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GC-MS: Principle, Technique and its application in Food Science

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Abstract

GC/MS-a combination of two different analytical techniques, Gas Chromatography (GC) and Mass Spectrometry (MS), is used to analyze complex organic and biochemical mixtures. GC can separate volatile and semi-volatile compounds with great resolution, but it cannot identify them. MS can provide detailed structural information on most compounds such that they can be exactly identified and quantified, but it cannot readily separate them. Therefore, it was not surprising that the combination of the two techniques was suggested shortly after the development of GC in the mid-1950's. Gas chromatography and mass spectrometry are, in many ways, highly compatible techniques. In both techniques, the sample is in the vapor phase, and both techniques deal with about the same amount of sample (typically less than 1 ng). This article was prepared with an aim to review different aspects GC-MS, such as principle, types, instrumentation and applications in food science.

Keywords: Gas chromatography, Mass spectroscopy, Biochemical, Identification, Quantification

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Introduction

Gas Chromatography (GC), is a type of chromatography in which the mobile phase is a carrier gas, usually an inert gas such as helium or an un-reactive gas such as nitrogen, and the stationary phase is a microscopic layer of liquid or polymer on an inert solid support, inside glass or metal tubing, called a column. The capillary column contains a stationary phase; a fine solid support coated with a nonvolatile liquid. The solid can itself be the stationary phase. The sample is swept through the column by a stream of helium gas. Components in a sample are separated from each other because some take longer to pass through the column than others. Mass Spectrometry (MS), the detector for the GC is the Mass Spectrometer (MS). As the sample exits the end of the GC column it is fragmented by ionization and the fragments are sorted by mass to form a fragmentation pattern. Like the retention time (RT), the fragmentation pattern for a given component of sample is unique and therefore is an identifying characteristic of that component. It is so specific that it is often referred to as the molecular fingerprint. Gas chromatography-mass spectrometry (GC-MS) is an analytical method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. GC can separate volatile and semi-volatile compounds with great resolution, but it cannot identify them. MS can provide detailed structural information on most compounds such that they can be exactly identified, but it cannot readily separate them.

Principle of GC-MS: GC/MS-a combination of two different analytical techniques, Gas Chromatography

(GC) and Mass Spectrometry (MS), is used to analyze complex organic and biochemical mixtures (Skoog et al., 2007). The GC-MS instrument consists of two main components. The gas chromatography portion separates different compounds in the sample into pulses of pure chemicals based on their volatility Oregon State University, 2012) by flowing an inert gas (mobile phase), which carries the sample, through a stationary phase fixed in the column (Skoog et al., 2007). Spectra of compounds are collected as they exit a chromatographic column by the mass spectrometer, which identifies and quantifies the chemicals according their mass-to-charge ratio (m/z). These spectra can then be stored on the computer and analyzed (Oregon State University, 2012)

Instrumentation and Working of GC-MS

The Fig. 1 is the schematic diagram of GC-MS. Its different parts and their functions are discussed below.

