Analytical Regimen of Chemical Weapons Convention

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1. Introduction

Chemical weapons are considered to be loathsome amongst weapons of mass destruction. To save mankind from horrifying menace of chemical weapons, an international treaty, known as Chemical Weapons Convention (CWC) has come into existence since April 1997. CWC prohibits proliferation of chemical weapons, signatory states are obliged to abide provisions of the CWC i.e. not to develop, produce, store or use chemical weapons other than the provisions not prohibited in the convention. Signatory states to this convention are also obliged to destroy their chemical arsenal within the stipulated period of time.

India is also one of the 193 signatories of CWC, hence, obliged to implement the provisions of CWC. One of the most important aspects of CWC is its verification program which ensures implementation of the treaty by virtue of inspections of chemical industries and sites of chemical weapons storage, destruction and storage facilities. These inspections are conducted by the inspectors appointed by the OPCW, an organization known as Organization for Prohibition of Chemical Weapons, The Netherlands. The OPCW is administrative organization of CWC and maintains a global network of designated laboratories to undertake the off-site analysis of samples collected by its inspectors during systematic, routine or challenge inspections. In case of unresolved, during on-site analysis, the samples are submitted to at least two off-site designated laboratories. Thus role of 'designated laboratories' become crucial in deciding the compliance and/or non-compliance of CWC.

India, by virtue of its large chemical industry, is one of the fastest growing economies including defence sector; hence, required at least one 'designated laboratory' within its territory to play a critical role in case of any eventuality of chemical emergency and / or unresolved ambiguities during inspections by the OPCW.

DRDO is not only committed to meet the scientific and technological requirements of national defence, but also committed to play an international role as and when entrusted by the nation. In the context of CWC, a laboratory under the DRDO, namely Defence R & D Establishment (DRDE) located in the city of Gwalior (M. P.), was entrusted to obtain the status of designation from OPCW. Achievements of DRDE in establishing the the analytical technology of verification analysis of CWAs are delineated below.

2. Process of Designation:

Process to attain and maintain the status of designated laboratories is an uphill task, as it requires excellence in synthesis, analysis and identification of CWAs and their degradation products (so called markers). To examine the scientific capabilities of a laboratory, OPCW conducts international official proficiency tests (OPTs) twice in a year. And a laboratory aspiring to become and stay designated has to participate in these OPTs and perform as per the following stringent criteria.

i) Laboratory must have in place an internationally recognised quality system e.g. ISO17025: 2017 or equivalent.

ii) Laboratory must participate at least once in a year in the OPTs conducted by the OPCW.

iii) Laboratory must secure at least two ‘A’ and one ‘B’ grade in three consecutive OPTs.

Official proficiency tests are conducted by the OPCW with the help of two already laboratories. One laboratory prepares the samples for analysis and second laboratory evaluates the reports submitted by the participating laboratories. There are several intricacies of these OPTs and a laboratory has to prove its competence in following ways.

1. The time frame for OPTs is only fifteen days i.e. the laboratory has to analyse the samples and submit the report within the 15 days.

2. Samples may include soil, water, polymer or organic liquids containing traces of CWAs and / or their degradation products with high background chemicals to challenge the analysis.

3. Laboratory has to identify the structures of spiked chemicals with the help of GC-MS, LC-MS, FT-NMR and GC-FTIR analyses. Sample preparation laboratory may select the spiking chemicals from three schedules of chemicals as laid down in the CWC or their degradation products.

4. The spectral data of spiked chemicals may not be available in any of the commercially available data base or in the data base supplied by the OPCW. Thus laboratory must have high skills of interpretation of spectral data of unknown chemicals to elucidate their structures.

5. Laboratory must have efficient sample preparation methods / protocols to eliminate the background chemicals and extract and enrich the analytes of interest from samples.

6. Total numbers of CWC related chemicals are several hundreds of thousands, out of which types and numbers of spiked chemicals are not known to the participating laboratory.

7. Identified chemicals must be substantiated by submitting the spectral data of synthesized reference chemicals. Thus laboratory must have the capability to synthesize new chemicals with in the 15 days.

8. Laboratory must report only those chemicals which are relevant to the CWC. Reporting a chemical (though present in the sample) not relevant to CWC, is considered as an irrelevant chemical and constitutes failure in the test.

