Simple Field Test for Marijuana

C. A. LAU-CAM ˣ and V. PIZZITOLA

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Abstract □ A simple, rapid, sensitive, and specific field test for marijuana and its products is described. The test employs an extracting-eluting solvent and an inert adsorbent microcolumn coated with Fast Blue B salt as the reagent. One milligram or less of the suspect material can be tested within 1 min. Of >80 nonmarijuana plant samples tested, only nutmeg and its mace responded similarly to marijuana initially. A differentiation was possible by careful evaluation of the colored microcolumn and of the elution behavior of the colored products. The test also provides for a confirmatory step, based on spectrophotometric analysis of the colored eluates. The reagent microcolumn was very stable to environmental factors such as moisture, light, and air.

Keyphrases □ Marijuana—analysis, microcolumn, field test, compared to other plant substances □ Chromatography, microcolumn—analysis, marijuana, field test, compared to other plant substances

With the recent relaxation of the laws governing marijuana use and possession in several states, the illicit market will increase. Consequently, there is a pressing need for a field test that will enable law enforcement agents, custom officers, and other responsible personnel readily to obtain presumptive evidence on suspected sample identity.

BACKGROUND

Fast Blue B salt (3,3'-dimethoxybiphenyl-4,4'-bis-diazonium chloride) is probably the most widely used reagent for marijuana detection and identification. In addition to high sensitivity and characteristic chromogenic reaction with several cannabinoids, Fast Blue B also is reasonably selective for these substances. Its low cost and ready availability further contribute to its popularity.

Marijuana samples were field tested on pieces of filter paper by means of a solid Fast Blue B reagent (1, 2). Although quite specific, this method requires special precautions in reagent preparation, reaction performance, and interpretation of results (1, 3). A very specific field test for hashish was reported, which utilized a suspension of Fast Blue B in chloroform in conjunction with aqueous sodium hydroxide (4). However, this method requires a large apparatus and two liquid reagents.

A "breathanalyzer" for the breath analysis of marijuana smokers was described (5). The suspect was required to breathe onto pieces of filter paper or tissue freshly dampened with aqueous Fast Blue B. The reagent was unstable, and the color response was slow. Hashish also was detected with filter paper strips pretreated with methanolic Fast Blue B (6). The reagent papers were unstable and decreased in sensitivity upon long storage (3, 6).

This paper describes a marijuana field test that circumvents many problems encountered with existing procedures. In addition to using a stable Fast Blue B color reagent, it requires few steps, one liquid reagent, and little apparatus. The test capability to differentiate false-positive responses also was investigated.

EXPERIMENTAL

Reagents—Fast Blue B salt[1], neutral alumina for chromatography[2], analytical reagent grade solvents[2] (petroleum ether, bp 60–90°; methanol; and chloroform), collodion USP[2], and microcrystalline cellulose[3] were used as obtained from commercial sources.

Apparatus—Spectrophotometric curves were recorded using 1-cm semimicro quartz cells on a double-beam recording spectrophotometer[4] equipped with a digital readout accessory and a strip-chart recorder.

Reagent Preparation—Neutral alumina–microcrystalline cellulose (3:1) was intimately mixed by manual stirring and bottle tumbling. For every 12 g of this mixture, a suspension of 0.2 g of Fast Blue B salt in 25 ml of chloroform was added with stirring. The smooth slurry was transferred to a large glass petri dish and allowed to air dry inside a hood. The dry residue was homogenized to a fine powder and stored in an amber glass container.

Microcolumn Preparation—A small glass wool or absorbent cotton pledget was placed at the bottom of a glass tube (3 × 0.7 cm), approximately 3 mm away from one end. Small portions of the reagent were introduced into the tube with gentle tapping until an ~2-cm high column was obtained. The upper end of this column was closed with another glass wool or cotton pledget. A 1-cm space remained over the packed reagent column. Both ends of the glass tube were sealed by immersion into collodion followed by air drying.

The microcolumns were stored in an amber glass container.

Extracting-Eluting Solvent Preparation—Isopropanol, 10 ml, was added, with stirring, to 80 ml of petroleum ether, bp 60–90°, and 20 ml of anhydrous methanol. The solution was stored in a dropping bottle.

Field Test—The collodion seals were removed from both ends of a microcolumn. A small amount of suspect material, preferably powdered, was layered ~2 mm high on top of the microcolumn. The extracting-eluting solvent was added dropwise until the liquid reached the bottom of the microcolumn. The color that developed along the microcolumn within 1 min was recorded. A red, purple-red, or burgundy-red color, deepening with time and rapidly spreading downward, was interpreted as a positive test.

