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RESEARCH ARTICLE

Qualitative and Quantitative Chemometry as Stability-Indicating Methods for Determination of Dantrolene Sodium and Paracetamol

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Abstract: ***Background:*** Simultaneous determination of binary mixtures with simple and cost effective analysis is always of interest. Progressive advancement in chemometrics enables spectral resolution of drugs in the presence of their degradation products or impurities.

Methods and Result: Three stability indicating chemometric methods are applied for the simultaneous determination of Dantrolene sodium (DNT) and Paracetamol (PAR). Partial Least Squares (PLS), Concentration Residuals Augmented Classical Least Squares (CRACLS) and Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) were selected for that purpose. DNT and PAR were determined in the linearity range of (2 – 10 µg mL^{-1}) and (12 – 28 µg mL^{-1}), respectively, in the presence of their degradation products. The presented methods were compared for their qualitative and quantitative analyses and validated according to the ICH guidelines. Furthermore, statistical comparison between the results obtained by the proposed methods and the reported chromatographic method showed no significant differences.

Conclusion: The proposed multivariate calibrations were accurate and specific for quantitative analysis of the studied components. Furthermore, CRACLS and MCR-ALS methods succeeded in both quantitative and qualitative of the studied components and their degradation products.

Keywords: Dantrolene sodium, paracetamol, stability indicating methods, PLS, CRACLS, MCR-ALS.

1. INTRODUCTION

Combination of skeletal muscle relaxant and NSAIDS showed more efficiency for the treatment of musculoskeletal disorders rather than single agent [1]. Dantrolene sodium (DNT); 1-(5-p-nitrophenylfurfurylideneamino) hydantoin sodium [2] (Fig. **1**) is a muscle relaxant used for the treatment of muscle spasm [3]. Paracetamol (PAR); N-(4-Hydroxyphenyl) acetamide [2] (Fig. **1**) acts as analgesic and antipyretic with mild anti-inflammatory activity [3].

Literature review revealed several HPLC methods [4-11] and electrochemical methods [12, 13] for the determination of DNT alone or in the presence of its metabolites. Also, a kinetic study was reported for DNT degradation in the alkaline solution to form (DNT-DEG) [14], (Fig. **1**). Some analytical methods were reported for the determination of PAR either in combination with Para-Aminophenol (PAP), a primary hydrolytic degradation product of PAR [15], or with other drugs including UV spectrophotometric [16-22], HPLC [23, 24] and electrochemical methods [25, 26]. Reviewing the methods used for the analysis of DNT and PAR in their mixture, revealed spectrophotometric methods [27-29] and HPLC methods [27, 30, 31], while there was only two reported RP-HPLC method for the determination of the studied drugs in the presence of their degradation products [30, 31].

This study aimed to develop simple and accurate stability indicating methods for the analysis of quaternary mixtures of DNT and PAR in the presence of their degradation products, DNT-DEG and PAP. They were applied without the need of sophisticated instruments, expensive solvents or large number of samples as needed for the reported stability indicating chromatographic methods [30, 31]. Chemometric methods, namely, Partial Least Squares (PLS), Concentration Residuals Augmented Classical Least Squares (CRACLS) and Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) were developed and validated for that purpose

2. EXPERIMENTAL

2.1. Materials and Reagents

- Dantrolene sodium authentic sample was kindly supplied by Chemipharm Pharmaceutical Industries, at 6th

