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A high throughput dried DMSO Log *D* lipophilicity measurement based on 96-well shake-flask and atmospheric pressure photoionization mass spectrometry detection

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ABSTRACT

A rapid throughput octanol–water lipophilicity measurement based on 96-well shake-flask and LC/UV/APPI/MS is described. The method utilizes central liquid storage where compounds are stored as 10 mM solutions in dimethyl sulfoxide (DMSO). The DMSO is subsequently removed to generate solid like material used for Log *D* measurement. The removal of DMSO minimizes the concern for potential DMSO cosolvent effect on the measured value. Sample preparation is automated using a liquid handling workstation with 96-well pipetter. Both octanol and buffer phases are quantified using state of the art ultra-high pressure HPLC coupled with a superficially diffused core reversed-phase column and an atmospheric pressure photo ionization mass spectrometer. The throughput of the method is two days for a batch of 96 compounds. The method has been validated using 72 literature compounds with diverse ionization and Log *D* values ranging from −2 to +6. The observed coefficient of determination r^2 is 0.9973.

1. Introduction

Lipophilicity, commonly expressed as the octanol–water partition coefficient, is considered the most important physical property for drug design and deriving quantitative structure activity relationship (QSAR) and quantitative structure property relationship (QSPR) [1–3]. Numerous literature reports relate lipophilicity to undesirable ADMET (absorption, disposition, metabolism, excretion, and toxicity) properties, including poor solubility [4], poor bioavailability [5], high protein binding [6], high affinity to microsomes and hepatocytes [7,8], and *in vivo* toxicological observations [9]. Two lipophilicity terms, Log *P* and Log *D*, are commonly used. Log *P* refers to partitioning of neutral or unionized species. Log *D* is called the distribution coefficient, a pH dependent parameter used for compounds with ionization center. For neutral compounds, Log *P* is equal to Log *D*. The commonly measured Log $D_{7.4}$ refers to Log *D* values at buffer pH equals to 7.4. The calculated Log *P* values have acceptable accuracy and are incorporated in compound design with great success, but the calculated Log *D* values remain inaccurate due to difficulties in handling ionization in computation, hence, a measured value is often desired.

Two types of high throughput methods are widely used in Log *D* determination: a miniaturized shake-flask method and a column method based on a compound's retention, typically using reversed-phase chromatography [10]. The shake-flask method is a direct measurement of octanol-buffer partitioning and can be preferred. The column method [11,12] is an indirect method with potential undesirable ionic interactions between compound and column packing materials affecting determined Log *D* values [13,14]. The literature shake-flask method has trimmed down significantly in recent years from traditional greater than 100 mL volume in one of the phases [15] to less than 1 mL and using 96-well shake-flask [16–18]. To facilitate automation and increase throughput, compound concentrated solution in DMSO are commonly used instead of dry powder. The amount of DMSO cosolvent is usually maintained at ≤1% to minimize its potential effect on the measured Log *D* value. When a higher amount of DMSO is present, it was observed that the DMSO caused a downward shift in measured Log *D* values [19]. Extrapolation from greater than 10% DMSO to 0% has been employed to correct the DMSO cosolvent effect [20]. LC/UV, nitrogen detector, LC/ESI/MS, LC/ESI/MS/MS have been employed for compound quantitation. In the case of LC/MS/MS detection, sample solutions were diluted 250–10,000-fold to bring the concentration to near linear range. In our previous Log *D* protocol, we

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employed 96-well shake-flask and electrospray LC/MS/MS detection. Similar to the literature, the octanol layer was diluted 1000 and 10,000 times for the MS/MS detection. We observed a compound dependent linear responses range that could result in inaccurate Log *D* values. Repeated measurements were sometimes needed to confirm the Log *D* value. More recently, we switched sample quantitation from electrospray ionization to the recently introduced atmospheric pressure photo ionization (APPI) detection in mass spectroscopy [21]. Compared to ESI, APPI offers larger linear dynamic range and minimal ion suppression [22]. The technique has been used to detect diverse analytes with a high percentage of success [23]. Here we wish to report our success with using APPI/MS for Log *D* measurement. Our method also includes a DMSO removal step prior to addition of octanol and buffer partitioning solvents, thus minimizing potential DMSO cosolvent effects on the measured Log *D* values. By extension of our "dried-DMSO" solubility method [24], we call our current method the "dried-DMSO" Log *D* method.

