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Simultaneous Estimation of Tramadol Hcl, Paracetamol and Domperidone in Pharmaceutical Formulation by Thin-Layer Chromatographic-Densitometric Method

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Abstract

A simple, precise, rapid, selective, and economic high-performance-thin-layer chromatography (HPTLC) method has been established for simultaneous analysis of Domperidone (DMP), Paracetamol (PCM) and Tramadol Hcl (TMD) in tablet dosage forms. The chromatographic separations were performed on precoated silica gel 60_{254} plates with toluene-ethylacetate-butanol-ammonia 5:4:1:0.2(v/v) as mobile phase. The plates were developed in a 7.0 cm at ambient temperature. The developed plates were scanned and quantified at their single wavelength of maximum absorption at approximately 278 nm for DMP and PCM, respectively. Experimental conditions such as chamber size, chamber saturation time, migration of solvent front, slit width, etc. was critically studied and the optimum conditions were selected. The drugs were satisfactorily resolved with R_f 0.18 ± 0.02 for DMP, R_f 0.25 ± 0.02 for PCM and for TMD R_f 0.50 ± 0.02. The method was validated for linearity, accuracy, precision, and specificity. The calibration plot was linear between 100-600 ng / band for DMP, 3250-19500 ng/band based for PCM and 375-2250 ng/band based for TMD. The limits of detection and quantification for DMP were 9.95 and 30.16 ng/band, respectively; for PCM they were 64.30 ng and 194.87 ng/band and for TMD 5.51 and 16.70/band. This HPTLC procedure is economic, sensitive, and less time consuming than other chromatographic procedures. It is a user-friendly and importance tool for analysis of combined tablet dosage forms.

Keywords: HPTLC; Paracetamol; Domperidone; Tramadol HCl tablet; Validation

Introduction

PCM (PCM; N-[4-hydroxyphenyl] ethanamide; Figure 1b) is a widely used analgesic and antipyretic for the relief of fever, headaches, and other minor aches and pains, and is a major ingredient in numerous cold and flu remedies. In combination with nonsteroidal anti-inflammatory drugs (NSAIDs) and opioid analgesics, paracetamol is used also in the management of severe pain (such as postoperative pain) [1]. Tramadol Hcl (TMD; (+/-) cis-2-[(Dimethylamino) methyl]-1-(3-methoxyphenyl) cyclohexanol hydrochloride figure 1c) is a centrally acting analgesic, having agonist actions at the μ-opioid receptor and affects reuptake at the noradrenergic and serotonergic systems. TMD is a compound with mild and delayed μ-agonist activity [2]. Domperidone (DMP; 5-chloro-1-[1-[3-(2-oxo-2, 3-dihydro-1H-benzimidazol-1- yl) propyl]-piperidin-4-yl]-1, 3-dihydro-2H-benzimidazol-2-one; figure 1a) used as antiemetic drug. PCM is official in Indian Pharmacopoeia. This pharmacopoeia suggests titrimetric and UV spectrophotometric assay method for PCM in bulk and tablet formulations. DMP is official in Indian Pharmacopoeia where assay is described by titrimetric method. Tramadol is official in Indian Pharmacopoeia. This pharmacopoeia suggests titrimetric (potentiometric) assay method for tramadol in bulk. Literature survey revealed that various analytical methods like spectrophotometric [3-6], HPLC [7-14], GC [15] and HPTLC [16-19] have been reported for the determination of TMD, PCM and either individually or combination with some other drugs, but no HPTLC method was reported for simultaneous estimation of TMD and PCM and domperidol in combined dosage forms. Many methods [20-27] have been described in the literature for the determination of domperidol and pracetamol, individually. The analytical methods like HPLC [28] and HPTLC [29] for determination of domperidol and PCM in combined dosage form have been reported. The RP HPLC [30] method has been reported for estimation of TMD, PCM and Domperidol in tablet formulation. The review of literature prompted us to develop an accurate, selective and precise simultaneous method for the estimation of TMD, PCM and Domperidol in combined dosage forms.

