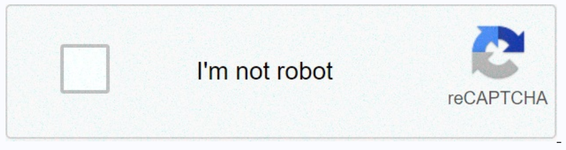

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Stability indicating. Stability indicating method.

Smela M J (2005) Regulatory considerations for stability indicating analytical methods in drug substance and drug product testing. Am Pharm Rev 8:51–54. Google Scholar ICH Q5 (1996) Stability testing of biotechnological/biological products. Google Scholar ICH Q1A (2003) Stability testing of new drug substances and products. Google Scholar ICH Q1E (2003) Evaluation of stability data. Google Scholar ICH Q3A (R) (2003) Impurities in new drug substances. Google Scholar ICH Q3B (R) (2003) Impurities in new drug products. Google Scholar ICH Q6A (2000) Guidance on specifications: test procedures and acceptance criteria for new drug substance and products: chemical substances. Google Scholar ICH Q2A (R) (1995) Guideline for industry, text on validation of analytical procedures. Google Scholar ICH Q2B (R) (1996) Guideline on validation of analytical procedures: methodology. Google Scholar Reynolds DW, Facchine KL, Mullaney JF, Alsante KM, Hatajik TD, Motto MG (2002) Available guidance and best practices for conducting forced degradation studies. Pharm Technol 26:48–56. Google Scholar Reynolds DW (2004) Forced degradation of pharmaceuticals. Am Pharm Rev 7:56–61 Google Scholar Thatcher SR, Mansfield RK, Miller RB, Davis CW, Baertschi SW (2001) Pharmaceutical photostability: a technical guide and practical interpretation of the ICH guideline and its application to pharmaceutical stability – Part I. Pharm Technol N Am 25:98–110.CAS Google Scholar Baertschi SW (2005) Pharmaceutical stress testing: predicting drug degradation. Taylor & Francis, Boca Raton. Google Scholar FDA (2003) Guidance for industry INDs for phase 2 and 3 studies; chemistry, manufacturing, and control information. KM, Martin L, Baertschi SW (2003) A stress testing benchmarking study. Pharm Technol 27:60–72.CAS Google Scholar Kats M (2005) Forced degradation studies: regulatory considerations and implementation. BioPharm Int 18:7. Google Scholar Alsante KM, Ando A, Brown R, Ensing J, Hatajika TD, Kong W, Tsuda Y (2007) The role of degradant profiling in active pharmaceutical ingredients and drug products.

