Spectrophotometric Determination of Diazepam

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Summary: Diazepam reacts with ninhydrin at pH range 12-12.5 to give a violet color having maximum absorbance at 530 nm wavelength. The reaction is specific for diazepam and provides a basis for its spectrophotometric determination. The color reaction obeys Beer's law from 0.1 to 2 mg/10 mL of diazepam. Coefficient of variation and confidence limit determination are 0.06 and 0.0041%, respectively. The relative standard deviation is 1.5%. The quantitative assessments of tolerable amount of other drugs have also been studied.

Keywords: *Diazepam , Ninhydrin, Spectrophotometric Determination.*

Introduction

Diazepam is benzodiazepines derivative. Benzodi-azepines are important group of sedatives, muscle-relaxants and anticonvalsant substances, members of which have been widely used in the treatment of anxiety, alcoholism and a variety of other disorders. A sedative drug decreases activity, moderates excitement and calms the recipient while hypnotic drugs produces drowsiness and facilitates the onset and maintenance of a state of sleep that resembles natural sleep [1-3]. Many analytical techniques have been employed for the detection and determination of Diazepam. An optimized capillary electrophoresis electron spray mass spectrometric method is presented for the identification and determination of diazepam and its metabolites [3-5]. A high performance liquid chromatographing (HPLC) method for the stimulate determination of five frequently prescribed benzodiazepines; diazepam, nitrazepam, miazolam and axazepam has also been developed [2, 3]. Methodology has been developed for the detection and quantization of diazepam in non-alcoholic carbonated beverages and fruit drinks which are adulterated for criminal motives. The determination of diazepam in 5 brands of spiked and stimulated cold drinks has been carried out at pH 8.5 by two different extended solvents di-ethylether and chloroform. The identification of diazepam was done on the basis of Rf values and *in-situ* UV spectra [1-3]. Diazepam has been evaluated in Argya Kalha by solvent extraction and high performance TLC with detection at 254 nm . Diazepam and related compounds have been determined in bulk and tablets by high performance TLC (HPTLC) densitometry at 254 nm. The recovery was 94-115%.

During a systematic study of pharmaceutical drugs, it was found that diazepam reacts with ninhydrin in an alkaline medium to give a violet color at λ_{max} value of 530 nm. The reaction is specific for diazepam and has 0.1 mg/mL as visual limit of identification. This indicates that this color reaction has not been reported previously. The present method is simple, not requiring many chemicals, is accurate, precise and other organic compounds like prioxicam, flauxitine HCl, chloromazine HCl, buscopan, phenobarbiturates do not interfere even if present as 18.2, 38.7, 21, 19.3, and 39.6, folds respectively.

Results and Discussion

Results of various investigations obtained in developing new spectrophotometric method for the determination of Diazepam are summarized in tables 1-9 and figures 1-7. The statistical parameters which show reproducibility, sensitivity, validity and reliability for the calibration curve were calculated and are summarized in the table-10. Diazepam reacts with Ninhydrin when heated for 60 seconds at 60 ^{o}C give violet colored complex, the absorption spectra under optimum condition lies at 530 nm. All measurements were carried out at wavelength 530 nm. It was found that heating for 60 seconds at 60 ^{o}C on water bath gave maximum color intensity and after 60 seconds there was no effect of further

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heating. It was found that 0.5 mL of 2% ninhydrin gave maximum color intensity along with sodium hydroxide. Above and below these concentrations color was decreased. Beer's law was obeyed when concentration ranges 0.1-2 mg. Effect of time on stability showed that the color is stable for more than 120 minutes. The readings were carried out within this time period. During all experiments total volume of test solution was made up to 10 mL with pure alcohol. The developed method was applied for the determination of Diazepam from pure solution, which shows the reproducibility, sensitivity, and validity of the method. It is also reasonably precise and accurate as the amount taken from identical samples is known and amount found by the above procedure does not exceed the standard deviation 1.5% which is the replicate of five determinations. The optimization has been done at lower analyte concentration.

