Journal of Pharmaceutical and Biomedical Analysis 86 (2013) 174–181

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Single column comprehensive analysis of pharmaceutical preparations using dual-injection mixed-mode (ion-exchange and reversed-phase) and hydrophilic interaction liquid chromatography

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ARTICLE INFO

Article history:
Received 29 May 2013
Received in revised form 1 August 2013
Accepted 3 August 2013
Available online 14 August 2013

Keywords:
Mixed-mode chromatography
Ion-exchange
HILIC
Active pharmaceutical ingredients
Excipients

ABSTRACT

The comprehensive separation and detection of hydrophobic and hydrophilic active pharmaceutical ingredients (APIs), their counter-ions (organic, inorganic) and excipients, using a single mixed-mode chromatographic column, and a dual injection approach is presented. Using a mixed-mode Thermo Fisher Acclaim Trinity P1 column, APIs, their counter-ions and possible degradants were first separated using a combination of anion-exchange, cation-exchange and hydrophobic interactions, using a mobile phase consisting of a dual organic modifier/salt concentration gradient. A complementary method was also developed using the same column for the separation of hydrophilic bulk excipients, using hydrophilic interaction liquid chromatography (HILIC) under high organic solvent mobile phase conditions. These two methods were then combined within a single gradient run using dual sample injection, with the first injection at the start of the applied gradient (mixed-mode retention of solutes), followed by a second sample injection at the end of the gradient (HILIC retention of solutes). Detection using both ultraviolet absorbance and refractive index enabled the sensitive detection of APIs and UV-absorbing counter-ions, together with quantitative determination of bulk excipients. The developed approach was applied successfully to the analysis of a dry powder inhalers (Flixotide®, Spiriva®), enabling comprehensive quantification of all APIs and excipients in the sample.

1. Introduction

Some pharmaceutical products are formulated to include multiple active pharmaceutical ingredients (APIs), typically with the aim of producing a synergetic effect, which is ideally more pronounced than using the component drugs individually. For example, in the treatment of asthma, some of the APIs that are known to be employed in such binary and ternary preparations include fluticasone propionate, salmeterol xinafoate, tiotropium bromide and ipratropium bromide. Salmeterol xinafoate and fluticasone propionate are frequently found in combinatorial formulations where the former component acts as a β2-agonist utilised for the treatment of chronic obstructive pulmonary disease (COPD), while the latter component is a corticosteroid possessing anti-inflammatory properties [1,2].

Traditionally, reversed-phase high performance liquid chromatography (RP-HPLC) has been the standard technique for the quantification of multiple APIs in pharmaceutical preparations, with either UV absorbance or mass spectrometric (MS) detection. For example, Murnane et al. [1] developed a RP-HPLC method for separating neutral hydrophobic APIs, with their respective lipophilicity parameters provided and expressed as log *P* values, such as fluticasone propionate ($\log P = 2.49$), salmeterol ($\log P = 3.20$) and the negatively charged (but relatively hydrophobic) xinafoic acid ($\log P = 3.42$), which was then applied successfully to the analysis of pressurised metered dose inhaler formulations. Van Eenoo et al. [2] determined salmeterol in the urine of horses after inhalation using RP-HPLC–MS, whilst Čápka and Carter [3] used the same analytical approach to study salmeterol in the presence of fluticasone. The use of RP-HPLC for the quantification of fluticasone has also been extensively reported, either singularly or in the presence of other APIs [4–6]. Tiotropium ($\log P = -1.12$) has been determined in human plasma by Wang et al. [7] and Ding

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http://dx.doi.org/10.1016/j.jpba.2013.08.003

et al. [8] using standard RP-HPLC, whilst Türck et al. [9] employed the same approach to analyse both plasma and urine samples for the drug. Using RP-HPLC–MS, Leusch et al. [10] developed a method for the simultaneous determination of positively charged tiotropium and ipratropium ($\log P = -3.71$) in biological fluids from both rats and dogs, whilst Jacobson and Peterson [11] simultaneously analysed a number of APIs in nebuliser solutions, including ipratropium bromide, using an ion-pair modulated RP-HPLC approach. It is clear from the above examples, and also from the broader literature, that the majority of chromatographic assays for APIs are based on the use of standard using octadecyl silica (C_{18}) reversed-phase columns [12–14]. It is also clear that such phases exhibit limited selectivity for medium to high polarity cationic and anionic solutes [15,16]. However, so-called 'comprehensive analysis' of pharmaceutical formulations requires the determination of all of the components therein, including APIs, their counter-ions, impurities, degradants and excipients, which excludes traditional RP-HPLC as a singular analytical solution. Some attempts have been made to develop quantitative assays capable of application to the above broad range of solutes, but simple, rapid and cost-effective methods have been very limited in number [13,17].

