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Discrimination of Human Insulin and Its Analogs by Flow Injection Analysis with Mass Spectrometry

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Abstract **Original Research Article**

Although insulin therapy is recognized as an effective treatment for diabetes, it can lead to serious side effects and medical accidents. Since living organisms contain many foreign components, the separation of these components by liquid chromatography (LC) is essential for the identification of insulin preparations. However, it is difficult to conclude that separation by LC is indispensable because multiple components are unlikely to be mixed in a sample obtained from a vial or syringe needle. Therefore, the introduction of flow injection analysis (FIA), in which samples are analyzed with the column removed, can potentially eliminate the sample separation process and reduce analysis time. In addition, tandem mass spectrometry (MS/MS), which allows detailed analysis, requires expensive equipment, and some facilities only allow analysis by single mass spectrometry (MS). The discriminatory ability of each analysis method should also be clarified. Here, we examined the analysis time and discriminatory ability using a combination of FIA, MS, and MS/MS. The results showed that FIA reduced the analysis time, FIA-MS could not discriminate when the molecular weights of the samples were the same or similar, and FIA-MS/MS could discriminate between different amino acid sequences. FIA-MS has limitations in discrimination, whereas FIA-MS/MS is considered an analytical method that can shorten the analysis time and discriminate formulations.

Keywords: Human insulin, Insulin analog, Insulin preparations, Flow injection analysis, Mass spectrometry.

INTRODUCTION

The incidence of diabetes mellitus is rapidly increasing worldwide and it is highly prevalent in Japan. Although diabetes requires lifelong treatment and self-management, maintaining good blood glucose control and preventing complications may lead to a daily life similar to that of people without diabetes (Yonezawa M *et al.*, 2008). Self-management of diabetes includes lifestyle management such as diet and exercise, and drug therapy such as oral medication and insulin self-injection. Among these, insulin therapy is used for various forms of diabetes including type 2 diabetes. As the prevalence of type 2 diabetes has been rising in recent years, the demand for insulin to treat this condition is expected to increase by more than 20% between 2018 and 2030 (Basu S *et al.*, 2019). Insulin preparations utilize human insulin and human insulin analogs with modified amino acid sequences, which differ only slightly in sequence, resulting in large differences in the duration of action.

Although insulin therapy is recognized as an effective treatment for diabetes mellitus, there is a risk of rapid hypoglycemia, hyperglycemia, and medical accidents owing to (1) the unique notation of dosage in units, (2) many types of formulations, (3) various administration methods, including self-injection, and (4) frequent dosage changes due to fluctuations in blood sugar levels. If an accident occurs in a medical institution, it is possible to promptly investigate the route of administration and the cause of the accident. However, it is difficult to identify the cause if an accident occurs at home or in other places, because there is little evidence left. In these situations, the analysis of human insulin or its analogs on medical devices left on-site, such as a vial or syringe needle, sometimes helps to investigate the cause of the accident (Ishii H, 2007).

Human insulin and its analog preparations differ only slightly in their amino acid sequences. Analytical methods based on mass spectrometry are effective for their identification, and many examples of analyses using liquid chromatography-tandem mass spectrometry (LC-MS/MS) have been reported (Vanhee C *et al.*, 2016; Ojanpera I *et al.*, 2013; Hess C *et al.*, 2012; Thevis M *et al.*, 2005). Identifying insulin preparations in vivo requires the separation of components by liquid chromatography (LC) because they contain many foreign components, and the separation provides a mass spectrum specific to insulin. However, samples obtained from vials or needles are unlikely to contain a mixture of multiple components, and it is difficult to conclude whether LC separation is essential. Therefore, the introduction of flow injection analysis (FIA), in which the sample is analyzed with the column removed, can potentially eliminate the sample separation process and significantly reduce analysis time. In addition, tandem mass spectrometry (MS/MS), which allows detailed analysis, requires expensive equipment, and some facilities only allow analysis by single mass spectrometry (MS); thus, the discriminatory ability of each analytical method should be clarified.

