

Fast detection of sildenafil in adulterated commercial products using differential scanning calorimetry

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Abstract Adulteration of drugs is a serious issue and can have a great impact on human health. It is mainly done with the intention of boosting the effects of products. For many years, the enforcement officers in Malaysia have had difficulty deciding whether some products should be confiscated as they have not been able to produce fast reliable evidence of adulteration. For that reason, we explored the use of differential scanning calorimetry (DSC) as a potential fast detection method of commercial products marketed in Malaysia that may be adulterated. We confirmed the outcomes qualitatively with high pressure liquid chromatography. DSC was set at a heating rate of $10\text{ }^{\circ}\text{C min}^{-1}$ and within a temperature range of $100\text{--}250\text{ }^{\circ}\text{C}$ with nitrogen as a purge gas at a flow rate of 20 mL min^{-1} to analyse a sildenafil reference standard (RS), sildenafil tablets and sildenafil adulterated commercial products. Four sildenafil adulterated commercial products were analysed. Since the melting point of sildenafil in the mixture tended to shift, the presence of sildenafil in three of these adulterated commercial products was confirmed using the spiking method and was re-analysed using DSC. The re-analysed results indicated that the enthalpy of fusion (ΔH) and the resolution peak of sildenafil increased accordingly depending upon the amount of spiked sildenafil RS. Apart from these results, the DSC curves also showed different patterns for sildenafil RS and the mixtures. Therefore, we concluded that DSC can potentially be used to detect sildenafil in adulterated commercial products.

KeyWords Sildenafil · Adulteration · Differential scanning calorimetry (DSC) · Thermal analysis

Introduction

Currently, there are many commercial products such as herbal preparations, health foods and dietary supplements available in the market. Most of these preparations seem to be popular among people who believe that products sourced from nature may give better health outcomes without any serious side effects as compared to modern pharmaceutical preparations [1]. They also believe dietary supplements are necessary as an additional element required by the body to enhance body functions and health.

Unfortunately, these were only assumptions that natural products are safe. A number of studies done proved that these herbal preparations, health foods and dietary supplements were adulterated by other active pharmaceutical ingredients and synthetic drugs such as anti-obesity and sex stimulant agent [2–4].

Adulteration of drugs is an illegal practice, since it may be dangerous for the health of consumers as the ingredients and the dosage of drugs are unknown. It may exacerbate conditions in people who already have an existing illness and also may cause drug–drug interactions in people who are taking pharmaceutical drugs concurrently. The Malaysian laws on poisons and sale of drugs [5] are gazetted to convict peoples who offend these laws to protect the Malaysian society from becoming victims of irresponsible persons.

Most of the traditional medicines and dietary supplements available in the Malaysia market are imported from other countries either by legal or illegal channel. Some of them are being manufactured locally. Enforcement officers can raid any premises that are suspected of selling or manufacturing adulterated products and seize any suspected products found there. At the custom point of entry, the enforcement officers need to approve and ensure that all imported drugs comply

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with the Malaysian Drug Control Authority requirement before being released to the market. One of the requirements is that any unregistered or registered traditional or dietary supplement products which consist of any undeclared poisons or substances that may be harmful to consumers should be seized. Under section 4, regulation 3 of Sales of Drug Act 1952, it clearly stated that the owner of premises, consignor, consignee or agent of the owner of products seized may redeem their product after 48 h if it has been confirmed that seized products are free from adulteration.

Through our literature search, we found studies using differential scanning calorimetry (DSC) to detect drug in pharmaceutical products [6] as well as to analyse the stability of active ingredients in formulations [7, 8]. DSC method is also used to detect adulteration in foods materials such as honey to ensure its authenticity [9]. The thermal behaviour of pure honey and pure syrups were determined and any differences of the thermal peak due to adulteration was detected and studied. In addition, DSC technique had also been used in other adulteration studies such as detection of adulteration in olive and virgin coconut oils [10, 11] and also detection of water adulteration in butter [12]. However, we could not find any article on the use of DSC in adulteration of pharmaceutical products. In this study, DSC will be used as a fast method of detecting adulteration of sildenafil in commercial product samples so that, decisions on confiscation of adulterated commercial product can be made within 48 h. A qualitative high pressure liquid chromatography (HPLC) analysis will also be carried out on the same samples for comparison.

Experimental

Materials

Reference standard of sildenafil citrate (Sildenafil RS) was purchased from Sigma Aldrich, USA. Lactose monohydrate was purchased from Aplab Saintifik, Malaysia whilst commercial products that were suspected of adulteration were obtained through the Malaysian National Pharmaceutical Control Bureau (NPCB).