The limitations of reversed-phase column selectivity for the above complex mixtures may potentially be addressed using the so-called 'mixed-mode' phases. A variety of mixed-mode columns have recently appeared and these stationary phases typically combine hydrophobic and ion-exchange functional group chemistries, thereby offering enhanced selectivity for mixtures of neutral, hydrophobic analytes and charged analytes having varying degrees of hydrophobicity. For example, Imtakt (Prospect Heights, USA) have developed mixed-bed chromatographic columns (marketed as the Scherzo column range), which are packed with a mixture of two types of silica particles, the first of which exhibits both hydrophobic and cation-exchange properties, whilst the second type combines hydrophobic and anion-exchange selectivity. This mixed-mode product is available as both high and low ion-exchange capacity columns, and with either weak or strong ion-exchange (IEX) properties [18]. SIELC Technologies (Philadelphia, USA) have also produced a number of new mixed-mode stationary phases, in the first instance based upon particles modified with a hydrophobic ligand with an embedded anion-exchange (AEX) functionality and a surface cation-exchange (CEX) group (Obelisc R range) [18,19]. A derivative of this phase containing an additional hydrophilic spacer is marketed under the name of Obelisc N [20,21]. In addition, this manufacturer also produces a range of other mixed-mode columns under the name of Primesep [21–24]. Recently, a mixed-mode agglomerated porous silica particle phase has been released under the name Acclaim Trinity P1 by Thermo Scientific (Sunnyvale, USA) [18,25–27]. The stationary phase particles consist of an inner core containing hydrophobic C_{10} chains with anchored weak anion-exchange end-groups, whilst the surface of the particle is agglomerated with electrostatically bound latex particles containing attached strong CEX groups [26]. This zwitterionic/hydrophobic column has been used for a number of pharmaceutical applications involving the simultaneous determination of APIs and their counter-ions, based upon combined IEX and hydrophobic interactions [18,21,25–27]. A further feature of the Acclaim Trinity column is its ability to retain hydrophilic molecules using mobile phases containing high levels of organic solvent through a hydrophilic interaction liquid chromatography (HILIC) mechanism, as demonstrated by Liu and Pohl [25]. This mode of application is important when considering the determination of hydrophilic carbohydrates used as excipients in pharmaceutical preparations. Most recently, the potential of the Trinity column has been further demonstrated in a 2-dimensional chromatographic system utilising a combination of size exclusion chromatography (1st dimension) with mixed-mode (Acclaim Trinity) chromatography (2nd dimension) [17]. In this example, the simultaneous assay of a protein API and several excipients in the form of anions, cations, a non-ionic hydrophobic surfactant and hydrophilic sugars was demonstrated.

The present study describes an approach to achieve single column, quantitative comprehensive pharmaceutical analysis in a rapid and cost-effective manner, using the Acclaim Trinity stationary phase. The goal was to design a method capable of the separation, detection and quantification of all APIs, degradants, impurities, counter-ions and excipients in pharmaceutical preparations. In addition, a robust approach was sought which would be readily transferable to a routine quality control or research and development laboratory, and which was also ideally compatible with electrospray ionisation MS (ESI-MS) detection.

2. Materials and methods

2.1. Chemicals

De-ionised water was obtained using a Millipore (Bedford, MA, USA) Milli-Q water purification system. Ipratropium bromide and trisodium citrate dihydrate were purchased from Sigma (Sydney, Australia). Fluticasone propionate, salmeterol xinafoate, xinafoic acid, Spiriva® inhalation powder (Boehringer Ingelheim, Berkshire, UK) and Flixotide® accuhaler (GlaxoSmithKline, Uxbridge, Middlesex) were donated by Pfizer Global Research and Development (Sandwich, England). All other chemicals were obtained from the following suppliers: acetonitrile (HPLC grade) and ammonia (28%) from Ajax Finechem (Sydney, Australia), hydrochloric acid from Merck (Germany), glacial acetic acid (99.7%) from BDH (Port Fairy, Australia), and tiotropium bromide from OChem Incorporation (Chicago, USA).

2.2. Instrumentation

A Waters (Milford, MA, USA) 2695 Alliance HPLC system equipped with a Waters 996 Photodiode Array Detector (PDA) was utilised for all HPLC experiments using photometric detection. For HILIC studies and detection of excipients, a Waters 410 Differential Refractometer Detector was used. All system controls, data collection and processing were carried out using Waters Empower Pro software. An Acclaim Trinity P1 (100× 3.0 mm I.D.; 3 μm) column was utilised throughout this study, sourced from Thermo Scientific (formerly Dionex Corp.), (Sunnyvale, USA). Plotting of chromatograms and baseline subtraction for gradient separations was conducted using Origin 8.6 software.

For dual injection separations, the workstation software used in this work (Millenium) did not allow the system to be 'programmed' to carryout two injections in a single run (although most modern chromatographic software allows this). However, sequential chromatographic data files were simply combined using Origin 8.6 software to overcome this limitation. A alternative approach is the use of a simple data logger from the detector, to collect chromatograms as data files over the full period covering two or more injections.

2.3. Methods

Mixtures of acetonitrile (ACN) and buffer solutions were filtered using nylon membranes (47 mm diam., 0.22 μm pore size). Solutions were degassed using an ultrasonic bath (Soniclean). The pH of each mobile phase was measured before the addition of the organic modifier. Gradient elution was performed using pump inlet lines A, B and C, where line A contained water, line B was a mixture of 95% ACN/5% water, and line C contained ammonium acetate (200 mM) buffer.