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Short communication

Degradation of components in drug formulations: a comparison between HPLC and DSC methods

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Abstract

Information about the stability of drug components and drug formulations is needed to predict the shelf-life of the final products. The studies on the interaction between the drug and the excipients may be carried out by means of accelerated stability tests followed by analytical determination of the active principle (HPLC and other methods) and by means of the differential scanning calorimetry (DSC). This research has been focused to the acetyl salicylic acid (ASA) physical–chemical characterisation by using DSC method in order to evaluate its compatibility with some of the most used excipients. It was possible to show, with the DSC method, the incompatibility of magnesium stearate with ASA; the HPLC data confirm the reduction of ASA concentration in the presence of magnesium stearate. With the other excipients the characteristic endotherms of the drug were always present and no or little degradation was observed with the accelerated stability tests. Therefore, the results with the DSC method are comparable and in good agreement with the results obtained with other methods.

Keywords: Drug formulation; Acetyl-salicylic acid; Degradation; Differential scanning calorimetry; Stability testing

1. Introduction

In drug formulation studies it is essential to evaluate the possible interactions between the active principle and the excipients, as the choice of the excipients should be performed in relation to the drug delivery, to their compatibility with the same drug and to the stability of the final product.

Differential scanning calorimetry (DSC) has been a standard method for the characterisation of solid drugs for many years in particular for preformulation studies [1–4]. The technique is used in preformulation studies because interactions between drug and excipients often result in shift, appearance or disappearance of endothermic or exothermic peaks and on the change of other enthalpic values on thermal curves obtained with DSC method.

The advantages of the method are due to its rapid response in order to evaluate the possible

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incompatibility between the formulation components.

The accelerated stability tests followed by analytical determination of the degradation of the active principle, i.e. HPLC, require, on the contrary, longer time and more sophisticated operations, but are able to offer qualitative and quantitative indications on the degradation pathway [5,6].

In the present paper DSC was used to evaluate the compatibility of acetyl salicylic acid (ASA) with some of the most used excipients (maize starch, microcrystalline cellulose (MCC), talc, lactose and magnesium stearate).

The effect of 1 or 2 months of ageing of the same samples at 35, 45 and 55 °C was also investigated by HPLC analysis in order to compare and to validate the DSC method.

2. Material and methods

2.1. Material

All the compounds utilised were Ph. Eur. F. U. IX: ASA, lactose (80 mesh), magnesium stearate, maize starch, talc, microcrystalline cellulose (Avicel PH 101).

2.2. Equipments

1. HPLC Water Mod. 700 Satellite WISP equipped with:
   - chromatographic column RP 18, 3.9 × 300 mm Waters;
   - automatic sampling injector Waters WISP 700
   - pump Mod. 600 E
   - UV-Vis detector Mod. 484
   - data recorder and processor PC-NEC Power Mate.
2. DSC 7 Perkin Elmer equipped with a control and calculation unit TAC 7/DX Perkin Elmer.
3. Thermostat Vismara equipped with continuous temperature recording.

2.3. Analytical methods

2.3.1. Accelerated stability study

The stability study has been performed using sample of: ASA alone, each single excipients and binary mixtures formed by ASA and only one excipient 1:1 w/w and mixture of ASA with all excipients in same w/w concentrations.

Samples were analysed immediately with HPLC method while other samples were stored for 2 months at 35, 45 and 55 °C and then analysed. It was calculated the ASA w/w percentage in samples at time 0 and after 2 months at all different temperature. Moreover, it was calculate the percentage of degradation with the following equation:

$$\%\ of\ \text{degradation} = \frac{(\text{recovery}\ \%\ at\ time\ 0 - \%\text{recovery}\ after\ 1\ or\ 2\ months)}{\text{recovery}\ \%\ at\ time\ 0} \times 100$$

2.3.2. HPLC method

The determination of ASA has been performed by using the method reported in the USP XXII/NF XVII (ASA tablets monograph) [7]. Briefly, elution was carried out at room temperature with a mobile phase made with 2 g of 1 sodium eptan sulphonate in a mixture of 850 ml of water and 150 ml of acetoniltrile; the pH was adjusted to 3.4 with acetic acid. The injecting volume was 20 μl, the flow rate was 2.0 ml/min and the detection was at 280 nm. In this condition the retention time of ASA was 7.00 min. The method was validate: accuracy was expressed as percent recovery of ASA and it was found to be 99%. Precision was expressed as percent coefficient of variation of the

method and it was found to be CV = 5.1% ($n = 6$). Linearity was tested at four concentration (0.2, 0.5, 1 and 2 mg/ml) and it was found to be $r^2 = 0.9998$. The limit of quantification was found to be 0.05 mg/ml (concentration at which R.S.D. = 10% with $n = 6$). No interfering peaks were pointed out.

