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RESEARCH ARTICLE

Simultaneous Determination of Furosemide, Carbamazepine, Diazepam and Carvedilol in Quaternary Mixture via Derivative Spectrophotometry

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Abstract

Quick and accurate quaternary mixture resolution of furosemide (FURO), carbamazepine (CARB), diazepam (DIAZ) and carvedilol (CARV) by using derivative spectrophotometric method was performed. FURO and CARV were determined by means of first (D1), second (D2), third (D3) and fourth (D4) derivative spectrophotometric methods, CARB was determined by using D1, D2, D3 derivatives, while D1 and D2 were used for the determination of DIAZ. The recommended methods were verified using laboratory prepared mixtures and then successfully applied for the pharmaceutical formulations analysis of the cited drugs. The results obtained revealed the efficiency of the proposed methods as quantitative tool of analysis of the quaternary mixture with no requirements for sample neither pretreatment nor preliminary separation of analytes from the pharmaceutical preparations.

Keywords: *Derivative spectrophotometry; Furosemide; Carbamazepine; Diazepam; Carvedilol.*

Introduction

Furosemide (FURO), Figure 1a [4-Chloro-2-[(furan-2-ylmethyl) amino] - 5-sulfamoyl benzoic acid] is a type of loop diuretics that get their name from loop shaped part of kidney where they have their effect. It is considered a powerful one and mainly used for the treatment of hypertension and edema. Furosemide is also used to treat fluid buildup caused by heart failure, liver cirrhosis, and chronic kidney failure [1, 2]. Carbamazepine (CARB), Figure 1b [5*H*-dibenzo [*b*, *f*] azepine-5-carboxamide] is an anticonvulsant primarily used in treatment of epilepsy and neuropathic pain. It is a lipophilic tricyclic compound used as a first-choice antiepileptic drug, and in the management of simple and complex seizures [2, 3].

Diazepam (DIAZ), Figure 1c [7-chloro-1-methyl-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one] is a medication of benzodiazepine derivatives, belongs to a group of psychoactive drugs used to treat a range of medical conditions like anxiety, which is the main indication for its use, also for acute alcohol withdrawal and status epilepticus and other convulsive states [2,4]. Carvedilol (CARV), Figure 1d [(2RS)-1-(9*H*-Carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy) ethyl] amino] propan-2-ol] is a nonselective beta-blocker/ alpha-blocker antihypertensive agent, widely used in the treatment of hypertension, congestive heart failure, cardiac arrhythmia, and angina pectoris.

It can be prescribed alone or together with other antihypertensive or with diuretic [5]. A review of the literature revealed that the analysis of FURO, CARB, DIAZ and CARV either alone or in the presence of other drugs has been reported through electrochemical [6, 9], GC-MS [10, 11], high performance liquid chromatography [12, 15], flow injection [16, 17] and differential derivative methods [18, 19].

No reported methods dealing with simultaneous determination of FURO, CARB, DIAZ and CARV in their quaternary mixture have been found. Derivative spectroscopy is an analytical technique based on differentiation of the original, zero-order spectrum. The result of derivatization is called the derivative spectrum, which represents the values of absorbance differentials as a function of wavelength (λ), and it can be expressed as:

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

Where n denotes the derivative order, ${}^nDx, \lambda$ represents the value of n-order derivative (i.e. the derivative amplitude) of the absorption spectrum of the analyte (x) at the given wavelength (λ), A-absorbance [20]. Derivative spectra often yield a characteristic profile where refined changes of curvature and gradient in the zero order spectrums are observed as distinctive bipolar functions. The first derivative represent the gradient at all points of the spectrum and can be used to detect hidden peaks, since dA/d λ is equal to zero at peak maxima. This bipolar function is typical of all odd-order derivative spectra.

The distinctive feature of the second derivative spectrum (as well as all even-order derivatives) is a negative peak with minimum at the λ_{max} of the normal spectrum. The derivative spectra are more complicated than the original spectra, and the generation of n^{th} derivative spectrum will produce (n+1) new signals with an intense main signal and weaker peaks, so called satellite signals [21]. This technique is often used in identifying weak absorption peaks obscured by large peaks, identifying closely adjacent absorption bands, and most importantly in performing quantitation assay of certain analytes in presence of other absorbing compounds [22].

For instance, derivative spectrophotometry is an analytical technique of great utility for extracting both qualitative and quantitative information from spectra composed of un-resolved bands, and eliminating the effect of baseline shifts and baseline tilts. This consists of calculating and plotting one of the mathematical derivatives of a spectral curve Therefore, the purpose of the present work is to study the utility of derivative spectrophotometric method in the estimation of the four drugs in bulk and pharmaceutical dosage forms.

Experimental

Instrumentation

All absorption spectra were recorded by Cecil CE7200 UV-Visible double beam spectrophotometer equipped with 1 cm quartz cells (Cambridge-England) on a range of 200-380 nm with scan speed of 10 $nm.sec^{-1}$, averaging of 1.0 nm, bandwidth of 1.8 nm, and data interval of 0.5 nm. The resulted absorption data were digitalized, plotted, and manipulated by Shimadzu 1800 software (UVProb 2.34) to obtain the first, second, third and fourth order derivatives.

Materials and Solvents

The standard grade powder (furosemide, carbamazepine, diazepam, and carvedilol) used in this study received in pure form (99.99%) as a gift from the State Company for Drug Industries and Medical Appliances Samara-Iraq (SDI). Methanol (99.9 %) for HPLC (Sigma Aldrich, Germany).Pharmaceutical formulations evaluated in this work were obtained from local pharmacies; Lasix 40 mg / tablet (SWI, France), Carbamazepine® 200 mg / tablet (TAVER, Cyprus), VALIAPAM 2 mg / tablet (SDI, Iraq), and Carvidol® 25 mg / tablet (Pharma International, India).

Preparation of Standard Solution

Standard solutions each containing 1000 $\mu g.mL^{-1}$ of FURO, CARB, DIAZ, and CARV were prepared by dissolving exactly 50 mg of each drug in methanol. For the preparation of working solutions, serial dilution was done to get 100$\mu g.Ml^{-1}$ and 50 $\mu g.Ml^{-1}$ of each drug.

Preparation of Pharmaceutical Solution

Ten tablets of each pharmaceutical product were separately weighed and finely milled. A portion of the milled tablets equivalent to 0.1994 gm, 0.3194 gm, 0.1504 gm, and 0.4756 gm for Lasix®, Carbamazepine®, VALIAPAM, and Carvidol® respectively were dissolved in methanol and completed the volume to 10 ml in a separate volumetric flask to get 1000 $\mu g.mL^{-1}$, serial dilution was done to get 100 $\mu g.mL^{-1}$ and 50 $\mu g.mL^{-1}$ of each drug.

General Procedure

Assay Procedure for Individual Determination of Furosemide, Carbamazepine, Diazepam, and Carvedilol

1.0 mL aliquots, of each drug standard solution containing 5-100 µg was transferred to a series of 5mL volumetric flask and diluted with methanol. The spectrum for each solution was recorded against the solvent blank. Zero-order spectrum was then manipulated for each to get its first (D1), second (D2), third (D3) and fourth (D4) derivative.

Assay of Laboratory Prepared Mixtures of Standard Furosemide, Carbamazepine, Diazepam, and Carvedilol

Mixtures containing different ratios of FURO, CARB, DIAZ and CARV over the concentration range of (0-20 $\mu g.mL^{-1}$) for each drug were prepared in 5-ml volumetric flask according to optimal mixture design (Simplex Lattice). The absorption spectrum for each solution was recorded against solvent blank and manipulated to get its D1, D2, D3 and D4 derivative.

