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Development and application of high throughput plasma stability assay for drug discovery

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Abstract

Plasma stability plays an important role in drug discovery and development. Unstable compounds tend to have rapid clearance and short half-life, resulting in poor in vivo performance. This paper examines the variables that affect the plasma stability assay results, including substrate concentration, %DMSO, plasma concentration, enzyme activity upon incubation and batch variation. The results show that plasma stability can accommodate a wide range of experimental conditions. Relatively minor differences in results are produced with major differences in conditions. Significant batch-to-batch variations were observed for rat plasma. We selected the following conditions: 1 μM substrate concentration, 2.5% DMSO, and 50% dilution of plasma in pH 7.4 buffer. Plasma stability can be used as a diagnostic assay when compounds are unexpectedly rapidly cleared, as a special assay when structural classes contain groups that may be susceptible to plasma enzyme hydrolysis, or as general screen for compounds if resources are available. Plasma stability assay has many applications in drug discovery: to alert teams to labile structural motifs, to prioritize compounds for in vivo studies and to screen prodrugs and antedrugs.
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Keywords: Plasma stability; Stability; Hydrolysis; Prodrugs; Enzymes; HPLC; LC–MS; High throughput

1. Introduction

Stability of drug candidates in plasma is essential for maintaining acceptable drug concentration and half-life in order to achieve desirable pharmacological effects. Compounds that are unstable in plasma tend to have rapid clearance, short half-life and poor in vivo performance. Furthermore, they cause difficulties for pharmacokinetic (PK) studies, because the compounds will continue to degrade even after the blood samples were taken from the animals. The PK study standards in plasma need to be prepared in the presence of hydrolyase inhibitors. Circulating hydrolytic metabolites complicate and slow down the drug development processes. Pharmaceutical companies tend to not advance compounds that rapidly degrade in plasma, with the exception of prodrugs, antedrugs and special cases. Screening of plasma stability provides useful information to prioritize compounds for in vivo studies and to alert researchers to the potential liabilities of

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key pharmacophores, so that structural modifications can be applied to improve stability (Borthwick et al., 2002, 2003; Breitenlechner et al., 2004). Furthermore, plasma stability is very useful for screening of prodrugs and antedrugs, where rapid conversion in plasma is desirable (Ettmayer et al., 2004; Sawa et al., 2002).

Several methods for plasma stability have been developed with an emphasis on improving efficiency of sample preparation and analysis, such as direct injection of plasma samples using LC–MS–MS with restricted access HPLC columns (Wang et al., 2002; Wang and Hsieh Yunsheng, 2002), an automated column switching HPLC method (Peng et al., 1999) and robotic sample preparation (Linget and du Vignaud, 1999). However, the literature procedures for plasma stability are quite diverse. There is a wide range of experimental conditions that are being used among different laboratories (Pop et al., 1999; Rautio et al., 1998; Kim et al., 2001; Udata et al., 1999; Nomeir et al., 1998; Greenwald et al., 2004; Geraldine and Jordan, 1998). For example, sample concentration in plasma varied more than 1000-fold from 3 μM to 6 mM (Pop et al., 1999; Rautio et al., 1998; Kim et al., 2001; Udata et al., 1999; Nomeir et al., 1998). The percent of organic solvent varied from 0 to 5% (Kim et al., 2001; Udata et al., 1999; Greenwald et al., 2004). Some laboratories remove all the organic solvent before adding the plasma and others add organic stock solutions directly into plasma. For plasma concentration, some laboratories use 100% concentration and others dilute the plasma with buffer to various concentrations (80–30%) before it is used (Rautio et al., 1998; Geraldine and Jordan, 1998). It is not clear how these drastically different conditions affect the results for plasma stability assays. In this study, we investigated the variables that affect the assay results, including substrate concentration, %DMSO, plasma concentration, and plasma batch-to-batch variation. Possible applications of the plasma stability in drug discovery were investigated and are reported.

2. Experimental

2.1. Materials

All reagents used were of the highest grade commercially available. The test compounds were obtained from Aldrich and Sigma Chemical Co. (St. Louis, MO), Fluka (Ronkonkoma, NY), and Wyeth compounds were obtained from Wyeth Research (Princeton, NJ). Sprague Dawley male rat plasma was heparinized and filtered (Bioreclamation Inc., Hicksville, NY).

2.2. Instrumentation and software

A Packard Multiprobe™ II EX HT (eight probes) robot with WinPrep™ software was used for sample preparation (PerkinElmer, Downers Grove, IL). The HPLC system was an HP1100 with PDA detector (Agilent Technologies, Piscataway, NJ). The mass spectrometer was a Waters ZQ (Waters, Milford, MA). Instruments were controlled by Masslynx software (Waters, Version 4.0).

2.3. HPLC conditions

Column: Aquasil C18, 50 mm × 2.1 mm, 5 μm (Thermo, Bellefonte, PA).
Mobile phase: A = 0.1% formic acid in water; B = 0.1% formic acid in acetonitrile.

