Research Article **Open Access**

Simplistic Application of 3-Methy-2-Benzothiazoline Hydrazone (MBTH), an Oxidative Coupling Chromogenic Reagent for Quantification of Metaxalone and Dabigatran Etexilate Mesylate Bulk Drug and Their Dosage Forms

Pani Kumar DA*, Archana G, Sunitha G, Rachel Paul K, Harika R and Sowndarya NSKR

Department of Pharmaceutical Analysis and Quality Assurance, Gokaraju Rangaraju College of Pharmacy, Osmania University, Hyderabad, India

Abstract

Purpose: A simple and sensitive spectrophotometric method in visible region has been developed and validated for quantification of metaxalone (MET) and dabigatran etexilate mesylate (DAB) in their bulk and pharmaceutical dosage forms.

Methods: The method is based on the oxidative coupling reaction of 3-methyl-2-benzothiazoline hydrazone (MBTH) with MET and DAB in the presence of ferric chloride to form green coloured chromogen with absorption maximum at 666 nm and 632 nm respectively.

Results: Beer's law is obeyed in concentration range of 4-20 and 1-6 µg/mL for MET and DAB respectively with correlation coefficient of 0.999. Limit of detection and quantification were 0.46 µg/mL and 1.518 µg/mL for MET and 0.0578 µg/mL and 0.298 µg/mL for DAB. When marketed formulations were analyzed, the results obtained by the proposed method were in good agreement with labelled amounts. The developed method was validated statistically as per ICH guidelines.

Conclusion: The developed method is simple, sensitive, specific and can be successfully employed in routine analysis of MET and DAB pharmaceutical dosage forms.

Keywords: MBTH; Metaxalone (MET); Dabigatran etexilate mesylate (DAB); Visible spectrophotometry

Introduction

Metaxalone (MET) which is chemically 5-(3, 5-dimethylphenoxymethyl)-1, 3-oxazolidin-2-one (Figure 1.A) is a muscle relaxant used to relieve pain caused by strain, sprains and other musculoskeletal conditions [1] Dabigatran etexilate(DAB) is chemically ethyl 3-(1-{2-[({4-[amino({[(hexyloxy)carbonyl]imino}) methyl]phenyl}amino)methyl]-1-methyl-1H-1,3-benzodiazol-5-yl}-N-(pyridin-2-yl)formamido)propanoate (Figure 1). DAB is oral anticoagulant. It is a pro-drug which is converted to its active form, by esterase-catalyzed hydrolysis in the plasma and liver. DAB is competitive and reversible direct inhibitor of thrombin.

A detailed literature survey revealed that few analytical methods have been reported for the determination of MET by UV Spectrophotometry, RP–HPLC [2-5], HPTLC [6], hydrotropic solubilisation technique using UV spectroscopy [7] and HPLC [8,9], Where as normal UV spectroscopic method [10] for the determination of DAB. It concluded that there is no visible spectrophotometric method available for quantification of MET and DAB. Even the reported spectrophotometric methods are not so sensitive; require high concentrations of organic solvents and extraction procedures. Therefore according to the need, this work presents a simple, specific and sensitive visible spectrophotometric method for the quantification of MET and DAB based on its oxidative coupling interaction with 3-methyl-2-benzothiazoline hydrazone (MBTH) [11,12]. The proposed method was validated as per ICH guidelines.

Methodology

Materials

MET standard gift sample was provided by Sun Pharma and marketed tablet dosage form Flexura 400 was acquired from local pharmacies. DAB pure drug was a gift sample provided by Neuland laboratories limited and commercial hard gelatin capsule dosage form Pradaxa -110 mg was acquired from local pharmacies.

Chemicals and reagents: All chemicals and reagents used were of analytical grade and were freshly prepared in distilled water.

