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A new sensitive and selective spectrophotometric method for the determination of catechol derivatives and its pharmaceutical preparations

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Abstract

A sensitive and simple spectrophotometric method for the estimation of certain catechol derivatives like pyrocatechol (PCL), dopamine hydrochloride (DPH), levodopa (LDP), methyl dopa (MDP) and adrenaline (ADH) in either pure form or in its pharmaceutical formulation is described. The method is based on the interaction of diazotised *p*-nitro aniline (DPNA) with catechol derivatives in presence of molybdate ions in acidic medium. Absorbance of the resulting red complex is measured at 500–510 nm, respectively, and is stable for 2–10 h. The method is highly reproducible and specific for these selected catechol derivatives. The common excipients used as additives in pharmaceuticals and phenol, hydroquinone, resorcinol, pyrogallol and phloroglucinol do not interfere in the proposed method. Analytical data for determination of the pure compound is presented together with the application of the proposed method to the analysis of some pharmaceutical formulations. The results compare favourably with those of official and reported methods. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: 4-nitroaniline; Diazotisation; Catechol derivatives; Molybdate ion; Spectrophotometry

1. Introduction

Aromatic vic-diols are catechol derivatives in which 3 or 4 position is unsubstituted and these positions are sterically blocked. These derivatives are drugs and now, are widely used in the treatment of bronchial asthma, hypertension, Parkinson's disease myocardial infarction and cardiac surgery. Dopamine, a neuro-transmitter is one of the naturally occuring catecholamines and its hydrochloride salt is being used in the treatment of acute congestive failure and renal failure [1]. Parkinson's is a debilitating disease that is associated with deficiency of dopamine in the brain. Levodopa (LDP) is used to treat the disease because unlike dopamine, it readily crosses the blood brain barrier and it is decarboxylated in the brain to dopamine [2]. Methyl-dopa (MDP) is converted to 1-methyl dopamine and 1-methyl norepinephrine and used in the treatment of hypertension. Adrenaline (ADH) (epinephrine) is a

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very potent vasoconstrictor and cardiac stimulant [3]. On the basis of this background, the determination of trace amount of catecholamines is increasingly important. In view of their importance, considerable work has been done on their detection and quantification. Various methods like spectrofluorimetry [4,5], gas chromatography [6,7], high performance liquid chromatography

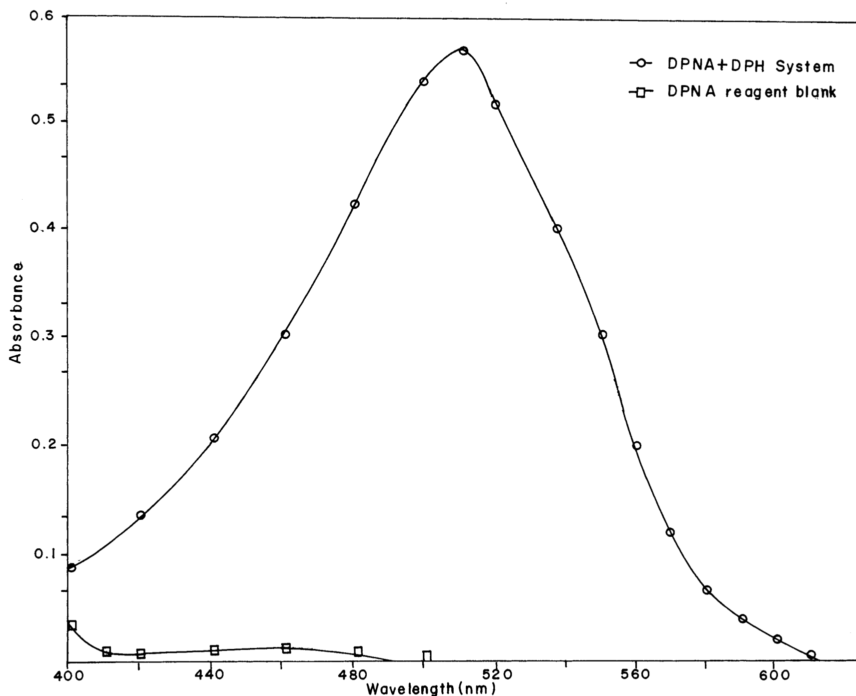

Fig. 1. Absorption spectra of DPH reaction product. Initial concentration of DPH was 2.0 μg ml^{-1}.

