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HPTLC monitoring of illegal antihypertensive and antihistamine compounds in herbal products from the Vietnam market

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Abstract. Given the growing craze for dietary supplements, it is essential to critically examine the safety concerns surrounding their usage. There have been several cases of contaminated food-related mishaps in spite of numerous rules and laws in existence. We thus made an effort to find the existence of five antihistamines and four hypertensive in herbal items from the Vietnamese market, given the rising severity of this issue. High-performance thin-layer chromatography (HPTLC) was used to screen 32 samples, which were typical of the many kinds of herbal products. The investigation included both intra- and inter-day precision as well as repeatability. For all analytes, the devised technique was proven to be exact, with %RSD lower than 6%. The LOD and LOQ of nine compounds were determined to be in the range of 0.15–0.30 mg/g and 0.50–1.00 mg/g, respectively (solid matrix sample); 0.075–0.150 mg/g; and 0.25–0.50 mg/g (liquid matrix sample). The intra-day variation's percentage RSD varies from 1.40 to 5.10. Nonetheless, the inter-day variation's percentage RSD fell between 3.10 and 6.60. As a result, the procedure may be used to analyze herbal dietary supplement samples that are in circulation in order to quality control the herbal products on the Vietnamese market.

Keywords: *HPTLC, antihistamine, antihypertensive, and herbal products*

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1. Introduction

Currently, herbal remedies and health foods are widely used in treatment, prevention, and healthcare around the globe (Bouldin et al., 1999). The tertiary function of food, or physiological regulation, is becoming more and more valued internationally above its primary and secondary functions, which is why health foods are gaining popularity in the food and health sectors (Hel et al., 2006). Oriental herbal remedies have a long history of research, development, and usage in Vietnam. The use of herbal remedies has confirmed its significance for the country's health. A planning viewpoint of the Vietnamese government includes developing herbal medicines in the direction of commodity production to meet market demand, promoting raw material consumption with products, and developing medicinal herb growing areas associated with the processing industry (Hung & Ernst, 2010).

Vietnam has seen a dramatic rise in the production of herbal medicine items in recent years, particularly nutritional supplements. There were only 143 nutritional supplement stores in the entire country in 2005, and 300 of those stores sold mostly foreign goods. With 5,000 dietary supplements from herbal sources, the number had soared to over 1600 enterprises in 2015. The market is filled with a wide variety of herbal medicine items, many of which have several components and are hard to monitor for quality and source. They are offered for sale via a variety of outlets, including pharmacies, health food shops, online

retailers, multi-level retailers, supermarkets, and more. Customers are therefore at a significant risk of experiencing adverse effects from this (Nguyen et al., 2021).

Illegal drugs such as hydrochlorothiazide, cyproheptadine, chlorpheniramine, loratadine, promethazine, cinnarizine, amlodipine, felodipine, furosemide, and nifedipine have been found in many places in the Vietnamese market, including retail stores and traditional medical clinics. In particular, several anti-hypertensive, anti-inflammatory, and eczema creams contained antihistamine chemicals such as promethazine, chlorpheniramine, and diphenhydramine (Miller et al., 2007; Moreira et al., 2013; Rocha et al., 2016). These results suggest that dietary supplement safety and purity criteria are not being maintained, particularly for herbal products. Therefore, in order to forbid the production and distribution of tainted goods and to safeguard the public's health and safety, drug administration organizations urgently need a comprehensive, rapid, and effective method of screening for tainted herbal medicines and health foods that are entering the market (Mosihuzzaman et al., 2012).

One of the more advanced methods for both qualitative and quantitative investigation of herbal medications is High Performance Thin Layer Chromatography (HPTLC) (Attimarad et al., 2011; Khatoon et al., 2011; Khatoon et al., 2014; Jain et al., 2014). It is utilized for the identification of phytochemicals and active components, the chemotypic, seasonal, and altitudinal fluctuation of secondary metabolites, and the batch-to-batch consistency of herbal products. There is currently little study being done to identify anti-histamine and anti-hypertensive medications that are illegally incorporated into oriental medicine concoctions from herbal products sold in Vietnam. Therefore, in order to evaluate and keep track of the quality of conventional medications that are now available on the market, this study will broaden its research subjects to include antihistamine and antihypertensive pharmaceutical compounds utilizing a variety of chromatography techniques, starting with basic HPTLC.

