Research Article

Advanced UV spectrophotometric method development and validation for simultaneous estimation of Azelnidipine and Telmisartan in Pharmaceutical Dosage Form

Pallavi Suthar[1], Rajashree Mashru[2]

From, [1] Assistant Professor, Department of Chemistry, Shree Swaminarayan Sanskar Pharmacy College, Gujarat, [2] Professor, HOD of Pharmaceutical Quality Assurance Department, Maharaja Sayajirao University of Baroda, India.

Correspondence to: Pallavi Suthar, Assistant Professor, Department of Chemistry, Shree Swaminarayan Sanskar Pharmacy college, Near Zundal Circle, S. P. Ring Road, Between Chandkheda-Adalaj, Zundal, Gandhinagar- 382421, Gujarat, India. **Email:** pallaveemali12@gmail.com

ABSTRACT

The presented research work aims to develop and validate three advanced UV spectrophotometric methods for the simultaneous estimation of Azelnidipine (AZL) and Telmisartan (TEL). These methods offer a higher degree of sensitivity than already-existing methods of analysis. By implementing advanced spectroscopic techniques such as the simultaneous method, Q-ratio method, and first derivative spectroscopy method. These methods offer a higher degree of sensitivity than already-existing methods of analysis. By implementing advanced spectroscopic techniques such as the simultaneous method, Q-ratio method, and first derivative spectroscopy method. The linearity of the three methods was in the range of 2 µg/ml to 12 µg/ml for AZL and 10 µg/ml to 50 µg/ml for TEL. The correlation coefficients for simultaneous estimation were 0.999 and 0.998 for AZL and TEL, respectively, and 0.9992 and 0.9989 for AZL and TEL, respectively, for the first derivative method, whereas the correlation coefficients for the Q-ratio method were 0.999 and 0.9988, respectively. The LOD values obtained by the simultaneo us estimation method were found to be lower as compared to those obtained by the first derivative method and the Q-ratio method, proving that the sensitivity of the simultaneous estimation method is high.

Key words: Method development, Validation, Azelnidipine, Telmisartan, Concentration.

The chemical formula for Azelnidipine (AZL) is 3(1diphenylmethylazetidin3-yl)-5-isopropyl-12amino-1,4-dihydro-6-methyl-4-(3-nitrophenyl)-3,5pyridine dicarboxylate. It is a dihydropyridine (DHP) type calcium channel blocker (CCB) used for the treatment of hypertension [1, 2]. AZL has two enantiomers due to an asymmetric carbon at the 4-position of the DHP ring. The pharmacological action of AZL resides in the (R) enantiomer. This is in marked contrast to other CCBs in which the (S) enantiomer is responsible for the biological activity (Figure 1) [3, 4]. The peculiar three-dimensional structure of the active enantiomer of AZL may be related to its unique pharmacological features that are not shared by other DHPs, such as a long-lasting reduction in blood pressure, decreased heart rate, and anti-atherosclerosis effect [6, 8]. AZL also shows a diuretic effect by increasing urine volume and thus reducing the retention of ions [9, 10].

Telmisartan [TEL] is a chemical compound that is 2-(4[4-methyl-6-(1-methyl-1H-1, 3-benzodiazol-2-yl)-2-propyl1H-1, 3-benzodiazol-1-yl] methyl phenyl) benzoic acid [1113]. It is an angiotensin II receptor antagonist used in the management of hypertension. Generally, angiotensin II receptor blockers (ARBs) such as telmisartan bind to the angiotensin II type 1 (AT1) receptors with high affinity, causing inhibition of the action of angiotensin II on vascular smooth muscle and ultimately leading to a reduction in arterial blood pressure [14–17]. Recent studies suggest that telmisartan may also have PPAR-gamma agonistic properties that could potentially confer beneficial metabolic effects [17–18]. After a literature review, it was known that numerous methods had already been described for the estimation of Telmisartan, such as visible spectrophotometric methods, stability-indicating UV spectrophotometric methods, and RP-HPLC methods. For the determination of telmisartan in human plasma, advanced techniques such as LC-MS were also found [19-20].

