FIRST DERIVATIVE SPECTROPHOTOMETRIC DETERMINATION OF DEMECLOCYCLINE AND MINOCYCLINE IN CLINICAL SAMPLES, URINE AND HONEY

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Abstract: The authors present simple and rapid method for the first derivative spectrophotometric determination of demeclocycline (DMC) and minocycline (MNC). The linear calibration function is established in the concentration range 10 to 50 µg/ml for DMC and 10 to 60 µg/ml for MNC. The proposed method is also applied for their resolution in synthetic binary mixtures. The resolution is based on the first derivative ultraviolet spectrophotometry with zero crossing measurement. The corresponding calibration function is linear in the rage 10 to 40 µg/ml for DMC and 10 to 50 µg/ml for MNC. The proposed methods are found to be selective, linear (R > 0.99), accurate (recovery = 98 to 104%) and precise (RSD < 1.1%) in the respective linear concentration ranges. The method is successfully applied for the analysis of these drugs in clinical samples, urine and honey without pretreatment.

Keywords: Demeclocycline; Minocycline; First derivative; Resolution; Applications.

1. INTRODUCTION

Tetracyclines are the broad spectrum antibiotics and are effective against nearly all gram positive and gram negative organisms. The antibacterial activity of tetracyclines is also attributed to their ability to form complexes (Albert, 1953). A number of spectrophotometric methods based on their complexation reactions with metal ions are reported in the literature (Basanti Rao et al.,. 1996; Siva Chandra et al., 1996 and Suryanarayana Rao and Rama Devi, 1993). In these methods tetracyclines show similar analytical behavior. Therefore it is not possible to achieve the resolution of binary mixtures of tetracyclines. A brief review of literature suggests that only few methods are reported for the simultaneous determination of tetracyclines (Ai, et al., 2009; Gaiping et al., 2009; Hai-Tao al., 2004; Rufino et al., 2009 and Yan et al., 2006). These methods are time-consuming, expensive, employ complicated procedure and are not suitable for routine analysis.

In the recent years, the interest in the analytical applications of derivative spectrophotometry has been increasing. The principle advantage of the derivative measurements is the improvement in the detectability of minor spectral peaks. Only few methods are reported in the literature for the derivative spectrophotometric determination of tetracyclines (Wahbi et al., 1985 and Salinas et al., 1989).

2. OBJECTIVES

2.1 General

The general objective of this investigation is to present simple and sensitive method for the analysis of demeclocycline and minocycline.

2.2 Specific

1. To extend the method for the determination of DMC and MNC in their synthetic mixtures.
2. To apply the proposed method for the analysis of said drugs in urine, honey and in pharmaceutical formulations.

3. EXPERIMENTAL

Spectral measurements are performed on an Elico SL UV-Visible spectrophotometer. The pH measurements are made using an Elico pH meter. All reagents used for the studies are analytical grade obtained from Merck. Double distilled water is employed for the preparation of solutions.

3.1 Preparation of sample solutions

3.1.1 Demeclocycline

The content of twenty tablets is finely powered. 50 mg of the sample is transferred into a 100 ml volumetric flask containing

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0.5 ml of 5M sulphuric acid. The solution is made up to the mark with double distilled water to get 0.5 mg/ml DMC solution.

3.1.2 Minocycline

The content of twenty capsules was finely mixed and 50 mg of the sample is taken into a 100 ml volumetric flask. The sample is dissolved in 100 ml of double distilled water so that final concentration of MNC solution is 0.5 mg/ml.

3.2 General Procedure

3.2.1 Analytical determination of demeclocycline

Different aliquots of 0.5 mg/ml demeclocycline solution are taken into the 10 ml standard flasks. To this, 5 ml of buffer solution of required pH is added and the solution is made up to the mark using double distilled water. The spectra are recorded against the blank solution containing no drug. Based on the method reported in the literature (Savitzky and Golay, 1964; Steinier et al., 1972) first derivative spectra are smoothened to the use of 24 experimental points.

3.2.2 Analytical determination of minocycline

Solutions are prepared in 10 ml standard flasks. The absorption spectra for solutions containing different aliquots of 0.5 mg/ml MNC, 5 ml of buffer solution of required pH and remaining amount of distilled water are recorded against a blank solution containing no drug.