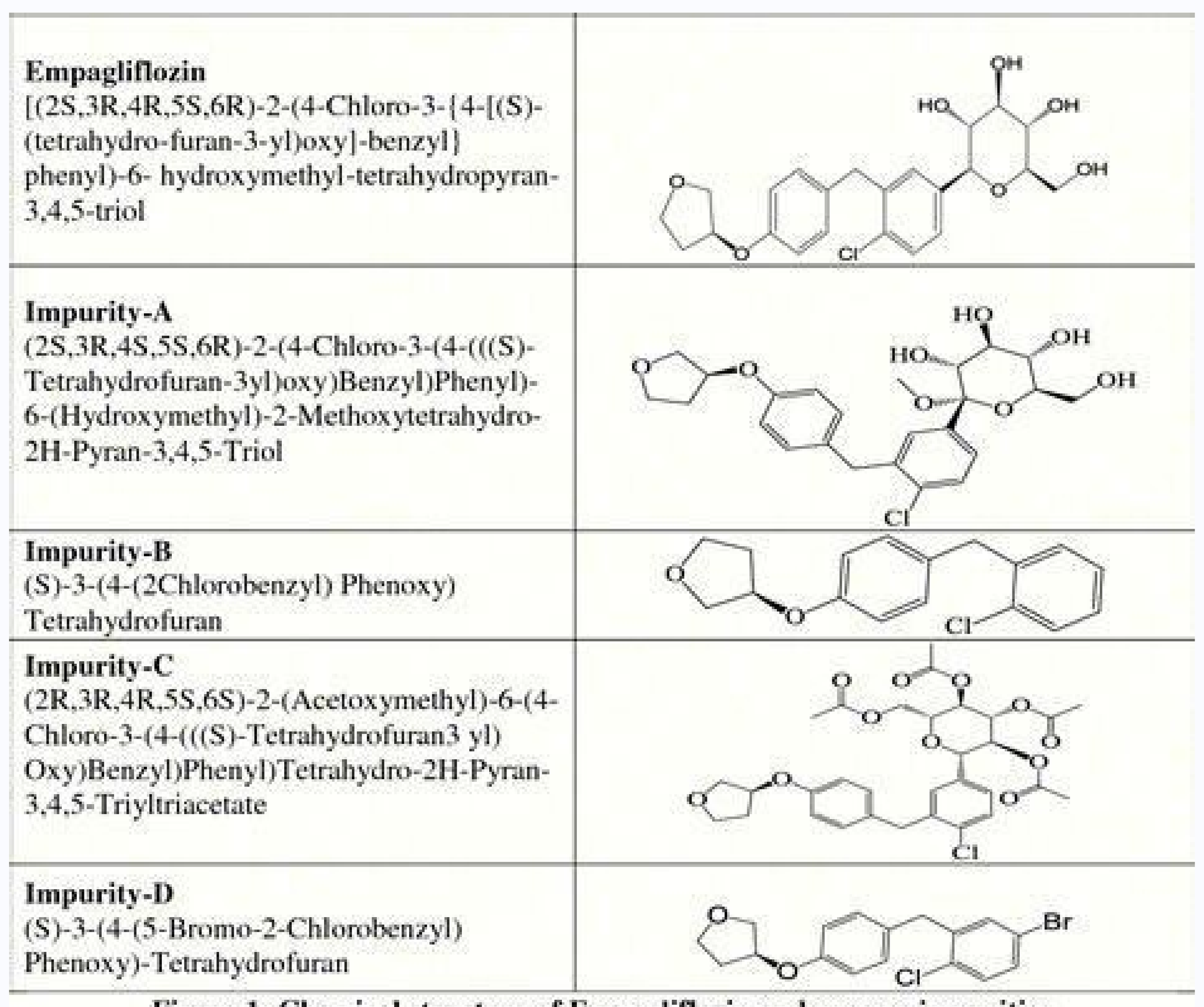

Figure 1: Chemical structure of Empagliflozin and process impurities

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Development and validation of a stability-indicating RP-HPLC method of cholecalciferol in bulk and pharmaceutical formulations: Analytical quality by design approach

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Key words:
Cholecalciferol, analytical quality by design, Taguchi orthogonal array design, Box-Behnken design, method validation, forced degradation studies.

ABSTRACT
The present article utilized analytical quality by design (AQbD) methodology to optimize chromatographic conditions for the routine analysis of Cholecalciferol (CHL). Taguchi orthogonal array design and Box-Behnken design were employed to screen and optimize critical method parameters for augmenting the method performance. The optimal chromatographic separation was attained on Eurosphere® 100-5, C8 (250 × 4.6 mm i.d., 5 μm) column in an isocratic elution mode using methanol:acetonitrile (50:50, % v/v) as mobile phase at a flow rate of 1.0 ml/minutes and photodiode array detection at 265 nm. The optimized chromatographic method was successfully validated as per International Council for Harmonisation Q2 (R_1) guidelines. The method was found to be linear (r^2 = 0.9991) in the range of 20–100 IU/ml. Limit of detection and limit of quantitation were found to be 10 and 20 IU/ml. The precision, robustness, and ruggedness values were within the acceptance limits (relative standard deviation < 2). The percent recovery of in-house developed 400 IU mouth dissolving tablets and marketed Tayo 60k tablets were found to be 99.89% and 101.46%, respectively. The forced degradation products were well resolved from the main peak suggesting the stability-indicating the power of the method. In conclusion, the AQbD-driven method is highly suitable for analysis of CHL in bulk and pharmaceutical formulations.

INTRODUCTION

During product development, quality assurance of pharmaceutical molecules is a matter of great concern in the pharmaceutical industry. Analytical methods are critical elements in product development due to their roles in assisting with process development and product quality control. Poor analytical methods can lead to inaccurate results, resulting in misleading information that may be detrimental to the drug development program. In an endeavor to address such plausible crucial issues, different Pharma regulatory agencies, such as International Council for Harmonisation (ICH) and U.S. Food and Drug Administration, have been transforming by adopting quality by design (QbD) principles to circumvent these quality crises. Recently, ICH has announced new guideline ICH Q14 on analytical procedure development and revision of Q2 (R_1) analytical Validation Q2 (R_1)/Q14 (ICH Assembly, Kobe, Japan, June 2018).

