FUNDAMENTAL PRINCIPLE OF ALCOHOL ANALYSIS USING HS-GC-FID INSTRUMENT AS A PROTOTYPICAL METHOD THAT UTILIZES FOR ROUTINE WORK IN FORENSIC TOXICOLOGY

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Abstract

Background

Gas chromatography (GC) is a significant method used widely to identify and quantify different types of analytes. The combination of high ability of separation power in GC with various types of detectors makes this method an important[)] .Gas chromatography is greatly used for volatile compounds that losses the analyte during sample preparation, have low molecular mass vapor pressure (under) less than 250 ^{0}C and decompose at 400^{0}CThe aim of this theoretical and practical laboratory is to introduce fundamental principle of HS-GC-FID instrument as a prototypical method that utilized for routine work in forensic toxicology.

Objective

- *The aim of this theoretical and practical laboratory is to introduce fundamental principle of HS-GC-FID instrument as a prototypical method that utilized for routine*
- *Understand the foundation of headspace gas chromatography, explain the function of each major part in the instrument and determine the concentration of ethanol in blood as it is important factor in forensic toxicology using data obtained from analysis.*

Method

Preparing calibration in different concentration (10,25,50,100,200,300,400) mg/dl, three controls 30, 80 and 300mg/dl and unknown sample. Each have DRF number by using compudil 300 diluter hook and tucker instrument to dilute 200mgl of calibrators, control and sample unknown with 500mgl of internal standard(n-propanol150mg/dl) and put in headspace vials then seal vial after that heat and shake vials to allow internal standard and any ethanol present to equilibrate between sample and headspace. Now the calibrators, controls and sample are ready to transfer to GC instrument CLARUS 500 Cofuigured with FID ionization and Turbomatrix 110 headspace sampler. The heater is 50 ^{0} C inside the turbo and 150C is the oven temperature CALRUS. The sample stay minutes in the heater then injected to oven at 150C and the temperature is constant while all samples are going through column.

Results

Shows that two out of three controls are within the range are lying between ±2SD when compared with QC chart in the appendix and just control 80 is out of range due to consult technical manager. The R^2 value from the graph (3) is 0.9999, according to criteria of acceptability is acceptable. In case of road traffic the CV for controls 80 and 300 and for the sample unknown is 0.8579,1.4217 and 0.7156 respectively are valid due to criteria of acceptability.

Conclusion

The investigation of the unknown blood sample 13/008 that determined by GC-HS-FID have two

detectors each detector give result and both results tested positive for alcohol and the dependable result was from combine both detectors to ensure the result and that also tested positive for alcohol.

Key words: *alcohol analysis, HS-GC-FID instrument, Gas chromatography*

INTRODUCTION

Gas chromatography (GC) is a significant method used widely to identify and quantify different types of analytes. The combination of high ability of separation power in GC with various types of detectors makes this method an important [(1)].

Gas chromatography is greatly used for volatile compounds that losses the analyte during sample preparation, have low molecular mass [(2),] vapor pressure (under) less than 250 ^{0}C [(3)] and decompose at 400^{0}C[(4)] .

Archer J. P. Martine and Richard L .M. Synge had a big role in GC development, when they anticipated that mobile phase could be gas instead of liquid and in this case the separation is notably shorter and the columns much more efficient. The first paper was published about gas chromatography in 1951by Marrine and James [(3)]. Traditionally, the identification of components based on peak retention time, while nowadays base on the nature of response get from detectors. The main target of analyst are to make compound appear in a distinct peak or band without overlap with other components, and the other target is to make these bands in one shape and narrow. These goals are achieved by careful choice of stationary phase column and by optimising the functioning conditions of the column. Furthermore, the introduction method of sample into the chromatograph, suitable type of detector and improve the volatility by chemical compound for modification, all these factors play role to changing a second-rate analysis into a first-class one [(4)] .

