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Research Article

TLC SIMULTANEOUS DETERMINATION OF VALSARTAN AND HYDROCHLOROTHIAZIDE IN PURE FORM AND IN TABLETS USING BUTYL-MODIFIED ALEPPO BENTONITE

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ABSTRACT

TLC simultaneous determination of valsartan (VAL) and hydrochlorothiazide (HCTZ) in pure form and in tablets using new butyl-modified aleppo bentonite (B_AC_4) with mobile phase of acetonitrile:water:acetic acid (49.35:49.35:1.3, v/v), at pH 3.2 and at wavelength λ= 260 nm was developed. The particles of Aleppo Bentonite which have diameter less than 45 μm were treated by concentrated HCl (B_A), after that grafted firstly by dimethyldichlorosilane, then secondly by Grignard reagent (butylmagnesium bromide). The surface properties of butyl-modified bentonite were studied by nitrogen adsorption at 77K. The retardation factors (R_f) of valsartan and hydrochlorothiazide were 0.49 and 0.78, respectively. Linearity for determination of VAL and HCTZ was in the range 2.00-20.00 and 1.00-10.00 μg/spot, respectively. The minimum determined concentration was 2.0 μg/spot for VAL and 1.0 μg/spot for HCTZ with percent relative standard deviation (RSD%) does not exceed 3.1% and 2.0%, respectively. The limits of quantification (LOQ) were 0.61 and 0.20 μg/spot, and the limits of detection (LOD) were 0.20 and 0.066 μg/spot for determination of VAL and HCTZ, respectively. The proposed method was novel, simple, accurate and successfully applied to simultaneous determination of VAL and HCTZ in pharmaceuticals with average recovery of 97.9 to 102.4%, the results obtained agree well with the contents stated on the labels.

Keywords: Butyl- modified Aleppo Bentonite; TLC; Hydrochlorothiazide (HCTZ); Valsartan (VAL).

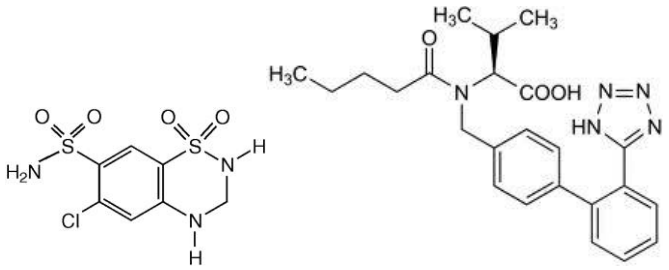
INTRODUCTION

Aleppo Bentonite is rocky clay which consists of 47% SiO_2, 14.4% Al_2O_3 and some other oxides as Fe_2O_3, MgO, CaO, Na_2O[1,2] and others. The thermal treatment causes decreasing of its specific surface area with increasing in the temperature of thermal treatment[3,4]. Bentonite clays are used in many industrial[5,6], and it can be used as chromatographic supports in gas chromatography to separate many mixtures after grafting with different methods[7]. Bentonite is used as stationary phase in thin layer chromatography to separate some metal ions and vitamins B_1, B_6, B_{12}[8-10].

Valsartan is N-(1-oxopentyl)-N-[[2-(1Htetrazol-5-yl) [1, 1-biphenyl]-4-yl] methyl]-l-valine. Valsartan is a potent, highly selective, and orally active antagonist at the angiotensin II AT1- receptor, mol. mass 435.519 g/mol, see Scheme 1 [**11-15]**.

Hydrochlorothiazide is 2H -1, 2, 4-Benzothiadiazine-7-sulfonamide, 6-chloro-3, 4-dihydro 1, 1-dioxide; Hydrochlorothiazide is the most famous thiazide diuretics, mol. mass 297.74 g/mol, see Scheme 1 [**11-15]**.

A new, simple, accurate, and precise high-performance thin-layer chromatographic (HPTLC) method has been established for simultaneous analysis of valsartan and hydrochlorothiazide in tablet formulations. Standard and sample solutions of valsartan and hydrochlorothiazide were applied to precoated silica gel G 60 F_{254} HPTLC plates and the plates were developed with chloroform–ethyl acetate–acetic acid, 5:5:0.2 (v/v/v), as mobile phase. UV detection was performed densitometrically at 248 nm. The retention factors of valsartan and hydrochlorothiazide were 0.27 and 0.56, respectively. The linear range was 800–5600 ng per spot for valsartan and 125–875 ng per spot for hydrochlorothiazide; the correlation coefficients, r, were 0.9998 and 0.9988, respectively. The method was validated in accordance with the requirements of ICH guidelines and was shown to be suitable for purpose. The method was successfully used for determination of the drugs in tablets. Tablet excipients did not interfere with the chromatography [16].



Hydrochlorothiazide **Valsartan**

Scheme 1: Chemical structure of Valsartan and Hydrochlorothiazide.

