Extractive Colorimetric Method for the Determination of Dothiepin Hydrochloride and Risperidone in Pure and in Dosage Forms

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Received January 12, 2008; accepted April 22, 2008; published online May 29, 2008

Three rapid, simple, reproducible and sensitive extractive colorimetric methods (A—C) for assaying dothiepin hydrochloride (I) and risperidone (II) in bulk sample and in dosage forms were investigated. Methods A and B are based on the formation of an ion pair complexes with methyl orange (A) and orange G (B), whereas method C depends on ternary complex formation between cobalt thiocyanate and the studied drug I or II. The optimum reaction conditions were investigated and it was observed the calibration curves resulting from the measurements of absorbance concentration relations of the extracted complexes were linear over the concentration range 0.1—12 μg ml^{-1} for method A, 0.5—11 μg ml^{-1} for method B, and 3.2—80 μg ml^{-1} for method C with a relative standard deviation (RSD) of 1.17 and 1.28 for drug I and II, respectively. The molar absorptivity, Sandell sensitivity, Ringbom optimum concentration ranges, and detection and quantification limits for all complexes were calculated and evaluated at maximum wavelengths of 423, 498, and 625 nm, using methods A, B, and C, respectively. The interference from excipients commonly present in dosage forms and common degradation products was studied. The proposed methods are highly specific for the determination of drugs I and II, in their dosage forms applying the standard additions technique without any interference from common excipients. The proposed methods have been compared statistically to the reference methods and found to be simple, accurate (*t*-test) and reproducible (*F*-value).

Key words colorimetry; ion pair; dothiepin hydrochloride; risperidone; pharmaceutical analysis

Dothiepin hydrochloride (I), (3(6*H*)-dibenzo[*b*,*e*]thiepin-11-ylidene) propyl dimethylamine hydrochloride, it is a tricyclic antidepressant with a noticeable action.[1] It is indicated in the treatment of depression and anxiety. Several methods have been reported for its determination, including derivative,[2] extractive colorimetric ion pair complex formation,[3] kinetic spectrophotometry using alkaline potassium permanganate or 4-chloro-7-nitrobenzofurazon,[4] charge transfer, and ion-associate complex formation.[5,6] Other techniques such as HPLC,[7,8] GC,[9] voltammetry,[10] and RIA[11] were also reported for dothiepin analyses.

Risperidone (II) is an atypical neuroleptic that has improved the quality of life of many schizophrenic patients. The drug is particularly important in the treatment of those patients that are non-responsive to treatment with haloperidol or other classical neuroleptic drugs, and/or suffer from extra pyramidal effects caused by these drugs.[12]

Recently, different analytical techniques, including liquid chromatography-electrospray tandem spectrometry,[13,14] HPLC-UV detection,[15] GC,[16] electrophoresis,[17] HPLC with electrochemical detection,[18] and spectrophotometry[19] were utilized for the determination of risperidone.

The present work describes different colorimetric methods utilizing methyl orange, orange G, and cobalt thiocyanate for the colorimetric determination of I and II in their pure and dosage forms. The proposed methods are simple, sensitive, rapid, and accurate; they also have the advantage of being cheaper than the reported methods.

Experimental

Apparatus All absorption spectra were obtained using a Shimadzu UV and Vis. Recording spectrophotometric UV 260 with matched 10 mm quartz cells. An Orion Research Model 601 A/Digital Ionalizer pH-meter was used to determine the pH values of a buffer solution prepared as previously recommended.[20]

Reagents All chemicals used were of analytical grade, and all solutions were freshly prepared in doubly distilled water.

Dothiepin HCl (99.6% purity) was obtained from Kahira Pharmaceutical and Chemical Industries Company, and used as received. Risperidone (99.8% purity) was purchased from October Pharma, S.A.E., Cairo, Egypt. A standard 5×10^{-3} M solution was prepared by dissolving an appropriate weight of drug I in a 100 ml measuring flask, whereas for drug II in least amount of 50% (v/v) acetic acid and then completed to the mark with water in a 100 ml measuring flask. Working solutions were obtained by further dilution of the stock solutions with water.

