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Simultaneous Determination of Ternary Mixture of Aspirin, Caffeine and Orphenadrine Citrate by Simple RP-TLC Spectrodensitometric Method

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Authors' contributions

This work was carried out in collaboration between all authors. Author NWA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MG and MRE managed the analyses of the study. Author MA managed the literature searches and made the final revision. All authors read and approved the final manuscript.

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ABSTRACT

Aims: A simple TLC Spectrodensitometric method was developed for analysis of Orphenadrine Citrate (OR), Caffeine (CAF) and Aspirin (ASP) either in pure form or in their pharmaceutical preparations.
Study Design: Validation study.
Methodology: In this method, The three drugs were separated on silica gel plate using ethyl acetate: acetone: methanol: triethylamine (6:3:1:0.2, by Volume) as mobile phase at room temperature. Many experimental parameters such as band size, slit width, different developing

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mobile phases and scanning wavelengths were examined and the optimum conditions were selected. The obtained bands were then scanned at 220 nm. The three drugs were adequately resolved with the R_f values of ASP (R_f = 0.08 ± 0.02), CAF (R_f = 0.55 ± 0.02) and OR (R_f = 0.35 ± 0.02). Validation parameters of the developed method were studied like linearity, accuracy, precision, and specificity.

Results: Linearity of the proposed method was found to be in the concentration ranges 0.4-2 μg/band for ASP, 0.4-2 μg/band for CAF and 0.3-3 μg/band for OR.

Conclusion: The suggested method was effectively used for analysis of ASP, CAF and OR in pure form and in their medicinal formulations. The method is proved to be specific, accurate and selective.

Keywords: TLC; spectrodensitometry; ternary mixture; aspirin; caffeine and orphenadrine citrate.

1. INTRODUCTION

Orphenadrine citrate (OR) Fig. 1a, chemically known as, N,N-Dimethyl-2-[(2-methylphenyl)-phenylmethoxy] ethanamine citrate [1]. It is a tertiary amine antimuscarinic actions and uses similar to those of trihexyphenidyl. OR is used as citrate to relieve pain due to skeletal muscle spasm. Combinations of OR with an NSAID have been used for the treatment of musculoskeletal and joint disorders [2].

Caffeine (CAF) Fig. 1b, is named chemically as, 3,7-Dihydro-1,3,7-trimethyl-1H-purine-2,6-dione [1]. CAF is a methylxanthine which inhibits the enzyme phosphodiesterase as theophylline and has an antagonistic effect at central adenosine receptors. It stimulate the CNS, particularly the higher centers. It can produce a condition of wakefulness and increased mental activity. CAF facilitates the performance of muscular work and increases the total work which can be performed by a muscle. It is also frequently included in oral analgesic preparations with aspirin, paracetamol, or codeine in unit doses of about 15–65 mg but its clinical benefits is debated [2].

Aspirin (ASP) Fig. 1c, is known as, 2-(acetyloxy) benzoic acid [1]. ASP is a salicylate NSAID and has analgesic, anti-inflammatory and anti- pyretic properties; it act as inhibitor of the enzyme cyclo-oxygenase, which results in the direct inhibition of the biosynthesis of prostaglandins. ASP is used for relieving of mild to moderate pains such as headache, dysmenorrhoea, myalgias, and dental pains. It has also been used in the management of pains and inflammation in acute and chronic rheumatic disorders such as rheumatoid arthritis, juvenile idio-pathic arthritis and osteoarthritis [2]. The drug combination of OR, CAF and ASP is formulated for symptomatic reliefs of mild to moderate pain of acute musculoskeletal disorders.

Different techniques have been mentioned before for analysis of CAF and ASP combination including, spectrophotometry [3–6], HPLC [7–14] and capillary electrophoresis [15] method. Besides that, different methods have been developed for determination of OR either alone or in combination with other drugs including spectrophotometry [16–22], GC [23], TLC [24] and HPLC [25–28] methods.

