The influence of sample preparation and the presence of other Active Pharmaceutical Ingredients (API) and excipients on the use of DSC method in quantification of paracetamol in commercially available tablets

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Abstract

The direct Differential Scanning Calorimetry method of the determination of paracetamol in commercially available drugs was developed. The method was based on calibration curves obtained from melting enthalpies ΔH of binary mixtures of paracetamol and commonly used excipients such as starch or microcrystalline cellulose in increasing weight ratios. In order to demonstrate how the technological processes of formulating the tablets affects the quantitative studies, the micronized and nonmicronized mixtures were used. The idea of using micronized mixtures was to simulate these technological processes. The appropriate paracetamol contents of the selected pharmaceutical preparations were calculated and compared. The final results demonstrated, that the contents of paracetamol obtained from micronized samples were much closer to those declared by the manufacturer than the nonmicronized. Excluding two drugs, the influence of starch or cellulose on quantification in the micronized group was not observed whereas in the nonmicronized group it was distinctly visible.

Keywords: Differential Scanning Calorimetry, DSC, paracetamol, acetaminophen, ibuprofen

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1. Introduction

The increasing use of paracetamol (PAR) as an effective and readily available analgesic makes its quantification as an important area of interest for the pharmaceutical industry. The review of the literature indicates many analytical techniques of its determination, both in pharmaceutical formulation and biological fluids, among the others, titrimetric method [1], spectrophotometric [2], electroanalytical [3-5], MS mass spectrometry [6], chromatographic LC [2, 7-14] and TLC [15], and differential scanning calorimetry DSC [16-18]. It is worth noting that DSC method is the only one performed in solid state. Some of these methods, however, are not very convenient for the pharmaceutical industry. For industrial quality control purposes, where large number of samples are analyzed, extended analysis, complex procedures, the use of expensive chemicals and the need for pretreatment of the sample, are limiting factors.

The Differential Scanning Calorimetry is a simple method, that does not require any prior sample preparations and relatively fast (usually each analysis takes at a heating rate 10°C/min approximately 20-30 minutes). That is why it is suggested that DSC method could be a useful tool in industrial quality control laboratories, not only for direct monitoring of drug manufacturing but also for quantification of current and final contents of the PAR in the presence of other APIs and/or declared excipients.

The quantitative DSC method is using a relationship between the signal value of analyte (enthalpy change ΔH) correlated with its concentration in the matrix. For this purpose binary mixtures of PAR, with commonly used excipients as Starch 1500 and Vivapur 12 were prepared. Tablet is the final product of several technological processes, namely grinding, granulating, compressing and others, that may cause several mutual interactions between drug and excipients [19-21] which affect the measured values of enthalpy. Described phenomenon concerning PAR and microcrystalline cellulose, both physical (nonmicronized) and grinded mixtures was

 Article submitted: 30.06.2016 Accepted: 20.10.2016

previously reported [22]. To demonstrate how the technological processes of formulating the tablets affects the quantitative studies, the micronized and nonmicronized mixtures were used in this study.

The ΔH enthalpies as a function of increasing concentrations of PAR were used to construct the appropriate calibration curves which were further applied to calculate the final content of PAR in the selected commercially available tablets. The tablets were different in terms of weight, type of excipient and composition. The obtained results were compared with those declared by the manufacturer. The influence of technological processes of formulating the tablets on quantitative studies is discussed.

The DSC method was validated according to ICH QR1 guidelines [23].

2. Experimental

2.1. Materials

Paracetamol powder – pure polymorphic form I with estimated melting temperature at T_{onset}=169.4°C (Lot: 6135999 B163) was supplied by POL.NIL. Warsaw Poland. The microcrystalline cellulose Vivapur 12 was obtained from J. Rettenmaier & Sohne, Weissenborn Germany (Lot: 5601290308) and the corn starch Starch 1500 was from Colcorcon Ltd. UK (Lot: 500075).

Paracetamol is a low potency, high dose drug. Its typical dose in one and poly pharmaceutical tablets is 500 mg, however, other concentration are also possible (as 325 mg in "Metafen").

