# Method for Determination of Pentobarbital in Dry Dog Food by Gas Chromatography/Mass Spectrometry

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A procedure has been developed and validated for measuring the concentration of pentobarbital residues in dry, extruded animal feed in the range of 3−200 ng/g (ppb) with an estimated limit of quantitation of 2 ppb. The method was developed for surveillance purposes: to measure the concentration of euthanizing agent which might be present in feeds incorporating rendered products which themselves might include some fraction of euthanized animals. A previously published qualitative procedure was modified by adding isotopically labelled pentobarbital as an internal standard. Dry feed was ground and extracted with methanol. The extract was loaded on a mixed-mode (C-18, anion exchange) solid-phase extraction cartridge designed for barbiturate residues. Pentobarbital was eluted and derivatized for gas chromatography/mass spectrometry in positive ion chemical ionization mode. Quantitation was based on the ratio of dimethyl-pentobarbital MH+ (*m*/*z* 255) vs dimethyl-pentobarbital-$d_5$ (*m*/*z* 260) in standards and extracts. Accuracy ranged from 112% at 3 ppb to 96% at 200 ppb, with relative standard deviations ranging from 4% at 3 ppb to 2% at 200 ppb.

**Keywords:** *Pentobarbital; quantitation; gas chromatography/mass spectrometry; feed; euthanasia*

## INTRODUCTION

It has been speculated that the presence of pentobarbital at low levels in some dog foods may result from processing a small proportion of euthanized animals with other tissues in the production of feed ingredients. Rendered products such as meat and bone meal (MBM) or animal fat (AF) are included in some feed formulations. It was shown by O'Connor et al that pentobarbital can survive the rendering process (*1*). Our laboratory previously developed two methods for the identification of pentobarbital in dry dog foods formulated with MBM. Adam and Reeves (*2*) validated a confirmatory method based on gas chromatography/mass spectrometry (GC/MS) for feed containing MBM over the range of 5−20 ppb. One example was given in which the presence of pentobarbital was confirmed in a retail feed (*2*). This method, which converts pentobarbital to a derivatized form, was corroborated with a method based on liquid chromatography/mass spectrometry (LC/MS) that did not rely on derivatization (*3*). In this case as well, several retail feeds were confirmed for the presence of pentobarbital.

To fully provide the information needed to ensure the safety of food products for animals, quantitative information is also needed. The extraction used for our qualitative GC/MS methods has now been modified by the inclusion of an internal standard to enable accurate measurement of concentration. The procedure was streamlined to enable somewhat faster sample processing. The method was validated by fortifying control feed at 1, 3, 8, 25, 75, and 200 ppb, and using a standard curve calibrated over the same range.

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Liu et al described an extraction for barbiturates in urine employing mixed mode (C-18, anion exchange) solid-phase extraction (SPE), derivatization by methylation, and GC/MS (*4*). This procedure was the basis of the Adam and Reeves method. Liu found that barbiturate levels could be measured by including isotopically labelled pentobarbital (pentobarbital-$d_5$). However, using GC/MS in electron ionization (EI) mode resulted in a small, systematic error in pentobarbital concentration due to low level interference by dimethyl-pentobarbital-$d_5$ on the major fragment ions from dimethyl-pentobarbital (*5*).

Hooierjink et al have developed methodology for pentobarbital and other such euthanizing agents that might also appear in MBM (*6*). They showed that positive ion chemical ionization (PICI) was a viable alternative to EI for the detection of methylated pentobarbital. Because the base peak (MH+) in PICI was also the highest mass ion produced by these compounds, it seemed reasonable that background interferences from complex samples such as animal feed would be minimized by using PICI−GC/MS for a quantitative method. The appropriate elements from these previous methods were combined in the development and validation of a sensitive quantitative method for pentobarbital in dry dog food.

