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Does Pool Water Speak in Deciding Ante Mortem and Post-Mortem Suspicious Drowning

Rakhi Khanna*

Deputy Director, State Forensic Science Laboratory, India

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***Corresponding author:** Rakhi Khanna, Deputy Director, State Forensic Science Laboratory, Rajasthan, Jaipur, India

Email: rakhi_khanna@yahoo.com

Abstract

A boy aged 25years was working in a club house as a cleaner. One day in the morning hours his body found drowned in the clubs swimming pool. The pool was very deep. It was highly questioned by the family members who alleged that he was murdered by the owner of the club using drowning as mode of murder. Such drowning cases are very crucial to decide when deciding the cause of death. Viscera material was sent for analysis to Forensic Science Laboratory for the presence of poison if any. After careful examination of crime scene and modification of extraction methodologies a SOP using Gas Chromatography and Mass Spectrometry was developed to get the presence of drugs in visceral samples. The samples were found positive for the presence of drugs as Phenobarbital, Valproic acid and Sodium Valproate. These are the drugs used in treatment of patient of epilepsy. The presence of these drugs provides direction to the investigative agencies to search for the case history, it was revealed by investigation further that the boy was a patient of epilepsy and was under treatment. Medical board constituted and postmortem was conducted; presence of severe froth indicates towards death due to drowning. During Gas-chromatography Mass Spectrometry forensic findings of Triazine herbicide along with drugs was observed and it becomes highly significant in absence of diatoms test results provides important insight and another way to decide ante-mortem and postmortem drowning in sudden epileptic attack and in cases of accidental drowning in epilepsy patients.

Keywords: Epilepsy; Antemortem- postmortem drowning; Valproic acid; Phenobarbitone; Triazine

Abbreviations: H: Herbicide; GS: Gas Chromatography; MS: Mass Spectrometry; SOP: Standard Operating Procedure; GC-MS: Gas Chromatography and Mass Spectrometry; TLC: Thin Layer Chromatography

Introduction

Drowning is one of the world's leading causes of unintentional deaths. Many studies in high-income countries have documented the association between drowning and alcohol, especially among adult males [1]. A nationwide study conducted in Sweden revealed that alcohol was involved in 44% of unintentional drowning deaths [2]. On the other hand, only limited information is available as to the role of drugs other than alcohol in unintentional drowning. The effects of many psychotropic drugs on cognition and judgment are, however, similar to those of alcohol [3]. Their impact on coordination, vision, balance, and other psychomotor functions is well documented, [4] especially for vehicle driving. Case studies have likewise reported individual drowning incidents involving drugs, and surveys on fatal accidental injuries have also included general data on drowning and drugs. However, in-depth studies addressing actual drug concentrations and aiming to assess the role of psychoactive drugs as a risk factor for drowning are lacking. Death of a victim found in water should not always be related to drowning [5]. It was assumed that 10% of the drowned humans die after laryngospasm or breath-holding without aspirating fluid [6]. For the electrolytes, the diagnosis of drowning was based on changes of these electrolytes between the blood samples taken from the right versus left ventricle [7,8]. Such electrolyte shifts were described in many other causes of death and do not provide reliable evidence of drowning. If water samples are not available, it is possible to compare the diatoms found in the organs. The validity of diatoms test is questionable because diatoms are present in soil and atmosphere, and samples are easily contaminated, beside Absence of diatoms does not rule out drowning. One of the signs of drowning would be large amounts of froth present around nostrils and mouth in freshly drowned bodies. This froth is also present in the upper and lower airways. Froth can also be observed in cases of edema of left ventricular failure but in drowning cases the volume of froth is generally much more abundant than in other origins. Hence there

are many factors related to and similar in epilepsy and drowning that makes it difficult to find the cause of death. In this case study a young boy aged about 25 years was working at club house. One day during working hours, he found dead in swimming pool.