2. Materials and Methods

2.1. Chemicals and reagents

2.1.1. Standard materials

Amlodipine besylate (AML) 100.43%, lot QT145090516; felodipine (FEL) 99.30%, water content 0.06%; lot WS.0107222; nifedipine (NIF) 99.68%, lot C0319200.03; furosemide (FUR) 99.51%, water content 0.07%, lot 0103128; Cyproheptadine hydrochloride (CYP) 100.64%, water content 7.6% lot 0102126; chlorpheniramine maleate (CLO) 99.75%, lot QT021070215; loratadine (LOR) 99.24%, water content 0.02%, lot 0108242; promethazine hydrocholoride (PRO) 99.65%, water content 0.13%, lot 0204085 (purchased from Vietnam National Institute of Drug Quality Control); Cinnarizine (CIN) 99.8%, lot QT082060916 (purchased from Ho Chi Minh Institute of Drug Quality Control). The molecular formulas and structures of the compounds are shown in Figure 1.

2.1.2. Standard materials

Solvents and reagents were LC-MS or HPLC grade, and deionized water had a conductivity ≥ 18 WM.

2.2. Preparation of samples and standard solutions

Subjects of this research were five antihypertensive compounds, including amlodipine (AML), felodipine (FEL), nifedipine (NIF), furosemide (FUR), spironolactone (SPI), and five antihistamine compounds: cinarizine hydrochloride (CIN), cyproheptadine hydrochloride (CYP), chlorpheniramine maleate (CLO), loratadine (LOR), and promethazine hydrochloride (PRO).

2.2.1. Blank sample

The blank sample used for method validation was prepared from 14 medicinal herbs that have an effect on antihypertension, sedation, diuretics, and vascular stability, including *Dioscorea persimilis* Dioscoreaceae, and *Paeonia suffruticosa* Paeoniaceae, *Rehmannia glutinosa* Scrophulariaceae, *Cornus officinalis* Comaceae, and *Alisma orientalis* Alismataceae, *Poria cocos* Polyporaceae, *Zea mays* Poaceae, *Prunella vulgaris* Lamiaceae, and *Morinda citrifolia* Rubiaceae, *Rehmannia glutinosa* Scrophulariaceae, *Plantago major* Plantaginaceae, *Styphnolobium japonicum* Fabaceae, *Achyranthes bidentata* Amaranthaceae, and *Ziziphus mauritiana* Rhamnaceae. The composition of the remedy and ratio of ingredients were chosen based on the principles of traditional medicine. These herbs were prepared in liquid extract (liquid form: L matrix) and solid extract (solid form: R matrix) according to the instructions in the Vietnamese Pharmacopoeia V (Ministry of Health Vietnam, 2018).

2.2.2. Spiked samples

Standards were spiked with different concentrations into the blank sample.

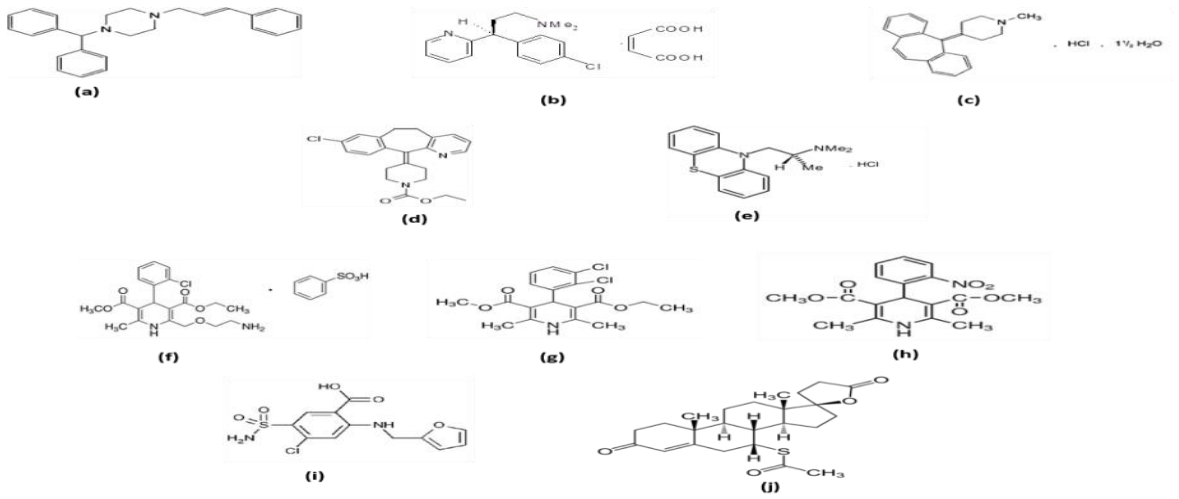

Figure 1. Chemical structure of 10 analytes [Antihistamine compounds (a-e): a. Cinnarizine; b. Clorpheniramine maleate; c. Cyproheptadinhydrocloride; d. Loratadine; e. Promethazine hydrocloride; antihypertensive compounds (f-j): f. Amlodipine besylate; g. Felodipine; h. Nifedipine; i. Furosemide; j. Spironolactone)]