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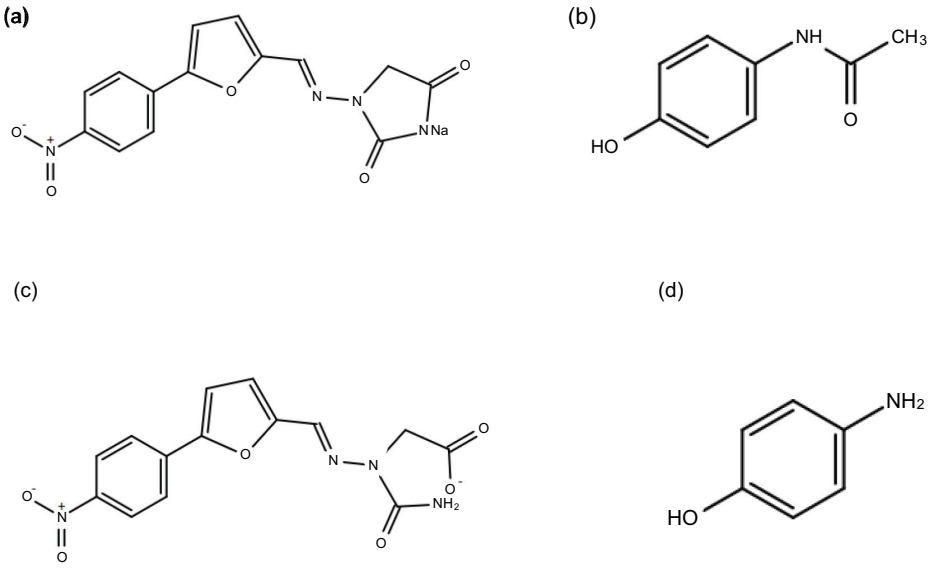

Fig. (1). Chemical structures of (**a**) Dantrolene sodium, (**b**) Paracetamol (**c**) Dantrolene degradation product and (**d**) p-aminophenol.

October, Egypt. Its purity was found to be 99.67 ± 0.913 according to the reported RP-HPLC method [30].

- Paracetamol authentic sample was obtained from El-Nile pharmaceutical Company, Cairo, Egypt. Its purity was found to be 100.04 ± 0.641 according to the reported RP-HPLC method [30].
- Para-Aminophenol Pure sample, labeled with purity ≥ 99%, was purchased from Sigma Aldrich, Darmstadt, Germany.
- Dantrelax Compound® capsules (Chemipharm Pharmaceutical Industries, 6th October, Giza, Egypt, batch No. 130859A and 131186A) were labeled to contain 25 mg Dantrolene sodium salt and 300 mg Paracetamol per capsule.
- All chemicals and reagents were of pure spectroscopic analytical grade including methanol (E. Merck, Darmstadt, Germany), hydrochloric acid, sodium hydroxide (El-Nasr Pharmaceutical Chemicals Co., Cairo, Egypt) and distilled water.

2.2. Instrument and Software

- SHIMADZU UV- 1605 PC (Japan), dual beam UV-visible spectrophotometer with two matched 1-cm quartz cells, connected to an IBM compatible (PC) and bundled UV-PC personal spectroscopy software version (3.7), were used to process the absorption spectra. The spectral band width was 0.2 nm with the wavelength scanning speed of 2800 nm min^{-1}.
- Matlab® (8.3.0.532) R2014a (The Mathworks, Natick, MA, USA) was used in the calculation of multivariate calibrations.
- PLS Toolbox 2.1 Eigenvector Research, Inc.2005 created by B.M. Wise and N.B. Gallagher for use with Matlab®.
- CRACLS function with previously designed codes [32].
- MCR-ALS Toolbox (free software available at http://www.mcrals.info).

2.3. Preparation of the Degradation product (DNT-DEG)

Based on the reported kinetic study [14], DNT-DEG was prepared by refluxing 50 mg of DNT powder with 50 mL of 2 M aqueous NaOH for 2 h followed by neutralization using 2 M aqueous HCl. The formed precipitate was filtered, washed with distilled water and dried. The residue was characterized by IR spectrometry as DNT-DEG.

2.4. Stock Standard Solutions (250 µg mL^{-1})

Stock standard solutions of DNT, DNT-DEG, PAR and PAP were prepared by accurately weighing 25 mg of bulk powder, separately into 100-mL volumetric flasks; 50 mL methanol was added to each, sonicated for few minutes and diluted to the volume with methanol.

2.5. Procedures

2.5.1. Spectral Characteristics of DNT, DNT-DEG, PAR and PAP

The zero order absorption spectra of different concentrations of DNT, DNT-DEG, PAR and PAP were scanned from 200 to 500 nm, against methanol as a blank.

