August 2008 Volume 7 Issue 8

Trade Science Inc.

Analytical CHEMISTRY
An Indian Journal

Full Paper

ACAIJ, 7(8) 2008 [652-658]

Simple and selective spectrophotometric methods for the determination of hydralazine hydrochloride in bulk drug and in pharmaceutical formulations based on the oxidation-reduction reactions

K.Siddappa*, Mallikarjun Metre, Tukaram Reddy, Mahesh Tambe
Department of Studies and Research in Chemistry, Gulbarga University, Gulbarga-585106, Karnataka, (INDIA)
E-mail: siddappa_65@rediffmail.com
Received: 13th June, 2008 ; Accepted: 18th June, 2008

ABSTRACT

Two simple, sensitive, accurate and cost effective spectrophotometric methods have been developed for the determination of Hydralazine Hydrochloride (HLH) in bulk drug and in pharmaceutical formulations. These two methods are based on their oxidation of 1-10 Phenanthroline (Method A) and 2, 2'- Bipyridyl (Method B). The colored complexes formed were measured at 510 and 523 nm respectively against the reagent blank. Reaction conditions have been optimized to obtain colored complexes of higher sensitivity. The absorbance was found to increase linearly with increase in concentration of HLH. The complexes obeyed Beer's Law in the concentration range of 0.1-1.0 μg/ml and 0.1-1.5 μg/ml for both the methods. The molar absorptivity, Sandell's sensitivity, detection limit, limit of quantification and correlation coefficient (r) of the methods were found to be 1.723×10^4, 0.1141 μg/ml 0.085 μg/ml, 0.2830 μg/ml and 0.9996 for method A and 1.253×10^4, 0.01569 μg/ml, 0.082 μg/ml, 0.2730 μg/ml and 0.9994 for method B respectively. The optimum reaction conditions and other analytical conditions were evaluated. The common excipients and additives did not interfere with their determinations. The results obtained by the proposed methods were further ascertained by parallel determination by a reference method.

KEYWORDS

Hydralazine hydrochloride;
1-10 Phenanthroline;
2, 2'- Bipyridyl;
Spectrophotometer,;
Pharmaceutical formulations.

INTRODUCTION

Hydralazine hydrochloride (HLH) is chemically 1 (2H)-phthalazinone hydrazone, 1-hydrazinophthala zine[1]. It is a potent vasodilator that has been used for many years, chiefly in the treatment of ambulatory patients with primary hypertension of moderate severity[2]. In therapeutic doses, hydralazine hydrochloride produces little effect on nonvascular smooth muscle or on the heart. Its pharmacological actions are largely confined to vascular smooth muscle and occur predominantly on the arterial side of circulations, many hydralazine derivatives and their formulations are official in World Health Organization (WHO), British Pharmacopoeia (BP) and Indian Pharmacopoeia (IP)[3-6]. The official methods involve potentiometric titration using 0.05 mol/l potassium iodide as a titrant[5] and High performance liquid chromatography (HPLC)[6]. The drug has been assayed by a variety of methods, such as . spectrofluorometry[7], oxidimetry[8], polarography[9] and High performance liquid chromatography (HPLC)[10]. However, the literature revealed that many spectrophotometric methods[11-20] have been reported for the determination of HLH. But none of these methods are

satisfactory for routine quality assurance for one or the other reasons. Some of these methods have low sensitivity[11], work out only at higher concentration of the drug[12] or have less stability[13] or involve extraction[14]. On the basis of this background, it was felt necessary to develop a rapid, low cost, accurate, simple and sensitive spectrophotometric methods, which do not suffer from the above limitations for the determination of HLH in bulk drug and in pharmaceutical formulations.

1-10 phenanthroline (1-10 PNL) and 2, 2' bipyridyl (2, 2'-BPL) forms a complex of low tinctorial value with Fe^{III} which in turn function as a better oxidant than Fe^{III}. Based on its complexing tendency and oxidizing properties, unhydrous ferric chloride, 1-10 PNL and 2, 2'-BPL were previously reported to be a sensitive reagent for spectrophotometric determination of a considerable number of amine and other functional groups containing medicinal compounds[21-26]. The proposed methods were based on the oxidation of drug, which reduces ferric ion (Fe^{III}) to ferrous ion (Fe^{II}) in presence of 1-10 PNL and 2, 2'-BPL. The colored complexes formed were measured at 510 and 523 nm.

