

Conducted by:

**HPBM**

Harlan Pharmacological & Biological  
Monitoring  
Kiryat Weizmann, Rehovot, Israel

Sponsored by:

**TavTech**

Katzrin, Israel

***Skin Treatment with JetPeel Device***  
***alamarBlue™ Oxidation or Caffeine***  
***Transport***

Experimental Report  
(Non-GLP)

November 2004

**Hagar Greif, Ph.D.**

## Preface

During October 2004, HPBM examined Oxygen- or Caffeine.

The experimental program included pressure treatment of skin samples obtained from pig ear, followed by either detection of alamarBlue oxidation or by Caffeine transport.

HPBM experimental program was conducted according to the experimental design that was agreed by the Sponsor.

## Table of Contents

<b>1</b>	<b>Executive Summary</b>	<b>4</b>
<b>2</b>	<b>Materials</b>	<b>5</b>
<b>3</b>	<b>Methods</b>	<b>6</b>
<b>4</b>	<b>Results</b>	<b>8</b>
<b>5</b>	<b>Results Summary</b>	<b>12</b>
<b>6</b>	<b>Appendix 1</b>	<b>13</b>

## List of Figures

Figure 1	Oxygen Treatment to Pig Skin samples.....	8
Figure 2	Oxygen Treatment to Rat Skin samples.....	9
Figure 3	Caffeine Treatment to Pig Skin samples.....	10

## List of Tables

Table 1	Caffeine permeability ( $P_{app}$ ) (following JetPeel device treatment) .....	11
---------	--	----

# 1 Executive Summary

## 1.1 Objectives

- To test the oxidation potential of skin samples following their treatment with oxygen.
- To test Caffeine transport parameters across skin samples following their treatment with Caffeine.

## 1.2 Main Findings:

- The signal of oxygen released from skin tissues was not specific to JetPeel treatment, probably due to high background signal from the tissue itself.
- JetPeel Caffeine treatment resulted in an almost instant permeation of the compound. Caffeine high concentrations levels were maintained for 20 hours.

## **2** Materials

### **2.1** Test Equipment

JetPeel device is intended for face skin peeling and rejuvenation. The treatment sprayer is connected to O<sub>2</sub> container and to dialyzed water (or drug solution) container.

### **2.2** Materials

alamarBlue™ – Serotec, Cat. No. BUF012B

Caffeine – Merck, Cat. No. 1.59692.0001

### **2.3** Skin Samples

Pig ears were obtained from the institute of animal research, Kibbutz Lahav. Pigs were stored at 4°C until use. Skin was peeled off the posterior ear side.

Rat abdomen skin (SD, Harlan) was freshly prepared prior to use.

## 3 Methods

### 3.1 JetPeel Device Treatment

Skin samples were stretched on cork surface and 2.8cm diameter was gently marked. Treatments were conducted within the marked circles at the indicated time points (see Results).

During treatment, the hand-piece was at 3-5 mm distance from the treated area and at an angle of about 70 degrees. The desired deepness was obtained through the velocity of hand-piece scanning motion.

### 3.2 alamarBlue™ Indicator

Following treatment (n=2), skin samples were cut with 2.8cm diameter template, washed briefly with phosphate buffered saline (PBS) and placed in 50ml tubes containing 1ml 10% alamarBlue™ in PBS.

Due to tube geometry, the basolateral side (body) of the skin circle was resting upon the top of the solution, while the apical side (air) was mostly exposed to the air.

100µl aliquots from the alamarBlue solution were removed at 0.5, 2.5, and 4 hours post-treatment. Aliquots were measured in fluorimeter (Fluorescan, Labsystems) at 544nm excitation and 590nm emission.

### 3.3 Caffeine Transport

#### 3.3.1 *Skin Treatment*

Caffeine was dissolved to 1mg/ml in water and used for JetPeel device treatment instead of water, for the duration of 1 minute. Treated and non-treated skin circles were cut along circle lines and placed in diffusion chambers.

#### 3.3.2 *Transport System*

5 cells of Diffusion Chamber System (Harvard) were filled with 3ml PBS at the basolateral side, and adjusted to 35°C on heat block for 15-30minutes.

At transport onset, apical side was loaded with 1mg/ml Caffeine in water.

Aliquots were removed at 0.5, 2, 6 and 20 hours and fresh PBS replaced the exact removed volume.

### 3.3.3 HPLC Analysis

Aliquoted samples were analyzed in HPLC according to the procedure described below.