Up to data, only three methods were mentioned for analysis of the studied ternary mixture which including, RP-HPLC [29] and spectrophotometric [30,31] methods.

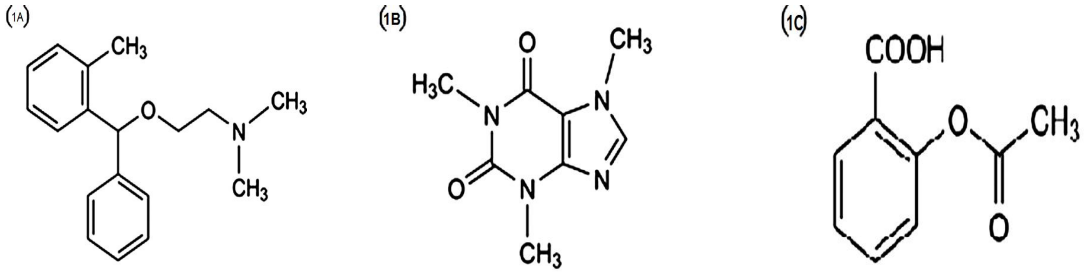

Fig. 1. Chemical structure of OR (A), CAF (B) and ASP (C)

The RP-HPLC chromatographic method [29] had the advantage of determination of this ternary mixture in presence of ASP degradation products, OR and CAF process related impurities, and excipients. The chromatographic method parameters were reversed-phase column [Phenomenex™ Luna ODS C18) with gradient elution based on; eluant [A]: 0.1% triethylamine in aqueous potassium dihydrogen phosphate buffer (50 mM; pH 3.0), while as, eluant [B]: acetonitrile, at a flow rate of 1.5 mL/min and UV detection at 215 nm. This method can be used for checking quality during stability studies of pharmaceutical preparations containing these drugs so it is considered as stability indicating method rather than simple analysis one.

The published spectrophotometric method [30] depends on solvent extraction of ASP prior to determination followed by assay of the remaining OR and CAF using first derivative and first derivative of ratio spectra methods. The other spectrophotometric method [31] uses double divisor ratio spectra derivative, area under curve of derivative ratio and mean centering of ratio spectra spectrophotometric methods for the analysis of the ternary mixture. It is clear that both methods lack simplicity and need complicated procedures that make them unsuitable for routine quality control analysis of the mixture.

No TLC method has been reported for simultaneous analysis of the three drugs. Therefore, the objective of this paper is to develop sensitive, selective and reproducible TLC method for simultaneous determination of OR, CAF and ASP for routine quality control analysis of these drugs in both bulk powder and pharmaceutical formulations.

2. EXPERIMENTAL

2.1 Apparatus

UV lamp with short wavelength 254 nm (Spectroline, Delaware, USA).

TLC scanner 3 densitometer (CAMAG, Muttenz, Switzerland).

The following factors were adjusted:

- Slit dimensions: 6.00x0.45, Micrometer - Scanning speed = 20 mm/s

Data resolution = 100 µm / step

Sample applicator for TLC Linomat IV with 100 µL syringe (CAMAG, Muttenz, Switzerland).

TLC plates (20x20 cm) coated with silica gel 60 F 254 with Layer thickness = 175 - 225 µm (Merck KGaA, Darmstad, Germany).

Bransonic ultrasonic cleaner (Branson, Danbury, USA).

2.2 Materials

2.2.1 Pure samples

Aspirin, Caffeine and Orphenadrine Citrate were kindly supplied by (Sigma for pharmaceuticals International (SPI), Egypt).

The purities of the studied drugs were found to be 100.84% ± 1.004, 98.96% ± 0.578 and 99.21% ± 0.602, respectively, according to the company analysis certificate (HPLC).

