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Breath and Urine Alcohol Level Analysis to Increase Student Awareness of Road Safety.

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Breath and Urine Alcohol Level Analysis to Increase Student Awareness of Road Safety (in collaboration with the Garda Road Safety Unit)

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February 2013

Thesis submitted in Partial Fulfilment of

Examination Requirements Leading to the Award

BSc (Forensic and Environmental Analysis)

Dublin Institute of Technology

Thesis Supervisor:

Dr. Claire McDonnell

Statement of Originality

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Name:

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Table of Contents

Abbreviations

BAC- Blood alcohol concentration

CRA- Chemical risk assessment

DIT- Dublin Institute of Technology

EU- European Union

GC- Gas chromatography

IR- Infrared radiation

MBRS- Medical Bureau of Road Safety

PSV- Personal service vehicle

RSA- Road Safety Authority

UAC- Urine alcohol concentration

Abstract

The aim of this project was to increase student's awareness of road safety and the relationship between alcohol and driving. This was carried out in conjunction with the Garda Road Safety Unit and the DIT interdisciplinary project known as CARS, College Awareness of Road Safety. This project was carried out last year by another student therefore the aim this year was to build and expand on the work that had been done so far. Increasing the student's awareness was achieved by speaking to class groups to explain the project and asking them to take part by supplying samples of breath and/or urine the morning after they had consumed alcohol. This was intended to demonstrate to the students that it is possible to be still over the limit the day after they have been drinking. To increase the student's awareness of the rules of the road, they were asked to take part in a survey and feedback was supplied on their answers both on the day of the student breathalyser event in DIT Kevin Street and by placing the results as well as the correct answers and links to road safety videos on the DIT website.

The basis of the work done throughout the project was to analyse urine samples supplied by student volunteers by gas chromatography the day after they had consumed alcohol. The results of these analyses were then compared to the new legal limits that were put in place in 2011. All analyses carried out were performed anonymously however, if requested by the volunteer, feedback was provided on the amount of alcohol that had been detected in their system at the time of giving the sample. Urine samples supplied from members aged 18 to 22 from a local football club, Greenhills Football club on a Sunday morning, were also analysed as a result of a recent Garda press release which stated that a new feature of the implementation of the lower limits was an increase in the number of people detected driving the morning after drinking particularly on Sundays around 11am.

In order to analyse the samples supplied by volunteers, ethanol working standards were prepared with the use of a propan-1-ol internal standard and run on the GC to establish a standard calibration curve. This curve was run once a week and the equation of the line obtained was used to calculate the ethanol content of any urine samples analysed that week. In previous years problems had been encountered with the lower concentration ethanol standards however accuracy, precision, linearity and reproducibility were demonstrated throughout the analysis therefore validating the method for these parameters. The coefficient of determination R^2, values obtained for the calibration of ethanol standards were 1.000, 0.9994 and 0.9997 respectively. In order to ensure the GC was fit for use each

morning, Diasys® check standards and standards provided by the Medical Bureau of Road Safety were analysed.

A total of 19 urine samples were analysed, 13 of these were supplied by student volunteers and 6 were supplied by Greenhills Football club. All samples supplied by Greenhills Football club were negative for alcohol suggesting that a recent Garda press release and campaign to target this sex and age group may have had an impact. Of the 13 samples supplied by student volunteers, 5 samples were over the limit for both a specified and full licence driver, 2 samples were over the limit for a specified driver but under the limit for a full licence driver and 1 sample contained alcohol but was under the limit for both a specified and full licence driver. The remaining 5 samples did not contain any alcohol. The legal limits for alcohol in urine can be seen in Chapter 1 Table 1.1.

Breath samples supplied by student volunteers were also analysed one morning in DIT Kevin Street using a commercially available breathalyser. A total of 48 student volunteers were breathalysed. Of these 48, 44 student volunteers were negative for alcohol however 4 student volunteers gave positive readings for alcohol. Three of these four volunteers were over the limit for a specified driver but under the limit for a full licence driver and one volunteer had alcohol in their system but was under the limit for both a specified driver and full licence driver. The legal limits for alcohol in breath, urine and blood can be seen in Chapter 1 Table 1.1. During this breath testing event, students were asked to complete a survey which questioned their knowledge of the new legal limits and the units in certain drinks. An online version of this survey was also generated and the survey was also provided in the urine sample packs. Overall, 98 surveys were completed which demonstrates that awareness was raised. Analysis of the surveys demonstrated that students were unsure of the amount of units contained in a bottle of wine and a pint of beer. With regards to the question about the legal limits for specified and full licence drivers, 29% of students answered correctly in both cases. 65% of students also agreed that more could be done to educate students on the relationship between drink and road safety.

1. Introduction

1.1.Background

This project was carried out in conjunction with the Garda Síochána Road Safety Unit and aimed to increase student's awareness of road safety and the relationship between alcohol and driving. This was achieved by speaking to class groups about the project and analysing breath and urine samples provided by volunteer students as well as asking students to complete a survey. Analysis of breath and urine samples supplied by student volunteers aimed to highlight the fact that it is possible to be over the limit the day after consuming alcohol. The survey was designed to test the students knowledge of current road safety laws and legal limits due to the introduction of the new lower legal limits in 2011. Under Sections 4 and 5 of the Road Traffic Act, the legal limit for drivers who have held a full driving licence for more than 2 years is now 50 mg of alcohol per 100 mL of blood, 22 mg of alcohol per 100mL of breath or 67 mg of alcohol per 100mL of urine. For specified drivers the new limits are 20 mg of alcohol per 100 mL of blood, 9 mg of alcohol per 100 mL of breath or 27mg of alcohol per 100 mL of urine. Specified drivers include those who are in possession of a learners permit, an individual that does not possess a licence, a disqualified driver, full licence drivers who have not held their licence for more than 2 years, PSV drivers, taxi drivers or drivers with a licence in class C, D, EB, EC, ED or W. Table 1.1 summarises the limits for drivers under the Road Safety Act 2010.[1]

Sample	Legal Limit for Full licence Drivers who have held their licence for more than 2 years (mg/100mL)	Legal Limit for Specified Drivers (mg/100mL)
Blood	50	20
Breath	22	9
Urine	67	27

Table 1.1 Legal alcohol limits for drivers as specified in Road Safety Act 2010[1]

Any amount of alcohol is said to impair driving and increase the risk of crashing. Those driving at the current legal limit of 50 mg of alcohol per 100 mL of blood are 6 times more likely to cause a collision and alcohol is estimated to contribute to 1 in 3 fatal crashes.[2] In 2006, 18,795 blood, urine and breath samples were analysed by the MBRS. Of these specimens, 2,167 were twice or more over the legal limit which at the time was 80 mg of alcohol per 100 mL of blood. In introducing these new limits Ireland is now on a par with most EU countries where research has shown that decreasing the legal limits has decreased the amount of deaths caused by drink driving.[2]

1.2 Alcohol

Alcohol is one of the most commonly consumed drugs. It is added to beverages in the form of ethanol also known as ethyl alcohol and is produced by a fermentation process in which yeast cells act on sugar in fruits or carbohydrates in grains and vegetables. Ethanol is a straight chain polar molecule therefore it is water soluble. Ethanol has the molecular formula C_2H_5OH and its chemical structure is shown in Figure 1.1. It is added to beverages in varying concentrations depending on the type of alcoholic drink being produced. Ethanol is a central nervous system (CNS) depressant which is directly related to a person's blood alcohol concentration (BAC). As BAC increases, impairment increases and decision making and motor function are affected. The BAC can be affected by food consumption, weight and gender. The BAC decreases as ethanol is eliminated from the body by the liver.[3]

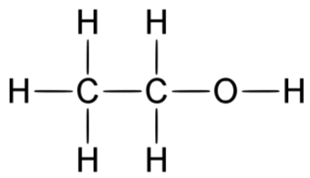

Figure 1.1: Chemical structure of ethanol

1.2.1 Alcohol in the body

Alcohol enters the body through ingestion. It is absorbed through the walls of the stomach and small intestine where it enters the bloodstream and is distributed to all body parts. Alcohol concentration is usually higher in parts of the body that have a high water content. As more alcohol is consumed, a maximum alcohol level is eventually reached and post absorption begins. Post absorption refers to the period where the alcohol concentration slowly decreases until it reaches zero. Alcohol is eliminated by oxidation and excretion. The majority of alcohol is oxidised in the liver to form carbon dioxide and water. Firstly the enzyme alcohol dehydrogenase converts it to ethanal also called acetaldehyde and then to acetic acid also called ethanoic acid which then forms carbon dioxide and water. The remaining alcohol in the body is excreted in the breath, urine and perspiration.[4] The metabolism of alcohol can be seen in Figure 1.2.[4]

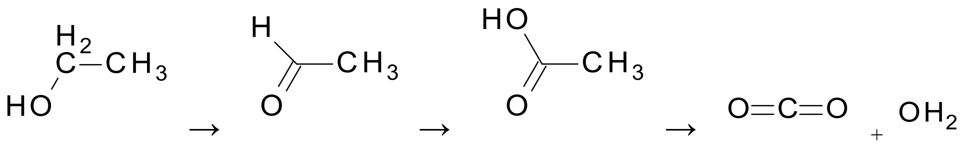

Figure 1.2: Metabolism of Alcohol[4]

Depending on the amount of alcohol consumed by an individual as well as their gender, height and weight different effects can occur. Table 1.2 summarises the effects caused by varying % BAC.[5]

% BAC	mg /100mL blood	Effects
0.02 – 0.03	20-30	Some individuals may experience impairment and slight euphoria
0.04 – 0.06	40-60	Inhibitions may be lowered, individual may begin to feel unwell and minor impairments can occur
0.07 – 0.09	70-90	Impairments of balance, speech and vision can occur. Reaction time and hearing are affected and self control is reduced
0.10 – 0.12	100-120	Motor co-ordination is impaired, balance, vision, speech and reaction time are further impaired
0.13 – 0.15	130-150	Loss of physical control and balance occurs, vision may become blurred. Euphoria reduces and *dysphoria begins
0.16 – 0.20	160-200	*Dysphoria predominates and nausea may occur
0.25	250	*Dysphoria continues and mental confusion can occur
0.30	300	Loss of consciousness
0.40 and upwards	400	Onset of coma and possible death

Table 1.2 Summary of the effects of alcohol on the body[5]

*Dysphoria refers to a state of feeling unwell, anxious and depressed.

