Health Canada Santé Canada

Part 8.2.2 Analytical methodology (parent compound and transformation products)

Common Name: Picarbutrazox

Product Name: Picarbutrazox Technical

Submission Number: 2017-5919

PCPA Reg. Number: Not yet assigned

Source Code: PBZ-NIN-2

Chemical structures:

Table 1. Chemical name, code and chemical structure for active and all major transformation products / metabolites

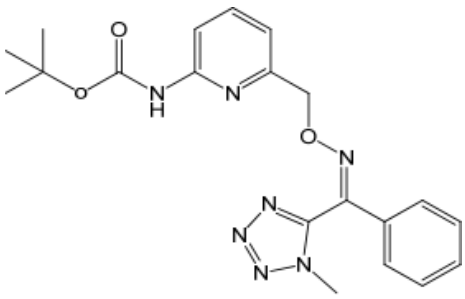
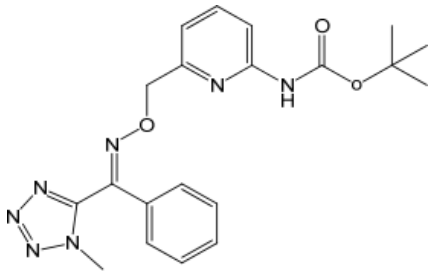
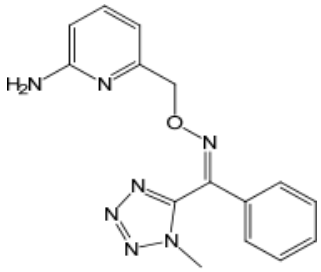
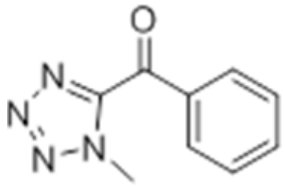
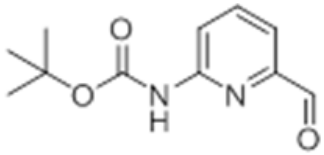
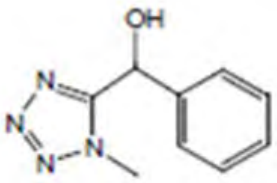
Chemical name	Code / CAS #	Chemical structure
tert-butyl (6-{[(*Z*)-(1-methyl-1*H*-5-tetrazolyl)(phenyl)methylene] aminooxymethyl}-2-pyridyl)carbamate MW = 409.44 g/mol	NF-171 (parent compound) / 500207-04-5	
tert-butyl (6-{[(*E*)-(1-methyl-1*H*-5-tetrazolyl) (phenyl)methylene]-aminooxymethyl}-2- pyridyl) carbamate MW = 409.44 g/mol	TZ-1E / 1253511-94-2	
(*Z*)-O-[(6-amino-2-pyridyl) methyl] (1-methyl-1*H*-5- tetrazolyl) (phenyl)methanone oxime MW = 309.33 g/mol	TZ-2 / 500206-79-1	

Table 1. Chemical name, code and chemical structure for active and all major transformation products / metabolites		
(1-methyl-1*H*-tetrazol-5-yl) (phenyl)methanone MW = 188.19 g/mol	TZ-4 / 33452-25-4	
carbamic acid, *N*-(6-formyl-2-pyridinyl)-, 1,1-dimethylethyl ester *(CAS)* *tert*-butyl (6-formylpyridin-2-yl) carbamate *(ChemSketch IUPAC)* MW = 222.24 g/mol	TY-3 / 956523-98-1	
1*H*-tetrazole-5-methanol, 1-methyl-α-phenyl- *(CAS)* (1-methyl-1*H*-tetrazol-5-yl) (phenyl) methanol *(ChemSketch IUPAC)* MW = 190.20 g/mol	TZ-5 / 33452-21-0	

Data Submission and Review History:

Table 2. Correspondence Dates, Data # and Content for Picarbutrazox Technical			
Date Received	Data #	Content Summary	Reviewer Officer #
2017-12-08	1	8.2.2 data for soil, sediment, water and fish	1160

The purpose of this Category A.3.2 application is to register a new fungicide active ingredient via a joint review between Canada and the U.S.; for the environmental analytical methods, Canada is carrying out the primary review while the U.S. is responsible for the secondary review.

