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EVALUATION OF INJECTABLE GENERIC DRUGS USING THERMOGRAVIMETRY AND THE INFLUENCE OF *d*-MANNITOL ON THE STABILITY OF GABEXATE MESILATE

Toru Otori^{1*}, Sumio Matzno¹, Masahiro Hyodo¹, Sara Sugimoto¹, Takayoshi Kimura², Kenji Matsuyama¹

¹Faculty of Pharmacy, Kinki University, 3-4-1 Kowakae, Higashi-Osaka 577-8502, Japan.

²Faculty of Science and Engineering, Kinki University, 3-4-1 Kowakae, Higashi-Osaka 577-8502, Japan.

Email : tohtori@phar.kindai.ac.jp

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Abstract

Gabexate mesilate has been mainly used in the treatment of disseminated intravascular coagration (DIC) in Japan since 1983. Recently, several generic versions of injectable gabexate mesilate with or without *d*-mannitol as an additive have been put on the Japanese market. In our study, thermo gravimetry (TG) did not show any differences between gabexate mesilate products with or without *d*-mannitol as an additive. However, differential thermal analysis (DTA) showed a significant change in absorption derived from endothermic reactions. Differential thermal analysis for M1, M2 and M3, with 100 mg of *d*-mannitol produced two endothermic peaks during the first rise in temperature from 30°C to 170°C, followed by an exothermic peak derived from the 170°C to 30°C cooling period. During the second rise in temperature from 30°C to 170°C another endothermic peak was produced. On the other hand, N1, N2, N3, and FOY without *d*-mannitol derived from gabexate mesilate produced only one endothermic peak, and were followed by no exothermic peaks during the cooling period. Interestingly, a glass transition was found at the second rise in temperature. In addition, we confirmed that gabexate mesilate undergoes hydrolysis in the presence of water due to the ester bonds in its chemical structure. The addition of *d*-mannitol seems to cause moisture absorption in the lyophilized processing of gabexate mesilate products, resulting in a higher content of hydrolyzed ethyl 4-Hydrobenzoate for M1, M2, and M3 by HPLC analysis. We propose that the addition of *d*-mannitol should be avoided in the production of gabexate mesilate formulation.

Keywords: Generic, gabexate mesilate, *d*-mannitol, Thermo gravimetry, Differential thermal analysis.

1. Introduction

Thermogravimetry (TG) measures the mass loss of a sample as the temperature of the sample is increased in a controlled manner. It can be applied to examine any physical (such as evaporation) or chemical process (such as thermal degradation) that causes a material to lose volatile gases.

On the other hand, differential thermal analysis (DTA) is concerned with the measurement of energy changes in materials. Differential thermal analysis measures Delta T against time, or more usually, a sample temperature with an endothermic (heat-absorbing, melting) and exothermic (heat-producing) peak. DTA is thus the most generally applicable to all thermal analysis methods, since every physical or chemical change involves a change in energy.

Gabexate mesilate is a non-peptide protein inhibitor, developed in Japan, which is administrated intravenously in the treatment of acute pancreatitis, and disseminated intravascular coagulation. Six generic gabexate mesilate drugs have been put on the Japanese market. Among them, three generic drugs M1, M2 and M3 each contain 100 mg of the additive *d*-mannitol. Whereas three generic drugs (N1, N2 and N3) and a brand-name drug (FOY) do not. In the case of freeze-dried products, different manufacturing methods may produce different crystal structures, and studies have shown that differences in the crystal structure can affect the stability and solubility of gabexate mesilate¹⁻⁶. Fig-1 shows a considerable amount of water in the bottom of a vial filled with gabexate mesilate containing *d*-mannitol (M1, M2 and M3). As shown in Fig-2, gabexate mesilate contains ester bonds in its chemical structure, and readily undergoes hydrolysis in the presence of water.

Fig-1 One Moisture uptake is observed in M1 vial (with *d*-mannitol), but not in FOY (brand-name drug without *d*-mannitol).