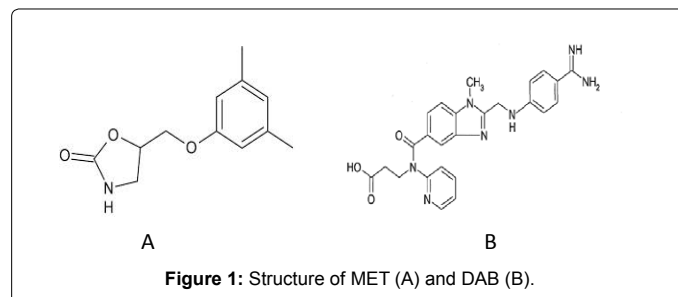**Preparation of 0.9%w/v of MBTH reagent:** The reagent 900 mg

Figure 1: Structure of MET (A) and DAB (B).

*Corresponding author: Panikumar DA, Gokaraju Rangaraju College of Pharmacy, Department of Pharmaceutical Analysis, Hyderabad, Telangana State -500090, India, Tel: 040-32912927; E-mail: panindrapharma@yahoo.co.in

was accurately weighed and dissolved in few mL of distilled water, further diluted upto 100 mL with distilled water.

Preparation of 3%w/v of ferric chloride solution: Ferric chloride 3 g was accurately weighed and dissolved in few ml of 0.1N HCl, further diluted upto 100 mL with 0.1N HCl.

Instrument: Double–beam Shimadzu UV–Visible Spectrophotometer 1800, with spectral bandwidth of 0.1 nm, wavelength accuracy ± 0.1 nm and a pair of 1 cm path length matched quartz cells were used to measure absorbance of the resulting solution.

Preparation of standard stock solutions

Preparation of MET standard stock solution: Accurately weighed 10 mg of MET (bulk drug) was dissolved in few mL of methanol in 10 mL volumetric flask and volume was made upto the mark with methanol to get 1000 µg/mL. From the above stock solution, 1 mL was pipetted out into 10 mL volumetric flask and the volume was made upto the mark with methanol water(50:50) to obtain final concentration of 100 µg/mL.

Preparation of DAB standard stock solution: 10 mg of DAB was weighed accurately and dissolved in few mL of methanol in 10 mL volumetric flask and volume was made upto the mark with methanol to obtain 1000 µg/mL solution. Further dilutions were done to obtain 100 µg/mL solutions.

Procedure for determination of MET and DAB: Aliquots of standard drug solutions of MET ranging from 0.4-2.0 mL and DAB ranging from 0.1-0.6 mL were taken into a series of 10 mL volumetric flasks and aqueous solution of 0.9% w/v MBTH (2 mL), 3 %w/v ferric chloride in 0.1 N HCl (2 mL) were added. The solutions were finally made up to the mark with water and were kept aside for 15 minutes and 60 min for MET and DAB respectively. The absorbance of the green coloured chromogen was measured at 666 and 632 nm for MET and DAB respectively against the corresponding reagent blank. The linearity of calibration curve (absorbance vs concentration) in pure solution was checked over concentration ranges of about 4-20 µg/mL for MET and 1-6 µg/mL for DAB. The mean ± standard deviation for the slope, intercept and correlation coefficient of standard curves (n=6) were calculated.

Assay of MET and DAB in their respective dosage forms: The proposed method was applied to commercially available marketed formulations of MET tablets (Flexura 400) and DAB capsules (Pradaxa 110). Accurate quantity equivalent to 10 mg of active ingredient was dissolved in methanol and the volume was made upto 10 mL with methanol. Subsequent dilutions of this solution were made to obtain the required concentration within the linearity range and similar procedure was followed as that of the standard. The absorbance of the green coloured chromogen was measured at selected wave length against the corresponding reagent blank and analyzed for drug content and their results were statistically validated. There are no official standard methods available to show a real amount of MET and DAB in the dosage form till now, so that self-punched tablets of DAB and MET were made with the common excipients (lactose, starch, microcrystalline cellulose, magnesium stearate, titanium dioxide and talc) of the pharmaceutical formulation. (as prescribed under hand book of excipients) and evaluated by the proposed methods in order to check if any component of the formulation could generate a response (absorbance) similar to the drugs.

