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Short Communication

High-performance liquid chromatographic method for the monitoring of the synthesis of the precursor for tetramisole

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ABSTRACT

The synthesis of the monochloro precursor $C_6H_2CH_2(OH)CH_2NHCH_2CH_2Cl$ is the crucial step in the synthesis of the important anthelmintic drug tetramisole. HPLC methods using mobile phases consisting of methanol, water and acetonitrile in various combinations together with ammonium chloride as an inorganic modifier and Nova-Pak C_{18} and μPorasil columns were developed for the monitoring and quantification of each step of the synthesis of the precursor. The methods provide important clues for increasing the yield of the product.

INTRODUCTION

Tetramisole hyldrochloride is a potent anthelmintic drug [1,2] with antidepressant activity. Several methods based on polarography [3], spectrophotometry [4] and HPLC [5] have been reported for the determination of this drug. In this work, however, our aim was to develop HPLC methods for monitoring and optimizing the yields of the various steps in the synthesis of tetramisole hydrochloride by a commercial route. In this synthetic route (Fig. 1), the formation of the monochloro precursor **III** is the crucial step, as the subsequent steps of condensation with thiourea followed by ring closure to give tetramisole proceed in a facile manner. We describe here efficient HPLC methods for monitoring and quantifying the yields of each step of the three-stage synthesis of the monochloro precursor **III**.

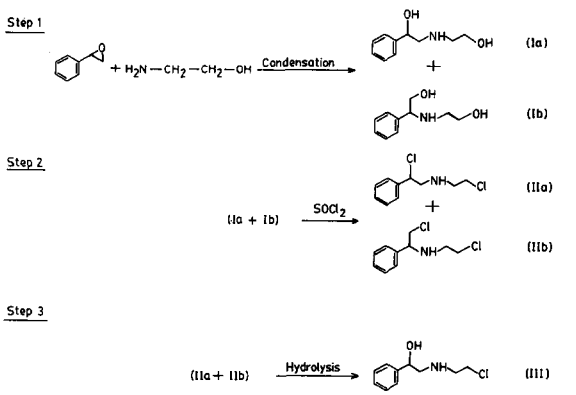

Fig. 1. Reaction scheme for the three-step synthesis of the monochloro precursor **III**.

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EXPERIMENTAL

Instrumentation

The HPLC system used has been described earlier [6] and consisted of a Model 590 alternating pump, a Nova-Pak C_{18} column (4 μm, 150 mm × 3.9 mm I.D.), a μPorasil column (10 μm, 300 mm × 3.9 mm I.D.), a Model R 403 refractive index detector (all from Waters, Milford, MA, USA), a Rheodyne injector with a 10-μl loop and a Hewlett-Packard Model 3394 A integrator.

Reagents and solvents

Styrene oxide, ethanolamine and thionyl chloride were obtained from Fluka (Buchs, Switzerland) and ammonium chloride, methanol and acetonitrile from BDH (Poole, UK). Authentic samples of **Ia**, **Ib**, **IIa**, **IIb** and **III** were synthesized and characterized in our laboratory.

Chromatographic analyses

The following chromatographic conditions were used for monitoring the different steps of the synthesis route:

Step 1: the mobile phase was methanol–water (65:35, v/v) containing 0.8 *M* NH_4Cl and with the pH adjusted to 8 with ammonia solution. The flow-rate was 1.0 ml/min and the column was μPorasil.

Steps 2 and 3: the mobile phase was methanol–water (17:83, v/v) containing 0.8 *M* NH_4Cl and with the pH adjusted to 3 with phosphoric acid. A Nova-Pak C_{18} column was used and the flow-rate was 0.8 ml/min.

The calibration and determination of the diol isomers **Ia** and **Ib** were done on a Nova-Pak C_{18} column using a mobile phase of acetonitrile–water (5:95, v/v) containing 0.4 *M* NH_4Cl at pH 8 with a flow-rate of 1.0 ml/min. The calibration and determination of the monochloro compound **III** were done using the conditions employed in step 2. A refractive index detector was used for all the chromatographic analyses.