Thorough investigation shows that the pool was very deep around 7.5 feet. Postmortem was conducted and lathery froth found inside the trachea indicative of death due to drowning. Water mixed with vomit emits from the dead body, when taken out of pool was found at tiles of floor outside the pool. But it was unanswered that how it is possible to connect the symptoms and evidence surrounded at the crime scene. Till toxicological examination report from Regional FSL Ajmer cleared the probable reason of drowning that converts it towards accidental drowning. His parents along-with others family members agitate at Govt. Collectorate for punishing the owner and demanding for some handsome compensation amount. Our study suggests that in such cases of drowning decision of ante-mortem and postmortem drowning needs to differentiate. By far the collection of sternum bone and brain part in place of lungs is also compulsory to send to FSL for Diatoms analysis. In this regard the water sample of pool needs to send to laboratory for diatoms analysis. But due to lack of knowledge in management of such cases it is not taken seriously and that creates lacunae in investigation. In this case, for the first time, the toxicological findings of Phenobarbital, Valproic acid clears that victim was a patient of Epilepsy and was under treatment at that time. The toxicological analysis of Triazine herbicide in liver, stomach and other visceral organs that tallied with that of Triazine in water sample collected from pool gives insight to case mystery that death occurs after falling in pool and the deceased was a patient of epilepsy had used drugs before death. Basic concept about Epilepsy and its symptoms: As we know, Epilepsy is a neurological condition. Some symptoms of epilepsy is repeated seizures and are mainly shown as:

a. Convulsion with no temperature (no fever).

b. Short spells of blackout, or confused memory.

c. Intermittent fainting spells, during which bowel or bladder control is lost, which is frequently followed by extreme tiredness.

d. The person becomes stiff, suddenly, for no apparent reason.

e. The person suddenly falls for no clear reason.

f. Peculiar changes in senses, such as smell, touch and sound etc.

Epilepsy become itself problematic when it is near water source, because even a person know how to swim sometime, not able to swim due to extreme tiredness. A complicated case of epilepsy and its connection to drowning is more cleared via analytical approach and toxicological findings.

Material and Method

Viscera sent for examination in the Regional FSL, was consist of Stomach and piece of small intestine and large intestine, piece of liver, spleen, kidney preserved in saline solution. Clothes of deceased, Tiles of floor containing vomit of deceased, water sample collected from pool were sent to FSL for examination. All chemicals and solvents used are of Analytical grade and of Merck. Acid Ether extraction was performed for separation of acidic drugs if any [11,12]. The tissue about 100gm is cut into small pieces, macerated mixed with 100ml of 5% acetic acid and taken into a beaker. Solid ammonium sulphate is added to it by frequent shaking. Again, 20gms of solid ammonium sulphate is added. The mixture is heated on a boiling water bath for three hours. The mixture is cooled slightly and filtered. The residue on the filter paper is again extracted with two portions of 100ml of 5% acetic acid and filtered as before. The filtrates are combined and taken into separating funnel. 100ml of diethyl ether is poured on the slurry left over the filter paper and received in a cold container. The ether fraction is added to the aqueous acidic extract in separating funnel and shaken for 5 minutes. Ether layer is separated and 100ml of ether is again added to the acidic layer shaken for 5 minutes and separated. Both ether layers are combined. The acidic extract is tested for drugs. The acidic ether extract is separated into three fractions; the first portion is shaken with 25ml of 5% sodium bicarbonate solution. The aqueous layer is removed taken into another separating funnel and is acidified with dilute sulphury acid and re-extracted with 25ml of ether. This ether fraction is passed through anhydrous sodium sulphate and dried just to dryness. The ether layer after washing with sodium bicarbonate is extracted twice with 25 ml portion of 1N sodium hydroxide. The aqueous layer is separated from ether layer combined and taken into another separating funnel. Made acidic with dil. sulphury acid and extracted twice with 25 ml portion of ether. This ether fraction is washed with 25ml of water and then dried passing through anhydrous sodium sulphate then evaporated to dryness. The residue contains barbiturates in relatively purified form. The ether layer after extraction with sodium hydroxide is washed with water and then evaporated to dryness. The residue may contain meprobamate, other carbamate and other neutral drugs.

