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## Determination of nitrofurantoin in urine by derivative spectroscopy

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### Summary

A simple procedure for the determination of nitrofurantoin in urine using second derivative spectroscopy is described. The method consists of direct extraction of the non-ionized form of the drug by the use of ethyl acetate, and determination of the nitrofurantoin concentration by measuring the amplitude of the second derivative peak. The method has a detection limit of 1.5  $\mu\text{g/ml}$ , with recovery of 99.3% and a standard curve that is linear to 50  $\mu\text{g/ml}$ .

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### Introduction

Nitrofurantoin is a urinary tract antibacterial agent whose clinical effectiveness depends on the high urinary drug levels encountered during therapeutic drug dosage. A variety of methods have been reported for the quantitative determination of nitrofurantoin in biological fluids (Bender et al., 1956; Buzard et al., 1956; Conklin and Hollifield, 1965; Burmicz et al., 1976; Mason and Sandmann, 1976; Aufrere et al., 1977; Hoener and Wolff, 1980; Juenge et al., 1985). Of the available techniques, that of Conklin and Hollifield (1965) has been established as the preferred method for determining nitrofurantoin in urine and is recommended by the American Pharmaceutical Association for use in nitrofurantoin bioavailability studies (Cadwallader, 1975). This method known as the nitromethane/Hyamine 10-X assay consists of

the direct extraction of nitrofurantoin from acidified urine into the solvent nitromethane, followed by the addition of the quaternary ammonium compound Hyamine 10-X hydroxide which yields a yellow-colored complex. The nitromethane/Hyamine procedure has been modified, to determine nitrofurantoin in the presence of its metabolites (Hollifield and Conklin, 1970), and increase its sensitivity for determining the drug in blood (Mattok et al., 1970).

Although the Hyamine 10-X assay has been proven to be a valuable method for determining nitrofurantoin, it has some disadvantages. Firstly, it is well known (Conklin and Hollifield, 1965; Mattok et al., 1970) that there is a gradual increase in absorbance with time of resulting complex and therefore absorbance measurements should be carried out rapidly after the addition of the Hyamine 10-X solution. Secondly, in our laboratory (Macheras, 1983; Macheras and Rappas, 1986) we encountered negative absorbance readings in urine samples of low drug concentra-

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tions. This is indicative of variations in urine sample blanks during the experimental period of bioavailability studies. The degree of the variable assay interference by urine constituents was not addressed in the literature and its contribution to the inaccuracy in determining nitrofurantoin bioavailability remains unexplored. However, when the Hyamine method was applied to blood samples, the possibility of an interfering blood constituent interacting with Hyamine was not excluded (Mattok et al., 1970).

The present study describes the application of derivative spectroscopy to the determination of nitrofurantoin in urine. This new and rapidly developing technique has been applied to forensic toxicology (Gill et al., 1982; Fell et al., 1981). It has also been used for the determination of benzodiazepines in plasma (Martinez and Gimenez, 1981). The technique is based on the principle that for any given wavelength and concentration interval, the fulfillment of the Lambert-Beer law for the  $n^{\text{th}}$  derivative is governed by the following equation:

$$\frac{d^n A}{d\lambda^n} = \frac{d^n \epsilon}{d\lambda^n} \cdot C \cdot \ell \quad (1)$$

where  $A$  = absorbance,  $\epsilon$  = molar absorptivity ( $l \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ ),  $C$  = concentration ( $\text{mol} \cdot l^{-1}$ ), and  $\ell$  = cell pathlength (cm).

## Materials and Methods

### Reagents

All solutions were prepared in deionized water from analytical reagent grade materials. Nitrofurantoin<sup>1</sup>, and other materials<sup>2-5</sup> were obtained from commercial sources.

### Apparatus

Double-beam spectrophotometer model Lamb-

da 7 (Perkin Elmer) with derivative mode was used. Operating conditions for derivative spectroscopy were: scan speed 480 nm/min; time response 5 s; and slit width 2 nm.

