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Research Article

Development and Validation of a Stability-Indicating RP-HPLC Method for Simultaneous Determination of Paracetamol, Tramadol HCl and Domperidone in a Combined Dosage Form

K Karunakaran[1*], G Navaneethan[1] and KP Elango[2]
[1]Department of Sciences, Sona College of Technology (Anna University), Salem 636 005, [2]Department of Chemistry, Gandhigram Rural Institute (Deemed University), Gandhigram 624 302, India

Abstract

Purpose: *To develop a stability indicating RP-HPLC method for a combination drug product containing a high dose of paracetamol (PR) and low doses of domperidone (DM) and tramadol HCL (TR).*
Methods: *The analytes are well separated by a reverse phase column and an isocratic mobile phase consisting of 0.1 %v/v trifluoroacetic acid: acetonitrile: methanol in the ratio 70:25:5 (v/v) with a flow rate of 1.0 mL/min. The effluent was monitored at 272 nm. The drug products were subjected to stress conditions of acid, base, peroxide, thermal and photolytic degradation and peak homogeneity of PR, TR and DM were obtained using photo diode array detector.*
Results: *The degradation products were well resolved from PR, TR and DM peaks, thus indicating the stability-indicating nature of the method. The assay was linear from 165 – 495 µg/mL for PR, 18.75 – 56.25 µg/mL for TR, and 5 – 15 µg/mL for DM. Although the tablet contained high and low doses of the drugs, the intra- and inter-day variations were < 2.0 %.*
Conclusion: *The proposed method was validated according to the ICH guidelines and proved suitable for stability and homogeneity testing, as well as for quality control of the combined drugs in pharmaceutical preparations.*

Keywords: *HPLC, Isocratic, Peak purity, Simultaneous determination, Paracetamol, Tramadol, Domperidone*

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Corresponding author: ***Email:*** *drkk@sonatech.ac.in;* ***Tel:*** *+91 427 4099998;* ***Fax:*** *+91 427 4099989,*

INTRODUCTION

Paracetamol (PR) has been in use as an analgesic for well over 30 years and is accepted as a very effective treatment for the relief of pain and fever in adults and children. Tramadol.HCl (TR) is an opioid analgesic. It also has noradrenergic and serotonergic properties that may contribute to its analgesic activity and is used for moderate to severe pain. Domperidone (DM) is an antidopaminergic drug and it is generally used orally, rectally or intravenously to suppress nausea and vomiting [1].Chemically PR is N-(4-hydroxyphenyl) acetamide, TR is (1RS,2RS)-2-[(Dimethylamino)methyl]-1-(3-methoxyphenyl) cyclohexanol hydrochloride and DM is 5-chloro-1-(1-[3-(2-oxo-2,3-dihydro-1H-benzoimidazol-1-yl)propylpiperidin-4-yl)-1H-benzoimidazol-2(3H)-one (Fig 1).

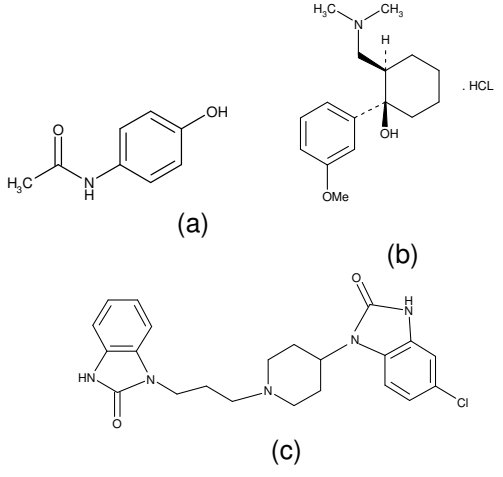

Fig 1: Structure of (a) paracetamol, (b) tramadol HCl and (c) domperidone

Chromatographic methods for the determination of PR, TR and DM as single entities are included in British Pharmacopoeia [2]. A survey of the literature revealed that only few methods have been reported for the determination of PR and TR or PR and DM in a combined drug formulations [3-9]. The literature also shows that assay methods for one of these drugs in combination with other drugs have been reported [10,11]. Recently, Arunadevi et al. reported the assay of PR and TR in a combination drug product in which DM was used as an internal standard [12]. A method has published which employed UV-spectrophotometry for the simultaneous determination of PR, TR and DM [13]. One HPLC method for the simultaneous determination of PR, TR and DM has been published [14]. In this method, the peak of PR showed tailing. Moreover, TR peak eluted in the void of paracetamol peak. Since the peak area of TR was very low, setting the integration parameter in HPLC system was difficult. Thus, quantification led to a bias result. In view of these flaws, the reported method is not suitable for analysis in pharmaceutical industry.

