Determination of Gabapentin in Serum Using Solid-Phase Extraction and Gas-Liquid Chromatography

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Abstract

A gas-liquid chromatographic method for the determination of gabapentin (Neurontin®) is described. The method involves extracting 0.5 mL of acidified sample by C18 solid-phase column, derivatization with MTBSTFA plus 1% tBDMCS, and analysis on an HP-1 column with a flame-ionization detector. Quantitation was performed with peak-height ratios of gabapentin to a gabapentin analogue [(1-aminomethyl-1-cycloheptyl) acetic acid] as the internal standard. The assay had a limit of detection of 0.2 mg/L and a linear range from 0.5 to 30.0 mg/L. Several compounds were analyzed for potential interference, and none interfered with the assay.

Introduction

Gabapentin [(1-aminomethyl-1-cyclohexyl) acetic acid] is an anticonvulsant agent prescribed alone or in combination with other anticonvulsant drugs in the treatment of adults and children with epilepsy. It is structurally similar to γ-aminobutyric acid (GABA), but does not interact with GABA receptors. It is not converted to GABA, nor does it inhibit GABA uptake or degradation. The mechanism by which gabapentin exerts its anticonvulsant activity is presently unknown (1). The pharmacokinetic profile of gabapentin includes rapid absorption following oral administration, an apparent volume of distribution of 58 L, and a plasma half-life of 5–7 h with elimination of up to 81% of the dose in urine as the parent drug in 96 h (2–5).

Several chromatographic methods for the determination of gabapentin in serum have been previously reported (6–8). Gabapentin is a zwitterion; hence, these methods involve labor-intensive extractions and cleanup steps prior to chromatographic analysis. High-performance liquid chromatographic (HPLC) methods require derivatization of gabapentin to ultraviolet absorbing chromophores (6,7). The gas-liquid chromatographic (GLC) method involves separate derivatizations of each functional group of the zwitterion (8).

We present a rapid GLC method for the determination of gabapentin in serum by solid-phase extraction followed by a single step derivatization of both functional groups of the zwitterion prior to capillary GLC analysis.

Materials and Methods

Reagents and standards

Gabapentin and a structural analogue (1-aminomethyl cycloheptyl acetic acid), the internal standard, were provided by Parke Davis Pharmaceutical Research (Ann Arbor, MI). One-milliliter C18 Clean Up® extraction columns (CUC18111) were provided by United Chemical Technologies (Bristol, PA). Ammonium hydroxide, glacial acetic acid, and hydrochloric acid were ACS reagent grade. Ethyl acetate, hexane, and methanol were GLC grade. The derivatizing reagent, n-methyl-n-t-butyldimethylsilyl trifluoroacetamide plus 1% /-butyldimethylsilane (MTBSTFA + 1% tBDMCS) was purchased from Regis Chemical (Morton Grove, IL).

Stock solutions of gabapentin and the internal standard (1 mg/mL) were prepared by dissolving 10.0 mg of each into separate 10-mL volumetric flasks with deionized water. Gabapentin plasma calibrator concentrations ranging from 0.2 to 30.0 mg/L were prepared by adding the appropriate aliquots of stock gabapentin to 0.5 mL drug-free serum.

Aqueous solutions of gabapentin and internal standard were stored at 4°C and had a 1-year stability period. Because of the nonavailability of commercial controls, in-house gabapentin controls of 1.1 and 4.5 mg/L in drug-free serum were prepared. These controls were stored frozen at −15°C and were stable for six months.

Sample preparation and derivatization

Five hundred microliters of sample, calibrator, or control was placed into a 10- x 75-mm disposable glass test tube, and 25 µL of internal standard (5.0 mg/L) was added. The tube was vortex mixed briefly. Then 500 µL of 20% acetic acid was added and the tube vortex mixed briefly again. The extraction column was prepared by sequential addition of one column

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volume of the following: methanol, deionized water, and 0.1N HCl. The sample was passed through the column, and washed with the sequential addition of one column volume of deionized water, ethyl acetate, and hexane. The vacuum was maintained at least less than 3 in. Hg, and increased for 30 s to remove excess water and dry the column before elution. Gabapentin and the internal standard were eluted from the column using 1.0 mL 2% ammonium hydroxide in methanol. The eluate was evaporated to dryness in a 40°C water bath under a constant stream of air.

