Research Article

DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING LC METHOD FOR SIMULTANEOUS ANALYSIS OF DROTAVERINE HYDROCHLORIDE AND DICLOFENAC POTASSIUM AND ITS APPLICATION FOR FORMULATION ANALYSIS

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ABSTRACT

Simple, sensitive, selective, precise stability indicating chromatographic methods for determination of Drotaverine Hydrochloride and Diclofenac Potassium from pharmaceutical tablets were developed and validated as per the ICH guidelines. A novel stability-indicating LC assay method was developed and validated for quantitative determination of Drotaverine and Diclofenac in bulk drugs and in pharmaceutical dosage form. An isocratic, reversed phase LC method was developed using an Qualisil BDS C8 column (250 mm x 4.6 mm, 5.0 µ particle size), using methanol: THF: acetate buffer (45:08:17 v/v) pH adjusted to 5.0 with acetic acid mobile phase, flow rate was 0.7 mL min^{-1} and column was maintained at 50 ^{0}C. The detection was carried out at 292 nm and injection volume was 20 µL. The peak purity was checked with the photodiode array detector. Drotaverine Hydrochloride and Diclofenac Potassium were subjected to acid, alkali and neutral hydrolysis, oxidation, photo degradation and dry heat and wet heat treatment. Degradation products were well separated from the analytes peaks. As the method could effectively separate the drug from its degradation products, it can be employed as stability-indicating one. The developed method was validated with respect to linearity, accuracy (recovery), precision, specificity and robustness.

Keywords: Drotaverine, Diclofenac, Stability indicating, Method Validation, Column Liquid Chromatography.

INTRODUCTION

Drotaverine hydrochloride (DRT), 1-[(3, 4-diethoxy phenyl) methylene]-6, 7-diethoxy-1, 2, 3, 4-tetra hydro isoquinolene, fig. 1 is an analogue of papaverine. It acts as an antispasmodic agent by inhibiting phosphodiesterase IV enzyme, specific for smooth muscle spasm and pain, used to reduce excessive labor pain. Drotaverine hydrochloride is official in Polish Pharmacopoeia. A few UV spectrophotometric[8-12] HPLC[15] and HPTLC[5] methods have been reported individually or in combination with other drugs for estimation of drotaverine hydrochloride. Diclofenac is chemically designated as 2[(2, 6-dichlorophenyl) amino] benzene acetic acid potassium salt, fig. 1. It is a NSAID, used in the management of osteoarthritis, rheumatoid arthritis, and ankylosing spondylitis. Diclofenac is official in United States Pharmacopoeia, British Pharmacopoeia and European Pharmacopoeia. Various UV, HPLC, HPTLC and stability indicating methods for diclofenac have been reported individually or in combination with other drugs.[4]

The literature contains no reports of stability indicating methods for simultaneous determination for DRT and DIC[14]. Therefore, this paper reports an attempt to develop and validate a novel stability indicating LC-PDA method for simultaneous analysis of DRT and DIC in commercial tablet dosage form. The method was developed and validated with the objective to be economical, selective and sensitive with a short run time and simple mobile phase composition. The method was validated for the important characteristics linearity, accuracy, precision, sensitivity, robustness, etc. in accordance with International Conference on Harmonization (ICH) guidelines.

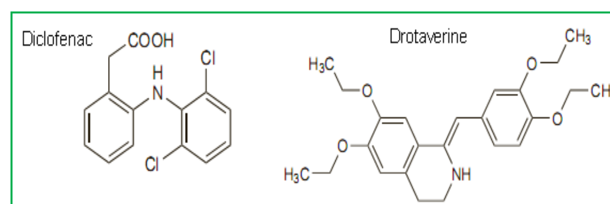

Figure 1: Structures of analytes

MATERIALS AND METHODS

Pure drug sample of DRT (% purity 98.5) and DIC (% purity 99.91) was kindly supplied as a gift sample by Alkem Pharmaceutical Ltd. Mumbai and Aarti Drugs Ltd., Mumbai, respectively. These samples were used without further purification. The formulations used were Tablet Formulation I - Brand: SMS-D (Lot No. AN9038) Sigman Wellness Ltd. and Tablet Formulation II - Brand : VERIN-D (Lot No. 059321) Corona Remedies Pvt Ltd. containing Drotaverine Hydrochloride (DRT) 80 mg and Diclofenac Potassium (DIC) 50 mg per tablet. HPLC grade Methanol and Tetrahydrofuran (THF) were procured from Merck and Qualigens fine Chemicals, respectively (Mumbai, India). AR grade ammonium acetate, acetic acid, HCl, NaOH and H_2O_2 were procured from Research Lab Fine Chem. (Mumbai, India). Double distilled water was made at lab scale only.

