# Brazilian Journal of Forensic Sciences, Medical Law and Bioethics



Journal homepage: www.ipebj.com.br/forensicjournal

# Importance of Ultra-pure Water for Sample Collection in Vitriolage Cases- Forensic Concern for Corrosives Examination

Pallavi Choudhary<sup>1,\*</sup>, Kanak Lata Verma<sup>1</sup>, Rohan Verma<sup>2</sup>, Rachna Chandela<sup>1</sup>

<sup>1</sup> Regional Forensic Science Laboratory, Chanakya Puri, Govt of NCT Delhi, New Delhi, India <sup>2</sup> Thermo Fisher Scientific India Pvt.Ltd., India \* Corresponding author. Email: pallavi.choudhary23@gmail.com

Received 5 May 2020

**Abstract.** Vitriolage, a heinous crime committed with intention to disfigure a person has come into view as an extremely brutal gender-based crime especially against young women in India and also another part of world mainly in South Asia over the past few years. India is facing an alarming situation of crime against women such as rape, domestic violence, dowry deaths etc. In recent year's cases of vitriolage are on the rise in India. By understanding the gravity of crime, section 326A and 326B was incorporated in the prevailing Indian Penal Code and came into effect from 3 February 2013. Forensic Science Laboratory, Delhi receives a number of cases pertaining to acid attacks every year. We present here five such cases which were received for forensic examination. The exhibits were analyzed using Ion Chromatography apart from classical tests in order to strengthen the forensic reports and play vital role in conviction of the accused. This paper encompasses the necessity and importance of packing of exhibits and role of investigating agencies/police in collection of exhibits, which play crucial role in proving the sanctity of the forensic examination.

Keywords: Vitriolage; Forensic; Exhibits; Ion-chromatography; Ultra-pure water; Police.

# 1. Introduction

Vitriolage or acid attack, an atrocious crime is an act of projecting acid which includes sulphuric acid, nitric acid, hydrochloric acid, hydrofluoric acid, phosphoric acid or another corrosive agent like alkali, juice of semicarpus on the body of human being especially women of age group 18 to 25 with purpose of mutilation of face and other body part which is mainly an act of revenge resulting into sometimes complete

disfiguring of face, burning of skin, hair, connective tissues, private parts and at times some bones as well<sup>1-3</sup>. There can be innumerable motives behind the heinous crime against women including rejection of marriage proposal, a failed love affair, denial of sexual favor or gratification, one sided obsessive love, revenge, jealousy, property dispute, dowry, other marital disputes etc<sup>1,2,4</sup>. Some mentally deranged men seem to adopt the dreadful act of vitriolage as a most rewarding method of slaughtering eves to boost their male ego<sup>1</sup>.

This frightful crime is not only restricted between the boundaries of India but has been committed against women across the world predominantly in South Asian countries like Pakistan and Bangladesh<sup>4</sup>. Reports of vitriolage have also been documented in UK, USA, Jamaica, Iran, Taiwan, Nepal, Colombia, China, South Africa, Saudi Arabia, Nigeria, Cambodia and Uganda<sup>5-7</sup>.

This brutal crime has shocked European countries also and revealed a deepseated culture of violence against women<sup>8</sup>. India scenario getting worst day by day, a Russian tourist encountered this unfortunate incident because she denied marriage proposal<sup>9</sup>. Time to time Indian girls come across talibanic decisions of sick mentality of certain section of societies like in Jharkhand there was a poster put on public display to warn the girls to not to wear jeans and other modern clothes otherwise ready to face serious consequences like acid attacks<sup>10</sup>.

According to Acid survivors' foundation India, 1500 people per year are affected across the world<sup>5</sup>. Data intelligence unit (DIU) of India Today has found that between 2014 and 2018, there have been 1483 victims of acid attacks in the country, which is in accordance to data released by National Crime Records Bureau. In 2017 and 2018, total 596 acid attack cases with 623 victims were reported but only 149 people were charge-sheeted in each year<sup>11</sup>.

Acid burns skin and flesh causing severe pain; sometimes it melt bones also. Severity of wound depends upon the concentration of acid and the exposure time. If acid is splashed over face it causes intense burning, and destroys facial features – eyelids, nose, lips, skin, bone on the skull, forehead, cheeks and chin. In the majority of cases, the targeted area is the face; nonetheless, some unusual cases, in which private parts such as vulval region were targeted, are also reported<sup>12</sup>. It causes blindness, deafness, etc<sup>1,13</sup>.

Consequences of such kind of crime are not only physical but also psychological, social and economic. In our society, victims are not acceptable and treated as guilty, which causes psychological problems in victims such as depression, insomnia, loss of self-confidence, fear of outside world. They lose their jobs as no one wants them as employees<sup>1</sup>.