A coding system was used to identify and recognise samples for analysis. All samples were coded as in Table 1.

Methods

DSC analysis

Perkin Elmer DSC 6000 (USA) used in this study was calibrated with zinc and indium. Samples were weighed using microbalance (Mettler Toledo, Switzerland) in the range of 0.5–1.0 mg and 40- μ L standard aluminium pans

Table 1 Description of samples for sildenafil analysis

Code	Type of sample	Description	Label claims	Dosage form
A1	Sildenafil RS	A white crystalline powder	–	Powder
A2	Sildenafil tablet	A film-coated tablet in blue rounded-diamond shape	–	Tablet
A3a	Adulterated commercial product A	A creamy crystalline powder	As a health drinks that contains herbs such as ginseng, goji and soya bean	Sachet
A3b	Adulterated commercial product B	A light brown powder enclosed in a hard capsule with brown cap and blue body	For sexual performance improvement and product contains herbs such as Habbatus-sauda and Tongkat Ali	Capsule
A3c	Adulterated commercial product C	A white powder enclosed in a pearly hard capsule and imprinted with Chinese word in red colour	For sexual performance improvement and product contains 100 % Chinese natural herbs such as ginseng and flowers	Capsule
A3d	Adulterated commercial product D	A brown powder enclosed in a clear hard capsule	–	Capsule

with lids were used. The DSC was programmed through the Pyris software (Perkin Elmer, USA) to heat the samples from 100 to 250 °C at a heating rate of 10 °C with nitrogen as a purge gas at a flow rate of 20 mL min⁻¹.

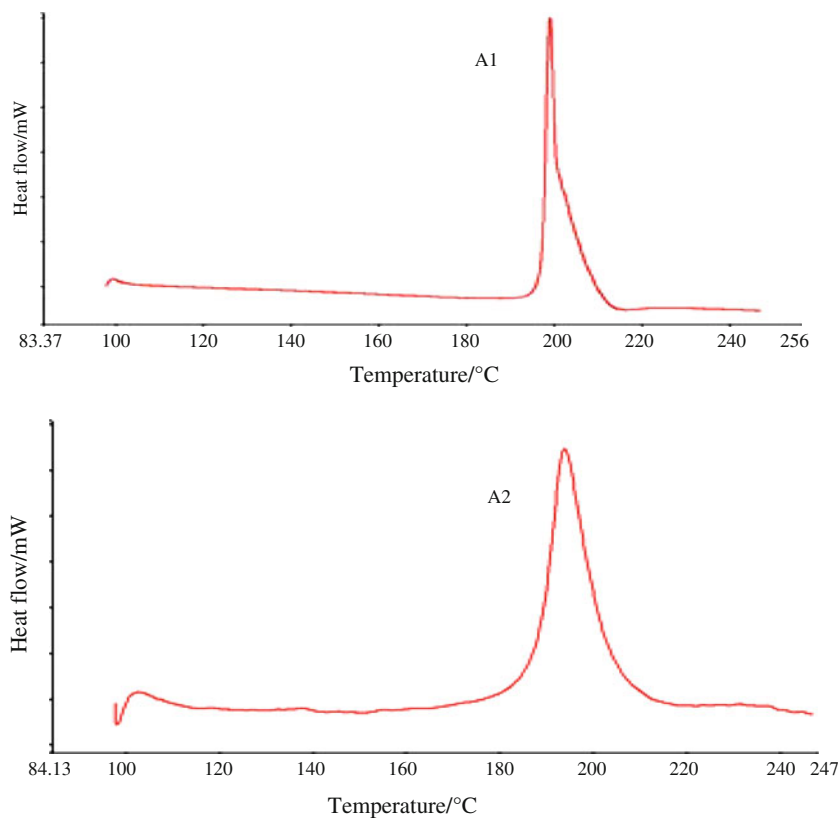
Adulterated commercial products were also used as a base and were spiked with about 30 and 50 % w/w sildenafil RS to confirm the presence of sildenafil in the mixture.

HPLC analysis

A qualitative HPLC analysis was performed using the NPCB in-house method for screening of sildenafil. A rapid resolution liquid chromatography (RRLC) 1200 series (Agilent, USA) with a photo-diode array detector (Agilent, USA) and an Agilent reversed-phase column of Zorbax

Table 2 Thermal characteristics of sildenafil RS and sildenafil tablets ($n = 5$)

Sample	Melting onset, $T_m/^\circ\text{C}$	Peak of melting, $H/^\circ\text{C}$	End of melting, $T/^\circ\text{C}$	Enthalpy of fusion, $\Delta H/\text{g}^{-1}$
A1	197.02 ± 0.16	198.83 ± 0.16	201.09 ± 0.12	339.66 ± 11.31
A2	188.53 ± 0.45	193.67 ± 0.44	201.68 ± 1.04	72.88 ± 10.09

Fig. 1 DSC curve comparison of sildenafil RS (A1) and sildenafil tablet (A2)

Eclipse XDB-C8 with dimensions of 4.6 mm \times 150 mm and a particle size of 5 μm for separation were involved in this experiment.