<u>Good Laboratory Practices Compliance Statement</u>:

The studies contained within this report were conducted in accordance with the Good Laboratory Practice Standards as specified in 40 CFR 160 (1989) and/or Japan MAFF (1999) and/or French Regulation Annexe II à l'article D523-8 du Code de l'Environnement (2007).

yes [x] no [ ] not stated / applicable [ ]

8.2.2 Analytical Methodology (parent compound and transformation products)

8.2.2.1 Soil

Reference:

1. PMRA #2808242; MRID 50218706. 2017, Method Validation of Picarbutrazox (NF-171) and Metabolites (TY-1, TY-2, TZ-1E, TZ-2, TZ-2E, TZ-4, and TZ-5) in Soil and NF-171 and Metabolites (TY-2, TZ-1E, TZ-5, TZ-2-B-Glc and TZ-5-Glc) in Grass Clippings using LC-MS/MS. Analytical Bio-Chemistry Laboratories, EAG Study #81543. (Data #1)
2. PMRA #2808241. 2017, Applicant DER - Method Validation of Picarbutrazox (NF-171) and Metabolites (TY-1, TY-2, TZ-1E, TZ-2, TZ-2E, TZ-4, and TZ-5) in Soil and NF-171 and Metabolites (TY-2, TZ-1E, TZ-5, TZ-2-B-Glc and TZ-5-Glc) in Grass Clippings using LC-MS/MS. Nippon Soda Co., Study RD-10116. (Data #1)
3. PMRA #2809344; MRID 50218707. 2017, Independent Laboratory Validation of an Analytical Method for the Determination of Picarbutrazox (NF-171) and Metabolites (TY-1, TY-2, TZ-1E, TZ-2, TZ-2E, TZ-4, and TZ-5) in Soil. EAG Laboratories-Hercules, Project ID 2912W. (Data #1)
4. PMRA #2809345. 2017, Applicant DER - Independent Laboratory Validation of an Analytical Method for the Determination of Picarbutrazox (NF-171) and Metabolites (TY-1, TY-2, TZ-1E, TZ-2, TZ-2E, TZ-4, and TZ-5) in Soil. (Data #1)

Ref. 1 contains the original validation of the soil method in two soils and Ref. 2 the applicant's corresponding data evaluation record (DER), for the parent compound and two transformation products (TZ-1E, TZ-2). The independent laboratory validation (ILV) of the same method in Ref. 3 (with the DER in Ref. 4) using a different soil also yielded acceptable results, all included in Table 4. Five other transformation products were also addressed in this method (TY-1, TY-2, TZ-2E, TZ-4, and TZ-5) but have not been included here since the environmental evaluator does not consider them to be relevant.

Table 3. Principle of the method

Items	Details
Details of sample used	Original: New York and Idaho soils, characterized in separate EAG Study #81547 ILV: Sandy loam/loam soil, characterized in Appendix C of Ref. 3
Sample preparation	5 g of sample was weighed into a 50-mL centrifuge tube followed by two stainless steel grinding balls, 1.25 g ammonium chloride and 25 mL methanol. The sample was extracted at 1200 rpm for 5 min, then centrifuged at 3000 rpm for 5 min. The supernatant was decanted into a clean 50 mL centrifuge tube. A second extraction was performed on the original soil using 15 mL of 75:25 (v/v) methanol / 0.1% formic acid, at 1200 rpm for 5 min, followed by centrifuging at 3000 rpm for 5 min. The two supernatants were combined and diluted to 50 mL with methanol. This was mixed by vortex and an aliquot filtered through a 0.2 µm nylon syringe filter. 1 mL of this filtrate was diluted to 4.0 mL with ultra-pure water in the original validation, or with 1 mL of methanol and 2 mL water in the ILV.

