SODIUM *O*-CARBOXYMETHYL CHITOSAN AS A NOVEL FIXATIVE FOR EAU DE COLOGNE

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MASTER OF SCIENCE
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ACKNOWLEDGEMENT

Chitosan used in this work was proudly granted by Assistant Professor Dr. Pornchai Rachtanapun, Chiang Mai University.

I would like to thank you Dr. Ampa jimthaisong and Dr. Nisakorn saewan for being my advisor and assist me for finding some correlation journal.

For any kind of admire in the future, I would like to donate to my father and mother including all of member in my family who always encourage me until I have make it through.

Surawut Wannaruemon

Independent Study Title Sodium *O*-carboxymethyl chitosan as a novel fixative for eau de cologne

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Degree Master of Science (Cosmetic Science)

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ABSTRACT

Sodium carboxymethyl chitosan (SCM-chitosan) was prepared by *O*-carboxymethylation reaction with monochloroacetic acid and use as a novel fixative for eau de cologne. SCM-chitosan is soluble in water but insoluble in ethyl alcohol, the optimum volume ratio between water and alcohol that still be able to soluble 0.1 percent of SCM-chitosan is 2:3. Water solubility of perfume compounds in eau de cologne affected the solubility of SCM-chitosan. The more water solubility of perfume compounds, the higher solubility of SCM-chitosan in formulation. Headspace analysis using gas chromatography is the method for evaluate fixative effect. SCM-chitosan has the significant fixative effect on *p*-cresyl methyl ether at concentration 10%in DPG eau de colonge. The fixative efficiency can rise to 92.19 percent at the temperature of 53 ^{o}C. However the fixative effect of SCM-chitosan on benzyl alcohol eau de colonge, ethyl acetoacetate eau de colonge and 94085 floral-fruity eau de cologne needs to future investigation due to the precipitation of the systems which might be cause by incompatibility between SCM-chitosan and the aroma material.

Keywords: Chitosan / carboxymethylation / Cologne / Fixative / Perfume

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ABBREVIATIONS AND SYMBOLS

O	Oxygen 3-position
CS	Chitosan
NAGA	β(1,4)-glucosamine (2-amino-2-deoxy-D-glucose) and -acetyl-D-glucosamine
β	Beta
IFRA	International fragrance association
EdP	Eau de Parfum
EdT	Eau de Toilette
EdC	Eau de cologne
%	Percentage
INCI	International Nomenclature of Cosmetic Ingredients
UV	Ultra violet ray
EO/PO/EO	Poly (ethylene oxide)/Poly (propylene oxide)/Poly (ethylene oxide)
CDs	Cyclodextrins
βCD	β-Cyclodextrin
2-HPβCD	2-hydroxypropyl-β-cyclodextrin
SARS	Severe acute respiratory syndrome
NaOH	Sodium Hydroxide
α	Alpha
γ	Gamma
HS-GC	Headspace gas chromatography
GC	Gas chromatography
FID	Flame ionization detector
BAL	Benzyl alcohol
PCME	*p*-cresyl methyl ether

ABBREVIATIONS AND SYMBOLS (continued)

DPG	Dipropylene glycol
EAA	Ethyl acetoaetate
SexG	Perfume compounds of 94085 floral-fruity
°C	Celcius degree
est	Estimate (in the term of weight)
SCM-chitosan	Sodium *O*-carboxymethyl chitosan
g	Gram
mL/ml	Millilitre
μL	Microlitre
min	Minute
ΔG	Free energy of activation
-OH bond	Hydroxy bond
ctrl	Controlled or without SCM-chitosan
scmt	With SCM-chitosan
$\%FX_{eff}/\phi$	Fixative efficiency
mm	Millimetre
Hg	Mercury
N	Nitrogen

CHAPTER 1

INTRODUCTION

1.1 Background and rationale

Eau de cologne is the product that consists of low quantity of perfume compounds but high volume of water and alcohol. Poor tenacity is the most important problem for cologne. It was compensated with satisfied scent of refreshing and low price. Long tenacity effect is one of the desirable characteristic. From a practical point of view, long tenacity should be gradually released or constantly volatile over a long period of time. Hence, many substances are brought to use as perfume sustained release agent and fixative such as resin, co-polymer, cyclodextrins and microcapsules. The mechanism of perfume sustained release still not clearly understood but the fixative involve the hydrogen bonding of fixative and perfume compound which is also known as fixation (Curtis & Williams, 2001, p.712). Then chitosan, which contains abundant hydroxy group on molecule, was used to study as perfume fixative in this work.

Chitosan (CS) is a linear biopolymer of natural amino polysaccharides having linked β(1,4)-glucosamine (2-amino-2-deoxy-D-glucose) and *N*-acetyl-D-glucosamine (NAGA) unique structure, which is a deacetylated form of chitin, a major component of crustacean outer skeletons. Chitosan has been reported to have many desirable properties of material science such as non-toxicity, biodegradability, biocompatibility, and anti-microbial characteristics. Moreover, its ability to sustained release effect of citronella oil is very interesting (Hsieh, Chang & Gao, 2006). However, chitosan is now established that the difficulty in solubilization is due to the structure of abundant hydrogen bonded and semi-crystalline (Pillai, Paul & Sharma, 2009). For using as perfume sustained release, soluble chitosan with the neutral solution is preferred.

Shrimp chitosan, which regarded as junk in food industry and inexpensive will be probably material used as fragrance fixative or sustained release carrier. It should be able to form micelle and decrease volatility. But solubility of chitosan is the important problem to formulate perfume product so water soluble chitosan could be the most appropriate chitosan for this work.

1.2 Objectives

1.2.1 To increase the tenacity of perfume by using *O*-carboxymethyl chitosan as the perfume fixative

1.2.2 To evaluate the perfume fixation efficiency of *O*-carboxymethyl chitosan

1.3 Scope

1.3.1 Synthesize the *O*-carboxymethyl chitosan

1.3.2 Develop the perfume formulation using *O*-carboxymethyl chitosan

1.3.3 Evaluate the volatility of perfume in the prepared formulas using head space analysis technique

1.4 Research question

1.4.1 How to synthesize water soluble chitosan?

1.4.2 How to evaluate the volatility of perfume?

1.4.3 Can water soluble chitosan control rate of volatility of perfume?

1.4.4 What is the relations of chitosan concentration and rate of volatility?

1.4.5 Is the water soluble chitosan valuable for use as perfume sustained release?

1.5 Benefit and application

1.5.1 Formulate the high tenacity perfume with natural derivative substance

1.5.2 Increase the value of chitosan and decrease demand of imported fixative

1.5.3 Reduce cost of fragrance manufacture

CHAPTER 2

LITERATURE REVIEW

2.1 Perfume

Perfume is a mixture of several aromatic materials such as essential oil, ester or wood resin. Fine Fragrance is the term for fashionable fragrance division, different in perfume concentration divided them to 4 classes. Perfume extract (Extrait) which have the concentration 15-40% of aromatic compounds, usually 20% by the IFRA (international fragrance association) commitment. Eau de Parfum (EdP) or Parfum de Toilette (PdT) which have the concentration 10-20% but typically around 15% of aromatic compounds, sometimes listed as "eau de perfume" or "millésime". Eau de Toilette (EdT) which have the concentration 5-15% but typically around 10% of aromatic compounds. Eau de Cologne (EdC) which have the concentration 3-8% but typically around 5% of aromatic compounds (Susan Irvine, 1995) The Eau de Parfum formulation contains 78% denatured ethanol was shown in Table 2.1

Table 2.1 The business Eau de Parfum formulation

Ingredient	%w/w
Ethanol (DEB100)	78.00
Fragrance	12.00
Purified water	8.50
PPG-20 methyl glucose ether	1.00
Benzophenone-2	0.50

The source of this ethanol is from synthetic or natural grain starch, sugar beet, or molasses alcohol can give rise to a different odor in the end product. Denaturants which deter people from ingesting the alcohol are required by legislation in many countries and are also useful to know, although less likely to have a significant effect on the finished product. In this case, a standard 99.7% v/v synthetic grade, containing 0.1% v/v t-butanol as a marker and denatured with 10 ppm of Bitrex, INCI name is denatonium benzoate, an extremely bitter substance, is likely to be used by the client. Given the high level of alcohol and consequent low level of water, there is unlikely to be any need for addition of solubilisers. Unless EdC formulation which has higher amount of water, solubliser will need. The formulation also contains an ultraviolet (UV) radiation absorber, benzophenone-2, to prevent degradation of the fragrance and any dyes by light. Although consumers are encouraged to keep fine fragrances in the dark, the manufacturer needs to protect the product from those customers who insist on storing it on a sunny windowsill. Long lasting effect is the important qualification of perfume but how to increase the tenacity of perfume without disturbing the whole scent still be limited by the number of fixatives. A moisturizing ingredient, which has additional fragrance-fixative properties (PPG-20 methyl glucose ether), is also incorporated in this instance (Pybus & Sell, 2006, pp.170-171).

2.2 Perfume fixative and perfume sustain released agent

2.2.1 Fixative

The fixation is the substance which has the fixation power. A fixative is used to prolong the effect of the more volatile ingredients in a perfume formula in an attempt to equalize the rate of evaporation of the component ingredients. Molecules with low vapour pressure are used; these are often resinous, crystalline or high relative molecular mass liquids e.g. oakmoss resinoid, macrocyclic musk, galbanum resinoid.

Figure 2.1 Galbanum resinoid

Figure 2.2 Musk

2.2.2 Perfume sustained release

Another way to increase the tenacity is to use perfume sustained release agent, it will function as surfactant but it still not clearly understood about mechanism (Suzuki, Saito, Tokuoka, Abe & Sato, 1997). For the example, hydrogenated castor is the well known substance. It is a non-ionic surfactant used as solubilizer for perfume oil of essential oil for a decade. Not only solubilizing property it has, it also a volatility retardment for the perfume or after shave products (Libraw Pharma, 2010).

Poly (ethylene oxide)/poly (propylene oxide)/poly (ethyleneoxide) copolymer has been reported in 1997 for its ability of decrease the volatilization. It was investigated by means of dynamic and static headspace analysis. Suppression of the volatility of perfume compounds by Poly (ethylene oxide)/Poly (propylene oxide)/Poly (ethylene oxide) or EO/PO/EO copolymer was markedly greater than by polyethylene glycol. This suppressive effect may be due to micelle and gel formation of EO/PO/EO copolymer. EO/PO/EO copolymer is expected to be useful as a novel sustained-release carrier that maintains constant release rates for the volatility of perfume compounds over a wide temperature range. The triblock copolymer still has a weak point that it will turn to gel upon the rising of temperature (Suzuki et al., 1997).

Cyclodextrin was use to increase the stability and water solubility of perfume compounds, to provide controlled release of linalool and benzyl acetate, and to convert these substances from liquid to powder form by preparing their inclusion complexes with cyclodextrins (CDs). The use of β-cyclodextrin (βCD) and 2-hydroxypropyl-β-cyclodextrin (2-HPβCD) for increasing the solubility of these two fragrance materials was studied and the results show that the solubility of the inclusion complex prepared with 2-HPβCD at a 1:1 molar ratio increase the solubility of linalool and benzyl acetate. The stability and in *vitro* release studies were performed on the gel formulations prepared using uncomplexed fragrance materials or inclusion complexes of fragrance materials at a 1:1 molar ratio. It was observed that the volatility of both fragrance materials was decreased by preparing the inclusion complexes with 2-HPβCD. Also, in *vitro* release data indicated that controlled release of fragrances could be possible if inclusion complexes were prepared (Numanoğlu et al., 2007).

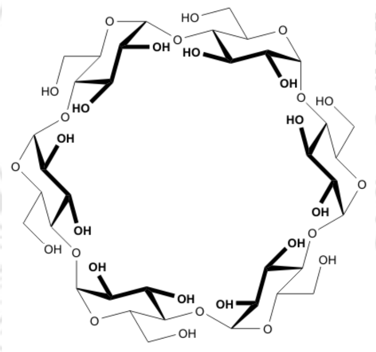


Figure 2.3 Cyclodextrin nomenclature

2.3 Chitin and chitosan

In 2006, when the earth spread of severe acute respiratory syndrome (SARS), Dengue Fever, and Avian Influenza (Bird Flu), the volatile essential oils that have efficacies of antibiosis, insect repelling and stress reducing are the alternative way to use among the trouble. Wen-Chuan

Hsieh et al. prepared the oil in water type chitosan encapsulated volatile citronella oil microcapsules and investigated the forming condition of microcapsules and the influence to sustained release effect of volatile citronella oil by applying thermal pretreatment to microcapsules. The result was depended on three factors (1) the concentration of chitosan was at least 0.2 wt%, (2) NaOH was greater than 0.1 wt%, and (3) addition of coconut oil as natural surfactant, product will perform the best forming microcapsule and disperse. If the microcapsules were thermal pretreated at 80 ˚C, the structure of chitosan wall membrane would shrink and thus achieve the effect of sustained release. The sustaining effect would increase along with treatment time increased (Hsieh et al., 2006).

So chitosan was selected to use and modified to water soluble chitosan for further study of sustained release effect or fixation.

Commercially chitosan is manufactured by deacetylation of chitin. About 45% of processed seafood consists of shrimp, the waste of which is composed of exoskeleton and cephalothoraxes. The chemical structure of chitosan is shown in Figure 2.4.

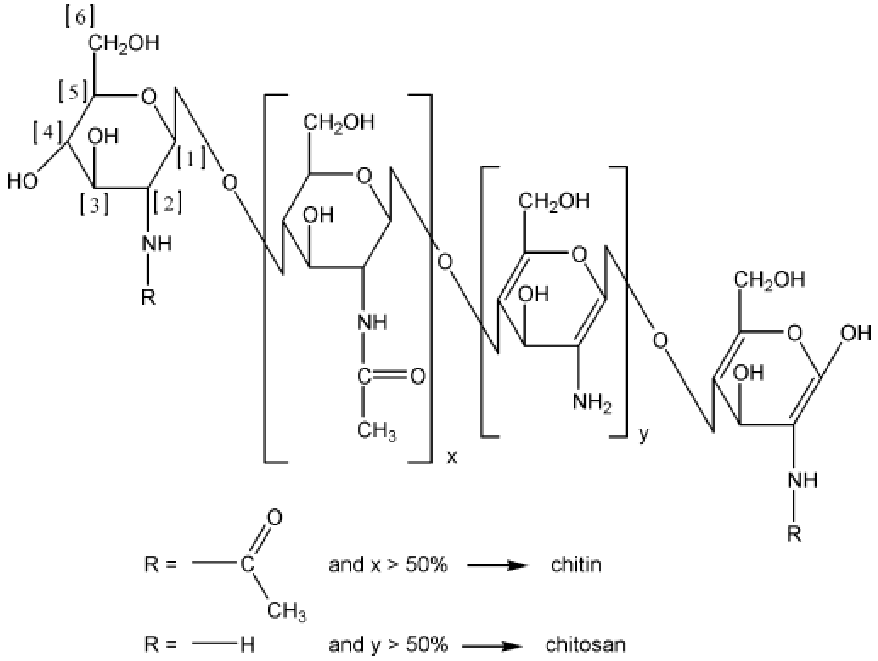


Figure 2.4 Structure of chitin and chitosan

According to the source, chitin is found in three polymorphic forms, different in the packing of adjacent chains in successive sheets. The α-form chains are arranged in an anti-parallel manner, strong intra- and inter-sheets hydrogen bonds occur. β-Chitin are aligned in a parallel manner, weak intra-sheets hydrogen bonds occur. The mixture of antiparallel and parallel chains that characterize the crystalline structure is a γ-form, Figure 2.5.

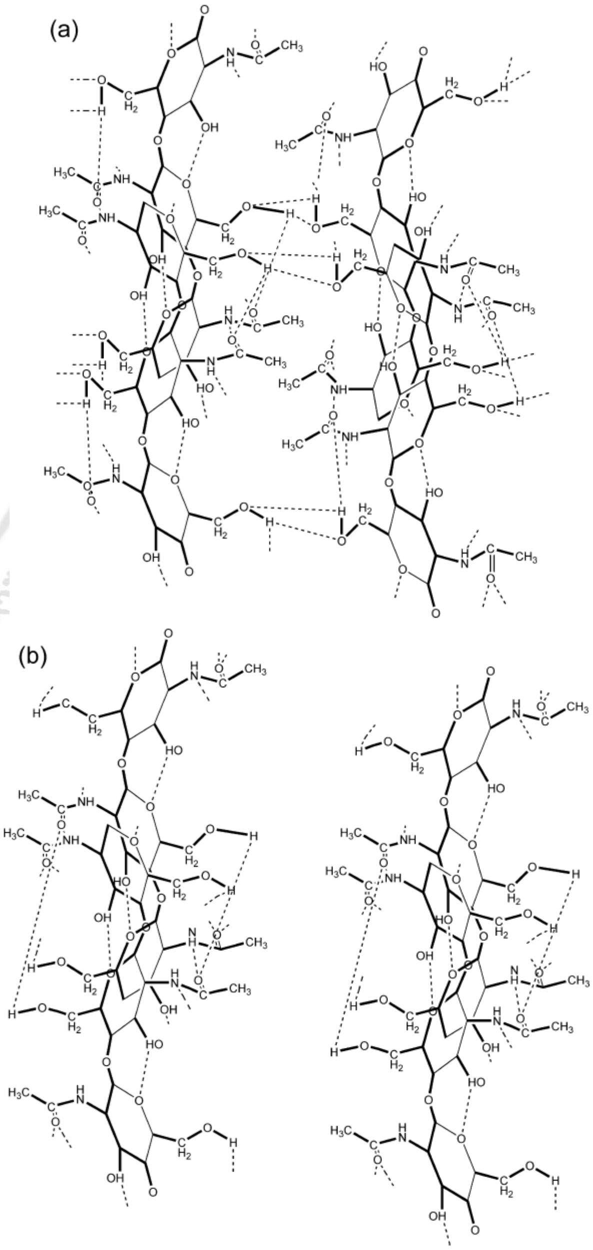


Figure 2.5 Molecular structure and hydrogen bonding in (a) α -chitin and (b) β –chitin

2.4 Static Headspace analysis

Static headspace gas chromatography (HS-GC) is a technique used for the concentration and analysis of volatile organic compounds. This technique is relatively simple and can provide sensitivity similar to dynamic purge and trap analysis. The popularity of this technique has grown and has gained worldwide acceptance for analyses of alcohols in blood and residual solvents in pharmaceutical products. Other common applications include industrial analyses of monomers in polymers and plastic, flavor compounds in beverages and food products, and fragrances in perfumes and cosmetics. Sample matrices like blood, plastic, and cosmetics contain high molecular weight, non-volatile material that can remain in the GC system and result in poor analytical performance. Many laboratory analysts use extensive sample preparation techniques to extract and concentrate the compounds of interest from this unwanted nonvolatile material. These extraction and concentration techniques can become time consuming and costly. Static headspace analysis avoids this time and cost by directly sampling the volatile headspace from the container in which the sample is placed (Restek Corporation, 2000).

Figure 2.6 shows two parts of interest for head space technique, gas phase or head space and sample phase. Gas phase is commonly referred to as the headspace and lies above the condensed sample phase. Sample phase is the compound(s) of the interest and is usually in the form of a liquid of solid in combination with a dilution solvent or a matrix modifier.

Once the sample phase is introduced into the vial and the vial is seal, volatile components diffuse into the gas phase until the headspace has reached a state of equilibrium as depicted by the arrow. The sample is then taken from the headspace by the syringe or auto-machine.

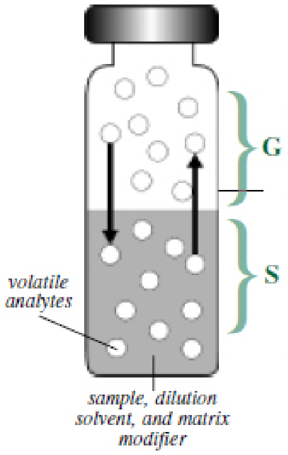


Figure 2.6 Static headspace illustrator

2.5 Instrumentation of gas chromatography (GC)

GC is a method of separation which employs a gas mobile phase and either a solid (GSC) or a liquid (GLC) or gas phase (Headspace) adsorbed on a solid as a stationary phase. Gas chromatography is capable of separating very complex mixtures and the selectivity can be adjusted to separate almost any given pair of solutes by judicious choice of the stationary phase. The major limitation of gas chromatography is the requirement that the solute have a reasonable vapor pressure at a temperature where it is still stable. The key parts of a gas chromatograph include: a source of gas as the mobile phase, an inlet to deliver sample to a column, the column where separations occur, an oven as a thermostat for the column, a detector to register the presence of a chemical in the column effluent, and a data system to record and display the chromatogram.

