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VALIDATED HPTLC METHOD FOR SIMULTANEOUS ESTIMATION OF TOLPERISONE HYDROCHLORIDE AND PARACETAMOL IN TABLET DOSAGE FORM

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ABSTRACT

A new, simple, precise and accurate HPTLC method was developed and validated for the simultaneous estimation of Tolperisone hydrochloride and Paracetamol in tablet dosage form. Chromatographic separation of the drugs were performed on aluminium plates precoated with silica gel 60 G F_{254} as the stationary phase and the mobile phase used was a mixture of Chloroform: Toluene: Methanol: Acetic acid (6.0: 3.0: 1.0: 0.5, v/v). Densitometric evaluation of the separated zones was performed using a UV detector at 254 nm in absorbance mode. The R_f values for Tolperisone hydrochloride and Paracetamol were found to be 0.65±0.01 and 0.47 ±0.01 respectively. The calibration curve was found to be linear between 200 to 1000 ng/spot for each of Tolperisone hydrochloride and Paracetamol. The accuracy and reliability of the method was assessed by linearity, precision (intraday % RSD and interday % RSD of Tolperisone hydrochloride and Paracetamol) and specificity in accordance with ICH guidelines. The limits of detection and quantification were found to be 60.02 ng/spot and 81.63 ng/spot for TOL, and 68.46 ng/spot and 92.72 ng/spot for PAR, respectively. The developed method can be successfully employed for the simultaneous determination of TOL and PAR in marketed tablet formulation.

KEYWORDS: Simultaneous estimation, Tolperisone hydrochloride (TOL), Paracetamol (PAR), High Performance Thin Layer Chromatography (HPTLC), Densitometry, Validation.

1. INTRODUCTION

Tolperisone hydrochloride (TOL), chemically, N-(2-[2-[(dimethylamino)methyl]thiazol-4-yl)methylthio]ethyl)-N-methyl-2-nitroethene-1,1-diamine (Fig.1) is a centrally acting muscle

relaxant, widely used as spasmolytic. It is official in Japanese Pharmacopoeia (JP). It is freely soluble in methanol and water. It is assayed by potentiometric methods as per JP [1]. Literature survey reveals Spectrophotometry [2], HPTLC [3] and HPLC [4] methods for estimation of Tolperisone either individually or in combination with other drugs.

Paracetamol (PAR), chemically, [N-(4-hydroxyphenyl)acetamide] (Fig.2) is an analgesic-antipyretic agent. It is effective in treating mild to moderate pain. It is soluble in methanol and sparingly soluble in water. It is official in Indian Pharmacopoeia (IP) [5], British Pharmacopoeia (BP) [6] and United States Pharmacopoeia (USP) [7]. It is assayed by titrimetric method. Literature survey reveals Spectrophotometry [8], HPTLC [9] and HPLC [10] methods for estimation of Paracetamol either individually or in combination with other drugs. Literature review also reveals that only few methods are available for simultaneous estimation of Tolperisone hydrochloride and Paracetamol in two component dosage forms.

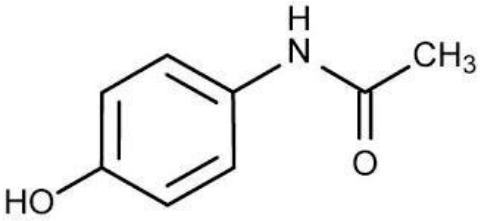


Fig. 1: Structure of Paracetamol.

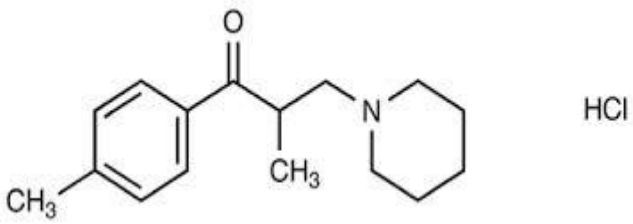


Fig. 2: Structure of Tolperisone Hydrochloride.

