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Original Article

# TLC SIMULTANEOUS DETERMINATION OF AMLODIPINE, ATORVASTATIN, ROSUVASTATIN AND VALSARTAN IN PURE FORM AND IN TABLETS USING PHENYL-MODIFIED ALEPPO BENTONITE

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**ABSTRACT**

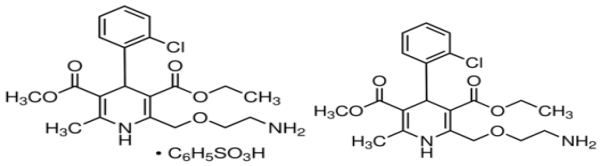
TLC simultaneous determination of amlodipine (AMD), atorvastatin (AT), rosuvastatin (RSV) and valsartan (VAL) in pure form and in tablets using phenyl-modified aleppo bentonite ($B_A$Phenyl) at λ=245 nm (for AT, RSV and VAL), and at λ=365 nm (for AMD) using Acetonitrile:Buffer(0.025M of $NaH_2PO_4.2H_2O$ in water) 45:55, v/v (as mobile phase) at pH 6.0 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 (phenylmagnesium bromide). The surface properties of phenyl-modified bentonite were studied by nitrogen adsorption at 77K. The retardation Factors ($R_f$) of AMD, AT, RSV and VAL were 0.27, 0.41, 0.62 and 0.78, respectively. Linearity for determination of AMD, AT, RSV and VAL was in the range 0.50-10.00 for AMD and 1.00-20.00 µg/spot AT, RSV and VAL. The minimum determined concentration was 0.5 µg/spot for AMD and 1.0 µg/spot for AT, RSV and VAL with percent relative standard deviation (RSD%) 4.0%, 3.8%, 3.2% and 4.2%, respectively. The limits of detection (LOD) and the limits of quantification (LOQ) were found to be 0.063 and 0.19, 0.125 and 0.38, 0.106 and 0.32, 0.142 and 0.43 µg/spot for AMD, AT, RSV and VAL, respectively. The proposed method was novel, simple, accurate and successfully applied to simultaneous determination of AMD, AT, RSV and VAL in pure form and pharmaceuticals with average recovery of 95.2 to 104.4%, the results obtained agree well with the contents stated on the labels.

**Keywords:** Phenyl-modified Aleppo Bentonite; TLC; Amlodipine (AMD); Atorvastatin (AT); Rosuvastatin (RSV); Valsartan (VAL).

## INTRODUCTION

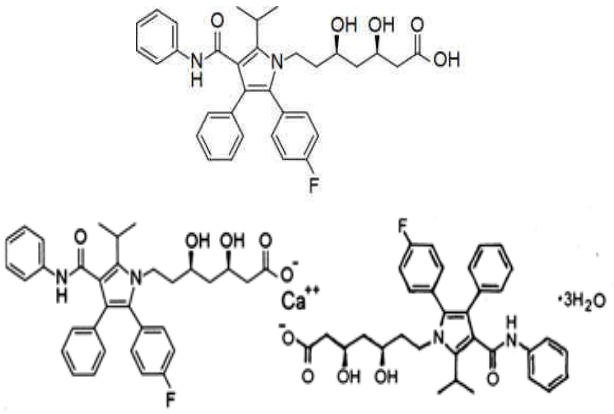
Aleppo Bentonite is rocky clay which consists of 47% $SiO_2$, 14.4% $Al_2O_3$ and some other oxides as $Fe_2O_3$, MgO, CaO, $Na_2O$[1,2] and others. The thermal treatment causes decreasing of its specific surface area with increasing in the temperature of thermal treatment[3,4]. Bentonite clays are used 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, vitamins $B_1$, $B_6$, $B_{12}$, Valsartan with Hydrochlorothiazide and Losartan with Hydrochlorothiazide [8-12]. Amlodipine Besylate ($C_{20}H_{25}ClN_2O_5$. $C_6H_5SO_3H$), chemically described as (R, S) 3- ethyl-5 -methyl-2-(2-aminoethoxymethyl) - 4 - (2-chlorophenyl) - 1, 4- dihydro - 6 -methyl-3,5-pyridinedicarboxylate benzenesulfonate; where the empirical formula of amlodipine (AMD) is ($C_{20}H_{25}ClN_2O_5$), mol. mass is 408.879 g, (Scheme 1), is a long acting calcium channel blocker (dihydropyridine class) used as antihypertensive. Amlodipine besylate is a white crystalline powder with a mol. mass of 567.1 g. It is slightly soluble in water and sparingly soluble in ethanol [13]. Atorvastatin calcium [14-15] is a calcium (bR, dR)-2-(r-fluorophenyl)-b,ddihydroxy-5-isopropyl-3-phenyl-4-(phenylcarbamoyl)pyrrole-1-hepatanoicacid (1:2) trihydrate. The empirical formula of atorvastatin calcium trihydrate is $C_{66}H_{68}CaF_2N_4O_{10}.3H_2O$ or $(C_{33}H_{34}FN_2O_5)_2Ca.3H_2O$, mol. mass is 1209.4 g; where the empirical formula of atorvastatin (AT) is $C_{33}H_{35}FN_2O_5$, mol. mass is 558.64 g (Scheme 2). Atorvastatin is a member of the drug class known as statins, used for lowering blood cholesterol [15-17].

Rosuvastatin calcium (RSV) $C_{44}H_{54}CaF_2N_6O_{12}S_2$ or $(C_{22}H_{27}FN_3O_6S)_2Ca$, a member of the class of statins, is the calcium salt of (E)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl (methylsulfonyl) amino] pyrimidin-5-yl] (3R,5S)-3,5-dihydroxyhept-6-enoic acid. Rosuvastatin is used to treat hypercholesterolemia and related conditions and to prevent cardiovascular disease. Rosuvastatin acts by inhibiting the activity of 3-hydroxy-3- methylglutaryl coenzyme A (HMG_CoA) reductase, the rate_limiting enzyme that converts 3-hydroxy-3-methylglutaryl coenzyme A to Mevalonate, a precursor of cholesterol, mol. mass 1001.14 g, while rosuvastatin (RSV) is $C_{22}H_{28}FN_3O_6S$ and its mol. mass is 481.539 g (Scheme3) [18-20].



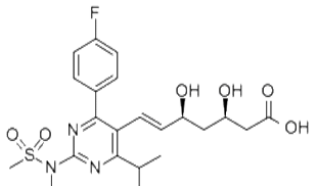
**Amlodipine Amlodipine Besylate**

**Scheme 1: Chemical structure of Amlodipine (Amlodipine Besylate).**

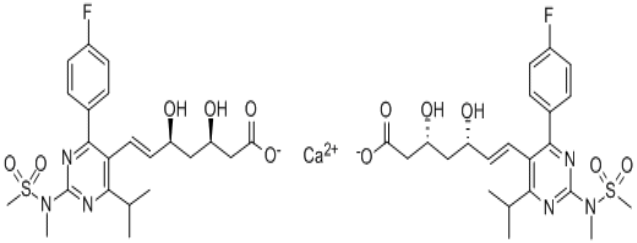


**Atorvastatin, Atorvastatin calcium**

**Scheme 2: Chemical structure of Atorvastatin and atorvastatin calcium trihydrate.**



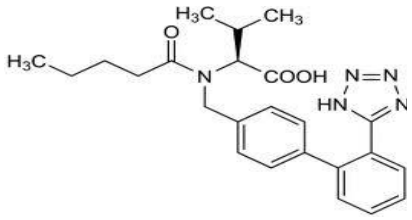
**Rosuvastatin $C_{22}H_{28}FN_3O_6S$**



**Rosuvastatin calcium$(C_{22}H_{27}FN_3O_6S)_2Ca$**

**Scheme 3: Chemical structure of rosuvastatin and rosuvastatin calcium.**

Valsartan ($C_{24}H_{29}N_5O_3$) 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 is 435.519 g [21-25], (Scheme 4).



