Analysis of Iboga and Voacanga Bark and Extracts by HPLC

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Appended Documents

Background

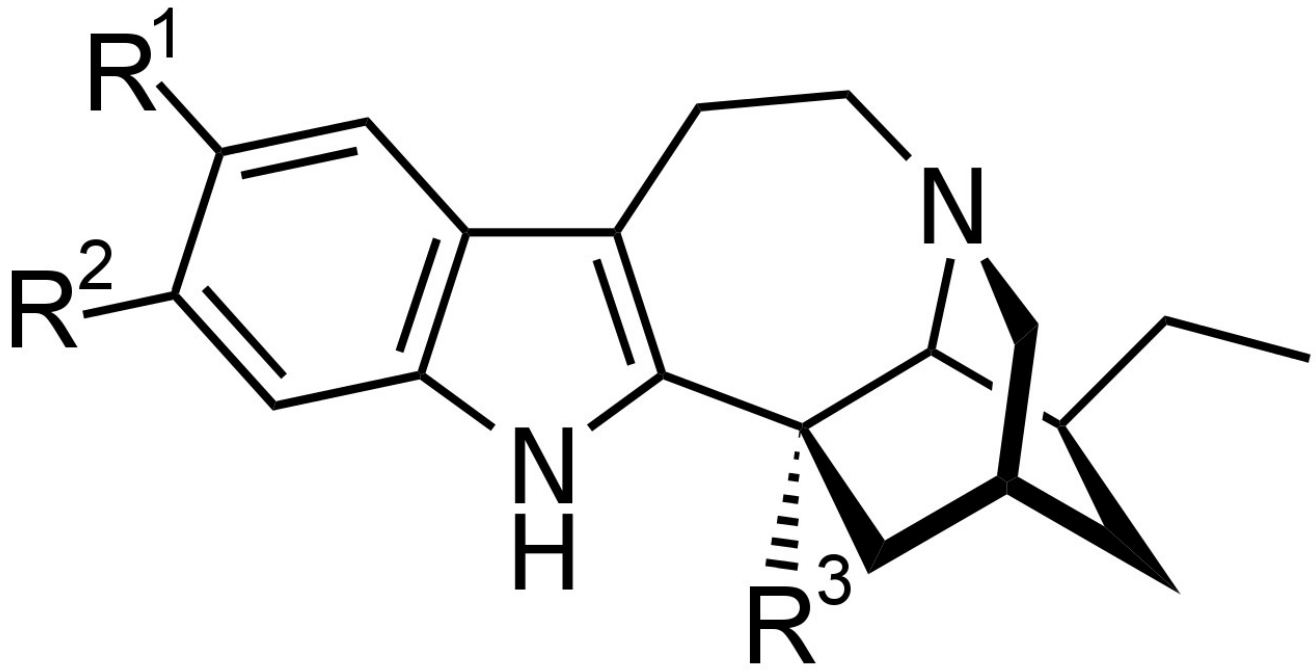
Since this analytical procedure is being written with the hope that iboga alkaloid analysis might be made widely available to anyone who needs it, this background section will explain the forms of iboga alkaloid containing material the analyst should anticipate encountering. These include root or bark of various species, especially *Tabernanthe iboga* and *Voacanga africana*, various purified fractions therefrom, and potentially semisynthetic ibogaine derived from voacangine.

In 1962, a man in New York who was addicted to heroin took a dose of ibogaine entirely to experience its reported visionary properties[1]. When the effects had worn off two days later, Howard Lotsof discovered that he was not debilitated by heroin withdrawal as expected, and even the daily desire to obtain heroin which had plagued him for years was gone, and did not return. After finishing college, Howard was awarded several patents for the use of ibogaine in treating drug addiction starting in 1985[2], and spent the rest of his life promoting the research and use of ibogaine for this purpose until his death in 2010.

Until the publication in 2002 of a low-tech procedure for the extraction and purification of the alkaloids in *Tabernanthe iboga* root bark[3], the best procedures[4] used conventional equipment and procedures intended for established laboratories, few of which offered ibogaine. This low-tech procedure uses acid-base chemistry and only a small amount of acetone to efficiently extract and isolate the solid iboga alkaloids and refine them.

The process begins with repeated extraction of milled bark with dilute acetic or hydrochloric acid, followed by precipitation of the total alkaloids (TA) using ammonium hydroxide or sodium hydroxide. The dried TA can be further purified in terms of increasing the percentage of ibogaine by extracting it with acetone and precipitating the purified total alkaloid (PTA) as the hydrochloride – a procedure originally reported by Dickel[4]. The acetone filtrate from this process still contains about half of the ibogaine, and lesser amounts of the similar alkaloids ibogamine, ibogaline, voacangine and probably tabernanthine. This recovered alkaloid (RA) mixture is isolated as the solid base by distilling and evaporating the acetone, dissolving the residue in water, filtering and precipitating the RA with base. The structures for the alkaloids discussed here are shown on the next page.

Iboga Alkaloids for Addiction Treatment Research



	R^1	R^2	R^3		R^1	R^2	R^3
Ibogamine	H	H	H	Coronaridine	H	H	CO_2CH_3
Ibogaine	OCH_3	H	H	Voacangine	OCH_3	H	CO_2CH_3
Tabernanthine	H	OCH_3	H	Isovoacangine	H	OCH_3	CO_2CH_3
Ibogaline	OCH_3	OCH_3	H	Conopharyngine	OCH_3	OCH_3	CO_2CH_3

History of Developing the Procedure

The development of this analytical method began with the conditions reported for a method intended for the forensic quantification of ibogaine and noribogaine in human tissues and fluids[5]. It says on page 436:

"Separation of the analytes was performed on a Zorbax eclipse XDB-C8 column (150 x 4.6-mm i.d., 5-µm particle size, Agilent Technologies, Palo Alto, CA). A C_{18} Symmetry column (20 x 3.9-mm i.d., 5-µm particle size, Waters, Paris, France) was used as a guard column. Mobile phase A was 0.02% (v/v) trimethylamine in acetonitrile, and mobile phase B consisted of 2 mM formate buffer (pH 3). The gradient started at 15% of phase A and then increased to 35% in 5 min. It increased to 50% in 6.2 min, then to 80% in 3.8 min. The column was then washed for 1 min with 80% of phase A, brought back to the initial conditions over 1 min, and re-equilibrated for 3 min. The flow rate started at 1 mL/min, then decreased to 0.5 mL/min from 1 to 5 min, and remained unchanged for 6.2 min. It increased to 1 mL/min over the next 4.8 min and then remained stable."

On page 435 the article explains that the formate buffer consists of 126 mg/L of ammonium formate in purified water. Although a UV detector was used, the wavelength was not specified. An older article[6] gives 278 nm as the absorption maximum of ibogaine, but it is unclear if this applies to the base or the salt, with the maximum absorption wavelength for the protonated form being slightly longer.

No C-8 column was thought to be available except an unopened package, so a 125 x 4.0 mm Prontosil Eurobond C-18 column with 5 µm particle size was used for most experiments. At the time this procedure was developed, the only HPLC instruments available which had reliable UV detectors used isocratic elution only. Therefore, the estimated composition of the gradient at the time ibogaine was eluted – at 10.5 minutes – was rounded down to 40% of phase A. This mixture gave an unacceptably long elution time for ibogaine, around 20 minutes, so the percentage of phase A was increased to 70. This mobile phase turned out to be difficult to further optimize.

Samples were also analyzed by GC/MS using a Hammilton HB5 column and a temperature gradient approaching 300ºC. The peak due to ibogaine was verified in PTA and RA samples by both GC and HPLC using 99% pure ibogaine HCl obtained from Phytostan Enterprises, Inc. This sample could not be used as an external standard for accurate quantitative analysis by HPLC because only a few milligrams were available. Ibogaine always produced the largest peak in the GC and HPLC analyses for all samples tested, consistent with the expectation of it having the highest concentration. The identity of the ibogaine peak was also verified by the

molecular weight of its parent ion ($C_{20}H_{26}N_2O$: 310.204) on GC/MS.

