Application Note
Drugs & Pharmaceuticals

The most reliable LC-EC applications for Drugs & Pharmaceuticals analysis

Antipsychotic drugs
Clozapine
Olanzapine
Risperidone

PET imaging tracer
Fluorodeoxyglucose (FDG)
FDG impurities

Pharmaceuticals, API
Acetaminophen
Artemether
Artemisinin, Dihydro-artemisinin
Betadex sulfobutyl ether sodium
Etoposide
Epinephrine
Heparin
mesna BNP7787
8-OH-DPAT
Vincristine
Sulfides
Glutathione
Aminothiols
Disulfides

Aminoglycoside drugs
Amikacin
Framycetin sulphate
Gentamicin sulphate
Kanamycin
Netilmycin
Neomycin sulfate
Spectinomycin
Lincomycin
Tobramycin

Ethylene Glycol in Hydroxyethyl Starch According to EP

- **European Pharmacopoeia 8.2 (2014)**
- **Impurity analysis**
- **Reproducible and robust**

Summary

The impurity analysis of ethylene glycol in hydroxyethyl starch (HES) was evaluated using the exact method and conditions described in the official EP 8.2 (2014) monograph [2]. The evaluation was performed using an ALEXYS® LC-EC analyzer for ethylene glycol, based on the DECADE Elite electrochemical detector and a SenCell with Au working electrode (WE) and HyREF (Pd/H_2) reference electrode. Detection of ethylene glycol was achieved by post-column addition of 250 mM NaOH followed by Pulsed Amperometric Detection (PAD) using a 4 step potential waveform. In this application note typical results obtained with the ALEXYS analyzer are reported, demonstrating its performance for the impurity analysis of ethylene glycol in HES bulk material used in blood volume expanders.

Ethylene Glycol in Hydroxyethyl Starch According to EP

Introduction

Hydroxyethyl starch (HES/HAES) is a nonionic starch derivative [1]. HES formulations in the form of colloidal solutions are mainly used as blood plasma expanders, since the 70'ties. In emergency situations an intravenous solution of hydroxyethyl starch is given to a patient with hypovolemia to prevent shock following severe blood loss by trauma, surgery, or other issues. After HES administration the blood volume is increase immediately restoring the capacity of the remaining red blood cells to deliver oxygen to the body. Commercial HES solutions are sold under the brand names Hespan, Hextend and Voluven. The use of Hydroxyethyl starch is under considerable debate with respect to safety and efficacy since its introduction. Recently, in 2013, the European Medicines Agency's issued an advice that HES should no longer be used in patients with sepsis (bacterial infection in the blood), burn injuries or in critically ill patients, due to the increased risk of kidney injury or mortality [3].

HES is commonly produced by acid hydrolysis of potato starch, followed by hydroxyethylation using ethylene oxide [4,5]. During this reaction ethylene glycol is formed as a side product. Ethylene glycol itself is moderately toxic. However, when ingested it is metabolized into a variety of toxic oxidation products such as glycolic acid and oxalic acid, which may lead to kidney failure and brain damage [6,7]. Since ethylene glycol do not contain chromophores or fluorophores, its direct detection by optical techniques, lacks sensitivity, which make it less suitable for trace analysis. Due to the presence of oxidizable hydroxyl groups in ethylene glycol, Pulsed Amperometric Detection (PAD) can be successfully utilized for the sensitive analysis of ethylene glycol [8-10]. The European Pharmacopoeia describes a compendial method for the impurity analysis of ethylene glycol in Hydroxyethylene Starches using PAD [2].

Method

The EP 8.2 compendial method for the analysis of impurity levels of ethylene glycol in HES is based on isocratic separation using an aqueous C18 reverse phase column with water as mobile phase, followed by post-column addition of 250 mM NaOH and pulsed amperometric detection on a gold (Au) working electrode.

Separation

In the monograph the use of the following column type is described for the separation of ethylene glycol: column with dimensions of 250 x 4.6 mm ID and stationary phase octadecylsilyl silica gel for chromatography (5 µm); precolumn with dimensions 10 x 4.0 mm ID and the same stationary phase. For this evaluation the Restek Ultra AQ C18 column and precolumn (see Table 1) were chosen as advised in the EP knowledge base. These columns match the exact criteria described in the monograph.

