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Pharmaceutical profiling method for lipophilicity and integrity using liquid chromatography–mass spectrometry

Edward H. Kerns[a,*], Li Di[a], Susan Petusky[a], Teresa Kleintop[a], Donna Huryn[a], Oliver McConnell[b], Guy Carter[c]

[a]*Wyeth Research, Chemical Sciences, Discovery Analytical Chemistry, CN8000, Princeton, NJ 08543-8000, USA*
[b]*500 Arcola Road, Collegville, PA 19426, USA*
[c]*401 N. Middletown Road, Middletown Road, Pearl River, NY 19426, USA*

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Abstract

A method is described for the simultaneous profiling of sample lipophilicity, integrity, and purity. The method is rapid and is applicable to high throughput profiling of pharmaceutical properties in drug discovery. A short Polaris C_{18} column is used with a rapid, wide-polarity mobile phase gradient, UV detection, and MS analysis. The lipophilicity of each component is estimated from a calibration curve using six drug or organic compounds and plotting their respective measured retention time versus Log $D_{7.4}$ (literature). The correlation of Log $D_{7.4}$ (literature) to Log $D_{7.4}$ (HPLC) for 60 structurally diverse drugs has a correlation coefficient r^2 of 0.89. The method is applicable to compounds with MW>200 and retention time>1.5 min for rapid, initial pharmaceutical profiling in drug discovery.

Keywords: Pharmaceutical profiling; Lipophilicity; Integrity

1. Introduction

Pharmaceutical profiling is the assessment of compound properties during drug discovery [1,2]. This function is valuable for reducing attrition and delays in development, resulting from poor biopharmaceutical properties, as well as providing property information that can be used by research teams to make informed decisions on discovery experiments. Properties such as lipophilicity, solubility, permeability, stability, and pK_a have a major effect on the concentration of drug delivered to the therapeutic target after dosing. Thus, properties affect the observed biological activity (pharmacology) in high throughput screening, enzyme/receptor assays, cell-based assays, animal models, and humans.

Lipophilicity is a physicochemical property of interest in drug discovery. This property provides discovery scientists with insights on the tendency of the compound to partition into lipid versus aqueous environments. Such insights are useful for correlation to important pharmaceutical processes such as gastrointestinal absorption, membrane permeation,

*Corresponding author. Tel.: +1-732-274-4520; fax: +1-732-274-4850.
E-mail address: kernse@wyeth.com (E.H. Kerns).

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solubility, volume of distribution, and protein binding.

Lipophilicity is commonly assessed via measurement of octanol–water partitioning [3–5]. The result is a value of Log *P* (the partition coefficient taken at a pH where, all of the compound is in the neutral state), or Log *D* (the distribution coefficient taken at a pH, where a significant portion of the compound exists in the charged state). The method has been scaled-down to 96-well plates, by Zaslavsky and co-workers, in order to increase throughput [6]. Lipophilicity has also been measured using microemulsion electrokinetic chromatography [7], pH-metric method [8], and HPLC [9–12]. Lombardo et al. [12,13] and Donovan and Pescatore [14] have described elegant methods for measuring Log *D* by HPLC. Valko and co-workers have described LC–MS methods for rapidly measuring hydrophobicity index [15,16].

LC–MS is commonly used in drug discovery to rapidly profile the integrity and purity of compounds [17]. Extending this methodology to also profile lipophilicity in the same analysis was investigated. A combined method would provide additional pharmaceutical profiling data for drug discovery without expending additional time or precious material. Retention time was calibrated to lipophilicity by using compounds for which Log *D* at pH 7.4 had been measured using octanol–water partitioning [12,13,18]. Performance of the method for integrity/purity profiling was also assessed. A rapid LC–UV-MS method that provides estimates of Log *D* at pH 7.4 and integrity/purity at the rate of 5.5 min/compound for application in pharmaceutical profiling is described.

2. Experimental

DMSO was from Aldrich (Milwaukee, WI, USA). Acetonitrile and water were HPLC grade from EM Sciences (Darmstadt, Germany). Ammonium acetate, acetic acid and ammonium hydroxide were from J.T. Baker (Phillipsburg, NJ, USA). Test compounds were from Sigma (St. Louis, MO), Fluka (Steinheim, Switzerland), and Aldrich.

