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COLORIMETRIC AND ATOMIC ABSORPTION SPECTROMETRIC METHODS FOR THE DETERMINATION OF NEFAZODONE HYDROCHLORIDE AND TRAZODONE HYDROCHLORIDE IN PURE FORM AND PHARMACEUTICAL PREPARATIONS

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ABSTRACT

Two sensitive methods, colorimetric and atomic absorption spectrometric (AAS) were developed for the determination of nefazodone HCl (I) and trazodone HCl (II). The methods are based on the formation of ternary complex between the cited drugs and molybdenum(V) thiocyanate in acid medium with the production of an orange red color followed by extraction with methylene chloride. The complex showed absorption maximum at 472 nm with apparent molar absorptivity 1.46×10^4 and 0.64×10^4 L mol^{-1} cm^{-1}, for I and II, respectively. Beer's law was obeyed in the range 2.5-32.5 and 2.5-30.0 μg ml^{-1} for I and II, respectively. The residue remained after evaporation of methylene chloride was dissolved in HCl and used for determination of molybdenum content by AAS as a direct method for the determination of the cited drugs. Results obtained by applying the proposed methods showed good recoveries of 99.9 ± 1.3% and 99.4 ± 1.6% for colorimetric method, whereas using AAS the recoveries were 98.7 ± 1.3% and 98.6 ± 1.8% for I and II, respectively. Results obtained by both methods showed good agreement with those of the official and reference methods. The developed methods were applied for determination of these drugs in their pharmaceutical formulations.

Keywords: Nefazodone HCl, trazodone HCl, spectrophotometric and AAS, ternary complex.

INTRODUCTION

Nefazodone HCl {2-[3-[4-(3-chlorophenyl)-1-piperazinyl]propyl]-5-ethyl-2,4-dihydro-4-(2-phenoxyethyl)-3H-1,2,4-triazol-3-one} is a phenylpiperazine while, trazodone HCl {2-[3-[4-(3-chloro-phenyl)-1-piperazinyl]propyl]-1,2,4-triazol[4,3-a]-pyridine-3-(2H)-one} is a triazolopyridine. Both drugs have antidepressant effect. They block the reuptake of serotonin at presynaptic neurones and have antagonist at postsynaptic 5-HT[1,2] receptors. Trazodone appears to have very significant anti-muscarinic properties but, nefazodone has no antimuscarinic properties[2]. The newly developed nefazodone HCl is used for the symptomatic treatment of all types of depressive illness and depression accompanied by sleep disturbances.

The reported methods for the determination of trazodone HCl were, HPLC[3-8], gas chromatographic[9-11], ion selective electrode[12,13], polarographic and differential pulse voltammetric[14,15], atomic absorption spectrometric[16] and spectrophotometric[17-20] methods. Nefazodone HCl have been determined by HPLC[21-24], mass spectrometry[25], differential pulse and square wave voltammetry[26] and capillary zone electro-phoresis[27].

Yet, no colorimetric or AAS methods have been reported in the literature for nefazodone. This study utilized the reaction between nefazodone HCl and/or trazodone HCl with Mo(V)-thiocyanate binary complex (which is non extractable with methylene chloride) to form orange-red colored ternary complexes which were extractable with methylene chloride. The methylene chloride extracts were used for the colorimetric and AAS, for direct determination of the cited drugs in either pure form or in their pharmaceutical formulations. The developed procedures are simple, accurate, precise, reliable and can be used in laboratories where modern and expensive instruments are not available.

EXPERIMENTAL

Apparatus

Cintra 5 UV and visible double beam spectrophotometer, equipped with 1.0 cm matched quartz cell, and Unicam atomic absorption spectrometer model AA 969, were used.

Materials and solutions

All reagents were of analytical reagent grade and used without further purification and water was doubly distilled.

Molybdenum(VI) solution, 1×10^{-3} M was prepared by dissolving appropriate weight of ammonium molybdate tetrahydrate in water.

