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VALIDATED VISIBLE SPECTROPHOTOMETRIC METHODS DEVELOPMENT FOR THE DETERMINATION OF BENIDIPINE HYDROCHLORIDE BASED ON COMPLEX AND INTERNAL SALT FORMATION REACTIONS

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ABSTRACT

Two simple, sensitive and cost effective visible spectrophotometric methods (M_1 and M_2) have been developed for the determination of benidipine hydrochloride from bulk and tablet dosage forms. The method M_1 is based on the formation of green colored coordination complex by the drug with cobalt thiocyanate which is quantitatively extractable into nitro benzene with an absorption maximum of 630 nm. The method M_2 involves internal salt formation of aconitic anhydride, dehydration product of citric acid [CA] with acetic anhydride [Ac_2O] to form colored chromogen with an absorption maximum of 565 nm. The calibration graph is linear over the concentration range of 10-50µg/ml and 8-24 µg/ml for method M_1 and M_2 respectively. The proposed methods are applied to commercial available tablets and the results are statistically compared with those obtained by the reference method and validated by recovery studies. The results are found satisfactory and reproducible. These methods are applied successfully for the estimation of the benidipine hydrochloride in the presence of other ingredients that are usually present in dosage forms.

INTRODUCTION

The Benidipine hydrochloride (BEN) (Fig.1) is a highly potent and long acting dihydropyridine (DHP) calcium channel blocker (L, N and T-type) and orally active anti anginal, antihypertensive agent which displace a wide range of activities in vitro and in vivo. Chemically it is known as (±) (R*)-3-[(R*)-1-Benzyl-3-piperidyl] methyl 1, 4-dihydro-2, 6-dimethyl-4-(3-nitrophenyl)-3,5pyridine dicarboxylate hydrochloride (1:1) base [1].

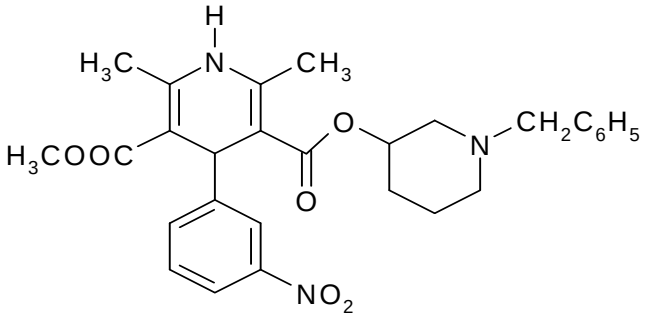


Fig.1: Chemical structure of BEN

It is mainly used clinically as a race mate for treat meant of hypertension, renal parenchymal hypertension, ischaemic heart disease, cardiac arrhythmias and angina pectoris. Also inhibits aldosterone-induced mineralo corticoid receptor activation and exhibits cardio protective and anti-atherosclerotic effects. BEN is listed in official monograph of Japan Pharmacopoeia [2] which describes potentiometric titration and chromatographic procedures for its assay in tablets. BEN tablet contain not less than 95.0% and more than 105.0 of the labeled amount of Benidipine. In literature several analytical methods such as HPLC[3], HPLC-ECD[4], UV-HPTLC[5], LC-MS[6], LC-tandem-MS[7], capillary GC-MS[8],GC-ECD[9], radio receptor assay[10], radio immuno assay [11], spectrophotometric (visible) [12-13] have been reported for the determination of BEN in biological fluids (considerable more) and formulations (less). Even though there are two visible spectrophotometric methods reported for the determination of the drug they are tedious, less specificity and extractive methods. Nevertheless, there still exists a need for development of sensitive accurate and flexible visible spectrophotometric methods for the determination of Benidipine in pharmaceutical preparations. The authors have made some attempts in this direction and succeeded in developing two methods based on the reaction between the drug and cobalt thiocyanate [14] (M_1) or drug and citric acid-acetic anhydride reagent [15] (M_2). These methods can be extended for the routine assay of BEN formulations.

MATERIAL AND METHODS (EXPERIMENTAL)

A Systronics UV/Visible spectrophotometer model -2203 with10mm matched quartz cells was used for all spectral measurements. A Systronics µ- pH meter model-362 was used for pH measurements. All the chemicals used were of analytical grade.

Reagents and chemicals

CTC ($2.50x10^{-1}$M, solution prepared by dissolving 7.25 g of cobalt nitrate and 3.8 g of ammonium thiocyanate in 100ml distilled water), Citrate buffer pH(2.0) (prepared by mixing 306ml of 0.1M trisodium citrate with 694ml of 0.1M HCl and pH was adjusted to 2.0) were prepared for method M_1.

