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Analysis of the antiepileptic drug keppra by capillary electrophoresis

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Abstract

A simple and rapid method for determination of the new antiepileptic drug keppra (levetiracetam) by capillary electrophoresis in borate buffer containing sodium dodecyl sulfate is described. The serum was injected without any treatment. The method compared well to high performance liquid chromatography. The mean of keppra in the serum of 35 patients was 25 mg/l (range 7–77 mg/l).

Keywords: Pharmaceutical analysis; Keppra; Levetiracetam

1. Introduction

Keppra (levetiracetam) is a new antiepileptic drug indicated as adjunctive therapy, in the treatment of partial onset seizures in adults with epilepsy. It is rapidly absorbed after oral administration. Food does not affect the extent of its bioavailability. Plasma half-life of the medication is approximately 6–8 h but is increased in the elderly and in patients with renal impairment. Steady state pharmacokinetics is achieved after 2 days of twice daily dosing. Keppra is not metabolized by the liver cytochrome P450 and does not affect the induction of this enzyme system. Two-thirds of the dose is excreted unchanged by the kidney with a small amount being metabolized to a carboxylic acid derivative. Thus, there is no active metabolite and it exhibits almost no protein binding (<10% bound). These factors indicate that this drug is unlikely to undergo any significant interactions with other medications and appears suitable for elderly patients and for conditions requiring complex pharmacotherapy [1–3].

In clinical studies, keppra was generally well tolerated by patients. However, it can be associated with the occurrence of central nervous system adverse events classified as somnolence and fatigue, coordination difficulties and behavioral abnormalities [2,3]. In controlled studies, when keppra was given with other antiepileptic drugs, the most frequently reported adverse events were somnolence, asthenia, infection and dizziness [2,4]. Adverse events were usually mild to moderate in intensity. Keppra may also reduce hyperactivity, impulsivity, mood instability, and aggression in autistic children with these problems [5].

Keppra has been analyzed by high performance liquid chromatography (HPLC) [6,7] and gas chromatography (GC) [7]. Both methods required sample clean up before analysis. From the clinical aspects, there is a lack of information about the therapeutic

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level of this drug in serum. Here we describe a simple capillary electrophoresis method without the need for sample extraction or pretreatment for quantification of this drug in serum.

2. Procedure

2.1. CE conditions

Serum was injected directly hydrodynamically for 15 s on an untreated capillary (50 μm×35 cm) (Polymicro Technologies, Scottsdale, AZ, USA). The sample was electrophoresed for 8.5 min in a buffer composed of 140 m*M* boric acid, final pH 8.9, containing 40 g sodium dodecyl sulfate (SDS), and 250 ml methanol/l. Iohexol (250 mg/l in water) can be mixed (1:1) with the sample and used as an internal standard. The capillary was washed for 1 min with 1 mol/l of NaOH followed by 1 min with the electrophoresis buffer between injections. Usually, the first two injections early in the run tend to have long migration time and therefore were omitted.

2.2. Instrument

A Model 2000 CE (Beckman, Fullerton, CA, USA) was set at 10 kV with detection at 214 nm.

2.3. HPLC conditions

For comparison serum samples (100 μl) were mixed with 100 μl of 10% trichloroacetic acid and centrifuged at 14 000 *g* for 15 s. A 20-μl aliquot of the supernatant was injected on a C_{18}, Varian Microsorb-MV column, 5 μm (250 mm×4.6 mm) (Varian, Walnut Creek, CA, USA). The drug was eluted with 10% acetonitrile containing 1 ml/l of phosphoric acid at a flow-rate of 1.0 ml/min with detection at 214 nm, Model 2151 (LKB, Bromma, Sweden).

2.4. Stock standard (1000 mg/l)

Keppra standard (UCB Pharma, Symrna, GA, USA) was prepared in distilled water and diluted further in serum or water to give 100 mg/l.

3. Results

Keppra has a pyrrolidine acetamide chemical structure, which essentially at the pH range used for analysis is uncharged. A micellar electrokinetic capillary chromatography thus was chosen for separating this drug from other compounds in the serum. The micelles of the SDS can solubilize the proteins and thus direct serum injection can be used in the analysis, simplifying the method. The migration time for keppra was about 6.5 min. Some samples contained an unknown interfering peak of varying height that migrated after the keppra peak (Fig. 1). Both the methanol concentration and the pH