Instrumentation and chromatographic conditions

The HPLC system consisted of a binary pump (model Waters 515 HPLC pump), auto sampler (model 717 plus Auto sampler), column heater, and PDA detector (Waters

2998). Data collection and analysis were performed using empower- version 2 software. Separation was achieved on Qualisil BDS C8 column (250 mm × 4.6 mm, 5.0 µ) columns maintained at 50°C using column oven. Isocratic elution with mobile ohase containing Methanol: THF: acetate buffer (45:08:17 v/v) pH adjusted to 5.0 with acetic acid at 0.7 ml/min flow rate of was carried out. The detection was monitored at 292 nm and injection volume was 20 µL. The peak purity was checked with the photodiode array detector.

Preparation of standard solutions and calibration curve

Standard stock solution of DRT and DIC (1000µg/ml) were prepared separately in methanol. To study the linearity range of each component, serial dilutions of DRT and DIC were made from 0.8 - 160 µg/ml and 0.5-100 µg/ml, respectively in mobile phase and injected on to column. Calibration curves were plotted as concentration of drugs versus peak area response. From the standard stock solutions, a mixed standard solution was prepared containing the analytes in the given ratio and injected on to column. The system suitability test was performed from six replicate injections of mixed standard solution.

Analysis of tablet formulation

Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 80 mg of DRT (50 mg of DIC) was weighed and dissolved in the 80 ml of methanol with the aid of ultrasonication for 10 min and solution was filtered through Whatman paper No. 41 into a 100 ml volumetric flask. Filter paper was washed with methanol, adding washings to the volumetric flask and volume was made up to the mark with methanol. From the filtrate, appropriate dilution was done in mobile phase to get a solution of 80 µg/ml of DRT and 50 µg/ml of DIC respectively and proposed method was followed. Concentrations in the samples were determined using multilevel calibration developed on the same HPLC system under the same conditions using linear regression equation.

Method validation[1]

The HPLC method was validated in terms of precision, accuracy, specificity, sensitivity, robustness and linearity according to ICH guidelines. Assay method precision (inter-day and intra-day) was determined using nine-independent test solutions. Assay method was evaluated with the recovery of the standards from excipients. Three different quantities (50%, 100% and 150%) of the standards were added to pre analyzed formulation powder and were analyzed using the developed HPLC method. Values of Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated by using σ (standard Deviation of response) and b (Slope of the calibration curve) and by using equations, LOD = (3.3 x σ)/ b and LOQ = (10 x σ)/ b. Calculated values were confirmed by repeated injection of samples containing amounts of analyte in the range of LOD and LOQ. To determine the robustness of the method, the final experimental conditions were purposely altered and the results were examined. The flow rate was varied by (±) 5%. Column temperature was varied by (±) 2°C and effect of column from different suppliers was studied. Measurement wavelength was varied by (±) 1nm, injection volume was changed (±) 2 µL, % organic changed by (±) 5%, buffer strength changed by (±) 5 mM. The stability of the drug solution was determined using the samples for short-term stability by keeping at room temperature for 12 h and then analyzing. The long-term stability was determined by storing at 4°C for 30 days. Auto-sampler stability was determined by storing the samples for 24 h in the auto-sampler.

Procedure for forced degradation study (SPECIFICITY)[2,3]

Stability testing is an important part of the process of drug product development. The purpose of stability testing is to provide evidence of how the quality of a drug substance or drug product varies with time under a variety of environmental conditions, for example temperature, humidity, and light, and enables recommendation of storage conditions, retest periods, and shelf life to be established. Forced degradation of the drug product was carried out under thermolytic, photolytic, acid/base hydrolytic and oxidative stress conditions. For photolytic stress, drug product in the solid state was irradiated with UV radiation with peak intensities at 254 and 366 nm with irradiation time 24 hours. A second photolytic stress test experiment with greater irradiation time, 48 hours, was performed to establish the specificity of the method. After subjecting to appropriate stress conditions the solutions were prepared / sample were diluted suitably to obtain solution containing 80 µg/ml of DRT & 50 µg/ml of DIC and then 20 µl of the solution was injected into the system. Analytes were subjected to following stressed conditions.

Acidic and Alkaline Degradation

Standard stock solution, 5ml each of DRT and DIC (1000 mg/ml) was transferred separately to a 10 ml volumetric flask and to it 2 ml each of 3N HCl and 3N NaOH was added separately. The mixture was left at 80°C for 1 hrs in a water bath then left to equilibrate to ambient temperature.

