Research Article

SIMULTANEOUS DETERMINATION OF DIDANOSINE AND NICOTINAMIDE IN BINARY MIXTURE BY FIRST DERIVATIVE UV SPECTROPHOTOMETRY

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ABSTRACT

A simple, rapid, precise, accurate and cost effective first derivative UV spectrophotometric method was developed for the determination of didanosine (DDI) and nicotinamide (NCT) in binary mixture without using any chemical separation step. The wavelengths at 260.8 nm for DDI and at 223.2 nm for NCT were the optimum working wavelengths for their determination without mutual interference. Linearity was obtained in the concentration range of 6-16 µg/mL for both drugs. Limit of detection and limit of quantitation of DDI at 260.8 nm were 0.33 and 1.01 µg/mL, respectively, while limit of detection and limit of quantitation of NCT at 223.2 nm were 0.48 and 1.44 µg/mL, respectively. The proposed procedure was successfully applied to the simultaneous determination of DDI and NCT in binary mixture with a good precision and accuracy.

Keywords: First derivative UV spectrophotometric, didanosine, nicotinamide.

INTRODUCTION

Didanosine (DDI), a synthetic analogue of dideoxypurine nucleoside inosine has been reported to inhibit the replication of human immunodeficiency virus (Fig. 1a). DDI act as competitive inhibitors of reverse transcriptase. The drug is approved by the U.S. Food and Drug Administration for the treatment of adults and children over age above 6 months.[1] However, there is a relevant issue related to the chemical stability of DDI. DDI hydrolyzed at the stomach pH, its low oral bioavailability and very short biological half-life.[2] In the solid state, DDI stable for at least 24months at 30°C[3] and degraded to hypoxanthine after heating at 200°C.[4]
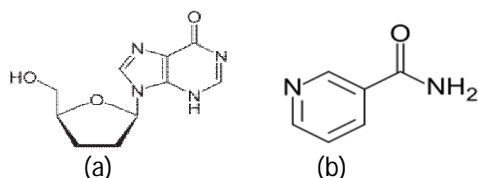

Figure   1: The structural formula of (a) DDI and (b) NCT

In the last years, the formation of pharmaceutical cocrystal has gained an increased interest as a means of optimizing the physicochemical properties of solid dosage forms, namely solubility, dissolution rate, hygroscopicity, and chemical stability.[5] Nicotinamide (NCT, Fig. 1b) is often used as a cocrystal former, because it can improve the physicochemical properties of drugs.[6-7] Research on the formation of binary cocrystal between DDI and NCT is currently being developed.

Various methods have been used for the determination of didanosine either alone or in combination including spectrophotometry[8] and HPLC[9-10]. The DDI-NCT binary mixture is not yet official in any pharmacopoeia. To my

knowledge, no analytical methods could be traced for the analysis of DDI-NCT combination in pharmaceutical dosage form. Therefore, a simple, rapid and reliable method for simultaneous determination of both drugs in mixture seemed to be necessary. Derivative spectrophotometry is a useful mean of resolving two overlapping spectra and eliminating matrix interference in the assay of two-component mixtures using the zero-crossing technique.[11-12]

The aim of this study is to develop a simple, rapid, precise, accurate, and cost effective analytical method for the simultaneous determination of DDI and NCT by first derivative UV spectrophotometric in binary mixture without the need to prior separation. The method had sufficiently good accuracy, precision and permitted a simple, time and money-saving assay of these compounds in mixtures. This method was tested and validated for various parameters according to ICH guidelines.

MATERIALS AND METHODS

Instrumentation

The Shimadzu 1601 PC double beam UV–VIS spectrophotometer with 1 cm quartz cuvettes, a fixed slit width (2 nm) connected to a PC computer loaded with Shimadzu UVPC software.

Standard and Chemical

All used chemicals were of analytical reagent grade, unless otherwise specified. Didanosine and nicotinamide was obtained from PT. Kimia Farma, tbk, Indonesia.

Preparation of standard solutions and calibration

Stock standard solutions of DDI and NCT were prepared separately by dissolving 50.0 mg of each drug in 50.0 ml of water. The standard solutions were prepared individually by dilution of the stock solutions with water for spectrophotometric experiments to reach concentration range of 6-16 µg/mL for both components.