Following poly pharmaceutical tablets were used: "Metafen" (325 mg of PAR and 200 mg of ibuprofen (IBU)) manufactured by Polpharma, Poland (LOT 016582), the mean weight of tablet 699.20 mg, composed of povidone, pregelatinized starch, microcrystalline cellulose, magnesium stearate; "Nurofen Ultima" (500 mg of PAR and 200 mg of IBU) manufactured by Reckitt Benckiser, Poland (LOT AB070), the mean weight of tablet 870.38 mg, composed of croscarmellose sodium, microcrystalline cellulose, anhydrous colloidal silica, magnesium stearate, stearic acid; "Panadol Femina" (500 mg of PAR and 10 mg of hyoscini butylbromidum) manufactured by GlaxoSmithKline, Poland (LOT TA3166), the mean weight of tablet 733.82 mg, composed of microcrystalline cellulose, starch, povidone K-30, sorbitol, talc, magnesium stearate, colloidal silica, Carboxymethyl cellulose sodium, hypromellose (HPMC), macrogol 6000; "Panadol Extra" (500 mg of PAR and 65 mg of coffeinum) manufactured by GlaxoSmithKline, Poland (LOT 100798), the mean weight of tablet 685.10 mg, composed of starch, povidone (K 25), potassium sorbate, talc, stearic acid, croscarmellose sodium; Dafalgan Codeine (500 mg of PAR and 30 mg codeini phosphas) manufactured by UPSA, France (LOT F3562), the mean weight of tablet 703.78 mg, no information on the composition were included.

2.2. Mixtures and tablet samples preparation

The set of PAR mixtures with starch or microcrystalline cellulose (1000 mg each) at concentrations of PAR from 30% to 90% (corresponding to 1.47-4.41 mg of PAR in the DSC sample) were prepared separately, gently homogenised and divided. One part (500 mg) was ready for further experiments; the other was micronized in an agate mortar and pestle with some drops of methanol for 10 minutes.

Twenty tablets of each medical product under the study were individually weighed and grounded in an agate mortar and pestle into fine powder.

The samples of mixtures or tablets of about 4.9 mg were accurately weighed in aluminium pans and sealed.

2.3. Method

The principle of the method is a relationship between the signal value of PAR (enthalpy change ΔH) and its concentration in the matrix. For this purpose both of starch or microcrystalline cellulose, micronized and nonmicronized sets of mixtures were measured by means of DSC. In this way four calibration curves with an increasing amounts of PAR were plotted. Appropriate calibration curves were further applied to calculate the final experimental contents of PAR in the tablets and were compared with those declared by the manufacturer.

2.4. Validation of DSC method

The method was validated in accordance with internationally accepted criteria [23]. The parameters evaluated were specificity, linearity, precision, limit of detection (LOD), and limit of quantification as well (LOQ) [23]. The calculations were made using statistical program STATISTICA v.10.

Specificity of the method was assessed by comparing the DSC heating traces of raw PAR,

Table 1. Effects of mixture composition on the area of melting peak ΔH (averaged from three determinations) and onset T_{onset} and maximum T_{max} temperatures

composition of the mixture	content of PAR in the sample [mg]	micronized mixtures			nonmicronized mixtures			ΔH differences between nonmicronized and micronized mixtures [mJ/mg]
		ΔH±SD of PAR* [mJ/mg]	T_{onset}±SD [°C]	T_{max}±SD [°C]	ΔH±SD of PAR* [mJ/mg]	T_{onset}±SD [°C]	T_{max}±SD [°C]	
PAR/cellulose	1.47	156.31±0.81	167.97±0.76	170.23±0.96	260.68±1.24	168.97±0.22	170.93±0.81	104.37
	1.96	276.36±0.77	168.27±0.12	170.77±0.37	343.65±0.89	169.03±0.31	171.50±0.47	67.29
	2.45	356.48±1.12	168.47±0.83	170.90±0.71	436.92±1.13	168.97±0.70	171.50±0.62	80.44
	2.94	472.36±1.09	168.63±0.35	171.40±0.69	537.04±1.03	169.03±0.66	172.00±0.91	64.68
	3.43	579.67±1.22	168.70±0.43	171.83±0.52	649.41±0.94	168.97±0.48	172.27±0.24	69.74
	3.92	676.20±0.95	168.77±0.42	172.53±0.47	736.96±0.99	169.57±0.44	172.23±0.28	60.76
	4.41	833.49±0.68	169.23±0.86	172.23±0.81	811.44±0.94	169.33±0.91	172.17±0.75	–22.05
PAR/starch	1.47	207.27±0.96	168.60±0.66	170.73±0.88	256.52±1.14	169.10±0.81	171.25±0.59	49.25
	1.96	307.72±0.70	168.70±0.48	171.53±0.72	352.80±1.01	168.95±0.46	171.50±0.64	45.08
	2.45	414.87±0.64	168.70±0.89	171.60±0.77	428.75±0.88	168.90±0.48	171.80±0.84	13.88
	2.94	473.34±1.10	168.73±0.81	171.97±0.72	514.50±0.94	169.25±0.73	171.90±0.54	41.16
	3.43	581.96±0.94	170.10±0.55	172.37±0.65	625.98±1.11	170.20±0.64	172.15±0.81	44.02
	3.92	661.17±1.01	170.43±0.61	172.17±0.52	691.88±1.14	169.73±0.28	172.07±0.76	30.71
	4.41	787.92±0.74	169.73±0.64	172.47±0.49	793.80±0.88	169.70±0.87	172.07±0.49	5.88

