

Degradation studies and thermodynamic parameters in aqueous solution of chemotherapeutic agents: Daunorubicin, doxorubicin and vincristine

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ABSTRACT: The degradation of daunorubicin (DNR), doxorubicin (DXR) and vincristine (VCR) as chemotherapeutic agents was investigated under stress conditions such as acidic and alkaline media, oxidation, photolytic and heating. The degradation pathway determined by high-performance liquid chromatography (HPLC). Daunorubicin, doxorubicin and vincristine completely disintegrated in the basic medium. It has been determined that these drugs are resistant to heat. Daunorubicin and doxorubicin are resistant to UV light. However, vincristine is not very stable such as the other two compounds against UV light. The kinetic and thermodynamic parameters of daunorubicin, doxorubicin and vincristine were investigated in neutral media with different temperatures by electrochemical technique. Enthalpy, entropy and Gibbs free energy parameters were investigated in pH 7.4 phosphate buffer (PB) solution due to stability of these drugs at this pH. The studied chemotherapeutic agents showed endothermic process on glassy carbon-based electrode surface. Daunorubicin, doxorubicin and vincristine showed strongly adsorption process in room temperature. However, it showed diffusion property from electrode to solution with increasing the temperature values.

KEYWORDS: Degradation; liquid chromatography; drug stability; chemotherapeutic agent; temperature effect.

1. INTRODUCTION

Stress tests have various degradation mechanisms such as photolytic, hydrolytic, oxidative, and heat degradation. To achieve the desired level of degradation, the exposure to oxidizing, acid or base agent concentration and exposure to conditions such as heat, light may be changed [1]. Drug products may deteriorate during manufacture, application and storage, as many of the drugs are sensitive to light [2,3]. The photostability of a drug is its response when exposed to the solar, UV, and visible light. There are many purposes of photostability studies such as internal structural properties of photostability, the physical and chemical changes caused by light effects, formation of photodegradation methods and mechanisms, the shelf life of the products, the light-induced biological effects, the change in formulation parameters, take precautions against negative situations that may occur during production, packaging, labelling, transportation and storage when exposed to light [4]. In this work, the degradation and stabilization of vincristine, daunorubicin and doxorubicin under different conditions were studied using the HPLC method.

Vincristine (Figure 1a), daunorubicin (Figure 1b) and doxorubicin (Figure 1c) are used in the wipe out of some species of cancer. Vincristine, a chemotherapeutic agent, is widely used in the treatment of solid tumours and acute leukaemia [5,6]. VCR has a great effect against Wilms' tumour, various lymphomas, acute leukaemia and rhabdomyosarcoma. The metabolic products and / or degradation products of the VCR can cause toxicity and antitumor [7].

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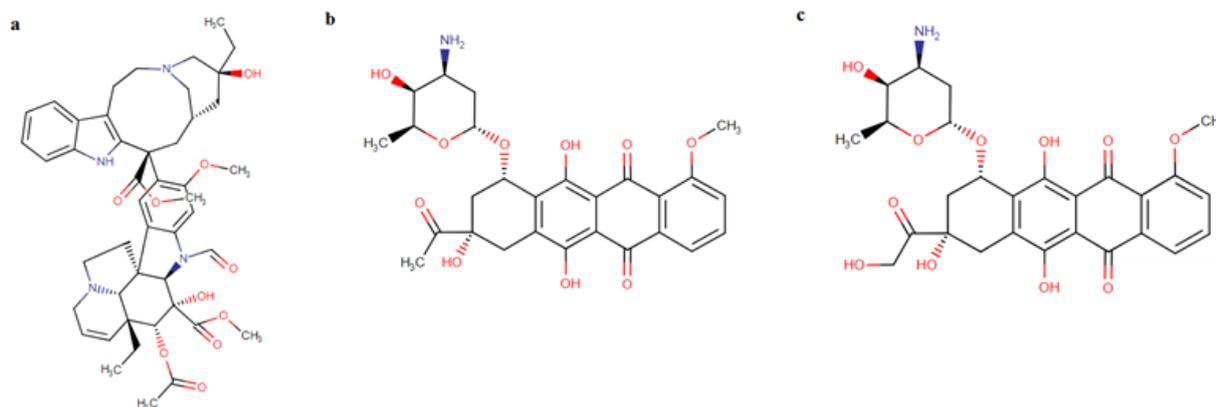


Figure 1. Structures of a) vincristine, b) daunorubicin and c) doxorubicin.

