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Article

Validated HPLC and HPTLC Methods for Simultaneous Determination of Colchicine and Khellin in Pharmaceutical Formulations

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The present work describes validated high-performance liquid chromatography (HPLC) and high-performance thin-layer chromatography (HPTLC) methods for the simultaneous determination of colchicine and khellin. The isocratic reversed-phase HPLC separation was performed on a 5 μm C18 column (Luna, Phenomenex, Torrance, CA). Good resolution between colchicine and khellin was achieved using a mixture of acetonitrile–10 mM NaH_2PO_4 (pH 3.0, 35:65 v/v) as a mobile phase. Quantitation was achieved with ultraviolet detection at 245 nm based on peak area. The HPTLC separation was conducted on Merck HPTLC aluminum sheets of silica gel 60 F254 as stationary phase using methylene chloride–methanol (95:5 v/v) as a mobile phase. Quantification was also achieved using densitometric measurements at 245 nm. Both methods revealed reasonable validation parameters concerning selectivity, linearity, accuracy, precision and limits of detection and quantitation.

Introduction

Colchicine (Figure 1) is a potent alkaloid isolated from various species of Colchicum belonging to family Liliaceae, primarily from *Colchicum automnale* L. It is a powerful anti-inflammatory agent used principally to treat acute flares of gout, pseudogout and acute gouty arthritis, specifically relieving the pain of acute attacks (1, 2). The alkaloid is also valuable in other inflammatory diseases such as scleroderma and amyloidosis secondary to Familial Mediterranean Fever (3). In gastroenterology, it may be used to slow the formation of fibrous tissue in the liver that occurs with conditions such as cirrhosis and primary biliary cirrhosis (4, 5). Additionally, the alkaloid possesses anti-neoplastic activity against breast cancer by arresting mitotic cell division in metaphase (6).

Khellin (Figure 1) is a furanochromone present in the *Ammi visnaga* (L.) fruis family Apiaceae (7). It is a potent vasodilator showing calcium channel blocking activity (8–10). It possesses many pharmacological activities including anti-inflammatory action (11), vascular smooth muscle relaxation (12), inhibition of renal crystal deposition and cell damage (13, 14) and vitiligo (15). Moreover, it is used for photochemotherapy of skin diseases (16).

A literature survey revealed that various analytical methods have been described for the analysis of colchicine in biological matrices, pharmaceuticals and plant material, including high-performance thin-layer chromatography (HPTLC) (17–21), gas chromatography (22), tandem mass spectrometry (23) and TLC densitometry (24).

Similarly, literature of khellin describes several analytical methods for its assay in formulations, *A. visnaga* extracts and biological matrices; for example, high-performance liquid chromatography (HPLC) (25–28), capillary electrophoresis (29) and gas chromatography (30).

Some phytopharmaceuticals from the Egyptian market combine colchicine and khellin and are prescribed for relieving urinary tract colic and spasm, and urinary stones. To evaluate these preparations, a simple and sensitive analytical method is required to determine the two drugs alone and in combination. The exhaustive literature survey revealed that none of the most recognized pharmacopoeias or periodicals includes the simultaneous determination of khellin and colchicine. In addition, no information is available regarding follow-up studies of these drugs during the shelf life of their phytopharmaceuticals.

The current study provides simple, sensitive and accurate HPLC and HPTLC methods for the simultaneous determination of colchicine and khellin alone or in combination in different phytopharmaceuticals available in the Egyptian pharmaceutical market. The proposed methods will be applied for qualitative and quantitative follow-up of these phytopharmaceuticals during their shelf lives.

Experimental

Chemicals and reagents

All reagents and solvents were of analytical and HPLC grade. These included hydrochloric acid, sodium hydroxide and sodium dihydrogen phosphate. Methanol for HPLC was purchased from Merck Ltd. (Mumbai, India) and acetonitrile for HPLC was purchased from Lab-Scan (Poland). Colchicine standard was purchased from Sigma Aldrich (St. Louis, MO). Khellin was provided by the Memphis Chemical Company (Cairo, Egypt). Colchicine and Khellin were certified to contain 98 and 99% of colchicine and Khellin, respectively. The water was deionized and filtered twice through a 0.45 μm filter (Millipore, Bedford, MA) and finally degassed under vacuum before use. For sample filtration, 0.45 μm Millex-HV Millipore filters were used. HPTLC plates (20×10 cm^2, precoated silica gel aluminium plates 60 F254, 0.25 mm) were purchased from E. Merck (Darmstadt, Germany).

Pharmaceutical products containing khellin and colchicine alone or in combination were purchased from the Egyptian pharmaceutical market. The pharmaceutical formulations are as follows:

(1) Colchicine tablets, batch numbers 14711, 194709, 364708, 114708, 304707 and 204706. Each tablet

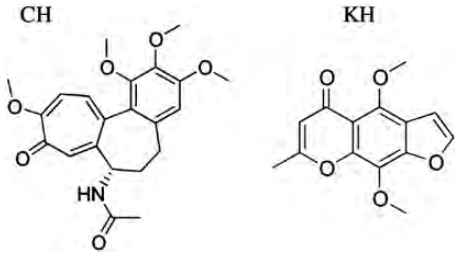

Figure 1. Molecular structures of colchicine (CH) and khellin (KH)

contains 0.5 mg colchicine, manufactured by El Nasr Pharmaceutical Chemicals Co. (Cairo, Egypt).