Ethanolic aqueous solutions (20% v/v) of methyl orange [5×10^{-3} M or 0.1% w/v) were prepared by dissolving an appropriate weight in the least amount of ethanol and then completion to the mark with water and ethanol (20% v/v) in a 100 ml measuring flask. A 5×10^{-3} M or 0.1% (w/v) stock solution of orange G was prepared by dissolving an accurate weight of the dye in the minimum amount of water and then completion to the mark in a 100 ml measuring flask with water. Tetrathiocyanate cobalt(II) stock solution was prepared by dissolving 28.2 g of ammonium thiocyanate and 13.6 g of cobalt nitrate in the minimum amount of water followed by completion to the mark in a 50 ml measuring flask with water.

General Procedures. Methods A and B Into 25 ml separating funnels, aliquots containing up to 120 μg ml^{-1} of drug solution were pipetted, and then 4.0 or 5.0 ml of 0.1% w/v of methyl orange and orange G for drug I or II was added. The solution was diluted to 10 ml with water after the addition of 2.0 ml of pH 2.7 or 3.4 for I or II in the case of method A only. Ten milliliters of chloroform for method A or 10 ml of dichloromethane using method B was added and then the mixture was mixed well. After shaking for 2.0 min, the mixture was centrifuged for 1.0 min at 2000 rev min^{-1}. After separating the organic layer, the absorbance of the extracts was measured at 423 and 498 nm for methods A and B, respectively, against a reagent blank prepared using the same method.

Method C Into 25 ml separating funnels, aliquots containing 5.0—800 μg ml^{-1} of drug I or II solution were pipetted, and 0.5 ml of cobalt thiocyanate was added. The solution was diluted to 10 ml with water and mixed with 10 ml of dichloromethane for I or 10 ml chloroform for II. After shaking for 2.0 min, the mixture was centrifuged for 1.0 min at 2000 rev min^{-1}. After separating the two layers, the absorbance of the extracts was measured at 625 nm, against a reagent blank prepared according to the same treatment.

Procedures for Dosage Forms For capsules ten capsules were emptied and an amount equivalent to 50 mg of I was weighed and dissolved in water, filtered if necessary, and completed to the mark in a 100 ml measuring flask with water. The assay of I content was completed as described above.

For Tablets Fifty tablets of II were powdered and a quantity of the pow-

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der equivalent to 20 mg of risperidone was dissolved by shaking with 2.0 ml of acetic acid [50% (v/v)] and 25 ml of water. The solution was stirred mechanically on a magnetic stirrer for 10 min, and the solution was then filtered and completed to the mark in a 100 ml measuring flask with water. The assay of II content was completed as described above.

Results and Discussion

The nitrogenous drugs are present in positively charged protonated forms and anionic dyes are present mainly in anionic form in acidic medium. So when treated with an acid dye such as methyl orange or orange G in acidic medium, a yellow orange ion pair complex which is extracted using dichloromethane or chloroform is formed. The absorption spectra of the ion pair complexes formed between I or II using methods A and B were measured at 340—560 nm against a blank solution. The developed methods were applied to dosage forms and the obtained results were evaluated statistically.

Optimization for Methods A and B The optimization of the reaction conditions of the proposed methods A and B was carefully studied to achieve the complete reaction, highest sensitivity and maximum color development. Reaction conditions or formation of the ion pair complexes were found by preliminary experiments by varying the pH of buffer solutions, nature of organic solvents, dyes concentration and shaking time for complete extraction of the formed ion pairs.

