QUANTITATIVE ANALYSIS FOR THE PRESENCE OF MELAMINE IN MILK POWDER USING GC-MS TECHNIQUE

By

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ABSTRACT

Milk powder is a widely consumed dairy product manufactured by evaporating milk to dryness. Purposes of drying are to preserve milk and reduce its bulk. Milk powder is available in the forms of whole milk powder and skim milk powder. Powdered milk is frequently used in the manufacture of infant formula, confectionery, baked goods, etc. In the 2008 infant milk scandal in China, melamine adulterant was found in infant milk powder formula, added to mislead tests into reporting higher protein content. Melamine may have been added to protein content tests after water was added to fraudulently dilute the milk. Because of melamine's high nitrogen content (66% by mass versus approx. 10-12% for typical protein), it can cause the protein content of food to appear higher than the true value.

Melamine is an industrial organic chemical with formula $C_3H_6N_6$ (1,3,5-Triazine-2,4,6-triamine). It is combined with formaldehyde to convert melamine to a very durable thermosetting resin. Also used as a fertilizer in Asia. Melamine by itself is nontoxic in low doses, but when combined with cyanuric acid it can cause fatal kidney failures due to the formation of insoluble melamine cyanurate. Recent adulterations with melamine and related analogues have created the need for more detailed testing of protein based food and feed ingredients. Sri Lankan government announced the maximum melamine content in the milk powder as 1mg per kilogram, as a resuit of widespread concern over its potential health hazards, thus there is a need to carry out analysis of melamine in milk powder in Sri Lanka, using routine analytical techniques.

The present research was aimed to determine the Melamine content in several milk powder brands in Sri Lanka in order to ensure the consumer safety. The first approach was to develop the method to quantify melamine. Most commonly used method for melamine analysis of milk is the HPLC method. But this method has limitations such as ppm levels of limit of detection (LOD) and difficulties in correct identification of melamine peak. Therefore the potential of using Gas chromatography mass spectrometry (GC-MS) technique to analyze samples was investigated. The GC-MS method was developed by referring to the FDA method for analyze melamine in pet foods. The method involves sampling, extraction, chemical derivatization, detection and confirmation of identity.

Results indicated that, the samples qualitatively and quantitatively satisfied for melamine levels. Recovery percentage was 84.08% and the (LOD) was 50ppb. Therefore developed method can effectively be used to analyze milk powder samples for their melamine content. Thirty commercially available samples (including different brands, varieties, and batches) were analyzed using the above method and none of the samples contained melamine. Therefore it can be concluded that the newly developed technique is effective and accurate for analyzing melamine in milk powder and milk powders marketed in Sri Lanka are safe with regard to melamine adulteration.

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ABBREVIATIONS

BSTFA; bis(trimethylsilyl)trifluoroacetamide

°C ; Celsius

DEA ; diethylamine

FDA ; Food and Drug Administration

GC ; Gas Chromatography

GC/MS; Gas Chromatography Mass Spectrometry

g ; gram

HPLC ; High Performance Liquid Chromatography

kg ; kilogram

LOD ; Limit of detection

LC/MS; Liquid Chromatography

MS ; Mass Spectrometry

µg ; microgram

µl ; micro liter

µm ; micro meter

mg ; milligram

ml ; milliliter

mm ; millimeter

min ; minutes

mp ; melting point

N_2 ; Nitrogen gas

ppb ; parts per billion

ppm ; parts per million(mg per kilogram)

rpm ; round per minute

sec ; second

tem ; temperature

TMCS ; Trimethylchlorosilane

TMS ; trimethylsilyl

U.S. ; United States

SYMBOLS

~ ; Approximately

@ ; At

% ; percent

CHAPTER 1

INTRODUCTION

1.1 Introduction

Milk powder is a widely consumed dairy product manufactured by evaporating milk to dryness. Purposes of drying are to preserve milk and reduce its bulk. Milk powder is available in the forms of whole milk powder and skim milk powder. Powdered milk is frequently used in the manufacture of infant formula, confectionery, baked goods, etc.

Recently, there have been incidents where food manufactures or supplies in China have added melamine to milk powder in order to deceive quality control analyses. Melamine may have been added to protein content tests after water was added to fraudulently dilute the milk. Because of melamine's high nitrogen content (66% by mass versus approx. 10-12% for typical protein), it can cause the protein content of food to appear higher than the true value. More recently, a wave of illness among Chinese infants was attributed to melamine-tainted infant formula. The FDA found that melamine binds easily with isocyanuric acid to generate a crystalline polymer, which is toxic to humans.

Melamine is an industrial organic chemical with formula $C_3H_6N_6$ (1,3,5-Triazine-2,4,6-triamine). It is combined with formaldehyde to convert melamine to a very durable thermosetting resin. Also used as a fertilizer in Asia. Melamine by itself is nontoxic in low doses, but when combined with cyanuric acid it can cause fatal kidney failures due to the formation of insoluble melamine cyanurate. Recent adulterations with melamine and related analogues have created the need for more detailed testing of protein based food and feed ingredients.

On October 3, 2008, the U.S. Food and Drug Administration said that up to 2.5 parts per million of melamine was safe for adults, but declined to set a standard for children. The FDA also implied it would not permit the sale of food deliberately adulterated (rather than accidentally contaminated) with melamine. Melamine content of the milk powder is one of the biggest issues in Sri Lankan food industry following the tragedy reported in China. All the milk products in Sri Lanka have undergone special laboratory tests and the authorities have confirmed most of them as free of

melamine.In Sri Lanka relevant authorities (SLSI, Health ministry, etc) recently engaged in developing standard melamine levels to ensure consumer safety. Sri Lankan government announced the maximum melamine content in the milk powders until further research is completed. Trade and Consumer Affairs Ministry pointed out that the maximum melamine content in milk powder and other foods, should be 1 mg per kilogram (1 parts per million) of the product.

Milk powder in the Sri Lankan market is also imported from China and Taiwan. This is mainly used as an ingredient in manufacturing several food items including chocolate confectioneries. Consumer needs to ensure the safety after using this milk powder and related products. Therefore determination of melamine in milk powder is important to carry out in Sri Lanka.

SGS (Societe Generale de Surveillance) is the world's leading inspection, verification, testing and certification company. SGS is recognized as the global benchmark for quality and integrity. The core services offered by SGS can be divided into three categories. There are Inspection, Testing and Certification services. SGS certifies that products, systems or services meet the requirements of standards set by governments (e.g. GOST R), standardization bodies (e.g. ISO 9000) or by SGS customers. SGS also develops and certifies its own standards at the certification services. SGS Lanka (pvt) ltd always tries to develop their method of testing to obtain correct and accurate result. So SGS Lanka (pvt) ltd wants to develop correct and accurate method to determine melamine adulteration in milk powder.

Most commonly used method for melamine analysis of milk is the HPLC method. But this method has limitations such as ppm levels of limit of detection (LOD) and difficulties in correct identification of melamine peak. There is matrix interference sometime; therefore retention time may be varying with several matrices. A simple, rapid, sensitive, and robust method for melamine that is free of matrix interference is essential for quantitative analysis. GC-MS technique provides a complete solution for melamine analysis in complex matrices.

Therefore the potential of using Gas chromatography mass spectrometry (GC-MS) technique to analyze samples was investigated. The GC-MS method is developed by referring to the FDA method for analyze melamine in pet foods. The melamine is not a volatile compound. In this respect the application of gas chromatography has

been greatly extended by the formation of volatile derivatives, by the use of silylation reagents. This derivatization also gives enhanced resolution from other components in a mixture and improved peak shape for quantitative analysis. Evaporation of water is done by using slow nitrogen flow. Otherwise samples cannot be injected to the GC-MS. The method involves sampling, extraction, chemical derivatization, detection and confirmation of identity. Appropriate methodologies are used to obtain minimum detection limit of method, percentage of recovery, and calibration curve.

Finally in this research, it is expected to find out whether milk powders marketed in Sri Lanka are safe with regard to melamine adulteration.

1.2 Objectives:

1.2.1 General objective

This study aims to determine the Melamine content in several marketed milk powders thus ensure the consumer safety.

1.2.2 Specific objectives:

1. To study about the adulterant melamine.
2. To conduct the qualitative analysis for melamine.
3. To Extract and quantify the melamine from milk powder samples.

CHAPTER 2

LITERATURE REVIEW

2.1 Milk powder

Powdered milk is a manufactured dairy product made by evaporating milk to dryness. One purpose of drying milk is to preserve it; milk powder has a far longer shelf life than liquid milk and does not need to be refrigerated, due to its low moisture content. Another purpose is to reduce its bulk for economy of transportation. Available as Dry Whole Milk (DWM), it is most commonly produced as Non-Fat Dry Milk (NFDM), also known as Dried Skim Milk (DSM) (Wikepedia 2008).

2.1.1 History and Manufacturing process of milk powder

While Marco Polo wrote of Mongolian Tatar troops in the time of Kublai Kahn carrying sun-dried skimmed milk as "a kind of paste", the first usable commercial process to produce dried milk was invented by T.S. Grimwade and patented in 1855, though a William Newton had patented a vacuum drying process as early as 1837. Today, powdered milk is usually made by spray drying nonfat skim milk or whole milk. Pasteurized milk is first concentrated in an evaporator to about 50% milk solids. The resulting concentrated milk is sprayed into a heated chamber where the water almost instantly evaporates; leaving fine particles of powdered milk solids.

Alternatively, the milk can be dried by drum drying. Milk is applied as a thin film to the surface of a heated drum, and the dried milk solids are then scraped off. Powdered milk made this way tends to have a cooked flavor, due to caramelization caused by greater heat exposure. Another process is freeze drying, which preserves many nutrients in milk, compared to drum drying. The drying method and the heat treatment of the milk as it is processed alter the properties of the milk powder (for example, solubility in cold water, flavor, and bulk density) (Wikepedia 2008).

2.1.2 Uses of milk powder

Powdered milk is frequently used in the manufacture of infant formula, confectionery such as chocolate and caramel, and in recipes for baked goods where adding liquid milk would render the product too thin. Powdered milk is also widely used in various sweets such as the famous Indian milk balls known as gulab jamun and

popular Pakistani sweet delicacy (sprinkled with desiccated coconut) known as Chum chum (made with skim milk powder).

Powdered milk is also a common item in UN food aid supplies, fallout shelters, warehouses, and wherever fresh milk is not a viable option. It is widely used in many developing countries because of reduced transport and storage costs (reduced bulk and weight, no refrigerated vehicles). As with other dry foods, it is considered nonperishable, and is favored by survivalists, hikers, and others require nonperishable, easy-to-prepare food. Reconstituting one cup of milk from powdered milk requires one cup of potable water and one-third cup of powdered milk. Powdered milk is also used in western blots as a blocking buffer to prevent nonspecific protein interactions, and is referred to as Blotto (Wikepedia 2008).

2.1.3 Nutritional value of milk powder

Milk powders contain all twenty standard amino acids (the building blocks of proteins) and are high in soluble vitamins and minerals. According to USAID the typical average amounts of major nutrients in the unreconstituted milk are (by weight) 36% protein, 52% carbohydrates (predominantly lactose), calcium 1.3%, and potassium 1.8%. Their milk powder is fortified with Vitamin A and D, 3000IU and 600IU respectively per 100g. Inappropriate storage conditions (high relative humidity and high ambient temperature) can significantly degrade the nutritive value of milk powder (Wikepedia 2008).

2.2 Melamine

Melamine was firstly found By German chemist, Justus Von Liebig in 1834. He produced melamine by using several reactions of Calcium cyanamide (The Taipei times 2008). Industrially melamine is produced by using urea.

2.2.1 Melamine and its physical properties?

Melamine is a white crystalline powder, without a smell and taste. It is an industrial chemical with formula $C_3H_6N_6$. Melamine is combined with formaldehyde to produce melamine resin, a very durable thermosetting plastic, used to make eating utensils, laminates, whiteboard wall paneling, flooring and Formica etc.

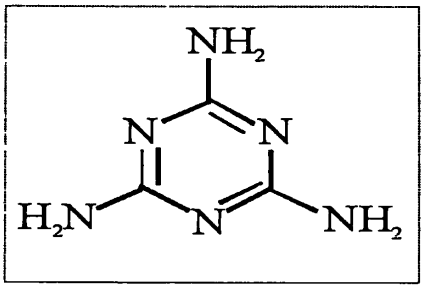


Figure 2.1 Structure of melamine

Melamine also named as 1,3,5-Triazine-2,4,6-triamine; 2,4,6- triamino-s-triazine; and cyanurtriamide. Molecular formula is $C_3H_6N_6$. Molecular weight is 126.13. C 28.57%, H 4.80%, N 66.64%. Usually prepared by heating dicyanadiamide, $H_2NC(=NH)NHC{\equiv}N$, under pressure mp < 250° ,sublimes d^{250} 1.573. Slightly soluble in water, very slightly soluble in hot alcohol and insoluble in ether. It is described as being harmful according to its MSDS (Material safety data sheet) sheet: "Harmful if swallowed inhaled or absorbed through the skin. Chronic exposure may cause cancer or reproductive damage. Eye, skin and respiratory irritant" (Budavari 1999).

2.2.2 Melamine scandal

Now melamine blamed for sickening thousands of infants in China has been found in dairy products, including powdered milk, baby formula, ice cream, candy and yoghurt etc. The contamination has been blamed for the deaths of four children and kidney ailments among 54,000 others. More than 13,000 children have been hospitalized due to melamine poisoning. More significantly, China's tainted milk products have contaminated fruits, vegetable, cosmetics and pet food. Approximately 8,500 dogs and cats died of kidney failure due to melamine poisoning. Based on the reports published by U.S. Food and Drug Administration (FDA) officials, who investigated melamine contamination in pet food last year, it is also used as a fertilizer in Asia because of its high nitrogen content. Melamine has been found in wheat/corn gluten and rice protein concentrate in South Africa, all products have been imported from China. Therefore, the milk powder scandal has dealt a severe blow to the "made in China" brands all over the world. Addition of melamine into food is not approved by the FAO/WHO Codex Alimentarius (food standard commission) or by any authorities (Nivas 2008).

2.2.3 Why melamine is mixed with milk powder?

The question is whether the introduction of melamine in food was intentional or accidental. By looking at the chemical structure of amino acids (the building blocks of proteins) and melamine, the answers should become clear. The average protein content in milk is around 3-4% and its nitrogen content is not more than 30%. Melamine is a white crystalline powder, without a smell and taste and contains 66% nitrogen by mass. Therefore melamine makes the protein content of the food is higher than it supposed to be. This is because of the testing procedures used to calculate % of protein by looking at the nitrogen content in crude protein (Paranagama 2008).

The industry standard test to measure % of protein in milk is Kjeldhal method, which measures the total nitrogen content in milk. In this method, nitrogen is released from protein and other nitrogen compounds in milk and converted to ammonia through acid digestion. Therefore crude protein is estimated by multiplying the N value by 6.38 to calculate the average N content in milk protein. Protein determined in this method is referred as crude protein because N comes from true protein and non-protein sources. Although, melamine is normally used to make plastics it can also make milk and other food products appear to have a higher protein content than they actually do leading to the use of melamine as a cheap "high-protein" fake to boost protein readings because melamine is relatively cheap industrial chemical. Additionally melamine is readily available, water soluble and most significantly, super-rich in nitrogen (Paranagama 2008).

2.2.4 What does melamine do in the body?

While the body can effectively eliminate certain toxins found in the environment, certain other toxins either cannot be readily eliminated from the body or are eliminated from the body very slowly causing damages at the cellular and sub-cellular level. This could be undetected until a considerable quantity has been accumulated. By the time damage is detected at the system level, the disease process initiated by the toxin has been in progress for quite some time. This is particularly true for melamine. Melamine is not a potent toxic to the body, but no food containing melamine should be ingested. When melamine enters the body, a portion of it is eliminated, and a portion of it participates in chemical reactions where it is converted to cyanuric acid, ammeline, and ammelide. Hydrolysis of melamine produces cyanuric

acid and it is mainly used as a precursor to *N* –chlorinated cyanurates, which are used to disinfect water and algicides for swimming pool water. Like melamine, cyanuric acid has low toxicity on its own but the two in combination can have serious effects. Cyanuric acid and melamine readily form hydrogen bonds, making and insoluble crystal lattice (figure 2.2) and this white solid is not fully dissolved in urine.

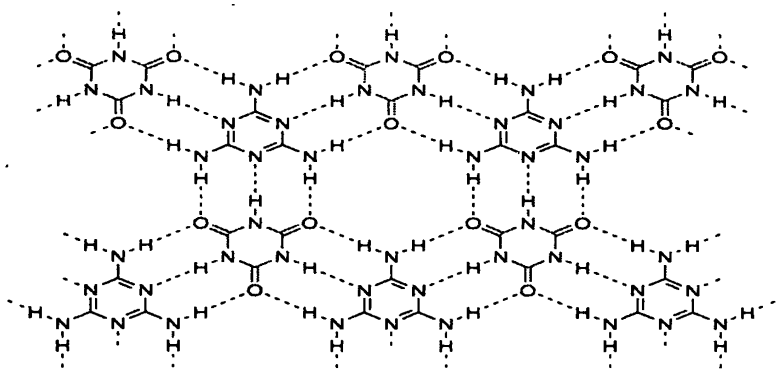


Figure 2.2 Insoluble crystal lattice form between melamine and cyanuric acid

In fact, the analytical test for cyanuric acid is precipitation with melamine. This can lead to kidney stones since the crystals block the tubules in the kidneys, leading to kidney failure. A serious health problem and this is, in fact, the mechanism that causes death. The deposition of crystalline lattice in the bodies of adults and infants recently resulted in deformities and deaths of many in China and other part of the world (Paranagama 2008).

None of the above chemicals are supposed to be found in the human body. There are two ways that these chemicals can enter the human body. They can be ingested along with the melamine or melamine can be subsequently converted to these compounds after ingestion. The metabolism of melamine in the human body to cyanuric acid, ammeline and ammelide is poorly understood by examining human biochemistry. The biochemical pathways in human metabolism to facilitate its conversion cannot be easily identified. It is believed that the large verity of microbial organisms inhabiting the intestinal tract metabolize melamine as microbes have a metabolism of their own, and have the capability to synthesize and break down chemical compounds that we, as humans, cannot. There are some bacteria that have specialized class of enzymes called amidohydrolase that can break down melamine into various components, such as cyanuric acid, ammeline, and ammelide. Two of

these specialized enzymes are Melamine deaminase and atrazine chlorohydrolase (Paranagama 2008).

The FDA has announced that there are traces of the industrial chemical melamine in United States infant formula and they reported that the melamine contamination in U.S. made formula had occurred during the manufacturing process, rather than by intentional contamination. According to the statement issued by FDA in October 2008, even though there are trace levels of melamine in infant formulae they do not raise public health concerns. Further several medical experts in USA also reported that trace concentrations would be diluted even in an infant, and are highly unlikely to be harmful. However there has been much fear and confusion on the part of parents and recently, FDA have introduced a "safe" level of melamine which happens to be higher than the levels that have been found in infant formula. In November 2008, the safe exposure level of melamine or one of its analogues alone has been set at 1ppm in infant formula. Further in a separate statement, the FDA reported infants may be more sensitive than adults for exposures to melamine because infant formula is the sole source of nutrition for infants and the exposure continues for up to 12 months. During this period renal function may be immature in infants compared to adults (FDA 2007).

2.2.4.1 Symptoms of the effects

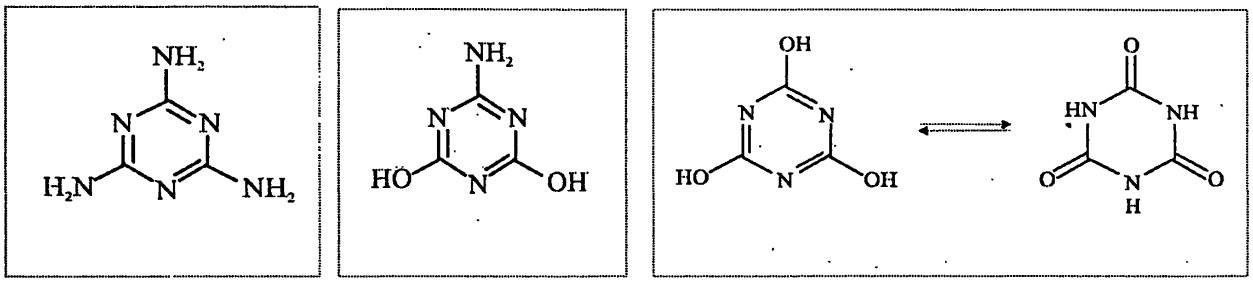
Include: stomach pain, vomiting, fever, lack of appetite, lethargy, irritability, or excessive crying; blood, crystals, or particles in urine; painful urination; little or no urine; swelling of hands, feet or face. And high blood pressure (WHO 2008).

2.2.4.2 Treatment

The standard treatments include injection of intravenous fluids to help flush the kidney off the toxins, medication to control blood pressure, nausea and anemia, special diets to reduce strain on kidneys, and frequent blood and urine tests to monitor the patient's kidney function. Treatment also depends on severity of the kidney effects. Treatment may include infusion of fluids and urine alkalinisation, correction of electrolyte and acid-base disturbance, haemodialysis or peritoneal dialysis, or surgical removal of kidney stones (Srilal 2008).

2.2.5 Determination of melamine

The use of melamine is an illegal food additive escapes routine detection because many commercial quality control processes merely test for the total nitrogen content of the proteins, not the protein itself. Other proven methods to detect melamine and other non-protein nitrogen are available but they are not part of routine checks. However, recent adulteration with melamine and related analogues has created the need for more detailed testing of protein based food and feed ingredients. This analysis is important because it detects common adulterants and works in conjunction with total nitrogen testing to verify the quantity and authenticity of protein in a food product. These methods should be focused on measuring true protein not crude protein, as true protein indicates the nutritional value. High visibility and the potential threat prompted the U.S. FDA to rapidly issue standard test methods for the analysis of melamine in protein materials (Jimmy et al. 2007). These methods, entitled GC-MS and LC-MS screen for the presence of melamine, ammeline (figure 2.3.a), ammelide (figure 2.3.b), and cynuric acid (figure2.3.c).



(a) **(b)** **(c)**

Figure 2.3 Structures of ammeline, ammelide and cynuric acid.

2.3 Chromatographic methods

Chromatography is the collective term for a family of laboratory techniques for the separation of mixtures. The complicated separations are achieved very rapidly and efficiently by chromatography. It involves passing a mixture dissolved in a "mobile phase" through stationary phase, which separates the analyte to be measured from other molecules in the mixture and allows it to be isolated (Bassett et al. 1989).

Chromatography may be preparative or analytical. Preparative chromatography seeks to separate the components of a mixture for further use (and is thus a form of purification). Analytical chromatography normally operates with smaller amounts of

material and seeks to measure the relative proportions of analytes in a mixture. The two are not mutually exclusive.

This method is so fruitful and common that by this time Chromatography has been used in almost every type of compounds and fields. Medicine, biology, art and painting and even intelligence departments have used this method to their greatest advantage (Skoog and West 1971).

2.3.1 Gas Chromatography (GC)

There are two types of gas chromatography:

- Gas-solid (adsorption) chromatography
- Gas-liquid (partition) chromatography

GC is widely used, particularly by organic chemists, and it undoubtedly ranks as one of the most important new analytical techniques since its development in 1952.The separation of benzene and cyclohexane (b.p.80.1 and 80.8^0c) is extremely simple by GC, but it is virtually impossible by conventional distillation. Very complex mixture can be separated by this technique (Tipler 1993).

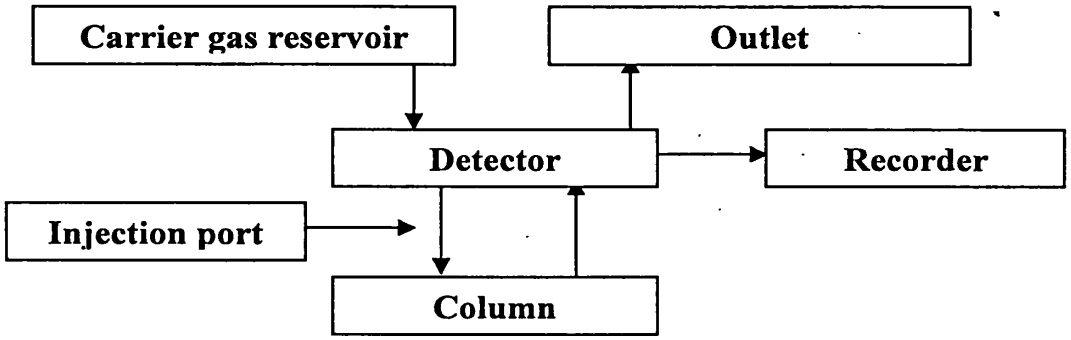


Figure 2.4 Block diagram of Gas Chromatography

2.3.1.1 Principles of GC

In GC, the sample is converted to the vapor state (if it is not already a gas) and the elute is a gas (the carrier gas). The stationary phase is generally nonvolatile liquid supported on an inert solid such as firebrick (Chromosorb-P or W, etc) or diatomaceous earth. There are a large number of liquid phases available, and it is by changing this phase rather than the mobile gas phase that different separations are accomplished.

A block diagram of a GC is given in Figure 2.4.The sample is rapidly injected by means of a hypodermic syringe through a rubber septum into the column. The sample injection port, column and detector are heated to temperature at which the sample has a vapor pressure of at least 10 torr. The injection port and the detector are usually kept somewhat warm than the column to promote rapid vaporization of the injected sample and prevent sample condensation in the detector. Liquid sample of 0.1 to 10 μl are injected, while gas sample s of 1 to10ml are injected, Gases may be injected by means of a gas-tight syringe or through a special gas inlet chamber of constant volume.

Separation occurs as the vapor constituent partition between the gas and the liquid phases in the same manner as in other chromatographic processes. The carry gas is a chemically inert gas available in pure form, such as Argon, Helium, Nitrogen, or Carbon dioxide. A high-density gas gives best efficiency, but a low-density gas gives faster speed. The choice of gas is often dictated by the type of detector.

The sample is automatically detected as it emerges form the column (at a constant flow rate), using a variety of detectors whose response is dependent upon the composition of the vapor. Usually, the detector contains a reference side and sampling side. The carrier gas is passed through the reference side before entering the column and emerges from the column through the sampling side. The response of the sampling side relative to the reference side signal is fed to a recorder where chromatographic peaks are recorded as a function of time. By measuring the retention time (the minutes between the time the sample is injected and the time the chromatographic peak is recorded) and comparing this time with that of a standard of the pure substances, it may be possible to identify the peak (agreement of retention time of two compounds does not guarantee the compounds are identical). The area under the peak is proportional to the concentration, and so the amount of substances can be quantitatively determined. The peak are often very shape and if so, the peak height is taken and compared with a calibration curve prepared in the same manner.

A gas can move much more rapidly through a packed column than a liquid, and so chromatographic separations require only minutes, as compared with much longer times for other chromatographic techniques. In addition, since the peaks are automatically recorded, the entire analysis time is amazingly short. This, coupled with the very small sample required, explains the popularity of the technique. This is not to

exclude the more important reason that many of the analyses performed simply cannot be done by other methods.

With complex mixtures, it is not a simple task to identify the many peaks. The individual fractions (each peak may be a mixture of components) can be collected by cooling the collecting vessel in a dry ice-acetone bath to condense the constituents, and then they can be analyzed by infrared spectrophotometer mass spectrometry to aid in their identification. Instruments are commercially available in which the gas effluent is automatically fed into a mass spectrometer where they are positively identified according to mass (formula weight and fragmentation pattern). This important analytical technique is called gas chromatography-mass spectrometry (GC-MS). The mass spectrometer is a very sensitive and selective detector, and when a capillary GC column (very high resolution) is used (capillary GC-MS), this technique is capable of identifying and quantifying unbelievably complex mixtures of trace substances (Srivasthava 1987).

2.3.2 Mass Spectrometry (MS)

MS analysis is commonly used in arson investigations, engine exhaust analysis, petroleum product analysis, and for blood monitoring in surgery. MS identifies substances by electrically charging the specimen molecules, accelerating them through a magnetic field, breaking the molecules into charged fragments and detecting the different charges. A spectral plot displays the mass of each fragment. A technician can use a compound's mass spectrum for qualitative identification. MS is a sophistication instrument technique that produces, separates, and detects ions in the gas phase (Christion 1994). The basic components of a MS are shown in Figure 2.5.

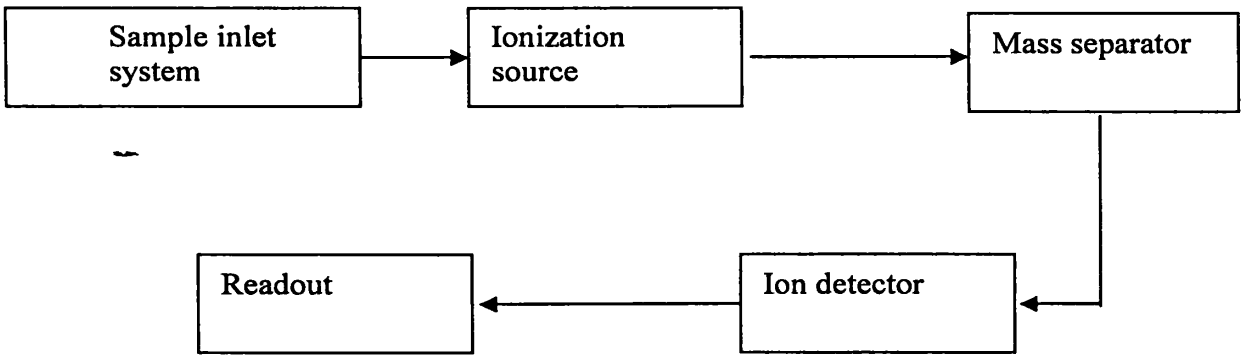


Figure 2.5 Block diagram of mass spectrometer

2.3.2.1 Principles of MS

The technician uses these fragment masses as puzzle pieces to piece together the mass of the original molecule, the "parent ion."

$$M + e^- \longrightarrow M^+ + 2e^-$$

M is the analyte molecule and M^+ is called the molecular ion or parent ion. The M^+ ions are produces in different energy states and internal energy (rotational, irrational, and electronic) is dissipated by fragmentation reactions, producing fragments of lower mass which are themselves ionized or converted to ion by further electron bombardment. The fragmentation pattern is fairly consistent for given condition (electron beam energy). Only a small amount or none of the parent ion may remain.

