IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

HYBRID TECHNOLGIES USED IN PHARMACEUTICAL ANALYSIS

Mansi Mahesh Patil*[1], Gayatri Rajendra Thube[1], Prerna sampat ugale[1], Kaveri Vaditake[1]

1 PRES's, College of Pharmacy (For Women), Chincholi,Nashik.

Abstract: A separation approach and an online spectroscopic detection technology were combined to create the hyphenated technique. Over the past 20 years, hyphenated analytical techniques have seen amazing advancements that have greatly expanded their scope of use in the study of biomaterials, particularly natural goods. This article discusses recent developments in the applications of various hyphenated techniques, such as GC-MS, LC-MS, LC-FTIR, LC-NMR, CE-MS, etc. in the context of crude extract or fraction pre-isolation analyses, isolation and online detection of natural products, chemotaxonomic studies, chemical fingerprinting, quality control of herbal products, dereplication of natural products, and metabolomic studies.

Key Words: Analytical Technics, TLC, HPLC, GC-MS, GC-MS, LC-MS, LC-FTIR, LC-NMR Etc.

1. INTRODUCTION

Hybrid approaches help identify and measure the components in a mixture by combining two or more analytical techniques. Some of the most well-liked hybrid analytical methods are gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), gas chromatography-infrared spectroscopy (GC-IR), and liquid chromatography-nuclear magnetic resonance spectroscopy (LC-NMR). Both chemistry and biochemistry frequently employ them.[1-3]

In latest years, many industries and medical disciplines have embraced the concept of excessive throughput experimentation to help the ever-increasing need for faster data era and acceleration of product improvement cycles. In pharmaceutical industry, HTE has been adopted in various areas, consisting of the invention of biomarkers and of new chemical entities in drug discovery, as a tool to boost up the characterization of new pharmaceutical compounds as well as small-molecule chemical technique development,[4-7] in bio therapeutics analysis,[8] forced degradation research of healing peptides,[9] and analytical technique qualification.[10]

Especially computerized structures which are able to swiftly running large numbers of experiments in parallel also require a concomitant boom within the fee at which the preferred analytical facts can be generated and processed, if analytics aren't to be the bottleneck.

For most analytical strategies, there exists a trade-off between the velocity at which statistics can be generated and the nice or accuracy of the data, and for any HTE workflow, one wishes to strike the right stability between those two elements.

Even though conventional analytical gear together with HPLC or NMR spectroscopy normally require measurement times inside the order of numerous mins in line with pattern, suitable excessive throughput analytical (HTA) strategies which might be capable of generating datasets in timeframes of less than a minute or seconds are critical for HTE workflows.

Earlier reviews had been published on HTA, masking a timeframe as much as 2022.[11, 12] This assessment objectives to affords a vital review of literature posted between early 2018 and September 2022. we've got surveyed the swiftly developing field of HTA, covering mounted chromatographic techniques in addition to recently brought spectroscopic and microfluidics-based techniques; the evaluate turned into established for that reason. The point of interest became directed to HTA structures and methods inside the context of small molecule drug discovery and chemical and pharmaceutical improvement.[13, 14]

2. THIN-LAYER CHROMATOGRAPHY

Even though TLC is one of the oldest, most effective, and most commonplace analytical strategies to determine the fee of conversion in a chemical response it has no longer acquired a great deal attention within the context of high throughput screening (HTS). Because of the restrained throughput and the truth that detection and quantification is frequently no longer straightforward, we however believe that it has little value inside the HTE area. The low throughput can but be increased to some extent through recognizing of multiple samples on a single TLC plate. Welch and co-workers[13] in addition stepped forward the throughput by using the usage of staggered parallel recognizing and automated computer photo processing equipment to calculate reaction yields and conversion.[14]

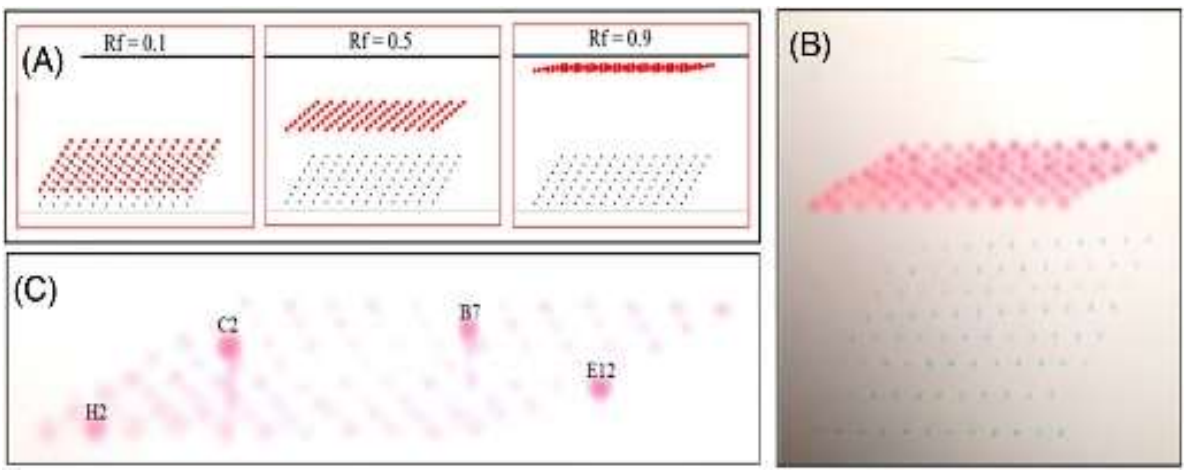

Fig: Thin Layer Chromatography

Modelling and initial research the usage of dyes from microplates spotted on TLC plates the use of a staggered spotting approach. A, Excel modelling displaying "candy spot" of Rf ~0.5 for simplest visualization. B, Elution of spots from a ninety six-well plate containing samples of methylene blue (Rf ~0) and methyl pink (Rf ~0.6) the use of 95% ethanol/water. C, evaluation of plate containing 4 wells spiked with additional methyl purple permits smooth determination of the "cope with" of the hits.[15-18]

3. LIQUID CHROMATOGRAPHY

Many HTE analytical workflows are the use of liquid chromatography as a key analysis technique. The best manner of growing throughput of the analytical platform is to reduce the dimension time in keeping with pattern. numerous strategies had been followed to shorten the LC evaluation time which include the software of high temperatures[14, 15] or the use of monolithic columns[16, 17] or parallel segmented flow columns.[18] Those diversifications provide especially modest gains in evaluation speed.

