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*Short Communication*

# The fluorimetric determination of salicylic acid using computer-based multicomponent analysis

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## Introduction

The use of laboratory microcomputers to perform complex mathematical operations has rapidly increased in recent years. With the interfacing of conventional spectroscopic instrumentation to microprocessors, data collection and reduction has become much simplified. The major advantage of spectroscopic analyses of multicomponent mixtures is their speed: there is no need for tedious separation steps and the analysis itself and subsequent decision-making is easily automated. This paper indicates the power of such a method for the analysis of low levels of one material in the presence of another with overlapping spectroscopic properties.

Salicylic acid (2-hydroxy benzoic acid) is present as a decomposition product in aspirin. The content of salicylic acid in pharmaceutical preparations of aspirin is limited to 0.3–0.75%, depending on their use. As a result, a large number of analytical methods have become available [1–7]. Many HPLC systems have been reported, but column chromatography and absorptiometry continue to be used [8]. These methods all require a separation step, with long analysis times and consequent errors. In the method of Shane and Stillman [9], salicylic acid was determined in the presence of aspirin by fluorimetry in a chloroform solution. The method combined formic acid treatment to liberate free salicylic acid, and an extraction into chloroform to yield a solution suitable for fluorescence measurements: it offered a simple, one-step procedure, but its sensitivity is limited since the salicylic acid and aspirin fluorescence spectra overlap substantially. Schenk *et al.* [10] demonstrated the effects of various aliphatic carboxylic acids on the fluorescence of salicylic acid and aspirin. Their results indicate that an acetic acid–chloroform (1:99 v/v) solvent optimizes sensitivity and emission band separation.

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## Experimental

*Instrumentation and software*

A Perkin–Elmer Model LS-5 luminescence spectrometer equipped with an RS232C serial interface was employed in all fluorescence excitation and emission studies: stoppered synthetic silica cuvettes (10-mm path-length) were used. Data were recorded using a Perkin–Elmer Model 7500 Professional Computer with its 'PECLS III' applications software providing instrument control, data manipulation and storage. Quantitative analyses were performed using the 'QUANT III' software. Hard copy was provided by a Perkin–Elmer Model PP1 printer/plotter.

*Chemicals*

Chloroform (G.P.R. grade), glacial acetic acid (AnalaR grade) and salicylic acid (AnalaR grade) were from B.D.H. Chemicals Ltd. Acetylsalicylic acid (2-acetyloxy benzoic acid) was from Sigma Chemical Co. All reagents were used as supplied. The aspirin preparation used was Evans aspirin BP BNF (Evans Medical Ltd., Greenford, Middlesex, UK) in tablet form.

*Spectroscopic measurements*

Stock solvent was prepared by adding 1% v/v acetic acid to chloroform. This mixture was kept in a closed container to minimize chloroform evaporation. Salicylic acid, acetylsalicylic acid and aspirin were dissolved directly in the chloroform–acetic acid solvent, and serially diluted to give solutions suitable for fluorescence measurements. Preparation of aspirin solutions involved grinding five tablets together and dissolving a weighed portion. Aliquots (2.5 ml) of the diluted solutions were placed in stoppered cuvettes and fluorescence excitation and emission spectra recorded (Fig. 1). Standard

