



Original article

Test article concentrations in the hERG assay: Losses through the perfusion, solubility and stability

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ABSTRACT

Introduction: Drug-induced prolongation of the electrocardiographic QT interval (long QT syndrome) has been associated with increased risk of a serious ventricular arrhythmia, torsade de pointes. Inhibition of hERG, a cardiac potassium channel that controls action potential repolarization, is the most common cause of QT prolongation by non-cardiac drugs. The ICH S7B describes preclinical safety testing required for new drugs, including the determination of the hERG IC₅₀. Actual and target concentrations may differ due to solubility, stability, or loss of compound. Significant differences will invalidate quantitative concentration–response curves which may be critical to interpretation of drug safety. To examine the frequency and significance of these differences, we conducted an analysis of studies where both the electrophysiology and the dose solution analysis were conducted in-house. We have investigated the actual concentrations of test article in vehicle solution as compared to the target concentrations in an attempt to determine the reasons behind differences between the two values. **Methods:** Studies that involved both electrophysiology and dose solution analysis performed at ChanTest Corporation were evaluated. The effects of stability, solubility and loss through the perfusion apparatus on actual dosing concentrations were investigated. **Results:** There was a large range in the loss of the test article attributed to the perfusion apparatus (range from 0 to 74% loss). For 12 of the 22 studies evaluated, the IC₅₀ was >2-fold more potent when using actual values as determined by HPLC versus the target concentrations. Twenty-two percent of the test articles were not stable 24 h after room temperature storage; 16% after 24 h frozen conditions. **Discussion:** The best practices when considering dose solution concentration verification of test article solutions are to: determine the solubility of the compound in a physiological buffer, analyze samples collected from the perfusion chamber, and analyze samples the same day as sample collection (e.g., same day as hERG assay).

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1. Introduction

Drug-induced prolongation of the electrocardiographic QT interval (long QT syndrome) has been associated with increased risk of a serious ventricular arrhythmia, torsade de pointes. Inhibition of I_{Kr} , a potassium current that controls cardiac action potential repolarization, is the most common cause of QT prolongation by non-cardiac drugs (Brown & Rampe, 2000; Weirich & Antoni, 1998; Yap & Camm, 1999). The cardiac potassium channel, hERG, is responsible for a rapid delayed rectifier current (I_{Kr}) in human ventricles (Curran et al., 1995; Sanguinetti, Jiang, Curran, & Keating, 1995). Drugs that inhibit hERG have the potential to prolong the cardiac action potential and the QT interval, and cause torsade de pointes. A number of drugs, including antibiotics, antipsychotics, and gastrointestinal prokinetic agents, have been withdrawn from the market due to delaying ventricular

repolarization and increasing the risk of torsade de pointes (Roy, Dumaine, & Brown, 1996; Suessbrich, Waldegger, Lang, & Busch, 1996; Mohammad, Zhou, Gong, & January, 1997; Rampe, Roy, Dennis, & Brown, 1997; Iannini, 2002; Ray et al., 2004). New chemical entities investigated for use in humans are now required to be evaluated for this potentially lethal side effect. The International Conference of Harmonisation issued the S7B guideline “The Non-Clinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals” in 2005 which describes non-clinical testing strategies to evaluate new chemicals for the potential to prolong the QT interval (www.ich.org). This document supports the evaluation of a new chemical entity in an *in vitro* electrophysiological hERG assay and in an *in vivo* QT assay. In the hERG assay, tests are conducted over a range of concentrations sufficient to determine the IC₅₀ or the maximum inhibitory effect at the highest achievable concentration within the physico-chemical limits of the assay (e.g., solubility or cytotoxicity).

