Asian Journal of Chemistry; Vol. 24, No. 1 (2012), 353-357

ASIAN JOURNAL OF CHEMISTRY

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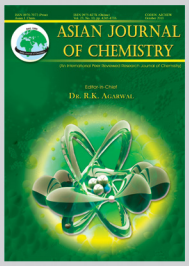

Simultaneous Determination of Losartan Potassium and Hydrochlorothiazide in Pure Form and in Tablets Using Octadecyl-Modified Aleppo Bentonite in TLC

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(*Received*: 31 December 2010; *Accepted*: 23 September 2011) AJC-10434

A new octadecyl-modified Aleppo bentonite (B_AC_{18}) in TLC was developed to determine losartan potassium and hydrochlorothiazide in pure form and tablets with mobile phase ammonium phosphate buffer (10 mmol L^{-1}; pH 3.2 with orthophosphoric acid) and acetonitrile (60:40, v/v), at wavelength λ = 260 nm. The particles of Aleppo bentonite with diameter less than 45 μm were treated by concentrated HCl (B_A), after that grafted firstly by dimethyldichlorosilane, then secondly by Grignard reagent (octadecylmagensium bromide). The surface properties of octadecyl-modified bentonite were studied by nitrogen adsorption at 77 K, IR and NIR techniques. The retardation factors (R_f) of losartan potassium and hydrochlorothiazide were 0.266 and 0.757, respectively. Linearity for determination of losartan potassium and hydrochlorothiazide was in the range 1.00-10.0 μg/spot. The minimum determined concentration was 1.0 μg/spot for losartan potassium and hydrochlorothiazide with per cent relative standard deviation (RSD %) does not exceed 3.08 and 1.74 %, respectively. The limits of quantification (LOQ) were 0.85 and 0.72 μg/spot and the limits of detection (LOD) were 0.28 and 0.24 μg/spot for determination of losartan potassium and hydrochlorothiazide, respectively.

Key Words: Aleppo bentonite, BET, TLC, Losartan potassium, Hydrochlorothiazide.

INTRODUCTION

Aleppo bentonite is rocky clay which originates from volcanic ash and consists of 47 % SiO_2, 14.4 % Al_2O_3 and some other metal oxides as Fe_2O_3, MgO, CaO and Na_2O[1,2]. Bentonite has large pore volumes and high specific surface area[3]. The thermal treatment causes decreasing of its specific surface area with increasing in the temperature of thermal treatment[4]. Bentonite clays are used in many industrial products and processes, drilling fluids, a certain lubricating grease[5,6] and it can be used as chromatographic supports in gas chromatography to separate many mixtures after grafting with different methods[7,8]. Bentonite is used as stationary phase in thin layer chromatography to separate some metal ions and vitamins B1, B6 or B12[9,10].

Losartan potassium (LOK) and hydrochlorothiazide (HCTZ) are the two active compounds of an oral pharmaceutical formulation, widely used in antihypertensive therapies. Losartan potassium, 2-butyl- 4-chloro-1-[*p*-(*o*-1*H*-tetrazol-5-ylphenyl)benzyl]imidazole-5-methanol monopotassium, is a selective non-peptide angiotensin II receptor antagonist which has an oral bioavailability of about 33 % and a half life of about 2 h. Hydrochlorothiazide, 6-chloro-3,4dihydro-2*H*-1,2,4-benzothiadiazine-7 sulphonamide-1,1-dioxide, is a widely used thiazide diuretic. It increases urinary excretion of sodium and water by inhibiting sodium reabsorption in the renal tubules[11-14]. Several methods have been reported for the analysis of LOK and its degradation products. In biological fluids, the active principle has been determined by high performance liquid chromatography (HPLC) methods[15-17]. For applications in pharmaceutical products, there are methods that make use of HPLC[18,19], high performance thin layer chromatography (HPTLC)[20,21], capillary electrophoresis (CE), capillary electrochromatography (CEC)[22] and spectrophotometry[23,24]. Recently, there have been reports in the literature of the employment of HPLC for bioequivalence studies of tablets containing LOK[25]. Hydrochlorothiazide is found in combination with many other drugs. Quantitative analysis of the samples containing HCTZ and benazepril is achieved by chemometry[26], spectrophotometry[27,28], HPLC[29,30], micro-bore liquid chromatography and thin layer chromatography[31]. The binary mixture of HCTZ and amiloride has been determined by spectrophotometry[32,33] and HPLC[34,35]. Hydrochlorothiazide-captoril mixture is analyzed by spectrophotometry[36,37] and HPLC[38].

