TWO DIFFERENT CHROMATOGRAPHIC METHODS FOR SIMULTANEOUS DETERMINATION OF COMMON COLD DRUGS AMBROXOL HYDROCHLORIDE, PSEUDOEPHEDRINE HYDROCHLORIDE, LEVOCETERIZINE DIHYDROCHLORIDE AND DESLORATADINE, IN PURE FORMS, AND IN PHARMACEUTICAL DOSAGE FORMS.

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ABSTRACT

Two chromatographic methods were developed for simultaneous determination of some drugs used in common cold. First method is TLC-Densitometric method for the simultaneous estimation of Ambroxol hydrochloride (AMB), Pseudoephedrine hydrochloride (PSE) and Desloratadine (DES) in pure form and in pharmaceutical dosage form, the best resolution was obtained by the use of mobile phase (Ethyl acetate: methanol: ammonia), (14: 0.8: 0.5, *v/v/v*), the scanning of spots was performed at 254 nm in concentration range (1-40 ug/spot), (5-35 ug/spot),(0.2-2) ug/spot for AMB, PSE and DES, respectively. With LOD 0.262, 0.358 and 0.006 ug/ml. And LOQ 0.793, 1.09 and 0.018 ug /ml for AMB, PSE and DES, respectively. Second method is HPLC-UV Method for the Simultaneous determination of Ambroxol hydrochloride(AMB),Pseudoephedrine hydrochloride(PSE), Desloratadine (DES) and levoceterizine dihydrochloride (LVC) in quaternary mixture by using mobile phase consisted of acetonitrile: 0.01 M phosphate buffer (PH 5.5) (50:50, v/v) in an isocratic mode through inertsil C8 (250mm x 4.6mm, 5 µ) column at a flow rate of 1.3 ml/min. UV detection was carried out at 215 nm in concentration range (1.6-40), (3.2-40) , (0.6-9), (2.4-8.4) ug/ml for AMB, PSE , DES and LVC, respectively .With LOD 0.428, 0.180, 0.125 and 0.124, ug/ml And LOQ 1.299 , 0.550, 0.379, and 0.375ug/ml for AMB, PSE , DES and LVC, respectively The validation of the proposed methods was done according to ICH guidelines.

Keywords: Ambroxol, Desloratadine Hydrochloride, levoceterizine dihydrochloride and pseudoephedrine hydrochloride, HPLC, UV, TLC.

Introduction:

Ambroxol hydrochloride **(AMB)** 4-[(2-Amino-3, 5-dibromophenyl) methylamino] cyclohexan-1-ol hydrochloride is a drug that breaks up phlegm, used in the treatment of respiratory diseases associated with viscid or excessive mucus. **Pseudoephedrine Hydrochloride (PSE)** (1S,2S)-2-(Methyl amino)-1-phenylpropan-1-ol hydrochloride is used to relieve nasal congestion caused by colds,allergies,and hay fever **Desloratadine Hydrochloride (DES)** 8-Chloro-11-(piperidin-4-ylidene)-6,11-dihydro-5H-benzo[5,6]cyclohepta[1,2-b]pyridine is an antihistamine. It is used to relieve the symptoms of hay fever and allergy symptoms. **Levocetrizine Hydrochloride (LVC**) 2-[2-[4-[(R)-(4-Chlorophenyl)-phenylmethyl]piperazin-1-yl]ethoxy] acetic acid; dihydrochloride is used to relieve runny nose and sneezing. Levocetirizine dihydrochloride and Desloratadine Are the most commonly used second-generation H_1 receptor blockers **B. G. Katzung, (2017)**, Fig (1).

All of the previous drugs are used in treatment of common cold which is caused by viral infection. The virus can spread through droplets in the air when someone who is sick coughs, sneezes and talks. Many types of viruses can cause a common cold. **K. Whalen, Lippincott, (2015).**

Many analytical methods have been published for the determination of Ambroxol (AMB), Desloratadine Hydrochloride(DES), levoceterizine dihydrochloride(LVC) and pseudoephedrine hydrochloride (PSE) alone, or in combination by TLC and HPLC **A. Dinakar , et al, (2010), A. Chaudhary , et al,(2012)** , **(B.P.)The British Pharmacopeia,(2020), Ch. Krishnaiah ,et al** , **(2012), Ch. Rao and L. Kalyani,(2017), D. Manjunatha, and N. Itagimatha (2019** ,**F. Kamau , et al, (2016), H. Hashem ,H. El-Say,(2020**], **J. Kumar ,(2013), M. Deshpande, et al, (2010), P. Jain,(2010**), **S. Lade and Y. Prasad,(2015)** , **U. S. P. C.(2015**), **for <u>AMB,</u> A. Reddy , et al**, **(2010),D. Sangeetha,et al,(2012)** ,**E. Khamis , et al,(2012), L. Latrous , et al,(2020**), **K. Patel, et al,(2015), <u>for DES,</u> A. Mirza, et al, (2010), A. Rote, V. Niphade, (2011), B. Ishaq, et al,(2015) J. Reddy, et al,(2011)<u>,for LVC</u>**, **A. Bozdoan,et al,(2011) D. Mohamed ,et al,(2019),E. Abdelaleem, F. Ibrahim, et al, (2015) N. Abdelwahab,(2017)** , **M. Loueslati,et al,(2009) S. Lade and Y. Prasad,(2015) <u>for PSE</u>** but no method in their mixtures.

The aim of the present work is to develop two simple, accurate, sensitive and precise methods for the determination of these antihistaminic drugs either alone or in combination with other related drugs.Also to the analyse choosen drugs in their pure form and in commercially pharmaceutical forms.

TLC-densitometry is commonly applied for the separation and analysis of many pharmaceuticals. The plan of this work also includes the use of this technique for the determination of AMB, PSE and DES in pure form and in their pharmaceutical products.

HPLC is a powerful analytical technique for the separation and quantitation of the pharmaceutical products. It is incorporated in the plan of the present work for determination of some H1 antihistaminic drugs including Desloratadine and

Levoceterizine in combination with other drugs including Ambroxol hydrochloride (AMB), and Pseudoephedrine hydrochloride (PSE).