**Valsartan**

**Scheme 4: Chemical structure of Valsartan.**

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/v), at pH 3.2 and at wavelength λ= 260 nm was developed. The particles of Aleppo Bentonite which have diameter less than 45 □ m 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 [11]. Simultaneous determination of losartan potassium (LOS) and hydrochlorothiazide (HCTZ) in pure form and in tablets by TLC using new butyl-modified Aleppo Bentonite ($B_AC_4$) by Grignard reagent with mobile phase of acetonitrile: water: acetic acid (44.35:54.35:1.3, v/v/v), at pH 3.2 and λ= 265 nm was developed. The surface properties of butyl-modified Bentonite were studied by nitrogen adsorption at 77K. The retardation factors ($R_f$) of LOS and HCTZ were 0.34 and 0.73, respectively. Linearity for determining of LOS 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 LOS and 1.0 µg/spot for HCTZ with percent relative standard deviation (RSD%) not exceed 3.0% and 1.8%, respectively. The limits of quantification (LOQ) were 0.60 and 0.18µg/spot, and the limits of detection (LOD) were 0.198 and 0.059 µg/spot for determining of LOS and HCTZ, respectively. The proposed method was novel, simple, accurate and successfully applied to simultaneous determination of LOS and HCTZ in pharmaceuticals with percentage of 98.6 to 104.8%. The results obtained agree well with the contents stated on the labels [12].

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/spot for valsartan and 125–875 ng/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[26].

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[9,27]. A simple, rapid, selective, and precise densitometric TLC method for simultaneous analysis of atorvastatin calcium and fenofibrate in pharmaceutical dosage forms has been established, and validated in accordance with ICH guidelines. Good resolution of atorvastatin calcium ($R_F$ 0.36 ± 0.02) and fenofibrate ($R_F$ 0.84 ± 0.02) was achieved on aluminum foil silica gel 60 $F_{254}$ plates with toluene:methanol:triethylamine 7:3:0.2 (v/v/v) as mobile phase. Detection and quantification were performed densitometrically at 258 nm. Polynomial regression data for the calibration plots showed there was a good linear relationship between response and amount in the range 100–800 ng per band for atorvastatin calcium ($R^2$ = 0.9983) and 1000–8000 ng per band for fenofibrate ($R^2$ = 0.9982). The method was validated for precision accuracy, ruggedness, and recovery. Statistical analysis proved the method enables repeatable, selective, and accurate analysis of the drugs. The method can be used for identification and quantitative analysis of atorvastatin calcium and fenofibrate in the bulk drug and in dosage forms [28].

A simple, precise, and accurate HPTLC method for simultaneous quantification of atorvastatin calcium and ezetimibe as the bulk drug and in tablet dosage forms was described. Chromatographic separation of the drugs was performed on aluminium plates precoated with silica gel 60 $F_{254}$, with toluene–methanol 8:2 (v/v) as mobile phase. Densitometric evaluation of the separated zones was performed at 240 nm. The two drugs were satisfactorily resolved with $R_F$ values 0.23 ± 0.01 and 0.39 ± 0.01 for atorvastatin calcium and ezetimibe, respectively. The accuracy and reliability of the

method was assessed by evaluation of linearity (0.4–2.4 µg/zone for both atorvastatin calcium and ezetimibe). The method can be used for analysis of ten or more formulations on a single plate and is a rapid and cost-effective quality-control tool for routine simultaneous analysis of atorvastatin calcium and ezetimibe as the bulk drug and in tablet formulations [29].

This paper described validated high performance liquid chromatographic (HPTLC) method for estimation of Rosuvastatin Calcium (RSV) and Ezetimibe (EZE) in tablet dosage form. The method involved separation of components by TLC on a precoated silica gel 60 $F_{254}$ using a mixture of n-butanol: methanol (3:1) as a mobile phase. Detection of spots was carried out at 274 nm and 230 nm for Rosuvastatin Calcium and Ezetimibe combinations, respectively. The mean retardation factor for Rosuvastatin Calcium and Ezetimibe were found to be 0.90 ±0.01, 0.82±0.05, respectively. The linearity and range was 0.1 to 0.5 µg/spot for two drugs. The method was validated for precision, accuracy and reproducibility [30].

A normal-phase TLC method has been established for the estimation of rosuvastatin calcium in its bulk drug and pharmaceutical formulations. Analysis was performed on silica gel $60F_{254}$ HPTLC plates. Aceclofenac was used as internal standard. The optimized mobile phase was toluene:methanol:ethyl acetate:formic acid, 6.0:1.0:3.0:0.1 (v/v/v/v). Quantitation was performed densitometrically at λ = 265 nm. A good correlation coefficient ($R^2$ = 0.9999) was obtained for the linearity for amounts of sample in the range 1.0 to 15.0 µg/spot. The accuracy of the method was found to be 100.62% and precision was found to be vary from 0.01% to 0.77%. The procedure was simple and rapid and the results were reliable [31].

In the present work, TLC simultaneous determination of amlodipine, atorvastatin, rosuvastatin and valsartan in pure form and in tablets using phenyl-modified aleppo bentonite ($B_A$Phenyl) was developed.

## MATERIALS AND METHODS

### 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), differential thermal analysis (DTA), LINSEIS type STA PT-1600, Germany 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

Amlodipine besylate pure substances were purchased from Sigma-Aldrich (St. Louis, MO, USA), its purity as amlodipine besylate was 98.0% and as amlodipine was 70.65%. Atorvastatin calcium trihydrate was supplied by ind-swift (India), its purity as atorvastatin was 92.0 %. Rosuvastatin calcium (98.6%) was supplied by BDR Pharmaceuticals International PVT. LTD. (INDIA), its purity as rosuvastatin was 94.66%. Valsartan (VAL) was purchased from Enaltec-India, its purity was (99.6%), were used. Methanol, acetonitrile, tetrahydroforan, dichloromethane, dimethyldichlorosilane, 1-bromophenyl, $NaH_2PO_4.2H_2O$, magnesium metal and fluorescent indicator $F_{254}$ for thin layer chromatography were purchased from Merck, Germany.

### A **stock solutions of pharmaceuticals**

An accurately weighed 1.4154, 1.0870, 1.0564 and 1.0040 g standard sample of AMD, AT, RSV and VAL were dissolved in methanol, transferred into a 50 mL standard flask and diluted to the mark with methanol to obtain 20.00 $mg.mL^{-1}$ of AMD, AT, RSV and VAL, stock solutions of pharmaceuticals.

