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# LABORATORY ANALYSIS REPORT

# for

# THE JOINT COMMITTEE OF EXPERTS ON TETANUS TOXOID VACCINE TESTING

# THE MINISTRY OF HEALTH AND KENYA CATHOLIC HEALTH COMMISION (KCCB)

| | |
|---|---|
| Date of Sampling - Lot 1: | 10th December 2014 |
| Date of Sampling - Lot 2: | 15th December 2014 |
| Date of Sampling - Lot 3: | 9th January 2015 |
| Date of Report: | 23rd January 2015 |

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## 1.0 EXECUTIVE SUMMARY

The Ministry of Health through the Division of Vaccines and Immunization aims to increase access to immunization services nationwide, in order to reduce morbidity and mortality due to vaccine preventable diseases. This is in acknowledgement of the proven benefits of immunization in the prevention, control and even eradication of life threatening diseases over the years. Of particular importance is the reduction of infant and child morbidity and mortality in line with the United Nations Millennium Development Goals (MDG). The other major consideration is to implement the World Health Organization / United Nations Children's Fund (WHO/UNICEF) Global Immunization Vision & Strategy (GIVS) which challenges national governments to immunize more people, from infants to seniors, with a greater range of vaccines.

Through the Catholic Health Commission of Kenya, the Catholic Church runs close to 45% of all healthcare facilities in Kenya. The Church has an expansive network which consists of 448 health units (54 hospitals, 83 health centres and 311 Dispensaries) and more than 46 Community Based Health and Orphaned and Vulnerable Children (OVC) Programs. In the Arid and Semi-Arid Areas, the Church has and manages mobile clinics for nomadic communities.

The Ministry of Health and the Catholic Health Commission established a joint committee to investigate the safety of the tetanus vaccine that was administered by the World Health Organization (WHO) / United Nations Children's Fund (UNICEF) in the last Kenyan vaccination campaigns.

The scope of the laboratory assessment was to determine the presence of beta human chorionic gonadotropin hormone (βhCG) in the samples submitted.

### 1.1 OBJECTIVES OF THE LABORATORY ASSESSMENT

The Laboratory assessment had the following objectives:

1.1.1 Analyze the content of the vials sampled for the presence of βeta human chorionic gonadotropin hormone (βhCG) in the samples.

1.1.2 Quantify the levels of βhCG for each of the samples where present

1.1.3 Full toxicological analysis of the vaccine, identifying any other substances apart from the immunogen, the detoxicant and diluents

1.1.4 Production of a written report that includes the procedure used for analysis, relevant references applied to the analysis, citing publication(s)

1.1.5 To inform and interpret to the Ministry of Health and the Catholic Health Commission (CHC), the findings from the laboratory analysis.

Further instructions from the joint committee through the Pharmacy and Poisons Board, Ministry of Health, dated 17th December 2014, directed the Laboratory to restrict its initial investigation to:

1.1.6 Determining if beta human chorionic gonadotropin hormone (βhCG) was present in the submitted vials.

1.1.7 Quantity of human chorionic gonadotropin hormone (βhCG) present in each of the vials detected.

The Laboratory restricted itself to these new directions in carrying out the analysis.

## 1.2 REFERENCES USED DURING THE LABORATORY ASSESSMENT

1.2.1 Purification of Human Chorionic Gonadotropin Hormone by Anion-Exchange High Performance Liquid Chromatography (HPLC)

1.2.2 Journal of Chromatography A, 847 (1999) - Determination of the molecular size distribution of type b tetanus toxoid conjugate vaccines by size exclusion chromatography

1.2.3 Dissociation of human chorionic gonadotropin hormone (hCG) into its sub units - Pierce, Morgan et al 1971

## 1.3 EXECUTION OF THE ASSESSMENT

The assessment was carried out at as follows:

1.3.1 Sampling and Sample Submission

Samples for the determination of βhCG were submitted as follows:

- Lot 1
  Sampling at the Kenya Expanded Program on Immunization (KEPI) National depot on 10th December 2014

- Lot 2
  Sampling at Upper Hill Medical Center on 17th December 2014.

- Lot 3
  Matching samples submitted to the Laboratory on 9th January 2015. The purpose of these samples was to obtain closed vials of batch numbers that were obtained from the Catholic Health Commission (CHC), as communicated by the Director of Medical Services on 8th January 2015.

### 1.3.2 Sample Description

The samples used for the Laboratory analysis were:

| Sampling Location | Batch No. | Expiry Date | Manufacturer | Comments |
|---|---|---|---|---|
| Lot 1 KEPI Stores | 019B4002D | Jan. 2017 | Serum Institute - India | Closed vial |
| | 019B4003A | Jan. 2017 | Serum Institute - India | Closed vial |
| | 019B4003B | Jan. 2017 | Serum Institute - India | Closed vial |
| | 019B4002C | Jan. 2017 | Serum Institute - India | Closed vial |
| | 11077A13 | Aug. 2016 | Biological E. Ltd | Closed vial |
| | 019B4002C | Jan. 2017 | Serum Institute - India | Closed vial |
| | 019B4002D | Jan. 2017 | Serum Institute - India | Closed vial |
| | 019B4003B | Jan. 2017 | Serum Institute - India | Closed vial |
| | 019B4003A | Jan. 2017 | Serum Institute - India | Closed vial |
| | 019L3001B | Feb. 2016 | Serum Institute | Open vial |
| | 019L3001C | Feb. 2016 | Serum Institute | Open vial |
| | 019L3001B | Feb. 2016 | Serum Institute | Open vial |
| | 019B4002D | Jan. 2017 | Serum Institute | Open vial |
| | 019B4003A | Jan. 2017 | Serum Institute | Closed vial |
| Lot 2 Upper Hill Medical Center | 019B4003A | Jan. 2017 | Serum Institute | Open vial |
| | 019B4002D | Jan. 2017 | Serum Institute | Open vial |
| | 019B4002D | Jan. 2017 | Serum Institute | Open vial |
| | 019B4002D | Jan. 2017 | Serum Institute | Open vial |
| Lot 3 Matching Samples | 019L3001B | Jan. 2017 | Serum Institute | Closed vial 10 (Pokot) |
| | 019L3001C | Jan. 2017 | Serum Institute | Closed vial 20 (Turkana) |
| | 019L3001B | Jan. 2017 | Serum Institute | Closed vial 10 (Turkana) |

1.3.3 Laboratory Analysis

Laboratory Analysis using High Performance Liquid Chromatography (HPLC) on:

- Lot 1 - analysis carried out on the 5th of January 2014
- Lot 2 - analysis carried out on the 5th of January 2014
- Lot 3 - analysis carried out on the 9th of January 2015

1.3.4 Reporting

Laboratory reporting and interpretation of the data on 9th of January 2015.

## 2.0 SUMMARY OF FINDINGS

Tetanus is acquired when the spores of the bacterium Clostridium Tetani infect a wound or the umbilical stump. Spores are universally present in the soil. People of all ages can get tetanus but the disease is particularly common and serious in newborn babies ("neonatal tetanus"). It requires treatment in a medical facility, often in a referral hospital. Neonatal tetanus, which is mostly fatal, is particularly common in rural areas where deliveries are at home without adequate sterile procedures.

Tetanus can be prevented through immunization with tetanus-toxoid (TT) - containing vaccines. Neonatal tetanus can be prevented by immunizing women of childbearing age with tetanus toxoid vaccine, either during pregnancy or outside of pregnancy. This protects the mother and - through a transfer of tetanus antibodies to the fetus - also her baby.

Human Chorionic Gonadotropin (hCG) hormone is synthesized by the chorionic tissue of the placenta and is found in urine during pregnancy. hCG comprises the glycoprotein hormone family. HCG is dimeric and is composed of two non-covalently bonded glycopeptides sub-units termed α (alpha HCG) and β (beta HCG).

A High Performance Liquid Chromatography (HPLC) method has been developed for the detection of hCG hormone sample in one chromatographic run using anion exchange chromatography. During the 60 minute linear gradient run, complete separation was accomplished in 40 minutes. The retention time for the hCG peak using this method was about 35 minutes.

Of the fifty nine (59) vaccine samples collected and subjected to Laboratory HPLC analysis, three of the samples were found to contain beta human chorionic gonadotropin hormone (βhCG):

- Two samples batch No. 019L3001B expiry date Jan. 2017 manufactured by Serum Institute
- One sample batch No. 019L3001C expiry date Jan. 2017 manufactured by Serum Institute

## 3.0 INTRODUCTION

Human chorionic Gonadotropin (hCG) hormone is synthesized by the chorionic tissue and is found in urine during pregnancy.
hCG together with luteinizing hormone (LH), follicle stimulating hormone (FSH, follotropin) and thyroid-stimulating hormone (TSH, thyrotropin) comprise the glycoprotein hormone family. All are dimeric and composed of two non-covalently bonded glycopeptide subunits termed α and β. The total number of amino acid residues in hCG for the α subunit is 92 and for the β 3 chain is 147. The amino acid sequences of both subunits of hCG have also been determined by Pierce and Morgan et al. Oligosaccharide and sialic acid chains are attached to both subunits. Each subunit is extensively crosslinked by intramolecular disulfide bonds.

A great number of immunological methods for HGC assays are available. These are based on haemagglutination, latex particle agglutination, complement fixation and radio immuno-reaction. The sensitivity and specificity of these methods make them potentially useful for the measurement of HCG hormone in urine concentration.

High separation of HCG has been obtained by simple methods of column chromatography on diethyl-aminoethyl (DEAE) Sephadex and Sephadex G-100 columns by Bell et al. Another method employing stepwise gradient with increasing NaCl concentration was reported by Yi-Han Chang et al. The isolation of HCG using these methods would be suitable but the procedures are time consuming, the standard column chromatographic procedures taking about two [2] weeks.

A rapid isolation of hCG can be accomplished in one day using anion exchange chromatography.

### 3.1 Description of the Analytical Method

The isoelectric point of the hCG molecule is about 4, solutions with higher pH contain HCG molecules in anionic form.

Eluent “A” was phosphate buffer [ph=6], while eluent “B” was 0.01M phosphate buffer [pH=3]. Buffer “B” contained 0.05M sodium sulfate and 0.05M sodium hydrogen sulfate. The pH of the solution was acidic due to the acidic character of the NaHSO4. Buffer “A” contained 0.01% v/v of “B” leading to the sulfate and hydrogen ion concentrations being 10,000 times smaller. This means that during a 40 min linear gradient both the hydrogen ion and the sulfate concentrations have been significantly increased, conditions that promote the elution and good resolution of HCG.

The retention time of the HCG peak was about 35mins.

## 3.2 HPLC Apparatus and Columns

### 3.2.1 HPLC Equipment

The analytical HPLC system used in this assessment consisted of a Shimadzu Class VP 10 system. A Dell computer connected to Shimadzu SCL 10A - system controller was used for gradient control of the two shimadzu pumps.

The preparative procedures were accomplished on Shimadzu LC-10AT Liquid Chromatograph connected to a Shimadzu 10AV UV VIS detector with a model 201 fraction collector and equipped with a 7125 sample injection valve with 1ml sample loop.

### 3.2.2 Column

Anion exchange separations were performed on an ODS 5µm 250mm x 4.6mm column.

### 3.2.3 Materials

Mobile phases contained analytical - grade sodium acetate, sodium bicarbonate, potassium chloride, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, sodium sulfate and sodium hydrogen sulphate.

HPLC grade acetonitrile and deionized water were used for preparing the eluents. Solvents were degassed ultrasonically.

### 3.2.4 Gradient Programs

The chromatographic run started isocratically by pumping 100% “A” mobile phase for 20min. A linear gradient from 0% “B” to 100% “B” mobile phase was employed at a 1.oml/min flow rate for 40 mins. The samples were dissolved in “A” eluent and injected at the beginning of the gradient run.

## 4.0 RESULTS

### 4.1 Chromatograms

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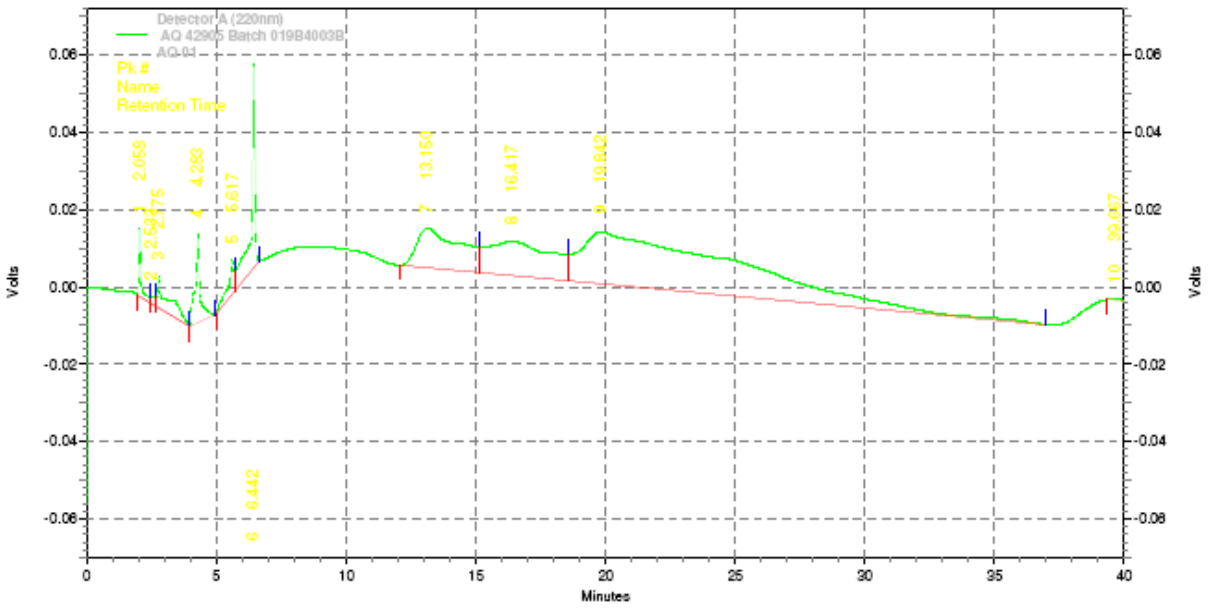