A simple, precise, accurate and rapid high performance thin layer chromatographic method has been developed and validated for the simultaneous estimation of valsartan and hydrochlorothiazide in combined dosage forms. The stationary phase used was precoated silica gel 60F_{254}. The mobile phase used was a mixture of chloroform: methanol: toluene: glacial acetic acid (6:2:1:0.1 v/v/v/v). The detection of spots were carried out at 260 nm. The method was validated in terms of linearity, accuracy, precision and specificity. The calibration curve was found to be linear between 300 to 800 ng/spot for valsartan and 100 to 600 ng/spot for hydrochlorothiazide. The limit of detection and the limit of quantification for the valsartan were found to be 100 and 300 ng/spot respectively and for hydrochlorothiazide 30 and 100 ng/spot respectively. The proposed method can be successfully used to determine the drug content of marketed formulation [17].

Simple, accurate, precise, sensitive, and validated HPLC and HPTLC-densitometric methods were developed for simultaneous determination of amlodipine (AML), valsartan (VAL), and hydrochlorothiazide (HYD) in combined tablet dosage form. Method A, the gradient RP-HPLC analysis was performed on a Phenomenex Luna C_{18} (4.60 mm × 150 mm, 5 μ particle size) column, using a

mobile phase consisting of 10 mM ammonium acetate buffer (pH 6.7) and methanol in solvent gradient elution for 20 min at a flow rate of 1 mL min^{-1}. Quantification was carried out using a photodiode array UV detector at 238 nm. The employment of a diode array detector allowed selectivity confirmation by peak purity evaluation. Method B, the HPTLC analysis was carried out on an aluminum-backed sheet of silica gel $60F_{254}$ layers using chloroform: glacial acetic acid:n-butyl acetate (8:4:2, v/v/v) as the mobile phase. Quantification was achieved with UV densitometry at 320 nm. These methods are applicable for simultaneous determination of AML, VAL, and HYD in pharmaceutical formulations and biological fluids[18].

Four methods namely, first-derivative of ratio spectra, bivariate, thin layer chromatography and high performance liquid chromatography were used to determine valsartan and hydrochlorothiazide simultaneously in their pharmaceutical dosage forms. The derivative ratio spectra method was based on measuring the peak amplitudes for valsartan at 233 nm and 253 nm using 0.4µg mL^{-1} hydrochlorothiazide as a divisor. Bivariate method is used for simultaneous determination of both drugs by measuring the absorbance at the selected wavelengths. A TLC separation with densitometric detection of both drugs was achieved using chloroform: methanol: ammonia [8:2:0.1, v/v/v] as developing solvent. Furthermore, a high performance liquid chromatographic procedure with ultraviolet detection at 225 nm was developed for the separation and determination of the studied drugs using a C18 column. The mobile phase is composed of 0.02 M phosphate buffer (pH 2.9): acetonitrile: methanol [50:40:10, v/v/v]. The proposed methods were successfully applied for the determination of the studied drugs in their mixtures and in pharmaceutical formulations containing them [19].

A new Octyl-modified Aleppo Bentonite (B_AC_8) in TLC was developed to determine valsartan and hydrochlorothiazide in pure form and pharmaceutical formations with mobile phase water: acetonitril: orthophosphoric acid (45:55:0.6, v/v/v) and wavelength λ= 260 nm. The retardation factors (R_f) of valsartan and hydrochlorothiazide were 0.473 and 0.747, respectively. Linearity for determination of valsartan and hydrochlorothiazide was in the range 2.00-20.0 µg/spot and 1.00-10.0 µg/spot, respectively. The minimum determined concentration was 2.00 µg/spot for valsrtan and 1.00 µg/spot for hydrochlorothiazide with percent relative standard deviation (RSD%) does not exceed 5.08% and 3.26%, respectively. The limits of quantitation were 1.76 and 0.64 µg/spot, and the limits of detection were 0.58 and 0.21 µg/spot for determination of valsartan and hydrochlorothiazide, respectively[9,20].

Several methods have been reported for the analysis of VAL and HCTZ in tablet dosage forms and in biological fluids, the active principle has been determined by high performance liquid chromatography (HPLC) and spectrophotometry methods[21-27].

In the present work, TLC simultaneous determination of valsartan and hydrochlorothiazide in pure and tablets dosage forms using new butyl-modified Aleppo Bentonite (B_AC_4) was developed.

METHODS AND MATERIALS

Apparatus

Surface area and pore size measurement (BET) were recorded using Micromeritics Gemini III 2375 under nitrogen atmosphere (USA). Scanner-densitometer CD60 (Desega, Germany), equipped with mercury, tungsten and deuterium lamps, infra red spectrophotometer type "TENSOR 27" (BRUKER, Germany), CAMAG Hand Operated TLC Coater for preparation of TLC plates (Switzerland), CAMAG UV Cabinet for assessing and marking thin layer chromatograms under UV light (Switzerland) and pH meter from Radio meter company model ion check were used. The diluter pipette model DIP-1 (Shimadzu), having 100 µL sample syringe and five continuously adjustable pipettes covering a volume range from 20 to 5000 µL (model PIPTMAN P, GILSON), a ultrasonic processor model POWERSONIC 405 (to sonicate the sample solutions) and electronic balance (Sartorius-2474; d=0.01 mg) were used.

Chemicals

Valsartan (VAL), mol.mass 435.519 g, Enaltec-India, and hydrochlorothiazide (HCTZ), mol.mass 297.74 g, New Century-China, were used. Methanol, acetonitrile, tetrahydroforan, dichloromethane, dimethyldichlorosilane, 1-bromobutane, acetic acid glacial, magnesium metal and fluorescent indicator F_{254} for thin layer chromatography were purchased from Merck, Germany.

