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The use of high-performance liquid chromatography technique for the simultaneous determination of amlodipine and valsartan

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***Abstract*---**This technique was used in the estimation of amlodipine and valsartan simultaneously. The method was sensitive and accurate, and the estimation was done in a short time. In this work, a separation column of type L1, 150 mm was used to separate and sold. The group also found the ultraviolet spectrophotometer that used a wavelength of 239 nm, the temperature of the column was 40 degrees Celsius, and the mobile phase, consisting of 50:50 (acetonitrile: methanol: drops of triethylamine, pH = 2.4) was long. Through what was reached, we find that the method is sensitive, accurate and medium in size (101.43-99.13) and (100.17-99.53), and linear punches (6-14 mcg/ml) and (48-112 mcg/ml) for the two drugs, respectively. The method was applied to a number of pharmaceutical preparations available in the local market, successful in the real-time quantification of amlodipine and valsartan, respectively.

***Keywords*---**high-performance liquid chromatography, amlodipine, valsartan.

Introduction

This technique was called by the scientist Tswett by the name of chromatography, which is a Latin word consisting of two parts chroma meaning color and graphein meaning to write, and because he was able to separate the components of the plant extract into its original components, it was called the technique of writing colors, and this name became applied to all chromatographic methods known at the present time and even Which does not analyze colored sections, and the

importance of chromatography comes in the first place because of its wide uses, for example, in determining the compounds that are included in a mixture qualitatively and quantitatively. In the study of complex structures such as carbohydrates, proteins, and complex phenols found in plants, chromatography is also a very important method in inorganic and organic chemistry. The work of chromatography depends on taking advantage of the difference in the polarity of the various molecules in the mixture. called the stationary phase, the solution containing the eye is added The mixture to be separated is transferred to the solvent (the mobile phase), which moves through the stationary phase, then the components of the mixture are separated based on their polarity with each of the stationary phase and the mobile phase. The least,[1-5]Classification of chromatography into four types: planar and perpendicular to the type of mobile phase and to the reaction on the basis based on the methods of operation [6] To understand the separation process, there are two important theories: plate theory and rate theory [7] The high-performance liquid chromatography technique is of a type Liquid chromatography is used to separate and quantify the amount of compounds that are dissolved in a solution, and then estimate the amount of a particular compound present in the solution [8-11]. This technique is widely used in the biomedical field for the separation and quantification of drug molecules, proteins, nucleic acids and amino acids [12].

In this technique, a liquid mobile phase is used to separate the components of the mixture after the components to be separated and quantified are dissolved in a suitable solvent and then passed (with the mobile phase) through the separation column (the stationary phase) under high pressure, and then the mixture is analyzed into its components inside the column and the amount of accuracy depends On the interaction between the solute components, the stationary phase (non-moving packing inside the column) and the liquid phase, the reaction of the solute with the two phases can be addressed through different choices for both phases [13].

This technique is divided into two main parts: the normal phase and the reverse phase. For the ordinary phase, a polar stationary phase (silica precipitates) is used to hold the polar analytes (dipole-dipole interactions), while the reversible phase separators depend on the forces between the nonpolar compounds and the nonpolar functional groups. which are bound to a silica support (hydrophobic and nonspecific interactions) [13-15]. Nowadays, most applications depend on reverse phase separation

Experimental part

- Amlodipine standard solution (2000 mcg/ml)
  Prepare by dissolving 0.2 g of the standard active ingredient of the drug in an amount of the mobile phase and then complete the volume to the mark in a volumetric bottle of 100 ml of the same solvent.
- Valsartan standard solution (2000 mcg/mL)
  Prepare by dissolving 0.2 g of the standard active ingredient of the drug in an amount of the mobile phase and then complete the volume to the mark in a volumetric bottle of 100 ml of the same solvent.
- Mobile phase solution (50:50 vol/v)

The mobile phase solution was prepared from mixing equal volumes of acetonitrile, methanol and drops of triethylamine and placed in an ultrasound machine for a period of time to get rid of bubbles.