3.1 Ultrahigh-Pressure LC

A considerable reduction of evaluation time was performed several years in the past by using the advent of ultrahigh-stress LC (UHPLC) systems and sub-2 µm diameter stationary section debris,[19] however extensive efforts continue to be directed towards enhancing the speed and throughput of the LC technique for HTA purposes.[20] by means of the usage of custom-made gadgets presenting very quick bed lengths, optimized geometries, and alertness of sign processing gear, Armstrong and co-employees have driven separation speeds down to the sub-second time-frame, for this reason drawing near sensor-like throughput.[21] in spite of the extensive evolution in commercially available column technology and instrumentation,[22] additional improvements that lessen the height dispersion added through frits, tubing, and other elements of the instrumentation are had to make such leaps in performance available to customers in the industry.[23] Recently published paintings at the gain that can be expected from extremely high-pressure separations[24, 25] showed that the separation speed may be doubled when systems are able to working at pressures as much as 3000 bar and by way of the use of columns packed with 1 µm diameter particles. The prediction that one can expect a component of 2 advantage in separation speed versus a threefold boom in pumping strain shows that any evolution on this discipline seems to stick to the "regulation of diminishing returns": an increasing number of larger effort is required to obtain decreasingly smaller or less massive improvements. The authors also stated that many similarly developments are needed, including the huge-scale production of well-packed 1–1.2 mm internal diameter columns, the production of uniform and routinely strong 1 µm particles, the layout of devices and detectors with a substantially reduced normal dispersion, pressure-tolerant column housings, connectors, and valves, as well as sufficiently specific stress-compliant glide meters. Pushing the speed of LC evaluation to timeframes of less than a minute using contemporary commercially to be had units and columns has been done by using very quick columns full of small diameter debris and the application of high float rates.[26-30]

3.2 Superficially porous particles

Besides using small-diameter particles, which require instruments capable of running at high pressures (> a 1000), using superficially porous debris (SPPs), also referred to as center-shell debris, has been a popular way to carry out speedy analyses without the want for high-strain contraptions. The gain of SPP columns lies in the truth that the stationary segment debris offer appreciably reduced plate heights, just like those of sub-2 µm debris, because of a reduction of the analyte diffusion course period. The latter impacts the A (eddy diffusion) and C (resistance to mass switch) terms of the van Deemter equation. Due to the bigger usual particle diameter, the higher separation efficiency is done without using high column pressures.

although conceptually dating from the Seventies, contemporary SPPs were added commercially only in 2006 with 2.7 µm Halo debris presenting a 1.7 µm nonporous silica middle and a 0.5 µm thick shell of porous silica.[31]

While evaluating FPP and SPP particles, maximum authors have determined that SPPs offer greater possibilities for elevated throughput than FPPs. Assessment of the chromatographic performance primarily based on van Deemter curves showed that columns full of SPP particles carried out better than FPP columns.[32-34] A kinetic plot-based assessment in addition established that SPP columns represent the maximum favourable compromise in terms of pace, performance, and stress drop, enabling sub-minute separations with tons lower strain drops. [35] studied the use of SPPs for the analysis of polycyclic aromatic hydrocarbons. They tested that, for similar levels of selectivity, fully porous 1.8 µm debris and superficially porous 2.7 µm particles showed similar efficiency however much less lower back stress turned into generated on the SPP column. The usage of the SPP column extended throughput and enabled them to split a sixteen-thing pattern mixture in a trifling minute time frame. The advantages of SPP columns have additionally been acknowledged for supercritical fluid chromatography (SFC) separations.

3.3 Open tubular columns

every other method to reduce the evaluation time is to step far away from the common packed-mattress layout hired in LC in choose of the open tubular (OT) column format, generally related to GC. The OT format, proposed within the Seventies[36] theoretically ends in lower plate heights due to the absence of eddy dispersion and doubtlessly gives better kinetic performance. The layout in no way became popular because of the tradeoff that exists among excessive performance (use of very slender capillaries) and the restrained mass load ability of such small internal diameter columns, main to troubles with detection.

Calculations through Causon et al supplied steering at the layout of OT columns, balancing their kinetic overall performance and loadability.[37] The loadability of OT columns has been progressed by using coating the internal floor of an OT capillary column with thin, porous (octadecylsilylated) silica layers,[38, 39] yielding column efficiency upgrades inside the order of 15%. Considering that then few authors have proven the capability of using OT columns for ultrafast LC separations. Xiang et al lately tested the idea using a 2.7 cm lengthy, 2 µm wide OT column. With this miniaturized column and an optimized laser-triggered fluorescence detection scheme, six peptides will be resolved and trypsin-digested cytochrome C separations can be completed in 10-50 s. [40]

4. SUPERCRITICAL FLUID CHROMATOGRAPHY

Fast or ultrafast separations have often been linked to the usage of SFC, and analysis instances within the order of mins or even seconds are indeed potential. [41-45] In most instances, the SFC-based technique yields better throughput than LC-primarily based approaches.[46] Armstrong and co-employees confirmed separations on the order of just a few seconds the usage of sub/supercritical CO_2 at the side of high-performance, slim particle-length distribution silica packed in short columns and using very excessive drift charges. [47] The authors did factor out that, as the analysis time is going down in the direction of the order of seconds, a few surprising peculiarities are visible which can be absent in ultrafast LC and which affect the obvious efficiency of the system. Such outcomes can be attributed to the compressible nature of the mobile segment and as a way to fully exploit the benefits of ultrafast SFC separations amazing care wishes to be taken in the layout of the gadget, the choice of tubing, and the again-stress regulator design. The theoretical limits of present day structures and column codecs have been notably studied in a latest paper via Desmet et al. and it appears there may be a fashionable consensus that, at this moment, column generation is ahead of instrumentation abilties and that any in addition increase in overall performance will likely want to come back from in addition tool optimization.[48, 49]

Berger suggested on the fast SFC separation of achiral solutes using short 20–30 mm columns full of sub-2 µm particles whilst addressing the need to reduce extra-column dispersion. With the aid of decreasing the gadget's greater-column dispersion from 80 to 5 µL2 he was capable of gain reduced plate heights as little as 2.2 and clear up 7 solutes in less than 8 seconds on a 2 cm long column. Essential factors influencing the performance in this experiment had been the choice of injection solvent and injection extent.

