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Electrochemical and liquid chromatographic analysis of triamcinolone acetonide in pharmaceutical formulations

Nuray Denizhan[1], Selehattin Yılmaz[1*], Gülşen Sağlıkoğlu[1], Emrah Kılınç[2], Çiğdem Yengin[3], Fatma Gülay Der[2]

[1] Çanakkale Onsekiz Mart University, Faculty of Science, Department of Chemistry, 17020, Çanakkale, Türkiye
[2] Ege University, Faculty of Pharmacy, Department of Analytical Chemistry, 35100, İzmir, Türkiye
[3] Ege University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 35100, İzmir, Türkiye

Abstract

The electrochemical reduction of triamcinolone acetonide on the pencil graphite electrode (PGE) surface was firstly investigated by cyclic voltammetry (CV). The dependence of the cathodic peak current and peak potential on different pHs and scan rates was investigated. The current type was determined as adsorption controlled. 0.067 M phosphate (pH 4.50 to pH 7.50), 0.2 M acetate (pH 3.50 to 5.50) and 0.04 M Britton Robinson (BR; pH 2.00 to 12.00) buffers were employed as supporting electrolytes. The scan rate studies were realized in the range of 25 – 1000 mV/s (vs. Ag/AgCl). The maximum peak current was observed in the 0.04 M BR buffer (pH 3.50). The peak current increased and shifted to more cathodic values with the increasing scan rate. The logarithm of the peak current (log I_P) versus the logarithm of the scan rate (log ν) showed linear regression with the equation log I_P (μA) = 0.8395 log ν (mV/s) - 0.8386 and the correlation coefficient (r = 0.9761). The slope of the log ν-log I_P curve was close to 1.0, which indicated that the electrode reaction was adsorption controlled, as desirable. The linear range was 1×10^{-7} – 5×10^{-5} M, the sensitivity was 1.3347 μA/M, and the limit of detection (LOD) and the limit of quantification (LOQ) were 3.18×10^{-8} M and 1.00×10^{-7} M, respectively. HPLC-PDA analysis was performed with H_2O:MeOH (28:72, v/v) as mobile phases A and B at a flow rate of 1 mL/min at 242 nm. The method validation studies were conducted in accordance with the ICH Q2(R1) guideline, and the corresponding results were summarized in tables. The HPLC-PDA method displayed linearity in the concentration range of 0.1 – 50 μg/mL (2.3×10^{-7} – 1.15×10^{-4} M) with LOD and LOQ values as 0.017 μg/mL (3.992×10^{-8} M) and 0.0561 μg/mL (1.29×10^{-7} M), respectively.

Keywords: Triamcinolone acetonide, voltammetry, HPLC-PDA, pencil graphite electrode

1. Introduction

Triamcinolone acetonide, 9-fluoro-11,16,17,21-tetrahydroxy-1,4-pregnadien-3,20-dion (TA), is a synthetic halogenated cyclic ketal pregnane corticosteroid used to treat various disorders such as skin inflammations, mouth sores, various joint conditions, and allergic rhinitis (Fig. 1).

The systematic administration of glucocorticoids is on the ban list by the World Anti-Doping Agency (WADA) and the International Olympic Committee (IOC) due to their feature of both creating toxicological risks and enhancing sports performance. Synthetic glucocorticoids, including TA, are also included in the list of the doping agents [1,2]. The analysis of pharmaceutical active substances from commercial forms is preferably conducted by analytical techniques. TA was determined in pharmaceutical dosage forms and biological samples by liquid chromatography (HPLC and LC-MS/MS) [3–8], gas chromatography (GC) [9], spectrophotometry [10–12], and electrochemical techniques (voltammetry) [13,14].

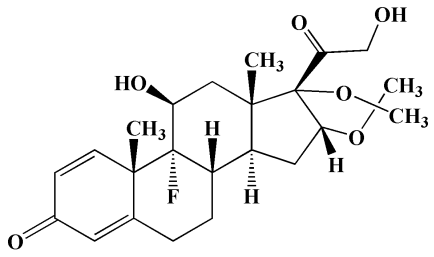

Figure 1. Structural formula of TA

It was shown in the literature that corticosteroids had 2 reducible electroactive groups, which are C-3 and C-20 carbonyl groups. Goyal et al. explained that the