By understanding the austerity of the issue, section 326A and 326B were incorporated in the prevailing Indian Penal Code and came into effect from 3 February 2013<sup>1</sup>. The FIR is registered under Section 326A and pertains to voluntarily causing grievous hurt by the use of acid; on the other hand, 326B pertains to voluntarily throwing or attempting to throw acid in someone else. They say that the First Information Report (FIR) should be registered by the victim's family; if it has not been done at the time of victim's hospital admission, the doctor is the responsible for informing the police. Further the trials take place at fast track courts and are to be decided within 6 months.But even after 8 years of introduction of 326 A and 326 B and recent introduction of 'The Prevention of Acid Attacks and Rehabilitation of Acid Attack Victims Bill, 2017, acid attacks still prevail in society and we come across such disheartening news very often. Despite the aforesaid the steep rise in the number of cases continues. The traumatic trials and lack of legal aid lawyers to assist survivors are the challenges that exist even today when it comes to implementation of the law.Recently two women were severely injured when an unidentified youth threw acid on them in a district of Haryana. Not only this, an 8 year old girl in Delhi's Shiv Vihar, sustained an injury in her right eye and severe burns on her face as of communal violence and riots that happened recently<sup>14-16</sup>. Rehabilitation programs by various organizations are operative these days and give a ray of hope to acid attack survivors to earn livelihood and lead a normal life. Some acid attack survivors are helping to rehabilitate other victims by their NGOs while some organizations are raising funds to launch initiatives that could make acid attack survivors earn their livelihood, thus saving them from emotional trauma and social stigma that follows an acid attack. Orange Café and Restaurant is one such example that is owned and run by acid attack survivors<sup>17,18</sup>. The reasons for such huge figures of acid attack are many: the patriarchal mindset, psychological immaturity, inability to accept denial and rejection, jealousy, hatred etc. The figures can be lowered by quick medical and forensic examination of victim, speedy trials and stringent laws with strict implementation. "A 21 year old acid attack survivor said that she was attacked by her husband and his parents when she failed to fulfill the dowry demands raised by them back in 2008. She was held down and acid was poured on her face. She was

struggling to get her attacker punished since 2008 and when years after a court sentenced him, it was only for 7 years and he is still out on bail and roams freely" <sup>1,16,19</sup>.

Forensic Science Laboratory, Govt. of NCT of Delhi receives a number of cases pertaining to acid attacks every year. We present here five such cases (Table no.1) which were received for forensic examination. The exhibits (clothes, skin, hair, footwear, soil etc.) were analyzed using Ion Chromatography apart from classical tests in order to strengthen the medico-legal reports and played vital role in conviction of the accused. The important analytical and instrumental techniques and their significance are mentioned below in nutshell.

Case	History	Injuries
no.		
1.	A 21 year old girl met an unfortunate acid attack while she was walking back home after completing her shift of work. On her way two bike riding boys crossed her and the boy sitting behind threw a corrosive agent on her.	Acid burn wound were found over her right arm, forearm, neck, cheeks and mandible.
2.	A 23 year old girl (student) was going to attend her English speaking classes in morning; two boys on motor cycle came and threw acid on her.	Mild burning on both legs anteriorly and laterally.
3.	On receiving information of acid throwing, when IO reached the scene of crime it was found that one girl and a women were taken to the hospital as they were attacked by two persons using acid.	Multiple superficial burns over both arms, right and left side of face, both foot and both legs of one victim and multiple superficial burns over breast were found on other victim.
4.	An information was registered by police that a 35 year old lady who worked as a sweeper at a hospital was poured with acid by her husband	Superficial burns all over body, face & chest wall, breasts, anterior abdominal wall, upper back, upper limbs and small patchy burn over ears were found.
5.	Information was registered by police that a lady was attacked by a person using urine mixed with acid by the intention of harming her.	Superficial burns on legs.

Table 1. Live case history of five cases received at FSL.

- **Titration:** Neutral pH water is required for titration to determine the precise values for different analysis of cations and anions present in water in order to get an idea about the presence of acid and bases in water<sup>20</sup>.
- HPLC/LCMS Analysis: In Forensics or any kind of sensitive analysis, Water is both the most widely used analytical laboratory reagent and the least-well characterized. While chromatographers take great care to assure the purity of salts, organic solvents, and other HPLC mobile phase components, they often take water quality for granted. High-purity water comprises by far the largest

mobile phase component for most reversed phase HPLC and LC-hyphenated methods. Because of its wide utilization and because of the volumes used in sample preparation and liquid chromatography, extreme care must be taken with the water quality. HPLC performance has been constrained by a system pressure limit of 6000 psi in most systems, which limited the particle size to 3 µm or larger, thereby limiting the speed and efficiency of separation that could be achieved. With the advent of UHPLC systems, the following benefits offered by smaller particle size can be achieved: higher resolution, faster analyses by using higher flow rates or shorter columns, higher sensitivity because the peaks are taller and narrower, easy method transfer from standard HPLC within the same column family, less eluent consumption per sample because equilibration and run times are shorter. So it's recommended to use high-purity water with the resistivity of 18.2 and TOC of less than 5 ppb so that no interferences would be the part of analysis which has been introduced by water<sup>20</sup>.