Differential Test—To a still wet or to a dry colored microcolumn, sufficient extracting-eluting solvent was added until the colored product emerged at the lower end of the tube and could be collected. A rapidly eluting red, purple-red, or burgundy-red liquid signified a positive test.

Spectral Analysis—The colored eluate from the differential step was transferred to a semimicro spectrophotometric cell, and its visible absorption spectrum was recorded against the extracting-eluting solvent serving as the blank. If the eluate separated into two phases, the addition of 1–2 drops of chloroform reestablished the single phase.

RESULTS AND DISCUSSION

Microcolumn—Several common chromatographic support media were tested for suitability in the microcolumn preparation. Among those investigated were microcrystalline cellulose, infusorial earth, neutral alumina, silica gel, nylon, and methylcellulose. Selection criteria were: (*a*) inertness toward Fast Blue B, (*b*) rate at which solvents such as petroleum ether, chloroform, and petroleum ether–methanol eluted when

Table I—Samples Tested

Part Tested	Name
Bark	*Cascara sagrada*, cinchona, cinnamon, quassia, sassafras, wild cherry
Fruit	Anise, black pepper, capsicum, caraway, cardamom, coriander, fennel, juniper, paprika, star anise
Leaf	Basil, belladonna, boldo, buchu, catnip, digitalis, eucalyptus, hamamelis, henna, hyoscyamus, jaborandi, marijuana (Mexican, New York), oregano, parsley, rosemary, rumex, sage, savory, senna, spearmint, stramonium
Root, rhizome	Aconite, aspidium, belladonna, gentian, ginger, golden seal, hellebore (American), ipecac, jalap, licorice, orris, rauwolfia, rhubarb, rumex, sarsaparilla
Seed	Betel, cacao, coffee (decaffeinated, ground, instant), colchicum, cumin, kola, mace, mustard (black), nutmeg, *Nux vomica*, strophanthus
Miscellaneous	Aloe, clove, curry, ergot, gum benzoin, gum kino, gum myrrh, nutgall, squill, tolu, turmeric

[1] Sigma Chemical Co., St. Louis, Mo.
[2] J. T. Baker Chemical Co., Phillipsburg, N.J.
[3] Avicel PH-105, FMC Corp., Newark, Del.
[4] Model DB-GT, Beckman Instruments, Fullerton, Calif.

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Table II—Differentiation of Samples Giving Similar Colors in the Field Test

Sample	Color on Microcolumn	Characteristics of Colored Product	Absorption Maxima, nm[a]
Marijuana	Red to burgundy red	Elutes very rapidly, red eluate	295 (300, 305), 475
Mace	Red to burgundy red	Remains at top of microcolumn, beige to light-orange eluate	343 (330), 390 (sh), 485 (490) (sh)[b]
Nutmeg	Red to burgundy red	Remains at top of microcolumn, beige to light-orange eluate	340, 390 (400) (sh), 485 (490) (sh)[b]
Coffee, decaffeinated	Lavender to light violet	Elutes very rapidly, lavender to light-violet eluate	335, 425 (sh), 480 (sh)
Coffee, ground	Lavender to light violet	Elutes very rapidly, lavender to light-violet eluate	313, 335 (sh), 485
Cascara sagrada	Red	Remains at top of microcolumn, yellow to greenish-yellow eluate	Not recorded
Rhubarb	Red	Remains at top of microcolumn, yellow eluate	Not recorded

[a] Values in parentheses indicate absorption maxima recorded for a different sample. [b] Eluted with chloroform–methanol (7:3)

Table III—Absorption Maxima of Fast Blue B–Marijuana and Fast Blue B–Cannabinoid Colored Products

Sample[a]	Absorption Maxima, nm[b]
Marijuana	295 (300, 305), 475
I	298 (300), 450 (465)
II	292 (295), 500 (495)
III	295, 460
I + II + III (1:1:1)	295, 475
I + II (2:1)	307, 480
I + II (1:2)	300 (305), 467 (470)
I + III (1:1)	306, 465
II + III (1:1)	298 (305), 490 (492)

[a] Values in parentheses indicate approximate ratios in the mixture. [b] Values in parentheses indicate absorption maxima recorded for a different sample.

passed through a microcolumn, (*c*) ease of handling and packing, and (*d*) provision of contrasting background for the color reaction interpretation.

Neutral alumina best fulfilled the requirements. However, to promote a better interaction between the cannabinoids contained in the mobile phase and the Fast Blue B present on the microcolumn, the elution rate was decreased by admixing the alumina with microcrystalline cellulose (3:1).