Result and Discussion

Normal mode spectra of FURO, CARB, DIAZ, CARV and the spectrum of their mixture (Figure 2) show a significant spectral-overlap that interfere with direct spectrophotometric determination of the studied drugs. Thus, derivative spectrophotometry was suggested for the simultaneous analysis of the titled drugs in their quaternary mixtures. Visual inspection was used for selecting the more convenient analytical wavelengths at which the multi-component system is analyzed by derivative spectrophotometry. The investigation reveals that FURO and CARV could be determined by all modes of derivative (i.e. first to fourth order); CARB could be determined by first, second, and third order; while only first and second modes of derivative could be used in the determination of DIAZ in presence of the other investigated drugs.

The overlaid spectra of the first, second, third, and fourth order derivatives of each of the cited drugs in the presence of the other studied drugs are shown in Figures 3, 4, 5 and 6 respectively. Calibration curves (Figures 7, 8, 9 and 10) were constructed over the concentration range (3.3-20 $\mu g.mL^{-1}$) for the four drugs and linear correlation was obtained between the measured D1, D2, D3 and D4 values (as signals) versus the respective drug concentrations.

Table 1 summarizes the analytical parameters for the selected methods that have been used in the determination of CARV, FURO, CARB and DIAZ. The accuracy and precision of the proposed methods were established by calculating the values of percentage of the relative error (RE %) and relative standard deviation percent (RSD %), for three replicate analyses at two different concentration levels of pure sample at the same day. The calculated analytical results show good accuracy with reasonable precision of the proposed methods as reported in Table 2.

Commercially available tablets of CARV, FURO, CARB and DIAZ (Carvedilol®, Lasix®, Carbamazepine® and Valiapam® respectively) were subjected to analysis by the proposed derivative procedures. The results obtained are in respectable agreement with the label claims and this indicates the applicability of the proposed methods for the simultaneous estimation of the cited drugs in real samples (Table 3).

Moreover, standard addition method was applied to analyze CARV, FURO, CARB and DIAZ in their pharmaceutical preparations to verify the efficiency of the proposed procedures. This study was performed by adding known amounts of pure drug (standard) to a given concentration of the commercial pharmaceutical solution. The resulting mixtures were analyzed by following the recommended procedures, and the total found amounts were calculated from the corresponding regression equation of each drug (Table 4).

Figures (11, 12, 13 and 14) represent the obtained derivative spectra by applying standard addition method for CARV, FURO, CARB and DIAZ respectively. Since there is no reported standard method for the simultaneous analysis of the quaternary mixtures of the mentioned drugs, the results of the proposed derivative spectrophotometry method were compared statistically with those of the developed RP-HPLC and PLS methods.

The comparison was performed by using student's t-test and F-test at 95 % confidence level with regards to recovery percentage. As shown in Table 5, the statistic t value and calculated F value for

the studied drugs are smaller than the critical ones, which indicate that there is no significant difference between the results of the proposed methods. Moreover, one-way ANOVA (or Anova: Single Factor), was applied further for variance comparison of the obtained results via the proposed methods for each drug. The results revealed that there is no significant difference between the three methods as the critical F-value is higher than the calculated one (Table 6).

Conclusion

Simultaneous determination of furosemide, carbamazepine, diazepam and carvedilol using derivative spectrophotometry is proposed in this work. The recommended derivative spectrophotometric method is suitable for the simultaneous analysis of multicomponent mixture due to its simplicity, low coast and short analysis time, nevertheless the effects of several instrument parameters on the derivative spectra causes a limitation in its application. The suggested method is simple, fast, inexpensive, and non-destructive and show good linearity and sensitivity. The recommended method enables the estimation of the cited drugs either in laboratory prepared mixtures or in pharmaceutical formulations without prior separation and other previous sample treatments.

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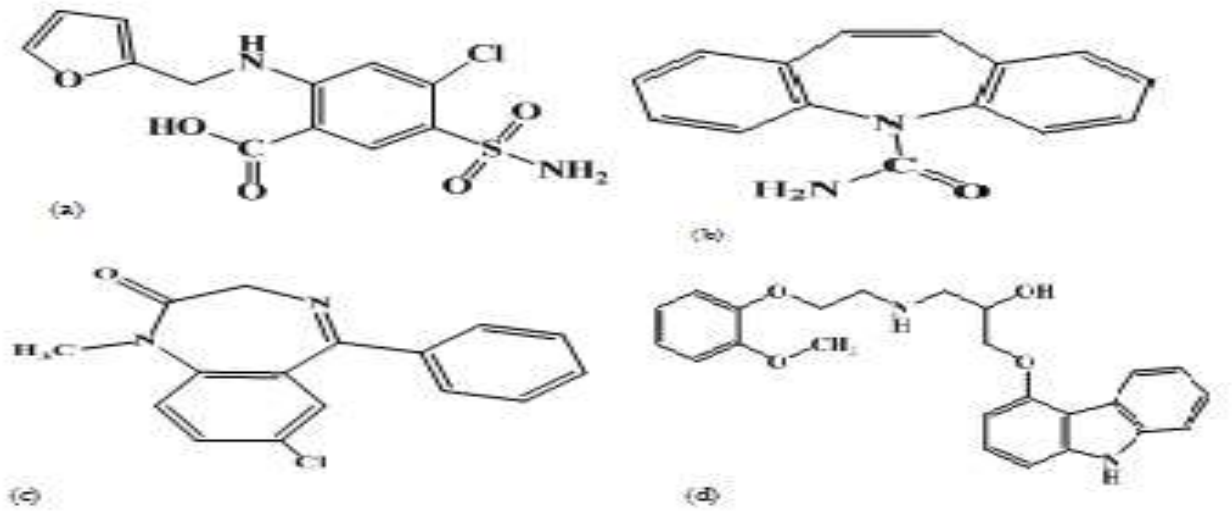


Figure 1: Chemical structure of (a) Furosemide, (b) Carbamazepine, (c) Diazepam and (d) Carvedilol

