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Short communication

Analysis of ibuprofen in serum by capillary electrophoresis

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Abstract

A rapid method for analysis of the analgesic drug ibuprofen in serum by capillary zone electrophoresis in a borate buffer 160 mmol/l pH 8.5 is described. The method involves deproteinization with acetonitrile to remove serum proteins followed by direct injection on the capillary. The recoveries of standards added to the serum were 84–92%. The method is suited for analysis of samples with concentrations >10 mg/l. Many other analgesics such as ketoprofen, daypro and salicylates can also be determined by this method.

Keywords: Ibuprofen

1. Introduction

Ibuprofen is a drug commonly available over the counter as an analgesic. It has a wide therapeutic window of 10–50 mg/l and a toxic level of >100 mg/l. Usually, monitoring the level of ibuprofen is not necessary. However, recently it has been shown that high doses, resulting in serum concentrations of 50–100 mg/l, which are close to the toxic level, can decrease pulmonary complications in patients with cystic fibrosis [1]. Under these conditions monitoring of the serum level becomes important [1].

Several methods based on HPLC have been used for measuring the level of this drug in serum [2–4]. These methods require, for drug elution, high concentrations of organic solvents in the mobile phase which are expensive and environmentally hazardous. Here we describe a simple and rapid method for measuring the level of this drug in serum by capillary electrophoresis (CE) based on sample deproteinization with acetonitrile. The method can be extended for measuring other drugs in serum too.

2. Experimental

2.1. Instrument

A Model 2000 capillary electrophoresis instrument (Beckman Instruments, Fullerton, CA, USA) was set at 17 kV, 24°C and 214 nm. The capillary was 60 cm×50 μm (I.D.) coiled in a cartridge with an aperture of 50×200 μm, in absence of the inner spool. A new capillary was washed for 30 min with 0.2 *M* NaOH, followed by 5 min with water. Between samples, the capillary was rinsed for 2 min with 0.2 *M* NaOH, 1 min with water and for 2 min with the electrophoresis buffer boric acid (160 m*M*) adjusted to pH 8.5 with sodium hydroxide (2 *M*). Samples were introduced by pressure injection for 20 s.

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2.2. Method

Serum, or standard, 50 μl, was vortex-mixed for 30 s with 100 μl acetonitrile containing an internal standard (3-isobutyl-1-methylxanthine), 30 mg/l. The mixture was centrifuged for 1 min at 14 000 *g* and the supernatant was injected into the capillary.

2.3. Chemicals

Ibuprofen was obtained from Sigma (St. Louis, MO, USA), and 3-isobutyl-1-methylxanthine from Aldrich (Milwaukee, WI, USA).

3. Results and discussion

Ibuprofen has a maximum light absorption around 221 nm. A filter at 214 nm gave satisfactory sensitivity and specificity. The separation of ibuprofen from other analgesics is illustrated in Fig. 1. Ketoprofen, another new over the counter analgesic, migrates slightly ahead of ibuprofen at 5.6 min. All these drugs can essentially be measured by this method or its modification. Acetaminophen and salicylates are measured more conveniently in clinical laboratories by immunoassays and color reactions, respectively. However, for daypro, keto-

