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Validated TLC-spectrodensitometric method for simultaneous determination of policresulen and cinchocain hydrochloride in combined dosage forms

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Abstract

TLC-spectrodensitometric method was developed and validated for the simultaneous determination of policresulen (POL) and cinchocaine hydrochloride (CIN). The drugs were separated on TLC aluminum plates coated with silica gel 60 F_{254}, using chloroform : methanol : ammonia (9.5:0.6:1.0, v/v/v) as a mobile phase and Dragendorff reagent as spot detection at 490 nm. The linearity ranges of the method were 0.8-20 and 0.2-3.5 µg band^{-1}, for POL and CIN, respectively. Limits of detection were 0.23 and 0.07 µg band^{-1} for POL and CIN, respectively. The proposed method was validated according to ICH guidelines and utilized for simultaneous determination of the cited drugs in their laboratory prepared mixtures and pharmaceutical preparations. The mean percentage recoveries were 100.46 ± 1.1 and 99.65 ± 0.49 for POL and CIN, respectively. The obtained results were statistically compared with that of the official or reported methods. Comparisons revealed that there is no significant difference regarding both accuracy and precision. The method was found to be precise, rapid, specific, and accurate. Thus the method can be applied for the routine analysis of POL and CIN in their pharmaceutical formulations without prior separation.

Key words
policresulen, cinchocain hydrochloride, TLC-spectrodensitometric, dosage forms

1. Introduction

Policresulen (POL) (2-Hydroxy-3,5-bis [(4-hydroxy-2-methyl-5-sulfo-phenyl) methyl] - 4 - methyl - benzenesulfonic acid, **Figure 1**) is a topical haemostatic and antiseptic drug. Cinchocaine HCl (CIN) (2-butoxy - N - [2-(diethylamino) ethyl] quinoline-4 carboxamide hydrochloride, **Figure 1**) has a role in the reduction of pain and itching of hemorrhoids [1]. Both drugs are used for the management of internal and external hemorrhoids and combined together in ointments or suppositories dosage forms [1].

A review of the literature showed that methods reported for the determination of CIN were spectrophotometry [2 , 5], HPLC [6 , 8], gas chromatography [9] and electrochemical methods [10 , 12]. Few methods were reported for POL which are spectrophotometry [13] and HPLC [14]. There is no reported method for the determination of both drugs either in their binary mixture or in the presence of their degradation products. Therefore, the objective of this work was to develop a sample and validated TLC-spectrodensitometric method for the concurrent determination of POL and CIN in bulk powders, laboratory prepared mixtures and pharmaceutical dosage forms.

2. Experimental

2.1. Apparatus and software

- TLC-spectrodensitometric system: CAMAG TLC scanner 3 S/ N 130319 operated with winCATS software, Linomat 5 autosampler (CAMAG, Muttenz, Switzerland), CAMAG micro syringe (100 µL).TLC aluminum sheets (20x20 cm) pre-coated with silica gel 60 F_{254} (Merck KgaA, Darmstad, Germany) were used. Calculations were performed using the Microsoft Excel program.
- The centrifugation system: Laboratory Centrifuge, Sigma 2-16KL, Sigma 2-16KHL, with order number 10350, 10353.

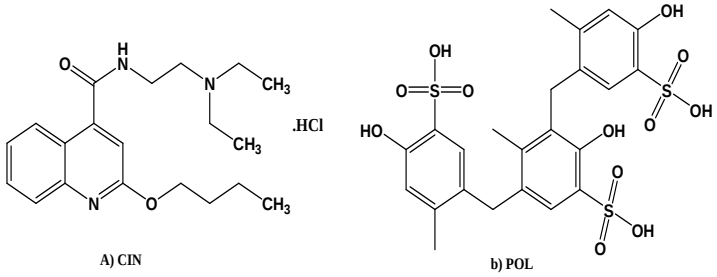

Figure 1: The chemical structures of (a) cinchocaine HCl, (b) policresulen

2.2. Chemicals and reagents

Policresulen (POL) powder with claimed purity of 99.15%, as to reported by HPLC method [14] was kindly supplied by AUG Pharma Co., Cairo, Egypt. Cinchocaine HCl (CIN) powder with claimed purity of 99.75%, according to an official method [15] was kindly supplied by AUG Pharma Co., Cairo, Egypt. The studied market dosage forms, FAKTU® ointment and suppositories (AUG Pharma Co., Cairo, Egypt), were labeled to contain 50 mg POL and 10 mg CIN per one gm ointment (batch

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No. 17289) and 100 mg POL and 2.5 mg CIN per one suppository (batch No. 18188). Methanol and chloroform (Analar grade), 32% ammonia solution were provided from (Adwic, El Nasr pharmaceutical Chemicals Co., Egypt). Dragendorff reagent was prepared as follow; solution A contain0.85 gm bismuth subnitrate, 10 mL acetic acid and 40 mL distilled water; solution B contain 8 gm potassium iodide, and 20 mL distilled water. To prepare 100 mL of the reagent, 5 mL of solution A , 5 mL of solution B and 40 mL of acetic acid were mixed together and completed to the mark with distilled water.

