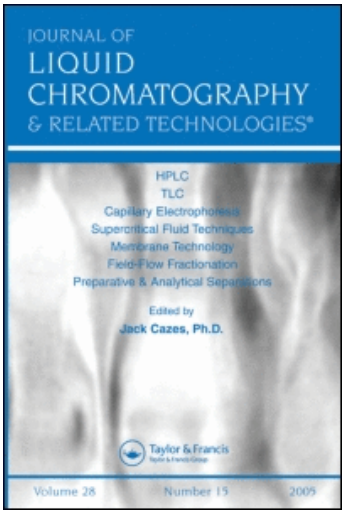

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Anti-Hormonal Agents. VI. Direct Plasma Analysis of Tamoxifen by HPLC Using an on-Line ISRP Extraction Cartridge

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ANTI-HORMONAL AGENTS. VI. DIRECT PLASMA ANALYSIS OF TAMOXIFEN BY HPLC USING AN ON-LINE ISRP EXTRACTION CARTRIDGE

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ABSTRACT

HPLC analysis of the anti-estrogen tamoxifen can be achieved by direct injection of diluted plasma samples without pre-treatment. The method uses an additional HPLC pump and two injectors and involves in-line filtration and sample clean up on a loading loop cartridge containing an internal surface reverse phase packing, followed by elution on an ODS-silica column. The minimization of sample handling and avoidance of extraction and adsorption losses result in a very rapid and accurate procedure suitable for routine assays.

INTRODUCTION

We recently reported [1] an HPLC method suitable for the rapid measurement of tamoxifen concentrations in a plasma samples from both women and monkeys treated

with the drug. Tamoxifen is a powerful anti-estrogen which is used extensively in the treatment of breast cancer [2-5] and the effects of tamoxifen on follicular development in the ovaries in women and non-human primates are also being investigated, following earlier evidence of anti-fertility effects in animals [6-8]. Previous papers had described the analysis of circulating levels of tamoxifen and its metabolites [9-13] by methods involving preliminary extraction followed by HPLC. In order to speed up the procedure and also avoid tamoxifen losses due to adsorption effects during sample handling, we developed a new method [1] in which the normal HPLC injection loop was replaced by an in-line filter and a cartridge containing an internal-surface reverse phase (ISRP) packing which absorbs drugs from water but excludes proteins [14]. This allowed the direct injection of plasma, after 1:1 dilution with water, by hand loading the injection valve with an ordinary HPLC syringe. After manually flushing with water using a large syringe, the valve was switched to the inject position and the tamoxifen retained in the ISRP cartridge eluted through an ODS-silica column. The procedure was successfully applied to the analysis of clinical samples of plasma [15] and of follicular fluid [16] from women receiving the drug.

More recently we found that changes in the nature of the commercially available ISRP cartridges had resulted in a significant increase in their resistance to flow. The greater back-pressure meant that it became impractical to hand-load the cartridges with plasma samples without substantial losses due to sample leakage from the syringe. Consequently, we have now modified the equipment design so that the plasma sample can be loaded first into a conventional injection valve

loop and delivered to the ISRP cartridge under pressure by means of a second HPLC pump. A simple switching technique using two injectors and the additional pump again allows the on-line filtration and extraction to be effected prior to reverse-phase HPLC. The modified method described in this paper gives rapid and reproducible assays of tamoxifen in plasma samples from women and monkeys treated with the drug.

MATERIALS AND METHODS

Blood samples from women and from tamoxifen-treated baboons were supplied by Dr. M.L. Swahn, Karolinska Hospital, Stockholm, Sweden and Dr. R.M. Eley, Institute of Primate Research, Nairobi, Kenya, respectively.

The HPLC equipment (see Figure 1) consisted of a Cecil 1100 pump connected to a Rheodyne 7125 injection valve fitted with a 200 ul loop. The outlet from this valve was connected via a Rheodyne in-line filter holder, with a 0.5 um steel filter element, to the injection port of a Negretti HPLC valve, whose loop was replaced by a 1 cm x 3 mm i.d. guard column containing an ISRP cartridge (Regis Chemical Co., Ill. USA). The Negretti valve outlet was connected in series to a 3 cm x 4.5 mm i.d. guard column packed with Co-Pel ODS, 30-38 um pellicular reverse phase (Whatman Ltd., UK), followed by a 25 cm x 4.5 mm i.d. column packed with 5 um ODS-Hypersil. Detection was at 260 nm x 0.002 AU fsd on a Cecil CE1200 UV monitor, connected to a Linseis recorder and Trivector TRIO integrator. The mobile phase, prepared from ultrasonically degassed, HPLC-grade solvents, was 75:20:5 v/v/v MeCN:buffer:THF, the buffer containing 0.01M KH_2PO_4 adjusted with conc. KOH solution to pH 7.8.

