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Short communication

Ketoprofen analysis in serum by capillary electrophoresis

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Abstract

A method for the quantification of ketoprofen, a new non-prescription non-steroidal anti-inflammatory drug, in serum, by capillary zone electrophoresis for therapeutic monitoring and emergency toxicology is described. Serum is deproteinized with acetonitrile in the presence of an internal standard, to remove serum proteins and to induce sample stacking. The migration time was about 10 min. The assay was linear between 1–10 mg/l without any interferences. The method compared well to an HPLC assay. The HPLC afforded a better detection limit, but the CE was less expensive to operate. This method demonstrates that capillary electrophoresis is a simple and effective method for determination of ketoprofen as well as other drugs in human serum at levels close to 1 mg/l.

Keywords: Ketoprofen

1. Introduction

Ketoprofen is a non-steroidal anti-inflammatory drug which has been recently released for non-prescription sale in USA. It is a derivative of propionic acid and is a cyclooxygenase inhibitor which also interferes with the bradykinin pathway and stabilizes lysosomal enzymes. It is rapidly absorbed after oral administration and reaches maximal concentrations in the serum in 1 to 2 h after ingestion. Its main side effects are gastrointestinal, but it may promote fluid retention and increased plasma creatinine in some patients [1]. Ketoprofen is eliminated mostly in the urine as the glucuronide metabolite. In patients with renal impairment, this metabolite may accumulate in the serum and be converted back to the parent compound [2], thus increasing circulating ketoprofen levels and increasing the possibility of significant side effects such as gastrointestinal bleeding.

Analysis of ketoprofen is important in two different areas: pharmacological studies where stereoselectivity is very important and in toxicological testing where the speed and ease of assay is more important. For this reason, two types of methods have been described for analysis of this drug by high-performance liquid chromatography (HPLC). In order to resolve the isomers for pharmacological investigations, solvent extractions and derivatizations were employed [3,4]. For therapeutic or emergency treatment, more simple methods were described [5,6]. The non-steroidal anti-inflammatory drugs in general are quite widely used [6]. We have shown that ibuprofen, another anti-inflammatory drug, can be analyzed by capillary zone electrophoresis for therapeutic monitoring [7]. However, ibuprofen is present in serum at levels of 10–100 mg/l [7], while ketoprofen is present at levels of only 1–3 mg/l.

The aim of this work was to devise, a simple and rapid assay for serum ketoprofen based on capillary

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zone electrophoresis (CZE), suitable for emergency toxicology for cases which might involve overdose treatment. We also demonstrate how the detection limits in CE can be enhanced by using acetonitrile. Using the previously described phenomenon that acetonitrile produces a special type of sample concentration on the capillary called 'stacking' in deproteinized serum [8–11], we are able to inject relatively large specimen volumes (10% of capillary volume) to enhance the detection without significant band spreading. This CE method is compared to another simplified HPLC assay also applicable to emergency toxicology. The advantages and disadvantages of the two methods are discussed.

2. Experimental

2.1. Reagents

All chemicals were obtained from Sigma Chemicals (St. Louis, MO, USA).

2.2. CZE

2.2.1. Instrumentation

A Model 2000 capillary electrophoresis instrument (Beckman Instruments, Palo Alto, CA, USA) was set at 12 kV, 30°C, and 254 nm. An untreated capillary, 50 cm (43 cm to the detector)×50 μm I.D., was filled by high pressure injection (13.79 kPa) for 2 min with the separation buffer: boric acid, 250 m*M* adjusted to pH 8.9 with 2 *M* sodium hydroxide. Acetonitrile 10 ml/l and β-cyclodextrin 1 g/l were added to the buffer. The current at the start of the run was 35 μA and increased to about 65 μA as the neutral molecules (acetonitrile) passed through the detector.

2.2.2. Procedure

To 100 μl of serum in a 0.5-ml microcentrifuge tube were added 200 μl of acetonitrile containing isobutyl methylxanthine, 10 mg/l as an internal standard. The samples were vortex-mixed thoroughly for 30 s and centrifuged for 30 s at 14 000 *g*. The supernatant was removed and introduced into the capillary by pressure injection for 99 s at a low pressure of 3447.4 Pa, and was subsequently electrophoresed for 12 min. After each run the capillary was rinsed at high pressure (13.79 kPa) for 2 min with 0.2 *M* NaOH and with the electrophoresis buffer. The samples were analyzed immediately after preparation to avoid acetonitrile evaporation.

