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Determination of ibuprofen in serum by capillary isotachophoresis

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Ibuprofen is a non-steroidal anti-inflammatory drug which has a wide therapeutic range of 5–50 mg/l (0.02–0.24 mmol/l) in serum and a toxic concentration of 100 mg/l (0.48 mmol/l) [1]. Gas chromatography [2–4], HPLC [5–7] and capillary zone electrophoresis [8] have been used to determine ibuprofen in serum, plasma and urine. Each of these methods requires a sample preparation based on simple acetonitrile deproteinization or extraction. The aim of this work was the development of a simple and sensitive isotachophoretic method for the determination of ibuprofen in serum samples.

Of several electrolyte systems tested, 10 mmol/l creatinine hydrochloride/creatinine buffer (pH 4.8) and 5 mmol/l MES were found to be the preferred leading and terminating electrolytes, respectively. In this system no endogenous compounds in serum interfered with analysis. This determination was achieved by the calibration method and statistically evaluated by linear regression. Seven calibration points repeated five times were measured with a control serum spiked with ibuprofen. Linearity was tested over the range 0.02–0.40 mmol/l. The regression line correlation (r^2), intercept (zone length) and line slope (zone length · l/mmol) were 0.9998, −0.11 and 48.59, respectively. Limit of detection and limit of determination were 0.007 and 0.02 mmol/l, respectively. The accuracy and precision (Table) of the method were evaluated by analysing five replicates of spiked serum at each concentration against a calibration curve. Accuracy was given by the % Bias (mean of measured − mean of added/mean of added) ×100. The bias was −0.5% (varied between −5.0 and +4.0%). The precision expressed as relative standard deviation (RSD) was 1.6% (range 0.4–5.1%). The results indicated that ±15% acceptance criteria are achieved [9]. The recovery of ibuprofen was determined by comparing the zone length from drug-free serum spiked with a known amount of ibuprofen with the zone length of the same concentration prepared in water. The mean recovery of ibuprofen was found to be consistent over the evaluated concentration range and was 96.5%. The procedure was applied to a set of patient's samples. An isotachopherogram of a real human serum sample containing ibuprofen is shown in the Fig. No interfering metabolite zones were observed in the serum. Drugs which were found not to interfere with the assay are: amiloride, diclofenac, fenoprofen, flurbiprofen, ketoprofen, labetalol, naproxen, metoprolol and verapamil.

Table: Accuracy, precision and recovery of the analytical procedure for ibuprofen

Added (mmol/l)	Found (mmol/l)	Accuracy Bias (%)	Precision RSD (%)	Recovery	
				Mean (%)	RSD (%)
0.0200	0.019	−5	5.1	94.8	5.4
0.0500	0.052	+4.0	1.5	98.3	2.0
0.1000	0.100	1 0.0 0.8		97.0	1.5
0.1500	0.147	−2.0	1.6	95.5	1.7
0.2000	0.198	−1.0	1.1	95.2	2.3
0.3000	0.302	+0.7	0.4	97.5	0.4
0.4000	0.399	−0.2	0.6	97.0	1.0
	Mean	−0.5	1.6	96.5	1.9

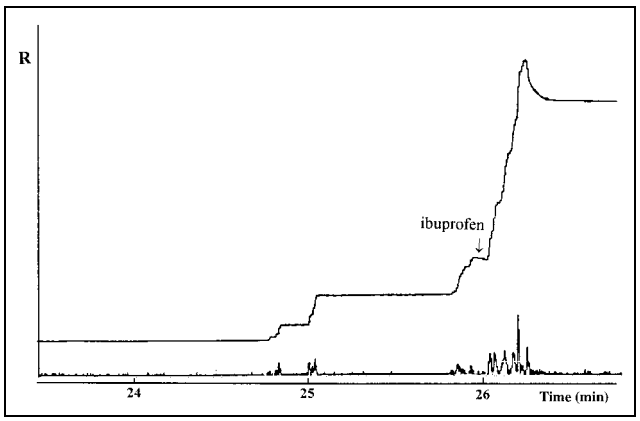

Fig.: Typical isotachopherogram from serum from an experimental subject 3 h after investigation of 400 mg of ibuprofen (5 µl injected)

Experimental

1. Apparatus

A ZKI 02 column-coupling isotachophoretic analyser (Villa Labeco) equipped with a conductivity detector and a column system consisting of a preseparation capillary (90 × 0.8 mm I.D.) and an analytical capillary (90 × 0.3 mm I.D.) was used. The current for the preseparation capillary was 100 µA and for the analytical capillary 30 µA.

2. Reagents

Ibuprofen was obtained from Sigma and control serum from Imuna (Slovak Republic). Hydrochloric acid (10 mmol/l) adjusted with creatinine to pH 4.8 plus 0.1% polyvinylpyrrolidone was used as the leading electrolyte and 5 mmol/l 4-morpholineethanesulfonic acid (MES) as the terminating electrolyte.

3. Procedure

A stock solution of ibuprofen (20 mmol/l) was prepared by dissolution of ibuprofen in 0.1 mol/l NaOH and diluted to volume with water and stored at 4 °C. Serum standards were prepared daily by adding a known amount of ibuprofen to drug-free control serum. Seven real samples were analysed by direct injection of serum (1.0–5.0 µl).

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