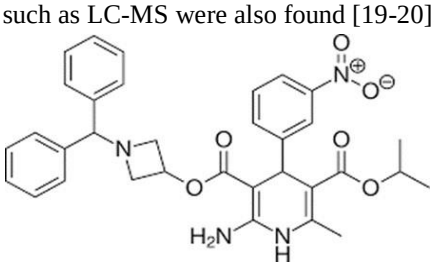

Figure 1 – Chemical structure of Azelnidipine

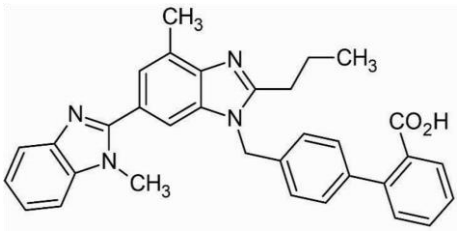

Figure 2 – Chemical Structure of Telmisartan

MATERIAL AND METHODS

Apparatus and Software: A Shimadzu UV-1700 doublebeam spectrophotometer connected to a computer with Shimadzu UV-Probe 2.10 software installed was used for all the spectrophotometric measurements. The samples were weighed on an electronic balance (A120) by Shimadzu.

Chemicals and Reagents: All the dilutions were made using Methanol as the diluent.

Preparation of Standard Stock Solution: The standard stock solutions of both AZL and TEL are prepared by taking 10 mg of the standard drug in a 10 ml volumetric flask and making up the volume using methanol as the diluent to achieve a concentration of 1000 µg/ml.

Preparation of Working Standard Solution: The working stock solution of AZL is prepared by taking 1 ml of the standard stock solution and transferring it to a 10 ml volumetric flask. The volume is made up to the mark using methanol to get a concentration of 100 µg/ml. Similarly, the standard working solution of TEL is prepared by taking 1 ml of the standard stock solution and transferring it to a 10 ml volumetric flask. The volume is made up to the mark using methanol to get a concentration of 100 µg/ml.

Preparation of Series for Calibration Curves for

Simultaneous Method, First Derivative Method, and QRatio Method: For preparing the solutions used in obtaining the calibration curve, a series of previously calibrated volumetric flasks were used. To prepare the linearity of Azelnidipine, 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, 1.0 ml, and 1.2 ml were withdrawn from the working standard solution of AZL and taken into separate volumetric flasks, and the volume was made up with methanol to prepare a series of solutions having concentrations in the range of 2 µg/ml to 12µg/ml. Similarly, to prepare the linearity of Telmisartan, 1 ml, 2 ml, 3 ml, 4 ml, and 5 ml were withdrawn from the working standard solution of TEL and taken into separate volumetric flasks, and the volume was made up with methanol to prepare a series of solutions having concentrations in the range of 10 µg/ml to 50 µg/ml.

Preparation of Sample Solutions (Test Solutions): Various pharmaceutical dosage forms are available for this particular drug combination, including the azova-T40 tablet dosage form. The dosage of each of these forms would vary according to the conditions of the patient. For the estimation of the azova-t40 formulation, one just needs to empty the content of azova-t40 into a 100ml volumetric flask and make up the volume using methanol. Then 2.5 ml of this solution is taken into another volumetric flask, and the volume is made up using methanol again. This solution is then placed in the UV spectrophotometer for quantitative analysis against a methanol blank.

Simultaneous Equation Method: This method uses the absorbance at two selected wavelengths, one at λ max of one drug where other drug also shows considerab le absorbance (λ2) and other being the wavelength at which the first drug has practically nil absorbance (λ1). Absorptivity of Azelnidipine and Telmisartan were calculated at both the wavelengths. The concentration of Azelnidipine and Telmisartan can be calculated from following equations:

Cy = A1ax2-A2ax1/ax2ay1 –ax1 ay2 … (1)

Cx = A2 ay1-A1ay2/ax2ay1- ax1ay2 … (2)

Where, A1 and A2 are the absorbance of mixture at λ1 and λ2 respectively, ay1 and ay2 are absorptivity of y at λ1 and λ2 respectively, ax1 is absorptivity of X at λ2, Cx is concentration of X, Cy is concentration of Y.

