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Identification of psychoactive alkaloids in ancient Andean human hair by gas chromatography/mass spectrometry

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ABSTRACT

Various ethnographic sources have demonstrated the symbolic and ritualistic importance of psychoactive plants in Native American societies. The social milieu of these mind- altering plants appears to be ancient. Archaeological evidence during the Tiwanaku empire expansion along the Atacama Desert of Chile, circa 500–1000 A.D., shows the presence of highly decorated snuffing tablets and tubes as grave goods. The preservation of mummified human bodies in the Azapa Valley, northern Chile, provided an opportunity to test the exact nature of the psychoactive plants used in this region. Using gas chromatography/mass spectrometry (GC–MS), here we show that ancient Andean populations from northern Chile consumed *Banisteriopsis*, a vine that contains harmine. This is the first direct archaeological evidence of hallucinogenic and medicinal ethnographic practices. Interestingly enough, this rainforest plant does not grow along the Atacama coast, thus our findings suggest extensive plant trade networks in antiquity as far as the Amazon.

1. Introduction

Ethnographic and ethnohistoric documentation indicate that consumption of psychoactive plants played an important role in the ritual lives of Native Americans (Montenegro, 2006; Furst, 1994; Galinier et al., 1995; Harner, 1976). However, Schultes and Hofmann (1980, 2000) pointed out the need for in-depth research on use and doses of psychoactive substances in therapeutic and hallucinogenic processes.

In the Azapa Valley, northern Chile, archaeological discoveries of several psychoactive kits, as grave goods of mummies and skeletons, suggest a considerable antiquity of drugs consumption. This was particularly true during the Middle Period or Tiwanaku Horizon (Berenguer, 2001; Chacama, 2001; Llagostera, 2006; Llagostera et al., 1988; Torres, 1994, 2000; Torres et al., 1991). To verify that ancient populations consumed psychoactive drugs, we analyzed hair from Azapa Valley mummies. Here we present chemical evidence suggesting *Banisteriopsis* consumption during the Tiwanaku Middle Period.

The Tiwanaku state rose in the Lake Titicaca area, Bolivia, and expanded its influence, into what is now Chile, by religious control or militaristic activities (Berenguer, 2000). The Tiwanaku art shows some of these characteristics: decorated textiles, ceramic, wood and stone iconography depicting trophy heads, plants and supernatural beings show a complex interplay of these elements with snuffing implements (tubes and tablets) (Llagostera, 2006; Torres, 1987, 1994, 2000) (Fig. 1). Various researchers in northern Chile have used this type of cultural material approach to suggest the consumption of psychoactive plants during the Tiwanaku period or even earlier (Chacama, 2001; Llagostera, 2006; Llagostera et al., 1988; Torres, 1994, 2000; Torres et al., 1991).

In San Pedro de Atacama under the Tiwanaku influence snuffing kits were abundant (Torres, 1987, 1994, 2000). Torres (2000: 432–434) reported 614 snuffing tablets, of which 61 were considered representative of Tiwanaku art. Also, Llagostera and colleagues (1988: 61) analyzed 20 cemeteries and 2754 tombs; he stated that 20% of the tombs had tablets. More recently, Llagostera (2006) reported 84 tablets with Tiwanaku art for the South Central Andes, of which 63 (75%) were found in San Pedro de Atacama. These later tablets represent only 17.5% of tablets with Tiwanaku art within San Pedro de Atacama populations. These numbers suggest that at least in view of the grave goods, the Tiwanaku people were using hallucinogenic drugs. Further chemical analysis of the snuffing tablets' powder from the Solcor-3 cemetery Tomb 112 (San Pedro de Atacama) showed the presence of *Anadenanthera colubrina* var. cebil (Torres et al., 1991: 643), a plant rich in hallucinogenic tryptaminic alkaloid such as 5-hydroxy-*N*,*N*-dimethyltryptamine (bufotenine),

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Fig. 1. Archaeological sniffing kits from Azapa Valley, northern Chile.

N,*N*-dimethyltryptamine (DMT), 5-methoxy-*N*,*N*-dimethyltryptamine (MeODMT) or tryptamine (Torres and Repke, 1996: 47). While the chemical analysis suggested the Solcor-3 people were familiar with this type of drug, it does not necessarily indicate ingestion.

