Journal of Chromatography, 89 (1974) 96–98

CHROM. 7068

Note

High-voltage electrophoresis of choline and its esters

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(Received September 11th, 1973)

Numerous methods have been described[1,2] for the identification and determination of choline in biological materials. Assay procedures are commonly based on precipitation by non-specific reagents (*e.g.* ammonium reineckate, phosphotungstic acid, cadmium chloride) followed by gravimetric, spectrophotometric or titrimetric determination. Other methods include colorimetric measurement after a complexing reaction.

More specific identifications employ chromatographic procedures. Those based on paper[3] and thin-layer[4] techniques are simple but time consuming, whilst gas chromatography requires either conversion to stable, volatile compounds[5] or pyrolysis[6]. This communication describes a more rapid method for the separation and identification of choline salts and some of its more important esters by high-voltage electrophoresis under a range of pH conditions. The method may be applied to quantitative determinations in biological extracts and pharmaceutical preparations.

EXPERIMENTAL

The Camag HVE system (Camag, Muttenz, Switzerland) was employed, separations being carried out on 40 cm × 20 cm strips of Whatman No. 3MM filter paper.

Qualitative identification

Paper strips were soaked in electrolyte and blotted in order to remove excess solution before application of 5 μl of 1% (w/v) solutions of choline salts and esters in distilled water. Conditions for the separations are shown in Table I. After drying at 110° for 5 min pherograms were sprayed with Dragendorff's reagent and R_M values calculated with respect to choline chloride.

Quantitative separations

Ten microlitres of solutions containing from 5 to 100 μg of standard were applied by means of Drummond Microcap pipettes (Drummond, Broomall, Pa., U.S.A.) to separate origins on paper strips impregnated with 8% formic acid solution (pH 1.8). Electrophoresis was carried out for 20 min at 2,500 V and pherograms were dried before spraying with Dragendorff's reagent. The outlines of the spots produced were carefully traced onto 1-cm graph paper and spot areas calculated.

TABLE I

MIGRATION VALUES, RELATIVE TO CHOLINE CHLORIDE, AND EXPERIMENTAL CONDITIONS

Electrolytes:

I= pH 1.8, formic acid 8% (v/v); 2,500 V, 20 min.
II= pH 3.1, ammonia (0.88) 20 ml, formic acid (90%) 50 ml and water to 2.5 l; 1,300 V, 30 min.
III= pH 4.2, 0.2 *M* potassium hydrogen phthalate 50 ml, 0.2 *N* sodium hydroxide 3.7 ml and water to 200 ml; 2,500 V, 25 min.
IV= pH 5.1, 1×10^{-5} *N* hydrochloric acid; 3,500 V, 15 min.
V= pH 5.8, ammonium chloride 0.05 g/l; 3,500 V, 15 min.
VI= pH 6.5, pyridine 50 ml, acetic acid 2 ml and water to 1 l; 3,500 V, 15 min.
VII= pH 7.2, collidine 4.7 ml, acetic acid 1.3 ml and water to 500 ml; 3,500 V, 15 min.
VIII= pH 10.4, 0.2 *M* sodium hydroxide 43.9 ml; 0.2 *M* boric acid 50 ml, 0.2 *M* potassium chloride 50 ml and water to 200 ml; 2,000 V, 30 min.

Compound	*Migration value*								*Colour with Dragendorff's reagent*
	I	*II*	*III*	*IV*	*V*	*VI*	*VII*	*VIII*	
Choline chloride	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	orange-red
Acetylcholine chloride	0.85	0.84	0.87	0.86	0.89	0.92	0.91	0.91	orange
Propionylcholine chloride	0.79	0.79	0.83	0.90	0.88	0.86	0.89	0.89	orange-yellow
Butyrylcholine chloride	0.74	0.70	0.80	0.82	0.81	0.81	0.82	0.81	orange-yellow
Methacholine	0.79	0.78	0.85	0.91	0.86	0.81	0.87	0.89	orange
Acetyl-β-methyl choline	0.81	0.82	0.87	0.81	0.87	0.86	0.86	0.86	orange
Choline dihydrogen citrate	1.04	1.04	1.04	1.00	1.04	1.04	1.03	1.05	orange-red
Choline theophyllinate	1.03	1.02	1.02	1.00	1.02	1.03	1.02	1.02	orange-red
Carbachol	0.84	0.88	0.91	0.87	0.92	0.89	0.89	0.92	orange-red

Calibration graphs were established for each pherogram by plotting the logarithm of the weight of choline salt or ester against the spot area.

Sample preparation was carried out by extraction and dilution with water to give a final concentration of choline salt or ester in the range 0.5–7.5 μg/μl.

RESULTS AND DISCUSSION

Qualitative separations

Migration values for choline salts and esters are given in Table I. The most satisfactory separation of choline salts from the esters occurred under low pH conditions, optimum resolution being obtained using 8% formic acid as electrolyte. It was found possible to resolve the acetyl, propionyl and butyryl esters and methacholine from each other but not carbachol, which exhibited similar migration values to acetylcholine. As the pH of the electrolyte was increased the R_M values tended to converge. The technique was also found valuable for rapid detection of the occurrence of decomposition products of choline esters during storage, both choline and acetylcholine being easily detected.

Quantitative determinations

Correlation coefficients were calculated for each pherogram and found to be close to unity in all cases (minimum value 0.97). A linear relationship was established between the limits 10 to 75 μg of choline salt or ester per spot, the minimum detection limit being 5 μg. This method is less sensitive than previously attained by paper chromatography[3]. Calibration graphs constructed for successive pherograms, although linear, were displaced and showed slightly differing slopes. As separations were carried out under identical conditions, these slight differences were attributed to variations in impregnation of the papers with electrolyte. Thus, it was found necessary to run sample and standard solutions on each paper strip and to construct calibration graphs for each pherogram.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the expert technical assistance provided by Mrs. N. Dale.

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