2.3.3. DSC method

Some preliminary tests have been performed in order to determine the main variables, which may influence the DSC methodology, i.e. the scanning rate, the weight, the granulometry and the degree of crystallisation of the sample. The oven was filled with nitrogen at pressure of 40 psi. The amounts of samples were of the order of 3.5–6.0 mg. To obtain the mixture, ASA and excipients were accurately weighed and mixed in a mortar with a gentle movement of the pestle. The scanning speed was of 10 °C/min. In such condition the results are reproducible, the peaks appear well defined and the melting temperatures are precise and accurate whether with crystalline or with milled ASA. The temperature range (45–240 °C) has been chosen in order to evaluate all enthalpic phenomena linked to ASA and the excipients. The resulted data (melting heat and temperature) were calculated by means of the suitable computer programme. The thermal analysis have been performed using sample of ASA alone; single excipients; binary mixtures formed by ASA and only one excipient 1:1 w/w and in other different ratios and mixture of ASA with all excipients.

3. Results and discussion

The ASA thermoanalytical curve shows only the melting endothermic peak at 143.40 °C (Fig. 1A) with a ΔT of 3.86 °C. Other enthalpic phenomena were not observed; therefore, no degradation occurs in these condition. The curves of maize starch and MCC are very similar with an endothermic lower unresolved peak, due to water evaporation. The lactose curve shows two melting endothermal points, the first corresponding to the melting of less stable (α-hydrate: 148.16 °C) and the second to the more stable form (α anhydrous: 214.50 °C). The talc does not show any type of signal in the observed temperature range. On the contrary it is difficult to interpret correctly the thermoanalytic curve of magnesium stearate for its chemical constitution of a mixture of long-chained aliphatic acids and for its variable content of humidity.

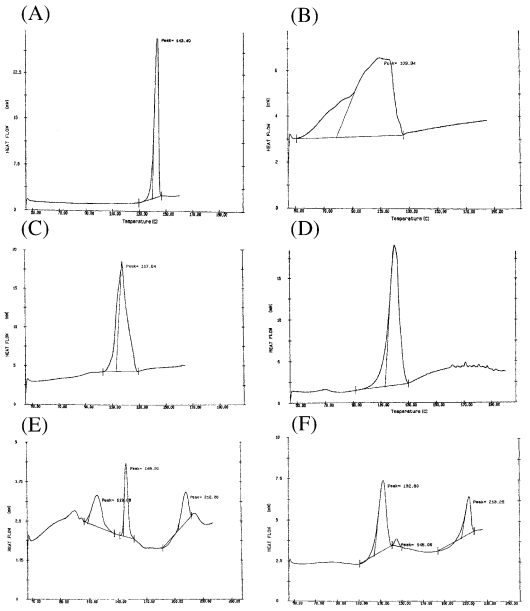

Fig. 1. Thermoanalytical diagram of (A) ASA. (B) ASA + Magnesium stearate 1:1 w/w. (C) ASA + Magnesium stearate 4:1 w/w. (D) ASA + Magnesium stearate 30:1 w/w. (E) ASA + all excipients. (F) ASA + all excipients excluding Magnesium stearate.

The data of the thermoanalysis of the binary mixtures and of the mixture formed by ASA and all excipients are summarised in Table 1.

The curve of ASA alone is compared with the curves obtained from the different mixtures and in case of superimposition no interaction and no incompatibility occur between ASA and the excipient. In the case of the binary mixture of ASA with talc, maize starch, MCC and lactose, the ASA endothermic melting peak is always observed. In particular with maize starch no interaction appears, while with talc and MCC a temperature shift is observed (12 °C in the presence of talc and

Table 1
Change of ASA melting peak in different mixtures

Mixture components	Melting peak of ASA (°C)	Other endothermal events occurring in the analysed temperature range (°C)
ASA	143.40	–
ASA + Talc (1:1)	131.29	–
ASA + Talc (9:1)	135.29	–
ASA + Maize Starch (1:1)	140.67	–
ASA + MCC (1:1)	133.36	–
ASA + Lactose (1:1)	136.77	205.31
ASA + Magnesium Stearate (1:1)	n.d.	109.94
ASA + Magnesium Stearate (4:1)	117.64	–
ASA + Magnesium Stearate (30:1)	120.40	–
ASA + Talc + Lactose + Magnesium stearate + Maize starch + MCC	n.d.	90.00, 119.08, 149.20, 212.20
ASA + Talc + Lactose + Maize starch + MCC	123.95	145.06, 213.25

10 °C in the presence of MCC). The shift is reduced by reducing the percentage of the excipient. Also in the case of ASA and lactose a temperature drop is observed for ASA and for the two lactose melting points. The peak at 136.77 °C includes both ASA and lower lactose peak, as the melting points are very close.