HPLC System: Waters 2790 HPLC system, with PDA 996 detector (Waters).

Solvents: A: Water  
B: Acetonitrile

Solid phase: LichroCART RP-Select B, 250-4, particle size 5µm, 250-4mm (Merck Cat No.165018) at RT.

Gradient:

Time (minutes)	A (%)	B (%)
0	95	5
10	5	95

Injection Volume: 40µl

Processing method: The data was processed using Millennium software. Processing wavelength was set to 273nm. The sample amount was calculated using derived calibration curves (see subsection 6.1).

### 3.3.4 Papp Calculations

An apparent permeability coefficient (Papp) of Caffeine was calculated from the following equation:

**Equation 1**

$$P_{app(t)} = (C_t V / t) * (1 / C_0 A)$$

In which  $C_0$  is the initial concentration (at  $t = 0$ ) of the compound on the donor (apical side) and  $C_t$  is the concentration at the calculated time point ( $t$ ) in the receiver chamber,  $V$  is receiver chamber volume,  $A$  is the surface area of the monolayer, and  $t$  is the elapsed time.

## 4 Results

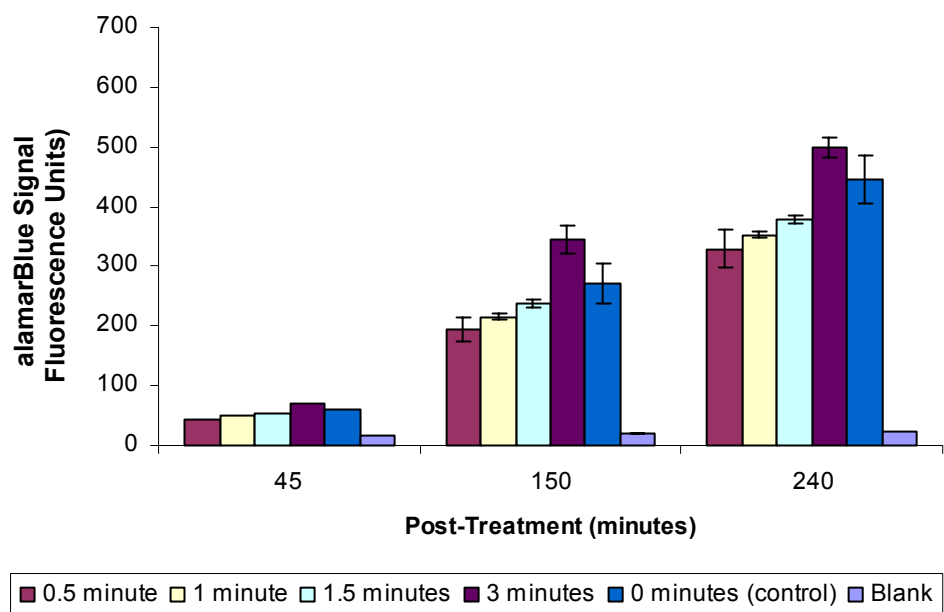
### 4.1 Oxygen Treatment

#### 4.1.1 Pig Skin

Skin was treated with oxygen-enriched water for 0.5 to 3 minutes. The excess oxygen was washed and the oxygen-soaked tissue was placed in indicator containing tube (AlamarBlue™). Figure 1 depicts indicator signals measured at several time points following treatment.

**Figure 1 Oxygen Treatment to Pig Skin samples**

Oxygen released from tissue samples was measured using AlamarBlue™ oxidative signal at 45, 150 and 240 minutes following JetPeel treatment. Fluorescent signal of AlamarBlue™ was measured at 544nm excitation and 590nm.



As can be seen, signal of non-treated skin samples was not significantly different than treated samples.

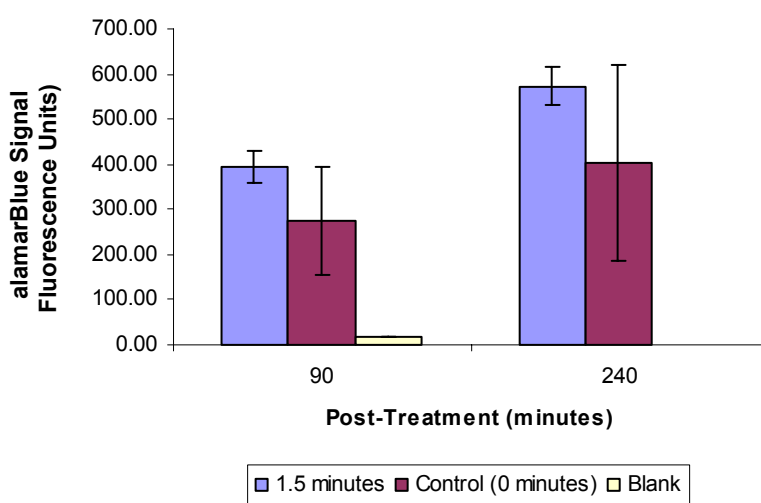
This observation is probably due to high metabolic rate of skin samples, resulting in high basal level of oxidation.

