Published in final edited form as:

Electrophoresis. 2010 June ; 31(11): 1886–1893. doi:10.1002/elps.200900761.

Determination of Salsolinol, Norsalsolinol, and Twenty-one Biogenic Amines Using Micellar Electrokinetic Capillary Chromatography – Electrochemical Detection

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Abstract

Micellar electrokinetic chromatography coupled to amperometric electrochemical detection was used to resolve and then quantify biogenic amines and metabolites within the fruit fly *Drosophila melanogaster*. A new separation scheme was devised to allow resolution of 24 compounds of interest. This was accomplished by precisely controlling the amount of base added to the background buffer, optimizing the resolution of the separation, and then calculating the pH. Here we focused on measurements of six of the analytes that are thought to be involved in the response to alcohol, dopamine, salsolinol, norsalsolinol, N-acetyloctopamine, octopamine, and N-acetyldopamine. These were identified and quantified within the fly head. We believe the identification of salsolinol and norsalsolinol in the fly brain is novel.

1 Introduction

Biogenic amines, such as catecholamines (dopamine, norepinephrine, octopamine, etc) and the indoleamine serotonin, serve as neurotransmitters and are widely distributed within both the central and peripheral nervous systems. They have been implicated to play important roles in a variety of physiological processes from learning and memory, emotion, motion, sleep, and appetite as well as disorders such as depression, Parkinson's Disease, and Huntington's Disease. Precursors and metabolites of biogenic amines can also play important roles within neuronal pathways helping to modulate the overall activity of the brain and can be used as biomarkers for these disease states [1,2]. As such, it is desirable to develop new methodologies to identify and quantify not only neurotransmitters within the brain but their related presynaptic and metabolic compounds.

The dopamine-derived tetrahydroisoquinolines, salsolinol and norsalsolinol, are putative metabolic neuromodulators that have been found to be present in both rat [3,4] and human [5,6] brains. These compounds modulate dopaminergic transmission and have been shown to be associated with neurotoxicity within cells and diseases such as alcoholism and Parkinsonism. Salsolinol can be formed either by condensation of dopamine with pyruvate followed by oxidative decarboxylation and reduction (Figure 1A) or enzymatic-condensation of ethanol to acetaldehyde with dopamine by the Pictet-Spengler mechanism (Figure 1B) [7–9]. Due to its direct chemical link to ethanol, studies on salsolinol have been

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in relation to alcohol use and abuse. Salsolinol has been found to be elevated in alcoholic patients [10,11] and induces reinforcement in self-administration experiments [12]. Norsalsolinol can be synthesized in vivo nonenzymatically by condensation of dopamine with formaldehyde, the oxidized metabolite of methanol (Figure 1B). It has been linked primarily with neurotoxicity, especially within Parkinson's Disease [13], but may be of interest in alcohol withdrawal due to increased methanol levels [9].

The fruit fly *Drosophila melanogaster* is a highly investigated model system for neuronal processes. In contrast to a rat or mouse model, *Drosophila* are smaller in size and have shorter life cycles making them easier to keep in larger quantities, while their fewer genetic functional redundancies and fully mapped out genome [14] have produced a plethora of genetic mutants available for study. Furthermore, many similarities exist between the genetics and function of *Drosophila* and mammals. Both systems contain similar molecular and cellular components, particularly neuromodulators such as dopamine, and physiological systems involving basic cellular function and signal transduction pathways [15–18]. Several separations-based techniques have been employed to study neuromodulators in *Drosophila*, including HPLC with electrochemical (EC) detection [19–23], GC-MS [24], and MEKC-EC [25–27]. Whereas both HPLC and GC have been shown to be viable methods to quantify the neurochemicals present in *Drosophila*, use of these techniques only allows a select few neuromessengers to be determined, usually the main neurotransmitters. Conversely, the separation and identification of fourteen biogenic-amine modulators and metabolites in the fly has been demonstrated [26].

While many studies have been performed to help better understand the precise roles salsolinol, norsalsolinol, and other neuronal metabolites play within the body, quantification of putative neuromessengers challenges our current analytical techniques due to small sample volumes and interferences from the complex biological matrix. A small-volume separation with high selectivity would be a valuable addition to the repertoire of methods available here.

