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Determination of minocycline by oxidative coupling and diazocoupling reactions in pharmaceutical formulations

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Abstract

Simple and sensitive spectrophotometric methods (M_1–M_4) by the application of oxidative coupling and diazocoupling reactions for the assay of minocycline (MC) in pure form and pharmaceutical formulations have been described. Methods M_1 and M_2 involve the oxidative coupling reactions of MC with 3-methyl-2-benzothiozolinone hydrazone (MBTH) (method M_1, λ_{max} 440 nm) or 4-aminophenazone (4-AP) (method M_2, λ_{max} 520 nm) in the presence of periodate. Methods M_3 and M_4 are based on the formation of diazocoupling products of MC with diazotised *p*-nitroaniline (DPNA) (method M_3, λ_{max} 420 nm) or diazotised sulfanilic acid (DSAC) (method M_4, λ_{max} 420 nm). Regression analysis of Beer's law plot showed good correlation in the concentration range of 8–48, 20–120, 4–20 and 8–40 μg ml^{-1} for methods A, B, C and D, respectively. The molar absorptivities fell within the range of 2.23×10^3–1.51×10^4 l mol^{-1} cm^{-1}.The recoveries range from 99.02 to 100.61%. © 2002 Published by Elsevier Science B.V.

Keywords: Minocycline; 3-Methyl-2-benzothiazolinone hydrazone; 4-Amino-phenazone; Sodium nitrite; *p*-Nitroaniline; Sulfanilic acid; Spectrophotometry

1. Introduction

Minocycline (MC) [1–3] (as hydrochloride) is an antibiotic and is primarily bacteriostatic through the inhibition of bacterial protein synthesis. It is chemically known as 2-naphthacenecarboxamide, 4, 7-bis (dimethyl amino)-1, 4, 4a, 5, 5a, 6, 11, 12a-octa hydro-3, 10, 12, 12a-tetra hydroxy-1, 11-dioxo-, monohydrochloride, (4S, 4a*S*, 5a*R*, 12a*S*)-]. Existing analytical methods reveal that relatively little attention was paid in developing visible spectrophotometric methods [4–7]. The analytically important functional group (phenolic hydroxyl) in MC has not been exploited so far and hence there is a need to

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develop sensitive and flexible visible spectrophotometric methods by exploiting phenolic hydroxyl group. The oxidative coupling procedure involving the use of couplers (3-methyl-2-benzothiozolinone hydrazone (MBTH) [8] or 4-AP [9]) in the presence of an appropriate oxidant to form highly coloured species were explored for the assay of drugs possessing functional groups such as phenolic hydroxyl, aldehyde, amine or diol in general. Diazocoupling reactions using diazonium salts (diazotised *p*-nitroaniline (DPNA) [10] or diazotised sulfanilic acid (DSAC) [11]) have opened the way to a very great number of characterisation and colorimetric determinations. This paper describes the applications of oxidative coupling reactions by using couplers (MBTH or 4-AP) in the presence of periodate and the diazocoupling reactions by using the diazonium salts (DPNA or DSAC) for the assay of MC. The relationship between the structure of MC due to the presence of phenolic hydroxyl and its conversion to coloured coupling product was explored in the present methods. The results for all these methods are statistically validated.

2. Experimental

2.1. Instruments

A Milton Roy Spectronic 1201 and Systronics 106 digital spectrophotometer with 1 cm matched quartz cells were used for the spectral and absorbance measurements in the UV and visible regions, respectively.

2.2. Reagents

All the chemicals and reagents used were of analytical grade and the solutions were freshly prepared. Aqueous solutions of MBTH (Fluka, 8.55×10^{-3} M), 4-AP (Ferak, 4.92×10^{-2} M), $NaIO_4$ (BDH, 9.35×10^{-3} M), PNA (Fluka, 7.24×10^{-3} M in 0.2 M HCl), SAC (Sd-fine, 5.77×10^{-3} M in 0.2 M HCl), $NaNO_2$ (E-Merck, 1.44×10^{-2} M) and NaOH (Loba, 1 M) were prepared using triply distilled water.