Fig-1: Moisture uptake is observed in M1 vial (with *d*-mannitol), but not in FOY (brand-name drug without *d*-mannitol)

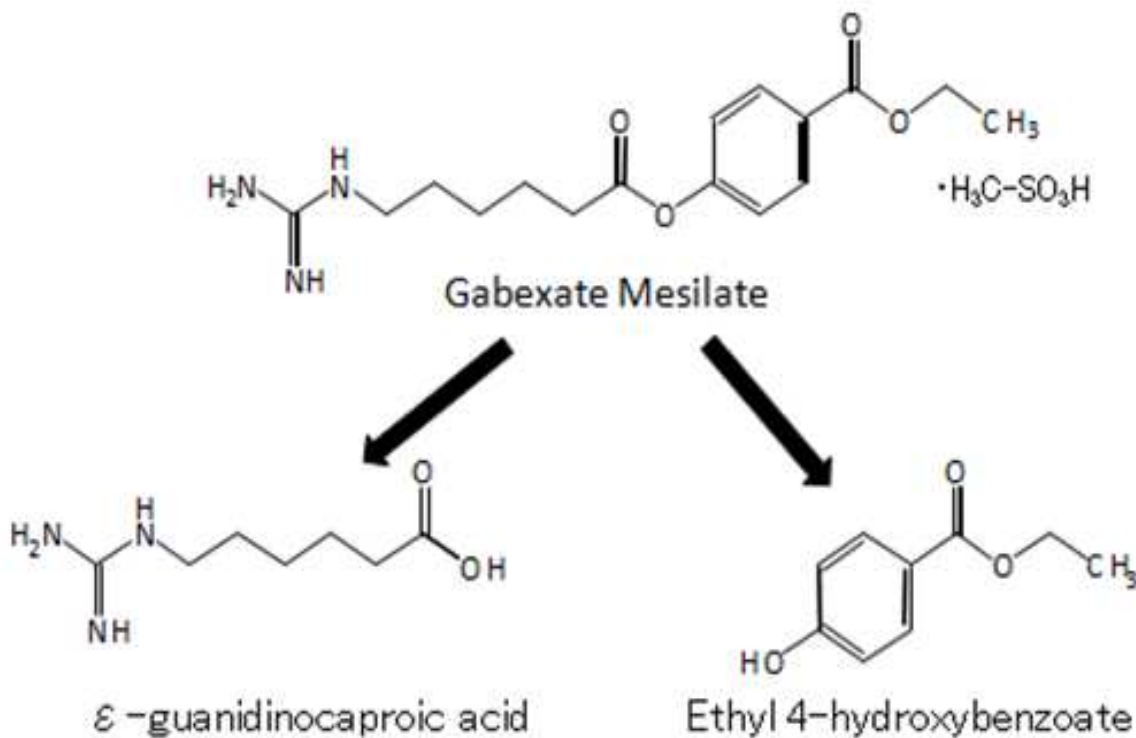


Fig-2: Hydrolysis of gabexate mesilate into ϵ -guanidinocaproic acid and ethyl 4-hydroxybenzoate.

According to these physical and chemical indicators, we conclude that the presence or absence of *d*-mannitol plays an important role in the content of water. To confirm this hypothesis, we put six generics and FOY under TG-DTA analysis, in order to measure the amount of water found in each drug.

The purpose of this study is to show the application of TG-DTA, for the analysis of evaluating generic drugs using gabexate mesilate with or without *d*-mannitol⁷⁻⁹.

Also, we determined the amount of ethyl 4-hydroxybenzoate derived from gabexate mesilate by HPLC for each vial, which confirms the effectiveness of TG-DTA for the assessment of the quality of gabexate mesilate products.

2. Material and methods

2.1. Test products

In this study seven injectable gabexate mesilate pharmaceutical products including a brand-name drug, FOY (the brand-name drug and six generic versions) are listed in Table-1. One hundred milligrams of injectable FOY was obtained from Ono Pharmaceutical Co., Ltd. The six generic products, M1, M2 and M3 contain 100mg of *d*-mannitol, whereas N1, N2 and N3 do not. Each product was purchased from each distribution vender.

Table-1: List of pharmaceutical products for gabexate mesilate.