2. MATERIALS AND METHODS

2.1. Materials

Gift samples of Tolperisone and Paracetamol reference standard (Themis Medicare Limited, Uttarkhand, India). HPLC grade solvents; Methanol (S D Fine-Chemicals Limited, Mumbai), Chloroform (Rankem Ltd, New Delhi), Toluene (Ranbaxy Fine-Chemicals Limited, New Delhi), Acetic acid (HiMedia Laboratories Pvt. Ltd, Mumbai). Commercially available tablets (MYO-MR PLUS, containing 150 mg TOL and 500 mg PAR) were obtained from local pharmacy.

2.2. Instrument and Software

Camag HPTLC system comprising of Linomat V automatic sample applicator, Camag Hamilton microlitre syringe (100 µl), Camag TLC scanner III, Camag wincats software with stationary phase precoated silica gel 60 G $F_{254,}$ Shimadzu analytical balance and Ultrasonicator were used.

2.3. Preparation of Standard Stock Solution

Standard stock solution of TOL and PAR were prepared by accurate weighing of 100.00 mg of each drug in the separate 100 ml volumetric flasks and dissolving in methanol and then made upto mark with methanol to obtain standard solution having concentration of TOL (1000 µg/ml) and PAR (1000 µg/ml). Accurately measured 10 ml of both the solutions were transferred to 100 ml volumetric flasks and diluted to the mark with methanol to obtain solution having concentration 100 µg/ml of TOL and PAR.

2.4. Preparation of Standard Mixture

50.00 mg of TOL and 166.66 mg of PAR reference standards were accurately weighed and transferred into 50 ml volumetric flask, mixed and dissolved in methanol and then made upto mark with methanol. 1 ml of the solution was transferred into 10 ml volumetric flask and diluted to the mark with methanol to obtain solution having concentration 100 µg/ml of TOL and 333.33 µg/ml of PAR.

2.5. Preparation of Sample Solution

Twenty tablets (MYO-MR PLUS, labelled to contain 150 mg of TOL and 500 mg of PAR per tablet, Themis Medicare Limited, Uttarkhand, India) were weighed and crushed to fine powder. An accurately weighed powder sample equivalent to 50.00 mg of TOL was weighed, transferred to 50 ml volumetric flask containing 20 ml methanol, sonicated for 30 min. The solution was then transferred to 50 ml volumetric flask through Whatman No.1 filter paper. The residue was further extracted twice with 10 ml each of methanol and passed through same filter paper and volume was made upto the mark with methanol. Accurately pipetted out 1.0 ml of resulting solution into 10 ml volumetric flask and diluted to the mark with methanol to obtain solution having concentration 100 µg/ml of TOL and 333.33 µg/ml of PAR.

2.6. Chromatographic Condition

Stationary Phase: Precoated silica gel 60 G F_{254} aluminium sheet (20 × 10 cm)

Mobile Phase: Chloroform: Toluene: Methanol: Acetic acid (6.0: 3.0: 1.0: 0.5, v/v)

Chamber saturation time: 30 min.

Development Distance: 8 cm

Temperature: Ambient Temperature

Wavelength: 254 nm

Bandwidth: 6 mm

Quantity of mobile phase: 20 ml

Scanning speed: 10 mm/sec.

Slit dimension: 5 mm × 0.45 mm

Application rate: 0.1µl/sec.

Detection: Densitometrically using a UV detector at 254 nm.

2.7. Selection of Mobile Phase

For HPTLC analysis, initially various mobile phases were tried in attempts to obtain the best separation and resolution between TOL and PAR. The mobile phase consisting of Chloroform: Toluene: Methanol: Acetic acid (6.0: 3.0: 1.0: 0.5, v/v) was selected, that gave satisfactory separation and gave two well resolved peaks for TOL and PAR which is shown in Fig.7 and Fig. 8. The R_f value for TOL and PAR was 0.65 and 0.47 respectively.