Section 58.113 of 21 CFR Part 58 (Good Laboratory Practice for Non Clinical Laboratory Studies) of the Food & Drug Administration Code of Federal Regulations requires that the uniformity, stability,

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and concentration of a test and/or control article in a solvent/vehicle be determined. This is to ensure that the actual concentrations of test article applied to the test system are comparable to the target concentrations. A validated analytical method for each test compound in the vehicle is developed to accurately measure the test article concentrations in the vehicle solution. Poor solubility, stability and/or binding to the study equipment can lower the actual drug concentration applied to the test system (Herron, Tower, & Templeton, 2004). Thus, if analytical methodology is not used to determine the actual concentrations in the vehicle solution applied to the test system, the possibility exists of overestimating or underestimating the concentrations applied. This would result in an inaccurate IC_{50} , with the reported IC_{50} being higher or lower than the actual value. In the case of the hERG safety test, errors would occur in the assessment of hERG risk and the calculated safety margin (Redfern et al., 2003).

We conducted an analysis of studies where both the electrophysiology and the dose solution analysis were conducted in-house. We have investigated the actual concentrations of test article in vehicle solution as compared to the target concentrations in an attempt to determine the reasons behind differences between the two values.

2. Methods

2.1. Cell culture

Human embryonic kidney (HEK293) cells were stably transfected with hERG cDNA. Stable transfectants were selected by coexpression with the G418-resistance gene incorporated into the expression plasmid. Selection pressure was maintained by including G418 in the culture medium. Cells were cultured in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (D-MEM/F-12) supplemented with 10% fetal bovine serum, 100 U/mL penicillin G sodium, 100 µg/mL streptomycin sulfate and 500 µg/mL G418. Cell culture stock plates were prepared each week. Cells for electrophysiological recording were cultured in 35-mm plastic dishes.

2.2. Vehicle solution

HEPES-buffered physiological saline solution (HB-PS) consisted of (composition in mM): NaCl, 137; KCl, 4.0; CaCl₂, 1.8; MgCl₂, 1; HEPES, 10; Glucose, 10; pH adjusted to 7.4 with NaOH (prepared weekly and refrigerated until use), supplemented with 0.3% DMSO. Canine Purkinje fiber buffer was composed of (composition in mM): NaCl, 131; KCl, 4.0; CaCl₂, 2.0; MgCl₂, 0.5; NaHCO₃, 18.0; NaH₂PO₄, 1.8; Glucose, 5.5, supplemented with 0.3% DMSO. Rabbit Purkinje fiber buffer was composed of (composition in mM): NaCl, 131; KCl, 5.4; CaCl₂, 2.0; MgCl₂, 0.5; NaHCO₃, 18.0; NaH₂PO₄, 1.8; Glucose, 5.5, supplemented with 0.3% DMSO.

2.3. Electrophysiology

Cells in plastic culture dishes were transferred to the stage of an inverted phase-contrast microscope. Cells were superfused with vehicle control solution (HB-PS). Micropipette solution for whole cell patch clamp recordings was composed of (mM): potassium aspartate, 130; MgCl₂, 5; EGTA, 5; ATP, 4; HEPES, 10; pH adjusted to 7.2 with KOH. Micropipette solution was prepared in batches, aliquoted, stored frozen, and a fresh aliquot thawed each day. The recording was performed at a temperature of 35 ± 2 °C using a combination of in-line solution pre-heater, chamber heater, and feedback temperature controller. Temperature was measured using a thermistor probe in the recording chamber. Micropipettes for patch clamp recording were made from glass capillary tubing using a P-97 micropipette puller (Sutter Instruments, Novato, CA). A commercial patch clamp

amplifier was used for whole cell recordings. Before digitization, current records were low-pass filtered at one-fifth of the sampling frequency.

Cells stably expressing hERG were held at -80 mV. Onset and steady state inhibition of hERG potassium current due to the test article were measured using a pulse pattern with fixed amplitudes (conditioning prepulse: +20 mV for 1 s; repolarizing test ramp to -80 mV (-0.5 V/s) repeated at 5 s intervals. Each recording ended with a final application of a supramaximal concentration of the reference substance (E-4031, 500 nM), to assess the contribution of endogenous currents. The remaining uninhibited current was subtracted off-line digitally from the data to determine the potency of the test substance for hERG inhibition. One or more test article concentrations were applied sequentially (without washout between test substance concentrations) in ascending order, to each cell. Peak current was measured during the test ramp. A steady state was maintained for at least 20 s before applying test article. Peak current was measured until a new steady state was achieved or 12 min of exposure time had elapsed.