2.3. Standard drug solutions

A 1 mg ml^{-1} solution was freshly prepared by dissolving 100 mg of pure MC in 100 ml of distilled water and this stock solution was diluted stepwise with distilled water to obtain the working standard solutions of concentrations of 100 μg ml^{-1} (for method M_3), 200 μg ml^{-1} (for methods M_1 and M_4) and 500 μg ml^{-1} (for method M_2).

3. Recommended procedures

3.1. For pure form

3.1.1. Method M_1

Aliquots of standard MC solution (1.0–6.0 ml, 200 μg ml^{-1}) were transferred into a series of 25 ml calibrated tubes. Then 2.0 ml of MBTH solution was added and kept aside for 10 min. After that, 0.5 ml of $NaIO_4$ solution was added and kept aside for 20 min. The volume was made up to the mark with distilled water. The absorbance was measured at 440 nm against a similar reagent blank. The amount of MC was computed from its Beer's law plot prepared with standard drug solution under identical conditions.

3.1.2. Method M_2

Aliquots of standard MC solution (1.0–6.0 ml, 500 μg ml^{-1}) were transferred into a series of 25 ml calibrated tubes. Then 3.0 ml of 4-AP solution was added and kept aside for 10 min. After that, 1.0 ml of $NaIO_4$ solution was added and kept aside for 5 min. The volume was made up to the mark with distilled water. The absorbance was measured at 520 nm against a similar reagent blank. The amount of MC was computed from its Beer's law plot prepared with standard drug solution under identical conditions.

3.1.3. Method M_3

Into a series of 25 ml calibrated tubes, 1.0 ml of PNA and 1.0 ml of $NaNO_2$ were added and kept aside for 10 min. Aliquots of standard MC solution (1.0–5.0 ml, 100 μg ml^{-1}) were added to these tubes. Then 2.0 ml of NaOH solution was added and the volume was made up to the mark

with distilled water. The absorbance was measured after 5 min at 420 nm against a similar reagent blank. The amount of MC was computed from its Beer's law plot prepared with standard drug solution under identical conditions.

3.1.4. Method M_4

Into a series of 25 ml calibrated tubes, 2.0 ml of SAC and 2.0 ml of $NaNO_2$ were added and kept aside for 10 min. Aliquots of standard MC solution (1.0–5.0 ml, 200 μg ml^{-1}) were added to these tubes. Then 2.0 ml of NaOH solution was added and the volume was made up to the mark with distilled water. The absorbance was measured after 5 min at 420 nm against a similar reagent blank. The amount of MC was computed from its Beer's law plot prepared with standard drug solution under identical conditions.

3.2. For pharmaceutical formulations

An accurately weighed amount of capsule powder equivalent to 100 mg of MC was extracted with isopropanol (4×15 ml) and filtered. The combined filtrate was evaporated to dryness and the residue was dissolved in 100 ml of distilled water to achieve a concentration of 1 mg ml^{-1}. The solution was further diluted stepwise with distilled water to get working standard solutions and analysed under procedures described for bulk samples.

The UV spectrophotometric method which was suggested for the identification of MC in USP [2] has been moulded for its assay and chosen as the reference method for ascertaining the accuracy of the proposed methods.

4. Results and discussion

4.1. Parameters fixation

The optimum conditions for the colour developments of methods (M_1–M_4) were established by varying the parameters one at a time keeping the others fixed and observing the effect produced on the absorbance of the coloured species [12]. The following experiments were conducted for this purpose and the conditions so obtained were incorporated in recommended procedures.