2.8. Selection of Wavelength for Measurement

After chromatographic development, the spots were scanned over the range of 200 - 400 nm and the spectra were overlain. It was observed that both drug showed considerable absorbance at 254 nm. So, 254 nm was selected as the wavelength for detection.

3. Validation of Method

The method was validated according to ICH Q2 (R1) guidelines[11]. The following parameters were used for validation of the proposed method:

3.1. Linearity

Linearity was checked by preparing standard solutions of both TOL and PAR at five different concentration levels. The calibration curves for TOL and PAR were drawn in the concentration range of 200-1000 ng/spot for both drugs. The calibration curves were constructed by plotting peak area versus concentration (Fig. 9 and Fig. 10). Each reading was

the average of three determinations. The correlation coefficient (r^2) for calibration curve of TOL and PAR was 0.9958 and 0.9980 respectively (Table 1).

3.2. Accuracy

The accuracy of the method was determined by calculating recoveries of TOL and PAR by the standard addition method. For that, known amounts of standard solutions at 50, 100 and 150 % level were added and analyzed by the proposed method, in triplicate (Table 2).

3.3. Precision

The precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analysis of three different concentrations (200 ng/spot, 600 ng/spot and 1000 ng/spot) for TOL and PAR six times on the same day. The intermediate precision of the method of the method was checked by repeating studies on three different days (Table 3).

3.4. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD and LOQ were calculated from the data obtained from the linearity studies. The slope of the linearity plot was determined. For each of the six replicate determinations, y intercept was calculated and the standard deviation of the y intercept was computed. From these values, Limit of detection (LOD) and Limit of quantitation (LOQ) were determined on the basis of response and slope of the regression equation. LOD and LOQ were calculated using following equation as per ICH guidelines. LOD = 3.3 × σ/S, LOQ = 10 × σ/S**,** where σ is the standard deviation of y intercepts of regression lines and S is the slope of calibration curves (Table 1).

3.5. Robustness

In order to establish the robustness of the method, small deliberate changes were made in the experimental conditions and chromatographic parameters like mobile phase composition (± 0.1 ml for each component), the plate activation time, chamber saturation time (± 10 % change from set time), volume of mobile phase (± 10 % change from set volume) and the development distance (± 10 % change from set distance). The time from spotting to development (0, 10, 20, 30 min) and from development to scanning (0, 10, 20, 30 min) was also varied. In the above changed conditions, stock solution was analyzed and results of robustness studies were expressed in term of % RSD of peak areas in each changed condition and were compared with similar results obtained in unchanged experimental conditions.

4. RESULTS AND DISCUSSION

A simple, accurate and precise HPTLC method was developed and validated for the simultaneous estimation of Tolperisone hydrochloride and Paracetamol. The proposed HPTLC method was optimized with several solvent systems. The mobile phase consisting of Chloroform: Toluene: Methanol: Acetic acid (6.0: 3.0: 1.0: 0.5, v/v) gave sharp and symmetrical peaks with the R_f values of 0.65 and 0.47 for TOL and PAR respectively. In order to reduce the broadening and tailing of peak, 0.5 ml of glacial acetic acid was added. Resolution of the peaks for mixture of standard drugs with clear baseline separation was obtained (Fig. 7) and for individual drugs (Fig. 5 and Fig. 6). Well defined spots were obtained when the chamber was saturated with mobile phase for 30 min at room temperature. A 3-D chromatogram showing peaks of TOL and PAR in different concentrations at 254 nm is depicted in Fig. 4. The calibration curves for TOL and PAR were constructed by plotting peak area and concentration which is shown in Fig. 9 and Fig. 10. The proposed HPTLC method was validated in terms of linearity, accuracy, precision, LOD, LOQ and robustness. The calibration curve was linear over the concentration range 200 to 1000 ng/spot for TOL and PAR with a correlation coefficient of 0.9958 and 0.9980 for TOL and PAR respectively. The method was found to be accurate with % recovery 98.54 % - 100.81 % for TOL and 99.65 % - 100.19 % for PAR. The method was found to be precise with % RSD 0.15 - 0.43 (for TOL) and 0.08 - 1.20 (for PAR) for repeatability (n = 6), and 0.59 - 1.60 (for TOL) and 0.19 - 0.49 (for PAR) for intraday (n = 3), and 0.15 - 0.67 (for TOL) and 0.87 - 0.98 (for PAR) for interday (n = 3) precision studies. LOD for TOL and PAR were found to be 60.02 ng/spot and 68.46 ng/spot, respectively. LOQ for TOL and PAR were found to be 81.63 ng/spot and 92.72 ng/spot, respectively. Summary of validation parameters is shown in Table 1. The proposed method was applied for the estimation of marketed formulations and assay results shown in Table 4.