### Nitrofurantoin standard solutions

A 100  $\mu\text{g}/\text{ml}$  solution is prepared by dissolving 50.0 mg of pure substance in 50.0 ml of *N,N*-dimethylformamide; then 10.0 ml of this solution is diluted to 100 ml with water. This stock solution, if kept in amber bottle in a refrigerator is stable for a long time. Working standard solutions in the range of 10–50  $\mu\text{g}/\text{ml}$  are prepared by appropriate dilutions of the stock solution with water. Internally corrected solutions of nitrofurantoin in urine were prepared, with 0.5 ml of urine, the appropriate volume of the standard stock solution in water, and 1.5 ml of 2 N hydrochloric acid to obtain nitrofurantoin concentrations of 10, 20, 30, 40 and 50  $\mu\text{g}/\text{ml}$ .

### Procedures

(a) *Hyamine method.* In a centrifuge tube, 0.5 ml of urine and 1.5 ml of 2 N hydrochloric acid are mixed thoroughly (vortexed for 1 min) with 5.0 ml of nitromethane. The tube is then centrifuged at 3000 rpm for 10 min. A 3.0 ml aliquot of the clear nitromethane layer is transferred to a test tube and mixed with methanolic hyamine (0.5 ml;  $0.05 \text{ mol} \cdot l^{-1}$ ). After 5 min the absorbance at 400 nm is measured, with nitromethane as the reference solution.

(b) *Second derivative method.* In a centrifuge tube, 0.5 ml of urine, and 1.5 ml of 2 N hydrochloric acid are mixed thoroughly (vortexed for 1 min) with 5 ml of ethyl acetate. The tube is then centrifuged at 3000 rpm for 10 min. The second derivative spectrum of the organic layer is then recorded from 300 nm to 500 nm and the amplitude between the maximum and minimum of the second derivative signal is measured graphically.

*In vivo study.* A single dose of a nitrofurantoin tablet (100 mg) was administered to a healthy informed volunteer after a light breakfast. Urine samples were collected at 0, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0 and 24 h with complete emptying of the bladder after taking the medication, and assayed for nitrofurantoin by the methods described above.

<sup>1</sup> Sigma Chemicals.

<sup>2</sup> Nitromethane from Fluka AG.

<sup>3</sup> Hyamine 10-X hydroxide from BDH Chemicals.

<sup>4</sup> Ethyl acetate from Merck.

<sup>5</sup> *N,N*-dimethylformamide from Fluka AG.

## Results and Discussion

### Standard curves

Fig. 1a shows the zero-order spectrum of urine extract containing nitrofurantoin at 50  $\mu\text{g}/\text{ml}$ . Fig. 1b shows the second derivative spectrum containing nitrofurantoin at levels of 10, 20, 30, 40 and 50  $\mu\text{g}/\text{ml}$ . The efficiency of the extraction procedure was established at 83.1% by comparing values of the amplitudes of the second derivative

signal obtained in setting up the calibration curve with values obtained with solutions in which the nitrofurantoin was dissolved directly in ethyl acetate. The statistical treatment of data for calibration curves are shown in Table 1. Data for the variation of precision shown in Table 2, reveal a coefficient of variation  $\pm 0.55$  to 1.60 for the range of 10–50  $\mu\text{g}$  added.

To find out if the variable assay interference by urine constituents could be appreciable, a number