A stock solutions of Valsartan (VAL)

An accurately weighed 1.00604 g standard sample of VAL (99.4%) was dissolved in methanol, transferred into a 25 mL standard flask and diluted to the mark with methanol to obtain 40.00 $mg.mL^{-1}$ of VAL (0.09184 $mol.L^{-1}$), stock solutions (a) of VAL.

A stock solution of hydrochlorothiazide (HCTZ)

An accurately weighed 1.00402 g standard sample of HCTZ (99.6%) was dissolved in methanol, transferred into a 25 mL standard flask and diluted to the mark with methanol to obtain 40.00 $mg.mL^{-1}$ of HCTZ (0.13434 $mol.L^{-1}$), stock solutions of HCTZ (b).

Standard solutions

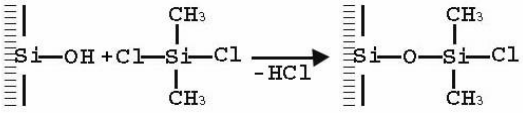
Volumes 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mL from stock solution (a) and 0.25, 0.5, 1.0, 1.5, 2.0 and 2.5 mL from stock solution (b) were transferred into six volumetric flasks (10 mL), respectively and completed to the mark with methanol (these solutions content: 2.0, 4.0, 8.0, 12.0, 16.0 and 20.0 $mg.mL^{-1}$ of VAL and 1.0, 2.0, 4.0, 6.0, 8.0 and 10.0 $mg.mL^{-1}$ of HCTZ, respectively).

Preparation of acidic treated Bentonite

Bentonite was crushed to obtain small pieces, which have diameter less than 45 µm, followed by washing with concentrated hydrochloric acid at boiling point for 30 hours to remove soluble oxides especially iron oxide. Then it was washed several times with distilled water and dried at 120˚C for 3 hours (B_A).

Chlorination Bentonite (B_A)

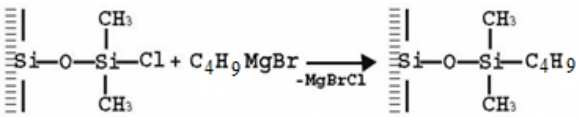
50g of treated Bentonite (B_A) is dispersed in 250 ml of dichloromethane and 10ml of dimethyldichlorosilane. The mixture is left under reflux during 3hours. The mixture is evaporated and dried at 280°C during 3hours. The chlorinated product was kept under inert atmosphere of Nitrogen (B_ACl).



Preparation of Butyl-modified Aleppo bentonite (B_AC_4)

Grignard reagent was prepared from reaction 11.0 ml of 1-Bromobutane with 2.4 g of clean and dry magnesium in 200 ml of anhydrous tetrahydroforan (THF) as according reaction*:*

The solution of Grignard reagent was added to chlorinated Bentonite (B_ACl) under inert atmosphere (N_2). The mixture was allowed to reflex for 3h. Then the heating was removed and contents were allowed to cool. The produce was filtered and washed with methanol and dried at 105 °Cfor 2hours (B_AC_4).



Preparation of TLC plates

For preparation of thin layer chromatography, 17.6 g of modified Bentonite (B_AC_4) was mixed with 1.4 g fluorescence substance (F_{254}), then the mixture was added to 40 mL water and methanol (7:1, v/v) containing 1.0 g corn starch as binder to obtain homogeneous slurry.

The slurry was spread over glass plates by an applicator, to form uniform thin layer 0.30 mm thick. The plates were dried at 105°C.

Mobile phase

The effect of mobile phase composition (Acetonitril:Water:Acetic Acid) as the follows: 39.35:59.35:1.3, 44.35:54.35:1.3, 49.35:49.35:1.3, 54.35:44.35:1.3 and 59.35:39.35:1.3 (v/v/v) were studied. It was found that, the mobile phase comprising of 49.35:49.35:1.3 (v/v/v) at pH 3.2 was better mobile phase, for using the development method.

Procedure (Chromatographic conditions)

One micro liter of standard solutions (or working solutions of pharmaceuticals) were spotted on TLC-glass plates 10×10 cm pre-coated B_AC_4 (F_{254} with 0.30 mm thickness). Mobile phase acetonitrile:water:acetic acid (49.35:49.35:1.3, v/v), at pH3.2 were used for development method, then the plates were dried at room temperature and the quantification was carried out densitometerically at λ = 260 nm. This process was repeated five times for each concentrations and calibration curves were obtained in the range 2.0-20.0 µg/spot for VAL and 1.0- 10.0 µg/spot for HCTZ.

