Table I—Change in Acid-Consuming Capacity during Aging at 25°

| Days | Percent of Theoretical Acid-Consuming Capacity | | |
|---|---|---|---|
| | Gel I | Gel II | Gel III |
| 13 | — | — | 78 |
| 15 | 78 | 81 | — |
| 64 | — | 61 | — |
| 66 | 63 | — | 57 |
| 113 | — | 53 | 41 |
| 161 | 55 | 34 | 28 |

crystalline aluminum hydroxide polymorph results in a decreased acid-consuming capacity. The behavior supports the earlier hypothesis (12–14) that anions present at the time of precipitation stabilize the gel structure. The removal of these stabilizing anions by washing results in an increased rate of development of order in the gel structure, with an associated decrease in the acid reactivity.

The sequence of thermograms showing the aging process of each gel (Figs. 1–3) indicates that, as aging proceeds, the intensity of the dehydration endotherm decreases and the intensity of the dehydroxylation endotherm increases. As the gel becomes ordered, less water is bound to the gel and the structural hydroxyl content increases. The bound water is probably replaced by structural hydroxyl in the form of double hydroxide bridges.

The proposed (15) polymer model for aluminum hydroxide suggests that particle growth occurs by a deprotonation–dehydration mechanism, which results in the conversion of bound water molecules into double hydroxide bridges. This process continues until a crystalline form of aluminum hydroxide is produced. With gibbsite, a highly crystalline form of aluminum hydroxide, the thermogram (Fig. 3) indicates that virtually no bound water is present.

In summary, it appears that differential thermal analysis is a useful technique for studying aluminum hydroxide gel. It is sensitive to the structural changes that occur during aging which are responsible for the decreased acid reactivity of the aluminum hydroxide gel. Analysis of the thermograms indicates that bound water in the gels was replaced during aging by structural hydroxyl groups in the form of double hydroxide bridges. This change results in a more ordered structure which is more resistant to reaction with acid. As aging progresses, the gel assumes the characteristic thermogram of a crystalline aluminum hydroxide.

## REFERENCES


(1) S. L. Nail, J. L. White, and S. L. Hem, *J. Pharm. Sci.*, **64**, 1166(1975).

(2) *Ibid.*, **65**, 231(1976).

(3) *Ibid.*, **65**, 1188(1976).

(4) *Ibid.*, **65**, 1192(1976).

(5) T. Sato, *Z. Anorg. Allg. Chem.*, **391**, 69(1972).

(6) R. C. MacKenzie, R. Meldeau, and J. A. Gard, *Min. Mag.*, **33**, 145(1962).

(7) J. M. Bracewell, A. S. Campbell, and B. D. Mitchell, *Clay Miner.*, **8**, 325(1970).

(8) H. C. Stumpf, A. S. Russell, and J. W. Newsome, *Ind. Eng. Chem.*, **42**, 1398(1950).

(9) T. Sato, *Z. Anorg. Allg. Chem.*, **391**, 167(1972).

(10) R. E. Grim, "Clay Mineralogy," 2nd ed., McGraw-Hill, New York, N.Y., 1968, pp. 285–297.

(11) "The United States Pharmacopeia," 18th rev., Mack Publishing Co., Easton, Pa., 1970, p. 26.

(12) S. L. Hem, E. J. Russo, S. M. Bahal, and R. S. Levi, *J. Pharm. Sci.*, **59**, 317(1970).

(13) G. J. Ross and R. C. Turner, *Soil Sci. Soc. Am. Proc.*, **35**, 389(1971).

(14) P. H. Hsu, *Int. Comm. Study Bauxites Aluminum Oxides-Hydroxides, 3rd*, **1973**, 613.

(15) J. D. Hem and C. E. Roberson, Geological Survey Water-Supply Paper 1827-A, U.S. Government Printing Office, Washington, D.C., 1967.


## ACKNOWLEDGMENTS AND ADDRESSES


Received July 2, 1975, from the *Industrial and Physical Pharmacy Department, School of Pharmacy and Pharmacal Sciences, and the ‡Department of Agronomy, Purdue University, West Lafayette, IN 47907

Accepted for publication November 26, 1975.

Supported in part by an American Foundation for Pharmaceutical Education Fellowship (S. L. Nail).

This report is Journal Paper 5946, Purdue University Agricultural Experiment Station, West Lafayette, IN 47907



ˣ To whom inquiries should be directed.


