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# REVIEW OF VALSARTAN ANALYSIS METHODS FROM 2000 TO 2020

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## ABSTRACT

Valsartan is used as a hypertension therapy that reduces blood pressure by inhibiting the activity of angiotensin II only at the AT1 receptor, hence it is also called ARB (angiotensin receptor blockers). The pharmacological effect of this drug is similar to the pharmacological effect of ACE inhibitors (angiotensin-converting enzyme), which results in dilatation of arterioles and veins and inhibits aldosterone sessions thereby reducing blood pressure and decreasing salt retention and water. valsartan is used as a hypertension therapy that reduces blood pressure by inhibiting the activity of angiotensin II only at the AT1 receptor, hence it is also called ARB (angiotensin receptor blockers). The pharmacological effect of this drug is similar to the pharmacological effect of ACE inhibitors (angiotensin-converting enzyme), which results in dilatation of arterioles and veins and inhibits aldosterone sessions thereby reducing blood pressure and decreasing salt retention and water. Angiotensin II type 1 receptor antagonists have been widely used in Indonesia for the treatment of various disorders such as hypertension, heart failure, myocardial infarction, and diabetic nephropathy. Over the years, various analytic methods have been developed. This review focuses on the latest developments in analytical techniques for estimating valsartan alone or in combination with other drugs in various biological media such as human plasma and urine. This review provides some insight into the analytical methods of UV spectroscopy, high-performance liquid chromatography (HPLC), ultra-performance liquid chromatography (UPLC), high-performance thin-layer chromatography (HPTLC). which is used for the determination of valsartan both single and combination.

**KEYWORDS:** Valsartan, spectrophotometry, potentiometric, HPLC, TLC, UPLC, voltammetry.

## INTRODUCTION

Hypertension is defined as having blood pressure exceeding the normal range of 120/80 mmHg. One of the more recent drugs to treat hypertension is valsartan, which belongs to the Angiotensin receptor blockers (ARB) group of drugs. Since its introduction in 1996, it has been widely used to treat hypertension due to its efficacy and is well tolerated by patients.[1]

Valsartan indications are for hypertension (can be used alone or in combination with other antihypertensive drugs); heart failure in patients who cannot tolerate ACE inhibitors (angiotensin-converting enzyme inhibitors). For information on the latest product names in Indonesia, see cekbpom.pom.go.id. Trade names circulating in Indonesia are Co Diovan, Valesco, Diovan, Valsartan Ni, Exforge.[2]

Valsartan occurs as a white powder. It is very soluble in methanol and ethanol (99.5 %) and practically insoluble in water. It has molecular formula $C_{24}H_{29}N_5O_3$; weight formula 435.52, and chemical name (2S)-3-Methyl-2-(N-{[2'-(1H-tetrazole-5-yl) biphenyl-4-yl]methyl}pentan-amido) butanoic acid. The structure of the valsartan was shown in Figure 1.[3]

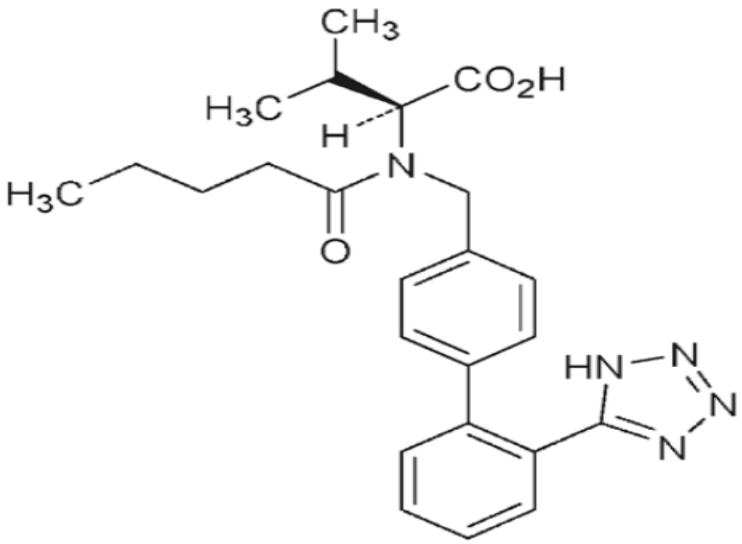


**Figure 1: Structure of valsartan[3]**

Several methods have been reported related to the analysis of valsartan in both pure compounds and pharmaceutical preparations and biological fluids. To date, many analytical methods have been developed for quantitative determination related to valsartan levels.

**Data collection**

In compiling this review article, the technique used is to use a literature study by finding sources or literature in the form of primary data in the form of official books and international journals in the last 20 years (2000-2020). Also, in making this review article a data search using online media with keywords was valsartan analysis in pharmaceutical preparations and biological matrices. The search for primary references used in this review article through a trusted web such as ScienceDirect, NCBI, Researchgate, Google Scholar, and other published and trustworthy journals.

