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FAST DETERMINATION OF DIPHENHYDRAMINE HYDROCHLORIDE IN RECONSTITUTABLE SYRUPS BY CWT, PLS AND PCR METHODS

BURCU DEVRİM[1]*, ERDAL DİNÇ[2] and ASUMAN BOZKIR[1]

[1]Department of Pharmaceutical Technology, [2]Department of Analytical Chemistry, Faculty of Pharmacy, Ankara University, 06100-Tandoğan, Ankara, Turkey

Abstract: Diphenhydramine hydrochloride (DPH), a histamine H1-receptor antagonist, is widely used as antiallergic, antiemetic and antitussive drug found in many pharmaceutical preparations. In this study, a new reconstitutable syrup formulation of DPH was prepared because it is more stable in solid form than that in liquid form. The quantitative estimation of the DPH content of a reconstitutable syrup formulation in the presence of pharmaceutical excipients, D-sorbitol, sodium citrate, sodium benzoate and sodium EDTA is not possible by the direct absorbance measurement. Therefore, a signal processing approach based on continuous wavelet transform was used to determine the DPH in the reconstitutable syrup formulations and to eliminate the effect of excipients on the analysis. The absorption spectra of DPH in the range of 5.0–40.0 µg/mL were recorded between 200–300 nm. Various wavelet families were tested and Biorthogonal1.1 continuous wavelet transform (BIOR1.1-CWT) was found to be optimal signal processing family to get fast and desirable determination results and to overcome excipient interference effects. For a comparison of the experimental results obtained by partial least squares (PLS) and principal component regression (PCR) methods were applied to the quantitative prediction of DPH in the mentioned samples. The validity of the proposed BIOR1.1-CWT, PLS and PCR methods were achieved analyzing the prepared samples containing the mentioned excipients and using standard addition technique. It was observed that the proposed graphical and numerical approaches are suitable for the quantitative analysis of DPH in samples including excipients.

Keywords: diphenhydramine hydrochloride, reconstitutable syrup formulation, continuous wavelet transform, quantitative analysis

Most of the population have not preferred the use of solid oral dosage forms due to their swallowing problem. In this reason, oral pharmaceutical syrup is one of the most favorable dosage forms for pediatric patients or patients unable to tolerate solid dosage forms. The liquid form has been preferred because it is useful for swallowing and flexibility in the administration of doses (1, 2).

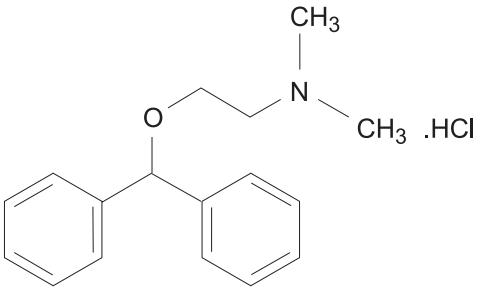

Figure 1. Molecular structure of diphenhydramine hydrochloride (DPH)

Diphenhydramine hydrochloride (DPH) named as 2-(diphenylmethoxy)-*N*,*N*-dimethylethylamine hydrochloride (Fig. 1), which is a first generation antihistamine, has been mainly used for the treatment of allergies and itchiness, insomnia, motion sickness, and extrapyramidal symptoms. Additionally, DPH has significant antitussive activity. The syrups containing DPH have been used as a cough suppressant for the control of cough due to colds or allergy (3). Recently, the use of DPH in combination with other drugs has been reported as antiemetic for the prevention of cisplatin-induced emesis in chemotherapy treatment. Furthermore, it has been used as sedative in dentistry for children and in local anesthesia (4–6). DPH oral syrups or elixirs are available commercially. However, previous studies described that DPH is more stable in solid form than

* Corresponding author: e-mail: bdevrim@pharmacy.ankara.edu.tr; phone: +90312 203 31 62, fax: +90312 213 10 81

that in liquid form (7). Therefore, we focused mainly on the preparation of a reconstitutable syrup formulation of DPH. DPH was distributed in dry mixture and reconstituted with required volume of water before administration to form oral liquid syrup.

Several methods including capillary electrophoresis (8–10), atomic absorption spectrometry (11), fluorometry (12), flow injection analysis (13, 14) and spectrophotometry (15–17) have been proposed for the determination of DPH in pharmaceutical preparations. Many chromatographic methods such as gas chromatography (18), liquid chromatography (19, 20) and high performance liquid chromatography (HPLC) (7, 21, 22) have been used for the analysis of DPH in samples.

