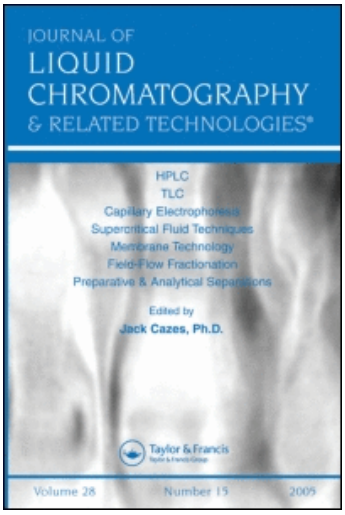

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SODIUM BOROHYDRIDE AND HYDROGEN PEROXIDE AS FLUOROGENIC SPRAY REAGENTS FOR THE DETECTION OF NALIDIXIC ACID AND FLUMEQUINE

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SODIUM BOROHYDRIDE AND HYDROGEN PEROXIDE AS FLUOROGENIC SPRAY REAGENTS FOR THE DETECTION OF NALIDIXIC ACID AND FLUMEQUINE

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ABSTRACT

A simple, sensitive and highly selective detection method for nalidixic acid and flumequine has been developed. A 1-μL aliquot of sample solution was spotted onto the thin-layer plate with the aid of a disposable micropipette, and after development, the plate was sprayed first with sodium borohydride and then hydrogen peroxide successively. The spots were visualized under longwave UV light. The detection limits were 30 pmol for nalidixic acid and 60 pmol for flumequine.

INTRODUCTION

Nalidixic acid (1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carboxylic acid)[1-2] and flumequine (9-fluoro-6,7-dihydro-5-methyl-1-oxo-1H,5H-benzo[ij]quinolizine-2-carboxylic acid)[3-4] are synthetic antibacterial agents. They are highly active against a wide spectrum of Gram-negative bacteria[5] and have been used for the treatment of urinary tract infections.

They have also been found to be applicable as effective aids in the control of cultivated fish such as salmon, trout, mackerel, carp, eel, yellowish, and chicken infection.[6-7]

Several methods such as fluorometry,[2] colorimetry,[6] high performance liquid chromatographic methods,[8-11] and gas chromatography[12] have been developed for the detection of the two compounds.

In this paper, we present a simple, selective, inexpensive, and sensitive fluorometric method for detection of the two compounds on TLC plates by using sodium borohydride and hydrogen peroxide as fluorogenic spraying reagents.

EXPERIMENTAL

Apparatus

The samples were separated on the pre-coated aluminium TLC sheets (silica gel 60, 0.2 mm layer) from E. Merck using disposable micropipettes (Blaubrand, Germany). The spots were visualized by irradiation of the plates with a long wavelength (366 nm) ultraviolet lamp (Camag).

Reagents and Chemicals

Nalidixic acid was purchased from Aldrich (Milwaukee, WI, U.S.A.). Cinoxacin, enoxacin, flumequine, norfloxacin, ofloxacin, pipemidic acid, and piromidic acid were obtained from Sigma (St. Louis, MO, U.S.A.). Hydrogen peroxide (30 %) was obtained from E. Merck (Darmstadt, G.F.R.).

Other reagents and solvents were of analytical reagent grade and were used as received. Sodium borohydride was obtained from E. Merck (Darmstadt, G.F.R.) and its solution (0.75 M) was prepared with 0.1 N sodium hydroxide.

Preparation of Standard Solutions

The standard solutions were prepared by weighing accurately the reference standard into a 100-mL volumetric flask and diluted to volume with methanol to make a solution containing 0.010-150.0 nmol of the standard per μL.

Thin-Layer Chromatography

On to a pre-coated aluminum TLC sheet (5 x 8 cm) without any treatment, one microliter of the standard solutions were spotted with micro-pipettes, and ascending chromatography was performed in a glass tank at room temperature. After developing with mobile phase ($CHCl_3$:MeOH:formic acid = 10 mL :2 mL :30 μL), the air-dried plate was sprayed successively with an aqueous solution of 0.75 M $NaBH_4$ and 15 % H_2O_2 in a fume-hood. The spots of nalidixic acid, flumequine, and other quinolone antibacterial agents were observed immediately under longwave UV light without heating.

