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SENSITIVE AND SELECTIVE EXTRACTION FREE ION-PAIR COMPLEXOMETRIC DETERMINATION OF METFORMIN IN BULK DRUG AND PHARMACEUTICAL FORMULATIONS

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Abstract

Two simple, accurate, rapid and fairly sensitive spectrophotometric methods are described for the determination of metformin hydrochloride (MET) in bulk drug and its tablets. The methods are based on the formation of yellow ion-pair complexes between MET and two sulphonphthalein dyes viz., bromophenolblue (method A) and bromothymol blue (method B) in chloroform medium. The chromogen was measured quantitatively at 420 and 415 nm respectively, for method A and method B. At these wavelengths, Beer's law is obeyed over the concentration range of 1.8–36.0 and 2–32.0 µg mL^{-1} for method A and method B respectively. The apparent molar absorptivity, limit of detection (LOD) and quantitation (LOQ) values are 2.04×10^3 l mol^{-1} cm^{-1}, 0.49 and 1.5 µg mL^{-1} for method A and 3.79×10^3 l mol^{-1} cm^{-1}, 0.04 and 0.11 µg mL^{-1} for method B. The reaction is extremely rapid at room temperature and the absorbance values remain unchanged up to 2 hrs and 4 hrs respectively for method A amd method B. All variables were studied in order to optimize the reaction conditions. The composition of the ion-pairs was found to be 1 : 1 by Job's method and the conditional stability constant (K_f) of the complexes have been calculated. The proposed methods were successfully applied to the analysis of the bulk drug and its tablets. No interference was observed from common pharmaceutical adjuvants. Statistical comparison of the results with those of an official method showed excellent agreement and indicate no significant difference in precision and accuracy.

Keywords:

Metformin HCl, Assay, Spectrophotometry, dyes, Pharmaceuticals, Tablets.

Introduction

Metformin hydrochloride (MET), chemically known as 1,1-Dimethylbiguanide monohydrochloride (Fig.1) is an oral antidiabetic drug in the biguanide class. It is the first-line drug of choice for the treatment of type 2 diabetes, in particular, in overweight and obese people and those with normal kidney function[1,2]. Metformin decreases hyperglycemia primarily by suppressing glucose production by the liver (hepatic gluconeogenesis)[3]. The drug is official in United State Pharmacopoeia[4] and European Pharmacopoeia[5], both recommend titrimetric assay for MET.

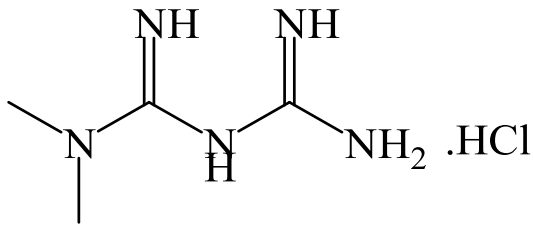


Fig.1: Structure of metformin HCl.

Analytical literature about the determination of this drug when it is present alone in dosage form is very scarce. Of many UV-specetrophotometric methods[6-18], only two procedures are applicable for single dosage forms[6,7]. Likewise, high performance liquid chromatography has been sparsely used for the assay of MET in single dosage tablets[19-22] compared to multi - component dosage forms[23-30]. MET and related substances[31] and combined dosage forms[32] have been assayed by ultra performance liquid chromatography. Capillary electrophoresis[33], spectroflurimetry[12,34] and ion-selective electrode (ISE) based potentiometry [34-36] are the other techniques reported for the assay of this drug in single component tablets. The above mentioned techniques, although sensitive, require several time consuming operations, sophisticated instruments and skilled personnel. Further, access to these techniques is limited for most clinical and quality control laboratories. It is apparent that less- cumbersome, rapid, simple, sensitive and inexpensive technique is required for the assay of drug in its dosage forms.

Visible spectrophotometry can be considered as potent alternative because of its inherent simplicity, sensitivity, accuracy and precision and fair selectivity. However, a perusal of literature reveals that there are only two reports dealing with the assay of MET by visible spectrophotometry, of which, the procedure based on ethylene chloride soluble 1:1 ion pair formation with bromythymol blue[37] has been applied for urine, thus leaving a solitary method [34] for pharmaceuticals in which the complex formed by the drug with Cu(II) in basic medium is measured at 540 nm. This

method is poorly sensitive with a linear range of 500-2000 µg mL^{-1}. The need for sensitive and reliable method for the determination of MET in pharmaceuticals is, thus clearly recognized.