In the existing interferences of pharmaceutical excipients, traditional spectrophotometric methods are not suitable for the quantitative resolution of single component and multicomponent formulations. In this case, the use of a separation method like HPLC is an answer in order to solve the mentioned problem. However, in some cases, HPLC approach may not give expected results due to similar chemical and physical properties of analytes with excipients. In addition, this separation procedure requires long period of analysis time, high cost and tedious procedures. It is clear that there is a need of new analytical powerful method for resolving these problems. Recently, wavelet transform methods have been used for the signal processing tools in many branches due to versatile and flexible properties (23–26).

In previous studies, some applications of the continuous wavelet transform (CWT) to the resolution of overlapped spectra for the quantitative determination of multicomponent mixtures were reported (27–32).

Chemometric calibration methods, partial least squares (PLS) and principal component regression (PCR) are popular methods for the quantitative resolution of the multicomponent mixture system. Recently, these chemometric methods have been used as alternative methods for several analytical problems from qualitative analysis to quantitative analysis in analytical chemistry and related branches (33–36).

The purposes of this study were to prepare a new pharmaceutical formulation and to improve new analytical methods for the quantification of DPH in its samples containing pharmaceutical excipients. The first aim is the preparation of reconstitutable syrup formulation of DPH. The second aim is the quantitative analysis of DPH in the prepared reconstitutable syrup formulation in the presence of pharmaceutical excipients, D-sorbitol, sodium citrate, sodium benzoate and sodium EDTA, by using CWT signal processing, PLS and PCR methods without requiring any separation step. We observed that the improved CWT, PLS and PCR methods gave satisfactory results for the quantitative estimation of the DPH content in a reconstitutable syrup formulation in spite of the spectral interferences of the pharmaceutical excipients.

EXPERIMENTAL

Reagents and equipment

Reagents were: diphenhydramine hydrochloride (DPH) (Bilim Pharmaceuticals, Turkey), D-sorbitol and sodium citrate (Merck, Germany), sodium benzoate and sodium EDTA (Sigma-Aldrich, Germany). All other chemicals were of analytical grade and distilled water was used for all experiments.

Equipment used: double-beam UV-visible spectrophotometer (Shimadzu UV-2550, Japan).

METHODS

Preparation of the dry mixtures for reconstitutable syrup formulation

In order to produce dry mixture for reconstitution, all the powder components were reduced to more or less the same particle size. To obtain a homogeneous mixture, ingredients were mixed based on

Table 1. Composition of the dry mixture for syrup formulation.

Component	g/100 mL
Diphenhydramine HCl (DPH)	0.250
D-sorbitol	24.29
Sodium citrate	1.27
Sodium benzoate	0.2
Sodium EDTA	0.0074

Table 2. Training (concentration) set of DPH in the presence of excipients.

No.	DPH	µg/mL			
		Sodium benzoate	Sodium citrate	Sodium EDTA	D-sorbitol
1	5	10	10	10	10
2	10	20	20	20	20
3	15	30	30	30	30
4	20	40	40	40	40
5	25	40	40	40	40
6	30	30	30	30	30
7	35	20	20	20	20
8	40	10	10	10	10

