

High-Throughput pKa with the Sirius Profiler SGA using a co-solvent approach

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Introduction

Spectrophotometric titration with the Sirius SGA is a powerful method for high throughput screening of ionization constants as it :

- ✓ Allows working with concentrations about 10-times lower than potentiometric titration,
- ✓ is faster (as it is based on a calibrated pH-gradient), 2-4min/sample vs. 20-40min/sample for potentiometric titration,
- ✗ but interferences due to sample precipitation still occur, as most lead optimization candidates are less soluble than generic drugs

→ We developed a co-solvent approach for our HT-pKa assay.

Principle of the Method

We have chosen a mixture of **Diethylene glycol monoethyl ether (DGME), Acetonitrile (ACN) and MeOH** in a 1:1:1 ratio as the co-solvent which best combines solubility enhancement and acceptable optical properties

One issue is that it is difficult to combine aqueous and co-solvent runs with a single instrument while maintaining a reasonable throughput.

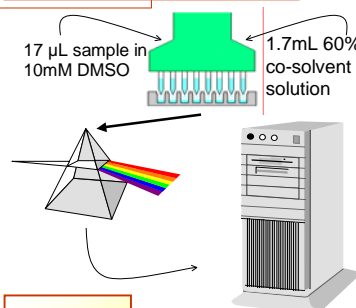
- Shift from aqueous to co-solvent runs require :
 - ✗ loading/unloading of the buffer reagents
 - ✗ recalibration of the instrument (too time consuming in the normal workflow of a high-throughput assay).

✓ However, it is possible to combine the 15 and 25% co-solvent runs without changing buffer reagents if

→ **the sample is loaded pre-diluted in the presence of 60% co-solvent.**

The acid and basic buffer reagents contain 15%co-solvent, thus an additional 10% co-solvent can be brought by the sample flow. We called this approach the 15+10%co-solvent method. At Novartis, we are routinely running this method for the HT-pKa assay, as most Lead Optimization Candidates are low soluble, otherwise it would be too time and sample consuming.

Sample Processing



The presence of the co-solvent induces a slight shift in the ionization constants of the reference standards. pKa values of these standards were measured separately and the co-solvent corrected values are used to construct the calibration curve. Samples are pre-dissolved in the dilution plate in the presence of 60% co-solvent.

To avoid sample precipitation and prior to titration :

- 1 mM HCl is added to the mixture when an Up method (pH 2-12) is selected (usually for Bases)
- 3 mM KOH when a Down method (pH 12-2) is selected (usually for Acids).

The use of the ACD/pKa DB software (ACD labs, Toronto, Canada) is required to calculate the ionization model, and approximate pKa values (seed values that will be used for data analysis).

→ **We process all samples with both Up and Down methods to avoid phantom pKa(s) due to precipitation.**

Results are refined once with automated TFA, then checked by the operator that adds/removes pKa(s) to get the correct result.

Results

60 generic drugs (27 bases, 20 acids and 13 ampholytes) were used in the study. Each compound was measured in aqueous medium and at two co-solvent concentrations (15 and 25%).

Table A	GlpKa		15% co-solvent			15+10% co-solvent		
	pKa1	pKa2	pKa1	pKa2	bias	pKa1	pKa2	bias
Acetaminophen	9.7		9.8		-0.1	9.7		0
Chlorothalidate	6.2	9.2	6.4	9.4	-0.2/-0.2	6.2	9.4	0/-0.2
Diclofenac	4.0	4.3			-0.3	4.2		-0.2
Estradiol		10.2				10.3		-0.1
Etoposide	9.6		10.1		-0.5	9.8		-0.2
Flurbiprofen	4.0	4.4			-0.4			
Ketoprofen	4.0	4.4			-0.4	4.5		-0.5
Mefenazone	10.3*	10			0.3	9.9		0.4
Nisiporen	4.7	4.2			0			
Phenylethanol	8.2					8.5		-0.3
Salicylic acid	2.9	3			-0.1	3		-0.1
Warfarin	4.8	5			-0.2	5.1		-0.3
Celluloxime	2.2	-	2.7		-0.5			
Entacapone	4.0	10.9*	4.3	10.4	-0.3/0.5	3.9	10.5	0.1/0.4
Furosemide	3.5	10.6	3.6	10.4	-0.3/0.2	3.7	10.4	-0.2/0.2
Hydrochlorothiazide	8.9	10.0	9.1	10.2	-0.2/-0.2	9.2	10.1	-0.3/0.1
Sulfasalazine	2.1	7.9	2.7	8.2	-0.6/-0.3	2.3	8.3	-0.2/-0.4
Average bias					-0.3			-0.3

Table B	GlpKa		15% co-solvent			15+10% co-solvent		
	pKa1	pKa2	pKa1	pKa2	bias	pKa1	pKa2	bias
Chlorpromazine	9.0		8.8		0.2	8.7		0.3
Clonidine	8.0		7.9		0.1			
Desipramine	10.1		9.8		0.3	9.7		0.4
Ganciclovir	9.4					9.4		0
Imipramine	9.3		9		0.3			
Prazosin	6.8		6.6		0.2	6.6		0.2
Propranolol	9.5		9.1		0.4			
Chloroquine	8.3	10.2	7.8	9.9	0.5/0.3			
Clozapine	3.7	7.5				3.6	7.1	0.1/0.4
Nicardipine	9.5	7.2				9.5	6.9	0.3
Quinidine	4.1	8.5	3.9	8.4	0.2/0.1	4	8.1	0.1/0.4
Quinine	4.2	8.6	3.9	8.4	0.3/0.2	3.9	8.3	0.3/0.3
Average bias					0.2			0.2

→ values must be corrected with the bias correction : -0.3 for acids and 0.2 for bases.

The number of successful determinations is significantly increased by the addition of co-solvent.

- with acids and ampholytes : **success rate increases** considerably in the presence of co-solvent.
 - with bases, the net effect of co-solvent addition was more modest : compounds which
 - **failed** in absence of co-solvent → **passed** when the co-solvent is added (desipramine, prazosin, nicardipine).
 - **passed** in absence of co-solvent → **failed** with the co-solvent as the UV signal is lowered or lost (alprenolol, diltiazem).
- 15% co-solvent was not enough to get good data for very low soluble compounds (estradiol, quinidine) → **passed** with 15+10%co-solvent. Other compounds (cefuroxime, clonidine) **passed** at 15% co-solvent but **failed** at 25% as UV signal was lowered or lost.

Conclusion

The systematic use of co-solvent appears to be the strategy of choice as it combines **good success rate** with **adequate throughput**. With the generic drugs tested, the overall success rate is 65-70%.

Ionization constants obtained using the combined co-solvent approach compare favorably with reference values and average bias corrections can be used to extrapolate back to aqueous.

This strategy is now routinely used to screen lead optimization compounds, which on average are significantly less soluble than generic drugs, with an **average success rate of 70%** (based on the number of ionizations identified by the ACD pKa DB software).