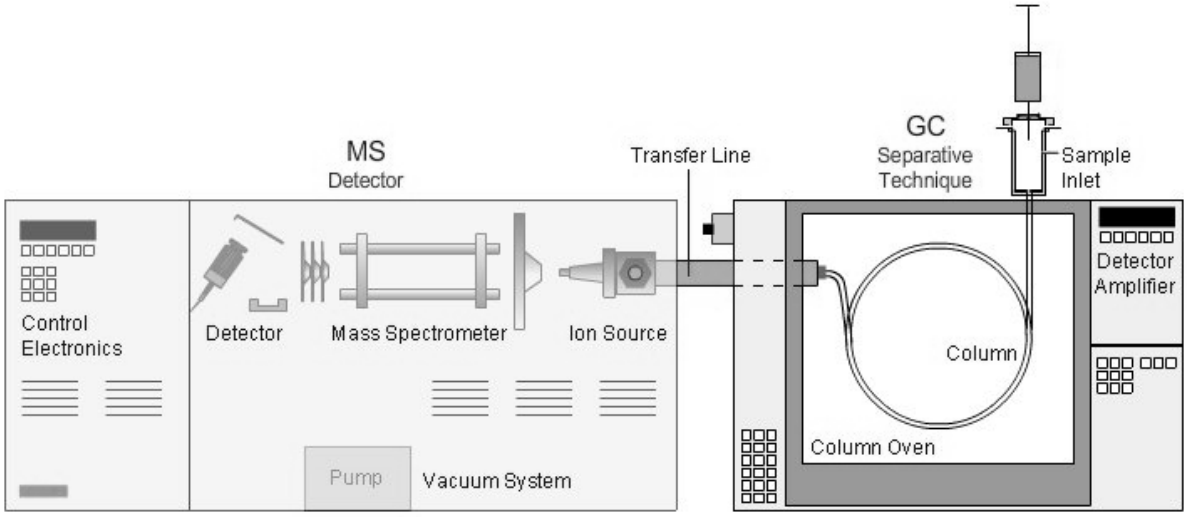

Fig. 1. Schematic diagram of GC-MS

Gas supply

Carrier gas is fed from the cylinders through the regulators and tubing to the instrument. It is usual to purify the gases to ensure high gas purity and gas supply pressure (Gas Supply and Pressure Control from theory and Instrumentation of GC-GC Channel).

Injector

Here the sample is volatilized and the resulting gas entrained into the carrier stream entering the GC column (Sampling Techniques and Sample Introduction from Theory and Instrumentation of GC-GC Channel).

Column

Gas Chromatography uses a gaseous mobile phase to transport sample components through columns either packed with coated silica particles or hollow capillary columns containing, the stationary phase coated onto the inner wall. Capillary GC columns are usually several meters long (10-120 m is typical) with an internal diameter of 0.10-0.50 mm, whilst packed GC columns tend be 1-5 meters in length with either 2 or 4mm internal diameter (GC columns from Theory and Instrumentation of GC).

Oven: Gas chromatography have ovens that are temperature programmable, the temperature of the gas chromatographic ovens typically range from 5℃ to 400℃ but can go as low as -25℃ with cryogenic cooling (GC Temperature Programming from the 'Theory and Instrumentation of GC').

Mass Spectrometer: The separation of the phase ions is achieved within the mass spectrometer using electrical and/or magnetic fields to differentiate ions.

Ion source: In the ion source, the products are ionized prior to analysis in the mass spectrometer.

Mass analyzer: There are several very popular types of mass analyzer associated with routine GC-MS analysis and all differ in the fundamental way in which they separate species on a mass-to-charge basis**.**

Vacuum system: Mass analyzers require high levels of vacuum in order to operate in a predictable and efficient way.

Detector: The ion beam that emerges from the mass analyzer, have to be detected and transformed into a usable signal. The detector is an important element of the mass spectrometer that generates a signal from incident ions by either generating secondary electrons, which are further amplified, or by inducing a current (generated by moving charges).

Control Electronics: The MS parameters can be selected and controlled from this panel. Modern instruments will also allow to control MS parameters from a computer by using specially designed software. The mobile-phase called as carrier gas, must be chemically inert. The helium gas is most commonly used, however, argon, nitrogen, and hydrogen are also used. These gases are held in pressurized tanks and use pressure regulators, gauges, and flow meters to control the flow rate of the gas. Flow rates usually range from 25-150 mL/min with packed columns and 1-25 mL/min for open tubular capillary columns, and are assumed to be constant if inlet pressure is constant. This is often accompanied by a molecular sieve to purify the gas before it is used. Samples are introduced as a plug of vapor. Liquid samples are introduced using calibrated micro syringes to inject sample through a septum and into a heated sample port which should be about 50°C above the boiling point of the least volatile constituent of the sample. After the sample is introduced, it is carried to the column by the mobile phase. The temperature of the column is an important variable, so the oven is equipped with a thermostat that controls the temperature to a few tenths of a degree. Boiling point of the sample and the amount of separation required determines the temperature the sample should be run with. As the mobile phase carrying the sample is passed through the stationary phase in the column, the different components of the sample are separated. After being separated, the sample is run through a detector (Skoog et al., 2007), which ionizes the sample and then separates the ions based on their mass-to-charge ratio. This data is then sent to a computer to be displayed and analyzed. The computer linked to the GC-MS has a library of samples to help in analyzing this data (Agilent Technologies, 2012). Data for the GC-MS is displayed in several ways. One is a total-ion chromatogram, which sums the total ion abundances in each spectrum and plots them as a function of time. Another is the mass spectrum at a particular time in the chromatogram to identify the particular component that was eluted at that time. A mass spectra of selected ions with a specific mass to charge ratio, called a mass chromatogram, can also be used.