#### 4.1.2 Pig Skin

Next, we tested whether fresh skin samples would give different oxygen-release signals. We used shaved SD rat skin and treated in the same manner as pig skin. Results are depicted in Figure 2.

**Figure 2** Oxygen Treatment to Rat Skin samples

Oxygen released from tissue samples was measured using alamarBlue™ oxidative signal at 45, 150 and 240 minutes following JetPeel treatment. Fluorescent signal of alamarBlue™ was measured at 544nm excitation and 590nm.



As can be seen and with accordance to pig skin results, signal of non-treated skin samples was not significantly different than treated samples. Due to high standard deviation in control samples, we repeated control treatment the next day. Control signals were essentially the same.

Similar to skin samples, high metabolic rate is observed with rat skin samples, resulting in high basal level of oxidation.

In order to detect oxygen release from metabolically active tissues, another examination method should be employed.

## 4.2 Caffeine Transport

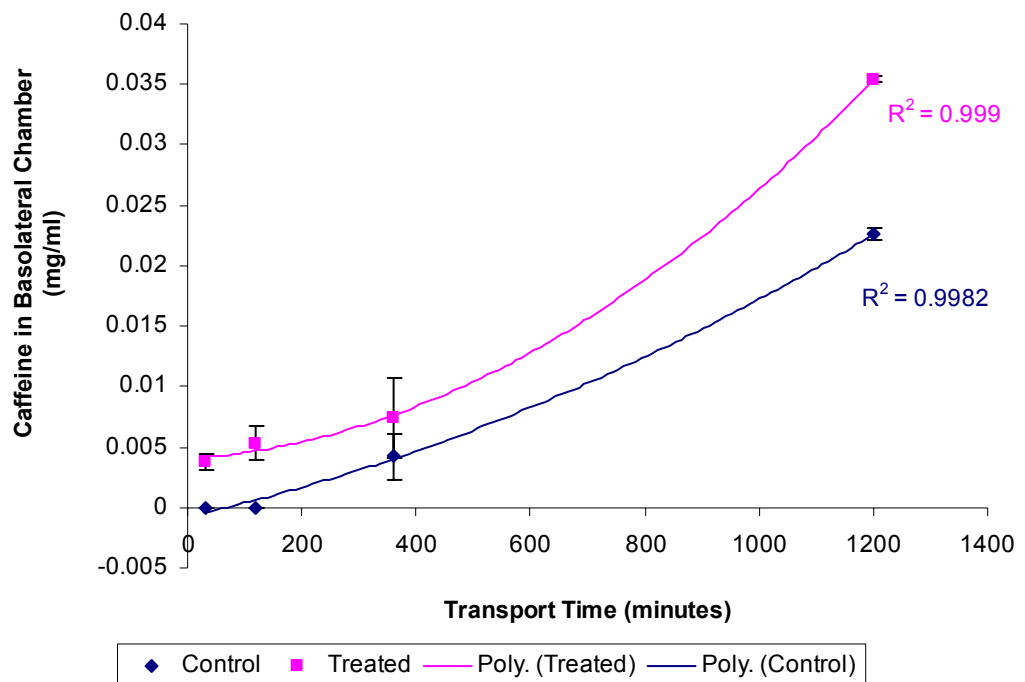
The JetPeel device may facilitate the transport of drugs across skin. To determine if indeed drugs are transported faster with device treatment, we utilized a model hydrophilic drug – Caffeine.

In the Caffeine transport experiment, 1mg/ml Caffeine solution replaced the water in the previous treatments. Following treatment, skin samples were stretched in diffusion chambers and Caffeine was loaded at their apical (air) side. Aliquots were removed at several time points from the basolateral (body) side of the diffusion chamber and analysed for Caffeine concentration with HPLC (Figure 3). Permeability coefficients were subsequently calculated (Table 1).

