Opinion

Chromatography—Mass Spectrophotometric Techniques for Air Pollutants: A Commentary

Sukesh Narayan Sinha1 and H. Venkatakrishna-Bhatt2

1Departments of Air-Pollution and 2Neurobehavioural Toxicology, National Institute of Occupational Health (NIOH), India

Key words: LCMS, GCMS, Air pollutants

Chromatography is an analytical technique employed for purification and separation of organic and inorganic substances including characterisation and quantitative measurement of compounds (table). Basically there are two types: 1. Gas chromatography (GC), 2. Liquid chromatography (LC). Although discovered in 1906 by Tshett, developed in the 1950s as the GC technique where the mobile phase are gases such as helium (He), nitrogen (N2), yet depends on temperature programming and the boiling point of the compound. Quantitative measurement of volatile organic compounds (VOC), polyaromatic hydrocarbons (PAH) and pesticides are controlled by the boiling point (BP) <400°C, being volatile and thermally stable in passing through the glass tubular column capillaries to ensure reliability.

Liquid chromatography

LC developed in 1960s and 1970s is a high performance liquid chromatographic (HPLC) technique. There are two modes of working by HPLC: 1. the normal phase and 2. the reverse phase. The reverse phase is the most conventional and suitable for the separation, purification, estimation and characterisation of the VOCs to assess the organic, biological and environmental particles in a mixture. The inorganic basic and acidic radicals such as nitrates (NO3–), sulphates (SO42–), carbonates (CO32–), chlorine (Cl–), bromine (Br–) and metal: copper (Cu2+), zinc (Zn2+), lead (Pb2+), etc, are analysed by ion LC techniques. Ion chromatography (IC) and HPLC differ in the column which is packed with suitable resin whereas HPLC has silica gel etc. Several other LC techniques such as thin layer chromatography (TLC), high performance thin layer chromatography (HPTLC), supercritical fluid chromatography (SFC) etc, are used for purification and separation of compounds. Columns with packing materials such as biogel, sephadex, silica gel, alumina and fluoroasil are also used in separation. Mass spectrometers (MS) are used for measuring the molecular weight and for structural formation of organic molecules of protein. MS analysis is for proteins, peptides11, mapping of oligonucleotides12 and air-pollutants. The industrial applications of MS are: 1) Pharmaceutical: testing and discovery of drugs, 2) Environmental: PAHs, (poly-chlorinated biphenyls) PCBs, VOCs, water and food contaminants, 3) Clinical: neonatal screening, haemoglobin analysis, drug testing and 4) Geological: oil composition.

Mass spectroscopy and modes

The different MS modes13 are used for qualitative studies of the organic molecules. In biological science, three modes of MS are used namely, matrix assisted laser desorption ionisation (MALDI-TOF)14 for molecular weight (MW) measurements, some reaction monitoring, Electro-spray ionisation (ESI-Q)15 for MW measurement, LC-MS for reaction monitoring of the structure of protein. Electro-spray-ionisation5/nanospray ionisation quadrupale time of flight (ESI/NS-Q-TOF) can be used for both molecular electron measurement reaction monitoring (MERM17), peptide nucleotide sequencing (PNS), protein and macromolecular structural determination (PMSD18) by the extended mass/charge (M/Z) ratio. Electron impact fails to give the MW of higher MW compounds. Fast atomic bombardment (FAB), chemical ionisation (CI) and secondary ion mass spectrometry (SIMS) are also used for qualitative as well as quantitative studies of different types of compounds. Gas chromatography-mass spectrometry (GC-MS) is now a well established routine technique where the MS is viewed as another detector. Interfacing of LC-MS is a challenge. Last decade mooted availability of systems by reliability10. SFC is complementary to GC and HPLC for the separation of mixtures16. Capillary electrophoresis (CE) is a family of related techniques that employ narrow bore (20–200 µm id) capillaries to perform high efficiency separation of large and small molecules, and the technique is particularly useful for highly polar and ionic compounds19. Interfacing of capillary zone electrophoresis-MS (CZE-MS) has been affected by using a capillary GC-MS interface with a directly heated restrictor11. Packed column SFC-MS offers faster analysis than the capillary approach and also enables quantification to be undertaken. In this area, interfacing has been effected by using a modified moving belt, particle beam and thermospray LC-MS interfaces. GC-MS, LC-MS, SFC, CZE-MS12 are used for quantitative as well as qualitative xenobiotic and air samples. LC-MS, a combination of HPLC with an MS system (like EI, CI, FAB, ESI and MALDI) is used for separation of mixtures and their characterisation on the basis of MW and fragmentation in a compound. It is used in...
mycotoxins, photographic products, drug industries, protein chemistry and natural products, etc. SFC is a complementary tool to GC and HPLC for the separation of mixtures. Packed column SFC-MS has advantages over LC-MS in short time and better chromatographic efficiency. Packed column SFC-MS is used in extraction which provides high recovery and selectivity.

