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DEVELOPMENT AND VALIDATION OF ANALYTICAL METHODS FOR SIMULTANEOUS ESTIMATION OF PARACETAMOL AND ONDANSETRON IN LUPISETRON-PLUS TABLET DOSAGE FORM

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ABSTRACT

Paracetamol (PCM) is the inhibition of cyclooxygenase (COX) and recent findings suggest that it is highly selective for COX-2 and used for headache, low backache. Ondansetron (OND) is a selective serotonin 5-HT2 receptor antagonist used in nausea, nausea and vomiting due to chemotherapy. A reversed phase high performance liquid chromatographic method was developed and validated for simultaneous estimation of Paracetamol and Ondansetron in tablet dosage form. The chromatographic separation was achieved using BDS hypersil C18 column (250 mm, 4.6 mm i.d., 5 µm) as stationary phase & Phosphate Buffer pH 5.5 : ACN (40:60 V/V). Detection was carried out at 297 nm. The average retention time for PCM and OND were found to be 3.727 and 6.037min. Linearity for PCM and OND were observed in the concentration range from 62.5-187.5 µg/ml (r2 = 0.999) and 0.5-1.5 µg/ml (r2 = 0.988). Accuracy of the method was studied by the recovery studies at three different levels 80%, 100% and 120% level. The recovery was found to be within the limits of acceptance criteria with average recovery of 99.62 – 99.87% for PCM and 100.32 – 100.62% for OND. The high precision of proposed method is confirmed by % RSD below 2.0 for repeatability. The proposed simple, accurate and pricise RP-HPLC method was successfully applied for determination of PCM and OND from Lupisetron-Plus tablet dosage form for routine analysis.

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INTRODUCTION

Paracetamol [24-28]

Paracetamol (PCM), also known as Acetaminophen, is commonly used for its analgesic and antipyretic effects. Its therapeutic effects are similar to salicylate, but it lacks anti- inflammatory, anti-platelet, and gastric ulcerative effects. Structure of Paracetamol shown in figure: 1[1, 2]. It is official in Indian, British and United State Pharmacopeia [3,4,5].

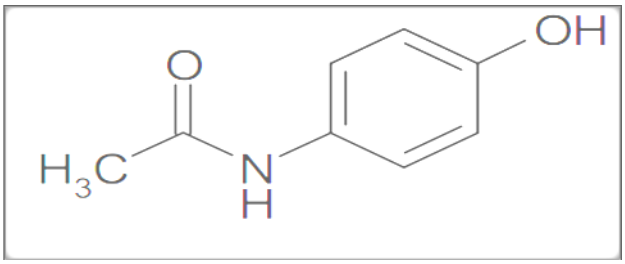

Figure: 1 Structure of Paracetamol

Mechanism of Action:

The mechanism of action of paracetamol is not completely understood. The main mechanism proposed is the inhibition of cyclooxygenase (COX), and recent findings suggest that it is highly selective for COX-2 [1,2].

Ondansetron

Ondansetron (OND) is a White or almost white powder. It is an effective anti-emetic agent, Although the high cost of brand name Ondansetron initially limited its use to controlling postoperative nausea and vomiting and chemotherapy-induced nausea and vomiting. Structure of Ondansetron shown in figure: 2 [6,7,8]. It is official in United State Pharmacopeia [5].

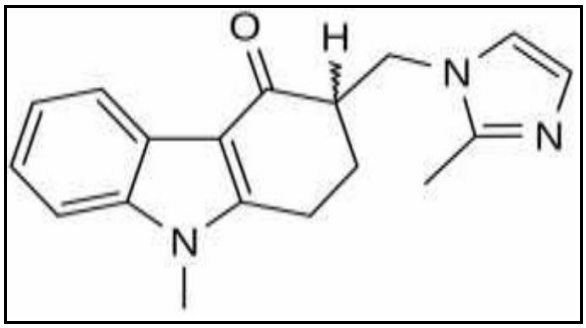

Figure: 2 Structure of Ondansetron

Mechanism of Action:

Ondansetron is a selective serotonin 5-HT3 receptor antagonist. The antiemetic activity of the drug is brought about through the inhibition of 5-HT3 receptors present both centrally (chemoreceptor zone) and peripherally (GI tract) [6,7,8].

