Research Paper

Simultaneous Estimation of Indapamide and Atenolol by Two Different Ultraviolet Spectroscopic Methods

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Two simple and rapid ultraviolet spectrophotometric methods were developed to estimate indapamide and atenolol in marketed tablet formulation. These ultraviolet methods are simple, economical and less time consuming as compared to other instrumental methods. The developed methods included the simultaneous equations method and absorbance ratio method. Methanol was used as a dissolving solvent and distilled water was used as a diluent. For the simultaneous equations method, the wavelengths selected were 241 nm (λ_{max} of indapamide) and 224.4 nm (λ_{max} of atenolol) and for absorbance ratio method, the two wavelengths selected were 233.8 nm (isosbestic point) and 224.4 nm (λ_{max} of atenolol). In both approaches, the linearity was proven over concentration ranges of 2-20 µg/ml for indapamide and 10-80 µg/ml for atenolol. The percentage purity in the marketed formulation was found to be 99.79 % for indapamide and 98.57 % for atenolol by simultaneous equations method and 99.56 % for indapamide and 100.0 % for atenolol by absorbance ratio method, which lies within the acceptance criteria i.e., 95 %-105 %. The methods were validated as per the International Council for Harmonisation guidelines and were found to be linear, precise, accurate, sensitive and robust and hence can be used for the estimation of indapamide and atenolol simultaneously in tablet formulation.

Key words: Indapamide, atenolol, isosbestic, International Council for Harmonisation, validation

Hypertension and heart related diseases are leading causes of death across the world. Therapy involving drugs in combined dosage form offers excellent results in reducing hypertension and indirectly decreasing cardiovascular issues. Indapamide (IND) is a diuretic drug used in treatment of high blood pressure, edema heart attack, stroke and heart failure in persons with high blood pressure[1]. Atenolol (ATN) is a selective Beta 1 (β1) receptor antagonist which decreases the formation of angiotensin II and secretion of aldosterone and helps in the treatment of cardiovascular disease such as angina, hypertension, cardiac arrhythmias, and myocardial infractions[2]. Literature survey revealed that many Ultraviolet (UV), High Performance Thin Layer Chromatography (HPTLC) and High Performance Liquid Chromatography (HPLC) methods have been developed on these drugs either alone[3-10] as such or in combination[11-22]. UV methods are simple to carry out, fast and economical. In this study we propose two simple and validated UV methods for the simultaneous estimation IND and ATN in marketed formulations. The validation of the methods has been carried out as per the International Council for Harmonisation (ICH) guidelines.

MATERIALS AND METHODS

Reagents and chemicals:

Active pharmaceutical ingredients IND and ATN were received as a gift samples from Cipla Pvt. Ltd. and Goa Zydus Lifesciences Ltd. Goa, respectively. The marketed tablet formulation, ATEN-D, comprising 2.5 mg of IND and 50 mg of ATN was purchased from a local pharmacy. Analysis was carried out on UV 1800 of Shimadzu as methanol was used as a solvent and distilled water as a diluent.

Choice of diluent:

The selection of diluent was made after assessing the solubility and stability of the drugs, IND and ATN, in different solvents. As both the drugs were

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soluble in methanol, it was used as solvent and distilled water was used as a diluent for further dilutions in the experiment.

Preparation of stock solutions (1000 µg/ml):

Stock solutions of IND and ATN were prepared by transferring 25 mg of each of the drug into a 25 ml volumetric flask and dissolving with sufficient amount of methanol. Further, volume was made up with methanol to get a concentration of 1000 µg/ml.

Preparation of working stock solutions (100 µg/ml):

The above stock solutions were appropriately diluted to give a concentration range of 2-20 µg/ml for IND and 10-80 µg/ml for ATN with distilled water.

Choice of wavelength:

Solutions containing 2 µg/ml of IND and of 10 µg/ml of ATN were prepared from the working stock (100 µg/ml) of ATN and IND respectively using distilled water as diluent and were scanned in the range of 200-400 nm. The two spectra were recorded and λ_{max} for both the drugs was determined. From the overlain spectra of both the drugs three wavelengths were selected, λ_{max} of IND 241 nm, λ_{max} of ATN 224.4 nm for the simultaneous equations method (method 1) and, λ_{max} of ATN 224.4 nm and isosbestic point 233.8 nm were selected for the absorbance ratio method (method 2).

