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Development of a semi-automated chemical stability system to analyze solution based formulations in support of discovery candidate selection

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Abstract

A semi-automated chemical stability system was developed, validated, and implemented to assess the chemical and physical stability (24 h) of intravenous and oral solution based formulations in support of preliminary in vivo drug discovery studies. The system utilizes a single Agilent 1100 LC and Xterra column with multiple UV wavelength monitoring. Mobile phase selection, either basic or acidic, is selected base upon on the physico-chemical properties of the test compound. The system was validated against 14 new chemical entities across multiple therapeutic areas. The results indicated that drug discovery compounds could be accurately quantified (<2% R.S.D.) in a wide range of formulation vehicles in greater than 90% of the test cases. This method can be used as a quantitative tool for triaging formulation variables and packaging configurations to quickly develop stable solutions for dosing.
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1. Introduction

Through the utilization of high throughput screening (HTS) a vast number of "leads" are identified on the basis of in vitro potency and selectivity [1]. These promising compounds are further evaluated in vivo against a targeted pharmacokinetic and safety profile [2–4], which requires each candidate to be rapidly formulated for either oral or intravenous administration [5–7]. In developing formulations, it is vital to deliver the lead compound at the target concentration, requiring solution state chemical and physical stability in the dosing vehicle. When stability is unknown, the formulation must be prepared at the time of dosing, increasing a typical rat pharmacokinetic study from an 8 h to 10–12 h workday. In addition, this operating model allows virtually no time for trouble-shooting when problems arise, resulting in study cancellations and timeline delays. In order to mitigate this potentially high-risk situation, a simple generic analytical method is needed to accurately quantitate the chemical stability of potential lead compounds in various formulation vehicles, commonly used for in vivo discovery screening.

In the search for a generic, robust analytical method, direct analysis is most desirable. However, such approaches as simple flow injection analysis utilizing UV spectrometry [8,9] are not possible due UV absorbing excipients, requiring pre-analysis sample extraction [10,11]. Other approaches such as MS offer a high degree of selectivity with little to no sample preparation, but in order to obtain sufficient accuracy an appropriate internal standard is required and typically not available at this very early stage of drug development [12,13]. To this end, the development of a simple standardized LC-UV method was explored.

This paper describes the semi-automated chemical stability system (SACSS) set-up, method validation, and use. Results indicated that SACSS can accurately (<2% R.S.D.) quantify the active pharmaceutical ingredient in greater than 90% of the test cases.

2. Experimental

2.1. Chemicals

Compounds **1–14** are proprietary compounds of Pfizer Global Research and Development (Ann Arbor, MI). Formic acid (85%, v/v) was purchased from Mallinckrodt Baker Inc. (Paris, KY).

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Ammonium hydroxide, acetonitrile, and water, all LC grade, were obtained from Sigma–Aldrich (St. Louis, MO). All formulation vehicle reagents used were obtained from Pfizer Global Research and Development (Ann Arbor, MI) or VWR International (Buffalo Grove, IL) and were research grade or better, and used without further preparation.

2.2. Instrumentation

The chromatographic system consisted of a model 1100 LC system with vacuum degasser, quaternary pump, thermostatted automatic sample injector, thermostatted column compartment and DAD from Agilent Technologies (Palo Alto, CA). The chromatographic separation was preformed on a 4.6 mm × 150 mm, 5 μm, XTerra® MS C_{18} column (Waters, Milford, MA) maintained at 25 °C. The system was controlled by Agilent ChemStation software (Rev A.08.04).

2.3. Chromatographic conditions

The acidic LC method employed a two component mobile phase system, 0.1% formic acid in water (v/v) (eluent A) and 0.1% formic acid in acetonitrile (v/v) (eluent B). A gradient elution was developed with eluent B increasing 10–20% over 8 min, holding B at 20% for 2 min, increasing to 80% B over 20 min, returning B to 10% in 5 min, and holding there for 10 min to stabilize the column. Flow rate was maintained at 1 ml/min and injection volume varied from 5 to 100 μl, allowing flexibility based on compound chromophore strength and target formulation concentration. Multiple wavelengths (214, 245, and 330 nm) were monitored to increase the probability of detecting a diverse set of compounds and potential degradents. All quantitative work was preformed at a wavelength appropriate for the selected compound. Total run time was 45 min.

