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# Validated stability-indicating densitometric thin-layer chromatography: Application to stress degradation studies of minocycline

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**Abstract**

A simple, stability-indicating high-performance thin-layer liquid chromatographic (HPTLC) method for analysis of minocycline was developed and validated. The densitometric analysis was carried out at 345 nm using methanol–acetonitrile–isopropyl alcohol–water (5:4:0.5:0.5, v/v/v/v) as mobile phase.

The method employed TLC aluminium plates pre-coated with silica gel 60F-254 as the stationary phase. To achieve good result, plates were sprayed with a 10% (w/v) solution of disodium ethylene diaminetetraacetic acid (EDTA), the pH of which was adjusted to 9.0. Compact spots of minocycline were found at $R_f = 0.30 \pm 0.02$. For proposed procedure, linearity ($r = 0.9997$), limit of detection (3.7 ng spot$^{-1}$), recovery (99.23–100.16%), and precision (% R.S.D. $\leq 0.364$) was found to be satisfactory. The drug undergoes acidic and basic degradation, oxidation and photodegradation. All the peaks of degradation products were well resolved from the pure drug with significantly different $R_f$ values. The acidic and alkaline degradation kinetics of minocycline, evaluated using this method, is found to be of first order.


*Keywords:* Minocycline; High-performance thin-layer chromatography; Stress degradation; Stability-indicating

## 1. Introduction

Minocycline (MC) is chemically (4*S*,4a*S*,5a*R*,12a*S*)-4,7-bis (dimethylamino)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a, 5,5a,6,11,12a-octahydrotetracene-2-carboxamide (Fig. 1). It is a semisynthetic tetracycline antibiotic used in the treatment of wide variety of extracellular and intracellular pathogens [1]. Since it is produced by chemical modification of demeclocycline, it contains several structurally related compounds as impurities such as 6-deoxy-6-demethyltetracycline (6-DODMTC), 7-didemethylminocycline (7-DDMMC), 7-monodemethylminocycline (7-MDMMC). 9-Minocycline (9-MC) is a side product while 4-epiminocycline (4-EMC) is a potential degradation product and the main impurity [2]. Liquid chromatography (LC) method using octylsilyl silica gel stationary phase for the analysis of minocycline is prescribed by the United State Pharmacopeia (USP) and the European Pharmacopoeia (EP) [3,4]. All the above mentioned impurities can be separated from the MC and from each other by LC on poly-(styrenedivinylbenzene) copolymer stationary phases with alkaline mobile phase as described by Naidong et al. [5]. Another improved LC method using a polymeric column with acidic mobile phase was described by Bryan and Stewart [6]. LC determination of MC with electrochemical detection was also reported for the separation of MC from other tetracyclines [7]. Recently, an isocratic liquid chromatographic method is described for the separation of minocycline and its impurities using XTerra RP-18, reversed phase stationary phase with reduced silanol activity [8]. Capillary zone electrophoresis [9] and thin-layer chromatography [10] using UV and fluorescence densitometry were also reported for the analysis of MC. For the quantification of minocycline in biological fluids, several methods based on high-performance liquid chromatography (HPLC) with UV detection [11], reverse-phase HPLC/UV [12], HPLC-tandem mass spectroscopy (MS–MS) analysis [13], HPLC/UV after liquid–liquid extraction [14], Because of high hydrophobicity of minocycline, the retention time is high and most of the methods are time consuming, complex, expensive and some even unable to separate minocycline from its degradation products. The determination of minocycline and

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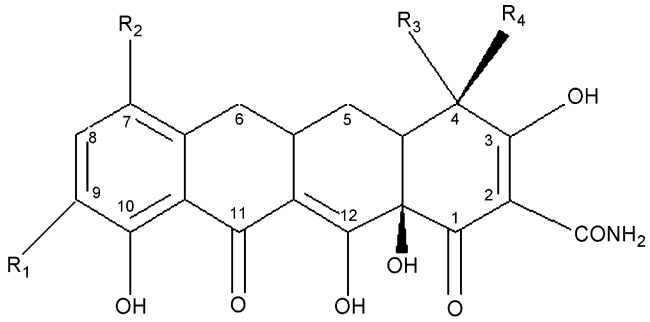