Applications of GC-MS in food science

GC-MS is widely used for research purposes in many fields and is emerging as an important technique in food science as well. Some of the recent applications of GC-MS in the food science have been summarized below:

Identification and quantification of volatile N-nitrosamines in meat products

N-Nitrosamines (NAMs), which can be present in the environment and in a wide variety of food are in most cases strongly carcinogenic (IARC, 1987). In all foods where they occur NAMs are formed from the reaction between a nitrosating agent and a secondary amine, and their formation can be the result of a chemical and/or microbial reaction. The contamination of NAMs in meat products occurs due to the use of nitrite and nitrate as curing agents (Sannino and Bolzoni, 2013). Sodium nitrite and sodium nitrate are very important not only as preservatives, but also for colour and flavour formation and antimicrobial effects (Lucke, 2003; Fischer et al., 2005; Honikel et al., 2008; Kabisch et al., 2008). Sodium nitrite and nitrate also play a considerable role in formation of the carcinogenic NAMs in meat, especially under the specific process conditions applied in the meat processing industry.

The chemistry of nitrosation is very complicated and shows a dependency on the pH, the basicity of the secondary amine and temperature (Mirvish, 1975; Wades and Coates, 1987; Patel and Jones, 2007). A sensitive and selective method was developed and validated for the determination of nine N-nitrosamines in meat products. The N-nitrosamines were extracted with NaOH/methanol, partitioned into dichloromethane on a ChemElut column and cleaned-up by solid-phase extraction. All samples were spiked with 2H isotope-labelled N-nitrosamine internal standard prior to extraction. After purification on a Florisil mini-column, the extracts were analysed by gas chromatography-chemical ionization tandem mass spectrometry (GC-CI/MS/MS) using ammonia as reagent gas. The presence of N-nitrosamines in samples was quantified by isotope dilution mass spectrometry. The method was validated for linearity and range, accuracy, precision and sensitivity. Recoveries were calculated at three levels of concentration (0.5, 1 and 10 lg/kg) spiked in raw pork meat. The values were found between 95 and 110% with relative standard deviation (RSD) values between 5 and 11%. The excellent selectivity and sensitivity allows quantification and identification of low levels of N-nitrosamines in meat products (limits of quantitation (LOQs) 0.3-0.4 l g/kg). N-Nitroso-dimethylamine was detected in all examined products in the range 0.3-1.1 l g/kg (Sannino and Bolzoni, 2013).

Determination of melamine and cyanuric acid in dairy products

Milk and milk products have made and will always make important contributions to the human diet. Cyanuric acid (CYA) and Melamine (MEL) were found to boost apparent protein content in dairy products (Chao et al., 2011). Melamine has low oral acute toxicity but excessive exposure in animals causes renal stones. When consumed by human beings, babies and children are affected the most because of the immaturity of their organs, which renders them vulnerable to chemical damage. Furthermore, the toxicity will be enhanced due to their mutual affinity when CYA and MEL are adulterated together in the milk (Pan et al., 2013). A method was described for simultaneous determination of cyanuric acid (CYA) and melamine (MEL) in dairy products. The samples were extracted by a mixture of acetonitrile/water and cleaned by graphite carbon/strong cation exchange (CARB/SCX) mixed-mode solid phase extraction column. The CYA and MEL were derivative with bis (tri-methylsily1) trifluoroacetamide (BSTFA)

containing 1% Trimethylchlorosilane (TMCS), and quantified with the internal standards of 15N3-cyanuric acid and 13C3-melamine by GC-MS. The results indicated that CARB/SCX mixed solid phase extraction column could be used for sample pretreatment. The fortification recoveries were 80.8-101.5%, and the relative standard deviations (RSDs) were 3.6-7.9%. The detection limits of CYA and MEL were reported to be 0.025 and 0.01 mg kg^{-1} respectively (Brokl et al., 2011; Apolinário et al., 2014).

