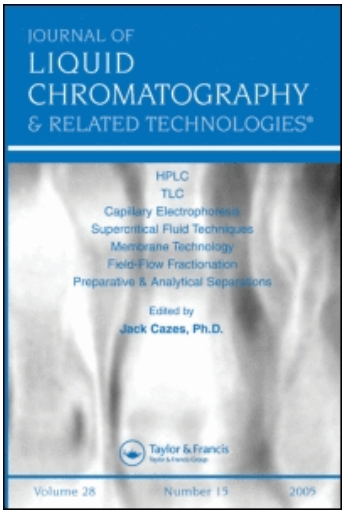

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Validated Method for the Determination of Deflazacort by a Flow-Injection Analysis with UV Detection: Application to Pharmaceutical Formulations

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ABSTRACT

A simple, precise, accurate, and fast flow-injection analysis (FIA) method employing UV detection is described for the determination of deflazacort (DEF) in pharmaceutical tablets. The best carrier solvent system consisted of EtOH: sodium dihydrogen phosphate (0.2 M) (20 : 80, v/v) (pH 6.0). Related parameters were elucidated and a flow-rate of 1.3 mL min^{-1} was used and the analyte was monitored at 247 nm. In the optimum condition, the repeatability was in the range of 0.27–0.41 as intra-day precision. The linearization was tested considering intra- and

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inter-day experiments in the range of 1.0×10^{-5}–5.0×10^{-5} M and good correlation coefficients were obtained. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated to be 2.35×10^{-7} and 7.04×10^{-7} M, respectively, as inter-day results. The proposed method was applied to the determination of DEF in pharmaceutical preparations. Conventional UV-spectrophotometry was used as a comparison method and their results were also compared to those of the FIA technique. Insignificant differences between FIA and UV-spectrophotometric results were observed ($p < 0.05$). In conclusion, the tablets provide the general official requirements, 101.3% and 102.3%, respectively. Therefore, the proposed method is suggested to the routine laboratories for the determination of DEF in tablets.

Key Words: Deflazacort; Flow-injection analysis; Pharmaceutical analysis; Validation.

INTRODUCTION

Deflazacort (DEF) [(11β, 16 β)-21-(acetyloxy)-11-hydroxy-2′-methyl-5′H-pregna-1,4-dieno[17, 16-d]oxazole-3,20-dione] is one of the glucocorticoid type drugs. Its chemical structure is shown in Fig. 1.

DEF has been used in many acute and chronic inflammatory therapies. It is used in therapies of rheumatoid arthritis, adrenal insufficiency, and many acute and chronic inflammatory disorders. Since DEF has low lipid solubility, it has been proposed that it has a smaller influence on phosphate-calcium, bone, carbohydrate metabolism than other corticoids.[1–3] Some sportsmen use corticoids to improve their performance. Therefore, the determination of corticoids is especially important for both clinical and doping control purposes.[4]

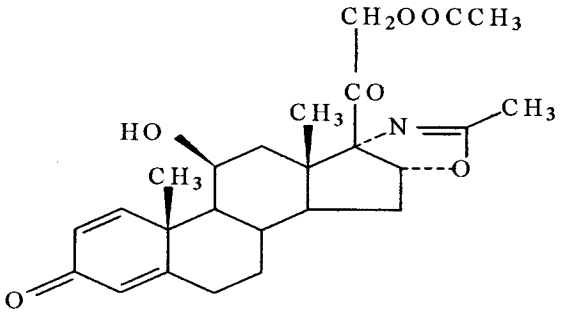

Figure 1. Chemical structure of DEF.

A few methods have been reported for the quantitative determination of DEF in the literature. These are determinations made in mostly biological samples such as serum, plasma, and urine using only HPLC methods with different detection system.[4–10]

There have been no reports concerning flow-injection analysis (FIA) of DEF in tablets using UV detection.

FIA is a new methodology characterized by its versatility, ease of automation, high sampling frequency, and minimum sample treatment prior to injecting into the system. The FIA techniques have recently found wide applications, mainly due to a reduction of the analysis time and consumption of reagents compared to conventional manual procedures.[11–13]

EXPERIMENTAL

Chemicals

Standard DEF was supplied from Aventis Pharma (Istanbul, Turkey). The commercial preparation of DEF, Filantadin® tablet each containing 30 mg, Aventis Pharma (Istanbul, Turkey) was purchased from the local drug store. Distilled ethanol and distilled water was produced in our laboratory using all glass apparatus. Other chemicals were of analytical grade and provided from Merck GmbH (Darmstadt, Germany).