Experimental

Chemicals and materials

TMD, PCM and DMP were procured from Cadila pharmaceuticals, Ahmedabad. Ethyl Acetate, Toluene, Ammonia and n-Butanol were used as solvents to prepare the mobile phase. All the reagents used were of Analytical reagent grade (CHEMDYES CORPORATION, Ahmedabad, india) and used without further purification. Tablet formulation A (Tramazac - PD, Zydus Cadila Healthcare Ltd.,

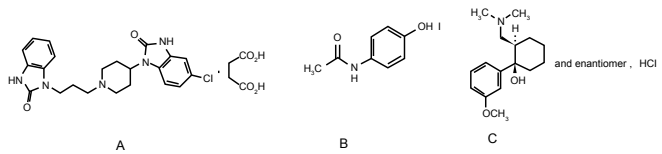

Figure 1: Structure Of Domperidone (A)Paracetamol (B) And (C) Tramadol HCl.

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Ahmedabad, India) and Tablet formulation B (RAMCET-D sundyota numandis pharma. Ahmedabad., India) containing labeled amount of 325 mg PCM, 37.5 mg TMD, and 10 mg DMP were procured from local market.

Chromatographic conditions

The samples were spotted in the form of bands of width 6 mm with a camag 100 µL sample (Hamilton, Bonaduz, Switzerland) syringe on precoated silica gel 60_{254} plates (10 × 10) with 250 µm thickness; (E MERCK, Darmstadt, Germany), supplied by using a camag Linomat V(Switzerland). The plates were prewashed by methanol and activated at 110°C for 5 minutes, prior to chromatography. A constant application rate of 0.1 µL/spot was employed and space between two bands was 5 mm. The slit dimension was kept at 6 mm × 0.45 mm and 10 mm/ spot scanning speed was employed. The monochromator band width was set at 20 nm and 320 cut off filter; each track was scan thrice and base line correction was used. The mobile phase consists of n-Butanol – ethylacetate - toluene – ammonia (1:4:5:0.1 v/v/v/v). Linear ascending development was carried out in 10 cm × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland). The optimized chamber saturation time for mobile phase was 20 min; at room temp. (25°C ± 2); at relative humidity 60% ± 5. The length of chromatogram run was 7 cm and approximately 40 min., subsequent to the development. TLC plates were dried in a current of air with the help of an air drier. Densitometric scanning was performed on Camag TLC scanner III in the reflectance-absorbance mode at 278 nm for all measurements and operated by WINCATS software version 1.3.0. The source of radiation utilized was deuterium lamp, continues emits UV spectrum between 200 nm to 400 nm [7-15].

Sample preparation

Twenty tablets of brand A were weighed and average weight is calculated. Powder equivalent to approximately 325 mg PCM, 10 mg DMP and 37.5 mg TMD was weighed accurately, transferred to a 100-mL volumetric flask, and methanol (100 mL) was added. The solution was sonicated for 15 min, then diluted to volume with methanol and filtered through a Whatman filter paper No.41.

Preparation of standard solution

A Combined standard stock solution containing 100 µg/mL DMP, 3250 µg/m PCM and 375 µg/ml TMD was prepared in methanol. Calibration was done by applying mix standard solution ranging from 1-6 µl by micro liter syringe with the help of automatic sample applicator Linomat V on TLC Plate, which gives concentration of 100-600 ng/spot of DMP , 3250-19500 ng/spot of PCM and 375-2250 ng/ spot of TMD. Each concentration was spotted six times on the TLC plates. The plate was developed on previously described mobile phase. The peak areas plotted against the corresponding concentrations to obtain the calibration graphs.

Method validation

The developed method was validated for linearity and range, specificity, accuracy, precision, Limit of detection, Limit of quantitation, robustness and solution stability as per ICH guidelines.

Linearity and range: Aliquots of standards 1-6 µl TMD, PCM & DMP solutions were loaded in the 10 × 10 Silica gel $60F_{254}$ TLC plate using 100 µl Hamilton syringe and Camag – Linomat -5 instrument.

