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Short communication

Determination of the 5-fluorouracil and N1(2'-furanidyl)uracil in the presence of tegafur by zero-crossing first derivative spectrometry

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Abstract

A first derivative spectrometric method has been developed for the determination of the 5-fluorouracil and N1(2'-furanidyl)uracil related substances and degradation products of tegafur. The wavelengths selected for the determination of 5-fluorouracil and N1(2'-furanidyl)uracil were 298 and 288 nm, respectively. At this wavelength, the calibration graphs between the amplitude of the signals and the concentration of each compound were linear up to 24.75 mg l^{-1} for 5-fluorouracil and up to 20.20 mg l^{-1} for N1(2'-furanidyl)uracil. The detection limits were 0.40 and 0.050 mg l^{-1} for 5-fluorouracil and N1(2'-furanidyl)uracil, respectively. The method is simple and rapid and does not require any preliminary treatment of the sample. The method was validated.

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1. Introduction

Tegafur [4-fluoro-1-(2-tetrahydrofuryl)-2,4(1H,3H)-pyrimidinedione] is a prodrug of 5fluorouracil (5-FU) and is converted into 5-FU by cytochrome P450 enzymes [1,2]. The use of tegafur in cancer treatment is due to its lower toxicity than 5-FU [3]. The quality control of tegafur raw materials requires the determination of 5-FU and N1(2'-furanidyl)uracil (NFU) as major impurities.

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For the assay of the tegafur thin layer chromatography [4], gas liquid chromatography [5,6], HPLC [7] and spectrophotometry [8] have been reported. The pharmacopeial method for the assay of tegafur requires a titrimetric method that involves a redox titration with sodium thiosulphate [9]. In order to determine the tegafur and its major metabolites in biological fluids HPLC, GLC-mass spectrometry, GC-MS and ¹⁹F magnetic resonance spectroscopy methods were used [10–16]. Even if the chromatographic methods are sensitive and selective they are expensive and time consuming.

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This paper reports a rapid, sensitive and highly selective UV derivative spectrometric method used for the simultaneous determination of 5-FU and NFU as major impurities in tegafur in the presence of each other as well as of the excipients. A critical research of the literature has revealed that no spectrometric and chromatographic method is reported for this purpose. It is for this reason that the derivative spectrometric method proposed is absolutely novel and very useful in quality control of tegafur. This method can be used for the control of tegafur during the stability studies.

2. Experimental

2.1. Apparatus

All the measurements were performed on a Unicam V 500 spectrometer coupled with a Hewlett–Packard PC computer, running the VI-SION spectrophotometric software supplied by Unicam. The spectrometer was validated. The measurements have been made in quartz cells of 1 cm pathlength and the optimal condition for recording the spectra were: wavelength range, 200–400 nm; scan speed, 100 nm min⁻¹; slit width, 2 nm; wavelength interval 1 nm; smooth, 5. For the ruggedness studies an UV–Vis spectrometer JASCO V 530 was used. The ORIGIN program (Micro Cal Inc., version 4.10.) was employed for the linear regression analysis.

2.2. Chemicals and reagents

All chemicals and solvents used were commercially available and were of analytical grade or better (obtained from Merck or Aldrich). The water used was double distilled. Tegafur (>99.9), 5-FU and NFU were obtained from Grindex. Stock solutions of tegafur ($2.50 \times 10^{-3} \text{ mol } 1^{-1}$), 5-FU ($4.00 \times 10^{-3} \text{ mol } 1^{-1}$) and NFU ($2.80 \times 10^{-3} \text{ mol } 1^{-1}$) were prepared by dissolving the appropriate quantity of each substance in water. Working solutions in water were prepared by dilution of the stock solutions. A series of working solutions containing a fixed quantity of tegafur and various quantity of 5-FU and NFU, respectively, were prepared.

2.3. Procedure

The UV spectra of the working solutions are recorded in the range 200–400 nm. The first derivatives of the above spectra were obtained by means of the software VISION. The concentration of 5FU and NFU were found to be proportional to the amplitude of the first derivative spectrum at 298 and 288 nm, respectively.

2.3.1. Linearity and range

Aliquots of 1 ml of the working solution of each compound were placed in a 25 ml volumetric flasks and the flasks were filled up with water. For the calibration graph successive dilutions were performed using 25 or 10 ml volumetric flasks. The first-derivative spectrum of each solution was recorded against a blank consisting of water.

2.3.2. Precision

Six spectra of different concentration of each compound were recorded on the same day and the values of R.S.D. were calculated to determine the intra-day precision. The same procedure was also performed on different days and the inter-days precision was determined.

2.3.3. Accuracy

Accuracy was evaluated by fortifying a mixture containing the analysed compounds with known concentrations of the tegafur. The recovery of each compound was calculated.

2.3.4. Ruggedness

The ruggedness was established through the spectrometric studies by different analysts on the same apparatus. A study was also performed on a different spectrometer on a different day.

3. Results and discussion

The conversion of tegafur in its principal impurities was accompanied by UV spectral changes and the reaction can be monitored by



Fig. 1. The absorption spectra of, tegafur;, 5FU and -, NFU.

identification and determination of 5FU and NFU the main products of degradation.