Daunorubicin is a cytotoxic agent used to protect against cancer cells. DNR is used for the treatment of many diseases such as Kaposi's sarcoma, acute lymphocytic leukaemia (ALL), chronic myelogenous leukaemia (CML) and acute myeloid leukaemia (AML) [8]. Doxorubicin, a hydroxylated similar of daunorubicin, is a natural anthracycline derivative. Doxorubicin is used for the treatment of many diseases such as neuroblastoma, Hodgkin's and non-Hodgkin's lymphomas, acute lymphoblastic leukaemia, soft tissue and bone sarcomas, transitional cell bladder carcinoma, breast carcinoma, bronchogenic carcinoma, ovarian carcinoma, gastric carcinoma, and thyroid carcinoma [9-11].

Activation energy (E_a), enthalpy (ΔH), entropy (ΔS) and Gibbs free energy (ΔG) are important parameters may help to explain the drug release from delivery systems. The changes in the thermodynamic parameters show drug release behavior, which are, at least, under the influence of the surrounding environment and the type of system. An enthalpy suggests that all systems present release processes of an endothermic nature ($\Delta H > 0$) or exothermic nature ($\Delta H < 0$) [12]. The systems' entropy is negative ($\Delta S < 0$), which characterizes a decrease in the system disorder, once the drug diffuses in the medium to try to reach an equilibrium between the amount inside and outside of the drug delivery [12]. In other way, entropy is positive ($\Delta S > 0$) which characterizes a increase in the system disorder. The thermodynamic of the reaction process is correlated to the variations in the ΔG , the most important thermodynamic parameter associated with the release kinetics [13]. Furthermore, ΔG reveals the spontaneity of the process when a negative value is calculated for this parameter. The positive ΔG ($\Delta G > 0$) shows non-spontaneous natural process [14]. Electrochemistry is very common method in analytical applications. The redox behavior and sensitive and rapid analysis of many electroactive compounds can be studied with this method. Our aim is to investigate physicochemical features of chemotherapeutic agents in neutral aqueous solutions. Up to date, no report found on the kinetic and thermodynamic parameters of daunorubicin, doxorubicin and vincristine by electrochemical methods.

2. RESULTS AND DISCUSSION

2.1. Degradation studies

The stress conditions employed for the degradation study included acid hydrolysis, base hydrolysis, oxidation, photolytic condition, and heating in the oven (at 100°C). For the hydrolytic and oxidative degradation studies, the solutions were prepared by dissolving the drugs directly in deionized water and then diluted with hydrogen peroxide, sodium hydroxide or hydrochloric acid solution, to achieve a concentration of 100 $\mu\text{g mL}^{-1}$ for each solution.

Acid, base and oxidation conditions were applied at room temperature during 2 hours. However, drugs could not degrade at this condition. Heating procedure is very practical pathway to investigate drugs behavior at acid, base and oxidation solutions [15,16,17]. Hence, further studies were applied at 75 °C during 30 min. Acid and alkaline hydrolysis of drug substance in solution state was conducted with 1.0 M HCl and 1.0 M NaOH at 75°C during 30 min. Sample solutions of drug substance and drug product were kept in 3% hydrogen peroxide at 75°C during 30 min for oxidative stress. Thermal and photo-degradation of drug substances carried out in solid state. After the degradation, the stock solutions were prepared by dissolving in deionized water to achieve a concentration of 100 $\mu\text{g mL}^{-1}$. For photolytic stress, drug samples as the solid state, was

irradiated with UV radiation having peak intensities at 360 nm for 6 and 24 h. For thermal stress, DNR, DXR and VCR put in a controlled-temperature oven at 100°C for 6 and 24 h.

Area values were found by injection from DNR, DXR and VCR standards at 100 µg mL⁻¹ concentration into the system. Percent degradation values were calculated by comparing the area values of the standard compounds of 100 µg mL⁻¹ with area values of degradation products. These results given in Table 1. Chromatograms obtained from the degradation studies for DNR, DXR and VCR were given in Figure 2, Figure 3 and Figure 4.

