High-Throughput Screening: Effective Tool in Drug Discovery

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Summary

The pharmaceutical industry faces a huge pressure nowadays to introduce new drugs in market as well as to increase productivity in short period of time with least investment through Research and Development (R&D) process which has increasing attrition rates and escalating costs. High-Throughput Screening (HTS) is most powerful tool with automation element that allows a researcher to quickly conduct millions of biochemical, genetic or pharmacological reactions. It has libraries or "decks" which can contain from 100,000 to more than 2,000,000 compounds.

Key words: Pharmaceutical industry, Research and Development (R&D), High-Throughput Screening (HTS).

Introduction

As we all know that pharmaceutical industry is nowadays under intense pressure to increase productivity and put new drugs into the market in short period of time. [1] Drug discovery is one of the most crucial components of the pharmaceutical industry's Research and Development (R&D) process and is the essential first step in the generation of any robust, innovative drug pipeline. Drug candidate pipelines are usually aimed at disease areas with high unmet medical need where the commercial reward for delivering first in class and/or best in class therapies can be enormous. The R&D process is an extremely lengthy, complex and strongly regulated activity that has increasing attrition rates and escalating costs. The use of combinatorial and high throughput research and development in heterogeneous catalysis is becoming well-established in industry and academics and has been exemplified in many recent publications.[2] Those companies that find the quickest way to put the first and best in class products on the market in a cost-efficient way will become the clear commercial winners in such a competition.

Use of robotics, data processing and control software, liquid handling devices, sensitive detectors and High-Throughput Screening or HTS allows a researcher to quickly conduct millions of biochemical, genetic or pharmacological reactions or tests. The results of these experiments offer groundwork for drug design and for understanding the interaction or role of a particular biochemical process in biology. In essence, HTS uses automation to run a screen of an assay against a library of candidate compounds. An assay is a test for specific activity: usually inhibition or stimulation of a biochemical or biological mechanism. Typical HTS screening libraries or "decks" can contain from 100,000 to more than 2,000,000 compounds. The key lab ware or testing vessel of HTS is the micro titer plate: a small container usually disposable and made of plastic which features a grid of small, open divots called wells. Modern micro plates for HTS generally have either 384, 1536 or 3456 wells. These are all multiples of 96, reflecting the original 96 well micro plate with 8 x 12 9mm spaced wells. Most of the wells contain experimentally useful matter, often an aqueous solution of dimethylsulfoxide (DMSO) and some other chemical compound, the latter of which is different for each well across the plate. To prepare for an assay, the researcher fills each well of the plate with some biological entity that he or she wishes to conduct the experiment upon, such as a protein, some cells or an animal embryo. After some incubation time period has passed to allow the biological matter to absorb, bind to or otherwise react or fail to react with the compounds in the wells and measurements are taken

across all the plate's wells either manually or by a machine. Manual measurements are often necessary when the researcher is using microscopy to look for changes or defects in embryonic development caused by the wells compounds and looking for effects that a computer could not easily determine by itself. Otherwise, a specialized automated analysis machine can run a number of experiments on the wells (such as shining polarized light on them and measuring reflectivity which can be an indication of protein binding). In this case, the machine outputs the results of each experiment as a grid of numeric values with each number mapping to the value obtained from a single well. A high-capacity analysis machine can measure dozens of plates in the space of a few minutes like this, generating thousands of experimental data points very quickly. Depending on the results of this first assay, the researcher can perform follow up assays within the same screen by "cherrypicking" liquid from the source wells that gave interesting results (known as "hits") into new assay plates and then re-running the experiment to collect further data on this narrowed set, confirming and refining observations.

Automation is an important element in HTS's usefulness. Typically, an integrated robot system consisting of one or more robots transports assay-micro plates from station to station for sample and reagent addition, mixing, incubation and finally readout or detection. An HTS system can usually prepare, incubate and analyze many plates simultaneously, further speeding the data-collection process. HTS robots currently exist which can test up to 100,000 compounds per day. The common theme in these approaches is the need to provide high-quality data at a faster rate to drive decision making process. Analytical chemists are challenged to find faster ways of delivering quality data across a range of project-driven needs.[3] A number of approaches are being employed to increase separation throughput including Ultra High performance liquid chromatography (UPLC).[4] UPLC was pioneered in the late 1900s by the Jorgenson and Lee groups and typically involves the use of 1-2mm particles at much higher pressure (15,000-100,000 psi) than conventional HPLC instruments (4000-6000 psi).The use of columns packed with high pressures allows dramatic decreases in analysis time with little compromise in column performance. The relative flat nature of the Van deemter plot for these small particles at higher linear velocities accounts for the ability to operate at higher flow rates without severe effects on column efficiency. Recently , commercial instrumentation capable of operating up to 15,000 psi combined with columns packed with 1.7 mm particles have become available in the form of the Waters ACQUITY UPLC system.[5-8]

Fig.: Waters ACQUITY UPLC system.

