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Ajithkumar and Pramod
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Short Communication

# RP-HPLC Method for Quantification of Doxorubicin in Presence of Folic Acid

K C Ajithkumar, K Pramod*
College of Pharmaceutical Sciences, Govt. Medical College, Kozhikode – 673008, Kerala, India.

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ABSTRACT

Colloidal delivery systems of doxorubicin (Dox) have been recently gained much importance for tumor targeted delivery. Many of the reported systems present Dox in delivery systems comprising of folic acid (FA). Herein we aimed for a reversed-phase high performance liquid chromatography (RP-HPLC) method for the quantification of Dox in presence of FA. The method employed a mixture of acetonitrile and water (containing 0.2% v/v formic acid) in the ratio 1:1 (v/v) as the mobile phase. It was observed that employing a detection wavelength of 480 nm eliminates any possible interference from FA. The developed method could be applied for quantification of doxorubicin during assay, drug loading and drug release studies where there are chances of folic acid interference.

**Key words:** HPLC; folic acid; acetonitrile; retention time, calibration plot

## 1. INTRODUCTION

Colloidal delivery systems of doxorubicin (Dox) have been recently gained much importance for tumor targeted delivery.[1] Many of the reported systems present Dox in delivery systems comprising of folic acid (FA).[2,3] Folic acid is mainly employed for targeting moiety.[4] It would be a challenge to quantify Dox in its delivery systems comprising of FA. Quantification of Dox during drug release studies is also a challenge wherein FA also could be released into the medium. In this context the aim to develop a selective method for the quantification of Dox in presence of FA gains much importance. So herein we report a a reversed-phase

**Corresponding author ***
**Dr. Pramod K**
Assistant Professor
Department of Pharmaceutics
College of Pharmaceutical Sciences
Govt. Medical College,
Kozhikode – 673008, Kerala, India.
E-mail address : pramodkphd@yahoo.com

high performance liquid chromatography (RP-HPLC) method for the quantification of Dox in presence of FA.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Folic acid and doxorubicin hydrochloride was obtained from from Sigma-Aldrich Co. (MO, USA). HPLC grade acetronitrile was obtained from Merck specialities Pvt. Ltd, Mumbai, India. All other reagents were of analytical-reagent grade, and Millipore ultra purified water was employed for solutions.

### 2.2. High pressure liquid chromatography (HPLC)

The HPLC method for the determination of doxorubicin was carried out on a Shimadzu UFLC (Shimadzu scientific instruments Inc., Kyoto, Japan) using photo diode array detector (Prominence SPD-M20A) with column oven (Shimadzu CTO)-10AS VP). The instrument was controlled by use of LC solutions software installed with equipment for data collection and acquisition. Compounds were separated on a C18G reverse phase column (250 × 4.6 mm, particle size 5 µm; Enable, Spinco Biotech Pvt. Ltd., Chennai, India) maintained at 35°C.

#### 2.2.1. Mobile phase

Acetonitrile and water (containing 0.2% v/v formic acid) in the ratio 1:1 (v/v) was chosen as the mobile phase.

#### 2.2.2. Chromatographic system

| | |
|---|---|
| Column | : C18G reverse phase column (250×4.6 mm, particle size 5 µm; Enable, Spinco Biotech Pvt. Ltd) |
| Flow rate | : 1.3 mL $min^{-1}$ |
| Retention time | : 2.149 ± 0.009 min |
| Detector | : PDA detector (Prominence SPD-M20A) |
| Detection wavelength | : 480 nm |
| Injection volume | : 20 µL |
| Temperature | : 35°C |
| Elution type | : Isocratic |
| Run time | : 5 min |

#### 2.2.3. Preparation of the mobile phase

HPLC grade water (containing 0.2% v/v formic acid) was mixed with HPLC grade acetonitrile in the volume ratio of 1:1. The prepared mobile phase was then filtered through a 0.22 µm nylon filter and sonicated in an ultrasonic bath for 15 min.

#### 2.2.4. Preparation of calibration plot

A stock solution of doxorubicin hydrochloride (10 mg $mL^{-1}$) was prepared in ultrapure water. From the stock solution, standard calibration solutions (20 - 200 µg $mL^{-1}$) for the assessment of linearity were prepared from this stock solution, by dilution with ultrapure water. The solutions were filtered through a 0.22 µm nylon filter. The filtered solutions were then injected into the HPLC system. The data of peak area versus drug concentration were treated by linear least square regression for assessment of linearity and range.

The quantitation limit (QL) and detection limit (DL) were determined based on the technique of signal-to-noise ratio[5] using the equations (Eqn. 1) and (Eqn. 2).

$QL = 10\ \sigma / S$ ---------- (Eqn. 1)

$DL = 3.3\ \sigma / S$ ---------- (Eqn. 2)

Where, σ is the standard deviation of the intercept of the calibration plot and S is the slope of the calibration curve.

## 3. RESULTS AND DISCUSSION

### 3.1. High pressure liquid chromatography (HPLC)

A method specific to Dox was required for the purpose of quantification of Dox at various possible stages of a study without the interference of folic acid. Interference of folic acid is a possibility during determination of Dox content and Dox release studies.

