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THE APPLICATION OF 19F-NMR IN THE ANALYSIS OF DRUG-RELATED SUBSTANCES OF LERITRELVIR (RAY1216) IN HUMAN URINE AND FECAL SAMPLES TO SUPPORT A PHASE I CLINICAL STUDY

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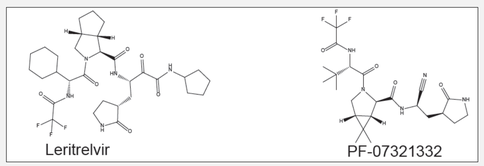
PURPOSE

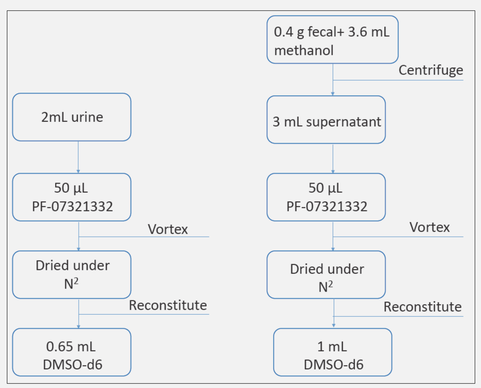
The human mass balance studies can provide critical data for evaluation of the absorption, distribution, metabolism, and excretion (ADME) properties of a new chemical entity (NCE). Traditionally, this has been performed by administration of radiolabeled drug substances, typically 14C or occasionally 3H, as detection methods for these isotopes allow absolute quantification of drug-related substances (DRMs) in blood, plasma, and excreta. However, the radiolabeling studies are usually costly and time consuming. As an alternative, Nuclear Magnetic Resonance (NMR) spectroscopy has recently emerged as a powerful analytical technique in mass balance study. Its non-destructive and non-invasive nature, coupled with advantage to provide detailed structural information, has made it an invaluable tool for quantitative analysis. Furthermore, the advent of high field magnets and cryoprobes significantly improve the sensitivity of NMR for its application in drug in vivo ADME research. Among the NMR based bioanalytical methods, 19F-NMR can provide qualitative insights for human ADME endpoints of fluorinated compounds, including mass balance, total DRM determination and metabolite profiling and identification in plasma and excreta. In this study, we developed 19F-NMR methods in urine and fecal samples in early clinical studies of Leritrelvir (RAY1216) to obtain preliminary data for human mass balance.

The structure of Leritrelvir and its internal standard PF-07321332:



METHOD

Sample Preparation



METHOD (CONTINUED)

NMR Conditions

Instrument	Bruker AVANCE NEO 400 MHz with 5mm BBO probe with a z-gradient system
Acquisition Parameters	TD:32668 SW: 7462 687 Hz D1: 5.0s NS: 1024 DS: 4 O1p: -73.2 ppm CPD2: waltz65
Data Process	Data were zero-filled to 65536 complex points to Fourier transformation and an exponential window function was applied with a line-broadening factor of 1.0 Hz. The spectra were manually phase and baseline corrected and referenced to the internal standard PF-07321332 (= -72.9 ppm)

RESULTS

During the method development options of internal standards were carefully evaluated

- The internal standard should have a distinct and well-separated chemical shift from Leritrelvir (RAY1216) in NMR spectrum to ensure the absence of signal overlap and interference to peak integration.
- To avoid off-resonance effects, the fluorine shift should not differ too much from Leritrelvir (RAY1216).
- Signal intensity of the internal standard should not differ more than 50-fold from the expected lowest and highest concentrations of Leritrelvir (RAY1216).

Typical NMR spectra of urine and fecal samples are shown in Figure 1 and 2, respectively.

Figure 1. *Typical spectrum for urine sample*

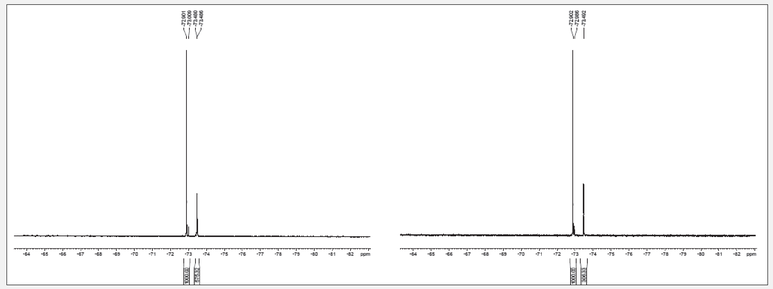


Figure 2. *Typical spectrum for fecal sample*

The standard curve and quality control sample for the urine and fecal matrix was prepared by addition of the working solution of Leritrelvir (RAY1216) into responding blank matrix. The methods were qualified before sample analysis.