Homogenous and Heterogeneous HTS:

HTS uses standard assay types which are known to most biological and biochemical scientists (e.g., ELISA, proliferation/cytotoxicity assays, reporter assays, and binding assays). However, adaptations of these assays have emerged to facilitate throughput and relieve robotic complexity. Screeners define assays as either homogeneous or heterogeneous. Homogeneous assays require only additions and incubations followed by reading (e.g. luciferase assay). Heterogeneous assays require steps that go beyond simple fluid additions, incubations and reading (e.g. filtration, centrifugation and plate washing steps). True homogeneous assays involve simply mixing all the assay ingredients in one step, incubating and reading the assay plates. However, most assays still referred to as homogeneous which require multiple additions and incubations at different times within a procedure followed by a final read step. In spite of the advantages of homogeneous assays, the HTS community continues to use heterogeneous assays (e.g., ELISA) in a good percentage of screens. Primary drivers for the use of homogeneous assays are their speed and simplicity. A simple assay will reduce the robotic complexity requirements for automation. Robotic processing of heterogeneous assays was far slower than that of homogeneous assays. Assembly- line-style robotics can process heterogeneous assays at the same rate as homogeneous assays. Thus, given the correct equipment environment, the advantages of moving to homogeneous assays are now fewer. For example, Tropix has performed a UHTS heterogeneous

kinase inhibitor screen that processed one 96-well micro plate/min for 24 hr. As mentioned above, the advantage of traditional heterogeneous assays is that their sensitivity and signal-to-noise ratios still exceed most homogeneous assay equivalents. The higher-quality assay data will result in fewer false negatives and false positives, reducing the potential miss of a hit and the expense of screening compounds that do not need to be rescreened. [9]

High-Throughput Screening Robotics (HTSR):

The nuptials of robotics and HTS have been important to achieve the desired screening rates as well as relieving scientific staff from tedious work. However, until the last few years or so, one could argue that robotics for screening has been more of a research undertaking that a true implementation of stable technology. Problems associated with screening robotics have included long design and implementation time, long manual to automated method transfer time, non-stable robotic operation and limited error recovery abilities. These problems can be attributed to robot integration architectures, poor software design and robot–workstation compatibility issues.[10, 11]

Fig.: High-Throughput Screening Robotics

These robot-centric HTS systems have a central robot with a gripper that can pick and place micro plates around a platform. They typically process between 40 and 100 micro plates in a single run (the duration of the run depends on the assay type). Similar to assembly-line

manufacturing, micro plates are passed down a line in serial fashion to consecutive processing modules). The importance of high-throughput robotics not only relates to being able to process very large libraries but also enables fast turnaround of screening data in a very short time (regardless of library size). This increases the speed of the overall drug discovery process, a crucial issue for all drug discovery companies.[12, 13]

Ultra-High Performance Liquid Chromatography (UPLC):

UPLC is a new category of separation science which builds upon well-established principles of liquid chromatography, using sub-2µm porous particles. These particles operate at elevated mobile phase linear velocities to produce rapid separation with increased sensitivity and increased resolution.[14]

