

DEVELOPMENT OF NEW VISIBLE SPECTROPHOTOMETRIC METHODS FOR DETERMINATION OF BENIDIPINE HYDROCHLORIDE IN BULK AND FORMULATIONS BASED ON OXIDATIVE COUPLING AND DIAZO COUPLING REACTIONS

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ABSTRACT

Three simple and sensitive visible spectrophotometric methods (A-C) have been developed for the estimation of Benidipine hydrochloride [BEN] from its bulk drug and dosage forms. The proposed methods are based on the reduction of the nitro group to amino group of the drug. This can be achieved by heating a mixture of an alcoholic solution of BEN, zinc powder and dilute hydrochloride in a water bath at $90^{\circ}\text{C} \pm 5$ for 15 minutes. The cold and clear filtrate reacts with 3-methyl-2-benzothiazolinonehydrazone hydrochloride in the presence of ferric chloride (Method A) or 4- amino phenazone –potassium ferri cyanide in the presence of weak base (Method B) or treated with nitrous acid for diazotization and then coupled with N-(1-Naphthyl) Ethylene Diamine dihydrochloride (NED) in acid medium (Method C) to form colored species with wavelength maximum at 620nm or 500nm or 525nm for methods A-C respectively. Beer's law obeyed in the concentration range of 10-30 $\mu\text{g/ml}$ for both methods (A and B) and 8-40 $\mu\text{g/ml}$ for method C. Commercial formulations were analysed, the results obtained by the proposed methods were in good agreement with the labeled amounts. Recovery in all the methods is found to be close to 99-100%.

KEYWORDS: Beer's law, Calcium channel blocker, MBTH, 4-AP, NED, Spectrophotometer.

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 antihypertensive agent which displace a wide range of activities in vitro and in vivo. Chemically it is known as ($\pm$) (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].

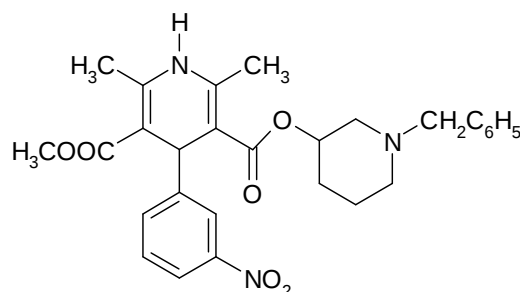


Fig.1: Chemical structure of BEN

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 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 three methods based on the color formation using the reagents such as MBTH-Fe(III) (method A) or 4-AP- $[K_3Fe(CN)_6]$ [14] (method B) or HNO_2 -NED [15] (method C) for determination of BEN after reduction with zinc and hydrochloric acid. Methods (A &B) were described the oxidative coupling of MBTH or 4-amino phenazone (4-AP) with aromatic amines in the presence of an oxidant ferric chloride or potassium ferricyanide respectively, resulting in the formation of an intensively colored species. Method C was described in the

Presence of amino group in reduced benidipine enable the diazotization with HNO_2 and coupling with NED to form colored species due to the formation of azo dye. These methods have been successfully extended to the pharmaceutical preparations.

Materials and Methods

Instrument: Systronics Double Beam UV/visible spectrophotometer model No.2203 used for optical measurements.

Reagents and chemicals: All reagents used were of analytical grade. 0.2%MBTH solution (Prepared by dissolving 200mg of MBTH in 100ml distilled water), 0.5%Ferric chloride solution (Prepared by dissolving 500mg of $FeCl_3$ in 100ml of 0.1N HCl). Zinc powder (LR grade), Hydrochloric acid, 0.1% 4-AP solution (prepared by dissolving 100mg of 4-AP in 100ml distilled water on slight warming in hot water bath and cooled to room temperature) and potassium ferricyanide solution (prepared by dissolving 1.0g of potassium ferricyanide in 100ml of distilled water), Sodium nitrite solution (Prepared by dissolving 250mg of $NaNO_2$ in 100ml distilled water), 2.5%aqueous solution of ammonium sulphamate, 3M aqueous solution of sodium acetate and 0.1%NED solution (Prepared by dissolving 100mg NED in 100ml distilled water) were used.