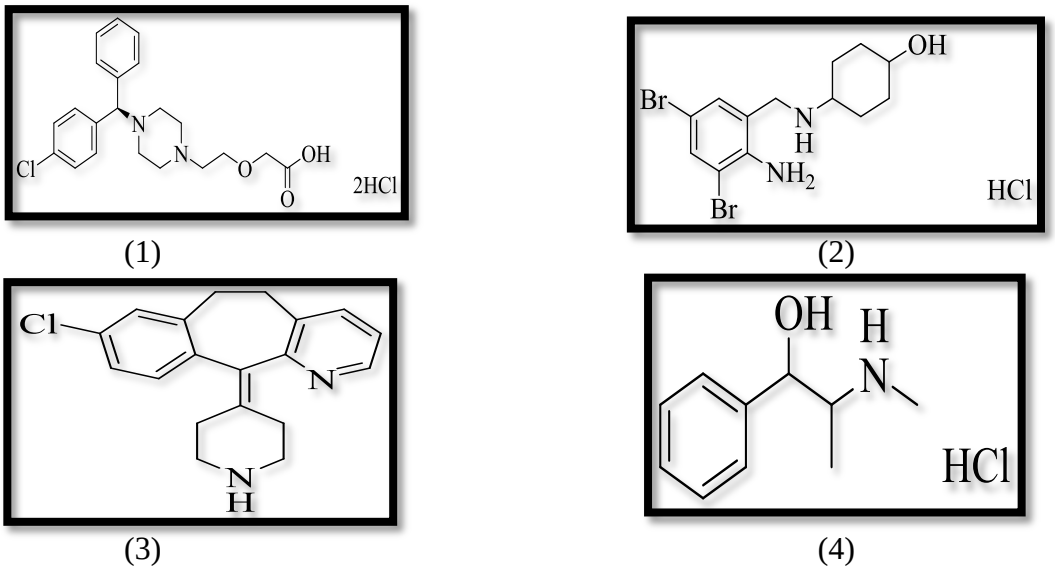

Figure (1) Chemical structure of (1) levoceterizine dihydrochloride (LVC) solubility =0.0658mg/ml in water (2) Ambroxol hydrochloride,solubility =5mg/ml in water ,(3) Desloratadine Hydrochloride (DES), Solubility = 30mg/ml in organic solvent (ethanol,DMSO,and DMF)(4) pseudoephedrine hydrochloride (PSE) solubility =8.26mg/ml in Water.

Experimental

1-Instrumentation

- A liquid chromatographic system (Thermo Fisher Scientific Dionex UltiMate 3000 Series, Germany) equipped with LPG-3400SD pump and MWD-3000 diode array (UV) detector was used. Data acquisition and instrument control was performed using Chromeleon V.7.2 SR2 Build 6394 data system software. Manual injector with 20 µl injector loop was used with an Agilent syringe, LC 50 µl, CA, U.S.A. The chromatographic separation was performed on inertsil C8 (250mm x 4.6mm, 5 µ).

- The pH measurements were carried out using a pH meter (Jenway glass electrode no. 924005-BO3-Q11C, Essex, UK).

- CAMAG TLC scanner 3 S/N 130319 with software winCATS (CAMAG, Muttenz, Swizerland), the scanning mode was absorbance mode, with scanning speed 20 mm/s. the source of radiation was deuterium lamp and the outputs were chromatogram and integrated peak area.

- Aluminum TLC sheets (20×20 cm) coated with silica gel 60 F_{254} (Merck, Darmstadt, Germany), with 0.25 mm thickness.

- Ultrasonic sonicator (Elma Elmasonic E30H, Germany).

- Centrifuge (OHAUS, frontier 5706, Germany).

- Magnetic stirrer, a Bandelin Sonorox, model Rx 510S (Budapest, Hungary)

2-Chemical and reagents

Methanol HPLC grade; Fisher Scientific (united kingdom), **Ethyl acetate analytical reagent**; El Nasr pharmaceutical chemicals Co., (Egypt); **phosphate buffer** (potassium dihydrogen phosphate, Sigma Aldrich, Germany); **Acetonitrile HPLC grade**, Carlo Erba, France.

Raw materials:

Ambroxol hydrochloride (AMB) 4-[(2-Amino-3, 5-dibromophenyl) methylamino] cyclohexan-1-ol hydrochloride (Its purity was found to be 99.7% (J.Kumar.etal), lot number; 1722124) was supplied by Medicare limited, India.

Desloratadine Hydrochloride (DES) 8-Chloro-11-(piperidin-4-ylidene)-6,11-dihydro-5H-benzo[5,6]cyclohepta[1,2-b]pyridine (Its purity was found to be 100.1% (B.P), lot number; 016FE030) was supplied by Glenmark, India.

Levocetrizine Hydrochloride (LVC) 2-[2-[4-[(R)-(4-Chlorophenyl)-phenylmethyl]piperazin-1-yl]ethoxy] acetic acid; dihydrochloride. (Its purity was found to be 99.97% (U.S.P), lot number; 05100018RM) was supplied by EGPI, Egypt.

Pseudoephedrine Hydrochloride (PSE) (1S,2S)-2-(Methyl amino)-1-phenylpropan-1-ol hydrochloride. (Its purity was found to be 100% (U.S.P), lot number; WS 02117040) was supplied by Embio Co., India

Market samples:

Nucope AD® tablet (labeled to contain 60 mg AMB,30mg PSE and 5mg DES) in Indian Market not in Egypt.

Mucosolvan® tablet (labeled to contain 30 mg of ambroxol hydrochloride, batch number: 01180201) was manufactured by Boehringer Ingelheim, Germany.

Deslorat® tablet (labeled to contain 5 mg of desloratadine, batch number: 1702527) was manufactured by Eva Pharma, Egypt.

Levcet® tablet (labeled to contain 5 mg of levocetirizine dihydrochloride, batch number: 1831918) was manufactured by Marcyrl, Egypt.

Decongess SR® capsule (labeled to contain 120 mg of pseudoephedrine hydrochloride, batch number: 6718929) was manufactured by Pharonia, Egypt.