Determination of zero-crossing DDI and NCT

Standard series of DDI and NCT in the linear concentration range of 6-16 µg/mL were obtained from the above stock solutions. A spectra of each solution was recorded between 210 and 290 nm. The absorption data was transformed into first derivative spectra with interval of Δλ=8 nm and factor=10 by using Shimadzu UVPC software. Zero-crossing wavelength of DDI and NCT were obtained from first derivative spectra of each component. The first derivative signals (dA/dλ) of DDI were measured at zero-crossing wavelength of NCT, otherwise the dA/dλ of NCT were measured at zero-crossing wavelength of DDI.

Interference test

Solution containing mixture of DDI and NCT with ratio of 10:6, 10:8, 10:10, 10:12, 10:14, and 10:16 µg/mL were measured at zero-crossing wavelength of NCT, while solution containing a mixture of NCT and DDI with same ratio were measured at zero-crossing wavelength of DDI.

Determination of linearity, LOD, and LOQ

Standard calibration graphs for DDI and NCT were constructed by plotting the dA/dλ values versus concentrations. Limit of detection (LOD) and limit of quantization (LOQ) of both components were calculated based on residual standard deviation of the response and the slope.

Determination of precision

To measure repeatability (intra-day) and intermediate precision (inter-days) of method, one concentration of each component was analyzed six times in the same day and in the two different days.

Determination accuracy

Preparation of a series synthetic binary mixture solution between DDI and NCTwas performed to determine the accuracy of the method. Solution containing mixture of DDI and NCT with nine ratio in the range 80-120% of components, namely 8:4, 8:6, 8:10, 10:4, 10:6, 10:8, 12:4, 12:6, and 12:8 µg/mL were measured at zero-crossing wavelength of DDI and NCT.

RESULTS AND DISCUSSION

Zero-crossing of DDI and NCT

As we know, the simultaneous analysis of combined mixtures is a very important task for analytical purpose. The absorption spectra of DDI and NCT overlap in the spectral region of 210-290 nm as shown in figure 2. So,

this indicates that small peaks of NCT exist against the higher peaks of DDI in the same spectral region. This is an analytical problem for the analysis of DDI and NCT. For these reasons, the simultaneous spectral analysis of binary mixtures of the two drugs is not possible by direct measurement of absorbance values, as well as, classical analytical approaches. Therefore, first derivative spectrophotometric was developed for the simultaneous determination of the above-mentioned compounds.
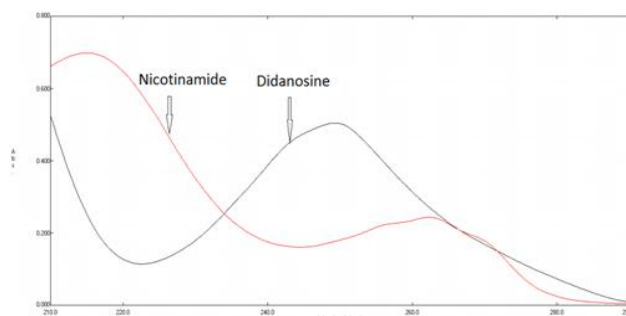

Figure 2: Zero order spectra of didanosine 10 µg/mL and nicotinamide10 µg/mL in water

Determination zero-crossing of each component was carried out by transform of absorption spectra of both components to first derivative spectra by using Shimadzu UVPC software. In figure 3, the first derivative spectra of each pure drug components showed zero-crossing points. Determination zero crossing is used to determine every component, because value of first derivative signal (dA/dλ) of every component at zero crossing is zero (not value). Both of them have two wavelengths zero-crossing. Zero-crossing of NCT were obtained at wavelength 244.8 nm and 260.8 nm, whereas zero-crossing of DDI were obtained at wavelength 223.2 nm and 248.3 nm. The values of dA/dλ at zero-crossing of NCT (244.8 nm and 260.8 nm) were measured for the determination of DDI, while values of dA/dλ at zero-crossing of DDI (223.2 nm and 248.3 nm) were measured for the determination of NCT.
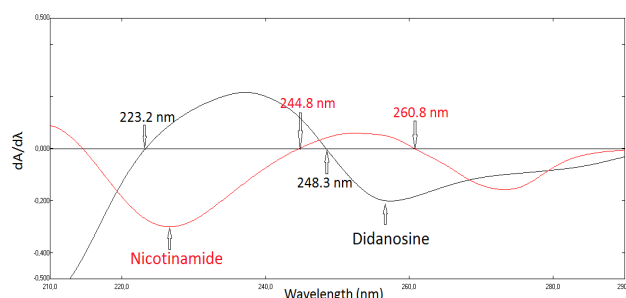