Extraction of Insecticides

Viscera and other articles were processed using sodium sulphate and acetone. Kept and heated for a period of one hour at water bath. Using principle of partial solubility of extractants in double liquids, then filtration was done, extraction was performed using separating funnels and Hexane was added. Hexane extract was separated and dried. Acetic acid ether extract was taken, and TLC performed using binary solvent system systems for TLC [11,12].

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Herbicide

Substituted phenoxy acids are commercially used as herbicides. Most of the commercial triazines pesticides are tri-substituted 1, 3, 5-triazines [12]. Solvent System-1 Chloroform: Acetone 60:40; Solvent System-2 Hexane: Acetone 80:20 is used for developing TLC. The plates were spryed using Dragondorffs and Palladium chloride sprys separately. Spots were observed but not confirmed with any pesticide. To get it confirmed Gas chromatography and mass spectrometry is done. SOP is developed to get separation of drugs and herbicide in the GC column [11,12] Figure 1-7.

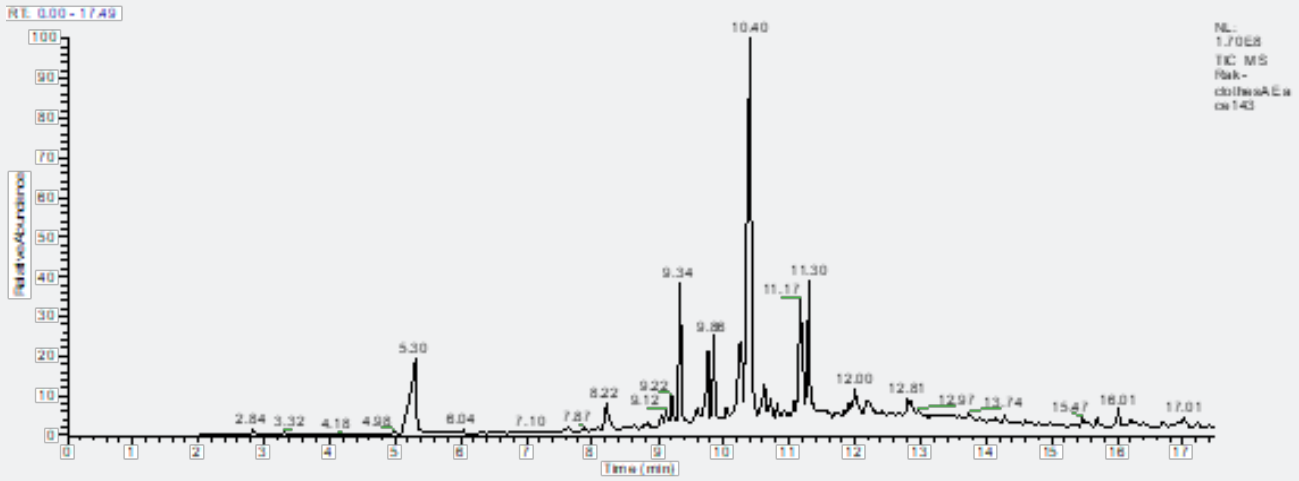

Figure 1: Gas chromatogram of washing of clothes of deceased shows peak at Rt- 5.30 and 10.40.