## MATERIALS AND METHODS

**Reagents.** Spectrophotometric-grade ethyl acetate, hexane, isooctane, and methanol were obtained from Burdick & Jackson (Muskegon, MI). Deionized water was purified through a Milli-Q system (Millipore, Marlboro, MA) to a purity >16 M-ohm/cm, and was used for all subsequent references to water. Silylation grade dimethyl sulfoxide (DMSO) packed under nitrogen was obtained from Pierce (Rockford, IL). Concentrated hydrochloric acid (HCl), (36.5−38%) was obtained from Fisher Scientific (Fairlawn, NJ). Iodomethane and

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**Table 1. Dilution of Injection Standards**

| level | μL 10 ppm pento | μL 1 ppm pento | μL 10 ppm pento-$d_5$ | actual ppb, pento | actual ppb, pento-$d_5$ | equivalent ppb, pento[a] | equivalent ppb, pento-$d_5$[a] |
|---|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 500 | 0 | 500 | 0 | 50 |
| 2 | 0 | 100 | 500 | 10 | 500 | 1 | 50 |
| 3 | 0 | 200 | 500 | 20 | 500 | 2 | 50 |
| 4 | 50 | 0 | 500 | 50 | 500 | 5 | 50 |
| 5 | 100 | 0 | 500 | 100 | 500 | 10 | 50 |
| 6 | 250 | 0 | 500 | 250 | 500 | 25 | 50 |
| 7 | 500 | 0 | 500 | 500 | 500 | 50 | 50 |
| 8 | 1000 | 0 | 500 | 1000 | 500 | 100 | 50 |
| 9 | 2000 | 0 | 500 | 2000 | 500 | 200 | 50 |

[a] Based on 10 g of feed sample.

tetramethylammonium hydroxide (TMAH) dissolved at 25% in methanol were obtained from Sigma Chemical (St. Louis, MO). Phosphate buffered saline (PBS) was obtained as a 10× concentrated solution and diluted 10-fold with water before use (BioWhittaker, Walkersville, MD). Anhydrous sodium sulfate and sodium acetate trihydrate (reagent grade) were obtained from J. T. Baker (Phillipsburg, NJ).

**Solutions.** A 1.0 N solution of HCl was prepared by diluting 83 mL of concentrated HCl to 1 L with water. This was further diluted with water to yield a 0.1 N solution. A 100 mM sodium acetate buffer was prepared by dissolving 13.6 g of sodium acetate trihydrate in about 800 mL of water, adjusting the pH to 7 with 1 N HCl and diluting to 1 L with water. The SPE wash solution was prepared daily by combining 2 mL of ethyl acetate with 38 mL of hexane. The SPE elution solution was prepared daily by combining 10 mL of ethyl acetate with 30 mL of hexane.

**Standards.** Pentobarbital stock standard prepared at 1.0 mg/mL (w/v) in methanol and pentobarbital-$d_5$ stock standard prepared at 0.1 mg/mL (w/v) in methanol were obtained from Radian International (Austin, TX). Pentobarbital standards at 10 μg/mL (ppm), 1 ppm, and 0.1 ppm were prepared by serial dilution of stock standard with methanol in 10-mL Class A volumetric flasks. The internal standard solution was prepared by diluting 2.5 mL of pentobarbital-$d_5$ to 25 mL with methanol in a 25-mL volumetric flask. Standard solutions were stored for 3 months at 4−8 °C. Calibration standards 1−9 were prepared according to the dilution scheme in Table 1. The appropriate volumes of pentobarbital and pentobarbital-$d_5$ were transferred to 10-mL volumetric flasks and diluted to the mark with methanol. To prepare injection standards, 0.5 mL of each calibration standard was evaporated to dryness, derivatized, and dissolved in 0.1 mL of ethyl acetate.

**Sample Preparation.** Dry extruded pet feed was ground to a uniform powder in an industrial-grade food processor (RobotCoupe, Ridgeland, MS). Approximately 3 cups of feed pellets were ground for 45 s at a blade speed of 2500 rpm to yield a uniform powder that could be easily handled for weighing without appearing oily or lumpy. The feed used for control and fortified control samples was Canine LabDiet 5007 (PMI Nutrition International, Brentwood, MO). This is a feed product formulated for research purposes that was analyzed repeatedly without showing any signals corresponding to pentobarbital at the 1 ppb level or higher. Also, several samples of retail feed containing MBM and AF on the ingredient statement were purchased locally. After screening these samples, two were chosen for use in method validation.

