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Short communication

Cumulative area pre-processing (CAP): A new treatment of UV data for the analysis of complex pharmaceutical mixtures

Michele De Luca*, Giuseppina Ioele, Gaetano Ragno

Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, Via P. Bucci, 87036 Rende, Italy

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ABSTRACT

A new approach to pre-processing of the UV spectral data in combination with chemometric techniques, aiming to obtain a significant amplification of the analytical information, is proposed. The single areas under the curve between two consecutive wavelengths were calculated along the full spectrum and therefore their cumulative sum was carried out. The method was called "cumulative area pre-processing" (CAP) and applied to multicomponent pharmaceutical formulations to test its performance in improving the accuracy of the analysis. The proposed procedure has demonstrated high ability in the quantitative determination of the components present in very low amount compared to other.

Three multicomponent drug formulations were analyzed by applying the partial least squares (PLS) algorithm to the UV data processed by CAP and the results compared with those carried out by using the same data without treatment and after derivative transformation. The best results in the determination of the components present in lower concentrations were obtained by applying the PLS models calculated on data processed by CAP and confirmed in the analysis of marketed drug products.

1. Introduction

Spectrophotometric analytical techniques remain particularly attractive in consideration of simplicity of execution, short analysis times, low cost and minimal sample preparation. All these features make them very suitable in the chemical, pharmaceutical and agro-food industries for routine and quality control analysis. Despite this success, the methods based on the ordinary spectrophotometry have proved to be often unsatisfactory for the analysis of complex mixtures, owing to their low resolution power [1,2].

Nowadays, the availability of computerized instrumentation and chemometric techniques has increased the possibilities for treatment of the spectral data and above all the ability to manage multiple measurements from a large number of calibration samples. The multivariate approach has demonstrated excellent performance in computing large data sets, by evaluating all the variables involved in a chemical process and then building mathematical models able to well describe the system. Chemometric procedures have been applied on spectral data of complex pharmaceutical systems with very reliable results [3–5]. The more used algorithms combined with spectrophotometry in the analysis of multicomponent pharmaceutical systems are the regression methods PCR (Principal Component Regression) and PLS (Partial Least Squares). Theory of these methods has been fully described by several authors [6,7] and successfully adopted in the analysis of several pharmaceutical formulations [8–11].

The pharmaceutical mixtures often show spectral signals overlapped and difficult to resolve, thus making hard the qualitative or quantitative determination of all species. In many cases, a pre-processing of the experimental data is necessary to eliminate or at least reduce the redundant or useless information, responsible of the decreasing of the predictive ability of the models. However, a consequence of a pre-processing can be also the removal of useful information from the spectral data. The selection of the appropriate pre-processing method often depends on the signal source, type of analytical technique and complexity of the data set.

Usually, the pre-processing methods are divided into three categories:

- *filtering and de-noising*, generally associated with the improvement of the signal-to-noise ratio (SNR). These methods are applied to the raw data before the construction of the multivariate model and reduce the influence of the random variance without altering that useful;
- *spectral normalization and differentiation*, such as the spectral derivation and the application of the Fourier transform. These procedures are also applied before the construction of the model;

* Corresponding author. Tel.: +39 0984493201; fax: +39 0984493201.
E-mail address: michele.deluca@unical.it (M. De Luca).

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- *selection of the variables and reduction of dimensionality of the data*. These methods are applied by multivariate modeling and therefore involve both the matrix **X** (independent variables) and the matrix **Y** (dependent variables). The most used method is *orthogonal signal correction* (*OSC*).

There are a number of excellent review articles providing guidance for application of the pre-processing techniques to the analytical signals [12–14].

In some pharmaceutical formulations one or more components are present in very low concentrations, so as to be hidden by the components more concentrated or by the instrumental noise. This increases the difficulty of building a mathematical model able to predict in a satisfactory manner all the species present in the mixture. In these cases, the data pre-treatment should provide to amplify the analytical information due to the components at lower concentration and at the same time to minimize the spectral noise that can interfere with their determination.

Undoubtedly, a good success in resolving these complex systems has been reached by applying the derivative transform to the original spectral data. The main features of this method are a better resolution of the overlapping signals and their amplification to increase the sensitivity. Derivative spectrophotometry has been used in qualitative and quantitative analysis of various pharmaceuticals mixtures [15–17]. Unfortunately, the differentiation signal-to-noise decreases with the increase of the derivation order and it is necessary to adopt some preliminary smoothing in the derivative calculation, such as the Savitzky–Golay approach [18,19].

In the present work, a new method for pre-processing the UV data is proposed with the aim of a significant amplification of the spectral signals. It was named "cumulative area pre-processing" (CAP) and consists in calculating the cumulative sum of all areas under curve between two consecutive wavelengths throughout the entire spectrum. The method was tested on the quantitative analysis of three common pharmaceutical formulations containing two, three and four drugs, respectively. The common difficulty in analyzing these matrices was the presence of some components in much lower amount than the others.

