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Spectrophotometric methods for the simultaneous determination of paracetamol and dantrolene sodium in pharmaceutical dosage form

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ABSTRACT

Accurate, precise and simple spectrophotometric methods have been developed and validated for the determination of paracetamol (PAR) and dantrolene sodium (DAS). Spectrophotometric methods including zero order, first derivative (^{1}D) and derivative ratio methods (^{1}DD) have been developed. The zero order spectrophotometric method was used for the determination of DAS in the range of 1-20 µg/mL by measuring the absorbance at 379 nm where PAR exhibits zero reading. ^{1}D and ^{1}DD methods were used for the determination of PAR in the range of 1.5-20.0 µg/mL by measuring the peaks amplitudes at 265.5 nm and 265.0 nm, respectively. The proposed methods were used to determine PAR in binary mixture with DAS in the laboratory prepared mixtures and in pharmaceutical dosage form. The results obtained were statistically evaluated and found to be accurate and precise and can be satisfactorily applied for the quality control analysis of the cited drugs.

1. Introduction

Paracetamol (PAR); *N*-acetyl-*p*-aminophenol; (Figure 1a) is an analgesic, antipyretic and weak anti-inflammatory drug [1]. Dantrolene sodium (DAS); 1-[5-(4-nitrophenyl)furfurylidene amino]imidazolidine-2,4-dione sodium salt (Figure 1b) is a muscle relaxant with a direct action on skeletal muscles [1]. DAS uncouples muscular contraction from excitation, probably by interfering with the release of calcium from the sarcoplasmic reticulum [1]. Most of the drugs used for treatment of skeletal muscle disorders are combined with analgesics such as PAR. PAR and DAS are formulated as capsule in the ratio of 12:1. This combination is used for myalgia, sprains and skeletal muscle spasms due to neurological disorder.

Several methods have been reported for the determination of PAR either alone [2-4], or with its metabolites in biological fluids [5-7], or in combination with other drugs [8-12]. DAS was also determined either alone [13] or in presence of its metabolites and impurities [14,15] or in biological fluids [16,17]. Literature search revealed only one high performance liquid chromatographic method with UV detection (HPLC-UV) for the simultaneous determination of PAR and DAS in dosage form [18]. Thus, the aim of this work was to develop and validate spectrophotometric methods for the determination of PAR and DAS in combination without sample pretreatment.

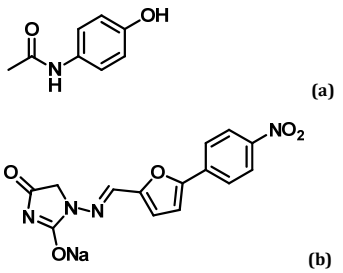

Figure 1. Chemical structures of paracetamol (a) and dantrolene sodium (b).

Among various techniques, spectrophotometric methods remain the simplest, fastest and less expensive for the determination of drug mixtures [19-21]. Direct UV-absorbance measurement (zero order) is subject to interference from degradation products and/or excipients. Therefore, derivative spectrophotometry has been applied to eliminate such interference.

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Table 1. Results obtained for the determination of dantrolene sodium by zero order method and paracetamol by ^{1}D and ^{1}DD methods in laboratory prepared mixtures.

Parameters	Ratio used		Amount recovered %		
	DAS	PAR	Zero order method	^{1}D method	^{1}DD method
	1	12	101.86	100.77	100.27
	1	12	99.71	98.53	98.06
	1	11	99.23	101.55	101.18
	1	10	99.36	100.70	99.84
	1	8	100.79	100.16	99.35
Mean (%)			100.19	100.34	99.74
S.D.±			1.000	1.007	1.030
S.E.±			0.44	0.45	0.46
R.S.D. (%)			0.9984	1.0030	1.0300

Two methods were developed and validated: first derivative (^{1}D) and first derivative ratio (^{1}DD) spectrophotometric methods to enhance the resolution of overlapping bands. The derivative spectrophotometry can be used for simultaneous determination of many drugs, it is applied by selecting a wavelength where the compound to be determined has a reasonable value while the contribution of the other compound is almost zero [19-21].

