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Original Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF TAPENTADOL HCL, ACECLOFENAC AND PARACETAMOL IN TERNARY MIXTURE

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ABSTRACT

Objective: To develop a accurate, precise and specific RP-HPLC method for simultaneous estimation of Tapentadol HCl (TAP), Aceclofenac (ACE) and Paracetamol (PCM) in its laboratory synthetic mixture.

Methods: The optimized method uses C8 Thermo quest, hypersil division, 250*4.60 mm; 5 µ column, mobile phase consisting of phosphate buffer (pH 3) and acetonitrile in the ratio of 40:60, flow rate 0.8 ml/min. The detection wavelength was set at 217 nm.

Results: The developed method resulted Paracetamol, Tapentadol HCl and Aceclofenac in eluting at 4.008 min 4.595 min and 6.073 min respectively. Linearity was observed over the concentration range of 52-130 µg/ml for PCM, 16-40 µg/ml for ACE and 12-30 µg/ml for TAP. The percentage recovery was found to be in the range of 98-102% at three different levels of a standard addition. The precision (intra-day, inter-day) of the method was within the limit (RSD<2%).

Conclusion: The proposed method was found to be accurate, precise, reproducible and specific and it can be used for qualitative and quantitative determination of Tapentadol HCl, Aceclofenac and Paracetamol in their combined dosage form in the pharmaceutical industry.

Keywords: Tapentadol HCl, Aceclofenac, Paracetamol, RP-HPLC, Isocratic, Simultaneous estimation.

INTRODUCTION

Paracetamol is N-acetyl-p-aminophenol (fig. 1 a) having analgesic, antipyretic with weak antiinflammatory action [1, 2]. Tapentadol hydrochloride is 3-[(1R, 2R)-3-(dimethyl amino)-1-ethyl-2 methylpropyl] phenol monohydrochloride (fig. 1 b). Agonists of the µ-opioid receptor are being used conventionally for the treatment of moderate to severe pain. Tapentadol hydrochloride (TAP) is centrally-acting oral analgesic [3, 4]. Aceclofenac is 2-[(2, 6-dichlorophenyl) amino] phenylacetoxyacectic acid (fig. 1 c) having potent analgesic and anti-inflammatory properties [5].

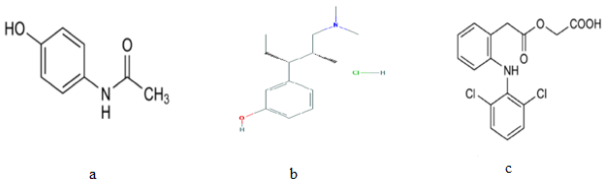

Fig. 1: Stucture of (a) Paracetamol, (b) Tapentadol hydrochloride (c) Aceclofenac

Survey of literature revealed that several methods have been reported for the individual analysis of Tapentadol hydrochloride [6-12], Aceclofenac [13-14] and Paracetamol [15-16] by UV Spectrophotometric and RP-HPLC methods. UV Spectrophotometric method and RP-HPLC methods are available in the literature for simultaneous determination of Aceclofenac and Paracetamol [17-20], Paracetamol and Tapentadol [21, 22]. However, as per our knowledge, no RP-HPLC Method has been reported for simultaneous estimation of Tapentadol hydrochloride, Aceclofenac and Paracetamol till date. The aim of the present work was to develop an easy, economic, accurate and specific HPLC method for simultaneous estimation of Tapentadol hydrochloride, Aceclofenac and Paracetamol in combined pharmaceutical formulation. The developed method was validated as per ICH guide line. [23].

MATERIALS AND METHODS

Chemicals and reagents

Pure drug sample of TAP was gifted by Ami Life Science Pvt. Ltd., Mumbai and reference drug samples of ACE and PCM were gifted by Theopharma Pvt. Ltd, Ahmedabad. The gift samples were used as standard without any purification. Methanol and acetonitrile (HPLC grade) were procured from Spectrochem Pvt Ltd. Mumbai. Double distilled water (Purified HPLC grade water) was obtained by filtering double distilled water through nylon filter paper 0.2 µm pore size and 47 mm diameter (Pall Life Sciences, Mumbai, India). Potassium dihydrogen orthophosphate purified was procured from S D Fine Chem. Ltd, Mumbai. Triethanolamine (HPLC grade) was procured from Spectrochem Pvt. Ltd., Mumbai. Ortho phosphoric acid HPLC grade was procured from Loba Chemie Pvt. Ltd., Mumbai.

