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Research Article

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Simultaneous estimation of tolperisone hydrochloride and paracetamol in combined tablet dosage form by validated normal phase HPTLC method

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ABSTRACT

A sensitive, simple, accurate, precise and selective validated HPTLC method for simultaneous determination of Tolperisone Hydrochloride and Paracetamol in tablet formulation with densitometric detection has been developed and validated. Chromatographic separation was achieved on Merck aluminum HPTLC plates precoated with silica gel 60 F_{254}. The solvent system comprised of toluene: ethyl acetate: methanol (1: 7: 3, v/v/v) and detection wavelength was 256 nm in reflectance-absorbance mode. The retardation factor for Tolperisone Hydrochloride and Paracetamol were found to be 0.39 ±0.01 and 0.79 ± 0.02, respectively. Results were found to be linear over a range of 50-800 ng band^{-1} and 100-800 ng band^{-1} for Tolperisone Hydrochloride and Paracetamol, respectively. The proposed densitometric method was applied for the analysis of tablet formulation. Percentage assay for Tolperisone Hydrochloride and Paracetamol were found to be 98.47 and 99.23 %, respectively. The proposed densitometric method was validated in accordance with International Conference on Harmonization guidelines [(ICH) Q2 (R1)]. The developed and validated densitometric method can be applied for routine quality control of Tolperisone Hydrochloride and Paracetamol in combined tablet dosage form.

Keywords: Tolperisone hydrochloride, Paracetamol, HPTLC, ICH

INTRODUCTION

Antispasmodic agent, Tolperisone Hydrochloride (TOLP) is (R, S) 2-methyl-l-(4 methyl phenyl)-3- (l-piperidyl) propan -l-one [1] and is official in Japanese Pharmacopoeia [2]. The literature survey showed that there are some analytical methods reported for Tolperisone Hydrochloride (Figure 1) like spectrophotometric [3-7], HPTLC [8, 9] and RP-HPLC [10 - 14] either individually or in combination with other drug/s.

Analgesic and antipyretic agent, Paracetamol (PCM) [15] is official in Indian Pharmacopoeia [16] and British Pharmacopoeia [17], and United States Pharmacopoeia [18]. Chemically, Paracetamol (Figure 1) is 4-hydroxyacetanilide [19]. Literature survey revealed that spectrophotometric [20 - 26], HPTLC [27, 28], and RP-HPLC [29 - 37] methods of analysis have been reported for PCM individually or in combination with other drug/s. The nature of the compound, its molecular weight and solubility decides the selection of the stationary phase [38, 39].

Comprehensive Literature survey revealed that, no HPTLC method has been reported for concurrent analysis of TOL and PCM in combined tablet dosage form. Hence, the present research manuscript describes a selective, simple, precise, accurate, and robust normal-phase high-performance thin layer chromatographic method, validated

in accordance with ICH guidelines Q2 (R1) [40] for the simultaneous quantification of TOL and PCM as a bulk drug and in their binary tablet dosage form.

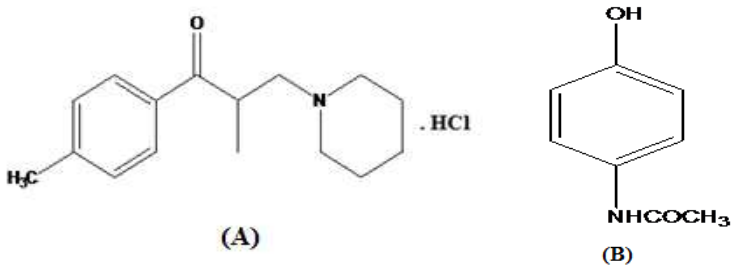


Figure 1. Chemical structures of (A) Tolperisone Hydrochloride and (B) Paracetamol

EXPERIMENTAL SECTION

Pharmaceutical grade TOLP and PCM were received as gift sample from Emcure Pharmaceuticals Ltd. (Pune. India). Marketed formulation, Myotop P Tablet (Emcure Zuventus Haelthcare Ltd., Pune, India) containing TOLP (150 mg) and PCM (500 mg) was procured from the local market. Analytical grade chemicals and reagents were procured from Merck Specialities Pvt. Ltd. (Mumbai, India). Precoated silica gel aluminium HPTLC plates 60 F_{254} (E. Merck, Darmstadt, Germany) were used in the study.

Instrumentation and Chromatographic Conditions

The HPTLC instrumentation consisted of a Linomat V sample applicator with a 100 µL Camag syringe and a TLC III scanner controlled by WinCATS software version 1.4.4. (Camag, Muttenz, Switzerland). The slit dimension was kept at 5 mm x 0.45 mm and a scanning speed of 10 mm/s. HPTLC plates were prewashed with methanol and activated at 120° C for 15 min previous to densitometric analysis.

