Spectrophotometric Estimation Of Drugs Using N-BromoSuccinamide And Indigo Carmine Couple

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ABSTRACT

Simple, specific, accurate and precise UV–visible spectrophotometric methods have been developed for the estimation of five drugs *viz.*, Cetrizine (CET), Esmolol (ESM), Rosuvastatin (ROS), Terazocin (TRZ) and Voriconazole(VOR). These methods involve the addition of a known excess of NBS to the drugs in acid medium followed by estimation of residual NBS by reacting with a fixed amount of Indigo Carmine and measuring the absorbance at 610nm. The proposed methods were found to be successful for the estimation of these drugs in bulk and their formulations. The results of analysis have been validated statistically for linearity, accuracy, precision, LOD and LOQ.

Keywords:Cetrizine, Esmolol, Rosuvastatin, Terazocin,UV-visible spectrophotometry, Validation,Voriconazole.

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INTRODUCTION:

CETRIZINE HYDROCHLORIDE (CET) (Fig.1a) is an orally active and selective H_1-receptor antagonist[1]. Its chemical name is (±) - [2-[4-[(4-chlorophenyl)phenylmethyl]-1-piperazinyl]ethoxy]acetic acid, dihydrochloride. It is a non-sedating type histamine H_1-receptor antagonist used in symptomatic treatment of seasonal rhinitis, conjunctivitis, perennial allergic rhinitis, and pruritusandurticaria of allergic origin. Various analytical techniques have been employed for the determination of CET in pharmaceutical preparations such as spectrophotometry[2],[3], high performance liquid chromatography (HPLC)[3],[4],[5],[6],[7],[8],[9] and liquid chromatography-mass spectrometry (LC/MS)[10],[11], RP-LC[12] and Capillary zone electrophoretic method[13].

ESMOLOL (ESM) (Fig.1b) hydrochloride is a class II antiarrhythmic and is chemically known as methyl (RS)-3-{4-[2-hydroxy-3-(propan-2-ylamino) propoxy] phenyl} propanoate hydrochloride. It is cardio selective beta1receptor blocker with rapid onset. It is used in the treatment for the rapid control of heart rate. ESM decreases the force and rate of heart contractions by blocking beta-adrenergic receptors[14] of the sympathetic nervous system. The methods which were reported in the literature for the determination of ESM includes HPLC[15],[16],[17],[18],[19], liquid chromatography-mass spectrometry (LC/MS)[20], Capillary zone electrophoretic method[21], Chiral column chromatography[22] and spectrophotometry[23],[24].

ROSUVASTATIN (ROS) (Fig.1c)Rosuvastatin (ROS),bis((E)-7[4-(4-Fluorophenyl)-6-isopropyl-2-(Methyl (methylsulfonyl) aminopyrmidin-5yl)(3R,5S)-3,5-dihydroxyhept-6-enoic acid) Calcium salt. It belongs to the class of drugs called statins which are employed to lower hypercholesterolemia and related conditions and to prevent cardiovascular diseases.[25] It is highly effective 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor.[1,3-8] In clinical trials, rosuvastatin achieved mark reduction in serum levels of LDL cholesterol, accompanied by modest increases in HDL cholesterol and reduction in triglyceride. The most important related compounds for rosuvastatin are anti isomer and lacton impurity. Literature survey reveals that few Stability-indicating spectrophotometry[26],[27],[28], liquid chromatography [29],[30],[31],[32], HPLC [33], Tandem mass spectrometry[34] and voltammetry[35] methods have been reported.

TERAZOCIN (TRZ) (Fig.1d) is chemically known as RS-1-(4-amino-6, 7-dimethoxy-2-quinazolinyl)-4-[(tetra-hydro-2-furanyl) carbonyl]-piperazinemonohydrochloride. It is a α_1-adrenoceptor blocker with a long lasting action. [36]. It is used in the management of hypertension and in benign prostate hyperplasia to relieve symptoms of urinary obstruction. Terazosin is rapidly and almost completely absorbed from the gastrointestinal tract after oral administration and is extensively metabolized in the liver to yield piprazine and three other

inactive metabolites. Literature review reveals that a few methods have been published for analysis of TRZ in the bulk form and in pharmaceutical preparations. Methods available include HPLC[37],[38],[39][40],Spectrophotometry[41],[42],[43],[44],[45],Visiblespectrphotometry[46] and potentiometry [47].