Effects of Extracted Solvents A number of organic solvents such as chloroform, dichloromethane, carbon tetrachloride, benzene and toluene were examined for extraction of the ion pair complexes in order to provide an applicable extraction procedure. Although dichloromethane is not an ecologically friendly solvent, it was preferred for its selective extraction for method B ion pair complexes due to the greater stability of the extracted ion pair (15 h) and considerably lower extraction abilities of the reagent blank. Chloroform is the optimum solvent for extraction of methyl orange (method A) ion pairs from the aqueous solution, in addition, the reagents were not extracted in these solvents. Reproducible absorbance readings were obtained after a single extraction with 10 ml of dichloromethane or chloroform. The overall extraction efficiency was 99.7%. Repeated extraction did not show any increase in the recovery percent. Using different solvents, a lower absorbance value of ion pairs formed is obtained, in addition to several extraction time.

Effect of Dye Concentration The effect of the dye concentration on the full color development at the selected wavelength and constant drug concentration was examined using different amounts (0.5—7.0 ml) of 0.1% (w/v) solutions of reagents. As shown in Fig. 1, the maximum absorbance for drug I was found with 4.0 ml, whereas for drug II it was found with 5.0 ml using both methods A and B.

Effect of pH The effect of pH was studied by extracting the formed ion pair in the presence of various buffer solutions. The maximum color development and constant absorbance values were found in acetate buffer solution for method A, whereas none of the examined buffer was studied for method B. It is evident that the absorbance of ion pair complex for drug I was found to be maximal at pH 2.7, while for drug II the absorbance was maximal at pH 3.4. Moreover, the optimum amount of buffer solution added to the aqueous layer was found to be 2.0 ml. For method B, aqueous solution without buffer solution gave the best results, although only marginally.

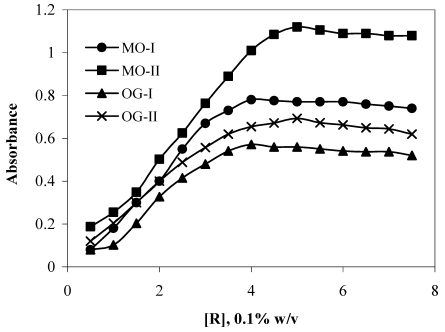

Fig. 1. Effect of Methyl Orange (MO) and Orange G (OG) Concentration (0.1% w/v) on the Absorbance of 8.0 μg ml^{-1} of the Studied Drugs Ion Pairs

Effects of Shaking Time and Temperature The ion pair complexes formed rapidly with both drugs at 25±2 °C. The extracted colored form attained maximum intensity instantaneously after shaking and centrifuging for 2.0 min. The colored ion pairs were stable for more than 15 h. Raising the temperature up to 50 °C did not change the absorbance of the ion pairs, while boiling decreased the color intensity with a blue shift in the wavelength of the formed ion pairs.

Effect of Sequence of Addition Although the sequence of mixing of the reaction components is not a fundamental factor, the most favorable sequence is drug-reagent-buffer-chloroform for the highest and most stable absorbance. The complexes obtained using this sequence of addition gave the highest absorbance and remain stable for at least 15 h.

Ternary Complex Formation Using Cobalt Thiocyanate Ternary complexes have been widely used in colorimetric analysis of many pharmaceutical drugs.[21—25] In the present study, the formed ternary complexes consist of the cited drug I or II as the main ligand, thiocyanate as a second ligand, and the metal ions. Different metal ions were examined (copper, nickel, iron, zinc and cobalt). Cobalt(II) was found to be the optimum metal ion to form highly stable ternary complexes and more sensitive ones in the determination of the studied drugs I and II. The ternary complex of I was extractable with dichloromethane, whereas that for drug II was extractable using chloroform with an absorption maximum at 625 nm. The binary systems (Co^{2+} : drug), (Co^{2+} : thiocyanate) and (drug : thiocyanate) have no absorbance in the visible region.