Table 1: Summary of validation Parameters.

Parameters		**HPTLC method**	
		TOL	PAR
Linearity (ng/spot)		200 - 1000	200 - 1000
Regression equation (y = mx+c)		y =7.8588x+118.49	y = 10.943x+1120.70
Slope		7.8588	10.943
Intercept		118.49	1120.70
Correlation coefficient (r^2)		0.9958	0.9980
Accuracy (% recovery)	Level I (50%) Level II (100%) Level III (150%)	98.54 100.81 99.66	99.74 100.19 99.65

Repeatability (% RSD, n = 6)	0.15 - 0.43	0.08- 1.20
Intraday (% RSD, mean of 3 determinations)	0.59 - 1.60	0.19 - 0.49
Interday (% RSD, mean of 3 determinations)	0.15 - 0.67	0.87 - 0.98
LOD (ng/spot)	60.02	68.46
LOQ (ng/spot)	81.63	92.72

Table 2: Results of Recovery studies.

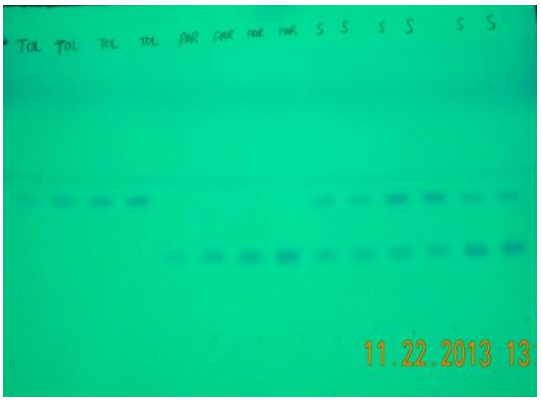
% Level	Amount of sample (µg/ml)		Amount added (µg/ml)		Amount found (µg/ml)		% Recovery		% RSD	
	TOL	PAR	TOL	PAR	TOL	PAR	TOL	PAR	TOL	PAR
50	100	333.33	50	166.66	147.81	498.74	98.54	99.74	0.48	0.15
100	100	333.33	100	333.33	201.62	667.98	100.81	100.19	0.81	0.56
150	100	333.33	150	499.99	249.16	830.46	99.66	99.65	0.63	0.38

Table 3: Results of Precision studies.

Drug	Amount of drug applied (ng/spot)	Repeatability (n=6)		Intermediate precision (n=3)			
				Intraday precision		Interday precision	
		Amount of drug found (ng/spot)	% RSD	Amount of drug found (ng/spot)	% RSD	Amount of drug found (ng/spot)	% RSD
TOL	200	197.46	0.15	204.09	0.76	201.68	0.67
	600	598.25	0.26	598.30	0.59	603.98	0.15
	1000	1011.19	0.43	996.93	1.60	998.83	0.28
PAR	200	198.68	1.20	198.98	0.49	199.08	0.98
	600	604.29	0.98	599.30	0.34	596.36	1.09
	1000	1028.90	0.08	1003.01	0.19	997.90	0.87

Table 4: Assay results for the combined dosage form using the proposed HPTLC method.