2.5.2. Construction of Calibration Models

The calibration (training) set was designed with 17 synthetic mixtures containing different concentration ratios from the four components. The mixtures contained DNT and DNT-DEG in the range of 2 - 10 µg mL^{-1} while PAR and PAP concentration ranges were 12 - 28 µg mL^{-1} and 6 - 14 µg mL^{-1}, respectively. The solutions were prepared by mixing different aliquots from their respective stock standard solutions (250 µg mL^{-1}) in 25-mLvolumetric flasks and then the volumes were completed with methanol. The spectra of the prepared solutions were recorded over the range 260 - 435 nm. The data points of spectra were transferred to Mat-

lab® and multivariate calibration models were constructed. All the spectral data were mean centered before calibration to construct PLS, CRACLS and MCRALS models.

In PLS model calibration, the number of latent variables (LVs) was optimized using leave-one-out cross validation and the optimum number of LVs was 6 corresponding to the lowest RMSECV value (Root Mean Square Error of Cross-Validation).

In CRACLS calibration model, optimization of the model was achieved through repetitive approximation of absorptivity matrix continued for several iterations. The number of iterations required in order to improve our model prediction was 17 iterations.

Applied constrains were the critical optimization parameter in MCR-ALS calibration; non-negativity constrains (non-negative least squares (fnnl)) were applied for both concentration and spectral profiles in order to obtain satisfactory parameters with the least number of iterations 50.

2.5.3. Validation of Calibration Models

The validation set was designed with eight samples of solution mixtures. Different aliquots from stock standard solutions (250 µg mL^{-1}) of the four components were mixed in 25-mL volumetric flasks and then the volumes were completed with methanol. The developed parameters for PLS, CRACLS and MCR-ALS models were used for determination of the drugs along with their degradation products in the external validation set.

2.5.4. Assay of Pharmaceutical Formulation

The contents of twenty Dantrelax Compound® capsules were evacuated and mixed carefully. Accurate amount of the powder equivalent to 2.5 mg of DNT and 30 mg of PAR was weighed, transferred into 250-mL beaker; 50 mL methanol was added, sonicated for 30 min then filtered into 100-mL volumetric flasks. The residue was washed 3 times each using 10 mL methanol and the solutions were completed to the mark with the same solvent. The prepared dosage form solution, containing 25 µg mL^{-1} of DNT and 300 µg mL^{-1} of PAR, then 0.8 mL was accurately transferred into 10-mL volumetric flask and diluted to the volume with methanol. The descried procedures under each method were followed for the determination of DNT and PAR concentrations.

3. RESULTS AND DISCUSSION

Chemometric methods for simultaneous determination of binary mixture or quaternary mixture having overlapped spectra, without previous chemical separation, are always of interest [33–37]. There is no need of sophisticated instruments, expensive solvents or large number of samples as needed for the reported chromatographic methods [30, 31].

In this work, multivariate data analysis was applied for resolving the severely overlapped spectra of the studied drugs and their degradation products (Fig. **2**). Three multivariate calibrations were applied for the analysis of DNT, DNT-DEG, PAR and PAP. 25 mixtures of 4 factors 5 levels were prepared using Brereton Design [38], where 17 quaternary samples were used as a training set and the other 8 samples were used as validation set (Table **1**). Upon optimization of data handling, it was found that the best results were obtained when the spectra were digitized each at 1 nm in the range 260 - 435 nm, where 176 experimental points were used in the calculations.

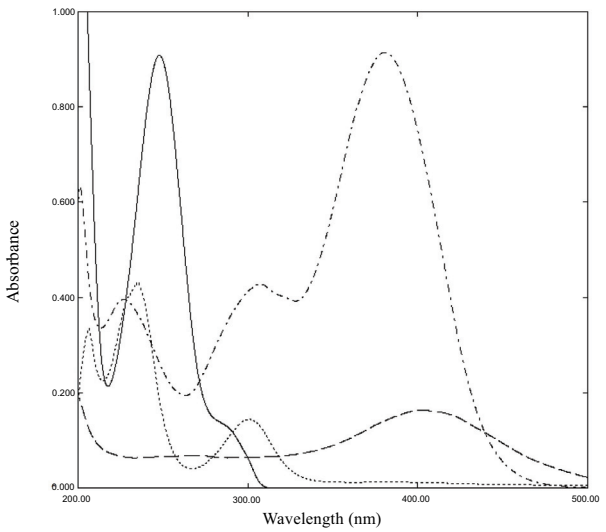

Fig. (2). The zero order absorbtion spectra of DNT 10 µg mL^{-1} (—··—··—···), DNT-DEG 10 µg mL^{-1} (— — — — —) PAR 10 µg mL^{-1} (————) and PAP 5 µg mL^{-1} (·····) separately, in methanol.