HPLC combinations

HPLC-ICP-MS is useful for plasma trace metal analysis. This is used as a detector for chromatography for its applications in the area of speciation. The elements namely Fe, Cu, As, Rh, Ni, I, Mn, Al, Mo, Pb, Sn, Co and Cd in blood can be analysed. The detection limit of ICP-MS is at the pg, ng level. With this technique identification and quantification of an element or metal in a certain oxidation state can be done. An excellent approach to obtain trace metal speciation is by combining an appropriate chromatographic technique such as LC, GC, SFC for the separation of metal containing species (precessions of 0.1 to 3% RSD). Detector selectivity is also desirable, because possible chromatographic interferences can be simplified by using the single ion-monitoring mode provided by the ICP-MS.

Hawari et al.14) used a combination of solid-phase microextraction-GC-MS and LC-MS techniques, for the identification of two distinctive cycles in the degradation of trinitro-toluene (TNT). One cycle was responsible for the stepwise reduction of TNT eventually to produce triaminotoluene (TAT) in relative high yield (160 µM). The other cycle of TAT is responsible for the production of azo derivatives, e.g., 2,2',4,4'-tetra-amino-6,6'-azotoluene and 2,2',4,4'-tetra-amino-4,4'-azotoluene (nomenclatures by International Union of Pure and Applied Chemistry) at pH 7.2. LC-MS to identify and verify TNT metabolites is performed on a micro-mass platform II benchtop single quadrupole mass detector fronted by a Hewlett Packard 1,100 series HPLC system. Analyte ionization was either done in the positive atmospheric pressure chemical ionisation (APCI+) mode with a cone voltage of 30V at 90° C. Chromatography is in an ion-pairing mode with a C18column (25 cm by 4.6 mm; 5-µm particles) and octanesulfonic acid as the ion-pairing reagent. The flow rate is 1 ml/min with a post-column split of 5:95. A cross-flow counter electrode scheme is used to prevent salt components in the mobile phase from entering the detector. Two powerful techniques, namely solid-phase microextraction (SPME) GC-MS and LC-MS, are applied to analyze the intermediates formed during TNT biodegradation anaerobic sludge. Both techniques are excellent for speed, sensitivity and detection limits.

Chromatography-MS (e.g. High Resolution GC-MS—Electron Impact (EI), Chemical Ionisation (CI) and
Negative Chemical Ionisation (NCI)\textsuperscript{14, 19} techniques were used for the determination of relevant environmental parameters (e.g. PO, SH\textsubscript{20}, KOW) of compounds available only in mixtures. Methods of organic trace analysis were used to study the chemistry of incomplete combustion and high temperature pyrolysis. The chemistry, analytical chemistry, emissions from the technical burning process, including the organic emissions of automobiles and their occurrence in environmental samples were studied for the groups of polychlorinated dibenzo-furans, biphenyls, diphenylethers, chlorophenols and microbial degradation of polychlorinated pesticides and arenes like the dibenzo-p-dioxins, dibenzofurans and naphthalenes.