Based on recent GC/MS analysis of many iboga products by another group[7], the alkaloids expected in PTA and RA were ibogaine, ibogamine, ibogaline and voacangine with trace amounts of apparent didehydroibogaine and ibogaine hydroxyindolenine based on the low-resolution molecular weights of the detected peaks. Tabernanthine, named after the *Tabernanthe* genus of *T. iboga*, was expected to be present based on published analyses but was not detected at either laboratory. If present it may have eluted at the same time as its isomer, ibogaine, buried beneath the large ibogaine peak. The didehydroibogaine, named entirely on it having a molecular weight two units less than ibogaine, was detected in all samples and was assumed to be created from ibogaine in the GC column due to the high temperature. The order of elution of the alkaloids from the GC column in that study, ibogamine, ibogaine hydroxyindolenine, ibogaine, voacangine, didehydroibogaine and ibogaline, was identical to that observed at BSC with the exception that didehydroibogaine was not detected. The identities of all peaks were confirmed by matching molecular weights calculated based on the most abundant isotopes: ibogamine ($C_{19}H_{24}N_2$: 280.193), ibogaine hydroxyindolenine ($C_{20}H_{26}N_2O_2$: 326.199), ibogaine ($C_{20}H_{26}N_2O$: 310.204), voacangine ($C_{22}H_{28}N_2O_3$: 368.209) and ibogaline ($C_{21}H_{28}N_2O_2$: 340.214). The assignment of ibogaine hydroxyindolenine to its peak rather than the other likely oxidation product of ibogaine, ibogaine pseudoindoxyl (iboluteine), was based on the fragments in its mass spectrum.

A comparison of the GC plots for PTA vs. RA (see appended pages) shows differences in the areas of the peaks which are interesting in terms of the chemistry used to prepare PTA and also provides a means to assign the peaks in the HPLC report. When the PTA HCl is precipitated from an acetone extract of TA using hydrochloric acid, apparently nearly all of the voacangine remains in solution to end up in the RA. The ibogaine hydroxyindolenine seems to do the same thing, or it could be forming from ibogaine in the further processing to isolate the RA. Finally, the proportion of ibogaline to ibogamine is significantly higher in RA than it is in PTA, allowing these peaks to be recognized on the HPLC report.

The order of elution of the peaks on the reverse phase HPLC column is expected to be the reverse of the order on GC. This is mostly true – the order of the peaks eluting from the HPLC is ibogaline, ibogaine, voacangine and ibogamine, with the tiny ibogaine hydroxyindolenine too difficult to distinguish to identify. While ibogaine and voacangine are not in the expected reverse order, small adjustments to the buffer composition were found to have a dramatic influence on the relative retention of voacangine compared with the other components. This may be explained by the observation that voacangine is a significantly weaker base than the other three alkaloids due to the carbomethoxy group it bears.

Analytical Condition Optimization

The following variations were tested to try to improve the peak separation, peak width or loading capacity:

Mobile Phase Tests (all performed on a 125 mm, 5 µm C18 column):

100 µL injection instead of 20 µL: Still clean resolution; ibogaine peak absorbance off scale.

Ammonium formate concentration increased 10x: Voacangine moves from 6.9 to 5.7 minutes. All other peaks flush out unseparated between 1.6 and 2.2 minutes.

Ammonium formate concentration decreased 10x: The retention times of ibogaline, ibogaine, voacangine and ibogamine increased by 0.4, 0.7, 0.6 and 1.0 minutes respectively, crowding the ibogaine and voacangine peaks.

Lowering the percentage of phase A (ACN/trimethylamine): Peaks are retained longer but tend to tail.

Eliminating the trimethylamine: The retention times of ibogaline, ibogaine, voacangine and ibogamine increased by 0.4, 0.2, 0.1 and -0.1 minutes respectively, crowding the ibogaine and voacangine peaks.

80% acetonitrile and no buffer: Only one peak eluted (4.4 min) within ten minutes. Presumably voacangine.

Methanol instead of acetonitrile: Every proportion, with or without buffer, gave severe tailing of peaks so that they could not be resolved. The best composition was 800 ppm trimethylamine in 100% methanol, giving sharp but poorly resolved peaks between 1.4 and 2.5 minutes.

Elution with 0.1% aqueous acetic acid (before I understood about pH vulnerability of reverse phase silica): All peaks eluted between 1.3 and 2.0 minutes unresolved.

40% acetonitrile, 40% methanol, 40 ppm ammonium formate: Gives a clean ibogaline peak at 3.85 minutes, a clean ibogaine peak at 4.50 minutes and an unresolved lump between 5 and 6 minutes. This was a promising alternative if it could be improved upon. Decreasing methanol to 30% led to peak tailing and overlap.

Replacement of buffer with 40 ppm ammonium acetate: All peaks except voacangine increase retention time by 0.7 – 1.0 minutes, causing ibogaine and voacangine to overlap.

Buffer is replaced with 30 ppm acetic acid and 50 ppm triethylamine: Retention times are very similar to those with the original buffer but peaks are much broader and poorly resolved.

Stationary Phase Tests (performed with 70% ACN buffer except where noted):

5 μm C8 column of the same (125 mm) length: All significant peaks come out as an unresolved mass between 3.3 and 5.5 minutes. Good resolution had been expected, and thinking the column was bad, another 5μm, C8, 150 mm column was tested with a similar poor result.

3 μm C18-AQ column, 125 mm: The ibogaline, ibogaine and ibogamine elute 0.8, 1.6 and 2.4 minutes earlier with broader peaks than given by regular C18 and the voacangine not separated.

5 μm Zorbax CN column, 150 mm: Four major peaks elute at 2.4, 8.6, 10.9 and 14.0 minutes that account for 30%, 26%, 26% and 10% of total peak area, with significant tailing of the first and third peaks. These areas are very different from the 13%, 39%, 15% and 11% resolved for this mixture by C18. However, the long times between peaks makes this column a possible promising alternative to C18.

10 μm Phenomenex Phenyl column, 300 mm: A lot of material had to be flushed from the column before use, and it gave unreproducible results where no more than two major peaks were visible. Maybe the identical ring systems of all four analytes makes them resolve poorly based on Pi bonding.

Detector Wavelength Tests:

An analysis was accidentally run with a detection wavelength at 326 nm. It appears that the ibogamine and ibogaine, and a normally minor peak at 6.3 minutes between the two, absorb strongly at this wavelength relative to the voacangine and ibogaline, making it possible to identify the compound responsible for a given peak. Total absorbance was only about 3% of that at 278 nm.

Absorbance at 254 nm was about 70% that at 278 nm. Ibogaline seemed to absorb more strongly than the other components at 254 nm than at 278 nm.

At 340 nm there was very little absorbance by the four alkaloids of interest, and the main peak is an impurity at 6.3 minutes.

Loading tests on the regular 125 x 4.0 mm, 5 μm C18 column:

Two milligrams of RA injected in 200 μL came back from off-scale between 5.0 and 5.2 minutes, showing the potential to separate ibogaline from the other alkaloids at this loading.

Quantification

The GC/MS and HPLC of PTA and RA (attached) gave the following peaks:

Table 1: Peak Areas and Percentages for PTA and RA by GC and HPLC

	Ibogamine		Ibogaine hydroxyindolenine		Ibogaine		Voacangine		Ibogaline	
	Response	%	Response	%	Response	%	Response	%	Response	%
GC PTA	28,582,282	2.4			1,051,908,096	88.9			103,047,752	8.7
HPLC PTA	131.461	3.0			3961.612	89.5			331.029	7.5
GC RA	157,447,904	13.9	25,530,088	2.3	569,963,200	50.3	192,061,264	17.0	187,333,520	16.5
HPLC RA	743.392	14.7	67.35	1.3	2493.071	49.4	883.443	17.5	854.436	16.9

From this and previous data it is possible to verify the identity of the ibogaine peak on the HPLC by matching it with the ibogaine standard and also by seeing a close match between the peak areas for the HPLC samples and the areas for the same peak on GC identified to be ibogaine by MS. Although no other standards were available in 2013, the successful identification of the other alkaloids listed above by MS and the matching of their peak areas between the GC and the HPLC establish the identities for all major peaks on the HPLC. The ibogaine hydroxyindolenine peak was too small to be confidently identified on the HPLC plot.