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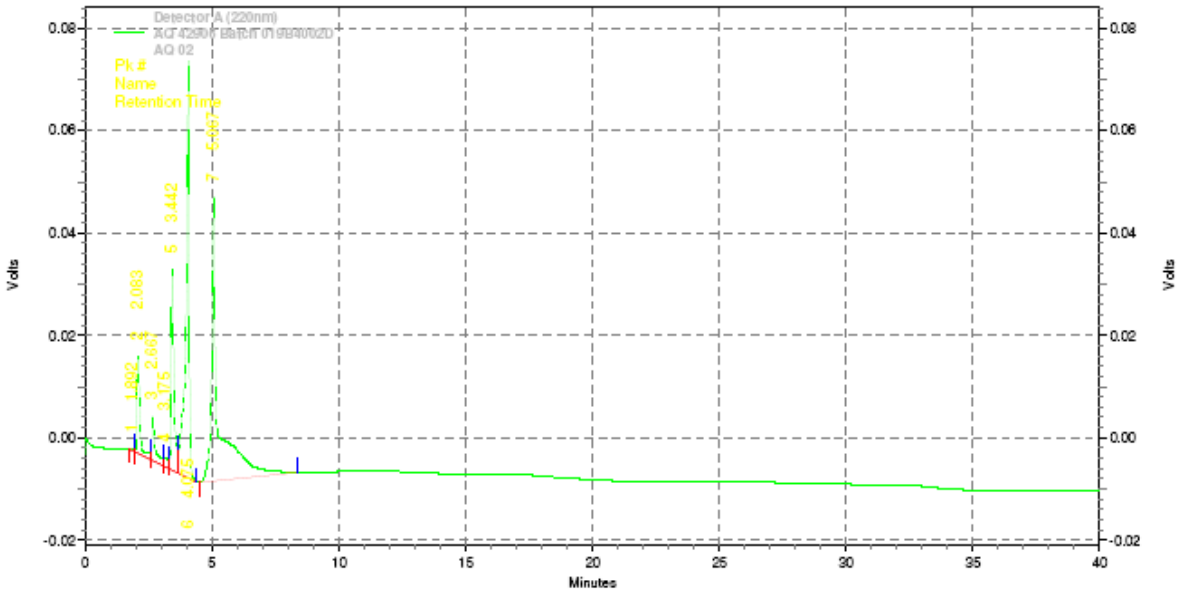


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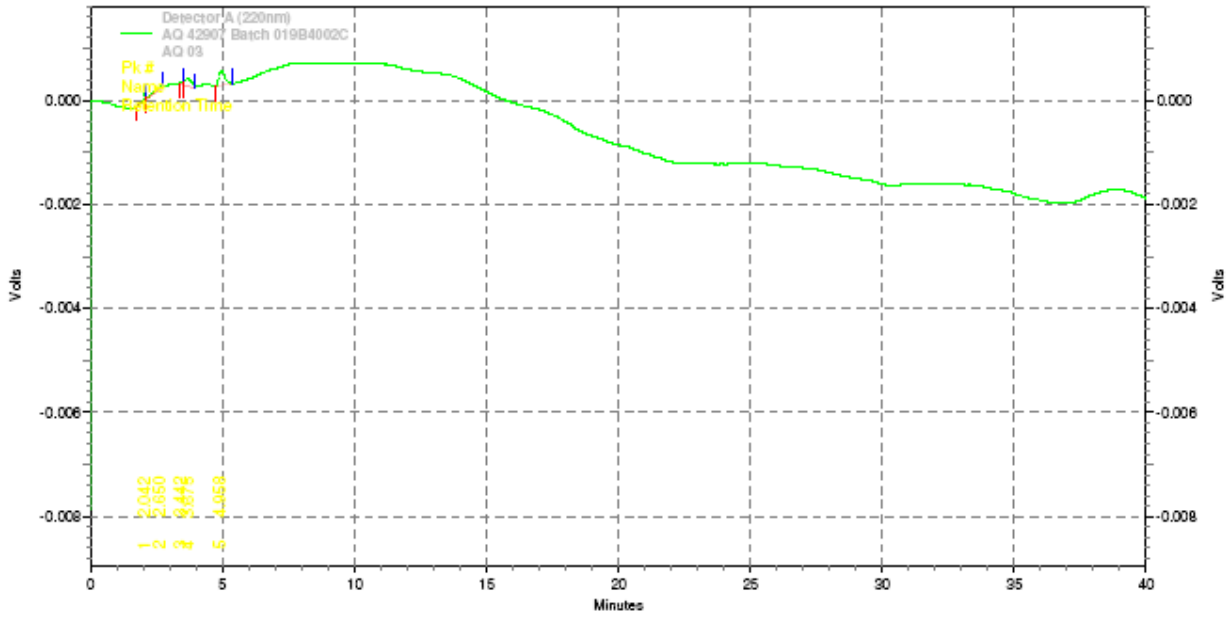


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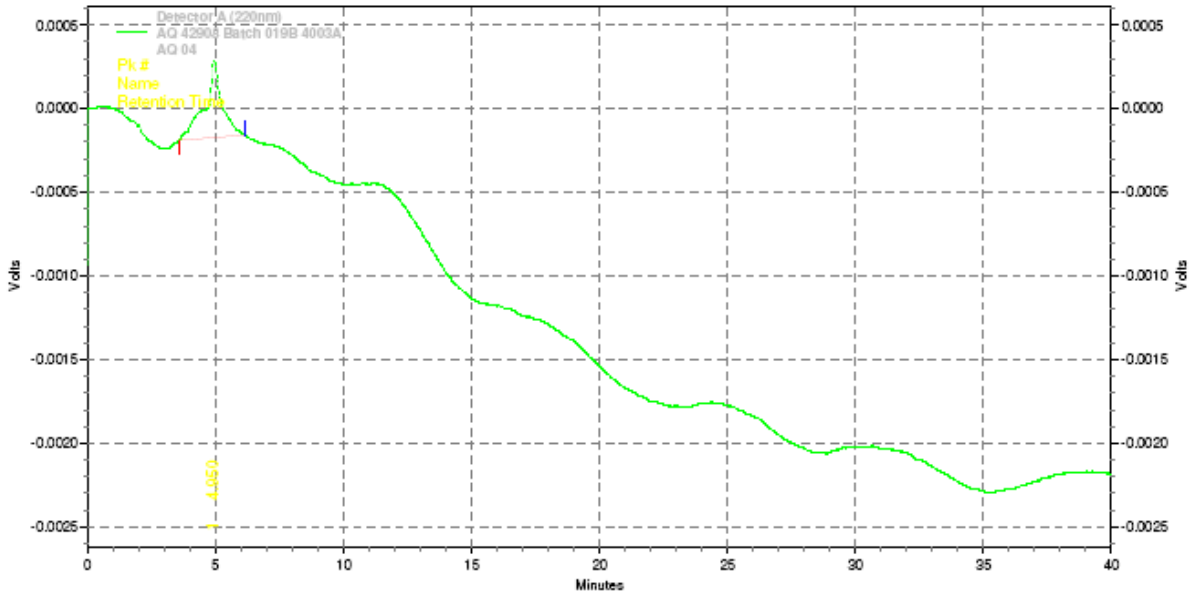


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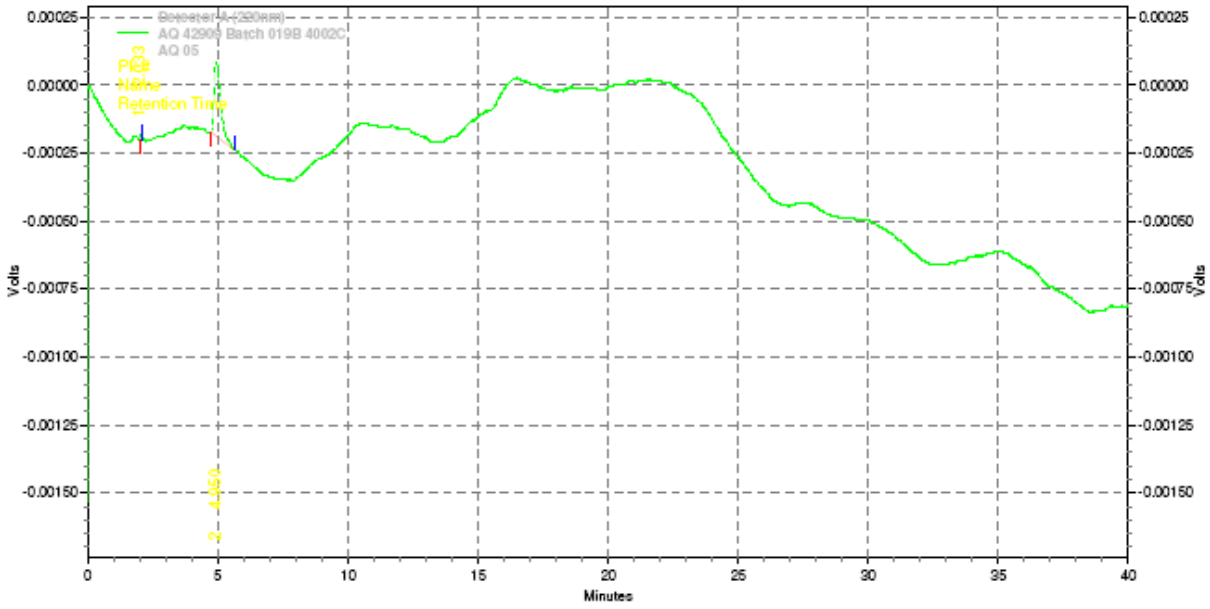


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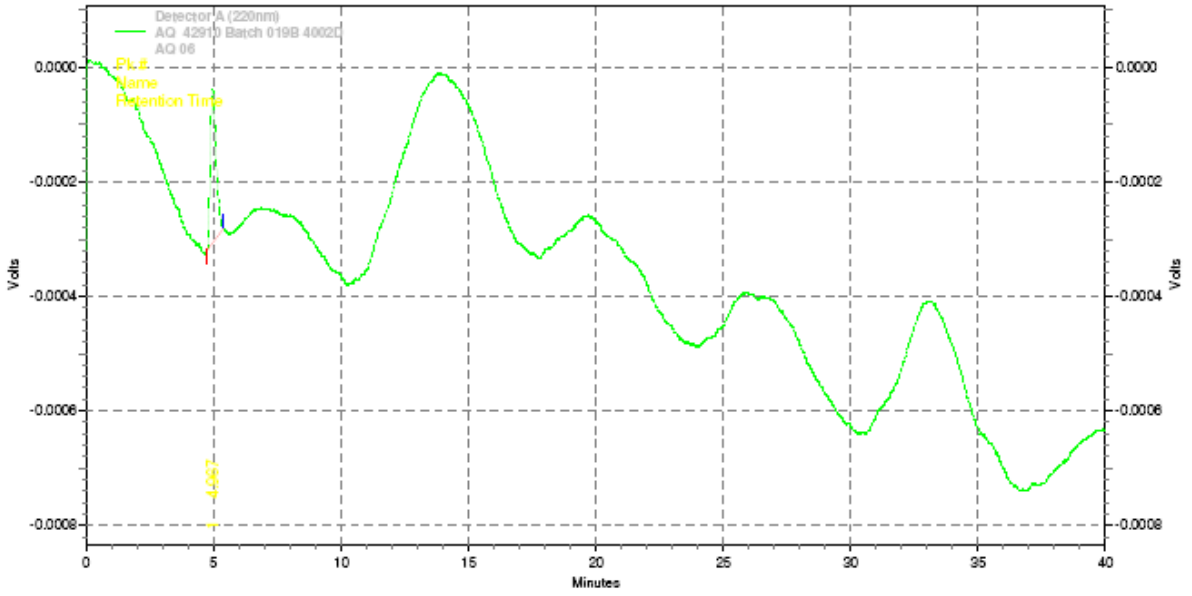


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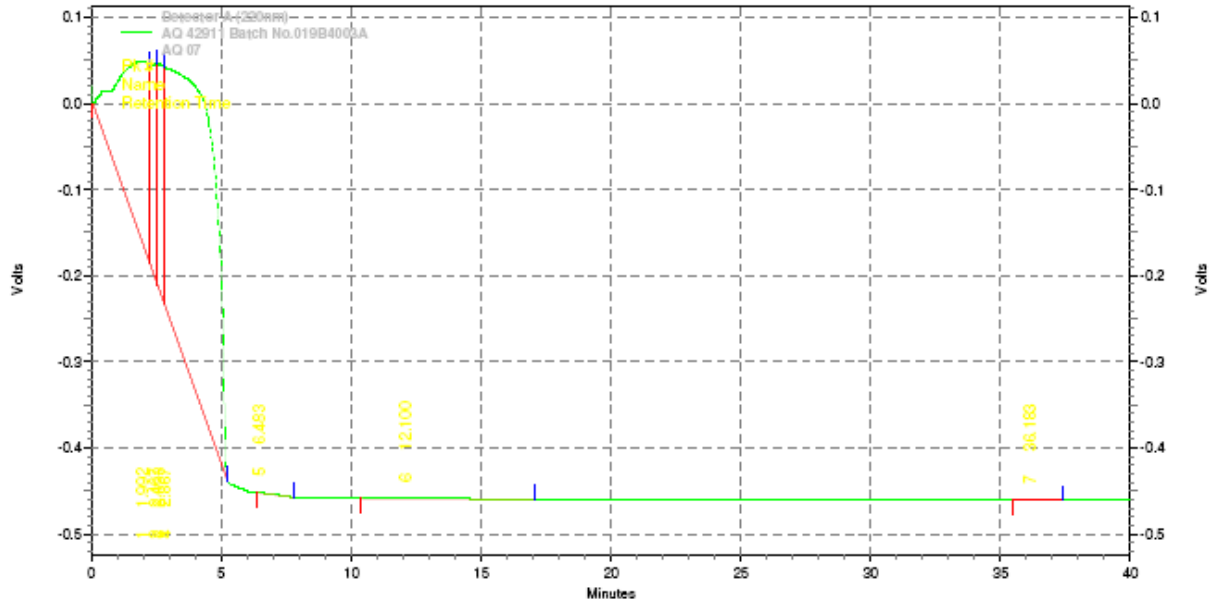