Table 1. The percent degradation values calculated for DNR, DXR and VCR.

Stress conditions	Degradation of DNR%	Degradation of DXR%	Degradation of VCR%
HCl (1.0 M)	96.37	30.39	65.20
NaOH (1.0 M)	100	100	100
H ₂ O ₂ (3%)	86.52	-	90.72
UV (6 h)	1.49	2.75	9.67
UV (24 h)	2.25	3.78	34.78
Heating (100 °C 6 h)	1.31	0.08	0.45
Heating (100 °C 24 h)	13.17	0.51	7.76

2.2. Physicochemical parameters of DNR, DXR and VCR

Electrooxidation of 0.1 mM DNR (anodic response at 0.5 V), 0.1 mM DXR (anodic response at 0.45 V) and 0.1mM VCR (anodic response at 0.65 V) onto glassy carbon electrode (GCE) studied in pH 7.4 PB solution by at various temperatures of 298, 303, 308 and 313 K for the determination of thermodynamic features. Anodic responses of DNR, DXR and VCR peaks increased with the increasing of temperature (Figure 5).

The kinetic rate constants (k_s) calculated at different temperatures by following equation (Table 2):

$$I_p = nFAk_s C \quad (\text{Eq. 1})$$

The values of ΔG evaluated from k_s using equation 2 (Table 2).

$$\Delta G = 5778.8 (5.096 - \log k_s) \quad (\text{Eq. 2})$$

The Arrhenius equation $k_s = Ae^{\frac{-E_a}{RT}}$ applied for the calculation of activation energy (E_a). The values of E_a acquired from the slope of the $\log k_s$ vs. $1/T$ plot inserted into equation (3, 4) for the evaluation of enthalpy (ΔH) and entropy (ΔS), respectively.

$$\Delta H^* = E_a - RT \quad (\text{Eq. 3})$$

$$\Delta S^* = \frac{\Delta H^* - \Delta G^*}{T} \quad (\text{Eq. 4})$$

An examination of exposes a decrease in ΔH and ΔS with increasing temperature. k_s and ΔG increased with increasing in temperature.

The decreasing of ΔG with rise in temperature shows the redox process to become more facile at higher temperature values. The positive ΔH of activation suggests the endothermic nature of the electrode process, while negative ΔS of activation can be attributed to the adsorptive characteristic of the oxidized product of the analyte. The decrease in ΔS with increasing temperature suggests the trend toward diffusivity of the oxidized product of DNR, DXR and VCR from the electrode surface.

