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APPLICATION OF TWO NOVEL SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS ESTIMATION OF LOSARTAN POTASSIUM AND CHLORTALIDONE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Objective: To develop two novel, simple, specific and accurate spectrophotometric methods for simultaneous estimation of Losartan Potassium and Chlortalidone without prior separation steps.

Methods: Ratio difference spectrophotometric method (RDSM) and mean centering of ratio spectra method (MCR).

Results: Calibration curves of the two methods are linear over the concentration range of 10-40µg/ml and 10-60µg/ml for Losartan Potassium and chlortalidone respectively, with good correlation coefficients. The methods were validated as per ICH guidelines; accuracy, precision and repeatability are found to be within acceptable limit. **Conclusion:** The two novel spectrophotometric methods are simple, accurate, precise, reproducible, economic and valid for application in laboratories lacking liquid chromatographic instruments.

KEYWORDS: Losartan Potassium, Chlortalidone, ratio difference spectrophotometric method, mean centering of ratio spectra.

INTRODUCTION[1, 2]

Losartan Potassium (LOS), chemically 2-Butyl-4-chloro-1-[(2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl)methyl]-1H-imidazole-5-methanol mono potassium salt(fig.1) is the first orally active Angiotensin II receptor antagonist available for the treatment of hypertension and Chlortalidone (CTD), 2-Chloro-5'-(1-hydroxy-3-oxo-1-isoindolinyl)benzene

sulfonamide(fig.2) is long acting thiazide like antihypertensive diuretic used in the treatment of edema associated with congestive heart failure.

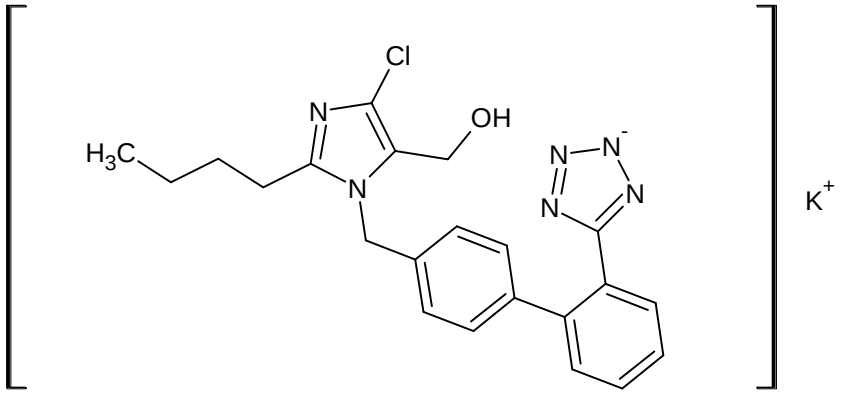

Fig.1.Structure of Losartan Potassium (LOS)

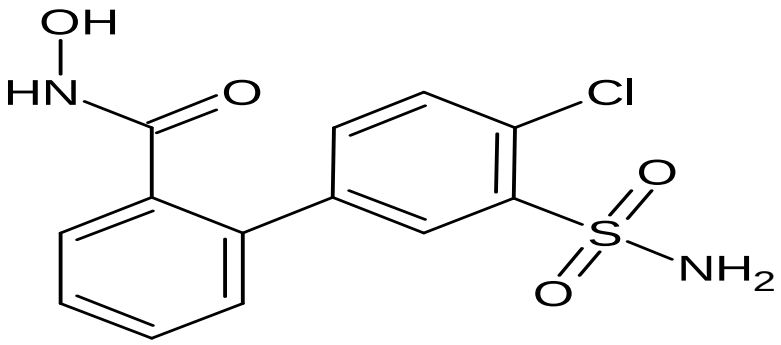

Fig.2.Structure of Chlortalidone (CTD)

The use of Angiotensin receptor blocker and diuretic in combination has the potential to afford additive blood pressure decrease by targeting different mechanisms involved in BP control. The literature review revealed that very few methods are reported for the simultaneous estimation of Losartan Potassium and Chlorthalidone which include high-performance liquid chromatography, ratio derivative method and area under curve method. The goal of present work is to develop two new simple spectrophotometric methods that could be applied in quality control laboratories for the simultaneous determination of both drugs.