Combined extraction, derivatization and analysis of S-PMA i.e., S-phenyl mercapturic acid and t\textsubscript{t} MA i.e., trans-mucoric acid, from urine, followed by derivatization using hydrochloric acid/methanol, LC-MS and GC-MS analysis. Ethyl acetate for liquid extraction recovery from 1 ml of acidified synthetic urine were 98 ± 6.2\% and 101 ± 4.2 \% for t\textsubscript{t} MA and 60\%/S-PMA. The limit of detection was 10 hg/ml for t\textsubscript{t}MA and 20 hg/ml for S-PMA. The coefficient of variation was less than 10\% for both t\textsubscript{t} MA and S-PMA. The best sensitivity for the combined analytes was obtained with positive ionization, although the negative ionization mode was more sensitive for t\textsubscript{t} MA alone\textsuperscript{20–22}.

A combined GC-MS technique is used in USA for the qualitative and quantitative analysis of methyl ter-butyl ether (MTBE) and benzene in gasolines\textsuperscript{23–25}. The GC-MS experiment demonstrates the use of internal standards to improve precision, standard additions and ion extraction/monitoring for the measurement of specific highly volatile organic compounds in air-pollution samples\textsuperscript{24}.

The thermal desorbed compounds were analysed by GC/FID (flame ionisation detector)/MS utilising a GC oven program ranging from 2°C (to prevent column plugging by ice) to 160°C and separation on a 30 m × 0.32 mm DB5-MS capillary column. VOCs are identified on the basis of retention time and mass spectral search, whereas quantification is based on FID response which should be calibrated weekly with VOC standards\textsuperscript{25, 26}. Structural characterization of PAC by combined GC-MS and GC/FTIR provides unambiguous identification of the isomers in imox samples.

**Structural analysis**

Detailed structural characterization of the diaromatic fraction of the imox is done by the GC-MS technique with a Hewlett-Packard MS-5970 MS. For this purpose, GC is used as an inlet system for MSD and component wise separation is achieved through high resolution capillary column of methyl silicone (25 m × 0.2 mm) under programmed temperature conditions from 50°C (5 min hold) at 5°C/min rate up to 180°C\textsuperscript{27}.

Air samples collected on sorbent media (e.g. OSHA i.e.Occupational Safety and Health Administration versatile sampler (OVS) tubes) will be analyzed for the target list of energetic analytes via LC-MS in accordance with the USEPA (United States Environmental Protection Agency) method 8321. The practical quantitation limit (PQL) for this method is reported to be ≤0.5 parts per billion by volume (ppbv). Polyurethane foam (PUF) cartridge samples were analyzed for dioxins and furans as per EPA test method TO-9\textsuperscript{28}. This method is designed for the analysis of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans in ambient air at high resolution GC/HRMS.

**Metabolites:** Different metabolites of benzene\textsuperscript{29} such as PH sulfate, PH blucuronide, S-phenylglutathione, CT, HQ, PH, benzene-1,2-dihydriodiol and metabolite G (presumably trans-trans-mucoric acid, are identified after preparative HPLC as described by Schrenk and Bock). Metabolites were detected with a Lambda-max 491 uv detector (Millipore, Dreieich, Germany) at 280 nm and a Beckmann 171 radioisotope detector. The radiodetector was set for liquid scintillation with Ultima Gold Scintillation at a flow rate of 3 ml/min. Alternative fractions of 0.3 min were collected and quantified by liquid scintillation counting. Two novel benzene metabolites separated by HPLC analysis of supernatants from mouse hepatocyte incubations were isolated by preparative HPLC. Fractions eluting at the respective retention intervals (0.3 min) were collected. After adjustment to pH 7.8, fractions were evaporated to dryness, kept under nitrogen and were analysed by fast atomic bombardment (FAB) MS with a Varian MAT 711 MS after dissolving the sample in a glycerol matrix.

The samples were analyzed in a Finnigan 4021 MS with EI MS after derivatization with N-methyl/N-(trimethylsilyl) trifluoroacetamide (MSTFA). Supersonic LCMS\textsuperscript{29, 30} were frequently used in an environmental as well as a biological field. Several thermally labile compounds that are not amenable for GC analysis were successfully analyzed, including underivatized steroids such as stanzolol and corticosterone, drugs such as beta-carotene for high quality liberally searchable EI-MS were obtained for all these compounds with no peak tailing injection analysis.

