*Journal of Chromatography*, 210 (1981) 536–539
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,724

## Note

# Fluorescence detection of 8-hydroxyquinoline and some of its halogenated derivatives using post-column derivatization in high-performance liquid chromatography

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(Received February 10th, 1981)

8-Hydroxyquinoline (oxine, Ox) and its halogenated derivatives are employed as fungicides or anti-infective agents. They have been determined colorimetrically after conversion into their iron complexes[1]. Recently, the high-performance liquid chromatographic (HPLC) separation and subsequent fluorescence detection of the aluminum chelate of Ox has been reported[2]. On the other hand, there is little information on the HPLC separation of 8-hydroxyquinolines. In this paper we describe an HPLC method involving the separation of four 8-hydroxyquinolines, *i.e.*, Ox, 5-chloro-8-hydroxyquinoline (5-Cl), 5,7-dichloro-8-hydroxyquinoline (Di-Cl) and 5-chloro-7-iodo-8-hydroxyquinoline (chinoform, CF), and their fluorescence detection after post-column derivatization to the corresponding metal chelates.

## EXPERIMENTAL

Ox was purchased from Tokyo Kasei (Tokyo, Japan). 5-Cl, Di-Cl and CF, which were kindly provided by Tanabe Seiyaku (Tokyo, Japan), were purified by recrystallization[3] and dissolved in methanol. Quinine sulphate was purchased from Nakarai Chemicals (Kyoto, Japan). The following compounds of guaranteed grade were used as sources of metal ions: $AlCl_3 \cdot 6H_2O$, $MgCl_2 \cdot 6H_2O$, $Y(NO_3)_3$, $In_2(SO_4)_3 \cdot 9H_2O$, $ZnCl_2$, $Cd(NO_3)_2 \cdot 4H_2O$, $CaCl_2 \cdot 2H_2O$ and $SnCl_2 \cdot 2H_2O$. They were dissolved in distilled water. All the other reagents were of analytical grade.

A Hitachi MPF-2A spectrofluorometer was used. Quinine sulphate in 0.1 *N* sulphuric acid was used as reference in the measurements of fluorescence intensity.

The liquid chromatograph consisted of two plunger type pumps (KSU-45H and KSD-16; Kyowa Seimitsu, Tokyo, Japan), a glass tube (18 cm × 3 mm I.D.) packed with Iatrobeads 6CP-2010 resin (10 $\mu$m, polystyrene type porous polymer; Iatron Laboratories, Tokyo, Japan), an Aminco-Bowman J4-7439 fluoro-colorimeter and a TOA EPR-100A recorder. For the fluorescence detection, a GE G4T4/1 (254 nm) lamp, a Shimadzu EX-2 primary filter and a Wratten 2A secondary filter were used.

0021-9673/81/0000–0000/$02.50 

## RESULTS AND DISCUSSION

### *Selection of metal ions for the formation of fluorescent chelates*

The fluorescence characteristics of various metal chelates are summarized in Table I. Excitation spectra showed two maxima around 270–280 nm and 380–400 nm. All of the chelates exhibited more intense fluorescence when excited at longer wavelengths. The relative fluorescence intensities thus obtained are given in Table II. Generally, intense fluorescence was observed when Mg(II), Al(III) or Y(III) was used as the metal ion. Since the last two are easily precipitated as hydroxides at alkaline pH values whereas Mg(II) is not, Mg(II) was employed.

TABLE I

EXCITATION AND EMISSION MAXIMA OF METAL CHELATES OF 8-HYDROXYQUINOLINES

To 50 $\mu$l of 1 m*M* 8-hydroxyquinoline solution were added 0.5 ml of 10 m*M* metal ion solution and 4.45 ml of methanol. The mixture was shaken well and the fluorescence maxima were measured within a few hours.

| *Metal* | $\lambda_{ex}/\lambda_{em}$ *(nm)* | | | |
|---|---|---|---|---|
| | *Ox* | *5-Cl* | *Di-Cl* | *CF* |
| Al | 266, 380/520 | 268, 398/526 | 273, 396/524 | 282, 396/531 |
| Mg | 267, 380/520 | 271, 398/532 | 274, 380/526 | 280, 402/526 |
| Y | 268, 380/520 | 272, 396/532 | 274, 396/528 | 281, 400/528 |
| In | 269, 384/530 | 270, 400/540 | 276, 400/540 | 284, 400/540 |
| Zn | 270, 390/536 | 273, 400/546 | 277, 400/546 | 282, 410/546 |
| Cd | 270, 390/530 | 272, 402/544 | 276, 400/540 | 281, 400/540 |
| Ca | 272, 390/530 | 276, 400/526 | 275, 400/532 | 280, 400/530 |
| Sn | 272, 384/530 | 273, 396/536 | 276, 400/540 | 280, 400/540 |

TABLE II

RELATIVE FLUORESCENCE INTENSITIES OF METAL CHELATES OF 8-HYDROXYQUINOLINES

To 50 $\mu$l of 1 m*M* 8-hydroxyquinoline solution were added 0.5 ml of 10 m*M* metal solution and 4.45 ml of methanol. The mixture was shaken well and the fluorescence intensity at the maximum wavelength was measured by exciting at the maximum wavelength around 380–400 nm, within a few hours.

| *Metal* | *Ox* | *5-Cl* | *Di-Cl* | *CF* |
|---|---|---|---|---|
| Al | 100* | 91 | 127 | 27 |
| Mg | 36 | 59 | 118 | 45 |
| Y | 45 | 66 | 127 | 50 |
| In | 39 | 39 | 55 | 19 |
| Zn | 26 | 28 | 39 | 15 |
| Cd | 19 | 27 | 45 | 18 |
| Ca | 10 | 41 | 50 | 20 |
| Sn | 5 | 8 | 11 | 4 |

* The fluorescence intensities are relative to that of the aluminium chelate of Ox taken arbitrarily as 100. The instrument was not calibrated.