**Figure 3 Caffeine Treatment to Pig Skin samples**

Caffeine was administered at the apical side of pig skin pieces with the JetPeel device (n=2 for treated and non-treated group). Samples were removed from the basolateral chamber at 0.5, 2, 6, and 20 hours following treatment, and analysed in HPLC. Typical chromatogram and summary of individual raw data are presented in Appendix 1.

Caffeine concentration in mg/ml with standard deviation is plotted vs. transport time in minutes. Trendline regression is presented adjacent to each graph.



As can be seen in Figure 3, JetPeel treatment resulted in almost immediate increase, as early as 30 minutes, in Caffeine transport to the basolateral chamber. The higher concentration levels were continual throughout the experiment. Only after two hours, Caffeine could be measured in the non-treatment samples.

These results demonstrate the ability of JetPeel device to cause Caffeine penetration into the skin, followed by its immediate release at the other side.

Permeability of compounds is usually expressed as the flow of a compound through surface area. Thus, we calculated permeability coefficients ( $P_{app}$ ) of Caffeine for each time point (Table 1).

**Table 1** Caffeine permeability ( $P_{app}$ ) following JetPeel device treatment

Permeability coefficients were calculated as described in Methods for each diffusion chamber at each time-point.  $P_{app}$  is presented as average  $\pm$  SD.

Transport Time (hours)	$P_{app}$ (cm/sec x $10^6$ )	
	<i>Caffeine-treated</i>	<i>Non-treated</i>
0.5	3.5 $\pm$ 0.6	0.0 $\pm$ 0.0
2	5.0 $\pm$ 1.3	0.0 $\pm$ 0.0
6	7.0 $\pm$ 3.1	4.0 $\pm$ 1.8
20	33.4 $\pm$ 0.3	21.3 $\pm$ 0.5

Permeability ( $P_{app}$ ) values of the Caffeine-treated skin samples are much higher all through the experiment.

$P_{app}$  values of Caffeine in non-treated skin are well correlated with its values in the literature (above  $1 \times 10^{-5}$ ).

An increase in  $P_{app}$  values along transport time with both treated and non-treated skin is observed. This phenomenon usually hints to active transport. We presume that the influence of Caffeine on transporters in the skin bring about this increase<sup>1</sup>.

<sup>1</sup> Franke H, Galla HJ, Beuckmann CT. An improved low-permeability in vitro-model of the blood-brain barrier: transport studies on retinoids, sucrose, haloperidol, caffeine and mannitol. Brain Res. 1999 Feb 6;818(1):65-71.

Sardao VA, Oliveira PJ, Moreno AJ. Caffeine enhances the calcium-dependent cardiac mitochondrial permeability transition: relevance for caffeine toxicity. Toxicol Appl Pharmacol. 2002 Feb 15;179(1):50-6.

## 5 Results Summary

In this reported study, JetPeel device treatment pressurized into the skin either oxygen with water, or oxygen with Caffeine.

The signal of oxygen released from skin tissues was not specific to JetPeel treatment, probably due to high background signal from the tissue itself.

JetPeel Caffeine treatment resulted in an almost instant permeation of the compound. Caffeine high concentrations levels were maintained throughout the experiment.

## 6 Appendix 1

### 6.1 Caffeine Chromatogram

Chromatogram of 2µg Caffeine.



