Original Article

Development and validation of UV-Visible spectrophotometric baseline manipulation methodology for simultaneous analysis of drotraverine and etoricoxib in pharmaceutical dosage forms

Abstract

Introduction: A simple, economical, precise, and accurate new UV spectrophotometric baseline manipulation methodology for simultaneous determination of drotaverine (DRT) and etoricoxib (ETR) in a combined tablet dosage form has been developed. **Materials and Methods:** The method is based on baseline manipulation (difference) spectroscopy where the amplitudes at 274 and 351 nm were selected to determine ETR and DRT, respectively, in combined formulation and methanol was used as solvent. Both the drugs obey Beer's law in the concentration ranges of 4–20 μg/mL for DRT and 4.5–22.5 μg/mL for ETR. **Results:** The results of analysis have been validated statistically and recovery studies confirmed the accuracy and reproducibility of the proposed method which were carried out by following the ICH guidelines. **Conclusion:** It has been concluded that a new simple and accurate UV spectrophotometric baseline manipulation method was developed for simultaneous do not declare DRT and ETR in a combined tablet dosage form has been developed.

Key words: Baseline manipulation (difference) spectroscopy, drotaverine, etoricoxib

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INTRODUCTION

Drotaverine (DRT) hydrochloride, 1-[(3,4-diethoxy phenyl)methylene]-6,7-diethoxy-1,2,3,4-tetra hydroisoquinolene, is an analogue of papaverine.[1] It acts as an antispasmodic agent by inhibiting phosphodiesterase IV enzyme, specific for smooth muscle spasm and pain, used to reduce the excessive labor pain.[2] DRT hydrochloride is official in Polish Pharmacopoeia.[3] A few UV spectrophotometric[4-8] and HPLC[9-13] methods have been reported for estimation of DRT hydrochloride.

Etoricoxib (ETR) a newer cyclo-oxygenase-2 inhibitor is mainly used in the management of osteoarthritis, rheumatoid arthritis, and acute gouty arthritis.[14] Chemically, ETR is a 5-chloro-6′-methyl-3-[4-(methylsulfonyl)phenyl]-2,3′-bipyridine, and is not official in any pharmacopoeia. Its impurity studies and HPLC/MS-MS methods in matrix have been reported.[15-18]

The combination of DRT and ETR is not included in any pharmacopoeia. Review of the literature revealed that there is no spectrophotometric method available for determination of this combination. The first-order derivative, ratio derivative, corrected absorbance spectrophotometric methods for the combinations were developed in the same laboratory. Therefore, the same combination was selected for application of newly developed baseline manipulation analytical methodology based on UV-visible spectrophotometry, so that the results of the

established methods can be compared with the new method and its validity can be proved. Therefore, the aim of the study was to develop simple, accurate, and economical new spectroscopic baseline manipulation methods for both the drugs in combined dosage forms. The proposed method was validated as per the International Conference on Harmonization (ICH) analytical method validation guidelines.

MATERIALS AND METHODS

Materials and reagents

Pure drug sample of DRT, % purity 98.80, and ETR, % purity 99.92, were kindly supplied as a gift sample by Alkem Pharmaceuticals Ltd., Mumbai and Mapro Pharmaceuticals Ltd., Vapi, respectively. These samples were used without further purification. Two batches of tablet formulations I and II (Batch no. JT901 and JT902, respectively) containing DRT 80 mg and ETR 90 mg per tablet, supplied by JCPL Pharma Ltd., Jalgaon, were used for analysis. Spectroscopic grade methanol supplied by Loba Chemicals Pvt. Ltd., Mumbai, was used throughout the study and double distilled water was made available at the lab scale.

Experimental instrumentation

A UV-visible double beam spectrophotometer (Varian Cary 100) with 10 mm matched quartz cells was used. A dual range electronic balance (Model Shimadzu AUW-220D) was used for weighing.

Methods

Preparation of standard stock solutions and calibration curve

A standard stock solution containing 100 μg/mL of DRT and 90 μg/mL of ETR were prepared separately in the methanol. Individual working standard solution containing 20 μg/mL of DRT was prepared form stock solution in double distilled water. The mixed standard solutions of these drugs containing 4–20 μg/mL of DTR and 4.5–22.5 μg/mL of ETR were prepared by serial dilutions of standard stock solutions in distilled water. Mixed standard solutions were scanned using 20 μg/mL of DRT solution as blank. Instrument response at 274 nm and 351 nm was measured for ETR and DRT, respectively, and used to prepare the calibration curve. Six replicates of five mixed standard solutions were used to prepare the calibration curve. Correlation coefficient is not true indicator of linearity therefore the Fischer variance ratio[19] (test of linearity) was used. Test of linearity was performed by using MIP Pharmasoft 1.0, software developed and validated at MAEER'S Maharashtra Institute of Pharmacy, Pune.

