Data Evaluation Record on method validation for metrafenone (BAS 560 F) and its product CL 377160 in soil

PMRA Submission Number {.....} EPA MRID Number 47267434

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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Final Reviewer: Lucy Shanaman
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Date: 01/02/09

Company Code:
Active Code:
Use Site Category:
EPA PC Code: 000325.

CITATION: Smalley, R. 2001. Method validation of RLA 12618.00 "LC-MS determination of CL 375839 and CL 377160 residues in soil". Unpublished study performed by BASF Agro Research, Gosport, Hampshire, United Kingdom; sponsored and submitted by BASF Corporation, Research Triangle Park, North Carolina. BASF Study and Report No.: 99305V, and Registration Document No.: 2001/7000247. Experimental start date August 31, 2000, and completion date April 26, 2001 (p. 10). Final report issued July 11, 2001.

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EXECUTIVE SUMMARY

A laboratory validation of a method (Method RLA 12618.01V) 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 (3-bromo-6-methoxy-2-methylphenyl)(3'-hydroxy-2',4'-dimethoxy-6'-methylphenyl)-methanone (CL 377160) in soil was conducted to support registration of metrafenone (p. 4; Appendix A, pp. 26-27). This method validation was conducted in compliance with USEPA GLP Standards 40 CFR, Part 160, with the exceptions that the validation was conducted in accordance with European Community Directives 87/18/EEC and 88/320/EEC, OECD guidelines (1997), and United Kingdom Health and Safety GLP Regulations (No. 3106, 1999, p. 5). Soil samples (ID No: 99-305-01-02 and 99-302-01-01) were obtained from two studies (BN-DK-99-305 and BN-GE-99-302), with no additional details or characterization provided (pp. 10-11). Aliquots (5 ± 0.1 g) of soil were separately weighed into microwave extraction vessels and fortified with metrafenone (purity 99.7%, Lot No.: AC11957-109B) and CL 377160 (purity 97%, Lot No.: AC12387-108), in acetonitrile, at 20 and 200 µg a.i./kg (ppb); six treated samples at the 20 µg a.i./kg rate and five at the 200 µg a.i./kg rate (pp. 10-12, 14-17). Metrafenone and CL 375816 were applied as a mixed fortification solution, with an application solution volume of 1 mL (Appendix A, pp. 31-33). Fortified soil samples were mixed (method not specified), allowed to stand for 15 minutes, then extracted once with acetonitrile:water:triethylamine (90:9.5:0.5, v:v) using a microwave extraction system under the following conditions: power 20% (two samples or fewer)/40% (four samples)/60% (six samples), pressure 50 psi, time to parameter not reported, time at parameter 3 minutes, temperature 125°C (Appendix A, pp. 29, 33). Following extraction the sample was allowed to settle and cool to approximately room temperature (Appendix A, p. 34). An aliquot (5 mL) of the extract was treated 1:1 (v:v) with 0.5% aqueous acetic acid (5 mL) and applied to a solid-phase extraction (SPE) cartridge (Isolute C18, 1g/6 mL) pre-conditioned sequentially with methylene chloride, methanol and water (one column volume each; Appendix A, pp. 27,). The loaded cartridge was rinsed with one column volume of water, dried under vacuum for *ca.* 1 minute, then residues were eluted with methylene chloride:methanol (90:10, v:v; Appendix A, p. 34). The collected eluate was taken to dryness under nitrogen using a Mini-Vap (*ca.* 60°C), and the resulting residues reconstituted in methanol (2 mL) with heating (*ca.* 60°C, 15 minutes) and vortex (10 seconds prior to and after heating). The sample was then diluted 1:1 (v:v) with water (2 mL) and vortexed (10 seconds). Samples were analyzed by reverse-phase LC/MS under the following conditions: Phenomenex-Ultracarb 5 ODS (30) column (4.6 x 100 mm), gradient mobile phase combining (A) 1.0% aqueous acetic acid and (B) 1.0% acetic acid in methanol [percent A:B at 0.0-11.0 min. 25:75 (v:v), 14.0-20.0 min. 0:100, 20.5 min. 25:75], column temperature ambient, injection volume 100 µL, flow rate 0.5 mL/minute, ThermoQuest aQa-Navigator MS, atmospheric pressure chemical ionization (APCI) positive ion mode, probe temperature 300°C, corona voltage 3.5 V, source voltage 20 V, SIM scan mode m/z $395^+/397^+$, 411^+ (Appendix A, pp. 29-30). Retention times for metrafenone and CL 377160 were *ca.* 16.5 and 8.9 minutes, respectively (pp. 18-19; Appendix A, p. 38). As a second confirmatory method (Specificity Method RLA 12636), samples were analyzed by LC/MS/MS as described above with the following modifications: gradient mobile phase percent A:B at 0.0-11.0 min. 25:75 (v:v), 14.0-20.0 min. 0:100, 21.0 min. 25:75], retention times of *ca.* 15.7 and 8.2 minutes for metrafenone and CL 377160, respectively, MicroMass Quattro triple quadrapole MS, electrospray ionization, positive ion mode, capillary voltage 3.97 V, cone voltage 17 V, source temperature 150°C, multiple reaction monitoring (MRM)