The mobile phase was prepared using acetonitrile HPLC grade (Fisher Scientific) and deionised water (1:1) to which 1 mL of trifluoroacetic acid HPLC grade (Fisher Scientific) was added. The chromatographic condition was designed with a flow rate of 0.8 mL min^{-1} , back pressure of 54 bar and the wavelength of photo-diode array detector was 254 nm at a temperature of 25 $^\circ\text{C}$. Data analysis was done using Agilent Lab Adviser software.

Standard preparation Sildenafil RS (Sigma Aldrich, USA) was diluted with the diluents to a concentration of 0.03 mg mL^{-1} and was filtered through a membrane filter with a pore size of 0.45 μm into a vial for HPLC analysis.

Sample preparation All the adulterated commercial products were extracted using the liquid–liquid extraction method (LLE) to remove any debris that may be present in the sample mixtures.

Samples of about 1.0 g were weighed using a balance (Precisa, Switzerland) and were placed into individual beakers. About 20 mL of deionised water was poured into the beakers, and the mixtures were sonicated for 15 min. The pH of the sample solutions were adjusted to pH > 11 using ammonia.

LLE using a chloroform solution was performed twice on the sample solutions. The extracts were filtered through filter papers grade 480 (Sartorius Stedim, USA) and were collected in individual evaporating dishes. The extracts were allowed to evaporate until dry at ambient temperature. Each dried samples was diluted in about 5 mL of diluents and filtered using a membrane filter with a pore size 0.45 μm before HPLC analysis.

Results and discussion

The thermal characteristic of the sildenafil RS (sample A1) obtained was found to be almost the same as in a previous study [13] with a melting onset (T_m) of 197.02 ± 0.16 $^\circ\text{C}$,

melting peak (H) of 198.83 ± 0.16 °C, end peak (T) of 201.09 ± 0.12 °C and enthalpy of fusion (ΔH) of 339.66 ± 11.31 J g⁻¹. Five different batches of sildenafil tablets (sample A2) were analysed using DSC to study the thermal characteristics of sildenafil in mixtures. The results precisely showed that sildenafil in the tablet started to melt at a temperature of 188.53 ± 0.45 °C and this concurred with other published literatures [14, 15]. According to Haines and Wilburn [16], the melting point of the substance in mixture should show the same thermal characteristic as the pure substance if there is no physical or chemical interaction, otherwise it may shift forward or backward from the original pure substance's melting point. This study showed that the sildenafil in the tablets had a lower T_m (about 9 °C) and H (about 5 °C) compared with the sildenafil RS while the T was no different (Table 2). These two samples of sildenafil RS and sildenafil in tablet form consumed different amounts of energy to melt as we can see from the difference in the results of ΔH and the DSC curve patterns of both (Fig. 1). These differences are

due to the excipient content of the tablet as it is not 100 % sildenafil.

To prove the H of sildenafil can shift at different concentrations, lactose was used as a base and was spiked with sildenafil RS between a range of 5–95 % w/w. The DSC curve of the pure lactose monohydrate consisted of two different endothermic peaks at 146–152 and 212–223 °C, respectively [17]. The first peak was the dehydration peak while the second peak was the melting peak of lactose which was followed by thermal decomposition [18]. As the result showed the T_m of sildenafil shifted from 196.98 °C (100 % w/w sildenafil RS) to 204.43 °C (5 % w/w sildenafil RS), this demonstrated that the T_m of sildenafil was influenced by increasing the amount of lactose (Table 3; Fig. 2a, b).

Table 4 summarised the thermal characteristics of four different adulterated samples. The adulterated commercial product A3a-1 showed that the T_m , H and the T shifted backward (about 14, 13 and 12 °C, consecutively) and consumed less energy to melt when compared to the

