*Research Article*

# DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF PHENYLEPHRINE AND KETOROLAC IN PHARMACEUTICAL DOSAGE FORMS

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**ABSTRACT**
A simple, precise and accurate reverse phase high performance liquid chromatographic method has been developed for the simultaneous determination of Phenylephrine in combination with Ketorolac using Kromasil C18 column (150mm x 4.60mm, 5μ) PDA(2996) with UV detector at 220 nm . The mobile phase consisting of buffer (pH 6.5), acetonitrile and methanol 30:60:10% v/v and at a flow rate of 1.0 mL/min. The method was linear over the concentration range of 20-120μg/ml and 6-36μg/ml for Phenylephrine and Ketorolac respectively. The % Mean recovery for Phenylephrine and Ketorolac are 100.18 and 100.09 respectively. The method was validated as per ICH guidelines and was successfully employed for the routine quantitative analysis of Phenylephrine and Ketorolac in combined Pharmaceutical dosage form.

**Keywords:** Phenylephrine, Ketorolac, RP-HPLC, Validation.

## INTRODUCTION
Phenylephrine (Fig. 1), chemically 3-[1-hydroxy-2-(methylamino) ethyl]phenol hydrochloride, is a selective $\alpha_1$-adrenergic receptor agonist used primarily as a decongestant, as an agent to dilate the pupil, and to increase blood pressure. Phenylephrine is marketed as a substitute for the decongestant Pseudoephedrine, though clinical studies differ regarding its effectiveness in this role.
Ketorolac (Fig. 2), chemically 5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid, is a non-steroidal anti-inflammatory drug (NSAID) in the family of heterocyclic acetic acid derivatives, used as an analgesic. Ketorolac acts by inhibiting the bodily synthesis of prostaglandins. Ketorolac in its oral (tablet or capsule) and intramuscular (injected) preparations is a racemic mixture of both (S)-(−)-Ketorolac, the active isomer, and (R)-(+)-Ketorolac.
Literature survey revealed few analytical methods[1-23] are reported for both the drugs in alone and in combination with other drugs. However, so far no HPLC method was reported for the simultaneous estimation of Phenylephrine and Ketorolac in Pharmaceutical dosage forms. The aim of the present study was to develop a simple, precise, reliable, sensitive and selective RP-HPLC method for the simultaneous determination of Phenylephrine and Ketorolac in combined dosage form.

## EXPERIMENTAL
### Chemicals and reagents
The pharmaceutical grade pure samples of Phenylephrine and Ketorolac were received as gift samples from Spectrum pharma research solutions, Hyderabad. Commercial formulations (Omidria injection) were purchased from the local pharmacy. HPLC grade acetonitrile, HPLC grade methanol and all other chemicals were obtained from Merck chemical division, Mumbai. HPLC grade water obtained from Milli-Q water purification system was used throughout the study.

## Apparatus and chromatographic conditions

Chromatography was performed with Alliance Waters 2695 HPLC provided with high speed auto sampler, column oven, degasser and & 2996 PDA detector to provide a compact and with class Empower-2 software. The chromatographic separation was carried out under the isocratic conditions. The mobile phase consists of buffer (pH 6.5), acetonitrile and methanol 30:60:10% v/v were allowed to flow through the Kromasil C18 column (150x4.6mm, 5$\mu$m) column at a flow rate of 1.0 ml/min for a period of 10 min at $30^0$C column temperature. Detection of the component was carried out at a wavelength of 220 nm. The retention time of the components were found to be 2.3 and 6.4 min for Phenylephrine and Ketorolac respectively.

## Preparation of standard and sample solutions

### Preparation of Standard Stock Solution

Standard stock solution was prepared by dissolving 80 mg of Phenylephrine and 24 mg of Ketorolac in a clean and dry 100 ml volumetric flask containing 60ml of diluent (water : methanol = 1:1), sonicated for 5 minutes, and volume was made up to 100 ml with diluent to get stock solution with a concentration of 0.8mg/ml and 0.24mg/ml of Phenylephrine and Ketorolac respectively.