Formulation	Labelled amount (mg)		Amount found (mg)		Amount found (%)	
	TOL	PAR	TOL	PAR	TOL	PAR
MYO-MR PLUS	150	500	149.13	498.86	99.42±0.67	99.77±0.96



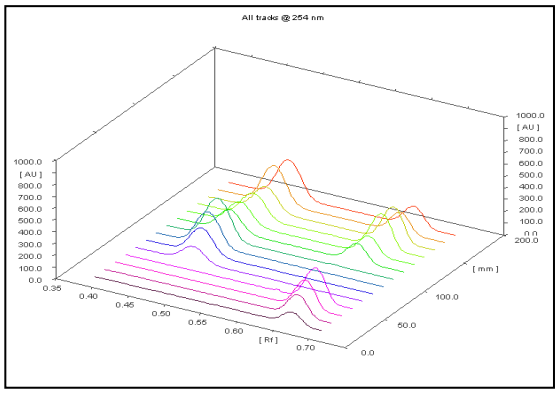


Fig. 3: Photograph of Developed HPTLC Plate **Fig.4:3DOverlain spectra of TOL and PAR**

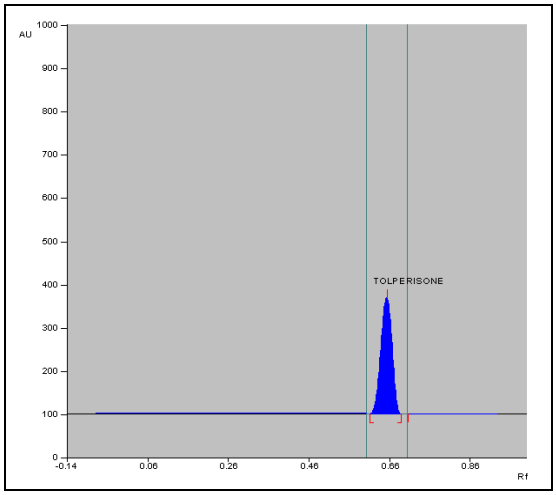


Fig. 5: Densitogram of Tolperisone

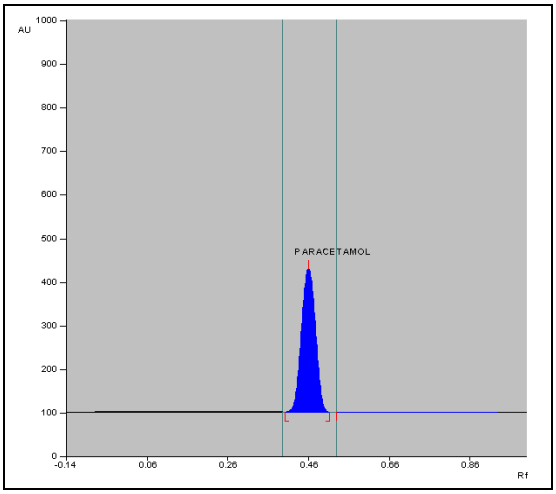


Fig. 6: Densitogram of Paracetamol

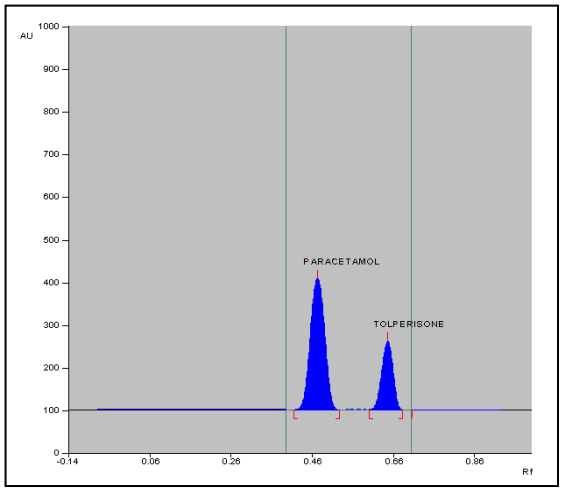


Fig. 7: Densitogram of mixed standard of TOL and PAR

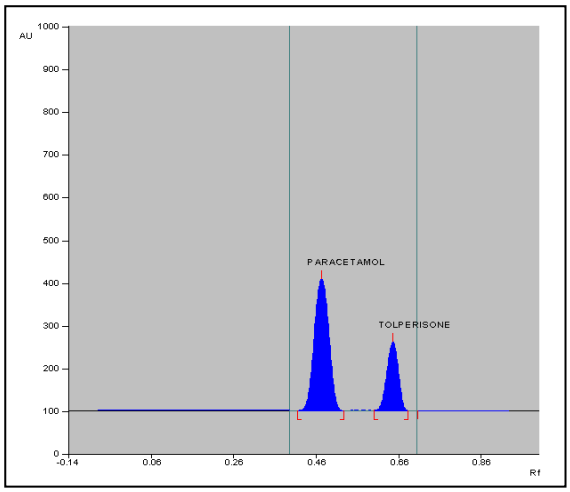


Fig.8: Densitogram of marketed formulation