4.1.1. Methods M_1 and M_2

In order to establish experimental conditions, the effect of various parameters such as volumes of MBTH or 4-AP, $NaIO_4$, waiting time, order of addition of reagents and the stability of coloured species were studied at room temperature. The applicability of MBTH or 4-AP in combination with various oxidising agents such as Fe(III), Ce(IV), Cr(VI), IO_4^-, $Fe(CN)_6^{3-}$ were examined. Preliminary investigations revealed that the cationic oxidants [Fe(III), Ce(IV), Cr(VI)] produced yellow colour with MC and enhanced the final colour. So further investigations were carried out with anionic oxidants as they do not produce any colour directly with MC. IO_4^- was found to be superior because of its sensitivity over $Fe(CN)_6^{3-}$. The laboratory temperature was found to be optimal for both the experiments. 1.8–2.2 ml of 8.55×10^{-3} M MBTH and 0.4–0.6 ml of 9.35×10^{-3} M $NaIO_4$ (for method M_1) or 2.0–4.0 ml of 4.92×10^{-2} M 4-AP and 0.6–1.4 ml of 9.35×10^{-3} M $NaIO_4$ (for method M_2) were found to be necessary for maximum colour development. So 2.0 ml of 8.55×10^{-3} M MBTH and 0.5 ml of 9.35×10^{-3} M $NaIO_4$ (for method M_1) or 3.0 ml of 4.92×10^{-2} M 4-AP and 1.0 ml of 9.35×10^{-3} M $NaIO_4$ (for method M_2) were preferred for further investigations. Addition of MC, MBTH or 4-AP and $NaIO_4$ in that order gave maximum absorbance. Altering this order of addition (i.e. MC + $NaIO_4$ + MBTH or 4-AP) resulted in decrease in absorbance. Final colour was achieved with 20 min and 5 min for methods M_1 and M_2, respectively. Both the colour products were stable for further 30 min and were measured at 440 nm (for method M_1) and 520 nm (for method M_2) (Fig. 1).

4.1.2. Methods M_3 and M_4

In order to establish experimental conditions, the effect of various parameters such as volumes of PNA (M_3) or SAC (M_4), sodium nitrite, NaOH, waiting time for diazotisation and for maximum colour formation and the stability of coloured species were studied at room tempera-

ture. Optimum volumes 0.8–1.2 ml each of 7.24×10^{-3} M PNA and 1.44×10^{-2} M $NaNO_2$ (for method M_3) or 1.6–2.4 ml each of 5.77×10^{-3} M SAC and 1.44×10^{-2} M $NaNO_2$ (for method M_4) were found to be adequate to produce diazonium chloride. Adding more than list volumes of PNA or SAC produced erratic results while $NaNO_2$ gave constant results. Ten minutes time was found be necessary for diazotisation. So 1 ml each of 7.24×10^{-3} M PNA and 1.44×10^{-2} M $NaNO_2$ (for method M_3) or 2.0 ml each of 5.77×10^{-3} M SAC and 1.44×10^{-2} M $NaNO_2$ (for method M_4) were preferred for further investigations. Minimum amount of 2.0 ml of 1 M NaOH was found to be suitable to maintain alkaline conditions in both the methods for the coupling of MC. The final colour (λ_{max} 420 nm) in each method (M_3 and M_4) was attained within 2 min and remained stable for 30 min (Fig. 2). The same results were obtained over the temperature range 10–30 °C. So it is not necessary to cool the solution in ice. However, low absorbances were observed at temperatures beyond 35 °C.

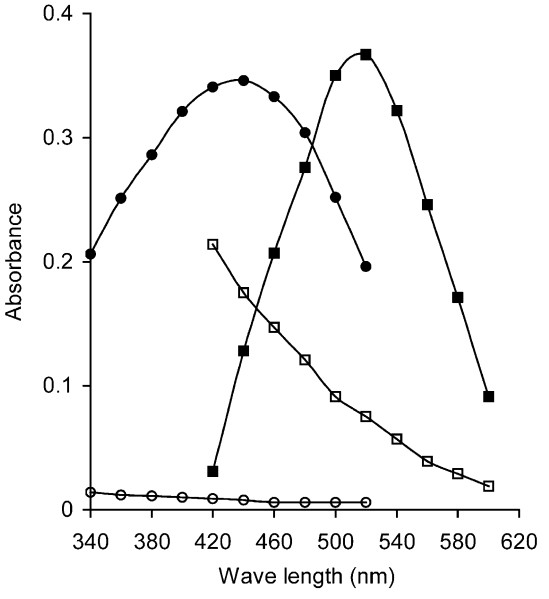


Fig. 1. Absorption spectrum of MC–MBTH–IO_4^- system (●–●) against reagent blank (○–○) vs. distilled water (M_1). [MC] = 6.47×10^{-5} M. [MBTH] = 6.84×10^{-4} M. [$NaIO_4$] = 1.87×10^{-4} M. Absorption spectrum of MC–4 AP–IO_4^- system (■–■) against reagent blank (□–□) vs. distilled water (M_2). [MC] = 1.61×10^{-4} M. [4 AP] = 5.90×10^{-3} M. [$NaIO_4$] = 3.74×10^{-4} M.

