Drug Development and Industrial Pharmacy, 27(1), 107–109 (2001)

COMMUNICATION

Determination of the Aqueous Solubility of Drugs Using a Convenient 96-Well Plate-Based Assay

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KEY WORDS: Solubility; Preformulation; High throughput screening; 96-well plate.

INTRODUCTION

The solubility of a drug in an aqueous medium is a key parameter in preformulation studies (1). Usually, the concentration at saturation is determined by adding an excess of drug substance into the medium, stirring the suspension during a defined period of time, filtering or centrifuging the suspension, and measuring the amount of drug dissolved (1). Generally, when performed in test tubes or vials, such a procedure is time-consuming, requires a large amount of compound, and cannot accommodate a large number of drugs. In this article, a novel, 96-well plate-based assay to measure drug solubility is presented. The drugs are introduced into the wells of a microtiter plate, and water is subsequently added to each well. Then, after stirring, the suspensions are filtered under vacuum in all wells at once and drug concentration is measured by UV spectrophotometry. The method is accurate, reproducible, and well suited for drug screening.

MATERIAL AND METHODS

Sample Preparation

The following drugs were used as models and assayed for solubility in pure water: niflumic acid (Hexachimie, Tonneins, France), acetaminophen (Rhône-Poulenc, Courbevoie, France), ketoprofen, aspirin, and caffeine (Sigma, St Quentin Fallavier, France). Two different protocols to introduce the sample in the 96-well plate were tested. In protocol A, approximately 3 mg of drug substance per well was directly weighted into a 96-well plate (Multiscreen®, MAHV N45 Millipore). Alternatively (protocol B), the drug substance was first dissolved in acetonitrile (ACN) at a concentration of 10 mg/mL. Then 0.3 mL of the solution was introduced in each well. Acetonitrile was subsequently evaporated under a nitrogen flux for 4 hr. In both protocols, 4 wells were used for each drug. Control wells without any drug were also prepared. Pure water (0.2 mL) and a 2-mm glass bead were

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then added to each well. The plate was covered with an aluminum foil to avoid light-induced degradation. The plate was then placed on a planar shaker (Denley, MedTec) for 24 hr at room temperature ($22 \pm 2^{\circ}C$). After 24 hr, the suspensions contained in the wells were filtered under vacuum all at once, using a plate filtration unit (MAVM 09601, Millipore). The filtrates were recovered in a new plate.

Determination of Drug Concentration

Each filtrate (0.075 mL) was transferred using a multichannel pipette to a quartz 96-well plate (Helma, Switzerland), and 0.025 mL of acetonitrile was added to each individual well. Standards with a known concentration were also prepared in the same vehicle (water/ACN 75/25 v/v) as follows: 10 mg of each drug was weighed in a 10-mL glass vial (Packard), and 1.25 mL of ACN was added to solubilize the drug (solution I). If the drug was totally solubilized, 0.1 mL was diluted with ACN and pure water to obtain 2 mg/mL and 0.2 mg/mL solutions in water/ACN 75/25 v/v. Each solution (0.2 mL) was then introduced in 2 wells of the plate. If the drug cannot be solubilized in pure ACN in solution I, it is advisable either to dissolve the drug in a mixture of ACN and water or to use a lower drug concentration.

Finally, the plate containing the test samples and the standards was placed in a multiwavelength UV spectrophotometer (Spectramax 190 Optics, Molecular Devices). The optical absorbance was measured between 200 and 400 nm against a blank (a mixture of 0.075 mL of water and 0.025 mL of acetonitrile). The optimal wavelength was selected according to the absorbance profile of each test compound. Depending on the absorbance value of the sample at this wavelength, the standard having the absorbance the closest from the test sample was choosen (either 2 mg/mL or 0.2 mg/mL). Then, the drug concentration in the test sample was calculated using the concentration and the absorbance of the standard, according to Beer's law.

