Simultaneous Determination of Paracetamol and Hyoscine-N- Butyl Bromide in Binary Mixture Using Derivative Spectrophotometry and Their Application for Pharmaceutical Samples

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Abstract

Derivative spectrophotometric (first, second, third and fourth derivative) were used for the determination of paracetamol (PAR) and hyoscine n-butyl bromide (HYO) the binary mixture by applying zero-crossing technique for pure synthetic mixture. Also simultaneous determination of PAR and HYO in (SPAZMOTEK PLUS) tablets was applied. PAR was determined by using ^{1}D and ^{2}D methods at 297.4 and 303.5 nm (zero crossing point of HYO) with linear concentration ranges 2-30 µg.mL^{-1}, with correlation coefficient r (0.9998, 0.9987), LOD (0.081, 0.250 µg mL^{-1}), relative error (0.68, 0.22) and RSD% (0.107, 0.400), respectively. HYO was determined by using ^{1}D method at 215.9 nm (zero crossing point of PAR) with concentration range 2-25 µg mL^{-1}, with correlation coefficient 0.9997 and LOD 0.091µg mL^{-1}, relative error 1.20 and RSD% 0.342. No interference found between both determined and those of matrices. A good accuracy and precision of simultaneous determination of PAR, and HYO were confirmed by statistical analysis. The percentage recovery of the individual drugs under the established conditions is ranged from 95.07% to 100.93%, the proposed procedures were successfully applied to the determination of these compounds in different ratio by synthetic mixtures and pharmaceutical tablets without requiring any separation step.

Keywords: Paracetamol; Hyoscine-n-butyl bromide; binary mixtures; derivative spectrophotometry; zero-crossing technique.

Introduction

Paracetamol (PAR), n-(4-hydroxyphenyl) acetamide, which has the empirical formula ($C_8H_9NO_2$), as shown in Fig.(1), its white, or almost white crystalline powder with molecular weight 151.2 g/mole, Sparingly soluble in water, freely soluble in alcohol, very slightly soluble in methylene chloride.[1]

Hyoscine–n-butyl bromide (HYO) (1R,2R,4S,5S,7s,9r)-9-Butyl-7-[[(2S)-3-hydroxy-2-phenylpropanoyl]oxy]-9-methyl -3-oxa-9- azoniatricyclo [3.3.1.02,4] nonane bromide, which has the empirical formula ($C_{21}H_{30}BrNO_4$), as shown in Fig.(2), its white, or almost white crystalline powder with molecular weight 440.4 g/mole, Freely soluble in water and in methylene chloride, sparingly soluble in anhydrous ethanol.[1]

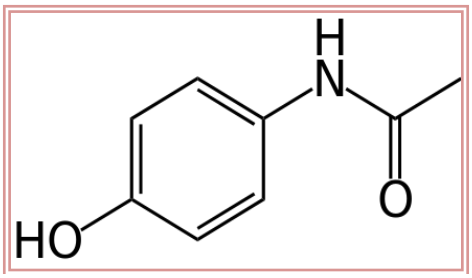

Fig.(1): Structure formula of paracetamol.

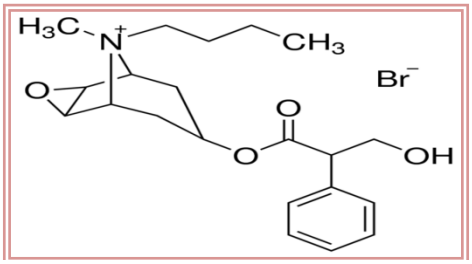

Fig.(2): Structure formula of Hyoscine –n-Butylbromide.

Paracetamol and Hyoscine–n-butyl bromide mixture

The combination of PAR and HYO are used for the relief of smooth muscle spasm (cramps) of the gastrointestinal and genitourinary system, it is effective in the

treatment of recurrent crampy abdominal pain.[2]

Various methods have been reported for the simultaneous determination of the combination of PAR and HYO in pharmaceutical formulations and biological fluids, including RP-TLC and HPLC [3-5], and UV spectrophotometry[2], Derivative spectrophotometry (DS) is widely applied for determination for inorganic ions [6,7] and organic analysis, toxicology and clinical analysis, analysis of pharmaceutical products [6-11], amino acids and proteins, in analysis of food and in environmental chemistry. In general, the application of DS is not limited to any particular case or filed, but it can be used whenever quantitative or qualitative investigations of broad spectra are difficult.

In this paper, simultaneous determination of PAR and HYO in spazmotek plus tablets DS[12]. An attempt was made to find suitable derivatives and wavelength for quantitative analysis for PAR and HYO at which both drugs show no interference. As no similar analyses were found in available literature it seems justifiable to develop a simple, quick and easily available spectrophotometric method for drug quality control purposes. This method differs from others it doesn't need any chemical treatment and both drugs can be determined directly in a binary mixture sample without using any separation process.

Experimental

Instruments and Equipments

Double-beam UV-Visible spectrophotometer model (UV-1650 PC) SHIMADZO (Japan), interfaced with computer via a SHIMADZU UV probe data system program (Version 1.10), using 1.00 cm quartz cells, (W. Germany)., Sartorius Handy 4digits Analytical Balance (GMBH, H110, Germany), and Micropipettes (200-1000µl) Swiss made.

Chemicals

1. Standards paracetamol (PAR) ($C_8H_9NO_2$; F.W. 151.2 g/mole) and hyocsine-n-butyl bromide (HYO) ($C_{21}H_{30}BrNO_4$; F.W. 440.4 g/mole) were purchased from the State Company of Drug Industries and Medical Appliances (IRAQ-SDI, Samarra). All drugs were used as working standards without further purification.
2. Pharmaceuticals drugs: Spazmotek plus tablet (SPAZMOTEK PLUS-500 mg PAR, 10 mg HYO) made by Bilim pharmaceutical limited company (Turkey).
3. Interferences material (Titanium dioxide) obtained from (BDH).