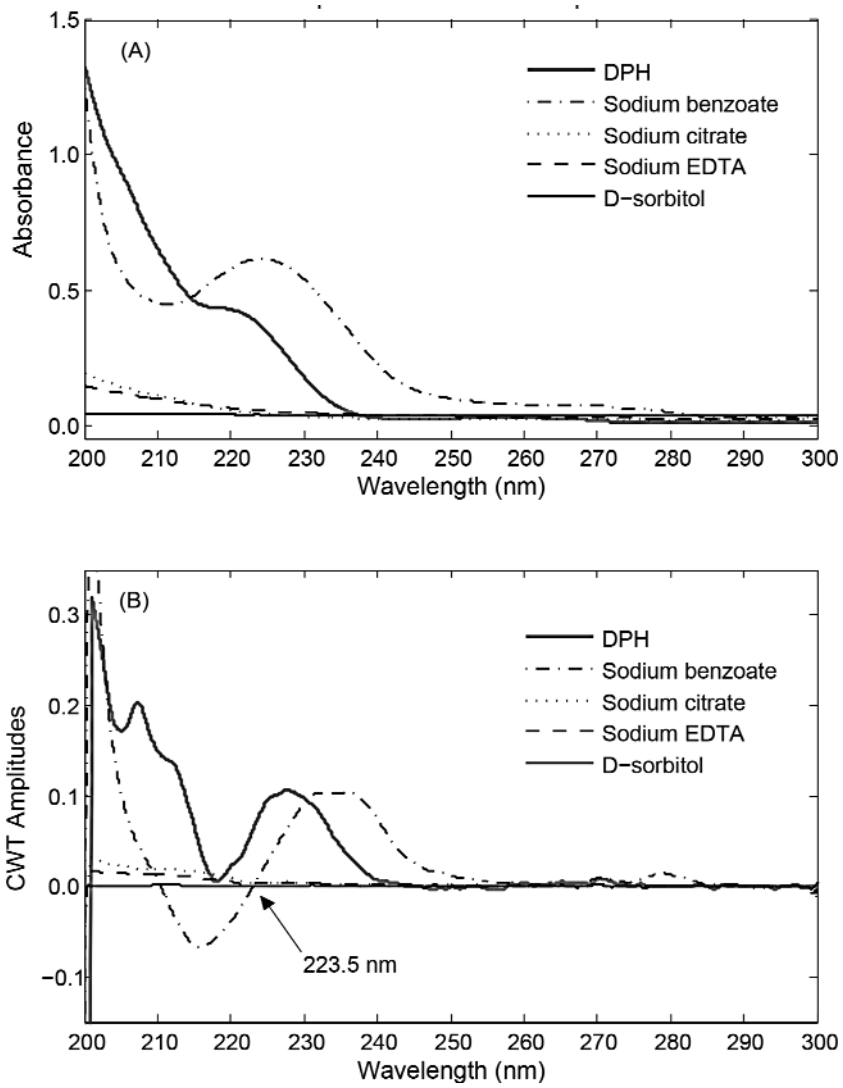

Figure 2. (A) Absorption spectra and (B) CWT spectra of DPH (——), sodium benzoate (– · –), sodium citrate (·····), sodium EDTA (– – –) and D-sorbitol (——) in distilled water

geometric dilution, which is a method used in mixing two or more ingredients of unequal quantities. Briefly, mixing process was started with the smallest quantity (sodium EDTA) and the equal of the other of the larger amount (sodium benzoate and DPH) were added. The same procedure was followed for sodium citrate and D-sorbitol until all ingredients were used up (37, 38). The representative formulation for the preparation of dry mixture is tabulated in Table 1.

For the preparation of the reconstituted syrup formulation, an appropriate amount of water was added to the dry syrup powder in two steps and stirred with spoon until a homogenous product was obtained.

Preparation of the standard solutions

The stock solution of DPH was prepared by dissolving 25 mg of DPH in 100 mL of distilled water. Additionally, the stock solutions of pharmaceutical excipients were prepared by dissolving an appropriate amount of each excipient in 100 mL of distilled water.

In case of the CWT signal processing method, the calibration solutions of DPH in the range of 5.0–40.0 µg/mL were obtained from the stock solution. In case of the PLS and PCR, a training (concentration) set of eight different mixtures containing DPH with pharmaceutical excipients was prepared (Table 2). An independent validation samples containing DPH with pharmaceutical excipients were prepared by using stock solution. Standard addition samples were obtained by adding the DPH stock solutions (at the concentration levels, 5, 10, 20, 30, 35 µg/mL) to the prepared syrup samples.