2.3. Standard solutions

Stock solutions of both POL and CIN of concentration 1 mg mL^{-1} were prepared using methanol as a solvent. Working solutions were freshly prepared by dilution of the stock solutions with methanol to have 500 µg mL^{-1} of both drugs.

2.4. Chromatographic conditions

TLC aluminum sheets 20 x 20 cm pre-coated with 0.25 mm silica gel 60 F_{254} were used. The samples were applied as bands (band width: 6 mm, bands were spaced 1 cm apart from each other and 1.5 cm from the bottom of the plate). The mobile phase was chloroform: methanol: ammonia (9.5:0.6:1.0 v/v/v). Linear ascending development was carried out in a chromatographic reservoir which was previously saturated with the mobile phase for 15 min. at room temperature to a distance of 8 cm from the baseline. The plates were dried with hair dryer, sprayed with dragendorff reagent and scanned at 490 nm. The identification was performed using Camage TLC scanner 3 operated in reflectance-absorbance mode. The slit dimension was kept at 3 mm x 0.45 mm and the scanning speed was 20 mm/s.

2.5. Procedure for calibration curve

Aliquot volumes (0.8–20.0 µg $band^{-1}$) of POL and (0.2–3.5 µg $band^{-1}$) of CIN were separately transferred from their working solutions (500 µg mL^{-1}) into 10 mL volumetric flasks and diluted to volume with methanol. Aliquot of 20 µL of each solution was applied to the TLC plate using a 100 µL syringe. The chromatographic separation were performed and the chromatograms were recorded. The calibration curves were constructed by plotting the recorded peak area versus the corresponding drug concentrations, from which the regression equations were calculated.

2.6. Assay of laboratory-prepared mixtures

Different aliquot volumes of both standard drug solutions were accurately transferred from their working solutions and mixed to prepare solutions of different ratios. Twenty µL of each solution was applied to a TLC plate using a 100 µL syringe. The chromatographic conditions were adopted for each laboratory-prepared mixture, and the concentrations of each drug were calculated from the corresponding regression equation.

2.7. Application to pharmaceutical preparations

Cream: A five-gram portion of the cream was transferred to a 50-mL volumetric flask, taking care to avoid sticking cream to the walls of the volumetric flask. A 35-mL of methanol was added to the flask, and the cream was allowed to melt by warming at 60 °C in a water bath with constant shaking. The solution was allowed to cool to room temperature. The volume was made up to the mark with methanol and mixed. The solution was centrifuged at 5000 rpm for 10 min, and the clear supernatant solution was obtained. A portion of the supernatant was diluted with methanol to obtain a final concentration 500 µg mL^{-1} of POL and 100µg mL^{-1} of CIN. Twenty µL of this solution was applied to a TLC plate and developed as described previously. The concentrations of each drug were calculated from the corresponding regression equation. When carrying out the standard addition technique, different known amounts of pure standard POL and CIN were added to the pharmaceutical dosage form content before proceeding in the previously mentioned method.

Suppositories: Five suppositories were accurately weighed, mixed with 30 mL of methanol, melted in a controlled water bath set at 60 °C, and then cooled while stirring. The volume was completed to mark with methanol and the solution was then centrifuged at 5000 rpm for 10 min, and the clear supernatant solution was obtained. The procedure was completed as mentioned under cream.

3. Results and discussion

This work was aimed to develop simple, selective and precise TLC-spectrodensitometric method for the simultaneous estimation of POL and CIN in their pure forms and pharmaceutical dosage forms. This method offers a simple way for quantification directly on TLC plate by measuring the optical density of the separated bands. The amounts of compounds are obtained by comparing to a standard curve of the standard drugs solutions chromatographed simultaneously under the same condition. To optimize the method conditions, it was necessary to test the effect of different variables. In order to separate the two drugs from each other, several ratios of different developing systems were checked. Finally it was found that the best separation of POL and CIN was obtained by applying the developing system containing chloroform: methanol: ammonia (9.5:0.6:1.0, v/v/v). R_f for POL and CIN were 0.29 ± 0.01 and 0.88 ± 0.01, respectively. Different scanning wavelengths were tried (254, 415 and 490 nm); on using 490 nm, the separated peaks were more sharp and symmetrical with minimum noise, as shown in **Figures (2 and 3).**

3.1. Method validation

Method validation was performed according to ICH guidelines [16] regarding linearity, range, precision, accuracy, limit of detection and limit of quantitation.

