Spectrophotometric and Conductometric Determination of Clomiphene Citrate and Nefazodone HCl

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Abstract: Two accurate, rapid and simple spectrophotometric and conductometric methods were developed for the determination of clomiphene citrate (CMP) and nefazodone HCl (NFZ), the proposed methods depends upon the reaction of ammonium reineckate with the two studied drugs to form stable precipitate of ion-pair complexes, which was dissolved in suitable solvent. The pink colored complexes were determined colorimetrically at 509, 523.6 nm, respectively. Using the conductometric titration, the studied drugs could be evaluated in 50% (v/v) acetone in the range 60.02-540.18 and 63.3-443.1 $\mu\text{g mL}^{-1}$ for clomiphene citrate and nefazodone HCl, respectively. While for spectrophotometric method the ranges were 0.2-1.8 and 0.2-1.6 mg mL^{-1} for clomiphene citrate and nefazodone HCl respectively. Various experimental conditions were studied. The results obtained showed good recoveries with relative standard deviations of 0.759 and 0.552%. The proposed procedures were applied successfully to the analysis of these drugs in their pharmaceutical preparations and the results were favourably comparable with the official and reference methods. The molar combining ratio reveal that (1:1) (drug : reagent) ion associates were formed.

Keywords: Conductometric titration, Spectrophotometric determination, Clomiphene Citrate, Nefazodone HCl.

Introduction

Clomiphene is used for induction of ovulation. It is a mixture of *E* and *Z* isomers of 2-{4-(2-chloro-1,2-diphenylvinyl)phenoxy} triethylamine dihydrogen citrate. Different methods were reported for its determination, either in pure or in dosage forms. It was determined by spectrophotometric¹⁻³, array-type DNA glass slide⁴, HPLC⁵⁻⁸, capillary electrophoresis⁹, potentiometric¹⁰ and NMR methods¹¹. Clomiphene was also determined in muco-adhesive oral formulation of high permeability / high solubility drugs¹².

Nefazodone hydrochloride belong to generation of antidepressant drugs, it appears to block both 5-HT₂ receptors. Scientific literature reports spectrophotometric method¹³, voltammetric technique¹⁴, HPLC methods for quantitative determination of nefazodone HCl in its metabolites in human plasma and in pharmaceutical formulations¹⁵⁻¹⁷, and MS technique¹⁸⁻¹⁹. Nefazodone HCl is not included in any pharmacopoeia. With its ever increasing use and the number of formulations entering into the market, there is always a need for simple, sensitive, accurate, rapid analytical method for the estimation of it in pure form and pharmaceutical preparations which can be easily adapted for routine in quality testing laboratories. In the present study, two different techniques for the simple and accurate determination of the two drugs mentioned above were investigated.

Ammonium reineckate was used to form ion-pair complex with many drugs *e.g.* thioridazine²⁰ and propranolol HCl²¹, the method based on precipitation of the ion-associates formed from the reaction of the drugs with ammonium reineckate. IR was reported to confirm the structure of the complex.

Experimental

Apparatus

The absorption spectra for all measurements were carried out using Shimadzu 260 recording spectrophotometer equipped with 10mm quartz cells. A CONSORT nv, Model (Parklaan 36, B2300 Tumbhout, Belgium) was used. The measurement range was 1.0–10.0 μ S with maximum error of $\pm 0.2\%$. The CONSORT nv model K410 dip-type cell was used with a cell constant, K_{cell} , of 1.0.

Reagent

Analytical grade reagents and double distilled water were used to prepare all solutions. Ammonium reineckate (Aldrich) was used. Clomiphene citrate pure drug and clomid[®] tablets, each tablet contains 50 mg of clomiphene citrate (Global Napi Pharmaceutical, under License of Aventis Pharma S.A.E.). Nefazodone HCl pure drug and serzone[®] tablets (produced by Bristol-Myers Squibb Pharm.Ind.-Egypt), each tablet contains 200 mg of nefazodone per tablet.

Preparation of sample solutions

Solution of 2 mg/mL was prepared by dissolving 50 mg drug in distilled water and made up to 25 mL in a volumetric flask for spectrophotometric procedure. For conductometric procedure, a stock standard solutions of 2.0×10^{-3} M nefazodone and clomiphene were prepared by dissolving an exact weight of the pure analytical reagent grade drug in 70 mL double distilled water, to which 0.01 M hydrochloric acid was added in a 100 mL measuring flask. The mixture was warmed at 50°C in water bath for 5 min and diluted to volume with double distilled water. It was agitated by an electrical shaker for 5.0 min then cooled to room temperature and diluted to volume with double distilled water.