Quantification of inulin type-fructans

Gas chromatography-Mass Spectrometry (GCMS) has been successful to obtain structural information on fructans mainly degree of polymerization (DP). Quantification of inulin type-fructans may be performed in the extracts so as to provide a preliminary assessment of plant fructan contents. Since fructans are usually found as complex mixtures of carbohydrates with different DP, monomer composition and glycosidic linkages, their analysis is a fundamental step to acquire basic information on the polysaccharide itself as well as to deepen understanding of its action mechanism, which is dependent on its chemical structure. However, the separation of complex mixtures of oligosaccharides is not straightforward, because of structural and molecular weight similarity; in addition, their identification is also hampered by the lack of available commercial standards (Arrizon et al., 2010). The effect of age of *A. tequilana* on its fructan content and structure for economical purposes was investigated by HPLC, HPAEC-PAD, MALDI-TOF-MS and GC-MS, with HPLC allowing for separation of polymerized and non-polymerized sugars, HPAEC-PAD for determination of distribution of fructans with approximate DP ranging from 3 to 6 and longer fructans compared with Dahlia tubers inulin, MALDI-TOF-MS for detection of differences in oligosaccharide distribution induced by plant age, and GC profile for linkages analysis (Altaki et al., 2007).

Detection of furan levels in foods: The determination of furan in food is not easy because of the extremely high volatility of furan (US FDA, 2014). The first quantitative method for furan in food was published by the FDA using head-space extraction coupled with gas chromatography-mass spectrometric (HS-GC/MS) determination (Sijia et al., 2014). A solid phase micro extraction-gas chromatography/mass spectrometry (SPMEGC/MS) method was developed for detection of the furan levels in 191 selected food products obtained from the Chinese markets. The performance of the analysis method was evaluated by some quality parameters such as limit of detection (LOD), limit of quantification (LOQ), linearity, recovery, and run-to-run (n = 6) and day-to-day (n = 18) precisions. The LOD and the LOQ of the method were 0.059 ng/g and 0.175 ng/ g, respectively. The recovery of the method in fruit juice, canned jam, and infant formula were found to range from 93.25 to 104.69%. The results from this study showed that furan was detected in almost all analyzed samples. Furan contents in different food samples were found to vary greatly according to the raw materials and processing conditions. The higher contents of furan were detected in traditional Chinese liquor (61.63 ng/g), coffee (71.36 ng/g), tea (68.28 ng/g) and pickle (85.63 ng/g). The dietary intake of furan was estimated to be 0.093 lg/kg body weight/day in adults and 0.333 lg/kg body weight/day in infants at 6 months (Sijia et al., 2014).

Screening, determination and confirmation of seafood, meat and honey: Studies on screening, determination and

confirmation of chloramphenicol (CAP) in 10 kinds of matrices, including seafood, meat, honey,etc. by enzyme-linked immunosorbent assay (ELISA), high-performance liquid chromatography (HPLC) with UV detector (HPLC-UVD) and gas chromatography in combination with electronic capture detector (GC-ECD) and mass spectrometry detector (GC-MS) in both electronic ionization mode (EI) and the negative ion chemical ionization mode (NCI) with selected-ion monitoring (SIM) acquisition method (GC-MS-EI-SIM and GCMS-NCI-SIM) have been carried out. Methods have been developed for both qualitative and quantitative detection of chloramphenicol (CAP). Extraction, clean-up, derivatization and analysis procedure have been optimized. The ELISA was carried out for screening, HPLC, GC and GC-MS were applied to determine and confirm CAP residue concentration in suspect samples. The ELISA procedure was carried out on an aqueous extract of the samples. Determination and confirmation of suspect samples were performed after extraction with phosphoric buffer solution (PBS, pH=6.88) /ethyl acetate, defatting with hexane, analyzed by HPLC or GC–ECD, GC–MS–EI, GC–MS–NCI method. Samples for GC analysis, were further clean-up with solid-phase extraction using LC-Si and LC-C18 cartridges and derivatized to form volatile derivatives by derivatization agent. These techniques are able to detect chloramphenicol residues at the level of 0.1-10 µg/kg. Overall recoveries were 75-120% with precision values at 5.4-8.1% (Shen and Jiang, 2005).

