Cassette Dosing in Drug Discovery: Advantages, Limitations, and Its Role in High-Throughput Pharmacokinetic Screening

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Abstract:
In early drug development, cassette dosing, also known as N-in-one dosing, has become more popular as a high-throughput pharmacokinetic screening technique. This method lets you quickly check the features related to absorption, distribution, metabolism, and excretion (ADME) by simultaneously giving several drug candidates to a single animal or a group of animals. Its main benefits are that it can efficiently screen vast chemical libraries, saving time and money and using fewer animals in preclinical trials. During the lead optimization stage, cassette dosing is beneficial when several analogs can be assessed to find viable options. It also facilitates biopharmaceutical classification and early toxicity assessments, which promote a more efficient medication development process. Despite its benefits, the method faces challenges, especially regarding potential compound interactions that may complicate data interpretation. Validating results thus requires careful study design and, when needed, additional single-compound trials. When paired with cutting-edge analytical methods such as LC-MS/MS, cassette dosing provides accurate, dependable, and reasonably priced pharmacokinetic screening. Cascade dosing is still a helpful approach in contemporary drug discovery despite several drawbacks. It facilitates the identification of promising drug candidates more quickly and helps pharmaceutical researchers make better-informed decisions at the beginning of the development process.

Keywords: Cassette Dosing, Pharmacokinetics, ADME, Drug Discovery, High-Throughput Screening.

Introduction

Drug discovery is one of the intensive processes to identify and develop a novel therapeutic agent with high efficacy and lesser toxicity. Drug discovery involves the pharmacokinetic parameters of a drug or target molecule to determine its safety efficacy and safe dosing regimen. These parameters define the journey of a molecule or drug from lab to market [1]. So, these parameters must be scrutinized thoroughly using advanced techniques such as high-throughput methodologies. Old school methods are necessary to define preliminary data, viz., testing each in an animal model is time-consuming, costly, and resource-intensive. One powerful tool or method will be utilized to simplify this process, known as cassette dosing or N-in-one dosing [2].
Cassette dosing is a process where multiple compounds are simultaneously used in the same group of animals. This method is faster, cost-effective, and reduces the usage of animals. Cassette dosing involves administering a cocktail of drug candidates (5-10 compounds) in a single dose to a group of animals. By analyzing the serum and plasma from this group of animals, ADME parameters can be estimated. Later, various spectroscopic methods were used to narrow down the measurement and differentiation of each compound within the mixture [3].

This technique has gained widespread popularity in preliminary drug discovery because of its ability to optimize time and resources while maintaining the accuracy required for pharmacokinetic profiling. The high throughput nature of cassette dosing is highly preferred when screening large compound libraries to identify those with favorable pharmacokinetic properties. This method helps identify compounds that are rapidly eliminated and identification of promising leads, which fastens the drug discovery process. However, cassette dosing offers not only advantages but also a lot of challenges [4].

The interaction of compounds in the cocktail mixture may affect PK parameters and protein binding differences. These reactions and interactions lead to accurate results and more accurate data. Moreover, the effective cassette dosing is highly dependent on the sensitivity of the bioanalytical technique used. Despite these challenges, cassette is a valuable tool in drug discovery, especially for high-throughput PK screening [5]. This review provides comprehensive knowledge about the specific advantages and limitations of cassette dosing along with its role in drug discovery efforts.

Cassette Dosing: An Overview

Cassette dosing is a scientific streamlined approach in PK studies designed to simultaneously evaluate ADME parameters of multiple drug molecules. In this method, a cocktail of several drug molecules, typically 5 to 10, are administered to the same group of animals. After dosing, bioanalytical testing is performed to test and determine the PK parameters of individual compounds in the mixture. This method is significant as there is a shift from the traditional single-compound dosing method to multiple compounds in one dosing, which requires a separate study for each compound, a more significant number of animals procurement, and time and resources Figure 1[3].

