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Assay of Guanfacine in Bulk and Its Pharmaceutical Formulations by Visible Spectrophotometry

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ABSTRACT

Three simple and sensitive visible spectrophotometric methods (M_1, M_2, and M_3) have been described for the estimation of Guanfacine (GUN). The methods are based on the formation of radical anion with the involvement of basic nitrogen in GUN (donor) and quinones [2,3-dichloro-5,6-dicyano-p-benzoquinone(DDQ), chloranilic acid (DHQ), 2,3,5,6-tetrachloro-p-benzoquinone (TQ)] (acceptor). The variable parameters in all these methods have been optimized and the chemical reactions involved are presented. The results obtained in the three methods are statistically validated and recoveries range from 99.7 to 100.3%. Common excipients used in additives in pharmaceutical preparations do not interfere in the proposed methods.

Keywords: Guanfacine, DDQ, DHQ, TQ, Spectrophotometric, Pharmaceutical formulations.

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INTRODUCTION

Guanfacine hydrochloride (GUN) is a centrally acting antihypertensive with *alpha*2-adrenoceptor agonist for oral administration and chemically known as N-Amidino-2- (2,6-dichlorophenyl) acetamide mono hydrochloride. A number of methods such as Spectroflurometric[1,2], Spectrophotometic[3,4] and GLC[5,6,7] were reported for the estimation of GUN. Literature survey revealed that only two visible spectrophotometric[3,4] methods were reported for its quantitative determination in bulk drug and pharmaceutical formulations. Hence there is a need to develop sensitive and flexible spectrophotometric methods for the assay of GUN.

A direct chemical analysis based on the reactivity of the intact molecule without cleavage is not frequently encountered. The methods that are based on the charge transfer complexation are usually rapid and simple to perform. π-acceptors such as 2,3-dichloro-5,6-dicyano-p-benzoquinone(DDQ), 2,3,5,6-tetrachloro-p-benzoquinone(TQ), chloranilic acid (DHQ) are known to yield charge transfer complexes with a variety of electron donors. The present work describes an improved direct simple analytical procedure that can be applied at quality control laboratories for the analysis of Guanfacine.

MATERIALS AND METHODS

Instrument

A Systronics model 117 UV – Visible spectrophotometric with 1cm matched quartz cells was used for spectral and absorbance measurements in the UV and visible regions respectively.

Materials and reagents

All reagents used were of Analytical Grade and freshly prepared solutions were always used. DDQ (Fluka, 4.4×10^{-3}M) solution in acetonitrile for Method A, DHQ (Sd-Fine, 0.1%, 4.785×10^{-3}M) solution in methanol for Method B, TQ (BDH, 0.1%, 4.067×10^{-3}M) solution in 1.4-dioxane for Method C were prepared.

Standard Drug Solution

Stock solution (1mg/1ml) of GUN was prepared by dissolving 100 mg of it in 100ml of methanol for method A & B and 100 mg of it in 100ml of acetonitrile for method C. The working standard solutions of GUN of the required strength were prepared by further dilution of stock solution of GUN with methanol (method A and B) and acetonitrile (method C).

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Method A (Bulk Sample)

Aliquots of standard drug GUN solution (0.5-2.5 ml, 200 µg /ml) in acetonitrile were delivered into 10 ml were delivered into 10 ml graduated tubes. Then 2ml of (4.4 $\times 10^{-3}$M) DDQ in acetonitrile was added and kept aside for 15 min (GUN). The volume was made up to 10 ml with acetonitrile and read at 440 nm against reagent blank during the stability period (15-60min). The amount of drug present was computed from the appropriate calibration curve

Method B (Bulk Sample)

Aliquots of standard drug GUN solution (1.0 – 3.0 ml, 200 µg/ml), was transferred into 10ml-graduated tubes. 2.0 ml of (4.785 x 10^{-3} M) DHQ in methanol was added and kept aside for 5 min. Then the volumes of the contents were made up to 10 ml with methanol and read at 520 nm for GUN against a blank reagent within 30 min. The amount of drug was computed from the appropriate calibration curve.

Method C (Bulk Sample)

Aliquots of standard drug GUN solution (0.5 – 2.5 mL, 400 µg/ml) in dioxan were delivered into 10 ml graduated tubes. Two ml of (4.067 $\times$ 10^{-3}M) TQ in 1,4-dioxan, followed by dioxan was added for bringing the volume to 7 ml. The final volume was brought to 10 ml with dimethyl formamide and the absorbance was measured against a reagent blank at 620 nm for GUN within the stability period (15-60min). The amount of the drug present was computed from the appropriate calibration graph.

Method A, B & C for Pharmaceutical Formulations

An accurately weighed amount of Guanfacine tablet powder equivalent to 100 mg of GUN was extracted with isopropanol (4 x 15ml) and filtered. The combined filtrate was evaporated to dryness and the residue was dissolved in methanol (method A & B) and acetonitrile (method C) to get 1mg/ml solution. The working standard solution of GUN of required strength prepared by further dilution of the stock solution of GUN with required solvent in the respective method and analyzed under procedure described for bulk samples.

