Egypt. J. Chem. **Vol. 66,** No. 3 pp. 271 – 280 (2023)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

First- and Second-Derivative Spectrophotometry for Simultaneous Determination of lorazepam and clonazepam in pharmaceutical formulations

Faeza H. Zankanah,[a*] Fatma A. A. Al Ani,[b] Ala'a R. Shaker,[a*] Ahmed b. Taha[a*], and Mohammed S. Abdulraheem [c]

[a] *College of Health & Medical Technology, Uruk University, Baghdad-Iraq.*

[b] *Department of Chemistry, College of Education for Pure Science/ Ibn Al-Haitham, Adhamiya, University of Baghdad, Baghdad-Iraq.*

[c] *Ministry of Education, Baghdad College, Baghdad-Iraq.*

Abstract

The present study describes employing zero-, 1st - and 2nd -order derivative spectrophotometric methods have been developed for determination of lorazepam (LORA) and clonazepam (CLON) in commercially available tablets. LORA was determined by means of 1st (D1), 2nd (D2) derivative spectrophotometric techniques using zero cross, peak height, and Peak area. D1 used for the determination of CLON by using zero cross and peak height while D2 (zero cross) was used for the determination of CLON. The method was established to be linear in concentration containing different ratios of LORA and CLON range of (20-200 mg/L) and (5-35 mg/L) at wavelength range (250 -370 nm), (210-370nm) respectively. The proposed techniques are highly sensitive, precise and accurate and can be used for the reliable quantitation of lorazepam and clonazepam in tablet formulation.

***Key words*:** first- and second derivative spectroscopy, lorazepam, clonazepam

1. Introduction

Lorazepam (LORA) and clonazepam (CLON) have the IUPAC name [1] ((3RS)-7-Chloro-5-(2-chlorophenyl)-3-hydroxy-1, 3-dihydro-2H-1, 4-benzodiazepin-2-one)) and (5-(2-Chlorophenyl) -7-nitro-1, 3-dihydro-2H-1, 4-benzodiazepin-2-one) respectively. Chemical formula can be seen in figure (1).

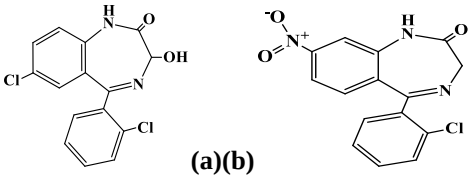
LORA and CLON [2 and 3] are benzodiazepines used for the management of anxiety disorders. LORA is also prescribed to treat insomnia, panic attacks, and alcohol withdrawal, while CLON is also known as Klonopin, is a drug that is used to prevent and treat epilepsy, panic attacks, and the movement disorder akathisia.

Numerous methods have been reported for the analysis of LORA and CLON, such as HPLC [4-7], Chromatographic [8, 9], Capillary electrophoresis [10], FTIR [11], Electrochemical [12, 13], and spectrophotometry [14- 19].

The absorbance values can be expressed as a function of the wavelength (λ) at the zero-cross and by the equation below:

$d^nA/d\lambda^n = {}^nDx,\lambda = f(\lambda)$

Where n, nDx λ , derivative order, value of derivative amplitude respictivlly of the absorption spectrum of the analyte (x) at the given wavelength (λ), A-absorbance [20].



(a)(b)

Fig. (1): Chemical structure of **(a)** Lorazepam and **(b)** Clonazepam

2. Experimental

Apparatus: A digital double beam spectrophotometer a type of Shimadzu 1800 UV-Visible (Shimadzu, Kyoto-Japan) had been used for all spectral and a Sartorius BL 210S balance, water bath (Memmert W-200 RING- Germany).

Materials and chemicals: All chemical reagents were of analytical grade, obtained from commercial suppliers, and used without further purification. LORA and CLON standard powder were donated by

*Corresponding author e-mail: dr.faizahazem@uruk.edu.iq.; (Faeza H. Zankanah).

Receive Date: 30 March 2022, Revise Date: 19 June 2022, Accept Date: 29 June 2022

DOI: 10.21608/EJCHEM.2022.130646.5753

Samara Co., Iraq (SDI). LORA (2 mg / tablet) (Ativan- IRAQ) and CLON (0.5 mg / tablet) (Rivotril – Switzerland) were obtained from local pharmacies. Absolute ethanol was supplied from Aldrich.

A stock solution of 250 mg/L LORA and CLON were prepared by dissolving accurately 0.025g of LORA and CLON in 40 mL ethanol and the volume was completed to 100 mL with DW.

Preparation of tablet sample solution: Ten of Ativan and Fifteen of Rivotril tablets were finely grounded and mixed. An accurately weighed containing 2.5mg of the drug (i.e. LORA, CLON) were dissolved in 10ml of ethanol by stirring for 5 minutes. the final volume was made to 25 mL with DW to obtain 100mg/L drug solution. After Then, insoluble materials were filtered by use Whatman filter paper.

GENERAL RECOMMENDED PROCEDURES

General method: Aliquots of solutions containing (100-1000µg) of LORA and (25-175µg) of CLON were transferred into a series of 5ml volumetric flasks and the volume was completed with (1:1) (DW: ethanol). The spectrum for each solution was recorded against the solvent blank. Apply the Beer-Lambert law and absorbance measurements to determine unknown concentration of a species in solution.

Derivative Spectrophotometric Method: Five milliter volumetric flask were used to prepare mixtures containing different ratios of LORA and CLON range of (20-200 mg/L) and (5-35 mg/L) respectively according to optimal mixture design (Simplex Lattice).

For the solutions as intended above the 1st and 2nd derivative spectra were registered with reference to the reagent blank in the wavelength range (250 -370 nm) for LORA and (210-370nm) for CLON.

