Spectrophotometric Determination of Dopaminergic Drugs Used for Parkinson's Disease, Cabergoline and Ropinirole, in Pharmaceutical Preparations

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Simple and reproducible spectrophotometric methods have been developed for determination of dopaminergic drugs used for Parkinson's disease, cabergoline (CAB) and ropinirole hydrochloride (ROP), in pharmaceutical preparations. The methods are based on the reactions between the studied drug substances and ion-pair agents [methyl orange (MO), bromocresol green (BCG) and bromophenol blue (BPB)] producing yellow colored ion-pair complexes in acidic buffers, after extracting in dichloromethane, which are spectrophotometrically determined at the appropriate wavelength of ion-pair complexes. Beer's law was obeyed within the concentration range from 1.0 to 35 μg ml^{-1}. The developed methods were applied successfully for the determination of these drugs in tablets.

Key words cabergoline; ropinirole; ion-pair extraction; pharmaceutical analysis

Cabergoline (CAB) and ropinirole hydrochloride (ROP) are dopamine agonists which are effective in improving motor function in Parkinson's disease.[1)]

CAB, *N*-[3-(dimethylamino)propyl]-*N*-(ethylamino)carbonyl-6-(2-propenyl)ergoline-8β-carboxamide, a long acting predominantly D2 receptor agonist is effective as adjunct therapy in advanced Parkinson's disease and also as monotherapy in *de novo* patients.[2,3)] A few high-performance liquid chromatographic (HPLC) methods have been developed for the determination of CAB in plasma using electrochemical detection,[4)] tandem mass spectrophotometry (TMS)[5—7)] or triple-quadrupole mass spectrometry.[8)] ROP, 4-[2-(dipropylamino)ethyl]-l,3-dihydro-2*H*-indol-2-one hydrochloride, is a specific D2 and D3 receptor non-ergoline dopamine agonist that is probably equally effective as L-dopa in mild, early Parkinson's disease.[9)] Very few HPLC methods in plasma were developed using ultraviolet,[10,11)] electrochemical[12)] or mass spectrometric[13)] detection. Capillary zone electrophoresis was used for the determination of the dissociation constants of ROP and five structurally related impurities potentially formed during its synthesis and for separation and quantification of these substances.[14)]

The present work aims to present a simple, rapid and sensitive method for the determination of cabergoline and ropinirole in tablets and can be used for the quality control and assurance of these drugs in industry. Extractive spectrophotometric procedures are popular for their sensitivity in the assay of drugs and, therefore, ion-pair extractive spectrophotometry has received considerable attention for the quantitative determination of many pharmaceutical compounds.[15—19)] The method is based on the formation of ion-associate between the cited drugs and methyl orange (MO), bromocresol green (BCG) and bromphenol blue (BPB) reagents. Different factors affecting these reactions are studied and established, then Beer's law is carried out. The constructed calibration curves were utilized in determining the concentration of these drugs in tablets. These methods are very simple in application and less expensive in comparison to the other techniques.

Experimental

Apparatus A Shimadzu UV-160 A UV–visible spectrophotometer with 1-cm matched glass cells was used for the absorbance measurements. pH measurements were determined using a WTW pH 526 digital pH meter, calibrated with pH 4.0 and 7.0 disodium hydrogen phosphate standard buffer solution.

Reagents and Solutions Cabergoline and its pharmaceutical preparation (Cabaser®) containing 4 mg of cabergoline per tablet were kindly supplied by Pfizer (Istanbul, Turkey). Ropinirole hydrochloride and its pharmaceutical preparation (Requip®) containing 2 mg of ropinirole per tablet were kindly supplied by GlaxoSmithKline (Istanbul, Turkey). Analytical-reagent grade chemicals/reagents and bidistilled water were used throughout the work. MO, BCG and BPB were obtained from Merck (Darmstadt, Germany). Stock solutions of CAB and ROP (1 mg ml^{-1} (for ROP, calculated as free base)) were prepared in acetonitrile and water, respectively, and diluted further with water to obtain standard solutions of 100 μg ml^{-1}. MO, BCG and BPB solutions of 2.2×10^{-3}, 1.4×10^{-3}, and 8.7×10^{-4} were prepared in 1% ethanol.