| Compound Name | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|
| Minocycline (MC) | H | $N(CH_3)_2$ | H | $N(CH_3)_2$ |
| 6-Deoxy-6-demethyl-tetracycline (6-DODMTC) | H | H | H | $N(CH_3)_2$ |
| 7-Didemethyl-minocycline (7-DDMMC) | H | $NH_2$ | H | $N(CH_3)_2$ |
| 7-Monodemethyl-minocycline (7-MDMMC) | H | $NHCH_3$ | H | $N(CH_3)_2$ |
| 9-Minocycline (9-MC) | $N(CH_3)_2$ | H | H | $N(CH_3)_2$ |
| 4-Epiminocycline (4-EMC) | H | $N(CH_3)_2$ | $N(CH_3)_2$ | H |

Fig. 1. Chemical structure of minocycline and related substances.

its degradation products has been found to be rather difficult because of structural similarity of the degradation products to minocycline and to each other consequently making separation problematic.

However to our knowledge, no information related to the stability-indicating high-performance thin-layer chromatography (HPTLC) determination of minocycline in pharmaceutical dosage forms has ever been mentioned in literature. The parent drug stability test guidelines (Q1A) issued by International Conference on Harmonization (ICH) requires that analytical test procedures for stability samples should be fully validated and the assays should be stability indicating [15]. An ideal stability-indicating method shall quantify the drug per se and also resolve its degradation products. Accordingly, the purpose of the present study is to put ICH recommendations into practice by subjecting minocycline to variety of suggested stress test conditions to establish inherent stability of the drug and to develop the validated stability indicating HPTLC assay. The proposed method is also employed for stability testing of commercial tablet and capsule formulation and for prepared polylactide co-glycolide (PLGA)-MC-nanoparticles. Furthermore, the developed HPTLC method was used to investigate the kinetics of acidic and alkaline degradation processes by quantification of drug at different temperature, to calculate the activation energy and half-life for minocycline degradation.

## 2. Experimental

### 2.1. Reagents and solvents

Minocycline was received as a gift sample form Ranbaxy Laboratories Ltd. (Gurgaon, Haryana, India) and certified to contain 99.62% (w/w) on dried basis. All other chemicals and reagents used were of analytical grade and were purchased from Merck Ltd. (Worli, Mumbai, India).

### 2.2. Instrumentation and chromatographic conditions

Precoated silica gel on aluminium Plates 60F-2 54 (20 cm × 10 cm, 200 μm thickness, E. Merck, Germany) were used after spraying with a 10% (w/v) solution of disodium ethylene diaminetetraacetic acid (EDTA), the pH of which was adjusted to 9.0 with a 10% (m/v) solution of sodium hydroxide [14]. The plates were dried in a horizontal position for at least 1 h at room temperature, and then in oven at 110 °C for 1 h, shortly before use. The samples were spotted in the form of bands of width 5 mm with Camag 100 microlitre syringe using a Linomat V (Camag, Muttenz, Switzerland) sample applicator. A constant application rate of 150 nL $s^{-1}$ was employed and the space between two bands was 10 mm. The slit dimension was kept at 5 mm × 0.45 mm and 20 mm $s^{-1}$ scanning speed was employed. The mobile phase consisted of methanol-acetonitrile-isopropyl alcohol (IPA)-water (5:4:0.5:0.5). Linear ascending development was carried out in 20 cm × 10 cm twin through glass chamber (Camag, Muttenz, Switzerland) and top of chamber was covered tightly with the lid. The optimized chamber saturation time for mobile phase was 30 min at room temperature (25 ± 2 °C) at relative humidity of 60 ± 5%. The length of chromatogram run was 8 cm. Subsequent to the development; TLC plates were dried in current of air with the help of an air dryer. Densitometric scanning was performed on Camag TLC scanner III in the absorbance mode at 345 nm and operated by winCATS software (Version 1.2.0). The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum in the range of 190–400 nm. Evaluation was done using linear regression analysis via peak areas.

### 2.3. Calibration curve of Minocycline

A stock solution of minocycline was prepared in methanol at 100 μg $mL^{-1}$. With proper dilution, standard solutions were