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
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, nitroacetic 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 Fe3+. 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 metallocomplexes [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, traced by metal traces. 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(NO₃)₂·2.5H₂O was from J.T. Baker (Center Valley, PA, USA); FeCl₃ (A.C.S reagent) was from Sigma-Aldrich (St. Louis, MO, USA); 20 mM FeCl₃ aqueous solution was prepared by dissolving 164.5 mg of FeCl₃ 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(NO₃)₂ (1.6 mM) in water/acetonitrile (75:25, v/v); the diluent for the DTPA was FeCl₃ 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 C₁₈ 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(NO₃)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(NO₃)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 FeCl₃ aqueous solution to obtain a standard solution of 0.0393 mg/mL (0.1 mM) DTPA–Fe³⁺ 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 FeCl₃ aqueous solution. The vial was closed and vortexed for at least 1 min. Both standard and sample
solutions were allowed to stand at room temperature for at least 30 min before analysis.

2.4. Chromatographic conditions

2.4.1. Determination of EDTA in Abilify<sup>©</sup> oral solution

A buffer solution of 24 mM tetrabutylammonium phosphate, pH 6.5 was prepared by diluting 60 mL of 0.4 M tetrabutylammonium hydroxide to 1000 mL with Milli-Q water and adjusting the pH to 6.5 using phosphoric acid solution.

A gradient mobile phase consisting of the tetrabutylammonium phosphate buffer, pH 6.5 (mobile phase A) and acetonitrile (mobile phase B) was used with the following profile: 25% B for 0–4 min, then linear gradient to 65% B at 10 min, followed by a linear return to 25% B at 15 min and a further re-equilibration to 20 min. The detection wavelength was set at 280 nm. The flow rate was controlled at 1.0 mL/min. The injection volume was 50 μL. The column temperature was controlled at 30 °C.

2.4.2. Determination of DTPA in Yervoy<sup>©</sup> intravenous injection solution

The chromatography for determination of DTPA in Yervoy<sup>©</sup> intravenous injection solution was performed using the same equipment as for the determination of EDTA in Abilify<sup>©</sup> oral solution. An isocratic run mobile phase consisted of premixed solution of 24 mM tetrabutylammonium phosphate buffer, pH 6.5 and acetonitrile in the ratio of 80:20 (v/v). The detection wavelength was set at 260 nm and injection volume was set at 50 μL. The flow rate was 1.0 mL/min and the runtime was 10 min. The column temperature was controlled at 30 °C.

3. Results and discussion

The determination of APCAs by direct UV detection is challenging since EDTA and DTPA do not contain a significant chromophore. In the Abilify<sup>©</sup> oral solution or the Yervoy<sup>©</sup> intravenous injection solution, the quantitation of EDTA and DTPA, respectively, by direct spectrophotometric methods becomes even impossible due to the lack of sensitivity or selectivity due to the addition of the excipients in the formulations.

Chromatographic methods including GC and LC are preferred for quality control (QC) analysis because both techniques are well-established and quite reliable. Since APCAs lack volatility, GC methods usually include a time-consuming derivatization step, in which APCAs are converted into their alkyl esters to enhance their volatility [6–8]. Considering the ruggedness and simplicity of operation as two important method attributes, ion-pairing reversed-phase HPLC coupled with UV detection presents a feasible method development option.

Ion-pairing chromatography is well understood and has been critically reviewed [24]. The determination of EDTA by ion-pairing HPLC using metallocomplexes has been known for some time [4,6,15–23]. In addition, the determination of EDTA in pharmaceutical formulations based on HPLC has been reported [5,20,21,23]. The use of metallocomplexes with ion-pairing chromatography introduces additional variables that require greater investigation during method development. Since pharmaceutical formulations often contain multiple excipient components which may interfere with APCA peaks in chromatography, selectivity is a critical consideration in method development. In literature, we have not seen any reports on controlling ion-pairing reagent concentration to modulate peak selectivity for APCA determination in drug formulations.

