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# High-Throughput Screening: The Hits and Leads of Drug Discovery- An Overview

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**ABSTRACT**

The mechanism-based approach which corresponds to the target-based approach screens for compounds with a specific mode of action. The highly effective nature of high-throughput screening (HTS) for identification of highly target specific compounds is attributed to its precise focus on single mechanism. This logical development of receptor technology is closely connected with the changes in strategy of chemical synthesis. The vast number of compounds produced by combinatorial chemistry and the possibility of testing many compounds, including natural products, in a short period of time by HTS attracted attention of many workers. Various detection techniques like fluorescence resonance energy transfer (FRET), Homogeneous time resolved fluorescence (HTRF), etc are available, and the screening of more than 100,000 samples per day is possible. With the introduction of robotics, automation and miniaturization techniques, it became feasible to screen 50,000 compounds a day with complex work-stations. High-throughput screening methods are also used to characterize metabolic and pharmacokinetic data about new drugs. With the use of Cassette dosing techniques even the pharmacokinetic data can be assessed for large number of drug candidates, though not free of drawbacks, yet an effective technique to further increase the drug discovery and development rate. The objective of this article is to give an overview to the High-Throughput screening methodologies used in industries as well as in academic research programmes.

***Keywords:*** High-Throughput Screening (HTS), Automation and Robotics, Miniaturization, Z-factor, Profiling, Errors

## INTRODUCTION

Of late High-Throughput Screening (HTS) a high-tech approach for drug discovery is more and more gaining popularity among industrial researchers as well as students doing their post-graduate and/or doctorate research projects. It is basically a process of screening and assaying huge number of biological modulators and effectors against selected and specific targets. The principles and methods of HTS find their application for screening of combinatorial chemistry, genomics, protein, and peptide libraries. The main purpose or goal of this technique is to hasten the drug discovery process by screening the large compound libraries with a speed which may exceed a few thousand compounds per day or per week. For any assay or screening by HTS to be successful several steps like target identification, reagent preparation, compound management, assay development and high-throughput library screening should be carried out with utmost care and precision. Methods commonly followed are: firstly selecting the target. Currently there are about 500 targets being used by companies. Of these, cell membranes receptors, mostly G-protein coupled receptors, make up the largest group (45% of the total), Enzymes make up the next largest group (28%), followed by hormones (11%), unknowns (7%), ion-channels (5%), nuclear receptors (2%), and finally DNA (2%) (**FIG 1**). Researchers anticipate that in near future human genome screening can add significant numbers to the aforementioned figures. The next concern is the library to be screened; they usually consist of microtiter plates with frozen or dried samples of compounds to be screened. High-Throughput Screening is a very vast area of study and development with many scopes including topics like enzyme testing, whole organ testing and even

whole animal testing via cassette dosing. Cassette dosing is a procedure for HTS enabling to rapidly assess the pharmacokinetics of large number drug candidates .Unlike other techniques to assess the pharmacokinetics in this procedure single animal is given simultaneously and blood samples collected to assay the same. The main advantage is that the pharmacokinetics of large number of compounds can be assessed rapidly and accurately. But the main disadvantage is that simultaneous administration can lead to drug-drug interaction.

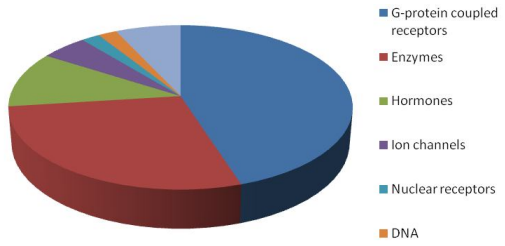


**Figure 1: Targets in HTS**

HTS is a novel method for drug discovery but it is not the only method. HTS not only helps in drug discovery but also in development of present drug moieties to optimize their activity. In past years many advances in science and technology and economic pressures have kept every researcher to develop speedy and precise drug development and screening technologies to combat the ever increasing diseases and many pathogens acquiring resistance to present available drugs. This also applies to screening the ever increasing compound libraries waiting to be screened due to increase in the parallel and combinatorial chemical synthesis. Research is also carried out so to cut the drug development costs, so that industries keep abreast with ever increasing competition. It is anticipated that with the introduction of human genomes as potential candidates the compound library will be as large as 100 million candidates that would require about $10^{12}$ assays to establish their Structure-Activity Relationship (SAR). Initially the assays were carried out in 96-well plates but with advancement now there are also 1586-well plates available. Typical HTS programs have potentials to screening up to10000 compounds per day, while some laboratories with Ultra High-Throughput Screening (UHTS) can perform 100,000 assays per day.

