Development of an automated in-line microfiltration system coupled to an HPLC for the determination of solubility

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Abstract

An automated in-line microfiltration system coupled to an HPLC was developed for the determination of solubility. Filtrations and subsequent solubility determinations are performed using a standard Gilson dilutor and autosampler and dual Rheodyne® valves equipped with a filtration assembly and configured to an HPLC system. The solubility data obtained using the automated in-line microfiltration system are in good agreement with the results obtained using conventional manual preparation techniques. Automating this labor-intensive and often variable portion of the solubility determination provides a reliable means of improving both the consistency and quality of solubility data. © 2001 Elsevier Science B.V. All rights reserved.

1. Introduction

The solubility of a drug continues to be a physicochemical property that is routinely measured during the identification and selection of lead compounds. In general, solubility data are used to support our understanding of the in vivo behavior of a drug and direct the synthesis of compounds whose structural features enhance the solubility and corresponding activity of a base structure [1–3]. In addition, solubility data are often used to target suitable formulations and salt-forms of drug candidates and enhance the understanding of the thermodynamic relationship between the solid-state forms of a compound [4–6]. During the past decade, significant advances have been made towards developing models that predict aqueous solubility [7]. However, these methods do not adequately deal with ionizable molecules and the variety of matrices typically encountered (e.g. acidic, basic, or organic media). With these limitations, scientists continue to rely on traditional methods for determining solubility.

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Automated methods for measuring the solubility of drugs have been applied in drug discovery settings [8–10]. However, these methods focus on the determination of kinetic solubility and involve quantitation using a non-specific (i.e. turbidimetric) analysis. Thermodynamic or equilibrium solubility measurements continue to be routinely performed early in the development process. These measurements often require significant human involvement, which can be a constant source of variability [11]. Automating the determination of equilibrium solubility is expected to improve consistency and increase efficiency by off-loading sample preparation, processing, and analyses onto a robotic system. This paper focuses on comparisons of data obtained using automated and conventional manual processing.

2. Experimental

2.1. Chemicals, reagents, and materials

HPLC grade acetonitrile was purchased from EM Sciences (Gibbstown, NJ). Purified water was obtained from a Milli-Q water purification system (Millipore Corporation; Bedford, MA). Spectroscopic grade trifluoroacetic acid was obtained from Aldrich Chemical Company (Milwaukee, WI). Phosphate buffered saline was obtained from Fluke (Switzerland). Citric acid, boric acid, trisodium phosphate, and solutions of hydrochloric acid were obtained from Aldrich.

Commercially available compounds used to probe the performance of the instrument were sourced from either Sigma Chemical (St. Louis, MO) or Aldrich. The remaining compounds were synthesized by Glaxo Wellcome.

2.2. Preparation of samples for equilibrium solubility determinations

Samples of solid and solvent were prepared by weighing 1 mg of solid into a standard 2 ml autosampler vial. Each vial received 1 ml of the solvent of interest, a Teflon®-coated stirring bar, and a cap having a piercable septum. The vials were stirred continuously while equilibrating at room temperature for at least 7 days.

2.3. Manual filtration and solubility determination

Manual filtrations were performed by first aspirating the suspension of the compound and solvent into a glass airtight syringe. The syringe was then equipped with a non-sterile Millipore Millex® HV syringe filter unit having a diameter of 13 mm and a pore size of 0.45 μm. The first 750 μl of filtrate was dispensed to waste, and the remaining 250 μl was dispensed into an autosampler vial. The amount of compound dissolved was determined by HPLC using a Hewlett-Packard 1050 HPLC system equipped with a diode-array detector (Palo Alto, CA).