2.2.2 Market samples

Relatic® tablet, (Batch No.71014), each tablet was claimed to contain 770 mg of Aspirin, 60 mg caffeine and 50 mg Orphenadrine Citrate were obtained from (manufactured by Sigma for pharmaceutical International (SPI), Egypt for Horizone Pharma, Egypt).

2.2.3 Reagents

All reagents and chemicals were of analytical grade and used without further purification.

Ethylacetate, acetone, triethylamine and methanol were purchased from (EL - Nasr Pharmaceutical Chemicals Co., Abu - Zabaal Cairo, Egypt).

2.3 Preparation of Standard Solutions

ASP, CAF and OR stock standard solutions (1 mg /mL).Weigh accurately 0.1 gram of each drug into a three separate 100-mL volumetric flask, add 50 mL methanol and shake to dissolve then complete the volume to the mark with methanol.

ASP, CAF and OR working standard solutions (100 µg /mL).Transfer accurately 10 mL of the stock solution of each drug into three separate 100-mL volumetric flasks and complete to volume with methanol to get 100 µg /mL working solution for each drug.

2.4 Procedures

2.4.1 Linearity and construction of calibration curves

Accurate aliquots equivalent to (0.4 - 2 µg /mL) of ASP, (0.4-2 µg /mL) of CAF and (0.3-3 µg /mL) of OR from its working solutions (l00 µg /mL) were applied to thin layer chromatographic plate (20x20 cm) as band using the Camage TLC sampler. A space of 1 cm between each band was left and 1.5 cm from the bottom edge of the plate. The plates were developed in a chromatographic tank previously saturated for one hour with the mobile phase consisting of ethylacetate: acetone: methanol: triethylamine (6: 3: 1: 0.2, by volume). The plates were developed by ascending chromatography through a distance of 17 cm by ascending chromatography at room temperature then the plates were dried in air.

The bands were detected under UV - lamp and scanned at 220 nm under the specified experimental conditions. The calibration curves were constructed for each compound by plotting the peak area/ 100 versus the corresponding concentration and then the regression equations were computed as shown from the following equations.

Parameters	Regression equation	Correlation coefficient (r)
ASP	Y1 =0.4067C1+0.3121	0.9991
CAF	Y2=0.5888C2 -0.0182	0.9992
OR	Y3=0.4107C3+0.3951	0.9990

Where Y1, Y2 and Y3 are the peak area /100, C1, C2 and C3 are ASP, CAF and OR concentrations in µg/band respectively and r1, r2 and r3 are the correlation coefficients.

2.4.2 Analysis of laboratory prepared mixtures

Mixtures containing ASP, CAF and OR were prepared in different ratios. Proceed as mentioned under linearity and construction of calibration curves. The concentrations of the three compounds were calculated from their corresponding regression equations as mentioned before.

2.4.3 Application of the proposed methods to pharmaceutical formulations

The contents of ten tablets of Relatic® tablet were thoroughly powdered and mixed then an amount of the powder equivalent to 770 mg of ASP, 60 mg of CAF and 50 mg of OR was weighed accurately in 250-mL beaker, 70 mL of methanol was added, stirred for about 30 min then filtered through filter paper into a 100-mL volumetric flask, the beaker and the funnel were washed and the volume was completed with methanol to get a concentration of 7.7, 0.6 and 0.5 mg /mL for ASP, CAF and OR respectively. Amount equal to 1 mL of filtrate was diluted with methanol in 50 mL volumetric flask then further dilution was made by taking 1mL from pervious flask into 10 mL volumetric flask and the volume was completed to the mark with methanol to bring up a concentration of 15.4, 1.2 and 1.0 µg /mL ASP, CAF and OR, respectively. Further diluation was done to get a concentration of 1.54, 0.12 and 0.10 µg /mL ASP, CAF and OR, respectively.

The proposed TLC spectrodensitometric method was applied for the analysis and calculation of ASP, CAF and OR concentrations.