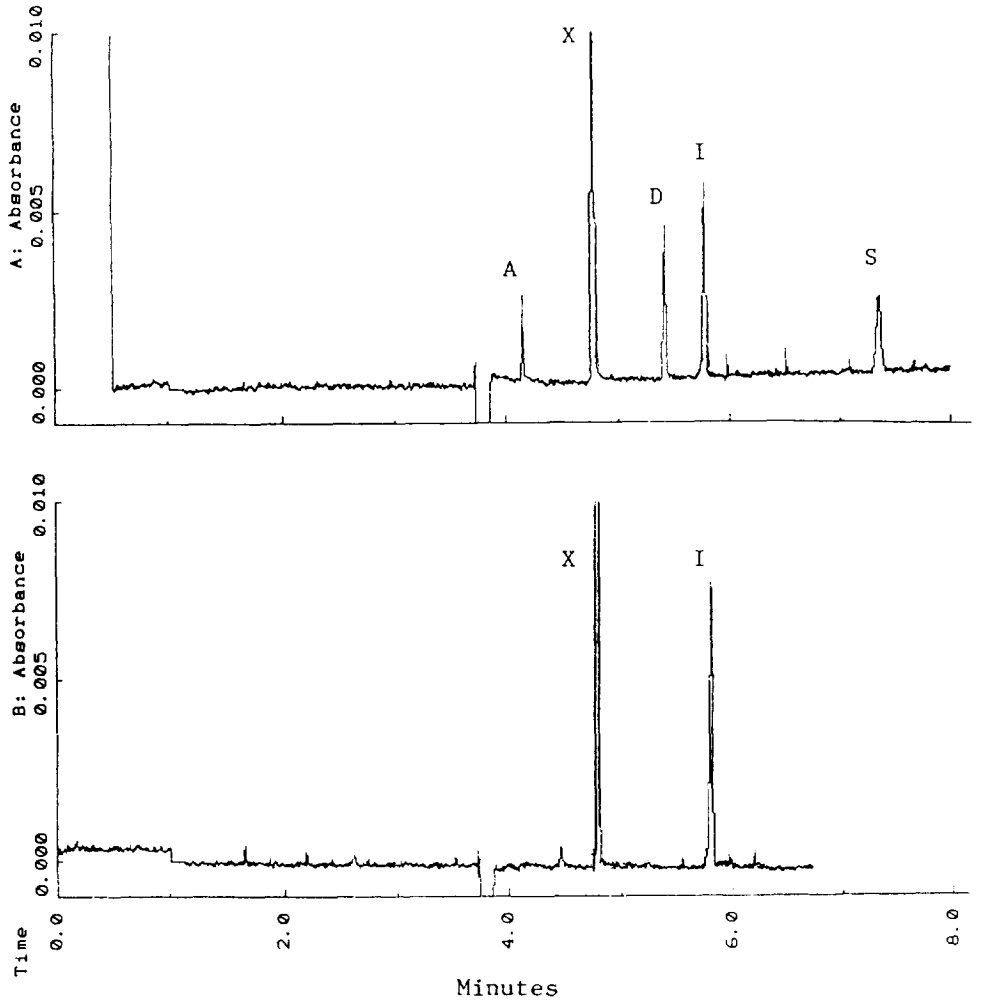

Fig. 1. Separation of ibuprofen and a few other analgesic drugs in 1% sodium chloride. (Top) A=acetaminophen, 10 mg/l; X=internal standard; D=daypro, 20 mg/l; I=ibuprofen, 50 mg/l; S=salicylate; 10 mg/l. (Bottom) Ibuprofen standard 75 mg/l, (I=ibuprofen; X=internal standard).

profen and ibuprofen no immunoassays are yet available and CE offers a good method for their analysis. Fig. 2 illustrates the detector response to a sample from a patient receiving this drug. The separation time is less than 10 min.

The method was linear over the range 8–120 mg/l (conc. mg/l=mA ×0.52+0.44, r=0.99). Acetonitrile treatment almost completely eliminates proteins and allows for a larger sample volume to be injected on the capillary leading to a better sensitivity [5]. The average recoveries of 23, 46 and 60 mg/l added to serum were 84, 88 and 85%, respectively, relative to standards prepared in 1% sodium chloride (n=3). The average recovery of standards at 23 and 46 mg/l added to the serum versus that added to the protein filtrate was 90% and 92% respectively, indicating that a small amount of the drug co-precipitates with the protein. To avoid differences in the recovery due to sample matrix, the standards were prepared in serum free from this drug [5,7]. The lowest detection limit was 8 mg/l (5 times baseline noise). The use of acetonitrile allows a larger volume of sample (up to 50%) to be injected on the capillary with an increase in sensitivity as described earlier [6,8]. However, in

