United States Department of Agriculture
Food Safety and Inspection Service, Office of Public Health Science

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Title: Identification of Poisons and Toxins by GC/MS		
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Contents

A. INTRODUCTION

1. Background

A generalized poison toxin screen is presented that is based on FDA's TO21 method. The focus is to extract as many compounds as possible, including unknowns, from the sample so that qualitative presence/absence information can be obtained. The method generates rapid but dirty acetonitrile extracts that are subsequently run on a GC/MS in full scanning mode. Further investigative results can be obtained from MS libraries which will result in tentative identifications. Alternately, this method may be used in a targeted approach using SIM mode for known compounds at the expense of investigative information.

2. Summary of Procedure

Various toxins, pesticides, and drugs are extracted from contaminated meat products using basic glycine buffer, acetonitrile, and sodium chloride. A small portion of the liquid extract is removed and analyzed in full scan mode by GC/MS. This method does not extract compounds that have strong or permanent charges, such as glyphosate, fluoroacetic acid, paraquat and diquat.

3. Applicability

This method is suitable for the identification of toxins in ground raw products, processed products and ready to eat products. It is also suitable for the identification of phenylbutazone in equine muscle. Refer to Section J.2.for specific analytes.

B. EQUIPMENT

Note: Equivalent equipment may be substituted.

1. Apparatus

a. Top Loading balance - Sensitive to 0.01 g, Cat. No. P1200, Mettler.

b. Analytical balance - Sensitive to 0.0001 g, Cat. No. AC1215, Sartorius.

c. 50 mL polypropylene centrifuge tubes - Cat. No. 352070, Falcon.

d. Pipettes - 1 mL and 10 mL electronic pipettes - Cat. No. E2-1000, EP-10 ML, Rainin.

e. Vortex mixer - Maxi Mix, Cat. No. M -16715, Thermolyne.

f. Centrifuge - Cat. No. T6000B, Sorvall.

g. Autosampler vial - 2 mL screw top, 12 x 32 mm, Cat. No. 27265, Supelco.

h. Volumetric flasks - Variable, Class A.

i. N-evaporator- N-Evap 112, Organomation

2. Instrumentation

a. Gas chromatograph/Mass spectrometer - Agilent 6890N/5975 Source – Electron Impact

b. Analytical column - Cat. No. DB-5MS, 30 m, 0.25 mm I.D., 0.25 µm film thickness, J&W.

c. Data System – ChemStation in the Environmental Mode and the Wiley/NIST Library.

C. REAGENTS AND SOLUTIONS

Note: Equivalent reagents / solutions may be substituted. The stability time frame of the solution is dependant on the expiration date of the components used or the listed expiration date, whichever is soonest.

1. Reagents

a. Acetonitrile (ACN) – Optima HPLC Grade, 0.2 µm filtered. CAT No. A996-4, Fisher.

b. Glycine - 99.5%, CAT No. 410225-50G, Sigma.

c. Methanol (MeOH) – Optima HPLC Grade, 0.2 µm filtered, CAT No. A454-4, Fisher.

d. Sodium chloride – 99.5% SigmaUltra, CAT No. S7653-1KG, Sigma.

e. Sodium hydroxide – 98% SigmaUltra, CAT No. S8045-500G, Sigma.

f. Water (H_2O) – Optima 0.5 µm filtered. CAT No. W7-4, Fisher.

2. Solutions

a. 1 M Glycine Buffer at pH 10:

Weigh 7.5 g of glycine and place into a 100 mL volumetric flask. Bring the volumetric flask up to volume with H2O. Adjust the pH to 10.00 (± 0.05) by the addition of sodium hydroxide pellets.

D. STANDARD(S)

Note: Equivalent standards / solutions may be substituted. Purity and counterions are to be taken into account when calculating standard concentrations. The stability time frame of the solution is dependant on the expiration date of the components used or the listed expiration date, whichever ends sooner.

1. Standard Information

   a. Reference standard materials are available from Fluka, Restek, Chem Service, Sigma-Aldrich, MP Biomedicals, and Cambridge Isotope Lab.