LC–MS analysis was performed using an Agilent 1100 HPLC and a Waters ZQ mass spectrometer system with electrospray ionization and alternating positive and negative ion quadrupole mass spectrometry and scanning m/z 100–1000 each second. The HPLC column was a Polaris C_{18}-A (2 mm I.D., 50 mm length, 3 μm particle size) from MetaChem Technologies (Torrance, CA, USA). A linear mobile phase gradient was used with mobile phase A as 100% 10 m*M* ammonium acetate (adjusted to pH 7.4 with ammonium hydroxide and acetic acid), and mobile phase B as 100% acetonitrile. The gradient table was: 0 min/0% B, 2.5 min/95% B, 4.0 min/95% B, 4.1 min/0% B, 5.5 min/0% B. Flowrate was 0.8 ml/min and column temperature was 40 °C. The sample was dissolved in DMSO at 0.5 mg/ml and 5 μl were injected. An Aquasil (2.1 mm I.D., 50 mm length, 5 μ*M* particle size) from Thermo Hypersil Keystone (Bellefonte, PA, USA) was used with the same mobile phase program for comparison purposes.

The retention time (t_R) for each compound was obtained by first confirming the identity of the compound using the mass spectrum. The relative area percent of each peak was obtained from the UV chromatogram at 254 nm. The log *D* was calibrated to t_R by running a mixture of six compounds (Table 1) and plotting t_R versus log *D* from the literature [12,13,18]. The log *D* values, used in this study, were obtained from the average values in the scientific literature, as referenced in Table 2.

A set of 70 structurally diverse drugs and organic compounds, including acids and bases, were chromatographed using the methods above. The data were compared to literature values for the same compounds. (Alternatively, Log *D* values were obtained from in-house measurement of partitioning between octanol (1 ml) and aqueous buffer (1 ml) at pH 7.4 in 10 m*M* phosphate buffer, followed by HPLC analysis of each phase to determine Log *D*).

Table 1
Set of compounds used to calibrate Log $D_{7.4}$ with t_R

Compound	Log $D_{7.4}$ [18]	t_R (min)
Atenolol	−1.38	1.86
Sulpiride	−1.15	2.02
Metoprolol	−0.06	2.25
Labetolol	+1.07	2.42
Diltiazem	+2.70	2.86
Triphenylene	+5.49	3.55

Table 2
Set of commercial drugs and organic compounds used in the study, with their data

