Journal of Chromatography, 102 (1974) 381–383

CHROM. 7737

SEPARATION OF SOME CHLORAMPHENICOL INTERMEDIATES BY HIGH-PRESSURE ION-EXCHANGE CHROMATOGRAPHY

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SUMMARY

High-pressure cation-exchange chromatography was used to separate three of the by-product compounds formed in the chloramphenicol production process. Baseline separation can be achieved within 8.5 min employing a 1-m column packed with Zipax SCX pellicular cation exchanger and 0.05 *M* sodium sulphate solution as eluent at pH 1.1 in a Varian Aerograph LC 4020 liquid chromatograph.

INTRODUCTION

This paper reports the separation of some chloramphenicol intermediates and by-products. These compounds are very polar, thermally labile and, owing to their low vapour pressure, cannot be analyzed by gas chromatography. A thin-layer chromatographic method has been applied for their analysis, employing a Kieselgel GF_{254} layer and chloroform–methanol–glacial acetic acid (90:5:5) as developing solvent[1]. However, good resolution and precise sub-microgram determinations of the compounds involved cannot be obtained. High-performance ion-exchange column chromatography was chosen in order to solve the separation problem.

EXPERIMENTAL

All experiments were carried out on a Varian Aerograph LC 4020 UV/RI liquid chromatograph (Walnut Creek, Calif., U.S.A.) equipped with some home-made units. Argon gas was used to pressurize the solvent container and to deliver the eluent. During the elutions, a high-pressure gas regulator (Lüedi, Zürich, Switzerland) kept the gas pressure constant. A 1 m × 1/16 in. O.D. stainless-steel capillary tube and a 0.5-μm stainless-steel porous frit was used to maintain a back-pressure in the detector cells and to prevent bubbling out of the dissolved gas. By this means, the eluent could be delivered for about 2 h before the gas pressure had to be released and the eluent degassed.

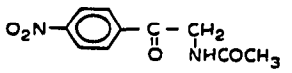
The starting-mark generating device[2] was used to determine the exact retention times of the compounds. The flow-rate was measured with a home-made bubble flow meter. Injection was made on-column through PTFE-laminated septa (Varian Aerograph) with a Hamilton HP 305 high-pressure microsyringe.

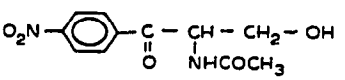
The columns were made of 1/8 in. O.D., 1.8 mm I.D. precision-bore stainless-steel tubing, equipped with a water jacket connected to a temperature-controlled water circulating bath (Type U10, MLW, Medingen, G.D.R.). The columns were dry-packed with Zipax SCX pellicular cation exchanger, (DuPont, Wilmington, Del., U.S.A.) employing a home-made machine similar to that described by Henry[3].

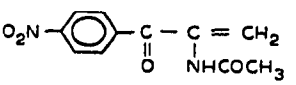
All chemicals used were of reagent grade and were obtained from Reanal (Budapest, Hungary). The compounds to be separated were prepared and supplied by the EGYT Pharmaceutical Factory (Budapest, Hungary) and were considered to be of the highest available purity.

RESULTS

The compounds to be separated were:

I 

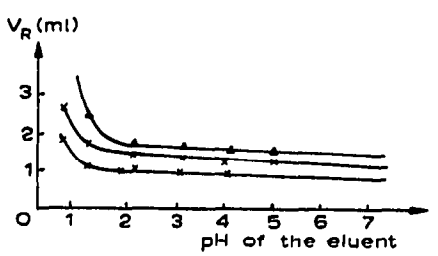
II 

III 

Earlier experiments[1] showed that when these compounds are exposed to prolonged daylight or temperatures above 60°, they decompose and form red-coloured compounds. Therefore, all separations were carried out at 30° and sample solutions were freshly prepared each day.

The flow-rate of the eluent was kept constant at 0.40 ml/min while the corresponding gas pressure was varied between 62 and 75 atm. The eluent was distilled water containing 0.05 mole/l of sodium sulphate. The pH was adjusted by adding sulphuric acid.

The pH of the eluent was varied systematically so as to change the retention of the compounds to be separated. The results are shown in Fig. 1. We measured the



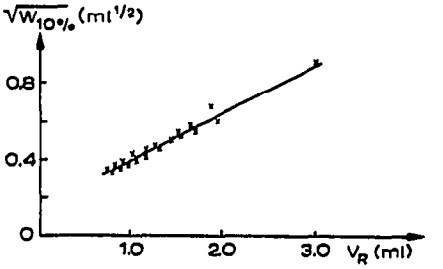


Fig. 1. Dependence of the retention volumes on the pH of the 0.05 *M* Na_2SO_4 eluent. Column: 1 m long packed with Zipax SCX, thermostatted to 30.0°, flow-rate 0.40 ml/min.

Fig. 2. Dependence of the square root of the peak width at 10% peak height on the retention volumes. Amounts injected were between 0.2 and 0.35 μg.

peak width at 10% height in each case. The change of the square root of this width as a function of the retention volume is shown in Fig. 2.

Down to pH 2, there was no significant change in the retention behaviour. The compounds were eluted so close together that their separation was not satisfactory.

Based on Figs. 1 and 2, we determined the pH of the eluent to be used in order to obtain resolution of at least 1.5 for each component pair. It was found that with an eluent of pH 1.1, the three compounds could be separated in 8.5 min. The chromatogram of the three components is shown in Fig. 3. The amounts injected were between 0.2 and 0.35 μg.

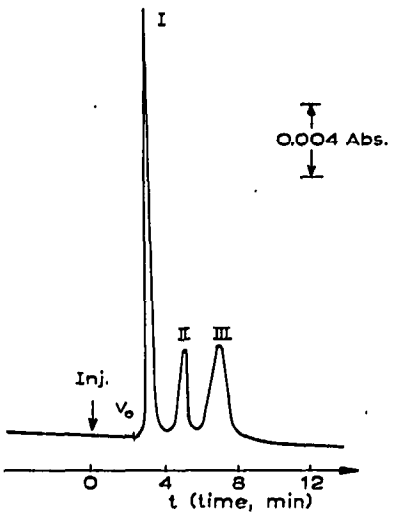


Fig. 3. Separation of compounds I, II and III. Amounts injected were 0.35, 0.20 and 0.25 μg in 3 μl respectively. Eluent: 0.05 *M* Na_2SO_4 at pH 1.1.

ACKNOWLEDGEMENT

The authors are grateful to the EGYT Pharmaceutical Factory for the samples and their permission to publish these results.

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