromatographic Method for Quantitative Determination of Amlodipine in Human Plasma and Pharmaceutical Dosage Form and Its Application to Pharmacokinetic Studies. *J Chromatogr Sci*. 2009;47(10):863–867.
- Li H, Wang Y, Jiang Y, et al. A liquid Chromatography/Tandem Mass Spec-trometry Method for the Simultaneous Quantification of Valsartan and Hydrochlorothiazide in Human Plasma. *Journal of Chromatography B*. 2007;852(1–2):436–442.
- Iriarte G, Ferreiros N, Ibarrondo I, et al. Optimization via Experimental Design of an SPE-HPLC-UV-Fluo-rescence Method for the Determination of Valsartan and Its Metabolite in Human Plasma Samples. *J Sep Sci*. 2006;29(15):2265–2283.
- Tian DF, Tian XL, Tian T, et al. Simultaneous Determination of Valsartan and Hydro-chlorothiazide in Tablets by RP-HPLC. *Indian J Pharm Sci*. 2008;70(3):372–374.
- Chitlange SS, Bagri K, Sakarkar DM. Stability Indicating RP-HPLC Method for Simultaneous Estimation of Valsartan and Amlodipine in Capsule Formulation. *Asian Journal of Research in Chemistry*. 2014;13(1):809–817.
- Iriarte G, Gonzalez O, Ferreiros N, et al. Validation of a Fast Liquid Chromatography-UV Method for the Analysis of Drugs Used in Combined Cardiovascular Therapy in Human Plasma. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2009;877(27):3045–3053.
- Shah NJ, Suhagia BN, Shah RR, et al. HPTLC Method for the Simultaneous Estimation of Valsartan and Hydrochlorothiazide in Tablet Dosage Form. *Indian J Pharm Sci*. 2009;71(1):72–74.
- Varghese SJ, Ravi TK. Quantitative Simultaneous Determination of Amlodipine, Valsartan, and Hydrochlorothiazide in ‘Exforge HCT’ Tablets Using High-Performance Liquid Chromatography and High-Performance Thin-Layer Chromatography. *Journal of Liquid Chromatography & Related Technologies*. 2011;34(12):981–994.
- Argekar A, Powar S. Simultaneous determination of atenolol and amlodipine in tablets by high performance thin-layer chromatography. *J Pharm Biomed Anal*. 2000;21(6):1137–1142.
- Alnajjar AO. Validation of a Capillary Electrophoresis Method for the Simultaneous Determination of Amlodipine Besylate and Valsartan in Pharmaceuticals and Human Plasma. *J AOAC Int*. 2011;94(2):498–502.
- Hillaert S, Van den Bossche W. Simultaneous Determination of Hydrochlorothiazide and Several Angio-tensin-II-Receptor Antagonists by Capillary Electrophoresis. *Journal of Pharmaceutical and Biomedical Analysis*. 2003;31(2):329–339.
- Hillaert S, Van den Bossche W. Optimization and Validation of a Capillary Zone Electrophoretic Method for the Analysis of Several Angiotensin-II-Receptor An-tagonist. *Journal of Chromatography A*. 2002;979(1–2):323–333.

29. Gonzalez L, Akesolo U, Jimenez RM, et al. Application of Capillary Zone Electrophoresis to the Screening of Some Angiotensin II Receptor An-agonists. *Electrophoresis* . 2002;23(2):223–229.
30. Yan J, Wang W, Chen L, et al. Electrochemical Behavior of Valsartan and Its Determination in Capsules. *Colloids Surf B Biointerfaces* . 2008;67(2):205–209.
31. Gazy AA. Determination of Amlodipine Besylate by Adsorptive Square-Wave Anodic Stripping Voltammetry on Glassy Carbon Electrode in Tablets and Biological Fluid. *Talanta*. 2004;62(3):575–582.
32. Altioikka G, Dogrukol-Ak D, Tuncel M, et al. Determination of Amlodipine in Pharmaceutical Formulations by Differential Pulse Voltammetry with a Glassy Carbon Electrode. *Arch Pharm (Weinheim)*. 2002;335(2):104–108.
33. ICH. Stability testing of new drug substances and products. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. 2003.
34. The United States Pharmacopoeia 31 National Formulary 26 (U.S.P. 31 N.F. 26). United States Pharmacopoeial Convention Inc. Rockville, USA. 2008.
35. [www.scribd.com/doc/.../ICH-Validation-of-Analytical-Procedures](http://www.scribd.com/doc/.../ICH-Validation-of-Analytical-Procedures)
36. ICH. Validation of analytical procedures: text and methodology. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use: ICH TriPLICATE Guidelines. 2005.