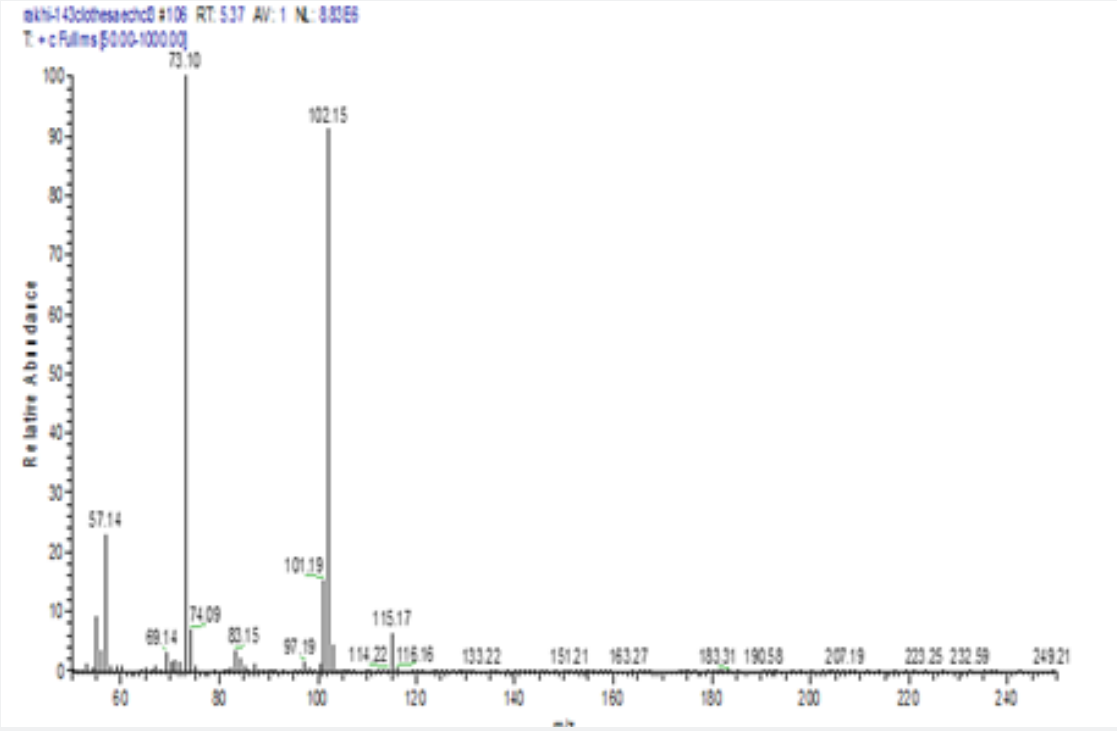

Figure 2: Mass Spectrum of peak at Rt-5.30 shows m/z values at 102,73,57,74,115 tallied with mass fragments of Valproic acid i.e Pentanoic acid.

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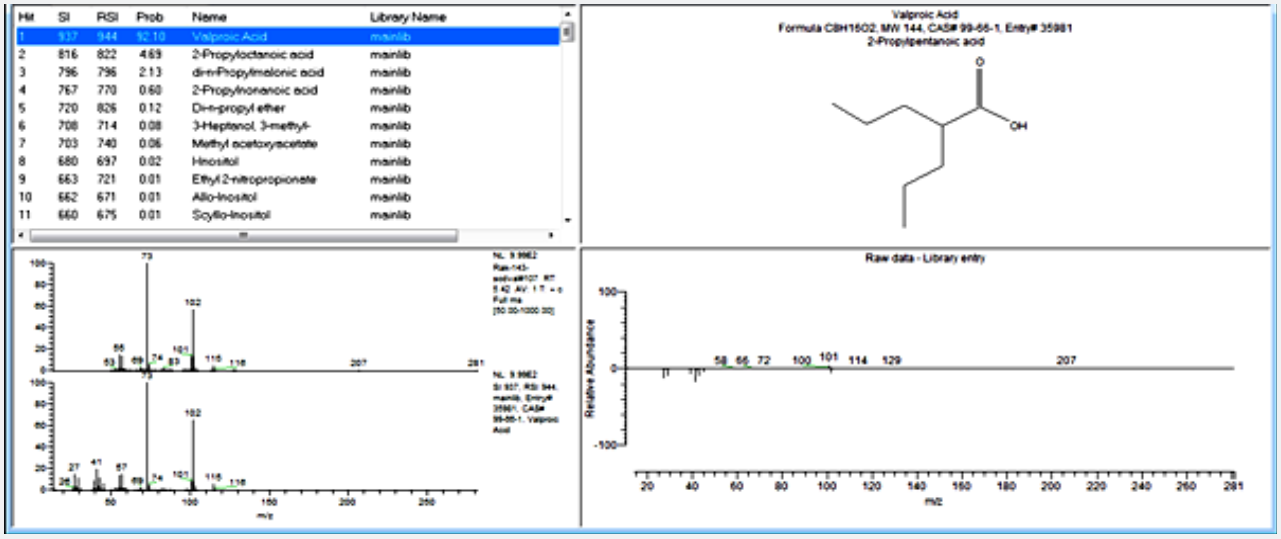

Figure 3: Mass spectrum of Peak at Rt-5.30 Gives Probability match of 92.37 with valproic acid.