**Extraction Procedure.** A subsample of 10 ± 0.4 g of ground feed was weighed in a 225-mL polypropylene centrifuge tube. The exact weight was recorded. Fortification with internal standard was performed by adding 50 μL of the 10 ppm pentobarbital-$d_5$ solution with a calibrated variable pipettor. To fortify at the desired level of pentobarbital, various amounts of pentobarbital solutions were added. For example, for 1 ppb fortification, 100 μL of the 0.1 ppm pentobarbital were added; for 10 ppb, 1000 μL of the 0.1 ppm pentobarbital solution were added; and for 100 ppm, 1000 μL of the 1 ppm pentobarbital solution were added.

A 100-mL portion of methanol was added to each sample tube. The tightly capped tubes were mounted at an angle and shaken overnight on a reciprocating shaker for at least 16 h at 200 rpm and ambient temperature. In the morning, samples were allowed to stand at least 5 min so particulates could settle. Approximately 50−65 mL of each extract was poured into 200-mL ground glass pear-shaped flasks. Methanol was removed by rotary evaporation at 2−5 Torr with a water bath set at 35 ± 5 °C until an oily dark residue remained.

The residue was dissolved in 5 mL of PBS and 2 mL of sodium acetate buffer. The flasks were placed at an angle on the reciprocating shaker for at least 15 min at 200 rpm to dissolve the residue. The flasks were rotated 180° and shaken at least another 15 min, so all surfaces were rinsed thoroughly. A yellow, waxy film remained on the interior of the pear-shaped flasks. The supernate solutions were poured into 15-mL disposable polypropylene centrifuge tubes. The tubes were centrifuged at 5500 rpm for 10 min in a benchtop centrifuge (Hermle Z230 A-MKII, National Labnet Co., Woodbridge, NJ). After centrifugation, typically, a loose precipitate was formed at the bottom and some fat floated on the top of a clear yellow liquid.

Bond Elut Certify II SPE cartridges (no. 1211-3051, 10 $cm^3$/200 mg, LRC, Varian, Harbor City, CA) were mounted on an SPE manifold. The cartridges were conditioned with 2 mL of methanol followed by 2 mL of 0.1 M sodium acetate buffer, pH 7.0. The SPE packing was not allowed to dry. Approximately 90−95% of the clear liquid extract was transferred to the SPE cartridge using a disposable Pasteur pipet, taking care not to transfer particulates from the bottom or the fat layer at the top.

Vacuum was adjusted to draw the extract through the SPE cartridge over about 2 min so the remaining liquid was even with the top of the SPE packing bed. The SPE cartridge was rinsed with 1 mL of 0.1 M sodium acetate buffer, pH 7.0. The cartridge was dried under vacuum of 15 in. Hg or greater for 5 min. Cartridges were washed with 2.0 mL of the hexane/ethyl acetate wash solution and all eluates were discarded. Labeled glass centrifuge tubes with conical bottoms (no. 73790) and with snap caps (no. 73837-2, Kimble Glass, Vineland, NJ) were placed under each SPE outlet. The SPE cartridges were eluted slowly (2−3 in. Hg) with 2.0 mL of hexane/ethyl acetate elution solution. The procedure could be stopped at this point if all tubes were capped tightly and stored in a refrigerator for up to 24 h.

**Derivatization**. The derivatization was based on a procedure described by Liu et al. (*4*). Dedicated glass syringes were used to transfer all reagents. A derivatization solution was prepared daily by adding 100 μL of 25% TMAH in MeOH to 2.0 mL of DMSO in a small vial with a Teflon-lined screw cap. To each tube containing dried residue, 100 μL of the TMAH reagent was added. The tubes were capped and vortexed for a minimum of 30 s and allowed to stand at least 2 min. A 25-μL aliquot of iodomethane was added to each tube. The tubes were capped, vortexed briefly, and allowed to stand at least 5 min. Then 0.4 mL of 0.1 N HCl was added to each tube, followed by 2 mL of isooctane. Tubes were capped and vortexed for 2 min with frequent stopping and starting of the vortex mixer.

