Data Evaluation Record on method validation for metrafenone (BAS 560 F) and its product CL 375816 in water

PMRA Submission Number {.....} EPA MRID Number 47267436

Data Requirement:	PMRA Data Code:	
	EPA DP Barcode:	D354164
	OECD Data Point:	
	EPA Guideline:	Non-Guideline

Test material:

Common name:	Metrafenone
Chemical name:	
IUPAC name:	(3-Bromo-6-methoxy-2-methylphenyl)(2,3,4-trimethoxy-6-methylphenyl)-methanone. Benzophenone, 3′-bromo-2,3,4,6′-tetramethoxy-2′,6-methyl-. 3′-Bromo-2,3,4,6′-tetramethoxy-2′,6-dimethylbenzophenone. 5-Bromo-6,6'-dimethyl-2,2',3',4'-tetramethoxy benzophenone.
CAS name:	(3-Bromo-6-methoxy-2-methylphenyl)-(2,3,4-trimethoxy-6-methylphenyl)-methanone. 3'-Bromo-2,3,4,6'-tetramethoxy-2'6-methyl-methanone. Methanone, (3-bromo-6-methoxy-2-methylphenyl)(2,3,4-trimethoxy-6-methylphenyl)-.
CAS No.:	220899-03-6.
Synonyms:	AC 375839, AC375839, CL 375839, CL375839, BAS 560 F, BAS 560F, BAS 560 00 F, Benzophenone, ROI-24, Reference Substance 1, BAS560, Reg. No. 4037710, Aladin request number 27274.
Smiles string:	C1=CC(Br)=C(C)C(C(=O)C2=C(C)C=C(O)C(O)=C2C)=C1C_O_CC_C_O_CC_CC_C_C_C (EPI Suite, v3.12 SMILES string from ISIS .MOL file).

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Date: 01/02/09

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Data Evaluation Record on method validation for metrafenone (BAS 560 F) and its product CL 375816 in water

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CITATION: Xing, J. 2002. BAS 560 F (CL 375839): laboratory validation of LC/MS determinative and LC/MS/MS confirmatory method M 3503 for the determination of BAS 560 F and CL 375816 residues in drinking and surface water. Unpublished study performed by BASF Agro Research, Princeton, New Jersey; sponsored and submitted by BASF Corporation, Research Triangle Park, North Carolina. BASF Study No.: 92533, Report No.: RES 02-009 and Registration Document No.: 2002/7005048. Experimental start date January, 30, 2002, and completion date February 13, 2002 (p. 5). Final report issued March 20, 2002.

Data Evaluation Record on method validation for metrafenone (BAS 560 F) and its product CL 375816 in water

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EXECUTIVE SUMMARY

A laboratory validation of a method (BASF Agro Research Method M 3503) used to detect and quantify (3-bromo-6-methoxy-2-methylphenyl)(2,3,4-trimethoxy-6-methylphenyl)-methanone (metrafenone, BAS 560 F) and its transformation product 2-methyl-3-bromo-6-methoxybenzoic acid (CL 375816) in drinking and surface water was conducted to support registration of metrafenone (p. 5; Appendix B, pp. 56-58). This method validation was conducted in compliance with USEPA GLP Standards 40 CFR, Part 160 (p. 3). Drinking and surface water samples (see Table 1 below for characterization) were obtained on site at the test facility, with no additional details regarding collection provided (p. 8). Surface water was filtered (GF/B Grade, glass microfiber filter paper, double-layered) prior to use (Appendix B, pp. 61, 68). Aliquots (100 mL) of the waters were fortified with metrafenone (purity 99.7%, Lot No.: AC11957-109B) and CL 375816 (purity 95%, Lot No.: AC11238-127), in methanol, at 0.05 and 0.50 µg a.i./L (ppb, five treated samples per application rate and water type; pp. 8-9; Appendix B, pp. 62, 68). Metrafenone and CL 375816 were applied as a mixed fortification solution; the application solution volume used was not specified, but reported as ≤1.0 mL (Appendix B, pp. 62, 69). Ammonium hydroxide (1 mL) was added to the fortified water sample, then the sample was partitioned once with methylene chloride (100 mL; Appendix B, p. 69). The organic phase, containing metrafenone, was evaporated to dryness by rotary evaporation (*ca.* 35°C), with the resulting residues reconstituted in methanol (1.0 mL), using sonication, and diluted 1:1 (v:v) with water (1.0 mL) for LC/MS analysis (Appendix B, p. 69). The remaining aqueous phase was treated with acetic acid (4 mL), then partitioned twice with methylene chloride (100 mL x 2; Appendix B, p. 69). The organic phases, containing CL 375816, were combined and evaporated to dryness/near dryness as described above (Appendix B, p. 69). Resulting CL 375816 residues were reconstituted in methanol (*ca.* 5 mL) and taken to dryness; this step was repeated to completely remove trace acetic acid, then the residues were reconstituted in methanol (2 mL), using sonication, and diluted 1:1 (v:v) with water (2 mL; Appendix B, pp. 69-70). The CL 375816 sample was applied to a solid-phase extraction (SPE) cartridge (Isolute SAX, anion-exchange, 1 g/6 mL) preconditioned with methanol (*ca.* 5 mL), followed by methanol:water (1:1, v:v, *ca.* 5 mL; Appendix B, pp. 60, 70). The sample flask was rinsed with methanol:water (1:1, v:v, 2 mL) and the rinsate also applied to the cartridge. The loaded SPE cartridge was rinsed once with methanol (*ca.* 5 mL), then CL 375816 residues were eluted with methanol:formic acid (99:1, v:v, Solution C, *ca.* 5 mL; Appendix B, pp. 59, 70). The CL 375816 eluate was taken to dryness under nitrogen using a Mini-Vap (*ca.* 40°C) and the resulting residues reconstituted in water:methanol:acetic acid (69:30:1, v:v, Solution B, 1.0 mL) for LC/MS analysis (Appendix B, pp. 59, 70-71).

