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Simultaneous quantitative determination of paracetamol and tramadol in tablet formulation using UV spectrophotometry and chemometric methods

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ABSTRACT

The UV spectrophotometric methods for simultaneous quantitative determination of paracetamol and tramadol in paracetamol-tramadol tablets were developed. The spectrophotometric data obtained were processed by means of partial least squares (PLS) and genetic algorithm coupled with PLS (GA-PLS) methods in order to determine the content of active substances in the tablets. The results gained by chemometric processing of the spectroscopic data were statistically compared with those obtained by means of validated ultra-high performance liquid chromatographic (UHPLC) method. The accuracy and precision of data obtained by the developed chemometric models were verified by analysing the synthetic mixture of drugs, and by calculating recovery as well as relative standard error (RSE). A statistically good agreement was found between the amounts of paracetamol determined using PLS and GA-PLS algorithms, and that obtained by UHPLC analysis, whereas for tramadol GA-PLS results were proven to be more reliable compared to those of PLS. The simplest and the most accurate and precise models were constructed by using the PLS method for paracetamol (mean recovery 99.5%, RSE 0.89%) and the GA-PLS method for tramadol (mean recovery 99.4%, RSE 1.69%).

1. Introduction

Paracetamol (*N*-(4-hydroxyphenyl)acetamide is one of the most popular and most commonly used analgesic and antipyretic drug with no anti-inflammatory activity. Its mechanism of action is complex and to date has not been completely elucidated. Due to a lack of significant peripheral action on prostaglandins, paracetamol is better tolerated than non-steroidal anti-inflammatory drugs (NSAIDs) and has no gastrointestinal side effects [1,2].

Tramadol, (1RS,2RS)-2-[(dimethylamino)methyl]-1-(3-methoxyphenyl)cyclohexanol hydrochloride, is a centrally acting atypical opioid analgesic consisting of two enantiomers, both of which contribute to its activity by different mechanisms. It inhibits noradrenaline (norepinephrine) and serotonin reuptake by binding to their neuronal reuptake sites resulting in the simultaneous reduction of afferent pain signalling and the amplification of efferent inhibitory signalling [3,4].

Orally administered fixed-dose combination of tramadol and paracetamol in the form of tablets is indicated for symptomatic treatment of moderate to severe pain. It is effective in providing pain relief in adult patients with postoperative pain after minor surgery, musculoskeletal pain, painful diabetic peripheral neuropathy, or migraine pain. As these two drugs have complementary modes of action and target multiple sites, their combination provides better analgetic action against several types and sources of pain [5].

Chemometric calibration techniques in spectral analysis are widely used in quality control of drugs, especially in the analysis of mixtures and multicomponent pharmaceutical formulations with overlapping spectra. The advantage of such techniques lie in the fact that separation procedures of the drugs are not required. Partial least squares (PLS) is a regression method long employed for the quantitative processing of spectroscopic data in order to reduce dimensionality of the variables and to extract only relevant information [6,7]. This method, coupled with a genetic algorithm (GA), is a useful tool for wavelength selection in spectral data analysis. The wavelength reduction in PLS calibration using genetic algorithm can provide valuable information about the system studied, improve accuracy and precision of the results obtained by applying mathematical models, and reduce their complexity [8,9].

Numerous quantitative analytical methods have been reported for the separate determination of tramadol and paracetamol in pharmaceutical products and biological materials. Descriptions of flow

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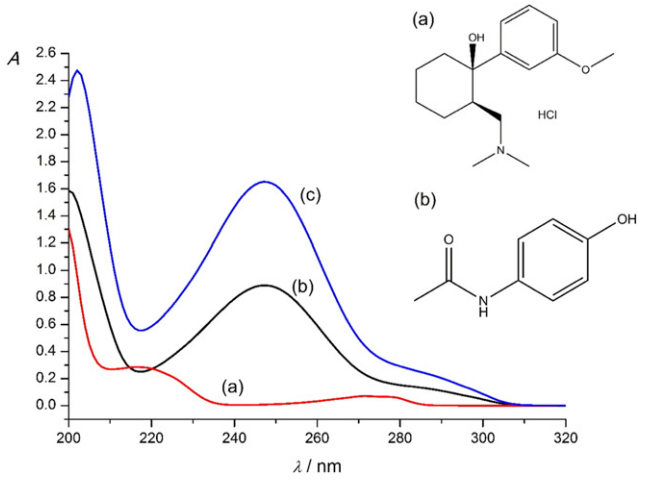

Fig. 1. UV spectra of (a) tramadol ($\gamma = 10$ mg mL^{-1}); (b) paracetamol ($\gamma = 10$ mg mL^{-1}), and (c) mixture of tramadol ($\gamma = 2$ mg mL^{-1}) and paracetamol ($\gamma = 20$ mg mL^{-1}) in 96% ethanol/0.1 mol L^{-1} HCl (1:1, volume ratio); $l = 1$ cm.

