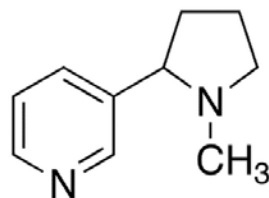


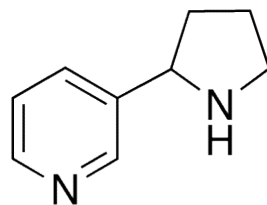
# Extraction efficiency of selected alkaloids from tobacco products

**Alexandra Martin  
Fraser Williamson**

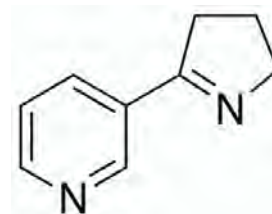
# Nicotine and Minor Alkaloids of Interest



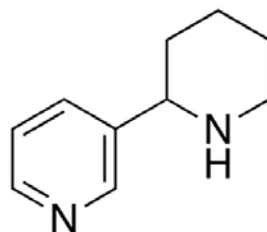
Nicotine



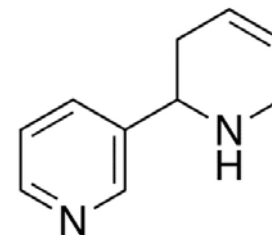
Nornicotine



Myosmine



Anabasine

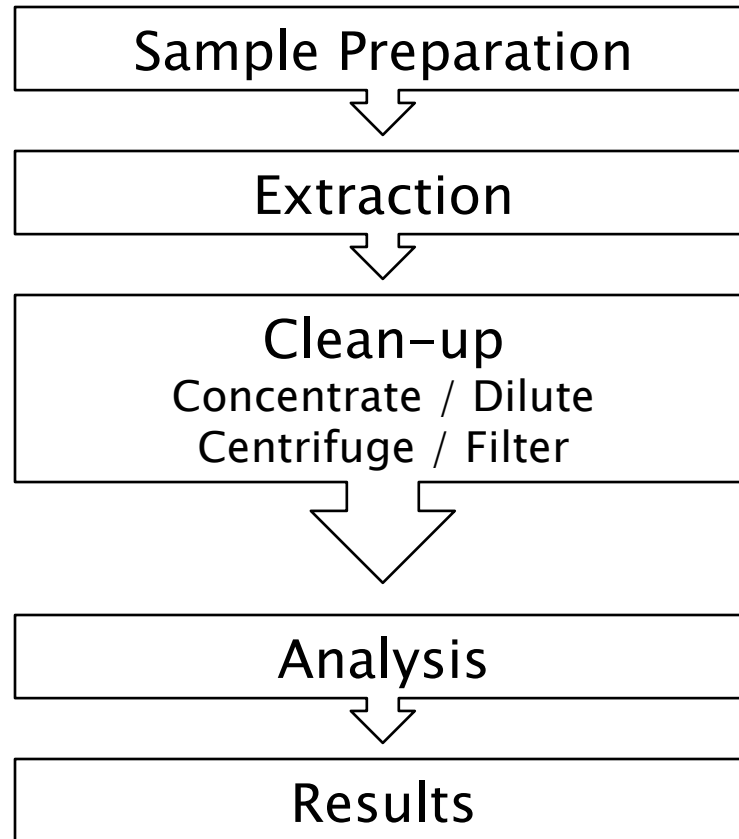


Anatabine

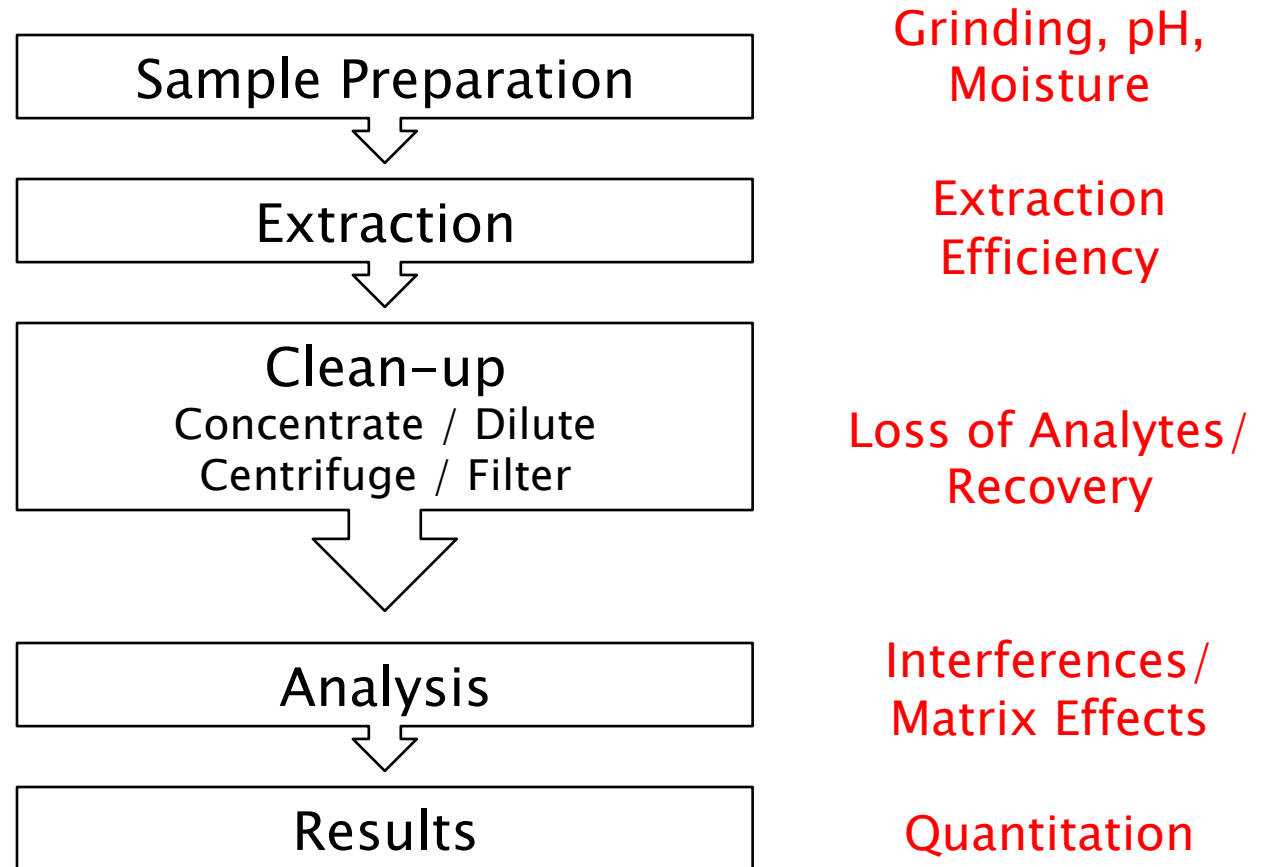
# Objectives

- ▶ To develop a method that meets the following criteria:
  - Capable of fully extracting nicotine and the four minor alkaloids from the matrix
  - Is applicable to a wide range of product and levels, with negligible interferences and without the use of standard additions

# Generalized Analytical Procedure



# Generalized Analytical Procedure



# Methodology

- ▶ **PMUSA Method**

Determination of tobacco alkaloids by GC with NPD, S.S. Yang, I. Smetena and C.B. Huang, *Anal. Bioanal. Chem.* (2002) 373:839–843.

- ▶ **CORESTA Recommended Method N° 62**

Determination of nicotine in tobacco and tobacco products by gas chromatographic analysis (*February 2005*).

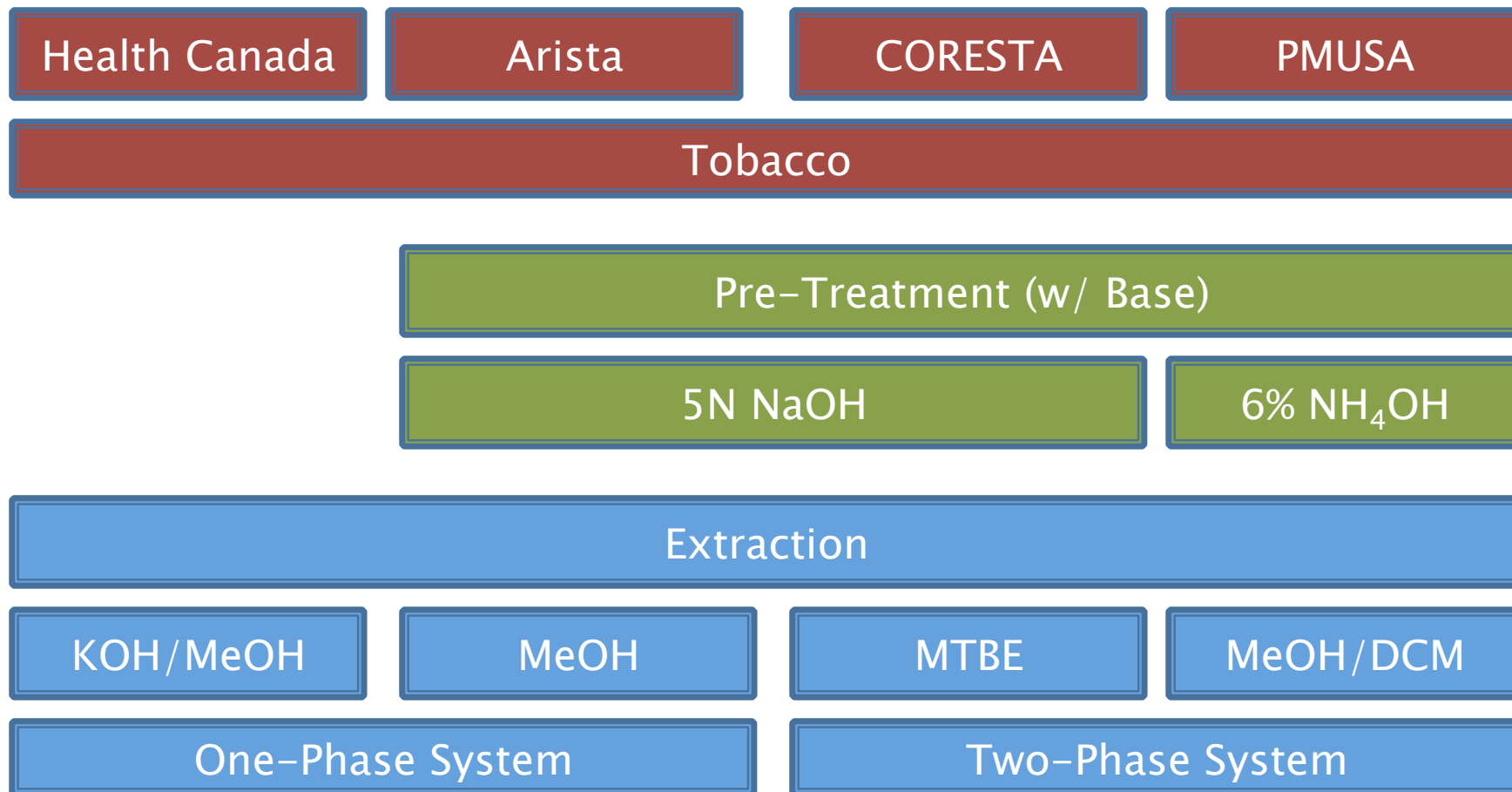
- ▶ **Health Canada Official Method T-301**

Determination of Alkaloids in Whole Tobacco, prepared by the Department of Health dated December 31, 1999.

