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Short communication

Application of differential scanning calorimetry and high performance liquid chromatography to determine the effects of mixture composition and preparation during the evaluation of niclosamide–excipient compatibility

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1. Introduction

Assessment of possible incompatibilities between an active drug substance and different excipients forms an important part of the preformulation stage during the development of a solid dosage form [1]. Successful compatibility studies require a good experimental design that furnishes the required information with the minimum of experimental effort. To achieve this requires that the questions to be answered by the experiments must be correctly formulated, a correct choice of experimental method must be made in the light of both the accuracy required and of the various experimental pitfalls which are likely to be encountered, and the general pattern of the experiments, i.e. the number, spacing and interrelation of the individual observations, must be correctly chosen [2].

For routine drug–excipient interaction studies, two methods are available; differential scanning calorimetry (DSC) and quantitative assay after isothermal stress tests [3–5]. DSC allows the fast evaluation of possible incompatibilities, because it shows changes in the appearance, shift or disappearance of melting endotherms and exotherms, and/or variations in the corresponding enthalpies of reaction [6]. Results obtained by studying the thermal behaviour of mixtures of the drug and excipients are then confirmed by analysing those mixtures indicating possible interactions, for degradation products [1].

In this study the compatibility between niclosamide and commonly-used tablet excipients was assessed prior to the formulation of a bolus for veterinary use. Drug–excipient mixtures were prepared in the ratio as in the bolus but, to enhance possible reactions, 1:1 mixtures and mixtures granulated with water and dried at an elevated temperature were also prepared. Both

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Table 1
Materials, and ratios as in boluses, used to prepare samples for DSC

Tablet ingredient	Composition (%)	Quantity per bolus (g)
Active ingredient Niclosamide (Sigma, St. Louis, MO)	30.7	2.80
Filler Lactose, mannitol, calcium sulphate, calcium carbonate (Saarchem, South Africa); lactitol monohydrate (Cape Sweeteners, South Africa); microcrystalline cellulose (Emcocel 50 M & 90 M, Mendell, UK); dibasic calcium phosphate monohydrate (Mendell, UK)	45.0	4.10
Disintegrating agent Methlycellulose, starch (Saarchem, South Africa); sodium starch glycolate (Explotab, Mendell, UK); croscarmellose sodium (Ac-di-sol, FMC, USA)	11.0	1.00
Binding agent Carboxymethylcellulose sodium, gelatine, starch, PEG 6000 (Saarchem, South Africa); Povidone (Kollidon K25, BASF, Germany)	11.0	1.00
Lubricant Magnesium stearate, sodium lauryl sulphate, stearic acid (Saarchem, South Africa); sodium stearyl fumarate (PRUV, Mendell, UK)	2.2	0.20
Glidant Colloidal silicon dioxide (Aerosil 200, Degussa, USA)	0.2	0.02
Total	100	9.12

DSC and high performance liquid chromatography (HPLC) were used to evaluate mixtures for possible interactions. Results were compared statistically to determine the effect of analytical technique, sample composition and preparation on the outcome of compatibility assessment.

2. Materials and methods

2.1. Materials

Niclosamide (2′,5-dichloro-4-nitrosalicylanilide) was obtained from Sigma (St. Louis, MO; USP or BP grade; purity above 98%). HPLC methanol from BDH (Poole, UK) and analytical-grade formic acid and dibasic ammonium phosphate (Saarchem, South Africa) were used. Excipients tested were obtained from companies distributing it in South Africa. Details are listed in Table 1.

2.2. Preparation of samples

The mixed samples consisted of niclosamide in a 1:1 w/w ratio with each of the excipients listed in Table 1. Mixture samples were also prepared in ratios to approximate the ratio of drug to excipient in the final dosage form as shown in Table 1. The same mixtures were made, but an equivalent amount of water was mixed with each of the samples. These samples were dried in an oven at 50°C for 1 h. Samples containing single components, subjected to the same stress conditions, included niclosamide and each of the excipients.