3- Preparation of stock and working solutions:

3.A. Stock solutions:

3-A. a Stock solutions for TLC:

1-AMB standard stock solution (12.5 mg/ml) In methanol.
2-PSE standard stock solution (12.5 mg/ml) In methanol.
3- DES standard stock solution (4 mg/ml) In methanol.

3.A.b. Stock solutions for HPLC:

1-AMB standard stock solution(1 mg/mL)in using water :acetonitrile (50: 50, v/v).

2- DES standard stock solution(1 mg/mL)in using water :acetonitrile (50: 50, v/v).

3- LVC standard stock solution(1 mg/mL)in using water :acetonitrile (50: 50, v/v).

4- PSE standard stock solution(1 mg/mL)in using water :acetonitrile (50: 50, v/v).

3.B.Working solutions:

3.B.a. Working solutions for TLC:

1-AMB working solution (10 mg/mL): An accurate aliquot (8 mL) of AMB stock solution was transferred into a 10 mL volumetric flask and the volume was completed with methanol.

2- PSE working solution (10 mg/mL): An accurate aliquot (8 mL) of PSE stock solution was transferred into a 10 ml volumetric flask and the volume was completed with methanol.

3-DES working solution (2 mg/mL): An accurate aliquot (5mL) of DES stock solution was transferred into a 10 mL volumetric flask and the volume was completed with methanol.

3.B.b Working solutions for HPLC:

Further dilution was carried out by transferring 40 mL of each stock solution of AMB and PSE and 30 mL of each stock solution of DES and LVC into four separate 100 mL volumetric flasks and completed to volume using mobile phase to obtain standard working solutions (400 µg/mL for AMB and PSE) and (300 µg/mL for DES and LVC).

3.C. Preparation of laboratory prepared mixtures:

For TLC method: Different aliquots of AMB, PSE and DES working solutions equivalent to (13-37 mg), (6.5-33 mg) and (1.08-1.6 mg), respectively were transferred into three series of 10 mL volumetric flasks and the volume was completed with methanol.

For HPLC method: Four different laboratory mixtures were prepared. Accurate aliquots from stock solutions equivalent to (1250-3400 µg), (250-750 µg), (250-690 µg) and (1250-3500 µg) of AMB, DES, LVC and PSE, respectively were transferred into a set of 100 mL volumetric flasks. The solutions were completed to volume with mobile phase.

4-Chromatographic conditions:

for TLC Method : was performed on precoated thin layer chromatographic plate (20 cm x 20 cm) coated with 60 F254 Silica as stationary phase. The developing system ethyl acetate: methanol: ammonia (14 : 0.8 : 0.5 , v/v/v) at room temperature , The spots were 10 mm apart and 10 mm from the bottom edge of the plate. The chromatographic tank was pre-saturated with. The plates were developed (over a distance of 80 mm) in an ascending order. The plates were removed, air-dried and the spots were scanned on a Camag TLC scanner operated in the absorbance mode at 254 nm.

for HPLC Method: was performed using inertsil C8(250mm x 4.6mm, 5 µ)column as Stationary phase and isocratic elution mode at room temperature (0.01 M phosphate buffer (PH 5.5):acetonitrile (50:50, v/v) as Mobile phase. The prepared mobile phase was filtered through 0.45 um nylon filter membrane and degassed by sonication at flow rate: 1.3 ml/min. Injection volume: 20 ul and UV detection at wavelength 215 nm.

Procedure

1. Construction of calibration curve:

(a) TLC Method

Accurately measured aliquots of AMB, PSE and DES working solutions equivalent to (1-40 mg), (5-35 mg) and (0.2 -2 mg), respectively were transferred into three series of 10 mL volumetric flasks and each flask was completed to volume with methanol. Ten µL of each solution were applied in triplicates to a thin layer chromatographic plate (10 cm x 10 cm) as spots using 50 µL Hamilton micro syringe. The spots were 10 mm apart and 10 mm from the bottom edge of the plate, having diameter of 3 mm. The chromatographic tank was pre-saturated with the developing system ethyl acetate: methanol: ammonia (14: 0.8: 0.5, v/v/v) for 30 minutes at room temperature. The plates were developed (over a distance of 80 mm) in an ascending order. The plates were removed, air-dried and the spots were visualized under a UV lamp at 254 nm. Calibration curves representing the relationship between area under peaks (AUP)and their corresponding concentrations in µg.spot^{-1} were constructed and the regression equations were then computed.

(b) HPLC Method

Accurately measured aliquots of AMB, DES, LVC and PSE working solutions equivalent to (16-400 µg), (6-90 µg), (24-84 µg) and (32-400 µg), respectively were transferred into four separate series of 10 mL volumetric flasks and each flask was

completed to volume with mobile phase. Twenty µL from each solution was injected onto the column and the chromatogram was recorded. The recorded PA of AMB, DES, LVC and PSE were then plotted versus the corresponding concentrations in µg/mL to obtain the calibration curves of AMB, DES, LVC and PSE. and the regression equations were then computed.

(2.A.) Application of the proposed TLC densitometric method for the determination of AMB, PSE and DES in bulk:

Different aliquots of AMB, PSE and DES working solutions equivalent to (5-36 mg), (10-32 mg) and (0.3-1.8 mg), respectively were transferred into three series of 10 mL volumetric flasks and the volume was completed with methanol. The PAs were recorded and AMB, PSE and DES concentrations were calculated using their regression equations

(2.B.) Application of the proposed HPLC method for the determination of AMB, PSE and DES in bulk:

Different aliquots of AMB, DES, LVC and PSE from working solutions equivalent to (80-940 µg), (30-210µg), (75- 165 µg) and (120-940 µg), respectively were transferred into four series of 25 mL volumetric flasks and the volume was completed with the mobile phase. The PA were recorded and AMB, DES, LVC and PSE concentrations were calculated using their regression equations

(3.A) Application of the proposed TLC densitometric method for the determination of AMB , PSE and DES in laboratory prepared mixtures:

The PA of the laboratory prepared mixtures were recorded at 254 nm, the method was completed as mentioned before and stored in the computer. The concentrations of AMB, PSE and DES were calculated using their regression equations.

