HPLC Analysis of Antidiabetic Drugs

Introduction

Drugs, including antidiabetic drugs, with different targets have various chemical structures. This makes it difficult for an HPLC method to simultaneously analyze all antidiabetic drugs which may contain peptides, sugars, and low-molecular weight compounds of different properties. Thus, analysis of each drug requires a specific method with an appropriate column and optimized analytical conditions. This technical article introduces application data for the analysis of four antidiabetic drugs using Shodex columns.

1. Analysis of an Oral Antidiabetic Drug (Metformin)

Metformin is an oral antidiabetic drug. It suppresses glucose production to lower blood sugar level as well as to elongate life expectancy. To understand its various actions and utilize the obtained information to the treatment, it requires highly sensitive and selective analysis methods.

Since polarity of metformin is very high, it is not retained by reversed phase mode. Meanwhile, HILIC mode is often suitable for the analysis of high polarity compounds. We analyzed metformin using a Shodex HILIC column and a triple quadrupole mass spectrometer, aimed for a highly sensitive detection.

Shodex Asahipak NH2P-40 2D is a column filled with polymer-based packing materials modified with amino groups. By using an eluent with a large acetonitrile concentration, we attempted to retain metformin under HILIC mode.

1.1 Experimental

Sample pretreatment

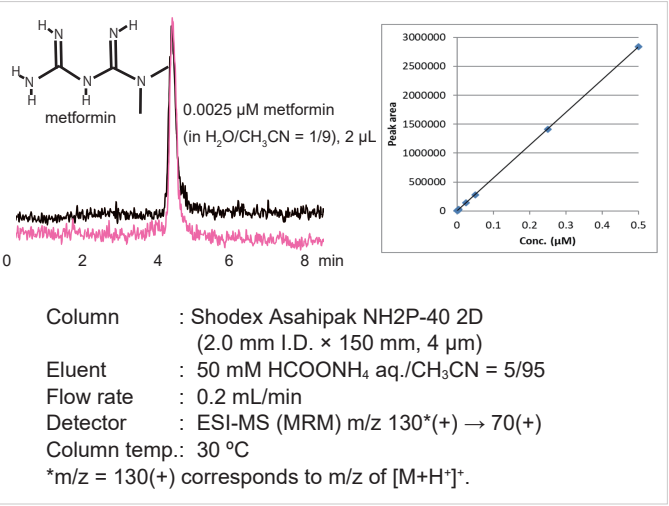
The test sample was prepared by mixing metformin standard and guinea pig serum following the below steps.

1. Add an equal volume of 0.05-μM standard solution to the serum.
2. Add 1+4 volume of CH_3CN to the mixture to let the protein precipitate.
3. Centrifuge the mixture. Add an equal volume of CH_3CN to the obtained supernatant and filter the mixture with a 0.45-μm membrane filter.

*The final test sample contains 90 % CH_3CN.

1.2 Results

The obtained chromatograms show sufficient retention of metformin that cannot be achieved by an ODS column (Fig. 1). The recovery rate was 103 %. This indicates that the metformin peak was not affected by ion suppression even under the presence of guinea pig serum.



Column : Shodex Asahipak NH2P-40 2D (2.0 mm I.D. × 150 mm, 4 μm)
Eluent : 50 mM $HCOONH_4$ aq./CH_3CN = 5/95
Flow rate : 0.2 mL/min
Detector : ESI-MS (MRM) m/z 130*(+) → 70(+)
Column temp.: 30 °C
*m/z = 130(+) corresponds to m/z of $[M+H^+]^+$.

Fig. 1. Chromatograms of (black) metformin standard and (pink) metformin standard with guinea pig serum

The test sample was quantified by standard addition method. A good linearity was obtained for the calibration curve with a coefficient of determination $R^2 \geq 0.99$. The MRM mode was used in this experiment. The selectivity and sensitivity of SIM is not as good as that of MRM, but it can also be used in place of a UV detector, by monitoring m/z 130(+).