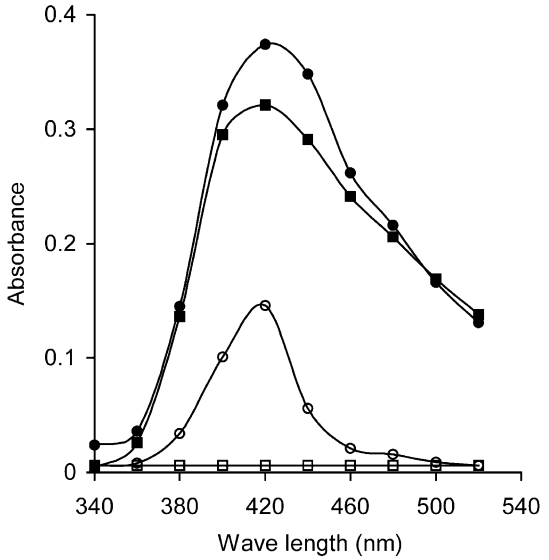


Fig. 2. Absorption spectrum of MC–PNA–HNO_2 system (●–●) against reagent blank (○–○) vs. distilled water (M_3). [MC] = 2.42×10^{-5} M. [PNA] = 2.89×10^{-4} M. [$NaNO_2$] = 5.76×10^{-4} M. [NaOH] = 8.00×10^{-2} M. Absorption spectrum of MC–SAC–HNO_2 system (■–■) against reagent blank (□–□) vs. distilled water (M_4). [MC] = 4.85×10^{-5} M. [SAC] = 4.61×10^{-4} M. [$NaNO_2$] = 1.15×10^{-3} M. [NaOH] = 8.00×10^{-2} M.

4.2. Analytical data

In order to test whether the coloured species formed in above methods adhere to Beer's law, the absorbances at appropriate lengths of a set of solutions containing varying amounts of MC and specified amounts of reagents (as given in the recommended procedures for each method) were recorded against the corresponding reagent blanks. Beer's law limits, molar absorptivity, Sandell's sensitivity and optimum photometric range for MC in each method developed with mentioned reagents were calculated. Least-square regression analysis was carried out for getting the slope, intercept and the correlation coefficient values. The precision of the proposed methods was ascertained from the absorbance values obtained by actual determination of six replicates of a fixed amount of MC in total solution (12 μg ml^{-1} for

method M_3; 24 μg ml^{-1} for method M_4; 32 μg ml^{-1} for method M_1 and 80 μg ml^{-1} for method M_2). The percent relative standard deviation and percent range of error (95% confidence limits) were calculated for the proposed methods (Table 1).

4.3. Analysis of formulations

The analytical utility of each method was verified by means of replicate assays of commercial formulations (capsules) containing MC. The values obtained by the proposed and reference methods for formulations were compared statistically with *t*- and *F*-tests and found not to differ significantly. The results were summarised in Table 2.

4.4. Interference studies

The effect of various substances that often accompany in various pharmaceutical formulations were studied separately in all the methods. To an aliquot containing 800 μg (for method M_1), 2000 μg (for method M_2), 300 μg (for method M_3) and 600 μg (for method M_4), MC different amounts of various ingredients and additives were added individually until a solution showed the same absorbance (± 0.01) as that of pure MC solution under experimental conditions as described under the procedure. The commonly used concomitants and additives in the preparation of formulation such as talc (up to 200-fold excess w/v), starch (150-fold), boric acid (150-fold), stearic acid (70-fold), cetyl alcohol (10-fold) and sodium lauryl sulfate (150-fold) did not interfere with the assay of MC by proposed methods. The other therapeutic components which do not contain free phenolic hydroxyl group also do not interfere in the colour development by proposed methods.

4.5. Stability studies

The stability of MC in aqueous solution is up to 6 h, while during actual analysis the stability of coloured species after maximum colour development remains stable for 30 min in all four methods. Hence these methods can be used for the stability studies.

