MedCrave
Step into the World of Research
Journal of Analytical & Pharmaceutical Research

Review Article

Open Access
CrossMark

Application of TLC densitometric method for simultaneous estimation of binary mixture of phenazopyridine hydrochloride and trimethoprim in their tablet dosage form

Abstract

In the present work; an accurate and precise TLC densitometric method has been developed for simultaneous determination of PHZ and TMP in their bulk and dosage form. The proposed method based on determination of the UV-visualized bands after TLC separation of PHZ and TMP. The studied drugs were quantitatively separated on 60 F_{254} silica gel plates using mobile phase consists of methanol: toluene: ethyl acetate (1:1:1, by volume)) with UV detection at 228nm over a concentration range of (0.5–6) and (1-10)µg/spot with mean percentage recoveries 99.66±0.984 and 99.72±1.232 for PHZ and TMP, respectively. The proposed method has been validated according to ICH guidelines. The validity of the proposed method was further evaluated by applying standard addition technique. The obtained results were statistically compared with the reported method, showing no significant difference with respect to accuracy and precision.

Keywords: phenazopyridine hydrochloride, trimethoprim, TLC, pharmaceutical analysis

Volume 7 Issue 2 - 2018

Nasr M El-Abasawy, Khalid AM Attia, Ahmad A Abo-serie, Ragab A Said, Ahmed A Almrasy
Department of Pharmaceutical Analytical Chemistry, Al-Azhar University, Egypt

Correspondence: Ahmed A Almrasy, Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Al-Azhar University, 11751 Nasr City, Cairo, Egypt, Tel +201019145212, Email ahmed.ali_83@yahoo.com

Received: February 24, 2018 | **Published:** April 03, 2018

Introduction

Phenazopyridine hydrochloride (PHZ), [2, 6-diamino-3-(phenylazo) pyridine hydrochloride] Figure 1A exerts an analgesic effect on the mucosa of the urinary tract and provides symptomatic relief of pain in conditions such as cystitis and urethritis. It is given in conjunction with an antibacterial agent for the treatment of urinary tract infections.[1] Trimethoprim (TMP), [5-(3,4,5-Trimethoxybenzyl) pyrimidine-2,4-diamine] Figure 1B exerts antibacterial effect that is used for the treatment of infections due to sensitive organisms, including gastro-enteritis and respiratory tract infections, and in particular for the treatment and prophylaxis of urinary tract infections.[1]

Few spectrophotometric[2] and chromatographic[3] methods have been reported for the determination of a mixture of PHZ and TMP while, multivariate spectrophotometric[4] and electrochemical[5] methods were used in combination with sulphamethoxazole in pharmaceutical dosage form. The aim of the present work was to develop simple, rapid, sensitive and selective TLC densitometric[6,7] method for the determination of binary mixture of PHZ and TMP in pharmaceutical formulation.

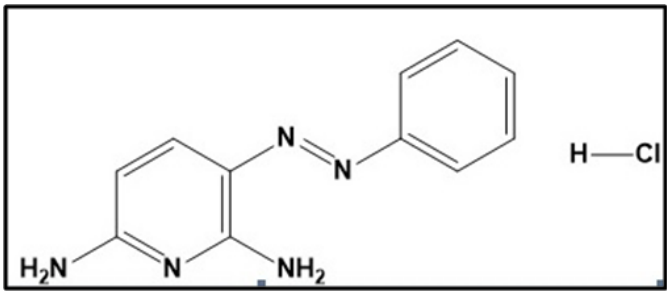

Figure 1 (A) Chemical structure of phenazopyridine hydrochloride.

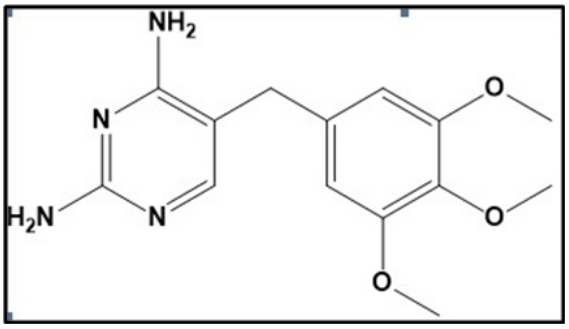

Figure 1 (B) Chemical structure of trimethoprim.