Product	<i>d</i> -mannitol	Lot. No	Price of a Vial
FO Y	-	949FA	\$11.09 (¥877)
N 1	-	10502	\$2.49 (¥199)
N 2	-	H 9A1	\$3.69 (¥295)
N 3	-	X03H	\$2.94 (¥235)
M 1	+	8101	\$2.35 (¥188)
M 2	+	8L22	\$2.35 (¥188)
M 3	+	CT1900	\$1.90 (¥152)
M 4	+	06K13C	\$11.09 (¥877)

\$1 = ¥80

2.2. Thermogravimetry and differential thermal analysis (TG-DTA)

Seven products were tested under Thermo plus TG8120 (Rigaku Co., Tokyo, Japan) for the evaluation of TG-DTA. The operating conditions conducted in an open aluminum pan system were as follows; sample weight 1.5 mg, heating rate 5°C /min, Airgas flow; 100ml/min and the temperature was set in the range of 20°C to 130°C then cooled to 40°C, then reheated to 130°C (*d*-mannitol (-)) or 20°C to 180°C then cooled to 40°C, and reheated again to 180°C (*d*-mannitol (+)).

2.3. HPLC analysis

The content of ethyl 4-hydroxybenzoate, a major hydrolysate of gabexate mesilate, was performed in each formulation by HPLC. Three vials were randomly extracted from one package of each drug product for this test. HPLC was done by assay of gabexate mesilate as described in *The Japanese Pharmacopoeia* (15th edition)¹⁰. Each gabexate mesilate (100mg/vial) was dissolved in 20 ml of mobile phase solvents (see below) and final concentration was adjusted to 5mg/ml. Next, 10ul of the sample solution were applied to HPLC under the following conditions; detector:

ultraviolet absorption spectrometer (measurement wavelength = 245 nm) SPD-20A (Shimadzu Corporation); column: Cosmosil Packed Column for HPLC 5C-18-AR-II 4.6 × 150 mm (Nacalai Tesque); column temperature: room temperature; mobile phase: methanol/sodium lauryl sulfate (1 → 1000)/1-heptane sulfonic acid sodium salt (1 → 200)/acetic acid (100) solution (540:200:20:1); flow rate: 1.5 ml/min; internal standard: butyl 4-hydroxybenzoate.

The peak area ratio of ethyl 4-hydroxybenzoate derived from gabexate mesilate and butyl 4-hydroxybenzoate as an internal standard was calculated by the automatic integration method. Also the content of hydrolysate was calculated.

2.4. Karl Fisher titration

Moisture content was used as an index of material losses in TG and was measured by Karl Fisher titration. Karl Fisher titration was performed by a moisture measuring device (CA-21, Mitsubishi Chemical Analytech Co., Tokyo, Japan), as described previously¹¹.

