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Spectrophotometric, spectrofluorimetric and LC determination of trazodone hydrochloride

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Abstract

Three methods are described for the determination of trazodone hydrochloride in pharmaceutical tablets. The spectrophotometric method was based on the formation of yellow ion pair complex between the basic nitrogen of the drug and bromophenol blue at pH 3.4. The formed complex was extracted with chloroform and measured at 414 nm. The spectrofluorimetric method was based on measurement of the native fluorescence of the drug in 50% acetic acid upon excitation at a maximum of 320 nm and the emission wavelength is 435 nm. The third method was based on the high performance liquid chromatographic determination of trazodone hydrochloride using a reversed phase, ODS column, with a mobile phase of acetonitrile–phosphate buffer at pH 4.5 (60:40, v/v). Quantization was achieved with UV detection at 250 nm based on peak area. The three methods were simple, accurate and suitable for quality control application.

Keywords: Reversed phase HPLC; Spectroflourimetry; Spectrophotometry; Trazodone hydochloride

1. Introduction

Trazodone HCl, 2-{3-[4-(3-chlorophenyl)-1-piperazinyl]propyl} - 1,2,4 - triazolo [4,3 - a] pyridin - 3 - (2H)-one monohydrochloride, is a anti-depressant. The official method for the determination of trazodone HCl is potentiometric non-aqueous titration with perchloric acid [1] and HPLC using octadecyl silane column and methanol–0.01 M ammonium phosphate buffer pH 6.0 (60:40) as mobile phase [2]. Various methods have been reported for the determination of trazodone HCl in pharmaceutical formulations using UV absorption measurement at 246 nm [3], ion-selective electrode [4,5], voltametry[6,7] and HPLC [2,8]. Different chromatographic methods have been reported for the determination of trazodone HCl in biological fluids including HPLC [9,10], capillary gas chromatography [11], gas chromatography-mass spectrometry [12] and instrumental thin-layer chromatography [13]. No colorimetric and spectrofluorimetric methods have been reported for determination of trazodone HCl in pharmaceutical tablets.

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For single component preparations, the simplest assay method involves the direct measurement of the UV absorption at the maximum. Trazodone HCl is relatively weak UV absorbing compound, therefore, the direct UV absorbance measurements at low concentration will be unreliable. In the present work, we develop rapid, accurate and sensitive colorimetric and spectrofluorimetric methods for the determination of trazodone HCl in tablet dosage form. For more specificity, a simple isocratic high performance liquid chromatographic method was developed.

2. Experimental

2.1. Instrumentation

A double-beam 1601 PC UV-visible spectrophotometer connected (Shimadzu, Japan) to a computer fitted with UVPC personal spectroscopy software version 3.7 (Shimadzu) was used. The spectral bandwidth was 2 nm and the wavelength scanning speed was 2800 nm per min. The absorption of test and reference solutions was recorded in 1 cm quartz cells.

Shimadzu spectrofluorophotometer, model RF-540 was used. The fluorescence spectra of test and reference solutions were recorded in 1-cm quartz cells.

The HPLC (Perkin–Elmer, Norwalk, CT, USA) instrument was equipped with a model series 410 LC pump, Rheodyne 7125 injector with a 20 μl loop and a LC-235 photodiode array detector. Separation and quantization were made on a 150 × 4.6 mm (i.d.) Phenomenex® Prodigy 5 μ ODS (5 μm particle size). The detector was set at λ, 250 nm. Data acquisition was performed on a model 1022 Pe Nelson (Perkin–Elmer).

2.2. Materials and reagents

Pharmaceutical grade of trazodone HCl was kindly supplied by Egyptian International Pharmaceutical Industrial Company (E.I.P.I. Co.), Egypt, and certified to contain 100.0%.

About 0.32% (w/v) bromophenol blue (E. Merk, Dermstadt, Germany) was prepared by dissolving 0.32 g in least amount of ethanol and completing the volume to 100 ml with distilled water.

Mcllvaine buffer solution (pH 3.4) was prepared by mixing 28.5 ml of 0.2 M disodium hydrogen phosphate and 71.5 ml of 0.1 M citric acid.

About 50%(v/v) acetic acid (Laboratory Raysan, France) was prepared in distilled water.

Phosphate buffer (pH 4.5) was prepared by dissolving 1.15 g of monobasic ammonium phosphate in 1000 ml of water. The pH was adjusted to 4.5 using 10% phosphoric acid and 1 N sodium hydroxide.

