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Der Pharma Chemica, 2012, 4 (3):847-853
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Study of stressed degradation behavior of drotaverine and development of a validated stability-indicating HPLC assay method

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ABSTRACT

A stability indicating HPLC method was developed for the analysis of Drotaverine in the presence of its degradable products using C_{18} column. Mixture of methanol and ammonium acetate (0.1 M, pH 5) in the ratio of 60:40 (v/v) was taken as mobile phase, at flow rate of 1.5 ml min^{-1}. Detection was carried out at wavelength 319nm using photodiode array detector. Drotaverine was subjected to different ICH prescribed stress conditions like acid, alkali, neutral hydrolysis, oxidation, dry heat, and Sunlight. In study it was observed that significant degradation was found in alkali, oxidation and photolytic conditions. The drug was labile under acidic and neutral hydrolytic conditions but stable to thermal stress conditions. The degradation products of Drotaverine in different conditions were well resolved from the pure drug with significant differences in their retention time values. The developed assay method was validated according to ICH guideline and found accurate, precised and specific as all the results were within the acceptance limit.

Keywords : Drotaverine , Stress testing , Stability-indicating assay , Validation , Reversed-phase HPLC

INTRODUCTION

In recent times, there is an increase tendency towards the development of stability-indicating assay method, using the approach of stress testing as mentioned in the ICH guidelines [1]. The International Conference on Harmonization (ICH) guideline entitled "Stability testing of new drug substances and products" requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. An ideal stability-indicating method is one that resolves the drug and its degradation products efficiently. The stress testing encompasses the influence of temperature, humidity, light, oxidising agent as well as susceptibility over a wide range of pH values [2,3]. The objective of present work to study degradation of drotaverine under different ICH recommended stress conditions, and to established a validated stability-indicating HPLC method. Chemically drotaverine (DRO) is 1-[(3,4-[Diethoxy phenyl) methylene]-6,7-diethoxy-1, 2, 3, 4-tetrahydro isoquinolene [4,5] [Figure 1]. It is an analog of papaver and is used mainly as an antispasmodic and smooth muscle relaxant in pain associated with gastrointestinal colics, biliary colics and postsurgical spasms [6] etc. DRO is not mention in any pharmacopoeia. From the literature survey it was revealed that DRO has been determined in human plasma [7] and urine by HPLC [8] and by UV-spectroscopic methods in single [6] and combine dosage forms [9,10]. Determination of DRO in tablets and plasma was carried out by the application of new membrane selective electrode [11]. Simultaneous estimation in combination using TLC [12] and selective differential spectrophotometric methods [13] were reported. Simultaneous quantitative estimation of drotaverine and omeprazole in tablet dosage form using UV-

spectroscopy and RP-HPLC were reported [14-16]. But no literature found regarding the development of stability-indicating assay method for DRO, hence it was felt to develop and validated stability-indicating HPLC assay method for analysis that separates the drug from its degradents formed under ICH suggested stress conditions (hydrolysis, oxidation, photolysis and thermal stress).

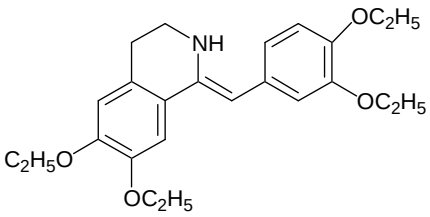


Figure 1. Chemical structure of DRO

MATERIALS AND METHODS

Materials

Working standards of pharmaceutical grade DRO was obtained as gift sample from Plethico Pharmaceutical Pvt. Ltd, Indore and was used without further purification. HPLC grade Water and methanol were purchased from Ranchem, New Delhi, India. All the buffer materials and reagents used were of HPLC grade and purchased from Spectrochem, Mumbai, India. Nylon filter paper of 0.2µm (Ultipor) was purchased from Pall Life Science, Mumbai, India.

Instrumentation

Precision mantel heater (Biotech, Mumbai) with temperature regulator equippted with a reflux condenser were used for degradation study in acid, alkali and neutral conditions. Dry air oven was used to study the effect of dry heat. Photolytic study was carried out by exposing the drug to direct sunlight for 4h.

