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USING ACHROMATOGRAPHIC SUPPORT PREPARED FROM SILANIZED LOCAL SYRIAN CLAY IN HPTLCFOR SYNCHRONOUSDETERMINATION OF CHLORPHENIRAMINE MALEATE, PHENYLEPHRINE HYDROCHLORIDE, AND PARACETAMOL IN TABLETS

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ABSTRACT

A New Chromatographic Supportwas prepared for high performance thin layer chromatography (HPTLC)from chemical modified (Silanized) Local Syrian Clay,through reacting dimethyldichloro-silane (DMDCS) with silanol group of the surface of acidic treated Clay, and using it for preparing high performance chromatographic thin layers (0.25 mm thickness) to separate and determinate mixture of Chlorpheniramine maleate, Phenylephrine Hydrochloride, and Paracetamol in pure form and in tablets. The separation carried out using mobile phase consisted ofIsopropanol – Isobutanol –Ethyl acetate– Toluene – Water - Acetic acid (15:15: 25:6:6:3) v/v. The specific surface area of silanizedLocalSyrian Clay was21 m^2/g. Quantification was carried out densitometerically at $\lambda = 220$ nm for Chlorpheniramine maleate, and Phenylephrine Hydrochloride, and at $\lambda = 250$ nm for Paracetamol. The retardation factors (R_f) of Chlorpheniramine maleate, Phenylephrine Hydrochloride, and Paracetamol were 0.44 , 0.61 , and 0.83 respectively. Calibration curves were obtained in the ranges of 0.5-4.0 µg/spot, 2.0-16.0 µg/spot and 5.0-40.0 µg/spot for standard solutions of Chlorpheniramine maleate, Phenylephrine Hydrochloride and Paracetamol respectively.The New Prepared Chromatographic high performance Thin Layers were successfully applied to the assay of commercial dosage forms (tablets) containing the drugs with average recovery 98.40 –102.00% with RSD not more than 4.93%.

INTRODUCTION

Local Syrian clay is rocky clay, it is considered as a porous cheap material naturally occurring in Syria. Local Syrian clay (Syrian Bentonite) constituents were determined, and Silica represents the major constituent of clay, in addition to several amounts of other metallic oxides. Local Syrian clay (Syrian Bentonite) consists of 47% SiO_2, 14.4% Al_2O_3 and some other oxides as Fe_2O_3, MgO, CaO, $Na_2O$and others [1- 4]. Bentonite has large pore volumes and high specific surface area. The thermal treatment causes decreasing of its specific surface area with increasing in the temperature of thermal treatment , and prolonged washing of bentonite by 6 N HCI removes all soluble oxides and about 67% of total iron oxide from the adsorbent, causing decrease of specific surface area and hydrolysis of siloxane groups on the surface of the support to yield more silanol groups [1- 4].Bentonite clays are used

in many industrial products and processes, drilling fluids, a certain lubricating grease [5], and it can be used as chromatographic supports in gas chromatography to separate different of chemical mixtures after grafting with different methods.Modification of the support surface by reaction with silanol groups was carried out by means of chlorosilane compounds as reactants, or by condensation of a suitable polymer as PEG-20M [6-10]. Bentonite is used as stationary phase in thin layer chromatography to separate some metal ions, and some drugs mixtures [11-15]. A mixture of paracetamol, phenylephrine hydrochloride, and chlorpheniramine maleate is widely used as an analgesic, antipyretic, antihistamine and antitussive [16, 17].

Paracetamol : is(Acetaminophen; N-Acetyl–p–aminophenol),$C_8H_9NO_2$**,** and it is a para-aminophenol derivative, has analgesic and antipyretic properties and weak anti-inflammatory activity [16-19].Paracetamol alone or in combination with other drugs is reported to be estimated by spectrophotometric method [19-22] , HPLC[18-23], TLC[24], HPTLC[25], LC-MS[26], FT-IR[27], amperometric determination[28], Fluorimetry[29], and Micellarelectrokinetic chromatographic method[30].

Phenylephrine hydrochloride: is[(R)-2-methylamino-1-(3-hydroxyphenyl) ethanol hydrochloride] , $C_9H_{13}NO_2$, and it is is a sympathomimetic with mainly direct effects on adrenergic receptors. It has mainly alpha-adrenergic activity and is without significant stimulating effects on the CNS at usual doses. Phenylephrine and its salts are most commonly used, either topically or by mouth, for the symptomatic relief of nasal congestion. It is frequently included in preparations intended for the relief of cough and cold symptoms such nasal congestion [16, 20]. Literature survey revealed that spectrophotometry[30], RP-HPLC[18 , 19, 32], electrophoresis[33], liquid chromatography[34], methods have been reported for the estimation of phenylephrine hydrochloride in pharmaceutical formulations.