RESULTS AND DISCUSSION

The optimum conditions for the colour development of method were established by varying the parameters one at a time in each method, keeping the others fixed and observing the effect produced on the absorbance of the coloured species.

The optical characteristics such as Beer's law limits, molar absorptivity for each method are given in Table 2. The precision of each method was found by measuring absorbance of six replicate samples containing known amounts of drug and the results obtained are incorporated

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in Table 2. Regression analyses using the method of least squares were made to evaluate the slope (b), intercept (a) and correlation coefficient (r) for each method and are presented in Table 2. The accuracy of each method was ascertained by comparing the results by proposed and reference methods (UV) statistically (Table 3). This comparison shows that there is no significant difference between the results of proposed methods and those of the reference ones. The similarity of the results is obvious evidence that during the application of these methods, the additives and excipients that are usually present in tablets do not interfere in the assay of proposed methods. As an additional check of accuracy of the proposed methods, the recovery experiments were performed by adding a fixed amount of the drug to the pre-analyzed formulations. The amount of drug found and the percentage of recovery was calculated in the usual way.

Table 1: Reaction time and intensity in polar solvent

Acceptor	Reaction time	Solvent	Absorption Maxima
TQ	10	Dimethyl formamide	620
DHQ	5	Methanol	520
DDQ	15	Acetonitrile	440

Table 2: Optical Characteristics, Precision and Accuracy of the Proposed Methods for GUN

Parameters	DDQ	DHQ	TQ
λ_{max} (nm)	440	520	620
Beer's Law limits (μg/mL)	10-50	20-80	20-100
Molar absorptivity (l $mol^{-1}cm^{-1}$)	4.331×10^3	3.357×10^3	1.921×10^3
Correlation coefficient (r)	0.9999	0.9996	0.9999
Sandell's sensitivity (μg/cm^2/ 0.001 absorbance unit)	0.065	0.084	0.147
Regression Equation (y = a + bc) (i)Slope (b)	0.01533	0.0118	0.0068
(iii) Intercept (a)	-0.0011	-0.0084	-0.0018
Relative Standard Deviation *	0.1931	0.2455	0.2498
% Range of error (confidence limits) (i) 0.05 level	0.162	0.205	0.209
(ii) 0.01 level	0.240	0.304	0.308

*Average of six determinations considered. **Average of three determinations.

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Table 3: Assay of GUN in pharmaceutical formulations

Sample	Labeled amount (mg)	Amount found by Proposed Methods*			Amount found by reference method	%Recovery by Proposed methods**		
		A	B	C		A	B	C
Tablet I	1	0.99± 0.001	0.99± 0.002	1.00± 0.004	0.99± 0.001	99.83± 0.15	99.73± 0.45	99.81± 0.004
Tablet II	1	1.00± 0.003	1.00± 0.005	0.99± 0.007	1.00± 0.002	100.38± 0.66	100.37± 1.00	99.94± 0.032
Tablet III	1	0.99± 0.005	0.99± 0.005	1.00± 0.011	0.99± 0.005	99.55± 0.56	99.81± 0.50	100.16± 0.79
Tablet IV	1	0.99± 0.010	1.00± 0.006	0.99± 0.009	1.00± 0.007	99.20± 1.09	100.29± 0.64	99.72± 0.11

*Average ± standard deviation of six determinations.
**After adding 3 different amounts of the pure labeled to the pharmaceutical formulation, each value is an average of 3 determinations.

Chemistry

The interaction of any of the investigated compounds with poly halo and polycyanoquinone π-acceptors in non polar solvents was found to produce colored charge-transfer complexes with low molecular absorptivity values. In polar solvents such as acetonitrile or methanol, the complete electron transfer from donor to the acceptor moiety takes place with formations of intensely colored radical ions with higher molar absorptivity values according to the following scheme.

$$D^{\cdot\cdot} + A \rightarrow \underset{\text{Complex}}{(D{-}A)} \xrightarrow{\text{Polar solvent}} A^{-\cdot} + D^{+\cdot}$$

The dissociation of the D-A Complex is promoted by the high ionizing power of the acetonitrile and the resulting bands of the named drugs with acceptors are similar to the maxima of radical anions of the pi-acceptors obtained by the iodide reduction method.
Acetonitrile was considered an ideal solvent as it afforded maximum sensitivity yield of radical anions in addition to its high solvating power of the reagents. Methanol gave maximum sensitivity in case of DHQ and DMF gave maximum sensitivity in case of TQ. The interaction of GUN with TQ, DHQ, DDQ, gave a colored chromogens with a strong absorption maxima in different solvents given in Table-1.

CONCLUSIONS

The proposed methods are applicable for the assay of drug (GUN) and have the advantage of wider range under Beer's law limits. The decreasing order of sensitivity and λ_{max} among the proposed methods are C > B > A respectively. The proposed methods are simple,

selective and can be used in the routine determination of GUN in bulk samples and formulations with reasonable precision and accuracy.

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