However, to the best of our knowledge, no work using an isocratic and stability-indicating RP-HPLC method has been applied for the simultaneous estimation of PR, TR and DM. This paper describes the development and validation of a precise, specific and reliable HPLC method with isocratic elution for the simultaneous determination of PR, TR and DM. in pharmaceutical formulations.

EXPERIMENTAL

Materials and reagents

Pharmaceutical grade PR, TR and DM were obtained as gifts from M/S GS Lab Baddi, India. A commercial combination drug product was tested. Each tablet contained 325 mg of PR, 37.5 mg of TR and 10 mg of DM. HPLC grade acetonitrile and methanol were purchased from Merck (India). Buffer materials and all other chemicals used were of analytical reagent grade. High purity water was generated from a Millipore Milli-Q plus purification system.

HPLC instrumentation and conditions

Chromatographic separation was performed on a Waters Alliance HPLC system equipped with a 2695 separation module and 2996 photo diode array detector as well as 2487 UV detector (Waters Corporation, Milford,

USA). Empower software build-2154 workstation was employed for data collection and processing.

The mobile phase consisted of a mixture 0.1 % trifluoroacetic acid, acetonitrile and methanol in the ratio 70:25:5 (v/v). The mobile phase was filtered and degassed through a 0.45µm membrane filter under vacuum. Peerless basic C_{18} (250 mm x 4.6 mm i.d., 5-µm) stainless steel analytical column was used as the stationary phase and was maintained at 30 ℃. The mobile phase was pumped at a constant rate of 1.0 mL/min and the effluent monitored at 272 nm. The injected sample volume was 10µL.

Preparation of solutions

Mixture of TR and DM standard stock solution

A mixture of TR and DM standard stock solution was prepared by transferring 37.5 mg of TR and 10.0 mg of DM into a 100 mL volumetric flask, and a 50 mL portion of diluent (mobile phase) was added, sonicated to dissolve and cooled to room temperature. The solution was made up to volume with diluent and mixed.

Standard solution of mixture of PR, TR and DM

PR (16.5 mg) was accurately weighed and transferred into a 50 mL volumetric flask and 20 mL of diluent (mobile phase) was added to dissolve it. Further, a 5 mL portion of TR and DM standard stock solution was added and the volume made up with diluent to obtain a solution containing 0.33 mg/mL of PR, 0.0375 mg/mL of TR and 0.01 mg/mL of DM. The solution was mixed, filtered through a 0.45 µm nylon syringe filter and 10 µL was injected.

Test solution

Five tablets were weighed and crushed in a mortar with a pestle and transferred into a 500 mL volumetric flask. The mortar and pestle thoroughly washed with the diluent and the washings transferred to the flask. Approximately 200 ml of diluent was added to the flask and the contents were sonicated for 30 min. The volume was made up to 500 mL with more diluent and mixed well. Five millliliters of the resulting solution was diluted to 50 mL with diluent to give concentrations of 0.33, 0.0375 and 0.01 mg/mL for PR, TR and DM, respectively. The resulting solution was filtered using a 0.45 µm nylon syringe filter; 10 µL of the resulting solution was injected for analysis.

Statistical analysis

Basic statistical data (standard deviation, mean, slope, intercept and correlation coefficient) were obtained by using Microsoft Excel 2003.

Method validation

The developed HPLC method was validated according to ICH guidelines in terms of precision, ruggedness, linearity, specificity, selectivity, robustness and accuracy [15, 16].

Precision

Assay method precision was assessed using six independent test solutions. The intermediate precision of the assay method was also evaluated using a different analyst, column and HPLC system on different days.

Linearity

Peak areas versus concentrations were plotted for PR, TR and DM at five different concentration ranges between 50 and 150 % of target level.

Specificity and selectivity

Stress studies of the drug product are utilized for identification of the possible degradation products and for the validation of the stability-indicating analytical procedure. It is the ability of the analytical method to measure analyte response in the presence of its degradation

products and sample matrix. Forced degradation studies were performed on the tablet samples using the following conditions: acid hydrolysis (0.1M HCl, 8 h), base hydrolysis (0.1M NaOH, 8 h), oxidation (3 % H_2O_2, 8 h), heat (80℃ for 48 h) and photolysis (UV 254nm, 48 h). All these samples were appropriately diluted with the diluent and injected into the HPLC. Peak purity test was carried out for PR, TR and DM by using PDA detector in the stress samples.

Limits of detection (LOD) and of quantification (LOQ)

The LOD and LOQ for PR, TR and DM were determined at a signal-to-noise ratio 3:1 and 10:1 respectively, by injecting series of dilute solution with known concentrations. Precision study was also carried out at the LOQ level by injecting six individual preparations and calculating the % RSD of the area.

Accuracy

The accuracy of the method was determined for PR, TR and DM by recovery experiments. Known amounts of PR, TR and DM bulk sample, in triplicate, at levels 80, 100 and 120 %, respectively, of the specified limits were taken for analysis.

