Spectrophotometric Assay of Clorazepate Dipotassium in Dosage Forms

[1]AMINA MUMTAZ, [1]ASRAR AHMAD KAZI*[y], [1]RABIA NAZIR,
[1]MUHAMMAD USMAN SABRI AND [2]MUHAMMAD NAEEM SHAHID
[1]*Pakistan Council of Scientific and Industrial Research Laboratories Complex, Ferozepur Road, Lahore-54600, Pakistan.*
asrarak_47sag@yahoo.com*
[2]*Department of Chemistry, Govt. College of Science, Wahdat Road, Lahore, Pakistan.*
[y]*Present Address: Kinnaird College for Women 93 Jail Road , Lahore 54000, Pakistan.*

(Received on 6th February 2007, accepted in revised form 23rd September 2010)

Summary: A new spectrophotometric method for the determination of clorazepate dipotassium in pure form and in pharmaceutical preparation has been developed. The method is based on the complex formation due to charge transfer between clorazepate dipotassium and alizarin sulphonic acid (sodium salt). The pink coloured complex is obtained after heating the reaction at 50 °C for 90s. It has maximum absorbance at 530 nm and is stable for more than 24 hours. The reaction obeys Beer's law from 5 to 250 µg/mL of clorazepate dipotassium with 5 µg/mL as the visual limit of quantitation. The molar absorptivity and relative standard deviation are 0.45 x 10^4 mol^{-1} cm^{-1} and 0.94 %, respectively. The quantitative assessment of tolerable amounts of other drugs not interfering was also studied.

Introduction

Clorazepate dipotassium (Fig. 1), an important benzodiazepine, is used for the symptom relief of anxiety associated with neurosis, psychoneurosis and as an adjunct in the disease states in which anxiety is a prominent feature. Drowsiness is the most adverse effect; less common untoward reactions include dizziness, various gastro intestinal complaints, nervousness, blurred vision, dry mouth, headache, mental confusion, insomnia, transient skin rashes, fatigue, irritability and slurred speech [1].

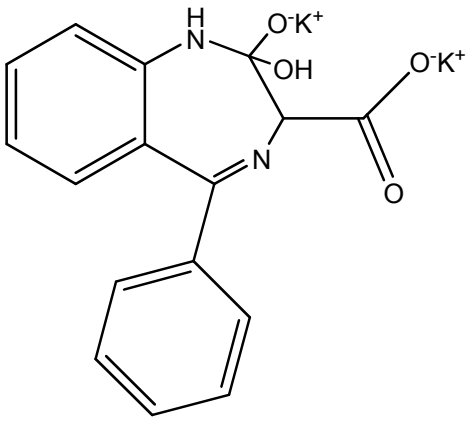

Fig. 1: Structure of clorazepate dipotassium.

The studies for the determination of clorazepate dipotassium have been carried out with many analytical techniques in the past. In the reversed phase HPLC [2-4] and HPLC with photodiode array detection [5], the hypersil ODS (5µm/ml) as stationary phase consisted of MeOH-Me*C*N-0.1M KH_2PO_4 (25:25:50 pH 2.4) as mobile phase [2] with detection of 254 nm [3] but have high standard deviation *i.e.* 1.7 [4] and long rinsing periods [5]. In wide bone capillary gas chromatography [6], the samples after heating in acid and centrifugation were passed through the sep-Pak C18 cartridges and eluted with chloroform [6]. Long and tedious methods are involved in thermospray and atmospheric pressure chemical ionization liquid chromatography-mass spectrometry [7] and liquid chromatography-electrospray-mass spectroscopy with GC and HPLC diode array detection [8].

In thin layer chromatography method a mixture of drugs having Clorazepate dipotassium was analyzed by separation and identification of the drugs in body fluid [9]. Miceller electrokinetic chromatography (MEKC) was employed to separate four benzodiazepines in Pharmaceutical preparations [10]. Simultaneous detection of benzodiazepines was also carried out by a gas chromatography-ion trap tandem mass spectrometry [11]. In human urine low dosed benzodiazepines were determined by solid phase extraction gas chromatography–mass spectrometry [12].