# Comparison of methods

|                               | <i>CORESTA</i>                  | <i>Health Canada</i> | <i>PMUSA</i>               | <i>Arista</i>                   |
|-------------------------------|---------------------------------|----------------------|----------------------------|---------------------------------|
| <b>Analyte</b>                | Nic only                        | Nic + MA             | Nic + MA                   | Nic + MA                        |
| <b>Tobacco Weight</b>         | 1.0 g                           | 0.025 g              | 0.150 g                    | 0.25 g                          |
| <b>Pre-treatment Solution</b> | 5N NaOH (aq); 15 min            | na                   | 6% NH <sub>4</sub> OH (aq) | 5N NaOH (aq); 30 min            |
| <b>Volume</b>                 | 5 mL                            | na                   | 0.3 mL                     | 4 mL                            |
| <b>Extraction Solvent</b>     | MtBE                            | 50 mM KOH in MeOH    | MeOH:DCM (1:3)             | MeOH                            |
| <b>Volume</b>                 | 50 mL                           | 1 mL                 | 11.7 mL                    | 40 mL                           |
| <b>Extraction Mechanism</b>   | mechanical shaking – horizontal | ultrasonic bath      | shaking                    | mechanical shaking – horizontal |
| <b>Extraction Duration</b>    | 120 min                         | 180 min              | 90 min                     | 30 min                          |
| <b>Filtering</b>              | allow to separate               | centrifuge           | filter (0.45 µm)           | centrifuge                      |
| <b>ISTD</b>                   | quinoline                       | 2,4'-dipyridyl       | 2,4'-dipyridyl             | quinoline and 7-methylquinoline |
| <b>Instrument</b>             | GC-FID                          | GC-TSD               | GC-NPD                     | GC-MS                           |
| <b>Liner</b>                  | Liner with glass wool           | split                | Carbowax-KOH on Chromasorb | Liner with glass wool           |
| <b>Column</b>                 | DB-5                            | CAM                  | DB-1701                    | CAM                             |

# Comparison of Methods – Extraction





# GC/MS Parameters

|                            |   |
|----------------------------|---|
| Analytical Column          | Agilent J&W CAM (30m x 0.25mm id x 0.25µm df) |
| Injection Port Temperature | 230°C   |
| Injection Port Liner       | Agilent Ultra Inert Split, Straight with Wool |
| Injection Volume           | 1 µL  |
| Injection Mode             | Split, 60:1                                   |
| Initial Oven temperature   | 110 °C hold for 1 minute                      |
| Temperature ramp           | 10 °C/min to 235°C, hold 4.5 minutes or more  |
| Transfer Line temperature  | 230°C   |
| MS Source temperature      | 230°C   |
| MS Quad temperature        | 150°C   |

# Masses and Retention Times

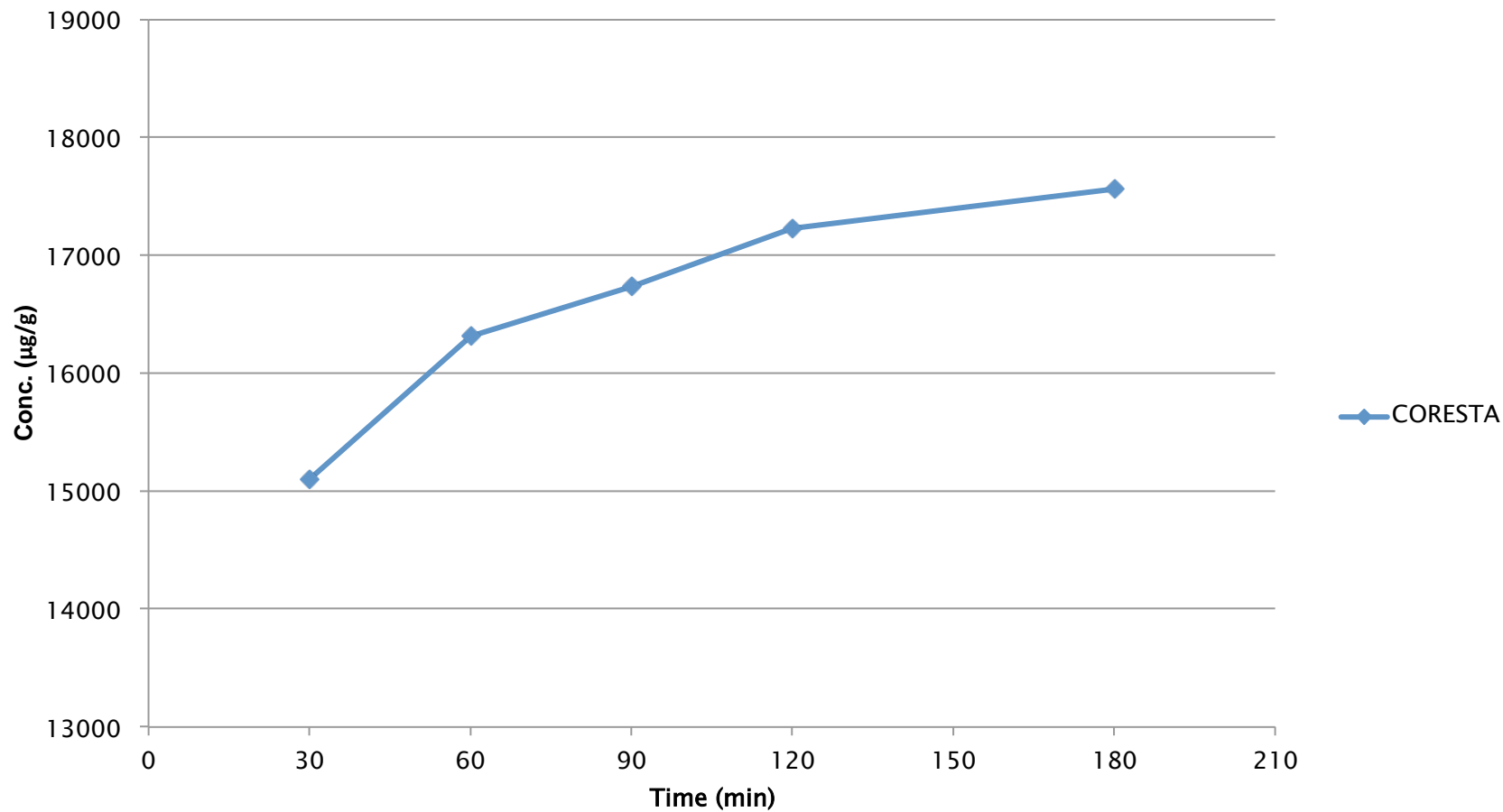
| Analyte           | MW    | Ret Time (min) | Quant Ion (m/z) | Qual Ion (m/z) | ISTD              |
|-------------------|-------|----------------|-----------------|----------------|-------------------|
| Quinoline         | 129.2 | 8.9            | 129             | N/A            | N/A               |
| Nicotine          | 162.2 | 8.1            | 84              | 162            | Quinoline         |
| 7-methylquinoline | 143.2 | 10.1           | 143             | N/A            | N/A               |
| Nornicotine       | 148.2 | 11.0           | 148             | 119            | 7-methylquinoline |
| Anabasine         | 162.2 | 11.3           | 162             | 119            | 7-methylquinoline |
| Myosmine          | 146.2 | 11.3           | 146             | 118            | 7-methylquinoline |
| Anatabine         | 160.2 | 12.7           | 160             | 118            | 7-methylquinoline |