Similar to the benefits for LC that were defined above, the gain of using SPPs in SFC mode has been confirmed by way of several authors. In a look at centered on the chiral analysis of pesticides and their stereoisomers, Hellinghausen et al.[50] said < 1 min separations for the general public of compounds that have been studied the usage of 2.7 µm SPPs. by using exploiting the low viscosity gain of the CO2/MeOH cell section, allowing the application of very excessive flow prices (up to fourteen mL/min), and the usage of high-efficiency 2.7 µm chiral SPPs, Roy and Armstrong were capable of do chiral separations in a timeframe of thirteen seconds.[51] The authors claimed that even faster separations may be completed if advances in SFC instrumentation ought to deal with a few shortcomings involving greater-column outcomes and pressure barriers. Such improvements could unleash the full capability of SPPs and different small particle helps. Recently, other corporations have additionally reported on the usage of SPPs in SFC situations for chiral analyses. Studied a set of 31 racemates of derivatized amino acids which will be resolved on a teicoplanin-primarily based chiral selector bonded on both 1.9 µm FPPs with a slender particle-length distribution or on 2.0 µm SPPs, attaining better consequences on the SPP segment versus the FPP version. Similarly, Folprechtova et al [53] have applied such teicoplanin-based totally SPP-packed chiral columns for SFC separations of phytoalexins, substituted tryptophan's, and ketamine derivatives. Ultrafast separations and screening of chiral compounds using OT columns in SFC mode changed into finished with the aid of Galietti et al [54] the usage of turbulent glide conditions. The authors used CO2 at noticeably high float and GC OT

columns to achieve turbulent go with the flow conditions. Upon transitioning from laminar go with the flow to the turbulent drift regime they determined a discount in plate peak main to a nearly threefold boom in top potential. The statement changed into defined with the aid of the more green mass transfer inside the cellular phase due to a flatter waft profile and quicker analyte dispersion throughout the OT column. Using turbulent flow conditions in OT columns allowed to separate four polycyclic fragrant hydrocarbons within a 2.2 s window[50-52].

5. MULTIPLE INJECTIONS IN A SINGLE EXPERIMENTAL RUN

A popular and user-friendly way of tracking a huge set of experiments with the aid of LC or LC-MS in an excessive throughput style is the so-called ‘multiple Injections in a single Experimental Run’ (MISER) approach developed via Christopher Welch at some stage in his time at Merck & Co.55 MISER chromatography lends itself nicely to kinetic analysis/profiling and relies on sequential pattern injections with minimal chromatographic separation of the analytes of hobby from interfering substances. The proportion of solvent used during a MISER run is chosen in the sort of way that there's minimum interplay with the stationary segment, consequently accelerating the passage of the analyte(s) through the column. The objective isn't to absolutely separate all peaks however only to solve matrix or interfering peaks and reduce/cast off ability ion-suppression or different matrix outcomes. Once the mobile phase has been optimized, the speed at which samples can be tested is basically most effective restrained by using the injection price of the auto sampler.

MISER evaluation is commonly used for evaluating related samples that comprise the equal compound of interest however were prepared the use of different situations.[56-58] the gathering of results is termed a ‘miser gram’ (discern eight) and allows for easy assessment of the information. The method has been very a hit in catalyst discovery wherein the screening of a large variety of reactions is required. Although most often used alongside LC or LC-MS, SFC- and GC-based totally MISER workflows were pronounced as properly. An instance is the MISER-GC-MS setup described via Knorrscheidt et al.[59] that's capable of reading a 96-well microplate inside 60 mins.

6. GAS CHROMATOGRAPHY

Gasoline chromatography (GC) is a common analytical device this is mainly useful for the analysis of greater volatile compounds, and it definitely has an area within the HTA field. Classical GC systems lack the required speed to aid HTE workflows, however some processes that permit greater speedy analyses had been advanced. These days achieved reasonably exact separations with cycle times of less than 1 min by way of combining low-stress (vacuum outlet) GC-MS (LPGC-MS) with low thermal mass (LTM) resistive heating for fast heating and cooling of the capillary column. They threaded the analytical column into LTM thin-walled metal tubing inside an "LTM fast GC” module that turned into installed onto a detector port of a classical GC system. The column inlet and outlet were related to the GC injector and MS transfer line, respectively (parent nine). The inlet operates at everyday GC pressures, however the analytical column is below vacuum, which will increase the superior helium provider fuel glide speed and the rate of full range

separations at the same time as maintaining an acceptable great of chromatographic separation. The LTM-LPGC-MS configuration provided a 64-fold advantage in velocity of analysis as opposed to popular GC-MS on the rate of a 4-fold loss in peak capacity and will lessen the analysis time from minutes to seconds in some not unusual packages.[60-67]

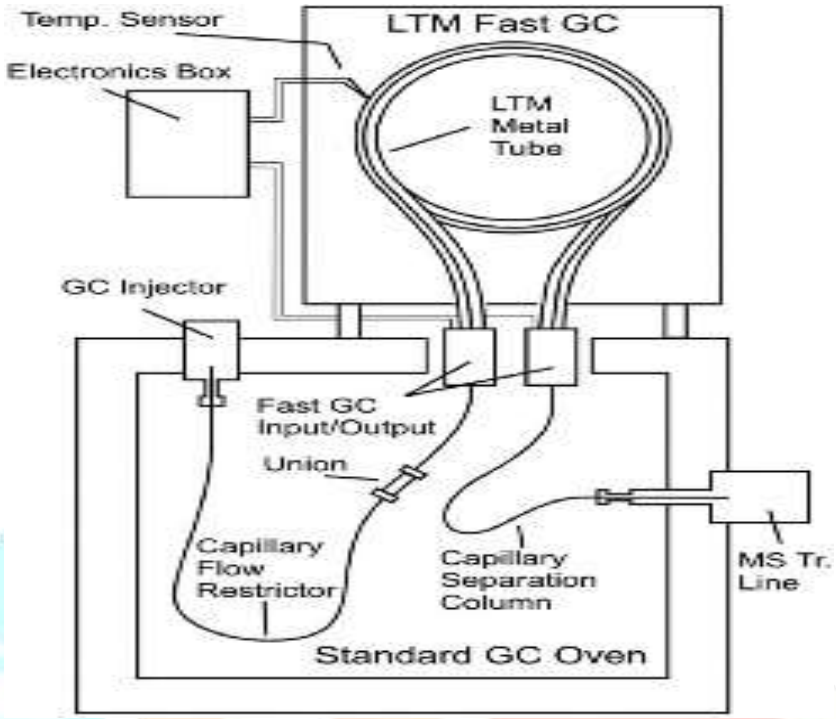

Figure: Open in parent viewer Schematic diagram of the low thermal mass speedy GC module hooked up on an to be had FID port atop an Agilent 7890 GC configured for GC-MS. notice the capillary column mixture in rapid LPGC-MS operation

7. MASS SPECTROMETRY

MS-primarily based analysis gives blessings exceedingly desirable in HTA, together with excessive pace mixed with the potential to identify and quantify compounds in a mixture. The capability to selectively screen the mass of a particular goal could be very beneficial for, for instance, hit and lead identification or chemical reaction screening. Those benefits have caused the rapid adoption and development of MS-based gear in HTE workflows inside the enterprise and have stimulated academic research efforts to expand innovative procedures. In conventional LC-MS setups counting on ionization strategies together with ESI, APCI, or APPI, [68] large gains in evaluation pace had been performed by means of automating the pattern education steps and the usage of optimized, short UHPLC strategies. [68-73] more specific opinions on novel MS-primarily based tools and procedures for HTA were written by way of Kempa et al [74] and Pu et al [75] and had been centered on methodologies that automate or get rid of sample instruction steps in hit and lead generation or response screening and optimization.