Hence, the already existing procedures for the determination of clorazepate dipotassium are lengthy and tedious and involve expensive instrumentation. Therefore, there is a need to design simple and cost effective method for the determination of clorazepate dipotassium salt in pure

*To whom all correspondence should be addressed.

and dosage forms. In this work the authors attempt to develop such a method that is simple and offers accurate and reliable determination of the drug in microgram levels.

Results and Discussion

Absorption Spectrum of the Coloured Complex

Clorazepate dipotassium reacts with alizarin sulphonic acid (sodium salt) when heated for 90 s at 50 °C to give pink coloured complex, the absorption spectrum of which under the optimum conditions lies at 530 nm (Fig. 2). The λ_{max} of the clorazepate dipotassium is 230 nm and that of alizarin is 424 nm. Hence, all measurements for the further studies were carried out at this wavelength.

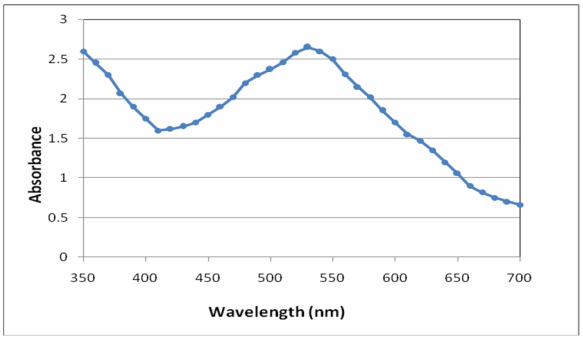

Fig. 2: Absorption spectra of dipotassium clorazepate with alizarine sulphonic acid.

Effect of Colour Producing Reagent

Sodium salt of alizarin sulphonic acid was used as colour producing reagent. It was found that 1500 µg/mL of 0.1% alizarin sulphonic acid gave a maximum colour (Fig. 3). When the concentration of the reagent was altered the colour intensity diminished and the colour became unstable.

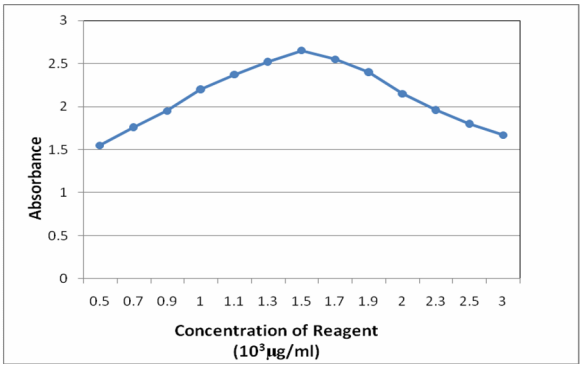

Fig. 3: Effect of reagent.

Effect of Temperature

The effect of temperature is shown in Fig. 4. It was found that with the rise of temperature the colour intensity increased and was maximum and stable at 50 °C. However, with the further increase in temperature the colour intensity decreased and the colour was unstable. The absorbance of the developed colour remained stable for more than 24 h. A water bath was used to carry out the temperature studies. The contents of the test tube were cooled to room temperature prior to dilution upto 10 ml with distilled water and measurement of the absorbance.

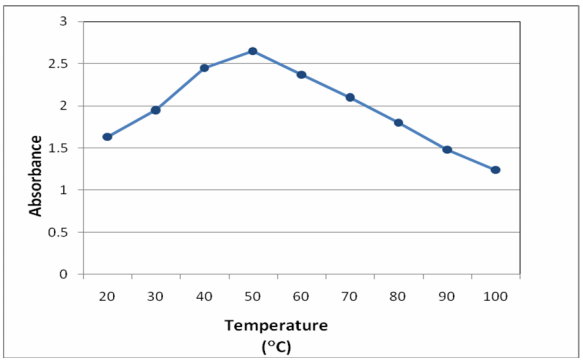

Fig. 4: Effect of temperature.