EXPERIMENTAL

Apparatus

A Elico SL model 164 UV-Visible double beam spectrophotometer with 1 cm matched quartz cell was used for recording spectra and absorbance measurements.

Reagents

All chemicals used were of analytical reagent grade or chemically pure grade and used without further purification, double distilled water was used for the dilution of reagents and samples. Freshly prepared solutions were used always. Analytical grade 1-10 PNL, 2, 2'-BPL, $FeCl_3$ and alcohol (S.D Fine Chem.Ltd., Mumbai) were used throughout the experiment. HLH bulk drug was obtained from Sequent Scientific Ltd., Mangalore (India). Pharmaceutical formulations of HLH were obtained commercially; these formulations containing only one drug and do not exist in combination with other drugs.

Standard drug solution

Stock solution of HLH (1000 μg/ml) was prepared by dissolving 100 mg of HLH in distilled water and diluting to the mark in a 100 ml volumetric flask. The solution was protected from light before use. Working standard solution (100 μg/ml) was prepared by further dilution of the above standard stock solution.

1, 10-PNL (0.001 mol/l): First, a 0.1 mol/l solution was prepared by dissolving accurately weighed 1.98 g of 1,10-PNL in alcohol and diluting to the mark in a 100 ml volumetric flask. This was again diluted stepwise to get 0.001 mol/l of 1,10-PNL.

2, 2' - BPL (0.002 mol/l): A 0.2 mol/l solution was first prepared by dissolving 3.12 g of 2,2'- BPL in alcohol and diluting to the mark in a 100 ml volumetric flask. The stock solution was diluted appropriately to get 0.002 mol/l of 2,2'-BPL with alcohol.

$FeCl_3$ (0.03 mol/l): A 0.03 mol/l solution was directly prepared by dissolving 0.486 mg of ferric chloride in distilled water and diluting to the mark in a 100 ml volumetric flask.

Procedure

For bulk drug sample

Spectrophotometry with 1-10 PNL (Method A)

Different aliquots of standard drug solution (0.1 to 1.0 ml) of 100μg/ml (1 ml=100 μg/ml) were accurately measured into a series of 10 ml volumetric flasks by means of a micro burette. To each flask, 0.5 ml of 0.03 mol/l ferric chloride solution and 2.5 ml of 0.001 mol/l alcoholic solution of 1-10 PNL solution were added. The flasks were stoppered, contents were mixed well and kept in a water bath (50-60±1^{0}C) for 10 min. Then immediately cooled to room temperature (25±1^{0}C) using a cold water bath. The flasks were made up to 10 ml with distilled water to get final concentration of 1-10 μg/ml. The absorbance of each orange red colored chromogen formed was measured against a reagent blank at 510 nm after 5 min.

Spectrophotometry with 2, 2'- BPL (Method B)

Varying aliquots of standard drug solution (0.1 to 1.5 ml) of 100 μg/ml (1 ml=100 μg/ml) were transferred into a series of 10 ml volumetric flasks and 0.6 ml of 0.03 mol/l of ferric chloride solution followed by

2.0 ml of 0.002 mol/l alcoholic solution of 2, 2'- BPL were added by means of micro burette. The flasks were stoppered, contents were mixed well and kept in a water bath (50-60±1^{0}C) for 15 min, then immediately cooled to room temperature (25±1^{0}C) using a cold water bath. The flasks were made up to 10 ml with distilled water to get a final concentration of 1-15µg/ml. The absorbance of each orange red colored chromogen formed was measured against a reagent blank at 523 nm after 5 min.

A calibration graph (for method A and B) was prepared by plotting absorbance versus concentration of drug and the concentration of the unknown was read from the calibration graph or computed from the regression equation derived from the Beer's law data. The calibration graph was then prepared by plotting the absorbance versus concentration of the drug.

Assay procedure for tablets

For analysis of HLH, 20 tablets were weighed and ground into a fine powder. An accurately weighed portion of the powder equivalent to 100 mg of HLH was transferred in to a 100 ml beaker containing small volume of water then it was shaken thoroughly for about 5-10 min. and filtered through a whatman filter paper no. 41 to remove the insoluble matter. The filter paper was washed with water and the washings were added to the filtrate and the final volume (100 ml) was made with double distilled water. 1 ml of the above filtrate was taken in a 10 ml volumetric flask and was treated as per procedure described in the above determination of pure HLH. The percentage recovery of drug was calculated from the corresponding linear regression equation.