3.1. Partial Least Squares (PLS)

Partial Least Squares is a well-established technique to build multivariate calibration models [39]. The PLS algorithm takes into account the information of responses and concentrations simultaneously. In the first step the matrix of spectral data **X** is decomposed into a matrix of latent variables scores (T) and a matrix of loadings (P).

$$X = TP + Ex \quad (1)$$

Where **Ex** was matrix of spectral residuals

In the next regression step, the scores of the latent variables are used in the regression equation as a substitute of the original variables.

$$C = Tq + E \quad (2)$$

Where **C** was the calculated concentrations matrix, while q and E were the regression coefficients and concentration residuals matrices, respectively.

In this study the cross validation method, leaving out one sample at a time, was used to select the optimum number of factors where 6 LVs provided the least significant prediction error (Fig. **3**).

3.2. Concentration Residuals Augmented Classical Least Squares (CRACLS)

CRACLS is an alternative method that uses both (A) and concentration (C) data matrices by a process of repetitive approximation to estimate absorptivity ($\hat{S}$) [32]. The initially calculated S is used to predict the concentration matrix (C')

Table 1. Concentration of DNT, PAR and their degradation products in the training and validation sets for the multivariate calibrations.

Mixture number	Concentrations (μg mL^{-1})			
	DNT	DNT-DEG	PAR	PAP
1	6	6	20	10
2[a]	6	4	16	14
3	4	4	28	6
4	4	10	12	14
5	10	2	28	10
6	2	10	20	6
7[a]	10	6	12	6
8	6	2	12	12
9	2	2	24	14
10	2	8	28	12
11	8	10	24	10
12	10	8	20	14
13[a]	8	6	28	14
14[a]	6	10	28	8
15	10	10	16	12
16	10	4	24	8
17[a]	4	8	16	10
18	8	4	20	12
19[a]	4	6	24	12
20[a]	6	8	24	6
21[a]	8	8	12	8
22	8	2	16	6
23	2	4	12	10
24	4	2	20	8
25	2	6	16	8

[a] samples used in the validation set

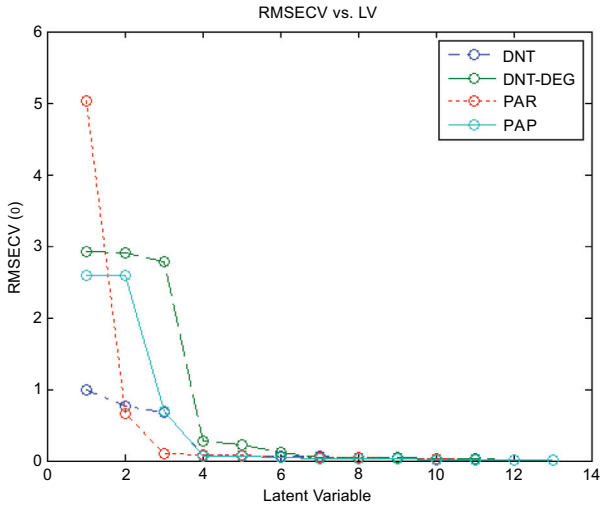

Fig. (3). RMSECV plot of the cross validation results of the training set as a function of the number of latent variables (LV's) used to construct the PLS calibration.

then Error (E) is calculated. One vector at a time of E is augmented to the original concentration matrix as (E) is considered a new component then calculation of absorptivity matrix is repeated using the augmented C matrix as follows:

$$\hat{S} = (C'C)^{-1} C'A \quad (3)$$

$$C' = A\hat{S}' (\hat{S}\hat{S}')^{-1} \quad (4)$$

$$E = C' - C \quad (5)$$

This iterative approach is continued until no further improvement in prediction. In this study 17 iterations were required in order to improve model prediction.

3.3. Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS)

Multivariate curve resolution is a factor analysis derived method which assumes a bilinear model [40]. In the MCR, data matrix (D) decomposition is carried out as follows:

$$D=CS^{T} +E \quad (6)$$

Where D is the data matrix of the measured spectra, C and S are the concentration and spectral profiles matrices of the pure components in the samples and E is a matrix of the experimental error.

