Spectrophotometric Determination of Lorazepam with Alizarin Sulphonic Acid in Pure and Pharmaceutical Preparations

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Summary:Lorazepam reacts with alizarin sulphonic acid (sodium salt) to give a pink colored complex, after heating at 50°C for 15s having maximum absorbance at 530 nm. The reaction is selective for lorazepam with 0.01 mg/10 ml as visual limit of quantitation and provides a basis for a new spectrophotometric determination. The reaction obeys Beer's Law from 0.01 to 3 mg/10 ml of lorazepam and the relative standard deviation is 0.68 %. The quantitative assessments of tolerable amounts of other drugs not interfering are also studied.

Introduction

Lorazepam is a derivative of benzodiazipine and is a common tranquilizer. It is used for insomnia, for anxiety associated with depression and for pre anesthetic medication to produce sedation. Its most common adverse effect is its physical and psychological dependence. Specially sedation followed by dizziness, weakness and unsteadiness. Less frequent adverse effects are disorientation, depression, nausea, headache, agitation, dermatological symptoms, eye function and gastrointestinal disturbances [1].

Many analytical techniques have been employed for the determination of lorazepam. In the HPLC [2], HPLC/MS [3], and HPLC/photo-diode array [4] procedures, four oxazolo compounds in a total ion chromatogram were not separated from each other [3] and liquid-liquid extraction with n-hexane: ethyl acetate [4], was carried out before photodiode detection. Similarly in the TLC in combination with UV irradiation [5], and micellar liquid chromatography [6] a number of solvents and reagents are also involved. In the thermal desorption gas chromatography [7] the relative standard deviation was high i.e. less than 4.1 %. In the spectrophotometric methods mostly UV procedures are followed [8,9]. In the immunoassay detection [10,11] of lorazepam none of the four immunoassays gave a positive response before hydrolyzation of the urine samples when a 200 mg/ml calibration cutt-off was used [10] and the analytical sensitives vary for the individual benzodiazepines [11]. Long and tedious procedures are involved in CSP/HPLC [12] and GC/MS [13] where a solid phase micro extraction is carried out with carbowax/DVB fiber coating [13].

During a systematic study of drugs of abuse [14, 15], it was found that lorazepam reacts with alizarin sulphonic acid (sodium salt) to give a pink colored complex having maximum absorbance at 530 nm. The reaction obeys Beer's Law from 0.01 mg to 3 mg/10 ml and has 0.01 mg/10 ml as visual limit of identification (Fig. I). The present method is simple, accurate, precise and sensitive. Percentages of tolerable limits of other drugs not interfering were also studied.

Results and Discussion

Absorption Spectrum of the Colored Complex

Lorazepam reacts with alizarin sulphonic acid when heated for 15s at 50°C to give a pink colored complex the absorption spectra of which under optimum conditions lies at 530 nm (Fig. 2).

Effect of color producing reagent

Alizarin sulphonic acid (sodium salt) was used as color producing reagent. It was found that 0.4 mg/10 ml of 0.1 % alizarin sulphonic acid gave maximum color (Fig. 3) above and below this concentration the color intensity diminished and the colour became unstable. The probable mechanism is that alizarin is an anthraquinone derivative containing phenolic moiety and a sulphonic group. It undergoes

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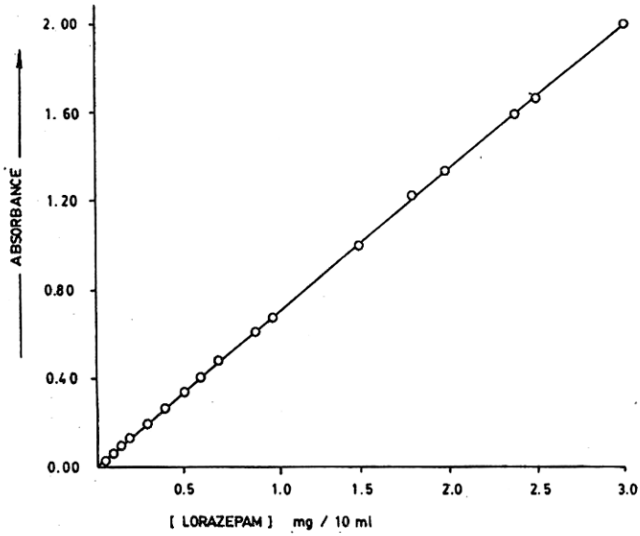

Fig. 1: Calibration Curve of Lorazepam with Alizarin Sulfonic Acid.

