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# An eco-friendly stability-indicating spectrofluorimetric method for the determination of two anticancer stereoisomer drugs in their pharmaceutical preparations following micellar enhancement: Application to kinetic degradation studies

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ABSTRACT

A new rapid and highly sensitive stability-indicating spectrofluorimetric method was developed for the determination of two stereoisomers anticancer drugs, doxorubicin (DOX) and epirubicin (EPI) in pure form and in pharmaceutical preparations. The fluorescence spectral behavior of DOX and EPI in a sodium dodecyl sulfate (SDS) micellar system was investigated. It was found that the fluorescence intensity of DOX and EPI in an aqueous solution of phosphate buffer pH 4.0 and in the presence of SDS was greatly (about two fold) enhanced and the mechanism of fluorescence enhancement effect of SDS on DOX was also investigated. The fluorescence intensity of DOX or EPI was measured at 553 nm after excitation at 497 nm. The plots of fluorescence intensity versus concentration were rectilinear over a range of 0.03–2 μg/mL for both DOX and EPI with good correlation coefficient ($r > 0.999$). High sensitivity to DOX and EPI was attained using the proposed method with limits of detection of 10 and 9 ng/mL and limits of quantitation of 29 and 28 ng/mL, for DOX and EPI, respectively. The method was successfully applied for the determination of DOX and EPI in biological fluids and in their commercial pharmaceutical preparations and the results were concordant with those obtained using a previously reported method. The application of the proposed method was extended to stability studies of DOX following different forced degradation conditions (acidic, alkaline, oxidative and photolytic) according to ICH guidelines. Moreover, the kinetics of the alkaline and oxidative degradation of DOX was investigated and the apparent first-order rate constants and half-life times were calculated.



## 1. Introduction

Doxorubicin (DOX, Fig. 1) and its stereoisomer, epirubicin (EPI, Fig. 1) are chemotherapeutic drugs that are administered for the treatment of different types of cancer. However, the clinical use of doxorubicin is limited due to its cumulative dose dependent irreversible chronic cardiomyopathic effect. This could subsequently lead to congestive heart failure, with an ultimate mortality rate of 20–40% [1]. As an anthracyclinic compound, doxorubicin can produce regression in disseminated neoplastic conditions such as leukemia, neuroblastoma, sarcomas and carcinoma [2]. The pharmacological effects of doxorubicin are related to the intercalation of the anthracycline moiety into the DNA double helix, which causes inhibition of replication and transcription of DNA in cancer cells [2]. Epirubicin differs from doxorubicin in the inversion of stereochemistry at C-4′ (daunosamine ring). Although the orientation of the 4′-hydroxyl group is equatorial in epirubicin and axial in doxorubicin, this deceptively small change does not apparently change their therapeutic efficacy. Epirubicin was shown to be effective as doxorubicin but with less cardiac toxicity at comparable doses [3]. Both DOX and EPI are official in the British Pharmacopoeia in which two liquid chromatographic methods are described for their assay in pharmaceutical preparations [4].

Literature survey revealed the sparsity of reports addressing the analysis of DOX and EPI in bulk or in pharmaceutical preparations. Only few methods were published for DOX including spectrophotometric, [5–7] electrochemical [2,8] and HPLC [9] methods while only one report was published for the determination of EPI in pharmaceutical preparations using HPLC [10]. On the other hand, many reports were published concerning the determination of DOX and EPI in biological fluids. For instant, DOX has been determined in biological fluids using spectrophotometry, [11,12] voltammetry [13], HPLC [14–18], LC-MS [19,20], UPLC [21], fluorometry [22,23] and synchronous fluorometry [24]. And EPI was determined using HPLC [3,15,25], LC-MS [26,27], HILIC [28] and UPLC. [29].

To the best of our knowledge, no spectrofluorimetric methods have been yet described for the determination of DOX or EPI in pharmaceutical preparations. In addition, no stability-indicating reports are available

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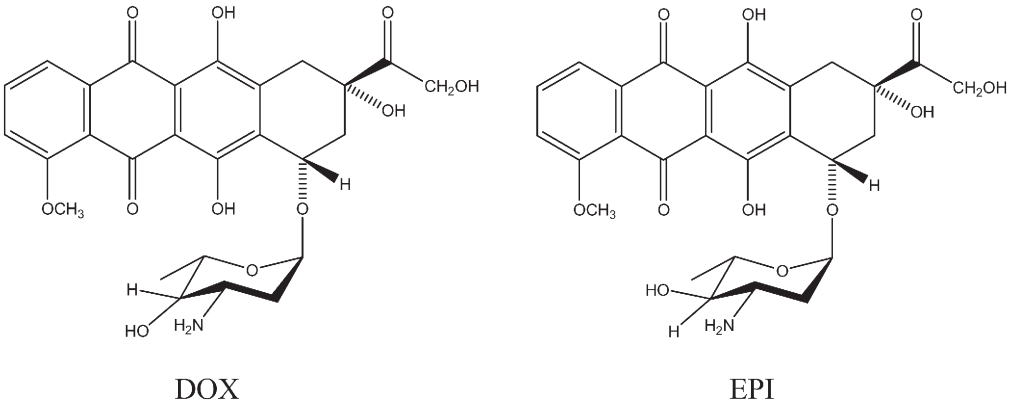