RESULTS AND DISCUSSION

Surface Properties of B_A and B_AC_4

Surface areas of B_A and B_AC_4 were determined by the adsorption of nitrogen at 77K (BET). For determination of textural properties, the adsorption was carried out until near saturation (P/Po ≈ 1.0), then the desorption was completed until closure of the hysteresis loop. Representative adsorption-desorption isotherms of nitrogen for B_AC_4 are shown in Figure 1. The isotherms are II and IV type of SING and BDDT classifications, which indicate to presence of mesoporous structure. Application of the linear BET equation to the nitrogen adsorption data was obtained within the range of relative pressures (0.02 – 0.25) was as the follows: y=0.0258x+0.000194 and y=0.04546x+0.0042 for B_A and B_AC_4, respectively. From these plots we found that the BET surface areas (S_{BET}) was 168.1 and 88.0 m^2/g for B_A and B_AC_4, respectively. The total pore volume v_p (0.441 and 0.261 mL/g) was determined from the adsorbed volume at P/Po = 0.95 in the liquid form. The mean pore radii r_a (52.47 and 59.32 Å), was determined from the equation: $r_a=2\times10^4\times v_p/S_{BET}$. The changes of surface area, total pore volume and mean pore radii during modification can be seen from Table 1.

The surface area and the total pore volume decreased from (168.1 m^2/g and 0.441 mL/g) to (88.0 m^2/g and 0.261 mL/g), respectively. The mean pore radii increased from 52.47 to 59.32 Å.

Spectrum infrared (IR)

The infrared spectrums were Studied for each B_A and B_AC_4. New peak appears in the spectrum of B_AC_4 in the region 2800-3100 cm^{-1} back to stretch C-H Figure 2. The information provided by IR that the surface of B_AC_4 has been modified with the alkenes groups.

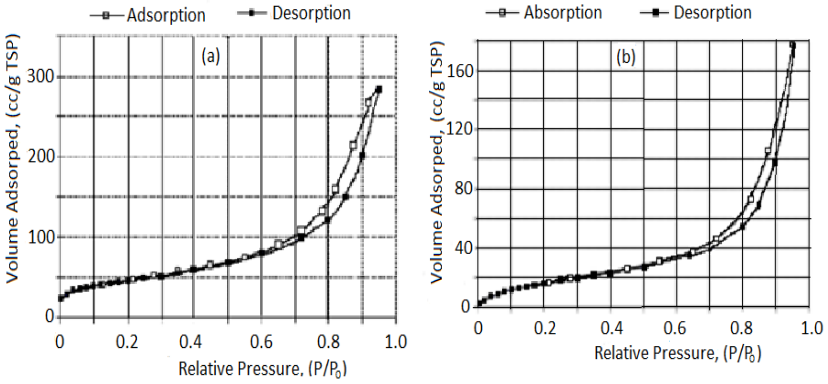


Fig. 1: Adsorption-desorption isotherm of nitrogen at 77K on B_A (a) and on B_AC_4 (b)

Table 1: Surface properties of B_A and B_AC_4

Support	S_{BET}, m^2/g	v_p, mL/g	r_a, Å
B_A	168.1	0.441	52.47
B_AC_4	88.0	0.261	59.32

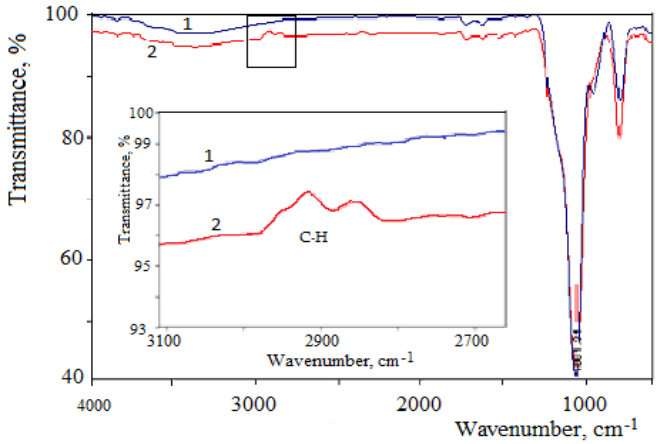


Fig. 2. IR spectra of B_A (1) and B_AC_4 (2).

Hydrophobicity

For the estimation of the changes in the hydrophobicity after modification, we compared dispersiblility of the B_A and B_AC_4 in water and benzene. As shown in Figure 3 the B_A disperses in the water layer only. Due to the presence of hydrophobic alkyl group on the external surface of B_AC_4, and the hydrophobicity of the rest the surface, the B_AC_4 was found in organic phase at the benzene-water boundary.

Chromatograms processing

The position of the spots from the front on the chromatographic plate for different concentrations 2.0 to 20.0 µg/spot of VAL and 1.0 to 10.0 µg/spot of HCTZ was studied. The retardation factors (R_f) were 0.49 and 0.78, respectively, see Figures 4 and 5.

The chromatogram of mixture of VAL and HCTZ (20.0 µg/spot of VAL and 10.0 µg/spot of HCTZ) can be observed with two peaks at different wavelengths (λ) at 200 to 300 nm. The first peak area of VAL remains constant to λ = 250 nm then decreases, where the second of HCTZ remains constant to λ = 250 nm, then sharply increases to λ = 265 nm, after that sharply decreases. Infer that, the best wavelength to determine the two material is 260 nm.