MEKC is a mode of capillary electrophoresis where surfactant exceeding the critical micelle concentration is added to the running buffer. The system benefits from fast separation times, high-resolution capabilities, and low mass detection limits from the capillary electrophoresis system, and the extra surfactant generates a second degree of separation that affords the separation based on hydrophobicity of the analyte. Moreover when combining this system with amperometric EC detection, one gains a highly sensitive and selective system that does not necessitate sample derivatization and addresses the aforementioned complexities of measurements in biological samples.

In this paper, we build upon previous the work to develop a separation scheme to investigate monoamine modulators, their metabolites, and related molecules (referred to as “metabolites” here) present in the *Drosophila* central nervous system. A sequential, more precise optimization of the borate/SDS micelle buffer using $[\text{OH}^-]$ is presented. Not only does this method improve upon resolution of the fourteen previously reported neurometabolites using borate complexation, but nine new metabolites are added to the separation, bringing the separation total to twenty-three compounds plus the internal standard. Standard additions were performed in order to quantify metabolites in CantonS wild-type *Drosophila* to verify the method in biological samples and to show the presence of salsolinol and norsalsolinol in the fly.

2 Materials and methods

2.1 Reagents

DL-tyrosine and sodium hydroxide were obtained from Fluka (Buchs, Switzerland), perchloric acid from Riedel-de Haën (Morristown, NJ, USA) and norsalsolinol from Acros organics (Geel, Belgium). N-acetylamine dopamine, N-acetylamine octopamine, and N-acetylamine tyramine were obtained from the The National Institutes of Mental Health (NIMH) chemical synthesis and drug supply program (Research Triangle Park, NC, USA). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). Separations were performed in 25 mM borate buffer containing 50 mM SDS and 2% 1-propanol with various concentrations of NaOH. All standards were prepared as 10 mM stock solutions in 0.1 M perchloric acid and were diluted to the desired concentration with additional 0.1 M perchloric acid.

2.2 *Drosophila* Strains

Canton-S wild-type *Drosophila melanogaster* strains were obtained from the Bloomington *Drosophila* Stock Center (Indiana University, Bloomington, IN, USA) and were maintained in the laboratory. Flies were cultured on standard potato meal/agar medium and collected for homogenization 4 and 5 days after emerging from pupal cages.

2.3 Homogenate Preparation

Flies were prepared for CE experiments based on a protocol previously described [25]. Flies were collected in 15-mL centrifuge tubes, plunged into liquid nitrogen, and vortexed for 60 s in order to separate the heads from the body. Approximately 100 heads were counted, collected, and homogenized in 0.1 M perchloric acid/100 μ M catecholamine (internal standard) at a volume of 0.5 μ L/head. The homogenized heads were centrifuged using an Eppendorf centrifuge with a fixed angle rotor (Brinkman Instruments, Westbury, NY, USA) and spun at 14,000 rpm for 5 min at 0 °C. The supernatant was collected and centrifuged (2 h, 14,000 rpm, 0 °C) through a 3-kDa centrifuge filter (VWR International, LLC, West Chester, PA, USA). The filtrates were dispensed in aliquots of 4.5 μ L and stored in the freezer (−80 °C) until use.

2.4 Instrumentation and Analysis

The CE system with end-column amperometric detection utilized in this study was built in-house and has been described previously [28]. Briefly, 45–50 cm of fused-silica capillary with an outer diameter of 151 μ m and inner diameter of 14 μ m (Polymicro Technologies, Phoenix, AZ) was used for the separations. Amperometric EC detection was carried out with a two-electrode format in which a 5- μ m carbon fiber microelectrode was held +0.75 V versus a Ag/AgCl reference electrode (Harvard Apparatus, Holliston, MA, USA). The current was measured using a Keithly model 427 current amplifier (Cleveland, OH, USA) and recorded at 10 Hz using a LabView 8.0 (National Instruments, Austin, TX, USA) interface, written in-house. Microsoft Excel (Redmond, WA, USA) was used to generate the electropherograms and statistics. Data analysis was performed using Peakfit Separation and Analysis Software v4.11 (SPSS Inc., Chicago, IL, USA).

