Verification of A Tear Gas and A Pesticide At Trace Levels in A Water Sample.

IMTIAZ AHMED.
National Physical & Standard Laboratory
16, Sector H/9, Islamabad, Pakistan

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Summary: Tear gas like CR and pesticide Malathion present at trace level in a water sample has been analyzed by gas chromatography and liquid chromatography techniques. Solid phase extraction method was used for extraction of suspect agents from water samples. Retention Index monitoring method was applied for identification. Mass spectrometry was used for verification of suspect agents.

Introduction

Tear gases are poisonous or noxious compounds and are characterized by their toxic effects that made them suitable for police use when filled into muntion and other devices. They are common riot control agents, which cause strong irritation and pain in eyes producing a flow of tears [1]

e.g., Chlorobenzylene malononitrile, " CS ".

and Dibenz (b.f) (1, 4) oxazepine, " CR ".

Pesticides are compounds useful for mitigation control or elimination of plants or animals detrimental to human health [2].

e.g., Malathion, S [1, 2 bis (ethoxycarbonyl) ethyl] O, O-dimenthylphosphorodithioate.

It is a yellow liquid used as an insecticide. Water contaminated with these toxic compounds is hazardous to human health if these compounds are present more than prescribed limits. Thus it becomes essential to identify these toxic compounds if suspected in water before human consumption. Water sample suspected to be polluted with the above-mentioned agents was analyzed. Modified analytical techniques has been used, which are very sensitive, selective and able to monitor the suspect agents in water samples up to parts per billion level [3].

Results and Discussion.

The HPLC chromatogram (Fig. 1) revealed one extra peak between K_3 and K_4 RIM Standards.

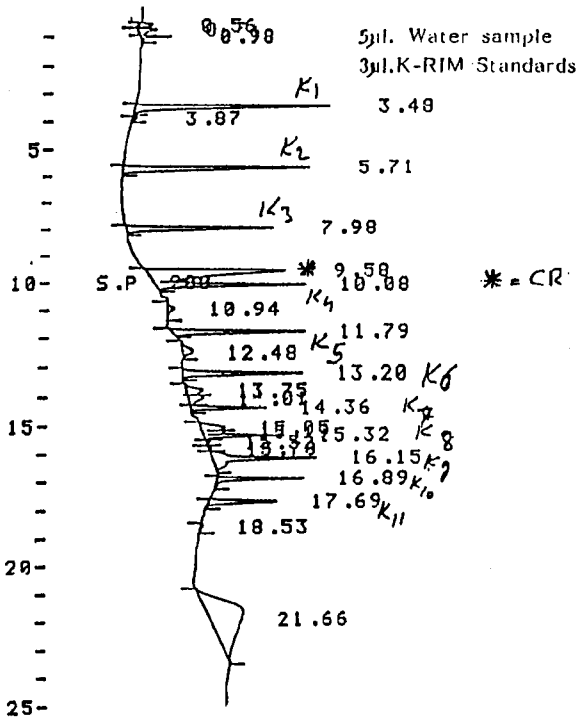


Fig. 1

The Retention Index (RI) value was calculated for the extra peak, i.e. RI = 376.19. This value was close to reference RI value of CR i.e. 375.8. To further verify the presence of CR in the sample, another run was made on HPLC by injecting 1 µl of pure CR standard with K – RIM standards. The RI value was found to be 376.19. The HRGC chromatogram (Fig. 2) for two channel with ATD detectors, the RI values were 1466 and 1363.2 for CR on OV – 701 and NB