# Control of Experimental Variability

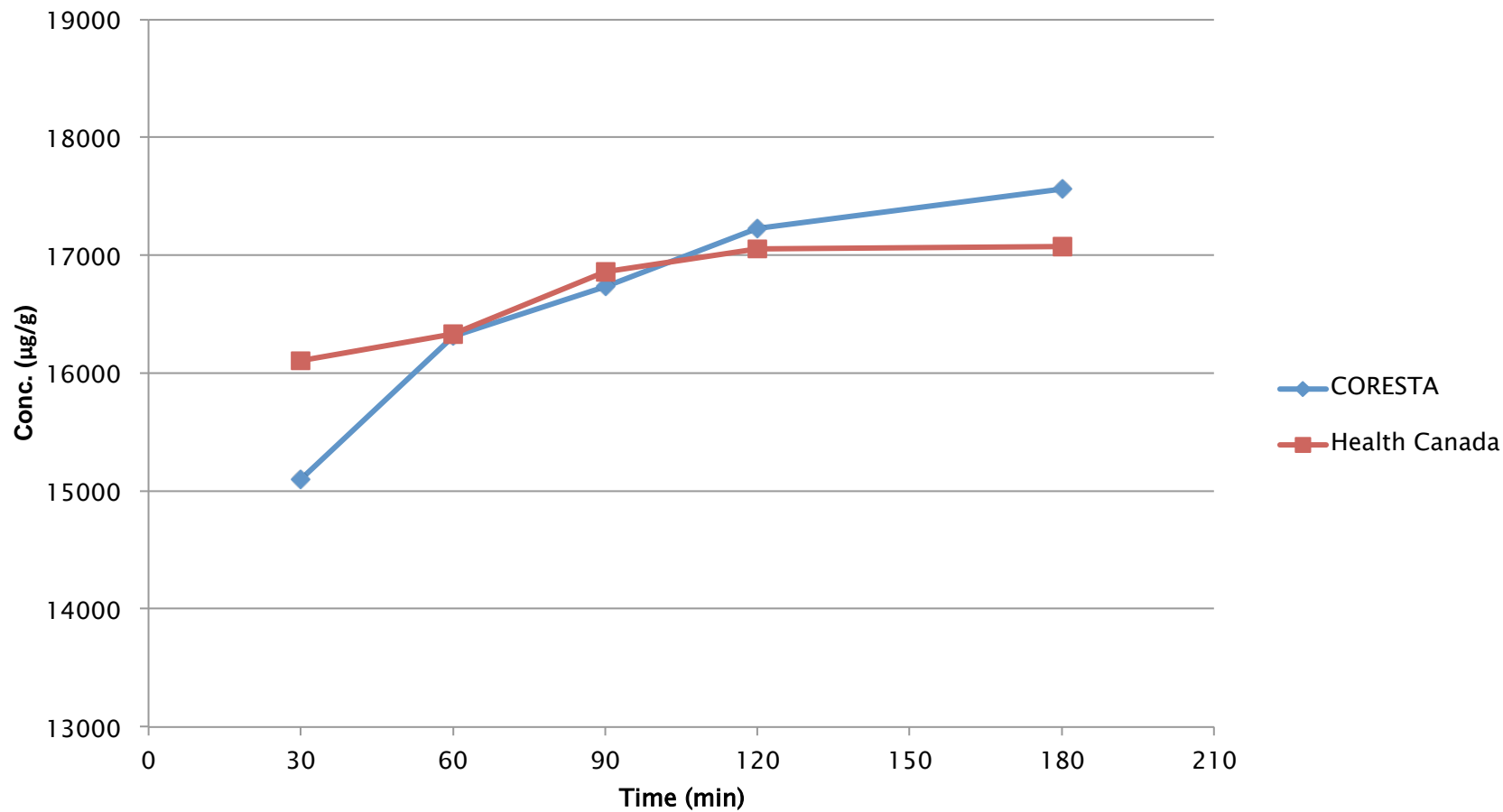
- ▶ Same stock solutions and internal standards
- ▶ Same GCMS, column, liner, temperature
- ▶ Adjusted sample weights to same order of magnitude for all methods
- ▶ Same lots of CRP2 and ground 3R4F
- ▶ Independent samples (5) at each time point
- ▶ All times 30, 60, 90, 120 and 180 minutes
- ▶ All results on an “as received” basis