Citric acid monohydrate (Prepared by dissolving 1.2 grams of (1.2%, $6.245X10^{-2}$M) Citric acid in 5 ml methanol initially followed by dilution up to 100ml with acetic anhydride) and Acetic anhydride **(**SD Fine chemicals) were used for Method M_2 **.**

Preparation of Standard and sample drug stock solution An accurately weighed quantity of BEN (pure or tablet powder) equivalent to 100mg was mixed with 5ml of 10% Na_2CO_3 solution and transferred into 125ml separating funnel. The freebase released was extracted with 3x15ml portion of chloroform and the combined chloroform layer was brought up to 100ml with the same solvent to get 1mg/ml BEN drug stock solution in free base form. This free base stock solution was further diluted step wise with the same solvent to get the working standard solution concentrations [M_1-400 µg/ml, M_2-200 µg/ml].

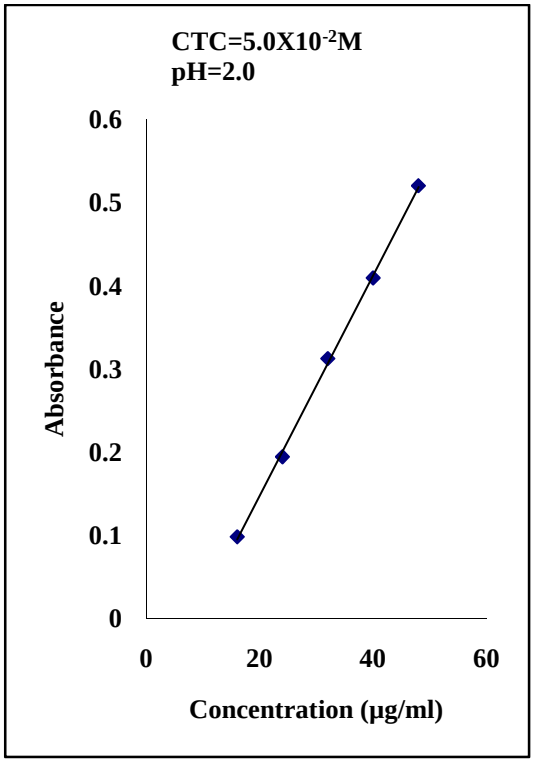
Assay

Method M_1

Aliquots of standard BEN solution (1.0ml-3.0ml, 400µg/ml in free base form) were delivered into a series of 125ml separating funnels. Then 2.0ml of buffer solution (pH 2.0) and 5.0ml CTC solution were added. The total volume of aqueous phase in each separating funnel was adjusted to 15.0ml with distilled water. To each separating funnel 10.0ml of nitrobenzene was added and contents were shaken for 2 minutes. The two phases were allowed to separate and absorbance of nitrobenzene layer was measured at 630 nm against a similar reagent blank.The colored product was stable for 1 hour. The amount of BEN in the sample solution was computed from its calibration graph (Fig-2 showing Beer's law plot).

Method M_2

Aliquots of standard BEN drug solution [1.0-3.0ml;200µg/ml in free base form] in chloroform were taken into a series of 25ml graduated tubes and gently evaporated in a boiling water bath to dryness. To this, 10ml of citric acid- Acetic anhydride reagent was added and the tubes were immersed in a boiling water bath for 30 minutes then the tubes were cooled to room temperature and made up to the mark with acetic anhydride. The absorbance of the colored solutions was measured after 15minutes at 565 nm against the reagent blank within the stability period of 15-60min.The amount of BEN was computed from its calibration graph (Fig-3 showing Beer's law plot).