General Procedure Zero point zero five to 2.0 ml aliquots of standard solutions were transferred into stoppered glass tubes and total volumes were brought to 2 ml with water. Two milliliters of reagent solutions and 2 ml of buffer solutions (for CAB and ROP respectively: for MO methods, pH 2.5 and 2 for BCG methods, pH 3.5 and 4 for BPB methods, pH 4 and 3.5) were added in each tube. The reaction mixtures were extracted with 5 ml of dichloromethane for 1 min using a vortex mixer. The two phases were allowed to separate, and the dichloromethane layers were passed through anhydrous sodium sulphate. The organic layers were transferred into separate 5 ml volumetric flasks, and the volume made up with dichloromethane. The absorbance of the yellow-colored dichloromethane extracts was scanned at 424 nm for the MO method, at 412 nm for the BCG method and 413 nm (for CAB), or at 410 nm (for ROP) for the BPB method against a corresponding reagent blank, prepared similarly except for addition of the drug substances. The absorbance (A) was then calculated by the least-squares method's. All measurements were performed under ambient laboratory conditions.

Sample Preparations All methods were developed using the same sample preparation procedure as explained below: twenty tablets were weighed and powdered using a mortar. An accurately weighed portion of the powder, equivalent to 10 mg of active ingredient, was transferred into a 10 ml calibrated volumetric flask. Then a 5 ml portion of solvent (as solvent, water and acetonitrile were used for CAB and ROP, respectively) was added to the flask containing the powdered drug substance. The mixture was shaken mechanically for 5 min, sonicated in an ultrasonic bath for 30 min, diluted to the volume with the same solvent, mixed, and filtered through a filter. An appropriate aliquot of the filtrate was further diluted with water to the respective volumes to obtain varied concentrations of calibration graphs and assayed as described above (General Procedure).

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Results and Discussion

Cabergoline and ropinirole are dopaminergic drugs used for Parkinson's disease. To date no method has been described for their determination in tablets. In this study for the first time visible-spectrophotometric methods were developed to assay these drugs in tablets. In the present investigation, MO, BCG and BPB being anionic dyes form with cabergoline and ropinirole in acidic pH yellow coloured ion-pair complexes which are soluble in dichloromethane and can be measured at ranges from 410 to 424 nm (Fig. 1). The colorless blanks have practically negligible absorbance.

The pH of the aqueous phase was studied by extracting the coloured complexes in the presence of phthalate buffers at different pH values. Optimum pH values for the ion-pair complexes are given in Table 1. The effect of different extraction solvents on the ion-pair complexes was studied. Dichloromethane was selected for extraction of the drug-dye complexes from the aqueous phase. It was observed that only one extraction with dichloromethane was adequate to achieve a quantitative recovery of the complexes. The effect of each of the reagents was studied separately by measuring the absorbances of final solutions resulting from reaction mixtures containing a fixed concentration of CAB and ROP and various amounts of the reagent. It was found that a 10-fold molar excess of MO and BCG, and 12-fold molar excess of BPB were sufficient for the maximum yield of the reactions. Excess of these dyes did not have any effect either on the colour of the complex or on the absorbance. Using a vortex mixer, shaking intervals of from 0.5 to 5 min were studied to determine the most efficient ion-pair complex formation. As a consequence, 1-min shaking time was observed to be the optimum for our study. The stoichiometric ratio of the formed products was investigated by Job's continuous variation method.[20] 10^{-4} M solutions of CAB and ROP were used with comparable solutions of the reagent (MO, BCG, or BPB). For each method, a series of solutions were prepared in which the total volume of the drugs and reagent was kept at 5 ml, and the procedure was completed as described under General Procedure. These measurements showed that 1 : 1 complex was formed with each reagent. The resulting complexes were found to be stable up to 24 h at room temperature in the dark.

Under the optimum conditions described above, the calibration graphs were constructed for the investigated drugs. The molar absorbtivity, Sandell sensitivity (*S*), concentration range, regression equation and correlation coefficient for each drug is tabulated in Table 1. A linear relationship was found between the absorbance at λ_{max} and the concentration of the drug substances within the range 1—35 $\mu g\,ml^{-1}$. Regression analysis of Beer's law plotted at λ_{max} reveals a good correlation (r^2=0.9986—0.9998). The graphs showed a neg-