The mixture ASA and magnesium stearate (1:1) shows the melting peak of magnesium stearate (109.94 °C) and the disappearance of ASA peak (Fig. 1B). Since in real formulations the drug and the excipients are present in ratios very different from 1:1 w/w, we have investigated the behaviour of ASA and magnesium stearate at other relative proportions [8]. By varying the ratio between ASA and magnesium stearate (4:1) a melting peak appears at 117.64 °C (Fig. 1C) and a similar behaviour has been observed when the ratio is changed to 30/1 (120.40 °C) (Fig. 1D). These peaks may be attributed neither to ASA nor to magnesium stearate, but they instead indicate an ASA degradation of difficult interpretation. The ASA main degradation products in the presence of magnesium stearate are salicylic acid, salilsalicyclic acid and acetyl salilsalicyclic acid, as it is known from the literature [9] and it was emphasised in our HPLC analysis.

The Table 1 and the Fig. 1E show the results obtained on a powder mixture of ASA with excipients at concentrations which could be suitable for a pharmaceutical formulation. The curve is complex and it is possible to emphasise:

- both lactose peaks are almost unmodified (149.20 and 212.20 °C);
- the peaks of talc, maize starch and MCC are not detectable;
- a peak at 119.08 °C is observed; this is similar to that one found in the mixtures ASA/magnesium stearate 4:1 and 30:1;
- a not well defined peak at about 95 °C may be attributed to magnesium stearate.

When the DSC analysis is repeated in the absence of magnesium stearate (Fig. 1F) the ASA melting peak (at 132.80 °C) and the two lactose peaks (145.06 and 213.25 °C) are observed. Therefore, the interaction of ASA is due only to magnesium stearate with consequent incompatibility with this excipient.

The accelerated stability tests are designed to increase the rate of chemical degradation or physical change of an active drug by using exaggerated storage conditions.

The HPLC analysis, carried out on mixtures preserved for 2 months, at the different tempera-

Table 2
HPLC data obtained for the mixtures preserved for 2 months at 35, 45, 55 °C

Mixture components	% Theoretic ASA	Initial		2 months at 35 °C		2 months at 45 °C		2 months at 55 °C	
		Found (mg)	Recovery (%)	Found (mg)	% of degradation	Found (mg)	% of degradation	Found (mg)	% of degradation
ASA	100	100.27	100.27	99.56	0.71	99.47	7.12	100	0.27
ASA+Talc	50	49.89	99.96	47.43	4.89	46.32	6.74	45.98	7.80
ASA+Maize Starch	50	46.91	100.3	44.6	4.93	46.91	10.28	45.86	2.24
ASA+Lactose (80 mesh)	50	51.63	107.87	51.63	11.26	46.32	2.98	48.24	6.56
ASA+MCC	50	47.06	103.86	47.06	12.75	45.66	61.68	46.98	0.17
ASA+Magnesium Stearate	50	45.36	90.72	29.45	28.44	15.77	72.03	17.52	57.43
ASA+all excipients	16.6	12.83	89.71	11.51	13.84	3.22	72.03	1,14	90.10
ASA+all excipients without magnesium stearate	20.0	19.1	101.06	19.1	6.49	18.5	0.03	17.9	6.29

tures of 35, 45 and 55 °C have given the results shown in Table 2.

As a matter of fact:

- The ASA, in binary mixture with maize starch, lactose, talc and MCC practically remains at the same initial percentage, showing that no interaction occurs between ASA and excipients; the changes in the obtained values fall in the analytical error.
- The percentage reduction of ASA when magnesium stearate is present, is quite high and it is accompanied by organoleptic modifications. The peak corresponding to salycilic acid derived from ASA hydrolysis, was observed in the HPLC chromatogram.
- A higher ASA degradation appears when all excipients are present; in this case the degradation of ASA is 90.10% at 55 °C, 72.03% at 45 °C and 11.51% at 35 °C.
- In the absence of magnesium stearate the ASA percentage remains almost unchanged in a similar powder mixture.

4. Conclusion

The results obtained in this work with the DSC method are comparable and in good agreement with the results obtained with the accelerated stability method. This method, which utilises the HPLC analysis, requires a long time, but it is able to offer qualitative and quantitative indications on the degradation pathway. The knowledge of the degradation pathway, however, is not so important in the preformulation studies, where it is essential the control of the stability at room temperature.

In our case it was possible to show, with the DSC method, the incompatibility of magnesium stearate with ASA; an unusual peak of difficult interpretation has been found and it is probably imputable to the formation of one eutectic product given by ASA and salicylic acid. The presence of the other excipients does not modify significantly the thermal curve of ASA.

The accelerated stability analysis confirms that the presence of magnesium stearate is responsible

of the degradation of ASA both in the binary mixture at different w/w ratio and in the typical pharmaceutical formulation; where the drug and the excipients are present at realistic concentration. With the other excipients the ASA concentration remains practically unchanged.

With the DSC analysis it is also possible to reveal the effect of the humidity present in the excipient in order to evaluate the future conservation of the pharmaceutical product.

In conclusion the application of the DSC method in studying solid drug substances and in particular their stability and their predictive shelf life appears of great interest in the preformulation studies for the screening of the excipients and confirms that this technique is a powerful tool in pharmaceutical technology for its high sensitivity and the rapid response which allows to identify the incompatibilities in a very short time.

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