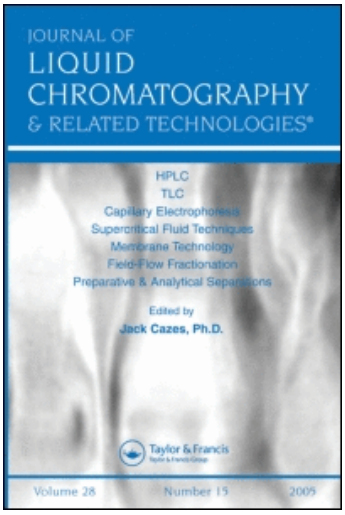

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High Performance Liquid Chromatographic Determination of Nalidixic Acid in Tablets

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF NALIDIXIC ACID IN TABLETS

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ABSTRACT

A rapid, specific and reliable high performance liquid chromatographic assay of Nalidixic acid in tablets has been developed. Reversed-Phase chromatography was conducted using a mobile phase of 0.05 M Ammonium acetate Methanol and acetonitrile, (65, 5, 30% v/v) pH 5 and detection at λ 254 nm. The recovery and coefficient of variation from six placebo tablets containing 100 mg of Nalidixic acid were 100.2% and 0.56 respectively. Replicate regression analyses of three standard plots in the concentration range 1 - 20 mcg/ml obtained on three different

days gave a correlation coefficient (0.99996) and the coefficient of variation of the slopes 0.089%. The assay was precise within day and between days as indicated by ANOVA test. It is suggested that the proposed HPLC method should be used for routine quality control and dosage fonn assay of Nalidixic acid.

INTRODUCTION

Nalidixic acid is 1-ethyl-1,4dihydro7methyl4-oxo-1,8 naphthyridine 3 carboxylic acid, a urinary antimicrobial agent[1].

Various methods have been developed for the determination of Nalidixic acid in pharmaceutical dosage forms. Fluorometry[2], polarography[3], UV spectrophotometry[4], Gas chromatography[5] and PMR spectrometry[6].

The purpose of this study, was to develop a simple and direct HPLC assay for the quantitation of Nalidixic acid in tablet formulations.

EXPERIMENTAL

Chemical and Reagents:

Nalidixic acid[7] and methyl paraben[8] were used without further purifications. Acetonitrile[9], Methanol[9] and water were HPLC grade. All other chemicals were of U.S.P. or A.C.S. quality and were used as received.

Chromatography:

A waters HPLC systems[10] was used consisting of the following components: One Model 45 pump, the WISP Model 710B autosampler, the Model 481 UV detector set at 254 nm at 0.05 AUFS, the model 730 data system. Chromatographic separation was accomplished using C_{18} column, 3.9 mm x 300 mm μBonda pack C_{18} column with 10um packing.

Chromatographic Conditions:

The eluting medium consisting of 0.05 M Ammonium acetate, methanol and acetonitrile (65, 5, 30% v/v, pH 5) was prepared and degassed by bubbling helium gas for 5 min prior to use. Column equilibrium with the eluting solvent was established by pumping the mobile phase at a rate of 0.2 ml/min overnight. The flow rate was set at 1.5 ml/min during analysis. The chromatogram was recorded and integrated at a speed of 0.2 cm/min.

Internal Standard:

A stock solution of methyl parahydroxy benzoate 1 mg/ml was prepared weekly and stored at 4°C.

Preparation of Standard Solution of Nalidixic Acid:

A stock solution was prepared by dissolving 10 mg of Nalidixic acid in 10 ml water. Ten aliquots equivalent to 0.5, 1, 2,

4, 6, 9, 12, 15, 18, and 20 ug of Nalidixic acid were added to one ml volumetric flask. After the aliquot of the internal standard equivalent to 20 ug was added to each 1 ml flask, the flasks were brought to volume by acetonitrile and mixed thoroughly. Three 20 uL injections of each standard solution of Nalidixic acid containing the internal standard were made to prepare standard plots. The peak area ratios of Nalidixic acid : methyl paraben were plotted against Nalidixic acid concentrations. Least square linear regression analysis was used to determine the slope, Y-intercept, and the correlation coefficients of the standard plots.

