Journal of Pharmaceutical and Biomedical Analysis 122 (2016) 126–140

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

A high throughput solubility assay for drug discovery using microscale shake-flask and rapid UHPLC–UV–CLND quantification

Baiwei Lin*, Joseph H. Pease

Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080-4990, USA

ARTICLE INFO

Article history:
Received 3 November 2015
Received in revised form 8 January 2016
Accepted 9 January 2016
Available online 13 January 2016

Keywords:
Rapid quantitative analysis
CLND—chemiluminescence nitrogen detector
HPLC hyphenated technique
Shake-flask solubility assay
Physicochemical properties

ABSTRACT

The rapid determination of key physical properties of lead compounds is essential to the drug discovery process. Solubility is one of the most important properties since good solubility is needed not only for obtaining reliable *in vitro* and *in vivo* assay results in early discovery but also to ensure sufficient concentration of the drug being in circulation to get the desired therapeutic exposure at the target of interest. In order for medicinal chemists to tune solubility of lead compounds, a rapid assay is needed to provide solubility data that is accurate and predictive so that it can be reliably used for designing the next generation of compounds with improved properties. To ensure speed and data quality, we developed a high throughput solubility assay that utilizes a single calibration UHPLC–UV–CLND method and a 24 h shake-flask format for rapid quantification. A set of 46 model compounds was used to demonstrate that the method is accurate, reproducible and predictive. Here we present development of the assay, including evaluation of quantification method, filtration membranes, equilibrium times, DMSO concentrations, and buffer conditions. A comparison of thermodynamic solubility results to our high throughput 24 h shake-flask solubility assay results is also discussed.

1. Introduction

Solubility is one of the most essential properties among all important physicochemical properties in drug discovery and development, where solubility plays crucial roles affecting *in-vitro* and *in-vivo* assay results [1–3]. Drug candidates need sufficient solubility for assessment in biochemical and cellular screening assays, microsomal and plasma stability assays, and physical property assays of lipophilicity and permeability. Furthermore, the oral bioavailability of a drug is highly affected by solubility [4]. As a drug discovery project progresses, the lead compounds are investigated *in-vivo* to gain a good understanding of its pharmacokinetics, pharmacodynamics, absorption, and toxicity. Throughout these tests, solubility is one parameter used to guide formulation design and suitable assay conditions. Poorly soluble compounds often have incomplete and variable oral adsorption and potentially have higher risk of failure in drug development due to variability in clinical response [5,6].