Fig.: Ultra-High Performance Liquid Chromatography

As the number of preclinical candidates increase, higher throughput chromatographic techniques are required to reduce cycle time. Ultimately, faster separation techniques without sacrificing quality (efficiency and resolution) are needed. Four different approaches have been used to shorten HPLC separation times for pharmaceutical analyses. First, shorter columns packed with 3–5micro meter particles were used to cut down separation time; however, column efficiency was sacrificed. To avoid loss in efficiency, a second approach used monolithic columns.[15-19] Monoliths with their high porosity and lower pressure drop; allow fast separations with high

efficiency. However, limited availability of different monolithic stationary phases and higher solvent consumption has been a concern. A third approach to achieve faster LC was to use elevated temperatures.[20-24] The reduced viscosity and higher diffusivity of the mobile phase at higher temperatures yields a lower resistance to mass transfer and thus higher efficiency. The lower viscosity results in lower backpressure allowing for higher flow rates to be used and thereby providing faster separations. High temperature separations are not common in pharmaceutical analyses due to potential on-column degradation of analyses and column stability.[25-29] The fourth approach is using columns packed with smaller particles to improve both speed and efficiency.[30, 31] From theory, the column efficiency is inversely proportional to the particle diameter and the retention time is directly proportional to the square of the particle diameter. Therefore, the smaller the particle diameter, the faster and more efficient the separation is. The relationship between the separation time and particle size is described by the following equation:

$$t_R = (1+k)\, N\, h / D_m\, V\, dp^2 \quad \text{.....} \quad (1)$$

Where,

t_R is the separation time,

k is the retention factor of an analyte,

N is the number of theoretical plates,

h is the reduced plate height,

dp is the particle diameter,

m is the reduced velocity and

D_m is the diffusion coefficient of an analyte in the mobile phase.

The limitation that arises from using smaller particles is higher backpressures. The pressure required to achieve the optimum linear velocity is inversely proportional to the square of the particle diameter when the required column efficiency and other parameters are kept fixed.[32, 33]

$$D_P \text{ (opt) is proportional to } 1/dp^2 \text{.....} \quad (2)$$

Reducing the particle size by a factor of 3 while keeping other variables constant increases the backpressure by a factor of 9, thus producing pressure significantly above any conventional HPLC pump capability (e.g., typical 10 cm column packed with 1.7 lm particles). This increased the interest in developing pumps that can operate at pressures higher than 6000 psi. Recently, several ultra high-pressure LC systems became commercially available. Some of these systems can operate at pressures as high as 15000 psi with excellent precision, accuracy, and reproducibility.[34]

Screening by UPLC:

Thirteen different active pharmaceutical ingredients (APIs) were screened on three stationary phases: C-18, phenyl and Shield RP 18 with column dimensions (150 mm, 62.1 mm, 1.7 lm) that should theoretically generate l35000 plates or l175% of the typical column plate count of a conventional 250 mm, 64.6 mm, 5 lm particle column. The "A" solvent was 0.1% phosphoric acid v/v and the "B" solvent was ACN. The gradient was 5–95% B in 30 min with a flow rate of 0.5 mL/min. The column temperature was set to 408˚C and 210 nm was used as detection wavelength. Solutions of APIs were prepared according to their respective conventional HPLC methods used for release testing. The sample tray was kept at 58˚C and injection volumes varied from 1 to 5 lL depending on the sample concentration.[35]

Table 1; Purity values using the three UPLC as well as various HPLC stationary phases

Project No.	HPLC*	UPLC stationary phase			HPLC column used*
	Lot data	C-18	Phenyl	RP shield	
1	99.5	99.5	99.5	99.6	Cadenza CD C-18
2	99.3	99.4	99.3	99.3	Zorbax ExtendC-18
3	98.8	98.7	98.7	98.6	Betasil C-18

4	99.0	98.7	98.7	98.6	Mac-Mod Ace C8
5	99.9	99.9	99.9	99.9	Waters Symmetry C-18
6	99.0	98.7	98.6	98.5	Thermo Betasil C-18
7	98.9	98.7	98.7	98.8	Zorbax SB Phenyl
8	99.7	99.8	99.7	99.7	YMC Pro C 8
9	99.5	99.5	99.6	99.5	Waters Symmetry C-18
10	99.5	99.4	99.1	99.4	Fluophase PFP
11	98.4	98.2	98.1	98.1	X-Terra RP 18
12	98.0	98.7	98.0	98.2	Mac Mod Ace C 8
13	99.6	99.2	99.4	99.4	Fluophase PFP
% Success (l0.2 Area %)	--	69.2	69.2	76.9	
% Success (l0.2 Area %)	84.6	Does not meet (l0.2 A %)			

***** The columns used for developing HPLC methods are listed. Success criterion is +0.2 area%. The numbers in bold did not meet.