Analysis of tablet formulation

Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to about 80 mg of DRT (90 mg of ETR) was dissolved in the 80 mL of methanol with sonication for 15 min, solution was filtered through Whatmann filter paper No. 41 into a 100 mL volumetric flask. The filter paper was washed by using solvent, filtrate, and washing transferred to a volumetric flask and the volume was made up to the mark. The solution was suitably diluted with distilled water to get of 12 μg/mL of DRT and 13.5 μg/mL of ETR. Instrument response for the analytes was measured by following the procedure described in preparation of standard stock solutions and calibration curve section.

Accuracy

Recovery studies were carried out by applying the method to drug content present in tablet dosage forms to which known amount of mixed standard of ETR and DRT was added at 50%, 100%, and 150% levels. At each of the levels, three determinations were performed.

Precision

The precision of repeatability was studied by six replicate analysis of tablet solutions containing 12 μg/mL of DRT. The precision was also studied in terms of intra-day changes in absorbance of drug solution on the same day and on three different days. The intra-day precision of the developed method was determined by preparing the tablet samples of the same batch in nine determinations with three concentrations and three replicate each on the same day. The inter-day precision was also determined by assaying the tablets in triplicate per day for consecutive 3 days. The intra-day and inter-day variations were calculated in terms of percentage relative standard deviation. Precision of the analyst was also determined by repeating the method by another analyst working in the lab. Three concentration used for the study were 6, 12, and 18 μg/mL of DRT and 7.25, 13.5, and 20.75 μg/mL of ETR. Precision data were also analysed by using experimental design based on two-way ANOVA.

Method sensitivity (LOD and LOQ)

The values of LOD and LOQ were calculated by using σ (standard deviation of response) and b (slope of the calibration curve) and by using equations, LOD = $(3.3 \times \sigma)/b$ and LOQ = $(10 \times \sigma)/b$.

Robustness

To determine the robustness of the method, the final experimental conditions were purposely altered and the results were examined. The parameters considered (±values) for the study were sonication (extraction) time of solution (±5 min), wavelength of measurement (±2 nm), and concentration of DRT in the reference cell (±2 µg/mL).

RESULTS AND DISCUSSION

Baseline manipulation method

Beer's and Lamberts law[20] is defined that when a beam of monochromatic radiation is passed through a solution of absorbing molecules, the rate of decrease of intensity of incident radiation with thickness (l) of the absorbing solution is proportional to the intensity of incident (I_0) radiation as well as the concentration (c) of the solution and mathematical expression of the law is,

$$\text{Log } I_0/I = \varepsilon \times c \times l = A \quad \ldots (1)$$

where I is intensity of transmitted light, ε is molar absorptivity, c is the concentration of solution in moles/litre, l is the path length, and A is absorbance (Log I_0/I). In the double beam spectrophotometer a blank is used to eliminate the contribution to absorbance by solvents. Under the situation the modified equation applicable is

$$A_{\text{observed}} = [\varepsilon \times c \times l]_{\text{sample}} - [\varepsilon \times c \times l]_{\text{blank}} \quad \ldots (2)$$

and on this basis the UV spectrum is obtained. By keeping solution of analyte(s) of appropriate concentration in the blank it is possible to obtain independent wavelength(s) in spectra for each analyte(s) that form the mixture which is the basis of the baseline manipulation method, newly developed analytical methodology by the authors.

For the simultaneous determination using the baseline manipulation method, solutions of suitable conc. of DRT and ETR were prepared from standard stock solution in distilled water. Mixtures of standard solutions were scanned in the range of 200–400 nm by keeping these solutions as blank. When DRT 20 µg/mL was used as blank, linear response of both the analytes was observed in different portions of the spectra. Wavelengths were selected suitable for each analyte, instrument responses were measured at the selected wavelengths and used for preparation of the calibration curve. It is easier to apply the baseline manipulation method when overlay spectra of analytes show well-resolved peaks.

Overlain spectra of ETR (E 9–45 µg/mL) and DRT (D 8–40 µg/mL) in methanol water are shown in Figure 1. A typical baseline manipulation spectrograph of ETR and DRT in combination with DRT 20 µg/mL used as blank is shown in Figure 2, and individual DRT spectra against solvent blank are also shown in Figure 2.

Types of baseline manipulation methods

Singular baseline manipulation

In this method composition of blank remains constant throughout the experiment. The method has certain advantages such as less time is required compared to other UV methods, less number of dilution(s) are required, and the method is suitable for binary mixtures of analytes.

