ATATURK UNIVERSITY PUBLICATIONS

Research Article

DOI: 10.5152/Pharmata.2023.23017

Simultaneous Spectrophotometric Determination of Dexketoprofen Trometamol and Thiocolchicoside by Using Principal Component Regression Multivariate Calibration Model in Combined Pharmaceutical Formulation

Nagihan KARAGÖL[1]
Fatma DEMİRKAYA MİLOĞLU[2]

[1]Department of Pharmaceutical Toxicology, Atatürk University, Faculty of Pharmacy, Erzurum, Turkey
[2]Department of Analytical Chemistry, Atatürk University, Faculty of Pharmacy, Erzurum, Turkey

ABSTRACT

Objective: The combination of the non-steroidal anti-inflammatory drug dexketoprofen trometamol (DXT) and the centrally acting muscle relaxant thiocolchicoside (TH) is used for symptomatic relief in various conditions, with the aim of alleviating associated symptoms. The study involves the development, validation, and application of the chemometrics-based spectrophotometric method.

Methods: An integral aspect of the investigation is the strategic application of experimental design techniques for calibration and validation mixtures to facilitate the assessment of factor effects within complex matrices. In this study, a factorial design was used to prepare calibration (25 samples) and validation (8 samples) sets comprising mixtures of DXT and TH within their linear ranges (2.5-25 µg/mL for DXT and 2-16 µg/mL for TH).

Results: Spectra of the acquired mixtures and samples were recorded at wavelengths between 220nm and 460 nm at $\Delta\lambda = 1$ nm intervals. Using regression models based on the principal component regression algorithm, the results obtained showed satisfactory performance, with a recovery rate of ≤98.54% for DXT and ≤98.88% for TH.

Conclusion: These models offer the potential for accurate identification and quantification of DXT and TH in pharmaceutical preparations

Keywords: Dexketoprofen trometamol, experimental design, PCR, thiocolchicoside

INTRODUCTION

Pain is often associated with potential tissue damage or described as a similarly unpleasant emotional experience. It is a major reason why people seek medical attention. Pain can be acute or chronic.[1] Physiologically, there are 3 types of pain: nociceptive (protective), inflammatory (tissue damage), and pathological (nervous system origin).[2] Today, one of the most preferred groups of analgesic drugs for the prevention of pain is the non-steroidal anti-inflammatory drugs (NSAIDs). This group of drugs works by inhibiting the enzyme cyclooxygenase. In this way, they prevent the synthesis of prostaglandins, which leads to peripheral sensitization, reduction of pain, and inflammation.[3,4]

Dexketoprofen is a ketoprofen (S+) enantiomer of NSAIDs. The chemical composition includes 2-amino-2-(hydroxymethyl) propane-1,3-diol and 2-(3-benzoylphenyl)propanoic acid.[5] Dexketoprofen trometamol (DXT) has analgesic, anti-inflammatory, and antipyretic properties and is more potent than ketoprofen.[6] Thiocolchicoside (TH) is used as a muscle relaxant. The chemical composition consists of N-[(7S)-3-(beta-D-glucopyranoxyloxy)-1,2-dimethoxy-10-(methylsulfanyl)-9-oxo-5,6,7,9-tetrahydrobenzo[a]heptalen-7-yl]acetamide and is a semi-synthetic colchicoside derivative.[7] Thiocolchicoside acts as a selective agonist by binding to the GABA-A receptor and the citrickinin-sensitive glycine receptor. It also has analgesic and anti-inflammatory properties.[8] There are many preparations of dexketoprofen that combine trometamol and TH. The combination of DXT and TH has a higher analgesic effect than DXT alone.[9] Dexketoprofen trometamol and TH, a centrally acting muscle relaxant, are used together in the symptomatic treatment of osteoarthritis, painful syndromes of the spine,

Received: 03.08.2023
Accepted: 19.09.2023
Publication Date: 06.10.2023

Corresponding Author:
Fatma DEMİRKAYA MİLOĞLU
E-mail: fdkaya@atauni.edu.tr

Cite this article as: Karagöl N, Demirkaya Miloğlu F. Simultaneous spectrophotometric determination of dexketoprofen trometamol and thiocolchicoside by using principal component regression multivariate calibration model in combined pharmaceutical formulation. *Pharmata* 2023;3(4):99-104.