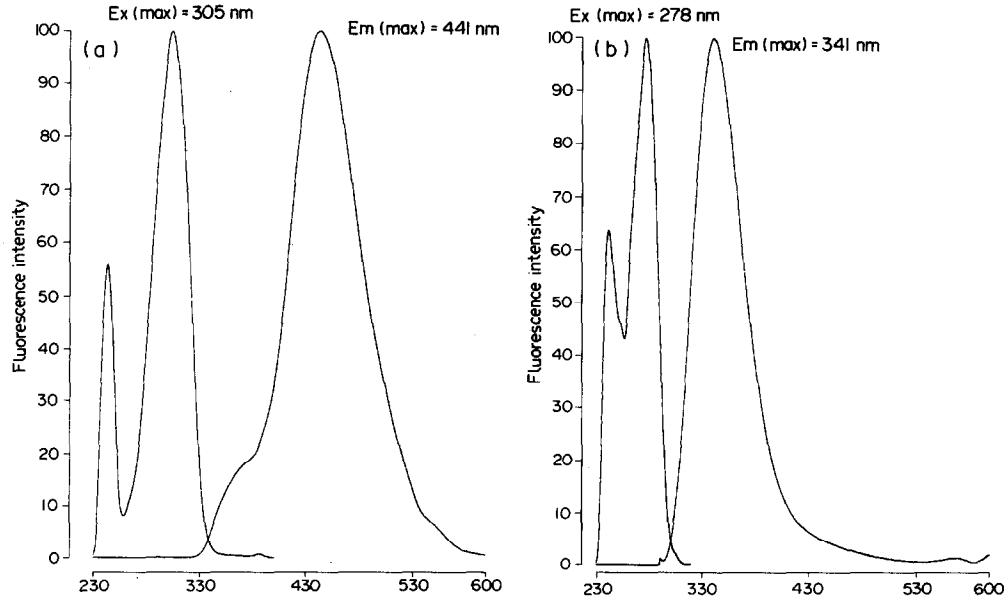


**Figure 1**
Excitation and emission spectra of salicylic acid (a) and acetylsalicylic acid (b).

conditions were chosen to record emission spectra for calibration and unknown data (Table 1). Calibration data were obtained by standard additions of salicylic acid to acetylsalicylic acid. These data, and data for aspirin samples recorded under identical conditions, were subjected to least squares quantitative analysis.

**Table 1**
Conditions for fluorescence spectrometry

| | |
|---|---|
| Solvent | 1% v/v acetic acid in chloroform |
| Slit widths | Excitation — 5 nm, emission — 5 nm |
| Fixed scale factor | 1000 |
| Emission scan range | 290–600 nm |
| Excitation wavelength | 278 nm |
| Scan speed | 4 nm/s |
| Response | 1 |

## Results and Discussion

Quantitative analysis in fluorescence spectroscopy is based on an adaptation of the Beer–Lambert law, viz.:

$$F = \Phi \cdot \epsilon \cdot c \cdot b \cdot I_0, \tag{1}$$

where $F$ is the total fluorescence intensity, $\Phi$ the fluorescence quantum yield, $\epsilon$ the molar absorptivity, $c$ the molar concentration, $b$ the optical path-length, and $I_0$ the intensity of the incident light. The simplest and most widely used fluorescence methods use characteristic excitation and emission wavelengths to determine each component. For mixtures of components having similar spectroscopic properties, this method is limited by spectral overlaps. However, if the signals from the sample components are additive equation (1) may be extended to include the fluorescence of each component at each of the analytical wavelengths, and standard matrix algebra can be used for multicomponent analysis [11].

The QUANT III software uses a different approach to the analysis of multicomponent mixtures with overlapping spectroscopic bands. Complete spectra or spectral regions of a series of standards are recorded and stored. These standards are generally mixtures of the components rather than pure components: this will compensate for non-linear deviations from equation (1). Sample spectra are then recorded. The software then attempts to analyse the data by obtaining the best least squares fit of the standard data to the sample data. Linear combinations of various proportions of the standard data are compared with the sample data, and the root mean square error (the square root of the sum of the squares of the deviations at each point in the spectrum) minimized. The results of the analysis (in the user's concentration units), the root mean square error and the sample spectrum derived by the least squares method are then output by the software. Because this method uses all the information in the spectra, better precision is achieved than if only a few wavelengths are used. Method development is simplified as it is not necessary to specify analytical wavelengths for each component. The fixed excitation or emission wavelength and the overall measurement range have to be defined, and the program can be instructed to ignore regions where there are bands from components not included in the analysis. Baseline estimation is handled automatically by the program.