9. A reported chemical must be identified by at least two spectroscopic techniques.

10. All the reported spectral data must fulfil the requirements of reporting criteria as formulated by the OPCW. Not meeting any one of these criteria is considered as incomplete information and data for that particular chemical are rejected.

Thus a laboratory willing to participate in OPTs must have team of skilled scientists and required infrastructure.

2.0 Status of DRDE:

DRDE Gwalior was tasked to participate in OPTs with an objective to achieve and sustain the status of 'designated laboratory'. DRDE started participating in OPTs and a division known as VERTOX (verification of toxic chemicals) laboratory was created and equipped with required infrastructure. A team of scientists was dedicated to accomplish this task. The Vertox laboratory launched its own R & D program on synthesis and analysis of CWC related chemicals and developed novel synthetic/analytical techniques which were published in reputed peer reviewed international journals. The new techniques which were developed in the Vertox laboratory include (i) SDME, (ii) INDEX, (iii) OMDEX, (iv) HF-LPME and many more. Simultaneously, the Vertox laboratory also participated in OPTs conducted by the OPCW. Like any scientific endeavour, with initial quirks, Vertox laboratory achieved 'A' grade in three consecutive OPTs and was first awarded the status of 'designated laboratory' in the year 2006. Continuing the regular participation in OPTs with securing the required grades, DRDE has so far (i.e. 2022) maintained the status of designation from OPCW. Thus, the Vertox Laboratory, DRDE remains the only laboratory of India designated by the OPCW.

3.0 Analytical Methods Developed:

The R & D program of Vertox laboratory has resulted in development of several analytical techniques including synthesis, spectroscopic characterization, sample preparation and mass-spectrometric analysis of CWAs and their markers. Entire program has culminated to approximately couple of dozens of publications in high IF international journals, twelve Ph.Ds and publications of many analytical methods in the form of recommended operating procedures' for the international community of scientists involved in the analytical verification work of the CWC. Some of the important analytical works are enumerated below.

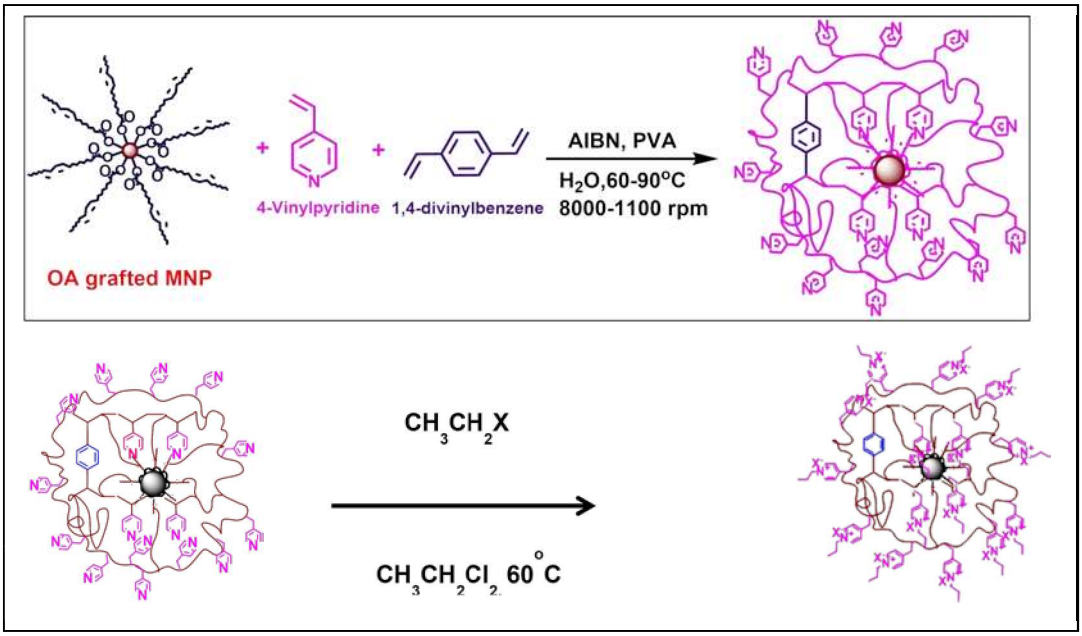
3.1 On-matrix derivatization extraction (OMDEX) of basic and acidic degradation products of CWAs from soil and water:

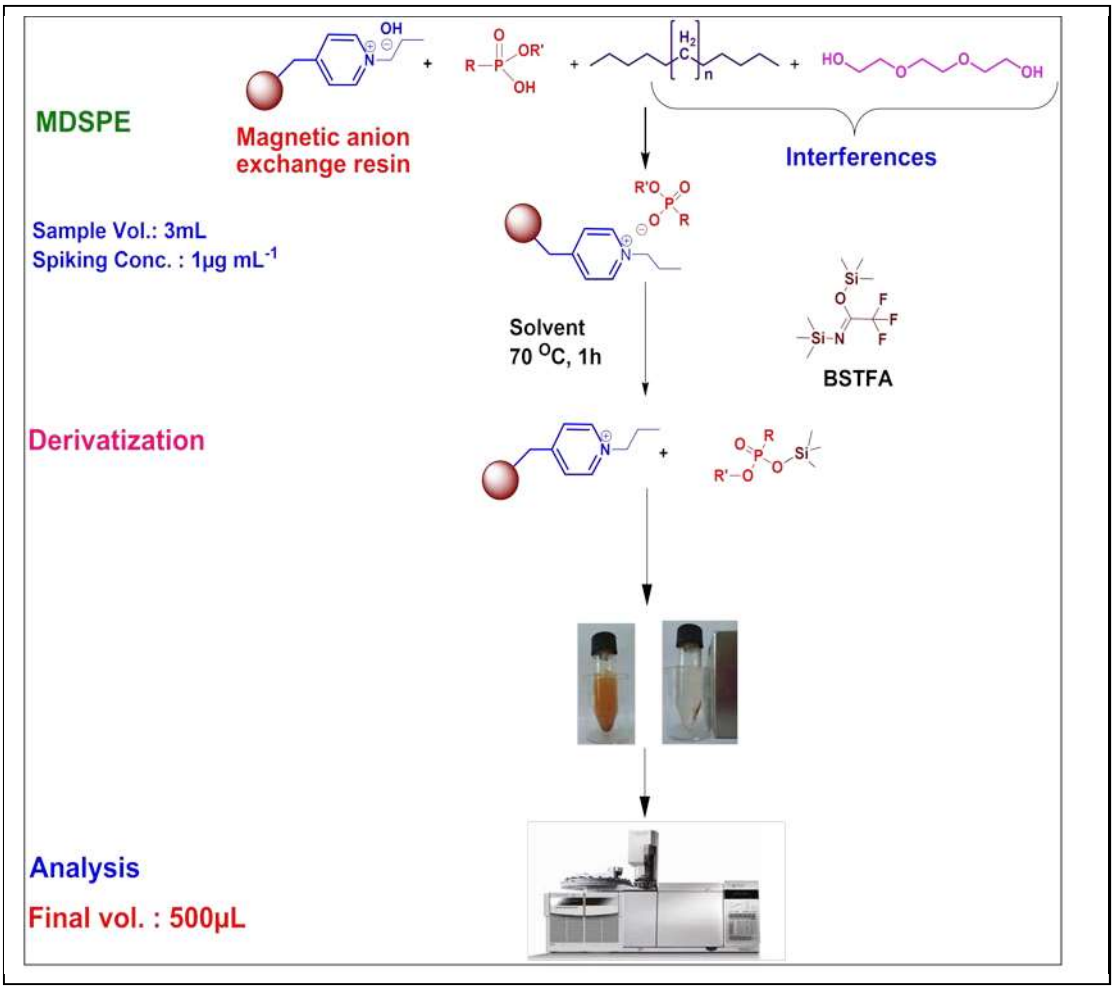
Derivatization in conjunction with extraction of aminoalcohols and phosphonic acids was developed. In this method, hexamethyldisilazane (HMDS) was combined with tert butyldimethylsilylation to derivatize and extract analytes from soil and diatomaceous earth. This procedure offered better recoveries than the one recommended by the OPCW in the form of

recommended operating procedures. The analytical technique used was gas chromatography–mass spectrometry. Recoveries of analytes ranged from 45-103% and the limits of detection (LOD) and limit of quantification (LOQ) ranged from 8-277 and 21-665 ng mL^{-1} respectively.