The water for HPLC was prepared by double glass distillation and filtration through 0.45 μm membrane filter. The acetonitrile used was HPLC grade (Honil, England). Other reagents were of analytical grade.

The commercial Trittico tablets used (Batch No. 984147) were manufactured by Egyptian International Pharmaceutical Industrial Company (E.I.P.I. Co), Egypt, under license from F. Angelini, Italy. Each tablet contains 100 mg trazodone HCl in addition to tablet excipients consisting of starch, sodium starch glycolate, povidone, lactose, microcrystalline cellulose, magnesium stearate, dibasic calcium phosphate and F.D. & C. yellow lack.

2.3. HPLC conditions

The mobile phase was prepared by mixing acetonitrile and phosphate buffer at pH 4.5 in a ratio of 60:40 v/v. The mobile phase was filtered using 0.45 μm membrane filter (Millipore, Milford, MA) and degassed by vacuum prior to use. The samples were also filtered using 0.45 μm disposable filters. The flow rate was 1 ml per min. All determinations were performed at ambient temperature. The injection volume was 20 μl.

2.4. Standard solutions and calibration procedure

The stock standard solution was prepared by dissolving trazodone HCl in distilled water to give a concentration of 125 μg ml^{-1}.

2.4.1. Spectrophotometric method

Into 50 ml separator aliquot, portions of trazodone HCl stock standard solution in the range of 1.5–6 ml were transferred, followed by 2 ml of McIlvaine buffer pH 3.4 and 3 ml of 0.32% bromophenol blue solution. The total volume of aqueous phase was adjusted to 11 ml with distilled water and the contents were shaken for 2 min. The ion pair colored complex was extracted with three successive portions, each of 10 ml chloroform, by shaking for 1 min. The chloroformic extracts were collected in 50 ml volumetric flask and the volumes was completed to 50 ml with chloroform and the absorbance was measured at 414 nm against the corresponding reagent blank in 1 cm quartz cell. The absorbance was plotted against the concentration. Linear relationship was obtained.

2.4.2. Spectrofluorimetric method

Further dilutions of the stock standard solution of trazodone HCl were made with 50% acetic acid to reach the concentration range of 0.125–0.75 μg ml^{-1}. The relative fluorescence intensity was measured at λ_{ex} 320 nm and λ_{em} 435 nm against 50% acetic acid as a blank in 1 cm quartz cell. Calibration graph was constructed by plotting relative fluorescence intensity versus concentration. Linear relationship was obtained.

2.4.3. HPLC method

Further dilutions of the stock standard solution of trazodone HCl were carried out with mobile phase to reach the concentration range of 0.125–0.75 μg ml^{-1}. Triplicate 20 μl injections were made for each concentration and chromatographed under the specified chromatographic conditions. Peak area values were plotted against concentrations. Linear relationship was obtained.

2.5. Sample preparation

Ten tablets were weighed and powdered. An accurately weighed quantity of the powder tablets equivalent to 25 mg trazodone HCl was extracted with 100 ml distilled water and filter. Further dilutions of the filtrate were made with distilled water (for spectrophotometric method), 50% acetic acid (for spectrofluorimetric method) and mobile phase (for HPLC method) to apply each method. The general procedures for the three methods described under calibration were followed. The absorbance, relative fluorescence intensity and peak area were measured as described under the calibration procedure and the concentration of trazodone HCl in sample was calculated from the regression equations.

2.6. Percent recovery study

This study was performed by addition of known amount of trazodone HCl to a known concentration of the commercial tablets (standard addition method). The resulting mixtures were assayed and results obtained were compared with expected results (Table 2).

3. Results and discussion

3.1. Spectrophotometric method

Trazodone HCl posses a relatively low absorption in the UV region. The presence of piperazine ring in its molecular structure, with two nitrogenous centers, offers a basic characteristic of the compound. So when treated with an acid dye such as bromophenol blue at pH 3.4, a yellow ion pair complex is formed, which is extracted with chloroform. The absorbance of the formed complex was measured at 414 nm (Fig. 1). The ion pair complex formation reaction was applied to enhance the sensitivity of trazodone HCl determination.