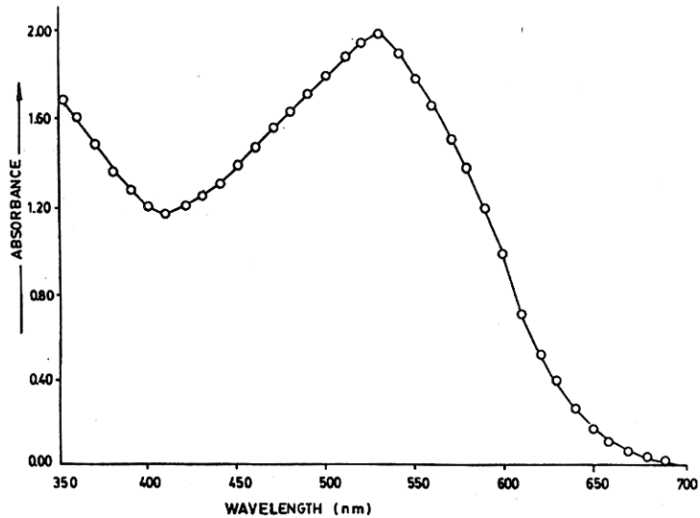

Fig. 2: Absorption Spectra of Lorazepam with Alizarin Sulfonic Acid.

intermolecular phenol amine association while interacting with lorazepam, which has an aromatic ring and amino groups having free electrons, thus producing a pink colored complex having a maximum absorbance at 530 nm. Structures of such complexes have been described as complicated network in which phenolate oxygen is involved in a multiple hydrogen bonding. Additional studies of crystalline phenol amine complexes by X-ray crystallography is very limited as stable products have been rarely isolated and identified [16]. However, charge transfer complexes are also formed by interaction between the basic N of lorazepam as electron donor (non bonding electron) and the electron acceptor reagent in this case is alizarin [17].

Effect of Temperature and Heating Time

The effect of temperature and heating time (Fig. 4) show that with the rise of temperature at

50°C and 15s of heating time the color intensity is maximum. The color was table after 5s and remained stable for more than 24 h. This is because the charge transfer complex formation is completed in 5s (explanation is given under color producing reagent). Above and below these timings and temperature the color was less intense and unstable. A water bath was used to carry out the temperature studies. The

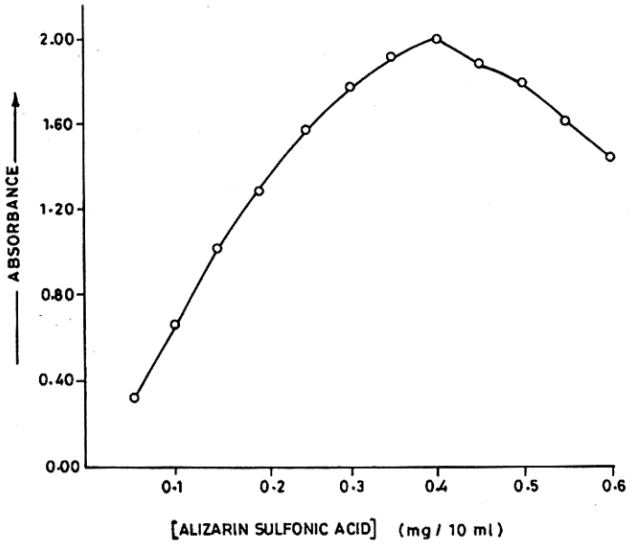

Fig. 3: Effect of Alizarin Sulfonic Acid Concentration on Absorbance.

