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Sample pooling to expedite bioanalysis and pharmacokinetic research

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Abstract

In the progression from drug discovery to development, not only pharmacokinetic (PK) characterization needed for lead compound selection often becomes a rate-limiting step, but also high volume of routine sample analysis ensued from numerous required biodisposition studies for the lead compounds and their back-ups often place a burdensome hurdle to the throughput of IND and NDA development phases. Higher throughput of PK screening via cocktail dosing has been reported to accelerate PK screening in the discovery phase. However, concerns on drug-drug interactions and other limitations associated with the cocktail M-in-One dosing (multiple compounds per dose per animal) has prompted the present investigation of sample pooling alongside One-in-One dosing strategy (one compound per dose per animal) as an alternative to the cocktail dosing approach. Using traditional HPLC for bioanalysis as an example, the present study illustrate the concept and usefulness of sample pooling that could facilitate the throughput of PK screening and characterization in both discovery and development phases. Six proprietary dopamine D_4 receptor antagonist preleads representing three different chemical classes, used as model compounds (C1–C6), were administered orally to rats. One rat received one compound and three rats were used for each compound. Six unknown plasma samples from six different rats at each time point were pooled. The pooled plasma samples were extracted by a one-step liquid–liquid extraction and concentrations of the six preleads were quantitated simultaneously. By sample pooling, a substantial amount of PK information was obtained at the same time for the six preleads, which requires much less workload than when bioanalysis is dealt with one compound at a time. For the first time in one aspect of innovative bioanalysis, the present investigation has demonstrated that sample pooling following One-in-One dosing can be utilized to enhance the throughput rate in PK screening in discovery phase. The sample pooling approach is likely to be useful in enhancing the throughput of PK characterization in development phase. With the advent of LC-MS and its becoming user-friendly, where separation of drug compounds is no longer an issue, the uniqueness of sample pooling may also pose a new way of thinking in regard to the old ways of handling bioanalysis for traditional PK research. © 1998 Elsevier Science B.V.

Keywords: Sample pooling; Bioanalysis; Pharmacokinetic screening; Throughput enhancement; One-in-One dosing; M-in-One dosing; HPLC; LC-MS; Rats

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1. Introduction

Advancement of three technologies, genomics (bioinformatics), combinatorial synthesis, and high throughput screening in pharmaceutical discovery research has resulted in the rapid identification of large numbers of prelead compounds. In the progression from discovery to development, pharmacokinetic characterization needed for lead compound selection often becomes a rate-limiting step. In addition, high volume of routine sample analysis ensued from numerous required biodisposition studies for the lead compounds and their back-ups often place burdensome hurdles to the throughput of IND and NDA development phases, which include enormous time consumption and prohibitive high cost for sample assay. These issues associated with drug discovery and development, in fact, are largely related to bioanalysis.

While numerous LC-MS (LC/MS or LC/MS/MS) examples have been shown to facilitate routine plasma sample assay, none except one has been reported to hasten pharmacokinetic screening in a broader sense via administering an intravenous cocktail dose of multiple compounds to experimental animals [1,2]. As the availability of LC-MS systems are limited, we have investigated an alternative throughput-enhanced approach, sample pooling, to characterize simultaneously the oral pharmacokinetics of multiple compounds utilizing traditional HPLC for illustrative purpose. To avoid complications that may result from drug-drug interactions due to cocktail administration (multiple compounds per dose per animal) [1,2], 'M-in-One dosing,' the present investigation features the idea of individualized oral dosing (one compound per dose per animal), 'One-in-One dosing,' followed by sample pooling for bianalysis. This throughput-enhanced approach was achieved by pooling individual samples from animals into one combined plasma sample per time point for simultaneous quantitation of drug concentrations.

For the first time in one aspect of innovative bioanalysis, the present investigation demonstrated the concept and practice of sample pooling with six proprietary psychiatric disorders discovery preleads, which belong to a class of dopamine D_4 receptor antagonists. The D_4 receptor has been reported to be an important target for new generations of antipsychotic agents [3,4], as the receptor is located in the limbic areas involved in control of cognition and emotion, where symptoms of schizophrenia may originate.