Ammonium thiocyanate and ascorbic acid (10 % w/v) aqueous solution was used.

3.0 M HCl was prepared by accurate dilution of concentrated HCl solution (11.5 M).

Nefazodone HCl and Serzone tablets containing 100 and 200 mg nefazodone HCl per tablet supplied by (Bristol-Mayer Squibb, Egypt). Trazodone HCl and Trittico tablets, containing 50 and 100 mg trazodone HCl per tablet manifested by Egyptian International Pharmaceutical Industries Company, Egypt (EIPICO) were used. Standard drug solutions of 50 mg of the investigated drugs were weighed into 50 ml calibrated flasks, dissolved and diluted to volume with 1.0 M HCl.

General procedure

1- Colorimetric method

Into 50 ml separating funnel, 1.5 ml ammonium molybdate reagent, 5.0 ml 3.0 M HCl, 1.5 ml ascorbic acid and 2.0 ml ammonium thiocyanate solution were mixed and left for 10 min at room temperature.

Appropriate volume of standard drug solution in the concentration range stated were added and left for another 10 min. Extract with 10 ml methylene chloride (twice, 5.0 ml portions), the mixture was shacked well for 1.0 min and allowed to separate into two phases. Methylene chloride extract was dried over anhydrous sodium sulphate and its absorbance was measured at 472 nm against reagent blank prepared similarly without the drugs.

2- AAS method

The dried methylene chloride extract, prepared as under procedure 1, was evaporated to dryness on a water bath of 60 ± 2°C. The residue was dissolved in 0.5 ml concentrated HCl, boiled, and completed to 2.0 ml volume with water. A reagent blank was performed under the same conditions. The absorption was measured at 313.3 nm, using lamp current of 9.0 mA, slit width 3.8 Å, air pressure = 10 L min^{-1}, acetylene pressure = 100 L min^{-1} and absorption sensitivity 0.77 ppm. The concentration of molybdenum content was calculated from calibration graph of standard ammonium molybdate solution.

Assay of tablets

Twenty tablets were powdered and mixed well. Accurately weighed amount of the powder equivalent to 25 mg drug was dissolved and completed to 50 ml with 1.0 M HCl in a 50 ml calibrated flask then, filtered. The assay was performed as stated above under procedure 1 and 2.

RESULTS AND DISCUSSIONS

Formation of ternary complexes between the tertiary amine group and Mo(V)-thiocyanate binary complex occurs via the protonated nitrogen atom. Mo(V) formed by reduction of Mo(VI) with ascorbic acid, combined with ammonium thiocyanate in 0.8 – 3.2 M HCl solutions[28]. In the absence of ascorbic acid, the same colour in methylene chloride was formed. Excess ammonium thiocyanate in acid medium may reduce Mo(VI) to Mo(V)[29].

In this study, ternary complexes were formed between the tertiary amino groups of nefazodone HCl or trazodone HCl and Mo(V)-thiocyanate binary complex via the protonated nitrogen atoms of the cited drugs. The formed ternary complexes were soluble in methylene chloride while Mo(V)-thiocyanate binary complex was insoluble. Double extractions were sufficient to extract the ternary complexes quantitatively into the organic phase. These ternary complexes showed absorption maximum at 472 nm in methylene chloride (Fig. 1).

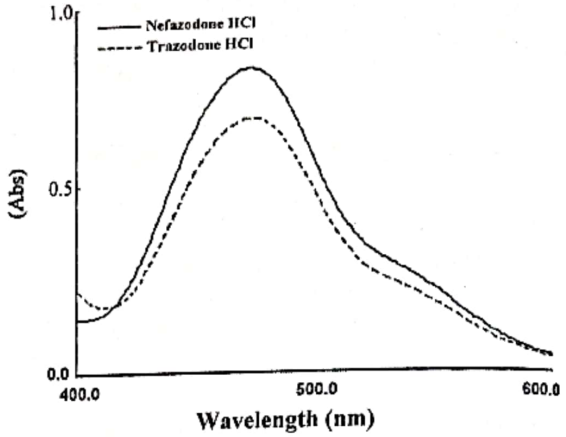

Fig. 1: Absorption spectra of nefazodone HCl and trazodone HCl complexes with $[Mo(CNS)_6]^-$