Quantitative evaluation: Summary of validation parameters as linearity range, regression equation of VAL:

y=81.514x+1.1918 (1)

and regression equation of HCTZ:

y=226.95x+1.6164 (2)

correlation coefficient (R^2): 0.9993 and 0.9995 for VAL and HCTZ, receptively, LOD, LOQ and RSD% for determination of VAL and HCTZ in pure forms by TLC-densitometric method using B_AC_4 at λ = 260 nm {mobile phase: acetonitrile:water:acetic acid (49.35:49.35:1.3, v/v/v), pH 3.2} included in Table 2.

The linear regression data for the calibration curves showed a good linear relationship and good correlation coefficient in the concentration range 2.0-20.0 µg/spot of VAL and 1.0-10.0 µg/spot of HCTZ with percent relative standard deviation (RSD%) does not exceed 3.1% and 2.0%, respectively, see Figure 6.

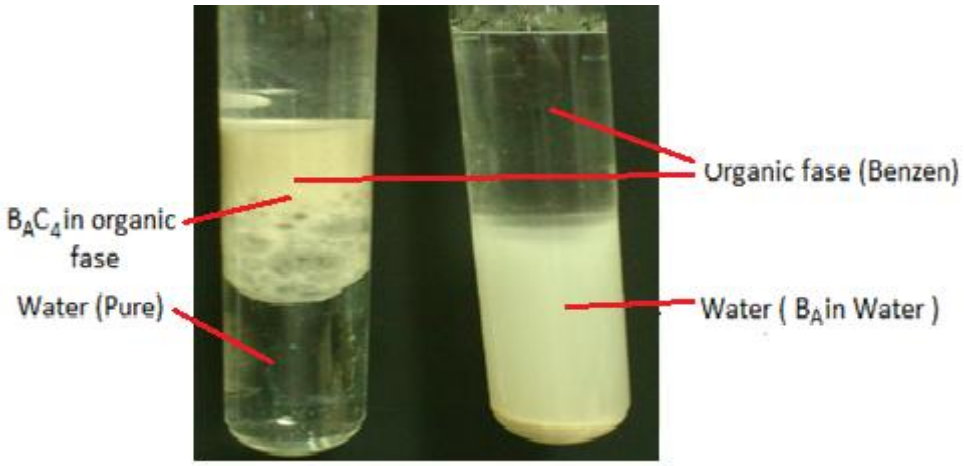


Fig. 3: B_A (right) and B_AC_4 (left) dispersed in water/benzene (organic phase) system.

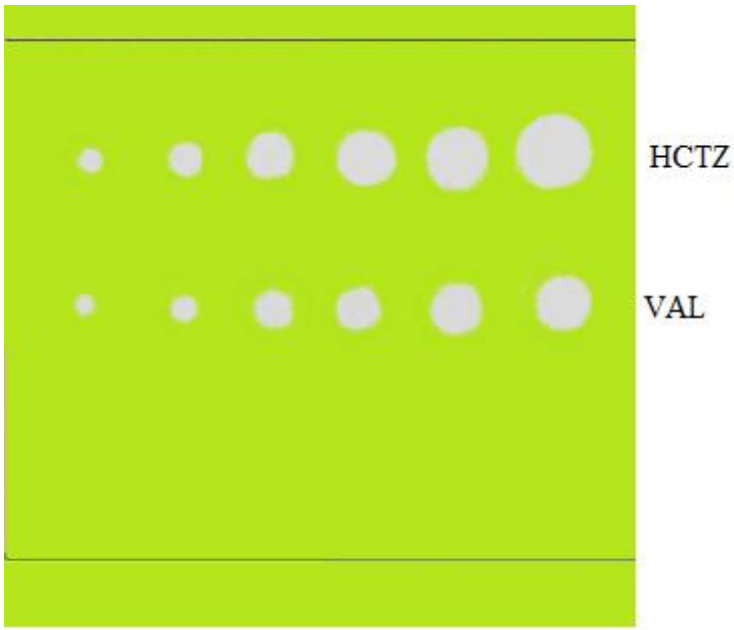


Fig. 4: TLC Plate of mixture standard VAL and HCTZ for concentrations: 2.0, 4.0, 8.0, 12.0, 16.0 and 20.0 µg/spot of VAL with 1.0, 2.0, 4.0, 6.0, 8.0 and 10.0 µg/spot of HCTZ, respectively {mobile phase: acetonitrile:water:acetic acid (49.35:49.35:1.3, v/v/v), pH 3.2}.