1.3 Effect of Alcohol on Driving

The consumption of alcohol even in very low volumes is known to affect driving ability due to the impairment of perception, reaction time and the ability to focus on more than one task at a time. Inability to control speed and decreased hazard perception are also likely to occur.[6] As the amount of alcohol in the system increases, these impairments increase and it takes a person longer to react in an emergency. It was noted as early as 1965 by the British Medical Association that driving with an alcohol level of 60 mg per

100 mL of blood doubles the potential of having an accident while having a level of 150 mg increases the potential by a factor of 25.[7]

1.3.1 Effect of drugs and alcohol on driving

Recently, investigations have been conducted into the effect of drink driving combined with the use of drugs. A study from 2013 documented in the Accident and Analysis Prevention Journal investigated the effects of two of the most commonly used drugs, alcohol and cannabis. The study comprised of 80 individuals, 31 of whom were female while the remaining 49 were male. These participants included regular and non- regular cannabis users. Alcohol was administered using a weight related dose method while cannabis cigarettes were administered using a controlled smoking procedure. A driving simulator known as the CyberCAR LITE driver training and evaluation simulator was used and participants observed a computer generated driving scene that was two dimensional which tested both day time and night time driving. Driving impairment and signal impairment scores were given to each participant depending on their driving. The results of this study showed that regular cannabis users showed significantly more signalling errors than non regular cannabis users. It was also shown that participants that had been administered cannabis combined with alcohol performed the worst with regards to signalling. The same affects were seen for participants who had low and high levels of cannabis combined with alcohol. This demonstrated that even a small amount of cannabis combined with alcohol can cause the participants driving performance to decrease. Therefore the study proved that consumption of alcohol combined with cannabis use significantly affected the ability of the participants to drive and signal particularly at night time.[6]

1.3.2 Effect of tiredness on driving

Tiredness has also been shown to effect driving specifically with regards to reaction time. A study carried out in 1996 documented in the Accident and Analysis Prevention Journal investigated the effect tiredness had on young males when driving between the hours of midnight and 6 a.m. The reaction time of 123 impaired drivers with an average BAC of 1.54 g/L and 240 sober drivers were tested. Levels of tiredness in the impaired drivers varied between tired and very tired. The results of this study showed that tiredness was a risk factor due to the effect it had on reaction times as impaired drivers performed significantly worse than sober well rested drivers.[8] A similar study carried out in 1993 investigated tiredness and reaction time among night time taxi drivers. This was a roadside survey carried out on a major highway in Copenhagen. The level of tiredness was selfassessed. 80 drivers declared themselves rested, 38 said they were tired while 2 said they were very tired. The results showed that tiredness had an effect on reaction time and also that some drivers self-assessment of their level of tiredness was wrong and they were more tired than they believed to be. These studies demonstrate the need for drivers to be more vigilant with regards to the amount of sleep they have before operating a vehicle.[9]

1.4 Detection of Alcohol in the Body

The most accurate way to determine the effect of alcohol on the body would be to obtain a sample of blood from an individual's brain. Due to the obvious safety issues with this procedure, scientists adopted the testing of an individual's breath in order to determine the BAC. Blood can also be taken from the individuals arm in order to determine the BAC. Although the BAC estimated from a person's breath and that measured directly from their blood differ the analysis of alcohol by breath is considered to be accurate.[10] However there are some limitations to be considered when obtaining a breath sample from an individual. One of these limitations is the false positive that can be given by the breathalyser for an individual who has Type 1 Diabetes. This false positive is given due to an increase in ketone bodies, namely acetone. High levels of acetone can occur due to diets low in carbohydrates or poorly treated Type 1 diabetic patients. In a study carried out in Jamaica by the Department of Paraclinical Sciences, 73.6% of respondents with Type 1 diabetes were breathalysed and shown to have a wobbly disposition. The results also showed a correlation between respondents with an unstable equilibrium and the period between their last meal. Therefore this needs to be considered when carrying out breath analysis.[11] BAC can also be determined by analysing a blood or urine sample from the body. In cases where blood or urine is unavailable, a water rich organ or fluid can be used to determine the ethanol concentration, for example the cerebrospinal fluid or brain fluid. [4]

1.4.1 Analysis of blood/urine for alcohol

BAC is tested more commonly than UAC. This is due to the fact that urine samples are less capable of providing reliable figures in comparison to blood samples. The errors associated with urine samples are due to the delay in elimination of alcohol body fluids into the bladder. If the person arrested on suspicion of drink driving has not recently deposited urine their sample may be partly full of alcohol free urine. In America this problem is overcome by discarding the first sample as it may not be representative of the actual BAC. A second sample is usually taken a half hour after the first sample and is more representative of the BAC. This is due to the fact that the second sample has been freshly eliminated.[12] In Ireland, only one urine sample is obtained and this is divided into two parts, one to be given to the suspect while the second part is sent to the MBRS for analysis.[13] Due to these errors which have been identified with the analysis of urine samples, it is much more common for blood samples to be analysed. Blood samples must be taken by a medical examiner. Once blood and urine samples have been provided they must be stored in the refrigerator until analysis is to take place.[12] BAC and UAC (Urine alcohol concentration) are measured using gas chromatography (GC). GC is based on separation of the components of a mixture. The sample is firstly vaporised and carried to the column by an inert flow gas which acts as the mobile phase. The inert flow gas is usually a mixture of two gases, the most common being helium and nitrogen. The column of the GC is coated in a thin layer of material providing the stationary phase. The polarity of the stationary phase determines the retention time of the components in the mixture. The greater the polarity of the substance passing through the column the more the substance is retained. The carrier gas carries the substances out of the column where they are sensed by a flame ionisation detector which is converted into an electrical signal.[14]

In the MBRS a headspace GC is used for the analysis of blood and urine samples.[13] Headspace GC is used when samples contain non volatile components which have the potential to block the injection port of the GC and cause damage to the column. Samples are placed in vials, sealed and heated for a specified amount of time. The volatile components present in the sample partition between the gas and sample phases in the headspace and are injected onto the column of the GC.[15]

1.4.2 Analysis of breath for alcohol

Breath samples can be analysed by the use of portable or stationary breathalysers. Both of these breathalysers measure alveolar breath to determine alcohol content. Alveoli are pear shaped sacs in the respiratory system which are located at the ends of the bronchial tubes. The walls of the alveoli are responsible for the exchange of oxygen and carbon dioxide in the blood. Carbon dioxide is discharged from the blood and oxygen passes through the alveoli walls and into the blood oxygenating it. Any volatile substances present in the blood will also pass into the alveoli during this exchange which allows these volatiles to be detected in alveolar breath.[4] The portable breathalyser which is used by the Garda Síochána is the Draeger model. These types of breathalysers incorporate a fuel cell which produces an electrical signal due to the conversion of a fuel with an oxidant. Alcohol which is the fuel is converted in the fuel cell to ethanoic acid by oxygen which acts as the oxidant. This conversion results in a current which is proportional to the amount of alcohol in the breath sample.[4] A schematic of a fuel cell can be seen in Figure 1.3. These types of instruments are used on the roadside by the Garda Síochána and display a PASS or FAIL reading.[4] The BACTrack breathalyser to be used throughout this experiment is similar to the Garda roadside breathalyser. It incorporates a fuel cell and is portable however this model gives a reading in mg/L, mg/100 mL or % BAC depending on the user's choice.[5]

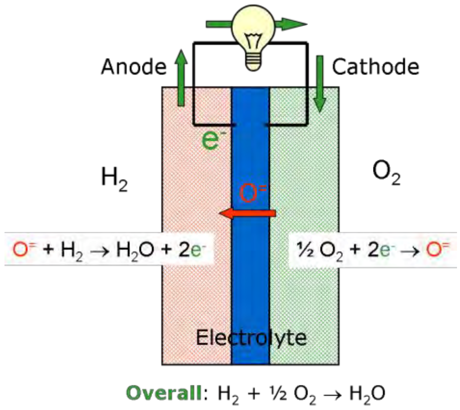

Figure 1.3: Schematic of a fuel cell[16]

Stationary breathalysers are used in the Garda station and are based on Infrared spectroscopy measurements. This involves aiming IR beams at the sample which contains the alcohol in order to measure the alcohol content. The subject blows into the instrument for a period of time which passes the breath sample into the breath chamber. A beam of infrared radiation is aimed at the breath sample where a filter is used to choose a specific wavelength where alcohol absorbs. The interaction of IR light with the alcohol in the breath sample causes a decrease in the intensity of the light which is measured by a photoelectric detector. This detector then produces a signal which is proportional to the amount of alcohol in the breath sample and the percent blood alcohol is given on a printout from the instrument.[4] The stationary breathalyser used by the Garda Síochána is the EvidenzerIRL (See Figure 1.4) and is produced by Lioniser. The final reading given by the EvidenzerIRL is measured against a standard which has ranges of; 7 ug of alcohol per 100 mL of breath, 11 ug of alcohol per 100mL of breath, 20 ug of alcohol per 100 mL of breath and 24 ug of alcohol per 100 mL of breath. Two printouts are generated by the instrument, one of which is given to the subject who provided the breath sample. The instrument is calibrated internally every 1-2 months and externally by the MBRS every 3-6 months.[17]

Figure 1.4: Image of the Evidenzer IRL in Store Street Garda station

1.5 Benefits of Lowering the Legal Limit

As discussed in Section 1.1 (Background) in 2010 Ireland lowered the legal limits for drink driving. This has become an international trend with the majority of countries reducing the legal limit for full licence drivers to 50 mg/100 mL. The effectiveness of this reduction has been investigated in fourteen independent studies in the United States. The results of these studies were documented in the Journal of Safety Research in 2006. It was found that lowering the limit from 100 mg/mL to 80 mg/100 mL had resulted in a 5 -16 % reduction in alcohol related crashes and fatalities in various states in America. Lowering of the limit to 50 mg/100 mL resulted in a further reduction of alcohol related fatalities. The study also showed that there is strong evidence to support lowering the limits to 20 mg/100 mL or lower for youths. The conclusion of the study was that the lowering of the limit had an impact on drivers and the changes in these laws had acted as a deterrent to drink driving.[18]

1.6 Legislation and Penalties

Legislation regarding drink driving can be found in Sections 4 and 5 of The Road Traffic Act 2010. This Act states that