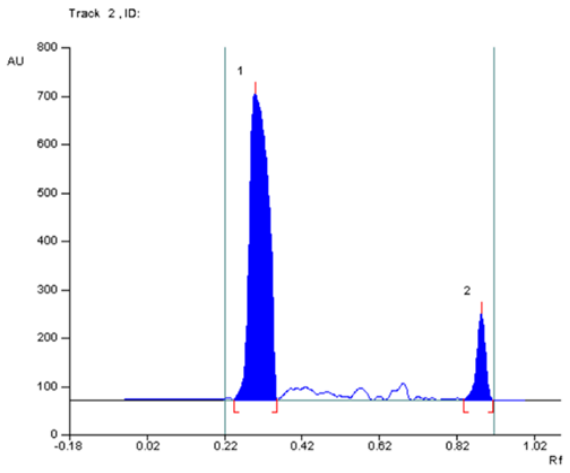

Figure 2: 2D Densitogram of FAKTU® ointment containing;15µg POL (R_f = 0.29±0.01) and3µg CIN (R_f = 0.88±0.01).

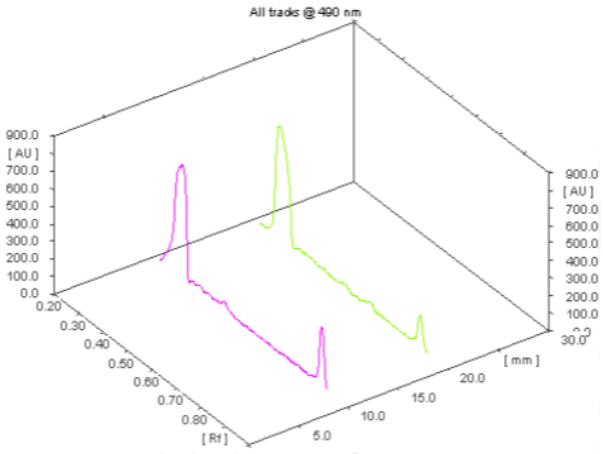

Figure 3: 3D Densitogram of FAKTU® ointment containing; 15µg POL and 3µg CIN.

3.1.1. Range and linearity

The linearity of the proposed method was evaluated by processing the different calibration curves. Analysis was carried out on a series of standard drug solutions, and the calibration curves were constructed between AUC and corresponding concentrations of bands. Linear regression analysis was applied and analytical parameters were calculated. The linear ranges were found to be 0.8-20 µg band^{-1} and 0.2-3.5 µg band^{-1} for POL and CIN, respectively. The method has a good linearity as indicated by the values of correlation coefficient The linear concentration ranges, calibration equations and other statistical parameters for the proposed method were listed in **(Table 1)**.

3.1.2. Limits of detection and quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) of the proposed method were calculated, for both drugs using a ratio of 3.3 and 10 standard deviations (SD) of the blank and the slope of the calibration line, **(Table 1).** The limits of detection were calculated as SD×3.3/slope. Whereas, limits of quantitation were calculated as SD×10/slope.

(Table 1): Assay parameters and method validation sheet obtained by applying the proposed TLC-spectrodensitometric method for determination of POL and CIN in binary mixture.

Parameter	POL	CIN
Concentration range (µg band^{-1})	0.8 – 20	0.2 – 3.5
Slope	46.81	99.64
Standard deviation of the slope (S_b)	0.2596	0.8754
Intercept	3.446	0.2857
Standard deviation of the intercept (S_a)	3.2863	1.9575
Standard deviation of the residuals (Sy/x)	4.387	2.316
Number of determinations	7	7
Correlation coefficient (r)	0.9998	0.9996
Determination coefficient (r^2)	0.9999	0.9998
Limit of detection, LOD (µg band^{-1})	0.232	0.065
Limit of quantitation, LOQ (µg band^{-1})	0.702	0.196

3.2. TLC system suitability

System suitability parameters were calculated and compared to reference value [17], **(Table 2)**.

(Table 2): System suitability parameters of the proposed TLC-spectrodensitometric method

Parameter	POL	CIN	Reference value [17]
R_f value	0.29 ± 0.01	0.88± 0.01	0.2 – 0.8
T (tailing factor)	1.15	1.11	T≤ 1.15 - 0.95
R_s *	4.23	2.18	R_s> 1.5

*experimental resolution

3.2.1. Accuracy

To study the accuracy of the proposed method, repeated analysis (three times) of different concentrations of POL and CIN within the linearity range were performed. The accuracy expressed as mean percentage recoveries and relative standard deviations (RSD) is shown in **(Table 1)**.

3.2.2. Precision

The inter- and intra-day precisions of the proposed method were determined by the analysis of three different concentrations of POL and CIN, within the linearity range. Three replicate analysis of three pure samples of both drugs were carried on a single day and three consecutive days, for the inter- and intra-day precisions, respectively. The results were expressed as mean percentage recoveries and RSD is illustrated in **(Table 3)**.

