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Molecular recognition of aromatic amines by coumarin substituted azacrown ether

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New chromogenic azacrown ether N[7-hydroxy-4-methyl-coumarin-8-methylene]-4-azadibenzo-18-crow-nether(HMCMADCE) was synthesised through the condensation reaction of 4-azadibenzo-18-crown-6ether, 4-methyl,7-hydroxy coumarin and formaldehyde was used to investigate its behaviour towards the aromatic amines. All the aromatic primary amines gave specific colours in aqueous medium. The functional group recognition behaviour is further extended to study the estimation of the aromatic primary amine in commercial drug samples. The amines can be estimated in the presence of several functional groups like phenolic-OH, secondary and tertiary aromatic amines as well as aliphatic amines.

Key Words: Chromogenic azacrown ether, primary aromatic amines, drug analysis, spectrophotometry.

Introduction

The chemistry of cation complexation with crown ethers and its analogues, such as podands, azacrowns, cryptands, has been well established.[1−2] However, studies focusing on the interaction of these macrocycles with neutral organic molecules have also been gaining importance. Aromatic primary amines are an active functional group of many drugs. There are reports of interaction of protonated amines with crown ethers and their analogues.[3−5] Separation and detection of amino compounds have been reported using many sophisticated and expensive instrumental techniques, such as HPLC,[6−7] HPTLC,[8] Capillary gas chromatography,[9−11] liquid-liquid-liquid microextractions (LLLME),[12−13] and solid phase extractions.[14] Potentiometric determination of amines by ion chromatography using macrocycle-based liquid membrane electrodes has been reported by Nagels and Poels.[15] Separation of 8 amino compounds by capillary electrophoresis (CE) in non-aqueous media has

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been examined.[16] Although many methods are reported for the separation and estimation of various amines by chromatographic or extraction techniques using crown ethers, there is still a need to develop simpler methods of detection and determination.

The present work demonstrates the ability of functionalised azacrown ethers (N [7-hydroxy 4-methyl-coumarin-8-methylene] 4-aza dibenzo 18-crown-6-ether) for molecular recognition of primary aromatic amines by change in the colour in aqueous media. This provides a simple, sensitive, and inexpensive method for the estimation of primary aromatic amines. There was no interference by secondary or tertiary amines. Various primary aromatic amines, such as aniline, toluidine, nitro anilines, and anthranilic, were studied. The study is further extended for the analysis of medicinal agents having primary amine group in the moiety. The method was applied to estimate primary amines in expectorant bromohexine hydrochloride, food supplement folic acid, anti-emetic meclopramide, and antibacterials, such as sulphadiazine, sulfaguanidine, trimethoprim and antileprotic dapsone.

Experimental

Apparatus

FT-IR spectra were recorded on a JASCO/410/FTIR spectrophotometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a DRX 300 spectrometer operating at 300 MHz, in $CDCl_3$ with TMS as internal standard. The FAB mass spectra were recorded on a JEOL SX 102/DA-6000 mass spectrometer/data system using argon/xenon (6 kV, 10 mA) as the FAB gas. *m*- Nitrobenzyl alcohol (NBA) was used as the matrix and the matrix peaks appeared at m/z 136, 137, 154, 289, and 307.

pH measurements were made on a Systronics pH-meter model 331 equipped with glass and calomel electrode. A Hitachi 3210 UV-Visible spectrophotometer with matched 10 mm quartz-cell was used for spectral measurements.

Chemicals and reagents

All reagents and solvents used are of A.R. grade of BDH and E.Merck unless otherwise specified. Buffer solutions are prepared as described elsewhere[17] and 0.1% reagent solution was prepared in ethanol.

Amine solutions

A standard 0.01 M solution of various aromatic amines was prepared by dissolving appropriate amount in 5 mL 1 M hydrochloric acid and diluted to 250 mL with distilled water. The concentration of the solution was determined by titrimetry in non-aqueous solvent using perchloric acid in glacial acetic acid and end point determined by potentiometry.[18]

Drug samples

The standard samples of bromhexine hydrochloride, dapsone, folic acid, metoclopramide, sulphadiazine, sulfaguanidine, and trimethoprim were prepared by dissolving appropriate quantities and standardized.[19]

The tablets are finely powdered and dissolved in ethanol and filtered. The filtrate is then diluted to 50 mL with ethanol and used.

