WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 9, 483-492. Research Article ISSN 2277– 7105

METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF PARACETAMOL, PHENYLEPHRINE HYDROCHLORIDE AND TRIPROLIDINE HYDROCHLORIDE IN BULK AND COMBINED TABLETS DOSAGE FORMS

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Article Received on
27 June 2017,
Revised on 17 July 2017,
Accepted on 07 August 2017
DOI: 10.20959/wjpr20179-9097

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ABSTRACT

A simple, selective, sensitive and precise, simultaneous high performance liquid chromatographic analysis of tablets containing Paracetamol, Phenylephrine Hydrochloride and Triprolidine Hydrochloride was described. Good chromatographic separation was achieved using a Prontosil C18 column (250 x 4.6mm, 5µm) and mobile phase consisting of acetonitrile: 0.1M potassium dihydrogen phosphate buffer (52:48) with 2.5ml of Triethyl Amine, adjusted to pH 3.2 with Orthophosphoric acid, at flow rate 1ml/min. The detector was set at 215nm. The retention time of Paracetamol, Phenylephrine Hydrochloride and Triprolidine Hydrochloride was found to be 2.910 min, 2.190 min and 3.377 min, respectively. The linear ranges for Paracetamol, Phenylephrine Hydrochloride and Triprolidine Hydrochloride were 260-910μg/ml, 8-28µg/mland 2-7µg/ml, respectively. The recoveries of Paracetamol, and Phenylephrine Hydrochloride and Triprolidine Hydrochloride in pharmaceutical preparation were all greater than 98% and their relative standard deviations were not more than 2.0%. The proposed method can be effectively applied for the simultaneous estimation of three drugs in bulk and in combined dosage form.

KEYWORDS: Paracetamol, Phenylephrine Hydrochloride, Triprolidine Hydrochloride, RP-HPLC, Validation.

INTRODUCTION

Combinations of decongestant, antihistaminic, and analgesicpreparations are widely used for cough and cold treatment. Severalmethods have been described for the quantitative determinationof these drugs. High-performance liquid chromatography (HPLC) methods have been investigated by many workers. Most of themwere based on ion-pair formation, and the detection methods were typically based on measuring the UV absorbance of the analytes.[1-5]

Acetaminophen (paracetamol) is analgesic and antipyretic.[6] As pain and fever are common, no home should be without some paracetamol, particularly homes with children. Acetaminophen is available in many different pharmaceutical preparations such as tablets, capsules, and liquid suspensions.[7] designed chemically as {N-(4-Hydroxyphenyl) acetamide

Triprolidine, 2-[(1E)-1-(4-methylphenyl)-3-(pyrrolidin-1-yl)prop-1-en-1-yl] pyridine, Antiallergic, Histamine H1 Antagonist that blocks the action of endogenous histamine, which subsequently leads to temporary relief of the negative symptoms brought on by histamine. It is used for the treatment of Seasonal or perennial allergic rhinitis or non allergic rhinitis, conjctivitis and mild uncomplicated allergic skin manifestations of urticaria and angioedema.[8-9]

Phenylephrine (PHE) is chemically (R) – 3[1-m-hydroxy-2-(methyl amino) methyl] benzyl alcohol hydrochloride used as decongestant. Oral phenylephrine is extensively metabolized by MAO enzyme in the gastrointestinal tract and liver. So compared to rally taken pseudoephedrine it has a reduced and variable bioavailability of only up to 38%. It is a direct selective alpha adrenergic receptor agonist; it does not cause release of endogenous noradrenalin, as pseudoephedrine does. So PHE has low side effects like CNS stimulation, irritability, insomnia, anxiety and restlessness[10-11].A successful attempt is made to estimate the three drugs simultaneously.

Therefore it was thought worthwhile to develop a accurate and rapid RP-HPLC method for simultaneous estimation of Paracetamol, Phenylephrine HCL and Triprolidine HCL from tablet formulations.