Introduction to dosage form[9]

Lupisetron Plus Tablet is used for Nausea and vomiting caused by chemotherapy or radiotherapy, Headache, Toothache, Ear pain, Joint pain, Periods pain and other conditions. Lupisetron Plus Tablet contains Ondansetron, and Paracetamol as active ingredients. Lupisetron Plus Tablet works by works on small intestine and brain that controls vomiting or nausea; increasing the pain threshold and increases the blood flow across the skin, heat loss and sweating [9].

Available marketed formulations:

Brand Name:

Lupisetron- PLUS

Manufacturer:

Lupin pharmaceutical Ltd.

Ratio of Drug:

Paracetamol: Ondansetron (500 mg: 4 mg)

The review of literature revealed that couple of analytical methods including UV-Spectrophotometry and HPLC have been reported for paracetamol and Ondansetron individually or with other combinations. But there is no HPLC validation method was reported for this combination of drugs. So, there is cospicuous regerance of interest to develop simple and cost effective RP-HPLC method.

MATERIALS AND METHODS

Determination of wavelength for maximum absorbance

125 µg/ml solution of Paracetamol and 1 µg/ml solution of Ondansetron were separately prepared in mobile phase. Each solution was scanned between 200-400 nm in Double beam UV-visible spectrophotometer (Shimadzu, model 1800). Wavelength was selected from the overlay spectra of Paracetamol and Ondansetron. Both the components show reasonably good response at 297 nm. (Figure 3)

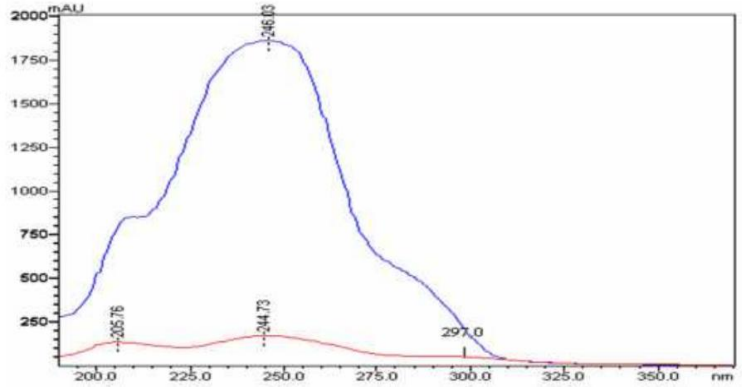

Figure 3: Overlay spectra of Paracetamol (125 µg/ml) and Ondansetron (1 µg/ml) for determination of wavelength for maximum absorbance

Chromatographic conditions

The column used for chromatographic separations was C_{18} (250 mm x 4.6 mm i.d., 5 µm particle size). The analytical wavelength was set at 297 nm and samples 20 µl were injected. The chromatographic separations were accomplished using mobile phase comprised of 0.02 M potassium dihydrogen phosphate Buffer (pH adjusted to 5.0 ± 0.1 using 1% orthophosphoric acid) and Acetonitrile (ACN) in the proportion of 60: 40 (%v/v) filtered through 0.45 µm filter (Millipore) and deaerated in ultrasonic bath. Mobile phase was pumped at a flow rate of 1.0 ml/min at ambient temperature.