The quantitative assessments of tolerable amounts of different organic compounds (W/V) under the experimental conditions have been worked out. Various amounts of diverse organic nature were added to a fixed amount of diazepam (1 mg/mL) and the recommended procedure for the spectrophotometer determination was followed. The proposed method is successfully applied for the quality control of pure diazepam and in pharmaceutical dosage.

The spectrophotometric method for the determination of diazepam is simple, reliable and sensitive.

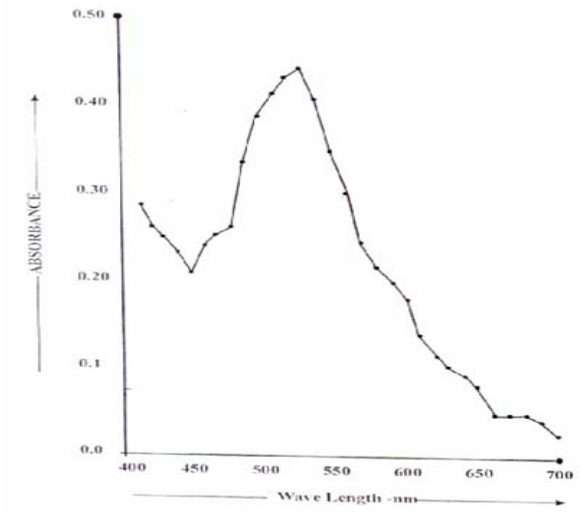