However, it is interesting to point out that computer axial tomography (CAT) analysis of ancient skulls from this area showed significant evidence of chronic perinasal damage in some cases, likely caused by frequent sniffing of *Anadenanthera*. This was particularly true for the individual in Solcor- 3, Tomb 112 (Casas et al., 2005: 66). In addition, it is interesting that this was the only Solcor-3 individual who had two snuffing kits as grave goods (Llagostera et al., 1988: 65).

In contrast, further north in the Azapa Valley the archeological evidence of tablets and tubes was minimal, even during the Tiwanaku Period (Chacama, 2001: 95). In fact, of 2018 archaeological burials excavated in the Azapa Valley, only 79 tombs or 3.91% presented evidence of snuff paraphernalia (Chacama, 2001: 94).

Excepting the Belmonte et al. (1994) and Molina et al. (1989) studies on *Erythroxylum coca*, a psychoactive and mild stimulant plant very common in the Andes, there is a paucity of psychoactive plant studies in pre-Hispanic Azapa Valley populations. Chemical analyses conducted by Cartmell et al. (1994) showed the presence of benzoylecgonine (BZE) metabolites in the hair of Azapa mummies. He provided the first evidence for direct consumption of coca leaves in northern Chile beginning 350–250 B.C. However, hair chemical analysis to establish direct proof of ingestions of *Anadenanthera* or *Banisteriopsis* plants along the Atacama Desert of Chile showed negative results (Castro et al., 2003).

2. Detection by gas chromatography/mass spectroscopy

The pharmacokinetic metabolic pathways are not well known, but cocaine alkaloids accumulate in the hair follicles (Cartmell et al., 1994). Thus, hair studies are important to bio-archaeological science, providing insight into the use and consumption of psychotropic substances in antiquity (Cartmell et al., 1994; Springfield et al., 1993). In our study, we tested the identification of psychotropic alkaloids in the hair of 32 pre-Hispanic mummies from northern Chile (Table 1), following analytical protocols presented by Castro et al. (2003) and Springfield et al. (1993). Some of the Arica mummies tested had significant psychotropic grave goods and items of social prestige (sniffing kits, four-points hat, pan pipes, and long-ear deformation, Allison et al., 1983).

Several hairs were cut directly from the head of the mummy and cleaned with a soft brush. Then, 200 mg of hair was weighed and treated with 2 ml of hydrochloric acid solution 0.1 M for 24 h at 37 °C. The solution was centrifuged at 4000 rpm for 10 min. The aqueous acid solution was neutralized with NaOH 0.1 N and adjusted with a buffer of phosphate pH 7. The alkaloids were extracted using a 3 ml Bond Elut cartridge C18 (SPE) and reconstituted for eluting in a methylene chloride matrix. The sample was then concentrated by evaporating under nitrogen.

Finally, the alkaloids were reconstituted in 40 μl of acetonitrile. The samples, at a volume of 2 μl, were injected into a Varian 3800 GC gas chromatograph, with a column SE-30 30 m ID 0.53 mm 1.2 μm film, in tandem on a Varian Saturn 2000 GC/MS mass spectrometer.

The sample was volatilized at the injection port and eluted through a capillary column under increasing temperature. The components of the sample were separated for affinity using the stationary phase of the column and identified by retention time (t_R). Each chemical component has a specific retention time under specific experimental conditions. A mass selective detector breaks the components into fragmented ions and these fractions are separated by the mass charge ratio and the mass charge ratio can be

Table 1
Archaeological samples analyzed and basic bio-archaeological information.

