Liquid Chromatography - Mass Spectrometry (LC-MS) – A review

Solleti Pranathi, Enjamoori Vijay Kumar, Vasudha Bakshi, Boggula Narender*

*Department of Pharmaceutical Chemistry, School of Pharmacy,
Anurag Group of Institutions, Venkatapur, Ghatkesar, Telangana, India.

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Corresponding author address
Narender Boggula
Assoc. Professor,
Department of Pharmaceutical Chemistry,
School of Pharmacy,
Anurag Group of Institutions,
Venkatapur, Ghatkesar,
Telangana, India-500088.

ABSTRACT

Liquid chromatography-mass spectrometry (LC-MS) is quick developing and it's the popular tool of liquid chromatographers. Liquid chromatography-mass spectrometry (LC-MS/MS) may be a technique that uses liquid chromatography (or HPLC) with the mass spectroscopic analysis. it's an analytical chemistry technique that mixes the physical separation capabilities of liquid action (or HPLC) with the mass analysis capabilities of mass spectroscopic analysis. (LC-MS/MS) is often utilized in laboratories for the qualitative and measure of drug substances, drug product and biological samples. This review describes temporary introduction to the methods of quantitative analysis that are one among the foremost valuable course in scientific training. an introduction to HPLC like stationary part used, applications of HPLC is concisely overviewed. the basic principle of mass spectroscopic analysis is shortly mentioned. the most objective of this review is to debate renowned and most generally used hyphenation technique, liquid chromatography in conjunction with mass spectrometry [LC-MS]. Interface plays a crucial role within the hyphenation technique; as eluent from liquid action is transferred to mass spectrometer with the assistance of interface. A outline of the key parts of LC-MS systems, similarly as an summary of major application areas that use this method as a part of the drug discovery process, are represented here.

Keywords: Liquid chromatography-mass spectroscopic analysis, applications, qualitation, drug discovery, quadrupole.

Introduction

Thousands of years of accumulation of experience on life and disease made by our ancestors finally translated into a modern pharmacy. Chinese herbs, with complex and various ingredients are usually put into practice by prescription, following the rules of monarch, minister, assistant, and guide. When applied, the number of single herbs and pharmaceutical formulations can vary, which may lead to changes in the interaction between the drugs and the active ingredient. In different drug application sites, there will be differences in ingredients [1].

Coupling of MS to chromatographic techniques has always been desirable due to the sensitive and highly specific nature of MS compared to other chromatographic detectors. The coupling of Gas Chromatography to MS (GC-MS) was achieved in the 1950s with commercial instruments available from the 1970s. Relatively cheap and reliable GC-MS systems are now a feature of many clinical biochemistry laboratories and are indispensable in several areas where the analysis of complex mixtures and unambiguous identification is required e.g. screening urine samples for inborn errors of metabolism or drugs. The coupling of MS with LC (LC-MS) was an obvious extension but progress in this area was limited for many years due to the relative incompatibility of existing MS ion sources with a continuous liquid stream. Several interfaces were developed but they were cumbersome to use and unreliable, so uptake by clinical laboratories was very limited. This situation changed with the development of the electrospray ion source by Fenn in the 1980s [1]. Manufacturers rapidly developed instruments equipped with electrospray sources, which had a great impact on protein and peptide biochemistry. Fenn was awarded the Nobel Prize in 2002 with Koichi Tanaka who developed matrix assisted laser desorption ionisation, another extremely useful MS ionisation technique for the analysis of biological molecules [2].

By the mid 1990s, the price and performance of LC-MS instruments had improved to the extent that clinical biochemistry laboratories were able to take advantage of the new technology. Biochemical genetics was one of the first areas to do so, and the analysis of neonatal dried blood spot samples for a range of inborn errors of metabolism was a major early application [3]. There are a number of other clinical applications of LC-MS, and the technique is more generally

applicable than GC-MS owing to the broader range of biological molecules that can be analysed and the greater use of LC separations in clinical laboratories. The reasons for choosing LC-MS over LC with conventional detectors are essentially the same as with GC-MS, namely high specificity and the ability to handle complex mixtures. Applications of electrospray MS were reviewed in The Clinical Biochemist Reviews in 2003 [4]. The current review focuses on the principles of LC-MS, practical considerations in setting up LC-MS assays and reviews some of the major applications in clinical biochemistry, concentrating on small molecule applications.