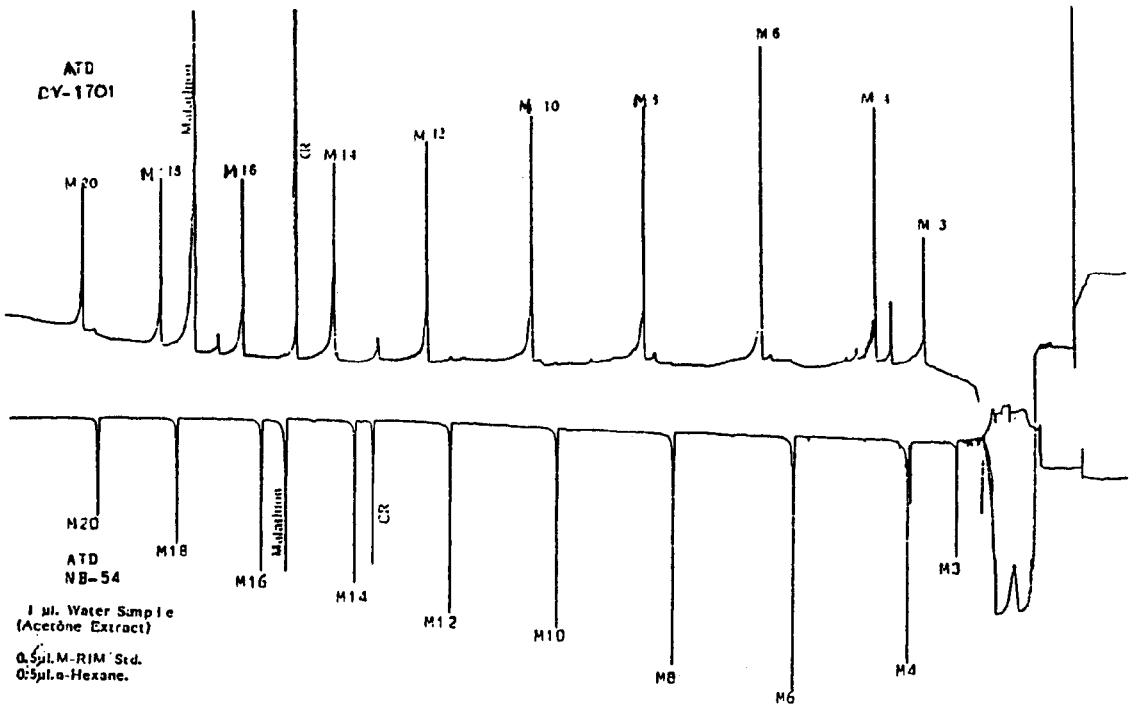


Fig. 2:

– 54 columns respectively identified the presence of CR. The RI values 1707 and 1547.8 on OV – 701 and NB – 54 column respectively identified the presence of Malathion in the sample. In another run having ATD on NB – 54 column and FID on NB – 54 column, the RI values for CR and Malathion were the same as on ATD, but the presence of Malathion could not be detected by FID probably due to very low concentration of Malathion in the sample, which is beyond the detection limit of FID. The small peaks in the HRGC chromatogram were ignored as impurities in the sample.

In HPLC Analysis the Malathion present in the water sample could not be identified due to fact that Malathion does not contain any UV absorbing groups. As we used UV detector in the HPLC analysis, only UV sensitive compounds could be detected by HPLC.

The two major compounds CR and Malathion present in water sample at trace levels were identified by HPLC and HGRC analysis, their presence was confirmed by GC Mass Spectrometer. The structural formulas for CR and Malathion is shown in Fig. 6. Verification of CR and Malathion by GC Mass Spectrometry was necessary because many other organic compounds have the same RI values as that of CR and Malathion. The EI mass spectrum gives not only information about the presence of a compound but also gives details of structure of that compound. According to rough estimates from the peak areas of the CR and Malathion on the chromatograms the concentration was about 15 and 20 ng/µl respectively in the water sample. It has been inferred from the experiments that only one analytical technique is not enough to verify a suspect agent in a sample. At least two authenticated analytical techniques should be used. The HPLC and HRGC techniques could reliably used to monitor pollutants at trace levels in environmental samples.

Experimental.

Sample preparation.

Samples of water of various origin can be subjected to chromatographic analysis in two basic procedures either by direct injection of the contaminated water or by concentration of

