DRUGS, COSMETICS, FORENSIC SCIENCES

Spectrophotometric Investigations of the Assay of Physiologically Active Catecholamines in Pharmaceutical Formulations

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Two simple, sensitive, and accurate spectrophotometric methods are proposed for the determination of levodopa (LD), methyldopa (MD), dopamine hydrochloride (DP), and pyrocatechol (PC) in pure and pharmaceutical preparations. The methods are based on measurement of the absorbances of tris(*o*-phenanthroline)iron(II) (method A) and tris(bipyridyl)iron(II) (method B) obtained by the oxidation of the catecholamines by iron(III) in the presence of 1,10-phenanthroline and 2,2′-bipyridyl at 510 and 522 nm, respectively. The absorbances were found to increase linearly with increases in the concentrations of the catecholamines, results which were corroborated by the calculated correlation coefficients (0.9990–0.9996). Beer's law was valid over the concentration ranges of 0.04–0.6, 0.06–0.75, 0.06–0.65, and 0.05–0.70 μg/mL in method A and 0.02–1.0, 0.04–1.3, 0.05–1.0, and 0.06–1.1 μg/mL in method B for PC, MD, LD, and DP, respectively. The common excipients and additives did not interfere in their determinations. The proposed methods were successfully applied to the assay of LD, MD, and DP in various dosage forms. The results were validated by statistical analysis.

Methyldopa (MD), levodopa (LD), and dopamine hydrochloride (DP) are vicinal dihydroxybenzene derivatives (catecholamines) in which either the 3- or the 4-position is unsubstituted, and these positions are not sterically blocked. These compounds are widely used in treating hypertension, bronchial asthma, and Parkinson's disease and in cardiac surgery. Expanding indications and more widespread use of these drugs have prompted many researchers to develop sensitive and accurate analytical methods for their determination, especially for routine quality control in the analysis of pharmaceutical products. These methods include the use of liquid chromatography (LC; 1, 2), spectrofluorimetry (3), and voltammetry (4). Because spectrophotometric assays offer significant economical advantages over chromatographic, electroanalytical, and spectrofluorimetric methods, many spectrophotometric methods (5–19) have been reported for the assay of catecholamines. All of these methods suffer from limitations (Table 1). For instance, some of these methods have low sensitivity (5–19), are less stable (6, 8, 9), require long standing for color development (5, 6, 18), or need extraction (19). The official methods suggested by various pharmacopeias (20–22) have been adopted worldwide for the assay of catecholamines; however, these methods are tedious and time-consuming for routine quality control. Consequently, we have developed simple, accurate, and highly sensitive spectrophotometric methods for the determination of MD, LD, DP, and pyrocatechol (PC) in pure and pharmaceutical preparations. The methods are based on measurement of the absorbances of tris(*o*-phenanthroline)iron(II) (method A) and tris(bipyridyl)iron(II) (method B) complexes at 510 and 522 nm, respectively.

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Experimental

Apparatus

A Hitachi Model U-2001 UV-Vis spectrophotometer with 1 cm matched quartz cells was used for spectral measurements.

Reagents

(**a**) *Standard catecholamine solutions.*—Aqueous solutions (1 mg/mL) of LD (Sun Pharmaceuticals Ltd., Baroda, Gujarat, India), MD (Indian Drugs and Pharmaceuticals Ltd., Hyderabad, Andhra Pradesh, India), DP (TTK Pharma Ltd. Chennai, Tamilnadu, India), and PC (AnalaR, Mumbai, Maharashtra, India) were prepared separately and stored in amber-colored bottles in a refrigerator. The solutions were diluted as needed.

(**b**) *Fe(III)-1,10-phenanthroline reagent (FPL).*—Prepared (23) by mixing 0.198 g 1,10-phenanthroline (PNL) with 2 mL 1M HCl and 0.16 g ferric ammonium sulfate dodecahydrate (FAS) and diluting with distilled water to 100 mL. This solution contains an Fe(III) concentration of 3.3182×10^{-3}M and a PNL concentration of 9.988×10^{-3}M.