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***Author of correspondence:** seletyilmaz@hotmail.com
Tel: +90 (286) 218 00 18 → 22191
Fax +90 (286) 218 39 17
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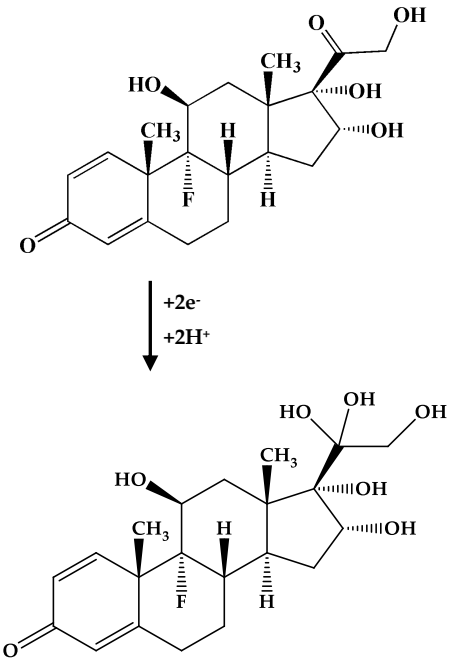

Figure 2. Tentative mechanism given in the literature [14,15] for the cathodic reduction of TA

carbonyl group of TA was being reduced and that this group was also activated by the C-16 and C-17 hydroxyl groups. Goyal proposed the following mechanism (Fig. 2), explaining that the reduction occurred with $2e^-$ and $2H^+$ as a result of experimental information [14,15].

Many studies were carried out in various supporting electrolyte to explain the reducing properties of corticosteroids. Kabasakalian and McGlotten studied prednisone, prednisolone, cortisone, and hydrocortisone in 50% ethanol solutions at pH of 5.5. They suggested that the value of n for the reduction of these compounds was between 0.9 and 1.0. In the reduction of the C-3 carbonyl group, dimerization occurred after radical formation [16]. The chemical structures of TA and the other investigated compounds in the literature are given in Fig. 3.

A few studies have been found in the literature for the analysis of TA by electrochemical methods. These are, in summary, as follows:

Hammam [13], have developed a determination of triamcinolone acetonide in pharmaceutical formulation and human serum by adsorptive cathodic stripping voltammetry (AdCSV). Under optimized conditions, the stripping voltammetric peak current of TA showed a linear dependence on TA concentration over the range $1 \times 10^{-9} – 9 \times 10^{-8}$ M. The detection limit (LOD) was 3×10^{-10} M, and the quantitation limit (LOQ) was 1×10^{-9} M.

Vehdi et al. [14], have developed a determination of triamcinolone asetonide steroid on glassy carbon electrode by stripping voltammetric methods. An adsorption-controlled well-defined reduction peak was observed in all pH conditions. A calibration plots

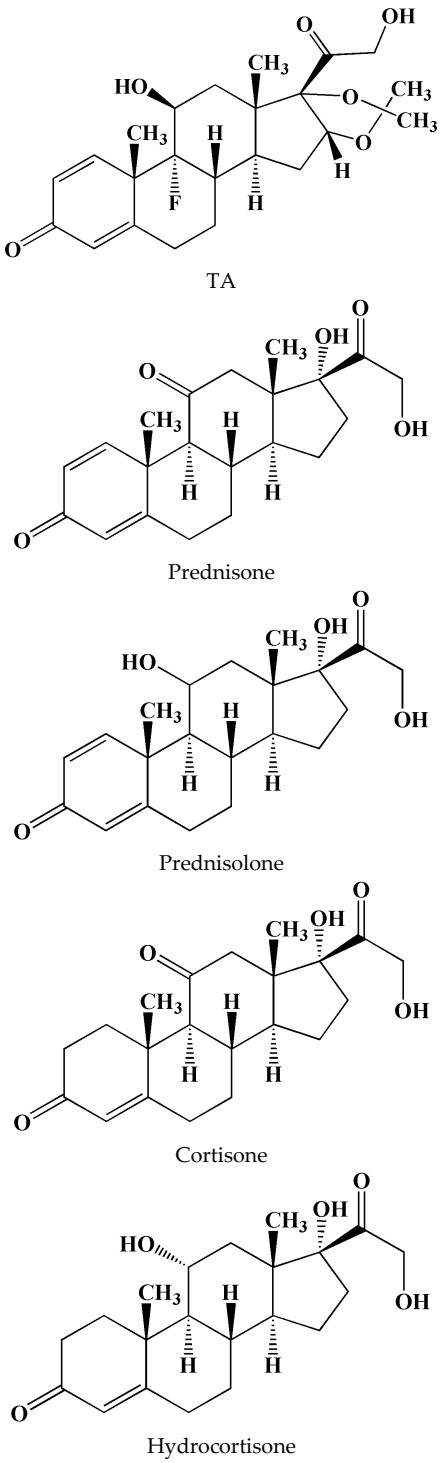

Figure 3. The chemical structures of TA and the other investigated compounds in literature

was derived, and the lower limit of determination observed are 0.1 μg/mL from DPSV and 0.01 μg/mL from SWSV.

