Quantitative Determination of Amorphous Nicardipine Hydrochloride in Long Acting Formula (NIC-LA®) Using Light Anhydrous Silicic Acid

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We investigated a method to quantitatively determine amorphous nicardipine hydrochloride (NIC) in the NIC-long acting formula (LA) model formulas prepared using NIC, light anhydrous silicic acid (LASA) and carboxymethylethylcellulose (CMEC). Consequently, since the quantity of total NIC in the formula can be determined by means of HPLC and crystal NIC can be determined by the differential scanning calorimetry (DSC) method because the heat of fusion (85.08 J/g) of NIC is constant and unaffected by excipients, we developed the HPLC-DSC method by which the quantity of amorphous NIC is calculated as the difference between the quantity of total NIC determined by HPLC and the quantity of crystal NIC determined by DSC. This practical HPLC-DSC method was confirmed to have good accuracy and reproducibility.

Key words nicardipine hydrochloride; long-acting preparation; amorphous; light anhydrous silicic acid (LASA); differential scanning calorimetry; assay method

Nicardipine hydrochloride (NIC) is a calcium channel-blocking agent that is effective in the treatment of mild to moderate hypertension, angina pectoris and cerebral disease. However, its conventional formulation undergoes rapid absorption and extensive biotransformation in the liver, and has a short elimination half-life (about 90 min)[1] Plasma NIC concentration often fluctuates significantly and adverse reactions such as syncope are consequently induced. Thus, for the purpose of alleviating adverse reactions due to over-absorption and improving dosing compliance, we investigated on the development of the long-acting formula of NIC-LA®. Sustained release formulations have already been developed for several drugs, and the following formulation techniques have been used: combining large amounts of a substance that hardly disintegrates in stomach or intestines, coating granules and tablets with a hydrophobic additive, coating a drug with a semipermeable membrane, and formation of a solid dispersion system with insoluble or hydrophilic polymer substances by means of mixing, adsorption and/or binding.[2] However, in the case of drugs like NIC, which is very insoluble in intestinal fluid, the above techniques merely reduce the bioavailability and cannot be expected to realize sustained release of the drug. Thus, Yuksel *et al.* have investigated formula to regulate the release of NIC in the stomach and to increase the solubility in intestinal fluid.[3—5] Ohmura *et al.*, on the other hand, have focused on the fact that the solubility in intestinal fluid is 43 to 47 mg/ml for amorphous NIC and 5 to 8 mg/ml for crystal NIC, and they found that sustained release could be obtained by making NIC amorphous without adding any excipients, thereby improving solubility in intestinal fluid.[6] Based on this notion, the NIC-LA® formula was developed, and by controlling the ratio of crystal NIC and amorphous NIC in this formula, suitable NIC plasma levels can be maintained (C_{max}=0.8 h and 6.0 h, and the elimination half-life=7.6 h), even when the frequency of administration is reduced.[6,7]

Drugs can be made amorphous by procedures such as grinding, lyophilization, spray drying, fusion and solvent evaporation.[8—10] Ohmura *et al.* developed the NIC-LA® by mixing enteric granules consisting of 100% amorphous NIC prepared by a vibration ball mill and conventional gastric granules consisting of 100% crystal NIC at a ratio of 7 : 3.[6] The amorphous NIC content of this formula, which affects the efficacy and safety of NIC-LA®, can be confirmed by controlling the quantities of gastric granules and enteric granules during the formulation process. On the other hand, in the NIC-LA® formula developed by Shibahara *et al.*, the sustained release granules, which are obtained by drying a suspension of light anhydrous silicic acid (LASA) and carboxy methylethylcellulose (CMEC) in a 20% aqueous ethanol solution dissolving about 40% of crystal NIC, were used as enteric granules, and these granules were coated with pulverized sustained release granules.[7] The amorphous NIC content of this formula is thought to correspond to the amount of NIC dissolved in the suspension if no re-crystallization occurs during the formulation process, but this has not yet been confirmed. A sustained release formula contains a large amount of a drug in one dosing unit when compared with conventional tablets and is intended for long-lasting release of the drug. Therefore, if process and quality controls are insufficient, not only could the target drug effect not be obtained but there is also a risk of adverse reactions due to plasma drug concentrations exceeding the safety margin as a result of dose dumping.[11] This is also the case for NIC-LA®, and the amorphous NIC content in NIC-LA® formula is very important from the viewpoint of quality control, but an appropriate determination method for amorphous NIC in formula has not been reported to date. The following are known to be techniques for determining the content of amorphous substances: Powder X-ray diffraction (XRD),[12] IR[13] or near infra-red spectroscopy (NIRS),[14] thermal analyses, such as specific heat capacity (C_p) at glass transition point (T_g)