No.	Compound	MW	Log $D_{7.4}$ (Literature)	t_R (min)	Log $D_{7.4}$ (from t_R)	Difference (Log $D_{7.4}$ Lit.− Log $D_{7.4}$ t_R)	Ref.
1	Acetylsalicylic acid[a]	180	−1.14	0.72	−6.33	5.19	[18]
2	Salicylic acid[a]	138	−2.11	0.90	−5.58	3.47	[18]
3	Acetaminophen[a]	151	0.51	1.50	−3.06	3.57	[12]
4	Amoxicillin	365	−1.35	1.78	−1.88	0.53	[18]
5	Theophylline[a]	180	−0.02	1.83	−1.67	1.65	[18]
6	Ceftriaxone	554	−1.23	1.75	−2.01	0.78	[18]
7	Terbutaline	225	−1.35	1.78	−1.88	0.53	[12,13]
8	Metoprolol	167	−0.06	2.25	0.09	−0.15	[18]
9	Atenolol	266	−1.38	1.86	−1.55	0.17	[18]
10	Sulpiride	341	−1.15	2.02	−0.87	−0.28	[18]
11	Cephalexin	347	−1.45	1.96	−1.13	−0.32	[18]
12	Pindolol	248	−0.21	2.1	−0.54	0.33	[18]
13	Nadolol	309	−1.21	2.06	−0.71	−0.50	[18]
14	Timolol	316	−0.047	2.22	−0.04	−0.01	[18]
15	Acebutolol	336	−0.29	2.21	−0.08	−0.21	[12,13]
16	Warfarin	308	1.12	2.37	0.59	0.53	[18]
17	Ketoprofen	254	−0.13	2.32	0.38	−0.51	[18]
18	Sulfasalazine	398	0.08	2.32	0.38	−0.30	[18]
19	Oxprenolol	265	0.32	2.36	0.55	−0.23	[18]
20	Labetalol	328	1.07	2.42	0.80	0.27	[18]
21	Flurbiprofen	244	0.91	2.66	1.81	−0.90	[18]
22	Ibuprofen	206	1.37	2.56	1.39	−0.02	[18]
23	Propranolol	259	1.26	2.48	1.06	0.20	[18]
24	Alprenolol	249	0.97	2.50	1.14	−0.17	[12,13]
25	Dexamethasone	392	1.83	2.61	1.60	0.23	[12,13]
26	Oxazepam	287	2.13	2.44	0.89	1.24	[18]
27	Corticosterone	346	1.82	2.63	1.69	0.13	[18]
28	Chloramphenicol	323	1.14	2.39	0.68	0.46	[12,13]
29	Lorazepam	321	2.51	2.51	1.18	1.33	[12,13]
30	Desipramine	266	1.28	2.64	1.73	−0.45	[12,13]
31	Promazine	284	2.52	2.76	2.23	0.29	[18]
32	Imipramine	280	2.4	2.77	2.27	0.13	[12,13]
33	Diltiazem	415	2.70	2.86	2.65	−0.05	[18]
34	Verapamil	455	1.99	2.91	2.86	−0.87	[18]
35	Triphenylene	228	5.49	3.55	5.55	−0.06	[18]
36	L-Dopa[a]	197	−2.57	0.28	−8.18	5.61	[18]
37	3,4,5-Trihydroxybenzoic acid[a]	170	−0.40	0.26	−8.26	7.86	–[b]
38	3,5-Dinitrobenzoic acid[a]	212	0.91	1.85	−1.59	2.50	–[b]
39	Pipemidic acid	303	−1.52	1.74	−2.05	0.53	[18]
40	2,4-Dihydroxybenzoic acid[a]	154	2.06	0.36	−7.84	9.90	[18]
41	Furosemide	331	−1.02	2.26	0.13	−1.15	[18]
42	Sulfamerazin	264	−0.12	1.87	−1.50	1.38	[18]
43	Sulfathiazole	255	−0.43	2.45	0.93	−1.36	[18]
44	Naproxen	230	0.30	2.30	0.30	0.00	[18]
45	Allopurinol[a]	136	−0.44	0.42	−7.59	7.15	[12,13]
46	Thiamphenicol	356	−0.27	2.07	−0.67	0.40	[12,13]
47	Caffeine	194	−0.07	1.97	−1.08	1.01	[12,13]
48	Metronidazole[a]	171	−0.02	1.63	−2.51	2.49	[12,13]
49	Nitrofurazone	198	0.23	2.04	−0.79	1.02	[12,13]

Table 2. Continued

No.	Compound	MW	Log $D_{7.4}$ (Literature)	t_R (min)	Log $D_{7.4}$ (from t_R)	Difference (Log $D_{7.4}$ Lit.− Log $D_{7.4}$ t_R)	Ref.
50	Prednisone	358	1.41	2.47	1.01	0.40	[12,13]
51	Carbamazepine	236	2.19	2.58	1.48	0.71	[12,13]
52	Testosterone	288	3.29	2.81	2.44	0.85	[12,13]
53	Estradiol	272	4.01	2.83	2.52	1.49	[12,13]
54	Bifonazole	310	4.77	3.16	3.91	0.86	[12,13]
55	Diethystilbestrol	268	5.07	3.02	3.32	1.75	[12,13]
56	Clotrimazole	345	5.20	3.16	3.91	1.29	[12,13]
57	Ephedrine	165	−1.48	1.85	−1.59	0.11	[18]
58	Sotalol	272	−1.35	1.84	−1.63	0.28	[12,13]
59	Sumatriptaon	295	−1.00	1.99	−1.00	0.00	[12,13]
60	Disopyramide	339	−0.66	2.33	0.43	−1.09	[12,13]
61	Atropine	289	−0.25	2.15	−0.33	0.08	[18]
62	Ranitidine	314	−0.29	2.13	−0.41	0.12	[12,13]
63	Procaine	236	0.33	2.16	−0.29	0.62	[18]
64	Triflupromazine	352	3.61	3.07	3.53	0.08	[12,13]
65	Clozapine	326	3.13	2.9	2.82	0.31	[12,13]
66	Thioridazine	371	3.34	2.99	3.20	0.14	[12,13]
67	Bupivacaine	288	2.65	3.03	3.36	−0.71	[18]
68	Chlorpromazine	319	3.38	2.96	3.07	0.31	[12,13]
69	Loratadine	383	4.40	3.18	3.99	0.41	[12,13]
70	Amiodarone	645	6.10	3.88	6.93	−0.83	[12,13]

[a] Outliers that were removed, for reasons discussed in the text, and re-plotted in Fig. 3.
[b] Log D measured at Wyeth Research.