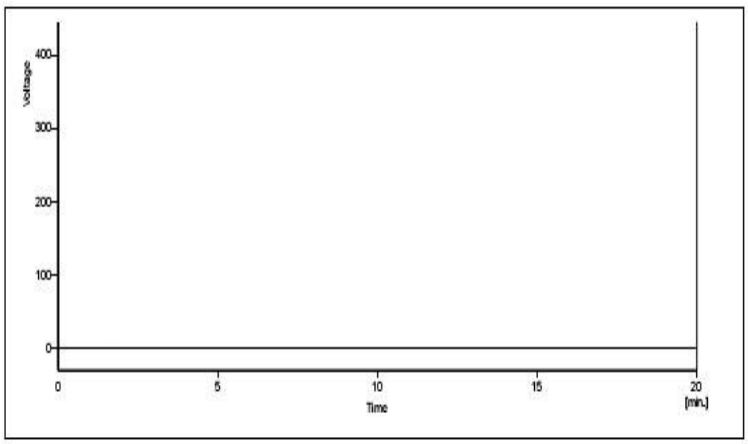

Figure: 4 HPLC blank chromatogram

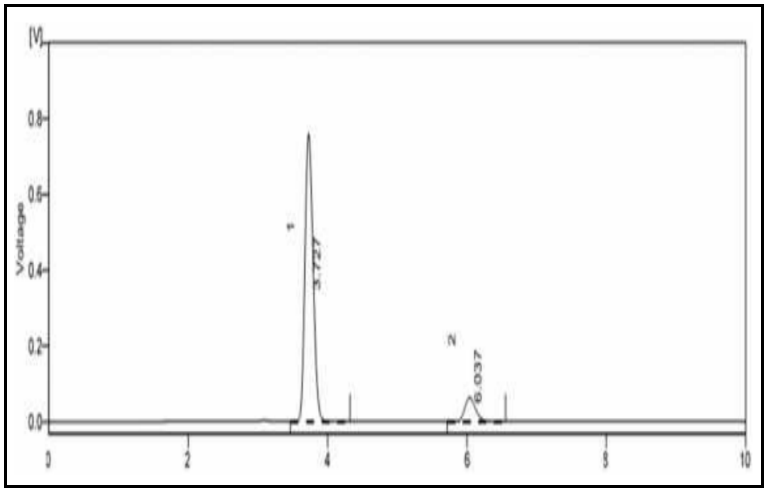

Figure: 5 HPLC chromatogram of Paracetamol (125 µg/ml) and Ondansetron (1 µg/ml)

Preparation of mobile phase

Buffer Solution : 2.72 g of Potassium dihydrogen phosphate was accurately weighed an dissolved in 1000 ml of HPLC water (0.02 M potassium dihydrogen phosphate) then pH 5.0 ± 0.1 was adjusted with 1% Ortho Phosphoric Acid.
Mobile Phase: Phosphate Buffer : ACN (60 : 40 %v/v)

Preparation of stock solution: Paracetamol (125 µg/ml) and Ondansetron (1 µg/ml)

An accurately weighed quantity of standard Paracetamol (125 µg/ml) and Ondansetron (1 µg/ml) were transferred to 100 ml volumetric flasks and volumes were made up to mark with methanol to get Paracetamol (125 µg/ml) and Ondansetron (1 µg/ml).

Preparation of calibration curve

Make the Series dilution with methanol to get final concentration of Paracetamol having concentration range 62.5-187.5 µg/ml (62.5,93.75,125,156.25,187.5 µg/ml) and Ondansetron 0.5-1.5 µg/ml (0.5,0.75,1.0,1.25,1.5 µg/ml). Plot the graph for area vs. time to get calibration curve.

Table 1: System suitability parameters

System Suitability Parameters	Paracetamol	Ondansetron
Retention Time (min)	3.727 ± 0.021	6.037 ± 0.033
Tailing factor	1.33 ± 0.018	1.38± 0.015
Theoretical plate	4328 ± 79.238	7268 ± 102.627
Resolution	9.06 ± 0.061	

Validation of the developed method

To be done by using following parameters as per International Conference on Harmonization (ICH Guidelines Q2R1) [10].

