



Stoichiometric Determination of Trifluoroacetate Counter-Ions in Drug Discovery



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Abstract

Purpose: A potentiometric titration method is described for determining drug:TFA stoichiometry.

Methods: A weighed sample with unknown trifluoroacetic acid (TFA) content is dissolved in water and analyzed by potentiometric acid-base titration using a Sirius GLpKa automatic titration system. A series of models is proposed which provide an initial estimate of the compound's stoichiometry and formula weight (e.g. B(TFA) = 1:1 stoichiometry; B(TFA)₂ = 1:2, with FW = 414 or 528 respectively, assuming B has MW = 300), and the data is refined to determine the sample's pK_a. The excess titratable acidity is determined by plotting a linear graph of the proposed number of TFA moieties vs. the acidity error from the refinement of each proposed model. This acidity is positive if the stoichiometry is underestimated or negative if overestimated. The correct stoichiometry is equivalent to the number of TFA when acidity error = 0.

Results: Preparative chromatography with TFA in the mobile phase is commonly used to isolate basic compounds in drug discovery. TFA counter-ions bind with bases in various stoichiometries, depending on the complexity and number of ionizable groups of the compound. TFA is a relatively heavy counter-ion (MW = 114) and can make a significant contribution to the weight of solid used in biochemical assays. Any concentration-dependent results should therefore factor in the proportion of TFA.

Conclusion: It has been shown that potentiometric titration can be used to determine the amount of trifluoroacetate incorporated into a sample during preparative chromatography.

Introduction

Trifluoroacetic acid (TFA) is widely used in HPLC mobile phases, primarily due to its low UV cutoff (210 nm at 0.1%, v/v) and effectiveness as an ion-pairing agent. TFA is therefore a common counter-ion with compounds isolated by preparative HPLC. These compounds may be used without further purification for various concentration-dependent biological assays in order to determine the efficacy of the drug discovery compound. The method of compound isolation and purification influences the final amount of counter-ion present, and given the relatively high molecular weight of TFA (114 g/mol), the association of just one molecule with a compound with a molecular weight of 500 would increase the formula weight by more than 20%. Clearly, the amount of TFA associated with a compound must be determined to avoid the introduction of a significant source of error into concentration-dependent assay results.

TFA is a strong acid, and a pK_a value of 0.52 has been determined at zero ionic strength [1]. We used a method based on Debye-Hückel theory to convert this to a pK_a value of 0.275 in 0.15M ionic strength solution. This low pK_a indicates that TFA exists only in anionic form during titrations at pH 1.8 and above.

The concentration of TFA may be measured by a number of techniques including headspace GC using derivatization to produce the methyl ester, ion chromatography, capillary electrophoresis (CE), ¹⁹F nuclear magnetic resonance (NMR), or fast CE- and NMR-based methods. The stoichiometry may then be deduced if the drug concentration is also known. However, the ratio of concentrations of drug to TFA may be measured **directly** by the simple titration experiment described here, which is a routine procedure to measure the pK_a of the drug.

References

[1] Kurz, J.L. Farrer, J.M. JACS, 1969, 91, 6057

Methods

Solid samples were accurately weighed and dissolved in 8mL aqueous 0.15M KCl solution. Compound 1 was dissolved in a solution containing 48.2% methanol; compound 2 was dissolved in purely aqueous solution. The weight of each sample was high enough to ensure that the contribution of the sample to the buffer capacity was at least five times higher than the contribution of the background electrolyte. Note that the higher the sample concentration, the more reliable the stoichiometry determination.

The pH was lowered below 1.8 by adding a measured volume of standardized 0.5M HCl solution. The solution was then titrated with standardized 0.5M KOH solution and the pH was recorded after each titrant addition together with the mL of KOH required to cause that pH change. The pK_a values of each sample were calculated from the pH/mL data using Sirius RefinementPro 2 software. The TFA content was deduced using a calculation made using Microsoft Excel. Titrations were done using a Sirius GLpKa instrument. Experiments were conducted at 25.0 ± 0.2°C.



Calculations

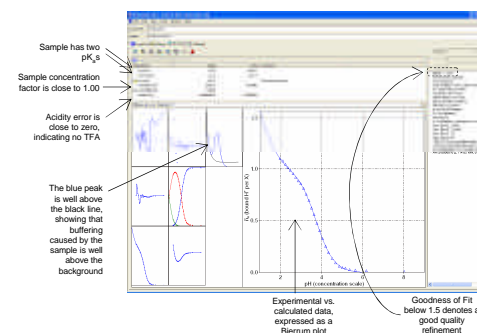
Measuring pK_a values

The pK_a values of each compound are determined by a mathematical analysis of its titration curve. In this process, the shape of the curve is predicted by calculating the pH of each point in a simulated titration, and comparing the experimental and calculated points. Each pH value is calculated by applying equations based on mass balance and charge balance. The parameters considered in these equations are:

Parameters which are fixed during refinement		Variable parameters
Initial volume	HCl and KOH concentrations	Sample concentration factor
Volume of HCl added	Sample weight and molecular weight (from which sample concentration is calculated)	Concentration of dissolved CO ₂
Volume of KOH added		Sample pK _a values
Calibration of pH electrode	Ionization model of sample (i.e. number of pK _a s, and whether acidic or basic)	Acidity error
Temperature and ionic strength point-by-point		

Refinement calculation

This picture shows a Summary Page from RefinementPro 2 with a result for Compound 1, a compound with two pK_as that contained no TFA. During the calculation, values for the variable parameters are systematically modified in a computerized calculation (called "refinement") until the experimental and calculated curves achieve the best agreement. The pK_a values required to achieve this good agreement are taken to be the correct measured pK_a values.



Measuring TFA concentration

The concentration of TFA is determined from the acidity error. Acidity error represents any excess strong acid or base present during the titration that is not accounted for by the acid or base introduced as titrants. Acidity errors determined in pK_a measurement of pure samples are usually very small (typically ±0.0001M or less) and often represents a correction for errors in the titrant standardization.

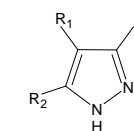
If a sample contains excess TFA left over from the synthesis or chromatographic purification, its effect on the data is to cause a large acidity error.

The acidity error for compound 1 is -0.000006M, indicating that compound 1 contains no TFA.

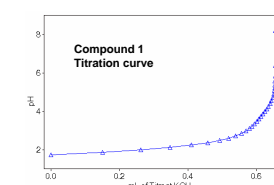
The acidity error for compound 2 is +0.001682. Because TFA was used in the sample preparation, this error was believed to be caused by TFA. To calculate the TFA content, the data must be refined as follows. First, the sample formula is entered as the unionised molecule (e.g. XH for a sample with one acidic and one basic pK_a). The molecular weight is also entered for that formula, the refinement is done and the acidity error is noted. Next, the formula and molecular weight are entered as if each molecule of sample was associated with one molecule of TFA, using the symbol A to represent TFA (e.g. XAH₂). Again the refinement is done and the acidity error is noted. The formula and molecular weight corresponding with XH associated with two, three or four molecules of TFA can also be used. A table is made in Excel showing acidity error vs. number of TFA molecules per molecule of XH. These terms are then plotted in an X-Y graph. The number of TFA molecules when the acidity error is zero denotes the stoichiometry of the compound.

Results

The method was used to measure TFA content of two similar compounds with low MW (188.19). Both compounds were ampholytes, with one acidic pK_a about 3.5 and one basic pK_a below 1.5. Although pK_as below 1.8 often cannot be measured by pH-metric techniques, they could be measured here because the other pK_a stabilized the refinement calculation, and because the concentration of sample was high. Plots of acidity error vs. number of TFA molecules per molecule of XH are shown below.



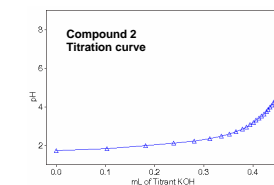
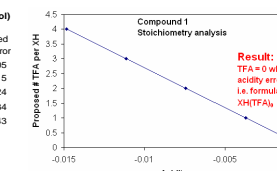
Partial structure of Compounds 1 and 2



Compound 1, titrated in 0.15M KCl (48% methanol)

Proposed #	Proposed formula	MW of proposed formula	Calculated Acidity error
0	XH	188.19	-0.000005
1	XAH ₂	302.21	-0.003715
2	XAH ₃	416.23	-0.007424
3	XAH ₄	530.25	-0.011134
4	XAH ₅	644.27	-0.014843

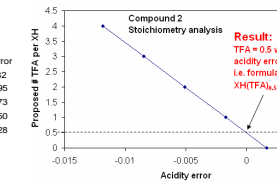
Weight of compound: 0.0056g
 Concentration of XH if no TFA: 0.00372M
 pK_a values determined from the data: 1.114, 3.738
 Goodness of fit: 1.11



Compound 2, titrated in aqueous 0.15M KCl

Proposed #	Proposed formula	MW of proposed formula	Calculated Acidity error
0	XH	188.19	0.001682
1	XAH ₂	302.21	-0.001695
2	XAH ₃	416.23	-0.005073
3	XAH ₄	530.25	-0.008450
4	XAH ₅	644.27	-0.011828

Weight of compound: 0.0071g
 Concentration of XH if no TFA: 0.00472M
 pK_a values determined from the data: 1.327, 3.675
 Goodness of fit: 0.86



Conclusion

Drug:TFA stoichiometry is easily determined as a by-product of pH-metric pK_a measurement.

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