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Determination of atenolol in human urine by emission–excitation fluorescence matrices and unfolded partial least-squares with residual bilinearization

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ABSTRACT

A second-order multivariate calibration method based on a combination of unfolded partial least-squares (U-PLS) with residual bilinearization (RBL) has been applied to second-order data obtained from excitation–emission fluorescence matrices for determining atenolol in human urine, even in the presence of background interactions and fluorescence inner filter effects, which are both sample dependent. Atenolol is a cardioselective beta-blocker, which is considered a doping agent in shoot practice, so that its determination in urine can be required for monitoring the drug. Loss of trilinearity due to analyte–background interactions which may vary between samples, as well as inner filter effects, precludes the use of methods like parallel factor analysis (PARAFAC) that cannot handle trilinearity deviations, and justifies the employment of U-PLS. Successful analysis required to include the background in the calibration set. Unexpected components appear in new urine samples, different from those used in calibration set, requiring the second-order advantage which is obtained from a separate procedure known as residual bilinearization (RBL). Satisfactory results were obtained for artificially spiked urines, and also for real urine samples. They were statistically compared with those obtained applying a reference method based on high-performance liquid chromatography (HPLC).

1. Introduction

Atenolol (RS)-4-(2-hydroxy-3-isopropylaminopropoxy)phenylacetamide is a beta-adrenoreceptor antagonist, a beta-1 blocker, because it is cardioselective and mainly affects the heart, competing for receptor sites on the cardiac muscle. This slows down the strength of the heart contractions and reduces its oxygen requirements and the volume of blood it has to pump. It is indicated in hypertension (high blood pressure), because of its ability to increase the diameter of blood vessels, allowing blood to flow under less pressure. It is also used to treat myocardial infarction (heart attack) and arrhythmias (rhythm disorders), angina (chest pain), disorders arising from decreased circulation and vascular constriction, including migraine and also panic attack. It is considered as a doping agent in competition in different sports as chez, aeronautical sports, bridge, gymnastics, bowling, shoot, ski, swimming, and weights. In shoot it is also considered a doping agent out of competition, since it reduces the cardiac frequency and minimizes tremors. It has been included in the list of prohibited drugs (as beta-1 blockers) by the World Anti-Doping Code belonging to the World Antidoping Agency (2010) [1].

In humans, absorption of an oral dose, usually of 25, 50 or 100 mg once a day is rapid and consistent but incomplete. Approximately 50% of an oral dose is absorbed from the gastrointestinal tract, reaching the peak plasma level concentration between 2 and 4 h after ingestion. This absorbed portion is eliminated primarily by renal excretion; therefore the total amount of atenolol excreted in urine can be used as a measure of bioavailability [2–4]. Thus, the determination of atenolol in urine can be useful for monitoring the drug, performing a suitable dosage adjustment. Moreover, in doping control, urine analysis is preferred.

Several analytical methods have been reported for the determination of atenolol in biological fluids based on gas chromatography [5–7], high-performance liquid chromatography (HPLC) [8–12], capillary zone electrophoresis [13] and voltammetry [14–16].

These methods have some advantages such as sensitivity and selectivity, although they also present some disadvantages: they may require expensive equipment, toxic and expensive solvents (mainly HPLC methods) and usually tedious sample pretreatment when used for analyzing biological samples. Spectroscopic methodologies may be useful and suitable for this kind of laboratories. A few analytical methods based on spectrophotometric and spectrofluorometric determination of atenolol in tablets or artificial samples have been reported in the literature, one of them based on

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fluorescence atenolol detection, using a molecular imprinted polymer, introducing a new approach for atenolol fluorescent analysis [17–20].

Indeed, spectrofluorimetry can be applied for determining atenolol since it presents natural fluorescence. Moreover, fluorescence spectroscopy is intrinsically sensitivity and instruments are easily available [21,22]. However, when spectrofluorimetric methodologies are applied for the determination of analytes in biological fluids such as urine, they may suffer the effects of the presence of potential natural fluorescence interferences, and also from other unexpected fluorescence sample components, so that sample pretreatment steps must be required. This problem can also be overcome combining spectrofluorimetry and multivariate calibration techniques, so that interferences could be mathematically removed [23–26]. First-order methods can handle the presence of potential interferences if they are represented during the calibration stage. A sample containing unexpected components is marked as an outlier due to the poor fit of its spectrum to the calibration model (first-order advantage) [27–29] but analyte prediction are inaccurate. Moreover, two spectrophotometric methods assisted by chemometrics have been reported for the determination of atenolol but in samples as pharmaceutical formulations, based on processing UV spectral data with different first-order methods [19].

Second-order data are suitable for the quantitative determination of analytes in complex multi-component samples such as urine. Processed with appropriate second-order multivariate calibration algorithms, concentration of calibrated analytes can be obtained even in presence of uncalibrated components, exploiting the so-called second-order advantage [24–26,30–32].