### Standard solutions

Volumes 0.250, 0.500, 1.000, 2.000, 3.000, 4.000, 5.000, 6.000 and 8.000 mL from stock solutions of pharmaceuticals were transferred into volumetric flasks (10 mL), respectively and completed to the mark with methanol (these solutions content: 0.50, 1.00, 2.00, 4.00, 6.00, 8.00, 10.00, 12.00, 16.00 and 20.00 $mg.mL^{-1}$ of each of AMD, AT, RSV and VAL).

### Sample preparations

A commercial formulations (as tablet) were used for the analysis of AMD, AT, RSV and VAL by using TLC developed method. The pharmaceutical formulations were subjected to the analytical procedures:

(1) ***Caduet*** 5/10 tablets, **PFIZER** Inc. USA, each tablet contains: 5 mg AMD and 10 mg AT.

(2) ***Caduet*** 5/20 tablets, **PFIZER** Inc. USA, each tablet contains: 5 mg AMD and 20 mg AT.

(3) ***Caduet*** 10/20 tablets, **PFIZER** Inc. USA, each tablet contains: 10 mg AMD and 20 mg AT.

(4) ***EXzar*** 10/160 tablets, **Asia** pharmaceutical industries- Aleppo – SYRIA, each tablet contains: 10 mg AMD and 160 VAL.

(5) ***EXzar*** 5/80 tablets, **Asia** pharmaceutical industries- Aleppo – SYRIA, each tablet contains: 5 mg AMD and 80 VAL.

(6) ***Norvek*** tablets, **ELSaad** pharma, Aleppo–SYRIA, each tablet contains: 5 mg AMD.

(7) ***Atorvex*** tablets, **Asia** pharmaceutical industries, Aleppo–SYRIA, each tablet contains: 10, 20 and 40 mg of AT.

(8) ***Atorvatin*** tablets, **Alpha**, Aleppo pharmaceutical industries, Aleppo–SYRIA, Each tablet contains: 10, 20 and 40 mg of AT**.**

(9) ***Lipito-med*** tablets, **Medico** labs., Homs–SYRIA, Each tablet contains:10, 20 and 40 mg of AT.

(10) ***Lipostatin*** tablets, **Ibn Al-Haytham** Pharma Industries Co., Aleppo–SYRIA, each tablet contains: 10, 20 and 40 mg of AT.

(11) ***Atoraz*** tablets, **Razi** pharmaceutical industries, Aleppo–SYRIA, each tablet contains: 10, 20 and 40 mg of AT.

(12) ***Rosuvastatin***-ElSaad tablets, **ELSaad** pharma, Aleppo–SYRIA, each tablet contains: 10, 20 and 40 mg of RSV.

(13) ***Rosuva*** tablets, **Unipharma**, Damascus–SYRIA, Each tablet contains: 5, 10 and 20 mg of RSV**.**

(14) ***Rosuvastatin Sandy*** tablets, **Sandy** pharmaceuticals, Aleppo – SYRIA, Each tablet contains:10, 20 and 40 mg of RSV.

(15) ***Turbovas*** tablets, **City Pharma Co.**, Aleppo–SYRIA, each tablet contains: 10 and 20 mg of RSV.

(16) ***Crostatin*** tablets, **Razi** pharmaceutical industries, Aleppo–SYRIA, each tablet contains: 5, 10 and 20 mg of RSV.

### Working solutions of pharmaceuticals

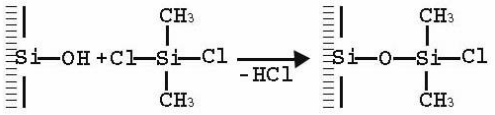
Ten tablets of each studied pharmaceutical formulations were weighted accurately, crushed to a fine powder and mixed well. Equivalent weight of one tablet, was solved in 8 ml methanol by using ultrasonic, filtered over a 10 mL flask and diluting to 10 mL with methanol.

### 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 ($B_A$).

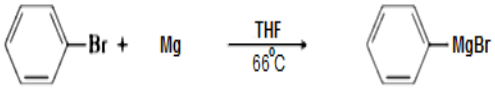
**Chlorination Bentonite ($B_A$)**

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 ($B_A$Cl).

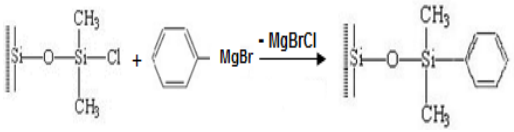



**Preparation of Phenyl-modified Aleppo bentonite ($B_A$Phenyl)**

Grignard reagent was prepared from reaction 10.76 ml of 1-Bromophenyl (its purity 98.0%) 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 ($B_A$Cl) 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°C for 2 hours ($B_A$Phenyl).




**Preparation of TLC plates**

For preparation of thin layer chromatography, 17.6 g of modified Bentonite ($B_A$Phenyl) 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 (Acetonitrile:Buffer; where buffer is 0.025M of $NaH_2PO_4.2H_2O$ in water) at pH 6.0 as the follows: 40:60, 45:55, 50:50 and 55:45 (v/v) were studied. It was found that, the mobile phase comprising of 45:55 (v/v) was better mobile phase, for using the development method.

**Wavelength scan**

The solution of AMD, AT, RSV and VAL of strength 1.5, 4.0, 4.0 and 5.0 $\mu g.mL^{-1}$, respectively, was prepared in methanol and scanned for dictating the maximum absorbance wavelength. The maximum absorbance of AT, RSV and VAL was found around 245nm and of AMD around 365 nm, see Figure 1.

**Procedure (Chromatographic conditions)**

One micro liter of standard solutions or working solutions of pharmaceuticals dose ≥20 mg/tab and 5µL for dose 5 and 10 mg/tab were spotted on TLC-glass plates 10×10 cm pre-coated $B_A$Phenyl ($F_{254}$ with 0.30 mm thickness). Mobile phase was used for development method, then the plates were dried at room temperature and the quantification was carried out densitometerically at λ= 245 nm for determination of AT, RSV and VAL, and at λ= 365 nm for determination of AMD, see Figure 2. This process was repeated five times for each concentrations and calibration curves were obtained in the range 1.0-20.0 µg/spot for AT, RSV and VAL and 0.5-10.0 µg/spot for AMD.