3. Results

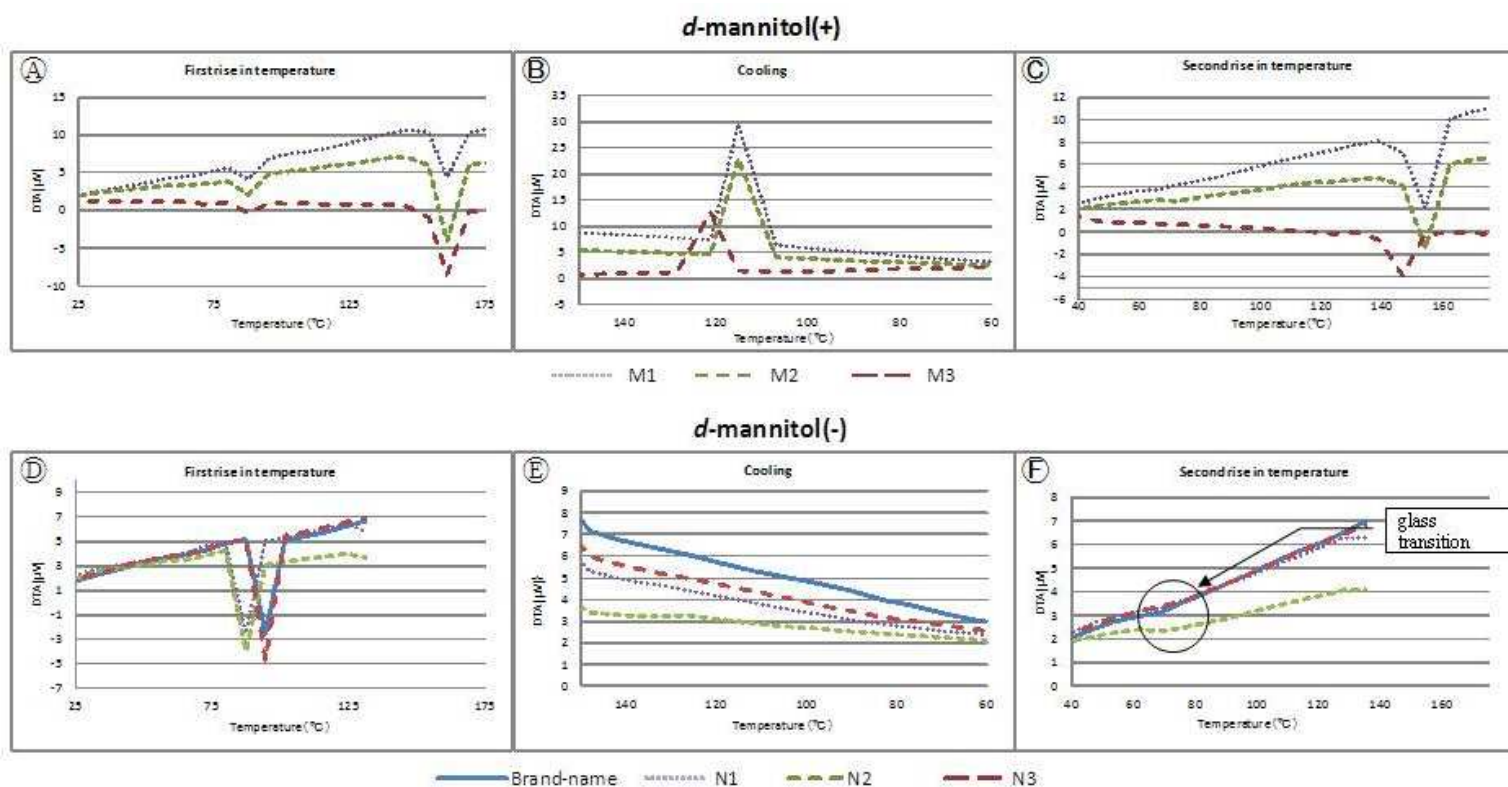
3.1. Thermal analysis

There were no significant changes in TG between each gabexate mesilate product. On the other hand, DTA showed a significant change in absorption derived from an endothermic reaction between products with *d*-mannitol and without *d*-mannitol. As shown in Fig- 3, M1, M2 and M3, with 100 mg *d*-mannitol, have two endothermic peaks during the first rise in temperature from 30°C to 170°C (panel A), followed by one exothermic peak from 170°C to 30°C (panel B). The second rise in temperature from 30°C to 170°C produced one endothermic peak (panel C). On the other hand, N1, N2, N3 and FOY without *d*-mannitol produced only one endothermic peak (panel D) derived from gabexate mesilate, followed by no exothermic peaks during the cooling period from 170°C to 30°C (panel E). Interestingly, glass transition (indicated arrow) was found at the second rise in temperature from 30°C to 170°C (panel F). These DTA findings are summarized in Table-2.

According to *The Japanese Pharmacopeia* (15th edition), the melting point of gabexate mesilate is 90–93°C; our result in Fig-3G confirmed the same melting point. Also, the results of thermal analysis of FOY was similar to generic products N1–N3 as illustrated in panel D, the endothermic peaks appeared at approximately 90°C, and all of the

observed peaks were found to be in the experimental error range. Considering the purity of generic drugs, interesting results were observed: The endothermic peak of N3 was almost at the same temperature as that of the brand-name drug. In contrast, N1 and N2 melted in a slightly lower temperature, suggesting that N1 and N2 contain a low level of impure substances and consequently their freezing point was depressed.

On the other hand, M1, M2 and M3, which contain *d*-mannitol, had two endothermic peaks. These peaks were different from FOY and N1, N2 and N3, the initial peak appearing near 85°C corresponds to be gabexate mesilate judging from the exothermic peak of an authentic reagent of gabexate mesilate (Fig-3G). The main peak of M1, M2 and M3, observed at 162°C, is related to be the endothermic peak of *d*-mannitol itself (Fig-3J). The exothermic peak derived from *d*-mannitol (panel K) was also found in the range of 120°C to 100°C in M1, M2 and M3 during cooling conditions (panel B). The endothermic peaks of M1, M2 and M3 observed at the second rise in temperature from 30°C to 170°C are thought to be that of *d*-mannitol judging from panel L. This difference indicates that the addition of *d*-mannitol causes a drastic change in the crystal system and subsequently, gives a shift in melting point as shown in Figure-3.