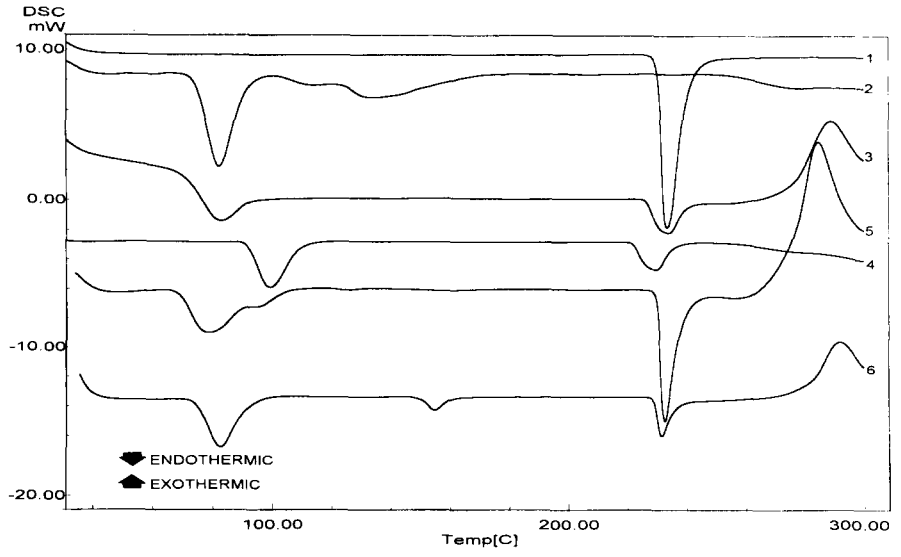

Fig. 1. DSC analysis of lactitol monohydrate–niclosamide combinations: (1) niclosamide; (2) excipient; (3) physical mixture ratio as in tablets and subjected to stress conditions; (4) 1:1 physical mixture; (5) 1:1 mixture subjected to stress conditions; (6) physical mixture with ratio as in tablets.

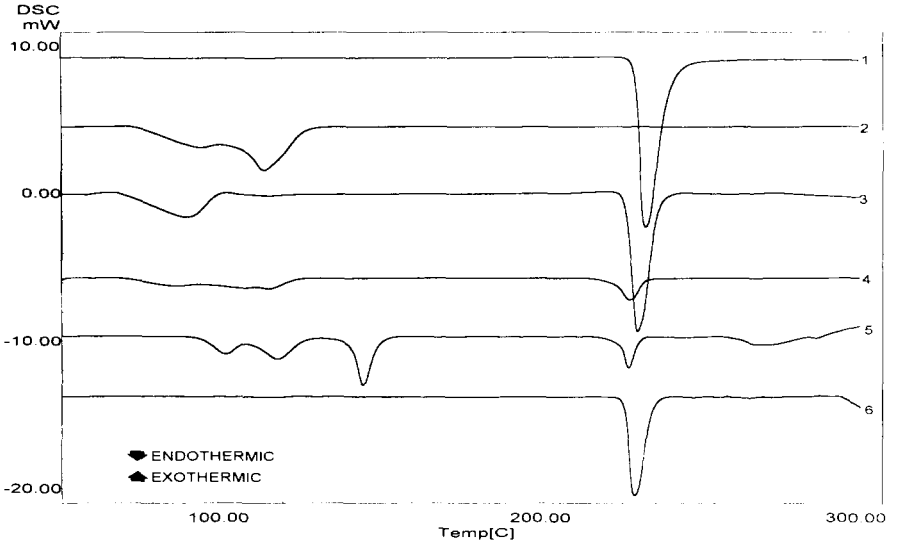

Fig. 2. DSC analysis of magnesium stearate–niclosamide combinations: (1) niclosamide; (2) excipient; (3) physical mixture ratio as in tables and subjected to stress conditions; (4) 1:1 physical mixture; (5) 1:1 mixture subjected to stress conditions; (6) physical mixture with ratio as in tablets.

2.3. DSC

DSC thermograms were obtained with a Shimadzu DSC-50 Differential Scanning Calorimeter at a heating rate of 10°C min^{-1} under nitrogen purge with a flow rate of 35 ml min^{-1}. The instrument was calibrated using indium as a standard (melting point 156.4°C). Samples (1–8 mg) were weighed to the nearest 0.001 mg and sealed in aluminium pans.

