TECHNICAL NOTE

T. R. Baggi,[1] *B.Sc., A.I.C.*

3-Methylbenzthiazolinone-2-Hydrazone (MBTH) as a New Visualization Reagent for the Detection of Cannabinoids on Thin-Layer Chromatography Plates

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ABSTRACT: The use of 3-methylbenzthiazolinone-2-hydrazone (MBTH) for the detection of cannabinoids on thin-layer chromatography (TLC) plates is reported. Because MBTH is readily available in analytical-grade preparations, is fairly stable, gives characteristic, specific colors with the various cannabinoids studied, and might not be a potent carcinogenic compound, it is suggested as an alternative spray reagent for the detection of cannabinoids on TLC plates.

KEY WORDS: toxicology, marihuana, 3-methylbenzthiazolinone-2-hydrazone

Cannabinoids represent the most important class of compounds present in marihuana. Most of these cannabinoids have a phenolic group and the tests indicating this group's presence are the basis for the identification of marihuana. The identification of these psychoactive phenolic compounds of marihuana can be achieved by using color tests, thin-layer chromatography (TLC), and gas chromatography. The microscopic examination and Duquenois test followed by TLC are the procedures of choice in the identification of marihuana in routine cases.

A number of TLC procedures with various solvent systems and spray reagents are reviewed in the literature [*1,2*]. Most of the spray reagents used in these TLC procedures aim at the detection of phenolic constituents. Many chromogenic reagents such as Beams reagent (5% ethanolic potassium hydroxide), Gibbs reagent (isopropanolic 2,6-dibromoquinone-4-chlorimide), Ghamrawy reagent (*p*-dimethylaminobenzaldehyde/sulfuric acid), Duquenois reagent (vanillin/acetaldehyde/hydrochloric acid), Blackie reagent (benzaldehyde/secondary butanol), Pauly reagent (diazotized sulfanilic acid), diazotized *p*-nitroaniline, 1% ethanolic 2,6-dichloroquinone chlorimide, Fast Blue Salt B [*1*], 1-nitroso-2-naphthol [*3*], and Fast Blue Salt 2B [*4*] have been reported in the literature, but the use of Fast Blue Salt B in aqueous, methanolic, or alkaline medium seems to be most common.

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[1]Assistant director, Central Forensic Science Laboratory, Bureau of Police Research and Development, Ministry of Home Affairs, Government of India, Hyderabad, India.

Although Fast Blue Salt B is widely used as a chromogen, batch-to-batch variations are reported to give variable intensities of color [5]. Furthermore, the safety of Fast Blue B dye is still sometimes questioned because of the presence of the potentially carcinogenic residual unreacted free amine in the diazonium salt [6]. Maunder [6] and Thornton and Nakamura [7] have investigated a large number of alternative dyes and have recommended Fast Red B, Fast Garnet GC (GR), and Fast Blue RR for field testing of marihuana, but no progress has been reported in replacing the popular Fast Blue Salt B used as a spray reagent in TLC. However, Hughes and Kessler [4] recently reported using an alternative spray reagent, Fast Blue 2B Salt, with good results.

The oxidative coupling of 3-methylbenzthiazolinone-2-hydrazone (also called 2-hydrazono-2,3-dihydro-3-methylbenzothiazole) (MBTH) with phenols to form highly colored products has been described by Hunig and Fritsch [3]. Several other applications of this reagent to the detection or determination of phenols have been reported [9]. Recently, Gasparic et al [10] carried out excellent investigations on the color reactions of various phenols with this reagent and reported that the colors obtained are highly dependent on the structure of the phenols, that is, the number and position of their substituents.

In this communication an alternative chromogenic reagent based on the oxidative coupling of MBTH [10] for the detection of cannabinoids on TLC plates is reported.

Materials and Methods

Reagents

The reagent used was a 0.05% w/v solution of MBTH (Merck & Co.). A 0.2% w/v aqueous solution of ceric ammonium sulfate (analytical reagent grade) containing 0.4% v/v sulfuric acid and ethanolic reference solutions of various cannabinoids were also used.

Extraction of Cannabinoids

Approximately 100 mg of marihuana sample was extracted with petroleum ether (boiling point, 40 to 60°C) as per the procedure of Merkus [2] or with benzene as per Clarke's method [11].

Thin-Layer Chromatography

Thin-layer chromatography of the extract was carried out either by the procedure reported by Korte and Sieper [1] or by the method of Aramaki et al [12]. In the former procedure the extract is applied to a 250-μm-thick silica gel G layer activated for 1 h at 105°C and impregnated with dimethyl formamide/carbon tetrachloride (6:4) and subsequently developed twice or three times in cyclohexane. In the latter method the extract is applied to a 250-μm-thick silica gel G plate activated for 1 h at 105°C and developed in a solvent system consisting of benzene/*n*-hexane/diethylamine (25:10:1). In both cases, reference samples of cannabinoids were also applied on the plates beside the samples being tested.

After the required development the plates were air-dried, sprayed with MBTH reagent, and allowed to stand for about 10 min. After this the plates were sprayed with acidic ceric ammonium sulfate solution and heated for about 5 min at 105°C in an oven. Various colored spots developed. The details of the spots are listed in Table 1 along with the sensitivity of the reagent.

TABLE 1—*Test results.*

Cannabinoid	Color of the Spot		Minimum Detectable Amount, μg
	Solvent System I [*1*]	Solvent System II [*12*]	
Cannabicyclol	orange brown	pinkish brown	2.5
Δ^8-Tetrahydrocannabinol	orange	orange brown	5
Δ^9-Tetrahydrocannabinol	orange	orange brown	5
Cannabidiol	yellow	violet brown	5
Cannabinol	pink	pink	2.5
Cannabigerol	yellowish brown	brownish yellow	5

Results and Discussion

This work was undertaken to develop an alternative spray reagent for visualizing cannabinoids on TLC plates. Gasparic et al [*10*] have reported that phenols with a free para position and at least one free ortho position give a mixture of colored products and that these colors are highly dependent on the structure of the phenolic compound, that is, the number, nature, and position of its substituents. Because the phenolic groups in most of the cannabinoids have a free para position and at least one free ortho position with specific structural features, they are expected to undergo the MBTH oxidative coupling and yield colored products. These reactions were proved by the experiments.

The colors of the spots developed from the two solvent systems studied vary slightly. The MBTH spray reagent is capable of developing the usual streak of seven or eight spots of different colors and shades for the marihuana extract. Six cannabinoids could be identified from these spots (Table 1). The other cannabinoids could not be identified because reference samples were not available to us. The color of the spots was stable for a number of days. However, the shades of the color either changed slightly or darkened, and some additional spots appeared after the plates had been stored at room temperature and exposed to daylight for a number of days. No fading of colors was observed in the chromatograms developed. If the developing solvent did not completely evaporate from the plate a greenish blue background developed after the acidified ceric ammonium sulfate spray was used. Different colored spots were visible with the sulfate spray, and therefore it is sometimes useful in visualizing the chromatograms in a better way. After a number of studies on the various parameters such as the concentration of reagents, the interval between the first and second spray, and the heating time, the conditions described above were found to be optimum. Both the MBTH reagent and the acidified ceric ammonium sulfate are stable for at least a week when stored in a refrigerator protected from light. No reference classifying MBTH as a potent carcinogen is available in the literature.

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Address requests for reprints or additional information to
T. R. Baggi
Central Forensic Science Laboratory
Bureau of Police Research and Development
Ministry of Home Affairs
Government of India
Hyderabad, India