GC is used for both of qualitative and quantitative analysis. The process of GC is shown in figure 2.7, a sample was vaporized in an injection port. Then carrier gas (mobile phase) carries the sample vapor to column (stationary phase) where sample was separated before go through detector. Separation principle is "like dissolve like" that depend on sample and column coating material polarity. Many detector of GC are ion detectors with varying methods of ionizing the components eluting from the GC's column. An ion detector is analogous to a capacitor or vacuum tube. It can be envisioned as two metal grids separated by air with inverse charges placed on them. An electric potential difference or voltage exists between the two grids. After components are ionized in the detector, they enter the region between the two grids, causing current to pass from one to the other. This current is amplified and is the signal generated by the detector. The higher the concentration of the component, the more ions are generated, and the greater the current. In this study flame ionization detector or FID was used as detector, figure 2.8. Sample will be ionized by flame before its ion conductivity was detected which vary to sample concentration and interpret to peak area (National Nanotechnology Center [NANOTEC], 2005).

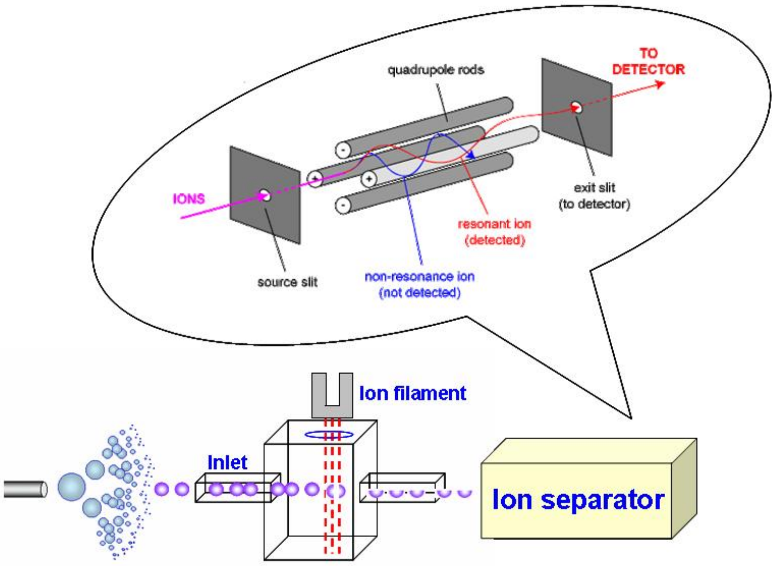


Figure 2.7 Instrument parts of gas chromatography diagram

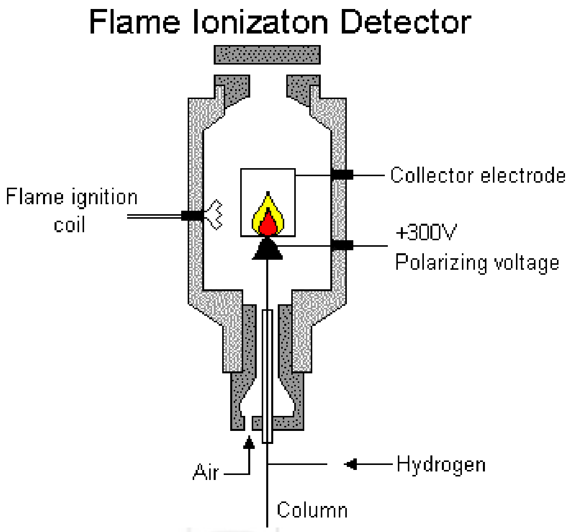


Figure 2.8 Flame ionization detector illustration

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Materials

Shrimp chitosan (polymer type) with degree of deacetylation > 85.00% and molecular weight (1,000,000 – 1,500,000 dalton) was purchased from Ta Ming Enterprisrs Co., ltd., Thailand. It was grinded by electric grinder to obtain the powder form. Monochloroacetic acid, isopropanol (cosmetic grade), ethanol (perfumer grade), sodium hydroxide (industrial grade), aroma materials: which are phenethyl alcohol, benzyl alcohol (BAL), linalool, geraniol, α-Ionone, β-Ionone, α-Methyl Ionone, citral, hydroxy citronellal, *p*-cresyl methyl ether (PCME) (10% in dipropylene glycol or DPG), diethyl malonate, ethyl benzoate, ethyl acetoaetate (EAA), phenyl ethyl acetate, benzyl acetate, 94085 floral-fruity (SexG) and distilled water were supplied by the Bangkok Perfumery, Thailand and used without further purification. Table 2.1 shows the chemical structures, molecular weights, and solubility in water (25°C) of the perfume compounds. (The Good Scents company, 2010).

Table 3.1 Chemical Structure, Functional group, and Solubility of Perfume Compounds

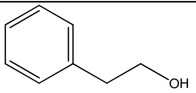
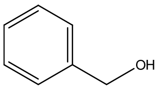
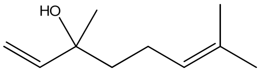
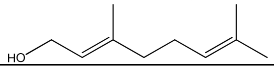
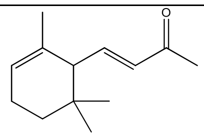
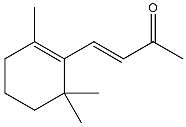
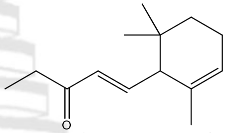
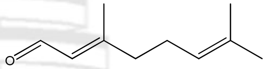
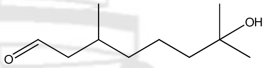
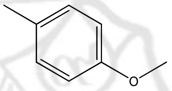
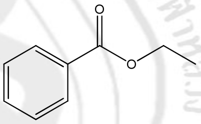
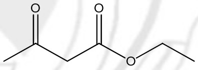
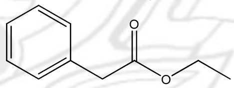
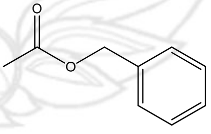
Perfume compounds	Functional group	Chemical structure	Solubility in water mg/L at 25°C (est)
Phenethyl alcohol	Alcohol		22200
Benzyl alcohol	Alcohol		42900
Linalool	Alcohol		1590
Geraniol	Alcohol		100

Table 3.1 (continued)

Perfume compounds	Functional group	Chemical structure	Solubility in water mg/L at 25°C (est)
α-Ionone	Ketone		24.67
β-Ionone	Ketone		25.16
α-Methyl Ionone	Ketone		slightly
Citral	Aldehyde		1340
Hydroxy citronellal	aldehyde		slightly
p-cresyl methyl ether	Ether		670
Diethyl malonate	Ester	Suddenly precipitate	99500
Ethyl benzoate	Ester		720
Ethyl acetoacetate	Ester		110000
Phenyl ethyl acetate	Ester		711
Benzyl acetate	Ester		3100
94085 floral-fruity	Compounds		1240

Note. est is stand for estimate (in the term of weight)

3.2 Sodium *O*-carboxymethyl chitosan (SCM-chitosan) synthesis

Carboxymethyl chitosan was prepared by the method of Liu, Guan, Yang, Li & Yao (2001). Chitosan (10 g), sodium hydroxide (13.5 g), water (20 ml) and isopropanol (80 ml) were added into a flask (500 ml) to swell and alkalized at 50 ^{o}C for 1 hour. The temperature was maintained in a water bath (Thermo-controller, Comabiotech. Co., Korea). The monochloroacetic acid (15 g) was dissolved in isopropanol (20 ml), and added into the reaction mixture dropwise for 30 min and reacted for 4 hour at the same temperature and then stopped by adding 70% ethyl alcohol (200 ml). The solid was filtered and rinsed in 70–90% ethyl alcohol to desalt and dewater, and dried at room temperature. The products were sodium carboxymethyl chitosan (SCM-chitosan). The yield was calculated by the following equation:

Yield (%) = [Water soluble chitosan (SCM-chitosan) (g)/Raw chitosan] x 100

Degree of substitution was not determined in this research.

3.3 Product formulation

The solubility of SCM-chitosan has been studied at ambient temperature by dissolve in the distilled water. Due to SCM-chitosan is an alcohol insoluble but the eau de cologne must contain high proportion of alcohol, so the optimal quantity of water in the formulation was investigated.

Eau de cologne was prepared by mixing 3% of each aroma material or perfume compound with water and ethanol at the optimal ratio. In the water phase, SCM-chitosan from 0.01-0.2% was for evaluation the solubility result. Microwave irradiation (medium-high, 2 min) was used to facilitate the solubility of SCM-chitosan. In the alcohol phase, aroma materials or perfume compounds were diluted with the alcohol after that slowly poured into the water phase, drop by drop was preferred. Name of aroma materials used in this step are shown in table 2.

Turbidity of sample was evaluated by observation of the precipitated matter in eau de cologne for 21 days.

3.4 Evaluation of fixative effect at zero-time

Static headspace analysis technique was used to evaluate the volatility of products. Eau de cologne of BAL, EAA, PCME10%inDPG and SexG (0.5ml) with and without SCM-chitosan were placed into a sample vial (1.5 mL) and sealed airtight with a silicon septum, and allowed each vial to equilibrate in a water bath at 25, 35 and 53 °C before injection into the gas chromatograph. Approximately 500 µL of vapor of perfume compound above the solution was drawn out from the vial with the gas-tight syringe. The sample volume was adjusted to 300 µL, and then the sample was injected into the gas chromatograph in splitless mode. Helium is a purge gas. The column temperature was 180°C by starting from 80 °C and increase 10°C per minute. The gas chromatograph was equipped with a DB-5 column, 60 m length, 0.25 mm inner diameter and 1.0 µm film thickness and flame ionization detector (FID) heated at 300˚C. The oven temperature was programmed from 80˚C (1 min hold) to 180˚C at a rate of 10˚C/min and final hold at 220˚C of 3 min.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Preparation of sodium *O*-carboxymethyl chitosan (SCM-chitosan)

This result was obtained by using the optimum condition for preparing SCM-chitosan. When swelling and alkalizing, shrimp chitosan turned into translucent and become opaque again after carboxymethylation step. SCM-chitosan obtained is the white powder with weak basicity (pH 8). Three replicate yields are shown in Table 4.1. Standard deviation equal 0.05

Table 4.1 Yields of SCM-chitosan samples prepared from carboxymethylation reaction

Batch no.	SCM-chitosan (g)	Yeild (%)
Batch no. 1	37.45	374.5
Batch no. 2	37.40	374.0
Batch no. 3	37.50	375.0
Mean	**37.45±0.05**	**374.5±0.5**

4.2 Product formulation

Microwave irradiation was used to facilitate the solubility of SCM-chitosan. The mechanism can be explained that involving free energy of activation (ΔG) degrades some weakly bonds especially –OH bond which have a dipole moment (Ramtohul, 2002). Lose of crystallinity from depression of hydrogen bond between molecule will occur and enhance more water solubility. However, the water solubility of SCM-chitosan was still limited by size of molecule, it can be explained in the same way of solubility of sugar in alcohol phenomenon. Although sugar and alcohol is close polarity, but sugar soluble in alcohol to some extent while sugar soluble in water very well. Both sugar and alcohol possess the hydrogen content in molecular structure that allows for bonding which also translates then to solubility through that hydrogen bonding. But carbon atoms of alcohol actually interfere with the hydrogen bonding between the sugar and alcohol molecules while water have not, so it seem to be sugar is insoluble in alcohol (Solomons & Fryhle, 2000, pp. 74-76). As the same way to SCM-chitosan which is cellulose derivative.

The optimal ratio of water phase and alcohol phase was 2:3 by volume, and the suitable percentage of SCM-chitosan is 0.10 %, some perfume compound was limited for formulation because they caused the turbidity after aging process. In order to evaluate product formulation, turbidity of formulation was observed overtime. The results are shown in the Table 4.2 where the "+" symbol mean turbidity level of solution ranking from 0-10.While "+" higher number mean higher turbidity and "+" lower number mean lower turbidity. Fig. 4.1 shows the level of turbidity by photograph.

Table 4.2 Solubility of SCM-chitosan in eau de cologne of various perfume compounds and stability

Perfume compounds	**turbidity**		
	Day1	**Day2**	**Day21**
Phenethyl alcohol	+	+	+
Benzyl alcohol	-	-	-
Linalool	+	+	+
Geraniol	+	+	+
α-Ionone	+	+	+
β-Ionone	+	+	+
α-Methyl Ionone	+	+	+
Citral	+	+	+
Hydroxy citronellal	+	+	+
p-cresyl methyl ether	-	-	-
Diethyl malonate		Suddenly precipitate	
Ethyl benzoate	+	+	+

Table 4.2 (continued)

Perfume compounds	turbidity		
	Day1	Day2	Day21
Ethyl acetoacetate	-	-	-
Phenyl ethyl acetate	+	+	+
Benzyl acetate	-	+	+
94085 floral-fruity	-	-	-

Note. EAA become little turbid after Day28

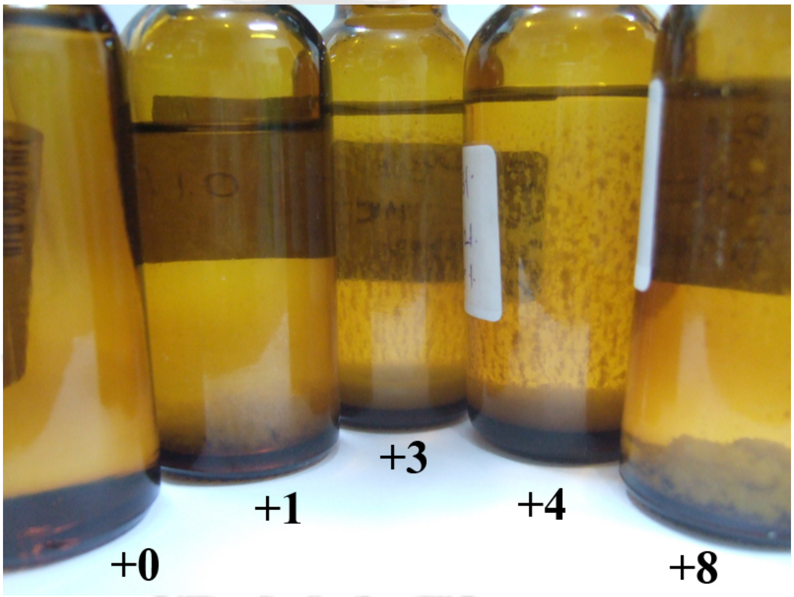


Figure 4.1 Turbidity level

According to the result obtained, benzyl alcohol, ethyl acetoacetate and p-cresyl methyl ether 10%inDPG and 94085 floral-fruity which was still clear after day 21 so they was selected to evaluating fixative effect.

4.3 Evaluation of fixative effect at zero-time

Determination of the perfume fixation was based on persistency of sodium carboxymethyl chitosan in the solution. Fixative effect will occur when SCM-chitosan solute in the media. After aging for 21 days, hydrogen bonding between SCM-chitosan and water or alcohol or perfume compound participated in the lower rate of volatility due to the reason of thermodynamic term. Table 4.3 shows the volatility matter calculated from sum of peak area of chromatogram. Ideally, benzyl acetate containing sodium carboxymetyl chitosan, ethyl acetoacetate containing sodium carboxymetyl chitosan, p-cresyl methyl ether 10%inDPG containing sodium carboxymetyl chitosan and 94085 floral-fruity containing sodium carboxymetyl chitosan (BALscmt, EAAscmt, PCMEscmt and SexGscmt) possed a lower volatility matter when compared with control-sample which are benzyl acetate, ethyl acetoacetate, p-cresyl methyl ether 10%inDPG and 94085 floral-fruity (BALctrl, EAActrl, PCMEctrl, SexGctrl).

Table 4.3 Volatility of volatile compounds in eau de cologne calculated from peak area at the different temperatures

Sample name	**Area (pA*s) at Temperature (^{o}C)**		
	25	**35**	**53**
BALctrl	$3.81228*10^5$	$3.16023*10^5$	$3.48614*10^4$
BALscmt	$2.86837*10^5$	$1.32879*10^5$	$2.15781*10^4$
EAActrl	$3.70841*10^5$	$4.16710*10^5$	9350.47528
EAAscmt	$2.96246*10^5$	$3.52931*10^5$	$1.53024*10^4$
PCMEctrl	$3.08051*10^5$	$2.59229*10^5$	$1.07468*10^5$
PCMEscmt	$2.95696*10^5$	$1.50083*10^5$	8393.03662
SexGctrl	$5.11710*10^5$	$2.99565*10^5$	$4.01783*10^4$
SexGscmt	$4.38149*10^5$	$3.78185*10^5$	$1.03591*10^5$

While BAL, EAA, PCME and SexG stand for benzyl alcohol, ethyl acetoacetate, p-cresyl methyl acetate 10% in DPG and 94085 floral-fruity. Ctrl and scmt after the capital letter mean the aroma materials or perfume compounds without SCM-chitosan and with SCM chitosan by the order.

To calculated fixative effects of SCM-chitosan, the following equation was used:

$$\% FX_{eff} = [(Area_{ctrl} - Area_{scmt}) / Area_{ctrl}] \times 100$$

Where $\%FX_{eff}$ is fixative efficiency compared with controlled sample, $Area_{ctrl}$ is peak area of controlled sample and $Area_{scmt}$ is peak area of SCM-chitosan sample. The calculated results are tabulated in Table 4.4

Table 4.4 Fixative efficiency when compare with control sample in percentage

Sample name	**Fixative efficiency ($\%FX_{eff}$)**		
	25 ^{o}C	**35 ^{o}C**	**53 ^{o}C**
BALscmt	24.76	57.95	38.10
EAAscmt	20.12	15.31	-63.65
PCMEscmt	4.01	42.10	92.19
SexGscmt	14.38	-26.24	-157.83

The relation between $\%FX_{eff}$ and temperature are graphically shown in Fig.3. It can be seen that PCMEscmt has a great $\%FX_{eff}$, which can be up to 92.19% when the temperature rising, this is also concerned as sustained release properties. BALscmt has the best fixative efficiency value at temperature of 37^{o}C which often be found in hot day. At temperature of 53^{o}C, fixative efficiency value of BALscmt drop to 38.10, this can be explained by heat energy destroy some of hydrogen lead to losing of fixation. Three breakpoints against ideal appeared on EAAscmt 53^{o}C, SexG 35^{o}C and SexG 53^{o}C. According to the precipitation of SCM-chitosan, reduce hydrogen bond between aroma materials or perfume compounds and hydro-alcoholic solution.

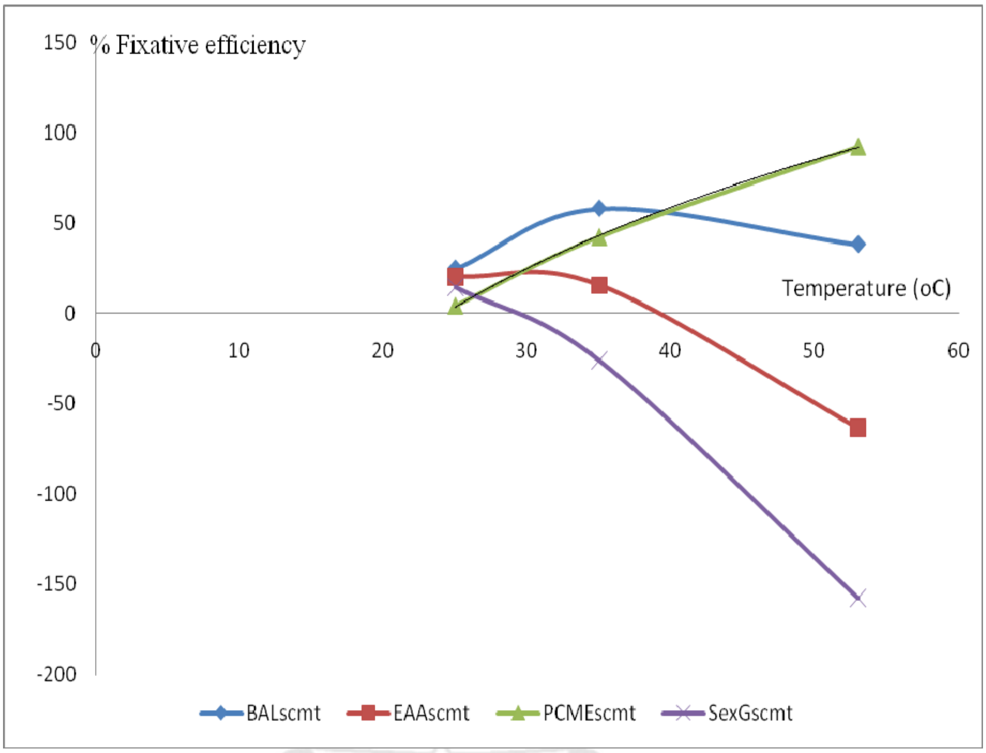


Figure 4.2 Sustained release efficiency of SCM-chitosan on eau de colonge as a function of temperature

Considering to breakpoints, it is interesting to see that EAAscmt and SexGscmt became turbid overtime (see Table 4.2) which indicates lower solubility of SCM-chitosan in the system. Thus system that contains less amount of SCM-chitosan would have lower fixative effect.

It can also be mentioned that EAAscmt and SexGscmt have negative value of % fixative efficiency. This may cause from sodium ion of SCM-chitosan interact with ethyl acetoacetate and turned into ethyl acetoacetate sodium salt which insoluble in ethanol and may precipitate out of the solution, and this will affect to the equilibrium of eau de cologne in the vial. Ethyl acetoacetate has the vapour pressure at 0.8 mm of Hg at 20^{o}C (Sciencelab.com, Inc., 2008) and ethanol has 40 mm of Hg at 19^{o}C (Weast, 1974). From this information, it can imply that if the system contains less amount of ethyl acetoacetate in ethanol, volatile content will higher than the system which contain more ethyl acetoacetate (ideal gas law).