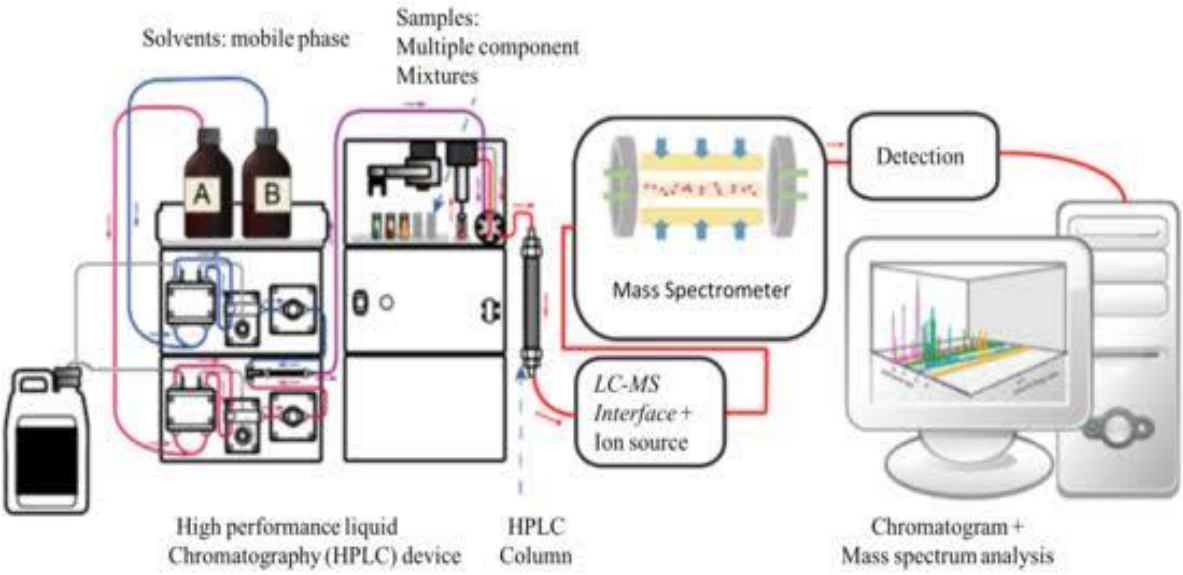

Fig 1: Direct electron ionisation Liquid Chromatography-Mass Spectroscopy

The interfacing mechanism is contained inside a common EI source, like that found in any GC-MS system. The liquid phase from a nano HPLC column is admitted from the capillary column port, where the connection tubing and the nebulizer are first introduced and sealed to prevent vacuum loss. The mechanism is based on the formation of an aerosol in high-vacuum conditions, followed by a quick droplet desolvation and final vaporization of the solute prior to the ionization. The completion of the process is quick and complete and reduces chances of thermal decomposition as reported in the Figure no:1, where a scheme of the interface is shown. The core of the interface is represented by the micro-nebulizer [5,6]. The nebulizer tip protrudes into the ion source so that the spray expansion is completely contained inside the ion volume. The eluate emerges as liquid phase at a flow rate of 300-500 nL/min, and any premature in-tube

solvent evaporation is prevented by a convenient thermal insulation of the nebulizer and the connecting tubing from the surrounding source heat. The high temperature of the ion source, between 300 and 400 ^{0}C, has a double function: to compensate for the latent heat of vaporization during the droplet desolvation, and to convert the solute into the gas phase. If all components of this simple interface are correctly placed and sized, then each substance separated by the nano-column is smoothly converted into the gas phase, the peak profile is nicely reproduced, and high quality mass spectra are generated [7,8].