Method Validation

The method was validated according to the ICH guidelines to evaluate the overall performance of qualitative analytical method with respect to certain parameters such as linearity, precision, accuracy, and sensitivity [13].

Linearity

Calibration curves were plotted for both MET and DAB. The linearity (absorbance vs concentration) in pure solution was checked over concentration ranges of about 4-20 µg/mL for MET and 1-6 µg/mL for DAB. The mean ± standard deviation for the slope, intercept and correlation coefficient of standard curves (n=6) were calculated.

Sensitivity

The sensitivity of the method was determined with respect to LOD and LOQ. LOD is the lowest amount of an analyte in a sample that can be detected and LOQ is the lowest amount of an analyte in a sample that can be quantified with acceptable precision and accuracy under the stated experimental conditions. The LOD and LOQ levels shall be predicted based on the standard deviation and slope of the constructed calibration curve.

Precision

Precision is the degree of agreement among the individual test results when the procedure is applied repeatedly to multiple portions of a homogeneous sample. Precision of the method was determined by intra-day and inter-day precision as per ICH guidelines. For both intra-day and inter-day precision of the samples containing 8, 12, 16 µg/mL for MET and 2, 4, 6 µg/mL for DAB were analyzed six times on the same day (intra-day precision)and for three consecutive days (inter-day precision). The % RSD was calculated.

Accuracy

The accuracy of the method was determined by calculating recoveries of both the drugs by standard addition method. Three different levels (80%, 100% and 120%) of standards were spiked to pre quantified samples in triplicate. The mean of percentage recoveries and % RSD were statistically calculated.
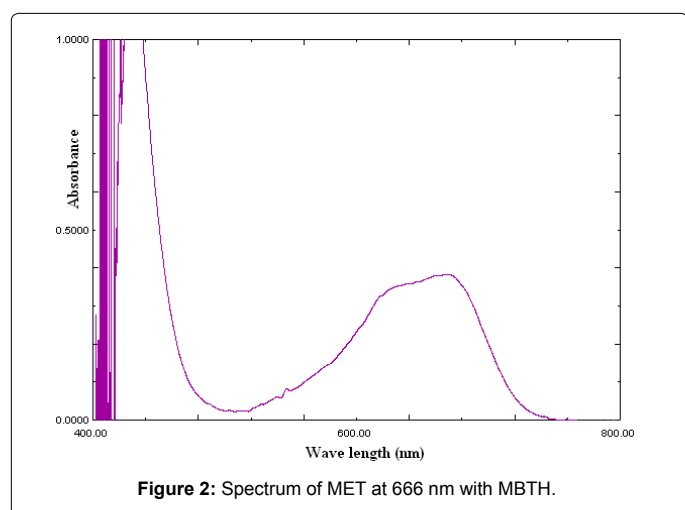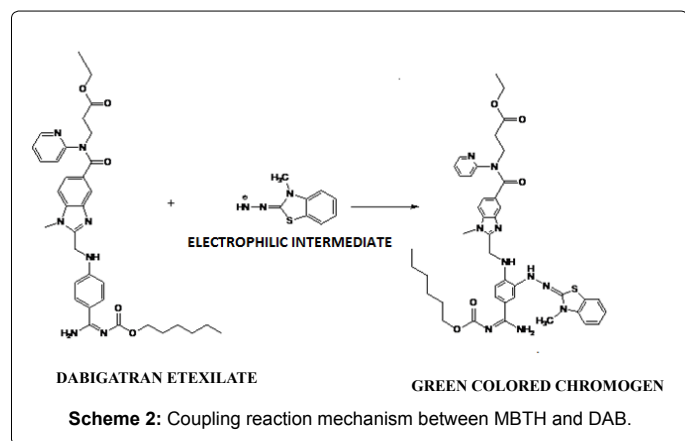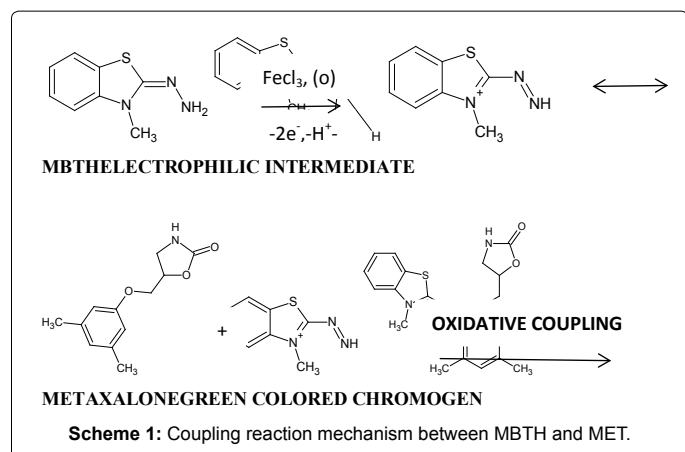
Results and Discussion