(3.B) Application of the proposed HPLC method for the determination of AMB, DES,LVC and PSE in laboratory prepared mixtures:

The PA of the laboratory prepared mixtures were recorded at 254 nm, the method was completed as mentioned before and stored in the computer. The concentrations of AMB, PSE and DES were calculated using regression equations.

(3.C) Application of the proposed TLC densitometric method for the determination of ambroxol , pseudoephidrin and desloratadine in Nucope AD ®tablet:

Corresponding amounts of Mucosolvan® tablet, Decongess® capsule and Deslorat® tablet were weighted, finely powdered and mixed to simulate the ratio of these drugs in the Indian drug Nucope AD® tablets which contain 60 mg AMB, 30 mg PHE and 5 mg DES. An accurately weighted powder equivalent to (60 mg of AMB, 30 mg of PSE and 5 mg DES) was accurately transferred into a 10 mL volumetric flask, 5 mL of methanol were added, and the mixture was sonicated for 30 minutes. The

volume was completed with methanol, then the mixture was mixed well and filtered, to prepare sample stock solutions (6 mg/mL of AMB, 3 mg / mL of PSE and 0.5 mg/mL DES). Different aliquots from the prepared sample stock solution were accurately transferred into a series of 5 mL volumetric flasks and the volume was completed with methanol in order to obtain concentrations of (12-30 µg/spot) of AMB , (6-15 µg/spot) of PSE and (0.6 – 1.67 µg/spot) of DES.

(3.D) Application of the proposed HPLC method for the determination of ambroxol,desloratadine hydrochloride, levoceterizin dihydrochloride and pseudoephedrine hydrochloride in their pharmaceutical dosage form:

Corresponding amounts of each of Mucosolvan® tablets, Deslorat® tablets, Decongess SR® capsules and Levcet® tablets were accurately weighed, finely powdered and mixed. Accurate amounts of the powdered dosage forms equivalent to (30 mg AMB, 5mg DES, 30 mg PSE and 5 mg LVC), respectively were weighted and transferred into a 50 mL volumetric flask, 30 mL of mobile phase was added, and the mixture was sonicated for 15 minutes. The volume was completed with solvent then the mixture was mixed well and filtered, to prepare sample stock solution of 600 µg/mL AMB, 100 µg/mL DES, 100 µg/mL LVC and 600 µg/mL PSE. Different aliquots from the prepared sample stock solutions equivalent to (50-200 µg) of AMB, (10-50 µg) of DES, (10-50 µg) of LVC and (50-200 µg) of PSE were accurately transferred into a series of 10 mL volumetric flasks and the volume was completed with the mobile phase. The experiment was repeated applying the standard addition technique and the regression equations (1), (2), (3) and (4) in Table (2) were used to calculate the recovered concentrations of labelled and the added AMB, DES, LVC and PSE. Table (13) shows that the mean percentage recoveries of AMB, DES, PSE and LVC in laboratory prepared mixtures were found to be 99.87 ± 0.94, 101.15 ± 0.38, 100.13 ± 1.41 and 99.22 ± 0.54, respectively.

5- Results and discussion

(A) TLC Method

Thin layer chromatography is a very useful technique because of the low cost of the analysis. TLC offers the possibility of automatic application of the samples, Compared to other chromatographic techniques, TLC is a simple, rapid and flexible technique allowing sensitive parallel processing of many samples on one plate. Also, quantitative TLC method proved to be an easy method for the determination of mixtures in quality control laboratories. In this section, a TLC densitometric method was suggested for the quantitative determination of AMB, PSE and DES, in bulk, in drug product and in laboratory prepared mixture of them similar to Nucope AD® tablet in the Indian market. The method is based on difference in R f values between AMB, PSE and DES.

Optimization of TLC method: Trials were carried out using different developing systems in order to achieve the best separation between AMB, PSE and DES. Finally the combination of [ethyl acetate: methanol: ammonia 14 :0.8 :0.5 V/V/V] resulted in well separated, compact spots which showed symmetrical peaks on the

densitogram. The Rf values of AMB, PSE and DES were 0.76, 0.48 and 0.14 respectively. After choosing the best developing system, the suitable wavelength for scanning the plate was investigated. The scanning wavelength was 254 nm. as shown in Figure (2).

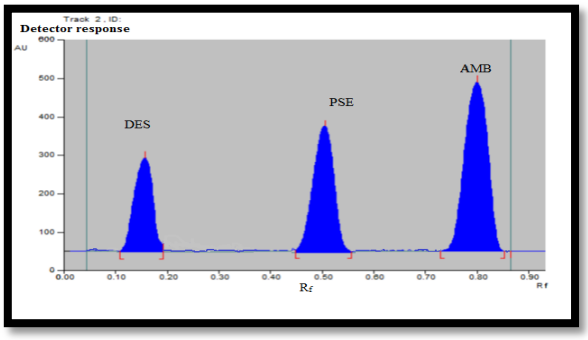

Figure (2): TLC chromatogram of a laboratory prepared mixture of DES (1.5 mg/spot), PSE (9 mg/spot) and AMB (18 mg/spot) using ethylacetate: methanol: ammonia (14 : 0.8 : 0.5, v/v/v) as developing system.

(B) HPLC Method

HPLC acquires a high degree of versatility not found in the chromatographic systems and it has the ability to easily separate a wide variety of chemical mixtures, it allows the determination of many individual components in a mixture using one single procedure.

In this work, HPLC method was developed and validated for the simultaneous determination of the previous mixture (AMB, DES, PSE). In addition to LVC as related drug was determine simultaneous by HPLC. Chromatographic separation was achieved on Inertsil C_8 (46 x 250mm, 5µm) column at room temperature, using a mobile phase consisting of 0.01 M phosphate buffer (pH 5.5):acetonitrile (50:50, v/v) using isocratic elution mode at flow rate 1.3 ml/min. UV detection was carried out at 215 nm.

Optimization of HPLC method: Many trials have been performed to achieve the best separation between the peaks of AMB, DES, LC and PSE Various mobile phase compositions containing different ratios of organic and aqueous phases were attempted in an isocratic mode and the ratio which gave the best separation was applied.

The addition of phosphate buffer was important to give sharp and symmetric peaks and it was more efficient than using unbuffered water.