No.	Sex	Age (years)	Significant cultural elements	Reference
1	Indeterminate	2–3		AZ6 T9
2	Female	20–22		AZ6 T19
3	Male	20–25	Elongated ear lobe	AZ6 T25
4	Male	35–45	Snuffing kit	AZ6 T41b
5	Male	25–30		AZ6 T122
6	Female	45–50		AZ6 T127
7	Male	35–40		AZ6 T177
8	Male	20–25	Elongated ear lobe	AZ6 T194
9	Male	35–40	Elongated ear lobe	AZ6 TMCA2
10	Female	40		AZ6 TMCA3
11	Male	Young adult	Trophy head	AZ70 T23
12	Male	Adult		AZ70 T7C4
13	Female	45–50		AZ71 T127
14	Male	25–30		AZ71 T300
15	Male	45–50		AZ71 T602
16	Female	20–25		AZ140 T10
17	Male	20		AZ140 T44
18	Male	44		AZ140 T64a
19	Female	30		AZ140 T73
20	Male	39	Elongated ear lobe	AZ140 T75
21	Female	20		AZ140 T79
22	Male	28–32		AZ140 T80
23	Female	50		AZ140 T93
24	Female	38–40		AZ140 T100
25	Male	47		AZ140 T105
26	Female	39–40		AZ140 T120
27	Male	40	Four-point hats, pan pipe	AZ140 T122
28	Female	50		AZ140 T126
29	Indeterminate	1–2	Snuffing kit	AZ141 T30[a]
30	Male	Adult	Snuffing kit, four-point hat, pan pipe	AZ141 T33[a]
31	Male	Infant		AZ 141 T34
32	Male	Young adult		AZ 141 T53

[a] Tested positive for harmine.

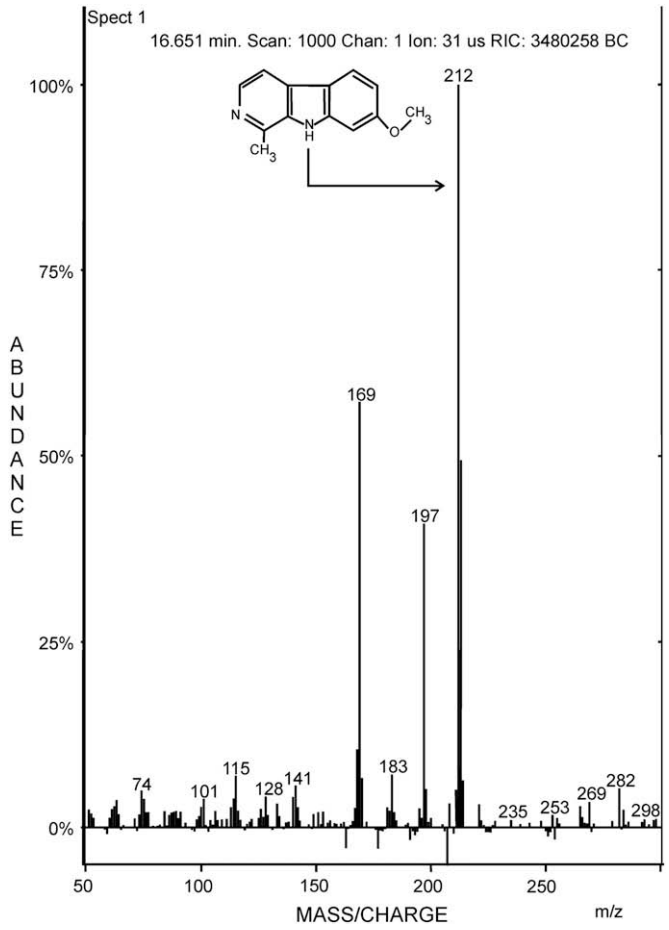

Fig. 2. Mass spectrum of harmine (9H-pyrido[3,4-*b*]indole, 7-methoxy-1-methyl-, MW: 212, related to *Banisteriopsis)* to t_R 16.651 min. Formula: $C_{13}H_{12}N_2O$. Ten largest peaks: 212 999 | 197 530 | 169 440 | 213 150 | 170 70 | 211 70 | 106 60 | 168 60 | 198 60 | 183 50 |.