RESULTS AND DISCUSSION

Determination of absorption maxima

The proposed methods undergo oxidation by Fe^{III} present in 1-10 PNL and 2,2'-BPL. The Fe^{II} so formed readily combines with 1-10 PNL or 2, 2' BPL to form a orange red colored complex, having absorption maximum at 510 and 523 nm. Under the experimental conditions each reagent blank showed a negligible absorbance at the corresponding λ_{max}. The absorption maxima of colored complexes of both the methods are shown in figure 1.

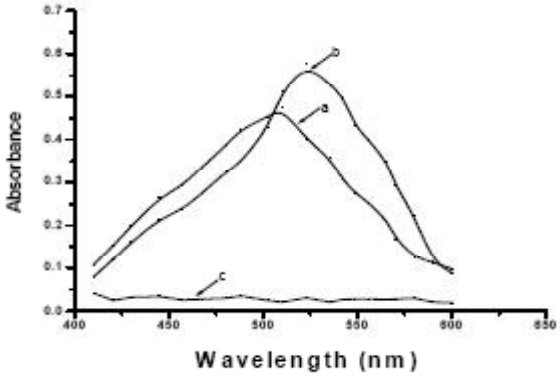


Figure 1: Absorption spectra of HLH at 4 and 5 µg per 10 ml of concentration for (a) Method A and (b) Method B with (c) reagent blank

Reaction sequence

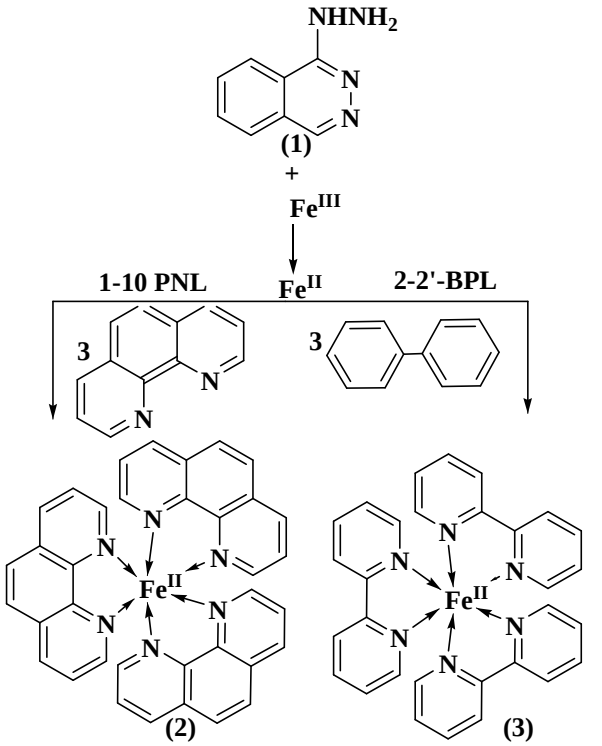
Ferric chloride play a prominent role in the spectrophotometric determination of many pharmaceutical drugs[21-27]. Acting as an oxidant, a ferric ion (Fe^{III}) gets reduced to ferrous ion (Fe^{II}) and this amount corresponds to drug concentration. The amount of Fe^{II} can be determined using reagents such as 1-10 PNL and 2, 2'- BPL. These properties have been utilized to develop spectrophotometric methods for the determination of HLH. In the proposed spectrophotometric methods, HLH was oxidized with excess of ferric chloride under specified experimental condition, the formed ferrous ion on reacting with 1-10 PNL and 2, 2' -BPL to form a orange red colored complex due to the acceptance of lone pair of electrons and forms six coordinate bond with ferrous ion, with absorption maxima at 510 nm and 523 nm for both the methods. (Shown in SCHEME)

Optimization of reaction conditions

The spectrophotometric properties of the colored complex formed with method A and B were extensively studied. The optimum conditions for the assay procedures have been established by studying the reaction as a function of heating time, temperature, concentration of reagents and stability of the colored complex.

