Determination of Melamine in Full Fat milk.

Introduction:
This document describes the first experiments to determine Melamine in raw cow milk (8-10% fat). Due to the limited time the application is divided in two steps. The first and main test is to determine the best on-line SPE conditions. This is decribed in this document.
In a later stage the second step has to be investigated; this is to find the best LC conditions for this compound.

Experiments:
The LogD (logP) indicates that Melamine is a very polar compound. As expected the tested reversed phase sorbents did not show any recovery of the compound.
Therefore the next step is to investigate the cation exchange sorbents. There are two cation exchange techniques available: strong and weak cation exchange. For both techniques are several cartridges available: MM-C, Oasis MCX, MM-Weak Cation, Oasis WCX, SCX and PRS.
All these are tested and the Oasis MCX gave the best peak shape and recovery.
This cartridge is used to clean the cow milk and validate the method.

The MS was tuned and the mass setting 127/85 was used for quantification purposes and the 127/68 was used for qualification purpose.

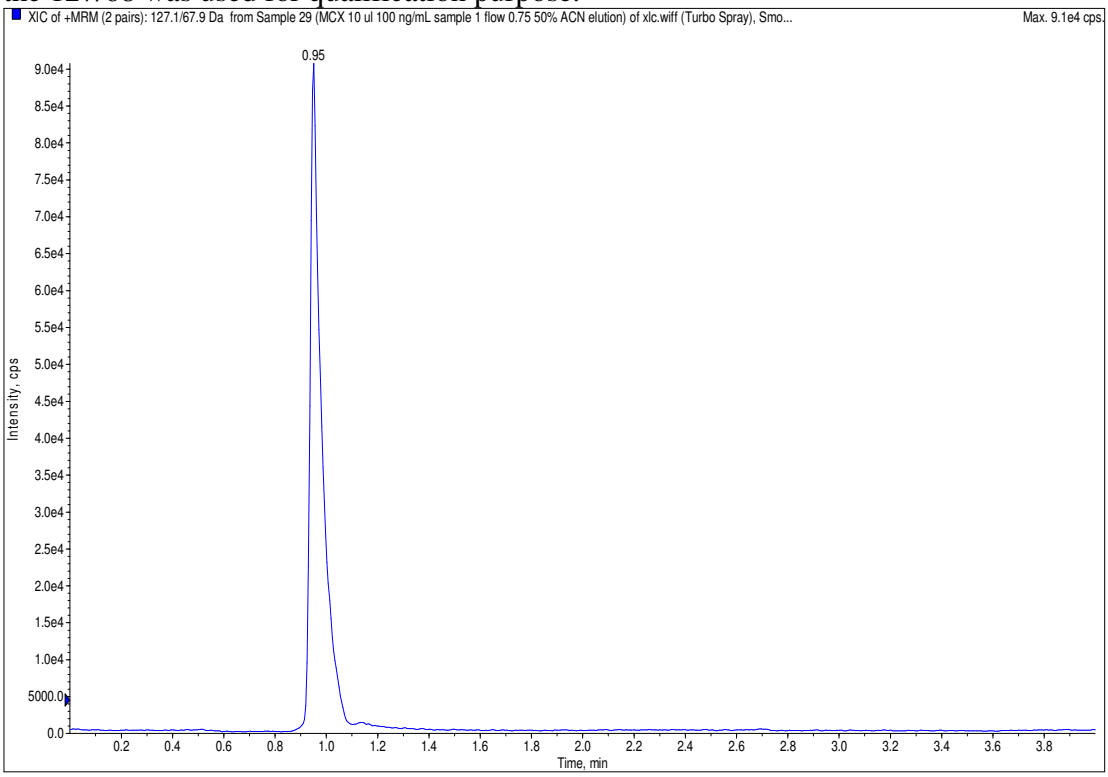


Figure 1: Melamine (127/68) spiked in raw cow milk. Concentration melamine is 100 ng/ml and 10 µL is injected