The parent ion is analogous to the picture on top of a puzzle box, a guide to the end result obtained by putting together the fragment masses, or puzzle pieces. From the molecular mass and the mass of the fragments, reference data is compared to determine the identity of the specimen. Each substance's mass spectrum is unique. Providing that the interpretation of the output correctly determines the parent ion, MS identification is conclusive.

Today many different types of MS instruments exist, each one using a different apparatus and process for producing mass spectra. This article's description of the MS process will limit itself to a basic description of a conventional large magnet mass spectrometer. Such a MS instrument contains a sample inlet, an ionization source, a molecule accelerator, and a detector.

MS analysis requires a pure gaseous sample. The sample inlet is maintained at a high temperature, up to 400°C (752°F), to ensure that the sample stays a gas. Next the specimen enters the ionization chamber. A beam of electrons is accelerated with a high voltage. The specimen molecules are shattered into well-defined fragments upon collision with the high voltage electrons. Each fragment is charged and travels to the accelerator as an individual particle. In the acceleration chamber the charged particle's velocity increases due to the influence of an accelerating voltage. For one value of voltage only one mass accelerates sufficiently to reach the detector. The accelerating voltage varies to cover a range of masses so that all fragments reach the detector.

The charged particles travel in a curved path towards the detector. When an individual charged particle collides with the detector surface, several electrons (also charged particles) emit from the detector surface. Next, these electrons accelerate towards a second surface, generating more electrons, which bombard another surface. Each electron carries a charge. Eventually, multiple collisions with multiple surfaces generate thousands of electrons, which emit from the last surface. The result is an amplification of the original charge through a cascade of electrons arriving at the collector. At this point the instrument measures the charge and records the fragment mass as the mass is proportional to the detected charge.

The MS instrument produces the output by drawing an array of peaks on a chart, the "mass spectrum." Each peak represents a value for a fragment mass. A peak's height increases with the number of fragments detected with one particular mass. As in the case of the GC detectors, a peak may differ in height with the sensitivity of the detector used (Christion 1994).

2.3.3 Gas chromatography-Mass spectrometry (GC/MS)

Gas chromatography ("GC") and mass spectrometry ("MS") make an effective combination for chemical analysis.

The GC device is generally a reliable analytical instrument. The GC instrument is effective in separating compounds into their various components. However, the GC instrument cannot be used for reliable identification of specific substances. The MS instrument provides specific results but produces uncertain qualitative results. When an analyst uses the GC instrument to separate compounds before analysis with an MS instrument, a complementary relationship exists. The technician has access to both the retention times and mass spectral data. Many scientists consider GC/MS analysis as a tool for conclusive proof of identity (Evershed 1993).

GC/MS analysis, where the effluent to the GC instrument is the feed to the MS instrument, is in wide use for confirmation testing of substances. Drug testing, manufacturing quality control and environmental testing are some typical uses.

Capillary GC, with thousands of theoretical plates, can resolve hundreds of molecules into separation peaks, and mass spectrometry, can provide identification.

Even if a peak contains two or more compounds, identifying peak can still provide positive identification, especially when combined with retention data (Christian 1994).

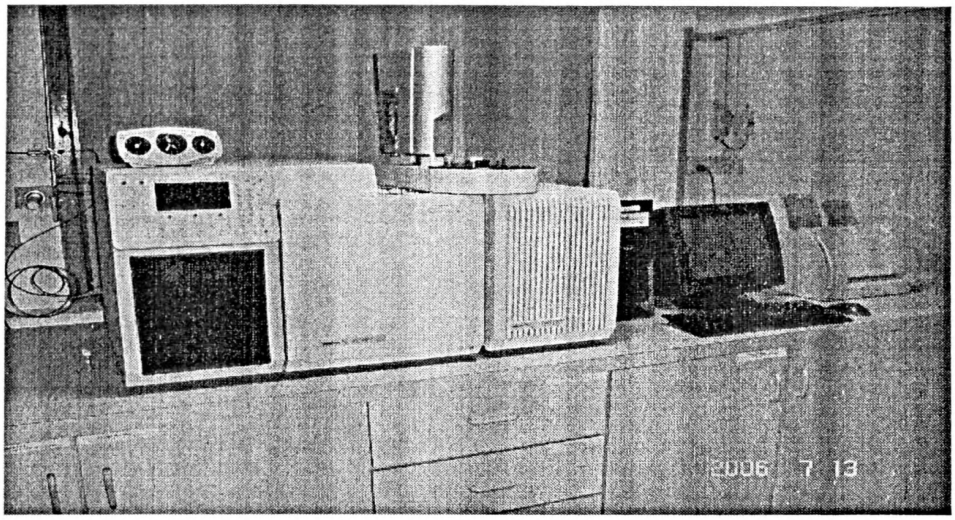


Figure 2.6 Gas chromatography-Mass spectrometry (GCMS)

2.3.4 Chemical derivatization in Gas chromatography

GC of volatile or non-polar compounds may be carried out without derivatizing the sample; indeed derivatives of compounds such as hydrocarbons cannot easily be prepared. It is possible to analyze polar compounds carboxylic acids and amines, without prior derivatization, on polar GC phases such as those based on polyethylene glycol. However, deerivatization is useful in many instances where it may:

(a) increase the volatility and decrease the polarity of polar compounds;

(b) stabilize compounds which are unsuitable at the temperatures required for GC;

(c) improve the separation of groups of compounds on GC;

(d) yield information with regard to the number and type of functionalities present in mixtures on unknown compounds.;

(e) improve the behavior of compounds towards selective detectors and mass spectrometry.

There are number of drawbacks in derivatization:

(a) the derivatizing agent may be difficult to remove and interfere in the analysis and this is particularly disadvantages when the purity of a compound is being assessed by GC;

(b) the derivatization conditions may cause unintended chemical changes in a compound for example dehydration;

(c) the derivatization step increases the time required for analysis.

For these reasons, GC with derivatization is less frequently employed in quality control applications, where the purity of a single substance or the components in a formulation are being determined, than for instance HPLC where derivatization is usually not necessary.

For quantitative accuracy in a derivatization procedure, it is best that reaction of the analyte with the derivatizing reagent is complete and that an internal standard is used. An internal standard may largely compensate for losses of analyte incurred during derivatization particularly if it is a close analogue of the substance being derivatized. This is particularly important for compounds with a degree of instability for example corticosteroids, catecholamines and other compounds prone to thermal elimination of water or oxidation. Derivatization reactions are usually simple chemical reactions which are likely to occur in nearly quantitative yield such as acylation, alkylation or silylation (Watson 1993).

2.3.4.1 Sample and reagent handling

Many of the reagents used in derivatization are highly reactive and contact with air and moisture must be kept to a minimum. The reagents are also often irritant and corrosive and must be handled in a fume cupboard.

The preferred method of transferring measured volumes of sample solutions or reagents is by use of a micro liter syringe. Small volume glass pipettes are a cheaper alternative, but require more skill in their use and allow more contact of reagents with the air. Contact of reagents and solvents with plastic tips is best avoided, especially when low amounts of analyte are being determined (Watson 1993).

2.3.4.2 Removal of derivatizing reagents

The simplest procedure for removing excess of derivatization reagent is by its evaporation under a stream of Nitrogen. For involatile reagents a chromatographic filtration step may be used. Strongly adsorbent chromatographic materials such as silica gel or alumina are usually not suitable for removal of reagents since they may degrade the product or irreversibly adsorb it. Sephadex LH20 or Sephadex LH60 may

be used for removal of reagents; these chromatographic materials are lipophilic but polar and will readily remove polar reagents (Watson 1993).

2.3.4.3 Silylation reactions (Trimethylsilyl (TMS) derivatives)

These derivatives may be prepared from a wide range of functional groups including hydroxyl, carboxylic acid, amine, amide, thiol, phosphate, hydroxime and sulphonic acid. The reaction is of the general type:

ROH		ROTMS
RNH_2		RNHTMS
RCOOH		RCOOTMS
RNH.COR'	Silylating reagent →	RNTMS.COR'
RSH		RSTMS
$ROPO_3H$		$ROPO_3TMS$
RNH_2OH		RN(OTMS)
RSO_3H		RSO_3TMS

In some instances, e.g. TMS derivatives of sulphonic acids and phosphates, the stability of the derivative is very low. There are a number of TMS donor reagents available but some have wider ranges of application than others. It is difficult in most instances to see advantages of many of the available commercial cocktails over simply using the neat reagents.

N, O-Bis (TMS) trifluroacetamide (BSTFA) and N-TMS-N-methyltrifluoroacetamide (MSTFA) may be substituted for BSA [N,O-bis(trimethylsilyl)acetamide]. These reagents are stronger silyl donors, who is important for less reactive groups such as thiols and amides, but are not as good solvents. They are also more volatile, while is important where they are being used to derivatize volatile compounds, e.g. short chain fatty acids.

Trimethylsilylmidazole(TMSIM) is the most powerful silylating reagent available and it may be used to prepare TMS derivatives from sterically hindered alcohols such as steroids with a side chain at C-17 and 17-hydroxy group. It may also be used in the silylation of carboxylic acids, but not in the silylation of amines. (Watson 1993)

2.3.5 High performance liquid chromatography (HPLC)

HPLC is a form of column chromatography used frequently in biochemistry and analytical chemistry. It is sometimes referred to as high pressure liquid chromatography but this is primarily a historical term and not widely accepted today. HPLC is used to separate components of a mixture based on a variety of chemical interactions between the substance being analyzed (analyte) and the chromatography column. HPLC allows separations and measurions to be made in a matter of minutes. Porous packing materials with particle sizesof 3-10μm are usually used in modern instrument,with plate counts of 60,000-90,000 per meter. (Skoog and West 1971)

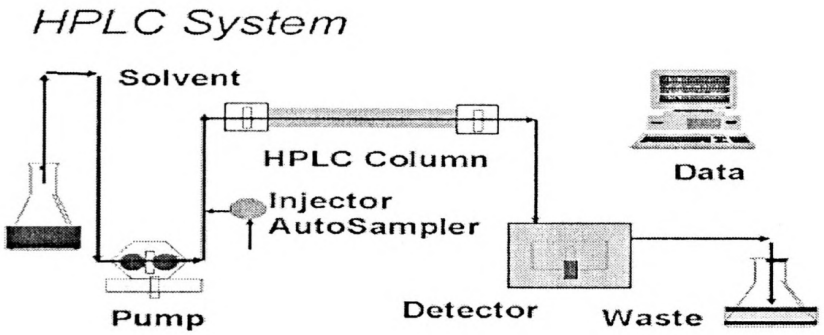


Figure 2.7 HPLC System

2.3.5.1 Principle of HPLC

In isocratic HPLC the analyte is forced through a column of the stationary phase by a liquid at high pressure. Use of pressure gives the components less time to diffuse within the column, leading to improved resolution in the resulting chromatogram. Solvents used include any miscible combination of water or various organic liquids (the most common are methanol and acetonitrile). Water may contain buffers or salts to assist in the separation of the analyte components.

A further refinement to HPLC has been to vary the mobile phase composition during the analysis; this is known as gradient elution. A normal gradient for reverse phase chromatography might start at 5% methanol and progress linearly to 50% methanol over 25 minutes, depending on how hydrophobic the analyte is. The gradient separates the analyte mixtures as a function of how well the changing solvent mobilizes the analyte. In this example, using a water/methanol gradient, the more hydrophobic components will elute (come off the column) under conditions of relatively high methanol; whereas the more hydrophilic will elute under conditions of

relatively low methanol. The choice of solvents, additives and gradient depend on the nature of the stationary phase and the analyte (Wikipedia 2008).

2.3.5.2 HPLC Determination of Melamine

Wheat gluten or rice protein concentrate was extracted with pH 2.5, 50 mM phosphate buffer by stirring vigorously for four hours at 60°C. The extract was injected onto a combination of two columns in series (a short strong cation exchange column and a C18 column) and eluted using a 50 mM phosphate pH 2.5 buffers. The limit of detection (LOD) and limit of quantification (LOQ) were estimated from the noise level to be approximately 100 μg/mL and 400μg/mL respectively for melamine, ammeline, and cyanuric acid in the original material. Ammelide LOD and LOQ were estimated at 10μg/mL and 40μg/mL respectively. Spike recoveries were 98% to110%. This method is useful for quantitation in the range of 0.1% to 5% for melamine and cyanuric acid, and from 400 ppm to 10,000 ppm for ammeline and from 50 ppm to 10,000 ppm for ammelide in the wheat gluten or rice protein (Jimmy et al. 2007).

In HPLC determinations retention time is the only identification of peaks. But this can be varying according to the flow rate, different packing materials of columns, etc. Therefore HPLC is not an efficient method for analysis melamine.

2.4 Physical properties of chemicals used in study.

2.4.1 Pyridine

Molecular formula: C_5H_5N(C 75.92%, H 6.37%, N 17.71%)

Molecular weight: 79.10

Flammable, colourless liquid; characteristic disagreeable odor; sharp taste. Flash point closed up 68°F; melting point-41.6 °F, boiling point 115.2-115.3 °F

Forms an azeotropic mixture with 3mols water, boiling at 92-93 °. volatile with steam, miscible with water, alcohol, ether, pet ether, oils and many organic and inorganic compounds. Weak base; forms salts with strong acid, pKa 5.19. pH of 0.2 molar solution in H_2O :8.5. LD_{50} orally in rats :1.58g/kg

Human toxicity: May cause CNS depression, irritation of skin and respiratory tract. Large dose may produce G.I. disturbances, kidney and liver damage (Budavari 1999).

2.4.2 Diethylamine

Molecular formula: $C_4H_{11}N$(C 65.68%, H 15.16%, N 19.15%)

Molecular weight: 73.14

Flammable, strongly alkaline liquid. Flash point <20°F; melting point-50 °, boiling point 55.5 °. miscible with water, alcohol, It is usually supplied as a solution. Keep well closed. LD_{50} orally in rats :540mg/kg (Budavari 1999).

2.4.3 Acetonitrile

Also named methyl cyanide; cynomethane; ethanenitrile

Molecular formula: C_2H_3N(C 58.51%, H 7.37%, N 34.12%)

Molecular weight: 41.05

Liquid. Ether like odor. Poisonous. Burns with a luminous flame. LD_{50} orally in rats: 3800mg/kg.

Caution: avoid breathing vapors. may cause skin irritation. (Budavari 1999)

2.5 Mass spectrum of TMS derivative of melamine

FDA published the method for analysis melamine and related compounds. Melamine tri-TMS derivative after react with pyridine and sylon BFT was identified as melamine presence. Therefore they published following mass spectrum. (Duan 2009)

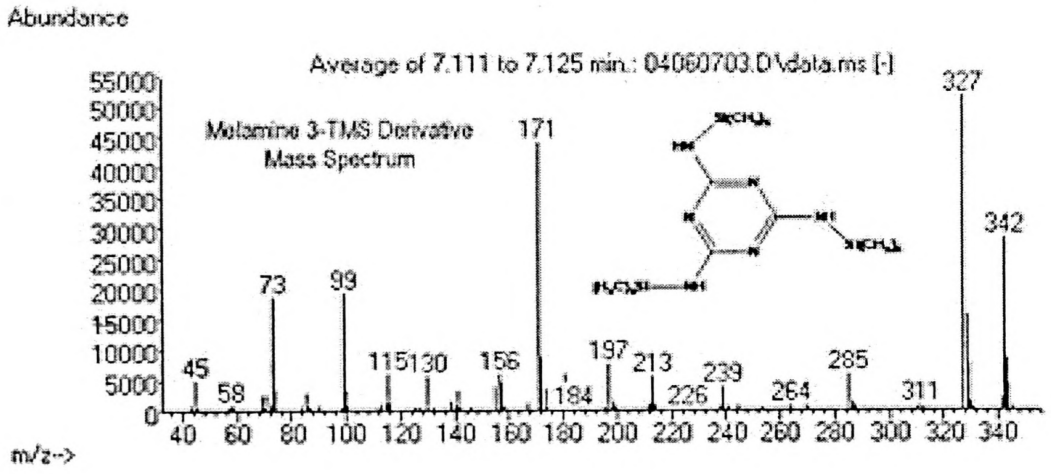


Figure 2.8 Mass spectrum of TMS derivative of melamine (sourse: Duan 2009).

CHAPTER 3

MATERIALS AND METHODOLOGY

3.1 Sample collection

All samples of milk powder were collected from the laboratories of SGS Lanka (pvt) ltd. They were including almost all brands of milk powders available in Sri Lankan market and also different batches and types.

3.2 Materials

3.2.1 Apparatus

Vials: 20ml

Volumetric flasks : Pyrex 10ml

Funnels

Micro pipette (10 µl -100 µl)

Pipette (1ml ± 0.05ml) /Bulb pipette (1ml)

GC vials

Polypropylene centrifuge tubes with screw caps (50ml)

Measuring cylinder (50ml, 100ml)

Teet pipette

Syringe : 25ml (TERUMO cor, TOREYO, JAPAN)

Syringe filter 25mm, 0.22 µm Nylon

N_2 gas flow

3.2.2 Equipments

- Vortex mixer (2800rpm)

Electronic balance (± 0.0001g)

Laboratory Centrifuge (5000rpm)

Hot plate (± 1°C)

Water bath (± 1°C)

3.2.3 Instruments

Gas chromatography Mass spectrometry (GCMS)

- Detector : MS ion trap
- Column: VF – 5 MS.
- Software : Varian MS Workstation 6.3

3.2.4 Reagents

1). Standard : Melamine CAS 108-78-1, Cat.240818-5g, Aldrich

2). Diethylamine (DEA), SigmaUltra, Sigma Chemical Co.

3). Pyridine, Certified A.C.S. Reagent, Fisher Scientific

4). Extraction Solvent: 10 / 40 / 50: DEA / Water / Acetonitrile

(Prepare a solution which consists of 10 parts (by volume) diethylamine, 40 parts water and 50 parts acetonitrile.) (This should be daily prepared)

5). Silylating Reagent: BSTFA with 1% TMCS: bis(trimethylsilyl)trifluoroacetamide with 1% Trimethylchlorosilane (e.g. Sylon BFT, Supelco)

3.3 Method

Melamine was analyzed by a modified method of U.S. FDA method of GC-MS screen for the presence of Melamine, Ammeline, Ammelide and Cyanuric acid (version 2.1). The details of the method development and analysis are given below.

3.3.1 Standards preparation

- To prepare 1000ppm of melamine standard solution; 10.00mg of Melamine was measured accurately and it was carefully transferred in to the 10ml volumetric flask.
- Then about 5ml of extraction solvent was added in to it. Volumetric flask was closed and shakes well until almost all Melamine was dissolved in solvent.

- Finally volume was made up to mark using extraction solvent.
- Other Melamine standard solutions were prepared by using serial dilution method. For example, 100ppm Melamine standard was prepared by using 1ml of 1000ppm melamine solution dissolved in extraction solvent and volume was made up to 10ml.

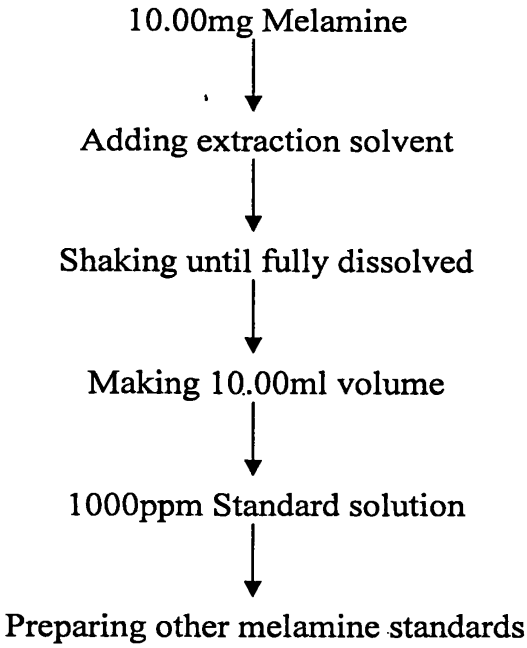


Figure 3.1 Flow diagram for the preparation of melamine standards.

3.3.2 Spiked sample analysis

A) Extraction procedure

- 5g of melamine free milk powder sample was weighed approximately and transferred to 50ml centrifuge tube.
- Required quantity of Melamine relative to the spike level was added to the milk powder sample.(as table below)

Table 3.1 Spiked quantity of standard for preparation of fortified samples

Spike level (ppb)	Melamine volume from 10 ppm Standard (µl)
0 (blank)	0
100	50
150	100
200	150

- Then 20ml of extraction solvent was added.
- Content was mixed well by using vortex mixer.
- Sample was centrifuged for 30minutes at 5000rpm.

B) TMS- Derivatization

- 200µl of supernatant was transferred to a GC vial.
- Content of GC vial was evaporated to dryness at 70°C by using hot plate maintained at 70°C and low flow stream of Nitrogen.
- 300µl pyridine was added to the GC vial and vortex well. 200µl BSTFA was added to the GC vial and vortex well.
- Then the content of GC vial was incubated at 70°C for 45minutes.If there any particles (insoluble material) present after incubation content was filtered to another GC vial using Syringe filter. After it was cooled to room temperature.

C) Analysis

- Then it was injected to the GC-MS, after setting following instrument parameters.

Instrument parameters

GC conditions

Column VF 5ms 50m*0.25mm*.25µm

Temperature 100°C 2min to 220°C at 10°C/min held 5min

Inject 260°C, splitless

Injected volume- 1µl

MS conditions

Transfer line 250°C

Trap- 180°C

Mode- selective ion system m/250 to 450

- Finally GC chromatograms and mass spectrums were collected.

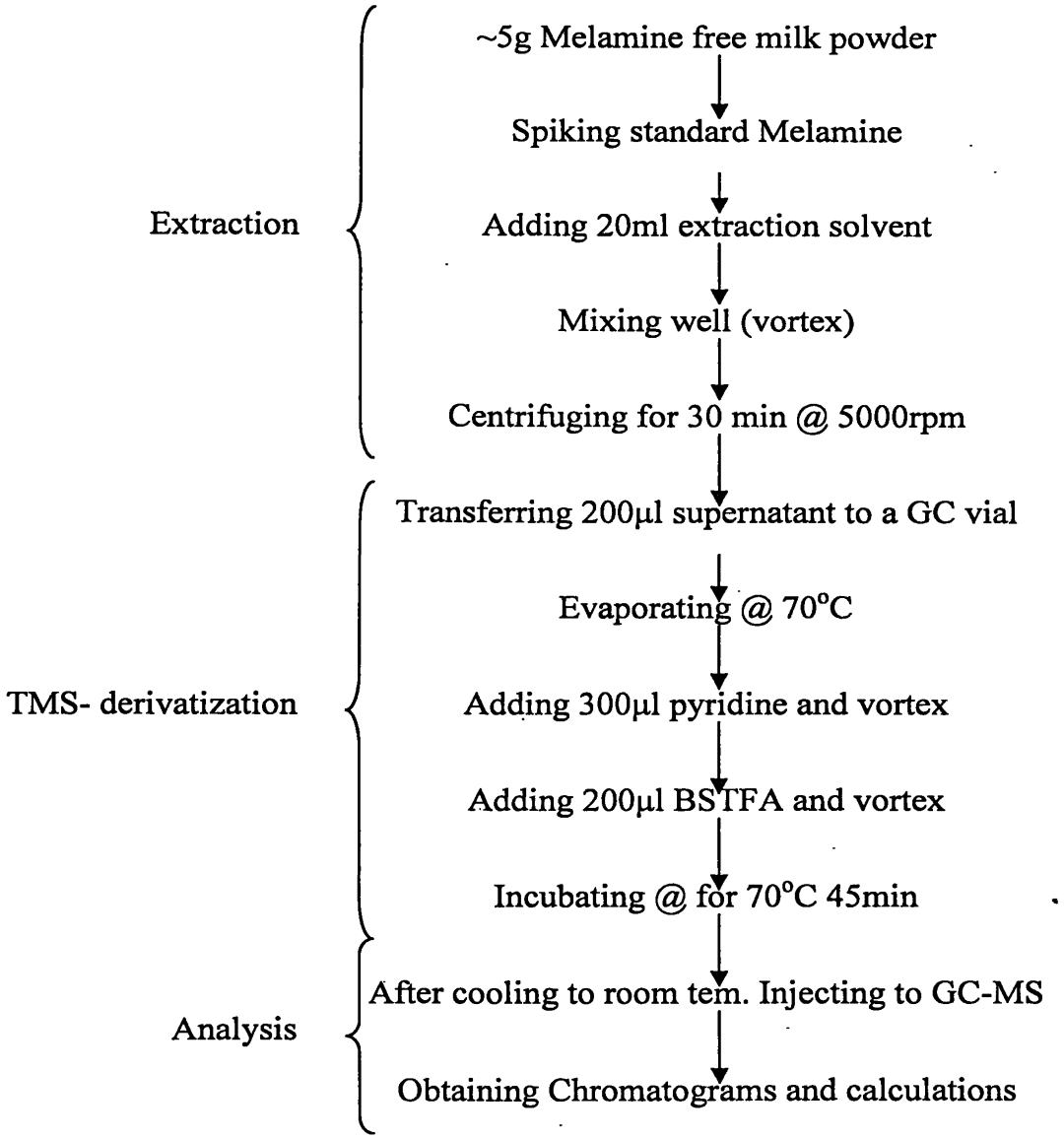


Figure 3.2 Flow diagram for the analysis of spiked samples.

3.3.3 Milk powder sample analysis

- 5g of commercially available milk powder sample was weighed approximately and transferred to centrifuge tube.
- 20 ml of extraction solvent was added into it.
- Other steps of procedure were same as above mentioned procedure in 3.3.2

3.3.4 Qualitative test method for Melamine

- 300µl pyridine and 200µl BSTFA were added to GC vial. It was incubated for 45min at 70°C. Content was injected to GC-MS and chromatogram and mass spectrum was obtained.
- 200µl of one of the melamine standard solution (Ex; 2ppm) was added to GC vial. TMS-derivatisation procedure (3.3.2.B) was done to that. Content of GC vial was injected to GC-MS and chromatogram and mass spectrum was collected.
- Two test portions with 5g of milk powder were obtained and significant amount of melamine standard was added to one sample and other was assumed to be melamine free control. Then procedure was followed as 3.3.2.

3.3.5 Quantitative test method for Melamine

3.3.5.1 Test for LOD

- Series of concentration of standard melamine solution were added into GC vial as follows.

Table 3.2 Preparation of melamine standards to LOD test

Concentration should be made	Added Melamine volume (µl)	
	From 10ppb	From 10ppm
25ppb	50	0
50ppb	100	0
100ppb	200	0
1ppm	0	200
2ppm	0	400

- TMS-derivatization was done according to the procedure in 3.3.2 B.
- Then series was injected to GC-MS after selection relevant GC-MS method.
- Chromatograms and mass spectrum were collected.

3.3.5.2 Preparing a standard curve for Melamine

- Standard series of melamine was prepared as 50 ppb, 100ppb, 150ppb, 200ppb and 250 ppb.
- After injecting those samples to GC-MS relevant peak areas were obtained

and regression analysis was done.

- Calibration curve was prepared.

3.3.5.3 Test for recovery percentage

- Four test portion of milk powder were weighed. Melamine standard was spiked according to the following table.

Table 3.3 Preparation of spiked samples for test of recovery

Concentration of melamine in Spiked sample (ppb)	Added volume of melamine from 10ppm (µl)
0	0
100	50
150	100
200	150

- Mentioned procedure in 3.3.2 was followed to samples.

3.3.5.4 Calculations

All calculations were done by using MINITAB 14 software.

3.3.5.4.1 Calculation of melamine concentrations

Melamine concentrations were calculated according to following equation obtained from regression analysis of peak area verses Melamine concentrations.

Peak area = 25.9 + 2.08 Concentration (ppb)

$$\text{Concentration} = \frac{\text{Peak area} - 25.9}{2.08}$$

3.3.5.4.2 Calculation of recovery percentage

$$\text{Recovery percentage} = \frac{\text{Calculated concentration} \quad * 100}{\text{Concentration of standard solution}}$$

Loss percentage = 100 - Recovery percentage

3.3.6 Analysis of milk powder samples for melamine

All milk powder samples were prepared and analyzed according to the procedure mentioned in 3.3.3.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Standards preparation

Melamine is white crystalline powder. Slightly soluble in water, very slightly soluble in hot alcohol and insoluble in ether (Paranagama 2008). Therefore before analysis, melamine standard solutions were prepared by dissolving melamine powder in extraction solvent. Solution which consist 10 parts (by volume) diethylamine, 40 parts distilled water, 50 parts acetonitrile. Above solution is used, because melamine powder is well dissolved in this solution than 20:80 mixture of diethylamine and distilled water. Selected extraction solvent turns yellow with time. It should be store in the dark. To prevent any interference to analysis from extraction solvent, solvent mixture was daily prepared.

