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Quantitative analysis of mitragynine, codeine, caffeine, chlorpheniramine and phenylephrine in a kratom (*Mitragyna speciosa* Korth.) cocktail using high-performance liquid chromatography

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ABSTRACT

A simple HPLC technique for determining mitragynine, codeine, caffeine, chlorpheniramine and phenylephrine in 'kratom cocktail' was developed. The analytical method for mitragynine, codeine and caffeine used an Eclipse XDB-C8 column. A Lichrospher CN column was using for analysing chlorpheniramine and phenylephrine. The correlation coefficient of each standard was between 0.9957 and 0.9993. The precision of the methods were between 0.700 and 7.108% RSD. The concentration of mitragynine, codeine, caffeine, chlorpheniramine and phenylephrine in 'kratom cocktail' was 90.021, 234.174, 73.986, 7.053 and 1.486 mg/L, respectively.

1. Introduction

Kratom (*Mitragyna speciosa* Korth.) in the family Rubiaceae, is a tropical tree indigenous to Thailand, Malaysia, Myanmar and other areas of South East Asia. The tree reaches heights of 50 ft with a spread of over 15 ft. Extracts from leaves of this tree contain many alkaloids that have psychoactive effects. There are two main kinds of kratom distinguished by the color of their leaf veins – red or green. The red veined variety is supposed to have a stronger effect. Over 25 alkaloids have been isolated from kratom, of which the indole alkaloid, mitragynine, is the most important. Mitragynine, chemically known as 9-methoxy-corynantheidine (Fig. 1), is the primary active alkaloid in the plant.

Kratom has been used by natives of Thailand and other regions of Southeast Asia as a herbal drug for decades and perhaps forever. Traditionally, kratom was mostly used as a stimulant by Thai peasants, laborers, and farmers to overcome the burdens of hard work. Thai natives chewed the leaves to make them work harder and feel good. Kratom was also used to alleviate opium withdrawal. A kratom user mostly likes to chew fresh leaves as the compounds in the leaf veins are then more easily extracted. Dried leaves can also be chewed, but since they are a bit tough, most people prefer to crush them up or powder them so that they can be swallowed. Heavy users may chew kratom between 3 and 10 times a day. While new users may only need a few leaves to obtain the desired effects, some users find that with time they need to increase the dose to 10–30 leaves or even more per day. Kratom has been reported to be a central nervous system stimulant, and also a depressant. It helps to increase work efficiency and tolerance to hard work under a scorching sun [1]. Kratom is also used to treat muscle ache and fatigue because it can produce muscle relaxation [2]. It can be a very effective treatment for diarrhea [3]. In 1979, the Thai government enacted the Narcotics Act B.E. 2522, placing kratom along with marijuana in Category V of a five category classification of narcotics. This means that it is illegal to buy, sell, import, or possess. This law makes planting the tree illegal and requires existing trees to be cut down. This law however is not fully effective, since the tree is indigenous to the country and native people have always used them. Hence, kratom remains a popular drug in Thailand, especially in the Southern regions.

From the national household survey in 2007, kratom is the most popular drug within southern Thailand, the second and third is marijuana and methamphetamine, respectively [4]. The pattern of

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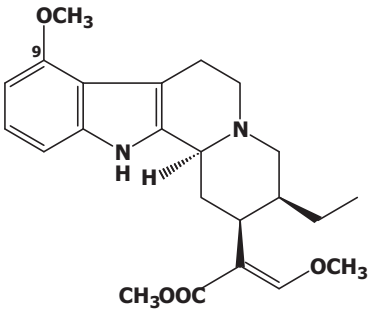

Fig. 1. Chemical structure of mitragynine.

use has more recently changed from chewing the fresh leaves to drinking a 'kratom cocktail' especially by teenager users. They often have a meeting everyday to drink this cocktail. The kratom cocktail (dubbed 4 × 100; 4 times 100) is a mixture of boiled kratom leaves, coke, antitussis syrup, coffee or codeine etc. and is served with ice. The origin of the name is unclear, but it likely came from its four or more ingredients. The users felt that kratom leaves and caffeine in soft drinks stimulated the nervous system, causing an increase in alertness, while the cough syrup and tranquilisers caused drowsiness. The above combination made the drinker high. 'Kratom cocktail' quickly spread to Bangkok where it is now popular in the poorer suburbs. Recently hitting the streets was the all new 5 × 100 and then quickly after the 6 × 100. New ingredients have included yoghurt, coffee or alprazolam, etc. The type and quantity of each ingredient was dependent on the users. People found with kratom leaves and antitussis syrup are still arrested by the police [5]. These cocktails may have an impact on certain health problems in long term users.

The aim of this study was to develop an analytical HPLC method to detect and quantify the main active substances in the kratom cocktail such as mitragynine, caffeine, codeine, chlorpheniramine and phenylephrine.

2. Experimental

2.1. Chemicals

Methanol, acetonitrile and isopropanol were supplied by J.T. Baker, Malaysia. Chlorpheniramine maleate, codeine, caffeine and phenylephrine were purchased from Sigma–Aldrich, USA. Pure mitragynine from kratom leaves was obtained by the described extraction procedure [6]. The pure compound had been analysed by MS (Thermofinnigan MAT 95 XL mass spectrometer: EIMS with a direct insert probe) and 1H-NMR and 13C-NMR (Varian Unity Inova 500 NMR spectrometer) spectra. When the obtained spectral data were compared with the published assignments [7,8], it was identified as mitragynine.