Table 1
Optical characteristics, precision and accuracy of the proposed methods for MC

Parameters	Methods			
	M_1	M_2	M_3	M_4
λ_{max} (nm)	440	520	420	420
Beer's law limits (μg ml^{-1})	8–48	20–120	4–20	8–40
Molar absorptivity (1 mol^{-1} cm^{-1})	5.30×10^{3}	2.23×10^{3}	1.51×10^{4}	6.66×10^{3}
Detection limit (μg ml^{-1})	0.646	1.177	0.284	0.369
Sandell's sensitivity (μg cm^{-2}/0.001 absorbance unit)	0.093	0.220	0.032	0.074
Optimum photometric range (μg ml^{-1})	10.1–42.6	25.7–102.3	4.5–17.8	9.4–38.4
Regression equation (*y*)[a]				
Slope (*b*)	1.08×10^{-2}	4.56×10^{-3}	3.04×10^{-2}	1.31×10^{-2}
Standard deviation on slope	7.47×10^{-5}	2.31×10^{-5}	2.17×10^{-4}	6.07×10^{-5}
Intercept (*a*)	-2.26×10^{-3}	6.66×10^{-5}	4.10×10^{-3}	5.10×10^{-3}
Standard deviation on intercept	2.32×10^{-3}	1.79×10^{-3}	2.88×10^{-3}	1.61×10^{-3}
Correlation coefficient (r)	0.9999	0.9999	0.9999	0.9999
Relative standard deviation (%)[b]	0.951	2.175	2.123	2.026
% Range of error (95% confidence limits)	0.998	2.281	2.230	2.127

[a] $y = a + bc$, where c is the concentration in μg ml^{-1} and y is the absorbance unit.
[b] Six replicate samples.

Table 2
Assay of MC in pharmaceutical formulations

Pharmaceutical formulations[a] (capsules)	Labelled amount (mg)	% Recovery by proposed methods (mg)[b]				UV reference method (mg)
		M_1	M_2	M_3	M_4	
C_1	100	99.67 ± 0.30 $t = 1.15$ $F = 1.00$	99.66 ± 0.36 $t = 1.37$ $F = 1.44$	99.93 ± 0.26 $t = 1.38$ $F = 1.33$	100.61 ± 0.30 $t = 1.08$ $F = 1.00$	99.86 ± 0.30
C_2	100	99.02 ± 0.13 $t = 1.06$ $F = 1.91$	99.08 ± 0.23 $t = 1.05$ $F = 1.63$	100.18 ± 0.22 $t = 0.89$ $F = 1.49$	99.72 ± 0.21 $t = 1.82$ $F = 1.36$	99.87 ± 0.18
C_3	100	99.61 ± 0.30 $t = 1.00$ $F = 1.33$	100.16 ± 0.33 $t = 0.56$ $F = 1.61$	99.74 ± 0.25 $t = 1.19$ $F = 1.08$	99.92 ± 0.31 $t = 0.84$ $F = 1.42$	99.72 ± 0.26
C_4	100	99.37 ± 0.22 $t = 1.00$ $F = 1.67$	99.82 ± 0.23 $t = 1.01$ $F = 1.83$	99.17 ± 0.18 $t = 1.72$ $F = 1.12$	100.02 ± 0.22 $t = 1.00$ $F = 1.67$	99.91 ± 0.17

[a] Four different batches of capsules from a pharmaceutical company.
[b] Average ($\pm$ RSD) of six determinations; the t- and F-values refer to comparison of the proposed method with the reference method. Theoretical values at 95% confidence limits, $t = 2.57$, $F = 5.05$.

4.6. Chemistry of the coloured species

4.6.1. Method M_1

When treated with periodate, MBTH undergoes oxidation with loss of two electrons and one proton, forming the electrophilic intermediate which has been postulated to be the active coupling species. The electrophilic intermediate couples with MC due to the presence of phenolic hydroxyl by electrophilic attack on the most nucleophilic site of the aromatic ring to the phenolic hydroxyl group [8] (*para* or *ortho* if *para* position to phenolic hydroxyl group is substituted) and the resulting intermediate species is spontaneously oxidised with periodate to form the coloured oxidative coupling product as shown in Scheme 1.