2.3. Chromatography

2.3.1. Instruments

Merck (Darmstadt, Germany) supplied the precoated TLC silica gel aluminum plates 60F254 (20 × 10 cm, 200 µm thickness) utilized in the investigation. Using a Camag Linomat IV sample applicator (Switzerland) and a Camag micro liter syringe under a nitrogen stream, samples were spotted on plates in the shape of 5 mm-wide bands. After applying the sample, 20 mL of the mobile phase, which included ethyl acetate, toluene, methanol, and amoniac (1.5:9:3:0.3, by volume) for antihypertensive compounds and cyclohexane, acetone, and triethylamine (8:2:0.5, by volume) for antihistamine compounds, was used to develop the plate in the linear ascending mode. The chromatogram run measured 135±2 mm in length. After development, the plates were left to dry in the air. They were then scanned densitometrically in the absorbance mode at 254 nm to find CIN, CLO, CYP, LOR, PRO, NIF, FUR, and SPI, and at 366 nm to find AML and FEL using a Camag TLC scanner III controlled by CATS software (V 3.15).

2.3.2. Preparation of calibration curve

A concentration of 1 mg/mL of ten analytes was prepared as methanolic stock solutions. The stock solutions were kept out of the light and at 4 ^{o}C in a refrigerator. Five dilutions (10, 20, 40, 80, and 100 µg/mL) were made from the stock solution and added to the HPTLC plate. This method was repeated six times. The calibration curve was made by using linear least squares regression on data that showed the relationship between the largest areas of the spots that were made and their concentrations after the plates were made and scanned.

2.3.3. Quantification of antihypertensive and antihistamine compounds

Ten micro liters of the standard and sample solutions were put in triplicate on an HPTLC plate. The plates were developed and scanned, as was previously indicated. Calibration graphs were produced using linear regression analysis when the resolved peak region was seen. By logging the peak areas of the corresponding peaks of the antihypertensive and antihistamine compounds, the calibration curve was utilized to calculate the concentration of each of the ten compounds.

2.4. Method validation

2.4.1. Repeatability for instrument precision

Repeated scanning (n = 6) of the same location of antihypertensive and antihistamine medications was used to verify the accuracy of the instrument. In order to measure peak areas, the analysis was conducted using 100 µg/mL of chemicals from the standard solutions. The findings were represented as the relative standard deviation (%RSD).

2.4.2. Specificity

By using the same thin-layer chromatography to examine the standards and samples, the technique's specificity was determined. By contrasting the Rf values and spectra of the spots with those of the standard, the spots for analytes in the samples were verified. Seven samples were analyzed simultaneously, including a mixed standard sample (C), a matrix solid sample (N1), a matrix liquid sample (N2), a blank matrix (N3), and three spiked samples (C/N1, C/N2, and C/N3). The concentration of each antihypertensive and antihistamine compound in the samples was 0.10 mg/mL. The results showed that the chromatograms of the spiked samples and the blank sample had spots and peaks with Rf equal to those found on the chromatogram of the standard sample. However, the chromatograms of the sample and the placebo sample did not have any peaks that matched the spots of the analytes.

2.4.3. LOD and LOQ

The instrument's sensitivity was assessed using LOD and LOQ. The LOD and LOQ were computed using the calibration curve as a guide. Using methanol as a control, different amounts of blood pressure and allergy medicines were used to test LOD and LOQ, which were then evaluated by looking at the signal-to-noise ratio. With s denoting the average standard deviation of the regression lines' y-intercepts and S denoting their average slope, the LOD and LOQ were determined to be 3.3 and 10 of S/N, respectively.

2.4.4. Inter-day and intra-day precision

Three distinct concentration levels were used for the inter-day and intra-day variances for the marker deduction. Parts of standard solutions that had 10, 50, and 100 µg/mL of CLO and 20, 50, and 100 µg/mL of CIN, CYP, LOR, and PRO were looked at to learn more about the process. The data were reported as %RSD and were collected on the same day (intra-day precision) and three separate days (inter-day precision).

