INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

NEW RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF PARACETAMOL AND TRAMADOL HYDROCHLORIDE IN BULK AND TABLET DOSAGE FORM

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Article Received on: 16/03/18 Approved for publication: 29/05/18

DOI: 10.7897/2230-8407.09694

ABSTRACT

The present work was focused on developing a new RP-HPLC method for the simultaneous estimation of paracetamol and tramadol hydrochloride in bulk and tablet dosage form and to validate it as per ICH and USP guidelines. The method involves use of water and acetonitrile in 9:1 ratio as mobile phase pumped at a rate of 1ml/min. The optimum wavelength selected for monitoring was 268nm. C_{18} column (4.6mm×250mm) of 5µ particle size was used as stationary phase. The method was finally validated, and parameters were reported. The system suitability parameters passed in which the asymmetric factors for Paracetamol and Tramadol were 1.54 and 1.09 respectively. Linearity ranges were found to be 20 to 100µg/ml with a correlation coefficient of 0.998. Accuracy studies reported a mean recovery of 98.7% for both the drugs. Faster retention times (1.1min and 4.1min) make the method simple and economic. Thus a validated and sensitive RP-HPLC method was developed for simultaneous estimation of Paracetamol and tramadol in bulk and tablet dosage form.

KEY WORDS: HPLC, Method, Paracetamol, Tramadol hydrochloride, Validation.

INTRODUCTION

Pain is an unpleasant sensation which can lead to distress and discomfort[1]. Pain can be acute or chronic. Drugs used to treat pain are called pain killers or analgesics. Paracetamol and Tramadol are commonly used analgesics. Paracetamol (Figure 1) is chemically N-(4-Hydroxyphenyl)ethanamide or N-(4-Hydroxyphenyl)acetamide. It is a cyclooxygenase-2 (Cox-2) inhibitor and it is used to treat fever and pain. Tramadol (Figure 2) is chemically trans-2-(Dimethylaminomethyl)-1-(m-methoxyphenyl)cyclohexanol. It is an Opioid receptor agonist, 5-HT inhibitor and it is used to treat mild to severe pain, depression. Both paracetamol and tramadol are practically freely soluble in water and methanol[2,3].

Literature survey reveals that much work is documented on the chromatographic (HPLC & HPTLC) estimation of these two drugs in combined pharmaceutical dosage forms[4-17]. However, they are tedious, time consuming and costly. Hence there is a need for the development of a relatively simple, precise, accurate, reproducible and cost effective HPLC method for the estimation of paracetamol and tramadol in tablets and to validate the developed method as per ICH and USP guidelines.

MATERIALS AND METHODS

Instrumentation

The analysis was carried out on a HPLC system (SPINCO BIOTECH) equipped with UV detector. Other apparatus and instruments used were electronic balance (Keroy). Digital pH meter (Systronics). Magnetic stirrer (Remi). Millipore (Direct Q UV3). Ultra sonicator (Pci). Micro pipette (Physio care). Membrane filters (Sartorins). UV- Spectro photometer (Shimadzu UV 1800) (Toshvin). Pipettes and volumetric flasks (Borosil). All instruments and glass-wares were calibrated

Materials

API of Paracetamol was obtained from MSN labs and Tramadol hydrochloride was obtained from NEQ Pvt. Ltd. Tablets (ULTRACET) were purchased from Local market. All chemicals and reagents used were of AR grade.

Chromatographic Conditions

The mobile phase consisted of water and acetonitrile. The chromatograph was operated in the isocratic mode starting at a mobile phase of water: acetonitrile (90:10 v/v). Eluent was delivered at a flow rate of 1 mL/min. Absorbance was monitored at 268 nm.

Preparation of Mobile Phase

Mix 90ml water and 10ml acetonitrile and degas in ultrasonic water bath for 15 minutes. Filter through 0.2 µ filter under vacuum filtration before injection.

Standard Solution preparation

Accurately weigh and transfer 20 mg each of paracetamol and tramadol hydrochloride standard drugs into a 10ml clean dry volumetric flask, add about 7ml of methanol and sonicate to dissolve it completely and make up the volume to the mark with methanol. From this stock solution, aliquots were transferred in

to different volumetric flasks and further dilutions like 20, 40, 60, 80, 100 and 120µg/ml were prepared using HPLC mobile phase.