Fig. 1: Change in absorbance with wavelength for solution of diazepam

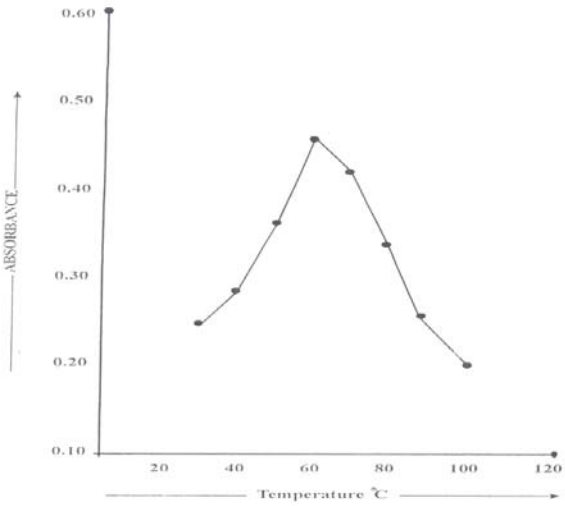

Fig. 2: Effect of temperature on stability of colour

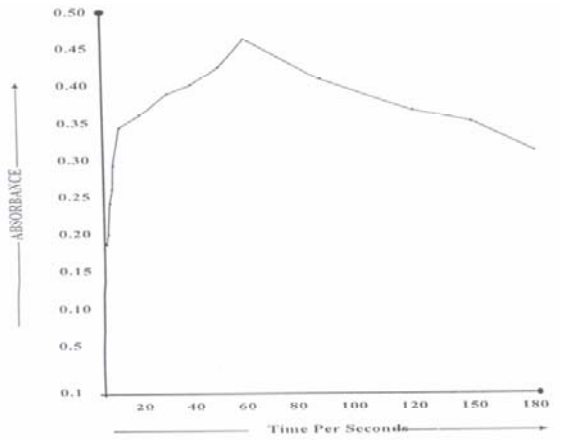

Fig. 3: Effect of time per seconds

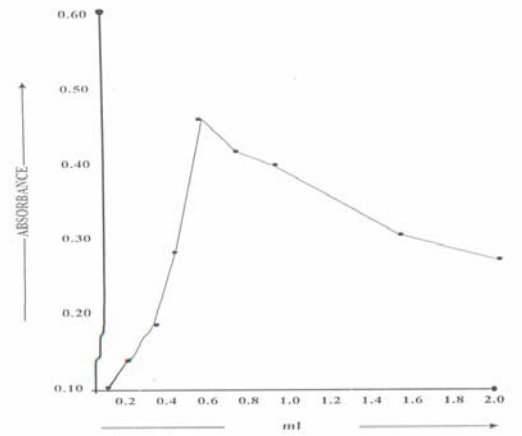

Fig. 4: Effect of Reagent ninhydrin

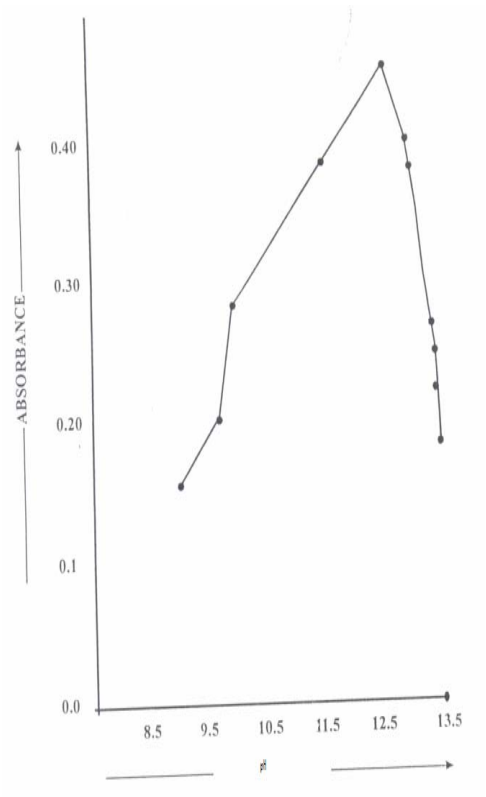

Fig. 5: Effect of pH.

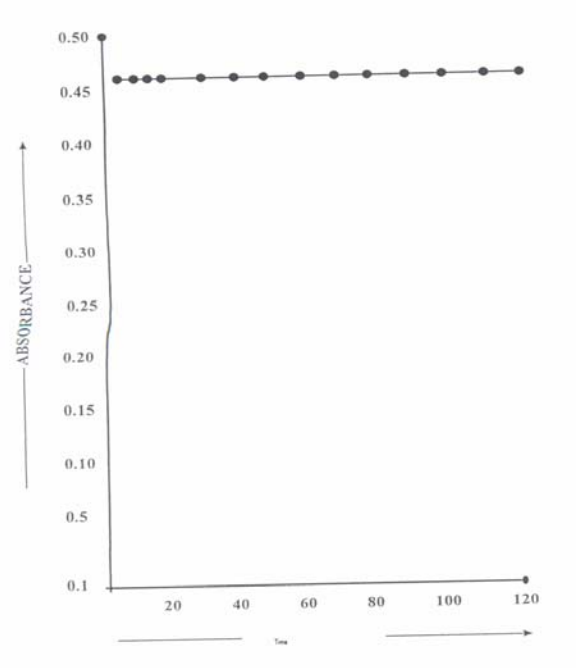

Fig. 6: Effect of time on color stability.

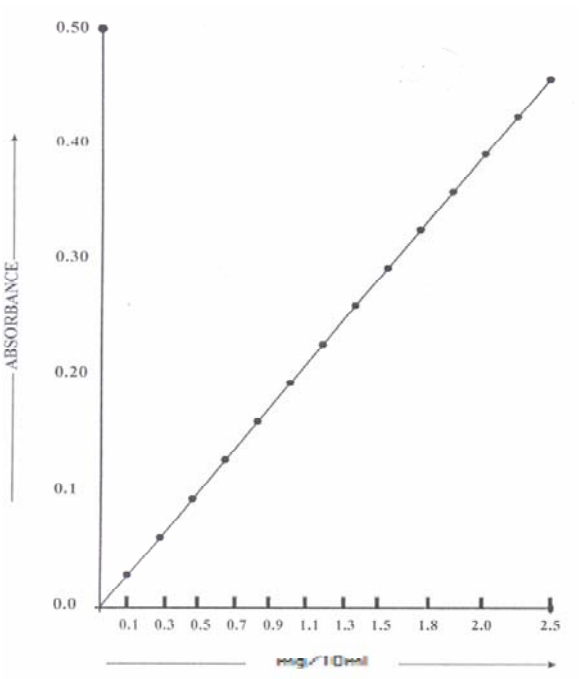

Fig. 7: Calibration curve of diazepam with ninhydrin.