2.2.3. Sample injected

The time to fill the capillary to (the detector) was determined experimentally by repeated injection (of 99 s) of the sample at the low pressure (3447.4 Pa) until the absorbance increased. Thus, the % sample injected of the total capillary volume=(sample injection time/capillary fill time×100).

2.3. HPLC

2.3.1. Instrumentation

For comparison studies with HPLC, a Beckman Model 112 solvent delivery module was used. The separation column was an Econosphere CN 5 μm, (150×4.6 mm I.D.) (Alltech Associates, Deerfield, IL, USA) with detection at 254 nm (Model 440, Waters Associates, Milford, MA, USA) at 0.010 AUFS. The sample was introduced through a 20-μl loop injector Model 7120 (Rheodyne, Berkeley, CA, USA).

2.3.2. Procedure

100 μl of serum in a 0.5-ml microcentrifuge tube was vortex-mixed with 200 μl of acetonitrile, and centrifuged as for CZE. An aliquot of 20 μl of the supernatant was injected on the column and eluted with a solvent consisting of 1000 ml of water, 40 ml of acetonitrile and 200 μl of phosphoric acid at a flow-rate of 1.5 ml/min.

3. Results and discussion

The aim of this work was to detect ketoprofen in serum at therapeutic levels of about 1–3 mg/l. In order to detect such low levels, the sample needs to be either concentrated on or outside the capillary. Previously we have found that acetonitrile treatment which is mainly used to remove serum proteins, also has the property of concentrating many small molecules directly on the capillary, 'stacking' [7,8] in such a way that the injected sample volume can be

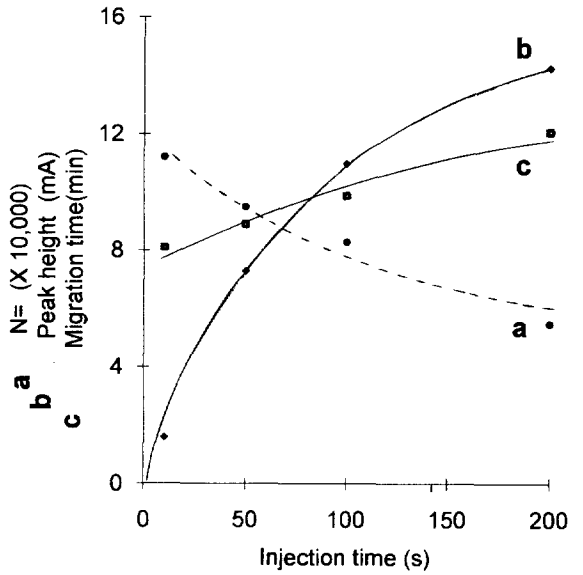

Fig. 1. Effect of injection time on peak height and migration time. Samples, spiked with 10 mg/l ketoprofen were injected at different periods of time (in s) and electrophoresed at 12 kV.

much higher than traditional 1% (of the capillary volume). Fig. 1 illustrates that the peak height increases non-linearly with an increase of sample volume. The plate number N, dropped slightly from 110 000, to 82 000, with an increase in the sample size from 10–100 s, respectively. A 99 s injection time (10% of the capillary volume to the detector, or 84 nl), produced a good sensitivity (peak height) without a significant drop in plate number, (Fig. 2 – bottom). A further increase in injection time did not significantly increase the peak height of ketoprofen.