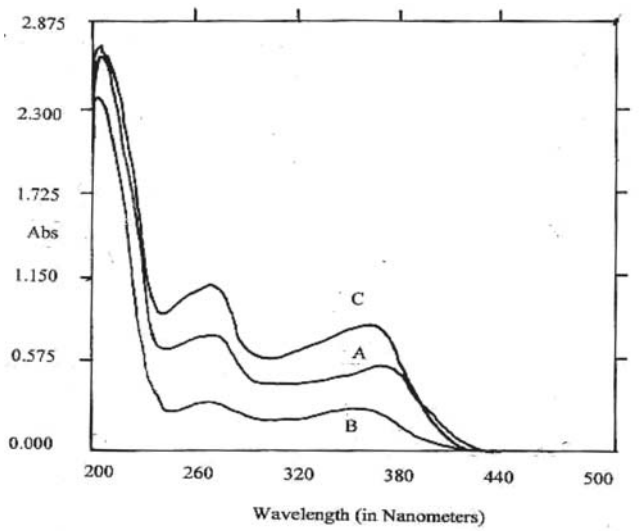

Figure 1: (A) Absorption spectrum of demeclocycline, (B) Absorption spectrum of minocycline, (C) Absorption spectrum of a mixture of demeclocycline and minocycline pH = 3; [DMC] = [MNC] = 25 µg/ml:

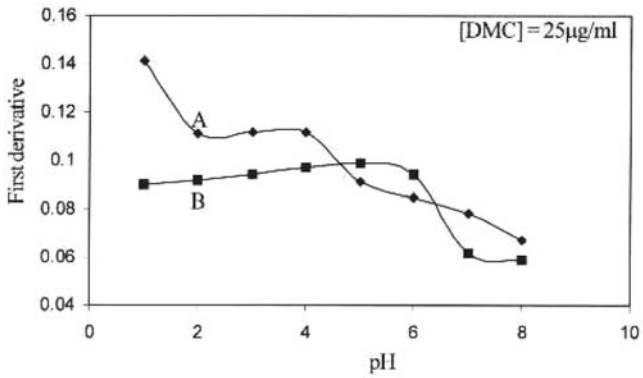

Figure 2: Effect of pH on first derivative amplitude (A) At 281.0 nm (B) At 382.0 nm

3.2.3 Resolution of demeclocycline and minocycline

5 ml of buffer solution of required pH and different aliquots of 0.5 mg/ml DMC and MNC solutions are taken in 10 ml standard flasks. The solution is made up to the mark with double distilled water. The spectra are recorded against the blank solution containing no drug.

4. RESULTS AND DISCUSSION

4.1 Effect of pH

The zero order spectra of DMC and MNC showed two absorption maxima in the entire pH range of study. As the absorption spectra of DMC and MNC are similar, spectrophotometric determination of one compound in the presence of the other is not possible using zero order spectra (Fig.1) The author employed derivative spectrophotometry for this purpose.

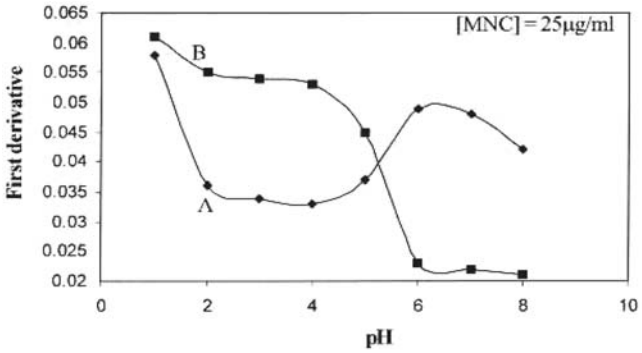

Figure 3: Effect of pH on first derivative amplitude (A) At 277.5 nm (B) At 378.5 nm

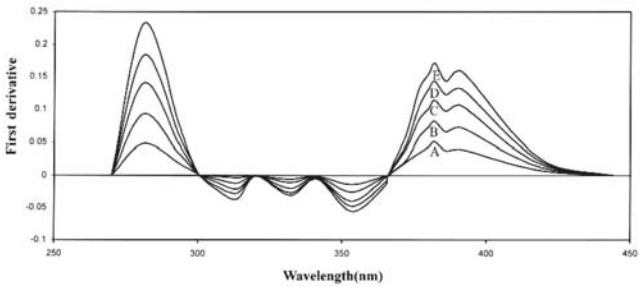

Figure 4: First derivative spectra at various concentrations of demeclocycline (A) 10 µg/ml; (B) 20 µg/ml; (C) 30 µg/ml; (D) 40 µg/ml; (E) 50 µg/ml