extra-articular rheumatism, painful muscle contractions, and the treatment of pain following trauma and surgery.[10,11] In order to carry out quality control studies in combination drug preparations, it is necessary to determine the concentrations of the drugs. Both DXT quantification methods, including spectrophotometric[12] and chromatographic[13,14] methods, and TH measurements in various combined preparations, utilizing spectrophotometric[15-17] and chromatographic[18,19] methods, have been employed in previous studies. In addition, it was found that the determination in the combined pharmaceutical preparations of DXT and TH was performed using Ultra Violet (UV) spectrophotometer,[20-23] High-Performance Liquid Chromatography (HPLC),[24] Reverse Phase High-Performance Liquid Chromatography with Photodiode Array Detection (RP-HPLC-PDA),[25,26] and High-Performance Thin-Layer Chromatography (HPTLC)[27,28] methods in the literature review. The spectrophotometric method is a simple and cost-effective method used in quality control studies of drugs. In recent times, a chemometric approach has emerged as a promising solution for tackling the issue of overlapping spectra in various media within mixtures. This approach involves the development of chemometric calibration models based on sample spectra, offering advantages such as reduced chemical consumption and sample preparation, elimination of time-consuming processes, and enhanced accuracy and precision values without the need for chromatographic elution. This technique has been explored in studies[17,29] to effectively address the challenges posed by spectral overlaps in complex samples. The most popular multivariate calibration algorithms in chemometrics are the classical least squares method or K-matrix method, the inverse least squares method or P-matrix method, the principal component regression (PCR) method, and the partial least squares regression (PLS) method, which is recognized as a straightforward and efficient calibration approach.[30]

METHODS

Instrument

The UV-visible measurements were performed using a Thermo Scientific Multiscan GO51119300 model instrument. In this study, spectrophotometric measurements were performed using a microplate reader with the following settings: data mode: absorbance, start wavelength (nm): 220, stop wavelength (nm): 460, scan speed: fast, bandwidth: 1 nm, and microplate temperature (°C): 25.

Materials

Dexketoprofen trometamol and TH were acquired from Sigma-Aldrich (Darmstadt, Germany). Methanol (Merck, Darmstadt, Germany) and double deionized water (Milli-Q water) were utilized as chemical reagents and were of analytical grade. Stock solutions of both analytes were prepared by dissolving the analytes in methanol at a concentration of 250 µg/mL for DXT and 40 µg/mL for TH. The commercial pharmaceutical preparation named "Dexplus effervescent tablet" was purchased from a pharmacy in Erzurum, Turkey.

Chemometric Method and Data Preprocessing

In this study, PCR, which is one of the chemometric calibration methods, was employed. Principal component regression is based on the decomposition of the measured absorbance[31] data of the concentration set into mutually orthogonal (orthogonal) axes. These obtained axes serve as the coordinate system for constructing the calibration.

Construction of the Training Set

Different working solutions of DXT (2.5-25 µg/mL) and TH (2-16 µg/mL) were prepared in methanol by using their stock solutions. A calibration batch of 25 mixtures was meticulously prepared following a well-established full factorial design with 5 levels in 2 categories. The UV absorption spectra of these resulting mixtures were meticulously recorded in the wavelength range of 220-460 nm at increasing intervals of 1 nm. Detailed concentration details can be found in Figure 1A.

Construction of the Validation Set

In order to assess the predictive capability of the PCR method, a validation set was generated. This set included 8 different laboratory-prepared mixtures, each containing different concentrations of DXT and TH, following a full factorial design with 3 levels in 2 categories (Figure 1B). Following the formulation process, absorbance measurements were taken within the 220-460 nm spectrum.

For data analysis, the PCR model was constructed using MATLAB R2019b with PLS-Toolbox software version 8.5.1. The calibration and validation sets were created using Design-Expert 12.0 software (Stat-Ease Inc., Minneapolis, Minn, USA). Additionally, statistical analyses were performed utilizing Microsoft Excel 2018.

Preparation of Pharmaceutical Formulations

In this study, the pharmaceutical formulation containing DXT and TH, namely Dexplus effervescent tablets, was meticulously carried out through the following steps:

Powdering of the commercial product: The commercial product containing DXT and TH was finely ground into a powder using a mortar and pestle.