Sample Solution preparation

10 tablets were weighed and finely powdered. The average weight was calculated. A portion of powder equivalent to the weight of one tablet was accurately weighed and transferred to a 100 ml volumetric flask. Add the mobile phase and degas in ultrasonic water bath for 15 minutes. Filter through 0.45µ filter under vacuum filtration into a 100 ml calibrated flask. Final volume was made up with methanol. Aliquots of the tablet solution were made up with the HPLC mobile phase (20-120µg/ml). The method was validated as per ICH guidelines.

Table 1: System Suitability Parameters for Paracetamol and Tramadol hydrochloride

Parameters	Paracetamol	Tramadol	Reference values
Theoretical plates	3442	5499	N > 2000
Capacity factor ($k^{'}$)	2.822	4.722	$1 < k^{'} < 10$
Tailing factor	1.543	1.092	-
Resolution (R_s)	2.944	2.586	$R_s \geq 2$

Table 2: Linearity Results for Paracetamol and Tramadol Hydrochloride

S.No	Concentration (µg/ml)	Peak area af Paracetamol	Peak Area of Tramadol hydrochloride
1	20	7600	2764
2	40	13723	3500
3	60	18696	4411
4	80	24937	5213
5	100	30608	6088
Correlation Coefficient(r^2)		0.998	0.998

Table 3: Accuracy Results for Paracetamol

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
80%	24937	7.95	7.92	99.62%	98.75%
100%	30608	9.98	9.9	99.19%	
120%	36237	11.8	11.5	97.45%	

Table 4: Accuracy Results for Tramadol

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
80%	5213	7.9	7.81	98.86%	98.71%
100%	6088	9.8	9.7	98.97%	
120%	7038	11.8	11.6	98.30%	

Acceptance Criteria: The % Recovery for each level should be between 98.0 to 102.0%.

Table 5: Results of Accuracy for Paracetamol and Tramadol hydrochloride

S.no	Concentration (mcg)	Peak area		Mean percentage recovery (%)	Standard deviation (SD)	Relative standard deviation (%RSD)
		Paracetamol	Tramadol			
1	80%	24937	5213	99.0	130	0.51
2	80%	25183	5271			
				98.6	80	0.49
3	80%	24985	5198			
4	100%	30608	6088	99	155	0.50
5	100%	30826	6102			
				99.2	28	0.45
6	100%	30909	6143			
7	120%	36237	7038	98.8	262	0.72
8	120%	36320	7092			
				98.75	44	0.62
9	120%	35830	7127			

Table 6: Results Of Inter-Day and Intra-Day Precision for Paracetamol and Tramadol Hydrochloride

Injection	Peak area of Paracetamol		Peak area of Tramadol	
	Intra day	Inter day	Intraday	Inter day
Injection-1	24937	24634	5213	5275
Injection-2	24985	24550	5271	5198
Injection-3	25183	24937	5198	523
Average	25035	24707	5227	5228
SD	130	203	38	40
%RSD	0.51	0.82	0.72	0.76

Table 7: Robustness in Flow Rate

S.no	Flow rate (ml/min)	Mobile phase (%v/v)	Detection wavelength (nm)	Remarks
1.	0.8	90:10	268	Peaks separated but not reproducible
2.	1	90:10	268	Good reproducible peaks appear
3.	1	90:10	268	Broad peaks appear

Table 8: Robustness in Mobile Phase

S.no	Mobile phase (%v/v)	Detection wavelength (nm)	Remarks
1.	85:5	268	Peaks separated but not reproducible
2.	90:10	268	Good reproducible Peaks
3.	95:5	268	Only paracetamol peak appears

Table 9: Robustness in Wavelength

S.no	Mobile phase (%v/v)	Detection wavelength (nm)	Remarks
1.	90:10	220	Peaks separated but not reproducible
2.	90:10	268	Good reproducible Peaks