1. Specificity
2. Linearity
3. Range
4. Accuracy
5. Precision
6. Detection limit
7. Quantitation limit
8. Robustness
9. System suitability testing

Specificity:

Specificity of an analytical method is its ability to measure the analyte accurately and specifically in the presence of component that may be expected to be present in the sample matrix. Chromatograms of standard and sample solutions of PCM and OND were compared.

Linearity and range

The linearity of the response for Paracetamol and Ondansetron was determined by preparing standard solutions with concentration range of 62.5-187.5 µg/ml (62.5, 93.75, 125, 156.25, 187.5 µg/ml) Paracetamol and 0.5-1.5 µg/ml (0.5,0.75,1.0,1.25,1.5 µg/ml) Ondansetron. The calibration curves of Paracetamol and Ondansetron shown in Figure and respectively indicate that the response is linear over the concentration range by correlation coefficient (r) value 0.999 for Paracetamol and 0.994 for Ondansetron.

Accuracy (n = 3)

It was carried out to determine the suitability and reliability of the proposed method. Accuracy was determined by calculating the %Recovery of Paracetamol and Ondansetron from the marketed formulation by the standard addition method in which, known amounts of standards powder of PCM and OND at 80%, 100% and 120% levels were added to the preanalysed samples. The recovered amounts of PCM and OND were calculate date ach level and %Recovery was reported.

Precision

It provides an indication of random error in results and was expressed as% Relative standard deviation.

Repeatability

The repeatability was checked by repeatedly (n=6) injecting125µg/ml PCM and 1µg/ml OND, sample and recording the responses.

Intraday Precision

Intraday precision was determined by assay of sample solution three times in a day for three different concentrations (Combined standard samples of concentrations 62.5,125,187.5µg/ml for PCM and 0.5,1.0,1.5µg/ml for OND).

Interday Precision

Interday precision was determined by an assay of sample solution on three different days for three different concentrations (Combined standard samples of concentrations 62.5,125,187.5µg/ml for PCM and 0.5,1.0,1.5 µg/ml for OND).

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

According to the ICH recommendation, the approach based on the standard deviation (SD) of the response and slope was use for the determining the LOD and LOQ values. LOD=3.3 σ/S LOQ=10 σ/S

Where,

σ =Standard deviation of response and

S= Slope of calibration curve.

Assay of pharmaceutical dosage form

- Twenty Tablets were weighed accurately. Powder equivalent to 125 mg of Paracetamol and 1 mg of Ondansetron was weighed and transferred in a 100 ml volumetric flask and mobile phase was added.
- This solution was sonicated for 15 minutes and final volume was made to the mark with mobile phase. The solution was filtered through Whatman filter paper No. 41.
- The filtrate 1 ml was transferred in a 10 ml volumetric flask and diluted to the mark with mobile phase and then, Take 1 ml was transferred in a 10 ml volumetric flask and diluted to the mark with mobile phase to obtain Paracetamol (125 µg/ml) and Ondansetron (1 µg/ml). Concentration was calculated by regression equation method and % Assay was calculated.
- Applicability of proposed method was tested by analyzing tablet formulations. The results are shown in Tables.

RESULTS AND DISCUSSION

Specificity

No interference of peaks were found in the chromatogram indicating that excipients used in the dosage form did not interfere with the estimation of the drugs by the proposed method for the simultaneous estimation of paracetamol and Ondansetron in the combined dosage form, hence the method is specific.