Chlorphenaminemaleate:is(RS)-3-(4-chlorophenyl)-3-(2-pyridyl) propyldimethylamine hydrogen maleate) ,$C_{16}H_{19}ClN_2$,$C_4H_4O_4$, an alkylamine derivative, and it is a sedating antihistamine that causes a moderate degree of sedation; it also has antimuscarinic activity, it is used for the symptomatic relief of allergic conditions. It is common ingredient of compound preparations for symptomatic treatment of coughs and the common cold [16, 20].For estimation of chlorpheniramine maleate, HPLC [18, 35], GC [19]**,** chemo metric-assisted spectrophotometric[19, 36] methods have been reported. Literature reveals that no analytical method is available for Synchronous determination of these three drugs in combination. In USP 30, and BP the determination of these components has also been performed with HPLC, but all of them were determined separately and the method does not involve Synchronous determination [18,19].So we communicate here rapid and cost effective quality-control tool for their routinequantitative analysis in pure and combined dosage forms by HPTLC with new chromatographic support.The aim of this study was to prepare a new chemical modifiedchromatographic support from DMDCSSilanized local SyrianClay and using it in high performance thin layer chromatography (HPTLC) to develop a validate analytical procedure for Synchronous determination of paracetamol, Chlorphenamine maleate, and Phenylephrine hydrochloride in a tablet and validated it.The developed analytical procedure was successfully used for routine analysis of paracetamol, Chlorphenamine maleate, and Phenylephrine hydrochloride in dosage form without any interference from involved excipients.

2. MATERIALS AND METHODS:

2.1Apparatus:Specific Surface area wasmeasured using a Spectrophotometric method depend on methylene Blue adsorption on JASCO V-650 dual beam UV-VIS spectrophotometer. Scanner-densitometer CS-9301PC (SHIMADZU) equipped with mercury, tungsten and deuterium lamps, CAMAG Hand Operated TLC Coater (Switzerland), CAMAG UV Cabinet for assessing and marking thin layerchromatograms under UV light (Switzerland), planetary ball mill , Vibration Sieves of size less than (20) µm (Germany) and different size of syringe (Hamilton,

Switzerland) were used. Ultrasonic processor model POWERSONIC 405 (to sonicate the sample solutions) and electronic balance (Sartorius-2474; d=0.01 mg) were used.

2.2 Reagents and Materials: Paracetamol (99.0 %, Jiangsu, China), ChlorpheniramineMaleate(99.74 %, Plethico Pharmaceuticals Ltd. India) and Phenylephrine HCl (99.46 %, Plethico Pharmaceuticals Ltd. India) were used. Ethanol,Isopropanol, Isobutanol, Ethyl acetate,Toluene, Acetic acid, Chloroform, Methanol, Dichloromethane, Concentrated Hydrochloric Acid, Dimethyldichlorosilane, $CaCl_2$, fluorescent indicator F254 for thin layer chromatography , Carboxymethylcellulose Sodium , and Iodine were of analytical grade, Merck, Germany. Water used was deionised and double distilled. Pharmaceutical.

3. SUPPORT PREPARATION:

3.1Preparation of acidic treated Syrian Clay (Bentonite): Bentonite was crushed, using a mortar and milled by planetary ball mill to obtain small pieces, which have diameter less than 10 µm,Further, the bentonite powder is activated with chemical reaction by Suspending 50 g of bentonite in 100 ml of HCl (6 M) for 24 hours, to remove soluble oxides especially iron oxide.The magnetic stirrer was employed to mix the solution at 300 rpm and temperature 85ºC. After that, the bentonite is washed several times using distilled water until the pH is neutral, and dried at 200 ℃ for 3 h (acidic treated Syrian Clay).

3.2 Preparation of DMDCS Silanized Syrian Clay (Silanized Syrian Bentonite): Chemical modification of yield acidic treated clay was done in glass flask including refluxing column, thermometer,and magnetic stirring. (250 ml) of methylene chloride dried with $CaCl_2$ were introduced in this glass flask, and (50 gr) of acidic treated clay was dispersed (immersed)in dichloromethane , and to a well stirred treated clay in dichloromethane (15 ml) of dimethyldichlorosilane was drop –wise introduced ,and the reaction mixture was well stirred at room temperature in presence of stream of dry pure nitrogen,then after constant stirring for (8hr) until hydrogen chloride stopped bubbling, the solvent was dried and the yield silanized clay was rinsed first with 3 volumes of chloroform and then with 3 volumes of anhydrous methanol,after being vacuum dried for (4hr) it was dried at (70 °C) before using it in preparing HPTLC plates.The new presented chromatographic support is (S.B DMDCS).

