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Determination of Losartan Potassium and Hydrochlorothiazide Mixture by AUC-CM and DRS_1 Spectrophotometric Methods

Prof. Dr. Saad Antakli [1,*]

ABSTRACT

Determination of losartan potassium LOS and hydrochlorothiazide HCTZ by two methods in raw material and tablets formulations. The first method is the area under the curve correction method (AUC-CM). The second one is the first derivative of the ratio spectra method (DRS_1). The determination by (AUC-CM) was at the wavelength's ranges (245 - 255) nm for LOS and (312 - 320) nm for HCTZ. The LOS linearity was to be between (2.00 – 48.00) µg/mL with $R^2 = 0.99994$, while the HCTZ linearity was to be between (7.50 – 100.00) µg/mL with $R^2 = 0.99987$. By using (DRS_1), wavelengths of 237.0 nm and 270.0 nm, used for the determination of LOS and HCTZ, respectively. The LOS linearity was to be between (1.50 – 50.00) µg/mL with $R^2 = 0.99998$, while the HCTZ linearity was to be between (1.00 – 20.00) µg/mL with $R^2 = 0.99996$. The validation of the two methods was estimated as linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, robustness, recovery and specificity. The two methods were successfully applied for the determination of LOS and HCTZ in tablets formulations in one Syrian pharmaceutical product LOTID in two doses (50/12.5 and 100/25) mg/tab. The proposed methods are not complicated, available in spectrophotometer programming, direct, and sensitive and does not require any pre-extraction process. Thus, the two methods could be ready to use in routine analysis and quality control.

KEYWORDS: Losartan Potassium, Hydrochlorothiazide, Spectrophotometry.

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1 Faculty of Farmacy, Al-Wataniya Private University, Hama, Syria.

* Corresponding author. E-mail address: Saad.Intakli@wpu.edu.sy

1. Introduction

Losartan potassium, Figure (1-a), ($C_{22}H_{22}ClKN_6O$, 461.00 g/mol, CAS No. 124750-99-8), has the chemical name (1H-Imidazole-5- methanol, 2-butyl -4-chloro-1-[[2-(1Htetrazol-5-yl) [1,1-biphenyl] -4-yl] methyl]-, monopotassium salt; 2-Butyl-4-chloro-1-[p-(o-1H-tetrazol-5-ylphenyl) benzyl] imidazole-5-methanol, monopotassium salt [1], is belongs to angiotensin-II receptor antagonist, which lowers the blood pressure and improves blood flow [2,3].

Hydrochlorothiazide, Figure (1-b), ($C_7H_8ClN_3O_4S_2$, 297.74 g/mol, CAS No. 58-93-5), with the chemical name 2H-1,2,4-Benzothiadiazine-7-sulfonamide, 6-chloro-3,4-dihydro-,1,1-dioxide [1], is a class of thiazide diuretics and is frequently prescribed globally for the treatment of essential hypertension in patients whose blood pressure cannot be controlled with LOS and HCTZ alone [4-6].

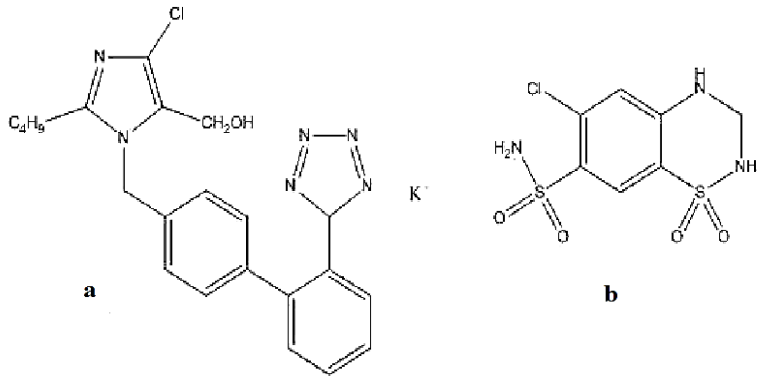

Figure (1): Chemical structures of losartan potassium (a), hydrochlorothiazide (b) [1].

2. Research aim

The aim of the research is to determine the combination of LOS and HCTZ using two methods. The first method is the area under the curve correction method (AUC-CM). The second one is the first derivative of the ratio spectra method (DRS_1) by UV spectrophotometry which considered to be simple, precise, accuracy and low cost compared to HPLC, another important aim that to determine the two spectrally overlapping drugs (LOS and HCTZ), making these two methods the solution for their determination.

3. Related Work or Literature Review

Various approaches have been described to estimate the dosage of LOS and HCTZ in some pharmaceutical preparations. Among these approaches, High-performance liquid chromatographic method (HPLC) [7–9], Reverse phase high-performance liquid chromatographic method (RP-HPLC) [10–15], Ultra-performance liquid chromatographic method (ULPC) [16], Spectrophotometric method (UV) [9,17–19] have been successfully applied to estimate the two compounds.