injection–Fourier transform infrared spectrometric method [10], Fourier transform infrared [11], near infrared [12–15], UV–Vis [16,17], and fluorescence spectroscopy [18], flow-injection spectrophotometry [19], HPLC [20], HPLC-MS [21], HPLC-MS-MS [22], GC [23,24], GC-MS [25,26], capillary electrophoresis with electrochemiluminescence detection [27,28], voltammetry [29,30], flow injection analysis system with square-wave voltammetric and amperometric detection [31], titrimetry [32], and flow injection with chemiluminescence detection [33] can be found in the literature. Derivative spectrophotometry [34,35] and spectrophotometry coupled with multivariate calibration techniques [36–38] have been used for the determination of paracetamol in the multicomponent mixture of drugs. Despite a large number of published papers describing individual determination of two analytes, there is a couple of papers on simultaneous quantification of two APIs in drug products and human plasma. A reversed phase high performance liquid chromatography [39], a second derivative spectrophotometry [40], and a differential pulse voltammetry with a glassy carbon electrode as a sensor [41] have been used for the quantitative analysis of tramadol and paracetamol in pharmaceutical products, whereas a high performance liquid chromatography–electrospray ionization-mass spectrometric method has been reported for the determination of both drugs in human plasma [42]. Due to a significant difference in amounts of the APIs in the tablet formulation (mass fraction of tramadol is 8.8% and that of paracetamol is 76.5%) as well as to their significant spectral overlapping, application of spectrophotometry for the quantitative analysis of tablets is very challenging. To overcome these issues, in the present work PLS and GA-PLS chemometric methods have been applied for the UV spectrophotometric data processing.

2. Experimental

2.1. Materials

The paracetamol-tramadol tablets (Paracetamol + Tramadol 325 mg + 37.5 mg tablets) were produced in Belupo's pilot production plant. The quality of all raw materials used for tablet production complied with Ph. Eur. quality. The tramadol was bought from Chemagis Ltd. (Israel) and paracetamol from Mallinckrodt Inc. (USA). Both substances were standardized according to the corresponding analytical procedures described in Ph. Eur. monographs and afterwards used as the working standards.

All the solvents and chemicals used were of analytical grade (Merck, Germany).

2.2. Instruments

The UV spectra were recorded by means of a Varian Cary 100 UV–Vis spectrophotometer using 1 cm quartz cells from 200 to 320 nm with a sampling interval 1 nm and integration time 0.5 s. The spectral data were processed by PLS Toolbox Solo (software version 7.1, demo). The statistical comparison of the results was carried out using OriginPro (software version 7.5).

The UHPLC analyses were performed by an Agilent 1290 Infinity LC system equipped with a quaternary pump, diode array detector, oven and automatic injector using a Zorbax SB C18, 50 × 2.1 mm, 1.8 μm (Agilent) column. The chromatographic conditions were: isocratic flow rate: 0.4 mL min^{-1}; detection: 274 nm for tramadol and 244 nm for paracetamol; run time: 4 min; injection volume: 3 μL, and column temperature: 35 °C.

Acetonitrile sodium dihydrogen phosphate ($c = 0.025$ mol L^{-1}, pH = 2.5) solution, with 3.5 mL of trimethylamine (15:85) was used as a mobile phase.

The data acquisition, chromatogram processing and all calculations were performed by means of an Empower Enterprise (Waters, USA) chromatographic software. The method was validated according to the current ICH guidelines on validation of analytical procedures [43].

2.3. Procedures

2.3.1. Preparation of standard and sample solutions

The standard stock solutions for spectrophotometric analyses were prepared by dissolving 50 mg and 20 mg of paracetamol and tramadol in 50 mL and 200 mL mixture of 96% ethanol and 0.1 mol L^{-1} HCl (1:1, volume ratio).

The combined working standard solutions of both drugs were prepared by transferring appropriate volume of each standard stock solution into a 20 mL volumetric flask and diluting to volume with the previously mentioned mixture of solvents. The prepared concentrations were in the range of 15–37 μg mL^{-1} for paracetamol and 1.7–4.3 μg mL^{-1} for tramadol. Two sets of standard solutions were prepared, the calibration set containing 25 solutions and the validation set containing 19 solutions.

Table 1
Composition of calibration solutions.

Calibration solution	γ/μg mL^{-1}	
	Tramadol	Paracetamol
1	3.10	16.0
2	1.70	18.0
3	3.90	24.0
4	2.10	20.0
5	2.90	31.0
6	3.70	35.0
7	4.20	21.0
8	3.80	35.0
9	1.70	32.0
10	4.30	36.0
11	4.00	28.0
12	1.90	25.0
13	2.20	18.0
14	1.90	33.0
15	4.00	18.0
16	2.50	32.0
17	1.90	17.0
18	2.50	15.0
19	2.60	34.0
20	3.20	26.0
21	4.00	37.0
22	4.00	35.0
23	1.70	32.0
24	4.00	28.0
25	3.80	37.0