Also 1×10^{-2} and 5×10^{-3} M ammonium reineckate (Aldrich) solutions were prepared by dissolving appropriate weight in 100 mL double distilled water for spectrophotometric and conductometric measurement respectively.

General procedures

Spectrophotometric procedure

Aliquots containing 0.2–1.8 and 0.2–1.6 mg/mL of clomiphene citrate and nefazodone HCl respectively (Tables 1 & 2) were quantitatively transferred into 10 mL measuring flasks. To each flask 4.3 mL of reagent for clomiphene and nefazodone were added. Solutions of clomiphene

were shaken well and left for 5 minute while that of nefazodone were left for 15 minute after shaking well. Then the solutions were filtered, the precipitate was washed with water and transferred with the appropriate solvent (acetonitrile for clomiphene citrate and acetone for nefazodone HCl) to 10 mL measuring flasks. Solutions were shaken well and made up to volume with the same solvent. Absorbance was measured at 509 and 523.6 against blank (Figure 1).

Table 1. Quantitative parameters for the spectrophotometric determination of clomiphene citrate and nefazodone HCl using ammonium reineckate

Items	Clomiphene citrate	Nefazodone HCl
Beer's law range, mg/mL	0.2 – 1.8	0.2 – 1.6
Apparent molar absorptivity*, mol ⁻¹ L cm ⁻¹	2.6×10 ²	2.5×10 ²
Sandell's sensitivity mg /mL per 0.001A	4.3 × 10 ⁻⁵	4.9 × 10 ⁻⁵
Regression equation		
Intercept (a)	0.04	0.123
Slope (b)	0.000377	0.000166
Correlation Coefficient (r)	0.9998	0.9999
Variance	0.57	0.30
Detection Limit	0.38	0.27

*Calculated on the basis of the molecular weight of the drug.

Table 2. Determination of clomiphene citrate and nefazodone HCl through complexation with ammonium reineckate.

Statistics	Clomiphene citrate		Nefazodone HCl	
	Taken, mg/mL	Recovery, %	Taken, mg/mL	Recovery %
	0.2	99.46	0.2	100.09
	0.28	100.41	0.28	99.40
	1.2	100.79	0.6	99.49
	1.4	100.79	0.8	99.92
	1.6	100.96	1.4	99.75
	1.8	99.17	1.6	100.05
Mean*±SD	100.26±0.761		99.95±0.55	
N	6		6	
SD	0.761		0.55	
RSD	0.759		0.55	
V	0.57		0.30	
SE	0.31		0.22	

*Mean of three different experiments.

Conductometric procedure

A suitable aliquot (up to 44.5 mL) of sample solution containing 2.5-30 mg of drug was transferred to a 50 mL calibrated flask and made up to the mark with 50% (v/v) acetone–water mixture. The contents of the calibrated flask were transferred to a beaker and the conductivity cell was immersed. 5×10⁻³ M ammonium reineckate solution was then added from a micro burette and the conductance was measured subsequent to each addition of

reagent solution and after thorough stirring. The conductance was measured after 2 min of each addition was corrected for dilution²² by means of the following equation, assuming that conductivity is a linear function of dilution.

$$\Omega_{correct}^{-1} = \Omega_{obs}^{-1} \left[\frac{v_1 + v_2}{v_1} \right]$$

Where Ω_{obs}^{-1} is the observed electrolytic conductivity, v_1 is the initial volume and v_2 is the volume of reagent added.

A graph of corrected conductivity *versus* the volume of added titrant was constructed and the end-point determined. 0.1 mL of 5×10^{-3} M ammonium reineckate is theoretically equivalent to 0.633 and 0.30 mg of nefazodone and clomiphenes respectively. The procedure takes 15–30 min in all.

Figure 1. Absorption spectra of the complex formed through reaction of 1.6 mg/mL clomiphenes and nefazodone with ammonium reineckate.

Assay of pharmaceutical preparations

The contents of 20 tablets of each of the studied drugs were thoroughly ground. A quantity equivalent to 50 mg drug was accurately weighed into a 100 mL volumetric flask. The mixture was warmed at 50°C in a water bath for 5.0 min, agitated by an electrical shaker for another 5.0 min, cooled to room temperature and diluted to volume with double distilled water, filtered and the procedures was completed as under the general procedures.