EXPERIMENTAL

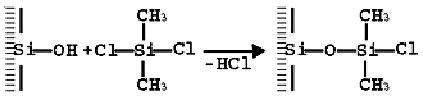
Surface area and pore size measurement (BET) were recorded using Micromeritics Gemini III 2375 under nitrogen

atmosphere (USA). Scanner-densitometer CD60 (Desega, Germany) equipped with mercury, tungsten and deuterium lamps, 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 different size of syringe (Hamilton, Switzerland) were used.

Losartan potassium (LOK), 99.4 %, Enaltec-India, hydrochlorothiazide (HCTZ), 99.6 %, New Century-China, were used. Methanol, acetonitrile, tetrahydroforan, dichloromethane, dimethyldichlorosilane, 1-bromooctadecane, orthophosphoric acid, ammonium dihydrogen phosphate, magensium metal and fluorescent indicator F_{254} for thin layer chromatography were purchased from Merck, Germany.

Preparation of acidic treated bentonite: Bentonite was crushed to obtain small pieces, which have diameter less than 45 μm, followed by washing with conc. hydrochloric acid at boiling point for 30 h to remove soluble oxides especially iron oxide. Then it was washed several times with distilled water and dried at 120 ºC for 3 h (B_A).

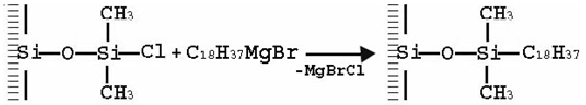
Chlorination: 50 g of treated bentonite (B_A) is dispersed in 250 mL of dichloromethane and 10 mL of dimethyldichlorosilane. The mixture is left under reflux during 3 h. The mixture is evaporated and dried at 280 °C during 3 h. Then the chlorinated product was kept under inert atmosphere of nitrogen (B_ACl).



Treatment with Grignard reagent: Grignard reagent was prepared from reaction 34.7 mL of 1-bromooctadecane with 2.4 g of clean and dry magnesium in 200 mL of anhydrous tetrahydrofuran (THF) as follows:

$$C_{18}H_{37}Br + Mg \xrightarrow[66\ ^\circ C]{THF} C_{18}H_{37}MgBr$$

The chlorinated bentonite (B_ACl) was added into solution of Grignard reagent under inert atmosphere. The mixture was allowed to reflux for 3 h. Then the heating was removed and contents were allowed to cool. The product was filtered and washed with methanol and dried at 105 °C for 2 h (B_AC_{18}).



Preparation of TLC plates: For preparation of thin layer chromatography, 17.6 g of modified bentonite (B_AC_{18}) 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 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 ºC.

Mobile phase: Ammonium phosphate buffer (10 mmol L^{-1}; pH 3.2 with orthophosphoric acid) and acetonitrile (60:40, v/v) were used for the development method as mobile phase.

Standard solutions: Stock solution was prepared by dissolving 1 g of each LOK and HCTZ in 25 mL mixture of distilled water and methanol (1:1), then transferred into 50 mL volumetric flask and the final volume was completed with the same mixture of solvents. Volumes 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mL from the former solution were transferred into 10 mL volumetric flasks and completed to the mark with the same mixture of solvents (these solutions content: 1.0, 2.0, 4.0, 6.0, 8.0 and 10.0 mg mL^{-1}, respectively for each material).

Sample preparation: Twenty tablets were weighed and the average tablet weight determined (each tablet contains: 50 mg LOK and 12.5 mg HCTZ). The tablets were finely powdered and a portion of powder equivalent to the weight of four tablets was dissolved in 20 mL mixture of distilled water and methanol (1:1) and vigorously shaken for a 20 min on a mechanical shaker, then filtrated and transferred into a 25 mL volumetric flask and the final volume was completed to 25 mL with the same mixture of solvents; this solution contains: 8.0, 2.0 mg mL^{-1} of LOK and HCTZ, respectively.

Procedure (chromatographic conditions): 1 μL of standard solutions were spotted on TLC-glass plates 20 × 10 cm pre-coated B_AC_{18} (F_{254} with 0.30 mm thickness). Mobile phase 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 1.0-10.0 μg/spot for LOK and HCTZ.