# Results

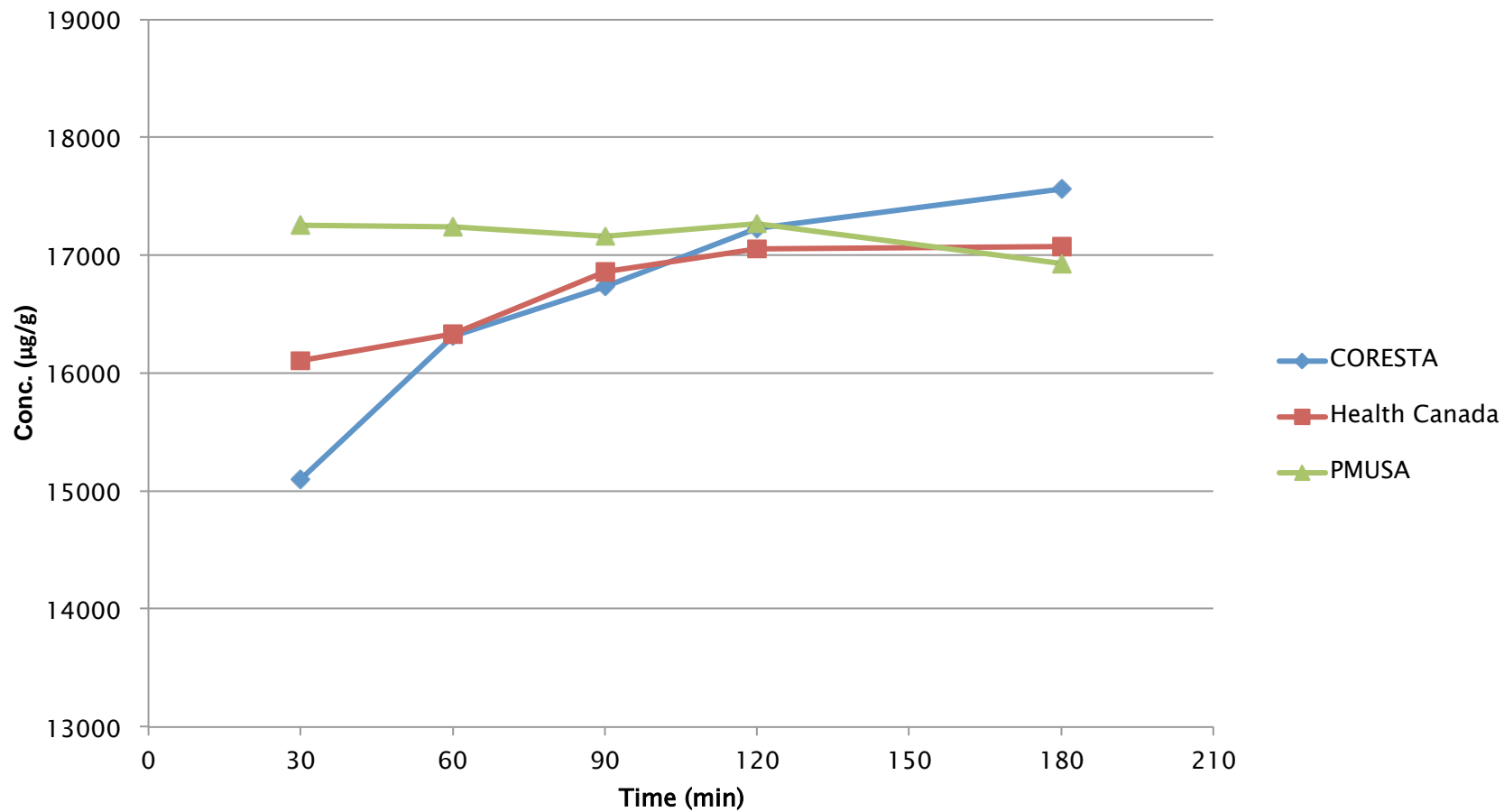
# Nicotine - 3R4F



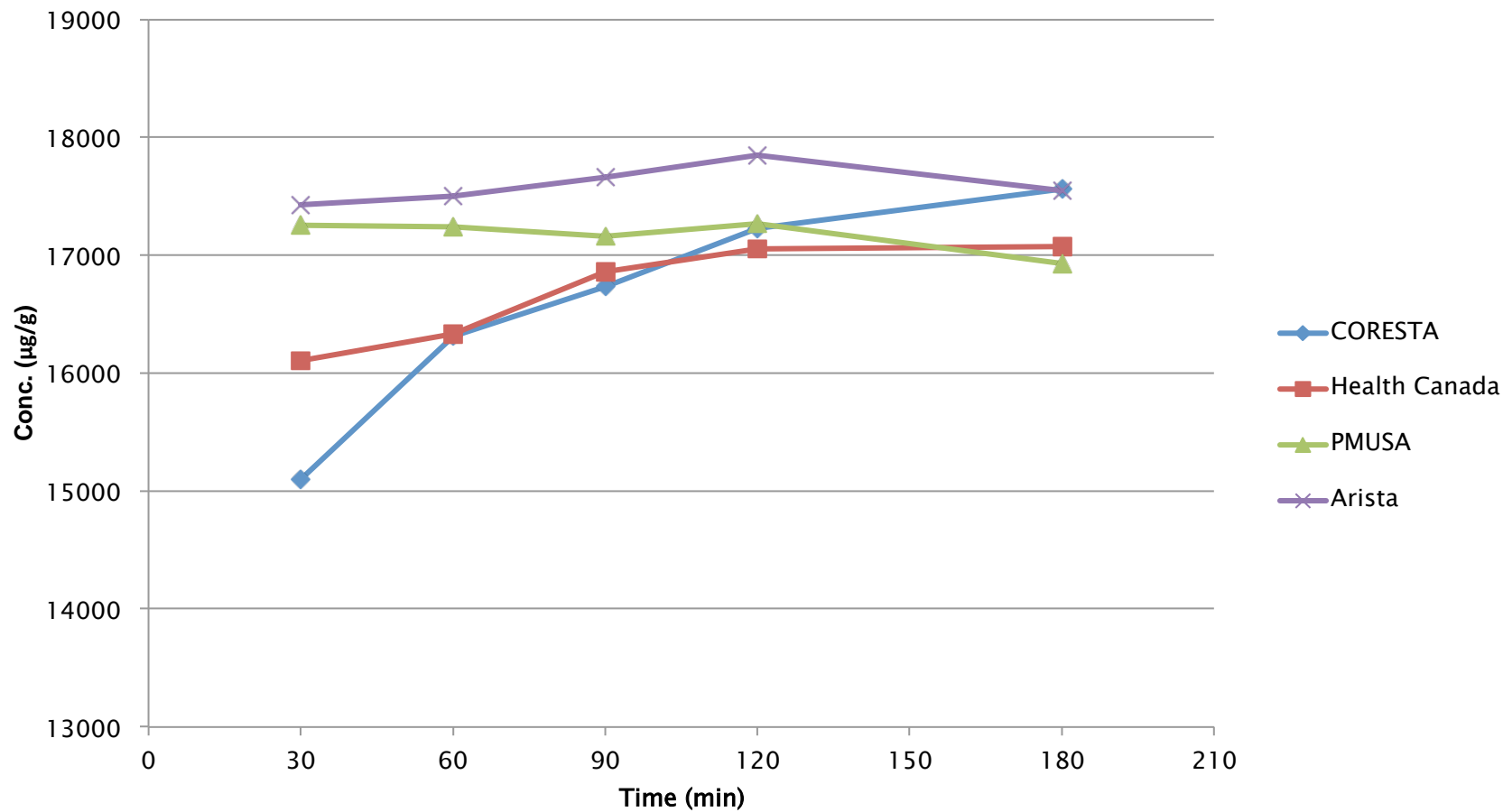
# Nicotine - 3R4F



# Nicotine - 3R4F



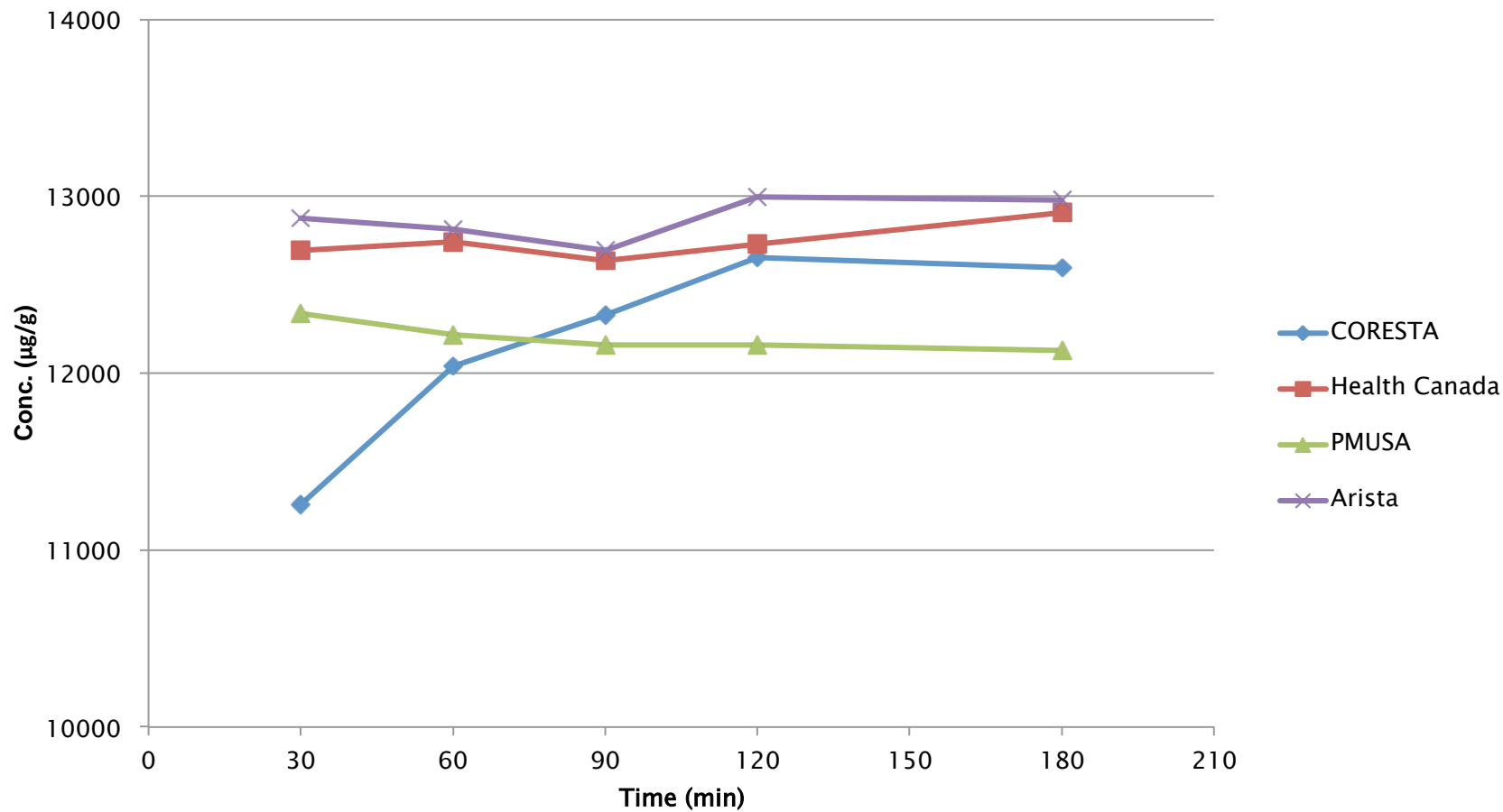
# Nicotine - 3R4F



Arista Laboratories, Inc.

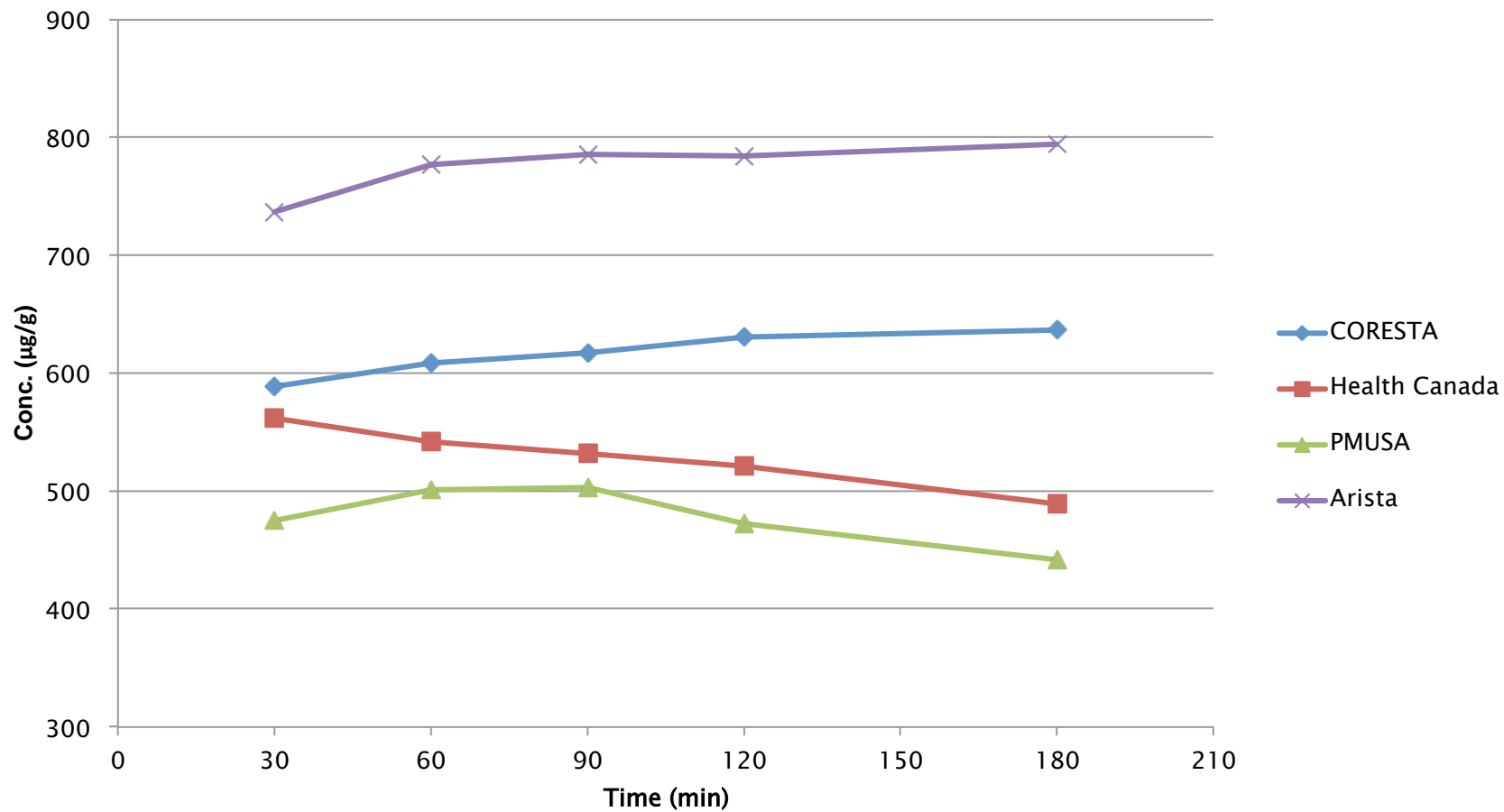


# Nicotine - CRP2



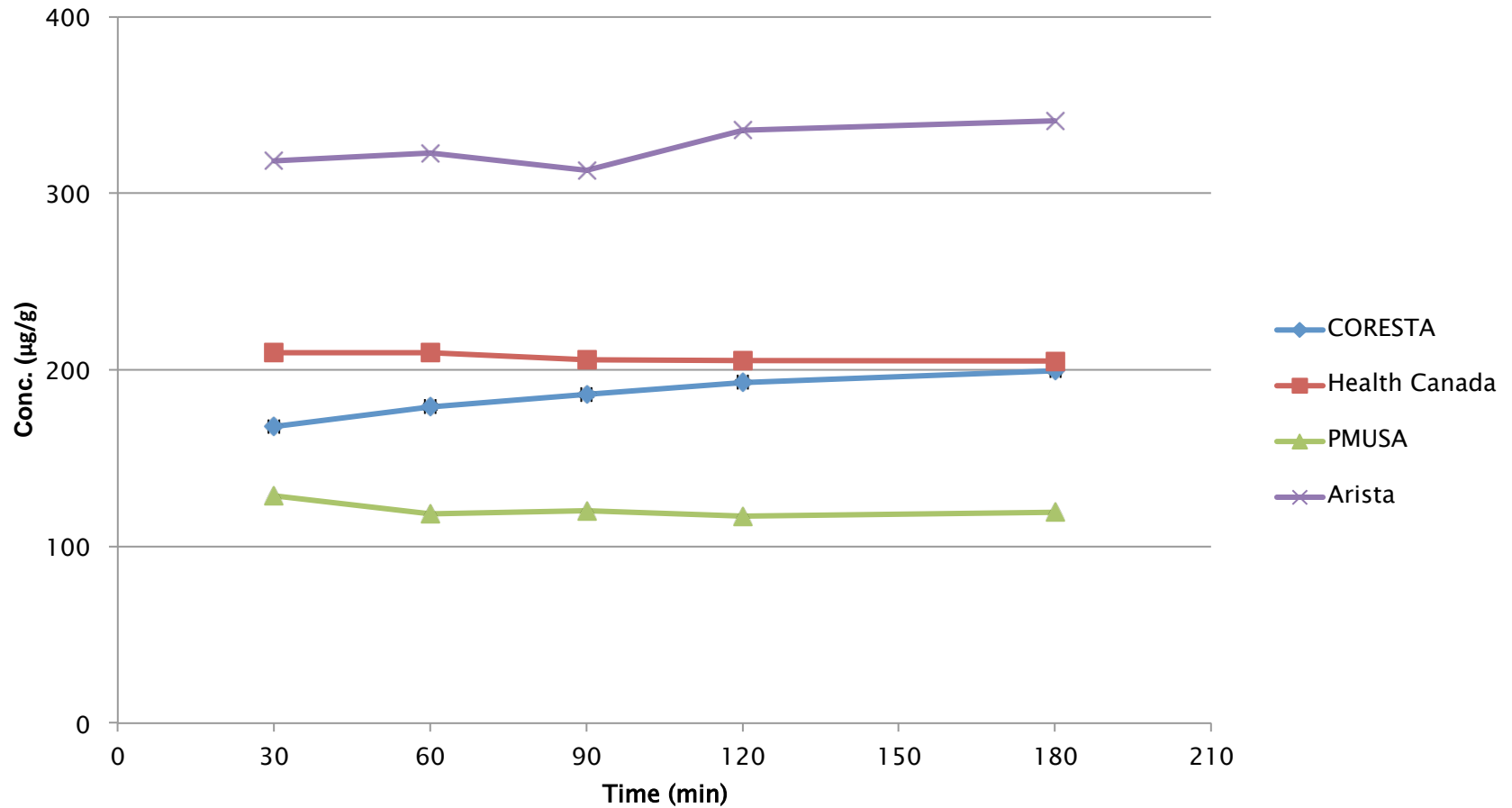
Arista Laboratories, Inc.