3. RESULTS AND DISCUSSION

3.1 Method Development and Optimization

The aim of this work is to develop a method that can be applied regularly for separation and quantification of the studied drugs.

Optimum experimental conditions were checked by investigating the effect of different variables. Different developing systems with different compositions and ratios were tried and the complete separation of ASP, CAF and OR was achieved by using ethylacetate: acetone: methanol: triethylamine (6:3:1:0.2, by volume) as developing mobile phase.

Also different scanning wavelengths were tested and the best sensitivity obtained at 220 nm.

Different band dimensions were tested to obtain sharp and symmetrical peaks. The optimum band length was 6 mm and the inter space between bands was 1 cm. The slit dimensions of the scanning light beam should ensure complete coverage of band dimensions on the scanned track without interference of adjacent bands. Different slight dimensions were tried where 6 mm x 0.45 mm provided the highest sensitivity.

The separation in this method is based on the difference in the Rf values of ASP (Rf = 0.08), CAF (Rf = 0.55) and OR (Rf = 0.35) as shown in Fig. 2.

3.2 Method Validation

Method validation was performed according to ICH guidelines [32].

Linearity of the proposed method was evaluated and it was evident in the concentration range of (0.4 - 2 µg /mL) for ASP, (0.4 - 2 µg /mL) for CAF and (0.3-3 µg /mL) for OR, Figs. 3-5. Good linearity was evident by the high value of the correlation coefficient and the low intercept value as mentioned before in regression equations in 2.4.1. linearity and construction of calibration curves.

Accuracy of the proposed method was checked by applying the proposed method for determination of different blind samples of ASP, CAF and OR. The concentrations were calculated from the corresponding regression equations. The results obtained as shown in Tables 1-2.

Accuracy of the method was assured by applying the standard addition technique on Relatic® tablets where good recoveries were obtained as shown in Table 3 revealing no interference from excipients and good accuracy of the proposed method.

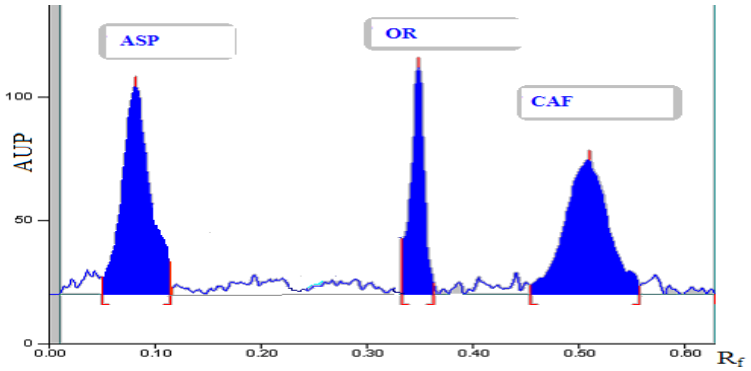

Fig. 2. A scanning profile of a TLC chromatogram showing an example of separated mixture of ASP, CAF and OR

