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Short communication

Testing for kavain in human hair using gas chromatography–tandem mass spectrometry

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Abstract

A sensitive, specific and reproducible method for the quantitative determination of kavain in human hair has been developed. The sample preparation involved a decontamination step of the hair with methylene chloride. The hair sample (about 50 mg) was incubated in 1 ml of methanol for 1 h, in an ultrasonic bath, in presence of 20 ng of methaqualone-d_7 used as internal standard. The methanolic solution was evaporated to dryness, and the residue reconstitued by adding 30 μl of methanol. A 2 μl aliquot of the extract was injected onto the column (Optima5-MS capillary column, 5% phenyl–95% methylsiloxane, 30 m × 0.25 mm i.d. × 0.25 mm film thickness) of a Hewlett-Packard (Palo Alto, CA) gas chromatograph (5890). Kavain was detected by its parent ion at *m/z* 230 and daughter ions at *m/z* 111 and 202 through a Finnigan TSQ 700 MS/MS system. The assay was capable of detecting 30 pg/mg of kavain (limit of detection (LOD)). Linearity was observed for kavain concentrations ranging from 100 to 2000 pg/mg with a correlation coefficient of 0.998. Intra-day precision at 400 pg/mg was 13.7%. The analysis of a segment of hair, obtained from an occasional consumer, revealed the presence of kavain at the concentration of 418 pg/mg. A higher concentration (1708 pg/mg) was detected in the corresponding pubic hair.

Keyword: Kavain

1. Introduction

Kava is a term used to describe both the plant *Piper methysticum* and the preparation obtained from its roots. This South Pacific plant is a robust, branching and perennial shrub growing best in warm, humid and sunny conditions, where it forms dense thickets [1].

Captain James Cook was the first to describe the use of kava during the religious and cultural ceremonies of the people of the South Sea Islands, where it was, and still is, prepared as a beverage and consumed for its intoxicating and calming effects that promote sociability [2]. Events typically accompanied by kava ceremonies included weddings, funerals, births, religious occasions, welcoming of guests and the exchange of gifts. The beverage was traditionally made by mixing grated, crushed or chewed fresh or dried root with cool water.

The psychotropic effects of kava are attributed to a group of substituted dihydropyrones called kavalactones. The main bioactive constituents include yangonin, methysticin and kavain [3]. Chemical structure of kavain, a lactone related to alpha-pyrone, is given Fig. 1.

It is believed that the components present in the kava resin are responsible for the central nervous system activities of kava including anxiolytic effect, sedation, hypnosis, analgesia and muscle relaxation [4].

As chronic use of kava has been associated with hepatotoxicity, this product cannot be sold any longer in France and is now a scheduled drug [5]. However, its chronic use is still a common way in the islands of South Pacific.

In order to document a long time exposure to a drug [6,7], the analysis of hair appears as the procedure of choice. We present here a sensitive method for testing kavain in hair using GC–MS/MS.

2. Material and methods

2.1. Specimens

Hair specimens, collected from head and pubic areas, were obtained from a occasional kavain consumer (frequency of consumption not known). The hair strand (>15 cm long) was taken at the surface of the skin from the vertex and stored in

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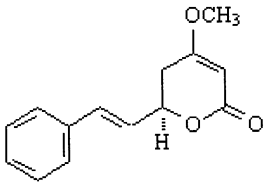

Fig. 1. Chemical structure of kavain.

an envelop at room temperature. The analysis was performed on the first 2 cm segment from the root weighing 29 mg, whereas 50 mg of pubic hair were analysed. Negative hair specimens ($n = 4$), obtained from laboratory personal, were used as control.

2.2. Chemicals and reagents

Methylene chloride and methanol were HPLC grade (Merck, Darmstadt, Germany). Kavain and methaqualone-d_7 were purchased from Extrasynthèse (Genay, France) and Promochem (Molsheim, France), respectively.

2.3. Kavain extraction

The hair was decontaminated twice using 5 ml of methylene chloride, for 2 min at room temperature, and then cut into small pieces (<1 mm).