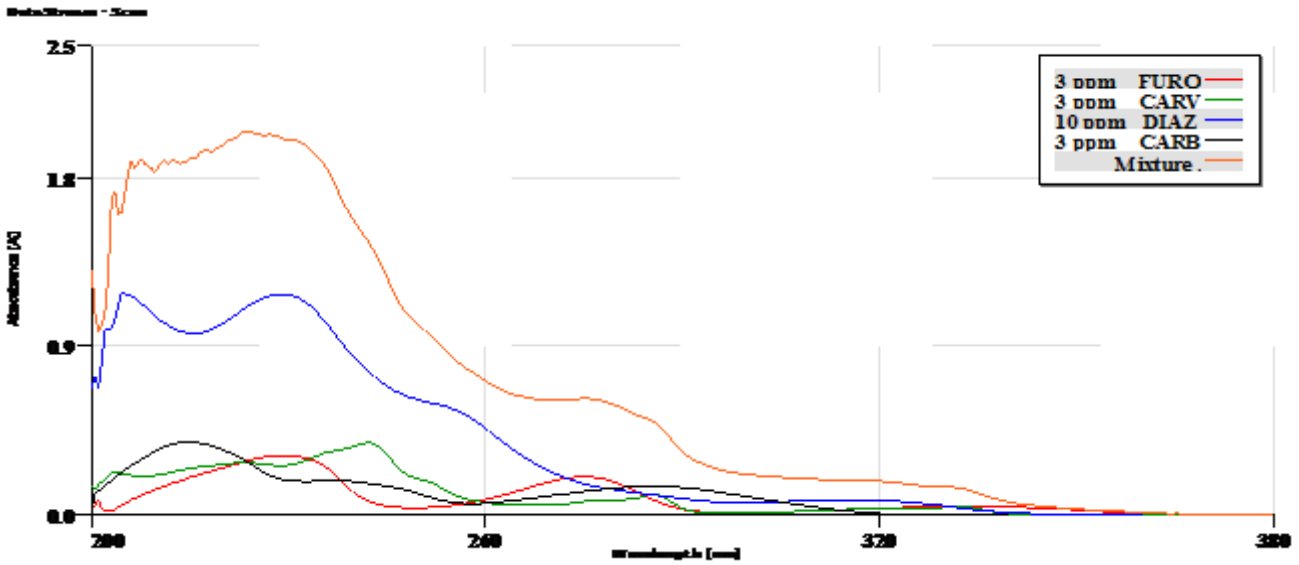


Figure 2. Zero-order absorption spectra of 3 µg.mL^{-1} CARV, 10 µg.mL^{-1} DIAZ, 3 µg.mL^{-1} CARB, 3 µg.mL^{-1} FURO, and quaternary mixture of 3.3 µg.mL^{-1} for CARV, CARB, FURO and 10 µg.mL^{-1} for DIAZ against methanol as a blank

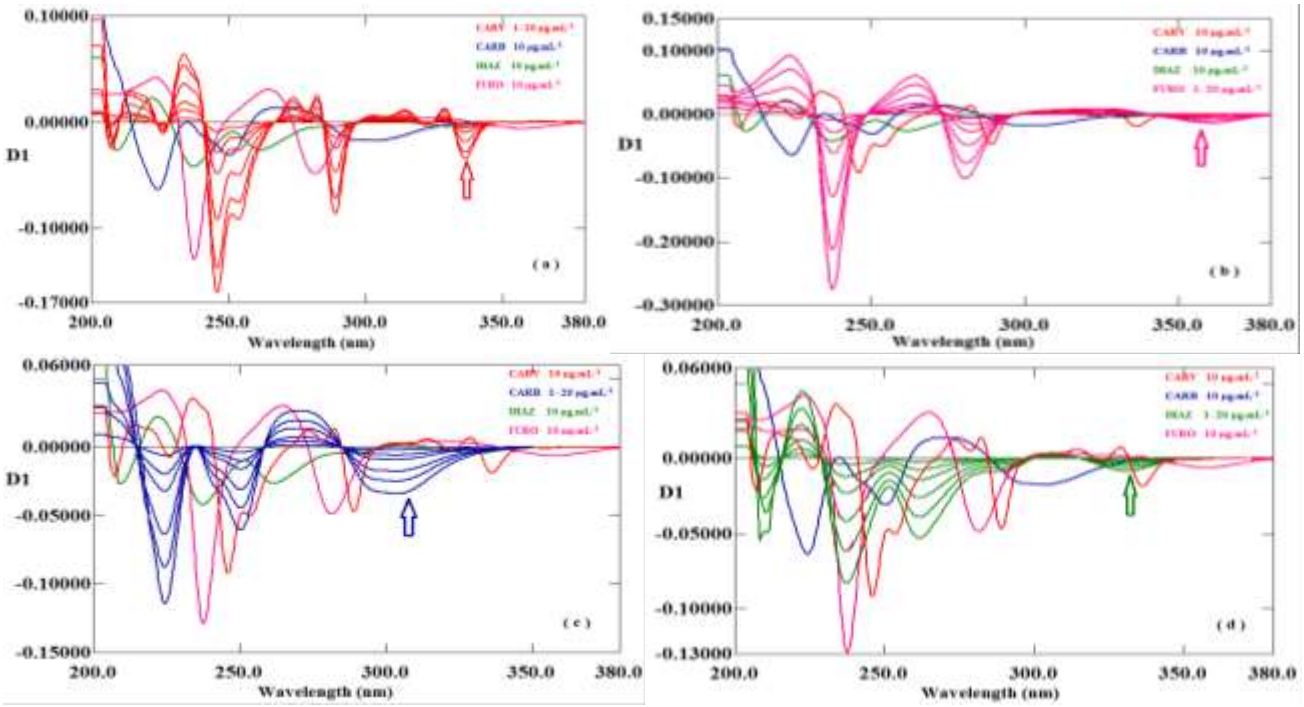


Figure 3: Overlaid first derivative spectra of: (a) CARV, (b) FURO, (c) CARB and (d) DIAZ

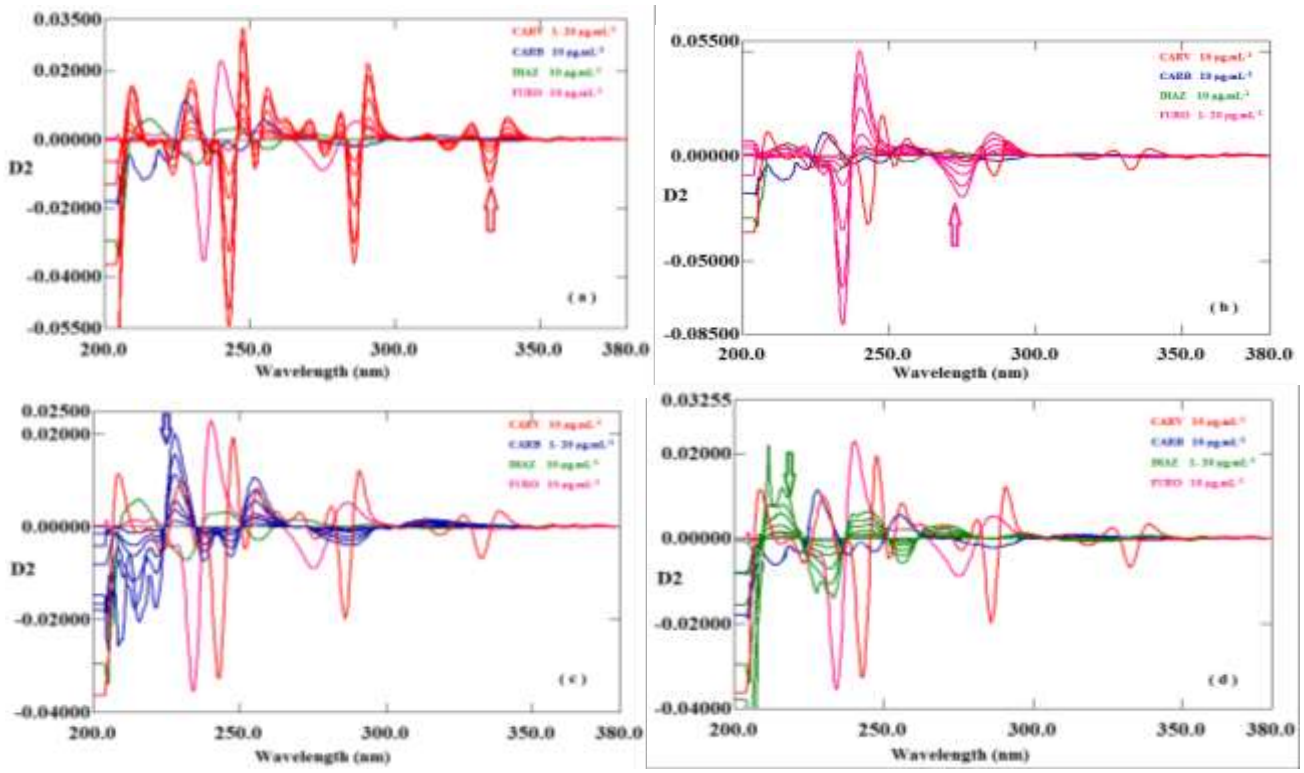
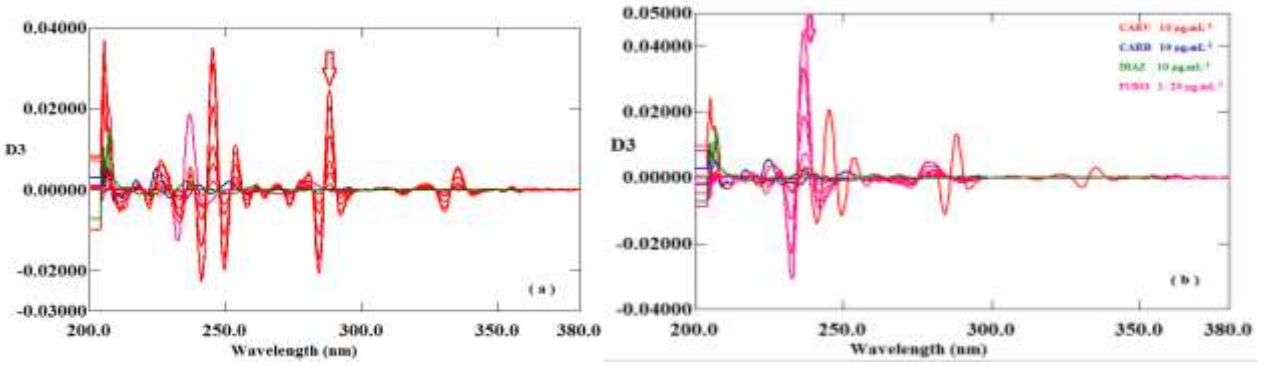