The ion pair complex formation reaction conditions were studied as a function of the pH, bromophenol blue concentration, reaction time, shaking time and type of organic solvent used for the extraction of ion pair complex. The described procedure gives maximum stability and sensitivity. The optimum pH value was found to be 3.4 ± 0.2. Maximum absorbance was obtained when 2.5 ml of 0.32% (w/v) of bromophenol blue was used, however, 3 ml of the dye was used during the experiment. The effect of mixing time of the reactants was studied by allowing the reac-

Table 1
Characteristic parameters for the regression equations of spectrophotometric (A); spectrofluorimetric (B); and HPLC (C); methods for determination of trazodone HCl

Parameters	A	B	C
Linearity (μg ml^{-1})	3.75–15.00	0.125–0.75	0.125–0.75
Regression equation (Y): slope (b)[a]	5.53×10^{-2}	100.66	18.38×10^{6}
S.D. of the slope (S_b)	6.59×10^{-4}	1.32	8.80×10^{4}
Relative S.D. of the slope (%)	1.19	1.31	0.48
Confidence limit of the slope[b]	5.38×10^{-2}–5.68×10^{-2}	97.72–103.60	18.19×10^{6}–18.57×10^{6}
Intercept (a)	1.52×10^{-2}	-6.6×10^{-3}	1.00×10^{4}
S.D. of the intercept (S_a)	6.46×10^{-3}	4.89×10^{-2}	4.11×10^{4}
Confidence limit of the intercept[b]	7.94×10^{-4}–2.96×10^{-2}	(-11.56×10^{-2})–10.24×10^{-2}	(-7.96×10^{4})–9.96×10^{4}
Correlation coefficient (r)	0.9997	0.9996	0.9999

[a] $Y=a+bC$,where C is the concentration of trazodone HCl in μg ml^{-1}; Y is the absorbance or relative fluorescence or peak area for A, B and C methods, respectively.
[b] 95% confidence limit.

tants to stand for different times up to 1 h, then extracted with organic solvent and absorbance was measured. It was found that the reaction is instantaneous and shaking time for about 2 min was sufficient to produce maximum absorbance that remained stable for about 1 h. Different organic solvents including chloroform, chlorobenzene and toluene were tried for extraction of the colored complex. Chlorobenzene and toluene were excluded due to the formation of emulsion during extraction while chloroform was found to be the most ideal solvent for extraction of colored complex yielding maximum absorbance intensity.

Application of Job's method of continuous variation [14] indicated 1:1 complexation ratio. The suggested mechanism of trazodone HCl–bromophenol blue ion pair complex formation at pH 3.4 is described in Scheme 1.

3.2. Spectrofluorimetric method

The spectrofluorimetric method was based on measurement of the native fluorescence of trazodone HCl in 50% acetic acid upon excitation at a maximum of 320 nm and the emission wavelength is 435 nm (Fig. 2). This permits the development of a sensitive method of assay for trazodone HCl in its tablets. Relative sensitivity, based on detection limit, was calculated. The spectrofluorimetric method was found to be 2–3 times more sensitive than the proposed HPLC and spectrophotometric methods, respectively.

3.3. HPLC method

The developed HPLC method based on using a reversed phase, ODS column, with a mobile phase consisting of acetonitrile–phosphate buffer pH 4.5 in a ratio 60:40 v/v with flow rate of 1 ml per min at ambient temperature. The effects of mobile phase composition and pH on the chromatographic separation of trazodone HCl were studied and optimized. Increasing acetonitrile concentration to more than 75% led to inadequate separation of trazodone HCl peak from injection peak. At lower acetonitrile concentration (less than

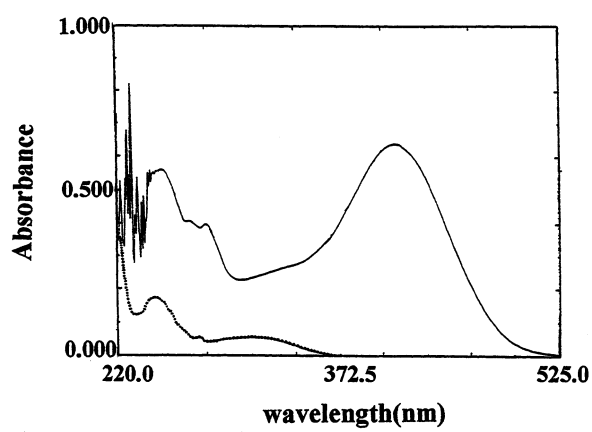

Fig. 1. Absorption spectra of 11.5 μg ml^{-1} of trazodone HCl in water before (- - - -) and after ion pair complexation with bromophenol blue in chloroform (—).

