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Spectrophotometric and TLC-densitometric determination of dantrolene sodium in presence of its acid degradates

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ABSTRACT

This work is concerned with determination of dantrolene sodium in presence of its acid degradates in bulk and in pharmaceutical formulations by two different techniques. The first one is the application of first derivative spectrophotometric technique which permits selective determination of dantrolene sodium at λ 420.9 nm without interference. Beer's law was obeyed in the concentration range of 2-22 μg mL^{-1} with mean percentage recovery of 99.67 ± 1.018. The second method depends on the quantitative densitometric evaluation of thin layer chromatogram of dantrolene sodium with ultraviolet detection at 388nm using chloroform/ acetone/ glacial acetic acid (85:15:1 by vloume) as a developing system with no interference of its acid degradates. The calibration graph was linear in the range of 0.1-0.6 μg/ band. The suggested methods were used to determine the drug in presence of its acid degradates in both pure form and commercial capsules. The obtained results were statistically compared with those obtained by the manufacturer's method, showing no significant difference with respect to accuracy and precision.

KEYWORDS

Dantrolene sodium;
Derivative spectrophotometry;
TLC-densitometry;
Acid degradation.

INTRODUCTION

Dantrolene sodium is 1-[[[5-(4-Nitrophenyl)-2-furanyl]methylene]amino]-2,4-imidazolidine-dione sodium hemiheptahydrate[1]. It is a directly acting skeletal muscle relaxant. It decrease the release of calcium ion by the sarcoplasmic reticulum and thereby block contraction of the skeletal muscle[2]. It is used to relax muscle spasm associated with multiple sclerosis, central palsy and spinal cord injury[3].

Ring opening reactions of various benzodiazepine derivatives and nitrofurantoin at azomethine bonds in acidic solutions at 37 °C have been examined[4]. It was found that the degradation products of dantrolene sodium are the precursors of its synthesis[5] so, determi-

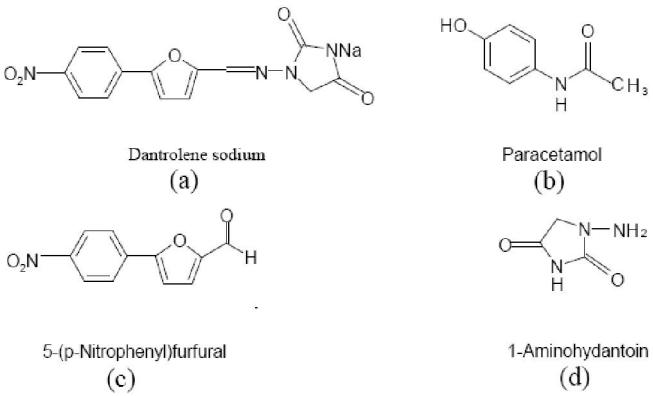

Figure 1 : The structures of dantrolene sodium (a), paracetamol (b) and its acid degradates (b&c)

nation of dantrolene sodium in presence of them is considered as stability and purity indicating method. Dantrolene sodium (DNT) is co-formulated with paracetamol (PCM) in the form of dantrelax® compound capsules. The structures of DNT, its acid degradates and PCM are shown in Figure 1.

Different techniques have been published for the quantitative determination of DNT in dosage form or in biological fluids in presence of its metabolites including; Spectrofluorimetry[6,7], high performance liquid chromatography[8-16], polarography[17-19]. A spectrophotomet[ic study of the changes accompanying the degradation of dantrolene in acidic medium is also reported[20]. To the best of our knowledge, no study has been described for the determination of DNT in presence of its acid degradates in pharmaceutical formulation. Therefore, it was desirable to develop a simple and fast procedure that could be applied in quality control laboratories for the quantitative determination of DNT in bulk powder, in pharmaceutical formulation and in presence of its degradates. In this work, two methods based on UV-derivative spectrophotometry (first derivative) and TLC-densitometry are described for the determination of the drug in presence of its degradates.