Figure 4: Overlaid second derivative spectra of: (a) CARV, (b) FURO, (c) CARB and (d) DIAZ



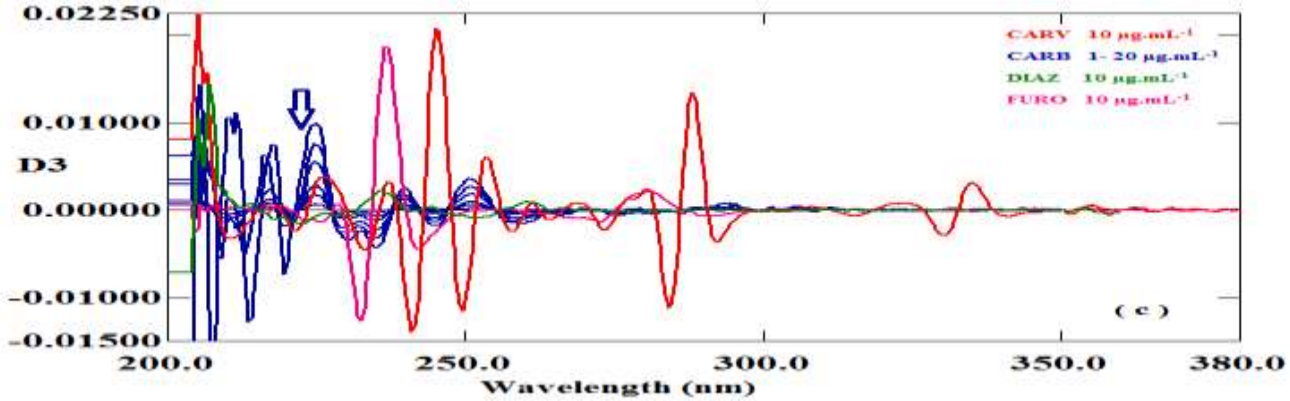


Figure 5: Overlaid third derivative spectra of: (a) CARV, (b) FURO and (c) CARB

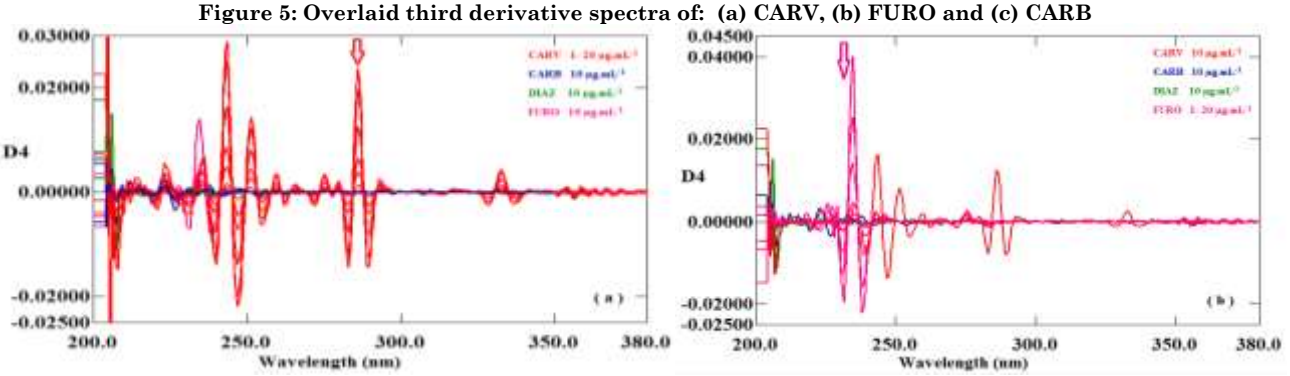


Figure 6: Overlaid fourth derivative spectra of: (a) CARV and (b) FURO

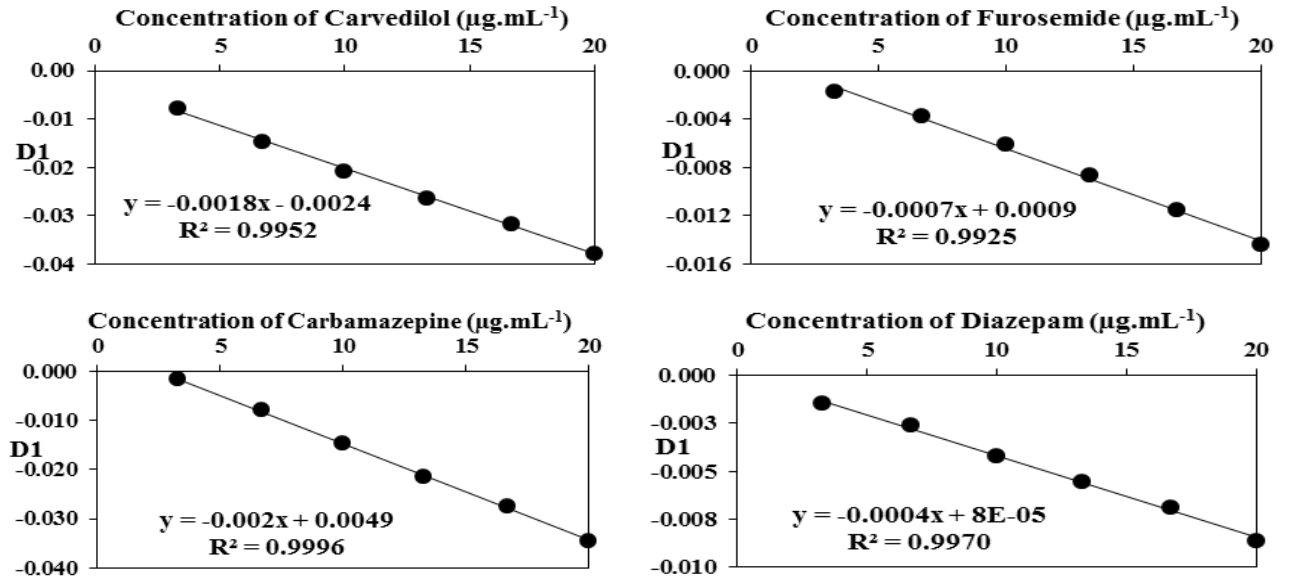


Figure 7: Calibration curves of first derivative for CARV, FURO, CARB and DIAZ

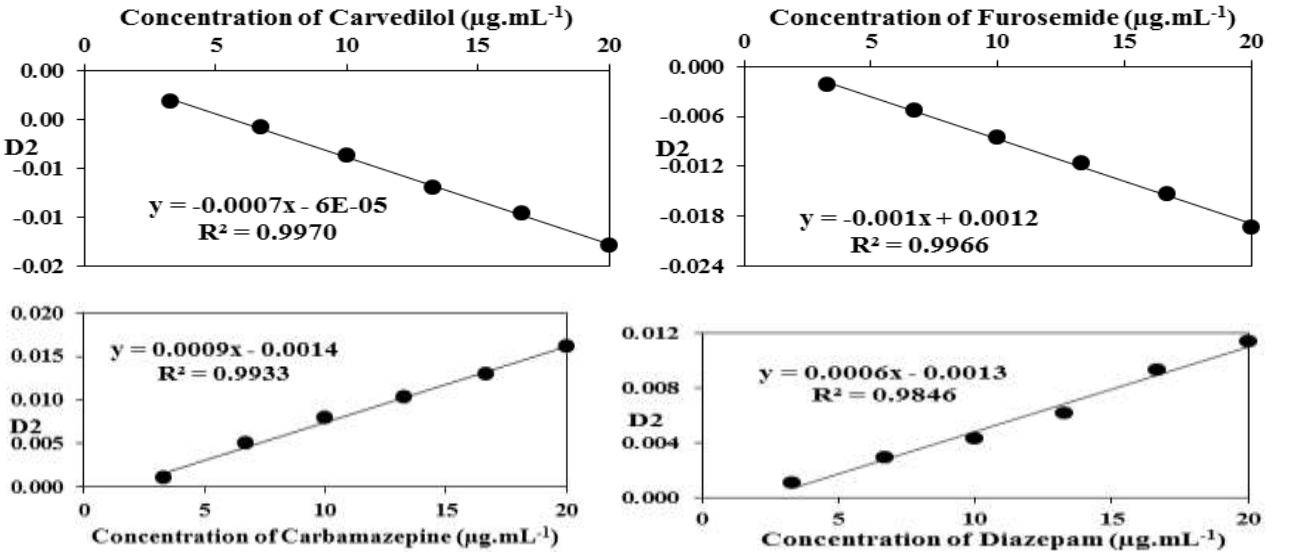