Table 1
Parameters for the spectrophotometric determination of catechol derivatives

Parameters	PCL	DPH	LDP	MDP	ADH
Colour	Red	Red	Red	Red	Red
λ max (nm)	500	510	510	510	510
Stability (h)	10	8	10	6	2
Beer's law range (μg ml^{-1})	0.05–3.2	0.05–3.4	0.1–4	0.25–9	0.25–7
Detection limit (μg ml^{-1})	0.03	0.03	0.05	0.15	0.15
Molar absorbtivity (l mol^{-1} cm^{-1})	5.42×10^4	5.39×10^4	3.74×10^4	2.2×10^4	2.13×10^4
Sandel's sensitivity (μg cm^{-2})	0.0020	0.0035	0.0052	0.0096	0.0085
Optimum photometric range μg ml^{-1})	0.1–2.8	0.2–3.0	0.5–3.5	0.8–8.0	8.0–6.0
Regression equation (Y)[a]	0.1109	0.0388	0.0410	0.0718	0.257
Slope (a) intercept (b)	0.037	0.011	−0.003	0.0031	0.0041
Correlation coefficient (r)	0.9990	0.9990	0.9958	0.002	0.9984
R.S.D. (%)[b]	0.78	0.98	1.21	1.41	1.58
% Range of error (95 % confidence limits)[b]	±0.24	±0.40	±0.31	±0.49	±0.51

[a] $Y=ax+b$, where x is the concentration (μg ml^{-1}) for catecholamine.
[b] calculated from five determinations ($n=5$).

Table 2
Effect of interfering ions and excipients on the determination of 2 μg ml^{-1} of catechol derivatives

Ions added	Tolerance limit (ppm)				
	PCL	DPH	LDP	MDP	ADH
Nitrate	5000	6000	4000	3000	5000
Chloride	800	300	900	850	250
Fluoride	1200	1400	1250	1210	800
Bromide	800	700	900	700	600
Sulphate	2000	3000	4000	4050	3000
Iodide	30	25	40	35	20
Oxalate	1500	1800	1600	1800	1400
Acetate	1300	300	500	400	500
Phosphate	80	70	90	70	60
Tartarate	500	600	800	400	800
Sulphite	1800	850	900	700	600
Dextrose	10 000	10 000	8000	9000	4000
Alginate	500	600	750	800	350
Ascorbic acid	16(80[a])	20(100[a])	24(120[a])	20(120[a])	10(50[a])
Phenol	5000	4000	3000	4000	4000
Resorcinol	400	350	400	450	250
Pyrogallol	400	350	400	550	200
Thiocyanate	120	100	140	160	100
Aluminium (III)	2000	2500	3000	4000	5000
Sulphide	400	400	500	400	250
Cobalt (II)	1000	2000	1500	1600	800
Zinc (II)	8000	8000	6000	8000	5000
Nickel (II)	5000	5000	4000	4000	3000
Cadmium (II)	4000	3500	4000	4500	2000
Fe(III)	80	60	100	120	25
Glucose	5000	6000	5000	4000	2500
Talc	5000	7000	6000	5000	4000
Starch	10 000	8000	7000	7000	6000

[a] Masked by Fe(III) ions.

(HPLC) [8–11], radio-immunoassay [12], chemiluminescence [13–15] and voltammetric determination [16,17] have been described in the literature for the determination of these compounds in various biological samples and pharmaceutical preparations. Most of these methods lack the simplicity needed for routine analysis. Many spectrophotometric methods have been proposed. Some of them either lack sensitivity and specificity [18,19] or require long heating [20] or involve non-aqueous media [20]. Some other spectrophotometric methods have very narrow limits of detection [21].