- Sodium hydroxide solution (1 molar)
  Prepare by dissolving 2 g of sodium hydroxide in an appropriate volume of distilled water and then complete the volume to the mark in a 50 ml volumetric vial with distilled water.
- Hydrochloric acid solution (1 mo)
  Prepare by mixing 4.16 ml of hydrochloric acid in an appropriate volume of distilled water and then complete the volume to the mark in a 50 ml volumetric vial with distilled water.

Solutions of pharmaceutical preparations

The pharmaceutical preparations used in this work are all in the form of tablets, and were treated in the same manner; As the tablets were well ground until we obtain a high homogeneity, then the weight of one tablet was taken and dissolved in the mobile phase solution acetonyl:methanol, adding drops of triethylamine, and after dissolving the mixture was filtered to get rid of impurities and then dilutions were made to obtain the desired concentration. It is within the linear range of the calibration curve.

EXFORGE 5mg/160mg

This preparation contains 5 mg amlodipine and 160 mg galsartan, and after handling it as described above, it was diluted to 100 ml in a volumetric vial to obtain 50 µg/ml of amlodipine 160 µg/ml, and then other dilutions were made to obtain the desired concentration.

VALSAR-AM 5mg/160mg

This preparation also contains 5 mg amlodipine and 160 mg galsartan, and after handling it as described above, it was diluted to 100 ml in a volumetric vial to obtain 50 micrograms/ml of amlodipine 160 micrograms/ml, and then other dilutions were made to obtain the desired concentration. work on it.

Amlodipine & Valsartan 5mg/160mg

This preparation also contains 5 mg amlodipine and 160 mg galsartan, and after handling it as described above, it was diluted to 100 ml in a volumetric vial to obtain 50 micrograms/ml of amlodipine 160 micrograms/ml, and then other dilutions were made to obtain the desired concentration. on him.

Covert 5mg/160mg

Covert contains 5 mg amlodipine and 160 mg galsartan, and after handling it as described above, it was diluted to 100 ml in a volumetric vial to obtain 50 µg/ml of amlodipine 160 µg/ml, and then other dilutions were made to obtain the desired concentration. on him.

Preliminary investigations

At the beginning, work was done by choosing initial conditions, such as choosing equal concentrations of both drugs 10 µg/ml, and this concentration was worked on in all choosing the optimal conditions, as the temperature at the beginning of the experiments was 25 degrees Celsius and the separation column that was used first was of type L1, 15 cm. The flow speed is 0.8 ml/min. The purpose of these experiments is to obtain an initial separation on the basis of which the best conditions can be selected to obtain a good and high-accuracy separation, in addition to the importance of the above conditions, but the role of the mobile phase is the most important among them, so first the use of A mobile phase consisting of 50:50 (methanol: water) by keeping the conditions chosen first as they are, but through the chromatogram obtained in Figure (2-1), we note that the separation of the two drugs did not succeed. Among many experiments, using various mobile phases, a mobile phase consisting of 50:50 (acetonitrile: methanol: drops of triethylamine) was used. An acceptable initial separation was obtained that can be improved by choosing the best conditions that lead to an acceptable and good separation. Figure (1).