So interaction of SCM-chitosan to some perfume compounds which may lead to sodium salt formation, may increase vapor pressure which lead to negative value of fixative efficiency when determine from sum of peak area.

The fixative efficiencies of SCM-chitosan on each perfume compound from Figure 3, zero-time determine were expressed by this logarithm equation:

BALscmt $\phi(\%) = 15.24 In(x) - 14.32$

EAAscmt $\phi(\%) = 114\ In(x) - 400.6$

PCMEscmt $\phi(\%) = 117.4\ In(x) - 374.6$

SexGscmt $\phi(\%) = -232.\ In(x) + 776.2$

Where ϕ represents the fixative efficiencies and “x” me ans temperature.

At equilibrium state, rate of volatile of each perfume compounds are constant so no need to concern.

It demonstrated that the analysis data of *p*-cresyl methyl ether10%inDPG and 94085 floral-fruity are quite reliable because the correlation coefficient in the logarithm function are 0.999 and 0.945 respectively, where analysis data of ethyl acetoacetate are moderate reliable because the correlation coefficient in the logarithm function is 0.839 and analysis data of benzyl alcohol is poor reliable because the correlation coefficient in the logarithm function is 0.111

CHAPTER 5

CONCLUSION AND SUGGESTION

5.1 Conclusion

From the results of this study, we have concluded that sodium carboxymethyl chitosan can be synthesized from chitosan by *O*-carboxymethylation process. By using of 0.10 percent of sodium carboxymethyl chitosan, the optimum volume ratio of water and alcohol is 2:3. Sodium carboxymethyl chitosan are soluble in the formulation of eau de cologne containing benzyl alcohol, ethyl acetoacetate, *p*-cresyl methyl ether10%inDPG and 94085 floral-fruity. Sodium carboxymethyl chitosan has a significant function as fixative for *p*-cresyl methyl ether10%inDPG and may possibly use as fixative for ethyl acetoacetate but it cannot use with 94085 floral-fruity. It needs to review the procedure of analysis for benzyl alcohol. Solubility of chitosan is the main factor which affected to the fixative efficiency, improvement of alcohol soluble chitosan should be studied.

5.2 Suggestions

5.2.1 Improvement of alcohol soluble chitosan

Mostly of water soluble chitosan has poor alcohol solubility which can be assesesed by the final step of synthesis, e.g. washing the product with ethanol. Although water and alcohol has close polarity but solubility of modified chitosans is not only polarity but crystallinity and space between chitosan sheet also involved. Reduction of the normal regularity of intermolecular hydrogen bonding of chitosan, resulting in derivatives with very good solubility in water. Obviously water doesn't have the carbon atoms that interfere with the hydrogen bonding, but alcohol does. So mostly of modified chitosan including *O*-carboxymethyl chitosan highly soluble in water but not soluble in alcohol. Polarity and intermolecular hydrogen bond must be concerned together to design the synthesis experiment of alcohol soluble chitosan. N. Kubota et al. has found the way to synthesis chitosan as shown in Figure 5.1 which soluble in aqueous organic solvent (Kubota, Tatsumoto, Sano, & Toya, 2000). Half N-acetylated chitosan that have the low molecular weight will dissolve in water and aqueous organic solvent.

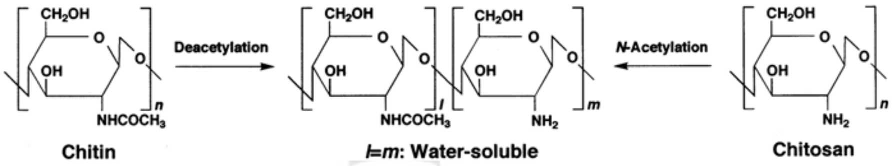


Figure 5.1 Synthesis route of half-N-acetylated chitosan

5.2.2 Improvement of analysis method

The method of analysis used in this research should be improved. Gas phase sample has too less analyte, chance of error by sensitivity limitation of GC instrument would increase. Solid-Phase Microextraction (SPME) should be applied in this research but SPME have the higher expense.

5.2.3 Change of chitosan type

β-type chitosan may be used instead of α-chitosan. Because of less intramolecular bond, increasing of water or alcohol soluble may be possible. But it should be beware about rate of reaction because β-chitosan has more reactivity.

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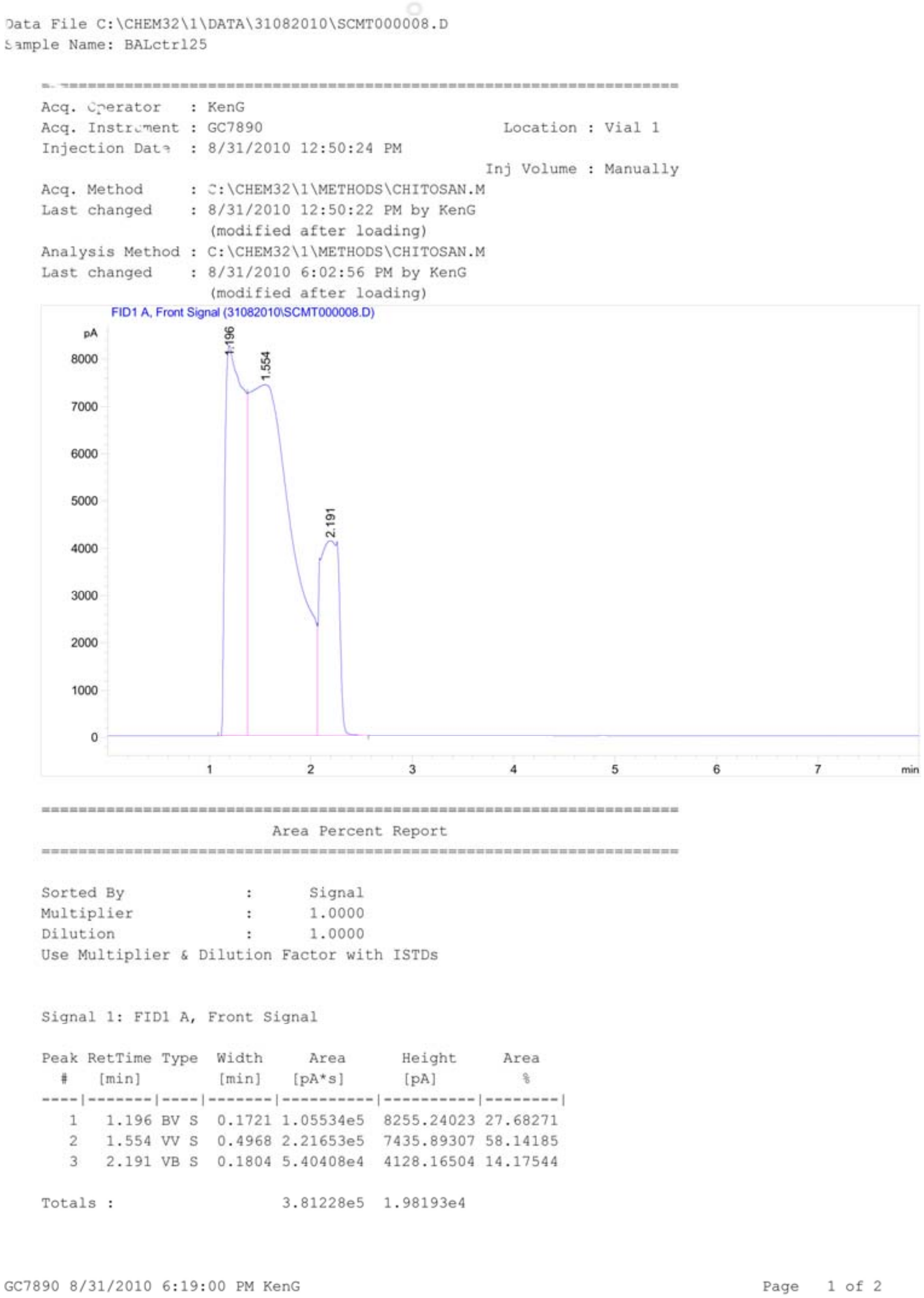
APPENDIXES

APPENDIX A

CHROMATROGRAM



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    Use Multiplier & Dilution Factor with ISTDs


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       1   1.196 BV S  0.1721 1.05534e5  8255.24023 27.68271
       2   1.554 VV S  0.4968 2.21653e5  7435.89307 58.14185
       3   2.191 VB S  0.1804 5.40408e4  4128.16504 14.17544

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GC7890 8/31/2010 6:19:00 PM KenG                                  Page    1 of 2
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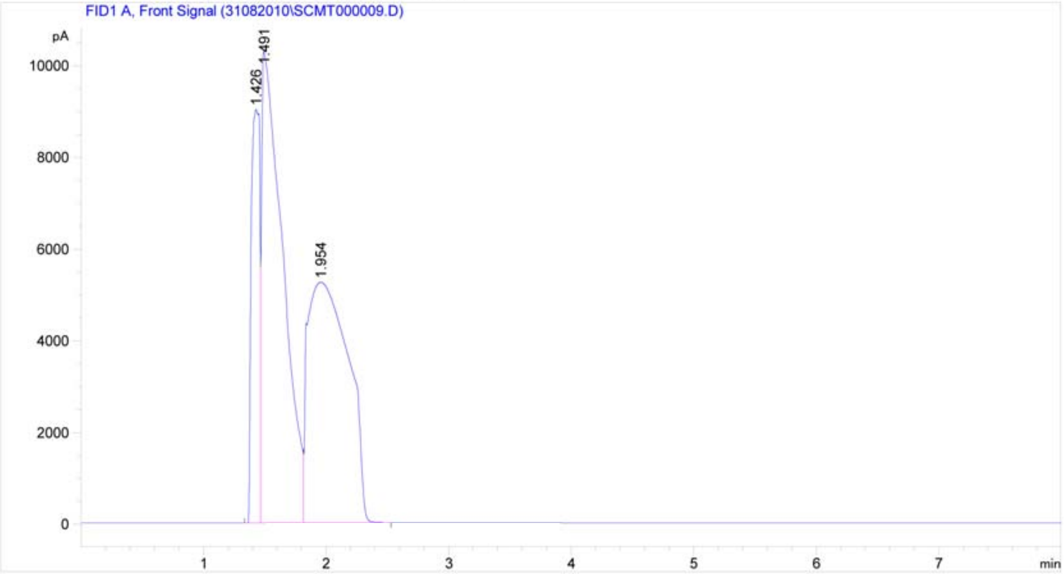

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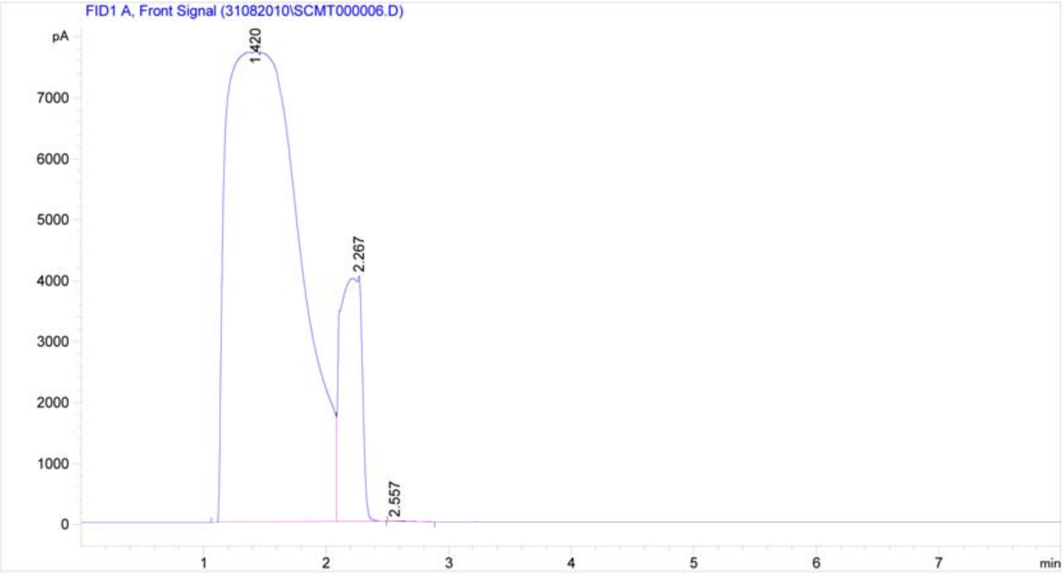

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       2   2.267 VB S  0.2038 4.92635e4  4028.73828 13.28429
       3   2.557 BB    0.1405   60.28455    5.31005  0.01626

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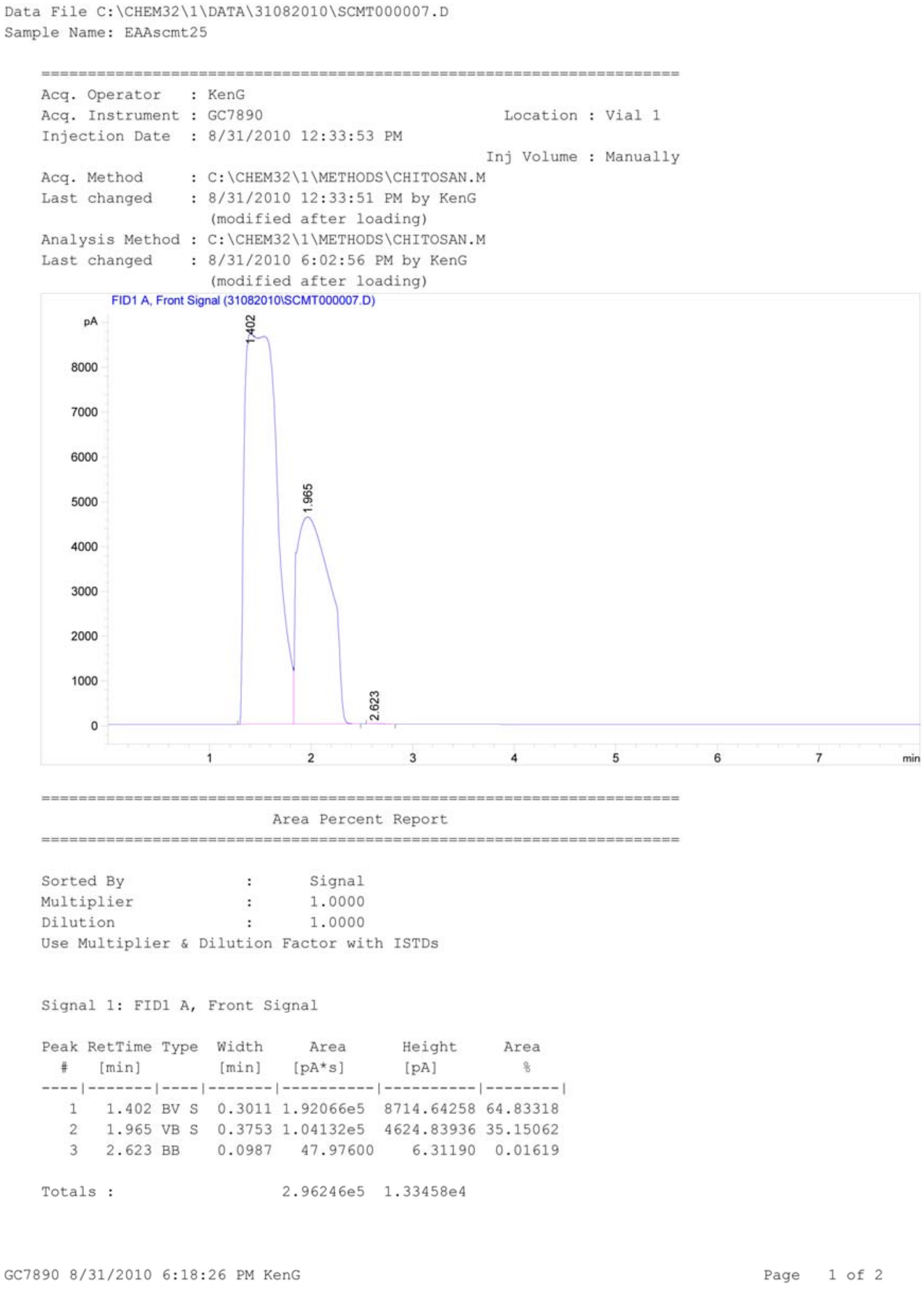

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       2   1.965 VB S  0.3753 1.04132e5 4624.83936 35.15062
       3   2.623 BB    0.0987   47.97600    6.31190  0.01619

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GC7890 8/31/2010 6:18:26 PM KenG                                    Page    1 of 2
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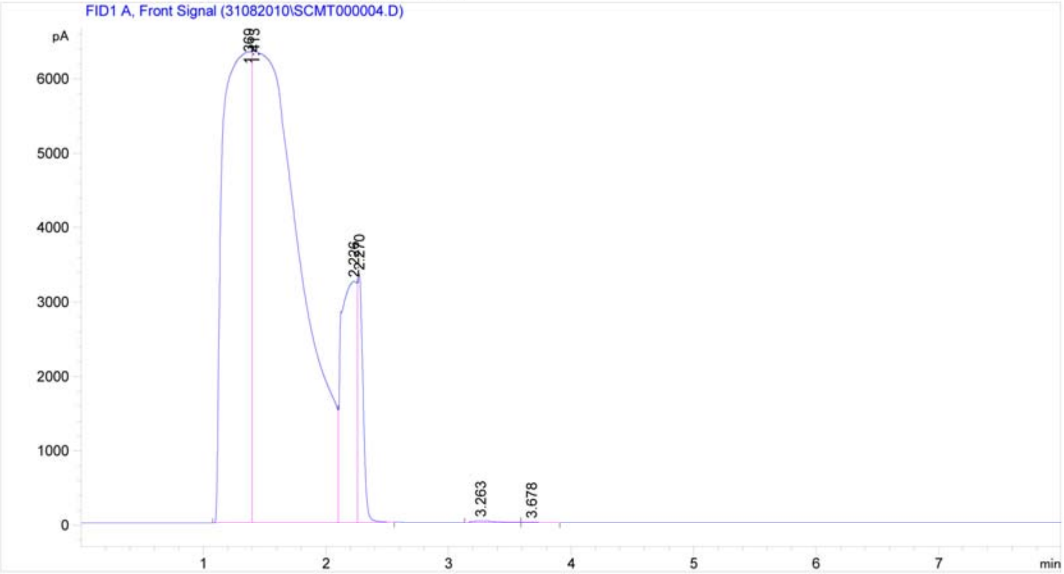

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       3   2.226 VV    0.1056 2.76794e4  3240.95801  8.98533
       4   2.270 VB    0.0463 1.03902e4  3336.75122  3.37290
       5   3.263 BV    0.1790  371.14349   25.62634  0.12048
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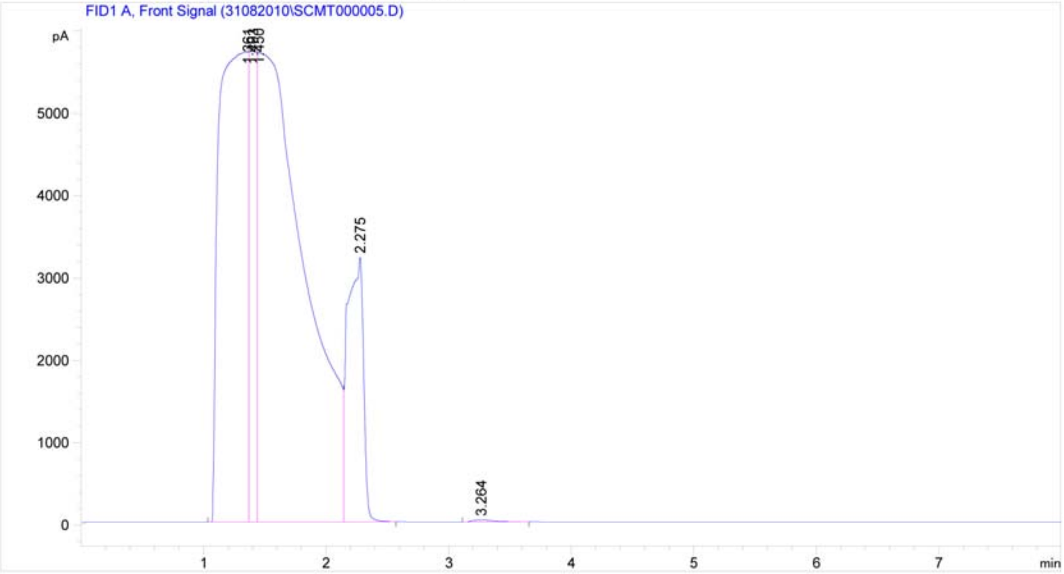
    Injection Date  : 8/31/2010 11:53:44 AM
                                                    Inj Volume : Manually
    Acq. Method     : C:\CHEM32\1\METHODS\CHITOSAN.M
    Last changed    : 8/31/2010 11:53:43 AM by KenG
                      (modified after loading)
    Analysis Method : C:\CHEM32\1\METHODS\CHITOSAN.M
    Last changed    : 8/31/2010 6:02:56 PM by KenG
                      (modified after loading)
```