Figure 3: First derivative spectra of didanosine10 µg/mL and nicotinamide10 µg/mL in water

Interference Test

The selection of the optimum wavelength is based on the fact that the absolute value of the total derivative spectrum at the selected wavelength has the best linear response to the analyte concentration. It is not affected by the concentration of any other component and gives a near-zero intercept on the ordinate axis of the calibration

curve. Interference test of NCT against DDI performed to determine the range of levels of NCT had no effect on DDI at a wavelength "zero crossing" of NCT and vice versa. In Fig. 4a, at a fixed concentration of DDI 10 µg/mL, the concentration of NCT up to 16 mg/mL did not affect the value of dA/dλ of DDI and at a wavelength of 260.8 nm, but affect the change of dA/dλ values at 244.8 nm. In Figure 4b, at a fixed concentration of NCT 10 µg/mL, the concentration of DDI up to 16 mg/mL did not affect the value of dA/dλ of NCT at wavelength of 223.2 nm, but affect the change of dA/dλ values at 248.3 nm. The wavelength at 260.8 nm for DDI and at 223.2 nm for NCT was the optimum working wavelengths for their determination. Therefore, at these selected wavelengths both drugs can be quantified without mutual interference in the presence of each other.
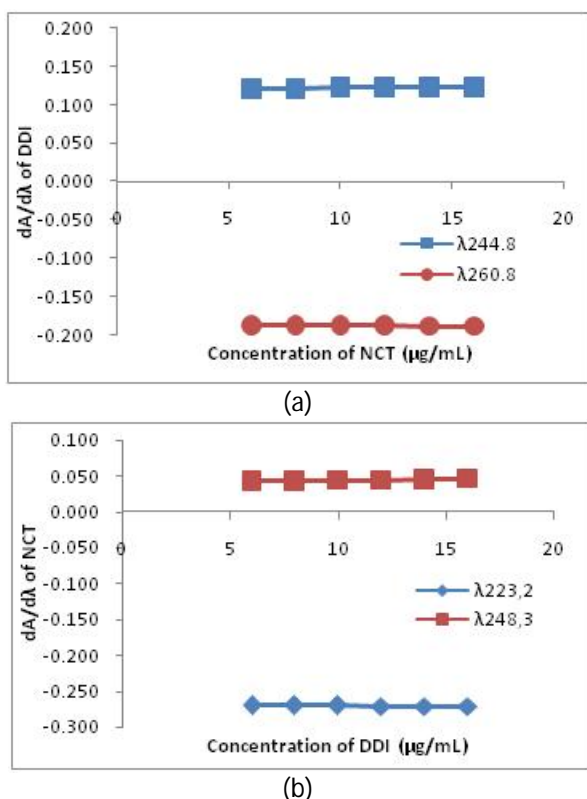

(b)

Figure 4: Graphs of (a) interference of NCT to dA/dλ of DDI, (b) interference of DDI to dA/dλ of NCT

Linearity, LOD, and LOQ

Linear relationships between dA/dλ and drug concentration were obtained over the concentration range of 6-16 µg/mL for both components (Fig.5). The correlation coefficients of DDI at 260.8 nm and NCT at 223.2 nm were 0.9997 and 0.9994 indicating good linearity. The linear regression equations together with correlation coefficients, limit of detection (LOD), and limit of quantitation (LOQ) obtained for each drug component are shown in table 1.