- Ion Chromatography (IC): Water samples collected for analysis by IC ideally should be collected in plastic containers, such as polystyrene or polypropylene bottles, as glass bottles can contribute ionic contamination when performing trace analysis and we have already described in detail the implications of this technique pertaining to its relevance in examination of exhibits related to vitriolage cases. So we consolidate the water quality has to be of very high quality like Ultra-pure Water to carry out such analysis and sample collection and preparation should only be in Ultra-pure water<sup>21</sup>.
- Molecular Biology: In forensic experiments, sensitive biological experiments, such as cell culture, require Ultra-pure water, free of contaminants like pyrogens. To carry out such analysis, water has to be pyrogen-free water, Technology present inside the water purification system, an initial germicidal 254 nm UV lamp is installed in-line in the pre-treatment system, and a second germicidal 254 nm UV lamp is set up in the storage reservoir. The role of both these lamps is to control and prevent bacterial contamination in the water purification chain. A dual wavelength [185 /254 nm] UV lamp is incorporated in polishing systems to reduce the TOC level via photo-oxidation processes. This UV lamp also emits UV at 254 nm and, therefore, additionally has a germicidal effect. Finally, monitoring of TOC levels is also performed by a dual wavelength UV lamp, installed at the outlet of the water purification system to ensure water quality. So carry out any molecular

biology experiment with pyrogen free application, it's recommended to use highly pure water<sup>22</sup>.

This paper encompasses the necessity and importance of controlled samples treated with the quality of water used in analysis, importance of packing of exhibits and role of investigating agencies/police in collection of exhibits, which play crucial role in proving the sanctity of the forensic examination. In the current study we are trying to bring focus towards the sample collection and importance of water in the entire process of sample analysis which starts from sample collection. We would be discussing the various cases having sample collections displaying importance of sample collection and becomes area of prominence. All the five cases were registered under section 269/270/294/323 IPC. On detail study of alleged history it was found in all cases, accused were known to the victim and there were earlier threats to kill them or disfigure their face by using acid.

In this study, we have tried to bring out the impact of sample collection with the right quality of water from the site where the alleged vitriolage took place, on the results of exhibits and finally to the implication it has on the victim/accused.

#### 2. Materials and methods

#### 2.1 Reagents and apparatus

Chemicals: Sodium hydroxide, Nitric acid, Hydrocholric acid, Sulphuric acid, Ammonium hydroxide, Silver nitrate, Barium chloride, Diphenylamine and Ultra-pure (Milli Q) water were obtained from MERCK. Glassware: Beaker, Conical and Funnel were obtained from BOROSIL.

# 2.2 Sample handling and preparation

The primary concerns when collecting samples for analysis using any measurement technique is that the sample collected is representative of the total sample matrix, and that no contamination occurs during the sampling process. Also, appropriate storage and preservation of the sample is required, in order that the final sample analysis is representative of the analyte concentrations present when the sample was originally taken. Solid samples, such as soils and sludge, are not directly amenable to IC analysis and require additional sample pretreatment. The choice of

extracting solution is dependent on both the sample matrix and the nature of the solute ions.

#### 2.3 Extraction from clothes

First possible area of having acid was identified and then cut 125mm piece from several places and chopped/shredded into fine pieces. After that chopped pieces were dipped into 20ml of Ultra-pure Water (Milli Q) at room temperature for overnight. Sample was filtered by using whatman no. 42 filter paper.

Other exhibits (bag, slippers, stone, mud etc.) were also extracted by above said procedure. After filtration all the exhibits were kept at 4<sup>o</sup>C and for stabilizing by NaOH at pH 12 for further analysis (holding time of 20-30 days).

# 2.4 Preliminarychemical tests<sup>23</sup>

#### 2.4.1 Test for chloride ions: silver nitrate test

1 ml of extract was added into 1 ml of  $AgNO_3$ solution, Yellow precipitate was obtained which was Insoluble in dilute  $HNO_3$  and soluble in dilute  $NH_3$  or KCN solution indicates presence of chloride.

#### 2.4.2 Test for sulphate ions: barium chloride test

Few drops of dilute HCl and 2 ml. of BaCl<sub>2</sub>solution were added to 2 ml of filtrate then white precipitate was obtained which was insoluble in warm dilute HCl and in dilute HNO<sub>3</sub> but moderately soluble in boiling concentrated HCl showed the presence of sulphate lons.

# 2.4.3 Test for nitrate ions: diphenylamine reagent test

Small amount of diphenylamine reagent was taken in a test tube then small portion of the filtrate was added carefully to the side of the test tube, blue ring at the junction indicates the presence of nitrate.

#### 2.4.4 pH paper test

The pH paper was soaked in ultrapure water and then again dipped in the ultrapure water extract of the exhibits. The pH less than 7 indicates the presence of acid.

# 2.4.5 Litmus paper test

Again for confirmation of presence of acidic moiety the blue litmus paper was dipped in ultrapure water extract of exhibits. The change in color from blue to red indicates the presence of acid.