2.5. Application of the proposed method

2.5.1. Antihistamine compounds

25 samples of traditional medicines and dietary supplements were collected from pharmacies, online shops, or traditional medicine clinics

2.5.2. Anti-hypertensive compounds

Seven samples of traditional medicines and dietary supplement products were collected from pharmacies and online shops.

3. Results and Discussion

3.1. Optimization of the mobile phase and the detection

3.1.1. Antihistamine compounds

All five medications were to be separated on 10 cm TLC plates in the preliminary studies. Survey solvents with suitable ratios for research pharmaceuticals, based on earlier findings (Srivastava et al., 2010; Ahmed et al., 2017): [n-hexane: dicloromethane: triethylamine], [cyclohexane: acetone: triethylamine], [acetone: n-hexane: amoniac], and [ethylacetate: methanol: amoniac] are the components of the mobile phases. The spots of PRO and CYP were persistent in the same positions in the mobile phases of [n- hexane: dicloromethane: triethylamine] and [ethylacetate: methanol: amoniac]. All analytes are fully separated at the spot in the mobile phase [acetone: n-hexane: amoniac], although the CLO spot is low toward the beginning.

The mobile phase [cyclohexane, acetone, and triethylamine] with a ratio of 80:10:10 features compact, fully separated spots with Rf ranging from 0.20 to 0.80.

To determine the best mobile phase for separating the five compounds, including CIN, CLO, CYP, LOR, and PRO, keep adjusting the ratio of the mobile phase (cyclohexane: acetone: triethylamine, 80:10:10). Examine the system of cyclohexane, acetone, and triethylamine with the following variations in the solvent ratios: The mobile phase (8:2:0.5) of cyclohexane, acetone, and triethylamine ultimately fully separated the five analytes. Ammonia seems to be a suitable addition to this mobile phase in order to eliminate spot tailing and improve peak form and clarity since the blockers have a fundamental center (Figure 2).

Each plate was scanned twice for the simultaneous determination of all five medicines in combination with regard to the selection of the detection wavelength. Owing to their structural resemblance, CIN, CLO, CYP, LOR, and PRO also exhibit comparable absorption properties, with a notable absorption of UV light in the 254–305 nm regions (Figure. 3). The absorption peaks for CIN are 254 nm, whereas those for CLO, CYP, LOR, and PRO are 254 and 305 nm, 224 and 250 nm, and 226, 253, and 287 nm. Because of its strong response and ability to reduce the impact of the sample matrix, the wavelength of 254 nm should be used to detect all 5 analytes (Figure 4).

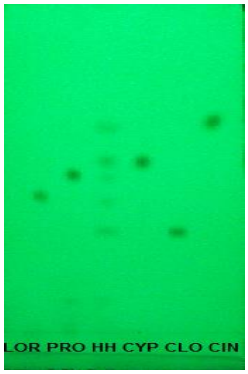

Figure 2. Chromatogram separating antihistamine compounds using the selected solvent system of the HPTLC method

3.1.2. Antihypertensive compounds

In the preliminary studies, five compounds were to be separated on 10-cm TLC plates. Survey solvents with suitable ratios for research compounds based on earlier investigations (Zhu et al., 2014). The mobile phases consist of the following: [ethyl acetate: toluene: methanol: ammonia], [ethyl acetate: methanol: amoniac], and [toluene: ethyl acetate: acetic acid]. Although the five analytes in the mobile phase of [ethyl acetate: toluene: methanol: ammonia, 8:20:6:0.6] are separated, the FEL and SPI spots are quite close to one another. The AML stain is still at the chromatography start line in the mobile phase of [chloroform: ethylacetate: glacial acetic acid, 6:5:0.2]. The NIF, FEL, and SPI three spots overlap. The FUR spot separates neatly and completely with Rf = 0.20–0.80. The mobile phases of AML, FUR, [ethyl acetate: methanol: amoniac, 8:2:0.1], and NIF, FEL, and SPI all stayed separate, but three analytes were mixed together. With the mobile phase [toluene: ethyl acetate: acetic acid, 17:13:1], AML does not move (Rf = 0).

