1.0 INTRODUCTION

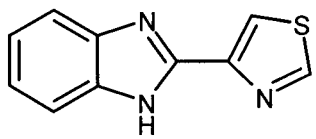
1.1 Scope and chemical structures

Analytical method GRM046.01A is suitable for the determination of thiabendazole (Figure 1) in water. The limit of quantification (LOQ) of the method has been established at 0.05 µg/L.

This method satisfies EU guidelines SANCO/3029/99 rev. 4, SANCO/825/00 rev. 7 and US EPA guidelines OPPTS 850.7100 and OPPTS 860.1340

Figure 1

Compound Name	:	Thiabendazole
Compound code number	:	MK360
CAS Number	:	148-79-8
IUPAC Name	:	2-thiazol-4-yl-1H-benzimidazole-
Molecular Formula	:	$C_{10}H_7N_3S$
Molecular Weight	:	201.3



1.2 Method summary

50 mL samples of water are acidified and taken through a solid phase extraction (SPE) procedure using Oasis HLB SPE cartridges. Thiabendazole is eluted from the cartridge with methanol and the eluate is evaporated to dryness. Samples are redissolved in acetonitrile/10 mM NH_4OAc (60/40 v/v, 2 mL). Final determination is by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS). The limit of quantification of the method is 0.05 µg/L.

2.0 MATERIALS AND APPARATUS

2.1 Apparatus

The recommended equipment and apparatus are listed in Appendix 1. Equipment with equivalent performance specifications may be substituted.

2.2 Reagents

All solvents and other reagents must be of high purity, e.g. glass distilled/HPLC grade solvents and analytical grade reagents. Particular care must be taken to avoid

contamination of the reagents used. Reagents of comparable purity may be substituted as long as acceptable performance is demonstrated. A list of reagents used in this method along with details of preparation of solutions is included in Appendix 2.

2.3 Preparation of analytical standard solutions

It is recommended that the following precautions should be taken when weighing the analytical materials.

1. Ensure good ventilation.
2. Wear gloves and laboratory coat.
3. Prevent inhalation and contact with mouth.
4. Wash any contaminated area immediately.

2.3.1 Stock solutions

Prepare a 200 µg/mL stock solution for thiabendazole by one of the following methods.

Weigh out accurately, using a five figure balance, sufficient thiabendazole analytical standard into separate amber "Class A" volumetric flask (50 mL). Dilute to the mark with methanol to give a 200 µg/mL stock solution of thiabendazole.

Alternatively, the appropriate volume of methanol to add to a known amount of standard material may be determined using the equation below. The standard concentration is corrected for its chemical purity.

$$V = \frac{W \times P}{C} \times 1000$$

P = Standard purity in decimal form (P(%)/100)
V = Volume of methanol required
W = Weight, in mg, of the solid analytical standard
C = Desired concentration of the final solution, (µg/mL)
1000 = Unit conversion factor

In this case, the standard material is weighed directly into an appropriate storage vessel.

2.3.2 Fortification solutions

Sample fortification solutions containing thiabendazole should be prepared by serial dilution in methaol. It is recommended that the following solutions are prepared: 10.0 µg/mL, 1.0 µg/mL, 0.1 µg/mL and 0.01 µg/mL. The preparation of LC-MS/MS calibration standards is discussed in Section 3.7.

2.3.3 Standard solution storage and expiration

All stock solutions should be stored in a refrigerator or freezer when not in use to prevent decomposition and/or concentration of the standard. Standard solutions should be allowed to equilibrate to room temperature prior to use.

An expiration date of two months for thiabendazole is recommended unless additional data are generated to support a longer expiration date.

2.4 Safety precautions and hazards

The following information is included as an indication to the analyst of the nature and hazards of the reagents used in this procedure. If in any doubt, consult the appropriate MSDS or a monograph such as 'Hazards in the Chemical Laboratory', edited by S G Luxon, The Chemical Society, London (Reference 2).