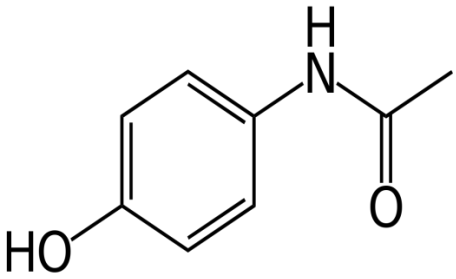

Figure 1: Structure of Paracetamol

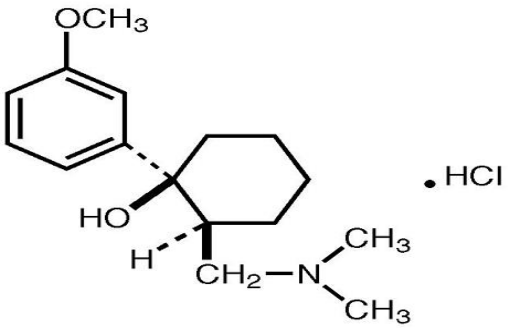

Figure 2: Structure of Tramadol Hydrochloride

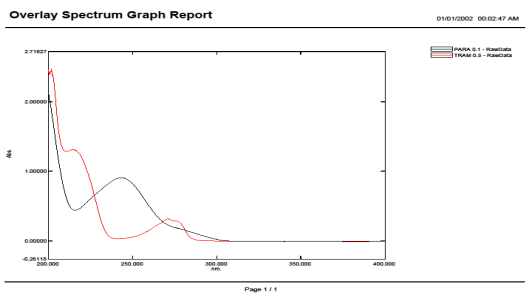

Figure 3: UV Spectrum of Paracetamol and Tramadol Hydrochloride

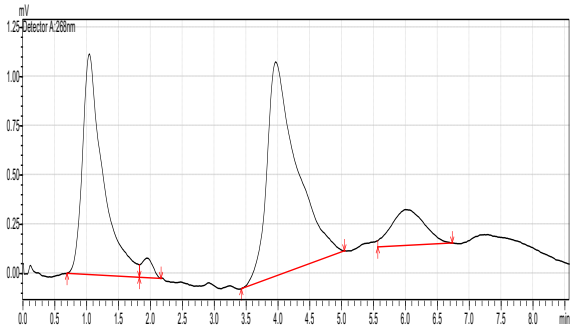

Figure 4: Chromatogram with respective retention times

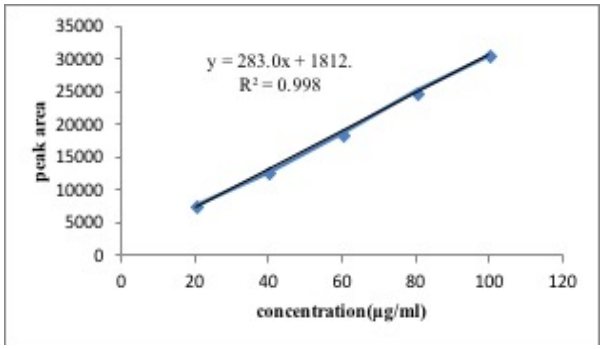

Figure 5: Standard Graph of Paracetamol

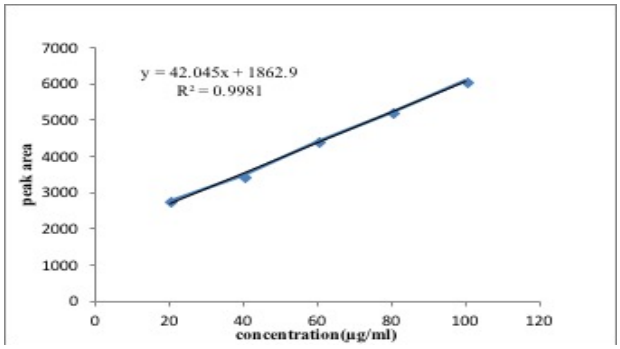

Figure 6: Standard Graph of Tramadol Hydrochloride

RESULTS AND DISCUSSION

Determination of absorption maxima (λmax) and Isobestic Point

The standard solutions of PCM (10µg/ml) and TMD (25µg/ml) drugs were dissolved in the HPLC mobile phase and they were scanned separately in the UV region of 200-400nm and the over lain spectra were recorded and the absorbance maxima was determined in Shimadzu UV spectrophotometer using mobile phase as blank.

The overlay spectrum (Figure 3) reveals that Paracetamol has a λ_{max} of 243nm and Tramadol has a λ_{max} at 271nm. The isobestic points were observed, one at 268nm and the other at 228nm. The isobestic point of 268nm was chosen for the study.