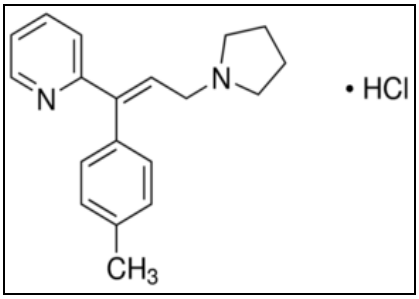

Fig 1: Structure of Triplodine hydrochloride

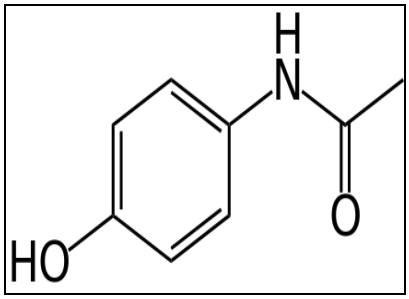

Fig 2: Structure of paracetamol

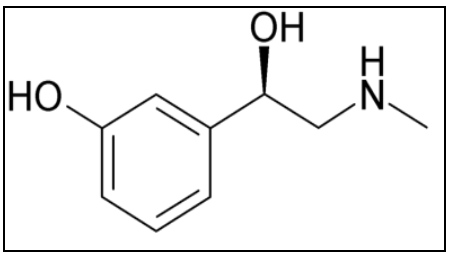

Fig 3: Structure of phenylephrine.

EXPERIMENTAL CONDITIONS

Materials and reagents

Paracetamol (SDFCL), Phenylephrine HCl was obtained from Yarrowchem Pvt Ltd and Triprolidine HCL was obtained as gift sample from Glenmark Pharmaceuticals, Mumbai. A commercial preparation (NOCOLD PLUS TABLET) used for analysis was procured from pharma market. Each tablet contains 325mg of PARA, 10mg of PHE and 2.5mg of TPE, HPLC grade acetonitrile (Thomas Baker) and water, Potassium dihydrogen phosphate (LOBA CHEM), Orthophosphoric Acid, Triethyl Amine (Thomas Baker).

Instrumentation

RP-HPLC was performed using Shimadzu HPLC system consisting of a pump LC-20AD, rheodyne sample injection port with 20 microlitre loop, SPD-20A UV-Detector, Spinchrom software, column used was Prontosil C18(250 x 4.6mm, 5µm), Weighing was done on Contech CA-123 balance and pH was adjusted using PCI analytics Digital pH meter 111.

Chromatographic Conditions

A reverse phase column [Prontosil C18 (250 x 4.6mm, 5µm particle size)], equilibrated with mobile phase consisting of acetonitrile: 0.1M potassium dihydrogen phosphate buffer (52:48) with 2.5ml of Triethylamine, adjusted to pH 3.2 with Orthophosphoric acid was used. Mobile phase flow rate was maintained at 1mL/min and effluents were monitored at 215nm. The

sample was injected using 20 microlitre fixed loop rheodyne injector and run time was 10min.

Preparation of 0.1 M Potassium dihydrogen orthophosphate plus 2.5ml TEA (pH 3.2)

About 13.605g of Potassium dihydrogen orthophosphate was accurately weighed and transferred to 1000ml volumetric flask and dissolved in 900ml of water, 2.5ml Triethyl amine was added to the solution. The pH with o-phosphoric acid to 3.2, volume was made upto 1000ml using mobile phase. The solution was then filtered using 0.45µ membrane filter.

Preparation of Mobile Phase

The pH of (0.1M) Potassium dihydrogen orthophosphate was adjusted to 3.2 with Orthophosphoric acid, and mixed with Acetonitrile in the ratio 48:52 and was sonicated.

Standard Solution preparation

About 100mg of Paracetamol, 100mg of Phenylephrine HCL and 100mg of Triprolidine HCL of each standard drug was weighed accurately and transferred to 100 ml volumetric flask and dissolved in mobile phase with and final volume was made up to the mark with mobile phase. Final concentration of Paracetamol, Phenylephrine HCL and Triprolidine HCL of 520µg/ml, 16µg/ml and 4µg/ml are made by suitable dilutions.