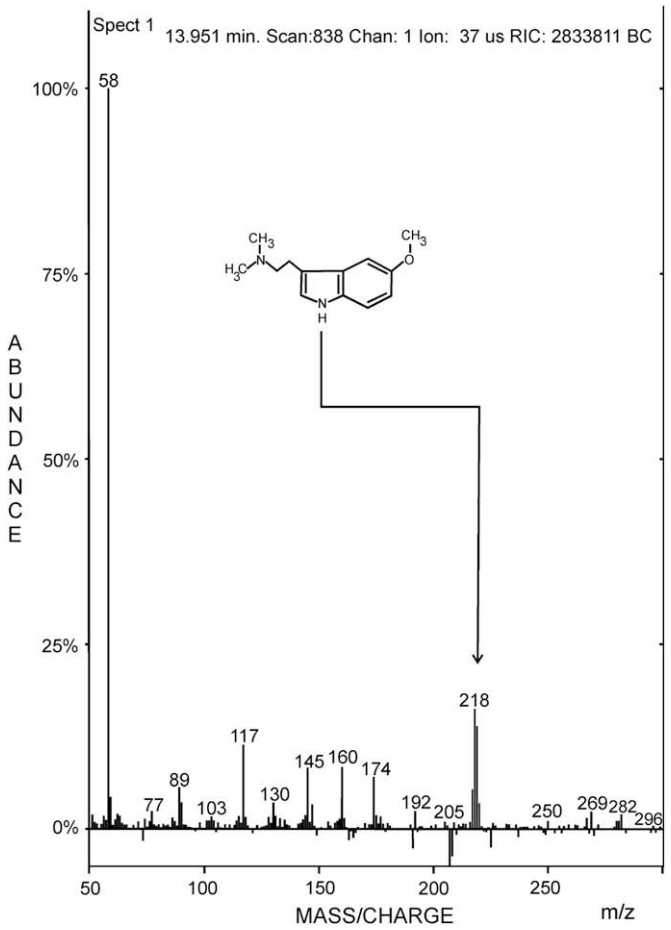

Fig. 3. Mass spectrum of 5-methoxy-*N*,*N*-dimethyltryptamine (3-(2-dimethylaminoethyl)-5-methoxyindole, MW: 218, related to *Anadenanthera*) to t_R 13.951 min. Formula: $C_{13}H_{18}N_2O$. Ten largest peaks: 58 999 | 218 146 | 160 60 | 117 50 | 42 43 | 145 40 | 59 38 | 89 29 | 130 29 | 90 23 |.

used to identify compounds. The intensity of the signals shows their abundance. The resulting mass spectrum is a unique "fingerprint."

As part of the protocol, before running the archaeological samples it was necessary to establish the lowest detection experimental value. This was undertaken by running a series of harmine and 5-methoxy-*N*,*N*-dimethyltryptamine samples with several diluted concentrations in both alkaloids. The lowest detectable concentration value obtained was 10 mg/l. Finally, three modern local hair samples [child, 12 years old; young adult (PESH), 24 years old, and adult (CARM), 45 years old (PES)] were used as controls.

Archaeological samples are precious, thus only two archaeological samples and two standard references were tested in triplicate runs to determine, qualitatively, if we could replicate the method. Also, our standardization analysis was qualitative and considered three analytical criteria for identification: retention time (experimental range 16.500 to 16.900 min), mass spectrum with largest peaks above 1% of relative intensity and a similarity index. An index of 1 indicates a 100% coincidence.

To determine whether a harmine or 5-methoxy-*N*,*N*-dimethyltryptamine test was positive, we used experimental data shown in Figs. 2 and 3, respectively.

3. Results and discussion

The results of 32 mummies' hair samples showed that none of the samples tested positive for 5-methoxy-*N*,*N*-dimethyltryptamine alkaloid. This information is extremely useful, because it shows the snuffing kits used in Azapa Valley were not related to *Anadenanthera* consumption. Our findings are controversial regarding the classical archaeological interpretations of snuffing kits and chemical evidence of snuff powder found in San Pedro de Atacama (Torres et al., 1991).

In our sample, two cases tested positive for harmine. However, before discussing these results we need to first consider the hair matrix information. The analysis of chromatographic and spectrometric data showed several mass spectrum peaks, 168 *m/z*, 179 *m/z* and 227 *m/z*, that need to be considered in the interpretation of the results. The peaks 168 *m/z* and 179 *m/z* showed an interference that appeared immediately before the signal of harmine, a chromatographic analytical signal in all modern hair samples. On the archaeological samples that show these peaks the value is 8.9%, suggesting that interference is modern or unstable in time. The peak 227 *m/z* appears after the signal related with harmine. This interference peak has a 59.4% presence in the archaeological samples. The point to highlight here is that these data created noise, likely masking the harmine peaks.