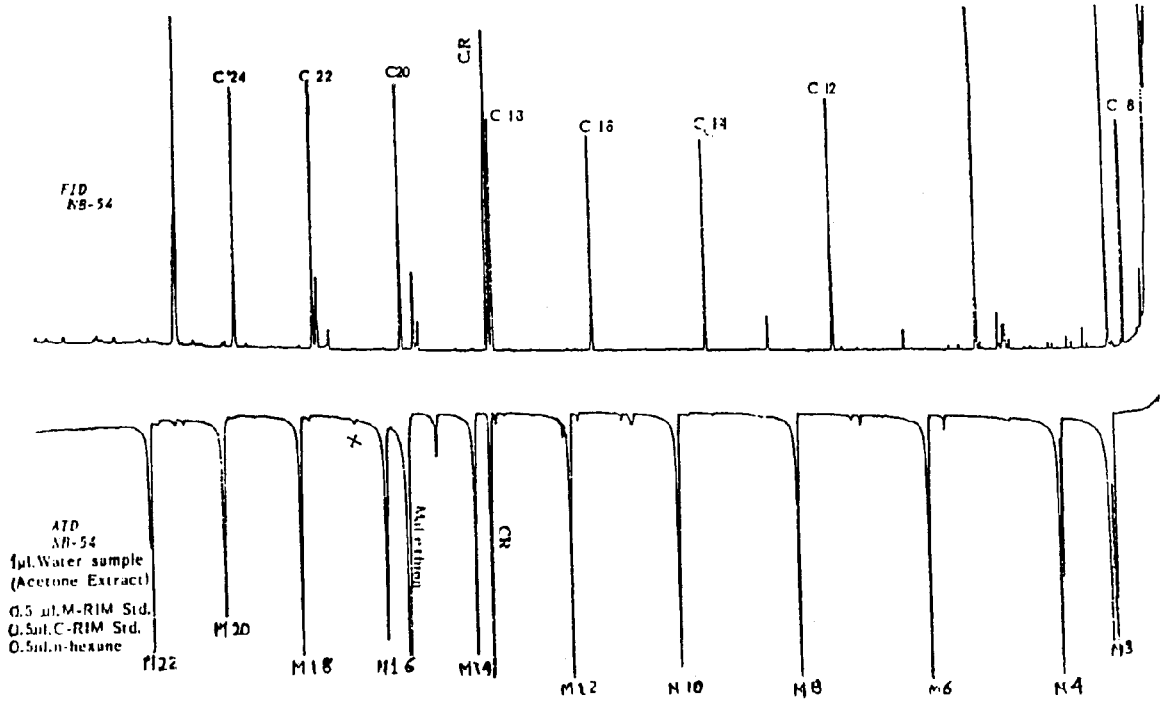


Fig. 3:

contaminants in the water prior to the proper analysis. With the liquid chromatography water can be analyzed directly but with gas chromatography sample preparation has to be carried out first to extract the analytes into a more suitable solvent. The extraction of suspect CR and Malathion present in water sample into acetone was carried out by solid phase extraction technique [4]. Materials used for this purpose were

(1). Bond Elut C18 Cartridges.
(2). Plastic Syringes.
(3). n-hexane.
(4). Methanol.
(5). Acetone.

All the solvents used were of chromatographic grades Bond Elut C18 cartridges was washed with n-hexane and then with deionized water. The adsorbent was activated with methanol and again rinsed with deionized water [5]. Contaminated water sample of about 20 ml was passed through the cartridge. The suspected compounds absorbed in the cartage were eluted with 1 ml of acetone. The eluent i.e., acetone containing suspected compounds was kept in well-stoppered glass vial and placed in a refrigerator. A clear portion of the water sample was kept as such for direct analysis on reversed phase liquid chromatography.

Identification and Verification.

Identification and verification of suspected CR and Malathion was carried out by

High Performance Liquid Chromatography (HPLC).

High Resolution Gas Chromatography (HRGC).

Gas Chromatography Mass Spectrometry (GCMS).

The instrument used in HPLC analysis was

HPLC, Make Merck Hitachi Model.L-6200 Germany.

UV detector, chromointegator D-2500 and HPLC column was C-18 24 cm long.