Robustness

Robustness was established by analyzing system suitability standard (n = 5) and sample (n = 3) at 25 and 35 ℃ (nominal = 30 ℃), at flow rates of 0.9 and 1.1 of the nominal flow (i.e., 1.0 mL/min) and ± 10 % change of buffer, acetonitrile and methanol (nominal ratio = 70:25:5 v/v). Tailing factors as well as theoretical plates of PR, TR and DM were evaluated.

Analysis of commercial tablet formulation

The ability of the optimized method to determine PR, TR and DM simultaneously in a commercial drug product was tested using six individual preparations. The combination tablet contained 325 mg of PR, 37.5 mg of TR and 10 mg of DM was employed in the present study (Decotram-PD®, Worth Medicine, Chandigarh, India).

RESULTS

Method validation

System suitability

To check the system and column performance, the standard solution was injected five times and the following parameters were monitored. System suitability results are shown in Table 1. Tailing factor (≤ 2.0), theoretical plates (≥ 8000 for PR, not less than 12000 for TR and ≥ 15000 for LR), %RSD of PR, TR and LR (≤ 2 %) were obtained.

Table 1: System suitability results

Compound	Retention Time (min)	USP tailing factor	Theoretical Plates
PR	3.87	1.2	11392
TR	9.85	1.2	16724
DM	22.40	1.1	18703

Precision

The %RSD values of PR, TR and DM for method precision were 1.1, 0.9 and 1.3, respectively but 0.8, 0.5 and 1.2, respectively, for intermediate precision. Low RSD values (< 2 %) showed the suitability of the method for the determination of PR, TR and DM in a combination tablet formulation.

Linearity

PR, TR and DM showed linearity in the range 165 – 495, 18.75 – 56.25 and 5 –15 µg/mL, respectively. The results obtained are represented by the following linear regression equations: Y_{PR} = 71017x - 4800 (r^2=0.9991), Y_{TR} = 1149.9x +500.2 (r^2=0.9998) and Y_{DM} = 574.21x - 460.2 (r^2=0.9993), for PR, TR and DM, respectively.

Specificity and selectivity

The results obtained from forced degradation study are summarised in Table 2. In acidic

conditions, the degradation product was formed at a retention time (RT) of 8.115 min; in basic condition, the degradation product formed was at RT of 4.557 min which had > 3.0 resolutions from PR. In peroxide condition, the degradation products formed were at RTs of 3.284, 4.175 and 6.116 min; the close eluting impurity at 4.175 min had > 1.3 resolutions from PR. No degradation occurred in heat and photolysis stressed samples.

In all degradation samples, purity angles were less than threshold, indicating the purity of the peaks and stability-indicating nature of the developed HPLC method.

Limits of detection (LOD) and of quantification (LOQ)

The LOD for PR, TR and DM were 0.15, 0.4 and 0.2 µg/mL, respectively, and LOQ for PR, TR and DM 0.5, 1.0 and 0.5µg/mL, respectively. The LOQ level %RSD for the six preparations < 5 for all three drugs.

Accuracy

The results for accuracy of the method are given in Table 3. Recoveries of PR, TR and DM in bulk drug samples were between 98.0 and 102.0 %, indicating the good accuracy of the developed method.

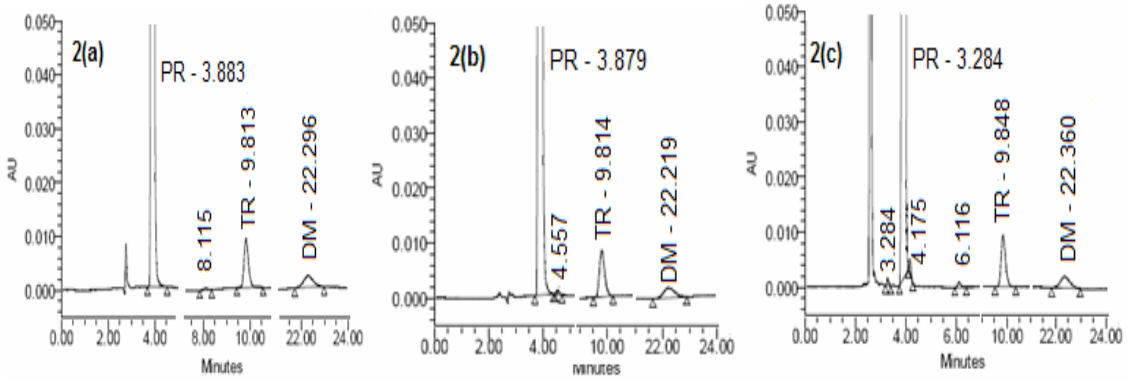

Fig 2: Typical LC chromatograms of a sample obtained after (a) acid degradation (b) base degradation and (c) peroxide degradation