(2) Colmetidine tablets, batch numbers 081425, 0711460, 0710282, 0711460 and 81283. Each tablet contains 0.5 mg colchicines, manufactured by El Kahira Pharmaceutical and Chemical Industries (Cairo, Egypt).

(3) Solvaure sachets, batch number 0803329. Each sachet contains 0.3 mg colchicines, manufactured by Jedco International Pharmaceutical (Cairo, Egypt).

(4) Ur-aid sachets, batch numbers 3348286, 3348109, 3340601, 3348017 and 3346293. Each sachet contains 0.3 mg colchicine, manufactured by Pharaonia Pharmaceuticals (Alexandria, Egypt).

(5) Urocoline sachets, batch numbers 906975, 810508, 806801, 810508 and 709104. Each sachet contains 0.5 mg colchicine, manufactured by EVAPharma for Pharmaceuticals and Medical Appliances (Cairo, Egypt).

(6) Urosolvine sachets, batch numbers 1211005, 129065, 128517 and 128173. Each sachet contains 0.3 mg colchicine, manufactured by The Nile Company for Pharmaceuticals and Chemical Industries (Cairo, Egypt).

(7) Kellagon sachets, batch numbers 171008, 440408 and 100606. Each sachet contains 20 mg khellin, manufactured by Arab Company for Pharmaceuticals and Medicinal Plants (Mepaco) (Cairo, Egypt).

(8) Coli-urinal effervescent granules, batch numbers 702088 and 728038. Each 100 g contains 35 mg khellin, manufactured by Misr Company for Pharmaceutical Industries (Cairo, Egypt).

(9) Uricol sachets, batch numbers 11067, 11080 and 9385. Each sachet contains 1.83 mg khellin, manufactured by Pharco Pharmaceuticals (Alexandria, Egypt).

(10) Jedcorine sachets, batch number 0903015. Each sachet contains 1.5 mg khellin, manufactured by Jedco International Pharmaceutical (Cairo, Egypt).

(11) Uricol Plus sachets, batch numbers 1644, 1227 and 1068. Each sachet contains 1.83 mg khellin and 0.3 mg colchicine, manufactured by Pharco Pharmaceuticals (Alexandria, Egypt).

(12) Urivin sachets, batch numbers 104331, 091136, 090058 and 08548. Each sachet contains 1.75 mg khellin and 0.3 mg colchicine, manufactured by Amoun Pharmaceutical Co. (Cairo, Egypt).

Instrumentation

The HPLC instrument (Shimadzu, Kyoto, Japan) was equipped with a model series LC-10 ADVP pump, SCL-10 AVP system controller, DGU-12 A Degasser, Rheodyne 7725i injector with a 20 μL loop and a SPD-10AVP UV-VIS detector; separation and quantitation were performed on a 250 × 4.6 mm (i.d.), 5 μm C18 column (Luna, Phenomenex, Torrance, CA). The detector was set at λ 245 nm. Data acquisition was performed on class-VP software.

A Camag (Wilmington, NC) Linomat IV sample applicator was used to dispense the aliquots of the standard stock solution and the prepared samples. The plates were saturated in a twin trough chamber with slit dimension settings of length 6 and width 0.1 mm, monochromator band width 20 nm and scanning rate of 10 mm/s. Zones were quantified by using a Camag TLC Scanner III densitometer controlled by CATS version 4.X software in the absorption mode using a deuterium source and a filter with a wavelength of 245 nm.

Chromatographic conditions

The isocratic reversed-phase HPLC separation and quantitation were performed on a 250 × 4.6 mm (i.d.), 5 μm C18 column (Luna, Phenomenex). Good resolution was achieved between colchicine and khellin using a mixture of acetonitrile–10 mM NaH_2PO_4 (pH 3.0, 35:65, v/v) as a mobile phase at a flow rate of 1 mL/min. Quantitation was achieved with UV detection at 245 nm based on peak area. The detector was set at λ 245 nm. All determinations were performed at ambient temperature. The injection volume was 20 μL. The samples were filtered through 0.45 μm pore size disposable filters. Data acquisition was performed with class-VP software.

For optimal sensitivity of the HPTLC method, solutions of the testing samples and standard were applied to the silica plates as 4 mm long bands, and 5 μL of sample was applied to each band. The bands were separated by a distance of 8 mm apart and 10 mm from the bottom of the plate. The development chamber was saturated with mobile phase. The HPTLC plates were developed in the ascending manner with methylene chloride–methanol (95:5 v/v) as a mobile phase. After developing over a distance of 8 cm, the HPTLC plates were air dried and scanned at 245 nm. The scan length and width were adjusted to cover the entire band.