In this study, human insulin and its analogs were measured using a combination of FIA and MS or MS/MS, and the analysis time and discriminatory ability of these methods were investigated and reported.

MATERIAL AND METHODS

Preparation of Reagents and Test Samples

Human insulin was purchased from Fujifilm Wako Pure Chemicals Co. For analogs, insulin lispro (Humalog®), insulin aspart (Novolapid®), insulin glulisine (Apidra®), insulin degludec (Tresiba®), insulin glargine (Lantus®) and insulin detemir (Levemir®). All other reagents were of liquid chromatography-mass spectrometry grade. Samples of human insulin or analogs were diluted accordingly in 0.1% formic acid solution and prepared to concentrations of 50 µg/mL for MS measurements and 100 µg/mL for MS/MS measurements.

Instrument Conditions

The analytes were identified using an AB SCIEX QTRAP® 6500 system (Applied Biosystems, Foster City, CA, USA). Samples (10 µL) were subjected to liquid chromatography using an autosampler not equipped with LC columns. A mobile phase of deionized water and acetonitrile (50:50, v/v) containing 0.1% formic acid was used at a flow rate of 0.2 mL/min. The turbo ion spray interface was operated in the positive ion mode at 5500 V under the following operating conditions: ion source temperature, 300 ºC; ion source gas 1, 60 psi; ion source gas 2, 60 psi; and curtain gas, 40 psi. Mass spectra were recorded in the mass range of 500 to 2000 *m*/*z* for MS analysis. The product ion spectra were recorded with suitable precursor ions and collision energies in the mass range of 100 to 500 *m*/*z* for MS/MS analysis. The molecular weights, selected precursor ions and collision energies of analytes are shown in Table 1.

Table 1: Molecular weights, precursor ions, and collision energies of analytes

Analyte	Molecular weight (Da)	Precursor ion (*m*/*z*) and their charge state	Collision energy (CE)
Human insulin	5808	1162.5 (5+)	50
Insulin lispro	5808	1162.5 (5+)	50
Insulin aspart	5826	1165.9 (5+)	55
Insulin glulisine	5823	1165.5 (5+)	40
Insulin degludec	6104	1221.6 (5+)	55
Insulin glargine	6063	867.1 (7+)	35
Insulin detemir	5917	1184.2 (5+)	45

RESULTS AND DISCUSSION

Analysis Time by FIA

In this study, FIA was employed to shorten the analysis time, and the analysis was performed without a column attached to the LC so that the sample introduced through the inlet reached the mass spectrometer without being retained in the column. The total ion current chromatogram obtained for the analysis of human insulin using MS is shown in Figure 1. The horizontal axis in Figure 1 shows the time elapsed since the introduction of the sample. It has been reported that separating LC components takes several minutes to several tens of minutes to detect insulin (Vanhee C *et al.*, 2016; Ojanpera I *et al.*, 2013; Hess C *et al.*, 2012; Thevis M *et al.*, 2005). However, under the present analytical conditions, human insulin was detected within 10–20 s after sample injection, suggesting that FIA can complete the analysis within 10–20 s for any insulin formulation, because the analyte is not retained in the column, thereby reducing the analysis time.

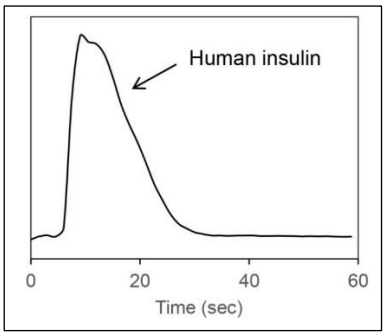

Figure 1: Total ion current chromatogram of human insulin obtained from FIA-MS analysis