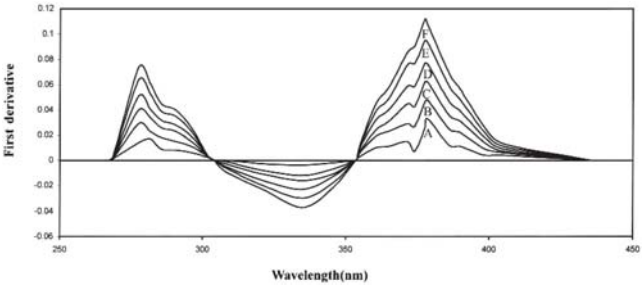

Figure 5: First derivative spectra at various concentrations of minocycline (A) 10 µg/ml; (B) 20 µg/ml; (C) 30 µg/ml; (D) 40 µg/ml; (E) 50 µg/ml; (F) 60 µg/ml

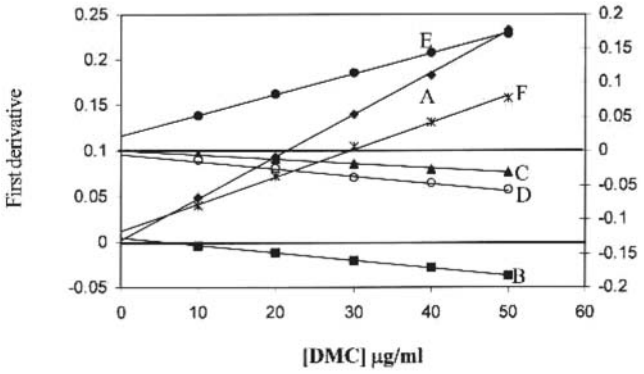

Figure 6: Calibration plots of demeclocycline at different wavelengths
Primary Y-axis: (A) 281 nm; (B) 313.5 nm; (F) 391.5 nm
Secondary Y-axis: (C) 332.0 nm; (D) 353.5 nm; (E) 382 nm

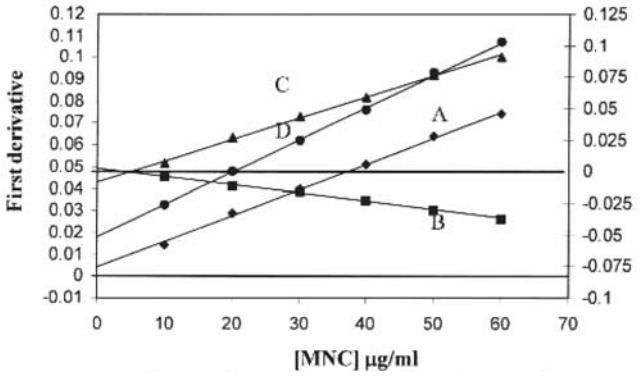

Figure 7: Calibration plots of minocycline at different wavelengths
Primary Y-axis: (A) 277.5 nm; (D) 378.5 nm;
Secondary Y-axis: (B) 335.0 nm; (C) 374.0 nm

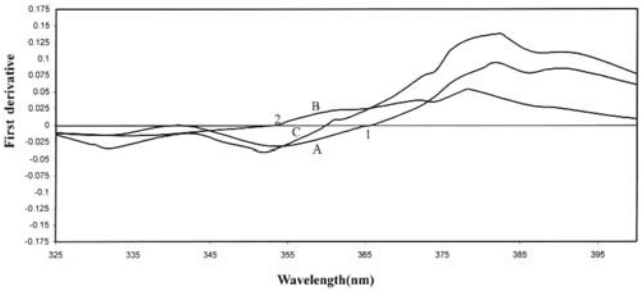

Figure 8: (A) First derivative spectrum of demeclocycline (25 mg/ml)
(B) First derivative spectra of minocycline (25 mg/ml)
(C) First derivative spectra of binary mixture of demeclocycline (25 mg/ml) and minocycline (25 mg/ml)
Zero cross points: 1-demeclocycline (366 nm) and 2-minocycline (353.5 nm)