The effect of pH on the separation of the analytes was studied. Different pH values were tested (3, 5.5, 6.5 ,8). It was found that pH 5.5 was the most suitable one as it achieved the best separation between the four analytes in the reasonable run time (< 5 min) and with good resolution between all peaks.

The effect of the flow rate on the retention times was also investigated and a flow rate of 1.3 ml/min was optimal for good separation in an analysis time of less than 5 min.

Proper choice of the detection wavelength is crucial for sensitivity of the method. Quantitation was achieved with UV detection at 215nm based on reproducible peak area for each of the analyzed drugs along with the highest sensitivity.

It was therefore concluded that a mobile phase consisting of 0.01 M phosphate buffer (pH 5.5): acetonitrile (50:50, v/v) using isocratic elution mode at flow rate 1.3 ml/min gave optimum resolution, clear baseline separation with reasonable retention times and no tailing of peaks of the studied drugs. UV detection was carried out at 215 nm. Good resolution was achieved with retention times 2.54, 3.21, 3.617, 4.04 min for PSE, LC, AMB, DES, respectively, Figure (3)

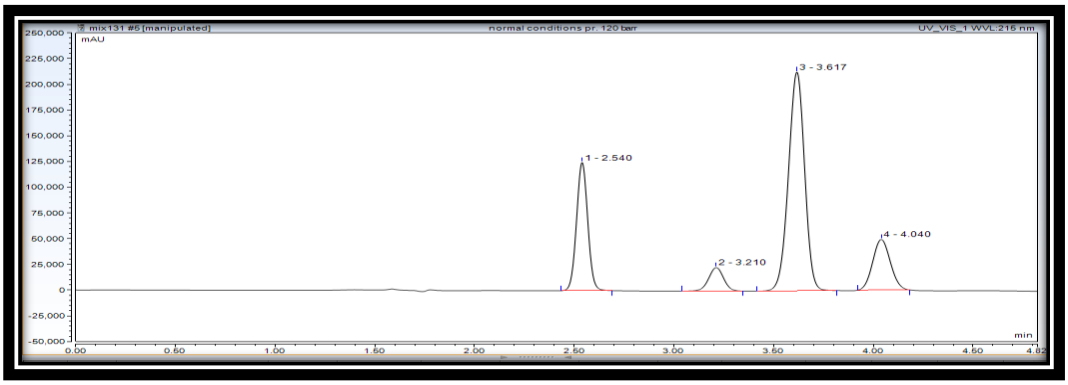

Figure (3): HPLC chromatogram of PSE (30 µg/mL) (tR= 2.540 min), LVC (5µg/mL) (tR = 3.210 min), AMB (30 µg/mL) (tR = 3.617 min) and DES (5 µg/mL) (tR = 4.040 min)

4- Method Validation

Method validation was applied according to the International Conference on Harmonization (ICH) guidelines. Including linearity, specificity, accuracy, precision, robustness, limit of detection (LOD) and limit of quantification (LOQ). **(ICH Steering Committee, 1996).**

Linearity and range:

FOR TLC method Linear relationships were obtained over the ranges of (1- 40 µg/spot) , (5-35 µg/spot) and (0.2- 2 µg/spot) for AMB , PSE and DES, respectively , and the calibration parameters are demonstrated in Table (1).

Table (1): Validation parameters and results obtained by the TLC-densitometric method proposed for the simultaneous determination of AMB, PSE and DES

Parameters	AMB	PSE	DES
Retention factor (R_f)	0.76	0.48	0.14
Measurement wavelength (nm)	254		
Linearity range (µg/spot)	1-40	5-35	0.2-2
LOD (µg/spot)	0.262	0.358	0.006
LOQ (µg/spot)	0.793	1.09	0.018
Regression coefficient (r)	0.9997	0.9995	0.9999
Slope	1990.4	157.0666667	7407.87
Standard deviation of slope (S_b)	6.52	0.72	1.50
Confidence limit of slope	1990.407±44.324	157.067 ± 4.876	7407.87±78.192
Intercept (a)	417.9631097	-4.883	9.018070053
Standard deviation of intercept (S_a)	157.8797681	17.067	13.11745977
Confidence limit of intercept	417.963± 1073.72	-4.883 ± 116.073	9.018±89.21
Standard error of estimation	524.84	42.42	43.58
Intraday precision[(a)]	0.19,0.47,0.29	0.88, 1.05, 0.18	0.582, 0.524, 0.276
Interday precision[(b)]	0.30, 0.62, 0.27	0.50, 0.86, 0.37	0.11, 0.39, 0.18

(a) The intraday (n=3) % RSD of three concentrations of AMB (16.4, 20.5, 24.6 µg/spot), PES (16, 20, 24 µg/spot) and of DES (0.88, 1.1, 1.32 µg/spot) which repeated three times within the day.

(b) The interday (n=3), % RSD of three concentrations of AMB (16.4, 20.5, 24.6 µg/spot) , PES (16, 20, 24 µg/spot) and of DES (0.88, 1.1, 1.32 µg/spot) which repeated in three consecutive days**.**

FOR HPLC method attained linearity over concentration range (1.6-40 µg/mL) for AMB, (0.6 -9 µg/mL) for DES, (2.4-8.4 µg/mL) for LVC and (3.2 -40 µg/mL) for PSE Figures (7), (8) (9) and (10). and the calibration parameters are demonstrated in Table (2).

Table (2): Validation parameters and results obtained by the RP-HPLC method proposed for the simultaneous determination of AMB, DES, PSE and LVC

Parameter	PSE	LVC	AMB	DES
Retention time (min)	2.540	3.210	3.617	4.040
Wavelength of detection (nm)	215			
Range of linearity (µg / mL+)	3.2-40	2.4-8.4	1.6-40	0.6-9
Regression equation	PA=265.402C_{PSE} +102.65199	PA =325.11 C_{LVC} +91.944	PA =717.85 C_{AMB}-275.8	PA =765.32 C_{DES} +352.4
Regression coefficient (r)	0.9992	0.9990	0.9992	0.9990
LOD (µg / mL)	0.180	0.124	0.428	0.125
LOQ (µg / mL)	0.550	0.375	1.299	0.379
S_b	3.772	5.179	9.92	12.388
S_a	87.652	29.916	22.548	71.16
Confidence limit of the slope	265.402±10.475	325.11±14.38	717.85±27.544	765.32±34.4
Confidence limit of the intercept	102.65199±243.36	91.944±83.06	-275.8±634.55	352.4±197.58
Standard error of the estimation	131.08	26.00	361.25	95.76
Interday [(a)](%R.S.D.)	0.10	0.21	0.10	0.17
Intraday [(b)](%R.S.D.)	0.24	0.21	0.27	0.30

[a] The inter-day(n=3), %RSD of three concentrations of AMB (24, 30 and 36), DES (4, 5, 6), LVC (4, 5 and 6) and PSE (24, 30 and 36) repeated three times in three successive days.

