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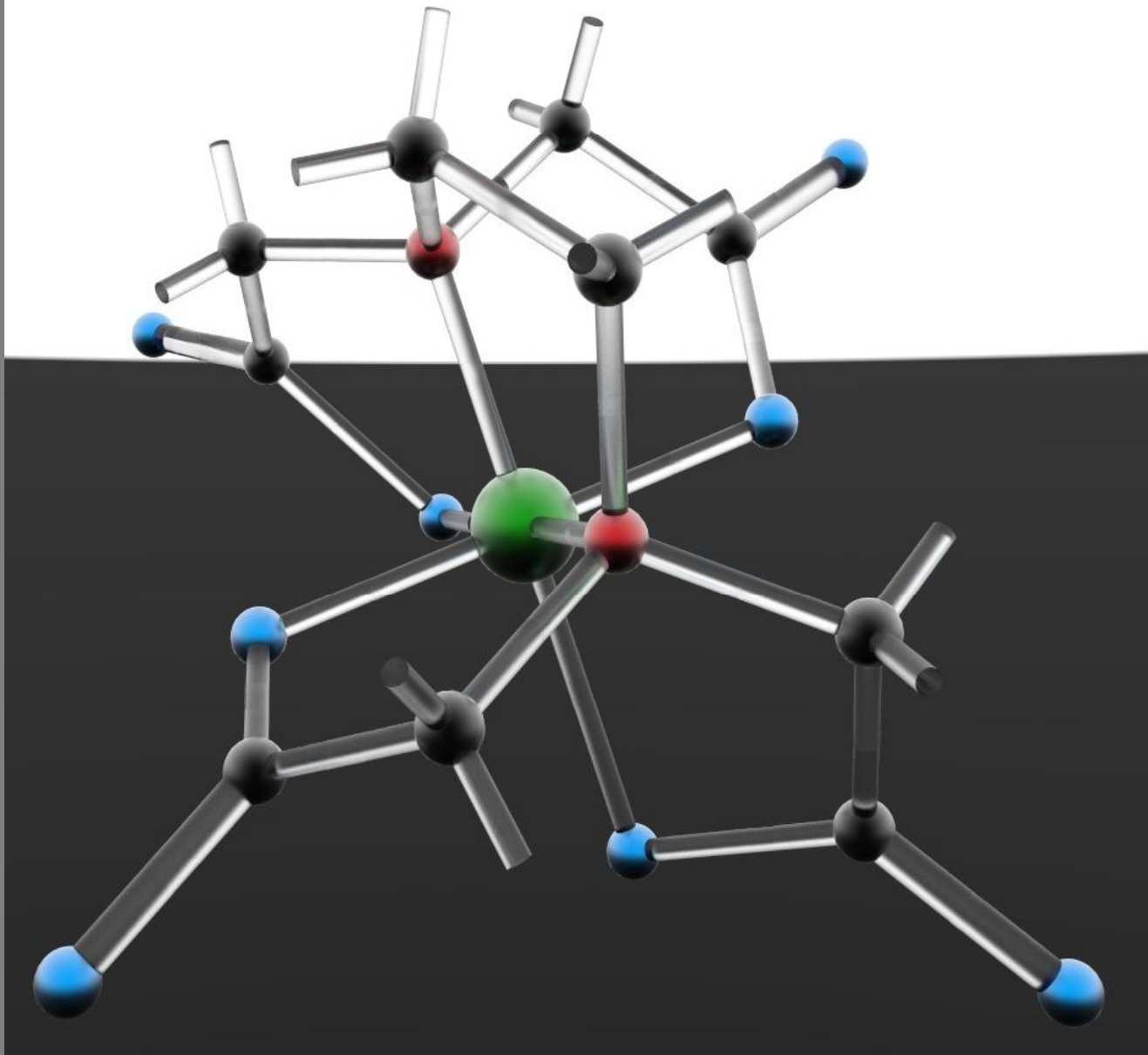
MEASURING EDTA USING HPLC