MTBSTFA plus 1% tBDMCS reagent (50 µL) was added to the residues for derivatization (Figure 1). The tubes were capped and placed into a water bath at 70°C for 30 min. The tubes were removed and allowed to cool for 5–10 min. The resultant extracts were transferred to autosampler vials or injected manually into the gas chromatograph (GC) for analysis.

GLC

Chromatographic analysis was performed with a Hewlett Packard 5880A GC equipped with a flame ionization detector (FID) and 7673A autosampler (Hewlett-Packard, Avondale, PA). The column was a 10 m x 0.53-mm internal diameter, 0.5-µm thickness, 100% methyl silicone megabore capillary column. The carrier gas was helium at a flow rate of 16.4 mL/min, and the detector gases were hydrogen at 30 mL/min and air at 300 mL/min. The column oven temperature was initially set at 170°C for 0.1 min, then programmed at 10°C/min to 240°C, then at 20°C/min to 280°C, and held for 0.5 min. The injector and detector temperatures were 235 and 300°C, respectively. Under these conditions, the retention times of gabapentin and the internal standard were 4.30 and 5.08 min, respectively (Figure 2). Quantitation was performed using linear regression extrapolation of a four-point calibration curve (0, 1.0, 5.0, and 20.0 mg/L gabapentin) using the peak-height ratios of gabapentin to internal standard.

Assay validation

Linearity of the assay was verified on three separate days by the analysis of gabapentin spiked into serum at 0.0, 0.2, 0.5, 1.0, 5.0, 10.0, 20.0, and 30.0 mg/L. Within-run precision was determined by repetitive analyses of the low- and high-control samples (n = 10, each). Between-run precision was determined over 16 days using these two controls. The recoveries of gabapentin and the internal standard were determined by comparing peak heights obtained from extracted aliquots of each drug in drug-free serum with those of unextracted aliquots. The specificity and potential interferences of the assay were determined by analyzing a commercial control that contained over 40 drugs at therapeutic concentrations (chemTRAK®-TDM,

Figure 1. Chemical structure of gabapentin and the internal standard before and after derivatization.
Medical Analysis Systems, Carmarillo, CA). Additionally, p- 
amino benzoic acid, γ-hydroxy butyrate, and m-hydroxy benzoic 
acid were analyzed for possible interference with the assay.

**Results and Discussion**

The assay for gabapentin in serum had a limit of detection 
(LOD) of 0.2 mg/L, a limit of quantitation (LOQ) of 0.5 mg/L, 
and was linear up to 30 mg/L. Calibrators spiked at 0.0, 0.2, 0.5, 
1.0, 5.0, 10.0, 20.0, and 30.0 mg/L gabapentin were analyzed 
one per day for three days. The upper limit of linearity was 
determined by the highest concentration calculated from the 
linear regression of the calibrators that was within 10% of the 
target value. LOQ was determined by the lowest concentration 
calculated that was within 10% of the target value. LOD was de- 
termined as the calibrator that was less than the LOQ, but 
could be quantitated and differentiated from the 0.0 calibrator. 
Gabapentin serum concentrations following therapeutic 
administration range from 2 to 10 mg/L (2).