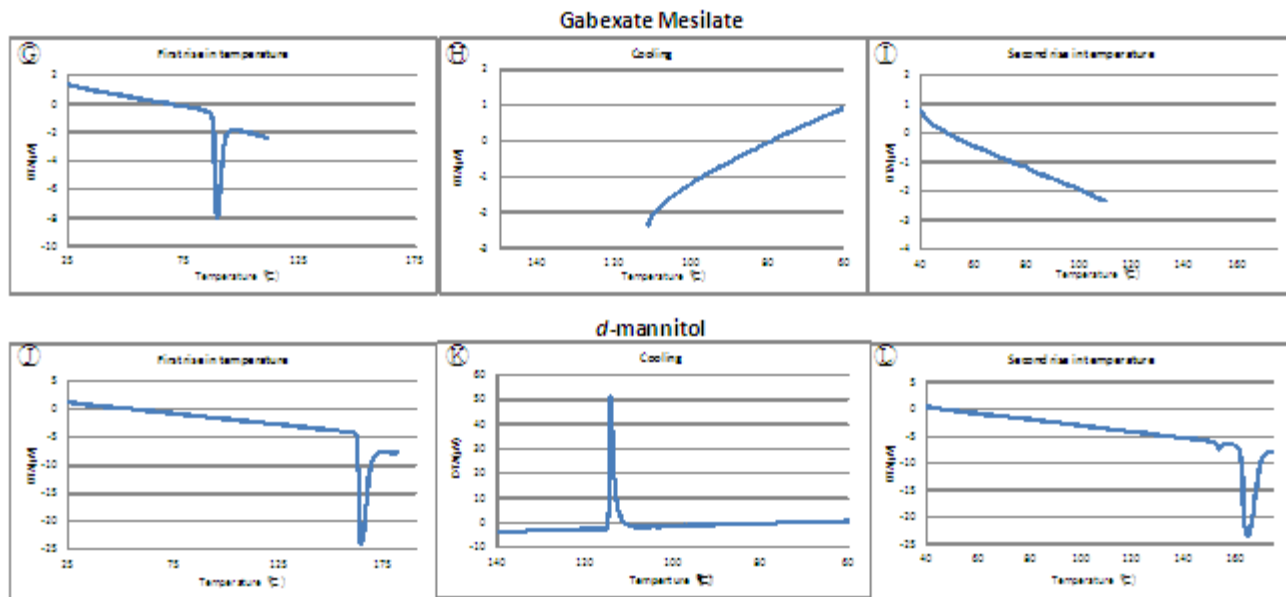


Fig-3: Differential thermal analysis of gabexate mesilate products.

Temperature was increased from 30°C to 170°C (A, D, G and J), decreased to 30°C (B, E, H and K) and then raised again to 170°C (C, F, I and L).

A, B, C: Products with *d*-mannitol, D, E, F: Products without *d*-mannitol.

G, H, I: gabexate mesilate (plain reagent), J, K, L: *d*-mannitol (plain reagent)

Table-2: Summary of DTA in gabexate mesilate products.

Peak energy shifts were represented in each column (KJ mol⁻¹)

		First rise in temperature from 30°C to 170°C			Cooling from 170°C to 30°C	Second rise in temperature from 30°C to 170°C
Product	<i>d</i> -mannitol	87°C	90°C	162°C	118°C	151°C
gabexate mesilate		-67.04				
<i>d</i> -mannitol				-277.45	140.05	-252.78
F0 Y	-	None	-75.57	None	None	None
N1	-	None	-78.51	None	None	None
N2	-	None	-108.32	None	None	None
N3	-	None	-81.93	None	None	None
M1	+	-23.10	None	-152.46	142.80	-144.81
M2	+	-24.35	None	-166.45	148.28	-152.07
M3	+	-24.84	None	-165.04	142.46	-148.70