The traditional liquid chromatographic method development for any drug molecule was performed by a trial and error approach, for example, by varying one-factor-at-a-time and examines the resolution of the result until the best method was found. It is a time-consuming process and required a large amount of manual data interpretation. This approach requires typically some experimental trials, and in some circumstances, the established method requires further modification in method or a supplementary purification stage when scaled up, consequently slackening the drug development process (Monks et al., 2011; Peraman et al., 2015). Moreover, this type of method development provides a limited understanding of a method's capabilities and robustness. This can be overcome by applying QbD principles to the analytical method development as it uses a statistical experimental design to

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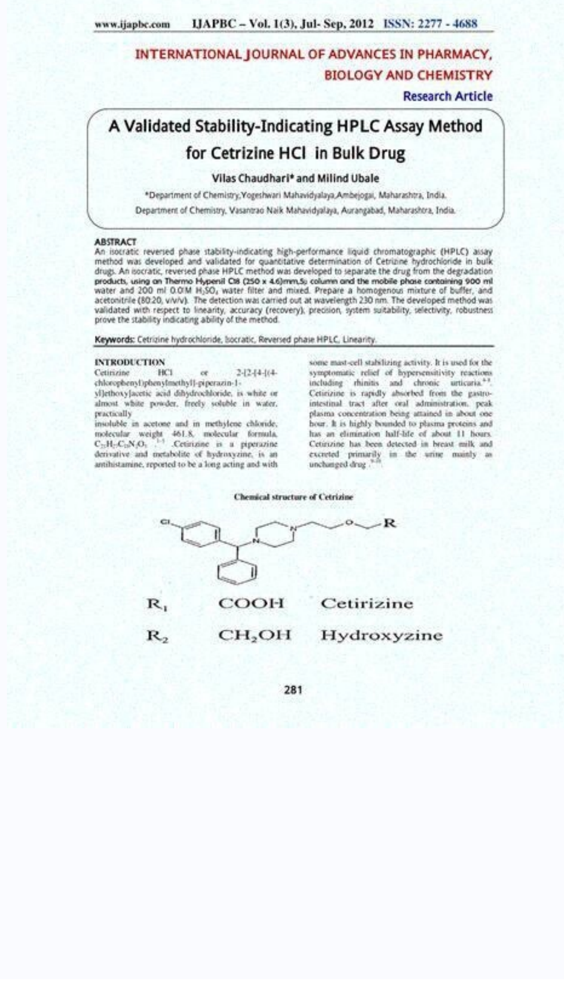
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Research Article

A Validated Stability-Indicating HPLC Assay Method for Cetrizine HCl in Bulk Drug

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Department of Chemistry, Vasantrao Naik Mahavidyalaya, Aurangabad, Maharashtra, India.

ABSTRACT
An isocratic reversed phase stability-indicating high-performance liquid chromatographic (HPLC) assay method was developed and validated for quantitative determination of Cetrizine hydrochloride in bulk drugs. An isocratic, reversed phase HPLC method was developed to separate the drug from the degradation products, using an Thermo Hypersil C8 (250 x 4.0mm,5μ column and the mobile phase containing 900 ml water and 200 ml 0.0M H_2SO_4 water filter and mixed. Prepare a homogenous mixture of buffer, and acetonitrile (80:20, v/v/v). The detection was carried out at wavelength 230 nm. The developed method was validated with respect to linearity, accuracy (recovery), precision, system suitability, selectivity, robustness prove the stability indicating ability of the method.

Keywords: Cetrizine hydrochloride, Isocratic, Reversed phase HPLC, Linearity.