Zagrzewski et al. [17], have developed an electrochemical behavior of TA on the carbon paste electrodes for its voltammetric determination.

The calibration plot was found linear in the concentration from 2 to 46 μmol/L, the detection limit was 1.5 μmol/L. Unlike the literature, PGE was applied for the first time in this study.

In the current study, the electrochemical and chromatographic analysis of triamcinolone acetonide was carried out in commercial pharmaceutical dosage forms. The aim of the voltammetric study is to determine TA for the first time using a pencil graphite electrode (PGE) because of its advantages such as being easily accessible, inexpensive, disposable, and high reproducibility compared to the literature. The chromatographic method was also applied for the accuracy of the electrochemical method.

2. Materials and methods

2.1. Reagents

TA standard and its pharmaceutical forms were obtained from DEVA Inc.. All chemicals used were analytical-reagent grade. Methanol, which was used as a HPLC mobile phase component, was of gradient grade for liquid chromatography (LiChrosolv, 99.9%) and was obtained from Merck KGaA (Germany). Ultrapure water used in the preparation of aqueous mobile phase B had a total organic carbon level <10 ppb and a total ion resistance > 18.2 MΩ cm and was produced freshly and used without storage by Elix Milli-Q Gradient A10 Ultrapure Water System (Millipore company).

2.2. Apparatus

A Metrohm 757 VA Trace Analyzer (Herisau, Switzerland) model instrument was used for voltammetric measurements, with a three-electrode system consisting of PGE as the working electrode, a platinum wire as the auxiliary electrode, and Ag/AgCl (KCl 3 mol/L, Metrohm) as the reference electrode. Tombow 0.5 mm HB model mechanical pencil refills were used as the PGE working electrode. Argon (Ar) gas was passed from supporting electrolyte solution (5 min) and after addition each sample (60 s) to remove oxygen. pH measurements were carried out with EZDO-5011A model (Herisau, Switzerland) pH-meter at 15 to 20 °C for laboratory temperature.

The HPLC-PDA hardware used during the chromatographic analysis was Ultimate-3000 series HPLC system of Thermo-Dionex Company, and consisted of solvent tray (SR3000), integrated online degasser and high-pressure gradient pump (LPG3400SD), derivative autosampler (WPS3000TSL), column compartment (TCC3000SD) and PDA detector (DAD3000) modules. The HPLC hardware was operated with the Chromeleon (v.6.80 SR13) chromatographic data acquisition software. The analytical column used for separation was Macherey-Nagel MN 250/4.6 Nucleosil C18 100-5µ (Serial No: 2065339, Batch No. 21302092).

2.3. Procedure

2.3.1. Electrochemical analysis

The electrochemical properties of TA were investigated by voltammetric techniques. In order to detect the experimental conditions for the analysis of the electrochemical reduction of TA, 0.067 M phosphate (pH 4.50 to pH 7.50), 0.2 M acetate (pH 3.50 to 5.50) and 0.04 M BR (pH 2.00 to 12.00) buffers were used to support the electrolytes. Kenacort-A ampoule was supplied from Deva Inc. (İstanbul, Turkey). 1×10^{-2} and 1×10^{-3} M TA stock solutions were prepared in methanol and stored in the refrigerator. Kenacort-A ampoule was dissolved in methanol. 1×10^{-2}, 1×10^{-3}, and 1×10^{-4} M of stock solutions were prepared in methanol. The solutions liquor was put into cell. The amount of TA in the Kenacort-A ampoule was calculated from the corresponding equations in the calibration plots. The drug sample analysis was performed by differential pulse adsorptive stripping voltammetry DPAdSV. Pulse amplitude 50 mV, pulse time 0.04 s and voltage step 0.009 V were selected for DPAdSV parameters. Potential step 10 mV and scan rate were selected in the between 25 – 1000 mV/s for CV.