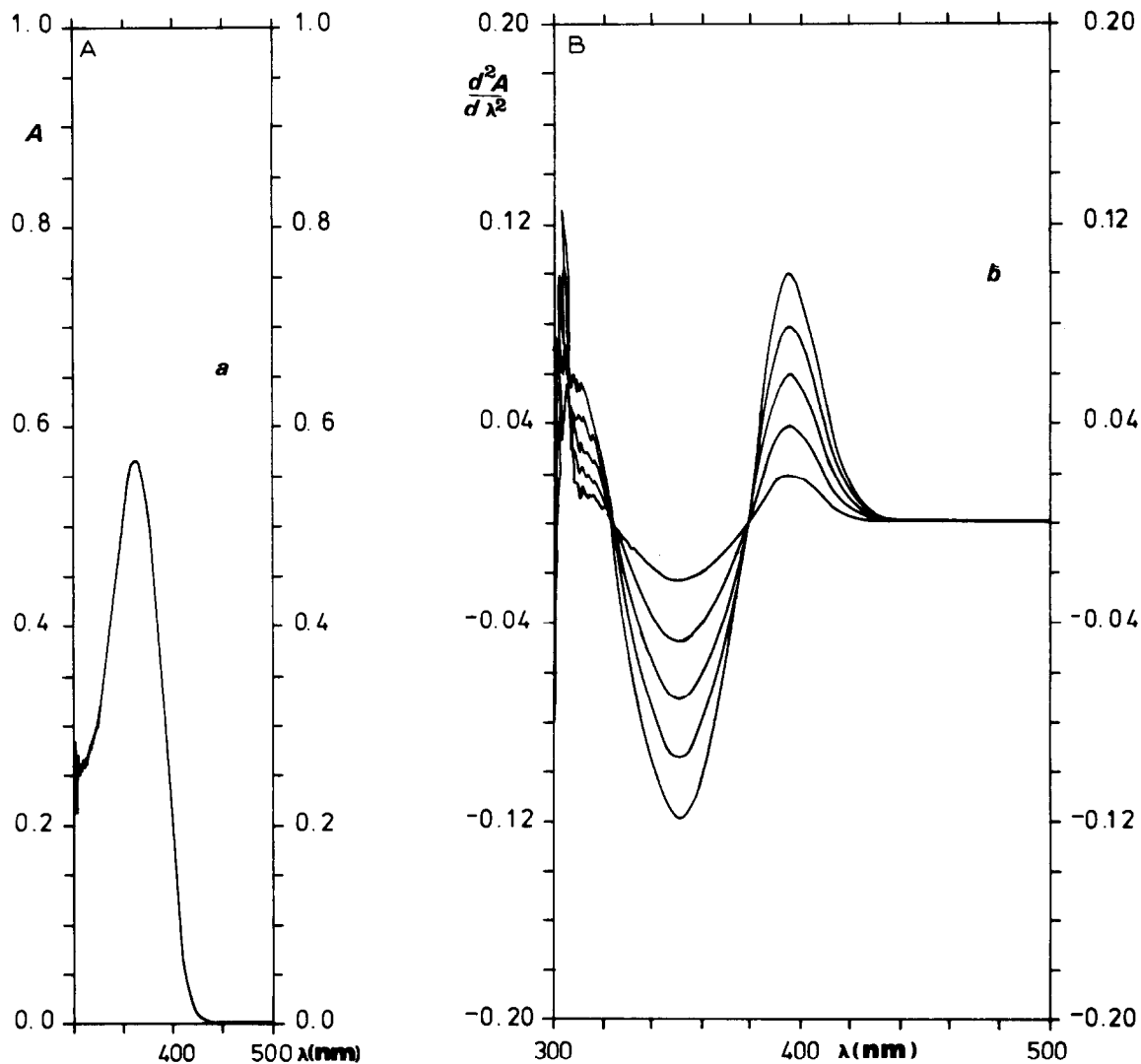


Fig. 1. Zero order spectrum and second derivative spectra of urine extracts containing in ethyl acetate: (a) nitrofurantoin 50  $\mu\text{g}/\text{ml}$  (zero order); (b) nitrofurantoin 10, 20, 30, 40, and 50  $\mu\text{g}/\text{ml}$  (2nd derivative).

TABLE 1  
CALIBRATION CURVES OF NITROFURANTOIN FOR RECOVERY STUDIES

| Fluid         | Calibration equation <sup>a</sup>          | S.E.E. <sup>b</sup> | r <sup>c</sup> |
|---------------|--|---------------------|----------------|
| Ethyl acetate | $y = -3.71 (\pm 0.83) + 15.15 (\pm 0.13)C$ | 0.796               | 0.9999         |
| Water         | $y = -3.20 (\pm 0.54) + 12.50 (\pm 0.08)C$ | 0.516               | 0.9999         |
| Human urine   | $y = -0.61 (\pm 0.77) + 12.70 (\pm 0.11)C$ | 0.730               | 0.9998         |

<sup>a</sup> Amplitude of the second derivative signal vs nitrofurantoin concentration in  $\mu\text{g}/\text{ml}$ ; 5 standards.

