DS5
Standard COMPOUND TABLE **NG/ML(10-3)**

		channel	RT	**ul in 1L**
ASC	Ascorbic Acid	3&4	7.2	**15000**
URIC	Uric acid	4	11.6	**10000**
CYS	Cysteine	14	11.7	**400**
XAN	Xanthine	11 or 12	14.4	**200**
HX	Hypoxathine	16	17.0	**400**
VMA	Vanillylmandelic Acid	7	18.9	**100**
GSH	Glutathione(reduced)	13-16	22.4	**500**
Xanth	Xanthosine	14	25.9	**200**
MHPG	Methoxy-Hydroxyphenly Glycol	7	24.6	**30**
HGA	Homogentisic Acid	1	26.7	**30**
7MXAN	7-Methylxanthine	15-16	27.1	**150**
MET	Methionine	15	31.1	**500**
NE	Norepinephrine	3	29.9	**30**
GR	Guanosine	13/14	28.8	**200**
LD	L-Dopa	3	30.4	**50**
GSSG	Glutathione(oxidized)	16	27.9	**400**
G	Guanine	10 or 11	31.8	**250**
3OHKY	3-Hydroxykynurenine	3	35.0	**30**
EPI	Epinephrine	3	34.5	**50**
TYR	Tyrosine	12	35.6	**5000**
DOPAC	Dihydroxyphenylacetic Acid	2 or 3	37.9	**60**
4HPLA	4-Hydroxyphenyllactic acid	10	38.9	**400**
3OHAN	3-Hydroxyanthranilic Acid	3	41.1	**30**
paraxanthine	1,7-diMethylxanthine	14-16	39.8	**100**
5HTP	5-Hydroxytrptophan	4	41.9	**50**

theophylline	1,3-diMethylxanthine	14-16	41.7 **150**
4HBAC	4-Hydroxybenzoic acid	13	44.9 **200**
3OMD	3-o-Methyldopa	7	40.2 **50**
5HIAA	5-Hydroxyindoleacetic acid	4	45.2 **60**
KYN	Kynureinine	13	45.9 **400**
NMN	Normetanephrine	7	42.7 **50**
DA	Dopamine	3	46.3 **50**
MN	Metanephrin	7	46.1 **50**
NA5HT	Acetylserotonin (N)	3	47.1 **50**
HVA	Homovanillic Acid	7	52.7 **60**
4HPAC	4-Hydroxyphenylacetic acid	10	48.5 **200**
TYRA	Tryamine	11	52.3 **1000**
2HPAC	2-Hydroxyphenylacetic Acicd	12	55.7 **200**
5HT	5-Serotonin	4	54.9 **50**
3MT	3-Methoxytyramine	7	55.1 **30**
NM5HT	Methyserotonin (N)	4	55.7 **50**
TRP	Tryptophan	12	56.2 **5000**
MEL	Melatonin	10	65.8 **300**
TPOL	Tryptophol	12	67.9 **200**
I3AA	Indole-3-acetic acid	9	68.2 **300**
I3PA	Indole-3-propionic acid	9	71.8 **200**
d toco	(+)-γ–Tocopherol		101 **150**
g toco	(+)-δ-Tocopherol		102.9 **200**
a toco	(±)-α–Tocopherol		104.5 **250**

The oxidation potential of each metabolite relates to the observed channels, which are held between 0 and 900 mV at 60 mV increments.

LC-EC Profiling Method[12]:

Separation was performed by gradient elution on two reversed-phase C_{18} META 250 x 4.6, 5µm columns (Thermo Scientific) connected in tandem with PEEK tubing. The column temperature was held at 32 ^{o}C and the conditions were as follows:

1. 1.00 min 0%B at 1.0 ml/min : start file
2. 30.00 min 12%B at 1.0 ml/min
3. 35.00 min 35%B at 1.0 ml/min
4. 55.00 min 48%B at 0.7 ml/min
5. 90.00 min 100%B at 0.99 ml/min
6. 95.00 min 100%B at 1.2 ml/min
7. 101.00 min 100%B at 1.2 ml/min
8. 101.10 min 0%B at 1.2 ml/min
9. 103.00 min 0%B at 1.2 ml/min
10. 105.00 min 0%B at 1.00 ml/min
11. 112.00 min 0%B at 1.00 ml/min : end file

The mobile phase A consisted of 100% water modified with 60 mM PSA, 0.1% methanol, and 1 mg/L citric acid adjusted to pH 3.1 with acetic acid. Mobile phase B consisted of 80:10:10 MeOH: ACN: IPA modified with 40 mM lithium acetate and 2.0% acetic acid in 10 mg/L citric acid. 50 µL of sample was injected per run and detection was performed on an ESA (ThermoFisher Scientific, Chelmsford, MA) LC-EC system with 16-channel coulometric array detector operated with potentials incremented in 60 mV steps (0–900 mV). All LC-EC system functions were controlled by CoulArray® software (CoulArray® for Windows® software version 3.10)