Standard solutions and calibration

For the HPLC method, stock standard solutions of colchicine and khellin were prepared by dissolving 25 mg of each compound in 25 mL methanol. The standard solutions were prepared by further dilution of the stock standard solution with the specified mobile phase to reach the concentration range of 0.5–20 μg/mL for both compounds.

For the HPTLC method, stock standard solutions of colchicine and khellin were prepared by dissolving 25 mg of each compound in 25 mL methanol. The standard solutions were prepared by further dilutions of the stock standard solutions with methanol to reach the concentration range of 0.7–10 μg /band for colchicine and 0.8–20 μg /band for khellin.

Five μL of each standard solution was applied to the corresponding HPTLC plate. Triplicate applications were made for each concentration. The corresponding plates for each concentration were developed using the previously described mobile phase. The peak areas were measured for the calibration.

Sample preparation

Formulations containing colchicine or khellin

Twenty tablets or sachets were weighed and finely powdered. A portion of the powder equivalent to 2.5 mg of colchicine or khellin was accurately weighed, transferred to a 25 mL volumetric flask, and extracted with approximately 22 mL (90:10 methanol–water) in an ultrasonic bath for 30 min. The previously mentioned solvent used for extraction was achieved by scientific trials and errors. The extract was cooled to room temperature and the solution was diluted to 25 mL with methanol and then filtered through a 0.45 μm membrane filter (Millipore).

Formulations containing colchicine and khellin

Twenty sachets were weighed and finely powdered. A portion of the powder equivalent to 0.6 mg colchicine and 3.66 mg khellin for Uricol Plus sachets and equivalent to 0.6 mg colchicine and 3.5 mg khellin for Urivin sachets were accurately weighed, transferred to a 25 mL volumetric flask, and extracted with approximately 22 mL (90:10 methanol–water) in an ultrasonic bath for 30 min. The extract in each case was cooled to room temperature, and the solution was diluted to 25 mL with methanol and then filtered through a 0.45 μm membrane filter (Millipore).

For the HPLC method, further dilutions of the sample solution were conducted with the mobile phase to reach the linearity range specified for colchicine and khellin. The general procedures described previously were followed and the corresponding concentrations of colchicine and khellin were calculated.

For the HPTLC method, 5 μL of the sample solution was applied to the HPTLC plate. Each plate was developed using the previously described chromatographic conditions. The general procedures described previously were followed and the corresponding concentrations of colchicine and khellin were calculated.

Results and Discussion

For simultaneous determination of colchicine and khellin, it is necessary to adjust the HPLC and HPTLC systems to avoid overlapping and provide optimum separation of the peaks. The organic ratio of the mobile phase should be adjusted to achieve good separation of the tested compounds with less baseline noise. The experimental variables such as the effect of the mobile phase composition and pH were optimized to result in simple, sensitive and accurate estimation methods.

HPLC method optimization

The developed HPLC method was applied for the simultaneous determination of colchicine and khellin. To optimize the HPLC assay parameters, the mobile phase composition and pH of 10 mM NaH_2PO_4 were studied. A satisfactory separation was obtained with a mobile phase consisting of acetonitrile–10 mM NaH_2PO_4 (pH 3.0, 35:65, v/v). Increasing acetonitrile concentration to more than 45% led to early elution of the colchicine peak. At lower acetonitrile concentration (<25%), separation occurred but with excessive tailing for khellin. The pH of the mobile phase considerably affects the chromatographic behavior of each compound. At pH 5.5–6.5, the peaks of colchicine and khellin appeared broad. However, at pH 3, optimum resolutions were observed with reasonable retention time. Quantitation was achieved with ultraviolet (UV) detection at 245 nm based on peak area. The specificity of the HPLC method is illustrated in Figure 2, which shows complete separation of the studied compounds. The average retention time ± standard deviation for colchicine and khellin were found to be 4.72 ± 0.007 and 10.61 ± 0.020 min., respectively, for 10 replicates. The characteristic parameters of the linear regression equation of both compounds are shown in Table I.

HPTLC method optimization

Experimental conditions such as mobile phase, wavelength of scanning and slit dimensions were optimized to provide accurate, precise and reproducible results for the determination of colchicine and khellin. Slit dimension settings of length 6 and width 0.1 mm, monochromator band width 20 nm and scanning rate of 10 mm/s gave reproducible results. Different trials for optimization of the developing systems resulted in the selection of methylene chloride–methanol (95:5) as the best for the separation of both colchicine and khellin from each other and from the other components of the matrix. The wavelength achieving maximum sensitivity was 245 nm. The specificity of the HPTLC method is illustrated in Figure 3, which shows complete separation of colchicine and khellin. The R_f values of colchicine and khellin were found to be 0.17 ± 0.01 and 0.56 ± 0.02, respectively, using the specified developing system. The relationship between the concentration of each compound and its corresponding peak area of the band was investigated. The linear relationship was tested and found to be acceptable for colchicine and khellin. The characteristic parameters of the linear regression equation of colchicine and khellin are shown in Table I.