Oxidative Degradation

Sample stock solution, 5ml each of DRT and DIC (1000 mg/ml) was transferred separately to a 10 ml volumetric flask and 3 ml 30% H_2O_2 was added. The mixture was left for 4 hrs at ambient temperature.

UV Degradation, Thermal Degradation and Neutral Hydrolysis

For thermal degradation approximately 10 mg solid drug, DRT and DIC was left at 80°C for 24 hrs separately. For UV degradation study, the solution of DRT and DIC separately and in mixture was exposed to short UV radiation (254 nm) and long UV radiation (366 nm) for 24 and 48hrs. For Neutral Hydrolysis study, the solution of DRT and DIC,

separately and in mixture prepared using distilled water were kept at 80°C for 1 hr. As per ICH guidelines above procedure was used for stress degradation of combined formulation of analytes.

RESULTS AND DISCUSSION

Optimization of the Chromatographic Conditions

A well-defined symmetrical peak was obtained upon measuring the response of eluent under the optimized conditions after thorough experimental trials that can be summarized. Two columns were used for performance investigations, including Kromasil C18 (5 micron 4.6×250mm) and Qualisil BDS C8 (5 micron 4.6×250mm), the second column was the most suitable one since it produced symmetrical peaks with high resolution. The UV detector response of DRT and DIC was studied and the best wavelength was found to be 292 nm showing highest sensitivity, Fig. 2. Development studies revealed that Methanol: THF: acetate buffer pH 5 (45:08:17 v/v) mobile phase at the flow rate of 0.7 ml/min were suitable conditions for a stability-indicating method for study of the degradation of DRT and DIC. DRT and DIC were well resolved having retention time 5.0 and 7.76 respectively, Fig. 3.

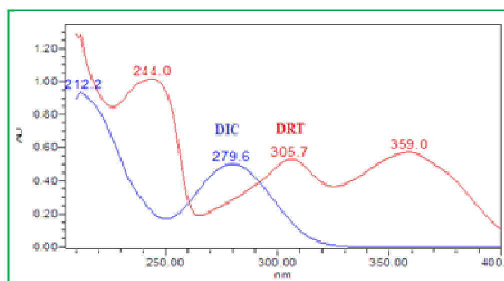

Figure 2: Online overlain PDA spectra of DRT and DIC

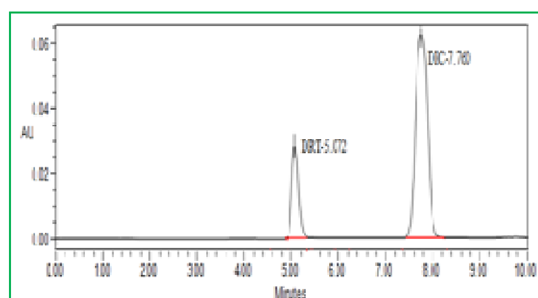

Figure 3: Typical chromatogram of DRT and DIC

Method Validation[1,2]

The following method validation characteristics were addressed for DRT and ACE: accuracy, precision, specificity, limit of quantitation, limit of detection, linearity, range and robustness. Standard calibrators were used to establish linearity and range. Robustness was established using the system suitability standard.

Linearity, Range and Method Sensitivity and System Suitability

Linearity was determined for DRT and DIC in range of 0.8-160 µg/ml and 0.5-100 µg/ml respectively in mobile phase and injected on to column. Calibration curves were plotted as concentration of drugs versus peak area response. The results obtained are shown in Table 1.

Precision

Precision of the repeatability was determined by repeating the method six times. The intra-day precision of the developed LC method was determined by preparing the tablet samples of the same batch in nine determinations with three concentrations and three replicate each on same day. The inter-day precision was also determined by assaying the tablets in triplicate per day for consecutive 3 days. The result obtained for Intraday and Inter day variations are shown for DRT and DIC in Table 1.

Table 1: Sensitivity, Linearity, precision, recovery and SST data for analytes

Parameter / Analytes (t_R)	DRT	DIC
Method sensitivity		
Limit of Detection (LOD, µg/ ml)	0.266 µg/ml	0.166 µg/ml
Limit of Detection (LOQ , µg/ ml)	0.8 µg/ml	0.5 µg/ml
Concentration Range (µg/ ml)	0.8 - 160	0.5 - 100
Regression equation (Y= b × Concentration ± a) data		
Intercept (a)	10273.88	8907.33
Slope(b)	16245.53	33417
Correlation coefficient (r)	0.9997	0.9998
Precision data, % RSD (n=6)		
Intra-day precision, n=3x3 times	1.64, 0.18, 0.19	1.54, 0.28, 0.59
Inter-day precision, n=3x5 days	1.20, 0.13, 0.92	1.20,0.17, 0.98
Recovery (accuracy) data at selected recovery levels		
50 % (% RSD)	0.58	0.72
100 % (% RSD)	0.87	0.47
150 % (% RSD)	0.39	0.88
System suitability Data		
Area	1296693	1647530
Theoretical plates[a] (T.P.) ± SD	5751	6217
Peak Tailing	1.21	1.07
USP resolution	-----	7.7
K prime	3.91	6.78
Assay, % R.S.D.	0.85	0.57
Purity Angle	0.191	0.126
Purity Threshold	0.266	0.272