In fact, application of sample pooling approach goes beyond drug discovery phase and may also find its usefulness in development phase, as the approach is independent of either M-in-One or One-in-One dosing strategies, and of either traditional HPLC or LC-MS. In addition to detailed illustration of HPLC assay with sample pooling, which was applied after the One-in-One dosing scheme, brief discussions were attempted throughout the text of the present report to contrast with those associated with the cocktail dosing, the M-in-One dosing scheme, and to contrast with those associated with LC-MS.

2. Materials and methods

2.1. Chemicals, reagents, and apparatus

Six proprietary dopamine D_4 receptor antagonists as model compounds (C1, C2, C3, C4, C5, and C6) and the internal standard (IS) used for HPLC quantitation were obtained from Parke-Davis Pharmaceutical Research (Ann Arbor, MI). Polyethylene glycol 400 was obtained from Sigma (St. Louis, MO); absolute ethanol from Aaper Alcohol (Shelbyville, KY); and *n*-butyl chloride (B and J Brand High Purity) from Baxter Scientific Products (McGaw Park, IL). HPLC grade water, methanol, and acetonitrile were obtained from Mallinckrodt (Paris, KY). Sodium carbonate, sodium bicarbonate, triethylamine, and phosphoric acid (all GR grade) were obtained from EM Science (Gibbstown, NJ). Heparinized rat plasma was obtained in-house from male Wistar rats. The HPLC system consisted of a Waters Associates Model 600 Multisolvent Delivery System (Milford, MA), a Waters Model 712 WISP autosampler, and a Milton Roy SpectroMonitor 3100 W detector (Rochester, NY). Data were collected using a ChromJet integrator interfaced

with a Chrom Station/2 data system from Spectra Physics. Evaporation of plasma extracts was done with a Turbovap LV from the Zymark (Hopkinton, MA).

2.2. Standard and quality control preparation

Separate stock solutions for each of the six antagonists were prepared by dissolving 15 μmoles of each prelead compound in 10 ml HPLC grade methanol. Instead of μg, 1 μmole was used for accurate comparative purpose between compounds of diverse molecular weights (Table 3). An intermediate solution containing all six compounds at 75 nmole ml^{-1} in 50% methanol/50% water was then prepared by appropriate dilutions. The intermediate solution was used to prepare quality control samples in heparinized rat plasma at 450, 1125, and 2250 pmole ml^{-1}. These quality control samples, which contained all six compounds, were stored at −20°C until assay. Plasma-based standards at 150, 300, 600, 900, 1500, and 3000 pmole ml^{-1} were also prepared from this intermediate solution. These standards were freshly made before each assay. A stock solution of the internal standard was prepared in methanol and diluted in 50% methanol/50% water.

2.3. One-in-One dosing protocol and pharmacokinetic analysis

One animal received one prelead compound (One-in-One dosing). Three rats were dosed at 22 μmole kg^{-1} for each compound. In this study, a total of 18 fasted male Wistar rats received oral doses by gavage. Separate dosing solutions of 4.2 μmoles ml^{-1} in 25% ethanol/50% PEG 400/25% water were made for each of the six D_4 receptor antagonists. One day prior to the study, jugular cannulae were surgically implanted in each animal and all rats were fasted overnight prior to dosing. Serial blood samples were collected from the jugular vein into heparinized tubes from each rat at 0.5, 1, 2, 4, and 8 h postdose to characterize plasma profiles of the prelead compounds. Plasma was separated from the red blood cells by centrifugation and stored at −20°C until analysis. After oral dosing, areas under the plasma concentration–time curves (AUC) were calculated using the trapezoidal rule with extrapolation to infinite time. The apparent terminal elimination half life is given by $t_{1/2}: = 0.693/\lambda$, where λ is the rate constant of the terminal phase. Peak concentration (C_{max}) and the corresponding peak time (t_{max}) were recorded from the observed data.

2.4. Sample pooling and liquid–liquid extraction

Sample pooling technique was used in an attempt to expedite the process of pharmacokinetic screening. An example of the pooling at each time point is as follows: 100 μl of one of the three 30 min samples for each prelead (C1–C6) were randomly mixed with 100 μl of one of the three 30 min samples for each of the other five compounds. Each pool had a total of 600 μl. Similarly, other pooled samples were formed so that there were 15 pooled plasma samples—three pooled samples ($n = 3$) for each of the five sampling time points. In preparing standards and quality control samples, 100 μl of standard or quality control sample was mixed with 500 μl of blank plasma so as to keep the same overall plasma volume between standards, quality control samples, and unknowns.