Weighing correct amount, measuring correct volumes are the critical steps of the standard preparation, otherwise loss the accurate results. Store stock solutions of standards in the refrigerator to retard hydrolysis. It has not been established how rapidly the solutions degrade but the potential does exist (FDA 2007). Therefore analysis of prepared stock solutions was carried out within a day.

4.2 Spiked sample analysis

4.2.1 Extraction

After weighing milk powder sample, melamine solution and extraction solvent were added. The mixture should be mixed well to ensure thoroughly wet the entire milk powder sample. Then sample is centrifuged for more than 30 minutes at 5000rpm to extract all the melamine to the extraction solvent.

4.2.2 Chemical derivatization

Melamine is not a volatile compound itself. Therefore chemical derivatization of melamine is very important in GC-MS analysis.

200µl of supernatant are taken to the GC vial. A smaller aliquot may be used provided that the necessary sensitivity level (10 µg/g of sample) is achieved. Reducing the amount of matrix present improves the general performance of the evaporation/derivatization step and saves wear and tear on the instrument.

Taking the filtrate completely to dryness is a critical step in the derivatization process. The presence of water prevents formation of TMS derivatives of the analytes. If the internal standard response is much lower than usual (less than 30%), there may have been problems associated with the derivatization step. In addition, if the vial warms significantly to the touch after addition of the derivatization reagents, residual water was present and a new aliquot of filtrate must be prepared.

The solution was mixed and then allowed to react for 45min at 70°C to complete derivatization. The derivatization of melamine is shown in figure 4.1.

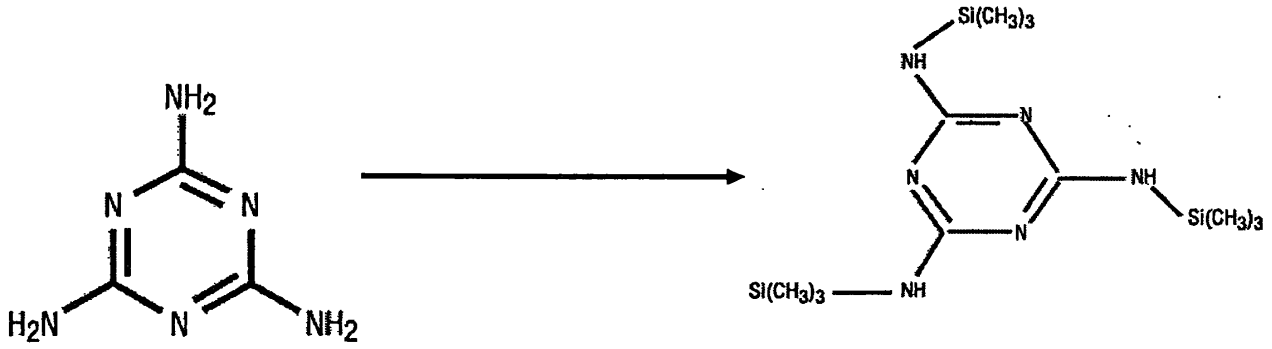


Figure 4.1 Formation of melamine derivative using silylating reagent.

Accepted safety measures should be employed when working with chemicals and pressurized gases. It is advisable to work in a fume hood whenever the procedure calls for the use of diethylamine and pyridine. Use caution with diethylamine as it is a volatile strong base and can cause chemical burns (FDA 2007). The preferred method of transferring measured volumes of sample solutions or reagents is by use of a micro liter syringe. Small volume glass pipettes are a cheaper alternative, but require more skill in their use and allow more contact of reagents with the air. Contact of reagents and solvents with plastic tips is best avoided, especially when low amounts of analyte are being determined (Watson 1993).

4.2.3 GC-MS analysis

The performance of the method may change when different equipment and supplies are used or when different sample matrices are encountered.

The multiple reactions monitoring trace of derivatized melamine spiked in milk powder is shown in figure 4.2.

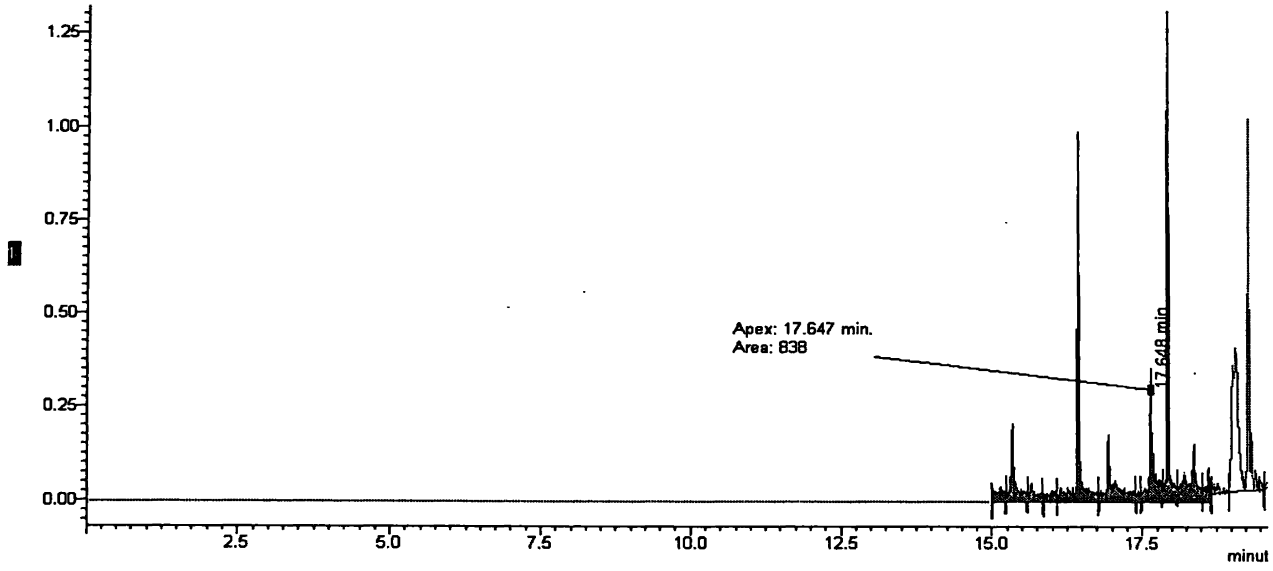


Figure 4.2 Gas chromatogram of milk powder spiked with 2ppm melamine.

The product ion spectrum obtained from derivatized melamine is shown in figure 4.3. Product ion ratios can be used to obtain qualitative confirmation.

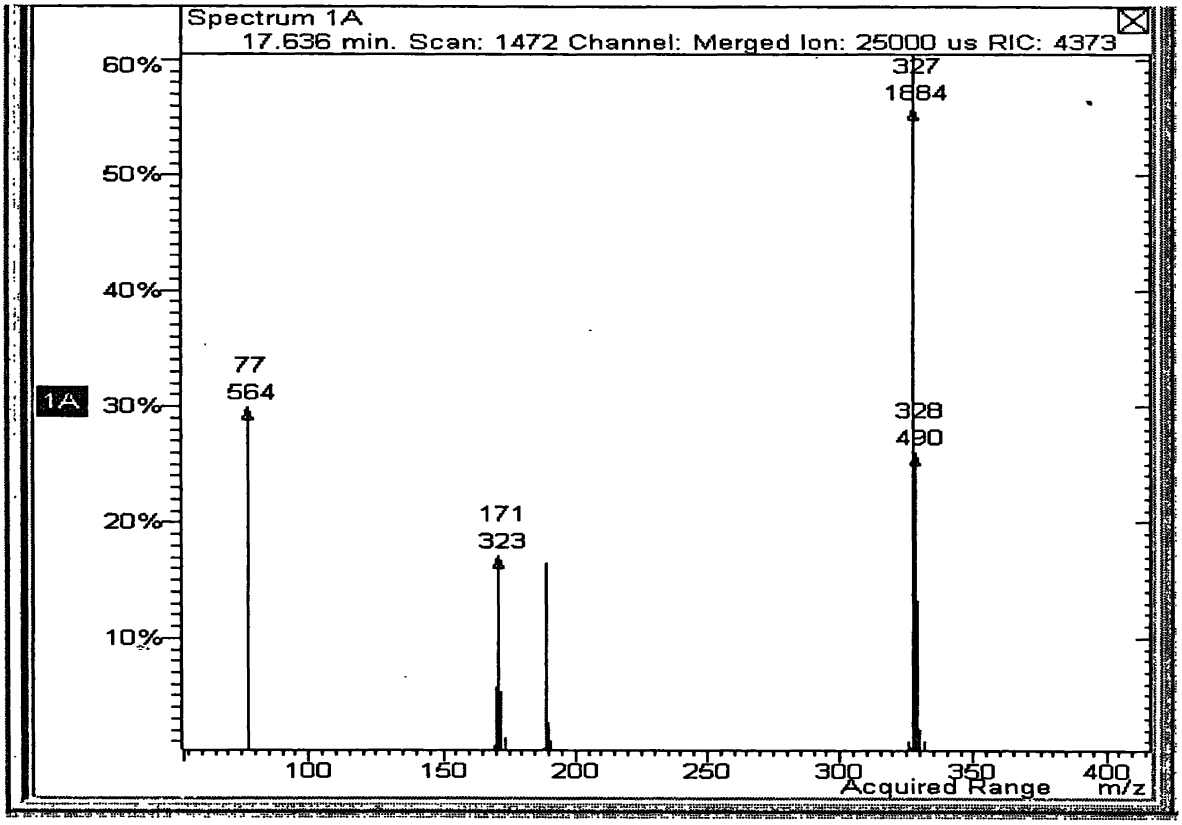


Figure 4.3 MS/MS product ion spectrum of melamine derivative in powdered milk.

4.3 Qualitative test for Melamine

According to qualitative test method for melamine, the blank was injected. After the injection a chromatogram was obtained (appendix 01). There was no any peak relevant to the melamine.

Using standard solution of melamine the retention time of melamine was identified (Appendix 01). The retention time of melamine was around 17.64 minutes for the standard solution. Retention time can vary in this range according to the pump pressure and quality of mobile phase.

According to result of qualitative test for melamine, a comparison test was carried out between controlled and spiked samples. As a result a peak area corresponding to melamine was observed for the spiked sample. Where as no corresponding peak area was observed for the Controlled sample.

4.4 Quantitative test for Melamine

4.4.1 LOD Test

At the results of minimum detection limit, the corresponding peak areas of melamine for 50ppb, 100ppb, 1ppm and 2ppm standard solutions were present in chromatograms. There was the corresponding peak area of melamine for 25ppb standard solutions, but Signal to noise ratio is not an acceptable level. Determination of the signal to noise ratio is comparing measured signals from samples with known low concentrations of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably detected. A signal to noise ratio between 3:1 or 2:1 is generally acceptable for estimating the detection limit (SPRING Singapore 2002). And also resulted peak is deviated from other peaks.

4.4.2 Calibration curve of Melamine

Concentration of melamine was calculated by a standard curve (calibration curve). Standard curve was plotted using peak area of five different standard solutions of melamine, 50ppb, 100ppb, 150ppb, 200ppb and 250ppb (Appendix 03). The MINITAB 14 statistical software was used to plot standard curve. Graph was concentration of melamine standard Vs resulted peak area (Appendix 04).

Table 4.1 Peak area at different concentration given by GC-MS

Melamine Concentration (ppb)	Resulted peak area
50	157
100	224
150	278
200	485
250	547

Calibration curve (appendix 04) demonstrates excellent linearity of the ion trap for the analysis, with R^2 value of 0.9450.

4.4.3 Recovery percentage

At the result of recovery test, three test portions resulted in different peak area. The peaks areas were proportional to the spiked concentration.

Table 4.2 Peak area at different spike concentration given by GCMS

Spiked melamine concentration	Results (peak area)
50ppb	122
100ppb	189
150ppb	280

Recovery percentage was calculated by using calibration curve (Appendix 05). Finally thirty commercially available milk powder samples were analyzed including different brands, types, and batches. Results are in appendix 06.

4.5 Limitations

This analysis requires hazardous solvents (e.g. pyridine, acetonitrile, etc) and derivatization. Also require more sample preparation time and run time.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

GC-MS method for melamine analysis was developed according to facilities, prevailing at SGS Lanka (pvt) Ltd laboratory.

Qualitative and quantitative test methods for melamine were satisfied. The limit of detection was 50ppb. Recovery percentage was 84.08% and loss percentage was 15.92%, indicating suitability for usage.

The detection limit of GCMS machine is 1ppb. The residue level may be lower than that; it can not be detected with this instrument.

The LOD of this method is comparatively lower than HPLC method and also less sample preparation time and run time than routine HPLC method.

The research results conclude that, the developed technique is effective and accurate for analyzing melamine in milk powder.

Among thirty commercially available samples tested (including different brands, varieties, and batches) none of the samples contained melamine.

Therefore it can be concluded that milk powders marketed in Sri Lanka are safe with regard to melamine adulteration.

5.2 Recommendations

- It is recommended to access the facility of a sonicator to fine tune the extraction procedure of melamine.
- It is recommended to study the derivatization procedure of melamine in deep to identify other possible derivatives.
- Due to the hazardous nature of chemicals used for the study and the need of much sample preparation and run time to this technique, LC-MS method would be the preferable method to carry out this analysis.

REFERENCES

Bassett, J., Denvey, R.C., Jeffery, G.H. and Mendham, J. (1989) Vogel's text book of quantitative chemical analysis, 5th edition, English language book society Longman. 877p.

Bartle, K.D. Introduction to the theory of chromatographic separations with reference to gas chromatography, 1-15pp edited by Baugh, P.J.(1993) Gas chromatography A practical approach, Oxford university press, 427p.

Bradley, D. (2008) Melamine in milk (Available from: http://www.sciencebase.com/science-blog/melamine-in-milk.html accessed on: 13th December 2008)

Budavari, H. (1999) The Merck index, 11th edition, published by Merck and company. Inc, 1606p.

Christion, G.D. (1994) Chromatographic methods in Analytical chemistry, 5th Ed, johnwiley and sons.INC, 809p.

Duan,A.(2009) Infant milk powder contaminated with toxic melamine, (Available from:http:malcolm4chemist.blogspot.com/2008/09/infants accessed on 04th March 2009).

Evershed, P.R. Combined gas chromatography mass spectrometry, 359-393pp edited by Baugh, P.J.(1993) Gas chromatography A practical approach, Oxford university press, 427p.

Jimmy, W.,Joan, N. and Robert, S .(2007) Determination of Melamine, Ammeline, ammelide, and Cyanuric Acid Contamination in Wheat Gluten and rice Protein Concentrate (Available from: http://acs.confex.com/acs/mwrm07/techprogram/P51682.HTM accessed on 31st January 2009).

LankaPage (LLC) (2008) Sri Lanka sets limits for melamine content in milk products,(Available from: http://www.colombopage.com/archive_08/November13144108RA.html, accessed on 14th November 2008).

Nivas, R.H. (2008) the global horror of melamine (Available from: http://www.dailynews.lk/2008/10/04/news23.asp accessed on 10th October 2008).

Paranagama, P.A. (2008) Melamine and the milk powder scandal, CCS news letter, vol. 7 No. 2, 4-5pp.

Rahman, M.A. (Dr.) (2008) Melamine menace (Available from: http://www.sciencebase.com/science-blog/melamine-in-milk.html accessed on 8th November 2008.)

Skoog, D.A. and West, M.D. (1971) principles of instrumental analysis, Rinehart and Winston,Inc 710p.

Smith, S.E. (2008) what is melamine? (Available from: http://www.wisegeek.com/what- is-melamine.htm accessed on 10th October 2008).

SPRING Singapore (2002) Method validation for chemical testing, guidance notes C&B ENV 001, SPRING Singapore , 6-8pp

Srilal de Silva (Dr.) (2008) Fragile Food Safety Regulations and Contaminated Milk Powder (Available from: http://www.dailymirror.lk/DM_BLOG/Sections/frmNewsDetailView.aspx?ARTID=29854 accessed on 10th October 2008).

Srivasthava,V.K. and Srivasthawa, K.K. (1987) Introduction to chromatography Theory and Practices, 3rd edition, S.Chand and company(pvt)Ltd,132p.

Tipler, A. gas chromatography instrumentation, operation, and experimental considerations, 15-71 edited by Baugh,P.J.(1993) Gas chromatography A practical approach, Oxpord university press, 427p.

The Taipei times (2008) (Available from: http://www.taipeitimes.com/News/taiwan/archives/2008/09/28/2003424438 accessed on 08th November 2008).

U.S. Food and drug administration Laboratory Information Bulletin No. 4396 May 2007 (Available from:http://www.cfsan.fda.gov/~frf/lib4396.html accessed on 10th November 2008).

U.S. Food and drug administration GC-MS screen for the presence of Melamine and Cyanuric acid (25 April 2007) (Available from: http://www.fda.gov/cvm/MelaminePresence.htm accessed on 12th November 2008).

Watson, D.G., Chemical derivatization in gas chromatography, 133-171pp edited by Baugh, P.J. (1993) Gas chromatography A practical approach, Oxford university press, 427p.

Wikipedia Foundation, Inc (2008) Melamine, (Available from: http://en.wikipedia.org/wiki/Melamine accessed on13th November 2008).

Wikipedia, The free encyclopedia. (2008) Chromatography, (Available from: http://en.wikipedia.org/wiki/chromatography accessed on13th November 2008).

Wikipedia. (2008) High performance liquid chromatography in: the free encyclopedia, GNU free documentation license, (Available from http://Highperformanceliquidchromatography-Wikipedia,thefreeencyclopedia.htm Accessed on 25th February 2009).

Wikipedia, The free encyclopedia. (2008) powdered milk (Available from: http://en.wikipedia.org/wiki/Powdered_milk accessed on15th February 2009).

World health organization (2008) melamine contamination event (Available from: http://www.who.int/foodsafety/fs_management/infosan_events/en/index.html accessed on 10th October 2008).

World health organization (2008) questions and answers on melamine (Available from: http://www.who.int/foodsafety/publications/micro/pif2007/en/index.html accessed on 10th October 2008).

Appendix 01

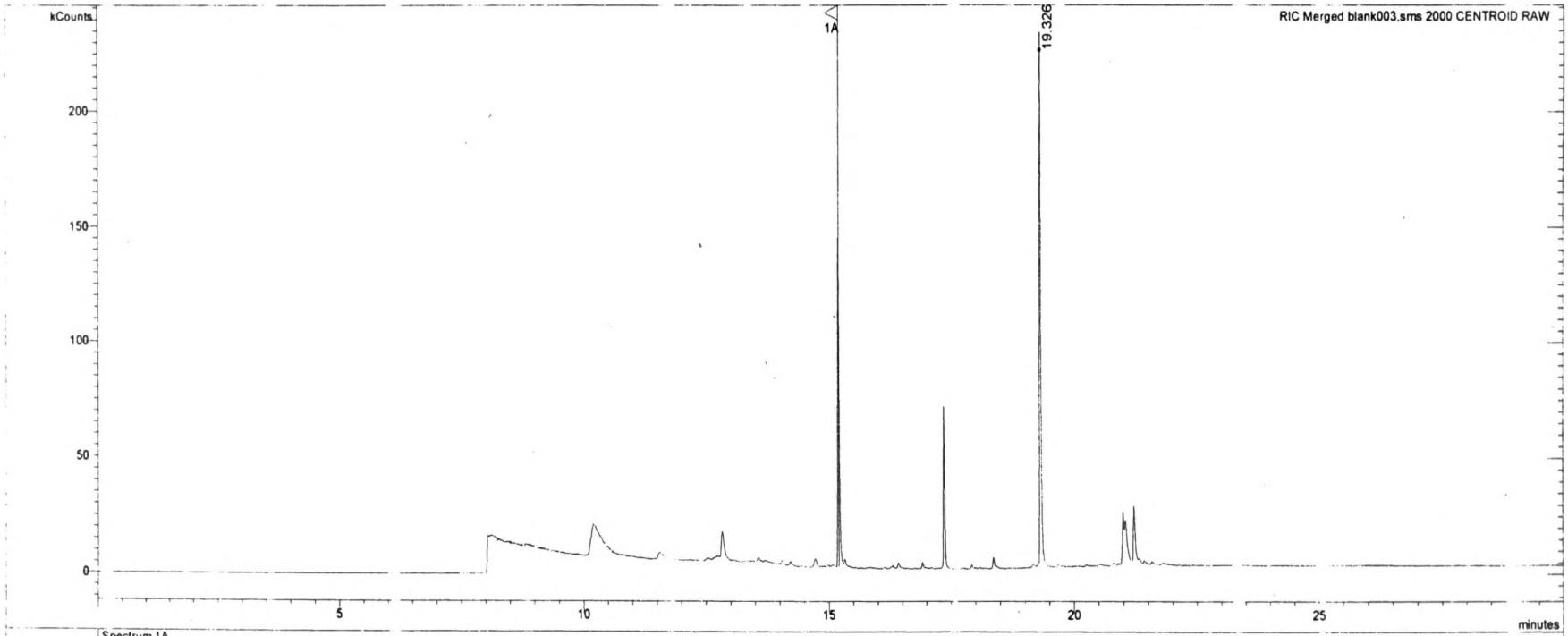
Chromatograms and mass spectrum plots of qualitative test.

- (a) Chromatogram of blank (pyridine + BSTFA)
- (b) Mass spectrum for first peak of blank
- (c) Mass spectrum for second peak of blank
- (d) Mass spectrum for third peak of blank
- (e) Chromatogram of Melamine standard solution
- (f) Mass spectrum of Melamine standard solution
- (g) Chromatogram of Melamine free milk powder sample
- (h) Chromatogram of Melamine spiked milk powder sample
- (i) Mass spectrum of Melamine spiked milk powder sample

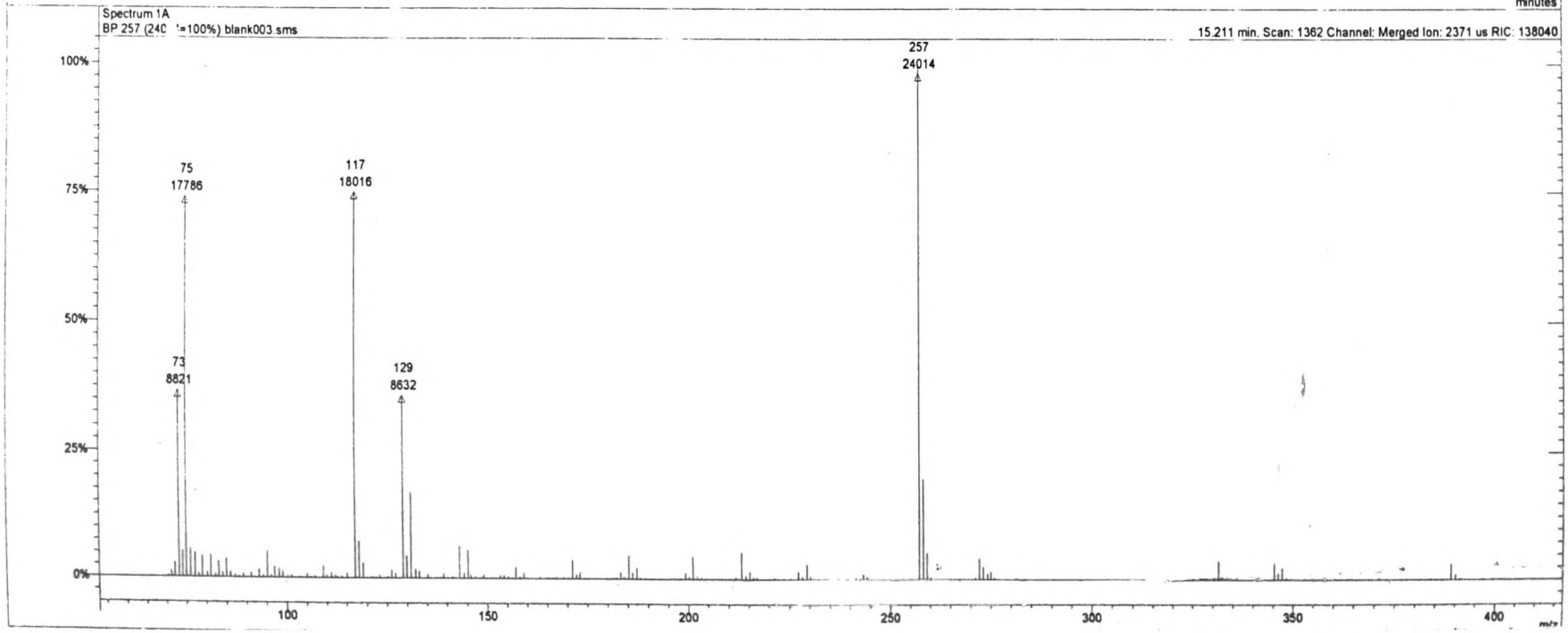
Print Date: 25 Feb 2009 16:08:52

MS Data Review Active Chromatogram and Spectrum Plots - 2/25/2009 4:08 PM

(a)



(b)



Print Date: 25 Feb 2009 16:10:30

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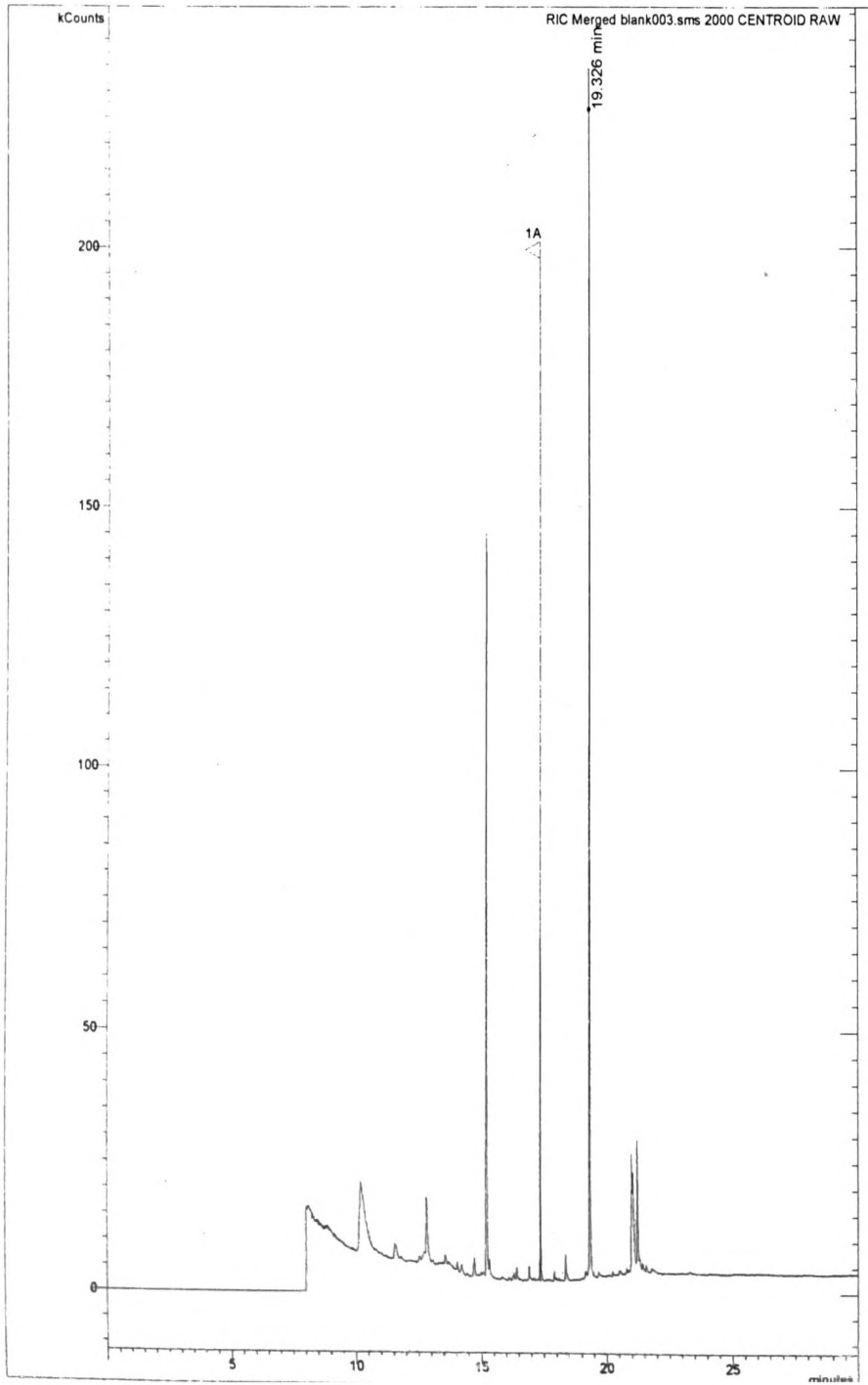
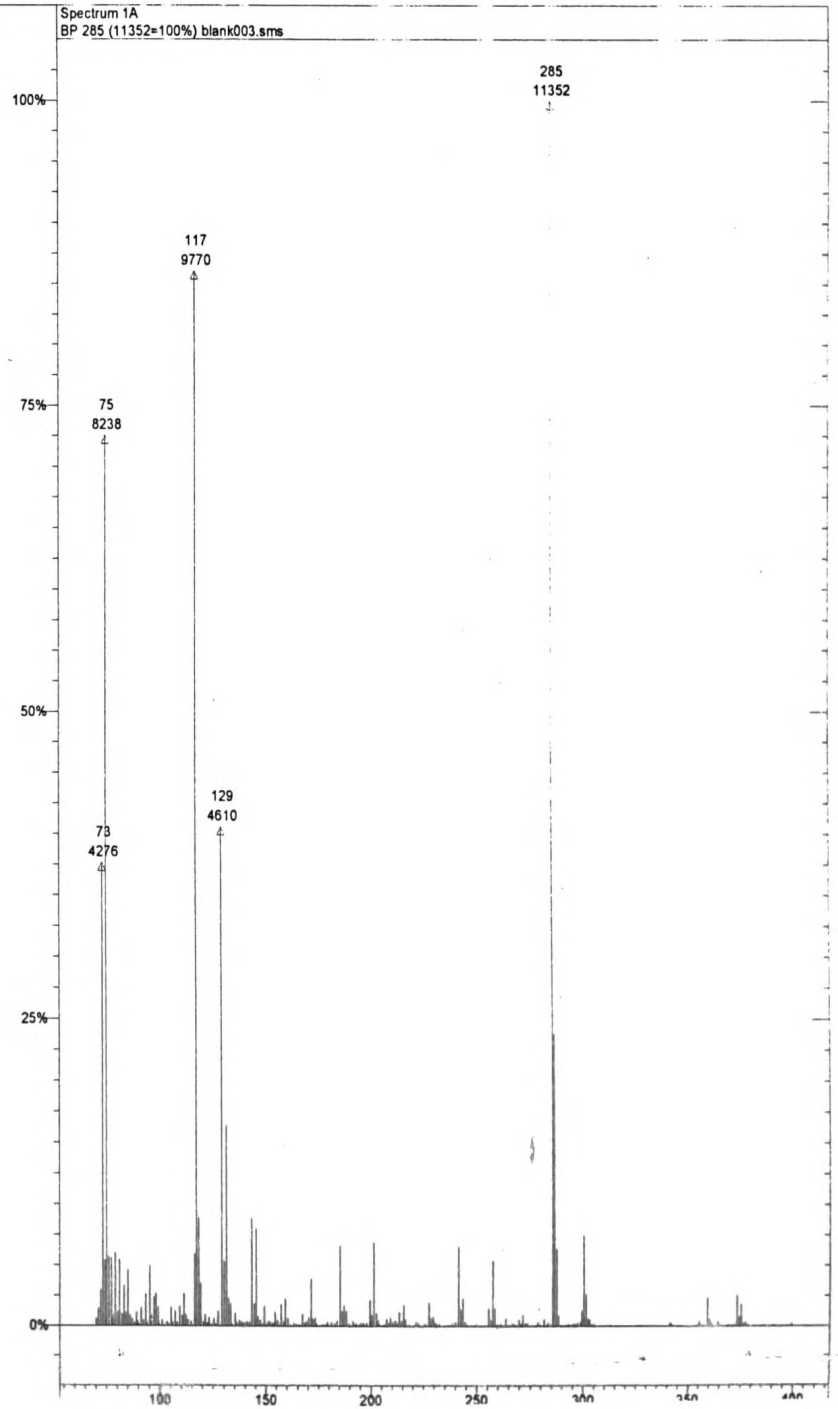