Solvent and Reagent hazards

	Methanol	Acetonitrile	Ammonium acetate	Hydrochloric acid	Formic acid
Harmful Vapour	✓	✓	✓	✓	✓
Highly Flammable	✓	✓	✗		✗
Harmful by Skin Absorption	✓	✓	✓	✓	✓
Irritant to respiratory system and eyes	✓	✓	✓	✓	✓
Causes severe burns	✗	✗	✗	✓	✓
Syngenta Hazard Category (SHC)	SHC-C, S	SHC-C, S	N/A, S	SHC-C, S	SHC-C, S
OES Short Term (mg/m^3)	310	105	N/A	7	N/A
OES Long Term (mg/m^3)	260	70	N/A	N/A	9

N/A not known

Thiabendazole has been assigned a Syngenta Hazard Classification SHC-A. The Syngenta Hazard Category scale rates highly toxic chemicals as category SHC-E and non-toxic chemicals as category SHC-A. An additional hazard category of S indicates the compound is a severe skin and eye irritant.

In all cases avoid breathing vapour. Avoid contact with eyes and skin.

3.0 ANALYTICAL PROCEDURE

The method is summarized in flow chart form in Appendix 8.

3.1 Modifications and potential problems

a) Bottled HPLC grade ultra pure water is used to prepare the LC mobile phase, which produces a lower background noise in the MS/MS chromatograms than water taken from a laboratory water purification system.

3.2 Sample preparation

If water samples are received deep frozen they should be allowed to defrost completely at room temperature. Defrosted samples should be shaken thoroughly to ensure sample homogeneity prior to analysis.

3.3 Sample fortification

a) Transfer 50 mL of the water sample to be analysed into a polypropylene centrifuge tube (50 mL size). Sample fortification, if required, is to be carried out at this time. Cap the tubes securely and shake gently to mix.

At least one untreated control and two control samples fortified with a known amount of thiabendazole should be analysed alongside each batch of samples to demonstrate acceptable performance of the method and allow recovery corrections to be made if desired.

b) Add concentrated hydrochloric acid (50 µL) to each sample. Cap the tubes and shake gently to ensure thorough mixing. Check that the pH is pH 2 -2.5 using suitable indicator paper.

3.4 Solid phase extraction procedure.

a) Take one Waters Oasis HLB SPE cartridge (200 mg, 6 mL size) for each sample to be analysed and place on a suitable vacuum manifold (e.g. IST Vacmaster). Add methanol (2 mL) and allow to percolate through under gravity or draw through under vacuum to the level of the top frit at a rate of approximately 1 mL/min, discarding the column eluate. Do not allow the cartridges to become dry. Add ultra pure water (2 mL) to the top of each cartridge and allow to percolate through under gravity or draw through under vacuum to the level of the top frit at the same rate, again discarding the column eluate. Do not allow the cartridges to become dry.

b) Using a suitable column connector, attach a column reservoir (70 mL capacity) fitted with a frit to prevent blockage of the SPE cartridge with any particulate material in the extract.

c) Load the acidified water from Section 3.3 (b) onto the SPE cartridges via the column reservoir and allow to percolate through under gravity or under low vacuum, at a rate of approximately 1-2 mL/min, to the level of the top frit. Do not allow cartridges to become dry.

d) On completion of loading, remove the column reservoir and connector. Add ultra pure water (1 mL) to the top of the SPE cartridge and allow to percolate through under gravity or draw through under vacuum to the level of the top frit at the same rate, again discarding the column eluate.

e) Dry the cartridge under high vacuum for 15 minutes.

f) Place suitable disposable, plastic, graduated centrifuge tubes (15 mL size) under each port, as required, in the manifold rack. Add methanol (2 mL) to the top of each cartridge and allow to percolate through under gravity. Collect the column eluate containing thiabendazole. Remove the excess solvent from the cartridges by application of positive pressure or vacuum, collecting the column eluate.

g) Evaporate the collected eluates to dryness under a stream of air in a sample concentrator with the heating block set at 40 °C.

h) Redissolve the residue in acetonitrile/10mMNH4OAc (60/40 v/v, 2 mL) and mix sample thoroughly by ultrasonicating the contents of centrifuge tube briefly.

i) Transfer the samples into suitable autosampler vials ready for final determination by LC-MS/MS. The final sample concentration is 25 mL/mL.

3.5 Time required for analysis

The methodology is normally performed with a batch of 20 samples. One person can complete the analysis of 20 samples in 1 day (8 hour working period).

3.6 Method stopping points

The analytical procedure can be stopped at various points for overnight and weekend breaks unless otherwise specified in the analytical procedure. Acceptable method recoveries will validate any work flow interruptions. Samples should be stored refrigerated in sealed containers where the analysis cannot be completed in a single day.

