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# RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF PARACETAMOL AND ONDANSETRON IN BULK & ORAL SUSPENSION

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## ABSTRACT
A RP-HPLC method is developed for simultaneous estimation of paracetamol and ondansetron in bulk and oral suspension. The mobile phase used was a combination of potassium dihydrogen orthophosphate buffer: acetonitrile (adjusted to pH 3.0 using orthophosphoric acid) 60:40 v/v. The reversed phase column used was Thermo Hypersil BDS C8 (250 X 4.6 mm i.d., 5µm) at ambient temperature. The detection of the combined dosage form was carried out at 258 nm and a flow rate employed was 1.2 ml/min. The retention time for paracetamol and ondansetron was found to be 3.23min and 4.48min respectively. The method was validated in terms of linearity, range, accuracy, precision, limit of detection (LOD) and limit of quantitation (LOQ). The linearity for paracetamol and ondansetron was found in the concentration range of 31.25- 187.5 µg/ml and 0.5 - 3.0 µg/ml respectively. The percentage recoveries of paracetamol and ondansetron were found to be 100.29 and 100.39 respectively. The percentage relative standard deviation (% RSD) of paracetamol and ondansetron for intraday precision were found to be 0.165 and 0.169 respectively. The LOD and LOQ values for paracetamol and ondansetron were calculated. Thus the proposed method was successfully applied for simultaneous estimation of paracetamol and ondansetron for routine analysis.

***Keywords:*** *Paracetamol; Ondansetron and RP-HPLC.*

## INTRODUCTION
Paracetamol is chemically 4-Hydroxyacetanilide[1]. It functions as a weak inhibitor of the synthesis of ostaglandins (PGs). However, the in vivo effects of paracetamol are similar to those of the selective cyclooxygenase-2 (COX-2) inhibitors (Fig.1). Ondansetron Hydrochloride is chemically (3RS)-9-Methyl-3-[(2-methyl-1*H*-imidazol-l-yl)methyl]-1,2,3,9 - tetrahydro-4*H*-carbazol-4-one hydrochloride dihydrate[2]. It functions as selective 5-$HT_3$ receptor antagonist. It is used in the management of nausea, vomiting induced by cytotoxic chemotherapy and radio therapy and also in post-operative conditions (Fig.2). The review of literature revealed that various analytical methods involving UV-Spectrophotometry[3-7] and HPLC[8-17] have been reported for paracetamol and ondansetron individually or with other combinations. But there is no HPLC method was reported for this combination of drugs. Hence the necessity of developing simple and cost effective RP-HPLC method always a continuing interest.

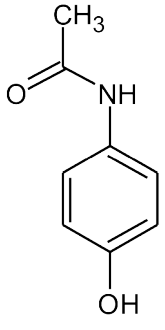


***Fig.1:*** *Structure of Paracetamol*

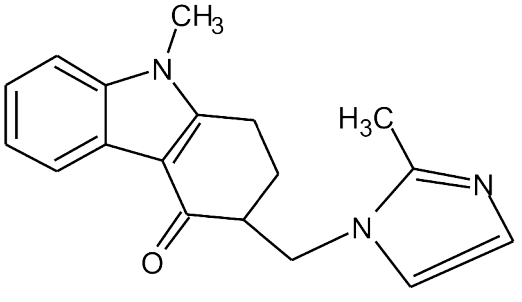


***Fig.2:*** *Structure of Ondansetron*

## MATERIALS AND METHODS
### *Instrumentation*
Waters HPLC System equipped with Empower software-2. Waters HPLC 2695 series consisting four pumps. Auto sampler with five racks, each with twenty four vials holding capacity and temperature control. Auto injector having capacity to inject 5µL to 500µL. UV-Vis Detector with PDA. Thermostat column compartment connected, it has a capacity to maintain 5°C to 60°C column temperature. Thermo Hypersil BDS C8 (250 x 4.6 mm i.d.,5µm) column was used.

### *Experimental conditions*
The HPLC system was operated isocratically at flow rate of 1.2 ml/min at 30°C ±0.5°C for 30 min. The mobile phase found to be most suitable for analysis was potassium dihydrogen orthophosphate buffer: Acetonitrile 60:40%v/v, detection was carried out at 258 nm.

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***Mobile phase***

Solvent-A: Phosphate buffer pH3.0 (pH adjusted to 3.0 with diluted orthophosphoric acid solution) and Solvent-B: Acetonitrile were used as mobile phase which was filtered through a 0.45µ membrane filter.

***Diluent*** **:** Mobile phase.

***Preparation of standard stock solution***

Accurately weighed 125 mg of paracetamol and 2 mg of ondansetron working standards were transferred to separate 100ml standard volumetric flask. Add 50ml of diluent and then the solution was sonicated for 5 min and the volume was made upto the mark with diluent to give a stock solution. Transferred 1ml of standard stock solution into 10ml volumetric flask and diluted to volume with diluents (2mcg/ml of ondansetron).