Table 1
Selected ion (*m*/*z*) and retention times for kavain and the internal standard

Analyte	Retention time (min)	Ions (*m*/*z*)
Kavain	8.60	230–111, 230–202
Methaqualone-d_7	8.36	257–242

Fifty milligrams of decontaminated hair were incubated in 1 ml of methanol for 1 h, in an ultrasonic bath, in presence of 20 ng of methaqualone-d_7 (final concentration 400 pg/mg) used as internal standard (IS). After centrifugation, the organic phase was removed, evaporated to dryness and the residue was reconstitued by adding 30 μl of methanol.

2.4. GC–MS/MS procedure

A 2 μl aliquot of the extract was injected onto the column of a Hewlett-Packard (Palo Alto, CA) gas chromatograph (5890). The carrier gas (helium, purity grade N 55) through the column (Optima5-MS capillary column, 5% phenyl–95 % methylsiloxane, 30 m × 0.25 mm i.d. × 0.25 μm film thickness) was at a pressure of 68.9 kPa.

The injector temperature was 215 °C. Splitless injection was employed with a split valve off-time of 1.0 min.

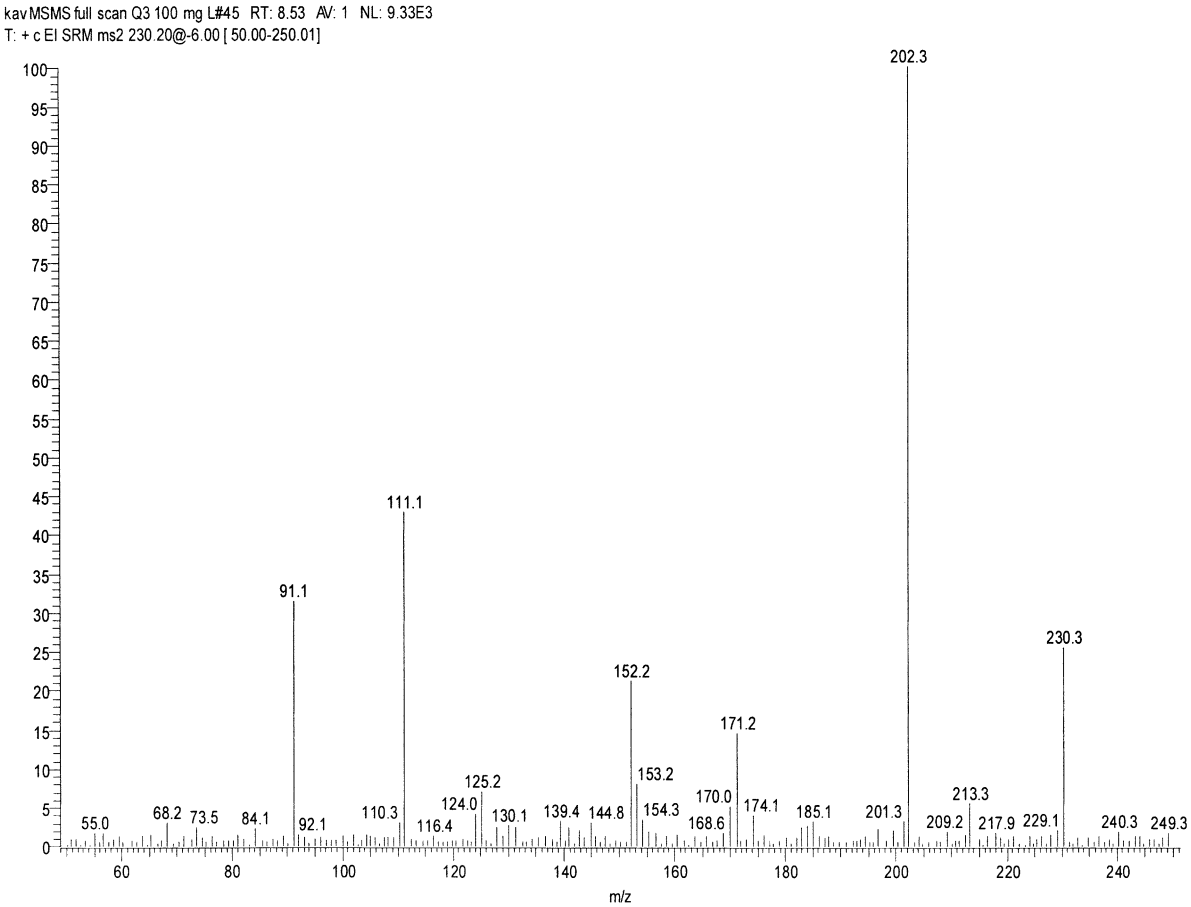

Fig. 2. Full scan daughter ion mass spectrum of kavain.