Experimental

Instruments

a. Camag Linomat autosampler (Muttenzl, Switzerland), a Camag microsyringe (100µL) and a Camag 35/N/30319 TLC scanner with win CATS software; an ultraviolet (UV) lamp (Desaga, Wiesloch, Germany).

b. Aluminum TLC plates precoated with silica gel 60 GF_{254} (20×20cm), (Merck, Darmstadt, Germany).

c. Chromatographic tank (25×25×9cm).

Materials and reagents

Pure standard

Standard PHZ (certified to contain 99.94%) and TMP (certified to contain 99.91%) were kindly supplied by kahira for pharmaceuticals, Cairo, Egypt.

Pharmaceutical preparation

Carmurit-T® tablets: (batch number 314467) each tablet is claimed to contain 25mg phenazopyridine hydrochloride and 100mg trimethoprim, manufactured by Memphis company for pharmaceuticals and chemical industries, purchased from local market.

Reagents and solvents

Methanol, acetonitrile, ethyl acetate and toluene, all of analytical grade, (Sigma-Aldrich, Germany).

Standard solutions

A standard solutions of (1mg/ml) was prepared by dissolving 0.1g of PHZ and TMP in 50ml methanol and complete to 100ml with the same solvent.

Procedures

Construction of calibration curves

Into two separate series of 10 ml volumetric flasks, aliquots of standard solutions equivalent to (0.5-6mg) and (1-10mg) of PHZ and TMP respectively, were transferred separately and diluted to volume with methanol. So the series of flasks contain (50-600)µg/ml and (100-1000) µg/ml of each solution were applied to a TLC plate using Camag Linomat auto sampler with micro syringe (100µl). The plate was then developed by the ascending technique using methanol: toluene: ethyl acetate (1:1:1, by volume) as a mobile phase. The plate was then removed and air-dried. The chromatograms were scanned at 228nm. Calibration curves representing the relationship between integrated peak area and the corresponding concentrations of each drug were plotted.

Application to pharmaceutical formulation

Ten Carmurit-T® tablets were grinded to a fine powder with the help of mortar and pestle. An amount of the powder equivalent to 25mg PHZ and 100mg trimethoprim was transferred into 100-ml volumetric flask, dissolved in methanol, shaken for about 10 minutes and filtered through filter paper to obtain a solution labeled to contain (0.25mg/ml) of PHZ and (1mg/ml) of TMP. Transfer 4 ml into 10-ml volumetric flask then complete to volume with methanol to obtain a solution labeled to contain (100µg/ml) of PHZ and (400µg/ml) of TMP. The proposed method was applied for the determination of the pharmaceutical preparation solution using the procedure described above under the construction of the calibration curve. The cited drugs concentrations were calculated from the corresponding regression equations.

Reported method[2]

The reported method depends on direct spectrophotometric determination of PHZ at 429nm while TMP was determined by first derivative spectrophotometry and measuring the amplitude at 241nm.

Results and discussion

This study is concerned with the simultaneous determination of PHZ and TMP using TLC densitometric method. Various systems were tried, where complete separation of PHZ and TMP was achieved using methanol: toluene: ethyl acetate (1:1:1, by volume) as the mobile phase. The R_f values were 0.33 for TMP and 0.69 for PHZ as shown in Figures 2 & 3.

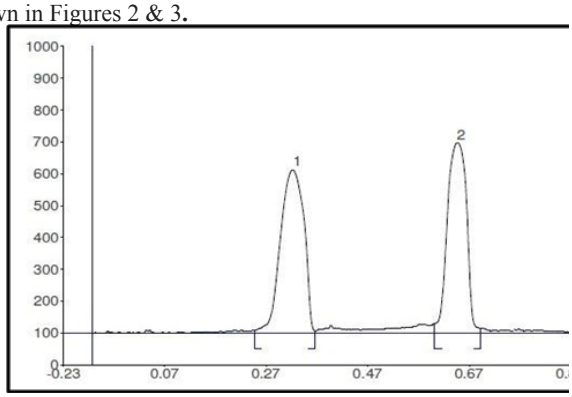

Figure 2 Two-dimensional densitometric chromatogram of (1) TMP (6µg/spot) and (2) PHZ (4µg/spot) at 228nm.