Synthesis of reagent

Synthesis of N-tosyl 4-aza dibenzo 18-crown-6

To the mixture of bis[2-(o-hydroxy-phenoxy) ethyl]ether,[20] 29 g (0.1 mol) in 500 mL acetonitrile and powdered potassium carbonate 28 g (0.2 mol), solution of ditosylate of N-tosyldiethanolamine[21] 19 g (0.1 mol) in 100 mL acetonitrile was added in 3 h. The mixture was refluxed for 18 h. The solvent was removed in vacuo and the product was dissolved in hot ethanol, filtered, and triturated. White powder thus obtained was recrystallized in ethanol and gave 20 g (63%) of white solid with mp 208-209 °C. The results obtained for elemental analysis of $C_{27}H_{31}NO_7S$ (MW. 513) C, 63.12; H, 6.05; N, 2.71; S, 6.25% is comparable to the calculated values of C, 63.14; H, 6.08; N, 2.73; S, 6.24%.

Detosylation of N-tosyl 4-aza dibenzo 18-crown-6

A mixture of N-tosyl 4-aza dibenzo 18-crown-6 and 10 g (19 mmol) 10 mL 4 M sulphuric acid was heated at 60 °C for 30 min. The mixture was neutralized with saturated sodium bicarbonate solution. Precipitated product was washed thoroughly with water and on recrystallization from ethanol gave 4.0 g (57%) of white solid with mp 170-172 °C.[24] The results obtained for elemental analysis of $C_{20}H_{25}NO_5$ (MW. 359) C, 66.82; H, 7.00; N, 3.91% is comparable with the calculated values of C, 66.83; H, 7.01; N, 3.90%.

Synthesis of (N [7-hydroxy 4-methyl-coumarin-8-methylene] 4-aza dibenzo 18-crown-6-ether) (HMCMADCE)

To the solution of 4-aza dibenzo 18-crown-6 ether 2.0 g (2.78 mmol) in 50 mL tetrahydrofuran (THF), 7-hydroxy 4-methyl coumarin 0.49 g (2.78 mmol) in 5 mL THF was added and followed by 0.22 g (2.78 mmol) formaldehyde. The mixture was stirred for 7 days at room temperature. The solvent was removed in vacuo and the mixture obtained was purified with column separation. Compound on recrystallization from ethanol gave 2.0 g (67%) of white solid with mp 196-198 °C. The results obtained for elemental analysis of $C_{31}H_{33}NO_8$ (MW. 547) C, 67.97; H, 6.05; N, 2.54% is comparable with the calculated values of C, 67.99; H, 6.07; and N, 2.56%.

IR(cm^{-1}) :3500 (O-H stretching), 1670 (C=O stretching), 1340(C-N stretching), 1255 (asymmetrical C-O-C stretching), 1060 (symmetrical C-O-C stretching)

^{1}HMR(ppm,$CDCl_3$) : 8.26 (s,1H,-OH); 7.09-6.50 (m,8H,Ar H); 6.13(s,1H,=CH, coumarin), 4.13-2.91(m,18H, all CH_2-); 1.3(s,3H, -CH_3).

^{13}C NMR(ppm, $CDCl_3$): 53.4(C1); 71.4(C2); 146.9 (C3); 114.8 (C4); 121.2 (C5); 69.1 (C6); 40.9(C7); 115.3 (C8); 145.6 (C9); 112.3(C10); 126.7(C11); 113.4(C12); 152.8(C13); 112.2(C14); 160.9 (C15); 151.7(C16); 21.2(C17).