[b] The intra-day(n=3), %RSD of three concentrations of AMB (24, 30 and 36), DES (4, 5, 6), LVC (4, 5 and 6) and PSE (24, 30 and 36) repeated three times within the day.

Limit of Detection and limit of quantification:

LOD and LOQ values were achieved on applying the TLC densitometric method were found in Table (1).

Low LOD and LOQ values were achieved on applying the HPLC method and were found in Table (2).

Accuracy :

The accuracy of the presented TLC method was assessed by recovery study of the cited drugs in their bulk powder as shown in Table (3) and the mean percentage

recoveries of AMB , PSE and DES drugs were 99.60±0.733 ,100.76± 0.35 and 100.28 ± 0.39 , respectively.

Table (3): Determination of AMB, PSE and DES in bulk using TLC-densitometric method

Concentration Taken (µg / spot)			**Concentration found (µg / spot)**			**Recovery %**		
AMB	**PSE**	**DES**	**AMB**	**PSE**	**DES**	**AMB**	**PSE**	**DES**
5	10	0.3	4.95	10.13	0.30	99.00	101.30	100.00
12	18	0.5	12.00	18.12	0.50	100.00	100.67	100.00
20	22	0.8	20.14	22.18	0.80	100.70	100.82	100.00
28	28	1.2	27.78	28.19	1.21	99.20	100.68	100.83
36	32	1.8	35.68	32.11	1.81	99.11	100.34	100.55
					Mean	**99.60**	**100.76**	**100.28**
					SD	**0.73**	**0.35**	**0.39**
					%RSD	**0.733**	**0.347**	**0.389**

The mean percentage recoveries of added standard were 99.62±0.79,100.23±1.23 and 99.67±0.82respectively for AMB,PSE and DES.Table (4).

Table (4): Determination 0f AMB, PSE and DES in mixed dosage forms applying standard addition technique using the proposed TLC-densitometric method

AMB (µg/spot)	**Added**	**Found (µg/spot)**	**Recovery %**	**PSE (µg/spot)**	**Added**	**Found (µg/spot)**	**Recovery %**	**DES (µg/spot)**	**Added**	**Found (µg/spot)**	**Recovery %**
Tablet				**Tablet**				**Tablet**			
12	10	10.07	100.70	6	6	5.95	99.16	1	0.40	0.40	100
	15	14.95	99.67		10	10.19	101.88		0.60	0.60	100
20	12	11.91	99.21	10	12	11.93	99.38	1.67	0.20	0.20	100
	18	17.99	99.92		16	16.27	101.67		0.30	0.30	100
30	5	4.99	99.87	15	15	14.89	99.27	0.6	0.50	0.49	98
	9	8.85	98.34		18	18.00	100.04		0.90	0.90	100
		Mean	99.62			**Mean**	**100.23**			**Mean**	99.67
		SD	0.79			**SD**	**1.23**			**SD**	0.82
		RSD	0.79			**%RSD**	**1.23**			**%RSD**	0.82

The accuracy for HPLC method was checked by applying the method for the determination of AMB, DES, LVC and PSE in pure form and the mean percentage

recoveries were calculated and found to be confirmed by recovery studies from tablets different levels of standard additions; the percentage recoveries of added were 100.61± 0.43, 100.83± 1.58, 99.52± 0.27 and 99.95± 1.67 for AMB, DES, LVC and PSE respectively, Tables (5).

Table (5): Determination of AMB, DES, LVC and PSE in bulk using HPLC method.

Concentration taken (µg/mL)				Concentration found (µg/mL)				Recovery %			
AMB	**DES**	**LVC**	**PSE**	**AMB**	**DES**	**LVC**	**PSE**	**AMB**	**DES**	**LVC**	**PSE**
3.20	1.20	3.00	4.80	3.18	1.22	2.99	4.80	99.38	101.67	99.67	100.00
6.40	2.40	4.20	8.00	6.38	2.41	4.17	8.04	99.69	100.42	99.29	100.50
25.60	4.80	5.04	25.60	25.52	4.82	5.02	25.78	99.69	100.42	99.60	100.70
30.08	5.04	5.40	30.08	29.90	5.06	5.37	30.31	99.10	100.40	99.44	100.76
37.60	8.40	6.60	37.60	37.50	8.41	6.56	37.88	99.73	100.12	99.40	100.74
							Mean	**99.52**	**100.61**	**99.48**	**100.54**
							SD	**0.27**	**0.61**	**0.15**	**0.32**
							%RSD	**0.271**	**0.606**	**0.151**	**0.318**

For HPLC method the accuracy of the method was also confirmed by recovery studies from tablets different levels of standard additions; the percentage recoveries of added were 100.41± 0.91, 100.03± 1.05, 99.45± 0.46 and 99.93± 1.28 for AMB, DES, LVC and PSE respectively, Tables (6). Good results obtained proved that there was no interference from the co-formulated drug or the frequent encountered tablet excipients.