2. Experimental

2.1. Instrumentation

LC/UV/MS was performed using Agilent 1200 binary pumps (G1312B), a 1200 autosampler (G1367C), 1200 Variable Wavelength Detector (G1314C), and an Agilent LC-MSD fitted with a Syagen™ Atmospheric Pressure Photionization source. Sample preparation and liquid handling were performed using a TomTec™ Quadra 96 workstation. Dimethyl sulfoxide (DMSO) was removed using a Genevac HT4 centrifugal evaporation instrument. Sample mixing was performed using an Eppendorf ThermomixerR™ fitted with a 96-well plate adaptor or Enviro-Genie™ inverted shaker (Scientific Industries Inc.). Centrifugation was performed using an Eppendorf centrifuge Model 5810R. An Atlas™ chromatography system was used in processing LC/UV data. An Agilent ChemStation was used in acquiring and processing LC/MS data. Microsoft Excel and HBase™ were used for polling LC/UV and LC/MS data and entering data into the AstraZeneca central database.

2.2. Chemicals and materials

All compounds mentioned in the present study were obtained from the AstraZeneca in-house collection. HPLC grade acetonitrile was purchased from Fisher Scientific. Na_2HPO_4 and NaH_2PO_4 used for buffer preparation were obtained from Fisher Scientific. The 96 glass vial well plates (FlexTier™), 1.5-mL flat bottom glass vial inserts, and PTFE coated plate-mat, were obtained from Analytical Sales & Products Inc. Polypropylene 2 mL collection plates and plate mats were purchased from Phenomenex. The Alligator tumble stirrer (VP710SM), StirStix (28 mm) and its 96-well dispenser were purchased from V&P Scientific Inc.

2.3. Sample preparation of the Log D method

A volume of 20 μL of compound as a 10 mM DMSO solution was dispensed into a 96-well FlexTier™ plate by AstraZeneca central liquid dispensary. The plate was placed in a Genevac where DMSO was removed at 40 °C under full vacuum for 30 min. After drying, StirStix were added to the plate using a 96-well dispenser. Using a TomTec™ Quadra 96®, 435 μL of octanol was added. The plate was stirred using an Alligator tumble stirrer for 5 min to dissolve compound in octanol. The plate was returned to the TomTec where 435 μL of pH 7.4, 0.01 M sodium phosphate buffer, presaturated with octanol, was added. The plate was capped using a PTFE lined cap mat and the solution mixed through inversion at 20 rpm and 25 °C for 5 h. After mixing, the plate was centrifuged at 3000 rpm for 30 min. Using the TomTec, the 350 μL top octanol layer was transferred to a polypropylene deep-well plate. The remaining solution in the FlexTier™ plate, capped using a slitted-Teflon cap mat, and the newly made plate containing the octanol layer were subjected to LC–UV/APPI–MS quantitation. Typical throughput of the method was one plate per day with a two days turnaround time. Two literature compounds, propranolol (Log *D* = 1.2), and chlorpromazine (Log *D* = 3.4), were used for quality control and run with each batch of compounds.

Table 1
Generic gradient for LC–MS quantitation.

Time (min)	%A	%B
0	100	0
0.3	100	0
3.0	25	75
3.1	100	0
3.7	100	0

2.4. TomTec volume calibration

The accuracy of octanol and buffer volume transfer by the TomTec™ Quadra 96 was determined by weighing the amount added. For TomTec programming, a three times mixing and a ten times blow out volume were added prior to aspirating and after dispensing. Based on four independent weighing of a 435 μL programmed volume, the actual added octanol volume was 442.6 ± 0.7 μL with %RSD = 0.18%, and buffer volume was 442.4 ± 0.4 μL with %RSD = 0.12%.

2.5. Phase equilibrium of octanol and buffer

Reproducibility of Log *D* values was determined for the set of literature compounds and more extensively using two high Log *D* compounds, chlorpromazine and amiodarone. Two different mixing methods, either shaking or inversion, were used. The shaking was carried out using an Eppendorf shaker at shaking speed of 1400 rpm for 2 h, resting for 1 h, and centrifuging for 30 min. The mixing via inversion was carried out using Enviro-Genie fitted with a custom plate holder for 3 h, 5 h, or 16 h.

