Measurement of Felbamate by Wide-Bore Capillary Gas Chromatography and Flame Ionization Detection

Nader Rifai,1,2,5 David Fuller,1 Terence Law,1,4 and Mohamad Mikati3

Felbamate, a newly developed antiepileptic agent, has been demonstrated to control partial and generalized seizures effectively. We have developed a gas-chromatographic method for the determination of felbamate, using a wide-bore capillary column, a flame ionization detector, and a simple extraction procedure. The assay day-to-day precision (n = 20) was 5.2% and 3.6% for drug concentrations of 50 and 150 mg/L, respectively; average recovery over a wide range of felbamate concentrations was 95%; the detection limit was 5 mg/L; and assay linearity extended to 300 mg/L. Although 9 of the 27 drugs tested were coextracted with felbamate, they exhibited significantly different retention times and showed no interference. A short-term stability study showed that plasma felbamate is stable at 4, −20, or −78°C for at least 1 month. Plasma felbamate concentrations in 66 pediatric patients ranged from 7 to 154 mg/L (mean ± SD 44 ± 24.7). We consider the method ideally suited for therapeutically monitoring of plasma felbamate concentration.

Indexing Terms: monitoring therapy/anticonvulsant drugs/pediatric chemistry/chromatography, capillary

Felbamate, 2-phenyl-1,3-propanediol dicarbamate, a newly introduced antiepileptic agent (1), decreases the frequency of partial and generalized seizures (2, 3). It also appears to be effective in treating Lennox–Gastaut syndrome (4), a form of early childhood epilepsy characterized by myoclonic and atypical absence seizures, generalized spike-wave discharges at a rate of 2/s, and regression in intellectual functions. Although the exact mechanism by which felbamate exerts its anticonvulsant activity is currently uncertain, it appears from animal studies that this drug has properties similar to existing anticonvulsants (5, 6).

Felbamate can be used either alone or in combination with other antiepileptics (7, 8). However, this drug can exert substantial effects on other anticonvulsants (9–11): Coadministration with phenytoin or valproate increases their steady-state plasma concentrations by as much as 47% and 23%, respectively; coadministration with carbamazepine decreases the plasma concentration of carbamazepine by ≤30% and increases carbamazepine epoxide by ≤57%. In contrast, phentoin and carbamazepine increase the clearance of felbamate at steady-state by ~100% and ~50%, respectively. The complex interactions between the antiepileptic agents create a particular challenge to clinicians in terms of dosage adjustment.

Therapeutic target concentrations for felbamate have not yet been clearly established in children. In clinically controlled adults, felbamate concentrations of 40–90 mg/L were seen 3.3–4.6 h after an oral dose (7).

Methods for measuring felbamate by HPLC have been reported (12, 13). Here, we describe a gas-chromatographic method for determining the plasma felbamate concentration by using a wide-bore capillary column, a flame ionization detector, and a simple extraction procedure.

Materials and Methods

Reagents

Felbamate was donated by Wallace Laboratories, Cranbury, NJ. The internal standard, 5-methylphenyl-5-phenylhydantoin (MPPH), was obtained from Aldrich Chemical Co., Milwaukee, WI; n-butyl acetate, from Eastman Kodak, Rochester, NY. Hydrochloric acid and methylene chloride were purchased from Fisher Scientific, Fair Lawn, NJ. For the interference study, brompheniramine, chlorpheniramine, dextromethorphan, phenylpropanolamine, acetaminophen, caffeine, acetylsalicylic acid, and ibuprofen were obtained from Sigma Chemical Co., St. Louis, MO; carbamazepine, phenytoin, and 5-ethyl-5-(4-hydroxyphenyl)-barbituric acid were from Aldrich; carbamazepine-10,11-epoxide was a gift from Roche Diagnostic Systems, Branchberg, NJ; phenobarbital, ethosuximide, methylsuccinimide, phenoxime, mephentoin, methaximide, phenacemide, mephabarbital, mephobarbital, paramethadione, trimethadione, and clonazepam were from United States Pharmacopeial Convention, Rockville, MD; valproic acid and primidone were from ICN Biochemicals, Cleveland, OH; and theophylline was from Pfaltz & Bauer, Stamford, CT.

Calibrators (25, 100, and 200 mg/L) and controls (50 and 150 mg/L) were prepared by supplementing filtered plasma with methanol-based stock felbamate solution (10.0 g/L). Calibrators and controls were aliquoted and stored at −70°C until use. The extraction mixture was prepared by adding 400 μL of MPPH (16 g/L), the internal standard, to 1 L of methylene chloride. The internal standard solution was stored at −70°C. The extraction mixture is stable in amber glass at room temperature for at least 1 month.