Regarding the three local modern samples, two female hair samples (child, 12 years old coded PESH and adult, 45 years old coded PES) tested positive for harmine alkaloid. Both had peaks representative of harmine fragmentation at t_R 16.768 min (PESH) and t_R 16.884 min (PES). The similarity indexes were 0.9612 and 0.9416, respectively. PES data were similar to harmine standard, but PESH did not show the peak 212 *m/z* as base peak; instead it

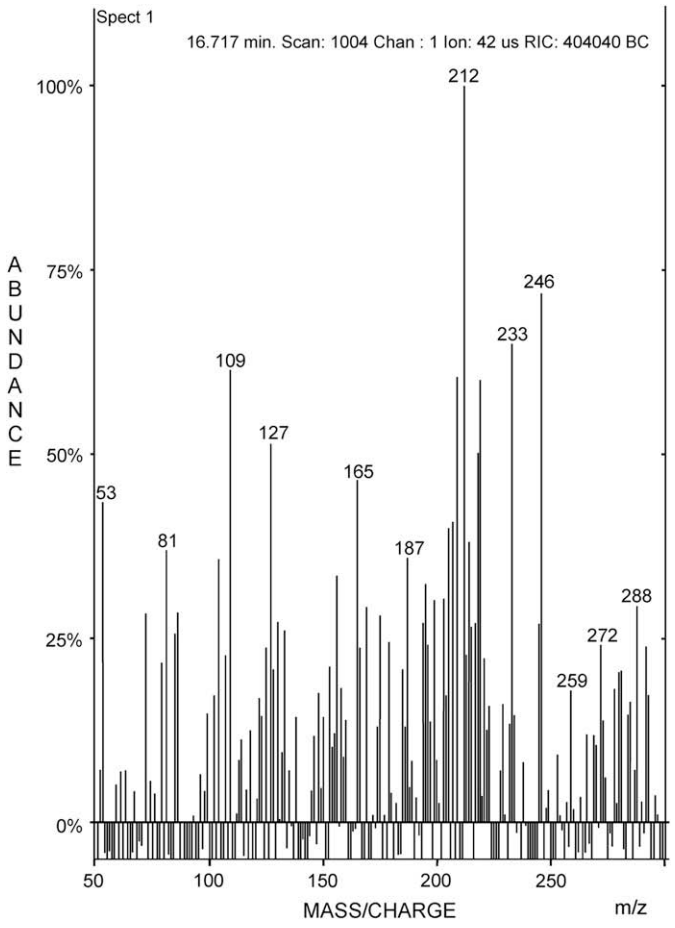

Fig. 4. Mass spectrum of AZ141T30 to t_R 16.717 min.

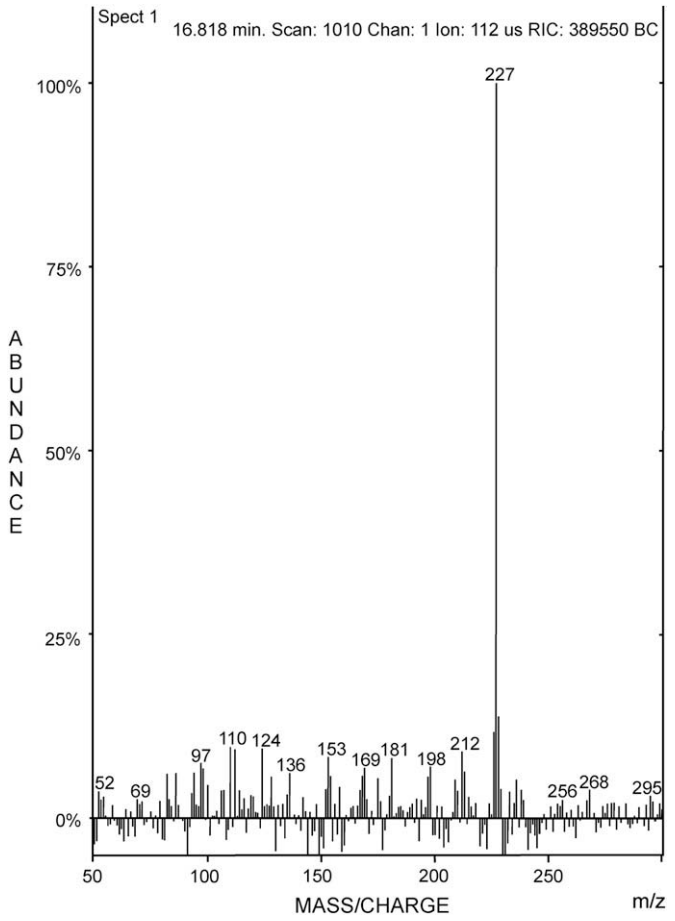

Fig. 5. Mass spectrum of AZ141T33 to t_R 16.818 min.