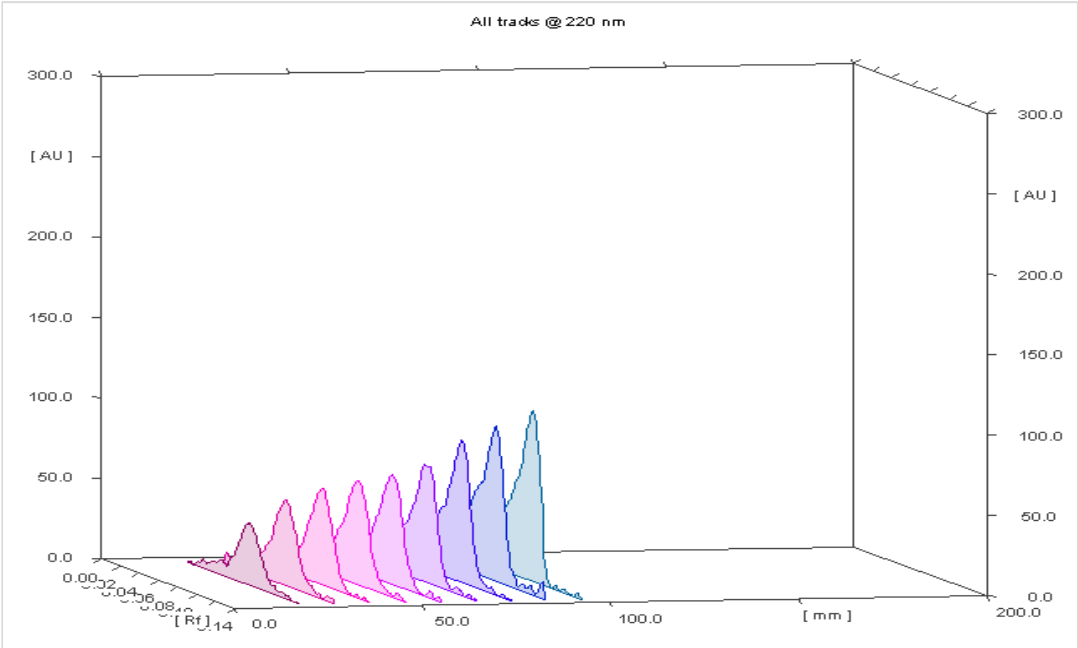

Fig. 3. A 3D diagram showing separation of ASP (Rf =0.08) over a concentration range 0.4 ─ 2.0 µg/band at 220 nm

Specificity of the proposed method is evident from the TLC-spectrodensitometric chromatogram as shown in Fig. 2.

Precision of the proposed TLC-spectrodensitometric method was evident as shown in Table 4.

Robustness of the proposed method was evaluated in the development phase by making small changes in the composition of mobile phase and detection wavelength. The low value of %RSD shows that the method is robust and that deliberate small changes in the studied factors did not lead to a significant change in Rf values, area or symmetry of the peaks.

System suitability tests are based on the concept that the equipment, electronics, analytical operations and samples constitute an integral system that can be evaluated as whole. System suitability is used to ensure system performance before or during the analysis of the drugs. System suitability was checked by calculating the capacity factor(K'), symmetry factor, the selectivity factor(γ) and resolution(Rs), where the system was found to be suitable as shown in Table 5.

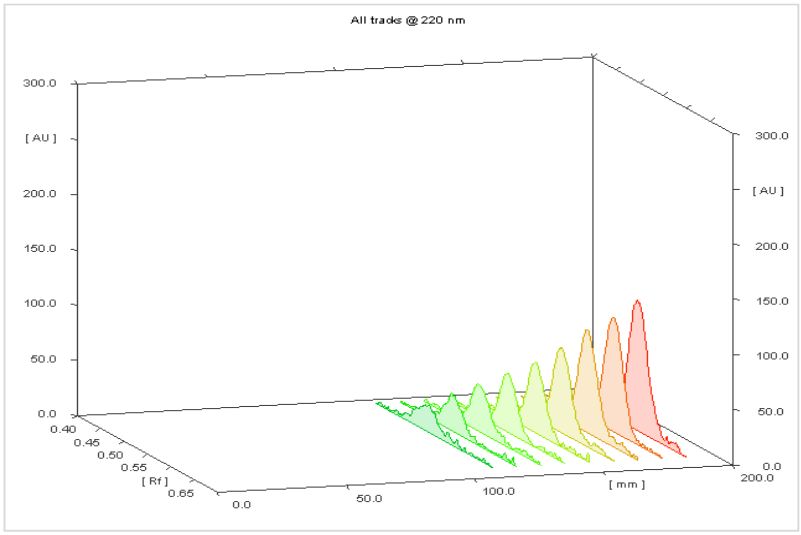

Fig. 4. A 3D diagram showing separation of CAF (Rf =0.55) over a concentration range 0.4 ─ 2.0 µg/band at 220 nm