2.4. Electrophysiology patch clamp data analysis

Data were stored on the ChanTest computer network (and backed-up nightly) for off-line analysis. Data acquisition and analyses were performed using the suite of pCLAMP (Version 8.2) programs (MDS-AT, Sunnyvale, CA). Steady state was defined by the limiting constant rate of change with time (linear time dependence). The steady state before and after test article application was used to calculate the percentage of current inhibited at each concentration. Concentration–response data were fit to an equation of the form:

$$\% \text{ Inhibition} = \{1 - 1/[1 + ([\text{Test}]/IC_{50})^N]\} * 100$$

Where [Test] is the test article concentration, IC_{50} is the test article concentration at half-maximal inhibition, N is the Hill coefficient, and % Inhibition is the percentage of current inhibited at each test article concentration. Nonlinear least squares fits were solved with the Solver add-in for Excel 2000, or later (Microsoft, WA). The IC_{50} was calculated if the test article produced greater than 50% inhibition at the highest concentration.

2.5. Analytical chemistry methods

Analytical methods were developed for each test article. High Performance Liquid Chromatography (HPLC) instruments using UV detection from Waters Corporation were used. The test method procedure was validated to establish stability, linearity, accuracy and intra-assay precision (repeatability), and lack of interference in the chromatographic region of interest. Data collection was performed and peak areas of the test articles were measured using Empower software from Waters Corporation. Descriptive statistics including arithmetic mean (average) and standard deviation, % CV (coefficient of variance) and % RE (relative error) were conducted using Microsoft Excel, version 2000, or later (Microsoft, WA). The accuracy of the dose solution samples was measured as the % RE using the equation % RE = 100 × (nominal concentration - actual concentration) / nominal concentration. The % change was used for the calculation of the stability of the samples using the equation % change = 100 × (measured concentration at T_n - mean concentration at T_0) / mean concentration at T_0 , where T_n = time of the non-zero time point. The acceptance criterion for accuracy of the dose solution concentrations was defined as ± 15% or in some studies, ± 10% of the target concentration. Likewise, the acceptance criterion for the stability of the formulations was defined as ≤ 15%, or in some studies, ≤ 10% change in concentration between time points.

3. Results

Our previous experience when dose samples were sent off-site for analysis suggested that a variety of factors lead to differences between the nominal and actual concentration of dosing formulations. Therefore, we conducted a review of studies where both the electrophysiology and the dose solution analysis were conducted in-house.

3.1. Decrease in actual concentration due to exceeding the solubility limit

We encountered many instances where test article solubility had been determined previously in solvents like DMSO or ethanol, but solubility in physiological buffer (pH=7.2–7.4) was not known. Solutions with visible precipitate can interfere with the gigaohm seal needed for patch clamp assays making the hERG assay impossible. Some solutions, though, have precipitate that is not visible even microscopically. When testing such formulations, the actual concentrations may differ significantly from target concentrations. Fig. 1 demonstrates an example of a compound where precipitation was not visible, but concentrations of 2, 3, and 10 μM inhibited hERG current to the same extent. This result suggests limited solubility in the hERG buffer or partial antagonism. The formulations were later tested for solubility by nephelometry. The solubility limit of the test article in hERG buffer was determined to be 1.8 μM ; precisely where the effect on hERG current began to level off. The limited solubility could explain the inability to obtain full inhibition of the hERG channel.