INTRODUCTION

Chemical structure of Cetrizine

R_1	COOH	Cetirizine
R_2	CH_2OH	Hydroxyzine

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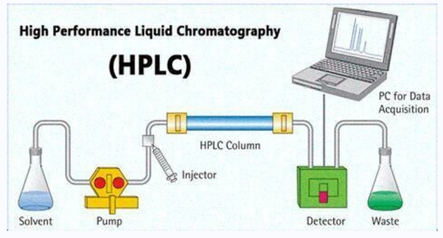

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Full Length Research Paper

Stability- indicating HPLC method for the determination of efavirenz in bulk drug and in pharmaceutical dosage form

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Accepted 1 December, 2009

A rapid and accurate isocratic HPLC method was developed and validated for the assay of Efavirenz (EFA) in bulk and pharmaceutical dosage forms. The chromatographic conditions comprise of a Novapak phenyl column. A mixture of phosphate buffer and Acetonitrile was used as mobile phase. Quantitation was achieved by UV detection at 247 nm. A linear response ($r^2 \geq 0.999$) was observed in the range of 0.05 - 0.15 mg/mL. The method was validated for accuracy and precision. The proposed method can be used for quality control assay of EFA in bulk and in finished dosage form and for the stability studies as the method separates EFA from its degradation products and excipients.

Key words: Efavirenz, stability indicating HPLC, dosage form, UV detection.

INTRODUCTION

Chemically, efavirenz is (S)-6-chloro-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2H-,1-benzoxazin-2-one. EFA is a non-nucleoside reverse transcriptaseinhibitor (NNRTI) and is used as a part of highly active antiretroviral therapy for the treatment of a human immunodeficiency virus (Figure 1). The drug is used in combination with other anti retroviral agents for the treatment of HIV-1 infection in children and adults. The usual dose of EFA is 600 mg per day (usually given at bed time).

Several methods have been reported for determination of efavirenz. Careri et al. (1993) achieved separation of alkynes by reversed phase HPLC using ruthenium complexes. Gita et al. (2008) and Agnes et al. (2008) reported separation of efavirenz in human plasma by using reversed phase HPLC technique using C18 column. So far in our knowledge only one stability indicating method has been reported using cyano column for the determination of efavirenz (Montgomery ER et al., 2001). The disadvantage of the method is that its run time is about 15 min and gradient separation. The Indian pharmacopoeia (Indian Pharmacopoeia, 2007) Iso published isocratic HPLC method for the assay of EFA.

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The Run time is about 15 min. The present work describes a stability indicating LC method using phenyl column. The method is rapid, accurate and precise. The run time is of 6.6 min and the drug is well separated from all of its degradants. Therefore the method can be employed as stability -indicating one.

EXPERIMENTAL

Chemicals

A sample of EFA, assigned purity 99.3% of pharmaceutical grade was received from Arobindo Pharmaceuticals, Hyderabad, India. EFA film coated tablet of strength 600 mg Sustiva (Bristo-Myers Squibb-Germany) and Efavir (Cipla Ltd-India) were procured from the market. Potassium dihydrogen orthophosphate, dibasic sodium phosphate and sodium hydroxide of analytical grade were purchased from Qualigens (Mumbai India). HPLC grade acetonitrile were purchased from Merck (Mumbai, India) High purity water was prepared by Millipore milli Q plus purification system. (Millipore-France)

Equipment

The M/S Shimadzu Japan HPLC system with a photodiode array detector system (SPD –M20A) was used for the method development and forced degradation studies. The LC system used for method validation was Shimadzu HPLC LC- 2010CHT with