## ANALYTICAL METHODS

### Spectrophotometry

There are several spectrophotometric methods used to determine valsartan levels both as raw materials and pharmaceutical preparations (Table 1).

**Table 1. Valsartan analysis using spectrophotometry.**

| No | Sample | Solvent | Mode | Wavelength | Range of Concentration | Reference |
|---|---|---|---|---|---|---|
| 1 | Tablet | Ethanol | Zero-order | 205.6 nm | 2.0 – 10.0 µg/mL | 4 |
| 2 | Tablet | Ethanol | Second-order | 221.6 and 231.2 nm | 0.5 – 4.0 µg/mL | 4 |
| 3 | Bulk, tablet | Methanol | Zero-order | 250.0 nm | 10 – 50 µg/mL | 5 |
| 4 | Bulk, tablet | Methanol | Second-order | 241.0 nm | 10 – 50 µg/mL | 5 |

UV- and second derivative-spectrophotometric methods for the determination of valsartan in the pharmaceutical formulation have been developed. For the first method, UV spectrophotometry, standard solutions were measured at 205.6 nm. The linearity changes were found to be 2.0 - 10.0 µg/mL in ethanol and the regression equation was $A = 1.05 \times 10 C + 4.26 \times 10^{-2}$ ($r = 0.9997$). For the second method, the distances between two extremum values (peak-to-peak amplitudes), 221.6, and 231.2 nm were measured in the second-order derivative-spectra of standard solutions. Calibration curves were constructed by plotting $d^2A/d\lambda^2$ values against concentrations, 0.5 - 4.0µg/mL of valsartan standards in ethanol. Regression equation of linear calibration graph was calculated as $D = 2.9 \times 10^{-2} C - 2.37 \times 10^{-3}$ ($r = 0.9996$).[4]

Two simple, precise, and accurate UV spectrophotometric methods have been developed and validated for the estimation of valsartan (VAL) in bulk and tablet dosage form. The zero-order spectra of valsartan in methanol show $\lambda_{max}$ at 250.0 nm and estimation was carried out by A (1 %, 1 cm) and by comparison with a standard (Method I). The second-order spectra showed $\lambda_{max}$ at 241.0 nm where n = 2 and estimation were carried out by comparison with a standard (Method II). Calibration graphs were found to be linear ($r^2$ = 0.999) over the concentration range of 10 - 50 µg/mL. The proposed methods were validated for its accuracy, precision, specificity, ruggedness, and robustness. Both methods can be adopted in their routine analysis.[5]

**Voltammetry**

Stripping voltammetric determination of valsartan using a hanging mercury drop electrode (HMDE) was described. The method was based on the adsorptive accumulation of the species at HMDE, followed by the first harmonic alternating current AC stripping sweep at pH 6. The behavior of adsorptive stripping response was thoroughly studied under various experimental conditions, e.g. type of supporting electrolyte, pH, accumulation time, scan rate, and mode of the sweep. In Britton-Robinson buffer solution, a quasi-reversible reduction process involving transfer two electrons and two protons was taken place. The response was linear over the concentration range of 0.08 - 0.64 mg/mL with regression coefficient 0.999 and limit of detection 0.02 mg/mL. The average of determinations of the cited compound in oral dosages with its standard deviation was 101.11 ± 4.38%. The result obtained by the proposed method was compared with that obtained by the UV-spectrophotometric technique. Furthermore, the proposed method was successfully applied as a stability-indicating method for determining valsartan in the presence of its acid-induced degradation products.[6]

**High-performance liquid chromatography (HPLC)**

Some HPLC methods have been used for valsartan analysis, either as raw material, in pharmaceutical dosage forms, and biological matrices (Table 2).