3.7 Preparation of calibration standards for LC-MS/MS

No significant suppression or enhancement of the instrument response for thiabendazole has been observed in the water types tested using the above procedure during method validation and non-matrix standards should normally be used for calibration.

For example, to prepare a 1.25 µg/L calibration standard in acetonitrile/10 mM NH_4OAc 60/40 v/v, transfer 1 mL of a 0.0125 µg/mL thiabendazole standard in methanol into a 10 mL volumetric flask. Adjust to the 10 mL mark with acetonitrile/10 mM NH_4OAc 60/40 v/v. Stopper the flask securely and shake gently to mix thoroughly. Transfer an aliquot to a suitable autosampler vial for analysis by LC-MS/MS.

A calibration curve may also be generated to quantify thiabendazole residues. Standards over an appropriate concentration range should be prepared as described above, using the requisite volumes of thiabendazole in methanol.

4.0 FINAL DETERMINATION

The following instrumentation and conditions have been found to be suitable for this analysis. Other instrumentation can also be used, though optimisation may be required to achieve the desired separation and sensitivity. The operating manuals for the instruments should always be consulted to ensure safe and optimum use. The method has been developed for use on an Applied Biosystems API4000.

4.1 Instrument description

Pump	:	Perkin Elmer Series 200
Degasser	:	Perkin Elmer Series 200
Column Oven	:	Perkin Elmer Series 200
Detector	:	Applied Biosystems API 4000 triple quadrupole mass spectrometer with Analyst™ software
Autosampler	:	Perkin Elmer Series 200

4.2 Chromatography conditions for thiabendazole

Column	:	Partisil SCX 10 µm 150 x 4.6 mm
Column Oven Temperature	:	40°C
Injection volume	:	10 µL
Stop Time	:	9.1 minutes
Injection protocol	:	Analyse calibration standard after 3 to 4 sample injections
Mobile phase	:	Isocratic acetonitrile/10 mM NH_4OAc 60/40 v/v
Flow rate	:	1 mL/min
Stop time		5.5 min

Notes : The column eluate is diverted to waste for the first 0.8 min to prevent ionic material from the sample contaminating the mass spectrometer front plate. A secondary pump providing flow of mobile phase to the mass spectrometer when the column eluate is switched to waste has been found to be unnecessary. Under these conditions the retention time of thiabendazole is 3.3 minutes.

4.3 Mass spectrometer conditions for thiabendazole

Interface	:	TurboIonSpray
Polarity	:	Positive
Curtain gas (CUR)	:	Nitrogen set at 25 (arbitrary units)
Temperature (TEM)	:	500°C
Ionspray voltage	:	5500V
Collision gas setting (CAD)	:	Nitrogen set at 4 (arbitrary units)
Gas 1 (GS1)	:	Air set at 50 (arbitrary units)
Gas 2 (GS2)	:	Air set at 60 (arbitrary units)
Interface heater (ihe)	:	On
Scan type	:	MRM

MRM Conditions		**Thiabendazole primary transition**	**Thiabendazole confirmatory transition**
Q1 *m/z*	:	202	202
Q3 *m/z*	:	175	131
Dwell time	:	300 ms	300 ms
Resolution Q1	:	Unit	Unit
Resolution Q3	:	Unit	Unit
Declustering potential (DP)	:	130 V	130 V
Entrance potential (EP)	:	10 V	10 V
Collision energy (CE)	:	35 V	45 V
Collision cell exit potential (CXP)	:	17 V	12 V

Typical chromatograms are shown in Appendix 4.

5.0 CALCULATION OF RESULTS

5.1 Single point calibration procedure

Thiabendazole residues may be calculated in µg/L for each sample using a mean standard response from each of the injections bracketing the sample as follows.

a) Make repeated injections of a standard containing thiabendazole at an appropriate concentration into the LC-MS/MS operated under conditions as described in Section 4. When a consistent response is obtained, measure the peak areas obtained for thiabendazole.

b) Make an injection of each sample solution and measure the areas of the peaks corresponding to thiabendazole.

c) Re-inject the standard solution after a maximum of four injections of sample solutions.

d) Calculate the thiabendazole residues in the sample, expressed as µg/L using a mean standard response from each of the injections bracketing the sample as follows.