Monitoring of autoxidation in LCPUFA-enriched lipid microparticles

Electronic nose and solid phase microextraction (SPME)-GCMS were used to monitor the autoxidation in long chain polyunsaturated fatty acid (LCPUFA)-enriched lipid microparticles produced by spray congealing with ultrasonic nebulization, during storage at 20°C up to 6 weeks with sufficient air supply and limited air supply. Conjugated dienes and peroxide value as well as secondary lipid oxidation products were analysed to follow the course of autoxidation. Principal Component Analysis evidenced that only Metal Oxide Semiconductor (MOS) sensors but not Metal Oxide Semiconductor Field Effect Transistors (MOSFET) sensors contributed to the discrimination of the samples and facilitated the ability of the electronic nose to distinguish the LCPUFA-enriched lipid microparticles into two groups according to the different oxidative status. The selected MOS sensor responses correlated well with quantitative dominating volatile compounds (propanal and hexanal) and with volatile compounds which have been associated with fishy and rancid off flavour (1-penten-3-one, 1-penten-3-ol, 2,4-heptadienal and 2,6-nonadienal). Bread mix supplemented with the LCPUFA-enriched microparticles was analysed as an example for a LCPUFA supplemented food. Data from the present study indicate that the electronic nose can be used as a sensitive tool to evaluate the lipid oxidative status of LCPUFA-enriched microparticles. In supplemented foods like bread mix, matrix-related changes, which occur in supplemented and non-supplemented samples, make a clear distinction more difficult (Benedetti et al., 2009).

Mapping the structural requirements of inducers and substrates for decarboxylation of weak acid preservatives by the food spoilage mould Aspergillus niger: Moulds are able to cause spoilage in preserved foods through degradation of the preservatives using the

Pad-decarboxylation system. This causes, for example, decarboxylation of the preservative sorbic acid to 1,3-pentadiene, a volatile compound with a kerosene-like odour. The Pad-decarboxylation system, encoded by a gene cluster in germinating spores of the mould *Aspergillus niger*, involves activity by two decarboxylases, PadA1 and OhbA1, and a regulator, SdrA, acting pleiotropically on sorbic acid and cinnamic acid. The structural features of compounds important for the induction of Pad-decarboxylation at both transcriptional and functionality levels were investigated by RTPCR and GC-MS. Sorbic and cinnamic acids served as transcriptional inducers but ferulic, coumaric and hexanoic acids did not. 2, 3, 4, 5, 6-Pentafluorocinnamic acid was a substrate for the enzyme but had no inducer function; it was used to distinguish induction and competence for decarboxylation in combination with the analogue chemicals. The structural requirements for the substrates of the Pad-decarboxylation system were probed using a variety of sorbic and cinnamic acid analogues. High decarboxylation activity, ~100% conversion of 1 mM substrates, required a mono-carboxylic acid with an alkenyl double bond in the trans (E)-configuration at position C_2, further unsaturation at C_4, and an overall molecular length between 6.5 Å and 9 Å. Polar groups on the phenyl ring of cinnamic acid abolished activity (no conversion). Furthermore, several compounds were shown to block Pad-decarboxylation. These compounds, primarily aldehyde analogues of active substrates, may serve to reduce food spoilage by moulds such as *A. niger*. The possible ecological role of Pad-decarboxylation of spore self-inhibitors is unlikely and the most probable role for Pad-decarboxylation is to remove cinnamic acid-type inhibitors from plant material and allow uninhibited germination and growth of mould spores (Stratford et al., 2012).

Pesticide residue analysis in routine food control

Five food matrices (cucumber, sweet pepper, grapefruit, wheat flour and curry powder) were compared using single ion monitoring (SIM) and multiple reaction monitoring (MRM). The samples were analyzed by the Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method (Anastassiades and Lehotay, 2003) in combination with GC-MS/ (MS) using a Programmed Thermal Vaporizing (PTV) injector to inject 3 µl of the extracts in solvent vent mode. The Waters Quattro micro tandem quadrupole mass spectrometer was used in electron impact (EI^+) mode in SIM and MRM mode (collision gas pressure of 3×10^{-3} mBar). The ion source was operated at 180 °C with electron energy of 70 eV and a trap current of 200 µA. In SIM mode three masses were acquired for each residue while in MRM mode it was two transitions. The confirmation criteria chosen were in accordance with the Qualitiy Control Procedures for Pesticide Residue Analysis. For a mixture of 32 pesticides calibration curves were constructed using matrix matched calibration standards, 0.005 to 0.5 mg/kg. Good correlation coefficients were obtained in all five matrices in both SIM and MRM modes, however in MRM mode the percentage of pesticide residues that could be screened and confirmed at 0.005 mg/kg was higher than in SIM mode, especially in complex matrix. The limits of determination obtained were in the range between 0.01 and 0.001 mg/kg (for some pesticides even lower) (Wauschkuhn et al., 2006).