# Nornicotine - 3R4F



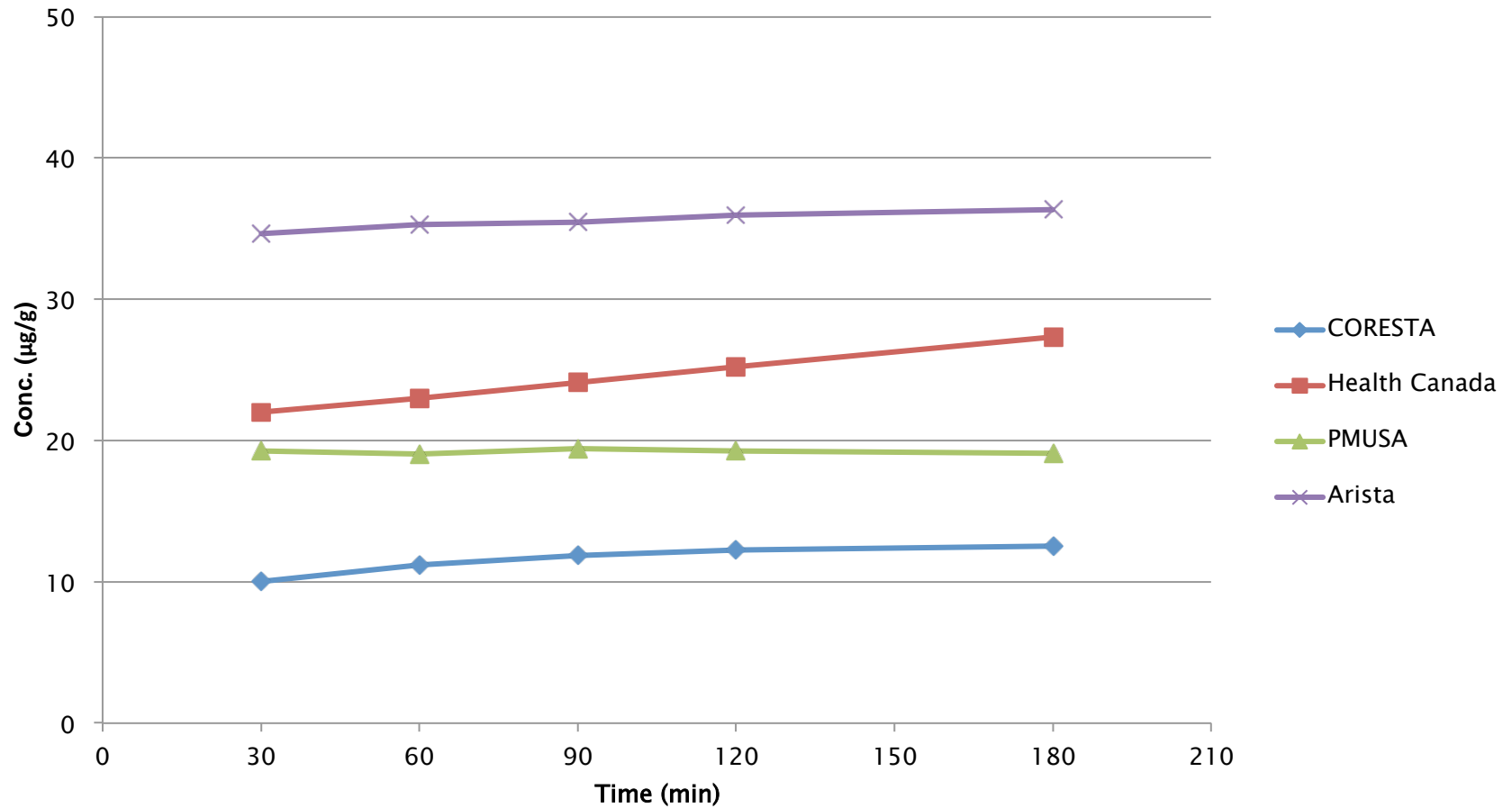
Arista Laboratories, Inc.

# Nornicotine - CRP2



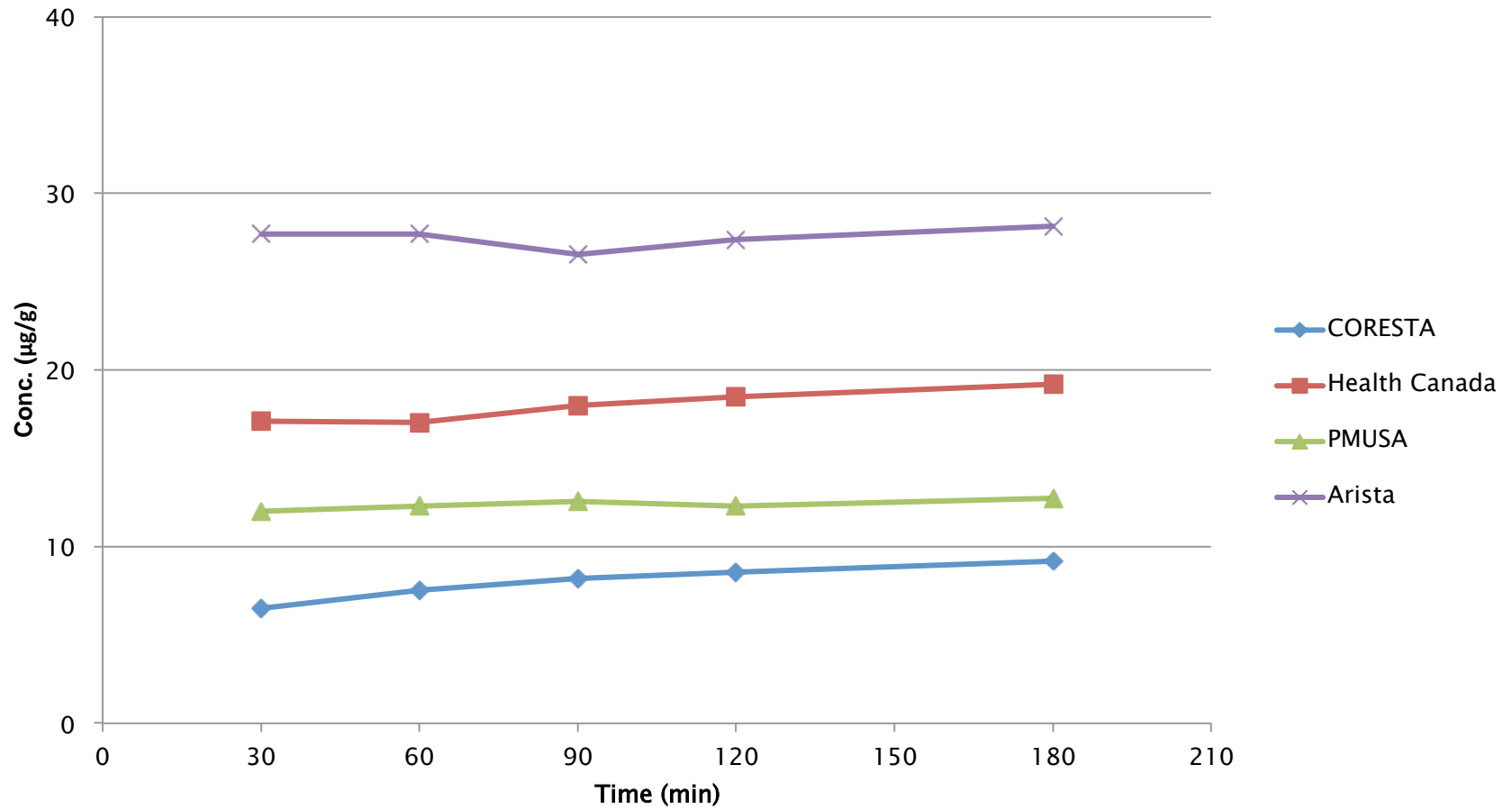
Arista Laboratories, Inc.