*t_{tab} = 1.895 for DP1 and MPS (p = 0.05; df = 7); [a] = standard deviation (SD) of intercept, [b] = SD of slope; [c] = resolution with respect to IS HTZ peak

Robustness

Robustness study was performed at 80 and 50 µg/ml concentrations of DRT and DIC, respectively as per procedure described previously. For each deliberate change, analysis was performed three times and % assay was calculated, % RSD for assay was always within 1.3. All other system suitability parameters were within limit indicated method robustness.

Forced Degradation Study (METHOD SPECIFICITY)

Analytes and its degradation product were well separated during all the selected stress conditions. Although the conditions used for forced degradation were attenuated to achieve degradation in the range 10–30%, this could not be achieved for thermal and photolytic degradation even after prolonged exposure. The drugs were extensively degraded by acid hydrolysis (Fig. 4 & 5), alkaline hydrolysis (Fig. 6 & 7) and oxidative stress conditions (Fig. 8 & 9). Chromatographic peak purity data were obtained from the spectral analysis report. Purity angle values which were always less than purity threshold Values is indicative of a homogeneous peak thus established the specificity of the assay.

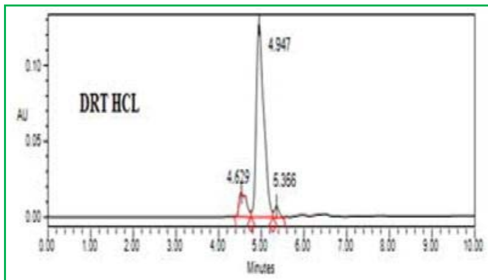

Figure 4: Chromatogram of acid degradation of DRT (3N HCl for 1 hr at 80°C)

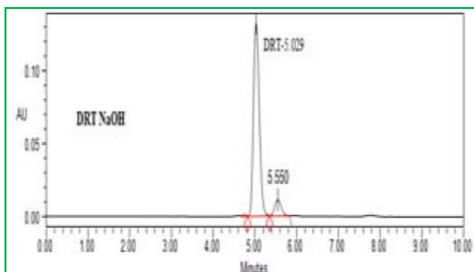

Figure 5: Chromatogram of base degradation of DRT (3N NaOH for 1hr at 80°C)

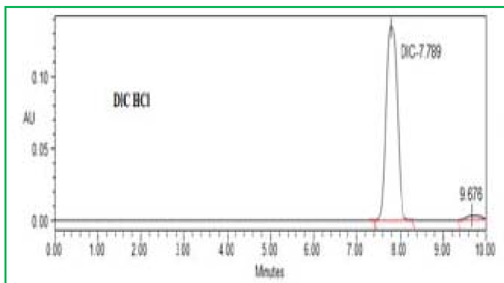

Figure 6: Chromatogram of acid degradation of DIC (3N HCl for 1 hr at 80°C

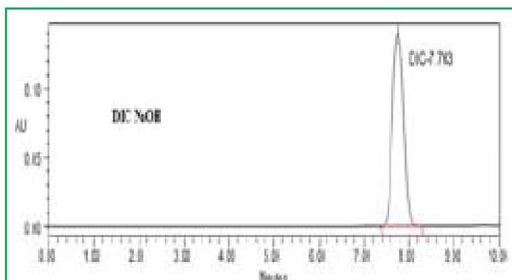

Figure 7: Chromatogram of base degradation of DIC (3N NaOH for 1hr at 80°C)

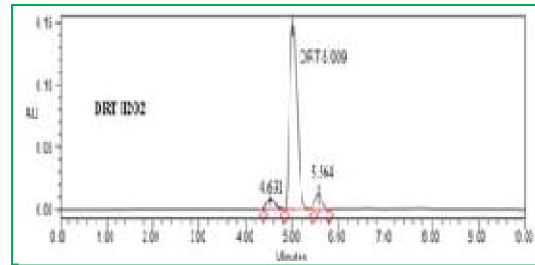

Figure 8: Chromatogram of DRT H_2O_2 degradation (30% H_2O_2 for 4 hr at RT)