Preparation of Standard Solutions

1. Stock solutions of 250 µg mL^{-1} standard for PAR and HYO were prepared by dissolving an accurately weighed amount 25 mg of the studied drugs in distilled water and made up to100 mL volumetric flask with distilled water. Two series of pure single standards (2-30 µg mL^{-1}) for PAR and HYO were prepared by diluting stock solutions with distilled water.
2. Solutions for binary mixtures of standard drugs PAR and HYO solutions were prepared by two series. First series of mixture solutions were prepared by using a fixed concentration (25 µg.mL^{-1}) of PAR with different concent- rations (0.5, 1, 2, 4, 6, 8, 10, 15, 20, and 25 µg mL^{-1}) of HYO, While the second series of mixture contains a fixed concentration (0.5 µg mL^{-1}) of HYO with different concentration (2, 4, 6, 8, 10, 15, 20, and 25 µg mL^{-1}) of PAR.
3. Stock solutions (250 µg mL^{-1}) of interferences were prepared by dissolving an accurately weighed amount (0.0125 g) of interferences (titanium dioxide) in 50 mL distilled water; the other working solutions were prepared by dilution.

Preparation of pharmaceuticals samples

Ten tablets of the pharmaceutical HYO-PAR (SPAZMOTEK PLUS-500mg PAR, 10mgHYO) were weight and grind to fine powder accurately to be found 6.8345 g, then 0.0683 g of this powder was dissolved in 100 mL distilled water then filtered, the clear solution was taken and filed up to 100 mL.

The resultant solutions may be contained 500 mg/L (PAR) + 10 mg/L (HYO), The other working solutions were prepared by dilution.

Standard addition method for the pharmaceutical HYO-PAR (SPAZMOTEK

PLUS-500mgPAR, 10mgHYO) were prepared by taken fixed volume 5ml from 0.5 µg mL^{-1} HYO from pharmaceutical sample to 7 volumetric flask with different concentrations (0, 5, 8, 10, 12, 15 and 18 µg mL^{-1}) of standard HYO in 10 mL volumetric flask.

Results and Discussion

Selection of Optimum Instrumental Conditions

The scaling factor affecting only on the derivative amplitude, weak derivative amplitude needs to high scaling factor to give a good high peak, the suitable scaling factor that chosen to give good peak were 6, 25, 75 and 150 for ^1D, ^2D, ^3D and ^4D, respectively for PAR and HYO. However, if the value of $\Delta\lambda$ is too large, the spectral intensity signal of the first derivative deteriorates,[13]. The suitable $\Delta\lambda$ that optimized to give a good selectivity were 2, 4, 8 and 16 for ^1D, ^2D, ^3D and ^4D, respectively for PAR and HYO.

Binary Mixture: PAR with HYO Mixture

The zero order spectra of standard PAR and HYO were found to be overlapped making the determination unthinkable, as shown in Fig.(3-a).

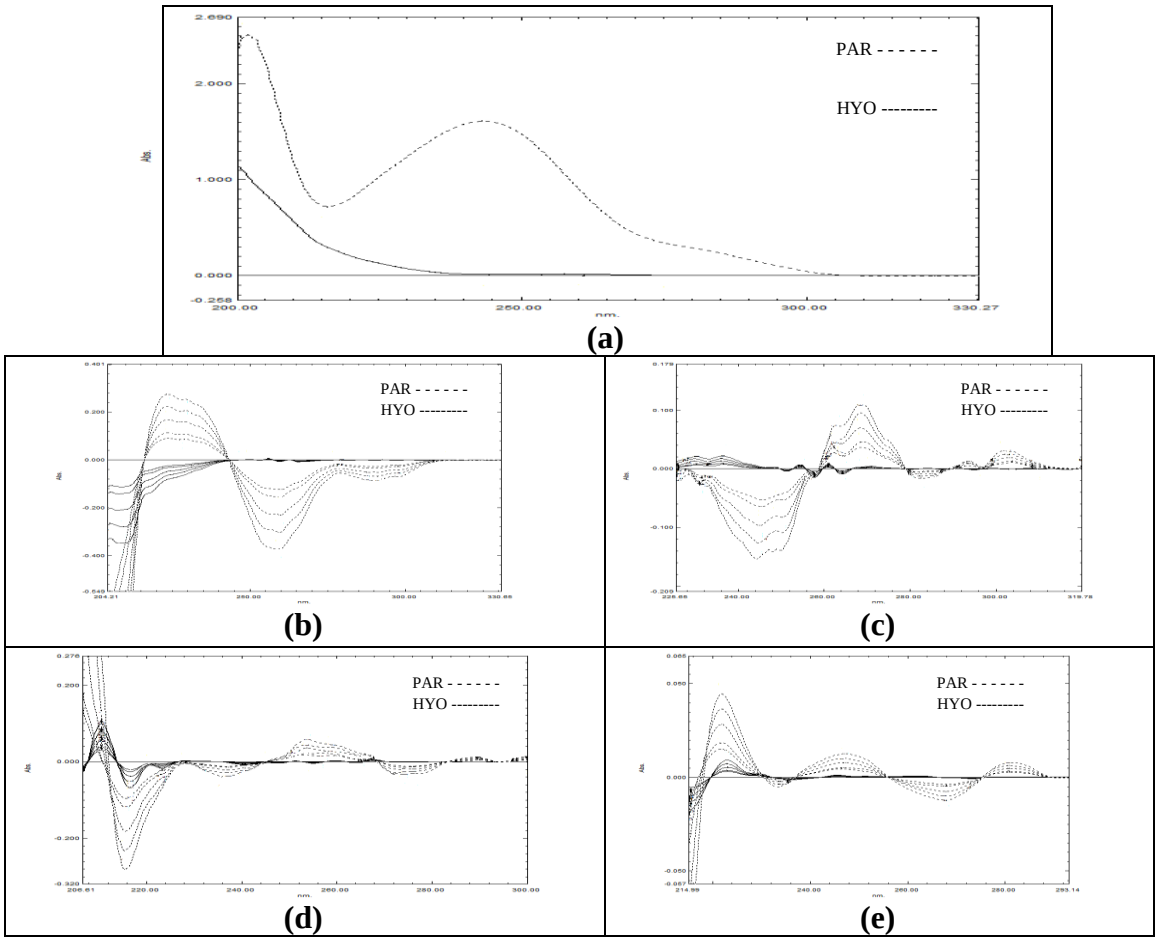

Fig.(3): Spectra of 8-25 µg.mL^{-1}PAR 8-25 µg.mL^{-1}HYO
a- normal spectrum of 25 µg mL^{-1} for each PAR and HYO.
b- first derivative(S=6,λ=2).
c- second derivative(S=25,λ=4).
d- third derivative (S=75,λ=8).
e- fourth derivative(S=150,λ=16).