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Table 1. Composition and Formulation Procedure of NIC-LA Model Formulas and Related NIC-LASA Physical Mixture

Model formula	Composition and ratio			Formulation procedure
	NIC crystal	LASA	CMEC	
A: NIC-LA Sustained Release Granule	1.0	1.0	1.5	Suspension in 20% aqueous ethanol solution
Placebo	0	1.0	1.5	
B: Related Model Formula	0.4	1.0	1.5	
C: Related Model Formula	1.0	1.0	0	
D: NIC-LASA physical mixture	1.0	1.0	0	Physical mixture

method[15] and DSC,[16] and solid-state NMR.[17] However, there have been few reports concerning the content of amorphous substance in formula.

In the present study, for the purpose of primarily developing a quantitative determination method for amorphous NIC without being affected by excipients, we attempted various analytical methods to determine the amorphous NIC content in sustained release granules prepared according to the NIC-LA® formulation method developed by Shibahara *et al.* In addition, we discuss the characterization of amorphous NIC based on the results obtained by multiple analytical methods.

Experimental

Materials NIC was manufactured by Yamanouchi Pharmaceutical Ireland Corporation, Ireland. The standard sample of NIC was carefully recrystallized from cold acetone and used as 100% crystalline standard sample. LASA (Adsolider 101) and CMEC were obtained from Freund Corporation, Japan.

Preparation of NIC-LA Model Formula The sustained release granules (Model Formula A) were prepared according to the NIC-LA formulation method developed by Shibahara *et al.*[7] In addition, the related model formulas (placebo, Model Formulas B to D) including a physical mixture of NIC and LASA were also prepared for reference. The composition and formulation procedure of those Model Formulas are shown in Table 1. Sustained Release Granules (Model Formula A); 427.5 g CMEC was dissolved in 855 ml of water containing ethanol (water 20% v/v), and viscous mucilaginous solution was obtained. After 285 g NIC was mixed with this solution until homogeneous, 285 g LASA was added using a kneader and an aqueous slurry was obtained. The slurry was dried into granules at 40 °C in air. The 0.5-mm to 1.0-mm fraction was used.

HPLC HPLC measurements were performed in order to determine Total NIC content of the formulas and solubility of NIC in suspension using an HP-1090M HPLC system (Hewlett-Packard Corporation, U.S.A.) on a 4.6-mm i.d.×15-cm column containing 5-mm octadecylsilanized silicagel (L-column, Chemicals Evaluation and Research Institute, Japan) and a photodiode array detector according to the internal standard method with di-*n*-butyl phthalate as the internal standard. The mobile phase was a mixture of 0.01 mol/l KH_2PO_4, methanol and acetonitrile (25 : 55 : 20), and the flow rate was 1.0 ml/min. The detection wavelength was 254 nm. All other chemicals and solvents were of analytical reagent grade and deionised water (Millipore Elix 5 system) was used throughout the study. Since NIC is a light sensitive, almost all experiments were carried out in a darkroom under yellow light (Philips Powertone SON E27), in order to avoid photodecomposition. When this photo protection was impossible to achieve, all samples containing NIC were protected from light by wrapping the vials with alminium foil.