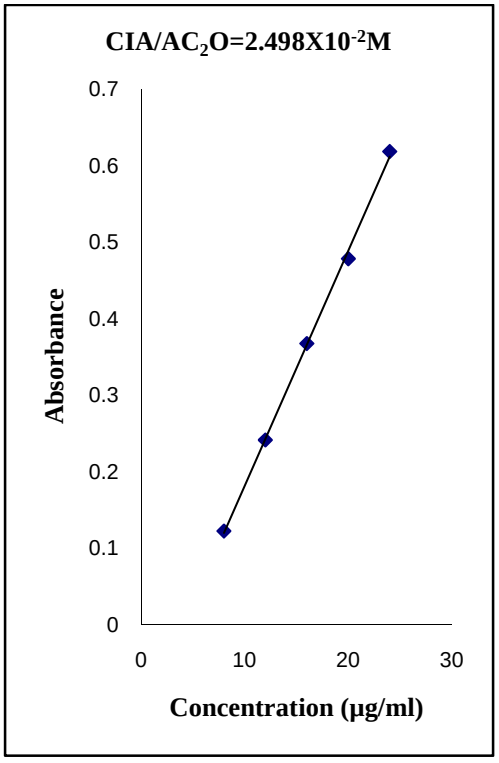


Fig.2: Beer's Law plot of BEN-CTC **Fig.3: Beer's Law plot of BEN-CIA/AC_2O**

RESULTS AND DISCUSSION

In developing these methods, systematic studies of the effects of various parameters were undertaken by varying one parameter at a time and controlling all others fixed (OVAT method). The effect of various parameters such as time, volume and strength of reagents, pH buffer solution and order of addition of reagents, stability period and solvent for final dilution of the colored species were studied and the optimum conditions were established. Among the various

water immiscible organic solvents (C_6H_6, $CHCl_3$, dichloro methane, nitro benzene, chloro benzene and CCl_4) tested for the extraction of colored coordinate complex into organic layer, nitrobenzene was preferred for selective extraction of colored complex from organic phase in method M_1. Different solvents like acetic anhydride, acetic acid, methanol, ethanol, and isopropanol were also used as diluents but acetic anhydride was found to be ideal for final dilution in method M_2. The ratio of organic to aqueous phase was found to be 1:1.5 by slope ratio method for method M_1. The optical characteristics such as Beer's law limit, Sandell's sensitivity, molar absorptivity, percent relative standard deviation, (calculated from the six measurements containing $3/4^{th}$ of the amount of the upper Beer's law limits) were calculated and the results are summarized in Table-1.

Table- 1: Optical characteristics, precision and accuracy of proposed methods

Parameter	**Method M_1**	**Method M_2**
λ_{max}(nm)	630	565
Beer's law limit(µg/ml)	16-48	8-24
Sandell's sensitivity (µg/cm^2/0.001 abs. unit)	0.097799511	0.021798
Molar absorptivity (Liter/mole/cm)	5542.1545	24865.17
Correlation coefficient	0.999	0.998
Regression equation (Y)*		
Intercept (a)	-0.117	-0.126
Slope(b)	0.013	0.03
%RSD	0.458	0.87
% Range of errors(95% Confidence limits)		
0.05 significance level	0.458	0.92
0.01 significance level	0.754	1.44

*Y= a + b x; Where Y= absorbance, x= concentration of BEN in µg/ml.

Commercial formulations containing BEN were successfully analyzed by the proposed methods. The values obtained by the proposed and reference methods for formulations were compared statistically by the t-and F-test and found not to differ significantly. As an additional demonstration of accuracy, recovery experiments were performed by adding a fixed amount of the drug to the pre analyzed formulations at three different concentration levels. These results are summarized in Table-2.

Table 2: Analysis of BEN in pharmaceutical formulations by proposed and reference methods.

Method	*Formulations	Labeled Amount (mg)	Found by Proposed Methods			Found by Reference Method ± SD	#% Recovery by Proposed Method ± SD
			**Amount found ± SD	t	F		
M_1	Caritec TAB	4	3.977± 0.015	0.293	2.72	3.971± 0.025	99.42 ± 0.38
M_2	Caritec TAB	4	3.98± 0.013	1.2	3.56	3.971± 0.025	99.49 ± 0.33

*Average ± Standard deviation of eight determinations, the t- and f-values refer to comparison of the proposed method with reference method (UV). Theoretical values at 95% confidence limits t =2.57 and F = 5.05.

Recovery of 10mg added to the pre analyzed sample (average of three determinations).

Reference method (reported UV method) using methanol (λ_{max}=238nm).

Recovery experiments indicated the absence of interference from the commonly encountered pharmaceutical excipients present in formulations. The proposed methods are found to be simple, sensitive and accurate and can be used for the routine quality control analysis of BEN in bulk and dosage forms.

Chemistry of colored species:

In method M_1 the green color species formation is the coordination complex of the drug (electron donor) and the central metal of cobalt thiocyanate, which is extractable into nitro benzene from aqueous solution and in method M_2 red-violet color internal salt of aconitic anhydride is formed when BEN was treated with CTC or CIA/Ac_2O reagents. The formations of colored species are due to the presence of the cyclic tertiary nitrogen in bis benzyl piperidine portion. It is based on the analogy of tertiary amine as given in scheme (Fig-4&5).