Validation of the methods

Linearity

The linearity of the HPLC and HPTLC methods for the determination of colchicine and khellin was evaluated by analyzing a series of different concentrations of each drug. In this study, seven concentrations were chosen in which reasonable linearity was achieved in the range of 0.5–20 μg/mL for both compounds using the HPLC method, and 0.7–10 μg/band for colchicine and 0.8–20 μg/band for khellin using the HPTLC method. Each concentration was repeated three times to provide information on the variation in peak area values between samples of the same concentration. The linearity of each calibration graph was validated by the high value of the correlation coefficient (Table I).

Precision

For intra-day precision, three concentrations for colchicine and khellin were analyzed seven times on the same day, whereas the same drug concentrations were analyzed on three different days for inter-day precision. Intra-day precision was expressed

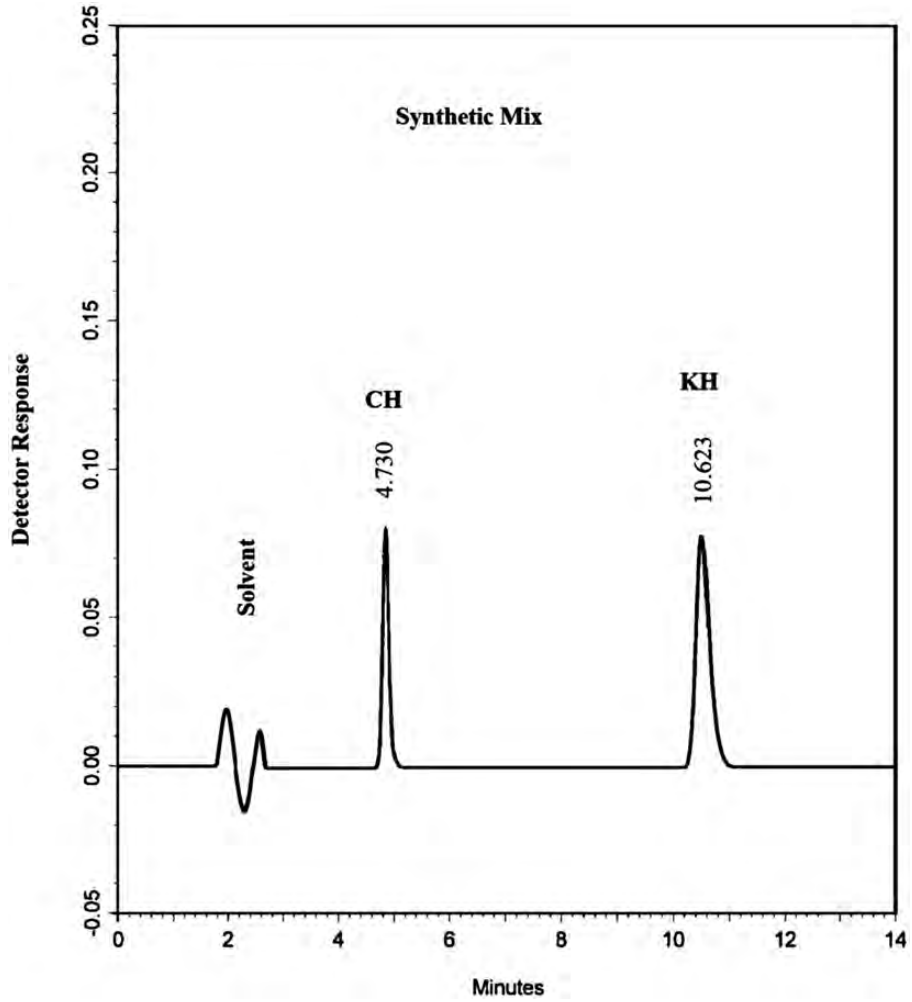

Figure 2. Typical HPLC chromatogram of 20 μL injection of laboratory-prepared mixture of 10 μg mL^{-1} of CH and 10 μg mL^{-1} of KH.

Table I
Characteristic Parameters for the Regression Equations of the HPLC and HPTLC Methods for the Determination of Khellin and Colchicine

	HPLC method		HPTLC method	
Parameters	Khellin	Colchicine	Khellin	Colchicine
Calibration range (μg/mL for HPLC or μg/band for HPTLC)	0.2–20	0.2–20	0.8–20	0. 7–10
Regression equation (Y)* Slope (*b*)	3.19×10^3	1.44×10^3	21.43×10^2	12.36×10^2
Standard deviation of the slope (S_b)	22.18	17.58	34.40	22.36
Relative standard deviation of the slope (S_b)	0.70	1.22	1.59	1.81
Confidence limit of the slope†	$3.17 \times 10^3 - 3.20 \times 10^3$	$1.42 \times 10^3 - 1.45 \times 10^3$	$21.11 \times 10^2 - 21.18 \times 10^2$	$12.14 \times 10^2 - 12.57 \times 10^2$
Intercept (*a*)	− 84.21	−30.42	87.38	−49.45
Standard deviation of the intercept (S_a)	224.41	177.92	432.96	111.96
Confidence limit of the intercept b	− 278.35 − 109.93	−184.34 − 123.51	−333.28 − 508.04	−158.22 − 59.33
Correlation coefficient (*r*)	0.9999	0.9998	0.9998	0.9998
Detection limit (μg/mL for HPLC or μg/band for HPTLC)	0.016	0.028	0.04	0.04
Quantitation limit (μg/mL for HPLC or μg/band for HPTLC)	0.053	0.093	0.12	0.14