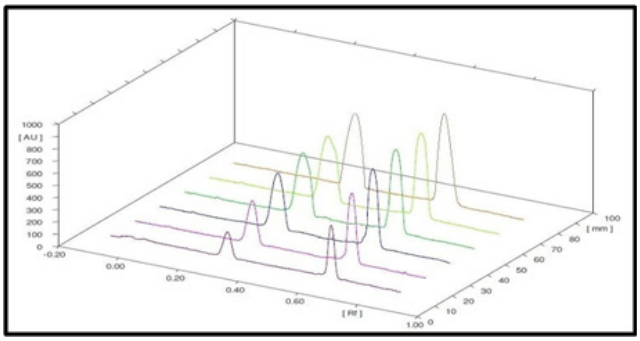

Figure 3 Three-dimensional densitometric chromatogram of TMP (1–10µg/spot) and PHZ (0.5–6µg/spot) at 228nm.

Method validation

Method validation was performed according to the International Conference on Harmonization (ICH) guideline[7] for the proposed method as follows:

Linearity and range

TLC-Densitometric technique permits selective determination of PHZ and TMP in the range of 0.5–6 and 1–10µg/spot, respectively. The linear regression equations were calculated and found to be:

y=4605.73 x- 66.22 (r=0.9996) for PHZ at 228nm.

y=3099.62 x-206.19 (r=0.9997) for TMP at 228nm.

Where y is the peak area at 228 nm, x is the drug concentration in µg/spot and r is the correlation coefficient.

Calibration graphs were constructed by plotting the area under peak versus drug concentrations.

Limits of detection and quantitation

The limits of detection (LOD) and the limits of quantitation (LOQ) were determined according to ICH guidelines from the following equations:

$LOD=3.3\sigma/S$ $LOQ=10\sigma/S$

Where σ is the residual standard deviation of the regression line and S is the slope of the calibration curve. LOD and LOQ values were mentioned in Table 1.

Parameters	PHZ	TMP
Wavelength (nm)	228	228
Linearity range	0.5-6(µg/spot)	1-10(µg/spot)
LOD	0.153(µg/spot)	0.225(µg/spot)
LOQ	0.465(µg/spot)	0.684(µg/spot)
- Regression Equation	$y^{a} = b\,x^{b} + a$	$y^{a} = b\,x^{b} + a$
- Slope (b)	4605.73	3099.62
- Intercept (a)	-66.22	-206.19
Correlation coefficient (r)	0.9996	0.9997
Accuracy (% R)	99.66	99.72
Precision (% RSD)		
Repeatability[c]	0.988	1.236
Intermediate precision[d]	1.191	1.417
Robustness (% RSD)		
- Mobile phase contents ratio (±2%)	1.413	1.655

Table 1 Regression parameters and results of determination of pure samples of PHZ and TMP by the proposed method

[a] Peak area.

[b] Concentration in µg/spot.

[c] The intraday (n=3), Average of three determinations for three concentrations (2, 4 and 6µg/spot), for PHZ and TMP repeated three times within the day.

[d] The interday (n=3), Average of three determinations for three concentrations (2, 4 and 6µg/spot), for PHZ and TMP repeated three times in three days.

Accuracy

The accuracy of the proposed method was calculated by the average of three determinations for three concentrations for TMP or PHZ repeated three times as mentioned in Table 1.

Precision

The Precision was evaluated by calculating intraday (repeatability) and interday (Intermediate precision) after repeating measuring of the three different concentrations three times in the same day and in three successive days using the proposed method. The calculated RSD% values were listed as mentioned in Table 1 indicating acceptable precision of the proposed method.

Robustness

The robustness of the method was evaluated by slight changes in the chromatographic parameters such as working wavelengths (±2nm) and the mobile phase contents ratio (±2%). In each case only one parameter was changed while other parameters were kept constant. These minor changes did not have any significant effect on the peak area or separation of both drugs and % RSD of the responses were <2% confirming robustness of the procedure, as mentioned in Table 1. The validity of the proposed procedures was assessed by applying the standard addition technique showing no excipients interference as mentioned in Table 2. The results obtained by applying the proposed method were statistically compared with the reported method[2] and no significant difference was found regarding accuracy and precision as mentioned in Table 3.