Differential Scanning Calorimetry (DSC) The enthalpy of fusion, based on the melting point of NIC crystal and the T_g of NIC amorphous, were measured using a DSC 2910 differential scanning calorimeter (TA Instrument Corporation, U.S.A.) under a constant flow of dry nitrogen gas (50 ml/min). About 5 mg of sample was loaded into a closed aluminium pan and measured at a heating rate of 10 °C/min under nitrogen gas flow (50 ml/min). Temperature and enthalpy were calibrated using indium as standard. All T_g measurements were made at a primary scan rate of 10 °C min^{-1} up to 130 °C, after which, the sample was cooled to room temperature, and at a secondary scan of 10 °C/min in the range of 30—200 °C.

The ΔC_p of sample was taken as the base line drift of transition in the DSC thermo grams.

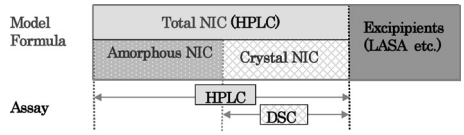

Fig. 1. Concept of HPLC-DSC Method

Fourier Transform-Infrared Spectroscopy (FT-IR) Fourier Transfer-infrared spectroscopy measurements were carried out by the diffuse reflectance method. FT-IR spectra were recorded with Perkin Elmer IR-2000 FT-IR spectrometer (Perkin Elmer Corporation, U.S.A.).

Powder X-Ray Diffraction (XRD) Powder X-ray diffraction patterns were obtained using a Rigaku RINT-400 diffractometer with Cu$K\alpha$ radiation (Rigaku Corporation, Japan) at ambient temperature. The measurement conditions were as follows: voltage, 30 kV; current, 15 mA; scanning speed, 0.067° s^{-1} (4°/min); 2θ collection range, 5—35°. Rising temperature XRD patterns were obtained using a Philips X'Pert MPD PW3050 temperature-controlled diffractometer (Philips Corporation, Netherlands) at ambient temperature to 164 °C. The measurement conditions were as follows: voltage, 40 kV; current, 55 mA; scanning speed, 0.067° s^{-1} (4°/min); 2θ collection range, 5—30°.

Solid-State ^{13}C- and ^{15}N-NMR Solid-state ^{13}C- and ^{15}N-NMR spectra of Crystal and Amorphous NIC were recorded on a CMX-300 NMR spectrometer (Chemgnetics Corporation, U.S.A.) by mean of CPMAS at ambient temperature. The sample (*ca.* 50 mg) was contained in a cylindrical ceramic probe and spun at 5.0 to 10.5 kHz. The durations of 90° pulse, contact time, and repetition time were 4.0 ms, 2.0 ms, and 30 s (^{13}C)/10 s (^{15}N), respectively. The ^{13}C and ^{15}N chemical shifts were calibrated using hexamethyl benzene and NH_4NO_3 respectively.

Results and Discussion

Concept of the HPLC-DSC Method For determining amorphous NIC in NIC-LA Model Formula without being affected by such excipients as LASA, we focused on crystal NIC in the formula. The heat of fusion due to melting of crystal NIC was found to be constant (85.08 J/g), and unaffected by excipients. Since total NIC can be determined by HPLC, the amorphous NIC in NIC-LA Model Formula can be calculated by subtracting the crystal NIC quantity determined by the DSC method from the total NIC quantity determined by HPLC. The concept of this HPLC-DSC method is shown in Fig. 1.

HPLC-DSC Method Chromatograms on NIC standard and NIC-LA sample solution are shown in Fig. 2. A DSC thermogram of Model Formula A is shown in Fig. 3. The methods were validated by applying the strategy proposed by the Commission of the SFSTP (Société Française des Sciences et Techniques Pharmaceutiques) for the validation of analytical methods and which complies with the ICH recommendations (International Conference on Harmonization, FDA).[18] In the HPLC method, the response fits a linear regression model with a good coefficient of determination

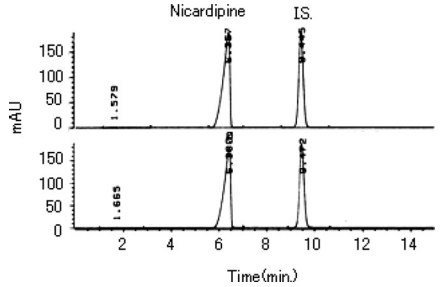

Fig. 2. Chromatograms of Nicardipine HCl Standard Solution and Sample Solution for Model Formula A

Upper: Standard solution: nicardipine HCl 0.5 mg/ml, internal standard 1.66 mg/ml. Lower: Sample solution to preparation.

