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Original Article

Ion-pairing HPLC methods to determine EDTA and DTPA in small molecule and biological pharmaceutical formulations ☆

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ABSTRACT

Ion-pairing high-performance liquid chromatography–ultraviolet (HPLC–UV) methods were developed to determine two commonly used chelating agents, ethylenediaminetetraacetic acid (EDTA) in Abilify® (a small molecule drug with aripiprazole as the active pharmaceutical ingredient) oral solution and diethylenetriaminepentaacetic acid (DTPA) in Yervoy® (a monoclonal antibody drug with ipilimumab as the active pharmaceutical ingredient) intravenous formulation. Since the analytes, EDTA and DTPA, do not contain chromophores, transition metal ions (Cu^{2+}, Fe^{3+}) which generate highly stable metallocomplexes with the chelating agents were added into the sample preparation to enhance UV detection. The use of metallocomplexes with ion-pairing chromatography provides the ability to achieve the desired sensitivity and selectivity in the development of the method. Specifically, the sample preparation involving metallocomplex formation allowed sensitive UV detection. Copper was utilized for the determination of EDTA and iron was utilized for the determination of DTPA. In the case of EDTA, a gradient mobile phase separated the components of the formulation from the analyte. In the method for DTPA, the active drug substance, ipilimumab, was eluted in the void. In addition, the optimization of the concentration of the ion-pairing reagent was discussed as a means of enhancing the retention of the aminopolycarboxylic acids (APCAs) including EDTA and DTPA and the specificity of the method. The analytical method development was designed based on the chromatographic properties of the analytes, the nature of the sample matrix and the intended purpose of the method. Validation data were presented for the two methods. Finally, both methods were successfully utilized in determining the fate of the chelates.

1. Introduction

Trace heavy metal ions can be harmful to human health and are of serious concern when existing as contaminants in food, drinking water, cosmetics and pharmaceutical products [1,2]. In addition, heavy metal ions can often catalyze oxidations and many other degradation reactions, leading to detrimental effects on the quality and shortened shelf life of products [3]. APCAs are the most commonly used and frequently studied synthetic chelating agents which can form stable complexes to "sequester" a wide variety of metal ions. These APCAs, including widely used EDTA and DTPA, can enhance the stability and shelf life of food, cosmetics and pharmaceutical formulations by chelating with metal ions and consequently deactivating the degradation reaction pathways mediated by the metal ions [3–5]. As a result, the stability studies must demonstrate that the level of APCAs in pharmaceutical formulations will ensure the desired product stability and quality. Therefore, development of appropriate analytical methods to quantitate the chelating agents in various pharmaceutical formulations is becoming more necessary and often poses analytical challenges.

A large number of analytical methods have been reported for the analysis of APCAs in a variety of sample matrices, including environmental samples, biological fluids, cosmetics, food and pharmaceutical products. These are usually chromatography or electrophoresis based methods including gas chromatography (GC), HPLC, ion chromatography (IC) and capillary electrophoresis (CE). The determination of APCAs by GC analysis often involves pre-treatment of samples by derivatization of the carboxylic group using acidified alcohol to convert APCAs into its methyl, ethyl, propyl or butyl esters to gain

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volatility. The sample preparation procedures can be quite complicated and time consuming [6–8]. HPLC methods usually utilize detection of UV or mass spectrometry (MS). Highly sensitive methods using LC/MS [9] and LC/MS/MS [10] were reported. In both articles, ion-pairing chromatographic approach was applied to determine APCAs at very low concentrations with detection of MS or MS/MS. Limit of detection (LOD) of μg/L was reached without any pre-concentration of samples. Since APCAs carry ionizable amino- and carboxylate groups, at appropriate pH, IC can also be applied for their quantitation. A Dionex Application Note described the determination of the μg/L concentrations of EDTA, nitriloacetic acid (NTA), DTPA and ethylene-bis(oxyethylenenitrilo)tetraacetic acid (EGTA) in municipal drinking water and waste water samples [11]. Harmsen et al. [12] reported determination of EDTA in water using anion exchange HPLC coupled with UV detection at 258 nm after complexing EDTA with Fe^{3+}. Pozdniakova et al. [13] developed a CE method to determine free EDTA through pre-capillary complexation to convert EDTA to Ni (II)-EDTA followed by CE determination of the negatively charged chelate using UV detection. Laamanen et al. [14] reported simultaneous determination of DTPA, EDTA and NTA by a CE/UV method using copper (II) complexation. A number of articles reported determination of trace level of APCAs in natural water, food and environmental samples by using ion-pairing reversed-phase HPLC coupled with UV-visible detector for quantitation of APCA metellocomplexes [4,6,15–23].

