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Comparative chromatography–mass spectrometry studies on the antiretroviral drug nevirapine—Analytical performance characteristics in human plasma determination

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ABSTRACT

A contrast between the analytical performance characteristics using gas chromatography–mass spectrometry (GC–MS) liquid chromatography–mass spectrometry (LC–MS) and liquid chromatography–ultraviolet (LC–UV) detection for the determination of the antiretroviral drug (ARV) nevirapine (NVP) in fortified human plasma after QuEChERS extraction has been made. Analytical performance characteristics, i.e. linearities, instrument detection limits (IDLs), limits of quantitation (LOQs), method detection limits (MDLs), % mean recoveries and the corresponding relative standard deviations (%RSDs) were estimated using techniques above. Using GC–MS, the correlation coefficients (r^2) were ≥0.990, which were deemed acceptable linearities. The MDLs ranged between 11.1–29.8 μg/L and 13.7–36.0 μg/L using helium and hydrogen carrier gases respectively. The LOQs ranged between 16.5–66.7 μg/L and 28.4–98.7 μg/L using helium and hydrogen carrier gases respectively with a % mean recovery of 83% and %RSD of 4.6%. Using LC–MS and LC–UV, the correlation coefficients (r^2) were ≥0.990. The MDLs were ranged between 3.14 and 47.1 μg/L. The LOQs ranged between 2.85 and 90.0 μg/L respectively. The MDLs using GC–MS, LC–MS and LC–UV were below the therapeutic range for NVP in human plasma is considered to be between 2300 μg/L (C_{min}) and 8000 μg/L (C_{max}). This study also demonstrated that helium can be substituted with hydrogen which is relatively cheaper and easily obtainable even by use of a generator.

1. Introduction

As a result of concerted efforts by researchers worldwide, 25 antiretroviral drugs have been approved by regulatory authorities to be used for the treatment of HIV/AIDS [1] in the last three decades. To date six different classes of antiretroviral drugs can be found on the market. These classes are represented by eight nucleoside (nucleotide) reverse transcriptase (RT) inhibitors (NRTIs), four non-nucleoside RT inhibitors, ten protease inhibitors (PIs) and one integrase inhibitor. The NRTIs were the first class of antiretroviral to be accessible to HIV/AIDS patients, starting with zidovudine (AZT) in 1985, administered for monotherapy [2]. However, virus resistance developed quite rapidly to monotherapy. The development of other classes of antiretroviral drugs, and particularly of PIs (1995) allowed combination therapy and considerably increased antiretroviral efficacy. The antiretroviral drug combination therapy became known as Highly Active Antiretroviral Therapy (HAART), and at least three drugs from two different classes started to be used in combination. Nevirapine (NVP) was the first non-nucleoside reverse transcriptase inhibitor (NNRTI) approved by the Food and Drug Administration (FDA) of the United States of America (USA) in 1996. Shortly thereafter, efavirenz (EFZ) was approved in 1998. These earlier NNRTIs are known as first generation drugs and have low genetic barrier to resistance. This led researchers to develop new NNRTIs which included etravirine (ETR), rilpivirine (RPV), RDEA806, IDX899 and lersivirine [3–5]. Discovered by researchers at Boehringer Ingelheim [5], NVP is a dipyridodiazepinone inhibitor of HIV-1. It is a white to off-white crystalline powder with a molecular formula $C_{15}H_{14}N_4O$. Fig. 1 shows the structure of nevirapine.

Accurate determination of plasma drug (ARVs) concentration levels is of potential clinical importance [6], as for instance, severe skin rashes and hepatoxicity widely reported in HIV/AIDS patients taking NVP may be related to drug over-exposure [7–9]. On the other hand sub-optimal ARV drug levels can cause development of viral resistance [10–13]. Most HIV/AIDS patients in Sub-Saharan

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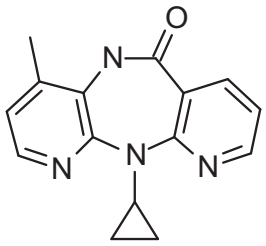

Fig. 1. The structure of nevirapine.

African countries are reportedly abandoning prescribed medication in preference to traditional herbal treatments and herbal supplements some of which can interact with drugs used in the treatment of HIV/AIDS [14]. Non-adherence to prescribed antiretroviral drugs, their toxicity and interactions with many other drugs used to treat opportunistic infections and coexisting morbidities have led to the development of therapeutic drug monitoring (TDM) of antiretrovirals. The issue of the routine use of TDM is still debated because although an effective TDM program could help to improve efficacy and lower toxicity, the considerable costs and the infrastructure needed are a huge problem particularly in developing countries.