Optimization of the reaction variables

It was stated that, the formation of Mo(V)-thiocyanate and drug ternary complexes were formed only in acid medium. However, the absorbance readings of methylene chloride extract from (1.5 - 4.0 M) HCl gave the maximum results. Hydrochloric acid has been selected as the suitable medium for the ternary complex formation and extraction[29]. 5.0 ml of 3.0 M HCl was found sufficient for the formation of Mo(V)-thiocyanate-nefazodone or trazodone complexes.

The effect of ammonium molybdate tetrahydrate on the ternary complex formation was studied. It was found that 1.5 ml of 1×10^{-3} M reagent was sufficient to produce maximum absorbance for the stated concentration ranges for drug I and II. After that, the absorbance was nearly constant. 1.5 ml ascorbic acid (10 % w/v) and 2.0 ml ammonium thiocyanate (10 % w/v) were found to be sufficient to produce maximum absorbance for the studied drugs. In this method complete formation of the ternary complexes needs 10 min before extraction with methylene chloride at room temperature.

Solvents like benzene, cyclohexane, acetone, dimethylformamide, carbon tetrachloride, diethyl ether and petrolium ether failed to extract the ternary complex[29]. On the other hand methylene chloride, chloroform, and dichloroethane extract the ternary complexes quantitatively. Methylene chloride was found to be more suitable with respect to stability and high solubility of the ternary complex in it. Moreover, double extraction with 10 ml (5.0 ml portion) and 1.0 min shaking time gave quantitative results.

Reaction of Mo(VI) with ammonium thiocyanate in 3.0 M HCl, in the presence of ascorbic acid and subsequent reaction with drug I or II is suggested to be as follows:

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$$Mo(VI) \xrightarrow[3.0\ M\ HCl]{ascorbic\ acid} Mo(V)$$

$$\xrightarrow{6\ SCN} [Mo(SCN)_6]^-$$

$$[Mo(SCN)_6]^- + 2\ drug\ I\ or\ II \longrightarrow$$

$$[Mo(SCN)_6][drug\ I\ or\ II]_2$$

ternary complex

The stoichiometry of the Mo(V) to each drug in the presence of excess ammonium thiocyanate was determined by the continuous variation method. Results indicated a 1:2 ratio between Mo(V) and nefazodone HCl or trazodone HCl ternary complexes (Fig. 2).

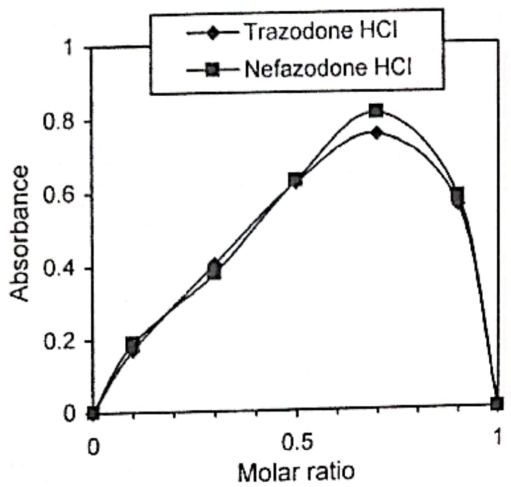

Fig. 2: Continuous variation method for the ternary complex formed between nefazodone HCl and/or trazodone HCl using $[Mo(CNS)_6]^-$

Calibration graphs were constructed under the optimum reaction conditions of the studied drugs I and II. Beer's law was obeyed over the concentration range of 2.5 - 32.5 and 2.5 - 30 μg ml^{-1} for I and II, respectively. The molar absorptivities for the formed ternary complex in methylene chloride were 1.46 × 10^4 and 0.64 × 10^4 L mol^{-1} cm^{-1} for nefazodone HCl and trazodone HCl, respectively at λ_{max} 472 nm. Moreover Sandell sensitivity, slope, intercept, and correlation coefficient for each drug were calculated (Table 1).