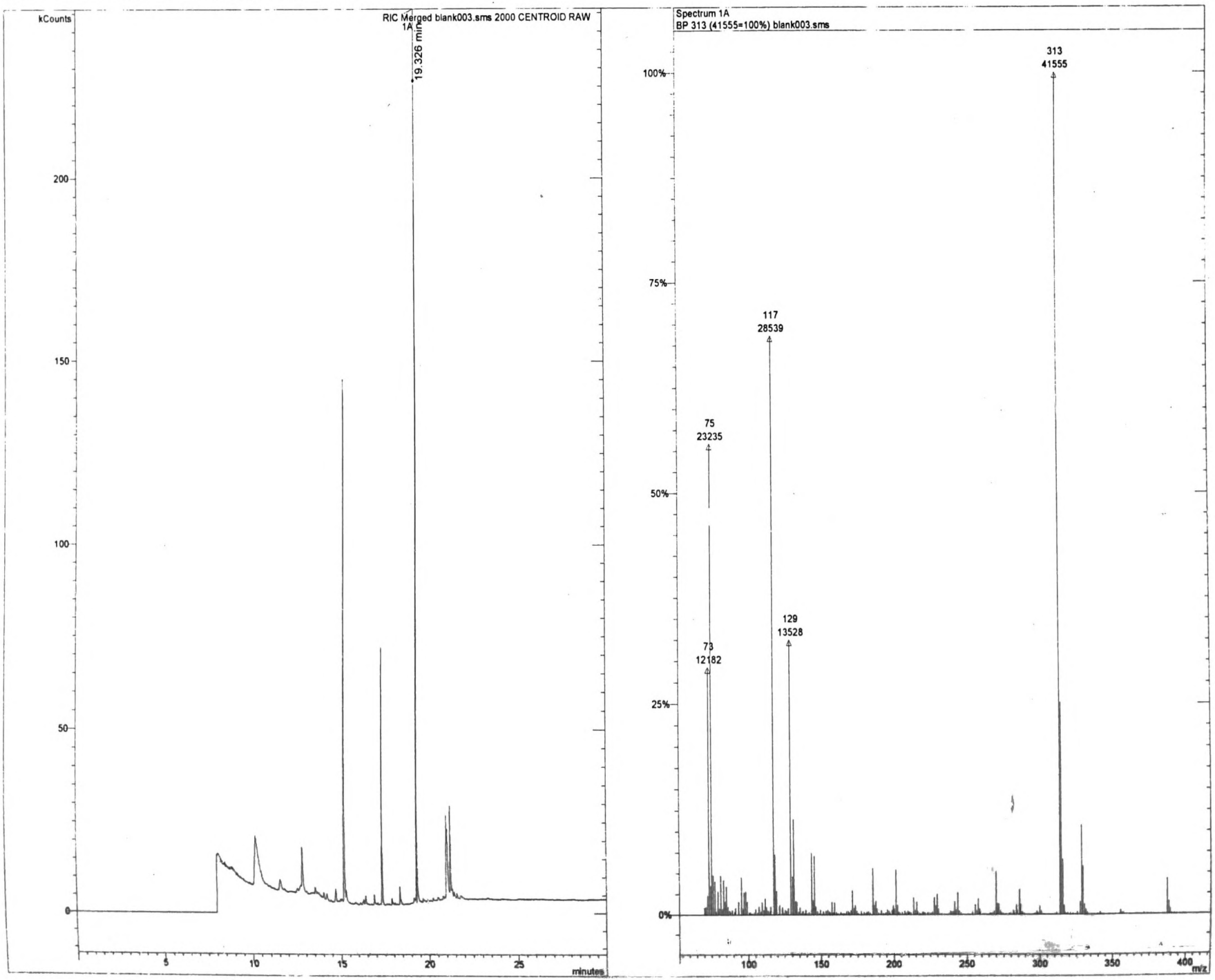


(c)

42

Print Date: 25 Feb 2009 16:12:15

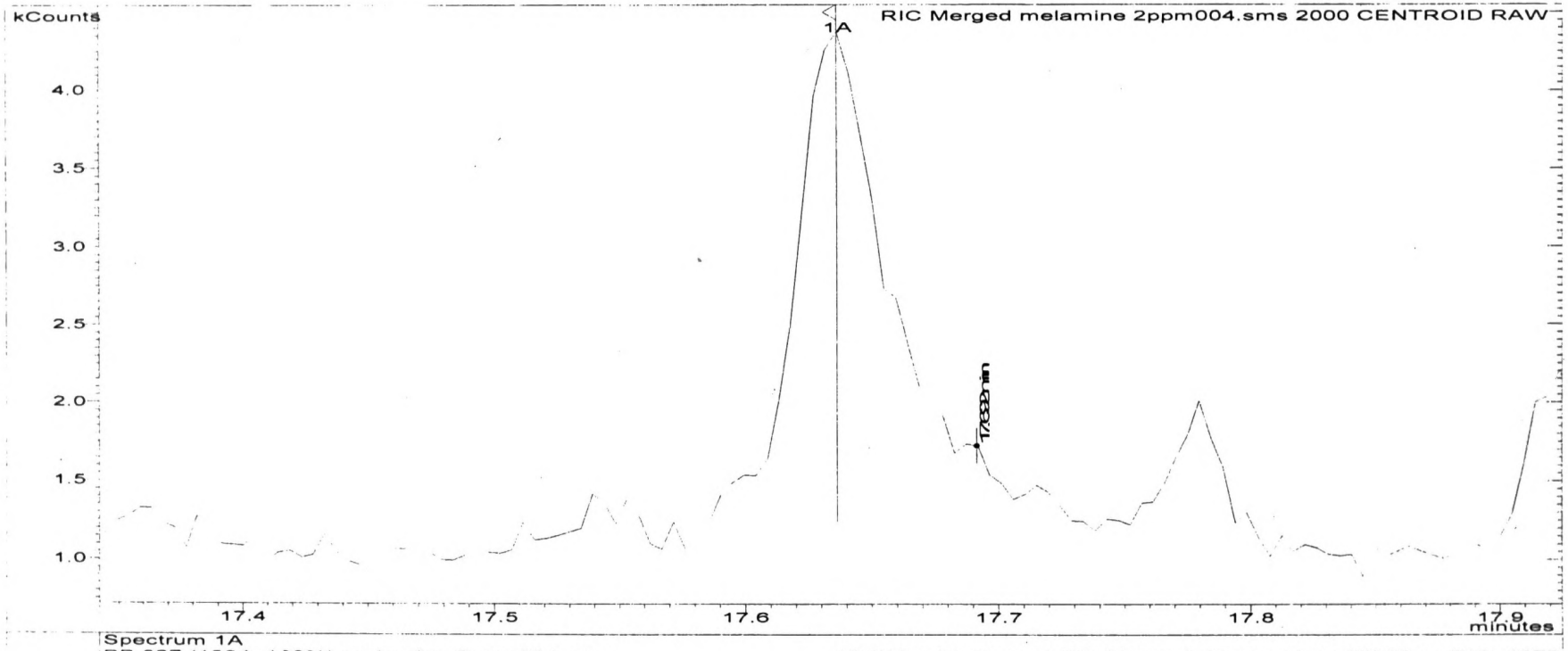
MS Data Review All Plots - 2/25/2009 4:12 PM



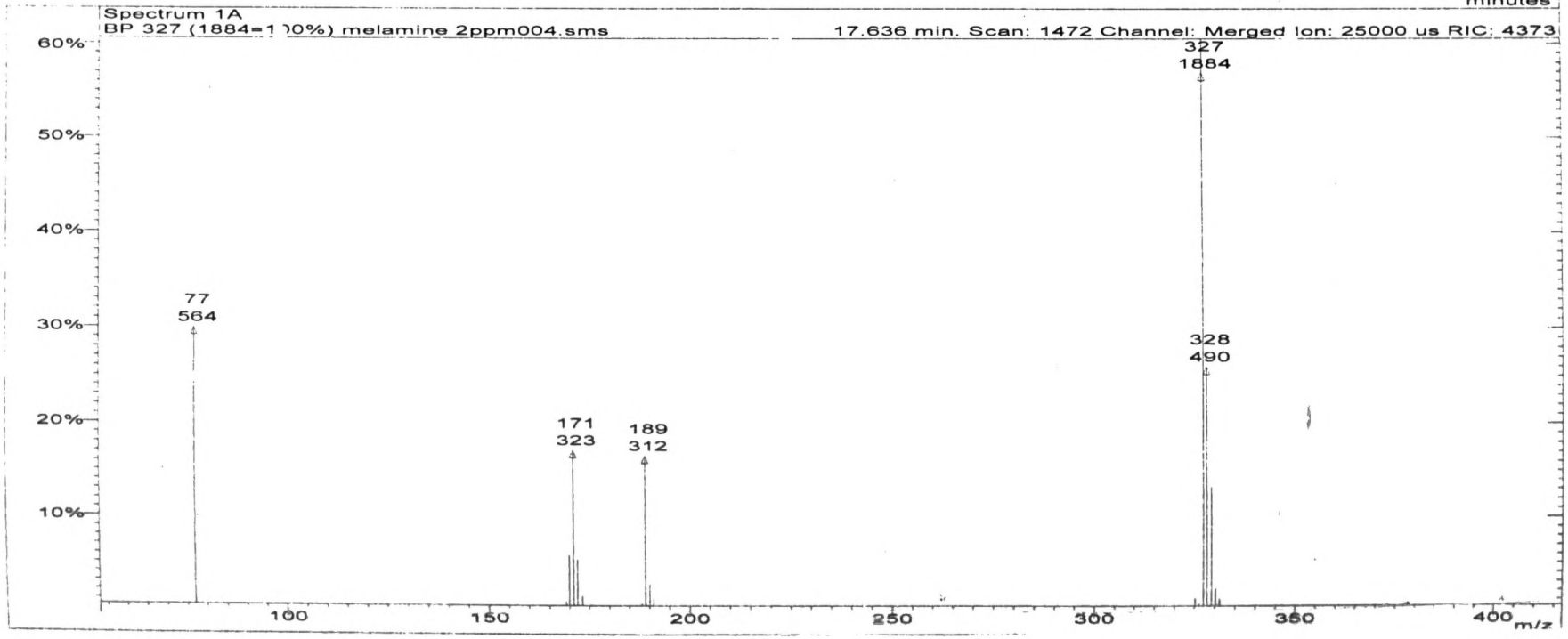
(d)

MS Data Review Active Chromatogram and Spectrum Plots - 1/22/2009 1:49 PM

(e)

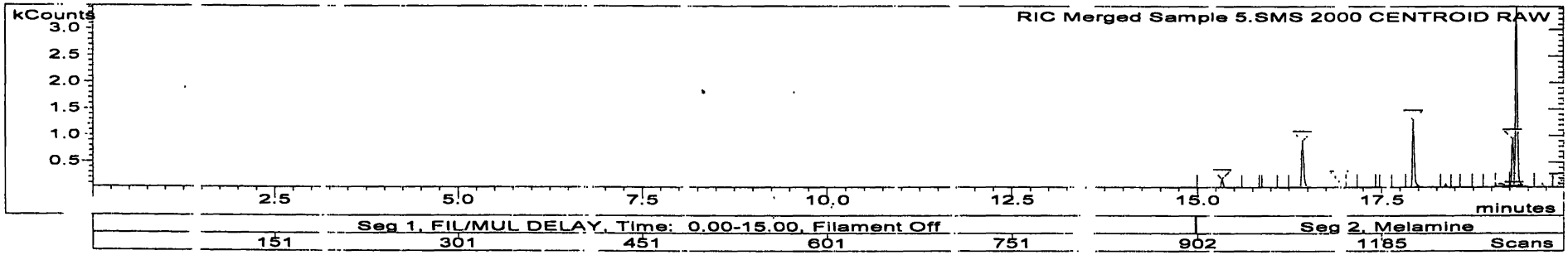


(f)



SGS Lanka /Analysis of Melamine

Acquisition Date:	1/22/2009 7:48 PM	Sample ID:	Sample 5
Data File:	...md\tasl\sample 5.sms	Method:	...amine 327 target.mth
Instrument ID:	Gc-Ms	Last Calibration:	1/8/2009 3:11 PM
Calculation Date:	1/22/2009 8:08 PM		
Inj. Sample Notes:	None		
Identified Compounds:	0		



(9)

Target Compounds

#	RT	Compound Name	Res Type	Quan Ions	Area	Amount
1	17.648	Melamine	Miss.	327.4	0	0.000 ppm

Unidentified Peaks

None

****

Calibration Log

****

Injection Time/Date	Recalc Time/Date	Calibration Log File
1/8/2009 1:00 PM	1/8/2009 1:20 PM	...m coloumn\melamine\1 cal .5ppm.sms
1/8/2009 1:28 PM	1/8/2009 1:48 PM	...0m coloumn\melamine\1 cal 1ppm.sms
1/8/2009 1:55 PM	1/8/2009 2:15 PM	... coloumn\melamine\1 cal 1.5ppm.sms
1/8/2009 2:23 PM	1/8/2009 2:43 PM	...0m coloumn\melamine\1 cal 2ppm.sms
1/8/2009 2:51 PM	1/8/2009 3:11 PM	... coloumn\melamine\1 cal 2.5ppm.sms

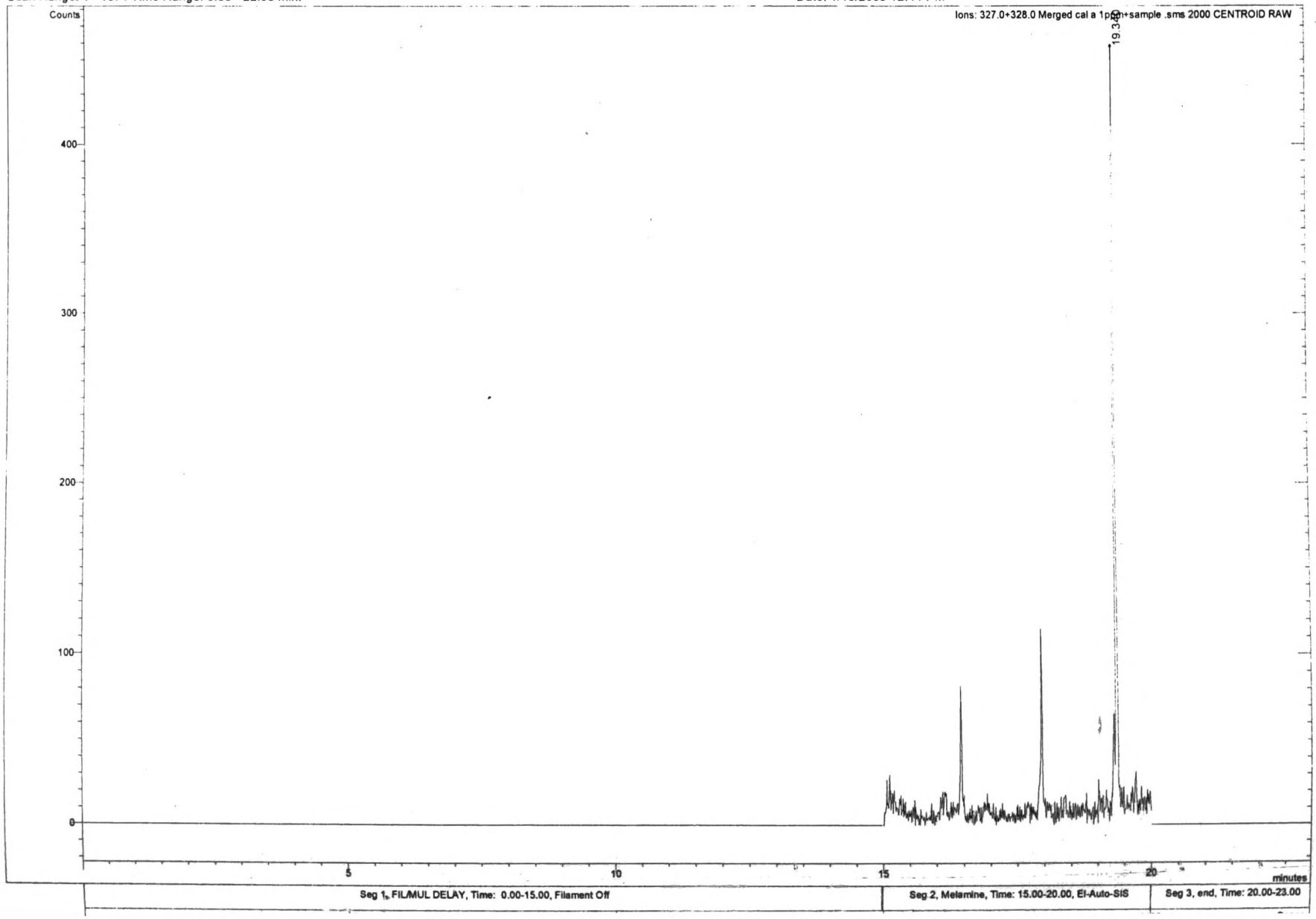
45

Print Date: 18 Feb 2009 16:24:02

Chromatogram Plot

File: ...ianws\data\standerd for 50m coloumn\melamine\cal a 1ppm+sample .sms
Sample: Cal A 1ppm+sample
Scan Range: 1 - 1674 Time Range: 0.00 - 22.98 min.

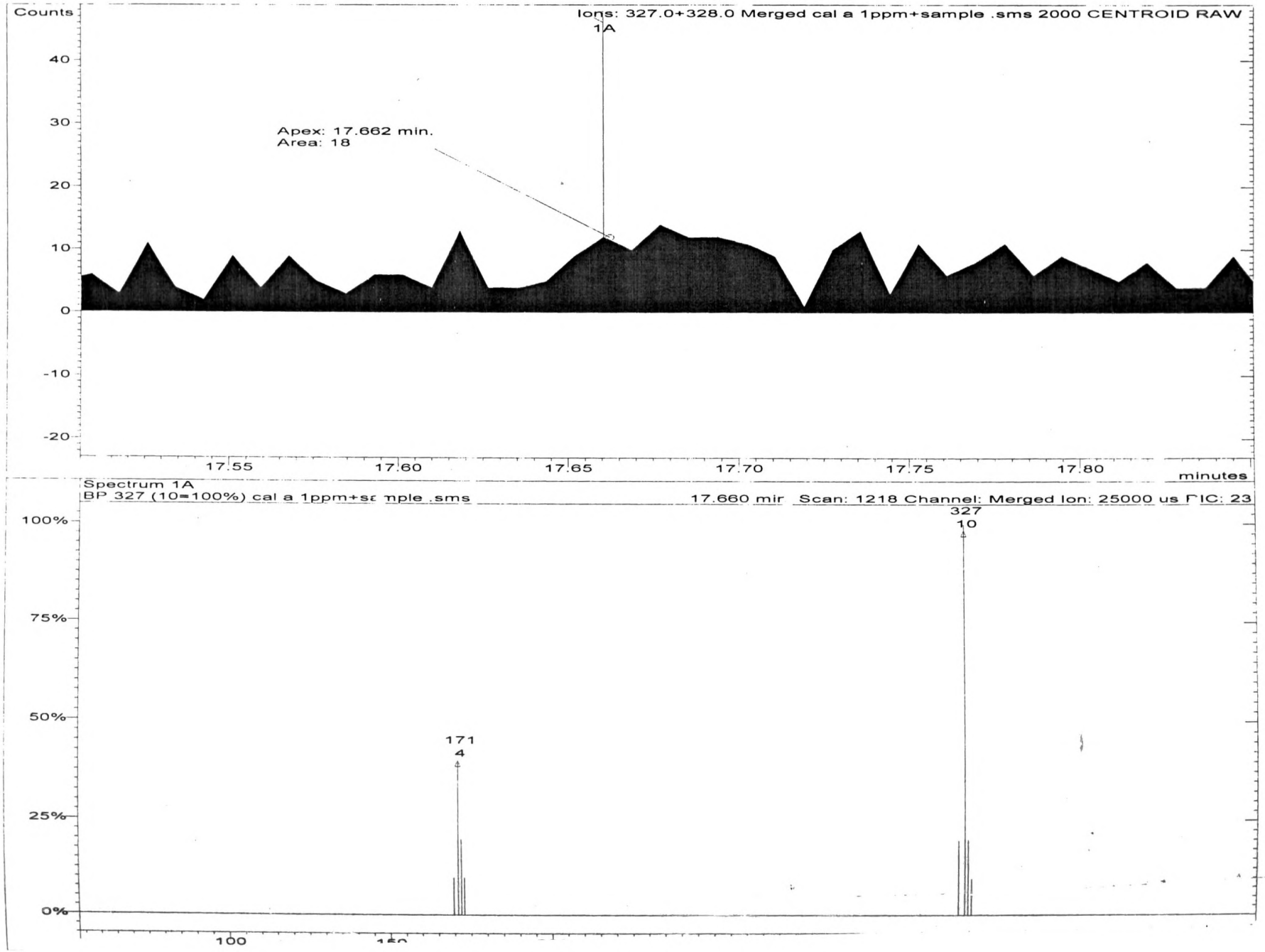
Operator: Darshana SGS
Date: 1/10/2009 12:44 PM



(H)

Print Date: 18 Feb 2009 16:15:47

MS Data Review All Plots - 2/18/2009 4:15 PM



47

Appendix 02

Chromatograms and mass spectrum plots obtained for LOD test.

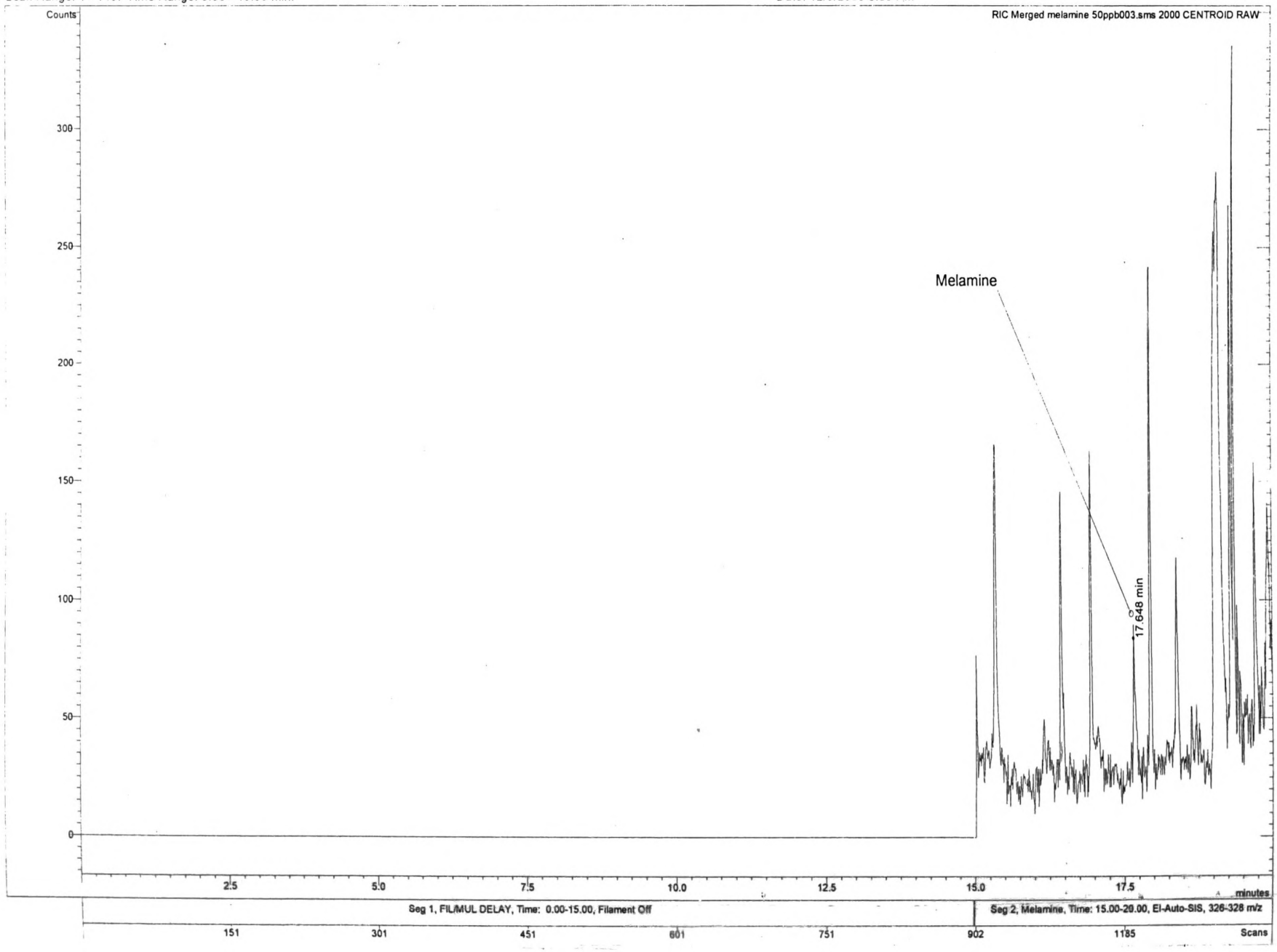
- (a) 50ppb standard
- (b) 100ppb standard
- (c) 2ppm standard

Print Date: 22 Jan 2009 13:24:27

Chromatogram Plot

File: ...rianws\data\standerd for 50m coloumn\melamine\melamine 50ppb003.sms
Sample: Melamine 50ppb
Scan Range: 1 - 1467 Time Range: 0.00 - 19.99 min.

Operator: Darshana
Date: 12/9/2008 3:39 PM



(a)

Print Date: 22 Jan 2009 13:34:21

Chromatogram Plot

File: ...ianws\data\standerd for 50m coloumn\melamine\melamine 100ppb002.sms
Sample: Melamine 100ppb
Scan Range: 1 - 1467 Time Range: 0.00 - 19.99 min.

