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Calix[6]arene derivative as chromogenic sensor for anti-hypertensive drugs

S K Menon*, P Jose, U Harikrishnan & U Pal
Department of Chemistry, School of Sciences, Gujarat University
Navrangpura, Ahmedabad 380 009, India
Email: sobhanamenon@rediffmail.com

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A simple, rapid, and sensitive spectrophotometric method has been developed for the determination of atenolol, propranolol hydrochloride and metaprolol tartarate. The method is based on the reaction of these drugs as n-electron donors with acceptor groups on macrocyclic ring of calixarene. Due to the rapid development of color at ambient temperatures, the chromogenic calix[6]arene derivative can be used for the determination of these β-adrenergic blocking drugs. The association constants (K_c^{AD}) and free energies for the complexes have been determined. The proposed method can be used to determine the drugs in pharmaceutical tablets and urine.

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Anti-hypertensive (β-adrenergic blocking) drugs, such as atenolol, propranolol hydrochloride, metaprolol tartarate, and methyl dopa are chemical agents that exert their principle pharmacological and therapeutic effects by acting at peripheral sites of the sympathetic nervous system[1]. β-Adrenergic blocking drugs are used mainly in angina pectoris, certain arrhythmia, systematic hypertension, and other cardiovascular disorders such as arterial fibrillation, myocardial infarction, and sinus tachycardia[2].

Several methods have been described for the quantitative determination of the β-adrenergic blocking agents including spectrophotometric[3-8], flourimetric[9,10] chromatographic[11-17] and electrochemical methods[18-21]. However, no method is reported using macrocyclic compounds like calixarenes, cyclodextrins, crown ethers, etc. These macrocyclic compounds have been extensively used to separate alkali, alkaline earth cations, heavy metals, and amines either by solvent extraction or by liquid membranes[22-24].

We report herein a simple, sensitive, accurate and precise spectrophotometric method for the determination of atenolol, propranolol hydrochloride, metaprolol tartarate via reaction of these drugs with coumarin calix[6]arene hydroxamic acid (CoCHA). Calixarenes are popular host molecules for small organic molecules. But till now, there have been very few reports of their inclusion complexes with drug molecules[25,26].

Experimental

Absorbance measurements were made on Hitachi 3210 UV-vis spectrophotometer with matched 10 mm quartz cells. *p*H measurements were made on Systronics *p*H-meter model 335 equipped with glass and calomel electrodes.

All solvents used were obtained from E. Merck (AR Grade) and used without further purification. Atenolol, propranolol hydrochloride and metaprolol tartarate were obtained as gift samples from Cadila Pharma, Ahmedabad. The standard stock was prepared by dissolving 200 mg of drug in 50 mL ethanol in a standard flask. Working solutions were subsequently prepared by appropriate dilution of the stock solution. CoCHA reagent solution (0.01%) solution was prepared in dichloromethane.

The nitro benzyl ester derivative of calix[6]arene was synthesized by stirring together methyl calix[6]arene (21.6 g, 0.03 mol) in dioxane(100 mL) and *p*-nitrobenzoyl chloride (5.6 g, 0.03 mol) in dichloromethane(20 ml) at 15-20°C for about 1 h. The yellow precipitate obtained was collected by filtration. Yield 12 g (75%).

For the partial reduction, nitro ester derivative of methyl calix [6] arene (12 g, 0.007 mol) was dissolved in dioxane:water (8:2, *v/v*); 4-5 g of Raney nickel (W-2) was added, followed by dropwise addition of hydrazine hydrate (80%) (20 mL, 0.41 mol). The reaction mixture was stirred below 0°C for 1 h, filtered and used directly for further synthesis. The partially reduced nitro ester derivative of calix[6]arene, thus prepared, was coupled directly with coumarin-3-carbonyl chloride to give the final compound, CoCHA. It was recrystallized using chloroform-heptane mixture. Yield 3 g (41%), m.pt.>300°C (decom.). (Found: C 68.25, H 4.03, N 4.30. Calc. for $C_{110}H_{78}O_{28}N_6$: C 68.39, H 4.04, N 4.35%). (KBr)/cm^{-1} 3200, 1700, 1620, 1580, 1370. $_H$ ($CDCl_3$) 10.3(2H, s, N-OH), 6.74-7.98 (46H, m, aromatic protons), 4.81 (4H, d, CH_2), 4.2 (4H, d, CH_2), 3.8 (2H, d, CH_2), 3.5 (2H, d, CH_2), 2.8 (6H, s, CH_3), 2.5 (12H, s, CH_3), *m/z* (FAB) 1930(M^+).