## ASSAY DESIGN

The primary screens re less quantitative than traditional biological assays. Often, compounds are only tested in duplicate now-a-days many companies are using singlet, with molar concentrations ranging as low as 1-10 micromolar for combinatorial chemistry synthesis. If a positive result or "HIT" is discovered in primary screen a more accurate and precise secondary screening is performed and accordingly quantification and $IC_{50}$ calculation are done. The assay procedures are no different from those known to biological and biochemical scientists like ELISA, reporter-gene assays binding assays etc. These simpler assay methods have however facilitated throughput and to help reduce the robotic complexity in case of automation.

Assays are mainly of two types either heterogeneous or homogeneous. Heterogeneous assays are bit complex requiring additional steps like filtration, centrifugation etc. beyond the usual steps like fluid addition, incubation and reading. Homogeneous assays are simpler consisting of the latter three usual steps, this may also be called true homogeneous assay. However at times homogeneous assays could be complex due to the need for multiple addition and different incubation times. Though advantageous, many companies prefer or continue to use heterogeneous assay, eyeing their better precision over its counterpart, though it is true only in few number of cases. The driving force for use of homogeneous assays is its simplicity of having less number of steps, which will help reduce assay cost. This simplicity may also reduce robotic complexity requirement for automation.

## BIOCHEMICAL ASSAYS:

Homogeneous biochemical assays in miniaturized formats are most frequently carried out using scintillation proximity assay (SPA) or fluorescence detection techniques because of the requirement for increased sensitivity as assay volume shrinks. The choice of detection technology employed is dependent on the particular class of assay target being investigated. Because of the high binding and low receptor densities required, binding assay for cell surface receptors are usually carried out using SPA techniques. For the use of miniaturized assay formats, emphasis should be given on proper assay design in order to get firm, reproducible and statically significant results.

In a study a high-throughput assay measuring the accumulation of the ABCG2 substrate pheophorbide a in ABCG2-overexpressing NCI-H460 MX20 cells were used to screen libraries of compounds. In another research series of CCR5 antagonists have been identified , using leads from high-throughput screening which was further modified based on results from competitor molecules. Lead optimization was achieved by balancing opposing trends of metabolic stability and potency.

Fluorescence-based detection methods are inherently sensitive due to the short duty cycle of most fluorophores (the fluorescence lifetime of fluorescein is ~4 ns) and consequently high emitted photon fluxes that can be achieved even with modest excitation light sources. This property, combined with the variety of different fluorescence modes that can be exploited to advantage in homogeneous assay formats, makes fluorescence detection highly amenable to many HTS applications.

Some techniques of biochemical assays are summarized below [Table 1]:

*Fluorescence resonance energy transfer (FRET):* It is the non-radioactive transfer of energy between appropriate energy donor and acceptor molecules.

**Table 1: Single Molecule Detection Techniques**

| Name of Technique | Abbreviation | Parameter/ species resolved | Hardware requirement | Amenability to current HTS |
|---|---|---|---|---|
| Fluorescence correlation spectroscopy | FCS | Translation diffusion | 1 detection CW laser | Limited |
| Fluorescence cross-correlation spectroscopy | FCCS | Colour | 2 detection CW laser | NO |
| Fluorescence intensity distribution analysis 2D | FIDA | Brightness<br>Anisotropic and Brightness | 1 detection CW laser | YES |
| Fluorescence intensity multiple distribution analysis | FIMDA | Brightness and diffusion time | 1 detection CW laser | NO |
| Confocal fluorescence lifetime analysis | cFLA | Fluorescence lifetime | 1 detection pulsed laser | YES |
| Fluorescence intensity and lifetime distribution analysis | FILDA | Fluorescence lifetime and brightness | 1 detection pulsed laser | YES |
| Confocal time-resolved anisotropy | cTRA | Fluorescence lifetime and anisotropy | 2 detection pulsed laser | YES |
| Combination cTRA + 2-D FIDA | FIDTRA | Fluorescence lifetime, anisotropy and brightness | 2 detection pulsed laser | In development |

*Fluorescence polarization (FP):* Its measurements allow one to measure changes in the rotational diffusion coefficient of small labeled probes upon binding to larger molecules.