MATERIALS AND METHODS

Instrumentation

Spectrophotometric analysis was carried out on a JASCO V 560 double beam spectrophotometer with fixed slit width (5nm) using a pair of 1cm matched quartz cells.

Software

Microsoft excel was used for handling data and processing calculations.

Materials

Reference standards of Losartan Potassium and Chlortalidone. Methanol HPLC grade obtained from S D Fine - Chemicals Limited, Mumbai. Marketed combination product of Losartan Potassium and Chlorthalidone (Covance-CT containing 50mg Losartan Potassium and 12.5mg Chlortalidone marketed by Ranbaxy Laboratories Pvt. Ltd.)

Stock and working standard solutions

Stock standard solutions

Losartan Potassium and Chlortalidone stock standard solutions (both are 1mg/ml), prepared by dissolving 50mg of LOS and CTD, respectively, in a few milliliters of methanol into a 50ml volumetric flasks and then completing to volume with the same solvent.

Working standard solutions

LOS and CTD working standard solution (both 100µg/ml), prepared by transferring 5ml of each LOS and CTD stock solutions, into 50ml standard flask and complete to volume with the same solvent.

PROCEDURE

Spectral characteristics and wave length selection

The absorption spectra of 10µg/ml of LOS and 10µg/ml CTD were recorded over the spectral wavelength range of 220-350nm using methanol as blank.

Linearity and construction of calibration curves

Accurately pipetted out 1-4ml of LOS and 1-6ml of CTD from their working standard solution into two series of 10ml standard flasks and volume was made upto the mark using methanol. The spectra of the prepared standard solutions were scanned from 220-350nm and stored in the computer.

For ratio difference spectrophotometric method (RDSM)

The stored spectra of LOS were divided by the spectrum of 60µg/ml CTD while CTD spectra were divided by 40µg/ml of LOS.

Calibration curves of LOS and CTD were constructed by plotting the difference between the amplitudes of ratio spectra at 263 and 280nm for LOS and 286 and 277nm for CTD, versus the corresponding concentrations and the regression equations were computed.

For mean centering of ratio spectra method (MCR)

The scanned spectra of LOS were divided by the normalized absorption spectrum of CTD (1µg/ml) and that of CTD were divided by the normalized spectrum of LOS (1µg/ml) and the obtained ratio spectra of both LOS and CTD were mean centered.

The calibration curves for LOS and CTD were constructed by plotting the mean centered values at 250 and 279.5nm respectively, versus the corresponding concentrations and the regression equations were computed.

Application of RDSM and MCR method for the determination LOS and CTD in laboratory prepared mixtures

40 mg of Losartan Potassium RS and 10 mg of Chlorthalidone RS were weighed separately and transferred into a 100ml standard flask. The drug mixture was allowed to dissolve in sufficient quantity of methanol by shaking for 15 min and the volume was made upto the mark with methanol to obtain a mixture with concentration of 400µg/ml of Losartan Potassium and 100µg/ml of Chlorthalidone.1 ml of this solution is accurately pipetted out into a 10 ml standard flask and made upto the volume with methanol to get a concentration of 40µg/ml and 10µg/ml Losartan Potassium and Chlorthalidone respectively.

Zero order absorption spectra of laboratory prepared mixture were recorded from 220-350nm using methanol as blank and the procedure under linearity for each method was then followed. Concentration of LOS and CTD in the prepared samples was calculated from the corresponding computed regression equations.

Application to pharmaceutical preparation

Twenty tablets of Covance-CT (each tablet contain 50mg LOS and 12.5mg CTD) were weighed; average weight of one tablet was determined and finely powdered with the help of mortar and pestle. A quantity of powder equivalent to 50mg of LOS, and 12.5mg of CTD was accurately weighed, transferred to a stoppered flask and extracted with 20ml of methanol initially by shaken vigorously for 15 minutes. The solution was transferred to a 50 ml standard flask through a Whatman No. 1 filter paper. The residue was then further extracted twice with 10ml methanol and transferred to the same standard flask through the same filter paper. The volume was finally made upto 50 ml with methanol. The resulting solution had a concentration of 1000µg/ml of LOS, and 250µg/ml of CTD. From this, a solution containing

40µg/ml LOS and 10µg/ml CTD was prepared after suitable dilutions. The general procedure described above under each method was followed to determine the concentration of both drugs in the prepared dosage form solution. The analysis was done in six times. Concentration of LOS and CTD in the prepared samples was calculated from the corresponding computed regression equations.