"A person shall not drive or attempt to drive a mechanically propelled vehicle in a public place while he or she is under the influence of an intoxicant to such an extent as to be incapable of having proper control of the vehicle"

The legal limits stated in this Act can be seen in Table 1.1. Table 1.3 summarises the penalties for full licence and specified drivers when they are found to be over the drink driving limits.[1]

	Limit (mg/100 mL)	Fine	Penalty
Blood	50-80	€200	3 penalty points
Urine	67-107		
Breath	22-35		
Blood	80-100	€400	6 months disqualification
Urine	107-135		
Breath	35-44		
For Specified Drivers			
Blood	20-80	€200	3 months disqualification
Urine	27-107		
Breath	9-35		

Table 1.3 Summary of the penalties as stated in the Road Traffic Act[1]

If a driver fails to provide their licence they will be tested at the lower limit, 20 mg, until they can produce their licence.[1]

1.7 Statistics and Recent Developments

In a recent press release the Garda Síochána stated that as of December 13th 2012, 153 people have died on the roads and although this is 22 less than the same date in 2011, more still needs to be done. The Garda Síochána began a Christmas Enforcement Campaign to tackle areas such as drink driving, speeding, non use of seatbelts and use of mobile phones behind the wheel. This campaign also aimed to highlight the changes that had been made to drink driving limits in 2011. As part of this campaign the Garda Síochána carried out a review of the lower drink driving limits for a period of one year between 28th October 2011 and 27th October 2012. In this period 9,771 drink driving incidents were detected for which 1,260 fixed charge notices were given. In examining these incidents it was found that; there was a decline in the number of arrests for all groups except for females age 58-67, a large number of the incidents involved male drivers driving late at night or early on weekends, the highest proportion of excessive BAC levels were associated with offenders aged 38-47 and there was an increase in the number of people testing positive for alcohol the morning after consuming alcohol. The Garda Síochána aim to use these statistics as areas to focus on for campaigns in the near future. [19]

1.8 Focus of the Project

In this project, urine samples will be collected from volunteers the day after consumption of alcohol for analysis by GC. The method to be used is one that has been established in previous years in DIT in which standards are used to establish a calibration curve for quantitation of urine samples. Propan-1-ol is used as an internal standard in order to compensate for any variations in volumes throughout preparation and injection.[14] The results of these samples will then be compared to the new legal limits introduced in 2011. Breath samples will be collected from student volunteers using the BACTrack breathalyser which incorporates a fuel cell as discussed in section 1.4.2. Analysis of these samples along with breath samples is aimed at increasing awareness of the relationship between road safety and drinking.

1.9 Aims of the Project

The aims of the project were:

- To complete a risk assessment of all chemicals to be used throughout the project.
- To produce calibration curves using prepared standards and improve accuracy and precision in comparison to the project undertaken last year for lower concentration ethanol standards.
- To analyse all urine samples using gas chromatography and apply them to the legal limits set up in 2011.
- To modify the information sheet, consent form and survey that was used for the previous project.
- To prepare sample packs for students containing a sample bottle for depositing urine, an information sheet, consent form and questionnaire and place these packs in a location where students could anonymously obtain them to take part in the project.
- To compare the results from the survey of the previous project to this year's project.
- To design an online survey and circulate it to the students in the School of Chemical and Pharmaceutical Sciences.
- To expand the number of volunteers by speaking to 1st, 2nd and 3rd year science and engineering classes and asking them to participate.
- To contact a local football team to obtain urine samples on the morning of a match to see if any of the players are over the legal drink driving limit.

- To contact Drinkaware and obtain leaflets for distribution to students.
- To carry out breath analysis on students in DIT Kevin Street after a student night out.
- To visit the Medical Bureau of Road Safety to observe their procedures for analysing alcohol in blood, breath and urine.
- To visit Store Street Garda Station to witness the operation of the Evidenzer 600IRL stationary breathalyser.
- To organise a meeting with Garda Derek Cloughley to discuss the aims of the project and his suggestions.

2. Experimental

A Chemical Risk Assessment (CRA) for the use of ethanol, propan-1-ol and sodium fluoride was carried out and signed off before work in the laboratory began. This CRA can be seen in Appendix 14.

These reagents shown in Table 2.1 were used in varying concentrations:

Chemical	Manufacturer	CAS Number	Concentration used	Frequency
Ethanol	Merck	CAS 64-17-5	Absolute diluted to 10 %v/v and 1 %v/v to produce varying concentrations of ethanol standards	Frequent
Propan-1-ol	Romil	CAS 71-23-8	Absolute diluted to 10 %v/v and 1 %v/v to produce 0.3 %v/v	Frequent
Sodium Fluoride	Merck	CAS 7681-49-4	≈ 0.150 g	Occasional

Table 2.1 Manufacturer details, CAS number, concentration and frequency of use for chemicals used.

2.1 Wine Analysis by GC

A range of ethanol standards were prepared and analysed along with a wine sample containing a known percentage of ethanol in order to gain familiarity with the use of the GC and to ensure that the GC system was operating as normal. The procedure for this method is outlined in the 3rd Year School of Chemical and Pharamaceutical Sciences Laboratory Manual.[20]

The reagents used, dilutions and final concentrations are presented in Table 2.2:

mL of Ethanol (12%w/w) (14.7%v/v) added	mL of propan-1-ol (10%v/v) added	Final volume (Deionised water mL)	Final working volume of ethanol standards (%v/v)
1	2	50	0.294
2	2	50	0.588
3	2	50	0.882
4	2	50	1.176

Table 2.2 Volumes of ethanol and propan-1-ol used for preparation of wine standards for GC analysis and their final concentrations

As summarised in Table 2.2, 1, 2, 3 and 4 mL of ethanol were added to 50 mL volumetric flasks along with 2 mL of propan-1-ol which was used as an internal standard. The same amount of internal standard was added to each volumetric flask. These ethanol standards were made up to the mark with varying amounts of deionised water. Wine samples were prepared in triplicate by pipetting 2 mL of the wine sample provided and adding 2 mL of the internal standard propan-1-ol to each 50 mL volumetric flask and making it up to the mark using various amounts of deionised water.

GC Conditions:

GC make and model: Shimadzu GC- 8A

1ul of each ethanol standard and wine sample was injected into the GC port. The following conditions were applied:

Injector/Detector temperature:	150ºC
Column temperature:	80ºC
Carrier gas:	Nitrogen with air and hydrogen flow gas
Detector:	Flame Ionisation Detector
Stop time:	4 minutes
Column:	Packed, 10% Carbowax
Injection:	Splitless

2.2 Preparation of Ethanol and Propan-1-ol Standards for Urine Analysis

The ethanol and propan-1-ol standards were prepared and analysed once weekly to ensure that satisfactory calibration curves were obtained with R^2 values greater than 0.99 and %RSD (Relative standard deviation) less than 5%. (See Section 2.2.1 for the specifications of the method). Ethanol and propan-1-ol standards were prepared as summarised in Tables 2.3 and 2.4.

Concentration of Stock standard (%v/v)	**Volume of stock removed (mL)**	**Diluted with deionised water (mL)**	**Working Concentation (%v/v)**
Absolute	10	100	10
10	10	100	1.0
1.0	6.0	10	0.60
1.0	4.0	10	0.40
1.0	2.0	10	0.20
1.0	5.0	50	0.10
0.10	5.0	10	0.05
0.10	2.0	10	0.02

Table 2.3 Preparation of ethanol standards for urine analysis

Concentration of stock standard (%v/v)	Volume of stock removed (mL)	Diluted with deionised water (mL)	Final Working Concentration (%v/v)
Absolute	10	100	10
10	10	100	1.0
1.0	15	50	0.3

Table 2.4 Preparation of propan-1-ol standards for urine analysis

5ml of each working concentration standard was placed in 10 mL volumetric flasks and 5 mL 0.3% v/v prop-1-anol was used to make the solution up to the mark. Table 2.5 shows the final working concentration of the ethanol standards as % v/v and mg/100 mL. An example of this type of calculation can be seen Results and Discussion 3.7.

Final working concentration (%v/v)	Corresponding final concentration (mg/100 mL)
0.01	7.89
0.025	19.73
0.05	39.45
0.10	78.90
0.20	157.80
0.30	236.70

Table 2.5 Final working concentrations of ethanol standards for urine analysis in % v/v and mg/100 mL

2.2.1 Specifications of the analytical method

The specifications used for the GC analysis of ethanol standards and urine samples were based on the ISO 17025 standard. For the method to fall within specification it was required to demonstrate linearity, accuracy, precision and reproducibility. Linearity was based on the coefficient of determination, R^2, produced by the calibration curve which was required to be greater than 0.99. Accuracy was examined with the use of the Diasys® and MBRS check standards. For these standards to be deemed accurate the % accuracy was required to fall within 90-110%. Precision was determined for both the check standards and the urine samples by calculation of the % RSD (Relative Standard Deviation) which was required to be less than 5%. Reproducibility was also examined based on the repetition of the method over a short period of time.[21]

2.3 Preparation of Diasys® Check Standards

Three check standards supplied by Diasys® Ltd. were prepared the concentrations of which were 100, 200 and 300 mg/100 mL. The ampoules containing the standards were cracked open and poured into small sample bottles (15mL). 0.5 mL of each standard was pipetted into a GC sample vial and 0.5 mL of 0.3% v/v propan-1-ol was added. The solution was shaken to mix it. 1ul of each standard was then injected onto the GC column before analysis of samples to ensure that the system was stable and operating as normal. These standards were prepared once a week and stored in the refrigerator.

2.4 Preparation of European Reference Standards Supplied by MBRS

Five check standards were supplied on the visit to the MBRS, the concentration of which were 19.9, 49.5, 80.0, 106.5 and 199.96 mg/100ml. 5 mL of each of these standards was placed in 10 mL volumetric flasks and made up to the mark with 5 mL of 0.3% v/v propan-1-ol. These standards were prepared once a week and stored in the refrigerator. The Reference Certificates for these standards can be seen in Appendix 15.