```
    =====================================================================
                             Area Percent Report
    =====================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: FID1 A, Front Signal

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [pA*s]      [pA]        %
    ----|-------|----|-------|----------|----------|--------|
       1   1.361 BV    0.1902 9.12181e4  5715.27246 30.84863
       2   1.403 VV    0.0515 2.33000e4  5721.22070  7.87972
       3   1.450 VV    0.3149 1.52063e5  5705.54395 51.42544
       4   2.275 VB    0.1155 2.87932e4  3213.52051  9.73745
       5   3.264 BB    0.1559  321.61145   24.70094  0.10876

    Totals :                  2.95696e5  2.03803e4
```

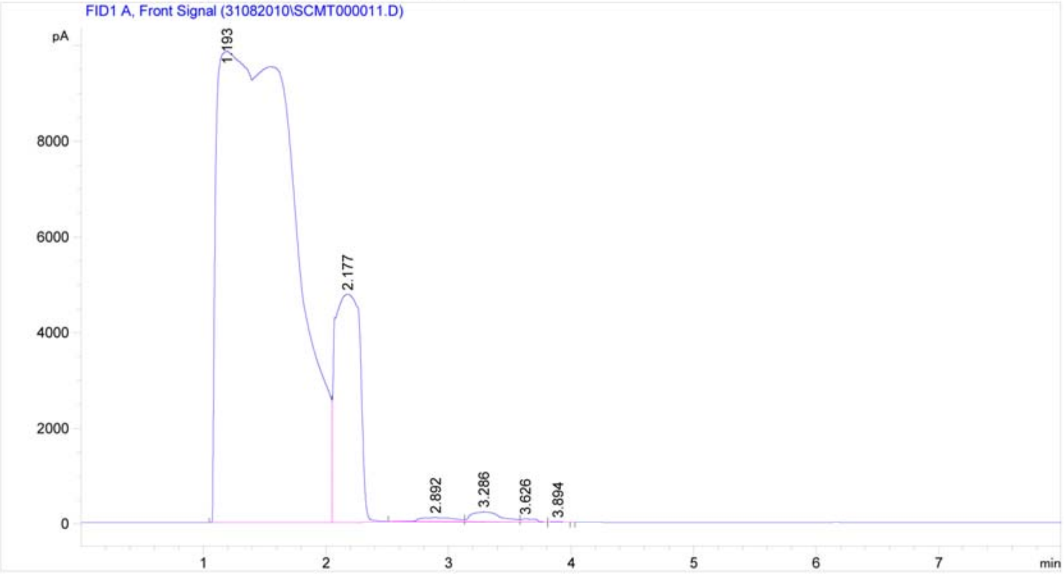
```
Data File C:\CHEM32\1\DATA\31082010\SCMT000005.D
Sample Name: PCMEscmt25

    =====================================================================
                             *** End of Report ***
```

```
GC7890 8/31/2010 6:16:23 PM KenG                                                    Page  2 of 2
```

```
Data File C:\CHEM32\1\DATA\31082010\SCMT000011.D
Sample Name: SexGctrl25

    =====================================================================
    Acq. Operator   : KenG
    Acq. Instrument : GC7890                          Location : Vial 1
    Injection Date  : 8/31/2010 1:32:09 PM
                                                    Inj Volume : Manually
    Acq. Method     : C:\CHEM32\1\METHODS\CHITOSAN.M
    Last changed    : 8/31/2010 1:32:06 PM by KenG
                      (modified after loading)
    Analysis Method : C:\CHEM32\1\METHODS\CHITOSAN.M
    Last changed    : 8/31/2010 6:02:56 PM by KenG
                      (modified after loading)
```



```
    =====================================================================
                             Area Percent Report
    =====================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: FID1 A, Front Signal

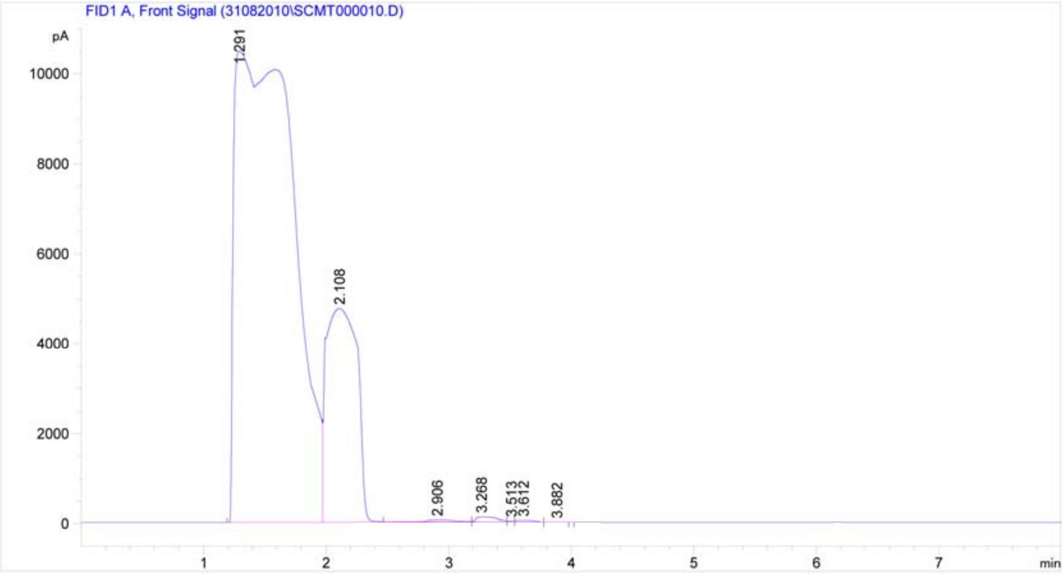
    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [pA*s]      [pA]        %
    ----|-------|----|-------|----------|----------|--------|
       1   1.193 BV S  0.5809 4.37062e5  9849.07129 85.41204
       2   2.177 VB S  0.2399 6.86966e4  4773.05664 13.42492
       3   2.892 BV T  0.2413 1676.42920   82.78647  0.32761
       4   3.286 VV T  0.2039 3599.19727  211.16568  0.70337
       5   3.626 VV X  0.1137  613.06671   70.29948  0.11981
       6   3.894 VB X  0.0957   62.66410    8.24436  0.01225
```

```
    Totals :                 5.11710e5  1.49946e4


    =====================================================================
                              *** End of Report ***
```

```
Data File C:\CHEM32\1\DATA\31082010\SCMT000010.D
Sample Name: SexGscmt25

    =====================================================================
    Acq. Operator   : KenG
    Acq. Instrument : GC7890                         Location : Vial 1
    Injection Date  : 8/31/2010 1:18:28 PM
                                                   Inj Volume : Manually
    Acq. Method     : C:\CHEM32\1\METHODS\CHITOSAN.M
    Last changed    : 8/31/2010 1:18:26 PM by KenG
                      (modified after loading)
    Analysis Method : C:\CHEM32\1\METHODS\CHITOSAN.M
    Last changed    : 8/31/2010 6:02:56 PM by KenG
                      (modified after loading)
```



```
    =====================================================================
                             Area Percent Report
    =====================================================================

    Sorted By              :      Signal
    Multiplier             :      1.0000
    Dilution               :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: FID1 A, Front Signal

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [pA*s]      [pA]        %
    ----|-------|----|-------|----------|----------|--------|
       1   1.291 BV S  0.4447 3.51136e5  1.04714e4  80.14079
       2   2.108 VB S  0.2951 8.41506e4  4752.57422 19.20595
       3   2.906 BV T  0.2163  762.83392   42.52377  0.17410
       4   3.268 VV X  0.1454 1468.07214  120.25696  0.33506
       5   3.513 VV X  0.0443  124.69770   35.21703  0.02846
       6   3.612 VV X  0.1224  461.28894   45.07664  0.10528
       7   3.882 VB X  0.1081   45.32081    5.07927  0.01034
```

```
Data File C:\CHEM32\1\DATA\31082010\SCMT000010.D
Sample Name: SexGscmt25

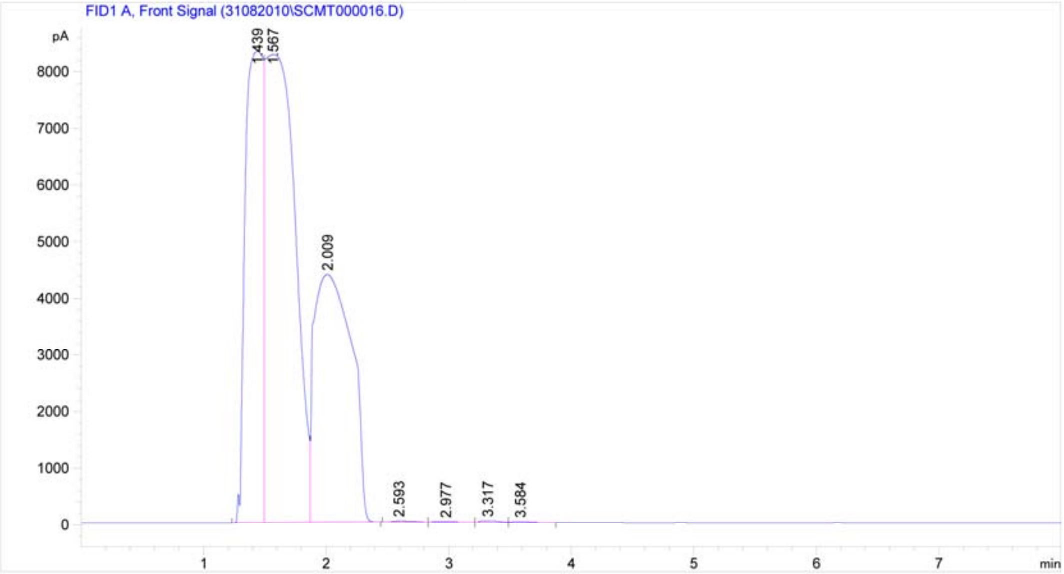
    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [pA*s]       [pA]        %
    ----|-------|----|-------|----------|----------|--------|
    Totals :                  4.38149e5  1.54721e4



    ======================================================================
                              *** End of Report ***
```

```
Data File C:\CHEM32\1\DATA\31082010\SCMT000016.D
Sample Name: BALctrl25

    =====================================================================
    Acq. Operator   : KenG
    Acq. Instrument : GC7890                         Location : Vial 1
    Injection Date  : 8/31/2010 3:11:10 PM
                                                   Inj Volume : Manually
    Acq. Method     : C:\CHEM32\1\METHODS\CHITOSAN.M
    Last changed    : 8/31/2010 3:11:09 PM by KenG
                      (modified after loading)
    Analysis Method : C:\CHEM32\1\METHODS\CHITOSAN.M
    Last changed    : 8/31/2010 6:02:56 PM by KenG
                      (modified after loading)
```

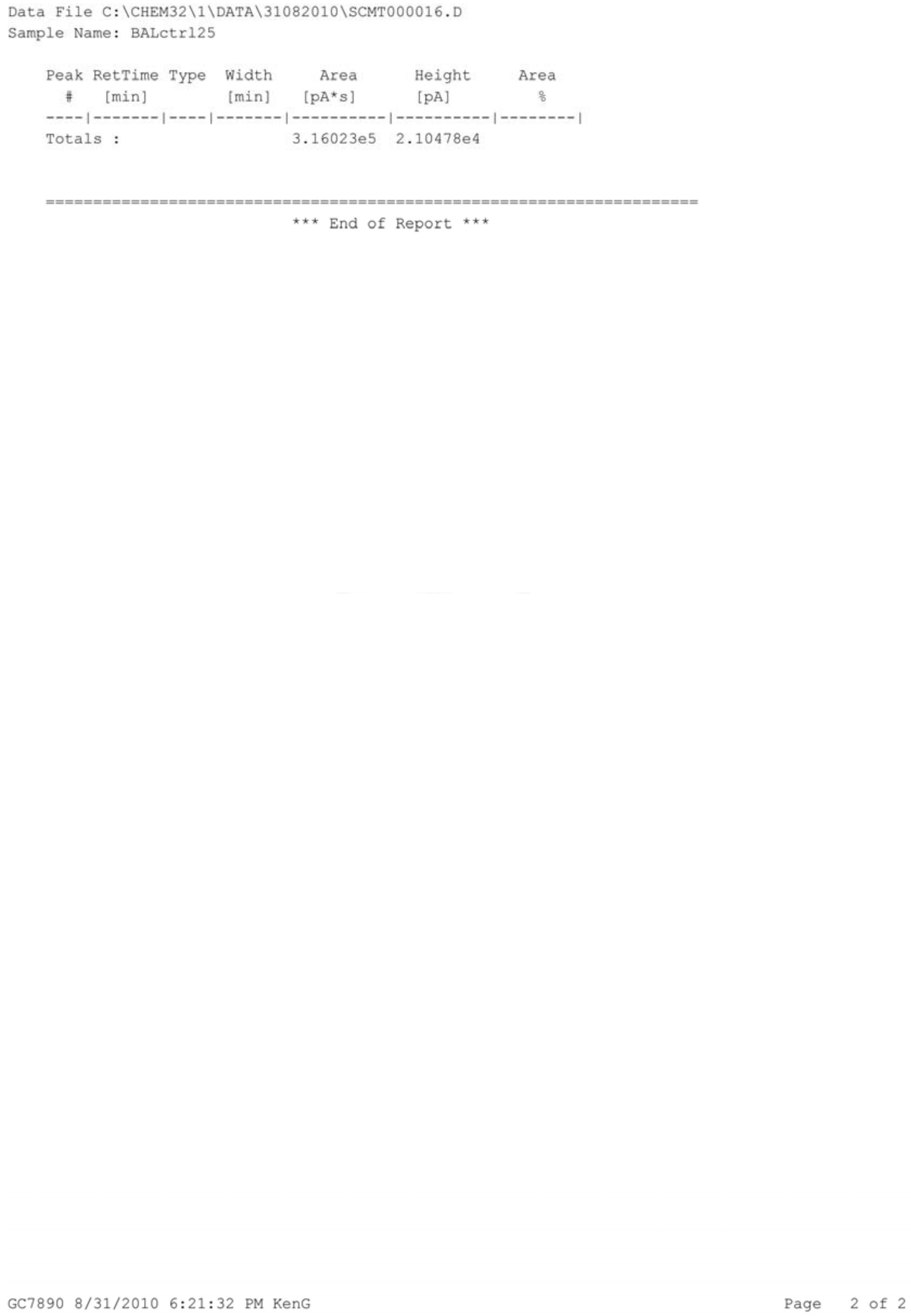


```
    =====================================================================
                             Area Percent Report
    =====================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: FID1 A, Front Signal

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [pA*s]      [pA]        %
    ----|-------|----|-------|----------|----------|--------|
       1   1.439 BV    0.1335 8.23911e4  8325.01758 26.07121
       2   1.567 VV    0.2023 1.39901e5  8270.74609 44.26931
       3   2.009 VB S  0.2656 9.29568e4  4379.63135 29.41452
       4   2.593 BV    0.1437  225.62062   19.84244  0.07139
       5   2.977 VV    0.1573  120.36100    9.28330  0.03809
       6   3.317 VV    0.1184  292.33762   29.81348  0.09251
       7   3.584 VB    0.1215  135.79936   13.48235  0.04297
```



```
Data File C:\CHEM32\1\DATA\31082010\SCMT000016.D
Sample Name: BALctrl25

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [pA*s]      [pA]        %
    ----|-------|----|-------|----------|----------|--------|
    Totals :                  3.16023e5  2.10478e4

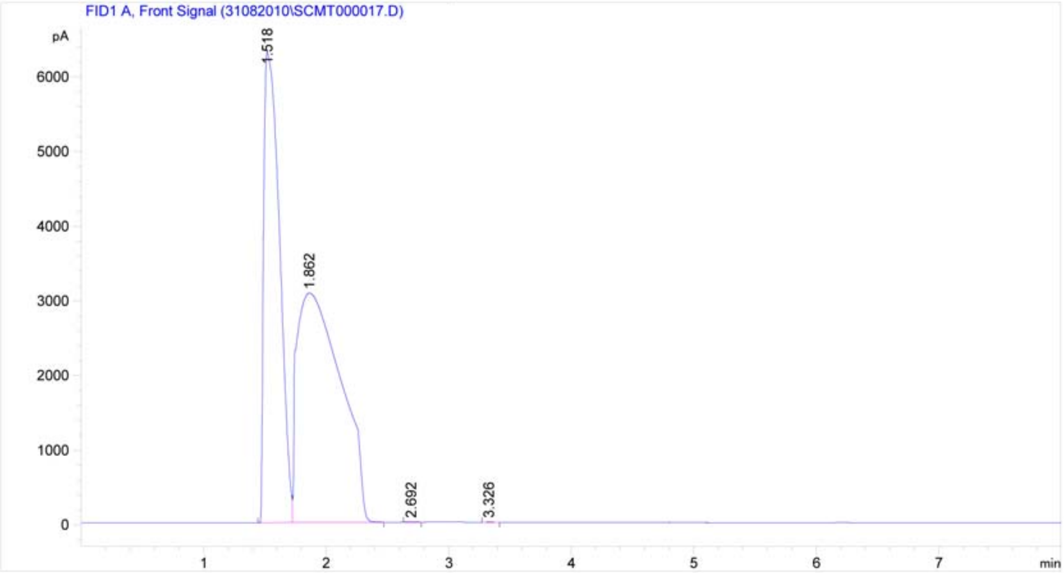


    =====================================================================
                              *** End of Report ***
```

```
GC7890 8/31/2010 6:21:32 PM KenG                                    Page    2 of 2
```

```
Data File C:\CHEM32\1\DATA\31082010\SCMT000017.D
Sample Name: BALscmt35

    =====================================================================
    Acq. Operator   : KenG
    Acq. Instrument : GC7890                        Location : Vial 1
    Injection Date  : 8/31/2010 3:24:55 PM
                                                  Inj Volume : Manually
    Acq. Method     : C:\CHEM32\1\METHODS\CHITOSAN.M
    Last changed    : 8/31/2010 3:24:53 PM by KenG
                      (modified after loading)
    Analysis Method : C:\CHEM32\1\METHODS\COOLDOWN.M
    Last changed    : 8/25/2010 5:27:45 PM by Ya
```



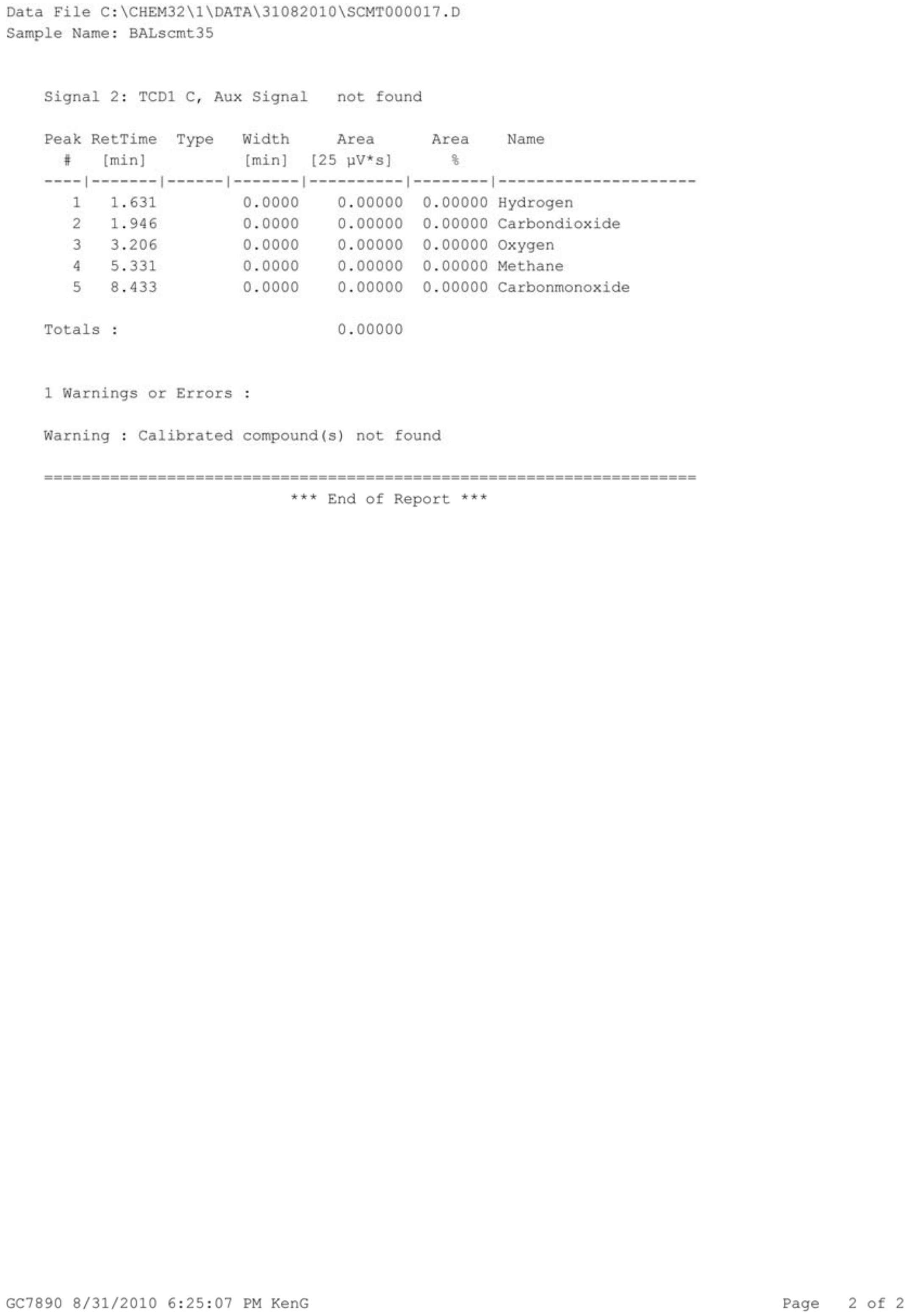
```
    =====================================================================
                             Area Percent Report
    =====================================================================

    Sorted By             :      Signal
    Calib. Data Modified  :      1/3/2008 3:31:33 PM
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: FID1 A, Front Signal

    Peak RetTime  Type  Width    Area      Area    Name
      #   [min]         [min]   [pA*s]      %
    ----|-------|------|-------|----------|--------|---------------------
       1   1.518 BV S    0.1179 5.62213e4  42.31005 ?
       2   1.862 VB S    0.4155 7.66191e4  57.66064 ?
       3   2.692 BB      0.0656   14.29434  0.01076 ?
       4   3.326 BV      0.0562   24.65319  0.01855 ?