Figure 8: Calibration curves of second derivative for CARV, FURO, CARB and DIAZ

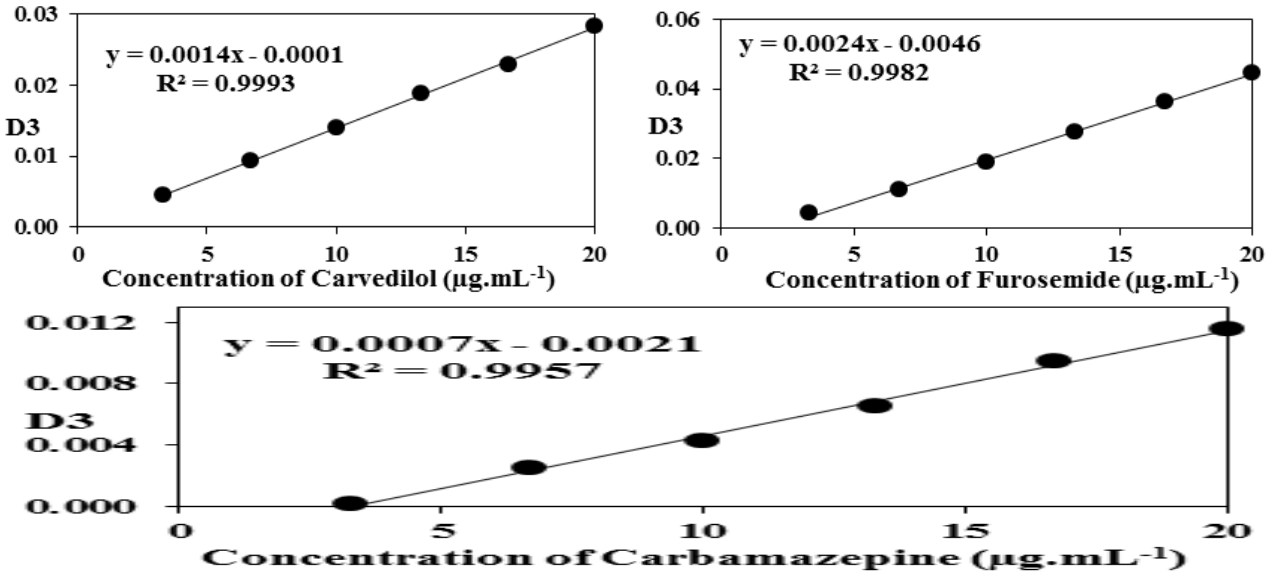


Figure 9: Calibration curves of third derivative for CARV, FURO and CARB

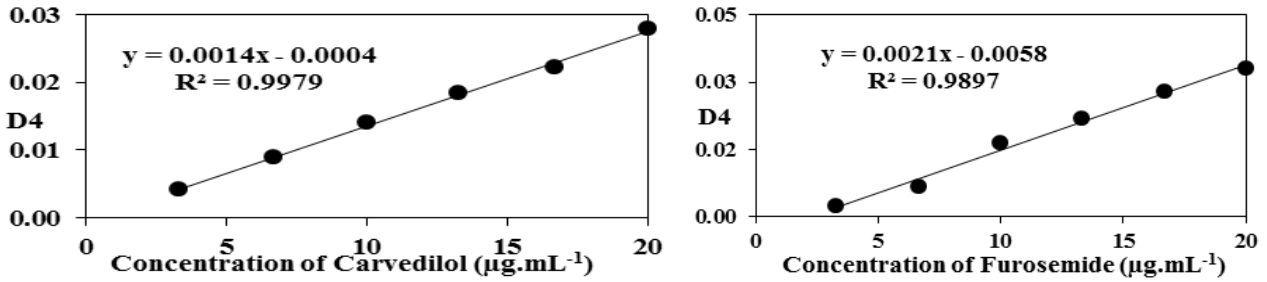


Figure 10: Calibration curves of fourth derivative for CARV and FURO

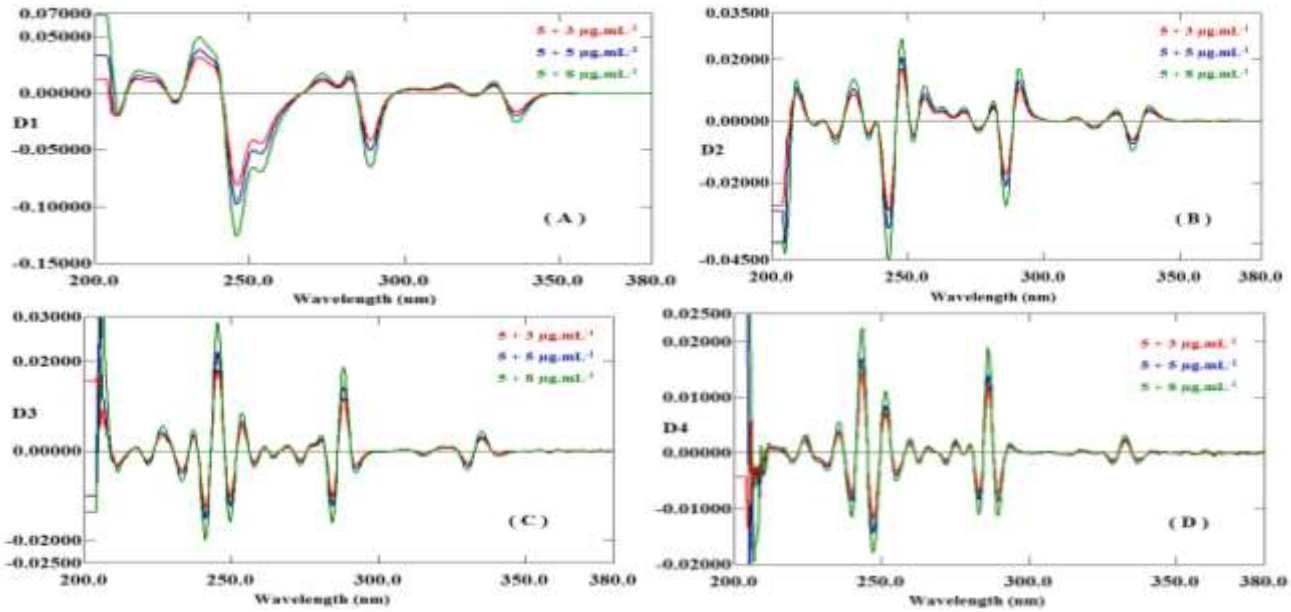
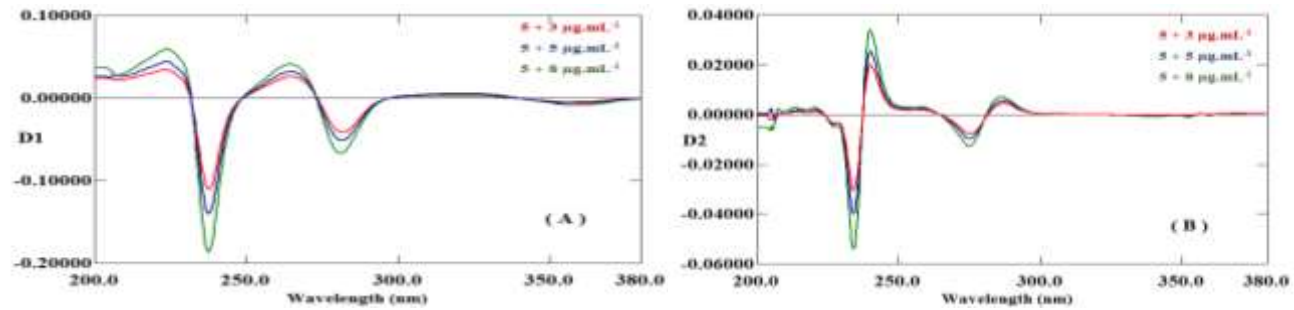


Figure 11: Derivative spectra of pure CARV (3, 5 and 8 µg.mL^{-1}) and commercial tablet (5µg.mL^{-1}) (A) 1st derivative, (B) 2nd derivative, (C) 3rd derivative and (D) 4th derivative