* ΔH recalculated to the content of PAR in the sample; SD stands for standard deviation

Table 2. Validation of the DSC method

validation parameters	micronized mixtures		nonmicronized mixtures	
	PAR/cellul.	PAR/starch	PAR/cellul.	PAR/starch
specificity	specific	specific	specific	specific
slope ($a \pm SD_a$)	222.6±7.2	190.7±6.0	193.3±4.2	181.3±3.7
intercept ($b \pm SD_b$)	-175.8±22.3	-70.0±18.5	-28.7±12.9	-9.52±11.4
r	0.9974	0.9976	0.9988	0.9990
r^2	0.9948	0.9951	0.9977	0.9980
LOD [mg]	0.28	0.27	0.18	0.17
LOQ [mg]	0.84	0.81	0.56	0.52
linearity [mg]	0.84-4.41	0.81-4.41	0.56-4.41	0.52-4.41
precision (n=6) %RSD	1.76	1.84	1.85	1.99

$y = ax + b$; SD_a – standard deviation of slope; SD_b – standard deviation of intercept

Table 3. PAR contents of the analgesics drugs under study, based on calibration curves obtained from the different micronized or nonmicronized PAR/cellulose and PAR/starch mixtures; Δ(%) stands for relative error of method, calculated from equation [(experimental content – nominal content)/nominal content]×100

analgesic drug	PAR/tablet declared [mg/tablet]	ΔH* ±SD [mJ/mg]	% of component/tablet			PAR content ±SD [mg/tablet]			
			PAR	second API	excip.	micronized mixtures		nonmicronized mixtures	
						PAR/cellul.; Δ(%)	PAR/starch; Δ(%)	PAR/cellul.; Δ(%)	PAR/starch; Δ(%)
Metafen	325	357.86±0.61	46.48	28.60	24.92	335.25±5.49; 3,2	313.75±6.41; −3.5	279.65±6.32; −14.0	283.37±6.74; −12.8
Nurofen Ultima	500	441.26±0.96	57.45	22.98	19.57	482.55±6.45; −3.5	466.69±7.52; −6.7	423.22±7.42; – 5.4	432.82±7.91; −13.4
Panadol Femina	500	519.04±0.95	68.14	1.36	30.5	467.47±4.08; −6.5	462.58±4.76; −7.5	424.36±4.70; −15.1	436.60±5.01; −12.7
Panadol Extra	500	606.81±0.46	72.98	9.49	17.53	491.56±7.90; −1.7	496.22±9.21; −0.8	459.67±9.09; −8.1	475.31±9.70; −4.9
Dafalgan Codeine	500	533.83±1.01	71.05	4.26	24.69	457.88±6.07; −8.4	454.79±7.08; −9.0	417.98±6.98; −16.4	430.45±7.45; −13.9

* ΔH recalculated to the content of PAR in the sample; SD stands for standard deviation

raw excipients and obtained both micronized and nonmicronized mixtures. The T_{onset}, T_{max} temperatures, and presence of new chemical individuals were taken into account.