The HPLC system equippted with an LC-10 AT VP solvent-delivery system with universal loop injector (Rheodyne 7725 i) of injection capacity of 20 µL. Detector consists of photodiode array detector SPD-10 AVP UV-Visible detector. Separation was carried out on a Phenomenex Luna C_{18} (5µm×25cm×4.6mm i.d) under reversed phase partition chromatographic conditions. The equipment was controlled by a PC workstation. The work was carried out in an air-conditioned room maintained at temperature 25 ± 2^0C. Chromatograms were recorded using a CLASS-VP software (Shimadzu, Kyoto, Japan).

Forced Degradation studies

Forced degradation studies were performed to provide an indication of the stability indicating property of the drug. Drug at a concentration of 1mg ml^{-1} was used in all degradation studies. The pH of the ammonium acetate buffer was checked before and after reaction and no change was observed. Conditions employed for stability studies were as follows.

Hydrolytic studies

For acid hydrolysis studies, 1mg ml^{-1} solution of the drug was prepared by diluting required amount of drug in 0.1N HCl and the solution was refluxed for 8h and then for 12h. Same concentration of drug solution was subsequently prepared in 1 N HCl and refluxed for 12h. Studies were also performed in 5 N HCl and refluxed for 12h. Hydrolysis study in alkali conditions was done at a drug concentration of 1mg ml^{-1} in 0.1 N NaOH and the solution was refluxed for 8h. For neutral condition 1mg ml^{-1} solution of the drug was prepared in water and refluxed initially for 12h and subsequently for 24h.

Oxidative studies

For oxidative degradation study, initially 1mg ml^{-1} strength of drug was prepared in 3% H_2O_2.The drug was kept under the conditions of room temperature for a period 12h and then for 24h. Subsequently the drug was exposed to 30% H_2O_2 at room temperature for a period of 72h. **Photolytic studies**

Photolytic study was done by exposing the dry drug to direct sunlight for 4h (17).

Thermal (dry heat) studies
Susceptibility of the drug to dry heat was studied by exposing the solid drug to 60^0 C for 15 days in a hot air oven. Sampling was carried out every day to study its degradation behaviour.

For all the stability study, the formation of degradable product was conformed by compairing the chromatogram of the degradable mixture with the blank solvent stored under normal condition and control drug solution kept under normal condition.

Preparation of samples for HPLC analyses
For hydrolysis study during 0.1N HCl, 0.1 N NaOH and oxidative study during 3% H_2O_2 the samples were diluted 10 times with water to a concentration of 10 µg ml^{-1} where as the samples were diluted 100 times with water during higher acidic, higher alkali and 30 % H_2O_2 conditions to a concentration of 10 µg ml^{-1}. The solution for neutral, thermal and photolytic degradation study were suitably diluted with water to a concentration of 10 µg ml^{-1}.

Separation studies on stressed samples
In all HPLC runs, the mobile phase was filtered through 0.2µm nylon membrane under vaccum and degassed before use. The injection volume was 20µl and the mobile phase flow rate was 1.5 ml min^{-1}, the analytical wavelength selected was 319nm.

HPLC studies were carried out on all reaction solution individually. Initially analysis were performed C_{18} column and mobile phase composed of methanol : ammonium acetate (0.1 M, pH 5 adjusted with orthophosphoric acid). As the satisfactory resolution of the drug and the degradation products was not achieved, hence to get good resolution the method was further optimized by increasing the ratio of methanol and it was found good resolution in the ratio of 60:40 (v/v) of methanol : ammonium acetate.

Validation of the method
Validation of the optimized HPLC method was done with respect to following parameters as per ICH norms [18].

Linearity and range
A stock solution of the drug (1mg ml^{-1}) was prepared in water. From this stock solution seven concentrations of the drug were prepared in water within the concentration range of 5-100µg ml^{-1}. The solutions were injected in hexaplicate into the HPLC column, keeping all the conditions constant.

Precision
Precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analyses of three different concentration of (30,50,80 µg ml^{-1}) drug in hexaplicate on the same day. Intermediate precision of the method was checked by repeating the studies on same day at an interval of one hour (intraday precision) for three hours and on three different days (interday precision).

Accuracy
Accuracy of the method was tested by fortifying a mixture of decomposed reaction solutions with three concentration of the drug and determining the percentage of recovery of added drug.

Specificity and selectivity
The specificity of the method towards the drug was established through study of resolution factor of the drug peak from the nearest resolving peak. Where as selectivity was established through determination of purity for each degradation product peak using PDA detector.