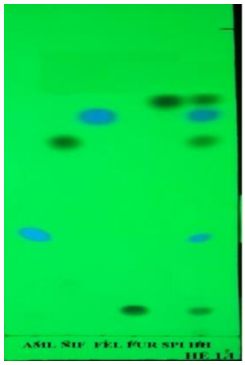

Figure 3. Chromatogram separating antihypertensive substances using the selected solvent system of the HPTLC method

Select the mobile phase of [ethyl acetate: toluene: methanol: ammonia, 8:20:6:0.6] to continue researching. Change the ratio of solvents in this mobile phase to obtain the best separation efficiency for the five analytes. Through the survey results, the system ethyl acetate: toluene: methanol: ammonia (1.5:9:3:0.3) was chosen to separate the 5 research substances (Figure 3).

Each plate was scanned twice for the simultaneous determination of all five medicines in combination with regard to the selection of the detection wavelength. The maximum absorption of AML is 366 nm that of NIF is 240 nm that of FEL is 364 nm with a strong response at 366 nm, that of FUR is 236 nm, and that of SPI is 245 nm. As a result, use a wavelength of 366 nm to measure AML, NIF, and FEL, and 240 nm to assess FUR and SPI (Figure 4). AML, FEL, FUR, and NIF are the four substances that showed up as four peaks on the chromatogram at 366 nm wavelength; SPI was unable to identify them. The FEL peak and the SPI peak overlap at 240 nm; hence, there will be a significant mistake when integrating the peak area. As a result, four analytes, including AML, FEL, FUR, and NIF, will be the subject of the subsequent validation.

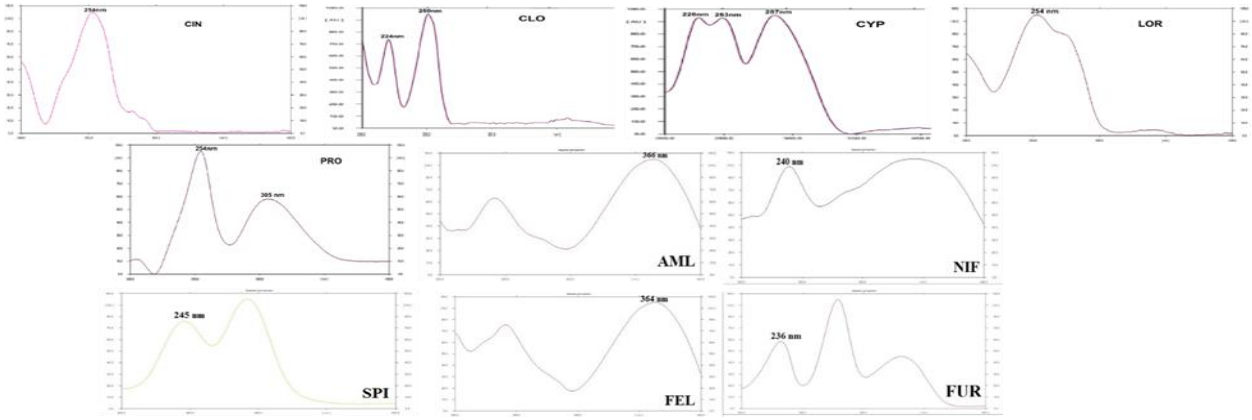

Figure 4. UV absorption spectrum of antihistamine and antihypertensive compounds

3.2. Method validation

In terms of instrument accuracy, range, linearity (Table 1), specificity (Figure 5), LOD, and LOQ (Table 2), the densitometric approach was verified. The accuracy investigation included both intra- and inter-day fluctuations as well as repeatability (Table 3). For all analytes, the devised technique was proven to be exact, with %RSD lower than 6%. According to Table 2, the LOD and LOQ of nine compounds were determined to be in the range of 0.15–0.30 mg/g and 0.50–1.00 mg/g, respectively (solid matrix sample) and 0.075–0.150 mg/g and 0.25–0.50 mg/g (liquid matrix sample).

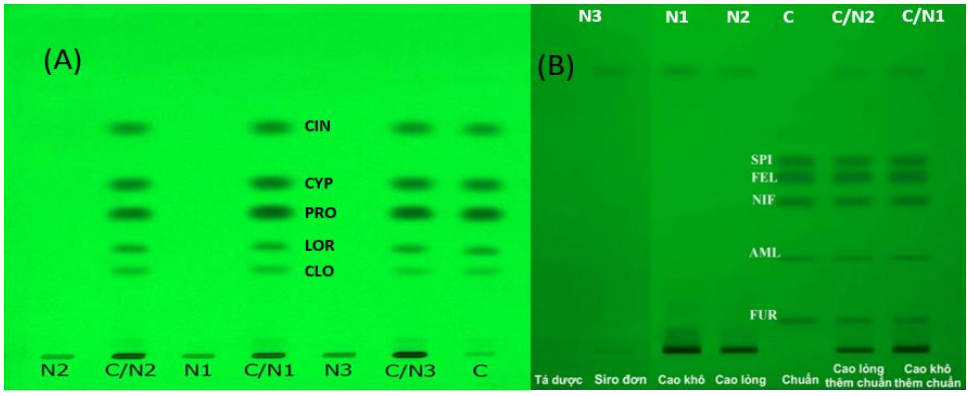

Figure 5. Chromatograms for specificity (A. Histamine compounds; B. Antihypertensive compounds)