Metrafenone. Samples were analyzed by reverse-phase LC/MS under the following conditions: TosoHaas (currently Tosoh Bioscience LLC) TSK-GEL Super-ODS LC column (4.6 x 100 mm, 2 µm), guard filter and holder (TosoHaas No.: 18207 and 18206, respectively), gradient mobile phase combining (A) 0.05% aqueous acetic acid and (B) 10mM ammonium acetate in methanol [percent A:B at 0.0 min. 70:30 (v:v), 6.0-8.0 min. 0:100, 9.0-10.0 min. 70:30], column temperature ambient, injection volume 100 µL, flow rate 1.0 mL/minute, retention time 6.2-6.8 minutes, Finnigan MAT LCQ Deca MS with atmospheric pressure ionization interface, atmospheric pressure chemical ionization (APCI) positive ion mode, capillary temperature 150°C, vaporizer temperature 400°C, sheath gas flow 80 mL/minute, auxiliary gas flow 20 mL/minute, source voltage 6 kV, source current 5 µA, capillary voltage 41 V, MS full scan mode at m/z 200^{+}-500^{+} and SIM scan mode m/z

411$^+$/IsoW = 1.0 (pp. 9-11; Appendix B, pp. 64-65). As a second confirmatory method, samples were analyzed by LC/MS/MS as described above with the following modifications: MS/MS full scan mode m/z 150$^+$-450$^+$, SIM scan mode m/z 411$^+$/IsoW = 1.5 (pp. 11-12).

CL 375816. Samples were analyzed by reverse-phase LC/MS as described above for metrafenone with the following modifications: gradient mobile phase [percent A:B at 0.0 min. 70:30 (v:v), 5.0 min. 45:55, 6.0-9.0 min. 0:100, 10.0-12.0 min. 70:30], flow rate 0.75 mL/minute, retention time 4.7-5.2 minutes, APCI negative ion mode, source voltage 5 kV, source current 80 µA, capillary voltage -16 V, MS full scan mode m/z 180$^-$-250$^-$, SIM scan mode m/z 243$^-$/IsoW = 1.0 (pp. 9-11; Appendix B, pp. 65-66). Samples were analyzed by LC/MS/MS with the following modification: MS/MS full scan mode m/z 180$^-$-220$^-$ (pp. 12-13).

Metrafenone and CL 375816 extracted from the fortified samples were identified against reference standards (Figure 3, p. 31; Figures 6-8, pp. 34-36; Figures 11-13, pp. 39-41; Figures 15-16, pp. 43-44; Figure 18, p. 46). The limits of detection (LOD) and quantitation (LOQ) were 0.010 and 0.050 µg a.i./L (ppb), respectively, for both compounds (Appendix B, p. 57).