METHOD VALIDATION

Linearity:

To determine the linearity range for IND and ATN, serial dilutions having concentration of 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 µg/ml and 10, 20, 30, 40, 50, 60, 70 and 80 µg/ml were prepared from the respective working stock solution of both the drugs. The absorbance of these solutions was recorded at predetermined wavelengths and the calibration curve was constructed by plotting absorbance versus concentration for both the methods. The linear regression equation and correlation coefficient (r^2) were determined for method 1 and method 2.

Precision:

Precision was evaluated by performing repeatability, intraday precision and interday precision. Repeatability was evaluated by performing the assay procedure for six times and recording the absorbances at the respective wavelengths. Intraday precision was evaluated by analysing the working sample solution of tablet formulation in triplicate at different intervals on one day and interday precision was evaluated by analysing the working sample solution of tablet formulation in triplicate for 3 consecutive d. The standard deviation and percentage relative standard deviation with respective to the absorbances were calculated at each stage for both the methods.

Accuracy:

To establish the accuracy, recovery studies were performed at three different concentration levels, i.e., 80 %, 100 % and 120 % in triplicates. A working sample solution of tablet formulation equivalent to 30 µg/ml of ATN and 1.5 µg/ml of IND was added into three sets (consisting of three volumetric flasks each of 10 ml capacity). To all these flasks, working sample solution of tablet formulation equivalent to a concentration of 30 µg/ml of ATN and 1.5 µg/ml of IND was added. To the first set of three flasks, 0.12 ml of IND and 2.4 ml of ATN was added. To the second set of three flasks, 0.15 ml of IND and 3 ml of ATN was added. To the third set of three flasks 0.18 ml of IND and 3.6 ml of ATN was added. Later diluent, i.e., distilled water was added up to the mark.

Limit of Detection (LOD) and Limit of Quantitation (LOQ):

LOD and LOQ were calculated by using slope and standard deviation response of calibration curve of the drugs, IND and ATN.

Robustness:

Robustness was established by performing the assay procedure under some deliberately varying conditions like changing the instrument, analyst and wavelength by ±2 nm and measuring the absorbances. The standard deviation and percentage relative standard deviation were calculated.

Analysis of tablet formulation:

Ten tablets of ATEN-D containing 2.5 mg of IND and 50 mg of ATN were weighed, average weight

determined and triturated to a fine powder. Tablet powder equivalent to 25 mg of ATN was accurately weighed and transferred into 25 ml volumetric flask containing about 15 ml of methanol and sonicated for 15 min. Using the same solvent dilution was made up to the mark and further dilutions were made with distilled water to obtain the final concentration of 2 µg/ml for IND 50 µg/ml of ATN. The assay procedure was repeated three times. The absorbances of the resulting solution were recorded at predetermined wavelengths and the percent purity of both the drugs were calculated for method 1 and method 2.

RESULTS AND DISCUSSION:

The structure of IND and ATN was confirmed by taking the Infrared (IR) spectra of both the drugs as shown in fig. 1 and fig. 2 respectively. Fourier Transform Infrared (FTIR) spectra of pure IND showed significant bands at 3317 cm^{-1} and 3217 cm^{-1} due to N-H stretching, at 1658 cm^{-1} due to C=O stretching, 1598 cm^{-1} due to aromatic C-H stretch. FTIR spectra of pure ATN showed significant bands at 3352 cm^{-1} due to O-H stretch, at 3172 cm^{-1} and 2966 cm^{-1} due to N-H stretching, at 1633 cm^{-1} due to C=O stretching and 1242 cm^{-1} due to aromatic C-O ether stretch. The solvent was selected based on the solubility of both the drugs. Methanol was used as a solvent and distilled water was used as a diluent. The solutions of IND and ATN were scanned by UV spectrophotometer in the range of 200-400 nm. The two spectra were recorded and λ_{max} for IND was found to be 241 nm and λ_{max} of ATN was found to be 224.4 nm as shown in fig. 3 and fig. 4 respectively. From the overlain spectra of IND and ATN (fig. 5), it was found that both the drugs, absorbs at the λ_{max} of the other. Hence, 241 nm and 224.4 nm were selected as λ_1 and λ_2 respectively, for simultaneous equations method and 224.4 nm and 233.8 nm (isosbestic point) were chosen as λ_1 and λ_2 for absorbance correction method.