First Derivative

First derivative method can be used to determine each of PAR and HYO in their mixtures, as shown in Fig.(3-b). In Fig.(4), PAR can be determined at V = 257.5 and V = 297.4 nm, while HYO have no any contribution. The calibration curve of ^{1}D spectra for standard PAR at 257.5 and 297.4 nm were constructed, as shown in part one. The linear equation, correlation coefficient and concentration range for the calibration curves are listed in Table (1). On the other hand, HYO can be determined at V=215.9 nm, where PAR absorbance was nil (zero crossing point of PAR), as shown in Fig.(5). The calibration curve of ^{1}D spectra for standard HYO at 215.9 nm were constructed, as shown in part one. The linear equation, correlation coefficient, and concentration range for the calibration curve are listed in Table (1). The results of the relative error % and recovery % for the determination of PAR and HYO in their mixtures are listed in Tables (2), (3) and (4).

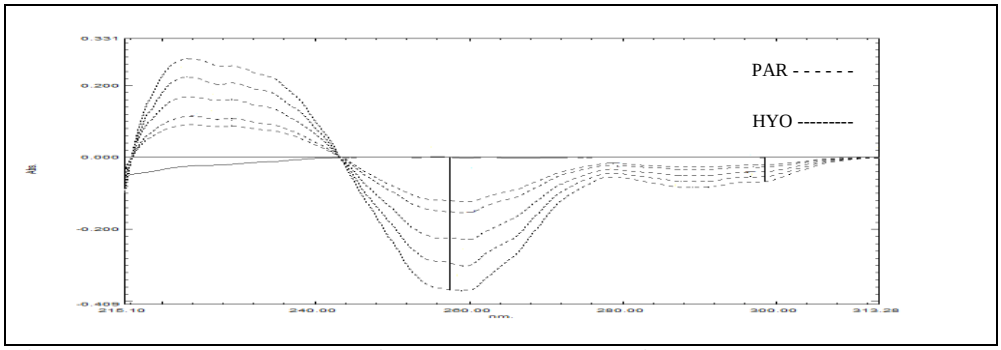

Fig.(4): ^{1}D spectra for 8-25 µg mL^{-1}PAR and 8 µg mL^{-1}HYO (zero crossing) at 257.5 and 297.4nm.

Table (1)
The parameters obtained from the calibration curves of PAR and HYO.

Method		Conc. range µg.mL^{-1}	λ (nm)	Equation	r
PAR	^{1}D	2-30	V=257.5	Y=-0.01439×-0.00542	0.9996
	^{1}D	2-30	V=297.4	Y=-0.00278×+0.00012	0.9998
	^{2}D	2-25	V=245.4	Y=-0.00595×-0.00333	0.9985
	^{2}D	2-30	P=268.2	Y= 0.00468×-0.00091	0.9990
	^{2}D	2-30	P=303.5	Y= 0.00111×+0.00151	0.9987
	^{3}D	2-35	V=237.1	Y=-0.00145×-0.00041	0.9983
	^{3}D	2-25	V=214.0	Y=-0.00910×-0.00626	0.9986
	^{4}D	2-20	P=219.7	Y= 0.00135×+0.00073	0.9976
	^{4}D	2-30	V=266.3	Y=-0.00049×-0.00000	0.9991
HYO	^{1}D	2-25	V=215.9	Y=-0.00602×+0.00111	0.9997

Table (2)
The relative error % and recovery % for the determination PAR in the presence of HYO at 257.5 nm using ^{1}D method.

PAR and HYO mixtures	PAR found* $\mu g.mL^{-1}$	Relative error %	Recovery %	PAR and HYO mixtures	PAR found* $\mu g.mL^{-1}$	Relative error %	Recovery %
30PAR+ 0 HYO	29.639	-1.20	98.80	25PAR+15 HYO	27.75	11.00	111.00
20PAR+ 0 HYO	20.195	0.98	100.98	25PAR+20 HYO	27.68	10.72	110.72
10PAR+ 0 HYO	10.191	1.91	101.91	25PAR+25 HYO	27.75	11.00	111.00
4 PAR + 0 HYO	3.755	-6.13	93.88	2 PAR+0.5 HYO	2.006	0.30	100.30
25PAR+0.5HYO	27.47	9.88	109.88	4 PAR+0.5 HYO	4.245	6.13	106.13
25PAR+ 1 HYO	27.61	10.44	110.44	6 PAR+0.5 HYO	6.553	9.22	109.22
25PAR + 2 HYO	27.401	9.60	109.60	8 PAR+0.5 HYO	8.792	9.90	109.90
25PAR +4 HYO	27.47	9.88	109.88	10PAR+0.5HYO	10.89	8.90	108.90
25PAR +6 HYO	27.61	10.44	110.44	15PAR+0.5HYO	16.697	11.31	111.31
25PAR +8 HYO	27.261	9.04	109.04	20PAR+0.5HYO	21.524	7.62	107.62
25PAR+ 10HYO	22.084	-11.66	88.34	25PAR+0.5HYO	27.47	9.88	109.88

Table (3)
The relative error % and recovery % for the determination PAR in the presence of HYO at 297.4 nm using ^{1}D method.