Time (min)	Gradient	
	(%) A	(%) B
0	100	0
2.5	10	90
4.0	10	90
4.1	100	0
5.5	100	0

Flow rate: 0.8 mL/min, split 0.2 mL/min to MS,
Detection: ESI+, SIM,
Injection: 20 μL,
Column temp.: ambient.

2.4. Stability procedure investigation

2.4.1. Effect of substrate concentration

Compounds were dissolved in DMSO to the concentrations of 40, 80, 200, 400, 600 and 800 μM and 100 μL were added to the shallow 96-well plates (350 μL, polypropylene, Corning Incorporated, Corning, NY). This sample stock plate was placed on the robotic deck. The robot delivered 195 μL of rat plasma

Table 1
Dilution protocol for the study of DMSO effect

Final DMSO in assay (%)	200 μM sample stock (μL)	DMSO (μL)	PBS pH 7.4 (μL)
0.5	25	0	975
1	25	25	950
2.5	25	100	875
5	25	225	750
10	25	475	500
20	25	975	0

and 5 μL of sample stock to deep 96-well plates (1 mL polypropylene, Corning Incorporated, Corning, NY) to produce final compound concentrations of 1–20 μM. All the plasma was centrifuged at 3000 rpm for 10 min at 10 °C to remove particulates before use. The plate was sealed with a plate mat (National Scientific Company, Duluth, GA), vortexed and placed on a 37 °C shaker (Armalab, Bethesda, MD) and shaken gently for 3 h. At the end of the incubation, 600 μL of cold acetonitrile were added to each well to stop the reaction. The solution was mixed and centrifuged at 3000 rpm for 15 min. The supernatant (400 μL) was transferred to a deep 96-well plate for LC–MS analysis. For the time 0 plate, samples were quenched with cold acetonitrile right after mixing with plasma.

2.4.2. *Effect of DMSO*

Compounds were dissolved in DMSO to 200 μM and then diluted to a concentration of 5 μM, according to Table 1, with the appropriate amount of DMSO and phosphate-buffered saline (PBS pH 7.4, isotonic, Invitrogen Corporation, CA). Two-hundred microlitres of each compound solution were added to the shallow 96-well plate. The robot delivered 160 μL of rat plasma and 40 μL of the different 5 μM sample stocks having different percentages of DMSO, to the deep 96-well plate. The plate was sealed, vortexed and placed on a 37 °C shaker and shaken gently for 3 h. At the end of the incubation, 600 μL of cold acetonitrile were added to the wells to stop the reaction. The solution was mixed and centrifuged at 3000 rpm for 15 min. The supernatant (400 μL) was transferred to a deep 96-well plate for LC–MS analysis.

2.4.3. *Effect of plasma concentration*

Samples are dissolved at a concentration of 40 μM in DMSO. Rat plasma was diluted with PBS (pH 7.4) to 0, 20, 40, 50, 60, 80 and 100% plasma. The robot delivered 195 μL of each of these diluted rat plasmas and 5 μL of sample stock to deep 96-well plates. The plate was sealed, vortexed and placed on a 37 °C shaker and shaken gently for 3 h. At the end of the incubation, 600 μL of cold acetonitrile were added to the wells to stop the reaction. The solution was mixed and centrifuged at 3000 rpm for 15 min. The supernatant (400 μL) was transferred to a deep 96-well plate for LC–MS analysis.

2.4.4. *Effect of incubation time on plasma enzyme activity*

Male rat plasma was incubated at 37 °C for 0, 1, 3, 6, 9 and 22 h before the assay. This batch of plasma was different than those used in the previous studies. Samples were dissolved at a concentration of 40 μM in DMSO. The pre-incubated plasma was diluted with PBS (pH 7.4) with a 1:1 ratio. The robot delivered 195 μL of each of these diluted rat plasmas from different batches and 5 μL of sample stock to deep 96-well plates. The plate was sealed, vortexed and placed on a 37 °C shaker and shaken gently for 3 h. At the end of the incubation, 600 μL of cold acetonitrile were added to the wells to stop the reaction. The solution was mixed and centrifuged at 3000 rpm for 15 min. The supernatant (400 μL) was transferred to a deep 96-well plate for LC–MS analysis.

2.4.5. *Batch-to-batch variation of rat plasma*

Samples are dissolved at a concentration of 40 μM in DMSO. Male rat plasma of different batches from the same vendor was diluted with PBS (pH 7.4) with a 1:1 ratio. The study proceeded as described in the above section on effect of incubation time on plasma enzyme activity.

3. Results and discussions

Eight commercial drugs with various plasma stabilities were selected for method development. The structures of the eight test compounds are shown in Fig. 1. The compounds all contain ester or amide functional groups that are susceptible to hydrolysis by plasma enzymes.