*Y = a + bc, where c is the concentration of the substance in μg/mL for HPLC or μg/band for HPTLC and Y is the peak area.
†95% confidence limit, $n = 7$.

through relative standard deviation (RSD) of seven repeated assays of samples at three concentration levels. Inter-day precision was determined by analyzing the same set of samples on three different days. RSD in the precision study for the colchicine and khellin assay was less than 2.0%, which confirmed that the method was highly precise. Results of the precision study for colchicine and khellin by the proposed HPLC and HPTLC methods are given in Table II.

Range

The calibration range was established through consideration of the practical range necessary to give accurate, precise and linear results, according to each compound concentration present in the pharmaceutical product. The calibration range of the proposed methods is given in Table I.

Detection and quantitation limits

According to the International Conference on Harmonization (ICH) recommendations (31), the approach based on the standard deviation (SD) of the response and the slope was used for determining the detection and quantitation limits. The theoretical values were assessed practically and given in Table I.

Selectivity

Method selectivity was achieved by preparing eight laboratory-prepared mixtures of the studied compounds at various concentrations within the linearity range. The laboratory-prepared mixtures were analyzed according to the previously described procedure. Satisfactory results were obtained (Table V), indicating the high selectivity of the proposed methods for the simultaneous determination of colchicine and khellin.

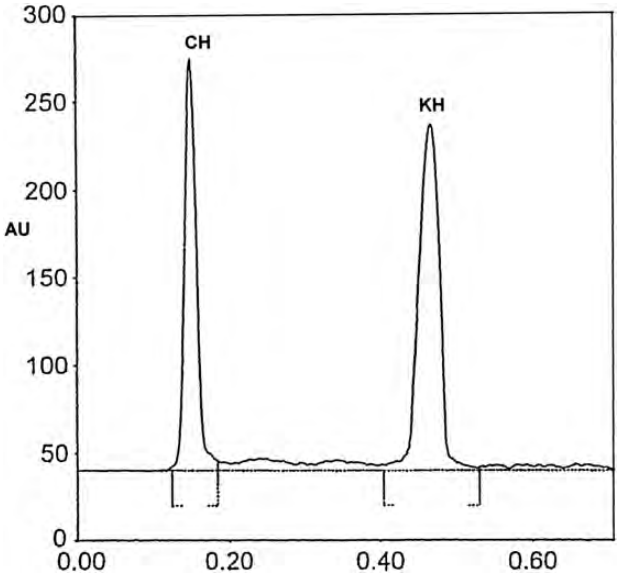

Figure 3. Typical HPTLC chromatogram of laboratory-prepared mixture of 5 μg band^{-1} of CH and 3 μg band^{-1} of KH.

Accuracy

The interference of excipients in the pharmaceutical formulations was studied in detail by the proposed methods. For this reason, the standard addition method was applied to three commercial pharmaceutical formulations containing these compounds. In the application of the standard addition method to three commercial pharmaceutical formulations, the mean percentage recoveries and their standard deviations were calculated for the proposed methods for three replicates. According to the obtained results, satisfactory accuracy was observed for these methods.

Robustness

Variation of the organic strength of the mobile phase by $\pm 2\%$ did not have significant effect on chromatographic resolution in the HPLC and HPTLC methods. Variation of the pH of the 10 mM NaH_2PO_4 of the mobile phase by ± 0.2 units did not have a significant effect on chromatographic resolution in HPLC method.

Analytical solution stability

Colchicine and khellin analytical solutions in methanol exhibited no changes for two days when kept at room temperature, or for 10 days when stored refrigerated at 4°C. Solutions of the studied compounds in the mobile phase exhibited no changes for six hours when kept at room temperature.

Analysis of pharmaceutical products

The proposed methods were first applied for the determination of colchicine alone in Colchicine tablets, Colmetidine tablets, Solvaure sachets, Ur-aid sachets, Urocoline sachets and Urosolvine sachets. Second, they were utilized in the determination of khellin alone in Kellagon sachets, Coli-urinal effervescent granules, Uricol sachets and Jedcorine sachets. Finally, colchicine and khellin were determined simultaneously via these methods in Uricol Plus sachets and Urivin sachets. Seven replicate determinations were made. Satisfactory results were obtained for each compound, which agreed with label claims (Tables III, IV and V).

