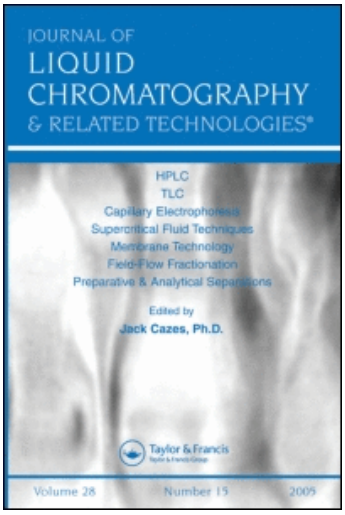

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Shihabi, Z. K. , Rocco, M. V. and Hinsdale, M. E.(1995) 'Analysis of the Contrast Agent *Iopamidol* in Serum by Capillary Electrophoresis', Journal of Liquid Chromatography & Related Technologies, 18: 18, 3825 — 3831
To link to this Article: DOI: 10.1080/10826079508014627
URL: http://dx.doi.org/10.1080/10826079508014627

JOURNAL OF LIQUID CHROMATOGRAPHY, 18(18&19), 3825-3831 (1995)

ANALYSIS OF THE CONTRAST AGENT *IOPAMIDOL* IN SERUM BY CAPILLARY ELECTROPHORESIS

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ABSTRACT

A rapid method for the analysis of the contrast agent iopamidol (isovue)in serum by CE is described. The method involves deproteinzation with acetonitrile to remove serum proteins followed by direct capillary zone electrophoresis. It is rapid(about 10 min) and sensitive. Other contrast agents and compounds used for renal function tests such as iohexol, iothalamic acid and p-aminohippuric acid can be detected by the same method. The use of acetonitrile in this method to remove serum proteins results in sample stacking allowing the detection of levels less than 1 mg/L.

INTRODUCTION

Iopamidol (Isovue, Squibb Diagnostics, Princeton, NJ) is an iodinated, non-ionic radiographic contrast medium similar to iohexol in chemical structure(1,2).

It is used frequently in cardiology for angiography. Because it is a non-metabolizable compound, does not bind to serum proteins, and is cleared by the kidney; it can also potentially be used as a measure of renal function. It is usually administered in large quantities, therefore it can cause contrast nephropathy (2,3) especially in patients with pre-existing risk factors. These patients exhibit delayed clearance of this compound.

In order to perform such studies and to access its use as a renal function measure a simple and rapid method for the assay of this compound is important. Previously this agent has been analyzed by HPLC (4) Here we show that this compound can be analyzed easily by capillary zone electrophoresis.

MATERIALS AND METHODS

Instrument

A Model 2000 capillary electrophoresis instrument (Beckman Instruments, Palo Alto, CA) was set at 8 KV, 24 C and 254 nm. The capillary was 42 cm X 50 um (i.d.). The electrophoresis buffer was boric acid (175 mmol/L) adjusted to pH 9.4 with sodium hydroxide(2 mol/L). Samples were introduced by pressure injection for 8 s (2.5% of the capillary).

Method

Serum, or standard, 50 uL was deproteinized by mixing for 15 s with 100 uL acetonitrile containing an internal standard 80 mg/L of 3-isobutyl-1-methyl-

xanthine The mixture was centrifuged for 30 s at 15,000 rpm and the supernatant was injected on the capillary.

Chemicals

Iopamidol, was obtained from Squibb Diagnostics, Princeton, NJ; iohexol, from Winthrop Pharmaceuticals, New York, NY; iothalamic acid, from Malinckrodt,St. Louis, MO; p-aminohippuric acid from Sigma Chemicals, St. Louis, MO ; and 3-isobutyl-1- methyl-xanthine from Aldrich Chemical Co., Milwaukee, WI).

Stock Standard

Iopamidol 3000 mg/L of water containing 9 g/L sodium chloride.

Working Standard

Dilute the stock standard in serum.

Calculation

Peak height was used for calculation.

