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Evaluation of LC–MS for the analysis of cleaning verification samples

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Abstract

The cleaning verification of pharmaceutical manufacturing equipment prior to further use is a cGMP requirement. Typically, relevant data are generated by HPLC with UV detection using methods individually developed and validated for each product. This work describes the use of HPLC with mass spectrometry to analyse cleaning verification samples, a novel means of utilising this analytical technology. The initial aim was to produce a single, generic method capable of quantifying a broad range of pharmaceuticals. Ultimately, however, a more effective strategy, in terms of efficiency and reliability, proved to be application of a well-defined approach to the rapid generation of compound specific methods. Results of studies to optimise the sample preparation for a basic compound in drug development (compound 1), together with experimental results for two further compounds are presented. These demonstrated that the combination of a well defined approach to chromatographic method development and mass spectrometric detection provided methodology with advantages in terms of sensitivity. Additionally, and by virtue of its potential for general applicability, the approach proposed has the potential to improve the overall efficiency with which methods for cleaning verification samples can be developed and applied.

Keywords: Cleaning verification; Liquid chromatography–mass spectrometry; Stable isotope labelled internal standards

1. Introduction

LC–MS is widely used in the pharmaceutical industry for applications such as the identification of potential drug candidates in pharmacological screens, the identification of impurities and degradation products obtained during clinical development and the quantification of drugs in biological media, both in vitro and in vivo [1] In the latter case, the technique's key advantages of improved sensitivity of detection and selectivity with consequent reduction in analysis times have led to it becoming widely adopted as the quantitative technique of choice [1]. For those laboratories not concerned with bioanalysis but with establishing the overall quality of active pharmaceutical ingredients (APIs) and their formulated products LC–MS equipment is, today, essential but is most often associated with qualitative rather than quantitative applications. However, given the technique's advantages, it seems likely that LC–MS has similar, as yet unrealised, potential in respect of quantitative work in these laboratories. Accordingly, in this work, the suitability of LC–MS for the quantification of API residues during the cleaning verification of pharmaceutical manufacturing equipment has been briefly assessed using three compounds under development in our laboratory.

After the manufacture of a pharmaceutical formulation has been completed it is a cGMP requirement that the equipment be cleaned prior to being used for the manufacture of a different product [2]. Various analytical methods have been used to verify the success of cleaning operations; including HPLC–UV, which is the most commonly applied [3,4], ion mobility spectrometry (IMS) [5] total organic carbon (TOC) [6] and HPLC with evaporative light scattering detection (ELSD) [7]. Both IMS and TOC have the advantage of speed with respect to HPLC–UV methods but the latter would not be specific for the compound of interest and the former is not generally available at pharmaceutical manufacturing facilities. Similarly, although it allows for the sensitive detection of compounds, including those with a poor chromophore, ELSD has not found general applicability in this area. Recently the reduction in LC–MS equipment prices and the increasing number of applications, have led to much greater access to this type of equipment within facilities where cleaning

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verification is routinely performed. Because of this and the techniques's potential advantages, including improved sensitivity, an investigation into the feasibility of using LC–MS for the analysis of cleaning verification samples was considered appropriate.

Following equipment cleaning, different procedures may be used to confirm the operation's success. These fall into two categories, rinse and direct surface sampling. Rinse samples are obtained by passing a volume of solvent (generally aqueous) through or over the cleaned equipment, which is then analysed for the compound of interest. By contrast direct surface sampling involves the use of swabs (for small surface areas) and wipes (for larger surface areas) which are moistened with the solvent of choice and rubbed over the surface to be monitored. This technique is generally preferred as it allows the use of a relatively small volume of organic solvent, which not only results in greater removal of compounds from the equipment surface, but also avoids excessive dilution of these species prior to analysis [2]. For this reason, within the general aim of testing the feasibility of using LC–MS for the analysis of cleaning verification samples, the scope of the experimentation was limited to the validation of direct surface sampling methodology and the use of the most common surface type, stainless steel.

Validation data required to support the determination of trace API levels during cleaning verification can be viewed as somewhat intermediate in nature between those that would be required to support quantification of an impurity in an API and those required for a limit test [8]. Because of this only validation data sufficient to show the approach to be feasible were produced recognising that, were the proposed approach to be adopted routinely, additional validation data may be necessary. Consistent with the above, methods were developed and applied to a piperidinyl derivative (compound 1), a substituted isoquiniline (compound 2) and a modified pyridazino species (compound 3) which were under development for differing pharmaceutical applications.