Table 2 Recovery study of the studied drugs by adopting standard addition technique using the proposed method

PHZ					TMP				
Taken (µg/spot)	Found	Pure added (µg/ml)	Pure found (µg/ml)	% Recovery	Taken (µg/ml)	Found	Pure added (µg/ml)	Pure found (µg/ml)	% Recovery
1	1.01	1	0.98	98.82	2	1.99	2	2.03	101.73
		2	2.03	101.75			3	3.01	100.13
		3	2.98	99.48			4	3.92	98.01
		4	4.07	101.72			15	4.93	98.64
Mean				100.44					99.63
% RSD				1.511					1.668

Table 3 Statistical comparison for the results obtained by the proposed and the reported methods for the analysis of PHZ and TMP in Carmurit-T® tablets

Parameters	TLC method		Reported method*[2]	
	PHZ	TMP	PHZ	TMP
Number of measurements	5	5	5	5
Mean	100.28	100.40	100.33	99.77
SD	1.586	1.677	0.874	1.172
Variance	2.516	2.812	0.764	1.374
Student- t-test*	0.059(2.306)	0.684(2.306)	——	——
F-value*	3.291(6.388)	2.047(6.388)	——	——

*The values in the parenthesis are the corresponding theoretical values of *t* and *F* at (P = 0.05).

Conclusion

In this study; sensitive and selective TLC-densitometric procedure for the simultaneous determination of PHZ and TMP in their pure form and in their pharmaceutical preparation has been developed and validated. The developed method is time saving where many bands can be run at the same time. This method is also economic since a small quantity of mobile phase as a developing system was used unlike HPLC procedures. Finally we can conclude that the described TLC-densitometric procedure can be used in routine analysis of PHZ and TMP in their pure forms and pharmaceutical dosage form without previous separation.

Acknowledgement

Authors are deeply thankful to ALLAH, the Almighty, by the grace of whom this work was realized. Deep gratitude and appreciation to pharmaceutical analytical chemistry department, faculty of pharmacy Al Azhar University, for providing facilities and continuous support.

Conflict of interest

The author declares that there is no conflict of interest.

References

1. Martindale. *The Complete Drug Reference*. 33rd ed. Pharmaceutical Press; 2002.
2. Ramadan NK, Mohamed HM, Moustafa AA. Simultaneous determination of phenazopyridine hydrochloride and trimethoprim. *Bulletin of Faculty of Pharmacy*, *Cairo University*. 2008;46(1).
3. El–Abasawy NM, Attia KA, Abo–Serie AA, et al. HPLC method for determination of binary mixture of phenazopyridine hydrochloride and trimethoprim in bulk and pharmaceutical preparation. *World journal of pharmacy and pharmaceutical sciences*. 2018;7(3):1186–1193.
4. Ribone ME, Pagani AP, Olivieri AC. Simultaneous multivariate spectrophotometric analysis of binary and ternary mixtures of sulfamethoxazole, trimethoprim and phenazopyridine in tablets. *Analytical letters*. 1999;32(7):1389–1401.
5. Pereira PF, da Silva WP, Muñoz RAA, et al. A simple and fast batch injection analysis method for simultaneous determination of phenazopyridine, sulfamethoxazole, and trimethoprim on boron–doped diamond electrode. *Journal of electroanalytical chemistry*. 2016;766:87–93.
6. El–Ghobashy MR, Fayez YM, Goda ZM, et al. HPLC and TLC chromatographic methods for simultaneous determination of binary mixture of isoconazole nitrate and diflucortolone valerate. *Bulletin of Faculty of Pharmacy, Cairo University*. 2016;54(1):33–37.
7. Salama FM, Attia KA, Abouserie AA, et al. Application of TLC Densitometric Method for Simultaneous Estimation of the Newly Co–formulated Antiviral Agents Ledipasvir and Sofosbuvir in Their Tablet Dosage Form. *Analytical Chemistry Letters*. 2017;7(2):241–247.
8. QB International Conference on Harmonization (ICH). Federal Register, 1997.