Sample Preparation:

Individual tablets containing 500 mg Nalidixic acid were pulverized using a mortar and pestle, and completely transferred to 250 ml volumetric flask. Ten ml of deionized water was added and the flask was swirled for 2-3 min. The volume was adjusted to 250 ml with methanol and the flask was mechanically shaken for five min. Five ml of the solution was removed into a centrifuge tube and centrifuged at 3000 r.p.m. for 5 min. One hundred ul of the supernatant was transferred to a 10 ml volumetric flask containing two hundred ul of methyl paraben stock solution, and the volume completed with the mobile phase. Twenty ul was injected onto the column for quantitation. Ten replicate commercial tablets were analyzed for statistical evaluation of the assay.

Quantitation:

The amount of Nalidixic acid per dosage form was determined from the following equation.

$$Q = [R/A + B] \times \text{dilution factor}$$

were Q is the mg Nalidixic acid per dosage form, R is the peak area ratio (drug/internal standard), A is the slope of the calibration curve and B is the y-intercept.

Recovery of Nalidixic Acid from the fabricated placebo tablets:

The reference tablets containing 500 mg of Nalidixic and 50 mg each of starch and lactose were prepared and subjected to the described HPLC assay and B.P. 88 to measure the accuracy and precision.

RESULTS AND DISCUSSION

Figure 1 shows typical chromatograms obtained following analysis of Nalidixic acid in tablets. Using the chromatographic conditions described, Nalidixic acid and Methyl paraben were well separated and their retention times were 7.27 and 4.0 min, respectively. For both compounds sharp and symmetrical peaks were obtained with good baseline resolution and minimal tailing, thus facilitating the accurate measurement of the peak area ratios. No interfering peaks were found in the chromatogram due to table t excipients. Figure 2 shows a calibration plot for the peak area ratios

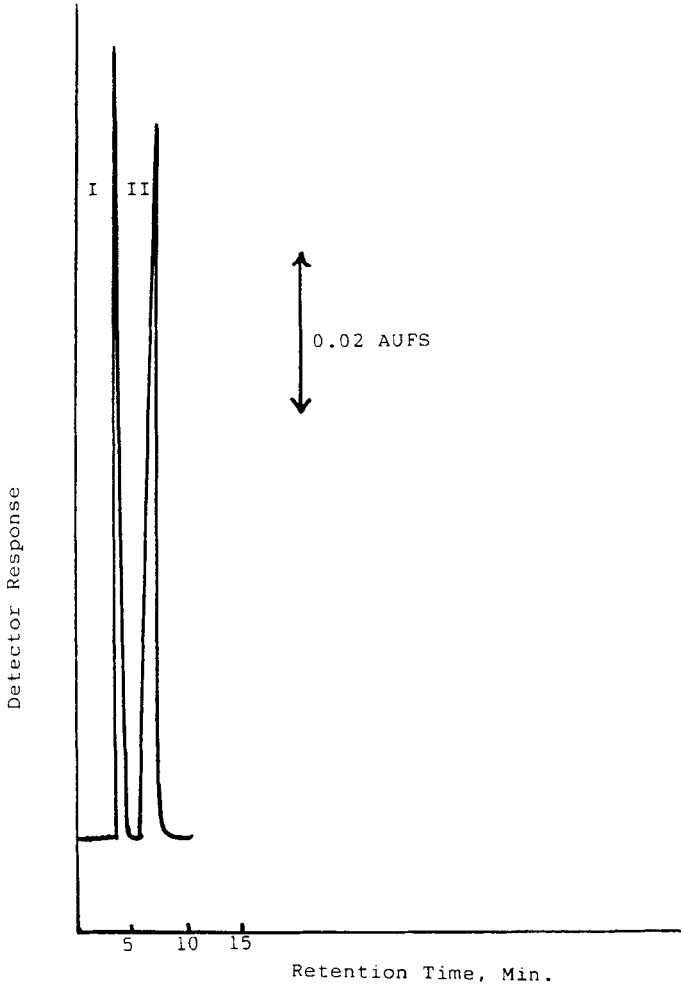

Figure 1: Chromatogram of Nalidixic Acid tablet.