<sup>b</sup> Standard error of the estimate.

<sup>c</sup> Correlation coefficient.

TABLE 2  
MEAN RECOVERY OF NITROFURANTOIN FROM HUMAN URINE SPIKED WITH THE DRUG

| Added ( $\mu\text{g}$ ) | Measured <sup>a</sup> ( $\pm$ S.D.) ( $\mu\text{g}$ ) | C.V. <sup>b</sup> |
|-------------------------|---|-------------------|
| 10                      | 9.96 ( $\pm$ 0.15)                                    | 1.48              |
| 20                      | 19.86 ( $\pm$ 0.32)                                   | 1.60              |
| 30                      | 30.38 ( $\pm$ 0.48)                                   | 1.57              |
| 40                      | 39.84 ( $\pm$ 0.22)                                   | 0.55              |
| 50                      | 51.14 ( $\pm$ 0.50)                                   | 0.98              |

<sup>a</sup> Mean of 5 replicate samples; standard deviation in parentheses.

<sup>b</sup> Coefficient of variation.

of calibration curves were constructed. Both the intra- and inter-subject variability was evaluated (Table 3). A comparison based on the means of

slopes of calibration curves reveals that the inter-subject variability is remarkably higher than the intra-subject variability. However, the Student's *t*-test showed no statistical significance ( $P > 0.05$ ) between the means of slopes of the regression lines. These results clearly indicate that the intra-subject and inter-subject variable assay interference by urine constituents is not considerable, and therefore a unique calibration curve can be reliably applied for all measurements in a bio-availability study.

#### Comparison of two methods

In order to compare the second derivative method with the Hyamine method, urine samples spiked with the drug were assayed by both methods. To mimic the application of the methods to an actual bioavailability study, two calibration

TABLE 3  
CALIBRATION CURVES OF NITROFURANTOIN IN HUMAN URINE FOR THE EVALUATION OF INTRA- AND INTER-SUBJECT VARIATION ASSAY INTERFERENCE

| Subject | Sampling time | Calibration equation <sup>a</sup>         | S.E.E. <sup>b</sup> | r <sup>c</sup> |
|---------|---------------|---|---------------------|----------------|
| 1       | 08.30 h       | $y = 0.20 (\pm 1.01) + 2.70 (\pm 0.03)x$  | 0.966               | 0.9998         |
| 1       | 11.00 h       | $y = 1.91 (\pm 1.07) + 2.57 (\pm 0.03)x$  | 1.016               | 0.9997         |
| 1       | 14.00 h       | $y = 2.32 (\pm 0.63) + 2.51 (\pm 0.02)x$  | 0.605               | 0.9999         |
|         | Mean slope:   | $2.59 (\pm 0.10) - 3.74^d$                |                     |                |
| 2       | 11.00 h       | $y = -0.82 (\pm 0.38) + 2.92 (\pm 0.01)x$ | 0.365               | 0.9999         |
| 3       | 11.00 h       | $y = -0.33 (\pm 0.83) + 2.61 (\pm 0.02)x$ | 0.795               | 0.9998         |
| 4       | 11.00 h       | $y = -0.61 (\pm 0.76) + 2.54 (\pm 0.02)x$ | 0.730               | 0.9998         |
|         | Mean slope:   | $2.69 (\pm 0.20) - 7.51^d$                |                     |                |

<sup>a</sup> Amplitude of the second derivative signal vs amount of nitrofurantoin in  $\mu\text{g}$ ; for each calibration curve human urine collected at the time quoted in column two of the table was spiked with the drug and used as blank solution too.

<sup>b</sup> Standard error of the estimate.

<sup>c</sup> Correlation coefficient.