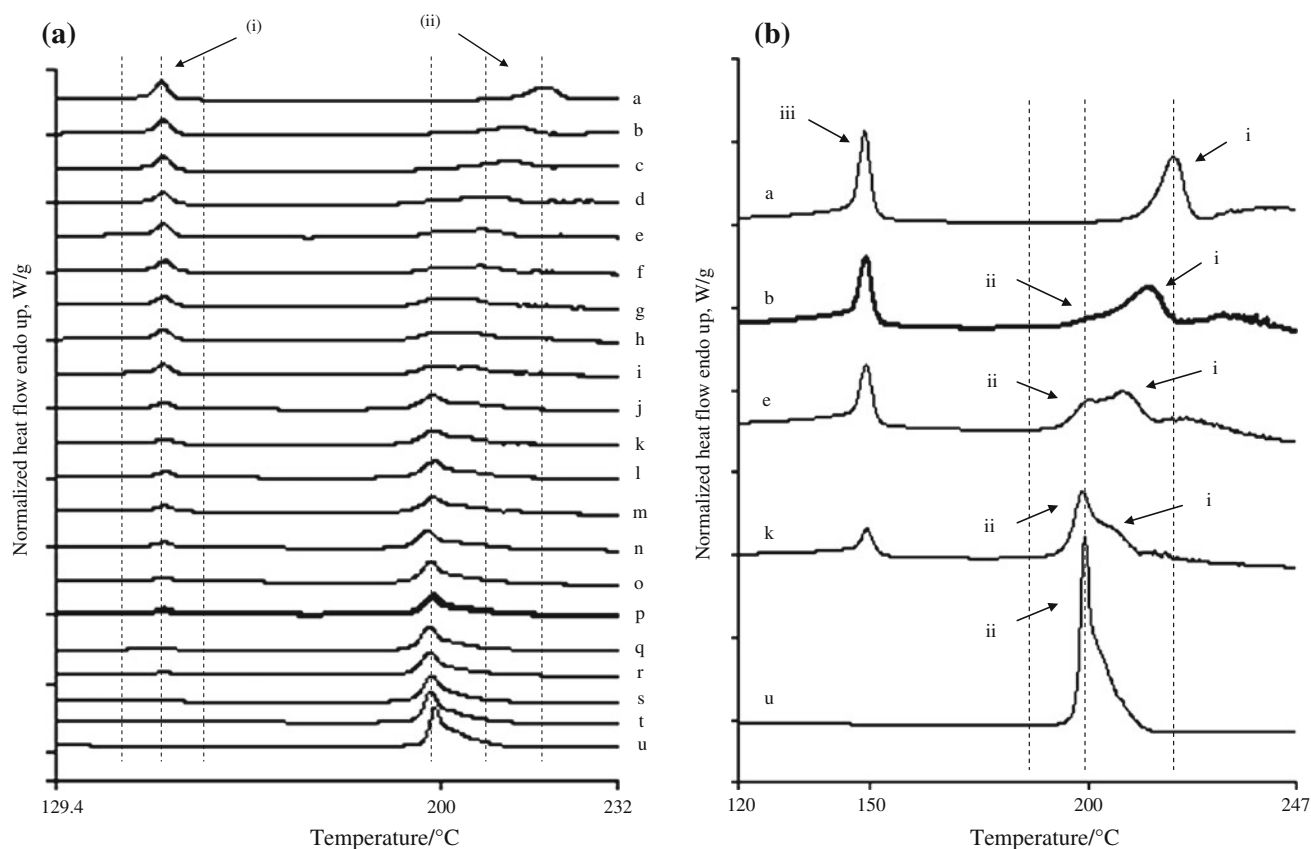


Fig. 2 a Representative curves of different percentage of pure sildenafil (0–100 %) in lactose. **b** Comparison of sildenafil and lactose peaks in different % w/w of sildenafil–lactose mixture. i, Dehydration peak of lactose; ii, melting peak of lactose; a, pure lactose; b, 5 % w/w of sildenafil; c, 10 % w/w of sildenafil; d, 15 % w/w of sildenafil; e, 20 % w/w of sildenafil; f, 25 % w/w of sildenafil; g, 30 % w/w of sildenafil; h, 35 % w/w of sildenafil; i, 40 % w/w of

sildenafil; j, 45 % w/w of sildenafil; k, 50 % w/w of sildenafil; l, 55 % w/w of sildenafil; m, 60 % w/w of sildenafil; n, 65 % w/w of sildenafil; o, 70 % w/w of sildenafil; p, 75 % w/w of sildenafil; q, 80 % w/w of sildenafil; r, 85 % w/w of sildenafil; s, 90 % w/w of sildenafil; t, 95 % w/w of sildenafil; u, pure sildenafil; i, peak of lactose; ii, peak of sildenafil and iii, lactose dehydration peak

Table 3 Thermal characteristics of different percentage of sildenafil RS (0 to 100 % w/w) in lactose