Effect of heating time

To study the effect of heating time for maximum color development, 0.5 ml and 0.6 ml of 0.03 mol/l $FeCl_3$ solution was mixed with 2.5 ml of 0.001 mol/l



SCHEME

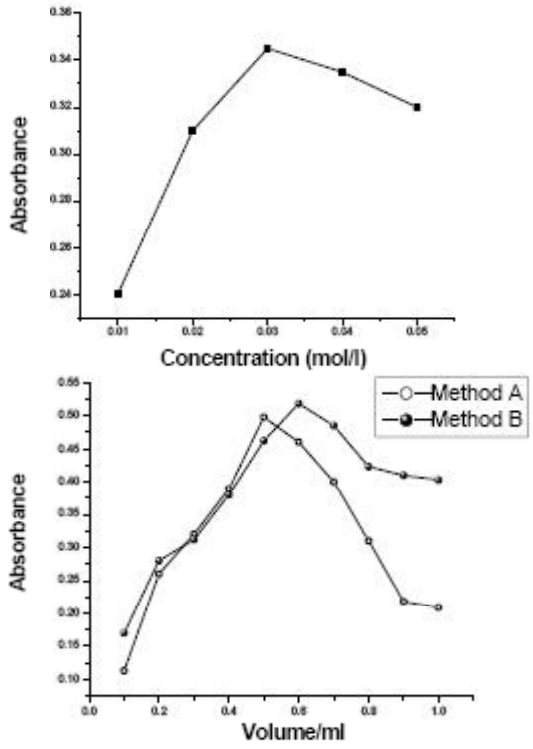


Figure 2(a): Effect of the $FeCl_3$ concentration on the absorbance of 4 μg/ml HLH; (b): Effect of the volume of $FeCl_3$ (0.03 mol/l) on the absorbance of 5μg/ml HLH

1-10 PNL and 2.0 ml of 0.002 mol/l 2, 2'- BPL reagents (Method A and B). The contents of the mixture were heated up to 30 min. for both the methods, in a water bath at 30-60^{0}C. It is apparent from investigations that the maximum intensity of color was attained after 8 min. and remains constant up to 12 min. for method A. For method B, the maximum intensity of color was attained at 12 min and remains constant up to 18 min. Therefore, the optimum heating time was fixed to 10 and 15 min. for Method A and B respectively throughout the experiment.

Effect of concentration and volume

$FeCl_3$: The effect of ferric chloride concentration was studied (for method A and B), by using an aliquot of drug containing 4 μg/ml (100 μg/ml) followed by varying concentration of ferric chloride in the range of 0.01 to 0.05 mol/l. As can be seen from figure 2(a), the analytical signal increased with an increase in reagent concentration up to 0.03 mol/l and thereafter decreases with an increase in reagent concentration. Therefore the concentration of ferric chloride selected was 0.03mol/l. The influence of volume of ferric chloride (0.03 mol/l) was observed during the formation of orange red colored product. To study this, an aliquot of drug containing 4 μg/ml (100 μg/ml) was used by varying volumes of ferric chloride (0.1-1.0 ml for mathod A and B) and 2.5 ml of 0.001 mol/l 1, 10- PNL (Method A) and 2.0 ml of 0.002 mol/l, 2, 2'-BPL (Method B). It is evident from figure 2(b) that the highest analytical signal was attained with 0.5 ml (Method A) and 0.6 ml (Method B) of 0.03 mol/l ferric chloride. Above this volume, the analytical signal remains unchanged. Therefore 0.5 ml and 0.6 ml of 0.03 mol/l ferric chloride was used in all further measurements for method A and method B respectively.

1-10 PNL AND 2, 2'-BPL: The effect of 1,10- PNL and 2, 2'- BPL concentration was studied by using an aliquot of drug containing 4 μg/ml (100 μg/ml). 0.5 ml and 0.6 ml of 0.03 mol/l of ferric chloride was added to the drug followed by different concentration of 1-10 PNL and 2, 2'- BPL in the range of 0.0005 to 0.004 mol/l. As can be seen from figure 3(a), the analytical signal increased with an increase in reagent concentration up to 0.001 mol/l for Method A and 0.002 mol/l for Method B, above which it remained virtually constant. Therefore the concentration selected was 0.001