Extractions were carried out in 16×100 mm borosilicate screw-cap tubes as follows: to 600 μl of plasma (pooled as above), were added 50 μl of internal standard (IS) working solution (1000 ng ml^{-1}), 200 μl 1.0 M sodium carbonate (pH 9.7), and 9 ml n-butyl chloride. Tubes were capped with Teflon-lined caps and rocked for 30 min on a Lab Quake shaker (Labindustries, Berkeley, CA) and then centrifuged for 10 min at 3000 RPM to separate the phases. The upper organic layer was transferred to a 13×100 mm borosilicate tube and evaporated at 50°C in a Turbovap evaporator. Dried extracts were then reconstituted with 200 μl mobile phase A, and 150 μl was injected onto the HPLC column.

2.5. Chromatographic separation, detection, and data acquisition

The six analysis and internal standard were

analyzed by an HPLC gradient method that utilized a 0.46×15 cm, 5 micron Ultrasphere ODS column from Beckman Instruments (Fullerton, CA) protected by a Brownlee 3.2×15 mm, 7 micron RP-18 Newguard guard column from Applied Biosystems (Foster City, CA). The gradient system consisted of two mobile phases; mobile phase A was 0.4% triethylamine, pH adjusted to 3.0 with H_3PO_4/acetonitrile (90/10) and mobile phase B was 0.4% triethylamine, pH 3.0/acetonitrile (20:80). The separation gradient was operated from 90% A/10% B to 75% A/25% B in 45 min. Flow rate was 2.0 ml/min. Between injections the column was washed with 100% mobile phase B for 10 min and then re-equilibrated at the initial conditions of 90% A/10% B for 10 min before the subsequent injection. All analyses were detected with ultraviolet absorbance monitoring at 210 nm. Chromatographic peak responses were integrated and peak height ratios (drug/internal standard) were calculated. The height ratio-concentration standard curve was constructed for each of the six analyses using weighted (1/concentration^2) linear regression. Concentrations in unknown samples and quality control samples were calculated from the standard curve. Limit of quantitation was 100–150 pmole ml^{-1}.

2.6. Stability tests

Stability of the six D_4 antagonists and the internal standard in rat plasma was tested by incubating freshly prepared quality control samples at 37°C for 2 h. Stability of these compounds in mobile phase A (the injection solvent) for 27 h at room temperature was also tested. Change in absolute peak height, compared to the peak height value at time zero was used as an index of stability.

3. Results and discussion

3.1. Chromatographic results and stability

An HPLC chromatogram of control rat plasma spiked with six dopamine D_4 receptor antagonist prelead compounds and the internal standard is shown in Fig. 1(a). Due to the proprietary status, chemical structures of these preleads are not shown. The chromatogram of blank control plasma (600 μl) is given in Fig. 1(b), where it is shown that no endogenous peaks interfere with prelead compound peaks. It is worth noting that the present HPLC operating conditions allow quantitation at UV 210 nm, a wavelength that is often associated with interfering peak responses. Few of these prelead compounds are fluorescent and good absorbance at UV higher than 210 nm is not available for any of the compounds. In fact, the six preleads represent three different chemical classes, suggesting that chemically unrelated compounds can be combined for assay using the present gradient HPLC approach.

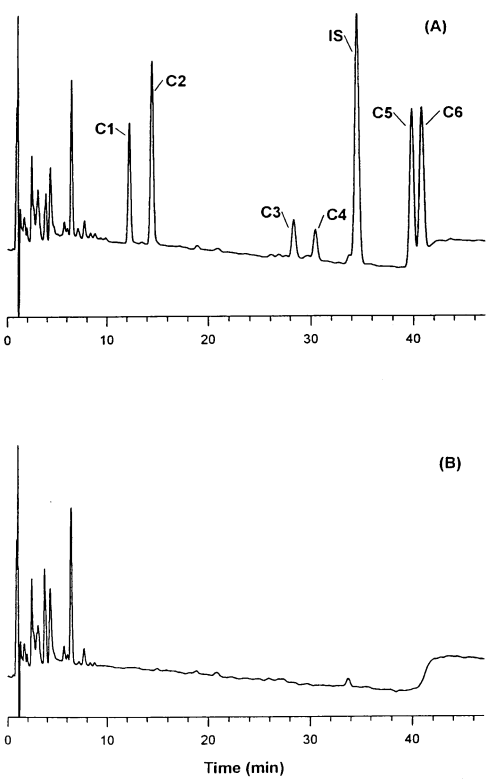

Fig. 1. HPLC chromatograms: (A) six preread compounds (C1–C6) spiked in rat plasma and the internal standard, IS; and (B) blank rat plasma (600 μl).