Preparation of Bulk and Sample solution:

About 50mg of BEN [pure or formulation] was accurately weighed and dissolved in 10ml methanol and treated with 10ml of 3N HCl and 50mg of zinc dust and then heated on a water bath at $90^\circ C \pm 5$ for 15 minutes. The solution was filtered through cotton wool, the residue was washed with 3x5ml portion of methanol and the total volume of the filtrate was brought to 50ml

with methanol (1mg/ml). The final concentration of reduced Benidipine was brought to 100µg/ml for methods (A and B), 200µg/ml for method C with the same solvent.

Assay for method A:

Aliquots of standard solution of reduced Benidipine ranging from 1.0-3.0ml, (100µg/ml) were transferred in to a series of 10ml graduated test tubes. A 1.5ml portion of MBTH solution, 5ml methanol were added and heated on water bath for 2minutes then 1ml of ferric chloride solution was added and kept aside for 10 min. and dilute to the mark with distilled water. Deep green colored species was formed and stable for 30 minutes. The absorbencies were measured at 620nm against the reagent blank. The amount of BEN was computed from its calibration graph.

Method B:

Aliquots of standard solution of reduced Benidipine ranging from 1.0-3.0ml, (100µg/ml) were transferred into a series of 10ml graduated tubes. To each, 0.5ml pyridine, 2.0ml of 4-AP and 1.5ml of potassium ferricyanide were added successively and the total volume in each flask was brought up to 9.0ml with distilled water and kept aside for 5 min. then made up to the mark with distilled water. The absorbance of the colored species was measured at 500nm against the reagent blank. The colored species was stable for 30 min. The amount of BEN was computed from its calibration graph.

Method C:

To aliquots of neutral solution of reduced BEN (1.0-3.0ml, 200µg/ml), 1.0ml of 2N HCl and 1.0ml of NaNO₂ solutions were added successively into 25 ml calibrated tubes and kept aside for 15 min. after that 1.0ml of 2.5% aqueous solution of ammonium sulfate, 2.0ml 3M aqueous solution of sodium

acetate and 1.0ml of 0.1% aqueous NED solutions were added successively. Purple colored species was formed and stable for 30 minutes. The absorbance was measured at 525nm against the similar reagent blank. The amount of BEN was computed from its calibration graph.

Results and Discussions

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. The effect of various parameters such as time, temperature, nature and concentration of oxidant, volume and strength of reagents, order of addition of reagents on color development and solvent for final dilution on the intensity and stability of the colored species were studied and the optimum conditions were established. Among the various oxidants (NaIO₄, K₂Cr₂O₇, Chloramine-T, potassium hexacyanoferrate (III), Ce (IV) and Fe (III) tried in combination with MBTH for oxidative coupling reaction. Ce (IV) and Fe (III) were responded for color development with MBTH. But MBTH-Fe (III) was found to be the best by virtue of high ϵ_{max} values and stability considerations for method A. Among the various oxidants (NaIO₄, potassium ferricyanide or K₂S₂O₈ tried in combination with 4-AP for the oxidative coupling reaction, potassium ferricyanide-4-AP was found to be the best suited with regard to sensitivity and stability of the colored species formed. Various basic substances (pyridine, Na₂CO₃ or NaOH) were tried for maintenance of alkalinity for the oxidative coupling reaction. Among these, pyridine and Na₂CO₃ were responded. But pyridine was found to be the best by virtue of high ϵ_{max} values and stability considerations for method B. Other water miscible solvents like methanol, ethanol, propan-2-ol and acetonitrile were found to provide no additional advantage. So distilled water is

selected as a solvent for final dilution of the colored species for all three methods. The optical characteristics such as Beer's law limit, Sandell's sensitivity, molar absorptivity, percent relative standard deviation (calculated from the six measurements containing 3/4th of the amount of the upper Beer's law limits), Regression characteristics like standard deviation of slope (S_b), standard deviation of intercept (S_a), standard error of estimation (S_e) and % range of error (0.05 and 0.01 confidence limits) were calculated and are shown in Table-1. Commercial formulations (caritec tablets) were successfully analysed by the proposed

methods .The values obtained by the proposed and reference method [reported UV in methanol $\lambda_{max}=238nm$] for pharmaceutical formulations were compared statistically by the t -and F-test [Table2] and found not to differ significantly. As an additional demonstration of accuracy recovery experiments were carried out by adding known amount of drug to the already analysed formulation. The results are presented in Table2. Recovery experiments indicated the absence of interference from the commonly encountered pharmaceutical excipients present in formulations.