Specificity: The specificity of the method was ascertained by analyzing PCM, DMP and TMD in presence of excipients commonly used for tablet formulations. The bands of PCM, DMP and TMD were confirmed by comparing R_F values and respective spectra of sample with those of standards. The peak purity of PCM, DMP and TMD was assured by comparing the spectra at three different levels, that is, peak start, peak apex and peak end positions, as listed in table 1.

Accuracy (% Recovery): The recovery study was carried out at three levels, 80%, 100% and 120%. To the powdered formulation, the standard drugs of TMD and PCM and DMP were added at 80 %, 100 % and 120% levels, dilutions were made and analyzed by the method. The % recovery and % RSD were calculated and found to be within the limit, as listed in table 2.

Method precision (repeatability): Repeatability of measurement of peak area was checked by repeated scanning of the same spot (*n* = 6) of DMP (400 ng/band), PCM (13000 ng/band) and TMD (1500 ng /band) without changing the position of the plate. Repeatability of sample application was assessed by spotting DMP (400 ng / band), PCM (13000 ng /band) and TMD (1500 ng /band) six times on an HPTLC plate, developing the plate, and recording peak area for the spots. The precision of the method was evaluated by calculating the percent relative standard deviation (% RSD) of mean peak areas obtained from each spot of sample.

Intermediate precision (reproducibility): The intra-day and interday precision of the method was determined by estimating the corresponding response three times on the same day and on three different days over a period of one week for three different concentrations of DMP (200, 400, and 600 ηg / band), PCM (6500, 13000, and 19500 ηg / band) and TMD (750, 1500 and 2250 ηg / band). Again here, the precision of the method was evaluated by calculating the percent relative standard deviation (% RSD) of mean peak areas obtained from each spot of sample.

Limits of detection (LOD) and limits of quantitation (LOQ): A working standard solution of 10 ng/µl and 100 ng TMD and 10 ng PCM and DMP were prepared in methanol. Series of 1-6 µl Standard TMD and PCM and DMP solutions were loaded in the 10 × 10 Silica gel $60F_{254}$ TLC plate using 100 µl Hamilton syringe and Camag – Linomat - 5 instrument.

Robustness : In order to establish the robustness of the method, small deliberated changes were made in the experimental conditions and chromatographic parameters like change in plate activation time, chamber saturation time (± 20% change from set time), volume of mobile phase (± 10% change from set volume) and development distance (± 10% change from set distance). 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

Sample	Correlation of center and slope spectra	
	r (s, m)	r (m, e)
PCM	0.991	0.998
PCM tablet formulation A	0.997	0.997
PCM tablet formulation B	0.995	0.996
DOM	0.998	0.995
DOM tablet formulation A	0.996	0.996
DOM tablet formulation B	0.996	0.995
TMD	0.995	0.998
TMD tablet formulation A	0.996	0.997
TMD tablet formulation B	0.998	0.996

Table 1: Peak purity correlation results of pcm, dom and tmd in two formulations at peak start, middle and end.

compared with similar results obtained in unchanged experimental conditions.

Solution stability : The solutions at analytical concentration (PCM-6500 µg mL^{-1}, DMP-200 µg mL^{-1} 750 µg mL^{-1} TMD) were prepared and stored at room temperature for 24 h and analyzed at interval of 0, 6, 12 and 24 h for the presence of any band other than that of PCM, DMP and TMD and the results were simultaneously compared with the freshly prepared standard solution of PCM, DMP AND TMD standard solution of the same concentration in the form of change in %RSD of the response obtained.

Application of validated method to pharmaceutical formulation

To determine the content of PCM, DMP and TMD in combined dose capsule formulation twenty tablets of each brand were weighed. Average weight was calculated, the tablets are crushed and powder equivalent to about 325 mg PCM, 10 mg DMP and 37.5 TMD was transferred to 100.0 mL volumetric flask, 20.0 mL of methanol was added and content of the flask were ultrasonicated for 30 minutes, volume was made up to the mark with methanol. The solution was mixed and filtered through Whatman filter paper No. 41. From the filtrate, 1.0 mL was diluted to 10.0 mL with methanol. On TLC plate two bannds of standard and four bands of sample solution, were applied and the plate was developed and scanned under the optimized chromatographic conditions. After scanning, the peaks obtained for standard and sample bands were integrated. Amount of the drugs present in applied volume of sample solution was obtained by comparison between peak area of standard and sample bands. Six samples were prepared and analyzed in similar manner. Results of analysis of capsules are shown in table 5.