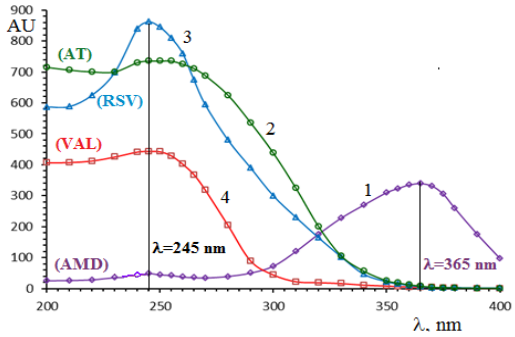



**Fig. 1: The effect of wavelength scan on dictating the maximum absorbance: 1-AMD 1.5 µg/spot, 2- AT 4.0 µg/spot, 3- RSV 4.0 µg/spot, 4- VAL 5.0 µg/spot using Acetonitrile:Buffer 45:55, v/v, as mobile phase (pH 6.0).**

**RESULTS AND DISCUSSION**

**Surface properties of $B_A$ and $B_A$ phenyl**

Surface areas of $B_A$ and $B_A$ Phenyl 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 $B_A$ Phenyl are shown in Figure 3. 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=0.0256x+0.00022 and y=0.05267x+0.0046 for $B_A$ and $B_A$Phenyl, respectively. From these plots we found that the BET surface areas ($S_{BET}$) was 169.2 and 76.3 $m^2/g$ for $B_A$ and $B_A$Phenyl, respectively. The total pore volume $v_p$ ( 0.442 and 0.250

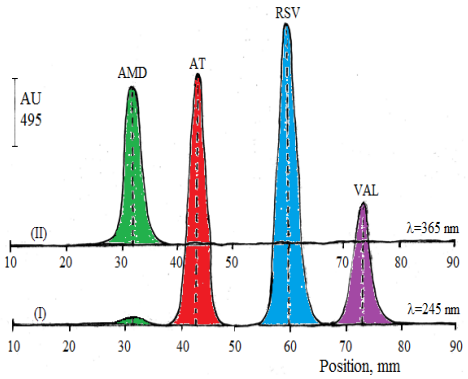



**Fig. 2: The chromatograms of mixture of AMD, AT, RSV and VAL disposed at concentrations: 10.0 µg/spot for AT, RSV and VAL, and 5.0 µg/spot for AMD were scanned for dictating the maximum absorbance wavelength at λ =245 nm (I) and at λ =365 nm (II) using Acetonitrile:Buffer 45:55, v/v, as mobile phase(pH 6.0).**

mL/g) was determined from the adsorbed volume at P/Po = 0.95 in the liquid form. The mean pore radii $r_a$ (52.25 and 65.53 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

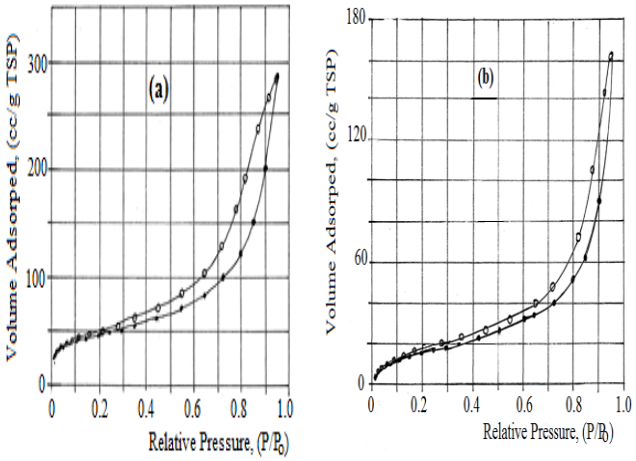



**Fig. 3: Adsorption-desorption isotherm of nitrogen at 77K on $B_A$ (a) and on $B_A$Phenyl (b)**

The surface area and the total pore volume decreased from (169.02 $m^2$/g and 0.442 mL/g) to (76.3 $m^2$/g and 0.250 mL/g), respectively. The mean pore radii increased from 52.25 to 65.53 Å.

**Differential Thermal Analysis (DTA)**

A useful method for the characterization of $B_A$ and $B_A$Phenyl was measured by differential thermal analysis (DTA ) technique in air atmosphere using 40 mg bentonite with α-$Al_2O_3$ as reference and heating rate 10°C/min. Figure 4 shows that, the DTA trace of the $B_A$ and $B_A$Phenyl. It exhibited two endothermic peaks.

The first appears at 145°C, which is corresponding to the loss of water of hydration. The second, which occurred at about 610°C, is related to the burning of hydrocarbon (Phenyl) in $B_A$Phenyl support.

**Hydrophobicity**

For the estimation of the changes in the hydrophobicity after modification, we compared dispersiblity of the $B_A$ and $B_A$Phenyl in water and benzene. The $B_A$ disperses in the water layer only. Due to the presence of hydrophobic phenyl group on the external surface of $B_A$Phenyl, and the hydrophobicity of the rest the surface, the $B_A$Phenyl was found in organic phase at the benzene-water boundary.

**Table 1: Surface properties of $B_A$ and $B_A$ Phenyl**

| Support | $S_{BET}$, $m^2$/g | $v_p$, mL/g | $r_a$, Å |
|---|---|---|---|
| $B_A$ | 169.2 | 0.442 | 52.25 |
| $B_A$Phenyl | 76.3 | 0.250 | 65.53 |

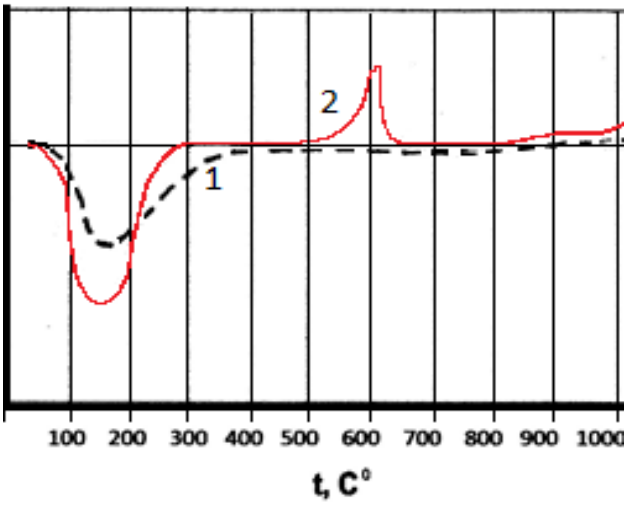



**Fig. 4: Differential thermal analysis curve for $B_A$ (1) and $B_A$Phenyl-modified (2) in air atmosphere using 40 mg bentonite with α-$Al_2O_3$ as reference and heating rate 10°C/min.**

**Chromatograms processing**

The position of the spots from the front on the chromatographic plate (BAPhenyl) for different concentrations 1.0 to 20.0 µg/spot of AT, RSV and VAL, and 0.5 to 10.0 µg/spot of AMD using mobile phase (Acetonitrile:Buffer 45:55, v/v) at λ=245 nm for determination of AT, RSV and VAL and λ=365 nm for determination of AMD were studied. The retardation factors (Rf) were 0.27, 0.41, 0.62 and 0.78 for AMD, AT, RSV and VAL, respectively.