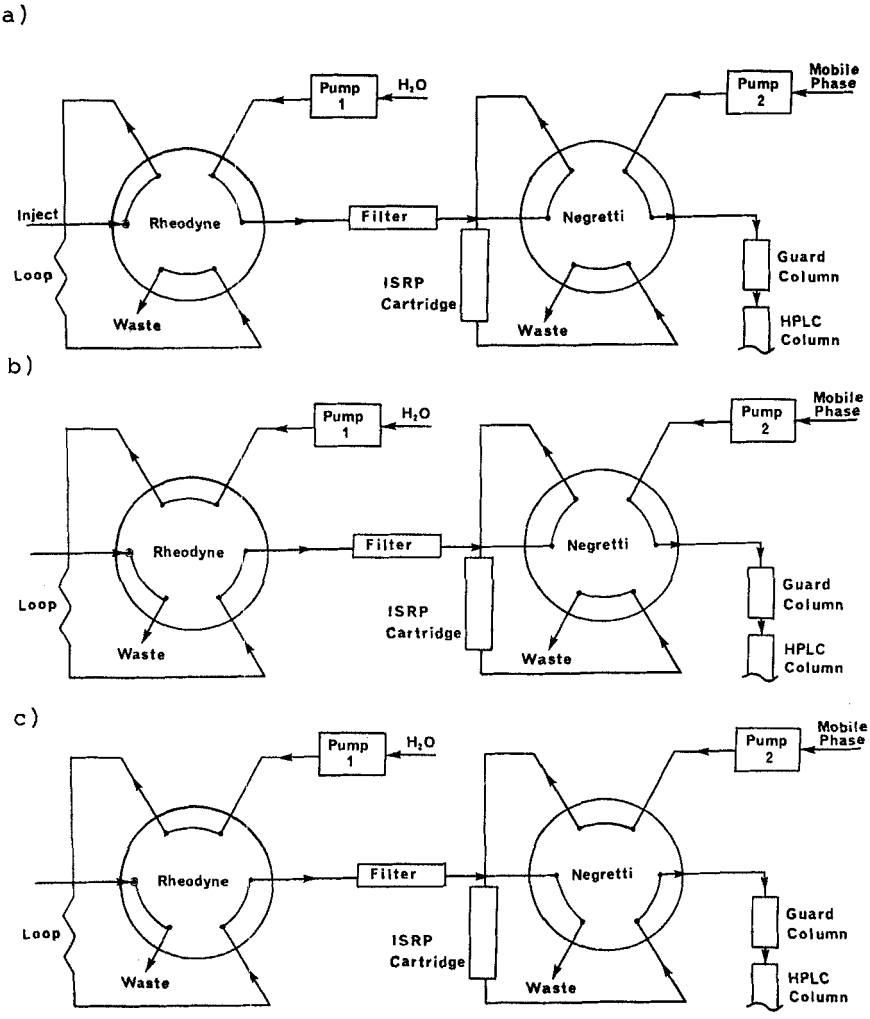

Figure 1 Valve switching technique for on-line plasma extraction

a) Sample loading into 200 ul loop in Rheodyne valve

b) Sample flushing with water from Pump 1, via filter into Negretti valve fitted with ISRP cartridge

c) Material retained by ISRP cartridge during loading is eluted through HPLC guard column and column with mobile phase supplied by Pump 2.

The analysis procedure is as follows:

a. Initial conditions: Distilled water is delivered at 2 ml/min from Pump 1, via the Rheodyne valve, through the ISRP cartrige to waste, i.e. Negretti valve in Load position. Simultaneously, Pump 2 is delivering HPLC mobile phase at 2 ml/min to the guard column and reverse phase column via the Negretti valve.

b. Sample loading and clean-up: The plasma sample is diluted 1:1 with distilled water and 200 ul loaded by syringe into the Rheodyne valve loop, which is then switched to the Inject position so that the sample is delivered through the filter to the ISRP cartridge and the protein and other unretained plasma components flushed via the cartridge to waste.

c. After 3 min. elution with water, the Negretti valve is switched to the Inject position, so that the tamoxifen is eluted via the HPLC column to the detector.

d. After a further 7 min. the Negretti valve is switched back to the Load position and water elution continued so that the initial conditions are re-established by the time the analysis is complete and the system is then ready for the next injection.