# Myosmine - 3R4F



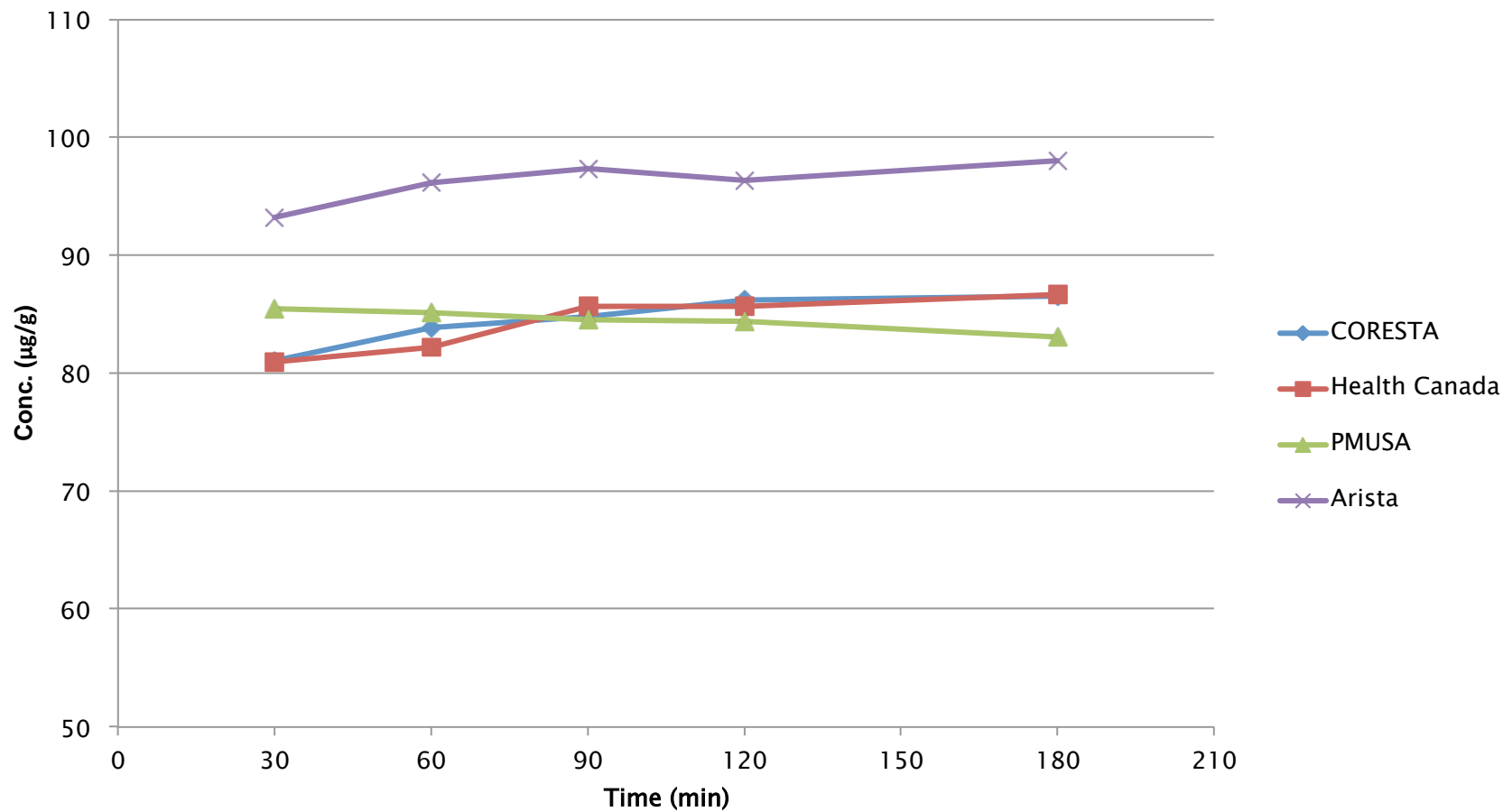
Arista Laboratories, Inc.

# Myosmine - CRP2



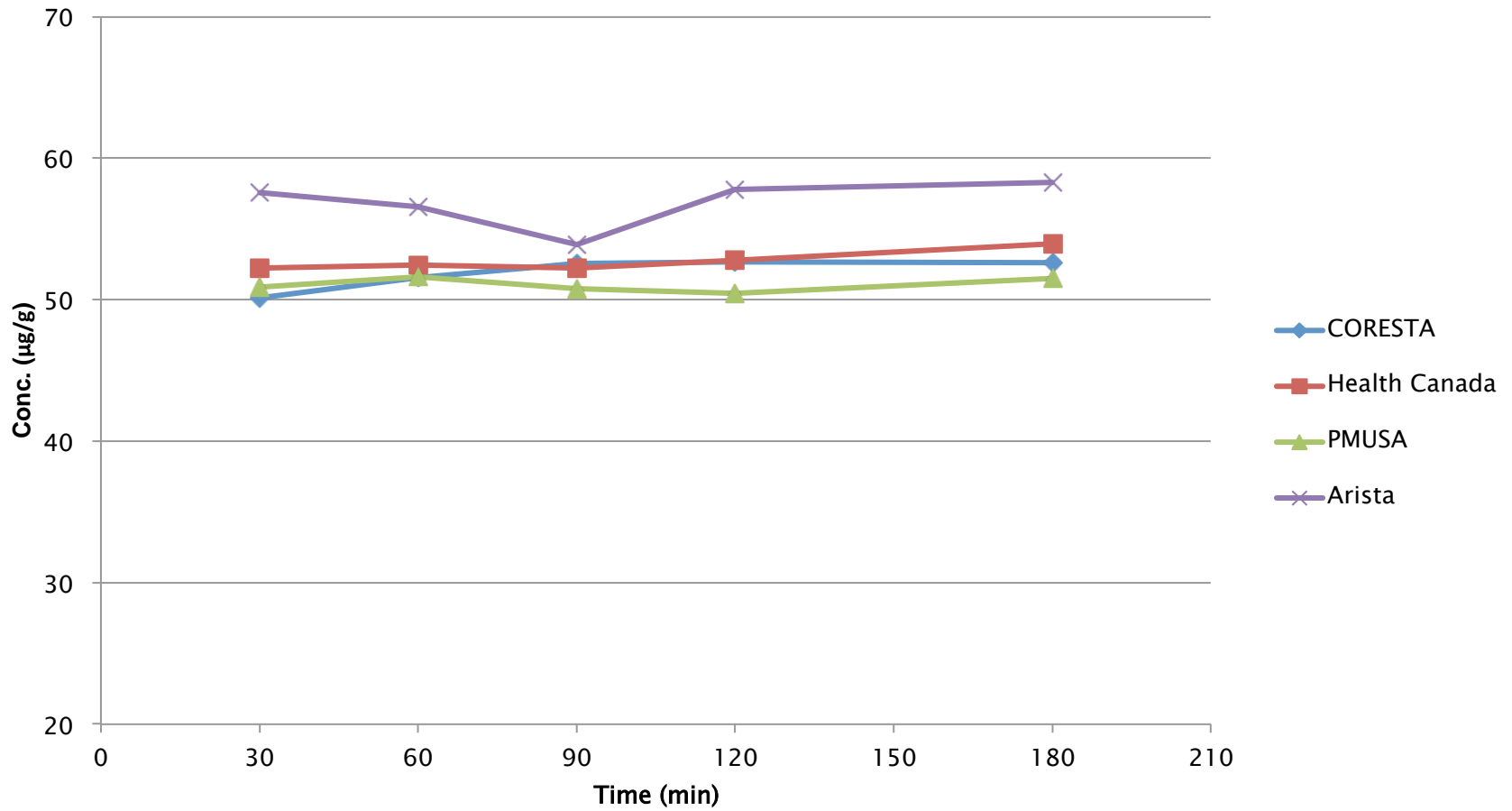
Arista Laboratories, Inc.

# Anabasine - 3R4F



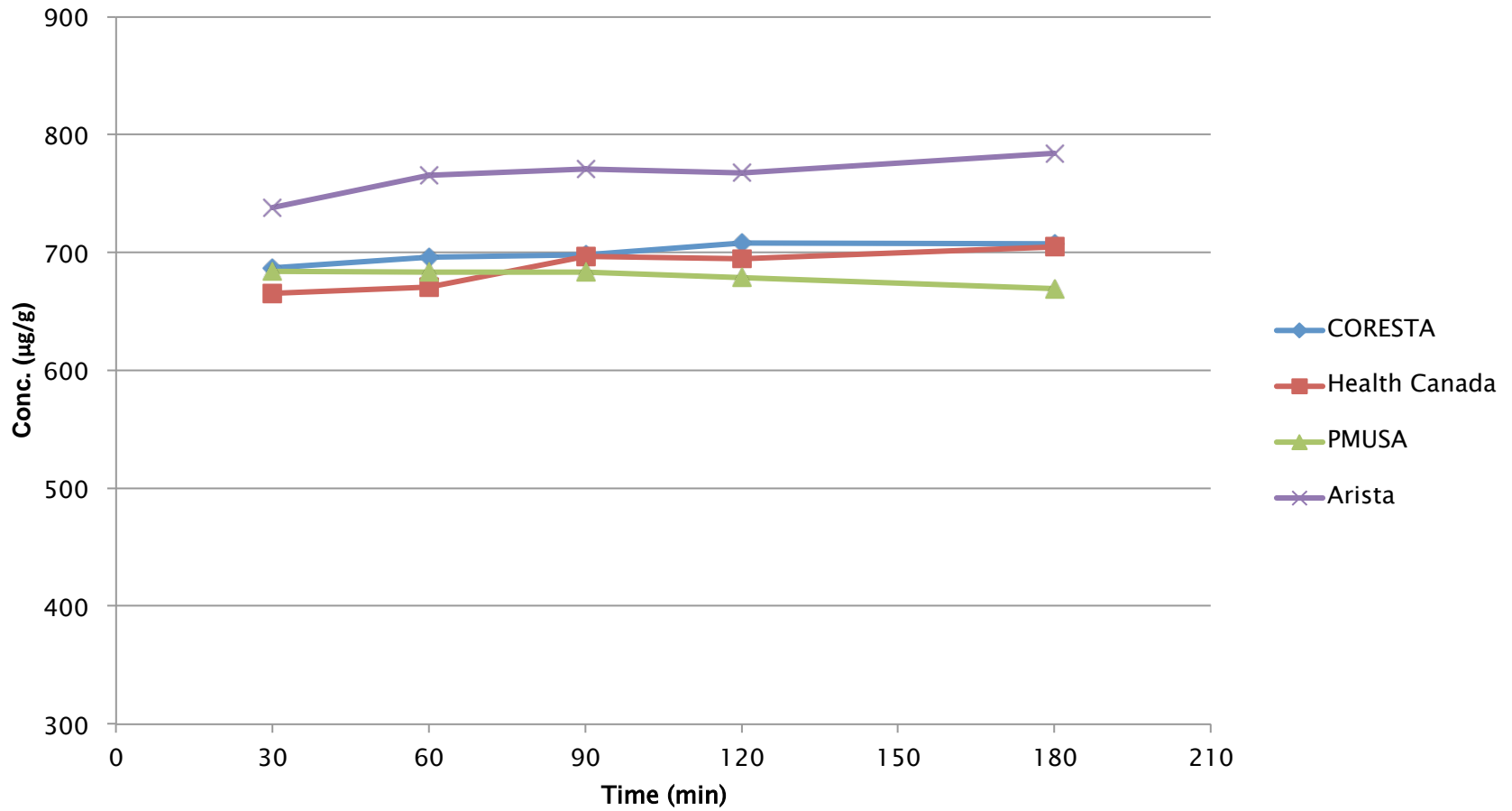
Arista Laboratories, Inc.