HPTLC plates were then developed in a Camag 20 x 10 cm twin trough chamber (Camag, Muttenz, Switzerland) with 20 mL mobile phase comprising of toluene: ethyl acetate: methanol (1: 7: 3, v/v/v). The optimized chamber saturation time for solvent system was 15 min at room temperature (25 ± 2° C) and relative humidity of 60 ± 5 %. The length of chromatographic run was 80 mm. After chromatographic development, plates were dried in an air current. Densitometric scanning was performed in reflectance-absorbance mode at 256 nm by Camag TLC scanner III using winCATS software version 1.4.4.

Preparation of standard stock solutions

Standard stock solutions of TOLP and PCM were prepared individually by dissolving 5 mg each of standard drug in 10 mL methanol to get concentration of 500 µg/mL and used for further studies.

Selection of detection wavelength

After chromatographic development, bands were scanned over the range of 200 - 400 nm and the spectra were overlain. Both compounds showed significant absorbance at 256 nm and thus was selected for densitometric analysis (Figure 2)

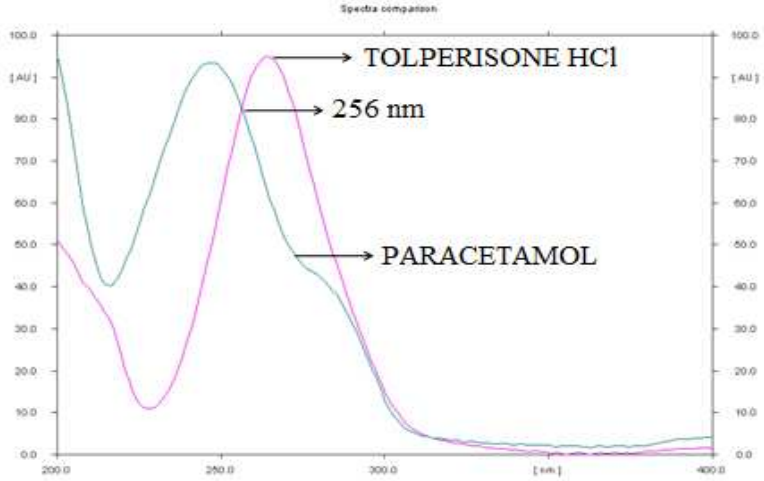


Figure 2. Densitogram obtained from mixed standard solution of TOLP and PCM

Preparation of sample solutions
For analysis of marketed tablet formulation, twenty tablets were accurately weighed; the average weight was estimated and tablets were finely powdered. Powder equivalent to 50 mg of TOLP was weighed, transferred to 50 mL of volumetric flask containing 30 mL methanol, sonicated for 15 min and diluted up to the mark with methanol. The resulting solution was filtered through Whatman filter paper No.41 and used for further analysis.

Assay validation
The developed densitometric method was validated as per the International Conference on Harmonization [(ICH) Q2 (Rl)] guidelines for selectivity, robustness, linearity, range, limit of detection (LOD) and limit of quantitation (LOQ), accuracy, and precision.

Linearity and Range
Stock solutions were applied on the HPTLC plate in the range of 50 - 800 ng band^{-1} and 100 - 800 ng band^{-1} for TOLP and PCM respectively, to evaluate linearity. Peak area versus concentration was plotted, subjected to least square linear regression analysis and the correlation coefficient, slope, intercept for the calibration were estimated. To verify linearity, residual analysis was also performed.

Sensitivity
Limit of detection and limit of quantitation was estimated using formula 3.3 σ/S and 10 σ/S, respectively, where σ is the standard deviation of the response (y-intercept) and S is the slope of the linearity plot.

Specificity
TOLP and PCM standard solutions and the sample solutions were applied on a HPTLC plate, developed and scanned as described above. The peak purity of drugs was checked by comparing the UV spectrum of TOLP and PCM at peak start, peak apex and peak end positions of the band.

Precision studies
Precision was studied by intra and inter-day precision studies. TOLP (100 ng band^{-1}) and PCM (333.33 ng band^{-1}) sample was prepared and analyzed six times on the same day in order to trace any variations in the results. For inter-day variation study, the above mentioned drug samples were analyzed on three successive days.

Accuracy studies
The accuracy of the method was determined by estimating recoveries of TOLP and PCM by the standard addition method. The samples were spiked with 80, 100 and 120 % of 100 ng band^{-1} of TOLP and 333.33 ng band^{-1} of PCM standard solutions. Recovery was estimated from the following equation:

[(spiked concentration - mean concentration) / spiked concentration] x 100.