### Preparation of Sample Solution

Twenty Omidria injections were poured into a clean dry beaker and mixed well. From the above solution, 1 ml was taken in to a 10 ml volumetric flask and made up to the volume with diluent. From the above solution, 0.8ml was pipetted out and transferred into another 10ml volumetric flask and made up to the volume with diluent and labeled as sample working solution with concentration of 80 µg/ml for Phenylephrine and 24 µg/ml for Ketorolac respectively.

## RESULTS AND DISCUSSION

Analytical method validation is a process that demonstrates the suitability of the proposed procedures for the intended purpose. More specifically, it is a matter of establishing documented evidence providing a high degree of assurance with respect to the consistency of the method and results to evaluate the product against defined specifications. The validation parameters viz., specificity, accuracy, precision, linearity, limit of detection, limit of quantitation, robustness, system suitability have to be evaluated as per the ICH[24]

guidelines for all analytical methods developed by HPLC.

## System suitability

This is an integral part of development of a chromatographic method to verify that the resolution and reproducibility of the system are adequate enough for the analysis to be performed. It is based on the concept that the equipment, electronics, analytical operations and samples constituting an integral system could be evaluated as a whole. Parameters such as plate number (N), asymmetry or tailing factors (As) and retention time (RT) (Table 1). These parameters were determined during the analysis of a sample. System suitability parameters were determined and compared with the recommended limits (Fig. 3).

## Specificity

Specificity is the ability of the method to measure the analyte response in presence of impurities. The specificity of the developed HPLC method was performed by injecting blank solution and standard solution separately. The chromatogram of drug was compared with the blank chromatogram, to verify the blank interference. No peak was observed at the retention time of Phenylephrine and Ketorolac. Hence the method is specific for the determination of Phenylephrine and Ketorolac.

## Linearity

The linearity for HPLC method was determined at six concentration levels ranging from 20 - 120 µg/mL for Phenylephrine and 6 - 36 µg/mL for Ketorolac. The calibration curve was constructed by plotting response factor against respective concentration of Phenylephrine and Ketorolac. The plots of peak area Vs respective concentration of Phenylephrine and Ketorolac were found to be linear in the range of 20 - 120 µg/mL and 6 - 36 µg/mL with coefficient of correlation ($r^2$) 0.999 and 0.999 for Phenylephrine and Ketorolac respectively (Table 2). The linearity of this method was evaluated by linear regression analysis. The slope and intercept calculated for Phenylephrine and Ketorolac were given in Fig. 4 and Fig. 5.

## Accuracy

Accuracy indicates the deviation between the mean value found and the true value. The accuracy of the method was determined by standard addition method by means of recovery experiments. A known amount of standard drug was added to the fixed amount of pre-analyzed tablet solution. Percent

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recovery was calculated by comparing the area before and after the addition of the standard drug. The standard addition method was performed at 50%, 100% and 150% level. The solutions were analyzed in triplicate at each level as per the proposed method. The percent recovery and %RSD at each level was calculated and results were presented in Table 3. Satisfactory recoveries ranging from 99.27 to 101.24 for Phenylephrine and 99.00 to 101.33 for Ketorolac respectively were obtained by the proposed method. This indicates that the proposed method was accurate (Fig. 6, 7 & 8).

### Sensitivity
The limit of detection (LOD) was determined as lowest concentration giving response and limit of quantification (LOQ) was determined as the lowest concentration analyzed with accuracy of the proposed RP-HPLC method. The limit of detection (LOD) and limit of quantification (LOQ) were found to 0.13µg/ml and 0.38µg/ml for Phenylephrine and 0.03µg/ml and 0.08µg/ml for Ketorolac. The LOD and LOQ showed that the method is sensitive for Phenylephrine and Ketorolac.

### Precision
The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision was determined as repeatability and intermediate precision, in accordance with ICH guidelines.

### Repeatability
Six replicates injections in same concentration of Phenylephrine and Ketorolac were analyzed in the same day for repeatability and the % RSD for Phenylephrine and Ketorolac found to be 1.1 and 0.7 respectively and % RSD for Phenylephrine and Ketorolac found to be within acceptable limit of ≤2 and hence, method is reproducible.