**Quantitative evaluation**

Validation parameters determination of AMD, AT, RSV and VAL in pure forms by TLC-densitometric method on $B_A$Phenyl plates at λ=245 nm (for AT, RSV and VAL), and at λ=365 nm (for AMD) using mobile phase: Acetonitrile:Buffer 45:55, v/v, (pH 6.0) are included in Table 3. The LOD and LOQ were found to be 0.063 and 0.19, 0.125 and 0.38, 0.106 and 0.32, 0.142 and 0.43 µg/spot for AMD, AT, RSV and VAL, respectively. Summary of validation parameters as linearity range, regression equation and correlation coefficient ($R^2$) of AMD, AT, RSV and VAL were as the follows:

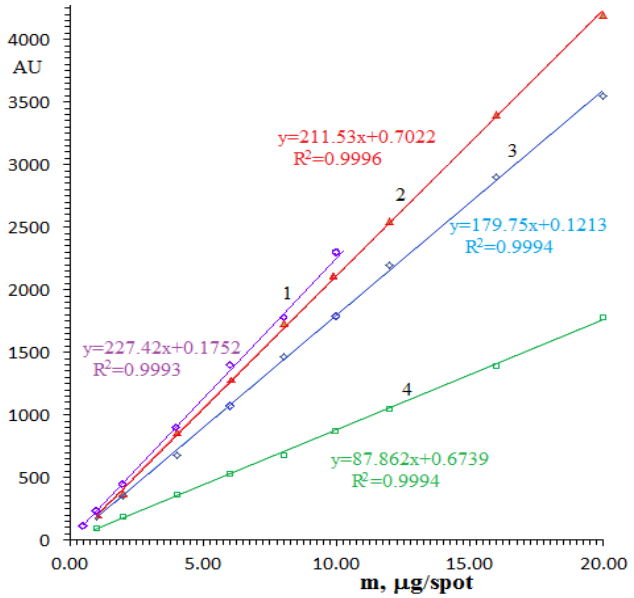



**Fig. 5: Calibration curves for determination 0.5 to 10.0 µg/spot of AMD (1) and 1.0 to 20.0 µg/spot of AT (3), RSV (2) and VAL (4) using Acetonitrile : Buffer 45:55, v/v, as mobile phase (pH 6.0) at λ=365 nm (1) and at λ=245 nm (2-4).**

**Table 2: Summary of validation parameters for determination of AMD, AT, RSV and VAL in pure forms by TLC-densitometric method on $B_A$Phenyl using the calibration curves at λ=245 nm (for AT, RSV and VAL), and at λ=365 nm (for AMD) using Acetonitrile : Buffer 45:55, v/v, as mobile phase (pH 6.0).**

| Parameter | AMD | AT | RSV | VAL |
|---|---|---|---|---|
| Linearity range (µg/spot) | 0.5-10.0 | 1.0-20.0 | 1.0-20.0 | 1.0-20.0 |
| Scanned λnm | 365 | 245 | 245 | 245 |
| Correlation coefficient ( $R^2$) | 0.9993 | 0.9994 | 0.9996 | 0.9994 |
| Regression equation: | | | | |
| Slope | 227.42 | 179.75 | 211.53 | 87.862 |
| Intercept | 0.1752 | 0.1213 | 0.7022 | 0.6739 |
| Limit of detection (µg/spot) | 0.063 | 0.125 | 0.106 | 0.142 |
| Limit of quantification (µg/spot) | 0.19 | 0.38 | 0.32 | 0.43 |
| RSD% | 4.0 | 3.8 | 3.2 | 4.2 |

**Table 3: Determination mixtures of AMD, AT, RSV and VAL in pure forms by TLC-densitometric method on $B_A$Phenyl plates at λ=245 nm (for AT, RSV and VAL), and at λ=365 nm (for AMD) using Acetonitrile : Buffer 45:55, v/v, as mobile phase pH 6.0 (n=5, t=2.776).**

| 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% |
|---|---|---|---|---|---|---|
| 0.50 | AMD | 0.48±0.019 | 4.0 | 0.008 | 0.48±0.024 | 96.0 |
| 1.00 | AMD | 1.01±0.037 | 3.7 | 0.017 | 1.01±0.046 | 101.0 |
| | AT | 0.99±0.038 | 3.8 | 0.017 | 0.99±0.047 | 99.0 |
| | RSV | 1.00±0.032 | 3.2 | 0.014 | 1.00±0.040 | 100.0 |
| | VAL | 1.02±0.043 | 4.2 | 0.019 | 1.02± 0.053 | 102.0 |
| 2.00 | AMD | 2.01±0.072 | 3.6 | 0.032 | 2.01±0.089 | 100.5 |
| | AT | 1.99±0.074 | 3.7 | 0. 033 | 1.99±0.092 | 99.5 |
| | RSV | 1.97±0.060 | 3.0 | 0.027 | 1.97±0.074 | 98.5 |
| | VAL | 2.02±0.082 | 4.1 | 0.037 | 2.02± 0.102 | 101.0 |
| 4.00 | AMD | 4.03±0.14 | 3.5 | 0.063 | 4.03±0.174 | 100.8 |
| | AT | 4.01±0.14 | 3.6 | 0.063 | 4.01±0.174 | 100.3 |
| | RSV | 3.99±0.12 | 3.0 | 0.054 | 3.99±0.149 | 99.8 |
| | VAL | 4.00±0.16 | 4.0 | 0.072 | 4.00± 0.199 | 100.0 |
| 6.00 | AMD | 6.02±0.20 | 3.4 | 0.089 | 6.02±0.249 | 100.3 |
| | AT | 5.99±0.21 | 3.5 | 0.094 | 5.99±0.261 | 99.8 |
| | RSV | 6.01±0.17 | 2.9 | 0.076 | 6.01±0.211 | 100.2 |
| | VAL | 5.98±0.23 | 3.9 | 0.103 | 5.98± 0.286 | 99.7 |
| 8.00 | AMD | 7.90±0.29 | 3.6 | 0.130 | 7.90±0.360 | 98.8 |
| | AT | 7.95±0.27 | 3.4 | 0.120 | 7.95±0.335 | 99.4 |
| | RSV | 8.01±0.22 | 2.8 | 0.098 | 8.01±0.273 | 100.1 |
| | VAL | 8.02±0.30 | 3.8 | 0.134 | 8.02± 0.372 | 100.3 |
| 10.00 | AMD | 9.70±0.45 | 4.6 | 0.201 | 9.70± 0.559 | 97.0 |
| | AT | 9.90±0.34 | 3.4 | 0.152 | 9.90±0.422 | 99.0 |
| | RSV | 10.0±0.28 | 2.8 | 0.125 | 10.0±0.348 | 100.0 |
| | VAL | 10.1±0.37 | 3.7 | 0.165 | 10.1± 0.460 | 101.0 |
| 12.00 | AMD | 11.3±0.72 | 6.4 | 0.322 | 11.3± 0.894 | 94.2 |
| | AT | 12.1±0.41 | 3.3 | 0.183 | 12.1±0.509 | 100.8 |
| | RSV | 12.0±0.32 | 2.7 | 0.143 | 12.0±0.397 | 100.0 |
| | VAL | 11.9±0.44 | 3.7 | 0.197 | 11.9± 0.546 | 99.2 |
| 16.00 | AMD | - | - | - | - | - |
| | AT | 16.2±0.56 | 3.5 | 0.250 | 16.2±0.695 | 101.3 |
| | RSV | 16.1±0.48 | 3.0 | 0.215 | 16.1±0.596 | 100.6 |
| | VAL | 15.7±0.62 | 3.9 | 0.277 | 15.7± 0.770 | 98.1 |
| 20.00 | AMD | - | - | - | - | - |
| | AT | 20.6±0.82 | 4.1 | 0.367 | 20.6±1.018 | 103.0 |
| | RSV | 19.8±0.70 | 3.5 | 0.313 | 19.8±0.869 | 99.0 |
| | VAL | 19.5±0.94 | 4.7 | 0.420 | 19.5± 1.167 | 97.5 |