2.6. LC/UV/MS quantitation

An Agilent 1200 HPLC coupled with an Agilent MSD single quadruple mass spectrometer was used for sample quantitation. Both the octanol and aqueous phases were quantified. The generic gradient shown in Table 1 was used: mobile phase (A) consists of 95% H_2O, 5% ACN, 0.1% TFA; and mobile phase (B) 4%H_2O, 96% ACN, 0.08% TFA. The flow rate was 0.9 mL/min and was split post UV detector to feed 300 μL/min into the mass spectrometer. The run time was 3.7 min. The detector wavelength was 220 nm. The injection order was blank buffer, buffer aqueous phase, and octanol phase. The injection volume was 30 μL of blank buffer, 1 μL of octanol phase and a variable volume of aqueous phase according to Table 2 derived from the ACD Log $D_{7.4}$ predicted value. The column used was a Halo (or Ascentis) C18 30 mm × 2.1 mm, 2.7 μm, (e.g. Mac-Mod or Supelco). Overlapping injection with needle wash was used. Mass spectrometer conditions: drying gas flow, 5.0 L/min;

Table 2
Injection volume of aqueous phase.

ACD Log $D_{7.4}$ predicted value	Buffer phase injection volume (μL)
<0	5
>0 and <4	30
>4	80

nebulizer pressure, 60 psi; drying gas temperature, 350 °C; vaporizer temperature: 350 °C; capillary voltage, 1500 V; MSD signal setting: single ion monitoring with positive polarity. No dopant was used.

Log *D* value was calculated according to the following:

$$\text{Log}\,D_{7.4} = \text{Log}_{10}\left(\frac{\text{peak area of octanol phase/injection volume of octanol phase}}{\text{peak area of buffer phase/injection volume of buffer phase}}\right)$$

2.7. Linearity of APPI response

The linearity of APPI response was determined for two quality control compounds: propranolol and chlorpromazine. Solutions were prepared by serial dilution of a 1 mM stock solution in 2-methoxyethanol to a final concentration of 500, 250, 100, 50, 25, 10, 5, 1, 0.5, and 0.1 μM using 40% ACN/60% H_2O.

2.8. Data reporting

The LC/UV and APPI–MS data were imported into an Excel worksheet for Log *D* calculations and data entry into AstraZeneca central database. For compounds with a Log *D* < 3, LC/UV data was used. When there was interference from coeluting impurities, MS data was used. For compounds with Log *D* ≥ 3, LC/MS data was reported. When the determined Log *D* value was greater than 4.0, the data was reported as greater than the measured value. A true value was reported when the high Log *D* value was confirmed from repeated measurement. When the compound eluted at the octanol peak retention time, a measured value of greater than 4.0 was reported as ">4.0".

3. Results and discussion

3.1. General description of the Log D method

A rapid throughput Log *D* method based on shake-flask principle for octanol–water partitioning has been developed (Fig. 1). The method utilizes DMSO stock solutions stored in the central liquid dispensary where each solution was prepared automatically upon new compound submission. After DMSO removal, the compound was redissolved in octanol prior to addition of buffer. By removing DMSO and generating solid material, the method minimizes concern of a potential DMSO effect on the measured Log *D*. The sample preparation was fully automated with minimal manual intervention involving moving the plate from one instrument to another. Both octanol and aqueous layers were quantified using LC/UV/APPI/MS. The combination produced a detection system capable of measuring Log *D* from −2 to +6 without sample dilution. For one plate of compounds, the method consumed two sets of disposable tips, one 96-well plate with glass tube inserts and one polypropylene plate, a nearly 50% savings compared to our previous method based on LC/ESI/MS/MS detection. The typical throughput of the method was one plate per day with a two-day turnaround time, a throughput comparable with the column based Log *D* method when measuring 96 compound batches. Details of the method development are discussed below.