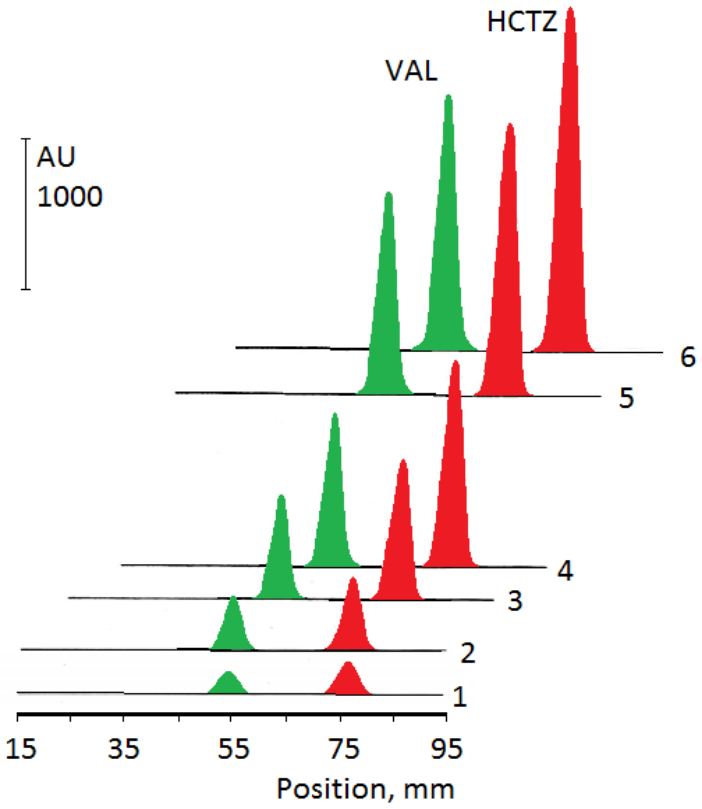


Fig. 5: The chromatograms of mixture of VAL and HCTZ disposed at concentrations: 1- 2.0 and 1.0; 2-4.0 and 2.0; 3-8.0 and 4.0; 4- 12.0 and 6.0; 5-16.0 and 8.0; 6- 20.0 and 10.0 µg/spot, respectively. {mobile phase: acetonitrile:water:acetic acid (49.35:49.35:1.3, v/v/v), pH 3.2}.

Table 2: Summary of validation parameters for determination of VAL and HCTZ in pure forms by TLC-densitometric method using B_AC_4 at λ =260 nm {mobile phase: acetonitrile:water:acetic acid (49.35:49.35:1.3, v/v), pH 3.2}.

Parameter	VAL	HCTZ
Linearity range (µg/spot)	2.0-20.0	1.0-10.0
Correlation coefficient (R^2)	0.9993	0.9995
Regression equation:		
Slope	81.514	226.95
Intercept	1.1918	1.6164
Limit of detection (µg/spot)	0.20	0.066
Limit of quantification (µg/spot)	0.61	0.20
RSD%	3.1	2.0

Validation parameters determination of VAL and HCTZ in pure forms by TLC-densitometric method using B_AC_4 at λ =260 nm with mobile phase: acetonitrile:water:acetic acid (49.35:49.35:1.3, v/v), at pH 3.2 are included in Table 3. The LOD and LOQ were found to be 0.20 and 0.61 µg/spot for VAL and 0.066 and 0.20 µg/spot for HCTZ, respectively.

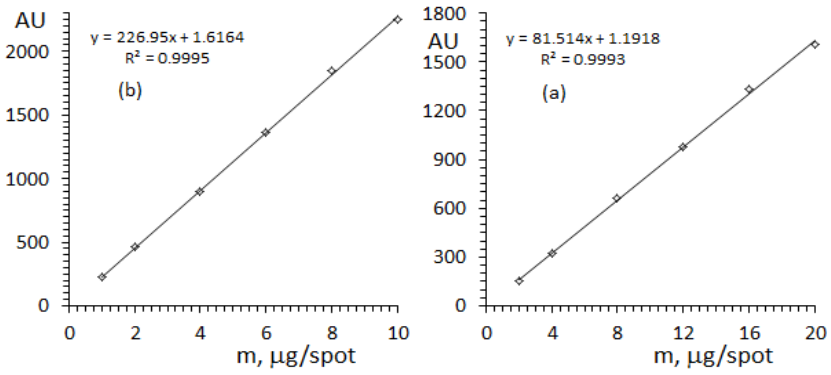


Fig. 5: Calibration curves for determination of VAL (a) and HCTZ (b) in pure forms by TLC-densitometric method using B_AC_4 at λ = 260nm {mobile phase: acetonitrile:water:acetic acid (49.35:49.35:1.3, v/v), pH 3.2 }.

Table 3: Determination of VAL and HCTZ in pure forms by TLC-densitometric method using B_AC_4 at λ =260 nm{mobile phase: acetonitrile:water:acetic acid (49.35:49.35:1.3, v/v), pH 3.2}.

Taken standard m, µg/spot	Material	Found $\overline{m} *\pm$ SD, µg/spot	RSD%	$\frac{SD}{\sqrt{n}}$, µg/spot	$\overline{m} \pm \frac{SD}{\sqrt{n}} \times t$, µg/spot	Recovery%
2.00	VAL	2.00±0.061	3.1	0.027	2.00±0.074	100.0
1.00	HCTZ	0.984±0.020	2.0	0.009	0.984±0.024	98.4
4.00	VAL	3.97±0.12	3.0	0.053	3.97±0.147	99.5
2.00	HCTZ	2.02±0.040	2.0	0.018	2.02± 0.050	101.0
8.00	VAL	8.08±0.23	2.9	0.103	8.08±0.286	101.1
4.00	HCTZ	3.96±0.076	1.9	0.034	3.96±0.094	99.0
12.00	VAL	12.01±0.34	2.8	0.152	12.01±0.422	100.1
6.00	HCTZ	5.99±0.113	1.9	0.051	5.99±0.142	99.8
16.00	VAL	16.30±0.43	2.7	0.192	16.30±0.532	101.9
8.00	HCTZ	8.14±0.14	1.8	0.063	8.14±0.174	101.8
20.00	VAL	19.74±0.56	2.8	0.250	19.74±0.694	98.7
10.00	HCTZ	9.91±0.18	1.8	0.081	9.91±0.225	99.1

* n=5, t=2.776.