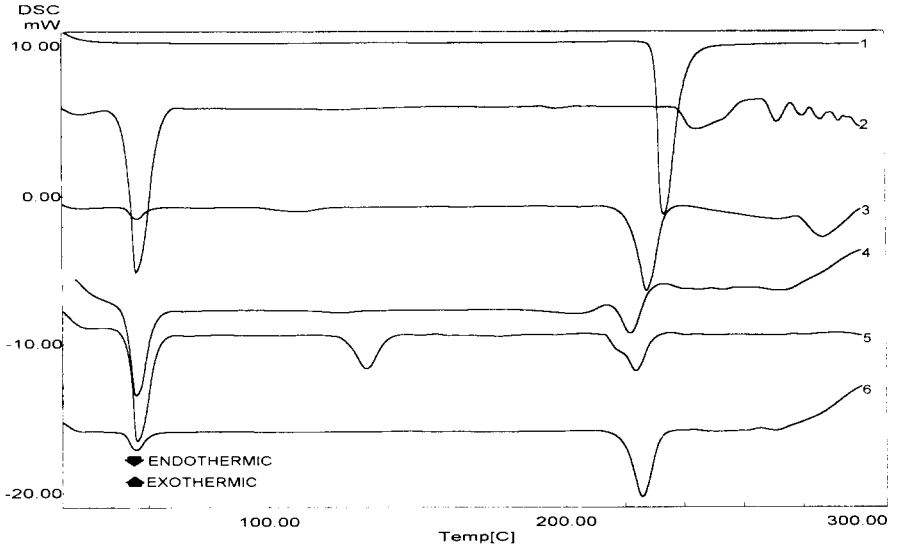

Fig. 3. DSC analysis of stearic acid niclosamide combinations: (1) niclosamide; (2) excipient; (3) physical mixture ratio as in tablets and subjected to stress conditions; (4) 1:1 physical mixture; (5) 1:1 mixture subjected to stress conditions; (6) physical mixture with ratio as in tablets.

2.4. HPLC analysis of samples

The HPLC system consisted of a Hewlett Packard HP1050 system with a HP3395 integrator (Hewlett Packard, Waldburg, Germany). A Nova Pak C_{18} cartridge (Waters, Bedford, MA; 150 mm × 3.9 mm i.d., 4 μm particle size) was used and UV detection was at 254 nm. To prepare the mobile phase phosphate buffer was prepared by dissolving dibasic ammonium phosphate (6.6 g, 0.05 M) in deionized water (1000 ml) filtered with a Milli-Q50 system (Millipore, Bedford, MA) and adjusting to pH 3.6 with concentrated phosphoric acid [7]. The optimum mobile phase consisted of a mixture of phosphate buffer and methanol (75:25, v/v) with a pH of 3.6. The flow rate employed was 1 ml min^{-1}. The isocratic system was operated at ambient temperature. The method did not require complex sample extraction procedures. Method validation included linearity, repeatability, accuracy and precision determination. The method was also stability-indicating because it was possible to distinguish between niclosamide and minute concentrations of degradation products [7].

To prepare standard solutions, accurately measured amounts of niclosamide were dissolved in methanol containing 10% formic acid. Two samples were weighed and then used to prepare two stock solutions. Dilutions made from both these solutions were injected into the chromatograph and the linearity of peak area versus concentration was calculated. Solutions were diluted with the mobile phase. Samples taken from mixtures of niclosamide and the excipients were similarly prepared such that the drug concentration in the samples injected into the chromatograph fell within the range of standards assayed.

2.5. Calculations and statistical analysis

Mean HPLC results were compared according to the Student–Newman–Keuls multiple range test (Statistica CSS 3.1, Statsoft, USA). A 95% confidence level ($p \leq 0.05$) was considered satisfactory for indicating significant differences.