Results and Discussion

Clomiphenes citrate and nefazodone HCl were found to react with ammonium reineckate to form stable ion pair complexes. These complexes are sparingly soluble in aqueous solution, but are readily soluble in acetone or acetonitrile. Investigations were carried out to establish the most favourable conditions for the ion pair complex formation of the two drugs with ammonium reineckate to achieve sharp end point and/or maximum color development, in the determination of the drugs the influence of some variables on the reaction was tested as follow:

Conditions for spectrophotometric method

Effect of PH: The effect of PH on the precipitation of the drug-reineckate complexes was studied, different buffers were tried, it was found that buffer had no effect on the reaction.

Effect of reagent volume: 4 and 3 mL of 0.01 M ammonium reineckate solution were sufficient to give best results with clomiphene citrate and nefazodone HCl, respectively (Figure 2).

Figure 2. Effect of reagent volume on the absorbance of the complex formed with 1.2 and 1.4 mg/mL clomiphene citrate and nefazodone HCl, respectively.

Effect of solvent: Distilled water, acetone, (acetone : water), ethanol and acetonitrile were tried. Acetonitrile and acetone were practically used to dissolve (clomiphene-reineckate) and (nefazodone – reineckate) complexes respectively.

Effect of precipitating time: 5 and 15 minute were sufficient to give complete precipitation, increasing time than this had no effect on absorption (Figure 3).

Effect of temperature: Temperature had no effect on the absorbance, so experiments were done at room temperature.

Composition of the complex: The stoichiometric ratio of the studied compounds to reineckate in the complexes were determined by applying Job's method²³, the results showed that drugs and reagent react in (1:1) ratio, Figure 4.

Condition for conductometric method

Conductometric analysis can be used in many titration procedures when ionic solutions are involved. As the conductance of a solution is related to the total ionic content, it can be applied to follow reactions that result in a change in this quantity.

Conductance measurements are used successfully in quantitative titration of systems in which the conductance of the solution varies before and after the equivalence point. In these cases, the titration curve can be represented by two lines intersecting at the end point.

Figure 3. Effect of precipitation time on the absorbance of the complex formed through reaction of 1.2, 1.4 mg/mL clomiphene and nefazodone with ammonium reineckate.

Figure 4. Continuous variation plot for 0.01M clomiphen citrate or nefazodone HCl and 0.01 M ammonium reineckate.

Investigations were carried out to establish the most favourable conditions for the ion pair complex formation of nefazodone and clomiphen with ammonium reineckate to achieve sharp end point and/or maximum colour development, in the determination of the drug. The influence of some variables on the reaction has been tested as follow:

The optimum conditions for performing the titration in a quantitative manner were elucidated as described below. Titrations in different media were attempted to obtain the best results. Preliminary experiments in:

- (i) Aqueous drug solution with aqueous reagent solution,
 - (ii) Ethanol drug solution with ethanol reagent solution,
 - (iii) Drug solution with reagent solution, both in ethanol–water (50%, v/v) mixture
 - (iv) Acetone drug solution with acetone reagent solution and
 - (v) Drug solution with reagent solution, both in acetone–water (50% v/v) mixture.
- Preliminary experiments showed that procedure acetone–water (50% v/v) media was the most suitable for successful results, because in other procedures precipitates were formed which caused some errors.

The reagent concentration in each titration must be not less than ten times that of the drug solution in order to minimize the dilution effect on the conductivity through the titration. The optimum concentration of the reagent was 5×10^{-3} M ammonium reineckate in titration of the two studied drugs to achieve a constant and highly stable conductance reading within 1-2 min of mixing. Concentrations less than these limits led to unstable readings and more time was needed to obtain constant conductance values. On increasing the temperature to 50°C, no change in the conductance reading was observed, whereas above which, the conductance value changed and so changed the shape of the conductometric titration curve.

Representative titration curves are shown in (Figure 5). Two straight lines are obtained, intersecting at the end-point, the first branch ascending and the second has conductance values would remain constant or slightly increase after the equivalence point. The increase of conductance may be attributed to the formation of ion-pair in solution as a result of the complexation reaction. After the end-point, the titration curves indicate a constant or slightly increase value of conductance, despite the excess of the reagent. This may be due to further ionic condensation, leading to species of lower mobility.

Figure 5. Conductometric titration curves of (a) 6.5 mL (2.0×10^{-3} M) NFZ and (b) 6.9 mL (2.0×10^{-3} M) CMP vs (5.0×10^{-3} M) ammonium reineckate.