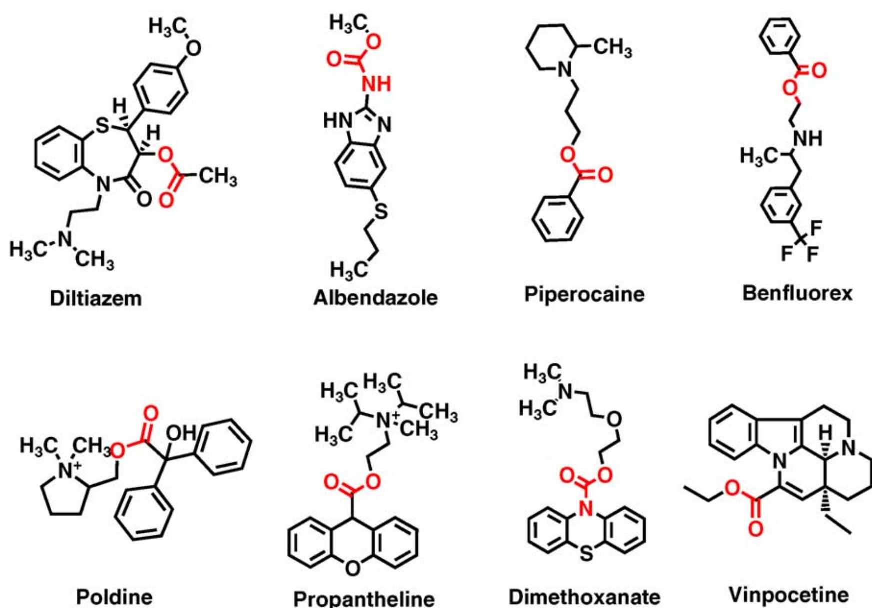

Fig. 1. Structures of the eight test compounds.

3.1. Method development

The effect of substrate concentration on plasma stability was explored at the concentrations of 1, 2, 5, 10, 15 and 20 μM. The results are shown in Fig. 2. There is no significant difference in % remaining for most of the compounds over the concentration range of 1–20 μM. The results suggest that drug stability in plasma was not sensitive to drug concentration up to 20 μM and plasma enzymes had high catalytic

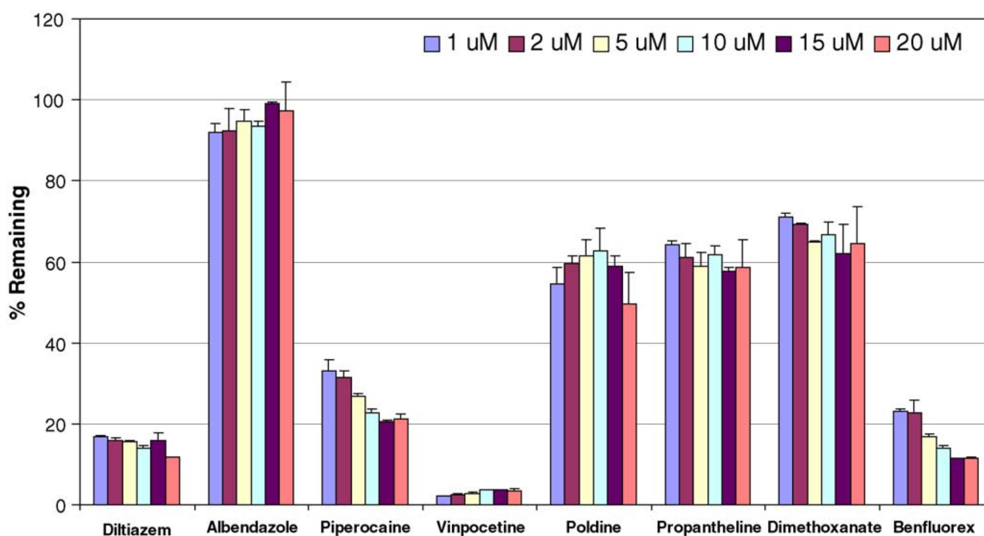

Fig. 2. Effect of substrate concentration on plasma stability. Experimental conditions: DMSO, 2.5%; plasma concentration, 100%; incubation time, 3 h.