Effect of Heating Time

The effect of heating time on colour intensity is shown in Fig. 5. It was found that heating for 90s at 50 °C gave maximum colour. Above and below this time the colour intensity decreased and become unstable.

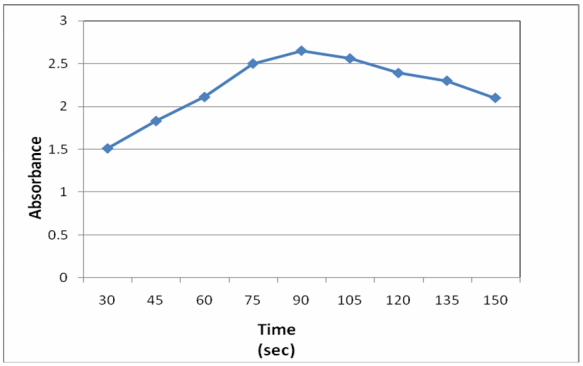

Fig. 5: Effect of heating time.

Effect of Organic Solvents

Different organic solvents such as isopropyl alcohol, *n*-hexane, xylene, methyl ketone, diisopropyl ether, benzene, dichloromethane, dioxane, formaldehyde and tetrahydrofuran were tested for colour extraction and stability. Since none of them were found to be effective, therefore, no organic solvent was employed.

Mechanism

The probable mechanism of this colour reaction is that nitrogen of clorazepate having the free lone pairs of electrons got associated with the sulphonic group of alizarin sulphonic acid resulting

in the formation of pink sulfonamide complex having maximum absorbance at 530 nm [13].

Sensitivity

The results of the determination of clorazepate dipotassium are shown in Tables-1 and 2 which reveal the sensitivity, validity and repeatability of the method.

Table-1: Determination of clorazepate dipotassium from pure solution.

Clorazepate dipotassium Taken(µg/mL)	Clorazepate dipotassium Found (µg/mL)*	Relative Standard Deviation (standard deviation) (%)
10	10.2	0.94 (0.001)
15	15.1	0.60 (0.001)
20	20.3	0.50 (0.001)
30	29.0	0.39 (0.001)
50	50.4	0.31 (0.001)
100	104.2	0.10 (0.001)
120	121.0	0.08 (0.001)
150	151.5	0.06 (0.001)

*Every reading is the average of five independent readings

Table-2: Optical characteristics, precision and accuracy of the proposed method.

Parameters	Values
λ_{max} (nm)	530
Beer's Law Limit (µg/ml, C)	5-250
Molar absorptivity ($mol^{-1}cm^{-1}$)	0.45×10^4
Regression equation Y*	
Slope (b)	0.9219
Intercept (a)	0.0737
Regression coefficient of determination (r^2)	0.9878
Relative standard deviation (RSD)** (%)	0.94
%Range of error (Confidence Limits) at 95% confidence level	1.25±0.024

*Y= a+ bC, where C is the concentration of analyst (µg/mL) and Y is the absorbance unit.
**Calculated from five determinations.

It is shown that method is reasonably precise and accurate, as the amount taken for the identical samples is known and the amount found by the above procedure does not exceed the relative standard deviation of 0.94 % which is replicate five independent measurements (cf Table-1). The optimization has been done at lower analyte concentration. The calibration graph is linear in the range of 5 to 250 µg/ml. The apparent molar absorptivity calculated was 0.45 x 10^4 mol^{-1} cm^{-1} and the regression of equation [14], was calculated by the method of least squares from twelve points, each of which was the average of five determinations. The regression coefficient of determination (r^2) comes out to be 0.9878.

Interferences

The quantitative assessment of tolerable amount of different organic compounds under experimental condition is given in Table-3. Various amounts of diverse interfering compounds having similar action was added to fixed amount of clorazepate dipotassium (1 mg/mL) and recommended procedure for the spectrophotometric determination was followed [15, 16].