EDTA Analysis Background

Ethylenediaminetetraacetic acid (EDTA) has multiple industrial, medical, biological and research applications. It presents a number of difficult problems when one attempts to analyse it using HPLC:

- It has no retention in reversed-phase chromatography
- It possesses multiple charges at acidic and neutral pH, which are most common in HPLC
- EDTA molecules have low UV absorption above 230 nm
- It has strong chelating properties and gets retained by all metal components of the HPLC system
- It is usually a part of the formulation, mixed with surfactants and other components, which can complicate separation and detection

SIELC developed a new selective methodology to address these difficulties and offers a simple and reliable method for EDTA quantitation in most liquid samples. The method is based on forming a complex of EDTA with Fe(III), which has strong UV absorption maximums at 260 nm (Fig. 1).

This complex can be measured by a UV-Vis detector and can be separated from the signal of complex-free Fe(III) using Newcrom BH HPLC column (Fig. 2).

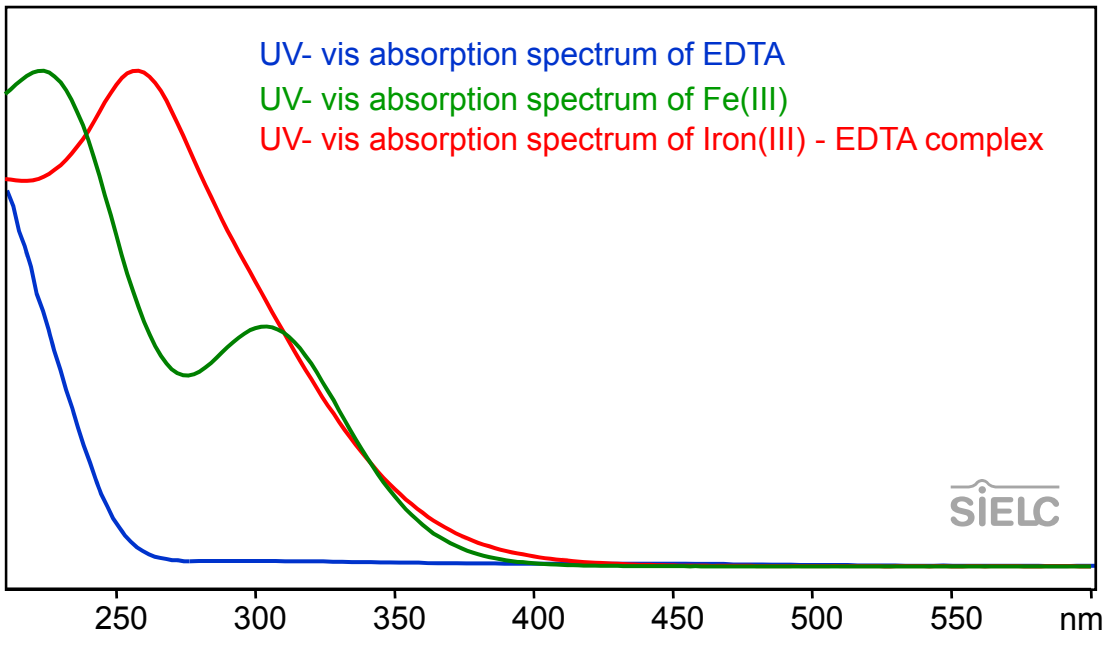


Fig. 1. UV spectra of EDTA • Fe(III) complex, $FeCl_3$ solution, and EDTA solution.

EDTA Analysis

EDTA Standards Solution A

For the preparation of EDTA standard solution, 5 mg of EDTA were accurately weighed, transferred into a 5 mL volumetric flask and dissolved in 0.001M NaOH aqueous solution using sonication or magnetic stirrer. The EDTA stock solution (1.0 mg/mL) should be stored in a cold dark place and can be used for a week to prepare standards of required concentration.