Sample extraction: Approximately 1 tablet of the powdered commercial product, equivalent to 25 mg DXT and 8 mg TH, was accurately weighed and placed in a volumetric flask.

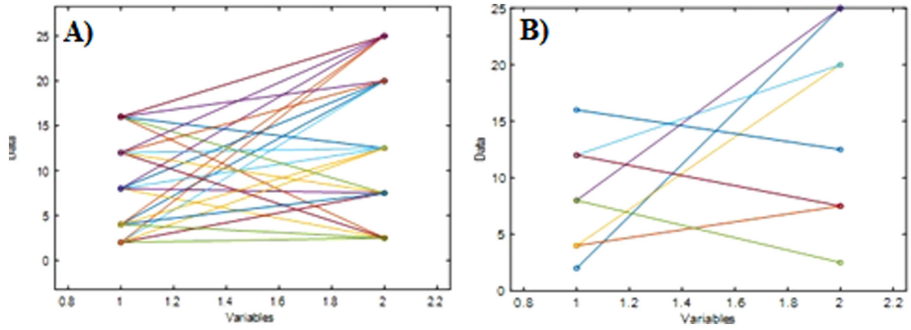

Figure 1. (A) Calibration and (B) Validation sets with varied dexketoprofen trometamol and thiocolchicoside concentrations.

Dissolution and solution preparation: The weighed tablet powder was dissolved in a methanol solution, and the volume was adjusted to 100 mL to ensure thorough mixing.

Ultrasonication and cooling: The prepared solution was ultrasonicated for 15 minutes to enhance dissolution. After this step, the solution was allowed to cool to room temperature.

Dilution and spectral recording: To ensure accurate measurements, the resulting solution was diluted accordingly. Spectra in the UV-visible region of the diluted solutions were recorded for further analysis.

RESULTS AND DISCUSSION

Optimization of Spectrophotometric Method

According to previous studies, it has been reported that compounds DXT and TH are insoluble in organic solvents, while they exhibit good solubility in water and methanol.[16] Based on this information, it was observed that compounds DXT and TH dissolved effectively in a methanol solution. After determining the optimal spectroscopic conditions, individual spectra of compounds DXT and TH were obtained within the wavelength range of 220-460 nm in the methanol solution. Regarding standard DXT and TH, the respective maximum absorbances are observed to occur at 258 nm and 370 nm, respectively (Figures 2A and 2B). It was noted that both compounds exhibited a linear wavelength range. Examination of the absorption spectra of DXT and TC (Figure 2C) revealed a significant overlap between the 2 compounds, which posed a challenge to their direct quantification. Therefore, the primary objective of this study was to implement highly sensitive and accurate analytical techniques to enable the simultaneous quantification of DXT and TH in their individual forms, laboratory-prepared mixtures, and pharmaceutical dosage forms without the need for prior separation.

Principal Component Regression Multivariate Calibration Model

Spectral Data Processing and Calibration Model Development

Recently, spectrophotometric analysis with chemometric calibration models has proven to be a robust method for the quantification of binary drug-active substances with overlapping spectra. This approach has gained popularity due to its effectiveness in solving the challenges posed by the spectral overlap of these compounds. Chemometric calibration models have been used to obtain accurate quantitation. In particular, the PCR model was developed to provide simultaneous and precise quantitative analysis of each drug in the binary mixture. But, careful preparation of calibration and validation data matrices is necessary for a comprehensive chemometric analysis. In this context, this study involved the pooling of several samples containing different concentrations of DXT- and TH-active pharmaceutical ingredients (2.5-25 µg/mL for DXT and 2-16 µg/mL for TH) for the preparation of a calibration set. By including a wide range of concentrations, the calibration model effectively captured spectral variations across the entire concentration spectrum, resulting in a more robust and reliable prediction model. This set included absorbance values measured at 241 points with $\Delta\lambda = 1$ nm intervals in the wavelength range of 220-460 nm. For each sample, the measured absorbance values were associated with the corresponding compound concentrations. In this context, a pair was created for each sample: the measured absorbance value and the known compound concentration. The prepared calibration sets were subjected to PCR algorithm-based modeling. The variance–covariance matrices of absorbance and concentration values within the calibration set were calculated, thereby forming a fundamental basis for the PCR calibration models. By processing absorbance data through a composition process in the variance–covariance matrix, a mathematical relationship was established between absorbances and concentrations, laying the foundation for robust calibration.