AAS studies

It was not practical to aspirate the methylene chloride extract of the ternary complex into the atomic absorption spectrometer. The chlorine/carbon ratio would lead to the formation of large quantity of HCl in the flame, which would damage the instrument[31,32]. Therefore, the organic extract was evaporated to dryness, the residue was dissolved in 0.5 ml concentrated hydrochloric acid and boiled to dissolve. Then the mixture was diluted to 2.0 ml with water. The aqueous solution was applied for the atomic absorption determination of molybdenum content. Results obtained by AAS were recorded (Table 2).

Table 1: Analytical characteristics of the proposed colorimetric procedures

Parameter	Drug	
	I	II
λ_{max} / nm	472	472
Stability / hrs	12.0	12.0
Beer's conc. range / μg ml^{-1}	2.5-32.5	2.5-30
Ringbom optimum range / μg ml^{-1}	4.0-30.0	5.0-27
Detection limits / μg ml^{-1}	0.81	0.75
Quantification limits / μg ml^{-1}	2.48	2.42
Molar absorptivity / L mol^{-1} cm^{-1}	1.46×10^4	0.64×10^4
Sandell sensitivity / μg cm^{-2}	0.0346	0.064
Regression equation*		
Slope	0.092	0.011
RSD % of slope	0.018	0.013
Intercept	-0.009	0.007
RSD % of intercept	0.043	0.036
Correlation coefficient	0.9996	0.9998
RSD %	1.25	1.42
Recovery %	98.6-101.2	98.3-101.5
Calculated t- value	1.56 (2.228)**	1.84 (2.228)**
Calculated F- value	3.15 (5.05)**	3.54 (5.05)**

* A = a + bC, where C is the concentration in μg ml^{-1}

** Values in parentheses are the tabulated values for t- (at 10 degrees of freedom) and F- (at 5,5 degrees of freedom) values at 95% confidence limits.

Table 2: Linear regression analysis for the studied drugs using AAS method.

Parameters	Drug	
	I	II
Optimum concentration / μg ml^{-1}	10-100	10-100
Regression equation		
Slope	0.142	0123
RSD % of slope	0.018	0.013
Intercept	0.005	-0.004
RSD % of intercept	0.037	0.031
Correlation coefficient	0.9998	0.9996
Relative standard deviation / %	1.65	1.55
Student's t-value	1.67 (2.228)[a]	1.88 (2.228)[a]
Calculated F-value	2.84 (5.05)[a]	3.17 (5.05)[a]

[a]: Values in parentheses are the tabulated values for t- (at 10 degrees of freedom) and F- (at 5,5 degrees of freedom) values at 95% confidence limits.

$$Mo(VI) \xrightarrow[3.0\ M\ HCl]{ascorbic\ acid} Mo(V)$$

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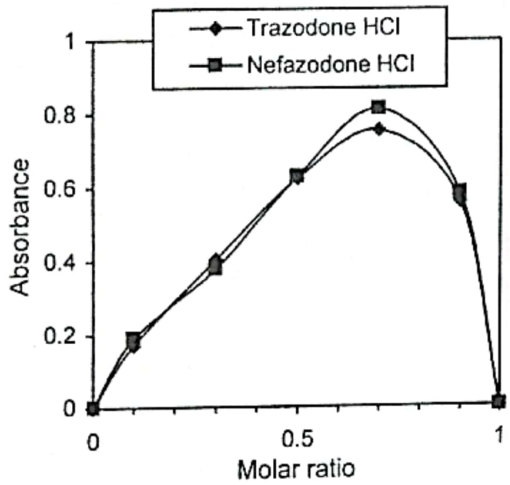

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Results of the proposed procedures were statistically compared with those of the official B.P. (non-aqueous titration), USP [30, 33] and reference methods (HPLC)[24]. Results of the proposed method showed good agreement with those obtained by the official and the reference methods. The proposed methods were successfully applied to the determination of the cited drugs in pharmaceutical dosage forms. The obtained results were accurate and precise as recorded in table (3).