VORICONAZOLE (VOR) (Fig.1e)chemically is (αR,βS)-(2,4-difluorophenyl)-5-fluoro-β-methyl-α-(1H-1.2.4-triazol-1-yl-methyl)-4-pyrimideethanol (Figure 1). It is a second generation broad spectrum triazole introduced to treat fungal infections[48] like invasive aspergillosis, disseminated infections caused by fluconazole resistant candida, fusarium infections and febrile neutropenia not responding to antibacterial therapy1 and its primary mode of action is by inhibition of the fungal cytochrome P450dependent 14α-steroldimethylase, an essential enzyme in ergosterol biosynthesis. Up to now there are many methods developed like HPLC[49],[50],[51][52],[53],HPTLC[54],[55],[56], LC-MS[57],[58],Colorimetry[59], UPLC-MS[60] and UV spectrophotometric [61] methods developed.

A comparision of various techniques used for estimation of above drugs in terms of sensitivity and reproducibility are presented in Table-1.

Thorough survey of literature revealed that simple spectrophotometric methods are not yet reported for the above drugs.In this communication we present simple, accurate, precise methods for the quantification of above drugs.

MATERIALS AND METHODS

The pharmaceutical grade drugs were supplied by Aurbindo Pharmaceuticals and Hetero drugs Pvt. Ltd, Hyderabad. IndigoCarmine, HCl were purchased from S.d fine chem. Pvt. Ltd, Mumbai, India. N-Bromosuccinamide (NBS) is purchased from SRL chemicals, Mumbai, India. Whatman filter paper no.42 was used for filtration purpose. All the reagents used were of analytical-reagent grade and distilled water was used throughout the investigation. Tablets were purchased from the local market.

All absorbance measurements were recorded on an Elico 210 double beam spectrophotometer, Systronics 117 spectrophotometer and also on ELICO 159 UV-VIS single beam spectrophotometers using quartz cells of 10 mm path length. A high precision Analytical (Dhona 200 single pan electrical) balance was used for weighing the reagents.

Preparation of Standard stock solutions:

N-Bromosuccinamide (NBS): An approximately 0.01M solution was prepared by dissolving 0.1779 g of NBS in 100 ml distilled water. It is diluted to get 124µgmL^{-} of NBS.

Indigo Carmine: Stock solution was prepared by dissolving 0.0484g of Indigo Carmine in 100 ml distilled water. From this stock solution, 353µgmL^{-}test solution was prepared.

Hydrochloric acid solution: Conc.HCl is diluted appropriately with distilled water to get 1M HCl solution.

Drugs: A standard solutions of drugs were prepared by dissolving accurately weighed 50 mg of pure drug in water and diluted to the mark in 100 ml calibrated volumetric flasks. The stock solutions of CET, ESM, ROS, TRZ and VOR were diluted with water to obtain 20-140µgmL^{-} 1.0-7.0µgmL^{-}, 4.8-33.6µgmL^{-}, 1.0-7.0µgmL^{-}and 30-210µgmL^{-}respectively.

Conc. Hydrochloric acid (HCl):Conc. HCl is diluted appropriately with distilled water to get 1.0 M HCl solution.

Method Development:

Aliquots of pure drug solution (1.0-7.0ml) were transferred into a series of 10ml calibrated flasks. To each flask 1.0ml of 1*M*HCl acid was added followed by 1.0ml of NBS solution. The flasks are stoppered and contents were mixed and the flasks are set aside for 15 min under occasional shaking. Finally, 1.0 ml of Indigo Carmine solution was added to each flask and the volume was adjusted to the mark with water and mixed well. The absorbance of each solution was measured at 520nm after 5 min.

Construction of Calibration Curve:

Six replicate experiments were performed and the relative response *i.e.*, absorbance / concentration (µgmL^{-1}) was calculated. The points falling between 95% and 105% of average only are considered for the construction of calibration. A standard graph was prepared by plotting the absorbance versus the concentration of drugs(Fig.2). The standard deviation of six residual intercepts of the plots is used for calculating LOD and LOQ. Beer's Law is obeyed and calibration curves for CET, ESM, ROS, TRZ, and VOR over a concentration range of 20-140µgmL^{-}1.0-7.0µgmL^{-}, 4.8-33.6µgmL^{-}, 1.0-7.0µgmL^{-}and 30-210µgmL^{-}respectively, were plotted. The spectral and statistical characteristics are recorded in Table-2.

