# RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF CINITAPRIDE AND OMEPRAZOLE IN SOLID ORAL DOSAGE FORM

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## ABSTRACT

The present study involves the development of a simple, specific, accurate, rapid and cost effective RP-HPLC method assisted with UV detection for the estimation of Cinitapride (CNP) and Omeprazole (OME) in solid oral dosage forms. The method utilized $C_{18}$ column (250x4.6 i.d 5µ particle size) and a mobile phase consisting of 40:20:40 ratio of acetonitrile: methanol: phosphate buffer (pH7). CNP was detected at 262 and OME at 301nm with a retention time of 6.5 and 3.2 minutes respectively. The chromatographic condition and polarity of the mobile phase were optimized. The drugs showed good linearity CNP at 84-132µg/ml and OME at 176-242µg/ml with a correlation coefficient of 1. The recovery percentage was found to be 99-100%. Thus the proposed method is simple, fast, accurate and could be applied in the routine analysis of CNP and OME in pure and pharmaceutical dosage forms.

Keywords: Cinitapride, Omeprazole, RP-HPLC.

## INTRODUCTION

Cinitapride[1,2] (CNP) is a substituted benzamide gastroenteric prokinetic agent acting via complex, but synergistic effects on serotonergic 5-HT2 (inhibition) and 5-HT4 (stimulation) receptor and dopaminergic D2 (inhibition) receptors in the neuronal synapses of the myenteric plexi; it is used as an anti-ulcerative drug. Omeprazole[3] is a gastric proton pump inhibitor. Cinitapride is chemically 4-amino –N-[1-(3-cyclohexen-1-ylmethyl)-4-piperidinyl] -2-ethoxy-5-nitrobenzamide. Omeprazole[3] is chemically 5-methoxy-2-[[(4-methoxy-3,5dimethyl) sulfinyl] benzimidazole. RP-HPLC and UV spectrophotometric methods have been reported for CNP.[4,5,6] Similarly, HPLC-UV[7] in plasma, spectrophotometric[8] and first order derivative[9] methods have been reported for OME individually. Extensive literature survey reveals that no HPLC determination of the drugs in combination has been reported so far. Therefore an effort has been made to develop a reverse phase high performance liquid chromatography for the estimation of the drugs and also to validate the developed method. Thus the aim of the study is to develop a simple specific accurate rapid and economic RP-HPLC method for the estimation of the drugs in pharmaceutical dosage forms.

## Structures[10]
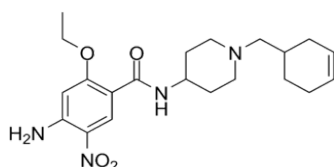


Cinitapride (Mol wt: 402.49)
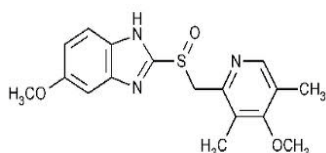


Omeprazole (Mol wt: 345.42)

## Instrumentation

A Shimadzu Prominence LC-20AT with SPD-20A detector was used for HPLC analysis. High performance liquid chromatographic method was developed using *Phenomenex C 18 Gemini (5 µ) 250 x 4.60 mm i.d* (internal dia) column. The instrument uses UV detector, and wavelength of 262 and 301 nm was selected to detect CNP and OME respectively. The chromatograph makes use of rheodyne injector and an injecting volume of 20 µl was employed.

## MATERIALS

All the reagents used for preparation of mobile phase were of HPLC grade. HPLC grade methanol, water, acetonitrile were acquired from Merck India ltd. The mobile phase after preparation was filtered through 0.2µ membrane filter.

### Preparation of mobile phase

The developed method employs a mobile phase consisting of acetonitrile, methanol and phosphate buffer (pH7) in the ratio of 40:20:40. Phosphate buffer was made from 0.2M potassium dihydrogen orthophosphate adjusted to pH7 with triethylamine. The mobile phase was filtered through 0.2µ membrane filter.

## METHODS

### Preparation of cinitapride standard solutions

Accurately weighed 30 mg of standard CNP in 25 ml volumetric flask, dissolved in methanol and made up to volume in methanol to get a concentration of 0.6mg/ml of cinitapride. The solution was filtered through 0.2µ membrane filter. Aliquots of the standard solutions were diluted with the mobile phase to get a concentration range of 84µg/ml to132µg/ml. The dilutions were injected one by one in the 20µl loop and the eluate was detected at 262nm and the chromatogram was recorded.