3.3Preparation of TLC plates: For preparation of High performance thin chromatographic layers, (10 g) of silanized clay was passed through a mesh vibrated sieves of size less than 10 µm, and then it was mixed with with 0.5 g fluorescence substance, and due to the hydrophobic nature of the silanized support it was mixed with (3 ml) of methanol, then the mixture was added to 20 mL hot water containing (0.5 g) Carboxymethylcellulose Sodium as binder to obtain homogeneous slurry. The slurry was spread over glass plates (10×20 cm) by an CAMAG Hand Operated TLC Coater, to form uniform thin layer 0.25 mm thick. The plates were dried at 105 °C.

3.4Mobile phase: Isopropanol :Isobutanol : Ethyl acetate : Toluene : Water : Acetic acid (15:15: 25:6:6:3) v/v. were used for the development method as mobile phase.

3.5Standard solutions: Stock solutionswere prepared by dissolving (2500 mg) of Paracetamol, (1000 mg) of Phenylephrine hydrochloride and (250 mg) of Chlorphenamine maleate in 40 mL of Ethanol, then transferred into a 50 mL volumetric flask and the final volume was completed to 50 mL with the same solvent. Volumes 1, 2, 3, 4, 5,6,7 and 8 mL from the former solution were transferred into 10 mL volumetric flasks and completed to the mark with the same solvent (Ethanol) (these solutions contain: 5, 10 , 15 , 20 , 25,30, 35, and 40 mg/ml , for Paracetamol , 2 , 4 , 6 , 8 , 10 ,12, 14 and 16 mg/ml , for Phenylephrine hydrochloride, and 0.5 , 1, 1.5 , 2 , 2.5 , 3 , 3.5 and 4 mg/ml for Chlorphenamine maleate).

3.6 Sample preparation (Anti Cold):

Twenty tablets were weighed and the average tablet weight determined (each tablet contains: 400 mg Paracetamol,20 mg Phenylephrine hydrochloride and 2 mg Chlorphenamine maleate). The tablets were finely powdered and

a portion of powder equivalent to the weight of one tablet was dissolved in 7 mL of Ethanol and vigorously shaken for a 20 min on a mechanical shaker, then filtrated and transferred into a 10 mL volumetric flask and the final volume was completed to 10 mL with the same solvent. This solution contains: 40 mg/ml Paracetamol, 2 mg/mlPhenylephrinehydrochlorideand 0.2 mg/mlChlorphenamine maleate).

4. PROCEDURE:

4.1 Chromatographic conditions: 1 µL of standard solutions were spotted on HPTLC glass plates (10 cm × 20 cm) pre-coated with DMDCS SilanizedBentonite (with 0.25 mm thickness). Mobile phase was used for development method, then the plates were dried at room temperature, and with suitable drier , then the spots were visualized under UV light in CAMAG UV Cabinet for assessing and marking thin layer chromatograms, or with Iodine vapor, and quantification was carried out densitometerically at λ = 250 nm for Paracetamol, λ = 220 nm for Phenylephrine hydrochloride, and Chlorphenamine maleate. This process was repeated five times for each concentration and calibration curves were obtained in the range 5-40 µg/spot for Paracetamol, 2-16 µg/spot for Phenylephrine hydrochloride and in the range 0.5 – 4 µg/spot for Chlorphenamine maleate.

4.2 Pharmaceutical formulations (Anti Cold): For Paracetamol quantification 1 µL of solution of tablets content, were spotted on HPTLC glass plates for separation of (Paracetamol, Phenylephrine hydrochloride, and Chlorphenamine maleate) and quantification was carried out densitometerically at λ = 250 nm for Paracetamol, then for Phenylephrine hydrochloride, and Chlorphenamine maleate quantification3 µL of solution of tablets content, were spotted on HPTLC glass plats for separation of (Paracetamol, Phenylephrine hydrochloride, and Chlorphenamine maleate) and quantification was carried out densitometerically at λ = 220 nm for Phenylephrine hydrochloride, and Chlorphenaminemaleate,The concentrations were calculated from the later mentioned standard curves.

5. RESULTS AND DISCUSSION

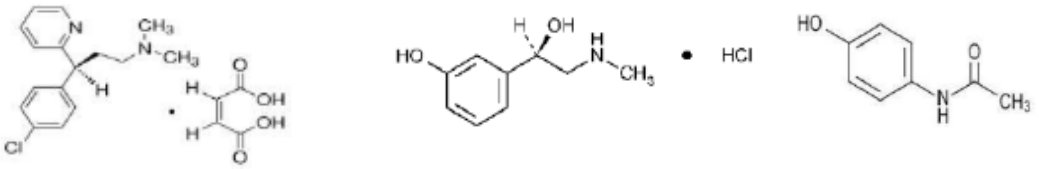
5.1 Specific Surface Area and particle size of Silanized Syrian Bentonite: Specific Surface Areaofsilanizedbentonite was determined by the adsorption of Methylene Blue; it is found that the surface areawas 21 m^2/g, and particles size distribution was determined by Zeta sizer, it is found that the prticles size of silanized treated bentonite was less than 10µm. The surface area decreased from 91 m^2/g for acidic treated Bentonite to 21 m^2/g for DMDCS Silanized Bentonite.