showed the 227 *m/z* base peak , but we considered this peak as the matrix interference. Thus, the similarity index was recalculated using peak 212 *m/z* as the base peak in the PESH case. It is likely these two modern samples were positive for the use of hair products containing harmine (Peña et al., 2006). Also, these two individuals (PES and PESH) are related (mother and young daughter) and use the same hair dyeing practices. The other modern sample, CARM, did not show the characteristic spectrum of harmine in our runs, but the peaks of interference were present.

In the archaeological runs, the first sample that tested positive (Fig. 4) was AZ141 Tomb 30, an infant, about 1 year old of unknown sex. The analysis of this sample showed only a few peaks of harmine fragmentation in a t_R 16.717 min. The similarity index was 0.8196, making this a solid case for harmine intake. This individual was buried with a tablet and a four-point Tiwanaku hat. These cultural materials also showed connotations of social prestige and have significant representations in Tiwanaku art (Berenguer, 1985, 1987, 1994, 2001; Pérez de Arce, 2004: 200). However, it is unlikely that this infant used a snuffing tablet for psychoactive plant consumption. Snuffing paraphernalia was also present in AZ6 Tomb 41b. The individual in that grave had a snuffing tablet, but tested negative for CAT lesions (Casas et al., 2005) and alkaloid chemical analyses.

The second sample testing positive (Fig. 5) was AZ141, Tomb 33, an adult male. This sample was made in triplicate and one sample was injected four times in a 12 h interval. During the last run we detected the presence of harmine. However, the time of retention of this analysis was displaced in one unit of time (circa 17 min). The review of the data of the first injections and/or duplicated runs showed spectral evidence confirming this result. The spectrum presented a retention time of 16.818 min, but the peaks of harmine fragmentation were masked with the matrix interference related to a 227 *m/z* peak. So the similarity index was calculated using corrected intensity relativity, resulting in a value of 0.8687. This value represents a solid case of harmine intake.

The grave goods were very significant: he had snuffing tablet, one four- point hat and the remains of another one, and several pan pipes. All of these cultural items are considered elements of social prestige by Tiwanaku Horizon researchers (Berenguer, 1985, 1987, 1994, 2001; Pérez de Arce, 2004). CAT analyses of his skull, to search for evidence of sniffing lesions, identified a sclerotic process on the perinasal area (Casas et al., 2005: 64). However, a chronic idiopathic inflammation is a possible cause of this pathology (Casas et al., 2005: 55).

The third sample that was analyzed for harmine (Fig. 6) is an adult male, about 39 years old, from AZ140 cemetery (Tomb 75). However, this sample satisfied the first two analytical criteria but the similarity index was low: 0.5256. Thus, we consider this individual that had artificially elongated ears to be negative for harmine intake.

The small number of samples that tested positive does not permit statistical conclusions. Chemical analyses conducted on individuals from San Pedro de Atacama identified *Anadenanthera* powder (Torres et al., 1991); however, samples tested from individuals in the Azapa Valley showed that they did not consume this plant, despite archaeological evidence of snuffing implements. This negative finding is important because a lack of tryptaminic alkaloids indicates the absence of hallucinogenic compounds during the Middle Period of the Azapa Valley.