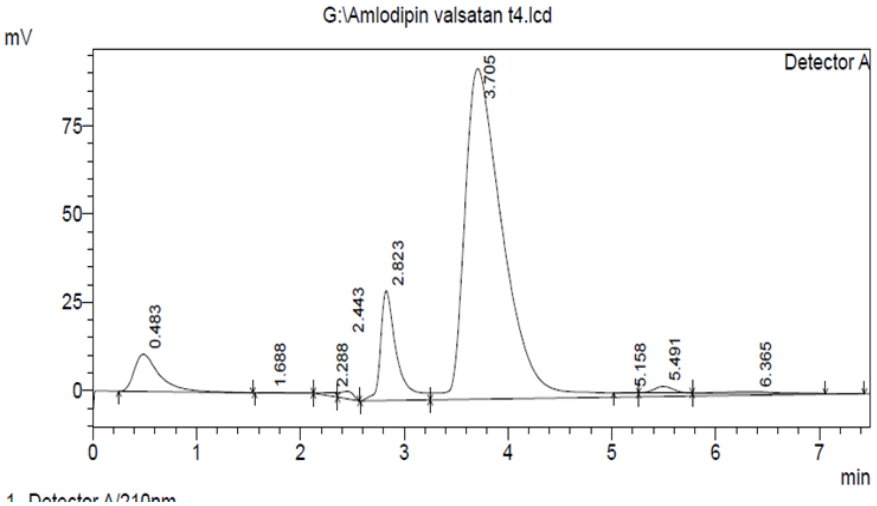

Figure 1: Chromatogram of the two drugs using 50:50 (acetonitrile: methanol: drops of triethylamine)

Results and Discussion

Simultaneous estimation of amlodopine and valsartan results
Wavelength selection

To show the most appropriate wavelength at which AML and VAL drugs are detected, a solution (10 µg/ml) of each of these two compounds was prepared in the same selected solvent (mobile phase), each solution was read by a UV spectrophotometer for the range of 200-400 nm and it showed Overlapping UV spectra indicates that the two drugs show acceptable and somewhat equal absorption values at 239 nm, which was used in preliminary tests. Figure (2)

shows the absorption spectrum of each drug against the mobile phase used as a mock solution.

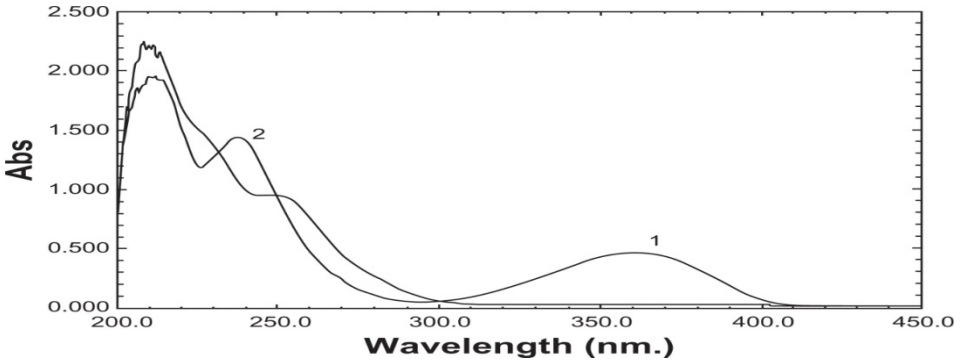

Figure 2: UV absorption spectrum of (1) AML. (2) VAL

Effect of wavelength

Choosing the optimum wavelength depends on the response chosen, the more the response is good in terms of the equivalent height of the theoretical plate or the efficiency (number of theoretical plates) and within the limits of the linear range of the detector (Detector), it is the optimum wavelength, and it should be noted here that the wavelength must be chosen The greater, λmax, because it reduces the spectral interference resulting from the measurement of the detector, and choosing the largest wavelength increases the sensitivity of the method more, and priority must be given when a mixture is determined simultaneously with the same experiment for the wavelength at which the particles are absorbed in a somewhat acceptable way and finding approximately equal areas in the spectra [17,16], and all this is because if the maximum wavelength of one material is chosen and the absorption of other substances is ignored, the quantification will not be accurate

Table (1): Optimum wavelength

Wavelength nm.	Drugs	Parameter	
		N	HETP
232	AML	--------	--------
	VAL	--------	--------
239	AML	3326	45.105
	VAL	8564	17.515
245	AML	3332	45.025
	VAL	5828	25.737

When looking at the recorded spectra of both properties, we find that the equal spectra range between 232-245 nm, so three wavelengths were chosen that fall within this range (232, 239 and 245 nm). Table (1) shows that the best wavelength was 239 nm in terms of HETP and N for the two drugs. Figure 3 shows the chromatograms for this study