Optimal Factor Determination and Cross-Validation

To ascertain the optimal factor numbers for the PCR algorithm, a cross-validation procedure was conducted, employing 25 calibration spectra. During each iteration of this process, a single calibration solution was treated as a sample. The procedure was repeated 25 times in total, and using 24 of the calibration spectra, PCR calibrations were conducted. The estimated concentrations of analytes were cross-validated by comparing them with the actual concentrations. The root- mean square error of cross-validation (RMSECV) was computed as an evaluation metric using the following formula:

$$\mathrm{RMSECV} = \sqrt{1/N}\left(\sum^{N_{i=1}}\left(C_{\mathrm{estimated}} - C_{\mathrm{actual}}\right)^2\right)$$

Optimal Factor Selection, Model Performance and Model Evaluation, and Performance

The selection of the optimal number of factors is crucial for ensuring accurate quantitation in PCR calibration. The chosen factor numbers are guided by criteria such as minimizing the prediction error sum of squares, RMSECV, or *F*-statistic. Figure 3 illustrates the variation of RMSECV as a function of the number of factors for DXT and TH. For both compounds, an optimal factor number of 3 was determined for accurate quantitation in mixtures containing DXT and TH. Among the selected models, the one with the lowest RMSECV value was preferred. The 5-factor PCR calibration yielded RMSECV values of 0.486 and 0.295 for DXT and TH, respectively.

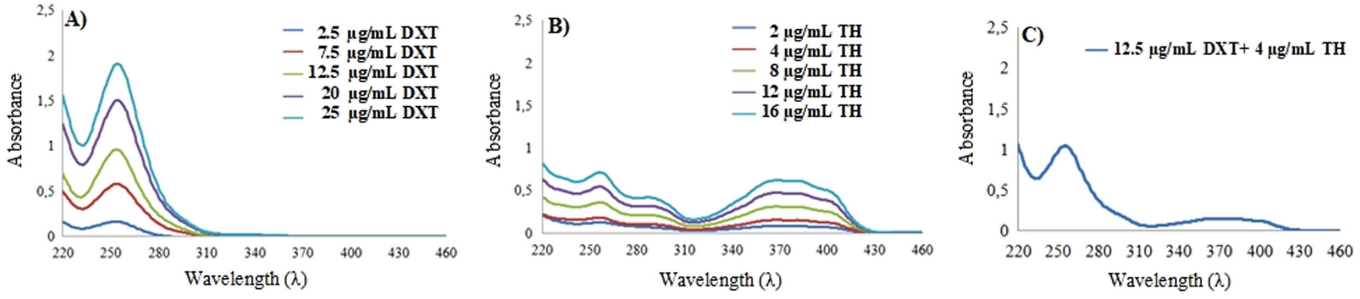

Figure 2. UV absorption spectra: (A) Ddexketoprofen trometamol (DXT) (2.5-25 µg/mL), (B) thiocolchicoside (TH) (2-16 µg/mL), and (C) spectral overlay of DXT (12.5 µg/mL) and TH (4 µg/mL).

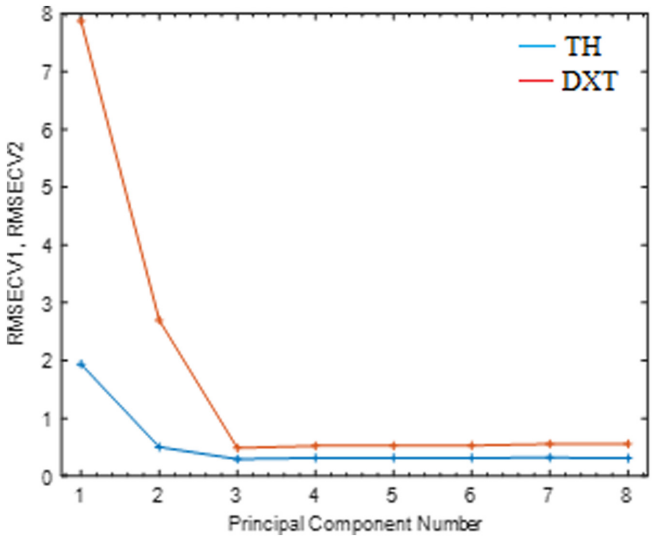

Figure 3. Principal component number curves versus root mean square error of cross-validation by Principal Component Regression multivariate calibration for DXT and TH."