Successive recrystallization of ibogaine and voacangine bases from methanol gave highly pure crystalline samples suitable for use as an external standard in quantitative analysis of these alkaloids. Here are all the 278 nm absorbance values gathered on these and similar pure samples by June 2014:

Ibogaine base analyzed on 28-Feb-2014:
6.2 µg ibogaine gave an absorbance of 9912.865 (right instrument)
9.8 µg ibogaine gave an absorbance of 13551.484 (right instrument)
8.7 µg ibogaine gave an absorbance of 12036.315 (right instrument)

Ibogaine base analyzed on 29-Apr-2014 (this is the reference standard left at BSC):
10.8 µg ibogaine sample #1 in 5 µL gave an absorbance of 14471.506 (left instrument)
* 21.6 µg ibogaine sample #1 in 10 µL gave an absorbance of 39806.323 (left instrument)
21.6 µg ibogaine sample #1 in 40 µL gave an absorbance of 29702.550 (left instrument)
10.8 µg ibogaine sample #1 in 5 µL gave an absorbance of 13329.325 (right instrument)
10.8 µg ibogaine sample #1 in 20 µL gave an absorbance of 15137.561 (right instrument)
20.7 µg ibogaine sample #2 in 10 µL gave an absorbance of 26994.420 (left instrument)
10.35 µg ibogaine sample #2 in 5 µL gave an absorbance of 13422.065 (right instrument)
10.35 µg ibogaine sample #2 in 20 µL gave an absorbance of 14134.449 (right instrument)
20.4 µg ibogaine sample #3 in 10 µL gave an absorbance of 26993.871 (left instrument)
10.2 µg ibogaine sample #3 in 5 µL gave an absorbance of 13162.794 (right instrument)
10.2 µg ibogaine sample #3 in 20 µL gave an absorbance of 13871.640 (right instrument)

* Excluded as an outlier due to an unusually high ratio of absorbance to sample weight.
Total ibogaine base injected: 160.9 µg
Total absorbance: 216,720.845
Absorbance at 278 nm per microgram of ibogaine: 1347
Absorbance at 278 nm per micromole of ibogaine (MW 310.441): 418,142

Ibogaine HCl from Phytostan analyzed on 27-Feb-2014 and 28-Feb-2014:
11.4 µg ibogaine HCl in 20 µL gave an absorbance of 13264.630 (right instrument)
11.4 µg ibogaine HCl in 20 µL gave an absorbance of 13947.183 (right instrument)
8.4 µg ibogaine HCl in 20 µL gave an absorbance of 11932.622 (right instrument)

Ibogaine HCl from Simon Loxton analyzed on 29-Apr-2014:
19.0 µg ibogaine HCl in 20 µL gave an absorbance of 28031.343 (right instrument)
9.5 µg ibogaine HCl in 10 µL gave an absorbance of 14102.774 (right instrument)

Ibogaine HCl from first Acidgaine production batch analyzed on 30-Apr-2014:
15.8 µg ibogaine HCl in 20 µL gave an absorbance of 19907.030 (right instrument)

Total ibogaine HCl injected: 75.5 µg
Total absorbance: 101,185.582
Absorbance at 278 nm per microgram of ibogaine HCl: 1340
Absorbance at 278 nm per micromole of ibogaine HCl (MW 346.902): 464,920

Voacangine base analyzed on 25-Feb-2014 (this is the reference standard left at BSC):
0.34 µg voacangine #1 in 20 µL gave an absorbance of 435.880 (right instrument)
0.68 µg voacangine #1 in 20 µL gave an absorbance of 880.002 (right instrument)
1.35 µg voacangine #1 in 20 µL gave an absorbance of 1745.296 (right instrument)
2.7 µg voacangine #1 in 20 µL gave an absorbance of 3347.310 (right instrument)
5.4 µg voacangine #1 in 20 µL gave an absorbance of 6681.294 (right instrument)
10.8 µg voacangine #1 in 20 µL gave an absorbance of 13490.342 (right instrument)
0.48 µg voacangine #2 in 20 µL gave an absorbance of 609.774 (right instrument)
0.96 µg voacangine #2 in 20 µL gave an absorbance of 1204.937 (right instrument)
1.92 µg voacangine #2 in 20 µL gave an absorbance of 2358.081 (right instrument)
3.84 µg voacangine #2 in 20 µL gave an absorbance of 4696.389 (right instrument)
7.68 µg voacangine #2 in 20 µL gave an absorbance of 9375.604 (right instrument)
15.36 µg voacangine #2 in 20 µL gave an absorbance of 19004.686 (right instrument)
0.48 µg voacangine #3 in 20 µL gave an absorbance of 638.952 (right instrument)
0.96 µg voacangine #3 in 20 µL gave an absorbance of 1253.535 (right instrument)
1.92 µg voacangine #3 in 20 µL gave an absorbance of 2501.842 (right instrument)
3.84 µg voacangine #3 in 20 µL gave an absorbance of 4840.036 (right instrument)
7.68 µg voacangine #3 in 20 µL gave an absorbance of 9698.396 (right instrument)
15.36 µg voacangine #3 in 20 µL gave an absorbance of 19380.312 (right instrument)

Total voacangine base injected: 81.75 µg
Total absorbance: 102,142.668
Absorbance at 278 nm per microgram of voacangine: 1249
Absorbance at 278 nm per micromole of voacangine (MW 368.478): 460,395

Quantitative analysis of the remaining iboga alkaloids may be possible without yet having accurately weighable standards available if the following three assumptions are accepted:

The absorption of 278 nm UV will be the same at the same molar concentration of the different iboga alkaloids. The response values for the GC/MS are based on MS detector counts which will be proportional to molar concentration, so the close agreement in response values for all analytes between the UV and MS detectors, along with the above molar absorbances being within five percent of each other, suggests that this assumption is justified.

With these assumptions, the absorbance values for ibogamine and ibogaline can be adjusted to account for their different molecular weights compared to ibogaine. Whether the sample to be analyzed is in base or salt form also needs to be accounted for when calculating absolute percentages from the peak areas.

Analytical Procedure

Prepare one liter of 70% acetonitrile buffer by diluting 350 µL of 40% aqueous trimethylamine (or 140 µL of neat trimethylamine) to 700 mL with acetonitrile. Add 37.8 mg of ammonium formate, dilute to one liter with water and dissolve the salt. Let the mixture warm back to room temperature to expel dissolved air before using it.

Accurately weigh (to 0.1 mg) approximately 50 mg of TA or 25 mg of PTA HCl, RA base or other semipurified form of iboga alkaloid base or salt and make a 50.0 mL solution of it in 70% acetonitrile buffer if sample quantity will permit. If the sample does not quickly dissolve completely (as it will not for TA), sonicate for ten minutes and filter into an autosampler vial.

Install a 125 x 4.0 mm column with 5-µm C-18 bonded silica on the HPLC. Set the detection wavelength to 278 nm, the flow rate to 1.0 mL/min and the run time to ten minutes. If any standards are available, verify that ibogaline elutes around 4.7 minutes, ibogaine elutes around 6.2 minutes, voacangine elutes around 6.9 minutes and ibogamine elutes around 7.8 minutes. The resolution of voacangine from the other alkaloids is very sensitive to the condition of the column and the buffer concentration. If clean peak separation is not achieved, try increasing the concentration of ammonium formate in the buffer by two or three fold. Inject 20 µL of the unknown sample and collect the peak area data.

Estimate the milligrams of ibogaine, ibogaine HCl or voacangine using the relevant absorption per microgram in the 20 µL injection volume. The milligrams of ibogamine or ibogaline base or HCl can be determined by using the molecular weight (Table 2) and area for the peak (absorption units) of the alkaloid in the following equation, along with the absorption per microgram for ibogaine or ibogaine HCl. Remember to select the molecular weight from the table based on the form of the product being analyzed – base (root bark, TA, RA) or salt (PTA HCl, ibogaine HCl).

$$3721.786\,UV^{278}\,absorption\,units \times \frac{1\,ug\,of\,ibogaine\,base}{1347\,UV^{278}\,absorption\,units}$$

= 2.76 µg ibogaine base

The percent of each alkaloid can then be calculated as follows for ibogaine, assuming 22.4 mg of RA was used to make the 50.0 mL of stock solution:

$$\frac{\frac{2.76\,ug\,ibogaine}{20\,uL\,injected\,stock\,solution}}{\frac{22.4\,mg\,RA}{50.0\,mL\,original\,stock\,solution}} * 100\% = 30.8\%\ \text{ibogaine}$$

The percent ibogaine (38.6%) in the attached analysis of BSC5 RA is incorrect because it was calculated before an external standard became available.