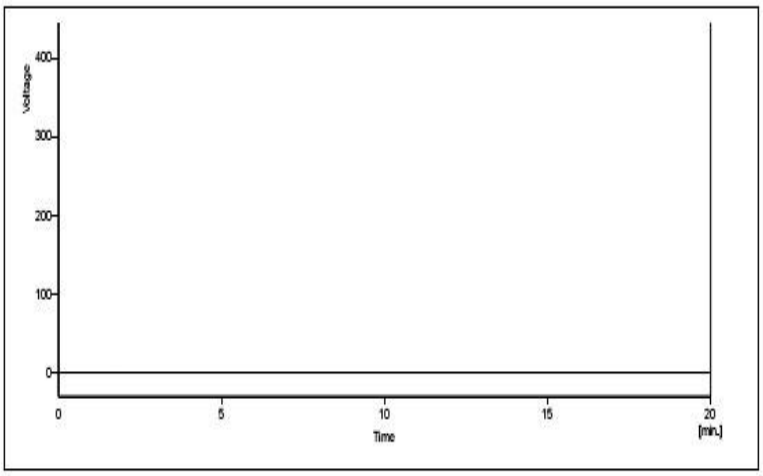

Figure: 6 Chromatogram of mobile phase

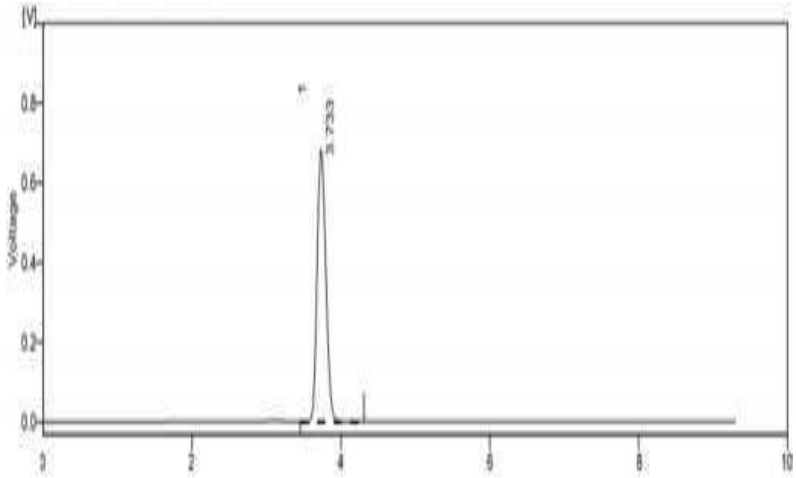

Figure: 7 Chromatogram of standard PCM

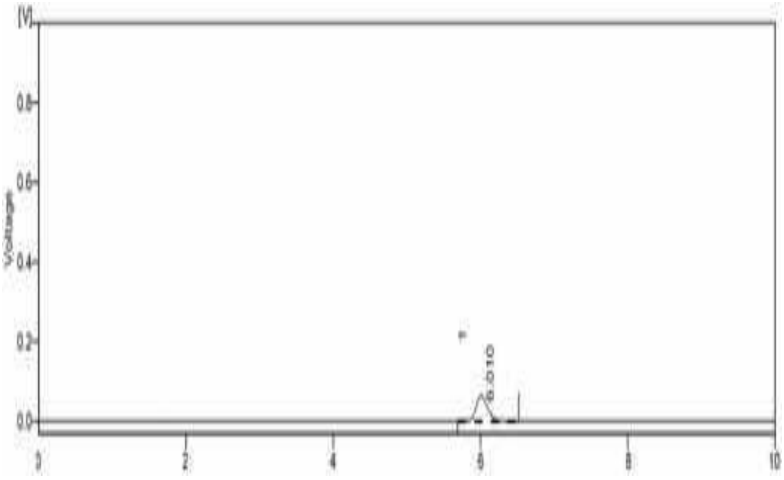

Figure: 8 Chromatogram of standard OND

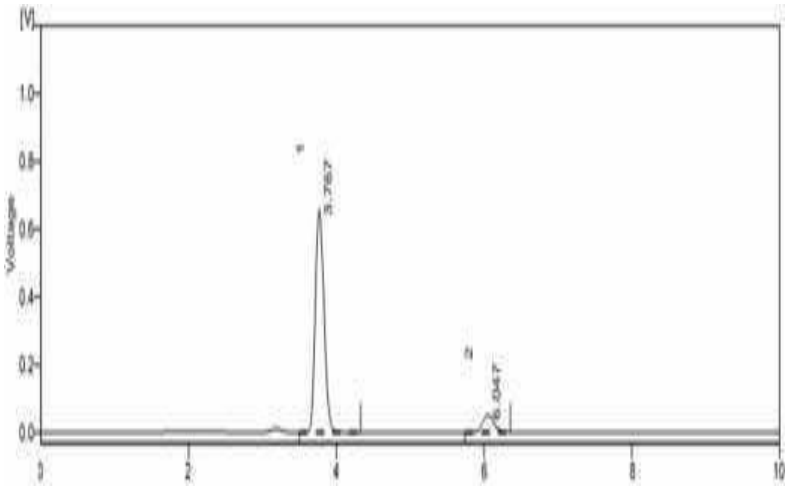

Figure 9: Chromatogram of sample containing PCM (125µ g/ml) and OND (1µ g/ml)

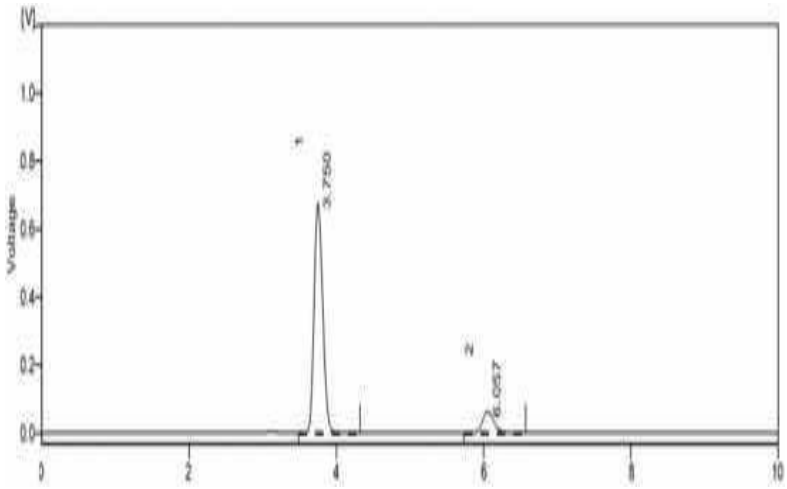

Figure 10: Chromatogram of combined PCM (125µg/ml) and OND (1µg/ml) from standard solution

Linearity and Range