**Table 4: Determination mixture of AMD, AT, RSV and VAL in pharmaceutical preparations by TLC-densitometric method on $B_A$Phenyl plates at λ=245 nm (for AT, RSV and VAL), and at λ=365 nm (for AMD) using mobile phase: Acetonitrile:Buffer 45:55, v/v, pH 6.0 (n=5, t=2.776).**

| Product | Compound and dose | Found $\overline{m} \pm$ SD, mg/tab. | RSD% | $\frac{SD}{\sqrt{n}}$ mg/tab. | $\overline{m} \pm \frac{SD}{\sqrt{n}}$ x t, mg/tab. | Recovery% |
|---|---|---|---|---|---|---|
| ***Caduet*** 5/10 tablets | AMD, 5 mg/tab. | $4.96 \pm 0.20$ | 4.0 | 0.09 | $4.96 \pm 0.25$ | 99.2 |
| | AT, 10 mg/tab. | $10.10 \pm 0.41$ | 4.1 | 0.19 | $10.10 \pm 0.51$ | 101.0 |
| ***Caduet*** 5/20 tablets | AMD, 5 mg/tab. | $5.03 \pm 0.20$ | 4.0 | 0.09 | $5.03 \pm 0.25$ | 100.6 |
| | AT, 20 mg/tab. | $19.98 \pm 0.80$ | 4.0 | 0.36 | $19.98 \pm 0.99$ | 99.9 |
| ***Caduet*** 10/20 tablets | AMD, 10 mg/tab. | $10.02 \pm 0.38$ | 3.8 | 0.17 | $10.02 \pm 0.47$ | 100.2 |
| | AT 20 mg/tab. | $19.98 \pm 0.80$ | 4.0 | 0.36 | $19.98 \pm 0.99$ | 99.9 |
| ***EXzar*** 10/160 tablets | AMD, 10 mg/tab. | $10.1 \pm 0.39$ | 3.9 | 0.18 | $10.1 \pm 0.49$ | 101.0 |
| | VAL, 160 mg/tab. | $159.2 \pm 5.9$ | 3.7 | 2.63 | $159.2 \pm 7.31$ | 99.5 |
| ***EXzar*** 5/80 tablets | AMD, 5 mg/tab. | $4.98 \pm 0.20$ | 4.0 | 0.09 | $4.98 \pm 0.25$ | 99.6 |
| | VAL, 80 mg/tab. | $80.0 \pm 3.0$ | 3.8 | 1.36 | $80.0 \pm 3.77$ | 100.0 |
| ***Norvek*** tablets | AMD, 5 mg/tab. | $5.01 \pm 0.20$ | 3.9 | 0.09 | $5.01 \pm 0.24$ | 100.2 |
| ***Atorvex*** tablets | AT, 10 mg/tab. | $10.26 \pm 0.42$ | 4.1 | 0.19 | $10.26 \pm 0.52$ | 102.6 |
| | AT, 20 mg/tab. | $20.87 \pm 0.81$ | 3.9 | 0.36 | $20.87 \pm 1.01$ | 104.4 |
| | AT, 40 mg/tab. | $41.03 \pm 1.60$ | 3.8 | 0. 70 | $41.03 \pm 1.94$ | 102.8 |
| ***Atorvatin*** tablets | AT, 10 mg/tab. | $9.82 \pm 0.40$ | 4.1 | 0.18 | $9.82 \pm 0.50$ | 98.2 |
| | AT, 20 mg/tab. | $20.05 \pm 0.80$ | 4.0 | 0.37 | $20.05 \pm 1.10$ | 100.3 |
| | AT, 40 mg/tab. | $39.03 \pm 1.48$ | 3.8 | 0.66 | $39.03 \pm 1.84$ | 97.6 |
| ***Lipito-med*** tablets | AT, 10 mg/tab. | $9.92 \pm 0.41$ | 4.1 | 0.18 | $9.92 \pm 0.51$ | 99.2 |
| | AT, 20 mg/tab. | $20.22 \pm 0.79$ | 3.9 | 0.35 | $20.22 \pm 0.99$ | 101.2 |
| | AT, 40 mg/tab. | $40.15 \pm 1.53$ | 3.8 | 0.68 | $40.15 \pm 1.89$ | 100.3 |
| ***Lipostatin*** tablets | AT, 10 mg/tab. | $10.28 \pm 0.41$ | 4.0 | 0.18 | $10.28 \pm 0.51$ | 102.8 |
| | AT, 20 mg/tab. | $20.37 \pm 0.79$ | 3.9 | 0.36 | $20.37 \pm 0.99$ | 101.9 |
| | AT, 40 mg/tab. | $40.50 \pm 1.54$ | 3.8 | 0.69 | $40.50 \pm 1.91$ | 101.3 |
| ***Atoraz*** tablets | AT, 10 mg/tab. | $9.75 \pm 0.39$ | 4.0 | 0.17 | $9.75 \pm 0.48$ | 97.5 |
| | AT, 20 mg/tab. | $19.87 \pm 0.78$ | 3.9 | 0.35 | $19.87 \pm 0.96$ | 99.4 |
| | AT, 40 mg/tab. | $40.18 \pm 1.49$ | 3.7 | 0.67 | $40.18 \pm 1.85$ | 100.5 |
| ***Rosuvastatin-ElSaad*** tablets | RSV, 10 mg/tab. | $9.80 \pm 0.38$ | 3.9 | 0.17 | $9.80 \pm 0.47$ | 98.0 |
| | RSV, 20 mg/tab. | $20.84 \pm 0.79$ | 3.8 | 0.35 | $20.84 \pm 0.98$ | 104.2 |
| | RSV, 40 mg/tab. | $39.70 \pm 1.43$ | 3.6 | 0.69 | $39.70 \pm 1.77$ | 99.3 |
| ***Rosuva*** tablets | RSV, 5 mg/tab. | $4.76 \pm 0.19$ | 4.0 | 0.09 | $4.76 \pm 0.24$ | 95.2 |
| | RSV, 10 mg/tab. | $9.95 \pm 0.39$ | 3.9 | 0.17 | $9.95 \pm 0.48$ | 99.5 |
| | RSV, 20 mg/tab. | $20.45 \pm 0.76$ | 3.7 | 0.34 | $20.45 \pm 0.94$ | 102.3 |
| ***Rosuvastatin Sandy*** tablets | RSV, 10 mg/tab. | $10.05 \pm 0.38$ | 3.8 | 0.17 | $10.05 \pm 0.47$ | 100.5 |
| | RSV, 20 mg/tab. | $19.20 \pm 0.71$ | 3.7 | 0.32 | $19.20 \pm 0.88$ | 96.0 |
| | RSV, 40 mg/tab. | $39.65 \pm 1.47$ | 3.7 | 0.66 | $39.65 \pm 1.82$ | 99.1 |
| ***Turbovas*** tablets | RSV, 10 mg/tab. | $10.10 \pm 0.39$ | 3.9 | 0.18 | $10.10 \pm 0.49$ | 101.0 |
| | RSV, 20 mg/tab. | $20.64 \pm 0.78$ | 3.8 | 0.35 | $20.64 \pm 0.97$ | 103.2 |
| ***Crostatin*** tablets | RSV, 5 mg/tab. | $4.81 \pm 0.19$ | 4.0 | 0.09 | $4.81 \pm 0.239$ | 96.2 |
| | RSV, 10 mg/tab. | $10.29 \pm 0.39$ | 3.8 | 0.18 | $10.29 \pm 0.49$ | 102.9 |
| | RSV, 20 mg/tab. | $19.90 \pm 0.74$ | 3.7 | 0.33 | $19.90 \pm 0.91$ | 99.5 |