Applications:

Researchers are used to making compromises and one of the most common scenarios involves sacrificing resolution for speed. With UPLC increased resolution in shorter run times can generate more information faster without sacrifices. Higher sample throughput with more

information per sample may decrease the time to market, an important driving force in today's pharmaceutical industry.[36]

Enhanced selectivity, sensitivity and rapid generic gradients made LC–MS the predominate technology for both quantitative and qualitative analyses. However, with the ever increasing numbers and diversity of compounds entering development and the complex nature of the biological matrices being analyzed, new analytical procedures and technology were required to keep pace with the testing demands. Unexpected, reactive or toxic metabolites must be identified as early as possible to reduce the very costly attrition rate. This quest for more accurate data meant improving the chromatographic resolution to obtain higher peak capacity, reducing the co-elution of metabolites, while enhancing the sensitivity and decreasing ion suppression in the MS.[37]

Drug Discovery Process-

UPLC can be used to significantly improve the success of the drug discovery process. Drug discovery is heavily dependant upon the early prediction of metabolic fate and interactions of drug candidate molecules. Factors such as metabolic stability, toxic metabolite production, p450 inhibition and induction are all routinely monitored to prevent "poor" candidates from progressing through the discovery process.**[38]** The power of the ACQUITY UPLC System when used in drug discovery can be illustrated by the analysis of the in-vitro metabolism of dextromethorphan. Dextromethorphan undergoes O-dealkylation in two positions leading to three major phase I metabolites. These products can be further metabolized via conjugation with glucuronic acid to form metabolites of masses MH_ _ 434 and 420. The chromatographic performance of the ACQUITY UPLC BEH 1.7-_m particles is significantly better than that produced by the 3.5 _m material. The 1.7-_m material gives peaks of width 4 s at the base, resulting in a peak capacity of over 100, whereas with HPLC the average peak width was 20 s at the base giving a total peak capacity of just 20, resulting in a 5-fold increase in the performance of the UPLC system. The extracted ion chromatogram m/z _ 258 and m/z _ 244 for the HPLC/MS analysis. Without the resolution generated by UPLC it would be possible to falsely assign the structure of a metabolite or miss a potential toxic moiety. The extra sensitivity produced by the UPLC system ensures more low concentration metabolites will be detected, helping to prevent potentially toxic compounds from progressing further into the drug discovery

process. This added sensitivity is extremely important when performing MS–MS experiments as it can make the difference between obtaining an interpretable spectrum or not.[39, 40]

Transfer of the USP Assay for Simvastatin to UPLC-

The USP HPLC-UV methodology for the assay of the cholesterol-lowering drug simvastatin has been successfully transferred to an ACQUITY UPLC method. The final methodology reduced the analysis time from analyte retention of 9.28 minutes with HPLC to analyte retention of just 1.41 minutes with UPLC (a seven-fold increase in throughput). The ACQUITY UPLC Console Calculator was used to easily guide the transfer methodology to ACQUITY UPLC using sub-2 mm particles. The calculator gave three separate options for UPLC methods: Equal Efficiency, Maximum Efficiency and Shortest Analysis Time conditions. The Equal Efficiency calculations gave an almost exact transfer of chromatographic performance when compared to the original HPLC conditions. With both the equal and maximum UPLC conditions, the USP assay criteria of efficiency, k' and tailing were met or exceeded. The assay reproducibility performance was determined for the Equal Efficiency method and was found to be less than 1% RSD for six replicate injections. Finally, the assay was transferred to a Shortest Analysis Time method on the ACQUITY UPLC system, which gave an analysis time of 0.3 minutes with an analyte retention of 0.23 minutes, resulting in an increase in throughput of 40-fold. The ease of HPLC to UPLC method transferability and the benefits that can be obtained in any time, resource and/or revenue-conscious laboratory environment where UPLC can significantly increase throughput with quality results. And with a variety of ACQUITY UPLC BEH column dimensions, scientists have the flexibility to tailor their UPLC separations to the goals at hand.[41]

Analysis of Soy Isoflavones from a Dietary supplement Using UPLC with PAD and SQ Detection-

Current interest in soy isoflavones is based on a vast literature reporting a wide range of biological properties for genistein and daidzein and on clinical studies supporting their potential health benefits. When using mass spectrometry in particular for quantification, it is important to have at least 10 data points across a peak for repeatable peak integration. For UPLC-based experiments where the peak widths are much smaller than comparable HPLC peaks, MS acquisition rates have to be faster to achieve this.[42-44]