PAR and HYO mixtures	PAR found* $\mu g.mL^{-1}$	Relative error %	Recovery %	PAR and HYO mixtures	PAR found* $\mu g.mL^{-1}$	Relative error %	Recovery %
30 PAR+ 0 HYO	30.433	1.44	101.44	25 PAR+15HYO	24.809	-0.76	99.24
20PAR + 0 HYO	19.922	-0.39	99.61	25 PAR+20HYO	24.809	-0.76	99.24
10PAR + 0 HYO	9.873	-1.27	98.73	25PAR +25HYO	24.809	-0.76	99.24
4 PAR + 0 HYO	3.974	-0.65	99.35	2 PAR +0.5HYO	2.031	1.55	101.55
25PAR+0.5HYO	25.171	0.68	100.68	4PAR +0.5 HYO	4.036	0.90	100.90
25PAR+ 1 HYO	24.809	-0.76	99.24	6 PAR +0.5HYO	6.111	1.85	101.85
25PAR + 2 HYO	25.171	0.68	100.68	8PAR +0.5 HYO	8.186	2.33	102.33
25 PAR +4 HYO	25.809	3.24	103.24	10PAR+0.5HYO	10.298	2.98	102.98
25PAR +6 HYO	24.446	-2.22	97.78	15PAR+0.5HYO	15.297	1.98	101.98
25PAR +8 HYO	24.446	-2.22	97.78	20PAR+0.5HYO	20.009	0.05	100.05
25PAR+10 HYO	24.809	-0.76	99.24	25PAR+0.5HYO	25.171	0.68	100.68

The results of Table (3) show that PAR can be determined with high accuracy by ^{1}D method at V = 297.4 nm, when the mixture contain (0 to 50% HYO).

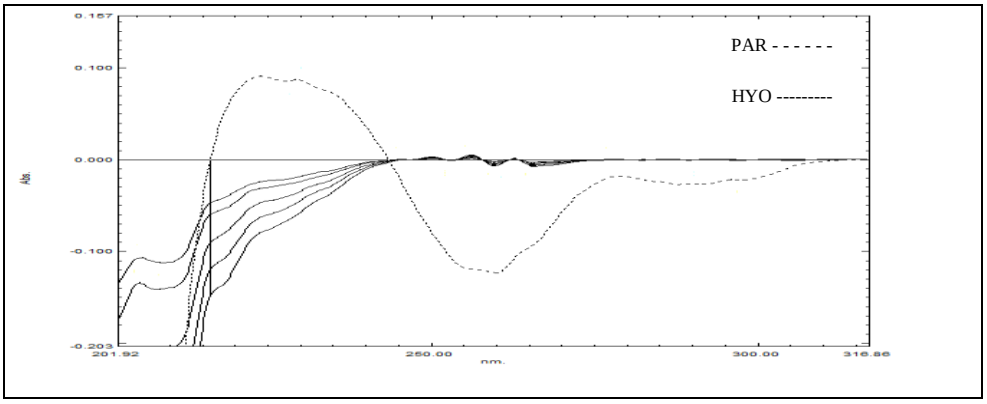

Fig.(5): ^{1}D spectra for 8-25 µg mL^{-1} HYO and 8µg mL^{-1}PAR (zero crossing) at 215.9nm.

Table (4)
The relative error % and recovery % for the determination HYO in the presence of PAR at 215.9 nm using ^{1}D method.

PAR and HYO mixtures	*HYO found* µg.mL^{-1}*	*Relative error %*	*Recovery %*	*PAR and HYO mixtures*	*HYO found* µg.mL^{-1}*	*Relative error %*	*Recovery %*
0 PAR+ 30 HYO	29.519	-1.60	98.40	25PAR +15HYO	15.387	2.58	102.58
0 PAR+ 20 HYO	20.281	1.40	101.41	25PAR +20HYO	20.402	2.01	102.01
0 PAR+ 10 HYO	10.103	1.03	101.03	25PAR +25HYO	25.126	0.504	100.504
0 PAR+ 4 HYO	4.056	1.40	101.40	2 PAR +0.5HYO	0.486	-2.80	97.20
25PAR+0.5HYO	0.506	1.20	101.20	4 PAR +0.5HYO	0.486	-2.80	97.20
25PAR+ 1 HYO	1.014	1.40	101.40	6 PAR +0.5HYO	0.512	2.40	102.40
25PAR + 2 HYO	2.023	1.15	101.15	8 PAR +0.5HYO	0.506	1.20	101.20
25PAR +4 HYO	4.118	2.95	102.95	10PAR+0.5HYO	0.512	2.40	102.40
25PAR +6 HYO	6.206	3.43	103.43	15PAR+0.5HYO	0.512	2.40	102.40
25PAR +8 HYO	8.143	1.79	101.79	20PAR+0.5HYO	0.512	2.40	102.40
25PAR+10 HYO	10.256	2.56	102.56	25PAR+0.5HYO	0.506	1.20	101.20

The results of Table (4) show that HYO can be determined with high accuracy by ^{1}D method at V = 215.9 nm, when the mixture contain (0 to more than 50% PAR).

Second Derivative

Second derivative method can be used to determine PAR only, because there is no suitable wavelength to determine HYO, as shown in Fig.(3-c). In Fig.(6), PAR can be determined at V=245.4, P= 268.2 and P=303.5 nm, while HYO have no any contribution; The calibration curve of ^{2}D spectra for standard PAR at 245.4,268.2 and 303.5 nm was constructed, as shown in part one. The linear equation, correlation coefficient and concentration range for the calibration curve are listed in Table (1). The results of the relative errors % and recovery % for the determination of PAR in the mixture are listed in Tables (5), (6) and (7).

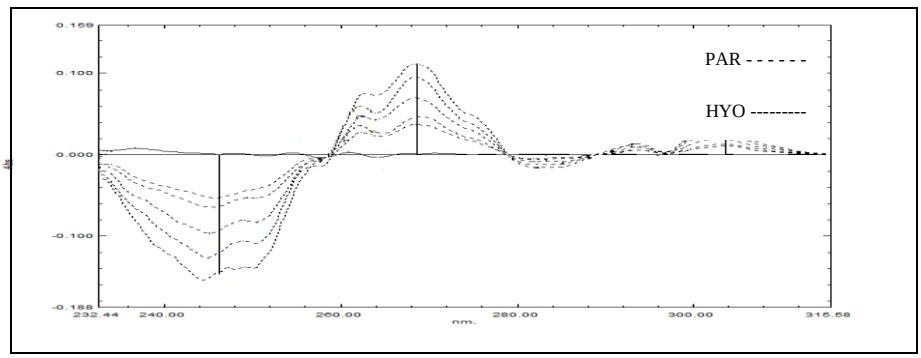

Fig.(6): ^{2}D spectra for 8-25 µg mL^{-1} PAR and 8 µg mL^{-1}HYO (zero crossing) at 245.4, 268.2 and 303.5nm.