The column oven temperature was programmed to rise at 30 °C/min from an initial temperature of 100 °C (maintained for 1 min) to 295 °C (maintained for 6 min).

The detector was a Finnigan TSQ 700 operated in electron impact mode used in selected reaction monitoring (SRM). The parent ions, *m*/*z* 230 and 257 for kavain and the IS, respectively, were selected in the first quadrupole. The corresponding daughter ions, *m*/*z* 111 and 202 for kavain and 242 for the IS, were selected in the third quadrupole after collision with argon at a cell pressure at 0.1 Pa. The collision offset voltage was 6 eV. The electron multiplier was operated at 1900 V.

2.5. Method validation

A standard calibration curve was obtained by adding 5 ng (100 pg/mg), 10 ng (200 pg/mg), 20 ng (400 pg/mg), 50 ng (1000 pg/mg) and 100 ng (2000 pg/mg) of kavain to 50 mg of control hair (negative for kavain).

Within-batch precisions for kavain was determined using negative control hair spiked with kavain at the final concentration of 400 pg/mg ($n = 8$). The limit of detection (LOD) was evaluated with decreasing concentrations of kavain until a response equivalent to three times the background noise was obtained.

It is acknowledged that the use of spiked specimens may not fully substitute for the use of real hair as a matrix. However, it was not possible to find reference material with authentic kavain incorporated. Moreover, the submitted amount of hair was not enough to perform the precision and LOD evaluations.

As the method was a simple methanolic incubation, there was no attempt to evaluate recovery. However, due to alkaline instability of kavain, it was not possible to obtain a complete disintegration of the hair matrix, and then there is no way to know if all the kavain has been recovered from hair.

3. Results and discussion

Under the chromatographic conditions used, there was no interference with the analytes by any extractable endogenous materials present in hair. There was no blank effects.

In order to obtain optimum selectivity, the SRM technique was applied. It is desirable to produce an intense ion signal which is characteristic for the target compound. Selectivity