Instrumentation

The HPLC was performed on a Shimadzu (Shimadzu Corporation, Kyoto, Japan) chromatography system equipped with Shimadzu LC-20AT pump and SPD-20AV UV detector. Samples were injected through a Rheodyne 7725 injector valve with fixed loop at 20µl. The column C8 Thermo quest, hypersil division (250*4.60 mm, 5µm particle size) was used. Data acquisition and integration were performed using Spin-chrome software.

Preparation of synthetic laboratory mixture

The Combined Dosage Formulation of TAP, ACE and PCM is of Orbit Life Sciences Pvt. Ltd., Mumbai[24] which was not available in the local market, so a synthetic laboratory mixture (laboratory sample) was prepared using the excipients mentioned in the literature[25-27]. Laboratory sample contained Tapentadol HCl (75 mg), Aceclofenac (100 mg), and Paracetamol (325 mg) with excipients microcrystalline cellulose, Lactose monohydrate, Povidone, Magnesium stearate and this laboratory sample was used for further analysis.

Preparation of mobile phase

Accurately weighed 0.27 g of potassium di hydrogen orthophosphate was transferred into a 100 ml volumetric flask and about 90 ml of

water was added. The mixture was sonicated and finally diluted with water up to 100 ml. Then 0.1% v/v Triethylamine was added and pH 3 was adjusted with ortho-phosphoric acid. This buffer was filtered through 0.2 µm filter paper and mixed with acetonitrile in the ratio of 40:60 into a mobile phase bottle. The prepared mixture was sonicated for 5 min and then used as the mobile phase.

Preparation of stock solution

Stock solutions of PCM (1000 µg/ml), ACE (1000 µg/ml) and TAP (1000 µg/ml) were prepared in acetonitrile solvent. Then primary working solutions of all three drugs PCM, ACE, TAP (200µg/ml) were prepared in the mobile phase. From primary working solutions aliquots ranging from 2.6 ml to 6.5 ml for PCM, 0.8 ml to 2 ml for ACE and 0.6 ml to 1.5 ml for TAP were taken in 10 ml volumetric flasks respectively, sufficient amount of mobile phase was added and sonicated, then the volume was made up to 10 ml with mobile phase that gave final concentrations 52, 65, 78, 91, 104, 117, 130 µg/ml of PCM, 16, 20, 24, 28, 32, 36, 40 µg/ml of ACE and 12, 15, 18, 21, 24, 27, 30 µg/ml of TAP as standard solution of mixtures respectively.

Preparation of synthetic laboratory mixture solution

Powder equivalent to 325 mg of PCM, 100 mg of ACE and 75 mg of TAP from a synthetic laboratory mixture was transferred to 100 ml volumetric flask. Sufficient amount of acetonitrile was added and sonicat, the volume was made up to 100 ml with acetonitrile. The Solution was filtered through Whatman filter paper (No. 42) to get a stock sample solution. An aliquot of 10 ml was pipetted out of a stock sample solution and diluted up to 50 ml with the mobile phase. An aliquot of 1 ml was taken from the above solution and diluted up to 10 ml with the mobile phase to obtain 65µg/ml PCM, 20µg/ml ACE and 15µg/ml TAP. This solution was injected in to chromatographic system to obtain chromatogram which was quantified for PCM, ACE and TAP.

Selection of detection wavelength

The Analytical wavelength for the analysis of PCM, ACE and TAP was selected by scanning standard solutions in the range of 200-400 nm. At the selected wavelength 217 nm, three drugs are having appreciable absorbance. Overlay UV spectra of PCM, ACE and TAP are shown in fig. 2.

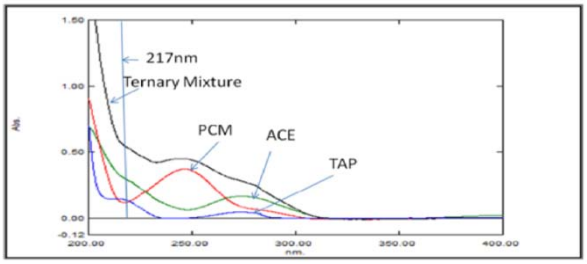

Fig. 2: Selection of wavelength

RESULTS AND DISCUSSION

HPLC method development

HPLC method was optimized for efficient separation and simultaneous quantification of PCM, ACE and TAP. The pKa value reported for PCM, ACE and TAP are 9.38, 4.7 and 9.34 respectively, whereas, log p values for the three drugs are 0.46, 2.17 and 2.87 respectively. Because of this, the chromatographic separation of the three drugs on the basis of polarity or pH of the mobile phase was a challenge. A number of solvents in different ratio over a wide range of pH were tried, but either peak shape was broad or resolution was not good. Repeated trials to obtain good, sharp peak with efficient resolution between two peaks of Paracetamol and Tapentadol done on C18, phenomenex column in isocratic HPLC did not give satisfactory results. The peak shapes were good in some trials but Retention time was not in the acceptable range while gradient elution was tried on C18 column. The run time was high in gradient trial on the C 18 phenomenex column. So, further trials were taken on C8, thermo quest column in isocratic HPLC. The mobile phase consisting of phosphate buffer (pH 3) and acetonitrile in the ratio of 40:60 and C8 Thermo quest, hypersil division, 250*4.60 mm 5 µm columns, flow rate 0.8 ml/min and detection wavelength 217 nm gave the satisfactory results in terms of retention time, resolution, symmetry and sensitivity.