The present paper describes two spectrophotometric methods that can be applied as an alternative to more time consuming complicated and expensive methods[6-36]. These methods are based on the formation of chloroform soluble ion pair complexes between MET and two sulphonthalenein dyes; bromophonol blue (method A) and bromothymol blue (method B), where the complexes were measured without extraction in contrast to the procedure applied for urine[37].

These methods offer better performance characteristics (higher sensitivity and better detection limits) than those reported previously including the lone visible spectrophotometric method[37].

Experimental

Apparatus

All absorbance measurements were made on a Systronics model 106 digital spectrophotometer (Ahmedabad, India) equipped with1-cm matched quartz cells.

Materials and reagents

All chemicals and reagents used were of analytical-reagent grade. The solvents used were of HPLC-grade. Reagents used included bromophenol blue (BPB) and bromothymol blue (BTB) supplied from Loba Chemie, Mumbai, India. Chloroform and sodium hydroxide was provided by Merck, Mumbai, India. The water was always twice distilled from all glass equipments. Redistillation was carries out from alkaline permanganate solution.

Standard metformin base (MEB) Solution

Pharmaceutical grade MET certified to be 99.9% was kindly provided by Sanofi Aventis, Mumbai, India. A stock standard solution containing 200 µg mL^{-1} MEB was prepared as follows: pure metformin hydrochloride (25.6 mg) dissolved in 20 mL water in a 125 mL separating funnel, 5 mL of 10% NaOH was added followed by 20 mL of chloroform. The content was shaken for 15 minutes. The lower organic layer was collected in a beaker containing anhydrous sodium sulphate. The water-free organic layer was transferred into a 100 mL calibrated flask and diluted to the volume with the same solvent to get 200 µg mL^{-1} with respect to MEB (hydrochloride free metformin). This solution was diluted to get 45 and 40 µg mL^{-1} of MEB solution for method A and method B respectively.

Two brands of tablets containing MEB, Glyciphage 500 mg manufactured by Franco Indian Pharmaceuticals pvt. Ltd. Mumbai, India, and Cetapin XR 500mg manufactured by Sanofi Aventis, Mumbai, India, used in the investigation were purchased from local commercial sources.

BPB and BTB (both 0.2 %) solution

Transferred 200 mg each of BTB and BPB in two different 100 mL volumetric flasks. Added about 20 mL each of chloroform separately, shaken gently until the dyes dissolved completely and made up with chloroform.

General procedures

Method A: Aliquots of 0.20, 0.5, 1.0, 2.0, 3.0 and 4.0 mL MEB standard solution in chloroform (45 µg mL^{-1}) were measured accurately and transferred into a series of 5 mL calibrated flasks. To each flask, 1 mL of 0.2 % BPB solution was added, diluted to the mark with chloroform and mixed well. The absorbance of the resulting yellow color chromogen was measured at 420 nm against reagent blank.

Method B: Varying aliquots, 0.25, 0.5, 1.0, 2.0, 3.0 and 4.0 mL of 40 µg mL^{-1} standard MEB solution in chloroform were measured accurately and transferred into a series of 5 mL calibrated flasks. To each flask was added 1 mL of 0.2 % BTB solution. The content was mixed well and diluted to the mark with chloroform. The absorbance of each solution was measured at 415 nm against reagent blank.

In both methods, a calibration graph was prepared by plotting the increasing absorbance values *versus* concentration of MEB. The concentration of MEB was computed from the respective regression equation derived using the Beer's law data.

Procedure for tablets

Ten tablets or content of ten capsules were weighed accurately and ground into a fine powder. A portion of the powder equivalent to 20 mg MEB was accurately weighed and transferred into 125 mL separating funnel. The procedure of conversion of MET to MEB was followed as done for pure MEB. The resulting solution of 200 µg mL^{-1} MEB was diluted to get working concentration 45 µg mL^{-1} (method A) and 40 µg mL^{-1} (method B), and suitable aliquots were analyzed following the procedures described above.