Mass(FAB): 547 (M^+)

General procedure for the analysis of amines

An aliquot of amine (aniline 8-252 μg, *o*-toluidine 12-284 μg, *m*-toluidine 16-298 μg, *p*-toluidine 14-226 μg, *o*-nitro aniline 23-327 μg, *m*-nitro aniline 24-333 μg, *p*-nitro aniline 25-305 μg,25-305 μg, anthranilic acid 24-273 μg, *p*-amino benzoic acid 17-255 μg) was transferred into a 20 mL glass beaker. The pH was adjusted to 5.5-7.0 with phosphate buffer or universal buffer[22]. Reagent solution (HMCMADCE, 5 mL) in ethanol was added and the contents were shaken gently. The mixture was transferred into a 10 mL volumetric flask and diluted to the mark with distilled water. The absorbance was measured at 480 nm for aniline; 492 nm for *o*-toludine, 484 nm for *m*-toludine, 489 nm for *p*-toludine, *o*-nitro aniline, 475 nm for *m*-nitro aniline, 472 nm for *p*-nitro aniline, 470 nm for anthranilic acid and 474 nm for *p*-amino benzoic acid) spectrophotometrically against the reagent blank.

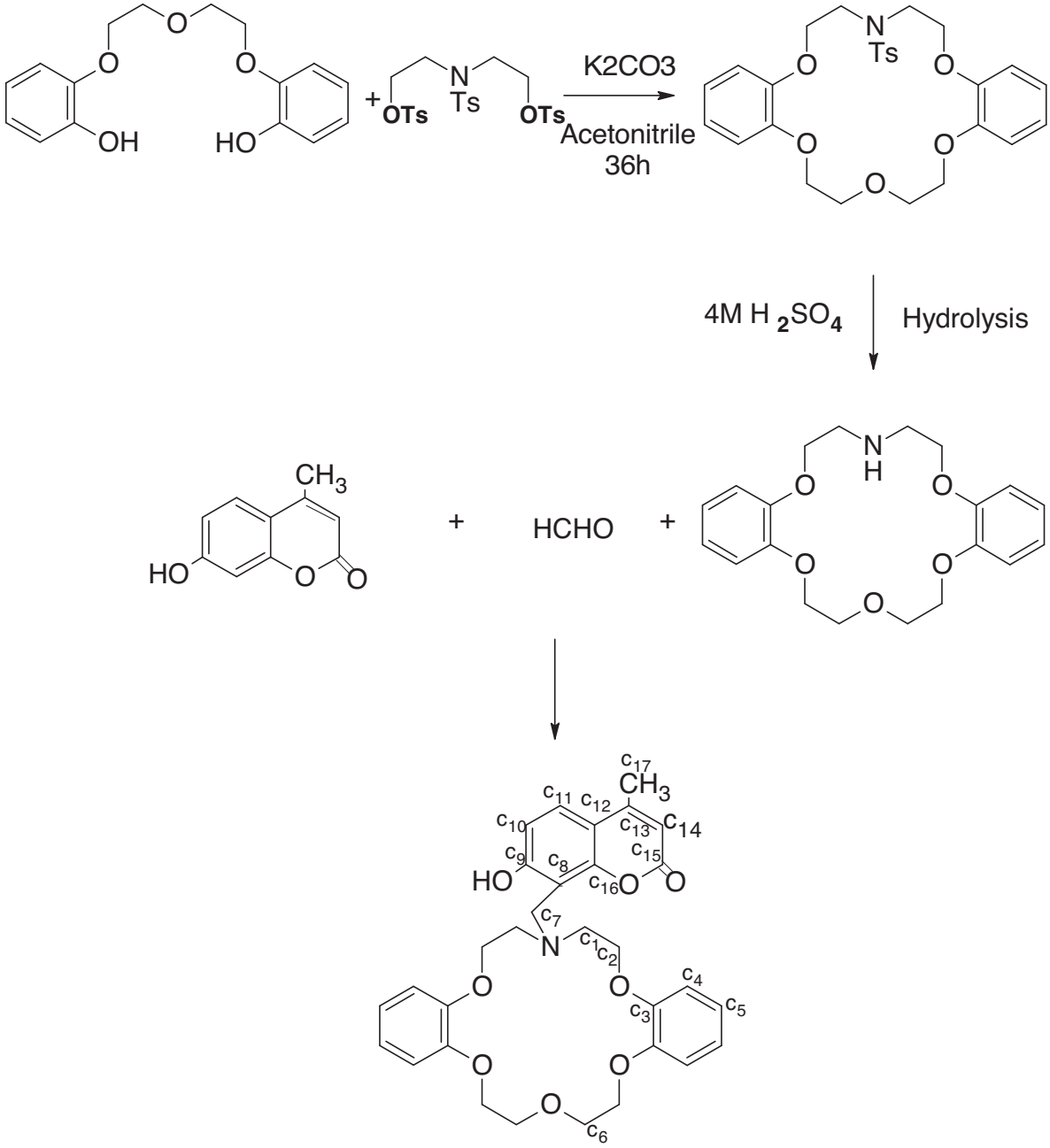
General procedure for analysis of amines in drugs

