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New methods for the assay of 5-isosorbide mononitrate and its validation

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Abstract: Differential scanning calorimetry (DSC) is a method which has been applied to obtain thermal information about both raw material and the determination of the content of 5-ISMN in dry mixtures with lactose. The main advantage this offers, compared to other methods, is that it is not necessary to use a standard, it only being necessary to know the data of its fusion heat.

HPLC and TLC-densitometry are considered for the determination of 5-ISMN: lactose and pharmaceutical dosage forms. Linearity test, repeatability and accuracy were satisfactory in both methods. Recovery data in pharmaceutical formulations (expressed as the percentage of the label claim) from HPLC and TLC did not give any significant difference (P = 95%). The results show that the chromatographic methods are simple, fast and reliable procedures for the determination of 5-ISMN.

Keywords: *5-Isosorbide mononitrate (5-ISMN); differential scanning calorimetry (DSC); high-performance liquid chromatography (HPLC); quantitative thin-layer chromatography (TLC); pharmaceutical dosage forms.*

Introduction

Isosorbide 5-mononitrate (5-ISMN) is the active metabolite of the vasodilator agent isosorbide dinitrate (2,5-ISDN), being also responsible for long-term effects. It has been found recently that 5-ISMN is more active and less toxic than other coronary vasodilators that are useful in the treatment of angina pectoris and ischemia of skeletal muscles.

For the identification and quantitation of the organic nitrate esters several methods have been reported such as polarographic [1], GC [2–4], TLC [5, 6] and HPLC [7–11]. This communication describes and compares HPLC, TLC and calorimetric methods for the determination of 5-ISMN suitable for the control of raw materials and commercial dosage forms.

Experimental

Samples

Raw materials. 5-ISMN (reference sample), 2-ISMN, 2,5-ISDN and dry mixture of 5-ISMN with lactose (label claim 90:10) were gifts from Nipa Laboratories Ltd (Inc. Graesser Laboratories Limited).

Finished products. Different commercial samples were purchased from retail pharmacies. For the purpose of this work, the trade marks of the samples are not mentioned.

Reagents

All of these were analytical grade or HPLC grade from Merck Igoda (Madrid, Spain).

Equipment

Differential scanning calorimetry (DSC). A Perkin–Elmer DSC system is composed of a DSC-7, instrument controller TAC7/7 and a professional computer 7700.

High-performance liquid chromatography (HPLC). A Waters HPLC system is composed of a sample processor 715 Ultra-Wisp, solvent delivery system 600E, photodiode array detector 990 and a personal system/2.

The column was Hypersil ODS 5 μm (200 × 2.1 mm) (Hewlett–Packard), the mobile phase water–methanol (80:20, v/v) and the detector was adjusted to 210 nm.

Thin-layer chromatography–densitometry (TLC–densitometry). A Camag TLC system is composed of an automatic sample II 27220, TLC scanner II and a laboratory data system HP 310 with Camag TLC software 86.

The plates were precoated HPTLC Silicagel

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60 F254, 20 × 10 cm (Merck, Darmstadt, Germany). The mobile phase was chloroform–acetone (23:2, v/v).

Differential scanning calorimetry

Purity study. Different samples of 5-ISMN (reference sample) from 1 to 3 mg were measured in aluminium pans hermetically sealed using as reference an empty sealed aluminium pan. Each sample was heated from 80 to 95°C. Thermograms were obtained by heating at a constant rate of 1°C per min.

Quantitative assay. The content of 5-ISMN in raw materials (dry mixture of 5-ISMN with lactose) was determined. Thermograms were obtained by heating at a constant rate of 5°C per min from 80 to 95°C and fusion heats of both samples and references were compared.

High-performance liquid chromatography

Raw materials, reference samples and finished products were dissolved in water at a concentration of about 0.5 mg ml^{-1} and then filtered. A 5 μl aliquot was injected into the liquid chromatograph at room temperature, using the following flow gradient for time and flow, respectively: 0 min, 0.4 ml min^{-1}; 3 min, 0.4 ml min^{-1}; 3.5 min, 0.6 ml min^{-1}; 13 min, 0.6 ml min^{-1}; 13.5 min, 0.4 ml min^{-1}.

DSC was used for establishing the reference sample content.

Thin-layer chromatography–densitometry

Raw materials, reference samples and finished products were dissolved in water at the same concentration (2 mg ml^{-1}) and then filtered. A 1.5 μl (3 μg) aliquot was applied to the HPTLC plates in spots at 10-mm intervals in a nitrogen stream at a rate of 25 nl s^{-1}. Chromatography was performed in saturated twin-through chamber. The developing path was 7 cm. Scanning by absorbance at 205 nm, deuterium lamp, monocromator bandwidth 10 nm, slit dimension 0.4 × 6 mm, scanning speed 4 mm s^{-1}, evaluation via peak area.