Full Paper

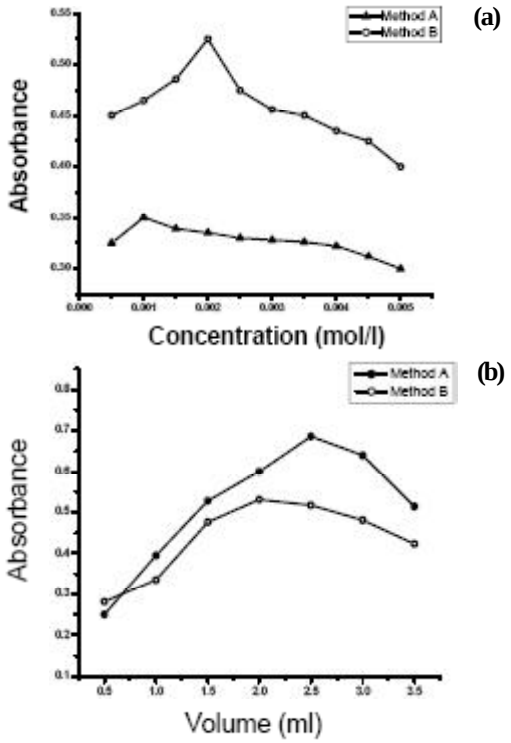


Figure 3(a): Effect of concentration of 1,10- PNL and 2,2'- BPL; (b): Effect of the Volume of 0.001 mol/l 1,10-PNL and 0.002 mol/l 2,2'- BPL

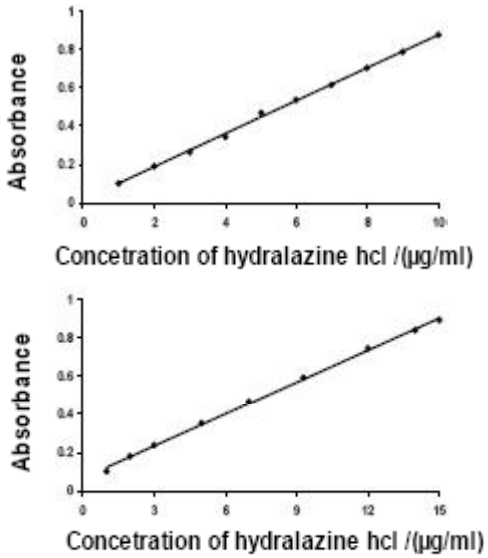


Figure 4(a): Calibration graph for the determination of HLH under the optimum conditions for method A; (b): Calibration graph for the determination of HLH under the optimum conditions for method B

mol/l and 0.002 mol/l for method A and method B respectively. To investigate the effect of volume of 0.001 mol/l 1, 10- PNL and 0.002 mol/l 2, 2' - BPL reagent for color development, different volumes (0.5- 3.5 ml for Method A and B) were mixed with 4µg/ml HLH drug of 100 µg/ml and 0.5 ml of 0.03 mol/l ferric chloride solution for Method A and 0.6 ml of 0.03 mol/l ferric chloride solution for Method B. The results are presented in figure 3(b) which reveals that the addition of 2.5 ml of 1,10- PNL for Method A and 2.0 ml of 2, 2'- BPL for Method B gave the highest analytical signal. Therefore 2.5 ml of 1,10- PNL and 2.0 ml of 2, 2'-BPL reagents were selected for determination of HLH through out the investigation. However, the complexes formed were stable for more than 1 h at room temperature.

TABLE 1: Results of optimization of the parameters

Methods	Concentration			Temp. (^{0}C)	Time (min.)
	$FeCl_3$ 0.03 mol/l (ml)	1,10 PNL 0.001 mol/l (ml)	2,2?BPL 0.002 mol/l (ml)		
A	0.5	2.5	-	55	10
B	0.6	-	2.0	55	15

Effect of temperature

After optimization of chemical variables, the influence of temperature on the colored complexes was studied at different temperature (30-80^{0}C); it was observed that the obtained colored complexes were stable up to 60^{0}C. However, no considerable improvements were occurred above 60^{0}C therefore 55^{0}C was selected as optimum temperature for both the methods.

The optimum chemical conditions investigated in reaction are summarized in TABLE 1. These values allowed to obtain good sensitivity and high precision of HLH determination.

Analytical data and calibration graphs

Under optimized experimental conditions, linear calibration curves (figures 4(a and b)) were obtained over the concentration range of 0.1-1.0 and 0.1-1.5 µg/ml HLH with molar absorptivities of 1.723×10^4 and 1.253×10^4 l/mol/cm for Method A and B respectively.