The linearity of the response for Paracetamol and Ondansetron was determined by preparing standard solutions with concentration range of 62.5-187.5 µg/ml Paracetamol and 0.5-1.5 µg/ml Ondansetron. The calibration curves of Paracetamol and Ondansetron shown in Figure and respectively indicate that the response is linear over the concentration range by correlation coefficient (r) value 0.999 for Paracetamol and 0.994 for Ondansetron.

Table 2: Linearity data for PCM

Conc. (µg/ml)	Area
62.5	2727.71
93.75	4022.60
125	5506.61
156.25	6780.15
187.5	8249.43

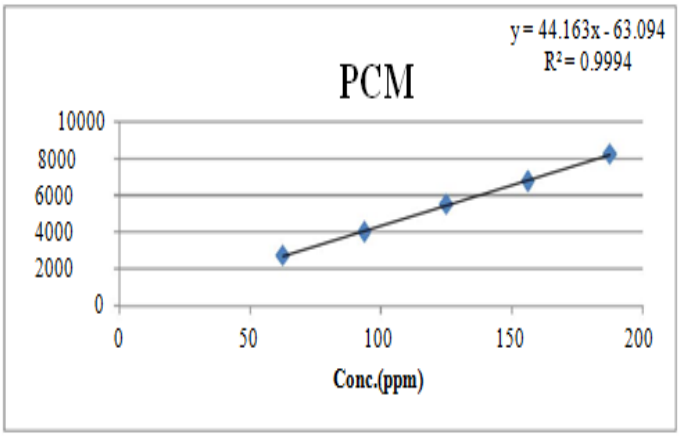

Figure: 11 Calibration curve of Paracetamol (62.5-187.5µg/ml)