**Table 2. GC Conditions**

| time after injection, min | oven temperature, °C | temperature ramp, °C/min | hold time, min |
|---|---|---|---|
| 0 | 40 | | 1 |
| 1 | 40 | 20 | |
| 6 | 140 | 3 | |
| 19.33 | 180 | | 3 |
| 22.33 | 180 | 30 | |
| 24.67 | 250 | | 8.33 |
| 33 | 250 | | |

The phases were allowed to separate on standing for 5−10 min. The upper layer was transferred with a disposable pasteur pipet to a clean, disposable conical glass tube. It was very critical to avoid transferring any of the lower aqueous phase, as this would interfere with the subsequent evaporation step.

The solvent was evaporated to dryness under a nitrogen stream in the N-Evap with water bath at 35 °C. It was very critical to remove the tubes immediately after they reached dryness. If samples sat in the nitrogen stream after going dry, the GC/MS response was found to decrease. This loss of sensitivity resulted in the inability to detect dimethyl-pentobarbital in the low level standards, and would compromise the ability to measure accurately down to 3 ppb. Extracts were reconstituted in 100 $\mu$L of ethyl acetate that had been dried over sodium sulfate. Extracts were vortexed briefly and transferred to GC vials containing glass inserts for small-volume injections. Extracts were stable for quantitative purposes if analyzed by GC/MS within 48 h at ambient temperature if the solvent had not evaporated, or up to 4 days after preparation if stored at or below −20 °C after derivatization.

Standards were prepared the same day as extracts or up to 4 days in advance, if stored at or below −20 °C after derivatization.

**Gas Chromatography.** The mass spectrometer was an HP5989A equipped with an HP5890 series II gas chromatograph, a split/splitless injector, the Chemstation data system, and a series 7673 autosampler (Agilent Technologies, Palo Alto, CA). The GC column was a DB-5: 30-m, 0.25-$\mu$m film thickness, 0.25-mm. o.d. (J&W Scientific, Folsom, CA). A 2-mm i.d. quartz injection port liner was used. The injector temperature was 270 °C. Carrier gas flow of chromatography grade helium was set at a linear velocity of approximately 30 cm/sec. The GC/MS interface temperature was 280 °C. The GC oven temperature program is shown in Table 2. The autosampler was programmed to inject 1 $\mu$L following two sample pumps with the syringe. After injection, the autosampler automatically washed the syringe five times from each of two ethyl acetate wash solutions. Carryover of standards from one injection to the next was prevented by preparing fresh autosampler wash solutions each day and changing the GC injector septum each day.

**Mass Spectrometry.** The mass spectrometer was tuned and calibrated for PICI according to the manufacturer's instructions. The reagent gas was methane. Methane pressure was adjusted so the instrument's source pressure was 1.0−1.1 Torr (as measured by the thermocouple gauge in the GC/MS transfer line). Tuning was optimized for the perfluorotributylamine (PFTBA) calibrant ions in the mass range of dimethyl-pentobarbital: *m/z* 169, 219, and 264. During tuning and calibration the peakwidths were adjusted to 0.6 Da at half-height, but during data acquisition the option for "low resolution" was selected to maximize sensitivity. The source was retuned prior to each day's analyses. Selected ion monitoring (SIM) was used for $MH^+$ and fragment ions: *m/z* 255.2, 184.1, and 169.1 for dimethyl-pentobarbital and *m/z* 260.1 for dimethyl-pentobarbital-$d_5$. Dwell time was 50 ms per ion.

The system suitability was evaluated each day by analyzing standard level 2 (equivalent to 1 ppm pentobarbital + 50 ppb pentobarbital-$d_5$ in feed) and an ethyl acetate blank. If the peak-to-peak signal-to-noise ratio was 5:1 or greater for dimethyl-pentobarbital, and if carryover signals from dimethyl-pentobarbital-$d_5$ did not appear in a subsequent blank chromatogram, the system was considered suitable.