Table (5)
The relative error % and recovery % for the determination PAR in the presence of HYO at 245.4 nm using ^{2}D method.

PAR and HYO mixtures	*PAR found* µg.mL^{-1}*	*Relative error %*	*Recovery %*	*PAR and HYO mixtures*	*PAR found* µg.mL^{-1}*	*Relative error %*	*Recovery %*
25 PAR+ 0 HYO	23.984	-4.06	95.94	25PAR+15 HYO	24.656	-1.38	98.62
20PAR + 0 HYO	20.118	0.59	100.59	25PAR+20 HYO	24.656	-1.38	98.62
10PAR + 0 HYO	9.863	-1.37	98.63	25PAR+25 HYO	24.152	-3.39	96.61
4 PAR + 0 HYO	3.643	-8.93	91.08	2 PAR +0.5HYO	1.896	-5.20	94.80
25PAR+0.5HYO	24.825	-0.70	99.30	4 PAR +0.5HYO	3.834	-4.15	95.85
25PAR + 1 HYO	25.497	1.99	101.99	6 PAR +0.5HYO	5.997	-0.05	99.95
25PAR + 2 HYO	27.01	8.04	108.04	8 PAR +0.5HYO	8.35	4.38	104.38
25PAR + 4 HYO	26.337	5.35	105.35	10PAR+0.5HYO	10.199	1.99	101.99
25PAR + 6 HYO	26.169	4.68	104.68	15PAR+0.5HYO	15.411	2.74	102.74
25PAR + 8 HYO	24.993	-0.03	99.97	20PAR+0.5HYO	20.286	1.43	101.43
25PAR+ 10HYO	26.842	7.37	107.37	25PAR+0.5HYO	24.825	-0.70	99.30

Table (6)
The relative error % and recovery % for the determination PAR in the presence of HYO at 268.2 nm using ^{2}D method.

PAR and HYO mixtures	*PAR found* µg.mL^{-1}*	*Relative error %*	*Recovery %*	*PAR and HYO mixtures*	*PAR found* µg.mL^{-1}*	*Relative error %*	*Recovery %*
30 PAR+ 0 HYO	30.015	0.05	100.05	25PAR+15 HYO	23.714	-5.14	94.86
20PAR + 0 HYO	20.02	0.10	100.10	25PAR+20 HYO	23.062	-7.75	92.25
10PAR + 0 HYO	9.807	-1.93	98.07	25PAR+25 HYO	23.062	-7.75	92.25
4 PAR + 0 HYO	3.723	-6.93	93.08	2 PAR +0.5HYO	2.419	20.95	120.95
25PAR+0.5HYO	25.017	0.07	100.07	4 PAR +0.5HYO	4.592	14.80	114.80
25PAR + 1 HYO	23.931	-4.28	95.72	6 PAR +0.5HYO	6.113	1.88	101.88
25PAR + 2 HYO	23.496	-6.02	93.98	8 PAR +0.5HYO	8.124	1.55	101.55
25PAR + 4 HYO	23.496	-6.02	93.98	10PAR+0.5HYO	9.985	-0.15	99.85
25PAR + 6 HYO	24.366	-2.54	97.46	15PAR+0.5HYO	15.239	1.59	101.59
25PAR + 8 HYO	24.148	-3.41	96.59	20PAR+0.5HYO	20.889	4.45	104.45
25PAR+ 10HYO	24.800	-0.80	99.20	25PAR+0.5HYO	25.017	0.07	100.07

Table (7)
The relative error % and recovery % for the determination PAR in the presence of HYO at 303.5 nm using ^{2}D method.

PAR and HYO mixtures	*PAR found* µg.mL^{-1}*	*Relative error %*	*Recovery %*	*PAR and HYO mixtures*	*PAR found* µg.mL^{-1}*	*Relative error %*	*Recovery %*
25 PAR+ 0 HYO	29.648	18.59	118.59	25PAR+15 HYO	25.055	0.22	100.22
20PAR + 0 HYO	19.542	-2.29	97.71	25PAR+20 HYO	25.055	0.22	100.22
10PAR + 0 HYO	10.155	1.55	101.55	25PAR+25 HYO	24.136	-3.46	96.54
4 PAR + 0 HYO	3.924	-1.90	98.10	2 PAR +0.5HYO	2.032	1.60	101.60
25PAR+0.5HYO	25.055	0.22	100.22	4 PAR +0.5HYO	4.087	2.17	102.18
25PAR + 1 HYO	25.055	0.22	100.22	6 PAR +0.5HYO	5.924	-1.27	98.73
25PAR + 2 HYO	25.973	3.89	103.89	8 PAR +0.5HYO	8.162	2.03	102.03
25PAR + 4 HYO	25.055	0.22	100.22	10PAR+0.5HYO	10.218	2.18	102.18
25PAR + 6 HYO	25.055	0.22	100.22	15PAR+0.5HYO	15.211	1.41	101.41
25PAR + 8 HYO	24.136	-3.46	96.54	20PAR+0.5HYO	19.705	-1.48	98.53
25PAR+ 10HYO	24.136	-3.46	96.54	25PAR+0.5HYO	25.055	0.22	100.22

The results of Table (7) show that PAR can be determined with high accuracy by ^{2}D method at P = 303.5 nm, when the mixture contain (0 to 50% HYO).

Third Derivative

Third derivative method can be used to determine PAR only, because there is no suitable wavelength to determine HYO, as shown in Fig.(3-d). In Fig.(7), PAR can be determined at V = 237.1, V=214.0 nm, while HYO have no any contribution; The calibration curve of ^{3}D spectra for standard PAR at 237.1, and 214.0 nm was constructed, as shown in part one. The linear equation, correlation coefficient and concentration range for the calibration curve are listed in Table (1). The results of the relative error % and recovery % for the determination of PAR in the mixture are listed in Tables (8) and (9).