EXPERIMENTAL

Instruments

(a) Spectrophotometer: Double beam UV-Visible spectrophotometer (SHIMADZU, Japan) model UV-1601 PC with quartz cells of 1cm pathlength, connected to IBM compatible computer and HP 680 inkjet printer. The bundled software was UVPC personal spectroscopy software version 3.7. The spectra bandwidth was 2 nm and wavelength scanning speed 2800 nm/min.

(b) TLC aluminum plates, precoated with silica gel 60 F_{254} 20x20 cm and 0.25 mm thickness (E. Merck, Germany).

(c) Camag Linomat autosampler (Switzerland), Camag microsyringe (100 µL) and Camag TLC Scanner 3S/N/30319 with win CATs software.

(d) Chromatographic tank 20x21x9 cm (Dessaga).

(e) UV short wavelength (254) lamp (Dessaga, Germany).

Reagents

All chemicals and reagents are of pure analytical grade.

(1) DMF of spectroscopy analytical grade (E.Merck, Germany).

(2) Acetonitrile/hydrochloric acid (1 N), aqueous solution (90: 10 by volume) mixture (ACN/HCl mixture), (E.Merck, Germany).

(3) Sodium hydroxide, 0.2%, aqueous solution (Adwic).

(4) Chloroform, acetone and acetic acid of HPLC grade (E.Merck, Germany).

Samples

(a) **Pure standard**: DNT and PCM pure substances were kindly supplied by Chemipharm Pharmaceutical Industries, Egypt. S.A.E. The purity of DNT was found to be 99.56% according to the manufacturer's method and the purity of PCM was checked spectrophotometrically according to the official method and found to be 99.01%.

(b) **Degraded sample**: 5-(p-Nitrophenyl)furfural (NPF) and 1-aminohydantoin hydrochloride (AHD) were purchased from Sigma-Aldrich (Steinheim, Germany). The purity of NPF and AHD were labelled to be 98% for both. It was also confirmed by evaluating their melting points and comparing them to that found in literature (204-206 and 201-205 °C, respectively)[21].

(c) **Pharmaceutical dosage form**: Dantrelax® compound capsules were purchased from Egyptian market. Each capsule is claimed to contain 25 mg of dantrolene sodium and 300 mg of paracetamol batch number 060341, 061227 and 056018. Dantrelax® compound capsules are manufactured by Chemipharm Pharmaceutical Industries, Egypt. S.A.E.

Standard solutions

(1) Stock standard solutions of DNT (0.04 mg mL^{-1}), PCM (0.40 mg mL^{-1}), NPF (0.0217 mg mL^{-1}) and AHD (0.0152 mg mL^{-1}) in ACN/HCl mixture for the derivative spectrophotometric method.

(2) Stock standard solutions of DNT (0.05 mg mL^{-1}), PCM (0.20 mg mL^{-1}), NPF (0.0271 mg mL^{-1}) and AHD (0.0189 mg mL^{-1}) in ACN/HCl mixture for

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TABLE 1 : Analytical data for the determination of dantrolene sodium

(Linear regression, A = bC + a)*				
Methosd	**Concn range**	**Intercept (a)**	**Slope (b)**	**Correlation coefficient(r)**
First derivative	2.00-22.00 μg mL^{-1}	0.0233	0.0587	0.9999
Densitometry	0.100 – 0.600 μg/band	0.31622	2.0289	0.9998

***a is the analytical signal and C is the concentration**

the TLC-densitometric method.

Laboratory prepared mixtures

Prepare mixtures contain different ratios of DNT and PCM including the market ratio and different concentrations of the degradates which were mixed in the ratio of 1:1 according to their molecular weights to simulate the natural process of degradation.

Procedures

Construction of calibration graphes

a. First derivative (^{1}D) spectrophotometric method

Transfer accurate aliquots equivalent to 20 – 220 μg of DNT from its stock solution (0.04 mg mL^{-1}) into a series of 10-mL volumetric flasks and complete to mark with ACN/HCl mixture.. Record the first derivative spectra using scaling factor = 50 and $\Delta\lambda = 4$.