$395^+/195^+$, $397^+/195^+$, $409^+/209^+$, $411^+/228^+$ (Appendix B, pp. 40, 43-45, 50-52). Metrafenone and CL 377160 extracted from the fortified samples were identified against reference standards (pp. 18-19, 23). The limit of quantitation (LOQ) was 20 µg a.i./kg (ppb) for both compounds; limits of detection (LOD) were not reported (p. 12).

Overall recoveries from soil fortified at 20 and 200 µg a.i./kg averaged (n = 11) 104.2 ± 3.9% (range 95.7-109.3%) of the applied for metrafenone and 93.9 ± 4.6% (87.0-101.2%) for CL 377160 (DER Attachment 2). Recoveries were comparable at the two fortification levels for metrafenone; however, average recoveries of CL 377160 were lower at the 20 µg a.i./kg fortification level (90.6 ± 2.2%, 87.0-94.5%) as compared to the 200 µg a.i./kg level (97.8 ± 3.5%, 91.5-101.2%). Detector responses were linear (r^2 = 0.9987-0.9988) for both compounds over a range of 2.5-50.0 µg/L (ppb; Figure 2, p. 20). Chromatograms of unfortified control soil indicated there were no significant interfering peaks at the retention times for metrafenone or CL 377160, and there were no measurable residues in the control soil (pp. 16-17; Figure 1, p. 18; Figure 5, p. 23). Chromatograms of reagent blank samples were not provided.

A second soil method validation study (MRID 47267435) was submitted in this data package. No statement was provided to verify that the more current method (Method M 3441) supersedes the latter (Method RLA 12618.01V). Differences between the two methods are listed in the table below.

Table 1: Differences between Method RLA 12618.01V (MRID 47267434) and BASF Agro Research Method M 3441 (MRID 47267435).[1]

Parameter	Method RLA 12618.01V (MRID 47267434)	Method M 3441 (MRID 47267435)
Fortification levels	20 and 200 µg a.i./kg.	5 and 50 µg a.i./kg.
Fortification solution volume	1 mL.	Not to exceed 0.1 mL.
Processing after fortification	Fortified soil samples mixed (method not specified), allowed to stand for 15 minutes, then extracted.	Fortified soil samples were extracted directly.
During microwave extraction	Time to parameter not reported.	Time to parameter 10 minutes.
Following SPE clean-up	Eluate was taken to dryness and the resulting residues reconstituted in methanol.	Eluate was taken to dryness, the residues reconstituted in methylene chloride:methanol (90:10, v:v) and again taken to dryness, then the residues were reconstituted in methanol.
Prior to LC/MS analysis	No preparation.	Sample filtered (Millex-HV filter unit, 0.45 µm), if needed (solution cloudy or particulate matter present).
HPLC column	Phenomenex-Ultracarb 5 ODS (30) 4.6 x 100.	TSK-GEL Super-ODS 4.6 x 50 mm, 2 µm.
HPLC gradient mobile phase combining (A) 1% aqueous acetic acid and (B) 1% acetic acid in methanol	Percent A:B at 0.0-14.0 min. 25:75 (v:v), 14.0-20.0 min. 0:100, 20.5 min. 25:75.	Percent A:B at 0 min. 60.40 (v:v), 13-14 min. 20:80, 15 min. 60:40.
LC/MS/MS analysis	Conditions were reported and spectra from analyses provided.[2]	Conditions were reported; however, no spectra or results from analyses were provided.[2]

Information obtained from p. 12; Appendix A, pp. 29-30, 33-34; Appendix B, pp. 40-52 in MRID 47267434; and p. 11; Appendix B, pp. 39, 41-46, 48-50 in MRID 47267435.

1 Cited page numbers are respective to MRID.

2 Equipment and conditions differed for the two methods, but are not detailed since the LC/MS/MS analysis was not validated for Method M 3441 with soil.