Preparation of Sample solution

10 tablets were weighed and powdered. The quantity of powder equivalent to 325 mg of Paracetamol, 10mg of Phenyephrine Hydrochloride and 2.5mg of Triplodine Hydrochloride were transferred into a 500ml volumetric flask. The volume was made up using the mobile phase, mixed and filtered through 0.45µ PVDF filter. Final concentration of Paracetamol, Phenylephrine HCL and Triprolidine HCL of 520µg/ml, 16µg/ml and 4µg/ml are made by suitable dilutions.

Validation of HPLC method

The proposed RP-HPLC method was validated as per ICH guidelines.

Assay

The amounts of PARA, PHE and TPE per tablet were determined by extrapolating the values of area from the respective calibration curve. Results are reported in **Table 1.**

Selectivity and Specificity

To assess the selectivity of the developed method solutions of all three drugs were injected into the system then observe three sharp peaks of PARA, PHE and TPE were obtained at retention time of 2.910min, 2.190 min and 3.377min respectively in reference to standard solution. Specificity was determined by comparison of the chromatogram of mixed standards and sample solutions. As the retention time of standard drugs and the retention time of the drugs in sample solutions were same, so the method was specific. The parameters like resolution (Rs) and asymmetric factor were calculated. Good correlation was found between the results of mixed standards and sample solutions. Results are shown in the Table 2.

Precision

Precision study was performed to find out intraday and interday variations. The intraday andinterday precision study of PARA, PHE and TPE was carried out by estimating the correspondence response 3 times on the same day and on 3 different days for 3 different concentrations of PARA, PHE and TPE and the results are reported in terms of % relative standard deviation (%RSD) however, all results fall within acceptance limits (RSD < 2), as shown in Table 3.

Linearity

Linearity studies of PARA, PHE and TPE were performed using a standard solution in the range of 260-910µg/ml, 8- 28µg/ml and 2-7 µg/ml respectively. Results are shown in table 4.

Accuracy

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 75%, 100% and 125%. The recovery studies were carried out by adding known amounts of standard PARA, PHE and TPE were added to pre-analyzed samples and they were subjected to proposed HPLC method. The recoveries results of PARA, PHE and TPE in pharmaceutical preparation are shown in the Table 4.

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

The LOD and LOQ were separately determined based on the calibration curves. The limit of detection (LOD) and limit of quantification (LOQ) of developed method were determined by injecting progressively low concentrations of standard solutions using the developed RP-HPLC method. The limit of detection (LOD) and limit of quantification (LOQ) were calculated as 3.3Ə/S and 10Ə/S, were respectively as per ICH guidelines Ə is

standard deviation of response (y–intercept) and S is the slope f calibration plot. Results are shown in the **Table 3**.

Robustness

The robustness study was done by making small changes in the optimized method parameters like ±1% change in mobile phase ratio, column temperature and ±1% change in pH. There was no s significant impact on the retention time and tailing factor.

RESULTS AND DISCUSSIONS

Table 1: Analysis of tablet Formulation.

Brand		% Amount Found
NOCOLD PLUS (Para 325mg + P.E 10mg+ Trip 2.5mg)	**PARA**	98.069%
	PHE	97.216%
	TRIP	99.520%

Table 2: System suitability parameters.

System Suitability Parameters	PARA	PHE	TRIP
Retention time (min)	2.910	2.190	3.377
Resolution	2.215	-	2.466
Theoretical plates	3652	4520	5220
Asymmetric factor	0.576	1.150	1.444

Table 3: Results of precision, LOD & LOQ.

Parameters	PARA	PHE	TRIP
Precision			
Intra-day (n=3)	0.52	0.48	0.53
Inter-day (n=3)	0.23	0.20	0.25
Limit of Detection	5.16	0.42	0.105
Limit of Quantification	15.63	1.28	0.321

Table 4: Linearity studies.

PARAMETERS	PARA	PHE	TRIP
Linearity range	260-910 µg/ml	8- 28 µg/ml	2-7 µg/ml
Slope	13.47	19.25	27.35
Intercept	26.29	1.158	0.701
Correlation coefficient	0.999	0.999	0.999

Table 4: Results of Recovery studies.