Extracting-Eluting Solvent—The solvents investigated were those commonly used for cannabinoid extraction from marijuana samples, *i.e.*, petroleum ether, chloroform, benzene, and toluene. None of these solvents by themselves produced a color reaction following incorporation into a microcolumn loaded with marijuana, probably due to the insolubility of the Fast Blue B in the liquid phase. Addition of a small amount of methanol to the solvents brought about azo dye solubilization and interaction with the cannabinoids. Based on cost, organoleptic properties, and human safety, petroleum ether was the most satisfactory solvent. Furthermore, mixtures of methanol with chloroform, benzene, and toluene were less selective in extracting exclusively cannabinoids than were the corresponding mixtures with petroleum ether.

Petroleum ethers of bp 30–75°, 60–90°, and 90–120° were admixed with methanol in 9:1, 8:2, and 7:3 (v/v) ratios, respectively. A 9:1 ratio was inadequate in producing readily discernible marijuana color reactions. A 7:3 ratio yielded the strongest colors but at the same time produced more false-positive reactions with samples other than marijuana. Of the three petroleum ethers, the lower boiling solvent was the only one completely miscible with methanol in the stated ratios; those with higher boiling points gave a two-phase system. The former, however, occasionally separated from methanol upon storage. The possibility that the field test would be carried out in hot climates also discouraged further testing of this type of petroleum ether.

Perfect and stable solutions of methanol in petroleum ether of bp 60–90 or 90–120° in an 8:2 ratio could be obtained by the addition of a small quantity of isopropanol. Petroleum ether (bp 60–90°)–methanol–isopropanol (8:2:1) was preferred over a similar combination containing petroleum ether of bp 90–120°, because the former was more compatible with methanol. Mixtures of any of these two petroleum ethers with the alcohols in a 7:3:1 ratio invariably separated into two phases upon storage. On the other hand, combinations of all three types of petroleum ether with isopropanol in 9:1, 8:2, and 7:3 ratios did not produce distinguishable color reactions.

Test Selectivity—Table I lists the plant samples tested by the proposed field test. A positive reaction was when red, purple-red, or burgundy-red zones developed almost immediately at the top of the microcolumn and, on further addition of extracting–eluting solvent, rapidly moved downward as a red band. Of the >80 samples investigated, only those listed in Table II were initially confused with marijuana, but they were ultimately differentiated by careful evaluation of the colored microcolumn characteristics. For example, nutmeg and its mace gave red zones in a hue almost identical with those obtained for marijuana samples. Nevertheless, on repeated addition of the extracting–eluting solvent mixture, the colored products barely moved downward and the eluate emerging at the other end was colored light brown to light orange.

Rhubarb and *Cascara sagrada* also yielded red bands, even when Fast Blue B was absent from the microcolumn, due to extraction of certain red to brown-red constituents by the mobile phase. Their eluates were usually yellow to greenish yellow in contrast to the red tones recorded for marijuana. Various ground and instant coffees gave lavender to light-violet zones, which could be eluted rapidly out of the microcolumn as lavender to light-violet solutions.

Spectrophotometric Analysis—When a more conclusive identification of the field-tested material is needed, a confirmatory step may be added. The colored product may be eluted into a spectrophotometric cell, and the visible absorption spectrum may be recorded. Tables II and III summarize the spectral characteristics of those samples submitted to the spectrophotometric analysis. Marijuana samples typically exhibited two absorption maxima, at ~300 and ~475 nm. Entirely different spectral patterns were recorded for coffee, nutmeg, and mace, which initially approximated the marijuana chromogenic response.

The absorption spectra of the three main cannabinoids, *i.e.*, cannabi-

Table IV—Comparison of Various Field Tests for Marijuana Using Fast Blue B Salt

Characteristic	de Faubert Maunder (Refs. 1 and 2)	Watanabe (Ref. 4)	Woodhouse (Ref. 6)	McCarthy and van Zyl (Ref. 5)	Proposed
Number of reagents	3	2	3	1	2
Number of liquid reagents	2	2	2	1	1
Steps required:					
Extraction	+	+	+	–	+
Filtration	+	–	–	–	–
Addition of color reagent	+	–	–	–	–
Addition of alkali	+	+	+	–	–
Differential step	None	None	None	None	Included
Interferences reported	2	None	Several	None	None
Stability of Fast Blue B reagent	Stable	Stable	Unstable	Unstable	Stable

diol (I), cannabinol (II), and tetrahydrocannabinol (II), demonstrated that the marijuana absorption curve was primarily contributed by a combination of these three substances (Table III) rather than by any single cannabinoid or cannabinoid pair. The fact that these constituents occur only in marijuana confers high specificity and diagnostic value on the spectrophotometric analysis.