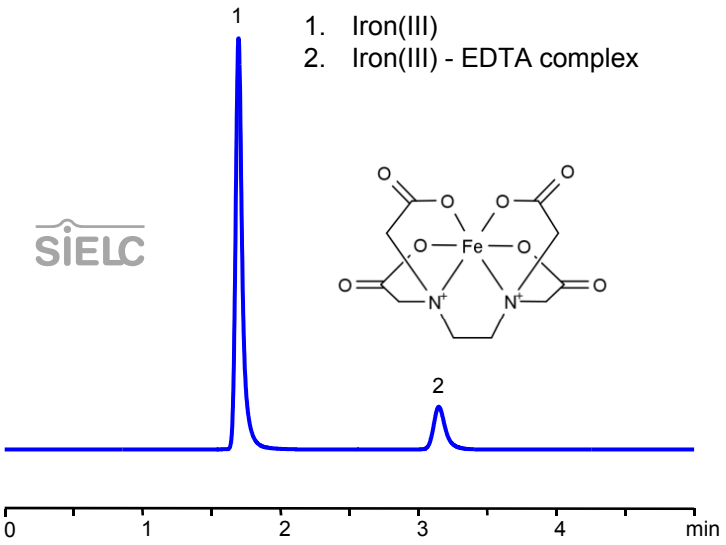


Fig. 2. Chromatogram of EDTA complex with Fe(III). HPLC column: Newcrom BH, 4.6 x 150 mm, 5 µm. Flow rate: 1.0 mL/min. Mobile phase: 2% MeCN in water containing 0.1% H_2SO_4. Detection: UV 260 nm.

Iron(III) chloride Solution B

The standard stock solution of Iron(III) chloride (10 mg/mL) was prepared in water. 50 mg of Iron(III) chloride hexahydrate were accurately weighed, transferred into a 5 mL volumetric flask, dissolved in water and sonicated, if needed.

General procedure for Iron(III) - EDTA complex analysis

Mix 100 µL of Solution A (or unknown sample) with 300 µL of Solution B and 600 µL of water. Place this mixture in a plastic HPLC vial for analysis. HPLC conditions described in Fig. 2.

For situations when a sample has more than one component that needs to be analyzed, gradient conditions can be used as well, in the same run. Example below (Fig. 3) shows isocratic conditions for EDTA testing only. The next example (Fig. 4) shows gradient run when other ingredients of the formulation can be measured at the same time.

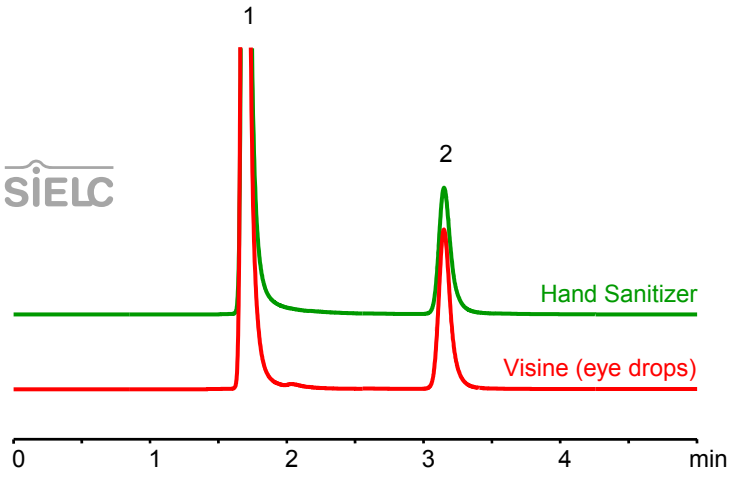


Fig. 3. Isocratic chromatogram of EDTA complex with Fe(III). HPLC column: Newcrom BH, 4.6 x 150 mm, 5 µm. Flow rate: 1.0 mL/min. Mobile phase: 2% MeCN in water containing 0.1% H_2SO_4. Detection: UV 260 nm.