2.5 Collection of Urine Samples

Sample packs were prepared for collection by volunteers wishing to take part in the project. Each sample pack contained an information sheet (Appendix 3), a questionnaire (Appendix 1), a consent form (Appendix 2) and a 100 mL plastic sample bottle for collection of the urine sample which was placed in a sealable plastic bag. The information sheet, questionnaire and consent form were designed in the previous year but required some changes. The information sheet and consent form required minor changes such as dates, names and locations. The questionnaire included previous questions such as how many units were in certain beverages, if the student believed they were over the legal limit and if they knew what the legal limits were for drivers. A new true or false question on whether a driver is tested at the 20 mg limit if they do not have a licence with them when stopped (even though they have their licence for more than 2 years) was also added. The information sheet explained the aims and objectives of the project and was to be kept by the volunteer. The consent form assured the volunteer that the results of the analysis would remain in the confidence of the researcher and was to be signed by the volunteer to indicate that they had been provided with sufficient information about the study and returned with the urine sample. The questionnaire was to be filled out by the volunteer and returned to the researcher. Each volunteer was asked to deposit approximately 15 mL of urine into the sample bottle, wipe the outside of the bottle with tissue and place it in the sealable plastic bag.

2.6 Preparation of Urine Samples

Urine samples were prepared in duplicate in glass sample bottles which held approximately 30 mL of liquid and had a metal lid. Approximately 0.150g of sodium fluoride preservative was added to each sample bottle. This was done in the fume hood. Using a glass pipette 10 mL of the urine sample was placed in the sample bottle and it was inverted to mix the contents. 5 mL of the urine sample was removed with a glass pipette and transferred to a second glass sample bottle. 5 mL of 0.3% v/v propan-1-ol was added to each glass sample bottle and inverted. Samples were prepared in the fume hood and all surfaces were cleaned with bleach afterwards. All samples were stored in the refrigerator until they were ready to be analysed.

2.7 Washing Glassware and Disposal of Urine Samples

A dilute solution of bleach was prepared to clean all glassware that came into contact with urine. 5 mL of bleach was placed in a beaker and diluted with 250 mL of water. A pasteur pipette was used to rinse the 10 mL pipette which was used to prepare all urine samples. This pipette was rinsed with bleach and deionised water in between preparation of each sample to prevent inaccurate results and contamination. The sample bottles in which the urine samples were stored were rinsed with bleach and deionised water. All urine samples and washings were poured into a dedicated plastic waste bottle in the fume hood which was then disposed of down the toilet. Any materials that came into contact with the urine samples such as urine sample collection pots, gloves etc were disposed of in a clinical waste bin in the fume hood. The protocol for handling urine samples can be seen in Appendix 4.

2.8 Calibration of Automated and Glass Pipettes

Before calibration of the pipettes the balance was calibrated to ensure it was fit for use. Both automated and glass pipettes were calibrated at 0.5 mL and 1 mL using a pre-weighed beaker and measuring the difference in weight when a specific volume of deionised water was added. This was repeated ten times at both volumes for both types of pipette.

2.9 Use of the BACTrack Breathalyser

The BACTrack breathalyser was used throughout the project along with 50 new mouthpieces which were purchased for the breath analysis of students. The instrument was turned on by pressing the "Start" button. On start up the instrument displays the number of tests previously conducted. A countdown is displayed on screen. At 2 seconds the subject should take a deep breath in and at 0 seconds begin to exhale into the instrument for a period between 8 to 10 seconds until the BACTrack breathalyser beeps to indicate that an adequate sample has been provided. The units of measurement could be changed to mg/L of breath, mg/100ml and % BAC by starting the countdown and pressing the mode button for 5 seconds. The accuracy of this sensor is 100 ± 5 mg and it has a detection range of 0 – 400 mg/100 mL. [5]

2.10 Breath Analysis of Students Following a Student Event in DIT Kevin Street Using the BACTrack Breathalyser

On the 22nd of February, the BACTrack Breathalyser was used between 9.30 and 12.00 in DIT Kevin Street to take breath samples from student volunteers. This day and time was chosen as Wednesdays are a popular student night out and the earlier the students were approached the more chance there was of alcohol still being found in their system. A stand was placed in the main entrance of the annex in Kevin Street in order to approach students before and after they made their way to lectures. A poster board was used to display a College Awareness of Road Safety (CARS) poster and one was also hung from the table. Questionnaires and consent forms were placed on the table along with biros for students to complete the survey. Anyone that volunteered to provide a breath sample was informed about the project and asked to sign the consent form and take part in the survey. Each volunteer was told to inhale a deep breath when the countdown of the BACTrack breathalyser reached 2 seconds and begin to exhale for a period of eight to ten seconds until the BACTrack breathalyser beeped to show that a sample had been taken and was being processed. A total of 48 students were breathalysed. In four cases the BACTrack breathalyser took longer to read the breath samples provided. It was detected by the BACTrack breathalyser that these four samples contained alcohol.

Figure 2.1: Breathalyser event in DIT Kevin Street

2.11 Investigation into Drink Driving Myths

The BACTrack breathalyser was used to investigate the myth that mints or chewing gum could be used to cheat the breathalyser and affect the reading it gave. Eight volunteers who had been drinking were breathalysed. Four of these volunteers were asked to chew on a piece of chewing gum for 2 minutes while the other 4 volunteers were asked to suck on a mint. The volunteers were then breathalysed again. In another experiment two volunteers were breathalysed 20 minutes after consumption of alcohol and then asked to chew a piece of chewing gum for 2 minutes and were breathalysed again after another 20 minutes. This second experiment was carried out due to the interferences caused by mouth alcohol in the first experiment.

2.12 Contact with Drinkaware

Drinkaware was contacted via email in order to see if they could send any leaflets or other materials which could be given to students on the day of the breathalyser analysis. A reply was received from Emily Burke, the media and communication officer at drinkaware, and she agreed to send 100 'Morning After' cards which gave information on what a standard drink is and how long it takes for units of alcohol to be eliminated from the body. These were placed on the table for students to take when the breathalyser analysis took place and the results of the breath analysis were also written on the card and supplied to students who took part.

2.13 Contact with Greenhills Football Club

A meeting was organised and conducted with Greenhills Football club on Sunday the 17th of February. The project was explained to members of the club before a league match at 12 a.m. in which the club were taking part. Players were asked to take part in the project by supplying a urine sample for alcohol analysis to see if any of the players were over the limit. Players were also asked to sign a consent form. Seven samples were supplied however one contained an insufficient amount of urine for analysis. The six samples supplied were analysed for alcohol by GC.

2.14 Visit to Medical Bureau of Road Safety (MBRS)

A visit to the Medical Bureau of Road Safety was organised and carried out on the 6th of February. The procedures used for the analysis of blood and urine samples were explained by Helen Kearns who runs this section and a tour of the blood and urine analysis laboratories was also provided. Protocols with regards to sample handling and storage and the chain of custody were also explained. Five check standards were provided by the MBRS and were used in the analysis of urine samples. The certified reference certificates for these standards were also supplied and can be seen in Appendix 15.

2.15 Visit to Store Street Garda Station

A visit to Store Street Garda Station was organised and carried out on the 8th February to meet with Garda Colm Reid and witness the operation of the on-site breathalyser, the Evidenzer 600IRL. During this visit a simulation was run as well as my own sample and I was also given the opportunity to set up the instrument as would be done in a real life situation when a person is arrested on suspicion of driving over the legal limit. This was done by typing in the person's details such as gender, name, date of birth and setting the instrument to test at the right limit for that individual e.g a specified or full licence driver. After a sample has been provided the instrument generates a printout which contains the details about the suspect that had been entered into the instrument and a breakdown of the concentration of ethanol in the sample. This printout can be seen in Appendix 13. It was explained that one printout from the instrument is kept by the Garda while another printout is given to the suspect. Garda Reid also explained the current legislation and drink driving limits which were also placed on the wall beside the instrument for a suspect to view. It was also explained that there are certain time limits to be considered when obtaining samples and by law a suspect must be observed for no less than 20 minutes before a sample is taken. During the visit a tour of the station was also given where I had the opportunity to view the new emergency call system in place in the station where a large screen is used to display the location of all Guards stationed at Store Street so that they can be called to the scene of an emergency in a more efficient time.

2.16 Meeting with Garda Derek Cloughley

A meeting with Garda Derek Cloughley from the Garda Road Safety Unit was conducted on the 14th February to discuss the aspects of the project which had been carried out and any suggestions that Garda Cloughley had for the remainder of the project. Garda Cloughley suggested that the results of the student survey completed during the breathalyser analysis on students this year be compared to the project from the previous year to see if students were more or less aware.[22] It was also suggested that an online survey should be generated and circulated to the students in the School of Chemical and Pharmaceutical Sciences and that a true or false question be added on whether a driver is tested at the 20 mg limit if they do not have a licence with them when stopped (even though they have their licence for more than 2 years). Garda Cloughley advised that the findings of the surveys could be communicated to Michael Rowland, Director of Education in the Road Safety Authority when the project is completed as well as posting the results and links to relevant Garda traffic Youtube videos on the DIT website for students to observe. It was proposed that drink driving myths could also be investigated such as the effects that mints have on the reading given by the breathalyser.

3. Results and Discussion

3.1 Analysis of Wine by GC

The results obtained from the GC analysis of a wine sample and the calculations involved can be seen in Appendix 5. This analysis was performed to ensure that the GC was operating normally and that the analysis of urine samples could be performed on it. This analysis was repeated three times. Results from Experiment KD1 determined that the wine sample had half the concentration of ethanol present than that stated as the true value on the bottle. As this was an old sample, it was concluded that a percentage of the ethanol had evaporated and so a new sample was provided. The results from Experiment KD4 showed variations in retention times and peak shapes which indicated that a leakage of flow gas was occurring through the septum. The septum on the GC was replaced and the analysis was repeated to obtain acceptable results as seen in Experiment KD5 Appendix 5.

