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Note

High-performance liquid chromatographic assay for vigabatrin and its primary degradation product in a pharmaceutical tablet formulation

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γ-Aminobutyric acid (GABA) has been established as a major inhibitory neurotransmitter in the central nervous system[1,2]. The defficiency of GABA in the brain has been inplicated to cause neurological and psychiatric disease, *e.g.*, Huntington's disease[3], tardive dyskinesia[4], parkinsonism[5], schizophrenia[6], and epilepsy[7].

Vigabatrin (γ-vinyl-γ-aminobutyric acid) (Fig. 1) is the first antiepileptic drug developed from current knowledge about the pathophysicology of epilepsy. Compared with the previous antiepileptic drugs, vigabatrin is more selective and less toxic and therefore has more therapeutic benefit[8–11]. Although the analysis of vigabratrin in biological fluids has been published[12,13], the assay of this drug in dosage forms has not been reported. This paper describes a high-performance liquid chromatographic (HPLC) produce to determine vigabatrin and its primary degradation product, 5-ethenyl-2-pyrrolidinone (MDL 17 637), in a pharmaceutical tablet formulation consisting of polyvinylpyrrolidinone (PVP) and other common excipients.

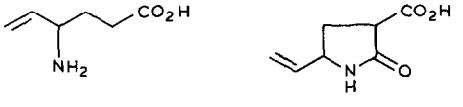

Fig. 1. The structure of (a) vigabatrin and (b) its primary degradation product 5-ethenyl-2-pyrrolidinone.

EXPERIMENTAL

Materials

Vigabatrin 500-mg tablets, placebo tablet mixtures, vigabatrin and 5-ethenyl-2-pyrrolidinone (MDL 17 637) were prepared at the Merrell Dow Research Institute. Acetonitrile, methanol and water were HPLC grade purchased from J. T. Baker (Phillipsburg, NJ, U.S.A.). Other reagents used were commercial reagent grade. The filter media used was a Whatman (Hillsboro, OR, U.S.A.) GF/F filter.

Apparatus and HPLC conditions

The HPLC system consisted of a Beckman (Berkeley, CA, U.S.A.) 344 gradient

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liquid chromatograph, a Micromeritics (Norcross, GA, U.S.A.) 725 autoinjector, a Valco (Houston, TX, U.S.A.) AH 60 sample valve equipped with a 20 μl sample loop, and a Whatman Partisil SCX column (10 μm particle size, 25 cm × 4.6 mm I.D.).

The mobile phase was 0.025 *M* potassium phosphate buffer (pH 2.8)–methanol–acetonitrile (1000:40:4) pumped at a flow-rate of 1.5 ml/min. Effluents were monitored at 210 nm using a Beckman 165 variable-wavelength detector. Chromatograms were recorded and integrated by an automated laboratory data system (Computer Inquiry Systems, Englewood Cliffs, NJ, U.S.A.).

Standard and sample preparations

Standard. About 100 mg of a vigabatrin standard were accurately weighed into a 50-ml volumetric flask, then dissolved in and diluted to volume with mobile phase.

Tablet samples. A representative sample, consisting of twenty tablets, was weighed to calculate the average tablet weight. The sample was ground in a glass mortar to a fine powder. An accurately weighed portion of the powder, equivalent to one tablet, was transferred to a 100-ml volumetric flask. About 90 ml of mobile phase were added to the flask and the sample solution was stirred for 10 min. The samples solution was then bropught to volume with mobile phase. After thorough mixing, about 15 ml of the sample solution were filtered through a Whatman GF/F filter. A 10-ml aliquot of the filtrate was pipetted into a 25-ml volumetric flask and diluted to volume with mobile phase. The normal concentration of vigabatrin in this final solution is about 2 mg/ml.

Procedure

The HPLC system was equilibrated using the conditions previously described. The standard solution was injected and the chromatogram was recorded for about 10 min. Similarly, the sample solution was injected and the chromatogram was recorded. The result for vigabatrin in the tablet was calculated by comparison of the peak areas obtained from the sample and standard chromatogram. The amount of MDL 17 637 present in the samples was calculated as mg per tablet after correction of the peak area using a response factor of 44 that was predetermined for this system.

RESULTS AND DISCUSSION

Fig. 2 illustrates a typical chromatogram of vigabatrin tablets assayed by the described procedure. A small peak that eluted in about 4 min appeared to be the only possible source of interference. It was confirmed as coming from the tablet excipient polyvinylpyrrolidinone (PVP). Under the HPLC conditions presented, the potential degradastion product of vigabatrin, MDL 17 637, was adequately separated from vigabatrin and PVP, as shown in Fig. 3. When required for regulatory purposes, a system suitability test ensuring the separation of these three components is included in the procedure.