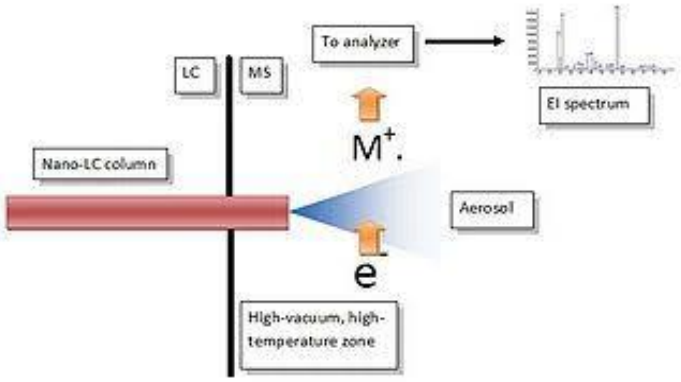

Fig 2: Mechanism (LC-MS)

Principle

In principle, the mixture of components to be separated is loaded on an autosampler, injected into an LC stream of mobile phase and separated on a column. The eluting fractions are then detected by a mass analyser (or commonly an in-line UV/DAD detector followed by a mass analyser) via ionisation in one of a number of techniques (EI, ESI, APCI, APPI, ESCI etc.). With respect to the LC setup, this can be HPLC or UPLC depending on the analytical method and there are obvious merits for each. There are a multitude of options on the characteristics of the mass analyser [9-12].

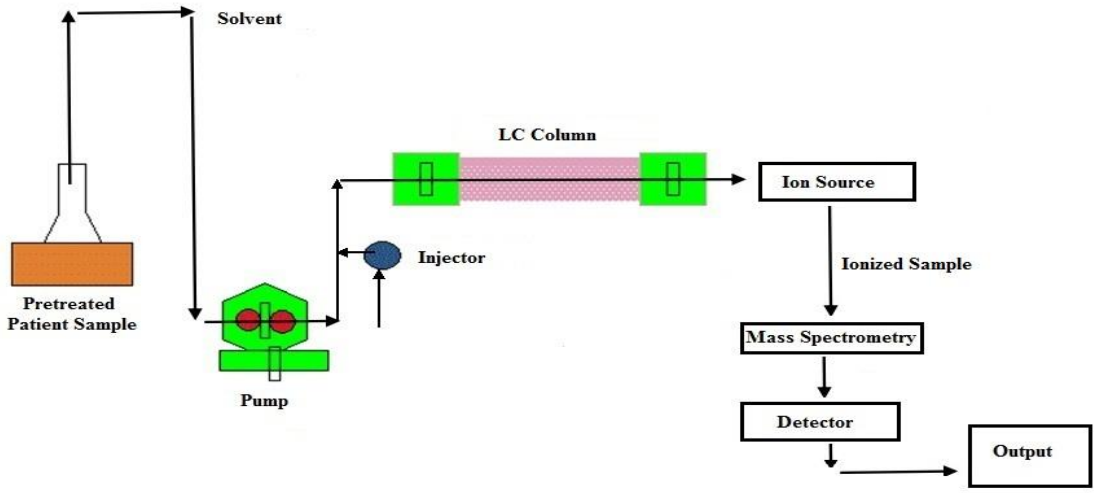

Fig 3: Principle-Liquid Chromatography-Mass Spectroscopy (LC-MS)

LC

Chromatography separates the mixture using the differences of the distribution coefficient between the two phases (mobile and stationary phase). According to the state of the mobile phase, chromatography can be divided into gas chromatography, liquid chromatography, and supercritical fluid chromatography, while, according to the geometric forms of the stationary phase, chromatography can be divided into column chromatography, paper chromatography, and thin layer chromatography. The most commonly used LC method is column chromatography which regards liquid as a mobile phase. High performance liquid chromatography (HPLC) is modified based on the classic liquid column chromatography [13-15].

The application of LC is divided into two categories. One of them is qualitative or quantitative for a particular composition. Qualitation is managed according to the consistency between the sample and the target component in the peak time. Quantitation is performed according to the standard curve generated after standards are injected at different concentration levels. The other one is a fingerprint which refers to the notion that, after the fingerprint sample has been disposed of in some way, we can obtain chromatogram or spectrogram labelled chemical characteristics by using certain methods of analysis. LC has a great advantage on the capability of separating complex samples, so it is the most effective option when applied to separate mixtures, but not suitable to obtain structural information of the material. Qualitation

finished by the contrast between the peak positions of unknown compounds and the standards is not available for monitoring of unknown compounds [16-20].