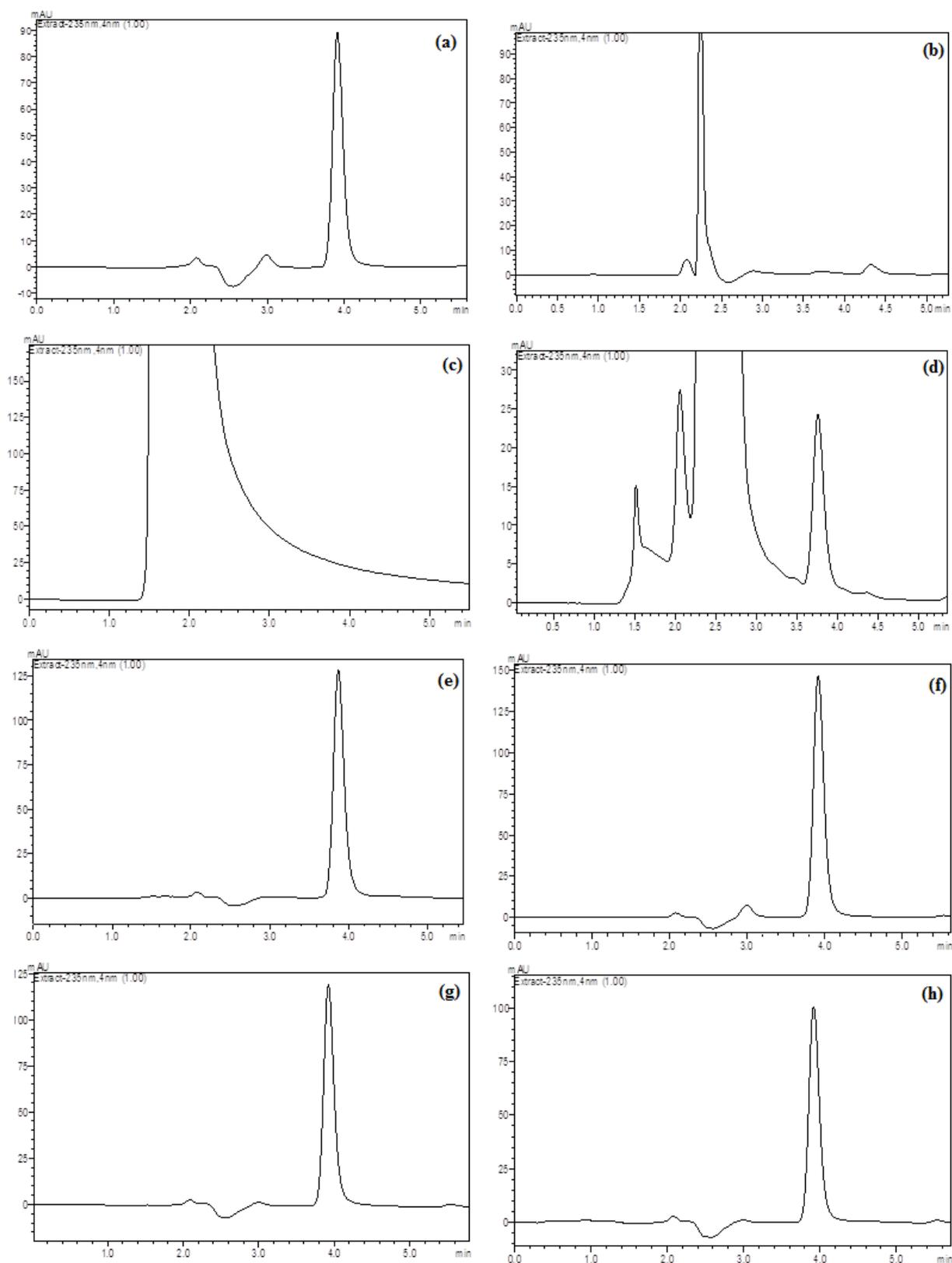


Figure 2. Typical LC chromatograms of DNR under drastic stressed conditions: (a) 100 $\mu\text{g mL}^{-1}$ DNR, (b) 1.0 M HCl at 75°C after 30 min, (c) 1.0 M NaOH at 75°C after 30 min, (d) H_2O_2 3% at 75°C after 30 min, (e) in UV light (360 nm) after 6 h, (f) in UV light (360 nm) after 24 h, (g) at 100°C after 6 h and (h) at 100°C after 24 h.