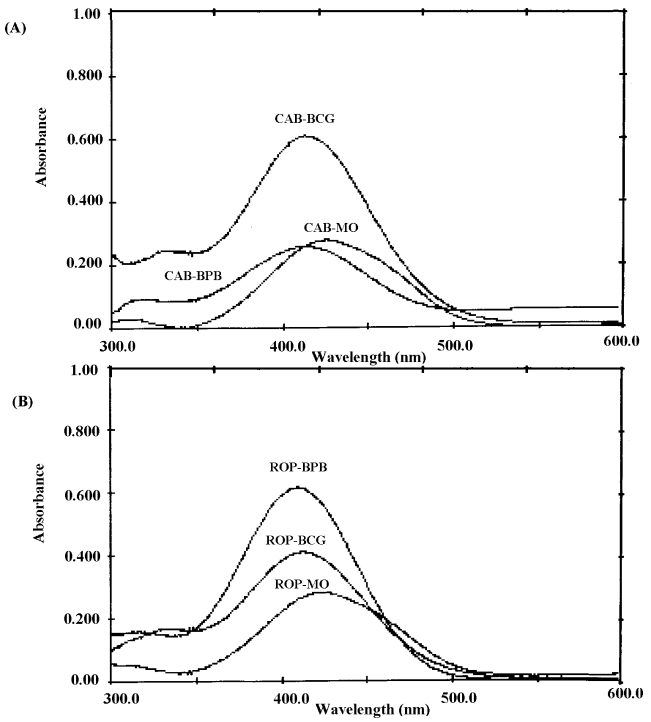

Fig. 1. Absorption Spectra of the Ion Pairs with (A) Cabergoline (CAB-MO, 11 $\mu g\,ml^{-1}$), (CAB-BCG, 10 $\mu g\,ml^{-1}$) and [(CAB-BPB, 9 $\mu g\,ml^{-1}$)] and (B) Ropinirole [(ROP-MO, 16 $\mu g\,ml^{-1}$), (ROP-BCG, 5.5 $\mu g\,ml^{-1}$) and (ROP-BPB, 14.5 $\mu g\,ml^{-1}$)] against Reagent Blanks

Table 1. Optical Characteristics and Statistical Data of the Regression Equations for the Drug Reaction with MO, BCG and BPB

Parameter	Cabergoline			Ropinirole		
	MO	BCG	BPB	MO	BCG	BPB
pH	2.0	3.5	4.0	2.5	4.0	3.5
λ_{max} (nm)	424	412	413	424	412	410
Beer's law limit[a] ($\mu g\,ml^{-1}$)	8—22	1.0—15	5.0—22	10—35	1.5—15	2.5—20
Molar absorptivity ($l\,mol^{-1}\,cm^{-1}$)	1.74×10^4	2.47×10^4	1.80×10^4	6.11×10^3	1.76×10^4	1.17×10^4
Sandell's sensitivity ($\mu g\,cm^{-2}$ per 0.001 absorbance unit)	0.026	0.018	0.025	0.042	0.014	0.022
Regression equation						
Slope±S.D.	0.0518±0.0002	0.0452±0.0004	0.0475±0.0002	0.0277±0.0002	0.063±0.0003	0.0421±0.0002
Intercept±S.D.	−0.2986±0.0020	0.1556±0.0017	−0.1736±0.0017	−0.153±0.003	0.0603±0.0005	0.0136±0.0002
Correlation coefficient, r^2±S.D.	0.9992±0.0003	0.9980±0.0007	0.9988±0.0004	0.9987±0.0005	0.9988±0.0005	0.9982±0.0002
LOD ($\mu g\,ml^{-1}$)	0.120	0.111	0.106	0.316	0.025	0.0162
LOQ ($\mu g\,ml^{-1}$)	0.398	0.371	0.352	1.054	0.083	0.054

a) Average of five determinations.

Table 2. Determination of Drugs in Dosage Forms by the Proposed Methods

Preparation	Labeled amount (mg/tablets)	Proposed methods					
		Recovery (%)			RSD (%)		
		MO	BCG	BPB	MO	BCG	BPB
Cabaser®	4	99.79	99.78	100.38	0.61	0.95	0.47
Requip®	2	99.56	100.57	100.05	0.81	0.68	0.48

Five independent analyses.

ligible intercept, which was calculated by the least-squares method's regression equation, $A=a+bC$ (where A is the absorbance of 1 cm layer, b is the slope, a is the intercept and C is the concentration of the measured solution in $\mu g\,ml^{-1}$). The high molar absorptivities of the resulting colored complexes indicated high sensitivity of the methods (1.74×10^4—2.47×10^4). The CAB-BCG method was found to be the most sensitive of all these methods with high ε value. The Limit of Detection (LOD) and Limit of Quantitation (LOQ) values were determined using the formula: LOD or LOQ$=\kappa$S.D./b, where $\kappa=3$ for LOD and 10 for LOQ, S.D. and b stand for standard deviation of the intercept and slope, respectively. The results are as shown in Table 1.

The precision of the proposed method was investigated by intra-day and inter-day determinations of two different concentrations of drug solutions (10, 15 $\mu g\,ml^{-1}$). The intra-day studies were performed in one day (for each level $n=5$) and inter-day studies in five days over a period of two weeks. The intra and inter-day precision expressed as relative standard deviation values (RSD %) were found to be within 0.45—1.12% and 0.65—1.45%, respectively (Table 2). The data proved the good precision of the developed method. To check the accuracy of the proposed method, the standard addition method was applied by adding known amounts of drugs to the previously analyzed tablet solution. The average percent recoveries obtained of 97.82—98.22% indicate good accuracy of the method.