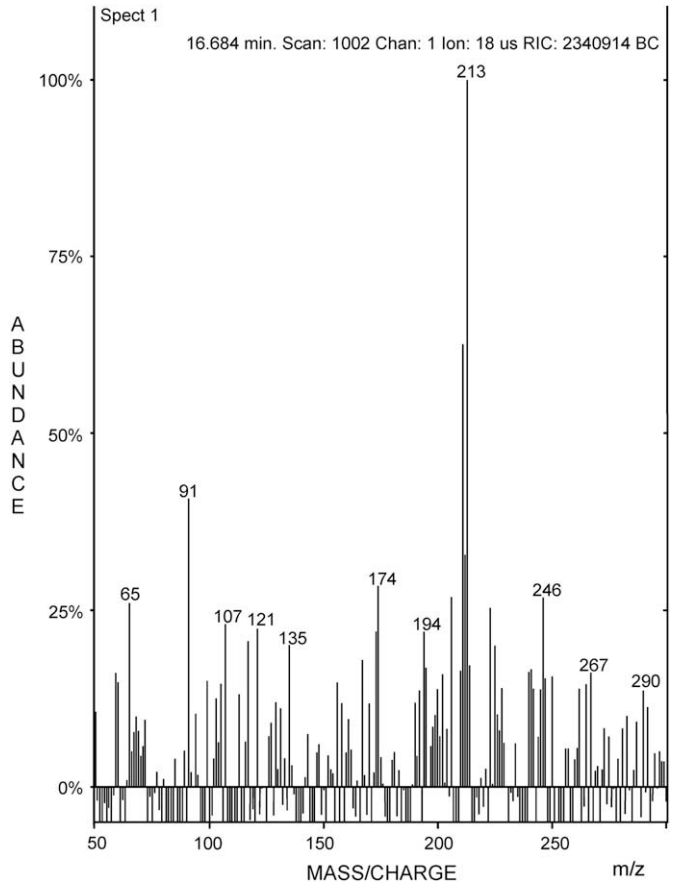

Fig. 6. Mass spectrum to of AZ140T75 to t_R 16.684 min.

However, our identification of harmine in the hair of two ancient Azapa Valley mummies provides direct and conclusive evidence of consumption of psychoactive substances. The *Banisteriopsis* vine is the only South American species studied that contains harmine. We believe this plant was not used to prepare hallucinogenic drinks in Azapa Valley because we did not find tryptaminic alkaloids and harmine is not hallucinogenic in its pure form (Petrie, 2002: 276), but an IMAO (inhibitor for monoaminooxidase).

In *Ayahuasca* hallucinogenic drinks, the *Banisteriopsis* is mixed with *Psychotria viridis*, a plant that contains a hallucinogenic compound, DMT or 5-MeODMT. When taken orally hallucinogenic alkaloids become inactive by the action of the monoaminooxidase (MAO) stomach enzyme. However, the harmine inhibits the action of this MAO enzyme promoting the absorption of the hallucinogenic mixture (Schultes and Hofmann, 1980: 175–180). Therefore, testing negative for tryptaminic alkaloids suggests that the *Banisteriopsis* was not necessarily used as a hallucinogenic admixture, and perhaps was used in therapeutic practices.

Finally, the *Banisteriopsis* vine is widely prepared and consumed in Amazonian areas today for medicinal and therapeutic purposes, often under the guidance of a medicine man (Furst, 1994; Harner, 1976; Schultes and Hofmann, 1980, 2000). Thus our data suggest ancient medicinal practices and extensive trade networks in antiquity, with plant import networks reaching from the Azapa Valley to as far away as the Amazonian region. This form of trade was possible under the vast network of Tiwanaku Horizon economic and religious interactions.

4. Conclusions

We believe the methodological approach used in this study is very useful for alkaloid detection, but there are some analytical limitations. The major problem is the small quantity of archaeological hair sample available and the destructive nature of the analysis.

Our research revealed that the snuffing paraphernalia was not directly associated with *Anadenanthera* in the Azapa Valley. This contrasts sharply with San Pedro de Atacama where the plant used was *Anadenanthera* (Torres et al., 1991). Certainly, the Azapa Valley mummies presented evidence of consumption of *Banisteriopsis* during the Tiwanaku Horizon; this is the first direct evidence of the consumption of the *Banisteriopsis* plant in this population. However, we believe the consumption of *Banisteriopsis* was part of a medicinal practice, perhaps as Ayahuasca infusion.

It is possible that *Banisteriopsis* consumption, an Amazonian plant, coincided with snuffing kits as elements of social differentiation. However, the perinasal CAT analyses (Casas et al., 2005) and phytochemical literature suggest that most snuffing kits were not used for *Banisteriopsis* ingestion (Ogalde, 2007: 67–71).

Finally, it is worth pointing out that the chemical methods presented here allow us to investigate psychotropic consumption in the Andean past. Archaeo-chemical analysis can be a useful tool to test archaeological propositions on shamanic and medicinal plants consumed or traded in antiquity. As long as the archaeological powders deriving from sniffing kits and bio-archaeological samples are well preserved in museum collections, these archaeo-chemical methods can provide factual data and shed light on the social milieu of this complex psychotropic subject.

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