Table 1

LC-EC conditions, European Pharmacopoeia 8.2 (2014)	
LC system	ALEXYS analyzer (180.0040) with kit for ethylene glycol
LC Column	Analytical column: Restek Ultra AQ C18 5µm 250 x 4.6 mm, Guard column: Restek Ultra AQ C18 10 x 4.0 mm
Mobile phase (A)	Water (resistivity > 18 Ohm-cm, TOC free)
Rinsing solution (B)	Acetonitrile/water 20/80 v/v%
Post-column eluent (C)	1.5 M NaOH carbonate-free in water
Flow rate	1 mL/min (mobile phase), 0.2 mL/min (post-column eluent)
$V_{injection}$	20 µL (full loop)
Temperature	30°C (separation & detection)
Pressure	Around 90 bar (column), around 50 bar (post-column mixer)
Flow cell	SenCell™ with Au WE and HyREF™ (Pd/H_2) RE, AST setting 2
Potential waveform (4-step)	E1, E2, E3, E4: +0.1, -2.0, +0.6, -0.1 V ts, t1, t2, t3, t4: 0.2, 0.4, 0.02, 0.01, 0.07 s
I-cell	0.5 - 1 µA
ADF	0.05 Hz
Range	2 µA/V

The post-column eluent was carefully prepared using a commercially available carbonate-free 50% NaOH solution, and diluted to a concentration of 1.5 M using deionized water (resistivity >18 MΩ-cm, TOC-free). The post-column eluent was stored in a plastic bottle instead of glass, NaOH is a strong etching agent and will react with the inner glass wall resulting in the release of silicates and borates. A separate isocratic

LC pump was used for the delivery of post-column eluent via a post-column mixer assembly. This tubing assembly consist of 7 meter 127 µm ID PEEK tubing to generate sufficient back pressure for optimal solvent delivery, in combination with a mixing tee.

Table 2

Column regeneration gradient program*		
Time (min)	Mobile phase A (%)	Rinsing solution B (%)
0	75	25
15	75	25
20	0	100
25	0	100
30	100	0
100	100	0

*) The column should be washed with the program after a maximum of 8 sample injections.

Detection

For detection of ethylene glycol the EP monograph specifies that a pulsed amperometric detector should be used, without any further specifications about the type of cell, working electrode or potential waveform settings. A DECADE Elite electrochemical detector is used in combination with a SenCell™. This wall-jet type flow cell has an Au working electrode (WE), maintenance-free HyREF (Pd/H_2) reference electrode and is using the proprietary [11] adjustable spacer technology (AST). This flow cell without polymeric gaskets, combines a high detection sensitivity with ease of use. As mentioned previously, In the EP monograph no specific potential waveform for detection is described, therefore we applied an optimized 4-step potential waveform as shown in figure 1. This particular waveform resulted in an excellent reproducibility and minimal electrode wear [12]; i.e. resulting in less flow cell maintenance and system down time. The cell current was typical about 0.5 – 1 µA under the specified conditions. The temperature for separation and detection was set to 30°C as specified in the monograph.

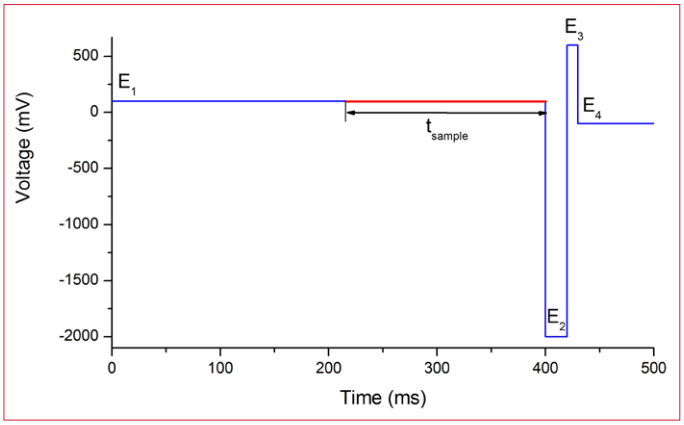

Figure 1: 4-step PAD potential waveform for the detection of Ethylene glycol.