2.4. In-line filtration and solubility determination

Sample volumes were manipulated using a Gilson 232 autosampler equipped with a Gilson 401 dilutor (Gilson Inc., Middleton, WI). The system is equipped with a septum-piercing needle mounted to an arm that can move in the $x, y,$ and z dimensions. The autosampler possesses an injection port connected to two resident, two-position, six-port, Rheodyne® valves. These valves work in concert to filter the sample, sample a fixed volume of the filtrate, inject the filtrate onto an HPLC system, and direct the rinse solvent used to clean the system. Filtration is conducted using a 0.5 μm Peek® frit sandwiched between two flangeless ferrules using a low-pressure standard union (Upchurch Scientific Inc., Oak Harbor, WA).

The fluid paths between the injection port, valves, and gradient HPLC system are shown in Fig. 1. Filtration of samples is performed in-line by aspirating a volume of sample from a vial into the autosampler needle and dispensing it into the injection port and through the filter. The valve configuration used for the filtration and sampling process is shown in Fig. 1A. Following a rotation of Valve 2, the filtered sample is introduced onto the HPLC column using the configuration shown in Fig. 1B. Finally, the

filtered solid is cleansed from the system by placing the valves in the configuration shown in Fig. 1C and dispensing rinse solvent through the injection port via the autosampler needle.

The gradient HPLC system used for quantitation employed two Spectroflow 400 pumps (Kratos Analytical, Ramsey, NJ) and an ABI 783A variable wavelength detector and gradient controller (Applied Biosystems Inc., Ramsey, NJ). Column temperature was controlled using a FIAtron TC-50 column heater (Fiatron Laboratory Systems, Oconomowoc, WI).
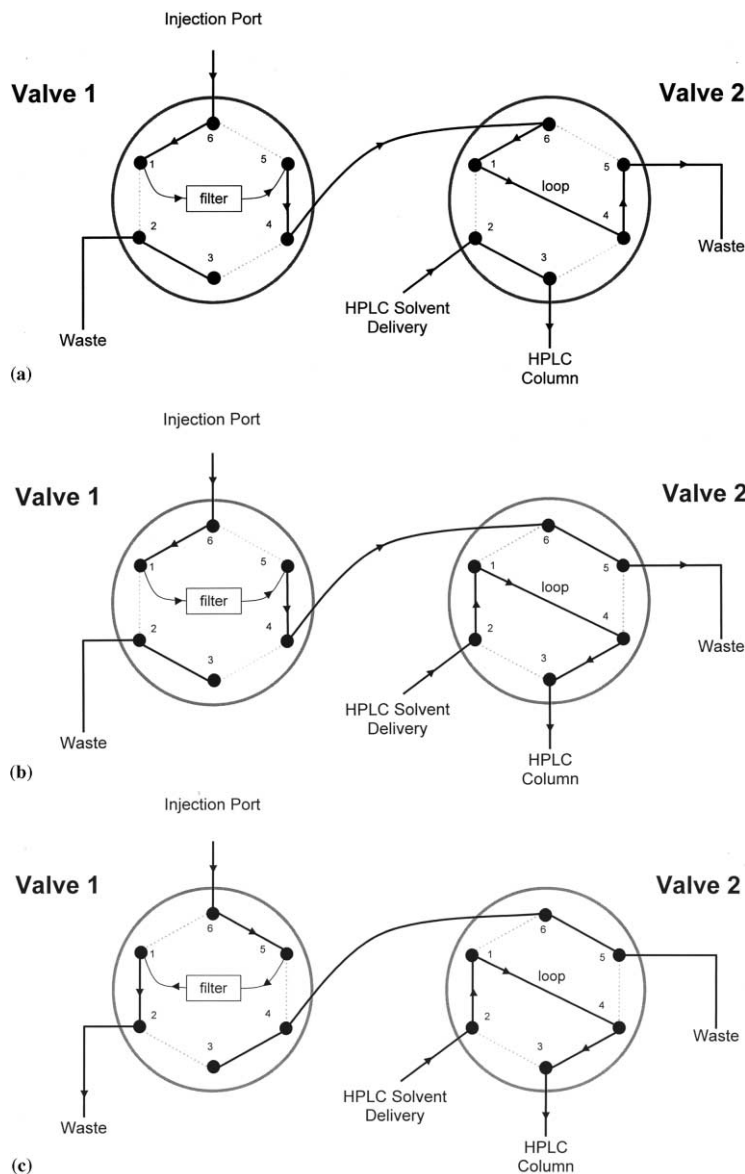