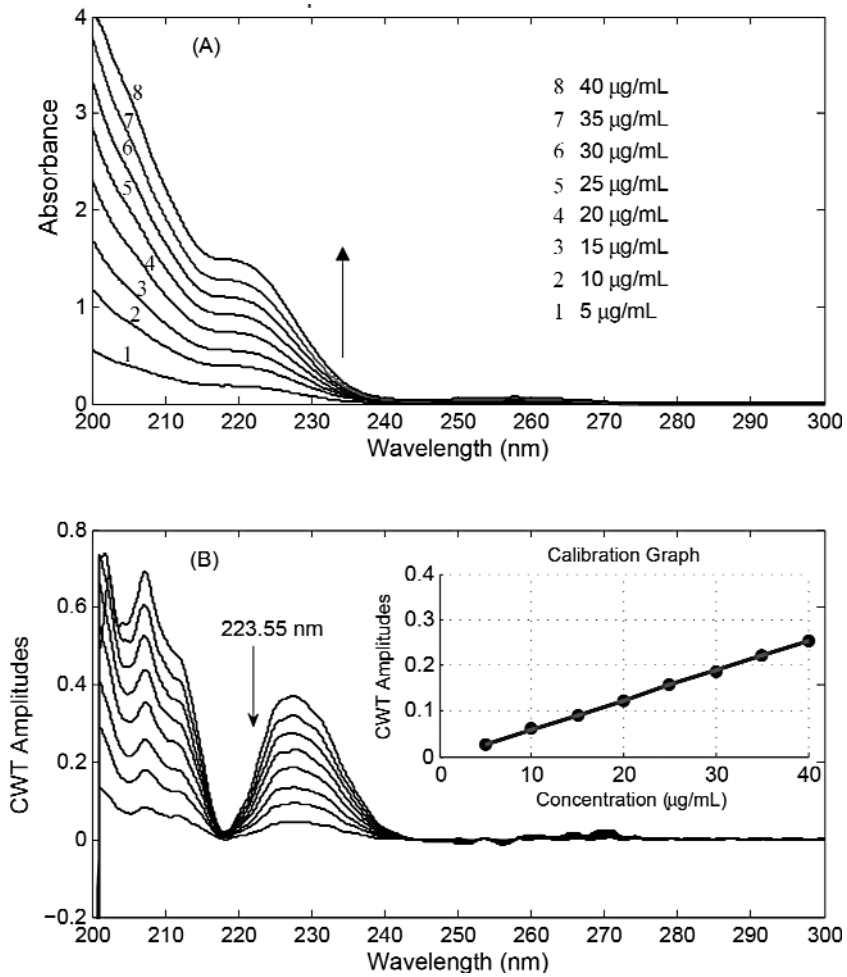

Figure 3. (A) Absorption spectra of DPH in the concentration range of 5.0–40.0 µg/mL in distilled water, (B) CWT spectra and calibration curve of DPH in the concentration range of 5.0–40.0 µg/mL in distilled water

Table 3. Calibration parameters and limits of detection, LOD and LOQ in μg/mL for Bior1.1-CWT calibration.

Parameters	
Concentration range (μg/mL)	5.0–40.0
λ (nm)	223.55
m	6.4×10^{-3}
n	6.4×10^{-3}
r	0.9998
SE (m)	0.0000
SE (n)	1.1×10^{-3}
SE (r)	1.4×10^{-3}
LOD	1.44
LOQ	4.80

*m = slope of the regression function, n = intercept of the regression function, r is correlation coefficient of the regression function, SE (m) = standard error of the slope, SE (n) = standard error of the intercept, SE (r) = standard error of the correlation coefficient, LOD = limits of detection, LOQ = limits of quantification.

Analysis procedure of dry powder and syrup formulation

The samples taken from three different points of dry syrup powder equivalent to 25 mg of DPH were weighed and dissolved in distilled water, filtered into a 100 mL calibrated flask to remove the insoluble matter and diluted to volume with water. The obtained solutions were diluted to the working concentration range with distilled water. The same sample preparation procedure was applied to the reconstituted syrup formulation. Absorption spectra of the resulting solutions of both dry powder and syrup formulation were recorded as well as calibration solutions.

RESULTS, DISCUSSION AND CONCLUSION

As it can be seen from Figure 2a, the absorption spectra of DPH and pharmaceutical excipients strongly overlap in the spectral region of 200–300 nm. Due to the mutual spectral interferences of DPH and pharmaceutical excipients in the same spectral region, the direct spectrophotometric determination of DPH in its syrup samples is not possible by traditional spectral analytical methodologies (13, 18). For the rapid and reliable resolution of this problem, we focused mainly on the application of the CWT signal processing approach to quantitative analysis of DPH in dry powder and syrup forms. Additionally, PLS and PCR calibrations were used

Table 4. Results obtained by the application of Bior1.1-CWT, PLS and PSR methods to the standard addition samples.

	DPH (n = 3)													
	BIOR1.1-CWT					PLS method					PCR method			
Added	Predicted	SD	RSD	BIAS	Recovery (%)	Predicted	SD	RSD	BIAS	Recovery (%)	Predicted	SD	RSD	BIAS	Recovery (%)
5	4.87	0.08	1.66	–2.60	99.8	4.99	0.04	0.76	–0.17	99.8	5.07	0.12	2.38	1.37	101.4
10	10.08	0.20	1.99	0.80	99.7	9.84	0.06	0.64	–1.60	98.4	9.89	0.19	1.88	–1.13	98.9
20	19.91	0.28	1.41	–0.45	99.9	18.97	0.41	2.17	–5.14	94.9	19.01	0.46	2.43	–4.97	95.0
30	30.38	0.19	0.64	1.26	100.7	28.99	0.85	2.93	–3.37	96.6	28.79	0.54	1.86	–4.03	96.0
35	34.93	0.02	0.04	–0.20	100.0	33.02	0.54	1.62	–5.65	94.3	33.39	0.82	2.45	–4.61	95.4

*SD = standard deviation, RSD = relative standard deviation.

for comparison of the assay results provided by the improved CWT approach with those obtained by applying PLS and PCR (32). The proposed methods gave us sensitive, selective, accurate and precise results for the fast analysis of DPH in samples.