An aliquot of drug containing bromhexine hydrochloride 30-300 μg; folic acid 25-267 μg, metoclopramide hydrochloride 22-198 μg; sulphadiazine 25-250 μg; sulfaguanidine 18-198 μg; dapsone 25-250 μg, and trimethoprim 18-198 μg was transferred into a 20 mL glass beaker and the pH was adjusted between 5.5 and 7.0 using universal buffer[22] or phosphate buffer. Reagent solution (5 mL) in ethanol was added and the contents were shaken gently. The mixture was transferred into a 10 mL volumetric flask and diluted to the mark with water. The absorbance was measured at 493 nm for bromhexine hydrochloride; 487 nm for folic acid, 490 nm for metoclopramide hydrochloride, 480 nm for sulphadiazine, 497 nm for sulfaguanidine, 483 nm for dapsone, and 483 nm for trimethoprim against the reagent blank.

Results and discussion

The new coumarin based azacrown ether (HMCMADCE) was synthesised and used to investigate its molecular recognition behaviour towards amines. The coumarin moiety was attached to azacrown moiety using methylene group as a spacer.[23] The 4-aza-dibenzo-18-crown-6-ether was synthesised by the detosylation of N-tosyl-4-aza-dibenzo-18-crown-6, which in turn was synthesised by reacting the podant, bis[2-(o-hydroxy phenoxy)-ethyl]ether with ditosylate of N-tosyldiethanol amine in the presence of potassium carbonate in acetonitrile medium (Scheme). This modification in the synthesis of azacrown resulted in better overall yield compared to the reported method[24] in which N-alkyl bis(2-chloro-ethyl)amine is reacted with bis[2-(o-hydroxy phenoxy)-ethyl]ether to prepare the azacrown. Further, the detosylation of the modified method is simpler than the reported method.

The primary aromatic amines like aniline, *o*-toluidine, *m*-toluidine, *p*-toluidine, *o*-nitro aniline, *m*-nitro aniline, *p*-nitro aniline, anthranilic acid, and *p*-aminobenzoic acid showed colour reaction at the optimum conditions, whereas secondary, tertiary aromatic amines and aliphatic amines did not interact with the chromophore. A bathochromic shift in the absorbance of HMCMADCE (λ max 350 nm) was observed in addition to various aromatic primary amines in the aqueous medium, which is exploited for their quantitative determination in the presence of secondary and tertiary aromatic amines, aliphatic amines as well as in the presence of phenol.



Scheme. Synthesis of the reagent.

The change in the colour is attributed to the strong interaction of $-NH_2$ of aromatic amines with –OH group present in the coumarin moiety by exchanging proton and charge stabilization by the hetero atoms of azacrown moiety and the phenyl ring itself (Figure 1). The bulkier secondary and tertiary amine does not interact because of the hindrance of bulkier alkyl group and the small cavity size of the azacrown ether. In the case of aliphatic amines, the shift in the absorption is very little and it may be because the charge of $-NH_3^+$ cannot be stabilized by the alkyl moiety as in the case of aromatic amines.

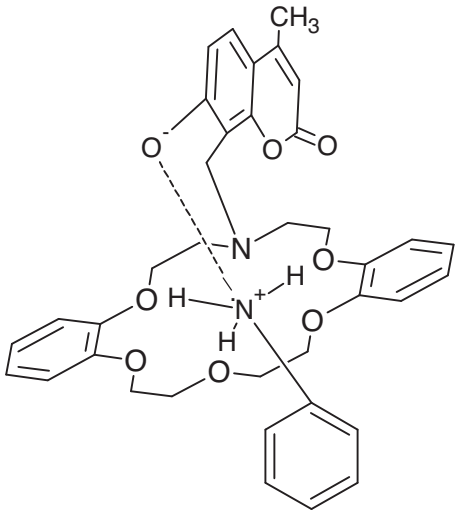


Figure 1. Probable structure of the complex.

