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Application of time-resolved fluorescence to the determination of metabolites

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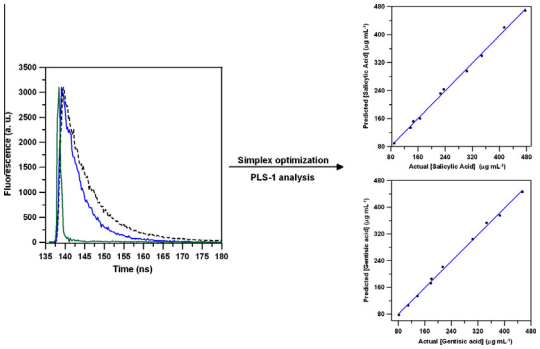
HIGHLIGHTS

- The fluorescence decay curves are used to resolve their mixture.
- A simplex optimization procedure was used to select the instrumental variables.
- The method combines time resolved measurements and multivariate calibration.
- Two major metabolites of acetyl salicylic were determined in acid human urine.

GRAPHICAL ABSTRACT



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ABSTRACT

A simple fluorescent methodology for the simultaneous determination of two major metabolites of acetylsalicylic acid – salicylic and gentisic acids – in pharmaceutical preparations and human urine is proposed. Due to the overlapping between the fluorescence spectra of both analytes, the use of the more selective fluorescence decay curves is proposed. Values of dependent instrumental variables affecting the signal-to-noise ratio were fixed with a simplex optimization procedure. A calibration matrix of thirteen standards plus two blank samples was processed using a partial least-squares (PLS) analysis. To assess the goodness of the proposed method, a prediction set of nine synthetic samples was analyzed, obtaining recovery percentages between 95% and 106%. Limits of detection, calculated by means of a new criterion, were 3.49 μg L^{-1} and 1.66 μg L^{-1} for salicylic and gentisic acids, respectively. The method was also tested in three pharmaceutical preparations containing salicylic acid, obtaining recovery percentages close to 100%. Finally, the simultaneous determination of both analytes in human urine samples was successfully carried out by the PLS-analysis of a matrix of thirteen standards plus five analyte blanks. Although spectra of analytes and urine overlap strongly, no extraction method neither prior separation of the analytes were needed.

Introduction

Acetylsalicylic acid is a non steroidal anti-inflammatory drug widely administrated all over the world. After the oral intake, the drug is mainly metabolized to its active form, salicylic acid (SA), which possesses anti-inflammatory, analgesic and antipyretic

Abbreviations: SA, salicylic acid; GA, gentisic acid; DW, Durbin-Watson parameter; PRESS, predict error sum of squares; SEP, standard error of prediction; REP, relative error of prediction; S_d, standard deviation; RES, spectra residual.

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properties because it inhibits the cyclooxygenase enzymes, responsible for the synthesis of prostaglandins. Due to the gastrointestinal adverse effects of salicylic acid, the oral intake of its derivatives – such as acetylsalicylic acid – is highly recommended. However, salicylic acid (and not derivatives) is commonly used as a treatment for skin diseases such as acne, calluses, warts or psoriasis. If orally administrated, SA and other metabolites, principally saliciluric acid and gentisic acid (GA), are mainly excreted in urine.

The large number of published methods for the determination of salicylic and gentisic acids is not only an indication of the great interest in the analysis of these compounds, but also of the problems found in their determination regarding directness, specificity, sensitivity or simplicity. Most methods applied to the simultaneous analysis of salicylic and gentisic acids are based on high performance liquid chromatography (HPLC) [1–3], gas chromatography [4] or capillary electrophoresis [5–7]. These methods, however, require complex and expensive instrumentation, separation of the analytes prior their determination and frequently a previous step of derivatization or extraction.

As an alternative, some spectrofluorometric methods have also been reported. The problem of the extensive overlap between the fluorescence spectra of SA and GA has been overcome by the use of non-conventional method such as synchronous fluorescence spectrometry [8–10], matrix isopotential synchronous fluorescence spectrometry [11] or linear variable-angle scanning fluorescence spectrometry [12]. The multivariate analysis of tridimensional fluorescence spectra [13–16] or synchronic fluorescence spectra at different pH [17] have also been reported.

Multivariate calibration methods are used in this work too, but for the analysis of the fluorescence decay curves of SA and GA. The main advantage of the use in multivariate calibration of the decay curves instead of excitation–emission matrices is that the determination can still frequently be carried out even if the analytes have fluorescence spectra that overlap completely, providing that the lifetimes of the compounds are different enough.

