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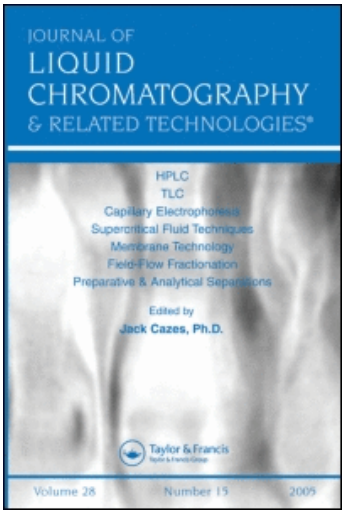

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THIN LAYER CHROMATOGRAPHIC DETERMINATION OF ALPHA-CAROTENE ON MAGNESIUM OXIDE LAYERS

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ABSTRACT

Procedures for the separation, identification, confirmation, and quantification of alpha-carotene on magnesia layers are described. The absence of this carotene isomer in spinach, iceberg and romaine lettuce, and Helisoma trivolvis (Pennsylvania and Colorado strains) and Biomphalaria glabrata snails is demonstrated.

INTRODUCTION

In an earlier study (1), seven types of high performance bonded silica gel plates were compared for the separation of chloroplast pigments, and the optimum system, C-18 bonded silica gel developed with petroleum ether-acetonitrile-methanol (2:4:4), was used for the qualitative and quantitative determination of pigments in spinach, lettuce, and snail samples. All of these samples were found to contain beta-carotene, but the absence or presence of alpha-carotene could not be confirmed because standards of the two

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compounds gave identical R_f values on all of the layers that were examined. The assumption was made that only beta-carotene was present, and quantification was carried out on C-18 layers by comparing sample zones to aliquots of a pure beta-carotene standard. The purpose of the present study was to develop methods for the separation, identification, and quantification of alpha-carotene on activated magnesium oxide (magnesia) layers and to test for the presence of this carotene isomer in spinach, lettuce, and snail samples.

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EXPERIMENTAL

Biomphalaria glabrata and *Helisoma trivolvis* (Pennsylvania and Colorado strains) snails were maintained on a diet of boiled lettuce and Tetramin (1:1) and were removed from food for at least 4 hours prior to sample preparation. Pigments were extracted from 1-2 g samples of spinach and lettuce leaves and snail bodies by blending with acetone and transferred into petroleum ether by addition of saturated NaCl solution as previously described (2), except that the upper layer was poured out through the top of the separatory funnel into the round bottom flask through Whatman (Clifton, NJ) 1PS phase separating paper to remove traces of water. The evaporated extract was prepared for TLC analysis by reconstituting it with 0.5-1.0 ml of petroleum ether. TLC standard solutions with concentrations of 1.0, 0.10, and 0.010 ug/ul levels were prepared by appropriate dilution of an alpha-carotene standard (Sigma, St. Louis, MO) with methylene chloride.

Solutions were applied to 20 x 20 cm precoated, glass-backed MgO/kieselguhr (1:1) layers containing 6% calcium sulfate binder and UV-254 fluorescent indicator (Analtech, Newark, DE) using a 25 ul Drummond (Broomall, PA) digital microdispenser in aliquot volumes ranging from 1-8 ul for standards and 5-70 ul for reconstituted samples. The plates were developed for a distance of 12 cm beyond the origin in a paper-lined, solvent vapor-saturated glass chamber wrapped in aluminum foil. In situ spectra (370-700 nm) and zone areas were measured immediately after TLC using a Shimadzu (Columbia, MD) CS-930 densitometer.

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RESULTS AND DISCUSSION

The best solvent systems for the separation of alpha- and beta-carotene on magnesia layers were petroleum ether (30-50°C)-benzene (3:1) (R_f 0.94 and 0.74, respectively) and petroleum ether-acetone (97:3) (R_f 0.61 and 0.48). As is typical in adsorption TLC, R_f values increased with the amount of extract spotted when the layer was overloaded. In the three systems, the xanthophylls and chlorophylls present in sample extracts (1), namely lutein, violaxanthin, neoxanthin, and chlorophylls a and b, did not migrate from the origin. Magnesia exhibits great selectivity for isomeric carotenoids that differ in the cis-trans arrangement of substituent groups, for isomeric carotenoids that differ in the arrangement (conjugation) and/or number of double bonds, and for various carotenoids that differ in the number and function of the substituent oxygen atoms (strain). The

separation of alpha- and beta-carotene is a vivid example of the selectivity of magnesia TLC, since the structures of compounds differ only in the position of one of 11 double bonds in each of the molecules.

In addition to their characteristic R_f values, the carotenes can be differentiated on the basis of their in situ visible spectra on magnesia layers. The spectrum of beta-carotene showed a central absorption maximum at 456 nm, with shoulders at 437 and 479 nm, while the corresponding wavelengths for alpha-carotene were 447 nm maximum and shoulders at 428 and 472 nm. These values compare to maxima at 420, 442, 472 for alpha-carotene and 425, 450, and 476 for beta-carotene in hexane solution (3).

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The absolute sensitivity limit for visual detection and precise densitometric scanning of alpha-carotene was 10 ng. The regression line for a series of standard zones between 10 and 80 ng scanned at 447 nm was represented by the following equation: peak area = (weight in ug) $(5.1 \times 10^4) + (-34.7)$, with a linearity correlation coefficient (r) value of 0.99.

It has been reported that many seed plants contain only beta-carotene, while in others the beta-carotene is accompanied by smaller quantities of the isomeric alpha-carotene (4). Magnesia TLC did not detect alpha-carotene in any sample we tested, including spinach, iceberg and romaine lettuce, and Helisoma trivolvis (Pennsylvania and Colorado strains) and Biomphalaria glabrata snail bodies. Assuming a 2 g sample, 100% recovery of pigments, reconstitution in 0.5 ml, and spotting of a 50 ul aliquot, the 10 ng sensitivity limit represents a minimum detectable concentration of 5 x

10^{-6}%, the level at or above which we can state alpha-carotene was not present.

Chromatograms of Biomphalaria glabrata extract developed with the petroleum ether-acetone mobile phase contained four contiguous yellow zones with R_f values ranging from 0.01 to 0.1. These were possibly carotenes other than alpha or beta, e.g., delta- or gamma-carotene or lycopene, which are known to migrate between beta-carotene and lutein (5), but the identity of the zones could not be confirmed since standards of these pigments were not available and the zones were not sufficiently separated to allow in situ spectra to be measured.

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