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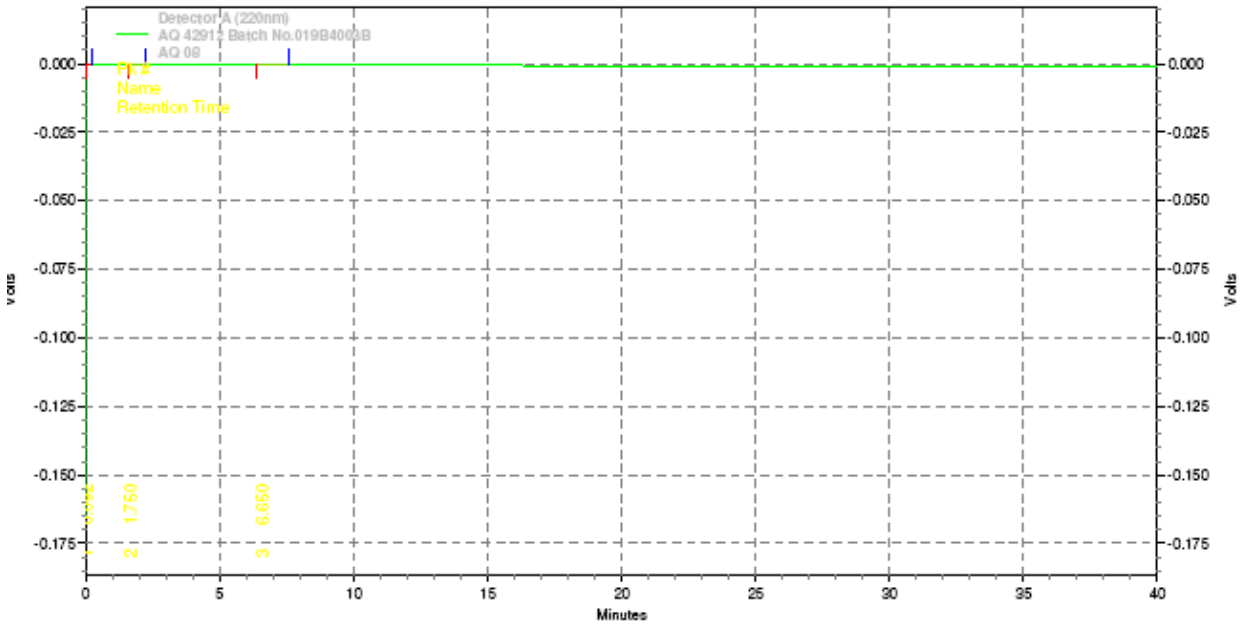


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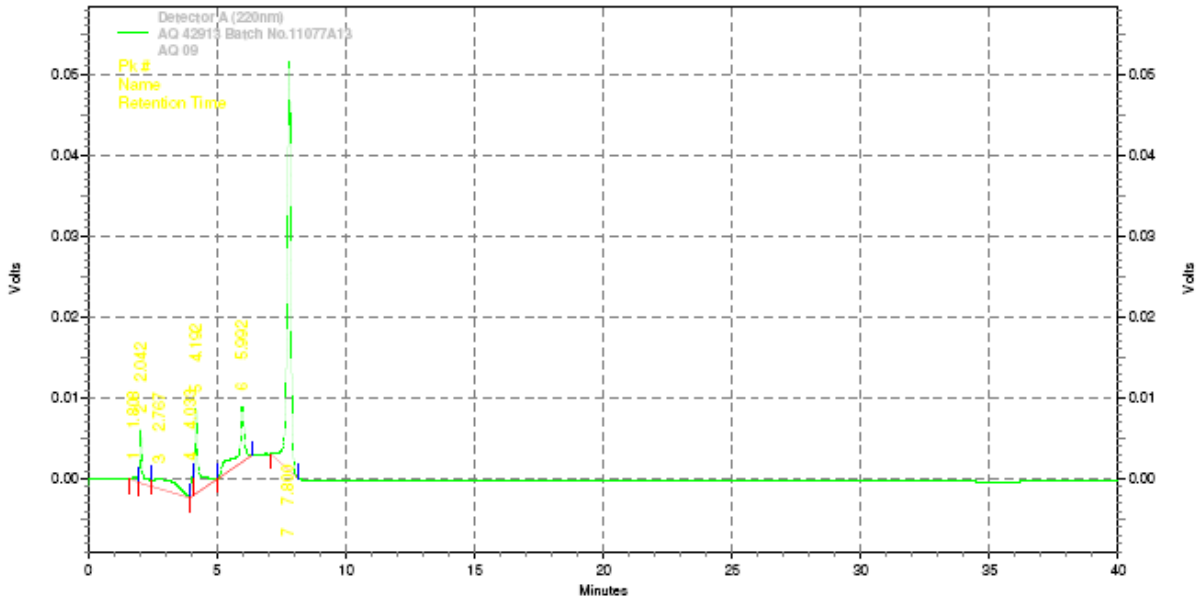


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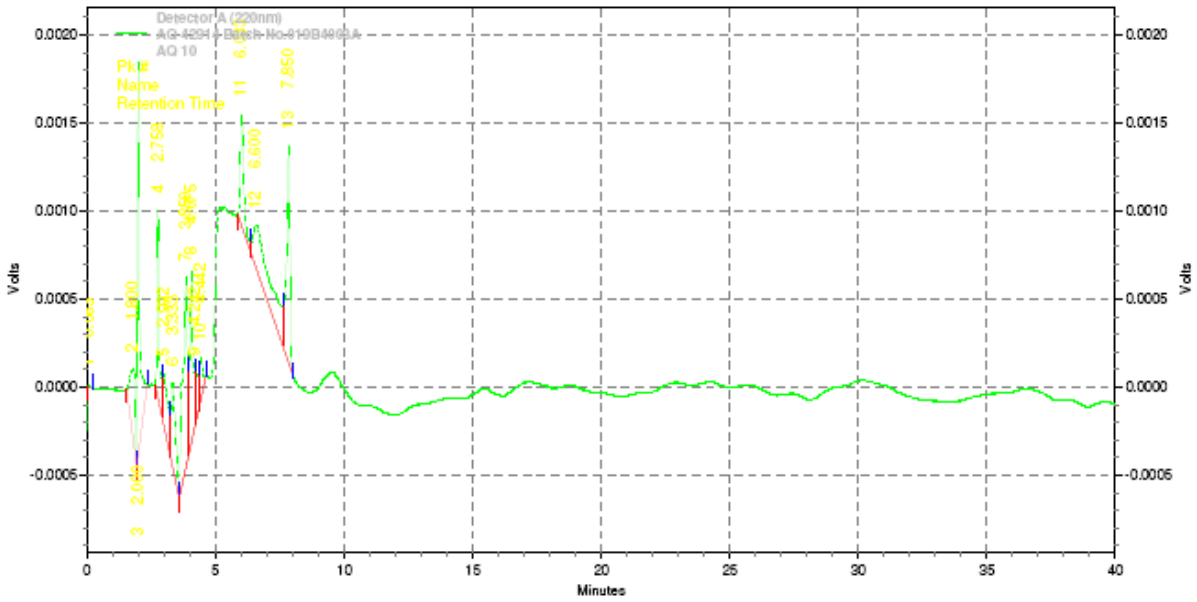


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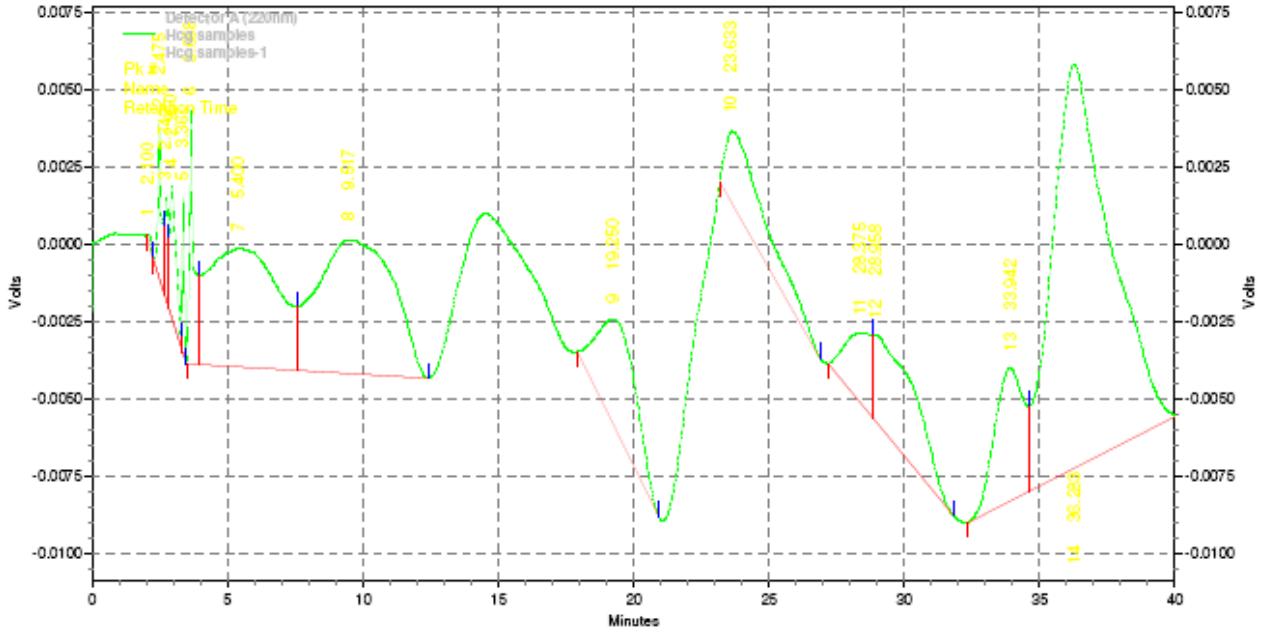


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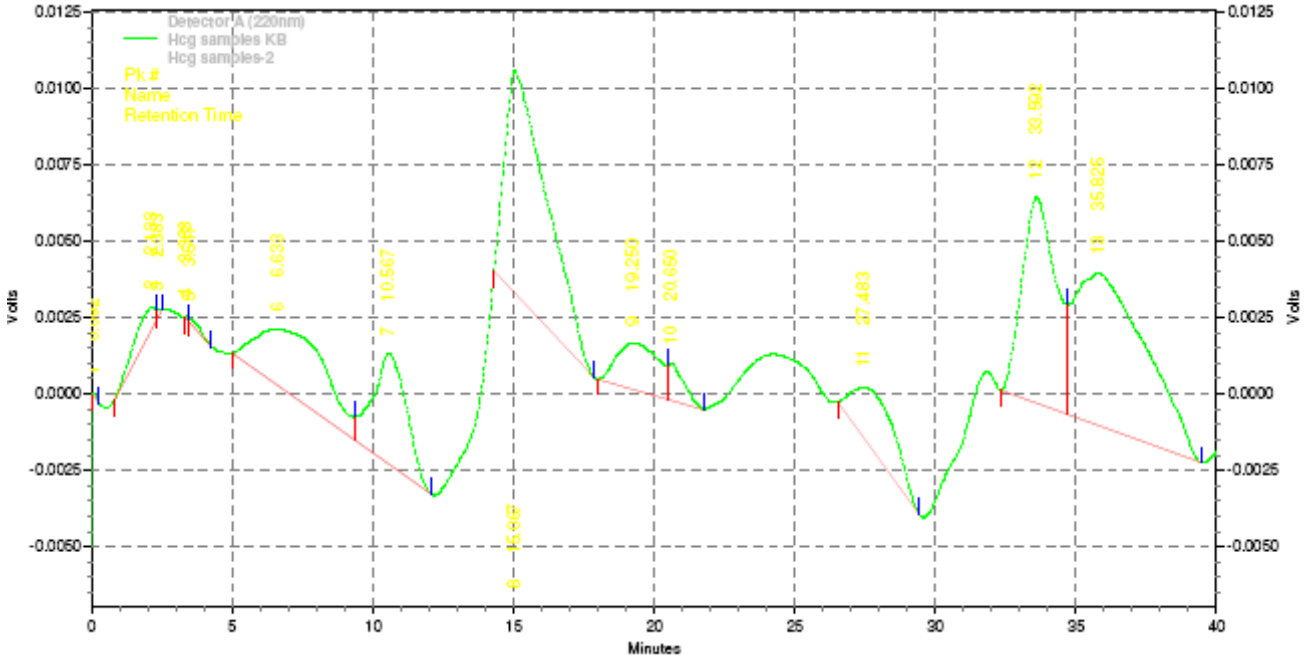

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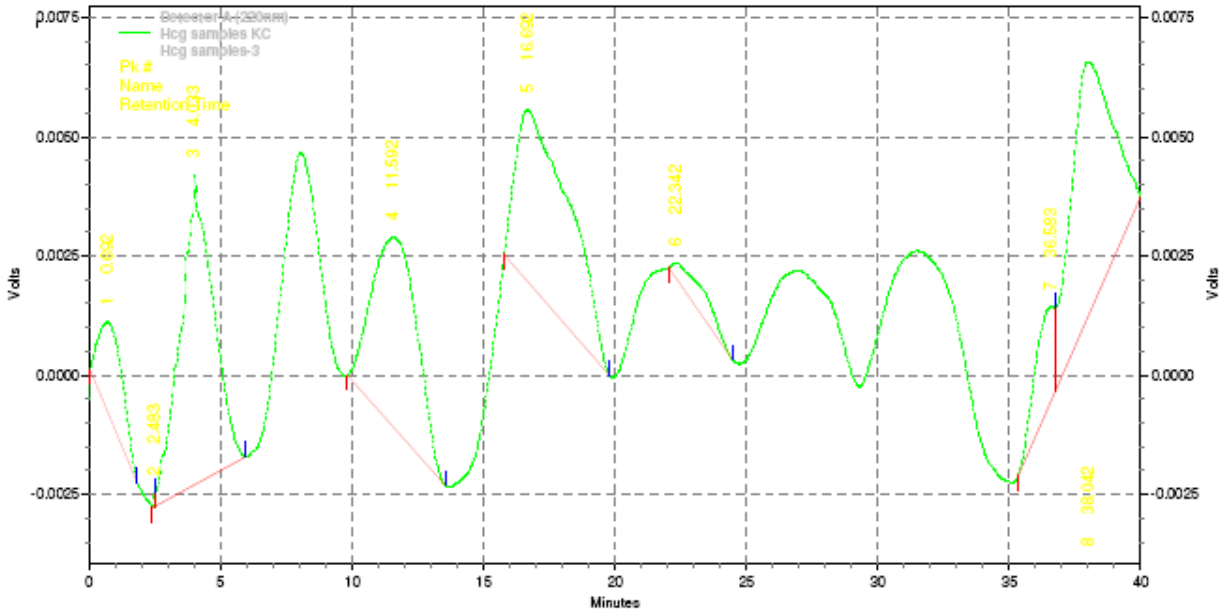