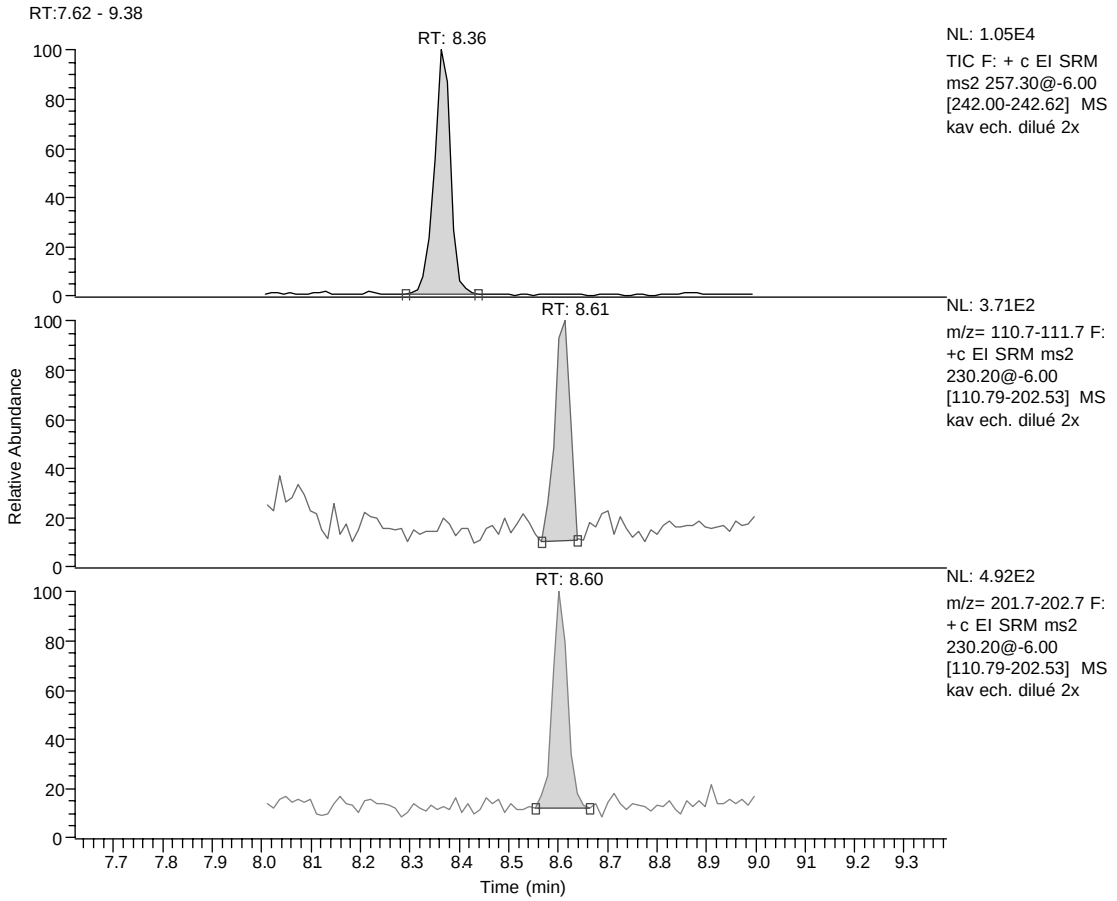

Fig. 3. SRM chromatogram obtained after methanolic incubation by the established procedure of a 29 mg hair strand specimen of a kavain consumer. Kavain was quantified at the concentration of 418 pg/mg. Top: methaqualoneone-d_7 with its daughter ion at *m*/*z* 242; middle: kavain with the daughter ion at *m*/*z* 111; bottom: kavain with the daughter ion at *m*/*z* 202.

and sensitivity are extraordinarily increased by almost completely suppressing the noise level. Selected ions and retention times of kavain and the IS are reported in Table 1. The parent ion of kavain (*m*/*z* 230) corresponds to the molecular ion; the two daughter ions (*m*/*z* 111 and 202) were chosen based upon criterion of specificity and abundance (Fig. 2). Less than 5% of kavain was converted into decarboxylated kavain in the injection port.

The calibration curve corresponds to the linear regression between the peak-area ratio of kavain to IS and the final concentration of the substance in spiked hair. Responses for kavain were linear in the range 100–2000 pg/mg with a correlation coefficient of 0.998.

The within-batch precision was 13.7%, as determined by analysing eight replicates of 50 mg of hair spiked with a kavain final concentrations at 400 pg/mg. The limit of detection of kavain was 30 pg/mg. The limit of quantitation was the first point of the calibration curve, that is 100 pg/mg.

The analysis of a 2 cm hair segment, obtained from an occasional consumer revealed the presence of kavain at the concentration of 418 pg/mg (Fig. 3). A higher level was observed in pubic hair (1708 pg/mg). Due to a lack of literature data, it was not possible to compare these concentrations with previous reports.

Sample preparation is very simple. Methanol extraction and direct detection with GC-MS is known since the early 1990s, but was not published until 1996 by Kauert and Röhrich [8]. These authors have used methaqualone as IS and that was retained in our procedure, due to the lack of deuterated kavain commercially available. The methanolic incubation can be considered as universal. Based on our experience, this procedure is particularly useful when ionic binding of the drug to the hair is weak. This is the case with kavain, as the drug does not possess a nitrogen atom, which would have interacted with the negatively charged melanin.

The distribution of concentrations between hair of head and pubic regions is similar to that previously reported for other drugs [9] as it is generally observed higher concentrations in pubic hair when compared with head hair.

4. Conclusion

The sensitive, specific and reproducible method developed seems to be suitable for the detection and quantification of kavain in human hair. Kavain was measured in hair for the first time, thus demonstrating that the drug is incorporated in this matrix. It will extend the number of drugs that can be detected in hair.

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