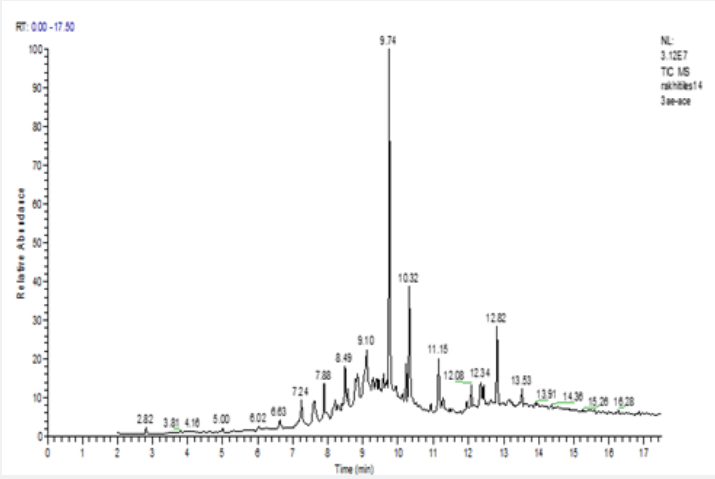

Figure 4: Gas chromatogram of washing of tiles contains omitted material acidic ether extract shows peak for 2-pentanone 4 hydroxy-4 methyl metabolite shows phenobarbitone.

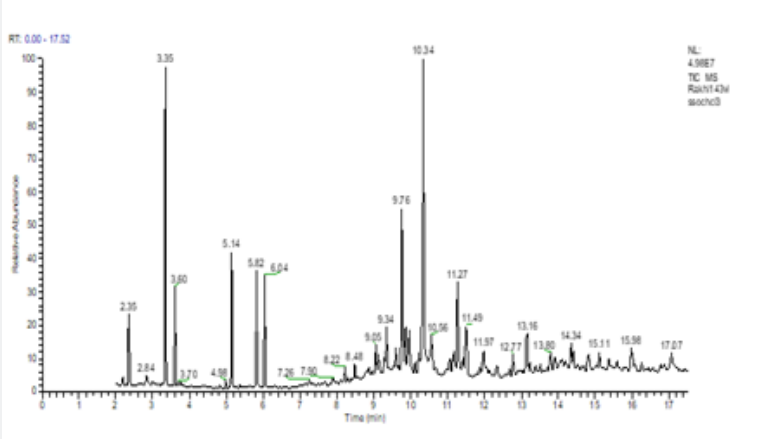

Figure 5: Gas Chromatogram of visceral extract in chloroform ether system of Phenobabitone, m/z 239,241,178,143,62,87,75,90,89,63.

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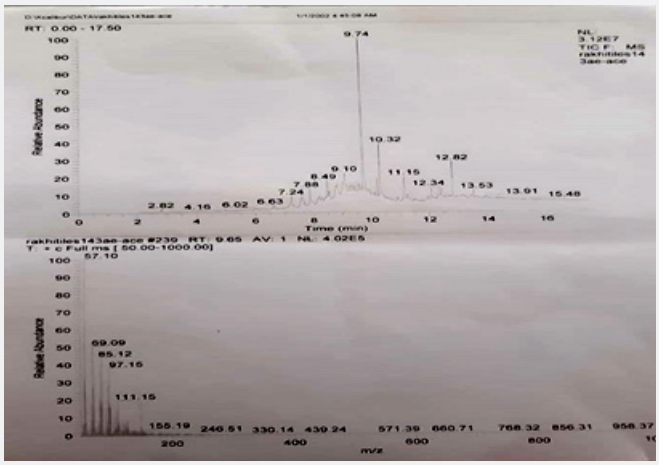

Figure 6: Gas chromatogram and Mass Spectra of extacts of Tiles shows peak at Rt- 9.74.