The standard curve ranges for urine and fecal are 3.50-175 µg/mL and 35.0-3500 µg/g, respectively. The response ratio of Leritrelvir (RAY1216) to internal standard is plotted against the concentration of Leritrelvir (RAY1216) in the standard curve.

The standard curve for urine and fecal is $y=0.03711x-0.008574$ ($r^2=0.9980$) (shown in Figure 3) and $y=0.003321x-0.02395$ ($r^2=0.9998$) (shown in Figure 4), respectively.

Figure 3. *Standard curve for Leritrelvir (RAY1216) in urine matrix*

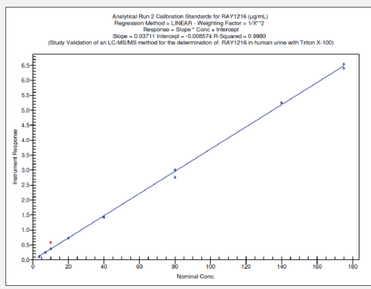
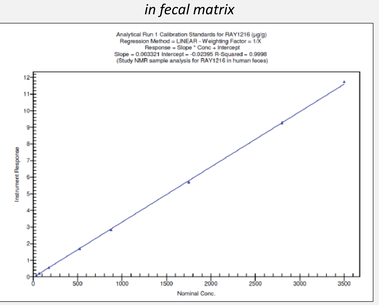


Figure 4. *Standard curve for Leritrelvir (RAY1216) in fecal matrix*



RESULTS (CONTINUED)

The accuracy of quality control samples for urine and fecal method are within 0.6 to 3.1% and 0 to 2.6%, respectively. (shown in Table 1).

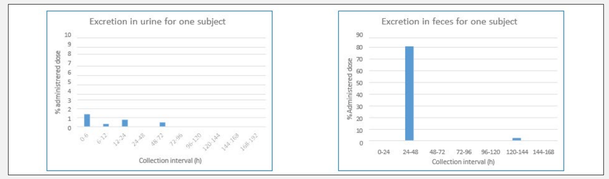
There was no matrix effect observed from different lots of matrix or between matrix and neat solution for urine and fecal matrix.

Table 1. *The Accuracy and Precision results for urine and fecal matrix*

Specification	Urine matrix				Fecal matrix			
	LLOQ	LQC	MQC	HQC	LLOQ	LQC	MQC	HQC
Nominal Conc. µg/mL	3.5	10.5	70	135	3.5	10.5	140	270
Mean of measured Conc. µg/mL (n=6)	3.61	10.8	70.4	138	3.59	10.5	141	271
CV(%)	16	5.6	3.4	3.2	5.4	2.8	0.4	2
Bais(%)	3.1	2.9	0.6	2.2	2.6	0	0.7	0.4

The urine and fecal samples in phase I clinical trial have been analyzed using the qualified method. The percentage of dose excreted in each collection interval, for both urine and feces, are shown in Figure 5. For urine, the percentage of dose excreted up to 192 hours post dose was about 3%. For feces, up to 168 hours post dose, about 82% of the dose was excreted.

Figure 5. *Excretion of Leritrelvir in urine and fecal for one subject*



CONCLUSION

In this study, sensitive and specific 19F-NMR bioanalytical methods in urine and fecal matrix have been developed and qualified to support a Phase I clinical trial of Leritrelvir (RAY1216) to obtain preliminary human mass balance data. The established 19F-NMR methods can be used as fast and reliable alternatives to traditional radiolabeled human mass balance for fluorinated compounds.

This qualified method has been applied to support urine and fecal samples analysis in clinical trial titled: A randomized, double-blind, placebo-controlled Phase I trial evaluating the tolerability, pharmacokinetics, and food effects of Leritrelvir (RAY1216) tablets in adult subjects.

REFERENCES

1. James, Alexander David, et al. Comparison of 19F-NMR and 14C measurements for the assessment of ADME of BYL719 (Alpelisib) in humans. DOI: 10.1124/dmd.117.075424
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