*Homogeneous time resolved fluorescence (HTRF):* It is a hybrid technique that takes advantage of the long fluorescence lifetimes of europium crytates and the large apparent Stokes shift (the difference between the peak excitation and peak emission wavelengths of a fluorophores) obtained by exploiting energy transfer between the europium donor and suitable acceptors. In an recent study HTRF as a screening application was used for the assay of tyrosine kinase and screening against tumor necrosis factor receptor in a 384-well microplate format.

*Fluorescence correlation spectroscopy (FCS):* FCS measurements are carried out using confocal optics to provide the highly focused excitation light and background rejection required for single molecule detection.

*Fluorescence intensity distribution analysis (FIDA):* It yields information on changes in fluorophore quantum yield or spectral shift, and can also be used to monitor binding events when the binding interaction influences these properties.

Severe acute respiratory syndrome associated coronavirus main protease (SARS-CoV $M^{pro}$) has been proposed as a prime target for anti-SARS drug development. Two novel small molecule inhibitors of the SARS-CoV Mpro were identified by high-throughput screening using an internally quenched fluorogenic substrate. The identified inhibitors have Ki values at low lM range with comparable anti-SARS-CoV activity in cell- based assays.

**Cell-based assays**

Cell-based assays for HTS can be classified under following classes:

*Second messenger assays*: It monitors signal transduction from activated cell-surface receptors. Second messenger assays typically measure fast, transient fluorescent signals that occur in matter of seconds or milliseconds. Many fluorescent molecules are known to respond to changes in intracellular Calcium ion concentration, membrane potential and various other parameters, hence they are used in development of second messenger assays for receptor stimulation and ion-channel activation. The development of hydrophobic voltage-sensitive probes and FRET-compatible microplate instrumentation has helped the advancement of the screening technique for ion-channel drug discovery.

*Reporter gene assays*: It monitors cellular responses at transcription/translation level. It indicates the presence or absence of a gene product that in turn reflects changes in a signal transduction pathway. The quantification of the reporter is usually carried out by biochemical methods viz by measuring the enzymatic activity. Plasmids are typical reporter genes employed. An entirely in vitro study was carried out by Suang Rungpragayphan et al. for generation and screening of combinatorial protein library in array format. This studied employed virtues of polymerase chain reaction (PCR) and in vitro coupled reporter gene assay.

*Cell proliferation assays*: It monitors the overall growth/no growth responses of the cell to external stimuli. These are quick and easy to be employed for automation.

*Yeast complementation assay*: *S. cerevisiae* is the typical model organism used for the two-hybrid technique's inception. It has several characteristics that make it a sturdy organism to host the interaction, including the ability to form tertiary protein structures, neutral internal pH, enhanced ability to form disulfide bonds and reduced-state glutathione among other cytosolic buffer factors, to maintain a favourable internal environment. Yeast systems are tolerant of diverse culture conditions and harsh chemicals which cannot be applied to mammalian tissue cultures. Proteins from as small as eight to as large as 750 amino acids have been studied using yeast cultures.

In a study conducted on human-derived and rat derived *Pneumocystis carinii* dihydrofolate reductase (DHFR) was expressed in *Saccharomyces revisiae* strain whose growth depends on complementation by this enzyme. Using quantitative assay measure the sensitivity of this yeast strain to DHFR inhibitors was found out. This assay is also useful for identifying new inhibitors of human-derived *P. carinii* DHFR.

*High content screening*: High content screening (HCS) is analysis of cells using fluorescence based reagents with the ArrayScan system to extract spatial and temporal information of target activities within cells. HCS yields information that will permit more efficient lead optimization before the in vivo testing. There are two types of HCS (1) using fixed cells with fluorescent antibodies, ligands, and/or nucleic acid probes, and (2) using live cells with multicolor fluorescent indicators and biosensors. A high content screen has also been explored for multiparametric measurement of apoptosis, which provides information on parameters such as nuclear size and shape changes, nuclear DNA content, mitochondrial potential, and actin-cytoskeletal rearrangements during drug-induced programmed cell death.

## HIGH-THROUGHPUT ASSAY TECHNIQUES FOR ION-CHANNEL

Ion channels represent a class of membrane-spanning protein pores that mediate the flux of ions in a variety of cell types. To date, more than 400 ion channels have been cloned and characterized, and some of these channels have emerged as attractive drug targets. The technologies used presently are binding assays, ion flux assays, fluorescence-based assays, and automated patch-clamp instrumentation. Technologies based on flux assays are available in a fully automated high-throughput format for efficient screening. This application offers sensitive, precise, and reproducible measurements giving accurate drug rank orders matching those of patch-clamp data.