The intra-day variation's percentage RSD varies from 1.40 to 5.10. On the other hand, the inter-day variation's %RSD fell between 3.10 and 6.60 (Table 3). It was found that the calibration plot was linear across the concentration range of 10–100 ng/mL for the reference standards that were studied (Table 1). All of the analytes had a correlation value of 0.9972-0.9998.

Table 1. Method validation parameters for the quantitation of analytes

Parameters	CLO	CYP	CIN	LOR	PRO	AML	NIF	FEL	FUR
R_f	0.27	0.56	0.74	0.35	0.46	0.29	0.47	0.55	0.10
Repeatability (n=6) RSD	1.89	1.47	1.40	1.46	1.37	1.76	0.87	0.00	0.00
Concentration range(ng/ml)	20-100	10-100	10-100	10-100	10-100	10-100	10-100	10-100	10-100
Intercept (a)	125.1	221.7	222.3	178.8	214.4	42.5	82.8	99.1	56.0
Slope (b)	-207.7	123.0	711.8	1530.5	2245.3	777.3	1155.3	868.6	953.1
Correlation coefficient (r)	0.9969	0.9966	0.9983	0.9983	0.9972	0.9990	0.9998	0.9973	0.9989

Table 2. LOD, LOQ using the proposed HPTLC method

Analytes	Solidmatrix (N1)				Liquidmatrix (N2)			
	LOD		LOQ		LOD		LOQ	
	Conc. (µg/mL)	mg/ g matrix sample	Conc. (µg/mL)	mg/ g matrix sample	Conc. (µg/mL)	mg/ g matrix sample	Conc. (µg/mL)	mg/ g matrix sample
CIN	3	0.15	10	0.50	3	0.075	10	0.25
CLO	6	0.30	20	1.00	6	0.150	20	0.50
CYP	3	0.15	10	0.50	3	0.075	10	0.25
LOR	3	0.15	10	0.50	3	0.075	10	0.25
PRO	3	0.15	10	0.50	3	0.075	10	0.25
AML	3	0.15	10	0.50	3	0.075	10	0.25
NIF	3	0.15	10	0.50	3	0.075	10	0.25
FEL	3	0.15	10	0.50	3	0.075	10	0.25
FUR	3	0.15	10	0.50	3	0.075	10	0.25

Table 3. Intra-day and inter-day precision, accuracy using the proposed HPTLC method

