

I. INTRODUCTION

A. Purpose of the Study

The purpose of this study is to perform an Independent Laboratory Validation (ILV) of BASF Analytical Method L0091 "THE DETERMINATION OF RESIDUES OF BAS 650F AND ITS METABOLITES, M650F01, M650F02, M650F03, AND M650F04 IN SOIL SAMPLES" and to demonstrate that the Method L0091 can be performed with acceptable recoveries at an outside facility.

B. Summary of the Results

The independent laboratory validation of the BASF method was successfully completed. The first trial met the criteria for a successful validation of the method for soil for BAS 650F and its metabolites. Communication between the performing laboratory and the study monitor was not needed for successful completion of the method validation.

II. SAMPLE HISTORY

Homogenized control soil, BASF sample code RSN R080484-0018 (Study No. 250678) was provided by BASF. The test system has BASF sample code R080484-0027 (Study No. 250678) and is used for the study. Soil with RCN R080484 is from Ontario and has been identified as sandy loam. This soil is collected from 18-24 inches and was chosen for this study so that the matrix will be harder to work with in comparison to other soils. The sample was received on September 02, 2009. Upon receipt of this sample, Alliance Pharma did the inventory and stored the sample in the freezer of -20 °C. In addition, the aliquots taken for control and fortification purposes were also documented accordingly.

III. PROCEDURE - METHOD SYNOPSIS

BASF method L0091, version 01, TP (technical procedure) 02 (Reference 2) as obtained by the sponsor, using LC-MS/MS was employed. The procedure is briefly described as following:

The analytes were extracted from soil by shaking with acetonitrile followed by acetonitrile/water (50:50 v/v). An aliquot of the extract is diluted with acetonitrile/water (10:90 v/v). The final determination of BAS 650 F and its metabolites M650F01, M650F02, M650F03, and M650F04 was performed by LC-MS/MS. For each analyte, two MRM parent-daughter ions for quantification and quantitative confirmation were monitored by LC-MS/MS. The validated method achieved a limit of quantification (LOQ) of 0.01 mg/kg and a limit of detection (LOD) of 0.002 mg/kg.

Method validation was accomplished by analyzing each of the five analytes in a validation set consisting of 2 blank control specimens, 5 replicate specimens fortified at LOQ, and 5 replicate specimens fortified at 10xLOQ.

IV. LIMIT OF QUANTITATION AND DETECTION

The limit of quantitation (LOQ) for residues of BAS650F and its metabolites M650F01, M650F02, M650F03, and M650F04 in soil is 0.01 ppm for each analyte. The limit of detection (LOD) is 20% of the LOQ, equivalent to 0.002 ppm for each analyte.

V. CALIBRATION, CALCULATIONS AND STATISTICS

Residues of BAS 650F and its metabolites M650F01, M650F02, M650F03, and M650F04 were quantitated by external standards. A calibration curve for each analyte was generated by plotting the detector's response in peak area versus the amount (ng) of standard injected. The data system derived an equation for the fit of the standard curve and this equation was used to calculate intercept and slope of the linear regression curve.

The calibration curve was obtained by direct injection of 50 µL of the mixed BAS650F and its metabolites M650F01, M650F02, M650F03, and M650F04 standards onto LC-MS/MS in the range of 0.02 ng/mL to 5.0 ng/mL. In a given injection run, the same injection volume was used for all samples and standards.

Peak integration and quantitation were performed using Applied Biosystems' Analyst software with version 1.4.2. PPM calculations and Recovery results were computed for each set of samples by Microsoft's Excel® and reported in a spreadsheet data report. Equations used for quantitation are presented in Figure 2.

FIGURE 2 Typical Calculations for the Quantitation of Residues in Soil

For the calculation of residues the following formula was used:

$$R = C_{\text{End}} \times (V_{\text{Ex}} \times V_{\text{End}} / V_{\text{I}} \times W) \text{ ng/g}$$
$$= C_{\text{End}} \times \text{Multiplier } M$$

Where:

R: Analyte residue in mg/kg.

C_{End} : Final concentration of analyte in extract in ng/mL.
(where multiple injections were evaluated: mean).

W: Soil dry mass: 5 g.

V_{Ex} : Volume of extraction solvent: 50 mL.

V_{I} : Aliquot of V_{Ex} : 0.10 mL.

V_{End} : Volume of final extract used for LC/MS/MS: 1.0 mL.

M: Multiplier: 0.10.

Recoveries (Rec.) were calculated for the fortified specimens as follows:

$$\text{Rec.} = (R / R_{\text{fortified}}) \times 100 \%$$