Almost all spectrophotometric methods based on the application of different oxidising agents on oxidation of catechol derivatives to *o*-benzoquinone [22,23] or oxidation followed by coupling with compounds having electron-donating groups [24,25], suffers from several disadvantages of critical oxidant concentration, instability of the coloured species, lack of sensitivity, and requires heating or extraction. D.W Barnum has reported a method [26] for the spectrophotometric determination of aromatic vic-diols by nitration with sodium nitrite in the presence of molybdate or tungstate ions in buffer medium. This method suffers from close control of pH, the optimum time for nitration (2–40 min) depends on the diphenol, the absorbance measurements were made within 20 min after adding sodium hydroxide and application in pharmaceuticals have not been reported. Only one diazotisation method has

been reported for the determination of dopamine by the formation of an azo red compound with sulphamic acid in the presence of alkali [27]. The reported broad Beer's law range is 10–50 μg ml^{-1} with low molar absorptivity 4.2×10^3 1 $mole^{-1}$ cm^{-1} Peroxidase based spectrophotometric method has been used for the determination of ascorbic acid, norepinephrine, epinephrine, dopamine and levodopa [28]. The method is based on the inhibitory action of the named compounds on oxidative coupling of *p*-chlorophenol and 4-amino antipyrine at pH 6.6, in the presence of hydrogen peroxide (H_2O_2) and horse-radish peroxidase (HRP) catalyst. Calibration graphs were linear in the range of 0.075–20 μg ml^{-1}. Berzas et al. [29] has reported stopped flow spectrophotometric determination of dopamine and MDP. The method is based on the aerial oxidation in sodium hydroxide at 60°C and determination of variation in absorbance at 360 nm at 30 s, after injection of the sample, calibration graphs were linear upto 4000 μg ml^{-1}. The present communication reports on the investigations based on the interaction of DPNA in the presence of molybdate ions with catechol derivatives in acidic medium. This method offers the advantages of simplicity, rapidity, specificity without the need of extraction or heating besides having higher sensitivity range than most of the existing spectophotometric methods. Moreover, the method is totally free from the twin disadvantages of interference and instability of the coloured species, typical of most spectrophotometric methods.

2. Experimental

A JASCO MODEL UVIDEC-610 UV–VIS spectrophotometer with 1.0 cm matched cells was used.

Pharmaceutical grade DPH, PCL, LDP, MDP and ADH were all purchased from Sigma (USA) and were used as working standards without further treatment. Molybdic acid and *p*-nitroaniline were purchased from Merck (Darmstadt, Germany) All other reagents and solvents were of analytical grade. Commercial dosage forms were purchased from local sources.

Table 3
Determination of catechol derivatives in pharmaceutical preparations

Drug	Label claim (mg)	% Recovery[a] ± % R.S.D.		
		BP method	Reported method [b]	Proposed method
Injections				
DPH[c]	200/5 ml	98.85 ± 1.25	99.04 ± 1.10	99.90 ± 0.42
DPH[d]	200/5 ml	99.20 ± 0.82	100.09 ± 0.99	99.85 ± 0.75
ADH[e]	200/5 ml	99.40 ± 0.55	99.30 ± 0.80	98.85 ± 0.65
ADH[f]	200/5 ml	100.00 ± 0.90	99.9 ± 0.49	99.4 ± 0.95
Tablets				
LDP[g]	500	98.78 ± 1.10	97.83 ± 0.96	99.80 ± 0.90
MDP[h]	250	98.78 ± 1.10	Not reported	98.84 ± 1.02

[a] Average of six determinations. R.S.D. relative standard deviation ($n = 6$).
[b] Reported by EL-Kommos et al.
[c] Marketed by TTk Pharma.
[d] Marketed by Trioka Paren.
[e] Marketed by Harson Laboratories.
[f] Marketed by Wallace Pharma.
[g] Marketed by Sun Pharma.
[h] Marketed by Merind Pharma.

2.1. Solutions

Stock solutions of DPH, PCL, LDP, MDP and ADH were prepared by dissolving the pure substances in water at concentration between 10 and 25 μg ml^{-1}. The resulting solutions were standardised by the reported method [30]. The solutions of 0.5% *p*-nitroaniline in 1 M HCl, 1% aqueous sodium nitrite, 2% aqueous sulphamic acid and 1 M hydrochloric acid were used. A 2% molybdic acid (dissolved in 2 ml of 5 M sodium hydroxide and neutralised with hydrochloric acid to get clear solution) was freshly prepared.