Fig. 1. Chemical structures of analytes (EDTA and DTPA) and APIs (aripiprazole and ipilimumab) in the two drug products, Abilify<sup>©</sup> oral solution and Yervoy<sup>©</sup> intravenous solution.
The method reported in the following studies utilizes gradient and isocratic mobile phases in the determination of an APCA in a small molecule and a biological formulation, respectively. In the two examples presented here, the use of metallocomplexes with ion-pairing reversed-phase chromatography provides the ability to achieve the desired sensitivity and selectivity. The analytical methods are designed based on the chromatographic properties of the analytes, the nature of the sample matrix and the intended purpose of the methods.

3.1. Analytical method for the determination of EDTA in Abilify® oral solution

Ion-pairing reversed-phase HPLC methods, because of the complexity of the interactions among analyte, stationary and mobile phases during the process of separation, tend to be difficult to reproduce, transfer between laboratories, and troubleshoot [25]. The slow equilibration of the column with ion-pairing reagents can also create problems especially when a gradient is used. Retention time may be irreproducible due to perturbations to the equilibration between column stationary phase and mobile phases. Problems with baselines, separation and quantitation may arise over the course of testing. However, recent studies demonstrate that a full equilibrium of the ion-pairing reagent on the column may not be necessary in developing a robust and selective gradient elution ion-pair chromatographic method [26].

A gradient HPLC method was developed for EDTA in Abilify® oral solution with an ion-pairing reagent, TBA, in a phosphate buffer solution at pH 6.5 as the aqueous component of the mobile phase. The sensitivity to determine EDTA is achieved by adding copper (II) nitrate (Cu(NO₃)₂) into the diluent which combines the chelation and sample dilution steps. In addition, a short C₁₈ column of 50 mm was chosen to expedite the equilibrium and the analysis.

Abilify® oral solution causes several challenges for the chromatographic method development. The oral solution contains reagents which have extremely differing retention behavior when using a conventional reversed-phase LC separation method. The EDTA-Cu²⁺, an ionic analyte, has very little retention on reversed-phase LC. The preservatives, methylparaben and propylparaben, are significantly retained in comparison to the EDTA. The aripiprazole, the active pharmaceutical ingredient (API) in the formulation and with high hydrophobicity, is expected to elute later in a reversed-phase LC. As an initial approach, isocratic systems were utilized to gain a better understanding of the retention behavior as a function of the concentration of TBA to achieve appropriate separation of the analyte and the sample matrix. As shown in Fig. 2, EDTA-Cu²⁺ elutes at the solvent front when no ion-pairing reagent is added, i.e., only 24 mM phosphate buffer as the mobile phase (Chromatogram 1). However, as the TBA buffer concentration in the mobile phase increases from 8 mM to 20 mM (Chromatograms 2–5), the retention of EDTA-Cu²⁺ decreases due to the competition of the interaction with the stationary phase from the ions in the solution. To further establish the separation condition for the critical pair peaks of EDTA-Cu²⁺ and methylparaben, Chromatograms 6 and 7 in Fig. 2 were collected with a solution containing the mixture of these two compounds. When the concentration of the TBA buffer was at 8 mM, the elution of the EDTA complex approached a retention of 3 min, causing its co-elution with methylparaben (Chromatogram 6). As noted, the EDTA-Cu²⁺ complex eluted with a retention time of less than 2 min when the TBA buffer was at 24 mM. In this condition, the peaks of EDTA-Cu²⁺ and methylparaben were well separated (Chromatogram 7). It was also noted that the peak shape of the EDTA complex deteriorated with decreasing ion-pairing concentration. In an effort to enhance peak shape and achieve the necessary retention, the concentration of the TBA buffer in the mobile phase was set at 24 mM. With the optimization of the gradient, the analysis time was set at 20 min (including re-equilibration). The elution order of the analytes is shown in Fig. 3, which was achieved with the final method conditions as provided in the experimental section. It should be noted that this method has the potential to analyze the preservatives in
Table 1
Summary of validation of the method for EDTA determination.

| Validation parameters | Results                                                                 |
|-----------------------|-------------------------------------------------------------------------|
| Specificity           | Separation of EDTA-Cu<sup>2+</sup> from excipients (methylparaben and propylparaben), aripiprazole and solvent blank is demonstrated [Fig. 3] |
| Linearity (0.01–0.08 mg/mL) | Slope = 21.291,615, R<sup>2</sup> = 0.9997                              |
| Repeatability (n=3)   | Results from three preparations: 0.5017, 0.5077, 0.5010 mg/mL; Mean 0.5035 mg/mL; %RSD 0.73 |
| Accuracy (n=3)        | 100.4%, 101.0%, 101.5%; Mean 101.0%; %RSD 0.55                          |
| Sensitivity           | LOD 1.5 μg/mL; LOQ 4.5 μg/mL                                            |
| Solution stability    | When standard and sample solutions were stored at room temperature, room light, they were good for 7 days (area change from initial < 2.0%) |