Analysis of Pesticide Residues in Baby Food-

The European Union residue monitoring program, establishes the need to cover 55 active ingredients in various foods, including baby foods. Twenty of these pesticides are suitable for multi-residue LC/MS analysis; only one has a negative polarity in electro spray mode, normally requiring two injections (one in each polarity ion mode). Consequently compounds with negative polarity are often excluded from monitoring programs. Ideally, these should be determined in a single analysis with polarity switching a fast and simple UPLC method involving polarity switching of MRM transitions has been successfully transferred to the ACQUITY TQD for the determination of 52 pesticides. Of these, 21 pesticides and 7 metabolites are included in the EU residue monitoring program. The ACQUITY TQD was capable of very fast polarity switching, allowing the analysis of positive and negative compounds in a single injection. The use of very short dwell times of 5 ms was found to have no effect on signal intensity, indicating that sensitivity can be maintained as the number of residues is increased.[45]

UPLC/MS/MS Bioanalytical method validation of Acebutolol and pindolol using an analogue internal standard-

Validated UPLC/MS/MS method for the analysis of Pindolol and Acebutolol in human plasma over the range of 0.2 - 150 ng/mL. Statistics for accuracy and precision were within the FDA guidelines for bioanalytical method validation. The data generated by UPLC/MS/MS were comparable to that generated by HPLC/MS/MS, however, it was shown that by using UPLC, a 4-fold increase in signal-tonoise ratio for the LLOQ, a 2-fold decrease in run time, and an increase in resolution was achieved. This equates to doubling the throughput of this method, as well as enabling the acquisition of meaningful data for lower sample concentrations. This has several benefits, for example, as it would allow more accurate measurement of the lower part of the PK curve.[46]

Peptide Separation Technology (Quantitative Aspects of UPLC Peptide Mapping)-

Throughout the development of a biopharmaceutical protein, peptide mapping is used to demonstrate genetic stability and to confirm the integrity of the protein. Changes in retention time, often in combination with MS or MS/MS detection, reveal changes in the primary structure of the protein. Modifications such as oxidation, deamidation, deletions, sequence clips and

glycosylation all affect chromatographic behavior. The modified peptides must be separated from the native peptides for a peptide mapping method to be useful. The presence of a modified peptide in the map of a sample reflects the presence of modified protein in the original sample. In the initial characterization of a protein, it is important to develop a peptide mapping method that resolves modified peptides from native peptides so that all possible modifications may be detected. As development of the biopharmaceutical advances, these peptides must be quantitated. Quantitation is generally expressed as area or height percent of the native peptide. In this way, the peptide map can provide information on the mixture of protein forms in each sample so that the safety and efficacy of the preparation may be assured. Methods must, therefore, exhibit excellent sensitivity and linearity for quantitative work. Ultra Performance Liquid Chromatography (UPLC®) has demonstrated significant advantages compared to HPLC for peptide mapping. UPLC gives increased resolution, higher sensitivity, excellent peak shapes for glycopeptides, and the potential to increase throughput. In this application note, we focus on the quantitative aspects of UPLC peptide mapping with UV detection. The technique is evaluated with respect to both chromatographic and detection linearity since the altered and normal peptides occurs at extreme molar ratios. Reproducibility of the area measurement at these extreme ratios is also examined. Results from a mixture of peptide standards and from a digest spiked with an amount of peptide are shown.[47]

LC/MS-based Differential Proteomics of the Mitochondria of (PSI+) & (PSI-) Saccharomyces cerevisiae Strains-

Proteomics focuses on the high throughput study of the expression, structure, interactions, and, to some extent, function of complex sets of proteins. Differential proteomics aims at finding differences between two or more multi-protein samples, which is imperative for the understanding of many biological problems. [PSI+] is a protein-based heritable phenotype of the yeast Saccharomyces cerevisiae, which reflects the prion-like behavior of the endogenous Sup35 protein release factor. Previous work has shown that the presence of a prion form of this protein in the cytosol can cause respiratory deficiency by decreasing level of mitochondrial-encoded Cox2 protein1. The main goal of the presented work is to identify proteins, which are present at different levels in the mitochondrial fractions of [PSI+] and [psi-] yeast strains. The latter should allow for the identification of the molecular mechanism of prion-dependent switching between