3.2 Design, synthesis, evaluation and validation of magnetic micro- / nano-spheres for extraction, enrichment, derivatization and identification of CWAs and their markers:

Extraction, enrichment and identification of CWAs and their markers from environmental samples was achieved by the development of novel materials based on magnetic nano-spheres. Magnetic nano-spheres were custom functionalized with hydrophilic-lipophilic balance (HLB) polymers, cation- and anion-exchange functionalities. Magnetic particles possessed super-paramagnetic character with high surface area. These properties endowed high mass transfer of analytes from matrix and simple retrieval of magnetic particles with external magnate. Factors influencing the extraction and enrichment of analytes such as solvent, time, amount of sorbent, washing solvent, derivatization conditions, reusability of material, temperature and interfering materials were optimized. Developed methods were compared with the reported and hitherto used methods. Magnetic spheres based methods surpassed the performance in terms of recoveries of analytes, removal of interfering materials, linearity, limits of detection, limits of quantification, time and ease of performance. Analytical figures of merit were much better than those required by the verification analysis. For example, for aminoalcols, linearity range was 5-200 ng mL^{-1}, LOD and LOQ were 2-6 ng mL^{-1} and 5-19 ng mL^{-1} respectively. Developed materials as well as methods were validated with real environmental samples and application was tested with the blind samples supplied by the OPCW during international proficiency tests. Synthesis of functional magnetic nano-particles (MNPs) and their application as extractant of markers of CWAs are exemplified with cation-exchange MNps using phosphonic acids as target analytes.



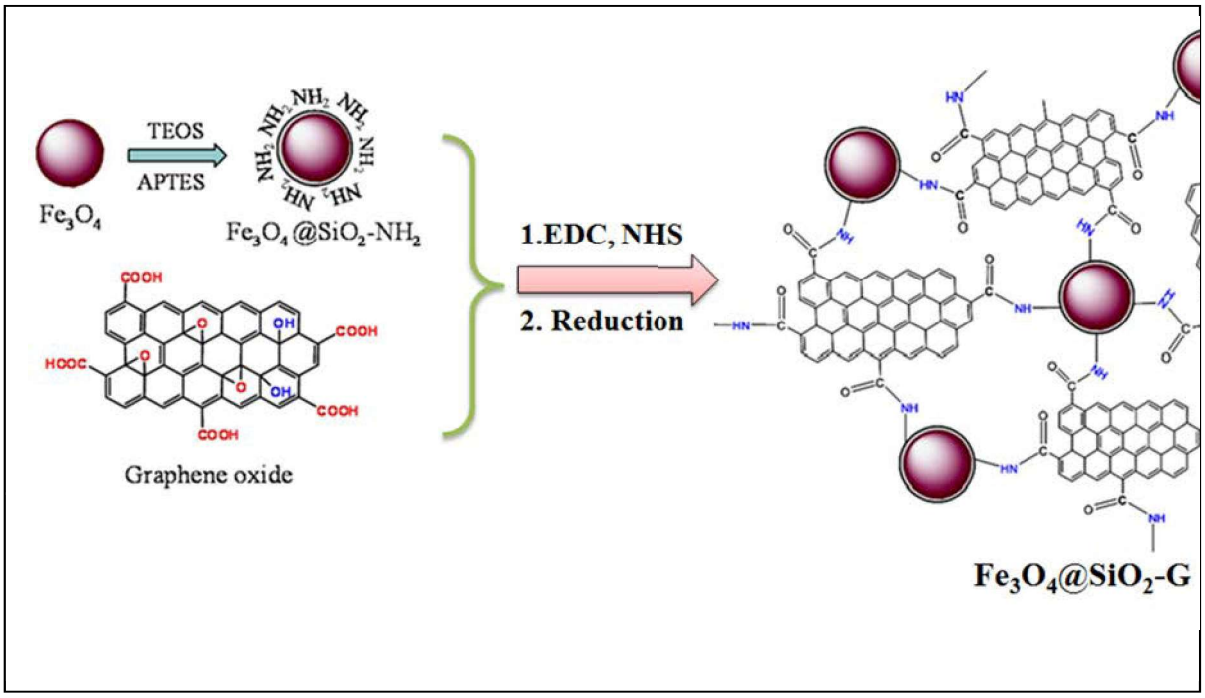


i) 05 publications in J. Chromatogr A (IF 4.18).