<sup>d</sup> Coefficient of variation.

curves (one for each method) were constructed from urine collected at 08.30 h and used throughout the study. Urine samples collected at 10.00, 13.00 and 17.00 h were spiked with the drug. The results shown in Table 4 clearly demonstrate that the second derivative method is superior to the Hyamine method in respect of precision and accuracy. Based on the results of Tables 3 and 4 the detection limit was about 1.5  $\mu\text{g}/\text{ml}$  (concentration corresponding to second derivative signal equal to twice the standard deviation of the most dilute standard).

#### *In vivo study*

Fig. 2 shows a linear relationship between the cumulative urinary excretion values based on the Hyamine and second derivative assay results. Although there is an apparent linear relationship, none of the 4 urinary excretion values determined by the Hyamine method were within  $\pm 10\%$  of the values determined by the second derivative method. Another point which requires mentioning

TABLE 4  
COMPARISON OF THE SECOND DERIVATIVE METHOD WITH THE HYAMINE METHOD

| Added ( $\mu\text{g}$ ) | Sampling time       | Measured <sup>a</sup> $\pm$ (C.V.) <sup>b</sup> - P.E. <sup>c</sup> |                             |
|-------------------------|---------------------|---|-----------------------------|
|                         |                     | Second derivative method <sup>d</sup>                               | Hyamine method <sup>e</sup> |
| 10                      | 10.00 h             | 9.96 (1.47) - 0.40  | 11.54 (1.71) - 15.40        |
| 10                      | 13.00 h             | 8.92 (1.50) - 10.80   | 8.45 (3.86) - 15.50         |
| 10                      | 17.00 h             | 8.84 (1.91) - 11.60   | 12.08 (2.48) - 20.80        |
|                         | Mean <sup>f</sup> : | 9.24 (0.62) - 7.60  | 10.69 (1.96) - 6.90         |
| 30                      | 10.00 h             | 30.38 (1.57) - 1.27   | 36.17 (0.94) - 20.57        |
| 30                      | 13.00 h             | 28.35 (0.95) - 5.50   | 32.25 (1.35) - 7.50         |
| 30                      | 17.00 h             | 27.56 (1.58) - 8.13   | 36.67 (3.91) - 22.23        |
|                         | Mean <sup>f</sup> : | 28.76 (1.45) - 4.13   | 35.03 (2.42) - 16.77        |
| 50                      | 10.00 h             | 51.14 (0.98) - 2.28   | 62.27 (1.66) - 24.54        |
| 50                      | 13.00 h             | 47.77 (0.43) - 4.46   | 55.36 (2.00) - 10.72        |
| 50                      | 17.00 h             | 46.31 (0.94) - 7.38   | 59.70 (2.10) - 19.40        |
|                         | Mean <sup>f</sup> : | 48.41 (2.48) - 3.18   | 59.11 (3.49) - 18.22        |

<sup>a</sup> Mean of 5 replicate samples.

<sup>b</sup> Coefficient of variation.

<sup>c</sup> Percent error.

<sup>d</sup> The calibration curve used was  $y = -0.33 + 2.61x$ .

<sup>e</sup> The calibration curve used was  $A = -0.0014 + 0.00958x$ .

<sup>f</sup> S.D. in parentheses.

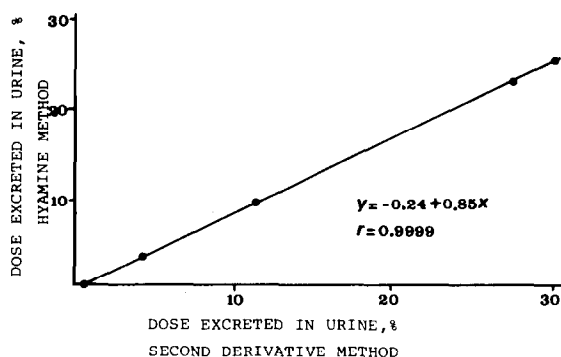


Fig. 2. Relationship between the percent of nitrofurantoin dose excreted in urine as determined by Hyamine and second derivative method.

is that no drug was detected by both methods in the urine samples of 1, 12 and 24 h after dosing. Moreover, the application of the Hyamine method resulted in negative absorbance reading for the urine sample of the 2nd hour while the second derivative method yielded for the same sample a concentration of 2.67  $\mu\text{g}/\text{ml}$  corresponding to an excretion value of 0.19 mg.