# Extraction and GLC Analysis of Pyrithyldione in Highly Putrified Human Postmortem Samples


**FRANK K. MARTENS ˣ, MARK A. MARTENS, JOHAN DEMETER, and AUBIN HEYNDRICKX**



**Abstract** □ Pyrithyldione was isolated from highly putrified human brain and small intestine samples by ether extraction followed by alumina column cleanup. The total yield was 90 ± 5%. No derivatization was necessary prior to GLC analysis.



**Keyphrases** □ Pyrithyldione—GLC analysis, putrified brain and intestine □ GLC—analysis, pyrithyldione in putrified brain and intestine □ Sedatives—pyrithyldione, GLC analysis in putrified brain and intestine


In forensic toxicology, many methods have been reported for extracting pyrithyldione and glutethimide from postmortem samples. Most of these methods consist of a single extraction step with chloroform, petroleum ether, or methylene chloride followed by TLC, UV spectrophotometry, or spectrofluorometry (1–4).

Impurities may be removed by gel permeation chromatography (5) or direct analysis by GLC, using various liquid phases (6, 7). If impurities interfere too much after direct extraction, ethylation with tetraethylammonium hydroxide may solve this problem (8). Three-month-old postmortem samples are difficult to analyze, and special cleanup requirements may be necessary for final unambiguous identification. To prevent interference with barbiturates and other hypnotics or sedatives, OV-275 was selected for the GLC analysis.

Table I—Concentrations of Pyrithyldione in 3-Month-Old Postmortem Samples from a Suicide Case

| Samples Analyzed | Concentration Found, ppm ± *SD* |
|---|---|
| Stomach content | 6.70 ± 0.15 |
| Blood | 3.50 ± 0.25 |
| Liver | 3.50 ± 0.20 |
| Small intestine content | 1.60 ± 0.17 |
| Kidney | 3.55 ± 0.20 |
| Brain | 0.75 ± 0.13 |
| Urine | 4.25 ± 0.22 |

## EXPERIMENTAL

**Reagents**—Peroxide-free ether was prepared by refluxing over potassium hydroxide and distillation and was stored in bottles protected from light. The methanol-deactivated neutral alumina[1] was prepared according to the method of McClure (9) and applied in forensic toxicology according to Martens *et al.* (10).

Dry sodium sulfate and extrapure seasand[1] were used to grind the postmortem tissues. Reference standards were pyrithyldione[2] and methyprylon[3]. For routine analysis, 1- and 0.1-mg/ml pyrithyldione solutions in ethanol and a 0.1-mg/ml methyprylon solution in ethanol were prepared as internal standards.

**Apparatus**—A research gas chromatograph[4] equipped with a dual flame-ionization detector was used. A Pyrex glass column, not silanized (4 mm i.d., 6 mm o.d. × 1.90 m) contained 3% OV-275 on Chromosorb W-AW, not silanized[5]. About 0.07-m glass beads were placed in the injection port of the column.

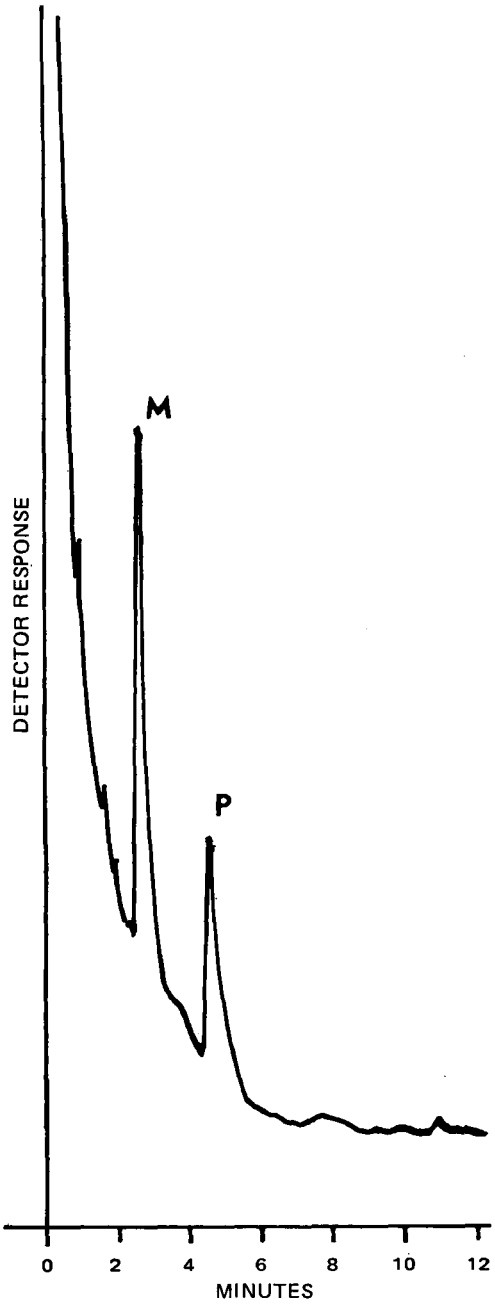


**Figure 1**—*GLC analysis of an extract of pyrithyldione from a 250-mg brain sample. An OV-275 column was used. Key: M, methyprylon internal standard; and P, pyrithyldione.*

