FIRST- AND THIRD-DERIVATIVE SPECTROPHOTOMETRY FOR SIMULTANEOUS DETERMINATION OF DEXAMETHASONE AND CYTARABINE IN PHARMACEUTICAL FORMULATIONS AND BIOLOGICAL FLUIDS

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The derivative spectrophotometric method was established for simultaneous determination of dexamethasone and cytarabine (cytosine arabinoside). Measurements were made in the zero-crossing wavelengths at 268.0 nm (first derivative) and 264.0 nm (third derivative) for determining dexamethasone and cytarabine, respectively. The calibration graphs were linear in the concentration ranges 0.10 to 10 μg/mL of dexamethasone and 0.25 to 50.0 μg/mL of cytarabine. The limits of detection 0.08 and 0.10 μg/mL and relative standard deviations 3.0 and 1.0% were obtained for dexamethasone and cytarabine, respectively. The possible interfering effect of other substances was also studied to investigate selectivity of the developed method. The proposed method was applied satisfactorily for the simultaneous determination of both drugs in the pharmaceutical formulation and biological fluid samples.

Keywords: *dexamethasone, cytarabine, derivative spectrophotometry, pharmaceutical formulation, biological fluid*.

ОДНОВРЕМЕННОЕ ОПРЕДЕЛЕНИЕ ДЕКСАМЕТАЗОНА И ЦИТАРАБИНА В ФАРМАЦЕВТИЧЕСКИХ СОСТАВАХ И БИОЛОГИЧЕСКИХ ЖИДКОСТЯХ С ПОМОЩЬЮ СПЕКТРОФОТОМЕТРИИ ПЕРВОЙ И ТРЕТЬЕЙ ПРОИЗВОДНЫХ

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Предложен деривационный спектрофотометрический метод для одновременного определения содержания дексаметазона и цитарабина (цитозин-арабинозида). Проведены измерения на длинах волн пересечения нуля 268.0 нм (первая производная) и 264.0 нм (третья производная) для определения дексаметазона и цитарабина. Калибровочные кривые линейны в диапазонах концентраций 0.10–10.00 мкг/мл дексаметазона и 0.25–50.00 мкг/мл цитарабина. Для дексаметазона и цитарабина пределы обнаружения 0.08 и 0.10 мкг/мл, относительные стандартные отклонения 3.0 и 1.0%. Для исследования селективности разработанного метода изучено возможное влияние других веществ. Предлагаемый метод успешно применен для одновременного определения обоих веществ как в лекарственных средствах, так и в образцах биологической жидкости.

Ключевые слова: *дексаметазон, цитарабин, деривационная спектрофотометрия, лекарственное средство, биологическая жидкость*.

Introduction. Dexamethasone was first synthesized in 1957 and it is on the World Health Organization's List of Essential Medicines. Dexamethasone is a representative of synthetic steroids, characterized by a huge anti-inflammatory and immunosuppressive effect. Due to its antiproliferative effect, it was primarily used to treat cancer, leukemia, rheumatic and skin diseases, cerebral edema, multiple sclerosis, and asthma [1–5].

Cytarabine, also known as cytosine arabinoside, is a chemotherapy drug used to treat acute myeloid leukemia, acute lymphocytic leukemia, chronic myelogenous leukemia, and non-Hodgkin's lymphoma. It is an antimetabolite that selectively inhibits DNA synthesis. It is injected into a vein, under the skin, or into the cerebrospinal fluid. Common side effects include bone marrow suppression, vomiting, diarrhea, liver problems, rash, ulcer formation in the mouth, and bleeding. Other serious side effects include loss of consciousness, lung disease, and allergic reactions. Use during pregnancy may harm the baby [6–10]. Dexamethasone and cytarabine are available in a single dosage form. They are combined in fixed doses and used for the treatment of cancer patients. Because of the wide range of the drug's therapeutic usage, the development of methods for the simultaneous determination is required.