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

Drugs	Maximum amount not interfering* (%)
Aspirin	100
Lorazepam	100
Diclofenac sodium	200
Clobazam	200
Diazepam	200
Mefenamic acis	100
Pheniramine maleate	200
Chloroquin phosphate	100
Metamizole sodium	200
Paracetamol	100

*The value is percentage of the drug with respect to 100µg/ml of clorazepate dipotassium that causes ±0.01 change in absorbance

Application

The proposed method is successfully applied for the quality control of pure clorazepate dipotassium and in pharmaceutical form as shown in Table-4.

Experimental

Apparatus and Reagents

A CE 2041 Cecil spectrophotometer with quartz cells of 10mm thickness was used to measure the absorbance and graduated pipettes were employed. All chemicals used were of analytical grade and doubly distilled water was used throughout the study. A standard solution (10^3 µg/ml) of clorazepate dipotassium (Searle Pakistan Ltd. Karachi, Pakistan) was prepared by dissolving 10^5 µg of drug in 100 ml of distilled water to give a stock solution which was diluted further as required. Also a 0.1 % (w/v) solution of sodium salt of alizarin sulphonic acid (E. Merck) was prepared in distilled water.

Table-4: Determination of clorazepate dipotassium in dosage forms.

Trade Name	Amount present (Manufacturer's specification) (mg)	Amount found (mg)	Recovery (%)
Tranxene (Searle Pakistan Ltd. Karachi Pakistan)	5	4.989	99.78
Tranxene (Searle Pakistan Ltd. Karachi Pakistan)	10	10.020	100.20
Tranxene (Searle Pakistan Ltd. Karachi Pakistan)	15	14.970	99.80

*Every reading is the average of five independent readings

General Procedure

To an aliquot of clorazepate dipotassium solution containing 5 to 250 µg/mL was added 105 ml of 0.1 % alizarin sulphonic acid (sodium salt) solution and the contents were heated for 90s in water bath at 50 ^{o}C. After cooling the volume was made upto 10 ml with distilled water. The absorbance of the resulting pink colour was measured at 530 nm. A blank was also run employing all reagents except clorazepate dipotassium. The experiment was repeated with different volumes of standard clorazepate dipotassium solution and a calibration curve was prepared (Fig. 6). The colour reaction obeys Beer's Law from 5 to 250 µg/mL of clorazepate dipotassium.

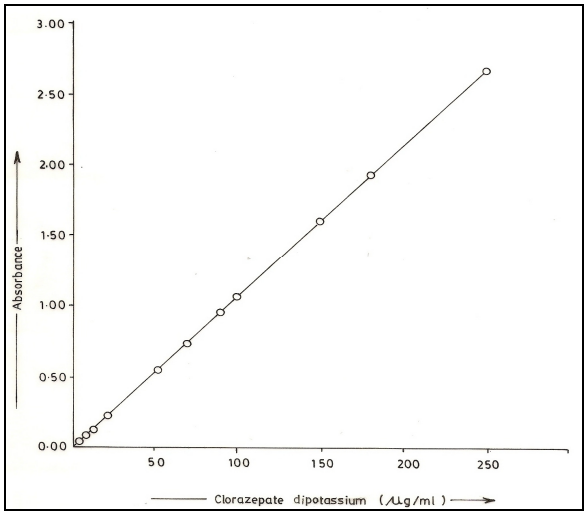

Fig. 6: Calibration curve of clorazepate dipotassium with alizarine sulphonic acid (sodium salt).

Procedure for Studying the Interfering Compounds

To an aliquot containing 10^2 µg/mL of clorazepate dipotassium different amounts of various organic compounds were added individually until the solution showed the same (±0.01) absorbance as that of the pure clorazepte dipotassium solution as before the addition of the interfering organic compound under experimental conditions as described in the general procedure. The value was calculated as the percentage of interfering compound with respect to the amount of clorazepate dipotassium.