3.2. Decrease in actual concentration due to loss of test article in the perfusion system

Analytical results from thirty-nine (39) studies were reviewed to determine the prevalence of a decrease in actual concentration of test article applied to the test system due to loss of test article in the perfusion system. Results were calculated as % change from the test article concentration in the solution reservoir to the test article concentration after perfusion through the delivery system (Table 1). There was a large range in the loss of the test article attributed to the perfusion apparatus (range from 0.0% to 73.9% loss; Table 1 and Fig. 2). There were examples where loss of the test article was consistent across all the concentrations tested (e.g., Study C) and

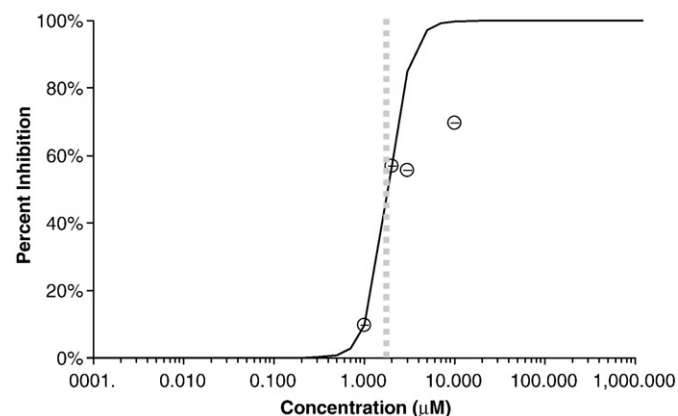


Fig. 1. Example of a test article where the effect reached saturation before 100% inhibition was observed. Circles represent the percent hERG current inhibition observed. The solid line represents a fit to an equation of the form $\% \text{ Inhibition} = \{1 - 1 / [1 + ([\text{Test}] / \text{IC}_{50})^n]\} * 100$. The dashed vertical line represents the solubility limit of the compound in physiological buffer (1.8 μM).

Table 1

Loss of test article in the perfusion apparatus

Study number	%Loss of test article in the perfusion apparatus				
	Concentration				
	1	2	3	4	5
A	0.0	1.4	-14.9	N/A	N/A
B	-0.9	-1.1	-3.4	N/A	N/A
C	3.9	-1.0	-0.3	-5.5	N/A
D	0.0	-0.6	N/A	N/A	N/A
E	0.0	0.0	0.0	0.0	N/A
F	0.6	0.2	N/A	N/A	N/A
G	2.0	0.0	0.8	2.3	N/A
H	7.7	-3.8	N/A	N/A	N/A
I	-1.1	-0.3	0.6	-1.3	N/A
J	0.0	0.0	0.1	1.2	N/A
K	1.7	-11.7	2.5	0.0	N/A
L	-13.1	-10.5	-6.5	-4.7	N/A
M	-3.3	1.1	1.8	-0.6	N/A
N	3.2	12.4	N/A	N/A	N/A
O	0.0	-33.3	N/A	N/A	N/A
P	-20.8	-25.5	N/A	N/A	N/A
Q	-0.8	0.0	0.0	-0.6	N/A
R	0.1	0.5	0.1	0.1	N/A
S	-0.3	0.5	1.1	0.3	0.5
T	0.0	-4.1	-2.0	-0.8	N/A
U	-5.8	-1.0	-0.4	-3.7	N/A
V	-12.5	-1.7	-5.5	0.5	N/A
W	-6.4	-0.4	N/A	N/A	N/A
X	1.0	-1.3	N/A	N/A	N/A
Y	-1.0	0.4	N/A	N/A	N/A
Z	8.5	1.0	-0.2	N/A	N/A
AA	-9.6	-10.5	-6.9	-8.9	N/A
BB	-1.9	-1.9	N/A	N/A	N/A
CC	0.0	-1.0	2.0	N/A	N/A
DD	-73.9	N/A	N/A	N/A	N/A
EE	0.0	1.1	1.0	-15.2	N/A
FF	-14.9	N/A	N/A	N/A	N/A
GG	0.0	3.6	1.0	-5.1	-8.6
HH	2.4	15.1	2.9	1.3	N/A
II	-3.7	-0.6	-0.6	N/A	N/A
JJ	0.0	0.6	-4.8	0.0	N/A
KK	-15.9	-5.7	-6.0	-0.3	N/A
LL	-32.2	-5.5	N/A	N/A	N/A
MM	-8.5	N/A	N/A	N/A	N/A