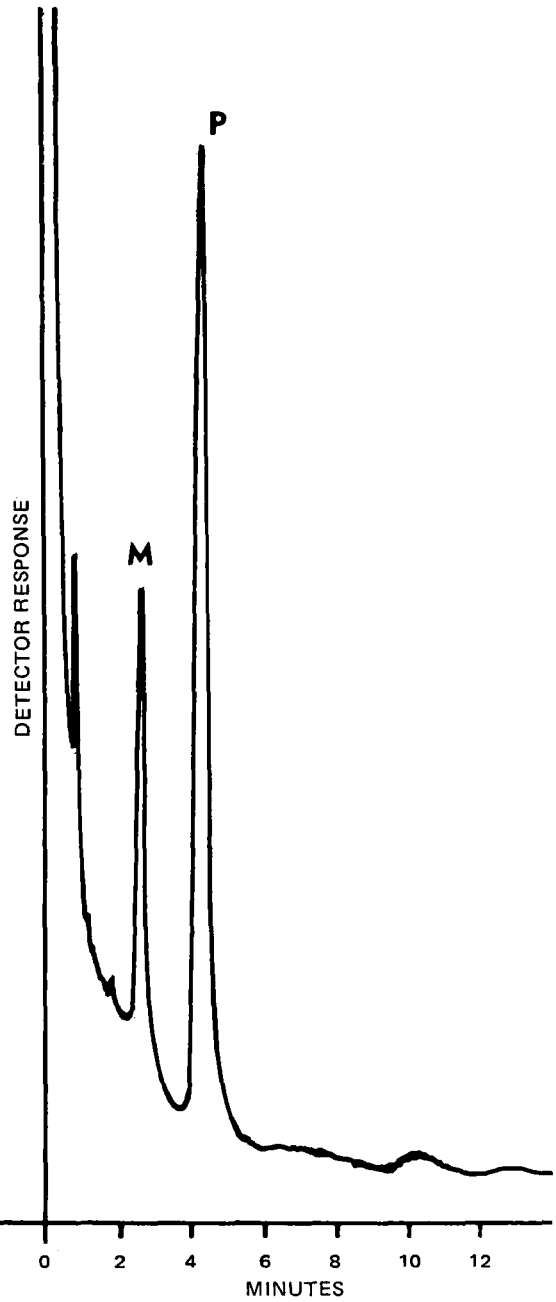


**Figure 2**—*GLC analysis of an extract of pyrithyldione from a 230-mg small intestine sample. An OV-275 column was used. Key: M, methyprylon internal standard; and P, pyrithyldione.*

[1] E. Merck, Darmstadt, Germany.
[2] Persedon, Roche, Basel, Switzerland.
[3] Noludar, Roche, Basel, Switzerland.
[4] Model 5750 B, Hewlett-Packard.
[5] Supelco, lot C-1249.

Table II—Relative Retention Times on OV-275

| Compound | Relative $R_t$ |
|---|---|
| Pyrithyldione | 1.00 |
| Methyprylon | 0.61 |
| Glutethimide | 1.77 |
| Methsuximide | 0.58 |
| Phensuximide | 1.29 |
| Mephenytoin | 2.42 |

This column was conditioned as follows: 30 min, a 10-ml/min carrier gas flow at room temperature; 2.30 hr, no flow at 100°; 1.30 hr, no flow at 150°; 2.00 hr, no flow at 220°; and 24 hr, a 10-ml/min carrier gas flow at 240°. During analysis, the oven temperature was maintained at 200° isothermal, the injection port at 240° and the detector at 255°. The column showed a top performance at 37 ml/min. Injections were made with a 10-μl syringe[6]. Chromatographic cleanup columns consisted of Pyrex glass, 0.7 × 20 cm.

**Procedure**—In a mortar, 5–10 g of forensic sample (brain, liver, kidney, stomach content, small intestine content, blood, and urine) are thoroughly mixed and shattered with sand and sufficient sodium sulfate to obtain a free flowing dry powder. This dry mixture is transferred into a 500-ml erlenmeyer flask containing 200 ml of ether and shaken for 30 min. The extraction residue is washed twice with 20 ml of ether.