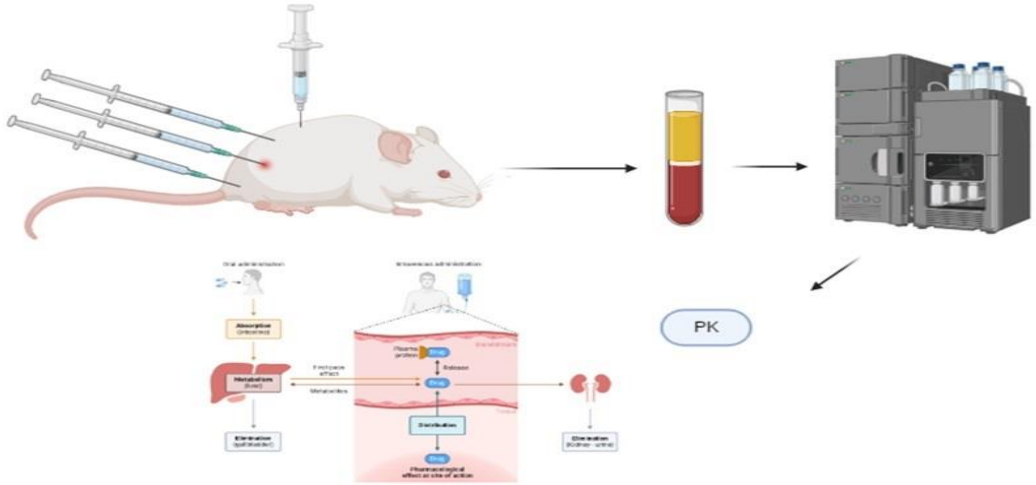

Figure 1: Process of Cassette Dosing

The Methodology

The primary goal of this methodology is to speed up the drug discovery process without compromising the safety and efficacy of drug candidates.

1. Compound Selection and Preparation

Compound selection was based on structural similarity to reduce analytical interference with other compounds. Metabolic profile is the second important criterion for avoiding interaction with other compounds in the same pathway. Finally, the physicochemical properties of compounds in the cassette should have similar solubility and stable profiles to have a homogenous formulation for accurate dosing. After selection,

compounds are dissolved in the appropriate vehicles to create a uniform mixture to administer to animals. The concentration of each compound should be carefully adjusted to ensure optimal PK analysis [6].

2. Administration of the Compound Mixture

After cassette dosing is ready, the dose is administered to animals by a traditional method, viz., the oral route of administration, which allows the study of the first-pass metabolism of compounds—and IV administration, where the focus of the study should be on bioavailability. The dose of every drug molecule in the cassette should be standardized according to the pre-established PK goals. Doses are chosen to minimize the drug-drug interactions between drug substances while permitting measurable quantities in plasma. The compound mixture is administered to the same group of animals to guarantee consistency [7].

3. Sample Collection and Time Points

Biological samples are collected at predefined time intervals. These time points should be selected based on PK profiles of interest, depending on the half-lives of the compounds. Usually, early time points are estimated in the first hour to estimate drug absorption and peak plasma concentration. Mid to late time points are used to calculate the distribution and metabolism of compounds. The terminal phase is to determine the elimination half-life and clearance of the compounds. Multiple samples from each animal are advised to determine the comprehensive profile of the behavior of each compound in the body. Blood volume depends on the type of species used in the study [8].

4. Bioanalytical Testing (LC-MS/MS)

Bioanalytical testing methods such as Liquid chromatography-tandem mass spectrometry (LC-MS/MS) are the golden standard for this type of analysis. LC-MS separates compounds based on physicochemical properties, such as size, charge, or polarity. Metabolite identification can also be performed using LC-MS/MS. This chromatographic separation ensures that each compound in the mixture can be analyzed individually. Another method is Mass spectrometry (MS/MS), which separates each compound from ionized and detected based on its mass-to-charge ratio. This bioanalytical method must be validated to ensure that it can accurately measure each compound in the presence of others without interference [9].

5. Data Analysis and Pharmacokinetic Profiling

PK parameters for each compound include Cmax (maximum plasma concentration, Tmax (time to reach Cmax), AUC (area under the curve, t1/2 (half-life), CL (clearance), and Vd (volume of distribution). These parameters allow us to study the absorption, distribution, metabolism, and excretion (ADME) of each compound in the cassette. The data can then be compared to identify compounds with the most favorable pharmacokinetic properties for further development.

