Original Article

Multiple responses optimization in the development of a headspace gas chromatography method for the determination of residual solvents in pharmaceuticals

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A B S T R A C T

An efficient generic static headspace gas chromatography (HSGC) method was developed, optimized and validated for the routine determination of several residual solvents (RS) in drug substance, using a strategy with two sets of calibration. Dimethylsulfoxide (DMSO) was selected as the sample diluent and internal standards were used to minimize signal variations due to the preparative step. A gas chromatograph from Agilent Model 6890 equipped with flame ionization detector (FID) and a DB-624 (30 m × 0.53 mm i.d., 3.00 µm film thickness) column was used. The inlet split ratio was 5:1. The influencing factors in the chromatographic separation of the analytes were determined through a fractional factorial experimental design. Significant variables: the initial temperature (IT), the final temperature (FT) of the oven and the carrier gas flow rate (F) were optimized using a central composite design. Response transformation and desirability function were applied to find out the optimal combination of the chromatographic variables to achieve an adequate resolution of the analytes and short analysis time. These conditions were 30 °C for IT, 158 °C for FT and 1.90 mL/min for F. The method was proven to be accurate, linear in a wide range and very sensitive for the analyzed solvents through a comprehensive validation according to the ICH guidelines.

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1. Introduction

Residual solvents (RS) are volatile organic chemicals (VOCs) that are used or produced during the manufacturing process of active pharmaceutical ingredients (APIs) or excipients and cannot be completely removed. RS analysis of pharmaceutical products is necessary not only because they represent a potential risk for human health, due to their toxicity and their undesirable side effects, but also because they may affect the physicochemical properties of pharmaceutical products. Therefore, it is a mandatory requirement for health authorities in the world to accurately determine the levels of RS that are present in APIs or excipients [1–3].

The International Conference on Harmonization (ICH) in their guideline Q3C (RS) [4] classifies the regularly used solvents into three different classes based on their toxicity: Class 1 (solvents that should be avoided due to their known carcinogenic effect on human), Class 2 (solvents that should be limited in order to protect patients from potential adverse effects), and Class 3 (solvents regarded as less toxic and of a lower risk for human health). According to ICH guidelines, the levels of Class 1 and 2 solvents should be restricted to the concentration limits established by the guideline. As regard to Class 3 solvents, amounts of up to 0.5% (w/w) are considered acceptable. Moreover, the European Pharmacopoeia (Ph. Eur.) and the United States Pharmacopoeia (USP) establish the maximum allowable limits of the RS in the APIs and excipients, in accordance with the ICH guidelines.

The most appropriate analytical technique to determine RS and organic volatile impurities is the capillary gas chromatography (GC). The reasons why GC is highly recommended to this purpose are its excellent separation ability, low detection limits and the possibility of analyzing liquid or solid samples of variable and complex nature. Most of the detectors used in GC, and most of them are based on the formation of ions by one means or another. Among them, the flame ionization detector (FID) becomes the most popular [5]. Mass spectrometers can also be used as detectors, properly coupled to the chromatograph. The combination of GC with mass spectrometry has become a very popular and powerful tool [6].
Sampling techniques such as static headspace gas chromatography (SHGC) have gained ground against direct injection, mainly because of the many disadvantages associated with the direct injection of sample solution into the GC system [7]. In the SHGC procedure, the liquid or solid sample is placed in a sealed vial and thermostated until a thermodynamic equilibrium between the sample and the gas phase is reached. A known aliquot of the gas phase is then injected into the gas chromatograph and analyzed. Therefore, any potential interference, from non-volatile substances, is removed or minimized.

It is worth noting that sample diluent has an important influence on SHGC, affecting sensitivity, equilibration temperature and time. In addition, the diluent should be able to dissolve a large variety of samples, present a high boiling point and an acceptable stability [8]. There are several commonly used sample diluents for HSGC analyses, such as water, dimethylsulfoxide (DMSO), N,N-di-methylformamide (DMF), N,N-dimethylacetamide (DMA), benzyl alcohol (BA), 1,3-dimethyl-2-imidazolidinone (DMI) and mixtures of water/DMF or water/DMSO [9]. Water is a good diluent for water soluble samples, because it is clean, stable and inexpensive. However, many organic synthetic drug substances and drug products have low water solubility. When mixtures of water/DMF or water/DMSO are used as sample diluent, the solubility of many drug substances or drug products increases and the partition coefficient of the analytes decreases, resulting in a better transfer of analytes from the liquid to the gas phase. However, if the sample solution is equilibrated at or above the boiling point of the diluent, the inner pressure of the vial is dangerously increased [8]. This means that if water or water mixtures are chosen, the headspace (HS) equilibration temperature must be below 100 °C, leading to poor volatilization of a large number of solvents with higher boiling points. In contrast, the use of pure solvents such as DMSO, DMF, DMA or DMI generally provides an adequate solubilization of most of drug substances, and gives the possibility to incubate at temperatures above 100 °C.