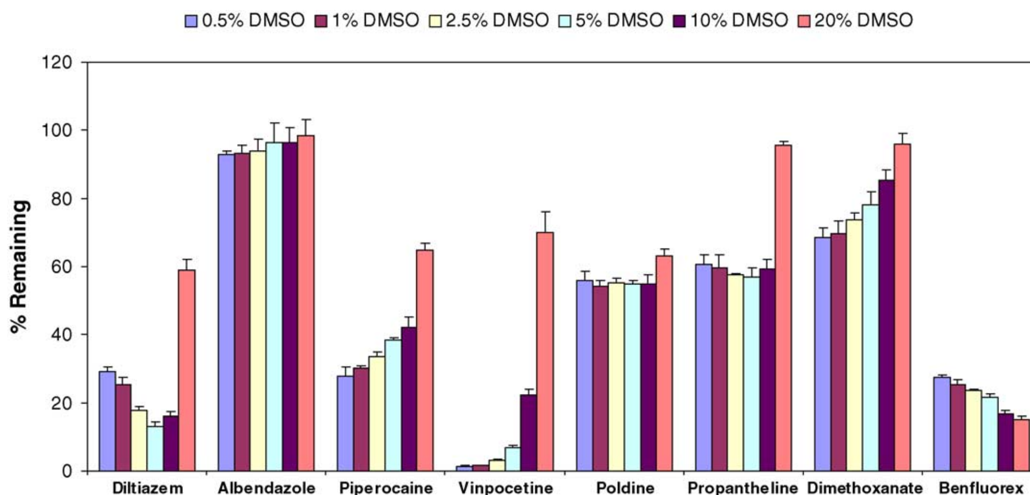

Fig. 3. Effect of DMSO on plasma stability. Experimental conditions: substrate concentration, 1 μM; plasma concentration, 80%; incubation time, 3 h.

capacity. This is quite different than microsomal stability, where CYP450 enzymes were easily saturated and give a higher % remaining at higher substrate concentration (Di et al., 2003). Saturation of plasma enzymes was previously observed at 100 μg/mL substrate concentration (Nomeir et al., 1998). Two compounds (piperocaine and benfluorex) showed a slight decrease in stability with increasing substrate concentration. This could potentially be due to plasma protein binding. At higher concentration, more free drug is available for enzyme hydrolysis, resulting in lower % remaining. In practice, the selection of substrate concentration should reflect in vivo drug concentration at low μM concentration. For this reason, we selected 1 μM as the assay concentration in our final method.

The effect of %DMSO on plasma stability is shown in Fig. 3, with %DMSO varying from 0.5 to 20%. Most compounds did not show significant changes up to 2.5% DMSO, which is quite a contrast to CYP450 metabolism by microsomes (Di et al., 2003; Busby et al., 1999; Chauret et al., 1998). At high %DMSO, compounds showed higher % remaining than at low %DMSO. This could potentially be due to denaturing of the plasma enzymes resulting in a decrease in enzyme activity. Benfluorex showed decreasing % remaining with increasing in %DMSO, even at 20% of DMSO. This could potentially be due to decrease in plasma protein binding. DMSO could release more bound drug to increase the free drug concentration for enzymatic hydrolysis. Diltiazem has an interesting profile. The % remaining decreases with increasing %DMSO up to 5% and then increases as DMSO increases to 20%. This effect could potentially result from the combination of decreasing protein binding and decreasing enzyme activity with increasing %DMSO. A %DMSO of 2.5% was selected for our final method.

The effect of plasma concentration on plasma stability is shown in Fig. 4. The plasma percentages in buffer in this study were: 100, 80, 60, 50, 40, 20 and 0%. The results showed that plasma concentration did not significantly impact hydrolytic rate of the compounds down to 40–20% plasma. This, again, suggests that plasma enzymes have high catalytic capacity. Several compounds showed significant degradation in the pH 7.4 buffer controls without any enzymes (vinpocetine and benfluorex). Both chemical and enzymatic hydrolysis must, therefore, contribute to degradation of these compounds. It has been reported previously that plasma pH increased to pH 8–9 during long-term storage. It is advisable to dilute plasma with pH 7.4 buffer to adjust the pH to 7.4, so that stability results are not complicated by the pH effect (Stella, 2005). Furthermore, dilution of plasma reduces viscosity, increases pipetting accuracy, and reduces the cost and animal use. Therefore, 50% plasma in buffer was selected for the method.