Traditional Single-Compound Dosing vs. Cassette Dosing

Before, PK studies involved administering a single compound to a test animal, followed by extensive sampling and analysis. Each drug candidate required a separate set of animals, dedicated resources, and additional time. When large compound libraries needed to be screened, this method became extremely resource-intensive, often requiring hundreds of animals and prolonged study duration.

Cassette dosing, in contrast, allows multiple compounds to be evaluated simultaneously in a single animal or group of animals. This reduces the number of animals required for pharmacokinetic studies and shortens the time needed to generate critical PK data. By condensing what would typically be several individual studies into one, cassette dosing can provide a faster and more cost-effective way to assess the ADME properties of multiple compounds at once [10].

Advantages of Cassette Dosing

Cassette dosing offers a range of advantages that make it a powerful tool in early drug discovery and pharmacokinetic screening. Simultaneously, testing multiple compounds significantly accelerates evaluating

drug candidates, saving time and cost, reducing animal usage, and improving decision-making efficiency.

1. High-Throughput Screening

The primary benefit of cassette dosing is its ability to speed up the PK screening. This high throughput approach decreases the time to assess the PK of multiple compounds from weeks or months to days. This is one of the most efficient methods to run parallel analyses, where data from various compounds can be acquired simultaneously, facilitating quicker decision-making. This acceleration is especially critical in the lead optimization phase, where drug developers must screen large numbers of analogs to refine a lead compound's pharmacokinetic profile.

2. Cost-Effective

Cassette dosing is quite economical in terms of managing resources and direct costs. Each molecule needs its own animal models, reagents, dose studies, and bioanalytical testing for traditional single-compound dosage. Cassette dosing results in significant cost savings by lowering the number of tests and animals required:

- **Less research on animals:** By doing several pharmacokinetic experiments concurrently on a single animal group, fewer animals are utilized overall, which lowers the expense of housing and animal care.
- **Less reagent and material consumption:** By using the same set of reagents, sample-collecting materials, and bioanalytical evaluations for several substances at once, these resources are consumed as little as possible.
- **Efficient resource allocation**: Cassette dosing enables researchers to concentrate on more essential tasks and eliminate unnecessary procedures by condensing PK evaluations into fewer tests.

Pharmaceutical organizations that need to balance the high expenses of drug development with the need to screen large compound libraries quickly will find it an attractive choice due to these cost benefits.

3. Early Decision-Making

The capacity of cassette dosing to speed up early decision-making in the drug development pipeline is another significant benefit. Researchers can quickly identify weak candidates with potentially damaging ADME features, such as poor absorption, rapid metabolism, or limited bioavailability, by screening several drugs for their pharmacokinetic qualities.

- **Deprioritizing bad candidates:** By addressing compounds with inadequate pharmacokinetics early on, time and resources can be saved that would otherwise be wasted on compounds unlikely to be successful in later stages of development.
- **Concentrating on promising leads:** Cascade dosing enables researchers to focus on honing and optimizing the most promising candidates, expediting their advancement through the development pipeline via early identification of favorable pharmacokinetic profiles.

4. Reduction of Animal Usage

Cassette dosing complies with ethical guidelines meant to reduce the number of animals used in research. Researchers are encouraged by the 3Rs principle (Replacement, Reduction, and Refinement) to limit harm by replacing animal models when practical, reducing the number of animals used, and refining study methods. Cassette dosing helps achieve these objectives by lowering the quantity of animals needed for pharmacokinetic research:

- **The decline in the number of animals:** Cascade dosing eliminates the requirement for separate animals to test different chemicals simultaneously in a single group of animals. This decrease can effectively address ethical issues regarding animal testing by reducing the number of animals utilized in drug discovery.
- **Improved study designs:** Because cassette dosing is so effective, fewer animals are exposed to experimental chemicals, and those that are utilized undergo fewer procedures overall, which improves their well-being and reduces their suffering. This decrease in the use of animals not only enhances

preclinical research's ethical aspects but also aids businesses in complying with laws that support more sustainable and humane scientific methods.