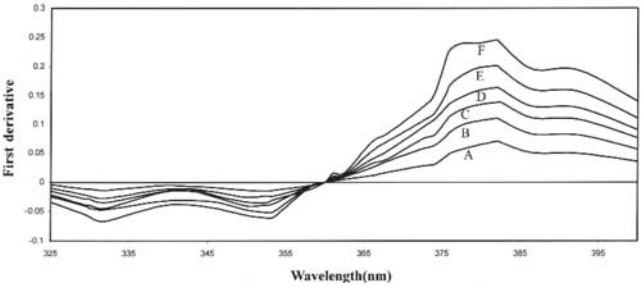

Figure 9: First derivative spectra of binary mixture of demaclocycline and minocycline at different concentrations - (A) 10 µg/ml; (B) 20 µg/ml; (C) 30 µg/ml; (D) 40 µg/ml; (E) 50 µg/ml of each.

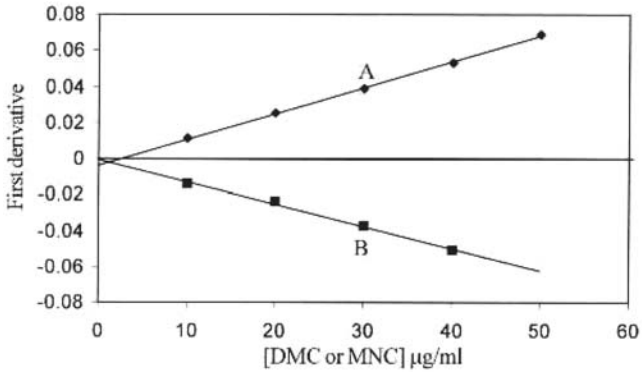

Figure 10: Effect of DMC/MNC concentration on first derivative amplitude in their mixtures
(A) Calibration plot for MNC at 366.0 nm
(B) Calibration plot for DMC at 353.5 nm

The first order amplitudes are recorded for the two tetracyclines DMC and MNC. They are plotted against corresponding pH values and shown in the Fig. 2 and Fig.3 respectively. It is evident from the figures that the first order amplitude is almost constant in the pH range 2 to 4. So a pH of 3 considered optimum for the further studies.

4.2 Selection of wavelengths for the determination of DMC and MNC

The first derivative spectra of DMC and MNC at different concentrations are shown in the Fig. 4 and Fig. 5. The analytical wavelengths for DMC and MNC are 281, 313.5, 332, 353.5, 382, 391.5 nm and 277.5, 335, 374 and 378.5 nm respectively. The calibration plots drawn between first order amplitude and the concentration of tetracycline are shown in the Fig. 6 and Fig. 7 for DMC and MNC respectively. The corresponding regression parameters are shown in the Table 1 and Table 2 respectively.

4.3 Resolution of DMC and MNC in their binary mixtures

The first derivative spectra of DMC, MNC and a mixture containing both are shown in the Fig.8. It is evident that, the zero crossing wavelengths for the DMC and MNC are 366

Table 1: Regression parameters for determination of demeclocycline C = [DMC] in µg/ml

Regression equation	Correlation coefficient
$^1D_{281.0}$ = 0.0046C + 0.0018	0.9998
$^1D_{313.5}$ = -0.0008C + 0.0042	0.9994
$^1D_{332.0}$ = -0.0007C + 0.0008	0.9927
$^1D_{353.5}$ = -0.0011C + 0.005	0.9924
$^1D_{382.0}$ = 0.003C + 0.0219	0.9997
$^1D_{391.5}$ = 0.003C + 0.0119	0.9983

Table 2: Regression parameters for determination of minocycline C = [MNC] in µg/ml

Regression equation	Correlation coefficient
$^1D_{277.5}$ = 0.0012 C + 0.0037	0.9986
$^1D_{335.0}$ = -0.0006 C + 0.0023	0.9975
$^1D_{374.0}$ = 0.0017 C - 0.0079	0.9990
$^1D_{378.5}$ = 0.0015 C + 0.0179	0.9996

nm and 353.5 nm respectively. So the analytical determination of MNC and DMC can be carried out respectively at these wavelengths. The first derivative spectra at different concentrations of DMC/MNC are shown in the Fig. 9. The linear proportionality is observed when the graphs are plotted between the first order amplitudes and respective drug concentrations (Fig.10). The corresponding regression parameters are shown in the Table 3.

