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Impurity profile tracking for active pharmaceutical ingredients: Case reports

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Abstract

Tracking the impurity profile of an active pharmaceutical ingredient (API) is a very important task for all stages of drug development. A systematic approach for tracking impurity profile of API is described. Various real pharmaceutical applications are presented through successful examples of impurity profile tracking for three different novel APIs. These include MK-0969, an M3 antagonist; MK-0677, an oral-active growth hormone secretagogue and API-A, a cathepsin K inhibitor. A general strategy including selection of a reversed phase high performance liquid chromatographic (RP-HPLC) impurity profile method based on screening various stationary phases and changing the pH of the mobile phase and elucidation of impurity structures through the utilization of LC–MS, preparative-LC and NMR is demonstrated. A series of studies were conducted on the peak purity check by using the LC-UV diode-array and LC–MS detections. The advantages and disadvantages of each technique in the evaluation of peak purity are discussed.

Keywords: Active pharmaceutical ingredient (API); Impurity profile tracking; Reversed phase high performance liquid chromatographic (RP-HPLC); Elucidation of impurity structures; Peak purity check

1. Introduction

Most active pharmaceutical ingredients (API) are produced by organic chemical synthesis. Various components, including residual solvents, trace amounts of inorganic, and organic components can be generated during such a process. Those components remaining in the final API are considered as impurities. Profile tracking for such impurities, especially for the organic impurities is a very important task for the development of an API from early to late stage in order to ensure its safety, efficacy, purity, stability and quality.

The organic impurities present in the API could be process-related impurities such as starting materials, intermediates, by-products, reagents, ligands, and process degradation products. They could also be formed as a result of degradation experienced under storage conditions. Prior to clinical studies, safety toxicology studies on animals are required for organic impurity qualification. Qualified impurities will have levels consistent with those used in safety and clinical batches. The International Conference on Harmonization (ICH) guidelines for the API indicate that new impurities at levels >0.15% for a ≤2 g/day daily dose or those >0.05% for a >2 g/day daily dose should be qualified or reduced by purification of the batch prior to use in clinical studies. If there are known human relevant risks of an identified impurity, the impurity level will need to be reduced to lower safe levels [1].

A chromatographic method, especially a reversed phase high performance liquid chromatographic (RP-HPLC) method with UV detection is commonly developed for controlling trace amount of organic impurities. This is done through a systematic approach that screens different stationary phases, changes the pH of the mobile phase, varies the organic modifiers and column temperature [2]. Each individual unqualified impurity exceeding the 0.15% level is identified by different techniques, such as authentic sample spiking, HPLC with diode-array detection (LC-diode-array), HPLC with mass spectroscopic detection (LC–MS), HPLC with NMR (LC-NMR), and isolation by preparative-LC followed by NMR. In addition, to prevent possible impurity peak co-elution with the main compound peak, a peak purity of the main compound is often assessed by using

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LC-UV diode-array or LC–MS detections. Although all these techniques are very useful for the structural identification of the impurity, the most important aspect is still to have a clear understanding of the chemistry used for the synthetic route and for the chemical degradation process to elucidate a reliable impurity structure with a clear mechanism.

In the past two decades, there are several publications on the topic of API impurity profiles [3–6], but in this paper, we focused on modernized and streamlined practices of pharmaceutical industry for impurity tracking process based on state-of-art techniques through various successful examples of real pharmaceutical cases. Three novel and distinct APIs and their related impurities are tracked based on the synthetic chemistry, the selected RPLC method, data collected by utilizing LC–MS and preparative-LC followed by NMR. These include MK-0969, an M3 antagonist [7]; MK-0677, an oral-active growth hormone secretagogue [8] and an API-A, a cathepsin K inhibitor [9]. A series of studies were conducted on peak purity check by using LC-UV diode-array and LC–MS. The advantages and disadvantages of each technique for the evaluation of peak purity are discussed.

2. Experimental

2.1. Instrumentation

In the RPLC mode, an Agilent LC 1100 (Hewlett Packard Co., Wilmington, DE, USA) system was used. Data collection and analysis were performed with a PE Nelson data system equipped with Turbochrom software (PE Nelson, Cupertino, CA, USA).