The relative response factor of MDL 17 637 to vigabatrin was found to be 44 in this system; it was determined by spiking the standard solution of vigabatrin with MDL 17 637 at levels of 0.1, 1.0 and 2.0% (w/w).

This response factor has been found to vary depending on the specifics of the

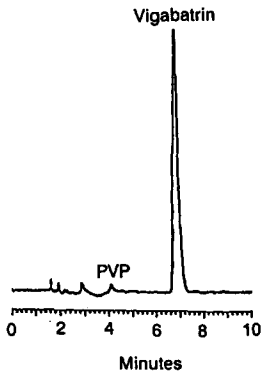

Fig. 2. HPLC chromatogram of vigabatrin tablet.

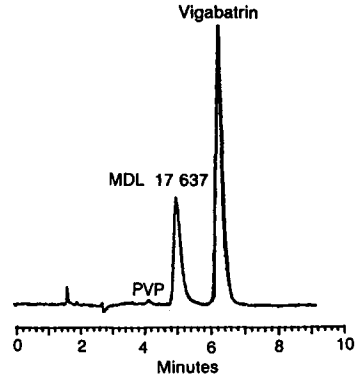

Fig. 3. HPLC chromatogram of vigabatrin tablet spiked with 1% (w/w) of MDL 17 637.

mobile phase, columns, etc., being used. Because the strong MDL 17 637 absorbance is being measured at 210 nm on the steep side of the end absorption band the differences resulting from detector geometry and bandpass size are much larger than normally encountered.

The small amount of acetonitrile (0.4%) used in the mobile phase is merely to improve the peak shape of vigabatrin and MDL 17 637. Varying the methanol ratio in the mobile phase, as expected, will change retention times but will not improve the resolution of the peaks significantly. Decreassing the pH of mobile phase will improve the resolution of vigabatrin and MDL 17 637 slightly, however, it is not recommended because of the negative long term effects on the column.

The response of vigabatrin measured at 210 nm was linear from 20% to 120% of the normal amount injected. The correlation coefficient was 0.99990 at the 95% confidence level. The recovery of vigabatrin from the tablets was determined in duplicate using placebos spiked with vigabatrin at the 80%, 100%, and 120% levels. Each sample was then carried through the assay procedure. The average recovery obtained was 99.8% with a relative standard deviation of 1.4%. The recovery study for MDL 17 637 was done by spiking the synthetic vigabatrin tablets with MDL 17 637 at relative levels of 0.10, 050 and 1.00% (w/w). The average percents found were 0.10, 0.51 and 0.99, respectively.

TABLE I

ASSAY OF VIGABATRIN AND MDL 17 637 IN VIGABATRIN TABLETS STRESSED IN 0.1 *M* SODIUM HYDROXIDE AT 80°C

Time (h)	*Assay value (mg/tablet)*	
	Vigabatrin	*MDL 17 637*
Initial	498	0.2
22	490	7.8
70	443	45.7

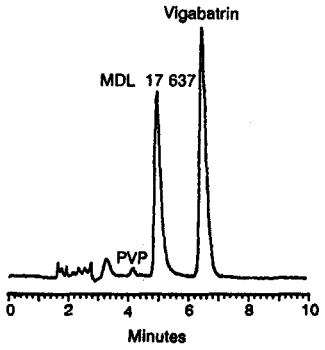

Fig. 4. HPLC chromatogram of vigabatrin tablet stored for 16 weeks at 60°C.

In order to demonstrate the stability indicating ability of the method, the vigabatrin tablets were stressed in 0.1 *M* sodium hydroxide solution. The solution was then stored in a capped bottle at 80°C and periodically assayed for vigabatrin and MDL 17 637 with this method. The results are given in Table I.

Since this method was developed, it has been successfully applied for the analysis of vigabatrin and MDL 17 637 in the tablets used for long-term stability studies. An example of a chromatogram obtained from a tablet stored for 16 weeks at 60°C is shown in Fig. 4. This chromatogram represents a loss of 10.1 mg of vigabatrin and formation of 8.4 mg of MDL 17 637 in the tablet. Due to its specificity and simplicity, this method will also be used for routine analysis in quality assurance for acceptance testing during the production of vigabatrin tablets.

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