Analysis Results by FIA-MS

Human insulin and its analogs were measured using FIA-MS, and the results are shown in Fig. 2 (A)–(G). In all the spectra, multiple peaks derived from multiple charged ions with different charges were observed. However, it is difficult to distinguish between them in the insulin formulations described below. (1) The spectra of human insulin (Fig. 2 (A)) and insulin lispro (Fig. 2 (B)) showed almost identical *m*/*z* values for multiple peaks. This is because human insulin and insulin lispro are identical in molecular weight, with only a partial difference in amino acid sequence, and there is no significant difference in the peak intensity ratio, making it difficult to distinguish them. (2) Insulin aspart (Fig. 2 (C)) and insulin glulisine (Fig. 2 (D)) showed almost identical *m*/*z* values for multiple peaks. This was because the difference in molecular weights was approximately a few Da, although the molecular weights were not identical. No significant differences were observed in the peak intensity ratios. Therefore, it was difficult to identify.

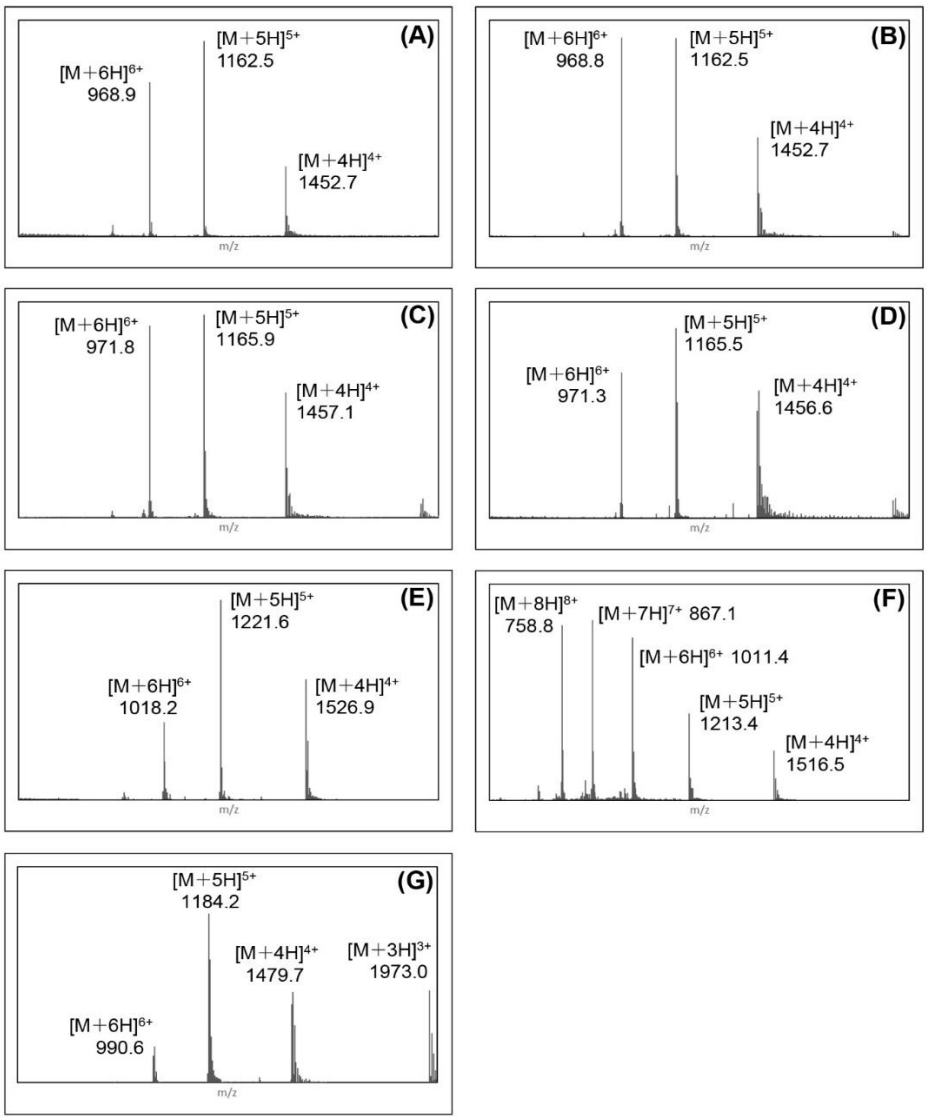

Figure 2: Mass spectra of human insulin (A), insulin lispro (B), insulin aspart (C), insulin glulisine (D), insulin degludec (E), insulin glargine (F) and insulin detemir (G) obtained from FIA-MS