**Table 2: Valsartan analysis using high-performance liquid chromatography.**

| No. | Sample | Column | Mobile Phase | Detector | Chromatographic Condition | Ref. |
|---|---|---|---|---|---|---|
| 1 | Capsule | C18 column | Acetonitrile, phosphate buffer | UV(265nm) | Not available | [4] |
| 2 | Human plasma | Atlantis C18 100 mm x 3.9 mm id, 100 Ä, 3 µm column | ACN with 0.025 % TFA and a 5 mM phosphate buffer with 0.025 % TFA at pH 2.5 | UV-fluorescence | Flow rate: 1.30 mL/min Temperature: 40 ± 0.2 $^{o}$C | [7] |
| 3 | Human plasma | RP C18 Atlantis 100 mm × 3.9 mm column | ACN 0.025 % TFA and phosphate buffer (5 mM, pH = 2.5) | fluorescence detector at 234 and 378 nm excitation and emission | Flow rate:1.30 mL/min | [8] |
| 4 | Human plasma | octadecyl silica column (50 mm × 4 mm, 5 µm particles) | acetonitrile -15 mM dihydrogen potassium phosphate, pH 2.0 (45:55, v/v) | the fluorimetric detector at 234/374 nm | Not available | [9] |
| 5 | Human plasma | Phenomenex® Luna C18 column | 42 % acetonitrile with 15 mM potassium dihydrogen phosphate in water (pH 2.0; adjusted with phosphoric acid) | UV Detector | Flow rate: 1.2 mL/min | [10] |
| 6 | Pure, tablet | RP-HPLC | 0.01 M $NH_4H_2PO_4$ (pH 3.5) buffer: methanol [50:50] | Detection at 210 nm | Flow rate: 1 mL/min | [11] |
| 7 | Tablet | Thermo-hypersil ODS column (150 mm × 4.6 mm i.d., 5 µm particle size) | Water: acetonitrile: glacial acetic acid (500:500:01) | UV 273 nm | Flow rate:1.0 mL/min | [12] |
| 8 | Tablet | C18 column (250 x 4.6 mm) | ammonium dihydrogen phosphate buffer : methanol (33.5 : 66.5) adjusted to pH 3 with formic acid | detection at 265 nm | Flow rate:1.0 mL/min | [13] |

The method was based on high-performance liquid chromatography on C18 column using acetonitrile, phosphate buffer as a mobile phase and losartan as internal standard. Detection was carried out at 265 nm with a UV detector. The assay was linear over the concentration range at 1.0 - 5.0 µg/mL and regression equation was found to be $A = 2.74 \times 10^{-1} C + 2.06 \times$

$10^{-2}$ ($r$ = 0.9991). Commercial capsules containing 160 mg valsartan were analysed by the developed methods and the results obtained were compared statistically at 95 % confidence level.[4]

A chemometric approach was applied for the optimization of the extraction and separation of the antihypertensive drug valsartan and its metabolite valeryl-4-hydroxy-valsartan from human plasma samples. Due to the high number of experimental and response variables to be studied, fractional factorial design (FFD) and central composite design (CCD) were used to optimize the HPLC-UV-fluorescence method. First, the significant variables were chosen with the help of FFD; then, a CCD was run to obtain the optimal values for the significant variables. The measured responses were the corrected areas of the two analytes and the resolution between the chromatographic peaks. Separation of valsartan, its metabolite valeryl-4-hydroxy-valsartan and candesartan M1, used as internal standard, was made using an Atlantis C18 100 mm x 3.9 mm id, 100 Ä, 3 µm chromatographic column. The mobile phase was run in gradient elution mode and consisted of ACN with 0.025 % TFA and a 5 mM phosphate buffer with 0.025 % TFA at pH 2.5. The initial percentage of ACN was 32 % with a stepness of 4.5 %/min to reach the 50 %. A flow rate of 1.30 mL/min was applied throughout the chromatographic run, and the column temperature was kept to 40 ± 0.2 $^{o}$C. In the SPE procedure, experimental design was also used in order at achieve a maximum recovery percentage and extracts free from plasma interferences. The extraction procedure for spiked human plasma samples was carried out using C8 cartridges, phosphate buffer (pH 2, 60 mM) as conditioning agent, a washing step with methanol-phosphate buffer (40:60 v/v), a drying step of 8 min, and diethyl ether as eluent. The SPE-HPLC-UV-fluorescence method developed allowed the separation and quantitation of valsartan and its metabolite from human plasma samples with an adequate resolution and a total analysis time of 1 h.[7]

A simple and fast method for the simultaneous determination of the antihypertensive drug valsartan and its metabolite in human plasma has been validated. The proposed method deals with SPE, followed by an HPLC separation coupled with fluorimetric and photometric detection. The optimization of the SPE-HPLC method was achieved by an experimental design. The separation was performed on an RP C18 Atlantis 100 mm × 3.9 mm column. The mobile phase consisted of a mixture of ACN 0.025 % TFA and phosphate buffer (5 mM, pH = 2.5) was delivered in gradient mode at a flow rate of 1.30 mL/min. The eluent was monitored with a fluorescence detector at 234 and 378 nm excitation and emission

wavelengths, respectively, and at 254 nm using a photometric detector. The full analytical validation was performed according to the Food and Drug Administration (FDA) 'guidance for industry: bioanalytical method validation' and the recoveries obtained for Valsartan and its metabolite ranged from 94.6 to 108.8 %. The validated method was successfully applied to 12 plasma samples obtained from patients under antihypertensive treatment with valsartan.[8]

