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# A novel flow injection spectrophotometric method using plant extracts as green reagent for the determination of doxycycline

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ABSTRACT

A novel flow injection spectrophotometric method was developed for the determination of doxycycline in pharmaceutical preparations using iron(III) contained in extracts from plants. The assay was based on the complex formed between doxycycline and iron(III) characterized by an absorption maximum at 435 nm. The calibration graphs obtained over the doxycycline concentration range 5–250 μg $mL^{-1}$ gave correlation coefficients of 0.9979, 0.9987 and 0.9987 with the three green reagents prepared from *Senna alata* (L.) Roxb. (*S. alata*), *Polygonum hydropiper* L. (*P. hydropiper*) or *Diplazium esculentum* (Retz.) Sw. (*D. esculentum*), respectively. The relative standard deviations of the repeatability was <2.00%. The percentage recoveries were in the range of 98.27–101.03%. Doxycycline contents obtained by this new method and by the reference methods reported in literature were in agreement at 95% confidence level with the paired *t*-test. The sample throughput was 36 $h^{-1}$ for each green reagent.



## 1. Introduction

Doxycycline is a semisynthetic tetracycline antibiotic obtained by the modification of oxytetracycline. It is used world wide as a prophylactic and therapeutic agent in the prevention and treatment of infections caused by gram positive and gram negative bacteria [1,2].

Several methods have been used to quantify the tetracycline family in pharmaceutical products and biological fluids: UV–Visible spectrophotometry [3,4], high performance liquid chromatography(HPLC) [5–7], flow injection analysis(FIA) [8,9], spectrofluorimetry [10] and capillary electrophoresis [11]. HPLC is the official method recommended for the quantitative determination of doxycycline in raw materials, tablets and capsules [12]. Although highly sensitive and selective for quantitative determination of doxycycline, HPLC is unsuitable for use by itinerant and local controllers due to the high cost of instrumentation and the need for skills and training in operation. The objective of our work was therefore to develop a simple, cost-effective and environmental friendly method for quality control of medicines. The ultimate aim is that the method may be performed on common, inexpensive equipment with a minimum of training.

Previous studies have reported on the use of green reagents prepared from *Morinda citrifolia* [13], *Clitoria ternatea, Dendrobium Sonia, Beta vulgaris* subsp. *vulgaris* [14], green tea [15] and guava leaf [16] for assaying pharmaceutical compounds or metal ions. *Lawsonia inermis* extract was also used as natural reagent for the determination of cefadroxil [17].

In this context, antibiotics of the tetracycline family have the property of forming complexes with metal ions such as iron(III). The stoichiometry of the [iron(doxycycline)$_2$] complex (Fig. 1) has been determined with the continuous variation method as reported [18,19]. Thus, it is possible to use the specific UV–Visible absorption of the complex to quantify this antibiotic in pharmaceutical preparations. We report here a method based on flow injection analysis (FIA), to assay doxycycline using a green reagent containing iron(III) obtained from *Senna alata* (L.) Roxb. (*S. alata*), *Polygonum hydropiper* L. (*P. hydropiper)* or *Diplazium esculentum* (Retz.) Sw. (*D. esculentum)* (Fig. 2). Analytical criteria for evaluation of the new method are also given.

## 2. Experimental

### 2.1. Chemicals

Double-distilled deionized water was used to prepare all solutions. All chemicals were analytical grade and used without pretreatment. Doxycycline standard was purchased from Sigma (St. Louis, MO, USA). Hydrochloric acid, sulfuric acid, acetonitrile and methanol (HPLC grade) were purchased from Merck (Darmstadt, Germany), perchloric and nitric acid were purchased from J.T. Baker (Avantor, Center Valley, PA, USA) and iron(III) nitrate was purchased from Ajax Finechem Pty Ltd. (NSW, Australia).

Solutions of hydrochloric acid, nitric acid, perchloric acid and sulfuric acid (1.0 mol $L^{-1}$) were prepared with double-distilled deionized