An aliquot of the sample solution (0.5-20 μg of atenolol, 5-120 μg of propranolol hydrochloride and 2-150 μg of metaprolol tartarate) was transferred to 10 mL volumetric flask and mixed with 5 mL of the 0.01% reagent solution. *p*H of the solution was maintained at 5-6 using appropriate buffers. The colored solution was diluted to the mark with dichloromethane and measured at 440 nm, 427 nm, and 432 nm for atenolol, propranolol hydrochloride, and metaprolol tartarate, respectively, against the reagent blank.

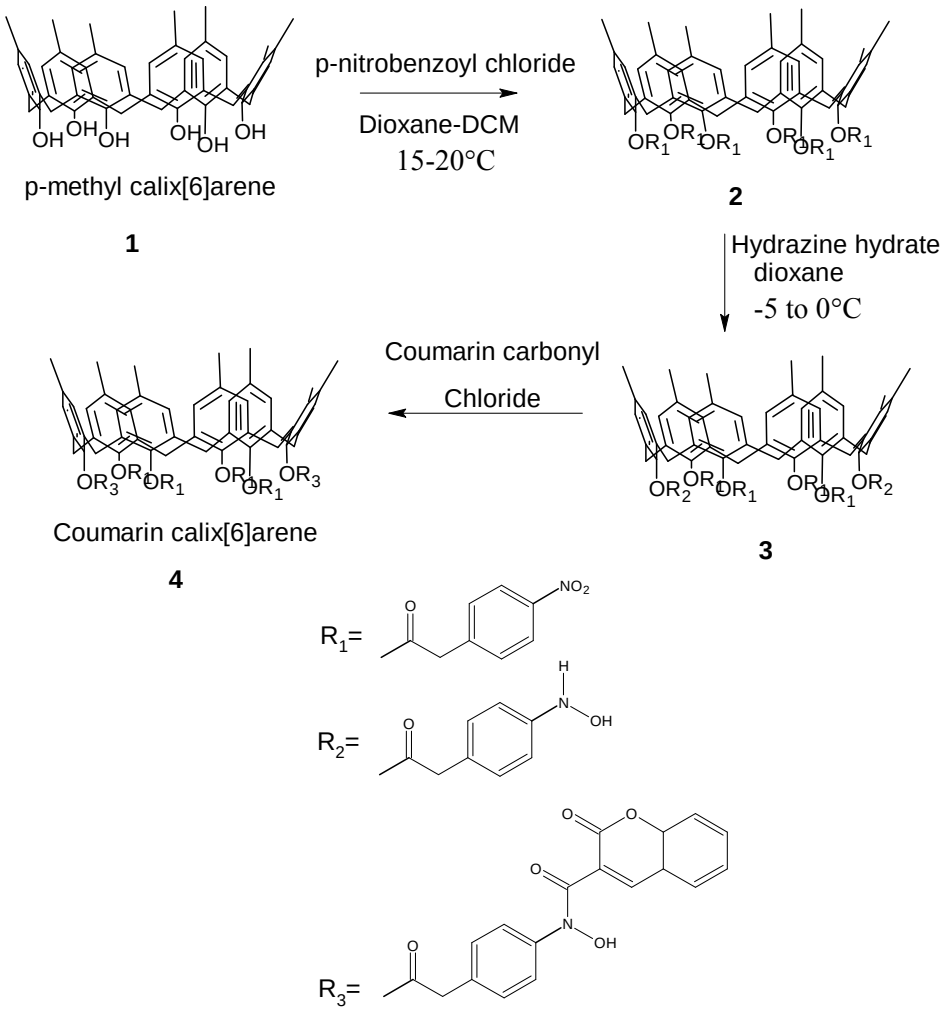
A quantity of the powdered tablets equivalent to 50 mg of drug was dissolved in ethanol and filtered to remove the insoluble contents like binders and other excipients.