A high-performance liquid chromatographic (HPLC) method for the determination of valsartan in human plasma is reported. The assay is based on protein precipitation with methanol and reversed-phase chromatography with fluorimetric detection. The preparation of a batch of 24 samples takes 20 min. The liquid chromatography was performed on an octadecylsilica column (50 mm × 4 mm, 5 μm particles), the mobile phase consisted of acetonitrile -15 mM dihydrogen potassium phosphate, pH 2.0 (45:55, v/v). The run time was 2.8 min. The fluorimetric detector was operated at 234/374 nm (excitation/emission wavelength). The limit of quantitation was 98ng/mL using 0.2 ml of plasma. Within-day and between-day precision expressed by relative standard deviation was less than 5 % and inaccuracy did not exceed 8 %. The assay was applied to the analysis of samples from a pharmacokinetic study.[9]

The primary objective of the study was to validate a simple and sensitive method of determining valsartan concentration in human plasma samples using high performance liquid chromatography (HPLC) combined with ultraviolet (UV) detection. Methanol appeared to be the best with a high recovery efficiency compared to other solvents such as acetonitrile, ethyl acetate and methyl-tert-butyl ether. After a simple protein precipitation using methanol, the analytes were separated on a Phenomenex® Luna C18 column using 42 % acetonitrile with 15 mM potassium dihydrogen phosphate in water (pH 2.0; adjusted with phosphoric acid) as the mobile phase at a flow rate of 1.2 mL/min. The standard calibration curve constructed in the concentration range of 50 - 2000 ng/mL showed good linearity ($r^2$ > 0.9997). Spironolactone was used as an internal standard (IS). Valsartan and IS eluted at 10.25 and 12.17 min, respectively. The intra-day and inter-day precision and accuracy were satisfactory with relative standard deviations of less than 15%. No interference peaks or matrix effects were observed in human plasma. Valsartan concentration in human plasma was well established following a single 80 mg oral dose (Diovan® capsule) to eight healthy volunteers. The current determination of valsartan concentration by protein precipitation using methanol

followed by analysis using HPLC with UV detection was rapid and sensitive, and provide an alternative to the analysis of valsartan by studying its clinical applications.[10]

A simple, rapid and accurate and stability indicating RP-HPLC method was developed for the determination of valsartan in pure and tablet forms. The method showed a linear response for concentrations in the range of 50 - 175 µg/mL using 0.01 M $NH_4H_2PO_4$ (pH 3.5) buffer: methanol [50:50] as the mobile phase with detection at 210 nm and a flow rate of 1 mL/min and retention time 11.041 min. The method was statistically validated for accuracy, precision, linearity, ruggedness, robustness, forced degradation, solution stability and selectivity. Quantitative and recovery studies of the dosage form were also carried out and analyzed; the % RSD from recovery studies was found to be less than 1. Due to simplicity, rapidity and accuracy of the method, we believe that the method will be useful for routine quality control analysis.[11]

A simple, rapid, sensitive, reverse phase isocratic RP-HPLC method was developed for determination of Valsartan in tablet dosage form. The method was carried out using Thermo-hypersil ODS column (150 mm × 4.6 mm i.d., 5 µm particle size) with mobile phase comprised of water: acetonitrile: glacial acetic acid (500:500:01). The flow rate was set at 1.0 mL/min and effluent was detected at 273 nm. The retention time of valsartan was found to be 4.6 minute. The method was validated for specificity, accuracy, precision, linearity, limit of detection, limit of quantification, robustness and solubility stability. LOD and LOQ were found to be 2.72µg/mL and 8.25µg/mL respectively. The calibration curve was linear in the concentration range of 40-140 µg/ml with coefficient of correlation 0.9990. The percentage recovery for the valsartan was found to be 99.0 - 100.2 and the % RSD was found to be less than 2 %. The proposed method was successfully applied for quantitative determination of valsartan in tablet dosage form.[12]

A simple, specific, rapid, precise and robust HPLC method has been developed for the quantitation of valsartan in tablet dosage form on a C18 column (250 x 4.6 mm) using a mobile phase consisting of ammonium dihydrogen phosphate buffer: methanol (33.5: 66.5) adjusted to pH 3 with formic acid at a flow rate of 1.0 mL/min and detection at 265 nm. The retention time of valsartan was found to be at 11.9 min. The validation of above method was also done. Percentage label claim of the tablet formulations were found to be 100.8 %. So the proposed method provides a faster and cost effective quality control tool for routine analysis of valsartan from formulations.[13]

**Ultra-performance liquid chromatography**

A simple, precise, accurate stability-indicating gradient reverse phase ultra-performance liquid chromatographic (RP-UPLC) method was developed for the quantitative determination of purity of valsartan drug substance and drug products in bulk samples and pharmaceutical dosage forms in the presence of its impurities and degradation products. The method was developed using Waters Aquity BEH C18 (100 mm × 2.1 mm, 1.7 µm) column with mobile phase containing a gradient mixture of solvents A and B. The eluted compounds were monitored at 225 nm, the run time was within 9.5 min, which valsartan and its seven impurities were well separated. Valsartan was subjected to the stress conditions of oxidative, acid, base, hydrolytic, thermal and photolytic degradation. Valsartan was found to degrade significantly in acid and oxidative stress conditions and stable in base, hydrolytic and photolytic degradation conditions. The degradation products were well resolved from main peak and its impurities, proving the stability-indicating power of the method. The developed method was validated as per international conference on harmonization (ICH) guidelines with respect to specificity, linearity, limit of detection, limit of quantification, accuracy, precision and robustness. This method was also suitable for the assay determination of valsartan in pharmaceutical dosage forms.[14]