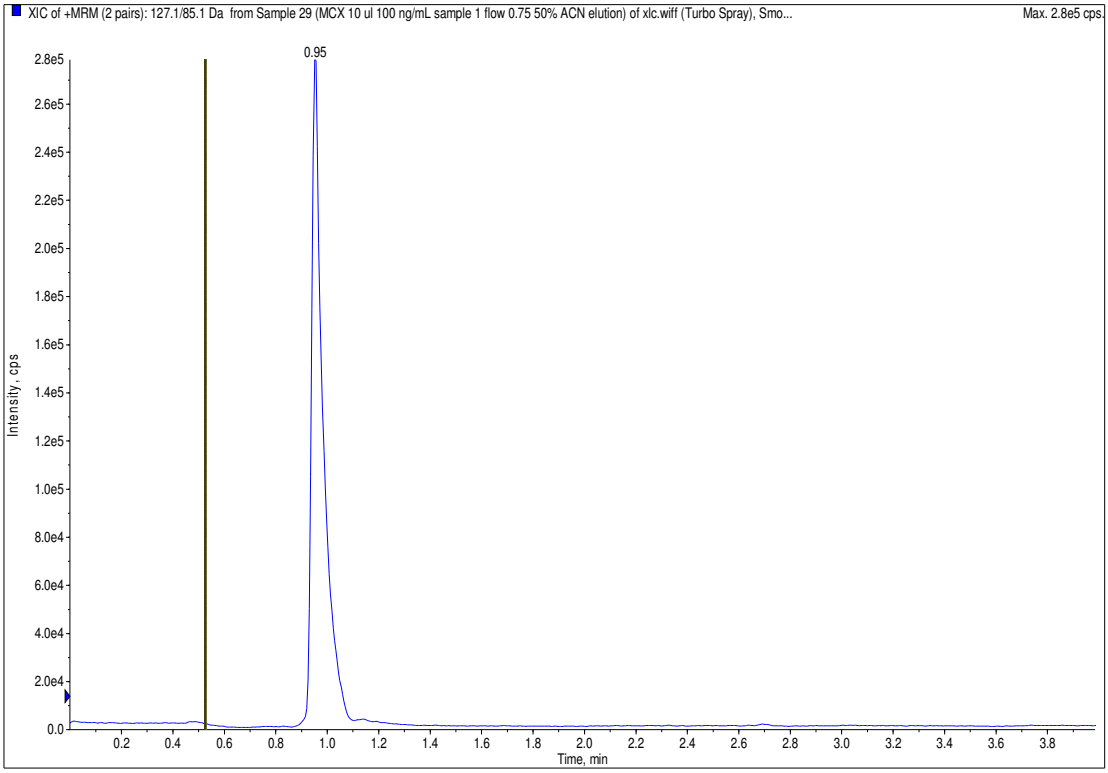


Figure 2: Melamine (127/85) spiked in raw cow milk. Concentration melamine is 100 ng/ml and 10 µL is injected

Sample preparation:

A pH 5.8 buffer is added to the milk in a 1 to 1 ratio.
This pH 5.8 buffer is made out of 10 mM Ammonium acetate and set to the right pH with Acetic acid.
25 µL of this diluted milk is loaded on the Waters Oasis MCX cartridge.

Method Data:

Symbiosis Pico

MethodType:	XLC
Alias	
Injection Mode:	Partial loopfill
Speed:	Normal
Flush volume:	2.0 x Needle volume
Needle height:	2.5 mm
HeadSpace pressure:	Off
Air segment:	Off
Wash solvent 1:	40% ACN 0.2% FA in water

Wash method

Wash volume (ul)	Wash solvent
700	40% ACN 0.2% FA in water

eXtraction unit

Conditioning solvent:	**ACN**
Conditioning flow:	**5000 ul/min**
Conditioning volume:	**1000 ul**
Equilibration solvent:	*5% ACN 0.2% FA*
Equilibration flow:	*5000 ul/min*
Equilibration volume:	*1000 ul*
Sample extraction solvent:	**5% ACN 0.2% FA**
Sample extraction flow:	**2000 ul/min**
Sample extraction volume:	**2000 ul**
Cartridge wash flow:	*5000 ul/min*
Cartridge wash volume:	*1500 ul*
Mix cartridge wash solvent A:	*ACN; 75 %*
Mix cartridge wash solvent B:	*5% ACN 0.2% FA; 25 %*
Clamp flush solvent2:	**50% ACN 2% NH4OH**
Clamp flush flow2:	**5000 ul/min**
Clamp flush volume2:	**75 ul**
Elution mode:	*Focusing (HPD)*
Focusing solvent:	*50% ACN 2% NH4OH*
Focusing flow:	*200 ul/min*
Focusing volume:	*400 ul*

Elution pump

Aqueous (pumpA):	Water 0.2% FA (pump A1)
Organic (pumpB):	ACN 0.2% FA (pump B1)
Pump Maximum pressure:	350 Bar
Pump Minimum pressure:	0 Bar
Equilibration Time:	00:02

Pump time table

Pump time	Pump flow (ml/min)	Pump Fraction A %	Pump Fraction B %
00:00:01	0.75	95	5
00:00:30	0.75	95	5
00:02:00	0.75	95	5
00:03:30	0.75	5	95
00:03:45	0.75	5	95
00:04:00	0.75	95	5
00:05:00	0.75	95	5