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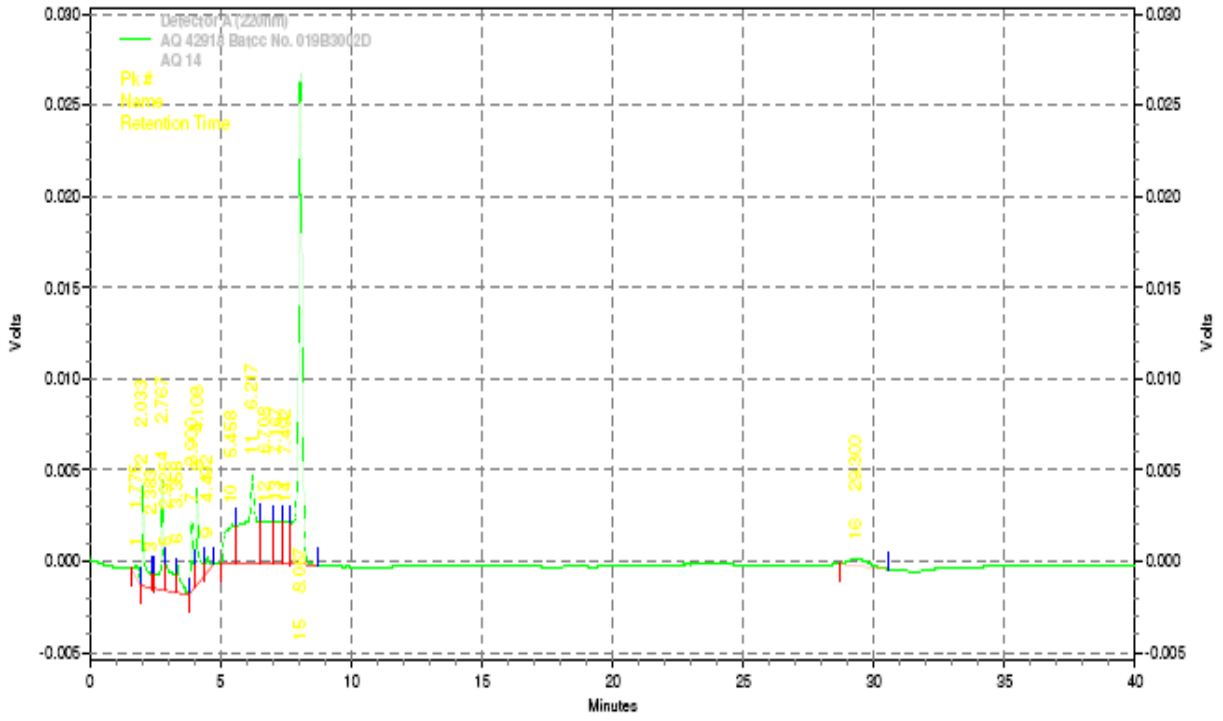

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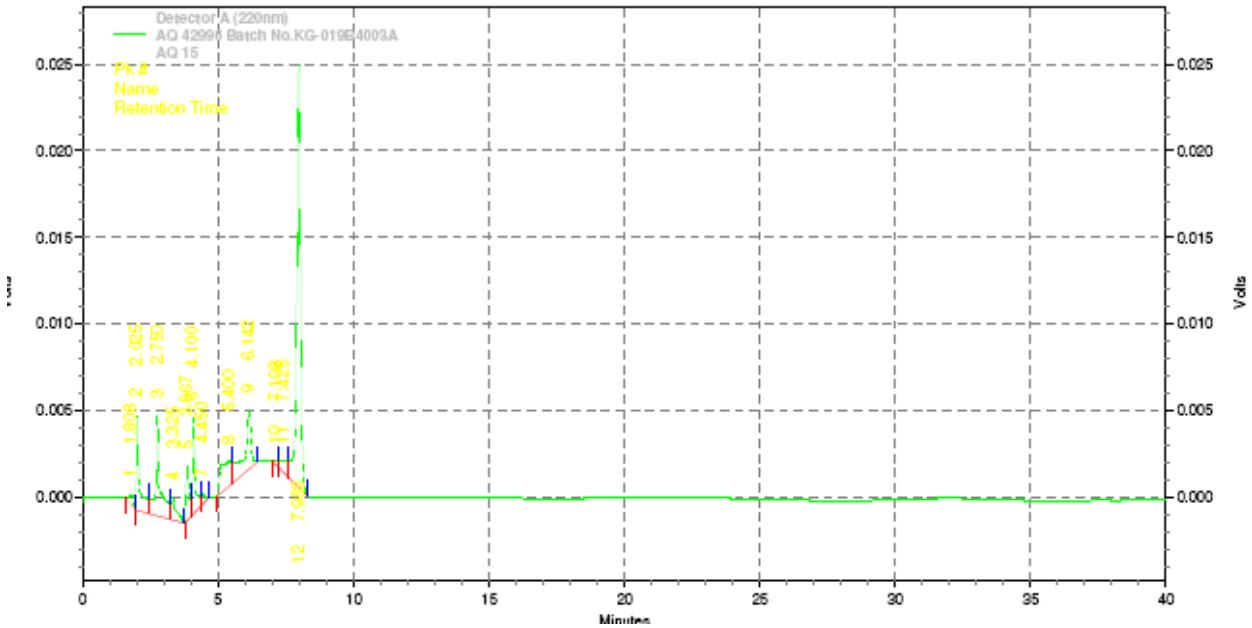


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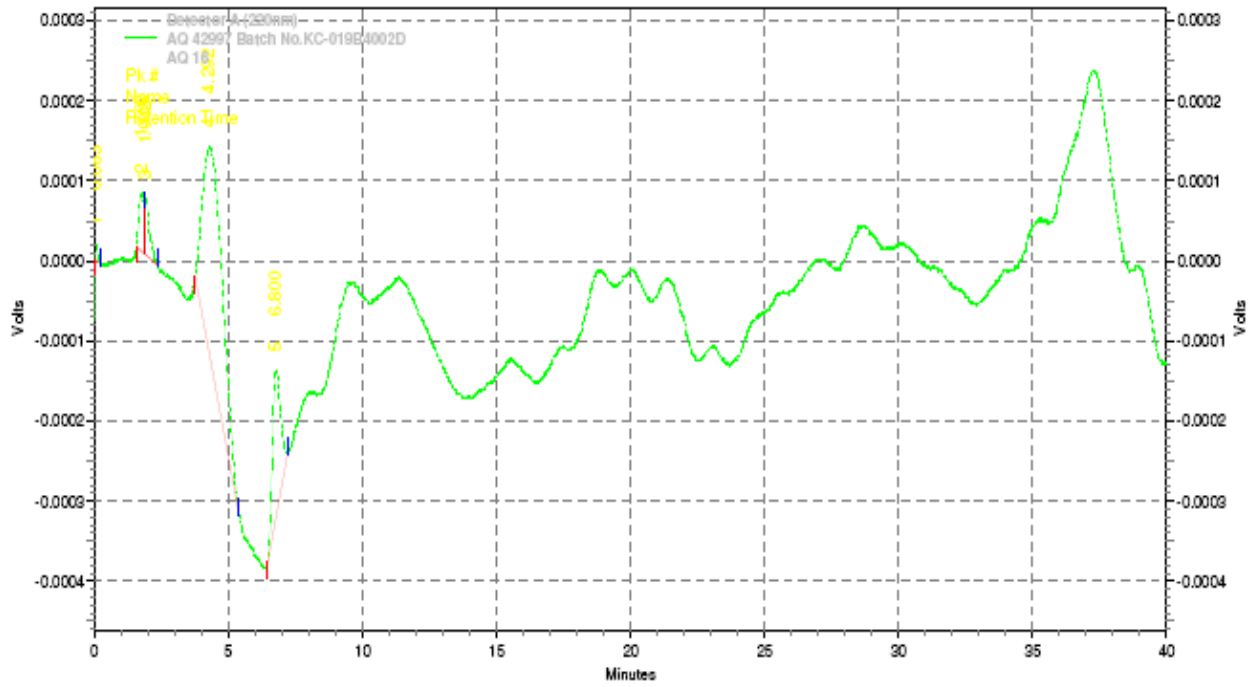

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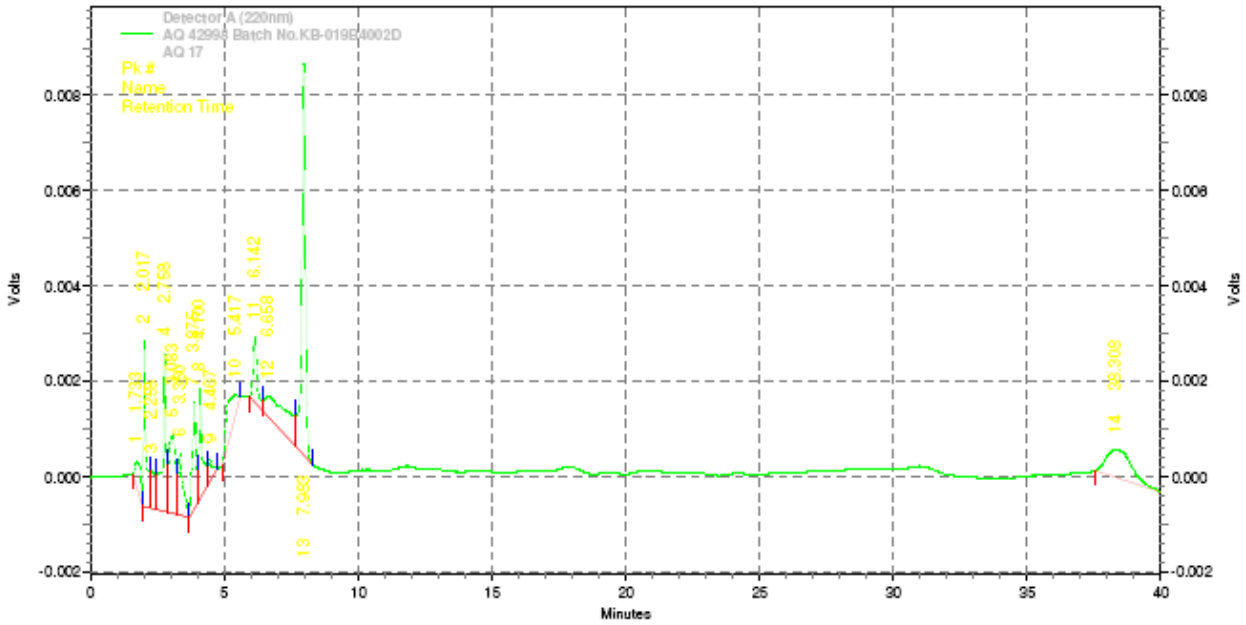


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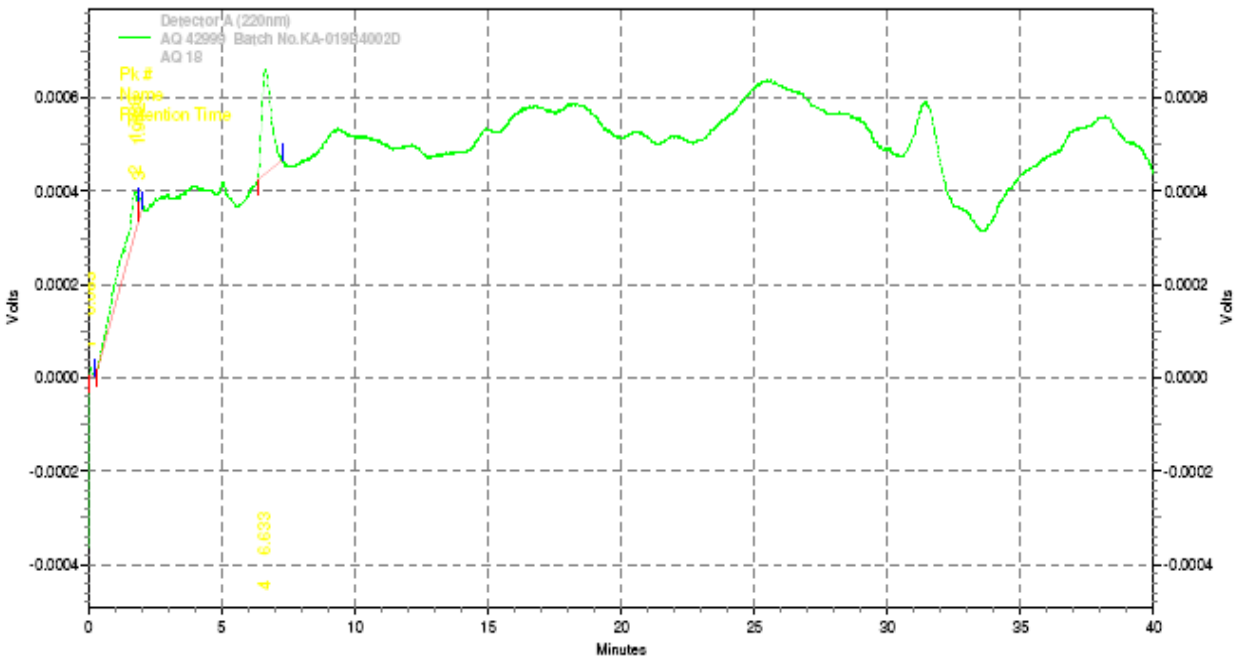