Table 1
Intra-assay validation for the six tested preread compounds (C1–C6) in rat plasma: observed concentration (precision, %RSD) (accuracy, %RE) of three quality control samples (450, 1125, and 2250 pmole ml^{-1}) $n = 3$

Preleads	Observed concentrations (pmole ml^{-1}), mean (%RSD), (%RE)		
C1	443 (8.1%) (−1 5%)	1114 (7.1%) (−1.0%)	2263 (3.9%) (+0 6%)
C2	451 (3.0%) (+0.2%)	1109 (4.3%) (−1.4%)	2130 (0.8%) (−5.4%)
C3	437 (10.0%) (−2.9%)	1072 (6.8%) (−4.7%)	2228 (1.7%) (−1.0%)
C4	460 (2.9%) (+2.2%)	1143 (1.1%) (+1.6%)	2430 (4.1%) (+8.0%)
C5	395 (3.3%) (−12.3%)	1062 (12.3%) (−5.6%)	2046 (2.8%) (−9.1%)
C6	393 (4.8%) (−12.7%)	1058 (4.4%) (−6 0%)	2052 (0.9%) (−8 8%)

Stability in plasma (or other biological matrices) and in relevant solutions is the first issue that should be addressed prior to development/validating and applying an HPLC method for pharmacokinetic (PK) screening. Moreover, results of a stability study may serve as an initial screening tool in the event that one or more of the compounds under evaluation are labile. Compared to the peak height at time zero, all six prelead compounds were found to be stable in rat plasma for at least 2 h at 37°C or in the HPLC injection solvent for 27 h at room temperature. No degradation component(s) was detected for any of the six compounds.

3.2. HPLC method validation

The present gradient HPLC method was validated simultaneously for all six compounds in plasma through the sample pooling approach, instead of six validations for six compounds. The method validation was confirmed in a one-day pre-study, which may be considered minimum optimal under the time constraint. Pre-study validation was acceptable for all six preleads as evidenced by intra-assay precision and accuracy obtained for the three quality control samples (Table 1). The overall intra-assay precision (%RSD) ranged from 0.8% to 12.3% and intra-assay accuracy (%RE) ranged from −12.7% to 8.0%. Similar values of %RSD and %RE for the same three quality control samples were also observed on the second occasion when the in vivo samples were analyzed in one day (batch). Limits of quantitation for all six preleads were in the range of 100–150 pmole ml^{-1} at the wavelength 210 nm. To further expedite the throughput rate, method validation and presence of quality control samples that come along with the calibration standards may be skipped altogether, depending on the status of GLP (good laboratory practice) or non-GLP for the studies.

3.3. Pilot samples

For each of the six compounds we picked one representative sample out of three replicates collected at 2 h postdose for pilot screening. The purpose was two-fold: firstly, to timely modify the existing HPLC system and make it suitable for accurate assay should any potential metabolise(s) show interference; and secondly, to obtain early information for each prelead regarding absorption and metabolism. It should be noted that use of LC-MS could greatly facilitate the method development as the chromatographic interference would be minimum. Selection of the 2 h sample for all prelead compounds is a compromise, assuming that this is a rough time point where peak drug and metabolise concentrations might occur. Without resorting to use of LC-MS, the present traditional HPLC method had quickly disclosed substantial amount of information simultaneously for all six compounds on both absorption and metabolism, based on just a few pilot runs.