### Intermediate Precision
Six replicates injections in same concentration were analyzed on two different days with different analyst and column for verifying the variation in the precision and the % RSD for Phenylephrine and Ketorolac is found to be 0.9 and 1.6 respectively and it is within acceptable limit of ≤2. Hence, the method is reproducible on different days with different analyst and column. This indicates that the method is precise.

### 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. To evaluate the robustness of the developed HPLC method, few chromatographic conditions were deliberately altered. The robustness was established by changing the flow rate, column temperature and composition of the mobile phase within allowable limits from actual chromatographic conditions. It was observed that there were no marked change in mean $R_t$ and RSD is within limit of ≤2. The tailing factor, resolution factor and number of theoretical plates were found to be acceptable limits for both Phenylephrine and Ketorolac. Hence, the method is reliable with variations in the analytical conditions.

### Stability
In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24 hr at room temperature. The results show that for both solutions, the retention time and peak area of Phenylephrine and Ketorolac remained almost similar (% R.S.D. less than 2.0) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 24 hr, which was sufficient to complete the whole analytical process.

### Assay
The percentage assay of labeled claim of Phenylephrine and Ketorolac present in the Omidria injection (Phenylephrine and Ketorolac: 1/0.3 % v/v) were 99.76±1.14 % and 99.63±0.66% respectively. RSD values for both Phenylephrine and Ketorolac are within limit of ≤2 and the results were shown in Fig. 9 and Table 4.

### CONCLUSION
A simple, specific, sensitive, rapid, accurate and precise RP-HPLC method has been developed for simultaneous estimation of Phenylephrine and Ketorolac. The result of the study follows the protocol of ICH guidelines and it can be successfully applied for the simultaneous estimation of the marketed products of Phenylephrine and Ketorolac and in combined tablet formulations.
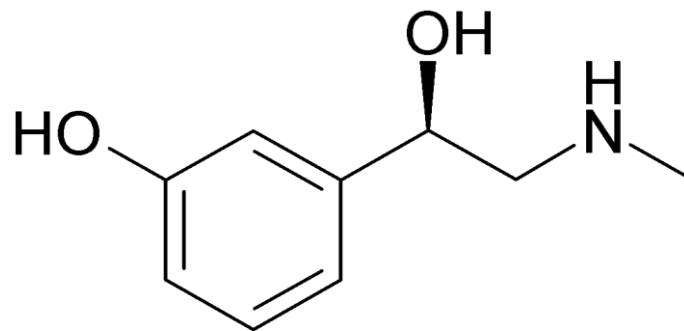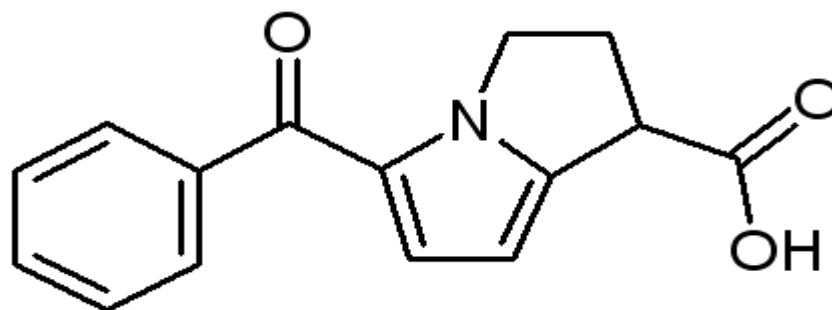
**Fig. 1: Chemical structure of Phenylephrine**
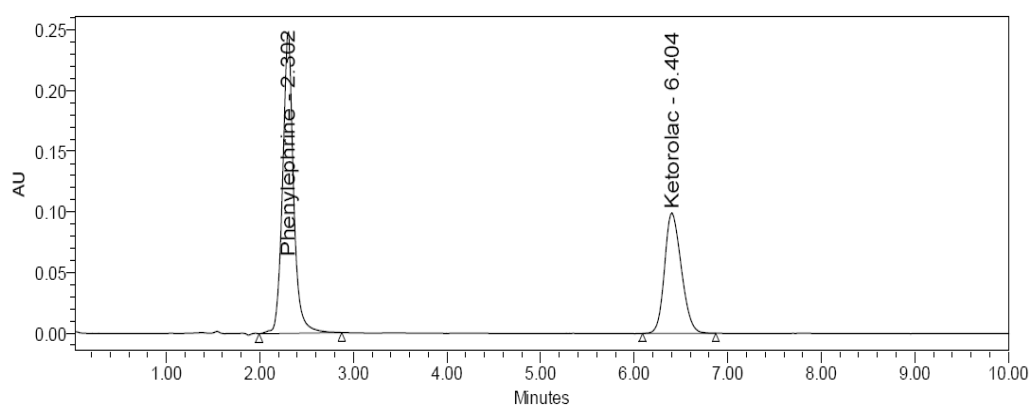