Table (6): Determination 0f AMB, DES, LVC and PSE in mixed dosage forms applying standard addition technique using the proposed HPLC method

AMB µg/ml Tablet	Added	Found µg/ml	Recovery%	DES µg/ml Tablet	Added	Found µg/ml	Recovery %	LVC µg/ml Tablet	Added	Found µg/ml	Recovery %	PSE µg/ml Tablet	Added	Found µg/ml	Recovery%
5	20	19.83	99.15	1	3	2.97	99.00	3	2	2.00	100.00	5	20	20.07	100.35
	5	5.06	101.20		5	5.03	100.60		5	4.96	99.20		5	4.93	98.60
15	10	9.95	99.50	3	2	1.99	99.50	4	2	1.98	99.00	15	10	9.82	98.20
	20	20.29	101.45		5	5.02	100.40		1	0.99	99.00		20	20.07	100.35
20	10	10.06	100.60	5	2	1.98	99.00	5	2	1.99	99.50	20	10	10.16	101.60
	15	15.08	100.53		3	3.05	101.67		3	3.00	100		15	15.07	100.47
		Mean	100.41			**Mean**	**100.03**			**Mean**	99.45			**Mean**	99.93
		SD	0.91			**SD**	**1.05**			**SD**	0.46			**SD**	1.28
		RSD	0.91			**%RSD**	**1.05**			**%RSD**	0.46			**%RSD**	1.28

Precision:

The precision of the analytical procedure was performed by analyzing three concentrations of AMB ,PSE and DES three times in a day for intraday precision (Repeatability) and in three successive days for interday precision (Reproducibility) in terms of % RSD. Results in Table (1) for TLC and table (2) for HPLC indicates high precision of the two method.

Selectivity:

The selectivity of the proposed TLC method was proved by the analysis the proposed laboratory prepared mixtures containing different concentrations of AMB, PSE and DES and satisfactory results were obtained Table (6).

Table (7): Determination of AMB, PSE and DES in laboratory prepared mixtures using TLC.

Concentration taken(µg/spot)			Concentration found (µg/spot)			Recovery %		
AMB	PSE	DES	AMB	PSE	DES	AMB	PSE	DES
13	6.5	1.08	13.05	6.61	1.09	100.38	101.69	100.93
18	9	1.50	17.97	9.15	1.51	99.83	101.67	100.67
24	21	0.35	24.01	21.23	0.35	100.04	101.10	100
29	27	0.90	29.05	27.45	0.90	100.17	101.67	100
37	33	1.60	37.17	33.13	1.61	100.46	100.39	100.63
				Mean		100.18	101.30	100.45
				SD		0.26	0.57	0.42
				RSD%		0.260	0.563	0.418

Selectivity of the HPLC method was confirmed by analyzing different laboratory prepared mixtures of AMB, DES, LVC and PSE. The mean percentage recoveries presented in Table (8).

Table (8): Determination of AMB, DES,PES and LVC in laboratory prepared mixture using HPLC.

Concentration taken (µg/ ml)				Concentration found (µg/ ml)				Recovery%			
AMB	DES	PES	LVC	AMB	DES	PS	LC	AMB	DES	PES	LVC
12.5	2.5	12.5	2.5	12.283	2.54	12.44	2.51	98.26	101.5941	99.51	101.27
14	2.8	14	2.8	14.05	2.89	13.97	2.84	100.37	103.0895	99.76	99.39
20	4.1	22	4.5	20.08	4.15	21.84	4.46	100.39	101.2859	99.26	98.88
29	6.4	27	5.8	29.14	6.62	26.56	5.7	100.5	103.4575	98.37	97.88
34	7.5	35	6.9	33.94	7.56	34.70	6.7	99.84	100.835	99.15	99.31
							Mean	99.87	102.0524	99.21	99.35
							SD	0.933	1.154288	0.527	1.232
							%RSD	0.936	1.131073	0.532	1.241

Robustness

The robustness of the HPLC method was investigated by introducing small changes in the flow rate (±0.1), in the pH (5.5±0.2) and in the organic ratio (± 5%) of the mobile phase, one parameter was changed at a time. The resolution between all peaks remained more than 2, also these changes has no significant effect on peaks shape and peak areas Table (9).

Table (9): Robustness studies of the HPLC method propoed for the simultaneous determination of AMB, DES, LVC and PSE.

Parameters	PSE		LVC		AMB		DES	
	+	-	+	-	+	-	+	-
Normal conditions	-		5.64		2.87		2.72	
Change in flow rate (± 0.1)	-	-	5.53	5.76	2.74	2.93	2.65	2.71
Change in pH (5.5 ± 0.2)	-	-	6.38	6.24	3.75	3.05	2.35	2.01
Change in organic ratio (±5%)	-	-	4.96	6.48	2.85	2.74	2.06	3.43

System suitability

Tests were carried out to ensure that the complete testing system is suitable for the intended application. The system suitability tests included number of theoretical plates, resolution, peak tailing, capacity factor and selectivity factor. Results are revealed in Table (10).

Table (10): System suitability tests of the HPLC method proposed for the simultaneous determination AMB, DES, PSE and LVC

Parameter	PSE	LVC	AMB	DES	Reference Value
N	9762	8980	9516	9734	The higher the value, the more efficient the column is
R	5.64	2.87	2.72		>2
T	1.02	1.01	0.95	1.06	≤ 2
K'	1.12	1.675	2.01	2.37	1-10
α	1.50	1.2	1.18		≥1

Where, N= Number of theoretical plates; R= Resolution; T=Tailing factor; K' = Capacity factor; α = Selectivity factor.

Statistical comparison with the reported methods

The comparison between results obtained by the proposed TLC and HPLC methods for AMB, DES, LVC and PSE and the reference methods showed no significant difference between them concerning accuracy and precision, Table (11,12).

Table (11): Statistical comparison between the proposed TLC densitometric method for the simultaneous determination of AMB, PSE and DES and the reference method

Statistical term	AMB		PSE		DES	
	Reference method**	TLC method	Reference method***	TLC method	Reference method****	TLC Method
Mean	98.99	99.59	100.26	100.76	100.40	100.28
SD	0.54	0.73	0.42	0.35	0.34	0.39
%RSD	0.55	0.73	0.42	0.35	0.34	0.39
n	5	5	5	5	5	5
V	0.29	0.53	0.18	0.12	0.12	0.15
t (2.306) *		1.51		2.04		0.52
F (6.390) *		1.83		1.5		1.25

*Figures in parenthesis are the theoretical t and F values at (p=0.05).

Method of AMB determination [H. Hashem ,H. El-Say,(2020**](HPLC,BDS Hypersil C8(250x4.6mm,5µm)RP-column, 25 mM of KH_2PO_4 (pH 3.5) in aqueous mobile phase, methanol (35:65, v/v) .