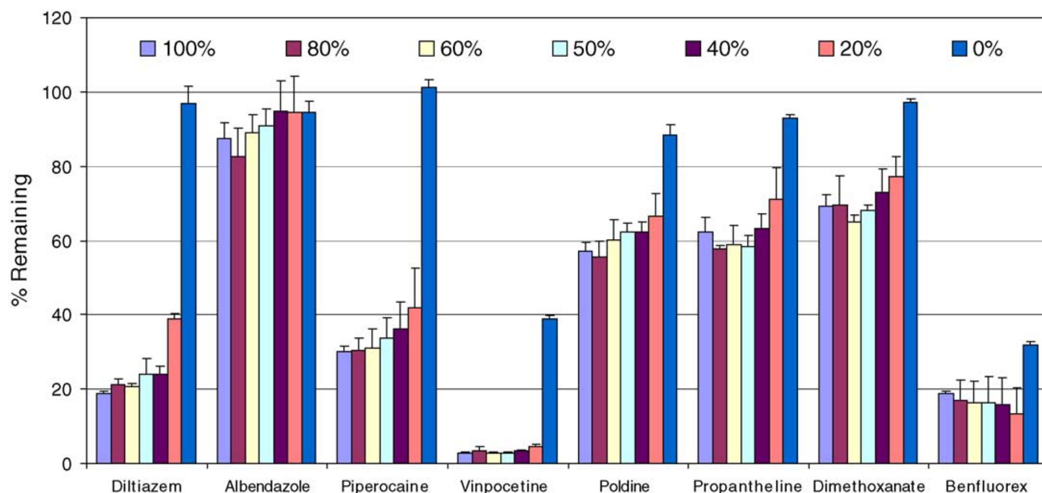

Fig. 4. Effect of plasma concentration on plasma stability. Experimental conditions: substrate concentration, 1 μM; DMSO, 2.5%; incubation time, 3 h.

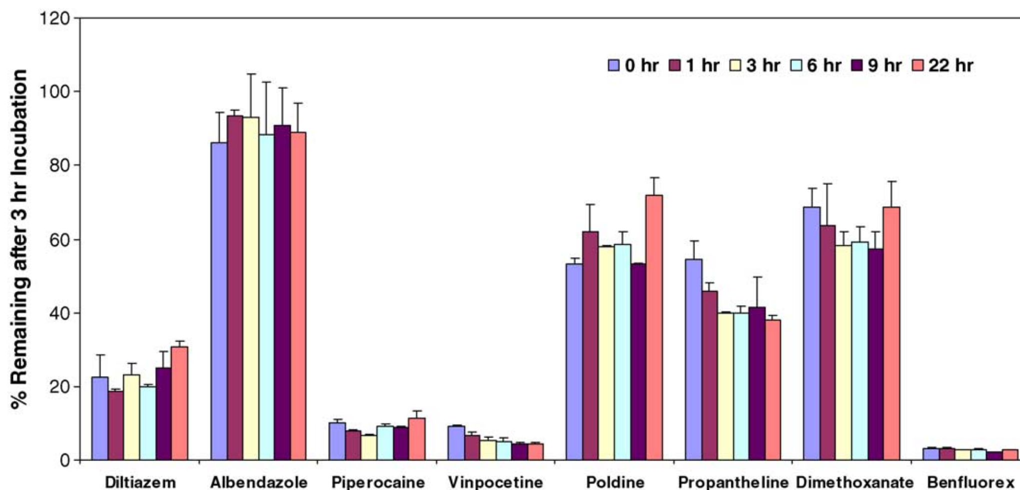

Fig. 5. Effect of incubation time on plasma enzyme activity to plasma stability. Experimental conditions: substrate concentration, 1 μM; DMSO, 2.5%; plasma concentration, 50%; incubation time, 3 h.

The effect of incubation time on enzyme activity to plasma stability is shown in Fig. 5. There is no significant loss of enzyme activity to plasma stability even after 22 h incubation at 37 °C. This is quite a contrast to the CYP450 enzymes. The robustness of the plasma enzymes allows a long incubation time to be used for monitoring slow degradation processes.

The final assay conditions for plasma stability are shown in Table 2 for screening in drug discovery.

Table 2
Final assay conditions for plasma stability

Assay parameters	Conditions
Sample concentration	1 μM
Plasma concentration	50% in pH 7.4 buffer
%DMSO	2.5%
Incubation time at 37 °C	3 h

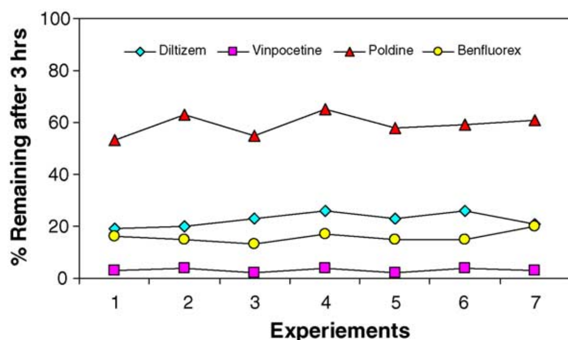

Fig. 6. Results of QC standards on different assay plates.

The 3-h time point is selected for screening to cover the predicted time period used in typical drug discovery settings (6 h study), since the maximum predictive half-life is 6 h based on a 3-h incubation (Di et al., 2004). The results of some QC standards for experiments are shown in Fig. 6, representing experiments performed on different days with the same batch of plasma. The assay is quite reproducible. Typically three QC standards with different stabilities are used to monitor the performance of the assay each time it is run.