The effects of reagent concentration, pH, temperature, time, order of addition of reagents and extracted solvents with respect to maximum sensitivity, selectivity, adherence to Beer's law and stability, have been studied through control experiments. The optimum conditions were established by varying one variable at a time and observing its effect on the absorbance of the colored ternary complexes. The optimum conditions were recorded into the general procedure.

Composition of the Complexes In order to establish the molar ratio between I or II, on one side and reagent used on the other, Job's method of continuous variation was applied. In this method, 5×10^{-3} M solutions of drug and reagent were mixed in varying volume ratios in such a way that the total volume of each mixture was the same. The absorbance of each solution was measured and plotted against the mole fraction of the drug. This procedure showed that a (1 : 1) complex was formed through the electrostatic attraction be-

tween the positively charged drug, D^+ ions and negatively charged reagent, R^-, ions (Fig. 2). The extraction equilibrium can be represented as follows:

$$D_{aq}+R^- \rightleftarrows D^+R^-_{aq} \leftrightarrows D^+R^-_{org},$$

Where D and R represent the protonated drug and the anion of the reagent, respectively, and the subscripts "aq" and "org" refer to the aqueous and organic phases, respectively. The stability constant ($-\log K$) was calculated and recorded in Table 1, applying the data obtained from the continuous variation method.[26)]

Interference Studies The effects of common excipients that often accompany the studied drugs in various pharmaceutical dosage forms were tested for possible interference in the assay. An attractive feature of the procedure is its relative freedom from interference by the usual tablet diluents and excipients such as talc, sucrose, starch, gelatin, lactose, and magnesium stearate. Amounts far in excess of their normal occurrences in dosage forms were added, and no effects due to these excipients were noted in the experimental procedure. Moreover, no interference due to the degradation products of the studied drugs I and II was observed.

Quantification A linear correlation was found between absorbance and concentration in the ranges given in Table 1. The correlation coefficients, slopes, and intercepts for the calibration data for the two cited drugs I and II were calculated using the least-squares method. The detection and quantification limits were calculated from the standard deviation of the absorbance measurements obtained from a series of 13 blank solutions for each procedure. The limits of detection ($K=3.0$) and of quantification ($K=10$) were established according to the IUPAC definitions.[27)]

In order to determine the accuracy and precision of the proposed methods, solutions containing three different concentrations of I and II were prepared and analyzed in six

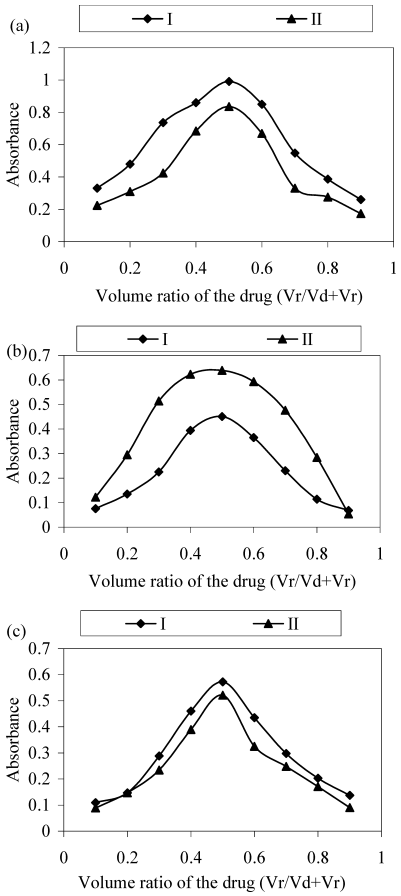

Fig. 2. Continuous Variation Plots for the Ion-Pair Complexes of I and II with Reagents

(a) Methyl orange, (b) orange G, (c) $[Co(SCN)_4]^{-2}$, [drug]=[dye]=5.0×10^{-3} M. Where, Vd and Vr are the volumes of added drug and reagent, respectively; (Vd+Vr)=1 ml.