Pre-analyzed sample solution[µg/ml]	Sample concentration [µg/ml]	Excess drug added [µg/ml]	Amount recovered [µg/ml]	% Recovery
PARA	260	130	394.74	101.23%
	260	260	519.01	100.19%
	260	390	641.21	101.37%
PHE	8	4	11.95	100.37%
	8	8	16.29	98.16%
	8	12	20.23	98.86%
TRIP	2	1	3.027	99.10%
	2	2	4.000	100.0%
	2	3	5.025	99.50%

CHROMATOGRAMS

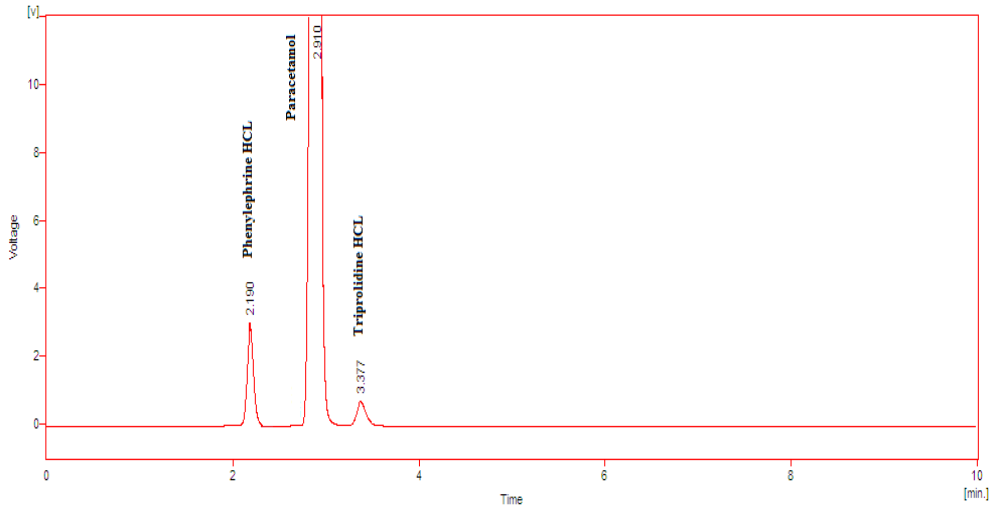

Fig 4: Chromatogram of standard solution.

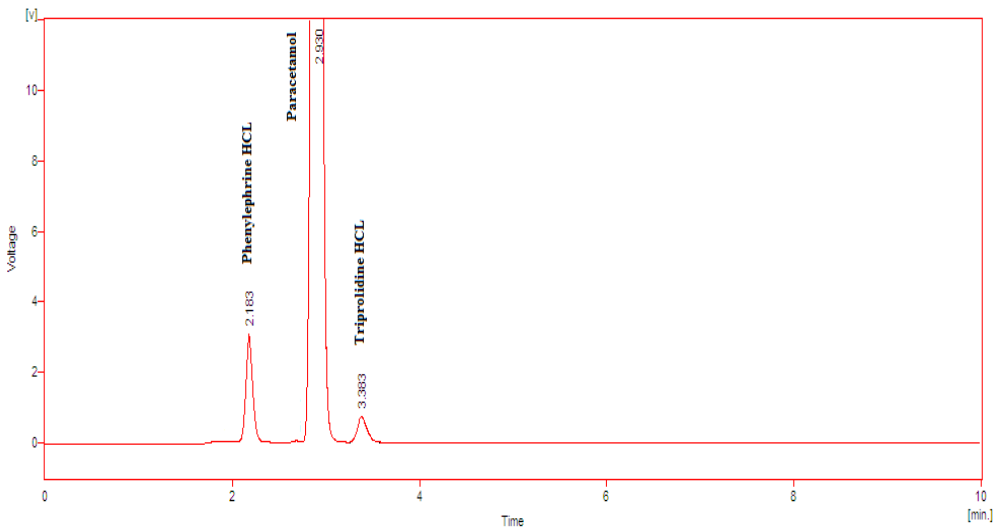

Fig 5: Chromatogram of standard solution.

