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# Flow Injection Spectrophotometric determination of Doxorubicin Hydrochloride in Urine samples

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**Abstract :** A simple and fast FI colorimetric procedure for determination of doxorubicin hydrochloride (DXH) was proposed. It is based on the redox reaction between iron (III) and DXH, resulting in an intensely red colored complex in the presence of 1, 10-orthophenantroline which can be followed by spectrophotometric measurement at  $\lambda_{max}$ = 510 nm. This is an automatic way to determine doxorubicin hydrochloride concentration which is applicable to the clinical laboratories for surveying curing processes. Optimum conditions for determining doxorubicin hydrochloride were investigated by univariate method. Under the optimum conditions a linear calibration graph was obtained over the range 0.1-70 µg ml<sup>-1</sup>. The detection limit (3 $\sigma$ ) and the quantification limit (10 $\sigma$ ) were 0.03 and 0.10 µg ml<sup>-1</sup> of doxorubicin hydrochloride respectively. The relative standard deviation of the proposed

Method for fifteen replicate injection of 40  $\mu$ g ml<sup>-1</sup> doxorubicin hydrochloride was 0.42%. The sample throughput was 80 h<sup>-1</sup>. The proposed method has been successfully applied to the determination of doxorubicin hydrochloride in urine samples.

Keywords: doxorubicin hydrochloride, flow injection analysis, spectrophotometry, urine samples.

## Introduction

Doxorubicin hydrochloride known chemically as (8scis)-5, 12 naphthacenedione,10- ((3-amino-2,3,6trioxy-L-lyxo-hexopyranosyl)oxy)- 7,8,9,10 tetra hydro-6,8,11-tridroxy-8-(hydroxyl acetyl)-1-methoxy hydrochloride (figure 1) and is official in the united states Pharmacopoeia (1).

DXH is a cytotoxic, anthracycline antibiotic used in antimitotic chemotherapy. It is infused intravenously to treat neoplastic diseases such as acute lymphoblastic leukemia, Wilm's tumor, soft tissue and osteogenic sarcomas, Hodgkin's disease, non- Hodgkin's lymphomas, Ewing's sarcoma, and bronchogenic, genitourinary, breast, and thyroid carcinoma (2).

The literature reveals several methods for determination of DXH in different methods. Among these methods there are flourimetry, (3,4) voltametry, (5) differential polarography, (6) liquid chromatography (7,8) thin layer chromatography (9,10) and high pressure liquid chromatography.(11-16) But these methods can not be automated easily and also be used for routine analysis in clinical laboratories. The objective of this research is accomplishing such work which can be regularly and routinely used in clinical laboratories.





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To measure micro amounts of DXH by flow injection (FI) method, the need for a rapid and facile reaction is important, so finding such reaction is a key point in this procedure. Ferric amoniom sulphate, ferric chloride or ferric nitrate plays a prominent role in the colorimetric determination of organic compounds. Acting as an oxidant, ferric salt is converted into ferrous salt and it can be detected easily by the usual reagent for divalent iron, 1, 10-orthophenantroline (PTL) (17). We have applied the

Above reaction (Fe, PTL) for determination of DXH in the pharmaceutical preparations. The objective of this work was the development of a simple, inexpensive and rapid FIA method for the routine determination of DXH in urine samples.

# Exprimental

#### Apparatus

The setup consisted of a FIA system (Perkin-Elmer) which was equipped with a four way injection valve. All system was managed by a home-made control system which was programmed in a special computer. The speed of pump and also the time of injection were managed by this system. All connectors, tees and adaptors were purchased from Supelco (USA). The spectrophotometer was from Perkin-Elmer (Lambda1)

that was prepared for FI analysis. All mixing coils were made from Polytetrafluoroethylene (PTFE) tubing in different lengths.

#### **Reagents and Solutions**

All solutions were prepared with analytical grade reagents and high purity deionized water. Iron (III) solutions were prepared by adequate dilution of a 0.02 M Fe (III) stock standard solution obtained by dissolution of exactly 8.08 g Iron (III) nitrate nanohydrate in 1000 ml of water in a volumetric flask. Aqueous solutions of PTL were prepared by dissolving 0.049 g of PTL in 100 ml of water in a volumetric flask and PTL was prepared daily. DXH solution as stock solution was 2  $\mu$ g ml<sup>-1</sup> and purchased from Sinadaroo (Iran). Different concentrations of buffer solution were produced by mixing different volumes of acetic acid and sodium acetate solution which both were 0.02 M.

### **Recommended Procedure**

DXH solutions were injected into carrier stream where PTL, buffer, and Fe (II) solution streams were merged with the carrier stream subsequently. The absorbance was measured at 510 nm. A calibration graph was prepared by plotting the maximum absorbance of peak versus DXH concentration.

Interferences	Species/DXH (w/w)	<b>Recovery %</b>
Cl	10:1	99
	100:1	96
Zn <sup>2+</sup>	10:1	98
	100:1	95
Cu <sup>2+*</sup>	10:1	98
	100:1	97
Ca <sup>2+</sup>	10:1	100
	100:1	102
Na <sup>+</sup>	10:1	102
	100:1	101
SO <sup>2-</sup>	10:1	99
$50_4$	100:1	97
PO <sub>4</sub> <sup>3-</sup>	10:1	98
	100:1	96
Urea	10:1	99
	100:1	98

Table 1. Effect of some species on the peak height of 2.0 µg ml<sup>-1</sup> DXH solution