3.2.3. Selectivity

Selectivity was ascertained by analyzing different mixtures containing both drugs in different ratios within the linearity range. Satisfactory results were shown in **(Table 4)**.

The interference of excipients in the pharmaceutical formulations was studied by applying standard addition method to the pharmaceutical formulation. Good accuracy proved that the excipients in pharmaceutical formulation did not interfere in the analysis of these excipients shown in **(Tables 5 and 6)**.

(Table 3) : Application of Intra-day and Inter-day technique to the analysis of POL and CIN in FAKTU® ointment by the proposed TLC–spectrodensitometric method.

Level	Drug	Conc. level (µg band^{-1})	*% Recovery ± SD	%RSD
Inter-day	**POL**	5	99.51 ± 0.65	
		10	98.31 ± 0.65	0.65
		15	100.04 ± 0.65	
	CIN	1	99.51 ± 0.79	
		2	100.75 ± 0.79	0.79
		3	98.44 ± 0.79	
Intra-day	**POL**	5	99.71 ± 0.98	
		10	101.82 ± 0.98	0.98
		15	99.54 ± 0.98	
	CIN	1	98.09 ± 0.92	
		2	99.54 ± 0.92	0.92
		3	100.79 ± 0.92	

*Average of three experiments

(Table 4): Determination of POL and CIN in laboratory prepared mixtures by the proposed TLC-spectrodensitometric method.

No.	Mix ratio, POL/CIN	POL	CIN
1	1:1	99.45	100.54
2	2:1	99.27	99.48
3	5:1	100.54	99.36
4	20:1	99.65	99.78
5	20:0.5	98.07	99.01
6	1:7	99.48	98.98
Mean		99.58	99.53
±SD		0.35	0.26

(Table 5): Analysis of POL an CIN in FAKTU® ointment and suppositories by the proposed TLC-spectrodensitometric method and those reported method.

Dosage forms	% Recovery ±SD	
	Proposed method	Reported method
FAKTU® ointment *		
POL	98.65 ± 1.45	99.15± 0.89
CIN	101.65 ± 1.13	99.75 ± 0.73
FAKTU® suppositories *		
POL	101.41 ± 0.98	99.15 ± 0.89
CIN	100.65 ± 0.91	99.75 ± 0.73

* The , batch No. for FAKTU® ointment and suppositories were 17289 and 18188

3.3. Application to pharmaceutical formulations

(Table 7) shows statistical comparison of the results obtained by the proposed method and reported method for POL [14] and official method for CIN [15]. The calculated t and F values were less than the theoretical ones indicating that there was no significant difference between the proposed, reported and official methods with respect to accuracy and precision.

(Table 6): Application of standard addition technique to the analysis of POL and CIN in FAKTU® ointment and suppositories by the proposed TLC–method.

Dosage forms	Drug claimed taken (µg band^{-1})	Added µg band^{-1}	Found µg band^{-1}	*% Recovery
FAKTU® ointment	**POL (10)**	5	5.05	101.00
		10	9.90	99.00
		15	15.09	100.60
		Mean ± SD		100.20 ± 0.8
	CIN (2)	1	0.99	99.00
		2	2.03	101.50
		3	2.97	99.00
		Mean ± SD		99.80 ± 1.1
FAKTU® suppository	**POL (15)**	10	9.96`	99.00
		15	15.09	100.60
		20	20	100.00
		Mean ± SD		99.86 ± 0.58
	CIN (2.5)	1.5	1.5	100.00
		2.5	2.45	98.00
		3.5	3.5	100.00
		Mean ± SD		99.30 ± 0.9

*Average of three experiments

(Table 7): Statistical comparison between the results obtained by the proposed TLC-spectrodensitometric method and the reported methods for the determination of POL and CIN in pure powder form.

Parameter	Reported method		TLC method	
	POL	CIN	POL	CIN
Mean	99.15	99.75	100.46	99.66
Standard deviation, SD	0.89	0.73	1.1	0.49
N	3	3	7	7
Variance	0.79	0.53	1.21	0.24
Student' t (2.306)			0.98	1.08
F (6.3338)			1.53	2.22

Conclusion

Despite of the complete overlapping between the UV spectra of the two drugs, TLC-spectrodensitometric technique was applied successfully for the determination of POL and CIN in their mixture and combined dosage forms. The advantage of TLC-spectrodensitometric method is that several samples can be run simultaneously, thus lowering analysis time and cost per analysis and providing high selectivity. The proposed method have been validated according to the ICH guidelines and applied for the determination of both POL and CIN in FAKTU® ointment and suppositories by applying the standard addition technique. The developed method could be used for routine analysis in quality control laboratories where economy and time are essential.

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