Analysis Results by FIA-MS/MS

Human insulin and its analogs were measured using FIA-MS/MS, and the results are shown in Fig. 3 (A)–(G). The spectra of human insulin (Fig. 3 (A)) and insulin lispro (Fig. 3 (B)) were considered similar and difficult to distinguish using MS; however, their spectra were different using MS/MS, suggesting that they could be distinguished. The spectra of insulin aspart (Fig. 3 (C)) and insulin glulisine (Fig. 3 (D)) were also considered difficult to distinguish by MS; however, using MS/MS, the spectra were different and were considered distinguishable. The spectra of the other insulin preparations were different, as in MS; therefore, it was considered possible to discriminate the other insulin preparations. From the above, it can be inferred that MS/MS analysis can discriminate in detail, even for insulin preparations that are thought to be difficult to discriminate by MS.

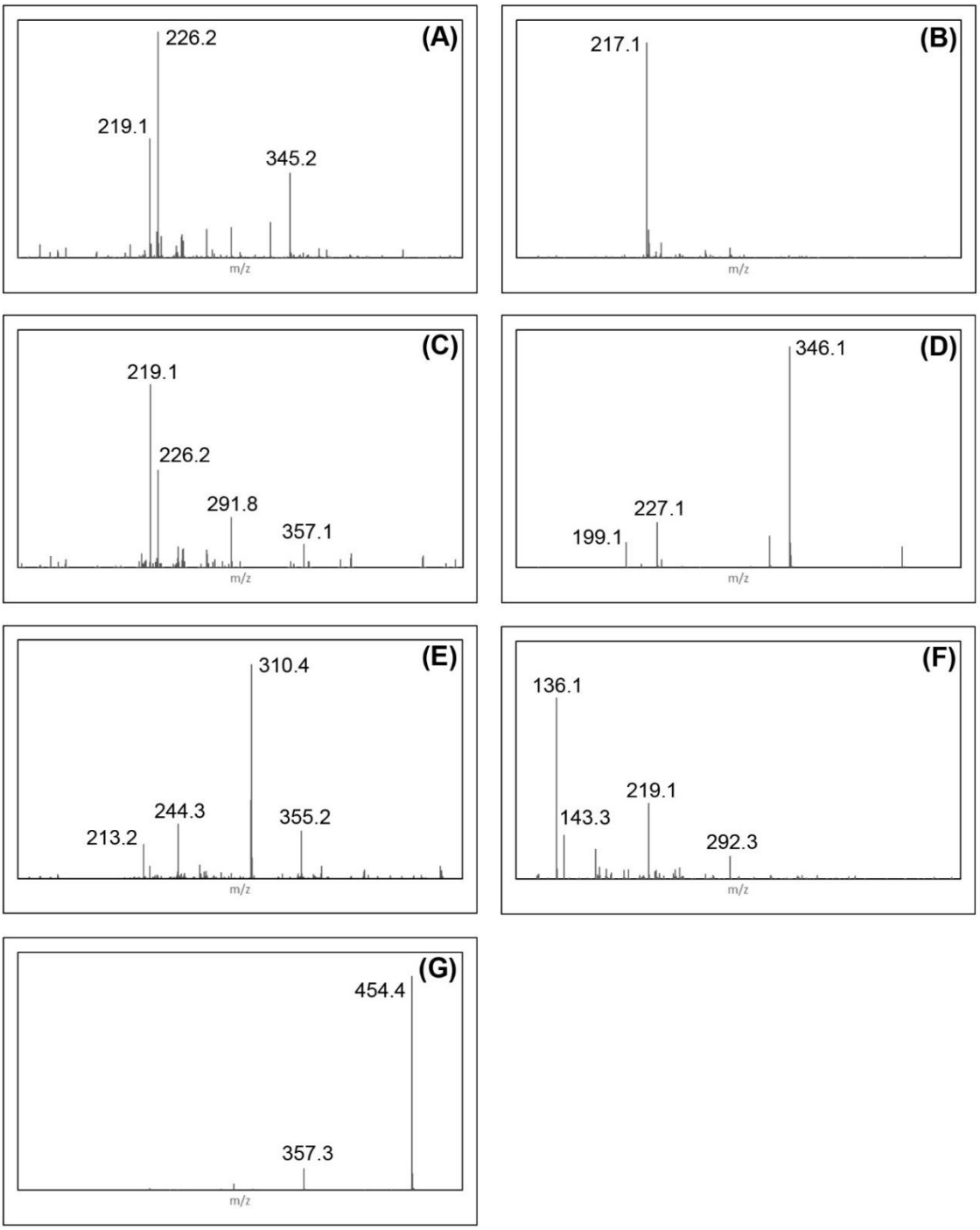

Figure 3: Product ion spectra of human insulin (A), insulin lispro (B), insulin aspart (C), insulin glulisine (D), insulin degludec (E), insulin glargine (F) and insulin detemir (G) obtained from FIA-MS/MS

CONCLUSION

FIA combined with MS or MS/MS has been used to analyze human insulin and its analogs. FIA was considered to reduce the analysis time, as the analysis time was only 10–20 s per sample. While FIA-MS had difficulty discriminating samples with identical or similar molecular weights, FIA-MS/MS could clearly identify insulin preparations because of differences in amino acid sequence. FIA-MS/MS is considered an analytical method that can shorten the analysis time of insulin formulations and clearly discriminate between formulations.

Conflict of Interest: The authors declare no conflict of interest.

REFERENCES