RESULTS AND DISCUSSION

Adherence of the system to Beer's law

1. **Zero-order method**

Figure 2, 3, 4 indicates the absorption spectra of LORA, CLON, the mixture about them in the wavelength reach about 200–370 nm. Zero-order absorption spectra of LORA and CLON in reagent blank gave rise to extreme peaks at 317 nm and 309 nm, respectively. Those absorption spectra of the two parts inside a wavelength reach of 200–370 nm was less overlapped, so it was chosen for the analysis of LORA and CLON. Beer's law was obeyed by LORA and CLON in the range 20 – 200 mg/ L and 5-35mg/L respectively.

2. First order derivative method

Figure (5) depicts the overlaid first derivative spectra of 20 mg/L solutions of LORA and CLON and for different concentrations (20-200 mg/L) of LORA and (5-35 mg/L) of CLON. Several points unwanted at zero crossing have discarded except the zero–cross point at 282 nm and 308nm of CLON while 293nm and 317nm of LORA.

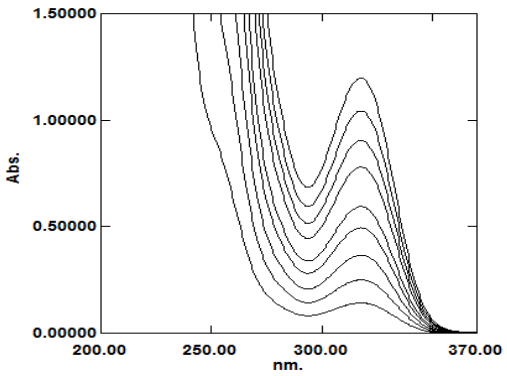


Fig. (2): Absorbance spectra of LORA (20-200 mg/L)

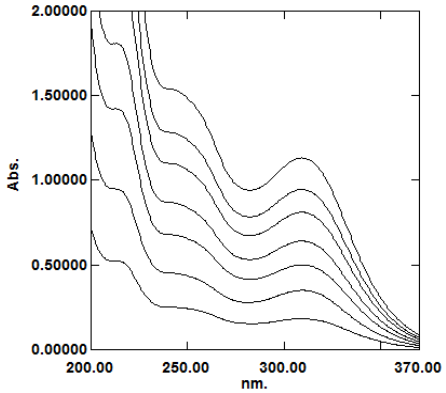


Fig. (3): Absorbance spectra of CLON (5-35 mg/L)

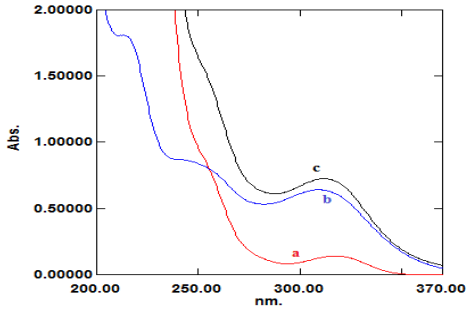


Fig. (4): Zero-order absorption spectra of (a) 20 mg/L CLON, (b) 20 mg/L LORA, and (c) binary mixture of 20 mg/L for LORA and CLON.

The point at 282 nm, 308 nm, 293nm and 317 nm are located almost in the center of the peak in the 1st derivative spectra of cited drugs. Figure (6) shows the calibration ranges was obtained for the assay of LORA and CLON in the existence of other at λ by plotting D1 against the concentration of drugs.

In order to select the suitable wavelength is the one that is responsible for the Highest Sensitivity, or the Highest Absorbance, derivative spectra have investigated of LORA and CLON figure (7). A wavelength of 261.5 nm and 296.0 nm were selected

for determination LORA and CLON respectively. So, it was determined peak height measurements at 261.5 nm and 296.0 nm for LORA and CLON respectively. Figure (8) displays the calibration curve at the λmax.

Several area under peak between two wavelengths at the point of maximum absorbance were found for LORA and CLON. All of these areas were proven to be un useful because of overlapping with each other except the area under peak 293 nm to 317 nm was found to be in proportion to the LORA amount, figure (9). Therefore, the Beer's law calibration curves for the LORA in the presence of CLON were establish toward those specified wavelengths by plotting the values of area under peak against the concentration of LORA as appeared in figures (10).

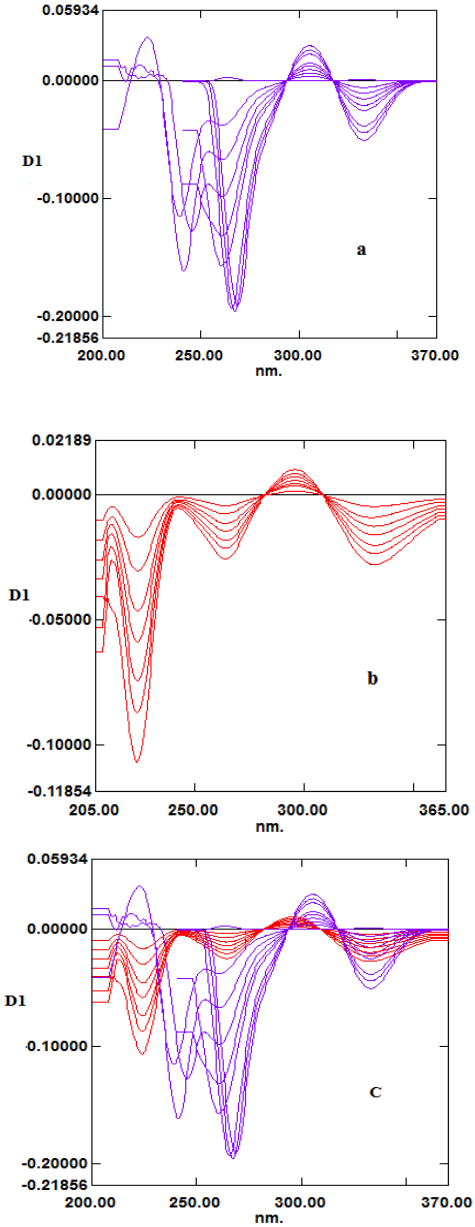


Fig. (5): Overlaid first derivative spectra of: (a) LORA (b) CLON (c): mixture LORA and CLON

