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Glass-fibre paper chromatography of ascorbic acid and related compounds

Existing procedures involving the use of thin-layer chromatography for the separation of ascorbic acid (AA) and its oxidation products, dehydroascorbic acid (DHA) and 2,3-diketogulonic acid (DKG), are generally unsatisfactory, as the separation requires too much time so that destruction of the compounds by oxidation on the plate cannot be excluded and tailing results.

In this communication, a glass-fibre paper chromatographic method is presented for the separation of these closely related compounds as well as of some derivatives and of one of the isomeric forms. For this purpose, glass-fibre paper impregnated with silica gel was used.

Materials

The solvents used were of the highest analytical grade (Merck, Fluka). Aqueous solutions (1–5 μl) of the compounds containing 1–5 μg were applied with a micropipette on to glass-fibre paper (Gelman Instruments, Ann Arbor, U.S.A.). Ascorbyl palmitate (AAP) was spotted in alcoholic solution. Spots were air-dried. AA, isoascorbic acid (iAA), 2-ketogulonic acid and AAP were products of Hoffmann-La Roche & Co., Ltd. (Basle, Switzerland). DHA was always prepared immediately before use by oxidation with bromine[1]. DKG was obtained from an aqueous solution of its calcium salt by treatment with Dowex 50-W (H^+-form) ion-exchange resin[2]. The calcium salt was prepared by oxidation of an aqueous solution of AA with potassium iodate and subsequent precipitation by addition of calcium iodide[3]. The O-methyl esters of AA were prepared by methylation of AA with diazomethane[4,*].

Acetonitrile–butyronitrile (60:30) was used as the basic solvent system. In order to change the polarity of this system, various volumes of ethanol and/or water were added, as indicated in Table I. Chromatograms were allowed to run

TABLE I

CHROMATOGRAPHIC SEPARATION ON GLASS-FIBRE PAPER

Basic solvent system: acetonitrile–butyronitrile (60:30). I contained in addition 8.5 ml of water, II 4.0 ml of water and III 12 ml of ethanol and 2 ml of water. Experimental details are given in the text.

Compound	R_F *values*		
	I	*II*	*III*
Ascorbic acid	0.64	0.56	0.62
Dehydroascorbic acid	0.85	0.80	
2,3-Diketogulonic acid	0.54		
2-Ketogulonic acid	0.45	0.35	0.39
Ascorbyl palmitate	0.92	0.71	
Isoascorbic acid	0.66	0.59	0.68
2-O-Methylascorbic acid			0.69
3-O-Methylascorbic acid	0.94	0.93	0.97

15 cm above the starting line (20–22°, relative humidity *ca.* 60 %). They were sprayed with concentrated sulphuric acid and compounds were made visible by burning the glass-fibre paper on a porcelain hot-plate (Corning, New York, U.S.A.).

Results and discussion

In Table I, the R_F values are listed in the solvent systems used. A good separation of all the compounds examined was achieved with the exception of iAA, which exhibited in systems I and II almost the same R_F values as AA. Solvent system III, however, could be used for the separation of these two isomers.

The described method gave an excellent separation within only 10–15 min, thus limiting the destruction of the compounds during chromatography to a negligible extent. In addition, it had a high sensitivity (1–5 μg) and a good reproducibility, emphasising that this method is of great advantage compared with thin-layer chromatographic methods[5,6].

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