2.3.2. HPLC measurements

Various mobile phase solvent ratios, wavelengths, flow rates and retention time were studied for the optimization of the chromatographic parameters. 1×10^{-3} M stock solution of TA was prepared in methanol. The diluted standard solutions were prepared by diluting the stock solution with appropriate volume of the mobile phase. The optimum conditions among them were determined as A and B during the HPLC-PDA analysis were H_2O:MeOH (28:72, v/v) at a flow rate of 1 mL/min. 190 – 400 nm spectrums were collected while the absorption was acquired at 242 nm with the PDA detector and the retention time was assigned as 3.52 min. Then, standard solutions in the range of 0.1 – 50 ppm were prepared from stock solutions, and the calibration curve with 9 points (0.1, 0.25, 0.50, 1.0, 2.5, 5.0, 10.0, 25.0, and 50.0 ppm) was plotted. 2D and 3D chromatograms of 5 ppm TA standard is given Fig. 9, where the retention time was assigned as 3.52 min. The developed method was validated in accordance with the ICH Q2(R1) guideline, and the corresponding results were summarized in tables. The HPLC-PDA method displayed linearity in the concentration range of 0.1 – 50 µg/mL (2.3×10^{-7} – 1.15×10^{-4} M) with limit of detection (LOD) and limit of quantification (LOQ) values as 3.992×10^{-8} and 1.29×10^{-7} M, respectively. Two different products (Kenacort-A ointment and Kenacort-A injection) were used for sample analysis. While preparing the ointment sample, 1.0 grams of ointment was weighed and dissolved in 50 mL of methanol. It was stirred for 3 hours at room temperature with a magnetic

stirrer in a sealed container at 400 rpm. Thus, 20 ppm sample solution was prepared and vialed, and injected into HPLC after filtering through coarse filter paper. While preparing the ampoule sample, 50 µL suspension was taken directly from the prepared ampoule and completed to 50 mL with methanol. Thus, a sample solution of 40 ppm was prepared, and this solution prepared was injected into HPLC. Different extraction methods, such as magnetic stirrer and ultrasonic bath, were used in drug analyzes.

3. Results and discussion

3.1. Electrochemical analysis of TA

The electrochemical reduction of TA was studied in various supporting electrolytes (pH 2 – 12). The stock solution of TA (1×10^{-3} M) was prepared with bi-distilled water. DPAdSV voltammograms of 5×10^{-5} M TA was taken in these electrolytes. The maximum peak signal was observed in the 0.04 M BR buffer (pH 3.50). Therefore, this buffer and pH was chosen for further studies. The CV measurements were performed with 5×10^{-5} mol/L TA at scan-rates of 25 – 1000 mV/s on a PGE in 0.04 mol/L BR buffer at pH 3.50 (Fig. 4) The peak current values increased with the increase of the scan rates. The peak potential values shifted to more positive values with the increase of the scan rate. This indicates that the reduction is easier at higher scan rates. In order to determine the type of current, the logarithm of the peak current (log I_P) versus the logarithm of the scan rate (log ν) was plotted. The linear regression equation was obtained as log I_P (µA) = 0.8395 log ν (mV/s) - 0.8386 with the correlation coefficient of (r) 0.9761. The slope of this equation was close to 1.0. This value indicates that the electrode reaction was adsorption controlled.

3.2. The effect of deposition time and deposition potential

The effect of deposition time and deposition potential on the peak current was given in Fig. 5 and Fig. 6, respectively.

The adsorption parameters were selected for the deposition time as 15 s and for the deposition potential as -250 mV/s according to Fig. 5 and Fig. 6. Subsequent studies were carried out according to these parameters.

3.3. Formation of calibration plots

The DPAdSV voltammograms of TA within the range of 1×10^{-7} – 5×10^{-5} M (0.1 – 15 µM) in 0.04 BR (pH 3.5) at PGE and the inset calibration plot are given in Fig. 7.

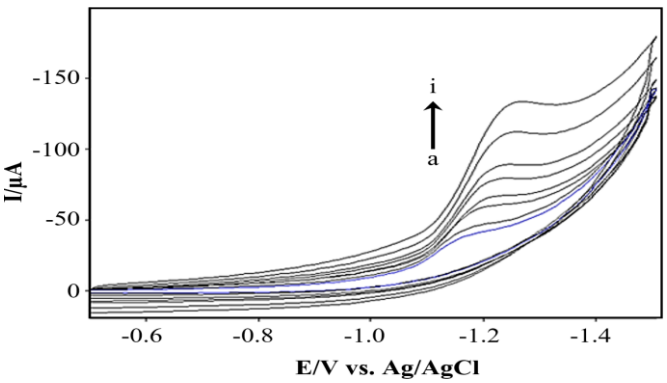

Figure 4. Dependence of cyclic voltammograms of 5×10^{-5} M TA on scan rates blank (a), 75 (b) 100 (c), 200 (d), 300 (e), 400 (f), 500 (g), 750 (h), and 1000 (i) mV/s in 0.04 M BR buffer (pH 3.50) at PGE Experimental details are given in subsection 2.3.1