LC–MS experiments were performed using an HP 1100 LC and a quadrupole ion trap mass spectrometer (LCQ, Finnigan MAT, San Jose, CA, USA) equipped with an electrospray ionization (ESI) or an atmospheric pressure chemical ionization (APCI) interface. LC conditions were the same as used for regular LC except that mobile phase A was 0.1% (v/v) aqueous formic acid. Parameters for ESI or APCI ion source were selected based on automatic tune with studied compound. The acquired mass range for full scan experiment was from *m/z* 50 to 1500 at 3 s per scan. MS data acquisition and analysis were performed with Xcalibur® software Version 1.2.

Preparative-LC was carried out using a Waters® LC system (Milford, MA, USA) equipped with a Waters Symmetry C18 column (5 cm × 25 cm). The mobile phases were 0.1% (v/v) aqueous formic acid (A) and acetonitrile (B). A 500 mL MK-0677 API solution was injected into the LC system and isocratically (75/25 v/v acetonitrile/0.1% formic acid) eluted at 50 mL/min. UV detection was set at 220 nm wavelength and desired fractions were collected and analyzed by analytical LC to check identity and purity. A rotary evaporator (Büchi, Meierseggstrasse, Switzerland) with a 40 °C water bath was used to remove acetonitrile in the LC fraction under a 15 Torr vacuum. Then, the fraction was lyophilized. Afterwards, a small aliquot of the collected solid was reconstituted in 50/50 (v/v) acetonitrile/water and reanalyzed by analytical LC to confirm the retention time.

In NMR mode, an AVANCE DRX-400 system (Bruker, Boston, MA, USA) was used. The frequency was set 399.9 MHz for ^{1}H and 100 MHZ for ^{13}C.

2.2. Chromatographic columns

The columns used in RPLC mode were Zorbax XDB-C8, Zorbax Rx C8, Zorbax CN, Zorbax phenyl (Agilent Technologies, Wilmington, DE, USA); SymmetryShield™ RP18, SymmetryShield™ RP8, Symmetry C18, Symmetry C8, Xterra-RPC18, Xterra-RPC8, YMC-AQ ODS, YMC basic, YMC pro-C18 (Waters, Bedford, MA, USA). All columns were 25 cm long and 4.6 mm in ID with a particle size of 5 μm.

2.3. Chromatographic conditions

All LC separations, except where specified, were performed at a temperature of 25 °C. Each mobile phase gradient profile was varied. The flow rate was 1.0 mL/min, the injection volume was 10 μL; UV detection wavelength was at 210–230 nm.

2.4. Reagents

MK-0969, MK-0677, API-A and all related authentic samples were synthesized by Process Research chemists at Merck & Co., Inc. (Rahway, NJ). HPLC grade potassium di-phosphate, potassium mono-phosphate and phosphoric acid were obtained from Aldrich Chemical Co. (Milwaukee, WI, USA). HPLC grade acetonitrile (MeCN) was obtained from EMS Scientific (Springfield, NJ, USA). Deionized (D.I.) water was obtained from a Milli-Q system (Millipore, Bedford, MA, USA).

3. Results and discussions

3.1. Selection of impurity profile methods

The most common impurity profile method for organic impurities is RP-HPLC with UV detection. Several parameters such as screening various stationary phases, changing the pH of the mobile phase, varying organic modifiers and column temperature are important for the development of an accurate, precise, reproducible, and rugged RP-HPLC impurity method. However, in this paper, we only focus on column and mobile phase pH, two most critical parameters.

3.1.1. Column selections

Numerous RP-HPLC columns are commercially available now. Each individual stationary phase would provide different levels of selectivity for different impurities due to various modifications, such as end capping, mixed-modes, amide bone-linkages, and phenyl and cyano bonding. The goal of an RP-HPLC impurity profile method is to separate different impurities from the main API peak and from each other as well. Thus, it is recommended that different stationary phases that possess large differences in polarity, wettability, and retention ability should be screened. This increases selectivity since the properties of the APIs and each impurity will differ greatly. Such