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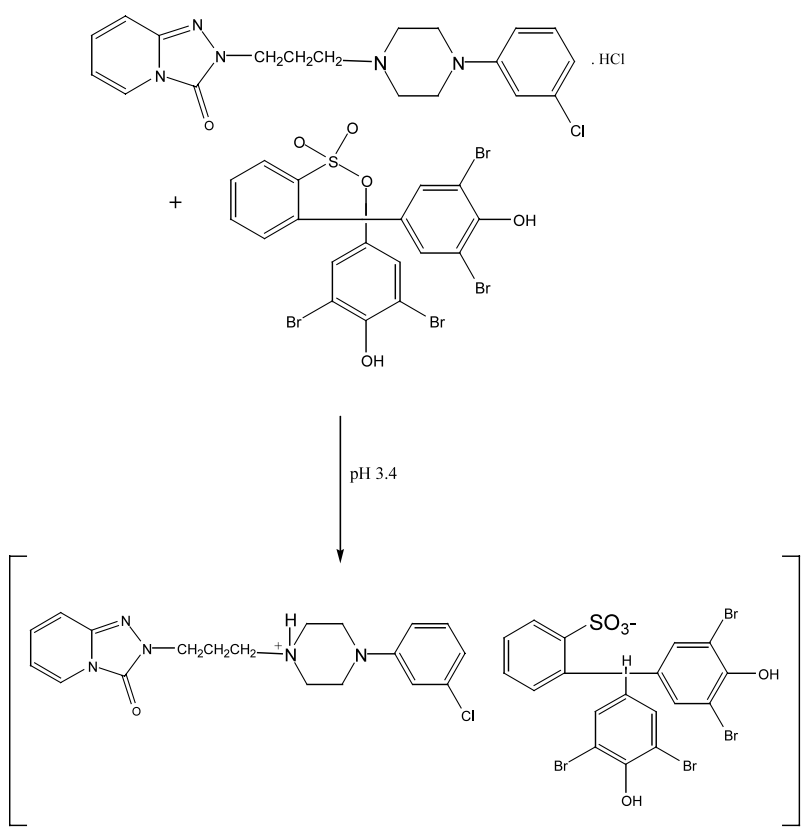

Scheme 1. Suggested mechanism of trazodone hydrochloride–bromophenol blue ion pair complex formation.

40%), separation occurred but with excessive tailing and increased retention time for trazodone HCl peak. Variation of apparent pH of the mobile phase resulted in maximum k' value at apparent pH 7.5 with loss of peak symmetry for trazodone HCl. At lower apparent pH value (2.5–3.5), bad resolution was observed for trazodone HCl peak and injection peak. At apparent pH 4–5, improved resolution was observed. At apparent pH 4.5, optimum resolution without peak tailing was observed. The effect of temperature on the separation of trazodone HCl was studied by changing the temperature in steps of 2°C from 20 to 30°C. Variations in temperature did not have a significant effect on separation and peak shape. Quantization was achieved with UV detection at 250 nm based on peak area. Under the described chromatographic conditions, sharp peak was obtained for trazodone HCl. The average retention time $\pm$ S.D. for trazodone HCl was found to be 1.8 ± 0.009 min, for ten replicates.

Under the described experimental conditions of the above mentioned three methods, plots of absorbance, relative fluorescence and peak area values versus concentrations within the range stated in the Table 1 show linear relationships. Linearity was checked for 3 consecutive days for the same concentration range, each of eight plots. The regression analysis of these plots using the method of least squares was made (Table 1). The linearity of the calibration graphs was proved by the high values of correlation coefficients of the regression equations.

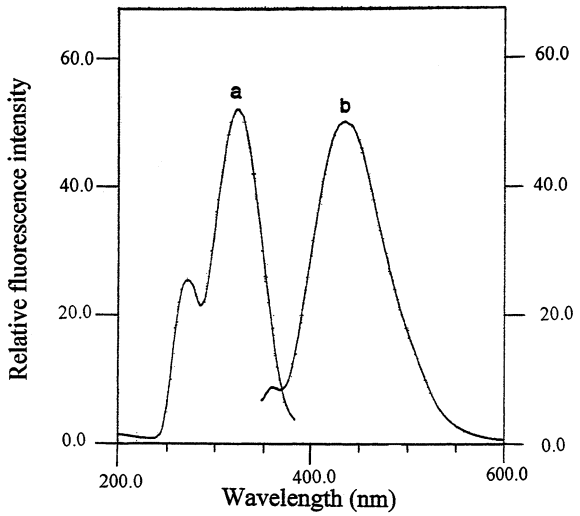

Fig. 2. Fluorescence (a) excitation ($\lambda_{ex} = 320$ nm); and (b) emission ($\lambda_{em} = 435$ nm) spectra of 0.5 μg ml^{-1} of trazodone HCl in 50% acetic acid.