2. Experimental

2.1. Instrumentation

A Jenway 6800 double beam ultraviolet/visible spectrometer (Keison International Ltd., U.K), connected to an IBM compatible computer using 1 cm quartz cell and supported with Jenway flight deck software was used.

2.2. Reagents and reference samples

Pharmaceutical grade of PAR (certified to contain 99.80%) and DAS (certified to contain 100.1%) and Dantrelex-Compound® capsules nominally containing 300 mg of PAR and 25 mg DAS per capsule (Batch no. 110201A) were kindly provided by Chemipharm Pharm. Ind. (6th October city, Egypt). Solvents used are tetrahydrofuran HPLC grade (Tedia Company, Fairfield, United States), glacial acetic acid and absolute ethanol analytical grade (El-Nasr Pharmaceutical Chemicals Company, Gesr El Suez, Cairo, Egypt.).

The standard stock solutions of PAR and DAS (100 µg/mL) were prepared by separately dissolving 10 mg of each drug in 100 mL of the solvent mixture which was further diluted by absolute ethanol to reach the required serial dilutions. The solvent mixture was prepared by mixing 10 mL of tetrahydrofuran, 2 mL glacial acetic acid and 88 mL absolute ethanol.

2.3. General procedures and calibration

2.3.1. Zero order spectrophotometric method

Aliquots from DAS standard stock solution equivalent to 10-200 µg were accurately measured and transferred into a series of 10 mL volumetric flasks and then completed to volumes with absolute ethanol. For each solution, the zero order absorption spectra was recorded at 379.0 nm against absolute ethanol as a blank, and then plotted against its corresponding concentration. The regression equation was computed.

2.3.2. ^{1}D method

Aliquots from PAR stock standard solutions equivalent to 15-200 µg were accurately measured and transferred into a series of 10 mL volumetric flasks and then completed to volumes with absolute ethanol. For each solution, the zero order absorption spectrum was recorded against absolute ethanol as a blank, and then ^{1}D spectrum were computed applying scaling factor of 100. The amplitudes at 265.5 nm were measured and plotted against their corresponding concentrations. The regression equation was computed.

2.3.3. ^{1}DD method

The previously scanned zero order absorption spectra of PAR were divided by the spectrum of DAS (2 µg/mL) which was the chosen divisor. ^{1}D of the obtained ratio spectra was computed. The amplitudes at 265.0 nm were measured and plotted against their corresponding concentrations. The regression equation was computed.

2.4. Assay of laboratory-prepared mixtures

Laboratory-prepared mixtures were prepared using known concentration of DAS and PAR in the ratio of 1:12, 1:10, 1:11 and 1:8, respectively (Table 1). The absorption spectra of the laboratory prepared mixtures of DAS and PAR were recorded against absolute ethanol as a blank. The zero order absorption spectra at 379.0 nm were used for the direct determination of DAS. On the other hand, the ^{1}D and the ^{1}DD methods were applied for the determination of PAR. The amplitudes of 1D spectrum of the laboratory prepared mixtures were measured at 265.5 nm. For the ^{1}DD method, the previously scanned zero order absorption spectra for the laboratory prepared mixtures were divided by the spectrum of DAS (2 µg/mL). The amplitudes were measured at 265.0 nm. The concentrations of DAS and PAR were calculated from their corresponding regression equations (Table 1).

2.5. Assay of Dantrelex compound ® capsules

Twenty capsules were emptied, weighted and finely powdered. An accurate amount of the powder equivalent to 10 mg of DAS and 10 mg of PAR were weighed separately, each transferred into a 100 mL calibrated flask, dissolved in solvent mixture, sonicated for 30 min then completed to volume with the solvent mixture. The solution was filtered and diluted with absolute ethanol to the required concentration for each experiment. The procedure was continued as mentioned before under general procedures and calibration. The concentrations of DAS and PAR were calculated from their corresponding regression equations (Table 2).

3. Results and discussion

The development of spectrophotometric methods for the determination of PAR in binary mixture with DAS was of interest as there were no spectrophotometric methods have been reported for this mixture.