4.6.2. Method M_2

4-AP in the presence of periodate yields N-substituted quinone imine which in turn is known to spontaneously react with phenolic compounds [9] to yield a red coloured antipyrine dye. The phenolic hydroxyl group present in MC renders it an extremely suitable substrate for this reaction. The coloured oxidative coupling product formation based on analogy is presented in Scheme 1.

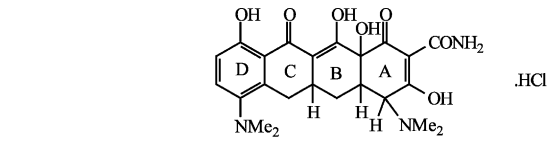
4.6.3. Methods M_3 and M_4

The diazocoupling reaction may be considered as a proton eliminating condensation of a diazonium salt with another compound possessing an active hydrogen atom. Coupling of a diazonium salt formed from aromatic primary amine (e.g. *p*-nitro aniline [10], sulfanilic acid [11]) takes place in mild acid conditions. Substitution usually proceeds to *para* position to the active group or to *ortho* if *para* is substituted. Both the methods involve two steps. In the first step, *p*-nitroaniline (M_3) or sulfanilic acid (M_4) is treated with nitrous acid (from sodium nitrite and HCl) to get diazotised *p*-nitroaniline (DPNA) or diazotised sulfanilic acid (DSAC). In the second step MC is added to DPNA or DSAC to get diazocoupled product as shown in Scheme 1.

5. Conclusions

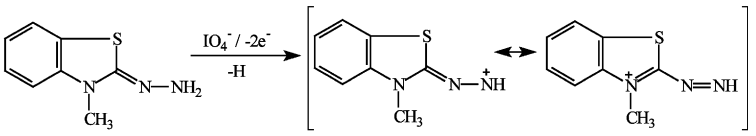
The proposed methods exploit the applications of oxidative coupling and diazocoupling reactions due to the presence of phenolic hydroxyl in MC. The differences between proposed and reported methods are dependent on the nature of reagents, type of reactions and coloured species formed.

The decreasing order of sensitivity and the increasing order of λ_{max} among the proposed methods are $M_3 > M_4 > M_1 > M_2$ and $M_3 = M_4 < M_1 < M_2$, respectively. Thus all the proposed methods are simple and sensitive with good precision and accuracy and can be used as alternatives for the assay of MC in pharmaceutical formulations.

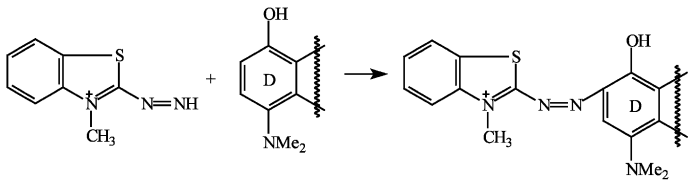


MC

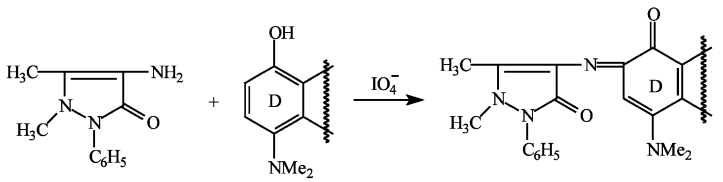
Method M_1:



MBTH



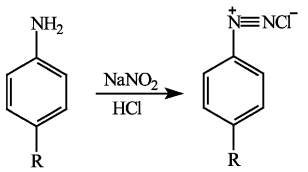
Method M_2:



4-AP

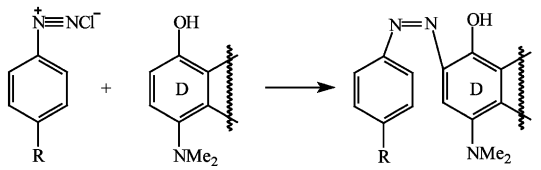
Methods M_3 and M_4:

Step-1:



where
R = NO_2 for PNA
R = SO_3H for SAC

Step-2:



Scheme 1.

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