Analysis of Drugs in the Pure form for Precision and Accuracy Studies:

As mentioned, six replicate experiments were performed to ascertain the precision of the methods. The results differed only in a small range of experimental errors.

The accuracy of the proposed methods was evaluated by percentage recovery studies on the drugs. The %RSD was ≥2, showing high degree of accuracy of the proposed methods. The effect of excipients on the methods developed was also tested and found that excipients do not interfere much. The results of the method lie within the prescribed limits showing that method is free from interference from excipients. The results of the recovery studies together with other statistical parameters are reported in Table-3.

Analysis of commercial Dosage forms:

A quantity of finely ground powder of tablets of equivalent to 50 mg of drug CET (Citrazine), ESM (Minibloc), ROS (Crestor), TRZ (Hytrin) and VOR(Vorzu),were accurately weighed and taken in 60 ml distilled water in 100 ml volumetric flask and left for 10 min for complete dispersion and then filtered through Whatman filter paper. First 10 ml portion of the filtrate was rejected and a convenient aliquot of filtrate was further diluted for the analysis within the limits of Beer's law.

Four different solutions of each drug were analyzed through recovery studies, using the calibration curves constructed. Excellent recovery was observed Table-4.

RESULTS AND DISCUSSION :

N-Bromosuccinamide (NBS) has been used widely as a brominating and oxidizing agent for organic compounds. The proposed methods are indirect and are based on the oxidation and bromination reaction between drug and NBS and determination of residual NBS after allowing the reaction between drug and measured amount of NBS to be complete. The amount of NBS reacted corresponds to the drug content in all the methods.

Drug + known excess of NBS --------» Reaction product of the drug + Unreacted NBS

Unreacted NBS+ Fixed amount of Indigo Carmine-------» Absorbance measured at 520nm.

Method validation:

The proposed methods were validated according to guidelines of International Conference on Harmonization (ICH). Under the described experimental conditions, standard experimental conditions, standard calibration curves for the studied drugs were constructed by plotting absorbance versus concentration. Conformity with Beer's law was evident in the concentration range cited in Table-2.The linear regression equations, molar absorptivity, Sandell's sensitivity, limits of detection (LOD) and limits of quantification (LOQ) were listed in it. Standard deviation, relative standard deviation, variance and standard error were calculated.

The accuracy of the method was established by analyzing the pure drug at four levels (within working limits) and the precision was ascertained by calculating the relative standard deviation of six replicate determinations on the same solution containing the drug at three levels in Table-3. The analytical results for accuracy and precision showed that the proposed methods have good repeatability and reproducibility.

The percentage recoveries of the drugs in tablet using the proposed methods compared with that given by reference methods are illustrated in Table-4. The validity of the proposed method in

literature is evaluated by statistical analysis between the results obtained and that of reference methods. Student's t-test and variance ratio F-test are chosen for the comparison of the results. Values are within the permissible range reported in literature. The tablet formulations were also analyzed to check the applicability of methods.

CONCLUSION :

The obtained results from the method for the determination of mentioned drugs indicate that method is simple, accurate and precise. The method is economical compared to other sophisticated analytical instruments. Hence this method can be used for routine analysis of commercially available formulations. The method is suitable for the determination of these drugs in tablet formulation without interference from commonly used excipients. The solvents used for the method are inexpensive and simple to prepare, and could be used in a quality control laboratory for routine drug analysis.

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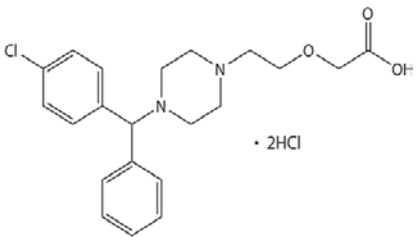
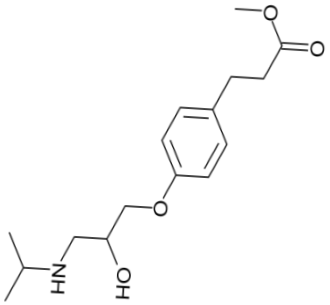
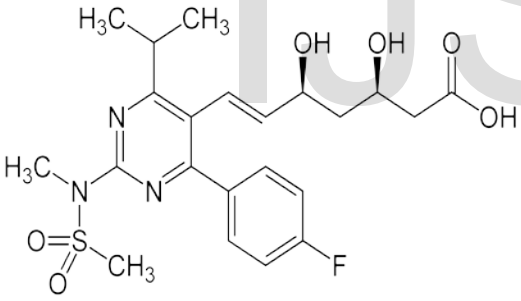
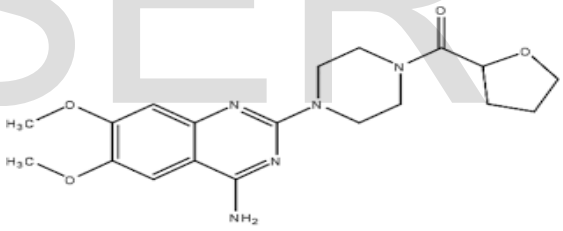
fig1.structure of drugs



