Pergamon

0731-7085(94)00060-3

Journal of Pharmaceutical & Biomedical Analysis, Vol. 12, No. 10, pp. 1333–1335, 1994

0731-7085/94 $7.00 + 0.00

Short Communication

Zero-crossing first and second derivative synchronous fluorescence spectroscopic determination of aspirin metabolites in urine

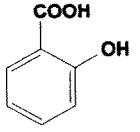
PATRICIA DAMIANI, GABRIELA IBÁÑEZ and ALEJANDRO C. OLIVIERI*

Departamento de Quimica Analitica, Facultad de Ciencias Bioquímicas y Farmacéuticas, Suipacha 531, Rosario (2000), Argentina

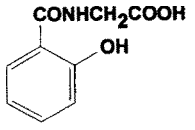
Keywords: *Determination of aspirin metabolites; second derivative fluorescence; synchronous fluorescence spectra.*

Introduction

Considerable interest has been devoted to the determination of acetylsalicylic acid (aspirin) metabolites: salicylic acid (SA), salicyluric acid (SU), and other minor metabolites in biologial fluids [1–13]. The subject is relevant regarding drug monitoring programs, and also in emergency determinations in cases of salicylate poisoning. The analysis of aspirin formulations regarding the content of SA has also attracted attention [14]. A variety of techniques have been applied to the determination of these compounds, including chromatography [1–3], spectrophotometry [4–6] and spectrofluorimetry [7–14]. The latter method seems to be of choice due to its simplicity, sensitivity and specificity. However, the main urine metabolites of aspirin (SA and SU) have fluorescent emission spectra which not only overlap with each other, but also with fluorescent normal urine components. This problem has been previously overcome by various sample pre-treatment methods (which eliminate the interferences) [7–12] together with synchronous fluorescence determinations (which increase the spectral resolution) [11, 12]. Recently, it has been suggested that SA and SU can be directly determined by a suitable selection of wavelengths [13]. The concentration of SA was previously measured as proportional to the first derivative synchronous fluorescence intensity at λ_{exc} = 325 nm (I^1_{325}), where the contribution of SU is zero (since at this λ SU presents a maximum). On the other hand, the concentration of SU was proposed to be determined by the intensity of the first derivative spectrum at λ_{exc} = 350 nm (I^1_{350}), where the fluorescence of SA has considerably decayed. However, this latter wavelength corresponds to one of the flanks of the SU first derivative synchronous fluorescence band, while it would be better to carry out the measurements at a spectral maximum. We now report the combined use of first and second derivatives of the fluorescence spectra of non treated urine samples. We show that a clear spectral maximum appears for SU in the second derivative mode, at a wavelength where the contribution of SA is not significant. This allows the direct and simultaneous determination of both SA and SU, avoiding tedious, error-prone sample pre-treatment procedures.



Salicylic acid



Salicyluric acid

* Author to whom correspondence should be addressed.

Experimental

Apparatus

All fluorescence measurements were done on a JASCO FP 770 spectrofluorometer equipped with a 150 W Xenon lamp. Synchronous fluorescence spectra were recorded by scanning both monocromators with a constant difference of 90 nm between excitation and emission. Usual experimental parameters were: scan speed, 200 nm min^{-1}; response time, 0.025 min; bandwidth, 5 nm. First and second derivative spectra were then obtained, and the zero crossing technique was applied for the independent determination of salicylic and salicyluric acids. The concentration of SA was measured as proportional to the first derivative intensity at λ_{exc} = 325 nm (I^1_{325}) whereas SU was determined from the corresponding second derivative intensity at 350 nm (I^2_{350}).

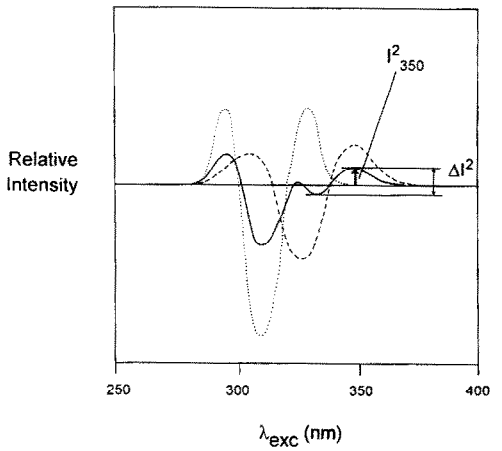


Figure 1
Second derivative synchronous fluorescence spectra of: (·····), salicylic acid (0.20 μg ml^{-1}); (-----), salicyluric acid (0.20 μg ml^{-1}); (——), a mixture of both acids (0.084 μg ml^{-1} of each acid). The value of I^2_{350} used to measure the concentration of salicyluric acid is shown, as well as the magnitude of $\Delta^2 I$.