3.2. Octanol-buffer phase equilibrium

Two common means of octanol-buffer phase equilibrium were employed in the literature, a gentle mixing through inversion and a more vigorous shaking using either vortex or shaker. Initially we used vigorous shaking for its purported short time. We found that while the mixing produced reproducible data (less than ±0.1) for compounds with a Log *D* less than 3, the reproducibility of compounds with a Log *D* greater than 3 was poor (greater than ±0.2). The buffer layer for some compounds appeared cloudy after 1 h standing post mixing. In the traditional literature, gentle mixing is preferred to avoid emulsion formation [25]. A gentler mixing through inversion was subsequently investigated. Mixing times of 3, 5, and 16 h were evaluated. Visual inspection of the buffer layer post mixing indicated that it was clear after all inversion time durations. Log *D* results showed that the accuracy and reproducibility were similar for either 5 h or 16 h mixing while reproducibility for 3 h was worse. The 5 h inversion was chosen for routine measurement.

3.3. LC–UV detection

A mid-range ultra-high pressure Agilent 1200 HPLC system coupled with a superficially porous reversed-phase column was used for sample quantitation. The column packing, made of solid core and porous shell particles, offered high efficiency similar to sub-2 μm columns but at much lower operating back pressure [26]. This combination produced excellent chromatography in both LC/UV and APPI/MS (Fig. 2). The LC run time was scheduled to allow maximum gradient spread to separate impurities from the parent and also to ensure completion of 96 compounds in one overnight run. The linear gradient to 75% B, sufficient for eluting literature high Log *D* compounds, was derived from an initial 90% B for reduced acetonitrile solvent usage. The UV signal was sufficient to measure compound with Log *D* less than 3. At Log *D* above 3, the compound concentration in the buffer phase is near the UV detection limit and LC/MS was used for quantitation. The sample carryover from the buffer phase injection was not detected by UV and MS, but the carry over from the octanol phase was significant at around 1% by both UV and MS. The carryover could not be minimized by varying autosampler parameters including needle wash volume or the choice of overlapping injection. When compounds with identical retention time (e.g. chiral isomers) were subsequently injected, this carryover could artificially increase the buffer peak area resulting in lower Log *D* values. To minimize this carry over effect, a blank buffer injection was inserted prior to injecting buffer phase of the next sample.

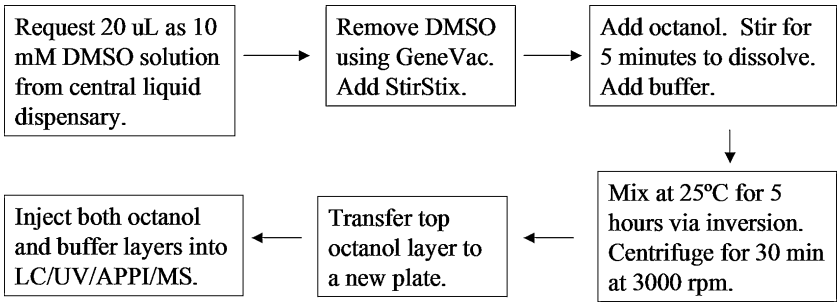

Fig. 1. Flow chart of the dried-DMSO Log *D* method.