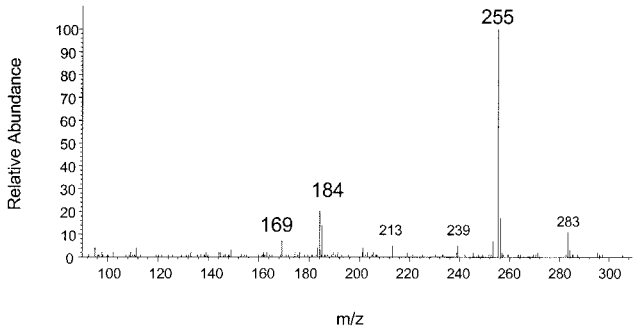


**Figure 1.** Full scan PICI mass spectrum of derivatized pentobarbital. Amount injected was 10 ng.

An ethyl acetate blank was injected before each unknown sample to reduce the possibility of carryover affecting the subsequent injection. The set of calibration standards was first analyzed from low to high concentration, then two ethyl acetate blanks were analyzed, then unknown samples were analyzed, each one followed by one ethyl acetate blank.

**Calculations.** Peak areas for *m/z* 255 and 260 were integrated. Integration parameters were used that separated the two peaks observed from dimethyl-pentobarbital-$d_5$ (as in Figures 3−7). The peak area ratios for *m/z* 255:260 in standards and extracts were calculated. A standard curve was calculated by linear regression using values for the concentration ratio based on equivalent concentrations shown in Table 1. It was a critical step to use 1/*x* weighting in the linear regression to properly calibrate the 1−10 ppb range as well as the 10−200 ppb range.

The pentobarbital concentration in unknowns was calculated by entering the peak area ratio into the equation for the standard curve. The ppb values found for retail samples were corrected by multiplying by 10/the starting weight of ground feed. This corrects for variance in the starting weight of feed, because 10 g was assumed in preparing the calibration curve.

**Safety.** Barbiturates are Drug Enforcement Agency (DEA) scheduled drugs. Exempted in vitro diagnostic drug standards are available for research. Access to the laboratory was controlled during analyses. Steps involving organic solvents were performed in the fume hood. Protective clothing, gloves, and safety glasses were worn during the extraction process.

## RESULTS AND DISCUSSION

Early in method development, ion trap LC/MS/MS methodology for pentobarbital (*3*) was tested along with the use of pentobarbital-$d_5$ as an internal standard to enable quantitation. This approach could have avoided the extra step of derivatization. However, as in the qualitative method, LC/MS/MS was found to be less sensitive than GC/MS for measuring pentobarbital in feed (*3*). The LC/MS/MS approach was not pursued further, in favor of GC/MS methodology.

The PICI mass spectrum of dimethyl-pentobarbital in GC/MS mode is shown in Figure 1. The $[M + H]^+$ ion appears at *m/z* 255, and the ion at *m/z* 283 is due to $[M + C_2H_5]^+$. The PICI spectrum of dimethyl-pentobarbital-$d_5$ is nearly the same except for mass shifts due to isotopic incorporation. Fragment ion structures for both compounds are shown in Figure 2. The side-chain fragmentation shown leading to *m/z* 169 for dimethyl-pentobarbital is supported by the appearance of *m/z* 171 for dimethyl-pentobarbital-$d_5$.

The GC temperature program was modified (Table 2) from that used in the qualitative method (*2*) to improve the separation between the two peaks we observed for pentobarbital-$d_5$. The rate of temperature ramp in the second stage was reduced significantly, which also