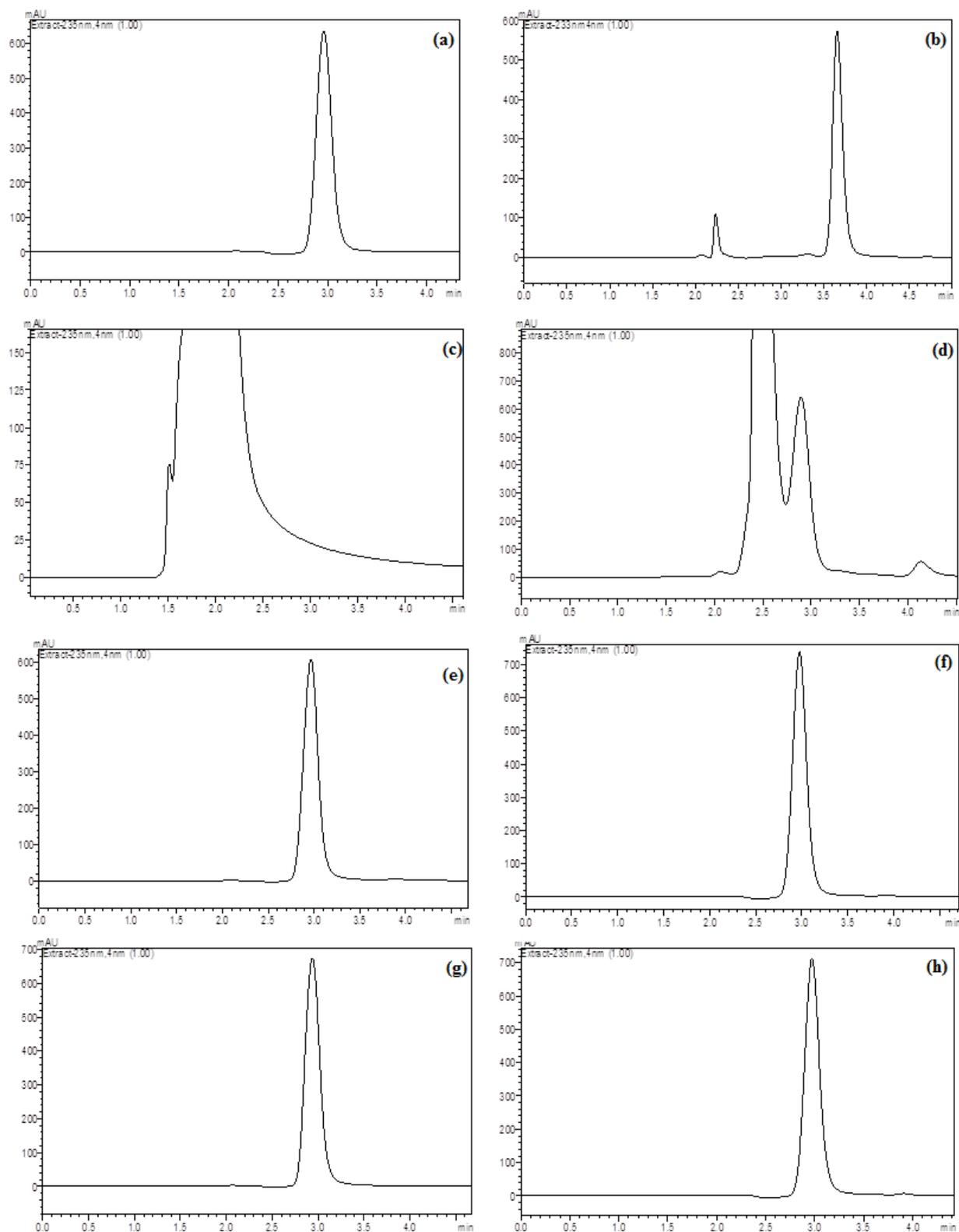


Figure 3. Typical LC chromatograms of DXR under drastic stressed conditions: (a) 100 $\mu\text{g mL}^{-1}$ DXR, (b) 1.0 M HCl at 75°C after 30 min, (c) 1.0 M NaOH at 75 °C after 30 min, (d) H₂O₂ 3% at 75°C after 30 min, (e) in UV light (360 nm) after 6 h, (f) in UV light (360 nm) after 24 h, (g) at 100°C after 6 h and (h) at 100°C after 24 h.