3.2. The content of the hydrolysate of gabexate mesilate in the samples as measured by HPLC analysis

The result of HPLC analysis shown in Fig-4. N1–N3 showed no significant increase in the content of ethyl 4-hydroxybenzoate (the hydrolysate) compared with the brand-name drug. However, the generic products M1–M3 showed greater ethyl 4-hydroxybenzoate content than the brand-name drug. Each drug product with a higher proportion of hydrolysate contains 200 mg of mannitol as an additive per 100 mg of gabexate mesilate. *The Japanese Pharmacopeia* (15th edition) prescribes less than 0.5% of the content of ethyl 4-hydroxybenzoate (500 μ g), but M3 was beyond that prescription.

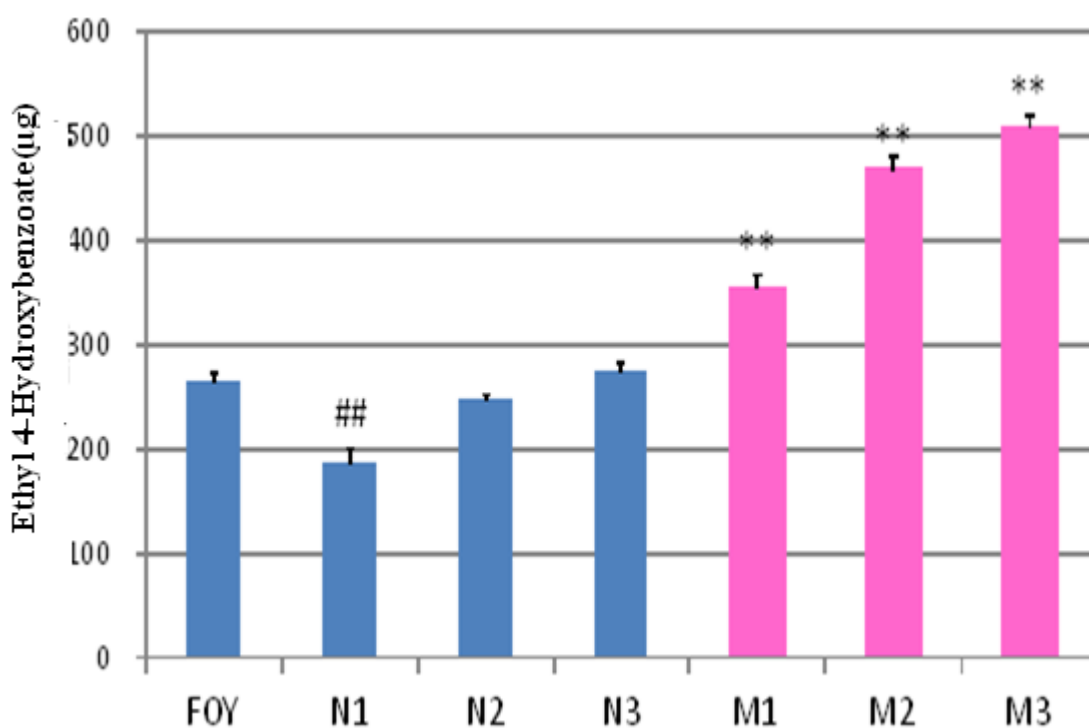


Fig-4: Content of Ethyl 4-Hydroxybenzoate in gabexate mesilate formulation.

Each bar represents the mean \pm S.D. (N=3),

* * ; $p < 0.01$, significantly increased from the brand-name drug by Dunnett's multiple comparison test.

##; $p < 0.01$, significantly decreased from the brand-name drug by Dunnett's multiple comparison test.

3.3. Moisture content measured by Karl Fisher titration

The results of Karl Fisher titration are shown in Fig-5. Generic products N1–N3 showed no significant increase in moisture content compared with the brand-name drug. However, generic products M1–M3 had significantly greater moisture content than the brand-name drug.

