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Extraction-Spectrophotometric Determination of Trace Amounts of Lorazepam in Pharmaceutical Formulation

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A simple and sensitive extraction-spectrophotometric method for the determination of lorazepam (LOR) in pharmaceutical formulations is reported. The $LORH^+$ cation which is formed in an acidic solution can form an ion-pair with Orange(II) anionic dye the $LORH^+$–$OR(II)^-$ ion pair is quantitatively extracted into dichloromethane solvent and its absorption was measured at 482 nm. The calibration graph is linear over the LOR concentration range of 1.0-25.0 μg mL^{-1} and the regression coefficient is 0.9999. The relative standard deviation (RSD) of 10 replicate determination of 10.0 μg mL^{-1} of LOR is 1.6 % and the limit of detection (LOD) of the method is 4.8×10^{-2} μg mL^{-1}. The method is successfully applied to the determination of LOR amount in pharmaceutical formations (1.0 and 2.0 mg tablets).

Key Words: Extraction, Determination, Ion pair, Lorazepam.

INTRODUCTION

Lorazepam (LOR) (Fig. 1) *i.e.*, 3-hydroxybenzodiazepine, one of the 1,4-benzodiazepine derivatives, is widely used or abused as an antianxietic and sedative agent. Benzodiazepines may exert antianxiety effects through the potentiation of the inhibitory neurotransmitter. Lorazepam is used clinically as an antianxietic or a sedative with central nervous system depressant activity[1]. Determination of low doses of this drug in pharmaceutical formulations is of great importance. This work presents a sensitive, simple and reliable analytical technique for determination of trace amounts of lorazepam in pharmaceutical formulation.

Several methods such as high performance liquid chromatography (HPLC)[2-16], gas chromatography (GC)[17-22], micellar electrokinetic capillary chromatography (MECC)[23], adsorptive-stripping voltammetry[24] have been reported for the determination of lorazepam in different samples. The

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liquid-liquid extraction[2-12,16] and solid phase extraction[5,13,14] was usually employed to perform matrix removal and analyte pre-concentration prior HPLC separation. The extraction-involved sample pre-treatment procedures could enrich the analyte by several folds even 1-2 orders of magnitude, allowing the HPLC or HPLC/MS to be used for determination of lorazepam in low ppb levels. But the procedures were complicated and tedious. The purpose of this study is to develop a fast and sensitive solvent extraction-spectrophotometric method for determination of lorazepam in pharmaceutical preparations.

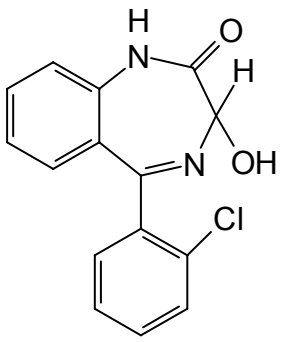

Fig. 1. Structure of Lorazepam

Solvent extraction is perhaps the most versatile of analytical techniques and invokes most of the physical and chemical principles used generally in analytical chemistry[25-27]. It therefore has pedagogic as well as practical values. Extraction methods using ions associated with a large ionic dye or counter ions, forming an ion-association complex with large molar absorptivities are still limited[28,29]. Many quantitative pharmaceutical determination methods such as HPLC suffer from large solvent consumption[30-35]. More investigation in this field could lead to sensitive methods for trace pharmaceutical determinations.

In this paper a simple and sensitive method for the quantitative extraction of lorazepam-Orange II ion pair [$LORH^+$-$OR(II)^-$] from an acidic solution into dichloromethane organic solvent, followed by spectrophotometric measurements at 482 nm is reported.

EXPERIMENTAL

All the chemicals were of the high purity and used without further purification. Double distilled deionized water was used throughout.

1000 μg mL^{-1} stock solution of LOR (purchased from FIS, 99.9 % pure) was prepared by dissolving 0.500 g of LOR powder in water and diluting to the mark in a 500 mL volumetric flask with distilled water. Working solutions were prepared by appropriate dilution of the stock solution with water.