respiratory competence and deficiency. In this study a label-free LC/MS-based approach was used where data is acquired in an alternating fashion, with low collision energy on the gas cell in the first function, switching to elevated energy in the alternate scan. In neither scan is a precursor ion isolated with the quadrupole, thus providing a parallel approach to ion detection and sequencing. The low energy portion of the obtained data sets is typically used for quantification of the proteins, whereas the combined low and elevated energy information are utilized for qualitative, identification purposes. Results obtained from yeast mitochondrial fractions allowed differentiation of proteins originating from [PSI+] vs. [psi-] strains, leading to the identification of a significant decrease of Phb1 and Phb2 (prohibitins) in mitochondria of the [PSI+] strain. The obtained results were confirmed by Western blotting experiments. Significant regulation between the investigated strains - [PSI+] and [psi-] - have been identified and quantified, Several proteins of interest, found to be down-regulated in the nanoscale UPLC/MSE dataset were validated by biological quantification methods, The Western blot analysis showed excellent correlation with the UPLC/MSE data, further investigation of the up- and down-regulated proteins from this study is being performed.[48]

Removal of Interferences and Easier Metabolite Detection by Mobility Mass Spectrometry-

The task of identifying drug metabolites from complex biological matrices such as bile, plasma, feces, and urine with traditional techniques can be difficult. One of the typical problems when running in vivo samples is that without the use of radio labeled compounds, there are no reference points to look for xenobiotics. Therefore in the vast majority of cases, the analyst relies heavily upon personal experience and customized analytical strategies to detect and identify low-level metabolites from high endogenous backgrounds. The complexity of this analysis could be significantly reduced by the use of an additional stage of separation, which is orthogonal to the LC and mass spectrometric separations, and occurs on a timescale that is intermediate between the two. A technique that possesses this capability is ion mobility spectrometry (IMS). IMS separates ionic species as they drift through a gas under the influence of an electric field. For any particular ion, the rate of drift depends on its mobility, which in turn is dependent on factors such as mass, charge state and the interaction cross-section of the ion with the gas. This additional dimension of separation fidelity leads to improved specificity and sample definition so that more information about the sample can be extracted. The multidimensional data produced by the

Waters® Synapt™ High Definition MS™ (HDMS™) system is visualized and manipulated using DriftScope™ Mobility Environment software. In this work, UPLC®/IMS-TOF-MS (Figure 1) analysis was conducted on a rat bile sample. By using the DriftScope software, the metabolites from this complex matrix were easily visualized, as the drift time was used to separate background ions from real drug-related metabolites. Further extracted ion chromatograms were also obtained by selecting specific ions with the software. As a result, the extracted ion chromatogram and MS spectrum for each metabolite were attained without interference from the endogenous compounds. Ion mobility capabilities with the Synapt HDMS system offer an additional fourth dimension of information in the analysis of very complex samples by UPLC/MS. The DriftScope Mobility Environment software is an essential tool to visualize the data and serves as a very powerful aid to extract the information required, allowing only relevant drug-related information to be extracted. IMS technology could significantly accelerate the identification of metabolite peaks in "cold" in vivo metabolism picture earlier on during drug discovery, where radio labeled material is usually not available.[49]

Environmental analysis-

Sensitivity, selectivity and analysis time (sample throughput) are also some of the challenges analysts face when analyzing environmental samples such as soil and water. Explosives residues in soil or environmental waters are of both forensic and environmental interest. These types of assays prove challenging because of the selectivity needed to resolve positional isomers. Typical HPLC analyses require viscous, buffered mobile phases operated at high temperatures and analysis times exceeding 30 min. In UPLC the separation of a complex mixture of explosive compounds in less than seven minutes, with a much simpler, more robust mobile phase than that commonly used in HPLC assays. The simpler no buffered mobile phase also is ideal for MS detection if desired.[50]

Discussion

High-Throughput Screening (HTS) is most integrated technique with automation element that allows one who conducts millions of biochemical, genetic or pharmacological reactions to generate faster results in short period of time and has libraries or "decks" which can contain from 100,000 to more than 2,000,000 compounds. Ultra-High Performance Liquid Chromatography

(UPLC) is one of the High-Throughput Screening (HTS) tools used for so many operations in Research and Drug Development process. Thus UPLC which is the modernized, integrated and with automation element reduces the investment in the development of new potent and most efficacies escort families.

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