3.1. Optimization of conditions for spectrophotometric methods

Table 2. Results obtained for the determination of dantrolene sodium by zero order method and paracetamol by ^{1}D and ^{1}DD methods in dosage form (dantrelex compound capsule) using standard addition technique.

Parameters	Amount taken (µg/mL) [a]		Amount recovered (%) by zero order method		Amount taken (µg/mL) [b]		Amount recovered (%) by ^{1}D method		Amount recovered (%) by ^{1}DD method	
	Dosage form	Standard added	Dosage form	Standard added	Dosage form	Standard added	Dosage form	Standard added	Dosage form	Standard added
	6	3	90.91	98.50	6	3	102.88	99.71	102.35	99.05
	6	4	90.91	99.13	6	4	102.88	100.55	102.35	100.23
	6	5	90.74	99.15	6	5	102.88	100.05	102.35	99.66
	8	6	90.52	101.28	8	6	103.24	100.55	102.57	100.34
	8	7	90.92	100.70	8	7	102.92	101.98	102.57	101.10
Mean (%)			90.80	99.75			102.96	100.57	102.44	100.08
S.D±			0.17	1.17			0.15	0.86	0.11	0.76
S.E±			0.07	0.52			0.06	0.35	0.04	0.31
R.S.D. (%)			0.03	1.17			0.15	0.86	0.11	0.76

[a] Amount taken of dantrolene sodium (µg/mL).
[b] Amount taken of paracetamol (µg/mL).

Table 3. Results obtained for the determination of dantrolene sodium by zero order method and for the determination of paracetamol by ^{1}D and ^{1}DD methods.

Parameters	Zero order method	^{1}D method	^{1}DD method
λ_{max} of measurements (nm)	379.0	265.5	265.0
Linearity (µg/mL)	1.0-20.0	1.5-20.0	1.5-20.0
Regression equation	$C_{\mu g/mL} = 0.0696\ A_{279} - 0.0018$	$H_{265.5} = 0.1989\ C_{\mu g/mL} - 0.0178$	$H_{265} = 0.0265\ C_{\mu g/mL} - 0.0017$
Regression coefficient (r^2)	0.9999	0.9999	0.9999
LOD (µg/mL)	0.26	0.28	0.25
LOQ (µg/mL)	0.89	0.96	0.86
S_b	1.89×10^{-4}	6.65×10^{-4}	8.9×10^{-5}
S_a	2.22×10^{-3}	7.809×10^{-3}	1.04×10^{-3}
Confidence limit of the slope	$0.0696\pm4.63\times10^{-4}$	$0.1989\pm1.620\times10^{-2}$	$0.0265\pm2.10\times10^{-4}$
Confidence limit of the intercept	$0.0018\pm5.44\times10^{-3}$	$0.0178\pm1.913\times10^{-2}$	$0.0017\pm2.54\times10^{-3}$
Standard error of the estimation	0.0037	0.01211	0.00162
Drug in laboratory prepared mixture (%)	100.19±1	100.34±1.007	99.74±1.03
Drug in dosage form (Dantrelex Compound®) (%)	90.8±0.17	102.96±0.157	102.44±0.116
Drug added (%)	99.75±1.17	100.57±0.868	100.08±0.769

3.1.1. Zero order method

DAS could be always determined using the zero order spectrum at 379.0 nm (Figure 2) without interference from PAR. DAS showed linear correlation between the absorption and their corresponding concentrations in the range of 1-20 µg/mL (Table 3). The characteristic parameters of the regression equation are given in Table 3.

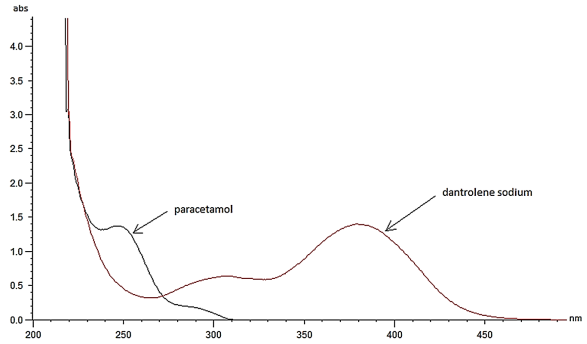

Figure 2. Zero order absorption spectra of dantrolene sodium (20 µg/mL) and paracetamol (14 µg/mL).

