

Development of a Simple Colorimetric Determination of Dexamethasone

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Received: 22nd March, 2020; Revised: 21st April, 2020; Accepted: 27th May, 2020; Available Online: 25th June, 2020

ABSTRACT

A simple, environmentally benign, cost-effective, and sensitive colorimetric determination for the pharmaceutical drug dexamethasone sodium phosphate (DXP) has been developed by the formation of a colored complex with fluoranil. The process was sensitive and linear over the range 1 to 40 µg/mL, excellent correlation coefficient 0.9989, recovery% 99.80 ± 1.3, limit of detection (LOD) and limit of quantification (LOQ) were 0.23 and 0.9 µg/mL, respectively, and good RSD ~1.63%. The experimental conditions were optimized after an intensive study. The approach was validated statistically for the quantification of the analyte in its pure and/or pharmaceutical form. Despite the proposed approach is selective, it still can be applied for the analysis of other pharmaceuticals.

Keywords: Colorimetry, Dexamethasone, Fluoranil, Oxime, Spectrometry.

International Journal of Drug Delivery Technology (2020); DOI: 10.25258/ijddt.10.2.12

How to cite this article: Al-Janabi KWS, Mahmood AK, Luaibi HM. Development of a Simple Colorimetric Determination of Dexamethasone. International Journal of Drug Delivery Technology. 2020;10(2):255-258.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

DXP, IUPAC nomenclature is “disodium;[2-[(8S,9R,10S,11S,13S,14S,16R,17R)-9-fluoro-11,17-dihydroxy-10,13,16-trimethyl-3-oxo-6,7,8,11,12,14,15,16-octahydrocyclopenta[a]phenanthren-17-yl]-2-oxoethyl] phosphate,”¹ a water soluble form of dexamethasone.

It is an effective artificial glucocorticoid medication. DXP is a corticosteroid² utilized for many years as immunosuppression and anti-inflammatory for the treatment of sensitivity, aggravation, intrinsic adrenal hyperplasia, and immune system conditions.³ An inflammatory study at cultural circumstances exhibited that treatment with dexamethasone reduces the secretion of interleukin IL-8.⁴

DXP and other derivatives of the glucocorticoid hydrocortisone family have been prohibited as per their abusing as doping drugs in sports to boost performance.⁵ Consequently, it is significant to improve a sensitive analytical approach to determine of DXP. Likewise, DXP and its photochemical derivatives have a toxic effect on aquatic organisms marina.^{6,7}

Several analytical techniques were adopted for DXP quantification. The British Pharmacopoeia adopted the blue tetrazolium colorimetric test for DXP, whereas United States Pharmacopoeia 1980 method officially adopted high performance liquid chromatographic (HPLC) practice for DXP assessment.^{8,9} DXP steroidal molecule has been studied

kinetically using gas chromatographer coupled with mass spectrometric detection gas chromatography–mass spectrometry (GC-MS)^{10,11} after derivatization to increase its thermal stability and to make it suitable for GC-MS analysis.¹² HPLC approach has been consistently employed for the quantification of corticosteroids in biological fluids¹³ and in dexamethasone containing drugs. HPLC technique has a privilege over gas chromatography (GC) that the corticosteroid can be analyzed directly without derivatization.^{14,15} Other common techniques were chemiluminescence,³ spectrophotometric,¹⁶ and voltammetric.¹⁷ Nevertheless, spectrometric approaches have privilege over almost all other analytical instrumentation for their low cost, low detection limit, and high sensitivity.

EXPERIMENTAL

Instruments and Reagents

- UV-vis spectrophotometer, Shimadzu UV-1800, Japan
- FTIR spectrometer, Shimadzu IRAffinity-1, Japan
- Chemicals and solvents were of analytical grades
- DXP drug tablets containing 0.5 mg of DXP per tablet
- DXP standard solutions were prepared by dissolving it in chloroform

Procedure

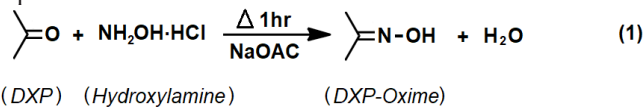
Fluoranil as a strong pi-acceptor and because its high electron affinity has been utilized along with iodine for spectrometric

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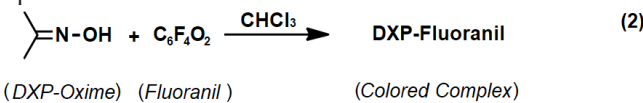
determination of corticosteroids. Fluoranyl and iodine can make a colored charge transfer complex with corticosteroids, which can be easily detected spectrometrically.