Reagents

Stock solutions of salicylic acid (Merck, 125 mg l^{-1}) and salicyluric acid (Aldrich, 100 mg l^{-1}) in distilled water were prepared. Standard solutions for fluorescence measurements were prepared by convenient dilution of these stock solutions.

Procedure for binary urine mixtures

Urine containing SA and SU in the range 40–200 ppm was diluted (1:20), 1 ml of the resulting mixture and 2 ml of NaOH were placed into a 50 ml volumetric flask and diluted with distilled water. In this way the final concentration range for both acids was 0.04–0.2 μg ml^{-1}.

Results and Discussion

We discuss the application of second derivative synchronous fluorescence spectroscopy to the simultaneous determination of SA and SU in urine samples. Figure 1 shows the corresponding results for SA, SU and a mixture of both acids. In principle, one could apply the usual method for evaluating derivative signals, according to which ΔI^n is measured:

$$\Delta I^n = (I^n_{Max} - I^n_{Min})_{\lambda 1,\lambda 2} \qquad (1)$$

where I^n_{Max} and I^n_{Min} are the maximum and minimum values of the nth. derivative ($I^n = d^n I/d\lambda^n$) within a relatively narrow spectral range (λ_1,λ_2). Both I^2_{Max} and I^2_{Min} for SA overlap with fluorescent normal urine bands (λ_{exc} at *ca.* 280–290 nm) and hence they are unsuitable for this purpose. On the other hand, the corresponding value for SU (ΔI^2 is also shown in Fig. 1) could be convenient for measuring its concentration. However, when applying this procedure for strongly overlapping bands it is frequently necessary to introduce correction factors [14]. Examination of both first and third derivative spectra did not lead to appropriate bands that could be used to safely apply equation (1).

A maximum appears at λ_{exc} = 350 nm in the second derivative of the synchronous fluorescence spectrum of SU, where the contribution of SA is negligible, and thus the zero-crossing technique seems to be preferable in this case. It involves the single measurement of I^2_{350}. No other zero-crossing point appears to be available in order to measure the concentration of SA through both second and third derivative spectra. This can be done by the previously discussed use of I^1_{325} [11–13]. Since both first and second derivative spectra are readily available from the spectrofluorimeter, the combined use of them should not present any complication. Furthermore, normal fluorescent urine components do not interfere at these wavelengths (see Fig. 2).

The use of I^2_{350} instead of I^1_{350} in order to determine the content of SU in urine should in principle give better results. On one hand, intensity measurements are more conveniently

Table 1
Calibration plot for salicyluric acid (as measured from I^2_{350}) and determination of SU in urine samples containing mixtures of SA and SU

Calibration plot*		Urine mixtures	
SU μg ml^{-1}	I^2_{350}†	SU:SA Taken μg ml^{-1}	SU found (Rec, %; RSD, %)‡ μg ml^{-1}
0.052	2.40	0.065:0.050	0.068 (105; 5.3)
0.078	3.73	0.065:0.100	0.071 (109; 5.3)
0.103	4.90	0.065:0.150	0.060 (92; 5.3)
0.153	7.22	0.129:0.050	0.120 (93; 5.0)
0.206	9.50	0.190:0.050	0.180 (95; 7.1)
0.258	11.90		

*These data give $I^2_{350} = A + BC$; $A = 0.11(6)$, $B = 46(4)$; C = concentration of SU in μg ml^{-1}; $r = 0.9998$; RSD = 5.9% for $C = 0.078$ μg ml^{-1}; 3.0% for $C = 0.153$ μg ml^{-1}.
†Arbitrary units.
‡The results are averages of three determinations.