TABLE 1: OPTICAL CHARACTERISTICS, PRECISION AND ACCURACY OF PROPOSED METHODS.

<i>Parameter</i>	<i>Method A</i>	<i>Method B</i>	<i>Method C</i>
λ_{max}	620	500	525
Beer's law limit($\mu g/ml$)	10-30	10-30	8-24
Sandell's sensitivity ($\mu g/cm^2/0.001abs.unit$)	0.058139535	0.044802867	0.041841004
Molar absorptivity (Litre/mole/cm)	9322.744	12097.8864	12954.278
Regression equation (Y)*	--	--	--
Intercept (a)	0.021	0.028	0.029
Slope(b)	-0.110	-0.146	-0.108
%RSD	0.6332	0.8548	0.693
% Range of errors(95% Confidence limits)	0.665	0.897	0.727
0.05 significance level	1.042	1.407	1.14
0.01 significance level			

*Y= a + b x; Where Y= absorbance, x= concentration of BEN in $\mu g/ml$.

TABLE-2 ANALYSIS OF BEN BY PROPOSED AND REFERENCE METHODS.

Method	Formulation	Labeled Amount (mg)	Found by Proposed Methods			Found by Reference Method $\pm$ SD	#% Recovery by Proposed Method $\pm$ SD
			*Amount found $\pm$ SD	t	f		
A	Tablet	4	3.98 $\pm$ 0.013	1.08	3.50	3.97 $\pm$ 0.025	99.47 $\pm$ 0.333
B	Tablet	4	3.98 $\pm$ 0.011	1.37	4.89	3.97 $\pm$ 0.025	99.56 $\pm$ 0.282
C	Tablet	4	3.97 $\pm$ 0.15	0.52	2.76	3.97 $\pm$ 0.025	99.39 $\pm$ 0.375

*Average $\pm$ 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).

Chemistry of colored species: Honing and Fritsch [16]described oxidative coupling of MBTH with aromatic amines or phenols in the presence of an oxidant under acidic conditions to form intense colored oxidative coupling products. In 1940 the highly sensitive color reaction of phenols with 4-aminophenazone was introduced into analytical practice by Emerson [17-18]. He postulated that the treatment of phenol, enol or amine with 4-AP and an oxidant under neutral or alkaline condition results in the formation of an oxidative coupling product (anti-pyrine dye) which is used for the determination of BEN in the present investigation, as reduced BEN

possesses primary amino group. The formation of oxidative coupling products for methods (A&B) may be represented in scheme (Fig.4 &5).

Bandelin and kemp [19] determined primary aromatic amines on the basis of diazotization and coupling with NED to yield a colored azo dye and the same was adapted by Siggia [20] in quantitative organic analysis via functional groups. The presence of amino group in reduced benidipine enables the diazotization with HNO_2 and coupling with NED in acid medium to form colored species for method C and may be represented in scheme (Fig.6).

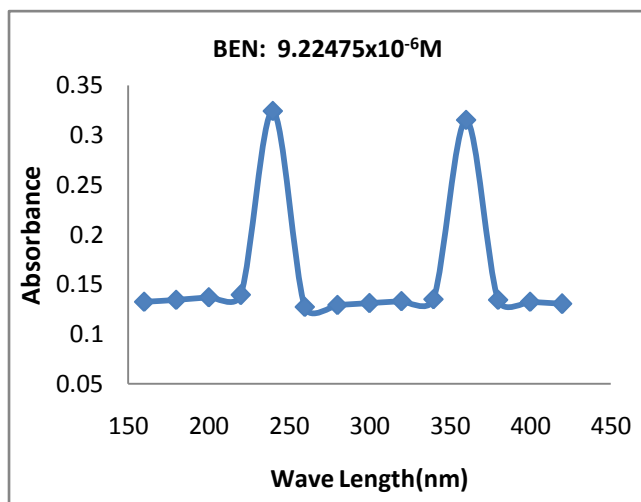


Fig.2: Absorption spectra of BEN in methanol (UV reference method)