i) for AMD ((λ=365 nm) y=227.42x+0.1752 (1)
$R^2$=0.9993 (2)

ii) for AT (λ=245 nm) y=179.75x+0.1213 (3)
$R^2$=0.9994 (4)

iii) for RSV (λ=245 nm) y=211.53x+0.7022 (5)
$R^2$=0.9996 (6)

iv) for VAL (λ=245 nm) y=87.862x+0.6739 (7)
$R^2$=0.9994 (8)

LOD, LOQ and RSD% for determination mixtures of AMD, AT, RSV and VAL in pure forms by TLC-densitometric method using $B_A$Phenyl at λ=245 nm (for AT, RSV and VAL), and at (λ=365 (for AMD) using mobile phase: Acetonitrile:Buffer 45:55, v/v, (pH 6.0) 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 1.0 to 20.0 µg/spot of AT, RSV and VAL, and 0.5 to 10.0 µg/spot of AMD with percent relative standard deviation (RSD%) does not exceed 4.0%, 3.8%, 3.2% and 4.2% for AMD, AT, RSV and VAL, respectively, see Figure 5.

**Applications**

**Determination of some pharmaceutical preparations**

TLC method using new phenyl-modified Aleppo bentonite ($B_A$Phenyl) plates at λ=245 nm (for AT, RSV and VAL), and at λ=365 nm (for AMD) using mobile phase: Acetonitrile:Buffer 45:55, v/v, (pH 6.0) were proposed as the follows:

**i) Simultaneous determination of AMD and AT in tablet dosage form: *Caduet*** 5/10 tablets, **PFIZER** Inc. USA, each tablet contains: 5 mg AMD and 10 mg AT; ***Caduet*** 5/20 tablets, **PFIZER** Inc. USA, each tablet contains: 5 mg AMD and 20 mg AT; ***Caduet*** 10/20 tablets, **PFIZER** Inc. USA, each tablet contains: 10 mg AMD and 20 mg AT.

**ii) Simultaneous determination of AMD and VAL in tablet dosage form:** : ***EXzar*** 10/160 tablets, **Asia** pharmaceutical industries- Aleppo – SYRIA, each tablet contains: 10 mg Amlodipine and 160 Valsartan; ***EXzar*** 5/80 tablets, **Asia** pharmaceutical industries, Aleppo – SYRIA, each tablet contains: 5 mg Amlodipine and 80 Valsartan.

***iii) Norvek*** tablets, **ELSaad** pharma**,** Aleppo – SYRIA, each tablet contains: 5 mg Amlodipine.

***iv) Atorvex*** tablets, **Asia** pharmaceutical industries, Aleppo–SYRIA, each tablet contains: 10, 20 and 40 mg of AT; ***Atorvatin*** tablets, **Alpha**, Aleppo pharmaceutical industries, Aleppo–SYRIA, Each tablet contains: 10, 20 and 40 mg of AT**;** ***Lipito-med*** tablets, **Medico** labs., Homs–SYRIA, Each tablet contains:10, 20 and 40 mg of AT; ***Lipostatin*** tablets, **Ibn Al-Haytham** Pharma Industries Co., Aleppo–SYRIA, each tablet contains: 10, 20 and 40 mg of AT; ***Atoraz*** tablets, **Razi** pharmaceutical industries, Aleppo–SYRIA, each tablet contains: 10, 20 and 40 mg of AT.

***v) Rosuvastatin***-ElSaad tablets, **ELSaad** pharma, Aleppo–SYRIA, each tablet contains: 10, 20 and 40 mg of RSV; ***Rosuva*** tablets, **Unipharma**, Damascus–SYRIA, Each tablet contains: 5, 10 and 20 mg of RSV**;** ***Rosuvastatin Sandy*** tablets, **Sandy** pharmaceuticals, Aleppo –SYRIA, Each tablet contains:10, 20 and 40 mg of RSV; ***Turbovas*** tablets, **City *Pharma Co.***, Aleppo–SYRIA, each tablet contains: 10 and 20 mg of RSV; ***Crostatin*** tablets, **Razi** pharmaceutical industries, Aleppo–SYRIA, each tablet contains: 5, 10 and 20 mg of RSV. The results of quantitative analysis for AMD, AT, RSV and VAL in these pharmaceutical preparations were calculated using the calibration curves were summarized in Tables 4. The proposed method was simple, economic, accurate and successfully applied to the determination of AMD, AT, RSV and VAL in pharmaceuticals with average recovery of 95.2 to 104.4%, the results obtained agree well with the contents stated on the labels.

**CONCLUSION**

TLC simultaneous determination of AMD, AT, RSV and VAL in pure form and in tablets using phenyl-modified Aleppo Bentonite ($B_A$Phenyl) at λ=245 nm (for AT, RSV and VAL), and at λ=365 nm (for AMD) using Acetonitrile:Buffer 45:55, v/v, as mobile phase (pH 6.0) 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 (phenylmagnesium bromide). The surface properties of phenyl-modified Bentonite were studied by nitrogen adsorption at 77K. The retardation Factors ($R_f$) of AMD, AT, RSV and VAL were 0.27, 0.41, 0.62 and 0.78, respectively. Linearity for determination of AMD, AT, RSV and VAL was in the range 0.50-10.00 for AMD and 1.00-20.00 µg/spot AT, RSV and VAL. The minimum determined concentration was 0.5 µg/spot for AMD and 1.0 µg/spot for AT, RSV and VAL with percent relative standard deviation (RSD%) 4.0%, 3.8%, 3.2% and 4.2%, respectively. LOD and LOQ were found to be 0.063 and 0.19; 0.125 and 0.38; 0.106 and 0.32; 0.142 and 0.43 µg/spot for AMD, AT, RSV and VAL, respectively. The proposed method was novel, simple, accurate and successfully applied to simultaneous determination of AMD, AT, RSV and VAL in pure form and pharmaceuticals with average recovery of 95.2 to 104.4%, the results obtained agree well with the contents stated on the labels.