One instance of a commercially available MS device for HTA is the Agilent 'RapidFire MS', in which an SPE-primarily based pattern-cleanup robotics workflow is coupled to ESI-MS detection and which allows analysis times as brief as 5-10 s according to sample. Such structures are typically used for screening or compound profiling obligations in which the preferred throughput is within the range of < 5000 samples/day. [76] but, the technology does now not meet the needs of screening assays that involve the evaluation of tens

of lots as much as thousands and thousands of samples in step with day. Those needs have caused the improvement of strategies along with surface-primarily based MS or microfluidics structures that require little to no pattern education and push the analysis speed into the sub-2nd time frame. Direct infusion (DI) MS techniques or go with the flow-injection (FI) MS, in which samples are without delay injected into the ionization supply, saw early uptake inside the HTA area, as an instance, for response screening.[77] A drawback of this technique is that samples are added without earlier separation or clean-up which might also result in ion opposition, lowering the sensitivity or obscuring the result. Sarvin et al[78] described an approach to triumph over this effect by using reading the distribution of ion m/z values and computationally determining a series of most desirable scan tiers in metabolomics and lipidomics analyses of serum samples.[78]

The maximum not unusual bottleneck in DI or FI analysis is the sampling rate that is frequently substantially slower than the real acquisition of MS data. To fully take advantage of the strengths of the MS system, the device need to be unexpectedly provided with small volumes of sample. Since the velocity of conventional LC-MS techniques is inherently constrained via the rate at which every test may be sampled by way of an autoinjector to introduce it into the MS ionization chamber, new approaches of sample advent have been advanced. This has led to the advent of a new magnificence of ionization strategies, usually referred to as "ambient ionization MS" (targets) wherein samples are ionized at atmospheric strain. Some floor/plate-based totally strategies require that samples are first deposited onto a suitable provider or integrated right into an appropriate matrix, after which fast in situ analysis is feasible. these tendencies have revolutionized the HTA area due to the fact ambitions techniques allow throughputs which are orders of importance higher than those of conventional LC-MS equipment, approaching analysis quotes which can be doable with fluorescence-primarily based strategies however without the want for fluorescent labels. numerous floor-primarily based MS techniques, such as matrix-assisted laser desorption/ionization (MALDI), direct analysis in real-time (DART), desorption electrospray ionization (DESI), secondary ion mass spectrometry (SIMS), and self-assembled monolayers coupled with desorption/ionization (SAMDI), are capable of direct ionization of analytes with little to no sample guidance and have been validated to be relevant in situations that require quick analysis times and very small pattern volumes.[79, 80] An in-intensity evaluation of the various pursuits tactics that have been reported to this point has been written by using Kuo et al.[81] beneath we are able to awareness on a number of the most commonplace techniques. Some objectives strategies, consisting of floor-assisted laser desorption/ionization (SALDI) mass spectrometry, had been used for on line monitoring of photocatalytic reactions and for ultrafast photocatalyst screening.[82] on this look at, a photocatalytic nanomaterial turned into used as the substrate to provoke and display the reactions concurrently. The measurements discovered a reaction acceleration effect: the interfacial reactions proceeded in seconds, versus hours for conventional reactions within the bulk phase; this is attractive for ultrafast reaction screening.

7.1 Matrix-assisted laser desorption/ionization

MALDI-MS is one of the oldest and maximum demonstrated implementations of ambient, floor-based totally MS. The analyte is co-crystallized on a surface collectively with a appropriate matrix and then subjected to UV laser light, which desorbs and ionizes the analytes.[74] MALDI coupled with time-of-flight (MALDI-TOF) MS has been used considerably for ultrahigh-throughput screening of large molecules, with many literature examples related to drug target identification proteomics and analysis of DNA/RNA, lipids, oligosaccharides, and synthetic polymers. Conventional MALDI-TOF-MS has various barriers in studying small molecules due to fragmentation of organic matrix molecules and matrix interferences in the decrease mass range. Therefore, its use for small molecule evaluation has no longer been evolved as exhaustively. despite the fact that, some agencies have validated the possibility of using MALDI for small molecule programs, even for the speedy analysis of crude response combos containing catalysts, salts and bases. [73] the use of a parylene-matrix chip, Park et al[84] had been able to use MALDI-TOF-MS for the high throughput quantification and evaluation of small-molecule cancer biomarkers. lately, Blincoe et al[85] posted a sensible guide for bench chemists on the way to broaden and compare high throughput MALDI-TOF-MS methods for the screening of chemical reactions in well plates without software of any MALDI plate amendment or product tagging (determine eleven). Simon and co-workers[86] confirmed the applicability of automated, direct MALDI-TOF-MS as a readout approach for big-scale drug discovery HTS campaigns. They used a MALDI-TOF-primarily based screening platform together with a 1536-properly format for figuring out inhibitors of human cyclic GMP-AMP synthase in a fast, strong, and accurate manner.

7.2 Direct analysis in real time/desorption electrospray ionization

From the MALDI technique, other MS methods which includes desorption electrospray ionization (DESI) and direct evaluation in actual time (DART) originated. because of its capability of fast screening of analytes in complex matrices with minimum pattern training, DART has come to be a popular tool in regions which includes food safety monitoring and environmental applications88 or to test for drug counterfeiting or contamination.[89] Khaled et al adopted an SPME-DART-based HTS workflow for screening multiresidue pharmaceutical tablets in bovine tissue samples.[90] They demonstrated that 53% of the ninety eight goal analytes might be efficiently ionized with the aid of DART and quantified at the desired stage, whilst the fully automatic sample education workflow allowed for general evaluation instances as quick as 1 min per sample. While DART showed restricted abilities in phrases of analyte coverage, their research highlights the capacity usefulness of SPME-DART-MS/MS as a way for rapid evaluation in food protection tracking applications.