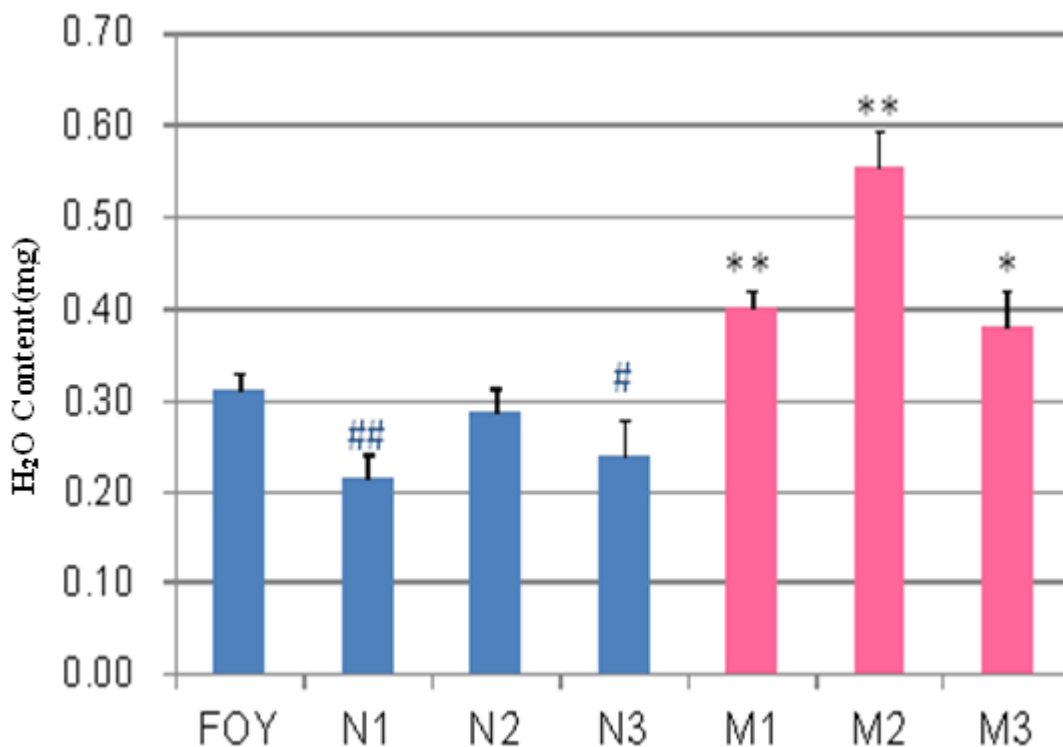


Fig-5: Moisture content in gabexate mesilate formulation.

Each bar represents the mean \pm S.D. (N=3),

*; $p < 0.05$, * *; $p < 0.01$, significantly increased from the brand-name drug by Dunnett's multiple comparison test.

#; $p < 0.05$, ##; $p < 0.01$, significantly decreased from the brand-name drug by Dunnett's multiple comparison test.

4. Discussion

The crystal structure of drugs is known to influence a variety of product characteristics, such as solubility, bioavailability, and stability^{3, 6, 12}. In this study, six generic versions of injectable gabexate mesilate were compared with a brand-name drug.

M1, M2 and M3, with 100 mg *d*-mannitol, had two endothermic peaks during the first rise in temperature from 30°C to 170°C (Fig-3A). This observation suggests that they were composed of two types of crystals in their vials. On the other hand, N1, N2, N3 and FOY showed only one peak, indicating that they had one crystal form. Figure-6 shows the correlation of moisture content and content of hydrolyzed ethyl 4-Hydrobenzoate in each vial of gabexate mesilate. We observed that the increment of moisture content in M1, M2 and M3 resulted in a greater content of hydrolyzed ethyl 4-Hydrobenzoate. The addition of *d*-mannitol is believed to cause the moisture absorption in the lyophilized process of gabexate mesilate products, resulting in a higher content of hydrolyzed ethyl 4-Hydrobenzoate.