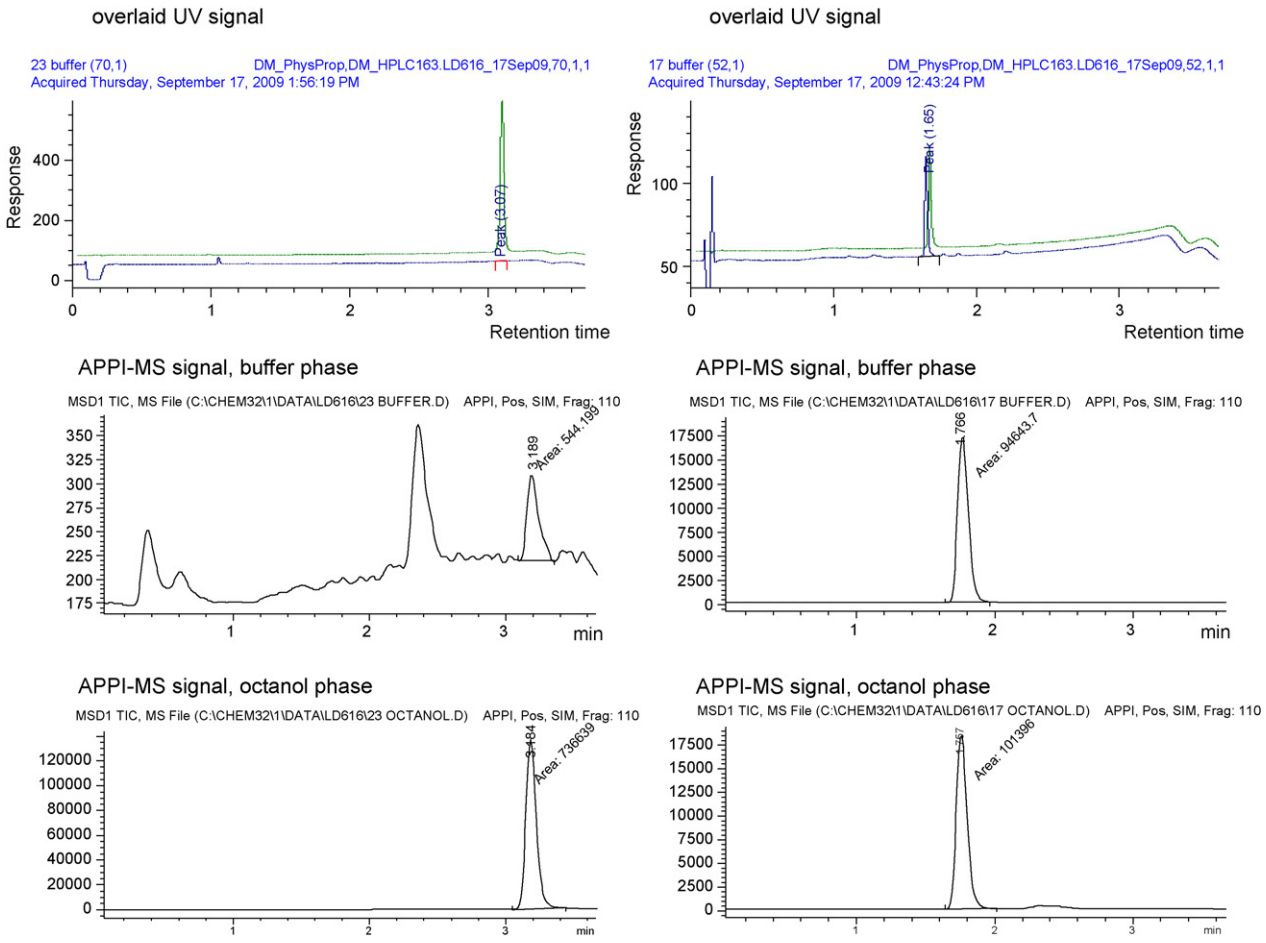

Fig. 2. LC/UV/APPI/MS chromatograms. (Left) A high Log *D* compound: tolnaftate (Log *D* = 5.4). Injection volume of the buffer phase was 80 μL. The peak eluding at 2.2 min in the buffer phase chromatogram is the "octanol" system peak. (Right) A mid-range Log *D* compound: hydrocortisone (Log *D* = 1.5).

3.4. LC–MS detection

The LC flow was split post UV with 0.3 mL/min going into the APPI/MS. Unlike ESI where the common mobile phase additive TFA can cause analyte ion suppression, TFA is tolerated by APPI, resulting in excellent chromatography. Dopant was not added for ease of operation. Based on greater than 3000 project compounds, greater than 99% compounds were detectable. The added advantage of no dopant is the low baseline noise observed enabling detection of very high Log *D* compounds (Fig. 2). The most abundant ion observed under single ion monitoring was $M+H^+$, presumably formed via proton extraction of the photoionized species from abundant mobile phase matrix [21].

The linearity range of APPI–MS response was determined for the two quality control compounds propranolol and chlorpromazine having Log *D* 1.2 and 3.4, respectively. Linear response was observed from 0.1 to 500 μM solution concentration or 0.5 to 2500 μM in the amount of compound injected (Fig. 3). The coefficient of determination r^2 for propranolol was 0.9999 and chlorpromazine 0.9992. At concentrations from 500 μM to 1 mM, there was slight downward shift of the curve (not shown), indicating MS signal saturation. This 3–4-fold MS linear range coupled with 1–2-fold autosampler injection variation implied 4–6 Log *D* ranges in direct determination possible without sample dilution. Assuming compound partitioned completely into one phase, the 500 μM upper linear range would cover the 500 μM maximum theoretical compound loading. Compared to ESI where compound dependent linear range from 0.8 to 100–200 μM was observed [17], the observed APPI linearity is at least one order of magnitude higher than ESI in single ion monitoring mode.