Any other ambient ionization approach that has attracted an awful lot interest is DESI. Given that its inception in 2004, it's been a famous studies topic inside the discipline of HTA. In its best shape, a DESI–MS setup uses an aqueous spray directed at an insulating sample or an analyte deposited on an insulating floor along with polytetrafluoroethylene (PTFE). The DESI-MS inlet is then moved across the floor the use of an x–y stage and a 2nd map of chemical records in the shape of full mass spectra may be generated. in

comparison to MALDI-MS, the DESI approach has the benefit that no matrix is needed to perform the experiment and that multiply charged ions can be generated, which has the benefit of extending the mass variety of the detector required for huge molecules or biological samples. The desorbed ions are sampled with a business ion trap mass spectrometer geared up with an atmospheric interface related to an extended, ideally flexible ion transfer line constructed from steel or an insulator. DESI has been efficiently used for the ionization of diverse compounds, along with peptides[91] and proteins gift on metallic, polymer, and mineral surfaces.[91, 92] The capability to report mass spectra of samples of their local environment that does not require sample practise by me of growing ions outdoor the device, enables extraordinarily speedy analysis with high sensitivity and high chemical specificity, characteristics which are especially in demand for HTA.

In recent years, the DESI method has been extensively implemented to HTA for small molecule response screening and optimization. For instance, chefs and co-people optimized amine alkylation reactions on PTFE membrane substrates the use of methanol because the DESI spray/analysis solvent.[93]

One form of DESI, known as “reactive DESI”, is used in the screening of chemical reactions and includes including reagents into the spray solvent which could selectively react with unique functional groups present in the aggregate. Reactions can occur all through the microdroplet surface collisions at better rates than in bulk answers.[94]

Loren et al used DESI-MS as a device for qualitatively predicting the consequences of microfluidics-based fast screening of N-alkylation reactions.[95] via the use of DESI-MS HTA they have been capable of quick slender down the wide variety of critical reaction parameters, which include the form of solvent. Comparable work however that specialize in Suzuki–Miyaura go-coupling and reductive amination reactions was posted through Fedick et al.[96] Their DESI-MS device applied microdroplet-based totally reaction acceleration, permitting more than one reagents, bases, and stoichiometries to be screened at fees close to 10,000 reaction combos consistent with hour (i.e., approximately three Hz). Chefs, Thompson, and co-workers optimized nucleophilic aromatic substitution reactions via HTE underneath go with the flow situations. They could compare 3072 unique reactions at a velocity of ~3.5 s in step with response using a gadget that covered both a liquid managing robot for response mixture instruction and a DESI-MS module. The reactions have been accomplished in microtiter arrays. The usage of in-residence evolved software program, heat maps had been generated from the MS facts and those facilitated speedy assessment and selection of the maximum promising situations. Authors from the identical research institution used a comparable method for screening 3840 specific reductive amination reactions.[94]

CONCLUSION:

A methodology known as a "hyphenated technique" was developed by fusing a separation approach and an online spectroscopic detection technology. Amazing developments in hyphenated analytical methods during the past 20 years have substantially increased their applicability to the study of biomaterials, especially natural products. In the context of purification and online detection of natural products, chemotaxonomic

studies, chemical fingerprinting, quality control of herbal products, dereplication of natural products, and metabolomic studies, this article discusses recent developments in the applications of various hyphenated techniques. The LC is specifically stressed as the separating tool in hyphenated methods.

REFERENCES:

1. Berenguel Hernandez AM, de la Cruz M, Alcazar-Fabra M, et al. Design of high-throughput screening of natural extracts to identify molecules bypassing primary coenzyme Q deficiency in *Saccharomyces cerevisiae*. *SLAS Discovery*. 2020; **25**: 299- 309.
2. Izquierdo M, Lin D, O'Neill S, et al. Development of a high-throughput screening assay to identify inhibitors of the major M17-leucyl aminopeptidase from trypanosoma cruzi using rapidfire mass spectrometry. *SLAS Discovery*. 2020; **25**: 1064- 1071.
3. Leavell MD, Singh AH, Kaufmann-Malaga BB. High-throughput screening for improved microbial cell factories, perspective and promise. *Curr Opin Biotechnol*. 2020; **62**: 22- 28.
4. Logsdon DL, Li Y, Paschoal Sobreira TJ, Ferreira CR, Thompson DH, Cooks RG. High-throughput screening of organic reactions in microdroplets using desorption electrospray ionization mass spectrometry (DESI-MS): hardware and software implementation. *Org Process Res Dev*. 2020; **24**: 1647- 1657.
5. Osipyan A, Shaabani S, Warmerdam R, Shishkina SV, Boltz H, Doemling A. Automated, accelerated nanoscale synthesis of iminopyrrolidines. *Angew Chem, Int Ed*. 2020; **59**: 12423- 12427.
6. Basuri P, Gonzalez LE, Morato NM, Pradeep T, Cooks RG. Accelerated microdroplet synthesis of benzimidazoles by nucleophilic addition to protonated carboxylic acids. *Chem Sci*. 2020.
7. Mennen SM, Alhambra C, Allen CL, et al. The evolution of high-throughput experimentation in pharmaceutical development and perspectives on the future. *Org Process Res Dev*. 2019; **23**: 1213- 1242.
8. Park H-M, Winton VJ, Drader JJ, et al. Novel interface for high-throughput analysis of biotherapeutics by electrospray mass spectrometry. *Anal Chem*. 2020; **92**: 2186- 2193.
9. Li Y, Hu Y, Logsdon DL, Liu Y, Zhao Y, Cooks RG. Accelerated forced degradation of therapeutic peptides in levitated microdroplets. *Pharm Res*. 2020; **37**: 138.
10. Kresge GA, Grosse S, Zimmer A, et al. Strategies in developing high-throughput liquid chromatography protocols for method qualification of pharmacopeial monographs. *J Sep Sci*. 2020; **43**: 2964- 2970.
11. Welch CJ. High throughput analysis enables high throughput experimentation in pharmaceutical process research. *React Chem Eng*. 2019; **4**: 1895- 1911.
12. Schafer W, Bu X, Gong X, Joyce LA, Welch CJ. High-throughput analysis for high-throughput experimentation in organic chemistry. *Comprehensive Organic Synthesis II*. Elsevier; 2014: 28- 53.
13. Boulgakov AA, Moor SR, Jo HH, et al. Next-generation TLC: A quantitative platform for parallel spotting and imaging. *J Org Chem*. 2020; **85**: 9447- 9453.
14. Pasch H, Heinz L-C, Macko T, Hiller W. High-temperature gradient HPLC and LC-NMR for the analysis of complex polyolefins. *Pure Appl Chem*. 2008; **80**: 1747- 1762.
15. Heinisch S, Rocca JL. Solvent selection in liquid chromatography. *J Chromatogr A*. 2009; **1216**: 642- 658.