### Preparation of omeprazole standard solutions

Accurately weighed 110mg of standard OME in 50 ml volumetric flask, dissolved in mobile phase and made up to volume with the mobile phase to get a concentration of 2.2mg/ml of OME. The solution was filtered through 0.2µ membrane filter. Aliquots of the standard solutions were diluted with the mobile phase to get a concentration range of 176µg/ml to 242µg/ml. The dilutions were injected in the 20µl loop separately; the eluate was detected at 301nm and the chromatogram was recorded.

### Assay of formulation

The capsule dosage form contains cinitapride as tablet and omeprazole as granules.

### Cinitapride

Twenty tablets of CNP from the formulation were weighed accurately. Tablet powder equivalent to 6mg of cinitapride was then accurately weighed, dissolved in methanol with the aid of

ultrasonication for 15minutes and made up to volume (10ml) with the methanol. The solution was then filtered through 0.2μ membrane filter and injected into the column. The eluate was detected at 262nm and the chromatogram was recorded.

**Omeprazole**

OME granules in twenty capsules were weighed accurately and crushed to fine powder and powder equivalent to 110mg of OME was accurately weighed. It was then dissolved in mobile phase with the aid of ultrasonication for 15minutes and made up to volume (50ml) with mobile phase. The solution was then filtered through 0.2μ membrane filter and injected into the column. The eluate was detected at 301nm and the chromatogram was recorded.

**Validation of the proposed method[11]**

Selectivity of the method was assessed on the basis of elution of CNP and OME using above mentioned chromatographic conditions and mobile phase. The developed method was validated according to *ICH Guidelines*. The specificity, linearity, accuracy, limit of detection, limit of quantitation and system suitability parameters for the proposed method has been validated.

**Linearity studies[12]**

The chromatogram of standard drugs reveals the retention time (RT) of CNP and OME as 6.5 and 3.2 minutes (fig 1&2) under the above said chromatographic conditions and mobile phase.
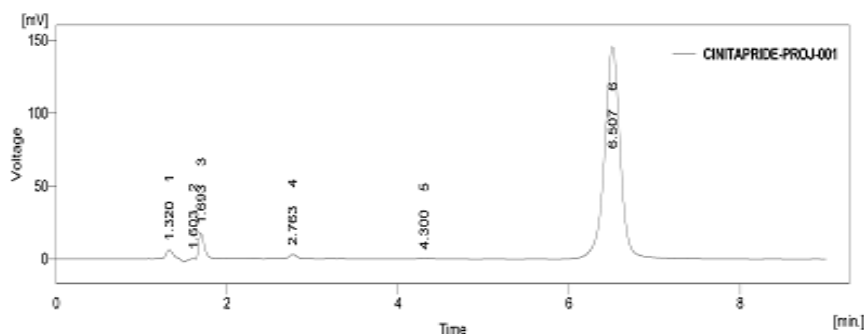


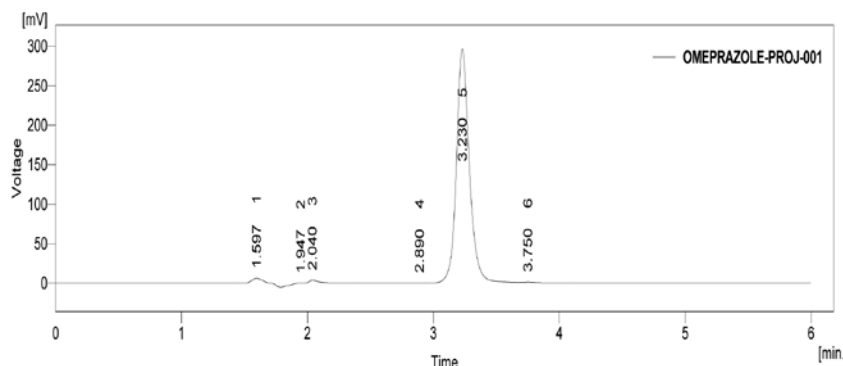**Fig. 1: Chromatogram of CNP– showing Rt = 6.5 minutes**



**Fig. 2: Chromatogram of OME– showing Rt = 3.2 minutes**

The peak areas obtained in the chromatogram of the standard dilutions at the said RT were plotted against concentration for both CNP and OME. A five point linearity chart was plotted for both the drugs. It was analyzed by regression analysis. CNP showed good linearity at 84-132 μg/ml and OME showed good linearity at 176-242 μg/ml (fig 3&4).
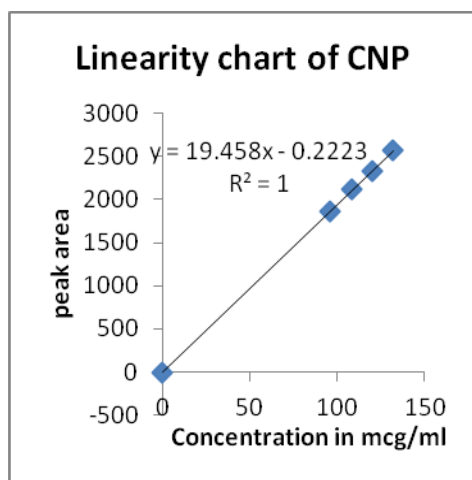