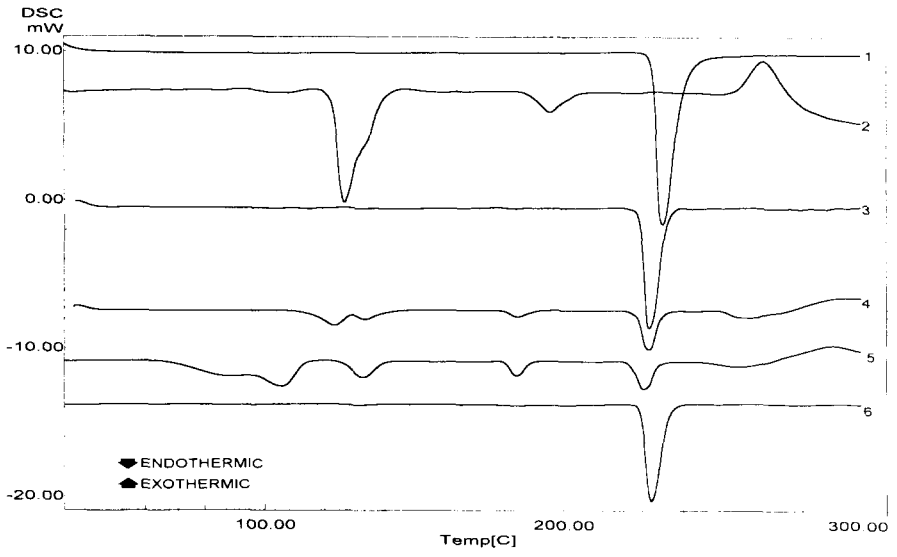

Fig. 4. DSC analysis of sodium stearyl fumarate–niclosamide combinations: (1) niclosamide; (2) excipient; (3) physical mixture ratio as in tablets and subjected to stress conditions; (4) 1:1 physical mixture; (5) 1:1 mixture subjected to stress conditions; (6) physical mixture with ratio as in tablets.

3. Results and discussion

Thermograms obtained by DSC analyses of individual components were compared with those of combinations of niclosamide and the excipients. Of the 20 commonly used tablet excipients that were tested, only six showed interactions with niclosamide. The excipients that showed interactions were lactitol monohydrate (Fig. 1), magnesium stearate (Fig. 2), stearic acid (Fig. 3), sodium stearyl fumarate (Fig. 4), Povidone (polyvinyl pyrrolidone) (Fig. 5) and polyethylene glycol (Fig. 6). Each Figure shows thermograms of (1) niclosamide, (2) the excipient and (3–6) mixtures of niclosamide and the excipient; (3) in a ratio as in the bolus, Table 1, granulated with water and dried at 50°C; (4) 1:1 physical mixture; (5) 1:1 physical mixture granulated with water and dried at 50°C; and (6) in a ratio as in the bolus not exposed to water and heat.

Changes in DSC thermograms, Figs. 1–6, observed for niclosamide–excipient combinations showing interactions, were large shifts in melting points signifying possible strong solid–solid interactions, changes in peak shape and height-to-width ratios, extra thermal effects in thermograms before the peak of the lower melting component and/or the disappearance of one of the component peaks. These changes may be indicative of interactions but they do not necessarily indicate incompatibilities because they could also be the result of possible differences in the sample geometry of the mixtures [1]. Such changes were not observed in the other mixtures studied, indicating that niclosamide was compatible with these excipients. This was confirmed by HPLC analysis results. For example, the mean assay for niclosamide–mannitol mixtures was $96 \pm 1.7\%$ and for niclosamide–starch mixtures it was $97 \pm 2.5\%$. These assay results were not significantly different from that of pure niclosamide treated in the same way as the mixtures.

Mixing lactitol monohydrate with niclosamide caused shifting of peaks, Fig. 1 (3, 5), and the appearance of extra peaks, Fig. 1 (6). The niclosamide peak also varied in size. Magnesium stearate reacted with niclosamide, causing the appearance of extra peaks, Fig. 2 (5), and the shifting of peaks, Fig. 2 (4, 5). These interactions were only present when 1:1 mixtures were studied and

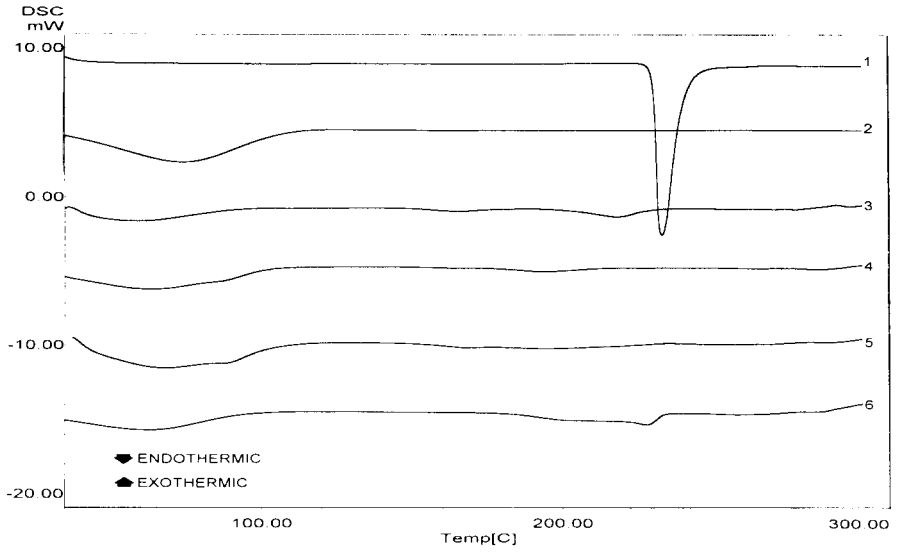

Fig. 5. DSC analysis of Povidone–niclosamide combinations: (1) niclosamide; (2) excipient; (3) physical mixture ratio as in tablets and subjected to stress conditions; (4) 1:1 physical mixture; (5) 1:1 mixture subjected to stress conditions; (6) physical mixture with ratio as in tablets.