16. Rashed NS, Zayed S, Abdelazeem A, Fouad F. Development and validation of a green HPLC method for the analysis of clorsulon, albendazole, triclabendazole and ivermectin using monolithic column: Assessment of the greenness of the proposed method. *Microchem J*. 2020; **157**:105069.
17. Dores-Sousa JL, Terryn H, Eeltink S. Morphology optimization and assessment of the performance limits of high-porosity nanostructured polymer monolithic capillary columns for proteomics analysis. *Anal Chim Acta*. 2020; **1124**: 176- 183.
18. Soliven A, Pareja L, Shalliker RA, Heinzen H. Perez-Parada, A. A high-throughput and high peak capacity narrow-bore parallel segmented flow column strategy for the liquid chromatography-tandem mass spectrometry analysis of organic contaminants in water. *Anal Methods*. 2020; **12**: 239- 246.
19. Mazzeo JR, Neue UD, Kele M, Plumb RS. Advancing LC Performance with Smaller Particles and Higher Pressure. *Anal Chem*. 2005; **77**: 460A- 467A.
20. Kaplitz AS, Kresge GA, Selover B, et al. High-Throughput and Ultrafast Liquid Chromatography. *Anal Chem*. 2020; **92**: 67- 84.
21. Patel DC, Wahab MF, O'Haver TC, Armstrong DW. Separations at the Speed of Sensors. *Anal Chem*. 2018; **90**: 3349- 3356.
22. Guiochon G. Separation science is the key to successful biopharmaceuticals. *J Chromatogr A*. 2011; **1228**: 2- 19.
23. Zhou Z, Desmet G, Pra M, Steiner F, Eeltink S. Assessing effects of ultra-high-pressure liquid chromatography instrument configuration on dispersion, system pressure, and retention. *J Chromatogr A*. 2020; **1634**:461660.
24. Broeckhoven K, Desmet G. Advances and Innovations in Liquid Chromatography Stationary Phase Supports. *Anal Chem*. 2020; **92**: 554- 560.
25. Sorensen MJ, Anderson BG, Kennedy RT. Liquid chromatography above 20,000 PSI. *Trends Analyt Chem*. 2020; **124**:115810.
26. Wahab MF, Wimalasinghe RM, Wang Y, Barhate CL, Patel DC, Armstrong DW. Salient Sub-Second Separations. *Anal Chem*. 2016; **88**: 8821- 8826.
27. Cebo M, Fu X, Gawaz M, Chatterjee M, Lammerhofer M. Micro-UHPLC-MS/MS method for analysis of oxylipins in plasma and platelets. *J Chromatogr A*. 2020; **1624**:461206.
28. Yoshikawa K, Furuno M, Tanaka N, Fukusaki E. Fast enantiomeric separation of amino acids using liquid chromatography/mass spectrometry on a chiral crown ether stationary phase. *J Biosci Bioeng*. 2020; **130**: 437- 442.
29. Gritti F, Martin M, Guichon G. Effect of the surface coverage of C 18 -bonded silica particles on the obstructive factor and intraparticle diffusion mechanism. *Anal Chem*. 2009; **81**: 3365- 3384.
30. Lesko M, Samuelsson J, Aasberg D, Kaczmarski K, Fornstedt T. Impact of methanol adsorption on the robustness of analytical supercritical fluid chromatography in transfer from SFC to UHPSFC. *J Chromatogr A*. 2020; **1625**:461076.
31. https://www.advanced-materials-tech.com/explore-fused-core-technology/.

32. Geibel C, Dittrich K, Woiwode U, et al. Comparison of small size fully porous particles and superficially porous particles of chiral anion-exchange type stationary phases in ultra-high performance liquid chromatography: effect of particle and pore size on chromatographic efficiency and kinetic performance. *J Chromatogr A*. 2019; **1603**: 130- 140.
33. Pantsulaia S, Targamadze K, Khundadze N, et al. Potential and current limitations of superficially porous silica as a carrier for polysaccharide-based chiral selectors in separation of enantiomers in high-performance liquid chromatography. *J Chromatogr A*. 2020; **1625**:461297.
34. Kresge GA, Wong J-MT, De Pra M, Steiner F, Grinias JP. Using superficially porous particles and ultrahigh pressure liquid chromatography in pharmacopeial monograph modernization of common analgesics. *Chromatographia*. 2019; **82**: 465- 475.
35. Godinho JM, Lawhorn J, Boyes BE. Rapid analysis of polycyclic aromatic hydrocarbons. *J Chromatogr A*. 2020; **1628**:461432.
36. Hibi K, Ishii D, Fujishima I, Takeuchi T, Nakanishi T. Studies of open tubular micro capillary liquid chromatography. 1. The development of open tubular micro capillary liquid chromatography. *J High Resolut Chromatogr*. 1978; **1**: 21- 27.
37. Causon T, Shellie R, Hilder E, Desmet G, Eeltink S. Temperature pulsing for controlling chromatographic resolution in capillary liquid chromatography. *J Chromatogr A*. 2011; **1218**: 8388- 8393.
38. Hara T, Futagami S, Eeltink S, De Malsche W, Baron GV, Desmet G. Very high efficiency porous silica layer open-tubular capillary columns produced via in-column sol–gel processing. *Anal Chem*. 2016; **88**: 10158- 10166.
39. Hara T, Izumi Y, Nakao M, et al. Silica-based hybrid porous layers to enhance the retention and efficiency of open tubular capillary columns with a 5 μm inner diame9ter. *J Chromatogr A*. 2018; **1580**: 63- 71.
40. Xiang P, Yang Y, Zhao Z, Chen M, Liu S. Experimentally validating open tubular liquid chromatography for a peak capacity of 2000 in 3 h. *Anal Chem*. 2019; **91**: 10738- 10743.
41. Regalado EL, Welch CJ. Pushing the speed limit in enantioselective supercritical fluid chromatography. *J Sep Sci*. 2015; **38**: 2826- 2832.
42. Chen J, Lou C. Applications of supercritical fluid chromatography technique in current bioanalysis and pharmaceutical analysis. *Bioanalysis*. 2020; **12**: 1347- 1351.
43. Pandya PA, Shah PA, Shrivastav PS. Facile separation of four co-formulated ternary antihypertensive drug combinations with a customized elution protocol using supercritical fluid chromatography. *Microchem J*. 2020; **159**:105594.
44. Novakova L, Sejkorova M, Smolkova K, Plachka K, Svec F. The benefits of ultra-high-performance supercritical fluid chromatography in determination of lipophilic vitamins in dietary supplements. *Chromatographia*. 2019; **82**: 477- 487.
45. Barhate CL, Joyce LA, Makarov AA, et al. Ultrafast chiral separations for high throughput enantiopurity analysis. *Chem Commun (Camb)*. 2017; **53**: 509- 512.