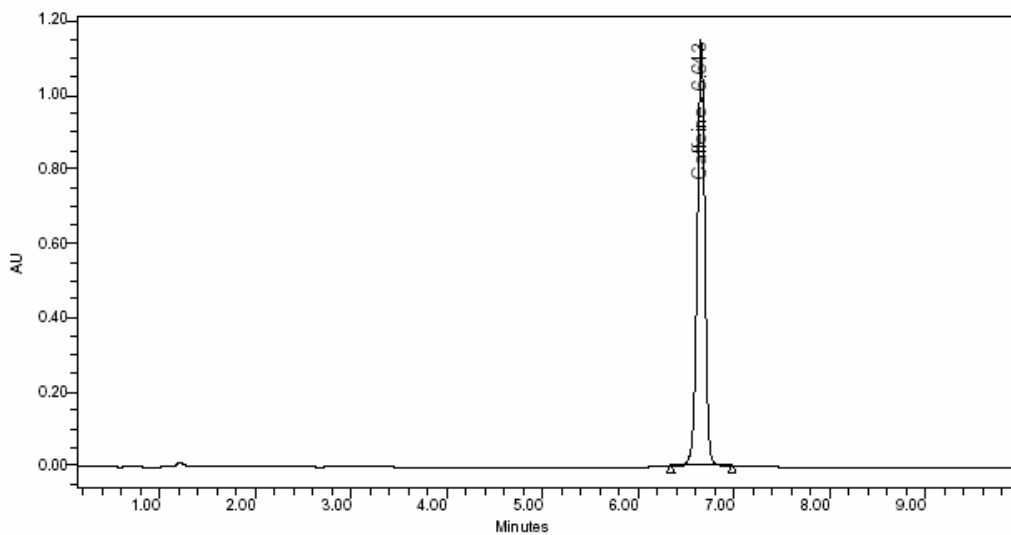
Reported by User: System

## Individual Sample Report

Project Name: Skin\_transport

### SAMPLE INFORMATION

Sample Name:	Caffeine 0.2mg/ml	Acquired By:	System
Sample Type:	Standard	Date Acquired:	10/24/04 3:46:07 PM
Vial:	1:A,1	Acq. Method Set:	Caffeine_5
Injection #:	1	Date Processed:	10/25/04 4:56:37 PM
Injection Volume:	10.00 ul	Processing Method:	Caffeine_1
Run Time:	10.0 Minutes	Channel Name:	WvlN Ch1
Sample Set Name:	transport_skin	Proc. Chnl. Descr.:	PDA 273.0 nm



	Peak Name	RT	Area	% Area	Height	Amount	Units
1	Caffeine	6.643	6363178	100.00	1141922	2.000	ug

## 6.2 Individual Results Summary



# Sample Summary Report

Reported by User: System

Project Name: Skin\_transport

Sample Name: Caffeine 0.2mg/ml  
Date Acquired: 10/24/04 3:46:07 PM

Vial: 1:A,1  
Inj. #: 1

Peak Name	Processed Channel	Retention Time (min)	Area	% Area	Height	Amount	Units
1 Caffeine	PDA 273.0 nm	6.643	6363178	100.00	1141922	2.0	ug

Sample Name: C1 time0  
Date Acquired: 10/24/04 5:25:35 PM

Vial: 1:A,4  
Inj. #: 1

Peak Name	Processed Channel	Retention Time (min)
1 Caffeine	PDA 273.0 nm	6.408

Sample Name: C2 time0  
Date Acquired: 10/24/04 5:36:44 PM

Vial: 1:A,5  
Inj. #: 1

Peak Name	Processed Channel	Retention Time (min)
1 Caffeine	PDA 273.0 nm	6.408

Sample Name: #1 time0  
Date Acquired: 10/24/04 5:58:58 PM

Vial: 1:A,6  
Inj. #: 1

Peak Name	Processed Channel	Retention Time (min)	Area	% Area	Height	Amount	Units
1 Caffeine	PDA 273.0 nm	6.489	1787106	100.00	142680	0.6	ug

Sample Name: #2 time0  
Date Acquired: 10/24/04 6:10:08 PM

Vial: 1:A,7  
Inj. #: 1

Peak Name	Processed Channel	Retention Time (min)	Area	% Area	Height	Amount	Units
1 Caffeine	PDA 273.0 nm	6.486	244220	100.00	20737	0.2	ug

Sample Name: #3 time0

Vial: 1:A,8

Report Method: Sample Summary Table

Printed 2:40:55 PM 11/19/04

Page: 1 of 4



# Sample Summary Report

Reported by User: System

Project Name: Skin\_transport

Date Acquired: 10/24/04 6:21:16 PM

Inj. #: 1

Peak Name	Processed Channel	Retention Time (min)	Area	% Area	Height	Amount	Units
1 Caffeine	PDA 273.0 nm	6.491	121096	100.00	9489	0.1	ug

Sample Name: c1 2hr

Vial: 1:B,1

Date Acquired: 10/24/04 6:32:27 PM

Inj. #: 1

Peak Name	Processed Channel	Retention Time (min)
1 Caffeine	PDA 273.0 nm	6.408

Sample Name: c2 2hr

Vial: 1:B,2

Date Acquired: 10/24/04 6:43:38 PM

Inj. #: 1

Peak Name	Processed Channel	Retention Time (min)	Area	% Area	Height
1	PDA 273.0 nm	5.369	50908	100.00	11879
2 Caffeine	PDA 273.0 nm	6.408			

Sample Name: #1 2hr

Vial: 1:B,3

Date Acquired: 10/24/04 7:05:52 PM

Inj. #: 1

Peak Name	Processed Channel	Retention Time (min)	Area	% Area	Height	Amount	Units
1 Caffeine	PDA 273.0 nm	6.496	2052203	100.00	155091	0.7	ug