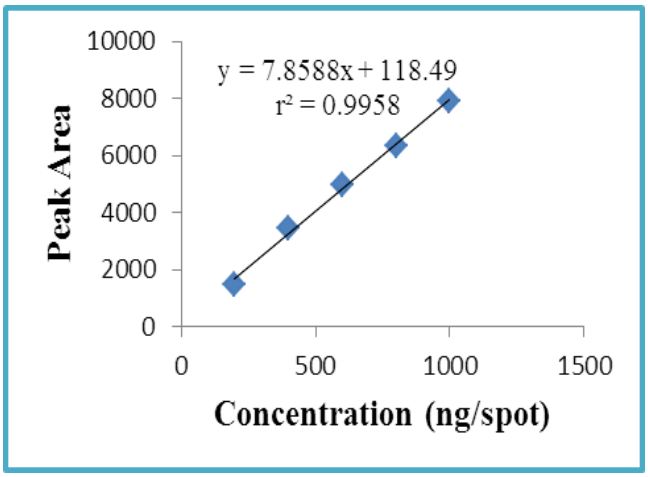


Fig. 9: Calibration curve of Tolperisone.

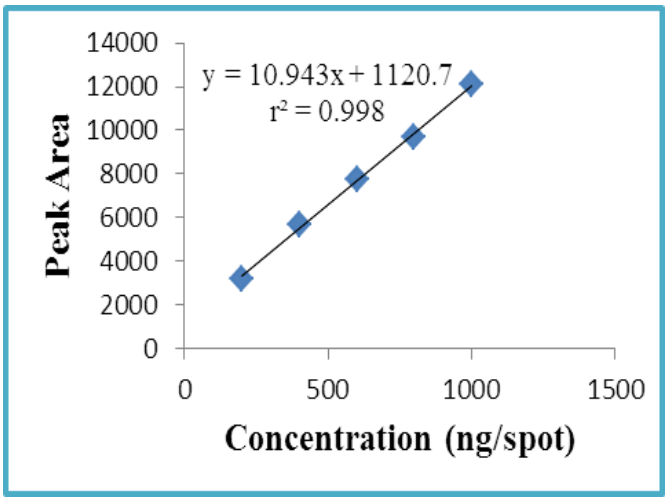


Fig. 10: Calibration curve of Paracetamol.

5. CONCLUSION

A sensitive, accurate, fast and precise HPTLC method has been developed for the simultaneous estimation of Tolperisone hydrochloride and Paracetamol in tablet dosage form. The proposed method does not suffer from any interference due to common excipients. Therefore the proposed method could be successfully applied to estimate commercial pharmaceutical products containing Tolperisone hydrochloride and Paracetamol.

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