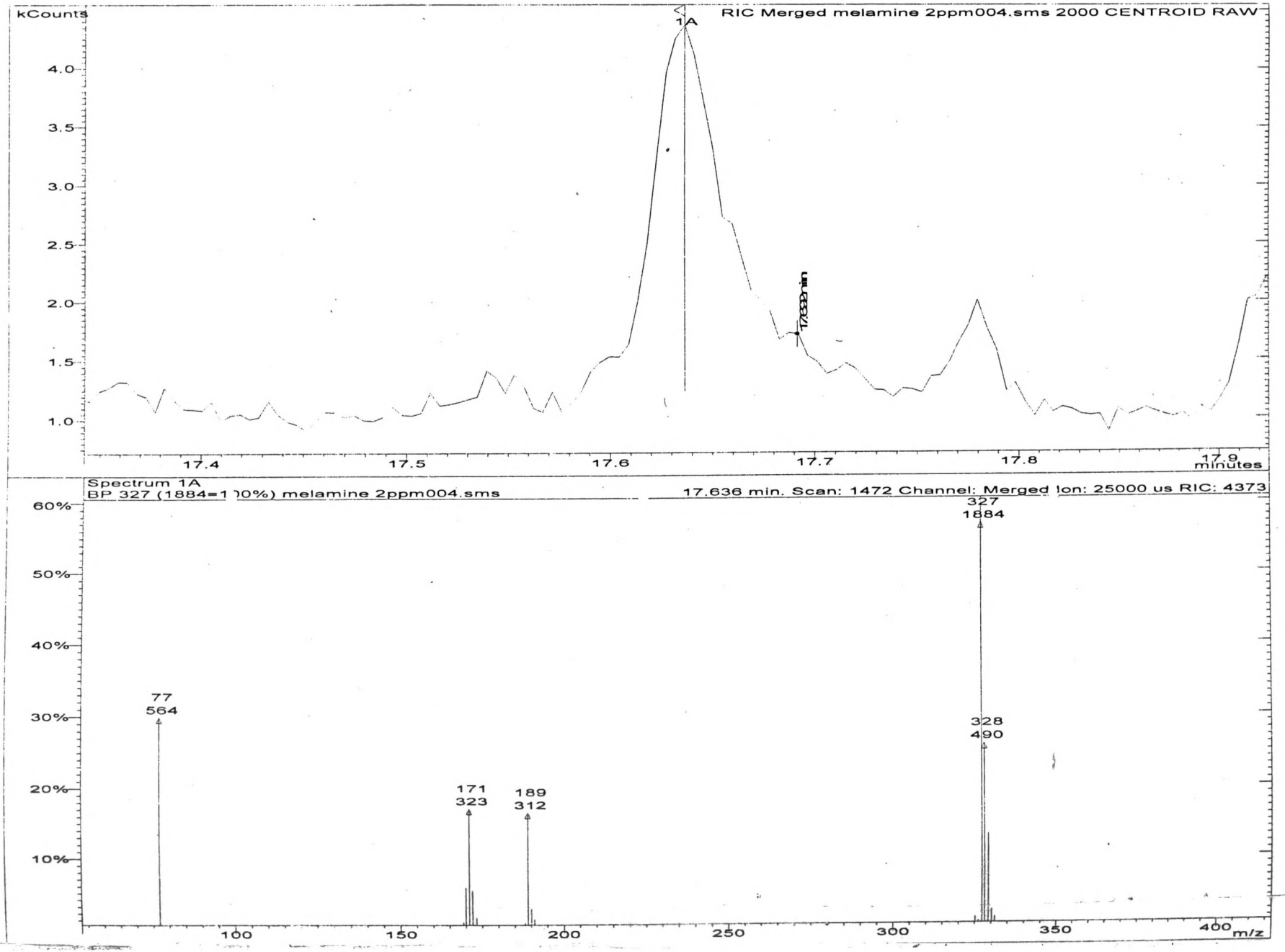
Operator: Darshana
Date: 12/9/2008 4:06 PM

RIC Merged melamine 100ppb002.sms 2000 CENTROID RAW

Print Date: 22 Jan 2009 13:49:09

MS Data Review Active Chromatogram and Spectrum Plots - 1/22/2009 1:49 PM

(c)



Appendix 03

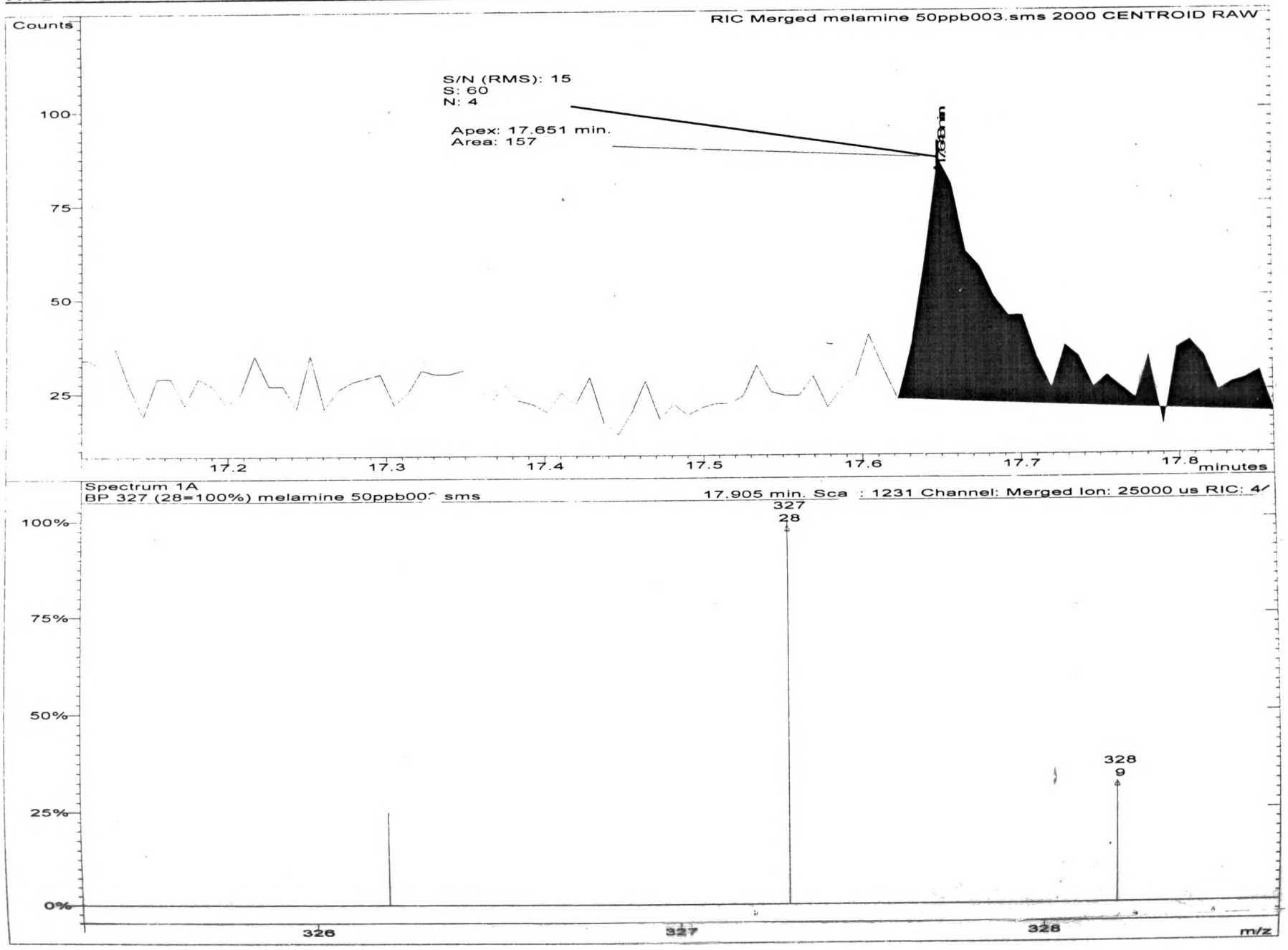
Chromatograms and mass spectrum plots obtained for Melamine standard curve.

- (a) 50ppb standard
- (b) 100ppb standard
- (c) 150ppb standard
- (d) 200ppb standard
- (e) 250ppb standard

Print Date: 22 Jan 2009 13:27:41

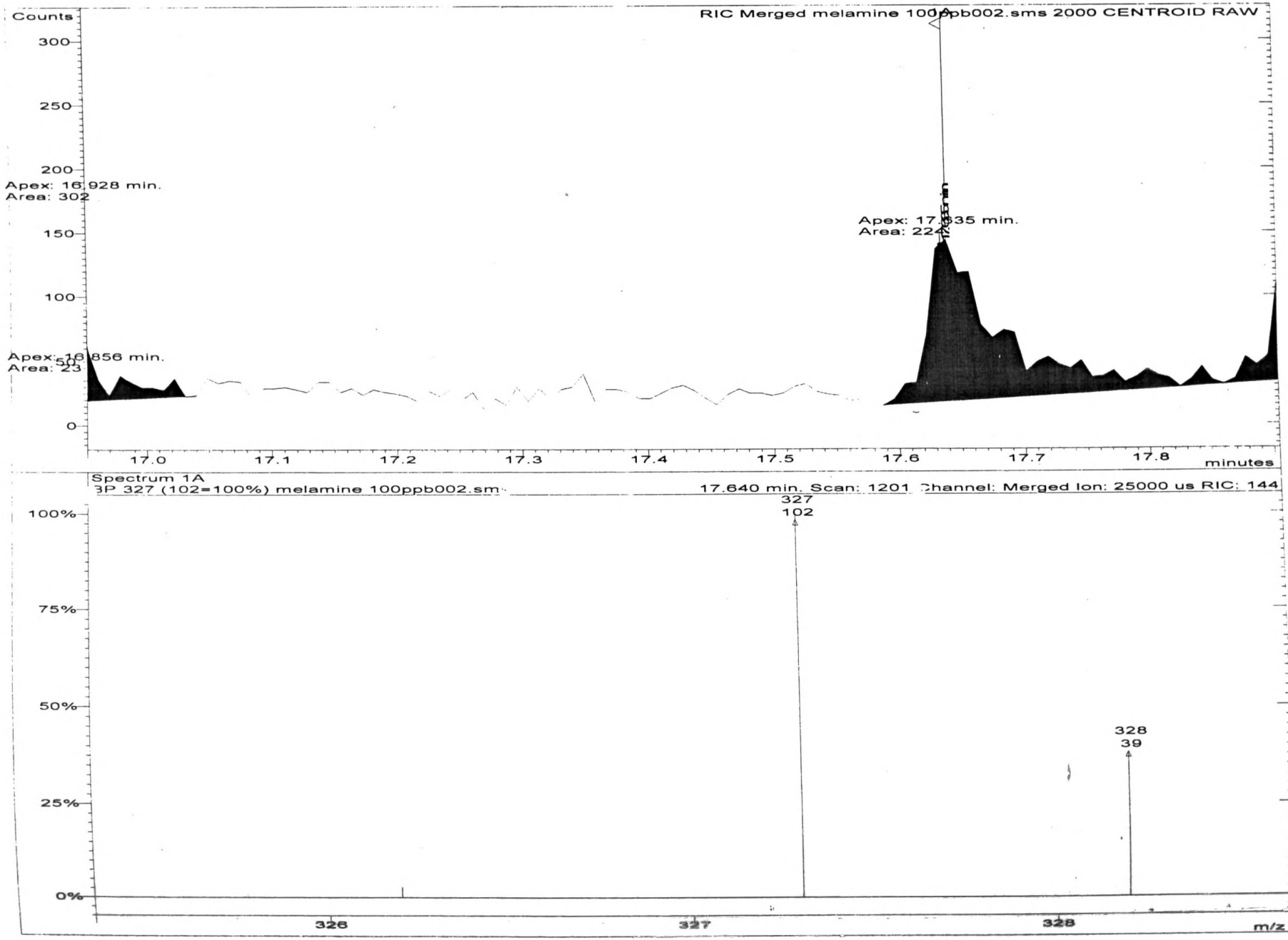
MS Data Review All Plots - 1/22/2009 1:27 PM

(a)



Print Date: 22 Jan 2009 13:35:24

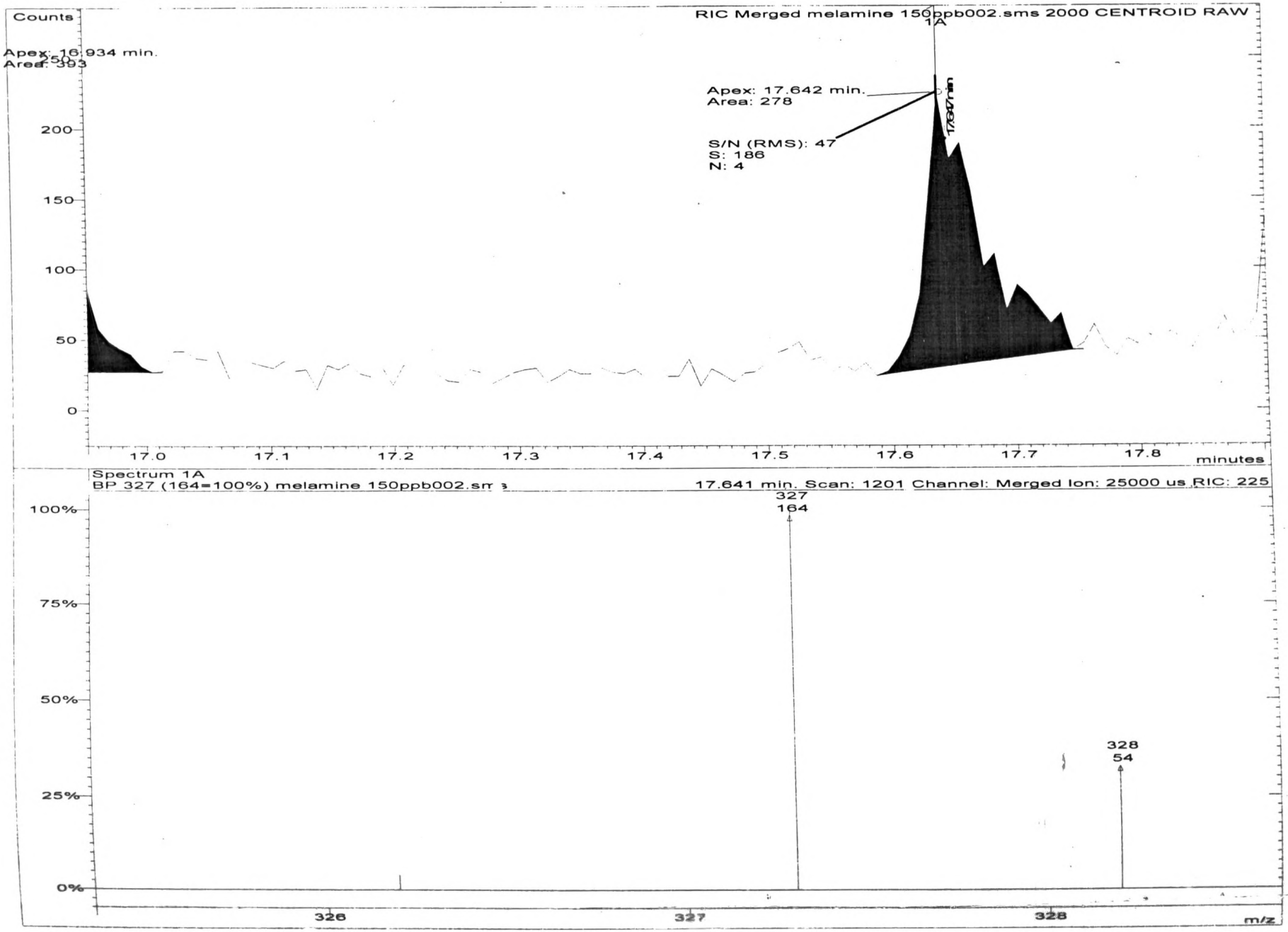
MS Data Review All Plots - 1/22/2009 1:35 PM



(b)

Print Date: 22 Jan 2009 13:38:25

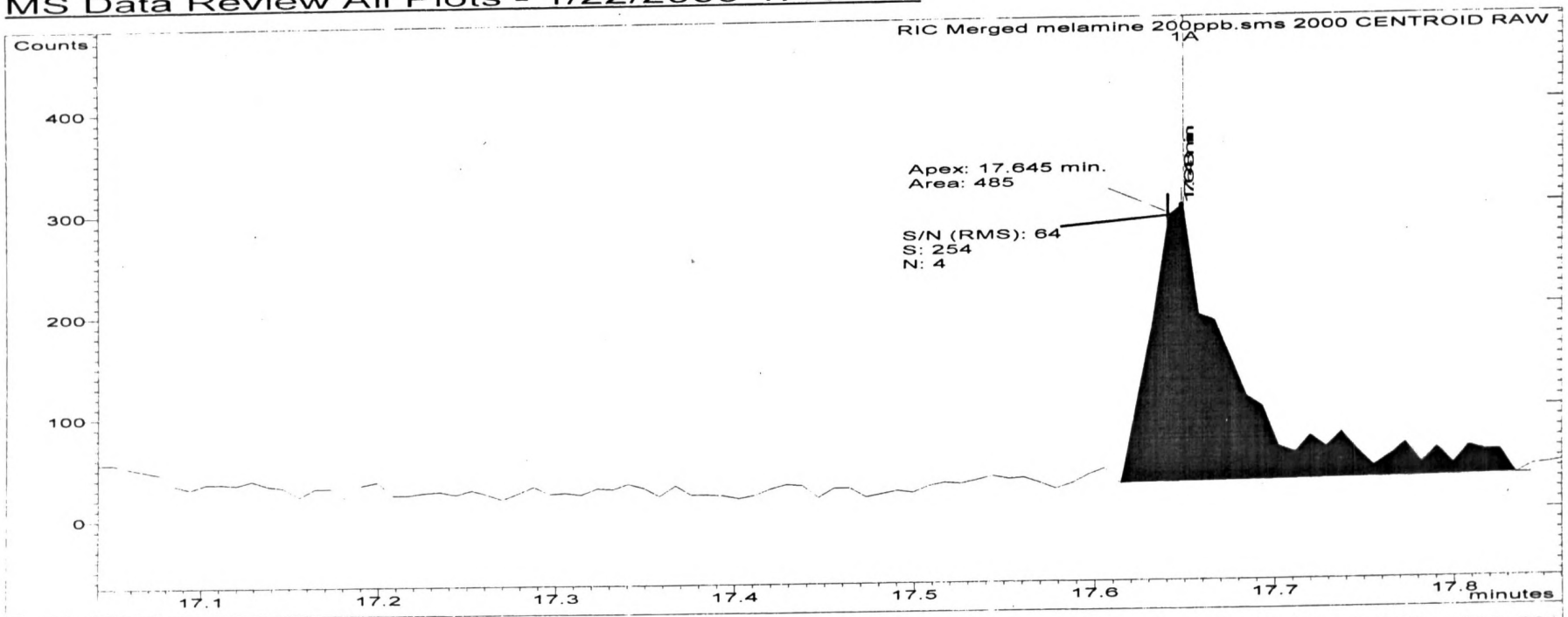
MS Data Review All Plots - 1/22/2009 1:38 PM

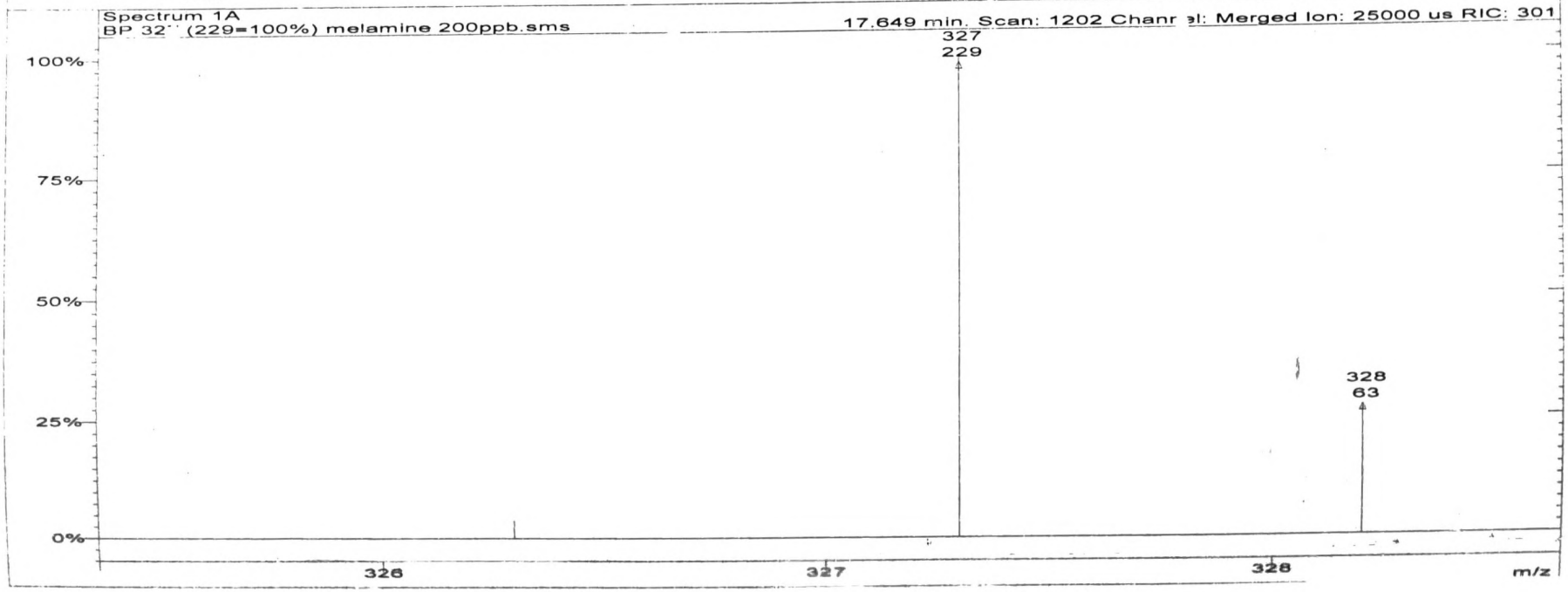


(c)

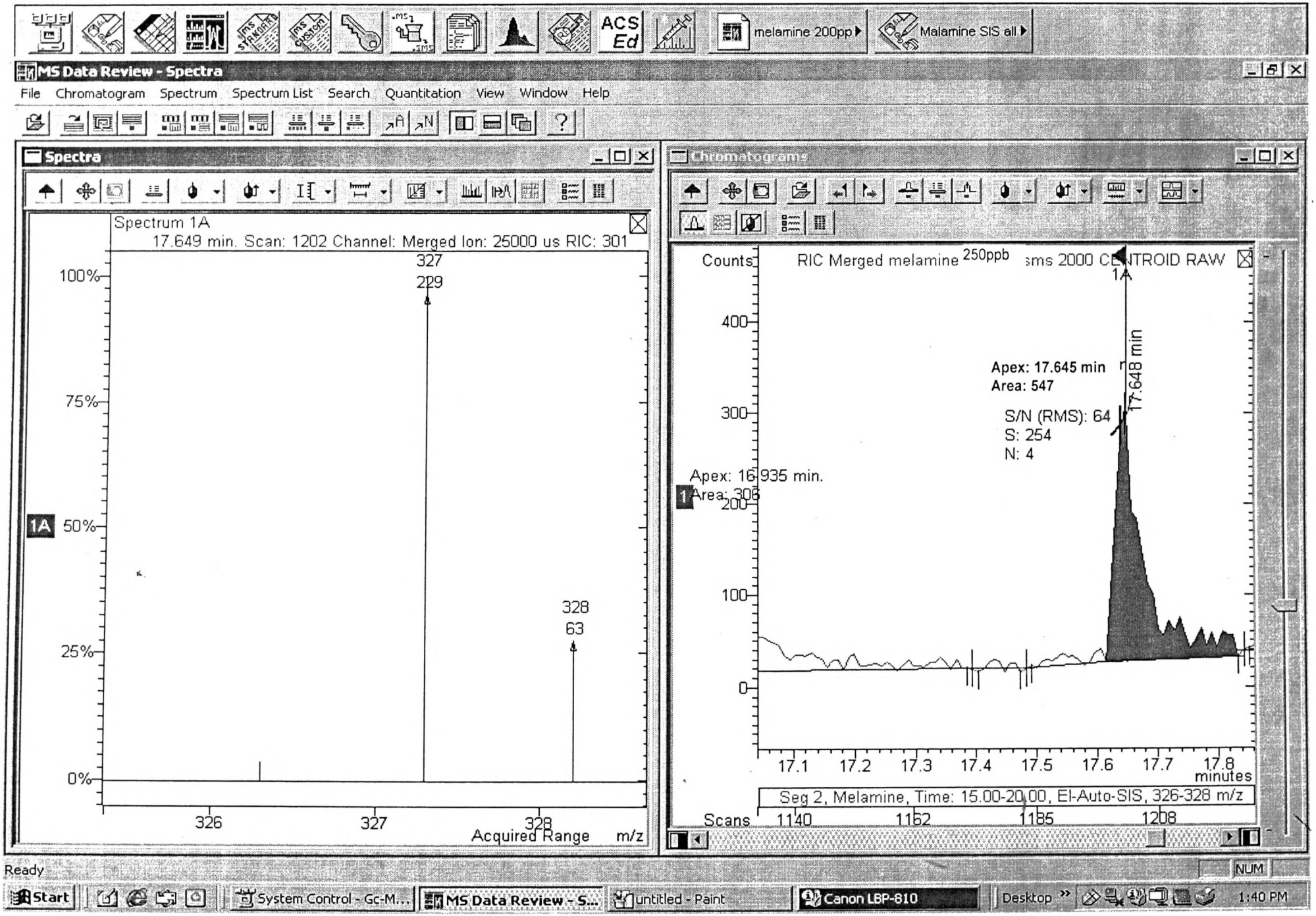
Print Date: 22 Jan 2009 13:40:29

MS Data Review All Plots - 1/22/2009 1:40 PM





(d)

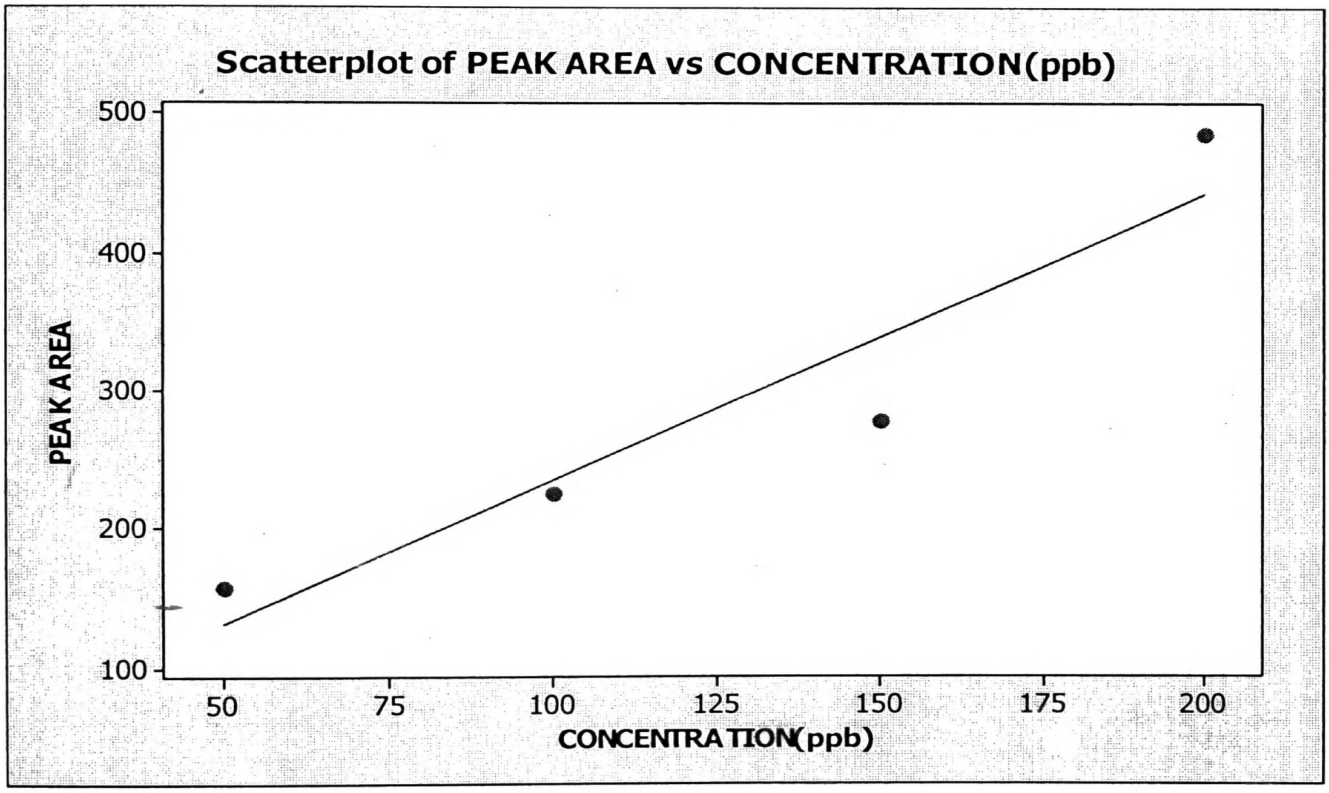

melamine 200pp
Malamine SIS all
MS Data Review - Spectra
File Chromatogram Spectrum Spectrum List Search Quantitation View Window Help
Spectra
Spectrum 1A
17.649 min. Scan: 1202 Channel: Merged Ion: 25000 us RIC: 301
100%
75%
50%
25%
0%
1A
327
229
328
63
326
327
328
Acquired Range
m/z
Chromatograms
Counts
RIC Merged melamine 250ppb
1A
400
300
200
100
0
Apex: 17.645 min
Area: 547
S/N (RMS): 64
S: 254
N: 4
17.648 min
Apex: 16.935 min.
Area: 306
17.1
17.2
17.3
17.4
17.5
17.6
17.7
17.8
minutes
Seg 2, Melamine, Time: 15.00-20.00, EI-Auto-SIS, 326-328 m/z
Scans
1140
1162
1185
1208
Ready
NUM
Start
System Control - Gc-M...
MS Data Review - S...
untitled - Paint
Canon LBP-810
Desktop
1:40 PM