Shown in Fig. 2 are three chromatograms representing dosing with compounds C5 and C2. C5 had a minor metabolise peak (C5M) (Fig. 2(a)). C2, which was negligible on the chromatogram, had two sizable metabolise peaks and one of them ($C2M_2$) was eluted closely to the parent compound (Fig. 2(b)). The presence of $C2M_2$ was not

confirmed until the same in vivo sample was spiked with the C2 parent compound and re-injected into the chromatograph (Fig. 2(c)), which revealed that $C2M_2$ eluted later than C2. These results suggest that C2 and C5 were significantly absorbed and that C2 was transformed into at least two major metabolises.

When comparing the 2 h sample chromatogram to a control plasma sample for each individual compound collected prior to dosing, a total of four drug compounds were found to form metabolises (Table 2). Note that there might exist conjugate metabolises that are not detectable by the present uv measurement. Retention time data (Table 2) suggested that metabolises would not interfere with the assay of parent compounds, and therefore pooling of a total of six plasma samples (100 μl per compound per time point postdose) would be workable for simultaneous quantitation. This is demonstrated in Fig. 3(a) which shows a chromatogram of a 1 h pooled sample with acceptable separation of analyses from metabolises and other endogenous peaks.

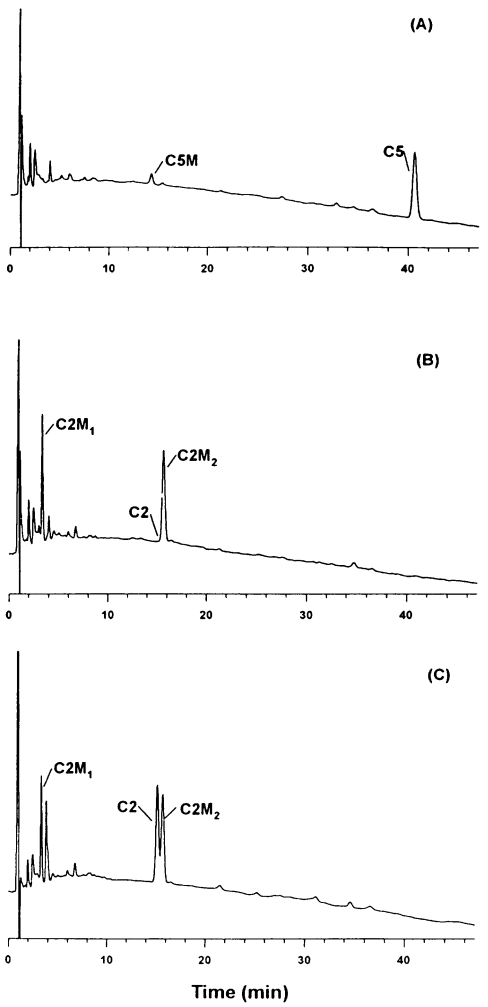

Fig. 2. HPLC chromatograms from plasma samples collected 2 h after oral dosing with compound C5 (panel A) which had one metabolite (C5M) or dosing with C2 (panel B) which had two metabolites ($C2M_1$ and $C2M_2$). Panel C is the result from spiking the same plasma sample in panel B with parent C2 to prove the presence of $C2M_2$.

It should be noted that separation of pooled parents and metabolises, which is normally one of a few big hurdlers in method development, would no longer be an issue with the advent of LC-MS and its gradually becoming user-friendly. This is because that the separation is based on molecular weight detection, and that, with quadruple (single or triple) or ion trap, the MS total ion scanning can allow detection of potential metabolises, conjugated and unconjugated, although the sensitivity of currently available LC-MS equipment toward the metabolises still has its limited capacity due to dilution effect on the detection intensity.

3.4. Pharmacokinetic (PK) profile under sample pooling

Substantial amount of PK information for all six preleads are simultaneously available. It is of interest to note that the 1 h sample (Fig. 3(a)) revealed peak responses of all parent compounds and metabolises (except for $C2M_2$) that are significantly larger than their corresponding peak responses at 4 h (Fig. 3(b)). This is a manifestation of both rapid oral absorption and rapid metabolise formation. The exception of $C2M_2$ indicates a slow formation of this metabolise which persisted in plasma at a high concentration for quite some time (Fig. 4). All parent compounds peaked at a time shorter than 1 h (Fig. 4), indicating rapid oral absorption. Apparent oral terminal elimination half lives were roughly estimated to be 0.4 to 2.8 h (Table 3). The plasma

Table 2
Retention times (min) of six dopamine D_4 antagonists (C1–C6) and their metabolites from a pooled plasma sample at 2 h postdose

Retention (min)	C1	C2	C3	C4	C5	C6
Parent	12.4	14.5	28.5	30.6	40.1	41.0
Metab.[a]	ND[b]	3.2, 15.1	35.9	ND	13.8	7.9, 11.8

[a] Only one or two metabolite peaks are discernable for each of four parent compounds.
[b] ND, not detectable.

