

## Introduction

The standard approach for assessing the mass balance of a compound in drug development involves administration of a radiolabeled (Carbon-14 or Tritium) drug to animals and humans because relatively simple and well established analytical methodologies can be employed for the detection of the total radioactivity derived from the drug and drug-related metabolites in excreta. When radiolabeled drug is not available, mass spectrometry-based techniques may be utilized to detect and quantify drug and its metabolites. However, this approach requires authentic reference standards for accurate assessment that are mostly unavailable especially at the early drug development stage. In addition, the results may not be reliable due to the lack of total metabolism information.

Fluorine-19 ( $^{19}\text{F}$ ) NMR provides an alternate means for quantitation of fluorine-containing drugs and metabolites without the prerequisite of synthetic reference standards or radiolabeled material.  $^{19}\text{F}$  NMR shares some attributes of radiochemical analysis with the following advantages:

- $^{19}\text{F}$  has a relative abundance of 100%, making measurements very fast (relatively sensitive, 15 min acquisition time at 10  $\mu\text{M}$ )
- $^{19}\text{F}$  yields sharp signals and has a wide chemical shift range
- Integrals of  $^{19}\text{F}$  signals are reliable with an accuracy of  $\pm 10\%$  (routine acquisition) or  $\pm 1\%$  (5x relaxation delay) due to the lack of an NOE (fluorine forms one bond only).
- Natural abundance of  $^{19}\text{F}$  is very low resulting in very low background
- Sample processing is relatively simple (urine and bile can be easily redissolved in deuterated oxide, and a simple sample work up is sufficient for feces).

## Overview

- The utility of  $^{19}\text{F}$  NMR spectroscopy as a means of measuring a compound's mass balance is described.
- The mass balance data by  $^{19}\text{F}$  NMR were compared to those obtained by radio-assay. The percent difference between the two methods was mostly  $< 10\%$ .
- The limit of quantitation for compounds with a  $\text{CF}_3$  group or a single fluorine atom was measured in a sensitive H, F Probe. The LOQ for a compound (MW 359) with a  $\text{CF}_3$  was  $0.31 \mu\text{g/mL}$ , and  $1.5 \mu\text{g/mL}$  for a compound (MW 595) with a single F.

## Experimental

**NMR:** Bruker 500 MHz  
**NMR Probe:** H, F Probe  
**Software:** Topspin 1.2.  
**Solvent:** 70%  $\text{CD}_3\text{OD}$  in  $\text{D}_2\text{O}$   
**Internal Standard** TA2(containing  $-\text{CF}_3$ )

### Sample Preparation:

Each 1 gram of fecal sample was extracted successively with 3 and 2 volumes of methanol:water (2:1) containing 0.1% formic acid. The supernatants were combined,  $\text{N}_2$ -evaporated to dryness, and reconstituted in 70%  $\text{CD}_3\text{OD}$  in  $\text{D}_2\text{O}$  with internal standard for NMR analysis.

## Results and Discussion

### 1. Preparation of Calibration Curve

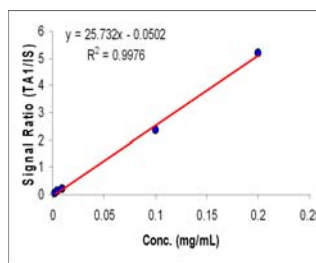
A series of calibration standards were prepared to determine the linearity of TA1. The integral ratios between TA1 and internal standard TA2 were plotted against the corresponding concentrations of TA1. The calibration curve was linear for TA1 over the range of  $2.5 \mu\text{g/mL}$  to  $0.2 \text{ mg/mL}$  (Figure 1).

Feces (h)	Percent of Dose Determined by		Difference (%)
	Radioassay	$^{19}\text{F}$ NMR	
S <sub>1</sub> 0-168	39.0	36.4	-6.67
S <sub>2</sub> 0-168	34.2	37.2	8.77
S <sub>3</sub> 0-168	39.3	35.7	-9.16
S <sub>4</sub> 0-168	34.1	36.3	6.45
S <sub>1-6</sub> 0-48	15.9	17.8	11.95
S <sub>1-6</sub> 48-96	14.6	15.4	5.48
S <sub>1-6</sub> 96-168	5.40	3.60	-33.3
S <sub>1-6</sub> 0-168 (sum)	35.9	36.8	2.51

**Table 1.** Comparison of %dose excreted in feces by radioassay and  $^{19}\text{F}$  NMR.

### 2. Comparison of Mass Balance Data by Radioassay and $^{19}\text{F}$ NMR

As shown in Table 1, the mass balance data obtained by  $^{19}\text{F}$  NMR for pooled 0-48-, 48-96-, and 96-168-hr feces from subjects 1 to 6, and 0-168-hr feces from subjects 1 to 4 were similar to those determined by radioassay. The difference was mostly within 10%.

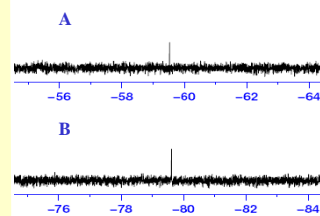


**Figure 1.** Calibration curve for the determination of TA1 in the range from  $2.5 \mu\text{g/mL}$  to  $0.2 \text{ mg/mL}$ .

### 3. Lower Limit of Quantitation (LLOQ)

A compound containing a  $\text{CF}_3$  with an MW 359 Da at  $0.31 \mu\text{g/mL}$  ( $0.65 \mu\text{M}$ ) showed a  $^{19}\text{F}$  signal with an S/N 2:1 (Figure 2A).

A compound containing a single F atom with an MW 595 Da at  $1.5 \mu\text{g/mL}$  ( $2.5 \mu\text{M}$ ) showed a  $^{19}\text{F}$  signal with an S/N 3:1 (Figure 2B).



**Figure 2.** Limit of Quantitation of compounds with a  $\text{CF}_3$  (A) or a single F atom (B).

Study No.	Urine (%dose)	Feces (%dose)	Extraction Recovery (%)
1	19.1	72.6	96.1
2	1.98	57.2	99.8
3	2.59	33.6	99.4
4	10.5	84.8	97.1
5	50	42	98.8
6	25.4	56	84.5
7	30.5	56.6	86.9
8	1.26	11.6	88.5
9	21.6	73.9	89.8
10	54.5	46.4	95.4

**Table 2.** %Dose recovered in urine and feces and fecal extraction recovery from 10 mass balance studies conducted at XBL.

### 4. Potential of $^{19}\text{F}$ NMR Application to Mass Balance Studies

The mass balance results from 10 XBL studies are summarized in Table 2, with an average recoveries of 53.5% and 21.7% of the dose in feces and urine, respectively. With a simple sample clean-up procedure, extract from 0.5 g feces is analyzable by  $^{19}\text{F}$  NMR, suggesting the feasibility of  $^{19}\text{F}$  NMR to any clinical samples with a dose  $\geq 5$  mg/subject (assuming 250 g of feces/day/subject).

## Conclusion

For fluorine-containing drugs,  $^{19}\text{F}$  NMR provides an alternate means for the determination of the mass balance without the prerequisite of synthetic reference standards or radiolabeled material.  $^{19}\text{F}$  NMR was demonstrated to be a selective and sensitive method to determine mass balance in feces.