Procedure for placebo blank and synthetic mixture analysis

A placebo blank of the composition: talc (43 mg), starch (35 mg), acacia (25 mg), methyl cellulose (40 mg), sodium citrate (25 mg), magnesium stearate (35 mg) and sodium alginate (30 mg) was made and its solution was prepared in 25 mL calibration flask as described under "Procedure of Tablets", and then subjected to analysis using the procedures described above.

To the 20 mg of the placebo blank described above, 25.6 mg of MET was added and homogenized. The solution of the synthetic mixture equivalent to 200 μg mL^{-1} of MEB was prepared as described under procedure for formulations. The resulting solution was assayed (n = 5) by the proposed methods after appropriate dilution in method A and B.

Procedure for stoichiometric relationship

Job's method of continuous variations of equimolar solutions were employed: 3.01×10^{-4} M each of MEB and BPB in chloroform (method A) solutions; and 3.09×10^{-4} M each of the MEB and BTB in chloroform (method B) solutions were prepared separately. Series of solutions were prepared in which the total volume of MEB and reagent was kept at 5 mL. The drug and reagent were mixed in various complementary proportions (0:5, 1:4, 2:3, 3:2. 4:1 and 5:0) and completed as directed under the recommended procedures. The absorbance of the resultant ion-pair complex was measured at 420 nm and 415 nm in method A and method B, respectively.

Results and discussion

Spectrophotometric technique based on extraction-free ion-pair complex formation reaction has received considerable attention in the recent years for the quantitative determination of many pharmaceuticals[38-42]. Since, MEB is an amino compound; attempts were made to determine it by applying ion-pair complex formation reaction. In the preliminary experiments, anionic dyes like BTB and BPB were found to give yellow colored ion-pair complexes with positively charged drug; based on this observation two spectrophotometric methods have been developed using BTB and BPB as chromogenic agents.

Absorption spectra

Absorption spectra of the yellow colored ion-pair complexes MEB-BPB and MEB-BTB are shown in Fig.2 with absorption maxima at 420 and 415 nm, respectively. The developed yellow color is due to the conversion of the dye into

an open quinoidal anionic derivative[13,43], which subsequently forms an ion pair with MEB as shown in Scheme 1.