Pharmaceutical formulations: 1 μL of solution for tablets was spotted on TLC-glass plats for determination of LOK and HCTZ and quantification was carried out densitometerically at λ = 260 nm. The concentrations were calculated from standard curves in the range 1-10 μg/spot for LOK and HCTZ.

RESULTS AND DISCUSSION

Surface properties of B_A and B_AC_{18}: Surface areas of B_A and B_AC_{18} were determined by the adsorption of nitrogen at 77 K (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_{18} are shown in Fig. 1. The isotherms are II and IV type of SING and BDDT classifications, which indicate the 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), representation linear BET plots for B_AC_{18} (Fig. 2). From these plots we found that the BET surface areas (S_{BET}) was 168.25, 68.27 m^2/g for B_A and B_AC_{18}, respectively. The total pore volume v_p (0.440, 0.242 mL/g) were determined from the adsorbed volume at P/Po = 0.95 in the liquid form. The mean pore radii r_a (52.3, 71.3 Å), were determined from the equation: $r_a = 2 \times 10^4 \times v_p/S_{BET}$. The changes of surface area, total pore volume and mean pore radii during modification can be seen from Table-1.

TABLE-1
SURFACE PROPERTIES OF B_A AND B_AC_{18}

	S_{BET} (m^2/g)	v_p, (mL/g)	r_a (Å)
B_A	168.25	0.440	52.3
B_AC_{18}	68.27	0.242	71.3

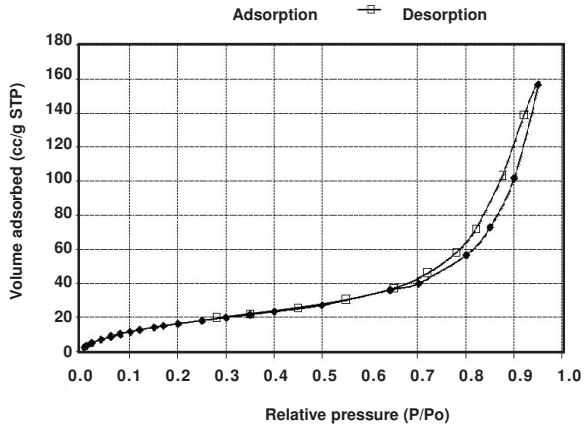


Fig. 1. Adsorption-desorptoin isotherm of nitrogen at 77 K on B_AC_{18}

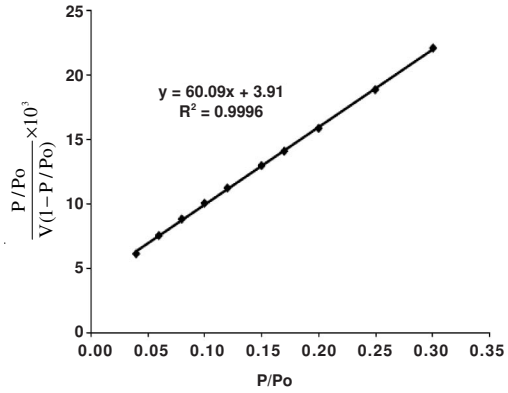


Fig. 2. BET plots for B_AC_{18}

The surface area and the total pore volume decreased from (168.25 m^2/g, 0.440 mL/g) to (68.27 m^2/g, 0.242 mL/g), respectively. The mean pore radii increased from 52.3 to 71.3 Å.

Infrared spectra (IR): The infrared spectra were studied for each B_A and B_AC_{18}. New peak appears in the spectrum of B_AC_{18} in the region 3100-2800 cm^{-1} back to stretch C-H (Fig. 3). The information provided by IR that the surface of B_AC_{18} has been modified with the alkenes group.

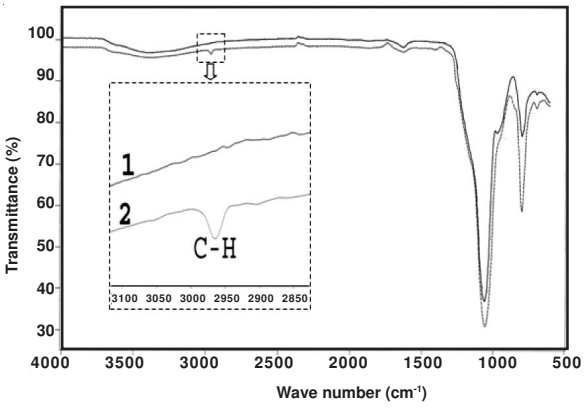


Fig. 3. IR spectra of B_A (1) and B_AC_{18} (2)

Near infrared spectra (NIR): Scan for each B_A and B_AC_{18} are show in Fig. 4, spectrum B_AC_{18} has signals in 4400-4200 cm^{-1} and 6000-5500 cm^{-1} regions. The signals were assigned to vibration of bond C-H in B_AC_{18}.