The proposed methods were applied for quantitative follow-up of these phytopharmaceuticals during their shelf lives. The study revealed that all phytopharmaceuticals maintained the concentrations of their active ingredients within an appropriate range throughout their shelf lives. However, the percent recovery in all phytopharmaceuticals decreased below the satisfactory limit (<95%) beyond the shelf life, except Kellagon

Table II
Precision Data of the Proposed RP-HPLC and HPTLC Methods

Drug	Actual concentration		Measured concentration*							
			Inter-day				Intra-day			
	HPLC (μg/mL)	HPTLC (μg/band)	HPLC (μg/mL)	RSD (%)	HPTLC (μg/band)	RSD (%)	HPLC (μg/mL)	RSD (%)	HPTLC (μg/band)	RSD (%)
Colchicine	2.00	2.00	1.97 ± 0.02	1.02	2.02 ± 0.02	0.99	2.10 ± 0.03	1.43	1.91 ± 0.03	1.57
	10.00	5.00	10.10 ± 0.11	1.09	5.02 ± 0.07	1.39	10.22 ± 0.19	1.86	4.92 ± 0.09	1.83
	20.00	10.00	20.23 ± 0.31	1.53	10.17 ± 0.12	1.18	20.11 ± 0.38	1.89	9.85 ± 0.15	1.52
Khellin	2.00	2.00	2.08 ± 0.02	0.96	2.05 ± 0.03	0.98	2.11 ± 0.04	1.90	2.12 ± 0.04	1.89
	10.00	10.00	10.27 ± 0.13	1.27	10.11 ± 0.12	1.19	10.22 ± 0.19	1.86	10.09 ± 0.20	1.98
	20.00	20.00	20.43 ± 0.35	1.71	20.28 ± 0.26	1.28	20.77 ± 0.40	1.93	20.34 ± 0.37	1.82

*Mean ± SD, $n = 7$.

Table III
Determination of Colchicine (Calculated as Percent Claimed) in Commercial Pharmaceuticals

Phytopharmaceuticals	Mean ± SD*		Student's *t*-value†	Variance ratio *F*-value†
	HPLC	HPTLC		
Colchicine tablets (El Nasr Co.)				
After 4 months (batch 14711)	99.20 ± 0.80	100.35 ± 1.30	1.99	2.64
After 9 months (batch 194709)	96.8 ± 0.62	95.96 ± 1.15	1.70	3.44
After 17 months (batch 364708)	98.45 ± 0.44	97.95 ± 0.85	1.38	3.73
After 22 months (batch 114708)	98.03 ± 0.17	96.45 ± 0.41	0.43	2.31
After 29 months (batch 304707)	97.08 ± 0.22	96.85 ± 0.42	1.28	3.64
After 36 months (batch 204706)	94.85 ± 0.70	95.75 ± 1.15	1.77	2.70
Colmetidin tablets (El Kahira Co.)				
After 11 months (batch 081425)	97.80 ± 0.42	98.45 ± 0.70	2.02	3.10
After 14 months (batch 0711460)	97.70 ± 1.58	96.2 ± 1.38	1.89	1.31
After 20 months (batch 0710282)	96.13 ± 0.85	97.10 ± 0.97	1.99	1.30
After 26 months (batch 0710282)	96.23 ± 0.32	95.95 ± 0.45	1.34	1.98
After 33 months (batch 0711460)	96.18 ± 0.59	95.63 ± 1.13	1.14	3.67
After 36 months (batch 81283)	93.20 ± 0.73	94.18 ± 1.25	1.79	2.93
Solvaure (Jedco)				
After 5 months (batch 0803329)	103.13 ± 1.31	104.04 + 1.76	1.10	1.81
After 33 months (batch 0803329)	85.50 ± 1.29	84.20 ± 1.24	1.92	0.92
Ur-aid effervescent (El Pharonia Co.)				
After 5 months (batch 3348286)	101.08 ± 0.99	102.07 ± 1.21	1.68	1.49
After 11 months (batch 3348109)	99.63 ± 0.62	100.51 ± 0.98	2.08	2.49
After 19 months (batch 3340601)	99.45 ± 0.31	99.84 ± 0.50	1.75	2.60
After 33 months (batch 3348017)	101.13 ± 1.72	100.95 ± 1.03	0.24	2.79
After 36 months (batch 3346293)	94.78 ± 0.51	94.45 ± 0.97	0.80	3.62
Urocoline effervescent (Eva Co.)				
After 2 months (batch 81283)	104.68 ± 0.46	105.21 ± 0.89	1.40	3.74
After 11 months (batch 81283)	103.95 ± 0.70	103.34 ± 0.99	1.33	2.00
After 21 months (batch 81283)	103.58 ± 0.38	104.12 ± 0.57	2.09	2.25
After 33 months (batch 81283)	98.10 ± 0.78	97.16 ± 1.12	1.82	2.06
After 36 months (batch 81283)	93.68 ± 0.31	92.55 ± 0.48	0.60	2.40
Urosolvine effervescent (El Nile Co.)				
After 6 months (batch 1211005)	95.65 ± 0.35	95.15 ± 0.62	1.85	3.14
After 12 months (batch 129065)	95.58 ± 0.44	96.10 ± 0.51	2.04	1.34
After 21 months (batch 128517)	95.49 ± 0.45	94.98 ± 0.85	1.40	3.57
After 33 months (batch 128173)	95.33 ± 0.44	94.95 ± 0.80	1.10	3.30
After 36 months (batch 128173)	93.60 ± 0.53	94.23 ± 1.07	1.40	4.08

*Mean ± SD, $n = 7$.
†Tabulated *t*-value at the 95% confidence level is 2.18; tabulated *F*-value at the 95% confidence level is 4.18.