(**c**) *Fe(III)-2,2′-bipyridyl reagent (FBL).*—Prepared (23) by mixing 0.16 g 2,2′-bipyridyl (BPL) with 2 mL 1M HCl and 0.16 g FAS and diluting with distilled water to 100 mL. This

Table 1. Comparison of proposed methods and reported for determination of catecholamines

Reagent used (ref.)	λ_{max}, nm	Beer's law limits, µg/mL	Molar absorptivity, L/mol/cm × 10^4	Remarks
Sulfamic acid in alkali (5)	540	10–50	0.42	Method has low sensitivity; required long time for complete color development
Cerium(IV)–3-methylbenzothiazoline-2-one hydrazone (6)	418	2–8	1.07	Method required long time for completion; has less stability
Tetrazolium violet chloride (7)	512	0.2–8.0	4.17	Method has low sensitivity
N-bromo succinimide–isoniazid (8)	480–490	0.8–16	0.396–0.838	Chromogen was less stable
Isoniazid in NaOH (9)	480	0.25–12	0.72–1.5	Chromogen was less stable and less sensitive
Ce(IV) nitrate (10)	510, 550	125–550	0.108–0.115	Method has low sensitivity
Nitration (11)	—	4–36	0.592	Method has low sensitivity
Thiosemicarbazide (12)	500	0.5–8	2.58	Method has low sensitivity
Molybdophosphoric acid (13)	600–900	—	2.4–3.1	Method has low sensitivity
Tetrazolium blue (14)	525	—	7.07	Method has low sensitivity
Chloranilic acid (15)	325	2–30	0.40	Method has low sensitivity
p-Aminoacetophenone (16)	440, 510	0.8–24	0.28–0.55	Method has low sensitivity
Sodium meta-periodate (17)	465–520	5.0–50	0.39–0.62	Method has low sensitivity
Folin and Ciocalteu phenol (18)	405–600	2.0–4.0	0.35–0.25	Chromogens were formed after long standing time
Chloranil (19)	325	2–30	0.4	Required extraction
FPL (present method)	510	0.02–1.25	11.3–23.5	Simple and more sensitive; does not involve extraction, and the chromogens were stable for >24 h
FBL (present method)	522	0.02–1.3	8.57–14.6	

solution contains an Fe(III) concentration of 3.3182×10^{-3}M and a BPL concentration of 1.024×10^{-2}M.

All chemicals used were analytical or pharmaceutical grade, and quartz-processed high-purity water was used throughout. Various commercial preparations were obtained from local sources.

Procedure

(**a**) *Assay of pure drugs*.—Aliquots of standard catecholamine solutions were transferred separately into a series of 10 mL calibrated flasks. To each flask was added 4 mL FPL (for LD and DP) in method A, 8 mL FPL (for MD and PC) in method A, 2 mL FBL (for LD and DP) in method B, 5 mL FBL (for MD) in method B, or 1 mL FBL (for PC) in method B. The contents were heated at 80°C on a water bath for 5 min for LD, 10 min for DP, and 15 min for MD and PC in method A, or 10 min each for LD and MD, 20 min for DP, and 15 min for PC in method B. The contents of each flask were cooled to room temperature and diluted to volume with distilled water. The contents were mixed well, and the absorbances of the red-colored complexes were measured at 510 nm (for method A) or at 522 nm (for method B) vs the corresponding reagent blank. Calibration graphs were plotted.

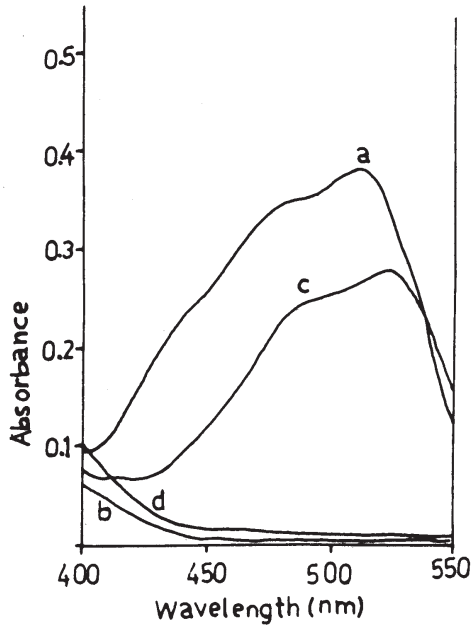

Figure 1. Absorption spectra of (a) the LD–FPL system, (b) the FPL reagent blank, (c) the LD–FBL system, and (d) the FBL reagent blank.