2.5 Procedures

Buffer solutions were filtered with a 0.2 μ m nylon filter (Alltech, Deerfield, IL) prior to use. Capillaries were filled with separation buffer using a stainless steel reservoir with applied N₂ pressure (3.5 MPa) and washed between changes in buffer with deionized water (18.0 M Ω) for ~15 min. Injections were then performed electrokinetically at 5 kV for 5 s to extract ~1 nL of homogenate from the homogenate. To enhance microelectrode placement, the

capillary inner diameter was enlarged via HF etching as previously described [28]. Approximately 2 mm of the polyimide coating was burned off from the capillary to expose the fused silica. The exposed portion of the capillary then was placed in HF for 15 min with a pressure of 3.5 MPa (N_2) through the capillary. Subsequently, the same segment of capillary was placed in a sodium bicarbonate solution to neutralize the acid and then washed with water to remove any excess salt. Typically, the same capillary was employed for any single set of experiments such as all the samples in the set of standard additions or calibration curve.

The buffers with small pH changes to optimize separations were made as follows. Five liters of 25 mM borax/50 mM SDS/2% 1-propanol were made and divided into equal 0.250 L portions. A small amount of OH^- was added to each portion sequentially increasing the concentration from 6.96 mM to 44.79 mM. The pH of the solution was then calculated based on the buffer present and amount of OH^- .

2.6 Safety Considerations

An in-house-built safety interlock box was utilized to protect the user from high voltage. Since HF could cause severe burns, it was used with extreme care in a fume hood with proper precautions taken.

3 Results and Discussion

3.1 Optimization of Borate/SDS Buffer With $[OH^-]$

Twenty-three electroactive biogenic amines and metabolites were selected for separation optimization and to better understand the possible roles they might play in the neurophysiology of *Drosophila*. Previous work applied separation buffers with 25 mM sodium tetraborate, 50 mM SDS, and 2% 1-propanol at pH 9.50 to investigate neurotransmitters and metabolites using MEKC-EC [26]. These included L-3,4-dihydroxyphenylalanine (L-DOPA), norepinephrine, epinephrine, octopamine, dopamine, tyramine, serotonin, 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxyindoleacetic acid, 3-methoxytyramine, homovanillic acid, N-acetyldopamine, N-acetylserotonin, N-acetyloctopamine, and N-acetyltyramine. Borate complexation with vicinyl hydroxyl groups helped to resolve a larger number of analytes. This adds a net negative charge to the borate-analyte complex and alters the overall migration behavior of the analyte in the electric field and its interaction with the SDS. Borate forms complexes with nine analytes in the separations. Six compounds, dopamine, norepinephrine, epinephrine, N-acetyldopamine, L-DOPA, and DOPAC have been used previously, while three salsolinol, norsalsolinol, and 3,4-dihydroxymandelic acid, are new.

Previously, borate concentration, SDS concentration, and pH were investigated to optimize the separation conditions of this running buffer [26]. Borate concentration had little effect on resolution, while increased SDS concentration increased resolution. Altering the pH of the separation buffer caused the resolution, peak profiles, elution time reproducibility, and elution times to vary. To prevent upsetting the sensitive balance previously obtained, initial experiments carried out here used the same pH and increased the concentrations of the SDS in the running buffer attempting to fine tune the resolution of all twenty-three analytes. This was, however, not enough to resolve these analytes, particularly dopamine, salsolinol, and norsalsolinol, which elute first from the capillary. As previously discussed, salsolinol and norsalsolinol are structurally similar metabolites of dopamine (Figure 1). As salsolinol and norsalsolinol are metabolites of dopamine and they differ from one another by only a methyl group, it is not surprising that these analytes have similar mobilities. A more powerful

method of optimizing the separation conditions was investigated to resolve these three analytes.

As many of the biogenic amines of interest have primary amines, their pK_a 's are between 9 and 10; it was surmised that small changes in the running buffer pH of 9.50 would have dramatic effects on the separation selectivity and resolution of specific species. The constituents of the buffer made pH changes of 0.01 difficult to measure, hence, we used a method where the pH was back calculated from the total amount of base added to the buffer and optimized relative to the separation of these analytes. Separations of dopamine, salsolinol, norsalsolinol, N-acetyloctopamine, and octopamine were then performed with incremental amounts of OH^- added to the running buffer. The capillary was washed with 18 MΩ H_2O for 15 min between each run to ensure removal of the old running buffer. Representative electropherograms of these separations are shown in Figure 2.