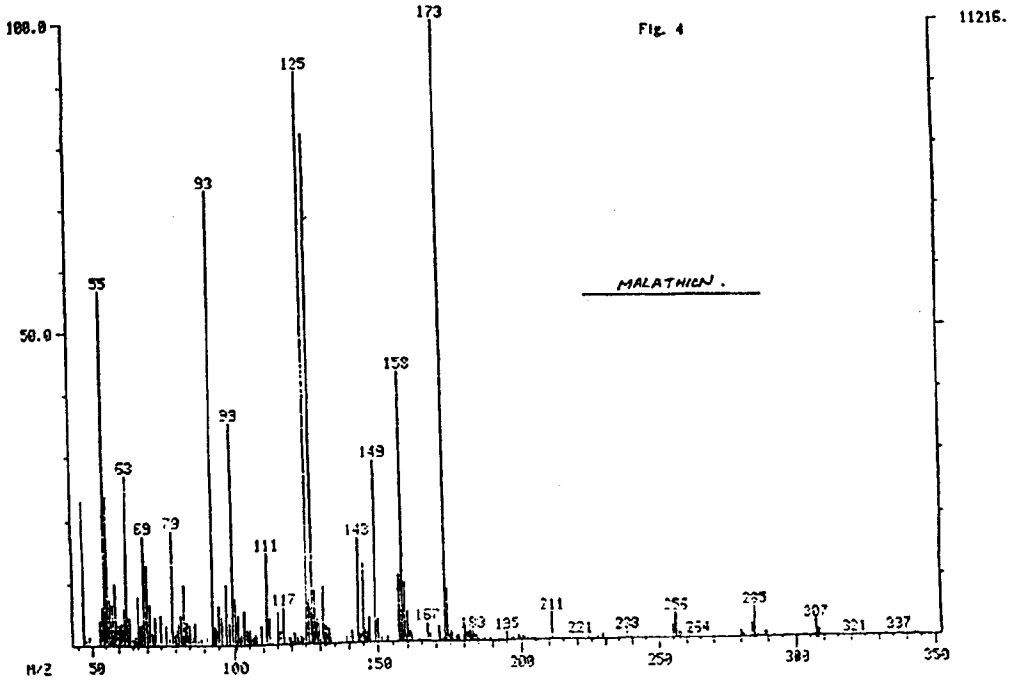


Fig. 4: EI mass spectrum of Malathion

Oven temperature was kept at 50°C the UV detector wavelength was adjusted at 255nm. Solvent used was methanol / water system. Step gradient of the eluent was employed in order to achieve the separation of compounds with a wide range of polarities and structure. Gradient programming used for the best results was:

60 % methanol. - water to 90 % methanol in 13 min.

90% methanol - water to 100 % methanol in 18 min.

100 % methanol - water to 40 % methanol in 25 min.

Absolute and relative retention times are widely used identification methods in HRGC and HPLC. These methods are laborious in routine analysis. Small changes in chromatographic run parameters, such as aging of columns and detectors significantly influence relative retentions and detector response. These effects are efficiently compensated by using Retention Index Monitoring (RIM) technique for identification of sample compounds, giving a better basis for automation of the analysis [6].

The suspect CR and Malathion were therefore identified by Retention Index Monitoring (RIM) technique. 5µl of water sample (direct) along with K Series RIM Standard i.e., 1 Phenyl-1-alknones. K series have strong chromophores, absorbing strongly in a wide wavelength range and having one strong absorption near 250nm. K_1 - K_{11} series were run on HPLC [7]. The HPLC Chromatogram is shown in Fig. 1. Only CR was identified by HPLC analysis.

The instrument used for HRGC analysis was MICROMAT HRGC 412 two channel [8], "Finland" equipped with two parallel capillary glass analytical columns 25m long, 0.32 mm Ø and inside coated with NB - 54 and OV - 1701 stationary phases respectively of 0.25 µ m thickness. The detectors used were:

Flame Ionization Detector. (FID).

Alkali Thermoionization Detector. (ATD).