$$\text{Residue } (\mu g/L) = \frac{\text{PK area (SA)}}{\text{PK area (STD)}} \times \frac{\text{Standard Conc.}}{\text{Sample Conc.}}$$

PK area (SA) = Peak response for sample
PK area (STD) = Average peak response for bracketing standards
Standard Conc. = Concentration of standard (µg/mL)
Sample Conc. = Sample concentration (L/mL)

If residues need to be corrected for average percentage recovery e.g. for storage stability studies, then the equation below should be used.

$$\text{Corrected Residue} = \frac{\text{Residue} \times 100}{\text{Average percentage Recovery}} \ (\mu g/L)$$

Although single point calibration may be used to quantify residues it is recommended that a calibration curve is generated with each analytical run to demonstrate the linearity of instrument response (Reference 3).

5.2 Multi point calibration procedure

Thiabendazole residues may be calculated in mg/kg for each sample as follows.

a) Prepare standard solutions over a concentration range appropriate to the expected residues in the samples (for example, 50% LOQ to 10 x LOQ). An appropriate number of different concentrations within this range should be prepared (at least four).

b) Make an injection of each sample solution and measure the areas of the peaks corresponding to thiabendazole. Calibration standard solutions should be interspersed throughout the analysis, after a maximum of four injections of sample solutions.

c) Generate calibration curve parameters using an appropriate regression package.

d) The following equation can be rearranged and used to calculate residues as follows:

$$y = mx + c$$

Where y is the instrument response value, x is the standard concentration, m is the gradient of the line of best fit ("X-variable 1" in MS Excel) and c is the intercept value. An example of this equation generated using the experimental values of m and c should be included in the raw data, as should the "R-Squared" value for the regression.

Re-arrangement for x gives

$$x = \frac{y - c}{m}$$

e) Alternatively (depending on the regression analysis software available) a quadratic equation may be used to fit the data. In this case the following general equation should be re-arranged and used to calculate residues:

$$y = a + bx + cx^2$$

Where y is the instrument response value, x is the standard concentration and a, b, c are constants.

f) Calculate the thiabendazole residues in the sample, expressed as µg/L, as follows

$$\text{Residue } (\mu g/L) = \frac{\text{Analyte found } (\mu g/mL)}{\text{Sample conc. (L/mL)}}$$

Where analyte found (µg/mL) is calculated from the standard calibration curve and sample conc. is the final sample concentration in L/mL.

If residues need to be corrected for average percentage recovery e.g. for storage stability studies, then the equation below should be used.

$$\text{Corrected Residue} = \frac{\text{Residue} \times 100}{\text{Average percentage Recovery}} \ (\mu g/L)$$

6.0 CONTROL AND RECOVERY SAMPLES

Control samples should be analysed with each set of samples to verify that the sample used to prepare recovery samples is free from contamination. A minimum of one control should be analysed with each batch of samples.

At least two recovery samples (control samples accurately fortified with known amounts of thiabendazole in methanol) should also be analysed alongside each set of samples. Provided the recovery values are acceptable they may be used to correct any residues found. The fortification levels should be appropriate to the residue levels expected.

Recovery efficiency is generally considered acceptable when the mean values are between 70% and 110% and with a relative standard deviation of $\leq$20%.

7.0 SPECIFICITY

It is recommended that reagent blank samples be included in a sample set if contamination is suspected.

7.1 Matrix

LC-MS/MS is a highly specific detection technique. Interference arising from the matrices tested has not been observed.

7.2 Reagent and solvent interference

Using high purity solvents and reagents no interference has been found.

7.3 Labware interference

This method uses mainly disposable labware. All reusable glassware should be detergent washed and then rinsed with HPLC grade methanol, acetone or acetonitrile prior to use.

8.0 METHOD VALIDATION

8.1 Recovery data and repeatability

Method validation has been carried out on the procedures described in Sections 3 and. The method validation data are reported in Eurofins ADME report number T001439-08-REG (Reference 1) and a summary is included in Appendix 3.

8.2 Limit of quantification (LOQ)

The limit of quantification of the method is defined as the lowest analyte concentration in a sample at which the methodology has been validated and a mean recovery of 70-110% with a relative standard deviation of ≤ 20% has been obtained. Generally, for accurate quantification, the response for an analyte peak should be no lower than four times the mean amplitude of the background noise in an untreated sample at the corresponding retention time.