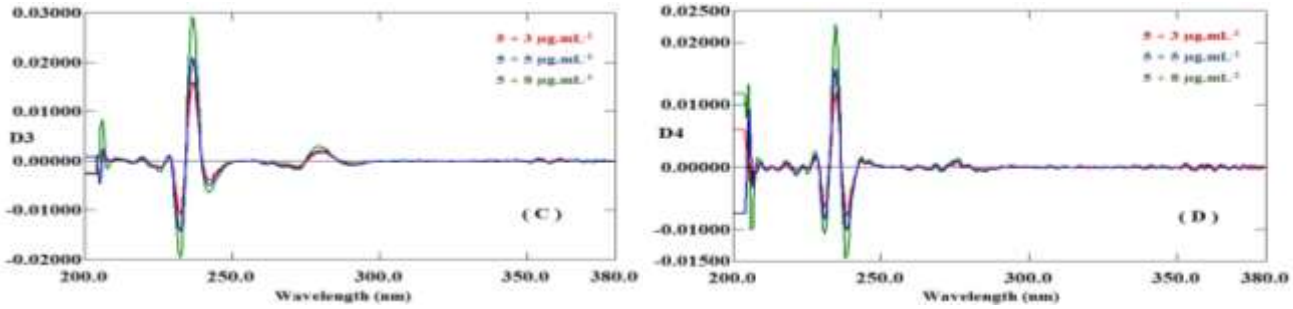
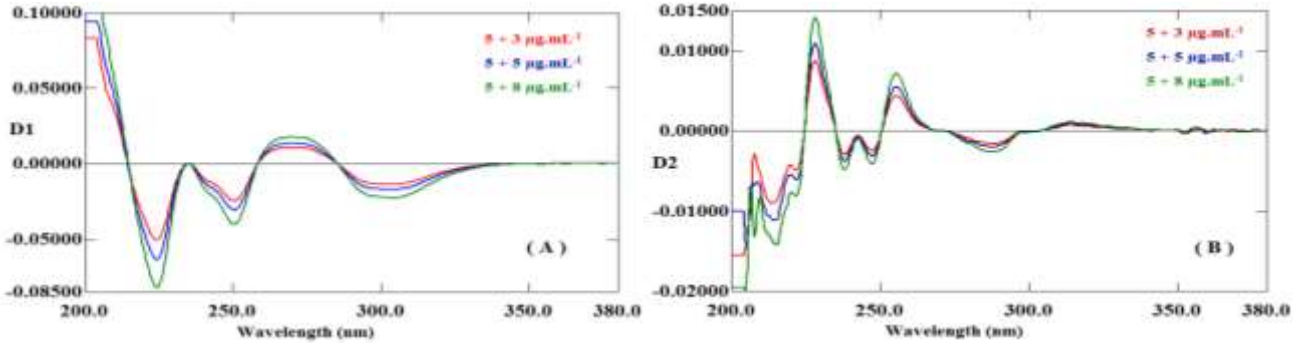


Figure 12: Derivative spectra of pure FURO (3, 5 and 8 µg.mL^{-1}) and commercial tablet (5 µg.mL^{-1}) (A) 1st derivative, (B) 2nd derivative, (C) 3rd derivative and (D) 4th derivative



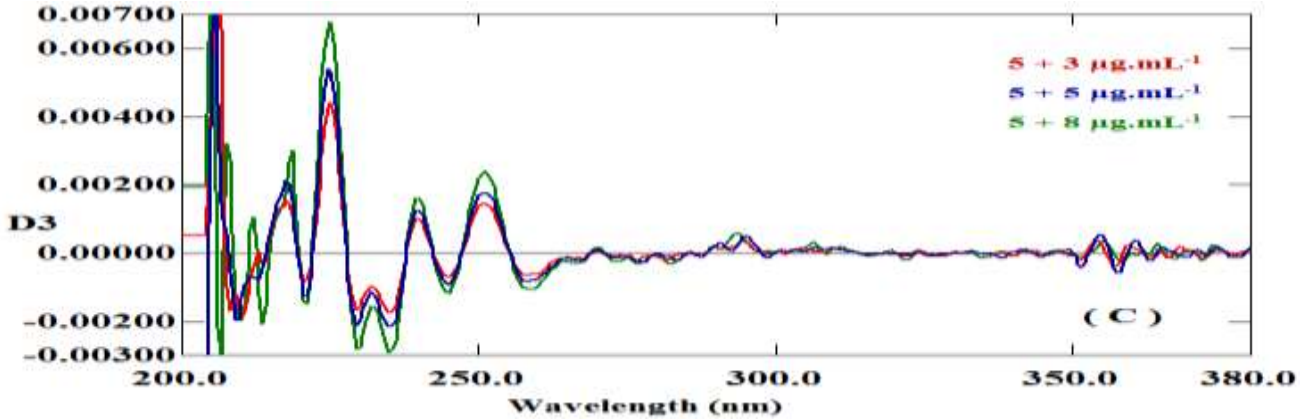


Figure 13: Derivative spectra of pure CARB (3, 5 and 8 µg.mL^{-1}) and commercial tablet (5µg.mL^{-1}) (A) 1st derivative, (B) 2nd derivative and (C) 3rd derivative

