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Current Trends in Pharmacy and Pharmaceutical Chemistry

Journal homepage: https://www.ctppc.org/

Review Article

A brief review of high throughput screening in drug discovery process

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ARTICLE INFO

Article history:
Received 19-07-2022
Accepted 16-08-2022
Available online 20-08-2022

Keywords:
High throughput screening
Drug discovery
Hits
FRET

ABSTRACT

The pharmaceutical industry is fast pace growing field in which companies are focusing on research and development to identify new therapies and novel compounds which can provide better alternatives and which acts on new targets. For that matter, they are in need of rapid compound testing and screening. High throughput screening (HTS) is a important tool in drug discovery process which enables to screen more than millions of compound in a short period of time. It comprises of various tools which includes microtiter well plates, robotic arms and detectors. The basic application of HTS is to find 'hits', a compound from compound library which shows the affinity with the target molecule.

1. Introduction

HTS (High throughput Screening) is a drug discovery method that involves the automated testing of a large number of chemical and/or biological substances for specific biological targets.

In the pharmaceutical sector, high-throughput screening technologies are widely used to quickly assess the biological or biochemical activity of a large number of compounds, mainly pharmaceuticals, using robots and automation. They help to speed up target analysis by allowing large scale compound libraries to be screened rapidly and cheaply. HTS can be used to evaluate pharmacological targets and pharmacologically profile agonists and antagonists for receptors (such as GPCRs) and enzymes.

The fundamental purpose of HTS is to find candidates that influence the target in the intended way, often known as "hits", through compound library screenings. Liquid handling equipment, robots, plate readers as detectors, and specific software for instrumentation control and data processing are commonly used to do this. [1]

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2. History

Early HTS methods screened libraries of hundreds and thousands of chemicals utilizing a 96-well plate format. The use of microplates in the laboratory was a ground-breaking innovation since it allowed numerous tests to be conducted concurrently in a standardized manner. Larger compound libraries were produced over the ensuing ten years employing improvements in natural compounds isolation and combinatorial chemistry, as well as parallel chemical synthesis and synthesis. Automation, assay downsizing, and the use of 384, 1536, and 3456 well plates made it possible to screen libraries with a million or more chemicals. Ultra-HTS (uHTS) is a term used to designate HTS techniques that can screen more than 1 lakh compounds per day. [2]

2.1. Process of drug discovery

The selection of appropriate drug targets is the usual first step in drug discovery programmes. These targets include biomolecules, which are typically proteins like receptors, enzymes, or ion channels. A sufficient level of assurance must be achieved during the sequential process of target

https://doi.org/10.18231/j.ctppc.2022.020

validation that the target is relevant to the disease under research and that modulation of the target will result in efficient disease treatment. Target validation typically begins in vitro and using animal models, but human clinical trials are the only way to reach the final stages of validation.

The identification of the target's modulators is required following target validation. Such modulators include enzyme activators, inhibitors, and openers or blockers of ion channels, as well as agonists and antagonists for receptors. The design and development of an appropriate assay to track the target under research marks the beginning of this so-called lead identification phase. The target is then exposed to a huge number of chemical compounds produced through high-speed parallel and combinatorial synthesis as a result of HTS. When a specific level of selectivity for the target under research can be demonstrated and the first favorable results in animal models are obtained, active substances that exhibit dose-dependent target modulation are referred to as lead compounds. Before they may be considered as candidates for drug development, such lead compounds are optimized for their potency, selectivity, and physicochemical qualities, as well as their pharmacokinetic and safety features.[2,3]

3. High Throughput Screening

Approximately one marketable medicine is discovered from one million tested compounds, according to the screening attrition rate used in the current drug discovery processes. As a result, there is pressure to screen bigger libraries in order to maintain the pipeline and advance HTS. From the end of the 1980s to the late 1990s, the HTS method was centered on small molecule libraries powered by genomics and combinatorial chemistry, and through three significant periods of evolution.