The QUANT program uses up to 15 standard spectra to match the spectrum of the sample, and can therefore analyse mixtures containing up to 15 components. When the number of standard spectra is greater than the number of components there is a theoretical possibility that a unique combination giving the closest fit cannot be found. In practice, noise components in the spectra, and deviations from equation (1), ensure that this problem does not arise. In matching the measured spectrum and the standard spectra the program can incorporate an artificial component to represent a non-specific background signal. This background can be zero, horizontal, linearly sloping or parabolic. For most samples the zero baseline component is the most appropriate, but offset, sloping or curved baseline correction may improve results.

The curve-fitting approach used in the QUANT program does not involve a calibration procedure, so no calibration curves are generated. The performance of a chosen method can be checked only by analysing samples of known composition. (The standard spectra cannot of course, be used in this test.) If the spectrum being analysed is identical to one of the standard spectra the program will obtain a perfect fit using the matching standard. When the number of standard spectra exceeds the number of sample components it is possible to remove one standard from the set and analyse it using the remaining standards: this procedure is useful in checking for any errors associated with the standard spectra.

The samples used to generate the standard spectra should span the range of compositions expected in the samples to be analysed. This minimizes the effects of molecular interactions between the various components. It is not necessary to confine the standards to a range of compositions over which equation (1) is obeyed. When the number of standards exceeds the number of components it is found that the major contributions to the synthesized spectrum always come from those standards which are closest in composition to the standard.

Calibration curves were prepared based on the analysis of acetylsalicylic acid plus salicylic acid standard additions. Spectra of the two solutions containing the least (pure acetylsalicylic acid) and the most ($1.2 \times 10^{-5}$ M, 1.6% w/w) salicylic acid were required by the QUANT III software as standards. Spectra of intermediate salicylic acid concentrations in acetylsalicylic acid were then analysed by treating each as an unknown sample. The equation of the line relating the salicylic acid concentration found ($y$) to the concentration used ($x$) was

$$y = 0.985x + 0.112,$$

with a correlation coefficient >0.998.

Aspirin sample spectra were then analysed for salicylic acid content. Five replicate samples of two aspirin preparations were analysed. One of these was over a year old, but the other preparation was purchased immediately prior to the analysis. The results show that both preparations contained less than the permissible limit of salicylic acid (0.3% w/w) (Table 2): the effect of storing aspirin for extended periods is also indicated.

Examples of the results page generated by the software, and comparison of a typical sample spectrum with its mathematically derived sample spectrum are shown in Fig. 2. Figure 3 shows the emission spectra of acetylsalicylic acid, and acetylsalicylic acid containing salicylic acid (1.6% w/w). The small differences would be very difficult to quantitate by traditional methods.

**Table 2**
Analysis of salicylic acid in aspirin preparations

| Aged aspirin salicylic acid | found (%) | Fresh aspirin salicylic acid | found (%) |
|---|---|---|---|
| 0.25 | | 0.02 | |
| 0.21 | mean = 0.218 | −0.10 | mean = 0.004 |
| 0.15 | | 0.09 | |
| 0.26 | S.D. = 0.038 | 0.06 | S.D. = 0.080 |
| 0.22 | | −0.05 | |