\* After adding 0.1M of 1,5 diphenil tio carbazone

Sample	Spiked Concentration	Found Concentration	RSD
Urine sample 1	$2 \ \mu g \ ml^{-1}$	$1.984\pm0.029 \ \mu g \ ml^{-1}$	0.58%
Urine sample 2	10 μg ml <sup>-1</sup>	9.979±0.031 μg ml <sup>-1</sup>	0.49%
Urine sample 3	$35 \ \mu g \ ml^{-1}$	$34.967 \pm 0.024 \ \mu g \ ml^{-1}$	0.51%

Table 2. DXH determination in real samples



Fig. 2. Flow system manifold employed for spectrophotometric DXH determination. S=sample, C=carrier (water), R1=Fe (III) solution, R2=buffer, R3=1, 10-orthohenantroline, P=peristaltic pump, IV=injection valve, CP=confluence point, RC=reaction coil, D=detector, W=waste



Fig. 3. The effect of pH

Fig. 4. The effect of iron (III) concentration



Fig. 5. The effect of PTL concentration

#### **Result and Discussion** Flow system

Initial experiments under continuous flow conditions were carried out to examine the manifold configuration and the approximate ranges of the tested parameters. The whole feature of the opted manifold is shown in figure 2.

The profitability of flow injection analysis (FIA) as an alternative to existing methods for determination of DXH is dependent on optimization of the system to reach maximum peak height with a short residence time and minimum dispersion. As a result, different FIA variables such as reaction coil length and flow rate and chemical variables such as acidity, DXH concentration, PTL concentration and iron (III) concentration were optimized by the univariate method in the continuous flow procedure.

#### **Optimization of FI system Influence of the FIA variables**

The effect of reactor length was studied. This length was varied from 10 to 50 cm for mixing coil length. The absorbance increased slightly with increasing the mixing coil length and the optimum value was selected at 30 cm.

The effect of flow rate on peak height was studied over the range 1.79 ml min<sup>-1</sup> to 5.70 ml min<sup>-1</sup>. Constant and maximum values of absorbance were obtained in the range 2.85 ml min<sup>-1</sup> to 4.80 ml min<sup>-1</sup> and then 4.52 ml min<sup>-1</sup> was selected for further studies.

#### Influence of chemical variables

The influence of pH was studied in a range of 2.5 and 6.0 (Fig. 3). The maximum value was obtained at

pH=4.0 as shown in fig. 3. Below pH=4.0 (because of PTL protonation) the read absorbance was low and beyond pH=4.0 (because ferrous ions precipitated as Fe  $(OH)_2$ ) the complex was not formed.

Influence of temperature was considered to be between 10°C to 60°C. The absorbance was maximum in 25°C and stayed relatively constant until 60°C and for simplicity; we selected 25°C for optimum temperature. Concentrations of iron (III) and PTL were investigated. Acquired data are shown in figures 4 and 5. When the concentration of iron (III) was considered up to 400  $\mu$ g ml<sup>-1</sup>, the absorbance increased and after that the absorbance was constant. The optimum concentration of iron(III) was 400  $\mu$ g ml<sup>-1</sup>.

The effect of concentration of PTL is the same as iron(III) concentration, therefore the concentration of 2000  $\mu$ g ml<sup>-1</sup> was selected for optimum concentration since the constant absorbance was seen in that concentration.

#### Features of analytical method

With the described manifold and under the selected experimental conditions of 0.001 M of iron (III) and 0.01 M of PTL and 2  $\mu$ g ml<sup>-1</sup> of DXH, linear calibration graph between 0.1-70  $\mu$ g ml<sup>-1</sup> was obtained. The typical equation found, was: A = 0.0053[DXH] + 0.0092 Where [DXH] is concentration of doxorubicin hydrochloride in  $\mu$ g ml<sup>-1</sup> with a correlation coefficient of 0.9998.

#### **Reproducibility of proposed method**

The limit of detection calculated was 0.03 of DXH and the quantification limit was  $0.10 \ \mu g \ ml^{-1}$ . The relative standard deviation of the proposed method calculated

from fifteen replicate injection of 40  $\mu$ g ml<sup>-1</sup> of DXH was 0.42%.

The sample throughput was also investigated. This rate was 80 samples per hour and we found that 80 samples can be analyzed per 1 hour.

#### **Influence of interferences**

There are some elements which can potentially be interfering in measuring DXH concentration in pharmaceuticals and urine.

Among these ions and species are  $Zn^{2+}$ ,  $Cl^-$ ,  $Ca^{2+}$ ,  $SO_4^{2-}$ ,  $PO_4^{3-}$  and also urea. Effect of these species on DXH determination was studied. Table 1 shows recovered percentage of peak height of each interfering agent by considering 10:1 and 100:1 (w/w) of species to DXH.

Almost all species tested caused interference  $<\pm5\%$  for determining the analyte of interest. However, only the most serious interference from copper (II) ions was observed. The possible masking reagent for reducing the effect of copper (II) was 0.1 M of 1, 5-diphenil tio carbazone.

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#### Examining the proposed method on real samples

The proposed method was applied to the real samples of urine and it was revealed that this procedure is applicable to real samples. The obtained results were compared favorably with those obtained by laboratory samples. As the results show in table 2 there is no significant error at the 95% confidence level (n=12).

#### Conclusion

The proposed FI spectrophotometric method has proved to be simple, fast and cost effective for determination of DXH in real samples. This FI method is based on complexation between iron (II) and PTL has been developed, in which a small volume of DXH was injected into a carrier stream of sample and results reducing iron (III) to iron (II) and afterwards reaction with complexation agent and monitoring at 510 nm. The wide linearity of the calibration graph is in the useful ranges for quantification of DXH in real samples, with detection limit of 0.03  $\mu$ g ml<sup>-1</sup>. This method is reasonably economic and provides a good sample frequency of 80 h<sup>-1</sup>, and should be useful for routine analysis of DXH in samples such as urine in clinical laboratories.

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