Optimizing the Analysis of Acrylamide in Food by Quadrupole GC/MS: Analytical methods for acrylamide

in food using the Finnigan, TRACE DSQ (Finnigan, DSQ and TRACE are trademarks of Thermo Electron Corporation) in positive electron ionization and positive and negative chemical ionization (PCI and NCI) modes were developed. The TRACE DSQ is a quadrupole mass spectrometer that incorporates a curved pre-filter which essentially eliminates non-chemical noise created by excited neutrals striking the detector. Two extraction methods with minimal sample preparation involved both derivatized and underivatized acrylamide. Selected Ion Monitoring (SIM) was chosen to increase sensitivity. A Finnigan TRACE GC Ultra with a PTV inlet provided the means for sample introduction and offered flexibility for optimized injections to accommodate sample matrix. Methane and ammonia were both used as reagent gases. The reagent gas flow rate was 2 mL/min. The TRACE DSQ is a valuable tool in the analysis of acrylamide in food due to its flexibility. In positive electron ionization SIM, acrylamide monomer was found to be linear from 5-500 ppb. Negative Chemical reagent gas (Robarge et al., 2003).

Detection of Chloropropanols

1,3-Dichloropropan-2-ol (1,3-DCP), 3-monochloropropane-1,2-diol (3-MCPD), 2-monochloropropane-1,2-diol (2-MCPD) are the most toxic chloropropanols found as contaminants in acid-hydrolysed vegetable protein. All resent methods for the determination of these toxicants are based on GC–MS after their previous derivatisation using phenylboronic acid, butaneborinic acid, heptafluorobutyric acid anhydride, heptaluorobutyrylimidazole, or *N*,*O*-bis(trimethylsilyl)trifluoroacetamide. Narrow bore nonpolar columns ((5%-phenyl)-methylpolysiloxane) are used for separation of chloropropanols with quadrupole (SIM) or ion trap analysers (MS/MS) for their detection (Chung et al., 2002; Abu-El-Haj et al., 2005).

Conclusion

GC-MS applications in the food sector are among the fastest developing fields in science and industry. The need for accurate molecular characterization of the food, demanded both by consumers and regulatory agencies, is leading the food industry to apply advanced techniques for detailed analytical assessment of food commodities. This shifts the food industry in the direction of the pharmaceutical sector, primarily with respect to analytical chemistry, but also in other aspects. Foods are complex mixtures of different components contained in varying amounts, making analysis a challenging task. GC-MS is in the forefront of this analytical challenge; being a unique tool for reliable characterization of complex mixtures. Its excellent figures of merit are a consequence of the combination of the separative power of gas chromatography to the power of mass spectrometry to identify molecular structure. It became possible to characterize any food at the molecular level. Desirable and undesirable molecules are often routinely identified and quantified in various foods. GC–MS is useful analytical method which allows simultaneous assessment of a variety of components in complex mixtures such as foods.

Nomenclature

GC Gas chromatography

MS Mass spectrometry

TMCS Trimethylchlorosilane

RSDs Relative standard deviations

CYA Cyanuric acid

MEL Melamine

BSTFA bis (trimethylsily1) trifluoroacetamide

LOQs Limits of quantitation

NAMs N-Nitrosamines

DP Degree of polymerization

LOD Limit of detection

LOQ Limit of quantification

CAP Chloramphenicol

ELISA Enzyme-linked immunosorbent assay

HPLC High-performance liquid chromatography

NCI Negative ion chemical ionization mode

SIM Selected-ion monitoring

ECD Electronic capture detector

PBS Phosphoric buffer solution

SPME Solid phase microextraction

LCPUFA Long chain polyunsaturated fatty acid

MOS Metal Oxide Semiconductor

MOSFET Metal Oxide Semiconductor Field Effect *Transistors*

PCR Polymerase chain reaction

MRM Multiple reaction monitoring

QuEChERS Quick, Easy, Cheap, Effective, Rugged and *Safe*

PTV Programmed Thermal Vaporizing

1,3-DCP 1,3-Dichloropropan-2-ol

3-MCPD 3-monochloropropane-1,2-diol

2-MCPD 2- monochloropropane-1,2-diol

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