Table 2. Evaluation of the optical characteristics and precision and accuracy data for the proposed methods

Parameter	Method A				Method B			
	LD	MD	PC	DP	LD	MD	PC	DP
λ_{max}, nm	510	510	510	510	522	522	522	522
Beer's law limits, μg/mL	0.06–0.65	0.06–0.75	0.04–0.6	0.05–0.70	0.05–1.0	0.04–1.3	0.02–1.0	0.06–1.1
Molar absorptivity, L/mol/cm × 10^4	23.5	19.0	13.1	16.2	14.6	13.1	8.5	12.0
Sandell's sensitivity, ng/cm^2	0.8386	0.1245	0.8405	0.9502	1.34	1.81	1.28	1.26
Correlation coefficient	0.9991	0.9993	0.9992	0.9996	0.9995	0.9992	0.9990	0.9991
Regression equation, y^a								
Slope, a	0.6379	0.8802	0.9046	0.9046	0.5631	0.5369	0.7510	0.5891
Intercept, b	0.0483	0.01513	0.0515	0.0493	0.0178	0.0037	0.0483	0.0746
Relative standard deviation, %[b]	0.91	0.89	0.87	0.76	0.93	0.88	0.84	0.79
Range of error, % (at $p = 0.05$)[b]	0.94	0.79	0.80	0.87	0.74	0.71	0.79	0.88

[a] $y = ax + b$, where x is the concentration of the drug in μg/mL.
[b] Average of 5 determinations.

(**b**) *Assay of tablets*.—Twenty tablets each of LD and MD were finely powdered separately. An amount equivalent to 25 mg drug was weighed accurately and transferred to a 100 mL beaker. By using a mechanical stirrer, the powder was completely disintegrated and dissolved in distilled water, and the solution was filtered. The filtrate was diluted to 100 mL with distilled water, and an aliquot of the drug solution was analyzed as described above for the pure drug.

(**c**) *Assay of injection*.—DP injection solution was appropriately diluted with distilled water to obtain the required concentration of the drug, and an aliquot of the solution was analyzed as described above for the pure drug.

Results and Discussion

Ferric salts play a prominent role in the spectrophotometric determination of many pharmaceutical drugs. Acting as an oxidant, a ferric salt is reduced to the ferrous salt, which corresponds to the drug concentration. The drugs can be determined by the usual reagents for Fe(II) such as PNL and BPL. These properties were exploited in the present investigation for the spectrophotometric determination of catecholamines.

PC or its derivative (catecholamine) undergoes oxidation by the Fe(III) present in FPL (method A) or FBL (method B). The Fe(II) so formed readily combines with the PNL of FPL or the BPL of FBL to form a red-colored complex, the ferroin, $[Fe(phen)_3]^{2+}$ or $[Fe(bipy)_3]^{2+}$, with an absorption maximum at 510 or 522 nm, respectively. The reagent blank showed a negligible absorbance at the corresponding λ_{max}. The absorption spectra of the colored complexes for LD, a representative member of the selected drugs, are shown in Figure 1.

The optimum reaction conditions for the determination of the selected drugs were established by a number of preliminary experiments. The effects of each of the reagents were studied separately by measuring the absorbances of solutions containing a fixed concentration of catecholamine and various amounts of the reagent. Constant and maximum color development of the complex was achieved with 3 mL FPL (for LD and DP) or 7 mL FPL (for MD and PC) in a total volume of 10 mL, or with 1.5 mL FBL (for LD and DP), 4 mL FBL (for MD), or 0.75 mL FBL (for PC) in a total volume of 10 mL. Although a larger volume of the reagent had no effect on the formation, stability, or sensitivity of the complex, the absorbances increased slightly because of the background of the reagent. Thus, an FPL volume of 4 mL for LD and DP or 8 mL for MD and PC in a total volume of 10 mL, or an FBL volume of 2 mL for LD and DP, 5 mL for MD, or 1 mL for PC in a total volume of 10 mL was used to ensure complete reaction. The formation of the colored complex was slow at room temperature (25°C) and required a longer time for completion. Thus, efforts were made to accelerate the reaction by performing it at higher temperatures. We observed that maximum color intensity was obtained by heating the reaction mixture at 80°C on a water bath for 5 min for LD, 10 min for DP, and 15 min each for MD and PC in method A, or 10 min each for LD and MD, 20 min for DP, and 15 min for PC in method B.