3.5. Correlation of dried-DMSO Log D with literature values

Using the present method, the Log *D* value was determined for 72 commercially available compounds with diverse ionization and lipophilicity values ranging from −2 to +6. Data are summarized in Table 3 and plotted in Fig. 4. A good agreement between the measured and literature values was obtained. The coefficient of determination r^2 was 0.9973. Reproducibility for compounds with Log *D* less than 4 was less than ±0.1 from duplicate measurements. The reproducibility for very high Log *D* compounds was slightly

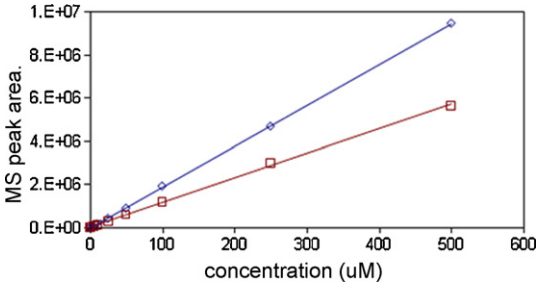

Fig. 3. Plot of peak area versus concentration of APPI/MS response. The injection volume was 5 μL. The fitted line is linear fit of the data. Blue diamond: propanolol; red square chlorpromazine.

Table 3
Summary of literature data and measured Log $D_{7.4}$ value of validation compounds used in the present study.