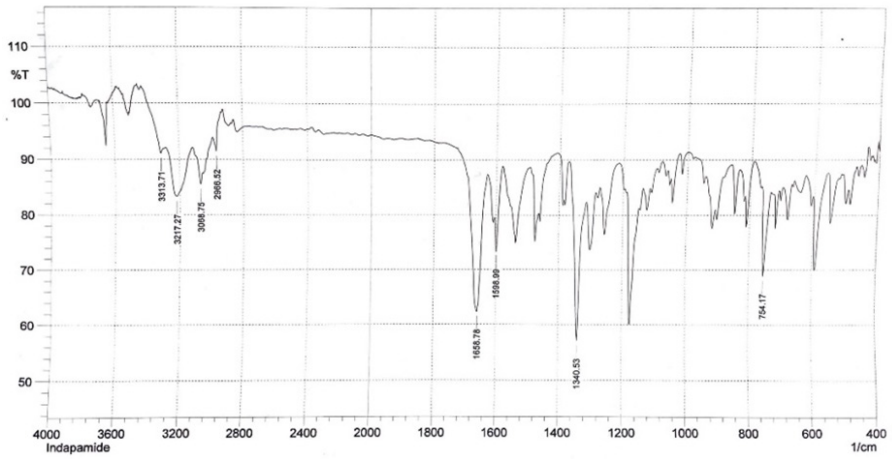

Fig. 1: IR spectra of IND

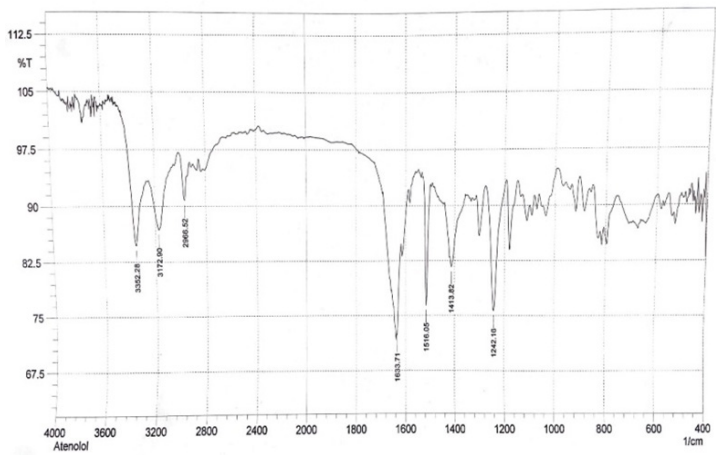

Fig. 2: UV spectra of ATN

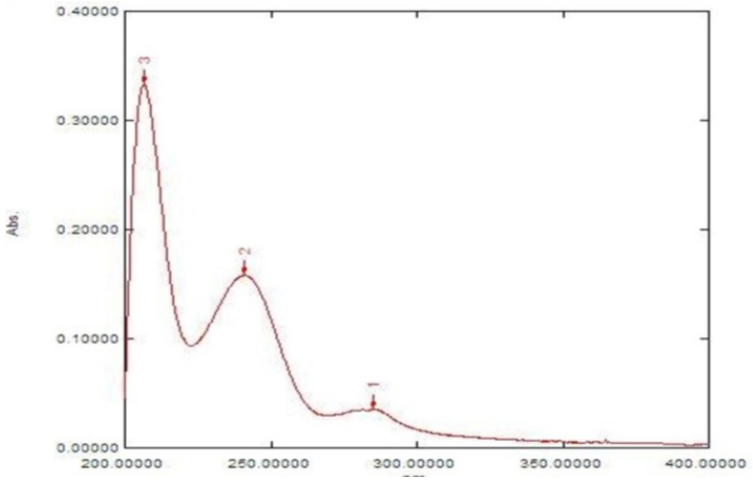

Fig. 3: UV spectra of IND

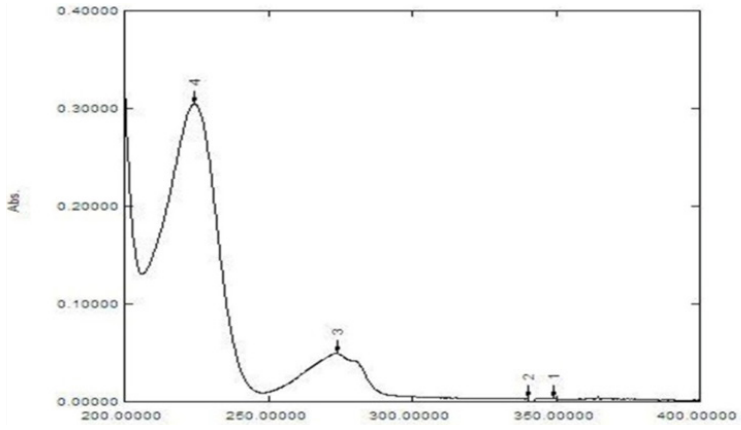

Fig. 4: UV spectra of ATN

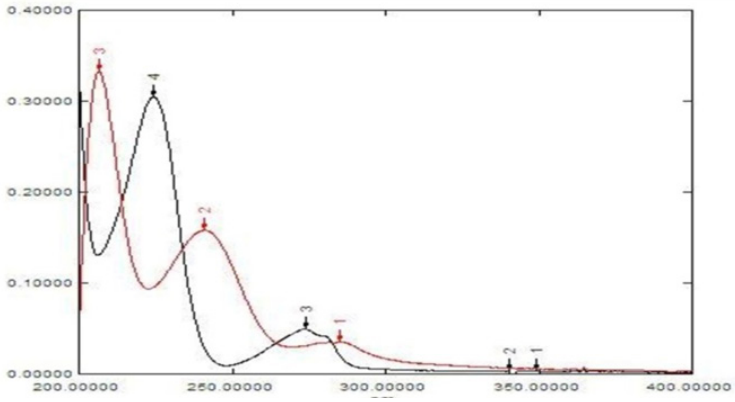

Fig. 5: Overlain UV spectra of IND and ATN

IND was linear in a concentration range of 2-20 µg/ml and ATN in a range of 10-80 µg/ml which is displayed in fig. 6-fig. 8 for both the methods. The correlation coefficient was 0.9993 and 0.999 for IND and ATN by method 1 and 0.9992 and 0.999 by method 2 respectively, which proved a good correlation between the absorbance and concentration. The results are displayed in Table 1.

Precision was carried out for by analysing the tablet formulation for repeatability, interday and intraday studies by both the methods. The results are depicted in Table 2 and Table 3. Accuracy of the methods was obtained by carrying out recovery studies at three different levels, 80 % 100 % and 120 %. The mean recovery for IND and ATN was between 98 %-101 % by method 1 and 100 %-101 % for IND and 99 %-102 % for ATN by method 2 as shown in Table 4. Robustness of the method was established by making deliberate changes in the instrument, analyst and wavelength and

the percentage RSD was found to be less than 2 % which was within the limits which is depicted in Table 5. From the LOD and LOQ values, the method was seen to be sensitive as seen in Table 6. Both the methods were applied for the analysis of marketed tablet formulation. The percentage purity in the marketed formulation was found to be 99.79 % for IND and 98.57 % for ATN by method 1 and 99.56 % for IND and 100.0 % for ATN by method 2, which lies within the acceptance criteria i.e., 95 %-105 % which is depicted in Table 7.

Two simple UV spectrophotometric methods have been developed for the estimation of IND and ATN in tablet dosage form. The results showed that both the methods were statistically equivalent. From the results of the validation it can be concluded that methods are suitable and meet the ICH requirement and it may be concluded that the result obtained by these two methods are in fair agreement with each other. Thus the analysis of solid dosage form of IND and ATN in combination may be successfully performed by the simultaneous ratio method and absorbance ratio method.

