Journal of Chromatography, 90 (1974) 233–235

CHROM. 7181

Note

Determination and micro-preparative separation of chlorocholine chloride by paper chromatography

S. KOUDELA and V. CIELESZKY

Institute of Nutrition, Department of Toxicological Chemistry, Gyáli ut 3/a, Budapest 1097 (Hungary)

(Received September 6th, 1973)

The specificity of detection of chlorocholine chloride (CCC) on chromatograms or its determination by spectrophotometry with dipicrylamine[1–3] or iodine[4,5] depends on the efficiency of the separation from choline (CC) and other positive compounds by use of paper or thin-layer chromatography (*e.g.* refs. 6–8), ion-exchange resins[2,3,9], alumina columns[1–3] or iodine extraction[10,11].

In our experiments, which were conducted in order to obtain more effective separations by using various combinations of solvent systems on buffered and salt-impregnated papers, it was found that the optimum separation of CCC from CC could be achieved on Whatman No. 1 paper impregnated with 3% sodium chloride solution by development with the supernatant of the solvent mixture isoamyl alcohol–*n*-propanol–3% sodium chloride solution (1:2:3) (Fig. 1). By decreasing the sodium chloride concentration, the CCC and CC spots became more satisfactorily resolved, but also became more diffuse.

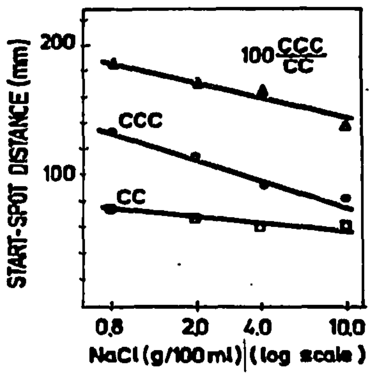
EXPERIMENTAL

Extraction and clean-up

The sample of the material to be tested (usually 50–100 g) was extracted with methanol and, after adding of water to the extract, the methanol was evaporated and the aqueous solution was purified by shaking with light petroleum (b.p. 40–70°). The aqueous solution was evaporated in a vacuum, the dry residue was redissolved by adding of 1 ml of water, 1 ml of methanol and 12 ml of acetone, and the solution obtained was transferred to an alumina column and eluted with acetone containing 25% of methanol[1,2]. From an acidic aluminium oxide (E. Merck, Darmstadt, G.F.R.) column, 20 cm long and 1 cm I.D., a total of 70 ml of eluate could be collected. Finally, the dry residue of the eluate was taken up in 0.2 ml of methanol.

Paper chromatography

A Whatman No. 1 paper sheet was immersed in 3% sodium chloride solution and dried at room temperature. An aliquot of the methanolic solution equivalent to one fifth of the sample extract and appropriate aliquots of stock CCC solutions were applied to the starting line. Development by the descending technique required 14–16 h. The air-dried paper was sprayed with potassium iodoplatinate solution



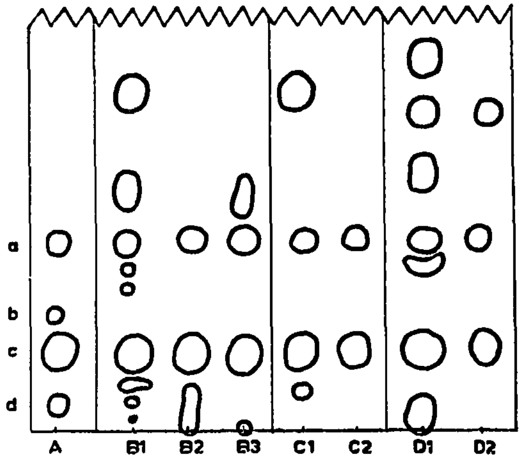


Fig. 1. Migration distance of CCC (○—○) and CC (□—□) spots, and the 100·CCC/CC distance ratio (△—△), plotted against the logarithm of the concentration of the impregnating sodium chloride solution.

Fig. 2. Paper chromatograms of some choline derivative standards (A) and of wheat seed (B), wheat flour (C) and grape (D) extracts from CCC-treated plants. Standards: (a) CCC; (b) acetylcholine; (c) CC; (d) betain. Clean-up: (1) Dowex 50 W-X8; (2) acidic alumina column; (3) extraction with iodine solution.

(1 g of platinum (IV) chloride and 10 g of potassium iodide were separately dissolved in water and the combined solutions were made up to 1 l with water). CCC and other detectable substances appeared as dark blue spots, and the chromatogram allowed a satisfactory semi-quantitative determination of the compound.

The efficiency of this separation technique was checked on some plant extracts, using for the clean-up an ion-exchange resin, aluminium oxide or extraction with iodine–potassium iodide solution (Fig. 2).

Micro-preparative separation

For the more sensitive and accurate spectrophotometric determination of CCC, paper chromatography can be used as a micro-preparative technique. The remaining four fifths of the methanolic extract were applied to the paper in two spots and developed as previously described. After location, the CCC spots (a piece of developed paper of a similar size served as a blank) were cut out and shaken with 10 ml of a 0.01% solution of dipicrylamine in dichloromethane[1] and the extracts were shaken with 5 ml of 0.03 *N* sodium hydroxide solution. The alkaline phases were discarded. The extracts were filtered through anhydrous sodium sulphate and finally evaporated to 5 ml. Spectrophotometric readings were made in 1-cm cells at 415 nm and corrected for the blank.

RESULTS

The procedure described permits the complete separation of CCC from CC even when the latter is present in a 1000-fold excess. Both substances can be easily detected in the 2–500 μg range for CCC and 10–1000 μg range for CC. With a sample size of 50 g, the detection limit is 0.2 ppm.

The recovery of CCC in preparative separations, involving regeneration from the paper and conversion into the dipicrylamine complex, is about 90%. The sensitivity of the spectrophotometric method is 1 μg per millilitre of solution, the measuring range being 5–50 μg. The detection limit for a 50-g sample is 0.05 ppm.

ACKNOWLEDGEMENT

The authors wish to thank Mrs. Irene Leder for technical assistance.

REFERENCES

1 R. P. Mooney and N. R. Pasarela, *J. Agric. Food Chem.*, 15 (1967) 989.
2 J. Jung and G. Henjes, *Z. Pflanzenernähr. Düng. Bodenk.*, 124 (1969) 97.
3 G. Petrosini, M. Businelli, F. Tafuri and L. Scarponi, *Analyst* (*London*), 94 (1969) 674.
4 H. Linser, H. Kühn and J. Bohring, *Z. Pflanzenernähr. Düng. Bodenk.*, 108 (1965) 57.
5 H. Bayzer, *Monatsh. Chem.*, 98 (1967) 1826.
6 H. H. Mayr and E. Presoly, *Planta*, 57 (1961) 478.
7 M. Červinka, *Rostl. Výroba*, 14 (1968) 313.
8 V. Jiráček, *Rostl. Výroba*, 14 (1968) 597.
9 H. Bier and H. Faust, *Z. Chem.*, 5 (1965) 386.
10 M. Drygas and A. Kotarski, in H. Dobrowolska (Editor), *Papers of CCC-symposium*, Institute of Organic Industry, Warsaw, 1972, p. 59.
11 F. Kretzschmann, in H. Dobrowolska (Editor), *Papers of CCC-symposium*, Institute of Organic Industry, Warsaw, 1972, p. 75.