Thus the validated method was used for analysis of PCM, TMD and DMP in their combined tablets dosage form (Brand A and B) [20-27].

Results and Discussion

Method development and optimization of chromatographic conditions

Selection of best solvent system is the critical step in HPTLC [16-19] method development. From the different solvent systems tried, mobile phase consisting of toluene : ethylacetate : n-butanol : ammonia in the ratio 5:4:1:0.15 (v/v/v) resulted in better separation which gave symmetric peaks of DMP with R_F of 0.18, PCM with R_F of 0.25 and TMD with R_F of 0.50 (Figure 2). Well defined bands were obtained when the chamber was saturated with mobile phase for 20 min at ambient temperature. For quantitative purpose, the densitometric

Level	Amount of drug added (mg)			Amount of drug found (mg)			Mean recovery [a] (%) (n=3)			%RSD		
	PCM	DOM	TMD	PCM	DOM	TMD	PCM	DOM	TMD	PCM	DOM	TMD
80%	260	8	30	585.5	17.52	67.55	99.92	99.79	100.02	0.48	0.59	0.33
	260	8	30	584.4	18.02	67.95						
	260	8	30	585.52	18.01	67.72						
100%	325	10	37.5	649.02	20.03	75.53	100.06	99.63	100.05	1.26	0.67	0.43
	325	10	37.5	651.05	19.94	74.48						
	325	10	37.5	648.04	20.92	75.55						
120%	390	12	45	715.06	22.06	82.52	99.96	100.13	100.08	0.46	0.57	0.37
	390	12	45	714.02	22.01	82.42						
	390	12	45	715.5	21.98	82.67						

Table 2: Results from accuracy study.

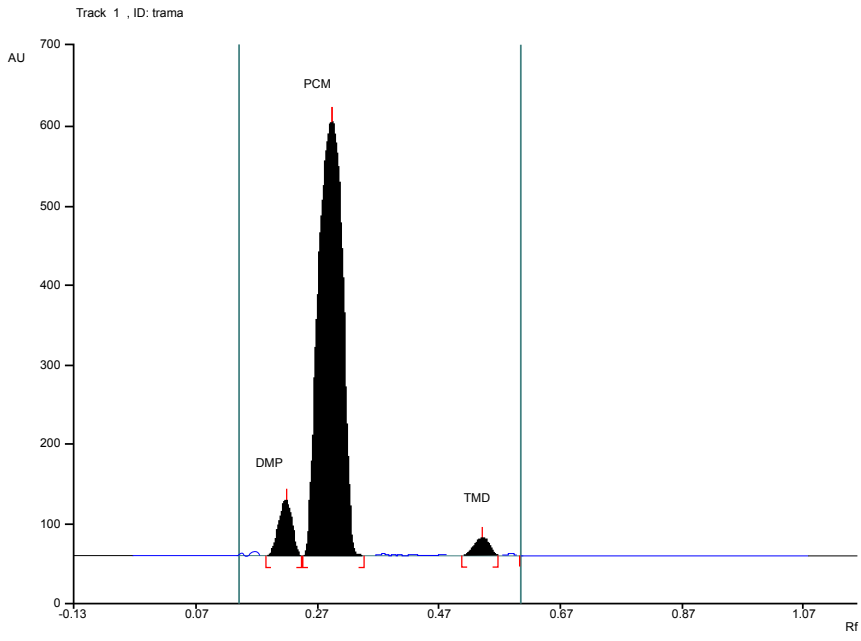

Figure 2: Hptlc Densitogram of Domperidone, Paracetamol and Tramadol hcl when Scanning At 278 Nm.

scanning was carried at wavelength 278 nm where PCM, DMP AND TMD exhibited good UV absorption.