    Totals :                    1.32879e5
```



```
Data File C:\CHEM32\1\DATA\31082010\SCMT000017.D
Sample Name: BALscmt35


    Signal 2: TCD1 C, Aux Signal   not found

    Peak RetTime  Type   Width     Area      Area    Name
      #   [min]          [min]  [25 µV*s]     %
    ----|-------|------|-------|----------|--------|---------------------
       1   1.631         0.0000    0.00000  0.00000 Hydrogen
       2   1.946         0.0000    0.00000  0.00000 Carbondioxide
       3   3.206         0.0000    0.00000  0.00000 Oxygen
       4   5.331         0.0000    0.00000  0.00000 Methane
       5   8.433         0.0000    0.00000  0.00000 Carbonmonoxide

    Totals :                        0.00000


    1 Warnings or Errors :

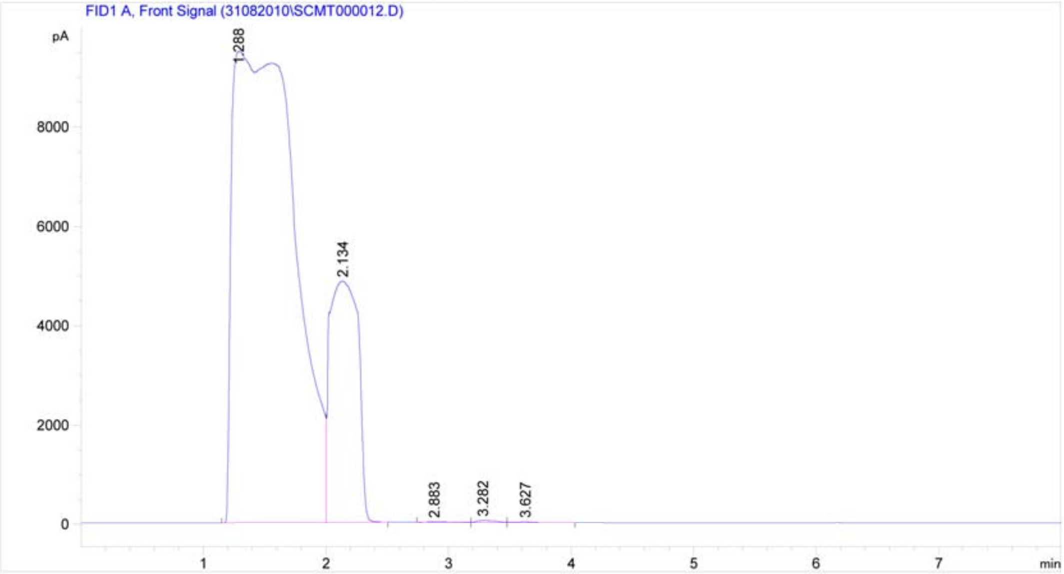
    Warning : Calibrated compound(s) not found

    ======================================================================
                              *** End of Report ***
```

```
GC7890 8/31/2010 6:25:07 PM KenG                                      Page   2 of 2
```

```
Data File C:\CHEM32\1\DATA\31082010\SCMT000012.D
Sample Name: EAActrl35

     =====================================================================
     Acq. Operator   : KenG
     Acq. Instrument : GC7890                         Location : Vial 1
     Injection Date  : 8/31/2010 2:13:43 PM
                                                    Inj Volume : Manually
     Acq. Method     : C:\CHEM32\1\METHODS\CHITOSAN.M
     Last changed    : 8/31/2010 2:13:41 PM by KenG
                       (modified after loading)
     Analysis Method : C:\CHEM32\1\METHODS\CHITOSAN.M
     Last changed    : 8/31/2010 6:02:56 PM by KenG
                       (modified after loading)
```



```
     =====================================================================
                              Area Percent Report
     =====================================================================

     Sorted By             :      Signal
     Multiplier            :      1.0000
     Dilution              :      1.0000
     Use Multiplier & Dilution Factor with ISTDs


     Signal 1: FID1 A, Front Signal

     Peak RetTime Type  Width     Area      Height     Area
       #   [min]        [min]   [pA*s]      [pA]        %
     ----|-------|----|-------|----------|----------|--------|
        1   1.288 BV S  0.4737 3.37642e5  9488.90723 81.02578
        2   2.134 VB S  0.2684 7.81714e4  4854.98975 18.75921
        3   2.883 BV    0.1990  185.88226   11.00658  0.04461
        4   3.282 VV    0.1433  493.42825   41.31087  0.11841
        5   3.627 VB    0.1655  216.67148   15.95104  0.05200

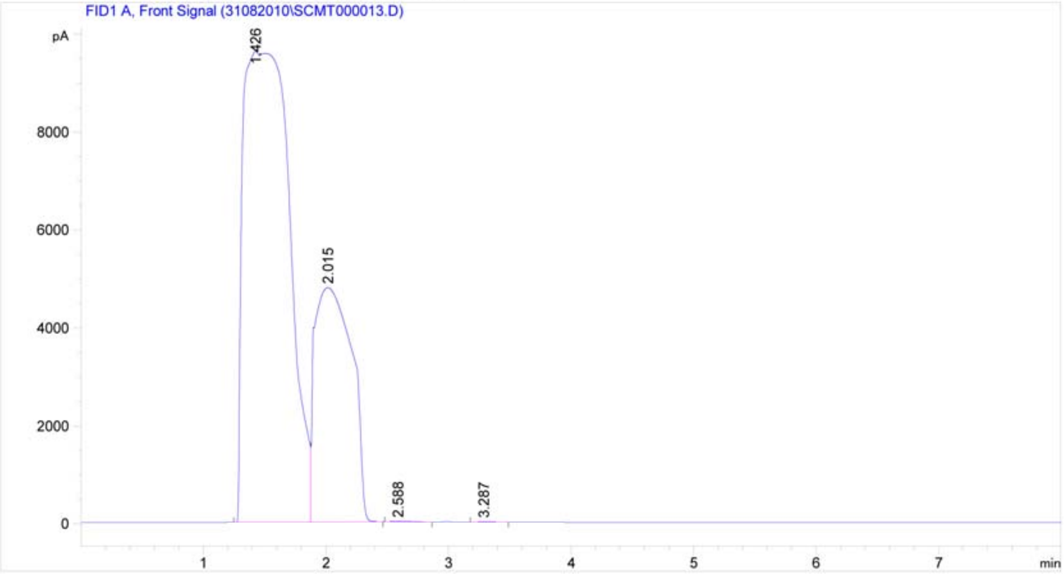
     Totals :                  4.16710e5  1.44122e4
```

```
Data File C:\CHEM32\1\DATA\31082010\SCMT000012.D
Sample Name: EAActrl35

    =====================================================================
                             *** End of Report ***
```

```
Data File C:\CHEM32\1\DATA\31082010\SCMT000013.D
Sample Name: EAAscmt35

    =====================================================================
    Acq. Operator   : KenG
    Acq. Instrument : GC7890                          Location : Vial 1
    Injection Date  : 8/31/2010 2:28:11 PM
                                                   Inj Volume : Manually
    Acq. Method     : C:\CHEM32\1\METHODS\CHITOSAN.M
    Last changed    : 8/31/2010 2:28:10 PM by KenG
                      (modified after loading)
    Analysis Method : C:\CHEM32\1\METHODS\CHITOSAN.M
    Last changed    : 8/31/2010 6:02:56 PM by KenG
                      (modified after loading)
```



```
    =====================================================================
                             Area Percent Report
    =====================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: FID1 A, Front Signal

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [pA*s]      [pA]        %
    ----|-------|----|-------|----------|----------|--------|
       1   1.426 BV S  0.3244 2.52140e5  9611.33789 71.44178
       2   2.015 VB S  0.3506 1.00589e5  4781.12500 28.50106
       3   2.588 BB    0.1263  121.75890   12.43255  0.03450
       4   3.287 BV    0.1137   79.96750    8.82434  0.02266

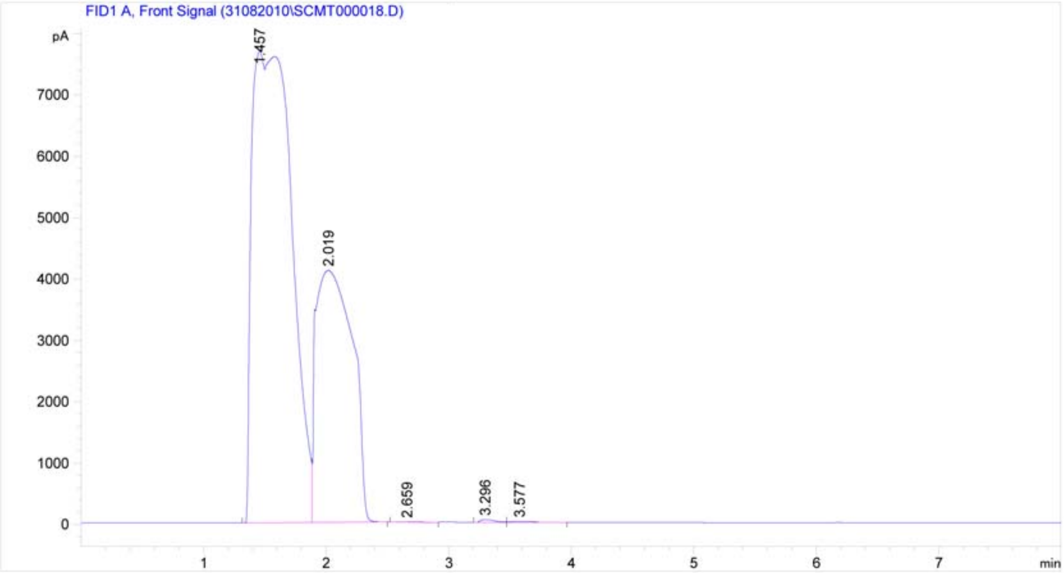
    Totals :                  3.52931e5  1.44137e4
```

```
Data File C:\CHEM32\1\DATA\31082010\SCMT000013.D
Sample Name: EAAscmt35

    =====================================================================
                          *** End of Report ***
```

```
Data File C:\CHEM32\1\DATA\31082010\SCMT000018.D
Sample Name: PCMEctrl35

    =====================================================================
    Acq. Operator   : KenG
    Acq. Instrument : GC7890                        Location : Vial 1
    Injection Date  : 8/31/2010 3:39:06 PM
                                                  Inj Volume : Manually
    Acq. Method     : C:\CHEM32\1\METHODS\CHITOSAN.M
    Last changed    : 8/31/2010 3:39:05 PM by KenG
                      (modified after loading)
    Analysis Method : C:\CHEM32\1\METHODS\COOLDOWN.M
    Last changed    : 8/25/2010 5:27:45 PM by Ya
```



```
    =====================================================================
                             Area Percent Report
    =====================================================================

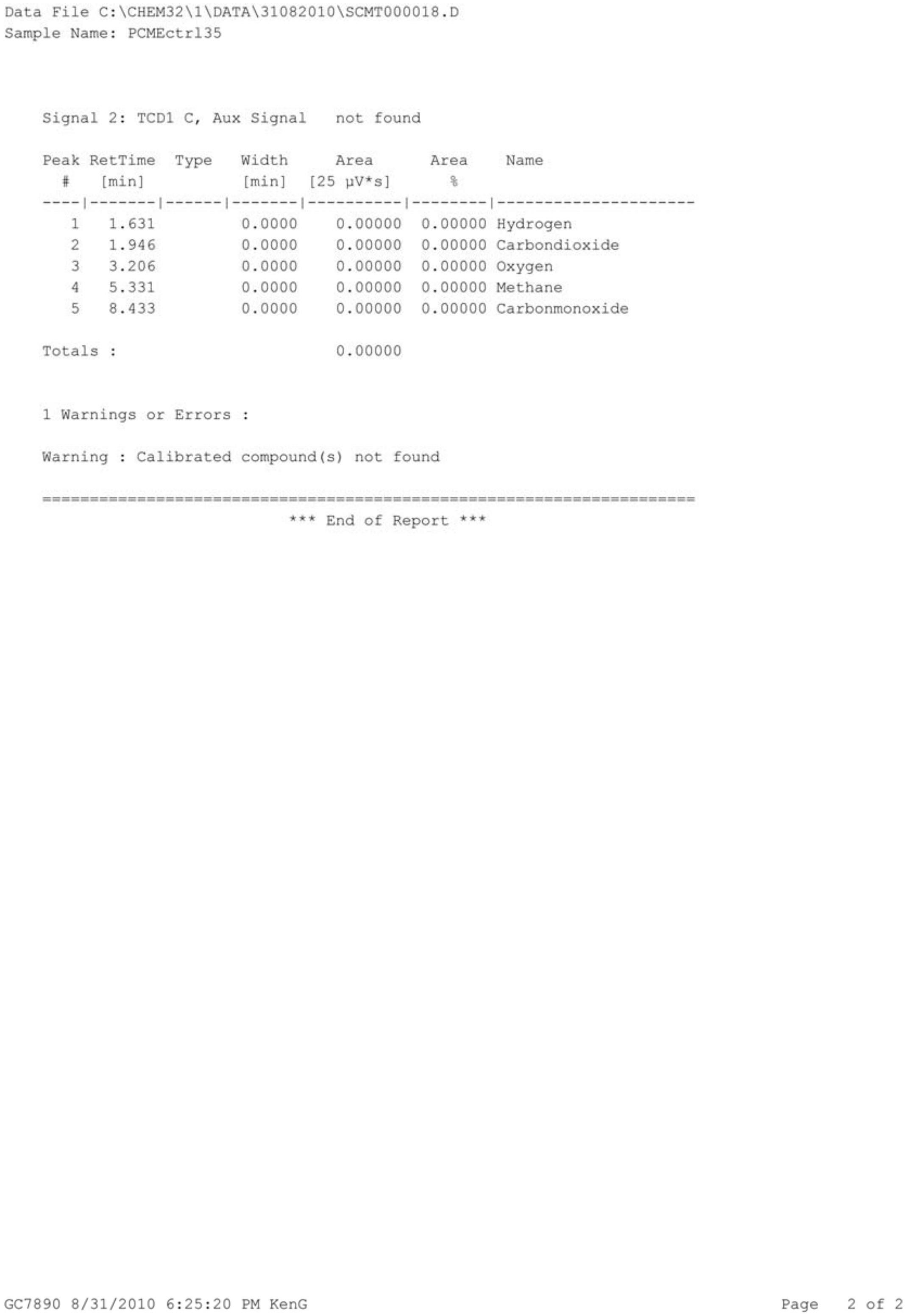
    Sorted By             :      Signal
    Calib. Data Modified  :      1/3/2008 3:31:33 PM
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: FID1 A, Front Signal

    Peak RetTime  Type   Width     Area      Area    Name
      #   [min]          [min]   [pA*s]        %
    ----|-------|------|-------|----------|--------|---------------------
       1   1.457 BV S    0.3019 1.73715e5  67.01238 ?
       2   2.019 VB S    0.3444 8.48904e4  32.74726 ?
       3   2.659 BB      0.0918   37.24281  0.01437 ?
       4   3.296 BV      0.1129  367.49738  0.14177 ?
       5   3.577 VB      0.1497  218.34688  0.08423 ?

    Totals :                    2.59229e5

GC7890 8/31/2010 6:25:20 PM KenG                                  Page   1 of 2
```



```
Data File C:\CHEM32\1\DATA\31082010\SCMT000018.D
Sample Name: PCMEctrl35


    Signal 2: TCD1 C, Aux Signal   not found

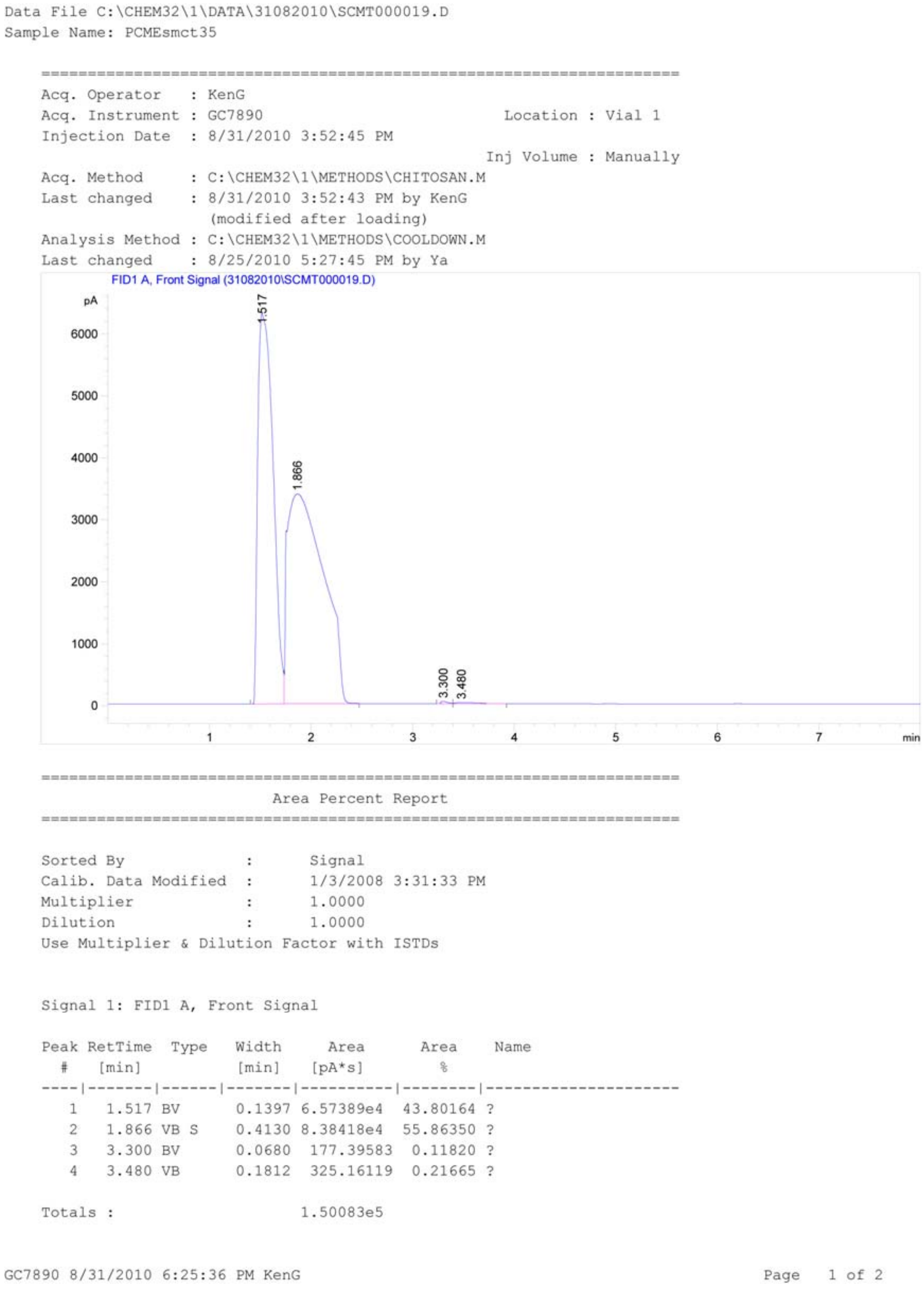
    Peak RetTime  Type  Width     Area      Area    Name
      #   [min]         [min]  [25 µV*s]     %
    ----|-------|------|-------|----------|--------|---------------------
       1   1.631         0.0000    0.00000  0.00000 Hydrogen
       2   1.946         0.0000    0.00000  0.00000 Carbondioxide
       3   3.206         0.0000    0.00000  0.00000 Oxygen
       4   5.331         0.0000    0.00000  0.00000 Methane
       5   8.433         0.0000    0.00000  0.00000 Carbonmonoxide

    Totals :                       0.00000


    1 Warnings or Errors :

    Warning : Calibrated compound(s) not found

    =====================================================================
                              *** End of Report ***
```



```
Data File C:\CHEM32\1\DATA\31082010\SCMT000019.D
Sample Name: PCMEsmct35

    =====================================================================
    Acq. Operator   : KenG
    Acq. Instrument : GC7890                        Location : Vial 1
    Injection Date  : 8/31/2010 3:52:45 PM
                                                  Inj Volume : Manually
    Acq. Method     : C:\CHEM32\1\METHODS\CHITOSAN.M
    Last changed    : 8/31/2010 3:52:43 PM by KenG
                      (modified after loading)
    Analysis Method : C:\CHEM32\1\METHODS\COOLDOWN.M
    Last changed    : 8/25/2010 5:27:45 PM by Ya
```

```
    =====================================================================
                             Area Percent Report
    =====================================================================

    Sorted By             :      Signal
    Calib. Data Modified  :      1/3/2008 3:31:33 PM
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: FID1 A, Front Signal

    Peak RetTime  Type  Width     Area      Area    Name
      #   [min]         [min]   [pA*s]       %
    ----|-------|------|-------|----------|--------|---------------------
       1   1.517 BV     0.1397 6.57389e4  43.80164 ?
       2   1.866 VB S   0.4130 8.38418e4  55.86350 ?
       3   3.300 BV     0.0680  177.39583  0.11820 ?
       4   3.480 VB     0.1812  325.16119  0.21665 ?