# 2.4.6 Test for sodium ions: uranyl zinc acetate test

Small amount of filtrate was taken in a test tube then neutralized it with acetic acid. After that, few drops of uranyl zinc acetate reagent was added, then stir with the glass rod. Absence of yellow colored precipitate indicated that sample did not contain sodium ions. (Presence of yellow precipitate indicates sodium ions).

# 2.4.7 Test for potassium ions: dipicrylamine reagent test

The filter paper was soaked in sodium dipicrylaminate reagent and dried. After that a drop of neutralized filtrate was placed. Then paper was put in 0.1N Nitric acid. No formation of red flakes or ring at site of the spot indicates absence of potassium. (Presence of red colored ring of formation of red colored flakes at the site of spot indicated presence of potassium).

# 2.4.8 Test for lead ions

One ml of dilute nitric acid and one ml of potassium iodide solution were added into one ml of filtrate in a micro test tube. Absence of bright yellow precipitate indicated absence of lead ions (a bright yellow colored precipitate appears, on boiling, precipitate dissolves out and on cooling golden yellow colored spangles appearsindicates presence of lead). Test for lead ion is carried out especially in case battery acids are used.

# 2.5 Instrumentation

# 2.5.1 Ion chromatography<sup>24</sup>

Conductivity detection: Conductivity detection is a non-selective detection principle; in this case both direct and indirect detection determinations are possible. As aqueous electrolytes are frequently used as the mobile phase in ion chromatography, the detector must be able to respond to the relatively small changes in the total conductivity of the eluent caused by the analyte ions. By the use of so-called suppression techniques the inherent conductivity of certain eluents can be dramatically reduced in the case of strong acid anions it is thus possible to considerably improve the sensitivity.

Table 2 shows the requirements for sample preparation and the holding times for cations and anions usually determined by IC.

Anions/Cations	Sample preservation	Holding time (days)
Bromide	None required	28
Chloride	None required	28
Fluoride	None required	28
Nitrate	Cool to 4 °C	2
Sulfate	None required	28

**Table 2.** Sample preservation and holding times for anions and cations commonly determined by IC.

Holding times can be increased by adjusting to pH 12 with sodium hydroxide (solution to be prepared in Ultra-pure water).

Filtration: As is the case with all liquid chromatographic methods, samples analyzed by IC should be free of particulates to avoid blockages or damage to connecting tubing, column end frits, and other hardware components. Samples are typically filtered through a 0.45 mm (or less) membrane based filter. Rinsing filters with 20mL of deionized water prior to filtration of the sample has been shown to remove most inorganic contaminants.

#### 2.5.2 Measurement with chemical suppression parameters

The following parameters have been set for measurements:

Column used: is Column v 6.1006.430 Metrosep A Supp 4 and Eluent used is 1.8 mmol/L Na2CO<sub>3</sub> / 1.7 mmol/L NaHCO<sub>3</sub> + 2% acetone. Conductivity (after chemical suppression) is approx. 14  $\mu$ S/cm. Certified reference standards TraceCERT@) are used for analysis: fluoride, chloride, nitrite, bromide, nitrate, phosphate and sulfate were used.Method used is: exp\_01\_s\_e2.mtw, System asupp.smt,

The Flow rate was fixed to 1.0 mL/min and Pressure 3 MPa and the total analysis time taken is 18 min (Loop 20  $\mu$ L) using Suppressor Regenerant: 50 mmol/L H2SO4, Ultra-pure water, Autostep with FillPolarity +.

# 2.5.3 Eluent preparation

Dissolve 191 mg sodium carbonate (anhydrous) and 143 mg sodium hydrogen carbonate in 980 mL Ultra-pure water. Then add 20 mL acetone.

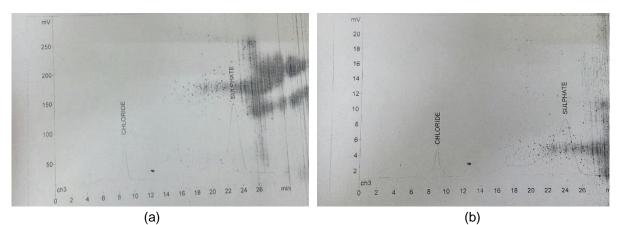
# 3. Results and discussion

Positive results for Hydrogen ion  $(H^{+})$  was observed by using pH indicator paper and litmus paper in all exhibits except exhibit D which is control sample in case no.2 &3. Further negative results for cations (Sodium, Potassium and Lead) were observed. Positive results for various anions: chloride and sulphate were obtained in different exhibits of the Victims through classical chemical test and sophisticated instrumental analysis. The preliminary identification of the ions were done by various chemical tests as described before, result of the test solutions were comparable in intensity and color with that of working standard solution. The blank test was run simultaneously which gave negative result confirming that our chemical test method was devoid of any cross contamination or false positive. The positive chemical test gave us the lead to move for confirmatory test of the target ions through confirmatory techniques like Ion chromatography. The ion chromatogram (Figures 1a-b, Figures 2a-b, Figures 3a-b, Figures 4a-b, Figures 5a-b, Figure 6 and Figure 7) of the exhibits, the standard and the blank were studied and the interpretations of the results confirmed the presence of HCl & H<sub>2</sub>SO<sub>4</sub> in exhibits and gave negative results for control samples (Table 3).