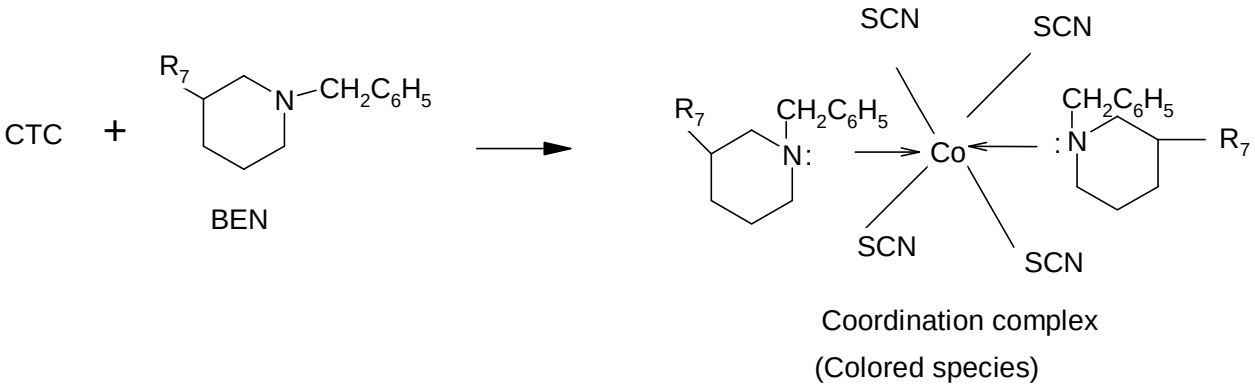
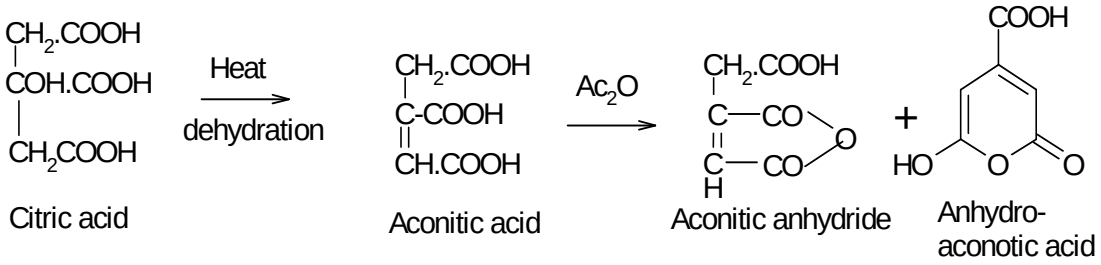
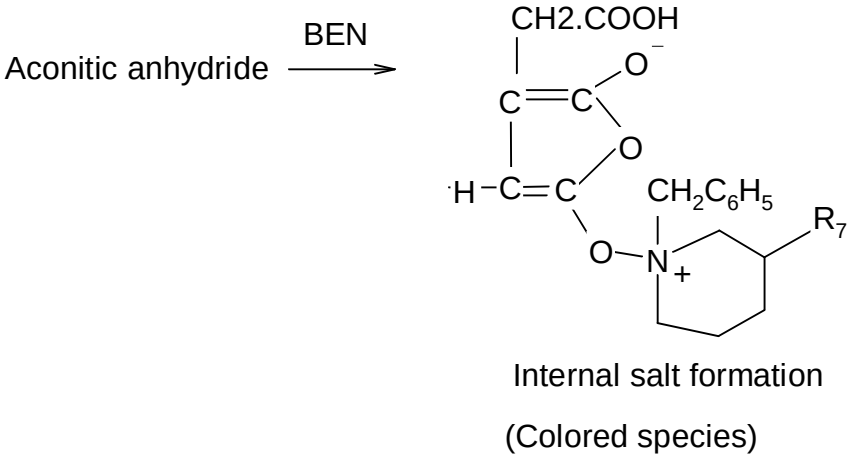


Fig.4: Probable Scheme of the reaction for method M_1





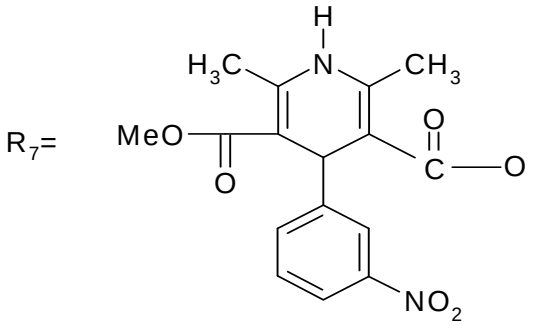


Fig.5: Probable scheme of the reaction for method M_2

CONCLUSION

The reagents utilized in the proposed methods are cheap, readily available and the procedures do not involve any critical reaction conditions or tedious sample preparation. The proposed visible spectrophotometric methods are validated as per ICH guide lines and possess reasonable precision, accuracy, simple, sensitive and can be used as alternative methods to the reported ones for the routine determination of BEN depending on the need and situation.

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