The determination of these two drugs by Spectrophotometric method is a frequent analytical problem in quality control in the pharmaceutical industries. The two drugs were be studied in this work showed strong absorption spectra overlap. Hence, their

simultaneous determination is hard when conventional spectrophotometric techniques are used, Therefore, the goal of this research is to develop simple, quick, and precise methods for the simultaneous estimation of LOS and HCTZ.
The simultaneous determination choice was by the spectral techniques: area under the curve correction method (AUC-CM) and derivative of the ratio spectra method (DRS_1).

4. Apparatus

T80+, UV/Vis. spectrophotometer PG instrument Ltd (UK), connected to computer, quartz cells 1 cm. Ultrasonic bath Daihan (China), and stirrer Velp Scientifica (Europe).

5. Chemical Reagents

Losartan potassium purity 100.1% and Hydrochlorothiazide purity 99.38% were obtained successively from Zhejiang Huahai Pharmaceutical **(**China**)** and Beijing Huawei Ruike Chemical (china), Sodium hydrogen carbonate assay (99.5 - 100.5) % from MERCK (Germany), and double distilled water was used.

6. Stock Solution

Stock solution 500 µg/mL of LOS and 250 µg/mL of HCTZ were prepared by separately dissolving appropriate weights of raw material (by taken the purity in consideration) in volumetric flask 50 mL and completed to volume with sodium hydrogen carbonate 0.1 M. The working standard solutions of each pharmaceutical sample is prepared by appropriate dilutions of stock solutions with sodium hydrogen carbonate 0.1 M to give concentrations between (1.50 - 50.00) µg/mL of LOS and (1.00 – 20.00) µg/mL of HCTZ.

7. Sample Preparation

One Syrian product LOTID in two doses (50/12.5 and 100/25) mg/tab were studied:

- Twenty tablets of LOTID (PHARMASYR), (LOS/HCTZ) 50/12.5 mg/tab were accurately weighed and finely powdered. An accurate weight equivalent of one tablet of 50 mg of LOS and 12.5 mg of HCTZ, dissolved in 0.1 M sodium hydrogen carbonate solution. The sample solution was filtered through a paper filter placed in a flask of 50 mL and adjusted to volume with 0.1 M of $NaHCO_3$. Then, 200 µL of this solution was taken to a 10 mL volumetric flask and adjusted to volume with 0.1 M of $NaHCO_3$, to obtain a solution that will have a concentration theoretically equivalent to 20.00 $\mu g.mL^{-1}$ of LOS [for the (AUC-CM) method and the (DRS_1) method]. And a solution that will have a concentration theoretically equivalent to 5.00 $\mu g.mL^{-1}$ of HCTZ [for the (DRS_1) method]. And 1000 µL of this solution was taken to a 10 mL volumetric flask and adjusted to volume with 0.1 M of $NaHCO_3$, obtain a solution that will have a concentration theoretically equivalent to 25.00 $\mu g.mL^{-1}$ of HCTZ [for the (AUC-CM) method]. Blank: 0.1 M $NaHCO_3$.
- Twenty tablets of LOTID (PHARMASYR), (LOS/HCTZ) 100/25 mg/tab were accurately weighed and finely powdered. An accurate weight equivalent of one tablet of 100 mg of LOS and 25 mg of HCTZ, dissolved in 0.1 M sodium

hydrogen carbonate solution. The sample solution was filtered through a paper filter placed in a flask of 100 mL and adjusted to volume with 0.1 M of $NaHCO_3$. Then, 200 µL of this solution was taken to a 10 mL volumetric flask and adjusted to volume with 0.1 M of $NaHCO_3$, to obtain a solution that will have a concentration theoretically equivalent to 20.00 $\mu g.mL^{-1}$ of LOS [for the (AUC-CM) method and the (DRS_1) method]. And a solution that will have a concentration theoretically equivalent to 5.00 $\mu g.mL^{-1}$ of HCTZ [for the (DRS_1) method]. And 1000 µL of this solution was taken to a 10 mL volumetric flask and adjusted to volume with 0.1 M of $NaHCO_3$, obtain a solution that will have a concentration theoretically equivalent to 25.00 $\mu g.mL^{-1}$ of HCTZ [for the (AUC-CM) method]. Blank: 0.1 M $NaHCO_3$.

8. Result and Discussion

The absorption spectra of LOS and HCTZ raw material in 0.1 M $NaHCO_3$ show that there is some degree of overlap between the two substances. The ratio of the ingredients of LOS and HCTZ is (4:1) respectively. In the absorption spectra of the ingredients of LOS and HCTZ, it is observed that HCTZ absorbs at 271.0 nm and 315.0 nm, whereas LOS absorbs at 250.0 nm deflection point. The zero order Spectral of LOS, HCTZ, and (LOS + HCTZ) Mixture is shown in Figure (2-a, b, c).