RESULTS AND DISCUSSION

Degradation behaviour of drotaverine
HPLC studies of sample obtained on stress testing of drotaverine under different conditions using methanol : ammonium acetate (60:40) as the mobile solvent system suggested the following degradation behaviour.

Table 1. Degradation study of DRO at different stress conditions

Standard DRO concentrartion (µg ml^{-1})	Stressed conditions	Intact DRO found	Degraded DRO found
10	Acidic degradation	87.38%	12.62%
10	Basic degradation	37.51%	62.49%
10	Neutral degradation	90.18%	9.82%
10	Oxidative degradation	44.86%	55.14%
10	Photolytic degradation	76.41%	23.59%
10	Thermal degradation	98.62%	1.38%

Table 2. Linearity Data of DRO

S.No	Concentration injected (µg ml^{-1})	[a]Average peak area ± S.D.	% COV	S.E.
1	5	51726 ± 110.910	0.214	45.288
2	10	117355 ± 101.022	0.086	41.250
3	20	221973 ± 153.241	0.069	62.573
4	40	450863 ± 176.318	0.039	71.996
5	60	671450 ± 207.696	0.030	84.808
6	80	898610 ± 173.611	0.193	70.890
7	100	1109501 ± 295.395	0.026	120.618

[a]Mean of six estimations; S.D. : Standard deviation; COV: Coefficient of variance; S.E.: Standard error

Table 3. Precision studies

Actual concentration (µg ml^{-1})	Measured concentration (µg ml^{-1}) ± S.D.					
	Repeatability (n=6)	% COV	Intra day precision (n=3)	% COV	Inter day precision (n=3)	% COV
30	30.658 ±0.227	0.751	30.056±0.203	0.677	29.661±0.301	1.016
50	50.428 ±0.392	0.780	50.144±0.417	0.832	49.713±0.191	0.384
80	79.528 ±0.402	0.502	79.980±0.434	0.543	79.348±0.249	0.313

S.D. : Standard deviation; COV: Coefficient of variance

Table 4. Recovery studies (n=6)

Added concentration (µg ml^{-1})	Concentration recovered (µg ml^{-1}) ± S.D	% COV	Recovery (%)
30	30.556 ± 0.288	0.943	101.85
50	50.715 ± 0.332	0.655	101.43
80	81.061 ± 0.314	0.388	101.32

S.D. : Standard deviation; COV: Coefficient of variance

Hydrolytic studies

Acidic condition.

It was observed that the drug gets slowly degraded about 12.62% in strongly acidic conditions over a period of time. On reflux in 0.1 N HCl (6h) and further for 12h, in 1.0 N HCl (12h) and 5.0 N HCl (12h), the height of the drug peak decreased with out corresponding rise in a new peak (Fig.2b). This indicates that the drug is hydrolysed under higher acid conditions, perhaps to a non-chromatographic compound. Hence the drug has liability in strong acidic conditions.

Degradation in alkali

In alkali, the drug was found to decompose almost 62.69% after refluxing for 8h in 0.1 N NaOH. As shown in chromatogram (Fig .2c), degradation of the drug resulted in the rise of three new peaks at 3.119 (I), 4.443 (II), 6.579 (III) mins with respect to the peak of DRO at 9.001 min.

Neutral (water) condition

In neutral condition, mild degradation only 9.82% was seen after reflux for 24h at 80^0C (Fig. 2d).

Oxidation studies

The drug was found to be stable in 3% H_2O_2 for 6h at room temperature. However about 55% drug degradation was observed on exposure to 30% H_2O_2 for 72h. One very small degradation product peaks at 6.32 min was seen, but there was no significant rise in the height of the peak with time (Fig.2e). This signifies that the drug was degraded in oxidative conditions to non-chromatographic compounds.

Photolytic studies

Photolytic study was carried out in dry form. Here the drug was directly exposed to the sunlight for 4h on a hot sunny day. Two very small degradation product peaks at 3.923 and 4.454 mins were appeared (Fig.3a). The peaks were found to increase with time. Around 23.59% of drug was degraded in this condition.