5.2 Spectrum infrared (IR): The infrared spectrums were Studied for each (Acidic treated Syrian Clay) (Acidic treated Syrian Bentonite), and (DMDCS Silanized Syrian Clay). New peak appears in the spectrum of (DMDCS Silanized Syrian Clay) in the region 2800-3100 cm^{-1} back to stretch C-H (Figs3 , 4) . The information provided by IR that the surface of (DMDCS Silanized Syrian Clay) has been modified with the alkenes groups.

5.3Hydrophobicity: For the estimation of the changes in the hydrophobicity after modification, we compared dispersion of the acidic treated Bentonite , and (DMDCS Silanized Bentonite) in water and Chloroform . As shown in Figure 5 the acidic treated Bentonite disperses in the water layer only. Due to the presence of hydrophobic alkyl group on the external surface of DMDCS SilanizedBentonite , the DMDCS Silanized Bentonite was suspended (found) in organic phase only at the Chloroform -water boundary.(Fig. 5).

5.4- Chromatograms processing:

The position of the spots from the mobile phase front after visualizing with Iodine vapor on the chromatographic plate for different concentrations (I) (0.5 to 4 µg/spot) of Chlorphenamine maleate ,(II) (2 to 16 µg/spot) of Phenylephrine hydrochloride, and (III) (5 to 40 µg/spot) of Paracetamol. was shown in Fig. 6.The chromatogram of mixture of Chlorphenamine maleate, Phenylephrine hydrochloride and Paracetamol (4 µg/spot for CPM, 16 µg/spot for PHE , and 40 µg/spot for PARA) can be observed with three peaks at different wavelengths (λ) at 200 to 300 nm (Fig. 7). The first peak for CPM increase to λ = 220 nm then decrease, the second for PHE increase to λ = 220 nm then decrease.



Chlorpheneramin maleate (CPM) Phenyephrine HCl (PHE) Paracetamol (PARA)

Figure .1Structures of Paracetamol , Chlorphenamine maleate ,and Phenylephrine hydrochloride.

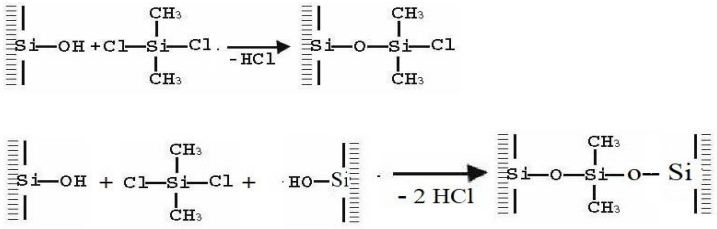


Figure .2Preparation of DMDCS Silanized Syrian Clay

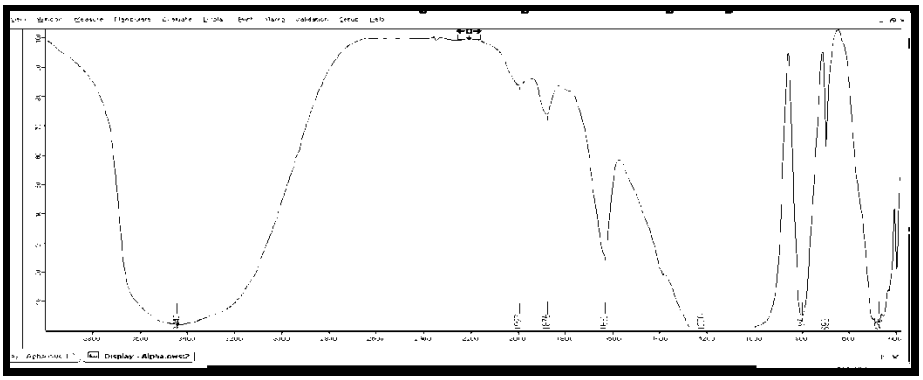


Figure .3 Infrared Spectrum of Acidic Treated Syrian Clay (Acid Treated Syrian Bentonite).