$AUC(0-\infty)$ of C2 is ranked low, while its two metabolises have high equivalent $AUC(0-\infty)$ values. Formation of metabolise $C2M_1$ is rapid, while that of $C2M_2$ is a slower process (Fig. 4). Note that C2 had two large metabolise peaks (Fig. 4).

On the other hand, compound C3 was not detected at any earlier and later time points and no metabolise peak(s) was apparent (Fig. 4), suggesting a negligible plasma availability for C3. Note that all six preleads are soluble in the PEG 400:ethanol:water co-solvent formulation and

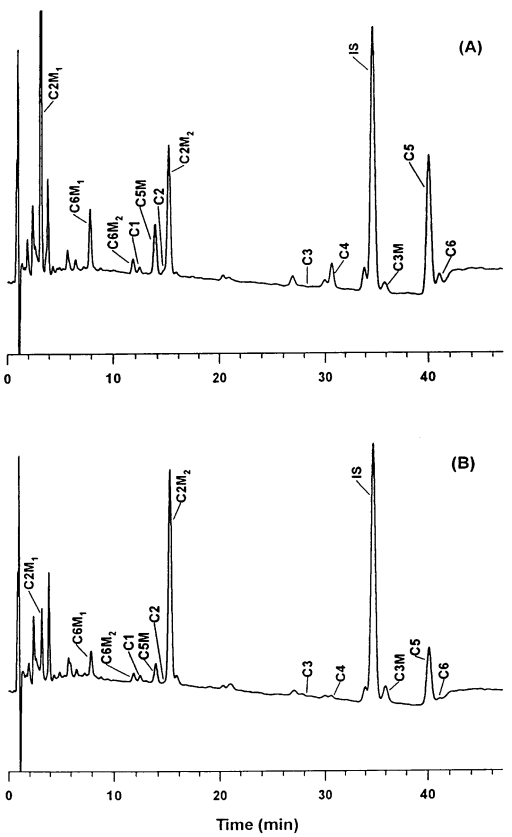

Fig. 3. HPLC chromatograms of pooled plasma samples from six individual rats, each received orally a single different compound: (A) samples collected at 1 h postdose; and B) samples collected at 4 h postdose. Six preread compounds (C1–C6) and six metabolites ($C2M_1$, $C2M_2$, C3M, C5M, $C6M_1$, and $C6M_2$).

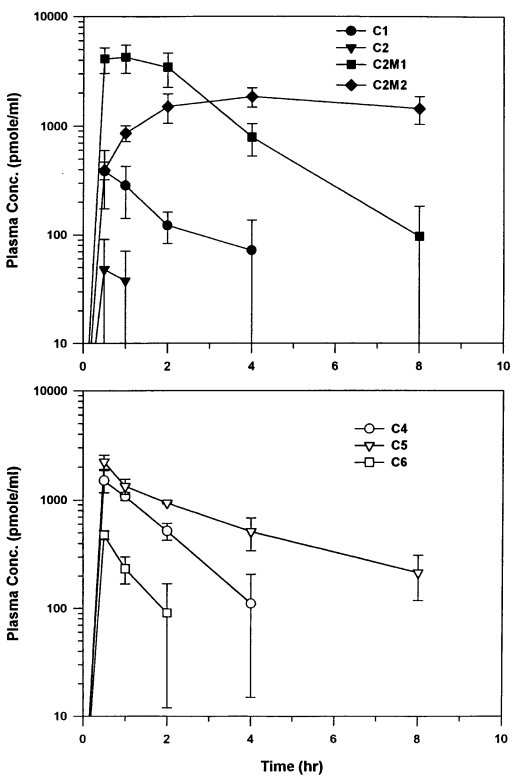

Fig. 4. Plasma concentrations of the six preread compounds (C1–C6) in male Wistar rats following a 22 μmole kg^{-1} oral dose. C3 (non-quantifiable) and minor metabolites such as C3M, C5M, $C6M_1$, and $C6M_2$ are not presented.