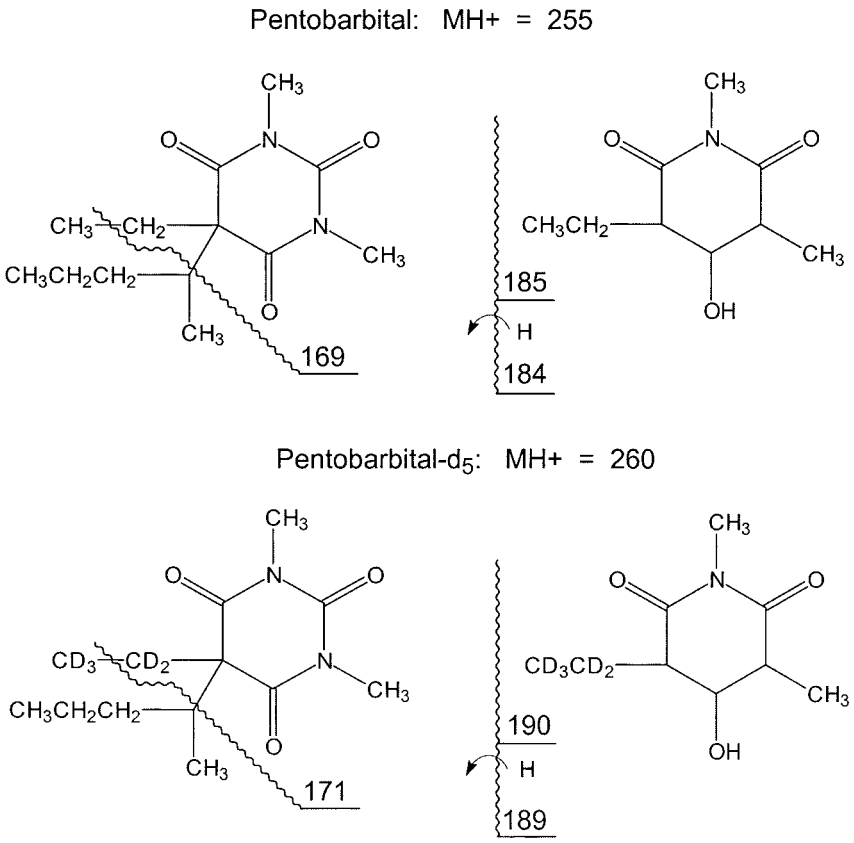


**Figure 2.** Structure and fragmentation pattern observed in PICI for derivatized pentobarbital and pentobarbital-$d_5$.

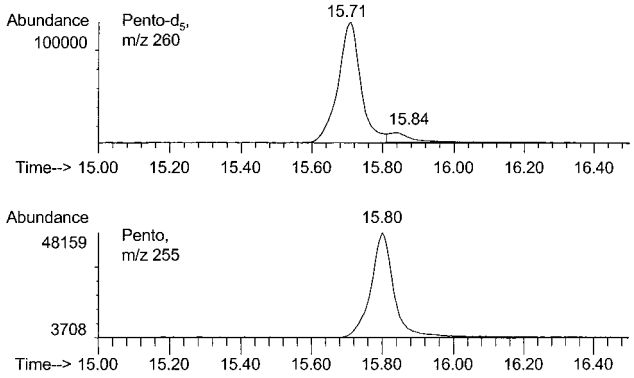


**Figure 3.** Ion chromatograms from PICI−GC/MS analysis of a level 6 standard, equivalent to 25 ppb pentobarbital and 50 ppb pentobarbital-$d_5$.

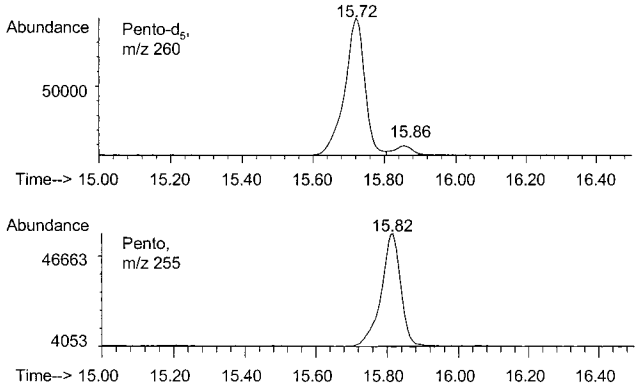


**Figure 4.** Ion chromatograms from PICI−GC/MS analysis of a 25 ppb fortified control feed. Pentobarbital-$d_5$ was added at 50 ppb as an internal standard.

increased the retention times. The retention time of dimethyl pentobarbital was shifted to 15.80 min. Dimethyl-pentobarbital-$d_5$ eluted at 15.72 min (Figure 3). A second, minor peak in the pentobarbital-$d_5$ chromatogram appeared at 15.86. This second peak was not included in the integrated peak area used for quantitation.