3.1. Spectrometric measurements

The method elaborated is based on the zerocrossing first derivative spectrometry. The absorption spectra of tegafur, 5FU and NFU are shown in Fig. 1. The spectra of the three compounds closely overlap; hence we circumvented the problem by making use of the first derivative spectra of 5FU and NFU. In Fig. 2 the first derivative spectra of 5FU and tegafur are presented. As is shown the zero-crossing wavelength at 298 nm was selected as the optimum working wavelength for determination of 5FU and the presence of tegafur. In Fig. 3 the first derivative spectra of NFU and tegafur are presented. As it is shown the zerocrossing wavelength at 288 nm was selected as the optimum working wavelength for determination of NFU in the presence of tegafur.

The achievement of reliable results is critical in the pharmaceutical field. In that sense the method proposed was validated [17]. The detection limit was calculated statistically [18].

3.2. Validation of the method

3.2.1. Linearity and range The first derivative spectra of a series of solution

containing a constant concentration of tegafur and various concentration of 5FU and NFU, respectively, were recorded (Figs. 3–5). The calibration graphs were achieved by plotting the values of the amplitude of the first derivative spectrum against the concentrations. The measurements were made at 298 and 288 nm for 5FU and NFU, respectively.

The equation of linear regression obtained for different concentration up to 24.75 mg 1^{-1} of 5FU is: $I_d = -8.90 \times 10^{-21} - 0.01C$, in which I_d is the intensity of the first derivative spectrum and *C* the concentration of 5FU in mg 1^{-1} . The correlation coefficient is 0.9998. The equation of linear regression obtained for different concentration up to 20.20 mg 1^{-1} of NFU is: $I_d = -2.42 \times 10^{-5} + 0.24C$ and the correlation coefficient is 0.9999.

The intercept is very small and the correlation coefficient close to unity. The values obtained show a good linearity and the fit of Beer's law. The



Fig. 2. The first derivative spectra of 5FU and tegafur.

Student's *t* distribution was calculated. The value calculated for *t* was 2.18 in the case of 5FU and 2.17 for NFU. These values do not exceed the tabulated data of 2.77 for a probability of 95% means that the intercept of regression line is not significantly different from zero hence the method is free from the procedural errors. Detection limit

(LOD) at a P = 0.05 level of significance, calculated statistically is 0.40 mg l⁻¹ for 5FU and 0.050 mg l⁻¹ for NFU.

3.2.2. Precision

The R.S.D. values for intra-day precision are 0.53% for tegafur, 0.94% for 5FU and 1.28% for



Fig. 3. The first derivative spectra of NFU and tegafur.



Fig. 4. The first derivative spectra of a series of solutions containing a constant concentration of tegafur and various concentration of 5FU.

NFU and for the inter-day precision are 0.87% for tegafur, 1.41% for 5FU and 1.62% for NFU. These results confirm that the method is precise.

3.2.3. Accuracy

In order to verify the accuracy and precision six replicate determinations were performed on each of five solid mixtures containing tegafur (100 mg),



Fig. 5. The first derivative spectra of a series of solutions containing a constant concentration of tegafur and various concentration of NFU.

Table 1 Determination of tegafur, 5FU and NFU in synthetic samples

	Quantity (mg)												
	Tegafur			5FU			NFU						
	Actual	Determined \pm S.D.; R.S.D.% ($n = 5$)	Recovery (%)	Actual	Determined \pm S.D.; R.S.D.% ($n = 5$)	Recovery (%)	Actual	Determined \pm S.D.; R.S.D.% ($n = 5$)	Recovery (%)				
Sample 1	100.0	99.98±0.10; 0.48	99.98	1.0	0.99±0.02; 1.66	99.00	1.0	0.99±0.02; 1.89	99.00				
Sample 2	80.0	$80.12 \pm 0.25; 0.61$	100.15	1.0	$1.02 \pm 0.02; 1.57$	102.00	1.0	$1.00 \pm 0.03; 1.96$	100.00				
Sample 3	120.0	119.48±1.28; 0.53	99.56	1.0	1.01 ± 0.03 ; 1.68	101.00	1.0	0.98±0.01; 1.42	98.00				

Table 2 Determination of tegafur, 5FU and NFU in synthetic samples by three different methods

	Quantity (mg)							
	Tegafur		5FU		NFU			
	Actual	Determined \pm S.D.; R.S.D.% ($n = 3$)	Actual	Determined \pm S.D.; R.S.D.% ($n = 3$)	Actual	Determined \pm S.D.; R.S.D.% ($n = 3$)		
Proposed method HPLC [19] Official method [9]	100.0 100.0 100.0	$\begin{array}{c} 99.97 \pm 0.10; \ 0.36 \\ 100.01. \pm 0.02; \ 1.01 \\ 101.75 \pm 3.28; \ 0.53 \end{array}$	1.0 1.0 1.0	$\begin{array}{c} 0.99 \pm 0.01; \ 1.54 \\ 0.99 \pm 0.01; \ 1.19 \\ - \end{array}$	1.0 1.0 1.0	$\begin{array}{c} 0.99 \pm 0.03; \ 1.64 \\ 1.02 \pm 0.01; \ 1.72 \\ -\end{array}$		

5FU (1 mg) and NFU (1 mg). The results obtained are presented in Table 1. Percentage recovery was calculated. As shown excellent recoveries were obtained.