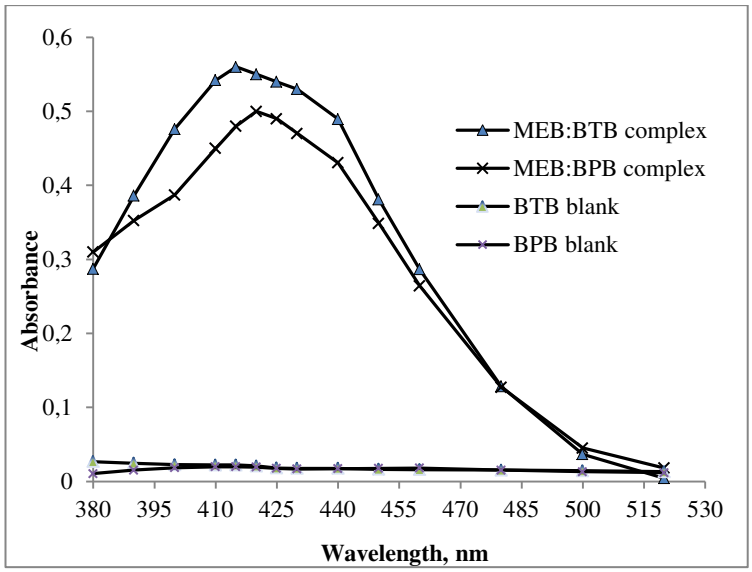
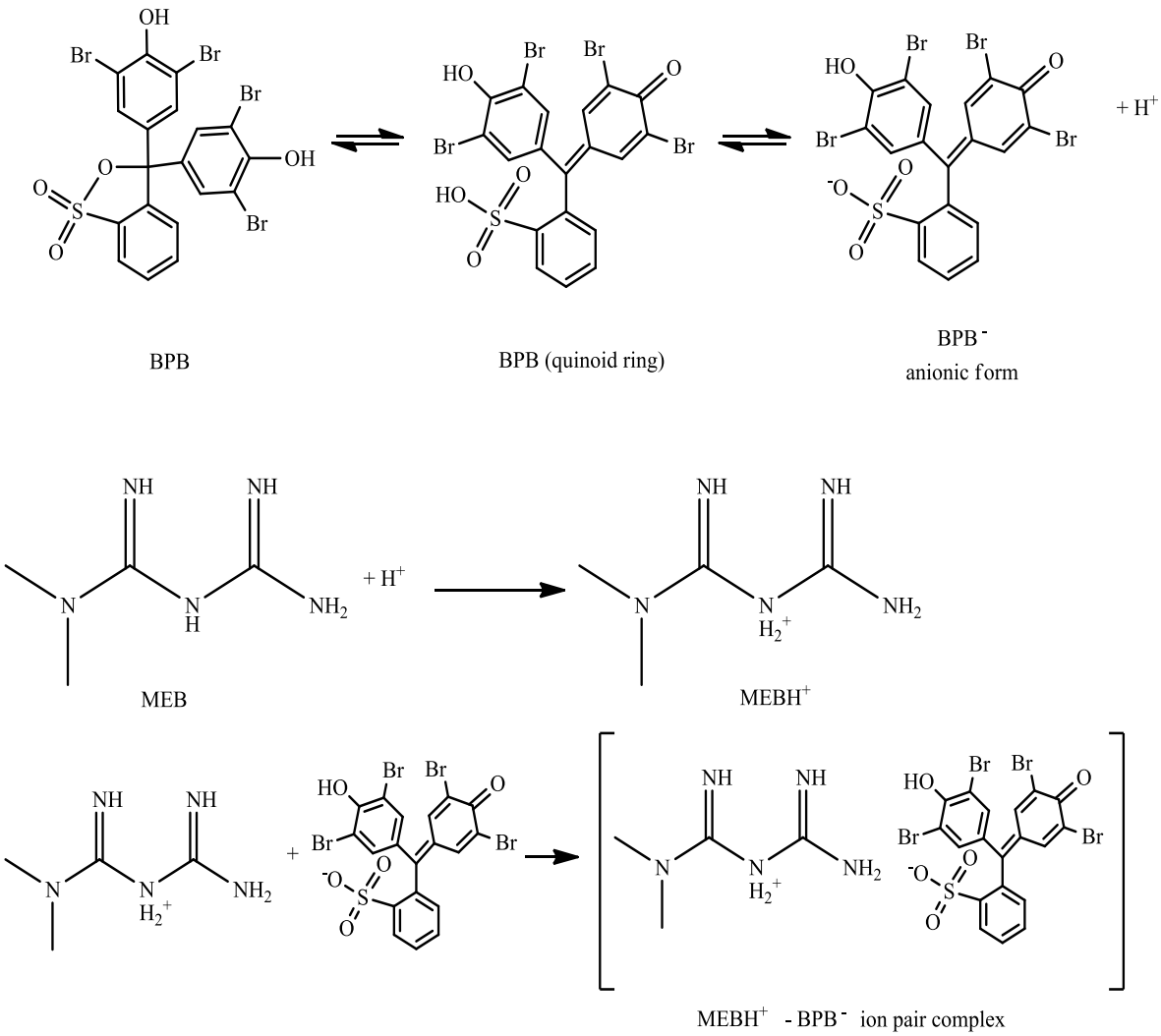
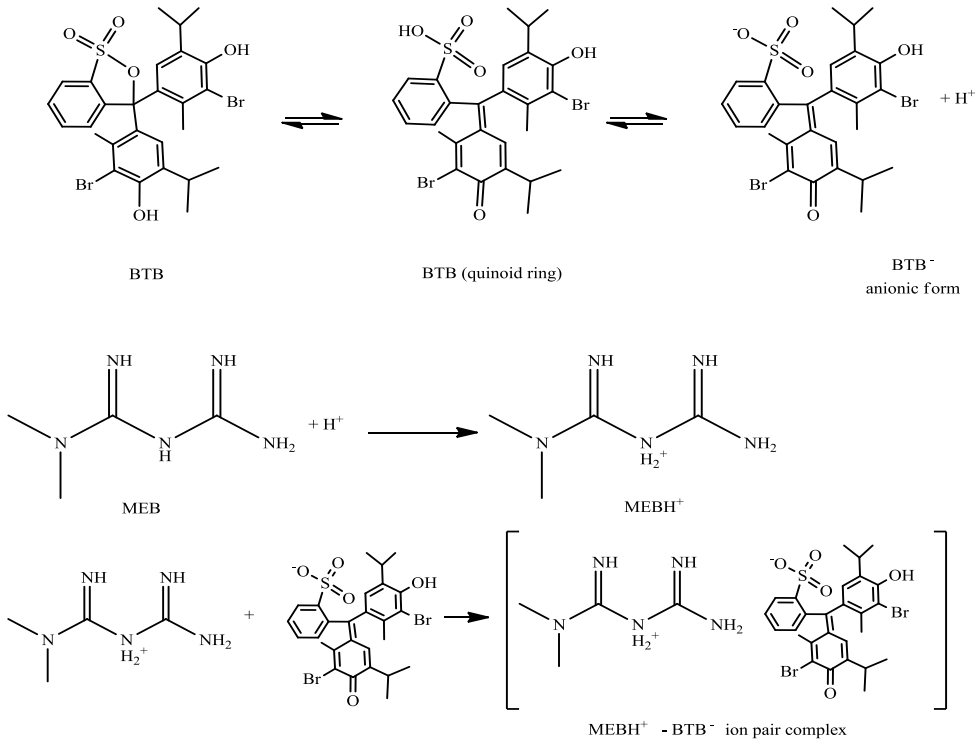