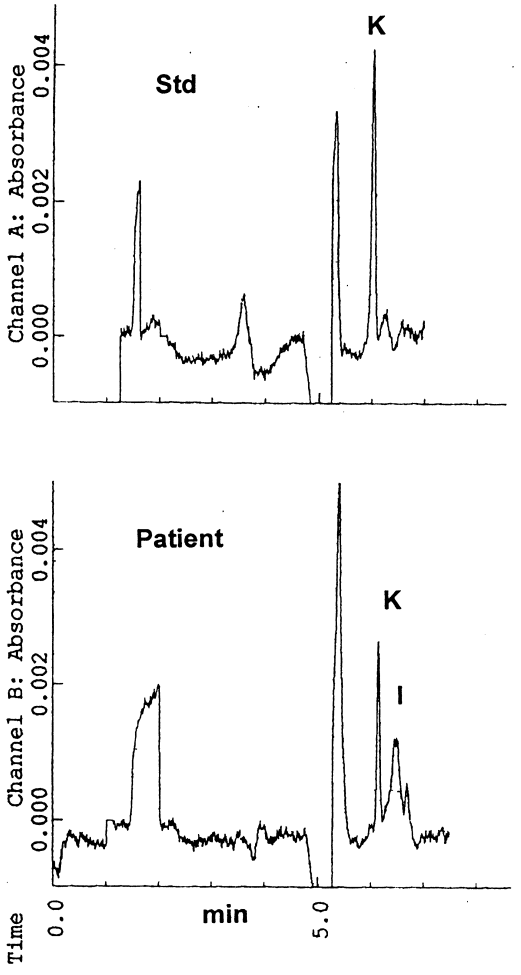

Fig. 1. Keprra (K) analysis by CE from a standard in serum (100 mg/l) and a patient 48 mg/l (I=interferences).

are important in separating this interference from the keppra. At pH values lower than 8.8 the unknown interfering peak migrated very close to the keppra. There is also a slight negative peak before the keppra peak, the source of this is unknown; however, it does not interfere or affect the calculation. On the other hand, it can be helpful as a marker. The test was linear between 10 and 100 mg/l. The minimum detection level at 214 nm was ~3 mg/l. This drug does not have a maximum for light absorption. However, it has three times more absorbance at 200 nm when compared to that at 214 nm. The latter wavelength was chosen because it is more common on most instruments. The RSD was 0.5% for migration time and 3.8% for peak height ($n=18$, mean=20 mg/l). Recovery in serum was about 96% compared to aqueous solutions. There was a slight difference in the migration time between the aqueous and serum based standards. In order to avoid this problem the stock standard was diluted in serum free from this drug for both the CE and the HPLC methods (as discussed later). None of the common drugs such as phenobarbital, phenytoin or carbamazepine interfered with the analysis. Iohexol can be added to the serum and used as internal standard. It elutes after the keppra peak. However, we did not find any advantage of including it to the method other than slowing the analysis time.

The blood therapeutic level of this drug has not been established. We found in this study that the mean serum level for 35 patients was 25 mg/l (range 7–77 mg/l). Patients in general tolerated this drug well with few minor side effects, mostly fatigue, sedation and impaired cognition.

This CE method was compared to a simple HPLC method based on serum protein deproteinization with trichloroacetic acid (Fig. 2). This procedure is simpler than methods requiring sample extraction [6,7]. CE was slightly faster than HPLC because a few interfering substances that eluted later slowed the HPLC method. The CE method is simpler than that of HPLC since no sample pretreatment was involved, and it was also much less expensive to operate. The regression of the CE to HPLC method is good (CE=0.987 HPLC+2.36, $r=0.98$). The HPLC has better signal to noise than the CE. However, because of interfering substances, the detection limits were similar in both the HPLC and CE methods. Much lower detection levels in both methods can be achieved if the sample was extracted and concentrated before injection and a lower wavelength chosen for analysis. However, both the described CE and HPLC methods have enough sensitivity for clinical routine use.

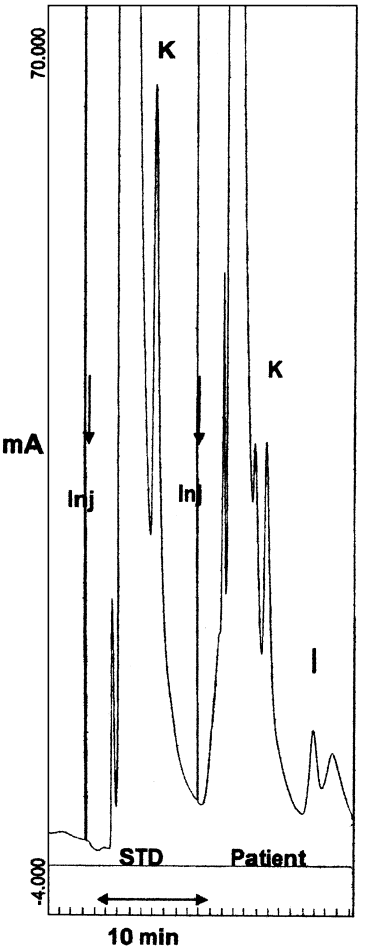

Fig. 2. Keppra (K) analysis by HPLC from a standard (100 mg/l) and a patient (48 mg/l) (I=interferences).

4. Conclusions

Some compounds tend to be more suitable for a particular type of analysis. Keppra is a good example of that. Because the serum level of keppra is relatively high it is well suited for analysis by the

CE. The MECC utilized here for the separation is well suited for direct serum injection, which leads to simplifying the analysis. Sample extraction is not well suited for routine analysis. In conclusion, the described method is rapid, simple and suitable for routine analysis since it does not require sample extraction or pretreatment for measuring the level of this new antiepileptic drug in serum.

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