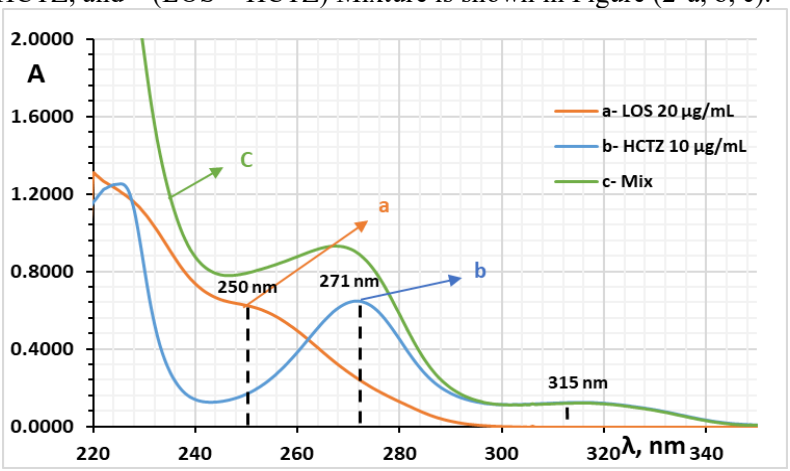

Figure (2): Zero-order spectra: a- los, b- hctz, c- los and hctz mixture.

8.1. Method (I): Area under the curve correction method (AUC-CM)

This method [20] developed to simultaneously determine LOS and HCTZ. Based on the theory of the absorption correction method (ACM) [21], a slight modification was made to the area under the curve method (AUC) [22]. This modification allowed the use of the area under the curve method instead of absorption, which helped to determine the components that have overlapping spectra by applying the least manipulation.

In the case of a binary mixture of LOS and HCTZ, where there is partial overlap of the spectra of the components at the region of (245 - 255) nm, there is no interference of the spectrum of LOS with HCTZ at the region of (312 - 320) nm, HCTZ can be determined directly by using the AUC at the region of (312 - 320) nm without

interference with the spectrum of LOS at this region, while the AUC of LOS at the region of (245 - 255) nm can be calculated by the following equation (1):

$$AUC_{LOS\,(245-255)\,NM} = AUC_{MIX\,(255-245)\,NM} - (F_{AUC} * AUC_{MIX\,(312-320)\,NM}) \quad (1)$$

Where; FAUC is area under curve factor of HCTZ raw material by the equation (2):

$$F_{AUC} = AUC_{HCTZ\,(245-255)\,NM} / AUC_{HCTZ\,(312-320)\,NM} \quad (2)$$

The area under curve factor was found to be equal to 1.7862. Standard curves of LOS and HCTZ were constructed by plotting the AUC values of LOS at the region of (245 - 255) nm and HCTZ at the region of (312 - 320) nm versus their concentrations in ($\mu g.mL^{-1}$).

8.2. Method (II): First derivative of the ratio spectra method (DRS_1)

Salinas created the method [23] that uses a standard divisor when the component spectra overlap. The analysis technique is based on dividing the mixture's spectrum into each analysis's standard spectra and then dividing the quotient to create an independent analyte concentration that can be utilized as a divisor. Experimental mistakes and background noise are reduced when a standard divisor is used. One benefit of ratio spectrum derivative spectroscopy over zero crossing derivative spectroscopy is that certain of the coexisting substances do not exhibit a maximum or minimum at zero crossing wavelengths.

The equation determines the combination of LOS and HCTZ from the absorption spectrum (3):

$$A_{Mix,\lambda 1} = \varepsilon_{LOS,\lambda 1}\, \ell\, C_{LOS} + \varepsilon_{HCTZ,\lambda 1}\, \ell\, C_{HCTZ}; \ell = 1 \quad (3)$$

Where;

$A_{Mix,\lambda 1}$ is absorbance value of the mixture at λ_1.

$\varepsilon_{LOS,\lambda 1}$, $\varepsilon_{HCTZ,\lambda 1}$ are molar absorptivity's of at λ_1.

C_{LOS}, C_{HCTZ} are molar concentrations of LOS and HCTZ.

ℓ is path length.

The equations are obtained by dividing the spectrum by the spectrum of a reference solution of one of the substances LOS of concentration $C°_{LOS}$ (4, 5):

$$\frac{A_{Mix,\lambda 1}}{\varepsilon_{LOS,\lambda 1} C°_{LOS}} = \frac{\varepsilon_{LOS,\lambda 1} C_{LOS}}{\varepsilon_{LOS,\lambda 1} C°_{LOS}} + \frac{\varepsilon_{HCTZ,\lambda 1} C_{HCTZ}}{\varepsilon_{LOS,\lambda 1} C°_{LOS}} \ldots \quad (4)$$

$$\frac{A_{Mix,\lambda 1}}{\varepsilon_{LOS,\lambda 1}} = C_{LOS} + \frac{\varepsilon_{HCTZ,\lambda 1}}{\varepsilon_{LOS,\lambda 1}} C_{HCTZ} \ldots \quad (5)$$