Table 2: Molecular Weights Based on Natural Isotope Abundance

	Base		Hydrochloride	
	Formula	MW	Formula	MW
Ibogamine	$C_{19}H_{24}N_2$	280.415	$C_{19}H_{25}N_2Cl$	316.876
Ibogaine hydroxyindolenine	$C_{20}H_{26}N_2O_2$	326.440	$C_{20}H_{27}N_2O_2Cl$	362.901
Ibogaine	$C_{20}H_{26}N_2O$	310.441	$C_{20}H_{27}N_2OCl$	346.902
Voacangine	$C_{22}H_{28}N_2O_3$	368.478	$C_{22}H_{29}N_2O_3Cl$	404.938
Ibogaline	$C_{21}H_{28}N_2O_2$	340.467	$C_{21}H_{29}N_2O_2Cl$	376.938

Using the formulas below, the base molecular weights from Table 2 and the peak areas from the attached analysis of BSC5 RA, the amounts and percents of the remaining alkaloids calculate to be:

$$1027.436\,UV^{278}\,absorption\,units \times \frac{1\,micromole\,of\,ibogamine}{418{,}142\,UV^{278}\,absorption\,units} \times \frac{280.415\,ug\,ibogamine\,base}{micromole}$$

= 0.689 µg ibogamine base

$$\frac{\frac{0.689\,ug\,ibogamine}{20\,uL\,injected\,stock\,solution}}{\frac{22.4\,mg\,RA}{50.0\,mL\,original\,stock\,solution}} * 100\% = 7.7\%\ \text{ibogamine}$$

$$1436.081\,UV^{278}\,absorption\,units \times \frac{1\,ug\,voacangine\,base}{1249\,UV^{278}\,absorption\,units}$$

= 1.15 µg voacangine base

$$\frac{\frac{1.15\,ug\,voacangine}{20\,uL\,injected\,stock\,solution}}{\frac{22.4\,mg\,RA}{50.0\,mL\,original\,stock\,solution}} * 100\% = 12.8\%\ \text{voacangine}$$

$$1248.014\,UV^{278}\,absorption\,units \times \frac{1\,micromole\,of\,ibogaline}{418{,}142\,UV^{278}\,absorption\,units} \times \frac{340.467\,ug\,ibogaline\,base}{micromole}$$

= 1.02 µg ibogaline base

$$\frac{\frac{1.02\,ug\,ibogaline}{20\,uL\,injected\,stock\,solution}}{\frac{22.4\,mg\,RA}{50.0\,mL\,original\,stock\,solution}} * 100\% = 11.3\%\ \text{ibogaline}$$

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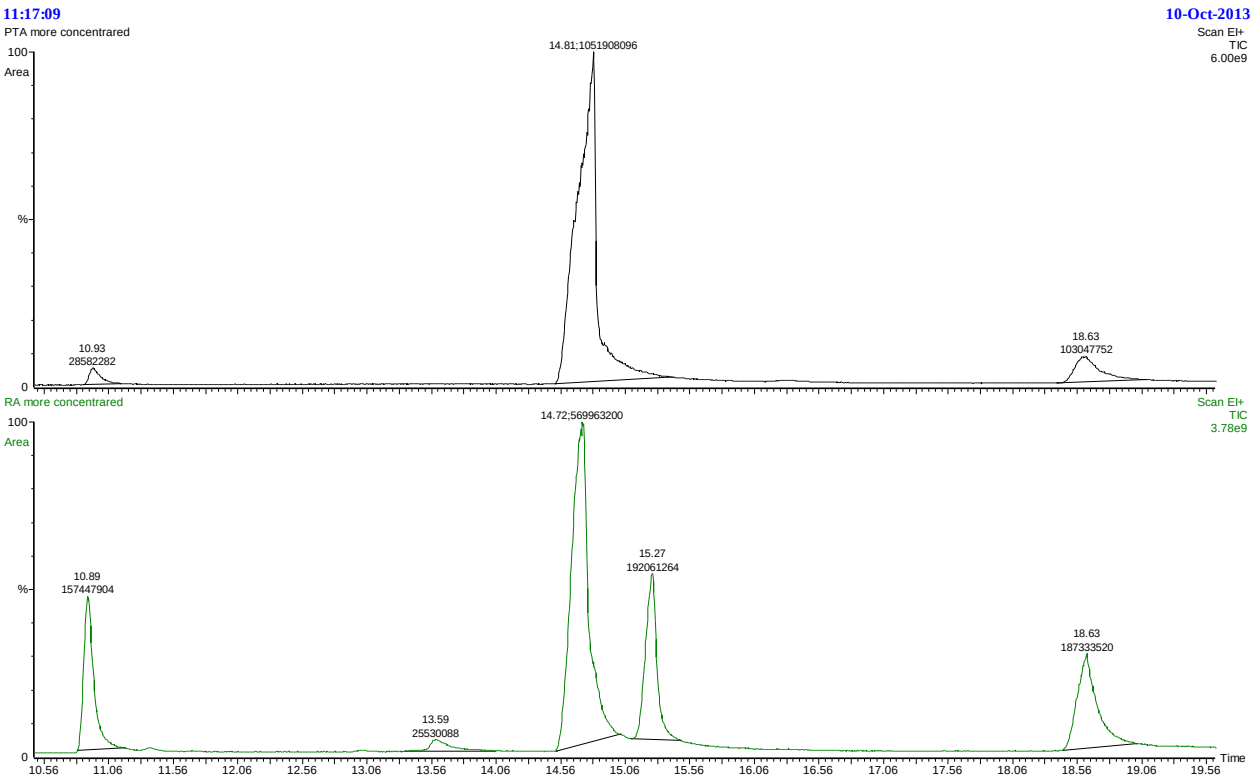
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18.56
19.06
19.56
Time

BSC

Biochemical and Scientific Consultants cc

Sample Info:

Sample ID	: PTA HCl 200 ppm	Amount	: 0
Sample	:	ISTD Amount	: 0
Inj. Volume [ml]	: 0.02	Dilution	: 1

Method	: Ibogaine	By	: valerie
Description	: Ibogaine		
Created	: 2013/10/04 12:16 PM	Modified	: 2013/10/10 11:31 AM
Column	: C18 reverse phase	Detection	: 278 nm
Mobile Phase	: 70% ACN with ammonium formate and trimethylamine	Temperature	:
Flow Rate	: 1.0 ml/min	Pressure	:
Note	:		

Autostop	: 10.00, min	External Start	: Start - Restart, Down
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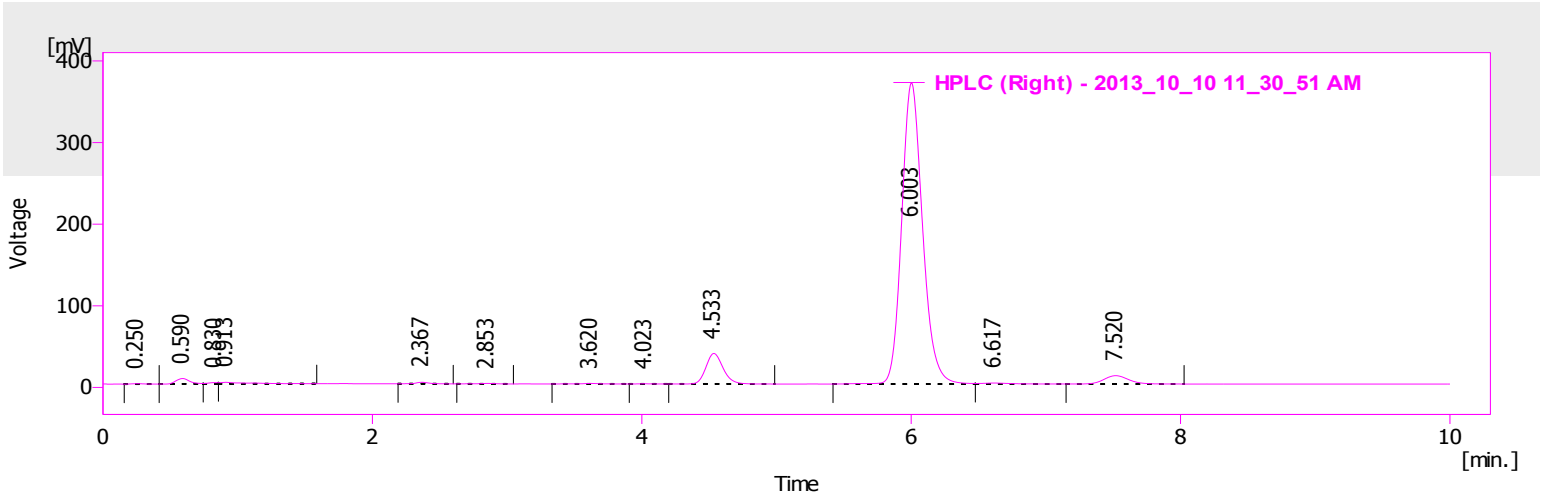
Subtraction Chromatogram	: (None)	Matching	: No Change