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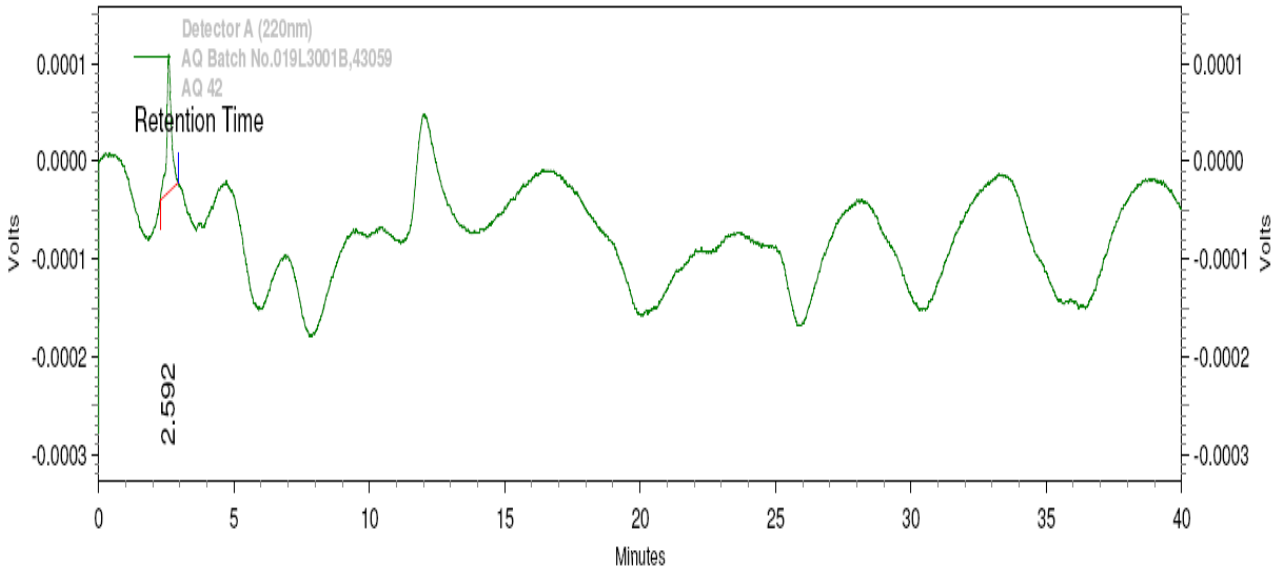


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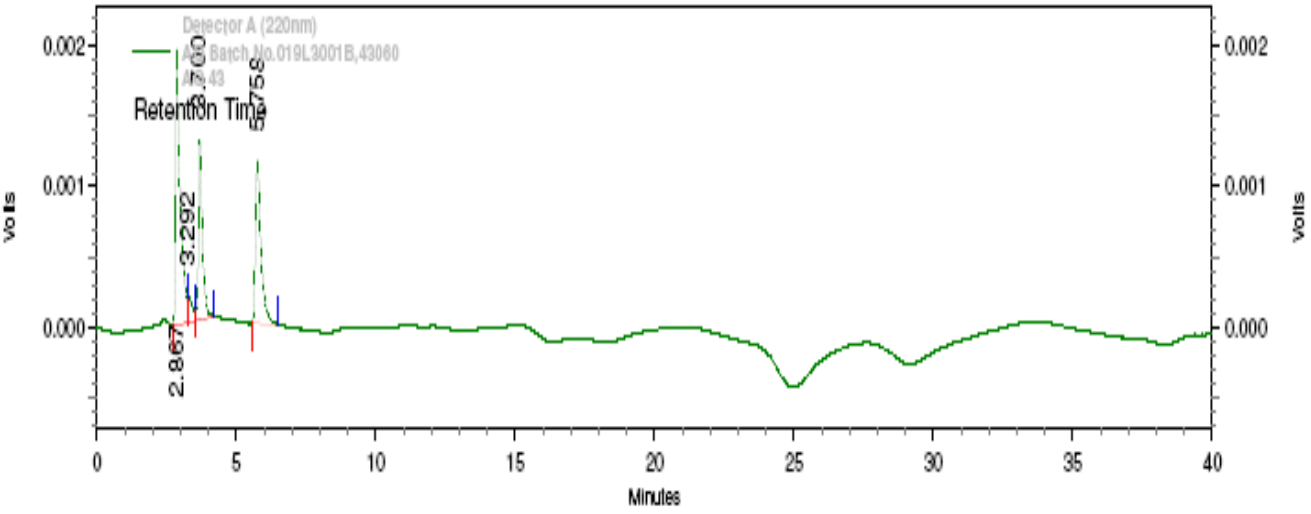

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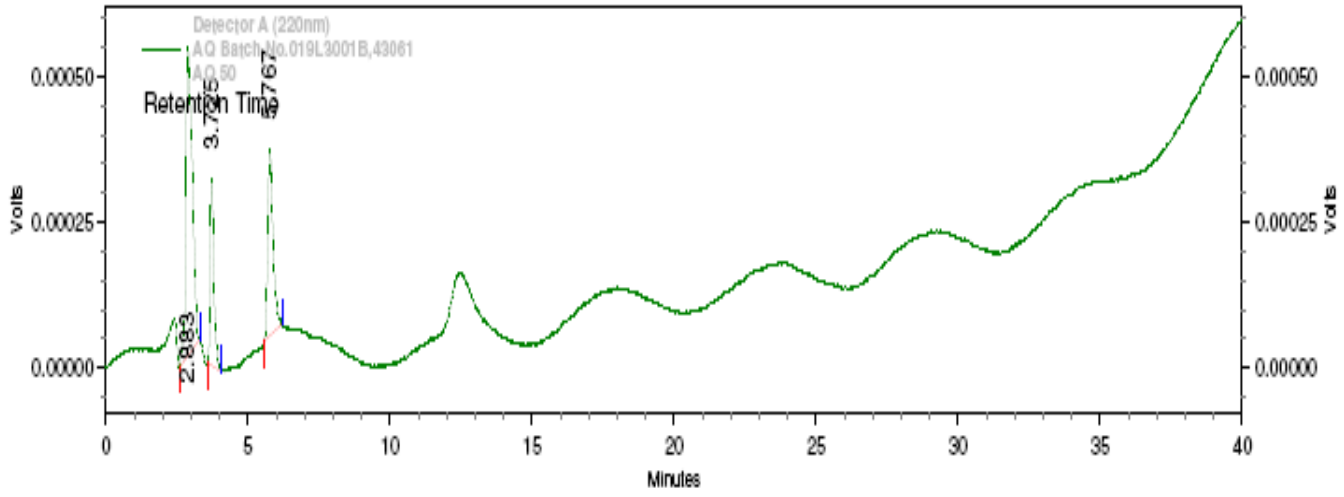


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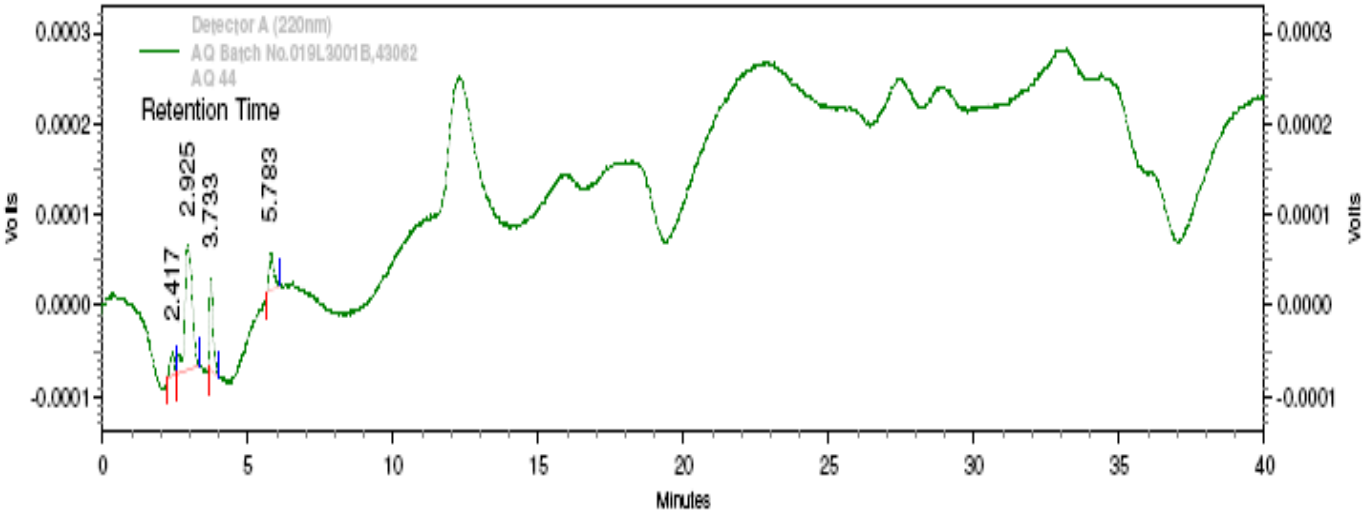

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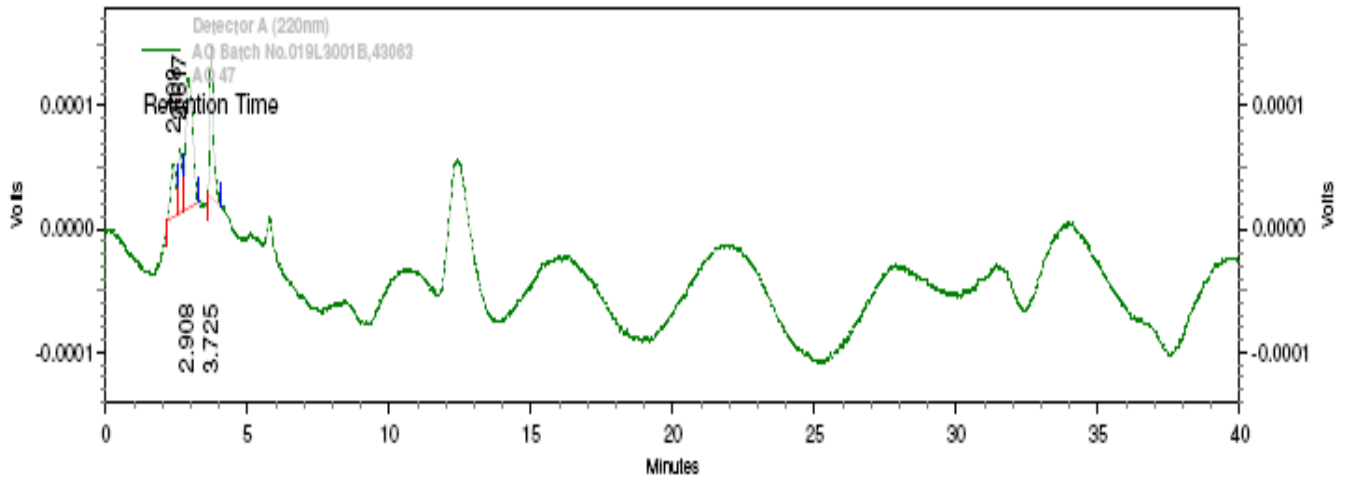


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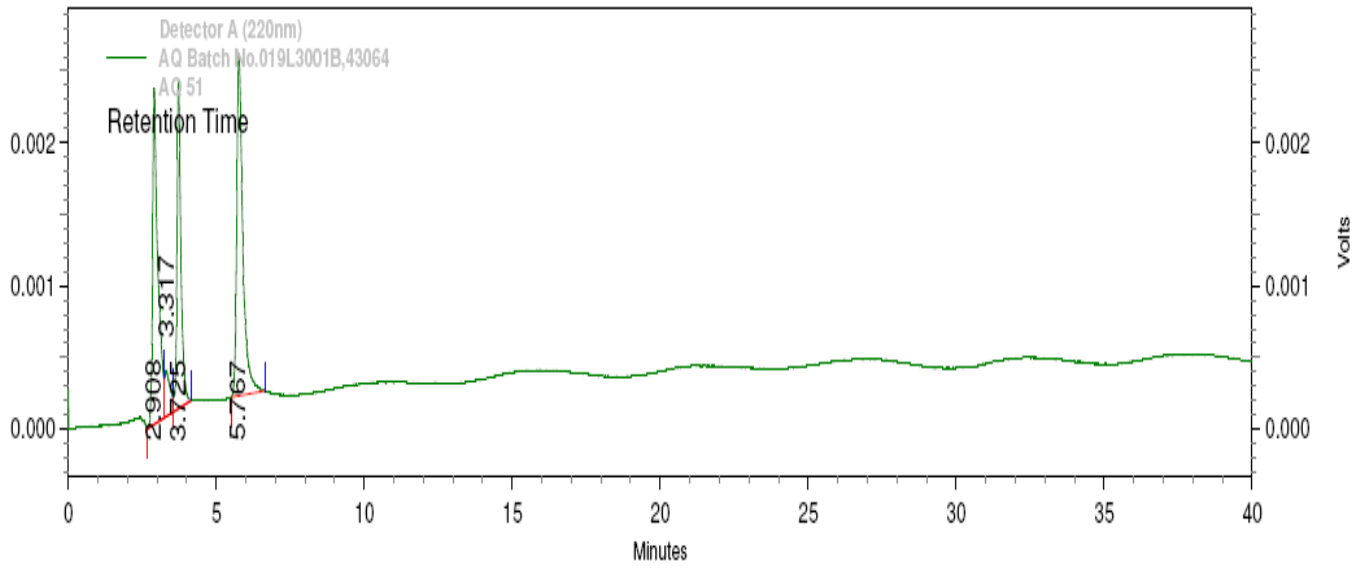