Fig. 1. (A) Rheodyne® valve positions and fluid path for filtration and loading the HPLC sampling loop; (B) Rheodyne® valve positions and fluid path for HPLC injection and analysis; (C) Rheodyne® valve positions and fluid path for system clean-up.

2.5. Chromatographic conditions

The chromatography was conducted on a Luna C18(2) column (Phenomenex, Torrance, CA) having a length of 100 mm and an internal diameter of 4.6 mm. The column was packed with 3 μm particles and was maintained at a temperature of 40°C. The mobile phase consisted of a gradient mixture of water and acetonitrile, both of which contained 0.05% v/v trifluoroacetic acid, delivered at a flow rate of 1.5 ml/min.

2.6. Precision and accuracy determinations

Precision was determined by assembling five individual samples of three development compounds and assessing the solubilities of these compounds using both manual and in-line filtration methods. Accuracy was determined by preparing 10 compounds in one or more of the following solvents: 0.1 N HCl, phosphate buffered saline (PBS), or Fasted-state Simulated Intestinal Fluid [12]. The solubilities of these samples were assessed using both manual and in-line filtration methods.

3. Results and discussion

3.1. Analytical performance

Five samples of three different development compounds having solubilities ranging from 30 to 170 μg/ml were examined using both manual and in-line filtration methods. The data from these analyses are presented in Table 1. The standard deviations of the samples filtered in-line ranged from 0.5 to 2.6 μg/ml. Similarly, the standard deviations of the samples filtered manually ranged from 0.3 to 2.4 μg/ml. Finally, there is no practical difference between the measured means.

The accuracy of the in-line filtration method was compared to that of the manual filtration method using a set of samples having solubilities ranging from 3 to 811 μg/ml. The data for these samples are given in Table 2. A log–log plot of

Table 1
Precision and accuracy of automated solubility determinations for three development compounds

Preparation number	Solubility, μg/ml	
	Manual	Automated
Compound A		
1	28.3	26.6
2	28.4	27.5
3	27.8	26.2
4	28.7	26.4
5	28.2	26.5
Mean	28.3	26.6
Standard deviation	0.3	0.5
RSD, %	1.1	1.9
Compound B		
1	63.2	71.4
2	62.2	69.7
3	63.6	70.5
4	61.8	67.9
5	60.9	68.1
Mean	62.3	69.5
Standard deviation	1.1	1.5
RSD, %	1.8	2.2
Compound C		
1	164.6	169.6
2	168.4	173.9
3	170.4	171.9
4	166.3	167.6
5	165.4	168.6
Mean	167.0	170.3
Standard deviation	2.4	2.6
RSD, %	1.4	1.5

these solubility data is given in Fig. 2. A straight-line fit to data yields a slope of 1.092 and an intercept that is not statistically different from the origin at a 95% confidence interval.

3.2. Operational performance

The in-line filtration requires only 0.1 ml of sample to achieve the accuracy and precision previously discussed. In comparison, the filter used for manual filtration was wetted/saturated with approximately 0.75 ml prior to sampling 0.25 ml of each solution for analysis. The wet-

ting procedure used for this filter is comparable to the manufacturer's guide, which recommends that 1 ml of solution be passed through the filter prior to sampling as a means of wetting the filter and saturating the potential adsorption sites.

