RESEARCH ARTICLE

A validated normal phase HPLC method for simultaneous determination of drotaverine hydrochloride and omeprazole in pharmaceutical formulation

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A simple, precise, specific and accurate normal phase HPLC method has been developed for the simultaneous determination of drotaverine hydrochloride and omeprazole in tablet dosage form. The chromatographic separation was achieved on HiQsil column using UV detector. The mobile phase consisting of n-heptane: dichloromethane: methanolic ammonia (5%): methanol at a flow rate of 1.0 ml/min was used. The method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision and robustness.

Keywords: *Drotaverine hydrochloride, Omeprazole, Normal phase HPLC.*

INTRODUCTION

Drotaverine hydrochloride is chemically known as 1-[(3, 4-[diethoxyphenyl) methylene]-6, 7 diethoxy-1, 2, 3, 4 - tetrahydroisoquinolene hydrochloride [1]. Omeprazole is chemically known as 6-methoxy-2-[(4-methoxy-3, 5-dimethylpyridin-2-yl) methylsulfinyl]-1H-benzimidazole. Drotaverine hydrochloride is highly potent spasmolytic agent [2]. Omeprazole is a used as an antiulcer drug and against other acid-related diseases [3]. Literature survey reveals that both USP 2007 [4] and IP 2007 [5] report HPLC method for assay of omeprazole. Several analytical methods that have been reported for estimation of drotaverine hydrochloride are spectrophotometry [6, 7, 8], HPLC [9, 10, 11], thin layer chromatography [12, 13] and voltametry [14]. Analytical methods reported for the estimation of omeprazole are HPLC [15-21], LC-MS [22] and HPTLC [23]. An RP-HPLC method has also been reported for this combination [24]. To the best of our knowledge, there is no published NP-HPLC method for this combination. The present paper describes a simple, accurate and precise method for simultaneous estimation of drotaverine hydrochloride and omeprazole in combined tablet dosage form. The present NP-HPLC method was validated following the ICH guidelines [25].

MATERIALS AND METHODS

Reagents and chemicals

Dichloromethane, n-heptane, methanol and water of HPLC grade were procured from Ashnoj specialties Pvt. Ltd., Navi Mumbai. Working standard of drotaverine hydrochloride and omeprazole was procured from Zydus Cadila Healthcare Ltd. India.

Equipment

Chromatographic separation was performed on a Jasco HPLC system equipped with a Jasco PU-2080 plus intelligent pump, Jasco UV-2075 plus UV detector and Rheodyne injector with 50 µl loop volume.

Chromatographic conditions

The mobile phase consisting of n-heptane:dichloromethane:methanolic ammonia (5%):methanol (50:25:1:4) at a flow rate of 1.0 ml/min was used. Mobile phase was prepared by first mixing the methanolic ammonia with methanol. Then dichloromethane was added to it followed by n-heptane. Precaution must be taken while mixing the solvents. The mobile phase was then filtered through membrane

filter and sonicated for 15 min in ultrasonic bath. The column HiQSil (4.6mm x 250mm) was used at ambient temperature.

Preparation of standard stock solution

Standard stock solutions of drotavarine hydrochloride and omeprazole of strength 1mg/ml were prepared using dichloromethane. Appropriate amounts of these stock solutions were then further diluted to get the required concentrations of standard stock solutions.

System suitability studies

The resolution, number of theoretical plates, retention time and peak asymmetry were calculated for the working standard solutions and is as shown in Table 1.

Table 1. It shows system suitability parameters

Parameters	Drotaverine hydrochloride	Omeprazole
Theoretical plates	34075.88	15192.87
Asymmetry Factor	1.01	1.18
HETP (cm)	0.00073	0.00164
Resolution*		4.53

* With respect to previous peak

The values obtained demonstrated the suitability of the system for the analysis of these drugs in combination. The typical chromatogram of standard solution is as shown in Figure 1.

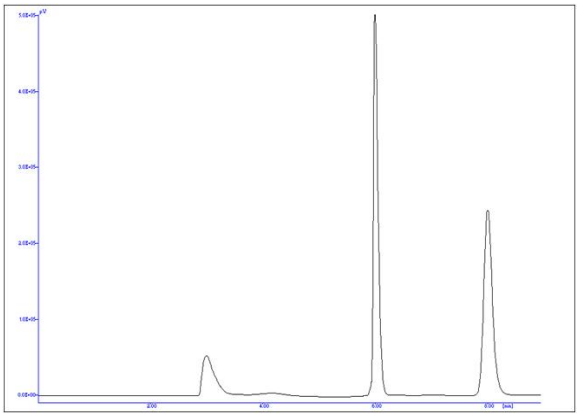

Figure 1. It shows a representative chromatogram of drotaverine hydrochloride (rt-6.0) and omeprazole (rt-8.01)

ASSAY

Preparation of sample solutions

Twenty tablets were weighed and powdered. Powder equivalent to 10 mg of omeprazole was weighed and transferred to 10 ml volumetric flask. Dicloromethane about 8 ml was added and sonicated for 10 min, volume was made up with the same solvent. This solution was then filtered through membrane filter paper. Further dilutions were made in dichloromethane to get concentrations in Beers law range. The retention times of drotaverine hydrochloride and omeprazole were found to be 6.0 ± 0.02 and 8.01 ± 0.03 respectively. The assay was calculated from the equation of regression line for each drug. The percentage assay of individual drug was calculated and presented in Table 2.