The limit of quantification has been set at 0.05 µg/L.

8.3 Limit of detection (LOD)

The limit of detection of the method is defined as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated sample at the corresponding retention time. An estimate of the LOD can be taken as three times background noise. Note that the LOD may vary between runs and from instrument to instrument. During method validation the LOD was estimated to be in the range 0.0003 - 0.001 µg/L.

8.4 Matrix effects

No significant suppression or enhancement of the instrument response for thiabendazole has been observed in the water types tested using the above procedure in this laboratory. Full details are presented in Table 4, Appendix 3.

8.5 Detector linearity

For accurate quantification of residue concentrations, analyses should be carried out within the linear range of the detector. For multi point calibration, detector range and linearity will be demonstrated within each sample set.

The linearity of the LC-MS/MS detector response for thiabendazole was tested in the range from 5 pg to 200 pg injected on column (equivalent to 0.5 µg/L to 20µg/L standards when using a 10 µL injection volume) and was found to be linear. The instrument response is linear over the range 50% LOQ – 16 x LOQ.

If a residue beyond the tested concentration range is expected, dilute the sample appropriately to bring it within the tested linear range prior to quantification.

Standards at 6 different concentration levels were injected and the response plotted against the standard concentration, using Microsoft Excel for both primary transition and confirmatory transitions for thiabendazole.

Detector linearity data are presented in Table 6 and linearity graphs are presented in Appendix 5.

8.6 Extract stability

Final water extracts in acetonitrile/10 mM NH_4OAc 60/40 v/v retained in vials and stored at a temperature of approximately 4°C were suitable for thiabendazole residue analysis, for storage periods of up to 11 days. See table 5 in Appendix 3.

9.0 LIMITATIONS

The method has been tested on representative water types. It can reasonably be assumed that the method can be applied to other water types not tested in this method provided successful recovery tests at the relevant levels validate the suitability of the method.

10.0 CONCLUSIONS

This procedure has been demonstrated to be a reliable and accurate procedure for the determination of thiabendazole residues in water. Only commercially available laboratory equipment and reagents are required. The analysis of a batch of 20 water samples for thiabendazole can be completed by one person in 1 day (8 working hour period). Untreated and fortified samples should be analysed with each set of samples to demonstrate absence of any interference and adequate recovery, if possible. The limit of quantification of the method is 0.05 µg/L.

This method satisfies EU guidelines SANCO/3029/99 rev. 4, SANCO/825/00 rev. 7 and US EPA guidelines OPPTS 850.7100 and OPPTS 860.1340.

APPENDIX 2 REAGENTS

UK suppliers

Solvents: Ultra pure water (HPLC grade), acetonitrile and methanol available from Rathburn Chemicals Ltd., Walkerburn, Scotland EH43 6AU

Analytical grade concentrated hydrochloric acid, formic acid and ammonium acetate available from Sigma-Aldrich, The Old Brickyard, New Road, Gillingham, Dorset SP8 4XT or www.sigmaaldrich.com

Thiabendazole analytical standard, available from GLP Testing Facility, Syngenta, CH-4333, Munchweilen, Switzerland.

US suppliers

Solvents: Analytical grade acetonitrile and methanol available from B & J Brand Solvents, from Scientific Products Division of Baxter Healthcare Corporation, USA.

Ultra pure water (HPLC grade) from e.g. Fluka via Sigma-Aldrich www.sigmaaldrich.com

Analytical grade concentrated hydrochloric acid, formic acid and ammonium acetate available from www.sigmaaldrich.com

Thiabendazole analytical standard, available from Syngenta Crop Protection, Inc., P.O. Box 18300, Greensboro, NC 27419-8300.

Preparation of reagents

a) 10 mM ammonium acetate pH 3.4:
Dissolve 0.7708 g ammonium acetate in ultra pure water in a 1 L volumetrixc flask. Adjust volume to 1 L with ultra pure water. Stopper flask securely and shake to mix thoroughly. Adjust the pH to pH 3.4 with formic acid.

b) Acetonitrile/10 mM ammonium acetate 60/40 v/v:
Add 600 mL acetonitrile to 400 mL 10 mM ammonium acetate pH 3.4 in a 1L volumetric flask. Stopper the flask securely and shake to mix thoroughly.