1.3 Conclusions

Metformin was retained and analyzed effectively using the Shodex Asahipak NH2P-40 2D. The use of MRM mode allows the quantification of metformin.

2. Analysis of Insulin

Since its discovery in the early 20th century, insulin has been taking a major role in the treatment of diabetes. Insulin forms dimers and hexamers; however, unlike insulin monomers, they do not have blood sugar lowering effects. Thus, it is important to separate and quantify monomers and other aggregates of insulin in insulin drugs.

Dimers of proteins, including insulin, are often separated by size exclusion chromatography. The US Pharmacopoeia (USP) General Chapters mentions size exclusion chromatography separation in <121.1> PHYSICOCHEMICAL ANALYTICAL PROCEDURES FOR INSULINS, LIMIT OF HIGH MOLECULAR WEIGHT PROTEINS. In this application, we examined a Shodex column's capability for fulfilling the system suitability.

2.1 Experimental

The USP method states to use a column filled with 5- to 10-µm diameter, L20 column packing material. The Shodex PROTEIN KW-802.5 is an aqueous SEC column filled with 5-µm modified silica gels, and this fulfills the requirement. The method also states to use an ambient temperature for the column temperature; however, to ensure obtaining the repeatable results, we set the column temperature at 25 °C. The "resolution solution" used for the system suitability test was prepared following the USP method. The test sample is required to contain more than 0.4 % "high molecular proteins" (i.e., dimers and hexamers). We prepared 4-mg/mL insulin (bovine) sample in 0.01 N HCl (aq). The injection volume used was 100 µL.

2.2 Results and Discussion

Figure 2 shows the obtained chromatogram. The sum of peak areas 1 and 2 was 9.1 % of the total peak area (sum of peak areas 1, 2, and 3). This fulfills the requirement stated in the "Resolution solution" (the sum peak area of peaks 1 and 2 to be ≥ 0.4 %). The system suitability requirements include the elution times of insulin aggregate, dimer, and monomer to be between 13 and 17 minutes, 17.5 minutes, and between 18 and 22 minutes, respectively. Also, the system suitability requires the ratio of dimer peak height and valley height (a valley between the dimer and the monomer peaks; peak-to-valley ratio) to be ≥ 2.0. The peaks 1, 2, and 3 in the figure 2 correspond to aggregate, dimer, and monomer, respectively. The observed retention times are very similar to the requirements. The obtained peak valley ratio was ≥10.0. The results demonstrated that the HPLC system using the Shodex PROTEIN KW-802.5 to fulfill all system suitability requirements.

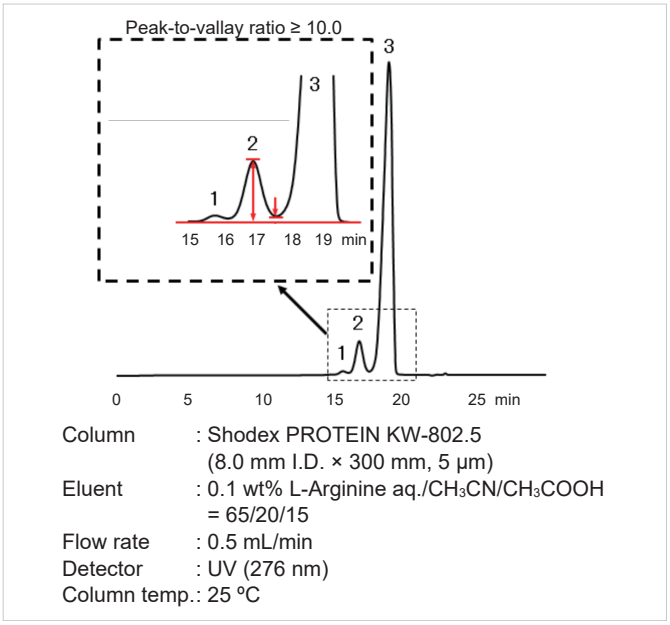


Fig. 2. Chromatogram of insulin (bovine)

2.3 Conclusions

The HPLC method using Shodex PROTEIN KW-802.5 fulfilled the system suitability stated in the USP <121.1> PHYSICOCHEMICAL ANALYTICAL PROCEDURES FOR INSULINS, LIMIT OF HIGH MOLECULAR WEIGHT PROTEINS.