2. Experimental

2.1. Reagents and chemicals

Compounds 1, 2 and 3 and their stable isotope labelled (SIL) versions (which all contained >99% labelled compound), lactose monohydrate, povidone, magnesium stearate and sodium starch glycolate were all supplied by sanofi-aventis. HPLC grade acetonitrile was purchased form Fisher Scientific Ltd. (Loughborough, Leicestershire, UK). HPLC grade ethanol and ammonium formate were purchased from BDH Laboratory Supplies (Poole, Dorset, UK). Purified water was produced in-house by use of an Elga Maxima system (Elga LabWater, High Wycombe, UK). Formic acid was purchased from Sigma–Aldrich Co. Ltd. (Poole, Dorset, UK).

2.2. Equipment

HPLC–MS was performed using a Micromass ZMD single quadrupole mass spectrometer (Waters-Micromass, Manchester, UK) coupled to an Agilent 1100 series HPLC system (Agilent Technologies UK Ltd., Stockport, UK). Solvent optimisation was performed using DryLab® chromatography optimisation software, Version 2.05 (LC Resources, Walnut Creek, CA, USA). Wipes used were Kimtex® Lite reference 7271 purchased from Kimberly-Clark Ltd. (Kent, UK) cut to 18 cm × 19 cm.

2.3. Chromatographic and mass spectrometric conditions

2.3.1. Single method for all compounds

For this methodology the HPLC column was a Waters XTerra™ C8 (3.5 μm particle size, 21 mm × 3 mm) purchased from the Waters Corporation (Watford, Hertfordshire, UK). The mobile phase consisted of acetonitrile–water, containing 20 mM formic acid (90:10 v/v) deliver at a flow rate of 2 ml min^{-1} and split 20:1 in favour of waste prior to the mass spectrometer. The column was held at 40 °C and 50 μl of each solution was injected.

The mass spectrometer was operated in electrospray mode with positive ionisation. The cone voltage was set to 30 V, the capillary voltage to 3.5 kV, the desolvation gas flow to 400 l h^{-1}, the source block temperature to 120 °C, and the desolvation temperature to 300 °C. The dwell time was 0.1 s. Each compound and its associated internal standard was monitored using SIM of the most abundant ion which, in each case, was the $[M + H]^+$ ion.

2.3.2. Well defined approach

Methods were developed for all three compounds. In each case two gradient analyses were carried out and DryLab® software used to predict the solvent ratio which would give a retention time of approximately 2 min. This allowed the analyte to be resolved from any interference at the solvent front.

For compounds 1 and 3 the HPLC column used was a Waters XTerra™ MS C8 (3.5 μm particle size, 50 mm × 4.6 mm) whereas for compound 2 a Waters XTerra™ RP C18 (3.5 μm particle size 100 mm × 4.6 mm) was used. Both columns were purchased from the Waters Corporation (Watford, Hertfordshire, UK).

For compounds 1 and 3 a mobile phase of acetonitrile–water, containing 20 mM formic acid (35:65, v/v) and (20:80, v/v) respectively was used at a flow rate of 2 ml min^{-1}. For compound 2 a mobile phase of 20 mM ammonium formate (pH 8)–acetonitrile (60:40, v/v) at a flow rate of 2 ml min^{-1} was used. All flow rates were split 20:1 in favour of waste prior to entering the mass spectrometer. The column was held at 40 °C in all cases. An injection volume of 100 μl was used for the extraction optimisation experiments and 50 μl for all other work.

The mass spectrometer conditions were optimised for each compound. In all cases the instrument was operated in electrospray mode with positive ion detection and a nitrogen gas flow of 400 L h^{-1}. The cone voltage was 30 V for compound 1 and 35 V for compounds 2 and 3; the capillary voltage was 3.5 kV for compounds 1 and 2 and 2.25 kV for compound 3. The source block temperature was 120 °C for compounds 1 and 3, and 150 °C for compound 2, the desolvation temperature was 300 °C for compounds 1 and 2 and 350 °C for compound 3, the dwell time was 0.1 s in all cases. Each compound and its asso-