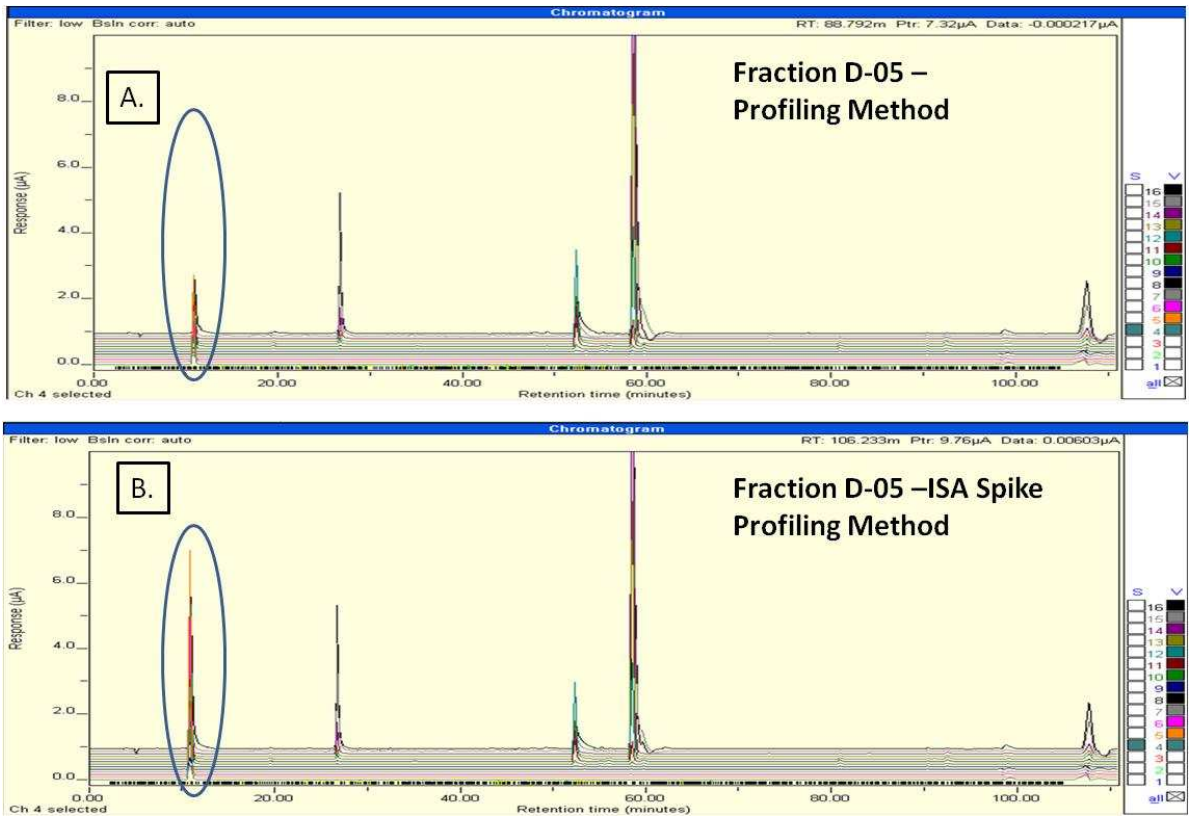
Fraction Analysis by LC-MS and LC-EC:

For the LC-MS and LC-EC analysis, the POOL fractions were reconstituted in either 100 or 50 µL of LC-MS mobile phase A (25 mM ammonium acetate in deionized water using a Millipore

water filtration system (Billerica, MA), adjusted to pH 3.1 using acetic acid) and separation was performed using two Shiseido C-18 columns (4.6 x 150 mm) connected in tandem and maintained at room temperature (or approximately 23 ^{0} C, without control). The gradient was as follows:

1. 1.00 min 0%B at 0.5 ml/min : start file
2. 08.00 min 20%B at 0.5 ml/min
3. 20.00 min 55%B at 0.5 ml/min
4. 35.00 min 48%B at 0.5 ml/min
5. 55.00 min 95%B at 0.5 ml/min
6. 65.00 min 95%B at 0.6 ml/min
7. 70.00 min 95%B at 0.6 ml/min
8. 80.00 min 0%B at 0.6 ml/min
9. 85.00 min 0%B at 0.5 ml/min
10. 120.00 min 0%B at 0.5 ml/min: end file

Supplementary Figure 1.



Supplementary Figure S1 Fraction D05 compared to the Fraction spiked with the ISA standard using LC-EC profiling. Panel A represents the fraction, D05, run using LC-EC profiling method with Panel B being the same fraction, spiked with the ISA internal standard. The circled peak is the ISA peak, which increases in height after the spike.

It should be noted that the ISA peak of interest, which is circled and increases in the spiked panel B chromatogram, has shifted in relation to the LC-EC chromatogram run using the LC-MS conditions (Figure 7, panel A). When run using the LC-MS conditions, ISA eluted at ~ 50.77 minutes, where as now it is coming out at 10.90 minutes. This change in retention is an aspect of the ion-pairing reagent used during fraction collection, TEA, being a cationic reagent which pairs strongly with the anion ISA compound. In the LC-EC profiling method, the ion-pairing reagent PSA, an anionic compound, is present at a high concentration in the mobile phase, displacing the ion-pair interaction, and preventing retention enhancement.