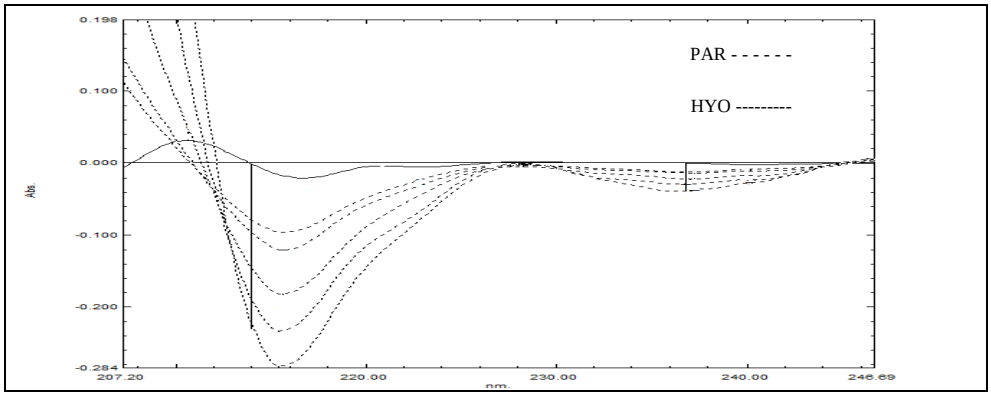

Fig.(7): ^{3}D spectra for 8-25 µg mL^{-1} PAR and 8 µg mL^{-1}HYO (zero crossing) at 237.1 and 214.0nm.

Table (8)
The relative error % and recovery % for the determination PAR in the presence of HYO at 237.1 nm using ^{3}D method.

PAR and HYO mixtures	*PAR found* µg.mL^{-1}*	*Relative error %*	*Recovery %*	*PAR and HYO mixtures*	*PAR found* µg.mL^{-1}*	*Relative error %*	*Recovery %*
25 PAR+ 0 HYO	28.969	15.88	115.88	25PAR+15 HYO	29.649	18.60	118.60
20PAR + 0 HYO	18.765	-6.18	93.83	25PAR+20 HYO	29.649	18.60	118.60
10PAR + 0 HYO	9.242	-7.58	92.42	25PAR+25 HYO	28.289	13.156	113.156
4 PAR + 0 HYO	3.799	-5.03	94.98	2 PAR +0.5HYO	2.239	11.95	111.95
25PAR+0.5HYO	26.928	7.71	107.71	4 PAR +0.5HYO	3.845	-3.88	96.13
25PAR + 1 HYO	28.969	15.88	115.88	6 PAR +0.5HYO	6.701	11.68	111.68
25PAR + 2 HYO	26.248	4.99	104.99	8 PAR +0.5HYO	8.742	9.28	109.28
25PAR + 4 HYO	24.207	-3.17	96.83	10PAR+0.5HYO	9.922	-0.78	99.22
25PAR + 6 HYO	27.609	10.44	110.44	15PAR+0.5HYO	14.684	-2.11	97.89
25PAR + 8 HYO	25.568	2.27	102.27	20PAR+0.5HYO	20.126	0.63	100.63
25PAR+ 10HYO	29.649	18.60	118.60	25PAR+0.5HYO	26.248	4.99	104.99

Table (9)
The relative error % and recovery % for the determination PAR in the presence of HYO at 214.0 nm using ^{3}D method.

PAR and HYO mixtures	PAR found* µg.mL^{-1}	Relative error %	Recovery %	PAR and HYO mixtures	PAR found* µg.mL^{-1}	Relative error %	Recovery %
25 PAR+ 0 HYO	24.26	-2.96	97.04	25PAR+15 HYO	21.073	-15.71	84.29
20PAR + 0 HYO	19.204	-3.98	96.02	25PAR+20 HYO	21.292	-14.83	85.17
10PAR + 0 HYO	10.082	0.82	100.82	25PAR+25 HYO	21.292	-14.83	85.17
4 PAR + 0 HYO	3.818	-4.55	95.45	2 PAR +0.5HYO	1.943	-2.85	97.15
25PAR+0.5HYO	23.710	-5.16	94.84	4 PAR +0.5HYO	3.987	-0.32	99.68
25PAR + 1 HYO	23.710	-5.16	94.84	6 PAR +0.5HYO	6.126	2.10	102.10
25PAR + 2 HYO	21.292	-14.83	85.17	8 PAR +0.5HYO	8.434	5.42	105.43
25PAR + 4 HYO	21.073	-15.71	84.29	10PAR+0.5HYO	10.192	1.92	101.92
25PAR + 6 HYO	23.710	-5.16	94.84	15PAR+0.5HYO	15.577	3.85	103.85
25PAR + 8 HYO	21.073	-15.71	84.29	20PAR+0.5HYO	20.523	2.62	102.62
25PAR+ 10HYO	21.292	-14.83	85.17	25PAR+0.5HYO	24.501	-2.00	98.00

Fourth Derivative

Fourth derivative method can be used to determine PAR only, because there is no suitable wavelength to determine HYO, as shown in Fig.(3-e). In Fig.(8), PAR can be determined at P = 219.7, V=266.3 nm, while HYO have no any contribution; The calibration curve of ^{4}D spectra for standard PAR at 219.7, and 266.3 nm was constructed, as shown in part one. The linear equation, correlation coefficient and concentration range for the calibration curve are listed in Table (1). The results of the relative error % and recovery % for the determination of PAR in the mixture are listed in Tables (10) and (11).