Results of batch-to-batch variation for plasma stability are shown in Table 3 with five different batches of male rat plasma. Significant batch variation was observed and it was compound dependent. The most pronounced effects were noted for diltiazem, piperocaine, vinpocetine and benfluorex with twofold to 20-fold difference in percent remaining due to different batches of plasma. This indicates that careful quality control is essential in order to generate meaningful and consistent results. Plasma batch variability could potentially be due to different expression levels of hydrolytic enzymes between individual animals. Certain compounds appear to be more sensitive to the particular enzymes that have the greatest variability among the individual animals resulting in their large variability. This could significantly impact compound ranking for discovery projects, in which assays are conducted over a long period of time, and different plasma batches are used. To minimize the effect of batch variability when switching to new plasma batch, selected compounds from the same project are tested again to reestablish the ranking scale. Alternatively, a large batch of plasma can be purchased for use over a long period of time in order to alleviate the impact of batch variability.

Table 3
Batch-to-batch variation on plasma stability

Compounds	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5
Diltiazem	28	8	22	20	10
Albendazole	86	97	97	94	90
Piperocaine	42	11	7	7	2
Vinpocetine	4	3	8	10	11
Poldine	69	65	64	64	33
Propantheline	65	64	61	53	53
Dimethoxanate	69	62	64	61	63
Benfluorex	23	6	3	2	1

3.2. Applications of plasma stability in drug discovery

Compounds with certain functional groups are more susceptible to hydrolysis by plasma enzymes than others. These include esters, amides, lactones, lactams, carbamides, sulfonamides and peptide mimetics. The plasma stability assay is designed to focus on these classes of compounds. It is commonly used as a secondary assay for special structural classes and not as a primary screening tool for all the discovery compounds. Due to species differences, screening of plasma stability in the most relevant animal species provides useful information for discovery teams. Plasma stability is an important member of the stability-profile for drug discovery (Fig. 7), which encompass microsomal stability (Di et al., 2003, 2004), buffer stability (Di et al., 2005) and others (Kerns and Di, 2004). Early screening of stability and other pharmaceutical properties provides important information for discovery teams to modify structures, diagnose in vitro and in vivo assay results and enhance the overall quality of development candidates (Kerns and Di, 2003; Di and Kerns, 2003; Kerns, 2001). The following are examples of the applications of plasma stability in drug discovery.

3.2.1. Application 1: provide early alert for plasma-labile structural motifs

In this example, a discovery team was developing two series (A and B in Fig. 8) with almost the same amount of resources. The plasma stability study revealed that Series A was stable in plasma, but Series

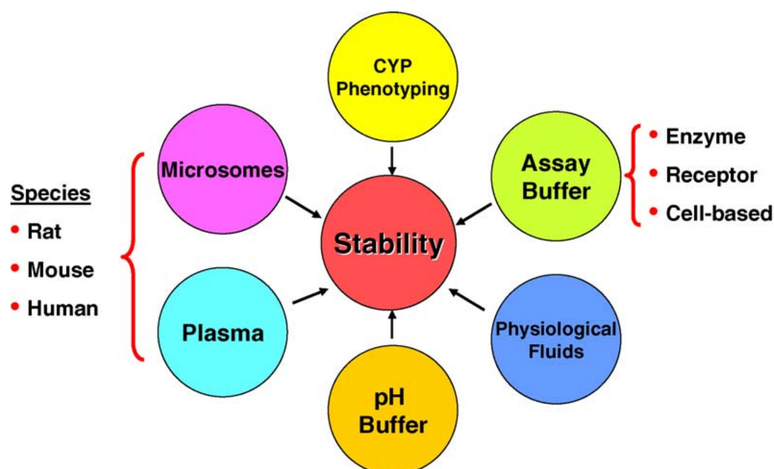

Fig. 7. Stability profiles in drug discovery.

B showed rapid degradation. Based on this information, the team decided to terminate the sulfone series and focused their efforts on the sulfide series. This example showed that plasma stability could be used to redirect synthetic efforts to the more promising series.

3.2.2. Application 2: prioritize compounds for in vivo animal studies

In this example, the team needed to select 20 out of 200 compounds for in vivo animal studies. The properties of the 200 compounds were very similar, which made selection difficult. However, the plasma stability of the compounds was very different (Fig. 9). By using the plasma stability information along with potency, structural diversity and other pharmaceutical properties, the team was able to select 20 compounds for in vivo animal studies.

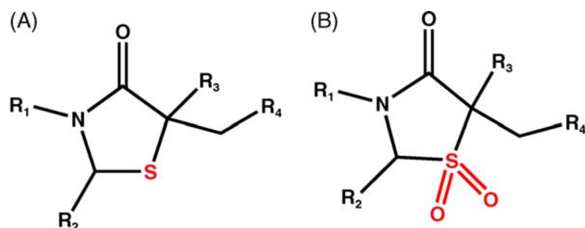

Fig. 8. Application of plasma stability in selecting compound series. Compound series A is stable in rat plasma. Compound series B rapidly degrades in plasma and resulted in termination of the series.