Result Table (Uncal - HPLC (Right) - 2013_10_10 11_30_51 AM)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	0.250	2.963	0.473	0.1	0.1	0.10
2	0.590	47.927	6.714	1.1	1.6	0.11
3	0.830	8.273	1.591	0.2	0.4	0.09
4	0.913	29.823	1.955	0.7	0.5	0.22
5	2.367	13.239	1.918	0.3	0.4	0.10
6	2.853	5.711	0.636	0.1	0.1	0.12
7	3.620	6.265	0.669	0.1	0.2	0.12
8	4.023	1.638	0.228	0.0	0.1	0.12
9	4.533	331.029	37.419	7.3	8.7	0.14
10	6.003	3961.612	368.960	86.9	85.4	0.17
11	6.617	18.781	1.192	0.4	0.3	0.26
12	7.520	131.461	10.230	2.9	2.4	0.20
	Total	4558.722	431.984	100.0	100.0	

BSC

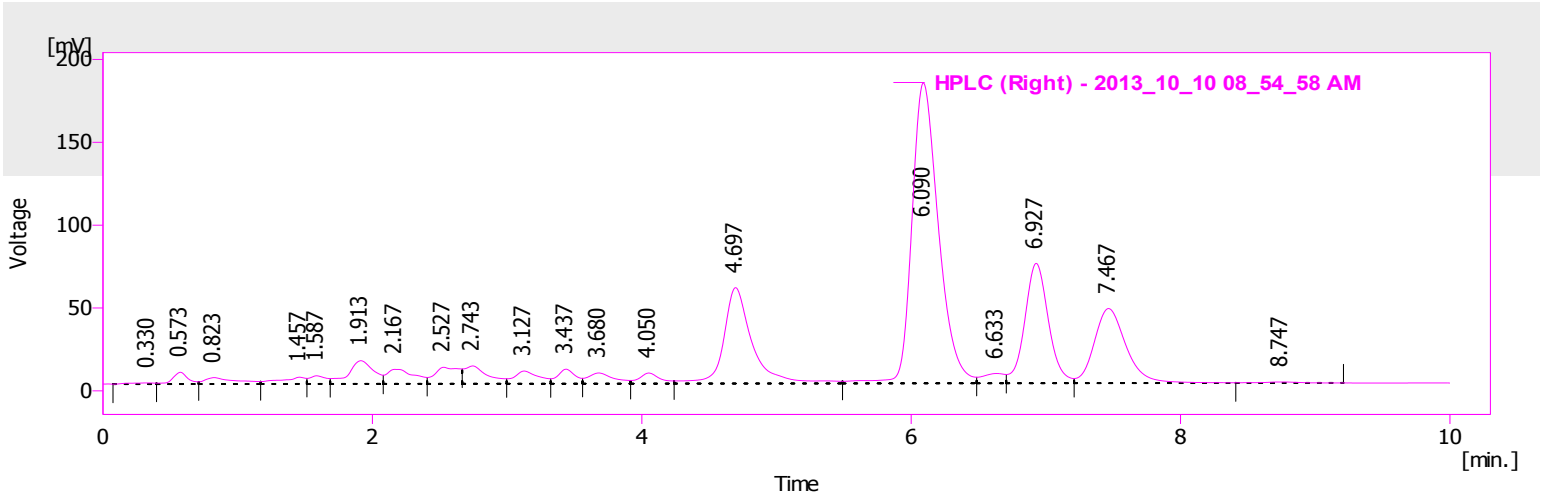
Biochemical and Scientific Consultants cc

Sample Info:

Sample ID	: RA base 200 ppm	Amount	: 0
Sample	:	ISTD Amount	: 0
Inj. Volume [ml]	: 25	Dilution	: 1

Method	: Ibogaine	By	: valerie
Description	: Ibogaine		
Created	: 2013/10/04 12:16 PM	Modified	: 2013/10/10 08:55 AM
Column	: C18 reverse phase	Detection	: 278 nm
Mobile Phase	: 70% ACN with ammonium formate and trimethylamine	Temperature	:
Flow Rate	: 1.0 ml/min	Pressure	:
Note	:		

Autostop	: 10.00, min	External Start	: Start - Restart, Down
Detector 1	: Signal 1	Range 1	: Bipolar, 1250 mV, 10 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result Table (Uncal - HPLC (Right) - 2013_10_10 08_54_58 AM)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	0.330	9.839	0.734	0.2	0.2	0.25
2	0.573	55.745	7.128	0.9	1.6	0.12
3	0.823	64.470	3.885	1.0	0.9	0.26
4	1.457	54.141	4.095	0.9	0.9	0.28
5	1.587	42.115	4.921	0.7	1.1	0.17
6	1.913	181.543	14.004	2.9	3.1	0.20
7	2.167	125.588	8.747	2.0	1.9	0.29
8	2.527	124.227	9.987	2.0	2.2	0.22
9	2.743	130.958	10.780	2.1	2.4	0.18
10	3.127	96.841	7.665	1.6	1.7	0.21
11	3.437	79.655	8.761	1.3	1.9	0.15
12	3.680	86.319	6.470	1.4	1.4	0.23
13	4.050	68.497	6.402	1.1	1.4	0.16
14	4.697	854.436	57.875	13.8	12.7	0.19

Result Table (Uncal - HPLC (Right) - 2013_10_10 08_54_58 AM)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
15	6.090	2493.071	181.359	40.4	39.7	0.21
16	6.633	67.350	5.921	1.1	1.3	0.22
17	6.927	883.443	72.352	14.3	15.8	0.18
18	7.467	743.392	45.051	12.0	9.9	0.25
19	8.747	12.801	0.642	0.2	0.1	0.27
	Total	6174.432	456.781	100.0	100.0	

BSC

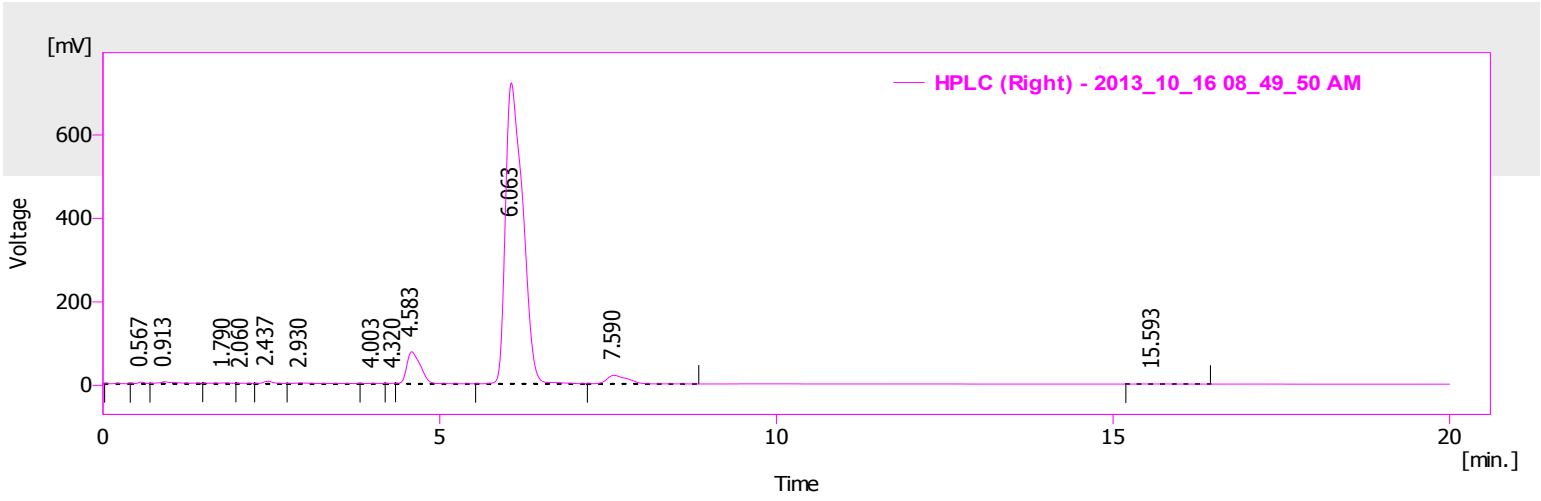
Biochemical and Scientific Consultants cc