MS

Mass spectrometry is widely used in the field of TCM research due to its high selectivity, high sensitivity, and capability of providing information including relative molecular mass and structural characteristics. MS completes the qualitation using molecular mass and relevant structural information and completes quantitation by the relationships of the peak and compound content which the peak represented. Atmospheric Pressure Ionization (API) of MS has Electro Spray Ionization (ESI) and Atmospheric Pressure Chemical Ionization (APCI). For many types of compounds, ESI has high sensitivity. Compared with ESI, APCI is suitable for the less polar compounds and the analysis of volatile compounds. Depending on the differences among mass analyzers used, common MS concludes Quadrupole Mass Spectrum (Q-MS), Time-Of-Flight Mass Spectrum (TOF-MS), and Ion Trap Mass Spectrometry (IT-MS) [21-25].

Tandem mass spectrometry refers to two or more MS working together. The most commonly used tandem mass spectrometry is triple-Quadrupole Mass Spectrometry (QQQ-MS). In order to use quadrupole to conduct multistage mass spectrometry, three quadrupoles are sequentially placed, which is triple quadrupole. Another type of tandem mass spectrometry, such as Quadrupole-Time-Of-Flight Mass Spectrometry (Q-TOF-MS) and Quadrupole-Ion Trap Tandem Mass Spectrometry (Q-IT-MS) also consists of a variety of quality analyzer series. Ion trap time series can achieve multistage MS scans sequentially at different times, so this study categorized IT-MS as tandem mass spectrometer. Tandem mass spectrometry can induce fragments of molecular ions generated by first-stage MS, according to which we can infer the relationship between child and parent, obtain structural information of the molecule and then suggest the structure of the compound, and conduct the qualitation analysis for known and unknown compounds more accurately. Although MS can provide structural information of a material, it requires higher purity for the sample. In TCM research, it is generally used in combination with LC [26-28].

LC-MS

LC-MS technique, using LC as a separation system and MS as a detection system, finally achieves the spectrum. When the LC and MS work together, they can carry out multistage MS to speculate the structure of the compound, thus finishing qualitative and quantitative analysis more accurately. Retrieving the papers on LC-MS in the application of TCM research literatures published during the past five years at home and abroad, we found that they could be separated into two categories, that is, LC-Q-MS and LC-MS/MS. In order to analyze the differences and the advantages and disadvantages of each method, papers were classified according to the difference of tandem mass spectrometry [29-34].

Mass spectrometry hardware

The variety of mass analysers capable of LCMS operation can be defined as the method by which mass is differentiated and by the experiment to be undertaken. In brief, a single quadrupole detector is commonly used for simple mass analysis; a triple quadrupole/ion trap for fragmentation analysis; a Time of Flight (TOF) for high resolution measurements and a Q-TOF for high resolution measurements of fragmentation patterns.

Simple mass analysis will give molecular ion data to the integer level and is very useful as a preliminary screen to see if the sample will ionise under the operating conditions. This is commonly undertaken to confirm the presence of an ion in a sample before looking at more elaborate techniques or is used in quantification. Fragmentation analysis is undertaken in order to aid identification of components within mixtures that separate in LC conditions. The data revealed by this process identifies the smaller fragments that when bonded together form the molecular ion seen in simple mass analysis. The use of a triple quadrupole or an ion trap instrument is associated with fragmentation experiments as there are two quadrupoles either side of a collision cell. The collision cell is where the ion fragmentation occurs but the ion beam can be scanning or selective before or after the collision cell. This essentially means the triple quadrupole is capable of scanning for molecular ions/fragments or selective monitoring of individual molecular ions/fragments. This is a very powerful technique as it can be used as a key determinant in the assessment of structure and again can be used for quantification [33,34].

The addition of a reference standard (or lock mass) and the use of a high resolution detector allows acquisition of mass to four decimal places and hence for elemental composition to be measured. This can be compared to that calculated and is a very common technique in the identification of unknowns. As a guiding principle this must fit to within 5ppm, above (though this is highly dependent on theoretical mass) and the technique works best if the sample history is well known as this will limit the potential parameters. The significant advantage of LCMS over other liquid chromatography detectors is the quality of information on detection of a peak and as such is commonly used as one of a suitable of techniques to determine structural information on a component [35-40].