In our preliminary experiments, folic acid was found to have a retention time of 4.057 min under another chromatographic conditions suitable for Dox [*Mobile Phase:* Acetonitrile (A) and water containing 0.2% v/v formic acid (B) with a binary gradient elution with A/B ratio of 15/85 changed linearly to final ratio of 50/50 by 5 min and continued up to 10 min with the ratio of 50/50; *Chromatographic system conditions:* Flow rate = 1.3 mL $min^{-1}$; Run time = 10 min, Retention time = 7.6 min for Dox]. Further, it has been observed that employing a detection wavelength of 480 nm eliminates any possible interference from folic acid. Folic acid does not show any absorbance at 480 nm in its UV-Vis spectrum. Figure 1 show the HPLC chromatogram obtained for a folic acid at detection wavelengths of 254 and 480 nm. Thus, it was inferred from the chromatograms that folic acid will not interfere in the detection of Dox at 480 nm.

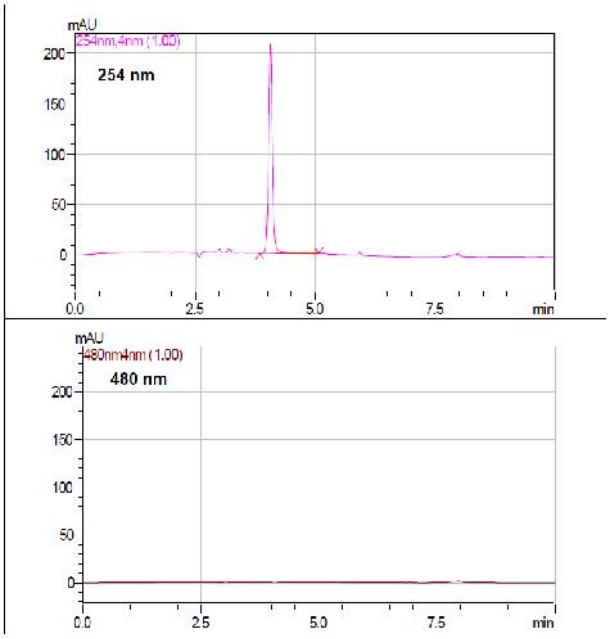


**Fig 1; HPLC chromatogram of folic acid at different detection wavelengths**

### 3.2. Calibration curve

A representative chromatogram of doxorubicin hydrochloride in the employed HPLC method is shown in

Figure 2. Insert shows UV-Vis spectrum obtained for the peak confirming that the peak is of Dox.

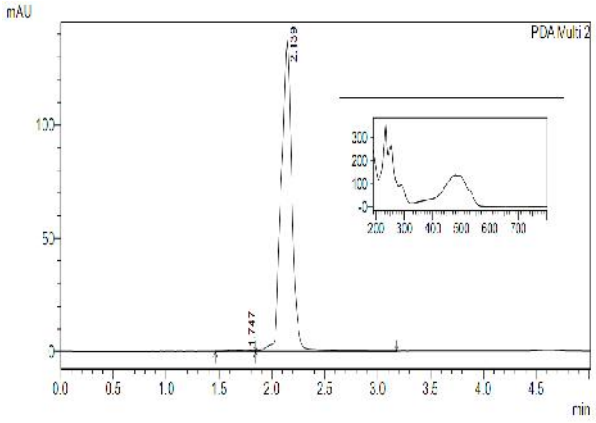


**Fig 2: HPLC chromatogram of doxorubicin hydrochloride at 480 nm (Insert shows UV-Vis spectrum obtained for the peak)**

The calibration curve for doxorubicin hydrochloride by the HPLC method is shown in Figure 3. The linear regression data for the calibration curve demonstrated a good linear relationship over the concentration range 2-200 µg $mL^{-1}$. No significant differences were observed in the slopes of standard curves as indicated by the low % RSD of 1.51. Table 1 displays the QL, DL and linear regression data for the calibration curve of doxorubicin hydrochloride.

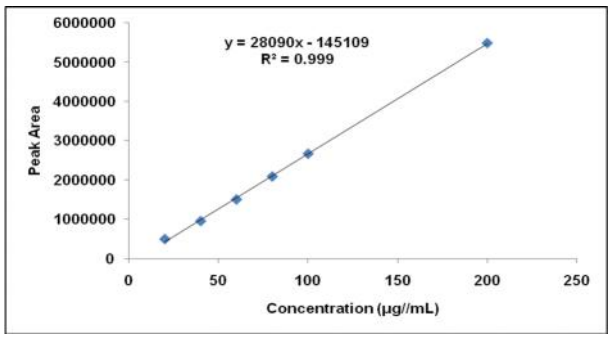


**Fig 3: Calibration curve of doxorubicin hydrochloride by HPLC method at 480 nm**

**Table 1: QL, DL and linear regression data for the calibration curve (n=3)**

| Parameter | Mean ± SD | % RSD |
|---|---|---|
| Linearity range (µg $mL^{-1}$) | 2 - 200 | ---- |
| Correlation coefficient ($R^2$) | 0.9996± 0.0002 | 0.02 |
| Slope | 28089.87± 423.83 | 1.51 |
| Intercept | -145108.87± 48894.41 | ---- |
| Quantitation limit, QL (µg $mL^{-1}$) | 17.41 | ---- |
| Detection limit, DL (µg $mL^{-1}$) | 5.74 | ---- |

## 4. CONCLUSION

Many of the reported systems present Dox in delivery systems comprising of FA. In this scenario, a method was developed for the selective quantification of Dox in presence of FA. The method employed acetonitrile and water (containing 0.2% v/v formic acid) in the ratio 1:1 (v/v) as the mobile phase. Further, it has been observed that employing a detection wavelength of 480 nm eliminates any possible interference from folic acid. A calibration plot was prepared for the developed method. The developed method could be applied for quantification of doxorubicin during assay, drug loading and drug release studies where there are chances of folic acid interference.

## 5. ACKNOWLEDGEMENT

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