**Fig. 2: Chemical structure of Ketorolac**



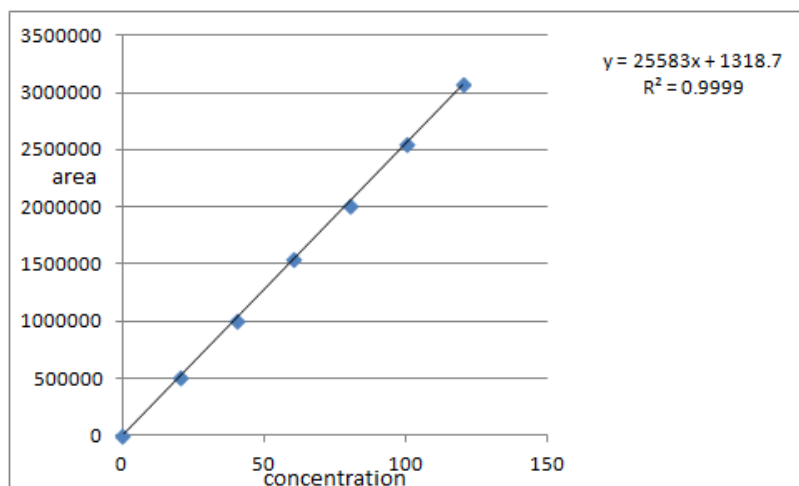**Fig. 3: Typical chromatogram of Phenylephrine and Ketorolac**

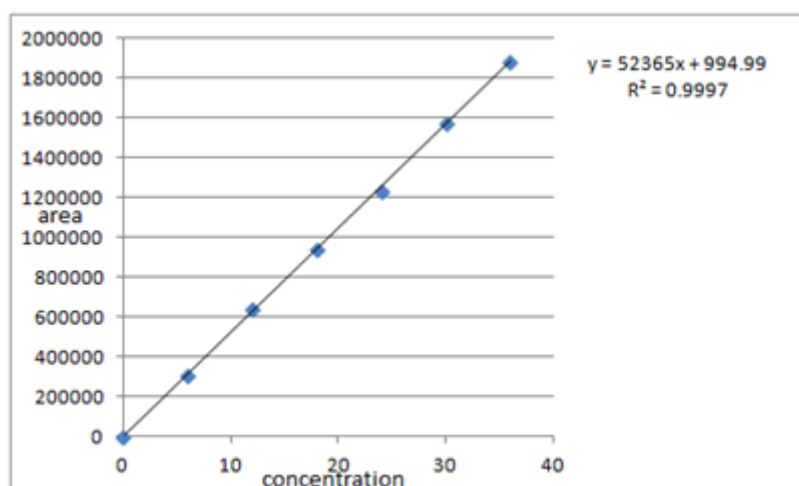**Fig. 4: Calibration curve for Phenylephrine**