Principle

The proposed colorimetric method is based on the oxidative coupling reaction between MBTH and drug molecule in the presence of oxidizing agent such as ferric chloride. Ferric chloride oxidizes MBTH to form an electrophilic intermediate by losing two electrons and one proton. The electrophilic intermediate forms green colored chromogen by coupling with the most nucleophilic site of MET and DAB as shown in Schemes 1 and 2. The absorbance of the green colored chromogen is measured at visible wavelength of 666 nm and 632 nm, Figures 2 and 3. The oxidative coupling reactions of DAB and MET with MBTH were time dependent, shown in Figure 4. The absorbance of DAB and MET were reached maximum at 60 min and 15 min respectively and stable upto 30 min.

Method validation

The method was validated according to the ICH guidelines.

Linearity

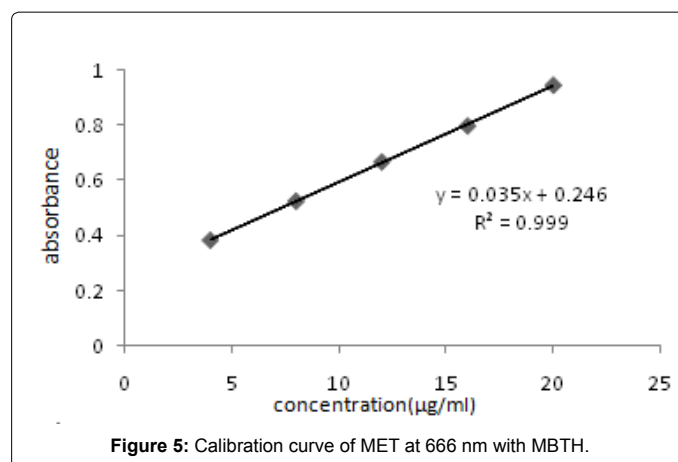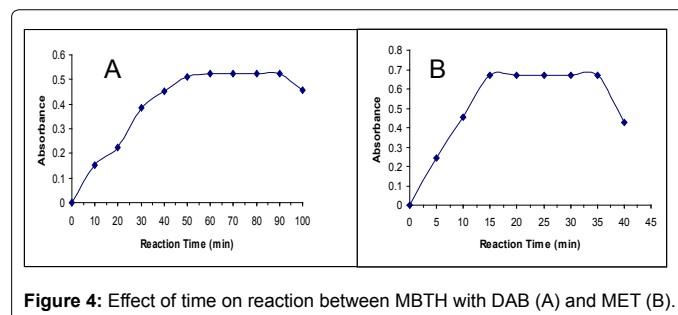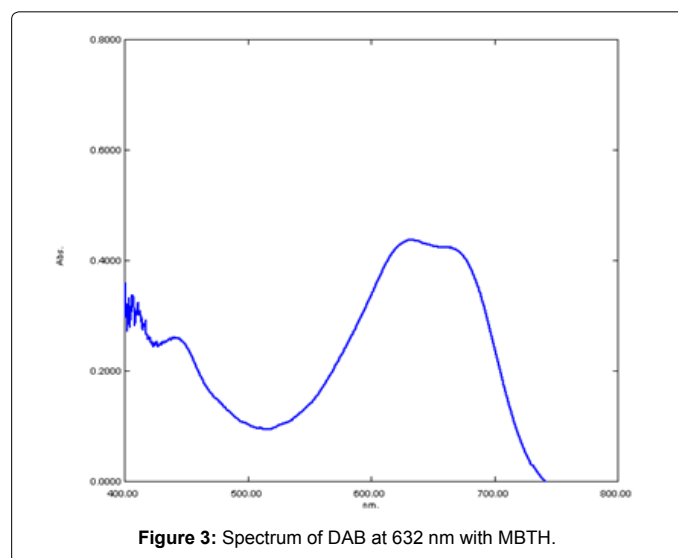The developed method follows the beer's law in the concentration