Table 3: Linearity data for Ondansetron

Conc. (µg/ml)	Area
0.5	337.08
0.75	498.73
1	682.49
1.25	782.53
1.5	1025.21

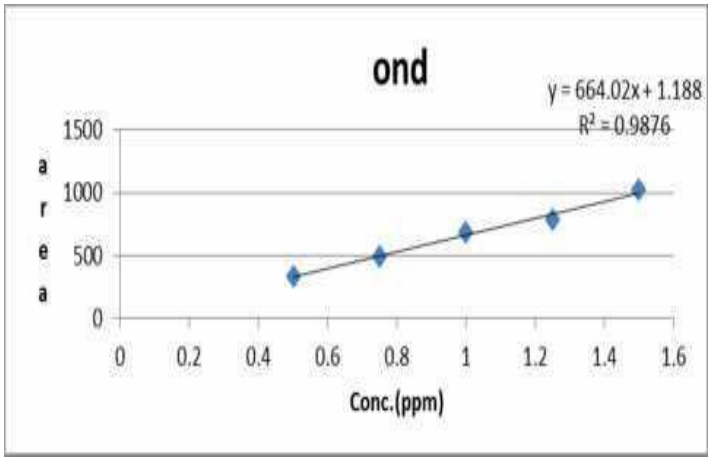

Figure no: 12 Calibration curve of Ondansetron (0.5-1.5µg/ml)

Table 4: Data of regression analysis of PCM and OND

Drug	Straight line equation of Calibration curve	Correlation coefficient
PCM	Y= 44.16x – 63.09	0.999
OND	Y= 664.0x + 1.188	0.988

Accuracy (%Recovery):

Accuracy of the methods was assured, involving analysis of formulation samples to which certain amounts of authentic drugs were added. The resulting mixtures were assayed, and the results obtained for both drugs were compared to those expected. The good recoveries prove the good accuracy of the proposed methods.

Table 5: Determination of Accuracy of PCM And OND (n = 3)

Drug	Amount Taken(µg/ml)	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery ± S.D (n=3)
Paracetamol	62.5	50	49.94	99.87±1.03
	62.5	62.5	62.27	99.62±0.58
	62.5	75	74.73	99.64±0.41
Ondansetron	0.5	0.4	0.401	100.62±1.12
	0.5	0.5	0.501	100.33±0.64
	0.5	0.6	0.602	100.32±0.49

Precision and Repeatability

The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intra-day studies, three repeated injections of standard solutions were made and the response factor of drug peaks and percentage RSD were calculated. In the inter-day variation studies, three injections of standard solutions were made for three consecutive days and response of drug peaks and percentage RSD were calculated. From the data obtained, the developed RP- HPLC method was found to be precise.

Table 6: Precision study of PCM

Drug	Conc. (µg/ml)	Intra-day precision		Inter-day precision	
		Mean ± S.D (n = 3)	% RSD	Mean ± S.D (n = 3)	% RSD
Paracetamol	62.5	2710.88 ±13.36	0.49	2707.48± 6.80	0.25
	125	5462.67 ±19.07	0.35	5462.67± 19.07	0.35
	187.5	8189.71 ±18.26	0.22	8189.71± 18.26	0.22
Ondansetron	0.5	332.58 ± 3.04	0.91	333.96 ± 2.48	0.74
	1	672.72 ± 11.72	1.74	673.49 ± 9.70	1.44
	1.5	1015.91 ± 8.52	0.83	1013.39 ±11.62	1.15