Analytes	m (µg)	Solidmatrix (N1)			Liquidmatrix (N2)		
		Intra-day RSD (%)	Inter-day RSD (%)	Accuracy % Recovery	Intra-day RSD (%)	Inter-day RSD (%)	Accuracy % Recovery
CIN	10	4.4	4.9	91.2 – 105.2	2.7	4.5	96.2 – 105.1
	50	3.2	3.6	95.4 – 102.7	3.5	2.3	95.3 – 103.1
	100	1.8	5.0	96.5 – 104.2	1.8	1.4	96.6 –101.2
CLO	20	5.1	6.7	92.8 – 105.7	3.8	4.6	95.1 –102.6
	50	3.6	4.3	96.2 – 103.3	2.5	3.3	98.0 – 103.5
	100	1.6	4.1	97.2 – 104.2	1.1	3.2	95.6 – 102.1
CYP	10	2.0	5.6	90.7 – 98.9	4.3	3.9	91.8 – 103.5
	50	1.5	4.5	96.1 – 103.8	3.4	3.3	98.2 – 103.8
	100	1.4	4.3	95.5 – 102.3	2.1	2.4	96.3 – 102.5
LOR	10	3.5	6.6	91.2 – 104.2	3.4	4.6	92.7 – 101.3
	50	1.5	5.2	97.2 – 104.6	1.9	2.6	97.1 – 102.4
	100	1.4	4.3	95.8 – 102.3	3.1	1.7	95.5 – 101.2
PRO	10	3.3	5.6	90.9 – 105.2	4.5	3.7	93.7 – 103.5
	50	2.9	4.8	95.3 – 103.6	2.1	1.8	95.3 – 100.9
	100	1.1	3.9	95.8 – 103.0	2.2	2.4	96.5 – 104.2
AML	10	4.5	5.6	92.3 – 106.0	5.1	5.3	93.2 – 104.1
	50	3.0	4.8	91.5 – 102.7	3,8	4.5	95.6 – 104.1
	100	2.5	3.1	96.5 – 104.2	2.9	3.0	96.1 –104.8
NIF	10	4.0	5.9	95.8 – 106.7	4.3	5.9	92.1 –106.6
	50	2.8	4.8	94.2 – 105.3	3.6	3.7	95.2 – 103.5
	100	3.2	5.1	94.2 – 105.2	2.5	3.9	95.3 – 104.2
FEL	10	4.6	5.2	92.7 – 103.9	4.8	6.2	91.0 – 106.2
	50	3.1	4.2	96.5 – 105.8	3.1	4.7	95.2 – 104.8
	100	3.5	4.6	95.8 – 104.3	2.6	4.2	96.0 – 104.5
FUR	10	4.6	5.1	91.2 – 104.2	4.1	5.2	93.7 – 105.2
	50	2.9	4.7	96.2 – 105.6	3.5	4.6	95.8 – 104.2
	100	3.1	3.9	95.4 – 103.3	3.0	4.5	95.2– 104.6

3.3. Method validation

3.3.1. Antihistamine compounds

Process test samples and analyze them according to established procedures. The results are presented in Table 3 and illustrated in Figure 5 and Table 5.

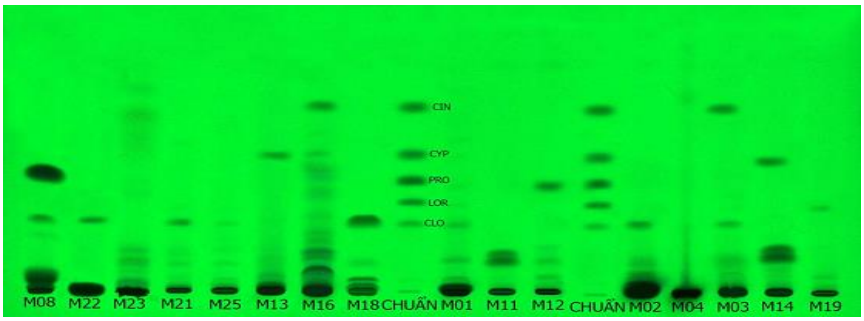

Figure 6. Chromatogram of real sample analysis to detect antihistamine group drugs using HPTLC method

Table 4. Results of analysis of positive samples of H1 antihistamine group drugs by HPTLC

No.	Dosage forms	Source	Concentration (mg/g)				
			CIN	CLO	CYP	LOR	PRO
1	Powder	Online	-	0.52	-	-	-
2	Powder	Online	-	0.84	-	-	-
3	Hard Capsule	Traditional medical clinic	0.79	0.94	-	-	-
4	Powder	Traditional medical clinic	-	2.06	-	-	-
5	Hard Capsule	Sample sent for testing	-	-	-	-	1.67
6	Powder	Sample sent for testing	-	-	0.65	-	-
7	Soft pill	Sample sent for testing	-	-	0.36	-	-
8	Hard pill	Sample sent for testing	0.65	-	+	-	-
9	Powder	Sample sent for testing	-	-	-	0.25	-
10	Powder	Sample sent for testing	-	2.08	-	-	-
11	Hard Capsule	Sample sent for testing	-	2.53	-	-	-
12	Powder	Sample sent for testing	-	+	-	-	-
13	Pill	Traditional medical clinic	-	2.140	-	-	-
14	Pill	Traditional medical clinic	-	0.691	-	-	-
15	Pill	Traditional medical clinic	-	0.691	-	-	-
16	Hard pill	Traditional medical clinic	-	0.408	-	-	-
17	Powder	Traditional medical clinic	-	1.149	-	-	-
18	Hard pill	Traditional medical clinic	-	1.259	-	-	-
19	Hard pill	Traditional medical clinic	-	1.054	-	0.364	
20	Hard pill	Online	-	0.626	-	-	-
21	Capsule	Online	-	1.033	0.369	0.110	-
22	Capsule	Online	-	1.407	-	-	-
23	Capsule	Online	-	4.245	-	-	-
24	Capsule	Online	-	3.287	0.753		-
25	Capsule	Online	-	6.762	-	-	-

(+): positive, but below LOQ; (-): below LOD

3.3.2. Antihypertensive compounds

Process test samples and analyze them according to established procedures. The results are presented in Table 6 and illustrated in Figure 7.