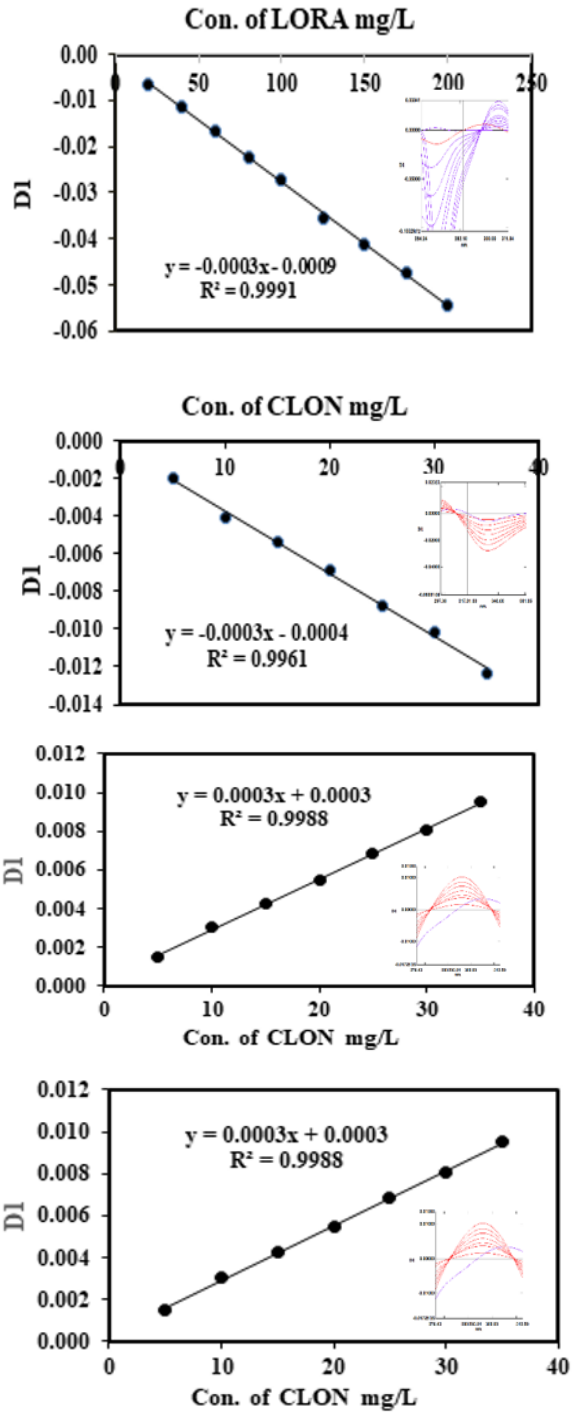


Fig. (6): Calibration curves of first derivative for LORA and CLON at zero cross.

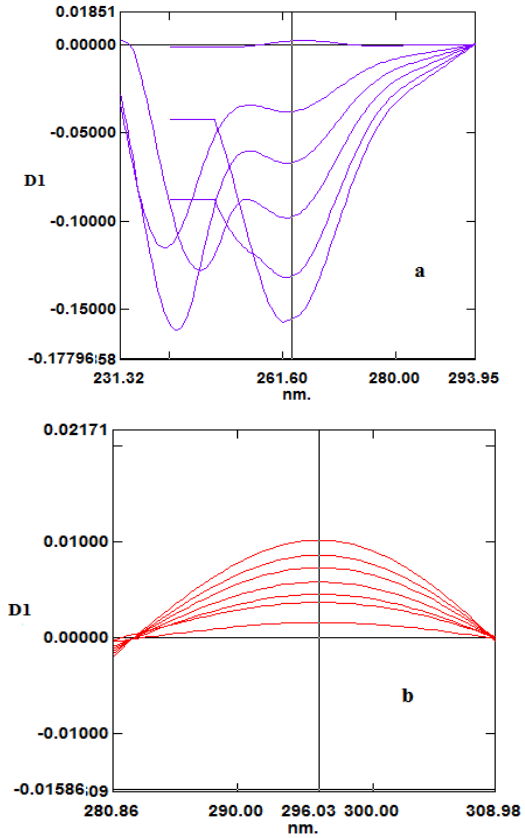


Fig. (7): Overlaid peak to baseline spectra of (a) LORA (b) CLON at 1st derivative

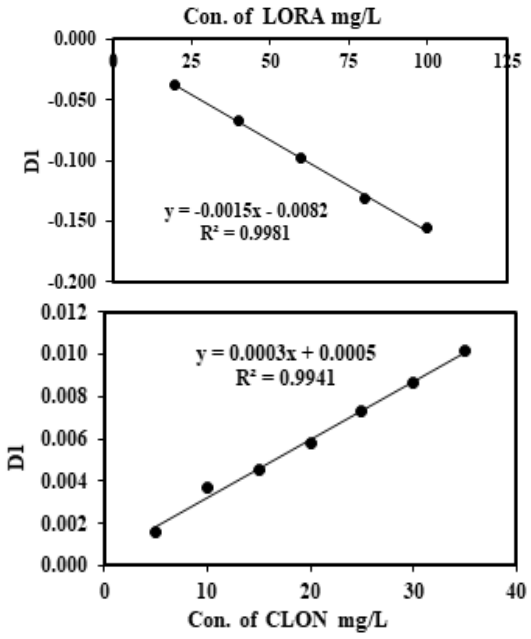


Fig. (8): Calibration curves of 1st derivative for LORA and CLON at peak to baseline