Construct the calibration curve relating the peak amplitudes of ^{1}D spectra at 420.9 nm to the corresponding drug concentrations. Compute the regression equation.

b. TLC-densitometric method

Into a set of 10-mL volumetric flasks, transfer accurately aliquots containing 50-300 μg from the standard stock solution of DNT (0.05 mg mL^{-1}), then complete to volume with ACN/HCl mixture. Apply 20 μL of each concentration using the autosampler to TLC plates. Bands were spaced 1.5 cm apart from each other and 1.5 cm apart from the bottom edge of the plate. Develop the plates by ascending chromatography in a chromatographic chamber presaturated with the mobile phase; chloroform/ acetone/acetic acid (85: 15: 1 by volume) for one hour. Dry the plate in air at room temperature, detect under UV lamp and scan at 388 nm. Record the area under the peak under the following conditions; source of radiation: deuterium lamp, scan mode: absorbance mode, slit dimension: 3 mm x 0.45 mm, scanning speed: 20 mm/S, band length: 4 mm.

Construct the calibration curve representing the relationship between the integrated peak area of the drug and its corresponding concentration in μg/band. Compute the regression equation

Analysis of laboratory prepared mixtures

a. First derivative (^{1}D) spectrophotometric method

Into a series of 10-mL volumetric flasks, transfer accurately, aliquots equivalent to 20 - 200 μg of DNT from its stock solution (0.04 mg mL^{-1}), add different aliquots equivalent to 280 - 960 μg of PCM from its stock solution (0.40 mg mL^{-1}) and then add aliquots of the degradation products (NPF and AHD) from their stock solutions (0.0217 mg mL^{-1} and 0.0152 mg mL^{-1}, respectively) to prepare mixtures containing the natural ratio according to their molecular weights, their percentage in the mixtures ranging from 25 - 125%. Complete to volume with ACN/HCl mixture, scan the spectra of the prepared mixtures and record the first derivative spectra. Calculate the concentrations of DNT directly from the corresponding regression equation.

b. TLC-densitometric method

Into 10-mL volumetric flasks, transfer accurately, aliquots equivalent to 50-200 μg from the corresponding stock solution of DNT (0.05 mg mL^{-1}) and 800-1400 μg from the standard stock solution of PCM (0.20 mg mL^{-1}). Add equal volumes of NPF stock solution (0.0271 mg mL^{-1}) and AHD stock solution (0.0189 mg mL^{-1}) according to their molecular weights to prepare mixtures containing different ratios of NPF and AHD mixture (1: 1 by mole). Complete to volume with ACN/HCl mixture. Proceed as under 2.6.1.b. Calculate the concentration of DNT from the corresponding regression equation.

Determination of dantrolene in dantrelax® compound

Evacuate the contents of ten capsules of Dantrelax® compound, weigh and mix well. Accurately, weigh an

amount of the powder equivalent to 4 mg DNT into a beaker, and then add 50 mL ACN/HCl mixture. Stir for 10 minutes using a magnetic stirrer then filter into a 100-mL volumetric flask. Wash the residue three times each with 10 mL ACN/HCl mixture and complete to volume with the same solvent. Make a suitable dilution to prepare solutions contain 6 μg mL^{-1} and 0.1 μg/band for ^{1}D and TLC-densitometric analysis, respectively. Proceed as under 2.6.2. a for ^{1}D method and 2.6.2.b. for TLC-densitometric analysis.

RESULTS AND DISCUSSION

The kinetic studies on the hydrolysis of dantrolene sodium in 0.1 N HCl at 37 °C were carried out spectrophotometrically[22]. It was found that DNT degrades at the azomethine bond to give NPF and AHD which are pharmacologically inactive and that reaction reaches equilibrium after 10 hours. The suggested mechanism for the degradation process is shown in Figure 2.