Scheme 1: Coupling reaction mechanism between MBTH and MET.

Scheme 2: Coupling reaction mechanism between MBTH and DAB.

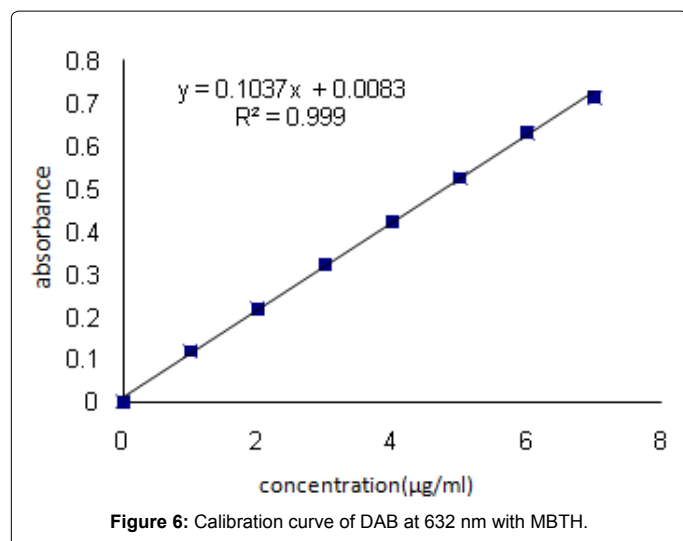**Figure 2:** Spectrum of MET at 666 nm with MBTH.

Sensitivity

The sensitivity of the method was determined with respect to LOD and LOQ. These were separately determined based on standard calibration curve using 3.3 σ/s and 10 σ/s, formulae respectively, where s is the slope of the calibration curve and σ is the standard deviation of y-intercept of the regression equation. Results are shown in Table 1.

Precision

Repeatability (intra-day) and intermediate precision (inter-day) were assessed using three concentrations and three replicates of each

Figure 3: Spectrum of DAB at 632 nm with MBTH.

Figure 4: Effect of time on reaction between MBTH with DAB (A) and MET (B).

Figure 5: Calibration curve of MET at 666 nm with MBTH.

range of 4-20 µg/mL with regression equation Y=0.035x + 0.246 and correlation value 0.999 for MET and concentration in the linearity range of 1-6 µg/mL with regression equation Y=0.1037x + 0.0083 and correlation value 0.9995 for DAB. The concentration vs absorbance plot is shown in Figures 5 and 6. The analytical data of the calibration curves including standard deviations for the slope and intercept and system suitability parameters are summarized in Table 1. This data indicates the linearity of the calibration graphs.

Figure 6: Calibration curve of DAB at 632 nm with MBTH.