**Liquid chromatography-tandem mass spectrometry (LCMS/MS)**

A sensitive liquid chromatography-tandem mass spectrometry (LCMS/MS) method for the determination of valsartan in human plasma was developed and validated. A 0.5 ml aliquot was extracted using solid-phase extraction in an Empore high performance extraction disk plate, universal resin 96-well format. The estimated calibration range of the method was 2 - 2000 ng/mL. The method was fully validated with intra-day mean accuracy and precision of 94.8 - 107 % and 2.19 - 5.40 % and inter-day mean accuracy and precision of 93.5 - 105 % and 1.87 - 5.67 %, respectively. No significant loss of valsartan in processed samples was confirmed in processed samples for up to 24 h at 10 °C. Sample dilution up to 50-fold with blank human plasma provided acceptable analyses. No interference peaks or matrix effects were observed. No effect of QC sample location results was observed in a 96-well plate. This LCMS/MS technique was found to improve quantitative determination of valsartan allowing its pharmacokinetic evaluation with clinically relevant doses.[15]

**Valsartan and ezetimibe mixture analysis**

A spectrophotometric method for the determination of valsartan and ezetimibe was developed using acidic dyes, namely, bromophenol blue (BPB) and bromocresol green (BCG). The method was based on selective ion-pair formation between valsartan and the acidic dye. The yellow coloured ion-pair induces a bathochromic shift in the spectrum with maximum absorbance at 425 and 428 nm for BPB and BCG, respectively. The developed method was validated as per ICH guidelines. With BPB, the ion-pair formed obeyed Beer`s law in the ranges 5 - 40 and 1 - 50 µg/mL for valsartan and ezetimibe, respectively. The assay data for valsartan and ezetimibe were, 99.39 ± 0.53 and 98.17 ± 0.91 %, respectively, for the commercial formulation, and 99.41 ± 0.48 and 98.16 ± 0.89 %, respectively, for the developed formulation. The method was validated and the correlation coefficient for valsartan and ezetimibe were 0.995 and 0.999, respectively. Recovery was in the range 99.3 - 100.3 %. The proposed method is reproducible, accurate, robust and suitable for the simultaneous quantitative analysis of the studied drugs in bulk and dosage formulation.[16]

A stability indicating method for the simultaneous estimation of valsartan and ezetimibe in combined tablet formulation using a RP-HPLC was developed and validated as per ICH guidelines using a symmetry C18 column with a mobile phase comprising phosphate buffer and acetonitrile (58:42 v/v, pH 3.15) with a flow rate of 0.8 mL/min at 230 nm. Stress degradation studies were performed in acidic, alkaline, oxidation and photolysis conditions to demonstrate the stability-indicating power of the method. The contents of valsartan and ezetimibe were in the range of 99.77 ± 0.10 % and 99.30 ± 0.43 % in the marketed formulation, 99.77 ± 0.08 % and 99.29 ± 0.38 % for the test formulation, respectively. The correlation coefficient for both valsartan and ezetimibe was 0.999 and recovery was in the range of 98 - 102 %. The limit of detection (LOD) was 0.2 and 0.3 µg/mL for valsartan and ezetimibe, respectively, while limit of quantification (LOQ) was 1 µg/mL for both valsartan and ezetimibe, respectively. The proposed method is simple, precise, accurate, reproducible, specific and reproducible used for the quantitative determination of valsartan and ezetimibe in bulk and dosage formulations.[17]

**Analysis of a mixture of valsartan and hydrochlorothiazide**

A method for the simultaneous determination of valsartan and hydrochlorothiazide in tablets is described. The procedure, based on the use of reversed-phase high-performance liquid chromatography, is linear in the concentration range 5.0 - 10.0µg/mL for valsartan and 0.5 -

2.0 µg/mL for hydrochlorothiazide, is simple and rapid and allows accurate and precise results. The limit of detection was 1.0 µg/mL for valsartan and 0.05 µg/mL for hydrochlorothiazide.[18]