RESULTS AND DISCUSSION

The chromatogram for step 1 of the synthesis route is shown in Fig. 2. The condensation of styrene oxide with ethanolamine gives rise to two isomeric diols (**Ia** and **Ib**) and the separation of these two isomers from each other and from the starting materials is clearly evident. Of the two isomers formed, **Ia** is the desired product. Consequently, the chromatographic analysis of this step was carried out not only for monitoring the completion of the reaction (through the disappearance of the peaks due to styrene oxide and ethanolamine) but also for minimizing the amount of the undesired isomer (**Ib**). The linearity of response for both the diol isomers **Ia** and **Ib** (Fig. 3) over the concentration range chosen is evident from Table I. The inter-assay precision of the method was established by triplicate analyses of synthetic mixtures of different compositions and the maximum error was found to be 1% (Table II). The results of the analysis of actual reaction mixtures for the synthesis of the diol isomers are shown in Table

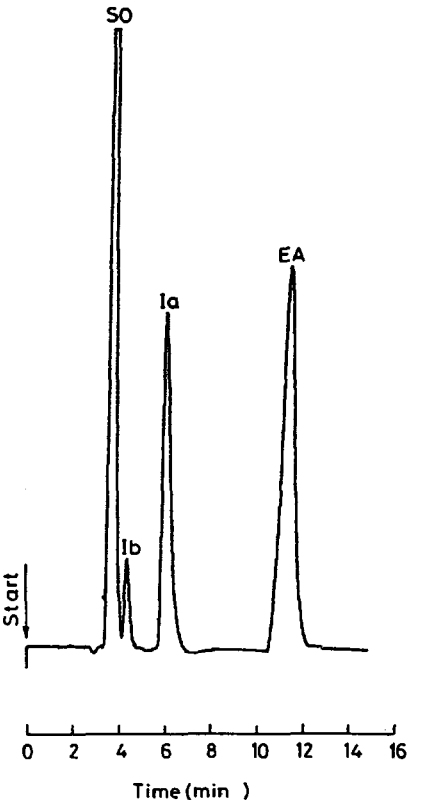

Fig. 2. Typical chromatogram for the reaction mixture in the synthesis of the diol isomers **Ia** and **Ib** from styrene oxide (SO) and ethanolamine (EA).

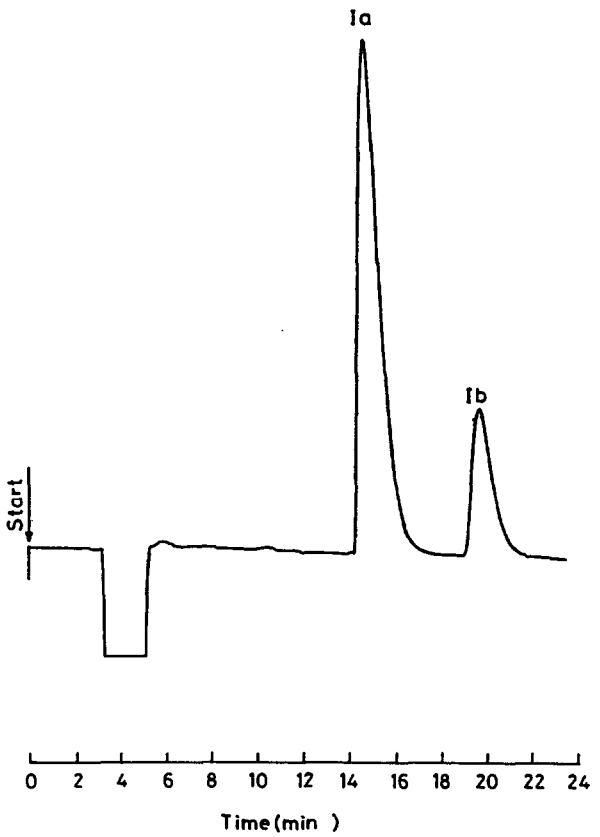

Fig. 3. Chromatogram of a synthetic mixture of diols **Ia** (15.51 μg) and **Ib** (3.51 μg) for calibration and quantification.

III and it was found that the purity of the diols synthesized in step 1 was lower than expected and varied with the reaction conditions. This was probably because of the formation of side-products such as the tertiary amine or the dimer of styrene oxide, which were not detected under the chromatographic conditions employed. However, it was possible to modify the reaction conditions of step 1 so as to improve the assay of the product from 73. 5% to 88.3% in the case of three different samples shown in Table III.

The conversion of the diol isomers to the corresponding dichloro derivatives (step 2) was also monitored chromatographically (Fig. 4) and it was found that there was total conversion of the diols to the dichloro compounds. It was also observed from the area percentages that the ratio of the two dichloro isomers (**IIa** and **IIb**) was similar to that of the two corresponding diols (**Ia** and **Ib**). These observations were in good agreement with the nearly quantitative isolated yields of **IIa** and **IIb**. As this reaction step was quantitative and the amounts of the dichloro isomers isolated were in good agreement with the amounts determined from the area percentages of the respective peaks in the chromatogram, no further quantification of this step was done.