High density microplates, homogeneous assays, high-performance microliter dispensers, imaging, and laboratory automation are only a few of the essential technologies that emerged during this time. More substances analyzed should, in theory, yield more positive molecules, or "hits." As a result, drug collections that were constructed with larger proportions of impure and non-drug like compounds were evaluated against novel targets of research drug's ability. Although the strategy produced some worthwhile hits, much effort was lost in the process.[4] On the basis of the target's enzymatic activity, simple readouts were put into place, primarily colorimetric. Additionally, developed were readouts for ATP consumption and bioluminescence. These tests have to be performed under extremely strict biochemical conditions despite their high sensitivity; several of them are still in use today. Combinatorial chemistry efforts were redirected in the late 1990s to produce libraries of pure drug-like molecules, and researchers concentrated on assay development and quality controls. However, there was very little chemical space covered. In contrast to the 10^{40}-10^{100} potential compounds that could exist in the small molecule universe, a drug development lab typically investigated only 10^5-10^7 molecules. Consequently, the enrichment of favored motifs was the main emphasis of combinatorial chemistry. In other words, after a hit is found, the screening compound collections could be enlarged to include specific family compounds. These collections should be "protodrug" space representations, including as many fundamental structures as possible.[3,4]

The pharmaceutical industry saw an opportunity to identify every potential human therapeutic target when the human genome sequence became available at the start of the twenty-first century. Picking the targets for the drug discovery programmes was a problem in addition to identifying the genes and creating a lengthy list of transcripts. Surveys conducted early on revealed that there were 500 molecular targets for current medicines, of which 45 percent were receptors, 20 percent were enzymes, 5 percent were ion channels, and 2 percent were nuclear receptors. More than 3000 targets were predicted using extrapolation to the human genome utilizing genomic databases.[5]

Due to the size of the target number, emphasis was put on ultra HTS (uHTS), a technology that allows for the rapid and inexpensive screening of a greater number of compounds. New technologies were required since uHTS was needed for medication development. Assay downsizing has advanced to the point where reliable assays are now carried out in 1556 well plates at volumes less than 2 μL, significantly lowering the assay's cost. However, to execute miniaturized assays at quantities of 2 μL or less, fluid handling apparatus that was dependable at the sub microliter volume range was needed.[4] Additionally, assay plates had to be created to accommodate large volumes. Flexible mechanisms that could move liquid samples from storage units to tiny wells were needed for the operation. DMSO or alcohols, which have fluidic qualities and can be difficult for many liquid dispensers, are frequently used to hold test chemicals. Speed is crucial in a relatively tiny volume. Variable assay results can result from the chemicals' evaporation while they are sitting in the plates. Additionally, a lot of assays have deadlines. The time at which the chemicals were added will vary depending on the assay, particularly those that use enzymes or evaluate equilibrium binding. A crucial prerequisite for HTS, and particularly uHTS, is the rapid transfer of chemicals to the test plates. Plates that can accommodate assay quantities in this range are also necessary for miniature screens that operate in the sub microliter volume range.[6,7]

The assay is directly impacted by the well's depth and shape. Potential evaporation can be reduced using deep wells. Sloped walls allow for accurate fluid accommodation in the well's center, minimizing the possibility of air bubble formation. Alcohols and volatile organics have a propensity

to climb the well walls when dispensed into small volume, high surface area wells with low surface tension. Wicking is a phenomenon that is made worse at corners of wells. Some companies have established "well-less" plates, which are glass plates coated with a highly hydrophobic matrix patterned with 1536 hydrophilic zones, to avoid these issues. In the hydrophilic zones, aqueous reagents position themselves on their own. In the hydrophilic zones, aqueous reagents position themselves on their own. The result of sandwiching two plates together is a 1536-well assay plate.

Parallel to this, the detection techniques that must adapt to greater sensitivity and durability permit simple and dependable downsizing. The assays must be carried out at a volume of one or two microliters without losing the response.[4]

4. Detection Methods Used in HTS

The two assay readouts for HTS that are most frequently employed are fluorescence and bioluminescence. Fluorescence signals, which are typically strong and cover a wide range, are particularly well suited for HTS. For HTS assays, a number of fluorescent detection techniques have been developed, including time-resolved fluorescence, which increases sensitivity by removing interference from library molecules and other assay components. These techniques include direct fluorescence measurement, fluorescence polarization, which enables the direct detection of changes in the rotational properties of the probe, fluorescence resonance energy transfer (FRET), which measures the transfer of energy between two fluorophores, and fluorescence resonance energy transfer. An illustration of a FRET-based assay is the b-lactamase gene reporter assay.[8,9]

5. Source of Funding

None.

6. Conflict of Interest

None.

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Cite this article: Kansagra JJ, Raval KY, Ganatra TH. A brief review of high throughput screening in drug discovery process. *Curr Trends Pharm Pharm Chem* 2022;4(3):120-122.