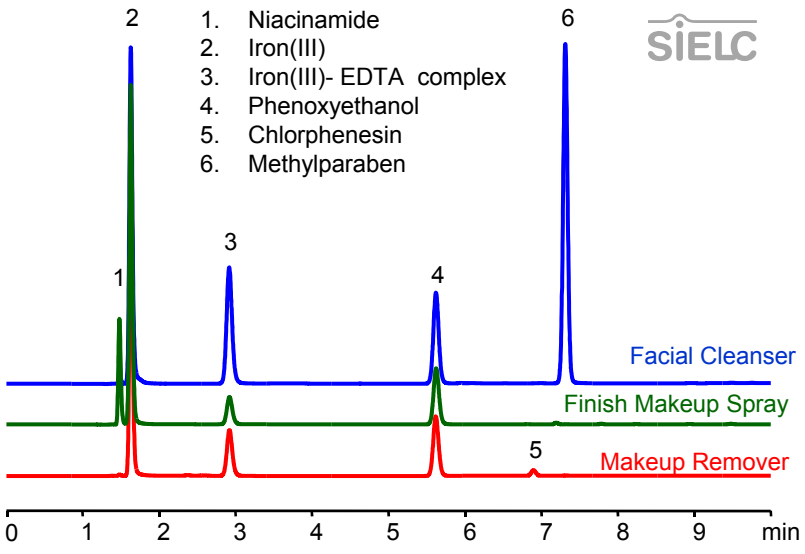


Fig. 4. Gradient method. HPLC column: Newcrom BH, 4.6 x 150 mm, 5 µm. Flow rate: 1.0 mL/min. Mobile phase: MeCN gradient from 2 to 70% in water in 10 min, mobile phase contains 0.1% H_2SO_4. Detection: UV 260 nm. Injection: 5 µL of sample.