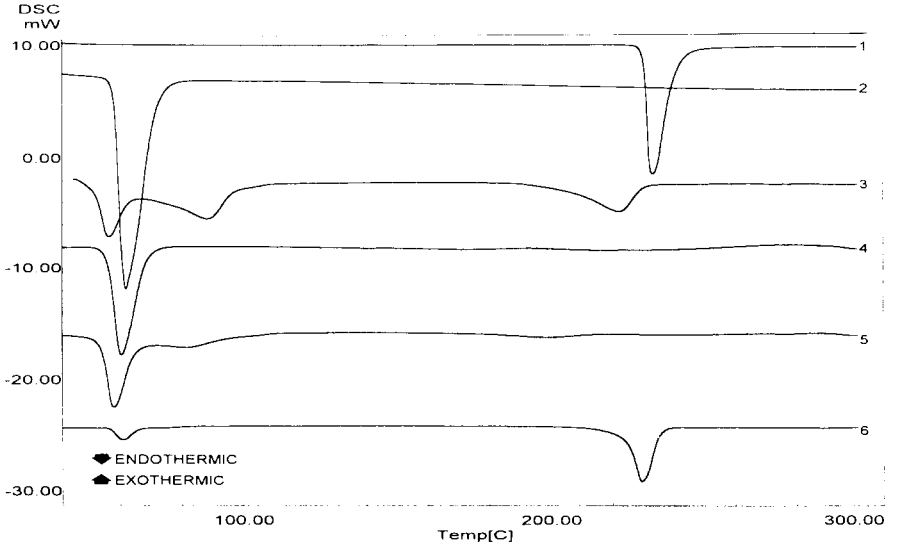

Fig. 6. DSC analysis of PEG 6000–niclosamide combinations: (1) niclosamide; (2) excipient; (3) physical mixture ratio as in tablets and subjected to stress conditions; (4) 1:1 physical mixture; (5) 1:1 mixture subjected to stress conditions; (6) physical mixture with ratio as in tablets.

were absent when mixtures containing the drug and excipient in the same ratio as in tablets were subjected to DSC analysis. DSC showed that stearic acid reacted with niclosamide in the 1:1 mixture and in the ratio as in tablets. The interactions were mainly changes in the peak shape and size, Fig. 3 (4, 5), and shifting peaks, Fig. 3 (3–6).

Sodium stearyl fumarate reacted with niclo-

Table 2
Calibration data for HPLC analysis of niclosamide

Concentration range ($g\ ml^{-1}$)	Regression coefficient	Slope	Standard error of slope	y intercept	Standard error of y intercept
5–25	0.997	19803	224	6560	4714

samide causing changes in the appearance of the niclosamide peaks and the appearance of additional peaks, Fig. 4 (4, 5). These reactions were again only present when studying 1:1 mixtures. Niclosamide reacted strongly with Povidone, causing the disappearance of the niclosamide peak in the DSC thermograms of mixtures, Fig. 5 (3–6). Polethylene glycol (PEG 6000) reacted with niclosamide, causing the disappearance of the niclosamide peak in the DSC thermogram of 1:1 mixtures, Fig. 6 (4, 5), and changes in the size and positions of the peak in mixtures with the same ratio as in tablets, Fig. 6 (3–6).

In Table 2 a summary of the calibration data for the HPLC assay for niclosamide is given. This method was found to be applicable to the assay of niclosamide in the presence of degradation products [7]. Assay results for pure niclosamide exposed and not exposed to stress conditions were not significantly different ($99.0 \pm 0.81\%$ compared to $99.5 \pm 1.77\%$, $p > 0.05$). Furthermore, comparison of HPLC assay results for niclosamide in combination with excipients and/or exposed to stress conditions showed that only two mixtures containing PEG 6000, the mixture in the ratio of the bolus and exposed to stress ($p = 0.0109$) and the 1:1 physical mixture ($p = 0.0002$), gave significantly lower results than the equivalent niclosamide samples not combined with excipients. These results are listed in Table 3. HPLC analysis results showed that sample composition and treatment, except for the two cases mentioned, did not cause the degradation of niclosamide.