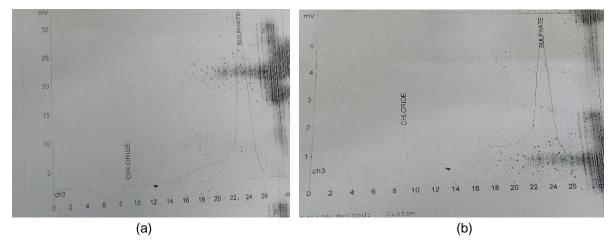
Case No.	Exhibits Description	Results				
		Indicator Paper Test( at 25 <sup>0</sup> C)		Preliminary Chemical Tests		lon Chromatography
		pH Value	Litmus Paper	Anion	Cation	
1.	<ul> <li>A. Salwar, Dupatta, Kurta and bag of victim.</li> <li>B. Trouser and T- shirt of accused</li> </ul>	4 in Exhibits A. 5 in Exhibits B	Blue to Red in Exhibits A & B.	Positive for Cl'& So4 <sup>2-</sup> in exhibits A & B	Negative for Na <sup>+</sup> , K <sup>+</sup> , Pb <sup>2+</sup> in exhibits A & B	Positive for CI <sup>®</sup> So4 <sup>2</sup> - in exhibits A & B
2.	A. Salwar of victim, B. Small stone lifted from the crime scene, C. Mud type liquid collected from the	5 in Exhibits A, B, &C 7 in Exhibits D	Blue to Red in Exhibits A, B, & C	Positive for CI & So4 <sup>2-</sup> in Exhibits A, B, & C	Negative for Na <sup>+</sup> , K <sup>+</sup> , Pb <sup>2+</sup> in Exhibits A, B, C	Positive for Cl <sup>*</sup> & So4 <sup>2-</sup> in Exhibits A, B, & C

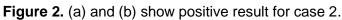
Table 3. Results of examination of exhibits.

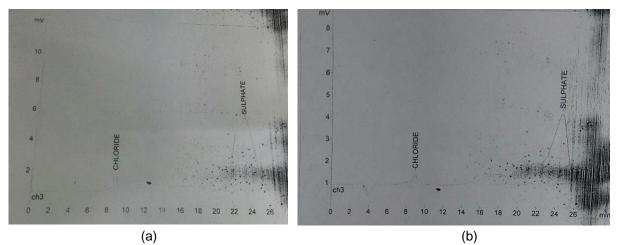
	crime scene D. Small stones lifted by Investigating Officer as control.		No Change in Exhibit D	Negative for Cl <sup>-</sup> So4 <sup>2-</sup> &NO <sub>3</sub> in Exhibit D	&D	Negative for Cl <sup>-</sup> ,So4 <sup>2-</sup> &NO <sub>3</sub> <sup>-</sup> in Exhibit
3.	<ul> <li>A .Glass bottle containing yellow colored liquid</li> <li>B. Acid stained earth control,</li> <li>C. Acid stained earth controlled under the glass bottle,</li> <li>D. Earth control from near the acid stained earth,</li> <li>E. Shawl of victim having acid burns,</li> <li>F. One suit, stole and sweater having acid burn marks</li> </ul>	4 in Exhibits A,B, C, E &F 7 in Exhibits D	Blue to Red in Exhibits A, B, C, E, & F No Change in Exhibit D	Positive for So4 <sup>2-</sup> in Exhibits A, &B Positive for Cl <sup>-</sup> & So4 <sup>2-</sup> in Exhibits C, E, &F Negative for Cl <sup>-</sup> So4 <sup>2-</sup> &NO <sub>3</sub> in Exhibit D	Negative for Na <sup>+</sup> , K <sup>+</sup> , Pb <sup>2+</sup> in Exhibits A, B, C D, E, & F	Positive for So4 <sup>2-</sup> in Exhibits A, &B Positive for Cl <sup>-</sup> & So4 <sup>2-</sup> in Exhibits C, E, &F Negative for Cl <sup>-</sup> So4 <sup>2-</sup> &NO <sub>3</sub> <sup>-</sup> in Exhibit D
4.	<ul> <li>A. One ladies purse (with some ladies item, one tobacco pouch, one spoon, one pocket diary)</li> <li>B. One empty lunch box</li> <li>C. One dupatta in burn condition</li> </ul>	4 in Exhibits A, B, &C	Blue to Red in Exhibits A, B, & C	Positive for So4 <sup>2-</sup> in Exhibits A, B, & C	Negative for Na <sup>+</sup> , K <sup>+</sup> , Pb <sup>2+</sup> in Exhibits A, B, C &D	Positive for So4 <sup>2-</sup> in Exhibits A, B, & C
5.	A.One burned salwar suit	4 in Exhibit	Blue to Red in Exhibit A	Positive for Cl <sup>-</sup> in Exhibits	Negative for Na <sup>+</sup> , K <sup>+</sup> , Pb <sup>2+</sup> in Exhibit A,	Positive for Cl <sup>-</sup> in Exhibits