To test the reproducibility of the method, the compounds were run again under the same conditions. To test the effect of mobile phase buffer pH on t_R, the samples were re-run at pH 6.9 and 7.9 and the t_R values were compared. Other columns and conditions were investigated, including the use of an Aquasil column. To test the capability of the method for performing integrity and purity profiles, several Wyeth Research compounds were obtained internally and analyzed using the method. To test the feasibility of combinatorial analysis, a mixture of 20 drugs was prepared and analyzed in a single injection. The retention times of these compounds were compared to their retention times when individually analyzed.

3. Results and discussion

A set of six compounds, having Log $D_{7.4}$ literature values from −2.00 to +5.50 were selected and used to calibrate t_R versus Log $D_{7.4}$. These compounds are listed in Table 1, along with the average literature Log $D_{7.4}$ value [18] and t_R, as determined using the method. The calibration line is shown in Fig. 1. A correlation coefficient of 0.994 was obtained.

Initial work with the method indicated that the volume of injection was critical for obtaining proper Log D predictions, especially for more polar or lower-molecular-mass compounds. For this reason, injections are performed with 5 μl or less of sample. Higher injection volume causes double peaks or the rapid elution of some compounds, presumably be-

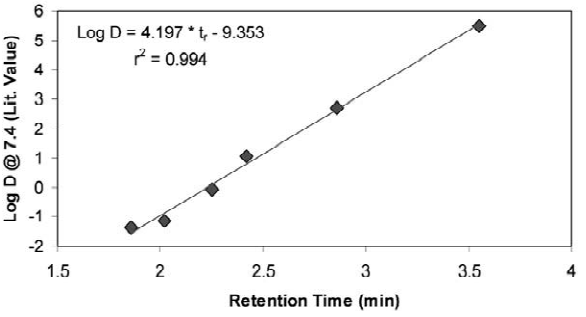

Fig. 1. Calibration line using the standards listed in Table 1 for calculating Log $D_{7.4}$ (HPLC) with test compounds.

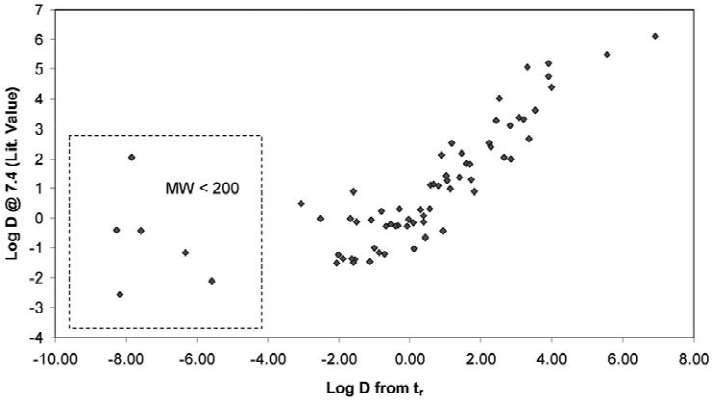

Fig. 2. Plot of literature Log $D_{7.4}$ versus predicted Log $D_{7.4}$ using the method for 70 compounds.

cause the local mobile phase in the region of the compound is greatly enhanced in organic content compared to the initial 100% aqueous mobile phase.

Test compounds, consisting of a set of 70 structurally diverse drugs and organic compounds, including acids and bases, were individually analyzed using the method and their respective predicted Log $D_{7.4}$ were calculated using the calibration line. The compounds are listed in Table 2, along with: their respective Log $D_{7.4}$ value from the literature, measured t_R, the predicted Log $D_{7.4}$, and the difference between the literature value and predicted value. The predicted value is plotted versus the literature value in Fig. 2. It is apparent that compounds with t_R<1.5 min are not well predicted. These compounds mostly have molecular masses less than 200. Thus, the Log $D_{7.4}$ of compounds with MW<200 or t_R<1.5 min should not be predicted using the method. These compounds may not have a sufficient number of partitions between the aqueous phase and the stationary phase. This shortcoming is not likely to be a significant problem in drug discovery because few compounds having MW<200 are studied.