Overall recoveries from water fortified at 0.05 and 0.5 µg a.i./L averaged (n = 20) 101.0 ± 3.4% (range 93.8-109.4%) of the applied for metrafenone and 83.0 ± 10.0% (66.6-104.4%) for CL 375816 (DER Attachment 2). For each water type, recoveries for metrafenone and CL 375816 averaged (n = 10) 101.2 ± 2.7% (98.4-105.2%) and 81.0 ± 10.4% (68.8-104.4%), respectively, for the drinking water, and 100.8 ± 4.0% (93.8-109.4%) and 85.0 ± 9.1% (66.6-96.2%), respectively, for the surface water. For both water types, recoveries were comparable at the two fortification levels for metrafenone; however, average recoveries of CL 375816 were lower at the 0.05 µg a.i./L fortification level (drinking water 76.9 ± 6.9%, surface water 77.8 ± 7.2%) as compared to the 0.5 µg a.i./L level (drinking water 85.1 ± 11.7%, surface water 92.2 ± 2.8%). Detector responses were linear for metrafenone (r^2 = 0.9996) over a range of 1.25-5.0 µg/L and for CL 375816 (r^2 = 0.9998) over a range of 2.5-10.0 µg/L (p. 13; Figures 1-2, pp. 29-30). Chromatograms of unfortified drinking water detected apparent residues of metrafenone and CL 375816 at <0.00194 µg a.i./L and <0.00445 µg a.i./L, respectively, and at <0.00201 µg a.i./L and <0.00690 µg a.i./L, respectively, in unfortified surface water (pp. 5, 21-28; Figure 5, p. 33; Figure 10, p. 38; Figure 14, p. 42; Figure 17, p. 45). Minor interferences for both compounds were also detected in reagent blank samples (Figure 4, p. 32; Figure 9, p. 37); quantitative results were not provided.

The study author reported that processed water samples were determined to be stable for at least 7 days prior to analysis under refrigerator storage (*ca.* 4 ± 2°C); however, no supporting data were provided (p. 6).

Table 1: Properties of the waters.[1]

Property	Control Drinking Water	Control Surface Water
Sample ID No.	AC12584-10	AC12584-12
pH	6.1	5.6
Dissolved organic carbon (mg/L)	Not determined.	
Total organic carbon (mg/L)	0.8	5.9
Hardness (mg $CaCO_3$/L)	102	47
Electrical conductivity (mmhos/cm)	0.42	0.19
Sodium Adsorption Ratio	0.94	0.69
Total Dissolved Solids (mg/L)	196	132
Turbidity (NTU)	1.32	4.92

Data obtained from p.8, Appendix C, pp. 98-99 of the study report.

1 Both water samples were obtained on site at BASF Agro Research, Princeton, New Jersey (p. 8); no additional details regarding collection were reported.

Attachment 1: Structures of the Test Material

Metrafenone [AC 375839, AC375839, CL 375839, CL375839, BAS 560 F, BAS 560F, BAS 560 00 F, Benzophenone, ROI-24, Reference Substance 1, BAS560, Reg. No. 4037710, Aladin request number 27274]

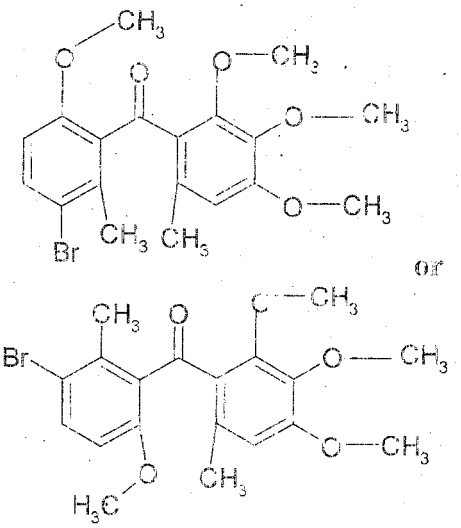
IUPAC Name: (3-Bromo-6-methoxy-2-methylphenyl)(2,3,4-trimethoxy-6-methylphenyl)-methanone.
Benzophenone, 3′-bromo-2,3,4,6′-tetramethoxy-2′,6-methyl-.
3′-Bromo-2,3,4,6′-tetramethoxy-2′,6-dimethylbenzophenone.
5-Bromo-6,6'-dimethyl-2,2',3',4'-tetramethoxy benzophenone.

CAS Name: (3-Bromo-6-methoxy-2-methylphenyl)-(2,3,4-trimethoxy-6-methylphenyl)-methanone.
3'-Bromo-2,3,4,6'-tetramethoxy-2'6-methyl-methanone.
Methanone, (3-bromo-6-methoxy-2-methylphenyl)(2,3,4-trimethoxy-6-methylphenyl)-.

CAS Number: 220899-03-6

SMILES String: C1=CC(Br)=C(C)C(C(=O)C2=C(C)C=C(O)C(O)=C2C)=C1C_O_CC_C_O_CC_CC_C_C_C (EPI Suite, v3.12 SMILES string from ISIS .MOL file).