Figure 3.1 shows the calibration curve obtained for analysis of wine sample by GC for Experiment KD5

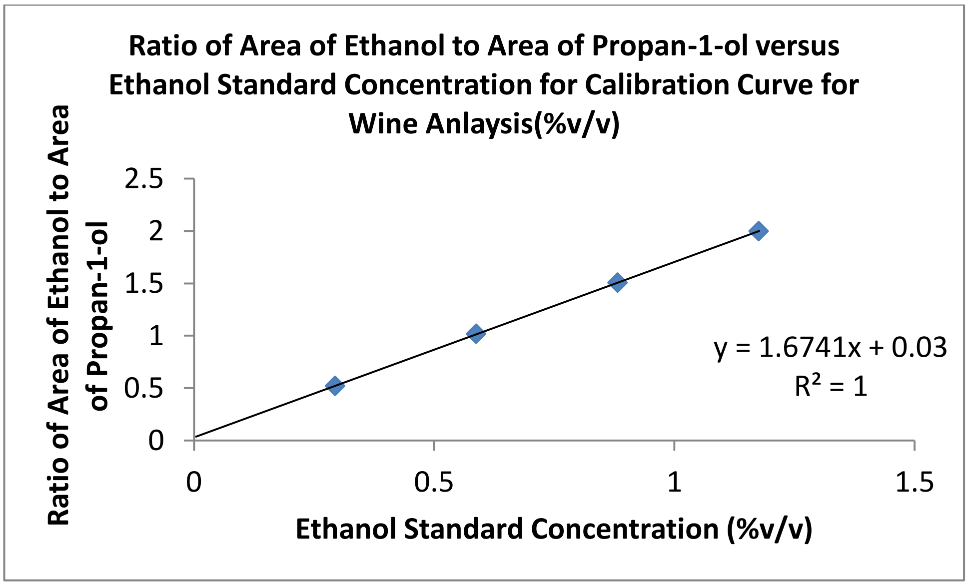

Figure 3.1 Calibration curve showing the ratio of the area of ethanol to the area of propan-1-ol versus ethanol standard concentration for wine analysis (Experiment KD5)

The results obtained in Experiment KD4 show that this analysis was necessary as the problem encountered with the seal in this case needed to be corrected for before analysis of urine samples could begin. Once the problems discussed had been corrected the results from the experiment showed that the method was acceptable statistically as the % error was low at 4%, the % RSD was less than 5 % at 0.45% and the R^2 for the calibration curve of the ethanol standards was greater than 0.99 at 1.[20] Therefore accuracy, precision and linearity were demonstrated.

3.2. Results for Pipette Calibrations

A glass pipette and auto pipette were calibrated as discussed in Section 2.8 in order to determine which was more accurate for use when measuring the Diasys ® check standards. The results of these calibrations and the associated Paired T-test can be seen in Appendix 9. The standard deviations were smaller for the glass pipette than the auto pipette indicating that the glass pipette was more precise. The results of the Paired T-test also showed that the methods differ significantly. A reason for this may be that the auto pipettes have not been calibrated since they were purchased and are also being stored incorrectly, lying down, when they should be standing up. For these reasons a glass pipette was used.

3.3 Calibration Curve for Ethanol Standards for Urine Analysis

Tables 3.1, 3.2 and 3.3 summarise the results obtained from the GC analysis of ethanol standards for urine analysis performed once a week over the three weeks. All standards were analysed in duplicate and the average of the ratios were used in the calibration curves.

% v/v of Standards	Ethanol Concentration of Standards mg/100 mL	Area of Ethanol peak	Retention Time (minutes)	Area of Propan-1-ol Peak	Retention Time (minutes)	Ratio of area of ethanol to area of propan-1-ol
0.01	7.89	5306	1.059	97049	1.804	0.0547
		5087	1.046	96196	1.790	0.0529
0.025	19.73	12529	1.056	96644	1.803	0.1296
		11940	1.063	91965	1.807	0.1298
0.05	39.45	26384	1.074	98336	1.822	0.2683
		26445	1.058	98264	1.807	0.2691
0.10	78.90	55581	1.060	100784	1.818	0.5515
		56319	1.049	102049	1.802	0.5519
0.20	157.80	111264	1.066	101352	1.819	1.098
		108918	1.058	99480	1.810	1.095
0.30	236.70	167305	1.045	101571	1.799	1.648
		160642	1.056	97315	1.810	1.651

Table 3.1 Retention times and peak areas of ethanol and prop-1-anol in ethanol standards obtained for urine analysis by GC (Experiment KD7, Week 2)

Figure 3.2 shows the calibration curve for ethanol standards for urine analysis obtained in Experiment KD7, week 2.

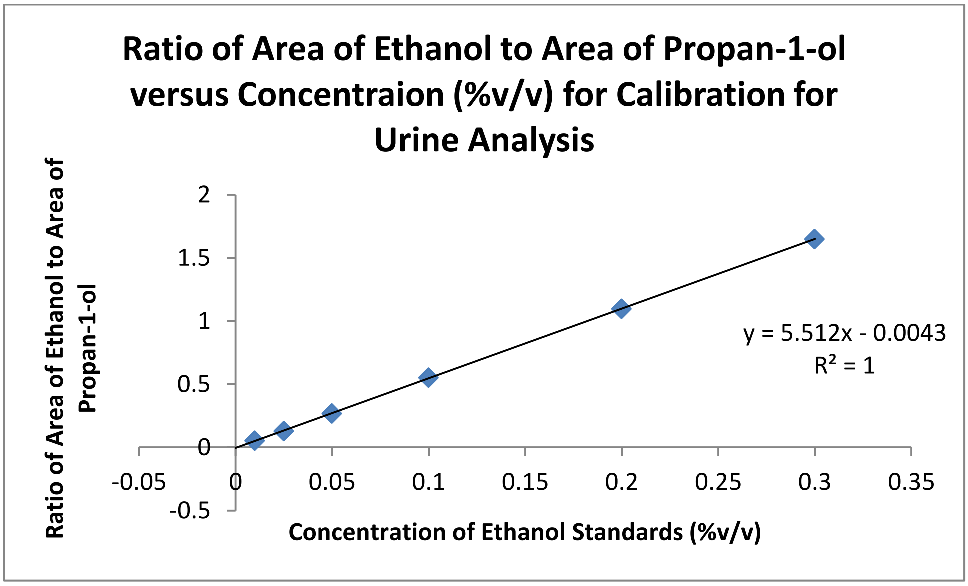

Figure 3.2 Calibration curve showing the ratio of the area of ethanol to the area of propan-1-ol versus ethanol standard concentration (Experiment KD7, Week 2)

% v/v of Standards	Ethanol Concentration of Standards mg/100 mL	Area of Ethanol peak	Retention Time (minutes)	Area of Propan-1-ol Peak	Retention Time (minutes)	Ratio of area of ethanol to area of propan-1-ol
0.01	7.89	5097	1.054	100997	1.797	0.0505
		5270	1.053	103793	1.796	0.0508
0.025	19.73	13046	1.061	100268	1.801	0.1301
		13205	1.064	102296	1.804	0.1291
0.05	39.45	26714	1.049	103023	1.790	0.2593
		25958	1.052	100154	1.794	0.2592
0.10	78.90	58017	1.062	102696	1.804	0.5649
		57060	1.053	100856	1.797	0.5658
0.20	157.80	112064	1.064	105509	1.810	1.062
		113773	1.051	107159	1.798	1.062
0.30	236.70	164651	1.067	101096	1.813	1.629
		166778	1.044	102163	1.791	1.633

Table 3.2 Retention times and peak areas of ethanol and prop-1-anol in ethanol standards obtained for urine analysis by GC (Experiment KD12, Week 3)

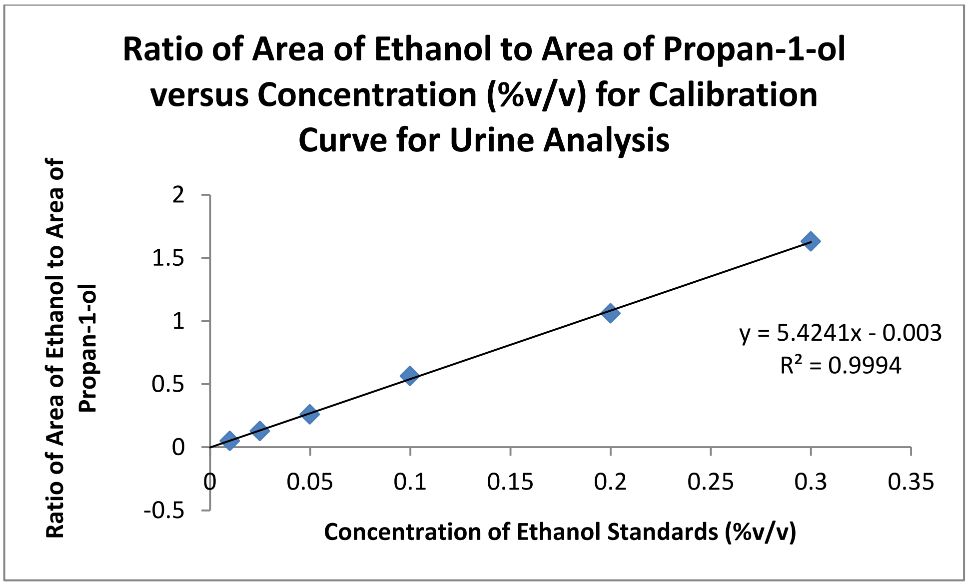

Figure 3.3 Calibration curve showing the ratio of the area of ethanol to the area of propan-1-ol versus ethanol standard concentration for urine analysis (Experiment KD12, Week 3)

% v/v of Standards	Ethanol Concentration of Standards mg/100 mL	Area of Ethanol peak	Retention Time (minutes)	Area of Propan-1-ol Peak	Retention Time (minutes)	Ratio of area of ethanol to area of propan-1-ol
0.01	7.89	5090	1.155	111094	1.944	0.0458
		5319	1.150	116612	1.941	0.0456
0.025	19.73	13306	1.141	116131	1.932	0.1146
		12989	1.144	113419	1.936	0.1145
0.05	39.45	27407	1.149	114869	1.943	0.2386
		27418	1.146	114904	1.940	0.2386
0.10	78.90	59114	1.139	114904	1.935	0.4932
		58350	1.141	119856	1.937	0.4926
0.20	157.8	116044	1.145	118464	1.942	1.0216
		112299	1.107	113596	1.905	1.0219
0.30	236.7	172089	1.145	109891	1.944	1.5042
		170656	1.152	113450	1.951	1.5042

Table 3.3 Retention times and peak areas of ethanol and prop-1-anol in ethanol samples obtained from GC analysis (Experiment KD16, Week 4)

Figure 3.4 shows the calibration curve for ethanol standards for urine analysis produced in week 4, Experiment KD16