Table 1. Quantitative Parameters for the Proposed Methods (A—C)

Parameter	Dothiepin HCl			Risperidon		
	A	B	C	A	B	C
pH	2.7			3.4		
λ_{max}	423	498	625	423	498	625
Beer's conc. range/μg ml^{-1}	0.2—12.0	0.5—10.0	3.2—80.0	0.1—10.0	0.6—11.0	4.0—80.0
Ringbom conc. range/μg ml^{-1}	0.5—11.4	0.8—9.3	5.0—77.5	0.25—9.7	0.9—10.5	6.5—76.0
Detection limit/μg ml^{-1}	0.06	0.15	0.98	0.031	0.18	1.15
Quantification limit/μg ml^{-1}	0.19	0.49	3.13	0.097	0.58	3.78
Molar absorptivity/l mol^{-1} cm^{-1}	3.04×10^4	2.32×10^4	3.60×10^3	6.05×10^4	2.89×10^4	4.11×10^3
Sandell sensitivity/μg cm^{-2}	0.0112	0.0143	0.092	0.0066	0.014	0.098
Stoichiometric ratio	1 : 1	1 : 1	1 : 1	1 : 1	1 : 1	1 : 1
Stability constant	3.69	6.74	5.50	7.06	6.64	5.65
Stability/h	15	15	15	15	15	15
Regression equation[a)]						
Slope	0.092	0.07	0.011	0.15	0.072	0.01
Intercept	−0.037	0.046	0.032	0.067	−0.025	−0.030
Correlation coefficient (r)	0.9996	0.9994	0.9985	0.9994	0.9996	0.9990
RSD % of slope	3.45×10^{-4}	6.13×10^{-4}	5.27×10^{-4}	7.32×10^{-4}	5.34×10^{-4}	8.09×10^{-4}
RSD % of intercept	1.67×10^{-4}	2.98×10^{-4}	2.67×10^{-4}	3.45×10^{-4}	2.65×10^{-4}	4.12×10^{-4}
Range of error	1.20	1.15	1.45	1.10	1.15	1.50
RSD %	0.91	0.78	1.17	1.15	1.07	1.28
Student t-value (2.57)[b)]	0.35	0.71	0.75	0.76	0.35	0.56
Variance ratio F-test (5.05)[b)]	1.72	3.07	2.92	1.02	2.20	1.49

a) $A=a+bC$, where C is the concentration in mg ml^{-1}. *b*) Values in parentheses are the theoretical values for t- and F-values at 95% confidence limits and five degrees of freedom.

Table 2. Accuracy and Precision of the Proposed Methods

Complex	Amount of drug		Recovery (%)	RSD[b] (%)	Relative error (%)	Confidence limits[c]
	Taken (μg ml^{-1})	Found[a] (μg ml^{-1})				
I-A	4.0	3.97	99.25	0.74	0.78	3.97±0.067
	8.0	8.05	100.63	0.91	0.96	8.05±0.033
	12.0	11.92	99.33	1.05	1.10	11.92±0.059
I-B	3.0	3.02	100.67	0.85	0.89	3.02±0.021
	6.0	6.05	100.83	0.77	0.81	6.05±0.032
	9.0	9.04	100.44	1.08	1.13	9.04±0.053
I-C	25.0	24.80	99.20	1.15	1.21	24.80±0.071
	50.0	50.45	100.90	1.23	1.30	50.45±0.084
	75.0	74.65	99.53	1.42	1.49	74.65±0.078
II-A	3.0	2.98	99.33	0.97	1.02	2.98±0.033
	6.0	6.04	100.67	1.04	1.09	6.04±0.021
	9.0	9.05	100.56	0.87	0.92	9.05±0.052
II-B	3.5	3.53	100.86	1.14	1.20	3.53±0.059
	7.0	6.95	99.29	0.96	1.01	6.95±0.043
	10.5	10.60	100.95	1.07	1.12	10.60±0.067
II-C	25.0	25.15	100.60	1.34	1.41	25.15±0.084
	50.0	49.70	99.40	1.25	1.31	49.70±0.059
	80.0	79.55	99.44	1.11	1.17	79.55±0.670

a) Average of six determinations. *b*) Relative standard deviation for six determinations. *c*) 95 % confidence limits and five degree of freedom.