Within-run precisions for control specimens containing 1.1 
and 4.5 mg/L were 7.0% (n = 10) and 3.4% (n = 10), respec- 
tively. These same control specimens analyzed over three weeks 
yielded between-run coefficients of variance of 12% (n = 16) and 
5.0% (n = 16), respectively. The absolute recoveries determined 
by analysis in triplicate serum specimens of gabapentin at 1.0, 
5.0, and 20 mg/L were 46, 57, and 51%, respectively. The abso- 
lute recovery of the internal standard was 69% (n = 9). Drugs 
commonly indicated for therapeutic monitoring and other 
serum constituents were found not to interfere with the

| Table I. Compounds Found Not to Interfere with 
| Gabapentin Assay |
| Acetaminophen | Amikacin |
| Amylase | Amitriptyline |
| Benzoylcyonine | BUN* |
| Caffeine | Carbamazepine |
| Chloramphenicol | Cholesterol |
| Creatinine | Cyclosporine |
| Digoxin | Disopyramide |
| Estradiol | Ethosuximide |
| γ-Hydroxy butyrate | Gentamicin |
| Glucose | hCG |
| LDH | Lidocaine |
| Lithium | Methotrexate |
| m-Hydroxy benzoic acid | NAPA |
| Netilmicin | Nortriptyline |
| p-Amino benzoic acid (PABA) | Phenobarbital |
| Phenytoin | Primidone |
| Propranolol | Quinidine |
| Salicylate | Theophylline |
| Thyroxine | Tobramycin |
| Triiodothyronine | Triglycerides |
| Uric acid | Valproic acid |

* Abbreviations: BUN, blood urea nitrogen; HCG, human chorionic gonado- 
trophin; LDH, lactate dehydrogenase; NAPA, n-acetyl procainamide.
extraction and chromatographic analysis of gabapentin and the internal standard (Table I). This was determined by extracting a serum-based drug mixture and observing no interfering substances in the chromatography.

Because gabapentin exists primarily as a zwitterion, it is difficult to obtain clean extracts from biological specimens without labor-intensive extractions. To address this problem, we adjusted the pH of the serum samples in order to suppress ionization of the carboxylic acid group. Presumably, this increased the proportion of drug present in the sample that attaches to the C18 column sorbent. The column was washed with water, ethyl acetate, and hexane. Ethyl acetate and hexane were used for washing because of gabapentin's lack of solubility in these solvents. The washing step also removed any potential interferes that may have been soluble in ethyl acetate and hexane. We eluted at an alkaline pH, thereby ionizing the acidic portion of the molecule and freeing gabapentin from the column sorbent. Gabapentin did not elute well from the column when only methanol was used. This was verified by collecting the column eluates after each washing and analyzing each eluate for gabapentin. The addition of 1–2% ammonium hydroxide to the methanol was sufficient to elute a consistent measurable amount of gabapentin. Increasing the concentration of ammonium hydroxide in the methanol elution solvent above 2% did not improve recovery.

We also evaluated underivatized gabapentin on our methylsilicone column and found it yielded a broad-tailing peak, which prevented acceptable gabapentin quantitation. Regisil® plus 10% TMCS was also evaluated as a potential derivatizing agent. The trimethylsilane-gabapentin derivative was not resolved from several other analytes listed in Table I. MTBSTFA reagent will harm the rubidium bead of a nitrogen-phosphorus detector (NPD); therefore, this method should not be attempted with a NPD system. The MTBSTFA derivatives increased the molecular weight of gabapentin and the internal standard, hence their increased retention times resolved them from potential interferences tested. Several incubation times were evaluated for completeness of MTBSTFA derivatization. The times evaluated were 30, 45, and 60 min. No difference in peak heights for either gabapentin or the internal standard was observed in the times tested. Once formed, the MTBSTFA-gabapentin derivative was very stable, and samples re-injected the day after preparation yielded less than 10% loss in response.

Conclusion
The presented GLC method for the determination of gabapentin in serum was sufficiently sensitive and reliable for routine therapeutic monitoring. The method was relatively simple and rapid to perform, requiring solid-phase extraction and one derivatization step prior to chromatography. The ease of extraction and stability of the gabapentin derivative permit the extraction, derivatization, and simultaneous analysis of a large number of serum specimens.

Acknowledgment
The authors wish to thank Lisa O'Dell and United Chemical Technologies for their helpful suggestions and for supplying the solid-phase extraction columns.

References