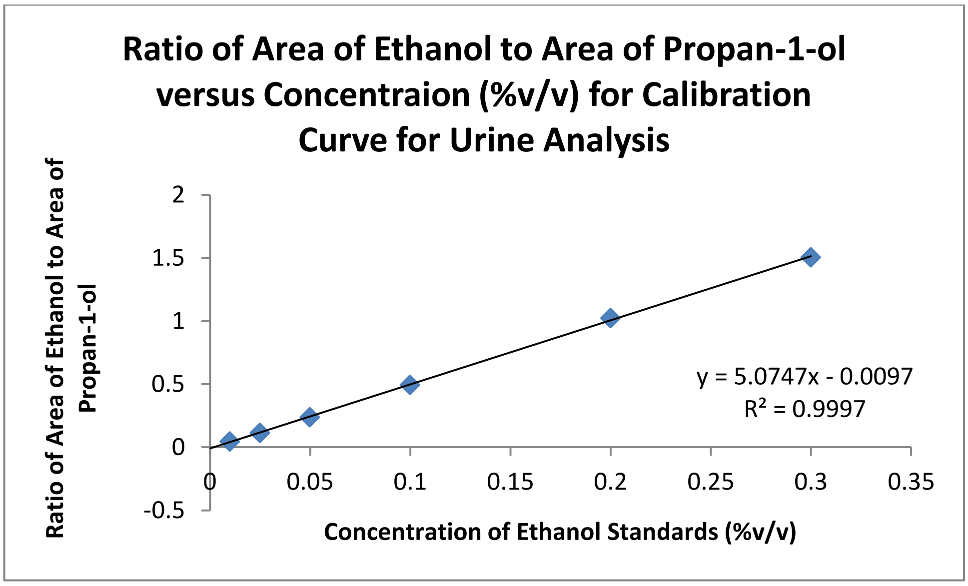

Figure 3.4 Calibration curve showing the ratio of the area of ethanol to the area of propan-1-ol versus ethanol standard concentration for urine analysis (Experiment KD16, Week 4)

Ethanol standards were run in duplicate at the beginning of each week. The equation of the line obtained from the weekly calibration curve was used to calculate the concentration of ethanol in any urine samples that were analysed during that week. The results obtained from all three calibrations carried out met specifications as all curves displayed linearity with R^2 greater than 0.99 at 1, 0.9994 and 0.9997. The method was deemed to be reproducible and reliable therefore it was successfully validated for the parameter of linearity and was deemed acceptable for use on all urine samples.[21]

3.4 Diasys® and MBRS Check Standards

As the ethanol calibration standards were only run once a week it was necessary to check that the system was stable for use every day before urine samples were run. This was done by running a range of Diasys® and MBRS check standards each morning. The results of these standard checks can be seen in Appendix 8. The percentage errors obtained for these checks varied daily. The highest percentage errors occurred for the Diasys® check standards. This may have been due to random errors occurring in their preparation as such small volumes (0.5mL) needed to be measured whereas for the MBRS standards a larger volume was measured (5mL). The highest percentage error was obtained for the 100 mg/mL Diasys® check standard at 25.7%. This check standard also fell outside specifications on three occasions as the % accuracy was greater than 110% at 113%, 125% and 126% (See Appendix 8)[21]. The lowest concentration MBRS reference material, 19.9 mg/mL, had a consistently low percentage error (2.0 – 3.5%) and a % accuracy that fell within specifications of 90 -110% with a range of 97-104%, throughout the daily analyses demonstrating that the method is suitable at low ethanol concentrations.[21] Throughout last year's project problems were encountered with the lower concentration check standards. These were improved upon this year and a reason for this may be that the MBRS check standards were made up to a total volume of 10 mL this year whereas last year they were made up to a total volume of 1 mL therefore errors would have been more likely to be associated with the smaller measurements. This is supported by the fact that difficulties were encountered again this year during preparation of the Diasys® check standards which are made up to 1 mL.[22]

3.5 Calibration Curves Generated from Check Standards from MBRS

The entire range of check standards supplied from the MBRS were prepared and analysed on two occasions in week 2 and week 4. The standards were analysed in duplicate and calibration curves were prepared. The retention times and peak ratios obtained from analysis of MBRS standards on week 2, Experiment KD10 and week 4, Experiment KD18 can be seen in Appendix 6.

Figure 3.5 shows the calibration curve obtained for GC analysis of MBRS check standards in Experiment KD10, Week 2.

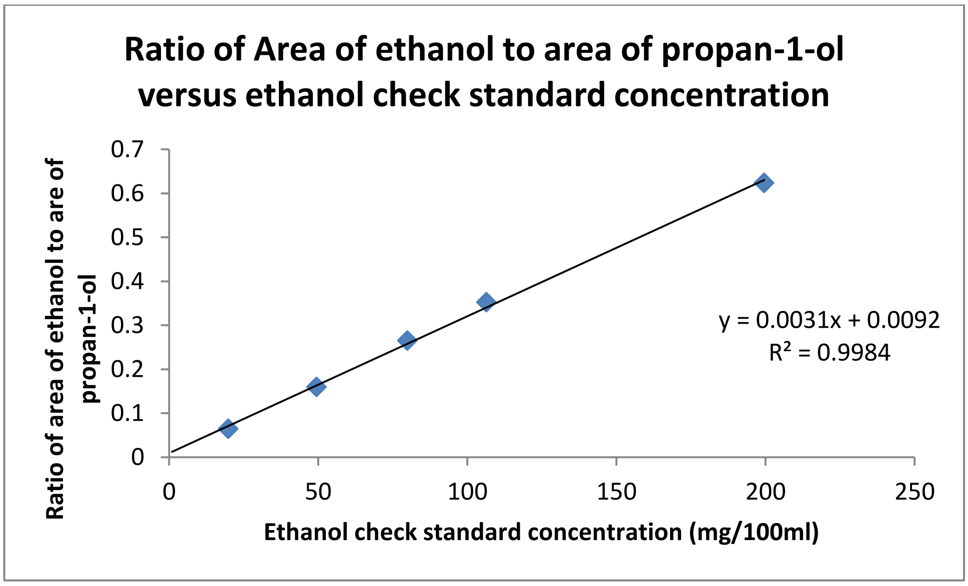

Figure 3.5 Calibration curve showing the ratio of the area of ethanol to the area of propan-1-ol versus ethanol standard concentration for MBRS check standards (Experiment KD10)

Table 3.4 shows the results obtained from GC analysis of MBRS check standards and associated percentage errors

Check Standard (mg/100 mL)	Ethanol Concentration (%v/v)	Ethanol Concentration (mg/100 mL)	Mean	Standard Deviation	% RSD	% Error	% Accuracy
199.6	0.2276	179.6	179.8	0.212	0.12	9.4	90
	0.2280	179.9					
106.5	0.1292	101.9	102.1	0.212	0.21	4.1	96
	0.1295	102.2					
80.0	0.0979	77.2	77.0	0.283	0.37	3.8	96
	0.0974	76.2					
49.5	0.0595	46.9	46.9	0.071	0.15	5.3	95
	0.0593	46.8					
19.9	0.0249	19.6	19.6	0.071	0.36	1.5	98
	0.0247	19.5					

Table 3.4 Ethanol concentration in % v/v and mg/100ml, mean, standard deviation, % RSD and % error for European Reference standards supplied by MBRS (Experiment KD10)

Table 3.5 summarises the data obtained from analysis of MBRS check standards in Experiment KD18. Figure 3.6 shows the calibration curve obtained from GC analysis of the MBRS check standards in Experiment KD18, Week 4.

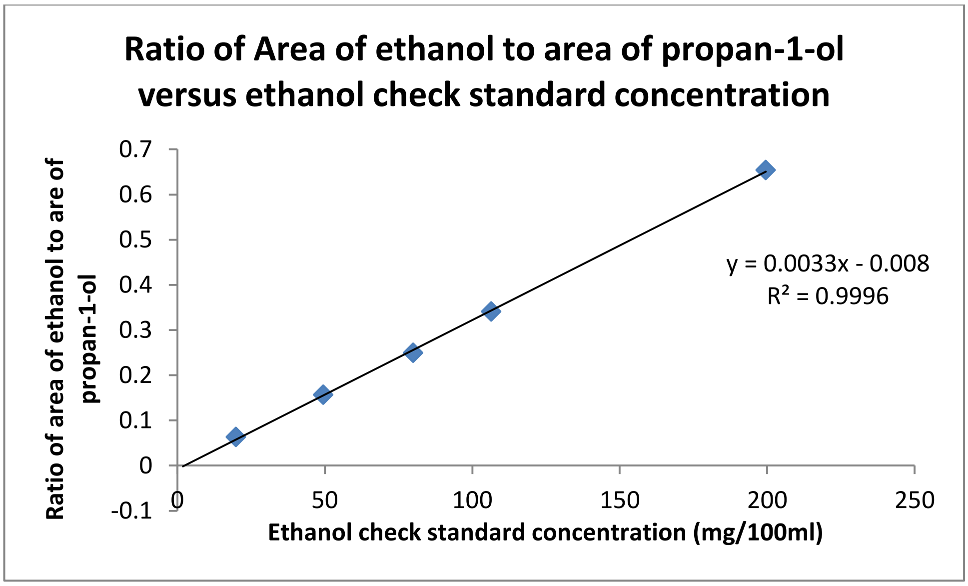

Figure 3.6 Calibration curve showing the ratio of the area of ethanol to the area of propan-1-ol versus ethanol standard concentration for MBRS check standards (Experiment KD18)

Check Standard (mg/100 mL)	Ethanol Concentration (%v/v)	Ethanol Concentration (mg/100 mL)	Mean	Standard Deviation	% RSD	% Error	% Accuracy
199.6	0.2616	206.40	206.4	0.057	0.03	3.4	103

	0.2615	206.32					
106.5	0.1383	109.12	109.0	0.170	0.16	2.3	102
	0.1380	108.88					
80.0	0.1021	80.56	80.5	0.113	0.14	0.6	101
	0.1019	80.40					
49.5	0.0653	51.52	51.6	0.057	0.11	4.2	104
	0.0654	51.60					
19.9	0.0285	22.49	22.5	0.057	0.25	13.1	113
	0.0286	22.57					

Table 3.5 Ethanol concentration in % v/v and mg/100 mL, mean, standard deviation and % RSD for European Reference standards supplied by MBRS (Experiment KD18)

On the first occasion (Experiment KD10) it was determined that the percentage error for the lowest ethanol concentration 19.9mg/100 mL was the smallest (1.5%) while the highest ethanol concentration had the highest percentage error (9.4%) which was unexpected. However in the second analysis (Experiment KD18) this was reversed with the 19.9mg/100 mL having a much higher percentage error (13.1%) than of that in the previous run which meant that it fell outside the specifications as the % accuracy was greater than 110% at 113%.[21] These results may be due to the occurrence of random errors in preparation of the standards. When the MBRS reference materials were analysed daily before urine analysis took place, the 19.9mg/100 mL reference was regularly used as it was the lowest concentration and it consistently had the lowest percentage error. The R^2 values for both calibrations performed using the MBRS standards were greater than 0.99 at 0.9984 and 0.9996 therefore the standards were deemed acceptable for quantitation of the urine samples. [21]

3.6 GC Analysis of Urine Samples

Overall 13 urine samples were provided by DIT student volunteers while 6 samples were provided by volunteers from Greenhills Football club. The concentration of ethanol in each urine sample was determined using the equation of the line that had been obtained from the weekly calibration of the ethanol standards prepared in house (See section 3.3). Urine samples were prepared and analysed in duplicate. The results obtained for the analysis of urine samples supplied by student volunteers are shown in Table 3.6. The results obtained for the analysis of samples supplied by Greenhills Football club can be seen in Appendix 7.