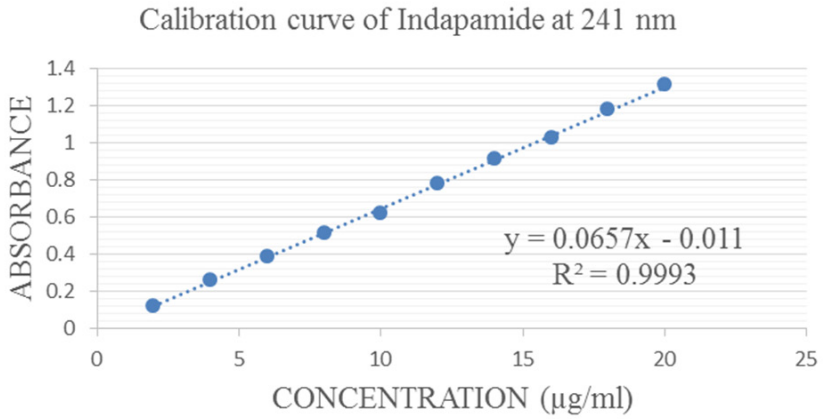

Fig. 6: Calibration curve of IND at 241 nm

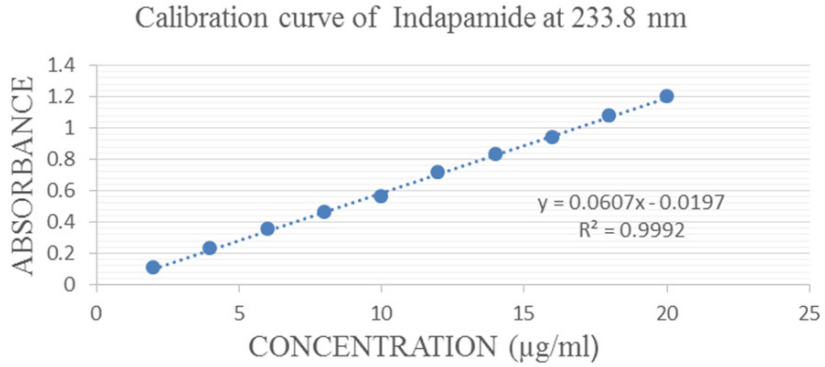

Fig. 7: Calibration curve of IND at 233.8 nm

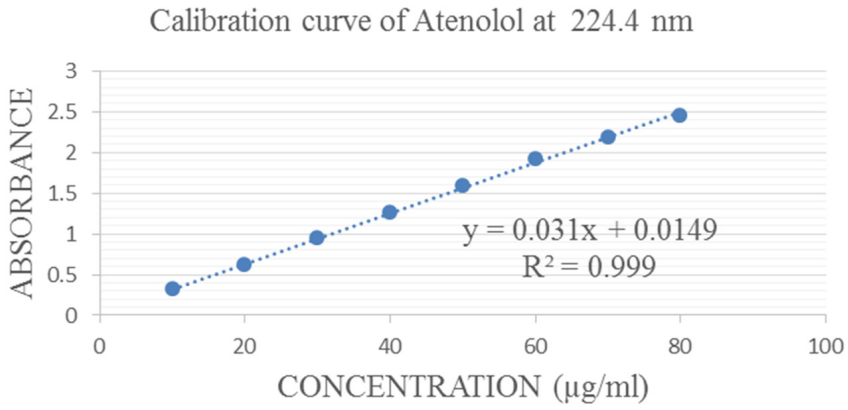

Fig. 8: Calibration curve of ATN at 224.4 nm

TABLE 1: LINEARITY DATA

Parameters	Method 1		Method 2	
	IND	ATN	IND	ATN
Wavelength selected	241 nm	224.4 nm	233.8 nm	224.4 nm
Beer's law range	2-20 µg/ml	10-80 µg/ml	2-20 µg/ml	10-80 µg/ml
Correlation coefficient (r^2)	0.9993	0.999	0.9992	0.999
Regression equation	y=0.0657x-0.011	y=0.031x+0.0149	y=0.0607x-0.0197	y=0.031x+0.0149
Slope	0.0657	0.031	0.0607	0.031
Intercept	0.0011	0.0149	0.0197	0.0149

TABLE 2: PRECISION DATA REPEATABILITY

S. no	Method 1		Method 2	
	Absorbance at 241 nm	Absorbance at 224.4 nm	Absorbance at 233.8 nm	Absorbance at 224.4 nm
Mean	0.339	1.662	0.941	1.761
SD	0.0029	0.0146	0.0142	0.0276
% RSD	0.8688	0.8837	1.5136	1.5691