Fig.2: Absorption spectra of method A (36 µg mL^{-1}) and method B (20 µg mL^{-1}).





Scheme-1: The Probable Reaction Scheme.

Optimization of reaction conditions

Preliminary investigations were carried out to establish the most favorable conditions to give a highly intense colour which could be used for the quantitative determination of the drug. Optimum conditions were fixed by varying one parameter at a time while keeping other parameters constant and observing its effect on the absorbance against respective blank. The influence of each of the following variables on the reaction was tested.

Choice of organic solvent

MEB is practically soluble in most of the organic solvents. However in some solvents, even the reagent blank gave an intense yellow color. Chloroform was found most suitable when compared with other solvents. In both methods, chloroform was found as the ideal solvent with high sensitivity and minimum blank absorbance.

Effect of dye concentration

The influence of the concentration of BPB and BTB on the intensity of the color developed at the selected wavelength was studied. In method A, the blank absorbance was found to increase with increasing concentration of BPB as shown in Fig.3. One mL of 0.2% BPB gave maximum absorbance with minimum blank reading (Fig.3). Hence, based on the sensitivity with minimum blank absorbance, 1 mL of 0.2% BPB was used. In method B, constant absorbance readings

were obtained when (0.5-2.0) mL of 0.2% BTB was used (Fig.3) and the respective blanks gave negligible absorbance values. Hence, 1 mL of 0.2% BTB was fixed in method B.

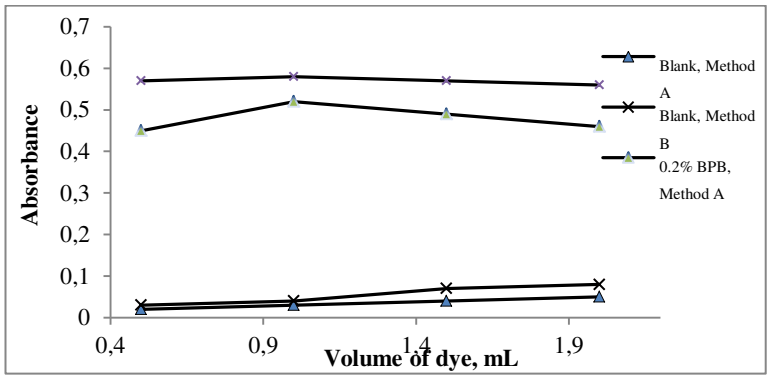


Fig.3: Effect of dye method A (36 µg mL^{-1}) and method B (20 µg mL^{-1}).