RESULTS AND DISCUSSION

The ISRP packing has internal pores whose surface area constitutes over 98% of the total surface and which are coated with a hydrophobic bonded reverse phase which retains drug molecules. Large molecules

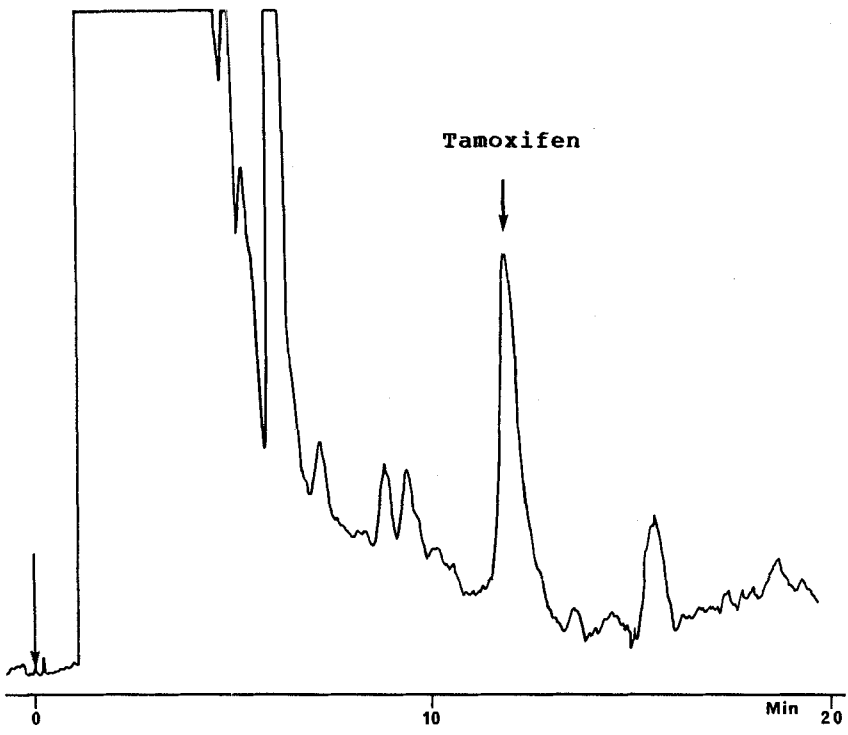

Figure 2 HPLC analysis of tamoxifen in human plasma
200 ul of 1:1 v/v plasma:water, pumped via 0.5 um steel filter into ISRP cartridge and eluted at 2.0 ml/min through 3 x 0.45 cm CO-PEL-ODS guard column and 25 x 0.45 cm 5 um Hypersil-ODS column, with 75:5:20 v/v/v MeCN:buffer:THF, (buffer: 0.01M KH_2PO_4 + conc. KOH soln. to pH 7.8). Detector 260 nm x 0.002 AU fsd.

such as proteins are excluded from the pores and are rejected by the chemically modified, hydrophilic external surface [14]. The combined use of an in-line filter and an ISRP cartridge in place of a conventional sample loop provides a very convenient means for handling plasma samples without pre-treatment [1]. The

flow scheme for the new method is illustrated in Figure 1. Samples (aqueous or plasma) containing tamoxifen are injected directly into the loading loop of an HPLC valve whose inlet is connected to a pump supplying distilled water. The outlet of this valve is connected, via a Rheodyne in-line filter holder fitted with a 0.5 um steel filter element, to the inlet port of a second HPLC valve, whose loading loop has been replaced with a guard column containing a cartridge of ISRP: the cartridge is then flushed well with water to remove non-retained plasma components before switching the valve position, so that the retained tamoxifen is carried directly to the ODS-Hypersil HPLC column.

Using the in-line filtration and extraction devices, good recoveries (>95%) and reproducible calibration graphs were obtained for standards and spiked human and baboon plasma blanks. There was a linear correlation (r = 0.9984) between peak area and amount of tamoxifen injected in spiked plasma and the detection limit for plasma tamoxifen was 6 ng/ml. Figure 2 shows a typical result for a clinical sample (200 ng/ml tamoxifen in plasma).

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