Like in the HPLC procedure, DSC was used for establishing the reference sample content.

Results and Discussion

Differential scanning calorimetry

Purity study. Eight different samples of 5-ISMN (reference sample) were tested. The purity values obtained were the following: 99.886, 99.879, 99.884, 99.852, 99.887, 99.883, 99.888, 99.888; mean value 99.880, RSD = 0.0121% (n = 8).

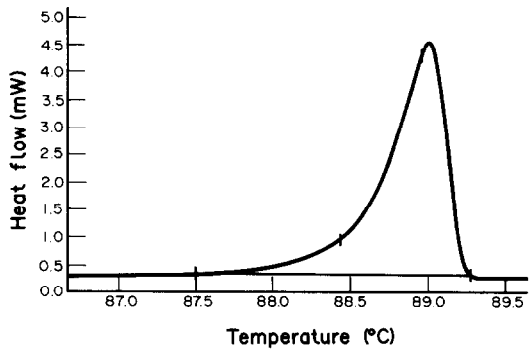

Figure 1
Thermogram of 5-ISMN (reference sample) in the purity study. ΔH = 24.184 kJ mol^{-1}, T_m = 88.741°C, T_o = 88.742 ± 0.002°C, X-correction = 6.147 ± 0.105%, purity = 99.886 ± 0.004%.

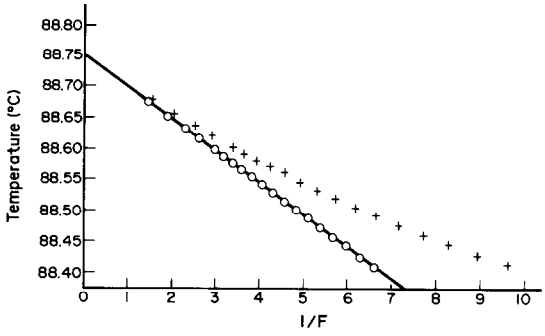

Figure 2
Graphical linearization of 1/F plot.

Figures 1 and 2 show examples of one thermogram and a Van't Hoff plot obtained in this purity study.

Quantitation assay. Eight different samples of 5-ISMN (reference sample) and raw material (dry mixture of 5-ISMN with lactose) were studied. The following fusion heats were found:

Reference sample (ΔH, J g^{-1}). 121.438, 121.706, 121.585, 121.704, 121.686, 120.623, 120.886, 119.512; mean value 121.140 J g^{-1}, RSD = 0.64% (n = 8).

Raw material (ΔH, J g^{-1}). 108.086, 107.607, 108.117, 107.711, 107.786, 107.644, 107.600, 107.993; mean value 107.814 J g^{-1}, RSD = 0.20% (n = 8).

The content of 5-ISMN in the dry mixture obtained from these data is 89.0%. Figure 3

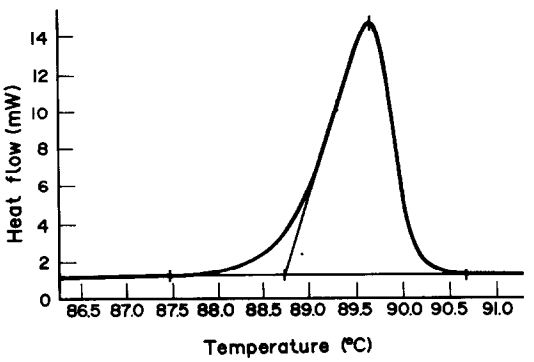

Figure 3
Raw material thermogram (dry mixture of 5-ISMN with lactose) obtained in the quantification assay. ΔH = 107.786 J g^{-1}.

shows an example of a raw material thermogram.

High-performance liquid chromatography

In order to demonstrate the selectivity of the method in both raw materials and pharmaceutical dosage forms, we prepared a mixture of 5-ISMN, 2-ISMN and 2,5-ISDN (the resolution obtained is shown in Fig. 4) and a mixture of 5-ISMN with all the excipients used in pharmaceutical preparations (Fig. 5 shows the suitability of the method).