Organge(II) (Fig. 2) stock solution (5.8×10^{-4} M) was prepared by dissolving 0.0200 g of the dyestuff sodium salt (Merck) in water and diluting to the mark in a 100 mL volumetric flask.

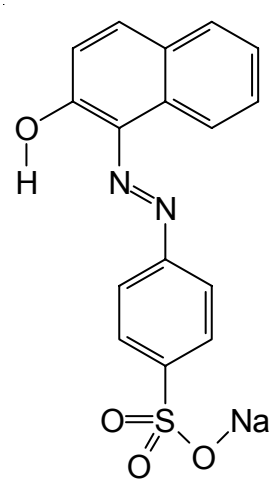

Fig. 2. Structure of Orange(II) dye

Sodium chloride stock solution (1.0 M) was prepared by dissolving 5.85 g of the NaCl (Merck) in water and diluting to the mark in a 100 mL volumetric flask.

The absorption spectra were recorded on a Jasco model 7850 UV-Vis recording spectrophotometer and a Perkin-Elmer model 550S spectrophotometer was used for absorbance measurements.

Recommended procedure: An aliquot of sample solution containing 10.0-250.0 μg of LOR was placed in a 10 mL volumetric flask. 2.5 mL of orange(II) dye solution (5.8×10^{-4} M), 1.0 mL of 5.0 M hydrochloric acid solution and 1.0 mL of NaCl (2.50×10^{-5} M) solution were added and then the solution was diluted to the mark with distilled water. The solution was trnasferred into a 50 mL separatory funnel and 5 mL of dichloromethane was added. The solution was shaken vigorously for 1 min. The phases were allowed to separate and the organic phase was separated and its absorbance was measured at 482 nm against a reagent blank. The concentrations of different species in final sample solution were as follow: HCl, 0.5 M, OR(II), 1.45×10^{-4} M and NaCl 2.50×10^{-4} M.

RESULTS AND DISCUSSION

Since $LORH^+$ cation forms a fairly stable ion-pair with OR(II) anionic dyestuff as counter ion in aqueous acidic solution, it is expected to results in a sensitive and useful spectroscopic method for trace LOR determination.

In preliminary experiments, it was found that the counter anion plays an important role in the formation of a stable ion-pair and among dyestuffs examined (methyl green, brilliant green, thionine, nile blue, methylene blue, safranine and orangeII) best results were obtained with orange(II) dyestuff.

Different common organic solvents such as dichloromethane, chloroform, carbon tetrachloride, methyl isobutyl ketone and benzene were examined as extracting solvent. It was found that the ion-pair is readily extractable in dichloromethane than other solvents.

The effect of HCl and OR(II) concentrations on the extraction of $LORH^+$–$OR(II)^-$ ion-pair were investigated and the results are shown in Tables 1 and 2, respectively. It has been observed that the absorbance of the organic phase increases with increasing HCl and OR(II) concentrations in the aqueous phase. Maximum extraction of $LORH^+$–$OR(II)^-$ occurs at 0.5 M HCl and 1.45×10^{-4} M OR(II) concentrations in the final solution. The results show that for acid concentrations more than 0.5 M, the absorbance difference (ΔA) decreases drastically and this is due to the formation of more hydronium ion, H_3O^+, which can form an ion-pair with $OR(II)^-$.