Table-1: Determination of Diazepam from Pure Solution.

No. of Obs.	Diazepam taken mg	Diazepam found mg	Standard Deviation.	Relative Standard deviation
1	0.1	0.101		
2	0.1	0.102		
3	0.1	0.103		
4	0.1	0.104		
5	0.1	0.105	0.516	1.5%

Table-2: Quantitative Assessment of Tolerable Amount of Other Drugs

Drugs	Maximum Amount* not Interfering (%)
Atenolol	2.50
Prioxicam	18.2
Procholorperazine	1.60
Flauxitine HCL	38.7
Choloromazine-HCl.	21.0
Buscopan	19.3
Phenobarbiturates	39.6
Ibuprofen	2.30

Table-3: Optical characteristics of the proposed method

Parameters	Values
λ max (nm)	530
Beer's Law Limits (mg/10 ml, C)	0.1-2
Molar Absorbtivity (mol^{-1} cm^{-1})	6.38×10^3
Correlation Coefficient (r)	0.06
RSDD** (%)	1.5
% Range of error confidence limits at 95% confidence level	2.52 ± 0.044
Optimum photometric range (mg/10 ml)	0.1-2

$y^* = a + bC$, where C so concentration of analyte (mg/ ml) *a* and *y are* absorbance units. (Calculated from five determinations).

Experimental

All chemicals i.e ninhydrin (E. Merk), diazepam (Roche), chloral hydrate, (E. Merk) Asprine, (Fazal Din and Sons (Lahore)), prochloroperazine (Fazal Din and Sons (Lahore)) acetyl salicylic acid, (E. Merk), barbituric acid, (BDH), atenolol, (Zafa) pentazocine (Searle Pak. Ltd. (Karachi)), phenobarbitone (E. Merk), nicotin (E. Merk) buscopan (Glaxo (Pak)), NaOH (E. Merk), fluxetine-HCl (Eli Lilly (England)) brufien(BASF Pharmas (Karachi)), indomethacine (TABROS pharma (Karachi)) were of analytical grade or comparable purity, 2 mg of ninhydrin was dissolved in sufficient quantity of water in the 100 mL measuring flask and the volume was made up to the mark with distilled water.

Physical Measurements

A HITACHIU-1100 spectrophotometer with 1cm silica cells was used to measure the absorbance.

Preparation of Reagents

All reagents and stock solution required for these spectrophotometer studies were prepared in double distilled water and ethyl alcohol, which were of analytical grade.

Diazepam Solution (1mg/mL)

100 mg of pure diazepam was dissolved in 100 mL of distilled water. The solution was stored in airtight container and protected from sunlight. This stock solution of diazepam was used as such or diluted throughout the experimental work.

Sodium Hydroxide (1N NaOH)

4 mg NaOH was dissolved in distilled water in a 100 mL volummatric flask and the volume made up to the mark with distilled water.

Stock Solution of Various Organic Compounds for Checking Interference

Stock solutions of strength 1 mg/mL of various organic compounds were prepared by dissolving 100 mg of the organic compounds in distilled water, ethyl alcohol, methyl alcohol etc, in 100 mL measuring flasks and the volume of the solutions were made up to the mark with above solvents in order to check the interference's of these compounds in the determination of diazepam.

Procedure

Diazepam reacts with ninhydrin and sodium hydroxide to give violet color. The reaction is specific for diazepam and this provides basis for a new spectrophotometric method for its determination in tablets. Effects of different reagents, heating time, temperature and color stability were studied along with interference of different organic compounds in the determination of diazepam.

Determination of λ_{max}

2 mL of diazepam (1 mg/mL) added to 0.5 mL of 2% ninhydrin and 0.5 mL of 1N NaOH. The mixture was shaked and heated for 60 seconds on water bath at 60 ^oC . The mixture was cooled down by adding distilled water and the whole volume was made up to 10 mL. Absorption of colored solution, was measured with a spectrophotometer at different wavelength in the visible region (400 to 700nm) using distilled water as a blank. The spectrum showed a maximum absorption at 530 nm.