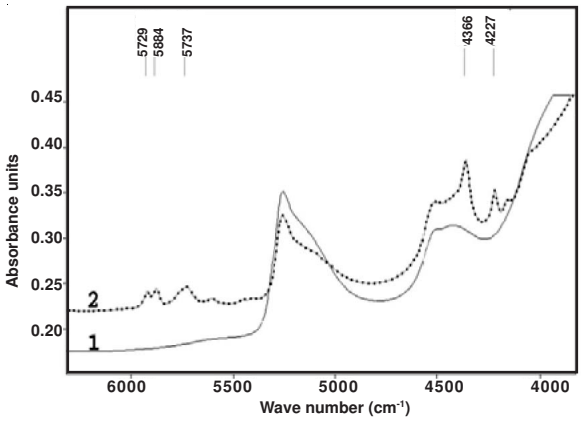


Fig. 4. NIR spectra of B_A (1) and B_AC_{18} (2)

Hydrophobicity: For the estimation of the changes in the hydrophobicity after modification, we compared dispersiblility of the B_A and B_AC_{18} in water and benzene. As shown in Fig. 5, the B_A disperses in the water layer only. Due to the presence of hydrophobic alkyl group on the external surface of B_AC_{18} and the hydrophobicity of the rest the surface, the B_AC_{18} was found in organic phase at the benzene-water boundary.

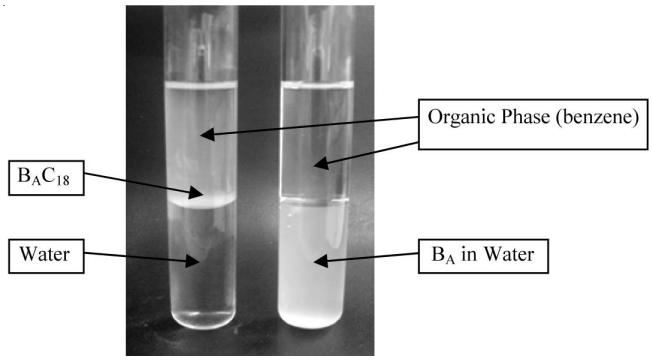


Fig. 5. B_A (right) and B_AC_{18} (left) dispersed in water/benzene (organic phase) system

Chromatograms processing: The position of the spots from the front on the chromatographic plate for different concentrations (1.0 to 10.0 µg/spot) of LOK and HCTZ, the retardation factors (R_f) were 0.266 and 0757, respectively (Fig. 6).

Fig. 6. TLC Plate of standard LOK and HCTZ for concentrations of each vitamin 1.0, 2.0, 4.0, 6.0, 8.0 and 10.0 µg/spot (mobile phase: phosphate buffer (10 mmol L^{-1}; pH 3.2) and acetonitrile (60:40, v/v)

TABLE-2
DETERMINATION OF LOK AND HCTZ IN PURE FORMS BY TLC-DENSITOMETRIC METHOD USING B_AC_{18} AT λ = 260 nm (MOBILE PHASE: PHOSPHATE BUFFER (10 mmol L^{-1}; PH = 3.2) AND ACETONITRILE (60:40, v/v)

Taken standard m (µg/spot)	Material	Found $\overline{m}$ * ±SD (µg/spot)	RSD (%)	$\frac{SD}{\sqrt{n}}$ (µg/spot)	$\overline{m} \pm \frac{SD}{\sqrt{n}} \times t$ (µg/spot)	Recovery (%)
1	LOK	0.959 ± 0.030	3.086	0.013	0.959 ± 0.037	95.9
	HCTZ	1.015 ± 0.018	1.747	0.008	1.015 ± 0.022	101.5
2	LOK	2.055 ± 0.060	2.924	0.027	2.055 ± 0.075	102.7
	HCTZ	2.078 ± 0.016	0.783	0.007	2.078 ± 0.020	103.9
4	LOK	4.076 ± 0.056	1.376	0.025	4.076 ± 0.070	101.9
	HCTZ	3.919 ± 0.034	0.855	0.015	3.919 ± 0.042	98.0
6	LOK	5.871 ± 0.091	1.552	0.041	5.871 ± 0.113	97.8
	HCTZ	5.960 ± 0.047	0.796	0.021	5.960 ± 0.059	99.3
8	LOK	7.997 ± 0.044	0.546	0.020	7.997 ± 0.054	100.0
	HCTZ	7.943 ± 0.016	0.203	0.007	7.943 ± 0.020	99.3
10	LOK	10.043 ± 0.067	0.670	0.030	10.043 ± 0.084	100.4
	HCTZ	10.085 ± 0.037	0.365	0.016	10.085 ± 0.046	100.9