The predictive capabilities of the models were assessed by graphing the predicted concentration against the known true concentration. The statistical results are presented in Table 1.

Validation of Chemometric Calibration

The validation of the developed chemometric model was conducted using the validation set depicted in Figure 1B. The validation set underwent the same analysis procedures as the calibration data set. The accuracy assessment was conducted by determining recovery values, which were found to be ≤99.71% for DXT and ≤98.73% for TH. To evaluate precision, the relative standard deviation values were in the range of 0.08%-3.63% for TH and 0.62%-2.21% for DXT. The statistical outcomes of this validation process are summarized in Table 2. Prior to the application of the PCR calibration method to actual commercial pharmaceutical preparation, recovery studies were conducted using the standard addition method. The pharmaceutical formulation, Dexplus effervescent tablets (8 mg TH and 25 mg DXT), as outlined in Section 2.3, was dissolved in methanol. Standard solutions were prepared at concentrations of 2-16 µg/mL for TH and 2.5-25 µg/mL for DXT. UV-visible spectra were obtained for each standard addition.

The PCR calibration method was applied, with formulation contributions subtracted. Percentage recovery values for both compounds within the added standards were calculated. The recovery percentages obtained from the PCR model were greater than 98.54% for DXT and 98.88% for TH, indicating a remarkable level of accuracy (Table 2).

Table 1. Summary of Statistical Parameters for Simultaneous Quantification of DXT and TH via PCR Multivariate Calibration Model

PCR Model/Cross-Validation Results	DXT	TH
Concentration (µg/mL)	2.5-25	2-16
Spectral region (nm)	220-460	220-460
Optimum number of factors	3	3
Calibration curves	1.0141x+0.0321	1.0039x+0.0068
R^2	0.9999	0.9999
RMSECV	0.486	0.295
RMSEC	0.420	0.254
Kalibrasyon bias	$-1.77.10^{-15}$	0

DXT, dexketoprofen trometamol; PCR, principal component regression; R, correlation coefficient; RMSEC, Root Mean Square Error of Calibration; RMSECV, root mean square error of cross-validation; TH, thiocolchicoside.

Table 2. The Prediction Results Obtained with Validation Set for the PCR Calibration Model

Drug Mixture (µg/mL)		Found (µg/mL)∓SD		Recovery %		RSD%	
TH	DXT	TH	DXT	TH	DXT	TH	DXT
4	7.5	4.06 ± 0.01	7.81 ± 0.05	101.44	104.17	0.08	0.62
4	20	4.13 ± 0.07	20.26 ± 0.28	103.20	101.31	1.76	1.40
8	25	8.15 ± 0.09	25.87 ± 0.17	101.85	103.48	1.12	0.65
8	2.5	8.09 ± 0.01	2.51 ± 0.03	101.09	100.41	0.18	1.18
12	20	11.93 ± 0.12	19.94 ± 0.24	99.42	99.71	1.03	1.21
12	7.5	11.96 ± 0.20	7.53 ± 0.08	99.69	100.35	1.70	1.09
2	12.5	2.01 ± 0.07	12.51 ± 0.20	100.69	100.06	3.63	1.64
16	12.5	15.80 ± 0.16	12.53 ± 0.28	98.73	100.21	1.00	2.21

DXT, dexketoprofen trometamol; PCR, principal component regression; RSD, relative standard deviation; SD, Standard Deviation, TH, thiocolchicoside.