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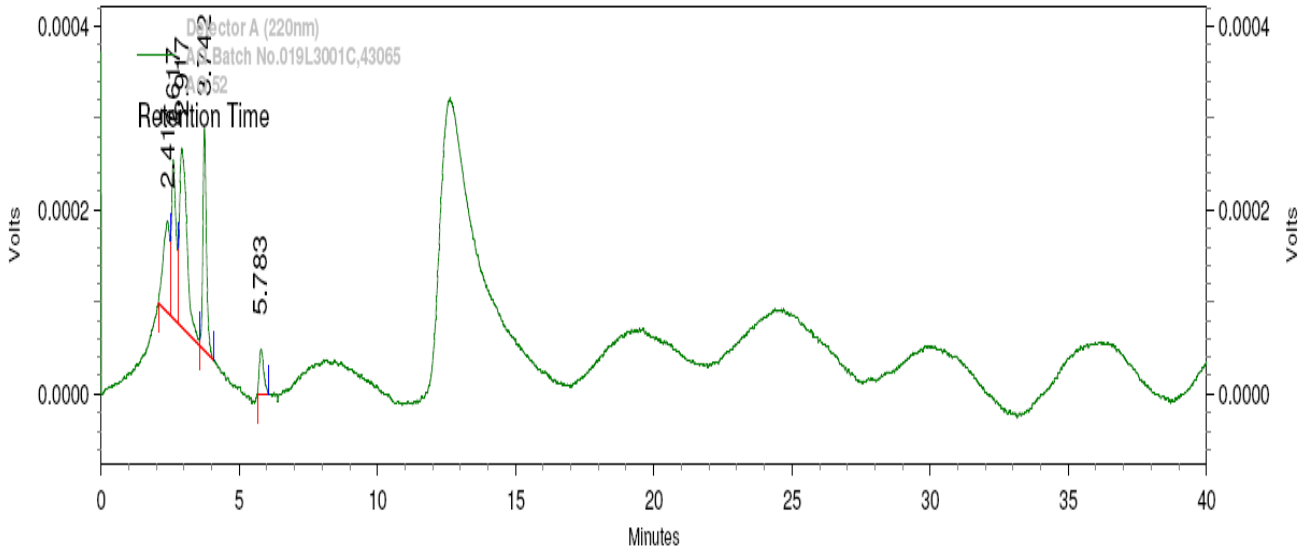


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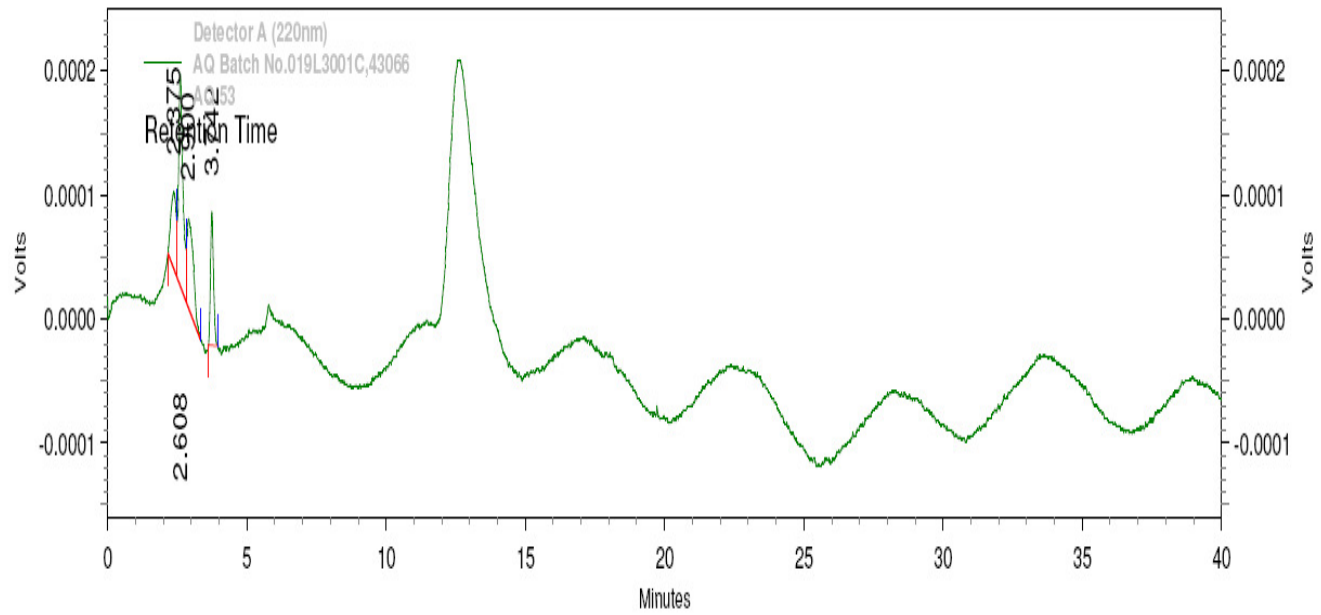

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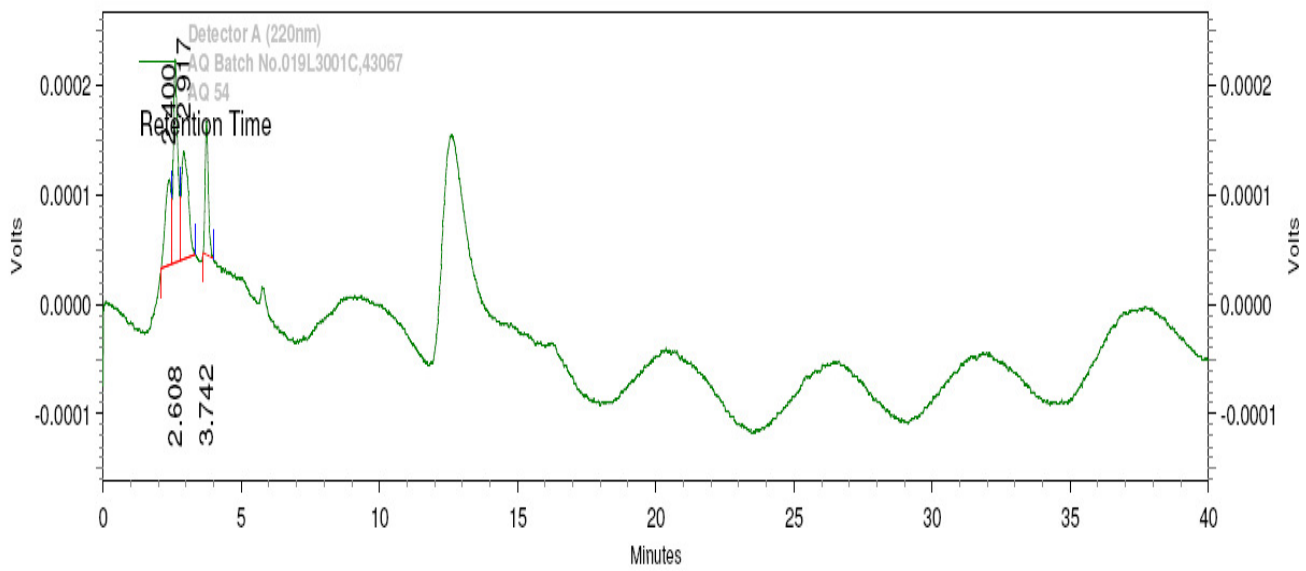


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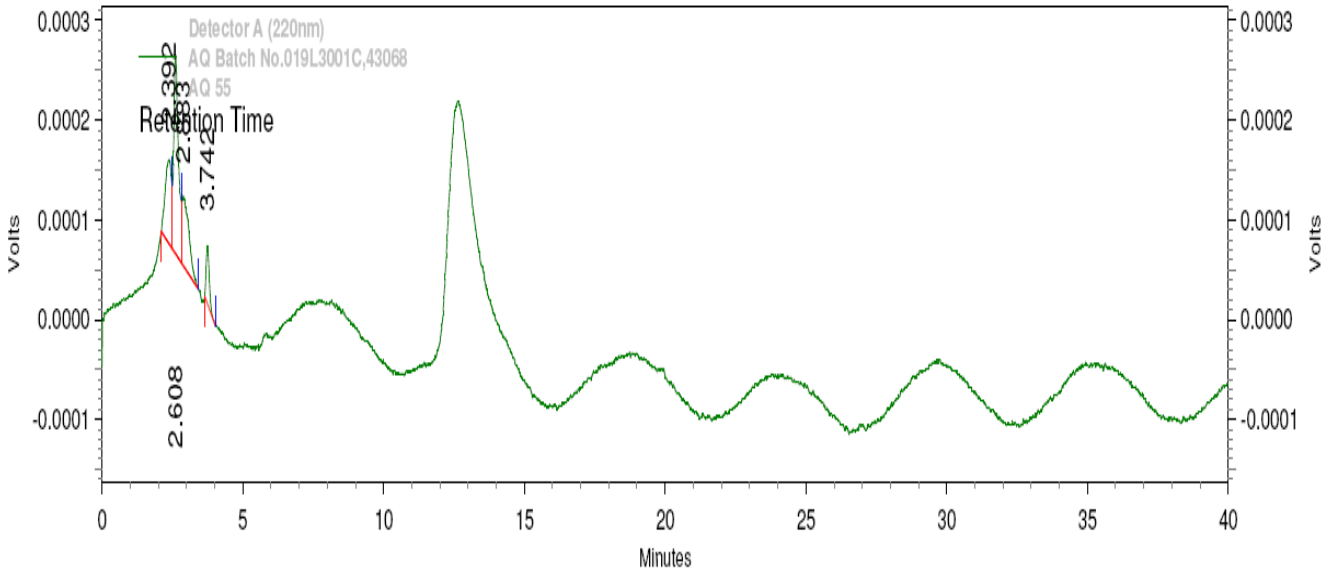

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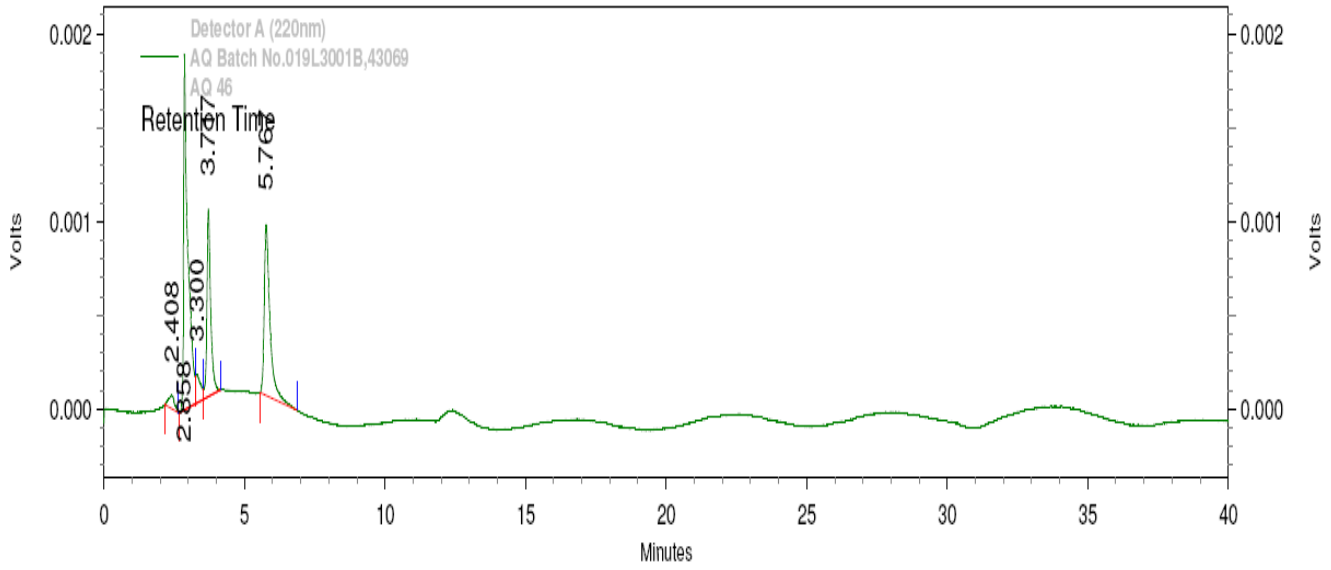


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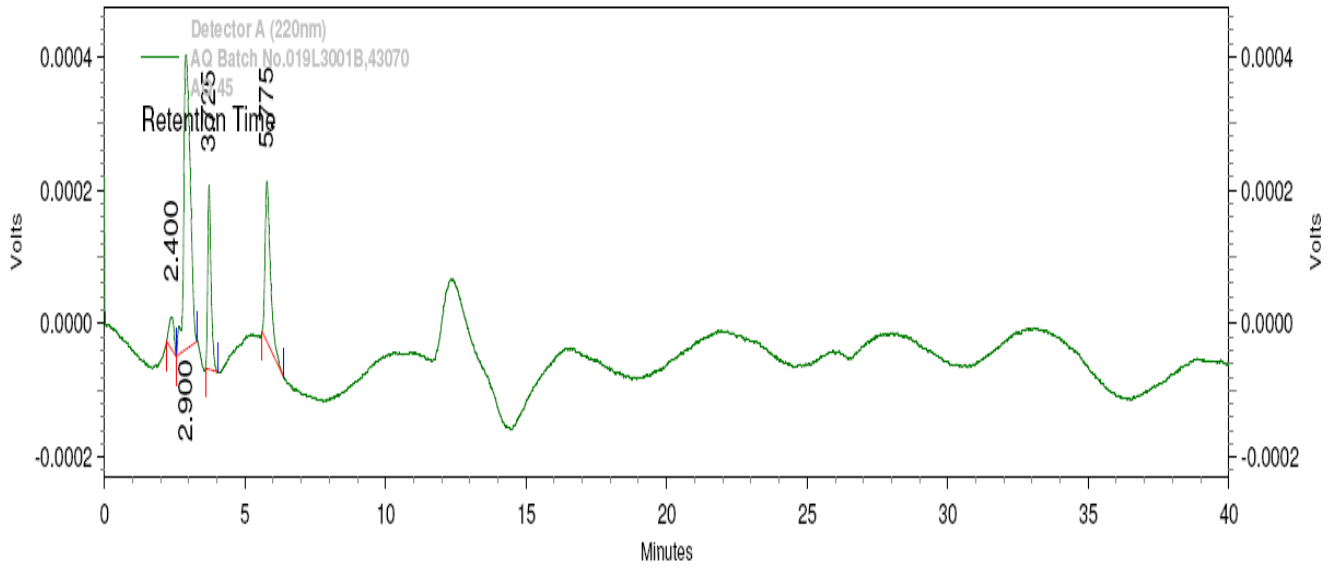