    Totals :                   1.50083e5


GC7890 8/31/2010 6:25:36 PM KenG                                    Page   1 of 2
```

```
Data File C:\CHEM32\1\DATA\31082010\SCMT000019.D
Sample Name: PCMEsmct35


    Signal 2: TCD1 C, Aux Signal   not found

    Peak RetTime  Type   Width     Area      Area    Name
      #   [min]          [min]  [25 µV*s]      %
    ----|-------|------|-------|----------|--------|---------------------
       1   1.631         0.0000    0.00000  0.00000 Hydrogen
       2   1.946         0.0000    0.00000  0.00000 Carbondioxide
       3   3.206         0.0000    0.00000  0.00000 Oxygen
       4   5.331         0.0000    0.00000  0.00000 Methane
       5   8.433         0.0000    0.00000  0.00000 Carbonmonoxide

    Totals :                        0.00000

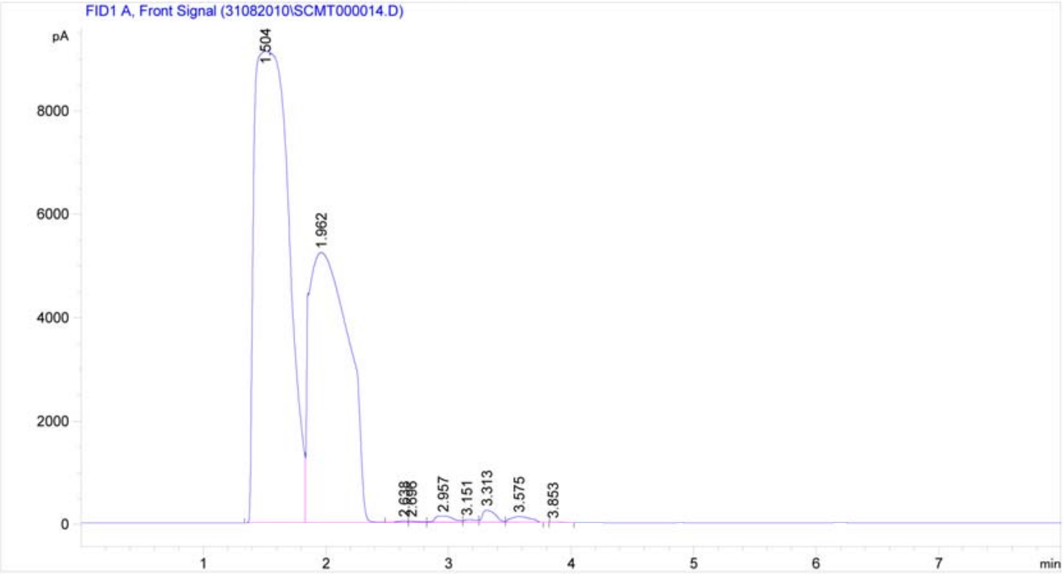

    1 Warnings or Errors :

    Warning : Calibrated compound(s) not found

    =====================================================================
                              *** End of Report ***
```

```
Data File C:\CHEM32\1\DATA\31082010\SCMT000014.D
Sample Name: SexGctrl35

    =====================================================================
    Acq. Operator   : KenG
    Acq. Instrument : GC7890                         Location : Vial 1
    Injection Date  : 8/31/2010 2:43:34 PM
                                                   Inj Volume : Manually
    Acq. Method     : C:\CHEM32\1\METHODS\CHITOSAN.M
    Last changed    : 8/31/2010 2:43:32 PM by KenG
                      (modified after loading)
    Analysis Method : C:\CHEM32\1\METHODS\CHITOSAN.M
    Last changed    : 8/31/2010 6:02:56 PM by KenG
                      (modified after loading)
```



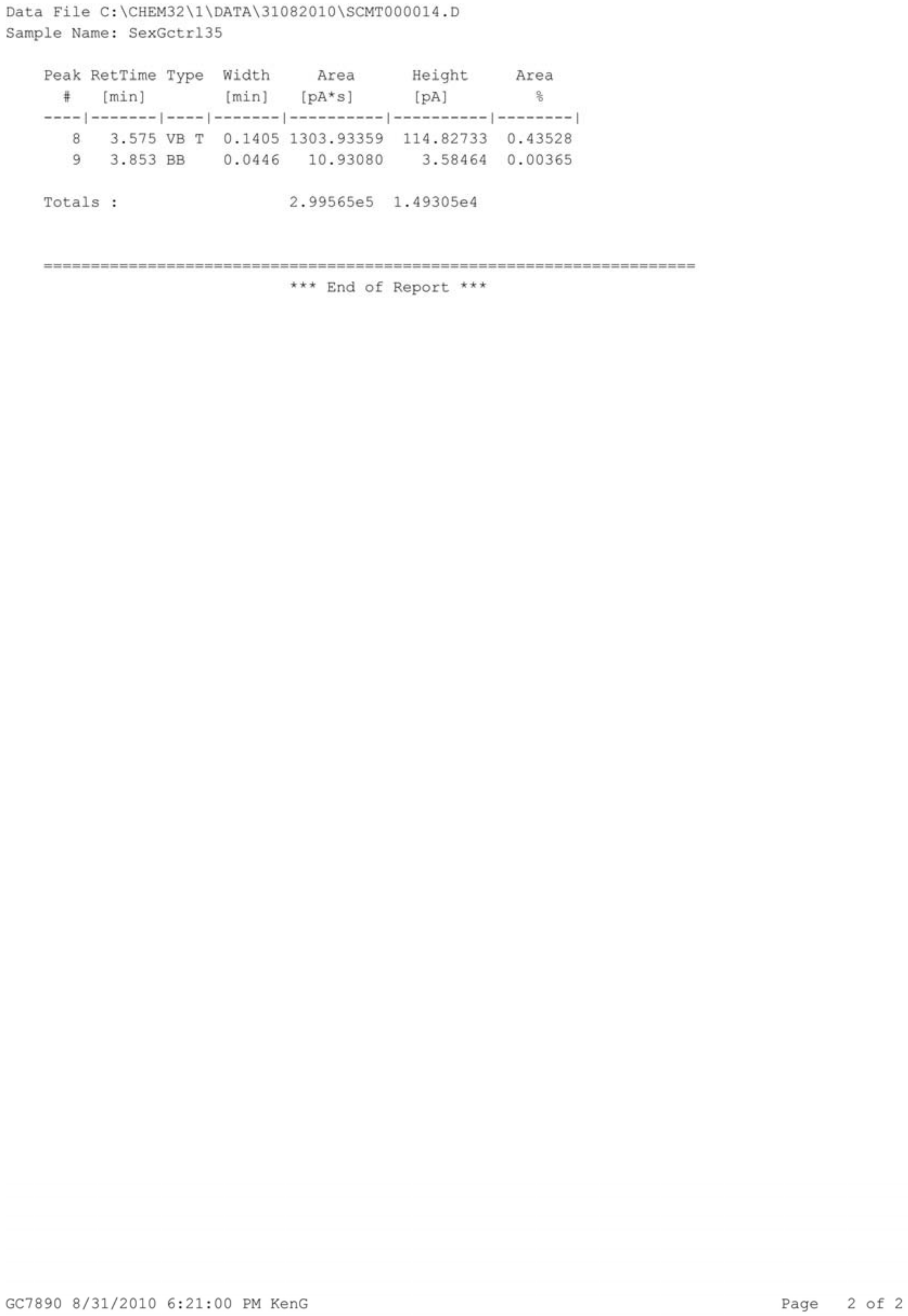
```
    =====================================================================
                             Area Percent Report
    =====================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: FID1 A, Front Signal

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [pA*s]      [pA]        %
    ----|-------|----|-------|----------|----------|--------|
       1   1.504 BV S  0.2362 1.77986e5  9117.25684 59.41463
       2   1.962 VB S  0.3714 1.16529e5  5228.72949 38.89943
       3   2.638 BV T  0.0816  163.13904   25.52239  0.05446
       4   2.696 VV T  0.0783  148.69731   25.78249  0.04964
       5   2.957 VV T  0.1292 1306.55750  126.82227  0.43615
       6   3.151 VV T  0.0884  368.95242   52.90666  0.12316
       7   3.313 VV T  0.1018 1748.28894  235.06125  0.58361

GC7890 8/31/2010 6:21:00 PM KenG                                          Page    1 of 2
```



```
Data File C:\CHEM32\1\DATA\31082010\SCMT000014.D
Sample Name: SexGctrl35

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [pA*s]       [pA]        %
    ----|-------|----|-------|----------|----------|--------|
       8   3.575 VB T  0.1405 1303.93359  114.82733  0.43528
       9   3.853 BB    0.0446   10.93080    3.58464  0.00365

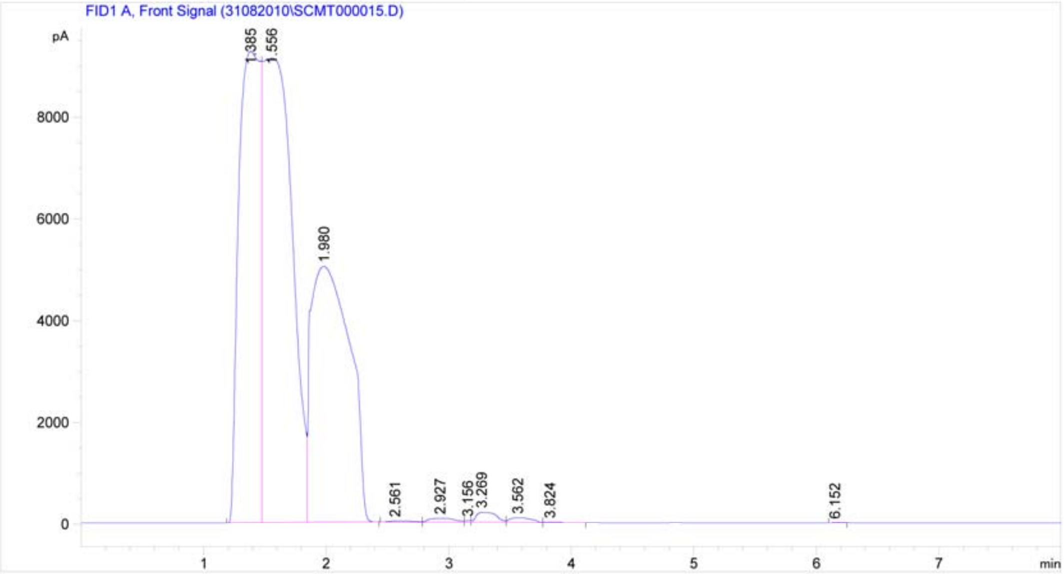
    Totals :                  2.99565e5  1.49305e4


    =====================================================================
                              *** End of Report ***
```

```
GC7890 8/31/2010 6:21:00 PM KenG                                        Page   2 of 2
```

```
Data File C:\CHEM32\1\DATA\31082010\SCMT000015.D
Sample Name: SexGscmt35

    =====================================================================
    Acq. Operator   : KenG
    Acq. Instrument : GC7890                          Location : Vial 1
    Injection Date  : 8/31/2010 2:57:23 PM
                                                    Inj Volume : Manually
    Acq. Method     : C:\CHEM32\1\METHODS\CHITOSAN.M
    Last changed    : 8/31/2010 2:57:22 PM by KenG
                      (modified after loading)
    Analysis Method : C:\CHEM32\1\METHODS\CHITOSAN.M
    Last changed    : 8/31/2010 6:02:56 PM by KenG
                      (modified after loading)
```



```
    =====================================================================
                         Area Percent Report
    =====================================================================

    Sorted By             :      Signal
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: FID1 A, Front Signal

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [pA*s]      [pA]        %
    ----|-------|----|-------|----------|----------|--------|
       1   1.385 BV    0.1850 1.12750e5  9261.87500 29.81330
       2   1.556 VV    0.1964 1.49732e5  9126.43066 39.59211
       3   1.980 VB S  0.3666 1.10657e5  5030.28125 29.26011
       4   2.561 BV    0.1751  293.77350   20.89121  0.07768
       5   2.927 VV    0.1745 1137.82166   77.41241  0.30086
       6   3.156 VV    0.0429  110.49923   35.95788  0.02922
       7   3.269 VV    0.1491 2277.12280  199.55804  0.60212
```

```
Data File C:\CHEM32\1\DATA\31082010\SCMT000015.D
Sample Name: SexGscmt35

    Peak RetTime Type  Width     Area      Height     Area
      #   [min]        [min]   [pA*s]      [pA]        %
    ----|-------|----|-------|----------|----------|--------|
       8   3.562 VV    0.1487 1166.36267   96.02589  0.30841
       9   3.824 VB    0.0699   45.36177    7.91318  0.01199
      10   6.152 BB    0.0516   15.86392    4.15320  0.00419

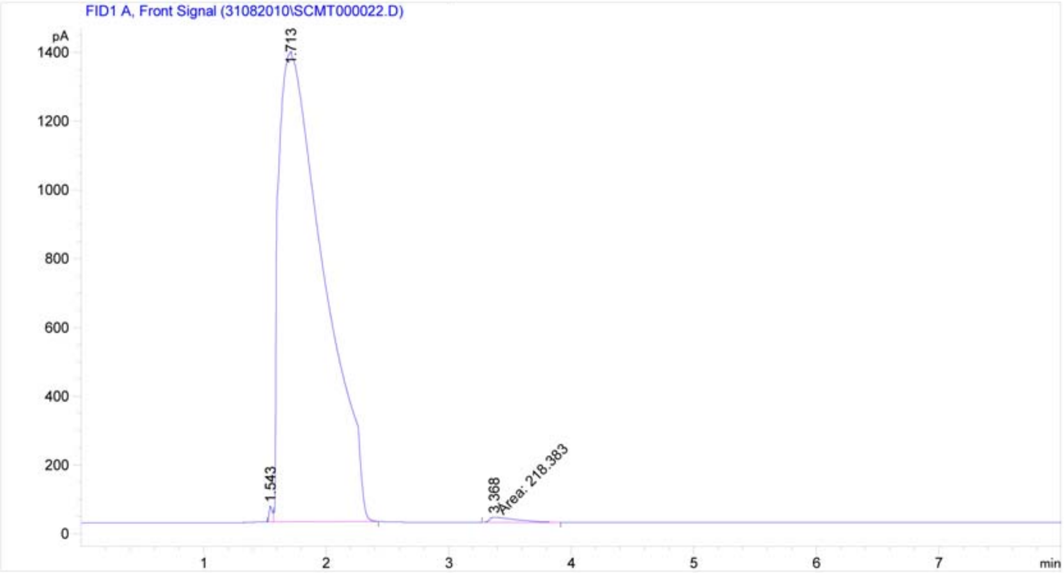
    Totals :                  3.78185e5  2.38605e4


    =====================================================================
                             *** End of Report ***
```

```
GC7890 8/31/2010 6:21:13 PM KenG                                    Page    2 of 2
```

```
Data File C:\CHEM32\1\DATA\31082010\SCMT000022.D
Sample Name: BALctrl53

    =====================================================================
    Acq. Operator   : KenG
    Acq. Instrument : GC7890                           Location : Vial 1
    Injection Date  : 8/31/2010 4:43:52 PM
                                                    Inj Volume : Manually
    Acq. Method     : C:\CHEM32\1\METHODS\CHITOSAN.M
    Last changed    : 8/31/2010 4:43:50 PM by KenG
                      (modified after loading)
    Analysis Method : C:\CHEM32\1\METHODS\COOLDOWN.M
    Last changed    : 8/25/2010 5:27:45 PM by Ya
```



```
    =====================================================================
                             Area Percent Report
    =====================================================================

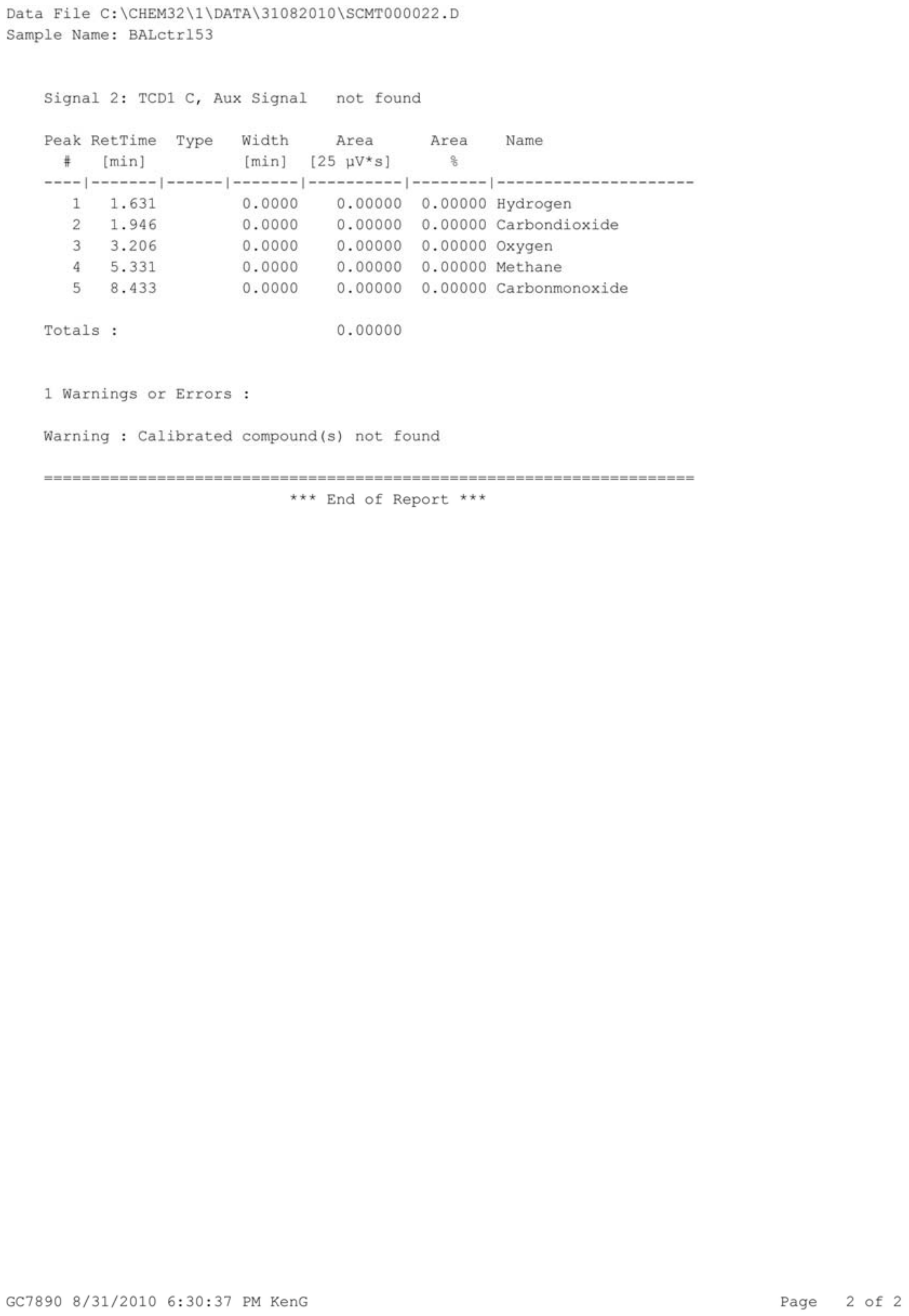
    Sorted By             :      Signal
    Calib. Data Modified  :      1/3/2008 3:31:33 PM
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: FID1 A, Front Signal

    Peak RetTime  Type   Width     Area      Area    Name
      #   [min]          [min]   [pA*s]       %
    ----|-------|------|-------|----------|--------|---------------------
       1   1.543 BV      0.0257   81.54475  0.23391 ?
       2   1.713 VB      0.2974 3.45615e4  99.13966 ?
       3   3.368 MM      0.2493  218.38278  0.62643 ?