3. Analysis of Liraglutide

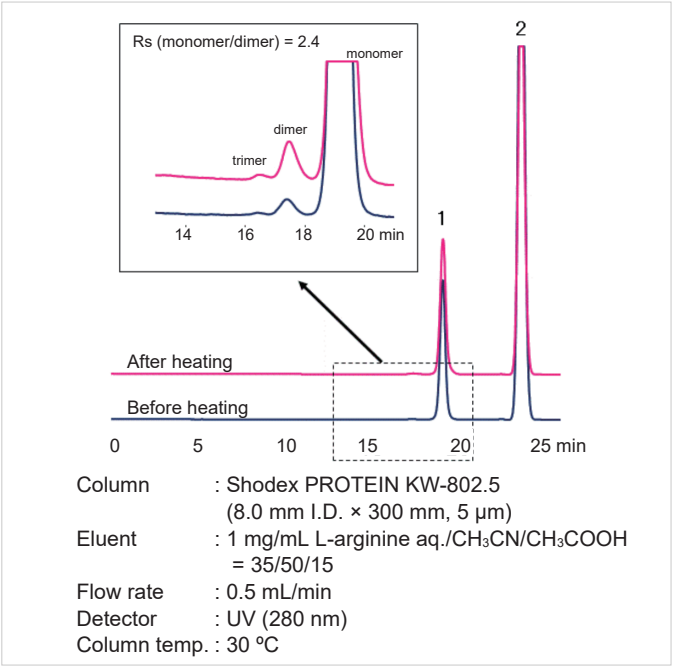
Liraglutide is a drug for treating type 2 diabetes. It promotes insulin secretion. It is an acylated peptide having a structure similar to GLP-1 secreted in a body. The molecular weight of liraglutide is 3,751 while that of GLP-1 is 4,170. Since size exclusion chromatography is known to effectively separate many peptide/protein drugs, including insulin and their dimers, liraglutide and its aggregates are also expected to be separated under size exclusion mode. Therefore, we analyzed liraglutide and its dimer and other aggregates using the Shodex PROTEIN KW-802.5.

3.1 Experimental

Shodex PROTEIN KW-802.5 is an aqueous SEC column filled with modified silica gel. The test sample was prepared by diluting the liraglutide five times with ultrapure water. We prepared another test sample by heating the liraglutide drug at 80 °C for 17 hours to purposely increase its dimer content. Prepared samples were used to test the method's capability for separating monomer and dimer. The heated sample was diluted five times with ultrapure water. The injection volume used was 5 µL.

3.2 Results and Discussion

Figure 3 shows the obtained chromatogram. The peaks 1 and 2 correspond to liraglutide monomer and phenol (an additive in the drug), respectively. The peaks eluted prior to liraglutide monomer are considered to be its dimer and trimer. The amount of those components increased after heating. The resolutions between the monomer and the dimer peaks in both heated and non-heated test samples were about 2.4. Each component was successfully separated for its quantification.



Column : Shodex PROTEIN KW-802.5 (8.0 mm I.D. × 300 mm, 5 µm)
Eluent : 1 mg/mL L-arginine aq./CH_3CN/CH_3COOH = 35/50/15
Flow rate : 0.5 mL/min
Detector : UV (280 nm)
Column temp. : 30 °C

Fig. 3. Chromatogram of liraglutide

3.3 Conclusions

The results demonstrated Shodex PROTEIN KW-802.5's effectiveness in separating monomer and aggregates of liraglutide as well as phenol, an additive, in the drug.