The methods available for the determination of dexamethasone are reported in [11–15]. Some analytical methods for the quantifying cytarabine are also described in [16–23]. The derivative spectrophotometric method has proven to be particularly useful for signal processing in resolving overlapping spectra and eliminating matrix interferences. The zero-crossing technique was successfully applied in derivative spectrophotometry for the quantitative determination of analytes in various mixtures. This approach allows one to resolve and determine binary mixtures of analytes by recording their derivative spectra at wavelengths where one of the components exhibits no signal. One can determine the absolute value of the derivative of the sum graph at an abscissa value (wavelength) corresponding to the zero-crossing of one of the components in the mixture. Therefore, zero-crossing measurements for each component of the mixture are the only function of the amount of the others. Many applications of derivative spectrophotometry for the simultaneous determination of different analytes have been published in the literature [24–28]. Since the method has advantages such as simplicity, easy operation, and low cost, it has been widely used in most laboratories to quantify various analytes.

To the best of our knowledge, no derivative spectrophotometric method has been reported for the simultaneous determination of dexamethasone and cytarabine. Therefore, it would be beneficial to develop a new method for this purpose. The present study aims to provide a simple and rapid analytical derivative spectrophotometric procedure, which can be used for the simultaneous determination of dexamethasone and cytarabine in pharmaceutical and biological samples.

Experimental. A double-beam UV-Vis spectrophotometer (Shimadzu 160-A, Japan) with 10 mm matched quartz cells was used to record the absorption spectra. It was developed to measure the spectral derivatives optically using wavelength modulation. The derivative spectra (the gradient $dA/d\lambda$ versus wavelength) were produced by processing the signal of the original absorption spectra (spectrophotometer output).The main instrumental parameters that affect the derivative spectra such as the scan speed and the wavelength increment were investigated and adjusted (200 nm/min and $\Delta\lambda$ = 6 nm). A pH meter (ISTEK Inc., South Korea) was applied to measure the acidity of the sample solutions. HCl (0.1 mol/L) and NaOH (0.01 mol/L) were used to adjust the pH. A thermostatic water bath (Heidolph, Germany) was also applied to maintain the temperature of the solutions at the required level. The aqueous stock solutions of the pure drugs in a concentration of 100 μg/mL were prepared freshly for analytical purposes. Aliquots of stock solutions of dexamethasone and cytarabine were simultaneously diluted in deionized water to obtain concentrations in the calibration ranges. All chemicals and solvents were of analytical grade.

The absorption spectra of the two compounds under investigation were obtained within 200–400 nm, directly against the solvent for standard solutions of dexamethasone and cytarabine. Then, the 1–4 orders of derivative spectra were obtained. By employing the zero-crossing technique, the wavelengths of the first- and third-derivative spectra were found at which no interference of measured quantities was observed in solutions of the drugs. The zero-crossing points of dexamethasone and cytarabine were 264.0 and 268.0 nm in the third and first derivative spectra, respectively. The characteristic of wavelengths for dexamethasone and cytarabine was confirmed by varying the concentration of both drugs. The absorbance in zero points versus the concentration of the standard solutions was plotted to obtain the calibration.

A series of working solutions was obtained by dilution and mixing of the stock solutions of dexamethasone and cytarabine. Blank human urine and serum samples were obtained from healthy volunteers and stored in the refrigerator until analysis. The matrices were spiked with drugs, and then a solid phase extraction technique with C_{18} cartridge (Supelco Inc.,100 mg) and liquid-liquid extraction (chloroform as extracting solvent) were used to extract and purify the drugs from the biological samples. A detailed description of the procedures can be found in [29, 30]. Finally, the concentration of drugs was measured by the developed method based on the zero-crossing approach.