The linear regression equation for both the methods have been evaluated by least square treatment of the calibration data (n=6). TABLE 2 summarizes Beer's law limit, linear regression equation, correlation coefficient, confidence limits and standard deviations for slope and intercepts at 95% (0.05) confidence level and detection limits for method A and B.

In each method, the correlation coefficient was high, indicating the excellent linearity of both the calibration

TABLE 2: Analytical parameters of spectrophotometric methods

Parameters	Method A	Method B
λ_{max} (nm)	510	523
Beer's Law limits (μg/ml)	1-10	1-15
Molar absorptivity (l/mol/cm)	1.723×10^4	1.253×10^4
Sandell's sensitivity (μg/ml)	0.1141	0.01569
Regression equation (Y=bx + c)**		
Slope(b)	0.0853	0.0556
Intercept(c)	0.0293	0.070
Correlation coefficient (r)	0.9996	0.9994
% Relative Standard Deviation (R.S.D)*	1.1055	1.9118
% Range of error (Confidence)*		
0.05 level	0.0074	0.0094
0.01 level	0.0110	0.0139
Limit of Detection (μg/ml)	0.0850	0.0820
Limit of Quantification (μg/ml)	0.2830	0.2730
Stability (h.)	2	2
Color	Orange red	Orange red

TABLE 3: Determination of HLH in pharmaceutical formulations

Method	Formulations (Tablets)	Labeled amount (mg)	Recovery [a] ± S.D (%)	
			Proposed	Reference[20]
A	Nepresol	25	99.22±0.28	101.03±0.49
B	Nepresol	25	98.87± 0.57	101.03 ± 0.49

[a]Average of six determinations ± S.D.

TABLE 4: Determination of HLH[a] in the presence of excipients

Excipients	Amount taken (mg/ml)	% Recovery +%RSD[b]
Glucose	20	99.5 +0.54
Sucrose	20	99.47+0.25
Lactose	25	99.64+0.56
Dextrose	20	100.1+0.21
Talc	25	98.96+0.82
Starch	20	99.20+0.38
Sodium alginate	15	100.25+0.64

[a]10μg/ml HLH were used, [b]mean value of six determinations

graphs. The low values of confidence interval at 95% confidence level for slope and intercept of the regression lines pointed towards high reproducibility of the proposed methods. Graphs of absorbance versus concentration showed zero intercept and are described by the regression equation Y=bX+c (where Y is the absorbance of 1 cm layer b is the slope, c is the intercept and X is the concentration of the drug in μg/ml) obtained by the least squares method.

Validation of the methods

The validity of Method A and B for the determination of HLH was examined by determining the precision and accuracy. These were determined by analyzing six replicates of the drug within the Beer's law limits. The low value of relative standard deviation (RSD) indicates good precision of the methods. To study the accuracy of these methods, recovery studies were carried out by standard addition method. For this, known quantities of pure HLH were mixed with definite amounts of pre-analyzed formulations and the mixtures were analyzed as before. The total amount of the drug was calculated by difference. The results are given in TABLE 3. The average percent recoveries obtained were quantitative (98.87±0.57 to 99.22±0.28), indicating good accuracy of the methods.

Interference studies

The effect of common additives and excipients were tested for their possible interferences in the determination of HLH. It was observed that starch, talc, dextrose, lactose, sucrose and sodium alginate did not interfere under the experimental conditions employed. To a known amount of drug (HLH 10 μg/ml) excipients in different concentrations were added and analyzed using method A and B. Results of the recovery analysis were found in the range of 98.96 to 100.25% and are presented in TABLE 4. The excipients up to 10 to 25 mg/ml concentrations did not interfere. In addition recoveries in most cases were 100% and the lower values of the RSD indicate the good precision of the method.

CONCLUSIONS

The proposed methods are simple, accurate, and selective and offer advantages of reagent availability and stability, less time consumption and high sensitivity. Although the color development at room temperature requires 2 h for completion, this can be shortened to 10 and 15 min by raising the temperature to 55^0C for both the methods. The proposed methods are suitable for determination of HLH in pharmaceutical formulations. Unlike the gas chromatography and high performance liquid chromatography procedures the UV-visible spectrophotometer instrument is simple and not of high cost, on the other hand in terms of simplicity and expense,

Full Paper

the method could be considered superior in comparison with the previously reported methods. The procedure do not involve any critical conditions or tedious sample preparation, moreover the methods are free from interferences by common additives and excipients.