DNT is co-formulated with PCM in dantrelax® com-

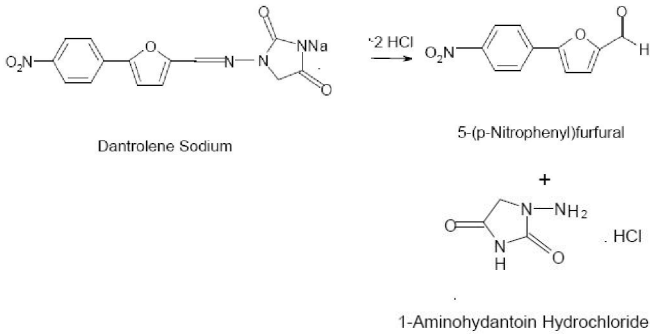

Figure 2 : The suggested mechanism for the degradation process of dantrolene sodium

pound, so it was involved in the analysis to ensure no interference during the application of the methods on its pharmaceutical formulation.

a. First derivative (^{1}D) procedure

The zero order absorption spectra of DNT and its degradates (NPF and AHD) show high degree of overlapping, Figure 3, that does not allow the use of direct spectrophotometric analysis of DNT in the presence of its acid degradates. The first derivative spectrophotometric technique permits selective determination of DNT at λ 420.9 nm without interference of neither PCM, NPF nor AHD as in Figure 4. A standard calibration curve was constructed by plotting the peak amplitude of ^{1}D

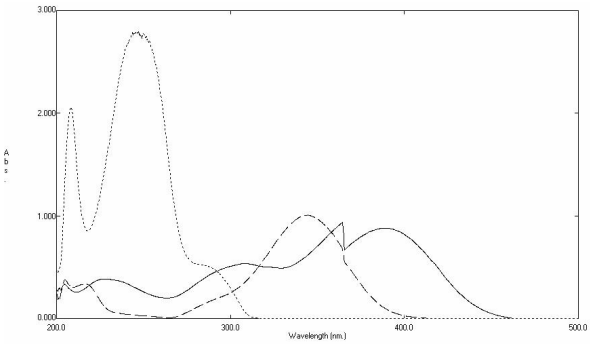

Figure 3 : Zero order spectra of dantrolene sodium (–), paracetamol (...), 5-(p-nitrophenyl)furfural and 1-aminohydantoin hydrochloride in mixture (1: 1 by mole) (---) using ACN/HCl mixture as a blank

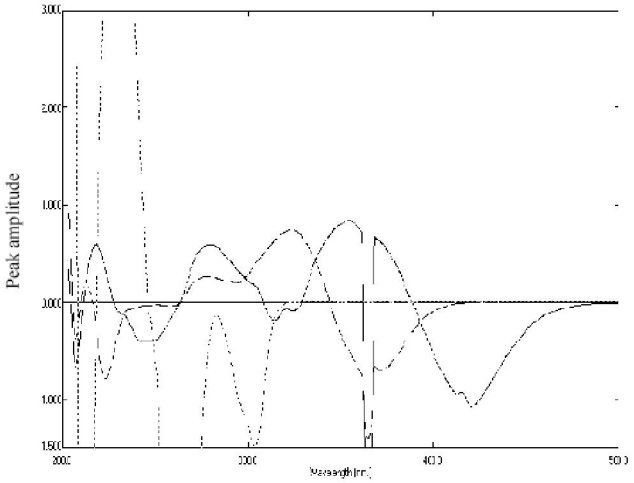

Figure 4 : First derivative spectra of dantrolene sodium (–), paracetamol (...), 5-(p-nitrophenyl) furfural and 1-aminohydantoin hydrochloride in mixture (1: 1 by mole) (---) using ACN/HCl mixture as a blank

versus concentration. Conformity with beer's law was evident in concentration range mentioned in TABLE 1.

b. TLC-densitometric method

TLC-densitometry is a useful technique for the resolution and in turn for the determination of drug mixtures, so it was described for the determination of DNT at 388 nm in the presence of its degradation products (NPF and AHD) and PCM in pure form and in pharmaceutical preparation without prior separation. Many trials were done to choose a developing system which can affect the separation. Satisfactory separation was obtained by using the developing phase chloroform/ acetone/acetic acid (85: 15: 1 by volume). The R_f values were 0.32, 0.2, 0.82 and 0.82 for DNT, PCM, NPF and AHD, respectively.