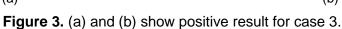


**Figure 1.** (a) and (b) show positive result for case 1.









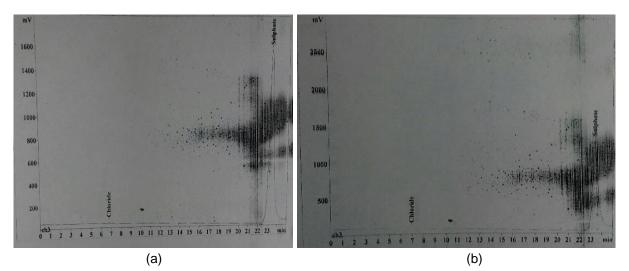


Figure 4. (a) and (b) show positive result for case 4.

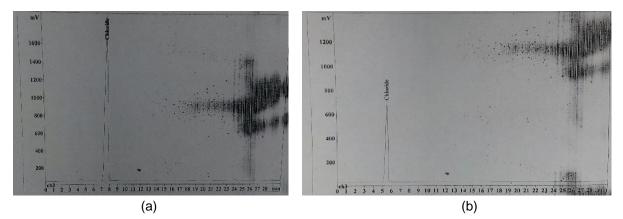


Figure 5. (a) and (b) show positive result for case 5.

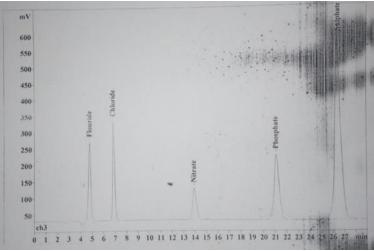


Figure 6. Chromatogram of the standard.

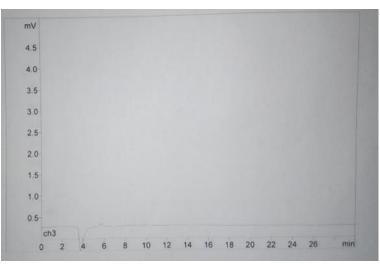


Figure 7. Chromatogram of Ultra -pure water.

We have observed that generally sample collected from such sites are pieces of clothes, skin swabs, hair etc. As per the guidelines, skin pieces or biological tissues, swabs have to be prepared in the Ultra-pure water to avoid any contamination and similarly other samples have to be taken care with specificity without introducing any contaminations which can easily come from the first time used water for cleaning the samples or swabbing. We are emphasizing on the water quality as this will be the first reagent which will come in contact with the sample of analysis and will decide the fate of further analysis. We can easily imagine that if the water quality is compromised at this stage, things can be really disguising in terms of results. As it is describes in Table 3, there are certain methodologies are required to have the analysis done for such sample and for which it becomes important keep a check on the water quality.

The information and knowledge about such sample collections at this level is either very low or not there at all and we see the impacts of such mishandlings of the samples on the results by the judiciary. In the entire process there will be a lot of analysis will be required to reach an decision so it becomes very important to have the samples analyze accurately and precisely at one go.

Information.					
	Nature of Physical Evidences	Nature of Examination conducted	Type of analytical information laboratory can provide	Analytical methodologies	
Cases of Vitriolage (Acid throwing)	Affected clothing material, containers seized at scene, pieces of stone, soil,clothes, hand bags,shoes, and other affected objects.	Color tests, In-organic chemical analysis.	<ul> <li>a) Nature of acid.</li> <li>b) Corrosive potency</li> </ul>	<ul> <li>(a) Titration</li> <li>(b) HPLC</li> <li>(c) Molecular Biology</li> <li>(d) LCMS/GCMS</li> </ul>	

**Table 3.** Nature of physical evidences and the examination conducted for different information.

# 3.1 Impact of Ultra-pure water on forensic analysis of exhibits<sup>25,26</sup>

Classically it has been observed, single/double distillation water has been used for titration but considering the critical nature of exhibits along with the forensic responsibility and stake of such analysis, to carry out the analysis where sample is less in quantity and results has to be accurate. Holistic approach here was to use the ultrapure water instead of distilled water was due to inconsistency of ions/organics/other contaminants levels in distilled water which will vary from location, weather, geography wise and even within the same location the sample taken for test with different lots of distilled water will introduced contaminant, which

will results in inconsistent results. Establishing how much ions are there in Distilled water is impractical and will not hold true universally due to inconsistent feed water used to produce distilled water due to various factor. Due to no monitoring mechanism (conductivity/TOC Monitor) on distillation technique, it will be difficult every time see the quality ensuring the good or bad water for sampling. Another factor is distilled water produced hot and has to cool down before use as heat may alter the results. Now, its important to note that during the cooling process, distilled water absorbs atmospheric gaseous like CO2, Nitrogenous, which will be detrimental for the pH of water and in turn, shall not be used for the sampling work. This amount of ions in distilled water even with one sample is less and other sample is high and that will create variation whereas the ultra-pure water eliminates the same inconsistency. Small practical example here is water in Deccan area like Hyderabad will be full of silica etc. but whereas water in northern region, water will have high organic load. Also, On the other hand, ultrapure water through proper laboratory water purification system is bound to give consistent grade of guality ensuring consistent results. This happens as system has their alerts and alarms to tell when water quality degrades.