Robustness studies
In the robustness evaluation, small, deliberate changes in the analytical parameters of the proposed method were done and its effect on the peak areas of the drugs was studied. Factors changed were amount of solvent system (± 5 %), solvent system (ethyl acetate) composition (± 0.1 mL), time from band application to chromatographic development (+ 10 min) and time from chromatography to scanning (+ 10 min). Single parameter was varied at a time. Concentration of 100 ng band^{-1} for TOLP and 333.33ng band^{-1} PCM in six replicates were used to study robustness of the method. The standard deviation of peak areas and % relative standard deviation (% RSD) were determined.

Solution stability
Solution stability of TOLP and PCM standard solutions (100 ng band^{-1}) was studied after 0, 6, 12, 24, 48 h of storage at room temperature. The stability of the solutions was estimated by comparing peak areas at each time hour against freshly prepared standard solutions.

RESULTS AND DISCUSSION

Optimization of HPTLC method
Different solvent systems comprising various ratios of toluene, dichloromethane, n-hexane, ethanol, methanol, water, ethyl acetate, and acetone were tried, to accomplish the R_f value in the range 0.2 - 0.8, and minimum resolution Rs ≥ 1.5. Finally, the mobile phase consisting of toluene: ethyl acetate: methanol (1: 7: 3, v/v/v) was selected for obtaining well resolved peaks. The optimum wavelength for densitometric analysis used was 256 nm. The retention factors were found to be 0.29 ± 0.02 and 0.79 ± 0.02, for TOLP and PCM, respectively (Figure 3).

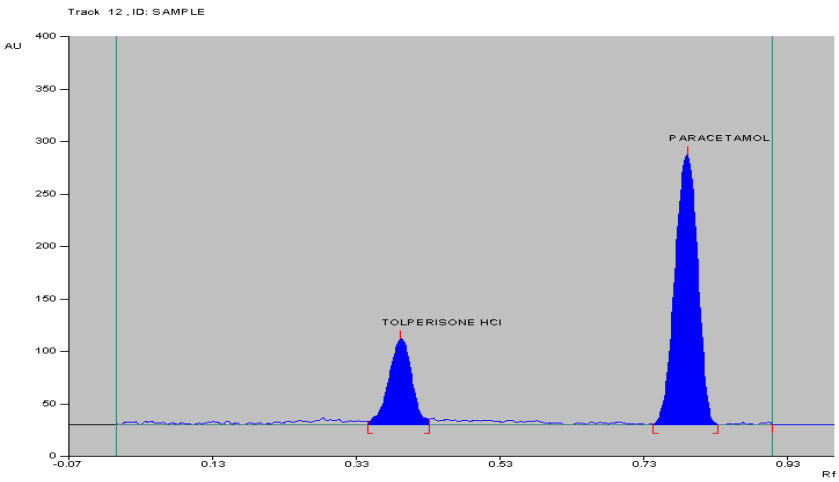


Figure 3. Densitogram obtained from mixed standard solution of TOLP and PCM scanned a 256 nm

HPTLC method validation

Linearity and Range

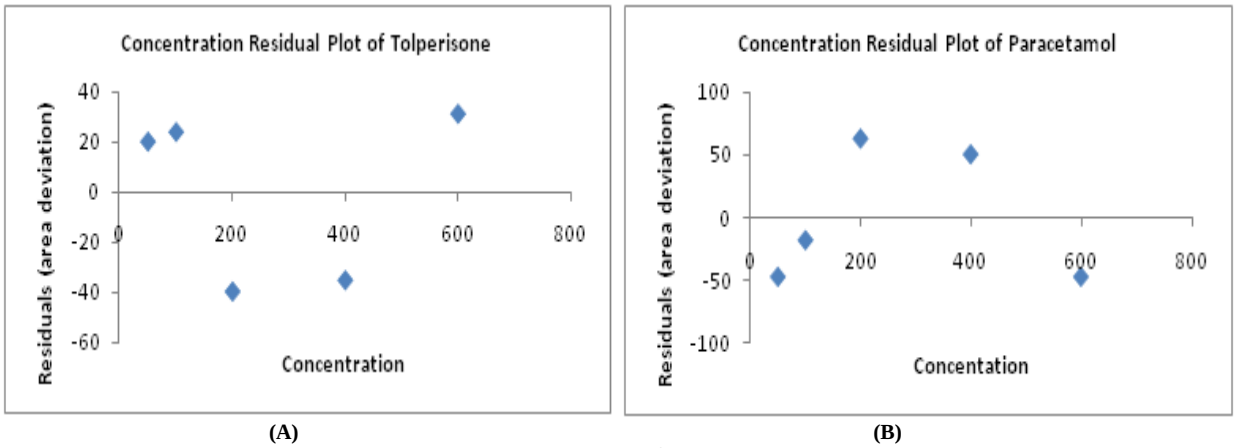
Linearity was studied by plotting standard drug concentration against peak areas obtained. The results were found to be linear over a range of 50 - 800 ng band^{-1} and 100 - 800 ng band^{-1} for TOLP and PCM, respectively (Table 1).