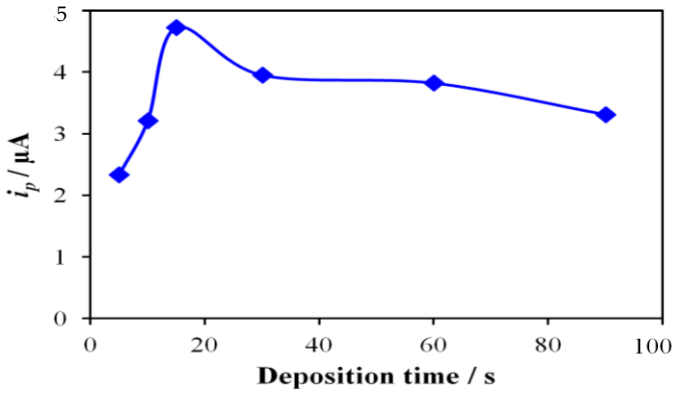

Figure 5. Variation of peak current with deposition time of 5×10^{-5} M TA with CV technique (pH 3.50 BR buffer)

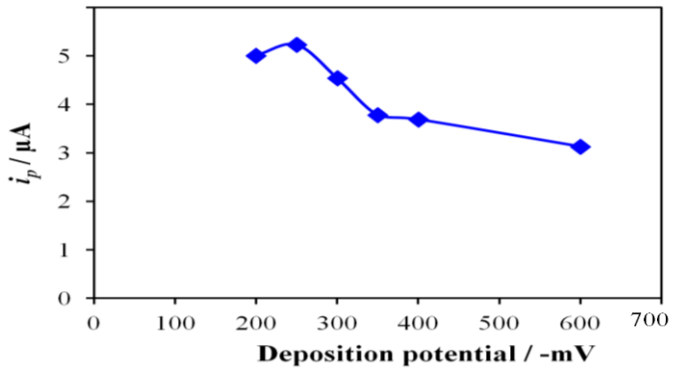

Figure 6. Variation of peak current with deposition potential of 5×10^{-5} M TA with CV technique (pH 3.50 BR buffer)

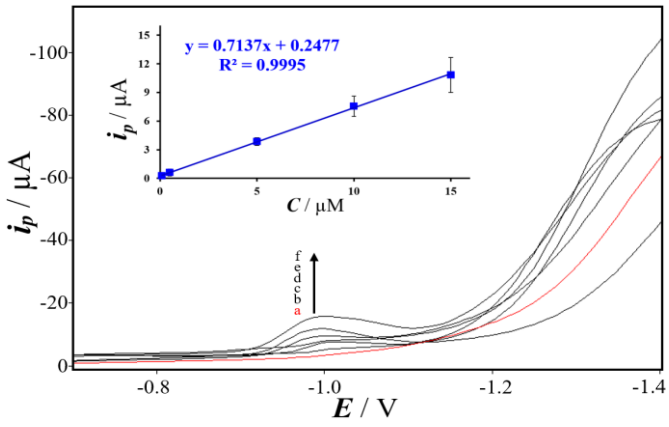

Figure 7. DPAdSV voltammograms of blank (a), and TA within the concentration range of 0.1 (b), 0.2 (c), 5.0 (d), 10 (e), and 15 (f) µM in pH 3.5 BR buffer at PGE, and inset calibration plot

The validation parameters and recovery date of the studied voltammetric are presented in Table 1.

Table 1. Regression data of the calibration curves of TA in pH 3.5 BR buffer at PGE

Validation Parameters	Results
Measurement potential (V)	-1.01
Linear concentration range (M)	$1 \times 10^{-7} - 5 \times 10^{-5}$
Slope (µA/µM)	0.7137
SD of slope	0.04
Intercept (µA)	0.2477
SD of intercept	0.06
r	0.9995
N	5
LOD (M)	3.18×10^{-8}
LOQ (M)	1.00×10^{-7}
Intra-day precision of PC/ RSD%*	1.50
Intra-day precision of PP/ RSD%*	2.00
Inter-day precision of PC/ RSD%*	3.30
Inter-day precision of PP/ RSD%*	2.50

* RSD%: Relative standard deviation (obtained from 5 measurements) r: coefficient of correlation, N: number of measurements, PC: peak current, PP: peak potential

The result of the analysis for TA in pharmaceutical preparations by voltammetry is given Table 2.

Table 2. Determination of TA in pharmaceutical preparations (Kenacort-A ampoule form) by DPAdSV

Parameters	Kenacort-A (Ampoule)
Labelled claim (mg)	40.00
Amount found (mg)*	43.00
RSD%	3.00
Amount added (mg)	10.86
Found (mg)*	10.58
Average recovered%*	97.50

The data for the electrochemical determination of TA was found comparable with the literature in terms of LOD, LOQ, and linear range (Table 3).