***Sample preparation***

To determine the content of drugs in pharmaceutical formulation, 5ml of suspension containing an amount equivalent to 125mg of paracetamol and 2mg of ondansetron was transferred into 100ml volumetric flask, about 70ml of diluent was added and sonicated for about 10 min. Then volume was made upto the mark with the diluent and filtered through a 0.45µ membrane filter. Finally different concentrations of tablet sample were prepared by serial dilution technique (1.5 & 2.0 mcg/ml of ondansetron).

## RESULTS

### Method Development and Optimization

Some important parameters like pH of the mobile phase, concentration of the acid or buffer solution, etc., were tested for a good chromatographic separation. Trials showed that an acidic mobile phase with reverse phase C8 column gives symmetric and sharp peaks. Mobile phase composition of potassium dihydrogen orthophosphate buffer: acetonitrile 60:40 (v/v) at a flow rate of 1.2 mL/min showed good resolution. When orthophosphoric acid was used as modifier, resolution between paracetamol and ondansetron was much better at pH 3.0. For the quantitative analytical purposes the wavelength was set at 258 nm. For the quantitative determination of paracetamol and ondansetron in formulations, initially mixed standard solution was injected into the column five times and the retention times of paracetamol and ondansetron were found to be 3.23 min and 4.48 min respectively (Fig.3).

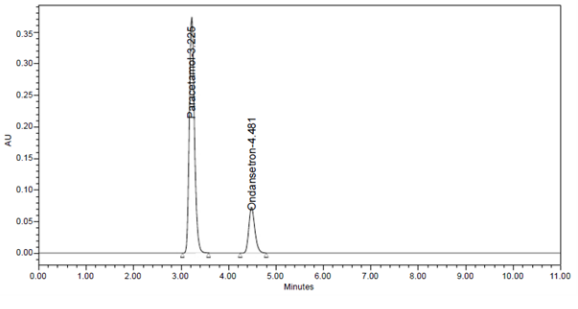


***Fig. 3:*** *Standard chromatogram of Paracetamol and Ondansetron*

### Validation[18]

The described method has been validated for the simultaneous estimation of paracetamol and ondansetron using following parameters.

***Linearity***

To establish linearity of the proposed method, six different sets of drug solution was prepared and analyzed. Standard curves were constructed in the concentration range of 31.25-187.50 µg/ml for paracetamol (Fig.4) and 0.5-3.0 µg/ml for ondansetron (Fig.5).

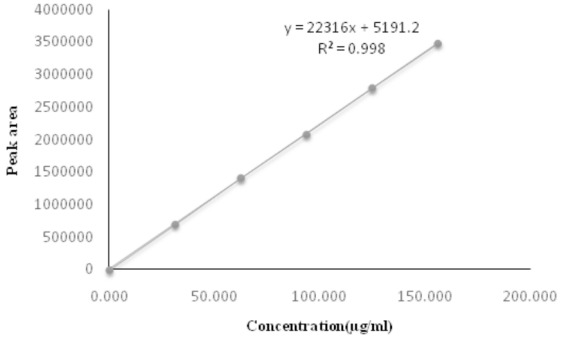


***Fig. 4:*** *Calibration curve of Paracetamol*

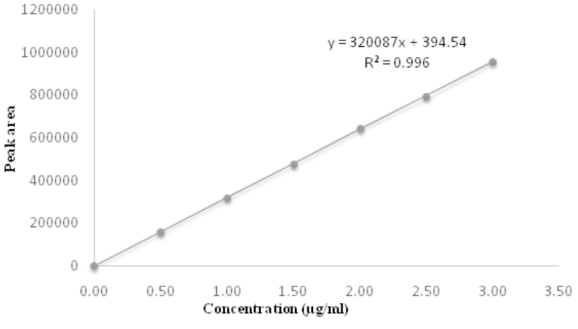


***Fig. 5:*** *Calibration curve of Ondansetron*

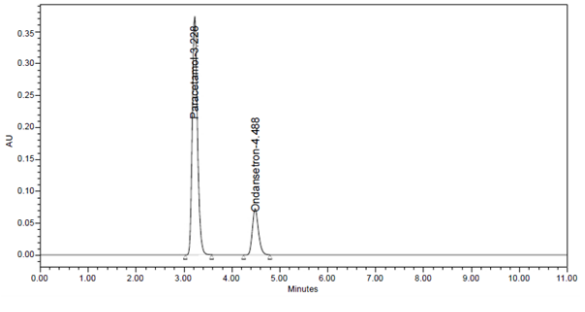


***Fig.6:*** *Sample Chromatogram of Paracetamol and Ondansetron in formulation*

***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.

### *Accuracy*

The accuracy of the method was demonstrated at three different concentration levels (80-120%) by spiking a known quantity of standard drugs into a previously analyzed sample in triplicate. The results of accuracy revealed that the method is more accurate. Results of accuracy are given in table 1.

