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ORIGINAL ARTICLE

Analytical determination of nicotine in tobacco leaves by gas chromatography–mass spectrometry

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Abstract A preliminary investigation using gas chromatography–mass spectrometry (GC–MS) to analyze the nicotine contained in tobacco leaves was carried out. Nicotine is an alkaloid and tobacco leaves was extracted with methanol and determined by GC–MS. The detection limit for nicotine was at the ppm level for non selective monitoring and the nanogram level for selective detection. This is a simple chromatography–mass spectrometry method for the analysis of nicotine in tobacco leave. Compared to other currently utilized methods for the detection of nicotine in tobacco leaves, the GC–MS provided advantages of high sensitivity, nicotine specific detection and lower instrumentation cost.

1. Introduction

Nicotine is an alkaloid found in the nightshade *Solanaceae* family of plants, predominantly in the leaves of tobacco, and in lower concentration in tomato, eggplant, and in green pepper. They are also found in the leaves of the coca plant. Most of the medicinal higher plants extractable organic compounds in sufficient quantities to be economically useful as chemical feed stocks or raw materials for various scientific, technological and commercial applications. Industrial oil, resins, tannins, saponins, nicotine, natural rubber gums, waxes, dyes, pharmaceuticals and many other products from economically important plants serve as sources (The Merck Index, 1989). Nicotine, 3-(1-methyl-2-pyrrolidinyl) pyridine is a colourless, less to pale yellow, hygroscopic oily liquid present in the leaves of *Nicotiana tabacum*.

Nicotine is one of the most highly toxic compounds belonging to the tobacco alkaloids (The Merck Index, 1989). Several chromatographic techniques have been applied to describe for the determination of nicotine in various plants extract (Burrows et al., 1971; Beckett and Triggs, 1996; Isaac and Rand, 1972; Feyerabend et al., 1995; Dow and Hall, 1978). Various solvent extraction techniques followed by gas chromatographic-mass spectrometric analysis (Thompson and Ho, 1982; Watson, 1977; Grubner et al., 1980) and liquid chromatography (LC) with ultra-violet absorbance detection (Davis, 1986; Moore et al., 1993) are the most useful techniques employed for the determination of nicotine in the leaves of tobacco. A most useful technique described for the determination of

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nicotine by Moore, et al. and cotinine in plasma after a single extraction procedure using gas chromatography-nitrogen selective detection. The electron capture detector used for the determination of nicotine after chemical derivatization using heptafluorobutyric anhydride. For the determination of nicotine and N-methylnicotinium ion at the picogram level using electrochemical detection by very useful and selective LC method has been developed (Mousa et al., 1985).

Bangladesh is an agricultural country. Vegetables, crops, tobacco and fruits are grown here in plenty, mainly in the winter season. Different types of tobacco herbs are the most commonly used because they are cheap and available all over the Bangladesh throughout the season. The concurrent use of tobacco with alcohol is one of the most common drug combinations in the United States. There is a general consensus that nicotine modifies the acute effects of alcohol.

The effects of nicotine are very important as sex-related differences have been noted. Generally, nicotine appears to be less reinforcer in women than in men for maintaining cigarette smoking and this may be due to sex differences in the sensitivity to nicotine's interoceptive cues (Perkins, 1999). Hormones are the most important factors for understanding many drug effects in women. As for example, cocaine and amphetamine are responsible to influenced by varying the level of estrogen and progesterone associated with the follicular and luteal phases (Evans et al., 2002; Justice and Wit de, 1999; Mitchell et al., 1995). Mello *et al.,* reported that cigarette smoking and alcohol self-administration in women and found that almost three-quarters of women increased smoking during the luteal phase of their menstrual cycle, as measured by inter-cigarette interval. On the other hand, additional important evidence for the influence of menstrual cycle phase on nicotine's effects is seen during withdrawal. Perkins *et al*. report that nicotine withdrawal symptoms for severity is greater during the luteal phase of the cycle than during the follicular, which may be explain why others have reported more smoking during this phase (Mello, 2007; Benowitz et al., 1988). Cigarette smoking has a relatively short duration of action of nicotine administration. The levels of nicotine peak for blood are achieved typically within the time it takes to consume the cigarette (5 to 10 minutes) and decline quickly (Pomerleau et al., 1994). However, alcohol via the oral route has a slower onset and longer duration of action. In this paper, a simple, chromatographic method is described for the determination of nicotine in the leave and stems of tobacco.