% w/w of Sildenafil	First peak				Second peak			
	Melting onset, $T_m/^\circ\text{C}$	Peak of melting, $H/^\circ\text{C}$	End of melting, $T/^\circ\text{C}$	Enthalpy of fusion, $\Delta H/\text{J g}^{-1}$	Melting onset, $T_m/^\circ\text{C}$	Peak of melting, $H/^\circ\text{C}$	End of melting, $T/^\circ\text{C}$	Enthalpy of fusion, $\Delta H/\text{J g}^{-1}$
0	146.15 ± 0.24	149.46 ± 0.27	152.50 ± 0.39	149.19 ± 7.90	211.91 ± 0.64	218.28 ± 0.42	222.82 ± 0.15	136.61 ± 8.74
5	146.23 ± 0.13	149.40 ± 0.15	151.90 ± 0.19	138.79 ± 9.64	204.43 ± 1.48	213.62 ± 0.32	218.85 ± 0.22	138.64 ± 10.67
10	146.31 ± 0.05	149.45 ± 0.09	152.07 ± 0.14	126.81 ± 12.20	199.76 ± 0.62	212.10 ± 1.14	217.64 ± 0.43	139.03 ± 6.77
20	146.31 ± 0.12	149.28 ± 0.08	151.98 ± 0.11	113.56 ± 9.68	193.68 ± 0.31	208.67 ± 0.83	214.25 ± 0.58	156.33 ± 4.83
30	146.38 ± 0.13	149.41 ± 0.09	152.10 ± 0.07	96.99 ± 7.68	192.75 ± 0.49	199.09 ± 0.16	213.196 ± 4.50	161.05 ± 5.75
40	146.44 ± 0.14	149.48 ± 0.10	152.17 ± 0.08	84.70 ± 8.96	192.74 ± 0.53	198.55 ± 0.22	209.11 ± 2.75	180.12 ± 14.24
50	146.50 ± 0.19	149.45 ± 0.09	152.14 ± 0.09	68.07 ± 1.47	192.82 ± 0.23	198.20 ± 0.24	203.85 ± 0.42	251.04 ± 12.54
60	146.61 ± 0.48	149.58 ± 0.13	152.28 ± 0.14	35.63 ± 2.14	193.89 ± 0.67	198.39 ± 0.42	203.31 ± 0.47	281.84 ± 17.32
70	146.81 ± 0.14	149.59 ± 0.11	152.23 ± 0.10	26.23 ± 2.28	192.95 ± 0.40	197.38 ± 0.19	201.73 ± 0.37	301.10 ± 8.56
80	146.85 ± 0.10	149.52 ± 0.11	152.14 ± 0.08	16.38 ± 5.77	194.30 ± 0.43	197.39 ± 0.35	201.34 ± 0.31	318.58 ± 24.50
90	147.11 ± 0.46	149.67 ± 0.1	152.21 ± 0.11	5.65 ± 2.63	194.24 ± 0.38	197.51 ± 0.24	201.17 ± 0.10	336.59 ± 15.93
95	146.99 ± 0.12	149.59 ± 0.12	152.15 ± 0.14	4.61 ± 1.07	195.65 ± 0.32	198.25 ± 0.15	201.46 ± 0.06	340.25 ± 4.23
100	—	—	—	—	196.98 ± 0.09	198.84 ± 0.18	201.21 ± 0.33	345.58 ± 12.67

Representative curves are as depicted in Fig. 2a. Each value was expressed as Mean ($n = 5$) ± SD

sildenafil in tablet form. The thermal characteristic of the adulterated commercial product A3b-1 was found to be similar when compared with the sildenafil in tablet form with a T_m of 188.37 ± 0.55 °C, H of 194.82 ± 0.12 °C and T of 200.73 ± 0.29 °C. The pattern of the DSC curve for A3b-1 was almost the same with the curve of sildenafil in tablet and the value of ΔH was only varied about 30 J g^{-1} . For sample A3c-1, the thermal characteristic shifted backward only about 2 °C in the T_m , H and T while ΔH varied about 40 J g^{-1} when compared to the sildenafil RS as opposed to the sildenafil in tablet form. For adulterated commercial product A3d-1, the DSC curve showed no peak around the thermal characteristic of sildenafil RS and sildenafil in tablet (Fig. 3).

As the shifting of the thermal peak varied among the adulterated commercial products, the spiking method was used to confirm that a particular thermal peak belonged to sildenafil. Adulterated commercial products were used as a base and were spiked with 30 and 50 % w/w sildenafil RS to observe any shifting in the thermal peaks.

From the analysis, the thermal characteristics of spiked samples A3a-2 and A3a-3 shifted slightly forward approaching the thermal characteristic of sildenafil in tablet form. The energy required to melt increased consistently with the incremental amount of sildenafil in the base (Fig. 4a).