In order to get the optimal wavelet analysis, various wavelet families at different scale factor (a) were applied the absorption spectra of DPH and pharmaceutical excipients (Fig. 2) and BIOR1.1-CWT (a = 40) among wavelet families was found to be an optimal signal analysis approach for the DPH. As illustrated in Figure 2b, the concentration of DPH is proportional to the BIOR1.1-CWT signal at the wavelength 223.55 nm, corresponding to the zero-crossing point for all pharmaceutical excipients (D-sorbitol, sodium citrate, sodium benzoate and sodium EDTA). The absorption spectra of the DPH calibration solutions were recorded between 200 and 300 nm as shown in Figure 3a and the BIOR1.1-CWT spectra were obtained using CWT procedure (Fig. 3b). An analogous procedure was applied to all samples. Calibration curve for the related compound was obtained by measuring the BIOR1.1-CWT amplitudes at 223.55 nm. The calibration curve and corresponding statistical results in the application of the linear regression analysis to the concentrations and BIOR1.1-CWT amplitudes were summarized in Figure 3b and Table 3. The content of DPH in samples was determined by the computed calibration curve.

In case of the PLS and PCR applications, a training (concentration) set of the mixture solutions in the concentration range of 5.0–40.0 μg/mL of DPH and pharmaceutical excipients (D-sorbitol, sodium citrate, sodium benzoate and sodium EDTA) in possible combinations were prepared to construct the PLS and PCR calibrations. Table 2 shows the training set of DPH in the presence of pharmaceutical excipients. The absorbance data matrix corresponding to the training set was obtained by measuring the absorbance values at the wavelengths set with the intervals of $\Delta\lambda$ = 0.05 nm in the spectral range 200.0–300.0 nm. Two chemometric calibrations based on the relationship between the training

Table 5. Stastistical parameters for PLS and PCR calibration.

	DPH	
Parameters	PLS	PCR
SEC	0.278	0.269
PRESS	0.540	0.577
SEP	0.894	0.832

*SEC = standard error of calibration,
PRESS = prediction residual error sum-of-squares,
SEP = standard error of prediction.

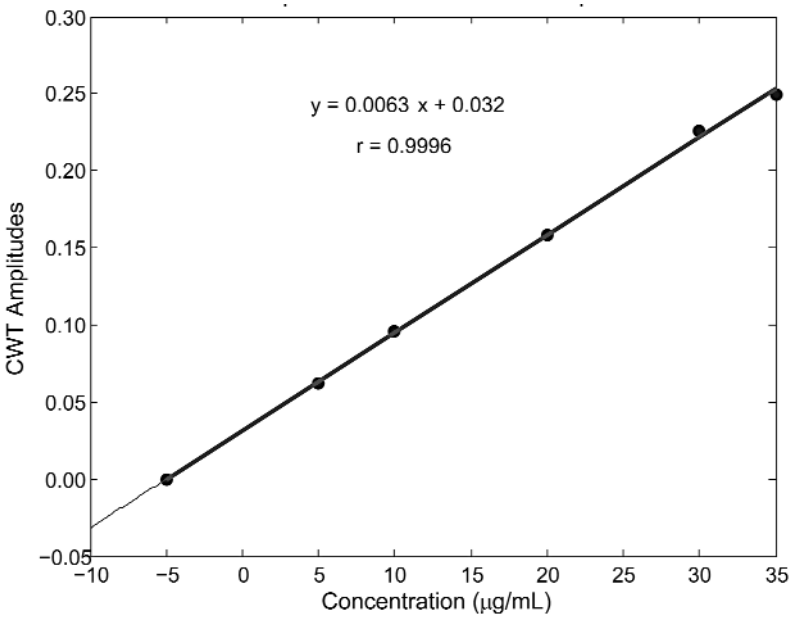

Figure 4. A plot of the standard addition samples, obtained by using the relationship between BIOR1.1-CWT amplitudes and concentration