Table 2
Stability constants (log K) of EDTA- and DTPA-metallocomplexes [27].

| Metal ion | EDTA | DTPA |
|-----------|------|------|
| Cu<sup>2+</sup> | 18.8 | 21.4 |
| Fe<sup>2+</sup> | 14.3 | 16.4 |
| Fe<sup>3+</sup> | 25.1 | 28.0 |
| Hg<sup>2+</sup> | 21.7 | 26.7 |
| Mg<sup>2+</sup> | 8.8  | 9.3  |
| Mn<sup>2+</sup> | 13.9 | 15.6 |
| Ni<sup>2+</sup> | 18.6 | 20.2 |
| Pb<sup>2+</sup> | 18.0 | 18.8 |
| Sr<sup>2+</sup> | 8.7  | 9.8  |
| Zn<sup>2+</sup> | 16.5 | 18.4 |

The formulation. However, this was not the intention of the method to quantitate the preservatives since the impurity profile of aripiprazole and the preservatives were quantitated by pre-existing analytical methods. Finally, the diluent contains copper nitrate which combines the chelation and sample dilution steps which simplifies the sample preparation. In addition, acetonitrile (25%) was added into the diluent to solubilize aripiprazole, which has very low aqueous solubility.

The validation of the method for EDTA determination demonstrated its specificity, linearity, accuracy, precision and solution stability. The specificity was demonstrated by the separation of EDTA from the methylparaben, propylparaben and aripiprazole. The linearity of the EDTA response was validated from 0.01 to 0.08 mg/mL with a correlation coefficient (R<sup>2</sup>) of 0.9997. The target concentration of EDTA in the formulation was 0.5 mg/mL. However, the working test solution concentration was 0.05 mg/mL. Therefore, the linearity covered from 20% to 160% of label claim. The repeatability data from three preparations resulted in a mean of 0.5035 mg/mL and %RSD of 0.73. The accuracy data from three preparations of spiking recovery from the sample matrix resulted in a mean of 101.0% and %RSD of 0.55. The sensitivity of the method (LOD and LOQ) was demonstrated at the μg/mL level, as shown in Table 1. The sample and standard solutions were stable for 7 days when stored at room temperature and room light. The validation data are summarized in Table 1.

3.2. Analytical method for the determination of DTPA in Yervoy<sup>®</sup> intravenous solution

Following the successful development of the method for EDTA determination for Abilify<sup>®</sup> oral solution, a method for DTPA determination in Yervoy<sup>®</sup> intravenous solution was required and a similar approach was initiated for the method development. The intent of the method was to quantitate the DTPA in the monoclonal antibody intravenous formulation. As previously stated, the DTPA is utilized as a preservative to ensure the quality of the product. Similar to EDTA, DTPA has a high affinity to metal cations and can form strong complexes with the cations involving the amino- and the carboxylate groups [27]. When Cu<sup>2+</sup> was initially tested as the chelating metal, it was found that the peak shape was distorted probably due to the interaction with the cations in solution by the extra charge on the Cu<sup>2+</sup>-DTPA complex formed. The type and quantity of metal ions as well as the anions involved in the process need to be considered since these factors affect the strength of the complex formed between the metal ion and the chelating agent. The stability or equilibrium constant (K), expressed as log K, was determined for the complexes of EDTA, DTPA and several metal ions as shown in Table 2 [27]. The greater the log K values, the more tightly the metal ion bounds to the chelating agent and the more likely that complexes are formed even in the presence of competing ions. Therefore, Fe<sup>3+</sup> was selected as the chelating metal ion for the complex with DTPA.

The sample preparation is simple and convenient. It consisted of pipetting a 2 mL Yervoy<sup>®</sup> intravenous solution into an HPLC vial to which 20 μL of 20 mM FeCl<sub>3</sub> aqueous solution was added. The vial was capped and vortexed. The standard and sample solutions were allowed to stand for at least 30 min before analysis.