The stationary phase in gas chromatography is a solid or polymeric liquid and called GSC and GLC respectively and the second one is the most popular. The most significant difference in GC with other method like HPLC is in the mobile phase, here mobile phase is gas as Martine and Synge suggested also called carrier gas to transport the analytes through the columns. Since Marrine and James work there have been many development in GC method as ability to separate complex sample of volatile analytes. The affinity of stationary phase with analyte and vapor pressure select the rate of partitioning

$$K_{C=}[C_S]/[C_M]$$

C_S = concentration of analyte in the stationary phase, C_M = concentration of analyte in the mobile phase. Large K_C effect to the analyte and have longer retention time, column temperature and chemical nature of stationary phase can control the K_C (distribution coefficient) [(3)].

structure of Gas chromatography

Gas Inlet: the main purpose is to filter gases to make sure of high purity of gas. Types of gases required in GC are carrier gas (N_2,H_e,H_2),make up gas(N_2,H_e,H_2)and detector fuel gas(Air,Ar,H_2,Ar-CH4,N_2).

Pneumatic controls: used to regulate the pressure or flow by regulate the gas inter into the instrument.

Injector: to inject the sample into the instrument and include many inlet types as the sample is volatilized to avoid decomposition such as split/pplitless,cool-on-column and programmed thermal vaporizing.

Columns: the column is responsible of separating analytes. Columns have different length and diameter, for packed column the standard dimension is 1.5m*4mm and for capillary column is 30m*0.32mm*0.1mm film thickness coated with immobilized liquid stationary and hallow(spread)with silica.

Column oven: is the responsible of temperature in the GC process and it heat to give good control and it is steady temperature during the GC process.

Detectors: from the physicochemical property of the analyte, the detectors can respond and by enlarge this respond can create an electronic signal to produce a chromatogram in data system, there are many types of detectors, such as Flame Ionization (FID), Flame Photometric (FPD), Electron

Capture (ECD), Mass Spectrometer (MS), Nitrogen Phosphorous (NPD) and Thermal Conductivity (TCD), the use of it is depend on application whether qualitative or quantitative data is needed.

Data system: is final steps of the process and give result that received from detectors as signal and digitizes it to form chromatogram.[3]

Headspace

Headspace gas chromatography (HS-GC-FID) is typical method has been used for identify and quantify alcohol in blood in forensic toxicology for many years [5] , the principle of headspace is depend on the release of volatile from sample into close space above the sample called headspace[6] figure1. Headspace gas chromatography is considered prototypical method for ethanol analysis because of the volatility, no need for extraction, sensitivity, specificity, accuracy and ease of automation [5]. in general HS is a suitable technique for sample has very light volatiles, is mostly used for complex matrices that can be placed directly to HS [7]. An advantage of HS technique is that these analyses can be detected without interference[8] .

This method consists of gas chromatography technique with static headspace instrument. Usually use FID detectors type.

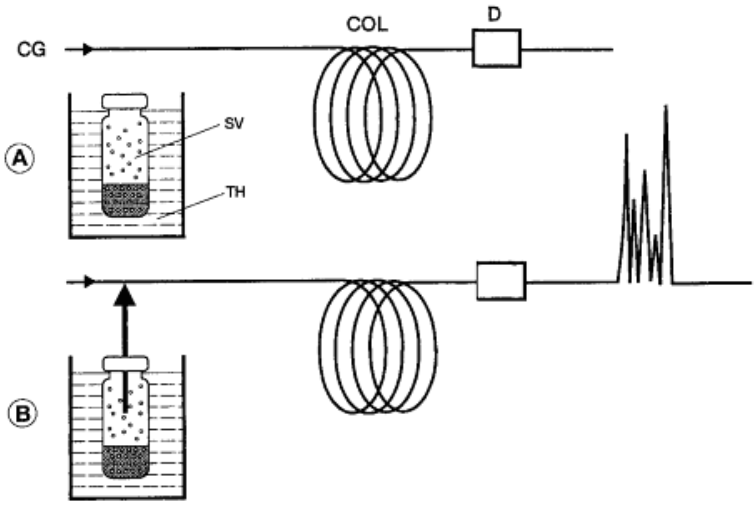


Figure 1[8] principle of static headspace-gas chromatography.(A)equilibration and (B) sample transfer. GC=carrier gas, SV=sample vial, TH=thermostat, COL=GC column, D=detector.