The basic LC method differs from the acidic LC method only in mobile phase composition. Utilizing a quaternary pump one LC system was used to run both the acidic and basic LC method. For the basic LC method, eluent C is 0.1% ammonium hydroxide in water (v/v) and eluent D is 0.1% ammonium hydroxide in acetonitrile (v/v). The gradient for basic LC method is the same as for the acidic LC method as described above, with the noted exception that eluent A is replace with C and eluent B is replaced with D.

The LC system was switched between the two methods by a simple gradient to equilibrate the column with the required mobile phase for analysis. To switch from the acidic LC method to the basic LC method, a gradient elution was developed with eluent C increasing 0–90% and eluent D increasing 0–10% over 15 min, holding C at 90% and D at 10% for 30 min to stabilize the column. The reversal of this method was applied to switch from the basic LC method to the acidic LC method. The column was always stored in the acidic conditions when not in use to prolong the life of the column.

2.4. Method optimization and validation

To maintain a generic chromatographic method, optimization was focused on two parameters, compound separation/retention and accuracy. A single method was developed, utilizing two different mobile phase compositions. Two conditions were required in order to maintain acceptable peak shape using compound standard stock solutions in acetonitrile–water (50:50, v/v). Selection between the two methods was based on compound ionization type, i.e. weak acids analyzed using acidic conditions, weak bases analyzed using basic conditions and non-ionizable/zwitterionic compounds analyzed using both method conditions, with final selection based on performance.

System precision was evaluated using the % R.S.D.'s calculated from repeat injections ($n = 3$) of the formulation. The resulting % R.S.D.'s (<2%) provided a high level of confidence in the reproducibility of the system. Linearity, detection limit and quantitation limit were not determined due to the very small quantitation range that was needed. As a result, single point standardization was used throughout the study.

2.5. Compound formulation and analysis

Compound formulation preparation was based on target exposures required by in vivo testing protocols, and were developed along similar guidelines as reported previously [5].

Formulations ranged from 0.05 to 1.00 mg/ml, requiring variable injection volumes. Parent remaining (%) is reported relative to the initial peak area ($t = 0$ h) and acceptable parent recovery was established at ≥95% remaining, as needed for dosing. Samples were stored at ambient conditions and injected every 6 h over a 24 h period. At the end of each run, the LC flow rate was reduced to 0.1 ml/min for 5 h to reduce mobile phase consumption (or up to eight samples can be monitored in series by staging each start time by 45 min). After 5 h the flow rate was increased to 1 ml/min allowing 15 min for column stabilization prior to injection.

3. Results and discussion

3.1. System optimization and validation

During the early phase of SACSS development, it was desirable to select a robust, versatile column capable of handling a wide-range of structurally diverse chemical matter, which typically emerges from discovery chemistry HTS. Of the columns evaluated, XTerra was chosen for its extended pH operating range, peak shape performance and analyte retention. In addition, acetonitrile proved superior to methanol with regard to peak shape and resolution. Several aqueous modifiers were considered but selection of a single condition to suit all needs could not be made, while maintaining an acceptable peak shape. Therefore, two separate mobile phase compositions were developed with formic acid and ammonium hydroxide selected as the aqueous modifiers for their optimum performance. These conditions provided an additional benefit, by being compatible with mass spectrometric ionization techniques; allowing preliminary degradant analysis, if desired.

To increase method applicability, a steep gradient was developed to handle compounds and their potential degradants across a wide log P range. The effects of flow rate, column tempera-