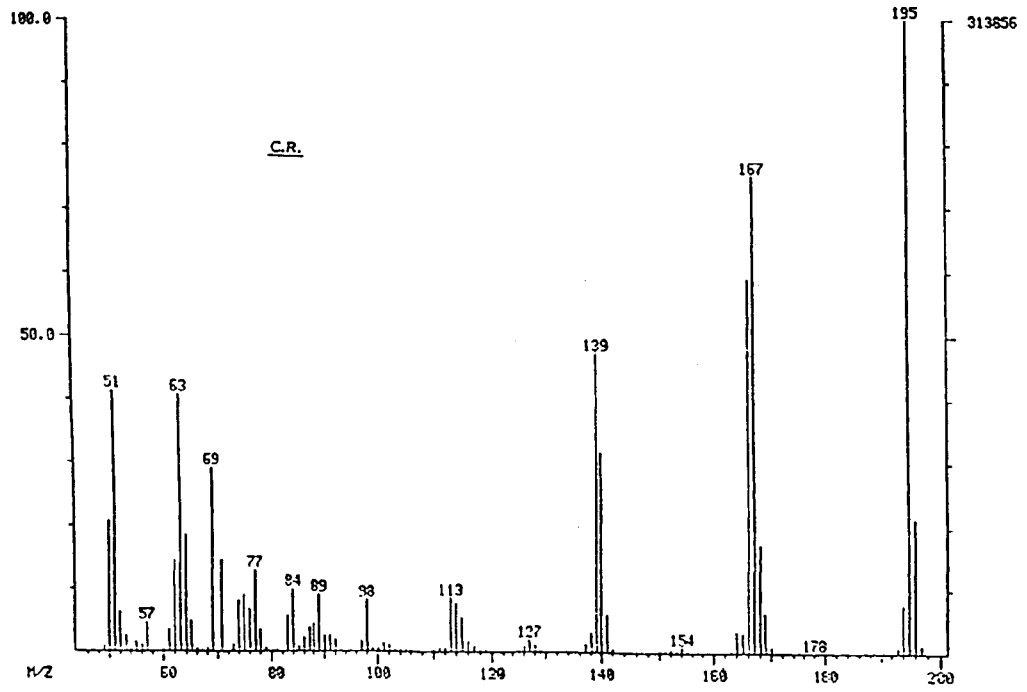


Fig. 5: EI mass spectrum of CR.

The HRGC was interfaced with Nordion RIM Data System and Micman software, carrier gas was Helium of 99.9 % purity. The running condition of HRGC were adjusted as:

Flow rate of air = 230 ml/min, H_2 = 30 ml/min and

He = 2 ml/min. Split Ratio for Split mode = 1/30.

Injection port temperature = 250°C, FID temperature = 280°C

For 1 Channel ATD Backing current 10.2

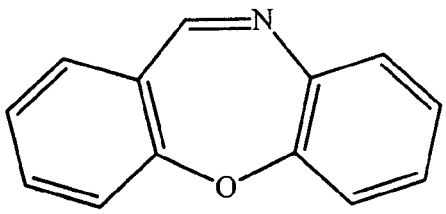
II Channel ATD Backing current 11.00

Two pen recorder speed 1 cm/min.

Temperature programming was adjusted: initial temperature 40°C for 1 min, temperature programming rate 10°C/min to 280°C for 10 min.

After optimizing the above conditions of HRGC, One µl of acetone extract from water sample along with 0.5 µ l of C – RIM standards i.e., $CH_3(CH_2)_n CH_3$ n – alkanes series from C_8 - C_{22} and 0.5 µl of

M series RIM standards i.e., $(CF_3)_2$ P(S) $(CH_2)_n CH_3$ Alkyl bis (Trifluoro methyl) Phosphine Sulphide series from M_4 - M_{22} were injected into HRGC. The two channel HRGC chromatogram for ATD / ATD is shown in fig - 2 and chromatogram for FID / ATD is shown in fig – 3. For verification of results, the acetone extract of water samples was analyzed by Mass Spectrometry [9]. The instrument used for this purpose was GC / MS Finning Mat TQS Mass Spectrometer. The GC / MS was first calibrated with FC-43, perflurotributyl amine and respective m/z peaks were adjusted. The GC was fitted with NB – 54 glass capillary column 25m length. The conditions of the GC were adjusted as that of previous HRGC analysis / experiments. Total ion chromatogram was obtained by injecting 1 µl of acetone extract of water sample along with M - Series RIM Standards. After confirmation of scanning numbers of compounds of interest, the Electron impact (EI) mode was used for selective ions. By choice of appropriate mass numbers of CR



Dibenz(bf)(1,4)Oxazepine (CR)

S [1,2 bis (ethoxycarbonyl) ethyl] *O,O*

dimethylphsphoordithioate (MALATHION)

Fig. 6:

and Malathion, the full EI mass spectrum for CR is shown in Fig. 4 and for Malathion in Fig. 5.

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References:

1. Krik – Othmer Encyclopedia of Chemical Technology, Wiley Interscience., 3rd Ed., 400 – 401, (1979).
2. McGraw *Hill Encyclopedia of Science and Technology*, **13**, 247, (1987).
3. Ahmed I., *Sci. Tech & Dev.*, **15**, 25 – 29, (1996).
4. Dressler M., *J.Chromatogr*, **165**, 167 – 208, (1979).
5. Hand Book of Solid Phase Extraction, Analytichem International, C.A., USA, (1990).
6. Yin H.F., and Sun Y.L., *Chromatographia*, **19**, 39, (1990).
7. Kuronen P., Ph.D. thesis, Development of a retention index monitoring method for reversed – phase high performance liquid chromatography of non-phosphorus chemical warfare agents, series A 11, 224 Dept. of Chemistry, University of Helsinki, 70 – 81, (1990).
8. Standard operating procedures for verification of chemical disarmament, series D – 2., The Ministry of Foreign Affairs of Finland, 87 – 165, (1990).
9. Jaeger H., Capillary gas chromatography mass spectrometry in medicine and phramacology, Huething, N.Y.,(1987).