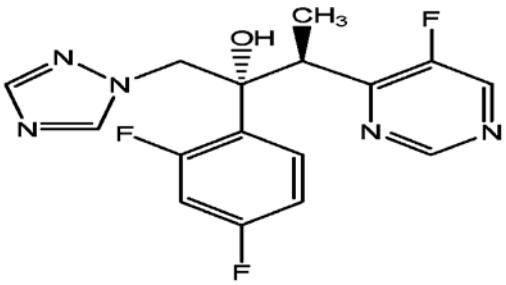


1a.cetrizine hydrochloride 1b.Esmolol



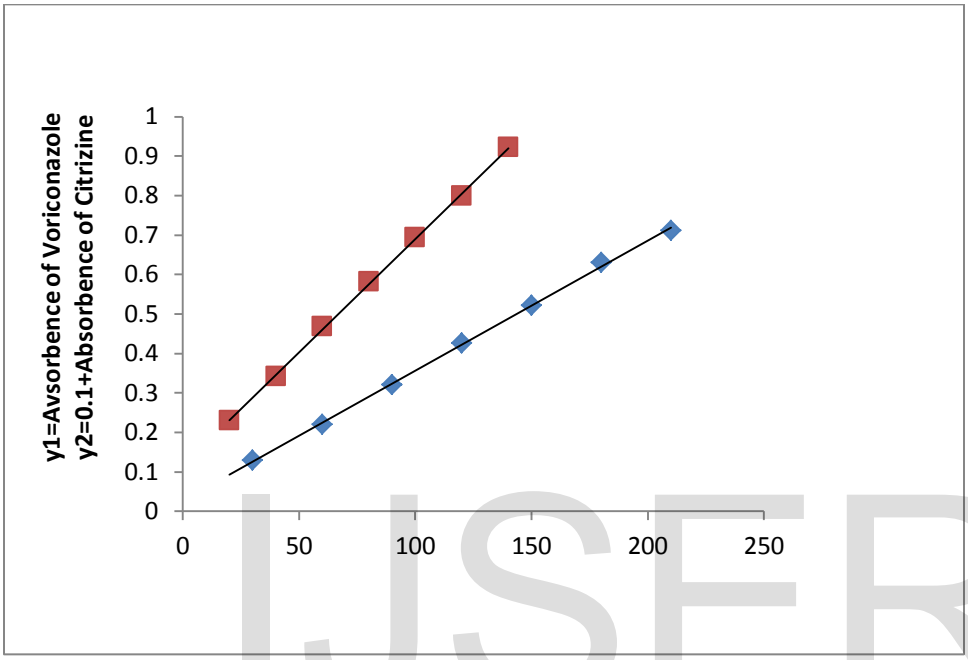


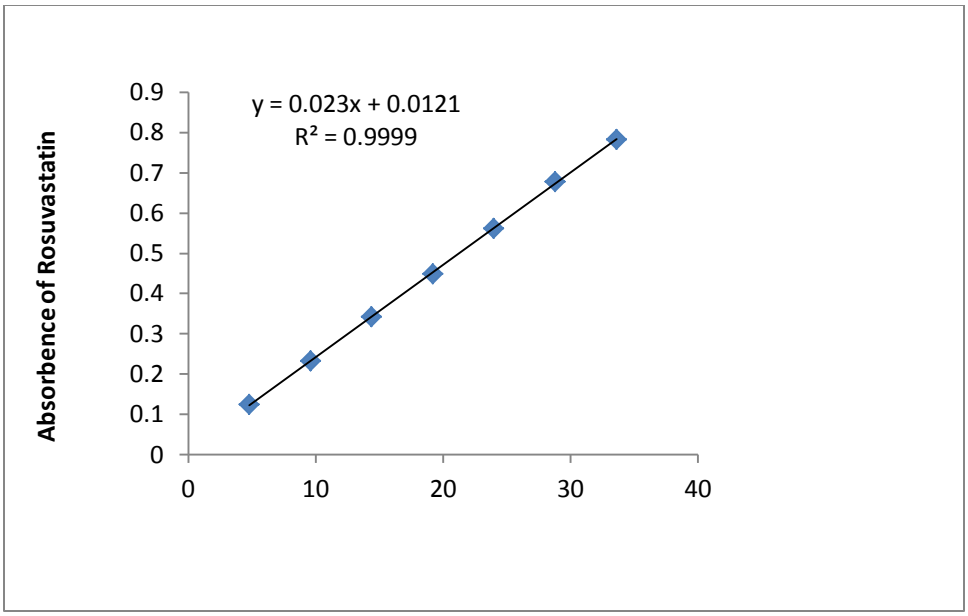
1c.Rosuvastatin 1d.Terazocin

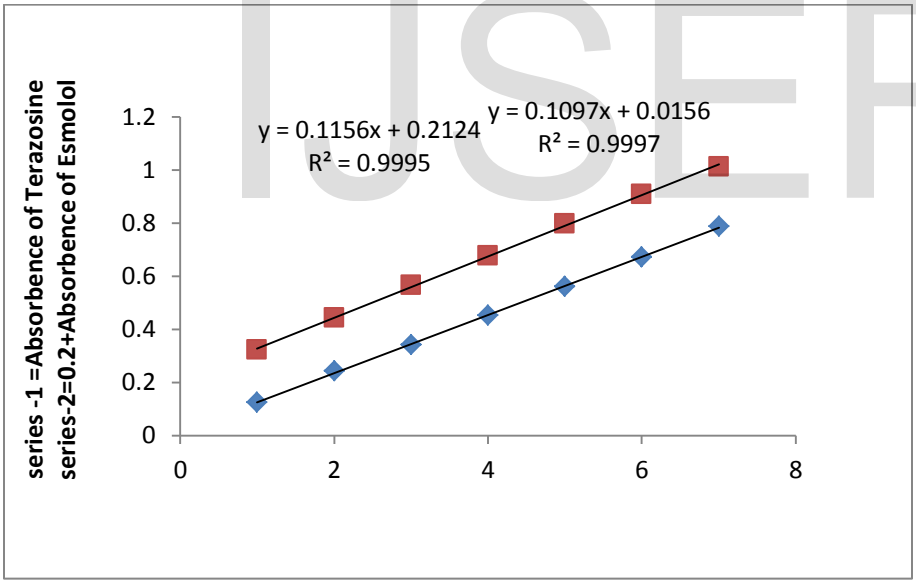


1e. Voriconazole

fig2.calibration curves




Absorbence of Rosuvastatin
y = 0.023x + 0.0121
R² = 0.9999
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40


series -1 =Absorbence of Terazosine
series-2=0.2+Absorbence of Esmolol
y = 0.1156x + 0.2124
R² = 0.9995
y = 0.1097x + 0.0156
R² = 0.9997
1.2
1
0.8
0.6
0.4
0.2
0
0
2
4
6
8

Method	Linearity range in	Sensitivity range	Precession %RSD	Accuracy %Recovery	Limitations
HPLC	0.05-2400μgmL^{-1}	0.1432-0.4849	0.613-1.899	98-101.54	Costly equipment
Electrochemical Methods	0.05-1000μM	----------	----------	98.65-100.76	Low sensitivity
Direct Spectrophotometry	1-400 μgmL^{-1}	0.0084-0.2186	0.34-1.96	98.74-101.54	Involve UV-light
Spectrofluorometrty	0.02-30 μgmL^{-1}	---------	----------	98.17-99.17	Rare equipment

Table 1 :Comparision of various techniques used for essay of the drus (range of parameters in general)

Table 2: Analytical parameters for determination of drugs by oxidation with NBS and Indigo Carmine