3.1.2. ^{1}D and ^{1}DD methods

However, PAR could not be determined by zero order spectrophotometry as its absorption spectrum exhibits overlap with that of DAS (Figure 2). ^{1}D and ^{1}DD methods have been applied to resolve the interferences between the overlapping spectra and to allow for the determination of PAR in the presence of DAS (Figure 3 and 4).

^{1}D technique showed that PAR could be determined by measuring the amplitude values at 265.5 nm (Figure 3). PAR showed linear correlation between amplitude values and their corresponding concentrations in the range of 1.5-20 µg/mL (Table 3).

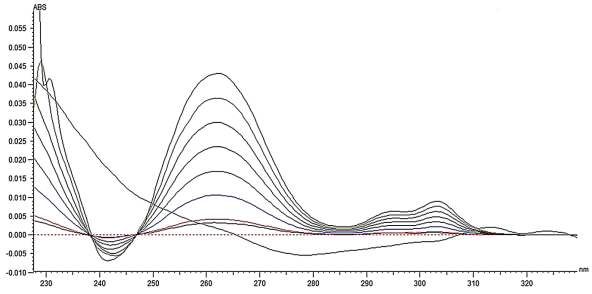

Figure 3. The first derivative spectra of paracetamol (1.5-20.0 µg/mL) at zero crossing wavelength (265.5 nm).

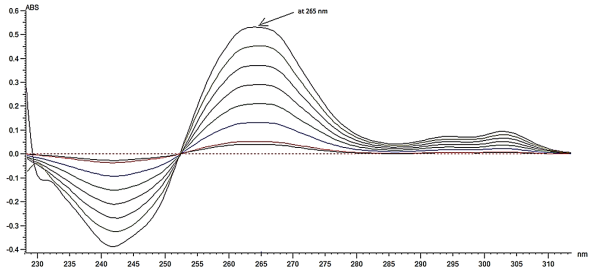

Figure 4. The first derivative ratio spectra (^{1}DD) of paracetamol (1.5-20.0 µg/mL) (Divisor: 2 µg/mL dantrolene sodium) at 265.0 nm.

Another method for resolving interferences between PAR and DAS spectra is the ^{1}DD method [19-21]. The zero order absorption spectrum of PAR is recorded, divided by the absorption spectrum of a standard solution of known concentration of DAS (2 µg/mL) and then the ^{1}D spectrum is obtained.

Table 4. The results obtained of intra-day and inter-day assays of 3 different concentrations of dantrolene sodium by zero order method and paracetamol by ^{1}D and ^{1}DD methods.

Parameters	Zero order method				^{1}D method		^{1}DD method	
	Concentration (µg/mL) [a]	Intraday (%)	Interday (%)	Concentration (µg/mL) [b]	Intraday (%)	Interday (%)	Intraday (%)	Interday (%)
	2	101.43	101.25	5	100.33	99.82	100.45	99.81
	5	99.51	99.43	11	99.99	100.45	99.89	100.08
	8	100.61	100.06	17	101.07	100.48	100.79	100.09
Mean (%)		100.51	100.25		100.46	100.25	100.38	99.99
S.D±		0.96	0.92		0.55	0.37	0.45	0.16
S.E±		0.55	0.53		0.32	0.21	0.26	0.09
R.S.D. (%)		0.96	0.92		0.55	0.36	0.45	0.16

[a] Concentration of dantrolene sodium (µg/mL).
[b] Concentration of paracetamol (µg/mL).

Table 5. Statistical comparison between the results of the spectrophotometric method and the reference method for the determination of Dantrolene Sodium.