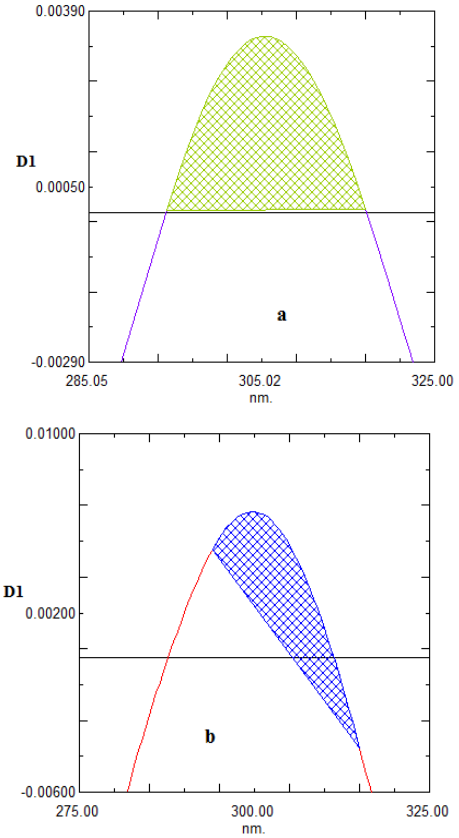


Fig. (9): 1st derivative spectra of (a) 20 mg/L LORA in the wavelength range 293-317 nm, (b) Mixture contain 20 mg/L LORA in the presence of 20 mg/LCLON for peak area.

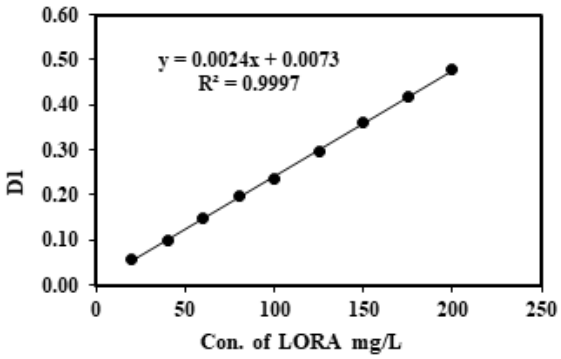


Fig. (10): Calibration curves of 1st derivative for LORA at peak area

3. Second order derivative method

Figures (11) show second order spectra for sets of solutions containing different amounts of LORA and CLON.

Zero-crossing wavelengths in the second-derivative spectra of LORA and CLON that can be used for their sensitive simultaneous determination are (264.62nm, 295.89nm) and 305.44 nm,

respectively and calibration curves can be seen by figures (12)

Calibration curves were constructed by peak to baseline (peak height) method at wavelength of 269 nm while 305.5nm to 333nm by peak area for determination LORA only. Figures (13 to 16)

Table (1) summarizes all the results for cited drugs analysis by using first and second derivative technique.

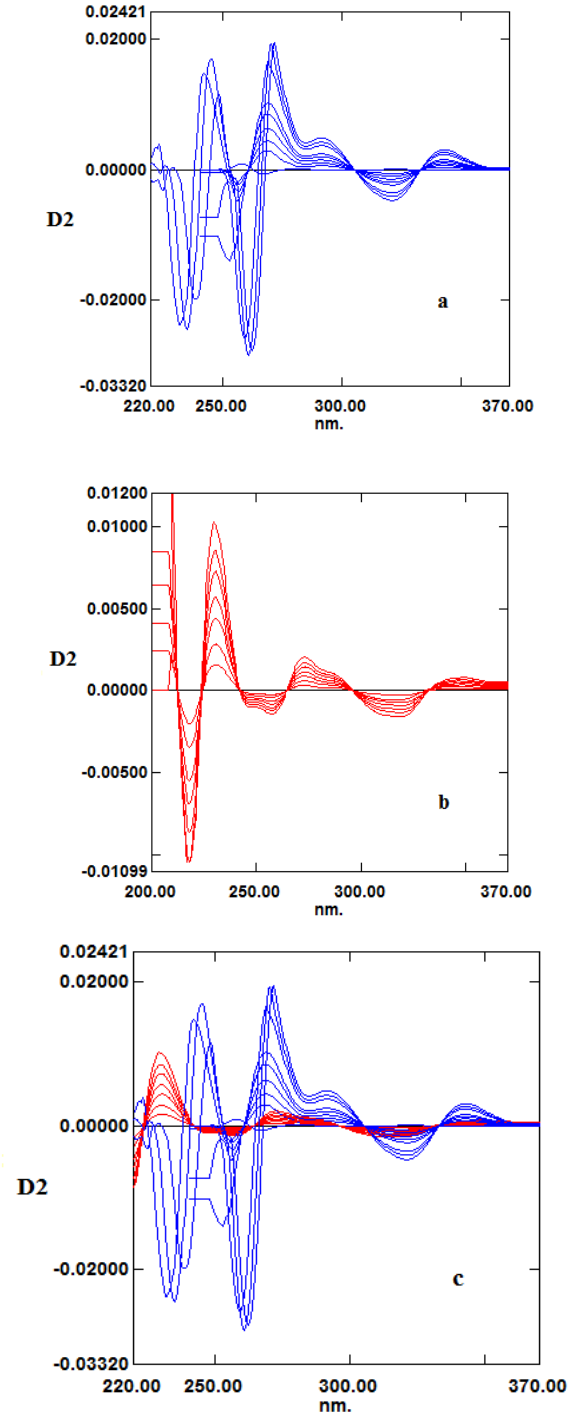


Fig. (11): Overlaid 2nd derivative spectra of: (a) LORA (b) CLON (c) Mixture LORA