Table 7 : Repeatability study of PCM and OND

Paracetamol			Ondansetron		
Conc. (µg/ml)	Mean ± S.D(n = 6)	% RSD	Conc. (µg/ml)	Mean ± S.D(n = 6)	% RSD
125	5485.16± 29.55	0.54	1	677.72± 9.18	1.35

Assay of formulation

Table 8: Assay result of Lupisetron- Plus

Formulation	Paracetamol			Ondansetron		
Lupisetron-PLUS	Amount Labeled (mg)	Amount Found (mg)	% Amount found SD (n = 3)	Amount Labeled (mg)	Amount Found (mg)	% Amount found SD (n = 3)
	500	486.50	97.30±0.45	4	3.89	97.28±1.16

RESULTS AND DISCUSSION

The objective of the proposed work was to develop and validate novel analytical method for simultaneous estimation of paracetamol and Ondansetron in pharmaceutical formulations according to ICH guidelines. A number of methods appeared in the literature, for estimation of individual drugs or combination with other drugs by UV and HPLC methods. So far there is no specific method for the simultaneous estimation of Paracetamol and Ondansetron. In view of the above fact, a simple RP- HPLC method was planned to develop with high sensitivity, accuracy, precision with costs effective.

Various compositions of mobile phase were used. The best results were obtained with 0.02 M potassium dihydrogen phosphate (pH adjusted to 5.5 ± 0.1 using orthophosphoric acid) Buffer and ACN in the proportion of 40: 60 (v/v) at 1.0 ml/min flow rate. The retention times were 3.727 min for PCM and 6.037 min for OND. The optimum wavelength for detection was set at 297 nm for better detector responses for both drugs. The proposed HPLC method was validated for precision, accuracy studies and the results were within the range thus the method is precise and more accurate. There is no significant changes in the results, thus the method is more robust. The optical regression characteristics and validation parameters are shown in Table 9.

Table 9: Optical Regression characteristics and validation parameters

Parameter	Paracetamol	Ondansetron
Calibration Range	62.5-187.5 µg/ml	0.5-1.5 µg/ml
Regression Equation	y = 44.16x –63.09	y = 664.0x +1.188
Slop (m)	44.16	664.0
Intercept (c)	63.09	1.188
Correlation co-efficient(r^2)	0.999	0.988
Intraday (% RSD, n = 3)	0.22 – 0.49	0.83 – 1.74
Interday (% RSD, n = 3)	0.22 – 0.35	0.74 – 1.44
Detection limit (LOD)	4.452 µg/ml	0.169 µg/ml
Quantification Limit (LOQ)	13.491 µg/ml	0.512 µg/ml

CONCLUSION

The proposed method was found to be simple, precise, accurate, linear, robust and rapid for simultaneous determination of paracetamol and Ondansetron in tablet dosage form labeled Lupisetron-PLUS. The developed method gave good resolution between paracetamol and Ondansetron with short analysis time (10 min). Results are in good agreement with claim which indicates there is no interference of routinely used excipients. The proposed RP-HPLC method was easily and conveniently applied for determination of PCM and OND from combined dosage form for regular monitoring, pharmaceutical manufacturing and research. The percentage of PCM and OND was found to be satisfactory, which is comparable with the corresponding label claim.

Conflict of interest

The author declared that there is no conflict of interest.

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Abbreviations

HPLC : High Performance Liquid Chromatography
TLC : Thin Layer Chromatography
UV : Ultra violet
mm : Millimetre
µm : Micrometre
LOD : Limit of Detection
LOQ : Limit of Quantitation
ICH : International Conference on Harmonisation
mL : Millilitre
µg/mL : Microgram/Millilitre
r^2 : Correlation coefficient
mg : Milligram
kg : Kilogram
% : Percentage
% RSD : Percent relative standard deviation
PCM : Paracetamol
OND : Ondansetron

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