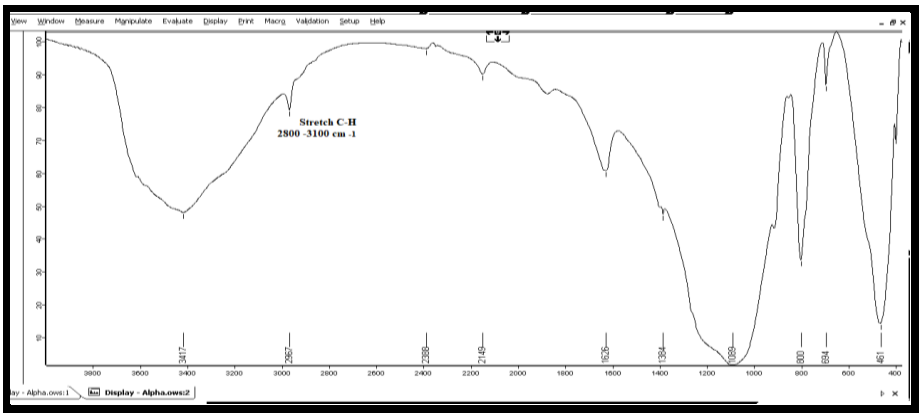


Figure .4 Infrared Spectrum of DMDCS Silanized Syrian Clay (Silanized Syrian Bentonite).

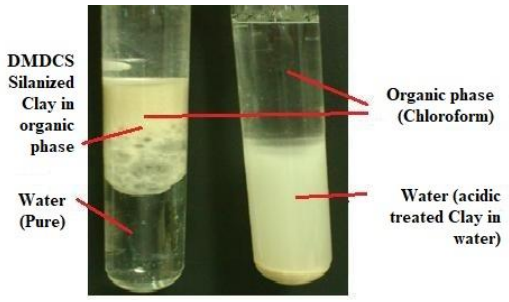


Figure 5 :Hydrophobicity of silanized clay

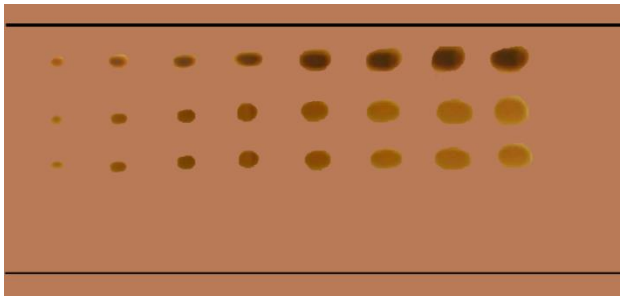


Fig. 6. HPTLC plate of standard mixtures of (I)0.5- 4 µg/spot of CPM, (II)2-16 µg/spot ofPHE and (III)5 -40 µg/spot of PARA.

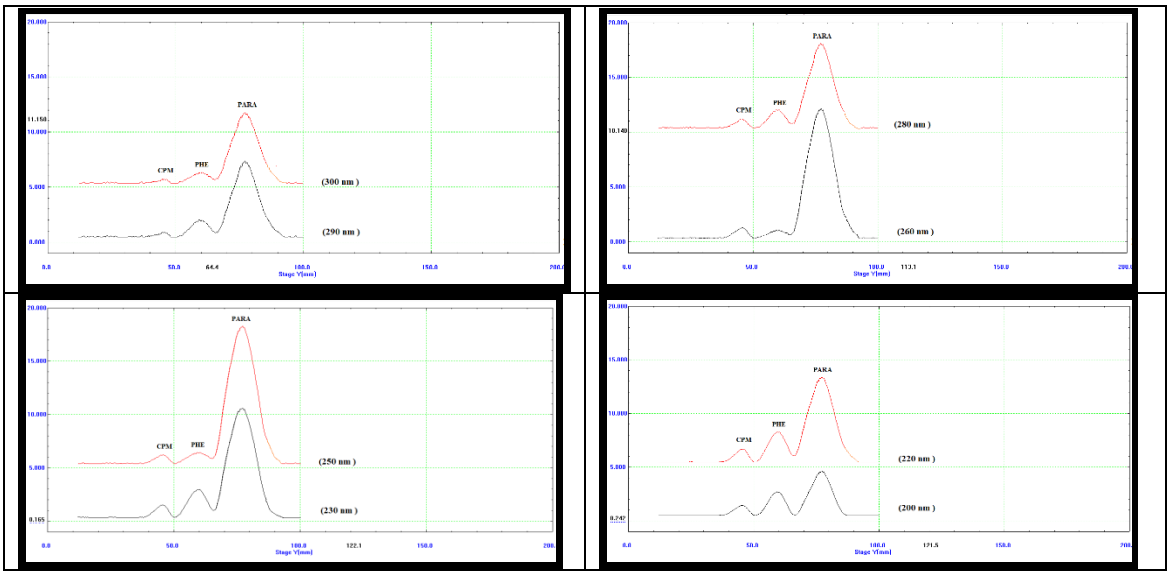


Fig.7 Chromatograms of mixture of CPM (4µg/spot), PHE (16µg/spot), and PARA (40µg/spot) at different wavelengths.