Attachment 1: Structures of Test Substances

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.
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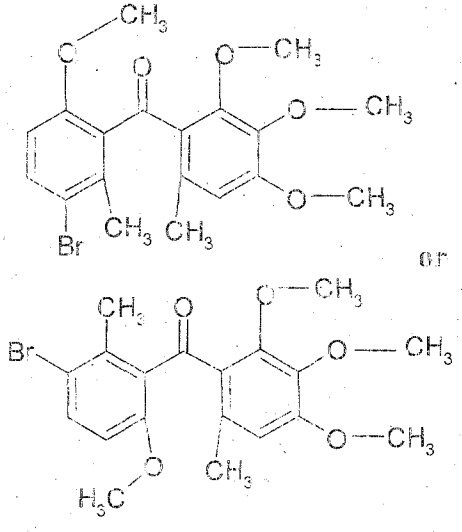
Methanone, (3-bromo-6-methoxy-2-methylphenyl)(2,3,4-trimethoxy-6-methylphenyl)-.

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:** $C_{19}H_{21}BrO_5$



CL 377160 [Reg. No. 4082230, CL377160, M23, ROI-19, LG, Aladin request number 27101, Reference Substance 4]

IUPAC Name: (3-Bromo-6-methoxy-2-methylphenyl)-(2,4-dimethoxy-3-hydroxy-6-methylphenyl)-methanone.
(3-Bromo-6-methoxy-2-methylphenyl)(3-hydroxy-2,4-dimethoxy-6-methylphenyl)methanone.
(3-Bromo-6-methoxy-2-methylphenyl)(3'-hydroxy-2',4'-dimethoxy-6'-methylphenyl)methanone.
((3-Bromo-6-methoxy-2-methylphenyl)(3'-hydroxy-2',4'-dimethoxy-6'-methylphenyl)-methanone).

CAS Name: (3-Bromo-6-methoxy-2-methylphenyl)(3-hydroxy-2,4-dimethoxy-6-methylphenyl)methanone.
Methanone, (3-bromo-6-methoxy-2-methylphenyl)(3-hydroxy-2,4-dimethoxy-6-methylphenyl)-.

CAS Number: Not reported.

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 (EPI Suite, v3.12 SMILES string from ISIS .MOL file).

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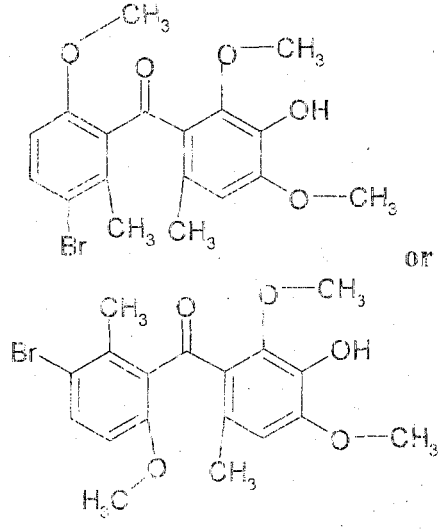

or

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]

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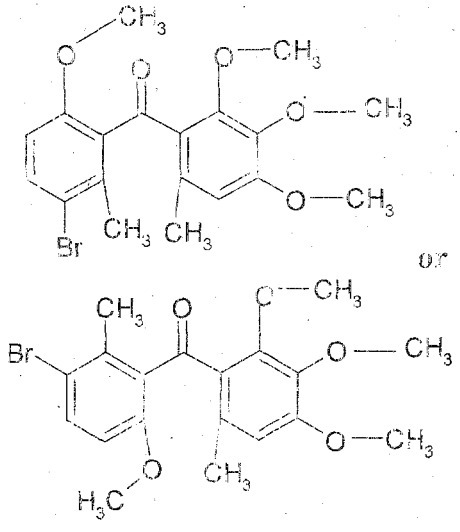

or

CL 377160 [Reg. No. 4082230, CL377160, M23, ROI-19, LG, Aladin request number 27101, Reference Substance 4]

IUPAC Name: (3-Bromo-6-methoxy-2-methylphenyl)-(2,4-dimethoxy-3-hydroxy-6-methylphenyl)-methanone.
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CAS Name: (3-Bromo-6-methoxy-2-methylphenyl)(3-hydroxy-2,4-dimethoxy-6-methylphenyl)methanone.
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CAS Number: Not reported.

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 (EPI Suite, v3.12 SMILES string from ISIS .MOL file).

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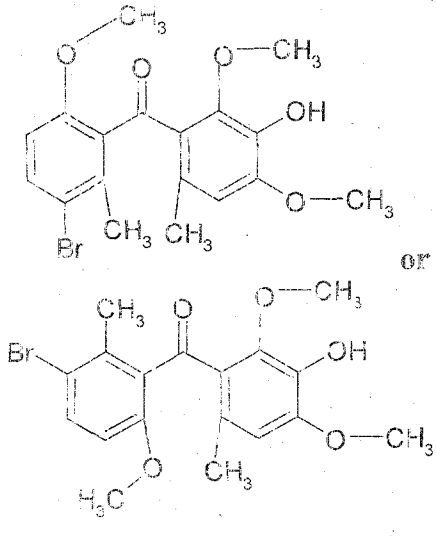

or