The sample pre-treatment involved in the SHGC procedure is a critical step that may lead to experimental errors that can invalidate the results of the analysis. A strategy used to overcome errors in the preparative step is the addition of an internal standard (IS) [5]. The IS may be used for two different purposes. On the one hand, it can be a substance or substances added to the sample solution prior to injection in order to minimize the variability due to the volume injected into the column. On the other hand, this substance or substances is added to the sample at the earliest possible point in an analytical scheme to compensate any loss during the extraction step [10]. The IS must meet several criteria: it should elute near the peaks of interest, but it must also be well resolved from them; it should be chemically similar to the analytes of interest, but it must not react with any sample component; and it must be available in high purity.

The IS is added to the sample in a concentration similar to that of the analyte(s) of interest. When several components are analyzed, it may not be possible to fulfill this condition and a concentration of IS between higher and lower concentrations of the analytes to be analyzed must be chosen. Moreover, if many analytes are to be determined simultaneously, several internal standards may be used to meet the preceding criteria [10]. The development of such a complex analytical method requires an appropriate optimization procedure.

When attempting to find the factors (k) that have a significant influence on the system under study and then optimize such a system, experimental design is a powerful tool that is increasingly being used [11]. The advantages of experimental design are well known by chemometricians in particular and, increasingly, by the scientific community in general. Especially, its use in separation science has increased in the last few years [12–17].

Response surface methodology (RSM) is a collection of statistical and mathematical techniques used to develop, improve and optimize processes. One of the strengths of RSM is that it may work well in cases where there is incomplete knowledge about the state and behavior of the system under study as long as the system is stable and there is reasonable correspondence between set points and actual conditions [18]. There are several experimental designs suitable for this purpose, which vary in the number of experiments required and in the complexity of the mathematical models that can be built to describe the relationship between the factors and the responses under study [11]. Using a factorial design in the screening phase followed by a central composite design (CCD) in the optimization stage is an effective tool in the optimization of a process with several parameters [19].

In addition, when different objective functions (responses) have to be optimized simultaneously, the so-called “Derringer’s desirability function” is a useful strategy. This function is based on the idea that the quality of a product or process that has many features is completely unacceptable if one of them is outside a “desirable” limit. Its aim is to find operating conditions that ensure compliance with the criteria of all the involved responses and, at the same time, to provide the best value of compromise in the desirable joint response. This is achieved by converting the multiple responses into a single one, combining the individual responses into a composite function followed by its optimization [20,21]. In the first step of this methodology, a partial desirability function (di) must be created for each individual response using the fitted models and establishing the optimization criteria. The most desirable ranges for each design factor or response are selected by the user, based on the prior knowledge of the system including the researcher’s priorities during the optimization procedure. This involves deciding if these factors or responses have to be maximized, minimized, maintained in the range or reach a target value. In addition, a weight (wi) or emphasis is given to each goal. After that, the global desirability function (D) is obtained using the following equation:

\[
D = \left( \frac{d_1^{x_1} d_2^{x_2} \ldots \times d_n^{x_n}}{\sum_{i=1}^{n} d_i} \right)^{1/\sum_{i=1}^{n}}
\]

where \( n \) is the number of variables included in the optimization procedure, and \( r_n \) is the importance of each factor or response relative to the others.

The \( n \) variables, transformed in desirability functions, are combined in a unique function (\( D \)) to find out the best joint responses. The optimization procedure implies maximizing \( D \).

Derringer’s desirability function allows the analyst to find the experimental conditions (factor levels) to reach simultaneously the optimal value for all the evaluated variables. When \( D \) reaches a value other than zero, all the variables which are being simultaneously optimized can be considered having a desirable value. Meanwhile, if one of the responses is completely undesirable, \( D \) will be zero.

In this work, an SHGC method was developed, optimized and validated for the simultaneous determination of methanol, ethanol, ethyl ether, acetone, 2-propanol, acetonitrile, methylene chloride, hexane, isopropyl ether, ethyl acetate, 2-butanol, chloroform, tetrahydrofuran, cyclohexane, benzene, heptane, isooctane, triethylamine, 1-butanol, trichloroethylene, 1,4-dioxane, propyl acetate, pyridine, toluene, ethylene glycol, carbon tetra-chloride, DMF, m-xylene, p-xylene, o-xylene and DMSO as RS in raw material.