Parameters	MET	DAB
Absorption Wavelength (nm)	666	632
Beers law range (µg/mL)	4-20	1-6
Molar absorptivity (L mol /cm)	0.00486	0.00075
Sandell sensitivity[a], (µg/cm)	0.1744	0.0083
Limit of Detection (µg/mL)	0.46	0.0478
Limit of Quantification (µg/mL)	1.158	0.248
Correlation coefficient (r^2)	0.999	0.9995
Slope (m)	0.035	0.1037
Intercept (c)	0.246	0.0083
Regression equation(y^b)	Y=0.035x + 0.246	Y=0.1037x+0.0083

[a]limit of determination as the weight in µg per mL of solution which corresponds to an absorbance of A=0.001 measured in a cuvette of cross sectional area 1 cm^2 and l=1 cm. y^b =a+bx, where y is the absorbance, x is concentration in µg/mL, a is intercept and b is slope.

Table 1: System Suitability Parameters of MET and DAB.

Concentration (µg/mL)	Intra-day precision		Inter-day precision	
	Concentration estimated (µg/mL) A.M ± SD[a]	% RSD[b]	Concentration estimated (µg/mL) A.M ± SD[a]	% RSD[b]
MET				
8	8.04 ± 0.01	0.166	8.06 ± 0.023	0.3833
12	12.12 ± 0.03	0.5	11.82 ± 0.017	0.2833
16	15.86 ± 0.05	0.833	15.88 ± 0.092	1.533
DAB				
2	2.02 ± 0.002	0.990	2.01 ± 0.004	0.199
4	3.98 ± 0.02	0.502	4.02 ± 0.011	0.273
6	6.00 ± 0.014	0.230	6.01 ± 0.012	0.199

[a]Mean value of 6 determinations
[b]Relative standard deviation (%)

Table 2: Precision for MET and DAB.

concentration. The results for precision studied are summarized in Table 2. The relative standard deviations were found to be very small (<2%) indicating reasonable repeatability and intermediate precision of the proposed method.

Accuracy

The accuracy of the method was determined by calculating

Brand name	Recovery level (%)	Theoretical content (mg)	Amount found (mg) ± SD*	% amount recovered	RSD (%)
FLEXURA 400(MET)	80	18	18.2 ± 0.045	101.1	0.9
	100	20	20.22 ± 0.078	100.6	1.56
	120	22	22.2 ± 0.068	101.4	1.36
PRADAXA 110 mg (DAB) (DAB)	80	9	8.91 ± 0.0702	99	0.78
	100	10	9.99 ± 0.014	99.9	0.14
	120	11	10.9 ± 0.0115	99.9	0.10

*Mean value of 3 determination

Table 3: Accuracy (Recovery studies) for MET and DAB.

Formulation	Active ingredient	Label claim (mg)	Amount found (mg) A.M ± SD[a]	% Assay	% RSD[b]
Flexura- 400	MET	400	396.2 ± 0.097	99.05	0.024
Pradaxa-110 mg	DAB	110	111 ± 0.11	100.9	0.099
[a]Mean of three determinations					
[b]Relative standard deviation (%)					

Table 4: Assay of MET and DAB using MBTH.

Formulation	DAB (110 mg)		MET (400 mg)	
	AM ± SD	% Assay	AM ± SD	% Assay
Bulk drug	110.5 ± 0.043	100.45	400.2 ± 0.027	100.05
Marketed Formulation	111.0 ± 0.11	100.9	396.2 ± 0.097	99.05
Self -punched tablets	110.9 ± 0.08	100.81	399.6 ± 0.105	99.90

Table 5: Comparison of assay values of marketed formulations with bulk drugs and self punched tablets.

recoveries of both the drugs by standard addition method. The % recovery values for the method are shown in Table 3. The satisfactory recovery percentage results for MET and DAB indicate the accuracy of the method.