Chromatograms for step 3, the conversion of the dichloro isomers to the monochloro deriva-

TABLE I

LINEARITY OF RESPONSE FOR THE ISOMERIC DIOLS **Ia** AND **Ib** FOR CALIBRATION BY THE EXTERNAL STANDARD METHOD

Sample No.	**Ia**[a]			**Ib**[b]		
	Amount (μg)	Area ($\times 10^7$)	Area/amount	Amount (μg)	Area ($\times 10^7$)	Area/amount
1	14.47	23.57	1.63	1.40	2.18	1.56
2	15.51	25.05	1.62	2.11	3.25	1.56
3	16.54	26.64	1.61	2.81	4.36	1.55
4	17.57	28.50	1.62	3.51	5.45	1.55
5	18.62	29.39	1.59	4.21	6.54	1.55
6	20.68	32.83	1.59	14.04	2.15	1.53

[a] Regression equation: $y = 0.1149 + 1.595C_{Ia}$. Linearity range: 14–21 μg.
[b] Regression equation: $y = 0.05525 + 1.526C_{Ib}$. Linearity range: 1–15 μg.

TABLE II

RESULTS OF THE ANALYSIS OF SYNTHETIC MIXTURES OF DIOLS

Average of triplicate determination.

Ia			Ib		
Taken (mg)	Found (mg)	Error (%)	Taken (mg)	Found (mg)	Error (%)
17.10	16.98	0.70	2.38	2.40	0.83
15.00	14.85	1.00	2.50	2.48	0.80
18.00	17.87	0.70	4.50	4.45	1.00

tive **III**, are shown in Fig. 5. It is interesting that the two dichloro isomers give rise to only one monochloro compound on hydrolysis. In fact, when the progress of this hydrolytic reaction was monitored it was evident (Fig. 5B–E) that initially only one of the dichloro isomers, **IIa**, was selectively being converted into **III**. However, when the reaction conditions were changed, the peak due to the other dichloro isomer (**IIb**) gradually disappeared with a corresponding increase in the intensity of the peak due to **III** but no additional peak was observed (Fig. 5F). It was apparent, therefore, that both the dichloro isomers on hydrolysis gave only one product **III**. In order to confirm this observation, the chromatographic conditions were modified to increase substantially the capacity factor of **III** and it was found still to give rise to only a single symmetric peak. The present HPLC results therefore provided a clue for increasing the yield of **III** by demonstrating the necessity for modifying the reaction conditions for converting **IIb** also into the desired product. In addition, the results also provide direct evidence for the mechanistically interesting conversion of both the dichloro isomers into a single monochloro compound.

The linearity of response for **III** over the concentration range chosen is shown in Table IV, and this was utilized for determining the assay of the final product of the three-step synthesis.

In conclusion, therefore, HPLC methods have been developed for each stage of the three-step synthesis of the monochloro precursor **III** and the methods have been used very effectively for the determination of the yield and purity of the product at each stage. Further, the analyses have provided useful clues for improving the yield of the product **III**.

Another aspect of this work is the use of NH_4Cl as a modifier in the mobile phase for a more efficient separation of the individual components in each step of the synthesis route. In particular, the separation of the two diol isomers

TABLE III

RESULTS OF THE ANALYSIS OF THE REACTION MIXTURE FOR THE SYNTHESIS OF THE DIOLS

Sample No.	Ia (mg)	Ib (mg)	Total (Ia + Ib) determined by HPLC (mg)	Total reaction product (mg)	Assay (%)
1	11.70	2.95	14.66	19.95	73.45
2	15.60	3.82	19.43	25.62	75.83
3	17.42	3.78	21.20	24.02	88.27