Table 3. Principle of the method

Items	Details
Method for quantitative analysis and identification of parent compound and transformation products	HPLC conditions: Column: Phenomenex Kinetex 2.6µm (50 mm x 2.1 mm) outfitted with a 0.5 µm pore size stainless steel frit attached pre-column Column Temperature: 40°C Injection Volume: 10 µL Mobile Phase: A) 0.01% ammonium hydroxide in HPLC Water B) 100% ACN Gradient Program: Time (min.) / Flow Rate (µL/min) / % A / % B 0.00 / 800 / 98 / 2 0.30 / 800 / 98 / 2 4.00 / 800 / 10 / 90 5.00 / 800 / 10 / 90 5.01 / 800 / 98 / 2 6.00 / 800 / 98 / 2 Retention times: TZ-2 2.14 (ILV 2.66) min TZ-1E 2.85 (ILV 3.34) min parent 2.93 (ILV 3.41) min MS/MS conditions: electrospray ionization in positive mode Analyte / Q1/Q3 m/z quantitation / Q1/Q3 m/z confirmation parent / 410/310 / 410/107 TZ-1E / 410/310 / 410/107 TZ-2 / 310/123 / 310/107
Chromatograms of spiked sample, control sample, blank and standard solution	Chromatograms provided for the reagent blank, matrix control blank, calibration standards and fortified samples show no interferences around the peaks of interest.
Quantitation	By external standard using a seven-point calibration curve. Standards from Nisso Chemical Analysis were supported with certificates of analysis: parent compound purity 98.8%, TZ-1E 98.0%, TZ-2 99.7%.
Criteria for setting LOD and LOQ	LOQ was pre-set at 0.01 mg/kg (ppm), and LOD was defined as ~30% of the LOQ.
Stability of parent and transformation products at various stages of analysis	Calibration standard solutions were found to be stable for at least 21 days.
Special problems encountered and/or precautions to be taken during analysis/handling/storage of samples	The soil sample was stored frozen when not in use. Matrix-based calibration standard solutions were used, which were stored refrigerated.
Total time for completion	For a set of 14 samples, one 8-hour day plus unattended LC analysis. ILV: For one set of samples (blank, controls, five samples fortified at each level), ~12 hours (1.5 calendar days) including data processing

The method validation data for the parent compound and the metabolites are summarized in Table 4, from the quantitation transitions.

Table 4. Method validation: Parent compound and transformation products in soil, from original validation (NY and ID soils) and ILV			
Parameter	**Parent compound**	**TZ-1E**	**TZ-2**
% Recovery at 0.01 ppm (LOQ)	NY: 97 - 102 ID: 98 - 104 ILV: 84 - 102	NY: 98 - 108 ID: 101 - 107 ILV: 76 – 102	NY: 83 - 91 ID: 87 - 95 ILV: 96 – 100
% Recovery at 0.10 ppm (10× LOQ)	NY: 84 - 93 ID: 73 - 81 ILV: 105 - 122	NY: 84 - 89 ID: 77 - 85 ILV: 92 - 118	NY: 81 - 86 ID: 84 - 86 ILV: 96 - 100
Mean % recovery, 2 levels	NY: 95 ID: 89 ILV: 94, 111	NY: 85 ID: 92 ILV: 90, 101	NY: 85 ID: 88 ILV: 98, 98
RSD %	NY: 5.9 ID: 14 ILV: 7, 6	NY: 9.8 ID: 13 ILV: 12, 11	NY: 3.7 ID: 5.0 ILV: 2, 1
Method linearity	0.1 – 5 ng/mL		
Correlation coefficient	r > 0.99		
LOD	0.003 ppm (mg/kg)		
LOQ	0.01 ppm (mg/kg)		

Conclusions/Other Comments: An HPLC-MS/MS method was developed for the determination of the parent and two transformation products in soil and was validated in two soils, as well as independently validated in another control soil. The recovery data were acceptable (means between 70-120%), and the LOQ was determined to be 0.01 ppm (mg/kg) for all analytes. The reported limit of quantitation (LOQ) was determined as the lowest level of method validation (LLMV). Further work could have been done to explore the actual LOQ. This means that concentrations can be reliably quantified at the LOQ (i.e., LLMV), but whether lower concentrations may also be reliably quantified is uncertain. This method is acceptable for use as a post-registration monitoring method.

EPA: This study is classified as **acceptable**.