Apparatus

A Model UV 2401 PC spectrophotometer (Shimadzu, Japan) and quartz cells were employed for the common UV-spectrophotometric measurement of absorbance. A Model LC 6A pump equipped with a 20 μL manual loop injector from Cotati (CA, USA), a Shimadzu Model SPD-A 10 UV variable wavelength detector and a Model C-R7A integrator (Kyoto, Japan) were used for FIA measurements. A Model Jenway 3320 pH-meter with a pH electrode (Essex, UK) was employed for pH measurements of the solution. A carrier solvent system was always sonicated in a Branson ultrasonic bath B-220 (CA, USA).

Procedures

Carrier Solvent

A carrier solvent was employed as aqueous solution of EtOH: sodium dihydrogen phosphate (0.2 M) (20 : 80, v/v) (pH 6.0). It was adjusted to

pH 6.0 by addition of 1 M NaOH. All the solutions were always degassed by a sonication.

Solutions

A stock solution of DEF (1.0×10^{-3} M) was prepared using EtOH. All dilutions were prepared by using a carrier solvent. The solution of 2.5×10^{-5} M DEF, during the optimization and validation studies and dilutions, in the range of 1.0×10^{-5}–5.0×10^{-5} M DEF for the study of linearization, were prepared and were used for the related experiments.

Certain DEF solutions, as in the FIA studies, were employed for the common spectrophotometric experiments.

FIA

FIA was performed by injecting the related solutions into the system as the carrier solvents are being pumped through in a fixed flow-rate, as mentioned above. The signals were detected at 247 nm where monochromatic light is maximally absorbed by DEF. Standard and sample solutions were injected into a 20-μL fixed volume of loop. The variation of flow-rate was examined in a wide range of 0.1–2.5 mL min^{-1}.

Application of the Method to DEF Tablets

The tablets were analyzed by obeying the pharmacopoeia procedures as given in USP XXIV.[14] For the analysis of DEF, ten Filantadin® tablets (each containing 30 mg DEF) were accurately weighed; average weight of a tablet was calculated. They were ground in a mortar to fine a powder. A sufficient amount of powder, equivalent to the average weight of a tablet, was accurately weighed, transferred to a flask, and 10 mL ethanol was added to dissolve the active material. It was sonicated for 15 min and it was made up to volume with carrier solvent. The solution was then centrifuged at 3000 rpm for 15 min. The supernatant was diluted with carrier solvent.

RESULTS AND DISCUSSION

Optimization of the Method

Certain attempts were tried to prepare a carrier solution. The solubility of the DEF was investigated to find out the optimum composition of carrier solvent, which will be employed during the analysis, and it was found that

20% ethanol is a suitable solvent. Then, the pH of the carrier solvent was adjusted to 6.0 to avoid probable hydrolysis of the molecule. In the mentioned conditions, there was no observation of any precipitation and turbidities in the solutions for at least 1 week.

Before examining the FIA parameters, the UV spectrum of DEF was recorded in the range of 200–360 nm and a sharp peak almost appeared at 247 nm. Carrier solvent was employed as a blank while they are in the quartz cells. The wavelength, where DEF absorbed the maximum light, is highly reasonable for the detection of the signals. Therefore, the signals were measured at the mentioned wavelength, throughout the study.

The effect of flow-rate on the area of the peak at 247 nm was investigated in the range of 0.1–2.5 mL min^{-1}. A logarithmic-type curve appeared, as was demonstrated in Fig. 2.

Large highly integrated areas of the peak were exhibited between 0.1 and 0.5 mL min^{-1}, and a high slope-curve was observed at 0.1 and 1.2 mL min^{-1} flow-rate, as well. The curve reached a plateau beginning from 1.2 mL min^{-1}. Therefore, 1.3 mL min^{-1} was an appropriate one, and that flow-rate was used for the rest of the experiments, because the peaks were almost symmetrical and appropriate for analytical consecutive injections.

Validation of the Method

Validation of the method was tested considering intra- and inter-day precision. Three sets of 2.0×10^{-5} M DEF dilutions were prepared and each one was injected for three consecutive days, and the results were

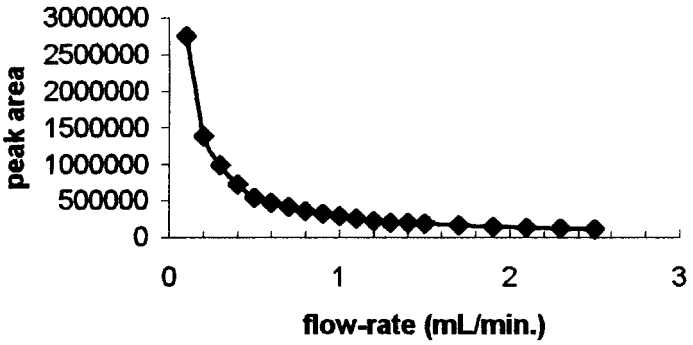

Figure 2. The variation of peak area vs. flow-rate of 1.0×10^{-5} M DEF detecting the signals at 247 nm.

evaluated statistically regarding mean ($\bar{X}$), standard deviation (SD), and relative standard deviation (RSD%). They are tabulated in Table 1.