A 2-mm Carbotrap tube was used to determine VOCs in ambient air. Such compounds were desorbed and thermally analyzed with GC-MS\textsuperscript{31}. Fisons MD 800 GC-MS\textsuperscript{31}, were used for identification of individual hydrocarbons. Separation of the compounds was achieved with a 60m × 0.25 mm, 1.50 \(\mu\)m film VOCOL capillary column (Supelco). This column has a programmed temperature of 35°C for 4 min and finally during analysis at a constant temperature of 200°C for 4 min. With the GC-MS technique 54 toxic\textsuperscript{32, 33} HC were
identified in the ambient air of Tehran and the average measured concentrations of benzene, toluene, m-and p-xylene, ethyl benzene and o-xylene were 127.6 µg/m³, 201.1 µg/m³, 110.7 µg/m³, 58.1 µg/m³ and 57.6 µg/m³, respectively (SD=3.8–51.7 µg/m³).

Quantiﬁcations

GC-MS was used to quantify tri-phenyl hydroquinol i.e. TPH (13) in environmental samples. Analysis of these data shows that there is no statistical difference between TPH values quantified with GC-MS and those derived from conventional TPH methods with GC/FID. Further the GC-MS analysis used in the study is readily adaptable to most environmental laboratories currently performing volatile and semi-volatile compounds. EPA analyses (i.e. 8260 and 8270 respectively; EPA 846 (14)). The strectic implications of these results are: (1) additional information (e.g. PAH) is often required when conducting a risk based assessment which can be derived from pre-existing site assessment data, thereby decreasing the cost and time required to obtain the additional samples and analytical information and (2) unique TPH distributions can be critically evaluated with mass spectra in order to ascertain the nature and potential source of the compounds present in the TPH mixture (15).

The SPME-GC-MS technique was used for bio-transformation of TNT (16, 17). For this purpose fused silica capillary fibres (1 cm) coated with either 85 µm of polyacrylate or 100 µm of polymethylsiloxane fitted to an autosampler assembly were used (Supelco, Bellfonte, Pa). The fiber was conditioned by placing it inside the injection port of a GCMS at 300 °C. Subsequently, aliquots (1 ml) from the TNT culture medium were spiked with the recovery standard 4-ethyl-dibenylthiopene (4-Et-DBT; 250 ppb) and filtered through a Millex-HP 0.45 µm–pore-size filter to remove suspended material including bio-mass. A 20-min adsorption time from the aqueous solution followed by a 10 min desorption inside the GC injector were found appropriate for reproducible analyses. GC (Varion 3400) fitted with a DB-5 column (30 m by 0.25 µm by 0.25 µm thick) by using He as carrier gas was used. The initial oven temperature was 90°C (2 min), increased to 165°C (10°C/min) and then incremented to 250°C (5°C/min). Mass spectral analysis was carried out with a Varian Saturn II system in the EI mode (70 eV) by using a mass range of 15 to 300 amu background mass of 14 amu, and a mass scan rate equal to 0.5 s/scan. A time study to monitor the formation and disappearance of metabolites was carried out at t=0 and at hourly intervals for the first 10 h and finally, daily sampling until the end of the experiment.

Acknowledgments: Facilities, support and encouragement by the Director of NIOH is gratefully acknowledged.

References

20. MG Menache, N Lian, JM Starr and RF Henderson:


29) D Schrenk, A Orzechowski, R Schwarz, R Synder, B Burechell, MI Sundberg and KW Bock: Phase II Metabolism of benzene. Env Hlth Persp. 104 (Sup.6) 1–17 (1996)


31) NIOSH. Manual of analytical methods, Cincinnati, OH 1997; (NIOSH).

32) USEPA: Compendium of methods for the determination of toxic organic compounds in ambient air, EPA–600 (4)–89–091, 1994; Chapel Hill, NC.


34) Robert H. TPH measurements; the advantage of using GCMS. 1995; Ph.D.Dissertation. ENTRIX INC JOHN MAC MURPHY ZYMAX ENVIRA TECHNOLOGY INC.