Validation of the method

Linearity: Linearity was observed over the concentration range of 100 - 600 ng / band for DMP, 3250 – 19500 ηg / band for PCM and 375 – 2250 ηg / band for TMD respectively. The regression equations (n = 5) were y = 10874x - 28.351 for DMP, y = 4394.7x - 1025.5, for PCM and y = 23810x + 76.43 for TMD respectively, where y is response and x the amount chromatographed. The correlation coefficients (r) were 0.9986, 0.9944, and 0.999 respectively, over these concentration ranges shown in figure 3.

Specificity: Specificity of the method for PCM, DMP and TMD was proved from the spectral scan (Figure 4), and peak purity correlation (r) results (Table 1) for PCM, DMP and TMD in two tablet formulations indicate that there is no merging or co-elution of interfering peaks with PCM, DMP and TMD, so there is no interference from any excipients present in tablet formulation.

Accuracy: Accuracy of proposed method was studied by preparing synthetic mixtures of capsule excipients having a known amount of PCM, DMP and TMD corresponding to approximately 80 – 120% of the label claim. Mean recovery was between 99.925 - 100.06% for PCM, 99.63 - 100.13% for DMP and 100.02 – 100.08% respectively indicating that the developed method was accurate for the determination of PCM, DMP and TMD in pharmaceutical formulations (Table 2).

Precision: For determination of precision of PCM, DMP and TMD by the proposed method, same homogenous samples of PCM, DMP and TMD were prepared repeatedly and analyzed. Intermediate precision was evaluated at different times on the same day, and on different days. Low values of RSD (less than 2%) obtained in the studies indicates that the method is precise and reproducible (Table 3).

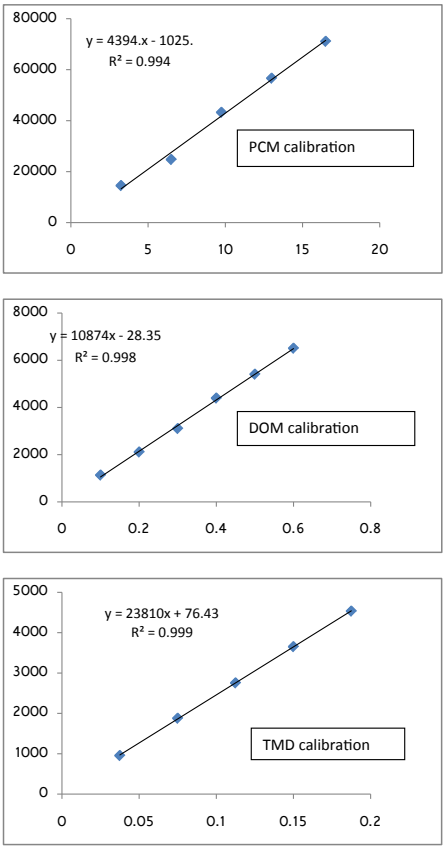

Figure 3: Calibration Plot of Pcm, Dom and Tmd by the Developed Hptlc Method.

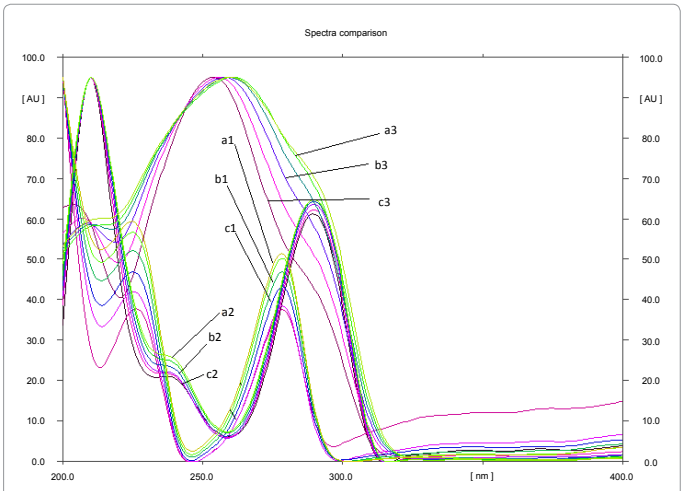

Figure 4: Spectra comparison of tmd, dom and pcm. (a1, a2 and a3) bulk of tmd, dom and pcm, (b1, b2 and b3) tablet formulation a and tmd, dom and pcm, (c1 ,c2 and c3) tablet formulation b respectively.