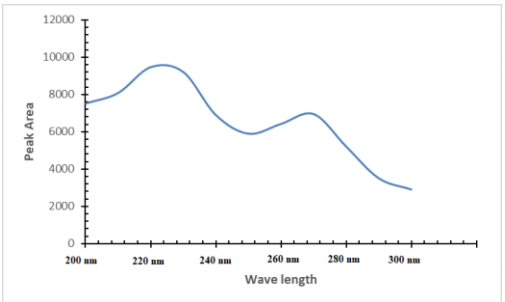


Fig.8 . Effect of wavelengths (λ) on peak areas of CPM

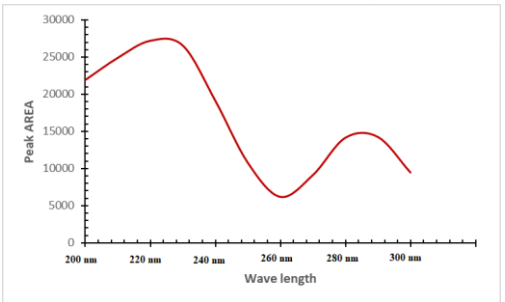


Fig.9 . Effect of wavelengths (λ) on peak areas of PHE

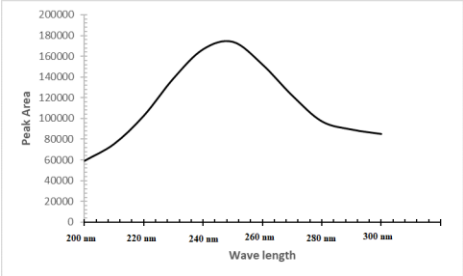


Fig.10 . Effect of wavelengths (λ) on peak areas of PARA

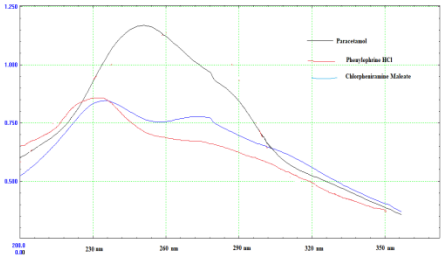


Fig.11 Spectrum Scan of Paracetamol, Chlorphenamine maleate, and Phenylephrine hydrochloride at different wavelengths.

TABEL 1 : analytical parameters of proposed method

parameter	CPM	PHE	PARA
Linearity range, µg/spot	0.5 - 4	2 – 16	5 - 40
Wavelength (nm)	220	220	250
Linear regression equation y=bx+c	Y=874.52X – 308.18	Y=1006.5X+1314.8	Y=714.66X+5802.6
Correlation coefficient R^2	0.9974	0.9932	0.9959
LOD (µg/spot)	0.1	0.02	0.02
LOQ (µg/spot)	0.33	0.07	0.07
RSD%	2.4 - 4.90	2.34 - 4.93	1.90 - 4.86

The third for PARA increase to λ = 250 nm then decrease, (Fig. 8), (Fig. 9) , (Fig. 10) & (Fig. 11). It is inferred from the (Figs. 7-11) that the best wavelengths to determination of CPM, PHE, and PARA were 220 nm, 220 nm, and 250 nm respectively, and the best wavelengths for Synchronous determination of CPM, PHE, and PARA was 220 nm.

The retardation factors (R_f) of CPM, PHE, and PARA were 0.44 , 0.61 , and 0.83 respectively.

Quantitative Evaluation: The method being validated through precision, linearity and accuracy for the determination of different standard mixtures of CPM, PHE and PARA in the range of 0.5 to 4.0 µg/spot for CPM, 2.0 to 16.0 µg/spot for PHE, and in the range of 5. 0 to 40.0 µg/spot for PARA using λ = 220 nm for Synchronous determination of three substances.The analytical parameters presented in table 1.

Application: The results of the validation verify the suitability of the proposed analytical procedure for the identification and quantitative determination of CPM, PHE and PARAin the mixture. Commercial product (that contained of the three studied Substance was analyzed in order to assaying the values of their contents using λ = 220 nm for CPM, PHE and PARA. The pharmaceutical formulation was selected for the study as the following, Tablets brand Anti Cold (manufactured by RAZI Pharmaceutical Industries, Aleppo-Syria).**:** Each tablet contains 2 mg CPM, 20 mg PHE and 400 mg PARA.

The three substances in the mentioned pharmaceutical formulation were Synchronous ly evaluated, on the same plate, reducing the time and the amount of the materials required for the analysis. The obtained results and their confidence intervals are listed in Table1.

The results are in good agreement with the results of HPLC. It can be observed that the difference between the results by HPLC and the found values by this method are less than 5% in and the relative standard deviation is not exceeding ± 5 %. The proposed method has been successfully applied to determine CPM, PHE and PARAin pharmaceutical formulations.