Repetitive estimations of **C** from $\mathbf{S}^T$ and vice versa by MCR were optimized by the ALS procedure. Since data matrix decomposition has no unique solution, the number of possible solutions can be minimized by applying constraints like; unimodality, closure or non-negativity. In order to start the optimization of the method, simple interactive self-modelling analysis (SIMPLISMA) [41] is applied to obtain a preliminary estimation of spectral profiles matrix which is used to calculate unconstrained least squares solution for the concentration profile as follow

$$C = D\ S\ (S^TS)^{-1} \quad (7)$$

In this work, a non-negativity constraint was applied for both concentration and spectral profiles, which obliged the spectra and concentration to be equal or greater than zero. The ALS optimization process ended up when reaching a definite convergence criterion (0.1%). The convergence was stopped at 50 iterations.

The calculated lack of fit (*%lof*) and variance percentages (R^2) were 0.16934 and 99.9997 which were satisfactory enough to assist the quality of the proposed MCR-ALS model.

3.4. Method Validation

The developed models were challenged with the spectra of the validation set in order to test their predictive ability. The predicted concentrations of the validation set were plotted against the true concentrations and the RMSEP (Root-Mean-Square Error of Prediction) was calculated for each component. The RMSEP was used as a diagnostic tool for examining the prediction errors and it indicated both accuracy and precision of the proposed models as shown in (Table **2**).

Moreover, both CRACLS and MCR-ALS models could estimate the spectral profiles of DNT, DNT-DEG, PAR and PAP. The resemblance was observed with the pure spectra and correlation coefficients were calculated that indicated the well correlation between the estimated and pure spectra for each component (Fig. **4**).

Validation of the proposed methods was performed according to the ICH guidelines [42]. Mean and RSD% values of the analysis of DNT and PAR in their lab prepared mixtures in the validation set by the proposed methods were satisfactory to evaluate their selectivity (Table **3**). Also, Linearity parameters along with LOD and LOQ were calculated (Table **3**). The same table showed accuracy results which assessed the high accuracy of the proposed methods.

The presented chemometric methods were applied for the determination of DNT and PAR in the Dantrelax Compound® capsules. The good percentage recoveries confirmed the suitability of the proposed methods for the routine determination of these drugs in their combined formulation (Table **3**).

Finally, statistical comparison between the results obtained by the proposed methods for the determination of DNT and PAR in bulk powder and those obtained by the reported RP-HPLC method [30] showed no significant differences, where the calculated t-values and F-values were less than the theoretical ones (Table **4**).

CONCLUSION

The present work emphasized the role of chemometry and suggested that it could be used for simultaneous determination of DNT and PAR binary mixtures with simple procedures and minimum manipulation. Moreover, the proposed methods could be used as stability indicating methods for the determination of the studied drugs and their degradation products. The studied methods were accurate and specific for quantitative analysis of the studied components. Also,

Table 2. Prediction of validation samples using the proposed chemometric methods.

Mixture No.	PLS				CRACLS				MCR-ALS			
	DNT	DNT-DEG	PAR	PAP	DNT	DNT-DEG	PAR	PAP	DNT	DNT-DEG	PAR	PAP
1	101.40	98.01	100.28	99.95	100.93	97.66	100.15	99.90	101.40	98.01	100.28	99.95
2	99.66	102.75	99.53	100.46	100.72	100.19	100.93	100.50	99.66	102.75	99.53	100.46
3	100.76	97.69	100.28	100.46	99.81	97.49	100.06	100.62	100.76	97.69	100.28	100.46
4	100.41	98.30	100.98	98.74	99.21	97.78	100.70	98.59	100.41	98.30	100.98	98.74
5	100.70	99.89	101.17	99.70	101.57	101.87	101.38	99.60	100.70	99.89	101.17	99.70
6	101.69	98.33	101.45	100.64	101.79	99.99	101.38	100.40	101.69	98.33	101.45	100.64
7	98.63	103.79	100.98	101.92	99.39	103.28	100.92	101.08	98.63	103.79	100.98	101.92
8	99.44	102.17	98.98	101.23	99.95	100.63	98.68	101.29	99.44	102.17	98.98	101.23
Mean	100.32	100.11	100.45	100.38	100.42	99.86	100.52	100.25	100.32	100.11	100.45	100.38
±RSD%	1.027	2.430	0.852	0.959	0.971	2.115	0.889	0.868	1.027	2.430	0.852	0.959
RMSEP	0.057	0.163	0.197	0.074	0.051	0.147	0.189	0.071	0.081	0.219	0.214	0.081