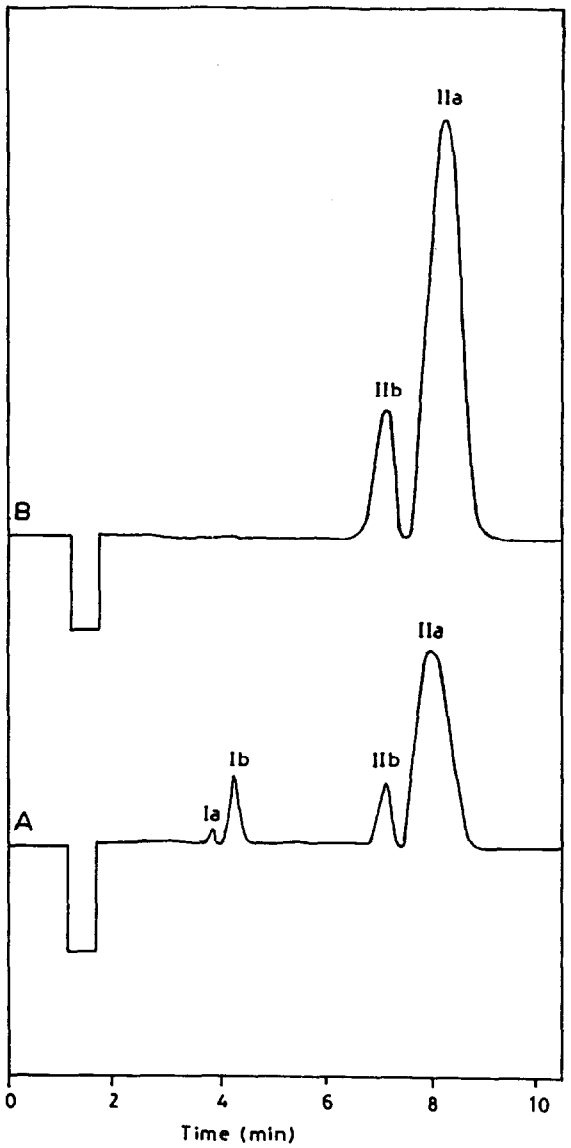

Fig. 4. Chromatograms for monitoring the conversion of the diol isomers **Ia** and **Ib** into the corresponding dichloro derivatives **IIa** and **IIb**: (A) on partial conversion, **IIa** = 19.11 μg, **IIb** = 2.46 μg and (B) on complete conversion, **IIa** = 63.12 μg, **IIb** = 15.41 μg.

Ia and **Ib** could be achieved only with the use of NH_4Cl in the mobile phase and the separation was not possible even with the addition to the mobile phase of conventional PIC reagents such as heptanesulphonic acid, which are known [7–9] to be effective in the separation of amines. We are currently engaged in a comprehensive study

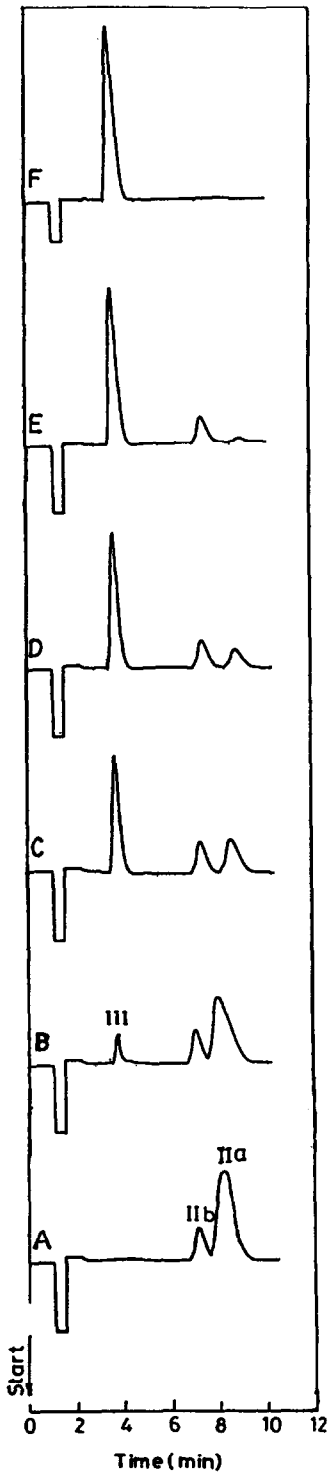

Fig. 5. Chromatograms for monitoring the gradual conversion of the dichloro isomers **IIa** and **IIb** into the monochloro precursor **III**: (A) at zero conversion, **IIa** = 106.8 μg, **IIb** = 26.2 μg); (B)–(E) gradual selective conversion of only **IIa**; (F) total conversion to **III** (120.3 μg).

TABLE IV

LINEARITY OF RESPONSE FOR THE MONOCHLORO PRECURSOR III FOR CALIBRATION BY THE EXTERNAL STANDARD METHOD

Regression equation: $y = (7.805 \times 10^5) + 2.005 \cdot 10^5 C_{III}$. Linearity range 40–400 μg.

Sample No.	Amount (μg)	Area ($\times 10^7$)	Area/amount ($\times 10^5$)
1	40.1	0.85	2.12
2	80.2	1.71	2.13
3	120.3	2.50	2.08
4	160.4	3.34	2.08
5	200.5	4.12	2.05
6	240.6	4.96	2.06
7	280.7	5.75	2.05
8	320.8	6.59	2.05
9	360.9	7.31	2.03
10	401.0	7.96	1.98

(the results of which will be published elsewhere) on the use of NH_4Cl as a mobile phase modifier in several different chromatographic separations and a comparison of its performance with other modifiers such as PIC reagents and other inorganic salts. This study, apart from its primary aim of developing suitable HPLC methods for monitoring the difficult steps in the synthesis of a commercially important compound and providing important clues for improving the yield of the product, is also a good example of the use of NH_4Cl as an efficient mobile phase modifier.

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