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[PMC free article] [PubMed] [CrossRef] [Google Scholar]Page 2HPLC chromatographic conditions of the current method.Chromatographic conditionsFlow rate1.0 mL/minWavelength (λ)268 nmStationary phaseRP18e, 5 µm, 250 × 4.6 mmColumn temperature25°CInjection volume20 µLRun time10 minutes High performance liquid chromatography (HPLC) method development can be a time-consuming process, particularly for stability-indicating analytical procedures of new chemical entities (NCEs). Most of the procedures for small-molecule drugs employ gradient reversed-phase liquid chromatography (RPLC) with ultraviolet (UV) detection.
These methods are designed to separate and quantitate the active pharmaceutical ingredients (APIs) and all process impurities and degradation products in drug substance (DS) and drug product (DP) samples. This important HPLC method category provides quality assessment data required in product release and stability studies in regulatory filings and procedures (Table 1). Information on the HPLC method development process and requirements are available in many textbooks (1–6), journal articles (7–13), regulatory guidance documents (14–16), and other resources (17).
In this instalment, we strive to provide a concise but comprehensive overview of the essential steps of the method development process, supported with case studies, common practices, regulatory guidance, and citations of critical references.Why Gradient RPLC with UV Detection for Stability-Indicating Methods?Let us start with the fundamentals and the rationale for the use of RPLC and UV methods in stability-indicating analytical procedures, which must separate the API from all impurities, and meet stringent regulatory requirements in method performance (6,14–16).First, the primary retention mechanism in RPLC is hydrophobic interaction, particularly useful for compounds with intermediate polarities, and an excellent match of most small-molecule drugs with oral bioavailability (2). The weak dispersive forces in RPLC ensure that all components in the sample can be eluted from the column using strong purging solvents at the end of the gradient, thus yielding 100% recovery of the injected sample.Second, the elution order in RPLC is highly predictable. It follows the “Linear Solvent Strength Model” (4). In most cases, the log k (retention factor) of the analyte is inversely proportional to %MPB (mobile phase B or % of the strong organic modifier). In addition, most drugs are basic, but have sufficient hydrophobicity to be retained in RPLC in the ionized forms in acidic mobile phases. The retention of the “solvated” analytes can be manipulated to provide increased resolution by changing the composition of the mobile phase (for example, water, organic solvents, and pH), that interacts with the analytes in the “solvated” state, and moderates their retention on the stationary phase (1,2). Gradient elution is commonly used in stability-indicating assays to yield higher peak capacity and sensitivity for both hydrophilic and hydrophobic components in the sample (2).Third, most NCEs are chromophoric, having one or more conjugated double bonds or aromatic moieties in their structures, thus providing high detection sensitivity with UV detectors. A peak area precision of 0.1–0.5% relative standard deviation (RSD) is routinely achievable in HPLC-UV assays, because the entire injected sample passes through the UV flow cell. This high precision performance is necessary for quality control applications for DS release testing (with typical specifications of 98.0–102.0%). In contrast, the precision performance of <1% is difficult to achieve with mass spectrometry (MS) detection, because only a small fraction of the nebulized eluent stream is ionized and detected (2). Furthermore, a UV detector offers a wide linear response range exceeding five orders of magnitude (1 × 10-5 to ~2 absorbance units [AU]), allowing the use of a single-point calibration curve for early-phase assays, and normalized area % analysis for the determinationof impurities (2).There are some notable exceptions to the adoption of RPLC-UV methods. For NCEs with no or low chromophoric properties, a gradient-compatible near-universal detector, such as a charged aerosol detector (CAD) or an evaporative light-scattering detector (ELSD), is typically employed (2). For the determination of enantiomers, a normal-phase separation using chiral LC or supercritical fluid chromatography (SFC) separation, is generally preferred, because the analytes are present in an unassociated and non-ionized state, which often leads to higher selectivity differences between the enantiomers (2).Another exception is the determination of potentially genotoxic impurities at parts-per-million levels (not discussed here), which may require a high-sensitivity analytical procedure, such as LC with mass spectrometry detection (LC–MS) (2).Objective of This ArticleThe objective of this instalment is to describe the traditional five-step strategy in HPLC method development, according to Snyder and associates (1), and illustrate the use of the selectivity-tuning for method optimization with case studies.The Traditional Five-Step Method Development ApproachA traditional five-step approach for HPLC method development was proposed by Snyder and associates, based on the concept of selectivity-tuning to increase the resolution of critical pairs (close-eluting peaks) for accurate quantitation (1,2). The five-step approach is outlined here.Step 1: Defining Method