2. Material and methods

2.1. Chemicals

Nicotine standard was purchased from Sigma–Aldrich Company with purity 99.9%. Methanol (BDH, UK), dichloromethane and water (Merck, Germany) were of HPLC grade. Anhydrous sodium sulfate (Merck, Germany) was cleaned by heating at 200 °C before use. Silica gel (60–120 mesh, Loba, India) was activated at 400 °C for 12 h prior to use.

2.2. Sample collection

There are different types of tobacco available in Bangladeshi markets all year round. The dried tobacco leaves samples were collected from one of the biggest tobacco industry at Dhaka Metropolitan City, Bangladesh in April 2009. Five fresh tobacco leaves samples were collected from southern district of Kushtia after harvest. The leaves samples were washed by tap and de-ionized water to remove dusts and any other foreign particles. After collection, the sample was kept in a polyethylene bag with aluminum foil protected cover and stored in refrigerator to avoid any deterioration. After harvesting, the apples are stored at 2 °C.

2.3. Isolation and preparation of crude extracts

After having washed, the leaves were cut into small pieces and dried by sunlight or oven below temperature 40 °C. The dried leaves samples were pulverized into powder form. The dried powder (0.1 g) was extracted three times with methanol (5 ml 3×) by sonication at 30 min. It was then filtered and the filtrate was evaporated near to dryness by Kuderna-Danish evaporator. The extract was passed through the cleanup column (i.d. = 1 cm), which was filled with cotton in the bottom. An activated silica gel (10 g) soaked with solvent was loaded into the cleanup column (5 cm), which was then topped with 1.5 cm of anhydrous sodium sulfate. Five milliliters of solvent were added to wash the sodium sulfate and the silica gel. The preconcentrated dried crude extracts, 1 ml of each extract sample, were then separately transferred into the column, and the vessel was rinsed twice with 2 ml loaded solvent, which was also added to the column. Sixty milliliters of loaded solvent were added to the column and allowed to flow through the column at a rate of 3–5 ml/min, and the eluent was collected. The collected eluent from the cleanup procedure was reconcentrated to 2 ml by using K-D concentrator. Finally the extract (2 ml) from leaves was filtered through a 0.45 lm Millex HA filter (Millipore, Molsheim, France) prior to GC–MS analysis.

2.4. GC–MS analyses

2.4.1. Preparation of samples from markets for GC–MS analyses

The methanol extract (1 ml) was diluted with 5 ml of methanol and the samples were filtered through 0.45 lm membrane filters (Molsheim, France) prior to GC–MS analysis.

2.4.2. Identification and quantification of marker in the leaves samples

The GC–MS analysis of the methanolic crude extract of tobacco leaves samples was performed using a Varian GC–MS (Model Varian CP 3800, Varian, Inc. Scientific Instruements, Lake Forest, CA 92630-8810, USA) equipped with a VF-5 fused silica capillary column (30 m × 0.25 i.d. mm film thickness 0.25 lm, Varian, USA). For GC–MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas was used as a carrier gas at a constant flow rate of 1 ml/min. Injector and mass transfer line temperature were set at 250 and 300 °C, respectively. The oven temperature was programmed from 50 to 200 at 8 °C/min, and then held isothermal for 20 min and finally raised to 300 °C at 10 °C/min. Diluted samples (1/100 v/v, in methanol) of 0.2 µl were manually injected in the split less mode. Identification of compounds of the methanolic crude extract was based on GC retention time on VF-5 capillary column, computer matching