Method

Using internal standard in GC make it the preferable method in forensic for testing drugs and alcohol [3]. The internal standard is a drug has the same chemical characteristics to the analyte drug and it adds to a sample at beginning steps before prepared so the internal standard IS has the same for preparing and analyzing like a sample drug. The signal produce by IS and sample compare to each other to help to quantify the analyte. The ideal IS is a deuterated drug [9].

Preparing calibration in different concentration (10,25,50,100,200,300,400) mg/dl, three controls 30, 80 and 300mg/dl and unknown sample. Each have DRF number by using compudil 300 diluter hook and tucker instrument to dilute 200mgl of calibrators, control and sample unknown with 500mgl of internal standard(n-propanol150mg/dl) and put in headspace vials then seal vial after that heat and shake vials to allow internal standard and any ethanol present to equilibrate between sample and headspace figure 2. Now the calibrators, controls and sample are ready to transfer to GC instrument CLARUS 500 Cofuigured with FID ionization and Turbomatrix 110 headspace sampler. The heater is 50 ^{0}C inside the turbo and 150C is the oven temperature CALRUS. The sample stay minutes in the heater then injected to oven at 150C and the temperature is constant while all samples are going through column.

Material and Equipment [6]

Table 1 material and equipment

Materials	Description
GC Instrument	CLARUS 500 Configured with FID ionization
Headspace Sampler	Turbomatrix 110
Diluter	Compudil 300 hook and tucker instrument

Standards and controls	Concentration Mg/dl	Lot number	Expire date
CAL 1	10	FN080612-04	Checked
CAL 2	25	FN081712-01	Checked
CAL 3	50	FN010912-01	Checked
CAL 4	80	FN042808-02	Checked
CAL 5	100	FN050312-01	Checked
CAL 6	200	FN032712-01	Checked
CAL 7	300	FN121510-01	Checked
CAL 8	400	FN040909-01	Checked
Control 30		02835-4	Checked
Control 80		10844-3	Checked
Control 300		CB547-1	Checked
N. Propanol IS	150		Checked
Unknown sample	**blood sample with an unknown concentration**		
Methanol	solution to wash/clean the Compudil diluter		

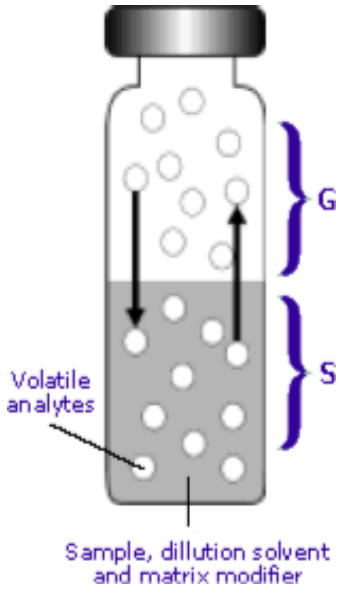


Figure 2[7] Phases of the headspace vial

Criteria of acceptability (lecture)

- **R^2** is at least **0.99**
- Control Minimum of 2 QCs must be within 2 SD for both left and right detectors. If not, consult technical manager.
- **%CV (RT ≤ 2 %, PM ≤ 10 %).**
- For **RT** If the result **≤ 100** mg/dl, **6 mg/dl** will be subtracted. If the result **> 100** mg/dl, **6%** will be subtracted, because of uncertainties of machine.

Result and Discussion

Results for right detector, table 1 provides the data of right detector for calibrations and table 2 provide result for controls and sample. The concentration of controls that is determined by the equation from graph (3)

y = 0.0046x - 0.0026 , y=PAR , X=concentration

Shows that two out of three controls are within the range are lying between ±2SD when compared with QC chart in the appendix and just control 80 is out of range due to consult technical manager. The R^2 value from the graph (3) is 0.9999, according to criteria of acceptability is acceptable. In case of road traffic the CV for controls 80 and 300 and for the sample unknown is 0.8579,1.4217 and 0.7156 respectively are valid due to criteria of acceptability, while the CV for control 30 is high therefore is not pass criteria of acceptability, the reason mostly for CV high refer to pipetting, sample poorly homogenised, and manufacture error, nevertheless, this criterion for other controls and sample for criteria passed, so this outlier did not influence the validation of the result because there were still to other control lying within the range. In road traffic case the cutoff is 80mg/dl[10]and the sample is 70.21mg/dl after subtracted therefore is not criminal offence and did not break any law. In case of post-mortem the all CVs for control and sample are pass criteria of acceptability because is less than limit of 10%.