46. Wang B, Liu X-H, Xue Z-Y, Yang X-Y, Fang Y-Y, Feng S-L. Comparative study of ultra-high-performance supercritical fluid chromatography and ultra-high-performance liquid chromatography to simultaneous determination of ten components in Radix hedysari. *Pharmacogn Mag*. 2020; **16**: 99- 110.
47. Barhate CL, Wahab MF, Tognarelli DJ, Berger TA, Armstrong DW. Instrumental Idiosyncrasies Affecting the Performance of Ultrafast Chiral and Achiral Sub/Supercritical Fluid Chromatography. *Anal Chem*. 2016; **88**: 8664- 8672.
48. Broeckhoven K, Desmet G. Methods to determine the kinetic performance limit of contemporary chromatographic techniques. *J Sep Sci*. 2020.
49. Berger TA. Packed column SFC. *Chromatographia*. 2019; **82**: 537- 542.
50. Hellinghausen G, Readel ER, Wahab MF, et al. Evaluation of nicotine in tobacco-free-nicotine commercial products. *Chromatographia*. 2019; **82**: 221- 233.
51. Roy D, Armstrong DW. Fast super/subcritical fluid chromatographic enantioseparations on superficially porous particles bonded with broad selectivity chiral selectors relative to fully porous particles. *J Chromatogr A*. 2019; **1605**:3603.
52. Mazzoccanti G, Manetto S, Ricci A, et al. High–throughput enantioseparation of Nα–fluorenylmethoxycarbonyl proteinogenic amino acids through fast chiral chromatography on zwitterionic-teicoplanin stationary phases. *J Chromatogr A*. 2020; **1624**:461235.
53. Folprechtova D, Kozlov O, Armstrong DW, Schmid MG, Kalikova K, Tesarova E. Enantioselective potential of teicoplanin- and vancomycin-based superficially porous particles-packed columns for supercritical fluid chromatography. *J Chromatogr A*. 2020; **1612**:460687.
54. Galietti MR, Peulon-Agasse V, Cardinael P, Fogwill MO, Besner S, Gritti FG. Turbulent supercritical fluid chromatography in open-tubular columns for high-throughput separations. *Anal Chem*. 2020; **92**: 7409- 7412.
55. Welch CJ, Gong X, Schafer W, et al. MISER Chromatography (Multiple Injections in a Single Experimental Run): the Chromatogram is the Graph. *Tetrahedron: Asymmetry*. 2010; **21**: 1674- 1681.
56. Zawatzky K, Barhate CL, Regalado EL, et al. Overcoming "speed limits" in high throughput chromatographic analysis. *J Chromatogr A*. 2017; **1499**: 211- 216.
57. Zawatzky K, Grosser S, Welch CJ. Facile kinetic profiling of chemical reactions using MISER chromatographic analysis. *Tetrahedron*. 2017; **73**: 5048- 5053.
58. Equitz TR, Rodriguez-Cruz SE. High-throughput Analysis of Controlled Substances: combining Multiple Injections in a Single Experimental Run (MISER) and Liquid Chromatography – Mass Spectrometry (LC-MS). *Forensic Chem*. 2017; **5**: 8- 15.
59. Knorrscheidt A, Puellmann P, Schell E, Homann D, Freier E, Weissenborn MJ. Development of 96 Multiple Injection-GC-MS Technique and Its Application in Protein Engineering of Natural and Non-Natural Enzymatic Reactions. *ChemCatChem*. 2020; **12**: 4788- 4795.
60. Ocvirk G, Verpoorte E, Manz A, Grasserbauer M, Widmer H. High-performance liquid-chromatography partially integrated onto a silicon chip. *Analytical Methods and Instrumentation*. 1995; **2**: 74- 82.

61. Waheed S, Cabot JM, Macdonald NP, et al. 3D printed microfluidic devices: enablers and barriers. *Lab Chip*. 2016; **16**: 1993- 2013.
62. Cocovi-Solberg DJ, Rosende M, Michalec M, Miro M. 3D Printing: the Second Dawn of Lab-On-Valve Fluidic Platforms for Automatic (Bio)Chemical Assays. *Anal Chem*. 2019; **91**: 1140- 1149.
63. Heiland J, Lotter C, Stein V, Mauritz L, Belder D. On-chip integration of organic synthesis and HPLC/MS analysis for monitoring stereoselective transformations at the micro-scale. *Anal Chem*. 2017; **89**: 3266- 3271.
64. Piendl SK, Raddatz C-R, Hartner NT, et al. 2D in Seconds: coupling of Chip-HPLC with Ion Mobility Spectrometry. *Anal Chem*. 2019; **91**: 7613- 7620.
65. Zheng R, Li L, Deng X, Tian M, Wang Z, Yang L. Fully automated chip-based nanoelectrospray ionization-mass spectrometry as an effective tool for rapid and high-throughput screening of 5α-reductase inhibitors. *Anal Bioanal Chem*. 2020; **412**: 1685- 1692.
66. Komendova M, Nawada S, Metelka R, Schoenmakers PJ, Urban J. Multichannel separation device with parallel electrochemical detection. *J Chromatogr A*. 2020; **1610**:460537.
67. Fialkov AB, Lehotay SJ, Amirav A. Less than 1 minute low-pressure gas chromatography - mass spectrometry. *J Chromatogr A*. 2020; **1612**:460691.
68. Beccaria M, Cabooter D. Current developments in LC-MS for pharmaceutical analysis. *Analyst (Cambridge, U K)*. 2020; **145**: 1129- 1157.
69. Musile G, Mazzola M, Shestakova K, Savchuk S, Appolonova S, Tagliaro F. A simple and robust method for broad range screening of hair samples for drugs of abuse using a high-throughput UHPLC-Ion Trap MS instrument. *J Chromatogr B: Anal Technol Biomed Life Sci*. 2020; **1152**:122263.
70. Margaryan T, Sargsyan M, Gevorgyan A, et al. Protein precipitation method for determination of clobazam and N-desmethylclobazam in human plasma by LC-MS/MS. *Biomed Chromatogr*. 2020; **34**:e4844.
71. Bian Y, Zheng R, Bayer FP, et al. Robust, reproducible and quantitative analysis of thousands of proteomes by micro-flow LC-MS/MS. *Nat Commun*. 2020; **11**: 157.
72. Li W-x, Zhang A-h, Zhou X-h, et al. High-throughput liquid chromatography mass-spectrometry-driven lipidomics discover metabolic biomarkers and pathways as promising targets to reveal the therapeutic effects of the Shenqi pill. *RSC Adv*. 2020; **10**: 2347- 2358.
73. Sobolevsky T, Ahrens B. High-throughput liquid chromatography tandem mass spectrometry assay as initial testing procedure for analysis of total urinary fraction. *Drug Test Anal*. 2020.
74. EE, Hollywood KA, Smith CA, Barran PE. High throughput screening of complex biological samples with mass spectrometry – from bulk measurements to single cell analysis. *Analyst (Cambridge, U K)*. 2019; **144**: 872- 891.
75. Pu F, Elsen NL, Williams JD. Emerging chromatography-free high-throughput mass spectrometry technologies for generating hits and leads. *ACS Med Chem Lett*. 2020.
76. Haslam C, Hellicar J, Dunn A, et al. The Evolution of MALDI-TOF mass spectrometry toward ultra-high-throughput screening: 1536-well format and beyond. *J Biomol Screen*. 2016; **21**: 176- 186.