Effect of reaction time and stability of ion pair complexes

The optimum reaction times were determined by measuring the absorbance of the complex formed upon the addition of reagent solution to MEB solution at room temperature. The reaction of MEB with BTB in method A and BPB in method B takes 5 min for complete color development. The absorbance of the resulting ion pair complexes remained stable for at least 2 hrs in method A and for about 4 hrs in method B.

Composition of ion-pair complexes

Job's continuous variation method[44] was applied to determine the stoichiometric ratio between MEB and the ion pair reagents. Fig 4 show that the interaction between this drug and reagents occur in equimolar basis, *i.e.* the two straight lines are intersected at [drug] : [reagents] = 1:1. Based on these findings, we propose a probable reaction mechanism for the formation of the complex as shown in Scheme 1.

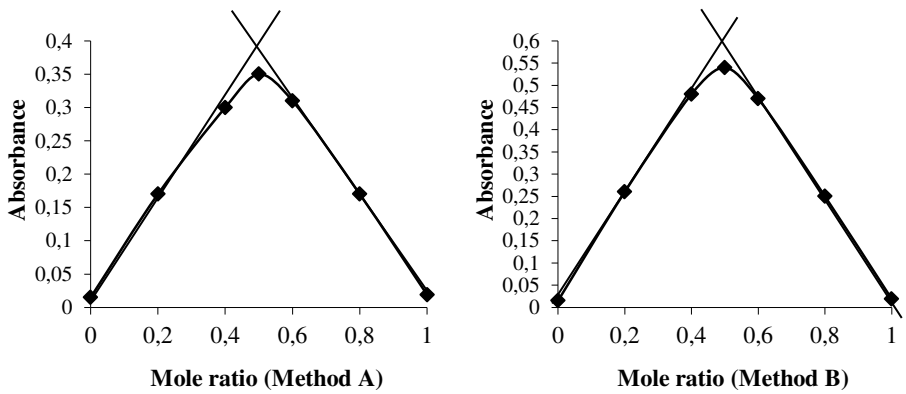


Fig.4: Job's Continuous - variations plots (a) method A and (b) method B.

Conditional stability constants (K_f) of the ion-pair complexes

The conditional stability constants (K_f) of the ion-pair complexes for MEB were calculated from the continuous variation data using the following equation:

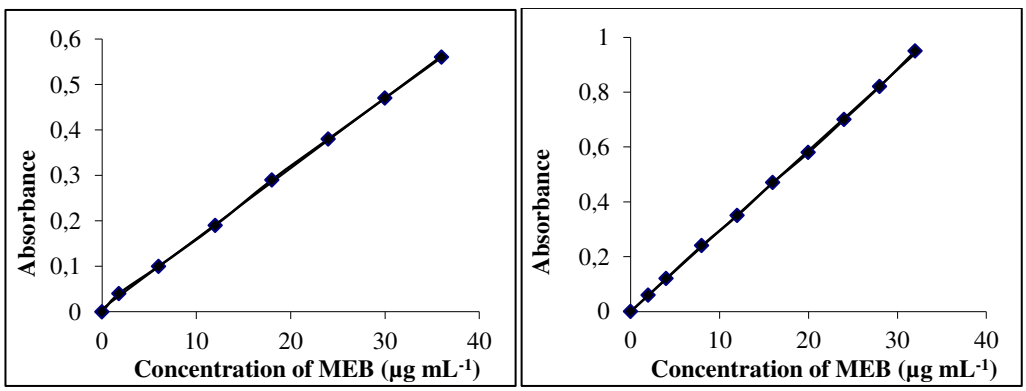
$$K_f = \frac{A/A_m}{[1-A/A_m]^{n+2} C_M(n)^n}$$

where, A and A_m are the observed maximum absorbance and the absorbance value when all the drug present is associated, respectively. C_m is the mole concentration of drug at the maximum absorbance and n is the stoichiometry with which dye ion associates with drug. The conditional stability constants were found to be 6.11 and 7.01 for method A and method B respectively.

Method validation: The proposed methods were validated for linearity, sensitivity, precision, accuracy, robustness, ruggedness, selectivity and recovery according to the International Conference on Harmonization (ICH)[45] guidelines.