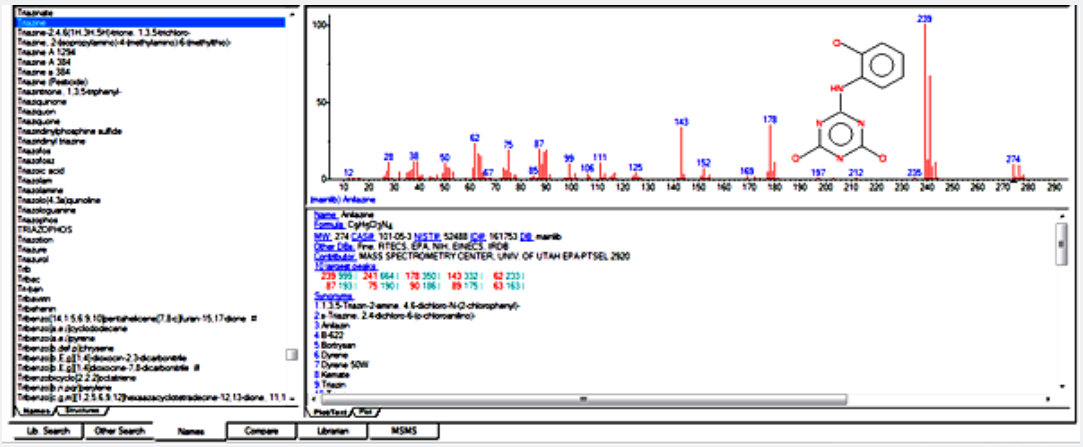

Figure 7: Mass Specta Library match tallied well with Triazine.

Phenobarbitone

Spots were observed after sprying the plates with potassium dichromate and acid.

Color Tests: Koppanyi-Zwikkers Test-Violet, Liebermann's Test- Red Orange, Mercurous Nitrate –Black [12] is obtained with acid ether extracts. TLC – System TD- chloroform: acetone [4:1] spot at 47, TE-ethyl acetate: methanol: strong ammonia spot at 28, TF- ethyl acetate spot at 65 for Phenobarbitone [12].

Spay reagent: Mercurous Nitrate Reagent; Mercuric chloride –Diphenyl carbazone Reagent; Zwicker's Reagent. Valproic acid and sodium valproate is anticonvulsant drug [12].

Color tests: Ferric chloride gives Orange color with Sodium Valproate [11,12]. As it is mentioned that sodium valproate spot remains at Rt-00 in TD, TE and TF solvent system. Hence For confirmation the dried extracts were taken for GC-MS analysis [11,12].

a. Instrument: Trace DSQ-Mass Spectrometer, Trace GC-2000 from Thermofinnigan, Italy.

b. Method: GC Oven temp starts from 60°C then hold time 1-min, followed by Rise in temp 20°C, till 300° C, Hold Time -4min.

c. General Parameters, Prep Run Time -10.00, Equilibration - 0.50, Max temp -350°C.

d. Injection temp split -280°C, Split flow/min 20, Split Ratio-20, Right Carrier-flow-constant- flow-1ml/ min, Ion Source Temp-200°C, Mode full scan 50-1000.

Column: TR-5MS, 30m x 0.25mm x 0.25um specification 5% diphenyl and 95% dimethylpolysilioxane.

Result

After getting these results the water sample from pool was checked extracted in similar way and Peaks of Triazine was observed in the water sample of pool (Table 1 & 2).