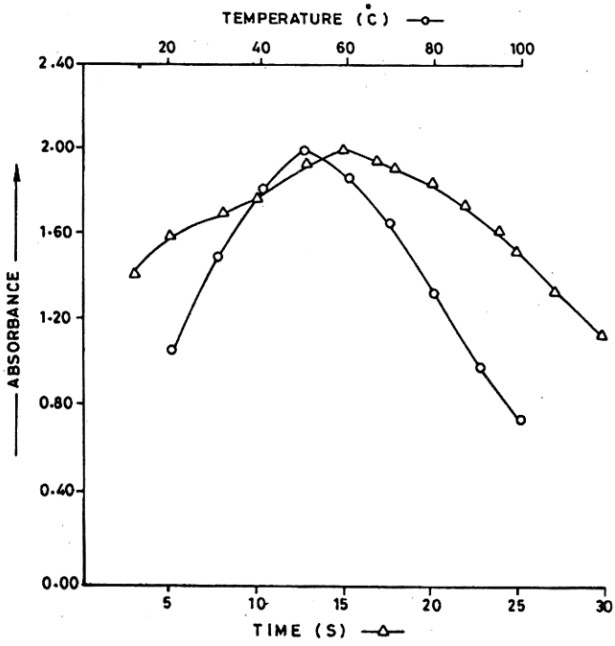

Fig. 4: Effect of Heating Time and Temperature on Absorbance.

contents of the test tube were cooled to room temperature prior to dilution upto 10 ml with ethyl alcohol and measurement of the absorbance.

Effect of Organic Solvents

Different organic solvents such as isopropyl alcohol, hexane, dichloromethane, diisopropyl ether, methyl ketone, carbon tetrachloride, xylene, trichlorobenzene and benzene were tested for color extraction and for stability. Since none were effective therefore no solvent was employed except for ethyl alcohol. The color was however, stable for more than 24 h without the addition of any solvent. Ethyl alcohol was used for dilution up to 10 ml of the colored complex so as to avoid any formation of an emulsion.

The results for determination of lorazepam are shown in Table 1 and 2, which show the sensitivity, validity and repeatability of the method. It is also reasonably precise and accurate, as the amount taken from identical samples is known and the amount found by above procedure does not exceed the relative standard deviation of 0.60 %, which is replicate of five determinations (cf. Table 1). The calibration graph is linear in the range of 0.01 to 3 mg/10 ml. The optimum photometric range (mg/ml) is the same because the relative error in this range is

Table-I:Determination of lorazepam from pure solutions

Lorazepam taken mg/10ml	Lorazepam found* mg/10ml	Relative standard deviation %
0.1	0.151	0.66
0.2	0.210	0.32
0.5	0.491	0.20
1.0	1.050	0.25
1.5	1.512	0.68
2.0	2.015	0.50
2.5	2.050	0.25
3.0	3.050	0.25

*Every reading is an average of five measurements.

minimum. The apparent molar absorptivity calculated was 0.2122×10^4 mol^{-1} cm^{-1}. The regression equation [18] was calculated by the method of least square sfrom ten points, each of which was the average of five determinations. The correlation between absorbance and concentration is 0.999 in terms of correlation coefficients (r).

Interferences

The quantitative assessment of tolerable amounts of different organic compounds (w/v) under

Table-2:Spectrophotometric parameters of the proposed method

Parameters	Values
λ_{max} (nm)	530
Beer's Law Limits (mg/10 ml, C)	0.01-3.0
Molar Absorptivity (mol^{-1} cm^{-1})	0.2122×10^4
Limit of Detection (mg/10 ml)	0.01
Regression Equation (Y)*	
Slope (b)	0.652
Intercept (a)	0.0192
Correlation Coefficient (r)	0.999
Relative Standard Deviation (RSD%)**	0.68
%Range of Error (confidence limits) at 95% Confidence Level	1.01±0.02

*Y = a + bC, where C is the concentration of analyte (mg/10 ml) and Y is the absorbance unit.
**Calculated from five determinations.

Table-3:Quantitative assessment of tolerable amounts of other drugs in Lorazepam determination.

Drugs	Maximum amount not interfering* (%)
Chlorpromazine	450
Ibuprofen	410
Trifluoperazine	400
Phenytoin sodium	400
Diclofenac sodium	380
Hydroxyzine	380
Fluoxetine-HCl	360
Acetyl salicylic acid	355
Chloral Hydrate	350
Barbituric acid	350
Paracetamol	300
Piroxicam	290
Aldomet	260
Mefenamic acid	260
Bethamidine	250
Promethazine	240
Thioridazine	240
Promazine	200

*The value is the percentage of the drug with respect to 1mg/10ml of Lorazepam that causes±0.01 change in absorbance.

the experimental conditions is given in Table 3. Various amounts of diverse interfering compounds were added to a fixed amount of lorazepam (1 mg/ml) and the recommended procedure for the spectrophotometric determination was followed. No compound examined showed any interference more so in case of trifluoperazine, chlorpromazine, hydroxyzine, ibuprofen, phenytoin sodium and diclofenac sodium.