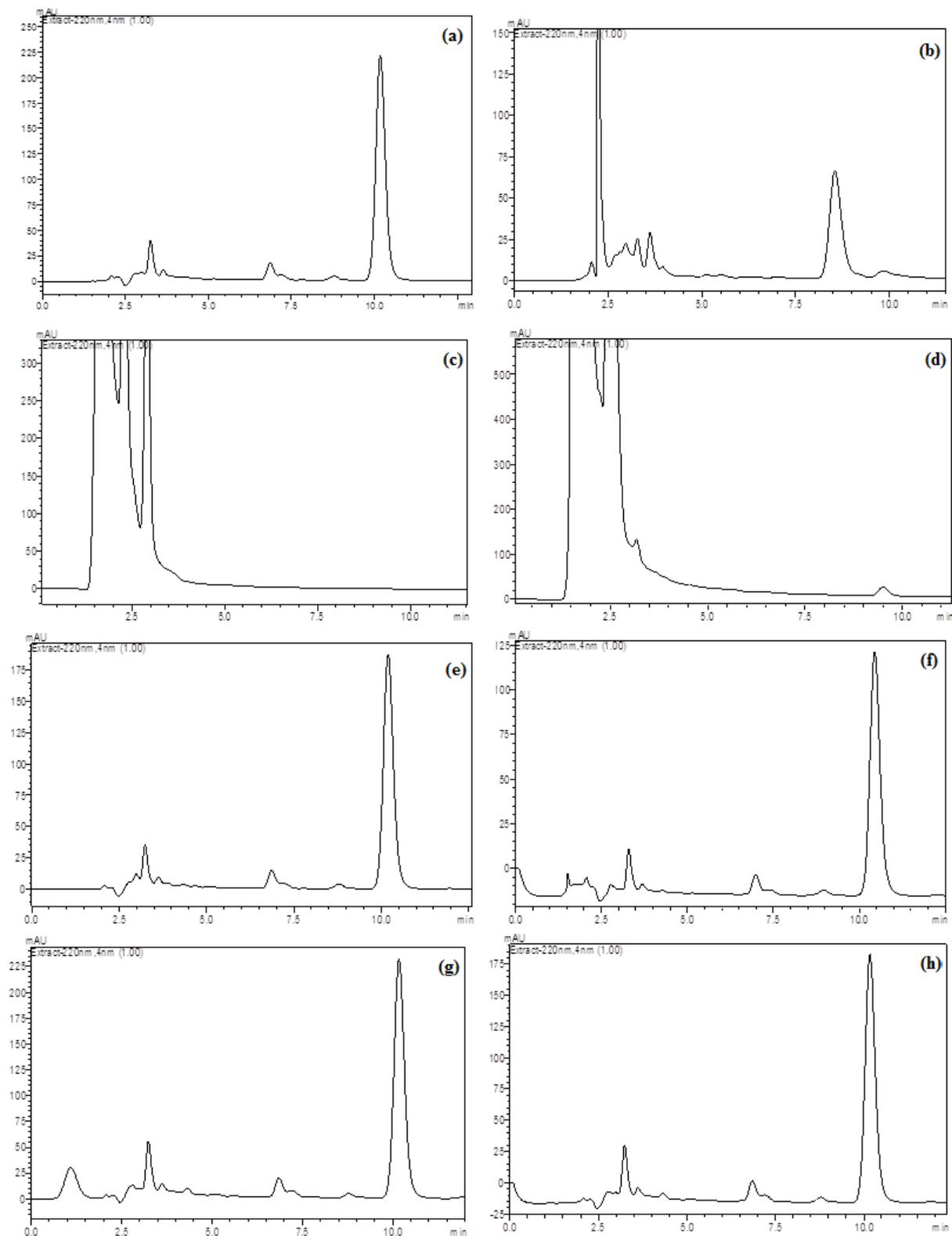


Figure 4. Typical LC chromatograms of VCR under drastic stressed conditions: (a) 100 µg mL⁻¹ VCR, (b) 1.0 M HCl at 75°C after 30 min, (c) 1.0 M NaOH at 75°C after 30 min, (d) H₂O₂ 3% at 75°C after 30 min, (e) in UV light (360 nm) after 6 h, (f) in UV light (360 nm) after 24 h (g) at 100°C after 6 h and (h) at 100°C after 24 h.