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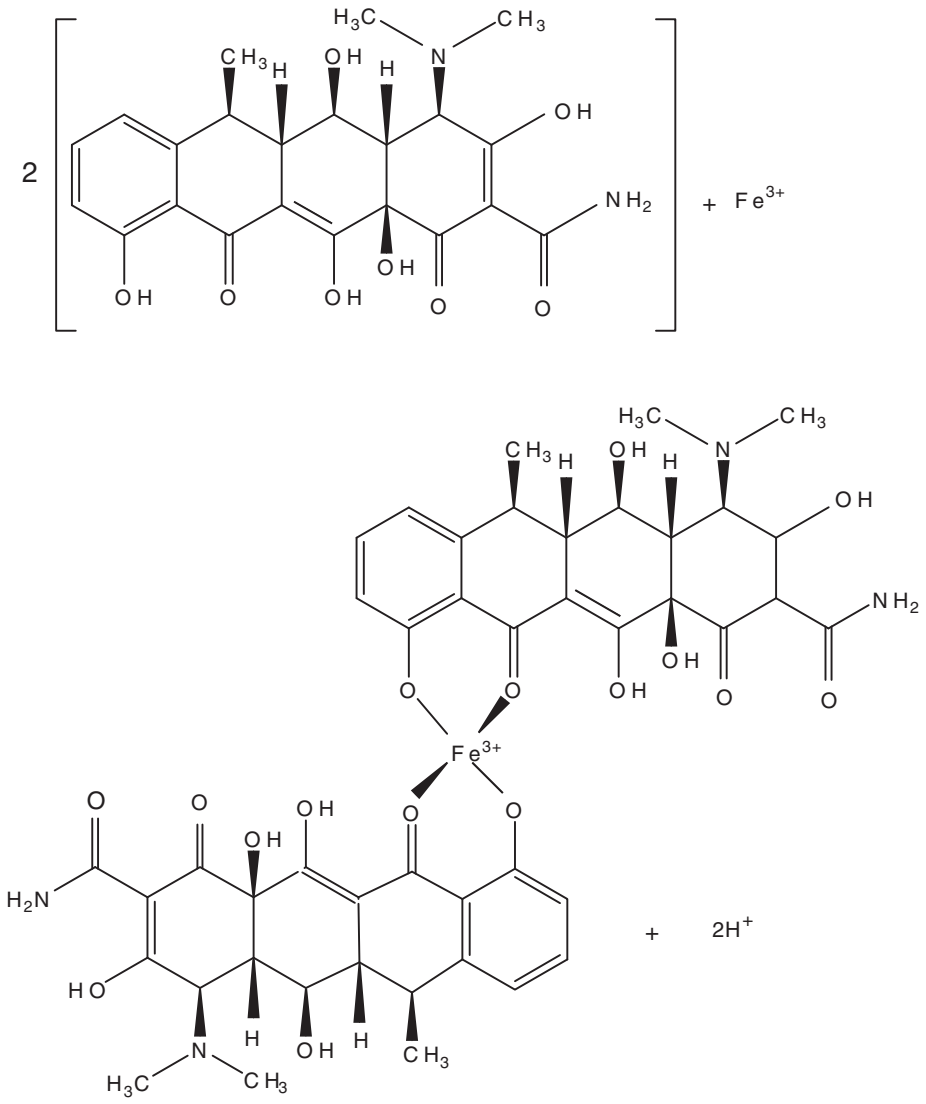

**Fig. 1.** Proposed mechanism of doxycyline and iron(III) complexation.

water. The iron(III) nitrate solution (500 μg $mL^{-1}$) was prepared in $5 \times 10^{-3}$ mol $L^{-1}$ nitric acid.

### 2.2. Instruments

The flow injection manifold consisted of a peristaltic pump (Eyela® MP3A, Tokyo Rikakikai Co. Ltd., Japan), and the standard or sample solution was injected via a four-way PTFE rotary valve with a 250 μL sample loop (Rheodyne® model 5041, Cotati, CA). PVC tubing (Elkay, Galway, Ireland) with 0.8 mm i.d. was used as a flow line for the green reagent prepared from *S. alata*, *P. hydropiper* or *D. esculentum* which was merged with the standard or the sample solution. The mixing coil was made from PTFE tubing, 0.8 mm i.d. and 150 cm in length for the recommended configuration. The FIA peak was acquired by using a UV–Visible detector (Thermo Seperation Product, TSP UV-2000, USA), coupled with a chart recorder (Kipp and Zonen® BD50, The Netherlands). A Shimadzu® UV–Visible spectrophotometer (model 1700, Japan) with matched quartz cells was used for scanning the UV spectra of doxycycline-metal complex. HPLC was Spectra series P1000 equipped with an isocratic pump, UV–Visible detector (Thermo Separation Products, TSP UV-2000, USA) and controlled by the Clarity Lite software. A reverse phase column (ACE® C18 column, 5 μm, 150 mm × 4.6 mm) was used.

### 2.3. Plants collection

Plant species were selected on the basis of consumption and availability in Thailand and Lao PDR and on iron level. Plant leaves were purchased from markets close to Khon Kean University in Khon Kaen Province, Thailand and collected from the natural forests around Vientiane Capital and in Vientiane Province, Lao PDR.

### 2.4. Extraction of iron(III) from plants

Fresh leaves were washed in tap water and cut into small pieces before being dried in hot air oven at 50 °C for 48 h. The dried leaves were ground to powder using a blender. The powder from each plant was placed in a receptacle and heated in a muffle furnace at 400 °C for 4 h. The resulting clean ash was stored in airtight containers at room temperature until required for use. In order to prepare the extracts containing iron(III), 5 g of *S. alata* ash was digested in a mixture of 25 mL 1.0 mol $L^{-1}$ nitric acid and 75 mL 1.0 mol $L^{-1}$ hydrochloric acid. For the *P. hydropiper* and *D. esculentum* extracts, 5 g of ash were digested in a mixture of 25 mL of 0.75 mol $L^{-1}$ nitric acid and 75 mL 0.75 mol $L^{-1}$ hydrochloric acid. The samples were heated on a hot plate for 30 min until the digestion process was completed. The