The equation is obtained from the ratio spectrum's first derivative (6):

$$\frac{d}{d\lambda}\left(\frac{A_{Mix(LOS+HCTZ),\lambda 1}}{\varepsilon_{LOS,\lambda 1}}\right) = C_{HCTZ} \frac{d}{d\lambda}\left(\frac{\varepsilon_{HCTZ,\lambda 1}}{\varepsilon_{LOS,\lambda 1}}\right) \ldots \quad (6)$$

According to equation (6), the derivative ratio spectrum of the binary mixture depends only on the value of C_{HCTZ}, regardless of the value of C_{LOS}. The LOS concentration was determined using the same technique.

The UV absorption spectra of standard solutions of LOS were divided by a standard spectrum of HCTZ (15.00 $\mu g.mL^{-1}$). The first derivative was calculated for the ratio

spectrum. The amplitudes at 237.0 nm were measured and found to be linear to the concentration of LOS. as seen in Figures (3, 4).

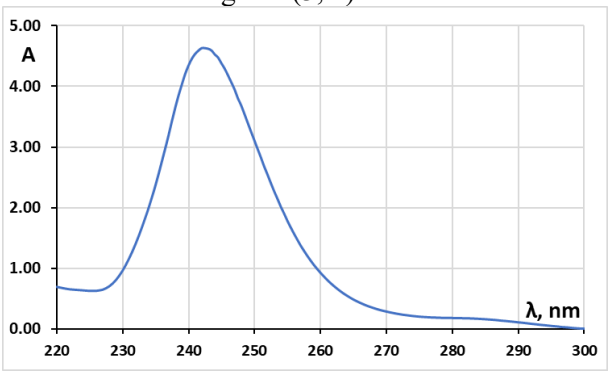

Figure (3): Ratio Spectra Of 20.00 µG/ML LOS, using A 15.00 µG/ML Hctz As Divisor.

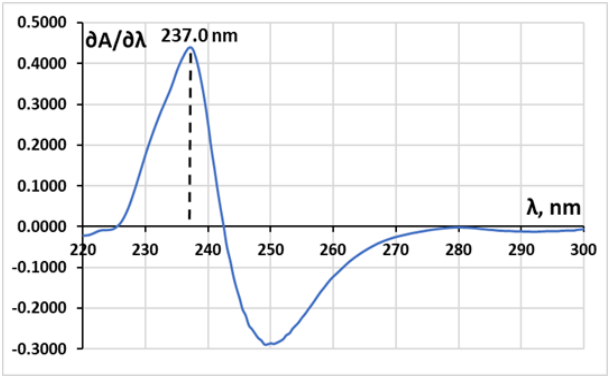

Figure (4): First Derivative Of The Ratio Spectra Of 20.00 µG/ML LOS, using A 15.00 µG/ML HCTZ AS DIVISOR.

For HCTZ, the UV absorption spectra of standard solutions of HCTZ were divided by a standard spectrum of LOS (µg.mL^{-1}). The first derivative was calculated for the ratio spectrum. The amplitudes at 270.0 nm were measured and found to be linear to the concentration of HCTZ. Figures (5, 6).

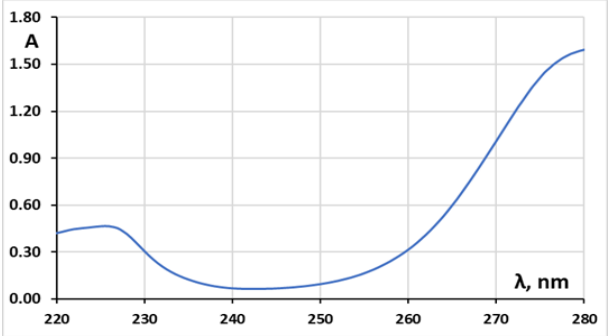

Figure (5): Ratio Spectra Of 10.00 µG/ML HCTZ, using A 40.00 µG/ML LOS AS DIVISOR.

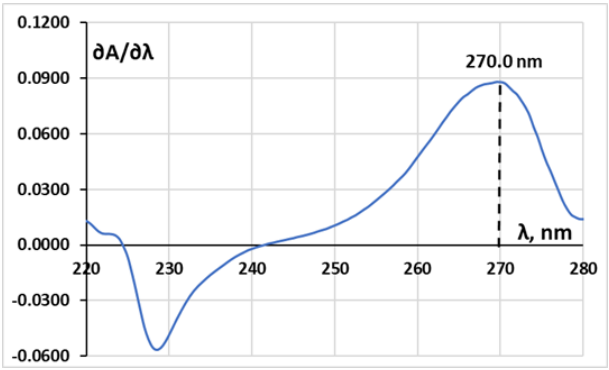

Figure (6): First Derivative Of The Ratio Spectra Of 10.00 µG/ML HCTZ, using A 40.00 µG/ML LOS AS DIVISOR.