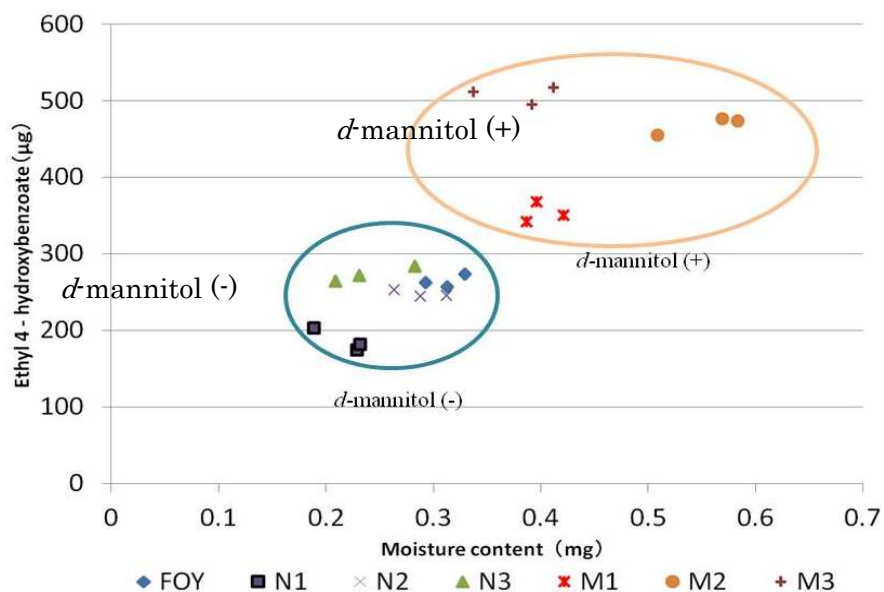


Fig-6: Correlativity of Ethyl 4-Hydroxybenzoate and Moisture using 2D discriminant analysis.

Products with (M1, M2, and M3) or without (FOY, N1, N2 and N3) *d*-mannitol are discriminated in 2D graph by solid and dashed circles, respectively.

Additional DTA results clearly demonstrate the purity differences in products without *d*-mannitol. Only N3 showed nearly the same pattern of that of FOY in reaction to calorimetry and hydrolyzed impurities as illustrated in Fig-7. Table-1 shows the list for the price of each product. Judging from both quality and economic points of view, we suggest that N3 is the best generic product among the seven products, including FOY.

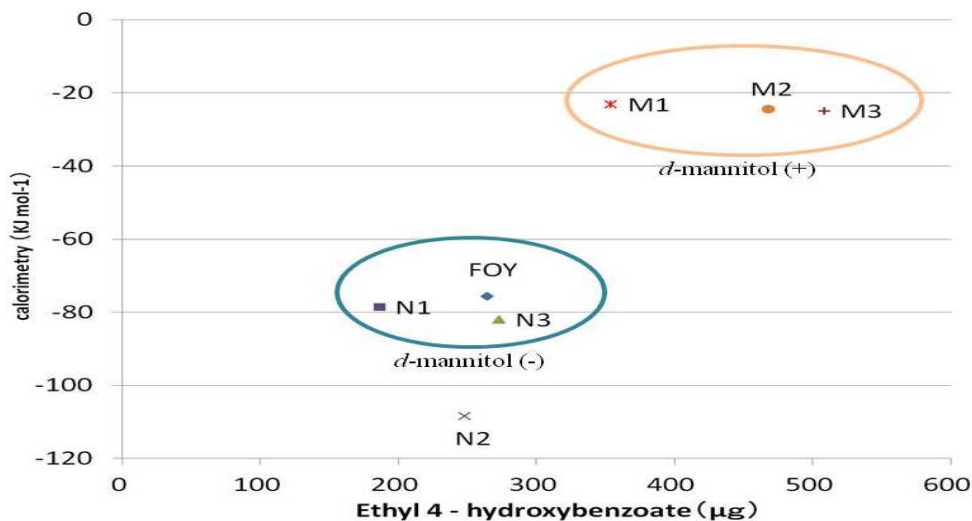


Fig-7: Correlativity of Ethyl 4-Hydroxybenzoate and Calorimetry using 2D discriminant analysis.

Products with (M1, M2, and M3) or without (FOY, N1, N2 and N3) *d*-mannitol are discriminated in 2D graph by solid and dashed circles, respectively.

5. Conclusion

In conclusion, DTA successfully identified the difference of each gabexate mesilate product including a brand-name drug. Our results indicate that the addition of *d*-mannitol during a lyophilized process causes moisture absorption and subsequent drug degradation. We propose that the addition of *d*-mannitol should be avoided in the production of gabexate mesilate formulation.

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Corresponding Author:

Toru Otori^{1*},

Email : tohtori@phar.kindai.ac.jp