Effect of Temperature

In order to study the effect of temperature on the colored reaction 2 mL of **(**1 mg/mL**)** diazepam was mixed with 0.5 mL of 2% ninhydrin and 0.5 mL of 1N NaOH. About 10 test mixtures were prepared and each mixture was heated for 60 seconds on water bath with varying temperature (20 to 100 $^{\circ}$C). The volume of each mixture was made up to 10 mL. Absorbance of the resulting colored solution was measured at 530 nm with a spectrophotometer.

Effect of Heating Time

To study the effect of heating time on the color reaction, 2mL of diazepam (1mg/mL) was mixed with 0.5 mL of 2% ninhydrin and 0.5 mL of 1N NaOH. About 9 test mixtures are prepared for different time intervals. The mixture was cooled by adding distilled water and volume of each solution was made up to 10 mL. The absorbance of these colored solutions were noted at 530 nm with spectrophotometer.

Effect of Color Producing Reagent

To study the effect of ninhydrin on the color reaction, various concentrations of ninhydrin were

mixed with 2 mL of diazepam (1 mg/mL) and 0.5 mL of 1N NaOH. The mixtures were heated at 60 ^{o}C on water bath for 60 seconds. The mixtures were cooled down by adding distilled water and volume of each solution was made up to 10 mL. The absorbance of the resulting violet colored solutions were noted at 530 nm, with spectrophotometer.

Effect of pH Change

To study the effect of pH on the color reaction, various concentration of 0.5 mL of 1N NaOH were mixed with

2 mL of diazepam (1 mg/mL) and 0.5 mL of 2% ninhydrin. About 10 test mixtures were prepared. The resulting mixture was heated at 60 ^{o}C in a water bath for 60 seconds. The absorbance of the resulting violet colored solutions were noted at 530 nm, with spectrophotometer.

Construction of Calibration Curve for Diazepam

The different concentration of diazepam (0.1 to 2.5 mg) were mixed with 0.5 mL of 2% ninhydrin and 0.5 mL

1N NaOH. These mixtures were heated at 60 ^{o}C on water bath for 60 seconds. The absorbance of the resulting violet colored solutions were noted at 530 nm, with spectrophotometer.

Procedure to Study the Effect of Time on Stability of Color

To study the effect of time on stability of color, 2 mL of diazepam (1mg/mL) was mixed with 0.5 mL of 2% ninhydrin and 0.5 mL 1N NaOH. These mixtures were heated at 60 ^{o}C on water bath for 60 seconds. The mixtures were then cooled down by adding distilled water and volume was made up to 10 mL. The absorbance of the solutions were noted after 2, 10, 20, 30, 40, 60, 120 minutes.

Determination of Diazepam from Available Pharmaceutical Preparations

The content of tablets containing diazepam were mixed in water and their solution was prepared by dissolving 500 mg of diazepam in 500 mL of water to get (1 mg/mL) solution. The solution was filtered and used for the determination of diazepam present in tablets using the developed method. The amount of diazepam determined by the developed method was compared by the amount present in different pharmaceutical preparations and percentage error was calculated.

Study of Interference's of Various Organic Compounds

2 mL of diazepam (1 mg/mL) was mixed with 0.5 mL of 2% Ninhydrin and 0.5 mL 1N NaOH and different amount of each interfering compound mixtures were heated at 60 ^{o}C on water bath for 60 seconds. The mixtures were then cooled down made up to 10 mL for each reading. The absorbance of the solutions were noted using distilled water as blank. Amount of each interfering compound was increased till it showed absorbance range of the various organic compounds. Which did not interfere during the determination of diazepam by developed method.

Calculation of Relative Standard Deviation

For the calculation of relative standard deviation, various concentration of diazepam were mixed with 0.5 mL of 2% Diazepam and 0.5 mL 1N NaOH. The mixtures were heated for 60 seconds in a water bath at 60 ^{o}C. The mixtures were cooled down by adding distilled water and the volume of each solution made up to 10 mL. Absorbance was measured at 530 nm with spectrophotometer using distilled water as blank. The absorbance was measured for five times after going through the same procedure and the amount of diazepam was found for the each determination.

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