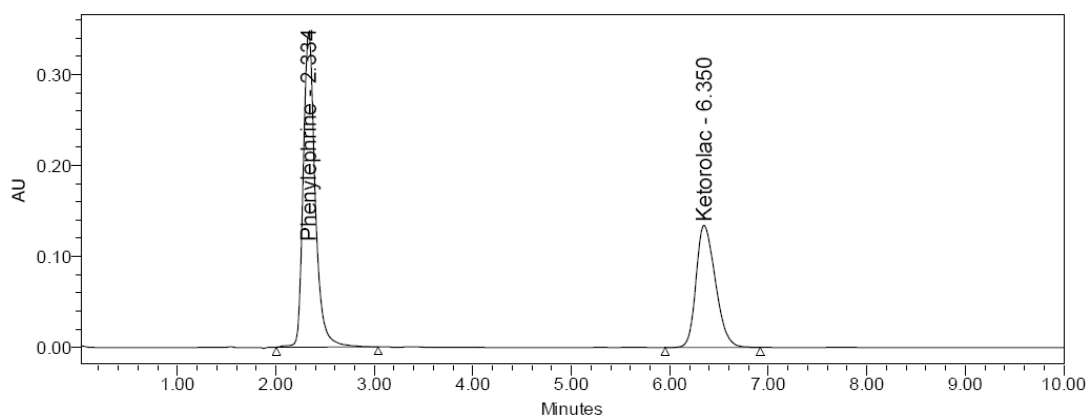**Fig. 5: Calibration curve for Ketorolac**


**Fig. 6: Accuracy 50% chromatogram of Phenylephrine and Ketorolac**
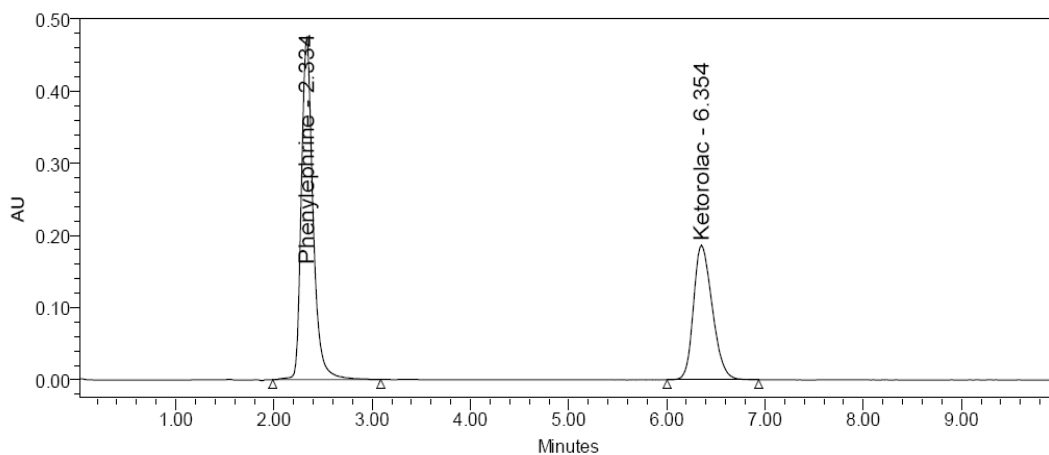
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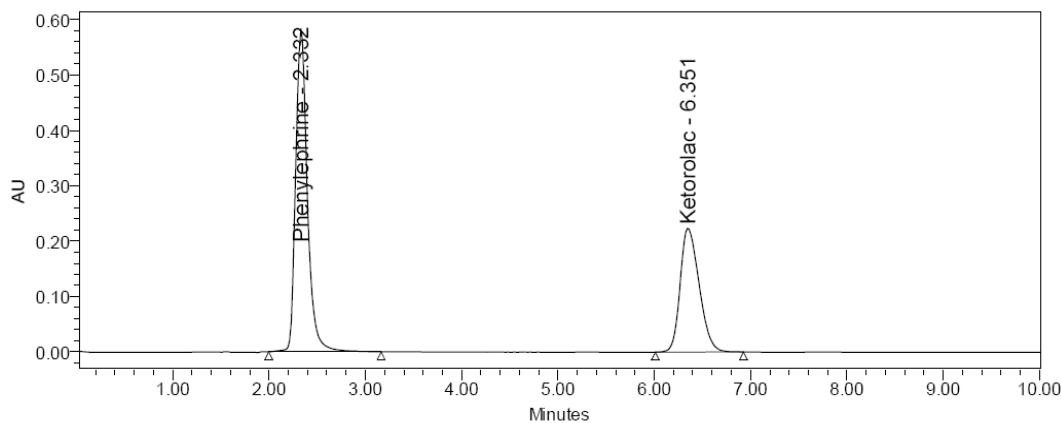**Fig. 7: Accuracy 100% chromatogram of Phenylephrine and Ketorolac**