The linearity of the method was tested using five solutions of 5-ISMN from 0.3 to 0.8 mg ml^{-1}, the results obtained were the following for concentration and area, respectively: 0.30 mg ml^{-1}, 0.039551; 0.40 mg ml^{-1}, 0.050346; 0.50 mg ml^{-1}, 0.061504; 0.60 mg ml^{-1}, 0.073211; 0.80 mg ml^{-1}, 0.093285. Results were evaluated by linear regression, the correlation coefficient obtained was 0.999 and the regression equation was:

$$y = 0.108215x + 0.007287$$
$$n = 5;\ r = 0.999.$$

The repeatability of the assay was determined analysing eight different samples of 5-ISMN prepared at the same concentration (0.45 mg ml^{-1}). The results (area of the peak) and RSD obtained were: 0.057250, 0.056953, 0.056346, 0.057110, 0.056451, 0.057259, 0.056190, 0.057331; $\bar{A}$ = 0.056778, RSD = 0.77% (n = 8).

The accuracy was determined by calculation of the percentage recovery of spiked amounts of 5-ISMN into a lactose matrix. Five milligrams of lactose were spiked with between 35.3 and 55.6 mg 5-ISMN and the mean recovery was 100.68%, RDS = 0.68% (n = 5).

Eight replicate analyses of raw material and five replicate analyses of five pharmaceutical preparations were performed. The content of

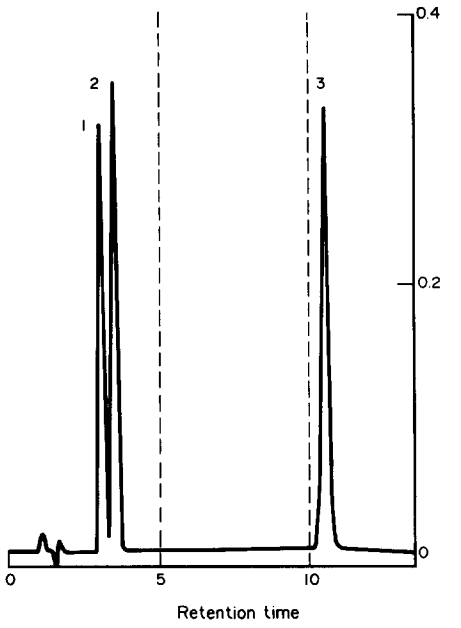

Figure 4
HPLC of a mixture of 2-ISMN, 5-ISMN and 2,5-ISDN at the same concentration (0.5 mg ml^{-1}). Peak 1: RT = 3.06, 2-ISMN; Peak 2: RT = 3.49, 5-ISMN; Peak 3: RT = 10.55, 2,5-ISDN.

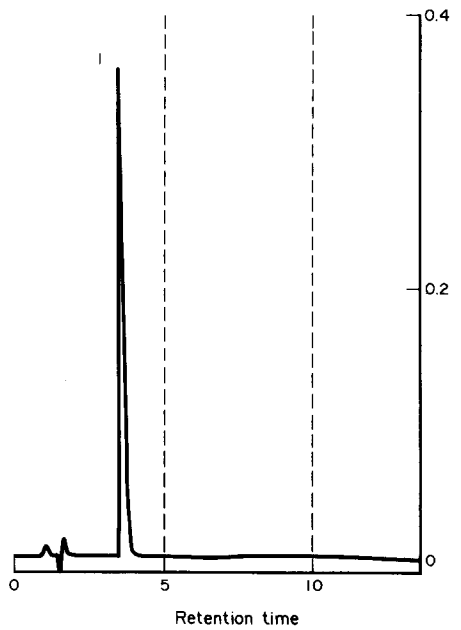

Figure 5
HPLC of a mixture of 5-ISMN with lactose and the excipients (concentration of 5-ISMN 0.45 mg ml^{-1}). Peak 1: RT = 3.49, 5-ISMN.

5-ISMN in the dry mixture (label claim 90% (p/p)) was 89.9%, recovery 99.9% (n = 8). Analysis of tablets containing 20 mg per tablet gave a recovery of 99.4% (value found 19.9 mg per tablet (n = 5)). Analysis of four different commercial samples containing 40 mg per tablet gave the following for recovery and value found: 100.1%, 40.0 mg per tablet; 103.7%, 41.5 mg per tablet; 100.0%, 40.0 mg per tablet; 98.7%, 39.5 mg per tablet (n = 5 for every value).

TLC–densitometry

In order to demonstrate the selectivity of the method in both raw materials and pharmaceutical dosage forms, a mixture of 5-ISMN, 2-ISMN and 2,5-ISDN was prepared (the resolution obtained is shown in Fig. 6) and a mixture of 5-ISMN with all the excipients used in pharmaceutical dosage forms (Fig. 7 shows the suitability of the method).