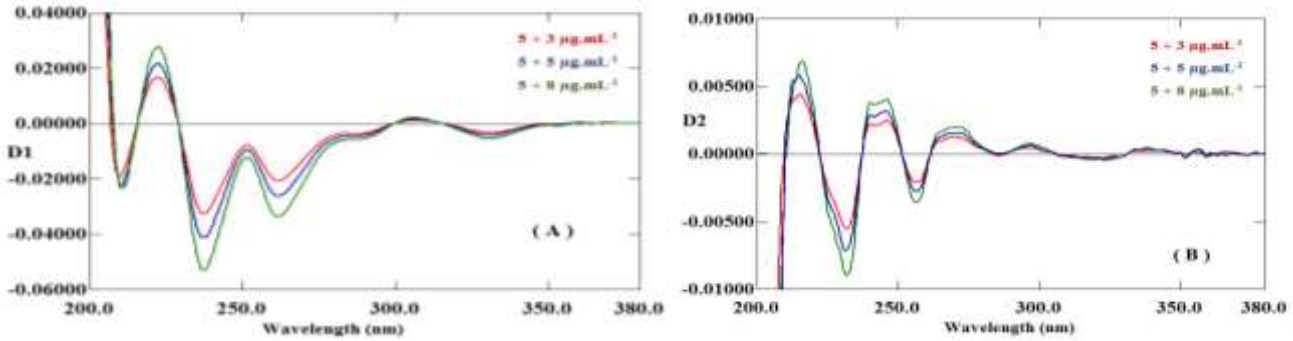


Figure 14: Derivative spectra of pure DIAZ (3, 5 and 8 µg.mL^{-1}) and commercial tablet (5µg.mL^{-1}) (A) 1st derivative and (B) 2nd derivative

Table 1: Summary of the selected methods for the determination of CARV, FURO, CARB and DIAZ and their analytical parameters

Drug	Taken range (µg.mL^{-1})	Derivative Mode	λ(nm)	Regression Equation	R^2	Detection limit* (µg.mL^{-1})
CARV	3.3 - 20.0	**D1** (*zero cross*)	336.5	y = -0.0018x - 0.0024	0.9952	0.30474
		D2 (*peak height*)	332.5	y = -0.0007x - 0.00006	0.9970	0.39386
		D3 (*peak height*)	288.0	y = 0.0014x - 0.0001	0.9993	0.07565
		D4 (*peak height*)	286.0	y = 0.0014x - 0.0004	0.9979	0.06147

FURO		**D1** (*zero cross*)	358.5	y = -0.0007x + 0.0009	0.9925	0.52135
		D2 (*zero cross*)	273.5	y = -0.0010x + 0.0012	0.9966	0.34104
		D3 (*zero cross*)	237.5	y = 0.0024x - 0.0046	0.9982	0.57262
		D4 (*zero cross*)	234.0	y = 0.0021x - 0.0058	0.9897	0.49775
CARB		**D1** (*zero cross*)	306.0	y = -0.002x + 0.0049	0.9996	0.10539
		D2 (*zero cross*)	226.0	y = 0.0009x - 0.0014	0.9933	0.59388**
		D3 (*zero cross*)	224.0	y = 0.0007x - 0.0021	0.9957	0.49068**
DIAZ		**D1** (*zero cross*)	331.5	$y = -0.0004x + 8 \times 10^{-5}$	0.9970	0.86439**
		D2 (*zero cross*)	218.5	y = 0.0006x - 0.0013	0.9846	0.93702**

*Detection limit = 3.3 (SD / slope), n = 5 measurements, ** n = 3 measurements

Table 2: Evaluation of accuracy and precision for the determination of CARV, FURO, CARB and DIAZ via derivative spectrophotometry

Drug	Derivative Mode	Taken ($\mu g.mL^{-1}$)	Found ($\mu g.mL^{-1}$)			Mean ($\mu g.mL^{-1}$)	RE%	RSD%
CARV	**D1**	3.3	3.2780	3.0830	3.1670	3.1760	-3.7576	3.0797
		13.3	13.1560	13.5220	13.6890	13.4557	1.1704	2.0261
	D2	6.7	6.5000	6.7140	6.4860	6.5667	-1.9900	1.9460
		10	10.0000	9.6860	10.2860	9.9907	-0.0933	3.0039
	D3	6.7	6.6930	6.7210	6.8070	6.7403	0.6020	0.8814
		13.3	13.4640	13.5290	13.3860	13.4597	1.2005	0.5319
	D4	3.3	3.3360	3.3070	3.2930	3.3120	0.3636	0.6622
		10	10.1640	10.3000	10.2640	10.2427	2.4267	0.6880
FURO	**D1**	3.3	3.0860	3.1290	3.2710	3.1620	-4.1818	3.0618
		6.7	6.5286	6.8000	6.8714	6.7333	0.4975	2.6861
	D2	3.3	3.2400	3.2300	3.3500	3.2733	-0.8081	2.0341
		10	9.5000	9.5200	10.2000	9.7400	-2.6000	4.0913
	D3	6.7	6.8630	6.8750	6.7500	6.8293	1.9303	1.0099
		10	9.9250	9.8130	10.3300	10.0227	0.2267	2.7137
	D4	3.3	3.3480	3.5240	3.2667	3.3796	2.4111	3.8917
		13.3	12.9330	13.1860	13.1330	13.0840	-1.6241	1.0198
CARB	**D1**	10	10.0600	10.0800	9.9600	10.0333	0.3333	0.6408
		13.3	13.2200	13.5550	13.4300	13.4017	0.7644	1.2632
	D2	10	10.1330	10.5670	9.6000	10.1000	1.0000	4.7955
		16.7	16.0780	15.9330	16.6667	16.2259	-2.8390	2.3946
	D3	6.7	6.8286	6.6000	6.5714	6.6667	-0.4975	2.1145
		13.3	12.8857	12.6286	13.1286	12.8810	-3.1506	1.9411
DIAZ	**D1**	6.7	6.8250	6.8250	6.1250	6.5917	-1.6169	6.1312
		10	10.0300	10.3500	9.7000	10.0267	0.2667	3.2415
	D2	10	9.3170	9.7000	10.5200	9.8457	-1.5433	6.2422
		13.3	13.6700	12.5500	12.7500	12.9900	-2.3308	4.5984

Table 3: Statistical validation data for quantitative assessment of commercial tablet formulation for CARV, DIAZ, CARB, and FURO