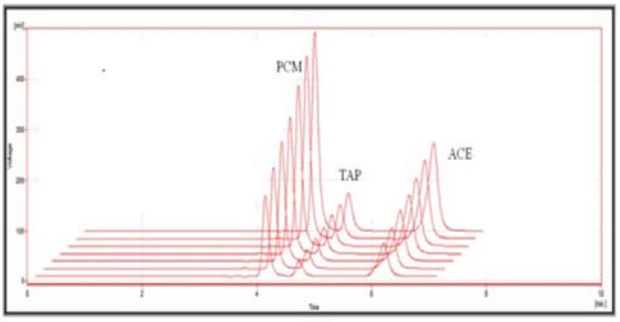

Fig. 3: Chromatogram of ternary mixture PCM, TAP and ACE

Method validation

As per ICH guideline, objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. The developed method was validated as per ICH guideline.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration of analyte in the sample. Minimum six concentrations were analyzed for linearity study. A linear response was obtained in the concentration range of 52-130 µg/ml for PCM, 16-40 µg/ml for ACE and 12-30 µg/ml for TAP. The linearity graphs were plotted using the concentration verses peak area for PCM, ACE and TAP in fig: 4, fig: 5 and fig: 6 respectively.

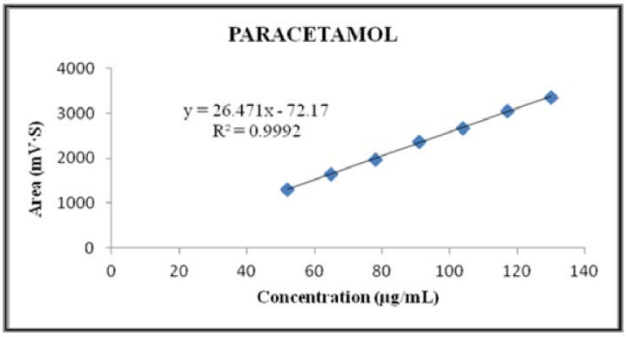

Fig. 4: Calibration curve for PCM in ternary mixture

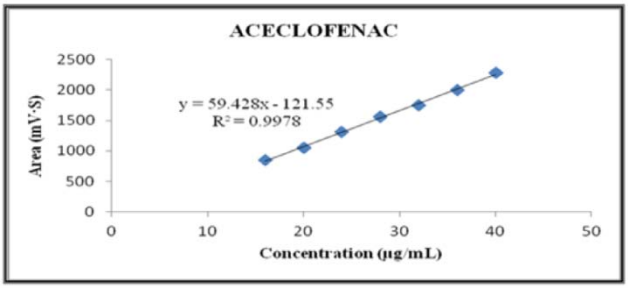

Fig. 5: Calibration curve for ACE in ternary mixture

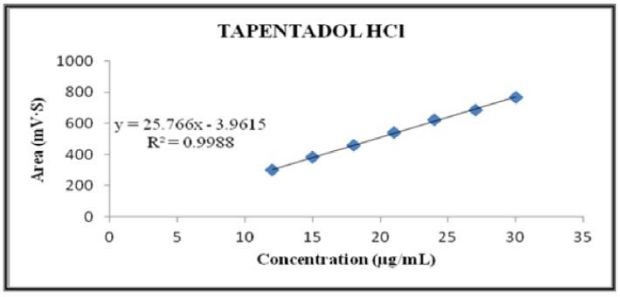

Fig. 6: Calibration curve for TAP in ternary mixture

Accuracy

The accuracy of the method was determined by recovery experiments using the standard addition method. The 50%, 100% and 150% of standard solution were added to previously analyzed solution of a laboratory sample. For the three drugs, the recovery studies were performed in triplicate and percentage recovery were calculated. The results are shown in table 1.

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision of the method was determined as Intra-day and inter-day. The experiments were repeated three times in a day for intra-day and on three different days for inter-day precision and results were reported as percentage RSD. The results are shown in table 2. From the data obtained, the developed RP-HPLC method was found to be precise.

Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from background levels. The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with acceptable accuracy, precision and variability. The LOD and LOQ of Tapentadol HCL, Aceclofenac and Paracetamol were determined by using the signal to noise ratio. The Limit of detection was 3.12µg/ml for PCM, 1.24µg/ml for ACE and 0.91µg/ml for TAP at a signal to noise ratio of 3.1. The limit of quantification was determined as 9.45µg/ml for PCM, 3.76µg/ml for ACE and 2.75µg/ml for TAP at a signal to noise ratio of 10:1.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness of the method was studied by deliberate variation in method parameter like changes in flow rate, mobile phase ratio and pH of the mobile phase. It was concluded that method is enough robust and will provide accurate results in normal quality control labs even if there is some sort of experimental error by human or system. None of the modifications caused a significant change in the resolution between the drugs, peak area % RSD. The results were shown in table 3.

Table 1: Recovery results for TAP, ACE and PCM

% spiked	Amount Added (µg/ml)			Amount Found* (µg/ml)			% Recovery (mean±SD)		
	TAP	ACE	PCM	TAP	ACE	PCM	TAP	ACE	PCM
50 %	3	4	13	2.98	4.04	12.85	99.5±0.93	101.08±0.8	99.13±1.38
100 %	6	8	26	6.05	8.01	25.53	100.84±0.49	100.71±1.44	99.34±1.29
150 %	9	12	39	9.02	12.14	39.42	100.27±0.98	100.66±0.9	99.73±1.89
Avg.							100.2±0.8	100.82±1.05	99.4±1.52

*Average of three determination

Table 2: Validation parameters of HPLC method of Tapentadol HCL, Aceclofenac, Paracetamol

Parameter	Tapentadol HCl	Aceclofenac	Paracetamol
Detection wavelength	217 nm	217 nm	217 nm
Linearity and range	12-30 µg/ml	16-40 µg/ml	52-130 µg/ml
Slope	25.766	59.428	26.471
Intercept	-3.9615	-121.55	-72.17
Correlation coefficient	0.9988	0.9978	0.9992
Precision (% RSD)			
Intraday precision	1.185	0.510	0.533
Interday precision	1.491	0.967	1.166

Table 3: Robustness Results for PCM, TAP and ACE

Factor	Area (mV·S)		
pH	**PCM**	**TAP**	**ACE**
2.9	1722.80	362.73	1184.47
3	1715.64	371.97	1224.97
3.1	1718.84	375.75	1187.48
%RSD	0.20	1.80	1.88
Mobile phase ratio			
62: 38	1712.87	374.43	1204.09
60:40	1715.64	371.97	1224.97
58: 42	1707.07	375.79	1191.55
%RSD	0.25	0.51	1.39
Flow rate			
0.7	1747.82	379.01	1244.25
0.8	1715.64	371.97	1224.97
0.9	1691.99	365.65	1198.22
%RSD	1.63	1.79	1.89

Table 4: System suitability parameters validated RP-HPLC method

Parameter	PCM	TAP	ACE
Retention time (min)±SD	4.008±0.0016	4.595±0.0019	6.073±0.0068
Theoretical plate per meter±SD	5902.7±216.80	5981.4±132.18	5836±69.98
Tailing factor±SD	1.4388±0.030	1.4115±0.026	0.770±0.013
Resolution±SD	-	2.533±0.024	5.917±0.078

Table 5: Assay of synthetic laboratory mixture sample

Synthetic Laboratory Mixture Labelled Claim: PCM: ACE: TAP (325 mg: 100 mg: 75 mg)			
% ASSAY	**PCM***	**ACE***	**TAP***
(mean*±SD)	101.29±0.38	101.30±0.31	100.74±1.32

* Average of three determination

System suitability parameters

System suitability parameters were studied to verify the optimum conditions. The System suitability test was performed as per USP guidelines on the chromatograms. Different parameters were evaluated such as resolution, tailing factor, theoretical plates. The results were shown in table 4.

Assay of synthetic laboratory mixture

The three drug combined dosage formulation is developed by Orbit Life Sciences Pvt Ltd, Mumbai and is shown on their website but not available in local pharmacies. A simulated laboratory mixture was used as the sample and analyzed using the proposed method. For the analysis, three replicates batches were assayed. The mean peak area of each drug was calculated and the drug content in the synthetic laboratory mixture was quantified. The results found were comparable with the corresponding true values and are shown in table 5.

CONCLUSION

The proposed RP-HPLC method is found to be simple, specific, accurate, precise, robust, rapid and economical. This method gives good resolution between all the three compounds with a short analysis time. The proposed RP-HPLC method can be useful for routine analysis of PCM, ACE and TAP in the combined dosage form.

CONFLICT OF INTERESTS

Declared None

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