RESULTS AND DISCUSSION

This paper describes the application of two recently developed spectrophotometric ratio-spectra methods for the simultaneous determination of LOS and CTD.

Ratio difference spectrophotometric method (RDSM)

The method comprises of two critical steps, the choice of the divisors and the selection of wavelength at which measurements are recorded. The selected divisors should compromise between minimal noise and maximum sensitivity. The divisor concentrations 60µg/ml CTD and 40µg/ml LOS gave the best results regarding the average percentage recovery when used for the prediction of LOS and CTD concentrations, respectively. The overlaid ratio spectra of LOS and CTD are shown in fig.3 and fig.4 respectively.

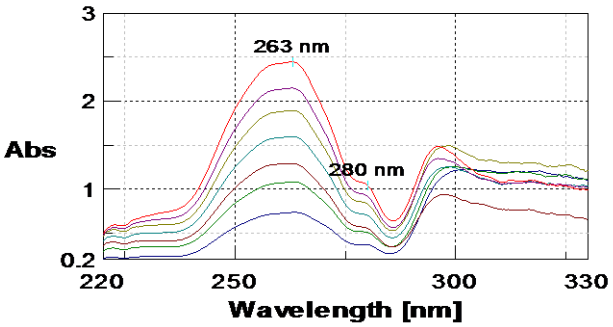

Figure.3: Ratio spectra of Losartan Potassium overlaid

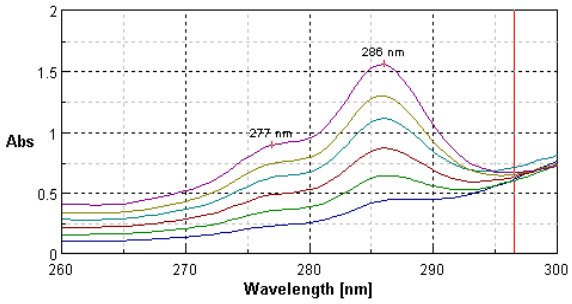

Figure.4: Ratio spectra of Chlorthalidone overlaid

Any two wavelengths can be selected provided that they exhibit different amplitudes in the ratio spectrum and a good linearity is present at each wavelength individually. Linear correlation was obtained between the differences in amplitude at 263nm and 280nm

(ΔP=263nm-280nm) for LOS and at 286nm and 277nm (ΔP=286nm-277nm) for CTD, against the corresponding concentrations of LOS and CTD, respectively. The calibration curves obtained are shown in fig.5 and fig.6 respectively.

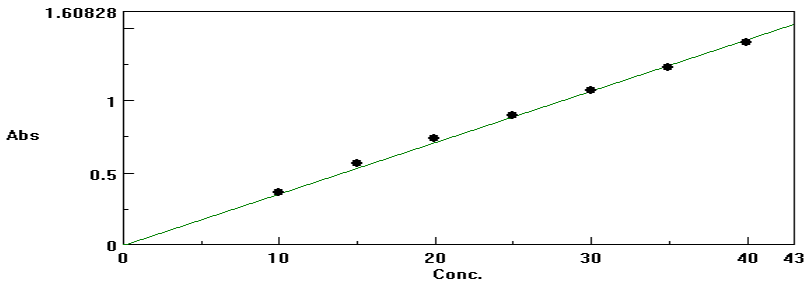

Figure.5: Calibration curve of Losartan Potassium using 60µg/ml Chlorthalidone as divisor.

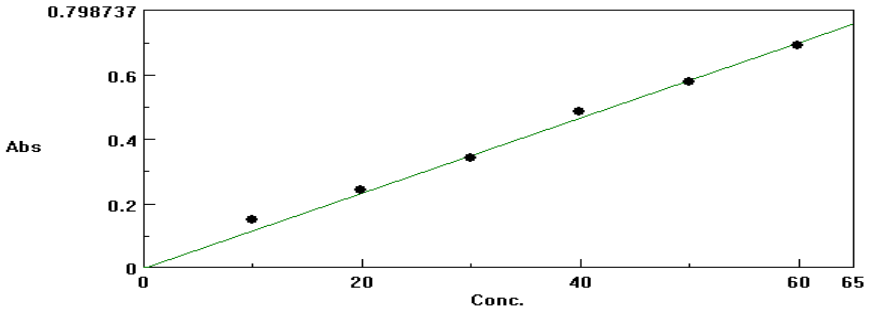

Figure.6: Calibration curve of ratio spectra of Chlorthalidone using 40µg/ml Losartan Potassium as divisor.

