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Establishment of a reference standard database for use in the qualitative and semi-quantitative analysis of pharmaceutical contact materials within an extractables survey by GC–MS

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ABSTRACT

The analysis of reference standards may be performed to enhance the qualitative and quantitative data generated by non-specific screening methods utilized in extractables studies performed on pharmaceutical contact materials. However, the establishment of a database containing relative response factor and retention index values obtained from these standards has not been published. In this study, the establishment of such a database for GC–MS, a methodology commonly included in extractables studies, on an intra-lab basis was investigated. A set of 154 organic compounds representing a diverse range of chemical functionalities and properties was analyzed at eight time points on four GC–MS instruments that represent the diversity of age and model at our laboratory. The results of this study have shown that any variance in relative response factor between instruments was not significant from a practical perspective as supported by the coefficient of variation values (n = 32), which were ≤15% and ≤10% for 75% and 45% of the compounds tested, respectively. Furthermore, the retention index of the compounds, as expressed by retention time and relative retention time, did not have more than a 2% coefficient of variation between instruments or columns in most cases. It was concluded that a database of these values could be established for future use in extractables studies on an intra-laboratory basis.

1. Introduction

Drug products come into contact with polymeric, elastomeric, and/or metallic materials during their manufacture, storage, and/or administration. These materials take the form of a wide range of components and/or systems including tubing, bags, vials, stoppers, bottles, syringes, and inhalers. While these components/systems ultimately serve beneficial and necessary functions, undesired interactions with the drug can occur. One such interaction involves the leaching of substances from the materials comprising the manufacturing/storage/delivery system(s) and into the drug formulation. Because these leached substances (*leachables*) may negatively impact the safety and/or efficacy of the drug product, their presence must be characterized, and if needed, justified via a toxicological assessment.

One of the first laboratory based exercises performed to assess the safety/compatibility of the materials comprising a pharmaceutical product's manufacturing, storage, and/or delivery system is an extractables study [1–3]. The purpose of this study is to generate a profile of compounds that may be extracted (*extractables*) from these material(s) using a range of solvents and contact scenarios. Based on the extractable profile obtained for the material(s), compounds that may be seen as leachables can be determined for further assessment.

Extractables studies are, by nature, investigational because it is not unequivocally known what substances are present in a given material. As such, these studies are primarily qualitative in nature and employ instrumentation that can support these needs; most commonly, a chromatographic system in tandem with a mass spectrometer. In addition to the qualitative aspect of the analysis, a preliminary quantitative evaluation of the data is performed to provide an estimate of the amount of each substance extracted from the material. This is achieved by the inclusion of a surrogate standard or standards to provide a generic response for quantification.

Abbreviations: TMS, trimethylsilyl; TFA, trifluoroacetyl; cv, coefficient of variation; RRF, relative response factor; RT, retention time; ANOVA, analysis of variance.

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The qualitative and quantitative data obtained from an extractables study can be enhanced by the analysis of authentic reference material of the tentatively identified compounds. Data obtained from this analysis allows for the determination of a compound's retention index and mass spectral fragmentation pattern for identification purposes, as well as a RRF which is used to correct for any difference in response between the surrogate standard and the compound.

It is apparent that a database of retention index and RRF values would be more convenient, less time consuming, and more cost effective than determining these values independently for every study. However, the reproducibility of these values over time on an intra-laboratory basis is unknown. To this end, it was hypothesized that the RRF and retention index values obtained from the analysis of organic compounds by gas chromatography used in tandem with a mass selective detector (GC–MS), a common methodology utilized as part of an extractables study, are sufficiently reproducible with variations in instrument age, model, column, chemist, and time to allow for the establishment of a database on an intra-laboratory basis.

The goal of this study was to test this hypothesis by comparing the variability of RRF and retention index values obtained with multiple instruments, analytical columns, and chemists over a period of time within a single laboratory. A GC–MS method previously established for the screening of semi-volatile extractables [4] was used to determine the RRF and retention index values for a set of 154 organic compounds representing a diverse range of chemical functionalities and physical properties. After analysis by the method, the RRF, retention time, and relative retention time (RRT) to acetophenone-d_5, the method's surrogate standard, were determined for each compound. An evaluation of the variability of the RRF and retention index on each instrument, and between all instruments, was then performed using statistical analyses in order to support or reject the hypothesis.