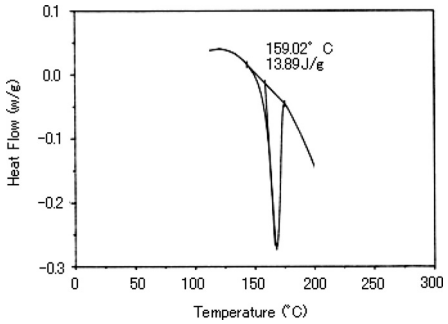

Fig. 3. DSC Thermogram of NIC-LA Model Formula A

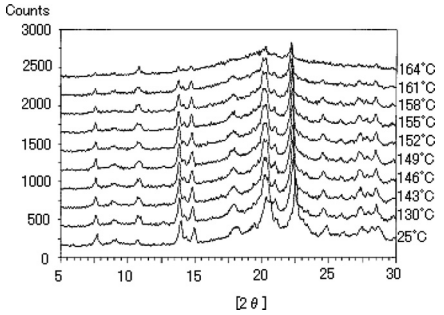

Fig. 4. Change in the XRD Pattern of Model Formula A by Heating

Table 2. Results of Amorphous Content Concerning Various NIC Model Formulas by HPLC-DSC Method

	Content (S.D. %, n=6)			
	A	B	C	D
Total NIC	28.5±0.2	13.7±0.1	50.1±0.1	50.0±0.1
Amorphous NIC	43.1±1.8, 42.6±2.0[a)]	99.5±1.7	69.1±2.0	40.2±3.8
Dissolved NIC in suspension	42.2±0.1	99.2±0.1	66.1±0.1	—

a) Stored at 40 °C, 75% RH for 3 months.

(γ^2=0.999). The reproducibility expressed by the R.S.D. measured in six independent samples was below 1%, and the intermediate precision was below 2.5%. The percentage recovery from placebo is 99.8±0.8% (n=6). In the DSC method, the heat of fusion based on melting of NIC was 85.08±1.8 J/g (n=6), the response fits a linear regression model having a good coefficient of determination (γ^2=0.995) within the calibration range (0.2—10 mg). Precision and accuracy of the method were studied at three concentrations: 2, 6 and 10 mg/ml. The reproducibility expressed by the R.S.D. measured in six independent samples was 3.1, 1.8 and 1.5%, respectively, and the intermediate precision was 5.9, 4.5 and 3.5%, respectively. The percentage recovery is 100% at the three levels of concentration, taking experimental error into account. The recovery rate of the heat of fusion when crystal NIC was added to placebo was 96.2±1.8% and nearly quantitative, and the recovery rate after thermal hysteresis of preheating to 150 °C was 98.1±3.1%, indicating that the heat of fusion is constant until melting without being affected by excipients. The XRD patterns obtained by varying temperature from room temperature to the melting point (164 °C) are shown in Fig. 4. Although NIC has crystal polymorphism of α type form,[19] crystal transition to α form was not observed during heating. It was confirmed from these results that amorphous NIC in NIC-LA formula could be accurately determined by the HPLC-DSC method.

Results of HPLC-DSC Method The results obtained by the HPLC-DSC method are shown in Table 2.

Though the amorphous NIC content in NIC-LA Model Formula A (43.1±1.8%) was similar to the amorphous NIC content in the Model Formula D (40.2±3.8%) in which the same amount of LASA was mixed as reference. The amorphous NIC in Model Formula A was derived from the dissolved NIC in suspension (42.2±0.1%), whereas the amorphous NIC in Model Formula D was produced by adsorption of NIC to LASA.[20] The results indicating that the NIC dissolved in the suspension corresponding to the amorphous NIC of Model Formula A was found to apply equally to Model Formula B (in which NIC was all amorphous), in which a reduced amount (40%) of crystal NIC was dissolved completely, and to Model Formula C, which contained no CMEC. The amorphous NIC content of Model Formula A did not change after storing at 40 °C, 75% RH for 3 months. Amorphous NIC in the formula did not re-crystallize during the drying process or in a stability study, despite the presence of crystal NIC acting as seeds to induce re-crystallization. It is conceivable that re-crystallization was prevented by LASA.