Carbonyl group of DXP can be modified by converting it into an oxime group, which contains a weak acidic hydroxyl group¹⁸ (Equation 1). The new derivative can be further derivatized by the reaction with fluoranyl to produce a colored complex that can be spectrometrically easily estimated¹⁹ (Equation 2).

Equation 1:



Equation 2:



A mixture of 20 mg standard DXP (carbonyl compound), 20 mg of hydroxylamine hydrochloride as a reactant to form oxime, and 20 mg sodium acetate as pH modifier²⁰ were liquefied into 25 mL ethyl alcohol (v/v% 75) and refluxed for 1-hour. The alcohol then evaporated at 70°C, while the residual was extracted out of two phases of 10 mL of chloroform and 10 mL of water. The organic layer of the chloroform containing the produced oxime was placed into a volumetric flask (50 mL) and filled with chloroform to the mark.

An aliquot of the collected extract that containing the produced DXP-oxime was complexed with fluoranyl solution in chloroform medium to form a colored complex. The resultant colored solution exhibited an absorbance wavelength maximum of 485 nm.

Twenty DXP tablets were crushed by a marble mortar to a fine powder. The extraction of 20 mg of the powder was accomplished by shaking with five 10 mL portions of ethyl alcohol successively. After heating up and filtration, the resultant alcoholic blend was poured into a 50 mL flask and completed with ethanol to the mark.

RESULTS AND DISCUSSION

A colored charge-transfer complex was formed upon mixing equal moles of DXP oxime with fluoranyl in chloroform. DXP-oxime has wavelength absorbance maximum λ_{max} at 243 nm in chloroform, and fluoranyl solution in chloroform absorbs at 258 nm. Accordingly, the absorbance of λ_{max} at 484.5 nm for the spectrum of the DXP-oxime with fluoranyl mixture in chloroform can only be attributed to the resultant charge transfer colored complex (Figure 1).

A mechanism may have proposed for the creation of the charge transfer DXP-fluoranyl is that the DXP-oxime has a lone pair of electrons on the heteroatom of nitrogen that can be shared in the complex formation. An electron can move down from the highest occupied molecular orbital HOMO of DXP-oxime toward fluoranyl lowest unoccupied molecular orbital LUMO. Due to its high electronegativity, fluoranyl has a higher ability to accept electrons than that of bromanyl and chloranyl.

By the application of the continuous variation method, the molar ratio of DXP-oxime to fluoranyl was found to be equal for the reactants (Figure 2). This was expected since the DXP-oxime has only one basic center that can be attached to one molecule of fluoranyl.

The formation reaction of the DXP-oxime complex was spontaneous and instant, as it reached the intensive color at room temperature within the first 2 minutes (Figure 3).

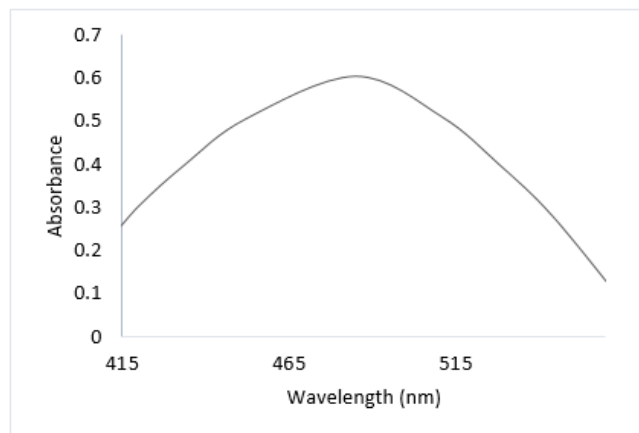


Figure 1: Spectrum of the charge-transfer DXP-fluoranyl complex resulting from the reaction of DXP-oxime with fluoranyl in chloroform