3.2.3. Application 3: screening of prodrugs

Plasma stability can be used to profile prodrugs. In this example, the team had made many different kinds of diester prodrugs to improve permeability and optimize oral bioavailability of the compound. Plasma stability of the prodrugs was studied. The results are shown in Table 4. Compound 1 was too stable in plasma to be a useful prodrug. There was no conversion in 24 h. Compound 6 was stable in gastric intestinal fluid but rapidly converted to diacid in plasma, thus it had a favorable prodrug profile. The compound was advanced to predevelopment.

3.2.4. Application 4: identification of hydrolytic products

In this example, the compound contains two carbamides in the molecule (Fig. 10). The team wanted

Table 4
Screening of diester prodrugs

Prodrugs	Diester at 3 h (%)	Monoester at 3 h (%)	Diacid at 3 h (%)
Wyeth 1	100	0	0
Wyeth 2	0.0	57.3	42.7
Wyeth 3	0.0	76.7	23.4
Wyeth 4	0.0	77.7	22.3
Wyeth 5	0.0	34.9	65.2
Wyeth 6	0.0	10.4	89.6
Wyeth 7	0.0	0.3	99.8

Percent of ester observed after a 3 h incubation in rat plasma.

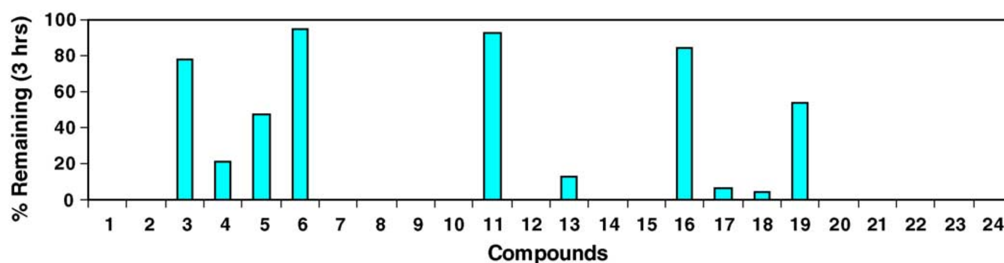

Fig. 9. Application of plasma stability in prioritizing compounds for in vivo animal studies. Relative plasma stability varied widely for these 24 representative compounds from the set of 200.

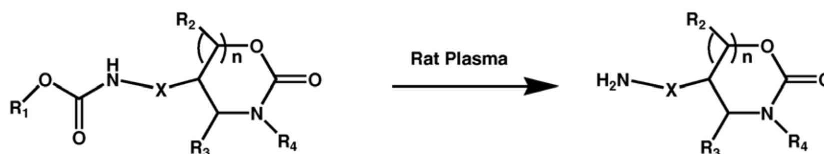

Fig. 10. Identification of hydrolytic products for plasma stability.

to find out the hydrolytic products of the compound so that modifications could be made to enhance stability. The degradation product of the plasma reaction of the compound was identified using LC–MS. The terminal carbamide was not stable, but the cyclic carbamide was stable. This type of information is very helpful for the team to optimize the stability of the compound.

4. Conclusions

Plasma stability is a very forgiving assay. It can accommodate a wide range of experimental conditions. Optimum method conditions were developed. Plasma stability has many applications in drug discovery: to alert teams to labile structural groups, to prioritize compounds for in vivo studies and to screen prodrugs and antedrugs.

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References

Borthwick, A.D., Davies, D.E., Ertl, P.F., Exall, A.M., Haley, T.M., Hart, G.J., Jackson, D.L., Parry, N.R., Patikis, A., Trivedi, N., Weingarten, G.G., Woolven, J.M., 2003. Design and synthesis of pyrrolidine-5,5′-trans-lactams (5-oxo-hexahydropyrrolo[3,2-b]pyrroles) as novel mechanism-based inhibitors of human cytomegalovirus protease. 4. Antiviral activity and plasma stability. J. Med. Chem. 46, 4428–4449.

Borthwick, A.D., Exall, A.M., Haley, T.M., Jackson, D.L., Mason, A.M., Weingarten, G.G., 2002. Pyrrolidine-5,5-trans-lactams as novel mechanism-based inhibitors of human cytomegalovirus protease. Part 3: potency and plasma stability. Bioorg. Med. Chem. Lett. 12, 1719–1722.

Breitenlechner, C.B., Wegge, T., Berillon, L., Graul, K., Marzenell, K., Friebe, W.G., Thomas, U., Schumacher, R., Huber, R., Engh, R.A., Masjost, B., 2004. Structure-based optimization of novel azepane derivatives as PKB inhibitors. J. Med. Chem. 47, 1375–1390.

Busby Jr., W.F., Ackermann, J.M., Crespi, C.L., 1999. Effect of methanol, ethanol, dimethyl sulfoxide, and acetonitrile on in vitro activities of cDNA-expressed human cytochromes P-450. Drug. Metab. Dispos. 27, 246–249.

Chauret, N., Gauthier, A., Nicoll-Griffith, D.A., 1998. Effect of common organic solvents on in vitro cytochrome P450-mediated metabolic activities in human liver microsomes. Drug. Metab. Dispos. 26, 1–4.