Repeatability, which is equivalent to the RSD, explains the precision of tests. In the table, the RSD% values as intra-day precision ranged between 0.27–0.41. These values state that the experiments have been achieved precisely.

Linearity

The linearity of the method was investigated as intra- and inter-day examinations. Three sets of calibration dilutions (1.0×10^{-5}–5.0×10^{-5} M) were prepared and they were also injected on consecutive days. Outputs of the data were computed and the results are presented in Table 2.

Here, a is slope, b is intercept, r is correlation coefficient, S_r is standard deviation of regression equation, and CL is confidence limit at the 95% level.

Limit of detection ($\mathrm{LOD} = 3.3\sigma/a$) and limit of quantification ($\mathrm{LOQ} = 10\sigma/a$) were calculated using the standard deviation values in the precision (σ) and slope values in the linearity (a), and it was found as inter-day results of 2.35×10^{-7} and 7.04×10^{-7} M, respectively.

Application of the Method to DEF Tablets by FIA and UV-Spectrophotometry

The determination of DEF in pharmaceutical tablets (Filantadin®, containing 30 mg DEF) was carried out by the FIA method and the results of this study were compared to those of UV-spectrophotometry.

The detail of the procedure for the FIA analysis of DEF tablets has been presented in the experimental section, under the pharmacopoeia principals.[14]

Table 1. The intra- and inter-day precision test of DEF (2.0×10^{-5} M) using FIA detected at 247 nm, employing the flow-rate of 1.3 mL min^{-1}.

	Intra-day precision (as area)			Inter-day precision (as area)
	First day ($n = 8$)	Second day ($n = 8$)	Third day ($n = 8$)	[whole-days ($n = 15$)]
Mean, $\bar{X}$	178,118	178,332	178,198	178,216
SD	518	730	489	570
RSD %	0.29	0.41	0.27	0.32

Table 2. Results of intra- and inter-day calibration studies for linearity of the method in the concentration of 1.0×10^{-5}–5.0×10^{-5} M DEF.

	Inter-day			Intra-day [Whole days ($n = 15$)]
	First day ($n = 5$)	Second day ($n = 5$)	Third day ($n = 5$)	
Slope, a	7.96×10^9	8.05×10^9	8.02×10^9	8.01×10^9
Intercept, b	2.46×10^3	7.58×10^2	1.19×10^3	1.48×10^3
Correlation coefficient, r	0.9999	0.9999	0.9998	0.9999
SD of regression equation, $\pm S_r$	1.26×10^5	1.27×10^5	1.27×10^5	1.17×10^5
CL ($p < 0.05$)	$\pm 1.57 \times 10^5$	$\pm 1.58 \times 10^5$	$\pm 1.58 \times 10^5$	$\pm 6.60 \times 10^4$

UV-spectrophotometry was chosen as a comparison method for the determination of DEF. A similar solvent system was also employed for the UV-spectrophotometric experiments. The signals detected at 247 nm were evaluated and the relationship between the absorbance and concentration was found to be [$A = 0.14265C(M) - 0.087$, $r = 0.9999$].

Here, C is the molar concentration of DEF and A is the absorbance of the DEF solution.

The assay results of DEF analysis carried out by FIA and UV-spectrophotometry, were tested by using statistical evaluations such as $\bar{X}$, SD, and RSD, in addition to confidence limits (CL) and t and F-tests of significance (all 95% probability level) and they were demonstrated in Table 3.

High reproducibility and insignificant differences between FIA and UV-spectrophotometry were observed at the 95% probability level. The tablets also provide the general official requirements as analyzed by FIA and

Table 3. Assay results of DEF by FIA and UV-spectrophotometry in pharmaceutical tablets (each tablet contains 30 mg DEF).

	FIA ($n = 6$)	UV-spectrophotometry ($n = 6$)
Mean, $\bar{X}$	30.4 (101.3%)	30.7 (102.3%)
SD	0.14	0.31
RSD%	0.45	0.99
CL ($p < 0.05$)	0.15	0.39
t-Test ($p < 0.05$)	1.89	$t_{0.05} = 2.26$ (table)
F-test ($p < 0.05$)	4.90	$F_{0.05} = 5.19$ (table)

UV-spectrophotometry 101.3% and 102.3%, respectively.[14] These results also indicate that there is good agreement between the two methods.

In conclusion, the proposed method is simple, accurate, precise, and rapid. Therefore, it seems a promising method for the analysis of DEF tablets regarding the time of analysis, consumption of solvents, and size of the sample required for a routine analysis of DEF.

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