Spectrophotometric determination of amines

The absorption spectra of various amine complexes with HMCMADCE are shown in Figure 2. A change in the colour from pale yellow to orange red was observed in the pH range of 5.5-7.0 for various amines. The data regarding the wavelength of maximum absorption, molar absorptivity, Sandell's sensitivity, relative standard deviation, and correlation coefficient for various amines are listed in Table 1. The reagent blank does not absorb at this wavelength.

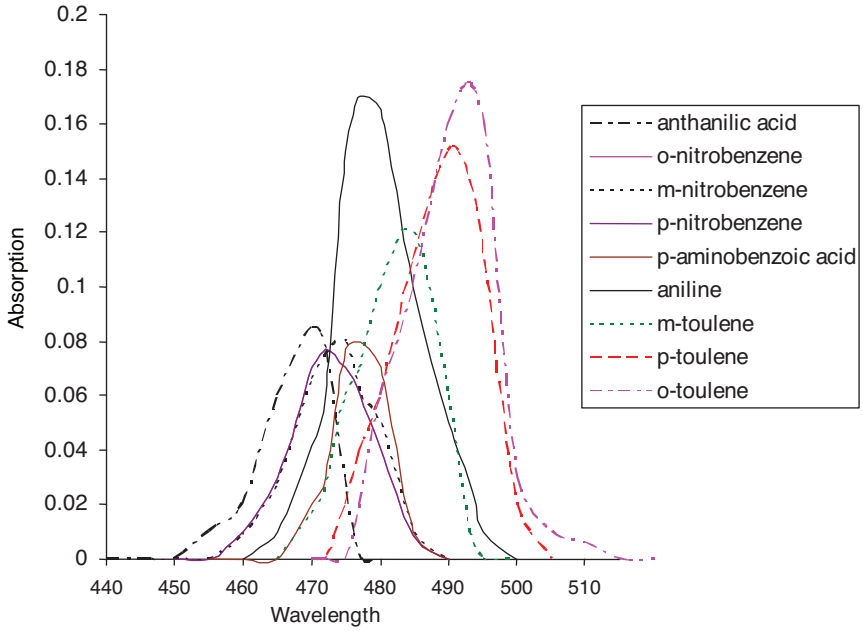


Figure 2. Interaction of amines with HMCMADCE.

Table 1. Absorption parameters of various amines.

Name of Amine	λmax nm	Molar absorptivity	Beers law range	Sandell sensitivity μg/mL	RSD*
Aniline	480	1.62xl0^3	0.8-25.5	.0574	±0.559
o-Toluidine	492	1.92xl0^3	1.2-28.4	.0574	±0.633
m-Toluidine	484	1.34xl0^3	1.6-29.0	0.0800	±0.678
p-Toluidine	490	1.67xl0^3	1.4-22.6	0.0641	±0.565
o-Nitro aniline	472	1.22xl0^3	2.3-32.7	0.1136	±0.573
m-Nitro aniline	475	1.28x10^3	2.4-33.3	0.1492	±0.754
p-Nitro aniline	472	l.l0xl0^3	2.5-30.5	0.1250	±0.578
Anthranilic acid	470	1.16x 10^3	2.4-27.3	0.1176	±0.648
p-Amino benzoic acid	476	1.07xl0^3	2.4-27.3	0.1282	±0.675

*Relative standard deviation is a mean of 5 determinations.

Effect of pH

Maximum absorbance is observed in the pH range of 5.5-7.0 (Table 2). With increase or decrease in the pH the colour intensity decreases. This may be due to incomplete dissociation of –OH group of coumarin at low pH and under alkaline condition amines do not accept proton to form $-NH_3^+$ resulting in decrease in interaction. The colour of the system remains stable for 1 h in all of the cases.