replicates. The analytical results obtained from this investigation are summarized in Table 2. The percentage standard deviation ($\leq$0.88) and the percentage range of error at the 95% confidence level ($\leq$0.975) can be considered as satisfactory. The performance of the methods was assessed by calculating the *t*- and *F*-values and then comparing them with the official methods[28,29] {based on non-aqueous titration using 0.1 M perchloric acid for I, and absorbance was measured at 238 nm in 0.1 N HCl for drug II}. The mean values were obtained in Student's *t*-test and the *F*-test at the 95% confidence level for five degrees of freedom.[30] The results show that the calculated *t*- and *F*-values did not exceed the theoretical ones.

Comparison of the results obtained with the proposed method A with those obtained earlier[3] for I using bromophenol blue, bromothymol blue, bromocresol purple and bromophenol red in acidic medium showed more sensitivity and higher accuracy in less time consumption with a lower range for microdetermination. The proposed method is simpler, highly precise and less time consuming than other HPLC methods.[7,8,13—18] Moreover, the proposed method could be used for the routine determination of I and II in pure form or in pharmaceutical formulations.

Analytical Applications The proposed methods were successfully applied to determine drugs I and II in their dosage forms using a standard addition method in which the variable amounts of the pure drug were added to the previously analyzed portions of pharmaceutical preparations. The results are presented in Table 3 and confirm that the proposed methods are not liable to interference by tablet fillers usually used with the drugs. The results obtained from the proposed methods were compared with those using official and reported methods. The accuracy (*t*-value) and the assessment of the precision (*F*-test) for six degrees of freedom and 95% confidence level were calculated and the results indicated that there is no significant difference between the characteristics of the proposed method and those of the official and reported methods. Moreover, the proposed methods provide more stable results (at least for 15 h) than the official and reported methods.

Table 3. Results for Determination of I and II in Dosage Forms Applying Standard Addition Technique

Dosage forms	Content	Taken (μg ml^{-1})	Added (μg ml^{-1})	Found[a] (μg ml^{-1})		
				A	B	C
Prothiaden[b]	25 mg dothiepin/cap.	2.0	—	1.99	2.02	—
			2.0	4.00	3.97	3.95
			4.0	5.96	6.05	6.10
			8.0	10.12	9.95	10.15
			16.0	—	—	18.15
			32.0	—	—	33.80
			64.0	—	—	66.50
Apexidone[c]	1 mg Risperidone/tab.	1.5	—	1.49	1.49	—
			3.0	4.52	4.47	4.45
			6.0	7.45	7.55	7.60
			12.0	—	—	13.50
			24.0	—	—	25.40
			48.0	—	—	49.85

a) Average of six determinations. *b*) Kahira Pharmaceutical Chemical Industries Company, Cairo, Egypt. *c*) October Pharma, S.A.E. 6th October City, Cairo, Egypt.

Conclusions

It is clear that anionic dyes such as methyl orange (method A) and orange G (method B) are highly sensitive reagents for the microdetermination of drugs I and II through ion pair complex formation, whereas cobalt thiocyanate is a reproducible reagent that forms ternary complexes with the drugs and is used for their determination. The proposed methods (A—C) were successfully utilized for determining these drugs in bulk, as well as in dosage forms, and proved highly sensitive, accurate, precise, simple and without interference from excepients and additives usually present in drugs. The Student's *t*-test and *F*-test values for the proposed methods gave lower values relative to the theoretical ones indicating

high accuracy and precision with no significant differences when compared to the official or reported methods. Therefore, these reagents can be safely used for quality control of I and II in their pure state and in their dosage forms.

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