Di, L., Kerns, E.H., 2003. Profiling drug-like properties in discovery research. Curr. Opin. Chem. Biol. 7, 402–408.

Di, L., Kerns, E.H., Yan, H., Kleintop, T.A., McConnell, O.J., Huryn, D.M., 2003. Optimization of a higher throughput microsomal stability screening assay for profiling drug discovery candidates. J. Biomol. Screen. 8, 453–462.

Di, L., Kerns, E.H., Gao, N., Li, S., Huang, Y., Bourassa, J.L., Huryn, D.M., 2004. Experimental design on single time point high-throughput microsomal stability assay. J. Pharm. Sci. 93, 1537–1544.

Di, L., Kerns, E.H., Chen, H., Petusky, S.L., 2005. Development and application of an automated solution stability method, in review.

Ettmayer, P., Amidon, G.L., Clement, B., Testa, B., 2004. Lessons learned from marketed and investigational prodrugs. J. Med. Chem. 47, 2393–2404.

Greenwald, R.B., Zhao, H., Yang, K., Reddy, P., Martinez, A., 2004. A new aliphatic amino prodrug system for the delivery of small molecules and proteins utilizing novel PEG derivatives. J. Med. Chem. 47, 726–734.

Geraldine, C., Jordan, M., 1998. How an increase in the carbon chain length of the ester moiety affects the stability of a homologous series of oxprenolol esters in the presence of biological enzymes. J. Pharm. Sci. 87, 880–885.

Kerns, E.H., Di, L., 2004. Accelerated stability profiling in drug discovery. Log P 2004 Proceeding.

Kerns, E.H., Di, L., 2003. Pharmaceutical profiling in drug discovery. Drug Discov. Today 8, 316–323.

Kerns, E.H., 2001. High throughput physicochemical profiling for drug discovery. J. Pharm. Sci. 90, 1838–1858.

Kim, D.K., Ryu, D.H., Lee, J.Y., Lee, N., Kim, Y.W., Kim, J.S., Chang, K., Im, G.J., Kim, T.K., Choi, W.S., 2001. Synthesis and biological evaluation of novel a-ring modified hexacyclic camptothecin analogues. J. Med. Chem. 44, 1594–1602.

Linget, J.M., du Vignaud, P., 1999. Automation of metabolic stability studies in microsomes, cytosol and plasma using a 215 gilson liquid handler. J. Pharm. Biomed. Anal. 19, 893–901.

Nomeir, A.A., McComish, M.F., Ferrala, N.F., Silveira, D., Covey, J.M., Chadwick, M., 1998. Liquid chromatographic analysis in mouse, dog and human plasma; stability, absorption, metabolism and pharmacokinetics of the anti-HIV agent 2-chloro-5-(2-methyl-5,6-dihydro-1, 4-oxathiin-3-yl carboxamido) isopropylbenzoate (NSC 615985, UC84). Pharm. Biomed. Anal. 17, 27–38.

Peng, S.X., Strojnowski, M.J., Bornes, D.M., 1999. Direct determination of stability of protease inhibitors in plasma by HPLC with automated column-switching. J. Pharm. Biomed. Anal. 25, 343–349.

Pop, M., Rachwal, S., Vlasak, J., Biegon, A., Zharikova, A., Prokai, L., 1999. In vito and in vivo study of water-soluble prodrugs of dexanabinol. J. Pharm. Sci. 88, 1156–1160.

Rautio, J., Taipale, H., Gynther, J., Vepsalainen, J., Nevalainen, T., Jarvinen, T., 1998. In vitro evaluation of acyloxyalkyl esters as dermal prodrugs of ketoprofen and naproxen. J. Pharm. Sci. 87, 1622–1628.

Sawa, M., Tsukamoto, T., Kiyoi, T., Kurokawa, K., Nakajima, F., Nakada, Y., Yokota, K., Inoue, Y., Kondo, H., Yoshino, K., 2002. New strategy for antedrug application: development of metalloproteinase inhibitor as antipsoriatic drugs. J. Med. Chem. 45, 930–936.

Stella, V., 2005. Personal communication.

Udata, C., Tirucherai, G., Mitra, A.K., 1999. Synthesis stereoselective enzymatic hydrolysis, and skin permeation of diastereomeric propranolol ester prodrugs. J. Pharm. Sci., 544–550.

Wang, G., Hsieh, Y, Lau, YY., Cheng, K.C., Ng, K., Korfmacher, W.A., White, R.E., 2002. Semi-automated determination of plasma stability of drug discovery compounds using liquid chromotography-tandem mass spectrometry. J. Chromatogr. B 780, 451–457.

Wang, G., Hsieh, Y., 2002. Ultilization of Direct HPLC–MS–MS for Drug Stability Measurement. American Laboratory, pp. 24–27.