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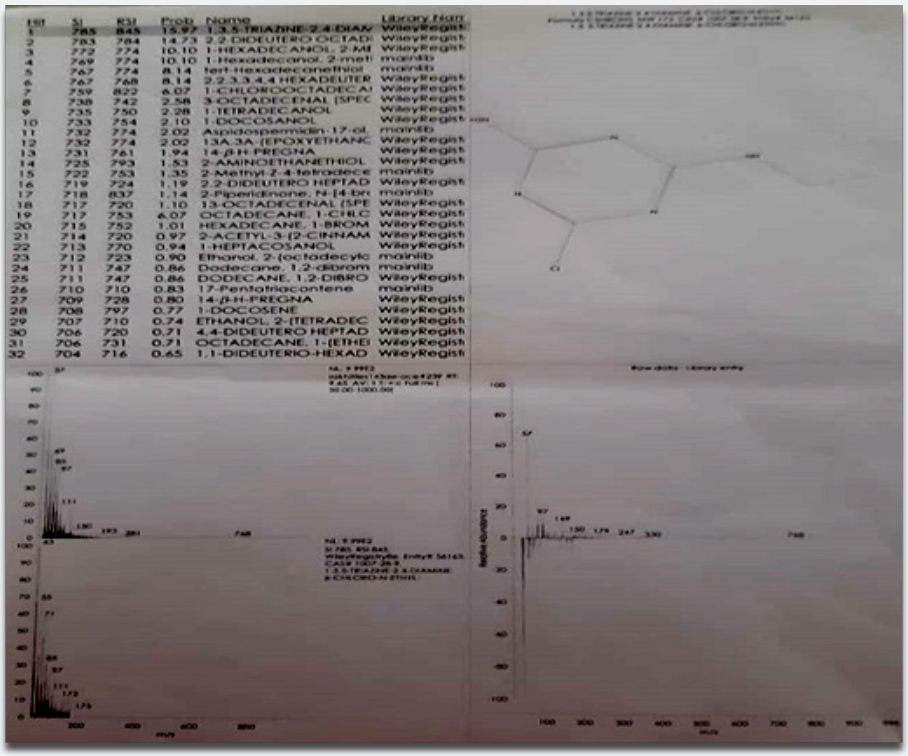

Figure 7a

Table 1: Shows type of content and tests performed and compared.

S.No	Content Tested	Technique	Diatoms Tests
1	Stomach	TLC, GC-MS, COLOUR TESTS	Not done
2	Small intestine	TLC, GC-MS, COLOUR TESTS	Not done
3	Large intestine	TLC, GC-MS, COLOUR TESTS	Not done
4	Liver	TLC, GC-MS, COLOUR TESTS	Not done
5	Spleen	TLC, GC-MS, COLOUR TESTS	Not done
6	Kidney	TLC, GC-MS, COLOUR TESTS	Not done
7	Blood	TLC, GC-MS, COLOUR TESTS	Not done
8	Clothes	TLC, GC-MS, COLOUR TESTS	Not done
9	Tiles	TLC, GC-MS, COLOUR TESTS	Not done
10	Water of pool	TLC, GC-MS, COLOUR TESTS	Not done

Table 2: Shows presence of valproic acid, phenobarbitone and triazine.

S.No	Content	Valpoic Acid	Phenobabitone	Triazine Hebicide
1	Stomach, Intestine	+++ve	++ve	+++ve
2	liver	+++ve	++ve	+++ve
3	Spleen	+++ve	++ve	+++ve
4	Kidney	+++ve	++ve	+++ve
5	Blood	+++ve	++ve	+++ve
6	Water	-ve	-ve	+++ve
7	Clothes	++ve	++ve	+++ve
8	Tiles	++ve	++ve	+++ve