The combined ether phases, loaded with fat, impurities, and the drug, are poured into an evaporation flask together with 25 ml of distilled water. The ether phase is slowly evaporated[7] at 50°. The remaining aqueous phase contains the drug while fatty material precipitates. This aqueous phase and two 20-ml portions of water, used for rinsing, are filtered through a prewetted filter[8] into a 250-ml separator. All combined aqueous phases are washed once with 50 ml of *n*-pentane to remove the remaining fats and free fatty acids.

Ether (2 × 100 ml) is used to extract the drug out of the aqueous phase. The combined ether phases are spiked with the appropriate amount of methyprylon as the internal standard. The volume of the ethereal phase is reduced[9] to not less than 0.5 ml. After adjusting the remaining volume to 2 ml with ether, 1 ml is pipetted onto the chromatography column containing 10 cm of methanol-deactivated alumina; the drug is completely eluted with 100 ml of ether. This volume is reduced[9] and further evaporated by a gentle jet of nitrogen at room temperature until dry. This final residue is dissolved in 10–50 μl of methanol and injected in the gas chromatograph.

## RESULTS

GLC data were quantified by their pyrithyldione–methyprylon peak ratios (Table I). The separation achieved is shown in Figs. 1 and 2. Methyprylon proved to be the best internal standard in all sample analyses.

[6] Hamilton Co., Reno, Nev.
[7] Buchi-Rotavapor.
[8] Whatman No. 1.
[9] Kuderna Danish evaporator.

## DISCUSSION

Recovery experiments were carried out on each extraction step. Volume–volume ratios, temperature conditions, and column conditions were optimized to obtain acceptable reproducibility. Total recovery was 90 ± 5% on 25-ppm pyrithyldione-spiked samples. The aqueous phase, containing the drug, gave a 97 ± 2% yield when extracted twice with equal volumes of ether; only 2–3% losses occurred during the *n*-pentane washing. Experiments on the influence of the aqueous phase pH demonstrated a higher extraction yield of pigments and impurities at low pH. Since the keto form of pyrithyldione prevails at neutral pH, no acidification was needed.

Methyprylon was chosen as the internal standard because of its superior properties compared to other hypnotics or sedatives of analogous molecular structure. Methyprylon eluted along with pyrithyldione from the alumina column in a 100% yield, and a constant pyrithyldione–methyprylon ratio was maintained over two evaporation steps. The coefficient of variation of this ratio during the alumina column cleanup ranged from 2.2 to 5.2%. However, close attention is required for the evaporation procedure, after which the final residues may not be heated over 40°. Therefore, glutethimide and phensuximide were not suited for use as internal standards. Most common hypnotics are separated on OV-275 (Table II). Other hypnotics such as brallobarbital, secobarbital, ethosuximide, phenytoin, primidone, phenobarbital, and mephobarbital do not interfere and appear at much higher $R_t$ values.

The developed method offers the possibility of extracting pyrithyldione with a high yield and purity. Because of its excellent performance with barbiturates and diketopiperidines, OV-275 allows a detection limit of 15 ng/g of sample with flame ionization.

## REFERENCES


(1) I. Sunshine, R. Maes, and R. Faracci, *Clin. Chem.*, **14**, 595(1968).

(2) O. Pribilla, *Arzneim.-Forsch.*, **6**, 138(1956).

(3) E. Hirschberg, M. F. Greenberg, E. De Ritter, and S. H. Rubin, *J. Am. Pharm. Assoc., Sci. Ed.*, **37**, 288(1948).

(4) P. Polatin, E. Hirschberg, S. Rubin, and L. Pirk, *Am. J. Med. Sci.*, **214**, 662(1947).

(5) M. Yoshihiko, *J. Forensic Sci.*, **15**, 92(1970).

(6) E. Watson and S. Kalman, *Clin. Chim. Acta*, **38**, 33(1972).

(7) P. Grieveson and J. S. Gordon, *J. Chromatogr.*, **44**, 279(1969).

(8) J. McGee, *Clin. Chem.*, **17**, 587(1971).

(9) V. McClure, *J. Chromatogr.*, **70**, 168(1972).

(10) M. A. Martens, F. K. Martens, and A. Heyndrickx, *Meded. Fac. Landbouwwet. Gent*, **39**(2), 1279(1974).


## ACKNOWLEDGMENTS AND ADDRESSES


Received August 22, 1975, from the *Department of Toxicology, State University of Ghent, Hospitaalstraat 13, B-9000 Ghent, Belgium.*

Accepted for publication October 25, 1975.

The authors are indebted to M. Creynen for technical assistance.



[x] To whom inquiries should be directed.