   Table 1 - Reference Standards

Phenanthrene-d10 (Internal Standard)	Codeine	Morphine	Nicotine	Pilocarpine
Triphenylphosphate (Internal Standard)	Parathion	Pentazocine	Phorate	Aldrin
Arecoline	Paraoxon	DDT	DDE	Tetramine
Scopolamine	Strychnine	Endrin	Fentanyl	
Diazinon	Terbuphos	Phenylbutazone		

2. Preparation of Standard Solutions

   Note: Specific examples of preparing standard solutions can be found in the Appendix.

   a. Stock Solutions:

   Weigh an appropriate amount of each reference material into a 5mL volumetric flask and bring to volume with MeOH (except for Tetramine--the diluent is ACN). Record the weight and calculate the actual concentration. These standard solutions are stable for 1 year refrigerated at 2 - 8 °C. The phenylbutazone solution is stable for 6 months stored at ≤ -10 °C.

   b. Spiking Solutions:

   Pipet the appropriate amount of each stock solution into an appropriate volumetric flask and dilute with MeOH to achieve the following concentrations (Table 2):

   Table 2 - Spiking Solution Concentrations

Analyte	Concentration
Diazinon	40 µg/mL
Phorate	60 µg/mL
Terbuphos	
Tetramine	
Nicotine	100 µg/mL
Paraoxon	

Analyte	Concentration
Parathion	100 µg/mL
Aldrin	
Endrin	
p,p,-DDT	
p,p,-DDE	
Pentacozine	
Scopolamine	
Codeine	
Fentanyl	
Strychnine	
Arecoline	200 µg/mL
Pilocarpine	240 µg/mL
Morphine	400 µg/mL
Phenylbutazone	15 µg/ml

Combining analyte solutions is acceptable provided that final concentrations reflect those in Table 2. These standard solutions are stable for 1 year refrigerated at 2 - 8 °C. The phenylbutazone solution is stable for 6 months stored at ≤ -10 °C.

Note: Morphine and Phenylbutazone solutions are made individually.

c. Internal Standard Solution (85 µg/mL):

Pipet appropriate amounts of triphenylphosphate and phenanthrene-d10 stock solution into a 5 ml volumetric flask to produce an 85 µg/ml solution. Bring the flask to volume with acetonitrile. This standard solution is stable for 3 months refrigerated at 2 - 8 °C.

3. Preparation of Reference Standard (X target level)

a. For raw ground product, processed products, and ready to eat products:

Pipet 250µL of each Spiking Solution excluding phenylbutazone (Refer to D.2.b, Table 2) and pipet 294µL of the Internal Standard Solution (85 µg/mL, Refer to D.2.c) into a 5 ml volumetric flask and bring to volume with acetonitrile. This standard solution is stable for 3 months refrigerated at 2 - 8 °C.

b. For Equine Muscle:

Pipet 166.7µL of the phenylbutazone spiking solution (Refer to D.2.b, Table 2) and pipet 294µL of the Internal Standard Solution (85 µg/mL, Refer to D.2.c) into a 5 ml volumetric flask and bring to volume with acetonitrile. This standard solution is stable for 3 months at < -10 °C.

Note: Stock Standards may be used instead of Spiking Solutions to make up the Reference Standard (X target level).

4. Preparation of External Calibration Curve (optional)

See the Appendix for an example preparation of the external calibration curve.

a. Matrix Match Standards (optional):

To prepare an X target level solution, pipet 42.5 µL of spiking solution excluding phenylbutazone (Refer to D.2.b, Table 2), 50 µL of the I.S. solution (Refer to D.2.c) and 757.5 µL of the blank sample extract (Refer to F.1.c) into an autosampler vial for a total volume of 850 µl. Prepare a matrix match standard for each matrix type in the sample set.

For Phenylbutazone, pipet 28.3 µL of the phenylbutazone spiking solution, (Refer to D.2.b, Table 2), 50 µL of the I.S. solution (Refer to D.2.c) and 771.7 µL of the blank sample extract (Refer to F.1.c) into an autosampler vial for a total volume of 850 µl.

Note: Negative controls using the same matrix type are to be analyzed in the sample set if matrix matched standards are used.

E. SAMPLE PREPARATION

Blend fresh and ready to eat meat products until homogeneous.

F. ANALYTICAL PROCEDURE

1. Preparation of Controls

a. Positive Control(s):

i. Raw ground product, processed products, and ready to eat products

(a) Weigh 3.0 +/- 0.04 g of representative blank sample into a 50 mL polypropylene centrifuge tube.

(b) Select a representative sample to be used as a positive control (recovery and check). The representative sample should be reflective of all samples in the set.