Key 1. Methyl-paraben
2. Nalidixic acid

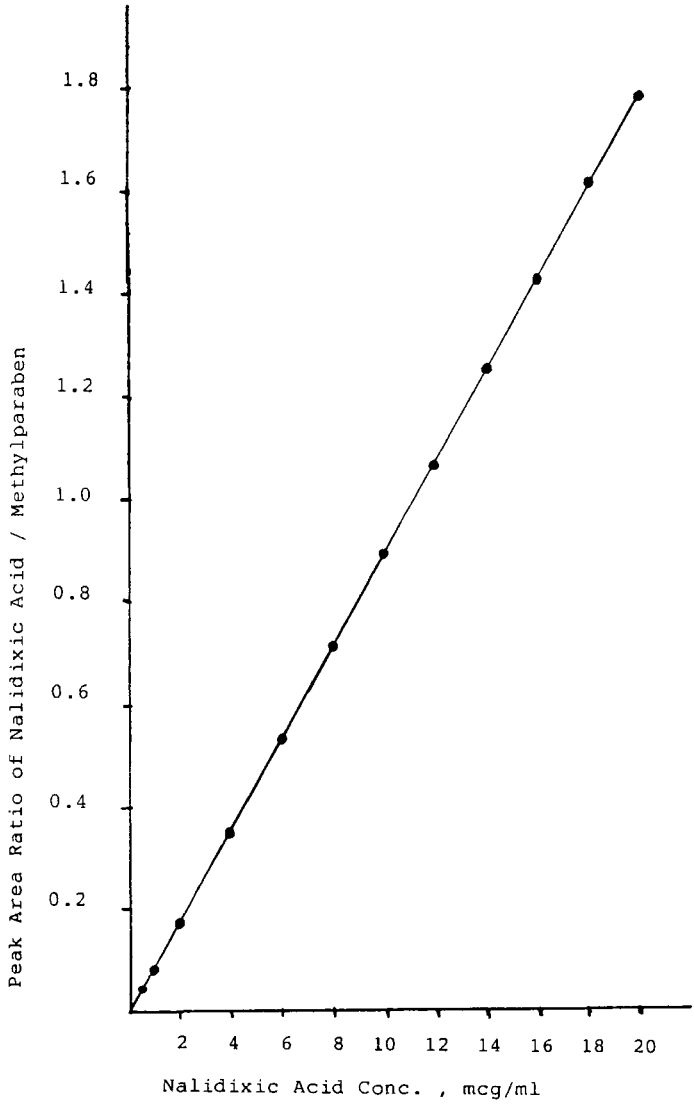

Figure 2: Standard calibration plot of Nalidixic acid

Table 1

Regression Analyses of the Three Standard Plots of Nalidixic Acid.

Standard[a]	Slope[b]	Intercept[b]	Correlation[b] Coefficient
1	0.09015	-0.0071	0.99986
2	0.09014	-0.0072	0.99979
3	0.09016	-0.0070	0.99989

a) obtained in 3 different days
b) The mean of 3 determinations at each drug concentration.

of varying amounts of Nalidixic acid (0.5-20 ug/ml) to a constant amount of methyl paraben (20 ug/ml). The plot was highly linear (r=0.99995) and the regression analysis of the data gave the slope and intercept as:

$$Y = 0.09015x - 0.0071$$

where y and x are the peak area ratio and Nalidixic acid concentration respectively. Three replicate analyses of Nalidixic acid at concentrations of 0.5 - 20 ug/ml were assayed at three different days over one week period. The results of this evaluation are summarized in Table I. The average correlation was 0.99984 and the coefficient of variation of the slopes of the three lines was 0.089%. Analysis of variance of the data showed no detectable difference in the slopes of the three standard plots ($F_{2.409}$, $P > 0.01$). The results, thus confirmed the excellent linearity of the calibration plots and high reproductivity of the assay.