Table 1
Test compound physical attributes and selected formulation vehicle

ID#	Ionization type	LC method	MW	$c\log P$	pK_a	Formulation (%, v/v/v)							
						DMA	NMP	Ethanol	PG	50 mM Tris base	50 mM Lactic acid	Saline	0.2% EDTA in 25 mM lactic acid
1	Acidic	Acidic	390.4	4.13	5.63	5			25	70			
2	Acidic	Acidic	431.4	2.18	5.36/8.24		5		25	70			
3	Acidic	Acidic	463.5	5.31	3.18					100			
4	Basic	Basic	403.4	3.33	4.92	5	5		40		50		
5	Basic	Basic	533.5	2.69	9.81/8.21	5			45			50	
6	Basic	Basic	267.3	3.05	7.61	5			45	50			
6r[a]						5			45		50		
7	Basic	Basic	504.4	2.95	7.4	5			45	50			
7r[b]						5			45	50			
8	Basic	Basic	360.3	3.09	8.79	5			25			70	
9	Basic	Basic	447.5	2.35	8.31/5.44							100	
9r[a]													100
10	Neutral	Basic	324.4	3.77	–	10	10		50			30	
11	Neutral	Basic	356.4	1.49	–	5	5	10	60	20			
12	Neutral	Acidic	388.4	2.86	–	5			45			50	
13	Zwitterion	Basic	303.3	1.52	3.88/10.46	5			25			70	
14	Zwitterion	n/a	173.2	−0.32	3.64/10.60							100	

[a] "r" denotes a reformulation of compound to improve stability.
[b] Formulation stored in amber vial.

ture, and gradient rate formation were not thoroughly evaluated and even though run times could potentially be reduced, sample through put was not the main objective of this study.

The SACSS was validated with 14 test compounds selected from recent formulation efforts in our laboratory. The test set represents a range of structurally diverse compounds from multiple therapeutic areas. The set is composed of three acids, six bases and five non-ionizable or zwitterions, ranging in MW from 173.2 to 504.4 and $c\log P$ values ranging from −0.32 to 5.31 (Table 1). During the initial validation, all compounds were analyzed as standard stock solutions with the objective of retention times greater than the void volume ($t_m = 2.2$ min) while maintaining sufficient peak shape ($T_f = 1.5$). Excellent system suitability was achieved as indicated by a 0.16% R.S.D., as averaged across all final formulations. Compound **14** was a noted exception, which lacked a strong chromophore. In such cases, utilization of alternate analytical techniques would be required to ensure chemical stability prior to dose preparation [14]. Compound **14** was formulated immediately prior to dosing to mitigate any stability risks.

To overcome vehicle interference, an isocratic hold time was incorporated to allow adequate separation of more hydrophilic compounds (i.e. compound **11**) from the void volume. Typical vehicle effects can be seen in Fig. 1 where vehicle 1 containing 5% DMA/45% propylene glycol/50% 50 mM Tris base (v/v/v) interfered for up to 4 min while monitoring at 214 nm and vehicle 2 containing 10% NMP/40% propylene glycol/50% 50 mM Tris (v/v/v) base under the same conditions interfered for up to 5 min.

3.2. Stability studies

The test compounds were formulated and analyzed by SACSS to assess their solution chemical and physical stability over a 24 h time period. Upon initial evaluation, four compounds (**6**, **7**, **9** and **11**) indicated a loss of parent over the monitored time period (Table 2). Therefore, SACSS was used as the quantitative tool for triaging formulation variables and packaging configurations to quickly develop stable solutions for dosing.

Compounds **6** and **7** both gave rise to several additional peaks that continued to grow over the 24 h time course (Figs. 2 and 3). Data from compound **6** (Table 2), suggested that the large % R.S.D. was due to rapid chemical degradation. This compound contains an ester group, which is known to undergo acid–base hydrolysis. In some cases, it has been noted that ester hydrolysis rates can be reduced if a slightly acidic environment (i.e. pH 3–5) is maintained. Therefore, compound **6** was reformulating in an weakly acidic vehicle (**6r**), which improved the chemical stability as indicated by an increase in parent recovery to 96.6% from 14.6% over the observed 24 h period. In the case of compound **7**, it was quickly determined that this compound was

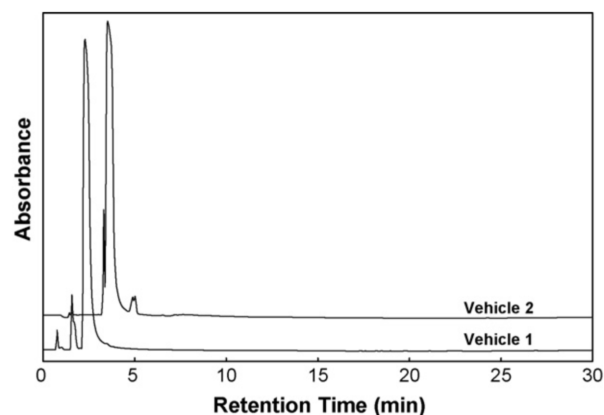

Fig. 1. Vehicle blanks monitored at 214 nm where vehicle effects are seen up to 5 min. Vehicle 1 (5% DMA/45% propylene glycol/50% 50 mM Tris base (v/v/v)) and Vehicle 2 (10% NMP/40% propylene glycol/50% 50 mM Tris base (v/v/v)).