- Basu, S., Yudkin, J. S., Kehlenbrink, S., Davies, J. I., Wild, S. H., Lipska, K. J., Sussman, J. B., & Beran, D. (2019). Estimation of global insulin use for type 2 diabetes, 2018-30: a microsimulation analysis. *The Lancet Diabetes & Endocrinology*, *7*(1), 25–33.
- Hess, C., Thomas, A., Thevis, M., Stratmann, B., Quester, W., Tschoepe, D., Madea, B., & Musshoff, F. (2012). Simultaneous determination and validated quantification of human insulin and its synthetic analogues in human blood serum by immunoaffinity purification and liquid chromatography-mass spectrometry. *Analytical and Bioanalytical Chemistry*, *404*, 1813–1822.
- Ishii, H. (2007). Determination of insulin by high performance liquid chromatography/mass spectrometry (LC/MS). *Japanese Journal of Forensic Science and Technology*, *12*(2), 207-215.
- Ojanpera, I., Sajantila, A., Vinogradova, L., Thomas, A., Schanzer, W., & Thevis, M. (2013). Post-mortem vitreous humour as potential specimen for detection of insulin analogues by LC-MS/MS. *Forensic Science International*, *233*, 328–332.
- Thevis, M., Thomas, A., Delahaut, P., Bosseloir, A., & Schanzer, W. (2005). Qualitative determination of synthetic analogues of insulin in human plasma by immunoaffinity purification and liquid chromatography-tandem mass spectrometry for doping control purposes. *Analytical Chemistry*, *77*, 3579–3585.
- Vanhee, C., Janvier, S., Moens, G., Deconinck, E., & Courselle, P. (2016). A simple dilute and shoot methodology for the identification and quantification of illegal insulin. *Journal of Pharmaceutical Analysis*, *6*, 326–334.
- Yonezawa, M., Hashimoto, C., & Matsushita, M. (2008). Self-care factors in poor glycemic control diabetics with self-injection of insulin. *Journal of Comprehensive Nursing Research*, *11*(1), 25–27.