Precision

Repeatability and intermediate precision were characterized by percentage of relative standard deviation (RSD) of difference between theoretical and

measured concentrations. RSD values are eligible if less than 2%. Repeatability and intermediate precision data were shown in Table 2-3. Percentages of RSD for intra-day of DDI at 260.8 nm and NCT at 223.2 nm were 0.66 and 0.90%, respectively. Whereas percentages of RSD for inter-days at the same wavelenths were 0.57 and 0.95%, respectively. These results indicate good precision for both drugs.

Accuracy

Accuray was characterized by mean recovery. Mean recovery in the range 98-102% showed good accuracy. Accuracy data was shown in Table 4. Mean recoveries of DDI at 260.8 nm and NCT at 223.2 nm were 100.81±1.03 and 100.55 ±1.13%, respectively. These result indicated a good accuracy of the method for determination of both drugs.
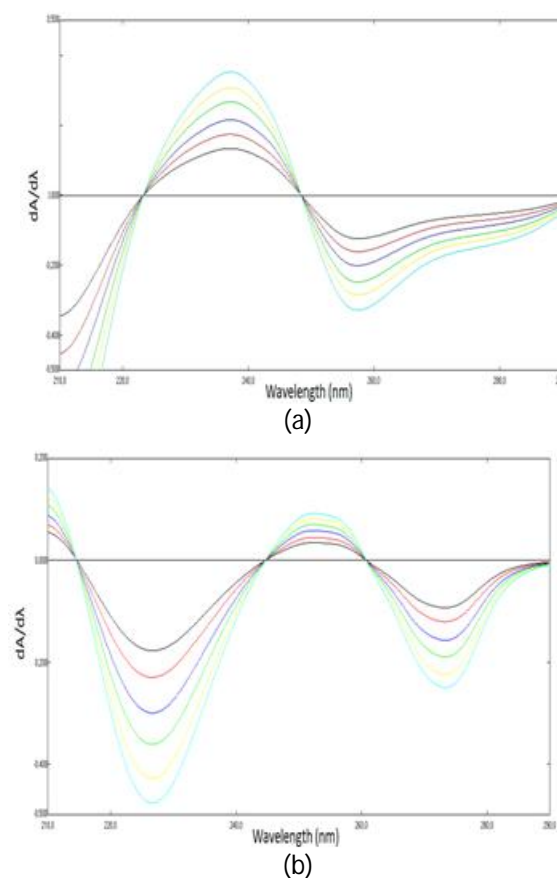

(a)

(b)

Figure 5: First derivative spectra of various concentration in linearity range of (a) DDI and (b) NCT

Table 1: Summary data of calibration curve of the DDI and NCT

Parameter	DDI λ260.8 nm	NCT λ223.2 nm
Range (µg/mL)	6-16	6-16
Calibration equation	$y= -0.0188x+0.0001$	$y=-0.0271x+0.001$
Slope	-0.0188	-0.0271
Intercept	0.0001	0.001
% RSD of slope	0.53	0.74
Correlation Coefficient (r)	0.9997	0.9994
LOD (µg/mL)	0.33	0.48
LOQ (µg/mL)	1.01	1.44

Tabel 2: Repeatability of method

	% found	
	Didanosine λ260.8 nm	**Nicotinamide** λ223.2 nm
	100.2	102.0
	99.4	101.4
	99.6	101.2
	101.2	101.8
	100.6	100.4
	100.2	99.6
Mean	100.20	101.07
SD	0.66	0.91
RSD (%)	0.66	0.90

Tabel 3: Intermediate precision of method

Day	% found	
	Didanosine λ260.8 nm	**Nicotinamide** λ223.2 nm
1	100.2	102.0
	99.4	101.4
	99.6	101.2
	101.2	101.8
	100.6	100.4
	100.2	99.6
2	99.2	101.6
	99.6	101.0
	99.6	100.4
	100.2	100.2
	99.8	99.4
	99.6	99.2
Mean	99.93	100.68
SD	0.57	0.96
RSD (%)	0.57	0.95