APPLICATIONS

Analysis of VAL and HCTZ in tablet dosage form

Many applications for the determination of VAL and HCTZ in some pharmaceutical preparations with a TLC method using new butyl-modified Aleppo bentonite (B_AC_4) and mobile phase of acetonitrile:water:acetic acid (49.35:49.35:1.3, v/v), at pH 3.2 and at wavelength λ= 260 nm were proposed.

Sample preparation

A commercial formulations (tablet) were used for the analysis of VAL and HCTZ by using TLC method. The following commercial formulations were subjected to the analytical procedures:

(1) **Valsartan HCT (80/12.5 tablet)**, Ibn Alhaytham pharma.Industries co., Aleppo – Syria, each tablet contains: 80 mg VAL and 12.5 mg HCTZ.

(2) **Valsartan HCT (160/12.5 tablet)**, Ibn Alhaytham pharma.Industries co., Aleppo –Syria, each tablet contains: 160 mg VAL and 12.5 mg HCTZ.

(3) **Valsartan HCT (160/25 tablet)**, Ibn Alhaytham pharma.Industries co., Aleppo – Syria, each tablet contains: 160 mg VAL and 25 mg HCTZ.

(4) **Valsartan plus (80/12.5 tablet)**, Asia pharmaceutical Industries, Aleppo – Syria, each tablet contains: 80 mg VAL and 12.5 mg HCTZ.

(5) **Valsartan plus (160/12.5 tablet)**, Asia pharmaceutical Industries, Aleppo – Syria, each tablet contains: 160 mg VAL and 12.5 mg HCTZ.

(6) **Vartan HCT(80/12.5 tablet)**, K.C. Pharma. for pharmaceutical Industry, Aleppo –Syria each tablet contains: 80 mg VAL and 12.5 mg HCTZ.

(7) **Vartan HCT(160/12.5 tablet)**, K.C. Pharma. for pharmaceutical Industry, Aleppo–Syria, each tablet contains: 160 mg VAL and 12.5 mg HCTZ**.**

Working solutions of pharmaceutical formulations

Crushed ten tablets {80 mg/tab VAL and 12.5 mg/tab HCTZ (type 1) or 160 mg/tab VAL and 12.5 mg/tab HCTZ (type 2) or 160 mg/tab VAL and 25 mg/tab HCTZ (type 3)} of each studied pharmaceutical formulations, mixed well and the average two tablets weight determined, solved it in 20 ml methanol by using ultrasonic, filtered over a 25 mL flask and diluting to 25 mL with methanol. The working solutions content: 6.40 $mg.mL^{-1}$of VAL and 1.00 $mg.mL^{-1}$ of HCTZ (S_1) or 12.8 $mg.mL^{-1}$ of VAL and 1.00 $mg.mL^{-1}$ of HCTZ (S_2) or 12.8 $mg.mL^{-1}$ of VAL and 2.00 $mg.mL^{-1}$ of HCTZ (S_3) for mentioned pharmaceuticals.

Regression equations and correlation coefficients were included in Table 4. Standard curves for determination of VAL and HCTZ in different pharmaceutical preparations were used. The amount (m) of VAL and HCTZ in one tablet calculated from the following relationship:

$$m = h.\ m' \quad (3)$$

where: m' is the amount of VAL or HCTZ in different working solutions of pharmaceutical formulations (by µg/spot) calculated from the standard curve according to the regression equations (1) and (2) for VAL and HCTZ, respectively, (m'= x), h conversion factor is equal to 12.5. The results of quantitative analysis for VAL and HCTZ in some pharmaceutical preparations were calculated using the standard curve were summarized in Tables 5. The proposed method was simple, economic, accurate and successfully applied to the determination of VAL and HCTZ in pharmaceuticals with average recovery of 97.9 to 102.4%, the results obtained agree well with the contents stated on the labels. The results obtained by this method were validated by HPLC**[22]**.

Table 4: Regression equations and correlation coefficients for determination of VAL and HCTZ in tablet by TLC-densitometric method using B_AC_4 at λ = 260 nm {mobile phase: acetonitrile:water:acetic acid (49.35:49.35:1.3, v/v), pH 3.2}.