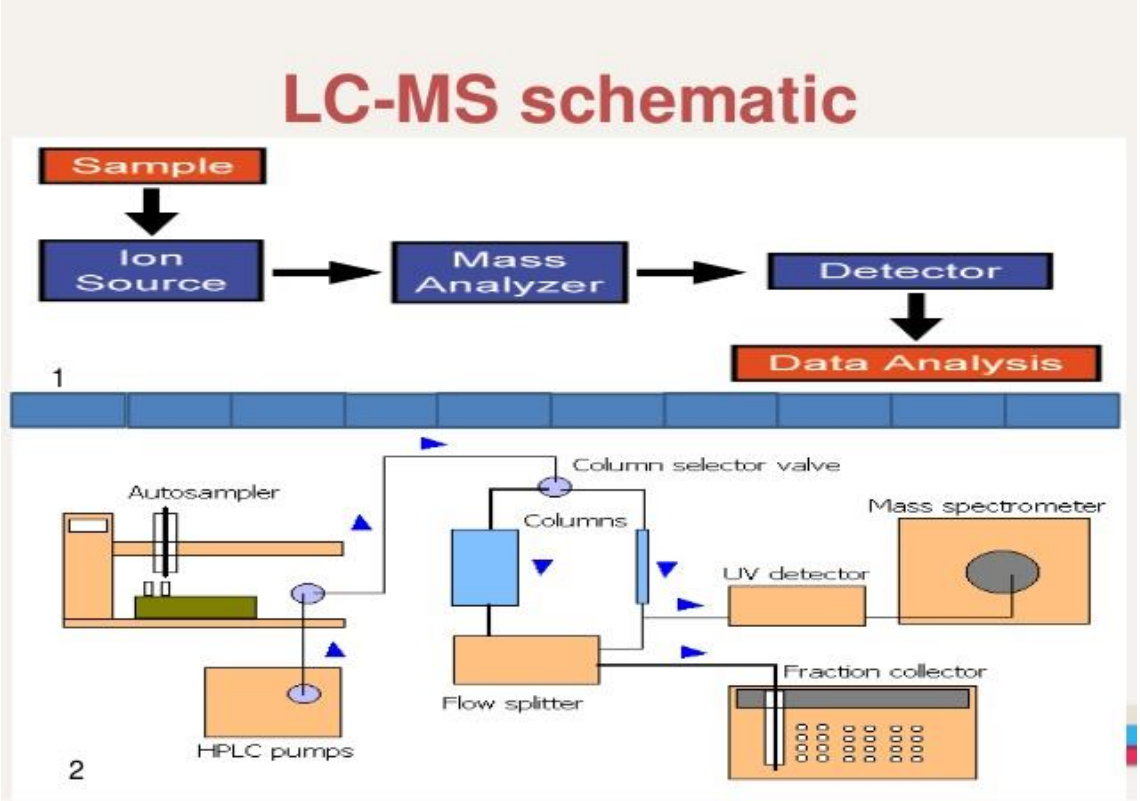

Fig. 4: Instrumentation diagram

The use of LCMS in pharmaceutical process development

The use of LCMS in a pharmaceutical setting is well established as analysts have always sought the best quality information from detectors and the outlay involved in set-up has significantly

decreased. The seeding point for much of what is described below has been in the industrial QA/QC laboratories (due to the obvious qualitative and quantitative data produced) but also in academic collaborations. Recently, the application of LCMS (in tandem with other qualitative and theoretical techniques) to process development has gained significant momentum. Outlined below are some recent developments in the use of LC-MS applied to process development change, a very significant area in pharmaceutical chemistry having achieved global investment in the past decade [41,42].

Pharmaceutical applications of LC-MS

In a recent publication from AstraZeneca, the process synthesis of a potent SRC kinase inhibitor (AZD0530) was revealed with the use of LCMS and molecular modelling key to the development. The preliminary synthetic route involved three successive nucleophilic aromatic substitutions and the key focus of this work was the final SnAr reaction which gave significant levels of by-product formation as identified by HPLC (and relatively poor isolated yield of only 63 per cent). Initial studies identified that by a change of base and solvent mixture from sodium t-amylate and t-amyl alcohol to sodium hydroxide and toluene resulted in a significant improvement in reaction profile. To test the hypothesis, the introduction of t-amyl alcohol to the toluene route significantly deteriorated the LCMS profile of the reactions with more hydrolysis products evident [43,44].