***Table 1:*** *Recovery study of Paracetamol and Ondansetron*

| Injection | Spike level | Amount present (µg/ml) | Amount recovered (µg/ml) | % recovery | Mean % recovery* | Acceptance criteria |
|---|---|---|---|---|---|---|
| Paracetamol | 80% | 100.00 | 100.47 | 100.47 | 100.29 | 100±2.0 % |
| | 100% | 125.00 | 125.20 | 100.16 | | |
| | 120% | 150.00 | 150.37 | 100.25 | | |
| Ondansetron | 80% | 1.60 | 1.60 | 100.27 | 100.39 | 100±2.0 % |
| | 100% | 2.00 | 2.01 | 100.55 | | |
| | 120% | 2.40 | 2.41 | 100.35 | | |

**Average of three determinations*

### *Precision*

The precision of the method was verified by repeatability and intermediate precision studies, three replicate were injected into the system on two different non-consecutive days, in each case %RSD was calculated. Results of precision are given in table 2, which indicated that the method was precise. Repeatability studies were performed by analysis of three different concentrations 3, 5, 7 µg/mL for paracetamol and 5, 7, 9 µg/mL for ondansetron six times on the same day. Repeating studies on three different days checked the intermediate precision of the method.

***Table 2:*** *Method precision for Paracetamol and ondansetron in combined dosage form*

| Parameter | Intraday Precision | | Interday Precision | |
|---|---|---|---|---|
| | Paracetamol | Ondansetron | Paracetamol | Ondansetron |
| Mean peak area* | 2800715 | 638493 | 2793264 | 637740.667 |
| SD | 4612.7 | 1080.99 | 4948.2405 | 520.3036 |
| %RSD | 0.1650.169 | 0.177 | 0.082 | |

**Average of six determinations*

### *Limit of detection (LOD) and Limit of quantization (LOQ)*

The limit of detection and limit of quantization for paracetamol and ondansetron were calculated from the linearity data using relative standard deviation of the response and slope of the calibration curve. The limit of detection of a compound is defined as the lowest concentration of analyte that can be detected. LOD value of paracetamol and ondansetron were found to be 0.6µg/ml and 0.01µg/ml respectively. The limit of quantization is the lowest concentration of a compound that can be quantified with acceptable precision and accuracy. LOQ value of paracetamol and ondansetron were found to be 2.06 µg/ml and 0.03 µg/ml, respectively.

### *Robustness*

In order to demonstrate the robustness of the method, system suitability parameters were verified by making out intentional method variations like mobile phase, flow changes, pH, mobile phase compositions and column oven temperature variations etc. The method was demonstrated to be robust over an acceptable working range of its HPLC operational parameters.

To ascertain the system suitability for the proposed method a number of statistical values such as theoretical plates, peak symmetry, resolution have been calculated with the observed readings and the results are tabulated in table 3.

***Table 3:*** *System Suitability Parameters*

| S. No. | Parameters | Paracetamol | Ondansetron |
|---|---|---|---|
| 1 | Retention time (min) | 3.226 | 4.485 |
| 2 | Theoretical plates | 4265 | 5880 |
| 3 | Tailing factor | 1.17 | 1.18 |
| 4 | Resolution | - | 5.82 |
| 5 | Range (mcg/ml) | 31.25- 187.5 | 0.5- 3.0 |
| 6 | Slope | 22316 | 320087 |
| 7 | Intercept | 5191.2 | 394.54 |
| 8 | Correlation coefficient | 0.999 | 0.999 |
| 9 | LOD(mcg/ml) | 0.6 | 0.01 |
| 10 | LOQ(mcg/ml) | 2.06 | 0.03 |

## 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 few 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 more economical.

A rapid and sensitive HPLC method was developed for the analysis of paracetamol and ondansetron in bulk drug and its Pharmaceutical dosage forms using thermo hypersil BDS C-8 column. Mobile phase and flow rate selection was based on peak parameters (height, capacity, theoretical plates), runtime and resolution.

The system with 0.01 M potassium dihydrogen orthophosphate and acetonitrile (60:40) v/v (pH 3 adjusted with ortho phosphoric acid) and 1.2 ml/min flow rate was quite robust. The optimum wavelength for detection was 258 nm at which the better detector response for the drugs was obtained. The run time was set for 10 min and the retention time for paracetamol and ondansetron was found to be 3.23 min and 4.48 min shown in Fig. 3. The amount of drugs presented in the marketed formulation was found to be 124.98 mg of paracetmol and 2.01 mg of ondansetron. The resulting chromatogram is shown in Fig 6.

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.

The LOD and LOQ were performed and percentage assay were calculated. The Robustness of the method was studied by testing influence of small changes in flow rate (±0.2 $mLmin^{-1}$), change in column oven temperature (±5$^0$C), and change in mobile phase composition with ±0.2 %. There is no significant changes in the results, thus the method is more robust. Summary of validation results are shown in Table 3.

## CONCLUSION

The proposed method was found to be simple, precise, accurate, linear, robust and rapid for simultaneous determination of paracetamol and ondansetron in bulk and its pharmaceutical dosage form. The developed method gave good resolution between paracetamol and ondansetron with short analysis time (10 min). Hence, the method can be easily and conveniently adopted for routine analysis of paracetamol and ondansetron in combined dosage forms.

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