GC-TOFMS Study on Components of the Amaryllidaceae Alkaloids, used in South African Traditional Medicine

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Key Words: GC-TOFMS

1. Introduction

Purpose of Analysis

Accurate identification of various components of the Amaryllidaceae alkaloid family of drugs is required in clinical and forensic studies. A method has been developed for analysis of some of these components, which have been implicated in the death of several patients during treatment with South African traditional medicines.

Background

The Amaryllidaceae form a large family of bulbous plants. The bulbs of these plants contain many alkaloids displaying a wide spectrum of bioactivity, both beneficial and harmful to humans.[1,2] In South Africa plant extracts containing these alkaloids have been used in male adolescent initiation rites and to induce visual hallucinations which are then interpreted by diviners, as well as for medicine to treat a number of ailments. However, the alkaloids are extremely toxic and even small overdoses can be fatal. Locally the bulbs of these plants are known by the Afrikaans term "gifbal" (poison ball), indicating the dangers associated with their use.

The three groups of samples submitted for analysis in this study were obtained from the organs of victims who had died as a result of alkaloid overdose.

2. Experimental Conditions

GC Parameters: Agilent 6890
Column:
DB-5; 20 m x 0.18 mm x 0.18 µm
Injector Temp: 240°C
Split Flow: Split, ratio 20:1
Oven Program:
150°C to 325°C at 25°C/minute, hold for 2 minutes
Flow Rate:
1.0 ml/minute Helium at constant flow.

MS Parameters: Pegasus III GC-TOFMS
Mass Range: 35 to 450 amu
Acquisition Rate: 20 spectra/second
Ion Source Temp: 220°C
Transfer Line Temp: 260°C
Total Acquisition Time: 9 minutes

Nine samples containing mixtures of the Amaryllidaceae alkaloids were analyzed for the Forensic Chemistry Laboratory (Johannesburg).

3. Results

Three sets of samples were analyzed. The first set, 681/02, consisted of samples obtained from the kidney, liver, and colon of a victim who had died within two hours due to an alkaloid overdose. In addition a crude extract of a plant bulb was included in this set. The second set, 682/02, consisted of samples obtained from the kidney and liver of a victim who had also died within two hours due to an alkaloid overdose. The final set, 808/01, consisted of samples obtained from the kidney and liver of a victim who had died within two days due to an alkaloid overdose, as well as a sample labeled 808/01 Buphanisine Poison.

As a representative example of the results obtained, the Selected Ion Chromatograms and the Peak Table for samples 681/02 Bulbs are shown below. During processing it was determined that the alkaloids eluted between 325 and 425 seconds using the GC conditions described above. Accordingly, to simplify the data display, only this portion of the chromatogram was processed, and only a limited number of ions—those with m/z 205, 229, 271, 285, 301, 315 and 331—were used to locate the position of alkaloid components. The display shown in Figure 1 corresponds only to the processed part of the chromatogram, i.e. between 325 and 425 seconds.

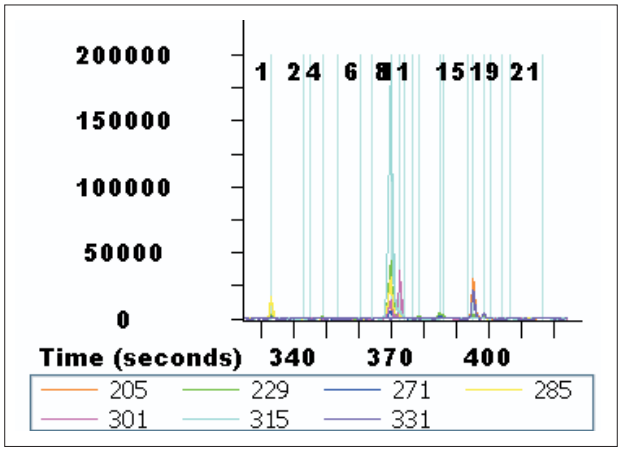

Figure 1. A Portion of the Selected Ion Chromatogram for sample 681/02 Bulbs.

Only those peaks which contained one of these ions with a signal-to-noise ratio (S/N) greater than 10:1 and a Peak Width greater than 2 seconds were added to the Peak Table (Table 1). Identifications are based solely on library search results, using the NIST and Wiley libraries.

Table 1. Peak Table for Sample 681/02 Kidney.