Table 3. Literature data for the electrochemical determination of TA

Method	Linear range (µM)	LOD (µM)	LOQ (µM)	Reference
HMDE	0.001 – 0.009	0.003	0.001	[13]
GCE	0.2 – 50	0.1	—	[14]
CPE	2 – 46	1.5	—	[17]
PGE	1 – 15	0.03	0.1	(This study)

As it was shown in Table 3, the limit of the detection and quantitation of PGE, used for the first time in this application, was close to HMDE and less than GCE and CPE. The advantages of it are that it is easily available, inexpensive, disposable, and highly reproducible.

Additionally, PGE electrode has some important advantages over the mercury electrode. While the mercury electrode is toxic, PGE is non-toxic. The mercury electrode is both expensive and difficult to clean. However, the PGE electrode is both disposable and economical. Therefore, PGE should be preferred.

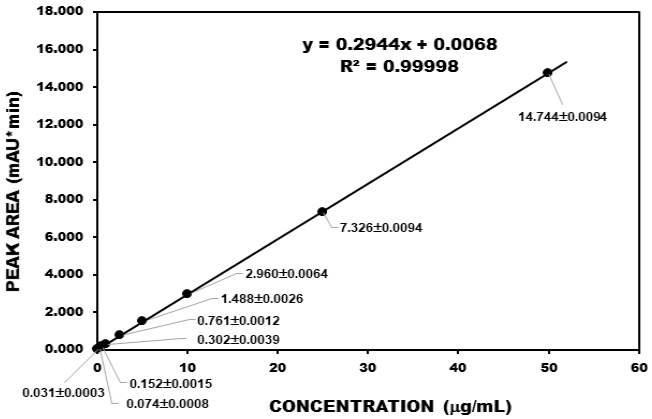

Figure 8. Calibration plot of HPCL-PDA method (Experimental details as in *HPLC measurements* subsection)

3.4. HPLC-PDA quantification of TA

The chromatographic analyses were carried out as described in 2.3.2. of the experimental section. Using the peak area, the HPLC calibration curve (Fig. 8) levels of TA in drug formulations were determined.

2D(A) and 3D(B) chromatograms of 5 µg/mL Triamincinolone acetonide (TA) standard were given in Fig. 9. 2D and 3D chromatogram taken to get a more detailed and clearer image. While 2D (A) gives dimensions such as height and width, depth is given in 3D (B).

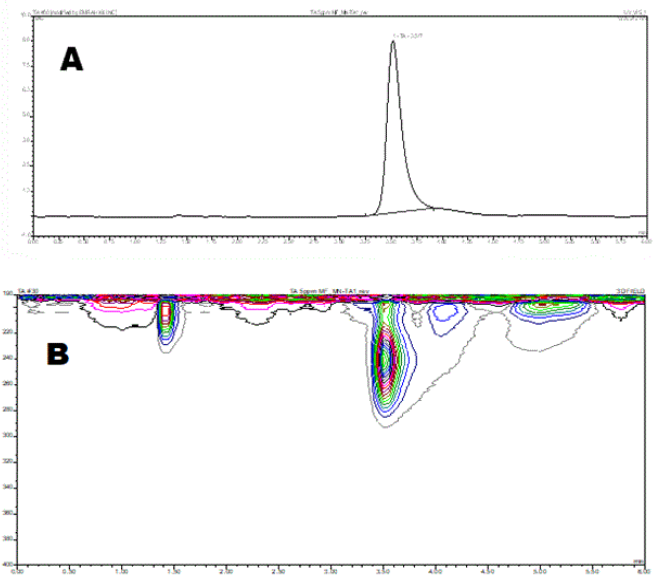

Figure 9. 2D (A) and 3D (B) chromatograms of 5 µg/mL Triamincinolone acetonide (TA) standard (Experimental details as in *HPLC measurements* subsection)

2D chromatogram of Kenacort-A ointment is given Fig. 10. The active ingredient in the composition of Kenacort-A ointment is 1 mg TA, and the excipients are Orahessive powder (Gelatin powder type-A, pectin, sodium carboxy methyl cellulose) and plastibase (mineral oil and polyethylene bakelite). 3D chromatogram of Kenacort-A ointment sample is given Fig. 10B.