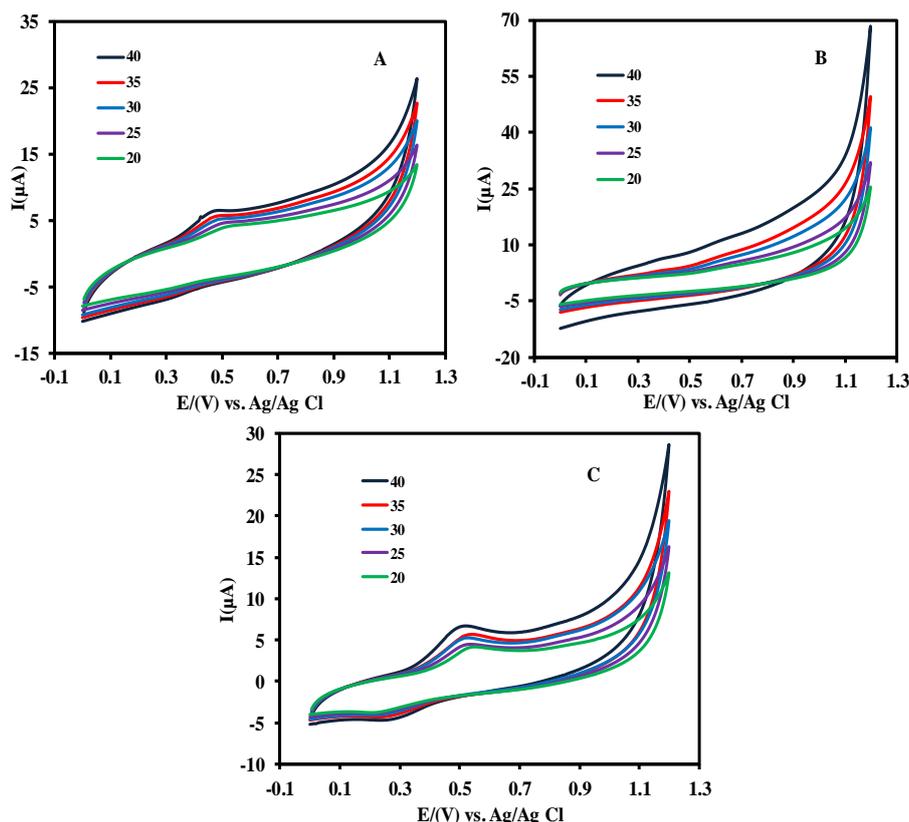


Figure 5. Cyclic voltammograms of 0.1 mM DXR (A), VCR (B) and DNR (C) in pH 7.4 PB solution at different temperatures (20, 25, 30, 35, 40°C) with GCE.

Table 2. Kinetic rate constant values of DNR, DXR and VCR at different temperatures.

T(K)	1/T(k ⁻¹)	DNR	DXR	VCR
		k _s (×10 ⁻⁵)	k _s (×10 ⁻⁵)	k _s (×10 ⁻⁵)
293	0.00341	133	53.1	2.22
298	0.00336	166	69.1	3.99
303	0.00330	201	86.0	5.99
308	0.00325	225	103	7.18
313	0.00320	276	130	9.25

3. CONCLUSION

When the studies on the degradation of the working compounds are examined, it was seen that DNR completely disintegrated in the basic medium and largely disintegrated in the acidic and peroxide medium. However, it has been determined that it is resistant to heat and UV light. It seems that DXR is completely degraded in basic medium, some in acidic medium. In the peroxide medium, the field value could not be determined due to the overlap of the DXR peak with the peroxide peak. However, it has been determined that it is resistant to heat and UV light. VCR is completely degraded in basic medium. According to the obtained results, the major part of VCR disintegrated in the peroxide medium. About more than half of VCR disintegrated in the acidic medium. It is not very stable such as the other two compounds against UV light. It has been determined that it is resistant to heat. Thermodynamic properties of three compound have been investigated in details with changing temperatures. Adsorption process is occurred at room temperature but, diffusion process began with increasing temperatures. These parameters are very important for drug release studies with nanomaterials. The kinetic profile of a drug can affect from temperature changes. The pharmacological answer of the drug might be affected, as a slower or faster release would change the drug plasmatic concentrations. In this study, we could show DNR, DXR and VCR behavior at changing temperatures at neutral solution.

Table 3. Thermodynamic parameters of the electrooxidation process of DNR, DXR and VCR.

Compounds	Temperature (K)	E _a (kJ mol ⁻¹)	ΔG (kJ mol ⁻¹)	ΔH (kJ mol ⁻¹)	ΔS (J K ⁻¹ mol ⁻¹)
DNR	293	11.67	46.1	9.23	-125.7
	298		45.5	9.19	-121.9
	303		45.0	9.15	-118.4
	308		44.8	9.11	-115.7
	313		44.2	9.07	-112.4
DXR	293	14.53	48.4	12.1	-123.8
	298		47.7	12.05	-119.7
	303		47.2	12.01	-116.0
	308		46.7	11.97	-112.8
	313		46.1	11.93	-109.2
VCR	293	22.9	56.3	20.46	-122.4
	298		54.9	20.42	-115.6
	303		53.8	20.38	-110.5
	308		53.4	20.34	-107.3
	313		52.8	20.30	-103.7