Table 2
Stability time course for all test compounds and subset of analytical values

ID#	Validation % R.S.D. ($n=3$)	λ (nm)	Retention time (min)	Parent remaining (%)			
				6 h	12 h	18 h	24 h
1	0.15	245	25.7	101.8	104.5	104.9	105.0
2	0.11	330	20.7	99.9	100.2	100.4	100.3
3	0.23	245	27.7	99.9	99.9	100.0	99.8
4	0.05	330	23.2	99.9	99.7	99.7	99.8
5	0.36	330	21.3	99.2	97.2	98.3	96.8
6	5.60	245	22.7	62.8	38.5	23.7	14.6
6r[a]	0.14	245	22.7	99.6	98.6	97.6	96.6
7	0.92	330	21.6	96.1	92.4	89.4	86.2
7r[b]	0.33	330	21.8	98.2	96.3	95.6	92.9
8	0.02	330	23.4	99.3	99.8	99.5	99.2
9	2.96	330	19.8	90.1	n.d.[c]	82.8	84.1
9r[a]	n.d.	330	19.8	n.d.	n.d.	n.d.	102.5
10	0.14	245	24.3	99.9	100.9	100.4	100.6
11	30.49	245	7.1	60.7	60.4	57.9	51.5
12	0.24	330	24.4	100.6	100.5	99.9	100.5
13	0.21	245	15.4	99.9	99.7	99.8	n.d.
14	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

[a] “r” denotes a reformulation of compound to improve stability.
[b] Formulation stored in amber vial.
[c] n.d.: not determined.

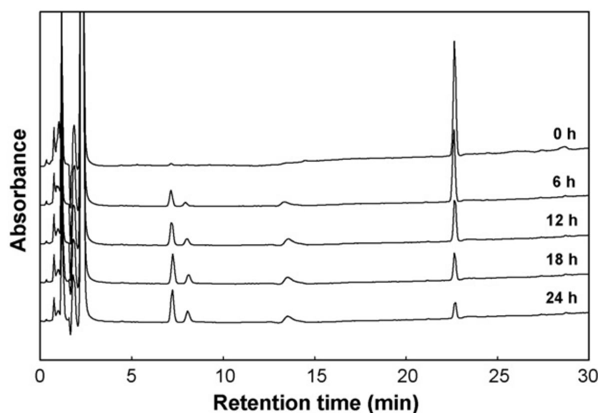

Fig. 2. Time course of compound **6** monitored at 245 nm over 24 h. Loss of parent and growth of degradation peaks at 7, 8 and 14 min are observed.

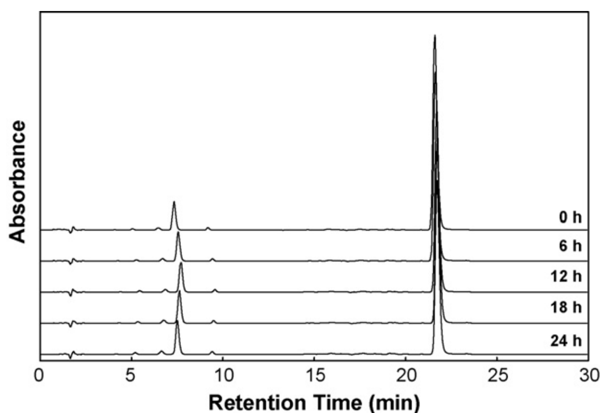

Fig. 3. Time course of compound **7** monitored at 330 nm over 24 h. Growth of degradation peaks between 5 and 10 min are observed.