We observed that standards and extracts showed different peak shapes. Standards showed peak tailing, whereas extracts showed more symmetrical peak shapes. As a result, extracts, rather than standards, showed better separation between the major and minor peaks in dimethyl-pentobarbital-$d_5$. This might have introduced a slight error, as dimethyl-pentobarbital-$d_5$ had to be integrated differently from dimethyl-pentobarbital because no following peak was observed in the unlabeled compound.

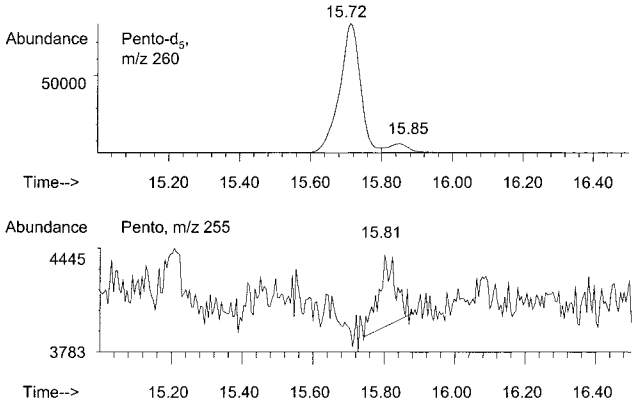


**Figure 5.** Ion chromatograms from PICI−GC/MS analysis of a control sample containing 50 ppb pentobarbital-$d_5$. A small matrix interference appears at 15.81 min for $m/z$ 255.

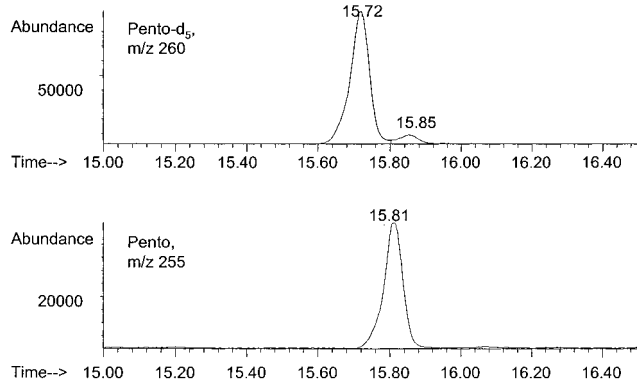


**Figure 6.** Ion chromatograms from PICI−GC/MS analysis of a retail dog food sample found to contain approximately 20 ppb pentobarbital. Pentobarbital-$d_5$ was added at 50 ppb.

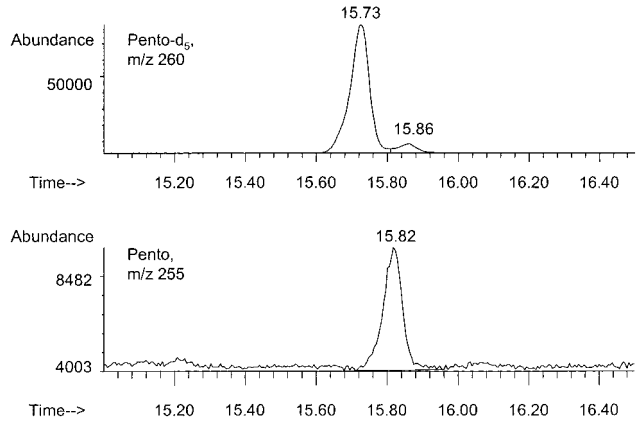


**Figure 7.** Ion chromatograms from PICI−GC/MS analysis of a 3 ppb fortified control feed.

Samples for method validation were analyzed over 6 days. No duplicate of any fortification level or retail feed was analyzed on the same day. Summary data from the method validation set are shown in Tables 3−5. Correlation coefficients for calibration curves were better than 0.99 in all cases. Table 3 includes data from samples fortified at 1−200 ppb. Because of the presence of an interference equivalent to approximately 0.3 ppb, the accuracy and signal-to-noise levels of 1 ppb fortified samples were unacceptable. Calculation of a conventional limit of quantitation (LOQ, Table 4) yielded a value of 1.2 ppb. For the purposes of this method it was stipulated that an error of ±15% would be acceptable at the LOQ. The 0.3 ppb bias yielded an LOQ of 2 ppb, at the point where the measurement error would exceed 15% (0.3/2 = 15%). The accuracy found at 3 ppb (112%) was influenced by the presence of this 0.3 ppb background peak. Above this concentration, accuracy ranged from 108% at 8 ppb to 96% at 200 ppb. Table 5 summarizes the results from analyses of five replicates each of two retail samples of dog food. For all measurements above LOQ in Table 3 or 5, the highest relative standard deviation in the results was 5.3%.