The linearity of the method was tested using eight solutions of 5-ISMN from 1 to 8 mg ml^{-1}, spots 1 μl (1–8 μg), the results obtained were the following for concentration and area: 1 μg per spot, 157.73; 2 μg per spot, 315.46; 3 μg per spot, 467.10; 4 μg per spot, 621.13; 5 μg per spot, 800.12; 6 μg per spot, 905.10; 7 μg per spot, 1056.20; 8 μg per spot, 1200.31.

Results were evaluated by linear regression, the correlation coefficient obtained was 0.999 and the regression equation was:

$$y = 148.747x + 21.032$$
$$n = 8;\ r = 0.999.$$

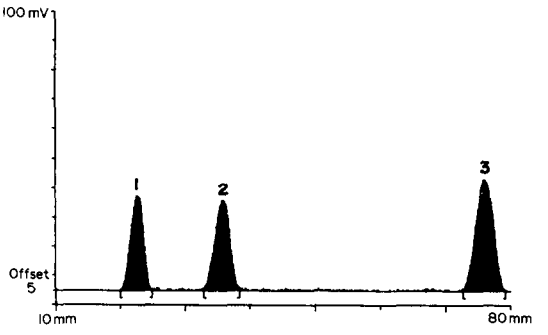

Figure 6
TLC of a mixture of 2-ISMN, 5-ISMN and 2,5-ISDN at the same concentration (3 μg per spot). Peak 1: R_f = 0.23, 2-ISMN; Peak 2: R_f = 0.33, 5-ISMN; Peak 3: R_f = 0.82, 2,5-ISDN.

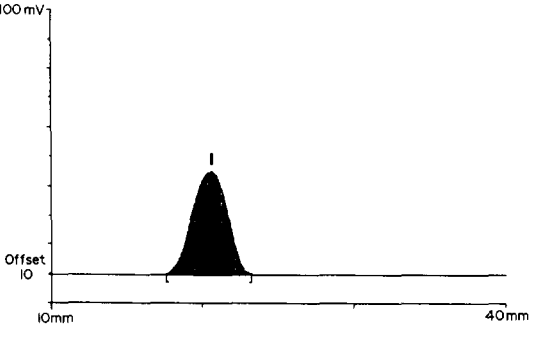

Figure 7
TLC of a mixture of 5-ISMN with lactose and the excipients. Peak 1: R_f = 0.33, 5-ISMN.

The repeatability of the assay was determined by analysing eight different samples of 5-ISMN prepared at the same concentration (2 mg ml^{-1}, spot 7 μg). The results (area of the

Table 1
TLC recoveries of spiked amounts of 5-ISMN into a lactose matrix

Lactose (mg)	5-ISMN spiked (mg)	5-ISMN found (mg)	% Recovery
20	10	10.3	102.8
20	20	20.8	103.9
20	30	30.5	101.7
20	40	40.0	99.9
20	50	49.8	99.6

Table 2
TLC results for raw materials and pharmaceutical preparations

	Label claim	Value found	%
Raw material	90% (p/p)	89.8% (p/p)	99.8
Finished products	20 mg per tablet	19.7 mg per tablet	99.4
	40 mg per tablet	39.2 mg per tablet	98.0
	40 mg per tablet	40.8 mg per tablet	102.0
	40 mg per tablet	40.4 mg per tablet	101.0
	40 mg per tablet	39.6 mg per tablet	99.1

peak) and RSD obtained were: 1041.47, 1042.33, 1045.88, 1045.24, 1047.69, 1047.30, 1042.22, 1038.91; $\bar{A}$ = 1043.88, RSD = 3.1% (n = 8).

Accuracy was determined by calculation of the percentage recovery of spiked amounts of 5-ISMN into a lactose matrix. The recoveries obtained are shown in Table 1.

Eight replicate analyses of raw material and five replicate analyses of five pharmaceutical preparations were performed. Table 2 shows the results obtained; average values are in good agreement with the stated amounts.

Conclusions

DSC appears to be an absolute method for establishing the purity of 5-ISMN in raw materials if the content is not less than 98.5%, DSC gives accurate results for 5-ISMN reference sample content. DSC, by means of comparing fusion heats of references and samples is a suitable method for quantitation of 5-ISMN in dry mixtures of 5-ISMN with lactose. Results obtained for the repeatability test were satisfactory.

Some advantages of this method are the short time of analysis required and the sample pretreatment elimination.

Chromatographic methods HPLC and TLC are suitable for the assay of 5-ISMN in raw materials, pharmaceutical preparations and stability studies. Selectivity, linearity, repeatability and accuracy tests were satisfactory.

Results in pharmaceutical preparations did not give any significant difference between both methods and are in good agreement with labelled amounts.

The advantage of HPLC is higher sensitivity. TLC–densitometry is suitable for routine determination and is faster and cheaper.

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