Sample Info:

Sample ID	: 548 ppm PTA HCl in ACN buffer	Amount	: 0
Sample	:	ISTD Amount	: 0
Inj. Volume [ml]	: 0.025	Dilution	: 1

Method	: Ibogaine	By	: valerie
Description	: Ibogaine		
Created	: 2013/10/04 12:16 PM	Modified	: 2013/10/16 09:04 AM
Column	: C18, 125 mm (good?)	Detection	: 278 nm
Mobile Phase	: 70% ACN with ammonium formate and trimethylamine	Temperature	:
Flow Rate	: 1.0 ml/min	Pressure	:
Note	:		

Autostop	: 20.00, min	External Start	: Start - Restart, Down
Detector 1	: Signal 1	Range 1	: Bipolar, 1250 mV, 10 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result Table (Uncal - HPLC (Right) - 2013_10_16 08_49_50 AM)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	0.200	5.202	0.565	0.0	0.1	0.13
2	0.567	26.422	3.917	0.2	0.5	0.10
3	0.913	103.485	5.071	0.7	0.6	0.23
4	1.790	44.841	2.017	0.3	0.2	0.49
5	2.060	20.871	1.408	0.1	0.2	0.28
6	2.437	73.940	6.056	0.5	0.7	0.16
7	2.930	67.398	2.070	0.5	0.2	0.36
8	4.003	21.284	1.229	0.1	0.1	0.31
9	4.320	10.109	1.322	0.1	0.2	0.15
10	4.583	1085.250	76.848	7.4	9.1	0.23
11	6.063	12848.505	721.163	87.1	85.6	0.30
12	7.590	438.925	20.536	3.0	2.4	0.36
13	15.593	10.816	0.277	0.1	0.0	0.61
	Total	14757.049	842.479	100.0	100.0	

BSC

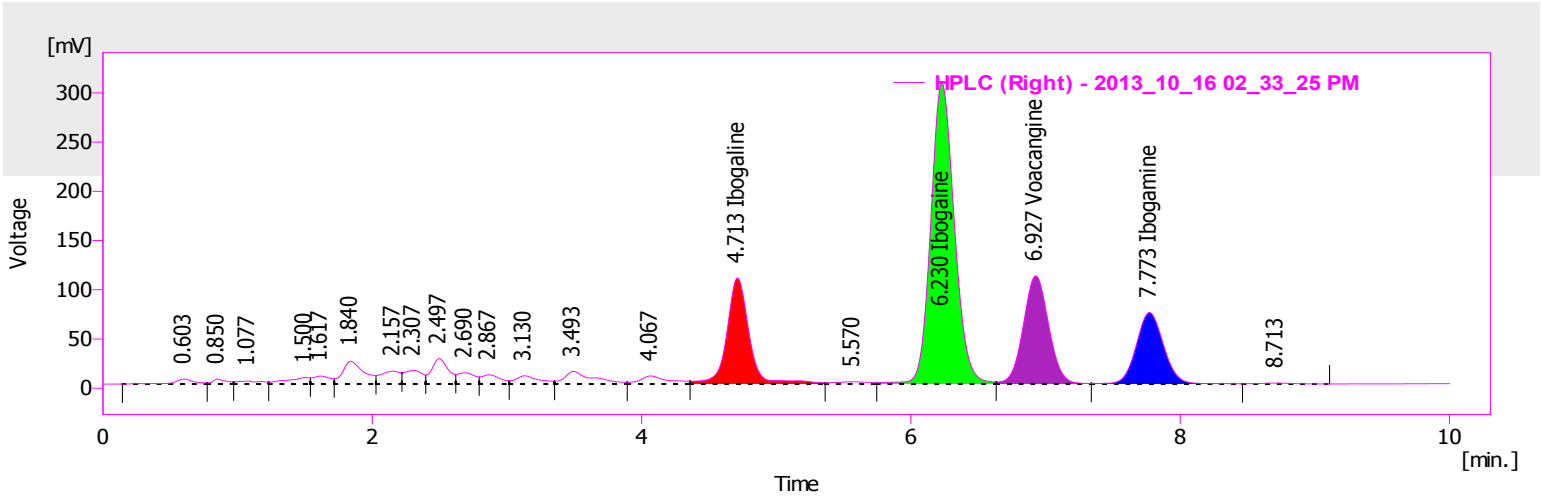
Biochemical and Scientific Consultants cc

Sample Info:

Sample ID	: 448 ppm BSC5 RA in ACN buffer	Amount [ug]	: 8.96
Sample	:	ISTD Amount	: 0
Inj. Volume [ml]	: 0.02	Dilution	: 1

Method	: Ibogaine	By	: valerie
Description	: Ibogaine		
Created	: 2013/10/04 12:16 PM	Modified	: 2013/10/16 02:33 PM
Column	: C18, 125 mm	Detection	: 278 nm
Mobile Phase	: 70% ACN with 38 ppm ammonium formate and 140 ppm trimethylamine	Temperature	: ambient
Flow Rate	: 1.0 ml/min	Pressure	: ~ 1430 psi
Note	: Sample was dissolved in straight acetonitrile		

Autostop	: 10.00, min	External Start	: Start - Restart, Down
Detector 1	: Signal 1	Range 1	: Bipolar, 1250 mV, 10 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result Table (ESTD - HPLC (Right) - 2013_10_16 02_33_25 PM)

	Reten. Time [min]	Response	RB	Amount [ug]	Amount [%]	Peak Type	Compound Name
1	0.603	54.816	A	0.000	0.0		
2	0.850	36.268	A	0.000	0.0		
3	1.077	39.654	A	0.000	0.0		
4	1.500	79.706	A	0.000	0.0		
5	1.617	73.032	A	0.000	0.0		
6	1.840	255.161	A	0.000	0.0		
7	2.157	132.179	A	0.000	0.0		
8	2.307	129.544	A	0.000	0.0		
9	2.497	225.057	A	0.000	0.0		
10	2.690	103.109	A	0.000	0.0		
11	2.867	92.108	A	0.000	0.0		
12	3.130	102.731	A	0.000	0.0		
13	3.493	191.800	A	0.000	0.0		
14	4.067	121.224	A	0.000	0.0		

Result Table (ESTD - HPLC (Right) - 2013_10_16 02_33_25 PM)

	Reten. Time [min]	Response	RB	Amount [ug]	Amount [%]	Peak Type	Compound Name
15	4.713	1248.014	A	1.160	12.9	Ordnr	Ibogaline
16	5.570	40.229	A	0.000	0.0		
17	6.230	3721.786	A	3.458	38.6	Ordnr	Ibogaine
18	6.927	1436.081	A	1.333	14.9	Ordnr	Voacangine
19	7.773	1027.436	A	0.954	10.6	Ordnr	Ibogamine
20	8.713	14.621	A	0.000	0.0		
	Total			8.960	77.1		