Table 3: Regression parameters for determination of MNC or DMC C = [MNC or DMC] in µg/ml

Tetracycline	Regression equation	Correlation coefficient
DMC	$^1D_{353.5}$ = -0.0012C - 0.0005	0.9973
MNC	$^1D_{366.0}$ = 0.0014C - 0.0038	0.9996

5. APPLICATIONS

5.1 Application to urine

It is found that the human body eliminated 30 to 60% of initially administered unchanged tetracyclines through urine during the first 24 hours. DMC and MNC in urine can directly determined by employing the above methods. The results pertaining to this analysis is shown in the Table 4. A dilution of 1+30 is performed in order to decrease the background of urine.

Table 4: Determination of demeclocycline in urine

Sample	Signal measured	Added (µg/ml)	Found (µg/ml)	Recovery (%)
Urine (1+30 dilution) + demeclocycline	$^1D_{382.0}$	16	15.8	98.7

Determination of minocycline in urine

Sample	Signal measured	Added (mg/ml)	Found (mg/ml)	Recovery (%)
Urine (1+30 dilution) + minocycline	$^1D_{378.5}$	15	15.2	103

Table 5: Determination of demeclocycline in honey

Sample	Signal measured	Added (µg/ml)	Found (µg/ml)	Recovery (%)
Urine (1+20 dilution) + demeclocycline	$^1D_{382.0}$	16	15.8	98.7

Determination of minocycline in honey

Sample	Signal measured	Added (mg/ml)	Found (mg/ml)	Recovery (%)
Urine (1+20 dilution) + minocycline	$^1D_{378.5}$	15	15.1	101

Table 6: Assay for DMC in pharmaceutical formulations (Average of ten determinations)

Sample	Signal measured	Labelled amount mg/tab or cap	Amount found mg/tab or cap	Recovery (%)
Ledermycin[a]	$^1D_{281.0}$	150	152	101
	$^1D_{313.5}$	150	148	99
	$^1D_{332.0, 353.5}$	150	155	103
	$^1D_{382.0,391.5}$	150	154	102.6

a = Wyeth Lederle Ltd., Gujarat, India.

Assay for MNC in pharmaceutical formulations (Average of ten determinations)

Sample	Signal measured	Labelled amount mg/tab or cap	Amount found mg/tab or cap	Recovery (%)
Cyanomycin[a]	$^1D_{277.5}$	50	52	104
	$^1D_{335.0}$	50	49	98
	$^1D_{374.0,378.5}$	50	51.5	103

a = Wyeth Lederle Ltd., Gujarat, India.

Resolution of demeclocycline and minocycline in synthetic mixtures by first derivative spectrophotometry

DMC to MNC ratio	DMC			MNC		
	Taken µg/ml	Found µg/ml	Recovery %	Taken µg/ml	Found µg/ml	Recovery %
2:6	10	9.8	98	30	31.2	104
2:8	10	9.9	99	40	41.3	103
10:6	100	98.1	98	30	29.5	98
4:5	20	20.2	101	25	24.2	97
6:4	30	31	103	20	20.4	102
5:2	25	25.2	100	10	9.7	97

5.2 Application to honey

To prevent the bacterial diseases of honeybee, their colony is usually treated with tetracyclines. Hence honey can become contaminated with tetracyclines (Corner et. Al., 1971; Khong et al., 2005). Now a days honey requires tetracycline free certification (Official journal of European Communities, 2001). Hence monitoring tetracyclines in honey is an important concern. The author successfully applied above methods for the determination of DMC and MNC in honey. A dilution of 1+20 is recommended to prevent the background resulting from honey. The results are shown in the Table 5.

5.3 Application to pharmaceutical samples

The method described by the author is successfully applied on the pharmaceutical compounds containing DMC and MNC. The recoveries are calculated in each case and are shown in the Table 6.

CONCLUSION

The author presents simple, sensitive and accurate methods for the determination of two tetracyclines namely DMC and MNC, when they are present independently and in their binary mixtures. A notable advantage of the proposed method is that the method can be applied for the analysis in urine, honey and in pharmaceutical formulations without any pretreatment of the samples.

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