Period 1 Experiment 1:

Scan Type:	MRM (MRM)
Polarity:	Positive
Ion Source:	Turbo Spray
Resolution Q1:	Unit
Resolution Q3:	Low
MR Pause:	5.0000 msec

Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop
127.13	85.10	150.00	CE	25.00	25.00
			CXP	14.00	14.00

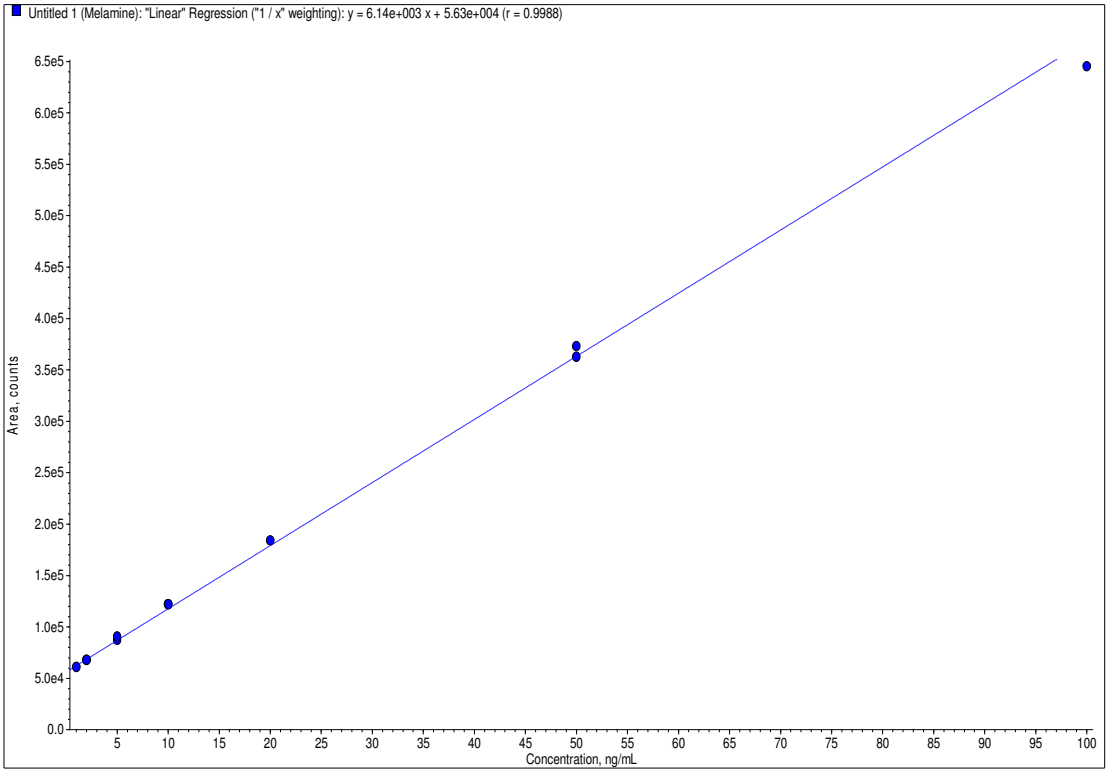
Q1 Mass (Da)	Q3 Mass (Da)	Dwell(msec)	Param	Start	Stop
127.13	67.90	150.00	CE	39.00	39.00
			CXP	12.00	12.00

Parameter Table(Period 1 Experiment 1)

CAD:	7.00
CUR:	15.00
GS1:	80.00
GS2:	40.00
IS:	5000.00
TEM:	450.00
ihe:	ON
IQ2	-18.00
DP	51.00
EP	10.00

Calibration:

The minimum acceptance level for milk in Europe is 0.5 ppm and for the US this limit is higher (1 ppm). This represents 0.5 mg Melamine per kg milk or milk powder.
1 Liter milk weights around 1 kg. Therefore for milk the limit is 0.5 mg/L or 0.5 µg/ml or 500 ng/mL.
The first experiments showed that the this detection limit is easy to reach. Therefore I created a calibration curve from 1 to 100 ng/ml in milk.



Calibration curve from 1 ng/ml till 100 ng/ml in fat milk. 1/X weighting and R=0.9989

Expected Concentration	%CV	Accuracy
1.000000	7.65	87.9
2.000000	1.68	95.6
5.000000	7.06	106.
10.000000	0.38	107.
20.000000	3.65	104.
50.000000	2.36	101.
100.00000	2.86	99.2

Two combined calibration curves.

N.B. No internal standard is used during this experiment