BSC

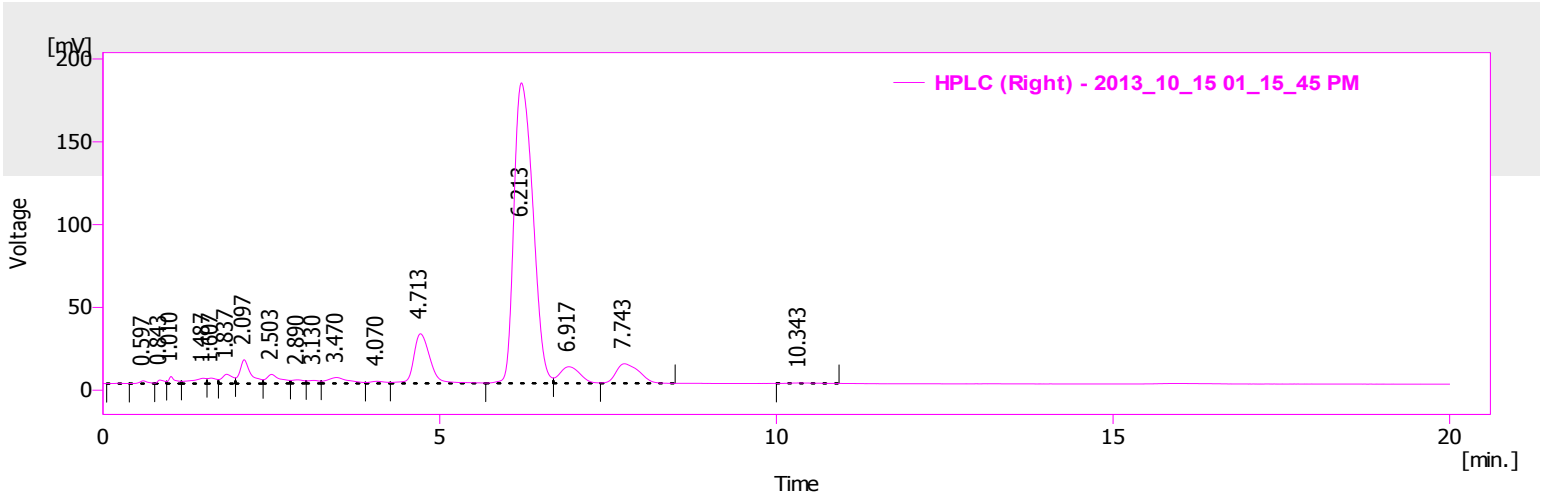
Biochemical and Scientific Consultants cc

Sample Info:

Sample ID	: 500 ppm TA in ACN	Amount	: 0
Sample	:	ISTD Amount	: 0
Inj. Volume [ml]	: 0.025	Dilution	: 1

Method	: Ibogaine	By	: valerie
Description	: Ibogaine		
Created	: 2013/10/04 12:16 PM	Modified	: 2013/10/15 01:26 PM
Column	: C18, 125 mm (good?)	Detection	: 278 nm
Mobile Phase	: 70% ACN with ammonium formate and trimethylamine	Temperature	:
Flow Rate	: 1.0 ml/min	Pressure	:
Note	:		

Autostop	: 20.00, min	External Start	: Start - Restart, Down
Detector 1	: Signal 1	Range 1	: Bipolar, 1250 mV, 10 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result Table (Uncal - HPLC (Right) - 2013_10_15 01_15_45 PM)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	0.233	2.087	0.263	0.0	0.1	0.10
2	0.597	14.363	1.687	0.3	0.6	0.12
3	0.843	15.068	2.135	0.3	0.8	0.14
4	1.010	29.568	4.260	0.6	1.5	0.09
5	1.487	49.617	3.220	1.0	1.1	0.28
6	1.607	30.315	3.301	0.6	1.2	0.17
7	1.837	63.445	5.589	1.2	2.0	0.22
8	2.097	161.790	14.343	3.1	5.1	0.15
9	2.503	80.779	5.512	1.6	1.9	0.22
10	2.890	27.785	2.214	0.5	0.8	0.23
11	3.130	22.761	1.901	0.4	0.7	0.22
12	3.470	79.708	3.623	1.5	1.3	0.34
13	4.070	21.455	1.344	0.4	0.5	0.33
14	4.713	509.292	30.009	9.8	10.6	0.25

Result Table (Uncal - HPLC (Right) - 2013_10_15 01_15_45 PM)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
15	6.213	3560.218	181.486	68.8	64.1	0.32
16	6.917	211.812	10.047	4.1	3.5	0.35
17	7.743	283.414	11.849	5.5	4.2	0.40
18	10.343	7.507	0.299	0.1	0.1	0.39
	Total	5170.984	283.083	100.0	100.0	

BSC

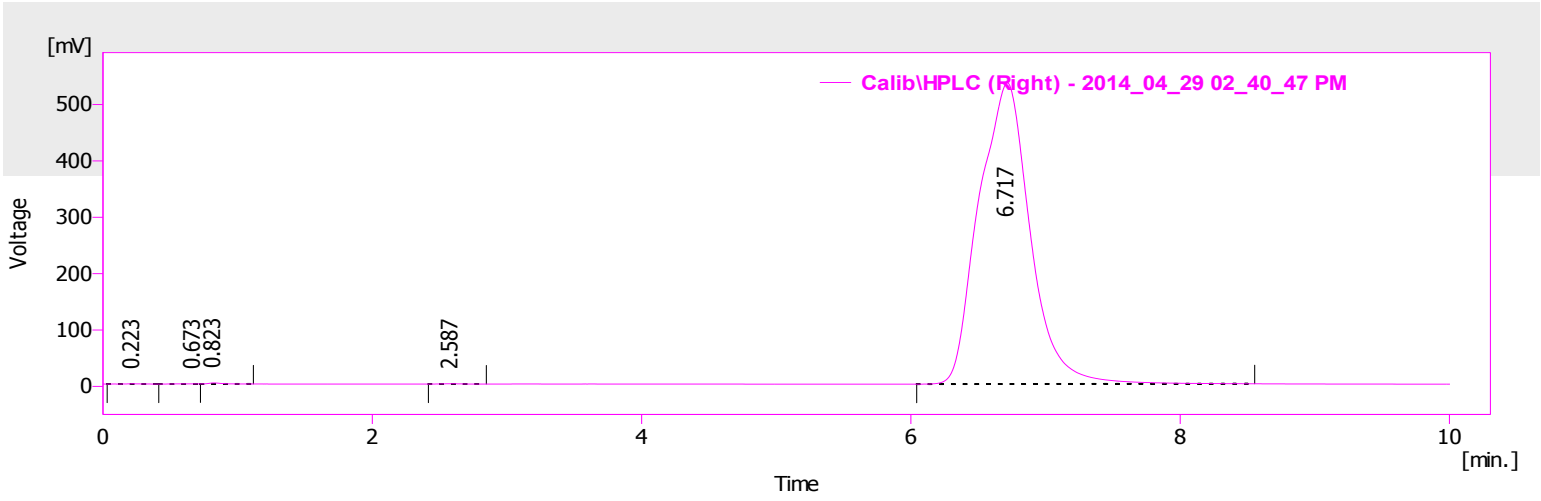
Biochemical and Scientific Consultants cc

Sample Info:

Sample ID	: analytical ibogaine base eluted with 70% ACN buffer	Amount [ug]	: 10.35
Sample	:	ISTD Amount	: 0
Inj. Volume [ml]	: 0.02	Dilution	: 1

Method	: ibogaine2	By	: chris
Description	: Ibogaine base standardization		
Created	: 2013/10/11 12:21 PM	Modified	: 2014/04/29 02:42 PM
Column	: C18 12.5 cm 5um (right)	Detection	: 278 nm
Mobile Phase	: 70% ACN with 38 ppm ammonium formate and 140 ppm trimethylamine	Temperature	: ambient
Flow Rate	: 1 mL/min	Pressure	: 1050 psi
Note	: Highly pure ibogaine base for reference		

Autostop	: 10.00, min	External Start	: Start - Stop, Down
Detector 1	: Signal 1	Range 1	: Bipolar, 1250 mV, 10 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



!!! Result Table (Uncal - Calib|HPLC (Right) - 2014_04_29 02_40_47 PM) No identified peak(s) !!!