(b) and CLON

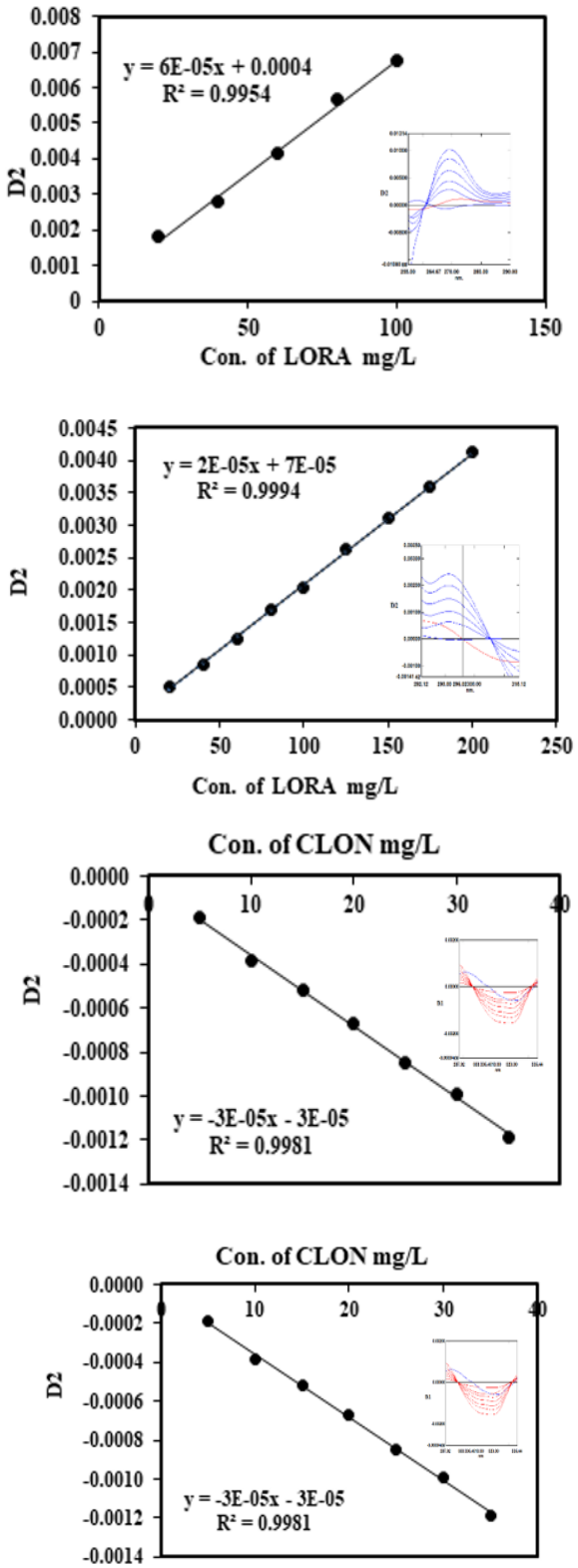


Fig. (12): Calibration curves of 2nd derivative for LORA and CLON at zero cross

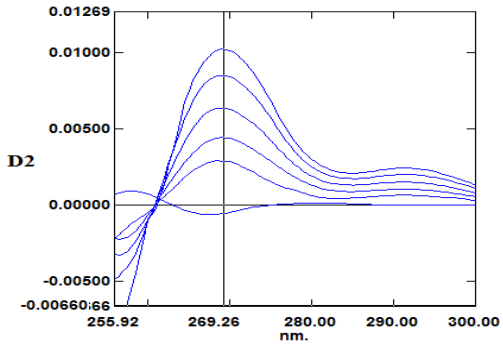


Fig. (13): Overlaid peak to baseline spectra of LORA at 2nd derivative

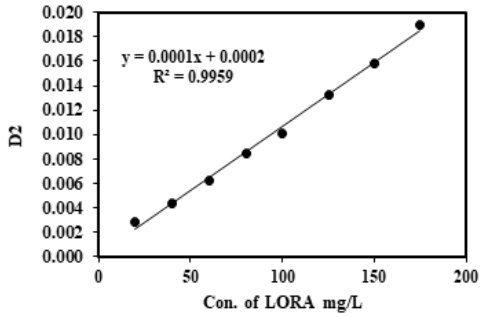


Fig. (14): Calibration curves of 2nd derivative for LORA at peak to baseline

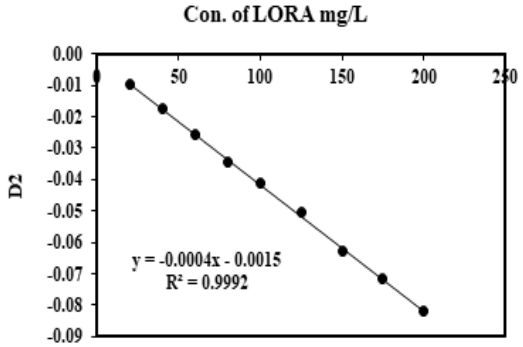


Fig. (15): Calibration curves of 2nd derivative for LORA at area under peak

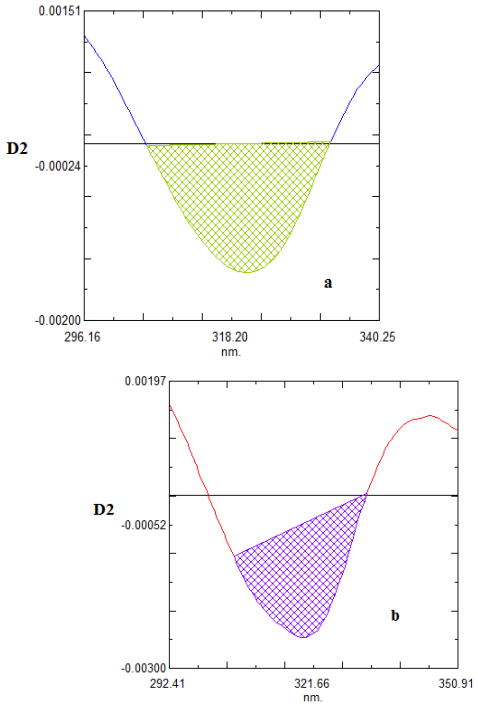


Fig. (16): 2nd derivative spectra of (a) 60 mg/L LORA in the wavelength range 305.5-333 nm, (b)Mixture contain 60 mg/L LORA in the presence of 35 mg/L CLON for peak area

ACCURACY AND PRECISION

The unwavering quality of the present techniques (precise %RSD and accuracy %RE) under trial conditions was controlled by playing out the standard investigation at various time interims around the same time (intraday) of the analytical methods. The intraday precision and accuracy were dictated by estimating three recreate analyses (n = 3) by adding a known amount of cited drug from the pre-analyzed tablet powder. The result is presented in table (2).