## ALTERNATE HIGH-THROUGHPUT SCREENING TECHNIQUES

At times it was so reported that assays for biological targets cannot be conveniently designed to fit with standard cellular or biochemical assay formats. For example, in the search for new antibacterial agents, genomic experiments have indicated a large number of proteins that are essential for the survival of the bacterium but their function in the cell is unknown. In this situation there is no known biological function that will allow the design a biochemical or cellular screen. To screen these types of target, an alternative to conventional bit chemical and cellular screen may be use. One alternative screening approach that does not require knowledge or analysis of the biological function of the target of choice is direct measurement of compound interaction with protein. A range of techniques are available to measure the direct binding events such as NMR and calorimetry These bio-physical techniques can yield important binding information, but the current form of the technology has low throughput and capacity limits so they cannot be used to screen large numbers of compounds. In addition, for this approach, large amounts of relatively pure protein need to be available.

## REAGENTS IN HTS

In any chemical synthesis or testing and screening, reagents play a major role, HTS is no exception to this. Reagents must be characterized and optimized before use. In a study it was found that Aptamers, nucleic acids that bind to other molecules with high affinity, can be used as versatile reagents in competition binding HTS assays to identify and optimize small molecule ligands to protein targets. The major advantages of using aptamers in HTS assays are speed of aptamer identification, high affinity of aptamers for protein targets, relatively large aptamer-protein interaction surfaces, and compatibility with various labeling/detection strategies. Aptamers may be particularly useful in HTS assays with protein targets that have no known binding partners such as orphan receptors.

Enzymes are often used as regents in HTS, an example Tyrosine Kinase was used to find its inhibitors. In this care must be taken that in reagent preparation there should not be any contamination with other kinases, phosphatases, and peptidases which may compete with Tyrosine Kinase to give false results. Other than kinase enzymes, generic reagents like biotinylated Deoxyuridine Triphosphate, Streptavidine-allophycocyanine, and Streptavidine-europium were used developed for determing the activity of HIV-Reverse Transcriptase.

Dimethyl sulfoxide (DMSO) is another widely used reagent as it is preferred vehicle for compound /sample delivery. The important point to remember during the use of DMSO is that its tolerance should be determined early during assay development stage so as to carry out further optimization during the screening stage.

## AUTOMATION AND ROBOTICS IN HTS

The union of robotics and HTS has been important to achieve the desired screening rates, as well as relieving scientific staff from tedious work. In recent years or so researchers could argue that robotics for screening has been more of a research endeavour 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 (e.g., microplate readers and liquid handlers). Traditionally, these integrated robot architectures have involved multiple layered computers, different operating systems, a single central robot servicing all peripheral devices, and the necessity of complex scheduling software to coordinate all of the above

The presently used robot-centric HTS systems have a central robot with a gripper that can pick and place microplates around a platform. They typically process between 40 and 100 microplates in a single run (the duration of the run depends on the assay type). The screener loads the robotic platform with microplates and reagents at the beginning of the experiment and the assay is then processed unattended. Robotic HTS systems often possess humidified $CO_2$ incubators and are enclosed for tissue culture work. Similar to assembly-line manufacturing, microplates are passed down a line in serial fashion to consecutive processing modules. Each module has its own simple pick and place robotic arm (to pass plates to the next module) and microplate processing device. Therefore, at each module, one step of the assay is completed. This arrangement, coupled with **Windows NT™** (Microsoft, Redmond, WA) and an Ethernet TCP/IP link between modules, provides a much simpler and more stable platform than robot-centric HTS systems.

## MINIATURIZATION IN HTS

The trend towards assay miniaturization arose simultaneously with move towards automation as a direct need to reduce development cost. Although at present most HTS is still carried out in 96-well plate format, the move towards 384-well and higher density plate formats is well under construction. Instrumentation for accurate, low-volume dispensing into 384-well plates is commercially available, so are sensitive plate-readers that accommodate this format. Many of the HTS studies are carried out in 384-well plates; yet, reformatting of 96-well compound plates into the higher density format can become a significantly difficult to implementing screens in this mode. Researchers have implemented their recombinase/luciferase reporter system for use in 864-well plates. As few as 560 cells per assay well were sufficient to measure dose response curves for ligand binding to the Glucocorticoid receptor. Some scientists have carried out luciferase reporter gene assays in human T-cells using a 1,536-well (3 microliter) plate format. The evolution and implementation of microplate-based screening in smaller volume, higher density formats (1,536-well plates and beyond) are challenged by numerous technical obstacles. Some assays may be difficult to implement in these formats due to sensitivity to final dimethylsulfoxide (DMSO) concentration as this a versatile and frequently used reagent which may build up additional cost. Oldenburg et al. demonstrated a assay in a 9600-well (0.2 microtiter) system.