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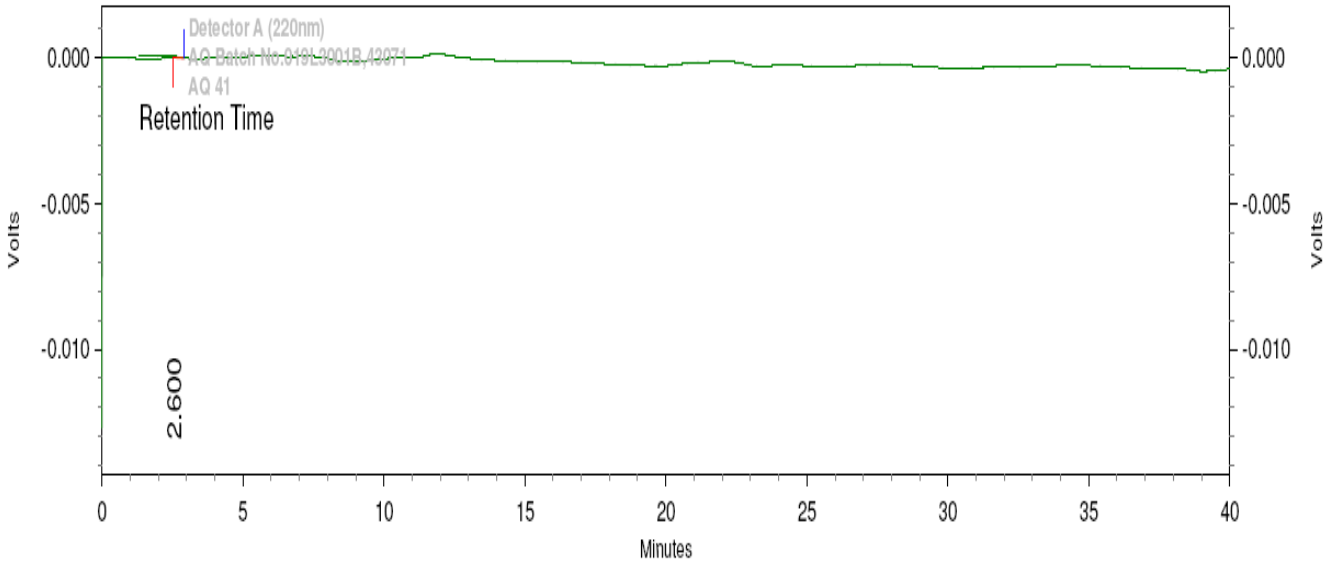


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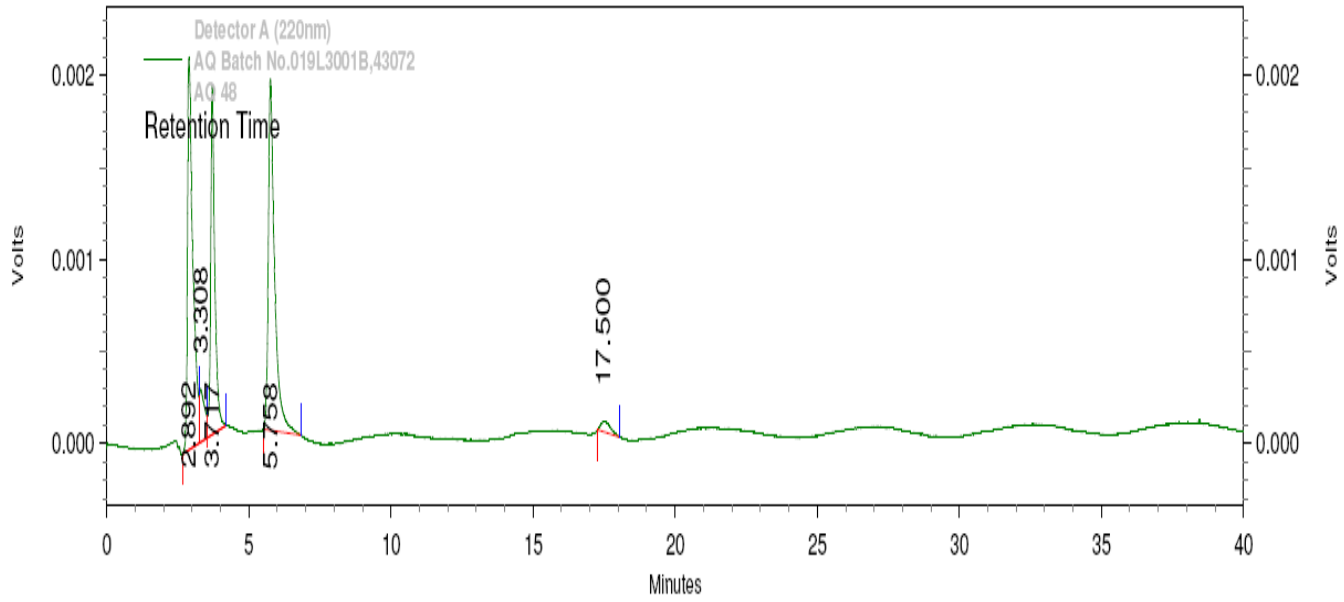

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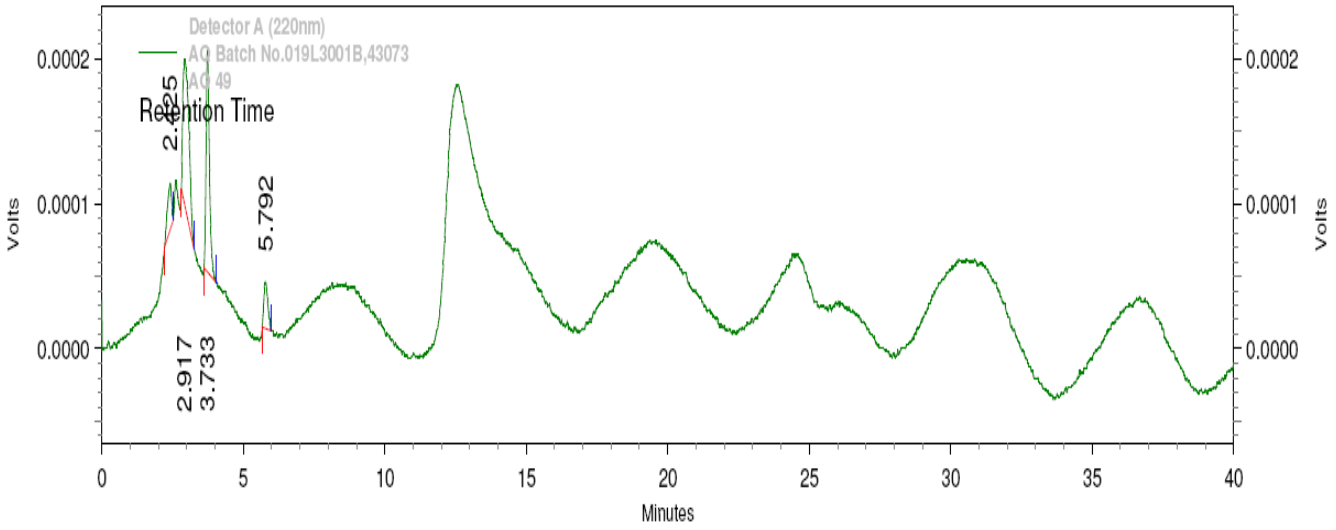


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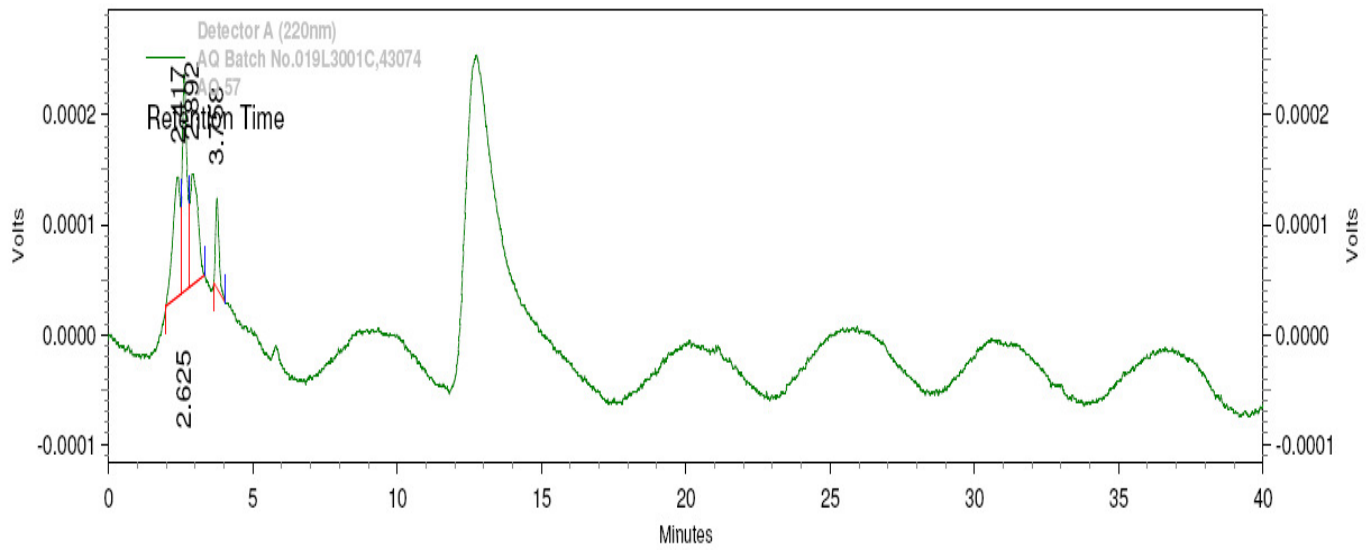

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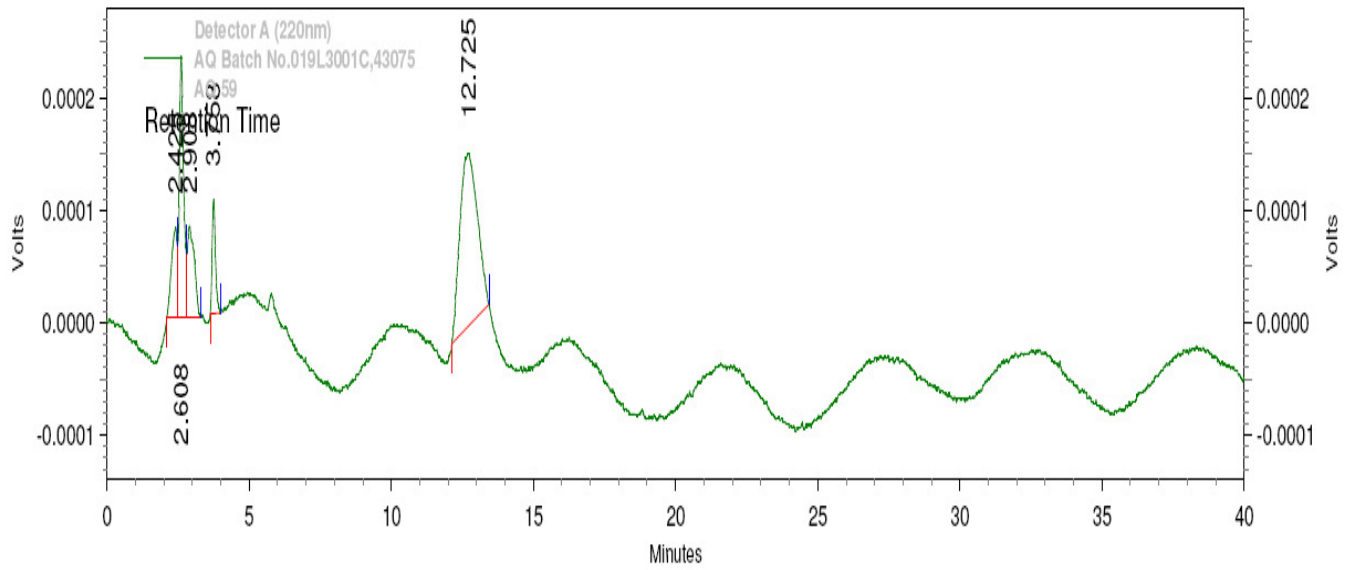