Table IV
Determination of Khellin (Calculated as Percent Claimed) in Commercial Pharmaceuticals

Phytopharmaceuticals	Mean ± SD*		Student's *t*-value†	Variance ratio *F*-value†
	HPLC	HPTLC		
Kellagon sachets (Mepaco Co.)				
After 5 months (batch 171008)	102.75 ± 1.71	103.12 ± 1.67	0.41	1.05
After 13 months (batch 440408)	100.20 ± 1.31	101.44 ± 1.45	0.63	1.68
After 20 months (batch 171008)	98.45 ± 0.44	97.84 ± 0.87	1.66	3.91
After 24 months (batch 440408)	97.63 ± 0.49	95.98 ± 0.93	1.64	3.47
After 33 months (batch 100606)	97.075 ± 0.22	96.83 ± 0.41	1.42	3.47
After 36 months (batch 100606)	97.15 ± 0.60	96.87 ± 1.19	0.56	3.93
Coli-urinal effervescent (El Kahira Co.)				
After 5 months (batch 702088)	104.175 ± 0.42	104.75 ± 0.71	1.83	2.86
After 12 months (batch 728038)	101.20 ± 0.84	101.78 ± 0.98	1.19	1.36
After 17 months (batch 702088)	99.15 ± 0.75	100.10 ± 0.96	2.06	1.64
After 27 months (batch 728038)	97.90 ± 0.82	97.97 ± 1.12	0.13	1.87
After 32 months (batch 702088)	96.18 ± 0.59	97.11 ± 1.10	1.97	3.48
After 36 months (batch 728038)	97.85 ± 0.75	96.95 ± 1.17	1.71	2.43
Uricol effervescent (El Pharonia Co.)				
After 3 months (batch 11067)	99.20 ± 0.56	98.29 ± 1.10	1.95	3.86
After 10 months (batch 11080)	98.38 ± 0.54	97.60 ± 0.99	1.83	3.36
After 22 months (batch 9385)	97.89 ± 0.43	98.19 ± 0.73	0.93	2.88
After 24 months (expired) (batch 9385)	92.94 ± 1.30	93.56 ± 1.87	0.72	2.07
Jedcorine effervescent (Jedco)				
After 16 months (batch 0903015)	100.97 ± 0.94	102.24 ± 1.38	2.01	2.16
After 36 months (batch 0903015)	90.91 ± 1.34	89.67 ± 1.13	1.87	1.41

*Mean ± SD, $n = 7$.
†Tabulated *t*-value at the 95% confidence level is 2.18; tabulated *F*-value at the 95% confidence level is 4.18.

sachets, Coli-urinal effervescent granules, Uricol Plus sachets and Urivin sachets. The chromatograms from commercial products showed satisfactory separation of colchicine and khellin peaks in different pharmaceutical formulations (Figures 4 and 5). No published methods have been reported for the simultaneous determination of the studied components of these mixtures. Therefore, the results of the proposed HPTLC method were compared with those of the proposed HPLC method. Statistical

Table V
Determination of Colchicine and Khellin (Calculated as Percent Claimed) in Commercial Pharmaceuticals

Phytopharmaceuticals	Mean ± SD*				Student's *t*-value†		Variance ratio *F*-value†	
	HPLC		HPTLC					
Uricol Plus effervescent (El Pharonia Co.)	Colchicine	Khellin	Colchicine	Khellin	Colchicine	Khellin	Colchicine	Khellin
After 3 months (batch 1644)	97.56 ± 0.65	97.82 ± 0.53	98.10 ± 1.05	98.22 ± 0.85	1.15	1.58	2.61	2.57
After 22 months (batch 1227)	97.08 ± 0.22	97.80 ± 0.42	96.71 ± 0.41	96.23 ± 0.69	1.65	1.87	3.47	2.70
After 36 months (batch 1068)	96.41 ± 1.13	95.53 ± 1.20	96.78 ± 1.15	94.52 ± 1.21	0.61	1.57	1.04	1.02
Urivin effervescent (Amoun Co.)								
After 6 months (batch 104331)	96.38 ± 0.59	97.53 ± 0.96	95.24 ± 1.02	98.12 ± 0.75	2.56	1.28	2.99	1.64
After 23 months (batch 091136)	96.37 ± 0.18	97.94 ± 0.42	96.64 ± 0.32	97.95 ± 0.89	1.95	0.92	3.16	3.54
After 28 months (batch 090058)	98.49 ± 0.37	98.45 ± 0.38	98.13 ± 0.41	97.91 ± 0.75	1.72	1.70	1.23	3.89
After 36 months (batch 08548)	95.33 ± 0.44	95.12 ± 0.94	94.96 ± 0.82	94.87 ± 1.23	1.05	0.42	3.47	1.23

*Mean ± SD, $n = 7$.
†Tabulated *t*-value at the 95% confidence level is 2.18; tabulated *F*-value at the 95% confidence level is 4.18.