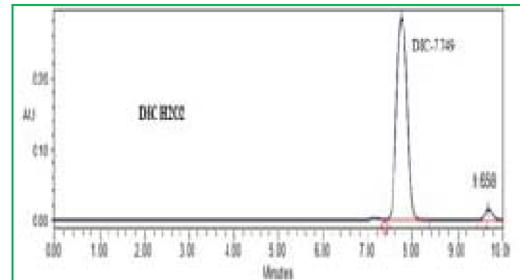

Figure 9: Chromatogram of DIC H_2O_2 degradation (30% H_2O_2 for 4 hr at RT)

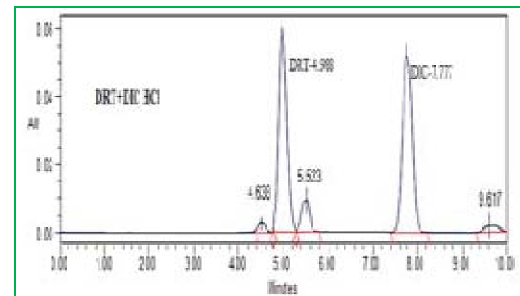

Figure 10: Chromatogram of acid degradation of DRT and DIC in tablet (3N HCl for 1 hr at 80°C)

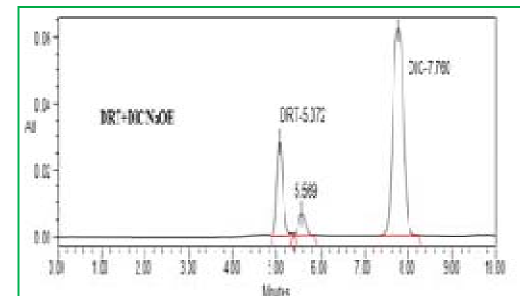

Figure 11: Chromatogram of base degradation of DRT and DIC (3N NaOH for 1hr at 80°C)

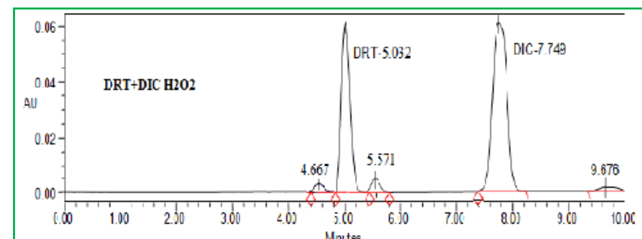

Figure 12: Chromatogram of H_2O_2 degradation of DRT and DIC sample.

The chromatogram of the DIC acid sample showed one additional peak at t_R 9.67 and DIC base sample showed no degradation (Fig. 5 and 6). The DRT sample degraded with 30 % w/v hydrogen peroxide showed two additional

peaks at t_R 4.631 and 5.564 min. (Fig.9) The DIC sample degraded with 30 % w/v hydrogen peroxide showed additional peak at t_R 9.658 min. (Fig.10). The chromatogram of DRT and DIC sample in tablet with its stress degradation peaks with HCl and NaOH are shown in Fig. 10 and 11, respectively. The chromatogram of DRT and DIC sample in tablet with its stress degradation peaks with H_2O_2 is shown in Fig. 12. Table 2 summaries stress conditions used, retention (t_R) time of the degraded products and % recovery of the analyte

Table 2: Summary of forced degradation result of DRT and DIC

Exposure Condition	DROTAVERINE*		DICLOFENAC*	
	t_r of degradation products	% degradation	t_r of degradation products	% degradation
Acid, 3N HCl, 80°C, 1 h	4.62,5.35	21.42	9.67	11.7
Base 3N NaOH, 80°C, 1h	5.55	13.57	No degradation	-
H_2O_2 (30%,v/v) 4 h	4.63,5.56,	24.71	9.65	16.12
Thermal, 80°C for 24 h	No degradation	-	No degradation	-
Short Wavelength UV, 24 h	No degradation	-	No degradation	-
Long Wavelength UV, 48 h	No degradation	-	No degradation	-
Neutral hydrolysis	No degradation	-	No degradation	-

CONCLUSION

The developed methods were found to be simple, sensitive, accurate, precise and reproducible and can be used for the routine quality control analysis of DRT and DIC in bulk drug and marketed formulation. As the method could effectively separate the drugs from their degradation products it can be employed as a stability indicating one. The method is sensitive enough for quantitative detection of the analytes in pharmaceutical preparations and can thus be used for routine analysis, quality control and for studies of the stability of pharmaceutical preparations containing the analytes.

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