Sample Number	**Area of Ethanol peak**	**Retention Time (minutes)**	**Area of Propan-1-ol Peak**	**Retention Time (minutes)**	**Ratio of area of ethanol to area of propan-1-ol**
1(I)	No ethanol	-	106323	1.824	No ethanol

	detected				detected
	No ethanol detected	-	103240	1.818	No ethanol detected
1(I)	No ethanol detected	-	107667	1.808	No ethanol detected
	No ethanol detected	-	103105	1.810	No ethanol detected
2(I)	43003	1.164	92480	1.964	0.4650
	44466	1.168	95573	1.968	0.4653
2(II)	46276	1.160	99937	1.961	0.4631
	46421	1.153	100263	1.953	0.4630
6(I)	No ethanol detected	-	82418	1.956	No ethanol detected
	No ethanol detected	-	83024	1.954	No ethanol detected
6(II)	No ethanol detected	-	83129	1.945	No ethanol detected
	No ethanol detected	-	82762	1.957	No ethanol detected
7(I)	No ethanol detected	-	84315	1.956	No ethanol detected
	No ethanol detected	-	79590	1.955	No ethanol detected
7(II)	No ethanol detected	-	88543	1.955	No ethanol detected
	No ethanol detected	-	90274	1.977	No ethanol detected
8(I)	45096	1.148	96857	1.947	0.4656
	47824	1.165	103130	1.965	0.4637
8(II)	45266	1.154	96392	1.954	0.4696
	46384	1.156	98856	1.956	0.4692
9(I)	4356	1.163	102525	1.958	0.0425
	4162	1.165	98886	1.960	0.0421
9(II)	4098	1.156	97637	1.950	0.0420
	4137	1.158	98657	1.953	0.0419
10(I)	21681	1.166	100139	1.964	0.2165
	21378	1.147	98729	1.946	0.2165
10(II)	21743	1.155	99439	1.952	0.2187
	21995	1.163	100727	1.960	0.2184
11(I)	13226	1.153	97935	1.951	0.1350
	13452	1.170	99722	1.968	0.1349
11(II)	13176	1.168	97969	1.967	0.1345
	12665	1.168	94399	1.965	0.1342
16(I)	No ethanol detected	-	90757	1.806	No ethanol detected
	No ethanol detected	-	91114	1.791	No ethanol detected
16(II)	No ethanol detected	-	95765	1.758	No ethanol detected
	No ethanol detected	-	99042	1.800	No ethanol detected
17(I)	49955	1.056	99304	1.801	0.5031
	50163	1.057	100445	1.803	0.4994
17(II)	51949	1.056	103018	1.802	0.5043
	48983	1.057	97006	1.803	0.5049

18(I)	49166	1.065	102928	1.812	0.4777
	51611	1.080	108418	1.825	0.4760
18(II)	49562	1.064	103596	1.809	0.4784
	47878	1.065	100316	1.809	0.4773
20(I)	No ethanol detected	-	104500	1.810	No ethanol detected
	No ethanol detected	-	107082	1.819	No ethanol detected
20(II)	No ethanol detected	-	104280	1.788	No ethanol detected
	No ethanol detected	-	100049	1.813	No ethanol detected
21(I)	45082	1.156	110388	1.955	0.4084
	45347	1.156	111240	1.955	0.4077
21(II)	44278	1.156	107445	1.955	0.4121
	44155	1.176	107219	1.977	0.4118

Table 3.6 Retention times and peak areas of ethanol and prop-1-anol obtained from GC analysis of urine samples supplied by volunteers (Experiments KD8, KD9, KD10, KD11, KD12)

Table 3.7 shows a colour key which identifies which calibration curve was used for determination of the UAC

Colour	Equation of the line from calibration curve used to calculate ethanol concentration in urine sample
(blue)	y= 2.756x – 0.0043 (Experiment KD7)
(red)	y= 5.4241x – 0.003 (Experiment KD12)
(green)	y= 5.0747x – 0.0097 (Experiment KD16)

Table 3.7 Colour key to identify calibration curve used for urine samples

Table 3.8 shows the UAC and BAC determined for the samples which tested positive for alcohol by GC analysis

Sample Number	Ethanol Concentration (%v/v)	Ethanol Concentration UAC (mg/100 mL)	Mean (mg/100 mL)	Standard Deviation	% RSD	Ethanol Concentration BAC (mg/100 mL) *
2(I)	0.1726	136.18	135.91	0.3667	0.27	104.55
	0.1727	136.26				
2(II)	0.1719	135.63				
	0.1718	135.55				
8(I)	0.1728	136.34	136.75	0.8276	0.61	105.19
	0.1721	135.79				
8(II)	0.1743	137.52				
	0.1741	137.36				
9(I)	0.0168	13.26	13.14	0.0800	0.61	10.10
	0.0166	13.10				
9(II)	0.0166	13.10				

	0.0166	13.10				
10(I)	0.0809	63.83	64.13	0.3422	0.53	49.33
	0.0809	63.83				
10(II)	0.0817	64.46				
	0.0816	64.38				
11(I)	0.0509	40.16	40.04	0.1033	0.26	30.80
	0.0508	40.08				
11(II)	0.0507	40.00				
	0.0506	39.92				
17(I)	0.1866	147.23	147.19	0.7482	0.51	113.22
	0.1852	146.12				
17(II)	0.1871	147.62				
	0.1873	147.78				
18(I)	0.1772	139.81	139.73	0.2949	0.21	107.48
	0.1766	139.34				
18(II)	0.1775	140.05				
	0.1771	139.73				
21(I)	0.1648	130.03	130.50	0.6891	0.53	100.38
	0.1645	129.79				
21(II)	0.1662	131.13				
	0.1661	131.05				

Table 3.8 Ethanol concentration in % v/v and mg/100 mL, mean, standard deviation and % RSD for urine samples which tested positive for alcohol

*Conversion of UAC to BAC using a factor of 1.3[23]

3.7 Example of Calculating the Concentration of Ethanol in a Urine Sample using Sample 2 as an Example

The concentration of ethanol in sample 2 was calculated using the equation of the line from Experiment KD12: y= 5.4241x – 0.003

A ratio of 0.4084 for the ethanol/propan-1-ol peak areas was calculated for the first run of urine sample 2

$0.4650 = 5.4241x – 0.003$
$0.4650 + 0.003 = 5.4241x$
$0.468 = 5.4241x$

$\frac{0.468}{5.4241} = x$

0.0863 %v/v = x

As 5 mL of the urine sample was diluted with 5 mL of propan-1-ol stock a dilution factor of 2 applies

0.0863 %v/v x 2 = 1.726 % v/v

Density of ethanol = 0.789 g/mL

1.726 % v/v x 0.789 g/ml = 1.3618 g/mL

1.3618 x 1000 = 136.18 mg/100mL

Samples 2, 8, 17, 18 and 21 were all found to be over the limit for urine alcohol levels for both a specified (27 mg/100 mL) and full licence driver (67 mg/100 mL). Samples 10 and 11 were over the limit for a specified driver but under the limit for a full licence driver. Sample 9 was under the limit for a full licence driver and a specified driver at 13.14 mg/100 mL. The legal limits for alcohol in urine can be seen in Chapter 1 Table 1.1. Samples 17 and 18 were taken after the volunteers had returned home from a night out rather than the next day and so the high results were to be expected and demonstrate that it is not safe to drive home after a night out. While the other samples were supplied anonymously and so the exact sampling time is not known however students were asked to deposit the samples the next morning after drinking. All samples supplied by Greenhills Football Club were negative for alcohol (See Appendix 7). This may suggest that the recent Garda press release[19], which stated that a large number of males were testing positive the day after consumption of alcohol at around the times 11-12am, has had a positive effect on this age group.