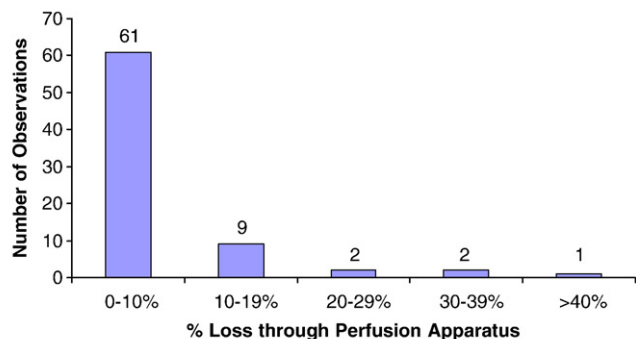
The % loss of the test article due to the perfusion system was calculated using $100 * (\text{concentration after perfusion} - \text{concentration before perfusion}) / \text{concentration before perfusion}$. Negative numbers indicate a loss of the test article in the perfusion system. N/A = concentration not tested in the assay.

examples where the loss was more extreme at either the lowest concentration tested (e.g., Study KK) or the highest concentration tested (e.g., Study EE). Overall, loss in the perfusion apparatus was observed more frequently when testing lower concentrations, with larger losses occurring too.

3.3. Decrease in actual concentration due to either loss of test article in the perfusion system or lack of stability of the test article in vehicle

Data from twenty-two (22) hERG assays in which concentration verification of the dosing solutions was performed were reviewed. These assays were not conducted following GLP guidelines, and therefore, the stability of the dosing solutions was not evaluated. Analytical methods were developed that had acceptable accuracy, precision, linearity, chromatographic non-interference, and robustness. Samples from each concentration of test article were collected from the perfusion chamber before application to the test system and analyzed using High Performance Liquid Chromatography (HPLC) the same day. For 12 of the 22 studies evaluated, the IC_{50} was >2-fold more potent when using actual values as determined by HPLC versus the target concentrations (Table 2).

A Low Concentrations



B High Concentrations

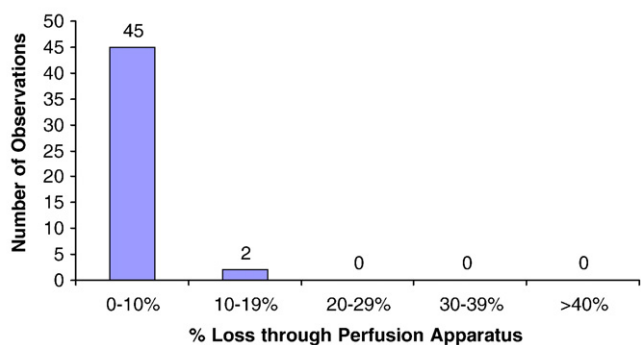


Fig. 2. Frequency distribution of the loss of test article in the perfusion apparatus.

3.4. Decrease in actual concentration due to lack of stability of the test article in vehicle

A series of thirty-seven (37) studies that were conducted following GLP guidelines was evaluated. In contrast to the studies previously described (Table 2), stability was evaluated under various conditions in this group of 37 studies. During analytical method validation, samples were prepared in hERG vehicle, stored for a time period under different storage conditions, analyzed for concentration after the storage period

Table 2
Differences in IC₅₀ when using actual versus nominal concentrations

Test article	Nominal IC ₅₀ (μM)	Actual IC ₅₀ (μM)
<i><2 fold</i>		
1	15.256	17.076
2	7.177	7.159
3	1.309	1.041
4	9.785	7.494
5	1.044	0.611
6	109.208	81.115
7	260.158	240.714
8	332.664	310.940
9	1.045	0.640
10	7.153	4.140
<i>>2 fold</i>		
11	1.278	0.260
12	0.853	0.405
13	10.660	5.399
14	3.584	1.350
15	143.459	71.464
16	0.821	0.375
17	0.372	0.123
18	0.195	0.082
19	0.952	0.388
20	155.685	57.922
21	6.474	2.698
22	4.559	1.964