Property Name of the Drug	CET	ESM	ROS	TER	VOR
λmax, nm	610	610	610	610	610
Beer's law limits µg mL^{-1}	20-140	1-7	4.8-33.6	1-7	30-210
Molar absorptivity, L mol^{-1} cm^{-1}	2.507×10^{3}	3.690×10^{4}	1.253×10^{4}	4.968×10^{4}	1.510×10^{3}
Sandell sensitivity* µg cm^{-2}	0.2000	0.0086	0.04 34	0.0091	0.3333
Slope (a)	0.016	0.012	0.012	0.015	0.027
Intercept (b)	0.005	0.115	0.023	0.109	0.003
Correlation coefficient (r)	0.999	0.999	0.998	0.999	0.999
Standard deviation of intercept (Sa)	0.0091	0.0114	0.0106	0.0109	0.0444
Limit of detection µg mL^{-1}	6.006	0.327	1.520	0.330	6.8467
Limit of quantification µg mL^{-1}	18.00	0.991	4.608	1.000	20.747
Regression equation Y=b+ax	0.005+0.016X	0.115+0.012X	0.023+0.012X	0.109+0.015X	0.003+0.027X

Table 3: Determination of accuracy and precision of the methods on pure drug Samples

Drug	Taken (µg/ml)	Found (µg/ml)	er (%)	Recovery (%)	RSD(%)	Proposed method Mean ± SD
CET	1.5 2.5 3.5	1.51 2.50 3.48	0.66 0.00 0.57	100.66 100 99.43	0.6153	100.03 ± 0.615
ESM	4.0 5.0 6.0	4.00 5.01 5.98	0.0 0.20 0.33	100.00 100.20 99.67	0.2677	99.95 ± 0.267
ROS	3.0 4.0 5.0	3.01 4.0 4.96	0.33 0.00 0.80	100.33 100 99.20	0.5819	99.84 ±0.581
TRZ	1.5 3.5 4.0	1.50 3.48 3.99	0.00 0.57 0.25	100 99.43 99.75	0.2865	99.72 ±0.286
VOR	2.0 4.0 6.0	1.97 4.04 5.98	1.50 1.0 0.33	98.50 101 99.67	1.2543	99.72 ±1.254

Table 4: Results of Assay of Tablets by the proposed methods (Standard amount of drug is added when the content of drug is very low.)

Tablets	Drug in tablet µg mL^{-1}	Drug added µg mL^{-1}	Total found µg mL^{-1}	er (%)	Recovery (%)	RSD (%)	Reference method Mean **± SD**	Propose method **± SD**	t-test	F-test
CET(Citrazine)	1.0 1.0 1.0 1.0 2.0 3.0	1.0 2.0 3.0 0.0 0.0 0.0	2.01 3.02 4.01 0.99 2.02 3.01	0.50 0.66 0.25 1.00 1.00 0.33	100.50 100.66 100.25 99.00 101.00 100.33	0.6839	99.50 ±1.10	100.29 ±0.685	0.565 (2.571)	0.387 (4.95)
ESM(Minibloc)	1.0 1.0 1.0 2.0 3.0 4.0	0.5 1.0 1.5 0.0 0.0 0.0	1.5 2.01 2.52 1.99 3.00 3.98	0.00 0.50 0.80 0.50 0.00 0.5	100.00 100.50 100.80 99.50 100.00 99.50	0.5241	100.40 ± 0.25	100.05 ±0.524	0.110 (2.477)	4.393 (4.28)
ROS (crestor)	1.0 1.0 1.0 2.0 4.0 6.0	1.0 2.0 3.0 0.0 0.0 0.0	2.02 3.00 3.99 2.01 3.98 6.03	1.00 0.00 0.25 0.50 0.50 0.5	101.00 100.00 99.75 100.50 99.50 100.50	0.5560	99.03 ±0.290	100.20 ±0.557	1.029 (2.571)	3.689 (4.95)
TERZ (Hytrin)	2.0 2.0 2.0 2.0 4.0 6.0	1.5 3.0 4.5 0.0 0.0 0.0	3.49 5.01 6.49 2.01 4.00 6.01	0.28 0.20 0.15 0.50 0.00 0.16	99.72 100.20 99.85 100.50 100.00 100.16	0.2775	100.38 ±0.58	100.07 ±0.277	1.154 (2.477)	0.228 (4.28)
VOR (Vorzu)	1.0 1.0 1.0 2.0 3.0 4.0	1.0 1.5 2.0 0.0 0.0 0.0	2.00 2.49 2.98 1.99 3.02 4.01	0.00 1.00 0.66 0.50 0.66 0.25	100.00 99.00 99.34 99.50 100.66 100.25	0.621	99.66 ±0.232	99.79 ±0.620	1.354 (3.182)	0.140 (4.75)

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