**Fig. 1.** Chemical structures of doxorubicin (DOX) and epirubicin (EPI).

for DOX or EPI that address the calculation of their rate constants and half-lives for oxidative and alkaline degradation reactions. Thus, the current study is aimed to develop and validate a simple, rapid and sensitive stability indicating spectrofluorimetric method for the determination of DOX or its stereoisomer, EPI, utilizing their native fluorescence following micellar enhancement in pharmaceutical preparations. It is noteworthy that one of the reported spectrofluorimetric methods [23] for the determination of doxorubicin in rabbit serum utilizes cetyl trimethyl ammonium bromide (CTAB) as a surfactant for micellar enhancement. Meanwhile the present study reported that sodium dodecyl sulfate (SDS) causes much better fluorescence enhancement effect on DOX compared to (CTAB) and the mechanism of the micellar-enhancement effect of SDS on DOX was also discussed in the present study.

The present study is simpler, more sensitive and more time saving, with no need for sophisticated software programs compared to the reported spectrofluorimetric methods for the analysis of DOX in biological fluids [22,24]. The proposed method is fully validated according to ICH guidelines, [30] and successfully applied for the determination of the study of these drugs in their pharmaceutical dosage forms. The method allows a quick determination of DOX and EPI in bulk drug and in pharmaceutical preparations without pretreatment of the sample with high accuracy and precision, and without interference from excipients. Furthermore, the proposed method is extended to establish the inherent stability of DOX under different stress conditions such as alkaline, acidic, oxidative and photolytic conditions. In addition, the proposed method was successfully applied for the determination of DOX and EPI in spiked human plasma and urine.

## 2. Experimental

### 2.1. Instrument

Fluorescence measurements were recorded using a Shimadzu (Kyoto, Japan) RF-1501 version 3.0 spectrofluorophotometer equipped with a 150 W xenon lamp and 1-cm quartz cells. The balance used for weighing is KERN & SOHN (GmbH Balingen, Germany). A J.P. SELECTA, S.A. (Spain) sonicator was used for sonication. All pH measurements were performed using Cyberscan (510) pH meter (Thermo Orion Beverly, MA, USA).

### 2.2. Materials and reagents

Pharmaceutical grade DOX (certified to contain 99.85%) and EPI (certified to contain 99.90%) were purchased from Selleckchem Chemicals, Houston TX, USA. The pharmaceutical formulations analyzed were Adriamycin® vials (Pfizer, UK) labeled to contain 50 mg DOX/vial and Farmorubicin® vials (Pfizer, UK) labeled to contain 10 mg EPI/vial. All solvents and materials used throughout this study were of analytical grade. Sodium dodecyl sulfate (SDS), cetyltrimethyl ammonium bromide (CTAB), hydroxymethyl cellulose (HMC), β-cyclodextrin (β-CD), Tween-80 and acetonitrile were all from Sigma-Aldrich (St. Louis, MO, USA). Sodium hydroxide, hydrogen peroxide (30% *w*/*v*), acetone, methanol, ethanol and boric acid were from (El-Nasr Chemical Ind. Co., Egypt.). Orthophosphoric acid, hydrochloric acid and disodium hydrogen phosphate were from (BDH Laboratory Suppliers, Poole, England). Borate buffer (0.1 M, pH 8–10) and phosphate buffer (0.21, pH 2.2–7) solutions were freshly prepared. SDS, CTAB, β-CD, HMC and Tween-80 were prepared as 2% *w*/*v* aqueous solutions; 6% *w*/*v* hydrogen peroxide, 0.1 M sodium hydroxide and 0.1 M hydrochloric acid were prepared for the forced degradation studies.

Plasma samples were obtained from Alexandria University Hospital (Alexandria, Egypt) and kept frozen until use after gentle thawing. Urine samples were obtained from a healthy volunteer (male, around 33 years old).

### 2.3. Standard solutions

Stock solutions 200 μg/mL of DOX and EPI were prepared in water. Working solutions of 25 and 2.5 μg/mL of each drug were prepared by further dilution of the stock solution with the same solvent to give the final desired concentration. Stock standard solutions were stored in deep freezer and diluted working solutions were stored at 4 °C in the dark.

### 2.4. Construction of the calibration graphs

Aliquots of DOX or EPI appropriate working solutions were transferred into two series of 5 mL volumetric flasks to give final concentrations of 0.03–2.0 μg/mL for both DOX and EPI and the volume was completed to 1 mL with water. Aliquots of 2 mL phosphate buffer pH 4 and 800 μL of 2% *w*/*v* SDS solution were added to each flask. The volume was completed with distilled water, the contents of the flasks were mixed well and the fluorescence intensity was measured at 553 nm after excitation at 497 nm. The corrected fluorescence intensity was plotted vs. the final drug concentration (μg/mL) to obtain the calibration graphs. Alternatively, the corresponding regression equations were derived.

### 2.5. Assay procedure for pharmaceutical preparations

An accurately measured volume of the Adriamycin® vial solution equivalent to 10 mg DOX, or of Farmorubicin® vial solution equivalent to 10 mg EPI was transferred into 50 mL volumetric flask and about 35 mL of water were added. The contents of the flask were sonicated for 10 min, completed to the mark with the same solvent to give 200 μg/mL solution of each drug. Further dilution with water was performed to get final concentration of 25 μg/mL of DOX and EPI. A