    Totals :                    3.48614e4


GC7890 8/31/2010 6:30:37 PM KenG                                   Page    1 of 2
```



```
Data File C:\CHEM32\1\DATA\31082010\SCMT000022.D
Sample Name: BALctrl53


    Signal 2: TCD1 C, Aux Signal   not found

    Peak RetTime  Type   Width     Area      Area    Name
      #   [min]          [min]  [25 µV*s]      %
    ----|-------|------|-------|----------|--------|---------------------
       1   1.631         0.0000    0.00000  0.00000 Hydrogen
       2   1.946         0.0000    0.00000  0.00000 Carbondioxide
       3   3.206         0.0000    0.00000  0.00000 Oxygen
       4   5.331         0.0000    0.00000  0.00000 Methane
       5   8.433         0.0000    0.00000  0.00000 Carbonmonoxide

    Totals :                       0.00000


    1 Warnings or Errors :

    Warning : Calibrated compound(s) not found

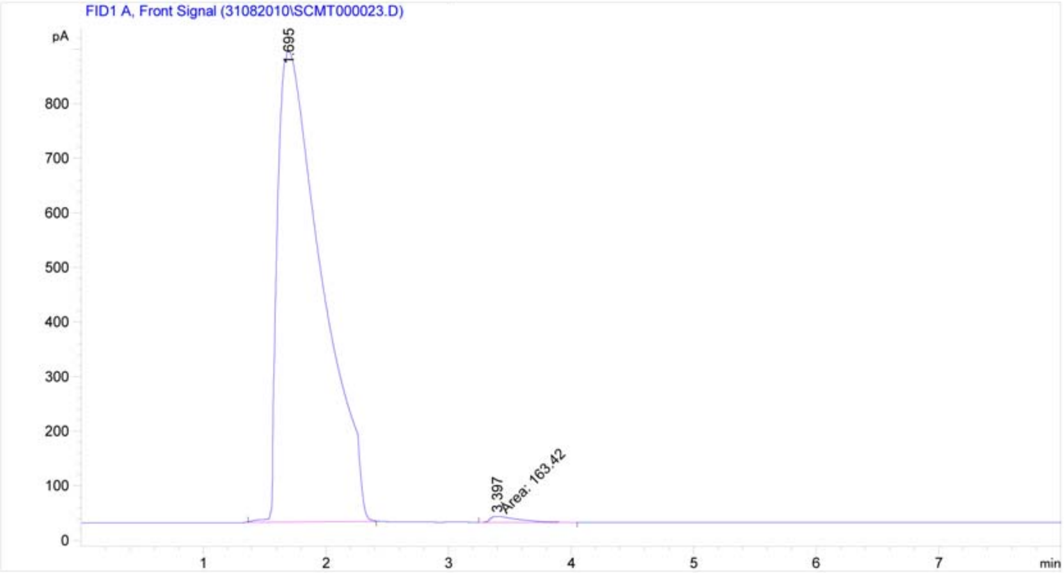
    =====================================================================
                              *** End of Report ***



GC7890 8/31/2010 6:30:37 PM KenG                                  Page   2 of 2
```

```
Data File C:\CHEM32\1\DATA\31082010\SCMT000023.D
Sample Name: BALscmt53

    =====================================================================
    Acq. Operator   : KenG
    Acq. Instrument : GC7890                          Location : Vial 1
    Injection Date  : 8/31/2010 4:57:29 PM
                                                    Inj Volume : Manually
    Acq. Method     : C:\CHEM32\1\METHODS\CHITOSAN.M
    Last changed    : 8/31/2010 4:57:26 PM by KenG
                      (modified after loading)
    Analysis Method : C:\CHEM32\1\METHODS\COOLDOWN.M
    Last changed    : 8/25/2010 5:27:45 PM by Ya
```



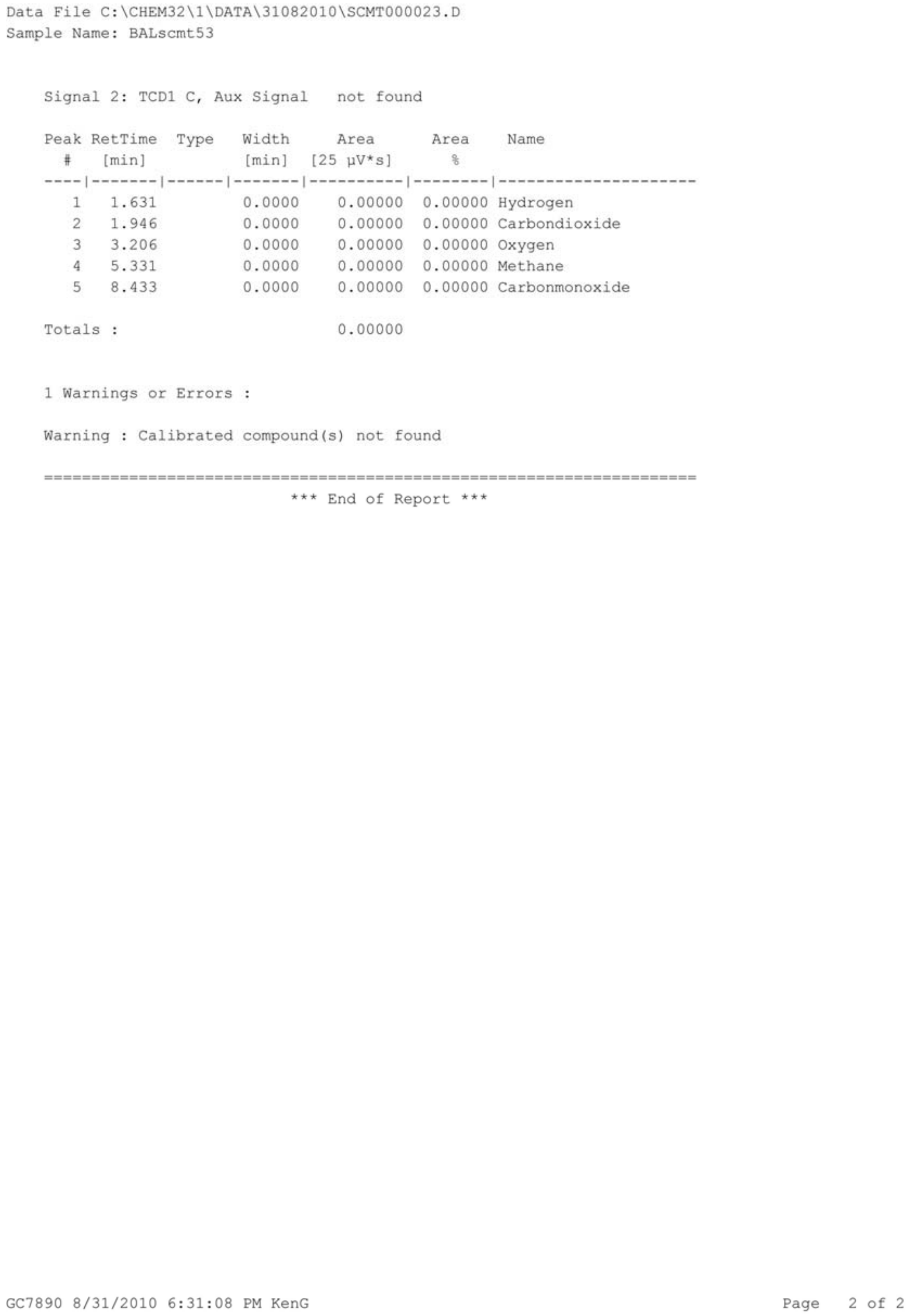
```
    =====================================================================
                             Area Percent Report
    =====================================================================

    Sorted By             :      Signal
    Calib. Data Modified  :      1/3/2008 3:31:33 PM
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: FID1 A, Front Signal

    Peak RetTime  Type  Width    Area      Area    Name
      #   [min]         [min]   [pA*s]      %
    ----|-------|------|-------|----------|--------|---------------------
       1   1.695 BB      0.3136 2.14146e4  99.24266 ?
       2   3.397 MM      0.2531  163.41959  0.75734 ?

    Totals :                    2.15781e4
```



```
Data File C:\CHEM32\1\DATA\31082010\SCMT000023.D
Sample Name: BALscmt53


    Signal 2: TCD1 C, Aux Signal   not found

    Peak RetTime  Type   Width     Area      Area    Name
      #   [min]          [min]  [25 µV*s]     %
    ----|-------|------|-------|----------|--------|---------------------
       1   1.631         0.0000    0.00000  0.00000 Hydrogen
       2   1.946         0.0000    0.00000  0.00000 Carbondioxide
       3   3.206         0.0000    0.00000  0.00000 Oxygen
       4   5.331         0.0000    0.00000  0.00000 Methane
       5   8.433         0.0000    0.00000  0.00000 Carbonmonoxide

    Totals :                        0.00000


    1 Warnings or Errors :

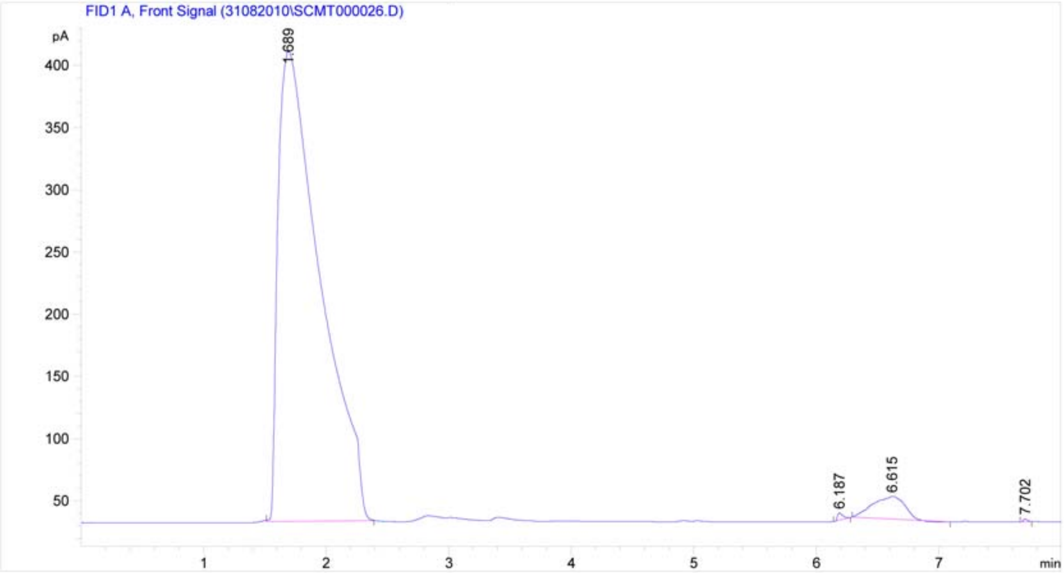
    Warning : Calibrated compound(s) not found

    =====================================================================
                              *** End of Report ***
```

```
GC7890 8/31/2010 6:31:08 PM KenG                                 Page   2 of 2
```

```
Data File C:\CHEM32\1\DATA\31082010\SCMT000026.D
Sample Name: EAActrl53

    =====================================================================
    Acq. Operator   : KenG
    Acq. Instrument : GC7890                        Location : Vial 1
    Injection Date  : 8/31/2010 5:38:27 PM
                                                  Inj Volume : Manually
    Acq. Method     : C:\CHEM32\1\METHODS\CHITOSAN.M
    Last changed    : 8/31/2010 5:38:26 PM by KenG
                      (modified after loading)
    Analysis Method : C:\CHEM32\1\METHODS\COOLDOWN.M
    Last changed    : 8/25/2010 5:27:45 PM by Ya
```



```
    =====================================================================
                             Area Percent Report
    =====================================================================

    Sorted By             :      Signal
    Calib. Data Modified  :      1/3/2008 3:31:33 PM
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: FID1 A, Front Signal

    Peak RetTime  Type  Width     Area      Area    Name
      #   [min]         [min]   [pA*s]       %
    ----|-------|------|-------|----------|--------|---------------------
       1   1.689 BB     0.3090 9009.52539 96.35366 ?
       2   6.187 BB     0.0442   17.06786  0.18253 ?
       3   6.615 BB     0.2119  318.41104  3.40529 ?
       4   7.702 BB     0.0355    5.47099  0.05851 ?

    Totals :                    9350.47528
```

```
Data File C:\CHEM32\1\DATA\31082010\SCMT000026.D
Sample Name: EAActrl53


    Signal 2: TCD1 C, Aux Signal   not found

    Peak RetTime  Type   Width     Area      Area    Name
      #   [min]          [min]  [25 µV*s]     %
    ----|-------|------|-------|----------|--------|---------------------
       1   1.631         0.0000    0.00000  0.00000 Hydrogen
       2   1.946         0.0000    0.00000  0.00000 Carbondioxide
       3   3.206         0.0000    0.00000  0.00000 Oxygen
       4   5.331         0.0000    0.00000  0.00000 Methane
       5   8.433         0.0000    0.00000  0.00000 Carbonmonoxide

    Totals :                       0.00000

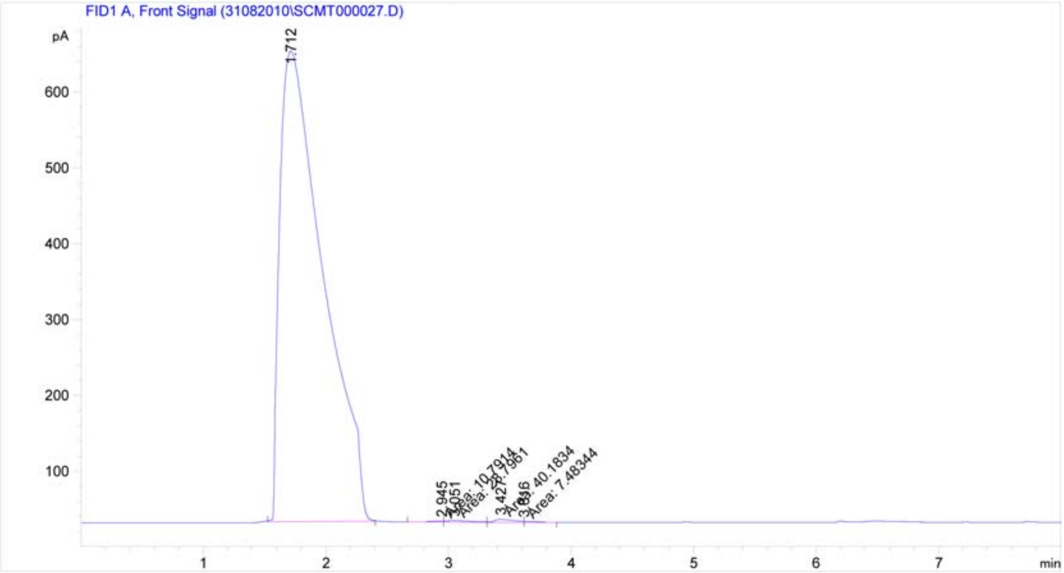

    1 Warnings or Errors :

    Warning : Calibrated compound(s) not found

    ======================================================================
                              *** End of Report ***
```

```
Data File C:\CHEM32\1\DATA\31082010\SCMT000027.D
Sample Name: EAAscmt53

    =====================================================================
    Acq. Operator   : KenG
    Acq. Instrument : GC7890                         Location : Vial 1
    Injection Date  : 8/31/2010 5:51:58 PM
                                                   Inj Volume : Manually
    Acq. Method     : C:\CHEM32\1\METHODS\CHITOSAN.M
    Last changed    : 8/31/2010 5:51:57 PM by KenG
                      (modified after loading)
    Analysis Method : C:\CHEM32\1\METHODS\COOLDOWN.M
    Last changed    : 8/25/2010 5:27:45 PM by Ya
```



FID1 A, Front Signal (31082010\SCMT000027.D)

```
    =====================================================================
                             Area Percent Report
    =====================================================================

    Sorted By              :      Signal
    Calib. Data Modified   :      1/3/2008 3:31:33 PM
    Multiplier             :      1.0000
    Dilution               :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: FID1 A, Front Signal

    Peak RetTime  Type  Width     Area      Area    Name
      #   [min]         [min]   [pA*s]       %
    ----|-------|------|-------|----------|--------|---------------------
       1   1.712 BB      0.3374 1.52151e4  99.42980 ?
       2   2.945 MF      0.1333   10.79143  0.07052 ?
       3   3.051 MF      0.2275   28.79612  0.18818 ?
       4   3.427 MF      0.1820   40.18338  0.26260 ?
       5   3.616 FM      0.1065    7.48344  0.04890 ?

    Totals :                    1.53024e4
```

```
Data File C:\CHEM32\1\DATA\31082010\SCMT000027.D
Sample Name: EAAscmt53


    Signal 2: TCD1 C, Aux Signal   not found

    Peak RetTime  Type  Width     Area      Area    Name
      #   [min]         [min]  [25 µV*s]      %
    ----|-------|------|-------|----------|--------|---------------------
       1   1.631         0.0000    0.00000  0.00000 Hydrogen
       2   1.946         0.0000    0.00000  0.00000 Carbondioxide
       3   3.206         0.0000    0.00000  0.00000 Oxygen
       4   5.331         0.0000    0.00000  0.00000 Methane
       5   8.433         0.0000    0.00000  0.00000 Carbonmonoxide

    Totals :                       0.00000

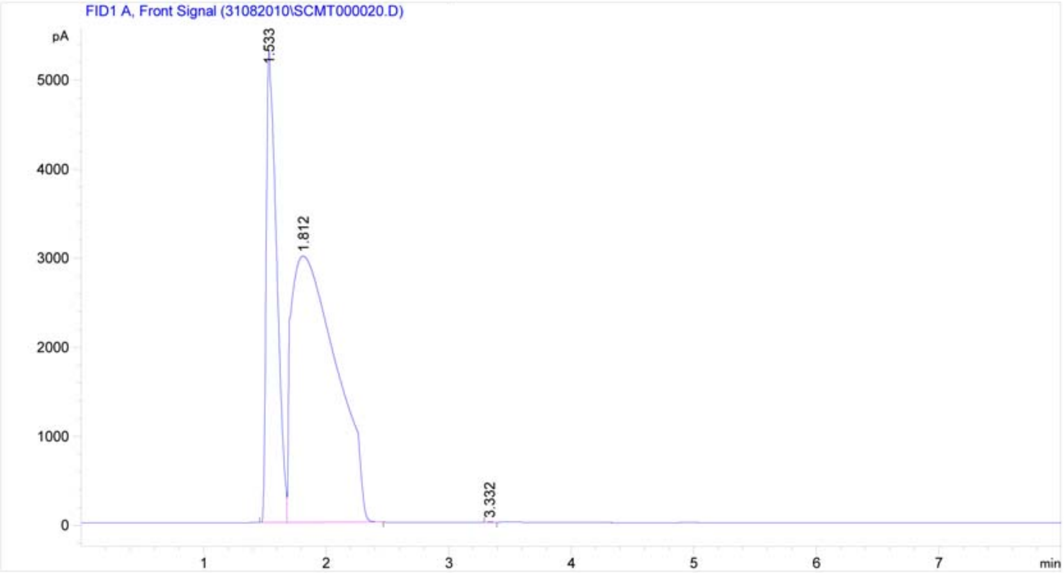
    1 Warnings or Errors :

    Warning : Calibrated compound(s) not found

    =====================================================================
                              *** End of Report ***
```

```
Data File C:\CHEM32\1\DATA\31082010\SCMT000020.D
Sample Name: PCMEctrl53

    =====================================================================
    Acq. Operator   : KenG
    Acq. Instrument : GC7890                        Location : Vial 1
    Injection Date  : 8/31/2010 4:14:22 PM
                                                  Inj Volume : Manually
    Acq. Method     : C:\CHEM32\1\METHODS\CHITOSAN.M
    Last changed    : 8/31/2010 4:14:20 PM by KenG
                      (modified after loading)
    Analysis Method : C:\CHEM32\1\METHODS\COOLDOWN.M
    Last changed    : 8/25/2010 5:27:45 PM by Ya
```



```
    =====================================================================
                             Area Percent Report
    =====================================================================

    Sorted By             :      Signal
    Calib. Data Modified  :      1/3/2008 3:31:33 PM
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: FID1 A, Front Signal

    Peak RetTime  Type   Width     Area      Area    Name
      #   [min]          [min]   [pA*s]       %
    ----|-------|------|-------|----------|--------|---------------------
       1   1.533 BV S    0.0798 3.11647e4  28.99906 ?
       2   1.812 VB S    0.4252 7.62847e4  70.98361 ?
       3   3.332 BV      0.0411   18.62075  0.01733 ?

