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Impurity profiling of drug candidates: Analytical strategies using reversed-phase and mixed-mode high-performance liquid chromatography methods☆

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ABSTRACT

The development of new active pharmaceutical ingredients (API) requires accurate impurity profiling. Nowadays, reversed-phase HPLC (RPLC) on C18 stationary phase is the method of first choice for this task and usually employed in generic screening methods. However, this method sometimes fails, especially when the target analyte is not sufficiently retained, making impurity analysis difficult or even impossible. In such cases, a second method must be available.

In the present paper, we compare the merits of RPLC on C18 phase to those of previously optimized alternative methods, based on the analysis of a large and diverse set of small-molecule drug candidates. Various strategies are considered: RPLC on C18 phase but with different mobile phase composition (acidic or basic), RPLC with a pentafluorophenyl stationary phase, or mixed-mode HPLC with both bimodal and trimodal stationary phases. First, method performances were compared in terms of response rate (proportion of compounds eluted) and peak shapes for a large set of synthetic drugs (140) with structural diversity and their orthogonality was evaluated. Then a subset of compounds (25) containing varied impurity profiles was used to compare the methods based on the capability to detect impurities and evaluate the relative purity of the API.

1. Introduction

Pharmaceutical manufacturers must guarantee the efficacy and limited toxicity of all synthesized products [1]. For this reason, the identification and quantification of residual solvents [2] and impurities (also called impurity profiling) requires strict control and high-performance chromatographic tools. For this task, while gas chromatography is useful to analyze volatile chemicals [3], reversed-phase high performance liquid chromatography (RPLC) with C18 stationary phase combined with UV and mass spectrometric (MS) detection modes is the gold standard in most companies [4–6], with about 90% of low-molecular weight pharmaceutical compounds carried out by RPLC [7]. A generic, universal method is usually desired in order to provide a fast response for any new product [8]. However, due to the complexity of some of the synthesized active pharmaceutical ingredients (API) causing a larger number of impurities and variety of polarities encountered, RPLC mode sometimes fails. Indeed, with C18 phases, one type of interaction between stationary phase and analytes dominates: the dispersion interactions. Besides these major interactions, secondary hydrophilic interactions can occur between residual silanols and analytes. These secondary interactions may cause increased retention of polar analytes, but they mostly cause peak shape deformation and loss of efficiency, especially for basic compounds. To avoid these problems, modern stationary phases include protection strategies against silanophilic interactions. As a result, RPLC mode is best suited for the separation of hydrophobic compounds but either fails to retain polar or charged compounds, or does retain them but with poor peak shape, yielding difficult or even impossible impurity profiling. It is then essential to turn to other analytical methods that offer different opportunities in terms of selectivity and specificity. Firstly, for ionizable species (acidic or basic API) with low polarity, the simplest and fastest method to implement is to adjust the pH of the mobile phase. When this

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strategy is not sufficient, the second easiest method may be to replace the C18 phase with a different sort of stationary phase. In this regard, column manufacturers have developed a variety of stationary phases, promising different selectivities. In particular, in the recent years, fluorinated ligands have been established as an alternative to octadecyl ligands, because of the unique selectivity [9,10] and orthogonality [11] they offer. In particular, pentafluorophenyl (PFP) phases have been found to offer superior selectivity and peak efficiency, compared to perfluoroalkyl phases [12]. The unique selectivity can be explained by the different interactions taking place between the stationary phase and the analyte: π–π interactions (between the analyte and electron acceptor PFP group), strong dipole–dipole and ionic interactions (related to the negative partial charges on the fluorine atoms [13]). A large number of PFP columns are now commercially available with most column manufacturers proposing these phases in their portfolio. PFP phases are particularly powerful for the analysis of basic [9,14], polar [15,16] and halogenated compounds [17] or to separate halogenated from dehalogenated compounds [6]. PFP phases also proved their worth for impurity profiling [18] and the separation of isomers [19,20].

Alternatively, other retention modes can be considered. Hydrophilic interaction liquid chromatography (HILIC) is adequate for polar species [21], thus not the preferred method when a large chemical space must be explored [22]. While compatibility to MS detection is excellent [23], equilibration times are excessively long. Mixed-mode HPLC (MM-HPLC) [24] involves the combined use of two (or more) retention mechanisms in a single chromatographic system (reversed-phase, ion exchange or hydrophilic interaction chromatography for example). MM-HPLC shows great flexibility, versatility and high orthogonality with RPLC in the separation of various polar and non-polar pharmaceutical compounds [25–28] and retains good compatibility to MS detection [29,30]. MM-HPLC covers a wide range of applications in the pharmaceutical field (APIs, impurities, synthetic intermediates and degradation products) and has shown possible applicability for impurity profiling of small molecules for therapeutic use [31–33].