The concentration of LOS and CTD in prepared mixtures and samples was determined from corresponding computed regression equations. Ratio spectra of sample solution using 60µg/ml CTD as divisor and 40µg/ml LOS as divisor are shown in fig.7 and fig.8.

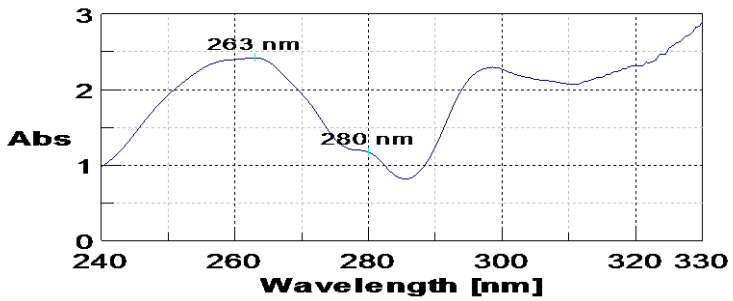

Figure .7: Ratio spectra of sample solution using 60µg/ml of Chlorthalidone as divisor.

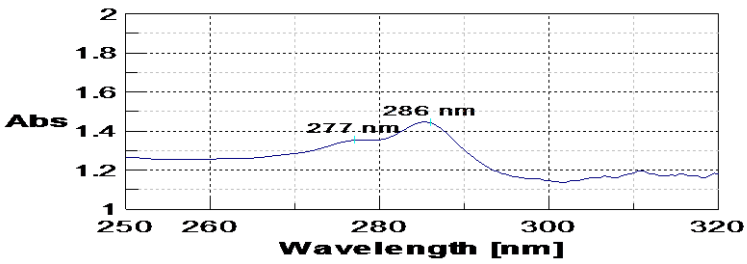

Figure.8: Ratio spectra of sample solution using 40µg/ml of Losartan Potassium as divisor.

Mean centering of ratio spectra

The absorption spectra of the standard solutions of LOS were recorded and divided by the normalized spectrum of CTD and the obtained ratio spectra were mean centered. Overlay of mean centered ratio spectra of LOS are shown in fig.9. Calibration curve was plotted (fig.10) by measuring the amplitude at 250nm (corresponding to a maximum in the MC of ratio spectra) versus corresponding concentrations.

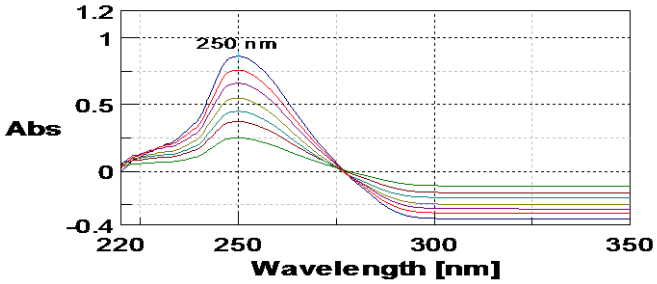

Figure.9: Mean centered ratio spectra of Losartan Potassium overlaid.

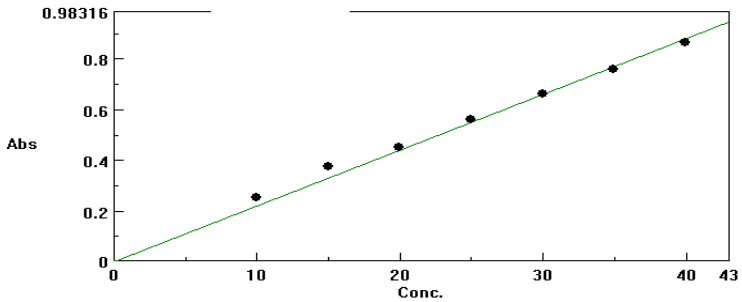

Figure.10: Calibration curve of mean centered Losartan Potassium corresponding to maximum wavelength at 250nm.

In the same way, the absorption spectra of CTD were divided by normalized spectra of LOS and then mean centered. Overlay of mean centered ratio spectra of CTD are shown in fig.11.