The aim of this study was to compare and contrast the analytical performance characteristics of optimized method parameters to be developed herein for methods used in the determination of NVP in human plasma meant for its TDM in HIV/AIDS patients. These methods were high performance liquid chromatography–mass spectrometry (HPLC–MS) and gas chromatography–mass spectrometry (GC–MS), the later rarely used in routine analysis for TDM. LC–UV which is widely used in many laboratories, perhaps due to the high cost of mass spectrometers would also be explored. Additionally, the suitability of QuEChERS, a Portmanteau word for Quick, Easy, Effective, Cheap, Rugged and Safe as a method of extraction was explored having been found suitable for extraction of drugs in matrices such as bovine milk in this laboratory [15]. The use of hydrogen as a carrier gas in GC–MS was also investigated and compared with helium which is the traditionally used carrier gas. This option arose due to the cost of hydrogen, which is lower than that of helium and would be of great benefit if it proved suitable in this geographical setting of the world. A comparison of three detection methods for the TDM of NVP would thus offer options in terms of cost, speed of analysis, robustness and simplicity for those who intend to apply the methods. The use of QuEChERS for human plasma sample extraction for NVP has not yet been reported in the literature and thus also offers a comparative basis with other extraction techniques such as protein precipitation (PPT) which is often used.

2. Experimental

2.1. Reagents and chemicals

HPLC grade NVP (11-cyclopropyl-5, 11-dihydro-4-methyl-6H-dipyrido-[3,2-b: 2′, 3′-e] diazepin-6-one) was purchased from Boehringer Ingelheim Pharmaceuticals (Ridgefield, CT, USA). HPLC grade chlorpromazine (99.2%), HPLC grade acetic acid, HPLC grade Acetonitrile and Acetone 99.9% purity were purchased from Sigma–Aldrich Co., St Louis (USA). Formic acid 85% was from Sigma–Aldrich (Saarchem, Muldersdrift, RSA). Formic acid was double distilled to increase its purity. All organic solvents used in this research work were filtered through 0.45 μm organic membrane filters, type HVLP, Millipore (Dublin, Ireland). Ultra high purity water was processed through a MilliQ Ultrapure Ionex Gradient A10 purification system (Millipore Cop., Bedford, MA, USA). Mass spectrometry calibration solutions Ultramark 1621b were from Alfa Aeser, Johnson Mathey Company, Haysham, Lancaster, Met-Arg-Phe-Ala, MRFA 98.5% (Barnegat, NJ, USA) and caffeine (99%) were obtained from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Apparatus

2.2.1. HPLC–MS

An Agilent 1100 series High Performance Liquid Chromatography HPLC (Agilent Technologies, Palo Alto, CA, USA), equipped with a degasser, quaternary pump, autosampler, thermo-stated column compartment and a diode array detector (DAD), was used in this study. This was coupled to a Thermo Scientific Finnigan LCQ-DECA Quadrupole Ion Trap (QIT) mass spectrometer with an electrospray ionization ESI source (Thermo Scientific, San Jose, CA, USA). LC–MS control and spectral processing was done using Xcalibur software, version 2.0 (Thermo Scientific, San Jose, CA, USA). All chromatographic separations were done on an ACE C_{18} 3 μm particle size, 3.0 mm × 50 mm, MAC-MOD (PA, USA).

2.2.2. GC–MS

An Agilent GC 7890A coupled to an Agilent 5975 C inert XL EI/CI MSD equipped with a Triple axis detector (Agilent Technologies, Palo Alto, CA, USA) was used. The acquisition software was Agilent MSD Productivity Chemstation. The system is equipped with and autosampler and can be operated in the EI/CI modes with CI further being operated in positive chemical ionization (PCI) and negative chemical ionization (NCI) modes.

2.2.3. Other materials and accessories

All calibration standards were weighed on a Sartorius super microbalance GmbH (Goettingen, Germany). The pH of the mobile phases was adjusted using a pH meter from HANNA instruments (Hanna instruments Inc, Romania). A Thermo Heraeus Multifuge 3S-R centrifuge was used for centrifuging (Thermo Scientific, San Jose, CA, USA). A glass syringe together with Ministart 0.45 μm acrodisc non-pyrogenic single use syringe filters (Goettingen, Germany) were used for filtering the sample extracts prior to GC–MS and HPLC–MS analysis. Autosampler vials and septa (Agilent Technologies, Palo Alto, CA, USA) were used in the GC–MS and HPLC autosampler rack. Disposable non-pyrogenic serological pipettes were used to transfer plasma into fluorinated ethylene propylene (FEP) centrifuge tubes after it was separated from whole blood (Aktiengesellschaft & Co., France). Plasma samples were stored in ARTIKO refrigerator of permitted range −75 °C to 85 °C (Arctiko Int., Lammefjordsyej, Denmark).

2.3. Sample preparation and handling

2.3.1. Human fluids sample handling

The school of Medicine of the University of Botswana, working in conjunction with the Botswana Harvard Partnership (BHP) Laboratory, organized and facilitated a course tailored at the safe handling and disposal of clinical specimens. The former provided theoretical training while the latter offered hands-on practical training in a state-of-the-art ISO 17025 accredited laboratory. Hazards associated with handling of body fluids such as blood, urine and saliva were taken care of through this undertaking. The safe disposal of clinical waste, the use of disinfectants and handling of spillages taken care of.

2.3.2. Sample collection and storage

Samples used in this study for method validation purposes were human plasma. Sample collection was done at the University of Botswana Clinic by the health staff. Blood specimens were drawn from two consenting adults who were HIV negative and hence not on antiretroviral therapy. The participant donated three 4 mL