ii) Developed methods are used as validated methods for the identifications of CWAs and their environmental degradation products in blind samples

3.3 Design, synthesis, evaluation and validation of magnetic graphene composites for extraction, enrichment, derivatization and identification of CWAs and their markers:

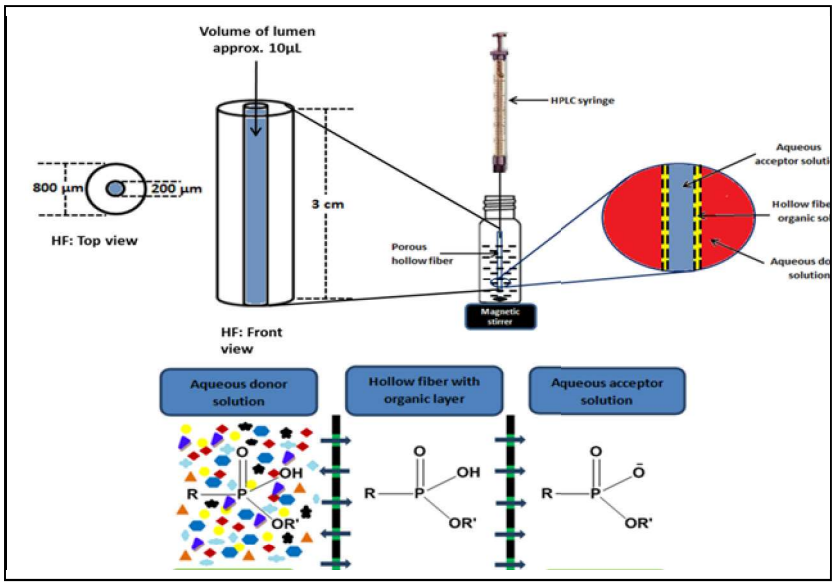
Graphene was selected due to its unique characters such as large surface area (theoretically 2630 m^2/g), extremely high surface to volume ratio, light weight nano-sheet structure and delocalized π – π electron system makes it an excellent sorbent material for analytical applications. Composites of graphene were prepared with magnetic nano particles, cationic and anionic polymers and were exploited for the extraction of real warfare agents and their acidic and alkaline degradation products respectively. The important findings of these studies are representatively illustrated with magnetic graphene. Nanocomposite, iron oxide-silica-graphene was prepared by anchoring silica and iron oxide onto the grapheme. Extraction parameters for chemical warfare agents were determined. Sample pH and ionic strength governed the extraction efficiency. Fo example, nerve agent Sarin showed maximum recovery at pH 5, while VX recovered best at pH 9. Salts raised recoveries to as high as 94% for SM. LODnfor sulphur mustard and VX were 0.11 ng $mL^{-1}$and 0.19 ng mL^{-1} respectively.



i) 03 publications in J. Chromatogr A (IF 4.18).