Removal of the 10 outliers, discussed above, produces the correlation of Log $D_{7.4}$ (literature) with predicted Log $D_{7.4}$ according to the equation: Log $D_{7.4}$=0.974(Log $D_{7.4}$)+0.20. With the guidelines of using the method for compounds of MW> 200 and t_R>1.5 min, the method produces predictions of Log $D_{7.4}$ with an r^2 correlation of 0.89.

The reproducibility of the method was studied using duplicate analyses of each compound. Fig. 3 compares the t_R values of the two separate runs. The r^2 correlation was 0.999, the average deviation in t_R was 0.0016 min and the standard deviation in t_R was 0.00856, indicating good reproducibility.

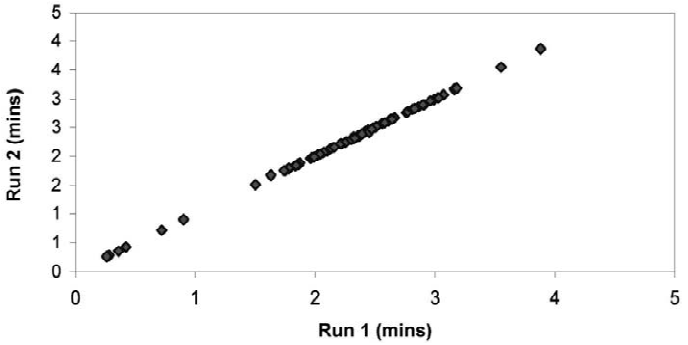

Fig. 3. Comparison of t_R of two separate runs of each test compound in Table 2.

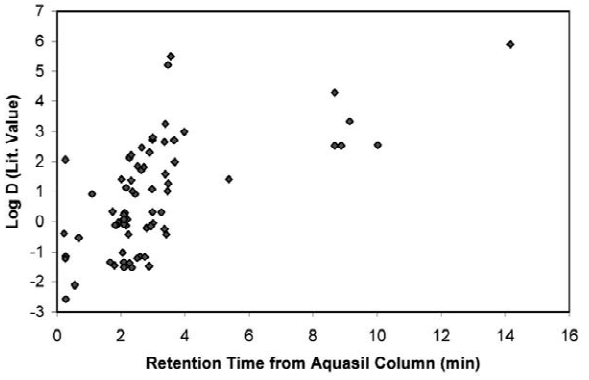

Fig. 4. Plot of literature Log$D_{7.4}$ versus predicted Log $D_{7.4}$ using an Aquasil column with the same mobile phase gradient.

The sensitivity of the method to pH variations was studied by analyzing each of the compounds under conditions in which the ammonium acetate was adjusted to pH 6.9, 7.4, or 7.9. At pH 6.9 the average deviation in t_R was 0.0037 min, with a standard deviation of 0.0202 min. At pH 7.9 the average deviation in t_R was −0.0071 min, with a standard deviation of 0.0288 min. It is apparent that small variations in the pH of the aqueous mobile phase have negligible effects on the measured retention times of the test compounds.

Other column conditions tested for this method did not produce as good of a correlation of t_R and literature Log $D_{7.4}$. One example is shown in Fig. 4 with an Aquasil column and the same mobile phase gradient. A large number of highly retained compounds were observed. In addition, there was much lower correlation of t_R and literature Log $D_{7.4}$ using the Aquasil column.

Use of the method in combination with integrity and purity profiling was investigated. Fig. 5 shows the results of typical integrity analyses using actual drug discovery compounds, in which minor components were observed. The method appears to be suitable for use in high throughput integrity profiling in addition to estimating Log $D_{7.4}$.

Another opportunity for increased pharmaceutical profiling throughput and efficiency is by combining compounds for analysis in a single run. The feasibility of using this method in a ''combinatorial'' mode was investigated by mixing 20 test compounds together and comparing each measured t_R, in the combinatorial mode, with the measured t_R, when individually measured. The results are shown in Fig. 6. It is apparent that the combinatorial approach does not compromise the results. The use of the mass spectrometer in this method allows for individual compounds to be de-convoluted. Thus, it is possible to save instrument analysis time by combining compounds for this analysis, when it is appropriate. A software routine for tagging each component and reporting retention times would be useful for the combinatorial experiment, in order that the bottleneck not shift to manual identification and instrument time by the analytical chemist.