Limits of detection (LOD) and Limits of Quantification (LOQ): The limits of detection (LOD) and quantification (LOQ) for PCM were 64.3ng and 194.87 ηg / band, respectively. For DMP the values were 9.95 ng and 30.15 ηg / band and for TMD 5.51 ng and 16.70 ηg respectively, are listed in table 3.

Robustness: Acceptable %RSD values obtained after making small deliberate changes in the developed HPTLC method indicate that the method is robust for the intended purpose (Table 4).

Solution stability: No significant change was observed in peak area of PCM, DMP and TMD when analyzed up to 24 h at different time intervals, (RSD 1.24, 1.15 and 1.10) which indicate the solution stability within the period of evaluation.

Method application

The proposed, developed and validated method was successfully applied to analysis of PCM, DMP and TMD in their marketed formulations (Brand A & B). There was no interference of excipients commonly found in tablets as described in specificity studies. The assay results obtained were satisfactory, accurate, and precise as indicated by the good recovery and acceptable standard deviation values (Table 5). The good performance of the method indicates that it can be used for the determination of PCM, DMP and TMD in pharmaceutical formulations [28-30].

Conclusion

This developed and validated method for simultaneous analysis of paracetamol, domperidone and tramadol HCl in pharmaceutical preparations is very simple, rapid, accurate, and precise. The method was successfully applied for determination of PCM, DOM and TMD in its pharmaceutical capsule formulations. Moreover it has advantages of short run time and the possibility of analysis of a large number of samples, both of which significantly reduce the analysis time per sample.

Validation parameters	PCM	DOM	TMD
Linear range (ηg per band)	3250-19500	100-600	375-2250
LOD (ηg per band)	64.30	9.95	5.51
LOQ (ηg per band)	194.87	30.16	16.70
Accuracy (%)	99.925 – 100.06	99.63 – 100.05	100.02 – 100.08
Repeatability of measurement of peak area (RSD %,*n* = 6)	0.6859	0.69	0.9229
Repeatability of sample application (RSD%, *n* = 6)	0.5823	0.78	0.2957
Precision (RSD %)			
Intraday (*n* = 3)	0.5296	0.57	0.3335
Interday (*n* = 3)	0.4783	0.48	0.7490

[b] = Limit of quantification
[c] =Relative standard deviation
n = number of determinations

Table 3: Summary of validation parameters of developed hptlc method.

Method parameter/Condition	Deliberate changes	%RSD of peak area (n = 3)		
		PCM	DOM	TMD
Plate activation time[a]	20 min	1.23	1.52	1.48
	30 min	1.33	1.49	1.68
Chamber saturation time[a]	16 min	1.59	1.27	1.74
	24 min	1.29	1.33	1.39
Volume of mobile phase[b]	8.2 Ml	1.37	1.46	1.25
	10.0 mL	1.91	1.93	1.73
Development distance from spot application[c]	7.7 cm	1.81	1.86	1.61
	6.3 cm	1.87	1.47	1.35

(a) ±20% change in set time.
(b) ±10% change in set volume.
(c) ±10% change in set distance.

Table 4: Results from the robustness study of method.

Capsule	Component	Label claim (mg)	% of lable claim (n = 5) ± % RSD (n = 5)
Brand A	PCM	325	99.293 ± 0.79
	DOM	10	100.01 ± 0.35
	TMD	37.5	99.52 ± 0.72
Brand B	PCM	325	100.21 ± 0.82
	DOM	10	99.89 ± 0.46
	TMD	37.5	99.75 ± 0.37

n = number of determinations

Table 5: Results from analysis of paracetamol, domperidone and tramadol hcl in the combined tablet dosage form.

Hence this method can be conveniently used for routine quality control analysis of PCM, DOM and TMD in its pharmaceutical formulations.

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