RESULTS AND DISCUSSION

Analytical Parameters for the Detection

Several parameters affecting the fluorogenic reaction, including the concentration of reagents, optimal reaction temperature and time, order of the spray reagents, have been investigated to optimize the conditions for detection of nalidixic acid and flumequine at 60 nmol level.

Detection Limits of Standard Solutions

In general, a large portion of quinolones emits native fluorescence under longwave UV light. The characteristic fluorescence of some quinolones on TLC plates are shown in Table 1. The table shows that nalidixic acid and flumequine fluoresce most weakly. To increase the detection sensitivity of the two compounds, the study has established a method by using sodium borohydride and hydrogen peroxide as fluorogenic spraying reagents.

The results, as can be seen in Table 1, demonstrate that after spraying with sodium borohydride and hydrogen peroxide, the fluorescence intensity of nalidixic acid and flumequine on TLC plates can be strongly potentiated.

Table 1

Detection Limits and Characteristic Fluorescence of Eight Quinolones

Compound	Limit of Detection (nmole) Native	After Spraying	Rf Value	Color of Fluorescence
Nalidixic acid	150	0.03	0.94	Yellow-blue
Flumequine	12.5	0.06	0.91	Orange
Norfloxacin	0.16	0.16	0.24	Blue
Ofloxacin	0.01	0.01	0.65	Yellow
Cinoxacin	0.31	1.25	0.72	Blue
Enoxacin	0.16	0.08	0.36	Blue
Pipemidic acid	0.31	0.16	0.30	Blue
Piromidic acid	0.16	0.16	0.96	Blue

The reactions of nalidixic acid and flumequine with sodium borohydride and hydrogen peroxide were very rapid without heating, because there was gradual decrease of the fluorescence intensity after heating. Although the fluorescence intensity of the spots diminished slowly, they remained detectable for 48 hours when the plates were kept in the dark, and the reagents retained their activities for at least 1 week.

CONCLUSION

This paper described a detection method by using sodium borohydride and hydrogen peroxide as fluorogenic spraying reagents for the detection of nalidixic acid and flumequine on TLC plates. The method was not only simple, but also inexpensive and rapid.

REFERENCES

1. G. Y. Lesher, E. J. Froelich, M. D. Gruett, J. H. Bailey, J. Med. Pharm. Chem., **5**, 1063 (1962).

2. E. W. McChesney, E. J. Froelich, G. W. Lesher, A. V. R. Crain, D. Rosi, Toxicol. Appl. Pharmacol., **6**, 292 (1964).

3. A. T. Andresen, K. E. Rasmussen, J. Liq. Chromatogr., **13**, 4051 (1990).

4. T. Agasosterand, K. E. Rasmussen, J. Chromatogr., **564**, 171 (1991).

5. A. King, L. Phillips, J. Microbial Chemother., Suppl. D, **18**, 1 (1986).

6. R. S. Browning, E. L. Pratt, J. Assoc. Off. Anal. Chem., **53**, 464 (1970).

7. H. K. L. Hundt, E. C. Barow, J. Chromatogr., **223**, 165 (1981).

8. P. P. Allen, K. M. Robert, B. T. Sherri, E. R. Jose, C. H. David, R. L. Austin, J. Assoc. Off. Anal. Chem., **78**, 1227 (1996).

9. Y. Kasuga, T. Sugiva, F. Yamada, J. Food. Hyg. Soc.Jpn., **23**, 344 (1982).

10. J. Guyonnet, M. Pacaud, M. Richard, A. Doisi, F. Spavone, Ph. Hellings, J. Chromatogr., **679**, 177 (1996).

11. K. M. Robert, B. T. Sherri, P. P. Allen, E. R. Jose, C. H. David, R. L. Austin, J. Assoc. Off. Anal. Chem., **78**, 343(1995).

12. K. Takatsuki, J. Assoc. Off. Anal. Chem., **75**, 982 (1992).

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