Paraquat in Marijuana

Because of very limited expertise currently available regarding the testing of Paraquat in contaminated marijuana, few laboratories are capable of determining the presence of Paraquat in the marijuana exported from Mexico. In view of the potentially health hazardous nature of the Paraquat, we wish to report a simple TLC procedure for determining its presence in marijuana or plant residue. The publication of this procedure will enable private laboratories to undertake its testing and thus alleviate future problems of a backlog of street specimens contaminated with Paraquat. The sensitivity of the procedure is less than 1 μg.

The procedure is as follows. Approximately 0.1–0.5 g of marijuana (plant material) is transferred to a 25- or 50-ml beaker (the plant material is not powdered or ground; the large size stems, if any, are cut into small pieces) and soaked for about 30 min with 5 ml of extraction solvent (methanol, 99 ml; concentrated hydrochloric acid, 1 ml). The contents are then boiled for about 30 sec on the water bath (if the extraction solvent has been absorbed by the plant material, an additional 3 ml is added prior to boiling the contents) and decanted into a 50-ml nongraduated conical centrifuge tube. This extraction process is repeated an additional three times, using 3–5 ml of the extraction solvent each time. All extracts are decanted into the same 50-ml conical centrifuge tube.

The color of the combined extracts is examined, and 1–2 teaspoonfuls of activated animal charcoal are added, depending on the color intensity of the extract. The test tube is heated to boiling for about 30 sec in the water bath with constant swirling, and the slurry of the extract and charcoal is filtered through Whatman No. 1 filter paper into another 50-ml nongraduated conical centrifuge tube. The tube is washed with 3 ml of boiling extraction solvent and poured through the same filter paper after the original solvent has been filtered.

Three such washings are performed, each with 3–5 ml of extraction solvent. Then the solvent is evaporated to about 50 μl in the drying oven having a horizontal air flow and maintained at 85–90°. The residue along the sides of the tube is washed with about 1 ml of methanol, the contents are mixed on a mechanical mixer, and the sides of the tube are again washed with an additional few drops of methanol. The solvent is evaporated to about 50 μl as described above. The contents are mixed on a mechanical mixer, and the entire extract is spotted on a 20 × 20-cm Gelman precoated silica gel glass microfiber sheet (ITLC type SA) with a layer thickness of 250 μm (if the solvent has entirely evaporated, 50–100 μl of methanol is added to the test tube, depending on the drug residue, the contents are mixed on a mixer, and the entire extract is spotted). A hair dryer or other means of drying may be used while spotting the entire extract onto the TLC plate to keep the size of the spot as small as possible.

Three spots each of 0.5-, 1.0-, and 1.5-μl size of the standard reference solution of Paraquat (1 mg of Paraquat/ml of methanol) are interspaced with five unknown specimens. If possible, two controlled marijuana specimens (0.2 g each) spiked with 1 and 2 μl of the Paraquat reference solution (equivalent to 1 and 2 μg of Paraquat) are carried through the procedure and spotted beside the unknown specimens to calculate semiquantitatively the concentration of Paraquat in unknown specimens. The plate is air dried for about 10 min and then dried in an oven at 85–90° for 5 min before it is placed in 100 ml of developing solvent (concentrated hydrochloric acid, 11 ml; water, 59 ml; acetic acid, 30 ml). The plate is developed up to 15 cm; if, however, a greenish spot or cloud from the plant material is below the 6–7-cm level, development is continued until the spot or cloud passes this level. From 1.5 to 3.5 hr or more may be required.

The plate is then removed from the developing tank and allowed to air dry for about 10–15 min. After heating the plate in the oven for 5 min at 85–90°, it is sprayed with iodoplatinate[1] followed by iodine–potassium iodide[1]. The plate is then allowed to air dry for 5 min and is again sprayed with iodoplatinate. Then it is covered with a paper towel for about 15 min and heated in an oven at 85–90° for about 4–5 min. The standard appears as an oval dark-gray (black) spot after iodoplatinate and as a white trail after heating in the oven for 4–5 min. The unknown specimen does not show the upper dark-gray spot but appears as a white decolorized spot of irregular or oval shape. The black or dark-gray color seen in the unknown specimens after iodoplatinate and iodine–potassium iodide sprays is ignored since it may be due to the reaction of iodoplatinate with plant material.

This solvent system is capable of separating Paraquat from Diaquat. The R_f value of Paraquat is about 0.25–0.40 and that of Diaquat is 0.35–0.45 when the solvent is allowed to travel a definite measured distance; if the solvent is allowed to run for several hours, Paraquat is seen at a distance of about 5.5–7.0 cm and Diaquat is seen at about 8.3 cm. Both of these herbicides form a white trail. However, the Paraquat dark spot is contained within the white trail; the dark spot of Diaquat is separate and is not contained within the white trail. We have been able to analyze 25 specimens/day using this procedure. The procedure is specific and does not give false positives.

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Received May 22, 1978.

We thank the Chevron Chemical Co., California, for their generous supply of Paraquat and Diaquat, and Jack Gilpin and the management of Dangerous Drugs Commission for their great interest in the Paraquat problem.

1 K. K. Kaistha and J. H. Jaffe, *J. Pharm. Sci.*, **61**, 679 (1972).

Carcinogenesis: A Coagulation–Coacervation Hypothesis

A recent critical response[1] to a single facet of a new carcinogenesis hypothesis proposed by Ecanow *et al.*[2] gives unwitting and unintended support for the hypothesis.

The essence of the overall concept proposed by Ecanow[3] and later developed with the help of coworkers is that the tumor matrix, which is rich in macromolecules, colloids, and electrolytes, combines with water to form a structured aqueous phase[4]. This phase is in a different thermodynamic state (a coacervated system) than is the normal extracellular aqueous phase[5]. This altered thermodynamic state now exists as a pathological aqueous matrix. It is more highly structured and less polar than the normal bulk water, polar, extracellular matrix[4–6]. When hydrophilic particles or cells with multilayers of strongly adsorbed water (low chemical potential) on their surfaces are aggregated so that the surface water layers are in contact (coagulated state), then the resulting matrix constitutes a coacervate phase[6]. The other aggregated state exists when the particles or cells are held in an open network structure in which the matrix is "normal" polar bulk water (flocculated state). Any event in the body that irreversibly converts the normal equilibrium thermodynamic states of the cells and the extracellular fluids to the coacervated coagulated state produces a pathological condition[4].

The first relevant sentence in the response[1] begins "The Class B particle complexes with the cellular material present. . . ." Thus, the critique of the pathological process begins with the unquestioned acceptance of the fact that the so-called inert particles are indeed capable of complexing with membranes. This is of particular importance because one major question in current oncology concerns the possible role of foreign inert particles found in the normal and