Sample	Weight labeled (mg/tablet)	Weight found (mg/tablet)				Mean (mg/tablet)	Recovery %	C.V. %
D1								
CARV (India) tablet 25mg	25	23.611	23.917	25.157	22.639	23.831	95.324	4.358
DIAZ (Iraq) tablet 2mg	2	2.170	2.010	2.020	2.040	2.060	103.000	3.611
CARB (Switzerland) tablet200mg	200	223.800	214.700	198.467	194.050	207.754	103.877	6.691
FURO (France) tablet 40mg	40	40.686	40.800	41.295	40.314	40.774	101.934	0.993
D2								
CARV (India) tablet 25mg	25	25.286	25.572	25.810	25.768	25.609	102.434	0.934
DIAZ (Iraq) tablet 2mg	2	2.347	1.940	1.878	1.830	1.999	99.931	11.827
CARB (Switzerland) tablet 200mg	200	224.440	191.778	183.704	170.000	192.481	96.240	12.014
FURO (France) tablet 40mg	40	41.680	40.360	41.093	41.380	41.128	102.821	1.375
D3								
CARV (India) tablet 25mg	25	25.679	25.589	25.774	25.563	25.651	102.604	0.373
CARB (Switzerland) tablet 200mg	200	228.572	203.428	191.619	185.286	202.226	101.113	9.447
FURO (France) tablet 40mg	40	41.680	40.360	41.093	41.380	41.128	102.821	1.375
D4								
CARV (India) tablet 25mg	25	25.929	25.804	26.476	26.295	26.126	104.503	1.198
FURO (France) tablet 40mg	40	41.680	40.360	41.093	41.380	41.128	102.821	1.375

Table 4: Application of standard addition method to the analysis of CARV, FURO, CARB and DIAZ using derivative technique

Drug	Derivative Mode	Amount of sample taken ($\mu g.mL^{-1}$)	Amount of standard added ($\mu g.mL^{-1}$)	Total amount found ($\mu g.mL^{-1}$)	Recovery %
CARV	**D1**	**5**	3	7.683	96.042
			5	9.611	96.111
			8	12.983	99.872
	D2	**5**	3	8.457	105.714
			5	10.343	103.429
			8	13.543	104.176
	D3	**5**	3	8.550	106.875
			5	10.286	102.857

			8	13.536	104.121
	D4	**5**	3	8.750	109.375
			5	10.386	103.857
			8	13.829	106.374
FURO	**D1**	**5**	3	8.329	104.107
			5	9.957	99.571
			8	13.171	101.319
	D2	**5**	3	8.130	101.625
			5	10.090	100.900
			8	12.850	98.846
	D3	**5**	3	8.575	107.188
			5	10.467	104.667
			8	13.813	106.250
	D4	**5**	3	7.638	95.476
			5	9.571	95.714
			8	12.038	92.601
CARB	**D1**	**5**	3	8.795	109.938
			5	10.470	104.700
			8	13.675	105.192
	D2	**5**	3	8.267	103.333
			5	10.200	102.000
			8	12.644	97.265
	D3	**5**	3	9.071	113.393
			5	10.714	107.143
			8	12.400	95.385
DIAZ	**D1**	**5**	3	8.750	109.375
			5	11.050	110.500
			8	13.850	106.538
	D2	**5**	3	8.150	101.875
			5	10.067	100.667
			8	12.333	94.872

Table 5: Statistical comparison of the results obtained by the three proposed methods

Furosemide							
	t-Test: Two-Sample with Equal Variances				**F-Test Two Sample for Variances**		
	Derivative	***HPLC****	***PLS****		***Derivative***	***HPLC****	***PLS****
Mean	101.5764	102.1806	100.2949	Mean	101.5764	102.18058	100.2949
Variance	4.6598	1.5103	3.5227	Variance	4.6598	1.51034	3.5227
Observations	7	6	4	Observations	7	6	4
df	11	8		df	6	5	3
| t Stat |	0.6044	1.9411		F	3.0852	2.3324	
P(T<=t) two-	0.2789	0.04410		P(F<=f) one-	0.1185	0.1911	
t Critical	2.2010	2.306		F Critical	4.9503	5.4095	
Carbamazepine							
	t-Test: Two-Sample with Equal Variances				**F-Test Two Sample for Variances**		
	Derivative	***HPLC****	***PLS****		***Derivative***	***HPLC****	***PLS****
Mean	96.9205	92.6575	95.8252	Mean	96.9205	92.0362	95.8252
Variance	11.26595	10.4970	5.8718	Variance	11.2659	13.6244	5.8718
Observations	6	5	5	Observations	6	5	5
df	9	8		df	5	4	4
| t Stat |	2.1300	1.7507		F	1.20934	2.3203	
P(T<=t) two-	0.0620	0.1181		P(F<=f) one-	0.4106	0.2175	
t Critical two-tail	2.2622	2.3060		F Critical one-tail	5.1922	6.3882	
Diazepam							

	t-Test: Two-Sample with Equal Variances				F-Test Two Sample for Variances		
	Derivative	***HPLC****	***PLS****		***Derivative***	***HPLC****	***PLS****
Mean	98.87778	99.7321	97.6249	Mean	98.8778	99.7321	96.3899
Variance	11.32466	7.3516	11.2826	Variance	11.3247	7.3516	11.9829
Observations	5	16	5	Observations	5	16	7
df	19	19		df	4	15	6
| t Stat |	0.5828	1.4381		F	1.5404	1.6300	
P(T<=t) two-tail	0.5669	0.1667		P(F<=f) one-tail	0.2410	0.2068	
t Critical two-tail	2.0930	2.0930		F Critical one- tail	3.0556	2.7905	
Carvedilol							
	t-Test: Two-Sample with Equal Variances				**F-Test Two Sample for Variances**		
	Derivative	***HPLC****	***PLS****		***Derivative***	***HPLC****	***PLS****
Mean	103.1806	103.5544	103.9956	Mean	103.1806	103.5544	103.9956
Variance	1.67606	1.5674	1.4472	Variance	1.6760	1.5674	1.4472
Observations	12	9	8	Observations	12	9	8
df	19	15		df	11	8	7
| t Stat |	0.6640	0.73851		F	1.0693	1.0831	
P(T<=t) two-tail	0.5147	0.4716		P(F<=f) one-tail	0.4745	0.46461	
t Critical two-tail	2.0930	2.1314		F Critical one-tail	3.3130	3.72571	

***** Ref. (23, 24)

Table 6: One-way ANOVA of the results obtained from the analyses of drugs mixtures by the proposed methods

Summary Statistics of **FURO** *determination*						
Method	***N***	***Sum***		***Mean***		***SD***
HPLC	6	613.0835		102.1806		1.2290
Derivative	9	902.225		100.2472		3.2334
PLS	5	507.0017		101.4003		2.9585
ANOVA						
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	13.9972	2	6.9986	0.9427	0.4090	3.5915
Within Groups	126.2029	17	7.4237			
Summary Statistics of **CARB** *determination*						
Method	***N***	***Sum***		***Mean***		***SD***
HPLC	5	460.1809		92.0362		3.6911
Derivative	6	581.5229		96.9205		3.3565
PLS	5	479.1258		95.8252		2.4232
ANOVA						
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	69.4122	2	34.7061	3.3591	0.0667	3.8056
Within Groups	134.3146	13	10.3319			
Summary Statistics of **DIAZ** *determination*						
Method	***N***	***Sum***		***Mean***		***SD***
HPLC	16	1595.7143		99.7321		2.7114
Derivative	5	494.3889		98.8778		3.3652
PLS	7	674.7293		96.3899		3.4616
ANOVA						
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	54.5049	2	27.2525	2.9952	0.0682	3.3852
Within Groups	227.4691	25	9.0988			
Summary Statistics of **CARV** *determination*						

Method	***N***	***Sum***	***Mean***	***SD***
HPLC	9	931.9896	103.5544	1.2520
Derivative	12	1238.1667	103.1806	1.2946
PLS	8	831.9644	103.9956	1.2030

ANOVA						
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	3.2024	2	1.6012	1.0128	0.3771	3.3690
Within Groups	41.1059	26	1.5810			

- Null Hypothesis: The means of all selected datasets are equal
- Alternative Hypothesis: The means of one or more selected datasets are different
- At the 0.05 level, the population means are not significantly different

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