(c) Fortify with 150 µL of the spiking solution(s).

ii. Equine muscle,

(a) Weigh 3.0 +/- 0.04 g of blank sample into a 50 mL polypropylene centrifuge tube

(b) Fortify with 100 µL of the phenylbutazone spiking solution to obtain a 0.5 µg/g positive control.

b. Check sample (if needed):

Weigh 3.0 +/- 0.04 g of representative blank sample, which is the same tissue used for the positive control, into a 50 mL polypropylene centrifuge tube and fortify it with an amount of the spiking solutions unknown to the analyst.

c. Negative Control (optional):

Weigh 3.0 +/- 0.04 g of representative blank sample which is the same tissue used for the positive control into a 50 mL polypropylene centrifuge tube.

d. Reagent Blank (Method Blank):

Prepare a reagent blank by delivering 3 mL of reagent water into a 50 mL polypropylene tube.

e. Reagent Spike (optional):

Prepare a reagent spike by delivering 3 mL of reagent water into a 50 mL polypropylene tube. Fortify with 150 µL of the spiking solution(s). This additional control may be useful when working with a new sample type, an unknown, or a problematic matrix type.

2. Extraction Procedure

a. Weigh 3.0 +/- 0.04 g of sample into a 50 mL polypropylene centrifuge tube.

b. Add 3 mL of Glycine buffer pH 10 to each tube to generate the base phase extract. Vortex till homogeneous. Wait 45 minutes.

Note: Liquid samples can proceed directly to the next step.

c. Add 6 mL of ACN to each tube. Vortex till homogeneous.

d. Add about 2 g of NaCl to each tube. Vortex till homogeneous.

e. Centrifuge the samples for approximately 5 min. at 3200 rpm.

f. Remove the upper solvent layer and place into a 15 ml Falcon tube.

g. Add 6 mL of ACN to each tube. Vortex till homogeneous.

h. Centrifuge the samples for approximately 5 min at 3200 rpm.

i. Remove the upper solvent layer and add to the previous 6 mL.

j. Blow down the samples to 3 mL under a stream of nitrogen at 65°C.

k. Transfer 0.80 mL of the upper layer into an autosampler vial.

l. Add 50 µL of the internal standard solution (conc: 85 µg/mL) to the autosampler vial.

m. Cap and mix.

n. Inject onto the GC-MS

3. Instrumental Settings

Note: The instrument parameters may be optimized to ensure system suitability.

a. Oven Parameters:

Init Temp - 65 °C

Init Time - 0.50 min

Rate1- 25.0 °C/min

Final Temp - 300 °C

Final Time - 8.00 min

Run Time - 17.9 Min

b. GC Parameters:

Injector Temp - 250°C

Splitless Injection

Injection Volume - 1µL

Injection liner - Double cyclo Gooseneck

Purge Valve open - 1.0 min

Column Flow Mode - Constant flow

Column Flow - ~ 1mL

c. Mass Spectrometer Parameters:

Solvent Delay - 3.0 min

Mass Scan Range: 50 - 500 amu

Ion Source Temp. - 230 °C

MS Quad Temp. - 150 °C

Transfer Line - 280° C

d. Tuning Requirement:

Mass Assignments for 69.00, 219.00 and 502.00:	± 0.2 amu
Peak Widths:	0.45 < Peak Width < 0.65
Absolute Abundance of mass 69:	~480000
Relative Abundance of mass 219:	> 35% and < 85%
Relative Abundance of mass 502:	> 2% and < 5%
Ratio of mass 70 to 69:	0.5% to 1.5%
Ratio of mass 220 to 219:	3.0% to 5.5%
Ratio of mass 503 to 502:	7.5% to 12.5%
Air and water to mass 69:	< 10%
The voltage for the Electron Multiplier (EM):	< 2500V.

e. Quantitation and Qualifying Ions:

Analyte	~Retention Time (min.)	Target Ion	Q1	Q2	Q3	I.S.
Arecoline	4.20	140	96	155	94	$Phend_{10}$
Nicotine	4.92	84	133	162	119	$Phend_{10}$
Phorate	6.61	75	121	260	97	$Phend_{10}$
Phenanthrene-d_{10} (IS)	7.11	188	189	184	160	-
Paraoxon	7.59	109	139	81	99	$Phend_{10}$
Parathion	7.88	109	97	291	155	$Phend_{10}$
Pilocarpine	8.22	95	96	109	208	$Phend_{10}$
Pentazocine	9.09	217	285	270	110	TPP
Scopolamine	9.29	94	138	154	303	TPP
Triphenylphosphate (IS)	9.37	326	325	233	77	-
Codeine	9.55	299	162	229	282	TPP
Morphine	9.75	285	162	215	268	TPP
Strychnine	13.32	334	335	130	162	TPP