Empirical formula: $C_{19}H_{21}BrO_5$ **Molecular formula:** $C_{19}H_{21}BrO_5$



or

AC 375816 [CL 375816, ROI-7, Reference Substance 3, AQ, Reg. No. 375816, Reg. No. 4074484, Aladin request number 27100]

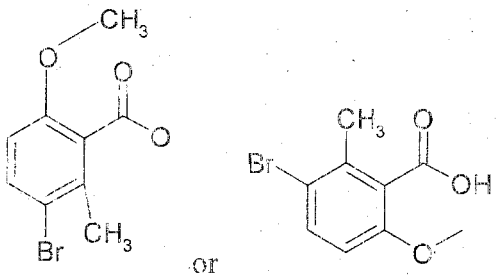
IUPAC Name: Not reported.

CAS Name: 2-Methyl-3-bromo-6-methoxybenzoic acid.
3-Bromo-6-methoxy-2-methylbenzoic acid.
Benzoic acid, 3-bromo-6-methoxy-2-methyl-.
Benzoic acid, (2-methyl-3-bromo-6-methoxy)-.

CAS Number: Not reported.

SMILES String: C1=CC(Br)=C(C)C(C(O)=O)=C1C_O_CC_C (EPI Suite, v3.12 SMILES string from ISIS .MOL file).

Empirical formula: $C_9H_9BrO_3$ **Molecular formula:** $C_9H_9BrO_3$



Identified Compounds

Metrafenone [AC 375839, AC375839, CL 375839, CL375839, BAS 560 F, BAS 560F, BAS 560 00 F, Benzophenone, ROI-24, Reference Substance 1, BAS560, Reg. No. 4037710, Aladin request number 27274]

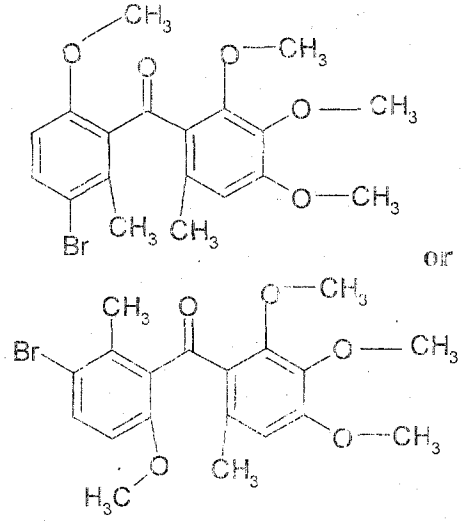
IUPAC Name: (3-Bromo-6-methoxy-2-methylphenyl)(2,3,4-trimethoxy-6-methylphenyl)-methanone.
Benzophenone, 3′-bromo-2,3,4,6′-tetramethoxy-2′,6-methyl-.
3′-Bromo-2,3,4,6′-tetramethoxy-2′,6-dimethylbenzophenone.
5-Bromo-6,6'-dimethyl-2,2',3',4'-tetramethoxy benzophenone.

CAS Name: (3-Bromo-6-methoxy-2-methylphenyl)-(2,3,4-trimethoxy-6-methylphenyl)-methanone.
3'-Bromo-2,3,4,6'-tetramethoxy-2'6-methyl-methanone.
Methanone, (3-bromo-6-methoxy-2-methylphenyl)(2,3,4-trimethoxy-6-methylphenyl)-.

CAS Number: 220899-03-6

SMILES String: C1=CC(Br)=C(C)C(C(=O)C2=C(C)C=C(O)C(O)=C2C)=C1C_O_CC_C_O_CC_CC_C_C_C (EPI Suite, v3.12 SMILES string from ISIS .MOL file).

Empirical formula: $C_{19}H_{21}BrO_5$ **Molecular formula:** $C_{19}H_{21}BrO_5$



AC 375816 [CL 375816, ROI-7, Reference Substance 3, AQ, Reg. No. 375816, Reg. No. 4074484, Aladin request number 27100]

IUPAC Name: Not reported.

CAS Name: 2-Methyl-3-bromo-6-methoxybenzoic acid.
3-Bromo-6-methoxy-2-methylbenzoic acid.
Benzoic acid, 3-bromo-6-methoxy-2-methyl-.
Benzoic acid, (2-methyl-3-bromo-6-methoxy)-.

CAS Number: Not reported.

SMILES String: C1=CC(Br)=C(C)C(C(O)=O)=C1C_O_CC_C (EPI Suite, v3.12 SMILES string from ISIS .MOL file).

Empirical formula: $C_9H_9BrO_3$ **Molecular formula:** $C_9H_9BrO_3$