Biomedical applications

The LC-MS technique is useful for the detection of steroid drugs in body fluids and in profiling endogenous steroids. Steroid sulfates have been detected with high sensitivity using this method. Plasmaspray has been used to test saliva for steroid hormones in patients suffering from congenital adrenal hyperplasia. Amino acids were one of the first compounds analyzed using LC-MS coupled with laser desorption and thermospray. Nucleosides, nucleotides, saccharides, peptides, and proteins were all analyzed and their molecular weights were determined using LC-MS coupled with electrospray. Bile acids have also been determined using LC-MS and thermospray. Ultra-pure additives and solvents such as acetic acid, acetonitrile, ammonium

acetate, ammonium bicarbonate, ammonium fluoride, and ammonium hydroxide are available from Sigma Aldrich for use in LC-MS systems [45,46].

Environmental applications

LC-MS is used in the analysis of diverse samples such as soil, drinking water or wastewater, air, and sludge. The samples may belong to many different chemical species ranging from non-polar hydrocarbons to ionic organometallic species. Several pesticides and herbicides including triazine derivatives, chlorophenols, phenoxyalkanoic acids, and sulfonylurea herbicides can be analyzed using LC-MS. Separation of polycyclic aromatic hydrocarbons and organometallic compounds is also possible using the technique [47-50].

Biochemical screening for genetic disorders

Blood samples of newborn babies are analyzed using LC-MS to detect metabolic disorders. Second-tier LC-MS testing has been used for confirming the results of first-tier immunoassays in newborn screening [50-52].

Pharmaceuticals

LC-MS is widely used in the determination of pharmaceutical compounds and especially in the separation of optically active drugs. Antibiotics and potential anti-malarials have been studied using thermospray. The use of LC-MS in the identification of bromazepam and similar drugs in case of intoxication has been successfully demonstrated. Detection, isolation, and purification of drug metabolites is another major application of LC-MS, as they are chemically or thermally labile, and need liquid chromatography. Separation and characterization of components in a crude mixture of natural products such as complex lipids, alkaloids, and hydroxylated or unsaturated fatty acids has been achieved using LC-MS. LC-MS systems from Shimadzu feature high sensitivity, provide precise quantification, and improve analytical throughput in food, pharmaceutical, chemical, and environmental analysis [53-56].

Therapeutic drug monitoring and toxicology

In drug monitoring, LC-MS assays have been developed for immunosuppressants including tacrolimus, cyclosporin, everolimus, sirolimus, and mycophenolic acid. Similar assays for aminoglycosides, anticancer drugs, and antiretrovirals have also been described. LC-MS assays can be multiplexed to measure several drugs and metabolites in a single run, thus simplifying lab workflows and providing additional information such as metabolite profiles in clinical biochemistry labs. LC-tandem MS is used in toxicology screening for the detection of a wide range of toxins, drugs, and metabolites. Thermo Fischer provides a range of LC-MS systems including ion trap LC-MS, triple and single quadrupole LC-MS solutions. This is in addition to LC-MS software and accessories suitable for the analysis of environmental pollutants, complex proteins, and drug metabolites [57].

Steroid hormones

LC-MS analysis has been helpful in steroid biochemistry studies where traditional immunoassays have not proved very effective. Highly sensitive LC-MS assays have been developed for the measurement of low dihydrotestosterone and testosterone levels in women and children. Urinary steroid profiling has been simplified using LC-MS methods as these steroids are excreted as glucuronide or sulfate conjugates which will need to be hydrolyzed and derivatized for GC-MS analysis [58].

Vitamins and related metabolites

LC-MS is a preferred method for the measurement of vitamin D and its metabolites. LC-MS assays have been developed for 25-hydroxyvitamin D2 and D3 in plasma and serum. Similar assays are also available for fat-soluble vitamins such as vitamin K and Vitamin E [59].

Conclusion

Current approaches to LC-MS correspondence are numerous and varied, yet share a host of drawbacks that must be considered during the design of next generation of algorithms. Because certain drawbacks are unavoidable with alignment approaches, correspondence-rather than warping functions-ought to be the focus of solutions. Methods with user-defined parameters need

analytical and automatic solutions. All methods ought to have bounds on run time. Reference samples and fixed RT and m/z comparison windows are undesirable. Because there are already so many correspondence algorithms, we strongly suggest that any new algorithms abide by the above-mentioned criteria.

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