Table 3
Mean (S.D.) pharmacokinetic parameters of six dopamine D_4 antagonists (C1–C6) in male Wistar rats following a 22 μmole kg^{-1} PO Dose ($n = 3$)

Parameters	C5	C4	C1	C6	C2[a,b]	C3[c]
Mol. wt. (g $mole^{-1}$)	392	301	337	338	305	327
t_{max} (h)	0.5 (0.0)	0.5 (0.0)	0.5 (0.0)	0.5 (0.0)	0.7 (0.3)	0 (0.0)
C_{max} (pmole ml^{-1})	2239 (322)	1514 (348)	386 (212)	477 (45)	66 (16)	0 (0.0)
$t_{1/2}$ (h)	2.8 (0.8)	1.1 (0.1)	1.8 (0.1)	0.8 (0.2)	0.4 (0.5)	0 (0.0)
AUC($0-\infty$) (pmole·h ml^{-1})	6460 (1128)	2714 (150)	903 (446)	612 (52)	70 (61)	0 (0.0)

[a] Parameters estimated for the first metabolite of C2 ($C2M_1$) are: t_{max}, 0.8 (0.3) h; C_{max}, 4286 (1177) pmole ml^{-1}; $t_{1/2}$, 1.3 (0.2) h; and AUC($0-\infty$), 12 455 (3377) pmole·h ml^{-1}.
[b] Parameters estimated for the second metabolite of C2 ($C2M_2$) are: t_{max}, 3.3 (1.2) h; C_{max}, 1928 (295) pmole ml^{-1}; $t_{1/2}$, 12.4 (4.4) h; and AUC($0-\infty$), 38 364 (14 943) pmole·h ml^{-1}.
[c] No C3 was detected at any time point and data were treated as zero.

they are stable in plasma for at least several hours. For C3 with negligible plasma concentrations of the parent and metabolises, this might be due to very little oral absorption and/or to an extremely large volume of tissue distribution that obliterated a possible significant appearance of C3 in plasma.

Although demonstrated with a setting of oral dosing, the same concept and practice of sample pooling can be applied to intravenous study where other PK parameters (such as clearance, volume of distribution, and bioavailability) can be obtained, if an appropriate common vehicle for IV dosing is available. Of course, more frequent blood sampling for a longer time duration is always desired for a more accurate PK characterization. Moreover, it should be noted that to rank the relative extent of oral absorption between the six preleads, information of oral plasma AUC is far from enough. For the purpose of ranking relative oral absorption between preleads, IV data are needed for such a characterization, namely, oral bioavailability. This is because that it is likely that a higher plasma AUC may be due to a lower volume of distribution, not to a higher oral absorption; and that a lower plasma AUC may be due to a higher volume of distribution, not to a lower oral absorption. This pitfall can only be easily overcome by a parallel separate IV study.

As the present investigation used solution preparation for the six preleads and applied sample pooling to assay work with a validated HPLC method, following the On-in-One dosing scheme (one compound per dose per animal), the PK parameters so obtained for each compound are tenable, while parameters obtained through a cocktail dosing containing these six compounds might be polluted to a certain degree, depending on the size of the combined dose. This concern associated with cocktail dosing may further become an issue when it is applied to multiple dosing, dose-proportionality or dose-rising studies, either IV or PO route, as the combined higher dose in the cocktail may be open to tout for formulation difficulty and saturation kinetics that could confound with drug-drug interaction.

3.5. Reduced assay workload and enhanced throughput of pharmacokinetic research

Sample pooling as described in Section 2 reduced the overall work load by several folds, when the pooling is compared to non-pooling. First, although total run time per pooled sample is longer than the individual sample, the number of unknown samples for a total of six compounds needed to be assayed was reduced from 90 to 15 samples (five time points per rat, three rats per compound) in the present case. Second, as a one-day assay validation process is considered a minimum requirement for accurate quantitation of plasma concentration, simultaneous validation (standard curves and quality control samples) and simultaneous quantitation by sample pooling of

six compounds on one same occasion gave additional workload reduction when compared to the total completion of the same job for each compound at six individual occasions. Reduction of workload would be more pronounced when massive number of samples from several different compounds are to be dealt with. Here it is open to debate if method validation and quality control samples are really needed when a good calibration standard curve is obtained and the non-GLP assay is to be dealt with, particularly in the discovery phase.