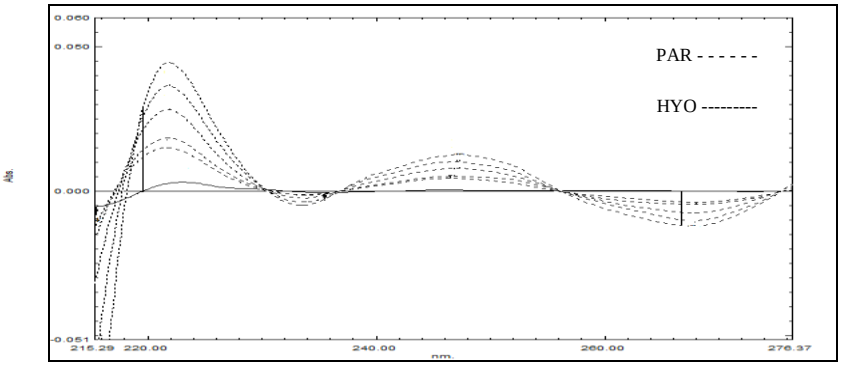

Fig.(8): ^{4}D spectra for 8-25 µg mL^{-1} PAR and 8 µg mL^{-1}HYO (zero crossing) at 219.7 and 266.3nm.

Table (10)
The relative error % and recovery % for the determination PAR in the presence of HYO at 219.7 nm using ^{4}D method.

PAR and HYO mixtures	PAR found* µg.mL^{-1}	Relative error %	Recovery %	PAR and HYO mixtures	PAR found* µg.mL^{-1}	Relative error %	Recovery %
20 PAR+ 0 HYO	19.429	-2.86	97.15	25PAR+15 HYO	20.168	-19.33	80.67
15PAR + 0 HYO	15.731	4.87	104.87	25PAR+20 HYO	20.908	-16.37	83.63
4 PAR + 0 HYO	3.897	-2.58	97.43	2 PAR +0.5HYO	1.765	-11.75	88.25
25PAR+0.5HYO	20.168	-19.33	80.67	4 PAR +0.5HYO	3.954	-1.15	98.85
25PAR + 1 HYO	20.908	-16.37	83.63	6 PAR +0.5HYO	6.221	3.68	103.68
25PAR + 2 HYO	20.168	-19.33	80.67	8 PAR +0.5HYO	8.411	5.14	105.14
25PAR + 4 HYO	20.908	-16.37	83.63	10PAR+0.5HYO	9.965	-0.35	99.65
25PAR + 6 HYO	20.168	-19.33	80.67	15PAR+0.5HYO	15.731	4.87	104.87
25PAR + 8 HYO	20.168	-19.33	80.67	20PAR+0.5HYO	19.65	-1.75	98.25
25PAR+ 10HYO	20.908	-16.37	83.63	25PAR+0.5HYO	20.168	-19.33	80.67

Table (11)
The relative error % and recovery % for the determination PAR in the presence of HYO at 266.3 nm using ^{4}D method.

PAR and HYO mixtures	*PAR found* µg.mL^{-1}*	*Relative error %*	*Recovery %*	*PAR and HYO mixtures*	*PAR found* µg.mL^{-1}*	*Relative error %*	*Recovery %*
30 PAR+ 0 HYO	30.238	0.79	100.79	25PAR + 15 HYO	26.449	5.80	105.80
20PAR + 0 HYO	20.127	0.63	100.64	25PAR + 20 HYO	26.449	5.80	105.80
10PAR + 0 HYO	10.092	0.92	100.92	25PAR + 25 HYO	26.449	5.80	105.80
4 PAR + 0 HYO	3.973	-0.68	99.33	2 PAR +0.5HYO	2.026	1.30	101.30
25PAR + 0.5HYO	24.414	-2.34	97.66	4 PAR +0.5HYO	4.062	1.55	101.55
25PAR + 1 HYO	26.449	5.80	105.80	6 PAR +0.5HYO	6.097	1.62	101.62
25PAR + 2 HYO	26.449	5.80	105.80	8 PAR +0.5HYO	8.132	1.65	101.65
25PAR + 4 HYO	24.414	-2.34	97.66	10PAR + 0.5HYO	10.167	1.67	101.67
25PAR + 6 HYO	26.449	5.80	105.80	15PAR + 0.5HYO	15.273	1.82	101.82
25PAR + 8 HYO	24.414	-2.34	97.66	20PAR + 0.5HYO	20.344	1.72	101.72
25PAR+ 10HYO	24.414	-2.34	97.66	25PAR + 0.5HYO	24.414	-2.34	97.66

Table (12) show that PAR can be determined in the presence of HYO by using ^{1}D method at 297.4 nm and ^{2}D method at 303.5nm, while HYO can be determined in the presence PAR by using ^{1}D method at 215.9 nm.

Table (12)
Statistical data for the calibration curve that used to determine PAR and HYO in their mixture.

Drug	***PAR***		***HYO***
Method	^{1}D	^{2}D	^{1}D
λ (nm)	V=297.4	P=303.5	V=215.9
Linearity range (µg.mL^{-1})	2-30	2-30	2-25
r	0.9998	0.9987	0.9997
Slope	-0.00278	0.00111	-0.00602
Intercept	+0.00012	+0.00151	+0.00111
LOD (µg.mL^{-1})	0.081	0.250	0.091
LOQ (µg.mL^{-1})	0.269	0.832	0.302
RSD (concentration)*	0.107	0.400	0.342
***SD**	0.027	0.100	0.002

**** n = 3, ** Concentration = 25 µg mL^{-1} for PAR and 0.5 µg mL^{-1}for HYO.***

Interferences Study

To find an effect of matrix constituents on the results of determination, and comparative analysis was carried out for standard solution containing active components at concentrations (25PAR+0.5HYO) µg.mL^{-1} comparable to those of the analyzed drug contain the same concentration, they show the same normal spectra Fig.(9-a).While Fig.(9-b) show comparable between standard solution containing active components at concentrations (25PAR+0.5HYO) µg mL^{-1} with interfering material (titanium dioxide) at ten time of concentrations (25PAR+0.5HYO) µg mL^{-1}.