Table 2. It shows assay of tablet

Drug	Amount present (mg/tab)	Amount found (mg/tab)	% label claim
Drotaverine Hydrochloride	40	39.70	99.25
Omeprazole	10	10.01	100.13

METHOD VALIDATION

As per ICH guidelines, the method validation parameters checked were specificity, linearity, precision, accuracy, limit of detection, limit of quantitation and robustness.

Specificity

A blank solution (mobile phase) was injected and the chromatogram showed no inferring peaks at retention time of the two drugs. The chromatogram of drotaverine hydrochloride and omeprazole extracted from the tablet were compared with those acquired from drotaverine hydrochloride and omeprazole standards, correlation was good (in terms of t_R and area) indicates specificity of method. Common tablet excipients like starch, lactose, magnesium stearate were dispersed in dichloromethane, filtered and injected. There was no interference found.

Linearity and range

Aliquots of standard stock solutions of drotaverine hydrochloride and omeprazole were taken in 10 ml volumetric flasks and diluted with dichloromethane to get final concentrations in range of 2.5-12.5 µg/ml for omeprazole and 10-50 µg/ml for drotaverine hydrochloride. Triplicate injections were made five times for each concentration for each drug separately and chromatographed under the conditions as described above. The plots of peak area versus respective concentrations of drotaverine hydrochloride and omeprazole were found to be linear in the concentration range of 10-50 µg/ml and 2.5-12.5 µg/ml respectively. The linear regression equations of the lines are:

For drotaverine hydrochloride -

$y = 48166x + 491336$, ($r = 0.9964$)

For omeprazole -

$y = 82804x + 163070$, ($r = 0.9953$)

Precision

Precision study was performed to find out intra-day and inter-day variations. The percent relative standard deviation for intra-day precision was 0.288% for drotaverine hydrochloride and 0.232% for omeprazole and inter-day precision was 1.107% for drotaverine hydrochloride and 1.312% for omeprazole. Both the values were well within the limit of 2% as per ICH guidelines.

Accuracy

The accuracy was determined by recovery studies. The recovery studies were performed by standard addition method, at 80%, 100%, 120% level. Percent recovered was calculated using regression equation. For both the drugs, recovery was performed in same way and in triplicate. The percentage recovery were calculated and presented in Table 3.

Limit of detection and limit of quantitation

The limit of detection (LOD) is the smallest concentration that can be detected but not necessarily quantified as an exact value.

$$LOD = \frac{3.3 \times \text{Standard deviation of y intercept}}{\text{Slope of calibration curve}}$$

Drotaverine Hydrochloride - 1.13 µg/ml

Omeprazole - 0.27 µg/ml

The LOQ is the lowest amount of analyte in the sample that can be quantitatively determined with suitable precision and accuracy.

$$LOQ = \frac{10 \times \text{Standard deviation of y intercept}}{\text{Slope of calibration curve}}$$

Drotaverine Hydrochloride - 3.42 µg/ml

Omeprazole - 0.81 µg/ml

Robustness

Robustness of the method was determined by making slight deliberate changes in chromatographic conditions like 1% change in ratio of mobile phase constituents, ± 1nm change in detection wavelength and 0.05% change in flow rate. It was observed that there were no marked changes in the chromatogram. It suggests that the developed method is robust.

Table 3. It shows recovery studies

Drug	Level of recovery	Amount present (mg)	Amount added (mg)	Amount recovered	% recovered
Drotaverine Hydrochloride	80	20	16	35.95	99.88 + 0.82
	100	20	20	40.47	101.17 + 0.28
	120	20	24	44.41	100.93 + 1.04
Omeprazole	80	5	4	8.83	98.15 + 0.11
	100	5	5	10.10	101.08 + 0.70
	120	5	6	10.88	98.98 + 1.00

RESULTS AND DISCUSSION

The proposed method was found to be simple and sensitive with linearity in the concentration range of 2.5 to 12.5 μg/ml for omeprazole and 10 to 50 μg/ml for drotaverine hydrochloride. The method was found to be accurate and precise as indicated by results of recovery studies and %RSD not more than 2%. LOD and LOQ for omeprazole were found to be 0.27 μg/ml and 0.81μg/ml respectively and for drotaverine were 1.13 μg/ml and 3.42 μg/ml respectively. The proposed method was found to be specific as there is no interference from common tablet excipients like lactose, starch etc.

CONCLUSION

Though the runtime per injection is slightly more by this method as compared to the reported RP-HPLC method, the developed NP-HPLC method leads to better resolution and peak symmetry. Hence the developed NP-HPLC method for the simultaneous determination of omeprazole and drotaverine hydrochloride can be used for routine analysis of both these components in combined dosage form.

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