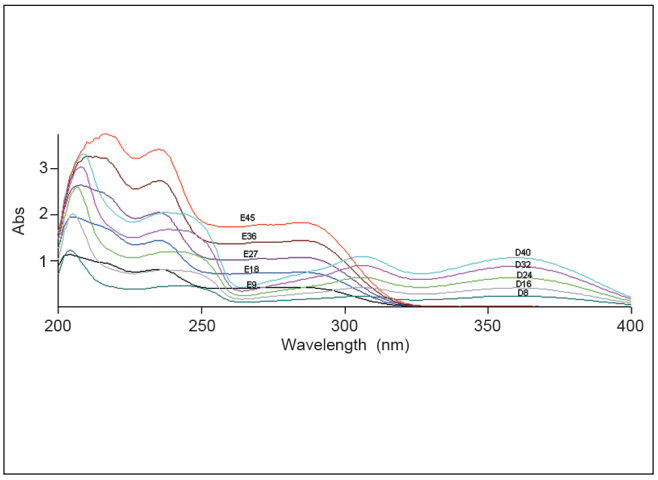

Figure 1: Overlain spectra of ETR (E 9–45 µg/mL) and DRT (D8–40 µg/mL)

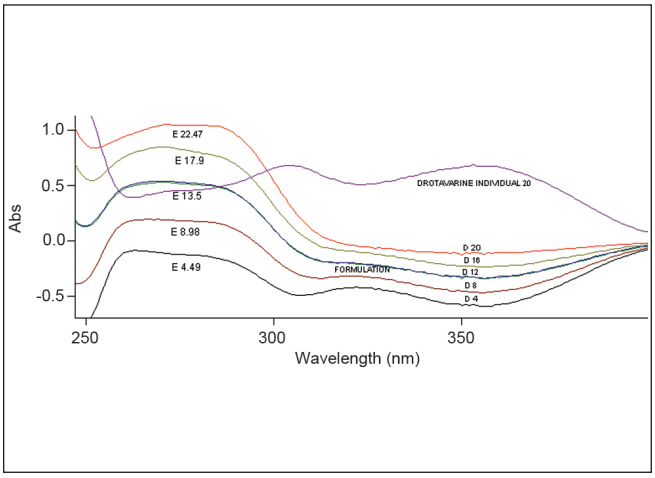

Figure 2: Typical baseline manipulation spectrograph of ETR and DRT in combination when DRT 20 µg/mL was used as blank, individual drotaverine spectra against solvent blank is also shown

Multiple baseline manipulation

Composition of the blank is changed to estimate different analytes form the mixture. The method has advantage over the Singular baseline manipulation (SBM) since it can be used for ternary mixtures of analytes. In these types of methods, the spectrum of sample-containing mixture of two drugs shows two different peaks, from which suitable analytical wavelength can be assigned to each analyte. This is free from inferences by other analytes in the mixture.

Method validation

The newly developed method was validated according to the ICH guidelines with respect to method sensitivity, linearity, range, accuracy, precision, robustness, and specificity.

Linearity and method sensitivity

Typically, the regression equations for the calibration curve was found to be $y = -0.02938X + 0.691341$ for DRT for a concentration range of 4–20 μg/mL and $y = 0.066701X - 0.39853$ for ETR for a concentration range of 4.5–22.5 μg/mL. The correlation coefficient ('*r*') values were >0.999 (n=6). In the test of linearity, i.e. Fischer variance ratio, calculated values of F (F_{cal}) for both the analytes were less than tabulated F values (F_{Tab}) which indicate linearity of response. Limits of detection (LOD) were found to be 0.42 μg/mL for DRT and 0.55 μg/mL for ETR. Limits of quantification (LOQ) were found to be 1.26 μg/mL for DRT and 1.65 μg/mL for ETR.

Formulation analysis and accuracy studies

Newly developed methodology was used to determine assay of the marketed tablets with present spectrographic conditions, and it was found to be accurate and reliable. The average drug content was found to be 99.98% for DRT and 100.12% for ETR of the labelled claim. No interfering peaks were found in the spectrograph, indicating that the estimation of drug free from inference of excipients. The results of the recovery study were in the range of 98.40–101.13% and % RSD was always less than 0.89. The results for formulation analysis and accuracy studies are presented in Table 1.

Precision

Assay values during the precision study were in the range of 98.8–100.7 and % RSD values were always less than 0.6. The results obtained for inter-day and analyst precision were statistically tested and presents in Table 2 for DRT. The method precision is shown by results of the ANOVA test for both the analytes where calculated F (F_{cal}) values were always less than tabulated F (F_{tab}) values for both the analytes.

Robustness

To determine the robustness of the method, the final experimental conditions were purposely altered and the results were examined. Effects of variation of experimental conditions were studied which shows that the assay and % RSD values were well within the limits [Table 3].