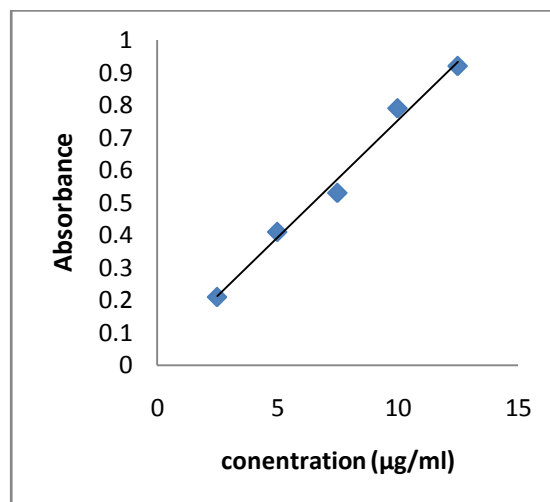
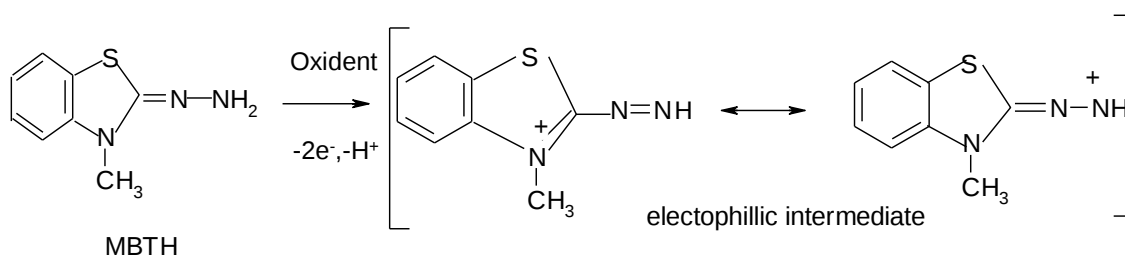


Fig.3: Beer's law plot of BEN in methanol (UV reference method)