*** Method of PSE determination [**U. S. P. C.(2015**)] (HPLC –Alcohol and 0.4 % ammonium acetate(17:3),4.6mm,25cm,flow rate 1.5 ml/min and Detector UV 254nm .

**** Method of DES determination [**(B.P.)The British Pharmacopeia,(2020)**]spectrophotometer at 250 nm.

Table (12): Statistical comparison between the HPLC method proposed for the simultaneous determination of AMB, DES, PSE and LVC and the reference method

Statistical term	AMB		DES		PSE		LVC	
	Reference method***	RP-HPLC method	Reference method****	RP-HPLC method	Reference method***	RP-HPLC method	Reference method*****	RP-HPLC method
Mean	98.99	99.58	100.40	100.61	100.26	100.54	99.57	99.48
SD	0.54	0.17	0.34	0.61	0.42	0.32	0.18	0.15
RSD	0.55	0.17	0.34	0.61	0.42	0.32	0.18	0.15
N	5	5	5	5	5	5	5	5
V	0.29	0.07	0.12	0.37	0.18	0.10	0.03	0.02
T (2.306*)		2.20		0.50		1.18		0.90
F ratio (6.39*)		4.14		3.08		1.80		1.50

*Figures in parentheses are the theoretical t and F values at (p=0.05).

HPLC method determination of AMB[**H. El-Sayed, H. Hashem,(2020] (HPLC,BDS Hypersil C8(250x4.6mm,5µm)RP-column, 25 mM of KH_2PO_4 (pH 3.5) in aqueous mobile phase, methanol (35:65, v/v)).

*** HPLC method determination of PSE [**U. S. P. C.(2015**)] (HPLC –Alcohol and 0.4 % ammonium acetate(17:3),4.6mm,25cm,flow rate 1.5 ml/min and Detector UV 254nm.

**** HPLC method determination of DES [**(B.P.)The British Pharmacopeia,(2020)**] spectrophotometer at 250 nm.

***** HPLC method determination of LVC[**U. S. P. C.(2015**)] HPLC method ,4.6mmx25 cm,5µm,flow rate 1ml/min, mobile phase Acetonitril,water and 1M sulfuric acid (9:0.6:0.4) and Detector UV230 nm .

Conclusion

The two methods described in this paper for simultaneous determination of AMB, DES and PSE by TLC and by addition of LVC to previous drugs by HPLC are found to be simple ,sensitive, accurate, precise, rapid, robust selective and economical .In their pure forms and in the pharmaceutical preparation. The method was validated according ICH guidelines. Thus the developed two methods can be proposed for routine analysis in Quality Control laboratories.

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طريقتين مختلفتين للتقدير المتزامن لبعض الادوية المستخدمة في نزلات البرد لكل من الامبروكسول هيدروكلوريد والديسلوراتادين والسودوايفيدرين هيدروكلوريد والليفوسيتريزين ثنائي الهيدروكلوريد بأستخدام طريقة كروماتوجرافيا الفصل للادوية في صورتهم النقية وفي أشكال مستحضراتهم الصيدلية

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الملخص العربى

تطوير طريقتين كروماتوجرافيين لتحديد متزامن لبعض الأدوية المستخدمة في نزلات البرد. الطريقة الأولى هي طريقة كورماتوجرافيا الفصل بالطبقة الرفيعة للتقدير المتزامن لـ من الامبروكسول هيدروكلوريد والديسلوراتادين والسودوايفيدرين هيدروكلوريد في شكل نقي وفي شكل جرعات دوائية ، تم الحصول على أفضل دقة باستخدام الطور المتحرك (إيثيل أسيتات: ميثانول: أمونيا) ، (١٤: ٠.٨: ٠.٥ ، حجم / حجم / حجم) ، تم إجراء مسح البقع عند ٢٥٤ نانومتر في نطاق التركيز (١-٤٠ ميكروغرام / بقعة) ، (٥-٣٥ ميكروغرام / بقعة) ، (٠.٢-٢) ميكروغرام / بقعة لـ AMB و PSE و DES على التوالي. مع LOD 0.262 و ٠.٣٥٨ و ٠.٠٠٦ ميكروغرام / مل. و LOQ 0.793 و ١.٠٩ و ٠.٠١٨ ميكروغرام / مل لـ AMB و PSE و DES على التوالي. الطريقة الثانية هي طريقة الكروماتوجرافيا السائلة عالية الاداءالمقترنة بجهاز المطيافية الضوئية .

لتقدير المتزامن لـ الامبروكسول هيدروكلوريد والديسلوراتادين والسودوايفيدرين هيدروكلوريد والليفوسيتريزين ثنائي الهيدروكلوريد في الخليط الرباعي باستخدام الطور المتحرك المكون من الأسيتونيتريل: ٠.٠١ M متعادل الفوسفات (50:50) (PH 5.5 ، ت / ت) في وضع متساوي من خلال عمود inertsil C8 250) مم × ٤.٦ مم ، ٥ µ) بمعدل تدفق ١.٣ مل / دقيقة. تم إجراء الكشف عن الأشعة فوق البنفسجية عند ٢١٥ نانومتر في مدى التركيز (٠.٦-٤٠) ، (٠.٢-٤٠) ، (٠.٦-٩) ، (٠.٢٤-٨.٤) ميكروغرام / مل لـ AMB، PSE، DES و LVC على التوالي. ٠.٤٢٨ ، ٠.١٨٠ ، ٠.١٢٥ و ٠.١٢٤ ، ميكروغرام / مل و LOQ1.299، 0.550، 0.379، 0.375 ميكروغرام / مل لـ AMB، PSE، DES و LVC ، على التوالي تم التحقق من صحة الطرق المقترحة وفقًا لإرشادات ICH.

الكلمات المفتاحية: أمبروكسول ، ديسلوراتادين هيدروكلوريد ، ليفوسيتيريزين ثنائي هيدروكلوريد ، سودوإيفيدرين هيدروكلوريد ، الكروماتوجرافيا السائلة عالية الاداءالمقترنة بجهاز المطيافية الضوئية .