Urine samples were collected during 48 h after the oral intake of a 100 mg single dose of each drug. Drug-free urine was also collected from two volunteers (male and female). Both types of samples were vortexed at 3800 rpm for 15 min and frozen until preparation for analysis. For the analysis of the urine samples, 10 mL of the sample was allowed to dry at ambient temperature and the residue was dissolved in ethanol for subsequent use.

Results and discussion

CoCHA was synthesized by the coupling of the partially reduced nitro ester derivative of methyl calix[6]arene with coumarin carbonyl chloride (Scheme 1). The synthesized compound CoCHA was characterized by its elemental analysis, FT-IR, ^{1}H NMR and FAB-MS.

The molecular interactions between electron donors and acceptors are generally associated with the



Scheme 1

Table 1—Comparative study of present method with other methods

Reagent	Molar absorptivity (L mol^{-1} cm^{-1})		
	Atenolol	Propranolol	Metaprolol
Hydroxamic acid[3]	5.3×10^2	-	-
Promethazine hydrochloride[31]	-	1.36×10^4	-
Methdilazine hydrochloride[31]	-	2.55×10^4	-
Chelation with Cu in CS_2[32]	-	6.89×10^3	1.08×10^4
Chloranil[6]	6.65×10^3	1.86×10^4	1.80×10^3
Iodine[6]	3.75×10^3	6.89×10^3	7.12×10^3
Bromanil[6]	1.05×10^4	1.04×10^4	1.36×10^4
Tetracyanoethylene[6]	4.88×10^3	3.96×10^3	7.53×10^3
7,7,8,8- Tetracyanoquinodimethane[6]	1.40×10^4	5.83×10^3	1.29×10^4
2,3-Dichloro-5,6-dicyano-1,4 –benzoquinone[6]	9.25×10^3	2.28×10^4	1.59×10^4
CoCHA (present method)	5.2×10^5	2.3×10^5	4.38×10^5

Table 2—Various parameters of the proposed method

Parameter	Atenolol	Propranolol	Metaprolol
Color developed	Red-orange	Yellow	Orange-yellow
Working *p*H	5-6	5-6	5-6
λ_{max}, nm	440	427	432
Beer's law range, μg/mL	0.05-2	0.5-12	0.2-15
Detection limit, μg/mL	0.023	0.18	0.076
Quantification limit, μg/mL	0.071	0.55	0.23
Molar absorptivity, l $mol^{-1}cm^{-1}$	5.2×10^5	2.3×10^5	4.38×10^5
Sandell sensitivity, μg cm^{-2}	0.0005	0.0001	0.0006
Regression equation[a]			
Intercept, a	- 0.0518	- 0.0089	- 0.0021
Slope, *b*	0.2035	0.0804	0.0788
Correlation coefficient	0.9990	0.9995	0.9991

[a]$A = a + b \,.\, C$, where A is absorbance and C is concentration in μg/mL

formation of intense colored charge transfer complexes, which absorb in the visible region. The photometric methods based on these interactions are usually simple and convenient because of the rapid formation of the complexes[27]. β-Adrenergic drugs are good non-bonding electron donors and form charge transfer complexes with σ or π-acceptors. The interaction of all studied drugs with CoCHA in dichloromethane at room temperature gave colored charge-transfer complexes. There was a complete one-electron transfer from the drug donor to the acceptor CoCHA.

There was an immediate change of the pale yellow color of the reagent in dichloromethane (409 nm) to orange-red upon addition of atenolol (440 nm), propranolol hydrochloride (427 nm) or metaprolol tartarate (432 nm). The π-π stacking interactions due to the aromatic rings attached to the drug molecules and CoCHA, and the strong hydrogen bonding by the hydroxamic acid moiety provide stability to the complex. A comparative study of this method with

Table 3—Evaluation of the accuracy and precision of the proposed method

Drugs	Conc. (μg/mL)	Recovery (%)	RSD[a] (%)	Lit. method[28]
Atenolol	0.5	100.10	0.90	100.20
	1.0	98.90	0.89	99.90
	1.5	99.00	0.94	100.10
	2.0	100.14	0.91	100.04
Propranolol	1.0	99.96	0.87	100.00
	2.0	98.99	0.88	98.97
	3.0	100.01	0.90	100.03
	4.0	99.90	0.84	99.96
Metaprolol	2.0	99.02	0.95	100.02
	4.0	99. 03	0.94	99.09
	6.0	99.01	0.92	100.00
	8.0	100.14	0.89	100.09