3.4 LC-MS/MS and appropriate sample preparation methods for CWAs:

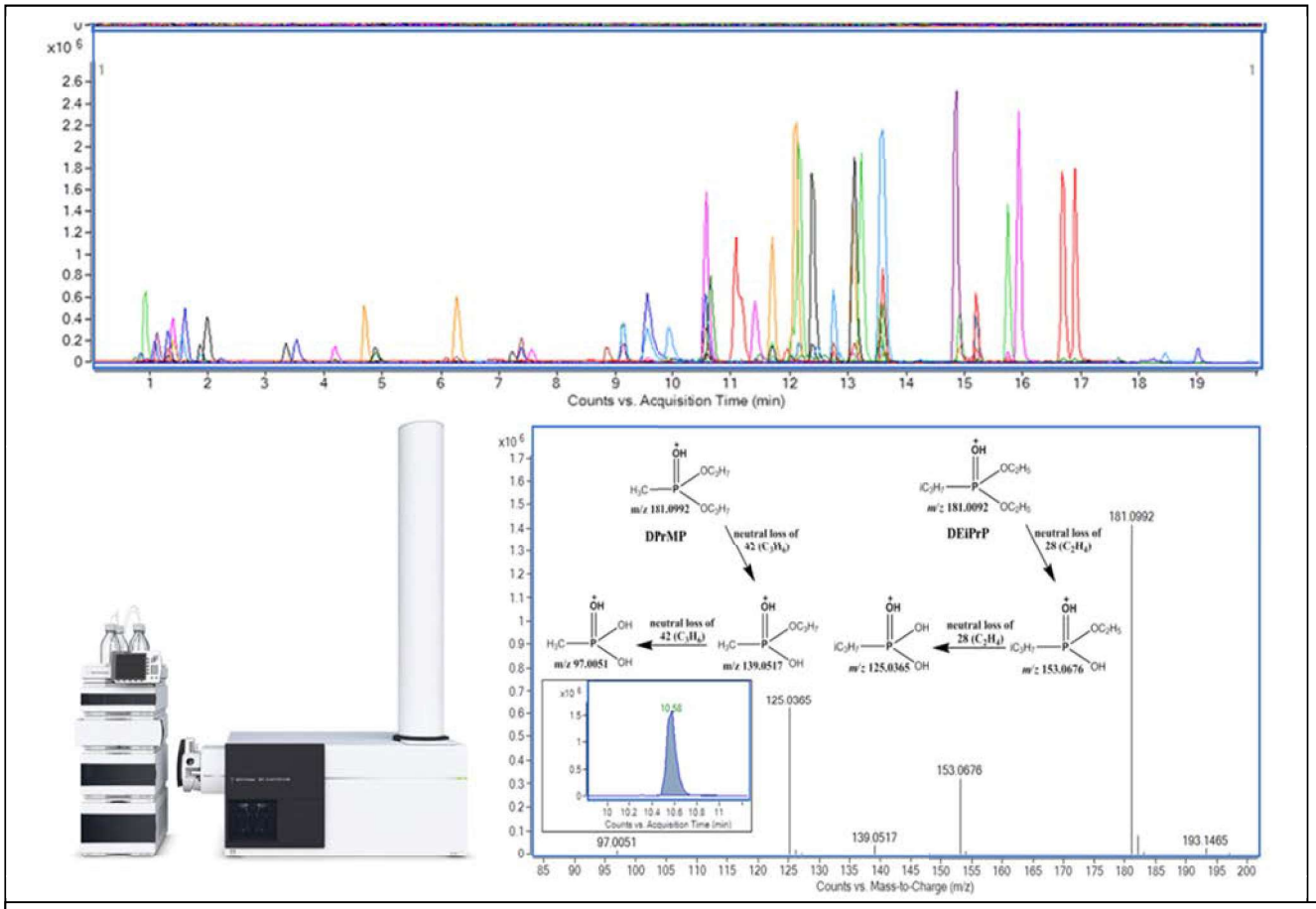
Liquid-liquid-liquid microextraction aided by hollow fibre (HFLLLME) was developed for extraction of phosphonic acids AAPAs from water samples. These were extracted from aqueous phase to organic phase through pores of hollow fibre and then concentrated to acceptor aqueous phase in it's lumen. Experimental conditions arrived were: 1-octanol in pores, pH in lumen 14; time, 60 min; pH of sample 1; and salt 10% (w/v). Lower limits of detection ranged from 0.1 to 100 ng mL^{-1}. After validation, the method was used for detection of AAPAs in the samples provided by the OPCW in subsequent proficiency tests.



Passive diffusion the analytes across the pores of hollow fibre was a limiting factor of this method, it was overcome by development of a simple and fast method through microfluidic electromembrane extraction (µ-EME). The edge provided by this method was efficiency (3 min) and sample size (10 micro liter).

Sensitivity of the method was enhanced by the post column addition of tricationic reagent which led to the formation of stable adducts with phosphonic acids. These adduct carrying positive charges were detected in positive ESI. 1,3-imidazolium-bis-(1-hexyl-benzyl-imidazolium)-trifluoride (IBHBI) was used as cationic reagent. Various parameters including solvent composition, concentration of IBHBI, effect of pH and interferences were optimized. Limit of detection (0.1 ng) achieved were better than those previously reported in literature.