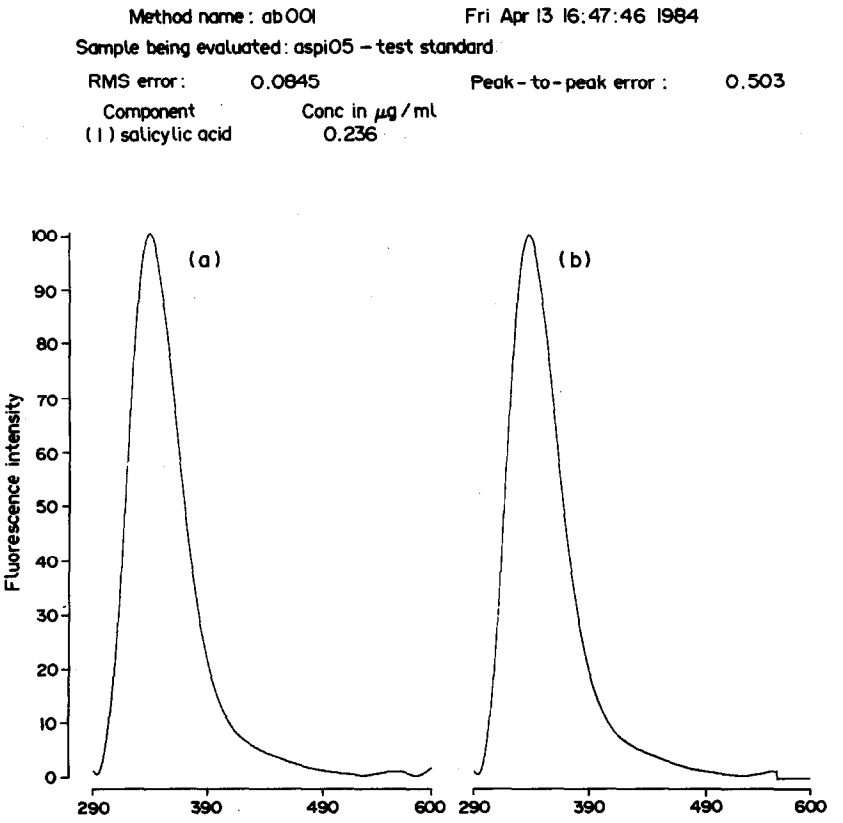


**Figure 2**
Sample emission spectrum (a) and its mathematically derived spectrum (b) with a typical results printout.

## Conclusion

One of the advantages of using computer-controlled techniques is that they are easily adapted to provide automated analyses. Once a method has been developed, it may be used repeatedly by personnel with little or no technical expertise. This is particularly simple with the QUANT III software since routines are incorporated for pass/fail decision-making. The present method indicates the power of this approach. Once standard solutions have been prepared, sample analyses may be performed in less than 2 min.

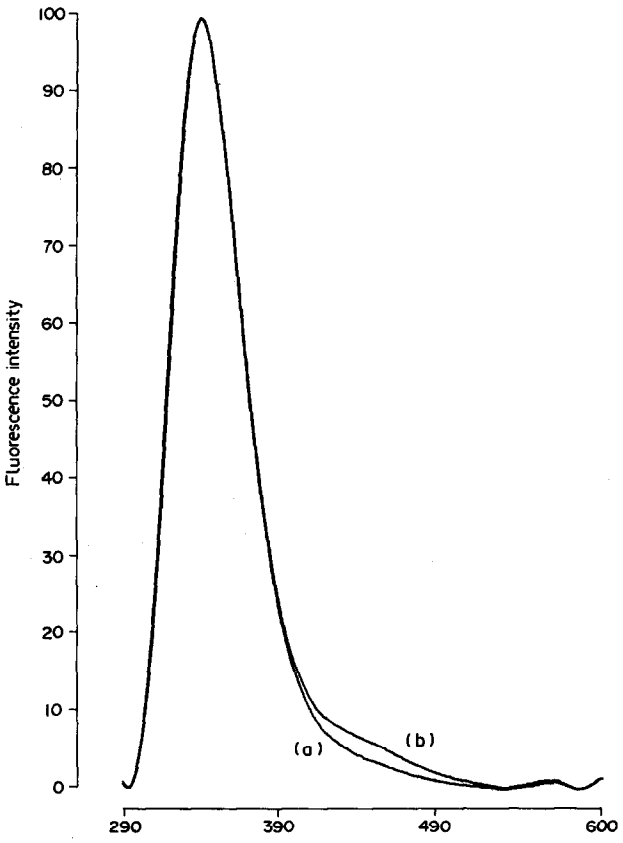


**Figure 3**
Emission spectra of acetylsalicylic acid (a) and acetylsalicylic acid containing 1.6% w/w salicylic acid (b).

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