CONCLUSION:

In the preceding method, determination of Chlorphenamine maleate, Phenylephrine hydrochloride and Paracetamol in pure form and Tablets pharmaceutical formulations by HPTLCdensitometric method using SilanizedBentonite and mobile phase: Isopropanol – Isobutanol – Ethyl acetate – Toluene – Water - Acetic acid (15:15: 25:6:6:3) v/v. has been applied.Quantification was carried out densitometerically at λ = 220

nm for Chlorphenamine maleate, λ = 220 nm for Phenylephrine hydrochloride and λ = 250 nm for Paracetamol, and λ = 220 nm for Synchronous determination of The three substances. The retardation factors (Rf) of Chlorpheniramine maleate, Phenylephrine Hydrochloride, and Paracetamol were 0.44, 0.61, and 0.83 respectively.Calibration curves were obtained in the range of 5.0- 40.0 µg/spot for Paracetamol, and were obtained in the range of 2.0- 16.0 µg/spot for Phenylephrine hydrochloride, and in the range of 0.5 - 4.0 µg/spot for Chlorphenamine maleate (at λ = 220 nm) for standard solutions, and Tablets pharmaceutical formulations.

REFERENCES

1- Ramadan A.A., Antakli S., Sakur A.A., Using Grafiting Aleppo Bentonite in Gas Chromatographic Analysis of Hydrocarbon Compounds C5 – C12 Some AromaricCompounds and Alcohols, Res. J. of Aleppo Univ.,1994,17, 142.

2- Ramadan A.A., Antakli S., Sakur A.A., Effect of chemical grafting on the surface structure of porous bentonite as a support in gas chromatography, Qatar Univ. Sci. J., 1994, 14 (C): 175-177.

3- Ramadan A.A., Antakli S., Sakur A.A., Gas Chromatographic Analysis of Some Normal Alcohols Using Column of Grafted Aleppo Bentonite, Res. J. of Aleppo Univ.,1995,19, 131.

4- SakurA.A., Gas chromatographic analysis using Aleppo bentonite columns deactivated by grafting, M. Sc. Thesis in Chem., Aleppo University, Syria, 1995.

5- F. BERGAYA, G. LAGALY, General introduction: Clays, clays minerals, and clay science, Cap.1 In: Bergaya F, Theng BKG, Lagaly G, editors. Handbook of Clay Science, Elsevier, Amsterdam, ,2006, p. 1.

6- Ramadan A.A., Antakli S., Sakur A.A., Characterization of the Grafted Bentonite Supports by Gas Chromatography, Asian Journal of Chemistry, 1999, 11 (4), 1343.

7- Sakur A.A., Studying of some chromatographic supports prepared from bentonite and using it in chromatographic analysis , Ph. D. Thesis in Chem., Aleppo University, Syria, 2000.

8- Ramadan A.A., Sakur A.A., and Lahmek M., Separation and Determination A Mixture of Cyclic and Aromatic Compounds Using Column of Bentonite Grafted With Silicone SE-52 by Gas Chromatography,Res. J. of Aleppo Univ., Syria, 2003, 39, 13-26.

9- Sakur A.A., Preparation of New Chromatographic Support from Bentonite Grafted by Silicone OV-101, Research Journal of Aleppo Univ., Medical Sciences Series, 2004, 49.

10- Lahmek M., Sakur A.A., Ramadan A.A., Modification of Algerian bentonite and using it as support in gas chromatography, Asian Journal of Chemistry, 2004 16 (1), 89.

11- Abdul Ghafour O., Thin layer chromatography using Aleppo bentonite.,M. Sc. Thesis in Chem., Aleppo University, Syria, 1998.

12- Ramadan A.A., Bodakji A., Mahmoud I., TLC-densitometric determination of vitamins B_1, B_6 and B_{12} in pure and pharmaceutical formulations using treated aleppo bentonite. *Asian J Chemy*, 2010; 22(4), 3283-3291.

13- Ramadan A.A., Al-Akraa H., Maktabi M., TLC Synchronous determination of valsartan and hydrochlorothiazide in pure form and in tablets using butyl-modified aleppo bentonite, Int J Pharm PharmSci, 2013; 5 (3): 762-769.

14- Sakur A. A., Mannaa F., ZeinEddin M. Y., Preparing a New Chromatographic Support from Local Syrian Clay and using It in Thin Layer Chromatography for Synchronous Determination of Paracetamol, Caffeine and Aspirin in Tablets,Research Journal of Pharmacy and Technology (RJPT) 2021,14(4).

15- Renger B., Planar J. ,Chromatogr, 1999, 12, 58 .

16-S.C. Seetman, editor. Martindale: The Complete Drug Reference. 37th Ed. London: The Pharmaceutical Press: London; 2011, 112. p. 1499.