2.2. Procedure

About 1.0 ml of *p*-nitroaniline working solution was transferred into each series of 25 ml graduated flasks and 1.0 ml of 1 M HCl was added to each. After cooling in an ice bath, 1.0 ml of sodium nitrite was added with swirling. The solutions were allowed to stand for 5 min, then 2.0 ml of sulphamic acid solution was added. The solutions were swirled and allowed to stand for 5 min. Then, 2.0 ml of molybdic acid was added. Aliquots of standard solution of PCL (1.25–80 μg), DPH (1.25–85 μg), LDP (2.5–100 μg) MDP (6.25–225 μg), and ADH (5–175 μg) were added to the flask and left for 10 min to develop pink precipitate. The solution was made upto the mark with 1:1 sulphuric acid and mixed thoroughly. The absorbance was measured at 500–510 nm against the corresponding reagent blank and calibration graphs were constructed.

2.3. Procedure for assay of catechol derivatives in commercial samples

Twenty tablets were weighed and finely powdered. The powder amount equivalent to 50 mg of LDP or MDP (for injection of DPH or ADH, an appropriate volume of the sample) was dissolved in water and filtered. The filtrate was made upto 100 ml and appropriate aliquots of the drug solutions were treated as described above for the determination of DPH, ADH, LDP or MDP.

3. Results and discussion

The method involves the reaction of catechol derivatives with diazotised *p*-nitroaniline in hydrochloric acid medium in the presence of molybdate ions to produce pink coloured precipitate, which turns to clear red colour after dilution with 1:1 sulphuric acid. The factors affecting the colour development, reproducibility, sensitivity and adherence to Beer's law were investigated with DPH as the model compound, since the other catechol derivatives behave similarly to it.

3.1. Spectral characteristics

A pink coloured precipitate is formed when DPH was allowed to react with DPNA in the presence of molybdate ions in hydrochloric acid medium, which turns to red coloured product with maximum absorption at 510 nm as shown in Fig. 1. The colourless reagent blank has practically negligible absorption at this wavelength.

3.2. Optimum reagents concentration

For the diazotisation coupling reaction, the use of hydrochloric acid as reaction medium was found to give more satisfactory results than sulphuric acid solution. It was found that 1 M solution of hydrochloric acid in the range of 0.5–1.5 ml, 1% solution of sodium nitrite in the range 0.5–2 ml, 2% solution of sulphamic acid in the range of 1–4 ml and 2% molybdate ion solution in the range of 1.5–3.5 ml were necessary to achieve maximum colour intensity. Hence, 1.0 ml of hydrochloric acid, 1.0 ml sodium nitrite, 2.0 ml sulphamic acid, were selected for diazotisation. A 2.5 ml of molybdate ion was used as complexing agent with catechol derivatives in the presence of DPNA to develop pink coloured precipitate. The excess of nitrite during diazotisation could be removed by the addition of sulphamic acid solution. An excess of sulphamic acid has no effect on colours.

Dilution of the pink coloured precipitate with different solvents like water, methanol, ethanol, acetic acid, hydrochloric acid, sulphuric acid and acetonitrile have been tested. Results showed that

1:1 sulphuric acid dissolves the pink coloured precipitate and turns to red colour with maximum intensity and stability of the colour.

3.3. Quantification

Table 1 shows the linear calibration ranges and equation parameters for this method. Separate determinations at different concentration levels of each drug gave coefficient of variation that did not exceed 2%.

3.4. Reaction sequence

In an acidic medium, nitrite reacts with *p*-nitroaniline to form diazonium salt. The salt is then coupled with catechol derivatives in the presence of molybdate ions to yield pink azodye complex, which turns to red coloured product, when dissolved in 1:1 sulphuric acid with maximum absorption at 500–510 nm.

3.5. Stability

The diazotisation of *p*-nitroaniline is complete within 10 min at room temperature. The diazotised salt is then stable for about 5 h, when kept in an ice-bath. The pink coloured product is formed immediately after the addition of the drugs in the presence of molybdate ions. However, 10 min is sufficient to complete the reaction. The azo product resulting from the suggested method was studied at different temperatures. It was found that the absorbance values remain constant in the temperature range 5–60°C. The red azo product was stable for about 2–10 h and results were reproducible.

3.6. Stability of catechol derivatives in stock solution

The stability of 1.0 mg ml^{-1} of PCL, DPH, LDP, MDP and ADH stock solutions were monitored for 2 weeks. The stock solutions were stored in dark bottles at room temperature. The appropriate amount of solutions (10–25 μg ml^{-1}) were sampled and analysed four times during 2 weeks period. The results obtained were compared using the analytical procedure. There was no difference between the results obtained with fresh and 2 weeks old samples. However, only DPH stock solution develops blackish brown colour on exposure to sunlight for more than 24 h.