Sample Name: #2 2hr

Vial: 1:B,4

Date Acquired: 10/24/04 7:17:02 PM

Inj. #: 1

Peak Name	Processed Channel	Retention Time (min)	Area	% Area	Height	Amount	Units
1 Caffeine	PDA 273.0 nm	6.506	527185	100.00	39276	0.3	ug

Sample Name: #3 2hr

Vial: 1:B,5

Date Acquired: 10/24/04 7:28:11 PM

Inj. #: 1

Report Method: Sample Summary Table

Printed 2:40:55 PM 11/19/04

Page: 2 of 4



# Sample Summary Report

Reported by User: System Project Name: Skin\_transport

Peak Name	Processed Channel	Retention Time (min)	Area	% Area	Height	Amount	Units
1 Caffeine	PDA 273.0 nm	6.514	271212	100.00	19060	0.2	ug

Sample Name: c1 6hr Vial: 1:B,6  
Date Acquired: 10/24/04 7:39:21 PM Inj. #: 1

Peak Name	Processed Channel	Retention Time (min)	Area	% Area	Height	Amount	Units
1 Caffeine	PDA 273.0 nm	6.507	76182	100.00	6715	0.1	ug

Sample Name: c2 6hr Vial: 1:B,7  
Date Acquired: 10/24/04 7:50:31 PM Inj. #: 1

Peak Name	Processed Channel	Retention Time (min)	Area	% Area	Height	Amount	Units
1 Caffeine	PDA 273.0 nm	6.511	432282	100.00	7266	0.2	ug

Sample Name: #1 6hr Vial: 1:B,8  
Date Acquired: 10/24/04 8:12:44 PM Inj. #: 1

Peak Name	Processed Channel	Retention Time (min)	Area	% Area	Height	Amount	Units
1 Caffeine	PDA 273.0 nm	6.530	7512957	100.00	542109	2.4	ug

Sample Name: #2 6hr Vial: 1:C,1  
Date Acquired: 10/24/04 8:23:53 PM Inj. #: 1

Peak Name	Processed Channel	Retention Time (min)	Area	% Area	Height	Amount	Units
1 Caffeine	PDA 273.0 nm	6.517	979351	100.00	68824	0.4	ug

Sample Name: #3 6hr Vial: 1:C,2  
Date Acquired: 10/24/04 8:35:06 PM Inj. #: 1

Peak Name	Processed Channel	Retention Time (min)	Area	% Area	Height	Amount	Units
1 Caffeine	PDA 273.0 nm	6.523	370672	100.00	26124	0.2	ug



# Sample Summary Report

Reported by User: System

Project Name: Skin\_transport

Sample Name: c1 20hr Vial: 1:C,3  
Date Acquired: 10/24/04 8:46:15 PM Inj. #: 1

Peak Name	Processed Channel	Retention Time (min)	Area	% Area	Height	Amount	Units
1 Caffeine	PDA 273.0 nm	6.528	2719764	100.00	217876	0.9	ug

Sample Name: c2 20hr Vial: 1:C,4  
Date Acquired: 10/24/04 8:57:25 PM Inj. #: 1

Peak Name	Processed Channel	Retention Time (min)	Area	% Area	Height	Amount	Units
1 Caffeine	PDA 273.0 nm	6.516	2627410	100.00	182413	0.9	ug

Sample Name: #1 20hr Vial: 1:C,5  
Date Acquired: 10/24/04 9:19:39 PM Inj. #: 1

Peak Name	Processed Channel	Retention Time (min)	Area	% Area	Height	Amount	Units
1 Caffeine	PDA 273.0 nm	6.513	15100150	100.00	1023323	4.7	ug

Sample Name: #2 20hr Vial: 1:C,6  
Date Acquired: 10/24/04 9:30:51 PM Inj. #: 1

Peak Name	Processed Channel	Retention Time (min)	Area	% Area	Height	Amount	Units
1 Caffeine	PDA 273.0 nm	6.533	4391824	100.00	320908	1.4	ug

Sample Name: #3 20hr Vial: 1:C,7  
Date Acquired: 10/24/04 9:42:00 PM Inj. #: 1

Peak Name	Processed Channel	Retention Time (min)	Area	% Area	Height	Amount	Units
1 Caffeine	PDA 273.0 nm	6.537	4342130	100.00	318291	1.4	ug