Separation of methionine and selenomethionine by thin-layer chromatography

Because of the role that selenium plays in animal nutrition much work has centred around the function of the selenoamino acids which have been identified in plant and animal protein[1–3]. PETERSON AND BUTLER[4] have shown that the chromatographic and electrophoretic behaviour of selenocystine and selenomethionine in all solvent systems tried was indistinguishable from the corresponding sulphur compounds. In addition these workers found that the selenium analogues of the common sulphur amino acids are unstable and mild solvents have to be used for their chromatography. Using ion-exchange resins, MCCONNELL AND WABNITZ[5] found that the selenium analogues of methionine and cystine do not have the same elution rates as the two sulphur containing amino acids. However, the use of ion exchange resins is time-consuming and identification of the eluate off the column is not always immediate. In a recent note SCALA AND WILLIAMS[6] describe a method for identifying selenoamino acids on paper chromatograms involving prior oxidation to the corresponding selenoxides with hydrogen peroxide and detection of these compounds by spraying with hydriodic acid. This method could not be used if the presence of nat-

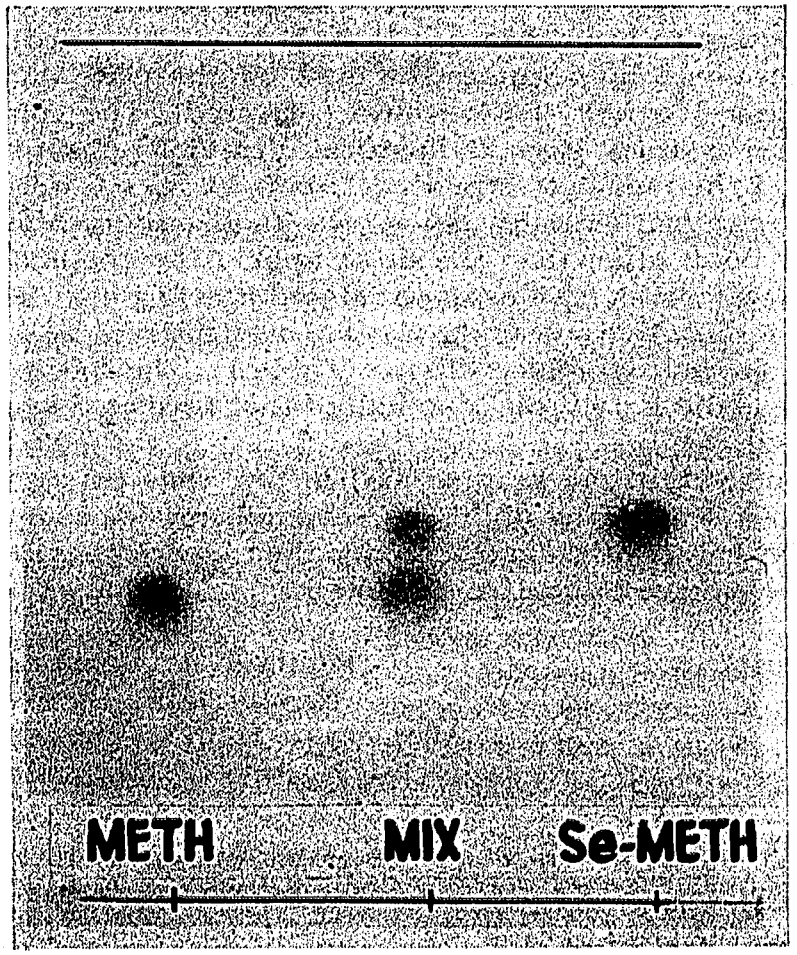

Fig. 1. Separation of methionine and selenomethionine on silica gel G with the mixture isopropyl alcohol–butanol–water (1:3:1).

urally-occurring selenoxides in a biological extract were suspected or if the seleno-amino acids were required for analysis after separation.

To overcome some of these difficulties a method has been developed to separate methionine and selenomethionine using thin-layer chromatography.

Thin-layer plates (20 × 20 cm) were prepared in the usual way with silica gel G (Merck) using the Desaga apparatus adjusted to give a 0.25 mm thick layer. The plates were oven dried at 110° for 60 min.

Several solvent systems were tried and the results are reported in Table I. The spots were detected by spraying with a 0.5 % solution of ninhydrin in *n*-butanol, the limit of detection being 0.2 μg.

TABLE I

R_F VALUES FOR MIXTURES OF METHIONINE AND SELENOMETHIONINE

Solvent	*Methionine*	*Seleno-methionine*
Ethyl alcohol–isobutyl alcohol–water (1:7:2)	0.22	0.30
Ethyl alcohol–*n*-butyl alcohol–water (1:7:2)	0.28	0.36
Isopropyl alcohol–*n*-butyl alcohol–water (1:3:1)	0.32	0.41
Methyl alcohol–*n*-butyl alcohol–water (1:7:2)	0.32	0.37
Ethyl alcohol–*n*-butyl alcohol–water (1:3:1)	0.37	0.41

The most satisfactory solvent was found to be a mixture of isopropyl alcohol, butanol, and water (1:3:1), separation being complete after the solvent had travelled 10 cm (Fig. 1).

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