FT-IR (Diffuse Reflectance FT-IR: DR-IR) DR-IR spectra were measured, the five characteristic absorption bands were picked up for amorphous content determination. The validation results regarding the five characteristic absorption bands are shown in Table 3.

The results of validation were satisfactory for any absorption band, but the carbonyl absorption band (1707 cm^{-1}/1679 cm^{-1}) showed the best accuracy and reproducibility. The amorphous NIC content in Model Formula A analyzed using the carbonyl absorption band was 47.0±1.8% which was slightly but significantly greater than the amorphous NIC content (43.1±1.8%) obtained by the HPLC-DSC method. This trend was also observed in Model Formula C.

When crystal NIC was physically mixed with LASA at a ratio of 1 : 1, a portion (37.4±2.5%) of crystal NIC became amorphous, and this content was slightly but significantly lower than the value obtained by the HPLC-DSC method (40.2±3.8%). Changes in amorphous NIC content measured by the FT-IR (DR-IR) method when heating hysteresis as in

Table 3. Validation Results and Amorphous NIC Content of Model Formulas by FT-IR (DR-IR) Method

	Wave number for characteristic absorption bands (cm^{-1})				
	1709/1679	880/871	856/849	711/704	609/601
Calibration range (%)	Amorphous; 0—100				
Analysis response function[a]					
Slope of the fitted straight line±S.D.	0.820±0.015	0.451±0.020	0.441±0.015	0.521±0.044	0.120±0.013
Intercept of the fitted straight line±S.D.	1.086±0.033	0.984±0.049	0.944±0.038	0.954±0.051	0.854±0.094
Coefficient of determination (γ^2)	0.984	0.985	0.991	0.999	0.981
Precision[b]					
40% Amorphous	1.4/4.1	3.6/9.7	3.1/6.8	4.8/11.2	6.8/17.1
Accuracy[c]					
40% Amorphous	100.7±1.8	104.2±4.0	97.3±3.1	93.6±5.3	84.5±7.5
Limit of detection (%)	2.1	4.2	3.4	8.4	10.5
Amorphous content of Model Formula (%)±R.S.D. (n=6)					
Model Formula A	47.0±3.0	43.8±4.7	45.9±4.6	ND[d]	ND[d]
Model Formula B	102.1±3.5	97.9±4.3	98.3±4.2	ND[d]	ND[d]
Model Formula C	71.2±2.6	77.2±2.6	77.1±3.0	59.0±4.8	80.0±2.2
Model Formula D	37.4±2.5	35.4±2.8	34.2±3.1	35.7±4.3	34.3±2.9

a) Regression line (n=15, 5 point×3 replicates). *b*) Repeatability/intermediate precision (R.S.D. %, n=6, 3 d replicates). *c*) Recovery±confidence interval (%, n=6, replicates). *d*) Interfered by CMEC.

Table 4. Influence of Heating Hysteresis Regarding Model Formula D (NIC/LASA Physical Mixture) with the (DR-IR) Method Using the Carbonyl Band (1707 cm^{-1}/1679 cm^{-1})

Temperature for thermal hysteresis	Amorphous content (%) (n=6)
Null	37.4±2.5
150 °C	39.1±2.2
164 °C	95.8±2.9

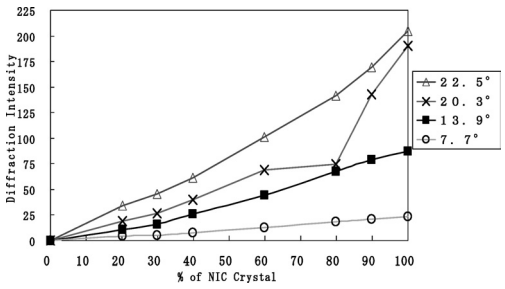

Fig. 5. Calibration Curves for NIC Crystal in NIC Amorphous/CMEC/LASA Physical Mixture with Klug–Alexane Correction

the DSC method was given to Model Formula D are shown in Table 4.