Application of the method to the marketed formulations

The proposed method was applied to standard drugs, self-punched and commercially available marketed formulations of MET (Flexura 400) and DAB (Pradaxa). The amount of drug was found to be 99.05 and 100.9 % for marketed formulations of MET and DAB respectively, as shown in Table 4. The sample recoveries in the formulation were in good agreement with their respective label claim and compared the assay values with standard drugs and self-punched tablets recoveries, which are reported in Table 5, revealed that there was no significant difference observed between them, which suggested no interference of formulation excipients in the estimation of the active ingredient.

Conclusion

It is concluded that the proposed method was found to be simple, sensitive, accurate and precise for the quantification of MET and DAB in pharmaceutical dosage forms. The assay values were in good agreement with their respective label claim. This spectrophotometric method has been found to be better because of its specificity, sensitivity, no extraction procedures and low concentration of solvent. These advantages encourage that the proposed method can be routinely employed in quality control for analysis of MET and DAB in the pharmaceutical dosage forms.

References

1. Brayfield, Alison (2009) Martindale: The Complete Drug Reference. London, UK: The Pharmaceutical Press.

2. Narsimha Rao R (2011) Method Validation of Metaxalone drug by using UV Spectroscopy. Int J Pharm Bio Sci 1: 284-302.

3. Mahipal reddy NV, Mohammed Isaq B, Thiruvengada Rajan VS (2013) Analytica Method development and Validation of Metaxalone in bulk and its pharmaceutical formulation by UV Spectroscopic method. Int Res J Pharm 4: 149-152.

4. Padmakana M, Arup RD, Ahmed S, Yeasmin N (2013) Development of analytical methods for the Estimation of metaxalone in pure and solid dosage Forms by UV - spectrophotometric and RP-HPLC Methods. Int J Pharm and Thera 4: 212-224.

5. Prafulla Kumar Sahu, M Mathrusri Annapurna, Sahoo Dillip Kumar (2011) Stability indicating RP – HPLC method development and validation for the determination of metaxalone in bulk and its formulations. J Chem 8: 439-447.

6. Milindkumar PR, Vishal Bharekar V, Savita Yadav S, Toufik Mulla S, Jahnavi Rao R (2011) Validated HPTLC method for simultaneous estimation of Diclofenac potassium and Metaxalone in Bulk Drug and Formulation. Int J Com Pharm 2: 1-4.

7. Priyadharisini J, Gigi GP, Niraimathi V, Jerad Suresh A (2011) Hydrotropic solubilisation technique of metaxalone by UV Spectroscopy and first order derivative spectroscopy. Res J Pharm Tech 4: 72.

8. Pradeep SG, Chandewar AV (2014) Validated stability-indicating high performance liquid chromatographic assay method for the determination of Dabigatran Etexilate Mesylate. Res J Pharm Bio Chem Sci 5: 1637-1644.

9. Sekhar Reddy BRC, Nallagatla, Vijaya B Rao (2014) A Stability indicating RP-HPLC method for estimation of dabigatran in pure and pharmaceutical dosage forms. Sou Pacific J Pharm Bio Sci 2: 81-92.

10. Ankit P, Sharad K, Ashim KS, Aarti Z, Seth AK (2014) Spectrophotometric method for estimation of dabigatran Etexilate in bulk and its pharmaceutical dosage form. Int J Pharm Sci 5: 31-39.

11. Panikumar AD, Kavitha A, Vijaya DD, Himabindu S, Sunitha G (2013) Spectrophotometric Quantification of Tadalafil bu Oxidative Coupling reaction with MBTH Reagent. Analytical chemistry – an Indian Journal 13: 11-16.

12. Michael E, Schartz IS, Krull (2004) Analytical method development and validation (3rd edn) John Wiley and sons, London, 25-46.

13. 2005 ICH Harmonised Tripartite Guideline. Validation of Analytical Procedure Text and Methodology Q2 (R1).