3.8 Comparing Results Obtained for Urine Samples Using MRBS Calibration Curve and Ethanol Standard Calibration Curve

Table 3.9 shows UAC obtained using the MBRS calibration curves from Experiment KD10 and KB18

Sample Number	Ethanol Concentration (mg/100 mL)	Mean (mg/100 mL)	Standard Deviation	% RSD
2(I)	128.77	129.29	0.7341	0.57
	128.55			
2(II)	129.97			
	129.87			
8(I)	147.23	147.69	0.9232	0.63
	146.61			
8(II)	148.52			
	148.39			
9(I)	10.74	10.62	0.0837	0.79
	10.61			
9(II)	10.58			
	10.55			
10(I)	66.87	67.20	0.3832	0.57
	66.87			
10(II)	67.58			
	67.48			
11(I)	40.58	40.47	0.1204	0.30
	40.55			
11(II)	40.42			

	40.32			
17(I)	154.88	154.83	0.7399	0.48
	153.77			
17(II)	155.24			
	155.42			
18(I)	147.18	147.08	0.3025	0.21
	146.67			
18(II)	147.39			
	147.06			
21(I)	126.18	126.67	0.6876	0.54
	125.97			
21(II)	127.30			
	127.21			

Table 3.9 Ethanol concentrations in % v/v and mg/100 mL, mean, standard deviation and % RSD for urine samples which tested positive for alcohol calculated using equation of the line from the MBRS check standard calibration curves (Experiment KD10 and Experiment KD18)

Table 3.10 compares the UAC determined using the MBRS calibration curve and the ethanol working standards calibration curve

Sample Number	U.A.C Ethanol Standards mg/100 mL	U.A.C MBRS Standards mg/100 mL	Mean U.A.C	Standard Deviation	% RSD
2	135.91	129.29	132.60	4.681	3.53
8	136.75	147.69	142.22	7.736	5.44
9	13.14	10.62	11.88	1.782	15.0
10	64.13	67.20	65.67	2.171	3.31
11	40.04	40.47	40.26	0.304	0.76
17	147.19	154.83	151.01	5.402	3.58
18	139.73	147.08	143.41	5.197	3.62
21	130.50	126.67	128.59	2.708	2.11

Table 3.10 Comparison of results obtained for urine analysis using ethanol standards and MBRS standards (Experiment KD10 and KD18)

From the comparison of results obtained for urine analysis using ethanol working standards and the standards supplied from the MBRS it can seen that there is some variation in the final concentration of ethanol determined with some of the samples having high standard deviations and % RSD. The largest difference was observed for the lowest level detected (15.0% RSD). From these results it has been concluded that the calibration curve obtained for the ethanol standards is more accurate than the MRBS check standard calibration. This is demonstrated by the R^2 values which are much more accurate for the ethanol working standards (1.000, 0.9994 and 0.9997) than the MBRS standards (0.9984 and 0.9996).[21] A possible reason for this may be due to the fact that only 5 MBRS standards were analysed whereas 6 ethanol working standards were analysed. Also the MBRS check standards didn't have a standard concentration at the lower range, 10 mg/100 mL which would also have contributed to the poorer R^2 values obtained for the calibration curves. However although the ethanol standard calibration curves have better R^2 values compared to the MBRS check standard calibration curves the results of a paired T-Test have shown that the methods do not give significantly different results which demonstrates that the calibration curves produced by the MBRS check standards are adequate for quantitation of urine samples. The results of the paired T-Test used to compare these methods can be seen in Appendix 10.

3.9 Breath Analysis of Student Volunteers the Morning After a Student Night Out Using the BACTrack Breathalyser

A total of 48 students were breathalysed in DIT Kevin Street the morning after a Wednesday night, which is popular with students for going out. Out of these 48 students the BACTrack breathalyser gave a reading of 0% BAC for 44 students while a positive reading was given for 4 students. The results for these 4 students and the answers they gave to the survey conducted are shown in Table 3.11.

Student	% BAC	mg/100 mL ethanol (BAC)	Sex	Age	How many drinks had they consumed	What types of drinks had they consumed	Did they expect to be over the limit	Do they have full licence	If so do they have their licence more than 2 years
1	0.037	37	Male	18-21	5-9	Guinness Heineken	No	No	N/A
2	0.026	26	Male	26-35	5-9	Beer	Yes	Yes	Yes
3	0.047	47	Male	18-21	5-9	Spirits	Yes	No	N/A
4	0.017	17	Female	18-21	5-9	Beer Spirits Wine	Yes	No	N/A

Table 3.11 Results obtained for 4 students who tested positive for ethanol in their breath on the day of breathalyser testing at DIT Kevin Street

Student 4 was under the limit for a specified (20 mg/100 mL) and full licence (50 mg/100 mL) driver. Students 1, 2 and 3 were over the limit for a specified driver (20 mg/100 mL) but under the limit for a full licence driver (50 mg/100 mL). All of these students had admitted that they had been drinking into the early hours of the morning and all of them except student 1 believed that they would be over the limit. The legal limits for alcohol in breath can be seen in Chapter 1 Table 1.1. The results of this analysis have demonstrated that it is possible to be over the limit the next morning after a night out. In doing this analysis, these particular students were made aware that it takes the body an hour to remove one standard drink and that it may not be safe for them to drive the next day.

3.10 Investigation into Interferences When Using the BACTrack Breathalyser

In the first trial when 8 volunteers were breathalysed, there was an insufficient period of time between consumption of alcohol and taking samples. This led to the breathalyser giving very high readings which corresponded to mouth alcohol.[1] Due to this, the experiment was repeated using 20 minute time gaps between taking samples and drinking alcohol. However as the length of time to do this experiment was much longer, 40 minutes it was difficult to get volunteers and the analysis was only carried out on two individuals. The results demonstrated that the %BAC was lower when the individual was breathalysed after chewing a chewing gum. However the experiment design needs to be considered as the decrease in % BAC is more likely to have been due to the 20 minute time gap than the effect of chewing a piece of chewing gum. If this experiment was to be repeated the volunteer should have one drink, wait 20 minutes and then be breathalysed, chew on a piece of chewing gum and then be breathalysed again 5 minutes later. A larger population would also need to be analysed for the results to be conclusive. Results from both experiments can be seen in Appendix 11.

3.11 Analysis of Surveys

Overall 98 students took part in the survey when the results of the online survey, surveys completed from the day of the breathalyser testing and surveys in the urine sample packs were combined. 50 of those who took part were male while the remaining 48 were female.

From the results of the survey it can be seen that a lot of work still needs to be done to educate students on the relationship between drinking and road safety and the rules of the road. Out of 94 people questioned only 29% knew that the driving limit for a driver that has held their licence for less than 2 years is 20 mg/100 mL. The majority believed it was lower at 10mg/100ml however 17% believed it was higher than 20 mg/100 mL. When asked the driving limit for drivers that had held their licence for over 2 years 29% of people out of 95 answered correctly (50 mg/100 mL). However 10% of respondents believed the limit was greater than 50 mg/100 mL while 61% people believed it was lower than 50 mg/100 mL. Of the 81 people that answered the question regarding the number of units contained in a bottle of wine (750ml), only 27% answered correctly while 62% believed there was less than 9 units and 11% believed there were more than 9 units in a bottle. Of the 84 people that answered the question regarding the number of units contained in a pint of beer, 61% answered correctly while 26% believed there was less than 2 units and 13% believed there were more than 2 units in a pint. When the students were questioned about whether they believed enough was being done to educate students on drinking and road safety, 65% believed that not enough was being done. In order to improve awareness, the results of this survey were placed on the DIT website along with the correct answers. Links to the Road Safety Authority and Garda website on drink driving were also provided for students to view. The results for some of these questions are presented as bar charts in Appendix 12.

3.12 Comparing this Year's Survey to Last Year's Results

This year a total of 98 people completed the survey while last year there was a total of 81. Of the 60 people that answered the question regarding the drink driving limit for those with a full licence longer than 2 years, only 10% answered correctly compared to 29% out of 95 answering correctly this year. Of 71 people that answered the question regarding the drink driving limit for specified drivers, 30% answered correctly compared to 29% out of 94 this year.[22] This demonstrates that students are still unaware of the limits and a lot more work still needs to be done to improve this. [22]

4. Conclusion

The aims set out in the introduction were achieved. The method was successfully validated for the parameters of accuracy, precision, linearity and reproducibility as the results for the lower ethanol standard concentrations were consistent and were successfully reproduced throughout the analysis. The calibration curves demonstrated linearity and reproducibility therefore ethanol standards were only run once a week as the results obtained were satisfactory. The R^2 values obtained for the calibration of ethanol standards were 1.000, 0.9994 and 0.9997 respectively. As the ethanol standards were only run once a week, Diasys® and MBRS check standards were run before analysis of urine samples in order to ensure that the system was stable. The percentage errors obtained for these standards varied depending on daily preparation. The highest percentage error was associated with the 100mg/100 mL Diasys check standard. This may have been due to random errors in preparation due to the small volumes being measured. When the lowest concentration MBRS check standard, 19.9 mg/100mL, was analysed daily it yielded the lowest percentage error. However when MBRS standards were prepared and analysed on two occasions to produce calibration curves, the 19.9mg/100 mL check produced a percentage error of 13.1% which was higher than the daily analysis had produced. As the MBRS check standards yielded less accurate and precise calibration curves than the ethanol working standard curves more work needs to be done to explore this. It may be that the lack of a check standard in the 10 mg/100 mL range contributed to this problem. As the MBRS standards were donated and not purchased, it was not possible to obtain a 10 mg/100 mL standard from this source.

Nineteen urine samples were analysed in total. Of these 8 tested positive for alcohol. Under the Road Safety Act five of these samples were found to be over the limit for both a specified driver and full licence driver while two were under the limit for a full licence driver but over the limit for a specified driver. The remaining sample contained alcohol but was under the limit for a specified driver and a full licence driver. The alcohol content in these samples was also calculated using the MBRS calibration curves and when compared to the results from the ethanol working standards a larger % RSD was determined therefore further demonstrating that the MBRS calibration curve was less reliable.

The analysis of urine samples provided by Greenhills Football Club added a new aspect to the project and was aimed at informing young males who would be driving early on Sunday morning of the need to ensure that they had processed alcohol in their system. This was in line with a recent Garda press release that stated that one of the most likely groups to be caught drink driving were young males on a Sunday morning who had been drinking the previous night. All samples supplied by Greenhills Football Club tested negative for alcohol. This may indicate that the recent Garda press release and focus on this age group and sex has had a positive effect although a wider would be necessary to make any conclusions.

The breathalyser event held in DIT Kevin Street was successful and a total of 48 students were tested however the number of volunteers was considerably higher last year in Bolton Street with 81 students taking part. Therefore if this study was to be carried out again more research needs to go into the day and venue for the event in order to improve the number of participants. Although it should also be considered that two breathalysers were used last year which allowed more tests to be carried out. In carrying out the breath analysis in Kevin Street 48 people were made aware of the new legal limits and the relationship between drink and road safety. Of the 48 people breathalysed four were found to have alcohol in their system. Student 4 was under the limit for a specified driver (20 mg/100 mL) and full licence (50 mg/100 mL) driver. Students 1, 2 and 3 were over the limit for a specified driver (20 mg/100 mL) but under the limit for a full licence driver (50 mg/100 mL). All but one of these students expected to be over the limit.

A total of 98 people were surveyed and therefore were made more aware of the rules of the road and road safety. The results of these surveys were also communicated on the DIT website along with links to Garda road safety videos therefore further promoting awareness of road safety. With regards to further work in this area more could be done to establish a more accurate calibration curve for the MBRS check standards. Also the effect of interferences on the BACTrack breathalyser could also be further investigated by trying to get results from more volunteers and modifying the testing protocol.

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Appendices