77. Truebenbach CS, Tong H, Huang N, Schnier PD, Siegel MM. *High throughput flow injection analysis-mass spectrometry for combinatorial chemistry using electrospray ionization, atmospheric pressure chemical ionization and exact-mass Fourier transform mass spectrometry*. Elsevier; 2004.
78. Sarvin B, Lagziel S, Sarvin N, et al. Fast and sensitive flow-injection mass spectrometry metabolomics by analyzing sample-specific ion distributions. *Nat Commun*. 2020; **11**: 3186.
79. Anderson SE, Fahey NS, Park J, O'Kane PT, Mirkin CA, Mrksich M. A high-throughput SAMDI-mass spectrometry assay for isocitrate dehydrogenase. *Analyst*. 2020; **145**: 3899- 3908.
80. Techner J-M, Kightlinger W, Lin L, et al. High-Throughput Synthesis and Analysis of Intact Glycoproteins Using SAMDI-MS. *Anal Chem*. 2020; **92**: 1963- 1971.
81. Kuo T-H, Dutkiewicz EP, Pei J, Hsu C-C. Ambient Ionization Mass Spectrometry Today and Tomorrow: embracing Challenges and Opportunities. *Anal Chem*. 2020; **92**: 2353- 2363.
82. Sun J, Jiang Y, Liu H, Huang X, Xiong C, Nie Z. Ultrafast photocatalytic reaction screening by mass spectrometry. *Anal Chem*. 2020; **92**: 6564- 6570.
83. Lin S, Dikler S, Blincoe WD, et al. Mapping the dark space of chemical reactions with extended nanomole synthesis and MALDI-TOF MS. *Science*. 2018; **361**:eaar6236.
84. Park J-M, Kim M-J, Noh J-Y, et al. Simultaneous Analysis of Multiple Cancer Biomarkers Using MALDI-TOF Mass Spectrometry Based on a Parylene-Matrix Chip. *J Am Soc Mass Spectrom*. 2020; **31**: 917- 926.
85. Blincoe WD, Lin S, Dreher SD, Sheng H. Practical guide on MALDI-TOF MS method development for high throughput profiling of pharmaceutically relevant, small molecule chemical reactions. *Tetrahedron*. 2020; **76**:131434.
86. Simon RP, Winter M, Kleiner C, et al. MALDI-TOF-based affinity selection mass spectrometry for automated screening of protein-ligand interactions at high throughput. *SLAS Discovery*. 2020; **25**: 372- 383.
87. Krenkel H, Hartmane E, Piras C, Brown J, Morris M, Cramer R. Advancing liquid atmospheric pressure matrix-assisted laser desorption/ionization mass spectrometry toward ultrahigh-throughput analysis. *Anal Chem*. 2020; **92**: 2931- 2936.
88. Zhang X, Mell A, Li F, et al. Rapid fingerprinting of source and environmental microplastics using direct analysis in real time-high resolution mass spectrometry. *Anal Chim Acta*. 2019.
89. Zhou L, Wang X, Liu W, Xiang P, Chen H. Advances in COVID-19: the virus, the pathogenesis, and evidence-based control and therapeutic strategies. *Anal Methods*. 2020; **12**: 1430- 1440.
90. Khaled A, Belinato JR, Pawliszyn J. Rapid and high-throughput screening of multi-residue pharmaceutical drugs in bovine tissue using solid phase microextraction and direct analysis in real time-tandem mass spectrometry (SPME-DART-MS/MS). *Talanta*. 2020; **217**:121095.
91. Han Y, Levkin P, Abarientos I, Liu H, Svec F, Fréchet JMJ. Monolithic superhydrophobic polymer layer with photopatterned virtual channel for the separation of peptides using two-dimensional thin layer chromatography-desorption electrospray ionization mass spectrometry. *Anal Chem*. 2010; **82**: 2520- 2528.
92. Takats Z, Wiseman JM, Gologan B, Cooks RG. Mass spectrometry sampling under ambient conditions with desorption electrospray ionization. *Science*. 2004; **306**: 471- 473.

93. Wleklinski M, Loren BP, Ferreira CR, et al. High throughput reaction screening using desorption electrospray ionization mass spectrometry. *Chem Sci*. 2018; **9**: 1647- 1653.
94. Girod M, Moyano E, Campbell DI, Cooks RG. Accelerated bimolecular reactions in microdroplets studied by desorption electrospray ionization mass spectrometry. *Chemical Science*. 2011; **2**: 501- 510.
95. Loren BP, Ewan HS, Avramova L, et al. High Throughput Experimentation Using DESI-MS to Guide Continuous-Flow Synthesis. *Sci Rep*. 2019; **9**: 1- 8.
96. Wouters B, Davydova E, Wouters S, Vivo-Truyols G, Schoenmakers PJ, Eeltink S. Towards ultra-high peak capacities and peak-production rates using spatial three-dimensional liquid chromatography. *Lab Chip*. 2015; **15**: 4415- 4422.