Appendix 04

- Calculations for calibration curve

Regression Analysis: PEAK AREA versus CONCENTRATION (ppb)

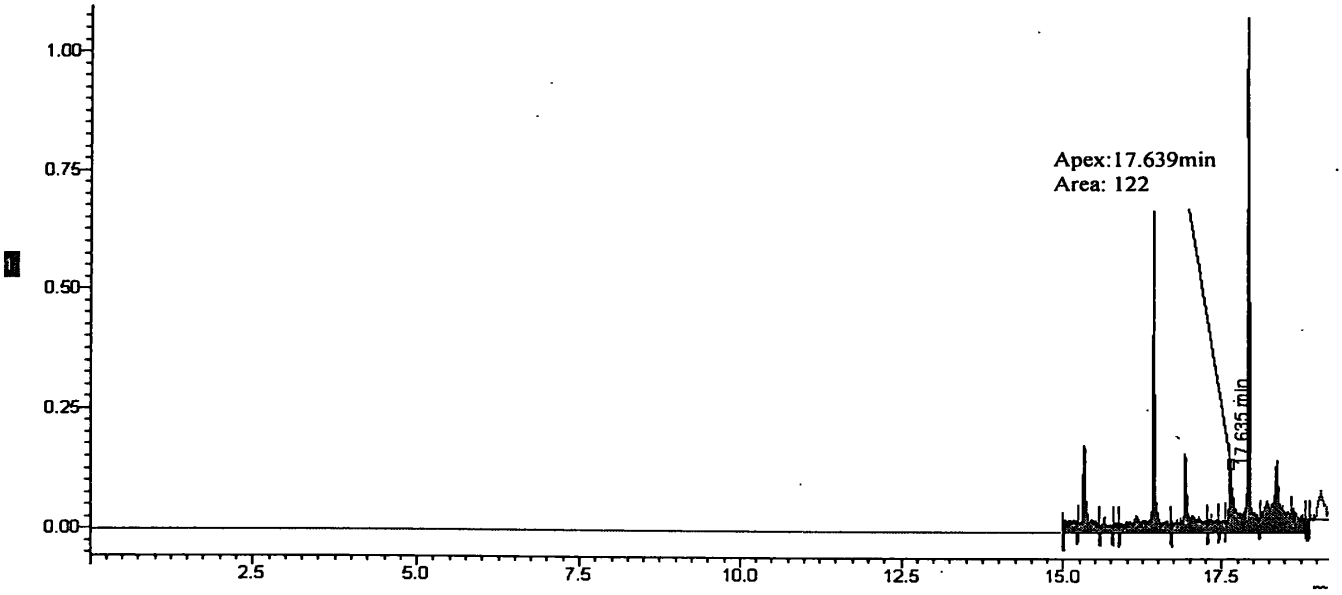
```
The regression equation is
PEAK AREA = 25.9 + 2.08 CONCENTRATION (ppb)


Predictor              Coef  SE Coef      T      P
Constant              25.90    47.98   0.54  0.627
CONCENTRATION(ppb)  2.0820   0.2893   7.20  0.006


S = 45.7482   R-Sq = 94.5%   R-Sq(adj) = 92.7%


Analysis of Variance

Source           DF      SS      MS      F      P
Regression        1  108368  108368  51.78  0.006
Residual Error    3    6279    2093
Total             4  114647


PEAK AREA     CONCENTRATION(ppb)
157           50
224           100
278           150
485           200
547           250
```



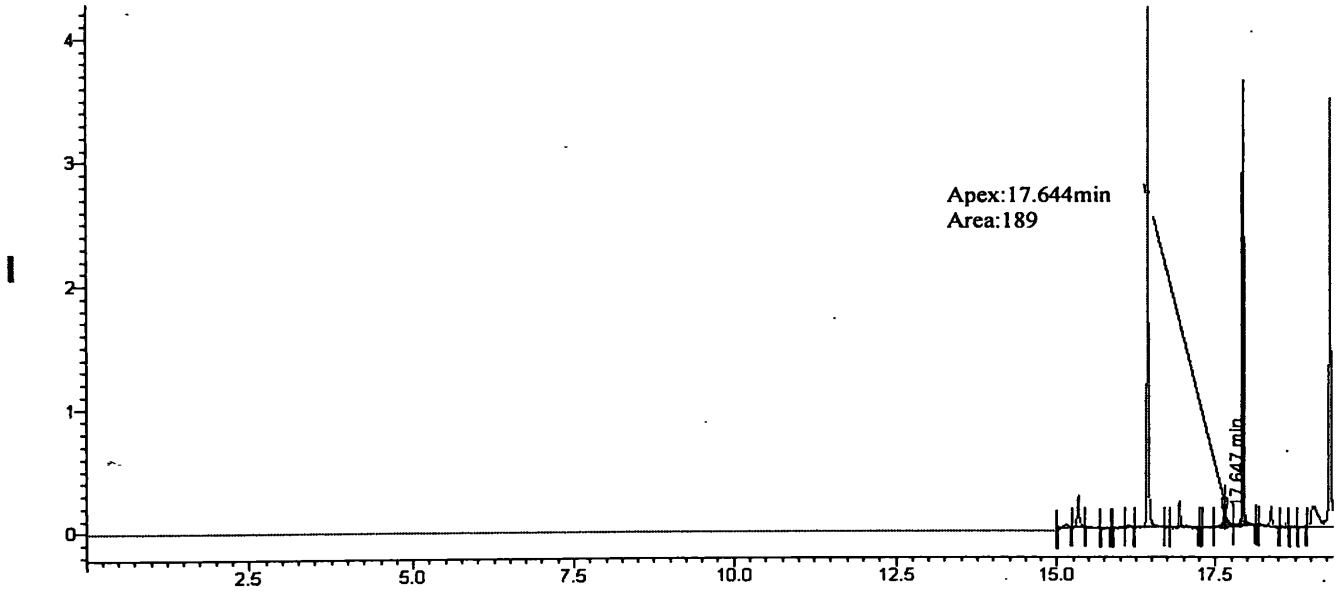
Appendix 05

Chromatograms of recovery test.

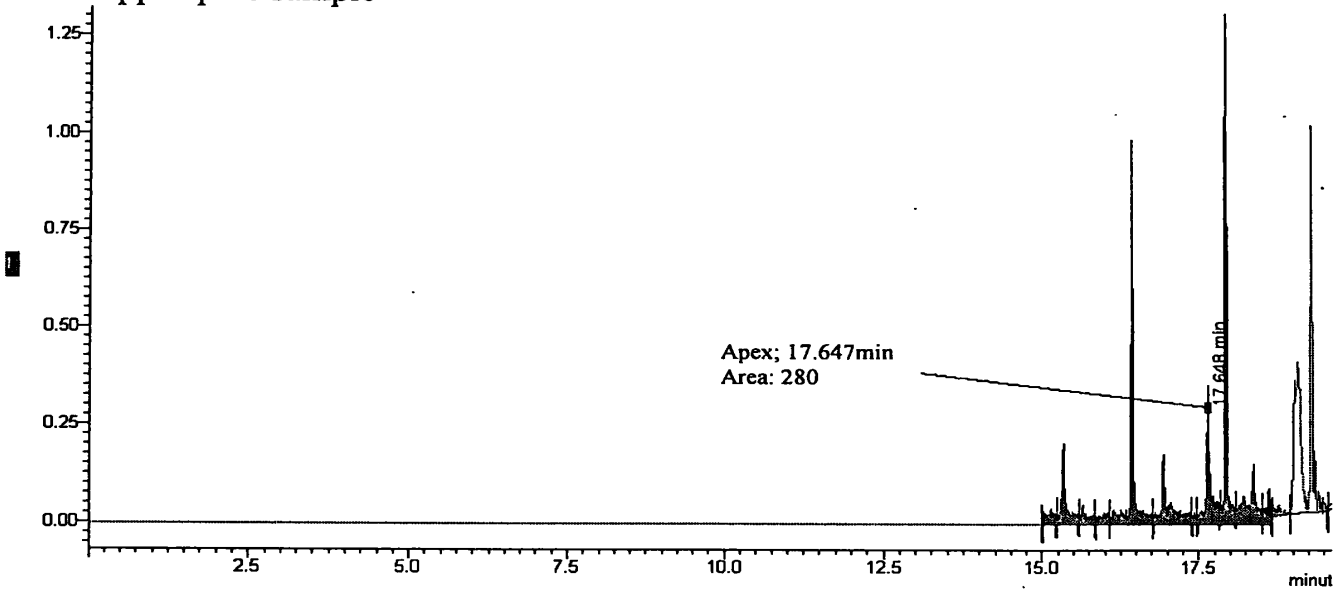
- 50ppb spike sample



- 100ppb spike sample



- 150ppb spike sample



- Calculations and results for recovery test

$$\text{Concentration} = \frac{\text{Peak area} - 25.9}{2.08}$$

Spiked concentration	Peak area	Calculated concentration
50ppb	122	46.20
100ppb	189	78.41
150ppb	280	122.16

Calculation of recovery percentage

$$\text{Recovery percentage} = \frac{\text{Calculated\_concentration}}{\text{Concentration of standard solution}} * 100$$

Loss percentage = 100 - Recovery percentage

Spiked concentration	Recovery percentage	Loss percentage
50ppb	92.40	7.6
100ppb	78.41	21.59
150ppb	81.44	18.56

Mean value for recovery percentage = 84.08%

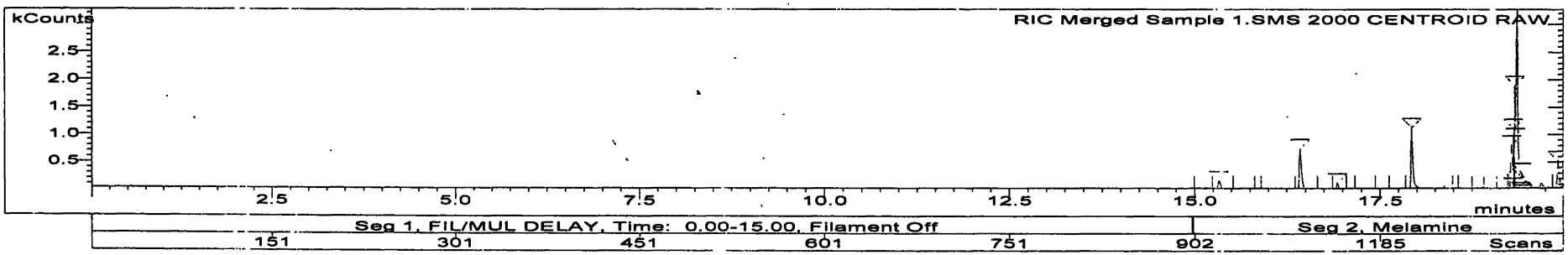
Mean value for loss percentage = 15.92%

Appendix 06

- Chromatograms and calculated results obtained for milk powder samples.

SGS Lanka /Analysis of Melamine

Acquisition Date:	1/22/2009 5:58 PM	Sample ID:	Sample 1
Data File:	...md\tasl\sample 1.sms	Method:	...amine 327 target.mth
Instrument ID:	Gc-Ms	Last Calibration:	1/8/2009 3:11 PM
Calculation Date:	1/22/2009 6:18 PM		
Inj. Sample Notes:	None		
Identified Compounds:	0		



Target Compounds

#	RT	Compound Name	Res Type	Quan Ions	Area	Amount
1	17.648	Melamine	Miss.	327.4	0	0.000 ppm

Unidentified Peaks

None

****

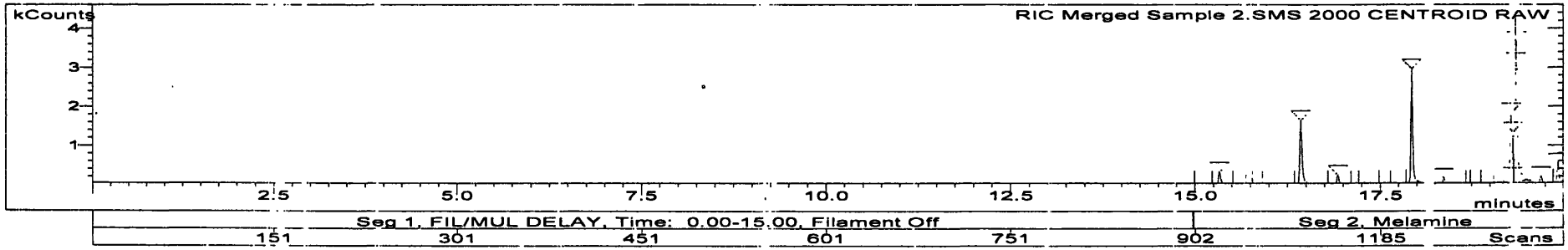
Calibration Log

****

Injection Time/Date	Recalc Time/Date	Calibration Log File
1/8/2009 1:00 PM	1/8/2009 1:20 PM	...m coloumn\melamine\1 cal .5ppm.sms
1/8/2009 1:28 PM	1/8/2009 1:48 PM	...0m coloumn\melamine\1 cal 1ppm.sms
1/8/2009 1:55 PM	1/8/2009 2:15 PM	... coloumn\melamine\1 cal 1.5ppm.sms
1/8/2009 2:23 PM	1/8/2009 2:43 PM	...0m coloumn\melamine\1 cal 2ppm.sms
1/8/2009 2:51 PM	1/8/2009 3:11 PM	... coloumn\melamine\1 cal 2.5ppm.sms

SGS Lanka /Analysis of Melamine

Acquisition Date:	1/22/2009 6:25 PM	Sample ID:	Sample 2
Data File:	...md\tasl\sample 2.sms	Method:	...amine 327 target.mth
Instrument ID:	Gc-Ms	Last Calibration:	1/8/2009 3:11 PM
Calculation Date:	1/22/2009 6:45 PM		
Inj. Sample Notes:	None		
Identified Compounds:	0		



Target Compounds

#	RT	Compound Name	Res Type	Quan Ions	Area	Amount
1	17.648	Melamine	Miss.	327.4	0	0.000 ppm

Unidentified Peaks

None

****

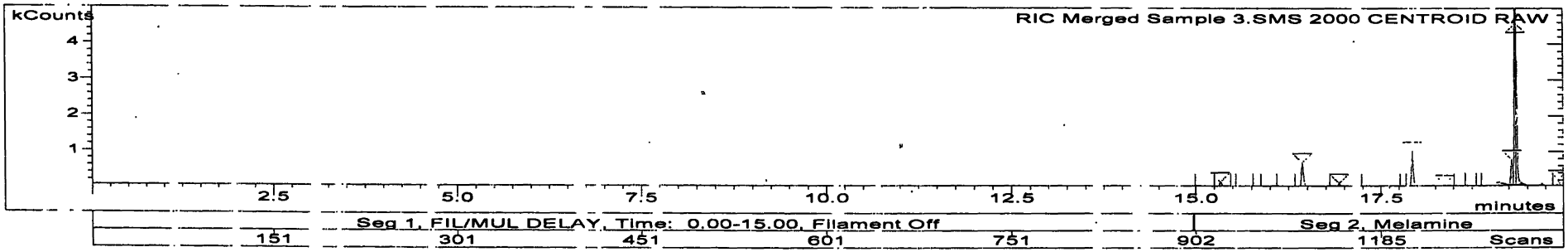
Calibration Log

****

Injection Time/Date	Recalc Time/Date	Calibration Log File
1/8/2009 1:00 PM	1/8/2009 1:20 PM	...m coloumn\melamine\1 cal .5ppm.sms
1/8/2009 1:28 PM	1/8/2009 1:48 PM	...0m coloumn\melamine\1 cal 1ppm.sms
1/8/2009 1:55 PM	1/8/2009 2:15 PM	... coloumn\melamine\1 cal 1.5ppm.sms
1/8/2009 2:23 PM	1/8/2009 2:43 PM	...0m coloumn\melamine\1 cal 2ppm.sms
1/8/2009 2:51 PM	1/8/2009 3:11 PM	... coloumn\melamine\1 cal 2.5ppm.sms

SGS Lanka /Analysis of Melamine

Acquisition Date:	1/22/2009 6:53 PM	Sample ID:	Sample 3
Data File:	...md\tasl\sample 3.sms	Method:	...amine 327 target.mth
Instrument ID:	Gc-Ms	Last Calibration:	1/8/2009 3:11 PM
Calculation Date:	1/22/2009 7:13 PM		
Inj. Sample Notes:	None		
Identified Compounds:	0		



Target Compounds

#	RT	Compound Name	Res Type	Quan Ions	Area	Amount
1	17.648	Melamine	Miss.	327.4	0	0.000 ppm

Unidentified Peaks

None

****

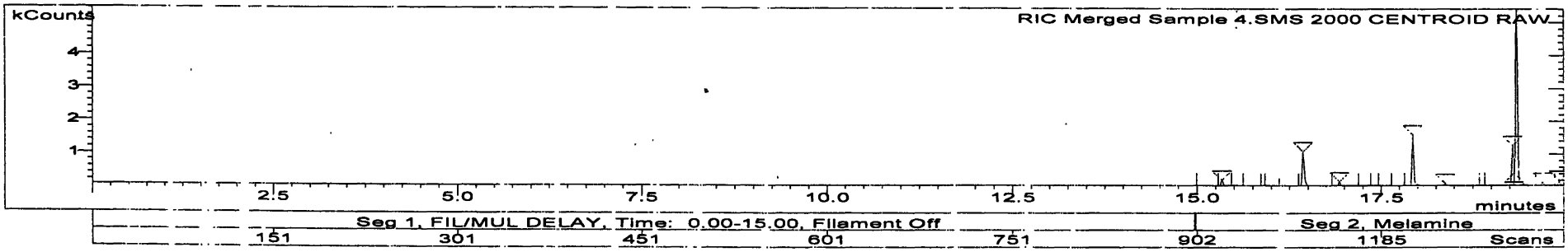
Calibration Log

****

Injection Time/Date	Recalc Time/Date	Calibration Log File
1/8/2009 1:00 PM	1/8/2009 1:20 PM	...m coloumn\melamine\1 cal .5ppm.sms
1/8/2009 1:28 PM	1/8/2009 1:48 PM	...0m coloumn\melamine\1 cal 1ppm.sms
1/8/2009 1:55 PM	1/8/2009 2:15 PM	... coloumn\melamine\1 cal 1.5ppm.sms
1/8/2009 2:23 PM	1/8/2009 2:43 PM	...0m coloumn\melamine\1 cal 2ppm.sms
1/8/2009 2:51 PM	1/8/2009 3:11 PM	... coloumn\melamine\1 cal 2.5ppm.sms

SGS Lanka /Analysis of Melamine

Acquisition Date:	1/22/2009 7:21 PM	Sample ID:	Sample 4
Data File:	...md\tasl\sample 4.sms	Method:	...amine 327 target.mth
Instrument ID:	Gc-Ms	Last Calibration:	1/8/2009 3:11 PM
Calculation Date:	1/22/2009 7:41 PM		
Inj. Sample Notes:	None		
Identified Compounds:	0		



Target Compounds

#	RT	Compound Name	Res Type	Quan Ions	Area	Amount
1	17.648	Melamine	Miss.	327.4	0	0.000 ppm

Unidentified Peaks

None

****

Calibration Log

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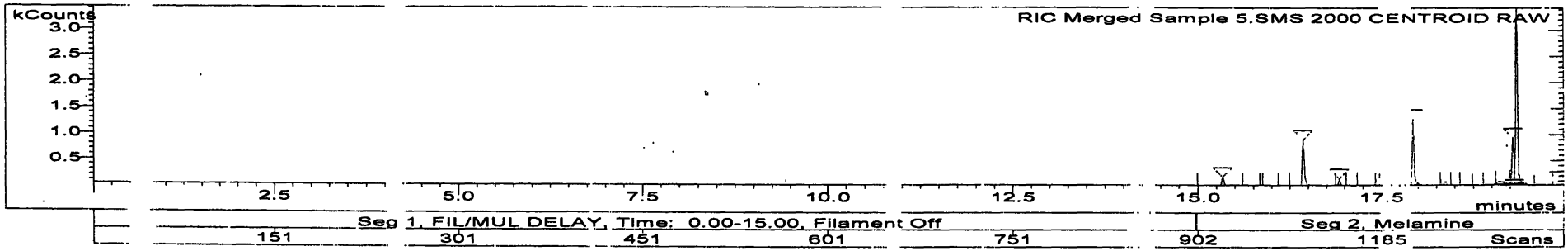
Injection Time/Date	Recalc Time/Date	Calibration Log File
1/8/2009 1:00 PM	1/8/2009 1:20 PM	...m coloumn\melamine\1 cal .5ppm.sms
1/8/2009 1:28 PM	1/8/2009 1:48 PM	...0m coloumn\melamine\1 cal 1ppm.sms
1/8/2009 1:55 PM	1/8/2009 2:15 PM	... coloumn\melamine\1 cal 1.5ppm.sms
1/8/2009 2:23 PM	1/8/2009 2:43 PM	...0m coloumn\melamine\1 cal 2ppm.sms
1/8/2009 2:51 PM	1/8/2009 3:11 PM	... coloumn\melamine\1 cal 2.5ppm.sms

SGS Lanka /Analysis of Melamine

Acquisition Date:	1/22/2009 7:48 PM	Sample ID:	Sample 5
Data File:	...md\tasl\sample 5.sms	Method:	...amine 327 target.mth
Instrument ID:	Gc-Ms	Last Calibration:	1/8/2009 3:11 PM
Calculation Date:	1/22/2009 8:08 PM		
Inj. Sample Notes:	None		
Identified Compounds:	0		



Target Compounds

#	RT	Compound Name	Res Type	Quan Ions	Area	Amount
1	17.648	Melamine	Miss.	327.4	0	0.000 ppm

Unidentified Peaks

None

****

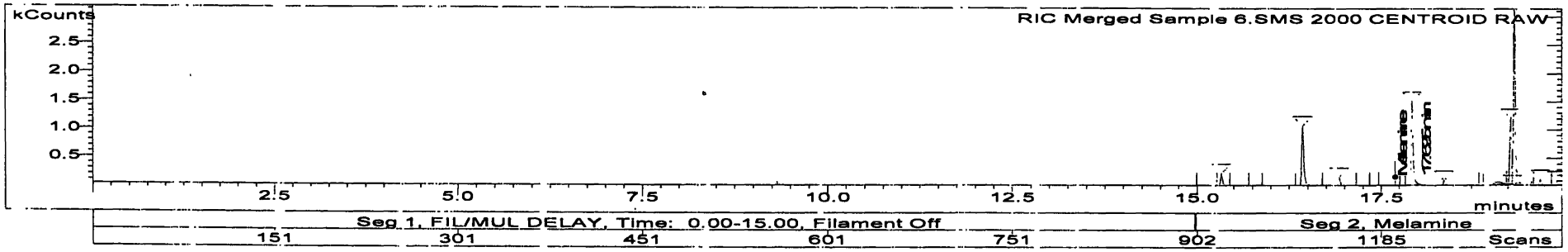
Calibration Log

****

Injection Time/Date	Recalc Time/Date	Calibration Log File
1/8/2009 1:00 PM	1/8/2009 1:20 PM	...m coloumn\melamine\1 cal .5ppm.sms
1/8/2009 1:28 PM	1/8/2009 1:48 PM	...0m coloumn\melamine\1 cal 1ppm.sms
1/8/2009 1:55 PM	1/8/2009 2:15 PM	... coloumn\melamine\1 cal 1.5ppm.sms
1/8/2009 2:23 PM	1/8/2009 2:43 PM	...0m coloumn\melamine\1 cal 2ppm.sms
1/8/2009 2:51 PM	1/8/2009 3:11 PM	... coloumn\melamine\1 cal 2.5ppm.sms

SGS Lanka /Analysis of Melamine

Acquisition Date:	1/22/2009 8:16 PM	Sample ID:	Sample 6
Data File:	...md\tasl\sample 6.sms	Method:	...amine 327 target.mth
Instrument ID:	Gc-Ms	Last Calibration:	1/8/2009 3:11 PM
Calculation Date:	1/22/2009 8:36 PM		
Inj. Sample Notes:	None		
Identified Compounds:	1		



Target Compounds

#	RT	Compound Name	Res Type	Quan Ions	Area	Amount
1	17.695	Melamine	Id.	327.4	33	32.745 Counts

Unidentified Peaks

None

****

Calibration Log

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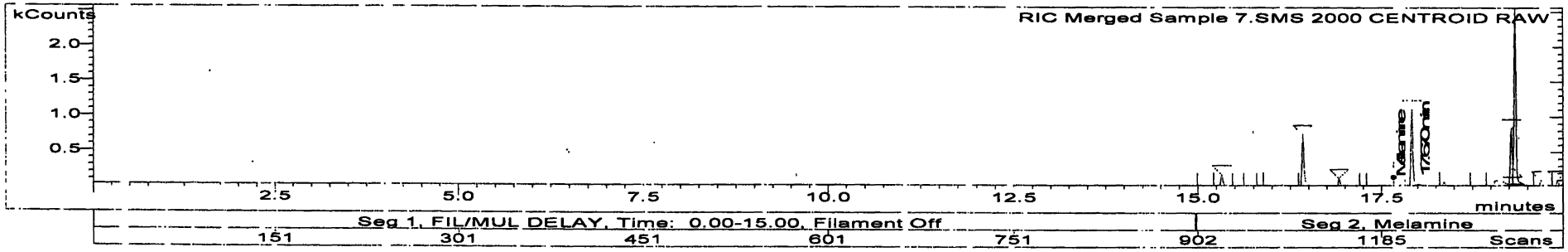
Injection Time/Date	Recalc Time/Date	Calibration Log File
1/8/2009 1:00 PM	1/8/2009 1:20 PM	...m coloumn\melamine\1 cal .5ppm.sms
1/8/2009 1:28 PM	1/8/2009 1:48 PM	...0m coloumn\melamine\1 cal 1ppm.sms
1/8/2009 1:55 PM	1/8/2009 2:15 PM	... coloumn\melamine\1 cal 1.5ppm.sms
1/8/2009 2:23 PM	1/8/2009 2:43 PM	...0m coloumn\melamine\1 cal 2ppm.sms
1/8/2009 2:51 PM	1/8/2009 3:11 PM	... coloumn\melamine\1 cal 2.5ppm.sms

SGS Lanka /Analysis of Melamine

Acquisition Date:	1/22/2009 8:44 PM	Sample ID:	Sample 7
Data File:	...md\tasl\sample 7.sms	Method:	...amine 327 target.mth
Instrument ID:	Gc-Ms	Last Calibration:	1/8/2009 3:11 PM
Calculation Date:	1/22/2009 9:04 PM		
Inj. Sample Notes:	None		
Identified Compounds:	1		



Target Compounds

#	RT	Compound Name	Res Type	Quan Ions	Area	Amount
1	17.670	Melamine	Id.	327.4	14	14.125 Counts

Unidentified Peaks

None

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Calibration Log

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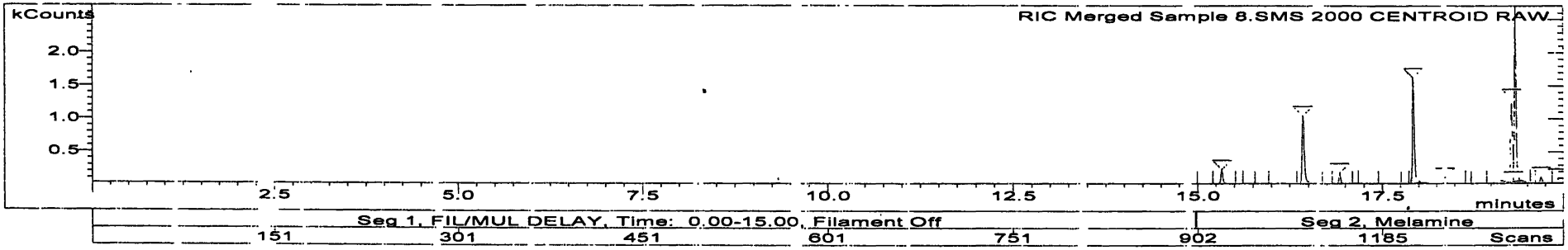
Injection Time/Date	Recalc Time/Date	Calibration Log File
1/8/2009 1:00 PM	1/8/2009 1:20 PM	...m coloumn\melamine\1 cal .5ppm.sms
1/8/2009 1:28 PM	1/8/2009 1:48 PM	...0m coloumn\melamine\1 cal 1ppm.sms
1/8/2009 1:55 PM	1/8/2009 2:15 PM	... coloumn\melamine\1 cal 1.5ppm.sms
1/8/2009 2:23 PM	1/8/2009 2:43 PM	...0m coloumn\melamine\1 cal 2ppm.sms
1/8/2009 2:51 PM	1/8/2009 3:11 PM	... coloumn\melamine\1 cal 2.5ppm.sms

SGS Lanka /Analysis of Melamine

Acquisition Date:	1/22/2009 9:11 PM	Sample ID:	Sample 8
Data File:	...md\tasl\sample 8.sms	Method:	...amine 327 target.mth
Instrument ID:	Gc-Ms	Last Calibration:	1/8/2009 3:11 PM
Calculation Date:	1/22/2009 9:31 PM		
Inj. Sample Notes:	None		
Identified Compounds:	0		