## STATISCAL PARAMETER FOR HTS

In validating a typical HTS assay, unknown samples are assayed with reference controls. The sample signal refers to the measured signal for a given test compound. The negative control (usually referred to as background) refers to set of individual assays from control wells that give minimum signals. The positive control refers to the set of individual assay room control wells that give maximum signals. In validating assay, it is critical to run several assay plates containing positive and negative control in order to assess reproducibility and signal variation at two extremes of the activity range. The positive and negative control data can then be used to calculate their means and standard deviations (SD). The difference between the mean of the positive controls and the mean of the negative controls defines the dynamic range of the assay signal. The variation in signal measurement for samples, positive control, and negative controls (i.e., SDs) may be different. The mean and SD of all the test samples are largely governed by the assay method and also by intrinsic properties of the compound library. Because the vast majority of compounds from an unbiased library have very low or no biological activity, the mean, and SD of all the sample signals should be close to those of the positive controls for inhibition/antagonist type assays and near those of the negative controls for activation/agonist types assays.

*Z-factor***:** The Z-factor is a measure of statistical effect size proposed for use in HTS to judge whether the response in a particular assay is large enough to warrant further attention. The Z-factor is defined in terms of four parameters: the means ($\mu$) and standard deviations ($\sigma$) of both the positive (p) and negative (n) controls ($\mu_p$, $\sigma_p$, and $\mu_n$, $\sigma_n$). Given these values, the Z-factor is defined as:

$$\text{Z-factor} = 1 - \frac{3 \times (\sigma_p + \sigma_n)}{|\mu_p - \mu_n|}.$$

In practice, the Z-factor is estimated from the sample means and sample standard deviations [Table 2]

$$\text{Estimated Z-factor} = 1 - \frac{3 \times (\hat{\sigma}_p + \hat{\sigma}_n)}{|\hat{\mu}_p - \hat{\mu}_n|}.$$

## ERRORS IN HTS

HTS suffers from type 1 and type 2 errors. Type 1 errors are false positives. In HTS, a poor candidate or an artifact gives an anomalously high signal, exceeding an established threshold. Type 2 errors are the false negatives. In HTS, a perfectly good candidate compound is not flagged as a hit, because it gives an anomalously low signal. Moreover, a low degree of relevance of the test may induce a high failure rate of type 2. Much more attention is given to false-positive (type 1) results than to false-negative results (type 2). Some of the false positives are promiscuous compounds that act

noncompetitively and show little relationship between structure and function.

**Table 2: The interpretations for the Z-factor**

| Z-factor | Interpretation |
|---|---|
| 1.0 | Ideal. Z-factors can never exceed 1. |
| between 0.5 and 1.0 | An excellent assay. Note that if $\sigma_p = \sigma_n$, 0.5 is equivalent to a separation of 12 standard deviations between $\mu_p$ and $\mu_n$ |
| between 0 and 0.5 | A marginal assay. |
| less than 0 | There is too much overlap between the positive and negative controls for the assay to be useful. |

## DATA ANALYSIS AND MANAGEMENT

owing to the large volume of data generated in HTS efficient data management is essential. Software packages for HTS (e.g. **Activitybase**, **Spotfire**) are available to carry out the principle tasks like

a) Storage of raw data

b) Quality control

c) Transformation of data into information

d) Documentation

e) Reporting

In HTS each biochemical experiment in a single well is analyzed by an automated device, typically a plate reader or other kind of detectors. The output of these instruments comes in different formats depending on the type of reader. Sometimes multiple readings are necessary, and the instrument itself may perform some initial calculation. These heterogeneous types of raw data are automatically transferred into the data management software.

In the next step raw data are translated in contextual information by calculating results. Data on percentage inhibition or percentage of control are normalized with values obtained from the high and low controls present in each plate. Values obtained depends on the method used (e.g. fitting algorithms used for dose-response curve) and have to be standardized for screens with a company. All the plates that fail against one or more quality criteria are flagged and discarded.

A final step in the process requires the experimenter to monitor visually the data that have been flagged, as a final check on quality. This is to ensure the system has performed correctly. In addition to registering the test data, all relevant information about the assay has to be logged, e.g. the supplier of reagents, storage conditions, a detailed protocol, plate layout, and algorithms for the calculation of results. Each assay run is registered and its performance documented. HTS will initially deliver hits in targeted assays. Retrieval of these data has to be simple, and the data must be exchangeable between different project teams to generate knowledge from mass of data.