Analyte	~Retention Time (min.)	Target Ion	Q1	Q2	Q3	I.S.
Terbuphos	8.092	231	153	97	288	$Phend_{10}$
Diazinon	8.139	199	179	152	304	$Phend_{10}$
Tetramine	7.839	212	240	132	121	$Phend_{10}$
Aldrin	8.129	263	293	186	66	$Phend_{10}$
Endrin	9.126	263	281	245	345	TPP
DDT	9.828	235	237	165	199	TPP
Fentanyl	12.545	245	146	189	-	TPP
DDE	10.139	318	315	246	248	TPP
Phenylbutazone	11.201	183	308	77	105, 184*	TPP

* if needed.

f. Data Processing

i. Identify the external standards to be used for a calibration and generate a calibration curve within Chemstation software. If running the method with a single point (ie the X level external standard), then generate a 1 point curve.

ii. Process the data so that a result is generated (quantitating the data to facilitate analyte identification). The process will allow the software to identify all of the known peaks in the chromatogram.

iii. Using the Qedit function, process the data so that false hits are deleted, leaving only the spectra of positive hits.

iv. Evaluate any unknown peaks of interest using the Library Search Compounds (LSC) function. This will tentatively identify an unknown in the sample.

Note: All identified unknowns using the LSC function are potential positive and must be evaluated using a known standard, appropriate matrix spikes and other controls.

4. Injection sequence / Sample Set

a. External standard at target level

b. External standard at ½X target, 2X target levels (optional)

c. Matrix match standard at target level (optional)

d. Reagent blank

e. Tissue blank (negative control) (optional, but required if matrix matched standards are used)

f. Positive control(s) at target level per matrix class

g. Intralaboratory Check Sample (as needed)

h. Samples (up to 20)

i. External standard at target level

G. CALCULATIONS / IDENTIFICATION

For an analyte to be identified as present in a sample, the following criteria must be met:

1. All monitored ions are present.

2. Retention time match within 3% between the analyte of interest and the target (X level) standard.

3. Two or more fragment ions must have a relative intensity within ± 30% of that of comparable ions in the target (X level) standard. This calculation is usually performed by the instrument software.

4. Internal standard area counts between the target level standard and the sample are to be within ± 50%.

5. Visual inspection that all target ions align up in retention time plot (All ions align).

6. Visual inspection that the spectrum is a match to a standard (visible spectral match).

H. SAFETY INFORMATION AND PRECAUTIONS

1. Required Protective Equipment — Gloves, safety glasses and laboratory coats.

2. Hazards

Procedure Step	*Hazard*	*Recommended Safe Procedures*
Acetonitrile	Flammable, toxic, may be fatal is swallowed, inhaled or absorbed.	Avoid heat, flames, ignition sources and incompatibles. Incompatibles: oxidizing materials, sulfuric acid, perchlorates.

Methanol	Flammable. Toxic by inhalation, ingestion, and skin absorption.	Use under the hood. Store in a tightly closed container, in a well ventilated area. Store away from heat, keep away from any source of ignition.
Sodium hydroxide	Corrosive. Skin contact may produce burns.	Store in a tightly closed container. Store with corrosives in a cool, well ventilated area, away from incompatibles. Incompatibles: metal, heat, water, and each other.
Analytes	Highly toxic.	It is advisable to have another analyst in the vicinity when working with all analytes.
Glycine	LOW	Use under the hood. Store in a tightly sealed container, in a well ventilated area.

3. Disposal Procedures

Follow local, state and federal guidelines for disposal.

Note: Some analytes may be considered P listed waste. Enhanced disposal guidelines may apply.

I. QUALITY ASSURANCE PLAN

1. Performance Standard
   a. No false negatives above the target level.
   b. No positives in the reagent blank (method blank) and negative control.
2. Critical Control Points and Specifications

Record	*Acceptable Control*
a. Sample Weight	3.0 +/- 0.04 g

3. Intralaboratory Check Samples
   a. System, minimum contents.

i. Frequency: One per week per analyst when samples analyzed.

ii. Records are to be maintained.

b. Acceptability criteria.

Refer to I. 1.

If unacceptable values are obtained, then:

i. Investigate following established procedures.

ii. Take corrective action as warranted.

4. Sample Condition upon Receipt

Except shelf stable products, all other samples frozen or cold.