Target Compounds

#	RT	Compound Name	Res Type	Quan Ions	Area	Amount
1	17.648	Melamine	Miss.	327.4	0	0.000 ppm

Unidentified Peaks

None

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Calibration Log

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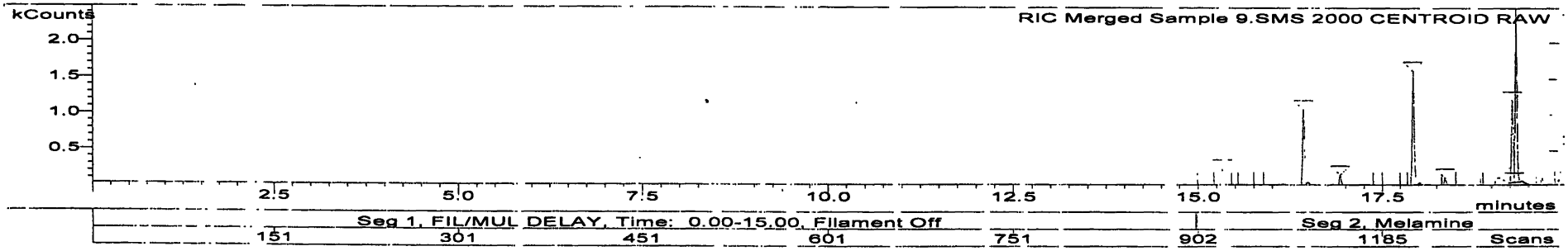
Injection Time/Date	Recalc Time/Date	Calibration Log File
1/8/2009 1:00 PM	1/8/2009 1:20 PM	...m coloumn\melamine\1 cal .5ppm.sms
1/8/2009 1:28 PM	1/8/2009 1:48 PM	...0m coloumn\melamine\1 cal 1ppm.sms
1/8/2009 1:55 PM	1/8/2009 2:15 PM	... coloumn\melamine\1 cal 1.5ppm.sms
1/8/2009 2:23 PM	1/8/2009 2:43 PM	...0m coloumn\melamine\1 cal 2ppm.sms
1/8/2009 2:51 PM	1/8/2009 3:11 PM	... coloumn\melamine\1 cal 2.5ppm.sms

SGS Lanka /Analysis of Melamine

Acquisition Date:	1/22/2009 9:39 PM	Sample ID:	Sample 9
Data File:	...md\tasl\sample 9.sms	Method:	...amine 327 target.mth
Instrument ID:	Gc-Ms	Last Calibration:	1/8/2009 3:11 PM
Calculation Date:	1/22/2009 9:59 PM		
Inj. Sample Notes:	None		
Identified Compounds:	0		



Target Compounds

#	RT	Compound Name	Res Type	Quan Ions	Area	Amount
1	17.648	Melamine	Miss.	327.4	0	0.000 ppm

Unidentified Peaks

None

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Calibration Log

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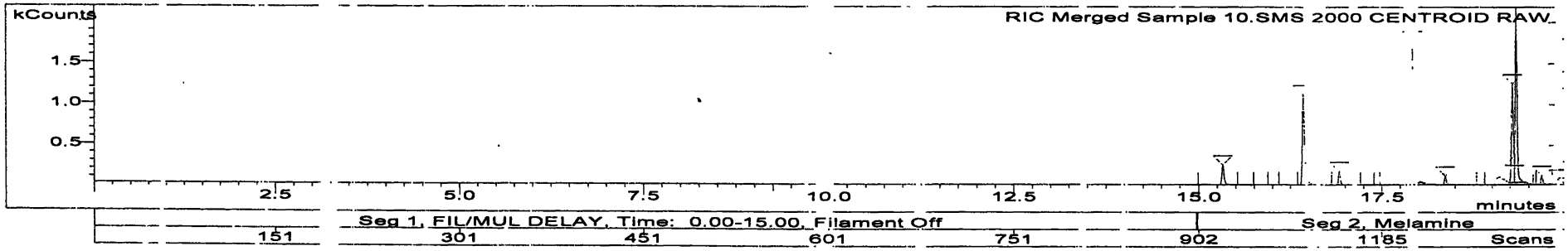
Injection Time/Date	Recalc Time/Date	Calibration Log File
1/8/2009 1:00 PM	1/8/2009 1:20 PM	...m coloumn\melamine\1 cal .5ppm.sms
1/8/2009 1:28 PM	1/8/2009 1:48 PM	...Om coloumn\melamine\1 cal 1ppm.sms
1/8/2009 1:55 PM	1/8/2009 2:15 PM	... coloumn\melamine\1 cal 1.5ppm.sms
1/8/2009 2:23 PM	1/8/2009 2:43 PM	...Om coloumn\melamine\1 cal 2ppm.sms
1/8/2009 2:51 PM	1/8/2009 3:11 PM	... coloumn\melamine\1 cal 2.5ppm.sms

SGS Lanka /Analysis of Melamine

Acquisition Date:	1/22/2009 10:06 PM	Sample ID:	Sample 10
Data File:	...d\tasl\sample 10.sms	Method:	...amine 327 target.mth
Instrument ID:	Gc-Ms	Last Calibration:	1/8/2009 3:11 PM
Calculation Date:	1/22/2009 10:26 PM		
Inj. Sample Notes:	None		
Identified Compounds:	0		



Target Compounds

#	RT	Compound Name	Res Type	Quan Ions	Area	Amount
1	17.648	Melamine	Miss.	327.4	0	0.000 ppm

Unidentified Peaks

None

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Calibration Log

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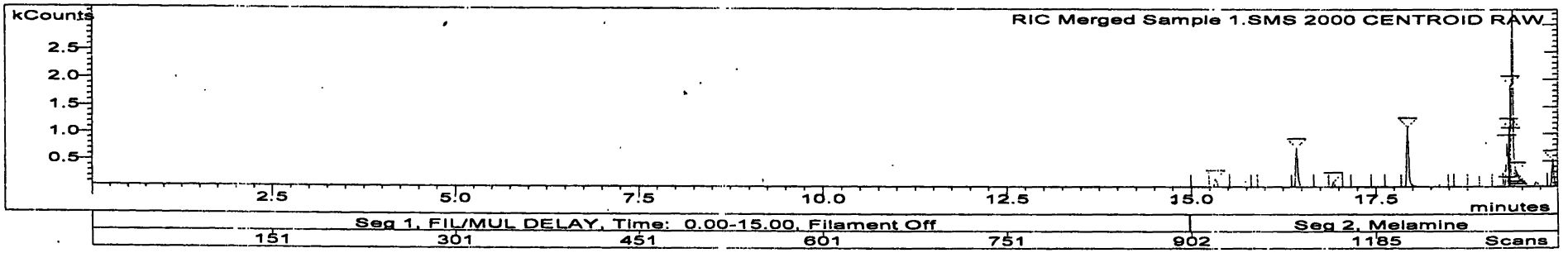
Injection Time/Date	Recalc Time/Date	Calibration Log File
1/8/2009 1:00 PM	1/8/2009 1:20 PM	...m coloumn\melamine\1 cal .5ppm.sms
1/8/2009 1:28 PM	1/8/2009 1:48 PM	...0m coloumn\melamine\1 cal 1ppm.sms
1/8/2009 1:55 PM	1/8/2009 2:15 PM	... coloumn\melamine\1 cal 1.5ppm.sms
1/8/2009 2:23 PM	1/8/2009 2:43 PM	...0m coloumn\melamine\1 cal 2ppm.sms
1/8/2009 2:51 PM	1/8/2009 3:11 PM	... coloumn\melamine\1 cal 2.5ppm.sms

SGS Lanka /Analysis of Melamine

Acquisition Date:	1/22/2009 5:58 PM	Sample ID:	Sample11
Data File:	...md\tasl\sample11.sms	Method:	...amine 327 target.mth
Instrument ID:	Gc-Ms	Last Calibration:	1/8/2009 3:11 PM
Calculation Date:	1/22/2009 6:18 PM		
Inj. Sample Notes:	None		
Identified Compounds:	0		



Target Compounds

#	RT	Compound Name	Res Type	Quan Ions	Area	Amount
1	17.648	Melamine	Miss.	327.4	0	0.000 ppm

Unidentified Peaks

None

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Calibration Log

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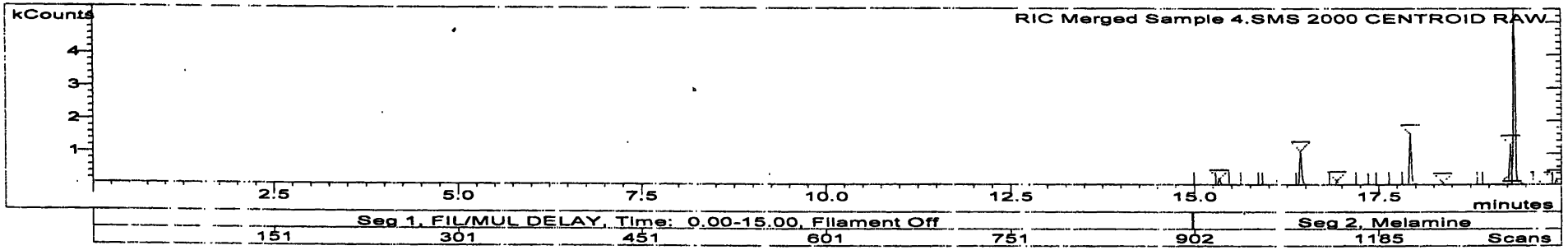
Injection Time/Date	Recalc Time/Date	Calibration Log File
1/8/2009 1:00 PM	1/8/2009 1:20 PM	...m coloumn\melamine\1 cal .5ppm.sms
1/8/2009 1:28 PM	1/8/2009 1:48 PM	...0m coloumn\melamine\1 cal 1ppm.sms
1/8/2009 1:55 PM	1/8/2009 2:15 PM	... coloumn\melamine\1 cal 1.5ppm.sms
1/8/2009 2:23 PM	1/8/2009 2:43 PM	...0m coloumn\melamine\1 cal 2ppm.sms
1/8/2009 2:51 PM	1/8/2009 3:11 PM	... coloumn\melamine\1 cal 2.5ppm.sms

SGS Lanka /Analysis of Melamine

Acquisition Date:	1/22/2009 7:21 PM	Sample ID:	Sample12
Data File:	...md\tasl\sample12.sms	Method:	...amine 327 target.mth
Instrument ID:	Gc-Ms	Last Calibration:	1/8/2009 3:11 PM
Calculation Date:	1/22/2009 7:41 PM		
Inj. Sample Notes:	None		
Identified Compounds:	0		



Target Compounds

#	RT	Compound Name	Res Type	Quan Ions	Area	Amount
1	17.648	Melamine	Miss.	327.4	0	0.000 ppm

Unidentified Peaks

None

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Calibration Log

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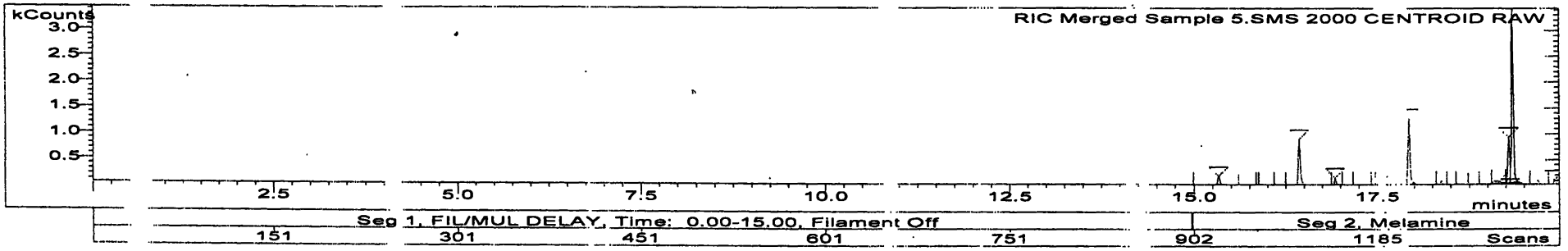
Injection Time/Date	Recalc Time/Date	Calibration Log File
1/8/2009 1:00 PM	1/8/2009 1:20 PM	...m coloumn\melamine\1 cal .5ppm.sms
1/8/2009 1:28 PM	1/8/2009 1:48 PM	...0m coloumn\melamine\1 cal 1ppm.sms
1/8/2009 1:55 PM	1/8/2009 2:15 PM	... coloumn\melamine\1 cal 1.5ppm.sms
1/8/2009 2:23 PM	1/8/2009 2:43 PM	...0m coloumn\melamine\1 cal 2ppm.sms
1/8/2009 2:51 PM	1/8/2009 3:11 PM	... coloumn\melamine\1 cal 2.5ppm.sms

SGS Lanka /Analysis of Melamine

Acquisition Date:	1/22/2009 7:48 PM	Sample ID:	Sample13
Data File:	...md\tasl\sample13.sms	Method:	...amine 327 target.mth
Instrument ID:	Gc-Ms	Last Calibration:	1/8/2009 3:11 PM
Calculation Date:	1/22/2009 8:08 PM		
Inj. Sample Notes:	None		
Identified Compounds:	0		



Target Compounds

#	RT	Compound Name	Res Type	Quan Ions	Area	Amount
1	17.648	Melamine	Miss.	327.4	0	0.000 ppm

Unidentified Peaks

None

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Calibration Log

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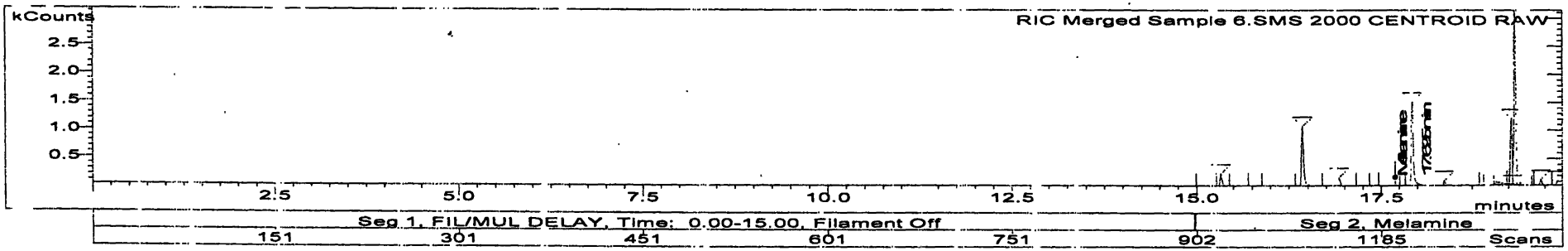
Injection Time/Date	Recalc Time/Date	Calibration Log File
1/8/2009 1:00 PM	1/8/2009 1:20 PM	...m coloumn\melamine\1 cal .5ppm.sms
1/8/2009 1:28 PM	1/8/2009 1:48 PM	...0m coloumn\melamine\1 cal 1ppm.sms
1/8/2009 1:55 PM	1/8/2009 2:15 PM	... coloumn\melamine\1 cal 1.5ppm.sms
1/8/2009 2:23 PM	1/8/2009 2:43 PM	...0m coloumn\melamine\1 cal 2ppm.sms
1/8/2009 2:51 PM	1/8/2009 3:11 PM	... coloumn\melamine\1 cal 2.5ppm.sms

SGS Lanka /Analysis of Melamine

Acquisition Date:	1/22/2009 8:16 PM	Sample ID:	Sample 14
Data File:	...md\tasl\sample14 sms	Method:	...amine 327 target.mth
Instrument ID:	Gc-Ms	Last Calibration:	1/8/2009 3:11 PM
Calculation Date:	1/22/2009 8:36 PM		
Inj. Sample Notes:	None		
Identified Compounds:	1		



Target Compounds

#	RT	Compound Name	Res Type	Quan Ions	Area	Amount
1	17.695	Melamine	Id.	327.4	33	32.745 Counts

Unidentified Peaks

None

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Calibration Log

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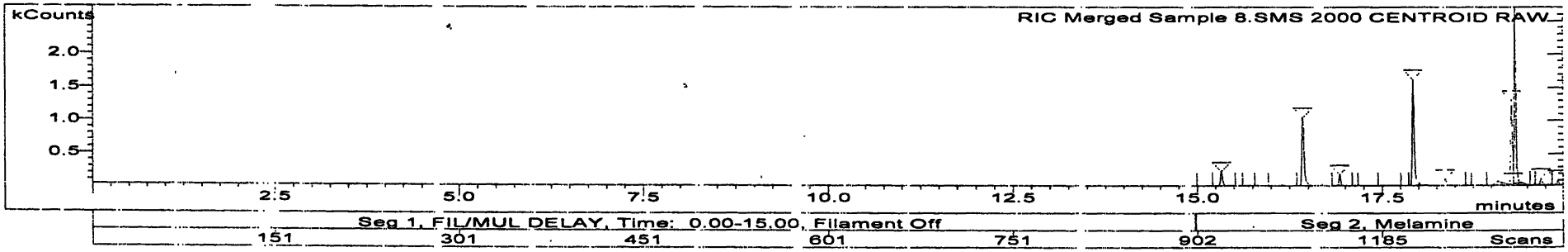
Injection Time/Date	Recalc Time/Date	Calibration Log File
1/8/2009 1:00 PM	1/8/2009 1:20 PM	...m coloumn\melamine\1 cal .5ppm.sms
1/8/2009 1:28 PM	1/8/2009 1:48 PM	...0m coloumn\melamine\1 cal 1ppm.sms
1/8/2009 1:55 PM	1/8/2009 2:15 PM	... coloumn\melamine\1 cal 1.5ppm.sms
1/8/2009 2:23 PM	1/8/2009 2:43 PM	...0m coloumn\melamine\1 cal 2ppm.sms
1/8/2009 2:51 PM	1/8/2009 3:11 PM	... coloumn\melamine\1 cal 2.5ppm.sms

SGS Lanka /Analysis of Melamine

Acquisition Date:	1/22/2009 9:11 PM	Sample ID:	Sample 15
Data File:	...md\tasl\sample15.sms	Method:	...amine 327 target.mth
Instrument ID:	Gc-Ms	Last Calibration:	1/8/2009 3:11 PM
Calculation Date:	1/22/2009 9:31 PM		
Inj. Sample Notes:	None		
Identified Compounds:	0		



Target Compounds

#	RT	Compound Name	Res Type	Quan Ions	Area	Amount
1	17.648	Melamine	Miss.	327.4	0	0.000 ppm

Unidentified Peaks

None

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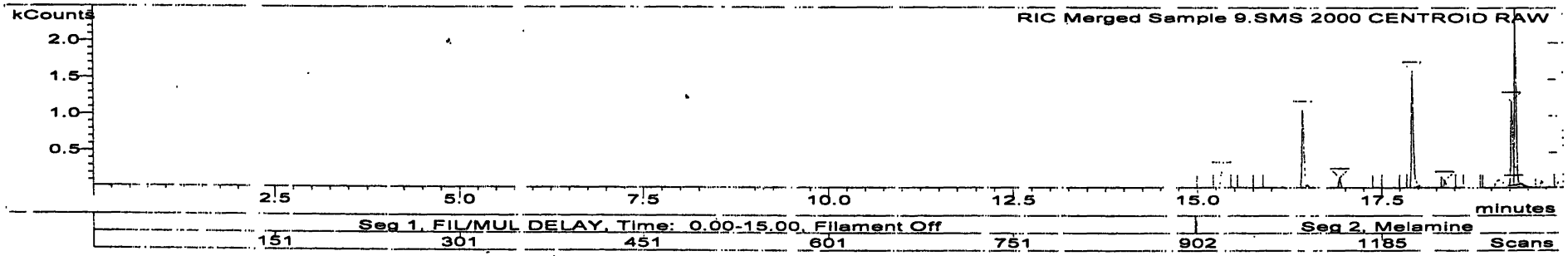
Calibration Log

****

Injection Time/Date	Recalc Time/Date	Calibration Log File
1/8/2009 1:00 PM	1/8/2009 1:20 PM	...m coloumn\melamine\1 cal .5ppm.sms
1/8/2009 1:28 PM	1/8/2009 1:48 PM	...0m coloumn\melamine\1 cal 1ppm.sms
1/8/2009 1:55 PM	1/8/2009 2:15 PM	... coloumn\melamine\1 cal 1.5ppm.sms
1/8/2009 2:23 PM	1/8/2009 2:43 PM	...0m coloumn\melamine\1 cal 2ppm.sms
1/8/2009 2:51 PM	1/8/2009 3:11 PM	... coloumn\melamine\1 cal 2.5ppm.sms

SGS Lanka /Analysis of Melamine

Acquisition Date:	1/22/2009 9:39 PM	Sample ID:	Sample 16
Data File:	...md\tasl\sample16.sms	Method:	...amine 327 target.mth
Instrument ID:	Gc-Ms	Last Calibration:	1/8/2009 3:11 PM
Calculation Date:	1/22/2009 9:59 PM		
Inj. Sample Notes:	None		
Identified Compounds:	0		



Target Compounds

#	RT	Compound Name	Res Type	Quan Ions	Area	Amount
1	17.648	Melamine	Miss.	327.4	0	0.000 ppm

Unidentified Peaks

None

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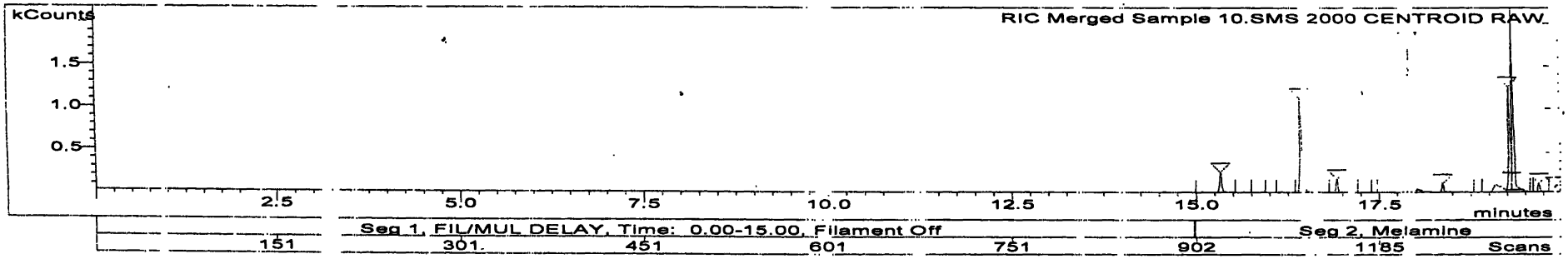
Calibration Log

****

Injection Time/Date	Recalc Time/Date	Calibration Log File
1/8/2009 1:00 PM	1/8/2009 1:20 PM	...m coloumn\melamine\1 cal .5ppm.sms
1/8/2009 1:28 PM	1/8/2009 1:48 PM	...0m coloumn\melamine\1 cal 1ppm.sms
1/8/2009 1:55 PM	1/8/2009 2:15 PM	... coloumn\melamine\1 cal 1.5ppm.sms
1/8/2009 2:23 PM	1/8/2009 2:43 PM	...0m coloumn\melamine\1 cal 2ppm.sms
1/8/2009 2:51 PM	1/8/2009 3:11 PM	... coloumn\melamine\1 cal 2.5ppm.sms

SGS Lanka /Analysis of Melamine

Acquisition Date:	1/22/2009 10:06 PM	Sample ID:	Sample 17
Data File:	...d\tasl\sample 17 .sms	Method:	...amine 327 target.mth
Instrument ID:	Gc-Ms	Last Calibration:	1/8/2009 3:11 PM
Calculation Date:	1/22/2009 10:26 PM		
Inj. Sample Notes:	None		
Identified Compounds:	0		



Target Compounds

#	RT	Compound Name	Res Type	Quan Ions	Area	Amount
1	17.648	Melamine	Miss.	327.4	0	0.000 ppm

Unidentified Peaks

None

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Calibration Log

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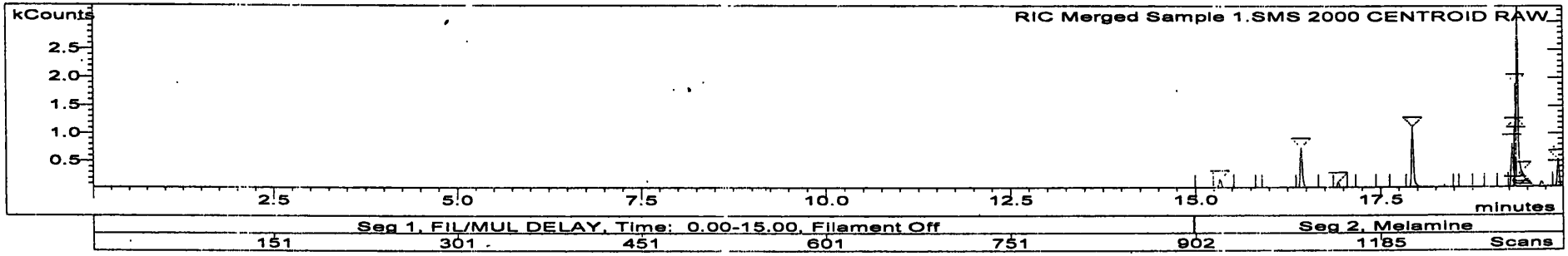
Injection Time/Date	Recalc Time/Date	Calibration Log File
1/8/2009 1:00 PM	1/8/2009 1:20 PM	...m coloumn\melamine\1 cal .5ppm.sms
1/8/2009 1:28 PM	1/8/2009 1:48 PM	...0m coloumn\melamine\1 cal 1ppm.sms
1/8/2009 1:55 PM	1/8/2009 2:15 PM	... coloumn\melamine\1 cal 1.5ppm.sms
1/8/2009 2:23 PM	1/8/2009 2:43 PM	...0m coloumn\melamine\1 cal 2ppm.sms
1/8/2009 2:51 PM	1/8/2009 3:11 PM	... coloumn\melamine\1 cal 2.5ppm.sms

SGS Lanka /Analysis of Melamine

Acquisition Date:	1/22/2009 5:58 PM	Sample ID:	Sample 18
Data File:	...md\tasl\sample18.sms	Method:	...amine 327 target.mth
Instrument ID:	Gc-Ms	Last Calibration:	1/8/2009 3:11 PM
Calculation Date:	1/22/2009 6:18 PM		
Inj. Sample Notes:	None		
Identified Compounds:	0		



Target Compounds

#	RT	Compound Name	Res Type	Quan Ions	Area	Amount
1	17.648	Melamine	Miss.	327.4	0	0.000 ppm

Unidentified Peaks

None

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Calibration Log

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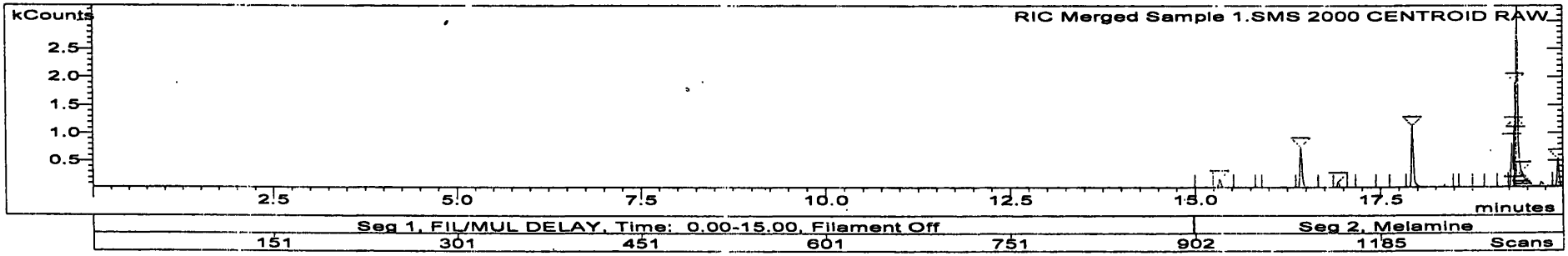
Injection Time/Date	Recalc Time/Date	Calibration Log File
1/8/2009 1:00 PM	1/8/2009 1:20 PM	...m coloumn\melamine\1 cal .5ppm.sms
1/8/2009 1:28 PM	1/8/2009 1:48 PM	...0m coloumn\melamine\1 cal 1ppm.sms
1/8/2009 1:55 PM	1/8/2009 2:15 PM	... coloumn\melamine\1 cal 1.5ppm.sms
1/8/2009 2:23 PM	1/8/2009 2:43 PM	...0m coloumn\melamine\1 cal 2ppm.sms
1/8/2009 2:51 PM	1/8/2009 3:11 PM	... coloumn\melamine\1 cal 2.5ppm.sms

SGS Lanka /Analysis of Melamine

Acquisition Date:	1/22/2009 5:58 PM	Sample ID:	Sample19
Data File:	...md\tasl\sample19.sms	Method:	...amine 327 target.mth
Instrument ID:	Gc-Ms	Last Calibration:	1/8/2009 3:11 PM
Calculation Date:	1/22/2009 6:18 PM		
Inj. Sample Notes:	None		
Identified Compounds:	0		