ACKNOWLEDGMENTS

The authors are grateful to M/s Sequent Scientific Ltd., Mangalore (India) for providing gift sample of HLH drug and Principal, H.K.E Society's College of Pharmacy, Gulbarga (India) for providing library and other necessary facilities.

REFERENCES

[1] M.J.O'Neil; 13th Ed., 'The Merck Index-An Encyclopedia of Chemicals, Drugs and Biologicals', Merck and Co. INC., 1946-1948 **(2001)**.
[2] D.F.Westfall, C.R.Craig, R.Stitzel; 'Modern Pharmacology', 347 **(1982)**.
[3] World Health Organization (WHO); 'The International Pharmacopoeia', 3rd Ed.,306 **(1988)**.
[4] British Pharmacopoeia and HM Stationary Office, London, **1**, **11**, 328-329, 941, 942 **(1993)**.
[5] 'Indian Pharmacopoeia', Controller of Publications, New Delhi, **1**, 368 **(1996)**.
[6] United States Pharmacopoeia-25, NF-20-US- Pharmacopoeial Convention, Rockville, MD, 846 **(2002)**.
[7] D.V.Naik, B.R.Davis, K.M.Minnet, S.G.Schulman; J.Pharm.Sci., **65**, 274 **(1976)**.
[8] O.M.Gaidukevich, T.V.Zhukova, M.A.Zarechensky, G.Sim; Farm-Zh-Kier., **43**, 49 **(1988)**.
[9] Z.Fijalek, E.Szysko; Acta Pol.Pharm., **40**, 343 **(1983)**.
[10] A.M.Di-pietra, P.Roveri, R.Gotti, V.Cavrini; II Farmaco., **48**, 1555 **(1993)**.
[11] M.C.Dutt, T.L.Ng, L.J.Long; J.Assoc.Anal.Chem., **66**, 1455 **(1983)**.
[12] J.T.Stewart, E.H.Parks; Int.J.Pharmaceutics, **17**, 161 **(1983)**.
[13] W.F.El-Hawar, A.F.Shoukry; Talanta, **71**, 179 **(2003)**.
[14] B.J.Marr, V.J.Manes, D.R.Garcia, M.G.Pous; J.Pharm.Sci., **80**, 672 **(1991)**.
[15] M.Bedair, M.A.Korany, F.A.El-Yazbi; Sci.Pharm., **54**, 31 **(1986)**.
[16] F.A.Ibrahim, M.S.Rizk, F.Belal; Analyst, **111**, 1285 **(1986)**.
[17] N.A.Zakaria, S.M.Hassan, Y.Elshabrawy; Drug Development and Industrial Pharmacy, **16**, 1554 **(1990)**.
[18] L.Martinez, Y.Miranda, Y.C.V.Aleyda; Rev.Cubana Form., **38**, 1 **(2004)**.
[19] A.A.Olaniyi, A.A.Fasanmade, A.A.Olaniyi; 'Principles of Drug Quality Assurance and Pharmaceutical Analysis', Mosuro Publishers; Ibadan, **(2000)**.
[20] B.H. Ahmed, O.O.Johnson; J.Pharmacy and Bioresources, **1**, 1 **(2004)**.
[21] S.Lakshmi, M.A.Mervin, L.Jayashankar, P.Rama, T.K.Raja; Indian J.Pharm.Sci., **2**, 249 **(2004)**.
[22] B.S.Nagaralli, J.Seetharamappa, M.B.Melwanki; J.Pharam.Biomed.Anal., **29**, 859 **(2002)**.
[23] B.H.M.Mruthyunjayaswamy, B.Hiremath, S.Appal Raju, S.M.Malipatil; J.Indian Council Chem., **23**, 1 **(2006)**.
[24] B.K.Prabhakar, M.S.Sobha, S.Appal Raju; Int.J. Chem.Sci., **31**, 517 **(2005)**.
[25] M.Vamsikrishna, D.Gowrishankar; E.J.Chem., **4**, 272 **(2007)**.
[26] F.Khalil, M.Ramin; Anal.Sci., **17**, 867 **(2007)**.