Using cassette dosing for pharmacokinetic screening and drug discovery has several substantial benefits. It offers a more effective and moral method of assessing medication candidates by sharply raising throughput, cutting expenses, facilitating early decision-making, and limiting the usage of animals. The technique is essential in contemporary pharmaceutical research because it enables quicker, more economical, and more accurate pharmacokinetic assessments. This is especially true in the early phases of drug development when high-throughput and resource optimization are crucial [11].

Limitations of Cassette Dosing

1. **Compound Interaction: The major** drawback is the potential for drug-drug interactions. The presence of multiple compounds in the cassette dosing may alter the interaction of lead compounds due to competition for metabolic enzymes, transporters, or binding proteins. This can lead to inaccurate or misleading data, complicating the interpretation of results [12].
2. **Quantitative Sensitivity:** To quantify PK parameters of each compound in a mixture may be limited due to the sensitivity of analytical methods adopted in the study. Analytical instruments such as liquid chromatography-mass spectrometry (LC-MS) must be sufficiently robust to detect and differentiate each compound in the presence of others.
3. **Metabolite Overlap:** Identifying and quantifying metabolites can also be challenging in cassette dosing studies, as overlapping metabolites from different compounds might confound the analysis. This makes it difficult to assess the metabolic profile of each compound accurately.
4. **Limited Dosing Range:** The dosing of each compound is constrained by the requirement to maintain similar concentrations in the mixture. If the pharmacokinetics of one compound in the cassette are vastly different from the others, adjustments may be needed, which could skew results.
5. **Non-Linear Pharmacokinetics:** In cases where compounds exhibit non-linear pharmacokinetics, the cassette approach might not accurately reflect their actual ADME properties, necessitating additional single-compound studies to resolve any ambiguities [13].

Role in High-Throughput Pharmacokinetic Screening

In high-throughput pharmacokinetic (PK) screening, cassette dosing is essential since it is an early filter during drug discovery. Large chemical libraries are frequently evaluated by researchers in the early phases of drug development to find compounds with advantageous pharmacokinetic qualities, such as excellent absorption, distribution, metabolism, and excretion (ADME) characteristics. With cassette dosing, several drugs can be tested concurrently in a single research, facilitating the quicker identification of weak candidates and concentrating attention on those with more promising characteristics.

Pharmaceutical companies often use cassette dosing with advanced analytical technologies such as LC-MS/MS, which allows for precise and high-throughput quantification of drug concentrations in biological samples. This combination of high-throughput dosing and advanced detection methods ensures that the pharmacokinetic profiling is efficient and reliable, provided compound interactions are appropriately managed.

However, one challenge of cassette dosing is the potential for compound interactions, where one drug may influence the metabolism or absorption of another. Compounds are carefully selected for cassette dosing to mitigate this risk based on their metabolic pathways to avoid interaction. Additionally, follow-up studies using single-compound dosing can be conducted to validate the results of cassette dosing and ensure that the pharmacokinetic data is not compromised by drug-drug interactions [14].

Applications in Drug Discovery

In the process of finding new drugs, cassette dosing has several significant uses that aid pharmaceutical researchers in streamlining and improving different phases of development. These uses, which include biopharmaceutical classification, toxicity testing, and lead optimization, demonstrate how adaptable this approach is for quickly and effectively assessing various therapeutic candidates.

1. Lead Optimization

The lead optimization stage of drug research is one of the primary uses for cassette dosing. To enhance lead compounds' pharmacokinetic and pharmacodynamic qualities, researchers modify their chemical structures during this phase. Here, cassette dosing is beneficial since it makes it possible to screen several analogs of a lead drug simultaneously. Researchers can rapidly assess pharmacokinetic variances such as absorption rates, bioavailability, metabolism, and excretion by evaluating various analogs in a single animal model or group. This facilitates the early removal of less desirable candidates by helping to identify the top-performing analogs based on their ADME characteristics. Lead optimization decisions may be made more quickly thanks to the effectiveness of cassette dosing, which makes it possible to identify candidates for clinical advancement. This program ensures that labor-intensive, individual dose trials are sufficient for optimization.