APPENDIX 3 METHOD VALIDATION DATA

Table 1: Water samples used for validation

Water Type	Source	pH	Silt Content (% w/w)	Dissolved Organic Carbon (DOC) (mg/L)	Total Hardness as $CaCO_3$ (mg/L)
Groundwater	Evian bottled mineral water	7.2	0.032	<1	366
Surface water	Le Rhony river, Vergèze	7.79	0.033	6.3	633
Drinking water	Tap water, Eurofins, ADME Bioanalysis building	7.3	0.057	<1	354

Determination of LC-MS/MS matrix effects

The effect of different water types on the LC-MS/MS signal was assessed by preparing standards in the presence of water matrix and comparing the peak area of thiabendazole against non-matrix standards at an equivalent concentration.

For example, to prepare a 1.25 µg/L matrix matched calibration standard, transfer add 125 µL of a 0.01 µg/mL thiabendazole standard in methanol to the evaporated sample at point in the method. Adjust the final volume to 2 mL with acetonitrile/10 mM NH_4OAc 60/40 v/v and Ultrasonicate briefly to mix thoroughly. Transfer an aliquot to a suitable autosampler vial for analysis by LC-MS/MS.

Table 4 : LC-MS/MS Matrix Effects

Water Type	Matrix Effect for Thiabendazole	
	m/z 202 → 175	*m/z* 202 → 131
Groundwater	-8.8	-9.4
Surface water	-2.0	-2.2
Drinking water	+2.5	+2.7

No significant suppression or enhancement of the instrument response for thiabendazole has been observed in the water types tested using the above procedure during method validation and non-matrix standards should normally be used for calibration. Any matrix effects observed may be compensated for by use of matrix matched standards, at the discretion of the study director.

Table 6 : **LC-MS/MS linearity data for thiabendazole standards in acetonitrile/10 mM NH_4OAc (60/40 v/v) (from groundwater analysis).**

Standard Concentration (µg/L)	Amount injected (pg) (10 µL injection volume)	Transition *m/z* 202.0→175.1	Transition *m/z* 202.0→131.2
		Response	Response
0.5	5	17115.2	7702.4
1.25	12.5	41096.7	20598.1
2.5	25	75967.3	36449.5
5	50	135327.5	67270.9
15	150	400054.0	201275.3
20	200	528404.2	265045.0

The coefficient of correlation (R^2) value for this data set was 0.9999 for the *m/z* 202.0 → 175.1 transition and 0.9998 for the *m/z* 202.0 → 131.2 transition respectively.

APPENDIX 6 LC-MS/MS TUNING PROCEDURE

Calibration of instrument

The instrument must be mass calibrated on a regular basis using polypropylene glycol (PPG) solutions according to the manufacturer's instructions. Calibrate both mass resolving quadrupoles (Q1 and Q3).

Tuning instrument for thiabendazole

Infuse a standard solution of thiabendazole (0.01 to 1.0 µg/mL) in mobile phase (see section 4) directly into the mass spectrometer interface at a rate at of approximately 10-20 µL/min. Roughly adjust interface parameters (sprayer position, spray, heater/auxiliary gas flows, as well as voltages of spray, orifice, and focusing ring) for a sufficiently high parent ion signal at *m/z* 202 for thiabendazole in positive ionisation mode.

Using the Analyst software quantitative optimisation routine, tune the instrument for thiabendazole, ensuring that the correct ion is selected. If desired, manual tuning of the ion optics and collision energy can be carried out to ensure maximum sensitivity.

Finally, connect the LC-pump via the autosampler directly to the MS/MS instrument. Perform repetitive flow injection of a thiabendazole standard using mobile phase at the flow rate to be used. Tune the interface parameters (sprayer position, spray and heater gas flows, spray, orifice, and focusing ring voltages) and the collision gas flow for maximum sensitivity.

In positive ionisation mode, the protonated molecular ion generated in the ion source (*m/z* 202) is selected and subjected to further fragmentation by collisional activation. The two most sensitive daughter ions (*m/z* 175 and *m/z* 131) are then selected and used for quantitative analysis.

The fragment *m/z* 175 corresponds to the loss of HCN from the parent molecule *m/z* 131 corresponds to the 2-methyl-benzimidazole fragment.

Final determination by LC-MS/MS with two transitions is considered to be highly specific; hence no confirmatory conditions are included.