## PROFILING

HTS has its first objective, the identification of a few "VALIDATED HITS" with large compound libraries. The decision as to whether a particular hits is worth pursuing as a chemical lead in a drug discovery project depends on several factors, important ones being chemical characteristics and its pharmacodynamics and pharmacokinetic properties.

The technology involved in miniaturization, automation and assay readouts needed for HTS is continuing to develop rapidly, and as it does so, the laboratory setups installed in HTS facilities are steadily broadening their capabilities beyond their primary function of identifying hits. As this happens it becomes possible for HTS techniques to be applied to more diverse compound profiling assays relating not only to the target selectivity of the compound libraries, but also to their pharmacokinetic characteristics. Increasingly, therefore, early compound profiling tasks on 'hit' compounds are being carried out in HTS laboratory where the necessary technological expertise is concentrated. Such assays are also very helpful in the 'lead identification' stage of a project, where focused synthetic compound libraries based on the initial hits need to be assessed, as this work generally involves testing small compound libraries, usually fewer than 1000 compounds at a time, in several different assays. Small dedicated robotic workstations are needed, rather than the fast but inflexible factory-style robotic assembles used for large-scale HTS. It is clear that pharmacological profiling will be an increasing activity of HTS units in future, and will help to add further value in the drug discovery chain.

### Screening expense and outsourcing screening:

Rarely any company wishes to screen 100,000 compounds per day in-house. The reasons behind this include drug discovery process restrictions, equipment/robotic requirements, infrastructure investment, and limited need to invest in changing technologies. Some specific costs related to screening are assay reagent costs (reagents, cell culture expenses, etc.), microplate costs, pipette tip box costs, screening employee costs, data handling/analysis time, database costs, robot purchase costs, and laboratory space costs. Due to the combined difficulties of the above, a growing number of contract screening companies are emerging (such as **Tropix; PanLabs** and **Evotec)**. The services provided by these companies usually include assay development and screening, data analysis, and other library support needs for HTS. Contract screening companies are also being used for their ability to provide assay data with very fast turnaround times. They achieve this by running 24-hr shifts and using HTS robotic technologies. Additionally, some companies choose to outsource primary screening, since they

are finding the need to move some of their screening personnel to growing secondary screening programs. This keeps the higher-value, more proprietary secondary screening in-house, and enables the maintenance of a high rate of hit generation derived from outsourced primary screening. The cost of completely screening such large compound library, a single assay may amount to over $300,000.

**RECENT ADVANCES:**

For the first time, a high-throughput mapping and sequencing of gangliosides in human fetal brain was performed by a novel mass spectrometry (MS)-based approach. Three GG mixtures extracted and purified from different regions of the same fetal brain in the 36th gestational week: **frontal neocortex (NEO36), white matter of the frontal lobe (FL36)** and **white matter of the occipital lobe (OL36)** were subjected to comparative high-throughput screening and multi-stage fragmentation by fully automated chip-based nanoelectrospray ionization (nanoESI) high capacity ion trap (HCT) MS. Using this method, in only a few minutes of signal acquisitions, over 100 GG and asialo-GG species were detected and identified in the three mixtures. Penicillin G acylase (PGA) is one of the most important enzymes for the production of semi-synthetic β-lactam antibiotics and their key intermediates. Purification of penicillin G acylase from fermentation broth with the aid of high-throughput screening (HTS) process was recently studied to speed up the process. Microtiter-plate was used for screening method to find appropriate purification conditions for the target protein. The screening method is based on a 96-well plate format.

**CONCLUSION**

The HTS field continues to dynamic and extremely competitive one, where a newer technique or method is being reported at a very frequent basis. The need to increase the throughput of drug-discovery screening operations while reducing development and operating costs is continuing to drive the development of homogeneous, fluorescence-based assays in miniaturized formats. The use of 384-well and higher density plates and commercially available plate-handling robotics has made HTS a reality, and has allowed some screening groups to achieve ultra-high throughput rates in excess of 100,000 samples per day. As the density of plate increases the volume of sample required for the assay is decreased drastically, as a result the assay of expensive drugs can be carried out at lower cost, which compensates the initial setup cost. The combination of nanoliter-scale liquid-handling, integrated devices for compound dilution and assay functionality, and state-of-the-art fluorescence detection techniques has the potential to revolutionize the drug discovery screening process.

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