The values for separation resolution for the DA-salsolinol and salsolinol-norsalsolinol pairs following different additions of OH^- (noted as calculated changes in pH) are shown in Figure 3A. Initially, the resolution of DA and salsolinol increased with increased pH going from an R of <0.5 to a maximum of 3.2 from pH 9.26 to 10.01. At even higher pH, the resolution then dropped dramatically, as all the available boric acid was converted to its singly deprotonated species. Conversely, salsolinol and norsalsolinol coeluted until pH 9.53. At higher pH, the resolution of these two species increased, peaked at $R = 1.2$, and dropped for the highest tested pH values. The separation resolution of N-acetyloctopamine with its two closest peaks, norsalsolinol and octopamine, were also calculated (Figure 3B). At the lowest and highest pH values tested, N-acetyloctopamine was highly resolved from octopamine ($R = 12-16$) but not as well resolved from norsalsolinol (< 2). As the pH shifted away from the most extreme values, the norsalsolinol-N-acetyloctopamine resolution increased and N-acetyloctopamine-octopamine resolution decreased. These resolution values reached both minimum and maximum values in the narrow range between pH 9.5 and 9.8 for the run buffer.

The pH = 9.66 value was chosen as optimal. Under this condition, we were able to resolve DA from salsolinol without being so close to the pH values where a very small change would lead to a large change in separation resolution. Similar logic applies to the ability to resolve salsolinol from norsalsolinol and N-acetyloctopamine from norsalsolinol and octopamine. This scheme produced a separation with sharper peak shapes and fewer impurity peaks, and helped to keep analysis times less than 30 min, all of which have impact on quantification.

3.2 Identification of Biogenic Amines and Metabolites

The optimized separation of twenty-three amines and metabolites is shown in Figure 4. Peak identities were elucidated by matching their elution times with that of each individual biogenic amine or metabolite measured under the same buffer conditions. The same capillary and electrode were used for all consecutive separations. Under these conditions, nine new analytes were separated and the elution order for the compounds examined previously [26] changed elution order. The elution position of two previously reported analytes, norepinephrine and homovanilic acid, changed elution order. Salsolinol and norsalsolinol eluted from the capillary second and third, between dopamine and epinephrine, followed by N-acetyloctopamine, octopamine, N-acetylserotonin, and norepinephrine. Although slight, the change in pH used was enough to move norepinephrine by two places in the elution order, whereas the newly added metabolite N-acetyltyramine eluted before N-acetyldopamine, 5-hydroxyindoleacetic acid, vanillylmandelic acid, and L-DOPA. The internal standard catechol switched positions with homovanilic acid from the original elution

order and these were followed by ascorbic acid, 3,4-dihydroxy mandelic acid, and p-hydroxymandelic acid, tyramine, 3-methoxytyramine, tyrosine, serotonin, and DOPAC.

The optimized separation conditions make possible the identification of previously unstudied compounds salsolinol, norsalsolinol, N-acetyltyramine, vanillylmandelic acid, 3,4-dihydroxy mandelic acid, p-hydroxymandelic acid, ascorbic acid, guanine, and tyrosine. Salsolinol and norsalsolinol are metabolites of dopamine associated with alcohol use and abuse, whereas N-acetyltyramine is the major metabolite of tyramine and a putative neuromodulator in the *Drosophila* central nervous system. Peaks corresponding to guanine (peak 19 in Figure 4), tyrosine (peak 22 in Figure 4), and DOPAC (peak 24 in Figure 4) are significantly smaller than those of the other analytes. The small peak size for guanine might result from poor solubility of this compound. Although guanine has been found to be soluble in acidic solutions [29] and standard solutions were made with 0.1M HClO₄, low solubility in the basic running buffer might result in stronger interactions with the SDS micelles. Similarly, the strongly polar amino acid group of tyrosine might lead to increased interactions with the SDS micelles and thus explain its low, broad peak. The peak for DOPAC is typically low in amplitude and broad in these separations as it elutes late and the highly anionic nature of the borate complex leads to poor electron transfer kinetics at carbon electrode such as those used here for detection. Even though some of these analytes have been studied using HPLC [20,22,23,30], these twenty-three biogenic amines have not been resolved in a single electrophoretic separation previously.