TypesThe first step is to define the method type. Is it a preparative, qualitative, or quantitative method? The most common method type is a stability-indicating analytical procedure (also known as an assay and impurities determination) for the quantitation of the API and impurities in pharmaceuticals. It is a challenging method development task to develop this type of method, because all key components in the sample must be physically separated in one chromatogram with method performance compliant to ICH guidelines (14). In comparison, other pharmaceutical methods for identification, limit tests, or performance assays, are simpler to develop, validate, and execute. Typically, the DS method is developed first for the NCE, and the DP method is then optimized based on the DS method.Step 2: Gathering Sample and Analyte InformationThe next step is to gather information on the sample and analytes. For NCEs, the structures and molecular formulae are well established, allowing the inference or calculation of physicochemical properties, such as pKa, logP, logD, polarity, numbers of acidic, basic, or aromatic functional groups, and chiral centres. These characteristics can be useful in the selection of columns, mobile phases, and sample diluents. The pKa values can be used to select a mobile-phase pH that ensures the compound is in a singly charged state. Knowledge of the acidic, basic, and aromatic functional groups can be helpful in column and mobile-phase selection, and provides forewarnings of potential stability or reactivity issues. Finally, knowing the presence and number of chiral centres is vital for planning an analytical procedure that includes diastereomeric separations. Though not entirely in the scope of this article, possible diastereomeric combinations may be separated with an achiral RPLC column (shown in the case study). Enantiomeric separation offers a different challenge that will require selecting the appropriate chiral-selective column for the determination of enantiomers of the API using a different analytical procedure. To gather analyte information, resources such as Certificate of Analysis (CoA) from suppliers or technical packages from the API manufacturers are invaluable in obtaining initial information regarding sample purity, methodologies, spectral and safety data, and other attributes of the API.Step 3: Initial Method DevelopmentThis is the first laboratory-based step in the development process, and it involves performing “scouting” runs to obtain the first chromatograms.
Details in column and mobile-phase selection are covered in many books (1–3). To illustrate the initial method development step, we will start here with the most common choice of a C18 column used with an acidified aqueous mobile phase and organic solvent. Step #3, outlined here, is extracted from a case study published in reference 2.Here, a sample of the API is dissolved in a default diluent (in this case, 1 mg/mL in 50% acetonitrile in water) and injected into an HPLC-UV system (with a photodiode array (PDA) detector and an MS instrument. A common broad-gradient RPLC method can be used (such as mobile phase A [MPA] = 0.1% formic acid in water; mobile phase B [MPB] = acetonitrile; C18 column: 3-µm, 100 mm × 3.0 mm i.d., 5–100% acetonitrile in 10 min at 1 mL/min and a column temperature at 30 °C). Full spectral data in UV (220–400 nm) and MS (100–1200 amu with positive ionization) are collected to allow reconstruction of chromatograms at any monitoring wavelengths.It is important to choose an MS-friendly mobile phase in this step if possible, to reduce the need for any method changes when using MS later. Results from the first scouting run can generate pertinent data, such as a “rough” sample impurity profile, estimation of purity and hydrophobicity of the API, maximum absorbance wavelength (λmax), (M+H)1+ data of the NCE, and any observed impurities. These initial data are used for determining the next logical steps in method fine-tuning.The process is often modified for polar or water-soluble NCEs, which may be better retained on an AQ-type-C18 or polar-embedded column (2). For drug candidates with low or no UV chromophoric activities, detectors such as CAD, ELSD, or MS may be employed. One crucial issue in the initial method development of NCEs is the rare availability of an “ideal test mix” sample containing the API and all key impurities or degradation products, deterring a systematic application of automation systems. This dilemma of sample availability will be discussed later.Step 4: Method Fine-Tuning and OptimizationThis is the most time-consuming step for the development of stability-indicating procedures. The process is typically reliant on “selectivity tuning” by changing selectivity (α) in a rational fashion by adjusting mobile phases (organic modifier, pH, buffer strength) and operating parameters (flow [F], gradient time [tG], column temperature [T]) (1–3). Often, changing to a column packed with different bonded phases other than C18 may be needed (such as, for example, phenyl, polar-embedded, or cyano) to achieve the separation of a critical pair of coeluting peaks.The next step is often to employ a “shallow middle gradient segment” indexed to the hydrophobicity of the API to increase the resolution around the main component (2). This use of a “multi-segment gradient approach” is preferred for complex NCEs and is discussed later.The method fine-tuning or optimization process typically takes a few days or 1–2 weeks, depending on the complexity of the NCE and the availability