17-Osol A., "Remington's Pharmaceutical Sciences". In *Analgesics and Antipyretics*. Philadelphia College of Pharmacy, Easton, PA, 1980, pp. 1056,1076–77.

18-The United States Pharmacopeia and National Formulary. The official compendia of standards, Asian ed., USP 30-NF 25, 2015.

19- British Pharmacopoeia Commission., *British pharmacopoeia*. London: Stationery Office,2014.

20- Budavari S, The Merck Index, 12th Edn, Merck Research Lab, Division of Merck and Co., Inc., Whitehouse Station, NJ, 1996, 9.

21- Nogowska M, Muszalska I, Zajac M, Synchronous spectrophotometric determination of acetyl salicylic acid, Paracetamol and caffeine in pharmaceutical preparations. Chem. Anal. (Warsaw), 1999, 44 (6), 1041-1048.

22- Dinc E, Yucesoy C., Onur F, Synchronous spectrophotometric determination of mefenamic acid and paracetamol in pharmaceutical preparations using ratio spectra derivative Spectrophotometry and chemometric methods. Journal of Pharmaceutical and Biomedical Analysis. 2002, 28 (6), 1091-1100.

23- Zarapkar SS, Hulkar UP, Bhandari NP, Reverse phase HPLC determination of ibuprofen, paracetamol and methocarbamol in tablets. Indian Drugs, 1999, 36 (11), 710-713.

24- Liang YR., Hu J, Wu HL, Le LY, Wang WJ, Determination of paracetamol and amantadine HCl tablets in pharmaceuticals by HPLC, TLC and CRM. YaowuFenxiZazhi (Chinese),
2006, 26 (3), 411-414.

25- Argekar AP, Sawant JG, Synchronous determination of paracetamol and mefenamic acid in tablets by HPTLC. Journal of Planar Chromatography, modern TLC, 1999, 12 (5), 361-364.

26- Godejohann M, Tseng LH, Braumann U, Fuchser J, Spraul M, Characterization of paracetamol metabolite using on-line LC-SPENMR-MS and a Cryogenic NMR-Probe.
Journal of Chromatography A , 2004, 1058 (12), 191-196.

27- Ekgasit S, Pattayakkorn N, Tongsakul D, Thammancharoen C, Kongyou T, A Novel ATR FTIR micro spectroscopy technique for surface contamination analysis without interference of substrate. Anal. Sci., 2007, 23 (7), 863-868.

28- Prabakar SJR, Narayanan SS., Amperometric determination of paracetamol by a surface modified cobalt hexacyanoferrate graphite wax composite electrode. Talanta, 2007, 72 (5), 1818-1827.

29- LlorentMarinez EJ, Satinsky D, Solich P, OregaBarrales P, Molina Diaz A, Fluorimetric SIA optosensing in pharmaceutical analysis Determination of Paracetamol. Journal of Pharmaceutical and Biomedical Analysis, 2007, 45 (2), 318-321.

30- Emre D, Ozaltin N, Synchronous determination of paracetamol, caffeine and propyphenazone in ternary mixtures by micellar electro kinetic capillary chromatography. Journal of Chromatography B; Analytical Technologies in the Biomedical and Life Sciences, 2007, 847 (2), 126-132.

31- Ivana S, Goran N, Vladimir B, Development and validation of spectrophotometric metho for phenylephrine hydrochloride estimation in nasal drops formulations. Macedonian Journal of Chemistry and Chemical Engineering, 2008, 27(2), 149–156.

32- Gary WS, David EH, Synchronous stabilityindicating determination of phenylephrine hydrochloride, phenylpropanolamine hydrochloride, and guaifenesin in dosage forms by reversed-phase paired-ion HPLC. JPS, 1983,72(1), 55–59.

33- María RG, Roberto AO Luis DM, María FS,Synchronous determination of dextromethorphan, diphenhydramine and phenylephrine in expectorant and decongestant syrups by capillary electrophoresis. Pharm Biomed Anal. 2002 15;30 (3), 791-9.

34- Ugo RC, Determination of phenylephrine hydrochloride, chlorpheniramine maleate, and methscopolamine nitrate in tablets or capsules by liquid chromatography with two UV bsorbance detectors in series. Journal of AOAC International, 2006, 89(1), 53-57.

35- Ruby R, Mary Mand John K, Full and fractionated experimental designs for robustness testing in the high-performance liquid chromatographic analysis of codeine

phosphate, pseudoephedrine hydrochloride and chlorpheniramine maleate in a pharmaceutical preparation. Journal of Chromatography A,2000,870 (1-2), 18, 45-51.

36- Nora HA, Determination of phenylephrine hydrochloride and chlorpheniramine maleate in binary mixture using chemometric-assisted spectrophotometric and high-performance liquid chromatographic-UV methods. Journal of Saudi Chemical Society, 2010, 14(1), 15-21.