*n = 5, t = 2.776.

The chromatogram of mixture of LOK and HCTZ (10 µg/spot for each material) was observed with two peaks at different wavelengths (λ) at 200 to 300 nm (Fig. 7). The first peak area (LOK) remains constant to λ = 250 nm then decreases, the second (HCTZ) remains constant to λ = 250 nm, then sharply increases to λ = 270 nm, after that sharply decreases (Fig. 8). Infer from the Figs. 7 and 8 that, the best wavelength to determine the two material is 260 nm.

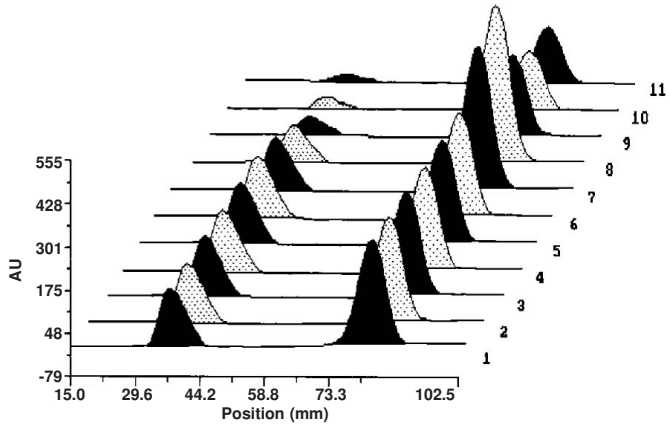


Fig. 7. Chromatograms of mixture of LOK and HCTZ (10 µg/spot) disposed at different wavelengths (λ) at 200 to 300 nm: (1) 200 (2) 210 (3) 220 (4) 230 (5) 240 (6) 250 (7) 260 (8) 270 (9) 280 (10) 290 and (11) 300 nm

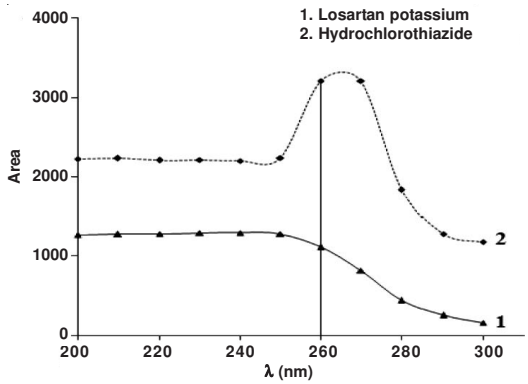


Fig. 8. Effect of wavelengths (λ) on peak areas of LOK and HCTZ by TLC-densitometric method using B_AC_{18} and mobile phase: phosphate buffer (10 mmol L^{-1}; pH 3.2) and acetonitrile (60:40, v/v)

Quantitative evaluation: The method being validated through, precision, linearity, accuracy, limit of detection (LOD) and limit of quantification (LOQ) for determination of different standard mixtures LOK and HCTZ. The repeatability of different standard mixtures and measurement of peak area were expressed in terms of relative standard deviation (RSD %) (Table-2). The linear regression data for the calibration curves is shown in Fig. 9, which showed a good linear relationship and good correlation coefficient in the concentration range 1-10 µg/spot. The limit of detection and limit of quantification of the proposed method are shown in Table-3, which were calculated according to 3.3 × SD/S and 10 × SD/S criterions, respectively, where SD is the standard deviation and S is the slope of the corresponding calibration curve. The LOD of LOK and HCTZ were found to be 0.28 and 0.24 µg/spot, respectively. These were the lowest amount of analyst in material that can be detected but not necessarily quantitated as an exact value. The LOQ of LOK and HCTZ were 0.85 and 0.72 µg/spot, respectively. These were the lowest concentration of drugs, accurately detected and integrated by the instrument.