3.4. Method validation

Spiked placebos were prepared according to the manufacturing formula. The spiked placebos were tested at five levels — 50, 75, 100, 125 and 150% of label claim for the drug. Assays were performed in duplicate on two samples at the five levels. This was repeated with a second instrument, standard and sample preparation and analyst on different days. The complete set of validation assays was performed for the drug, determined by the proposed methods. Spiked placebo assays were used to determine accuracy and precision of the proposed methods for determination of the drug. The recoveries ranging from 99.6 to 100.5% of the amount of active ingredient spiked into the placebo. The bias showed only minor variation in recovery at each level with 0.4% the maximum variation observed. The proposed methods were tested for repeatability, reproducibility, selectivity, specificity, robustness and ruggedness. Satisfactory results were obtained. The proposed methods complied with USP [2] validation guidelines.

The non-instrumental methods for determination of the detection limit and the quantization limit were applied [2], the limit of detection is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected. While the limit of quantization is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be determined with acceptable accuracy and precision. The detection limits of the proposed methods were found to be 0.01, 0.02 and 0.03 μg ml^{-1} for trazodone HCl, detected by spectrofluorimetric, HPLC and spectrophotometric methods, respectively. While the quantization limits of the proposed methods were found to be 0.05, 0.09 and 2.1 μg ml^{-1} for trazodone HCl, determined by spectrofluorimetric, HPLC and spectrophotometric methods, respectively.

Table 2
Determination of trazodone HCl in commercial tablets using spectrophotometric (A); spectrofluorimetric (B); HPLC (C) and USP24 methods

	Mean found ± S.D.[a]			
	A	B	C	USP24
Commercial tablets	99.6 ± 1.11	99.8 ± 0.72	99.5 ± 0.96	100.4 ± 0.78
	$t = 1.44$	1.38	1.78	(2.23)[b]
	$F = 2.03$	1.17	1.51	(5.05)[b]
Recovery[c]	99.8 ± 1.28	99.7 ± 0.44	99.9 ± 0.60	

[a] Mean and S.D. for six determinations, percentage recovery from the label claim amount.
[b] Theoretical values for t and F.
[c] For standard addition of 50% of the nominal content ($n = 6$).

The stability of trazodone HCl during the analytical procedures were studied and found to be stable. The analyte was stable for at least 24 h in solution.

3.5. Tablet analysis

The three proposed methods were applied to the determination of trazodone HCl in commercial tablets. Six replicates determination were made. Satisfactory results were obtained and were in a good agreement with the label claims (Table 2). Moreover, to check the validity of the proposed methods, the standard addition method was applied by adding trazodone HCl to the earlier analyzed tablets. The recovery of the drug was calculated by comparing the concentration obtained from the spiked mixtures with those of the pure drug. The results of analysis of the commercial tablets and the recovery study (standard addition method) of the drug (Table 2) suggested that there is no interference from any excipients, which are present in tablets such as starch, sodium starch glycolate, povidone, lactose, microcrystalline cellulose, magnesium stearate, dibasic calcium phosphate and F.D. & C. yellow lack. This was confirmed by the peak purity results obtained by photodiode array detector.

The results of determination of trazodone HCl in tablets obtained from the three proposed methods were compared with those of the USP 24 method, based on the HPLC separation using octadecyl silane column and methanol–phosphate buffer pH 6.0 (3:1, v/v) as mobile phase [2]. Statistical comparison of the results was performed with regard to accuracy and precision using Student's t-test and F-ratio at 95% confidence level (Table 2). There is no significant difference between the three proposed methods and USP24 method with regard to accuracy and precision.

4. Conclusion

The proposed spectrophotometric, spectrofluorimetric and HPLC methods provide simple, accurate and reproducible quantitative analysis for the assay of trazodone HCl in tablets. The spectrophotometric method was applied to enhance the sensitivity of trazodone HCl determination. While the spectrofluorimetric method has the greatest sensitivity, the HPLC method is more specific than the other two methods. In the proposed HPLC method, the trazodone HCl was faster separated with sharper peak than that in the USP method due to using acetonitrile in the mobile phase and lowering of the pH of the mobile phase.

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