Procedure for the Determination of clorazepate dipotassium in Pharmaceutical Preparations

Capsules containing 5, 10, 15 mg of clorazepate dipotassium were opened, contents weighed dissolved in distilled water and filtered. The filtrate was further diluted with distilled water to get a 1 mg/mL solution of clorazepate dipotassium. An aliquots containing 5 to 250 µg/mL were taken and the same procedure were followed as described previously and the absorbance was measured at 530 nm. The quantity per capsule was calculated from standard calibration curve.

Conclusion

In present studies, it was found that clorazepate dipotassium react with alizarin sulphonic acid (sodium salt) to give a pink coloured complex having maximum absorbance at 530 nm. The reaction obeys Beer's Law from 5 to 250 µg/mL of clorazepate dipotassium, 5 µg/mL being visual limit of identification (Fig. 6). The present method is simple, accurate, precise and sensitive. Percentages of tolerable limits of interfering drugs were also studied.

The spectrophotometric method for the determination of clorazepate dipotassium is simple, reliable, sensitive and less time consuming. The statistical analysis is in good agreement with those of the Official British Pharmacopeia 1988 and USPXIX. The colour reaction is selective for clorazepate dipotassium. The method can be successfully applied to micro determination of clorazepate dipotassium in pure or in pharmaceutical preparations. The colour reaction has 5 µg/mL as visual limit of identification. The advantage of the present procedure is that it does not require many solvents whereas the HPLC procedures [2, 3, 4, 5] are long, tedious and expensive, involving many reagents and solvents.

A significant advantage of a spectrophotometric determination is its application to the determination of individual compounds. This aspect of spectrophotometric analysis is of major interest in analytical pharmacy, since it offers a distinct possibility of quality control in the assay of formulation dosage formulations.

References

1. R. G. Alfonso, Remington's Pharmaceutical Sciences, 17th Edition , pp. 1061, Mack Publishing Co. Pennsylvania, 18042 (1985).
2. M. Wang and C. Gonnet, *Yaowu Fenxi Zazhi*, **6**, 142 (1986).
3. M. Montalto de Mecca, M. L. Oneto, M. L. Paviolo and R. S. Graells de Kempny, *Acta Bioquim. Clin*. **20**, 73 (1986).
4. K. Hazer and R. Barchet, *Journal of Chromatography*, **132**, 83 (1977).

5. W. He, N. Parissis and T. Kiratzidis, *Journal of Forensic Sciences*, **43**, 1061 (1988).
6. H. seno, O. Suzuki, T. Kumazawa and H. Hattori, *Journal of Analytical Toxicology*, **15**, 21 (1991).
7. M. Tatsuno, M. Nishikawa, H. Tsuchihashi, K. Igarashi, f. Kasuya and M. fukui, *Japanese Journal of Toxicology and Environmental Health*, **42**, 248 (1996).
8. H. Hattori, O. Suzuki, N. Sugiura and T. Yamada, *Masu Kenbyukai Koenshu*, **12**, 211 (1987).
9. A. Zevzikoviene, A. Zevzikovas and A. Bertulis, *Medicina* (Kaunas), **39**, 1100 (2003).
10. J. J. Berzas, G. Castaneda and M. J. Pinilla, *Talanta*, **57**, 33 (2002).
11. S. Pirmay, I. Ricordel, D. Libong and S. Bouchonnet, *Journal of Chromatography A*, **954**, 235 (2002).
12. D. Borrey, E. Meyer, W. Lambert, C. Van Peteghen and A. P. Dc Leenheer, *Journal of Chromatography B*, **765**, 187 (2001).
13. B. Cupareneu and J. Horak, *Research Communicatyion in Biological Psychology and Psychiatry*, **20**, 19 (1995).
14. G. D. Christian, Data Handling and Spread sheets in analytical chemistry, 6th edition, pp.102-106, john Wiley and Sons, New York (2004).
15. N. Sultana, M. S. Arayne and A. Waheed, *Journal of the Chemical Society of Pakistan*, **31**, 273 (2009).
16. N. Pourreza and B. Mostafavi, *Journal of the Chemical Society of Pakistan*, **31**, 462 (2009).