**REFERENCES**

1. Lahmek M, Gas chromatographic analysis by using Aleppo bentonite columns, *M. Sc. Thesis in Chem.*, Aleppo University, Syria, 1987.
2. AlHAJ Sakur A., Gas chromatographic analysis using Aleppo bentonite columns deactivated by grafting, *M. Sc. Thesis in Chem.*, Aleppo University, Syria, 1995.
3. Ramadan AA, Antakli S, Mahmoud I, Grafting thermal and acidic treatment Aleppo bentonite with silicon OV-1 and using it in gas chromatographic analysis of pseudoephedrine hydrochloride in pharmaceutical, *Res J Aleppo Univ*, Syria, 2007; 55: 297-312.
4. Martini M, Chromatographic applications using Aleppo Bentonite, *M. Sc. Thesis in Chem.*, Aleppo University, Syria, 1990.
5. Alhaj Sakur A, Studying of some chromatographic supports prepared from bentonite and using it in chromatographic analysis *Ph. D. Thesis in Chem.*, Aleppo University, Syria, 2000.
6. Sahlabji T, Development of some chromatographic supports prepared from natural bentonite and using them in some gas chromatographic applications *M. Sc. Thesis in Chem.*, Aleppo University, Syria, (2003).
7. Ramadan AA, Antakli S, Mahmoud I, Determination of lidocaine hydrochloride, carbinoxamine maleate and chlorphenramine maleate in some pharmaceuticals by gas chromatography using chromatographic support of grafted bentonite with silicon OV-1, *Res J Aleppo Univ*, 2007; 56: 97-116.
8. Abdul Ghafour O, Thin layer chromatography using Aleppo bentonite., *M. Sc. Thesis in Chem., Aleppo University, Syria, 1998.*
9. Mahmoud I, Preparation of chromatographic supports and using them in thin layer chromatographic analysis. Ph. D. Thesis in Chem., Aleppo University, Syria, 2011.
10. Ramadan AA, Bodakji A, Mahmoud I, TLC-densitometric determination of vitamins B1, B6 and B12 in pure and pharmaceutical formulations using treated aleppo bentonite. *Asian J Chemy*, 2010; 22(4), 3283-3291.
11. Ramadan AA, Al-Akraa H, Maktabi M, TLC simultaneous determination of valsartan and hydrochlorothiazide in pure form and in tablets using butyl-modified aleppo bentonite, *Int J Pharm Pharm Sci*, 2013; 5 (Issue 3): 762-769.
12. Ramadan, A.A., Al-Akraa, H., Maktabi, M., Simultaneous Determination of Losartan Potassium and Hydrochlorothiazide in Pure Form and in Tablets by TLC Using Butyl-modified Aleppo Bentonite, *Res J Aleppo Univ*, Syria, 2013; 89, Under press.
13. Abdallah OM, Badawey AM, Determination of amlodipine and valsartan in binary mixture using derivative- ratio spectrophotometric, chemometric and, *Int J Ind Chem*, 2011; 2(3): 131-139.
14. Reents S, Seymour J, Clinical Pharmacology; An Electronic Reference and Teaching Guide CD-ROM, Gold Standard Multimedia Inc., Tampa, FL, 1998.
15. Mohie MK Sharaf El-Din, Khalid AM Attia, Mohamed WI Nassar, Mohamed MY Kaddah, Colorimetric determination of simvastatin and lovastatin in pure form and in pharmaceutical formulations. *Spectrochimica Acta Part A*, 2010; 76 :423–428.2
16. Ramesh D, Ramakrishna S, New spectrophotometric methods for simultaneous determination of amlodipine besylate and atorvastatin calcium in tablet dosage forms. *Int J Pharm Pharm Sci*, 2010; 2(4): 215-219.3
17. Smita T Kumbhar, Swapnil D Jadhav, Neela M Bhatia, Manish S Bhatia, Development and validation of derivative

spectrophotometric method for estimation of atorvastatin calcium and amlodipine besylate in tablet dosage form. *Int J Pharm Pharm Sci*, 2011; 3, (4): 195-197.

18. Nissen S, Nicholls S, Sipahi I, Libby P, Raichlen JS, Ballantyne CM. Effect of very high-intensity statin therapy on regression of coronary atherosclerosis. The Asteroid Trial. *JAMA*, 2006; 295(13): 1556- 1565.
19. Lennernas H, Fager G. Pharmacodynamic and pharmacokinetics of the HMG_CoA reductase inhibitors. *Clinical Pharmacokinetics*, 1997;32: 403-425.
20. Asma Afroz, Tasnuva Haque, Md. Mesbah Uddin Talukder, S. M. Ashraful Islam, Spectrophotometric Estimation of Rosuvastatin Calcium and Glimepiride in Tablet Dosage Form. *Asian J Pharm Ana,* 2011; 1(4): 74-78.
21. Li NC, Lee A, Whitmer RA, *et al.,* Use of angiotensin receptor blockers and risk of dementia in a predominantly male population: prospective cohort analysis. *BMJ,* 2010; 340: 5465.
22. Wang J, Ho L, Chen L, *et al.*, Valsartan lowers brain β-amyloid protein levels and improves spatial learning in a mouse model of Alzheimer disease. *J Clin Invest*, 2007; 117 (11): 3393–3402.
23. Duarte JD, Cooper-DeHoff RM (June 2010). Mechanisms for blood pressure lowering and metabolic effects of thiazide and thiazide-like diuretics. *Expert Rev Cardiovasc Ther,* 2010; 8 (6): 793–802.
24. Dvorak MM, De Joussineau C, Carter DH et al., Thiazide diuretics directly induce osteoblast differentiation and mineralized nodule formation by targeting a NaCl cotransporter in bone. *J Am Soc Nephrol*, 2007; 18 (9): 2509–2516.
25. Johnson, KK; Green, DL, Rife, JP, Limon, L (2005 Feb). Sulfonamide cross-reactivity: fact or fiction. *The Annals of pharmacotherapy*, 2005; 39 (2): 290–301.
26. Kadam BR, Bari SB, Quantitative analysis of valsartan and hydrochlorothiazide in tablets by high performance thin-layer chromatography with ultraviolet absorption densitometry. *Acta Chromatographica*, 2007; 19: 260-269.
27. Ramadan AA, Bodakji A, Mahmoud I, Simultaneous determination of valsartan and Hydrochlorothiazide in Pure Form and Pharmaceuticals Using Octyl-modified Aleppo bentonite in TLC, *Res J Aleppo Univ*, 2010; 72: 127-147.
28. Shirkhedkar AA, Surana SJ, Simultaneous densitometric TLC analysis of atorvastatin calcium and fenofibrate in the bulk drug and in pharmaceutical formulations, *J of Planar Chromatography,* 2009; 22(5):355-358.
29. Dhaneshwar SS, Dhaneshwar S R, Deshpande P, Patil M, Development and validation of a method for simultaneous densitometric estimation of atorvastatin calcium and ezetimibe as the bulk drug and in tablet dosage forms. *Acta chromatographica*, 2007; 19: 141-148.
30. Sharma MC, Sharmaa Smita, Kohlib DV, Sharmac AD, A validated HPTLC method for determination of simultaneous estimation rosuvastatin calcium and ezetimibe in pharmaceutical solid dosage form, *Arch Apll Sci Res,* 2010; 2(1): 1-7.
31. Sane RT, Kamat SS, Menon SN, Inamdar SR, Mote MR, Determination of rosuvastatin calcium in its bulk drug and pharmaceutical preparations by high-performance thin-layer chromatography. *J of Planar Chromatography TLC*, 2005; 18:194-198.