TABLE 3: INTRADAY AND INTERDAY DATA

Wavelength	Method 1			Wavelength	Method 2		
	Mean	SD	% RSD		Mean	SD	% RSD
Intraday							
241 nm morning	0.343	0.003	0.771	233.8 nm morning	0.951	0.008	0.898
Afternoon	0.342	0.002	0.608	Afternoon	0.947	0.002	0.244
Evening	0.341	0.003	0.880	Evening	0.951	0.006	0.616
224.4 nm morning	1.676	0.015	0.879	224.4 nm morning	1.778	0.020	1.125
Afternoon	1.673	0.007	0.432	Afternoon	1.778	0.005	0.283
Evening	1.671	0.012	0.719	Evening	1.782	0.017	0.967
Interday							
241 nm day 1	0.337	0.003	0.745	233.8 nm day 1	0.935	0.005	0.535
Day 2	0.342	0.001	0.337	Day 2	0.942	0.008	0.858
Day 3	0.346	0.006	1.642	Day 3	0.946	0.013	1.379
224.4 nm day 1	1.654	0.007	0.425	224.4 nm day 1	1.749	0.010	0.580
Day 2	1.680	0.009	0.540	Day 2	1.762	0.016	0.887
Day 3	1.683	0.018	1.056	Day 3	1.774	0.027	1.517

TABLE 4: ACCURACY DATA

Spike level (%)	Amount present (µg/ml)		Amount recovered (µg/ml)		% Recovery		Mean % recovery	
Drugs	IND	ATN	IND	ATN	IND	ATN	IND	ATN
80	1.2	24	1.2	23.43	100	97.6	100.2	98.63
	1.2	24	1.19	23.49	99.16	97.6		
	1.2	24	1.22	24.17	101.6	101		
100	1.5	30	1.53	30.77	102	103	100.4	101
	1.5	30	1.51	30.17	100.6	101		
	1.5	30	1.48	30.08	98.66	100		
120	1.8	36	1.77	36.26	98.33	101	98.7	99.69

TABLE 5: ROBUSTNESS DATA

Parameters	Method 1				Method II			
Change of UV	UV 1		UV 2		UV 1		UV 2	
Wavelength (nm)	241	224.4	241	224.4	233.8	224.4	233.8	224.4
Mean	0.342	1.676	0.349	1.706	0.945	1.771	0.938	1.754
SD	0.002	0.015	0.001	0.005	0.008	0.019	0.007	0.016
% RSD	0.608	0.902	0.286	0.343	0.899	1.077	0.799	0.952
Change of analyst	Analyst 1		Analyst 2		Analyst 1		Analyst 2	
Mean	0.341	1.673	0.342	1.669	0.945	1.771	0.938	1.754
SD	0.002	0.0100	0.0055	0.0080	0.0085	0.0190	0.0075	0.017
% RSD	0.609	0.5984	1.6072	0.4793	0.8996	1.0772	0.7995	0.9522
Change in the wavelength	Wavelength +2		Wavelength -2		Wavelength +2		Wavelength -2	
Wavelength (nm)	243	226.4	239	222.4	235.8	226.4	231.8	222.4
Mean	0.278	1.617	0.437	1.662	0.728	1.720	1.192	1.764
SD	0.004	0.008	0.0070	0.009	0.006	0.024	0.013	0.019
% RSD	1.695	0.525	1.605	0.558	0.827	1.423	1.107	1.132

TABLE 6: LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION LOD AND LOQ

Drugs	IND		ATN	
Method I	241 nm	224.4 nm	241 nm	224.4 nm
LOD	0.190	0.168	0.907	0.480
LOQ	0.576	0.509	2.749	1.454
Method II	233.8 nm	224.4 nm	233.8 nm	224.4 nm
LOD	0.116	0.168	0.642	0.480
LOQ	0.503	0.509	1.945	1.454

TABLE 7: ASSAY OF THE FORMULATION

Method	Brand name	Label claim of IND (µg/ml)	Label claim of ATN (µg/ml)	Amount found for IND (µg/ml)	Amount found for ATN (µg/ml)	% Recovery for IND (n=3)	% Recovery for ATN (n=3)
I	Aten D	2.5	50	2.49	49.28	99.79	98.57
II	Aten D	2.5	50	2.48	50.01	99.56	100

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Conflict of interests:

The authors declared no conflict of interests.

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