Technical Note

Analytical Method for the Quantification of 2',3'-Didehydro-3'-Deoxythymidine, a New Anti-Human Immunodeficiency Virus (HIV) Agent, by High-Performance Liquid Chromatography (HPLC) and Ultraviolet (UV) Detection in Rat and Monkey Plasma

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INTRODUCTION

2',3'-Didehydro-3'-deoxythymidine (d4T or BMY-27857; Fig. 1) was synthesized in 1966 by Horowitz and coworkers at the Detroit Institute of Cancer Research (1). The potent anti-human immunodeficiency virus (anti-HIV) activity of d4T has been demonstrated by a number of investigators using a variety of assays (2–7). Biochemical studies indicate that, in common with other related nucleoside analogues, this compound acts at the level of reverse transcriptase of HIV and other retroviruses (8–13); following phosphorylation by cellular kinases, d4T-triphosphate is produced which preferentially inhibits viral reverse transcriptase activity, with relatively little inhibition of host cell DNA polymerases. Although the in vitro activity of d4T against HIV is almost comparable to that of 3'-azido-3'-deoxythymidine (AZT or zidovudine) (4,6), its toxicity to cells in culture, especially to human bone marrow progenitor cells, is markedly less than that of AZT (7).

Currently, d4T is undergoing preclinical evaluation. In anticipation of the analysis of plasma samples from toxicologic and pharmacokinetic nonclinical studies, a selective and sensitive high-performance liquid chromatographic (HPLC) method was developed for the quantification of d4T in rat and monkey plasma, respectively. In order to expedite the assay validation process, a simultaneous validation in rat and monkey plasma was carried out.

MATERIALS AND METHODS

Materials

D4T reference standard, Lot No. 26630-21A, 98.0% purity, and thymidine oxetane (internal standard, Fig. 1), Lot No. 29867-075, 98.2% purity, were supplied by the Reference Standards Department and Department of Chemical Processing and Development, respectively, Pharmaceutical Research and Development Division, Bristol-Myers Co., Syracuse, N.Y. The water was of Milli-Q quality (resistivity, >10 MΩ·cm) produced by the Milli-Q water purification system (Millipore Corp., Bedford, Mass.). HPLC-grade monobasic potassium phosphate and Optima-grade methanol were purchased from Fisher Scientific Co., Fair Lawn, N.J. Control Sprague-Dawley rat plasma with EDTA, Lot No. S-3515, and control cynomolgus monkey plasma with EDTA, Lot No. 880222, were purchased from Cocalico Biologicals, Inc., Reamstown, Pa. Rat plasma was obtained from an in-house rat colony.

Instrumentation and Accessories

The HPLC instrumentation consisted of a M-45 solvent delivery system (Waters Associates, Inc., Milford, Mass.), a WISP 710B autoinjector (Waters), and a UV detector, Lambda-Max Model 481 LC spectrophotometer (Waters). The detector wavelength was set at 254 nm. The chromatographic system was designed to provide a sensitive and specific determination of d4T in biological matrices. The use of optimized mobile phases and column conditions allowed for the efficient separation of d4T and the internal standard, thymidine oxetane. The chromatographic system was calibrated using standard solutions of d4T and thymidine oxetane. Calibration curves were constructed using peak areas and corresponding concentrations. The method was validated for linearity, precision, accuracy, and stability of the analytes in plasma samples.

![Chemical structures of 2',3'-didehydro-3'-deoxythymidine, d4T (1a), and thymidine oxetane, internal standard (1b).](image-url)

Fig. 1. Chemical structures of 2',3'-didehydro-3'-deoxythymidine, d4T (1a), and thymidine oxetane, internal standard (1b).

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graphic separations were performed at ambient temperature
on a 5-μm Apex octadecyl (250 × 4.6-mm i.d.) column
(Jones Chromatography, Littleton, Colo.) using a mobile
phase of 0.05 M potassium phosphate–methanol, 80:20 (v/v),
at a flow rate of 1.0 ml/min. The HPLC column was pre-
ceded by a guard column (Part No. C-135B, Upchurch Sci-
entific, Inc., Oak Harbor, Wash.) packed with Pellicular
ODS, 37–53 μm (Whatman, Inc., Clifton, N.J.). The chart
recorder (Model SE120, BBC Goerz Metrawatt, Broom-
field, Colo.) operated at a chart speed of 12 cm/hr and a
10-mV full-scale deflection. Data acquisition was done on
the HP 3357 Laboratory Automation System (Hewlett Pack-

Fig. 2. Typical chromatograms for d4T and internal standard. (a) Blank rat plasma containing 50 μg/ml of internal
standard; (b) rat plasma containing 50 μg/ml of d4T and internal standard. The approximate retention times of d4T
and internal standard are 6 and 8 min, respectively.