Product	Drug	h	m', µg/spot	Amount of VAL or HCTZ (m), mg/tab
Valsartan HCT (80/12.5 tablet)	VAL	12.5	6.528	$m_{VAL/tab}$ =12.5m'=**81.60**
	HCTZ	12.5	0.986	$m_{HCTZ/tab}$ =12.5m'=**12.24**
Valsartan HCT (160/12.5 tablet)	VAL	12.5	13.005	$m_{VAL/tab}$ =12.5m'=**162.56**
	HCTZ	12.5	0.993	$m_{HCTZ/tab}$ =12.5m'=**12.41**
Valsartan HCT (160/25 tablet)	VAL	12.5	13.11	$m_{VAL/tab}$ =12.5m'=**163.84**
	HCTZ	12.5	1.964	$m_{HCTZ/tab}$ =12.5m'=**24.55**
Valsartan plus (80/12.5 tablet)	VAL	12.5	6.534	$m_{VAL/tab}$ =12.5m'=**81.68**
	HCTZ	12.5	0.991	$m_{HCTZ/tab}$ =12.5m'=**12.39**
Valsartan plus (160/12.5 tablet)	VAL	12.5	12.57	$m_{VAL/tab}$ =12.5m'=**157.12**
	HCTZ	12.5	0.981	$m_{HCTZ/tab}$ =12.5m'=**12.26**
Vartan HCT(80/12.5 tablet)	VAL	12.5	6.278	$m_{VAL/tab}$ =12.5m'=**78.48**
	HCTZ	12.5	1.014	$m_{HCTZ/tab}$ =12.5m'=**12.68**
Vartan HCT(160/12.5 tablet)	VAL	12.5	12.54	$m_{VAL/tab}$ =12.5m'=**156.80**
	HCTZ	12.5	1.010	$m_{HCTZ/tab}$=12.5m'=**12.63**

Table 5: Determination of VAL and HCTZ in tablets by TLC-densitometric method using B_AC_4 at λ = 260 nm {mobile phase: acetonitrile:water:acetic acid (49.35:49.35:1.3, v/v), pH 3.2}.

Product	Compound and dose	Found $\overline{m} * \pm$ SD	RSD%	$\frac{SD}{\sqrt{n}}$	$\overline{m} \pm \frac{SD}{\sqrt{n}}{}_{x\,t}$	Recovery%
Valsartan HCT (80/12.5 tablet)	VAL 80 mg/tab.	81.60 ± 2.45	3.0	1.094	81.60 ± 3.036	102.0
	HCTZ 12.5 mg/tab.	12.24 ± 0.257	2.1	0.115	12.24 ± 0.318	97.9
Valsartan HCT (160/12.5 tablet)	VAL 160 mg/tab.	162.56 ± 4.71	2.9	2.103	162.56 ± 5.837	101.6
	HCTZ 12.5 mg/tab.	12.41 ± 0.273	2.2	0.122	12.41 ± 0.388	99.3
Valsartan HCT (160/25 tablet)	VAL 160 mg/tab.	163.84 ± 4.91	3.0	2.202	163.84 ± 6.112	102.4
	HCTZ 25 mg/tab.	24.55 ± 0.491	2.0	0.220	24.55 ± 0.611	98.2
Valsartan plus (80/12.5 tablet)	VAL 80 mg/tab.	81.68 ± 2.46	3.0	1.103	81.68 ± 3.062	102.1
	HCTZ 12.5 mg/tab.	12.39 ± 0.248	2.0	0.111	12.39 ± 0.309	99.1
Valsartan plus (160/12.5 tablet)	VAL 160 mg/tab.	157.12 ± 4.56	2.9	2.045	157.12 ± 5.677	98.2
	HCTZ 12.5 mg/tab.	12.26 ± 0.257	2.1	0.115	12.26 ± 0.320	98.1
Vartan HCT (80/12.5 tablet)	VAL 80 mg/tab.	78.48 ± 2.35	3.0	1.054	78.48 ± 2.909	98.1
	HCTZ 12.5 mg/tab.	12.68 ± 0.254	2.0	0.114	12.68 ± 0.316	101.4
Vartan HCT (160/12.5 tablet)	VAL 160 mg/tab.	156.80 ± 4.55	2.9	2.040	156.80 ± 5.663	98.0
	HCTZ 12.5 mg/tab.	12.63 ± 0.265	2.1	0.119	12.63 ± 0.330	101.0

* n=5, t=2.776

CONCLUSION

TLC simultaneous determination of valsartan (VAL) and hydrochlorothiazide (HCTZ) in pure form and in tablets using new C_4-modified aleppo bentonite (B_AC_4) with mobile phase of acetonitrile:water:acetic acid (49.35:49.35:1.3, v/v), at pH 3.2 and at wavelength λ= 260 nm was developed. The particles of Aleppo Bentonite which have diameter less than 45 μm were treated by concentrated HCl (B_A), after that grafted firstly by dimethyldichlorosilane, then secondly by Grignard reagent (butylmagnesium bromide). The surface properties of butyl-modified Bentonite were studied by nitrogen adsorption at 77K. The retardation factors (R_f) of valsartan and hydrochlorothiazide were 0.49 and 0.78, respectively. Linearity for determination of VAL and HCTZ was in the range 2.00-20.00 and 1.00-10.00 μg/spot, respectively. The minimum determined concentration was 2.0 μg/spot for VAL and 1.0 μg/spot for HCTZ with percent relative standard deviation (RSD%) does not exceed 3.1% and 2.0%, respectively. The limits of quantification (LOQ) were 0.61 and 0.20 μg/spot, and the limits of detection (LOD) were 0.20 and 0.066 μg/spot for determination of VAL and HCTZ, respectively. The proposed method was novel, simple, accurate and successfully applied to simultaneous determination of VAL and HCTZ in pharmaceuticals with average recovery of 97.9 to 102.4%, the results obtained agree well with the contents stated on the labels.

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