3.2.4. Selectivity

In order to check the selectivity of the method the results of the tegafur, 5FU and NFU spectrometric assay of different samples containing all three compounds and the excipients commonly used for oral dosage forms (lactose, starch, magnesium stearate) were compared with that obtained from individual samples of each compounds and excipients. With regard to the selectivity the samples containing excipients did not have a quantifiable absorbance at the working wavelengths.

3.2.5. Stability of the solutions

The stock solutions of the tegafur, 5FU and NFU were stored in light at room temperature. The appropriate amount of solution was sampled and analysed three times during 4 weeks. The results obtained by the proposed method were compared with those obtained using a HPLC method. No degradation products were observed during this time. Reproducible results were obtained in the temperature range 25–40 °C. The increase in temperature up to 60 °C did not affect the results.

3.2.6. Ruggedness

Ruggedness was performed to confirm that the assay of tegafur, 5FU and NFU, respectively, was satisfactory under condition external to the method. Good results were obtained during this study confirming that the method remained selective and precise for all components under tested conditions.

3.3. Application

A synthetic sample containing 100 mg of tegafur, 1 mg of NFU and 1 mg of 5FU has been analysed by three different methods. The results are shown in Table 2.

The method was used to determine the possible degradation of finished product that contain

tegafur during dissolution tests. No degradation products were observed and the results obtained have been confirmed by the HPLC method used for the assay of 5FU [19].

4. Conclusion

The official monograph for tegafur involves a titrimetric procedure for the assay and does not have any procedure for determination of related impurities. Until now, only HPLC methods are suitable for this purpose but are expensive and time consuming. The results obtained demonstrated that the zero-crossing first derivative method proposed could be used for determination of 5FU and NFU in the presence of tegafur. The proposed method is free from procedural errors, in particular those depending on the simultaneous presence of three compounds. The method does not require any preliminary treatment of the sample and is simple, precise and selective with respect to the excipients usually used for oral dosage forms. The proposed first derivative UV-Vis spectrometric method is suitable for rapid and reliable quality control of drugs containing tegafur as active substance. This method needs inexpensive apparatus and reagents.

References

- T. Komatsu, H. Yamazaki, N. Shimada, S. Nagayama, Y. Kawaguchi, M. Nakajima, T. Yokoi, Clin. Cancer Res. 7 (2001) 675–681.
- [2] T. Komatsu, H. Yamazaki, N. Shimada, M. Nakajima, T. Yokoi, Drug Metabol. Dispos. 28 (2000) 1457–1463.
- [3] H. Tomankova, I. Zyka, Microchem. J. 22 (1977) 70-74.
- [4] N.G. Blokhina, E.K. Vozny, A.M. Garin, Cancer 30 (1972) 390–396.
- [5] E.B. Hills, V.C. Godefroi, I.A. O'Leavry, M. Burke, D. Andrzejewski, W. Brukwinski, J.P. Horwitz, J. Pharm. Sci. 66 (1977) 1447–1451.
- [6] C. Pantarotto, R. Fanelli, S. Filippeschi, T. Facchinetti, F. Spreafico, M. Salmona, Anal. Biochem. 97 (1979) 232– 238.
- [7] N. Hobara, A. Watanabe, J. Chromatogr. Biomed. Appl. 3 (1979) 518–524.
- [8] J.L. Fabregas, J.E. Beneyto, Anal. Lett. 14 (1981) 119– 128.
- [9] Japan Pharmacopoeia, XIII, 654.

- [10] J.A. Benvenuto, K. Lu, T.L. Loo, J. Chromatogr. 134 (1977) 219–222.
- [11] S. Yoshida, T. Adachi, S. Hirose, Microchem. J. 39 (1989) 351–360.
- [12] S. Yoshida, K. Urakami, M. Kito, S. Takeshima, S. Hirose, J. Chromatogr. 530 (1990) 57–67.
- [13] V.R. Jarugula, F.D. Boudinot, J. Chromatogr. B Biomed. Appl. 677 (1996) 199–203.
- [14] M. Harada, H. Nishitani, K. Koga, I. Miura, Y. Umeno, Jpn. J. Cancer Res. 83 (1992) 387–391.
- [15] E. Matsushima, K. Yoshida, R. Kitamura, K. Yoshida, J. Chromatogr. B Biomed. Appl. 691 (1997) 95–104.
- [16] T. Marunaka, Y. Umeno, K. Yoshida, M. Nagamachi, Y. Minami, S. Fujii, J. Pharm. Sci. I 69 (1980) 1296–1300.
- [17] European Pharmacopoeia, third ed., 1997, pp. 1388-1389.
- [18] S. Okada, H. Nakahara, Chem. Pharm. Bull. 35 (1987) 2495–2503.
- [19] The United States Pharmacopoeia, XXIV, 5-Fluorouracil Monograph.