Table 2 data for right detector calibration

Level	mg/dl	Mean PA	Mean IS	PAR
BLK	0			

CAL 1	10	1064391.5	24781412.5	0.0429512
CAL 2	25	2799729.5	24870751	0.11257117
CAL 3	50	5747005	25258306.5	0.22752931
CAL 4	80	9157578	25093258	0.36494177
CAL 5	100	11399661.5	24947342	0.45694894
CAL 6	200	23340450.5	25135710	0.92857733
CAL 7	300	34647549.5	25180648	1.37595941
CAL 8	400	46371807.5	25177431	1.8418006

Table 3 result for controls and sample for right detector

Sample	mg/dl	PAR	CV	SD
CON 30	31.6603724	0.14303771	4.94802577	179196.415
CON 80	82.2329489	0.37567157	0.85798614	82399.8603
CON300	298.842281	1.37207449	1.42177678	490483.205
Unknown	76.2126788	0.34797832	0.71562859	60703.703

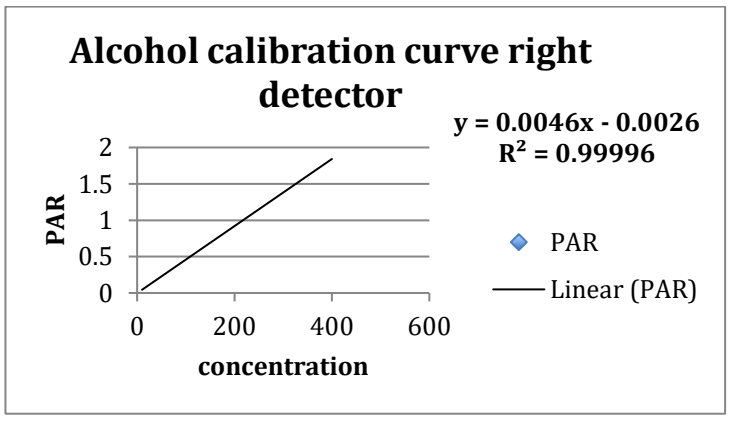


Figure 3 calibration curve for right detector

Results for left detector, table 3 and 4 provide data for left detector for calibrations, controls and sample unknown. From the graph 4 calculated the concentration of controls and sample by equation **y = 0.0047x - 0.01 ,Y=PAR, X=concentration**
show that the quality control for all controls and sample are valid and within the range of ± 2SD of QC chart in appendix. the R^2 value from the graph 4 is 0.9999 and is acceptable according to criteria. The CV for control 30 for left detector is 6.11434866% is not acceptable for road traffic case as is more than 2% but is valid for PM case, and CV for other controls and sample is undergo within the

criteria of acceptability in both case RT and PM. The concentration of alcohol is still less than cutoff limit in RT case is 70.19mg/dl.

Table 4:result for calibrations from left detector

Level	mg/dl	Mean PA	Mean IS	PAR
CAL 1	10	1064430	27465703.5	0.03875488
CAL 2	25	2976070.5	27576921	0.10791888
CAL 3	50	6326071	28010049	0.22585005
CAL 4	80	10118332.5	27746943.5	0.36466476
CAL 5	100	12732185.5	27604131	0.46124203
CAL 6	200	26206758	27892328.5	0.93956867
CAL 7	300	39115441.5	27857665	1.40411774
CAL 8	400	52412308.5	27892123.5	1.87910786

Table 5:result for controls and sample from left detector

Sample	mg/dl	PAR	CV	SD
CON 30	31.6201391	0.13861465	6.11434866	236811.475
CON 80	81.7925106	0.3744248	0.43658292	46340.95
CON 300	299.102128	1.39578	1.75450924	681838.32
Unknown	76.192178	0.34810324	0.43650161	40749.1496