During the chromatographic method development, it was initially envisioned to be a trifluoroacetic acid with gradient mobile phases which is typically utilized for the separation of
proteins. An initial gradient indicated that the monoclonal antibody eluted with a very low organic content. As a result, several isocratic runs were attempted with a low organic content from 10% to 30% acetonitrile. The final conditions yielded an isocratic mobile phase consisting of 20% acetonitrile and 80% aqueous with a pH of 6.5. With an isocratic flow, it was found that the antibody protein eluted at the solvent front, showed by a clear apex at 280 nm on the UV spectrum collected with a photodiode array detector. A possible explanation for this early elution of the monoclonal antibody is that the proteins are too large to penetrate the narrow pores of the column (100 Å) and just pass by the column with the mobile phase. Column clogging is a potential challenge for performing reversed-phase LC for proteins. Multiple injections demonstrated that column pressure did not increase. Based on these findings, the analytical method is extremely efficient and does not require additional sample preparations such as protein precipitation prior to separation. The concentration of the TBA in the mobile phase was established based on the learnings of the Abilify® case described in detail above. The retention behavior of the monoclonal antibody and the retained DTPA complex is shown in Fig. 4. As evident, the ipilimumab peak elutes in the void and the DTPA complex is well retained and elutes at 3 min.

The validation of the method for DTPA determination demonstrated its specificity, linearity, accuracy, precision, and solution stability. The specificity was noted by the separation of DTPA from the ipilimumab. The linearity of the DTPA response was validated from 0.01 to 0.08 mg/mL with a correlation coefficient (R²) of 1.000. The target concentration of DTPA in the formulation is 0.039 mg/mL. Therefore, the linearity covered from 25% to 200% of label claim. The repeat data from three preparations resulted in a mean assay of 0.0393 mg/mL and %RSD of 2.6. The accuracy data from three preparations of spiking recovery from sample matrix resulted in a mean recovery of 105.6% and %RSD of 1.4. The sensitivity of the method (LOD and LOQ) was demonstrated at the µg/mL level. The sample and standard solutions were stable for 24 h when stored at room temperature and under room light. All the validation data are summarized in Table 3.

4. Conclusion

In conclusion, two fits for purpose, ion-pairing reversed-phase HPLC methods were developed to determine EDTA in Abilify® oral solution and DTPA in Yervoy® intravenous solution drug products. TBA was added into the mobile phase to form ion pairs with metallocomplexes of EDTA and DTPA to enhance retention on reversed-phase HPLC columns. The analytical method utilized gradient and isocratic mobile phases in the determination of an APCA in a small molecule and a biological formulation, respectively. 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 allows UV detection with high sensitivity. Copper (II) was utilized for the determination of EDTA and iron (III) was utilized for the determination of DTPA. For the determination of EDTA, a gradient mobile phase separated the components of the formulation from the analyte. In the case of DTPA, the active drug substance, ipilimumab, was eluted in the void. To the best of our knowledge, there is no report on analytical HPLC method for APCAs by direct injections of monoclonal drug formulations. In addition, the optimization of the concentration of the ion-pairing reagent was discussed as a means of enhancing the retention of the APCAs 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.