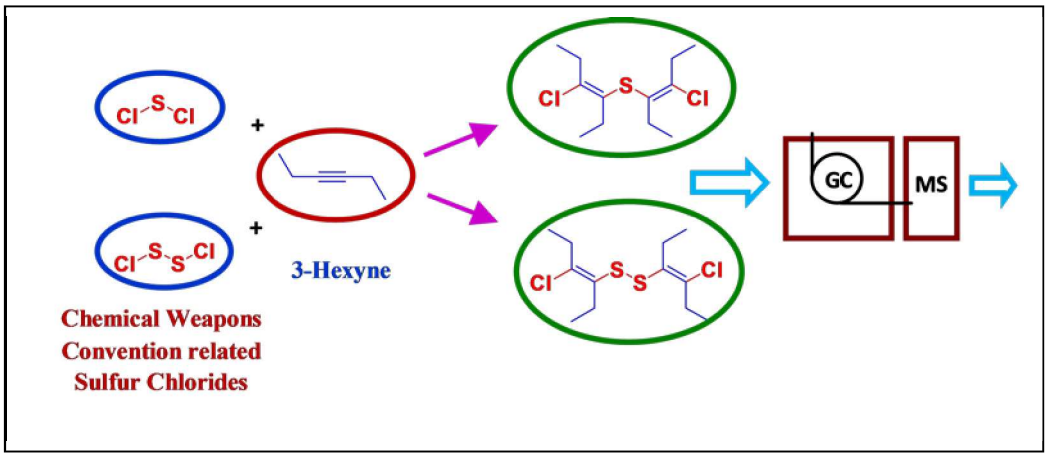
Structural diversity of CWAs and their associates is major challenge for analytical scientists. Operational parameters govern the spectral features in LC-MS; it makes more difficult to universalize the LC-MS method for CWAs. A generic LC-MS method was developed employing ultra-high performance liquid chromatography in combination with quadrupole time-of-flight mass spectrometer (UHPLC-QTOF). Accurate mass measurement with characteristic fragment ions of pseudo-molecular ions and retention time were resorted to identify the target analytes. Even the isomeric and isobaric compounds were distinguished by this method.



i) 03 publications in J. Chromatogr A (IF 4.18); 3 publications in Rapid Commun Mass Spectrom

3.5 Analysis of sulfur chlorides *via* electrophilic additionderivatization:

Sulfur monochloride (S_2Cl_2) and sulfur dichloride (SCl_2) are used as raw materials to manufacture the mustards. An electrophilic addition to 3-hexyne by these compounds was resorted to develop a novel analytical method. Various analytical conditions, including reaction conditions (time, temperature, stoichiometry), and the detection parameters were optimized. Analytical figures of merit of derivatives in GC-MS analysis were sufficient for the forensic analysis and the verification of these analytes if used in the manufacturing the sulphur mustards.



Published in Analytical Chemistry

4.0 Future Endeavours:

DRDE is committed to work as the nodal laboratory for the verification of CWAs, and fulfil the national obligation by sustaining the status of the 'Designated Laboratory'. The scientific developments on the synthesis and analysis of markers of CWAs involving computational chemistry, microfluidics, automation, material development including nano-technology, magnetic materials and miniaturisation shall continue. The laboratory is looking forward to develop the analytical methods to detect and identify the new threats emerging on horizon**.**

Acknowledgement:

Entire work is outcome of my dedicated, hard –working and wisdom full team of scientists and students. I profusely thank one and all of them for their un-tiring efforts, patience

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Biosketch of Dr. D.K. Dubey

Dr. D. K. Dubey is Director for Defence Research Development Establishment (DRDE), Gwalior being the nodal laboratory for the development of Chem-Bio defence technologies. Dr. Dubey is associated with this laboratory for more than 42 years and has made significant contributions in the development of efficient and up-scalable synthetic procedures, enzyme based nerve agent detection devices, decontamination formulations, state-of-the-art analytical procedures and materials for forensic and verification analysis of warfare agents. He is the founder of `Vertox Laboratory' which is the internationally recognized national centre for the off-site analysis of chemical warfare agents from environmental as well as biomedical samples. He is the recipient of many awards including the prestigious `DRDO Scientist' of the year and `Agni Award' for excellence in self-reliance.