Because of the large sample volume used here, ketoprofen added to low ionic strength phosphate buffer, 30 mM containing 66% acetonitrile (Fig. 2 – top), or added to those solutions which also contain 0.7% NaCl (Fig. 2 – middle) migrates differently from that added to serum. The presence of salts and acetonitrile together favor the stacking [8–11]. However, without sample adjustment to about pH 7.4, no peak can be detected for ketoprofen. This effect is due to the large sample volume [11]. Because of the large sample volume, the pH and other ions present in the serum (matrix effects) influence greatly the ionization and the overall separation, including the migration and the stacking [11]. Serum samples

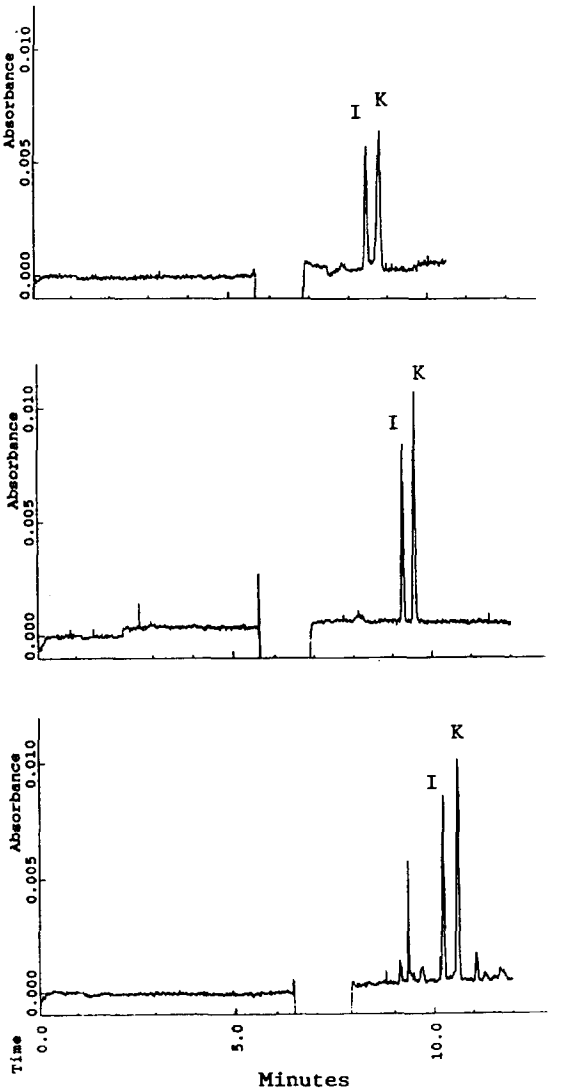

Fig. 2. Effect of sample matrix on analysis of ketoprofen. Stock solution of ketoprofen diluted in: (top) phosphate buffer pH 7.4 30 mmol/l, containing 66% acetonitrile; (middle) as in 1 but also containing 0.7% NaCl; and (bottom) serum deproteinized by acetonitrile to give a final concentration of 10 mg/l. Peaks: I=internal standard; K=ketoprofen. Sample injection is for 99 s; other conditions as in Fig. 1.

normally have a pH of 7.4 which happened to be suitable for ketoprofen stacking leading to a good peak shape (Fig. 2 bottom). For some drugs, such as the anti-epileptic drug lamotrigine, the pH of the deproteinized serum was not suitable for CE analysis but had to be adjusted before injection into the

capillary [12]. The addition of ketoprofen (10 mg/l) to the serum directly compared to that in the supernatant of the deproteinized serum gave a recovery of 87%, indicating that a small amount of ketoprofen binds to the serum proteins. Because of the difference in recovery and effects of sample matrix, the standard (10 mg/l) was prepared directly in serum. The peak was confirmed based on its migration relative to the internal standard, and occasionally by spiking. A standard curve prepared in serum of ketoprofen showed good linearity in the range of 1 to 10 mg/l, (mA=Conc×0.91+0.018, r=0.99). The minimum detectable level (3 times baseline noise) was 0.6 mg/l. Thus the method can detect therapeutic as well as toxic levels.

Boric acid buffer (250 m*M*, pH 8.8) produced good separation from the endogenous compounds in serum and the common drugs, (Fig. 3). β-Cyclodextrin and acetonitrile were added to the electrophoresis buffer to move some of the interfering compounds from the ketoprofen peak. No interferences were detected in 8 serum pools. The common drugs listed in Table 1 did not interfere either.