Summary

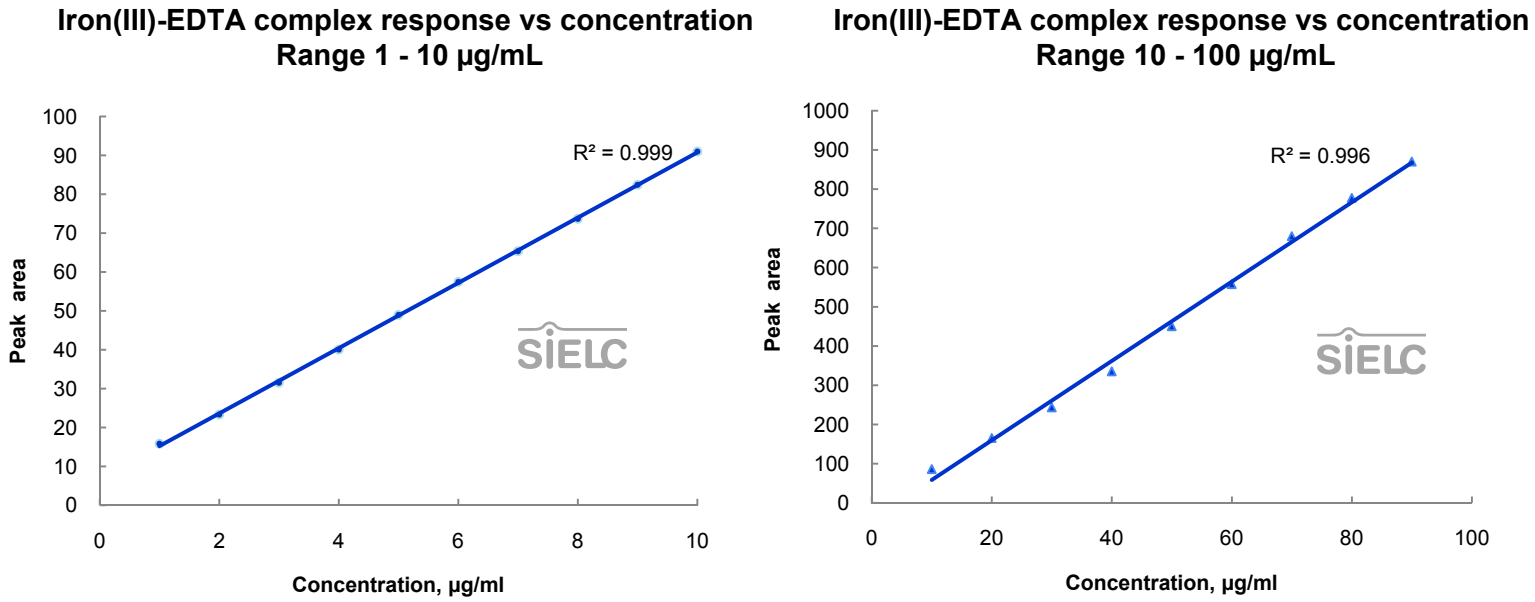


Fig. 5. Linearity study of the EDTA analysis quantitation method. Analytical column: Newcrom BH, 4.6 x 150 mm, 5 µm. Flow rate: 1.0 mL/min. Mobile phase: 2% MeCN in water containing 0.1% H_2SO_4. Detection: UV 260 nm. Injection: 5 µL of EDTA standards with $FeCl_3$ solution.

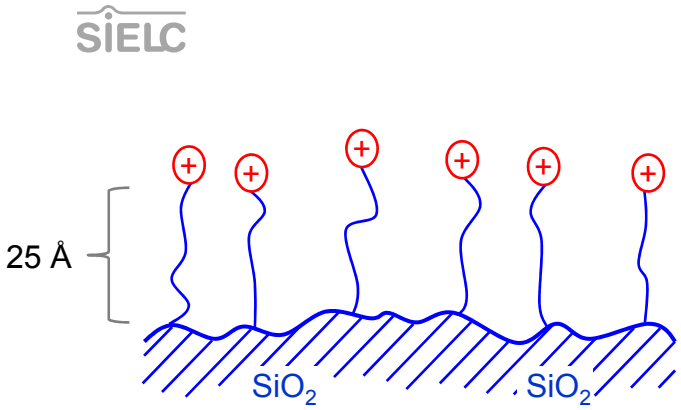


Fig. 6. Schematic structure of Newcrom BH column surface chemistry

Linearity of this method was investigated using increased concentrations of EDTA. Good linearity of the UV absorption response at 260 nm of EDTA complex with Fe(III) ions was observed within a wide concentration range.

Simple sample preparation includes mixing the unknown sample with Fe(III) stock solution followed by an HPLC separation. Sensitivity (LOQ) of down to 0.1 ppm of EDTA in samples has been achieved routinely.

Column surface chemistry is schematically presented in Fig. 6. It is comprised of a long alkyl chain with a terminal strong basic group carrying a permanent positive charge. This ligand is covalently bonded to the silica gel support. Silica gel employed is of spherical type, with fully porous particles of 5 µm in diameter and pore size of 120 Å.

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formerly Allsep Technologies

For decades liquid chromatography stationary phase design has been dominated by the goal to eliminate multiple, or “unwanted”, interactions and to obtain a simple and predictable retention mechanism. Unfortunately, the simplification of the retention process limits the ability to control elution order of the analytes and the scope of available applications this system can be used for. As a response to this limitation, hundreds of different reverse-phase columns were introduced in the last years to cover a variety of analytical situations.

In contrast, PrimesepTM stationary phases were intentionally designed with two major interactions offered on the same column. Both interactions are independently adjustable with mobile-phase composition producing unlimited states of the stationary phase. The hydrophobic interaction is controlled by the amount of organic modifier in the mobile phase (as in any reverse-phase column), while the ion-exchange interaction is controlled by the ion-strength and pH of the mobile phase (as in other ion-exchange columns). This unique property allows using one stationary phase for numerous applications, including analyses of polar and non-polar, ionizable and neutral, organic and inorganic compounds. The behavior of PrimesepTM columns is predictable and reproducible. The method development process is simple and versatile.

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