The time required to: (i) aspirate the sample volume used for analysis; and (ii) process the sample using the automated in-line filtration routine totals 1 min and is not significantly different than the time required to perform manual filtrations. Although the in-line filtration employs a backflush procedure to remove the filtered solid from the system, the backflush is conducted within the timeframe of the HPLC analyses (< 10 min) and does not negatively affect sample throughput. Similar to manual filtration methods, the in-line filtration process does

not effectively deal with gel-like substances. However, the process is effective at managing samples having a variety of geometric shapes (e.g. needles, plates, rods, etc.) and particle sizes (approximately 1 μm to greater than 500 μm).

4. Conclusions

Solubility data obtained using the automated in-line microfiltration system directly correlate with the results obtained using conventional manual preparation techniques. Because sample-processing techniques can vary between scientists and these variations can often lead to significant differences in the solubility values obtained, automating sample processing will improve the consistency of the solubility determination. Fi-

Table 2
Tabulated solubility data for samples filtered manually and in-line

| Compound	Solvent	Solubility, μg/ml	
		Manual	In-line
Acetazolamide	0.1 N HCl	608.2	663.5
Bendroflumethiazide	0.1 N HCl	16.7	13.9
	Fasted-state simulated intestinal fluid (FaSSIF)	34.3	44.4
	Phosphate buffered saline (PBS)	15.8	13.7
Benzocaine	PBS	811.3	886.8
Benzthiazide	0.1 N HCl	3.2	2.4
	PBS	10.4	18.8
	FaSSIF	18.7	13.0
Betamethasone	0.1 N HCl	61.3	62.3
	FaSSIF	73.6	90.4
	PBS	54.0	56.5
Butamben	PBS	144.7	156.2
Butylparaben	0.1 N HCl	185.5	202.6
	FaSSIF	629.9	684.6
	PBS	189.5	204.8
Chlorpropamide	0.1 N HCl	133.5	153.9
Indomethacin (Form 1)	PH 6.2 phosphate/borate/citrate buffer	110[a]	101.1
Phenytoin	PH 7.0 phosphate/borate/citrate buffer	20[b]	18.6

[a] From [13].
[b] From [14].
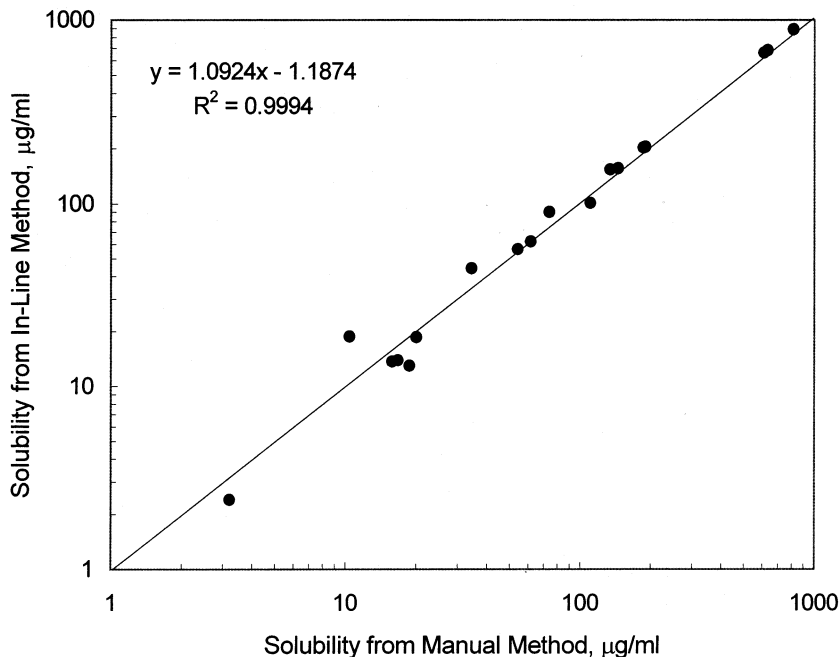
Fig. 2. Log–log comparison of solubility data for samples filtered manually and in-line. Line corresponds to a slope of 1 and an intercept of 0.

nally, off-loading the tedious work of sample processing onto a robotic system translates into a reduction of labor costs.

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