Abilify® is a psychotropic drug for the treatment of schizophrenia. In the Abilify® oral solution formulation, EDTA is used as a preservative to prevent the degradation of drug active ingredient, aripiprazole, induced by trace metals. Yervoy® is marketed to treat metastatic melanoma. Ipilimumab, the active ingredient in Yervoy®, is a monoclonal antibody with an approximate molecular weight of 148 kD. In Yervoy® intravenous solution formulation, DTPA serves as a stabilizer to prevent possible trace metals from denaturing or catalyzing the degradation of the drug active monoclonal antibody. The structures of aripiprazole, ipilimumab, EDTA and DTPA are shown in Fig. 1.

In this study, two ion-pairing reversed-phase HPLC–UV methods were used to determine the concentrations of EDTA used in Abilify® oral solution and DPTA in Yervoy® intravenous solution formulations, respectively. The approach is based on complexation of APCAs with metal ions before analysis by ion-pairing reversed-phase HPLC coupled with UV detection. The analytical method will be used to evaluate the use of a gradient versus an isocratic mobile phase in the determination of an APCA in the matrices of a small molecule and a biological formulation, respectively, to achieve the desired separation, specificity and quantitation. Discussions will include the choice of metal cations for complexation and the effects of the ion-pairing reagent on retention of the analytes and specificity of the sample matrix.

2. Experimental

2.1. Reagents and chemicals

Abilify® oral solution and Yervoy® intravenous injection solution were obtained from Bristol-Myers Squibb (Princeton, NJ, USA). The Abilify® oral solution contains 1.0 mg/mL of aripiprazole (active pharmaceutical ingredient), 0.5 mg/mL of EDTA, and other excipients such as fructose (200 mg/mL), glycerin, dl-lactic acid, methylparaben, propylene glycol, propylparaben, sodium hydroxide, sucrose (400 mg/mL), and purified water. The oral solution is flavored with natural orange cream and other natural flavors.

The Yervoy® intravenous injection solution contains 5.0 mg/mL of ipilimumab monoclonal antibody as the active pharmaceutical ingredient, 0.0393 mg/mL of DTPA (0.1 mM) and a few other inactive ingredients such as mannitol, polysorbate 80, sodium chloride, tris hydrochloride, and purified water.

Ultrapure water was obtained from a Milli-Q system (EMD Millipore, Bedford, MA, USA). Acetonitrile (HPLC Grade) was from EM Science (Gibbstown, NJ, USA); $Cu(NO_3)_2 \cdot 2.5H_2O$ was from J.T. Baker (Center Valley, PA, USA); $FeCl_3$ (A.C.S reagent) was from Sigma-Aldrich (St. Louis, MO, USA); 20 mM $FeCl_3$ aqueous solution was prepared by dissolving 164.5 mg of $FeCl_3$ in a 50 mL volumetric flask with water. Phosphoric acid aqueous solution (Analytical reagent for HPLC, ~0.66 M) was from Sigma-Aldrich. Tetrabutylammonium hydroxide (TBA) aqueous solution (0.4 M) was ordered from J. T. Baker as "Baker Analyzed"® HPLC reagent; EDTA and DTPA were American Chemical Society (ACS) reagent grade material and were both obtained from Sigma-Aldrich. The diluent for EDTA was $Cu(NO_3)_2$ (1.6 mM) in water/acetonitrile (75:25, v/v); the diluent for the DTPA was $FeCl_3$ aqueous solution.

2.2. Instrumentation

A Waters Alliance Model 2695 (Waters Corporation, Milford, MA, USA) HPLC system consisting of a membrane degasser, a quaternary gradient pump, an autosampler and a column thermostat was used. The system was equipped with a Waters Model 2487 UV–vis detector. The chromatogram collecting and processing were controlled with the Waters Empower software package. HPLC analysis was performed using YMC Pack Pro C_{18} column (50 mm × 4.6 mm, 3 μm; YMC Corporation, Allentown, PA, USA).

2.3. Preparation of standards and sample solutions

2.3.1. Preparation of standards and sample solutions for EDTA determination

An EDTA standard solution was prepared by dissolving 64.1 mg of EDTA disodium salt dihydrate in 1 L diluent (1.6 mM $Cu(NO_3)_2$ in water/acetonitrile (75:25, v/v)) to obtain an EDTA standard solution of 0.05 mg/mL.

Abilify® oral solution was diluted with the diluent (1.6 mM $Cu(NO_3)_2$ in water/acetonitrile (75:25, v/v)) by 10 fold to obtain a sample solution for EDTA determination. Both standard and sample solutions were allowed to stand at room temperature for at least 30 min before analysis.

2.3.2. Preparation of standards and sample solutions for DTPA determination

A DTPA standard solution was prepared by dissolving 39.3 mg of DTPA with Milli-Q water in a 1 L volumetric flask containing 10 mL of 20 mM $FeCl_3$ aqueous solution to obtain a standard solution of 0.0393 mg/mL (0.1 mM) DTPA-Fe^{3+} complex.

A 2 mL Yervoy® intravenous injection solution was transferred into an HPLC vial using a pipette and then spiked with 20 μL of 20 mM $FeCl_3$ aqueous solution. The vial was closed and vortexed for at least 1 min. Both standard and sample