9. Methods Validation

Methods validation consist of the linearity by regression equation, limit of detection (LOD), limit of quantification (LOQ), precision reported as RSD%, accuracy reported as percent%, robustness, recovery, and specificity were used to assess the validity and applicability of the suggested procedures.

9.1.Linearity

Linearity of LOS and HCTZ was checked by regression equation of LOS and HCTZ individually. The range of linearity of LOS is within (2.00 - 48.00) µg.mL^{-1} at (245.0 – 255.0) nm for (AUC-CM), and within (1.50 - 50.00) µg.mL^{-1} at 237.0 nm for (DRS_1). The range of linearity of HCTZ is within (7.50 - 100.00) µg.mL^{-1} at (312.0 – 320.0) nm for (AUC-CM), and within (1.00 - 20.00) µg.mL^{-1} at 270.0 nm for (DRS_1). Both methods show that the correlation coefficient values are closer to 1, which proves good linearity where the obtained results were considered accur,as disclosed shown in Table 1.

9.2.Limit of detection (LOD) and limit of quantification (LOQ)

Five distinct concentrations of both medications were examined in five repetitions in order to ascertain the LOD and LOQ of LOS and HCTZ. The following formulas were used to determine LOD and LOQ [24]:

$$LOD = \frac{3.3 \times SD}{m} \quad ; \quad LOQ = \frac{10 \times SD}{m}$$

Where SD is the standard deviation of y-intercepts (a) of regression lines and (m) is the slope of the equitation of calibration curve, y = a + m x, as presented show in Table 1.

9.3.Precision

Precision of the sophisticated methods was recognized by evaluating three different concentrations of concertation's (12.00, 24.00, 30.00) µg/mL for LOS and (8.00, 10.00, 12.00) µg/mL for HCTZ, which were analyzed as triplicates on the same day (Intra-day) and on three different successive days (Inter-day). Acceptable and

satisfying results RSD% which were less than 5% prove a good precision in both (Intra-day) and (Inter-day) as disclosed in Table 1.

Table (1): Linearity, LOD, LOQ, Precision for LOS and HCTZ.

Parameters	**(AUC-CM)**		**(DRS_1)**	
	LOS	HCTZ	LOS	HCTZ
Selected Wavelength (nm)	245.0 - 255.0	312.0 - 320.0	237.0	270.0
Linearity range µg/mL	2.00 - 48.00	7.50 - 100.00	1.50 - 50.00	1.00 – 20.00
Intercept (a)	0.03633	0.14122	0.00027	- 0.00002
Slope (m)	0.32629	0.09374	0.02211	0.00878
Regression equation	Y = 0.32629 X + 0.03633	Y = 0.09374 X + 0.14122	Y = 0.02211 X + 0.00027	Y = 0.00878 X - 0.00002
Correlation coef. (R^2)	0.99994	0.99987	0.99998	0.99996
LOD µg/mL	0.210	0.429	0.091	0.064
LOQ µg/mL	0.638	1.299	0.276	0.194
Intra-day* (RSD %)	1.20	1.44	1.09	1.05
Inter-day* (RSD %)	1.33	1.67	1.18	1.05

* The greatest RSD % average of five replicates, and three experiments.

9.4. Accuracy

Five repeated determinations were carried out on five distinct standard concentrations of LOS and HCTZ in order to verify the precision and accuracy of the two suggested procedures. The obtained results approximately border to 100% prove the accuracy of two methods, as seen as in Tables 2, 3.

Table (2): Accuracy and Precision for LOS determination by the two proposed methods.

Raw sample	**Method**	**Theoretical Concentration* µg.mL^{-1}**	**Observed Concentration* µg.mL^{-1}**	**Accuracy %**	**SD µg.mL^{-1}**	**Precision RSD %**
LOS	**AUC-CM**	6.00	6.01	100.17	0.18	3.00
		12.00	11.97	99.75	0.20	1.67
		24.00	23.98	99.92	0.24	1.00
		30.00	30.08	100.27	0.26	0.86
		36.00	36.16	100.44	0.19	0.53
	DRS_1	6.00	5.97	99.50	0.17	2.85
		12.00	12.05	100.42	0.23	1.91
		24.00	24.11	100.46	0.30	1.24
		30.00	30.07	100.23	0.38	1.26
		36.00	36.18	100.50	0.39	1.08

$\bar{x}$: mean of five replicated determinations,
Accuracy (%) = (observed concentration/theoretical concentration) x 100,
Precision (RSD %) = (standard deviation/mean concentration) x 100.

Table (3): Accuracy and Precision for HCTZ determination by the two proposed methods.