and compared to time 0. Samples were stored under two conditions (room temperature and frozen). Samples were analyzed after 8 h. A longer stability time period (24 h) was evaluated for some of the test articles (Table 3). Acceptable stability was defined as ≤15% change in concentration after storage. Of the 37 studies evaluated, there were 5 instances where stability of the test article failed (– indicator in column) when analyzed 8 h after storage at room temperature. Three of these failures were analyzed under shorter storage periods and found to be stable between 3 and 6 h when stored under room temperature conditions. Thus, instability occurred between 3 or 6 h and 8 h in approximately 10% of the cases. After 24 h of room temperature storage, 3 more test articles failed stability. Thus, 22% (8 of 37) test articles were not stable for 24 h at room temperature. When stored under frozen conditions, 4 test articles failed stability after 8 h, and two additional test articles failed after 24 h resulting in 16% of the test articles not being stable after 24 h under frozen conditions. Each of the test articles that failed stability after 24 h under frozen conditions also failed stability after 24 h room temperature. Therefore, the addition of the freeze–thaw cycle that occurs after frozen conditions does not account for the lack of stability.

4. Discussion

4.1. Decrease in actual concentration due to loss of test article in the perfusion system

The loss of test article in the perfusion apparatus was variable but could be significant. Due to this loss, concentration verification samples should be collected from the recording chamber, and not just the solution reservoirs, to provide the most accurate description of what

Table 3
Stability evaluation of test articles in hERG vehicle under various storage conditions

Study number	Room temperature			Frozen			
	8 h	24 h	Time (x)	8 h	24 h	Time (x)	Time (x)
1	–	–		–	–		
2	√	–		√	√		
3	√	√		√	√		
4	√	√		nd	nd		
5	√	√		√	√		
6	√	√		√	√		
7	√	√		√	√		
8	√	√		√	√		
9	√	√		√	√		
10	√	√		√	√		
11	√	–		√	–		
12	–	–	√(4 h)	–	–		
13	√	√		√	√		
15	–	nd		nd	nd		
16	–	–	√(4 h)	–	–		
17	√	√		√	√		
18	√	√		√	√		
19	√	√		√	√		
20	√	√		√	√		
21	√	√		√	√		
23	√	√		√	√		
24	√	√		√	√		
25	√	√		√	√		
26	√	nd		√	√		
27	√	√		√	√		
28	√	√		nd	nd		
31	nd	nd		nd	nd	√(3 h)	–(10 d)
32	nd	nd	√(4 h)	nd	nd		
33	√	√		√	√		
34	√	–	√(6 h)	√	–		
35	–	–	√(6 h)	–	–		
36	√	√		√	√		
37	√	√		√	√		

√: Stability passed.
–: Stability failed.
nd: not determined.

concentration was applied to the test system. For a series of nine compounds analyzed for concentration in non-clinical models of QT prolongation, four compounds (~45%) showed loss through the delivery system (Hanson et al., 2006). A comparison of samples taken from the solution reservoir with those taken from the recording chamber allowed determination of whether changes in concentration could be attributed to loss of test article in the testing equipment versus instability or dose preparation error. The current practice in our facility is to report nominal concentrations if the concentrations found in the recording chamber samples are within ± 10 –15% of the target concentration. If the values are outside of this acceptable range, the concentrations tested are corrected for the values determined by analytical chemistry and the IC₅₀ is recalculated using those values. This provides the most conservative IC₅₀ and is the preferred value in safety evaluations.