Table 2: Results of forced degradation study

Condition	PR			TR			DM		
	Assay (%)	***Purity angle***	***Purity threshold***	***Assay (%)***	***Purity angle***	***Purity threshold***	***Assay (%)***	***Purity angle***	***Purity threshold***
Unstressed sample	99.5	4.396	5.724	99.3	0.581	0.796	99.6	0.452	0.821
Acid Hydrolysis (0.1M HCl)	97.5	3.362	4.121	98.2	0.531	0.821	98.5	0.423	0.792
Base Hydrolysis (0.1M NaOH)	96.2	3.261	4.528	96.5	0.521	0.792	99.2	0.515	0.798
Oxidation (3 % H_2O_2)	92.3	4.321	5.251	98.2	0.532	0.732	97.5	0.457	0.812
Heat (80 ℃)	98.9	2.871	3.215	98.9	0.543	0.752	99.2	0.521	0.832
Light (254 nm)	99.1	3.526	4.512	99.1	0.535	0.825	99.3	0.535	0.752

Note: *Acceptance criteria: Purity angle is less than threshold*

Table 3: Results of recovery study

Theoretical (% of target level)	Added amount (in mg)	Recovered amount (in mg)	Recovery (%)	Mean recovery (%)	% RSD
PR					
80%	259.2	260.1	100.3	100.4	0.30
	253.5	255.2	100.7		
	260.1	260.3	100.1		
100%	324.5	325.1	100.2	99.7	0.46
	325.6	323.2	99.3		
	326.2	325.4	99.8		
120%	390.1	389.2	99.8	99.9	0.09
	390.5	390.3	99.9		
	391.1	390.5	99.8		
TR					
80%	30.2	30.1	99.7	99.9	1.02
	30.1	29.8	99.0		
	29.8	30.1	101.0		
100%	37.6	37.2	98.9	100.3	1.33
	37.4	37.5	100.3		
	37.3	37.9	101.6		
120%	45.2	44.5	98.5	99.6	1.86
	44.9	44.3	98.7		
	45.3	46.1	101.8		
DM					
80%	8.15	8.10	99.4	99.3	1.06
	8.02	8.05	100.4		
	8.05	7.91	98.3		
100%	10.20	10.02	98.2	99.5	1.49
	10.30	10.21	99.1		
	9.80	9.91	101.1		
120%	12.12	12.01	99.1	99.8	0.90
	12.30	12.25	99.6		
	11.95	12.05	100.8		

Robustness

In all the deliberate changes, no significant change of assay value was observed. However, the current method was robust for such deliberate changes. %RSD of the standard was ≤ 1.0 and the assay data of the drug components were in the range 98 – 102 %, indicating robustness of the method.

Analysis of commercial tablet formulation

The assay results for PR, TR and DM were 99.2, 98.9 and 100.2 %, respectively,; the assay values were close to the labelled claim for all the three drugs, indicating that interference by excipients was insignificant.

The low values of RSD (< 2 %) for the assay established the precision of the proposed method.

DISCUSSION

Optimization of chromatographic conditions

In order to develop a suitable and isocratic RP-HPLC method for the simultaneous determination of PR, TR and DM, different buffer pH and column chemistry were applied to achieve the separation of all three components. The main objective of the chromatographic method was to develop a single method for all three components. In

the case of DM, the dose is very low compared to PR. Finally, the mobile phase consisting of 0.1 % trifluoroacetic acid, acetonitrile and methanol in the ratio 70:25:5 (v/v) at a flow rate of 1.0 mL/min using Peerless basic C_{18} (250 mm x 4.6 mm i.d., 5-µm) column was found to be suitable, allowing good separation of PR, TR and DM.

In the optimized chromatographic conditions, the peak shape of PR, TR and DM were symmetrical with a satisfactory resolution. The main system suitability parameter of theoretical plates for PR was greater than that of a recently published method [14]. This indicates that PR had very good retention in the optimized condition and the resolution between PR and TR is > 15. The response of PR, TR and DM were adequate at 272 nm.

To the best our knowledge, this is the first isocratic and stability–indicating method that has been developed for a combination drug product containing PR, TR and DM. The value of % RSD, which was < 1.0, indicates that the developed method is a precise method

CONCLUSION

The developed isocratic HPLC method for the simultaneous determination of PR, TR and DM in a pharmaceutical dosage form is specific, precise, accurate, linear and robust. An excellent correlation exists between peak area and concentration for the three drugs. The developed method is a stability indicating method and can be conveniently used by quality control outfits to determine the contents of PR, TR and DM simultaneously in routine and stability samples. The optimized method can be conveniently adopted for testing the dissolution and uniformity of content of tablets incorporating PR, TR and DM.

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