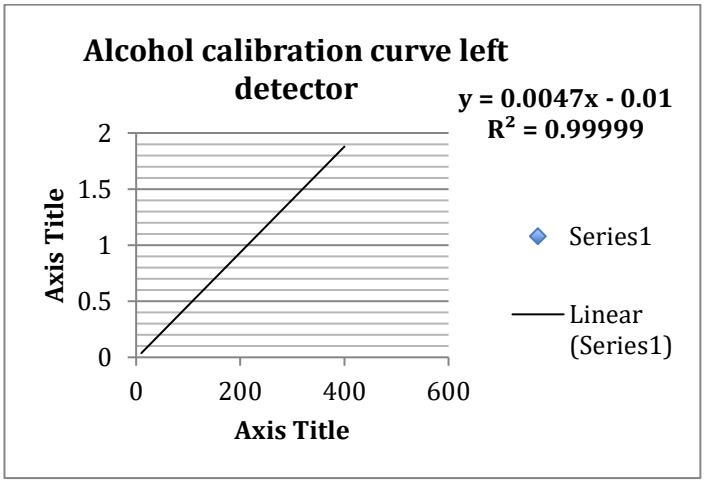


Figure 4 calibration curve for left detector

For both detectors combine, due to the fact that each sample ran in duplicate and was measured

by two detectors, four-peak area and internal stander peak area was received there should be calculate the mean for all data of both detectors to confirm the result. From table 8 shows the mean of concentrations for controls30, 80,300 and sample unknown for both detectors are 31.6402558, 82.2329489, 298.972469 and 76.2024284mg/dl respectively. The control 80 is not lying between ±2SD while other do as show in table 9 and the result is still valid as two controls are within the range and for criteria of acceptability minimum 2 controls must be within the range.

For CV of control 30 is an outlier with CV of 5.53118722% so this control do not pass the criteria of acceptability if the case is road traffic, and for other controls 80, 300 and sample unknown have CV% acceptable so one outlier sample can not effect the validation of result because there are still two other controls within the range, so the result is valid do depend on in our investigate. As known that is If the sample is part of a road traffic 6gm/dl must be subtracted for the sample concentration because of uncertainties of machine the therefore the final result is 70.202mg/dl, this concentration of ethanol in road traffic case is normal concentration as is under cutoff limit.

In post mortem case the presence of ethanol is refer to many factors, in appropriate condition ethanol can be produced in concentration up to, and afar, ethanol can also help as a substrate for many microorganisms such that ethanol concentration in blood and tissues may increase and then decrease. Production of post mortem ethanol not related with the degree of putrefaction. Many strictly decomposed sample may contain no ethanol, whereas other less strict decomposed may contain of 80mg/dl or higher. Other factors are when the stomach contains large amount of ethanol and this amount may diffuse through the stomach wall and diaphragm and enter into the heart and central blood. Sever trauma, adequate to rupture the stomach and diaphragm and may allow gastric contents to pass into the chest cavity. In such cases it may be difficult to find blood from the peripheral vessels.

Another factor is that the movement of gastric contents into the trachea and lungs and this could lead to raised blood ethanol concentration mainly in the central pulmonary and cardiac vessels and consequently to erroneous interpretation, for all these reasons a second test in post mortem cases in different specimen is required [(4)]. In general, the concentration in ethanol in sample 76.2024284 mg/dl is not fatal is just lead to Slight impairment of balance, speech, vision, reaction time, hearing and Euphoria. Reduced judgment and self-control. Impaired reasoning and memory [(11)].