To demonstrate the quantitative ability of this method, calibration curves of standards (concentrations from 1 μ M to 40 μ M) were plotted for all twenty-three analytes (Table 1). Each of the sets of standards were run sequentially three times using the same electrode and capillary on the same day, but a fresh electrode, capillary, and running buffer were prepared for each new set of standards. The use of an internal standard catechol compensates for variation due to sample handling, injection efficiency, and fouling of the working electrode. The resulting calibration plots were linear with correlation coefficients of 0.97 or greater for fifteen of the analytes, including the first 14 compounds eluting from the column in the separation. Two more compounds, ascorbic acid and 3,4-dihydroxymandelic acid, had correlation coefficients just under 0.97 at 0.92 and 0.95, respectively. The last six compounds eluting from the capillary (guanine, tyramine, 3-methoxytyramine, tyrosine, serotonin, and DOPAC) had correlation coefficients less than 0.90. This is not entirely unexpected as the increased time in the capillary might indicate stronger interaction with the SDS micelles, thus creating additional band broadening.

3.3 Identification of Salsolinol and Norsalsolinol in Wild-Type *Drosophila* Head Homogenates

To demonstrate the applicability of using this method in bioanalysis, dopamine, salsolinol, norsalsolinol, N-acetyloctopamine, octopamine, and N-acetyldopamine have been separated in head homogenates of the wild-type *Drosophila* strain Canton-S have been separated and quantified. We used standard additions of 40, 20, 10, 5, 1 and 0 μ M of these substances. Previous reports utilized comparisons of standard separations run before and after the homogenate separation, but standard addition was chosen for this analysis to reduce any possible matrix effects and to aid in peak identification within the multitude of unidentified electroactive species present in a homogenate sample. To prevent dilution of the head homogenates as much as possible and ensure consistency between samples, head homogenates were split into 4.5 μ L aliquots and spiked with 0.5 μ L of standard spiking solution with concentration corresponding to the correct final concentration of standard in the sample. Although no statistical difference in basal monoamine levels has been observed in the fly heads between genders [25], gender differences to alcohol sensitivity have occurred. Therefore, only male flies were used in fly homogenates.

Figure 5A shows a sample electropherogram of a full separation of a *Drosophila* homogenate including the internal standard catechol. Homogenates were divided into six samples and spiked with standards. Enlargements of the first 400 s of a set of standard additions (Figure 5B) consistently identified peaks corresponding to dopamine, salsolinol, norsalsolinol, N-acetyloctopamine, octopamine, and N-acetyldopamine as the amount of the standard increase in the spiked samples. Mobilities from standard electropherograms were then compared to verify these analyte positions.

Standard additions were performed on three independent preparations of CantonS head homogenates that have been analyzed by using this optimized borate/SDS MEKC-EC method, and these values are reported in Table 2. As reported in previous studies [25,27], some variability was present and expected between fly cultures possibly resulting from subtle differences in the environment of the fly culture or variability in sample homogenization efficiency, and sample handling. As before, the internal standard helped to compensate for variation due to sample handling, injection efficiency, and fouling of the working electrode, but not differences in the environment. Only the analysis of N-acetyldopamine proved to be difficult to complete (correlation coefficients for the three fly cultures ranged between 0.70 to 0.83) because of its proximity to several large unidentified electroactive peaks. The other analytes, dopamine, salsolinol, norsalsolinol, N-acetyloctopamine, and octopamine were identified and quantified consistently in all three cultures with correlation coefficients of 0.95 or greater. We have thus used the optimized separation scheme to measure previously reported as well as novel analytes in the fly head.

4 Conclusions

We have developed an improved separation scheme for the analysis of biogenic amines and metabolites present in *Drosophila melanogaster* samples, which should also be applicable to other biological systems. The separation is extremely sensitive to small changes in pH, hence, optimization of the separation by careful addition of known amounts of OH^- proved more reliable than the more common adjustments using a pH meter. Under these conditions, twenty-four standards of potential interest are resolved. These include key players that might play a role in alcohol tolerance and reward, such as dopamine, salsolinol, norsalsolinol, N-acetyloctopamine, and octopamine. These five biogenic amines and putative neuromodulators were identified and quantified in the *Drosophila* head using this methodology.