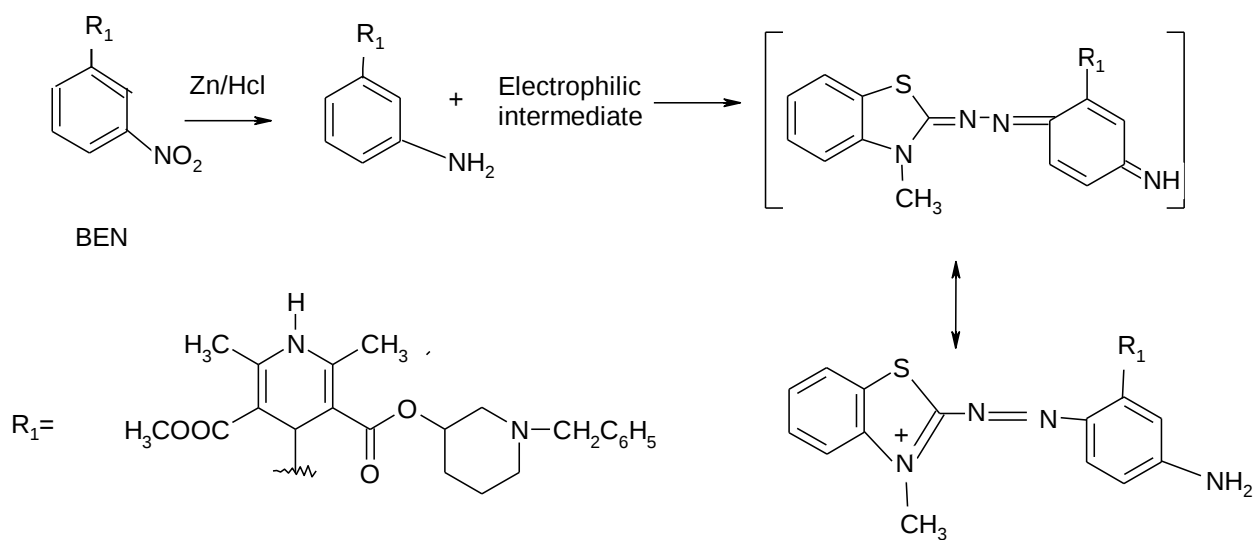


Fig. 4: Scheme for method A

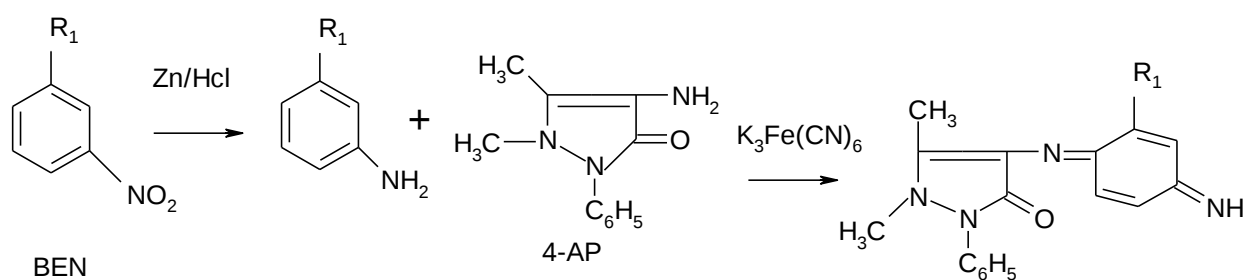


Fig.5: Scheme for method B

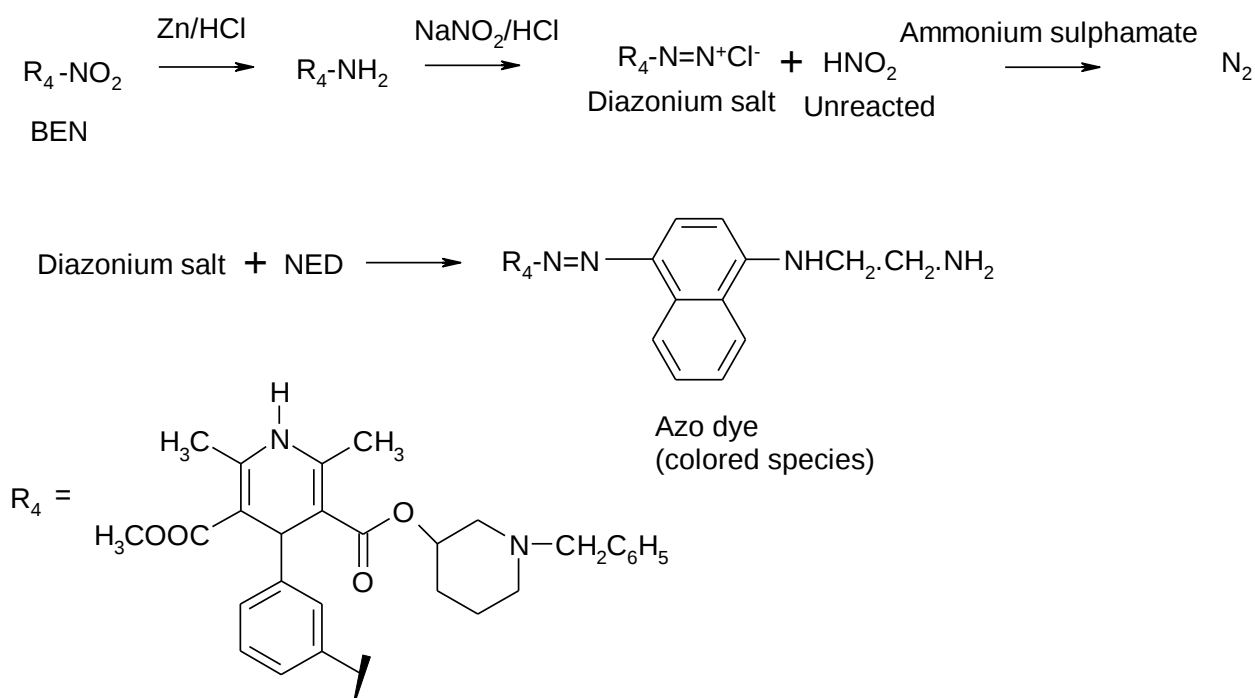


Fig.6: Scheme for method C

CONCLUSIONS

The reagents utilized in the proposed method are readily available and the procedure does not involve any critical reaction conditions or tedious sample preparation. The proposed analytical methods possess reasonable precision, accuracy. The methods offer the advantages of rapidity, simplicity, sensitivity and can be easily applied to resource-poor settings without the need for expensive instrumentation and reagents 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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REFERENCES