2. Experimental

2.1. Study design

Four GC–MS systems, four analytical columns, and four chemists were selected to obtain the RRF and retention index values for the purposes of this study. The relevant details for each GC–MS instrument can be found in Table 1. These specific systems were selected to encompass the range of age, make, and model of GC, MS, and auto-sampler components available at our laboratory, thus ensuring the experiment is evaluating the full variability of the parameters that may be encountered from one analysis to the next. The four analytical columns used in the study were identical in terms of their make and model. However, in order to represent a range of age and use, two of the columns were new/unused while the other two columns had been used for 200–300 injections prior to this study. The four chemists selected to perform the work had various levels of experience, but all were trained and capable of performing the analytical work associated with this study. This was considered to be the least impactful variable in this study because the chemist has no involvement in the performance of the instrument, but in the interest of being representative of this variable it was still accounted for in this study. Variation between chemists due to differences in sample preparation was eliminated by utilizing the same standard solutions throughout the study as opposed to having these prepared by each chemist.

The subjects of study for this experiment were a set of 154 organic compounds, which are listed in Table 2. These compounds were selected to represent a diverse range of volatility, polarity, and chemical functionality, as well as compounds known, or reported [3,5,6] to be commonly encountered as extractables from common materials used to construct pharmaceutical packaging, manufacturing, and delivery systems.

To generate a dataset for evaluation, the RRF and retention index values were measured 8 times on each instrument over the course of approximately 9 months, with each analysis performed approximately 1 month after completion of the previous time point. This testing schedule was utilized to determine if minor changes in the system over time, such as analyses executed under different mass spectrometer tune parameters, preventative maintenance cycles, and/or states of cleanliness impacted the response. For each analysis, the analytical column and chemist were randomized as described in Table 3 in order to distribute/randomize any variability that each factor/variable may contribute to the overall data set.

Within the context of this study, the RRF is defined as the response factor of a given substance relative to the response factor of the acetophenone-d_5 surrogate standard as defined in Eq. (1):

$$RRF = \frac{A_{Ext}}{A_{SS}} \times \frac{C_{SS}}{C_{Ext}} \quad (1)$$

Where A_{Ext} is the peak area response of the extractable, A_{SS} is the peak area response of the surrogate standard, C_{SS} is the concentration of the surrogate standard, and C_{Ext} is the concentration of the extractable.

The retention index was expressed as the compound's RT (in minutes) on the column, and its RRT was defined as the ratio of the compound's retention time divided by the acetophenone-d_5 retention time in each analysis.

Once the dataset was generated, it was evaluated using a statistical analysis to assess the variability of RRF and retention index within each instrument and between the instruments over the course of the study. This was accomplished using hypothesis testing, specifically an ANOVA, in addition to calculation of the means, standard deviations, and *cv*. An ANOVA was employed to provide an objective way of evaluating variance in the RRF values obtained between the instruments. However, an ANOVA analysis was not performed on the retention index values because these are potentially affected by both the instrument and column. Instead, the absolute level of variability is assessed to determine reproducibility, or lack thereof.

2.2. Chemicals and reagents

A majority of the reference standard material utilized in this study was obtained from Tokyo Chemical Incorporated – America (Portland OR) or Sigma-Aldrich (Saint Louis, MO). 7,9-Di-*tert*-butyl-1-oxaspiro[4,5]deca-6,9-diene-2,8-dione was synthesized at our laboratory.

Water was produced by an in-house water purification system at a resistivity of 18.2 MΩ cm. Solvents such as methanol or ethyl acetate were obtained from Sigma-Aldrich or Honeywell Burdick and Jackson. BSTFA:TMCS (99:1, N,O-Bis(trimethylsilyl) trifluoroacetamide: Trimethylchlorosilane) and MBTFA (N-methyl-bis(trifluoroacetamide)) derivatization reagents were derivatization grade purchased from Sigma-Aldrich.

2.3. Preparation of standard mixes

Stock solutions of the model compounds were prepared at 2000 μg/mL in ethyl acetate. Working standards were prepared at 10 μg/mL in ethyl acetate using a serial dilution from the stock. The acetophenone-d_5 surrogate standard was included in each working standard at a concentration of 10 μg/mL. In total, ten working solutions were prepared for analysis in this study to insure each compound was adequately resolved from the other compounds