RESULTS

Repeatability and Reproducibility

Niflumic acid was selected to assess the repeatability and the reproducibility of the procedure. Table 1 indicates the SD for each experiment (4 wells) as well as for a series of four or five independent experiments. The variation coefficient between wells in a single experiment was less than 11% and 20% for protocols A and B, respectively. Overall, the reproducibility between experiments was considered satisfactory because results agreed within 30 to 40% for four or five independent experiments.

Table 1.

Solubility at Saturation in mg/mL of Niflumic Acid in Pure Water at Room Temperature Using the 96-Well Plate-Based Assay

Experiment[a]	Protocol A[b]	Protocol B[b]
1	0.022 ± 0.001	0.028 ± 0.001
2	0.039 ± 0.003	0.043 ± 0.008
3	0.044 ± 0.005	0.026 ± 0.004
4	0.023 ± 0.002	0.051 ± 0.010
5	0.034 ± 0.002	0.033 ± 0.006
1 to 5 (20 wells)	0.032 ± 0.009	0.037 ± 0.015

[a] Results are the mean $\pm$ SD of 4 wells for each experiment.
[b] Please refer to "Material and Methods."

Accuracy

Results obtained using the 96-well plate-based assay were compared with those reported in the literature for six different analgesic drugs: niflumic acid, ibuprofen, ketoprofen, aspirin, acetaminophen, and caffeine (Table 2). When using protocol A, differences between experimental data and values found in the literature ranged from 6 to 17%, depending on the drug. However, solubilizing the drug in acetonitrile before the assay induced larger differences with reference solubility values. This was particularly the case for caffeine. Indeed, on acetonitrile evaporation, caffeine crystals were present outside the wells, leading to a loss of material. This observation can certainly be explained by the efflorescent nature of caffeine. Nevertheless, protocol B led to results similar to those

Table 2.

Solubility at Saturation in mg/mL of Different Drugs in Pure Water at Room Temperature Using the 96-Well Plate-Based Assay[a]

	Reference	Literature Value	Protocol A[b]	Protocol B[b]
Niflumic acid	2	0.035	0.032	0.037
Ibuprofen	3	0.070	0.082	0.087
Ketoprofen	3	0.180	0.189	0.229
Aspirin	4	3.30	2.73	2.86
Acetaminophen	5	12.0	12.8	11.4
Caffeine	6	19.0	16.1	2.90

[a] Results are the mean of 4 to 8 wells.
[b] Please refer to "Material and Method."

reported in the literature for the other molecules under investigation (Table 2).

DISCUSSION

Two protocols to measure the aqueous solubility under a 96-well plate format were investigated. When the drug powder was weighted directly into the wells (protocol A), solubility values were quite reproducible (variation coefficient below 28% for five experiments) and not significantly different from those reported in the literature. However, this procedure was rather cumbersome. To overcome the tedious weighting of the substance, another protocol was developed. The drug was first solubilized in acetonitrile, and an aliquot of the solution was pipeted into the well. Acetonitrile was subsequently evaporated before adding the water (protocol B). In that case, the results were more scattered than those using protocol A. In addition, this protocol is not suitable for efflorescent drugs, as seen with caffeine. However, for screening purposes, protocol B is easy to use and faster.

For both protocols, it is recommended to use at least 4 wells for each drug to test. Furthermore, it is advisable to add a reference compound (i.e., a compound for which the concentration at saturation is known) on each 96-well plate. If the measured concentration value of the reference compound is not significantly different from what is expected, the series of measurements for the plate can be validated. The novel method described in this article has a high throughput capacity to measure drug solubility. Approximately 15 drugs can be tested in a single 96-well plate. It is reproducible, accurate, and easy to use. It can also accommodate a large range of solubility (from approximately 0.003 to approximately 20 mg/mL in this study). Finally, the method is rather inexpensive. It uses a limited amount of drug, which is of particular interest when testing expensive, short-supplied new chemical entities.

It is believed that the method can be extended to other drugs than those described here, providing they absorb UV light and are not efflorescent (if protocol B is used). Indeed, the method described in this article may be of interest for preformulation scientists who are dealing with a large number of chemical entities for which little information is available.

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