Calibration curve was plotted (fig.12) by measuring the amplitude at 279.5nm (corresponding to the minimum in the MC of ratio spectra) versus corresponding concentrations.

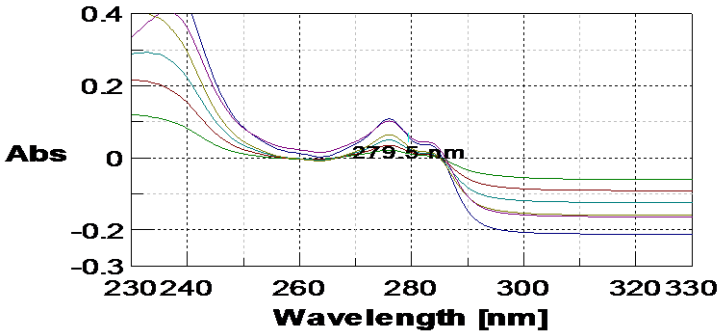

Figure.11: Mean centered ratio spectra of Chlorthalidone overlaid.

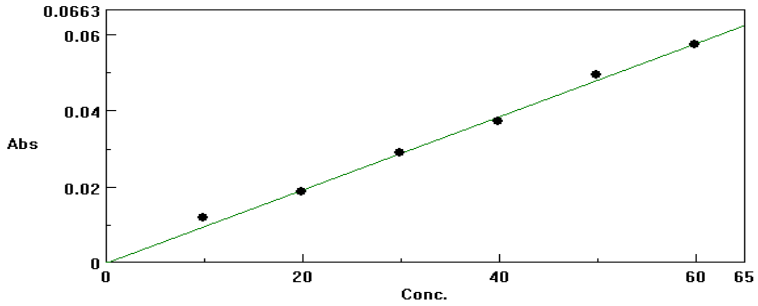

Figure.12: Calibration curve of mean centered Chlorthalidone at 279.5nm corresponding to minimum wavelength.

The absorption spectra of mixtures and samples were also divided by normalized spectrum of CTD and LOS and then mean centered (fig.13 and fig.14). Amplitude at 250nm and 279.5nm was measured and the concentration of LOS and CTD in prepared mixtures and samples was determined from corresponding computed regression equations.

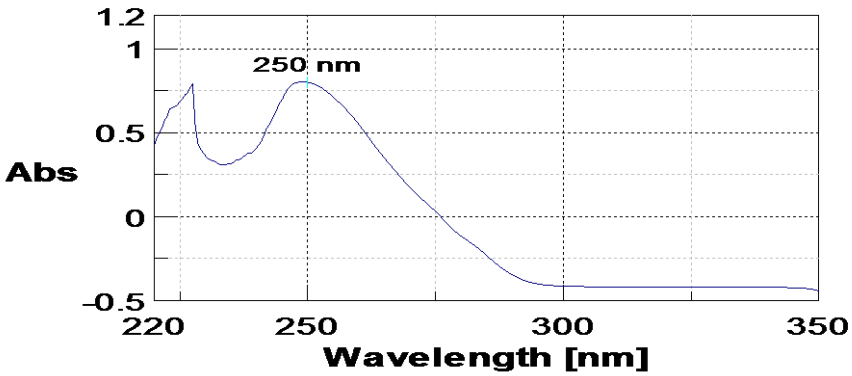

Figure.13: Mean centered spectrum of sample using normalized spectrum of Chlorthalidone as divisor.

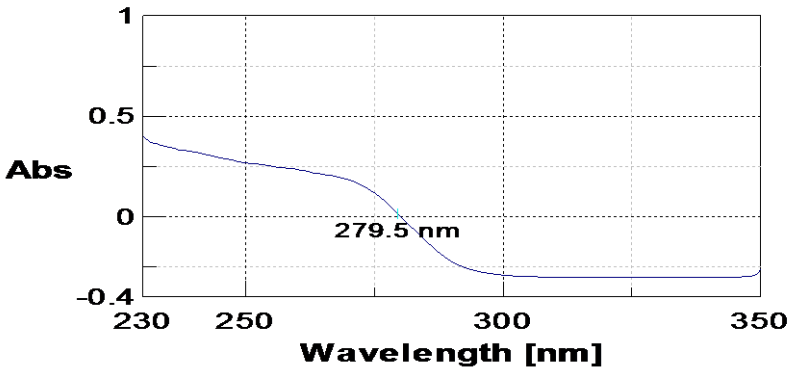

Figure.14: Mean centered spectrum of sample using normalized spectrum of Losartan Potassium as divisor

METHOD VALIDATION

Validation of the proposed methods was done according to ICH guidelines.