Table 3: Determination of Nefazodone HCl and Trazodone HCl in dosage forms, applying the standard addition technique

Dosage form	Supplier	Nominal value	Taken µg ml^{-1}	Added µg ml^{-1}	Color. Recovery %	AAS Recovery %	Official and reference Recovery %
Serzone tablets	Bristol-Mayer Squibb, Egypt	200 mg	2.0	-- 4.0 8.0 16.0	99.00 100.83 100.80 100.83 t^b=1.37 F^b=2.75	101.50 99.17 101.00 99.17 t^b=1.56 F^b=3.17	98.00 97.50 98.30 98.61
Trittico tablets	EIPICO	50 mg	4.0	-- 3.0 6.0 12.0 24 48	99.25 99.29 100.80 100.56 99.82 -- t^b=1.46 F^b=2.93	101.25 99.57 99.60 100.90 99.39 -- t^b=1.18 F^b=2.33	-- -- 102.00 98.44 98.21 98.75

[a]: Average of six determinations

[b]: Tabulated values for t- (at 10 degrees of freedom) and F- (at 5,5 degrees of freedom) at 95% confidence level are 2.228 and 5.05, respectively.

Conclusion

The proposed methods were advantageous over the reported methods with respect to sensitivity, simplicity, accuracy, precision and stability of the colored species. No interferences from associated excipients and additives were observed. The proposed methods can be applied for the routine analysis and in quality control laboratories for the quantitative determination of the studied drugs in pure form and in pharmaceutical formulations depending upon the availability of the chemicals and instruments.

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طريقة لونية وطريقة امتصاص ذري لتعيين نيفازودون هيدروكلوريد وترازودون هيدروكلوريد في صورتها النقية وفي مستحضراتها الصيدلية

جمال حسن رجب

قسم الكيمياء التحليلية - كلية الصيدلة - جامعة الزقازيق

يصف هذا البحث طريقتين الأولي لونية والثانية طريقة امتصاص ذري لتقدير نيفازودون هيدروكلوريد وترازودون هيدروكلوريد. تعتمد كلا من الطريقتين علي تكوين معقد ثلاثي بين الأدوية المذكورة والموليبدنم الخماسي والثيوسيانات في وسط حامضي. وقد تم استخلاص المعقد الثلاثي بواسطة كلوريد المثيلين وتم قياس اللون الأحمر البرتقالي الناتج عند طول موجة قدرة ٤٧٢ نانو متر. وكان معامل الامتصاص الجزيئي مساوياً ١,٤٦ × ١٠ ٤ و ٠,٦٤ × ١٠ ٤ لتر/ مول سم لكل من نيفازودون وترازودون علي التوالي. والطريقة المستخدمة تتبع قانون بير عند تركيزات ٢,٥ – ٣٢,٥ و ٢,٥ – ٣٠ ميكروجرام لكل ١ مليلتر بالنسبة لنيفازودون وترازودون على التوالى. ومن ناحية أخري تم في هذا البحث قياس كمية الموليبدنم في المعقد الثلاثي المستخلص بكلوريد الميثلين بواسطة طريقة الامتصاص الذري كطريقة مباشرة لتعيين العقارين. وقد كان هناك توافق جيد بين الطرق المستحدثة لتقدير العقاقير السابقة والطرق الدستورية والمرجعية لهذه العقاقير. وطبقت الطرق بنجاح لتقدير العقاقير السابقة في بعض المستحضرات الصيدلية المحتوية علي هذه العقاقير.