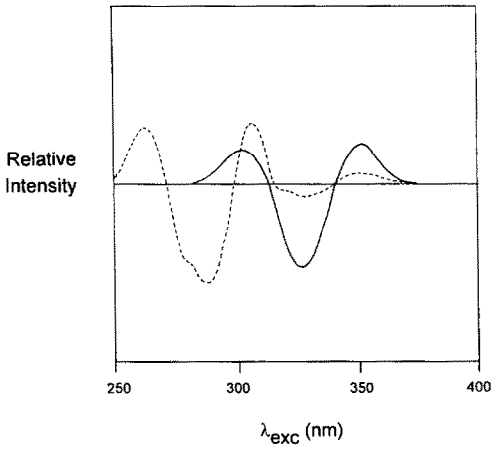


Figure 2
Second derivative synchronous fluorescence spectra of: (——), salicyluric acid (0.20 μg ml^{-1}) and (-----), a diluted human urine sample (see Experimental) containing salicyluric acid at the lowest concentration (0.052 μg ml^{-1}) used to construct the calibration plot.

done at spectral maxima rather than at flanks of spectral bands. On the other, for two overlapping gaussian bands of equal widths, centered at λ_1 and λ_2, the ratios of I^1 intensities at a distant wavelength λ_m ($\lambda_m \gg \lambda_1,\lambda_2$) is $[(\lambda_m - \lambda_1)/(\lambda_m - \lambda_2)]$ whereas for I^2 it is $\{[(\lambda_m - \lambda_1)^2 + 1]/[(\lambda_m - \lambda_2)^2 + 1]\} \approx [(\lambda_m - \lambda_1)^2/(\lambda_m - \lambda_2)^2]$, thus increasing the selectivity.

Urine samples containing mixtures of SA and SU were studied by the above procedure. The concentration of SU was measured from the intensity of the second derivative of the synchronous fluorescence spectrum of the sample at $\lambda_{exc} = 350$ nm, after a calibration plot was prepared. The results are summarized in Table 1.

In conclusion, it has been shown that the main urine metabolites of aspirin, salicylic and salicyluric acids, can be conveniently determined using spectrofluorimetric techniques. If first and second derivative synchronous fluorescence spectra are recorded, the direct and simultaneous determination of both acids is possible without sample pre-treatment procedures.

Acknowledgements — Financial support from the University of Rosario and Consejo Nacional de Investigaciones Cientificas y Tecnicas (CONICET) is gratefully acknowledged G.I. thanks the University of Rosario for a research student fellowship.

References

[1] I. Bekerski, H.G. Boxembaum, M.H. Whitson, C.V. Publisi, R. Pocelinko and S.A. Kaplan, *Anal. Lett.* **10**, 539–550 (1977).
[2] E.N. Amick and W.D. Mason, *Anal. Lett.* **12**, 629–639 (1979).
[3] C.M. Moore and I.R. Tebbett, *Forensic Sci. Int.* **34**, 155–158 (1987).
[4] P. Trinder, *Biochem. J.* **57**, 301–303 (1954).
[5] E.B. Truitt, A.M. Morgan and J.M. Little, *J. Am. Pharm. Assoc. Sci. Ed.* **44**, 142–148 (1955).
[6] A.M. Bermejo, M. Lopez-Rivadulla, P. Fernandez and A. Cruz, *Anal. Lett.* **24**, 1147 (1991).
[7] D. Schachter and J.G. Manio, *J. Clin. Invest.* **37**, 800–807 (1958); G. Pitet, J. Cros and R. Caujolle, *C.R. Acad. Sci. Paris Ser. D* **268**, 868 (1969).
[8] R.N. Gupta and M. Zamkanei, *Clin. Chem.* **36**, 1690–1691 (1990).
[9] J.C. Jacobs and M. Pesce, *Arthritis Rheum.* **21**, 129–132 (1978).
[10] M.P. Bailey, B.F. Rochs and C. Riley, *Anal. Chim. Acta* **201**, 335–338 (1987).
[11] F. Salinas, A. Muñoz de la Peña, I. Durán-Merás and M.S. Durán, *Analyst* **115**, 1007–1011 (1990).
[12] A. Muñoz de la Peña, F. Salinas and I. Durán-Merás, *Anal. Chem.* **60**, 2493–2496 (1988).
[13] P. Damiani, G. Ibánez and A.C. Olivieri, *Anal. Lett.* **26**, 247–257 (1993).
[14] D.G. Konstantianos, P.C. Ioanou and C.E. Efstathiou, *Analyst.* **116**, 373–378 (1991).

[Received for review 12 July 1993; revised manuscript received 4 January 1994]