The linearity was checked and calibration curve was

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TABLE 2 : Validation of the assay results using the proposed methods

Methods validation	First derivative	Densitometry
Accuracy		
Range of linearity	2.00 – 22.00 µg mL^{-1}	0.100 – 0.600 µg/band
Mean±RSD%	99.67±1.021	99.97±1.048
Specificity & selectivity	99.66±1.036	98.81±0.898
Precision		
(Mean ±RSD%)[a]	99.59±0.931	99.75±0.924
(Mean ±RSD%)[b]	100.85±1.103	100.18±1.035
(Robustness)	101.01±1.243	100.96±1.298

[a]Intraday precision (average of 3 different concentrations (n=3) within the same day)
[b]Interday precision (average of 3 different concentrations (n=3) repeated on 3 successive days)

constructed for the above linear relation relating the integrated peak areas to the corresponding concentrations.

The validity and applicability of the proposed methods were assessed regarding accuracy, repeatability, intermediate precision and robustness, TABLE 2. The validity of the methods was indicated by the application of the suggested methods for the determination of DNT in pharmaceutical formulation and the application of standard addition technique that revealed no interference from the excepients that may be found in the pharmaceutical preparation, TABLE 3.

Results of the determination of DNT in laboratory prepared mixtures of the intact drug, its acid degradates and PCM in different ratios are presented in TABLE 4, showing high sensitivity and selectivity of the proposed methods as stability-indicating methods. TABLE 5 shows the statistical analysis of the results in comparison with the manufacturer's method using student's t-test and F-ratio at 95% confidence limit and no significant difference was found.

TABLE 3 : Determination of dantrolene sodium in pharmaceutical formulation by the proposed methods and application of standard addition technique

Preparation	First derivative	Densitomery
Dantrelax® compound capsules 25 mg DNT /capsule Batch No. 061227	99.09±1.110	99.80±0.524
Standard addition		
Recovery±RSD%	99.76±1.054	99.53±0.922

TABLE 4 : Determination of dantrolene sodium in presence of its acid degradates in laboratory prepared mixtures by the proposed methods

Degradates %*	First derivative	Densitometry
25%	100.37	100.17
50%	99.01	98.00
75%	100.81	99.06
100%	100.50	98.80
125%	98.25	98.00
Mean±RSD%	99.79±0.988	98.81±0.813

***Degradates are mixed according to their molecular weights in the ratio 1:1**

TABLE 5 : Statistical comparison between results obtained by applying the proposed and manufacturer methods for the determination of dantrolene sodium in pure powder form

Parameters	First derivative	Densitometry	Manufacturer method[a]
Mean±SD	99.67±1.018	99.97±1.048	99.56±0.983
n	6	6	6
Variance	1.036	1.098	0.966
F	0.07(5.05)[b]	1.14(4.88)[b]	
Student's t-test	0.190(2.228)[b]	0.698(2.179)[b]	

[a]Direct spectrophotometric determination of standard solution of DNT in 0.2% NaOH/DMF mixture at 395 nm
[b]The figures in parenthesis are the corresponding tabulated values at P= 0.05

CONCLUSION

The proposed methods could be used as stability indicating methods, for the determination of DNT in pharmaceutical laboratories for routine analysis and quality control analysis in both pure and dosage forms and for checking the extent of its degradation in pharmaceutical formulations due to their simplicity, accuracy and sensitivity.

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