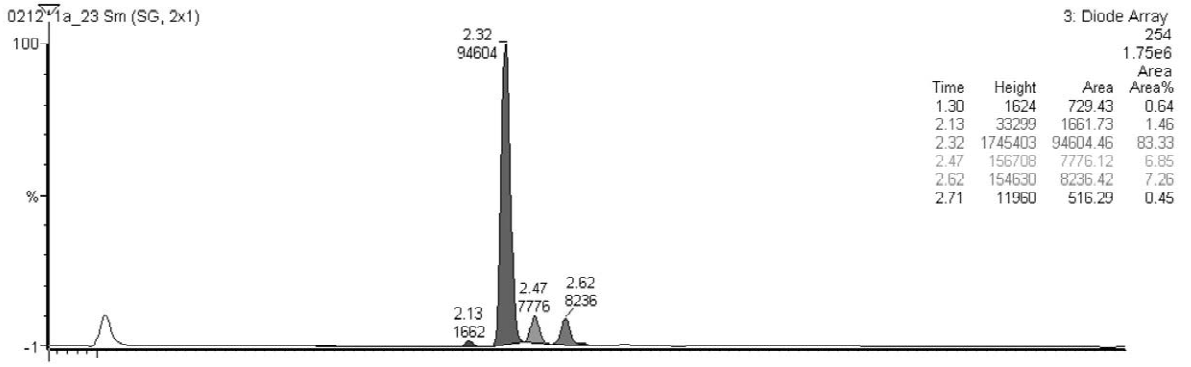

Fig. 5. Chromatogram of typical drug discovery sample using the method, indicating its suitability for integrity and purity analyses.

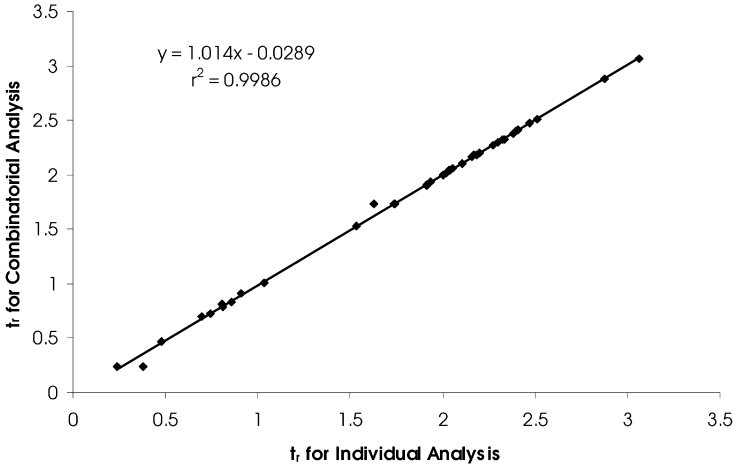

Fig. 6. Plot of t_R from combinatorial analysis of a mixture of 20 test compounds versus t_R from individual analysis of each compound.

4. Conclusion

Pharmaceutical profiling assays provide a rapid assessment of the properties of compounds in drug discovery. They are not intended to provide the precision and accuracy of established methods, typically used for in-depth analysis in late discovery or development. Their utility is to rapidly provide property information to increase medicinal chemist's early knowledge and assist informed decisions in drug discovery. Research teams typically assess libraries of thousands of compounds in a rapid manner with very low quantity of sample (mg level) available for property or activity assays. These constraints dictate the nature of the methods that can be applied for pharmaceutical profiling.

The rapid gradient reversed-phase method appears to be suitable for the initial profiling of lipophilicity of drug discovery compounds via Log $D_{7.4}$. The method shows good correlation to literature Log D values that were measured by more in-depth methods. The limitations of the method are that compounds having MW<200 or t_R<1.5 min are not likely to be well predicted. Log $D_{7.4}$ is predicted using a calibration of t_R to literature Log D at pH 7.4 for six drug or organic molecules. Good reproducibility from run to run was obtained. The method has little sensitivity to changes of 0.5 pH units. The data is also suitable for rapidly profiling the integrity, purity and Log D of a sample in a single analysis run, in order to produce greater efficiency. Another opportunity for increased efficiency is to combine compounds into mixtures for a single ''combinatorial'' analysis. The mass spectrometer allows deconvolution of the mixture to obtain information on individual compounds. The method is applicable for rapid, initial profiling of large numbers of compounds investigated in drug discovery and very definitive measurements should be made using other methods [12,13,18] as fewer compounds are under study in later drug discovery stages. Another benefit is that no additional time or material is required to obtain lipophilicity information, when integrity/purity profiling is already being performed. This provides significant savings of resources and precious materials that are often only available in small amount in drug discovery.

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