Tabel 4: Accuracy of method

DDI		NCT	
Added (µg/mL)	**% Found** λ260.8 nm	**Added (µg/mL)**	**% Found** λ223.2 nm
8.0	101.13	4.0	99.63
8.0	99.14	6.0	99.02
8.0	102.46	8.0	102.40
10.0	101.12	4.0	100.55
10.0	100.05	6.0	99.63
10.0	100.05	8.0	100.09
12.0	101.11	4.0	100.55
12.0	101.99	6.0	102.09
12.0	100.22	8.0	101.01
Mean Recovery (%)	**100.81**		**100.55**
RSD (%)	**1.03**		**1.13**

CONCLUSION

First derivative spectrophotometry method is a powerful signal processing technique for the overlapping peak resolution and for the significant peak identification. This tool was successfully applied to the spectrophotometric multicomponent analysis of didanosine and nicotinamide in binary mixture. Although the individual spectra of didanosine and nicotinamide overlap in the region of 210-290 nm, the first derivative spectrophotometry method gave successful results for the quantitative resolution of the binary mixtures consisting of two components. Zero-crossing NCT was obtained at wavelength 244.8 nm and 260.8 nm. Whereas zero-crossing DDI was obtained at wavelength 223.2 nm and 248.3 nm. The wavelength at 260.8 nm for DDI and at 223.2 nm for NCT was the optimum working wavelengths for their determination without mutual interference.

REFERENCES

1. AHFS Drug Information, 'Didanosine', American Hospital Formulary Service, American Society of Hospital Pharmacist, Bethesda, 1995, pp. 416–426.

2. Aungst, B.J., P-glycoprotein, secretory transport, and other barriers to the oral delivery of anti-HIV drugs, Adv. Drug. Deliv.Rev., 35,1999, pp. 105-116.

3. Nassar, M.N., Chen, T., Reff, M.J., Agharkar, S.N., Didanosine, In Analytical Profiles Drug Substances and Excipients, Vol 22, Elsevier, San Diego, 1991, pp.185-227.

4. Alatas, F., Soewandhi, S.N. Sasongko, L, and Ismunandar, Heating effect on the solid characteristics and solubility of anti-HIV didanosine, Int J Pharm Sci Rev Res, 13(1), 2012, pp. 9-12.

5. Almarsson, M. Zaworotko, Crystal engineering of the composition of pharmaceutical phases. Do pharmaceutical co-crystals represent a new path to improved medicines? Chem. Comm., issue 17,2004, pp.1889–1896.

6. Fábián, L., Hamill, N. Eccles, K.S., Moynihan, H.A., Maguire, A.R., McCausland, L., and Lawrence, S.E., Cocrystals of Fenamic Acids with Nicotinamide, Cryst. Growth Des., 11(8),2011,pp 3522–3528.

7. Lu, J. and S. Rohani, Preparation and Characterization of Theophylline-Nicotinamide Cocrystal, Org. Proc. Res. Dev., 13(6),2009,pp 1269-1275.

8. Sangshetti, J.N. Kulkarni, P.A and Shinde, D.B., Spectrophotometric Determination of Didanosine in Bulk and Tablet Formulation, Trends in Applied Sciences Research, 2(1), 2007, pp. 71-75

9. de Oliveira A.M., Löwen T.C., Cabral L.M., dos Santos E.M., Rodrigues C.R., Castro H.C., dos Santos T.C., Development and validation of a HPLC-UV method for the determination in didanosine tablets, J Pharm Biomed Anal., 38(4),2005, pp. 751-756.

10. Rebiere H, Mazel B, Civade C, Bonnet PA , Determination of 19 antiretroviral agents in pharmaceuticals or suspected products with two methods using high-performance liquid chromatography, J Chromatogr B 850(1-2,), 2007,pp. 376-83.

11. J.J. Berzas, J. Rodriguez, G. Castaneda, Simultaneous determination of ethinylestradiol and levonorgestrel in oral contraceptives by derivative spectrophotometry, Analyst, 122(1), 1997, pp. 41-44.

12. Hacioglu F, Onal A. Determination of Eprosartan Mesylate and Hydrochlorothiazide in Tablets by Derivative Spectrophotometric and High-Performance Liquid Chromatographic Methods, J Chromatogr Sci., 48(9), 2010, pp.704-713.

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