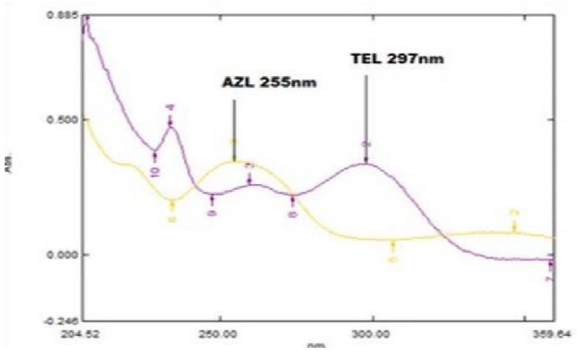

Figure 3 – Overlay Spectra of Azelnidipine and

Telmisartan

Calculation for Azelnidipine

Cx = A2 ay1-A1ay2/ax2ay1- ax1ay2

= 1.477*0.0282-1.466*0.356/0.084*0.282-0.366*0.356

= 7.92PPM

CALCULATION FOR TELMISARTAN

Cy = A1ax2-A2ax1/ax2ay1 –ax1 ay2

= 1.466*0.084-1.477*0.366/0.282*0.084-0.366*0.356

= 39.54PPM

Q-absorbance ratio method: This method, also called the "Absorption ratio method," is a modification of the simultaneous equation's method. According to this method, the ratio of absorbance at any two wavelengths for a substance, which obeys Beer's law, is a constant value independent of the concentration and path length. This constant is termed "Hufner's Quotient" or Qvalue.

The method involves the measurement of absorbance at two wavelengths, one being the λ max of one of the components (λ

2) and the other being a wavelength of equal absorptivity of the two components (λ 1), called the iso- absorptive point.

Cx = (Qm - Qy/Qx - Qy) *A/a1….(3)

Cy=(Qm-Qy/Qy-Qx) *A/a2….(4)

Where, Cx and Cy are the concentrations of x and y respectively, A is absorbance of sample at iso-absorptive wavelength and a1 and a2 are the absorptivity of x and y respectively at iso-absorptive wavelength.

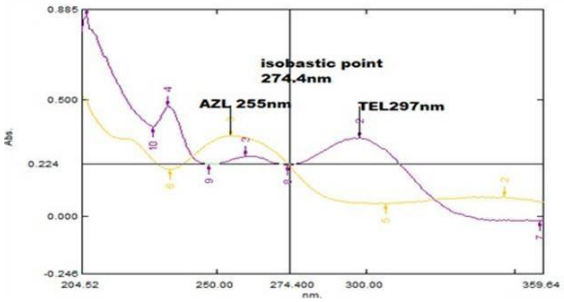

Figure 4 – Overlay Spectrum of Azelnidipine and

Telmisartan

First Derivative Zero Crossing Point Method: Derivative spectroscopy involves the conversion of a normal spectrum (a fundamental or zero-order spectrum) to its first, second, or higher derivative spectra by differentiating the absorbance of the sample with respect to the wavelength. The advantages of using derivative spectroscopy are that it leads to the separation of overlapped signals, the elimination of background caused by the presence of other compounds in a sample, and an improvement in the resolution of mixtures.

Sensitivity and Specificity: If the measured height of derivative peak of analyte is performed at those wavelengths at which the spectra of other components are undergoing zeroing (cross through the zero line), the measured amplitude is proportional only to concentration of the analyte in consideration – ZERO CROSSING TECHNIQUE.