The thermal characteristic of spiked samples A3b-2 and A3b-3 were found to be similar to the sildenafil in tablet form but the peak resolution increased consistently with the spiking amount of sildenafil RS when compared to the pure adulterated commercial product A3b-1. The DSC curves of spiked samples A3b-2 and A3b-3 showed the peak of spiked sildenafil RS merged together with the original peak of the adulterated product with ΔH value of 134.86 ± 8.71 and $212.64 \pm 8.82 \text{ J g}^{-1}$, consecutively (Fig. 4b).

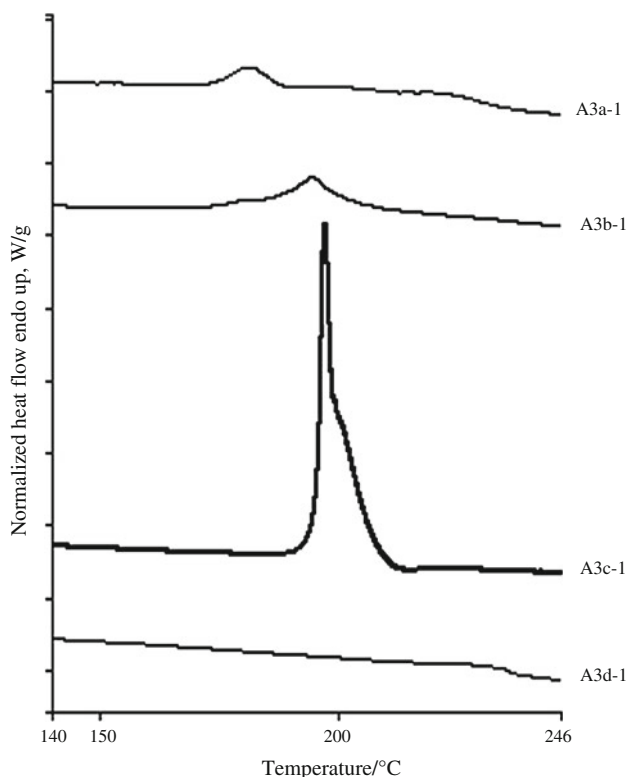
The DSC outcomes showed that the thermal characteristics of spiked samples A3c-2 and A3c-3 were identical to the adulterated sample A3c-1. Its T_m , H and T only differed about less than 2 °C and ΔH varied about 18 and 6 J g^{-1} for the spiked samples A3c-2 and A3c-3, respectively, when compared to the sildenafil RS (Fig. 4c).

For the adulterated commercial product A3d-1, no melting peak was observed in the DSC curve. Surprisingly, the H which is similar to the peak of sildenafil in tablet form emerged in the curves of spiked samples A3d-2 and A3d-3. The results showed that both spiked samples were shifted backward about 6 and 4 °C for T_m , shifted forward about less than 1 °C for H , and shifted forward about 3 °C for T when compared to the thermal characteristics of sildenafil in tablet form, while the ΔH value differed by about 55.44 and 96.99 J g^{-1} , respectively (Fig. 4d).

A qualitative analysis using the HPLC method was applied to all the adulterated commercial products to confirm the presence of sildenafil in these samples and to verify the

Table 4 Thermal characteristics of adulterated commercial product spiked with sildenafil RS

Adulterated product	Melting onset, $T_m/^\circ\text{C}$	Peak of melting, $H/^\circ\text{C}$	End of melting, $T/^\circ\text{C}$	Enthalpy of fusion, $\Delta H/\text{J g}^{-1}$
A3a-1	174.94 \pm 0.87	180.92 \pm 0.85	189.26 \pm 0.65	14.34 \pm 3.74
A3a-2	188.26 \pm 1.75	196.17 \pm 1.82	205.31 \pm 1.52	91.46 \pm 14.89
A3a-3	184.89 \pm 1.37	195.64 \pm 1.29	204.63 \pm 2.67	134.28 \pm 18.24
A3b-1	188.37 \pm 0.55	194.82 \pm 0.12	200.73 \pm 0.29	41.31 \pm 6.02
A3b-2	188.81 \pm 0.24	195.91 \pm 0.37	204.65 \pm 0.22	134.86 \pm 8.71
A3b-3	190.27 \pm 0.40	196.28 \pm 0.43	204.59 \pm 0.49	212.64 \pm 8.82
A3c-1	195.41 \pm 0.28	197.29 \pm 0.32	199.59 \pm 0.41	299.83 \pm 9.16
A3c-2	195.43 \pm 0.16	197.51 \pm 0.10	200.37 \pm 0.07	321.48 \pm 10.42
A3c-3	195.59 \pm 0.14	197.69 \pm 0.11	200.63 \pm 0.23	345.69 \pm 8.02
A3d-1	0	0	0	0
A3d-2	182.89 \pm 0.47	194.43 \pm 0.69	205.14 \pm 0.41	128.32 \pm 7.99
A3d-3	184.46 \pm 0.25	193.54 \pm 0.85	204.93 \pm 0.50	169.87 \pm 3.83