	Reten. Time [min]	Response	RB	Amount [ug]	Amount [%]	Peak Type	Compound Name
1	0.223	3.488	A	-	-		
2	0.673	3.554	A	-	-		
3	0.823	13.302	A	-	-		
4	2.587	5.779	A	-	-		
5	6.717	14134.449	A	-	-		
	Total			-	-		

BSC

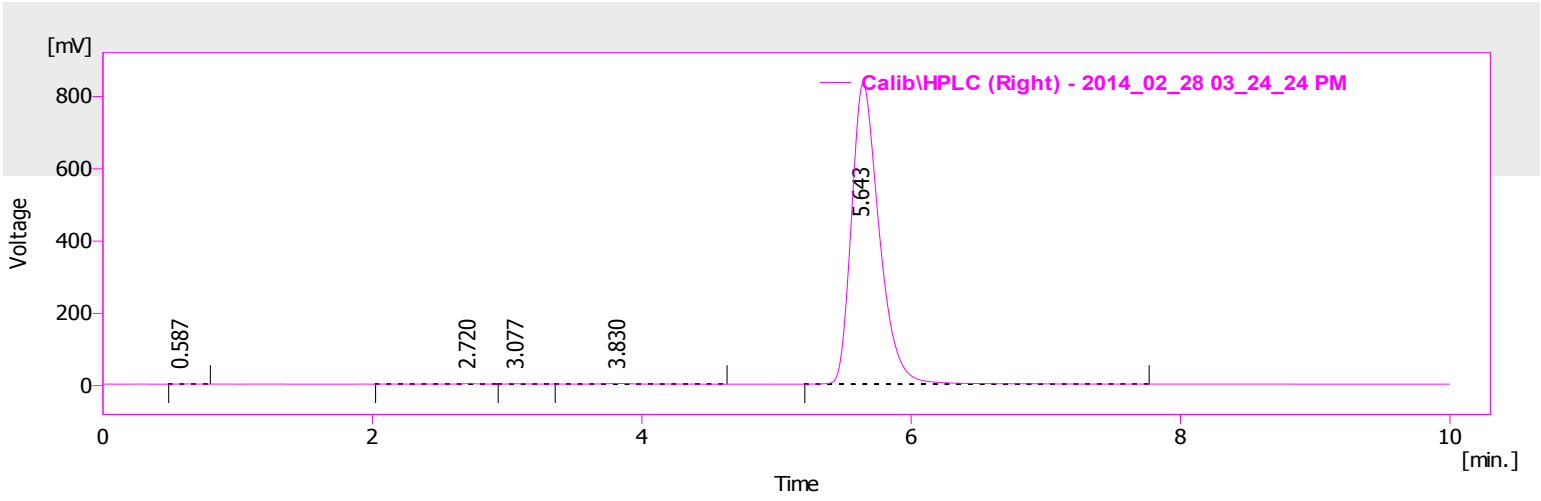
Biochemical and Scientific Consultants cc

Sample Info:

Sample ID	: Recrystallized ibogaine HCl, 4.2 mg in 10 mL 70% ACN Buffer, triple formate	Amount [ug]	: 8.4
Sample	:	ISTD Amount	: 0
Inj. Volume [ml]	: 0.02	Dilution	: 1

Method	: ibogaine2	By	: chris
Description	: Voacangine Quantitative Analysis		
Created	: 2013/10/11 12:21 PM	Modified	: 2014/02/28 03:24 PM
Column	: C18 12.5 cm 5um (right)	Detection	: 278 nm
Mobile Phase	: 70% ACN with 102 ppm ammonium formate and 140 ppm trimethylamine	Temperature	: ambient
Flow Rate	: 1 mL/min	Pressure	: 1050 psi
Note	: Samples from Voacanga bark extraction		

Autostop	: 10.00, min	External Start	: Start - Stop, Down
Detector 1	: Signal 1	Range 1	: Bipolar, 1250 mV, 10 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



!!! Result Table (Uncal - Calib|HPLC (Right) - 2014_02_28 03_24_24 PM) No identified peak(s) !!!

	Reten. Time [min]	Response	RB	Amount [ug]	Amount [%]	Peak Type	Compound Name
1	0.587	3.318	A	-	-		
2	2.720	12.337	A	-	-		
3	3.077	9.447	A	-	-		
4	3.830	31.259	A	-	-		
5	5.643	11932.622	A	-	-		
	Total			-	-		

BSC

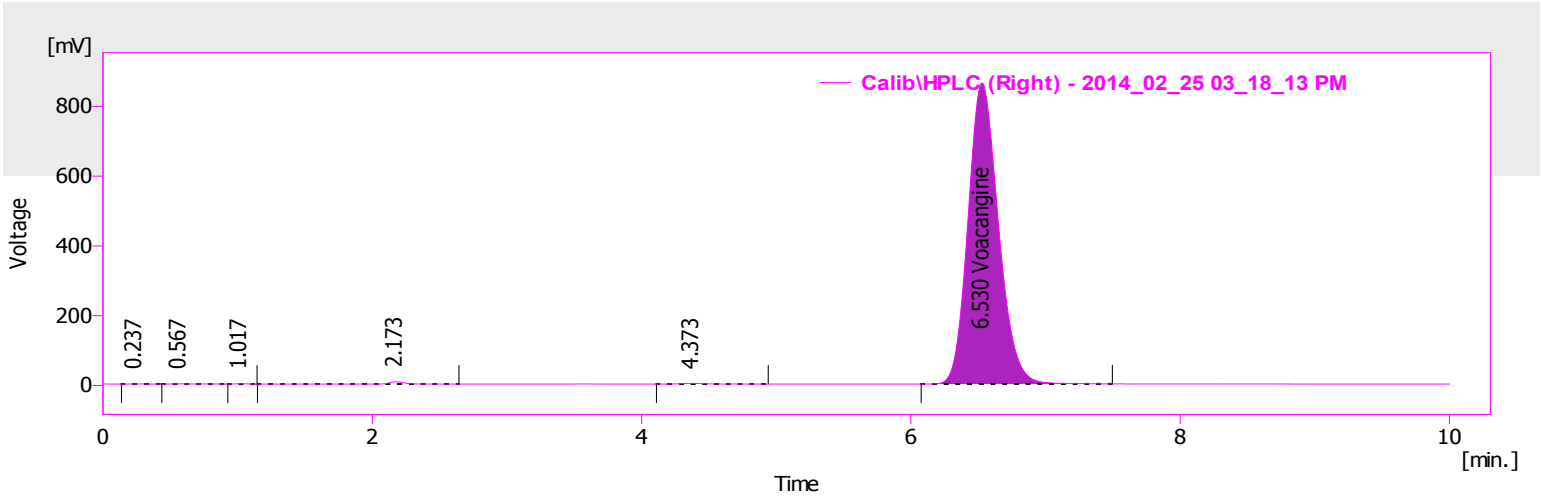
Biochemical and Scientific Consultants cc

Sample Info:

Sample ID	: 540 ppm voacangine standard 1 in 70% ACN Buffer	Amount [ul]	: 10.8
Sample	:	ISTD Amount	: 0
Inj. Volume [ml]	: 0.02	Dilution	: 1

Method	: ibogaine2	By	: chris
Description	: voacangine standard calibration		
Created	: 2013/10/11 12:21 PM	Modified	: 2014/02/25 03:18 PM
Column	: C18 12.5 cm 5um (right)	Detection	: 278 nm
Mobile Phase	: 70% ACN with 38 ppm ammonium formate and 140 ppm trimethylamine	Temperature	: ambient
Flow Rate	: 1 mL/min	Pressure	: 1050 psi
Note	: Three standard solutions of voacangine, diluted in half each run		

Autostop	: 10.00, min	External Start	: Start - Stop, Down
Detector 1	: Signal 1	Range 1	: Bipolar, 1250 mV, 10 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result Table (ESTD - Calib\HPLC (Right) - 2014_02_25 03_18_13 PM)

	Reten. Time [min]	Response	RB	Amount [ul]	Amount [%]	Peak Type	Compound Name
1	0.237	3.312	A	0.000	0.0		
2	0.567	11.808	A	0.000	0.0		
3	1.017	8.309	A	0.000	0.0		
4	2.173	61.373	A	0.000	0.0		
5	4.373	23.871	A	0.000	0.0		
6	6.530	13490.342	A	1.408	13.0	Ordnr	Voacangine
	Total			10.800	13.0		