Linearity

The linearity of the methods was evaluated by analyzing seven concentrations of LOS and six concentrations of CTD ranging 10-40µg/ml and 10-60µg/ml, respectively. Each concentration was repeated three times. The assay was performed according to the experimental conditions previously mentioned. The linear regression equations are summarized in Table 1.

Range

The calibration range was established through consideration of the practical range necessary according to adherence to Beer's law and the concentration of LOS and CTD present in the pharmaceutical preparations to give accurate, precise and linear results as shown in Table 1.

Sensitivity

The limit of detection and limit of quantitation were determined based on the standard deviation of response (y-intercept) and slope of the calibration curve according to ICH guidelines. (Table.1)

Precision

The intraday and interday precision was also evaluated using six determinations at 100% test concentration. The developed method is found to be precise as the % RSD values were less than 2% (Table.1)

Accuracy

Accuracy of the methods was assured by applying the standard addition technique where good percentage recoveries were obtained, confirming the accuracy of the proposed methods (Table.2). The recovery studies were carried out by adding known amount of standard to samples at 80, 100 and 120% level and analyzed by the proposed method, in triplicate.

Table 1: Assay parameters and method validation obtained by applying proposed methods.

Parameter	RDSM		MCR	
	LOS	CTD	LOS	CTD
Range (µg/ml)	10-40	10-60	10-40	10-60
Slope	0.034	0.011	0.0200	0.0011
Intercept	0.043	0.027	0.0550	0.0010
Correlation coefficient(r)	0.999	0.997	0.9980	0.9950
LOD (µg/ml)	0.0776	0.2100	1.4190	1.5000
LOQ (µg/ml)	0.2353	0.6364	4.3000	4.5455
RSD %[a]	0.0182	0.1088	0.4091	0.4652
RSD %[b]	0.0199	0.1135	0.3574	0.3785

a: intraday(n=6), concentrations repeated 3 times within the same day

b: interday(n=6), concentrations repeated 3 times in three consecutive days.

Table 2: Recovery studies of the proposed methods.

Level of % recov-ery	Amount of std added (mg)		% drug recovery				%RSD			
			RDSM		MCR		RDSM		MCR	
	LOS	CTD	LOS	CTD	LOS	CTD	LOS	CTD	LOS	CTD
80%	40	10	100.06	99.94	99.93	99.67	0.0556	0.1375	0.6510	0.7663
100%	50	12.5	100.02	99.95	99.8	100.00	0.0099	0.1114	0.6095	0.7000
120%	60	15	100.11	99.96	99.99	100.17	0.0152	0.0917	0.0153	0.4501

Application of methods in assay of tablets.

The proposed spectrophotometric ratio-spectra methods were applied for the determination of LOS and CTD in their combined pharmaceutical formulation (Covance-CT tablets) and the results are shown in table 3. The high percentage recoveries values confirm the suitability of the proposed methods for the routine determination of these components in combined formulation.

Table 3: Estimation of dosage form by proposed methods.

Formulation	Drug	Label claim	%Assay± SD		%RSD	
			RDSM	MCR	RDSM	MCR
Covance-CT tablet	LOS	50mg	100.05±0.31	100.05±0.84	0.3095	0.8395
	CTD	12.5mg	99.00±0.67	100.90±1.05	0.6697	1.0406

CONCLUSION

The proposed methods offer significant advantages over conventional methods because of its speed and ease of operation. The methods work without the need of preconcentration or extraction steps. Moreover, using Microsoft excel, for manipulation of spectral data during handling these methods, eliminates the need for using specific expensive software.

Regarding simplicity, the proposed methods showed minimal data manipulation; instead of applying a certain order derivative in the derivative ratio method or subtraction of a constant and re-multiplication by the divisor in the ratio subtraction method, simply measuring the maximum/minimum amplitude in mean centered ratio spectrum (MCR) or calculating the amplitude difference at any two wavelengths in the ratio spectrum (RDSM).

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