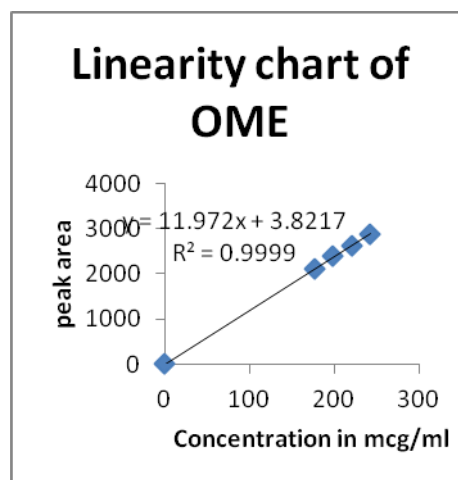**Fig. 3: Calibration chart of CNP**



**Fig. 4: Calibration chart of OME**

The correlation coefficient was calculated to be 1 and 0.999 for CNP and OME respectively which is within the limit (Table 1).

**Table 1: System suitability parameters**

| S. No. | Parameter | Cinitapride | Omeprazole |
|---|---|---|---|
| 1. | Theoretical Plates | 6497 | 5080 |
| 2. | Tailing factor | 0.93 | 1.27 |
| 3. | Capacity factor | 0 | 0 |
| 4. | Temperature of the column | ambient | ambient |
| 5. | Linearity (µg/ml) | 84-132 | 176-242 |
| 6. | Correlation coefficient ($r^2$) | 1 | 1 |
| 7. | Limit of Detection (LOD) | 1.5 | 3.3 |
| 8. | Limit of Quantitation (LOQ) | 5.3 | 11.1 |

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

The LOD and LOQ for CNP and OME were predicted based on the parameters of standard error of estimate and slope, calculated from linearity of the response data of CNP and OME.

### Accuracy[13]

The accuracy of the developed method was determined by performing recovery studies. A known amount of the standard drugs were added to the respective samples and the chromatogram was recorded for the same. The recovery studies were performed on spiked samples and injected in duplicate.

### Specificity[14]

The specificity test of the proposed method demonstrated that the excipients from dosage form do not interfere in the drug peak. Furthermore, well shaped peaks indicate the specificity of the method. Better resolution was found for the drug peak with no interference proved that the method was found to be selective to the drug.

### RESULTS AND DISCUSSION

RP-HPLC method was developed for the estimation of CNP and OME in bulk and oral dosage form. The chromatogram for CNP and OME obtained by the proposed method showed the retention time to be 6.5 and 3.2 respectively. The drugs presented a good linearity at the range of 84µg/ml to132 µg/ml and 176µg/ml to 242µg/ml for CNP and OME respectively which assures that the proposed chromatographic conditions and the mobile phase were suitable for the estimation. The chromatographic conditions were optimized using mobile phase of varied polarity. Finally a mobile phase consisting of acetonitrile, methanol and phosphate buffer (pH- 7) in the ratio of 40:20:40 gave good linearity of response and the shape of chromatogram was also perfect. The regression equation of the drugs' concentration over peak area was calculated. The system suitability parameters are presented in table 1. The number of theoretical plates for CNP and OME was found to be 6497 and 5080 respectively which confirms the good efficiency of the column for the drugs and nature of mobile phase. The low value of LOD and LOQ indicates the sensitivity of the method. The percentage purity of the CNP and OME by the proposed method was appreciable and presented in table 2.

**Table 2: Assay and recovery of CNP and OME**

| Drug | Assay* | | %Recovery ± SD* |
|---|---|---|---|
| | %Amount Present ± SD | %RSD | |
| CNP | 99.72 ± 0.7856 | 0.7878 | 99.44±0.4545 |
| OME | 100.55 ± 0.2151 | 0.2131 | 98.98±0.1414 |

*mean of three determinations

The good, acceptable percentage recovery of the drugs (table 2) ascertains the accuracy of the developed method. This confirms that the results of estimation by the proposed method are not affected by the excipients present in the dosage forms. The developed method was validated with respect to linearity, specificity, accuracy and system suitability and was inferred that the proposed method is perfect.

### CONCLUSION

A new analytical method has been developed. The results indicate that the proposed RP-HPLC method is specific, accurate, simple, cheap and less time consuming which could be applied for the routine analysis of CNP and OME in formulation.

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