Raw sample	Method	Theoretical Concentration µg.mL^{-1}	$\bar{x}$ Observed Concentration µg.mL^{-1}	Accuracy %	SD µg.mL^{-1}	Precision RSD %
HCTZ	AUC-CM	10.00	10.02	100.20	0.17	1.70
		20.00	20.06	100.30	0.22	1.10
		30.00	29.82	99.40	0.25	0.84
		40.00	39.83	99.58	0.33	0.83
		50.00	50.06	100.12	0.38	0.76
	DRS_1	1.50	1.52	101.33	0.03	1.97
		3.00	2.98	99.33	0.07	2.35
		6.00	6.04	100.67	0.09	1.49
		7.50	7.48	99.73	0.11	1.47
		9.00	9.05	100.56	0.12	1.33

$\bar{x}$: mean of five replicated determinations,
Accuracy (%) = (observed concentration/theoretical concentration) x 100,
Precision (RSD %) = (standard deviation/mean concentration) x 100.

9.5. Robustness

An analytical procedure's robustness serves as a gauge of its dependability during typical use by determining how well it can withstand even very little changes in certain parameters [20].

The parameters under study were slit range, scan speed, and wavelength, which were per-formed at three different concentrations (10.00, 20.00, 30.00) µg.mL^{-1} for LOS and (8.00, 10.00, 12.00) µg.mL^{-1} for HCTZ. The results obtained results in Tables 4 and 5 showed that there were no significant differences between very little changes of studied parameters.

Table (4): Robustness test for LOS and HCTZ by (AUC-CM) method.

Raw sample	parameter	Deviation	$\bar{x}$ (µg/mL)	Per %	SD (µg/mL)	RSD %	$\bar{x}$ (µg/mL)	Per %	SD (µg/mL)	RSD %	$\bar{x}$ (µg/mL)	Per %	SD (µg/mL)	RSD %
LOS at (245.0 – 255.0) nm	Slit range	2 nm	10.00	100.00	0.14	1.40	19.98	99.99	0.19	0.95	30.07	100.23	0.25	0.83
	2 nm	1 nm	9.96	99.60	0.17	1.71	19.92	99.60	0.21	0.81	29.68	98.93	0.17	0.57
	Scan speed (Fast)	Fast	10.00	100.00	0.14	1.40	19.98	99.99	0.19	0.95	30.07	100.23	0.25	0.83
		Slow	9.99	99.90	0.10	0.90	20.06	100.30	0.14	0.70	30.20	100.67	0.22	0.73
		Medium	10.03	100.30	0.13	0.81	19.92	99.60	0.19	0.95	30.18	100.60	0.25	0.83
	Wave length	+ 2.0 nm	6.48	98.90	0.11	1.11	19.71	98.55	0.17	0.86	29.61	98.70	0.24	0.81
		- 2.0 nm	6.53	101.70	0.14	1.38	20.31	101.55	0.22	1.08	30.42	101.40	0.25	0.82
HCTZ at (312.0 – 320.0) nm	Slit range	2 nm	8.02	100.25	0.12	1.50	9.99	99.90	0.17	1.70	12.02	100.17	0.10	0.83
	2 nm	1 nm	7.92	99.00	0.09	1.14	9.88	98.80	0.13	1.32	11.96	99.67	0.17	1.42
	Scan speed (Fast)	Fast	8.02	100.25	0.12	1.50	9.99	99.90	0.17	1.70	12.02	100.17	0.10	0.83
		Slow	8.04	100.50	0.13	1.62	10.13	101.30	0.14	1.38	12.09	100.75	0.11	0.91
		Medium	7.99	99.88	0.11	1.38	10.10	101.00	0.19	1.88	12.05	100.42	0.17	1.41
	Wave length	+ 2.0 nm	7.91	98.88	0.15	1.90	9.88	98.80	0.09	0.91	11.89	99.08	0.13	1.09
		- 2.0 nm	7.95	99.38	0.11	1.38	9.91	99.10	0.12	1.21	11.92	99.33	0.09	0.76

$\bar{x}$: mean of five replicated determinations.

Table (5): Robustness test for LOS and HCTZ by (DRS1) method.