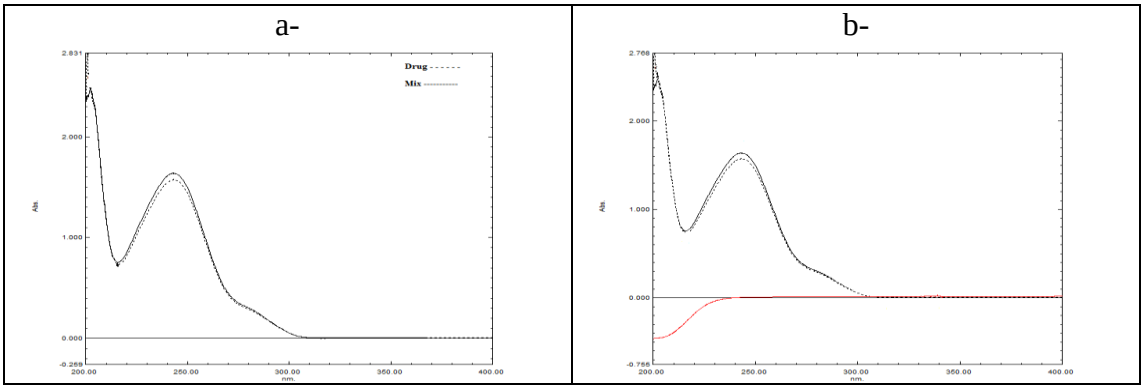

Fig.(9): Normal spectra for standard solution containing active components at concentrations (25 PAR+0.5 HYO) µg mL^{-1} comparative with.
(a) drug contain the same concentration.
(b) interfering material (titanium dioxide) at ten time of concentration of standard solution.

Analysis of Pharmaceutical Samples

SPAZMOTIC PLUS sample (25µg mL $^{-1}$) was measured by using ^{1}D, and ^{2}D methods, as shown in table (13).

Table (13)
The relative error% and recovery %for the determination of PAR in Spazmotic plus sampleb(25 µg mL^{-1} PAR + 0.5 µg mL^{-1} HYO) by DS.

Drugs	*Spazmotic plus (PAR)*	
Method	**^{1}D**	**^{2}D**
λ (nm)	V=297.4	P=303.5
Conc. found (µg.mL^{-1})	26.426	23.768
Er %	5.704	-4.926
RC %	105.704	95.073
$\mu=\overline{x} \pm (t\delta)/\sqrt{n}$	26.426±0.056	23.768±0.626
δn-1	0.045	0.503

Table (13) shows the results for the determination of PAR in Spazmotic plus by ^{1}D and ^{2}D methods. The suitable method that gave more accurate result was the ^{2}D method at 303.5 nm for PAR. While HYO cannot be determine by using direct method therefore standard additions method (SAM) was used to determine HYO as shown in Fig.(10). Table (14) shows the comparing between standard and commercial drug by ^{2}D for PAR and ^{1}D for HYO.

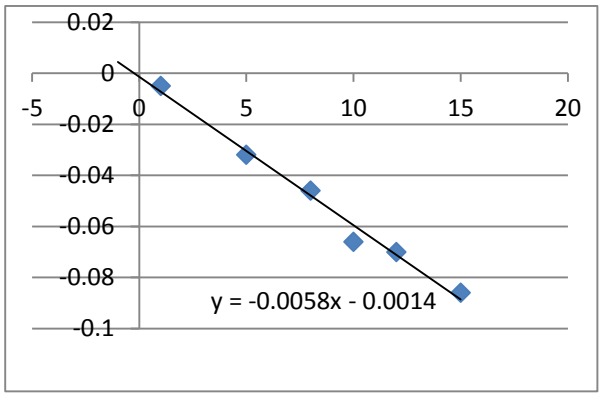

Fig.(10): Calibration carve for standard additions method for HYO by using ^{1}D method at V=215.9 nm.

Table (14)
Statistical data for the determination of PAR and HYO in their mixture in pure and Spazmotic plus tablets by ^2D for PAR and ^1D for HYO.

PAR+HYO Mixture	PAR				HYO			
	Found µg mL^{-1} λ=303.5nm	ER%	RC%	RSD%	Found µg mL^{-1} λ=215.9nm	ER%	RC%	RSD %
Standard								
25PAR+0.5HYO	25.055	0.22	100.22	0.552	0.506	1.20	101.20	0.342
Spazmotic plus								
25PAR+0.5HYO	23.768	-4.92	95.07	2.117	0.505	0.93	100.93	7.779

**** Each concentration represents an average of at least three measurements.***

Conclusions

A fast and accurate method for determining PAR and HYO was developed by using derivative spectrophotometry. The advantage of this method is that both constituents can be determined directly in binary mixture sample without the need to be separated. It was also found that D^1, D^2 is used for determining HYO and PAR, respectively in spazmotic plus tablets.

Acknowledgments

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الخلاصة

تم استخدام المشتقات الطيفية (الاولى, الثانية, الثالثة والرابعة) في تعيين الباراسيتامول والهايوسين بيوتل بروماید في مزيج ثنائي وبتقنية التقاطع الصفري للنماذج النقية والمستحضر الصيدلاني. تم تقدير الباراسيتامول و الهايوسين في مزيجهما باستخدام المشتقة الاولى والثالثة في (٢٩٧,٤ نانومتر) و (٣٠٣,٥ نانومتر) وكان مدى التراكيز الخطية (٢-٣٠ مايكروغرام/مل) بمعامل ارتباط r (٠,٩٩٩٨) و (٠,٩٩٨٧) وحد الكشف (٠,٠٨١ و ٠,٢٥٠ مايكروغرام/مل) والخطأ النسبي (٠,٦٨, ٠,٢٢) وانحراف معياري نسبي (٠,١٠٧, ٠,٤٠٠) للباراسيتامول على التوالي. اما الهايوسين فتم تقديره باستخدام المشتقة الاولى في (٢١٥,٩ نانومتر) ومدى التراكيز الخطية (٢-٢٥ مايكروغرام/مل) بمعامل ارتباط r (٠,٩٩٩٧) وحد الكشف (٠,٠٩١ مايكروغرام/مل) وكان الخطأ النسبي (١,٢٠) بمعامل الانحراف النسبي (٠,٣٤٢) على التوالي وتم تطبيقه على دواء (SPAZMOTEK PLUS-500PAR,10HYO mg) بدون تداخل بين مكونات الادوية. النتائج الاحصائية تساند دقة وصحة الطرق المستعملة حيث بلغت نسبة الاسترجاع بين (٩٥,٠٧%-١٠٠,٩٣%). هذه الطريقة سهلة لا تحتاج الى فصل او اي معاملة.