Target Compounds

#	RT	Compound Name	Res Type	Quan Ions	Area	Amount
1	17.648	Melamine	Miss.	327.4	0	0.000 ppm

Unidentified Peaks

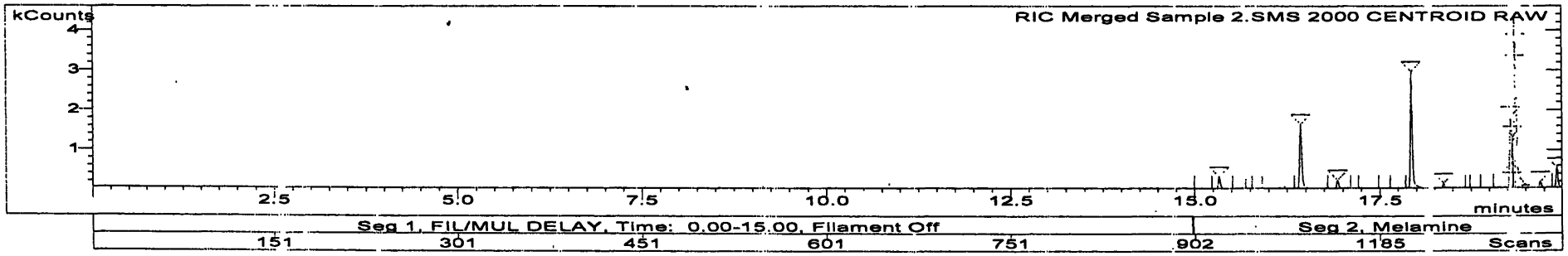
None

Calibration Log

Injection Time/Date	Recalc Time/Date	Calibration Log File
1/8/2009 1:00 PM	1/8/2009 1:20 PM	...m coloumn\melamine\1 cal .5ppm.sms
1/8/2009 1:28 PM	1/8/2009 1:48 PM	...0m coloumn\melamine\1 cal 1ppm.sms
1/8/2009 1:55 PM	1/8/2009 2:15 PM	... coloumn\melamine\1 cal 1.5ppm.sms
1/8/2009 2:23 PM	1/8/2009 2:43 PM	...0m coloumn\melamine\1 cal 2ppm.sms
1/8/2009 2:51 PM	1/8/2009 3:11 PM	... coloumn\melamine\1 cal 2.5ppm.sms

SGS Lanka /Analysis of Melamine

Acquisition Date:	1/22/2009 6:25 PM	Sample ID:	Sample 20
Data File:	...md\tasl\sample 20sms	Method:	...amine 327 target.mth
Instrument ID:	Gc-Ms	Last Calibration:	1/8/2009 3:11 PM
Calculation Date:	1/22/2009 6:45 PM		
Inj. Sample Notes:	None		
Identified Compounds:	0		



Target Compounds

#	RT	Compound Name	Res Type	Quan Ions	Area	Amount
1	17.648	Melamine	Miss.	327.4	0	0.000 ppm

Unidentified Peaks

None

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Calibration Log

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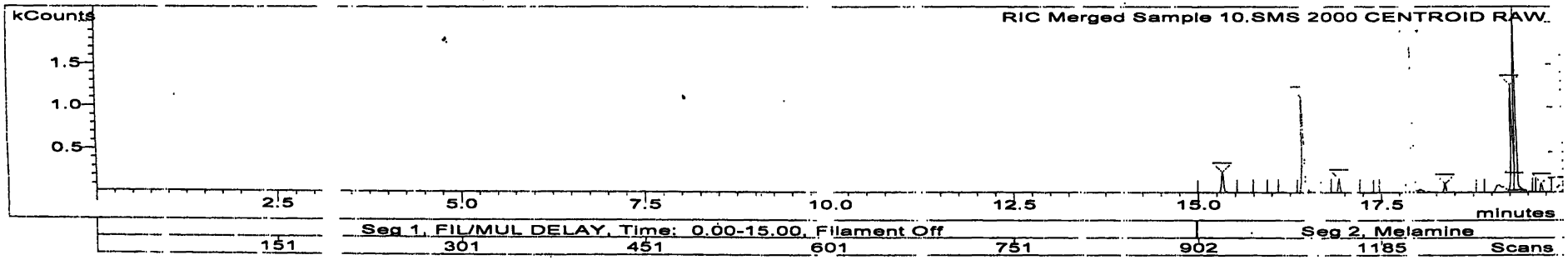
Injection Time/Date	Recalc Time/Date	Calibration Log File
1/8/2009 1:00 PM	1/8/2009 1:20 PM	...m coloumn\melamine\1 cal .5ppm.sms
1/8/2009 1:28 PM	1/8/2009 1:48 PM	...0m coloumn\melamine\1 cal 1ppm.sms
1/8/2009 1:55 PM	1/8/2009 2:15 PM	... coloumn\melamine\1 cal 1.5ppm.sms
1/8/2009 2:23 PM	1/8/2009 2:43 PM	...0m coloumn\melamine\1 cal 2ppm.sms
1/8/2009 2:51 PM	1/8/2009 3:11 PM	... coloumn\melamine\1 cal 2.5ppm.sms

SGS Lanka /Analysis of Melamine

Acquisition Date:	1/22/2009 10:06 PM	Sample ID:	Sample 21
Data File:	...d\tasl\sample 21.sms	Method:	...amine 327 target.mth
Instrument ID:	Gc-Ms	Last Calibration:	1/8/2009 3:11 PM
Calculation Date:	1/22/2009 10:26 PM		
Inj. Sample Notes:	None		
Identified Compounds:	0		



Target Compounds

#	RT	Compound Name	Res Type	Quan Ions	Area	Amount
1	17.648	Melamine	Miss.	327.4	0	0.000 ppm

Unidentified Peaks

None

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Calibration Log

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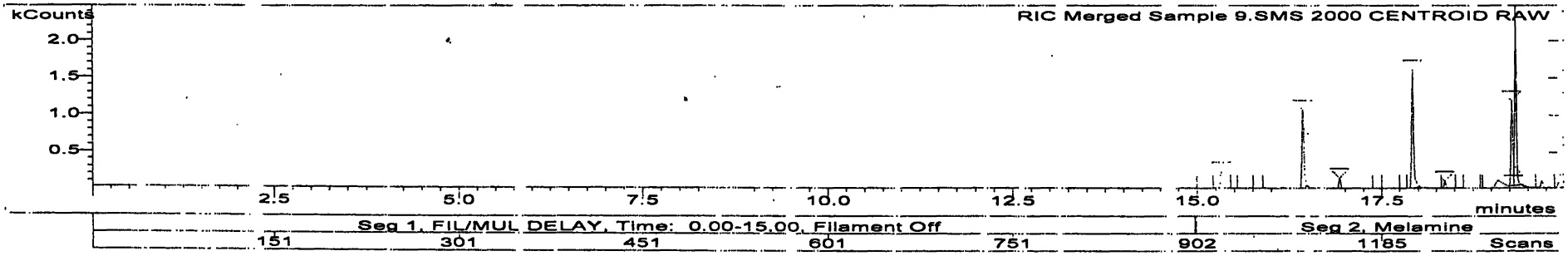
Injection Time/Date	Recalc Time/Date	Calibration Log File
1/8/2009 1:00 PM	1/8/2009 1:20 PM	...m coloumn\melamine\1 cal .5ppm.sms
1/8/2009 1:28 PM	1/8/2009 1:48 PM	...0m coloumn\melamine\1 cal 1ppm.sms
1/8/2009 1:55 PM	1/8/2009 2:15 PM	... coloumn\melamine\1 cal 1.5ppm.sms
1/8/2009 2:23 PM	1/8/2009 2:43 PM	...0m coloumn\melamine\1 cal 2ppm.sms
1/8/2009 2:51 PM	1/8/2009 3:11 PM	... coloumn\melamine\1 cal 2.5ppm.sms

SGS Lanka /Analysis of Melamine

Acquisition Date:	1/22/2009 9:39 PM	Sample ID:	Sample 22
Data File:	...md\tasl\sample22.sms	Method:	...amine 327 target.mth
Instrument ID:	Gc-Ms	Last Calibration:	1/8/2009 3:11 PM
Calculation Date:	1/22/2009 9:59 PM		
Inj. Sample Notes:	None		
Identified Compounds:	0		



Target Compounds

#	RT	Compound Name	Res Type	Quan Ions	Area	Amount
1	17.648	Melamine	Miss.	327.4	0	0.000 ppm

Unidentified Peaks

None

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Calibration Log

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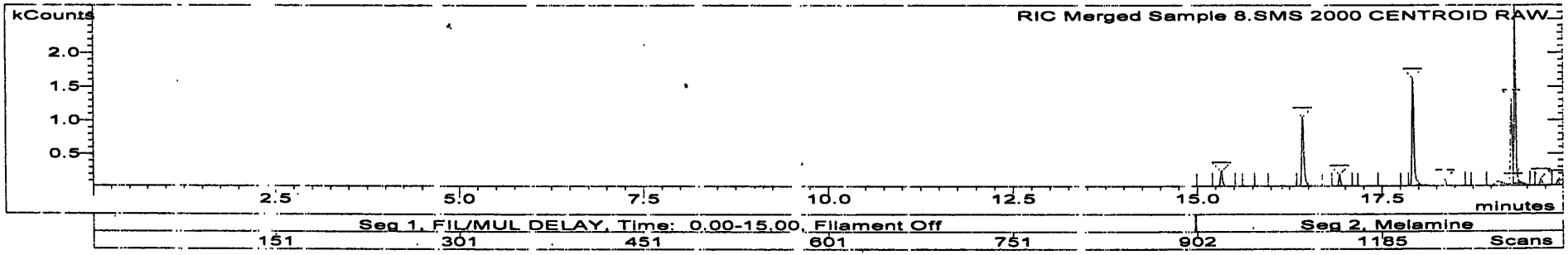
Injection Time/Date	Recalc Time/Date	Calibration Log File
1/8/2009 1:00 PM	1/8/2009 1:20 PM	...m coloumn\melamine\1 cal .5ppm.sms
1/8/2009 1:28 PM	1/8/2009 1:48 PM	...0m coloumn\melamine\1 cal 1ppm.sms
1/8/2009 1:55 PM	1/8/2009 2:15 PM	... coloumn\melamine\1 cal 1.5ppm.sms
1/8/2009 2:23 PM	1/8/2009 2:43 PM	...0m coloumn\melamine\1 cal 2ppm.sms
1/8/2009 2:51 PM	1/8/2009 3:11 PM	... coloumn\melamine\1 cal 2.5ppm.sms

SGS Lanka /Analysis of Melamine

Acquisition Date:	1/22/2009 9:11 PM	Sample ID:	Sample 23
Data File:	...md\tasl\sample 23.sms	Method:	...amine 327 target.mth
Instrument ID:	Gc-Ms	Last Calibration:	1/8/2009 3:11 PM
Calculation Date:	1/22/2009 9:31 PM		
Inj. Sample Notes:	None		
Identified Compounds:	0		



Target Compounds

#	RT	Compound Name	Res Type	Quan Ions	Area	Amount
1	17.648	Melamine	Miss.	327.4	0	0.000 ppm

Unidentified Peaks

None

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Calibration Log

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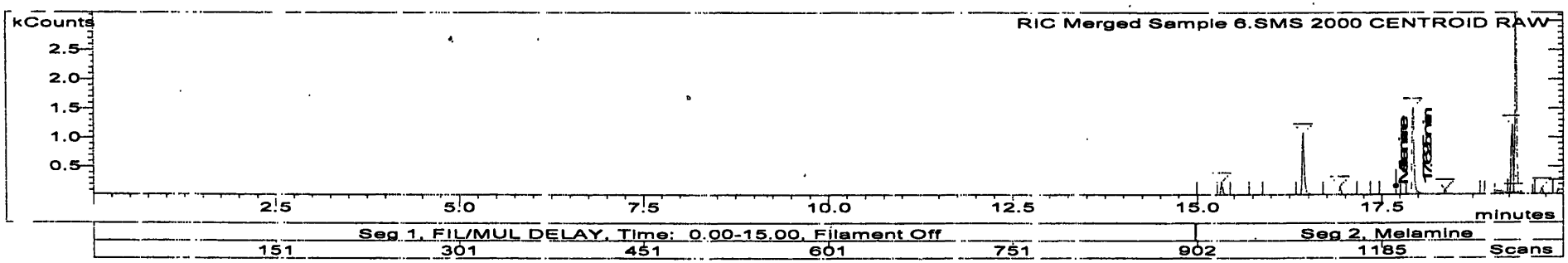
Injection Time/Date	Recalc Time/Date	Calibration Log File
1/8/2009 1:00 PM	1/8/2009 1:20 PM	...m coloumn\melamine\1 cal .5ppm.sms
1/8/2009 1:28 PM	1/8/2009 1:48 PM	...0m coloumn\melamine\1 cal 1ppm.sms
1/8/2009 1:55 PM	1/8/2009 2:15 PM	... coloumn\melamine\1 cal 1.5ppm.sms
1/8/2009 2:23 PM	1/8/2009 2:43 PM	...0m coloumn\melamine\1 cal 2ppm.sms
1/8/2009 2:51 PM	1/8/2009 3:11 PM	... coloumn\melamine\1 cal 2.5ppm.sms

SGS Lanka /Analysis of Melamine

Acquisition Date:	1/22/2009 8:16 PM	Sample ID:	Sample24
Data File:	...md\tasl\sample24.sms	Method:	...amine 327 target.mth
Instrument ID:	Gc-Ms	Last Calibration:	1/8/2009 3:11 PM
Calculation Date:	1/22/2009 8:36 PM		
Inj. Sample Notes:	None		
Identified Compounds:	1		



Target Compounds

#	RT	Compound Name	Res Type	Quan Ions	Area	Amount
1	17.695	Melamine	Id.	327.4	33	32.745 Counts

Unidentified Peaks

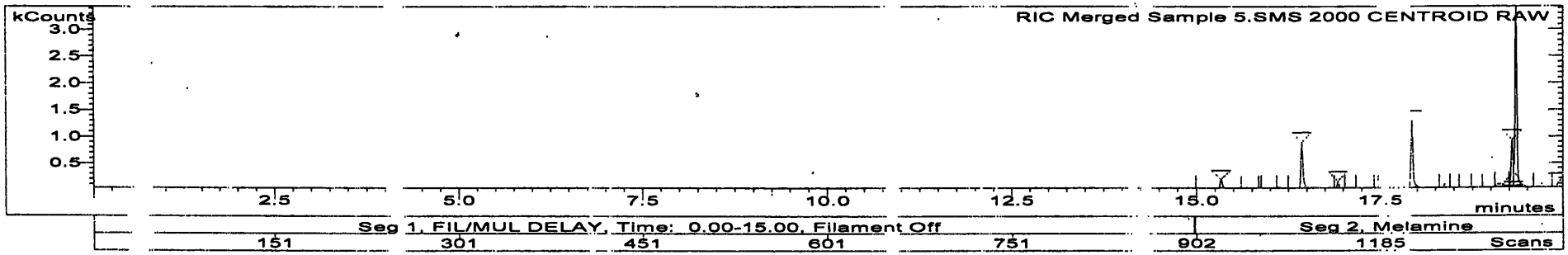
None

Calibration Log

Injection Time/Date	Recalc Time/Date	Calibration Log File
1/8/2009 1:00 PM	1/8/2009 1:20 PM	...m coloumn\melamine\1 cal .5ppm.sms
1/8/2009 1:28 PM	1/8/2009 1:48 PM	...0m coloumn\melamine\1 cal 1ppm.sms
1/8/2009 1:55 PM	1/8/2009 2:15 PM	... coloumn\melamine\1 cal 1.5ppm.sms
1/8/2009 2:23 PM	1/8/2009 2:43 PM	...0m coloumn\melamine\1 cal 2ppm.sms
1/8/2009 2:51 PM	1/8/2009 3:11 PM	... coloumn\melamine\1 cal 2.5ppm.sms

SGS Lanka /Analysis of Melamine

Acquisition Date:	1/22/2009 7:48 PM	Sample ID:	Sample 25
Data File:	...md\tasl\sample25.sms	Method:	...amine 327 target.mth
Instrument ID:	Gc-Ms	Last Calibration:	1/8/2009 3:11 PM
Calculation Date:	1/22/2009 8:08 PM		
Inj. Sample Notes:	None		
Identified Compounds:	0		



Target Compounds

#	RT	Compound Name	Res Type	Quan Ions	Area	Amount
1	17.648	Melamine	Miss.	327.4	0	0.000 ppm

Unidentified Peaks

None

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Calibration Log

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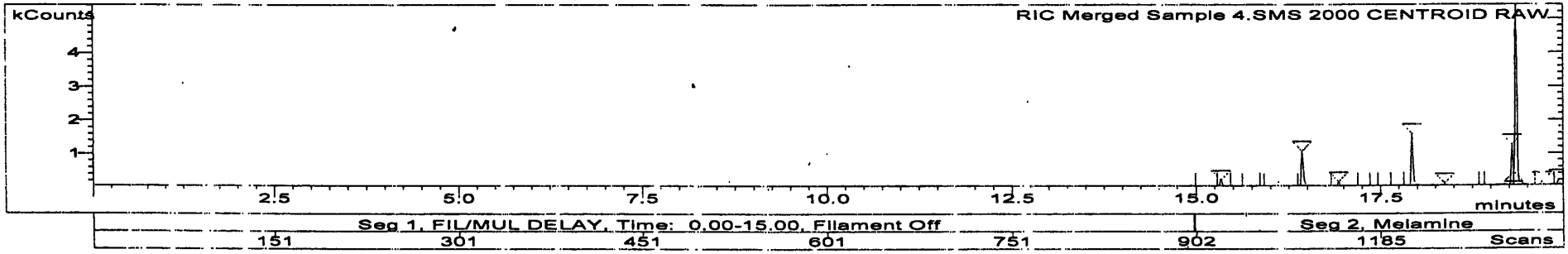
Injection Time/Date	Recalc Time/Date	Calibration Log File
1/8/2009 1:00 PM	1/8/2009 1:20 PM	...m coloumn\melamine\1 cal .5ppm.sms
1/8/2009 1:28 PM	1/8/2009 1:48 PM	...0m coloumn\melamine\1 cal 1ppm.sms
1/8/2009 1:55 PM	1/8/2009 2:15 PM	... coloumn\melamine\1 cal 1.5ppm.sms
1/8/2009 2:23 PM	1/8/2009 2:43 PM	...0m coloumn\melamine\1 cal 2ppm.sms
1/8/2009 2:51 PM	1/8/2009 3:11 PM	... coloumn\melamine\1 cal 2.5ppm.sms

SGS Lanka /Analysis of Melamine

Acquisition Date:	1/22/2009 7:21 PM	Sample ID:	Sample 26
Data File:	...md\tasl\sample 26.sms	Method:	...amine 327 target.mth
Instrument ID:	Gc-Ms	Last Calibration:	1/8/2009 3:11 PM
Calculation Date:	1/22/2009 7:41 PM		
Inj. Sample Notes:	None		
Identified Compounds:	0		



Target Compounds

#	RT	Compound Name	Res Type	Quan Ions	Area	Amount
1	17.648	Melamine	Miss.	327.4	0	0.000 ppm

Unidentified Peaks

None

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Calibration Log

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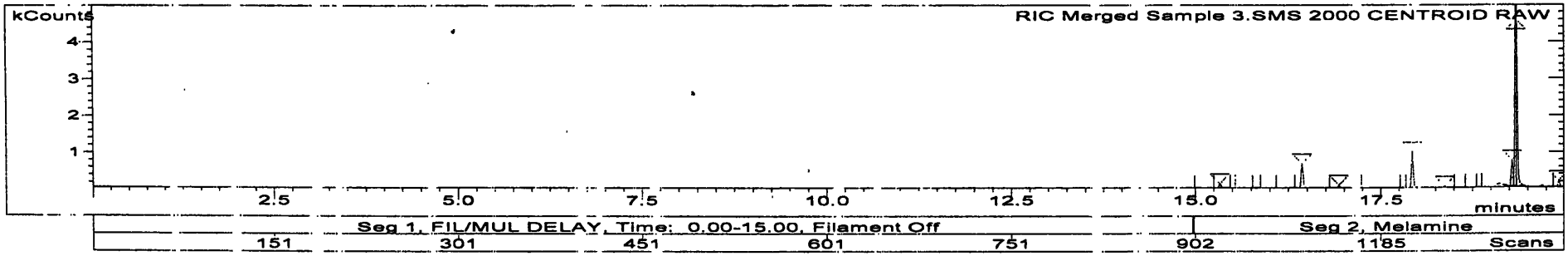
Injection Time/Date	Recalc Time/Date	Calibration Log File
1/8/2009 1:00 PM	1/8/2009 1:20 PM	...m coloumn\melamine\1 cal .5ppm.sms
1/8/2009 1:28 PM	1/8/2009 1:48 PM	...0m coloumn\melamine\1 cal 1ppm.sms
1/8/2009 1:55 PM	1/8/2009 2:15 PM	... coloumn\melamine\1 cal 1.5ppm.sms
1/8/2009 2:23 PM	1/8/2009 2:43 PM	...0m coloumn\melamine\1 cal 2ppm.sms
1/8/2009 2:51 PM	1/8/2009 3:11 PM	... coloumn\melamine\1 cal 2.5ppm.sms

SGS Lanka /Analysis of Melamine

Acquisition Date:	1/22/2009 6:53 PM	Sample ID:	Sample 27
Data File:	...md\tasl\sample27.sms	Method:	...amine 327 target.mth
Instrument ID:	Gc-Ms	Last Calibration:	1/8/2009 3:11 PM
Calculation Date:	1/22/2009 7:13 PM		
Inj. Sample Notes:	None		
Identified Compounds:	0		



Target Compounds

#	RT	Compound Name	Res Type	Quan Ions	Area	Amount
1	17.648	Melamine	Miss.	327.4	0	0.000 ppm

Unidentified Peaks

None

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Calibration Log

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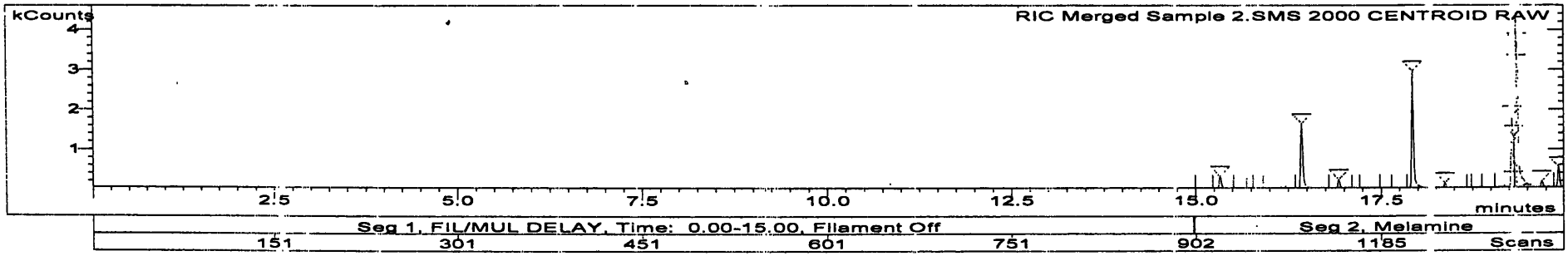
Injection Time/Date	Recalc Time/Date	Calibration Log File
1/8/2009 1:00 PM	1/8/2009 1:20 PM	...m coloumn\melamine\1 cal .5ppm.sms
1/8/2009 1:28 PM	1/8/2009 1:48 PM	...0m coloumn\melamine\1 cal 1ppm.sms
1/8/2009 1:55 PM	1/8/2009 2:15 PM	... coloumn\melamine\1 cal 1.5ppm.sms
1/8/2009 2:23 PM	1/8/2009 2:43 PM	...0m coloumn\melamine\1 cal 2ppm.sms
1/8/2009 2:51 PM	1/8/2009 3:11 PM	... coloumn\melamine\1 cal 2.5ppm.sms

SGS Lanka /Analysis of Melamine

Acquisition Date:	1/22/2009 6:25 PM	Sample ID:	Sample 28
Data File:	...md\tasl\sample 28.sms	Method:	...amine 327 target.mth
Instrument ID:	Gc-Ms	Last Calibration:	1/8/2009 3:11 PM
Calculation Date:	1/22/2009 6:45 PM		
Inj. Sample Notes:	None		
Identified Compounds:	0		



Target Compounds

#	RT	Compound Name	Res Type	Quan Ions	Area	Amount
1	17.648	Melamine	Miss.	327.4	0	0.000 ppm

Unidentified Peaks

None

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Calibration Log

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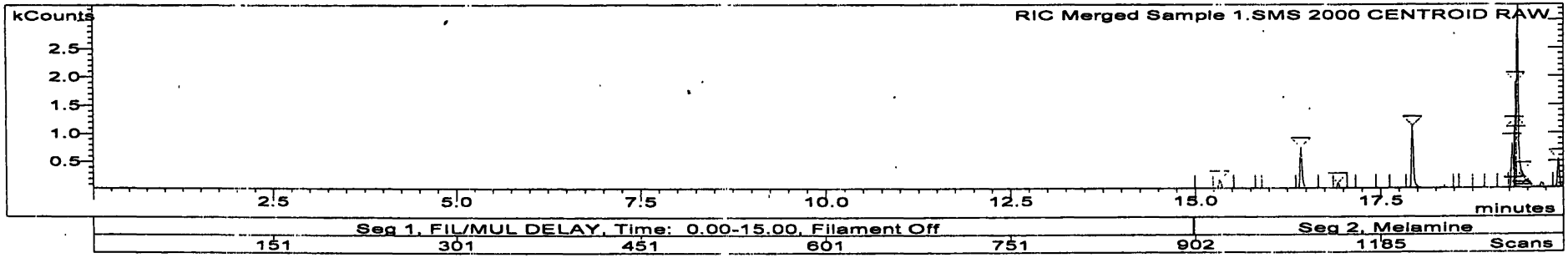
Injection Time/Date	Recalc Time/Date	Calibration Log File
1/8/2009 1:00 PM	1/8/2009 1:20 PM	...m coloumn\melamine\1 cal .5ppm.sms
1/8/2009 1:28 PM	1/8/2009 1:48 PM	...0m coloumn\melamine\1 cal 1ppm.sms
1/8/2009 1:55 PM	1/8/2009 2:15 PM	... coloumn\melamine\1 cal 1.5ppm.sms
1/8/2009 2:23 PM	1/8/2009 2:43 PM	...0m coloumn\melamine\1 cal 2ppm.sms
1/8/2009 2:51 PM	1/8/2009 3:11 PM	... coloumn\melamine\1 cal 2.5ppm.sms

SGS Lanka /Analysis of Melamine

Acquisition Date:	1/22/2009 5:58 PM	Sample ID:	Sample 29
Data File:	...md\tasl\sample29.sms	Method:	...amine 327 target.mth
Instrument ID:	Gc-Ms	Last Calibration:	1/8/2009 3:11 PM
Calculation Date:	1/22/2009 6:18 PM		
Inj. Sample Notes:	None		
Identified Compounds:	0		



Target Compounds

#	RT	Compound Name	Res Type	Quan Ions	Area	Amount
1	17.648	Melamine	Miss.	327.4	0	0.000 ppm

Unidentified Peaks

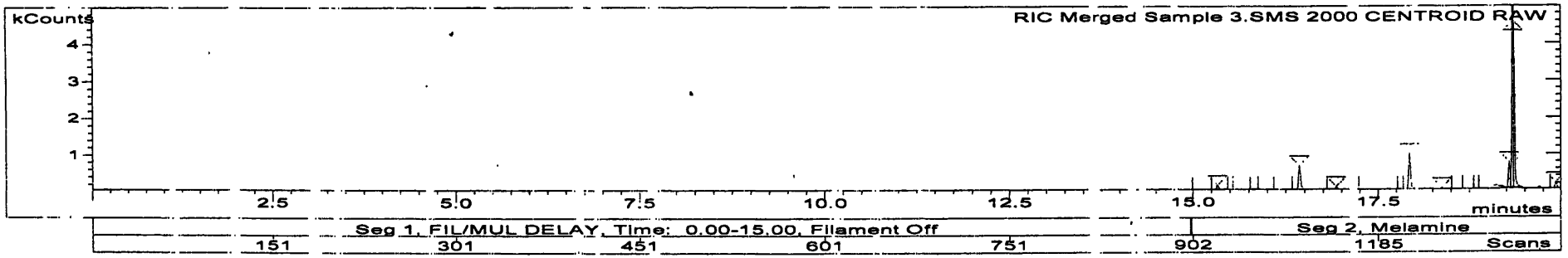
None

Calibration Log

Injection Time/Date	Recalc Time/Date	Calibration Log File
1/8/2009 1:00 PM	1/8/2009 1:20 PM	...m coloumn\melamine\1 cal .5ppm.sms
1/8/2009 1:28 PM	1/8/2009 1:48 PM	...0m coloumn\melamine\1 cal 1ppm.sms
1/8/2009 1:55 PM	1/8/2009 2:15 PM	... coloumn\melamine\1 cal 1.5ppm.sms
1/8/2009 2:23 PM	1/8/2009 2:43 PM	...0m coloumn\melamine\1 cal 2ppm.sms
1/8/2009 2:51 PM	1/8/2009 3:11 PM	... coloumn\melamine\1 cal 2.5ppm.sms

SGS Lanka /Analysis of Melamine

Acquisition Date:	1/22/2009 6:53 PM	Sample ID:	Sample 30
Data File:	...md\tasl\sample 30sms	Method:	...amine 327 target.mth
Instrument ID:	Gc-Ms	Last Calibration:	1/8/2009 3:11 PM
Calculation Date:	1/22/2009 7:13 PM		
Inj. Sample Notes:	None		
Identified Compounds:	0		



Target Compounds

#	RT	Compound Name	Res Type	Quan Ions	Area	Amount
1	17.648	Melamine	Miss.	327.4	0	0.000 ppm

Unidentified Peaks

None

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Calibration Log

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Injection Time/Date	Recalc Time/Date	Calibration Log File
1/8/2009 1:00 PM	1/8/2009 1:20 PM	...m coloumn\melamine\1 cal .5ppm.sms
1/8/2009 1:28 PM	1/8/2009 1:48 PM	...0m coloumn\melamine\1 cal 1ppm.sms
1/8/2009 1:55 PM	1/8/2009 2:15 PM	... coloumn\melamine\1 cal 1.5ppm.sms
1/8/2009 2:23 PM	1/8/2009 2:43 PM	...0m coloumn\melamine\1 cal 2ppm.sms
1/8/2009 2:51 PM	1/8/2009 3:11 PM	... coloumn\melamine\1 cal 2.5ppm.sms

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