Sample preparation

Reference solution: 800 mg of Ethylene glycol was accurately weighted and dissolved in 100 mL of water in a volumetric flask and mixed. 2 mL of the obtained solution was 100x diluted with water using a 200 mL volumetric flasks. Subsequently, 2 mL of this solution was 100x diluted in the same manner to obtain a final concentration of 0.8 mg/L. The reference solution is used to quantify the impurity level of ethylene glycol in HES and as system suitability standard to check the EP system performance criteria.

Sample preparation: Three commercial HES samples were obtained and analyzed:

1. Hydroxyethyl starch, high molecular weight (Mw > 1000 KDa), Sigma Aldrich, product code H6382
2. Hydroxyethyl starch, high Mw (500 kDa), EP standard, product code Y0001322
3. Hydroxyethyl starch, medium Mw (130 kDa), EP standard, Y0001277

All 3 sample test solutions were prepared in the following way: 1 g of the sample was accurately weighted and dissolved in 50 mL of water in a volumetric flask to obtain a final concentration of 20 g/L.

Results

System suitability

In figure 2 an example chromatogram obtained with the EP reference solution (0.8 mg/L Ethylene glycol in water). The retention time for the Ethylene glycol peak was 3.8 min, which is in correspondence with the retention time published in the EP monograph (about 4 min).

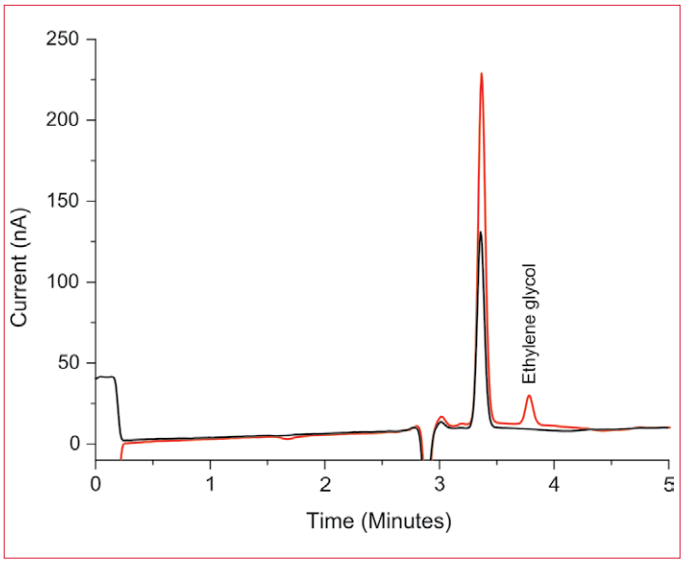

Figure 2: Chromatogram obtained with a 20 µL injection of the EP reference solution consisting of 0.8 mg/L Ethylene glycol dissolved in water (red curve). A blank injection of water is shown for reference (blue curve).

In the EP monograph for HES the following system suitability requirement are specified:

- Signal-to-noise ratio: 10 or higher for the principal peak.
- Repeatability: maximum relative standard deviation (RSD) of 10% for 6 replicate injections.

To evaluate the system suitability requirements the EP reference solution of 0.8 mg/L Ethylene glycol dissolved in water is used as described in the monograph.

Table 3

EP system suitability requirements		
Parameter	EP criteria	Measured
Retention time Ethylene glycol (EG)	About 4 min	3.8 min
Repeatability EG (n=6), peak area	≤ 10%	1.8%
Signal-to-noise ratio EG	≥ 10	66

The results of the system suitability test are shown in table 3. It is evident that the system suitability requirements are met for all parameters.

Linearity, repeatability & LOD

The linearity for Ethylene glycol was investigated in the concentration range of 0.1 mg/L – 0.8 mg/L and 1 mg/L – 8 mg/L in this concentration ranges the correlation coefficient for peak area was 0.999 or better. The relative standard deviation (RSD) of the retention time, peak area and height were determined for 6 replicate injections for the 0.8 mg/L Ethylene glycol reference solution. The RSD's were <0.2%, 1.8% and 0.8%, respectively for the Ethylene glycol peak. For a higher concentration of 8 mg/L Ethylene glycol the RSD's (n=6) for both peak area and height were better than 0.4%. The Limit of Detection (LOD) for Ethylene glycol, calculated as the analyte response corresponding to 3x the ASTM noise (average peak-to-peak baseline noise of 30 segments of 0.5 min), was about 30 µg/L (0.5 µM). The calculated LOD is more than 25 times lower than the limit specified by the EP for the maximum allowed concentration of Ethylene glycol in HES samples.