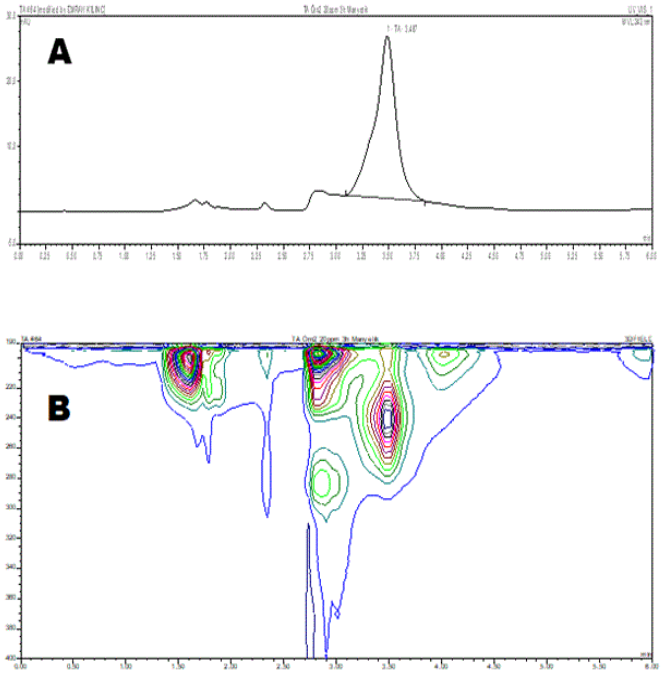

Figure 10. 2D (A) and 3D (B) chromatograms of Kenacort-A ointment sample (Experimental details as in *HPLC measurements* subsection)

The other studied commercial pharmaceutical formulation was Kenacort-A ampoule. 2D and 3D chromatograms of Kenacort-A ampoule are given in Fig. 11A and 11B, respectively. In the content of Kenacort-A ampoule, the active ingredient is 40 mg TA in every 1 mL of suspension, and the auxiliary substances are benzyl alcohol, polysorbate 80, sodium carboxymethyl cellulose, sodium chloride, and injectable water.

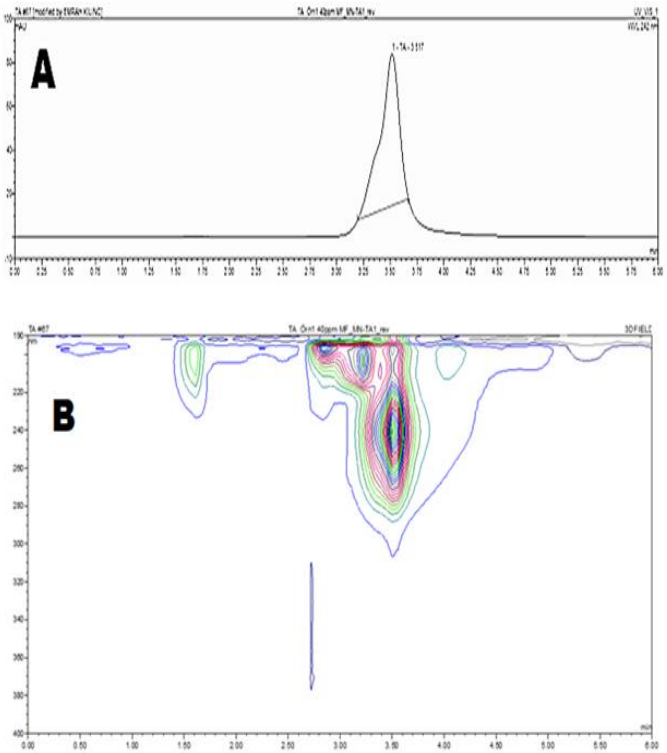

Figure 11. 2D (A) and 3D (B) chromatograms of Kenacort-A ampoule sample (Experimental details as in *HPLC measurements* subsection)

Different extraction methods were used for sample preparations prior to the drug analyses, while the optimum was found to be magnetic stirring for Kenacort-A ointment. HPLC results are given for the chromatographic analysis of TA in formulations (Table 4).

Table 4. The assay results of TA in Kenacort-A Ointment and ampoule with HPLC-PDA method (Experimental details as in Samples preparation and extraction and *HPLC measurements* subsections)

Dosage Forms	Spiked (ppm TA)	Determined (ppm TA)	Extraction Methods	Recovery (%)
Ointment	20	20.001 ± 0.042	Magnetic stirrer (3 hours at 400 rpm)	100.005
Ointment	20	18.166 ± 0.032	Ultrasonic bath (3 hours at 25°C)	90.83
Ampoule	40	48.736 ± 0.436	Filtered after dilution	121.84

The validation studies of the HPLC-PDA method for TA were executed performed in accordance with the ICH Q2(R1) guideline, and the obtained results were summarized in Table 5.

