International Journal of Pharmaceutics 474 (2014) 193–201

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Personalised Medicines

Routine application of Raman spectroscopy in the quality control of hospital compounded ganciclovir

Alexandre Amin, Philippe Bourget [1,*], Fabrice Vidal, Flavie Ader

Clinical Pharmacy Department, HU Necker-Enfants Malades, 149 rue de Sèvres, 75743 Paris cedex 15, France

ARTICLE INFO

Article history:
Received 10 July 2014
Received in revised form 14 August 2014
Accepted 17 August 2014
Available online 20 August 2014

PubChem classification:
Ganciclovir (PubChem CID: 3454)

Keywords:
Raman spectroscopy
Analytical quality control
HPLC
Safety
Hospital environment
Ganciclovir

ABSTRACT

This study compares the performance of a reference method of HPLC to Raman spectroscopy (RS) for the analytical quality control (AQC) of therapeutic objects. We assessed a model consisting of a widely used antiviral drug, i.e., ganciclovir, which was compounded in a medical device and then transferred in a vacuum glass vial prior to analyses. As the aim of the alternative RS method is to replace the destructive, time-consuming HPLC method, requiring sample preparation, it needs to be demonstrated that RS performs at least as good as the HPLC method. Therefore, the two methods were validated by calculating the accuracy profile and provided excellent results for the analytical validation key criteria, i.e., trueness, precision and accuracy, ranging from 0.8 to 10 mg/mL. The Spearman and Kendall correlation tests (p-value $< 1.10–15$) and the statistical studies performed on the graphs confirm a strong correlation in the results between RS and the standard HPLC under the experimental conditions. These results confirmed the potential of this option for future applications, owing to its analytical and practical quality and its contributions to the safety of the medication circuit. This method also greatly contributes to the protection of caregivers and their working environment.

1. Introduction

In France, central IV admixtures of chemotherapy treatments are required by law (Decree No. 2005-317 of 24 August, 2005). The preparation of these admixtures is currently performed under pharmaceutical liability, especially at hospitals. This requirement represents an important step forward in terms of both the quality and the safety of care as well as (a) a strong contribution to the standardization of prescription practices, (b) a lower exposure of caregivers to chemicals, (c) an improved organization of caregiver workloads and (d) a substantial cost savings (Martin et al., 2004).

In this multifaceted context, and as previously shown in various works and technical circumstances, the development of effective tools for the analytical quality control (AQC) of pharmaceutical therapeutic objects (TO) shaped at the hospital is highly relevant (Bouligand et al., 2004; Bouligand et al., 2005; Bourget et al., 2003; Gravel et al., 2005). In brief, it consists of verifying the following two key parameters: the identity and the nominal concentration of an active pharmaceutical ingredient (API) in the solution or suspension in a sterile medium. A therapeutic object is the product resulting from a compounding process, which is performed by specialized staff, and includes the following: (a) an active ingredient in solution or suspension in an appropriate medium, which is usually diluted in normal saline or a 5% dextrose solution, and (b) an immediately labeled package that is potentially pre-connected to an infusion set. The presence of secondary packaging may complete this definition.

Ideally, the purpose of AQC is to enable the analytical certification of the TO prior to its administration to a patient. In terms of hospital organization, the AQC should be fast, reliable, and fully integrated into the production process and treatment. This is particularly relevant for day care units. The most frequently used analytical techniques are the following: (a) chromatography methods coupled with appropriate detection systems, (b) high-performance thin layer chromatography methods, and (c) UV/visible light spectroscopic techniques coupled to a Fourier transform infrared spectroscopy detector. Chromatographic methods such as high-performance liquid chromatography (HPLC) are powerful; unfortunately, their

Abbreviations: GCL, ganciclovir; API, active pharmaceutical ingredient; AQC, analytical quality control; CMV, cytomegalovirus; HPLC, high-performance liquid chromatography; LLOQ, lower limit of quantification; LoA, limits of acceptability; LoAP, limits of acceptability weighted; PLS, partial least square regression; QC, quality control; RS, Raman spectroscopy; RSD, relative standard deviation; TO, therapeutic objects; ULOQ, upper limit of quantification.

* Corresponding author. Tel.: +33 1 42 11 51 88; fax: +33 1 42 11 52 00.
E-mail addresses: alexandre.amin@nck.aphp.fr (A. Amin), philippe.bourget@nck.aphp.fr (P. Bourget).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.ijpharm.2014.08.028
0378-5173/

implementation is costly and sometimes tedious, and they require specialized skills of the individuals performing them. The strengths and weaknesses of this reference option will not be detailed. According to our criteria, and despite substantial technical improvements, chromatographic methods remain unsuitable for use in high-throughput AQC. Raman spectroscopy (RS) allows for the qualitative and quantitative characterization of an API and its matrix. However, among the characterization parameters, both the specificity and reliability of the technique must be demonstrated through experimentation. Furthermore, some molecules are structurally similar (Bourget et al., 2012). In addition, it is worth noting that quantitative Raman studies of APIs in injectables currently remain few in number (Bourget et al., 2012; Bourget et al., 2014a,b; Bourget et al., 2013; Mazurek and Szostak, 2006).