Test Comparison—Table IV compares the proposed field test with those reported in the literature in terms of reagent requirements, steps involved, and reported interferences.

CONCLUSIONS

The proposed field test was rapid, simple, sensitive and selective. It required only one liquid reagent, which served as the extracting-eluting solvent. Color development and sample extraction were accomplished simultaneously. Colors appeared almost immediately and were easy to interpret against the white background of the microcolumn. The use of a dry absorbent such as alumina offered several advantages since it acted as a diluent for the color reagent and as a medium for the color reaction, and, more importantly, provided a means for differentiating marijuana from other plant materials such as nutmeg and its mace, rhubarb, and *C. sagrada*, whose colored products remained tightly adsorbed to the microcolumn and were not eluted by petroleum ether–methanol mixtures.

The dry microcolumn formulation of Fast Blue B resulted in a reagent that remained stable to ambient conditions such as light and air for periods over 6 months. Since both ends of the glass tube enclosing the microcolumn could be sealed with collodion, adverse effects from moisture, air, and contaminated atmospheres were avoided. The inclusion of differential and confirmatory steps improved the test from a simply presumptive one to a tool with diagnostic capability. None of the plant samples tested by this procedure was confused with marijuana.

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Metabolic Performance and GI Function in Magnesium-Deficient Rats

WENDELL E. LANDIN, FRANCIS M. KENDALL, and MARTIN F. TANSY ˣ

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Abstract □ A quantitative evaluation of the mass balance and GI motor effects of dietary magnesium deficiency in the adult male Sprague–Dawley rat is described. Seventy-seven animals were used. Both sham control and experimental groups were maintained on a commercial stock laboratory diet *ad libitum* for 30 days, after which the experimental rats were switched for 30 days to the magnesium-deficient diet *ad libitum*, containing a magnesium concentration of 16.2 ppm. Ten rats were used to determine the acetylcholine responsiveness of duodenal muscle segments *in vitro*. In all cases, the segments from the deficient rats were hyperresponsive to a fixed acetylcholine dose. Forty-six rats were used to determine the average intestinal transit rate, which increased significantly in 30-day magnesium-deficient rats. A final series employed 21 rats who were housed in individual metabolic cages. After 5 days on the deficient diet, the average daily fecal pellet counts and fecal weights were significantly reduced. It is concluded that chronic magnesium deficiency is associated with altered GI motor function in the adult male rat.

Keyphrases □ Magnesium deficiency—GI motility, metabolism, rats □ GI tract—magnesium deficiency, motility, metabolism, rats □ Nutritional disorders—magnesium deficiency, GI motility, metabolism, rats

With few exceptions, most of the existing literature concerning the effects of chronic magnesium deficiency in the rat considers the GI tract only as a defective intake route: magnesium deficiency resulting from malabsorption, failure of magnesium conservation in the gut, and loss of fluid and ions *via* the intestines.

Curiously, the effect of magnesium deficiency on the functioning of the GI tract itself was not considered until the motor effects from exposure of the small intestines of rats (1) and humans (2–4) to ionizing radiation were significantly associated with hypomagnesemia. The data also indicated that prophylactic administration of soluble magnesium salts prior to exposure had a mitigating effect in both cases. These observations led to the proposal that the GI symptomatology defined by the acute intestinal radiation syndrome in the rat was the result of acute hypomagnesemia (5, 6).

Another interesting relationship is that between magnesium deficiency and the GI secretory apparatus. The effect of magnesium on gastric structure and function has received some attention, but reported results have been inconsistent. Recent observations (7) showed that dietary magnesium deficiency in rats alters the ratio of chief to parietal cells. Additional evidence (8) indicated that rats treated with parathyroid hormone for 2 weeks exhibited a reduced basal gastric acid secretory rate associated with nonspecific gastric mucosal damage. Veilleux (9) demonstrated that mast cells increase in the duodenum and kidney of magnesium-deficient rats. Unfortunately, these latter studies were not extended to the gastric mucosa. The foregoing results and the finding that the inhibition of calcium-induced acid secretion by magnesium may be caused by blockade of a calcium effect directly on the parietal cell (10) preclude any simple explanation of the effect of magnesium on gastric secretion.

Practically speaking, sufficient attention has not been directed to the effect of magnesium on the GI system. Therefore, the purpose of this study was to determine whether the GI motor functions of rats maintained on a magnesium-deficient diet were different from similar functions in control animals.

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