Table 1
List of drugs tested for interference

Drug	Concentration (mg/l)
Acetaminophen	143
Amikacin	33
Caffeine	19
Carbamazepine	8
Digoxin	0.003
Disopyramide	6
Gentamicin	8
Ibuprofen	30
Lidocaine	8
N-acetylprocaineamide	12
Phenobarbital	50
Phenytoin	30
Primidone	16
Procainamide	12
probenecid	10
Quinidine	6
Salicylate	48
Theophylline	30
Tobramycin	8
Valproic Acid	135
Vancomycin	50

We compared the analysis of ketoprofen by CZE to that by a simplified HPLC method. We used sample deproteinization similar to the Streete method [6] to simplify and speed up the analysis. We chose a CN rather than a C_{18} column for the HPLC to reduce the amount of acetonitrile for elution of the drug. The k for HPLC was dependent on pH (Fig. 4). The

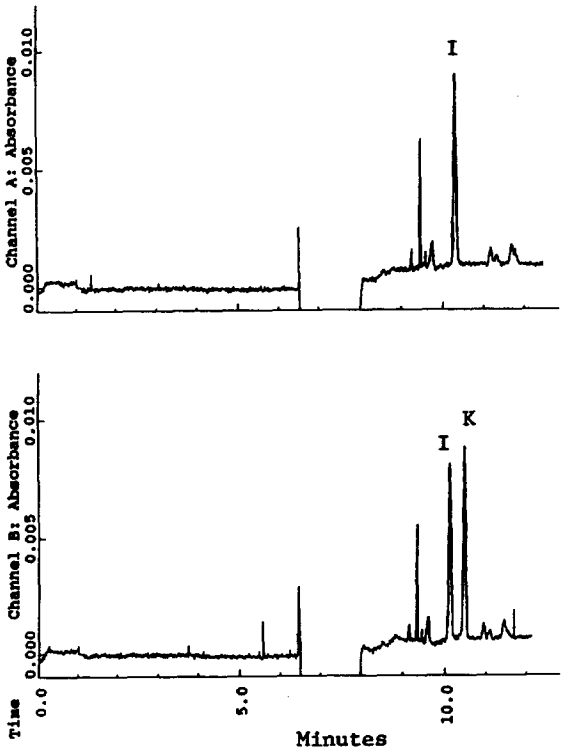

Fig. 3. Electrophoresis of serum without (top), and with (bottom) addition of 10 mg/l ketoprofen. Sample injected for 99 s. Peaks: I=internal standard; K= ketoprofen.

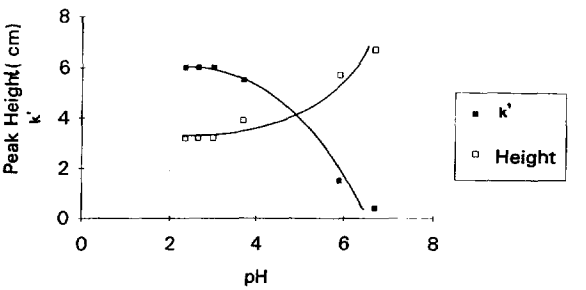

Fig. 4. Effect of pH on k and peak height for ketoprofen analyzed by HPLC. A spiked sample 10 mg/l of ketoprofen was deproteinized and injected.

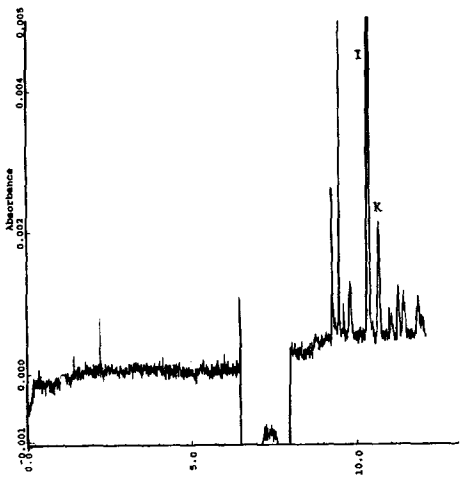

Fig. 5. Electropherogram of serum from a patient taking 25 mg of ketoprofen (serum level 1.6 mg/l), injected for 99 s. Peaks: I=internal standard; K=ketoprofen.

recovery of 10 mg/l of ketoprofen added to serum compared to that added to aqueous standards by HPLC was 93%. Fig. 5 illustrates ketoprofen from a sample of a patient receiving this drug analyzed by CZE with 10% injection of the capillary volume and in Fig. 6, the source sample analyzed by HPLC. The R.S.D. values of ketoprofen by HPLC and CE were 2.7% and 6.0%, respectively ($n=10$, mean=4.4 mg/l). This illustrates the importance of stacking. The minimum detectable level (3 times baseline noise) is 0.1 mg/l. The serum levels of ketoprofen in five samples, obtained 2 h after intake of 25 mg of ketoprofen orally (analyzed once) were 0.8, 0.9, 1.2, 1.7 and 2.1 for CE, and 0.9, 1.0, 1.4, 1.6, and 2.1 mg/l for HPLC, respectively, indicating a good correlation.