4.2. Decrease in actual concentration due to either loss of test article in the perfusion system or lack of stability of the test article in vehicle

For approximately half of the studies examined, the IC₅₀ was >2-fold more potent when using actual values as determined by HPLC versus the target concentrations. The most likely explanations for this difference are degradation and/or instability of the compound in the vehicle and/or loss of compound to adsorption to the perfusion apparatus (Gintant, Su, Martin, & Cox, 2006; Gintant 2008). In a similar analysis (Vargas et al., 2007; Vargas & Yu, 2008), 14/115 compounds (12%) demonstrated decreased concentrations as compared to nominal. The IC₅₀ for these compounds were 10-fold more potent when using actual concentrations in the calculation as compared to nominal. Vargas et al. (2007) speculated that the differences between actual concentrations and nominal concentrations may be related to the timeframe between sample collection during the assay and analysis of the samples by HPLC. They concluded that measurements of actual concentrations in non-GLP hERG assays were misleading, and therefore, unreliable. For their data set, analytical evaluation of the solutions occurred up to 48 h after collection. For our data set, the concentration verification assay was performed the same day as sample collection. In these circumstances, actual concentrations are more accurate and provide a faithful representation of the actual concentration–response relationship.

4.3. Decrease in actual concentration due to lack of stability of the test article in vehicle

Analysis of thirty-seven (37) studies that were conducted with stability analyses of the test article in vehicle solution determined that in 5 of the cases, the compound was stable less than 8 h when stored under room temperature. The eight hour evaluation time was chosen to mimic the typical experimental day where the formulated test article was used. In the instances where 8 h stability failed, samples were re-evaluated after shorter time points. When a known stability period was determined, our laboratory coordinated the electrophysiological data acquisition and dose sampling with the analytical evaluation of the samples such that all samples were analyzed within their stability limits. This entailed beginning the analytical portion of the study early in the day such that the calibration curve and quality check samples were injected into the HPLC before samples from the hERG assay needed to be injected. Such an analysis was possible only because the hERG assay and the analytical analysis were performed at the same facility. An additional 3 studies had test articles which were not stable after 24 h at room temperature. Thus, 22% (8 of 37) test articles were not stable for 24 h at room temperature. When stored under frozen conditions, 16% of the test articles were not stable after 24 h. The test articles that were found to be unstable after frozen storage also were not stable at room temperature, suggesting that the freeze–thaw cycle was not the reason for the instability.

In our experience when our clients outsource the analytical work to another laboratory, many analytical techniques involve the addition of organic solvents to the samples in order to validate the analytical method. The addition of the organic solvent stabilizes the test article in the vehicle which allows the accuracy and precision numbers to be acceptable. Such methodology is commonly needed when developing analytical methods for lipophilic compounds due to poor aqueous solubility (Herron et al., 2004). While this procedure can be used to validate analytical methods, stability of the test article in the vehicle prior to addition of the organic solvent is needed in order to determine the stability over the course of the experimental day of a hERG or Purkinje fiber assay (Sha et al., 2000). Organic solvents cannot be added to the test solutions prior to testing in the assay. Therefore, for the studies discussed above where there was limited stability of the test articles in vehicle (<8 h at room temperature), this procedure that allows for analytical methods to be validated by stabilizing the compound would not have corrected the recovery values.

4.4. Limitations of the analysis

One limitation of our data set is the lack of testing reference compounds in order to determine if compound structure can be correlated to the loss of certain compounds in the perfusion apparatus and/or the lack of stability of certain compounds. For the compounds discussed in this paper, any structure information that the client has provided is confidential. Typically, such information is not provided to us. To date, we do not have a database with comparable analytical data for reference compounds (e.g., positive controls used in our assays). Vargas et al. (2007) and Vargas and Yu (2008) determined that the compounds in their data set that had large differences between actual and nominal concentration had low solubility in aqueous buffers, were lipophilic, and had large clogP values. Since this chemical information is either not provided to outsourced laboratories or not known at the time of the hERG or Purkinje fiber assay, analyzing dosing solutions as suggested in the next section is recommended.

4.5. Conclusions

From the data that we have obtained, the best practices when considering dose solution concentration verification of test article solutions are:

- Determine the solubility of the compound in a physiological buffer, for instance, using a turbidimeter
- Analyze samples collected from the recording chamber
- Coordinate sample collection and analysis to remain within known stability limits (e.g., same day as hERG assay).

These methodologies will produce the most accurate values when determining safety evaluations.

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