LINEARITY DATA

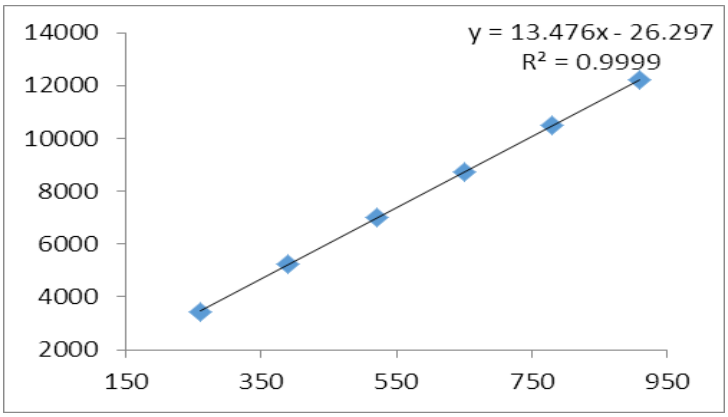

Fig 6: Calibration curve of PARA.

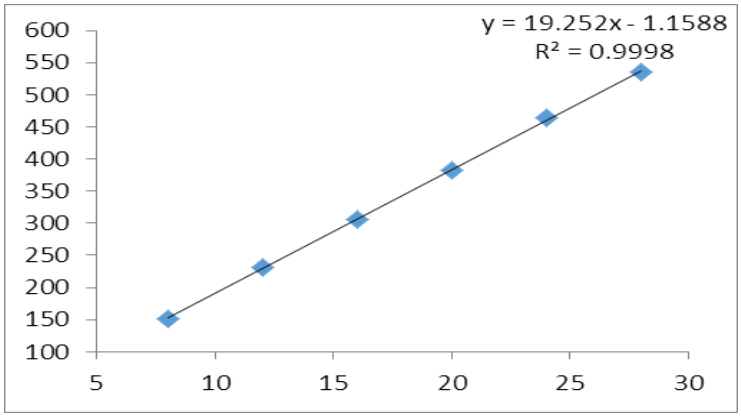

Fig 7: Calibration curve of PHE.

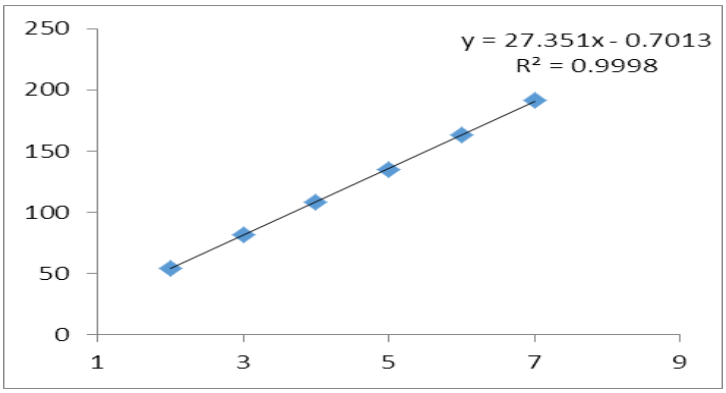

Fig 8: Calibration curve of TRIP.

CONCLUSION

The present paper describes proposed RP-HPLC method for the simultaneous estimation of PARA, PHE and TRIP in tablet dosage form is accurate, precise, linear, rugged, robust, simple and rapid. Acceptable regression values, RSD (%) and standard deviations which make it versatile and valuable for simultaneous estimation of three drugs in bulk and new

tablet formulation. Acceptable values of precision and accuracy have been obtained all levels by this method as per guidelines for assay validation. The results of this developed RP-HPLC method can be could be conveniently adopted for quality control analysis of PARA, PHE and TRIP simultaneously, from tablet dosage form.

ACKNOWLEDGEMENT

The author is acknowledged to Dr.Maraya Campos, Ms. Deepa Shetty & Sitec labs for their immense support & also to Dr.Sachin Ambirwar for providing with the API .

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