Acknowledgments

The authors thank the National Institutes of Health (grant 5R01GM078385-02) and the Swedish National Science Foundation (VR) for support. A.G.E. is supported by a Marie Curie Chair from the European Union's sixth Framework.

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Figure 1.

Biosynthesis and structures of the dopamine derived isoquinolines, salsolinol and norsalsolinol. (A) One pathway through which salsolinol can be formed *in vivo* involves dopamine condensing with pyruvate, removal of the carboxylate group, and reduction. (B) Salsolinol and norsalsolinol can both be formed by a Pictet-Spengler reaction. Initially an alcohol, either methanol or ethanol, is oxidized by alcohol dehydrogenase (ADH) to its respective aldehyde. That aldehyde then reacts with dopamine to form norsalsolinol or salsolinol (B).

Figure 2.

Standard separations of 100 μ M (1) dopamine, (2) salsolinol, (3) norsalsolinol, (5) N-acetylocopamine, and (6) octopamine with increasing pH (added aliquots of $[\text{OH}^-]$) to optimize separation resolution between analytes. Amounts of $[\text{OH}^-]$ aliquots correspond to calculated pHs of (a) 9.26, (b) 9.30, (c) 9.51, (d) 9.61, (e) 9.70, (f) 10.15, and (g) 10.40. All separations were run sequentially on the same 45-cm piece of capillary using the same electrode at 15 kV with a 15-min wash of deionized water between buffer changes.

Figure 3.

Resolution plots for the peaks from Figure 2. (A) Resolution for dopamine-salsolinol (circles) and salsolinol-norsalsolinol (diamonds) versus the calculated pH of the OH^- aliquots added. Values generally increased with increasing pH until a pH of 10.01 where the resolution dropped sharply. (B) Resolution calculations for N-acetyloctopamine and its two closest peaks, norsalsolinol (diamonds) and octopamine (circles), versus the calculated pH for OH^- aliquots added.

Figure 4.

Optimized separation of 100 μM standards for all 23 biogenic amines and metabolites using a buffer at pH 9.66 (corresponding to addition of 28.4 μM OH^-). Peak identities are: (1) dopamine, (2) salsolinol, (3) norsalsolinol, (4) epinephrine, (5) N-acetyloctopamine, (6) octopamine, (7) N-acetylserotonin, (8) norepinephrine, (9) N-acetyltyramine, (10) N-acetyldopamine, (11) 5-hydroxyindoleacetic acid, (12) vanillylmandelic acid, (13) catechol, (14) l-DOPA, (15) homovanilic acid, (16) ascorbic acid, (17) 3,4-dihydroxy mandelic acid, (18) p-hydroxymandelic acid, (19) guanine, (20) tyramine, (21) 3-methoxytyramine, (22) tyrosine, (23) serotonin, and (24) DOPAC. The separation was carried out in a 45-cm-long, 14-i.d. capillary at 15 kV, and the working electrode was held at +750 mV vs a Ag/AgCl reference electrode.

Figure 5.

(A) MEKC-EC separation of a *Drosophila* male head homogenate highlighting the internal standard catechol (peak 13) and breadth of the separation. The five large peaks (signals greater than 100 pA) were unidentified. The peaks for all identified biogenic amines and metabolites were closer to the baseline and represented perhaps medium level substances. (B) Enlargements of electropherograms examining times from 400–800 s of the separation shown in (A) with standard additions of the known substances. Final concentrations of spiking standard solutions were (a) 0.5 μ M, (b) 1 μ M, (c) 5 μ M, (d) 10 μ M, (e) 20 μ M, and (f) 40 μ M for the six analytes. Peaks correspond to the numbers used in Figure 4.

Table 1

Regression parameters for three independent calibrations of twenty-three biogenic amines and metabolites in the optimized borate/SDS buffer system. For intercepts where the variance was larger than the value calculated, values are reported as zero.