The amorphous NIC content produced by physical mixing (37.4%) did not increase, even when the formula was heated to 150 °C. This suggests that crystal NIC in the NIC-LA formula can be determined by the DSC method and is unaffected by excipients.

XRD Method In a physical mixture of crystal NIC, amorphous NIC, CMEC and LASA, the calibration curves plotting the amorphous NIC content at the characteristic diffraction peaks of crystal NIC, 2θ=7.7°, 13.9°, 20.3° and 22.5°, against the diffraction intensity are shown in Fig. 5. Since the calibration curves were influenced by the heavy atom in LASA, silicon, the curves were corrected by using the equation of Klug and Alexane.[21]

Validation results and amorphous NIC content in Model Formula A are shown in Table 5. The amorphous NIC content differed depending upon the diffraction peaks used for analysis. Since X-ray diffraction intensity was influenced by LASA, the XRD method showed no satisfactory accuracy and reproducibility, and was not suitable for quantitative analysis.

Specific Heat Capacity at Glass Transition Point (T_g) Results concerning the T_g and specific heat capacity of the NIC-LA Model Formulas and the amorphous substances prepared by various methods are shown in Table 6. Amorphous NIC showed several glass transition points (T_g) depending upon the preparation method used. It is obvious from the results of HPLC-DSC and DR-IR analyses that all Model Formulas A to D contained amorphous NIC at the quantities shown in Tables 2 and 3. However, no T_g was observed for Model Formulas A, C and D. Though only Model Formula B showed a T_g of 90.3 °C, and this T_g did not correspond with that of any amorphous substance. In amorphous substance 3 (Table 6), which was prepared by the same method for reference, the amorphous content calculated based on the specific heat capacity (0.327 mJ/deg mg) was only 80.4%. In Model Formulas A and B, CMEC coexisted at a constant ratio.

Since CMEC has a T_g of 139.9 °C, T_g on these model formula should be observed at the temperature corresponding to the presence ratio of compounds according to Gordon-Taylor equation[22,23] when both are simple mixtures. However, the all Model Formulas showed no T_g at the corresponding temperature without following the Gordon-Taylor equation. This suggests that amorphous NIC in NIC-LA Model Formula and that in the physical mixture with LASA are not simple mixtures but are actually interacting with excipients.

Solid-State NMR The chemical structure of NIC is shown in Fig. 6, and the observed values of the relaxation time (T_1) of individual signals due to ^{13}C and ^{15}N for crystal NIC and amorphous NIC are shown in Table 7. The T_1 values of crystal NIC (about 6 s for ^{13}C and 2 s for ^{15}N) with inferior S/N-ratios were employed for the purpose of quantitative analysis, and the pulse repetition time (PD) was employed at 30 s for ^{13}C and 10 s for ^{15}N (5-fold of T_1), which takes into

Table 5. Validation Results and NIC Amorphous Content by XRD Method

2θ	Calibration curve (γ^2)	Amorphous content (%)	R.S.D. (%) (n=6)
7.7°	$Y=0.0008X^2+0.1710X+0.052$ (0.980)	8.5	4.6
13.9°	$Y=0.0038X^2+0.5355X-1.250$ (0.928)	3.5	6.2
20.3°	Not obtained		
22.5°	$Y=0.0084X^2+1.1220X+3.392$ (0.897)	36.4	10.1

Table 6. The Glass Transition Point (T_g) and Specific Heat Capacity for the Various Amorphous Substances and NIC-LA Model Formulas (n=6)

Sample and Preparation Method			T_g (°C)	ΔC_p (mJ/deg mg)
Amorphous substance				
1	Dissolved in $CHCl_3$	Solvent dry up	94.8	0.352±0.011
2	Dissolved in EtOH/H_2O (8 : 2)	Solvent dry up	76.4	0.367±0.011
3	Dissolved in EtOH/H_2O (8 : 2)	Spray dry	86.5	0.327±0.010
4	Milling with a Vibration mill	—	88 (broad)	0.334±0.013
Model Formula				
A, C	Suspension in EtOH/H_2O (8 : 2)	Spray dry	N.D	—
B	Suspension in EtOH/H_2O (8 : 2)	Spray dry	90.3	0.263±0.008
D	Physical mixture	—	N.D	—