First-derivative ultraviolet spectrophotometry and high-performance liquid chromatography (HPLC) were used to determine valsartan and hydrochlorothiazide simultaneously in combined pharmaceutical dosage forms. The derivative procedure was based on the linear relationship between the drug concentration and the first derivative amplitudes at 270.6 and 335 nm for valsartan and hydrochlorothiazide, respectively. The calibration graphs were linear in the range of 12.0–36.1 µg/mL for valsartan and 4.0 - 12.1 µg/mL for hydrochlorothiazide. Furthermore, a high-performance liquid chromatographic procedure with ultraviolet detection at 225 nm was developed for a comparison method. For the HPLC procedure, a reversed phase column with a mobile phase of 0.02 M phosphate buffer (pH 3.2)-acetonitrile (55: 45; v/v), was used to separate for valsartan and hydrochlorothiazide. The plot of peak area ratio of each drug to the internal standard versus the respective concentrations of valsartan and hydrochlorothiazide were found to be linear in the range of 0.06 - 1.8 and 0.07 - 0.5µg/mL, respectively. The proposed methods were successfully applied to the determination of these drugs in laboratory-prepared mixtures and commercial tablets.[19]

Two new methods for the simultaneous determination of valsartan and hydrochlorothiazide in pharmaceutical dosage forms have been developed. The first method, based on compensation technique is presented for the derivative spectrophotometric determination of binary mixtures with overlapping spectra. By using ratios of the derivative maxima or the derivative minimum, the exact compensation of either component in the mixture can be achieved, followed by its determination. The second method, differential derivative spectrophotometry, comprised of measurement of the difference absorptivities derivatized in the first order ($\Delta D_1$) of a tablet extract in 0.1 N NaOH relative to that of an equimolar solution in methanol at wavelengths of 227.8 and 276.5 nm, respectively. Neither sample pre-treatment nor separation were required. These methods showed good linearity, precision, and reproducibility. The results obtained were compared with the results of reversed phase HPLC. It was found that the difference was not statistically important between these methods. The proposed methods were accurate, sensitive, precise, reproducible and could be applied directly and easily to the pharmaceutical preparations.[20]

A simple, reproducible and efficient reverse phase high performance liquid chromatographic method was developed for simultaneous determination of valsartan and hydrochlorothiazide in tablets. A column having 200 × 4.6 mm i.d. in isocratic mode with mobile phase containing methanol: acetonitrile: water: isopropyl alcohol (22: 18: 68: 2; adjusted to pH 8.0 using triethylamine; v/v) was used. The flow rate was 1.0 mL/min and effluent was monitored at 270 nm. The retention time (min) and linearity range (µg/mL) for valsartan and hydrochlorothiazide were (3.42, 8.43) and (5 - 150, 78 - 234), respectively. The developed method was found to be accurate, precise and selective or simultaneous determination of valsartan and hydrochlorothiazide in tablets.[21]

A new absorption ratio method was developed and validated for the determination of valsartan and hydrochlorothiazide in tablets. Calibration curves for valsartan and hydrochlorothiazide over concentration range of 2 - 20 µg/mL were plotted and molar absorptivity for both the drugs were calculated at both the wavelengths of 270.5 nm (λ-max of hydrochlorothiazide) and 231.5 nm (iso-absorptive point). The results of analysis have been validated statistically and by recovery studies. The value of standard deviation was satisfactory and recovery studies ranging from 99.05 - 102.23 % for valsartan and 97.42 - 100.22 % for hydrochlorothiazide were indicative of the accuracy and precision of the proposed method. The results of the assay are in good agreement with the label amount. The method was found to be simple, rapid, and accurate and can be adopted in routine analysis of these drugs in formulations. Due to these attributes, the proposed method could be used for routine analysis of these drugs in combined dosage forms.[22]

Simultaneous determination of valsartan and hydrochlorothiazide by the H-point standard additions method (HPSAM) and partial least squares (PLS) calibration is described. Absorbances at a pair of wavelengths, 216 and 228 nm, were monitored with the addition of standard solutions of valsartan. Results of applying HPSAM showed that valsartan and hydrochlorothiazide can be determined simultaneously at concentration ratios varying from 20:1 to 1:15 in a mixed sample. The proposed PLS method does not require chemical separation and spectral graphical procedures for quantitative resolution of mixtures containing the titled compounds. The calibration model was based on absorption spectra in the 200–350 nm range for 25 different mixtures of valsartan and hydrochlorothiazide. Calibration matrices contained 0.5–3 µg/mL of both valsartan and hydrochlorothiazide. The standard error of prediction (SEP) for valsartan and hydrochlorothiazide was 0.020 and 0.038

mg/mL, respectively. Both proposed methods were successfully applied to the determination of valsartan and hydrochlorothiazide in several synthetic and real matrix samples.[23]