Application to Commercial Pharmaceutical Preparation

In order to demonstrate the applicability of the developed and validated PCR method to pharmaceutical formulations, quantification analyses of active substances in pharmaceutical preparations containing DXT and TH were performed. The pharmaceutical preparation containing DXT and TH, namely Dexplus effervescent tablets, was dissolved in methanol as described in "Preparation of Pharmaceutical Formulations"Section. Subsequent dilutions were performed to prepare solutions with concentrations of DXT and TH ranging from 4 to 8 µg/mL and 12.5 to 25 µg/mL, respectively. The absorbance values of the prepared solutions, measured in the wavelength range 220-460 nm with $\Delta\lambda = 1.0$ nm intervals, were recorded (Figure 4).

The PCR algorithm described above was then applied to calculate the quantities of DXT and TH within the tablet content. The results presented in Table 3 underline the reliability and effectiveness of the PCR method for quantitative analysis of DXT and TH in complex pharmaceutical matric. This successful application further validates the practical value of the developed PCR method in pharmaceutical analysis, providing a robust and accurate approach to the quantification of active substances in commercial pharmaceutical formulations.

In this study, we developed, validated, and applied a chemometrics-based spectrophotometric method for the simultaneous quantification of DXT and TH in pharmaceutical formulation. An important aspect was our use of experimental design techniques for calibration and validation mixtures. This approach allowed us to study the effects of factors in the presence of varying levels of other factors, thereby increasing the efficiency of the method

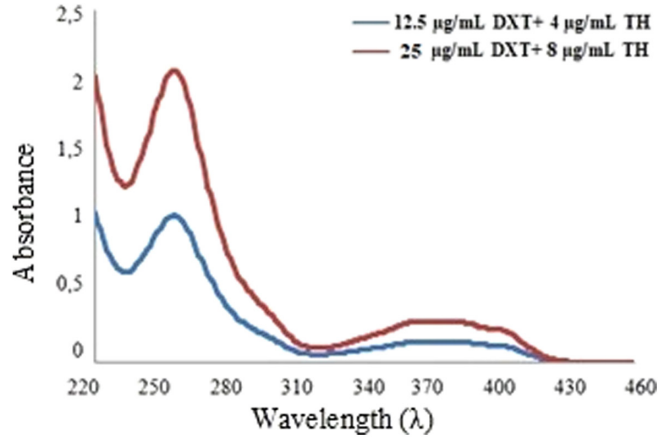

Figure 4. The UV absorption spectra of the drug (Dexplus effervescent tablets) solutions containing dexketoprofen trometamol (DXT) and thiocolchicoside (TH).

Table 3. Simultaneous Determination of DXT and TH in the Commercial Pharmaceutical Preparation

Commercial Pharmaceutical	Active Substance	Number of Analyses	Mean ± SD	Recovery %	RSD %
Dexplus effervescent tablets	TH (8 mg)	30	7.94 ± 0.015	99.43	0.195
	DXT (25 mg)	30	25.14 ± 0.54	100.54	2.15

DXT, dexketoprofen trometamol; RSD, relative standard deviation; SD,Standard Deviation, TH, thiocolchicoside.

in complex matrix analysis. The successful development of our chemometric calibration model using UV-visible spectra demonstrated the potential of the PCR method for accurate DXT and TH quantification. The model demonstrated remarkable precision and accuracy, confirming its effectiveness for complex matrix analysis. Validation, including accuracy, precision, and recovery studies, further confirmed the robustness of the method. Consistently achieved recovery percentages within the predefined range underscored the method's ability to recover true concentrations in the midst of complex matrices. Application of our method to a pharmaceutical formulation demonstrated its practical utility by accurately determining DXT and TH concentrations. This confirmed the applicability of the method for real-world pharmaceutical analysis.

Ethics Committee Approval: There was no need for ethics committee approval for this study. The research conducted did not involve human subjects, animal experimentation, or sensitive personal data. Therefore, ethical approval was not required in accordance with the established regulations and guidelines governing research ethics.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – F.D.M., N.K.; Design – F.D.M.; Supervision – F.D.M., N.G.; Resources – F.D.M., N.K.; Materials – F.D.M., N.K.; Data Collection and/or Processing – F.D.M., N.K.; Analysis and/or Interpretation – F.D.M., N.K.; Literature Search – F.D.M., N.K.; Writing Manuscript – F.D.M., N.K.; Critical Review – F.D.M., N.K.

Declaration of Interests: The authors declare that they have no competing interest.

Funding: The authors declared that this study has received no financial support.

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