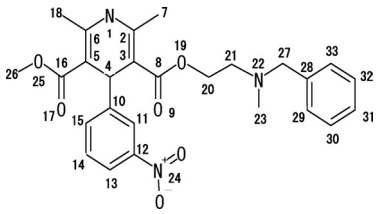

Fig. 6. Structure and Atomic Numbering for Assignment by $^{13}C/^{15}N$ Solid-State NMR

Table 7. $^{13}C/^{15}N$ Solid-State NMR Peak Assignments and T_1 of Crystal and Amorphous NIC

	Peak (ppm)	Assignment (Atom No.)	T_1 (s)	
			Crystal	Amorphous
^{13}C	19	7 or 18	5.9	1.9
	21	7 or 18	5.4	2.3
	42	4	5.7	2.3
	46	23	5.6	1.9
	51	26 and 21	5.3	2.0
	60	20 or 27	5.2	1.8
	64	20 or 27	6.1	2.2
	101	3 and 5	4.8	2.2
	122	11 and 13	4.1	1.8
	125	14	5.9	1.7
	130	28—33	5.5	1.8
	135	15	NT	NT
	149	2, 5 and 12	4.7	1.6
	155	10	5.4	1.5
	167	8 and 16	5.8	1.4
^{15}N	−3.5	24	2.1	0.4
	−236	22	—	0.4
	−237.9	22	2	—
	−323.2	1	1.8	0.3

NT: Not tested.

account the relative quantitative ability. As the contact time, 2 ms was employed for both ^{13}C and ^{15}N, since this contact time was suitable for measurement of amorphous NIC with relatively low sensitivity. Measurement was carried out using

Table 8. Amorphous Content of Model Formula A by ^{13}C and ^{15}N Solid-State NMR

	Atom No.	CT (ms)	PD (s)	Amorphous content (%)
^{13}C	All	2.0	30	29
	2, 5, 12	2.0	30	27
	10	2.0	30	33
^{15}N	22	2.0	10	42

the typical signals reflecting the characteristics of crystal NIC and amorphous NIC by the peak partition method, using a crystal/amorphous mixture (1 : 1) as a reference sample. The results obtained for NIC-LA Model Formula A are shown in Table 8. In ^{13}C-NMR, the amorphous content differed within a range from 27 to 33% depending upon the signal used, whereas the amorphous content (42%) obtained by ^{15}N-NMR supported the results obtained by the HPLC-DSC method.

Comparison of Each Methodology Concerning NIC-LA Model Formula A for Quantitative Determination of NIC Amorphous Content The results of validation of all the methods used for the quantitative determination of amorphous NIC content in the present study, the observed content of amorphous NIC in NIC-LA Model Formula, and the observed content of NIC dissolved in suspension are summarized in Table 9.

In addition, the percent dissolved NIC, and the percent amorphous NIC content as measured by the HPLC-DSC method and the FT-IR (DR-IR) method in NIC-LA Model Formulas A, B, C and D are shown in Table 10.

The HPLC-DSC method was superior to other methods in accuracy and reproducibility. The amorphous NIC content in NIC-LA Model Formula A, which was measured by the HPLC-DSC method, was 43.1% and was comparable to the content of dissolved NIC in suspension (42.2%). The XRD, ΔC_p, DR-IR and solid-state NMR methods showed relatively good quantitative ability in the binary system consisting of

Table 9. Summary of Results Concerning Validation and Amorphous Content about NIC-LA Model Formula A (Sustained Release Granules) by Various Testing Methods ($n=6$)