The shape of the titration curve depends on all the species present during the titration process and other factors such as viscosity, dielectric constant, solvation, ion-pair association and proton transfer. The conductometric titrations of different volumes of 5×10^{-3} M ammonium reineckate solution in acetone-water (50%, v/v) mixture was performed. The results show an obvious maximum in the conductance curve at drug-reagent molar ratio of (1:1). The reactions may be represented by the equations:

The conductance of the titrated solution is mainly due to the drug cations and chloride ions and partially to the hydrogen ions resulting from the dissociation of the protonated drug cation. It was expected that the conductance values would remain constant or slightly increase after the equivalence point. However, the conductance of the solution decreased. This may be due to interaction of the protons available in the titration medium with the added reagent. Measurements of the pH before and after reaching the end-point for nefazodone and clomiphen, respectively, supporting the above assumption. The results from the conductometric titrations are summarized in Tables 3-4. The data show that accurate results were obtained with good recoveries and low standard deviation values. The optimum concentration ranges for determination of the two drugs were in the range of 63.3–443.1 and 60.02–540.18 $\mu\text{g mL}^{-1}$ for nefazodone and clomiphen respectively. At such ranges, sharp inflections (Figure 5) and stable conductance reading were obtained.

Table 3. Analytical characteristic of conductometric procedure.

Parameters	NFZ	CMP
Optimum concentration, $\mu\text{g mL}^{-1}$	63.3-443.1	60.02-540.18
Shift or intercept of the regression line ^a	0.0699	0.0864
Slope of regression line	0.5044	0.7374
Correlation coefficient (r)	0.9998	0.9992
Relative standard deviation, %	1.90	2.08

^aObserved vs. theoretical; NFZ: Nefazodone HCl; CMP: Clomiphen citrate

In order to establish whether the proposed methods exhibit any fixed or proportional bias, a simple linear regression²⁴ of drug concentration (dependent variable) against the theoretical values (independent variable) (6 points) were obtained using a programmable calculator.

Table 4. The intra-day accuracy and precision data for the studied drugs obtained by conductometric method.

NFZ					CMP				
Taken $\mu\text{g mL}^{-1}$	Found $\mu\text{g mL}^{-1}$	Recovery %	RSD %	Er %	Taken $\mu\text{g mL}^{-1}$	Found $\mu\text{g mL}^{-1}$	Recovery %	RSD %	Er %
63.3	63.62	100.50	0.46	0.50	60.02	59.96	99.90	0.43	-0.10
126.6	126.09	99.60	0.53	-0.40	120.4	119.14	98.95	0.81	-1.05
189.9	188.48	99.25	0.84	-0.75	180.06	179.16	99.50	0.70	-0.50
253.2	251.35	99.27	0.90	-0.73	300.1	298.00	99.30	0.92	-0.70
316.5	313.81	99.15	0.43	-0.85	420.14	419.72	99.90	0.52	-0.1
443.1	442.21	99.80	0.72	-0.20	540.18	540.72	100.10	0.64	0.10

^a Average value of six determinations.

Quantification

Calibration graphs with good linearity were obtained as recorded before. The linear regression equations were also calculated. Correlation coefficient, intercept and slope values for the calibration data calculated, detection limit was also evaluated and recorded in Table 1. The Validity of the proposed methods was assessed by its application to the determination of the two drugs in their pharmaceutical preparations Tables 5-7. Student's *t*-test (at 95 % confidence level) was applied to the results obtained compared with that obtained when applying the official method for clomiphene citrate or reference one for nefazodone HCl, the results showed that it didn't differ significantly and there are no systematic differences between the proposed and official or reference methods. The results of different statistical treatment of the data are shown in Table 8.

Table 5. Application of the proposed spectrophotometric method for the analysis of clomiphene citrate drug in dosage form

Commercial product	Claimed amount, mg/mL	Authentic added, mg/mL	Recovery, %
	0.28	---	101.17
Clomid® tablets	---	0.2	100.79
Each tablets contain 50 mg	---	0.6	99.02
clomiphene citrate per tablet	---	0.8	101.12
	---	1.2	101.67
Mean*±SD	100.65±1.14		
N	4		
SD	1.14		
RSD	1.13		
V	1.29		
SE	0.57		

*Mean of three different experiments.

Table 6. Determination of nefazodone HCl in its pharmaceutical preparation by using spectrophotometric method.

Commercial product	Label claim, mg/mL	Found, %
Serzone tablets	0.20	100.30
	0.28	99.61
	0.40	101.35
	0.60	100.70
Mean $\pm$ SD	100.49 $\pm$ 0.728	
N	4	
SD	0.728	
RSD	0.725	
V	0.529	
SE	0.364	

*Mean $\pm$ SD (mean of three different experiments)** Theoretical values for *t* and *F*-values at five degree of freedom.**Table 7.** Application of the proposed conductometric method to the determination of the studied drugs in dosage forms.