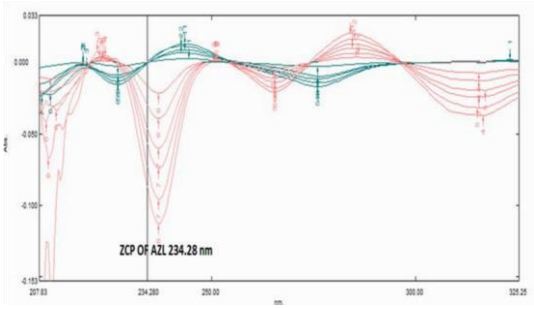

Figure 5 – Zero Crossing Point of Azelnidipine

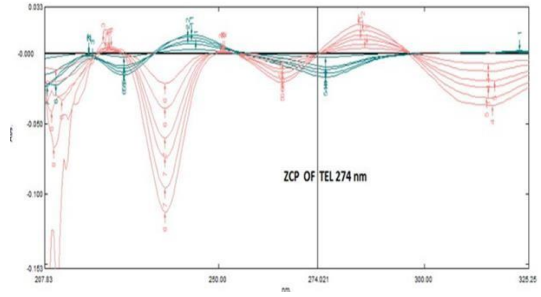

Figure 6 – Zero Crossing Point of Telmisartan

Validation Parameters

Linearity: In three of the developed methods, three sets of calibration curves were plotted between the absorbance and concentration. The calibration curve that showed the best values is represented below in Figures 7–8. The linearity of the simultaneous equation method was determined by the zero-order spectra of both drugs individually in the range of 200–400 nm. Thus, at 255 nm (the λ max of AZL), whereas at 297 nm (the λ max of TEL), the linearity of the simultaneous equation method was found in the range of 2 µg/ml to 12 µg/ml for AZL (r2 = 0.9996) and 10 µg/ml to 50µ g/ml for TEL (r2 = 0.9989).