Method and analytical parameters		Amorphous content (%)	Regression coefficient determination (γ^2)	Reproducibility R.S.D (%)
HPLC-DSC	HPLC	43.1	$y=0.0022x+0.025$ $\gamma^2=0.999$	1.8
	DSC		$y=1.068x-1.006$ $\gamma^2=0.995$	
DR-IR	1709/1679	47.0	$y=0.008x+1.086$ $\gamma^2=0.9844$	3.0
	880/871	43.8	$y=0.0045x+0.984$ $\gamma^2=0.985$	4.7
	856/849	45.9	$y=0.0044x+0.944$ $\gamma^2=0.991$	4.6
XRD	$2\theta=7.7°$	8.5	$y=0.0007x2+0.170x+0.0526$ $\gamma^2=0.980$	4.6
	13.9°	3.5	$y=0.0038x2+0.536x+1.2508$ $\gamma^2=0.928$	6.2
	20.3°		Not obtained	
	22.5°	36.4	$y=0.0084x2+1.122x+3.3922$ $\gamma^2=0.897$	10.1
ΔC_p			Not detected	
Solid-state NMR	^{13}C	27—33 ($n=2$)	Calculated by Peak Partition Method that used Reference Standard Sample	
	^{15}N	42 ($n=1$)	(Crystal : Amorphous=1 : 1)	
Dissolved NIC in suspension		42.2	HPLC method	0.1

Table 10. Summary of Results Concerning Dissolved NIC (%) and Amorphous Content (%) of Model Formulas by Various Testing Methods

Model Formula	Dissolved NIC (%)	Amorphous content (%)					
		HPLC-DSC	DR-IR; analytical band (cm^{-1})			ΔC_p	^{15}N Solid-state NMR
			1709/1679	880/871	856/849		
A	42.2±0.1	43.1±1.8	47.0±3.0	43.8±4.7	45.9±4.6	Not detected	42
B	99.2±0.1	99.5±1.7	102.1±3.5	97.9±4.3	98.3±4.2	Not detected	—
C	66.1±0.1	69.1±2.0	71.2±2.6	77.2±2.6	77.1±3.0	Not detected	—
D	—	40.2±3.8	37.4±2.5	35.4±2.8	34.2±3.1	Not detected	—

crystal NIC and amorphous NIC. However, in the NIC Model Formulas using LASA and CMEC as excipients, the amorphous content measured by these methods varied depending on the analysis parameters such as 2θ and analytical band in XRD and FT-IR method respectively. The glass transition point method was not suited for quantitative determination, because amorphous NIC interacted with excipients. The XRD, DR-IR and solid-state NMR methods are specific to the chemical structure of NIC, whereas HPLC and DSC in the HPLC-DSC method reflect the quantity of total NIC and properties of the entire NIC crystal system ("The enthalpy of fusion is intrinsic property for the substance"), respectively. In the system in which amorphous NIC interacted with excipients such as LASA and CMEC through adsorption, *etc.*, as in the present formula, the HPLC-DSC method reflecting the entire system is considered to be more reliable than structure-specific methods. In Model Formulas A and C, the amorphous content measured by the DR-IR method tended to be higher than that measured by the HPLC-DSC method, whereas the opposite tendency was observed in Model Formula D. Considering that the HPLC-DSC method reflects the amorphous content of the entire system and the DR-IR method reflects mainly the surface amorphous content, it is conceivable that the amorphous content of each Model Formula may be slightly different outside and inside. Although Model Formulas C and D contain NIC and LASA at the same mixing composition of 1 : 1, these Models were prepared by different methods; dissolution mixing for Model Formula C and physical mixing for Model Formula D. Since the amorphous content in each of these Model Formulas was different between the HPLC-DSC method and the DR-IR method, it is conceivable that the molecular state of amorphous NIC molecules was varies depending on the method of the formulation.

Conclusions

In the NIC-LA Model Formula using LASA and CMEC as excipients, the heat of fusion of crystal NIC (measured by the DSC method) was constant and unaffected by the excipients, and crystal NIC showed no transition to the α form, thereby the HPLC-DSC method, in which the crystal NIC measured by the DSC method is subtracted from the total NIC obtained by the HPLC method, was found to be reliable for the quantitative determination of amorphous NIC in NIC-LA formula. This method was confirmed to have sufficient accuracy and reproducibility for evaluating the content of amorphous NIC, which affects the efficacy and safety of NIC-LA.

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