RESULTS AND DISCUSSIONS

Iopamidol has a maximum light absorbency at about 240 nm and a strong signal at 254 nm. Iopamidol, iohexol, iothalamic acid, and p-aminohippuric acid are common compounds which can be used in angiography, or as a measure of renal function. All of these compounds have a strong absorption at 254 nm and can be separated at the same time by CE, Fig 1. Although we optimized the separation for iopamidol these compounds can also

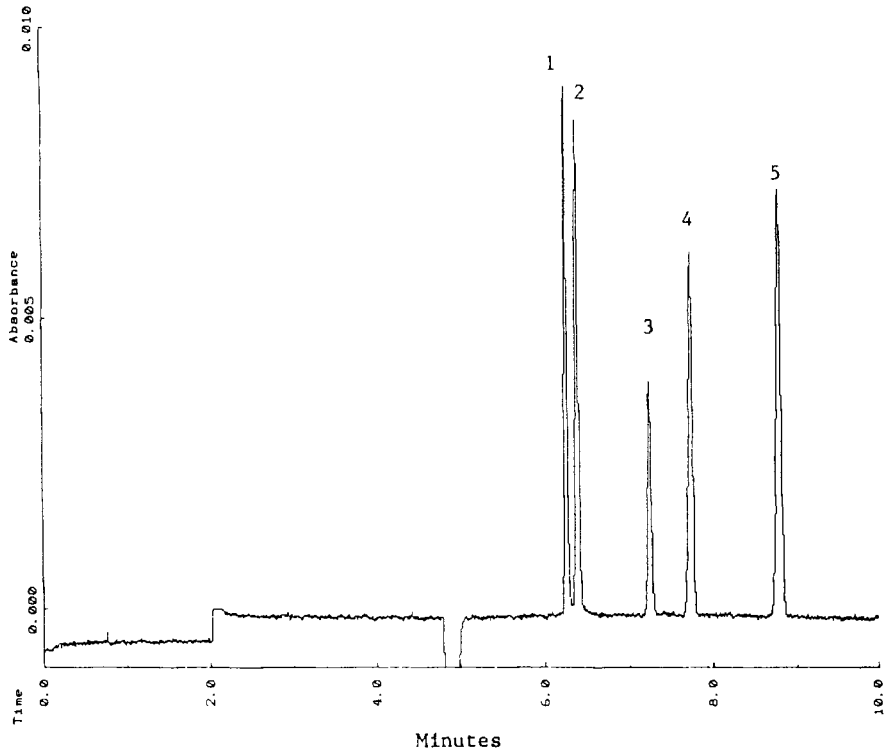
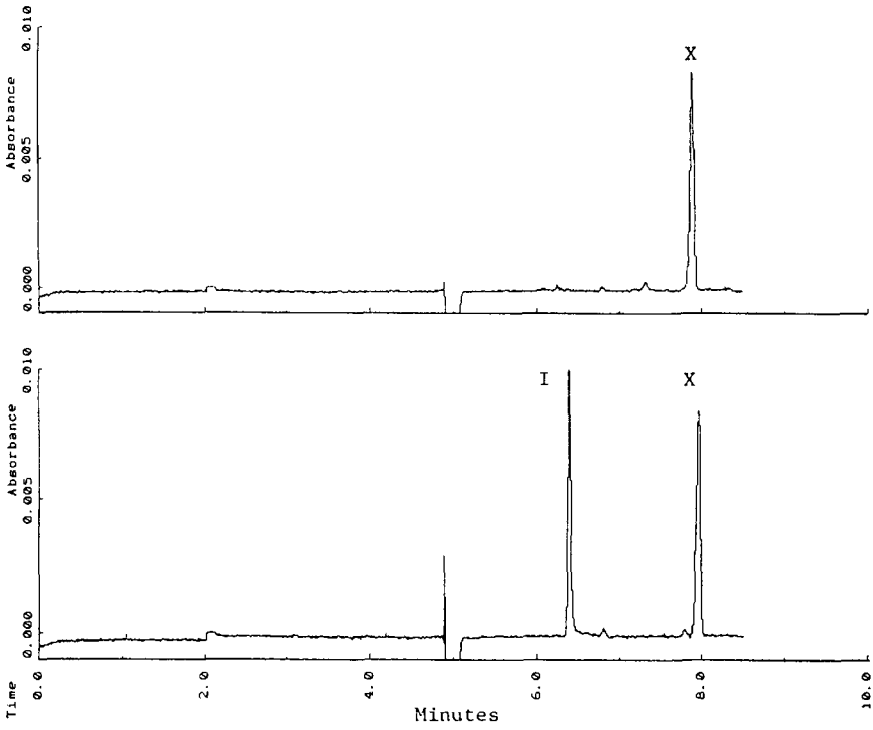


Fig 1. Separation of iopamidol and a few other related compounds in 1% sodium chloride.(1 =iopamidol 110 mg/L, 2 = iohexol 80 mg/L, 3= iothalamic acid 40 mg/L, 4 =internal standard, 5= p-aminohippuric acid 90 mg/L).

be analyzed by this method. Figure 2 illustrates the detector response to a sample from a patient receiving this drug. The electropherograms are relatively clean. Acetonitrile treatment eliminates proteins and allows for a larger sample volume to be injected on the capillary. The separation is fast and can be completed in less than 10 min.