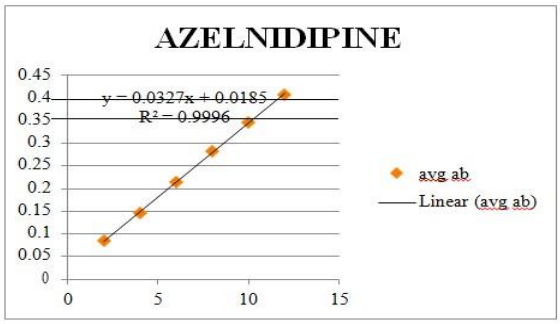

Figure 7 – Calibration Curve of Azelnidipine

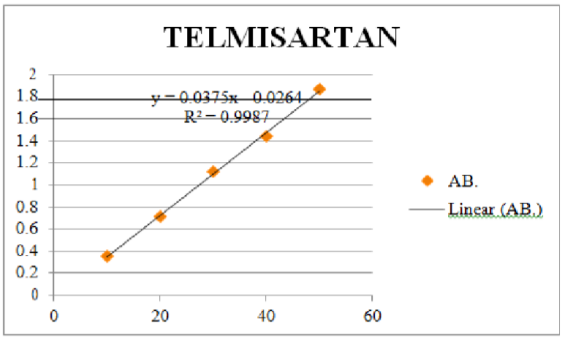

Figure 8 – Calibration Curve of Telmisartan

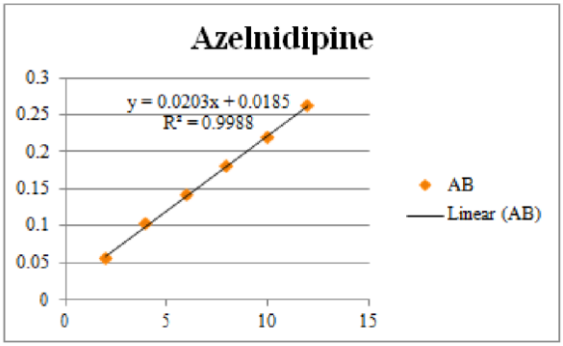

Figure 9 – Calibration Curve of Azelnidipine

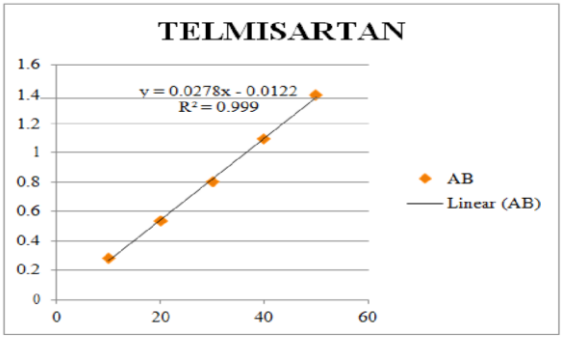

Figure 10 – Calibration Curve of Telmisartan

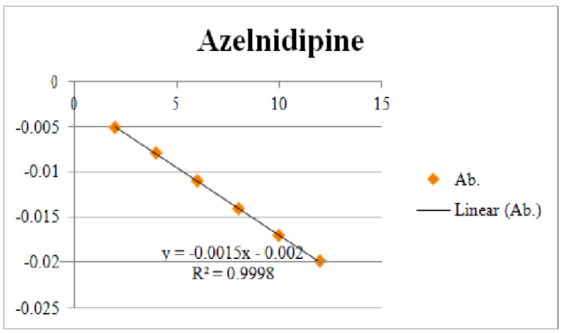

Figure 11 – Calibration Curve of Azelnidipine

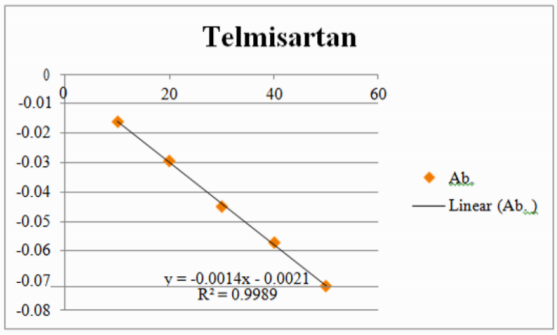

Figure 12 – Calibration Curve of Telmisartan

The absorbance ratio method compares absorbances at two different wavelengths, one being an isoabsorptive point and the other being the λ max of one of the two components. From the overlay spectra of two drugs, it is evident that AZL and TEL show an isoabsorptive point at 274.4 nm. The second wavelength employed is 297 nm, which corresponds to the λ max of TEL. The linearity shown in Figure 9 & 10. The Q-absorbance ratio method was found in the range of 10 µg/ml to 50 µg/ml for TEL (r2 = 0.999) and 2 µg/ml to 12 µg/ml for AZL (r2 = 0.998).

Then these zero-order spectra were converted into their respective first derivative spectra using the UV Probe software itself. A Δ = 5 nm and a scaling factor of 10 were used to convert zero-order spectra to first derivative spectra. Thus, here at 234 nm (the zero crossing point of TEL), the estimation of AZL is possible, whereas at 274 nm (the zero crossing point of AZL), the estimation of TEL is done as shown in Figures 11 and 12. The linearity of the First Order Derivative Method was found in the range of 10 µg/ml to 50 µg/ml for TEL (r2 = 0.9989) and 2 µg/ml to 12 µg/ml for AZL (r2 = 0.9989).

Limit of Detection and Limit of Quantification

The Limit of Detection and Limit of Quantification was calculated using the series of calibration curves plotted. The LOD and LOQ values were determined using the following equations and the data is represented in below Table 1.

Table 1 – Limit of Detection and Limit of Quantification for Azelnidipine and Telmisartan

Parameter	Drag	Simultaneous Method	Q-Absorbtion Method	First Derivative
LOD	AZL	0.35	0.16	1.8
	TEL	0.73	0.77	1.71
LOQ	AZL	0.927	0.048	5.4
	TEL	2.16	2.34	5.16

Precision

The precision of an analytical method expresses the closeness of agreement between a series of measurements which are obtained by performing multiple samplings of the same homogenous sample under the given conditions of the method. In this section of the article the two developed methods have been analyzed for precision at two levels:

1. Repeatability (precision under the same operating conditions over a short interval of time)
2. Intermediate precision (variations in the results obtained at different intervals)

From the results of precision, it may be concluded that both the methods developed are precise as the %RSD values are less than 2. It also may be concluded here that the Dual Wavelength Method is more precise than the 1st Derivative Method. In the data below represents the repeatability data, whereas Table- 2 (A) and Table- 2 (B) present the data for intra-day and inter-day precision respectively. Precision data are represented in terms of %RSD and the nominal concentration of Azelnidipine (TEL) was kept 8µg/ml and 10µ (AZL) and Telmisartan g/ml respectively.

Accuracy: Recovery studies for the UV-Spectrophotometric methods were conducted using the Standard Addition Method by taking a nominal concentration of 10µg/ml for Azelnidipine and 20µg/ml for Telmisartan from the formulation (test sample) and then spiking this solution by 80%, 100% and 120% of standard drug (API) (Table 3).

Assay of Marketed Formulation; 20 tablets of formulation (Azova-T40) containing 8 mg of Azelnidipine and 40 mg of Telmisartan were weighed accurately. The average weight of tablets was found and tablets were powdered. The tablet powder equivalents to 40 mg of Telmisartan was weighed and transferred into 100ml volumetric flask and volume is made upto the mark using methanol to get 400 µg/ml solution.