Table 3. Analysis of pharmaceutical preparations containing catecholamines by the proposed methods and comparison with the official methods (20–22)

Drug formulation	Label claim, mg/5 mL or mg/tablet	Recovery ± SD, %[a]		
		Method A	Method B	Official method
		DP		
Injection 1	200	98.89 ± 0.84; F = 1.19; t = 1.28	99.12 ± 0.98; F = 1.61; t = 1.28	99.05 ± 0.77
Injection 2	200	99.53 ± 0.92; F = 1.30 ; t = 1.11	99.27 ± 0.81; F = 1.68; t =1.11	99.13 ± 1.05
		LD		
Tablet 1	500	99.43 ± 1.07; F = 1.38; t = 1.24	98.87 ± 0.72; F = 1.59; t = 1.24	98.14 ± 0.91
Tablet 2	250	99.13 ± 0.94; F = 1.22; t = 1.84	98.91 ± 1.04; F = 1.49; t = 1.78	99.61 ± 0.85
		MD		
Tablet 1	250	98.87 ± 1.12; F = 1.11; t = 1.21	99.64 ± 0.97; F = 1.19; t = 1.21	99.21 ± 1.06
Tablet 2	250	99.36 ± 0.74; F = 1.38; t = 1.73	98.91 ± 0.97; F = 1.24; t = 1.68	98.94 ± 0.87

[a] SD = standard deviation; each value is the average of 6 determinations.

The absorbances remained constant at room temperature for >24 h.

Beer's law limits, molar absorptivity and Sandell's sensitivity values, the regression equation, and the correlation coefficient for all the systems were evaluated (Table 2). The results were also subjected to detection of heteroskedacity. The graphical method was followed for this purpose wherein e_i^2 (squared error term) values were plotted versus estimated $\hat{y}_i$ values. The graph revealed no set pattern of relationship between e_i^2 and $\hat{y}_i$, thereby indicating the absence of heteroskedacity.

The precision of the proposed methods was excellent, as indicated by the low relative standard deviations (<1.0%) calculated from 5 replicate analyses for each drug.

The validity of the methods for the assay of catecholamines was assessed by investigating the effects of common excipients and other substances. We found that talc, glucose, starch, lactose, sulfate, dextrose, acetate, and magnesium stearate did not interfere in the determination.

To determine the accuracy and reproducibility of the proposed methods, recovery experiments were performed by mixing known quantities of each pure drug solution separately with definite amounts of pre-analyzed formulations and determining the total amount of the drug by following the procedure as described earlier. The amount of added drug was calculated by difference.

The proposed methods were successfully applied to the determination of catecholamines in tablets and injections (24). The results obtained were compared statistically, by means of the Student's t-test and by the variance ratio F-test, with those obtained by official methods (20–22). The Student's t-values at the 95% confidence level did not exceed the theoretical value, indicating that there was no significant difference between the results obtained by the official and those obtained by the proposed methods. We also observed that the variance ratio F-values calculated for $p = 0.05$ did not exceed the theoretical value (Table 3), indicating that there was no significant difference between the precision of the proposed methods and the precision of the official methods.

Conclusions

Unlike the instruments needed for spectrofluorimetric, chromatographic, and electroanalytical techniques, the instrument used in the proposed methods is simple and inexpensive. Moreover, the proposed methods are simple, accurate, and highly sensitive, compared with the reported methods (Table 1). Thus, the proposed methods could be used as an alternative to the existing methods for routine quality control.

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