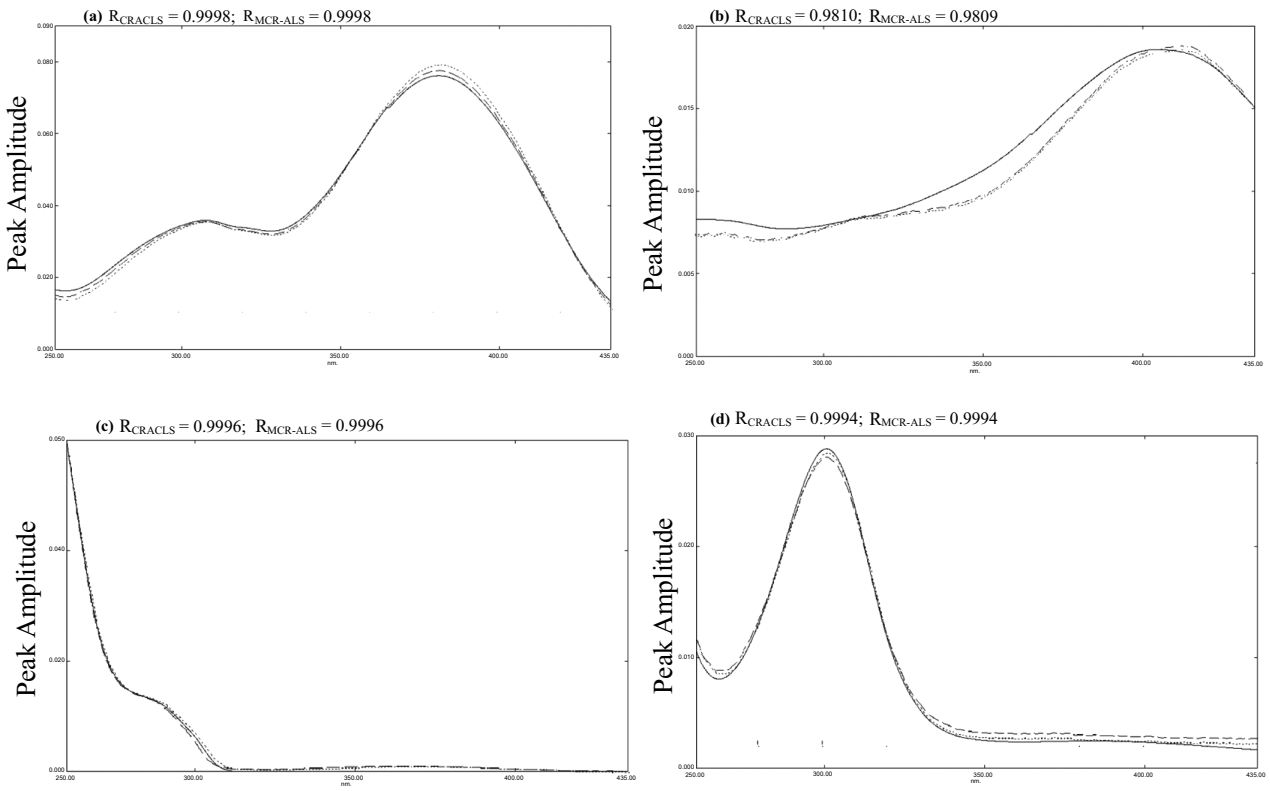

Fig. (4). Pure spectra (———) and estimated spectra by CRACLS (- - - - - -) and MCR-ALS (·····) for the four components with the corresponding correlation coefficients (R) of (**a**) DNT (**b**) DNT-DEG (**c**) PAR and (**d**) PAP.

Table 3. Validation parameters for the proposed chemometric methods and results of application analysis.