Specificity study

Specificity of the method was determined by comparing the absorbance values of the standard mixture of drugs and the formulation sample at specified wavelengths for both drugs. Mean of three absorbance values of the standard mixture and the formulation sample at 4–20 μg mL^{-1} for DRT and 4.5–22.5 μg mL^{-1} for ETR were compared by the *t*-test. Calculated t values (t_{cal} = 1.563) where values were

Table 1: Optical characteristics of the method and results of formulation analysis, recovery study, and method sensitivity

Parameters		Analyte name	
		DRT	ETR
λ_{max} (nm)		351	274
Range (μg/mL)		4–20	4.5–22.5
Regression coefficients	Slope	−0.02938	0.066701
	Intercept	0.691341	0.39853
Method sensitivity	LOQ	0.42	0.55
	LOD	1.26	1.65
Formulation analysis (% Assay, % RSD)	F I	99.65, 0.77	99.98, 0.56
	F II	101.3, 0.53	98.83, 0.71
Recovery study at the level (% recovery, % RSD)	50%	100.19, 0.59	98.40, 0.42
	100%	101.09, 0.97	101.13, 0.87
	150%	99.80, 0.31	99.79, 0.89

Table 2: Results of precision study

Source of variation	Sum of square	Degree of freedom	Mean of square	*F*-ratio	5% *F*-limit
Between time	65	2	32.5	−3.19672	5.14
Between time and analyst	94	3	31.333	−3.08197	4.76
Residual	-61	6	–10.167		
Total	98	11			

Table 3: Results of robustness study (n=3)

Factor	Level	Mean % assay, % RSD	
		DRT	ETR
Shaking time (min) (± 2 min)	10	99.18, 0.87	99.99, 0.06
	6	98.08, 0.20	100.16, 0.22
Measurement wavelength (nm) (± 2 nm)	272 (ETR), 349 (DRT)	99.45, 0.53	100.05, 0.77
	276 (ETR), 353 (DTR)	99.08, 0.47	99.30, 0.28
Concentration of DTR in blank (± 0.1 μg/mL)	21	99.09, 0.78	101.16, 0.96
	19	100.03, 0.18	100.04, 0.22

Table 4: Specificity study

Concentration (µg/mL) of mixture		Abs. standard (*n*=3)	Abs. sample (*n*=3)	Difference (D)	D-mean
DRT	ETR				
4	4.5	−0.579	−0.577	1.8×10^{-3}	1.66×10^{-6}
8	9	−0.456	−0.46	3.3×10^{-4}	3.24×10^{-8}
12	13.5	−0.326	−0.33	2×10^{-5}	2.4×10^{-7}
16	18	−0.225	−0.225	2×10^{-4}	9.61×10^{-8}
20	22.5	−0.123	−0.125	2×10^{-4}	9.61×10^{-8}

Table 5: Comparison of results by one way ANOVA

Number	Baseline manipulation	First-order derivative	Ratio derivative	Absorbance correction
1	99.3	98.8	101.2	100.3
2	100.4	101.5	99.4	100.7
3	101.1	100.5	100.5	99.5
4	99.5	99.6	100.2	99.8
5	98.5	100.4	99.6	101.4
6	100.3	99.2	99.2	100.6
Average	99.85	100	100.02	100.38
SD	0.92	0.989	0.76	0.68
% RSD	0.93	0.98	0.79	0.67

Table 6: ANOVA table for drotaverine

Source of variation	Degree of freedom	Sum of square	Mean square	F_{cal} value	F_{tab} value
Treatments (between methods)	3	0.924	0.308	0.3421	3.24
Residual (within methods)	16	14.411	0.900		
Total	–	15.336	–		

less than the tabulated *t* (t_{tab}, 2.785) values for both the analytes and this proves that there is no significant difference between the standard mixture of drugs and the formulation sample and thus the specificity of method. Overlay spectra of the standard mixture and formulation solution is similar as shown in Figure 1, which further proves the specificity of the method [Table 4].

STATISTICAL COMPARISON OF THE RESULTS

The results of the baseline manipulation method were compared with the laboratory developed first order-derivative method (FD), ratio derivative (RD), and absorption corrected (AC) methods (unpublished data). The results of ANOVA for DRT are shown in Table 5. Calculated *F* values (F_{cal}) were determined by MIP Pharmasoft 1.0, and these values for both the analytes were less than tabulated *F* (F_{tab}) values. As the F_{cal} values are less than the F_{tab} values for both drugs it can be concluded that there is no significance difference among these methods and hence the baseline manipulation method is equivalent to these three methods [Table 6].

CONCLUSIONS

The newly developed UV spectrophotometric baseline manipulation method was found to be simple, sensitive, accurate, precise, and specific and can be used for the routine quality control analysis of ETR and DRT in combination. The same concept can be extended for quantitative analysis of other binary and ternary combinations of the analytes in pharmaceuticals. As the method could effectively separate the drugs from each other in a single spectrometric scan, it reduces human efforts and errors as well.

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