The binary formulation consisted of cyproterone acetate (CIP) and 17α-ethynylestradiol (ESD) in a ratio of 57:1, used in the treatment of androgen-dependent diseases in women. The ternary formulation contained paracetamol (PAR), sodium ascorbate (ASC) and chlorpheniramine maleate (CHL), with a ratio among the drugs of 150:140:1. Quaternary mixture was contained paracetamol (PAR), caffeine (CAF), phenylephrine hydrochloride (PHE), chlorpheniramine maleate (CHL) in a ratio of 125:6.25:1.25:1. These last two mixtures are commonly used as analgesic and antipyretic pharmaceuticals.

The analytical performance of the procedure CAP was tested by applying the algorithm PLS on the matrices of the spectral data pretreated and comparing the results with those obtained by using the unprocessed data matrices and after mathematical derivative transformation.

2. Experimental

2.1. Chemicals

The compounds ASC (European Pharmacopeia reference standard (EP)) CAF (EP), CHL (99%, perchloric acid titration), CIP (EP), PAR (97.0%, HPLC), ESD (EP) and PHE (EP) were purchased from Sigma–Aldrich (Milan, Italy). Pure water and ethanol were of instrumental purity grade (J.T. Baker, Holland). The marketed drug products Diane® (composition: APIs – ESD and CIP; excipients – lactose, starch–maize, povidone, talcum, magnesium stearate, sucrose, polyethylene glycol, calcium carbonate, glycerol, montan acid glycol ester, titanium dioxide and yellow iron oxide; AIC Bayer SpA), Zerinolflu® (composition: APIs – PAR, ASC and CHL; excipients – citric acid anhydrous, sodium bicarbonate, sodium carbonate, sorbitol, povidone, aspartame, dimethicone, orange and lemon flavors, Boehringer Ingelheim SpA) and Dequa-Flu® (composition: APIs – PAR, CAF, CHL and PHE; excipients – starch–maize, silica colloidal anhydrous, cellulose-microcrystalline, povidone, croscarmellose sodium and stearic acid, Aspen Pharmacare SpA) were obtained commercially. All other reagents were of the highest purity commercially available.

2.2. Instruments

Absorption spectra of the samples in a 10 mm quartz cell were recorded on a Perkin-Elmer Lambda 40P spectrophotometer at the following conditions: wavelength range 200–350 nm; scan rate 1 nm s^{-1}; time response 1 s; spectral band 1 nm; data density 1 point nm^{-1}. Derivative spectra were elaborated by the Savitzky–Golay algorithm with a derivative order 1, polynomial order 2 and a $\Delta\lambda$ value fixed at 5 nm. The instrumental parameters were tested every day before spectra data acquisition. The software UV Winlab 2.79.01 (Perkin-Elmer) was used for spectral acquisition and elaboration. Application of PLS algorithm was supported by the software package "The Unscrambler X 10.2" (Camo Process As., Oslo, Norway).

2.3. Standard solutions

Stock solutions were separately prepared by dissolving in ethanol nearly 20.00 mg of each drug in 100 mL calibrated flasks. A first set of 25 binary calibration samples was built by combining five different concentrations of ESD in the range 0.51–10.20 mg L^{-1} and CIP in the range 5.17–31.05 mg L^{-1}. A second calibration set of 18 ternary mixture solutions was prepared with drug concentration in the range of 5.05–30.3 mg L^{-1} for PAR, 2.04–30.60 mg L^{-1} for ASC and 0.20–5.05 mg L^{-1} for CHL. A third calibration set of 36 quaternary mixture solutions contained the drugs in the concentration range of 5.10–30.60 mg L^{-1} for PAR, 0.50–5.00 mg L^{-1} for CAF, 0.20–2.01 mg L^{-1} for CHL and 0.21–2.10 mg L^{-1} for PHE.

The calibration samples of the binary mixtures were prepared by adopting a full experimental design whereas the ternary and quaternary mixtures were selected according to a simple latex design (SLD) by using five different concentration levels. In order to validate the multivariate models, three further independent external validation sets consisting of 10 binary, 12 ternary and 15 quaternary mixtures were prepared.

Pharmaceutical formulations were assayed by weighing ten tablets for binary system and five tablets for ternary and quaternary mixtures and reducing them to a fine powder. The powder was suspended in ethanol and made up to a volume of 100 ml. The suspension was sonicated for 10 min and then filtered through a PTFE 0.45 μm membrane filter. Samples for analysis were obtained after proper dilution of the filtrate with ethanol.

3. Data elaboration

3.1. Cumulative area pre-processing (CAP)

The CAP data treatment transforms an original UV spectrum, characterized by absorbance signals (absorptivity units), into a new curve built by using the area underlying the same spectrum. This elaboration is based on two mathematical steps.