Analysis of Pharmaceutical Preparations The proposed methods were applied to the determination of cabergoline and ropinirole in commercial tablets. Their applicability for the assay of these drugs in tablets was examined and the results are tabulated in Table 4. Five replicate determinations were made. Satisfactory results were obtained for each drug and were in good agreement with the label claims (Table 2); the results were reproducible with low RSD values. The average percent recoveries obtained were quantitative (99.56—100.57%), indicating good accuracy of the methods. The results of analysis of the commercial tablets and study of the recovery of drugs suggested that there is no interference from any excipients (such as starch, lactose, titanium dioxide, and magnesium stearate), which are present in tablets.

Conclusion

Although the HPLC method is sensitive and gives accurate results, its major disadvantages include the requirement for complex and expensive equipment, provision for use and disposal of solvents, a labor-intensive sample preparation procedure, and personnel skilled in chromatographic techniques. The significant advantage of the extractive spectrophotometric method is that it can be applied to determine individual components in a multicomponent mixture. This aspect of spectrophotometric analysis is of major interest in analytical pharmacy, since it offers distinct possibilities in the assay of a particular component in a complex dosage formulation. The high recovery percentage and low relative standard deviation reflect the high accuracy and precision of the proposed methods; moreover, the methods are easy, applicable to a wide range of concentration, besides being less time consuming and depending on simple and available reagents thus offering economic and acceptable methods for the routine determination of CAB and ROP in tablets.

References

1) Tuite P., Ebbitt B., *Sem. Neurol.*, **21**, 9—14 (2001).
2) Rinne U. K., Bracco F., Chouza C., Dupont E., Gershanik O., Marti Masso J. F., Montastruc J. L., Marsden C. D., Dubini A., Orlando N., Grimaldi R., *Neurology*, **48**, 363—368 (1997).
3) Del Dotto P., Colzi A., Musatti E., Strolin Benedetti M., Persiani S., Fariello R., Bonuccelli U., *Clin. Neuropharmacol.*, **20**, 455—465 (1997).
4) Pianezzola E., Bellotti V., La Croix R., Strolin Benedetti M., *J. Chromatogr. B*, **574**, 170—174 (1992).
5) Igarashi K., Hotta K., Kasuya F., Abe K., Sakoda S., *J. Chromatogr. B,* **792**, 55—61 (2003).
6) Kimball B. A., DeLiberto T. J., Johnston J. J., *Anal. Chem.*, **73**, 4972—4976 (2001).
7) Allievi C., Dostert P., *Rapid. Commun. Mass Spectrom.*, **12**, 33—39 (1998).
8) Hughes N., Winnik W., Dunyach J. J., Amad M., Splendore M., Paul G., *J. Mass Spectrom.*, **38**, 743—751 (2003).
9) Rascol O., Brooks D. J., Brunt E. R., Korczyn A. D., Poewe W. H., Stocchi F., *Mov. Disord.*, **13**, 39—45 (1998).
10) Ramji J. V., Keogh I. P., Blake T. J., Broom C., Chenery R. J., Citerone D. R., Lewis V. A., Taylor A. C., Yeulet S. E., *Xenobiotica*, **29**, 311—325 (1999).
11) Swagzdis J. E., Mico B. A., *J. Pharm. Sci.*, **75**, 90—91 (1986).
12) Swagzdis J. E., Gifford R., Mico B. A., *J. Chromatogr. B*, **345**, 203—208 (1985).
13) Bhatt J., Jangid A., Shetty R., Shah B., Kambli S., Subbaiah G., Singh S., *J. Pharm. Biomed. Anal.*, **40**, 1202—1208 (2006).
14) Coufal P., Stulík K., Claessens H. A., Hardy M. J., Webb M., *J. Chromatogr. B*, **720**, 197—204 (1998).
15) Onal A., Kepekci S. E., Oztunc A., *J. AOAC Int.*, **88**, 490—495 (2005).
16) Al-Ghannam S. M., *J. Pharm. Biomed. Anal.*, **40**, 151—156 (2006).
17) Rahman N., Hejaz-Azmi S. N., *J. Pharm. Biomed. Anal.*, **24**, 33—41 (2000).
18) Ramesh K. C., Gowda B. G., Melwanki M. B., Seetharamappa J., Keshavayya J., *Anal. Sci.*, **17**, 1101—1103 (2001).
19) Marona H. R., Schapoval E. E., *J. Pharm. Biomed. Anal.*, **26**, 501—504 (2001).
20) Christian G. D., Reilly J. E. O., "Instrumental Analysis," 2nd ed., Prentice Hall, Englewood Cliffs, NJ, 1986, p. 186.