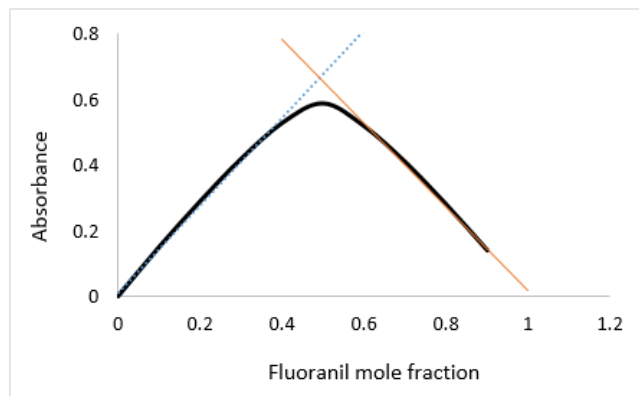


Figure 2: A plot showing the application of continuous variation method when fluoranyl added to DXP-oxime solution in chloroform; mole ratio found to be 1:1

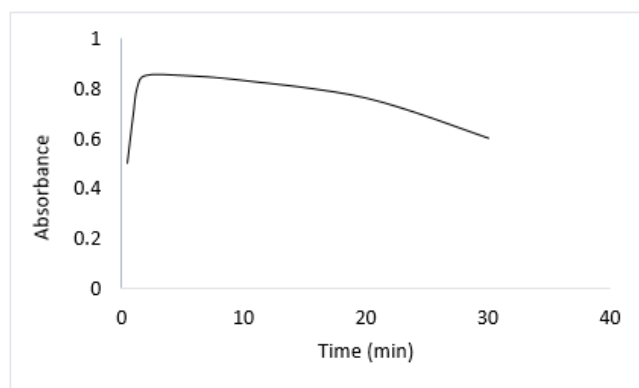


Figure 3: Kinetic study of the formation of DXP-oxime complex at room temperature

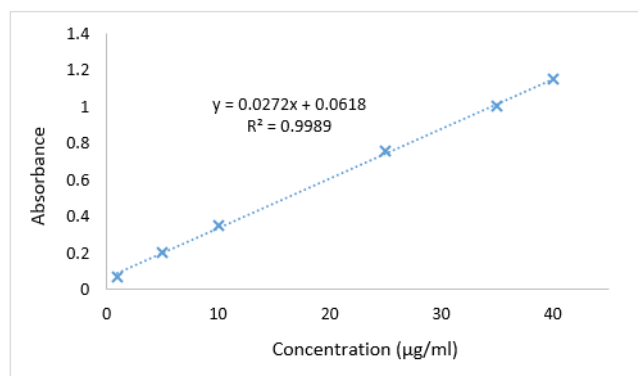


Figure 4: Calibration curve of DXP measured under optimum conditions

However, elevated temperatures, exceeding 50°C, cause decomposition for the colored complex.

Whereas, various solvents (methanol, ethanol, cyclohexane, acetonitrile, and chloroform) examined as a reaction medium for the charge transfer complexation, however, the latent exhibited the best results, since chloroform does not show any background signal within the absorbance range of measurement.

The method shows good sensitivity as per the quantification range for the determination of DXP by the developed approach was along with the range of 1 to 40 µg/mL. The LOQ and LOD were 0.9 and 0.23 µg/mL, respectively. A calibration curve was built up under optimum conditions (Figure 4).

The extraction of the crushed tablets by shaking with five 10 mL portions of ethyl alcohol before the step of complex formation increased the specificity, as well as, the recovery percentage by excluding interfered excipients. The offered technique was considered for the quantification of DXP, in its pure and pharmaceutical forms. The mean percentage recovery was $99.8 \pm 1.3\%$ for pure DXP, while the relative standard deviation RSD was 1.63%. Such outcomes verify the accuracy of the suggested approach and the least effect of interferences within the excipients.

CONCLUSION

The proposed approach was simple, eco-friendly, cost-effective, and sensitive spectrometric quantification of DXP. The method is highly reproducible since it has RSD less than 2%. Nevertheless, it can be applied to other pharmaceutical preparations. Method accuracy and precision were determined with five replicates of DXP for individual tests. The high level of method precision made it appropriate for routine analysis of DXP.

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