The purpose of this study was to develop and validate a method using RS as an effective tool for the non-intrusive pre-delivery AQC of ganciclovir (GCL) prescribed and shaped at the hospital, under pharmaceutical liability, in the same way as other cytotoxic agents (Decree No. 2005-317 of 24 August, 2005; National Institute for Occupational Safety and Health (NIOSH), 2012). The protocol was validated and compared to a reference HPLC method. For the very first time, using an automated bench, the solution was able to be non-intrusively validated and routinely applied.

Therapeutic objects of GCL were compounded in flexible pouches by production operators working in our injectable manufacturing unit. Then, 0.5 mL of each therapeutic solution was withdrawn under aseptic conditions and transferred to a sealed vacuum glass vial prior to analysis. We also examined how the use of one analytical method vs. another contributes to the security and safety of the administration of medication at the hospital.

2. Material and methods

2.1. Choosing a suitable API and working conditions

Ganciclovir is a widely used drug worldwide; it is also considered to be a potential carcinogen, teratogen, and mutagen as well as being considered as a likely cause of the inhibition of spermatogenesis in humans (American Society of Health-System Pharmacists, 2013). In brief, GCL is a nucleoside analog widely used to treat or prevent cytomegalovirus (CMV) infections, which mainly affect immunocompromised patients. Ganciclovir is also a synthetic analog of 2′-deoxy-guanosine. After phosphorylation by various viral and cellular kinases, the deoxyguanosine triphosphate (dGTP) is incorporated into the viral DNA leading to the inhibition of the viral DNA polymerases. Related to this activity, the use of GCL is mostly indicated for the following: (a) sight-threatening CMV retinitis diagnosed in severely immunocompromised people, (b) CMV pneumonitis diagnosed in bone marrow transplant recipients, and (c) the prevention of CMV diseases in bone marrow and solid organ transplant recipients. As a reminder, acute infections are commonly treated by intravenous drug doses given as a one hour infusion in saline in the following two steps: (a) the induction stage, i.e., 5 mg per kg of weight, administered intravenously every 12 h for 14 to 21 consecutive days and (b) a maintenance phase, i.e., 5 mg per kg of weight administered intravenously every day. In view of this treatment, the target therapeutic concentrations are between 2 and 8 mg/mL of GCL compounded exclusively in saline. To thoroughly test the analytical performance of the Raman spectroscopy and build robust calibration models, the range of concentrations was deliberately expanded to range from 0.8 to 10 mg/L, leading to the production of a large number of TO.

2.2. HPLC analysis

Chromatographic separation was performed on a Dionex Ultimate® 3000 series liquid chromatographic system equipped with a quaternary pump, a variable UV/visible detector, and an autosampler (Dionex, 78960 Voisins le Bretonneux, France). Chromatographic separation was performed on Lichrospher® C18 column (125 mm × 4 mm, dp = 5 μm; Merck, 69008 Lyon, France). The mobile phase for injection consisting of a mixture of water and acetonitrile was delivered at rate of 1.5 mL per min. The mobile phase was filtered through a 0.45 μm membrane (Millipore, 67120 Molsheim, France) and degassed prior to use. The separation was performed at ambient temperature, i.e., 21 °C, and the detection was performed at 254 nm. The injection volume was 5 μL with a run time of 3 min. The data were recorded, and the Dionex Chromeleon® (version 6.80) software was employed for data collection and processing. The chromatographic conditions were based on a HPLC method coupled with UV detection developed by Merodio et al. and Teshima et al. (Merodio et al., 2000; Teshima et al., 2003).

2.3. RS analysis

The RS analysis was performed between 50 and 3500 cm^{-1} using a DXR SmartRaman® spectrometer (Thermo Fisher Scientific, Courtaboeuf, France); Fig. 1 is a picture of the apparatus. The bench is equipped with the following: (a) a user-safe laser source, easily interchangeable and emitting at 532 nm with the aim of minimizing the fluorescence interference due to glass, and (b) a 180 degree sampling accessory design to accommodate vials. This light-tight accessory is equipped with a focal adjustment system, which is used for accurately setting the distance between the laser source, i.e., the extremity of the probe, and the vials for examination.

Fig. 1. Vacuum glass vial (ref 2C1702KD, Interchim).