# Anabasine – CRP2



Arista Laboratories, Inc.

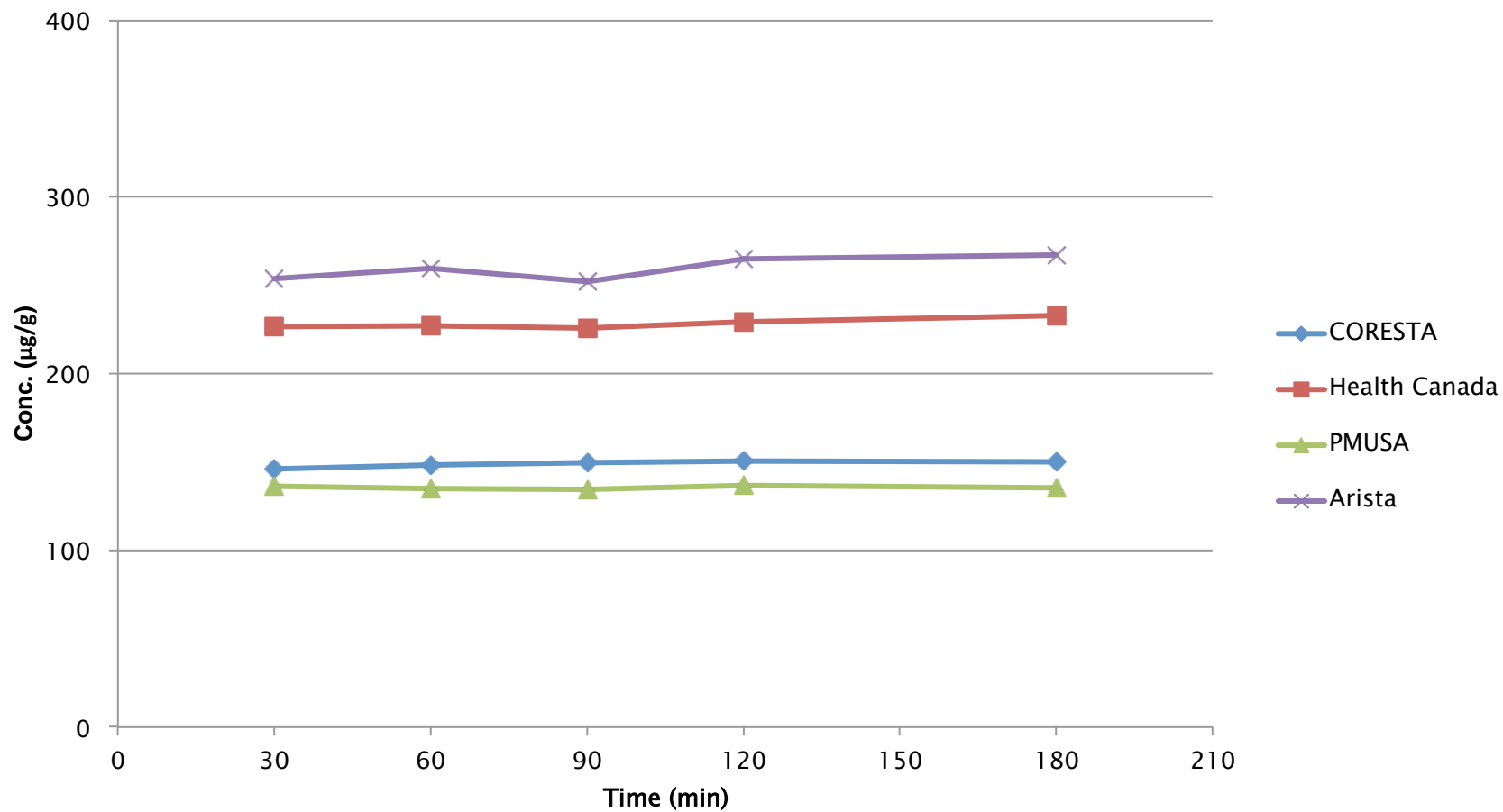
# Anatabine - 3R4F



Arista Laboratories, Inc.



# Anatabine - CRP2



Arista Laboratories, Inc.

# Arista Method Precision – Quest 3

|         | Nicotine<br>( $\mu\text{g/g}$ ) | Nornicotine<br>( $\mu\text{g/g}$ ) | Anabasine<br>( $\mu\text{g/g}$ ) | Myosmine<br>( $\mu\text{g/g}$ ) | Anatabine<br>( $\mu\text{g/g}$ ) |
|---------|---------------------------------|------------------------------------|----------------------------------|---------------------------------|----------------------------------|
| Average | 1994                            | 102                                | 8.9                              | 6.1                             | 70.0                             |
| Std Dev | 40                              | 9                                  | 1.3                              | 0.4                             | 5.1                              |
| %rsd    | 2.0                             | 9.2                                | 14.6                             | 7.0                             | 7.2                              |

# Matrix Recoveries – Arista method

|         | Nicotine (%) | Nornicotine (%) | Anabasine (%) | Myosmine (%) | Anatabine (%) |
|---------|--------------|-----------------|---------------|--------------|---------------|
| Average | 102.7        | 102.4           | 108.2         | 104.4        | 105.9         |
| Std Dev | 1.2          | 1.1             | 1.9           | 0.9          | 0.9           |
| %rsd    | 1.2          | 1.0             | 1.8           | 0.8          | 0.9           |

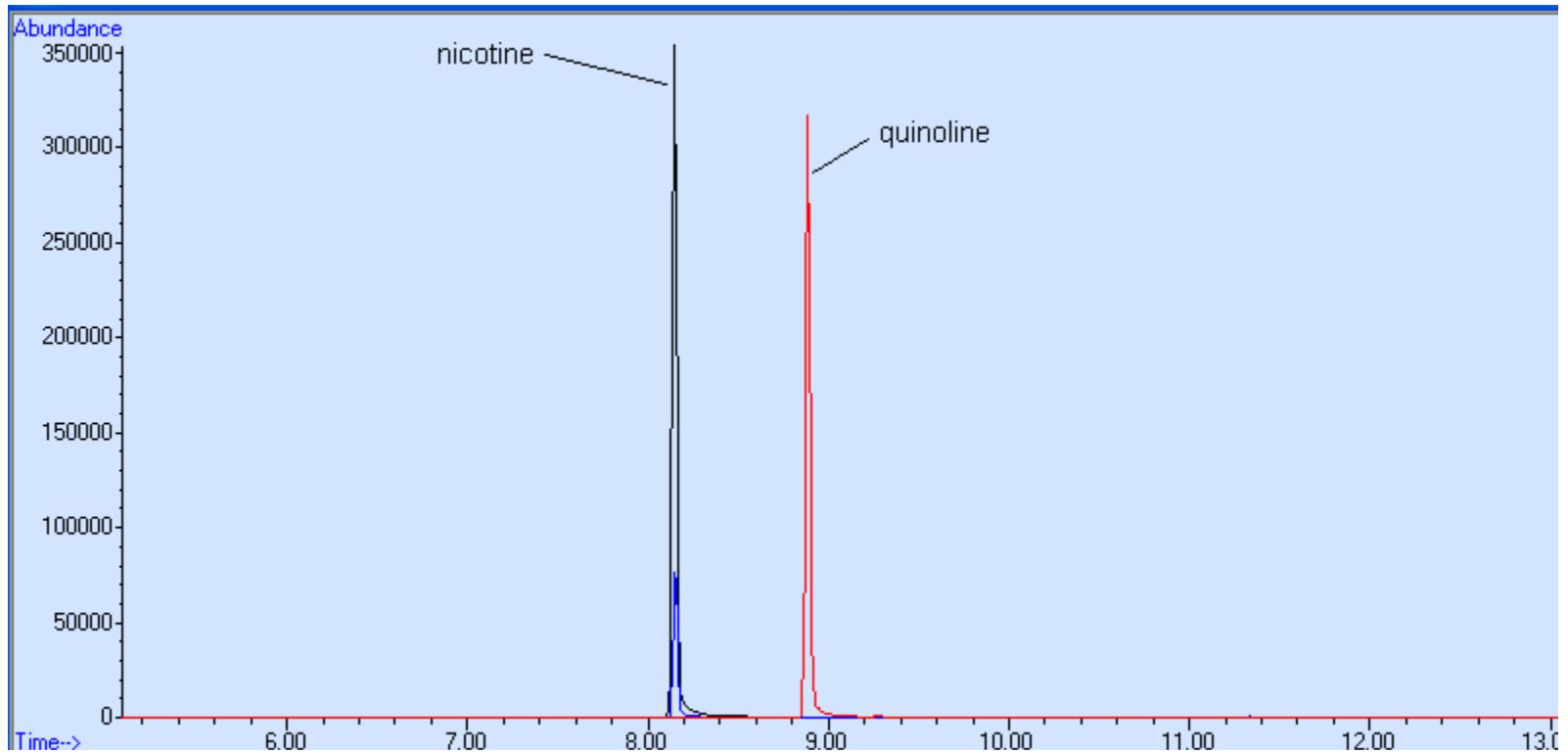
# Method Precision – Ground Tobaccos

|          |                 | Nicotine<br>(µg/g) | Nornicotine<br>(µg/g) | Anabasine<br>(µg/g) | Myosmine<br>(µg/g) | Anatabine<br>(µg/g) |
|----------|-----------------|--------------------|-----------------------|---------------------|--------------------|---------------------|
| Dark     | Average<br>%rsd | 19606<br>2.1       | 1587<br>1.9           | 144<br>0.7          | 109<br>3.0         | 830<br>1.2          |
| Burley   | Average<br>%rsd | 15360<br>1.0       | 2229<br>1.5           | 113<br>1.3          | 124<br>3.0         | 798<br>0.7          |
| Oriental | Average<br>%rsd | 25296<br>2.5       | 920<br>1.5            | 67.6<br>3.8         | 39.1<br>3.1        | 455<br>1.6          |
| Virginia | Average<br>%rsd | 29250<br>0.7       | 862<br>1.3            | 178<br>0.4          | 27.0<br>4.7        | 1633<br>0.6         |
| KY3R4F   | Average<br>%rsd | 17849<br>1.5       | 799<br>2.7            | 96.1<br>3.4         | 31.2<br>4.0        | 791<br>2.7          |