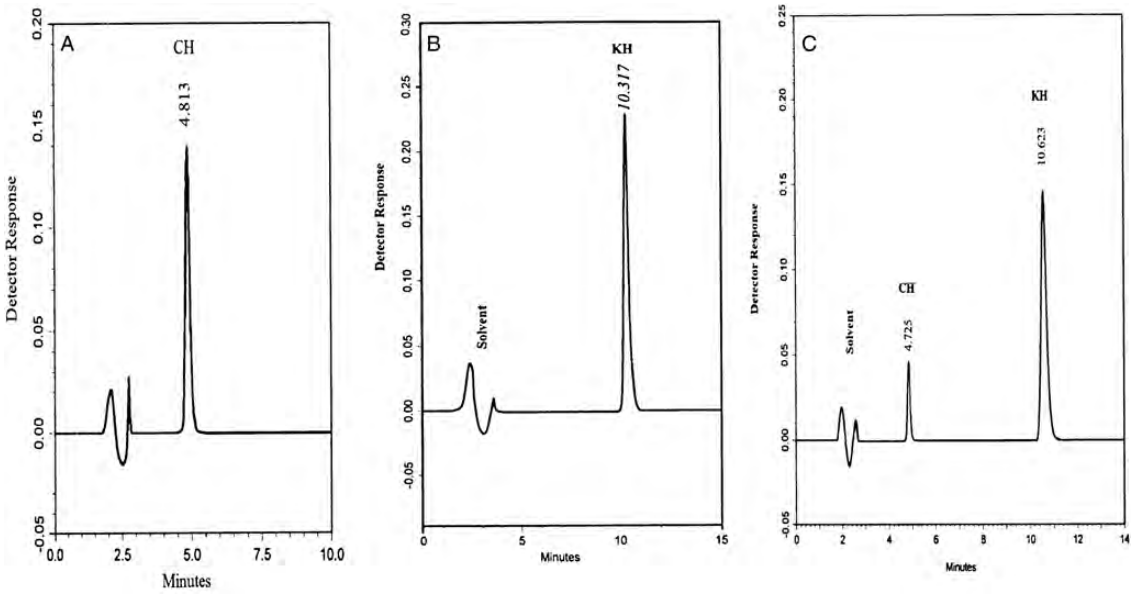

Figure 4. Typical HPLC chromatograms of 20 μl injection of (A) Colmetidine® tablets containing 20 μg mL^{-1} of CH; (B) Kellagon® sachets containing 20 μg mL^{-1} of KH and (C) Urivin® sachets containing 3 μg mL^{-1} of CH and 17.5 μg mL^{-1} of KH.

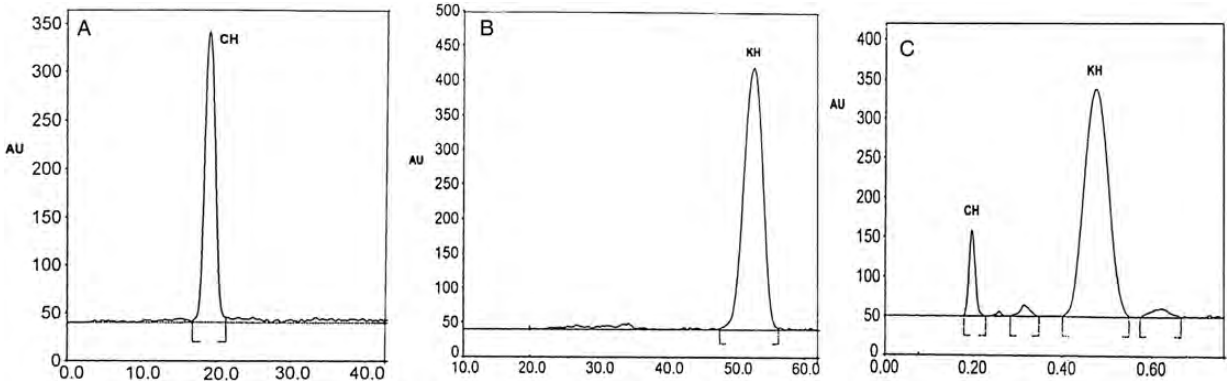

Figure 5. Typical HPTLC chromatograms of (A) Colmetidine® tablets containing of 6 μg $band^{-1}$ of CH; (B) Kellagon® sachets containing 5 μg $band^{-1}$ of KH and (C) Urivin® sachets containing 0.8 μg $band^{-1}$ of CH and 4.66 μg $band^{-1}$ of KH.

comparison between the results was performed with regard to accuracy and precision using Student's *t*-test and *F*-ratio at 95% confidence level (Tables III, IV and V). No significant difference was observed between the results.

Conclusion

The proposed HPLC and HPTLC methods provide simple, accurate and reproducible quantitative analysis for the determination of colchicine and khellin in pharmaceutical products, without any interference from the excipients. The HPTLC method is simple and uses a minimal volume of solvents compared to the HPLC method.

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