Statistical term	Reference method [a]	Zero order method	Binary mixture with PAR
Mean (%)	99.72	100.04	100.19
S.D.±	1.27	0.98	1
S.E.±	0.63	0.44	0.44
R.S.D. (%)	1.28	0.98	0.99
N	5	5	5
V	1.61	0.96	1
t (2.306) [b]		0.41	0.31
F (6.39) [b]		1.67	2.27

[a] Reference method: aliquots of standard solutions were prepared according to B.P 2011, containing 2-10 µg/mL of DAS and measured at 262 nm using C18 column.
[b] Figures in parentheses are the theoretical t and F values at (p = 0.05).

Table 6. Statistical comparison between the results of the spectrophotometric methods and the refrence method for the determination of Paracetamol.

Statistical term	Reference method [a]	^{1}D at 265.5 nm	^{1}DD at 265.0 nm	^{1}D of Binary mixture with DAS	^{1}DD of Binary mixture with DAS
Mean (%)	100.16	100.24	99.65	100.34	99.74
S.D.±	0.99	1.04	1.08	1.007	1.03
S.E.±	0.44	0.46	0.48	0.45	0.46
R.S.D. (%)	0.98	1.04	1.09	1.003	1.03
N	5	5	5	5	5
V	0.98	1.08	1.16	1.01	1.06
t (2.306) [b]		1.25	0.78	0.286	0.6598
F (6.39) [b]		1.1	1.18	1.03	1.08

[a] Reference method: aliquots of standard solutions in distilled water containing 1.5-20.0 µg/mL PAR were measured at 249 nm using water as a blank.
[b] Figures in parentheses are the theoretical t and F values at (p = 0.05).

In order to optimize the ^{1}DD method that was developed, the influence of different variables was studied such as the divisor concentration, smoothing factor and the wavelength of measurement.

The exact choice of these parameters was of great importance, so ten different concentrations of DAS (1, 2, 4, …, and 20 µg/mL) were tried as divisors. It was found that the minimum noise and the better selectivity were obtained upon using 2 µg/mL of DAS spectrum as a divisor and smoothing factor 15 and measuring the amplitudes at 265.0 nm (Figure 4). PAR showed linear correlation between the amplitudes values and their corresponding concentrations in the range of 1.5-20 µg/mL. The characteristic parameters of the regression equation of the ^{1}DD for the determination of PAR are given in Table 3.

3.2. Validation of spectrophotometric methods

Standard calibration curves were performed by measuring the response of serial dilutions of each of the two drugs separately and applying the previously suggested procedures. The calibration curves linearity was validated by the correlation coefficients good values (Table 3). Table 3 reveals the correlation coefficients along with standard deviation of the slope (S_b) and that of intercept (S_a). The regression equations of the calibration curves were utilized for the determination of concentrations of DAS and PAR in laboratory prepared mixtures (Table 1) and capsules (Table 2). The accuracy and the reproducibility of the proposed methods were evaluated by using laboratory prepared solutions of different concentrations of the suggested drugs separately and by determining the concentrations in the dosage form. The standard addition technique was used to validate the accuracy of the procedures for the estimation of capsules (Table 2). The precision was evaluated by performing intra-day and inter-day assays on 3 different concentrations of DAS and PAR separately using the suggested spectrophotometric methods (Table 4). The results obtained were accurate and precise (Table 4).

3.3. Limit of Detection and limit of quantification

According to the ICH recommendations [22,23], the approach based on the S.D. of the response and the slope was used for determining the limit of detection and limit of quantification (Table 3).

3.4. Statistical analysis

A statistical analysis of the obtained results by the proposed methods for the determination of DAS and PAR and those obtained by the reference methods was carried out by using "*SPSS statistical package version 11*". The one way ANOVA (F-test) at p = 0.05 was performed to test the significant difference between groups as shown in (Table 5 and 6). No significant difference was found among the methods.

4. Conclusion

The suggested methods for the simultaneous determination of DAS and PAR show simplicity, precision and accuracy. The ^{1}D and ^{1}DD methods enable the simultaneous quantitation of DAS and PAR either in laboratory prepared mixtures or in pharmaceutical dosage form without sample pretreatment or separation steps. Hence the two proposed methods could be

used for routine analysis in quality control laboratories for the mentioned drugs where economy and time are essential.

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