Table1. Linear regression data for the calibration curves (n = 6).

Parameters	TOLP	PCM
Linearity range (ng band^{-1})	50-800	100-800
r^2	0.9998	0.9997
Slope	11.24	12.64
Intercept	398.07	7.8
Confidence limit of slope[a]	10.97- 11.52	12.21–13.06
Confidence limit of intercept[a]	304.32–491.81	-135.83-152.10
$S_{y.x}$[b]	34.38	52.80

[a]95 % confidence limit; b - Standard deviation of residuals from line.

To ascertain linearity, residual analysis was also performed (Figure 4). Slope was significantly different from zero.



(A) **(B)**

Figure 4. Concentration versus Residual Plot of (A) Tolperisone and (B) Paracetamol

Sensitivity

The limit of detection and limit of quantitation were found to be 10.08 and 30.56 ng band^{-1} for TOL; and for PCM it was 13.78 and 41.77 ng band^{-1}.

Specificity

By comparing UV spectrum acquired at the start (S), apex (M), and end (E) of the peak obtained from the scanning of band the peak purity for TOLP and PCM was estimated, that is, r(S, M) = 0.998, 0.999 and r (M. E) = 0.998, 0.998, respectively. Data indicated that peaks obtained for TOL and PCM were pure.

Precision

As per ICH guidelines, RSD < 2 %, both intra and inter-day precision studies showed good precision (Table 2).

Table 2 Intra and inter day precision of the HPTLC method (n=3)

Drug	concentration [a]	Intra/Inter day concentration obtained [a]	(%)RSD
Tolperisone	100 ng band^{-1}	98.26/98.04	0.64/0.69
Paracetamol	333.33 ng band^{-1}	332.10/331.76	0.81/0.84

[a]Concentration in ng band^{-1}; RSD is the relative standard deviation

Accuracy

Recoveries of TOLP and PCM were found to be 98.61 – 101.48 % and 98.89 - 100.61 %, respectively which indicate that the proposed simultaneous densitometric method is reliable for the estimation of TOL and PCM in the marketed formulation used in the study (Table 3).

Table 3 Results of recovery studies (n=6)

Drug	Amount taken[a]	Amount added[a]	Amount found [a] ± S.D	% Recovery ± % RSD
	100	80	177.5 ± 1.073	98.61 ± 0.60
TOLP	100	100	202.96 ± 1.43	101.48 ± 0.70
	100	120	218.71 ± 1.33	99.41 ± 0.61
	333.33	266.66	603.7 ± 4.74	100.61 ± 0.78
PARA	333.33	333.33	669.91 ± 5.61	100.48 ± 0.83
	333.33	399.99	725.2 ± 5.81	98.89 ± 0.80

[a] Concentration ng band^{-1}; RSD is the relative standard deviation

Robustness studies

In robustness studies, after deliberate variation of the analytical parameters (Table 4), it was observed that areas of peaks of interest remained unaltered by small changes of the operational parameters (% RSD < 2) which indicates the robustness of method.

Table 4 Robustness testing (n =6, 100 ng band^{-1})

Parameter	SD of peak area		% RSD	
	TOLP	**PCM**	**TOLP**	**PCM**
Mobile phase (ethyl acetate) composition (± 0.1 mL)	11.1	25.95	0.71	0.66
Amount of mobile phase (± 5 %)	10.5	28.25	0.67	0.72
Time from band application to chromatography (+ 10 min)	9.46	23.57	0.61	0.60
Time from chromatography to scanning (+ 10 min)	12.1	29.01	0.78	0.74

Solution Stability

Stability of standard solution of TOLP and PCM was evaluated at room temperature for 48 h. The percentage relative standard deviation was found to be below 2.0 % indicating that both standard and sample solutions were stable up to 48 h at room temperature.

Analysis of marketed formulation

Proposed HPTLC method was applied for analysis of tablet formulation (Myotop - P tablets) in six replicate determinations. The percent assay was found to be 98.47 and 99.23 %, for TOLP and PCM, respectively in marketed formulation.

In the present research work, attempt has been made to develop and validate new, rapid, precise, accurate, selective and robust HPTLC method for simultaneous quantification of TOLP and PCM the tablet formulation. Results obtained indicate the reliability of proposed densitometric method.

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