Water should be free of unwanted ions and charged organics which can shield the ions of interest in titration analysis. So its been recommended to have ion free water like Ultra-pure Water.

# 3.2 Impact of contamination due to improper collection and packaging of the sample in vitriolage cases and its implications on Forensic Examination Results

In cases pertaining to Violence against women, it's necessary to collect and preserve the biological, non-biological/chemicals and other samples very carefully because any negligence in sample collection make alteration in nature of crime or may destroy the exhibits which would otherwise had been very useful in investigation. The importance of water is been also mentioned for the different applications during and after sample collection from the crime sites. As discussed earlier common physical evidences encountered in such cases are: container used to throw the chemical, clothes of the victim, clothes of the accused, swabs from the scene of crime etc. The acid or alkali recovered (if any) from the scene of crime must be taken in an all glass container, labelled with and must bear a caution in bold letters "CONTAINS ACID/ALKALI" and sealed by the Investigating Officer, put in duly labelled cloth bags. If the samples are packed in plastic containers then there is chance of leaching of plastic martial as it may react with samples which contains acid in itself and may alter the results. Each exhibit must be packed in separate container to avoid cross contamination.

#### 4. Conclusion

The authors detected the presence of HCl and H<sub>2</sub>SO<sub>4</sub> by versatile and reliable technique of Ion Chromatography and various chemical tests without any complex sample clean-up steps. To maintain the credibility of Forensic examination there is a need of training/awareness on the part of investigating agencies to understand the importance of collection, packaging and immediate depositing of exhibits at FSL for examination of such cases of crime against women on top priority basis In case of vitriolage it is very important/mandatory that Police should collect control samples from the crime scene within the circumference of ten meters to avoid false positive as well as false negative reports. For producing accurate and effective reports, it is equally important to have the right kind of reagent used in sample preparation, mobile phases and solution preparation for standards has to be prepared with the precise methodologies. To avoid false positive and cross contamination in case of vitriolage cases use of Ultra-pure water in analysis of exhibit, preparation of standards is indispensable as tap/distilled water contains different type of minerals and ions. Study was to demonstrate to bring the sensitiveness to the sample handling during the Vitriolage cases by using a right grade of water so that sampling of the forensic samples (Vitriolage) will not be diluted with contaminated water(distilled water) and show variation in results. Sensitivity of the analytical technique used are to demonstrate the results which were good and constitutes parity. To study the difference in Ultrapure water and distilled water, we would be need to introduce the Liquid Chromatography in tandem mass spectrometry as we have to touch the PPT levels. With the given resources, we could able to bring the attention to the topic. This will be wishfully our next step to introduce more sensitive techniques on the subject.

#### Acknowledgements

The Authors express their sincere gratitude to Ms. DeepaVerma, Director, Forensic Science Laboratory GNCT of Delhi and Shri K.C. Varshney, HOO, Regional Forensic Science Laboratory, Chanakyapuri New Delhi for their encouragement. The authors are thankful to Ms. Anusha Srivastava, Intern, Department of Chemistry, Banaras Hindu University, UP for literature review.

#### References

- Menon P,Vashishta S. Vitriolage & India- The Modern Weapon of Revenge. International Journal of Humanities and Social Invention. 2013;2(10):1-9.
- Sethi S S, Panda BB. Vitriolage and its current Law: Two case Reports. Scholars Journal of Medical Case Reports.2015;3(2):145-8.
- Chela A, Reig R, Sanz P, Huguet E, Corbella J. Death due to Hydrofluoric acid . American Journal of Forensic Medicine & Pathology. 1989;10(1):47-48. <u>https://doi.org/10.1097/00000433-198903000-00012</u>
- Akhter G A, Islam F. Acid Violence: A burning issue in Bangladesh. Ibrahim Medical College Journal. 2013;7(1):18-20. <u>https://doi.org/10.3329/imcj.v7i1.17744</u>
- 5. A worldwide problem. Acid Survivors Trust International.https://www.asti.org.uk/aworldwide-problem.html. Accessed 16 November 2019.
- Acid Violence in Uganda, A situational analysis, Acid Survivors Foundation Uganda in partnership with the Acid Survivors Trust International.https://www.acidviolence.org/assets/uganda\_asfu\_situational\_analysis\_rep ort\_final\_nov2011\_1.pdf. Accessed 16 November 2019.
- Byard RW. The manifestations of acid attacks (Vitriolage or Vitriolism). Forensic Science, Medicine and Pathology. 2020.16:387-8. <u>https://doi.org/10.1007/s12024-020-00241-4</u>
- Young American Women attacked in France with Acid as Similar Attacks increase Across Europe. https://www.thedailybeast.com/young-american-women-attacked-infrance-with-acid-as-similar-attacks-increase-across-europe. Accessed 16 November 2019.
- Russian tourist suffers acid attack in India's Varanasi, 13 November 2015, www.bbc.com/news/world-Asia-India. https://www.bbc.com/news/world-asia-india-34808054. Accessed 16 November 2019.
- Posters warn girls of acid attacks if seen in jeans in Ranchi. The Times of India, https://timesofindia.indiatimes.com/city/ranchi/Posters-warn-girls-of-acid-attacks-ifseen-in-jeans-in-Ranchi/articleshow/15396796.cms. Accessed 16 November 2019.