Sample	Reported or official method	Conductometric method
Serzone Tablets (200 mg NFZ/tablet)		
X $\pm$ SD ^a	99.80 $\pm$ 0.58	99.70 $\pm$ 0.58
<i>t</i> -value ^b		0.27
<i>F</i> -value ^b		1.0
Clomid Tablets (50 mg CMP/tablet)		
X $\pm$ SD ^a	99.67 $\pm$ 0.64	99.82 $\pm$ 0.73
<i>t</i> -value ^b		0.35
<i>F</i> -value ^b		1.30

*Mean $\pm$ SD (mean of three different experiments)** Theoretical values for *t* and *F*-values at five degree of freedom and 95 % confidence limit.**Table 8.** Determination of clomiphene citrate and nefazodone HCl through complexation with ammonium reineckate using Spectrophotometric method compared with official and reference one.

Statistics	Clomiphene citrate			Nefazodone HCl		
	Official method	Spectrophotometric method	Conductometric method	Reference Method ¹³	Spectrophotometric method	Conductometric method
Mean*,p=0.05	99.56	99.60	99.95	99.10	99.61	100.26
N	6	6	5	6	6	4
V	0.57	0.32	0.45	0.304	0.452	0.32
SD	0.81	0.552	0.673	0.74	0.761	0.57
<i>t</i> -test **		1.56 (2.306)	0.12(2.306)		2.14 (2.262)	0.743(2.262)
<i>F</i> -test **		1.80 (5.41)	1.69(5.41)		0.148 (5.19)	1.45(5.19)

*Mean $\pm$ SD (mean of three different experiments)** Theoretical values for *t* and *F*-values at five degree of freedom and 95 % confidence limit.

IR spectra (Figure 6&7) show that complexation between clomiphene citrate and ammonium reineckate (ammonium tetra thiocyanate diamine chromate) took place through ion pairing to produce clomiphene reineckate. IR chart of the resulting complex not only contain bands due to the reineckate part such as 3314 , 3237 cm^{-1} NH_2 and 2077 cm^{-1} SCN , but also enclose a band at 3442 cm^{-1} NH of clomiphene. Moreover, The absence of C=O absorption band at 1731 cm^{-1} and the broad OH absorption band at $3250\text{--}2500\text{ cm}^{-1}$ propose the absence of the citrate anion from the complex. As a conclusion from the above data, the complex is ion pair where protonated clomiphene replaces the ammonium cation of ammonium reineckate with a molar ration (1:1).

In the same way, IR spectra were analyzed to verify the structure of the complex formed between nefazadone HCl and ammonium reineckate. Again the complex is ion pairing between protonated nefazadone and reineckate with a molar ration (1:1). The IR spectrum of complex shows bands characteristic for functional groups of both parts of the salt, such as:

1. Presence of amidic C=O at 1678 cm^{-1} confirms the presence of nifazadone in the complex.
2. Presence of two bands at 3295 , 3236 cm^{-1} NHs , in addition to the very characteristic band at 2076 cm^{-1} SCN authenticates the presence of reineckate in the complex.

Figure 6. IR spectra of (a) ammonium reineckate, (b) Clomiphene citrate and (c) Clomiphene citrate and ammonium reineckate complex.

Figure 7. IR spectra of (a) ammonium reineckate, (d) Nefazodone HCl, and (e) Nefazodone HCl and ammonium reineckate complex.

Accuracy and precision

In order to determine the accuracy and precision of the proposed method, solutions containing six different concentrations of each drug were prepared and six replicate determinations were carried out for the pure form and the pharmaceutical preparation of the drugs under investigation. The analytical results obtained from this investigation are summarized in Table 4. The relative standard deviation (RSD %) as precision and percentage relative error (Er %) as accuracy of the suggested method was calculated. The percentage relative error calculated using the following equation:

$$\text{Er \%} = [(\text{found} - \text{added}) / \text{added}] \times 100$$

The intra-day precision and accuracy results are shown in Table 4. These results of accuracy and precision show that the proposed method have good repeatability and reproducibility.

Conclusions

The proposed methods have the advantages of being simple, rapid, accurate, highly reproducible and time saving, thereby encouraging its applications in quality control of these drugs in their pure form and in pharmaceutical preparations; conductometric method has the advantage over the spectrophotometric one of being more sensitive.

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