TABLE-3
VALIDATION PARAMETERS OF DETERMINATION OF LOK AND HCTZ IN PURE FORMS BY TLC-DENSITOMETRIC METHOD USING B_AC_{18} AT λ = 260 nm (MOBILE PHASE: PHOSPHATE BUFFER (10 mmol L^{-1}; pH = 3.2) AND ACETONITRILE (60:40, v/v)

Parameter	LOK	HCTZ
Linearity range (µg/spot)	1.0-10.0	1.0-10.0
Correlation coefficient (R^2)	0.9995	0.9996
Regression equation:		
Slope	99.94	318.18
Intercept	24.77	-10.20
Limit of detection (µg/spot)	0.28	0.24
Limit of quantification (µg/spot)	0.85	0.72
RSD % (1.0-10.0 µg/spot)	3.86-0.55	1.747-0.203

Analysis of losartan potassium and hydrochlorothiazide in tablet dosage form: The proposed method was applied to the determination of LOK and HCTZ in commercial pharmaceuticals. Tablet containing 50 and 12.5 mg of LOK and HCTZ, respectively, brand LOSARTIC (manufactured by Oubari pharmaceutical) and LOTID (manufactured by Pharmasyr

TABLE-4
DETERMINATION OF LOK AND HCTZ IN TABLETS BY TLC-DENSITOMETRIC METHOD USING B_AC_{18} AT λ = 260 nm
(MOBILE PHASE: PHOSPHATE BUFFER (10 mmol L^{-1}; pH = 3.2) AND ACETONITRILE (60:40, v/v)

Product	Compound and dose	Found $\overline{m}$*±SD	RSD (%)	$\frac{SD}{\sqrt{n}}$	$\overline{m} \pm \frac{SD}{\sqrt{n}} \times t$	Recovery (%)
Losartic (tablets)	LOK 50 mg/tab.	50.63 ± 0.49	0.957	0.217	50.63 ± 0.602	101.3
	HCTZ 12.5 mg/tab.	12.61 ± 0.12	0.974	0.055	12.61 ± 0.152	100.9
Lotid (tablets)	LOK 50 mg/tab.	50.14 ± 0.43	0.861	0.193	50.14 ± 0.536	100.3
	HCTZ 12.5 mg/tab.	12.36 ± 0.13	1.020	0.056	12.36 ± 0.156	98.9

*n = 5, t = 2.776.

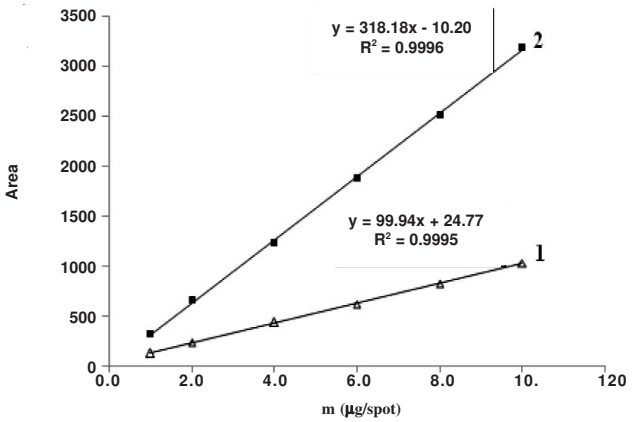


Fig. 9. Calibration curves for determination of LOK (1) and HCTZ (2) in pure forms by TLC-densitometric method using B_AC_{18} and mobile phase: phosphate buffer (10 mmol L^{-1}; pH 3.2) and acetonitrile (60:40, v/v) at λ 260 nm

pharmaceutical) (Table-4). Results of identification losartan potassium and hydrochlorothiazide in tablets and the name of the manufacturer and the drug dosage, where the results were set close to and consistent with the amounts declared on the product and this confirms that the method applied is valid for identification of these two compounds together in pharmaceutical.

Conclusion

The surface of B_A was grafted by two steps, the first one was a chlorination of Bentonite using dimethyldichlorosilane and the second by reacting Grignard reagent with octadecylmagensium bromide to obtain B_AC_{18}. The surface of B_AC_{18} by BET, IR and NIR was studied and used as support in thin layer chromatography. The developed TLC technique is a precise, specific, accurate and robust for the determination of losartan potassium and hydrochlorothiazide in pure form and tablets.

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