- India saw almost 1500 acid attacks in five years.https://www.indiatoday.in/diu/story/indiasaw-almost-1-500-acid-attacks-in-five-years-1636109-2020-01-12. Accessed 26 March 2020.
- Kaur AD, Sandhu HS, Aggarwal KK, Oberoi SS. An unusual case of vitriolage. Journal of Punjab Academy of Forensic Medicine & Toxicology. 2007;7(2):35-38.
- 13. Nair AR. Acid attack –violence against women 'Need of the hour'. Journal of innovative Research and solution. 2014;1(1):107-17.
- Two women injured in acid attack in Ambala. https://www.tribuneindia.com/news/haryana/two-women-injured-in-acid-attack-inambala-48956. Accessed 26 March 2020.
- 15. Delhi riots: 8 year-old girl attacked with acid, mother wonders if her child will fully recover.https://www.indiatoday.in/india/story/delhi-riots-violence-northeast-shiv-vihar-gokulpur-acid-girl-trauma-1653190-2020-03-06 accessed on 26 March 26, 2020
- 16. Why India's stringent new laws have not reduced acid attacks,https://www.newsclick.in/Why-India-Stringent-New-Laws-Have-Not-Reduced-AcidAttacks#:~:text=Many%20cases%20are%20not%20even,the%20world%2C%20suc h%20as%20ridicule.&text=Until%202013%2C%20acid%20attacks%20were,a%20separ ate%20offence%20in%20India. Accessed 26 March 2020.
- 17. Acid attack survivors are proud owners of a restaurant in Varanasi. https://www.indiatoday.in/magazine/care-today/story/20200316-acid-attack-survivorsare-proud-owners-of-a-restaurant-in-varanasi-1652630-2020-03-06. Accessed 26 March 2020.
- The acid attack survivor helped rehabilitate over 250 acid and burn victims, https://thelogicalindian.com/exclusive/acid-attack-survivor-rehabilitated-victims-20052. Accessed 26 March 2020.
- 19. India reports 300 acid attacks in a year, https://indicanews.com/2020/01/10/india-reports-300-acid-attacks-in-a-year/accessed on 26 March, 2020.
- Verma KL, Verma R, Sarin RK, Sharma M, Jaiswal A. Importance of Ultra-Pure Water in Analytical Methods For Determination of Drug of Abuse by Mass Spectrometry. SMU Medical journal. 2015;2(2):249-264.
- 21. Water
   Purification
   in
   the
   Chromatography
   Lab.

   http://files.alfresco.mjh.group/alfresco\_images/pharma//2017/07/27/f06083e7-c879 

   4c8d-abf1-ea53e0f188b6/LCGC0717\_LCGC%20Millipore\_3.pdf.
   Accessed
   20
   January

   2020.
- 22. UV technologies in water purification systems- The R&D Notebook 9 A publication of the Lab Water Division of Merck Millipore. http://merckcatalog.vakilspremedia.com/(S(nm2jiepdhmzjz2xrzrpnaqru))/pdf/literature/la

b-water/scientific-literature/UV-technologies-in-water-purification-systems.pdf. Accessed 20 January 2020.

- 23. Rao MS. Toxicological Manual, Directorate of Forensic Science, Ministry of Home Affairs Government of India, New Delhi, (2005) 1st Edn. Selective and Scientific Books.
- 24. Haddad PR, Jackson PE. Ion Chromatography Principles and Applications, 1st ed. 1990; 46, Elsevier Science.
- 25. Chaudhary R, Anjali, Verma R, Singh S, Singh RP. Study on the Comparative Performance Assessment of Distillation method by using a Conventional Method and Elix Technology.International Journal of Science, Technology and Society. 2018. https://doi.org/10.18091/ijsts.4130
- Elix® UV Technology Compared to Single and Double Distillation. https://www.labmanager.com/white-papers-and-application-notes/elix-uv-technologycompared-to-single-and-double-distillation-16718. Accessed 26 June 2020.