A simple, selective, rapid, precise and stability-indicating HPTLC method has been developed for the quantitative simultaneous estimation of valsartan and hydrochlorothiazide in combined pharmaceutical dosage form and validation was done. The proposed HPTLC method involves the use of HPTLC plates (Merck) pre-coated with silica gel 60 $F_{254}$ on aluminium sheets and a mobile phase comprising of chloroform: ethyl acetate: acetic acid (5:5:0.2 v/v/v). Densiometric analysis of both the drugs was carried out in the absorbance mode at 248 nm. The method has been successfully applied for estimation of valsartan and hydrochlorothiazide in combined tablets dosage form. Both the drugs were subjected to acid-alkali hydrolysis, oxidation and photolytic degradation and both of them were found to be susceptible to acid-alkali hydrolysis, oxidation and photolytic degradation. Linearity of valsartan was found to be within the range of 800 - 5600 ng/spot ($r^2$ = 0.9996) and for hydrochlorothiazide the range was found to be 125 - 875 ng/spot ($r^2$ = 0.9975), with significant high values of correlation coefficient for both the drugs. The limit of detection and limit of quatitation were found to be 124.71 ng/spot and 377.91 ng/spot for valsartan and 47.67 ng/spot and 114.46 ng/spot for hydrochlorothiazide. The percentage recovery of valsartan and hydrochlorothiazide was ranged from 99.93 to 99.99 and 99.97 to 99.98 respectively. The % R.S.D. values for intraday precision study were <1.0% and for inter-day study were < 2.0 %, confirming that the method was sufficiently precise. The validation studies were carried out fulfilling International Conference on Harmonisation (ICH) requirements. The method was validated for the precision, robustness and recovery. As method could effectively separate the drug from its degradation products, it can be employed as a stability indicating one.[24]

A new, simple, precise and stability-indicating UPLC (Ultra Performance Liquid Chromatography) method was developed and validated for the simultaneous determination of anti-hypertensive drug valsartan (VAL) and hydrochlorothiazide (HCTZ) in combined dosage forms. The method was developed using Kromasil eternity C-18 column (50 mm × 2.1 mm, 3.5 µm) with isocratic elution. Triethylamine buffer (0.1 % v/v) and methanol (75:25 v/v) were used as mobile phase with 0.6 mL/min flow rate at room temperature. The detection wavelength was fixed at 225 nm; the run time was within 2 min. The method was validated in terms of linearity, accuracy and reproducibility. Calibration plots were linear

over the range of 7 - 13 µg/mL for HCTZ and 56 - 104 µg/mL for VAL. Recovery was in the range of 98 - 102 % with the relative standard deviation of less than 2 % for both drugs. The limit of detection and the limit of quantification for the valsartan were found to be 0.8 and 2.4 µg/mL respectively and for hydrochlorothiazide 0.12 and 0.36 µg/mL respectively. The proposed method was also suitable for determination of VAL & HCTZ in bulk and pharmaceutical dosage forms.[25]

**Valsartan and nebivolol HCl mixture analysis**

A simple, fast and precise reversed phase high performance liquid chromatographic method is developed for the simultaneous determination of valsartan and nebivolol in tablet dosage form. Chromatographic separation of these drugs was performed on Kromasil C18 column (250 X 4.6 mm, 5µ) as stationary phase with a mobile phase comprising of 20 mM potassium dihydrogen orthophosphate: acetonitrile in the ratio of 43:57 (v/v) containing 0.1 % glacial acetic acid at a flow rate of 1 mL/min and UV detection at 282 nm. The linearity of valsartan and nebivolol were in the range of 40 to 96µg/mL and 2.5 to 6.0µg/mL respectively. The recovery was calculated by standard addition method. The average recoveries were found to be 99.32 % and 99.38 % for valsartan and nebivolol respectively. The proposed method was found to be accurate, precise and rapid for simultaneous determination of valsartan and nebivolol.[26]

**Valsartan and ramipril mixture analysis**

A simple, sensitive and validated HPLC method has been developed to determine valsartan and ramipril simultaneously in synthetic mixture. Chromatographic separation was achieved on a C-18 column using a mixture of acetonitrile and water in the ratio 55:45 (v/v), pH adjusted to 3.6 with 88 % orthophosphoric acid at a wavelength of 215 nm. Linearity of the method was found to be in the concentration range of 50-250µg/mL for valsartan and l00-500 µg/mL for ramipril with correlation coefficient greater than 0.999. The total eluting time for the two components is less than five minutes. The method can be used for simultaneous determination of valsartan and ramipril.[27]