It should be recognised that the random nature of the preparation of the mixtures means that variation in the assay results will occur. To compensate for this, assay results were compared using a 95% confidence interval, instead of 99%, for indicating significant differences. Under these conditions, interactions shown by DSC evaluation of mixtures were therefore not causing degradation of niclosamide and no correlation could be shown between results obtained from DSC analysis and HPLC analysis. This suggests that the method of sample preparation, i.e. the ratio of active component to excipient and the exposure of mixtures to water and heat, had a significant influence on results obtained by DSC but not by HPLC. This does not mean that DSC results should be ignored, because although interactions may not cause degradation of niclosamide they may deteriorate the performance of dosage forms, i.e. poorer disintegration times and a decrease in dissolution rate [1]. For example, the effect of Povidone on drug performance has been well described [8–10]. Generally, combination with Povidone increased the dissolution rate and solubility of drugs.

4. Conclusions

Thermal analysis proved useful for the determination of reactions between niclosamide and excipients, especially when combined with quantitative analysis of the drug with HPLC. Successful evaluation of compatibility using these methods required that experiments had to be correctly formulated and experimental methods and the general pattern of experiments had to be correctly chosen. To maximise the prediction of incompatibilities, drug–excipient mixtures should be exposed to stress, i.e. heat and moisture, and should be evaluated using more than one technique, i.e. HPLC and DSC. However, stress conditions should not exceed those which the drug may encounter during actual manufacturing and storing conditions.

Table 3
DSC and HPLC analysis of niclosamide in niclosamide–excipient mixtures ($p < 0.05$) indicates significant differences in niclosamide content of mixtures compared to pure niclosamide)

Excipient	Sample preparation[a]	Changes in DSC thermograms	Assay (% ± SD)	p
PEG 6000	1	Changes in size and position of peaks	88 ± 2.8	0.0109
	2	Disappearance of niclosamide peak	61 ± 1.43	0.0002
	3	Disappearance of niclosamide peak	100 ± 2.8	0.9990
	4	Changes in size and position of peaks	100 ± 2.1	0.9996
Povidone	1	Disappearance of niclosamide peak	94 ± 2.1	0.6705
	2	Disappearance of niclosamide peak	95 ± 3.5	0.8162
	3	Disappearance of niclosamide peak	100 ± 3.6	0.8498
	4	Disappearance of niclosamide peak	93 ± 2.8	0.5193
Sodium stearyl fumarate	1	No changes	100 ± 2.1	0.9999
	2	Changes in appearance of niclosamide peak Appearance of additional peaks	90 ± 9.9	0.0596
	3	Changes in appearance of niclosamide peak Appearance of additional peaks	100 ± 1.2	0.9999
	4	No changes	97 ± 2.1	0.9839
Stearic acid	1	Shifting peaks	94 ± 1.4	0.7686
	2	Changes in appearance of niclosamide peak Appearance of additional peaks	91 ± 0.7	0.8998
	3	Changes in appearance of niclosamide peak Appearance of additional peaks	92 ± 6.4	0.9692
	4	Shifting of peaks	96 ± 1.4	0.9729
Magnesium stearate	1	No changes	99 ± 0.7	0.9776
	2	Shifting of peaks	102 ± 2.1	0.9646
	3	Appearance of additional peaks Shifting of peaks	97 ± 2.1	0.9473
	4	No changes	97 ± 1.4	0.9819
Lactitol monohydrate	1	Shifting of peaks	91 ± 2.8	0.1559
	2	Shifting of peaks	91 ± 0.7	0.0845
	3	Shifting of peaks	101 ± 0.7	0.9998
	4	Shifting of peaks Appearance of additional peaks	99 ± 1.4	0.9776

[a] (1) Mixture in ratio as in bolus and exposed to stress; (2) 1:1 physical mixture; (3) 1:1 physical mixture exposed to stress; (4) mixture in ratio as in bolus.

When applying these principles, the reactions occurring between niclosamide and excipients could be measured more accurately. It was found that interactions shown by DSC depended on the ratio of the niclosamide–excipient mixture and the stress conditions the mixtures were subjected to, such as the addition of water and exposure to elevated temperatures. These interactions were not detected by HPLC analysis, illustrating that degradation did not take place. DSC and HPLC thus showed that although some reactions occurred, niclosamide was compatible with the majority of common tablet excipients tested.

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