J. APPENDIX

1. References

US FDA SOP T021(004) General Method to Examine Foods and Beverages for Volatile and Semi-Volatile Contaminants using Acidic and Basic Extraction with Capillary Gas Chromatography-Mass Spectrometry; Kevin J. Mulligan, May 12,2006.

2. Levels of interest

Analyte	Target Level (ppm)
Diazinon	2
Phorate	3
Terbuphos	3
Tetramine	3
Nicotine*	5
Phenanthrene d_{10} (IS)	5
Paraoxon	5
Parathion	5
Pentazocine	5
Scopolamine	5
Triphenylphosphate (IS)	5
Codeine	5
Strychnine	5

Analyte	Target Level (ppm)
Aldrin	5
Endrin	5
DDT	5
Fentanyl	5
DDE	5
Arecoline	10
Pilocarpine	12
Morphine	20
Phenylbutazone	0.5

*Note: Interferences may exist when analyzing some types of salami for nicotine.

3. Examples of Standard Preparation

   a. From Section D.2.a, Stock Solutions:

   To obtain a Stock Solution of ~2mg/mL weigh approximately 10mg of reference material into a 5mL volumetric flask and bring to volume.

   b. From Section D.2.b, Spiking Solutions:

   The following table is an example based on individual 2 mg/mL stock solutions:

Spiking Solution	Analyte	Volume (µL) of stock std	Bring to volume
Spiking Solution 1	Diazinon	100	5mL of MeOH
	Phorate	150	
	Terbuphos	150	
	Tetramine	150	
	Nicotine	250	
	Paraoxon	250	
	Parathion	250	
	Aldrin	250	
	Endrin	250	
	p,p,-DDT	250	

Spiking Solution	Analyte	Volume (µL) of stock std	Bring to volume
	p,p,-DDE	250	
	Pentacozine	250	
	Scopolamine	250	
	Codeine	250	
	Fentanyl	250	
	Strychnine	250	
Spiking Solution 2	Arecoline	500	5 mL of MeOH
	Pilocarpine	600	
Spiking Solution 3	Morphine	1000	5 mL of MeOH
Spiking Solution 4	Phenylbutazone	37.5	5 ml MeOH

c. From Section D.2.c, Internal Standard solution:

An example based on 2 mg/mL individual internal standard stock solutions is as follows:

Pipet 213 µL of Triphenylphosphate stock solution and 213 µL of Phenanthrene-d10 stock solution into a 5 ml volumetric flask and bring to volume with acetonitrile.

d. From Section D.4, Preparation of External Calibration Curve (optional):

The following table shows an example of the preparation of an external standard curve based on spiking solutions and the internal standard solution.

i. Raw ground product, processed products, and ready to eat products

Level	Volume (µL) of Spiking Solution (Refer to D.2.b, Table 2)	Volume (µL) of Internal Standard Solution(85 µg/mL)(Refer to D.2.c)	Bring to final volume
½ X	125	294	5mL of ACN
X	250	294	5mL of ACN
2X	500	294	5mL of ACN

ii. Equine muscle

Level	Volume (µL) of Phenylbutazone Spiking Solution (Refer to D.2.b, Table 2)	Volume (µL) of Internal Standard Solution (85 µg/mL)(Refer to D.2.c)	Bring to final volume
½ X	83.35	294	5mL of ACN
X	166.7	294	5mL of ACN
2X	333.4	294	5mL of ACN

e. From Section D.4.a, Matrix Matched Standards (optional):

The following table shows an example of the preparation of a matrix matched standard curve based on spiking solutions and the internal standard solution.

i. Raw ground product, processed products, and ready to eat products

Sample type	Spiking Solution (µL) (Refer to D.2.b, Table 2)	Internal Standard Solution (85 µg/mL) (µL) (Refer to D.2.c)	Tissue Blank extract(µL) (Refer to F.1.c)
½ X matrix matched std	21.25	50	778.75
X matrix matched std	42.5	50	757.5
2X matrix matched std	85	50	715

ii. Equine muscle

Sample type	Phenylbutazone Spiking Solution (µL) (Refer to D.2.b, Table 2)	Internal Standard Solution (85 µg/mL) (µL) (Refer to D.2.c)	Tissue Blank extract(µL) (Refer to F.1.c)
½ X matrix matched std	14.15	50	785.85
X matrix matched std	28.3	50	771.7
2X matrix matched std	56.6	50	743.4

K. APPROVALS AND AUTHORITIES

1. Approvals on file.

2. Issuing Authority: Director, Laboratory Quality Assurance Division.