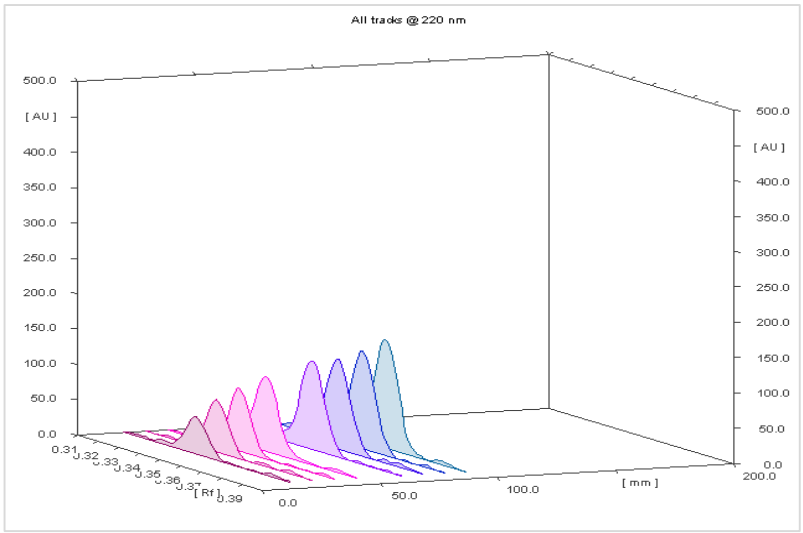

Fig. 5. A 3D diagram showing separation of OR (Rf =0.35) over a concentration range 0.3 ─ 3 µg/band at 220 nm

Table 1. Results of accuracy for determination of pure authentic of ASP, CAF and OR by the proposed TLC spectrodensitometric method

ASP			CAF			OR		
Taken (µg/band)	Found* (µg/band)	Recovery %	Taken (µg/band)	Found* (µg /band)	Recovery %	Taken (µg/band)	Found* (µg /band)	Recovery %
0.40	0.41	102.50	0.40	0.40	100.00	0.50	0.50	100.00
0.60	0.59	98.33	0.60	0.59	98.33	1.00	0.99	99.00
0.80	0.81	101.25	0.80	0.80	100.00	1.50	1.47	98.00
1.00	0.99	99.00	1.00	0.99	99.00	2.00	2.01	100.50
1.20	1.22	101.66	1.20	1.19	99.17	2.50	2.50	100.00
2.00	2.02	101.00	2.00	1.96	98.00	3.00	2.95	98.33
Mean±SD		100.62±1.614			99.08±0.829			99.31±1.014

** Average of three determinations*

Table 2. Determination of ASP, CAF and OR in laboratory prepared mixtures by the proposed TLC spectrodensitometric method

Mix. no.	Ratio ASP:CAF:OR	ASP			CAF			OR		
		Taken (µg/band)	Found* µg/band)	Recovery %	Taken (µg/band)	Found* (µg/band)	Recovery %	Taken (µg/band)	Found* (µg /band)	Recovery %
1	1:1:1	1.00	0.98	98.00	1.00	1.02	100.20	1.00	0.99	99.00
2	8 :6 :8	0.80	0.80	100.00	0.60	0.59	98.33	0.80	0.79	98.75
3	**1.5:0.1:0.1****	1.50	1.50	100.00	0.10	-------	------	0.10	------	------
	4.5:0.3:0.3**	4.50	-----	-------	0.30	0.30	100.00	0.30	0.30	100.00
4	8:10:10	0.80	0.79	98.75	1.00	0.99	99.00	1.00	1.03	103.00
5	10:5:5	1.00	0.98	98.00	0.500	0.50	100.00	0.50	0.49	98.00
6	2:2:3	2.00	1.96	98.00	2.00	2.04	102.00	3.00	3.02	100.67
Mean±SD				98.79±0.980			99.92±1.248			99.90±1.786