The content was filtered through the Whatman filter paper to get clear solution. From the clear sample stock solution dilutions 1 ml was withdrawn and taken into 10 ml volumetric

flask and volume is made upto the mark using methanol to obtain 40 µg/ml of Telmisartan and 8µg/ml of Azelnidipine. The resulting solutions were analyzed for drug content by spectrophotometric method at 255 nm and 297 nm for AZL and TEL, respectively. Assay was repeated 6 times and standard deviation was calculated. The drug content in AZL and TEL was found by three methods and results are mention in below tables (Table 4).

RESULTS

The two methods discussed above may be compared with each other by comparing their Limits of Detections and Limits of Quantifications as shown in the Table 5. ANOVA and the t-test were used to obtain the assay results from the simultaneous equation method and the QAbsorbance Ratio method, and the results are shown in Table 6.

Table 2 – Precision (A) Repeatability (B) Interday Precision

Repeatability									
Azelnidipine					**Telmisartan**				
Concentration	Parameter	Simultaneous method	Q-Ratio	First derivative	Concentration	Parameter	Simultaneous method	Q-Ratio	First derivative
8 µg/ml	Mean	0.0285	0.2803	0.0135	10 µg/ml	Mean	0.0155	0.1816	0.0155
	SD	0.00164	0.00136	0.0016		SD	0.00055	0.0013	0.0005
	%RDS	0.001	0.4872	0.0010		%RSD	0.001	0.7519	0.001

Table 3 – Accuracy of Azelnidipine and Telmisartan

	Simultaneous			Q-ratio			First derivative		
Interday – Azelnidipine									
Conc. µg/ml	**Mean**	**SD (n=3)**	**%RSD**	**Mean**	**SD (n=3)**	**%RSD**	**Mean**	**SD (n=3)**	**%RSD**
6	0.217333	0.00152	0.702	0.142	0.0017	1.219	0.0106	0.0011	0.0010
8	0.284667	0.00115	0.405	0.181	0.0015	0.840	0.049	0.0615	0.010
10	0.344	0.002	0.581	0.22	0.001	0.454	0.016	0.0017	0.001
Interday –Tel misartan									
Conc. µg/ml	**Mean**	**SD (n=3)**	**%RSD**	**Mean**	**SD (n=3)**	**%RSD**	**Mean**	**SD (n=3)**	**%RSD**
10	0.354	0.0015	0.283	0.280	0.0015	0.544	0.016	0	0
20	0.714	0.0020	0.280	0.537	0.0025	0.468	0.0303	0.0005	1.903
30	1.075	0.002	0.186	0.802	0.0011	0.143	0.044	0.0005	1.29

Table 4 – Assay Results

Drug		Concentration taken (µg/ml)	Concentration of µg/ml	%Recovery	%RSD
Assay Result-Simultaneous method					
AZL		8	7.93	99.12	0.0144
TEL		40	39.93	99.98	0.0724
Assay Result –Q Absorption ratio method					
AZL	8	7.98	99.75	0.0147	
TEL	40	39.24	98.1	0.0723	
Assa y Result –First derivative method					
AZL	8	7.8	97.5	0.147	
TEL	40	39.65	99.12	0.0145	

Table 5 – Comparison of Methods

Para-meter	Drug	Simultaneous method	Q-absorption ratio method	First derivative method
LOD	AZL	0.35	0.16	1.8
	TEL	0.73	0.77	1.71
LOQ	AZL	0.927	0.048	5.4
	TEL	2.16	2.34	5.16

Table 6 – Two way ANOVA

ANOVA						
Source of Variation	**SS**	**DF**	**MS**	**F**	**P-Value**	**F Crit**
Sample	0.59535	3	0.19845	0.710972	0.55952	3.238872

Columns	0.464816667	1	0.464816669	1.665263	0.215232	4.493998
Interaction	1.100816667	3	0.366938889	1.314604	0.304208	3.238872
Within	4.466	16	0.279125			
Total	6.626983333	23				

CONCLUSION

The simple, rapid, accurate, and precise simultaneous methods, Q absorbance ratio and first derivative methods of UV spectroscopy have been developed and validated for the routine analysis of AZL and TEL in API and pharmaceutical dosage form. This method was validated as per the ICH guidelines. The values of the standard deviation and coefficient of variation calculated were satisfactory, which indicates the suitability of the proposed methods for routine estimation of AZL and TEL. According to LOD, LOQ, and assay results, the simultaneous method is more accurate and precise than the Q-ratio method and the first derivative method. There was a significant difference between the three methods for AZL and TEL.

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