Apparatus

We used a Sigma 2000 gas chromatograph with a flame ionization detector and PE Nelson 1020 chroma-
tography data station (Perkin-Elmer, Norwalk, CT). A 15-m wide-bore (0.546 mm (i.d.)) capillary column with a permanently bonded, nonpolar stationary phase (DB-1701, 7% cyanopropyl, 7% phenyl polysiloxane, film thickness 1.0 μm) from J & W Scientific, Folsom, CA, was installed in the chromatograph. To evaporate the extraction solution, we used an N-Evap Analytical Evaporator (Organomation Associates, Northborough, MA).

Procedures
Add 100 μL each of patient's serum or plasma, calibrators, and controls to an extraction tube containing 20 μL of HCl (3 mol/L), vortex-mix, and then add a 2-mL aliquot of the extraction mixture (methylene chloride containing the internal standard) to each tube. Vortex-mix for 1 min and centrifuge the samples at 2000g for 10 min. After separating and evaporating the organic layers to dryness under nitrogen in a water bath at 30°C for 5 min (we used the N-Evap system), add 25 μL of n-butyl acetate to each tube. Again vortex-mix, and inject 1.0 μL into the gas chromatograph. The temperatures of the oven, injector, and detector should be 225, 250, and 265°C, respectively; the helium carrier gas flow 10 mL/min; and the air and hydrogen pressures for the detector 138 kPa each.

Results and Discussion
The absolute retention time of felbamate was 2.8 min. Fig. 1 shows a typical chromatogram of an extracted patient's specimen containing felbamate and the internal standard. This chromatogram shows the good resolution obtained with the 0.546 mm (i.d.) capillary column. The use of a 15-m wide-bore column combines the efficiency attainable with a typical 30-m narrow-bore (0.28 mm (i.d.)) capillary column (~50 000 theoretical plates) with a capacity approaching that of a typical 180-cm packed column (10–15 μg per component). Thus, excellent resolution can be obtained while avoiding problems associated with column overloading (shifts in retention time and deviation from linearity) (14). In addition, the use of a covalently bound, nonpolar stationary phase results in improved thermal stability and lower resistance to carrier gas. Higher flow rates can therefore be used, allowing shorter analysis times.

The calibration curve, with felbamate concentrations of 25–200 mg/L, was linear (slope = 1.025, r = 1.00, n = 23) and reproducible: mean CV for the calibrators = 4.11% (n = 23). Day-to-day precision was determined by assaying 20 replicates of quality-control materials with two different drug concentrations, using a newly extracted set of calibrators to calibrate the assay each day. For drug concentrations of 50 and 150 mg/L, the CV was 5.2% and 3.6%, respectively. A recovery study was performed after supplementing drug-free plasma with known amounts of the purified drug; we assayed 15 replicates for each concentration, except for the 10 mg/L concentration, for which only 5 replicates were assayed. At felbamate concentrations of 10, 25, 100, and 200 mg/L, the assay recoveries were of 91%, 87%, 100%, and 99%, respectively. The assay detection limit often was 5 mg/L, and the calibration curve was linear to 300 mg/L. These limits of analytical range more than encompass the felbamate concentration range encountered in our patients.

To further validate the accuracy of this assay, we conducted an interference study, using 27 commonly prescribed medications and antiepileptic agents (Table 1). Of the drugs tested, nine were extracted and detectable in this assay. However, none of these drugs had a similar retention time to felbamate. Mephobarbital, carbamazepine-10,11-epoxide, brompheniramine, dextromethorphan, phenobarbital, barbituric acid, and

![Fig. 1. Typical chromatogram of an extracted plasma felbamate sample: 1, felbamate; 2, internal standard (MPPH).](image)