These results clearly indicate that interferences by urinary constituents, which vary during the experimental period and with the extent of dilution of the urine specimen, can cause appreciable errors in nitrofurantoin bioavailability estimates based on the Hyamine method. The second derivative method is simple and rapid requiring non-expensive reagents. It has excellent precision and accuracy and therefore it can be used as the routine analytical method for urinary excretion-based bioavailability studies on nitrofurantoin.

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#### References

- Aufrere, M.B., Hoener, B. and Vore, M.E., High performance liquid chromatographic assay for nitrofurantoin in plasma and urine. *Clin. Chem.*, 23 (1977) 2207-2212.

- Bender, R.C., Nohle, E.G. and Paul, M.F., Nitrofurantoin estimation in urine with the aid of chromatography. *Clin. Chem.*, 2 (1956) 420-426.
- Burmicz, J.S., Smyth, W.F. and Palmer, R.F., An ultraviolet spectral and polarographic study of nitrofurantoin, a urinary-tract antibiotic. *Analyst*, 101 (1976) 986-991.
- Buzard, J.A., Vrablic, D.M. and Paul, M.F., Colorimetric determination of nitrofurazone, nitrofurantoin, and furazolidone in plasma. *Antibiot. Chemother.*, 6 (1956) 702-707.
- Conklin, J.D. and Hollifield, R.D., A new method for the determination of nitrofurantoin in urine. *Clin. Chem.*, 11 (1965) 925-931.
- Cadwallader, D.E., Nitrofurantoin. *J. Am. Pharm. Ass.*, 15 (1975) 409-412.
- Fell, A.F., Jarvie, D.R. and Stewart, M.J., Analysis of paraquat by second and fourth derivative spectroscopy. *Clin. Chem.*, 27 (1981) 286-292.
- Gill, R., Bal, T.S. and Maffat, A.C., The application of derivative UV-visible spectroscopy in forensic toxicology. *J. Forensic Sci. Soc.*, 22 (1982) 165-171.
- Hoener, B. and Wolff, J.L., High-performance liquid chromatographic assay for the metabolites of nitrofurantoin in plasma and urine. *J. Chromatogr.*, 182 (1980) 246-252.
- Hollifield, R.D. and Conklin, J.D., A method for determining nitrofurantoin in urine in the presence of phenazopyridine hydrochloride and its metabolites. *Clin. Chem.*, 16 (1970) 335-338.
- Juenge, E.C., Kreienbaum, M.A. and Gurka, D.F., Assay of nitrofurantoin oral suspensions contaminated with 3-(5-nitrofurfurylideneamino)hydantoic acid. *J. Pharm. Sci.*, 74 (1985) 100-102.
- Macheras, P.E., Comparative bioavailability studies of four commercial nitrofurantoin products, *Chimika Chronika, New Series*, 12 (1983) 63-73.
- Macheras, P.E. and Reppas, C.I., Studies on freeze-dried drug-milk formulations II: Effect of regenerated fluid volume on nitrofurantoin bioavailability. *J. Pharm. Sci.*, in press.
- Martinez, D. and Gimenez, M.P., Determination of benzodiazepines by derivative spectroscopy. *J. Anal. Toxicol.*, 5 (1981) 10-13.
- Mason, W.D. and Sandmann, B., Determination of nitrofurantoin by reduction at rotating platinum electrode. *J. Pharm. Sci.*, 65 (1976) 599-601.
- Mattok, G.L., McGilveray, I.J. and Charette, C., Improved nitromethane-hyamine method for the chemical determination of nitrofurantoin in whole blood. *Clin. Chem.*, 16 (1970) 820-823.