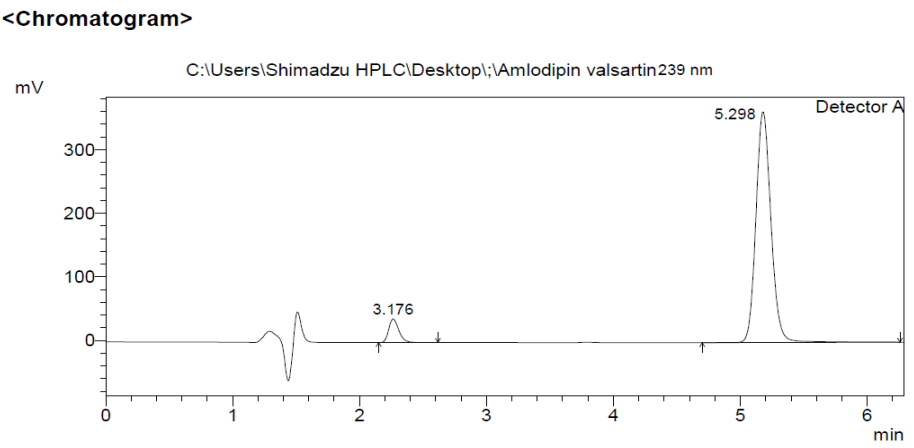

Figure 3: The effect of wavelength on the separation of the two drugs (AML and VAL)

Effect of mobile phase composition

An organic modifier must be added when using RP columns, meaning that water cannot be used alone. The most common modifiers in the mobile phase of these systems are methanol, acetonitrile and (to a lesser extent) tetrahydrofuran [18]. In this study, acetonitrile was used as an organic modifier, as the percentage of this modifier that was used in a mobile phase was evaluated. Several ratios were tried, and the degree of separation was followed by examining the values of the chromatographic variables in addition to the chromatographic shapes, and it was found that increasing the percentage of acetonitrile above 50% leads to a reduction in the retention time in addition to an increase in the height of the peak and also to an improvement in the quality of the separation [19]. The observed changes in selectivity with the increase of the organic composition are attributed to the change in the hydrogen function of the water part of the mobile phase in addition to the variation in the ionization state of the substances to be separated while continuing to change the organic composition [20]. The optimum composition of the mobile phase was determined to be 1:50:50 (acetonitrile:methanol:triethylamine), Table (2), which leads to good separation at an optimum wavelength of 239 nm. The HETP values for each drug change independently with the percentage change in the rate in the mobile phase, which leads to easy selection of the optimal ratio.

Table (2): HETP values and theoretical plate count according to mobile phase composition

Mobile phase (v/v) Acetonitrile: buffer solution	Drug	Parameter	
		N	HETP
30: 70	AML	--------	--------
	VAL	--------	--------
50: 50	AML	3912	38.342
	VAL	9860	15.214
70: 30	AML	106	1418.183
	VAL	2632	56.997

Effect of flow rate of mobile phase

The purpose of studying the optimum flow velocity is to obtain a short analysis time as well as to prevent the diffusion of solute, which in turn leads to a high efficiency of the column [21]. The mobile phase flow velocity has an important effect on the retention time of the material to be separated, and higher flow velocity leads to shorter retention time because the drugs move through the column faster after absorption and vice versa. Figure (4) shows the chromatograms of the mobile phase flow velocity from 0.8 to 1.5 ml/min. The flow velocity 1 ml/min was chosen, which reduced the analysis time from 28.660 to 7.102 min.