Entry	Name	Ion class	*C* Log *P*	ACD Log *D* pH 7.4	Measured Log *D*	St. dev.	Log *D* (literature)	buffer phase inj vol (μL)	Comments	Literature reference
1	3,5-Dinitrobenzoic acid	Neutral	0.63	0.55	0.80	0.01	0.91	30	No APPI signal	[27]
2	Acebutolol	Base	1.71	0.24	−0.31	0.02	−0.29	5		[28]
3	Albendazole	Neutral	3.46	3.06	3.24	0.01	3.29	30		[7]
4	Alprenolol	Base	2.65	1.11	0.91	0.03	0.97	30		[28]
5	Amiodarone	Base	8.95	6.91	6.20	0.17	6.1	80		[28]
6	Antipyrine	Neutral	0.2	0.27	0.23	0.01	0.4	30		[28]
7	Astemizole	Base	5.84	4	4.42	0.01	4.14	80		[7]
8	Atenolol	Base	−0.11	−1.67	−1.88	0.01	−1.82	30		[29]
9	Atropine	Base	1.3	−1.05	−0.61	0.02	−0.55	30		[29]
10	Bupivacaine	Base	3.69	2.8	2.41	0.01	2.65	30		[29]
11	Caffeine	Neutral	−0.04	−0.13	−0.03	0.01	−0.07	30		[28]
12	Carbamazepine	Neutral	2.38	2.67	1.65	0.01	1.54	30		[7]
13	Chlorpheniramine	Base	3.15	1.25	1.39	0.01	1.41	30		[28]
14	Chlorpromazine	Base	5.3	3.23	3.37	0.02	3.38	30		[28]
15	Chlorthalidone	Neutral	0.45	−0.74	0.96	0.01	1.11	30		[28]
16	Clozapine	Base	3.71	2.23	3.12	0.01	3.13	30		[28]
17	Cyclothiazide	Neutral	1.98	1.13	2.09	0.01	2.09	30		[28]
18	Disopyramide	Base	2.58	0.28	−0.52	0.03	−0.66	30		[28]
19	Flecainide	Base	3.66	−0.15	1.02	0.01	0.97	30		[28]
20	Flufenamic acid	Acid	5.53	1.89	2.03	0.01	2.02	30		[29]
21	Flurbiprofen	Acid	3.75	0.86	0.87	0.02	0.91	30	No APPI signal	[29]
22	Furosemide	Acid	1.9	−1.29	−1.10	0.02	−1.02	5		[29]
23	Glyburide	Acid	4.24	3.75	2.19	0.01	2.16	30		[7]
24	Griseofulvin	Neutral	2.05	3.53	2.23	0.02	2.18	30		[28]
25	Hydrocortisone	Neutral	1.89	1.43	1.51	0.01	1.55	30		[28]
26	Ibuprofen	Acid	3.68	0.73	1.02	0.01	0.98	30	No APPI signal	[7]
27	Imipramine	Base	5.04	2.75	2.40	0.02	2.4	30		[28]
28	Indomethacin	Acid	4.18	−0.33	0.93	0.01	0.91	30		[29]
29	Ketoconazole	Base	3.63	3.43	3.70	0.01	3.8	30		[30]
30	Labetalol	Zwitterion	2.5	0.64	1.06	0.01	1.07	30		[29]
31	Lidocaine	Base	1.95	1.2	1.65	0.03	1.7	30		[28]
32	Loratadine	Neutral	5.05	5.94	4.54	0.05	4.4	80		[28]
33	Methotrimeprazine	Base	4.83	3.01	2.92	0.01	2.77	30		[28]
34	Metoclopramide	Base	2.23	−0.05	0.54	0.02	0.64	30		[28]
35	Metoprolol	Base	1.49	0.01	−0.31	0.01	−0.16	5		[28]
36	Metronidazole	Neutral	−0.46	−0.01	−0.08	0.01	−0.02	30		[28]
37	Mexiletine	Base	2.57	0.95	0.57	0.01	0.47	30		[28]
38	Nifuroxime	Neutral	1.1	0.65	1.24	0.02	1.28	30	No APPI signal	[28]
39	Nizatidine	Base	−0.16	0.97	−0.50	0.01	−0.52	5		[28]
40	Oxprenolol	Base	2.09	0.52	0.13	0.01	0.32	30		[29]
41	Pentoxifylline	Neutral	0.12	0.32	0.40	0.02	0.29	30		[28]
42	Pipemidic acid	Zwitterion	−2.48	−2.51	−1.56	0.10	−1.52	5		[29]
43	Pirenzepine	Base	−0.35	−0.97	−0.48	0.02	−0.61	30		[28]
44	Prednisolone	Neutral	1.42	1.49	1.59	0.01	1.6	30		[28]
45	Procainamide	Base	1.42	−1.33	−1.06	0.10	−0.91	5		[28]
46	Propafenone	Base	3.64	2.03	1.63	0.02	1.81	30		[28]
47	Propranolol	Base	2.75	1.35	1.17	0.02	1.26	30		[29]
48	Risperidone	Base	2.71	2.29	2.01	0.01	2.04	30		[28]
49	Sotalol	Base	0.23	−1.49	−1.56	0.02	−1.52	30		[29]
50	Sulfathiazole	Acid	0.73	−0.26	−0.31	0.01	−0.43	5		[29]
51	Sumatriptan	Base	0.74	−1.24	−1.03	0.02	−1	5		[28]
52	Tamoxifen	Base	6.82	6.57	4.97	0.07	5.02	80		[7]
53	Terbutaline	Base	0.48	−1.33	−1.47	0.06	−1.35	30		[28]
54	Tetracaine	Base	3.83	2.61	2.24	0.01	2.29	30		[28]
55	Theophylline	Neutral	−0.03	−0.2	−0.03	0.01	−0.02	30		[29]
56	Thiamphenicol	Neutral	−0.1	−0.27	−0.26	0.01	−0.27	5		[28]
57	Thioridazine	Base	6	3.85	3.66	0.01	3.59	30		[7]
58	Tiapride	Base	1.3	−1.54	−0.87	0.01	−0.9	5		[28]
59	Tolnaftate	Neutral	5.34	5.15	5.40	0.18	5.4	80		[28]
60	Trazodone	Base	3.85	1.61	2.64	0.01	2.54	30		[28]
61	Triamterene	Neutral	1.61	1.31	1.20	0.01	1.21	30		[28]
62	Trichlormethiazide	Neutral	0.88	−0.18	0.44	0.02	0.43	5		[28]
63	Trifluoperazine	Base	4.69	4.38	4.20	0.02	4.01	80		[29]
64	Triflupromazine	Base	5.61	3.73	3.68	0.08	3.61	30		[28]
65	Trimethoprim	Base	0.98	0.58	0.63	0.01	0.6	5		[28]
66	Verapamil	Base	4.47	2.32	2.57	0.01	2.57	30		[7]
67	Warfarin	Neutral	2.9	0.52	0.87	0.03	0.75	30		[7]
68	Bifonazole	Base	4.74	4.78	>4		4.77	80		[28]
69	Estradiol	Neutral	3.78	4.13	>4		4.01	80	No APPI signal	[28]
70	Triphenylene	Neutral	5.66	5.91	>4		5.49	80	No APPI signal	[29]
71	Diethystilbestrol	Neutral	4.96	5.93	>4		5.07	80	No APPI signal	[29]
72	Clotrimazole	Base	5	5.42	>4		5.2	80	No APPI signal	[28]