System Suitability Parameters

Standard solutions of 1-6µg/ml of Paracetamol and Tramadol Hydrochloride was prepared from the 1mg/ml standard stock solution and injected six times into the HPLC system. The system suitability parameters were studied from standard chromatograms. The results are presented in Table 1.

Method development and Optimization

After numerous trails with various solvents, the mobile phase containing water and HPLC grade acetonitrile in the proportion of 90:10 v/v respectively was selected to estimate and validate Paracetamol and Tramadol hydrochloride in bulk and Tablet dosage form by RP-HPLC. Maximum resolution for Paracetamol and Tramadol hydrochloride was obtained with this mobile phase at the detection wavelength of 268nm. Mobile phase at a flow rate of 1.0 ml/min produced optimum separation with good resolution. A reverse phase Column C18, (250mmL. x 4.6mm i.d., 5µm) was used as stationary phase. The retention time of Paracetamol and Tramadol hydrochloride was found to be 1.1 and 3.9 minutes respectively (Figure 4).

Method Validation parameters

ICH guidelines were taken as criteria for validating the linearity, accuracy, precision, specificity and robustness, LOD and LOQ values for the developed method.

Linearity

Under the optimized experimental conditions, linear correlation between the peak area and applied concentration was found to be in the concentration range of 20-100 µg/ml, as confirmed by the correlation coefficient of 0.998. Linearity results for Paracetamol and Tramadol Hydrochloride are shown in Table 2.

Accuracy

The accuracy of the method was studied by determining the recovery of Paracetamol and Tramadol hydrochloride at three levels of concentrations. It was performed in three different levels for at 80%, 100%, 120% (Table 3, 4, 5). Samples were analyzed at each level in triplicate. From the results, % recovery was calculated.

Precision

Precision data on the intra-day and inter-day variation for three different concentration levels are summarized in Table 6. Both inter-day and intra-day R.S.D. were less than 2%, indicating a sufficient precision.

Limit of detection and quantification (LOD & LOQ)

The peak area (y) is proportional to the concentration of Paracetamol and Tramadol hydrochloride (x) following the regression equation y=283.0x+1812 (Figure 5) and y=42.04x+1862 (Figure 6). The LOD and LOQ for paracetamol and tramadol hydrochloride were found to be 0.72µg/ml, 1.8µg/ml and 1.1 µg/ml, 3.5µg/ml, respectively.

Specificity

Since bulk and tablet formulations are made of different components and excipients, the specificity was carried out through the comparison of the peak retention time of the formulations with Paracetamol and Tramadol hydrochloride standard drug sample and blank solution. No interference of the excipients was detected since no peak was detected in the same retention time of Paracetamol and Tramadol hydrochloride.

Robustness

For the robustness test, peak area dependence on the percentage of methanol and pH of the mobile phase; percentage of methanol and the flow rate of the mobile phase; and the temperature of the column. Effects of the selected factors were evaluated over a range of conditions by determining the maximum Paracetamol and Tramadol hydrochloride response (quantification). Finally it is concluded that chromatographic behavior was mostly influenced by the mobile phase composition. Among the studied factors, the flow rate had minor influence on Paracetamol and Tramadol hydrochloride peak area and was kept at the value of 1ml/min. The column temperature influences the retention time but has no significant impact on the peak area. It was therefore maintained at ambient temperature throughout the study. Robustness in flow rate, mobile phase and wave length are shown in Table 7, 8, 9.

CONCLUSION

From the above results, it can be concluded that the developed RP-HPLC method represents a good technique for the determination of Paracetamol and Tramadol hydrochloride content in tablet formulation with good sensitivity, precision and reproducibility. The sample preparation involving ultrasonic extraction is very simple and cost effective. Furthermore, the method can be used in determination of Paracetamol and Tramadol hydrochloride in other pharmaceutical preparations and in routine quality control of formulations.

ACKNOWLEDGEMENTS

The authors are highly thankful to the Principal and Management of Vaagdevi College of Pharmacy (affiliated to Kakatiya University), Warangal, Telangana, India, for providing all the necessary facilities to carry out this work.

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Cite this article as:

D.Kumara Swamy *et al*. New RP-HPLC method for the simultaneous estimation of paracetamol and tramadol hydrochloride in bulk and tablet dosage form. Int. Res. J. Pharm. 2018;9(6):82-86 http://dx.doi.org/10.7897/2230-8407.09694

Source of support: Nil, Conflict of interest: None Declared