In this study, we compare the performance of RPLC with a C18 phase (in acidic or basic conditions) to RPLC with a PFP phase, and to two mixed-mode HPLC methods (with bimodal and trimodal stationary phases combining reversed-phase and ion-exchange mechanisms) coupled to UV and MS detection for impurity profiling of drug candidates. First, method performances were compared in terms of response rate and peak shapes for a large set of synthetic drugs (140), then a subset of compounds (25) containing varied impurity profiles was used to compare the methods based on the capability to detect impurities and evaluate the relative purity of the API. Because these methods are intended for a laboratory analyzing drug candidates at an early stage of drug development, accurate quantitation is not desired but it is expected that the most abundant impurities (above 0.04%) should be separated from the main compound and detected.

2. Material and methods

2.1. Chemicals and solvents

For the evaluation of chromatographic performance and orthogonality, the set of 140 drug candidates was obtained from Servier Research Laboratories (Suresnes, France). The structures are confidential, but they were previously described [34]. Briefly, molecular weights ranged from 150 to 750 g/mol, and log P values varied between −1.9 and 7.5, with a large majority of positive values. Moreover, as is usual in compounds of pharmaceutical interest, a large portion of them have basic functions (80%). 14 mixtures of 10 compounds each were prepared at 1 mg/mL in acetonitrile-water (1:2 v/v) for RPLC methods (C18 and PFP phases) or in a 50:50 mixture of acetonitrile-ammonium acetate buffer (pH 7, 60 mM in water) for mixed-mode HPLC methods.

For the sample applications of impurity profiling, a subset of 25 diverse drug candidates was selected from the initial set. In this subset, molecular weights ranged from 200 to 670 g/mol and log P values varied between 0 and 7.5. 74% of them had a basic function and 12% had an acidic function. Considering that the larger selection was representative of the diversity of structures normally encountered in this research laboratory, this subset adequately represents the same diversity. They were selected so as to reflect the diversity of samples to be processed every day at the laboratory: they included some compounds with high purity (above 95%) and others with lower purity and a large number of impurities. They were injected individually (dilution solvent as described above for 10-component mixtures) to evaluate the relative purity of the main compound and provide an estimated quantification of major impurities. Two of them have a pair of diastereoisomers as principal ingredient thus 27 substances were considered for purity evaluation but 25 substances were considered for the number of impurities.

Water was obtained from a Milli-Q Purification System from Millipore (Millipore SAS, France), HPLC-grade acetonitrile was purchased from Merck (VWR international SAS, France), methanesulfonic acid, trifluoroacetic acid and ammonium acetate were provided by Sigma-Aldrich (Sigma Aldrich Chimie, France).

2.2. Instruments

The UHPLC systems used in all cases were ACQUITY UPLC® I-Class from Waters Corporation. They were equipped with a binary solvent delivery pump compatible with mobile phase flow rates up to 2 mL/min and pressures up to 827 bar, an autosampler that included partial loop volume injection system, 2-position column oven compatible with 150 mm length columns and a photodiode-array (PDA) detector. The extracted wavelength for UV detection was fixed at 210 nm. Frequency was set at 20 pts/s and resolution at 1.2 nm.

For analyses performed in RPLC on C18 phase and acidic conditions, an ACQUITY QDa® single-quadrupole mass detector (Waters Corporation) with electrospray ionization source was used. An isocratic solvent manager was used as a make-up pump and was positioned before the mass detector. The main flow stream was then split by the on-board flow-splitter assembly. With this system only 1/10th of the column flow enters the MS. For analyses performed in all other conditions, an ACQUITY SQD® single-quadrupole mass detector (Waters Corporation) with electrospray ionization source was used. MS operating conditions are specified below. Note that no comparison of the MS responses is used in the following, as the MS detection served only at identifying the peaks of API and major impurities.

2 μL of each sample (10-compounds mixture or single drug candidate) were injected with a 10 μL-loop and acetonitrile was used to rinse the system.

MassLynx® software (V4.1) was used for system control and data acquisition. Empower® 3 was used for integration of peaks for column efficiency measurements. Waters Data Converter (V2.1) was used to convert data from MassLynx to Empower.

2.3. Analytical methods

2.3.1. RPLC methods with C18 phase

At Servier Research laboratories, it is common practice to use two complementary reversed-phase UHPLC methods that were optimized several years ago to maximize the chances to identify and estimate correctly all impurities. Both methods make use of the

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