Table 5. Summary of method validation studies according to ICH Q2(R1) guideline

Validation parameters	Results		
Specificity	R < 1		
Linearity	Linearity range: 0.1 – 50 µg/mL (2.3×10^{-7} – 1.15×10^{-4} M) Regression equation: y = 0.2944x +0.0068 (r = 0.9998)		
Precision (Intra-day, RSD%)	**Sample No**	**Determined (%)**	**RSD%**
	1	100	1.6
	2	92.8	0.8
	3	97.2	0.7
	4	96.0	1.6
	5	97.6	0.5
Precision (Inter-day, RSD%)	**Sample No**	**Determined (%)**	**RSD%**
	1	101.6	0.3
	2	101.7	0.4
	3	100.2	01.
	4	99.8	0.1
	5	99.7	0.2
Accuracy	**δ ± SD:** 5.1 ± 0.14 **Rel. Error:** +0.2%		
LOD	0.017 µg/mL = 3.92×10^{-8} M		
LOQ	0.0561 µg/mL = 1.29×10^{-7} M		

The linearity range was obtained as 2.30×10^{-7} – 1.15×10^{-4} M (0.1 – 50 µg/mL). LOD and LOQ were achieved as 3.92×10^{-8} M (0.017 µg/mL) and 1.29×10^{-7} M (0.0561 µg/mL), respectively.

In the literature, there are studies related to HPLC and TA quantification, and their results are in harmony with the HPLC findings of the current paper. Literature and this study are given Table 6.

The chromatographic determination of TA was found comparable with the literature in terms of LOD, LOQ, linear range, and the other parameters are given in Table 6.

As it was shown in Table 6, in this study, retention time was close to that in literature studies.

Table 6. Literature data for the chromatographic determination of TA

Method	Linear range (μg/mL)	LOD (μg/mL)	LOQ (μg/mL)	Column	Temperature (°C)	Flow rate (mL/min)	Retention time (min)	Reference
HPLC-PDA	1 – 200	0.14	0.47	C8 (Hypersil 150 × 4.6 mm, 5 μm)	Ambient	1 – 2 (Gradient)	12.45	[18]
HPLC-UV	—	—	0.24	C18 (Nucleosil125 × 4 mm, 5μm)	30	0.8	4.8	[19]
HPLC-UV	1 – 50	0.19	0.62	C18 (Hypersil 150 × 4.6 mm, 5μm)	40	1.5	3.42	[20]
HPLC-UV	6.26 – 100.20	2.63	7.97	Supelcosil LC-ABZ (150 × 4.6 mm, 5μm)	37	—	2.68	[21]
HPLC-PDA	0.1 – 50	0.017	0.0561	C18 (Nucleosil 250 × 4.6 mm, 5 μm)	Ambient	1	3.52	(This study)

However, linear range, LOD and LOQ of HPLC-PDA was less than that of the previous methods. So, this indicates that the applied HPLC method was more suitable than previous HPLC methods.

Electrochemical method displayed the linear range was $1 \times 10^{-7} – 5 \times 10^{-5}$ M, and LOD and LOQ were 3.18×10^{-8} M and 1.00×10^{-7} M, respectively. The HPLC-PDA method displayed linearity in the concentration range of $2.3 \times 10^{-7} – 1.15 \times 10^{-4}$ M with LOD and LOQ values as 3.992×10^{-8} M and 1.29×10^{-7} M, respectively. Although these results obtained with both methods are close to each other, the electrochemical method has advantages such as being more economical, easier experimental procedures, working with less samples, taking less time and working with disposable electrodes. However, this study indicates that both techniques are good alternatives to each other.

4. Conclusions

In the current study, the electrochemical properties of TA were simultaneously determined by voltammetric techniques using first time PGE, and HPLC-PDA was developed for its analysis in commercial pharmaceutical dosage forms. Regression data of the calibration curves and analysis of TA were successfully carried out in Kenacort-A Ampoule form (Table 1 and Table 2) at PGE (easily available, inexpensive, disposable and highly reproducible) compared with the other electrodes in the literature. The electrode reactions were found to be adsorption controlled. The TA levels in various pharmaceutical formulations (Kenacort-A ointment and Kenacort-A Ampoule) were determined with HPLC-PDA methods. Different extraction methods were used for the preparation of Kenacort-A ointment samples prior to the analysis, while it was concluded that the optimum method was magnetic stirring (Table 4). Analytical method validation studies were performed, and the obtained experimental results were summarized in Table 5. This study indicates that the developed voltammetric and HPLC methods can be applied for the electrochemical and chromatographic analysis of other drug active materials.

Acknowledgement

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