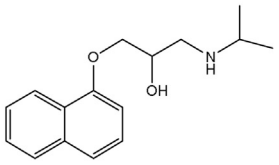
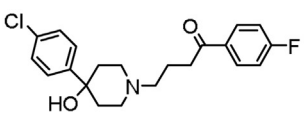
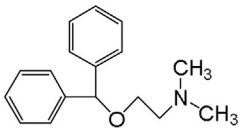
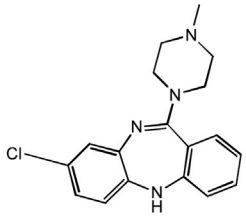
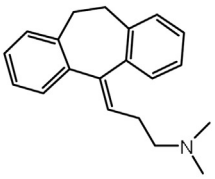
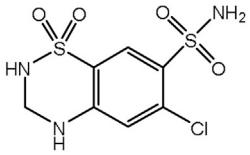
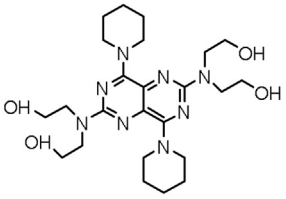
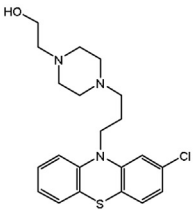
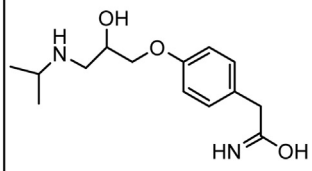
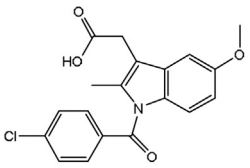
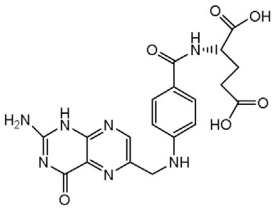
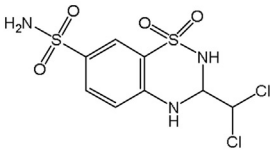
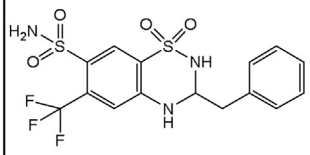
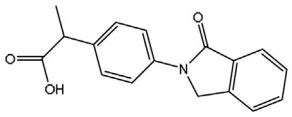
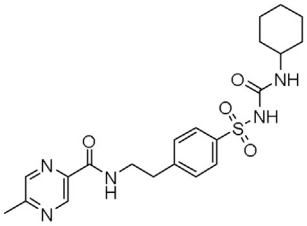
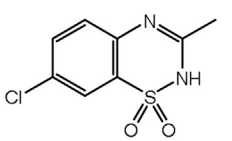
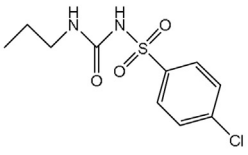
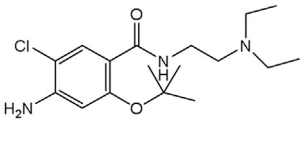
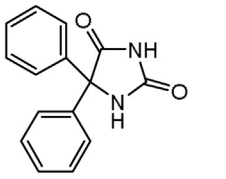
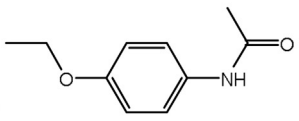
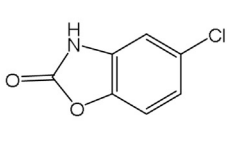
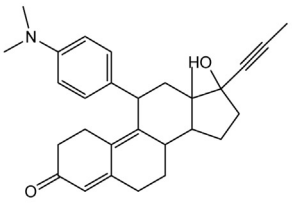
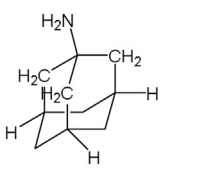
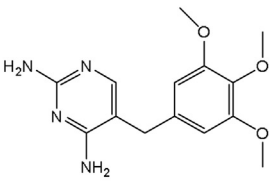
Solubility measurements are typically done using either solid material or from a compound DMSO stock solution. Thermodynamic solubility is measured from solid material and is the preferred solubility assessment during drug development, where it is used by preformulation groups for determining dosage composition and estimating ultimate bioavailability. Thermodynamic solubility is defined as the maximum quantity of a substance in a saturated solution where the solution and solid phases are in equilibrium. Unfortunately, this process is low throughput and requires a significant amount of material and thus is not well suited for drug discovery. Conversely, kinetic solubility is measured from a compound stock solution typically prepared in DMSO. Kinetic solubility assays are high throughput and require minimal amounts of material, usually a few micro grams, but do have limitations relative to thermodynamic solubility assays.

Many drug discovery *in-vitro* assays such as biological screening and pharmacokinetic assays are controlled under kinetic solubility conditions. Kinetic solubility is the compound concentration in solution shortly after the addition of DMSO stock solution into aqueous buffer, normally measured in a few minutes to two hours after addition [5,7]. This short-time solubility can be non-reproducible and have noticeable deviations from equilibrium solubility values owing to that kinetic solubility values are associated with metastable conditions. This unreliable kinetic solubility data may cause potential errors in interpretation of pharmacokinetic and *in-vitro* potency results [8]. Kinetic solubility using long

* Corresponding author. Fax: +1 650 225 2061.
E-mail address: Lin.baiwei@gene.com (B. Lin).

http://dx.doi.org/10.1016/j.jpba.2016.01.022
0731-7085/

Table 1
24 commercial compounds used for CLND response testing.

			
Propranolol	Haloperidol	Diphenhydramine	Clozapine
			
Amitriptyline	Hydrochlorothiazide	Dipyridamole	Perphenazine
			
Atenolol	Indometacin	Folic acid	Trichlormethiazide
			
Bendroflumethiazide	Indoprofen	Glipizide	Diazoxide
			
Chlorpropamide	Metoclopramide	Phenytoin	Phenacetin
			
Chlorzoxazone	Mifepristone	Amantadine	Trimethoprim