In the present report, a rapid, sensitive and selective method suitable for routine laboratories, based on chemometrics-assisted spectrofluorimetry, is developed for determining atenolol in urine. Second-order trilinear data (excitation–emission fluorescence matrices) have been recorded and processed by second-order algorithms achieving the second-order advantage, allowing the determination of atenolol in urine even in the presence of interferences [32]. The selection of a suitable second-order algorithm is discussed, since this is a peculiar analytical situation. Interactions occur between the analyte atenolol and the urine background, as well as fluorescence inner filter effects, and vary from sample to sample, so that the background must be included in the calibration set. All these facts cause trilinearity losses. This situation cannot be handled by most second-order algorithms, being appropriate those that take into account trilinearity deviations, such as unfolded partial least squares (U-PLS). On the other hand, although the background is included in the calibration set, algorithms achieving the second-order advantage must be applied to predict atenolol concentrations in urine samples different than those used in calibration, since unexpected components could be present. Parallel factor analysis (PARAFAC) could be one of these methods, but it requires trilinearity, hence in principle it is not applicable to this case [33,34]. Residual bilinearization (RBL) could be applied for modeling unexpected signals.

In conclusion, in the present case, methods taking into account deviations of trilinearity, and exploiting the second-order advantage, such as U-PLS/RBL, are applied for the determination of atenolol in complex urine samples even in presence of background interactions, inner filter effects and unexpected components and without sample pretreatments [35,36]. Predictive ability, figures of merit and accuracy are discussed.

To the best of our knowledge, this is the first attempt to apply U-PLS/RBL for this purpose.

2. Experimental

2.1. Equipment

All fluorescence measurements were done on an Aminco Bowman Series 2 spectrofluorophotometer, equipped with a 150 W Xe lamp, and connected to a microcomputer running under OS/2 (through a GPIB IEEF-488 interface). In all cases, 1.00 cm quartz cells were used, excitation–emission matrices (EEMs) were registered in the range λ_{em} = 270–340 nm each 1 nm and λ_{exc} = 200–250 nm each 3 nm, making a total of 17 × 71 data points per sample matrix. Excitation and emission slit widths were both 4 nm and the scan rate was 10 nm min^{-1}. The matrix data were then transferred to an IBM-compatible microcomputer with an Intel core duo T7100, 1.80 GHz microprocessor and 2.00 Gb of RAM and processed by applying chemometric analysis based on second order algorithms, tipically unfolded partial least squares with residual bilinearization (U-PLS/RBL), written in MATLAB 7.0 (The MathWorks Inc., Natick, MA, USA) and available at www.chemometry.com, including a graphical user interface data input and parameter setting [37].

High-performance liquid chromatography (HPLC) was carried out with a Waters liquid chromatograph equipped with a 515 Waters high-pressure pump, a Rheodyne injector and UV–visible detector, using: (a) column Zorbax SB C_{18} 4.6 mm × 150 mm (5 μm particle size), (b) mobile phase methanol: NaH_2PO_4 0.34% (w/w) adjusted to pH = 3.00 with H_3PO_4 (20:80), (c) flow rate of 1.00 mL min^{-1}, (d) temperature maintained at 25 ± 1 °C, and (e) detection wavelength 223 nm (Section 2.7).

2.2. Solutions

A stock 1000 mg L^{-1} solution of analytical grade atenolol (Sigma) was prepared by dissolving the compound in doubly distilled water, sonicating for a few minutes and storing in the dark at 4 °C. Working solutions were prepared by suitable dilutions of the stock solution with double distilled water. Buffer solution was prepared from KH_2PO_4·Na_2HPO_4 (Merck) at pH = 7.4.

2.3. Calibration sample set

A linear relationship between fluorescence intensity and atenolol concentration was previously checked to have an upper limit of 0.60 mg L^{-1} (~2 × 10^{-6} mol L^{-1}). Thus, calibration was performed using a pool of healthy human urine samples as matrix, spiked with different amounts of atenolol stock solution, considering the reference concentration values of atenolol in urine in accordance to usual oral doses.

Oral doses of atenolol are approximately 56% absorbed and subsequently eliminated by renal excretion. Most of an orally absorbed dose (85–100%) is eliminated in urine within 24 h. Thus, considering pharmacokinetic data such as renal clearance and distribution volume, atenolol urine concentrations may be from 10 mg L^{-1} to 40 or 60 mg L^{-1} [2–4]. Hence, spiked urine samples were diluted 1:100 in order to obtain equally spaced concentrations in the range 0–0.60 mg L^{-1}, according to the previously checked linear concentration range. The calibration set was prepared at pH = 7.4 adding KH_2PO_4/Na_2HPO_4 buffer solution as well as the surfactant sodium dodecyl sulfate (SDS) at a concentration 2.1 mmol L^{-1}, higher than the critical micelle concentration (cmc) of 1.4 mmol L^{-1}.

The analytical technique was performed as follows: in 10.0 mL volumetric flasks, suitable amounts of atenolol stock solution were added, in order to obtain concentrations in the range 0–60 mg L^{-1}, in accordance to reference concentration values of atenolol in urine, completing to the mark with urine pools. Then 100 μL of these pool urine samples, suitable spiked with atenolol, and 250 μL of SDS 0.087 mol L^{-1} were added in another 10.0 mL volumetric flasks