Table 3 shows no interferences were found using 1st and 2nd derivative mode for the determination of cited drugs.

Table 1: Summary of the selected methods for the determination of LORA and CLON and their analytical parameters

Drug	Order of derivative	Mode of calculation	λ (nm)	Regression equation	r	*D.L (μg. mL⁻¹)
Lorazepam	D1	Zero cross	282	Y=-0.0003x-0.0009	0.9995	0.5099**
			308	Y=0.0001x+0.0005	0.9995	0.3123**
		peak height	261.5	Y=-0.0015x-0.0082	0.9990	0.8083**
		Peak area	293 - 317	Y=0.0024x+0.0073	0.9998	0.4956**
	D2	Zero cross	264	Y=6E-05x+0.0004	0.9977	0.5803**
				Y=-0.0003x+0.0357	0.9994	0.3966**
			295	Y=2E-05x+7E-05	0.9997	0.1563**
		peak height	296	Y=0.0001x+0.0002	0.9979	0.6133**
		Peak area	305.5-333	Y=-0.0004x-0.0015	0.9996	0.8722**
Clonazepam	D1	Zero cross	293	Y=0.0003x+0.0003	0.9994	0.4449**
			317	Y=-0.0003x-0.0004	0.9980	0.5124**
		peak height	296	Y=0.0003x+0.0005	0.9970	0.6033**
	D2	Zero cross	305.5	Y=-3E-05x-3E-05	0.9990	0.5977**

*Detection limit = 3.3 (SD / slope), **n = 3 measurements

Table 2: Accuracy and precision study for synthetic mixture via derivative spectrophotometry

Drug	Derivative Mode	λ (nm)	Taken(mg/L)	Mean*(mg/L)	RE%	RSD%
LORA	D1 (*zero cross*)	282	**20**	20.4083	2.0417	2.4166
			50	47.5500	-4.9000	4.0308
			140	139.1111	-0.6350	0.2370
			200	199.2889	-0.3557	3.3525
		308	**50**	50.8000	1.6000	4.9213
			140	144.0843	2.9174	2.9138
			200	201.6667	0.8333	2.4415
	D1 *(peak height)*	261.5	**20**	21.1944	5.9722	0.1667
			50	52.2467	4.4933	3.5600
			80	81.6833	2.1042	0.9835
	D1 *(Peak area)*	293-317	**50**	52.0354	4.0708	2.5584
			80	81.8396	2.2995	3.1439
			140	138.7604	-0.8854	1.2657
			170	170.0528	0.0310	1.4420
			200	196.2938	-1.8531	0.8811
	D2 (*zero cross*)	264	**20**	19.2778	-0.2874	1.3206
			50	49.0000	-2.0001	2.7211
			100	97.4800	-2.5200	2.2022
			140	138.598	-1.0014	0.2391
			200	202.4444	1.2222	1.2696
		295	**20**	19.6667	-1.6667	3.8835
			50	46.7500	-6.5000	2.6738
			80	80.0375	0.0468	0.6299
			140	139.2500	-0.5357	0.8977
			170	167.2500	-1.6176	-1.6176
			200	193.1667	-3.4167	-3.4167
	D2 *(peak height)*	296	**20**	21.5667	7.8331	1.1669
			80	81.5100	1.8875	0.5030
			140	142.8500	2.0357	0.5950
			170	171.9500	1.1471	0.9014
	D2 *(Peak area)*	305.5-333	**50**	54.3375	8.675	4.7579
			140	144.400	3.1429	1.4889
			170	172.050	1.2059	0.3526
			200	198.1875	-0.9063	1.7083
CLON	D1 (*zero cross*)	293	**5**	5.1778	3.5556	2.6803
			15	14.375	-4.1667	0.5217
			35	33.5634	-4.1047	3.0886
		317	**5**	4.7533	-4.9333	6.7736
			15	14.9000	-0.6667	2.9083
			35	34.5333	-1.3333	0.8228
	D1 *(peak height)*	296	**5**	5.3917	7.8337	3.2454
			25	26.1574	4.6296	2.7289
			35	34.9500	-0.1429	2.7182
	D2 (*zero cross*)	305.5	**5**	4.9333	-1.3333	5.6531
			10	10.3333	3.3333	3.2258
			15	15.0370	0.2469	2.2574
			30	32.5000	8.3333	0.5128
			35	34.8333	-0.4762	1.4354

Table 3: Percent recovery for LORA and CLON in the presence of (500 µg. mL^{-1}) of excipients.

Excipients	40 µg. mL^{-1} of LORA presence of (500 µg. mL^{-1}) of excipients.		10 µg. mL^{-1} of CLON presence of (500 µg. mL^{-1}) of excipients.	
	*Conc. found of LORA (µg. mL^{-1})	Recovery%	*Conc. found of CLON (µg. mL^{-1})	Recovery%
Vanillin	20.0341	100.1705	10.2255	102.255
Glucose	19.6111	98.0555	9.9754	99.754
Lactose	19.8093	99.0465	9.5587	95.587
Starch	20.5223	102.6115	10.2199	102.199
Sucrose	19.7999	98.9995	10.1076	101.076

*Average of three measurements

APPLICATION

The described procedures were successfully applied to the determination of LORA and CLON in the in marketed formulation. The results obtained are tabulated in Table 4. In general, the recovery percentage determined in three replicate analyses is very close to the claimed amount by the manufacturers and the values of standard deviation indicate very good reproducibility.