Linearity and sensitivity

Under optimum conditions, linear relations were obtained between absorbance and concentration of MEB in the range of 1.8 - 36 µg mL^{-1} in method A and 2-32 µg mL^{-1} in method (Fig. 5)



a b

Fig.5: Calibration curves; a. method A and b. method B.

The calibration graph in each instance is described by the equation:

$$Y = a + b X$$

(Where, Y = absorbance, a = intercept, b = slope and X =concentration in µg mL^{-1}) obtained by the method of least

squares. Correlation coefficient, intercept and slope for the calibration data are summarized in Table 1. Sensitivity parameters such as apparent molar absorptivity and Sandell sensitivity values, the limit of detection (LOD) and the limit of quantification (LOQ) are calculated as per the current ICH guidelines[45] are compiled in Table 1 speak of the excellent sensitivity of the proposed methods. LOD and LOQ were calculated according to the same guidelines using the formulae:

$$LOD = 3.3\sigma/s \text{ and } LOQ = 10\sigma/s$$

Where σ is the standard deviation of five reagent blank determinations and s is the slope of the calibration curve.

Robustness and ruggedness

Method robustness was tested by making small incremental change in concentration of BPB in method A and BTB in method B. To check the ruggedness, analysis was performed by four different analysts; and on three different spectrophotometers by the same analyst. The robustness and the ruggedness were checked at three different drug levels. The intermediate precision, expressed as percent RSD, which is a measure of robustness and ruggedness was within the acceptable limits as shown in Table. 3.

Precision and accuracy

Intra-day precision and accuracy of the proposed methods were evaluated by replicate analysis (n = 7) of calibration standards at three different concentration levels in the same day. Inter-day precision and accuracy were determined by assaying the calibration standards at the same concentration levels on five consecutive days. Precision and accuracy were based on the calculated relative standard deviation (RSD, %) and relative error (RE, %) of the found concentration compared to the theoretical one, respectively (Table2).

Selectivity

The absorbances resulting from 18 μg mL^{-1} and 16 μg mL^{-1} (method A and method B) were nearly the same as those obtained for pure MEB solutions of identical concentrations. This unequivocally demonstrated the non-interference of the inactive ingredients in the assay of MEB. Further, the slopes of the calibration plots prepared from the synthetic mixture solutions were about the same as those prepared from pure drug solutions.

Application to tablets

In order to evaluate the analytical applicability of the proposed methods to the quantification of MEB in commercial tablets, the results obtained by the proposed methods were compared to those of the official method[5] by applying Student's t-test for accuracy and F-test for precision. The official method describes the ptentiometric titration of the pure drug in 4 mL anhydrous formic acid and 80 mL acetonitrile with 0.1M perchloric acid. The results (Table 4) show that the Student's t-and F-values[46] at 95% confidence level are less than the theoretical values, which confirmed that there is a good agreement between the results obtained by the proposed methods and the reference method with respect to accuracy and precision.

Recovery studies

The accuracy and validity of the proposed methods were further ascertained by performing recovery studies. Pre-analyzed tablet powder was spiked with pure MEB at three concentration levels (50, 100 and 150 % of that in tablet powder) and the total was found by the proposed methods. In both methods, the added MEB recovery percentage values ranged of 97.89-101.2% with standard deviation of 1.08–2.25 (Table 5) indicating that the recovery was good, and that the co-formulated substance did not interfere in the determination.

Table-1: Sensitivity and regression parameters.

Parameter	Method A	Method B
λ_{max}, nm	415	420
Color stability, min	120	240
Linear range, µg mL^{-1}	6-36	2-32
Molar absorptivity(ε), L $mol^{-1}cm^{-1}$	1.86×10^4	1.84×10^4
Sandell sensitivity*, µg cm^{-2}	0.0631	0.0340
Limit of detection (LOD), µg mL^{-1}	0.49	0.04
Limit of quantification (LOQ), µg mL^{-1}	1.5	0.11
Regression equation, Y**		
Intercept (a)	0.0153	0.0050
Slope (b)	0.0149	0.0288
Standard deviation of b (S_b)	0.0037	0.0186
Standard deviation of a (S_a)	0.2059	0.4022

Regression coefficient (r)	0.9988	0.9987

*Limit of determination as the weight in µg mL^{-1} 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=a+bX, where Y is the absorbance, X is concentration in µg mL^{-1}, a is intercept, b is slope.