Results and discussion. The original absorption spectra of dexamethasone and cytarabine showed that they overlapped completely, and each compound interfered in the spectrophotometric determination of the other one. Therefore, this technique cannot be used for the mixture analysis. The derivative method was selected and applied because of the spectral characteristics, good selectivity, and sensitivity. The derivative spectra allowed determining simultaneously two drugs. The first- and third-derivative spectra of dexamethasone and cytarabine are shown in Fig. 1. According to Fig. 1, the suitable zero-crossing wavelengths in the first-derivative spectrum of dexamethasone and in the third-derivative spectrum of cytarabine were 268.0 and 264.0 nm, respectively. In the zero-crossing approach, it is necessary that zero-crossing wavelengths do not change by varying the concentration of related compounds. These wavelengths were selected to quantify the two drugs in the presence of each other (without interference).

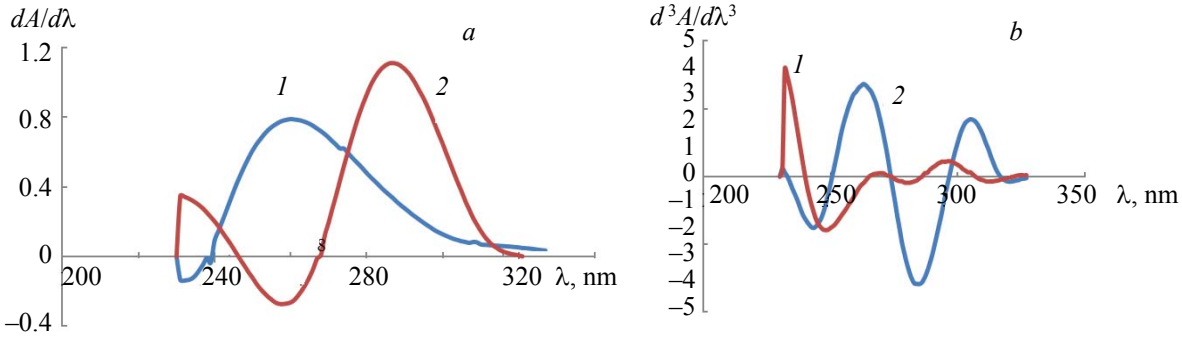

Fig. 1. The first (a) and third (b) derivative spectra of dexamethasone (1) and cytarabine (2).

To assess the optimum conditions, the influence of various parameters on the simultaneous determination of dexamethasone and cytarabine was investigated at 268.0 nm (1-derivative) and 264.0 nm (3-derivative), respectively. One at a time, the optimization procedure was evaluated for obtaining optimum conditions. One of the most important parameters was pH. Experiments in various pHs showed that the spectra of the two drugs were dependent on the solution pH. According to the results (Fig. 2), pH 5 was selected for subsequent studies. Also, optimization of temperature was performed spectrophotometrically. The appropriate temperature varied in the range 10 to 60°C at optimal pH. According to this investigation (data not shown), 25°C (room temperature) was obtained as the optimum temperature.