2.2. Sample

A sample of a 'kratom cocktail' (4 × 100) was obtainen from Pattani province, southern Thailand. It was checked for its volume and its pH was checked by a pH meter (Metler Toledo, USA). It was concentrated by a vacuum freezer drier (Corrosion Resistant Freezer Drier, FTS System Inc., USA). The dry sample was weighed and kept at −20 °C until assayed. The dry sample of 'kratom cocktail' was reconstituted in the column mobile phase and filtered through a nylon membrane (0.2 μm) before analysis.

2.3. Chromatographic instruments and conditions

The HPLC system consisted of an Agilent model 1100 series Diode array detector (Agilent, USA). The system was controlled and data analyses were performed with ChemStation software. The analytical method for mitragymine, codeine and caffeine was optimized using an Eclipse XDB-C8 column (5 μm, 150 mm × 4.6 mm i.d.; Agilent, USA). The analytical method for chlorpheniramine and phenylephrine was optimized using a Lichrospher CN column (LC) (5 μm, 250 mm × 4.0 mm i.d.; Agilent, USA). All solvents for the mobile phase and sample were filtered through a 0.20 μm Millipore filter.

HPLC analysis for mitragynine was performed by isocratic elution with a flow rate of 0.5 mL/min. The temperature was controlled at 35 °C. The detector wavelength was set at 221 and 291 nm. The mobile phase was methanol:water (80:20, v/v). A volume of 10 μL of each prepared solution and samples were injected into the column. The chromatographic run time was 10 min.

Codeine and caffeine was analysed by isocratic elution with a flow rate of 1.0 mL/min. The separation was carried out at 25 °C. The detector wavelength was set at 215 nm. The mobile phase was 0.01 M KH_2PO_4:methanol:acetonitrile:isopropanol (74:8:9:9, v/v/v/v). The injection volume of each prepared solution and samples was 20 μL. The chromatographic run time was 5 min.

The analytical method developed for chlorpheniramine and phenylephrine used the LC system. The temperature was controlled at 35 °C. The detector wavelength was set at 215 nm for chlorpheniramine and 270 nm for phenylephrine. Separation was carried out at 35 °C. The mobile phase was 10 mM phosphate buffer at pH 3.0: acetonitrile (96:4, v/v), with a flow rate of 1.5 mL/min. A volume of 10 μL of each prepared solution and sample was injected into the column. The chromatographic run time was 10 min.

2.4. Method validation

Validation of the analytical methods was performed in accordance with the ICH Harmonized Tripartite Guideline Topic Q2B (ICH Steering Committee, 1996).

Standard solutions of mitragynine containing (5.000–100.000 mg/L) were prepared in the column mobile phase. Caffeine and codeine were mixed (0.010–200.000 mg/L) in their mobile phase. Chlorpheniramine and phenylephrine containing 5.000–200.000 mg/L were prepared in acetonitrile:water (20:80, v/v). The five concentrations of standard were subjected to regression analysis to calculate the calibration equation and correlation coefficients. Linearity was obtained for compounds between peak-area ratios and concentrations of 1–1500 mg/L mitragynine, 0.010–200.000 mg/L codeine and caffeine and 0.400–500.000 mg/L chlorpheniramine and phenylephrine. The limit of detection (LOD) was established at a signal-to-noise ratio (*S*/*N*) of 3. The limit of quantification (LOQ) was established at a signal-to-noise ratio (*S*/*N*) of 10. LOD and LOQ were experimentally verified by 6 injections of compounds at the LOD and LOQ concentrations.

2.5. Suitability of the method

The chromatographic parameters such as resolution, selectivity and peak symmetry were satisfactory for the compounds analysed. The standard solution of standard mitragynine, codeine, caffeine, chlorpheniramine and phenylephrine was prepared at a concentration of 10.000 mg/L and was checked by injecting nine replicates. The relative standard deviation (RSD) was calculated according to the formula: RSD (%) = (standard deviation/mean value) × 100. The level of acceptance was not more than 15% of the RSD value.

2.6. Precision

The precision of the method (within-day variations of replicate determinations) was checked by injecting nine replicates of standard mitragynine, codeine, caffeine, chlorpheniramine and phenylephrine at their LOQ level. The precision is expressed as %age RSD at the LOQ level.

2.7. Accuracy

Working standard solutions of mitragynine, codeine, caffeine was prepared to give final concentrations of 5.000, 10.000, 40.000, 60.000, 80.000, 100.000 mg/L. Standard working solutions of chlorpheniramine and phenylephrine were prepared at final concentrations of 10.000, 40.000, 80.000, 100.000, 150.000, 200.000, 250.000, 300.000 mg/L. Each standard was injected with nine replicates. From the respective area of the eluant, the concentrations of mitragynine were calculated using the detector responses. The accuracy was defined in terms of the %age deviation of the calculated concentrations from the actual concentrations. The percentage recovered was calculated using the expression: Recovery (%) = (response after extraction/response after direct injection) × 100.

2.8. Ruggedness

The ruggedness of the HPLC method was evaluated by carrying out the analysis using a working standard solution in the same chromatographic system and column on different days. Only small differences in area-ratios were obtained and there was a good constancy in the retention times observed after a 48 h time period. The responses obtained on different days indicated that this method was capable of producing results with a high precision on different days.

3. Results

The 3D spectra of standard mitragynine and mitragynine in the sample were detected by a diode array detector shown in Fig. 2. The parameters of standard mitragynine codeine, caffeine, chlorpheniramine and phenylephrine were ckecked in a similar