2. Toxicity Testing

Cassette dosing can be used for preliminary toxicity assessment and pharmacokinetic profiling. Drug development requires early detection of potential toxicities or off-target effects since hazardous substances might have significant side effects or fail at a later stage of development, leading to expensive delays. Researchers can evaluate the safety characteristics of several drugs at once using cassette dosing. Researchers can screen for toxicity in the same study by simultaneously administering different chemicals. This allows them to identify potential side effects early on, such as aberrant biochemical markers, organ damage, or other physiological alterations. Time and money can be saved if a specific cassette chemical shows indications of toxicity early in the process. Furthermore, by avoiding the requirement for independent toxicity testing for every drug, this method conserves animal and material resources.

Although cassette dosing is mainly utilized for PK screening, its application in preliminary toxicity testing offers a double advantage by bringing safety and pharmacokinetic issues to light early, which is crucial in deciding if a chemical is worth developing further.

3. Biopharmaceutical Classification

The biopharmaceutical classification process, which involves classifying drug candidates according to their ADME properties, is another significant application of cassette dosing. Understanding the difficulties in drug administration and the specifications for each compound's formulation depends on this classification. Cascade dosing helps classify medications according to their permeability, solubility, bioavailability, and other pharmacokinetic characteristics by evaluating several substances simultaneously.

Based on a compound's permeability and solubility, the Biopharmaceutical Classification System (BCS) divides substances into four groups, influencing the compound's formulation and oral delivery potential. This classification is made more accessible by cassette dosing, which allows researchers to evaluate several chemicals at once and quickly determine which category each one falls into.

For example, substances with low solubility or permeability could need specific formulations like lipid-based systems or nanoparticles to improve absorption and bioavailability. Researchers can better prepare for the formulation development and drug delivery strategies necessary for effective clinical translation by recognizing these problems early on using cassette dosing. Cassette dosage can help with biopharmaceutical classification, interspecies changes in ADME features, and how drugs behave. This can help researchers anticipate human behavior based on evidence from animal models. This application takes care of potential formulation or delivery issues as early in the medication development process as possible. Table 1 contains previously done studies using this cassette dosing.

Title	Application	Key Focus	References
Cassette Dosing for Optimization of Toxico (pharmacy) Kinetic Investigations	Toxico/pharmacokinetic optimization	Optimization of PK/TK studies	[16]
The Application of Cassette Dosing for Pharmacokinetic Screening in Small-Molecule Cancer Drug Discovery	Pharmacokinetic screening in cancer drug discovery	PK screening of small-molecule cancer drugs	[17]
Murine Pharmacokinetic Studies	Murine pharmacokinetic studies	PK studies using cassette dosing in murine models	[18]
Optimizing DMPK Properties: Experiences from a Big Pharma DMPK Department	DMPK property optimization	Experience in a large pharmaceutical setting	[19]

Table 1: Studies which are conducted using cassette dosing

Conclusion

In high-throughput pharmacokinetic screening, cassette dosing has emerged as a crucial strategy that provides substantial benefits throughout the initial phases of drug discovery. Because of its capacity to assess the ADME characteristics of several compounds at once, researchers can quickly determine whether or not a therapeutic candidate is viable, ultimately saving time and money. This approach facilitates early toxicity assessments, biopharmaceutical classification, and lead optimization while reducing animal usage. Because of these uses, cassette dosing helps expedite drug discovery and accelerate the identification and development of promising candidates.

Cassette dosing has many drawbacks, though. Compound interactions can complicate the interpretation of results; therefore, proper study design is necessary to limit such effects. Retesting certain substances separately to validate pharmacokinetic findings can also be required. Despite these difficulties, cassette dosing improves the effectiveness of drug development pipelines when utilized effectively and in conjunction with single-compound trials, enabling pharmaceutical companies to bring safer and more effective medications to market faster.

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