of key impurities as reference materials (such as isomers) that are used as retention time markers. This process is often iterative, and is performed before or after the initial forced degradation studies where degradation products are generated to challenge the separation power of the initial method. Peak purity should be evaluated with PDA and MS. Quite often, the initial column used (for example, C18) is found to be inadequate in the separation of a critical pair (for example, API and the immediate synthetic precursor) (2). This would invariably trigger a column screening experiment to find a better column with a different selectivity (for example, phenyl, polar-embedded, or cyano) (2,3). The use of MS can be a valuable tool in column screening, as it can help to track known impurities from column to column as well as degradation products formed during forced degradation studies. Knowledge of the molecular weights helps to determine the molecular structures of the degradation products.For laboratories specializing in method development, the use of automated column and mobile-phase screening systems and other software platforms can expedite this time-consuming step. The last steps in the method development phase are the final minor method adjustments needed to improve sensitivity and peak shape (injection volume, sample concentration, diluent, monitoring wavelength), and analysis time.It should also be noted that the drug product will also require assays and impurity analysis throughout the life cycle of the NCE. The best and shortest approach to DP method development is to use the chromatographic conditions for the DS with a modified sample preparation procedure.Step 5: Method PrequalificationStep 5 for methods used to test regulated products is a method prequalification or prevalidation step. This step will ensure the method can be successfully validated by determining the potential to pass typical method validation criteria (2,16), including specificity, precision, linearity, sensitivity, and often accuracy also. This prequalification step can take from a few hours to 1 or 2 d for most methods.Examples of the Selectivity Tuning Approach by Changing Mobile Phase, Column, or BothFigures 1 to 3 show studies of the use of selectivity tuning by changing the mobile phase, column, or both. Figure 1 shows the separation of a 12-component test mixture of basic, acidic, and neutral drugs and the changes in peak spacings with the gradient separation on a C18 column by switching the organic mobile phase (MPB) from methanol to acetonitrile. Note that acetonitrile is a stronger organic modifier in RPLC; thus, the elution time is considerably shorter. The peak shapes are also sharper due to its lower viscosity (1,2). While the elution order remains the same without any peak crossovers, the band spacings are changed due to selectivity differences. Note that acetonitrile is an aprotic solvent, while methanol is capable of hydrogen bonding and polar interaction with the analytes. In cases of analytes that can hydrogen-bond or have polar interaction, the elution order may be different when changing from methanol to acetonitrile.Figure 2 shows eight comparative chromatograms of a 7-component mixture of basic drugs using columns packed with different types of C18, phenyl, cyano, and pentafluorophenyl phases from a single manufacturer. All columns have the same dimension and are packed with similar particle sizes of ~1.7 µm. Chromatograms show similar elution order but with many differences in band spacings for C18 phases, whose predominant retention mechanism is hydrophobic interaction. However, the elution order can be substantially different in columns packed with different bonded phases that have additional retention mechanisms from π-π, polar, and hydrogen bonding interactions. To find the best column for a specific separation, the most efficient approach is to use an automated column and mobile phase screening system (2,3,12).Figure 3 shows two comparative chromatograms in a case study on proactive phase-appropriate method development advocated by Rasmussen and associates (3,17). The top chromatogram shows the separation of a retention marker solution of an API spiked with available impurities that is analyzed by a primary stability-indicating method for a DS method using a C18 column and an MPA at pH 2.5. The bottom chromatogram shows the separation of the same test mix using the secondary orthogonal method on a polar-embedded phase at a neutral MPA pH. More discussion on the development of a secondary orthogonal method will be discussed in Part 2.AcknowledgementsThe authors express their gratitude to the following colleagues for their time and efforts to provide timely reviews of the manuscript to improve content accuracy and clarity: Tao Jiang of Mallinckrodt; Anissa Wong and Chris Foti of Gilead Sciences; Mike Shifflet of Johnson and Johnson Consumer Health Care; He Meng of Sanofi; Adrijana Torbovska of Farmahem; Alice Krumenaker of TW Metals, LLC; Mark Shapiro of MCS Pharma Consulting; Szabolcs Fekete of U. Geneva; Deidre Cabooter of KU Leuven; Tamara Andreoli of Medinova AG, Schweiz; Michael DeHart of CMP Pharma; Marc Foster of Mythctest.ReferencesL.R. Snyder, J.J. Kirkland, and J.L. Glajch, Practical HPLC Method Development (John Wiley & Sons, Hoboken, New Jersey, 1997), Chapters 1–2 and 8–9.M.W. Dong, HPLC and UHPLC for Practicing Scientists, (John Wiley & Sons. Hoboken, New Jersey, 2nd Edition, 2019), Chapters 2–6 and 9–11.S. 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