**Fig. 3** Representative curves of adulterated commercial products**Table 5** Peak areas of adulterated commercial products

Adulterated product	Peak area
Adulterated commercial product A	2871.77409 \pm 2.73242
Adulterated commercial product B	3207.45851 \pm 6.62770
Adulterated commercial product C	3900.55274 \pm 5.95988
Adulterated commercial product D	995.73925 \pm 0.62730

Representative chromatograms are as depicted in Fig. 5. Each value was expressed as Mean ($n = 3$) \pm SD

DSC outcomes as well (Table 5). Figure 5a showed that two peaks emerged in the chromatogram. Using a photo-diode array detector the first peak at retention time (RT) 2.076 min was confirmed as a sildenafil peak from its UV spectrum. The second peak shown in chromatogram was an unknown peak, and therefore sample A3a was suspected to contain a mixture of sildenafil and other compounds. This was because the DSC curves of the spiked samples A3a-2 and A3a-3 showed more than one peak merged together and were not well separated. The HPLC outcomes proved that sildenafil was present in samples A3b and A3c as the UV spectrum of the sildenafil RS exactly overlapped on the UV spectrum of samples A3b and A3c.

The chromatogram in Fig. 5d showed sildenafil presence in the sample A3d at RT 2 min. The RT of second peak emerged was identical to the RT peak of tadalafil and not sildenafil. This result was consistent with the DSC outcome, which was no peak appeared in the thermal curve around the T_m of sildenafil RS and sildenafil in tablet form.

The peak of sildenafil can be detected at amount of sildenafil more than 20 % w/w presence in the mixture. From the results obtained, it was found that DSC can detect sildenafil in sample A3a, A3b and A3c, but not in the sample A3d since the amount of sildenafil was too small as compared to other component in the sample. However, using the HPLC method, a small peak of sildenafil can be detected at RT 2 min in the sample A3d.

Detection of adulterants in samples which contain many excipients in dosage form such as sachet will be very challenging. This is clearly depicted in the curve of sample A3a. The melting point was shifted about 14 °C of the melting point of sildenafil in tablet form and this is may be due to the eutectic effect of the excipient.

It was also found that the HPLC method only showed us what type of analyte is present in the sample but did not

Fig. 4 Representative curves of **a** adulterated commercial product A, **b** adulterated commercial product B, **c** adulterated commercial product C and **d** adulterated commercial product D. 1, Adulterated commercial product; 2, adulterated commercial product spiked with 30 % w/w of sildenafil RS and 3, adulterated commercial product spiked with 50 % w/w of sildenafil RS