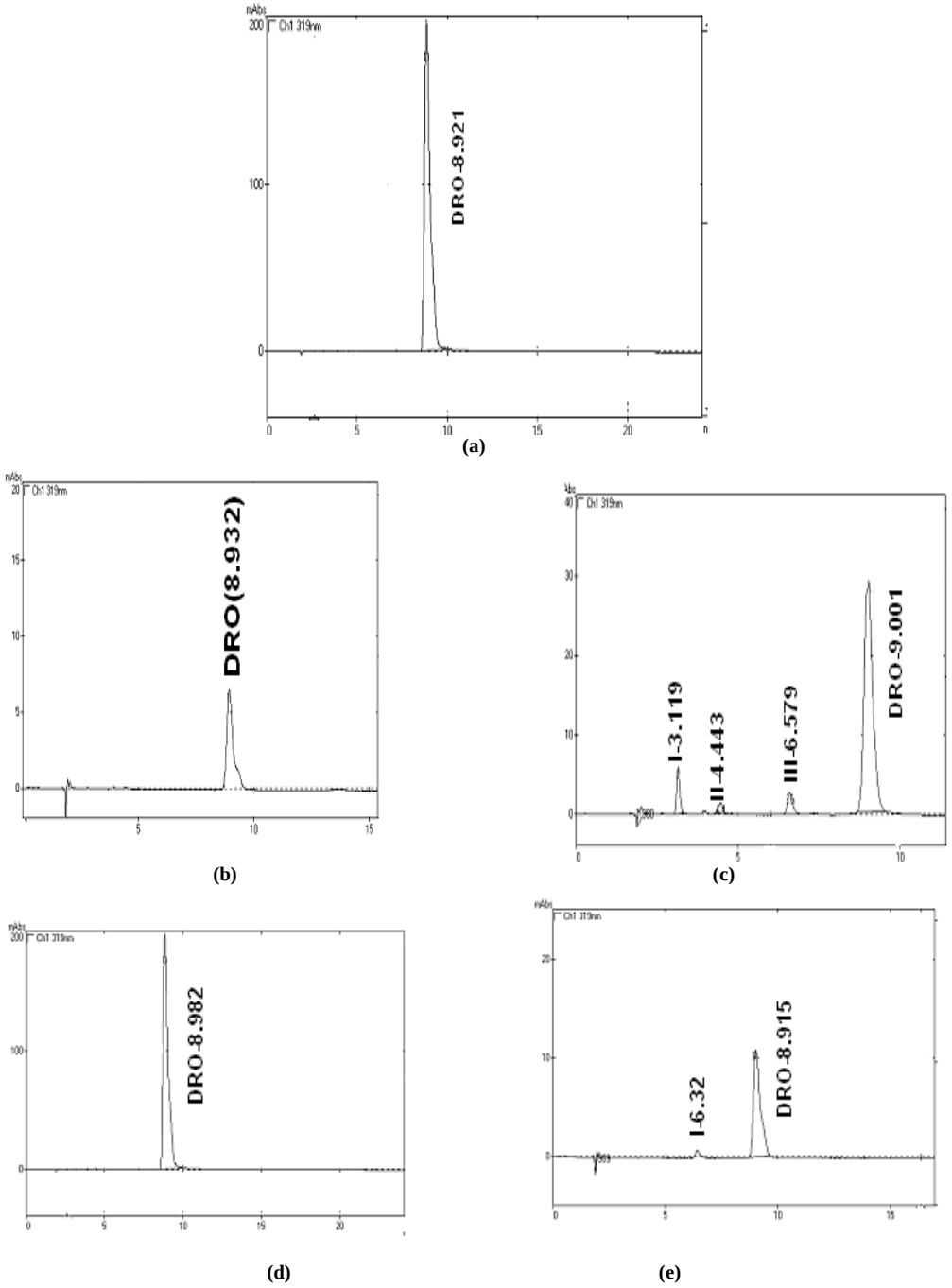
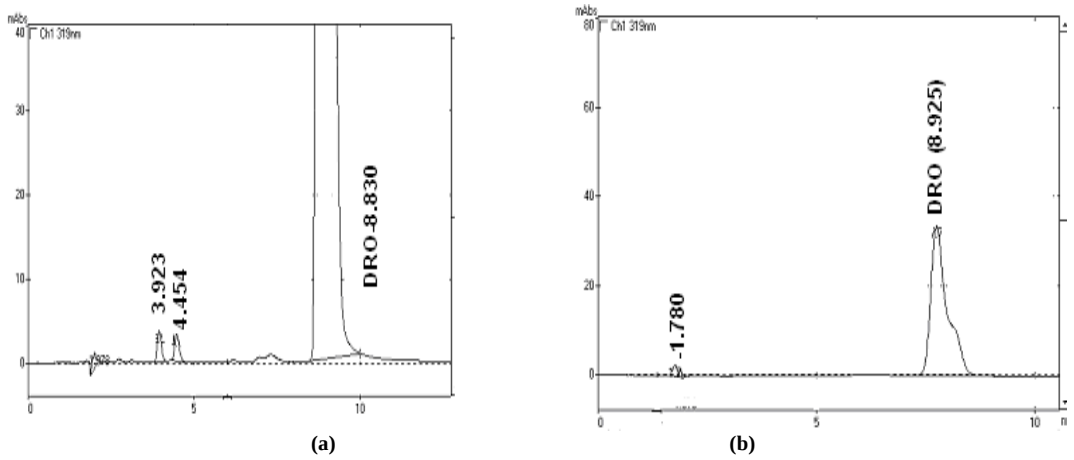