[a]Average of six determinations

Table 4—Physical parameters of the proposed method

Drug	Association constant/ formation constant	Free energy ΔG (kJ/mol)
Atenolol	3.8×10^2	-15 .0
Propanolol	3.4×10^4	-32.05
Metaprolol	3.8×10^3	-26.48

Table 5—Analysis of the formulations

Drug	Preparation	Nominal value (mg/tablet)	Recovery (%)	RSD[a] (%)	Lit. method[28]
Atenolol	Tenormin (Nicholas Piramal)	50	99.0	0.89	100.14
	Hipres-50 (Cipla)	50	98.5	0.78	98.00
	Coronol 50 (Sarabhai Chemicals)	50	99.0	0.43	99.30
Propranolol	Ciplar LA-40 (Cipla)	40	99.9	0.89	100.08
	Inderal (Nicholas Piramal)	40	101. 0	0.86	101.80
	Corbeta Sarabhai (Chemicals)	40	100.2	0.58	100.3
Metoprolol	Metolar –H (Cipla)	50	100.8	0.12	101.0
	Betaloc (Astra Zeneca)	50	100.1	0.91	100.6
	Lopresor (Novartis)	50	99. 9	0.89	100.0

[a] = average of six determinations; RSD=Relative standard deviation

Table 6—Analysis of urine samples

Drug	Sample	Amt. (mg)	RSD[b] (%)	Lit. method[28]
Atenolol	1	1.6	1.5	1.7
	2	2.8	0.6	2.8
	3	2.9	0.9	2.9
	4	1.8	0.6	1.9
	5	2.9	0.7	2.9
Propanolol	1	3.6	1.5	3.7
	2	3.8	0.6	3.8
	3	3.9	0.5	3.9
	4	3.8	0.6	3.8
	5	2.9	0.9	2.9
Metoprolol	1	2.6	1.5	2.7
	2	2.8	0.8	2.8
	3	1.9	0.7	1.9
	4	2.8	0.6	3.8
	5	2.9	0.9	3.9

[b]Average of six determinations

other reported methods is as shown in Table 1. All assays in the proposed method are performed in the visible region and do not have interferences from extracted excipients of dosage forms.

The complexation of the anti-hypertensive drugs was studied under the optimum conditions of *p*H, nature of solvent, and reagent concentration. Optimum *p*H range for complexation was found to be 5.0-6.0. Beyond this *p*H range, complexation of the drugs was incomplete. Common organic solvents like chloroform, dichloromethane, ethanol and carbon tetrachloride were studied. But, optimum absorbance was found when dichloromethane was used.

The amount of reagent needed to obtain maximum and reproducible absorbance was 5 mL for atenolol, 7 mL for propanolol and 5 mL for metoprolol tartarate.

The general spectral characteristics like Beer's law range, molar absorptivity, Sandell's sensitivity, regression equations, and correlation coefficients obtained by the linear treatment of the results are given in Table 2. The limit of detection and limit of quantification were calculated from the standard deviations of the absorbance measurements obtained from a series of 13 blank solutions for each procedure.

In order to compare the accuracy and precision of the proposed method with the official method, six replicate analyses were performed on four standards. The values obtained for the students t-test and the variance ratio F-test are less than the critical values suggesting that the proposed method is precise and accurate as the USP methods[28] (Table 3).

Interference studies

In order to examine the selectivity of the proposed method to pharmaceutical preparations and urine samples, effect of common additives, adjutants and excipients (glucose, lactose, talc, magnesium stearate and starch) as well as albumin (excreted in the urine) was experimentally studied. An error of not more than ±3 % in the absorbance was taken as interference. The results show that the common additives and excipients cause no interference in the proposed method.

Physical parameters

The association constants were calculated for the interaction of each drug with CoCHA using Benesi-Hildebrand equation[29].

The standard free energy changes of complexation (ΔG^{o}) were calculated from the association constants[30].

The association constants and the free energy reveal that the complexes formed with the drugs are stable (Table 4). The high values of association constants are common in n-electron donors where the intermolecular overlap may be considerable.

Analysis of pharmaceutical dosage forms and urine samples

The results were compared statistically with those obtained by applying the literature method[28] (Tables 5 and 6). The percentage recoveries were in the range of 98-100 implying that the method can be satisfactorily used for the spectrophotometric determination of the studied drugs.

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