**Table 1. Drugs in the interference study.**

<table>
<thead>
<tr>
<th>Antiepileptics</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barbituric acid (1.99)*</td>
<td>Acetaminophen</td>
</tr>
<tr>
<td>Carbamazepine (4.51)*</td>
<td>Acetylsalicylic acid</td>
</tr>
<tr>
<td>Carbamazepine-10,11-epoxide (1.26)*</td>
<td>Brompheniramine (1.30)*</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>Caffeine</td>
</tr>
<tr>
<td>Ethosuximide</td>
<td>Chlorpheniramine</td>
</tr>
<tr>
<td>Mephenytoin</td>
<td>Ibufrofen</td>
</tr>
<tr>
<td>Mephobarbital (0.98)*</td>
<td>Phenyipropanolamine</td>
</tr>
<tr>
<td>Methadone</td>
<td>Theophylline (2.01)*</td>
</tr>
<tr>
<td>Methsuximide</td>
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<tr>
<td>Methysuccinimide</td>
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<tr>
<td>Paramethadione</td>
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<tr>
<td>Phencemide</td>
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<tr>
<td>Phenobarbital (1.58)*</td>
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<tr>
<td>Phensuximide</td>
<td></td>
</tr>
<tr>
<td>Phenytoin (6.37)*</td>
<td></td>
</tr>
<tr>
<td>Primidone (5.26)*</td>
<td></td>
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<tr>
<td>Trimethadione</td>
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<tr>
<td>Valproic acid</td>
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* Drugs coextracted with felbamate (2.80). Retention time (min) is indicated in parentheses.
theophylline were eluted before felbamate (2.80 min), whereas carbamazepine, primidone, and phenytoin appeared between felbamate and the internal standard (8.30 min).

At present, the concentrations of antiepileptic agents, including felbamate, are determined in our laboratory by this chromatographic assay. A typical chromatogram of our antiepileptic panel is shown in Fig. 2. This assay can be assumed to be free of interference from several hundred other drugs that would not be coextracted under the conditions reported here or would have significantly different retention times.

Serum and plasma specimens, regardless of the type of anticoagulant used, are suitable for felbamate analysis. However, if specimen is collected in a red-top Vacutainer Tube (Becton Dickinson; Franklin Lakes, NJ), an interfering peak might appear just after the internal standard. Although the exact nature of this interfering peak is unknown, it probably results from the presence of a plasticizer. By increasing the separation time from 10 to 12 min or increasing the column temperature from 225 to 255°C (15°C/min) 3 min after initiating the separation, we could completely elute the interfering peak and alleviate this problem. The advantage of using the temperature program is the ability to keep the total separation time to 10 min.

A stability study was conducted to determine the adequate short-term storage conditions of plasma specimens. Felbamate concentration was determined in five plasma samples supplemented with the purified drug. Samples were then aliquoted and stored at 4, −20, or −78°C for up to 1 month. Our data indicate no change in plasma felbamate concentrations in samples stored at either temperature.

Our experience with this drug indicates that a relatively wide range of concentrations are tolerated. The drug concentrations measured in 66 pediatric patients ranged from 7 to 154 mg/L (mean ± SD 44 ± 24.7). However, some patients manifest certain adverse effects even at low concentrations. Of 32 patients on polytherapy being closely monitored for adverse effects and seizure frequency, 16 developed at least one adverse reaction: Anorexia, weight loss, and insomnia were the effects most commonly seen. In some patients, the occurrence of these side effects diminishes with the reduction of plasma felbamate concentrations. In others, however, adverse reactions persist, even when felbamate concentrations are low. For example, one 7-year-old patient with Lennox–Gastaut syndrome developed significant insomnia and irritability at a felbamate concentration of 55 mg/L. Despite the fact that he had been seizure free, his felbamate dose had to be reduced to decrease the severity of the side effects. The insomnia then decreased and became tolerable; however, the seizures recurred, so the felbamate was eventually discontinued and replaced by other antiepileptic agents. Other children did not exhibit any noted side effects, even at felbamate concentrations >75 mg/L.

Based on our limited experience with this drug, our guidelines currently include determining felbamate concentration once a stable dosing regimen has been achieved and periodically thereafter to monitor patient’s compliance. Many of the children on polytherapy under age 10 years have initial felbamate concentrations of 10–20 mg/L and are presumably receiving therapeutic doses of this drug. So when the original concentration is <25 mg/L, the dose is usually increased to achieve a higher plasma value. It remains to be seen, however, whether this concentration represents the lower limit of the therapeutic range in children. We do not believe the current available data allow for the determination of a definite upper limit for the therapeutic range in children. If seizures are not controlled, we titrate the felbamate dose upward until control of seizures is achieved or adverse reactions prevent further increases.

In conclusion, this assay combines the durability, capacity, efficiency, and speed of analysis of wide-bore capillary gas chromatography with a simple extraction procedure. The combination of these features has enabled the development of an accurate, precise, sensitive, and ideally suited method for the therapeutic monitoring of plasma felbamate concentration.

This work was supported in part by the Wark Epilepsy Research Fund.

References