Table-2: Evaluation of intra-day and inter-day accuracy and precision.

Method	MEB taken, µg mL^{-1}	Intra-day accuracy and precision (n=7)			Inter-day accuracy and precision (n=5)		
		MEB found, µg mL^{-1}	%RE	%RSD	MEB found, µg mL^{-1}	%RE	%RSD
A	18	18.102	0.57	0.79	17.992	0.04	0.99
	24	24.724	3.02	0.78	24.586	2.44	1.02
	30	30.549	1.83	0.31	30.533	1.78	1.03
B	8	8.047	0.59	1.07	8.079	0.14	1.77
	16	15.959	0.25	0.94	16.004	0.24	1.47
	24	23.795	0.86	0.82	23.871	0.28	1.19

% RE-Percent relative error, % RSD-relative standard deviation. n = Number of measurements.

Table-3: Method robustness and ruggedness expressed as intermediate precision (% RSD).

Method	MEB taken, µg mL^{-1}	Robustness	Ruggedness	
			Inter-analysts (%RSD), (n=4)	Inter-instruments (%RSD), (n=4)
A	18	0.62	0.21	1.38
	24	0.61	0.46	0.82
	30	0.66	0.56	1.25
B	8	0.39	0.35	0.76
	16	0.45	0.62	1.02
	24	0.68	0.77	1.19

*The volumes of Method A or Method B added were 1±0.2 mL

Table-4: Results of analysis of tablets by the proposed methods and statistical comparison of the results with the official method.

Formulation name	Labeled amount*	Found# (Percent of label claim ± SD)		
		Reference method	Method A method	Method B method
Glyciphage	500	98.92±1.20	99.98±0.54 t = 1.80 F = 4.94	99.45±1.21 t = 0.69 F = 1.02
Cetapin XR	500	99.41±1.45	99.22±1.43 t = 0.21 F = 1.03	98.79±1.36 t = 0.70 F = 1.14

*Amount in mg per tablet.
#Mean value of 5 determinations
Tabulated t-value at the 95% confidence level and for four degrees of freedom is 2.77 tabulated F-value at the 95% confidence level and for four degrees of freedom is 6.39.

Table-5: Results of recovery study *via* standard-addition method with tablet/injection.

Tablets studied	Method A				Method B			
	MET in tablet, µg mL^{-1}	Pure MEB added, µg mL^{-1}	Total found, µg mL^{-1}	Pure MEB recovered (Percent±SD*)	MEB in tablet, µg mL^{-1}	Pure MEB added, µg mL^{-1}	Total found, µg mL^{-1}	Pure MEB recovered (Percent±SD*)
Glyciphage	10.03	5	18.05	100.09±1.57	10.03	5	17.96	99.609±2.12
	10.03	10	23.74	98.807±1.19	10.03	10	23.61	98.269±1.15
	10.03	15	29.50	98.242±1.08	10.03	15	29.37	97.809±1.51
Cetapin XR	10.08	5	8.09	100.35±2.20	10.08	5	8.04	99.71±1.22
	10.08	10	16.17	100.67±2.25	10.08	10	16.26	101.24±1.32
	10.08	15	23.86	99.163±1.45	10.08	15	23.95	99.54±2.05

* Mean value of 3 determinations

Conclusions

The present communication reports the first spectrophotometric assay of MET in formulated drugs by ion pair reaction. The proposed methods are simple, rapid and selective compared to the published visible spectrophotometric methods of MEB in pharmaceuticals. Rapid and stable formation of the coloured complexes with no need for the extraction process is advantages of the developed method over the reported methods. The proposed methods are superior to the existing UV spectrophotometric methods in terms of selectivity as the interferences from many excipients present in the formulations will increases in the UV region compared with visible region. In addition, extractive spectrophotometric procedures are time consuming and required rigid pH control. Besides the simplicity and sensitivity of the procedures, apparatus and reagents demonstrate their advantageous characteristics. The methods are also useful due to high tolerance limit for common excipients found in drug formulations. These merits coupled with the use of simple and inexpensive instrument and high selectivity of the methods recommend the use of the methods in routine quality control laboratories.

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