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References

[1] W.G. Landis, R.M. Sofield, M. Yu, Introduction to Environmental Toxicology: Molecular Substructures to Ecological Landscapes, 4th ed., CRC Press, Boca Raton, Florida, 2010.
[2] R.C. Rowe, P.J. Sheskey, P.J. Weller, Handbook of Pharmaceutical Excipients, 4th ed., Science and Practice, London, 2003, pp. 225.
[3] J.O. McCord, A. Kilara, Control of enzymatic browning in processed mushrooms (agaricus bisporus), J. Food Sci. 48 (1983) 1479–1483.
[4] J. De Jong, A. Van Polanen, J.J.M. Driessen, Determination of ethylenediaminetetraacetic acid and its salts in canned mushrooms by reversed-phase ion-pair liquid chromatography, J. Chromatogr. 553 (1991) 243–248.
[5] A.S. Kord, I. Tumanova, W.L. Matier, A novel HPLC method for determination of EDTA in a cataract inhibiting ophthalmic drug, J. Pharm. Biomed. Anal. 13 (1995) 575–580.
[6] M. Sillanpää, M.L. Sihvonen, Analysis of EDTA and DTPA, Talanta 44 (1997) 1487–1497.
[7] J. Sorvari, M. Sillanpää, M.L. Sihvonen, Development of a gas chromatographic method for the simultaneous determination of trace amounts of ethylenediaminetetraacetic acid and diethylenetriaminepentaacetic acid in natural waters, Analyst 121 (1996) 1335–1339.
[8] Y. Nishikawa, T. Okumura, Determination of nitrilotriacetic acid and ethylenediaminetetraacetic acid in environmental samples as their methyl ester derivatives by gas chromatography-mass spectrometry, J. Chromatogr. A 600 (1995) 109–118.
[9] A. Dodi, V. Monnier, Determination of ethylenediaminetetraacetic acid at very low concentrations by high-performance liquid chromatography coupled with electrospay mass spectrometry, J. Chromatogr. A 1032 (2004) 87–92.
[10] J.B. Quintana, T. Reemtsma, Rapid and sensitive determination of ethylenediaminetetraacetic acid and diethylenetriaminepentaacetic acid in water samples by ion-pair reversed-phase liquid chromatography-electrospray tandem mass spectrometry, J. Chromatogr. A 1145 (2007) 110–117.
[11] DIONEX Application Note 268, Determination of chelating agents in drinking water and wastewater samples, Dionex, Sunnyvale, CA, 2011.
[12] J. Harmes, A. van den Toorn, Determination of EDTA in water by high-performance liquid chromatography, J. Chromatogr. 249 (1982) 379–384.
[13] S. Pozdniakova, R. Ragauskas, R. Dikcius, et al., Determination of EDTA in used sorbed EDTA species in water and sediments by HPLC, Anal. Chem. 68 (1996) 731–737.
[14] M. Lin, M. Royal, K. Hayenga, et al., Monitoring EDTA process residuals in reverse osmosis systems by ion-pair liquid chromatography-mass spectrometry, J. Chromatogr. A 1145 (2007) 561–566.
[15] R. Geschke, M. Zehinger, A new method for the determination of complexing agents in river water using HPLC, Fres, J. Anal. Chem. 357 (1997) 773–776.
[16] P.J.M. Bergers, A.C. de Groot, The analysis of EDTA in water by HPLC, Water Res. 28 (1994) 639–642.
[17] P. Laine, R. Matilainen, Simultaneous determination of DTPA, EDTA, and NTA by UV-visible spectrometry and HPLC, Anal. Bioanal. Chem. 382 (2005) 1601–1609.
[18] M. Lin, M. Royal, K. Hayenga, et al., Monitoring EDTA process residuals in recombiant protein manufacturing using liquid chromatography, J. Chromatogr. B 792 (2003) 205–215.
[21] E.L. Inman, R.L. Clemens, B.A. Olsen, Determination of EDTA in vancomycin by liquid chromatography with absorbance ratioing for peak identification, J. Pharm. Biomed. Anal. 8 (1990) 513–520.

[22] D.L. Venezky, W.E. Rudzinski, Determination of ethylenediaminetetraacetic acid in boiler water by liquid chromatography, Anal. Chem. 56 (1984) 315–317.

[23] R. Heydari, M. Shamsipur, N. Naleini, Simultaneous determination of EDTA, sorbic acid, and diclofenac sodium in pharmaceutical preparations using high-performance liquid chromatography, AAPS PharmSciTech 14 (2013) 764–769.

[24] T. Cecchi, Ion pairing chromatography, Crit. Rev. Anal. Chem. 38 (2008) 161–213.

[25] J.W. Dolan, Ion pairing—blessing or curse? LCGC Eur. 21 (2008) 258–263.

[26] J. Zhang, T. Raglione, Q. Wang, et al., Regeneration of tetrabutylammonium ion-pairing reagent distribution in a gradient elution of reversed phase ion-pair chromatography, J. Chromatogr. Sci. 49 (2011) 825–832.

[27] R.M. Smith, A.E. Martell, Critical Stability Constants, 6, Plenum Press, New York, 1989, pp. 1–58.