Parameter	PLS				CRACLS				MCR-ALS			
	DNT	**DNT-DEG**	**PAR**	**PAP**	**DNT**	**DNT-DEG**	**PAR**	**PAP**	**DNT**	**DNT-DEG**	**PAR**	**PAP**
Selectivity (Mean ±RSD%)	100.35 ±1.311	———	101.37 ±0.960	———	99.89 ±1.883	———	100.07 ±1.176	———	101.78 ±1.888	———	101.90 ±1.202	———
Linearity Range (μg mL^{-1})	2-10	2-10	12-28	6-14	2-10	2-10	12-28	6-14	2-10	2-10	12-28	6-14
Slope	0.98844	1.0112	1.0176	0.9983	0.9967	1.0090	1.0103	1.0008	0.9539	0.9539	1.0067	0.9964
SE of Slope	0.01084	0.03800	0.00685	0.00906	0.00990	0.03462	0.00837	0.00900	0.04078	0.04078	0.00956	0.01056
Intercept	0.08822	-0.0605	-0.2298	0.0464	0.0416	-0.0624	-0.0862	0.0125	0.2124	0.21240	0.0153	0.04715
SE of intercept	0.07356	0.27408	0.14370	0.09262	0.06715	0.24971	0.17567	0.09201	0.29413	0.29413	0.20067	0.10799
Correlation coefficient (r)	0.9993	0.9916	0.9997	0.9995	0.9994	0.9930	0.9996	0.9995	0.9994	0.9892	0.9995	0.9993
LOD (μg mL^{-1})	0.18	0.56	0.37	0.24	0.16	0.51	0.45	0.24	0.64	0.64	0.52	0.28
LOQ (μg mL^{-1})	0.55	1.70	1.11	0.73	0.50	1.55	1.37	0.72	1.93	1.93	1.57	0.85
Accuracy (Mean ± RSD%)	100.32 ±1.027	100.11 ±2.430	100.45 ±0.852	100.38 ±0.959	100.42 ±0.971	99.86 ±2.115.	100.52 ±0.889	100.25 ±0.868	100.32 ±1.027	100.11 ±2.430	100.45 ±0.852	100.38 ±0.959
Application analysis (Mean ± RSD%)	102.96 ± 0.586		101.38 ± 0.202		103.31 ± 1.169		102.65 ± 0.201		101.27 ± 0.784		103.78 ± 0.100	

Table 4. Statistical comparison between the proposed methods and a reported HPLC method for the determination of DNT and PAR in their bulk powder.

Value	Chemometric Methods						Reported Method [30][a]	
	PLS		CRACLS		MCR-ALS			
	DNT	PAR	DNT	PAR	DNT	PAR	DNT	PAR
Mean	100.32	100.45	100.42	100.52	100.33	100.45	99.67	100.04
SD	1.030	0.855	0.975	0.893	1.030	0.855	0.910	0.641
RSD%	1.027	0.852	0.971	0.889	1.027	0.852	0.913	0.641
n	8	8	8	8	8	8	5	5
Variance	1.062	0.732	0.952	0.798	1.062	0.732	0.828	0.411
Student's t Test (2.201)[b]	1.190	0.984	1.406	1.125	1.190	0. 984	-------	-------
F (6.09)[c]	1.52	1.87	1.14	1.94	1.52	1.78	-------	------

[a] Hadad et al; RP-HPLC using HS C18 column (250mm×4.6mmi.d., 5µm particle size), with gradient mobile phase; consisting of (A) 50 mmol L^{-1} sodium dihydrogen phosphate, 5 mmol L^{-1} heptane sulfonic acid sodium salt, pH 4.2 and (B) acetonitrile. The flow rate was 1.5 mL/min, UV detection at 214 nm.
[b] The corresponding theoretical values of t at (P = 0.05)
[c] The corresponding theoretical values of F at (P = 0.05)

CRACLS and MCR-ALS methods succeeded in estimating the spectral profiles of the four studied components; therefore they could be used for both quantitative and qualitative analysis.

Finally, the proposed methods were successfully applied and validated for simultaneous determination of DNT and PAR in pure powder form and in pharmaceutical formulation so they can easily be applied for the determination both drugs in quality control laboratories.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

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