4. MATERIALS AND METHODS

4.1. Chemicals and reagents

All reagents and solvents were analytical grade and used with no further purification. DXR, VCR, and DNR were from Sigma. HPLC grade acetonitrile (ACN) was purchased from Merck (Darmstadt, Germany). Orthophosphoric acid (85%, d:1.71 g mL⁻¹) was obtained from Merck (Darmstadt, Germany). Sodium hydroxide, hydrochloric acid (37%, d:1.19 g mL⁻¹) and hydrogen peroxide (35%, d: 1.13 g mL⁻¹) were supplied from Riedel-de Haen (Seelze, Germany). Ultra-pure water was produced in the laboratory with conductivity 18.2 IS cm⁻¹ was obtained using a Zeneer Power I water system (Human Corp. Korea) and pure water was used to preparation of all aqueous solutions. Phosphate buffer solution was prepared with sodium phosphate dibasic heptahydrate and sodium phosphate monobasic monohydrate in bidistilled water. 0.1 mM drug solutions were prepared with pH 7.4 phosphate buffer solutions.

4.2. Apparatus and conditions

HPLC was achieved on a Shimadzu HPLC system (Kyoto, Japan) with a pump (LC-20 AD), a DAD detector system (SPD-M 20A), and column oven (CTO 20 AC). The separation was done on a X-Terra RP-18 (250 × 4.60 mm ID × 5μ) (Waters Corp., Milford, MA, USA).

The mobile phase is 15 mM o-phosphoric acid in this study. The elution performed on isocratic solvent system using this mobile phase. The mobile phase was prepared as pH 9.5 using 1 M NaOH. The flow rate was set at 1.5 mL min⁻¹ with controlled temperature at 30°C [12]. DAD detector was set at the wavelength of 235 nm for DXR and DNR and 220 nm for VCR and injection volume was 20 μL for every sample.

Kinetic studies performed with electrochemical analyser 'PalmSens5.2' at changing temperatures (in pH 7.4 phosphate buffer solution). Glassy carbon, Ag/AgCl and platinum as working, reference and counter electrodes, respectively. Cyclic voltammetry was applied between 0 and 1.7 V with 0.1 mV/s scan rate.

4.3. Stock and working solutions of standard compound

Standard stock solutions of drugs (200 μg mL⁻¹) were prepared by dissolving for each drug in ultra-pure water and stored at +4°C.

4.4. Stress testing

4.4.1. Acid hydrolysis

There is an optimum pH value, which each material is stable. The drug changes at outside of this pH. This change can be in the form of degradation and hydrolysis of the molecule. Solutions were prepared at concentrations of 200 µg mL⁻¹ from DNR, DXR and VCR standards. 200 µg mL⁻¹ of the prepared solutions were diluted to 100 µg mL⁻¹ by adding 1 M HCl. Then, the prepared solutions put in a water bath adjusted to 75°C for 30 min.

4.4.2. Base hydrolysis

Solutions were prepared at concentrations of 200 µg mL⁻¹ from DNR, DXR and VCR standards. 200 µg mL⁻¹ of the prepared solutions were diluted to 100 µg mL⁻¹ by adding 1 M NaOH. Then, the prepared solutions put in a water bath adjusted to 75°C for 30 min.

4.4.3. Oxidation

Solutions were prepared at concentrations of 200 µg mL⁻¹ from DNR, DXR and VCR standards. 200 µg mL⁻¹ of the prepared solutions were diluted to 100 µg mL⁻¹ by adding 3 % H₂O₂. Then, the prepared solutions put in a water bath adjusted to 75°C for 30 min.

4.4.4. Photolytic condition

10 mg from DNR, DXR and VCR standards were weighed and then, samples were exposed to UV light having a wavelength of 360 nm for 6 hours and 24 hours. Standard solutions were prepared at 100 µg mL⁻¹ concentrations and 20 µL injection was made from these solutions into the system.

4.4.5. Heat in an oven

10 mg from DNR, DXR and VCR standards weighed, and these samples put in the oven adjusted to 100°C for 6 hours and 24 hours. Then, using these standards, 100 µg mL⁻¹ solutions were prepared and 20 µL solution was injected from these solutions into the HPLC system.

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