4. Analysis of Exenatide

Exenatide is a drug used for the treatment of type 2 diabetes. It promotes insulin secretion, similar to liraglutide mentioned in the previous section. The USP includes two related monographs for "Exenatide" and "Exenatide Injections". Each states different and multiple analytical conditions to test various related items. In this application, we examined the system suitability required for the "Assay" section of the "Exenatide Injection".

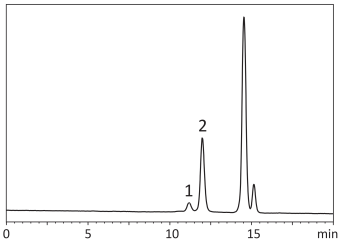
4.1 Experimental

Shodex PROTEIN KW-802.5 is an aqueous SEC column filled with modified silica gel. The samples tested were "system suitability solution" and "standard solution" stated in the monograph.

The diluent for the sample preparation contained 3.26-g sodium acetate trihydrate, 2.98-g L-methionine, and 2.06-mL glacial acetic acid, and it was prepared in 2-L ultrapure water. The 0.25-mg/mL standard solution was prepared by diluting exenatide with the diluent. The system suitability solution was prepared by mixing 30-µL soybean trypsin inhibitor stock solution and 1-mL standard solution using a vortex mixer. The soybean trypsin inhibitor stock solution was prepared by diluting the soybean trypsin inhibitor in ultrapure water to make its concentration at 1 mg/mL. The injection volume was 20 µL. The USP monograph states to set the autosampler temperature between 2 and 8 °C. We used 8 °C for the analysis.

4.2 Results and Discussion

Figure 4 shows chromatogram of the system suitability solution. The peaks 1 and 2 corresponds to soybean trypsin inhibitor and exenatide, respectively. The system suitability requires for the soybean trypsin inhibitor peak area to be 2.0 to 5.0 % of the total peak area. The soybean trypsin inhibitor's peak area detected was 2.8 % of the total peak area, fulfilling the requirement.

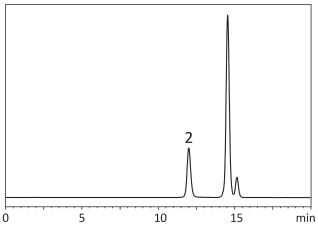


Column : Shodex PROTEIN KW-802.5 (8.0 mm I.D. × 300 mm, 5 µm)
Eluent : 0.13 M Na_2SO_4 aq./CH_3CN/trifluoroacetic acid = 1500/500/2
Flow rate : 0.8 mL/min
Detector : UV (214 nm)
Column temp.: 25 °C

Fig. 4. Chromatogram of the system suitability solution

The system suitability also requires the resolution of the exenatide and soybean trypsin inhibitor peaks to be ≥ 1.3 and the obtained data was 1.7, thus demonstrated to fulfill the requirement.

Figure 5 shows the chromatogram of the standard solution. The system suitability requires the tailing factor of exenatide to be between 0.8 and 1.4, and the relative standard deviation of its peak area to be less than 2.0 %. The obtained results were 1.1 and less than 0.1 %, respectively, and thus demonstrated to fulfill these requirements also. The molecular weights of soybean trypsin inhibitor and exenatide are about 20,100 Da and 4,200 Da, respectively. The obtained chromatogram showed that the elusion orders of them were from bigger (soybean trypsin inhibitor) to smaller (exenatide). This indicates that their separations were carried under size exclusion mode as the monograph intended.



Refer to Fig.4 for analytical conditions used.

Fig. 5. Chromatogram of the standard solution

4.3 Conclusions

The Shodex™ PROTEIN KW-802.5 demonstrated to fulfill the system suitability required for the Assay section of the USP Exenatide Injection.

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