

DIRECT DETERMINATION OF TAMOXIFEN AND ITS FOUR MAJOR METABOLITES IN PLASMA USING AN SPS NITRILE GUARD COLUMN IN A COLUMN-SWITCHING CONFIGURATION

Analytes: Tamoxifen; 4-hydroxytamoxifen; N-desdimethyltamoxifen; N-desmethyltamoxifen; tamoxifen-ol.

Sample Matrix: Human plasma

Sample Size: 50 μ L

Columns: 1: Loading: SPS Nitrile, 5 μ m, 100 \AA ; 1 cm x 3.0 mm I.D.
2: Analytical: Rexchrom Nitrile, 5 μ m, 100 \AA ; 25 cm x 4.6 mm I.D. (Guarded with a Rexchrom 1 cm x 3.0 mm C18 guard column).

Mobile Phase: Loading mobile phase: Deionized water
Eluting mobile phase: 20mM potassium phosphate buffer (pH=3.0)/Acetonitrile 65/35

Flow Rate: 1.0 mL/min

Detection: Fluorescence, Exc 250 nm, Em 370 nm

Discussion: A quick, reliable and fully automated method has been developed for the determination of tamoxifen and its metabolites in human plasma. The system is based upon an in-line extraction process combined with column switching to a coupled analytical column (See Fig 1.). The plasma sample is first deproteinated with acetonitrile and then injected onto a Semi-Permeable Surface (SPS) nitrile guard column. After briefly washing the guard column with water, the sample is eluted with a more polar mobile phase and directed through a nitrile analytical column and a photochemical reactor where the analytes are converted to highly fluorescent phenanthrene derivatives. The switching-valve positions used during chromatography and the functions of these positions are presented in Table 1. 4-hydroxytamoxifen, N-desdimethyltamoxifen, N-desmethyltamoxifen, tamoxifen and tamoxifen-ol are eluted in that order at a flow rate of 1.0 mL/min and detected by fluorescence. This method has been validated for use in a clinical study utilizing tamoxifen in the treatment of recurrent cerebral astrocytomas.

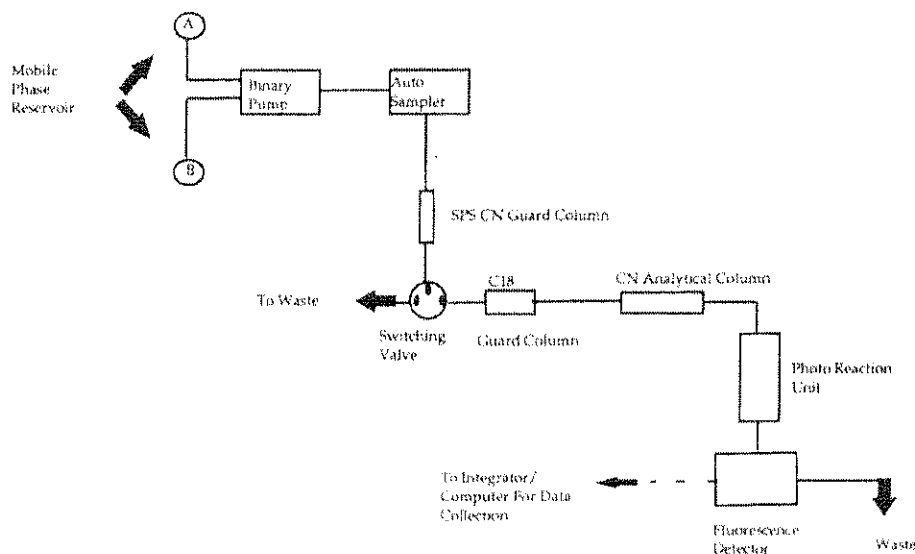


Fig. 1. HPLC schematic representation

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Discussion (cont.):

Table 1. Program of valve positions during chromatography

| Time (min) | SPS CN Column | Valve position | Analytical system |
|---------------|---------------------------------------|----------------------|---|
| Pre-injection | On-line; mobile phase B at 1.0 mL/min | To waste | Off-line; equilibrated with Mobile phase A; no flow through system |
| 0-2 min | On-line; mobile phase B at 1.0 mL/min | To waste | Off-line |
| 2-4 min | On-line; mobile phase A at 1.0 mL/min | To waste | Off-line |
| 4-75 min | On-line; mobile phase A at 1.0 mL/min | To analytical system | On-line; mobile phase A at 1.0 mL/min to photo-reactor and detector |

Figure 2 shows the structures of the known tamoxifen species and their photochemically converted fluorescent phenanthrene derivatives. Under the described chromatographic conditions, baseline separation of tamoxifen and its metabolites was accomplished in biological samples.