Table II

Analysis of Variance for Intra- and Inter day Precision

Day/Assay	1	2	3	4	5	6
1	500.9	500.6	499.3	502.2	503.1	499.1
2	502.7	500.8	498.5	500.4	500.9	497.3
3	501.8	500.5	497..7	503.7	499.2	502.1
4	502.1	498.5	500.3	501.9	498.1	499.3

Mean = 500.458
SD = 1.7356
CV % = 0.3468

ANOVA TEST

Source of Variation	DF	Sum of Squares	Mean of Squares	F ratio	P
Within day	5	31.914	6.38	2.614	0.05
Between day	3	3.699	1.233	0.505	0.05
Error	15	36.685	2.44		
Total	23	72.298			

Precision and Accuracy: Six placebo tablets containing 50 mg each of lactose and starch and 500 mg Nalidixic acid were assayed for four consecutive days for intra and interday precision studies. The average recovery shown in Table II was (500.458) with the coefficient of variation 0.341%. Estimation of day to day and

Table III

Recoveries From Spiked Placebo Tablets

Method	n	Amount Added	Amount Received	CV%
B.P.	6	100	99.05	1.40
HPLC	6	100	100.2	0.56

n = number of replicates

within day precision were calculated by ANOVA test. The calculated F values, $F_{0.05}(5, 15) = 2.614$ and $F_{0.05}(3, 15) = 0.505$ were smaller than the table values $F_{0.05}(5, 15) = 2.9$ and $F_{0.05}(3, 15) = 3.9$ respectively. Thus it was concluded that there was no significant difference for the assay which was tested within day and between days.

Recovery :

Table III compares the average recovery by the B.P. and the proposed HPLC method for placebo samples containing 100 mg Nalidixic acid and 50 mg each of the lactose and starch. the average % recovery was 100.2 and 99.05 for the HPLC and the B.P. method, respectively and their % coefficient of variation were 0.56 and 1.4, respectively. The values obtained using the HPLC method compared favorably with those obtained using the B.P. method. The smaller recovery by the B.P. method may have been caused by the loss of the drug during several sample preparation steps.

Table IV

Recovery of Nalidixic acid from Commercial Tablets by HPLC Method.

Table I	Amount found mg	% of Label Claim
1	510	102.0
2	508	101.6
3	512	102.4
4	505	101.0
5	509	101.8
6	506	101.2
7	500	100.0
8	504	100.8
9	502	100.4
10	503	100.6
		Mean 101.18
		CV% 0.71516

Analysis of Nalidixic acid in Tablets:

Table IV presents the results obtained from the HPLC analysis of 10 Nalidixic acid tablets (500 mg) commercially available. The mean percent recovery was 101.18% with 95% confidence limits of 503 to 509. Each of the tablets analyzed showed highly uniform Nalidixic acid content between 100-102.4% of the label claim. the requirements for content uniformity of

Nalidixic acid tablets in the B.P. specify that the potency must fall within 95-105% of the label claim. Thus the tablets selected randomly in this determination met the B.P. requirements for the content uniformity.

CONCLUSION

The HPLC method developed in this study has the advantages of simplicity, precision and convenience. It also allows for the direct determination of Nalidixic acid by passing several tedious steps involved in other assay methods. Therefore, the methods should be useful for routine analytical and quality control assay of Nalidixic acid in dosage forms.

REFERENCES

1. Martindale "The Extra Pharmacopoeia" 29th Ed., The Pharmaceutical Press, London (1989) p.1051.

2. R. Staroscik, J. Sulkowska, Acta-Pol. Pharm., 30, 499 (1973).

3 R. Starosik, I. Prochowska, J. Sulkowska, Pharmazie, 29, 387 (1974).

4. VG. Zubenko, IA. Shcherba, Farm. Zh. 30, 28 (1975).

5. Wu-H. Lung, T. Nakagawa, T. Uno, J. Chrom., 157, 297 (1978).

6. H.Y. Aboul-Eneim, K.A. Al Rashood, H.M. El-Fatatry, Pharm. Acta. Helv., 56, 262 (1981).

7. Fluka AG, CH- 9470 Buchs.

8. BDH Chemicals Ltd., Poole, England.

9. J.T. Baker Chemical Co., Phillipsburg, N.J., U.S.A.

10. Waters Associates, 34 Maple Street Milford, MA 01757, U.S.A.

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