Table 6:CV mean for both detectors

level	mg/dl	cv1	cv2	mean
CAL 1	10	0.5994246	0.03560678	0.31751569
CAL 2	25	1.04189623	1.38911353	1.21550488
CAL 3	50	0.20458955	0.16511641	0.18485298
CAL 4	80	1.78878925	1.8648123	1.82680078
CAL 5	100	1.58662726	2.01182055	1.79922391
CAL 6	200	0.39063109	0.76503255	0.57783182
CAL 7	300	1.90951314	2.11574482	2.01262898
CAL 8	400	2.47034449	2.43339925	2.45187187
CON 30		4.94802577	6.11434866	5.53118722
CON 80		0.85798614	0.43658292	0.64728453
CON 300		1.42177678	1.75450924	1.58814301

unkown		0.71562859	0.43650161	0.5760651

Table 7:SD mean for both detectors

level	mg/dl	SD1	SD	mean
CAL 1	10	6380.22449	379.009235	3379.61686
CAL 2	25	29170.276	41340.998	35255.637
CAL 3	50	11757.7716	10445.3814	11101.5765
CAL 4	80	163809.771	188687.909	176248.84
CAL 5	100	180870.136	256148.724	218509.43
CAL 6	200	91175.0555	200490.228	145832.642
CAL 7	300	661599.51	827582.927	744591.219
CAL 8	400	1145543.39	1275400.72	1210472.06
CON 30		179196.415	236811.475	208003.945
CON 80		82399.8603	46340.95	64370.4052
CON 300		490483.205	681838.32	586160.763
unknown		179196.415	236811.475	208003.945

Table 8:PAR mean for both detectors

level	mg/dl	PAR1	PAR2	mean 1+2
CAL 1	10	0.0429512	0.03875488	0.04085304
CAL 2	25	0.11257117	0.10791888	0.11024503
CAL 3	50	0.2275293	0.22585005	0.22668968
CAL 4	80	0.36494176	0.36466476	0.36480326
CAL 5	100	0.45694892	0.46124203	0.45909548
CAL 6	200	0.92857729	0.93956867	0.93407298
CAL 7	300	1.37595935	1.40411774	1.39003855
CAL 8	400	1.84180053	1.87910786	1.8604542
CON 30	-	0.14303771	0.13861465	0.14082618
CON 80	-	0.37567155	0.3744248	0.37504818
CON 300	-	1.37207444	1.39578	1.38392722

Unknown	-	0.34797831	0.34810324	0.34804078

Table 9:concentration means

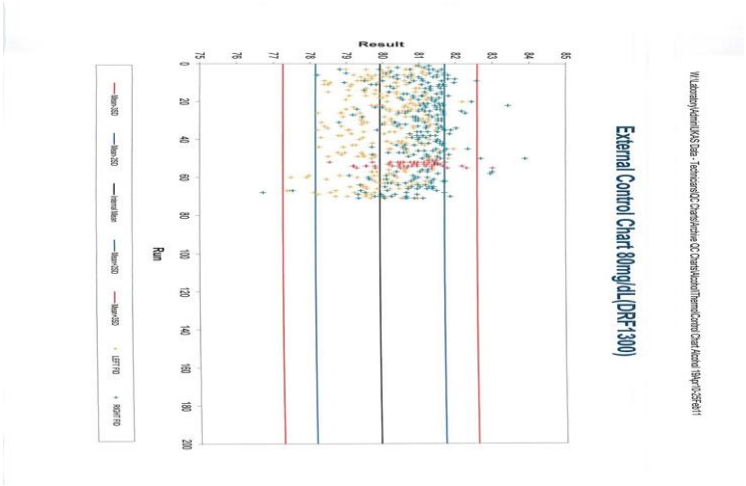
Sample	Concentration R	Concentration L	Mean concentration
CON 30	31.6603724	31.6201391	31.6402558
CON 80	82.2329489	81.7925106	82.0127298
CON 300	298.84281	299.102128	298.972469
unknown	76.2126788	76.192178	76.2024284