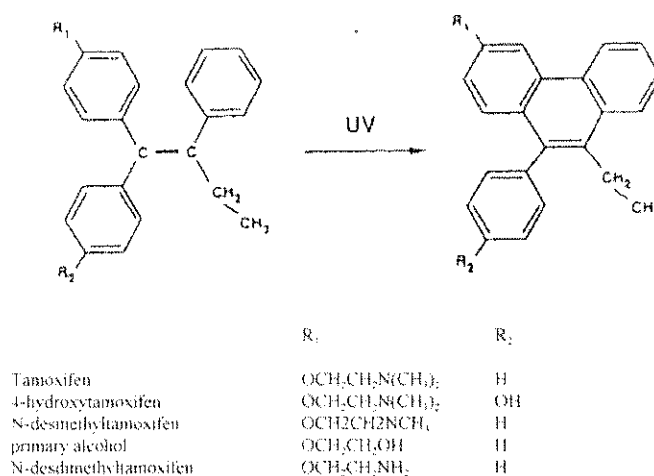


Fig. 2. Structures of tamoxifen and its metabolites along with their fluorescent phenanthrene derivatives.

Towards this end, the addition of a C18 guard column to the analytical system was necessary to prevent co-elution of an unidentified peak with tamoxifen in plasma samples from patients in this study. No interfering compounds were found in pre-dose blank plasma samples. There were, however, several unidentified peaks found in steady-state plasma

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samples but not in pre-dose plasma blanks. It is possible to conclude that some of these peaks are other metabolites of tamoxifen, such as 4-hydroxydesmethyltamoxifen but without reference samples it was not possible to establish the identities of these compounds. Trace A in Fig. 3. is a chromatogram of spiked blank plasma; trace B is a chromatogram of pre-dose plasma from a patient and trace C is a chromatogram of a steady-state sample from the same patient.

Conclusion: The assay described in this application note is rugged, utilizes commercially available components and is run at room temperature. Since the method is fully automated, large numbers of specimens can be processed within a short period of time with a minimal risk of photochemical degradation. The system also protects the analytical column from plasma components through the use of an SPS guard column which can be easily and inexpensively replaced. Thus, this analytical approach overcomes many of the disadvantages of other published methods.

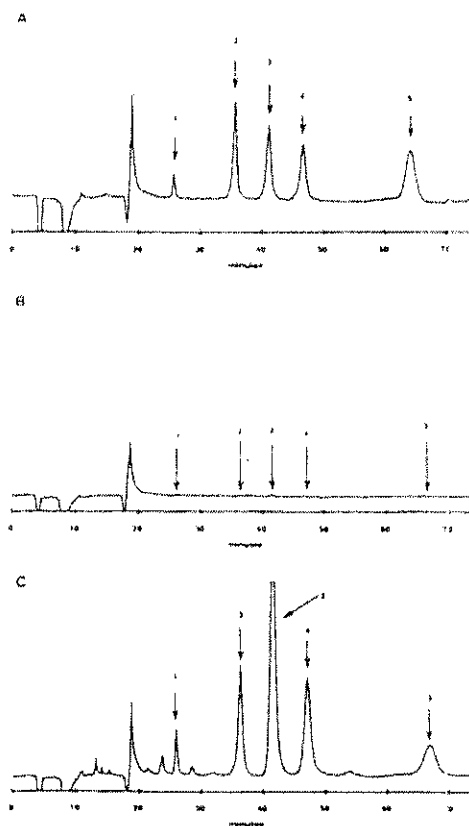


Fig. 3. (A) Chromatogram of drug-free plasma spiked with 50 ng/ml. of 4-hydroxytamoxifen and 500 ng/ml. each of N-desmethyltamoxifen, N-desmethyltamoxifen, tamoxifen, and tamoxifen-ol. (B) Chromatogram of drug-free plasma (C) Chromatogram of plasma from a patient after 4 weeks on a dose of 200 mg twice daily. Peaks: 1 - 4-hydroxytamoxifen; 2 - N-desmethyltamoxifen; 3 - N-desmethyltamoxifen; 4 - tamoxifen; 5 - tamoxifen-ol

Acknowledgements: Regis would sincerely like to thank Dr. Irving W. Wainer and Karen M. Fried for providing their manuscript and reviewing this application note.

References:

- (1) DIRECT DETERMINATION OF TAMOXIFEN AND ITS FOUR MAJOR METABOLITES IN PLASMA USING COUPLED COLUMN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY. Fried, K. M.; Wainer, I. W. *J. Chromatogr. B.* 1994, 655, 261-268.