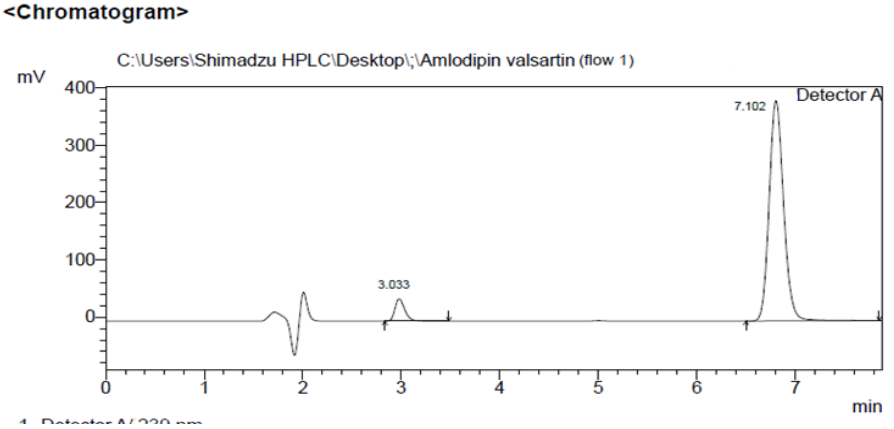

Figure (4) Chromatograms when changing the mobile phase flow velocity 1 ml/min

Effect of Column type

To improve the chromatographic separation, the experimental conditions must be changed so that the components of the mixture are separated in the least possible time, and one of these conditions is the quality of the separation column, which represents the main pillar in the separation process. By observing the chromatograms in Figure (5), it is clear that the best column for separation is L1 150 mm after using the above optimal conditions.

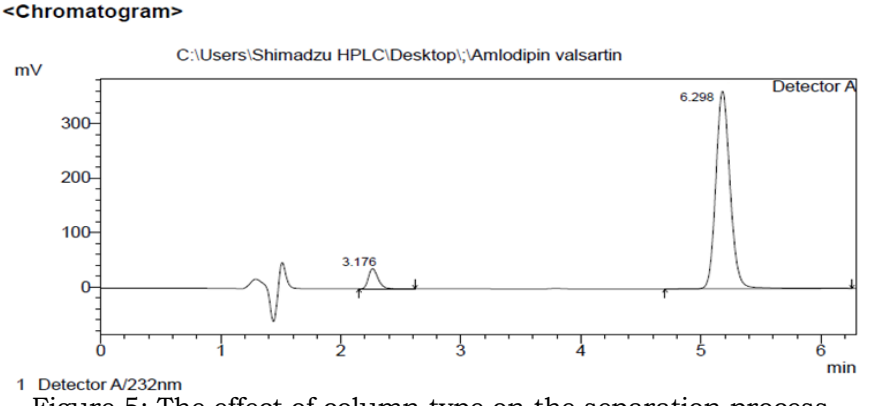

Figure 5: The effect of column type on the separation process

Calibration curves

According to the best experimental conditions, linearity was tested for a range of concentrations (0.1-500) µg/ml for a mixture of (AML and VAL). Separate calibration curves were built for the two drugs by plotting the height of the peak against their dependent concentrations as shown in Figure (6) and it was found that the linearity of each drug (AML) is within the range (6-14 µg/ml) while that of drug (VAL) is within the range (48-112 mcg/ml). Table (3) shows the regression equation, estimation factor, detection limit and quantitative limit