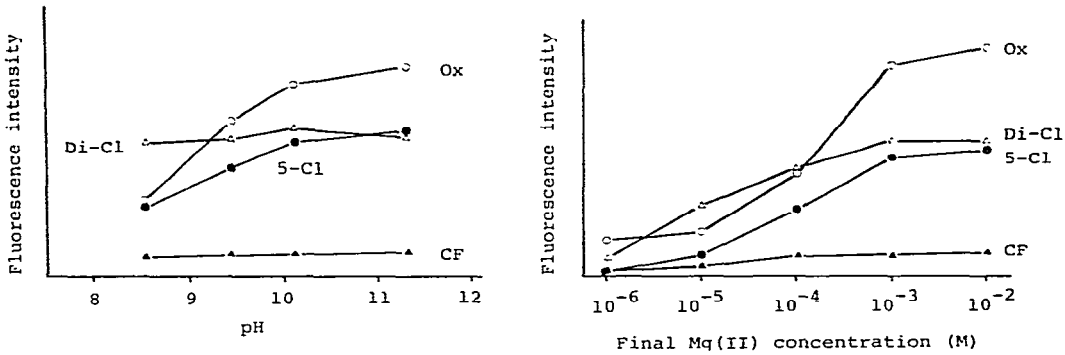


Fig. 1. Effect of pH on the fluorescence intensity of magnesium chelates of 8-hydroxyquinolines. To 0.5 ml of 0.1 *M* glycine–NaOH buffer at various pH values were added 50 $\mu$l of 0.1 m*M* 8-hydroxyquinoline solution and 0.5 ml of 0.1 *M* $MgCl_2$ in methanol and made up to 5 ml with methanol. After stirring, the fluorescence intensity was measured within a few hours at an excitation wavelength of 254 nm and an emission wavelength of 520 nm (Ox), 532 nm (5-Cl) or 526 nm (Di-Cl, CF).

Fig. 2. Effect of magnesium(II) concentration on the fluorescence intensity. To 0.5 ml of 0.1 *M* glycine–NaOH buffer (pH 9.5) were added 5 $\mu$l of 1 m*M* 8-hydroxyquinoline solution and 0.5 ml of $MgCl_2$ solution in methanol at various concentrations and made up to 5 ml with methanol.

Fig. 1 shows the relationship between the fluorescence intensities of the magnesium chelates of the 8-hydroxyquinolines and pH. The fluorescence intensities of Ox and 5-Cl increased with pH, while those of Di-Cl and CF did not increase. Above pH 12, a distinct white precipitate of magnesium hydroxide was formed. Therefore, a

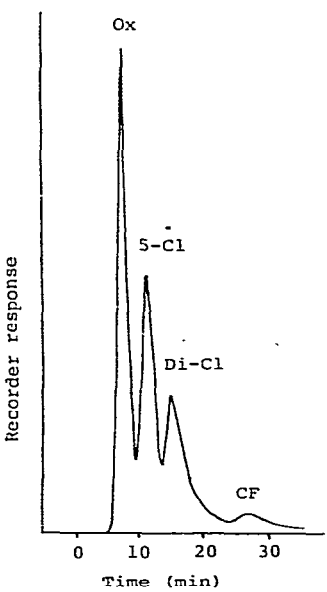


Fig. 3. Fluorometric detection of 8-hydroxyquinolines as their magnesium chelates after post-column derivatization. Column: Iatrobeads 6CF-2010 (10 $\mu$m, 180 × 3 mm I.D., 15°C). Eluent: 0.01 *M* glycine–HCl buffer (pH 3.0)–methanol (5:95 v/v); flow-rate 1.0 ml/min. Post-column reagent: 0.01 *M* $MgCl_2$ in 0.1 *M* glycine–NaOH buffer (pH 9.5); flow-rate 0.26 ml/min. Sample amount: 2.5 nmol of each compound.

pH of 9.5 was chosen for further experiments. The effect of the magnesium concentration on the fluorescence intensities of the chelates was examined (Fig. 2). At least $10^{-3}$ *M* Mg(II) was required to obtain intense fluorescence when the final concentration of 8-hydroxyquinoline was $10^{-6}$ *M*.

*HPLC of 8-hydroxyquinolines with fluorescence detection after post-column derivatization*

For the 8-hydroxyquinolines, the chromatographic conditions described previously[4] were adopted, and 0.1 *M* glycine–NaOH buffer (pH 9.5) containing 0.01 *M* $MgCl_2 \cdot 6H_2O$ was pumped into the column effluent for derivatization. However, *n*-hexane was omitted from the eluent because small bubbles were formed after addition of the alkaline solution. Fig. 3 illustrates the elution pattern of the 8-hydroxyquinolines.

The formation of metal chelates of the 8-hydroxyquinolines occurred very rapidly at room temperature. Such a mild and fast reaction is ideal for post-column derivatization in HPLC. The present post-column derivatization reaction may also be applied to the HPLC analysis of other compounds having a 8-hydroxyquinoline skeleton.

ACKNOWLEDGEMENT

The authors thank Mr. Kazuichi Hayakawa, Kanazawa University, for his suggestions.

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