**Valsartan and amlodipine mixture analysis**

A simple, precise and accurate stability indicating RP-HPLC method has been developed and subsequently validated for simultaneous estimation of Valsartan (VAL) and Amlodipine (AML) from their combination dosage form. A Shimadzu's HPLC (LC-2010-HT, Shimadzu, Singapore) equipped with UV-Visible and Diode Array detectors, with Class-VP software

was used. Column used was XTerra® RP18, 5 µm, 150 mm × 4.6 mm i.d., at 25 °C. Mobile phase consisted mixture of solution A (1000 mL Water + 0.2 mL Trifluoro Acetic Acid) and solution B (Water: Acetonitrile: Trifluoro Acetic Acid, 400:600:1, v/v/v) with flow rate of 1.5 mL/ min and UV detection was carried out at 237 nm and 265 nm for AML and VAL, respectively. VAL, AML and their combined dosage form were exposed to thermal, photolytic, oxidative, acid-base hydrolytic stress conditions, the stressed samples were analyzed by proposed method. Peak purity results suggested no other co-eluting, interfering peaks from excipients, impurities, or degradation products due to variable stress condition, and the method is specific for the estimation of VAL and AML in presence of their degradation products and impurities. The method was validated with respect to linearity, precision, accuracy, system suitability, and robustness. The described method was linear over the range of 1.6 - 240 µg/mL and 1 - 30 µg/mL for VAL and AML, respectively. The mean recoveries were 100.12 and 99.72 % for VAL and AML, respectively. The intermediate precision data were obtained under different experimental conditions and calculated value of the coefficient of variation (CV, %) was found to be less than critical value. The proposed method can be useful in the quality control of pharmaceuticals.[28]

In the present work a simple, accurate and precise method has been developed and validated for the simultaneous estimation of valsartan and amlodipine and in their combined dosage form by UV Spectrophotometric methods. The Method A employs estimation of drugs by simultaneous equation method (SEM) using 250.0 and 238.0 nm i.e. $\lambda_{max}$ values of VAL and AMD respectively. Method B employs the estimation of drugs by Absorption Correction method (ACM) at 360.0 i.e. $\lambda_{max}$ values of one drug and 236.0 nm an isobestic wavelength. VAL and AMD individually and in mixture follow Beer's law over the concentration range 5-30 µg/mL at all the selected wavelengths. Additivity study concluded that both the drugs do not interact with each other in solution. The percent recoveries of the drugs were found nearly 100 % representing the accuracy of the both methods. Validation of the proposed methods was carried out for its accuracy, precision, specificity, linearity and range, ruggedness, limit of detection according to ICH guidelines. The proposed methods can be successfully applied in routine work for the determination of valsartan and amlodipine in combined dosage form.[29]

The present study describes development and validation of simple, rapid, sensitive and stability indicating high-performance liquid chromatographic assay method for simultaneous

determination of amlodipine besylate and valsartan in bulk as well as in commercial formulation. Analytical separation was achieved with RP Waters Symmetry C18 Column [150 × 4.6 mm] using a combination of methanol and potassium dihydrogen phosphate buffer [0.01 M] pH 2.5, in ratio 60:40 v/v with flow rate of 1 mL/min. The retention times for amlodipine and valsartan were found to be 4.6 and 7.6 min respectively. Both the drugs were exposed to thermal, photolytic, hydrolytic, and oxidative stress conditions individually as well as in combination, subsequently samples were analyzed by the proposed method.

Detection was done by PDA detector at 238 nm. Method was found to be very sensitive as LOD found to be 20ng/mL and 44ng/mL for Amlodipine besylate and Valsartan respectively.

The method was found to be specific and stability indicating as no interfering peaks of degradation compounds and excipients were noticed. The developed method was validated with respect to linearity, accuracy, precision, specificity, robustness as per International Conference on Harmonization (ICH) guidelines. The proposed method hence useful for the application in quality control laboratories for quantitative analysis of both the drug individually or in combination, as it is simple and rapid method with excellent accuracy and precision.[30]

Anodic behavior of binary mixture of amlodipine besylate (AMD) and valsartan (VAL) was studied on glassy carbon electrode based on the irreversible oxidation signal of AMD at 0.95 V and that of VAL at 1.15 V versus Ag/AgCl at pH 5.0 in Britton-Robinson buffer.

Differential pulse voltammetric method was proposed to direct determination of AMD and VAL in pharmaceuticals and spiked human serum. Linearity for AMD was in the range from 1.0 µM to 35.0 µM and that for VAL was in the range from 1.5 µM to 32.0 µM when concentrations of AMD and VAL are increased simultaneously. Limit of detection and limit of quantification were found to be 0.31 µM and 1.03 µM, for AMD and 0.36 µM and 1.21 µM for VAL, respectively. The method was successfully applied with good recoveries between 90.8 % and 100.4 % with relative standard deviation less than 10 % for tablet samples and around 15 % for serum samples.[31]

## CONCLUSION

Overall, various analytic methods have been used to determine valsartan levels. Spectrophotometry, spectrofluorimetry, electroanalytic, are easy to apply. However, high-

performance liquid chromatography (HPLC), and ultra-performance liquid chromatography (UPLC) methods are often used in research on biological matrices because they can detect samples with low concentrations. In addition, the HPLC and high-performance thin layer chromatography (HP-TLC) methods can be applied in valsartan mixtures with other drugs.

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