**Fig. 8: Accuracy 150% chromatogram of Phenylephrine and Ketorolac**



**Fig. 9: Assay chromatogram of Phenylephrine and Ketorolac**
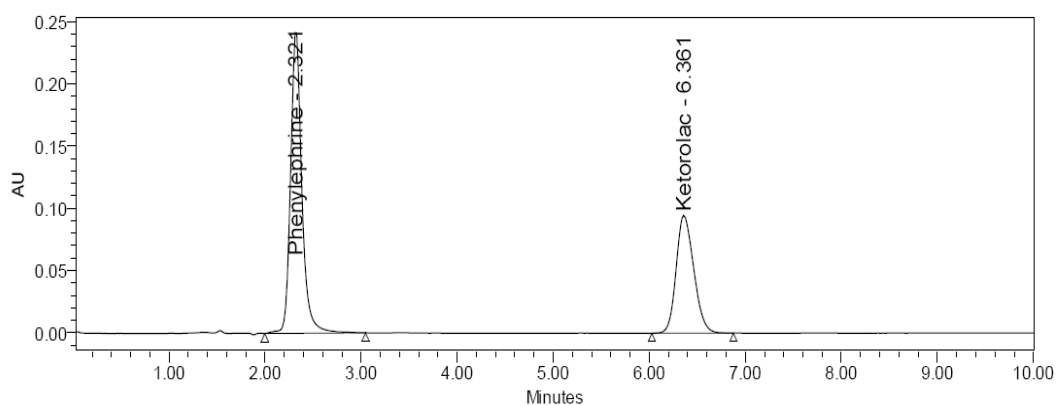
**Table 1: System suitability Parameters**

| Parameter | Phenylephrine | Ketorolac |
|---|---|---|
| Theoretical Plates | 2138 | 5996 |
| Tailing Factor | 1.20 | 1.21 |
| Retention Time (min) | 2.3 | 6.4 |

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**Table 2: Linearity data of Phenylephrine
and Ketorolac**

| Phenylephrine | | Ketorolac | |
|---|---|---|---|
| Conc (µg/ml) | Peak area Average(n=3) | Conc. (µg/ml) | Peak area Average(n=3) |
| 20 | 513997 | 6 | 312130 |
| 40 | 1019057 | 12 | 644838 |
| 60 | 1559788 | 18 | 941903 |
| 80 | 2027374 | 24 | 1235785 |
| 100 | 2555246 | 30 | 1580179 |
| 120 | 3078496 | 36 | 1890068 |

**Table 3: Results of Recovery Experiments of
Phenylephrine and Ketorolac**

| Preanalysed amount (µg/ml) | | Spiked Amount (µg/ml) | | % Recovered | |
|---|---|---|---|---|---|
| Phenylephrine | Ketorolac | Phenylephrine | Ketorolac | Phenylephrine | Ketorolac |
| 80 | 24 | 40 | 12 | 99.27 | 100.11 |
| 80 | 24 | 40 | 12 | 100.32 | 99.00 |
| 80 | 24 | 40 | 12 | 100.65 | 100.59 |
| 80 | 24 | 80 | 24 | 100.60 | 99.38 |
| 80 | 24 | 80 | 24 | 101.24 | 100.19 |
| 80 | 24 | 80 | 24 | 99.71 | 99.54 |
| 80 | 24 | 120 | 36 | 99.82 | 100.67 |
| 80 | 24 | 120 | 36 | 100.37 | 101.33 |
| 80 | 24 | 120 | 36 | 99.66 | 100.03 |
| | | | MEAN | 100.18 | 100.09 |
| | | | SD | 0.61 | 0.72 |
| | | | %RSD | 0.6 | 0.7 |

**Table 4: Assay of pharmaceutical dosage form**

| S. No. | Drug Name | Amount injected (µg/mL) | Amount found (µg/mL) | % Assay ± SD* |
|---|---|---|---|---|
| 1 | Phenylephrine | 80 | 79.81 | 99.76±1.14 |
| 2 | Ketorolac | 24 | 23.91 | 99.63±0.66 |

* n=6 for each parameter
[Brand Name: OMIDRIA [TM] Injection; Label claim: Phenylepherine and Ketorolac 1% / 0.3%].

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