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Discussion

Presence of common epileptic drugs Valproic acid, Phenobarbitone etc., used in Epilepsy treatments are found present in viscera and blood and washings of tiles and clothes of deceased. It indicates towards condition of boy before death that the boy was a patient of epilepsy. Common symptoms of Epilepsy seizures are cleared after getting treatment paper from hospital of the deceased. At this time due to toxicological findings it was clear that boy had taken drugs before drowning, but how did it happen. If he was murdered outside the pool and then put in the pool the forensic findings will be different to those if he falls and drowned. But during GC-MS ANALSIS the findings of specific molecule of triazine in visceral content and specifically in liver content and blood it makes the findings interesting and significant to search for same moiety in pool water. Surprisingly, pool water analysis shows presence of Triazine molecule. Swimming pool waters are often treated with herbicide to make the water clean from time to time. General Agents are Triazine is one among them. The presence of this chemical clearly indicates that the male fall in swimming pool and death occurs due drowning clears postmortem drowning. Generally in such complicated cases we approaches towards identifying various symptoms like frothing etc. and diatoms studies are studied, but all these are common in some diseases and still not decisive, hence when evidences based on diatoms are unavailable, even after then correlation of presence of dissolved chemicals especially herbicide/insecticide can be checked. In this case identification of Triazine herbicide provides a new way of solving such cases. The identification of Triazine herbicide in visceral content and in pool water not only solves typical case but gives new significant approach to solve such cases without going for diatoms analysis.

Conclusion

The correlation of alcohol and drowning emerges in several studies, but the effect of drugs' contribution to drowning has not been extensively studied [5]. In our study, we focused on psychotropic drugs (used as treatment in Epilepsy) and their potential involvement in unintentional drowning. Cumulative presence of the medicinal drugs, and their influence on drowning was evaluated. A better understanding of the actual role of psychotropic drugs would result from analysis, in the context of a safety investigation, of all the events underlying and leading to a single drowning event. In this case the analysis of valproic acid and sodium valproate, phenobarbitone and Triazine Herbicide was reported. Insecticides are major cause of poisoning and death but in this case, it was not the cause of poisoning but presence of herbicide Triazine was found which was not a lethal one [9,10]. Police investigation also queried and found that he was taking medication since some months or period before his death. However, the presence of drug and metabolites indicates that he was under treatment of epilepsy but the presence of Triazine herbicide strongly clears that the death occurred after falling and drowned in the pool. It was cleared that the water of pool contains herbicide, and that herbicide was found in the vomit material, viscera and blood sample. The case was very typical but the findings of valproic acid and Phenobarbitone drugs given for treatment of epilepsy strongly recommends the possibility of epileptic attack and fall in the pool accidentally as in day time when sun starts shining the patients have enormous possibility of attack and for a moment unbalancing may cause accidental fall . As there were no sign of struggle and no evidence of postmortem drowning the possibility of murder was rare. There was no other reason found of his death. In this regard the toxicological findings provide concrete base of the cause of death. Finally, based on our observations and case study, specific evaluation of toxicological findings becomes significant in victims of unintentional drowning, as well as the victims of unintentional injury deaths in general and those of intentional drowning. Even after conducting postmortem often the question remained unsolved in absence of chemical analysis. The actual cause of death and scenario of crime becomes clearer when chemical analysis and forensic aspect are considered, and significant results provides directions to investigation and allegations imposed on innocent can be removed. A new approach to solve cases of drowning by analyzing content of viscera and chemicals goes inside the body during drowning. In this case findings of dissolved herbicide of the pool water in the visceral content turns a typical case of drowning from homicidal postmortem drowning into antemortem accidental drowning. It becomes a rare case where due toxicological analysis three important aspect of forensic examination was explored.

a. Presence of same herbicide in water sample of pool and visceral samples was only detected via toxicological and instrumental analysis. Even if diatoms examination results are unavailable than also, we can explore type of drowning.

b. Toxicological examination goes into depth study of drugs like sodium valproate, valproic acid and phenobarbital often used for epileptic patients in viscera, washings of clothes and tiles etc proves that deceased was a patient of epilepsy and was under treatment and had used drugs before death.

c. There is similarity in postmortem symptoms of patients of epilepsy, other diseases and that of drowning, in such crucial condition specific methodologies were designed and Gas chromatography Mass spectrometry findings proves that not only the drugs were analyzed in a complete unknown case but forensic crime examination and toxicological approach provides new direction in cases of drowning after findings of herbicide in the pool water as well as in visceral contents significantly decide the cause of death and modus operandi of the case.

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