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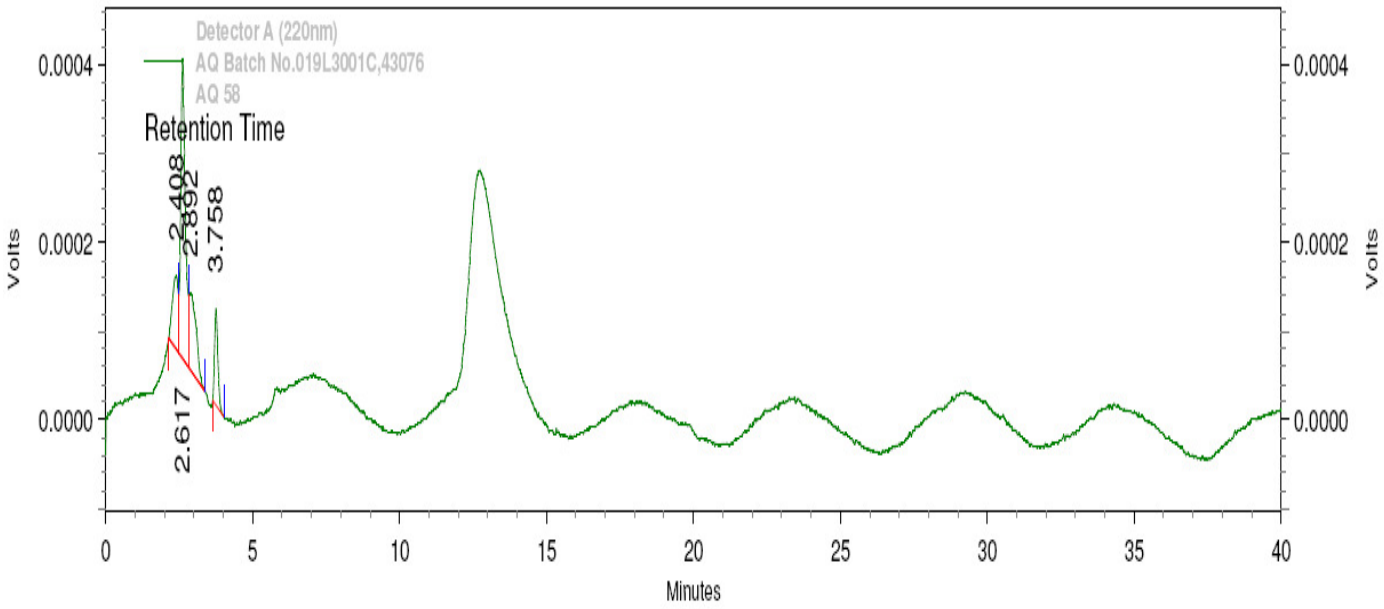

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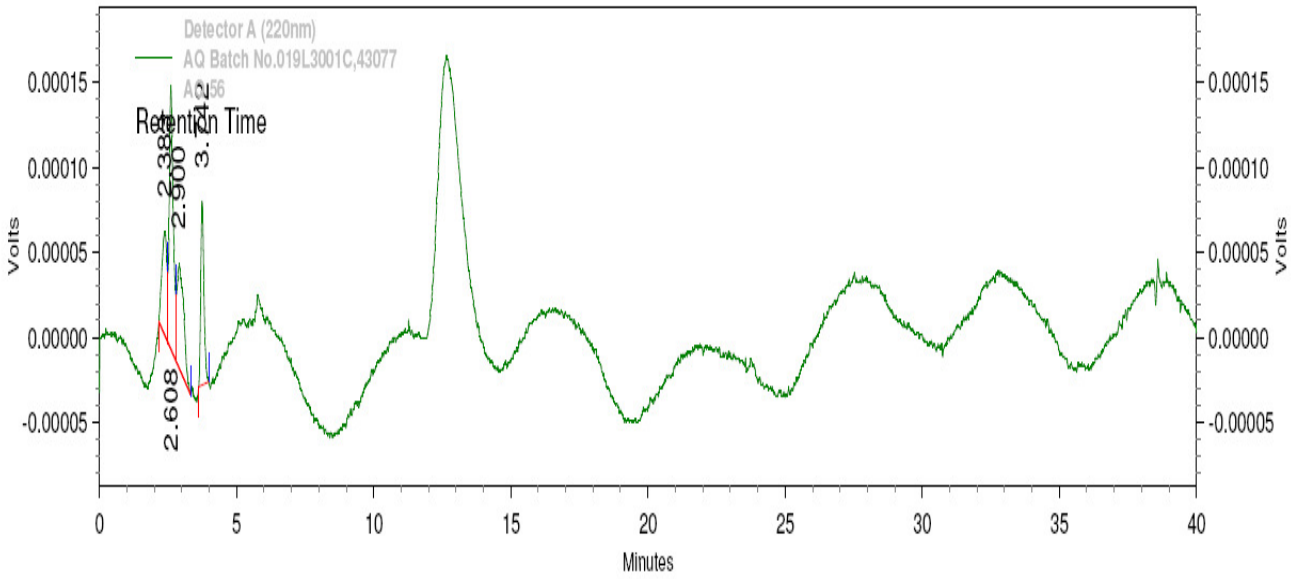


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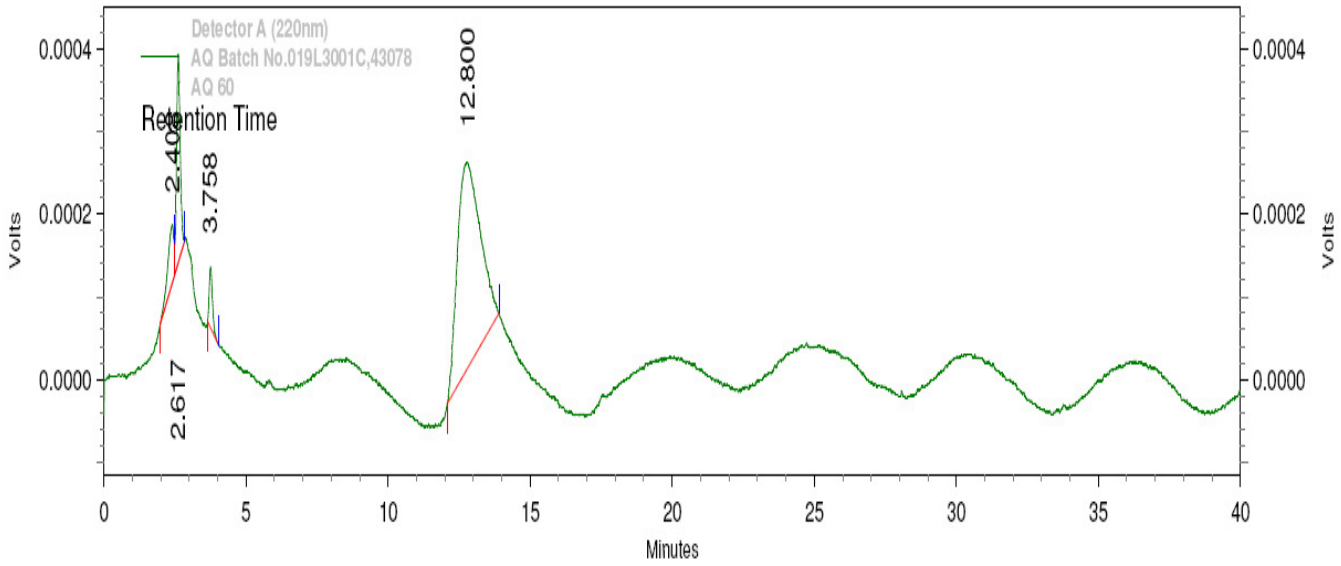


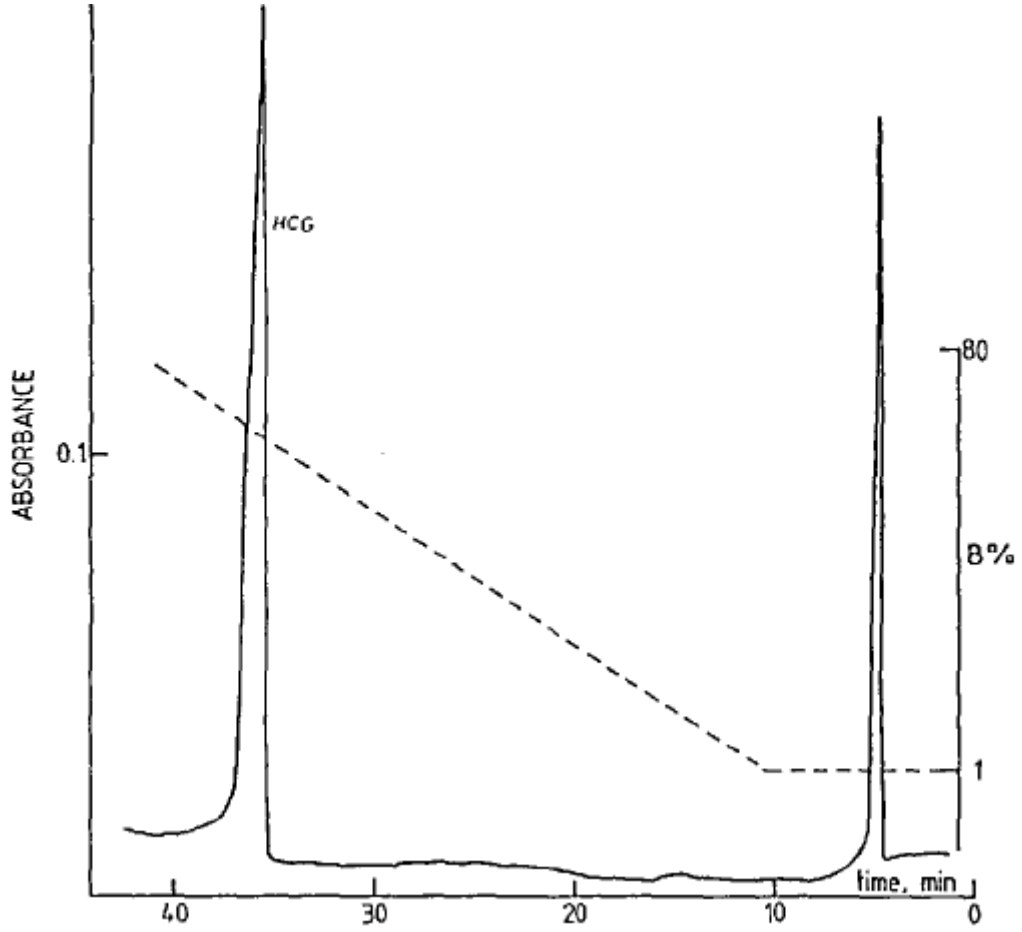
4.2 Summary of Laboratory Results

| Sampling Location | Lab Ref. No | Batch. No | Result |
|---|---|---|---|
| **Lot 1 KEPI Stores** | AQ42905 | 019B4003B | βhCG absent |
| | AQ42906 | 019B4002D | βhCG absent |
| | AQ42907 | 019B4002C | βhCG absent |
| | AQ42908 | 019B4003A | βhCG absent |
| | AQ42909 | 019B4002C | βhCG absent |
| | AQ42910 | 019B4002C | βhCG absent |
| | AQ42911 | 019B4003A | βhCG absent |
| | AQ42912 | 019B4003B | βhCG absent |
| | AQ42913 | 11077A13 | βhCG absent |
| | AQ42914 | 019B4003A | βhCG absent |
| | AQ42915 | 019L3001B | **βhCG present** |
| | AQ42916 | 019L3001B | **βhCG present** |
| | AQ42917 | 019L3001C | **βhCG present** |
| | AQ42918 | 019B3002D | βhCG absent |
| **Lot 2 Upper Hill Medical Center** | AQ42996 | 019B4003A | βhCG absent |
| | AQ42997 | 019B4002D | βhCG absent |
| | AQ42998 | 019B4002D | βhCG absent |
| | AQ42999 | 019B4002D | βhCG absent |
| **Lot 3 Matching Samples** | AQ44000 | 019L3001B | βhCG absent |
| | AQ44001 | 019L3001B | βhCG absent |
| | AQ44002 | 019L3001B | βhCG absent |
| | AQ44003 | 019L3001B | βhCG absent |
| | AQ44004 | 019L3001B | βhCG absent |
| | AQ44005 | 019L3001B | βhCG absent |
| | AQ44006 | 019L3001B | βhCG absent |
| | AQ44007 | 019L3001B | βhCG absent |
| | AQ44008 | 019L3001B | βhCG absent |
| | AQ44009 | 019L3001B | βhCG absent |
| | AQ44010 | 019L3001B | βhCG absent |
| | AQ44011 | 019L3001B | βhCG absent |
| | AQ44012 | 019L3001B | βhCG absent |
| | AQ44013 | 019L3001B | βhCG absent |
| | AQ44014 | 019L3001B | βhCG absent |
| | AQ44015 | 019L3001B | βhCG absent |

| Sampling Location | Lab Ref. No | Batch. No | Result |
|---|---|---|---|
| **Lot 3 Matching Samples** | AQ44016 | 019L3001B | βhCG absent |
| | AQ44017 | 019L3001B | βhCG absent |
| | AQ44018 | 019L3001B | βhCG absent |
| | AQ44019 | 019L3001B | βhCG absent |
| | AQ44020 | 019L3001C | βhCG absent |
| | AQ44021 | 019L3001C | βhCG absent |
| | AQ44022 | 019L3001C | βhCG absent |
| | AQ44023 | 019L3001C | βhCG absent |
| | AQ44024 | 019L3001C | βhCG absent |
| | AQ44025 | 019L3001C | βhCG absent |
| | AQ44026 | 019L3001C | βhCG absent |
| | AQ44027 | 019L3001C | βhCG absent |
| | AQ44028 | 019L3001C | βhCG absent |
| | AQ44029 | 019L3001C | βhCG absent |
| | AQ44030 | 019L3001C | βhCG absent |
| | AQ44031 | 019L3001C | βhCG absent |
| | AQ44032 | 019L3001C | βhCG absent |
| | AQ44033 | 019L3001C | βhCG absent |
| | AQ44034 | 019L3001C | βhCG absent |
| | AQ44035 | 019L3001C | βhCG absent |
| | AQ44036 | 019L3001C | βhCG absent |
| | AQ44037 | 019L3001C | βhCG absent |
| | AQ44038 | 019L3001C | βhCG absent |
| | AQ44039 | 019L3001C | βhCG absent |

## 5.0 INTERPRETATION OF LABORATORY ANALYTICAL RESULTS

Following the Laboratory assessment, three of the analysed vaccine samples submitted for analysis were found to contain human chorionic gonadotropin (HCG) hormone.




Standard chromatogram showing HCG presence after 35 mins

## List of References

Purification of Human Chorionic Gonadotropin Hormone by Anion Excahnge High performance Liquid Chromatography,

World Health organization (WHO) Technical Report Series, No. 800, Annex 2 of WHO Technical Report Series, No. 927 Recommendations to assure the Quality, Safety and Efficacy of tetanus vaccines (adsorbed)

Pharmacophore 2011 vol. 2 (3) 186 -194, Qualitative and Quantitative Analysis of Various Constituents of Vaccines using Analytical Techniques

## Appendices

### Sampling Photos

Vaccine storage at KEPI

Vaccine sampling at KEPI


Taking vaccine aliquots at KEPI

Vaccine sampling at UHMC