Peak #	Name	R.T. (sec.)	Similarity	S/N
1	Buphanisine	333.24	937.00	1608.20
2	Crinan-3-ol,1,2-didehydro-	343.44	784.00	41.88
3	Crinamine,6-methoxy-	345.09	686.00	45.89
4	Norpluvine	349.09	739.00	165.13
5	Galanthan-1-ol, 3,12-didehydro-9, 10-[methylenebis(oxy)]-	353.99	673.00	50.91
6	Flexinine	360.99	635.00	48.94
7	Morphinan-6-ol, 4,5-epoxy-3-methoxy-17-methyl-, acetate (ester)	364.39	713.00	79.50
8	Buphandrin	370.19	945.00	11401.00
9	Buphanamin	372.84	879.00	2052.20
10	Desmethoxy-oxo-bata-dihydroundulatine	374.44	883.00	292.11
11	Epibuphanamine	376.89	669.00	76.29
12	Powelline	378.79	873.00	94.22
13	Oxopowelline	385.19	806.00	606.91
14	Chlidanthine, 1,2-dihydro-	386.34	694.00	17.97
15	6-Acetyl-buphanidrine	393.74	605.00	55.30
16	Undulatine	395.39	907.00	1153.60
17	Ambelline	398.59	747.00	266.69
18	Morphinan-6-one, 3,4-dimethoxy-5,N-dimethyl-	400.89	617.00	90.91

As an example of the mass spectra obtained during the course of this study, the spectrum for the Amaryllidaceae alkaloid, buphanisine, is shown in Figure 2, below. The deconvoluted spectrum is shown, compared with the spectrum of the appropriate library match.

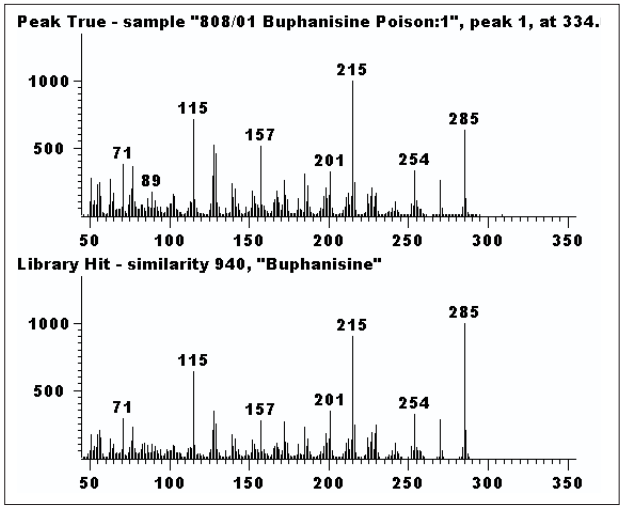

Figure 2. Recorded spectrum and library spectrum of Buphanisine.

As can be seen from the representative results shown above, the Amaryllidaceae alkaloids can be analyzed quickly and efficiently using the Pegasus III GC-TOFMS. The Peak Find software can be used to locate the components using a restricted ion set, which results in a clean display that is easily usable to study the required compounds. The Spectral Deconvolution software produces clean mass spectra for compounds.

Moreover, the analysis can be undertaken using rapid GC oven rates, thus shortening sample turn- over time, with no sacrifice in the quality of the results obtained. Important results can be obtained quickly and efficiently, a vital necessity in the pressurised analytical chemistry environment found today in many commercial and government laboratories.

4. Conclusions

The described work demonstrates the use of GC-TOFMS to locate and identify the Amaryllidaceae alkaloids. The use of a Time-of-Flight mass spectrometer in this work is an innovative approach that demonstrates a number of advantages over other types of mass spectrometers.

The strength of the Pegasus GC-TOFMS for the analysis of these sample types lies in its automated data handling capabilities. Peak finding, spectral determination (deconvolution), and library searching are all automatic. This is possible due to the high degree of spectral continuity generated, as well as the large data density allowed by the Pegasus GC-TOFMS system—up to 500 full mass spectra per second. Peaks are located and full range mass spectra obtained for all components, even when peaks coelute completely beneath other peaks, allowing confident structural determination.

5. References

[1]Machocho, S. C. Chhabra, F. Viladomat, C. Codina and J. Bastida, Phytochemistry, 1999, 51, 1185.

[2]Sener, M. Koyuncu, F. Bingol and F. Muhtar, Invited lecture presented at the International Conference on Biodiversity and Bioresources: Conservation and Utilization, 23-27 November 1997, Phuket, Thailand. (http://www.iupac.org/symposia/proceedings/phuket97/sener.html).

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