Raw sample	parameter	Deviation	$\bar{x}$ (µg/mL)	Per %	SD (µg/mL)	RSD %	$\bar{x}$ (µg/mL)	Per %	SD (µg/mL)	RSD %	$\bar{x}$ (µg/mL)	Per %	SD (µg/mL)	RSD %
LOS at 237.0 nm	Slit range	2 nm	10.02	100.20	0.16	1.60	20.10	100.50	0.19	0.95	30.11	100.37	0.26	0.86
	2 nm	1 nm	9.98	99.80	0.17	1.70	20.07	100.35	0.17	0.85	30.05	100.17	0.19	0.63
	Scan speed (Fast)	Fast	10.02	100.20	0.16	1.60	20.10	100.50	0.19	0.95	30.11	100.37	0.26	0.86
		Slow	10.03	100.30	0.12	1.20	20.05	100.25	0.19	0.95	30.14	100.47	0.28	0.93
		Medium	9.98	99.80	0.13	1.30	20.03	100.15	0.21	1.05	30.12	100.40	0.31	1.03
	Wave length	+ 0.5 nm	9.91	99.10	0.16	1.61	19.93	99.65	0.19	0.95	29.78	99.23	0.26	0.87
		- 0.5 nm	9.83	99.83	0.17	1.73	19.78	98.90	0.21	1.06	29.53	98.43	0.32	1.08
HCTZ at 270.0 nm	Slit range	2 nm	7.98	99.75	0.08	1.01	9.99	99.90	0.04	0.40	12.00	100.00	0.07	0.58
	2 nm	1 nm	7.86	98.25	0.04	0.51	9.93	99.30	0.07	0.71	11.97	99.75	0.09	0.76
	Scan speed (Fast)	Fast	7.96	99.50	0.08	1.01	9.94	99.40	0.04	0.40	12.00	100.00	0.07	0.58
		Slow	8.01	100.13	0.05	0.62	10.01	100.10	0.09	0.90	12.01	100.08	0.06	0.50
		Medium	7.99	99.88	0.06	0.75	10.01	100.10	0.11	1.10	11.94	99.50	0.10	0.84
	Wave length	+ 1.0 nm	7.84	98.00	0.17	2.17	9.83	98.30	0.16	1.63	11.79	98.25	0.17	1.44
		- 1.0 nm	7.95	99.38	0.12	1.51	9.98	99.80	0.09	0.90	11.91	99.25	0.10	0.42

$\bar{x}$: mean of five replicated determinations.

9.6. Recovery

For each dosage, the recovery was examined using three addition standards: 80%, 100%, and 120%. The recovery outcomes for the two doses of the Syrian trademark medication. Tables 6 and 7 demonstrate a good recoveries results, arrived to approximately 100%, which proved that the extraction of the drugs from the tablets product arrived to 100%.

Table (6): Recoveries of LOS and HCTZ for LOTID product (50/12.5) mg/tab.

Raw sample	Method	Sample µg/mL	Added µg/mL	Total Found $\bar{x}$ µg/mL	Recovery % $\bar{x}$	*SD (%)	*RSD %	Recovery Average %
LOS	AUC-CM	*19.91*	18.00	38.06	100.83	1.02	1.01	100.27
			20.00	39.94	100.15	0.99	0.99	
			22.00	41.87	99.82	0.74	0.74	
	DRS_1	20.19	18.00	38.35	100.89	1.09	1.08	100.95
			20.00	40.47	101.40	1.31	1.29	
			22.00	42.31	100.55	0.98	0.97	
HCTZ	AUC-CM	25.16	20.00	44.89	98.70	1.39	1.41	99.32
			25.00	50.07	99.69	1.22	1.22	
			30.00	54.94	99.30	0.89	0.90	
	DRS_1	5.02	4.00	9.03	100.25	0.89	0.89	99.44
			5.00	9.99	99.40	0.76	0.76	
			6.00	10.94	98.67	0.55	0.56	

$\bar{x}$: Mean for five replicates, *calculated from recovery.

Table (7): Recoveries of LOS and HCTZ for LOTID product (100/25) mg/tab.

Raw sample	Method	Sample µg/mL	Added µg/mL	Total Found $\bar{x}$ µg/mL	Recovery % $\bar{x}$	*SD (%)	*RSD %	Recovery Average %
LOS	AUC-CM	*20.34*	18.00	38.29	99.72	1.29	1.29	100.21
			20.00	40.47	100.65	1.22	1.21	
			22.00	42.40	100.27	0.97	0.97	
	DRS_1	20.25	18.00	38.26	100.06	1.82	1.82	100.21
			20.00	40.37	100.06	1.55	1.55	
			22.00	42.36	100.50	1.61	1.60	
HCTZ	AUC-CM	25.39	20.00	45.55	100.80	0.66	0.65	100.11
			25.00	50.24	99.40	0.74	0.74	
			30.00	55.43	100.13	0.41	0.41	
	DRS_1	5.01	4.00	8.94	98.25	0.46	0.47	98.51
			5.00	9.94	98.60	0.52	0.53	
			6.00	10.93	98.67	0.38	0.39	

$\bar{x}$: Mean for five replicates, *calculated from recovery.

9.7. Specificity

It was determined LOS and HCTZ in LOTID product without any interference with supposed tablets excipients as starch, lactose, talc, magnesium stearate and polyvinyl povidone. A good specificity was proved by demonstrating a good recovery result, arrived to approximately 100%, as seen in Tables 6 and 7. So, the two methods could be considered specific.

10. Application

The developed methods were used for quantitative analysis for LOS/HCTZ in the Syrian pharmaceutical tablet's product LOTID in two doses (50/12.5 and 100/25) mg/tab, for three different batches of each one. The results obtained using the quantitative analysis using the calibration curve are summarized in Tables (8 - 11). Generally, the doses of LOS and HCTZ in each of the products were in the range of the allowed doses according to USP legislation [1] (the tablets contain not less than 95.0 % and not more than 105.0 % of the labeled amounts of Losartan potassium and Hydrochlorothiazide), thus the obtained results are conformed to USP legislation. The two methods gave acceptable and borderline results where they be used both of them.