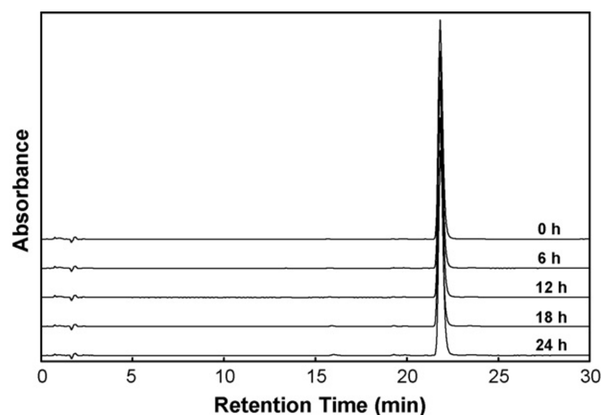

Fig. 4. Time course of compound **7r** monitored at 330 nm over 24 h. Formulation reformulated in an amber vial resulting in increased stability for at least 24 h.

light sensitive and was re-prepared (**7r**) in an amber vial (Fig. 3). Chemical stability improved over 24 h, however the 95% recovery criterion was not met. The light protected formulation had acceptable stability up to 18 h after preparation, whereas the unprotected formulation was only stable for 6 h. This package improvement did allow advance dose preparation (Fig. 4).

Compound **9** had a rapid disappearance of parent within the first 6 h to 90.1% followed by a deceleration in decomposition out to 24 h (Table 2). Growth in a potential degradant peak around 18 min from the initial sample ($t=0$ h) provided an indicator to evaluated chemical stability (Fig. 5). Two of the possible scenarios that can be theorized which may explain this aberrant profile are acid–base hydrolysis occurring in the formulation’s uncontrolled pH environment and as degradation proceeds the solution becomes more acidic or basic, promoting chemical stability [15] or compound oxidation [16] stemming from an impurity, which is consumed during the reaction [17]. To determine if acid–base hydrolysis is the mechanism, compound **9** was reformulated separately in both a lactic acid buffer and Tris base buffer. In addition to discourage potential oxidation, the compound was reformulated in the presence of EDTA. All formulations were evaluated for chemical stability over 24 h.

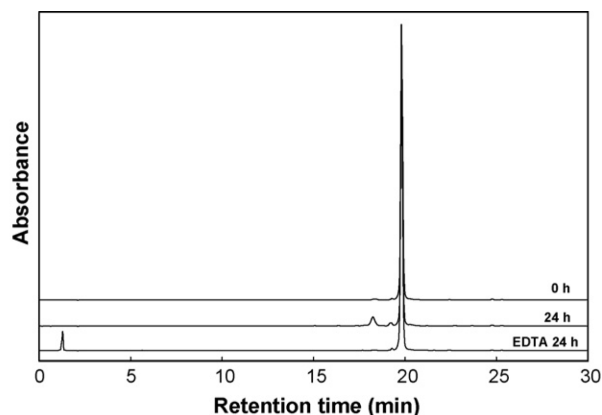

Fig. 5. Time course of compound **9** monitored at 330 nm over 24 h and compound **9r** at 24 h. Growth of degradation peaks from 0 to 24 h. Reformulation of the compound with EDTA improved the overall stability for at least 24 h.

Maintaining at a buffered pH had relatively no effect on the chemical stability of compound **9** (data not shown). However, 24 h chemical stability was attained with the addition of EDTA (Table 2), as confirmed by SCASS with no growth in the potential degradant peak around 18 min (Fig. 5).

The loss of compound **11** resulted in no observable degradant formation and upon visual inspection of the vial it was confirmed that the formulation was physically unstable with a noticeable precipitate formed. The ∼40% observable loss of parent by 6 h did not provide an adequate time window for dosing. Typically a compound that precipitates can be reformulated in a vehicle that contains a higher percentage of organic, however this formulation is only 20% aqueous and increasing the organic content could pose vehicle safety concerns. Therefore, compound **11** was prepared at the time of dosing using a high intensity light to visually confirm that the solution was clear prior to administration.

4. Conclusion

SACSS combines two generic analytical methods into one system that can effectively assess physical and chemical stability in parallel with formulation optimization, allowing formulators to deliver a quality product to Discovery scientist a day prior to scheduled dosing. This timeframe allows formulations that fail stability requirements to be reformulated without delaying the study start. In addition, SACSS is easily maintained with a simple set of mobile phases that requires limited analysis time and small sample quantities. This robust process has proven to be a good indicator of chemical and physical stability in greater than 90% of the compounds tested.

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