Table 4: Results from the analysis of LORA and CLON in tablet drugs by the proposed method

Pharmaceutical	Order (Mode of analysis)	Found amount (mg)	Con. (µg.mL^{-1})		*SD (n=3)	*C.V. % (n=3)	Recovery %
			Taken	*Found (n=3)			
Ativan 2 mg/tablet	**D1** (*zero cross*)	1.9867	**40**	39.7333	1.1504	2.8952	99.3333
		1.9844	**60**	59.5333	1.0599	1.7803	99.2222
		2.0013	**100**	100.0667	0.3055	0.3053	100.067
	D1 *(Peak area)*	1.9981	**40**	39.9625	0.4731	1.1838	99.9063
		2.0806	**60**	62.4181	0.5141	0.8237	104.031
		1.9725	**100**	98.6264	2.2829	2.3147	98.6264
	D2 (*zero cross*)	1.9750	**40**	39.5000	2.6458	6.6981	98.7500
		1.9944	**60**	59.8330	1.6073	2.6863	99.7222
		2.0600	**100**	103.000	1.5001	1.4563	103.000
Rivotril 2 mg/tablet	**D1** (*zero cross*)	2.0312	**10**	10.1556	0.2009	1.9785	101.556
		2.0578	**20**	20.5778	0.0839	0.4077	102.889
		2.0015	**30**	30.0222	0.0839	0.2794	100.074
	D1 *(peak height)*	2.0267	**10**	10.1333	0.5783	5.7070	101.333
		1.9767	**20**	19.7667	0.4807	2.4321	98.8334
		2.0022	**30**	30.0333	0.6658	2.2170	100.111
	D2 (*zero cross*)	2.0156	**10**	10.0778	0.3595	3.5675	100.778
		2.0711	**20**	20.7111	0.2694	1.3001	103.556
		2.0704	**30**	31.0556	0.2546	0.8198	103.519

ANALYTICAL METHODS

There are several papers referenced in the table below describing the method for the quantitative determination of the cited drugs in UV spectrophotometry, and can be compared with the current method by limits of detection (LOD) and quantification (LOQ). Table 5 shows some methods for the determination of LORA and CLON in pharmaceutical preparations.

Table 5: Usual limits of detection (LOD) and quantification (LOQ) of LORA and CLON by difference methods

Remarks	Linearity Range (µg. mL^{-1})	LOD µg. mL^{-1}	LOQ µg. mL^{-1}	Ref.
A Method for Rapid Determination of Lorazepam by High-Performance Liquid Chromatography	2.5 to 75 µg/L	0.5 µg/L	1.6667	**[21]**
Spectrophotometric Determination of Clonazepam in Pure and Dosage forms using Charge Transfer Reaction	5-40	3.2427	10.8089	**[22]**
Cloud-point extraction and spectrophotometric determination of clonazepam in pharmaceutical dosage forms	0.3-25	0.11	0.3667	**[23]**
A Synchronous Fluorescence Spectrofluorometric Method for the Simultaneous Determination of Clonazepam and Paroxetine Hydrochloride in Combined Pharmaceutical Dose Form	1-5	0.055	0.1690	**[24]**
Spectrophotometic determination of Lorazepam in pharmaceutical tablets using batch and reverse flow injection methods	2 to 40 and 25 to 400	0.61 and 2.29	2.0333 and 7.6333	**[25]**
Determination of Lorazepam in Drug Formulation and Biofluids Using a Spectrophotometric Method and Response Surface Methodology	0.3 –19.5	0.08	0.2667	**[26]**
First- and Second-Derivative Spectrophotometry for Simultaneous Determination of lorazepam and clonazepam in pharmaceutical formulations	**LORA D1** 308nm	0.3123	1.0410	**Current Study**
	LORA D2 295 nm	0.1563	0.5210	
	CLON D1 293 nm	0.4449	1.4830	
	CLON D2 305.5 nm	0.5977	1.9923	

CONCLUSION

The aim of this study was to develop simple, fast, validated and very economic methods for the simultaneous analysis of the binary mixtures of CLON and LORA by zero-derivative, **1st** derivative, and **2nd** derivative spectrophotometry are effective. Hence, the method could be used successfully for the routine analysis of the pharmaceutical dosage forms of LORA and CLON

REFERENCES

[1]. British Pharmacopeia, CD-ROM Her Majesty, Stationary office, London, (2013).

[2]. American Society of Health-System Pharmacists. June 29, 2016. Archived from the original on 5 June 2016. Retrieved 15 July 2016.

[3]. The American Society of Health-System Pharmacists. Archived from the original on 2015-09-05. Retrieved August 15, (2015).

[4]. P. M. Patil, S. B. Wankhede, and P. D. Chaudhari, "A validated stability indicating HPLC method estimation of clonazepam in the bulk drug and pharmaceutical dosage" form. Pharm. Anal. Acta. (2015).

[5]. M. Christine, H. Salem, S. M. Amer, & M. Nebsen,"Validated HPLC method for simultaneous determination of aripiprazole and co-administered clonazepam in spiked human plasma" J. Pharm. Appl. Chem, 3(1), (2017), 57-61

[6]. J. F. Feng, R. L. Shen, D. T. Zhang, Z. Y. Dong, D. D. Zhang, and H. Y. Wang, "Quantification of lorazepam in human plasma using HPLC: application to a pharmacokinetic study." latin american journal of pharmacy, 34, no. 3, (2015): 622-626.