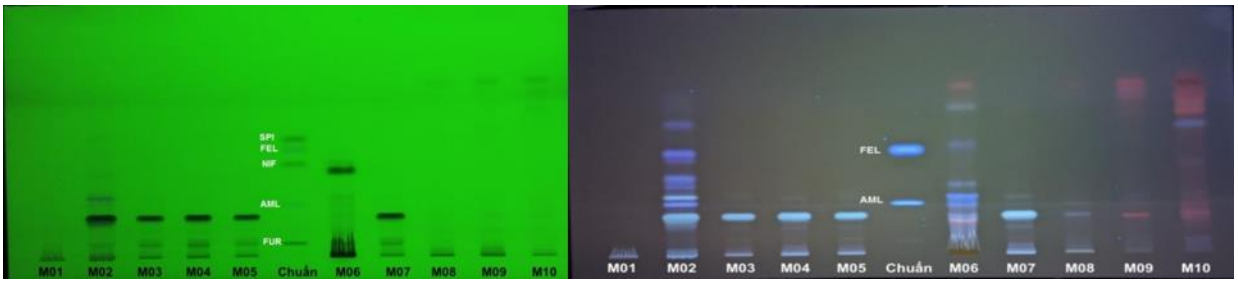

Figure 7. Chromatogram of real sample analysis to detect antihypertensive drugs using HPTLC method at wavelengths of 254 nm (a) and 366 nm (b)

Table 6. Qualitative results on real samples of antihypertensive compound by HPTLC

No.	Dosage forms	Source	Analytes			
			AML	**NIF**	**FEL**	**FUR**
1	Hard capsule	Pharmacy	-	+	-	-
2	Capsule	Pharmacy	+	-	-	+
3	Capsule	Pharmacy	-	-	-	+
4	Capsule	Pharmacy	-	-	-	+
5	Capsule	Online	-	-	-	+
6	Capsule	Online	+	-	-	+
7	Capsule	Online	-	-	-	+

(-):below LOD; (+): positive

AML, NIF, FUR, and SPI traces were visible at the Rf point in a few genuine samples. Analyze the spectrum at the analyte point on the chromatography plate and compare it to the standard's spectrum at the same spot. According to the results, the chemicals' spectra from the suspected samples differed from the reference spectra, and the match coefficient, which ranged from 0.10 to 0.76, was extremely low. Figure 6 provides an illustration of the results.

The primary benefit of HPTLC is its capacity to examine many samples concurrently under the same chromatographic conditions, which reduces analysis time and chemical requirements while also offering excellent reproducibility. HPTLC is a great method for screening analyses with a large number of samples since it can analyze up to 20 samples in a single chromatography session in roughly 30 minutes when using a thin plate measuring 20 by 10 cm. The popular and affordable thin plate Silica gel 60 GF254 was employed in the study, along with two chromatographic processes and readily available solvents, including cyclohexane, acetone, triethylamine, ethyl acetate, toluene, methanol, and ammonia, to successfully separate the substances of nine compounds with various groups of pharmacological effects.

Specifically, the maximum wavelength with contemporary equipment (Camag HPTLC equipment with scanner) has been established based on the spectrum data corresponding to each trace of analyte, resulting in a strong signal and enhanced selectivity for quantification. The spectral superposition findings in analyte identification enable the removal of false positive conclusions that may arise from a complex herbal preparation matrix, including signals other than those of the analyte appearing at the appropriate spot on the chromatography.

4. Conclusions

Set up an HPTLC procedure and method to look at four blood pressure-lowering and water-respiratory drugs (furosemide, amlodipine, felodipine, and nifedipine) that are used in eastern medicine to help treat allergies, rashes, colds, and the flu. The five antihistamines are cyproheptadine, chlorpheniramine, loratadine, promethazine, and cinnarizine. The AOAC 2016 requirements were satisfied when it came to specificity, linear range, precision, accuracy, LOD, and LOQ evaluation of these processes. When assessed in the National Institute for Food Control's laboratory, the assessment results were comparable. As a result, the procedure may be used to analyze test samples that are in circulation in order to support product quality control and testing.

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