Sample analysis

As an example three commercial Hydroxyethyl starch samples were analyzed (see the sample preparation section for details). Note that these samples are not specifically intended for the use in medical products. The samples are respectively abbreviated as sample H6382, Y0001322 and Y0001277 from this point onwards. The chromatograms obtain for the three sample solutions are shown in figure 3.

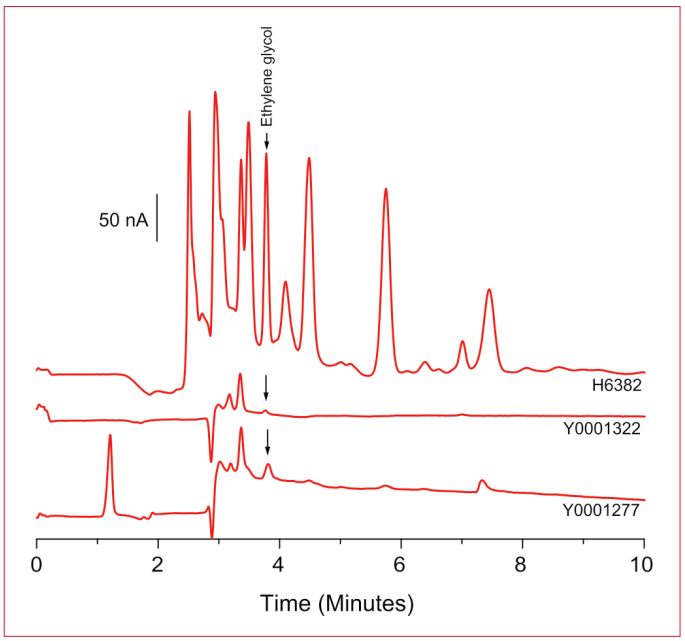

Figure 3: Overlay of the chromatograms obtained with 20 µL injections of the three Hydroxyethyl starch samples. Top: H6382, middle: Y0001322 and bottom: Y0001277. The Ethylene glycol peaks are indicated with an arrow in the overlay. A scale bar representing a current of 50 nA is shown as a reference.

Table 4

Limit of Ethylene glycol, EP

Hydroxyethyl starch sample	EP criteria (ppm)	Measured (ppm)
H6382, high molecular weight (Mw > 1000 KDa)	≤ 40	544
Y0001322, high Mw (500 kDa)		10
Y0001277, medium Mw (130 kDa)		60

The limit of Ethylene glycol impurities in the EP is specified in ppm (parts-per-million). The dry Hydroxyethyl starch bulk material used for medical HES formulations should not contain more than 40 ppm Ethylene glycol. In the EP monograph the peak area of the Ethylene glycol peak in the chromatogram obtained with the reference solution (0.8 mg/L EG) represents a concentration of 40 ppm when compared to a chromatogram of 20 g/L HES, the sample test solution (0.8 mg /20 g = 40 ppm).

The contents of Ethylene glycol in the Hydroxyethyl starch samples is calculated as specified in the EP monograph:

Contents (ppm) = (A_{sample} /$A_{reference}$) x 40 ppm

Where:

A_{sample} = Ethylene glycol peak area obtain from the chromatogram of the sample test solution

$A_{reference}$ = Ethylene glycol peak area obtain from the chromatogram of the reference solution

The results for all analyzed samples are listed in table 4. It is evident from table 4 that of the three tested HES samples only sample Y0001322 passed the EP acceptance criteria with respect to the allowed limit of Ethylene glycol impurities in Hydroxyethyl starch bulk material.

Conclusion

The ALEXYS HPLC-ECD Analyzer based on pulsed amperometric detection using the DECADE Elite detector offers a tailored solution for the impurity analysis of Ethylene Glycol in Hydroxyethyl starch using the official method of the European Pharmacopeia.

References

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Ordering information

180.0040	ALEXYS LC-EC analyzer, with kit for Ethylene glycol
250.1170	Restek Ultra AQ C18 5µm 250 x 4.6 mm analytical column
250.1172	Restek Ultra AQ C18 10 x 4.0 mm guard column

For research purpose only. The information shown in this communication is solely to demonstrate the applicability of the ALEXYS system. The actual performance may be affected by factors beyond Antec's control. Specifications mentioned in this application note are subject to change without further notice.