[7]. H. Miresan, C. Rosca, N. Matei, F. Roncea, R.Cazacincu, I. Iancu, E. Stefanescu, D.Enache, M.Bratu, and A. Popescu, "Simultaneous quantification of four benzodiazepines from whole blood by highperformance liquid chromatography in forensic toxicological analysis." Analele Universitatii" Ovidius" Constanta-Seria Chimie 25.1, (2014): 24-27.

[8]. C. John, P. Ghosh, K. M. Varshney, S. Kaur, S. K. Shukla, and S. Satyanarayana," Determination of CLONzepam in human hair and nail using liquid chromatography tandem mass spectrometry (LC-MS/MS)." Journal of Liquid Chromatography and Related Technologies, 37: (2014), 1917–1928.

[9]. D. Jing, S. Yintao, W. Junwei," Qualitative and quantitative analysis of CLONzepam and its metabolite 7-aminoCLONzepam in blood by LC-tandem QTOF/MS and LC-MS/MS", Forensic sci sem, 4(1), (2014):45-52.

[10]. A. Wo´zniakiewicz, R. Wietecha-Posłuszny, M. Wo´zniakiewicz, E.Bryczek, P. Ko´scielniak, "A quick method for determination of psychoactive agents in serum and hair by using capillary electrophoresis and mass spectrometry." Journal of Pharmaceutical and Biomedical Analysis 111, (2015), 177–185.

[11]. V. K. Sheeja, & A. S. Swapna,"Method development and validation for the simultaneous estimation of clonazepam and paroxetine in combined dosage form using FT-IR. Asian Journal of Research in Chemistry", 13(1), (2020), 12-14.

[12]. B. Rezaei, M. K. Boroujeni, and A. A. Ensafi, "A novel electrochemical nanocomposite imprinted sensor for the determination of lorazepam based on modified polypyrrole@ sol-gel@ gold nanoparticles/pencil graphite electrode." Electrochimica Acta, 123, (2014): 332-339.

[13]. M. Vafadar, E. Zarei, & A. Asghari, "Electrochemical Measurement of Noscapine and Lorazepam Using a Carbon Paste Electrode Modified with Multi-walled Carbon Nanotubes and Natural Deep Eutectic Solvent". Iranian Journal of Pharmaceutical Research: IJPR, 20(3), (2021), 490.

[14]. S. R. Fadhel, & N. M. Khamees,. "Development of Spectrophotometric Method for Estimating Clonazepam in its Pure Form and in Pharmaceutical Tablets". Oriental Journal of Chemistry, 34(3), (2018), 1604-1610.

[15]. N. S. Saddam, & K. H. Kadhim ,"Spectrophotometric determination of Drug Clonazepam in Pure form and Pharmaceutical Tablets by Oxidative Coupling Reaction with Chlorpromazine hydrochloride"International Journal of Pharmaceutical Quality Assurance (2019); 10(2); 342-348.

[16]. H. Hadi, "Spectrophotometric determination of CLONzepam in pure and dosage forms using charge transfer reaction." Iraqi J Pharm Sci, (2015) Vol.24 (1), 25-32.

[17]. J. U. Patel, H. Dalwadi, & P. Shah, Application of Ratio Derivative Spectrophotometry for Simultaneous Determination of Clonazepam and Paroxetine Hydrochloride in Tablet Dosage Form. Journal of Pharmaceutical Research and Reviews, 2, (2018).

[18]. H. S. Alteemi and K. H. Kadim," Spectrophotometric Determination of Clonazepam Drug in Pure form and Pharmaceutical Preparation by Diazotization Reaction" International Journal of Psychosocial Rehabilitation.Vol.24, No.5(2020), 3808-3817.

[19]. S. Ghorbanpoor, M. R. Shishehbor, A. Sheibani, M. Safaei, & A. Nazari ,"Determination of Lorazepam in Drug Formulation and Biofluids Using a Spectrophotometric Method and Response Surface Methodology". Journal of Applied Spectroscopy, 87(5), (2020),965-975.

[20]. M. Gallignani, R. A. Rondón, J. F. Ovalles, M. R.Brunettoa, Transmission FTIR Derivative Spectroscopy Estimation of Furosemide in Raw Material and Tablet Dosage Form, Acta Pharmaceutica Sinica B, 4(5), (2014), 376–383.

[21]. T. Kondo, D. C. Buss, & P. A. Routledge "A method for rapid determination of lorazepam by high-performance liquid chromatography". Therapeutic drug monitoring, 15(1), (1993). 35-38.

[22]. H. Hadi, "Spectrophotometric determination of clonazepam in pure and dosage forms using charge transfer reaction". Iraqi Journal of Pharmaceutical Sciences (P-ISSN: 1683-3597, E-ISSN: 2521-3512), 24(1), (2015),25-32.

[23]. H. H. Abdullah, "Cloud-point extraction and spectrophotometric determination of clonazepam in pharmaceutical dosage forms. Bulletin of the Chemical Society of Ethiopia, 31(3), (2017). 373-382.

[24]. J. U. Patel, & U. K. Chhalotiya," A Synchronous Fluorescence Spectrofluorometric Method for the Simultaneous Determination of Clonazepam and Paroxetine Hydrochloride in Combined Pharmaceutical Dose Form" Turkish Journal of Pharmaceutical Sciences, (2017).14(3), 251.

[25]. M. Jamal, & H. Hadi, "Spectrophotometic determination of Lorazepam in pharmaceutical tablets using batch and reverse flow injection methods." Egyptian journal of basic and applied sciences, 5(2), (2018).151-156.

[26]. S. Ghorbanpoor, M. R Shishehbor, A.Sheibani, , M. Safaei, , & A. Nazari,. "Determination of Lorazepam in Drug Formulation and Biofluids Using a Spectrophotometric Method and Response Surface Methodology". Journal of Applied Spectroscopy, 87(5), (2020), 965-975.