C Log *P* was calculated using V. 4.3. ACD Log *D* was calculated using V. 10.

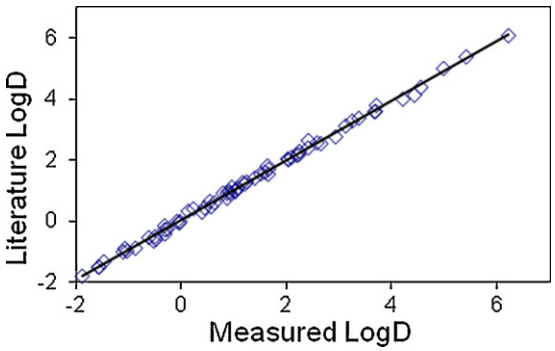

Fig. 4. Plot of measured Log *D* (pH 7.4) versus literature value.

worse, up to ±0.2 due to the small buffer phase peak area observed and potential interference from system carryover and other interferences. Extremely high Log *D* compounds such as amiodarone and tolnaftate tend to stay around in the LC/MS detection system affecting buffer phase peak area when repeated measurements were made, resulting in increased buffer peak area and apparent lower Log *D* values. Insertion of six blank injections between octanol and buffer phase of the next sample was found to be insufficient to remove the interference. Greater than 200 injections were necessary to minimize the interference. Another potential interference came from the "octanol" peak that was present in all injections under SIM conditions. The "octanol" peak appeared as a small peak with r.t. = 2.2 min in buffer phase injection and a broad peak with r.t. = 2.2–2.6 min in octanol phase injection (Fig. 2). For low- to mid-range Log *D* compounds, the "octanol" peak interference was minimal. But for high Log *D* compound such as bifonazole eluting at the identical retention time, the "octanol" peak area in buffer phase represented greater than 50% of the compound peak area resulting in increased buffer peak area and apparent low Log *D* value. Adjusting gradient conditions or changing column dimension could not separate the "octanol" peak from compound. Bifonazole was the only compound in the validation set that is adversely affected by the "octanol" peak. All other compounds in the validation set either have sufficient retention time separation or higher buffer phase concentration that the "octanol" peak effect was minimal. Under photo ionization conditions and positive polarity mode, some neutral and acidic compounds were not detected by APPI and UV data was used for Log *D* reporting. For several high Log *D* compounds where there was no APPI signal, approximate value based on UV signal was reported (entry 69–72, Table 3). These compounds are unlikely to appear in drug design space; their lack of detection was not seen to be crucial in Log *D* support.

4. Conclusions

A rapid throughput dried-DMSO Log *D* method is described. The method utilizes DMSO stock solution that is prepared and stored in a central liquid dispensary, which is shared by physical properties and other assays such as *in vitro* biology and *in vitro* ADME screenings. In this way, only microgram of material is used in the Log *D* measurement. By removing DMSO, the method minimizes potential concern of the DMSO effect on measured Log *D* values. The method is straight forward, fully automated in sample preparation, data acquisition, and data processing, and has been validated with literature compounds with Log *D* ranging from −2 to +6. We have used the method successfully to support all phases of CNS discovery projects where the target Log *D* range is 1–3 [31]; the extended range is 0–4. The large linear dynamic range and TFA compatibility by APPI/MS enabled the use of generic chromatography with good resolution. Compared to LC/ESI/MS/MS, the use of APPI has resulted in simplified sample preparation and greater than 50% savings in lab ware. The success of this APPI/MS application in Log *D* measurement has enabled our subsequent switching of solubility quantitation from LC/UV/ESI/MS/MS to LC/UV/APPI/MS.

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