DRUG DISCOVERY INTERFACE

Rapid Throughput Screening of Apparent K_{SP} Values for Weakly Basic Drugs Using 96-Well Format

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ABSTRACT: A rapid-throughput screening assay was developed to estimate the salt solubility parameter, K_{SP}, with a minimal quantity of drug. This assay allows for early evaluation of salt limited solubility with a large number of counter-ions and biologically promising drug leads. Drugs dissolved (typically 10 mM) in DMSO are robotically distributed to a 96-well plate. DMSO is evaporated, and drugs are equilibrated with various acids at different concentrations (typically <1 M) to yield final total drug concentrations around 2.5 mM. The plate is checked for precipitation. Filtrates from only those precipitated wells were subjected to rapid gradient HPLC analysis. An iterative procedure is employed to calculate all species concentrations based on mass and charge balance equations. The apparent K_{SP} values assuming 1:1 stoichiometry are determined from counter-ion and ionized drug activities. A correlation coefficient >0.975 for eight drugs totaling 16 salts is reported. Intra-day and inter-day reproducibility was <10%. Conventional apparent K_{SP} measurements were translated to 96-well format for increased throughput and minimal drug consumption (typically 10 mg) to evaluate at least eight different counter-ions. Although the current protocol estimates K_{SP} from 10^{-3} to 10^{-7} M, the dynamic range of the assay could be expanded by adjusting drug and counter-ion concentrations. © 2007 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 97:2080–2090, 2008

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INTRODUCTION

It takes over a decade on average for an experimental drug to travel from lab to medicine chest. Only five in 5000 compounds that enter preclinical testing make it to human testing and only one of these five ends up being approved by the FDA. It costs millions to develop each new drug.[1] Rapid screening assays for bioactivity and toxicity employed at the early stages of drug development have been shown to reduce these development costs.[1,2] Application of similar screening approaches to physiochemical property evaluations can potentially improve efficiency of drug lead identification, and increase probability of final success.

Drug leads are usually selected and designed around specific interaction with a target molecule leading to bioactivity. However, it is often the physiochemical parameters that influence biological

Abbreviations: K_{SP}, solubility product; API, active pharmaceutical ingredients.

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behavior, and possible dosage form strategies to provide adequate levels of drug at the target. Unfortunately, the evaluation of physicochemical properties early enough in development to impact lead selection is often limited by drug availability, and time constraints. Thus, physicochemical screening assays are playing an increasingly important role in early stage pharmaceutical discovery, and development.[1–4]

Solubility is a critical physicochemical factor that can significantly influence biological behavior, ranging from dissolution of a dosage form, and resulting oral absorption of the drug to safety concerns for intravenous administration.[5] In the case of oral drug formulations, both the dissolution, and absorption rates of a drug are directly proportional to the solubility of the drug, hence very low solubility can result in poor, and variable release from a dosage form, coupled with inadequate absorption. Precipitation of drug from solubilized formulation has always been a threat for parenteral drugs because of the high volume of dosage form delivered. Introduction of an ionizable group can significantly enhance drug solubility and enables isolation of solid crystalline salts. The solubility limit of the salt is now controlled by the counter-ion and its solubility constant, K_{SP}.

Knowledge of apparent K_{SP} values early in lead development can aid salt selection, as well as interpreting biological data generated using such salts.[6] Theoretically, free-base compounds may reach very high solubility as the pH of the dissolving media decreases. In practice, however, solubility is limited by the presence of counter-ions in the acidic medium, resulting in a solubility plateau as the salt form approaches its apparent K_{SP}. Early in the discovery process, *in situ* salt formation is often used to obtain highly concentrated drug solutions. Consequently, it is important to understand the limits imposed by various counter-ions on maximum obtainable solubility, thus influencing the selection of the acid for pH adjustment. Similarly, solubility, log P, and pK_a, along with K_{SP} values, can all influence salt selection for development.[7] Finally, the potential for weak bases to interact with endogenous counter-ions (chloride, citrate, etc.) resulting in drug precipitation is a concern during biological evaluations.

K_{SP} values of an ionizable drug in various counter-ions are key parameters in making salt selection decisions. We developed a 96-well based screen that can provide apparent K_{SP} values rapidly for many compounds with minimal amount of material requirement.

MATERIALS AND METHODS

Materials and Equipment

Terfenadine (98% pure), triameterene (99% pure), phenazopyridine (95% pure), papaverine hydrochloride (98% pure) were purchased from Sigma (St. Louis, MO), and used without further purification. PHA1, PHA2, PHA3, and PHA4 are weakly basic compounds synthesized by Pharmacia Medicinal Chemistry, listed in Table 1. Acetic acid, citric acid, hydrochloric acid, methanesulfonic acid, sulfuric acid, and phosphoric acid were purchased from Mallinckrodt (Paris, KY). Succinic acid and formic acid were purchased from Aldrich (Milwaukee, WI).

A Biomek 2000 laboratory automation workstation (Beckman Instruments, Inc., Fullerton, CA) was used in liquid dispensing. Drug concentration assays were performed by HPLC using an Agilent model 1100 (Santa Clara, CA) equipped with binary pump (G1312A), autosampler (G1313A), column compartment (G1316A), and photodiode array detector (G1315A). A liquid handler (Gilson, model 215, Middleton, WI) was used to inject samples directly from a 96-well plate. DMSO solvent was evaporated by centrifugal evaporation (Genevac Technologies, model HT-4, Valley Cottage, NY). Titer plate shaker (Lab-line Instruments, Barnstead International, Dubuque, IA) accompanied by coated parylene stir bars (V&P Scientific, San Diego, CA) were used in equilibration. Millipore multiscreen 96-well filter plates (0.4 μm, model MAHVN4550) were used for filtration. P250, and P20 pipette tips from Beckman Instruments, Inc., and/or Molecular Bioproducts, Inc. (San Diego, CA).

A Gilson injector was used in this study for autosampling from 96-well plates. It exhibited a dispensing variability of 4–8%, which was deemed acceptable for our rapid throughput-screening assay.

Drug Plate Preparation

Drugs were dissolved in DMSO at a high concentration (typically 10 mM). Drug stocks were robotically (Biomek 2000) distributed into 96-well flat bottom polystyrene plates (Costar 3595, Corning, NY). DMSO was then evaporated (Genevac, typical run time is ~2 h) and the drug