Table (8): Results of LOS and HCTZ in LOTID product (LOS 50 mg/tab. and HCTZ 12.5 mg/tab.), by (AUC-CM) method, for three different batches.

Number of batches	LOS 50 mg/tab				HCTZ 12.5 mg/tab			
	Result dose $\bar{x}$mg/tab	Per %	SD mg/tab	RSD %	Result dose $\bar{x}$mg/tab	Per %	SD mg/tab	RSD %
1	51.02	102.04	0.26	0.51	12.44	99.52	0.13	1.05
2	50.83	101.66	0.20	0.39	12.49	99.92	0.09	0.72
3	49.79	99.58	0.32	0.64	12.58	100.64	0.08	0.64

Range mg/tab	**49.79 – 51.02**	**12.44 – 12.58**
Range Per %	**99.58 – 102.04**	**99.52 – 100.64**

Table (9): Results of LOS and HCTZ in LOTID product (LOS 50 mg/tab. and HCTZ 12.5 mg/tab.), by (DRS_1) method, for three different batches.

Number of batches	**LOS 50 mg/tab.**				**HCTZ 12.5 mg/tab.**			
	Result dose $\bar{x}$mg/tab	**Per %**	**SD mg/tab**	**RSD %**	**Result dose $\bar{x}$mg/tab**	**Per %**	**SD mg/tab**	**RSD %**
1	50.19	100.38	0.54	1.08	12.42	99.36	0.19	1.53
2	50.16	100.32	0.32	0.64	12.35	98.80	0.11	0.89
3	50.49	100.98	0.24	0.48	12.55	100.40	0.16	1.27
Range mg/tab	**50.16 – 50.49**				**12.35 – 12.55**			
Range Per %	**100.32 – 100.98**				**98.80 – 100.40**			

$\bar{x}$: Five separate determinations were performed and calculated the mean.

Table (10): Results of LOS and HCTZ in LOTID product (LOS 100 mg/tab. and HCTZ 25 mg/tab.), by (AUC-CM) method for three different batches.

Number of batches	**LOS 100 mg/tab.**				**HCTZ 25 mg/tab.**			
	Result dose $\bar{x}$mg/tab	**Per %**	**SD mg/tab**	**RSD %**	**Result dose $\bar{x}$mg/tab**	**Per %**	**SD mg/tab**	**RSD %**
1	101.69	101.69	0.23	0.23	25.39	101.56	0.38	1.50
2	99.21	99.21	1.73	1.74	25.47	101.88	0.18	0.71
3	102.07	102.07	1.38	1.35	25.23	100.92	0.20	0.79
Range mg/tab	**99.21 – 102.07**				**25.23 – 25.47**			
Range Per %	**99.21 – 102.07**				**100.92 – 101.88**			

$\bar{x}$: Five separate determinations were performed and calculated the mean.

Table (11): Results of LOS and HCTZ in LOTID product (LOS 100 mg/tab. and HCTZ 25 mg/tab.), by (DRS_1) method, for three different batches.

Number of batches	**LOS 100 mg/tab.**				**HCTZ 25 mg/tab.**			
	Result dose $\bar{x}$mg/tab	**Per %**	**SD mg/tab**	**RSD %**	**Result dose $\bar{x}$mg/tab**	**Per %**	**SD mg/tab**	**RSD %**
1	101.26	101.26	0.96	0.95	25.06	100.24	0.70	2.79
2	99.87	99.87	0.51	0.51	25.03	100.12	0.40	1.60
3	99.86	99.86	1.16	1.16	24.92	99.68	0.38	1.52
Range mg/tab	**99.86 – 101.26**				**24.92 – 25.06**			
Range Per %	**99.86 – 101.26**				**99.68 – 100.24**			

11. Conclusion

This Method enables the simultaneous assessment of LOS and HCTZ in binary synthetic mixtures and pharmaceutical preparations using two methods: the first derivative of the ratio spectra method (DRS_1) and the area under the curve correction method (AUC-CM). Every UV spectrometric method used in this study is straightforward, economical, and environmentally benign.

The accuracy, precision, and sensitivity of all the approaches proposed in the present investigation were similar. This proves the potential supremacy of all the approaches proposed in the present investigation over the UV announced one. Additionally, the solvent used in this technique is 0.1 M $NaHCO_3$ is more environmentally friendly.

LOS and HCTZ were estimated in combination for one local pharmaceutical product LOTID in two different doses (50/12.5 and 100/25) mg/tab The levels of pharmaceutical compounds were within the permissible limits set by the USP legislation. The presence of active pharmaceutical compounds in real quantities in pharmaceutical products was conformed to the pharmacological LOS and HCTZ formulation.

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