1. The Martindale extra pharmacopeia, 30th ed., 343 (1993).

2. Japan Pharmacopoeia, XVth edition, 338-339, (2006).
3. Jane I, Mc kinnon, A and Flanagan,R. HPLC analysis of basic drugs on silica columns using non-aqueous ionic elements,II, applications of UV, fluorescence and electrochemical oxidation detection. Journal of Chromatography, 323: 191-225(1985).
4. Tokuma Y, Naguchi H. HPLC determination of benidipine in body fluids. Journal of Chromatography, 694, 181(1995).
5. Sane,R.T., Mrs. Mathura Phadke, P.S. Hijii, Miss Meghana Shah and P.H. Patel, UV- spectrophotometric and HPTLC determination of benidipine from its bulk drug. Indian Drugs, 35(2), 79-85(1998).
6. Kang, W., Dong-Jun Lee, Kwang-Hyeon Liu, Yu Eun, Sunwoo et al. Analysis of benidipine enantiomers in human plasma by LC-MS using a macrocyclic antibiotic (Vancomycin) chiral stationary phase column. Journal of Chromatography B, 814(1), 75-81(2005).
7. Kang, W., Yun, H.Y., Liu, K.H., Kwang-il Kwon and Jae Gook Shin, Determination of benidipine in human plasma using LC –tandem mass spectrometry. Journal of Chromatography B, 805(2), 311-314 (2004).
8. Magara, H., Kobayashi, H., Kobayashi, S., Determination of benidipine in human plasma by capillary column gas chromatography-negative chemical ionization mass spectrophotometry. Journal of Chromatography. Biomed. Appl., 617(1), 59-63(1993).
9. Kobayashi, H., Kobayashi, S., Inoue, A., Oka, T., Nakamizo, N., Gas chromatographic method for the quantification of the new antagonist benidipine in plasma using electron capture detection. Arzneimittel Forschung (Germany), 38(11A), 1730-1733(1988).
10. Ishii, A., Nishida, K., Oka, T., Nakamizo, N., Sensitive radio receptor assay of the calcium antagonist benidipine in plasma and urine. Arzneimittel Forschung (Germany), 38(11A), 1733-1737(1988).
11. Akinaga, S., Kobayashi, S., Inoue, A., Oka, T., Nakamizo, N., Determination of the calcium antagonist benidipine in plasma by sensitive radio immuno assay. Arzneimittel Forschung (Germany), 38(11A), 1738-1741 (1988).
12. Singhavi, I., Chaturvedi, S.C., Spectrophotometric method for the estimation of amlodipine and benidipine hydrochloride from tablets. Indian J. Pharm. Sci., 61(3), 190-191(1999).
13. Singhavi, I., Chaturvedi, S.C., Spectrophotometric methods for the estimation of benidipine hydrochloride from tablets. Indian J. Pharm. Sci., 60(2), 113-114 (1998).
14. Sastry CSP and Sastry BS. A review of MBTH chemistry in pharmaceutical analysis. The Eastern Pharmacist, 29(345):31(1986).
15. AVSS Prasad, SR Lakshmi, CSP.Sastry, UV Prasad. Determination of minocycline by oxidative coupling and diazocoupling reactions in pharmaceutical formulations. Journal

- pharmaceutical and biomedical analysis, 30(3): 491-498(2002).
16. Hunig S and Fritsch KH. Oxidative coupling reactions of phenols and amines with MBTH. Ann. Chim., 609:143(1957).
 17. Emerson E, U.S. Patent, 2, 194&201(1940).
 18. Emerson E, Beachem HH, Beegle LC, J. Org. Chem., 8: 417(1943)
 19. Bandelin FJ and CR Kemp, Ind. Eng. Chem., Anal ed., 18,470(1946).
 20. Siggia S, Quantitative organic analysis via functional groups, 3rd ed., John Wiley, New York, 1963,511.



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