Most applications of time resolved fluorescence techniques belong to the field of Biochemistry due to the fluorescent properties of many biological compounds, including amines [18], enzymes [19], nucleotides [20] or ADN [21,22]. Moreover, time resolved fluorescence has become an extremely useful tool in combination to common biochemical essays such as the Polymerase Chain Reaction (PCR) [23] or immunoassay [24,25]. In Analytical Chemistry, time-resolved measurements are becoming an important analytical tool as well, being widely applied to the determination of lanthanides, actinides and heavy metals [26–28] and compounds that can produce quelates in combination to these ions, such as fluoroquinolones [29,30], benzodiazepines [31] or fulvic acid [32].

Some methods that combine time resolved measurements and multivariate calibration have also been reported to analyze aromatic compounds [33,34], and mixtures of tetracycline and oxycicline in bovine serum [35] and salicylic acid and salicylamide in blood and urine [36]. These successful simultaneous determinations of very similar compounds speak for the huge selectivity obtained by the use of time-resolved measurements and multivariate methods together, an advantage that is profited in this work to use for the first time time-resolved measurements to determinate simultaneously SA and GA in pharmaceutical preparations and human urine, without prior separation, extraction or derivatization steps.

The dependent instrumental conditions affecting the fluorescent signal of SA and GA were here optimized using a simplex-based strategy. The original simplex method described by Spendley et al. [37] starts with the building of a geometrical figure of maximum symmetry with $k + 1$ vertexes, being k the number of dependent variables, and where each vertex implies certain values for the k variables. The analytical response is evaluated in each point and compared to each other. The point giving the worst response is rejected and reflected through the hyperplane formed by the remaining points, so that a new simplex is built. This procedure is repeated successively, each step approaching the simplex more and more to the optimal conditions, and ends when this point is reached or it is considered to be close enough. Nelder and Mead [38] described a new strategy, known as the Modified Simplex Method, in which the simplexes adapt their shape and dimensions depending on each response, in order to arrive more efficiently to the optimum experimental conditions. Later on, Ryan et al. [39] proposed the use of weighted reflections in their Centroid-Weighted Modified Simplex Method, and showed that it improved the performance of simplexes considerably. In this work, we use a combination of the contributions of Ryan and Nelder and Mead, a strategy that has proved to be the fastest and easiest way [40,41] to find simultaneously the optimum values for all the dependent instrumental variables involved in the method.

Materials and methods

Instrumentation

Measurements of pH were performed using a Crison 2001 pH-meter with a glass-saturated calomel combination electrode. A Selecta ultrasons bath was used for the preparation of the solutions from pharmaceutical preparations.

The luminescence decay curves of the analytes were obtained using a Photon Technology International (PTI) TimeMaster fluorometer equipped with a picoseconds nitrogen laser that emits light at 337 nm. A wavelength selector based on the use of different dyes combined with a frequency doubler can be used to modify the wavelength of the incident light. In this work, the dye PLD-609 – that contains Rhodamine B – was chosen to obtain a light beam, the maximum emission of which is centered around 305 nm. A stroboscopic detector is coupled to a Czerny-Turner monochromator on the emission port. The system is connected to a PC via Ethernet and is governed by the Felix32 software [42].

This equipment also possesses a Peltier based cuvette holder for Quartz cuvettes with a pathlength of 1.0 cm × 1.0 cm, the temperature of which can be controlled between −20 and 100 °C.

In order to carry out the PLS regression, the decay curves obtained in the Felix32 were exported as ASCII files and transferred to a PC fitted with MatLab 7.0.1 and the routine MVC1, designed by Olivieri et al. [43], which is freely available on the Internet.

Reagents

All solution were prepared using ultrapure distilled water with a total organic carbon (TOC) level <5 μg L^{-1}, which was obtained from a Milli-Q 185 plus system.

Stock solutions (50 mg L^{-1}) of both analytes (SA and GA) were prepared by dissolving the required amounts (Sigma Aldrich, St. Louis, USA) in NaOH 0.01 mol L^{-1} (Panreac, Barcelona, Spain), and were stored at 4 °C. These solutions were found to be stable for, at least, three weeks.

A buffer solution of acetic acid/acetate (2 mol L^{-1}, pH 5.5) was prepared using the appropriate amount of sodium acetate (Panreac, Barcelona, Spain). Solutions of NaOH and HCl were used for pH adjustments and were also obtained from Panreac.

Pharmaceutical preparations were bought in a local drugstore. Diprosalic (Farmacéutica Essex, S. A., Alcobendas, Madrid, Spain) is an ointment against psoriasis and dermatitis, 1 g of which contains 30 mg SA. Callicida rojo (ESCANED, Madrid, Spain) and Antiverrugas ISDIN (ISDIN, Barcelona, Spain) are liquid medical preparations containing 12 g SA/100 g and 16.7 g SA/100 mL,