** Average of three determinations*
*** The ratio present in Relatic® tablet*
--------- not calculated as it is out of linearity

Table 3. Application of standard addition technique to analysis of ASP, CAF and OR in dosage form by the TLC spectrodensitometric method

Relatic® tablet, Batch no.71014	Claimed (µg /band)	Found* (µg /band)	Found %	Pure added (µg /band)	Pure found** (µg /band)	Recovery %
ASP	1.54	1.56	101.30	0.100	0.101	101.00
				0.200	0.204	102.00
				0.300	0.300	100.00
				0.400	0.402	100.50
Mean ±SD						100.88±0.854
CAF	1.20	1.22	101.67	0.200	0.202	101.00
				0.400	0.402	100.50
				0.600	0.598	99.67
				0.800	0.799	99.87
Mean ±SD						100.26±0.607
OR	1.00	0.99	99.00	0.200	0.198	99.00
				0.300	0.304	101.33
				0.800	0.808	101.00
				1.000	1.006	100.60
Mean ±SD						100.48±1.032

** Average of six determinations*
*** Average of three determinations*

Table 4. Results of assay validation parameters of the proposed TLC spectrodensitometric method for the determination of ASP, CAF and OR in ternary mixture

Parameters	ASP	CAF	OR
Range (µg /band)	0.4-2	0.4- 2	0.3-3
Slope	0.4067	0.5888	0.4107
Intercept	0.3121	-0.0182	0.3915
Correlation coefficient (r)	0.9991	0.9992	0.9990
Accuracy (mean ± SD)	100.62±1.614	99.08±0.829	99.31±1.014
(RSD%)a*	1.223	1.701	1.249
(RSD%)b*	1.064	1.323	1.002

(RSD%)[a], (RSD%)[b]* the intra-day and inter-day relative standard deviations of the average of concentrations (0.4, 0.6 and 1.0 µg /band for each drug)*

Table 5. Statistical analysis of parameters required for system suitability testing of the proposed spectrodensitometric method

Parameters	For TLC-densitometric method			
	Obtained value			Reference value
	ASP	OR	CAF	
Resolution (R_s)	5.40	3.33		›1.5
Capacity factor(K')	11.5	1.86	0.82	0- 10 acceptable
Symmetry factor	1.00	1.12	1.20	≈ 1
Selectivity factor(γ)	6.18	2.27		› 1

Table 6. Statistical analysis of the results obtained by proposed method and reference method for the determination of ASP, CAF and OR

Parameter	Spectrodensitometric method			Reference method		
	ASP	CAF	OR	ASP	CAF	OR
Mean %	100.62	99.08	99.31	100.84	98.96	99.12
SD	1.614	0.829	1.014	1.004	0.578	0.602
n	6	6	6	6	6	6
Student 's t-test (2.23)[b]	0.284	0.277	0.209			
F-value (5.05)[b]	2.584	2.383	2.840			

[a]Manufactured method (HPLC) personal communications

[b]The values between parenthesis are the theoretical values for t and F at P=0.05

4. CONCLUSION

The mentioned TLC-spectrodensitometric method is considered simple, sensitive, accurate and reproducible method that can be used for simultaneous quantitative analysis of ASP, CAF and OR in bulk powder and pharmaceutical formulation without any interference from excipients. TLC-spectrodensitometric method has the advantages of that several samples can be run simultaneously using a small quantity of mobile phase and provides high sensitivity and selectivity. The separation power of chromatographic methods allows determination of mixture of drugs by any ratio in pharmaceutical formulations while spectrophotometric methods can determined the mixture only after complex procedures.

Statistical analysis was performed by comparing the results of the proposed method with those of manufacturer method. No significant difference was observed regarding accuracy and precision, as shown in above Table 6.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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