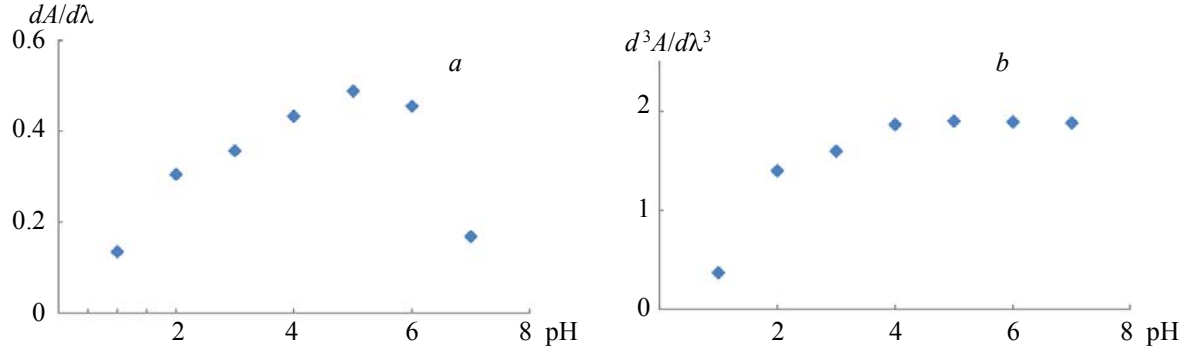

Fig. 2. Effect of pH on the absorbance of (a) dexamethasone at 268.0 nm from the first derivative spectrum and (b) cytarabine at 264.0 nm from the third derivative spectrum.

The developed method was validated for the simultaneous assay determination of dexamethasone and cytarabine using the analytical parameters. Two derivative spectrophotometric calibration graphs were constructed at the zero-crossing wavelengths (268.0 nm of 1-derivative for dexamethasone and 264.0 nm of 3-derivative for cytarabine) for the simultaneous determination of the two drugs. The obtained linear graphs were as follows: $dA/d\lambda = 2.3\times10^{-2} + 62.1C_{Dex}$ for dexamethasone in the concentration range of 0.1–

10 μg/mL with correlation coefficient of 0.982; $d^3A/d\lambda^3 = 4.2\times10^{-2} + 76.6C_{Cyt}$ for cytarabine in the concentration range of 0.25–50 μg/mL with a correlation coefficient of 0.982. The limit of detection (LOD) and the limit of quantification (LOQ) were determined based on the standard deviation of the blank and the slope of the regression equation. The LODs were 0.08 and 0.10 μg/mL and also LOQs were 0.25 and 0.30 μg/mL for dexamethasone and cytarabine, respectively. The precisions were also calculated and expressed as RSDs%, which were found to be 3% for dexamethasone and 1% for cytarabine.

The effect of foreign species on the derivative spectrophotometric determination of dexamethasone and cytarabine was investigated individually under optimum conditions. Solutions containing dexamethasone or cytarabine and various amounts of foreign species were prepared, and the proposed derivative method was followed. According to the Table 1, approximately all investigated species did not interfere. This showed the selectivity of the developed method to detect quantitatively the analytes in the presence of components that may be expected to be present in the sample matrix.

TABLE 1. Effect of Foreign Ions on the Individual Determination Using Derivative Spectrophotometry of Dexamethasone (8.0 μg/mL) and Cytarabine (20 μg/mL)

Foreign species	Tolerance limit for dexamethasone	Tolerance limit for cytarabine
Urea	8000	2000
Sucrose	7500	2000
Glucose	7500	2000
Cl^-	3700	1500
K^+	3500	1000
Na^+	2500	1000
NH_4^+	2500	1000
NO_3^-	<10	

The proposed method has been successfully applied to the simultaneous determination of dexamethasone and cytarabine in dosage forms and biological fluids (serum and urine). According to the Table 2, there is no significant difference between the results obtained by the proposed method with the prepared and spiked values.

TABLE 2. Determination of Dexamethasone and Cytarabine Using the Proposed Method in Different Samples

Sample	Spiked, μg/mL		Found (Recovery, %)	
	Dexamethasone	Cytarabine	Dexamethasone	Cytarabine
Pharmaceutical preparation	2.20	8.33	90	106
	4.40	16.00	93	106
	8.80	33	92	100
Serum	0.68	1.27	97.8	94.7
	0.82	1.63	93.7	108
	1.10	1.90	106	107
Urine	0.84	3.7	97.6	99
	0.93	2.5	91.4	93.3
	0.97	4.4	99	98

Conclusion. This paper demonstrates the potential of derivative spectrophotometry as an analytical technique and also its usefulness for the accurate and precise simultaneous determination of dexamethasone and cytarabine in pharmaceutical preparations and biological fluid samples. Furthermore, the developed method is simple and rapid, which can be suitable for routine analysis in quality control.

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