phyrin IX, 0.47; coproporphyrin III, 0.11; impure protoporphyrin IX prepared according to GRINSTEIN[1], 0.47, 0.32, 0.17. When samples were applied in streaks along the origin, the developed chromatogram could be eluted in zones for the determination of spectra.

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Separation of some oxine derivatives on thin layers impregnated with ferric chloride

The present methods available for the separation of oxine derivatives are inadequate. The method of CASTIGLIONI[1] will effectively separate oxine from iodochloroxine but does not distinguish between various halogenated derivatives. The method of KORZUN, BRODY AND TISHLER[2], using polyamide layers does not separate 5,7-dichloroxine and iodochloroxine, or 5-monoiodo- and 5,7-diiodo-oxine.

Previous work in this laboratory, CLEGG AND CAWTHORNE[3] using Kieselgel G impregnated with phosphate buffer pH 5 (0.1 *M*) gave similar R_F values for all the halogenated derivatives examined. The author has used a reverse phase system of Kieselguhr—5% liquid paraffin in petroleum ether–acetone. Whilst separation could be achieved with this system, streaking could not be prevented. In connection with this work, it was found that spraying with a saturated solution of magnesium acetate in methanol and then viewing under 350 mμ light showed the oxine derivatives as green fluorescent spots against the blue background. This method of detection was found to be superior to one using diazotised reagents.

The separation of oxine derivatives was attempted by impregnating plates with ferric chloride. The choice of ferric chloride for chelation was made because of the known colour reaction of this material with oxine derivatives[4].

Experimental

Preparation of plates. The suspension for five plates (20 cm × 20 cm) was prepared from 30 g Kieselgel G (E. Merck A.G., Darmstadt) or 30 g Kieselguhr (E. Merck A.G., Darmstadt) by the addition of 60 ml water containing 6 g ferric chloride hexahy-

drate. The mixture was ground in a mortar until uniform and then applied with a Desaga applicator to a thickness of 0.25 mm. The plates were dried at room temperature overnight and then placed in an oven at 50° for 30 min. It was found advisable to cover the inside of the oven with aluminium foil to prevent attack by ferric chloride. The plates were then placed in a desiccated cabinet over silica gel until ready for use.

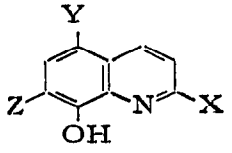
Development. The samples were dissolved in benzene and applied with micropipettes 1.5 cm from the bottom of the plate. The plates were developed in filter paper lined saturated tanks (27 cm × 8 cm × 22 cm) containing benzene–methanol (90:10) to a distance 15 cm from the origin. The plates were removed and the solvent allowed to evaporate in air. The development time was approximately 45 min.

Results and discussion

It was found that Kieselguhr was superior to Kieselgel G in that it gave more uniform results. This is probably because Kieselgel is more active and its degree of activation is more difficult to control than that of Kieselguhr. For this reason, only the results obtained using Kieselguhr are given.

TABLE I

R_F VALUES OF OXINE DERIVATIVES



Name	*X*	*Y*	*Z*	R_F
Oxine	H	H	H	0.06
2-Methyloxine	CH_3	H	H	0.19
5-Chloroxine	H	Cl	H	0.37
7-Chloroxine	H	H	Cl	0.25
5,7-Dichloroxine	H	Cl	Cl	0.57
5,7-Dibromo-oxine	H	Br	Br	0.68
Iodochloroxine	H	Cl	I	0.72
5,7-Diiodo-oxine	H	I	I	0.80
2-Methyl-5,7-dichloroxine	CH_3	Cl	Cl	1.0

The R_F values are given in Table I and the separation of mixtures shown in Fig. 1. The R_F values increased with increasing substitution in the nucleus. Separation of the monochloro isomers was achieved, the *ortho* derivative having the lower R_F value.

The results obtained suggest that the method may be used as a tool in determining the course of the reaction and the nature of the products in the preparation of oxine derivatives of pharmaceutical interest.

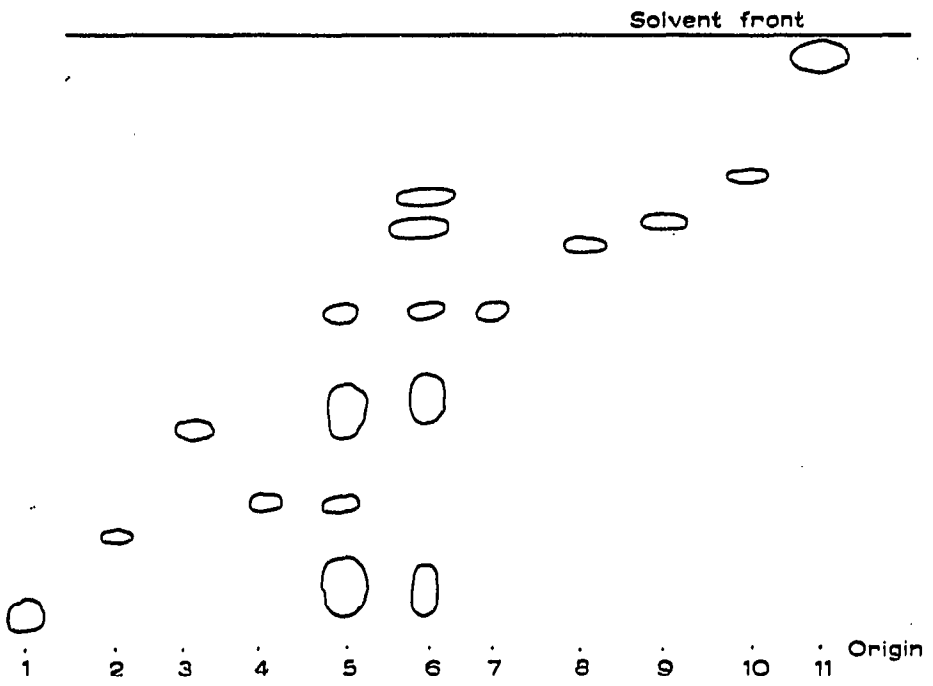


Fig. 1. 1 = Oxine, 0.2 μg; 2 = 2-methyloxine, 0.2 μg; 3 = 5-chloroxine, 0.2 μg; 4 = 7-chloroxine, 0.2 μg; 5 = mixture of oxine, 5-chloroxine, 7-chloroxine and 5,7-dichloroxine, 0.4 μg each; 6 = mixture of oxine, 5-chloroxine, 5,7-dichloroxine, 5-chlor-7-iodo-oxine and 5,7-diiodo-oxine, 0.4 μg each; 7 = 5,7-dichloroxine, 0.2 μg; 8 = 5,7-dibromo-oxine, 0.2 μg; 9 = 5-chlor-7-iodo-oxine, 0.2 μg; 10 = 5,7-diiodo-oxine, 0.2 μg; 11 = 2-methyl-5,7-dichloroxine, 0.2 μg.

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