Table 6. DPH content of dry powder and syrup formulation by Bior1.1-CWT, PLS and PCR approach.

	mg/100 mL											
	Dry powder sample 1			Dry powder sample 2			Dry powder sample 3			Syrup		
	BIOR1.1-CWT	PLS	PCR	BIOR1.1-CWT	PLS	PCR	BIOR1.1-CWT	PLS	PCR	BIOR1.1-CWT	PLS	PCR
Mean	25.692	25.261	25.144	27.385	26.330	26.199	26.639	25.389	25.284	25.539	25.549	25.482
SD	0.335	0.377	0.367	0.137	0.533	0.532	0.248	0.724	0.730	0.162	0.388	0.383
RSD	1.305	1.490	1.459	0.501	2.024	2.031	0.930	2.854	2.887	0.635	1.519	1.503

*SD is standard deviation for n = 10 observations, RSD is relative standard deviation.

set and absorbance data matrix were calculated by applying the PLS and PCR algorithms. PLS and PCR calibrations obtained were used for the prediction of the amount of DPH in its samples containing pharmaceutical excipients.

In case of the BIOR1.1-CWT process, a good linearity for regression equation was observed. Correlation coefficient was found to be 0.9998 as shown in Table 3. Limit of detection (LOD) and limit of quantitation (LOQ) were calculated to be 1.44 and 4.80 µg/mL, respectively (Table 3). The applicability and validity of the improved BIOR1.1-CWT were tested by analyzing the mixtures of DPH and pharmaceutical excipients. Recovery and standard deviation for the proposed CWT signal processing method were illustrated in Table 4. It is clear that these results indicate good accuracy and precision for the application of the improved CWT method to the analysis of the mixtures containing active compound and pharmaceutical excipients. For the observation of the pharmaceutical excipients' effects on the analysis of DPH, standard addition technique was applied by adding 5 different concentration levels (5, 10, 20, 30 and 35 µg/mL) to the syrup samples containing 5 µg/mL DPH. The concentration values of the standard addition samples were plotted against the values of the BIOR1.1-CWT amplitudes and a straight line was observed as shown in Figure 4. The results indicate that the linear regression slope of standard addition samples is close to the slope of the calibration equation of DPH (Table 3 and Fig. 4). This uncovers that no interference of pharmaceutical excipients was observed during the application of the proposed BIOR1.1-CWT approach to the standard addition samples, dry powders and syrup samples.

In order to test the applicability and validity of the PLS and PCR methods, the DPH samples containing pharmaceutical excipients were analyzed by PLS and PCR in the calibration and prediction steps. Predictive ability of the proposed chemometric calibration method was checked by the standard error of calibration (SEC), the predicted residual error sum-of-squares (PRESS) and the standard error of prediction (SEP). Table 5 shows the SEC, PRESS and SEP values calculated by using the actual and predicted concentrations. It was observed that the applicability and predictive validity of PLS and PCR gave satisfactory results. As a result, percent mean recoveries with the relative standard deviations were found as 94.3–99.8 ± 0.64–2.93% and 95.0–101.4 ± 1.86–2.45% for DPH using PLS and PCR methods, respectively (Table 4). These experimental results indicate that the proposed chemometric numerical and signal processing meth-

ods are suitable for the simultaneous determination of DPH in dry powder and syrup formulations.

The CWT, PLS and PCR methods were applied to analysis of the dry powder and syrup samples containing DPH and pharmaceutical excipients and the obtained results are presented in Table 6. A good agreement was observed for the assay results with the prepared amount of the DPH in the dry powder and syrup composition. Furthermore, it was observed that the determination results obtained from three different dry powder samples are very close to each other. These results showed that DPH and pharmaceutical excipients were mixed homogeneously. As reported in previous studies (37, 38), mixing of active and inactive compounds according to the principle of the geometric dilution resulted in homogeneous distribution of active compound in dry powder samples.

A reconstitutable syrup formulation of DPH containing D-sorbitol, sodium citrate, sodium benzoate, and sodium EDTA as pharmaceutical excipients was successfully prepared in this study. The proposed CWT, PLS and PCR chemometric methods are rapid, precise, and accurate for the simultaneous resolution of dry powder and syrup formulations containing DPH and pharmaceutical excipients having strongly overlapping spectra. The obtained results indicate that the CWT, PLS and PCR calibration methods are very suitable for the analysis of DPH in the presence of pharmaceutical excipients without using chemical pretreatments.

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