3.7. Interference

The extent of interference by common anions and other substances were determined by measuring the absorbance of a solution containing 2 μg ml^{-1} of catechol derivatives and various amounts of diverse ions. An error of 2.5% in the absorbance readings was considered tolerable. The substances that are tested and tolerance limits found are presented in Table 2. No interferences were observed in the determination of studied drugs in the presence of common excipients of the injections and tablets (e.g. sodium chloride, starch, talc, glucose and alginate). The influence of some ions on the determination of catecholamine drugs was tested. The ions Al(III), Zn(II), Co(III), Fe(III) and Cd(II) did not interfere but Cu(II), Ag(I) and CN^- interfere seriously. Ag(I) and CN^- forms white precipitate instead of pink precipitate and Cu(II) decreases the colour intensity. Oxidizing agents like $Cr_2O_7^{2-}$ Ce^{4+}, Chloramine-T, *N*-bromosuccinimide etc, seriously interfere without allowing the reaction to occur.

3.8. Application

The reproducibility of the method was checked by ten replicate determination at 2 μg ml^{-1} level of DPH and the standard deviation (S.D.) was found to be between 0.8 and 1.2%. The present method has been applied for the analysis of catechol drugs in various pharmaceutical preparations. The results of tablets and injections are presented in Table 3 and compared favourably with those reported by EL-Kommos et al. [31] and official method [30]. The proposed method is highly sensitive and gives reproducible results. The results of the method is compared with the other reported methods. Table 4 clearly indicates the proposed method to be highly sensitive and free from interferences of I^-, SO_3^{2-} and ascorbic acid, as reported in other methods.

Table 4
Comparison of visible spectrophotometric methods for the determination of catechol derivatives

Serial number	Reagent	Coloured species	Drug analysed	λ max (nm)	Range of determination (ppm)	ε (l mol^{-1} cm^{-1})	Remarks	Reference
1.	Nitration in the presence of molybdate or tungstate	Nitration product	PCL, DPH, LDP, ADH	465–520	Not reported	$0.89–1.39\times10^4$	Required 50 min to complete the reaction, readings are taken within 20 min	[26]
2.	Nitration in the presence of molybdate and CDTA	Nitration product	ADH	475	1.5–22	3.5×10^3	Wait for 1 h to develop colour	[19]
3.	Thiosemicarbazide	Coupled product	DPH	504	1–8	2.4×10^4	Required 45 min to complete the reaction	[33]
4.	Periodate in the presence of molybdate and sulphanilamide	Coupled product	ADH	520	0.2–1.2	9.6×10^4	Narrow Beer's law range, DPH, PCL, LDP MDP will not give the colour	[34]
5.	Isoniazid in the presence of NBS	Coupled product	PCL, DPH, LDP, MDP	480–490	0.8–17	$0.36–1.27\times10^4$	Vitamin C interfere seriously	[23]
6.	Barbituric acid	Coupled Product	MDP	540	10–250	2.9×10^3	Heating at 100°C for 20 min	[18]
7.	3-Methylbenzothia zoline-2-one hydrazone (MBTH)	Coupled product	MDP	593	10–35	Not reported	Tartrazine interfere	[35]
8.	Meta periodate	Aminochrome of oxidative cyclisation	DPH, LDP	465–520	5–45	$3.9–6.25\times10^2$	Heating at 60 ± 5°C in ethanol medium	[31]
9.	Polyphenol oxidase enzyme	Oxidation product	DPH, MDP	470–480	38–1400	Not reported	I^-, SO_3^{2-} interfere seriously, less sensitive and complicated procedure	[32]
10.	Diazotised with sulphamic acid in alkali	dye	DPH	540	10–50	4.2×10^3	Wait for 10 min to develop colour	[27]
11.	DPNA in the presence of molybdate	Dye complex	PCL, DPH, LDP, MDP, ADH	500–510	0.05–9	$5.42–2.13\times10^4$	Wait for 10 min to develop colour	This Work

4. Conclusions

The proposed method is found to be simple, selective and highly sensitive than any reported methods on DPH. The statistical parameters and the recovery study data clearly indicate the reproducibility and accuracy of the method. Thus, the method can be adopted as an alternative to the existing methods.

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