Analyte	Slope $\pm$ Standard Deviation	Intercept $\pm$ Standard Deviation	Correlation Coefficient (r)
Dopamine	2.07 ± 0.094	$-1\text{E-}05 \pm 1.5\text{E-}05$	0.98
Salsolinol	1.08 ± 0.067	$0 \pm 1.1\text{E-}05$	0.97
Norsalsolinol	1.7 ± 0.11	$0 \pm 1.7\text{E-}05$	0.97
Epinephrine	2.0 ± 0.11	$-2\text{E-}05 \pm 1.7\text{E-}05$	0.98
N-acetyloctopamine	0.81 ± 0.042	$-9\text{E-}06 \pm 6.8\text{E-}06$	0.98
Octopamine	1.47 ± 0.061	$6\text{E-}06 \pm 9.9\text{E-}06$	0.99
N-acetylserotonin	1.76 ± 0.078	$-4\text{E-}05 \pm 1.3\text{E-}05$	0.99
Norepinephrine	2.24 ± 0.077	$-1\text{E-}05 \pm 1.3\text{E-}05$	0.99
N-acetyltyramine	0.77 ± 0.035	$6\text{E-}06 \pm 5.8\text{E-}06$	0.98
N-acetyldopamine	0.70 ± 0.026	$0 \pm 4.2\text{E-}06$	0.99
5-hydroxyindole acetic acid	0.61 ± 0.038	$0 \pm 6.2\text{E-}06$	0.97
Vanillylmandelic acid	1.17 ± 0.056	$0 \pm 9.2\text{E-}06$	0.98
L-DOPA	2.2 ± 0.11	$-2\text{E-}05 \pm 1.7\text{E-}05$	0.98
Homovanillic acid	0.67 ± 0.035	$3\text{E-}06 \pm 5.8\text{E-}06$	0.98
Ascorbic Acid	0.54 ± 0.057	$1.6\text{E-}05 \pm 9.3\text{E-}06$	0.92
3,4-dihydroxymandelic acid	0.50 ± 0.041	$6\text{E-}06 \pm 6.6\text{E-}06$	0.95
p-Hydroxymandelic acid	1.84 ± 0.084	$0 \pm 1.4\text{E-}05$	0.98
Guanine	0.33 ± 0.056	$1.1\text{E-}05 \pm 9.1\text{E-}06$	0.82
Tyramine	2.0 ± 0.43	$3\text{E-}05 \pm 7.0\text{E-}05$	0.75
3-methoxytyramine	0.21 ± 0.030	$1.0\text{E-}05 \pm 4.9\text{E-}06$	0.87
Tyrosine	0.11 ± 0.055	$0 \pm 1.3\text{E-}05$	0.65
Serotonin	0.20 ± 0.036	$1.1\text{E-}05 \pm 5.9\text{E-}06$	0.81
DOPAC	0.07 ± 0.035	$4\text{E-}06 \pm 3.5\text{E-}06$	0.55

Quantification of dopamine, salsolinol, norsalsolinol, N-acetylsalsolinol, octopamine, and N-acetyloctopamine using standard addition of three separate wild-type *Drosophila* head homogenates. Values reported for each analyte include; the calculated concentration of the analyte (C), correlation coefficient of the standard addition regression (r), and mean and standard error of the mean (SEM) for the three fly cultures.

Table 2

Fly culture	Dopamine		Salsolinol		Norsalsolinol		N-acetyl-octopamine		Octopamine		N-acetyl-dopamine	
	C(μ M)	r	C(μ M)	r	C(μ M)	r	C(μ M)	r	C(μ M)	r	C(μ M)	r
1	1.8	0.99	2.08	0.99	2.0	0.99	5.3	0.99	12.6	0.99	16.5	0.70
2	1.5	0.99	2.21	0.99	2.3	0.98	4.8	0.99	10.9	0.99	18.1	0.73
3	1.1	0.99	2.23	0.98	0.8	0.98	4.2	0.97	9.2	0.96	19.8	0.84
Mean $\pm$ S.E.M.	1.5 $\pm$ 0.21		2.17 $\pm$ 0.048		1.7 $\pm$ 0.44		4.8 $\pm$ 0.32		10.9 $\pm$ 0.97		18.1 $\pm$ 0.95	