Table 2. Effect of pH on molar absorptivity of aniline.

pH	λmax nm	Absorbance Absorbance	Molar absorptivity ϵ 1 mol^{-1}cm^{-1}
3.0	480	0.027	2.51×10^2
3.5	480	0.032	2.98×10^2
4.0	480	0.124	1.15×10^3
4.5	480	0.147	1.37×10^3
5.0	480	0.162	1.51×10^3
5.5	480	0.174	1.62×10^3
6.0	480	0.174	1.62×10^3
6.5	480	0.174	1.62×10^3
7.0	480	0.174	1.62×10^3
7.5	480	0.121	1.13×10^3
8.0	480	0.043	4.00×10^2

Effect of reagent concentration

Studies with varying concentration of HMCMADCE shows that 5 mL of 0.1%(w/v) reagent is adequate for the quantitative determination of various aromatic primary amines. Lower concentration gives less absorption, which indicates incomplete molecular interaction, whereas higher concentration has no adverse effect.

Effect of various amines

The interference studies are made by measuring the absorbance of the mixture against the reagent blank and the tolerance limit is set as the amount of foreign amine causing a change of ± 0.02 in the absorbance. Most of the secondary and tertiary aromatic amines and aliphatic amines like N-methyl aniline, N,N-dimethyl aniline, methyl amine, diethyl amine, trimethyl amine as well as phenol do not interfere up to 20mg under mentioned conditions.

Application to drug analysis

The method is extended for the quantitative determination of certain drugs, such as bromhexine hydrochloride, dapsone, folic acid, metoclopramide, sulphadiazine, sulfaguanidine, trimethoprim in their pure forms as well as in formulations containing these. The data are given in Tables 3 and 4. The results are compared with the reported method.

Table 3. Drug determination range and molar absorptivity.

Drug	λmax nm	Molar absorptivity ϵ 1 $mol^{-1}cm^{-1}$	Beers law range ppm	Sandell μg/mL	RSD*
Bromhexine	493	1.45xl0^3	3.0-30.0	0.285	±0.675
Folic acid	487	1.51 xl0^3	2.5-26.7	0.2941	±0.675
Metoclopramide	490	1.67 xl0^3	2.2-19.8	0.2128	±0.675
Sulphadiazine	480	1.34 xl0^3	2.5-25.0	0.1852	±0.675
Sulfaguanidine	497	1.38xl0^3	1.8-19.8	0.1563	±0.675
Dapsone	483	1.56xl0^3	1.8-19.8	0.1567	±0.675
Trimethoprim	489	1.48x10^3	1.8-19.8	0.1961	±0.675

*Relative Standard deviation is mean of 5 determination

Table 4. Determination of drugs in various samples.

Drug	Company	Formulation	Label content mg	Present Method, mg*	Reported mg
Bromhexine	I pca	Bromhexine tab.	4.0	4.1	4.1
		Bromhexine syp.	4.0	4.0	4.0
	Seagull labs.Ltd.	Broncocet syp.	4.0	4.1	4.0
	Glaxo	Bronko tab.	4.0	4.1	4.0
		Bronko syp.	4.0	4.0	4.1
	Bannetpharma	Broxine	4.0	4.0	4.1
Folic acid	Zydus	Folic acid tab.	5.0	5.0	5.0
	Wings	Folic acid tab.	5.0	5.2	5.1
	Intas	Folic acid tab.	5.0	5.1	5.1
	Micro	Folcit	5.0	5.2	5.0
	Mars pharma	Folacin	5.0	5.1	5.0
	Burnet pharma	Fol-V	5.0	5.2	5.0
Metoclopramide Hydrochloride	Cipla	Metoclopramide tab.	10.0	10.2	10.1
	Ranbaxy	Metoclopramide inj.	10.0	10.1	10.1
	Rhone Poulnc	Sulphadiazine tab.	500.0	507.0	503.0
Dapsone	GSK	Dapsone tab.	100.0	100.0	100.0
Sulphadiazine	Nicholas	Sulphadiazine tab.	500	504.0	502.0
	Rhone-Poulnc	Sulphadiazine tab.	500.0	507.0	503.0
Sulfaguanidine	Alkem	Sulfaguanidine tab.	500.0	501.0	500.0
Trimethoprim	Alkem	Tprim tab.	80.0	80.0	80.0
		Tprim tab.	160.0	160.0	160.0
	GSK	Septran Tprim tab.	80.0	80.0	80.0
	Nichlolas	Antrima	80.0	80.1	80.1
	Cipla	Ciplin	80.0	80.0	80.0
	Bectrim	Piramal	80.0	80.1	80.1

*Average of 5 determination

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