In the absence of the stacking, the sample size would be limited to 1% and the peak height by CE would be about 10 times less. Thus, the ketoprofen peak in Fig. 5 would be almost undetectable. This illustrates the importance of stacking brought about by the acetonitrile which will be helpful for analysis of other drugs. Both the CE and the HPLC methods give similar results with comparable simplicity and speed and both are well suited to clinical use. Because the capillaries are cheaper than the HPLC column, CZE is less expensive to operate; however, the HPLC has better sensitivity and better reproducibility and is less affected by sample matrix. The CZE and HPLC can be used also as complementary methods to check for interferences or impurities.

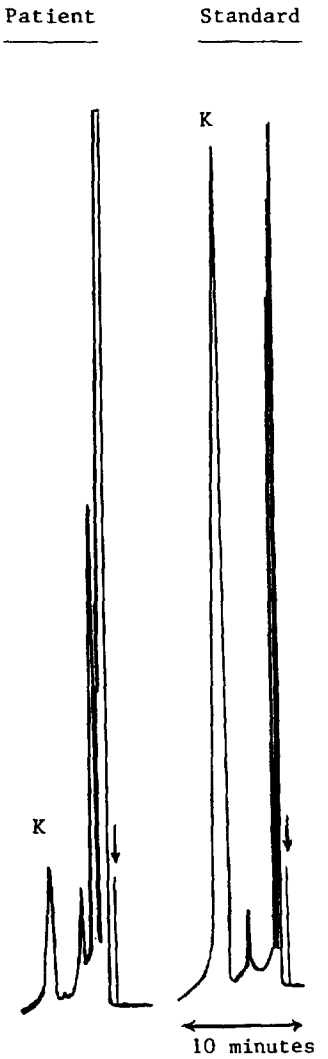

Fig. 6. Chromatogram of the same patient as in Fig. 5 by HPLC. Arrows indicate the injection. Peak: K=ketoprofen.

References

[1] R.H. Harris and I. Vavra, in K.O. Rainsford (Editor), Anti-inflammatory and Anti-rheumatic Drugs. Vol. II, CRC Press, Boca Raton, FL, 1985, pp. 163–165.

[2] L.R. Goldfrank, N.E. Flomenbaum, N.A. Lewin, R.S. Weisman, M.A., Howland and R.S. Hoffman (Editors), Goldfrank's Toxicologic Emergencies, Appleton and Lange, Norwalk, CN, 5th edition, 1994, pp. 516–518.
[3] K.E. Palylyk, F. Jamali, J. Chromatogr. 568 (1991) 187.
[4] R.T. Foster, F. Jamali, J. Chromatogr. 416 (1987) 388.
[5] R.A. Upton, J.N. Buskin, T.W. Guentert, R.L. Williams, S. Riegelman, J. Chromatogr. 190 (1980) 119.
[6] P.J. Streete, J. Chromatogr. 495 (1989) 179.
[7] Z.K. Shihabi, M.E. Hinsdale, J. Chromatogr. B 683 (1996) 115.
[8] Z.K. Shihabi, M.E. Hinsdale, J Chromatogr. B 669 (1995) 163.
[9] Z.K. Shihabi, J. Cap. Elec. 2 (1995) 267.
[10] Z.K. Shihabi, J. Chromatogr. A 744 (1996) 231.
[11] Z.K. Shihabi, in J.P. Landers (Editor), Handbook of Electrophoresis, CRC Boca Raton, FL, 1997, pp. 457–477.
[12] Z.K. Shihabi, K.S. Oles, J. Chromatogr. B 683 (1996) 119.