The end result of reduced assay workload is the enhanced throughput of PK characterization. Additional merit associated with sample pooling is the simultaneous reporting of six compounds with respect to their comparative PK profiles. Note that the number of compounds that can be pooled depends on the system capacity of HPLC or LC-MS, and even a pooling of only two or three compounds has certain benefits when a long list of prelead compounds are awaiting PK characterization in the discovery phase. Clearly, much longer time would be needed to complete the oral PK screening from the method development through the compilation of final job report for these six compounds if the traditional one-compound-at-a-time bioanalytical measure is used.

4. Conclusions

With the example of six dopamine D_4 receptor antagonist prelead compounds after oral dosing, the present investigation has demonstrated for the first time the usefulness of a simple concept of One-in-One dosing followed by sample pooling in accelerating PK screening. Of six preleads, compound C5 was found to have the highest plasma $AUC(0-\infty)$, while the two metabolises of C2 might have an equivalent $AUC(0-\infty)$ several-fold greater than that of C5. The lowest performer in the rank of $AUC(0-\infty)$ was identified to be compound C3. However, from a PK perspective, for selection of the highest orally bioavailable lead compound and its back-up for future development, it is required that the intravenous PK study be conducted by the One-in-One dosing followed by sample pooling analysis. Note that the drug-drug interaction associated with the M-in-One approach [1,2] would not occur with the One-in-One approach, although the latter consumes more animals and has more samples from a PK study than the former. And, it is recognized that application of LC-MS would further accelerate the PK process.

5. Implications

With advancement of three technologies, genomics (bioinformatics), combinatorial synthesis, high throughput (cell culture- and receptor-based) screening in drug discovery research, the demand from the discovery phase of pharmaceutical research to shorten the process of PK screening needed for lead compound has been strong. To meet the demand, the sample pooling following One-in-One dosing presented in this investigation and the M-in-One approach reported previously [1,2] may provide two potential solutions. These two different approaches may be further facilitated once LC-MS technology becomes more readily available, more user friendly, and more efficient and reproducible in detection. Issues associated with traditional HPLC such as run time, sensitivity, detection, and endogenous interfering would no longer be a concern when MS is hyphenated with LC. Namely, utilization of MS may further facilitate the method development process and assay work, and if the capacity of LC-MS is powerful enough and if concerns associated M-in-One dosing can be minimized, a combination of sample pooling and cocktail dosing with LC-MS would make the PK screening a truly high throughput manner.

The concept and practice of sample pooling technique described here may also find for itself a right niche beyond the discovery phase. During development stages of IND (Investigation of New Drugs) and NDA (New Drug Application), biological samples generated from numerous required biodisposition and toxicokinetic studies for the lead compounds and their back-ups often place a burdensome hurdle to the development throughput, which is, unfortunately, associated

with huge cost for sample assay, if jobs of sample assay are contracted out. In these cases, both the time constraint and the assay cost probably can be substantially relieved, and thus the throughput of PK characterization is enhanced, through the application of sample pooling when more than two drug compounds are under consideration or investigation. With LC-MS and its becoming popular for routine sample assay as a trend for the near future, this is highly possible as drug compounds of different chemical classes can be pooled and easily handled by MS based on the total ion scanning of molecular weight.

The concept of sample pooling may also pose new thinking to the old ways of handling bioanalysis for traditional PK characterization. The culture of one-compound-at-a-time PK in pharmaceutical industry may be changed when faced with the new challenge in the era of new technology. In fact, sample pooling is independent of either M-in-One or One-in-One dosing strategies, and of traditional HPLC or LC-MS. Therefore, sample pooling may have wider implications. In addition to the aforementioned benefits for increased throughput in PK screening and characterization, sample pooling can be applied to bile, urine, tissue, and other biological samples, be it radiolabelled or nonlabelled. From the standpoint of efficiency and cost reduction, sample pooling may be appealing to high volume routine sample analysis, and to any other studies requiring sample assay, be it in vitro or in vivo. Moreover, sample pooling may be also applicable to non-biological samples that depend on HPLC or LC-MS assay for quantitation.

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