Application

The proposed method is successfully applied for the quality control of pure lorazepam and in the pharmaceutical dosage in tablet form as shown in Table 4. The excipients and the coloring agents in the formulations did not interfere with the analysis.

Table - 4: Determination of Lorazepam in tablet form from different pharmaceutical sources

Trade Name	Amount Present (Manufacturer's Specification) (mg)	Amount Found* (mg)	Recovery (%)
Ativan (Wyeth Spencer	1	1.000	100.00
Pharma Pvt. Karachi Pakistan)	2	1.010	101.00
Avor (Popular Chemical Works, Lahore, Pakistan)	1	0.995	99.50
Orgalopam (Organon Pvt. Ltd	1	1.000	100.00
Karachi, Pakistan)	2	1.995	99.75
Tanzil (Acto Lab. Ltd. Pakistan)	1	0.999	99.90
	2	2.010	100.50
Tranquil (Pharmadic, Lahore, Pakistan)	1	1.000	100.00
Emotivon (Siza International	1	1.000	100.00
Ltd. Lahore, Pakistan)	2	0.999	99.95

*Every reading is the determination of five readings.

Experimental

Apparatus and Reagents

A Hitachi u-1100 spectrophotometer with 1 cm silica cells was used to measure the absorbance and graduated pipettes were employed. Analytical grade chemicals and doubly distilled water were used. Lorazepam (Wyeth Spencer Pharma (Pvt.) Karachi, Pakistan) standard solution (w/v) 1 mg/ml was prepared in ethyl alcohol (BDH) to get a stock solution, which was diluted further as required. 0.1 % (w/v) alizarine sulphnoic acid (sodium salt) (E-Merck) was prepared in distilled water.

General Procedure

To an aliquot of lorazepam, containing 0.01 to 3 mg/10 ml was added 0.4 ml of 0.1 % alizarin sulphonic acid and the contents were heated for 15s in a water bath as 50°C, cooled and the volume was made up to 10 ml with ethyl alcohol. The resulting absorbance of the pink color was measured at 530 nm employing all reagents, except lorazepam as blank. The experiment was repeated with different volumes of standard lorazepam solution and a calibration curve was prepared (Fig. 1). The colour reaction obeys Beer's Law from 0.01 to 3 mg/10 ml of lorazepam.

Procedure for Studying the Interfering Compounds

To an aliquot containing 1 mg/ml of lorazepam, different amounts of various compounds (1 mg/ml) (w/v) were added individually until the solution showed the same (±0.01) absorbance as that of pure lorazepam solution without the addition of the organic compound, under experimental conditions, as described in the general procedure. The value was calculated as the percentage of the organic compound with respect to the amount of lorazepam.

Procedure for the Determination of Lorazepam in Pharmaceutical Preparations

Tablets containing 1 and 2 mg of lorazepam were powdered, weighed, dissolved in alcohol and filtered. The filtrate was diluted further to get a 1 mg/ml (nominal) solution of lorazepam. An aliquot containing 0.01 to 3 mg/10 ml was taken, the procedure was followed as described above and the absorbance measured at 530 nm. The quantity per tablet was calculated from the standard calibration curve.

Conclusions

The spectrophotometric method for the determination of lorazepam is simple, reliable, sensitive and less time consuming. The statistical analysis is in good agreement with those of the Official British Pharmacopoeia 1988 and United States Pharmacopoeia XXII. It is selective for lorazepam. The method can be successfully applied to determine trace amounts of lorazepam either in pure or in pharmaceutical preparations. The color reaction has 0.01 mg/10 ml as visual limit of identification. The color reaction has not been reported in literature. The present method has the advantage over the different techniques reported for the determination of lorazepam, by not involving any form of extraction as in HPLC/photodiode [4] and in TLC [5] procedures neither involving pH control [9] nor many reagents as in chromatographic procedures [12, 13] and the relative standard deviation is 0.68% whereas in the thermal desorption procedure [7] the relative standard deviations is < 4.1%. Other compounds like chlorpromazine, ibuprofen, trifluoperazine, phenytoin sodium, diclofenac sodium and hydroxyzine do not interfere. A significant advantage of a spectrophotometric analysis is its application to the determination of individual

compounds. This aspect of spectrophotometric analysis is of major interest in the analytical pharmacy, since it offers a distinct possibility of quality control in assay of pharmaceutical dosage formulations.

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