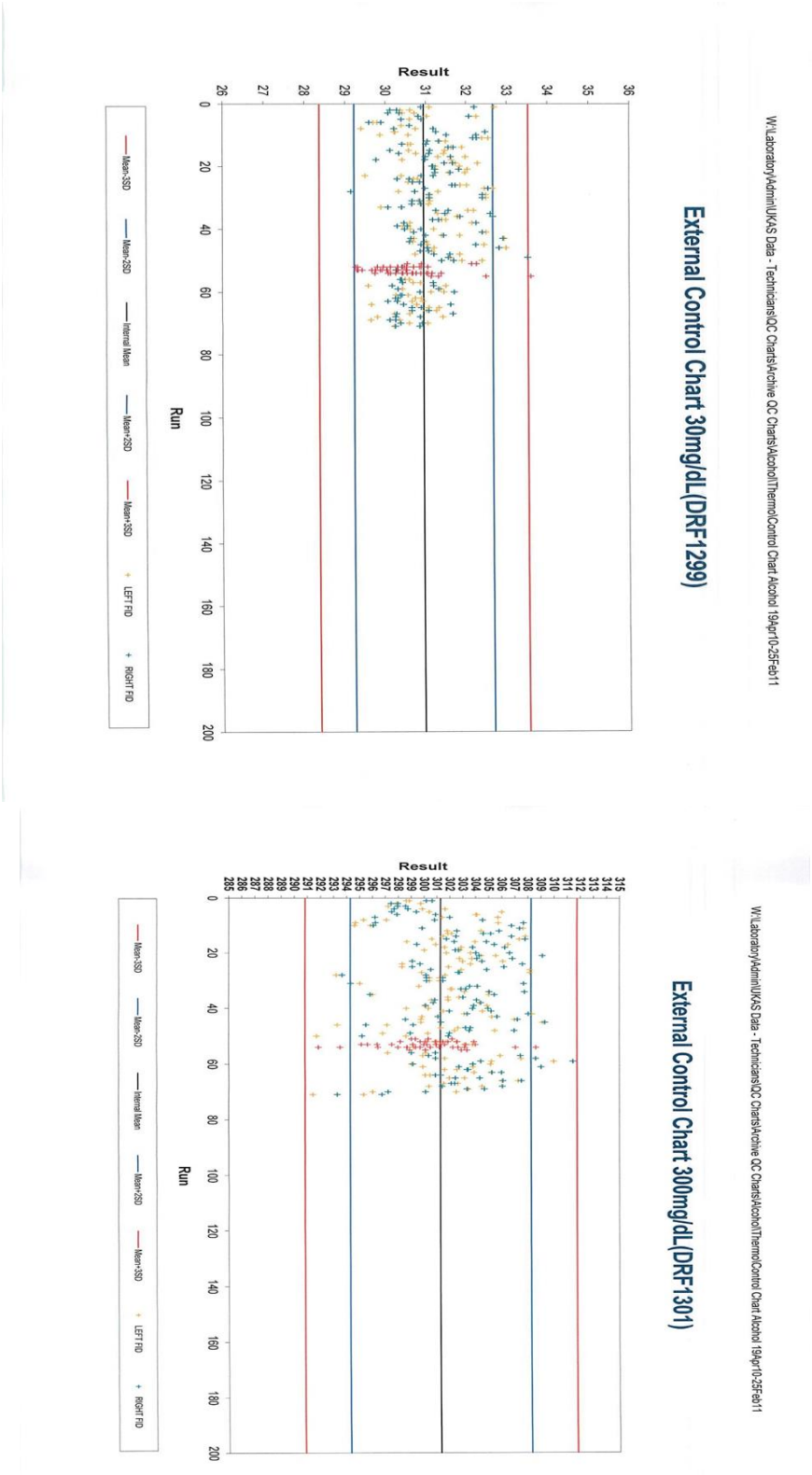
Table 10 acceptability criteria for controls

sample	Right detector	Left detector	Combine
CON 30	2SD	2SD	2SD
CON 80	>2SD	2SD	>2SD
CON 300	2SD	2SD	2SD

Conclusion

The investigation of the unknown blood sample 13/008 that determined by GC-HS-FID have two detectors each detector give result and both results tested positive for alcohol and the dependable result was from combine both detectors to ensure the result and that also tested positive for alcohol, the sample is considered to be 76.2024284mg/dl. In road traffic case the sample concentration was determined as 70.202mg/dl. Hence, at the same time of sample collection the person was under the limit of 80mg/dl which consider not above illegal limit with consider the sample just measured in duplicate not in quadruplicate so the result is not 100% valid. If the sample is belonged to a post-mortem case, all criteria of acceptability were valid.





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