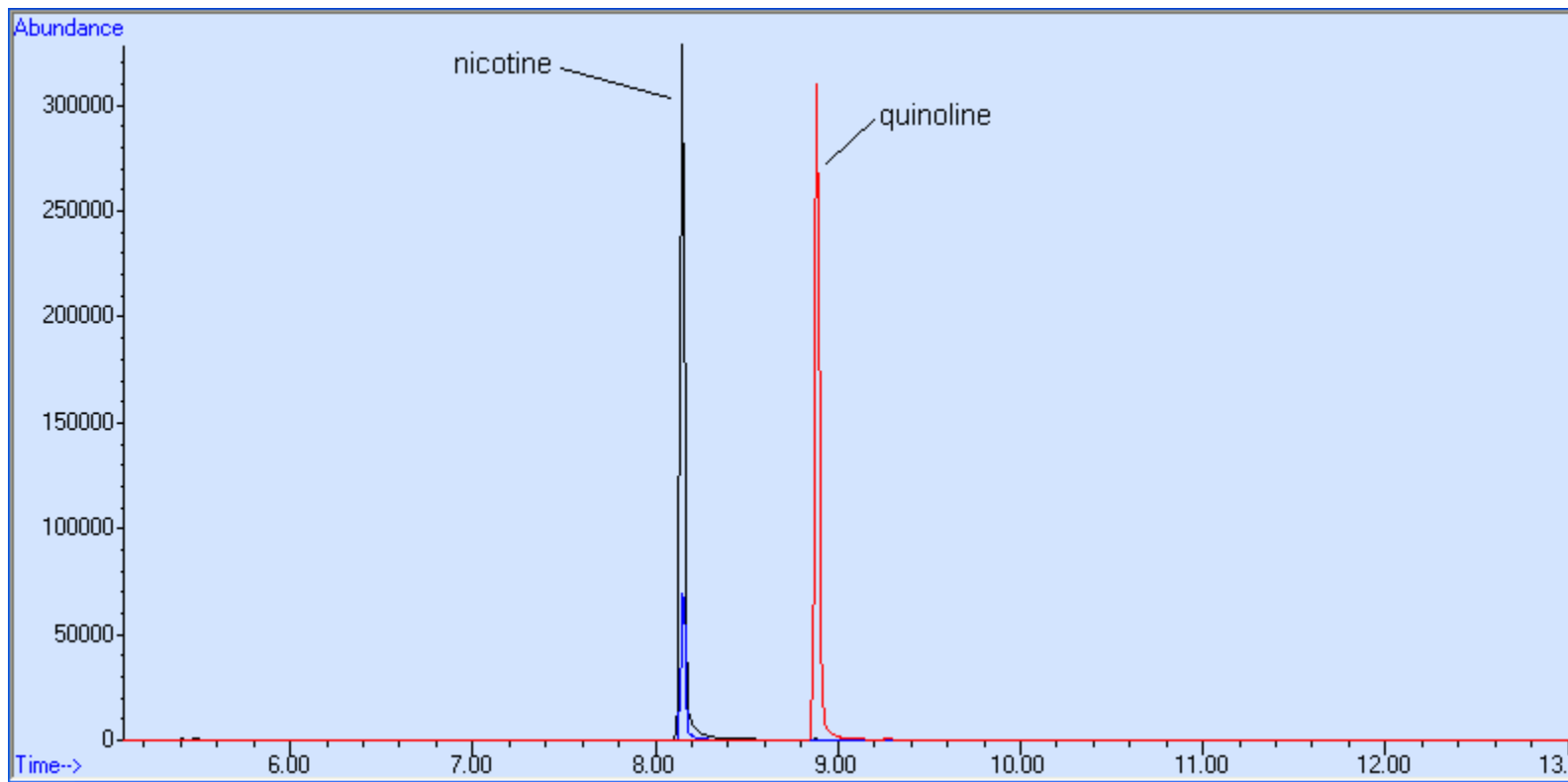
# Method Precision – Smokeless Tobaccos

|                       |                 | Nicotine<br>(µg/g) | Nornicotine<br>(µg/g) | Anabasine<br>(µg/g) | Myosmine<br>(µg/g) | Anatabine<br>(µg/g) |
|-----------------------|-----------------|--------------------|-----------------------|---------------------|--------------------|---------------------|
| CRP1<br>(snus)        | Average<br>%rsd | 9889<br>1.2        | 232<br>1.9            | 38.0<br>1.6         | 25.4<br>2.3        | 141<br>1.4          |
| CRP2<br>(moist snuff) | Average<br>%rsd | 12515<br>1.1       | 324<br>2.5            | 55.4<br>4.2         | 23.1<br>4.9        | 169<br>2.0          |
| CRP3<br>(dry snuff)   | Average<br>%rsd | 22066<br>0.9       | 668<br>1.5            | 97.9<br>3.6         | 64.4<br>1.5        | 588<br>1.5          |
| CRP4<br>(loose leaf)  | Average<br>%rsd | 10592<br>1.4       | 505<br>2.6            | 41.0<br>4.2         | 20.9<br>2.9        | 496<br>1.8          |
| Skoal LC<br>Spearmint | Average<br>%rsd | 13159<br>1.1       | 300<br>2.9            | 47.9<br>3.1         | 23.2<br>4.1        | 197<br>1.5          |

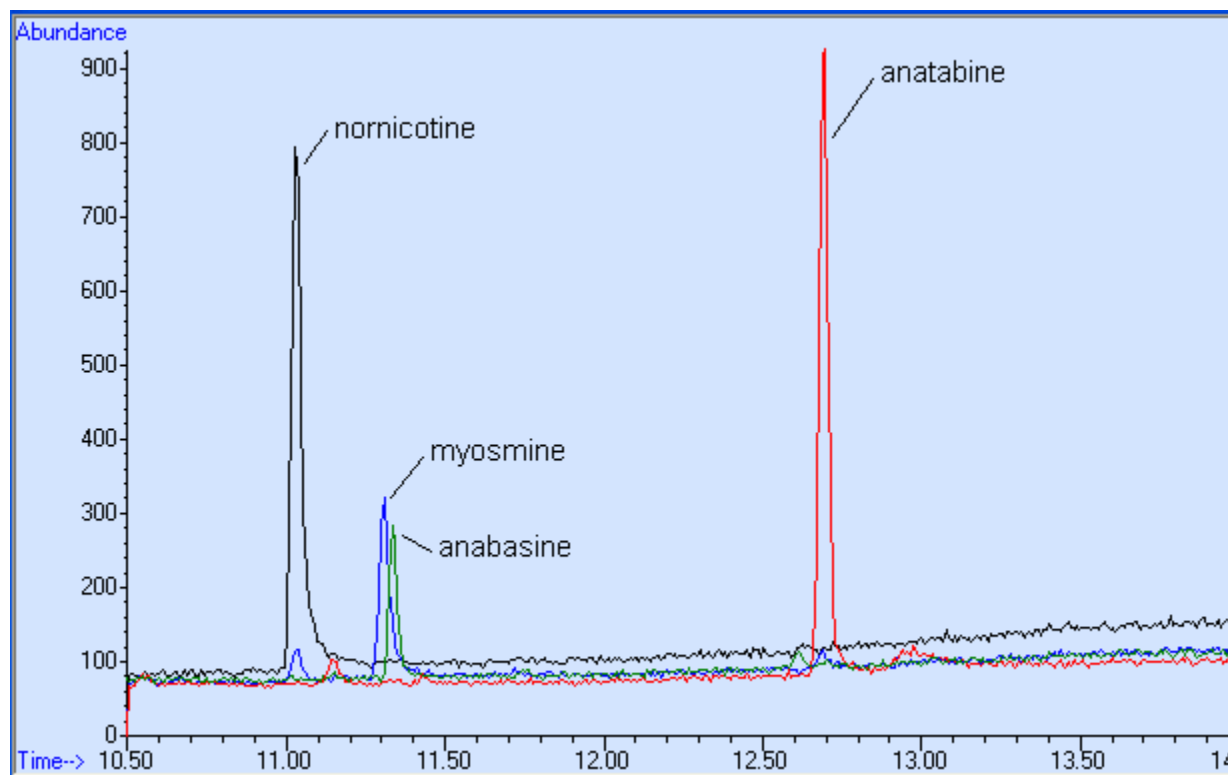
# Ion Chromatograms – Standard Solution



# Ion Chromatograms – Skoal LC Spearmint



# Ion Chromatograms – Skoal LC Spearmint





# Conclusions

- ▶ Extraction is a complicated multi-step process, involving:
  - Preparation of the matrix (grinding, pH adjustment, wetting)
  - Extraction of analytes of interest from the matrix into the aqueous phase
  - Transfer of the analytes of interest into a phase suitable for the analysis.
- ▶ Complete extraction cannot always be inferred by a plateau in the extraction profile

# Conclusions

- ▶ We have developed a method capable of fully extracting nicotine and the minor alkaloids within 30 to 60 minutes, from a wide range of products and levels.
- ▶ MS is a better choice for analysis due to its selectivity compared to other detectors.

Thank you