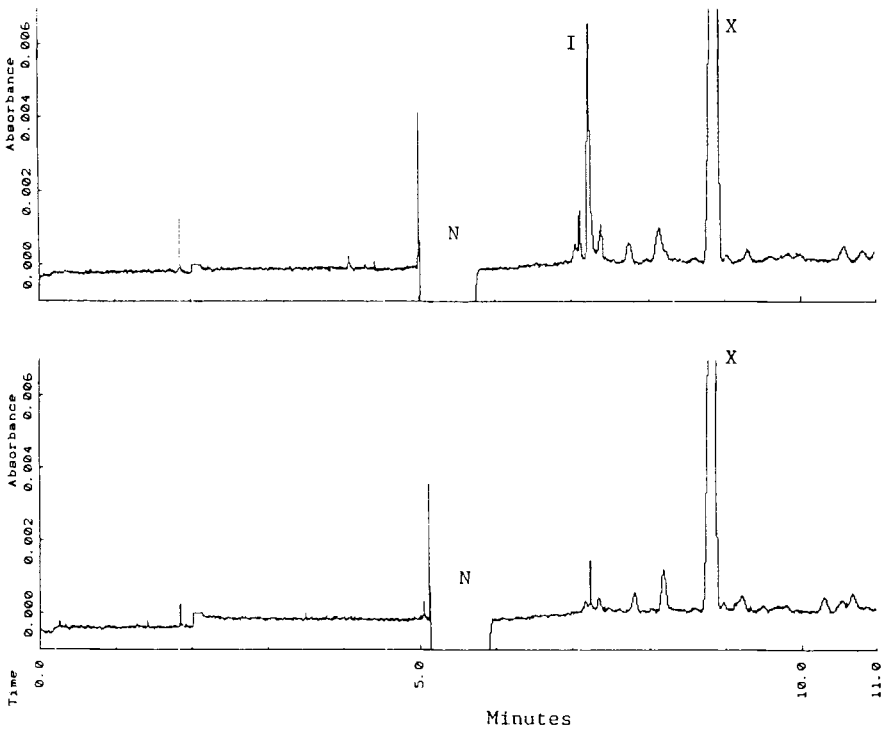
The analysis, by peak height is linear between 5 and 450 mg/L (Conc. mg/L= mA x 0.54+ 0.4, r=0.99). The



2-Electropherogram of (top) a patient free from iopamidol; and (bottom) a patient administered with the contrast agent (135 mg/L , I = iopamidol, X= Internal standard).

average recovery of 150 mg/L added to serum was 91 % relative to standards (n=4). To avoid the difference due to matrix the standards were prepared in serum free of this compound(6).

The lowest detection limit is 5 mg/L. Previously we have shown that samples deproteinized with acetonitrile can be concentrated by stacking(5)due to the low conductivity of the acetonitrile. Under such conditions, half of the capillary can be loaded with



3- Top) Serum sample spiked with 5 mg/L iopamidol; and bottom) same sample before spiking (I = iopamidol, X= Internal standard, N= neutral compounds).

sample(7). Prolonging the injection time to 40 s (12.5% of the capillary) increased the sensitivity of detection such that levels < 1 mg /L can be detected, Fig 3. However, at this high volume of sample the effective length of the capillary decreases. Some compounds which are present normally in trace amounts in serum concentrate too. Without better separations (e.g. longer capillary) they may interfere, Fig 3. We did not see any need for analyzing at the present time such low levels; however, this illustrates the

potential of this method to concentrate and detect low levels of samples in general by CE. We checked about 20 samples from normal individuals for possible interference. Hemolysis (1 g/L), bilirubin (100 mg/L) and none of the common drugs which we tested, such as salycilate, acetaminophen, and phenobarbital interfered with the test.

Several investigations(1-4) have established that iohexol is a reliable and convenient compound for GFR measurements(1-4,8). It is also possible to use iopamidol as another compound for GFR measurement. We analyzed 32 serum samples from patients received this agent as part of cardiac catheterization . The serum values had a wide range, depending on the time of sample collection and the dose, ranging from <5- 5100 mg/L, with a mean of 667 and SD of 1266 mg/L.

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Received: July 10, 1995
Accepted: August 6, 1995