We found that to minimize problems with carryover it was necessary to institute several procedures: change the ethyl acetate autosampler wash vials every day; use fresh blanks every day; change the GC septum every day; and use ethyl acetate blanks for the last two injections of the day. Otherwise, carryover of up to 2% of the previous injection might have been observed in the subsequent runs (worst case scenario). Also, if a syringe is not fully cleaned, derivatized pentobarbital can remain in the syringe at room temperature for many days. However, even when injections showed slight evidence of carryover, the blank injection was found to fully clean the syringe. It was shown during method development that negligible carryover follows through to the second injection, even if the septum and wash solutions are not changed.

Use of an isotopically labeled internal standard along with positive ion chemical ionization enabled a qualitative method (*2*) to be modified for quantitative purposes. The limit of quantitation of this method compares favorably to a procedure for pentobarbital residues in MBM (*6*). This validated method provides regulatory

**Table 3. Validation Data, Fortified Samples**

| sample type | sample ID | accuracy, % | mean, % | std dev | % CV |
|---|---|---|---|---|---|
| fortified 1 ppb | A | 115.3 | 134 | 12.4 | 9.3 |
| | B | 132.5 | | | |
| | C | 138.3 | | | |
| | D | 132.0 | | | |
| | E | 149.5 | | | |
| fortified 3 ppb | A | 117.2 | 112 | 4.3 | 3.8 |
| | B | 114.2 | | | |
| | C | 109.3 | | | |
| | D | 112.1 | | | |
| | E | 106.1 | | | |
| fortified 8 ppb | A | 110.9 | 108 | 2.0 | 1.8 |
| | B | 107.7 | | | |
| | C | 107.3 | | | |
| | D | 105.4 | | | |
| | E | 107.5 | | | |
| fortified 25 ppb | A | 107.5 | 103 | 3.5 | 3.4 |
| | B | 103.2 | | | |
| | C | 105.2 | | | |
| | D | 99.8 | | | |
| | E | 99.2 | | | |
| fortified 75 ppb | A | 104.9 | 101 | 2.9 | 2.9 |
| | B | 99.2 | | | |
| | C | 101.2 | | | |
| | D | 100.5 | | | |
| | E | 96.9 | | | |
| fortified 200 ppb | A | 97.6 | 96 | 1.6 | 1.7 |
| | B | 95.9 | | | |
| | C | 96.5 | | | |
| | D | 97.1 | | | |
| | E | 93.5 | | | |

**Table 4. Estimate of Limit of Quantitation**

| sample type | sample ID | apparent ppb | mean, ppb | standard deviation | LOQ, ppb [mean + (10 × std dev)] |
|---|---|---|---|---|---|
| control | A | 0.32 | 0.305 | 0.092 | 1.2 |
| | B | 0.38 | | | |
| | C | 0.22 | | | |
| | D | 0.26 | | | |
| | E | 0.21 | | | |
| | F | 0.44 | | | |

**Table 5. Analysis of Retail Feeds**

| sample type | sample ID | measured pentobarbital (ppb) | mean | std dev | %CV |
|---|---|---|---|---|---|
| retail 1 | A | 7.6 | 7.0 | 0.37 | 5.3 |
| | B | 7.1 | | | |
| | C | 6.6 | | | |
| | D | 7.0 | | | |
| | E | 6.8 | | | |
| retail 2 | A | 20.3 | 19.8 | 0.49 | 2.5 |
| | B | 19.0 | | | |
| | C | 19.8 | | | |
| | D | 19.9 | | | |
| | E | 20.2 | | | |

scientists with the means to survey dry dog food for the level of pentobarbital, if present.

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