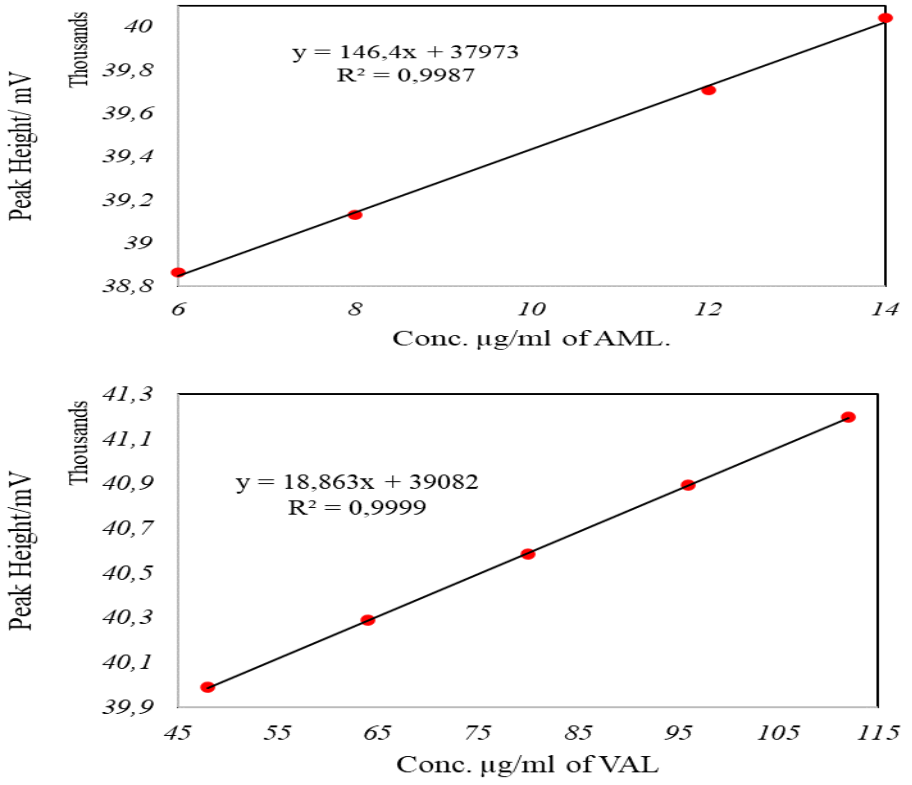

Figure 6: Calibration curves for the two drugs (AML and VAL)

Table (3): Results of the calibration curves for the two drugs under optimal conditions by HPLC technology

Drug	Linearity ($\mu g.mL^{-1}$)	Response	r^2	Slope	Intercept	LOD ($\mu g.mL^{-1}$)	LOQ ($\mu g.mL^{-1}$)
AML	6-14	Peak Height	0.9987	146.4	37973	0.475	1.426
VAL	48-112	Peak Height	0.9999	18.863	39082	0.713	2.140

Accuracy and precision

The accuracy was studied by calculating the percentage recovery and the harmonic by calculating the relative standard deviation, for the results of the studied drugs. Three different concentrations of each drug were selected within the linear range of the calibration curve, Table (4). The results of accuracy and agreement indicate that the proposed method is good for quantitative determination

Table (4): Accuracy and compatibility of the proposed method

Drug	Injected ($\mu g.mL^{-1}$)	Sample Conc. calculated from peak height	
		Mean*	Rec %
AML	6	6.09	101.43
	8	7.93	99.13
	14	14.13	100.90
VAL	48	48.08	100.17
	80	79.63	99.53
	112	112.12	100.11

Application of the method

The proposed method has been successfully applied to estimate two medically important drugs in some of their pharmaceutical forms that are available in the local market by direct method by straight line equations. Pharmaceutical solutions were prepared with different concentrations for each drug. It was then injected four times into the HPLC system at the optimum conditions chosen. The amount of drug was calculated and the results obtained were recorded as in Table (5). The good agreement between these results and the percentage return values within the permissible range shows that the application of the proposed method is effective for the determination of amlodipine and valsartan in their pharmaceutical preparations and that there is no interaction with the existing drug additives.

Table (5): Results of applying the method to some pharmaceutical preparations

Sample	Conc. taken ($\mu g.mL^{1}$) Of drug	Conc. calculated from peak height	
		Conc* found ($\mu g.mL^{1}$)	Recovery %
EXFORGE/ Tablets (5mg AML, 160mg VAL)	8	7.92	99.0
	50	49	98.0
VALSAR-AM/ Tablets (5mg AML, 160mg VAL)	8	7.92	99.0
	50	50	100.0
Amlodipine & Valsartan/ Tablets IP (5mg AML, 160mg VAL)	8	8.008	100.1
	50	49.75	99.5

Covert/ Tablets (5mg AML, 160mg VAL)	8	7.912	98.9
	50	49.5	99.0
Covert/ Tablets (10mg AML, 320mg VAL)	8	8.08	101.0
	50	50.6	101.2
Extor/ Tablets (5mg AML, 80mg VAL)	8	8.024	100.3
	50	49.85	99.7

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