TABLE-1
EFFECT OF HCl CONCENTRATION ON THE ABSORBANCE OF EXTRACTED $LORH^+$–$OR(II)^-$ ION-PAIR

HCl conc. (M)	Absorbance of extracted $LORH^+$–$OR(II)^-$ ion-pair respect to related blank (ΔA)
0.00	0.065
0.10	0.161
0.20	0.228
0.30	0.249
0.40	0.372
0.50	0.517
0.60	0.455
0.70	0.388
0.80	0.362
0.90	0.198

It must be mentioned that quantitative extraction of $LORH^+$–$OR(II)^-$ ion-pair is complete by 5 mL of dichloromethane in a single stage extrac-

tion process. This has been confirmed by observing further enhancement in the absorption of complex in the mixture of two 5 mL portions of the organic phase obtained from two successive extraction of an aqueous $LORH^+$–$OR(II)^-$ ion-pair solution, compared with that of a 10 mL organic phase obtained from a single stage extraction of the same complex solution under optimal experimental conditions. The absorbance reading for 10 mL organic phase containing the $LORH^+$–$OR(II)^-$ ion-pair is half of the absorbance from 5 mL dichloromethane from the first stage of extraction.

TABLE-2
EFFECT OF NaCl CONCENTRATION ON THE ABSORBANCE OF EXTRACTED $LORH^+$–$OR(II)^-$ ION-PAIR FROM AQUEOUS SOLUTION

NaCl conc. (M)	Absorbance of extracted $LORH^+$–$OR(II)^-$ ion-pair respect to blank (ΔA)
0.000	0.405
0.005	0.426
0.010	0.461
0.015	0.495
0.020	0.503
0.025	0.562
0.030	0.505
0.035	0.502

The effect of shaking time on the extraction of $LORH^+$–$OH(II)^-$ ion-pair was studied and shaking of 1 min was found sufficient for the extraction of ion-pair. By increasing the shaking time, the absorbance of related blank will increase and ΔA decreases.

Under the optimum conditions described above, linear calibration graph for LOR was obtained in the concentration range of 1.0-25.0 μg mL^{-1}. The regression equation for LOR is $\Delta A_{LOR} = 0.0084 \pm 0.0506 C_{LOR}$, (where ΔA_{LOR} is the absorbance difference between sample and related blank solution and C_{LOR} is the concentration of LOR in μg mL^{-1}) with a correlation coefficient of 0.9999. The relative standard deviation (RSD) of 10 replicate measurements is 1.6 % for 10.0 μg mL^{-1} of LOR solution. The limit of detection[36], LOD of the method is estimated to be 0.03 μg mL^{-1}.

In order to study the influence of various cations and anions on the determination of LOR, a fixed concentration of LOR, 10.0 μg mL^{-1}, was taken with different amounts of foreign ions and the recommended procedure was followed. A relative error of ± 3 % with respect of the absorbance difference for the LOR solution was considered tolerable. Tolerance limits are as follows: Cl^-, NO_3^-, Na^+ (120000 folds); SO_4^{2-}, Al^{3+} (400 folds); Ca^{2+}, Ba^{2+}, Sr^{2+}, Mg^{2+}, K^+, Li^+, Cu^{2+}, Zn^{2+}, Mn^{2+}, NH_4^+ (300 folds); Br^-, I^-,

CH_3COO^-, ClO_3^- (100 folds); Hg^{2+}, ClO_4^- (1 folds). The results showed that most of the cations are anions used have no considerable effect on the determination of LOR. However ClO_4^- and Hg^{2+} ions were found to interfere seriously with the LOR determination.

The proposed method was applied to the determination fo LOR content of 1 and 2 mg lorazepam tablets from Chemi Daruo Co. The results are shown in Table-3. There is a satisfactory agreement between the results of the proposed method and the reference value of LOR content of the formulations.

TABLE-3
THE RESULTS OF LOR CONTENT IN 1 AND 2 mg TABLET FORMULATION OBTAINED BY THE PROPOSED METHOD

LOR content	LOR obtained[a]	Recovery (%)
1.0	0.987	98.7
2.0	1.974	98.7

[a]Average of five determinations.

The method described provides a simple and reliable means of determination of trace amounts of LOR in real samples. The limit of detection of the proposed method seems to be very good. The method compares favourably in sensitivity and selectivity with most of the published methods[2-12] for the determination of LOR and it can certainly be placed among the most sensitive ones.

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