    Totals :                    1.07468e5
```

]

```
Data File C:\CHEM32\1\DATA\31082010\SCMT000020.D
Sample Name: PCMEctrl53


    Signal 2: TCD1 C, Aux Signal   not found

    Peak RetTime  Type   Width     Area      Area    Name
      #   [min]          [min]  [25 µV*s]     %
    ----|-------|------|-------|----------|--------|---------------------
       1   1.631         0.0000    0.00000  0.00000 Hydrogen
       2   1.946         0.0000    0.00000  0.00000 Carbondioxide
       3   3.206         0.0000    0.00000  0.00000 Oxygen
       4   5.331         0.0000    0.00000  0.00000 Methane
       5   8.433         0.0000    0.00000  0.00000 Carbonmonoxide

    Totals :                        0.00000

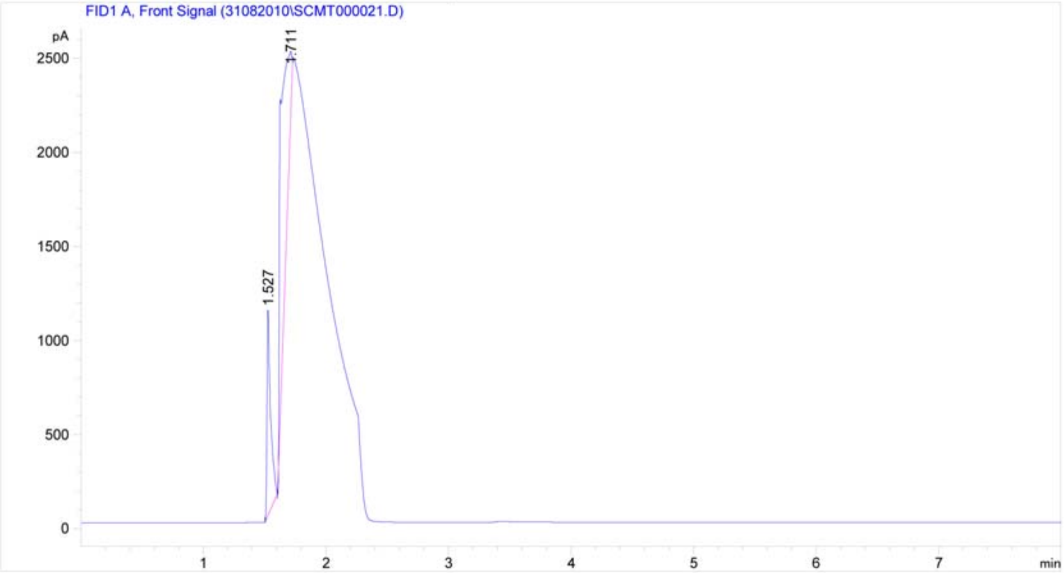

    1 Warnings or Errors :

    Warning : Calibrated compound(s) not found

    =====================================================================
                              *** End of Report ***
```

```
Data File C:\CHEM32\1\DATA\31082010\SCMT000021.D
Sample Name: PCMEsmct53

    =====================================================================
    Acq. Operator   : KenG
    Acq. Instrument : GC7890                         Location : Vial 1
    Injection Date  : 8/31/2010 4:29:21 PM
                                                   Inj Volume : Manually
    Acq. Method     : C:\CHEM32\1\METHODS\CHITOSAN.M
    Last changed    : 8/31/2010 4:29:19 PM by KenG
                      (modified after loading)
    Analysis Method : C:\CHEM32\1\METHODS\COOLDOWN.M
    Last changed    : 8/25/2010 5:27:45 PM by Ya
```



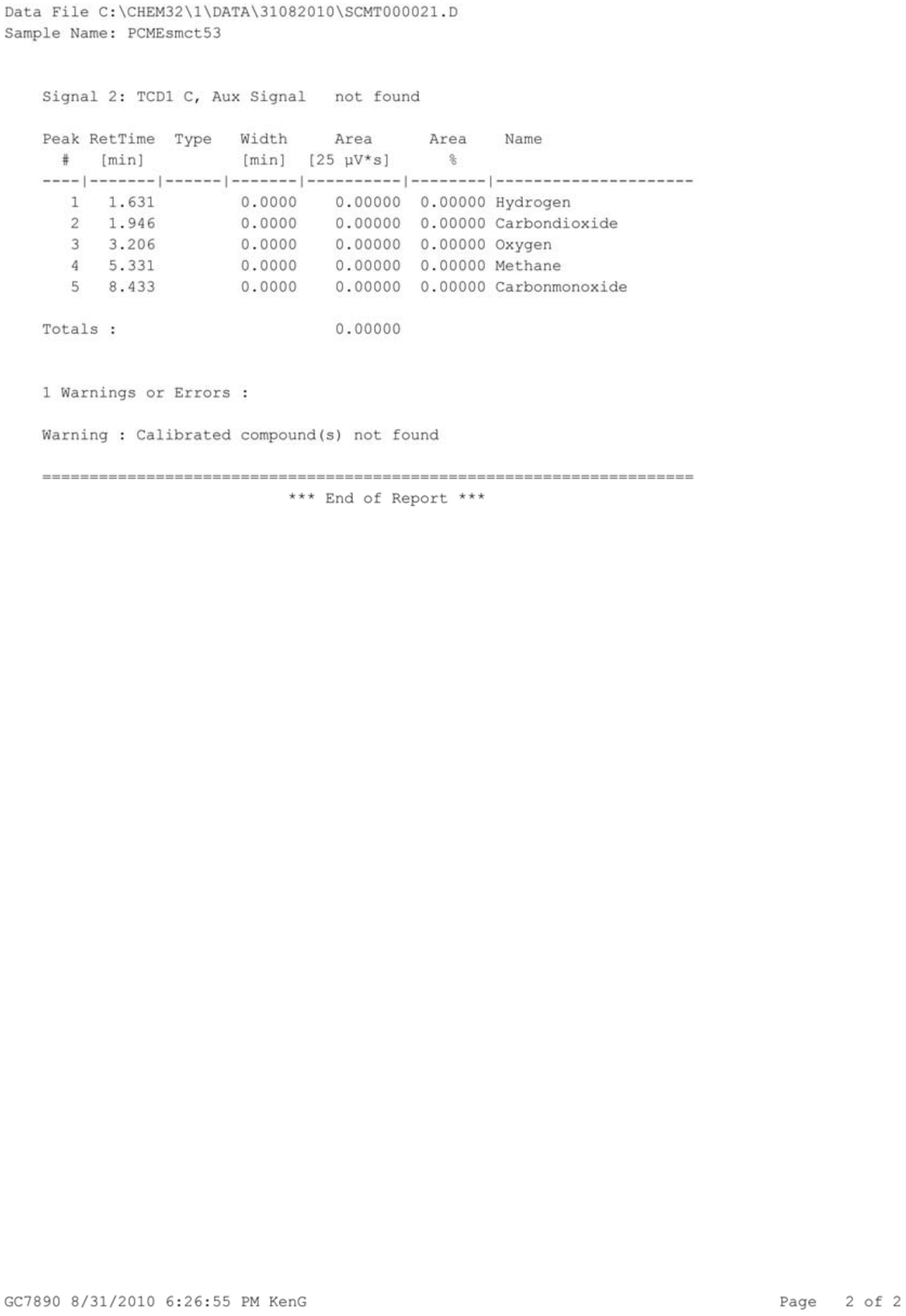
```
    =====================================================================
                             Area Percent Report
    =====================================================================

    Sorted By             :      Signal
    Calib. Data Modified  :      1/3/2008 3:31:33 PM
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: FID1 A, Front Signal

    Peak RetTime  Type   Width     Area      Area    Name
      #   [min]          [min]   [pA*s]       %
    ----|-------|------|-------|----------|--------|---------------------
       1   1.527 BV      0.0291 2260.16602 26.92906 ?
       2   1.711 VB S    0.2311 6132.87061 73.07094 ?

    Totals :                    8393.03662
```



```
Data File C:\CHEM32\1\DATA\31082010\SCMT000021.D
Sample Name: PCMEsmct53


    Signal 2: TCD1 C, Aux Signal   not found

    Peak RetTime  Type   Width     Area      Area    Name
      #   [min]          [min]  [25 µV*s]     %
    ----|-------|------|-------|----------|--------|---------------------
       1   1.631         0.0000    0.00000  0.00000 Hydrogen
       2   1.946         0.0000    0.00000  0.00000 Carbondioxide
       3   3.206         0.0000    0.00000  0.00000 Oxygen
       4   5.331         0.0000    0.00000  0.00000 Methane
       5   8.433         0.0000    0.00000  0.00000 Carbonmonoxide

    Totals :                       0.00000


    1 Warnings or Errors :

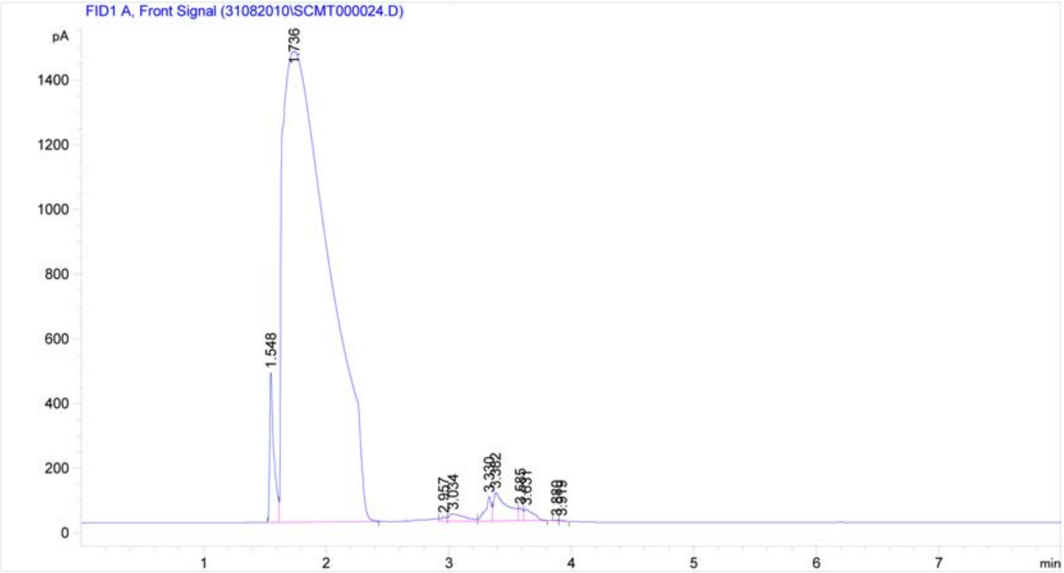
    Warning : Calibrated compound(s) not found

    =====================================================================
                              *** End of Report ***
```

```
GC7890 8/31/2010 6:26:55 PM KenG                                          Page    2 of 2
```

```
Data File C:\CHEM32\1\DATA\31082010\SCMT000024.D
Sample Name: SexGctrl53

    =====================================================================
    Acq. Operator   : KenG
    Acq. Instrument : GC7890                          Location : Vial 1
    Injection Date  : 8/31/2010 5:11:15 PM
                                                    Inj Volume : Manually
    Acq. Method     : C:\CHEM32\1\METHODS\CHITOSAN.M
    Last changed    : 8/31/2010 5:11:14 PM by KenG
                      (modified after loading)
    Analysis Method : C:\CHEM32\1\METHODS\COOLDOWN.M
    Last changed    : 8/25/2010 5:27:45 PM by Ya
```



```
    =====================================================================
                             Area Percent Report
    =====================================================================

    Sorted By             :      Signal
    Calib. Data Modified  :      1/3/2008 3:31:33 PM
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: FID1 A, Front Signal

    Peak RetTime  Type   Width     Area      Area    Name
      #   [min]          [min]   [pA*s]        %
    ----|-------|------|-------|----------|--------|---------------------
       1   1.548 BV      0.0323 1084.44250  2.69908 ?
       2   1.736 VB      0.3127 3.76255e4  93.64625 ?
       3   2.957 BV      0.0433   43.13305  0.10735 ?
       4   3.034 VV      0.1286  200.33215  0.49861 ?
       5   3.330 VV      0.0450  251.33864  0.62556 ?
       6   3.382 VV      0.0999  678.54987  1.68885 ?
       7   3.585 VV      0.0340   93.26061  0.23212 ?
```

```
Data File C:\CHEM32\1\DATA\31082010\SCMT000024.D
Sample Name: SexGctrl53

    Peak RetTime  Type   Width     Area      Area    Name
      #   [min]          [min]   [pA*s]        %
    ----|-------|------|-------|----------|--------|---------------------
       8   3.631 VB      0.0704  189.64441  0.47201 ?
       9   3.880 BV      0.0300    4.94901  0.01232 ?
      10   3.919 VB      0.0373    7.17758  0.01786 ?

    Totals :                    4.01783e4


    Signal 2: TCD1 C, Aux Signal   not found

    Peak RetTime  Type   Width     Area      Area    Name
      #   [min]          [min]  [25 µV*s]      %
    ----|-------|------|-------|----------|--------|---------------------
       1   1.631         0.0000    0.00000  0.00000 Hydrogen
       2   1.946         0.0000    0.00000  0.00000 Carbondioxide
       3   3.206         0.0000    0.00000  0.00000 Oxygen
       4   5.331         0.0000    0.00000  0.00000 Methane
       5   8.433         0.0000    0.00000  0.00000 Carbonmonoxide

    Totals :                       0.00000

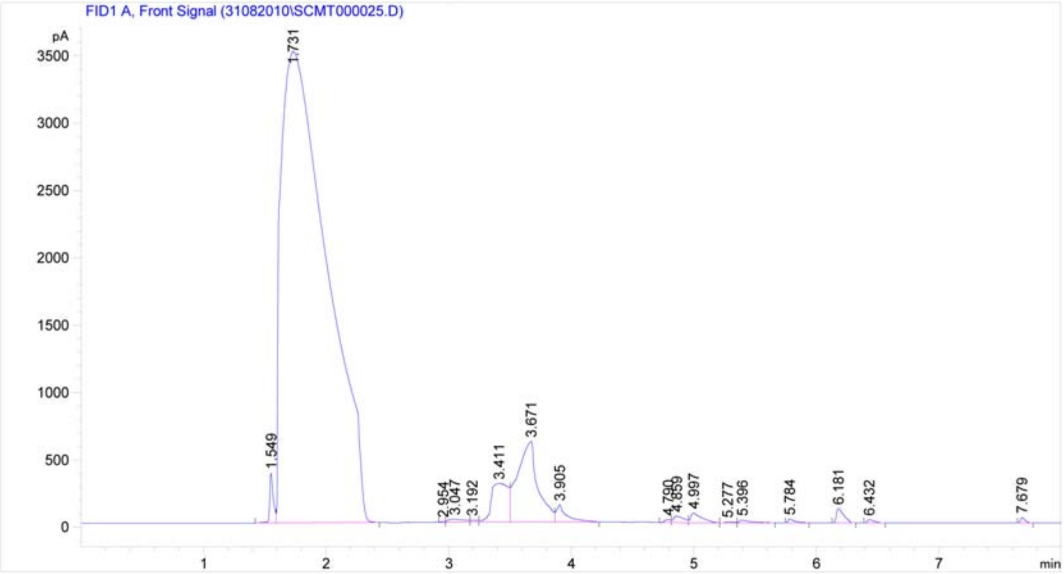

    1 Warnings or Errors :

    Warning : Calibrated compound(s) not found

    =====================================================================
                              *** End of Report ***
```

```
Data File C:\CHEM32\1\DATA\31082010\SCMT000025.D
Sample Name: SexGsmct53

    =====================================================================
    Acq. Operator   : KenG
    Acq. Instrument : GC7890                         Location : Vial 1
    Injection Date  : 8/31/2010 5:24:56 PM
                                                   Inj Volume : Manually
    Acq. Method     : C:\CHEM32\1\METHODS\CHITOSAN.M
    Last changed    : 8/31/2010 5:24:54 PM by KenG
                      (modified after loading)
    Analysis Method : C:\CHEM32\1\METHODS\COOLDOWN.M
    Last changed    : 8/25/2010 5:27:45 PM by Ya
```



```
    =====================================================================
                         Area Percent Report
    =====================================================================

    Sorted By             :      Signal
    Calib. Data Modified  :      1/3/2008 3:31:33 PM
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Use Multiplier & Dilution Factor with ISTDs


    Signal 1: FID1 A, Front Signal

    Peak RetTime  Type  Width     Area      Area    Name
      #   [min]         [min]   [pA*s]       %
    ----|-------|------|-------|----------|--------|---------------------
       1   1.549 BV     0.0295  742.05481  0.71633 ?
       2   1.731 VB S   0.3560 9.05888e4  87.44878 ?
       3   2.954 BV     0.0291   11.89526  0.01148 ?
       4   3.047 VV     0.1192  199.19984  0.19230 ?
       5   3.192 VV     0.0577   59.07988  0.05703 ?
       6   3.411 VV     0.1155 2726.28442  2.63179 ?
       7   3.671 VV     0.1429 6867.32324  6.62929 ?
```

```
Data File C:\CHEM32\1\DATA\31082010\SCMT000025.D
Sample Name: SexGsmct53

    Peak RetTime  Type   Width     Area      Area    Name
      #   [min]          [min]    [pA*s]       %
    ----|-------|------|-------|----------|--------|---------------------
       8   3.905 VB      0.0758  752.33887  0.72626 ?
       9   4.790 BV      0.0478   71.04162  0.06858 ?
      10   4.859 VV      0.0855  274.32675  0.26482 ?
      11   4.997 VB      0.0812  438.72449  0.42352 ?
      12   5.277 BV      0.0587   25.00184  0.02414 ?
      13   5.396 VB      0.0795  109.23034  0.10544 ?
      14   5.784 BB      0.0510   95.01575  0.09172 ?
      15   6.181 BB      0.0576  436.34766  0.42122 ?
      16   6.432 BB      0.0516   94.17446  0.09091 ?
      17   7.679 BB      0.0368   99.85268  0.09639 ?

    Totals :                     1.03591e5


    Signal 2: TCD1 C, Aux Signal   not found

    Peak RetTime  Type   Width     Area      Area    Name
      #   [min]          [min]  [25 µV*s]      %
    ----|-------|------|-------|----------|--------|---------------------
       1   1.631         0.0000    0.00000  0.00000 Hydrogen
       2   1.946         0.0000    0.00000  0.00000 Carbondioxide
       3   3.206         0.0000    0.00000  0.00000 Oxygen
       4   5.331         0.0000    0.00000  0.00000 Methane
       5   8.433         0.0000    0.00000  0.00000 Carbonmonoxide

    Totals :                        0.00000


    1 Warnings or Errors :

    Warning : Calibrated compound(s) not found

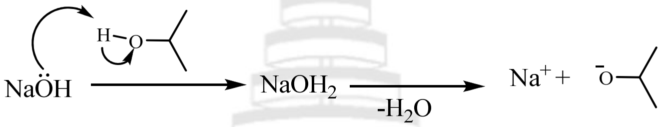
    =====================================================================
                             *** End of Report ***
```

APPENDIX B

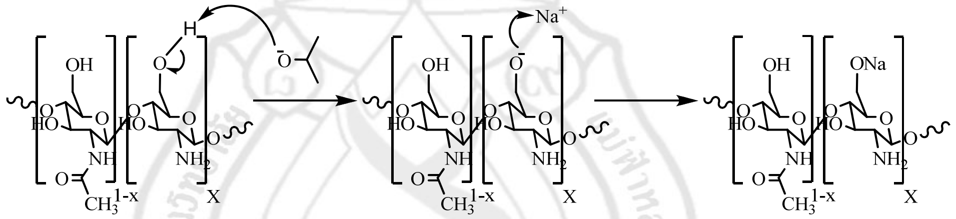
MECHANISM OF CARBOXYMETHYLATION PROCESS

Carboxymethylation of Chitosan

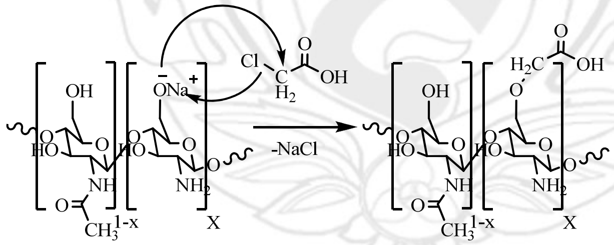
1. alkoxide generation



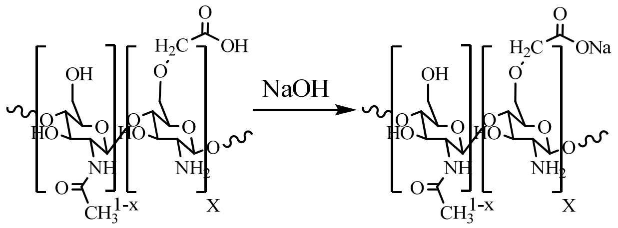
2. Abstract protron at 3-O-Position



3. SN_2 addition



4. Neutralising with excess NaOH



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WORK EXPERIENCE

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2007 - 2008	Flight Attendant Saudi Airlines, Saudi arabia
2006	QA Supervisor Dole, Chumporn, Thailand
2004	Analyst The Office of Atoms for Peace, Bangkok, Thailand