Figure 2. Representative HPLC chromatograms of DRO, (a) standard solution of drug (b) sample degraded in 0.1 N HCl, (c) sample degraded in 0.1N NaOH, (d) sample degraded in water, (e) sample subjected to oxidative degradation.



(a) **(b)**

Figure 3. Chromatogram of DRO (a) sample subjected to photolytic degradation in dry form.(b) sample subjected to dry heat.

Thermal stress study

DRO was found to be degraded in very negligible amount as a new peak of degradation product was appear at 1.780 min (Fig.3b). Only 1.38 % of the drug was found to be degraded hence DRO was almost stable after exposing the drug to 60^0 C for 15 days.

The results of percent degradation shown by DRO in different stressed conditions are given in Table I.

Development of stability-indicating method

It was observed that satisfactory resolution of DRO and its degradation products formed under various conditions was achieved when the analyses was performed by using a mixture of methanol and ammonium acetate (0.1 M, pH 5 adjusted with orthophsophoric acid) in the ratio of 60:40 (*v/v*) as mobile phase at a flow rate of 1.5 ml min^{-1}. Detection was carried by PDA detector at 319nm.

Validation of the developed stability-indicating HPLC method

The developed method was validated by using following criteria.

Linearity

The response curve of the drug was linear in the concentration range of 5-100 µg ml^{-1}. The mean values of slope, intercept and correlation coefficient were 11147, 1254.9 and 0.9997 (r^2) respectively. The linearity results are given in Table 2. From the result it was cleared that there was an excellent correlation existed between the peak area and concentration of the analyte.

Precision

The results of repeatability and intermediate precision study are given in Table 3. The developed method was found to be precise as the % COV values for repeatability and intermediate precision studies were < 2.

Accuracy

The accuracy of the developed HPLC methods was checked by performing recovery study. Here standard drug solutions of 30, 50 and 80 µg ml^{-1} were added to fortifying mixtures of decomposed reaction solutions and the percentage of drug recovery was calculated. The result of the recovery study is given in Table 4. Good recoveries (101.32-101.85%) of the drug and low % COV (< 2) were obtained at each added concentration, indicating that the method was accurate.

Specificity and selectivity

The method was specific for the drug and its degradents. The resolution for the drug peak was > 2 from the nearest resolving peak. The method was also selective to degradation products as the purity factor for all the degradents

formed under different stressed conditions were within the threshold limit checked by Shimanzu SPD-10 AVP, photo diode array detector demonstrated the analyte peak homogeneity.

CONCLUSION

In the present study, a stability-indicating assay method for drotaverine was established by following the ICH recommended stress conditions. The drug was found to be degraded extensively in alkaline medium, higher oxidative stress and in the presence of light where as mild degradation of drug occured in higher acidic conditions, neutral condition. But the drug was stable in thermal stress conditions.

A new, reversed-phase HPLC method was developed, which is simple, accurate, precise, selective and specific. The method was completely validated showing satisfactory data for all the method validation parameters tested. The developed method is stability indicating and can be helpful for analyse the drug in stability samples.

Acknowledgement
The authors are thankful to Plethico pharmaceutical PVT. LTD., Indore for providing gift samples and School of Pharmacy, DAVV, Indore for providing necessary facillities and chemicals. One of the author, Mr. Debadash Panigrahi is thankful to All India Council for Technical Education (AICTE) for providing junior research fellowship**.**

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