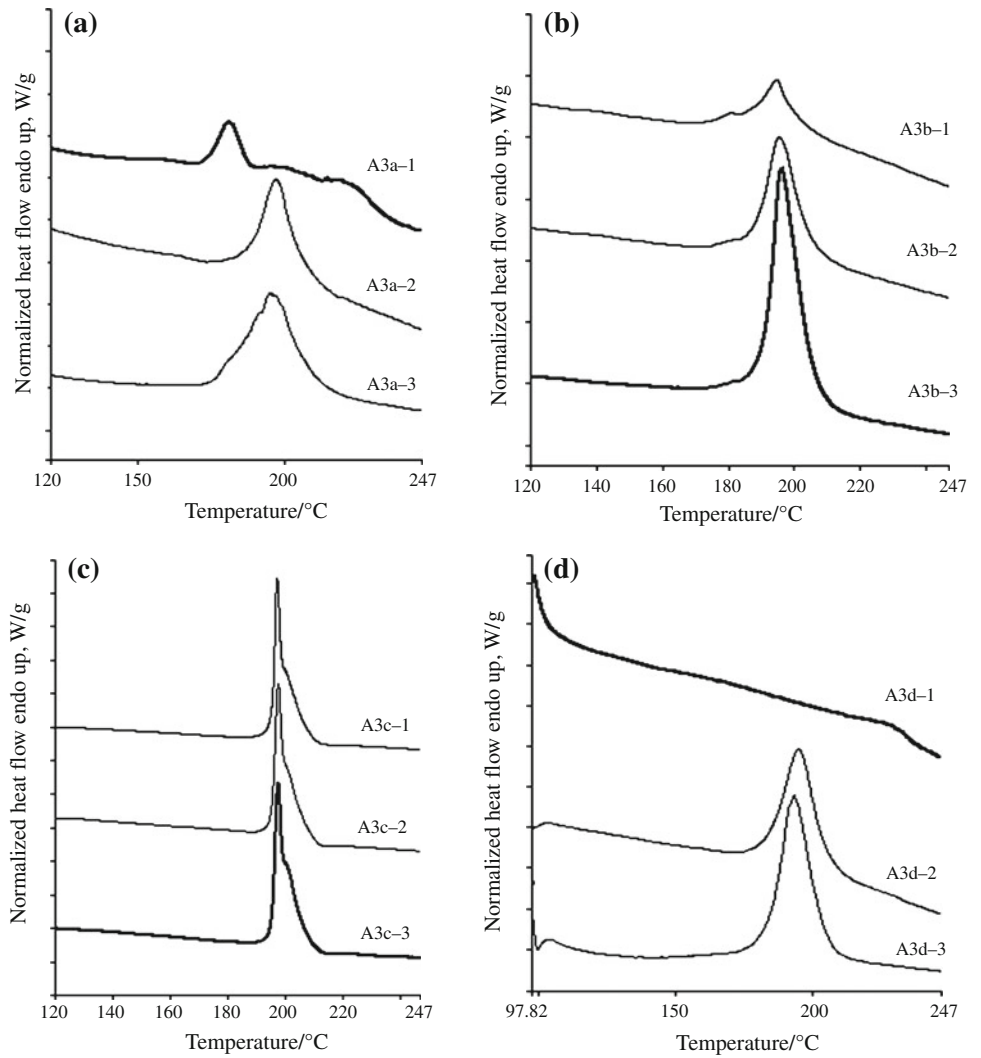
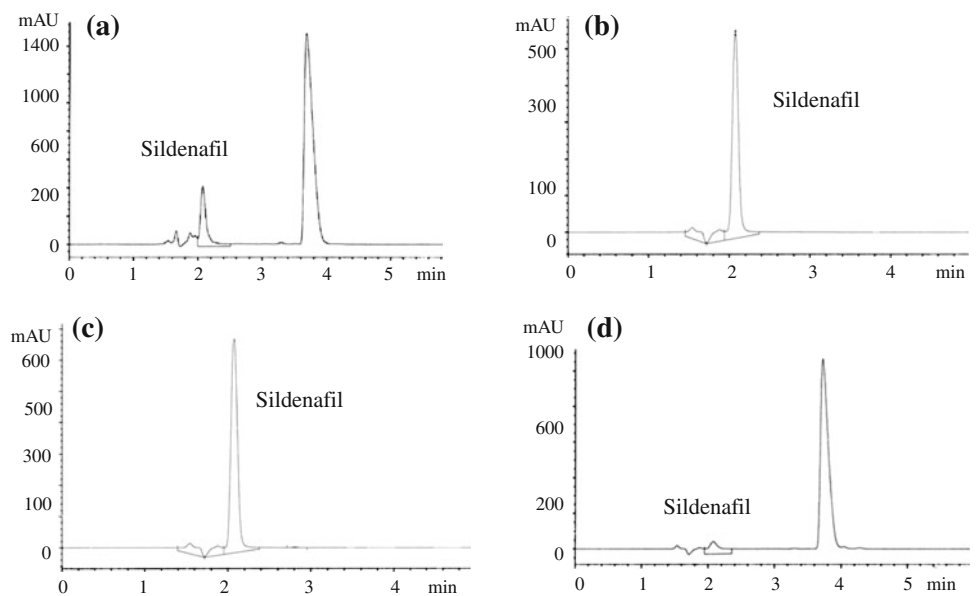


Fig. 5 Chromatogram of **a** adulterated commercial product A3a-1, **b** adulterated commercial product A3b-1, **c** adulterated commercial product A3c-1 and **d** adulterated commercial product A3d-1



show whether the analyte presence was in a pure or mixture form, whereas DSC could differentiate the analyte presence and show whether it is in a pure or mixture form. From observation on the pattern of the DSC curve for samples A3b and A3c, we can conclude that the sildenafil mixture present in the sample A3b may be similar to the mixture of sildenafil in the tablet form; while sample A3c-1 was suspected to contain pure sildenafil instead of a mixture since its DSC curve pattern was identical to the pure sildenafil.

Conclusions

The study shows that DSC detected sildenafil as an adulterant in commercial products. Sildenafil has a different shifting behaviour in different samples but the thermal curves of DSC are still reproducible for the sildenafil in pure and tablet form. The DSC curve itself shows the difference in patterns for sildenafil in pure form and mixture.

Therefore, it can be concluded that DSC can be used as a potential tool for fast detection of sildenafil in commercial products.

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