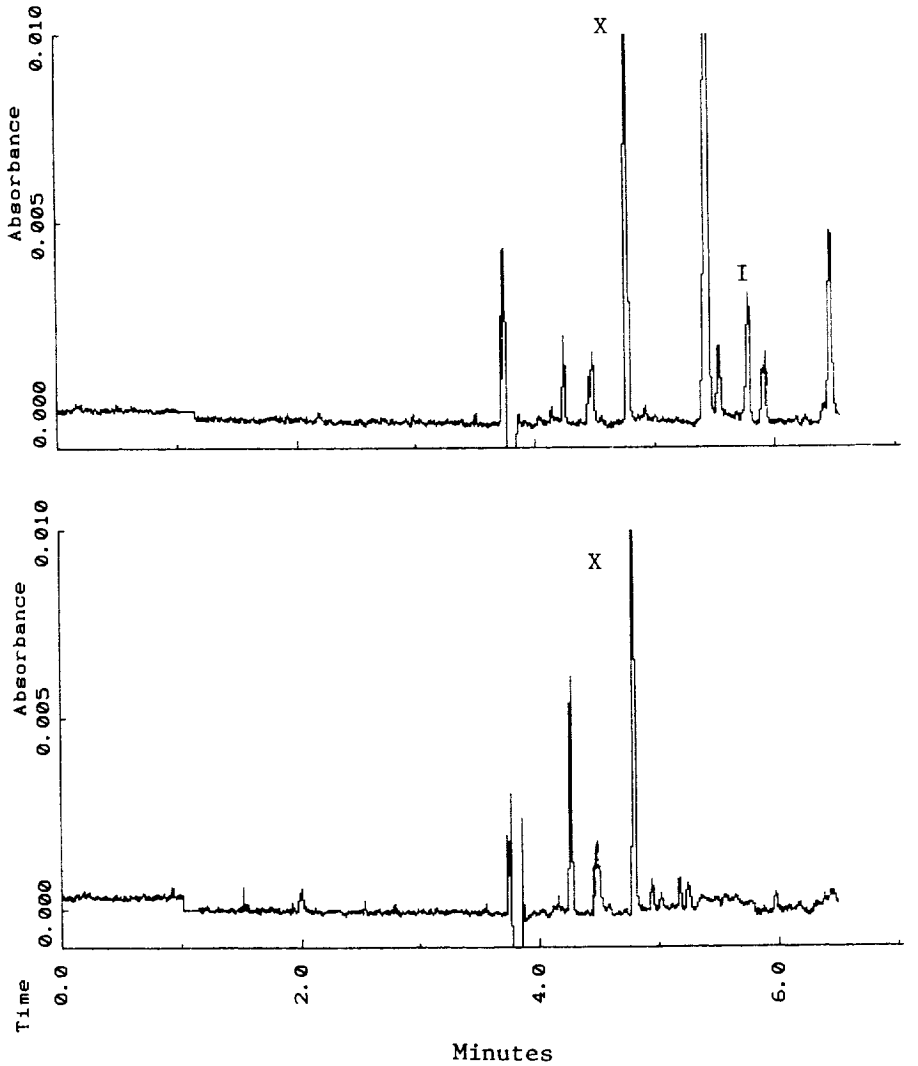

Fig. 2. Electropherogram of: (top) a patient on ibuprofen (31 mg/l); (bottom) a patient free from ibuprofen (I=ibuprofen; X=internal standard).

this analysis, the increase in the sample volume over 20 s causes some loss of resolution to the extent that some compounds interfere with the ibuprofen peak. Values <10 mg/l require extraction and/or assay at 225 nm. We do not see any need for analyzing such low levels at the present time.

A relatively high ionic strength buffer was necessary to separate ibuprofen from the common drugs and endogenous substances present in serum. We checked about 20 samples from normal individuals for possible interference. The following common drugs: theophylline, phenytoin, pentobarbital and phenobarbital have migration times of 4.7, 4.9, 5.2 and 5.6 min respectively, far from that of ibuprofen. Hemolysis (1 g/l), and bilirubin (100 mg/l) and uric acid also did not interfere with the test.

Racemic standard solutions of ibuprofen have been earlier resolved into enantiomers by CE [9]. However, here ibuprofen is analyzed in serum based on acetonitrile deproteinization. This method demonstrates that CE is a useful technique for measuring drug levels in serum based on the acetonitrile deproteinzation.

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