The calibration plots were constructed by analysis of seven (n = 7) different mixtures (both micronized and nonmicronized), corresponding to content of PAR ranging from 1.47 mg to 4.41 mg. Determination of linearity was made *via* three replicates and assessed as a relationship between the area of DSC melting peak ΔH and content of PAR in mg per sample.

Linearity was reported as the linear calibration equations (y=ax+b) and the correlation coefficients r and r2.

LOD and LOQ were calculated from the calibration curve slope (a) and the slope standard estimation error (S_e), using formulas LOD=3.3×S_e/a and LOQ= 10×S_e/a.

The repeatability of the method was determined by analysis of six (n = 6) replicates of samples from individual weighing. The study was done for one concentration level of 2.00 mg of PAR in the sample, and the results were expressed as the relative standard deviation (%RSD).

2.5. Thermal analysis

The DSC measurements were performed in nitrogen atmosphere with a flow rate of 50 ml/min using EXSTAR DSC 7020 apparatus (SII NanoTechnology Inc.) calibrated with indium and tin, and equipped with DSC7020 electric cooling unit.

The pans were equilibrated at 30°C for 15 min and afterwards the melting behaviour was analysed at heating rate of 10°C/min. All measurements were performed at least three times and averaged. The tablets were examined at least six times.

3. Results and discussion

Table 1 shows the influence of mixture composition on the area of melting peak ΔH (averaged from three determinations) as well as onset T_{onset} and maximum T_{max} temperatures of the micronized PAR mixtures with microcrystalline cellulose Vivapur 12 and starch Starch 1500 compared to the corresponding nonmicronized mixtures. The observed melting points depressions towards higher temperatures are typical for binary mixtures when the concentration of a analyzed component is increasing. However one can see the differences in the peaks surface areas. For the same content of PAR in the sample, nonmicronized mixtures show higher values of enthalpy than their micronized analogues regardless used excipients. The broadest differences were related to PAR/cellulose mixtures, and range from 104.37 mJ/mg to 60.76 mJ/mg. A similar, but smaller effects recur in PAR/starch mixtures, where the differences were in the range of 49.25 mJ/mg to 5.88 mJ/mg. The exception is PAR/cellulose sample with the highest concentration of PAR (4.41 mg), where the ΔH value is accordingly smaller. There were also differences between PAR/cellulose and PAR/starch mixtures. In the same concentration of PAR in the micronized group, the ΔH values obtained for PAR/starch mixtures were significantly higher than those measured for PAR/cellulose, but only up to 3.43 mg of PAR per sample. Over that concentration the ΔH values were lower. Similar dependence were not found in nonmicronized group where ΔH values measured for PAR/starch were lower in the whole range of PAR content.

In order to plot appropriate calibration curves, the obtained ΔH enthalpies of micronized as well as nonmicronized mixtures as a function of the increasing weights of PAR in a sample were used.

Validation of the developed method proved that it meets the acceptance criteria in the scope of parameters mentioned in the section 2.4. The appropriate data were summarized in Table 2.The validated method described above was successfully applied to quantitative determination of PAR in the commercially available drugs under study. The PAR contents obtained experimentally were reported in Tables 3.

During the analysis of Table 3 one can see that the experimental contents of PAR calculated from micronized mixtures were significantly different than nonmicronized. When 500 mg of PAR were declared by manufacturer, the values obtained for "Nurofen Ultima", "Panadol Femina" and "Panadol Extra" were ranging from 467.47 mg to 491.56 mg and 462.58 mg to 496.22 mg respectively for micronized PAR/cellulose and PAR/starch mixtures. The relative error of method Δ(%) calculated for those analgesics were on the level ranging from −7.5% to −0.8% and were comparable to those obtained in [16] that were ranging from −3.9% to 8.7%. However, it should be emphasized that the method found in that paper was applied to pharmaceutical formulations/mixtures not tablets and there are no other publication (with the exception of one

more paper from the same research team concerning determination of acetylsalicylic acid [24]) using the DSC in quantification of solid materials. The same parameter Δ(%) but calculated using other instrumental methods were showed in Table 4.

It can clearly be seen that the featured Δ(%) values didn't deviate significantly from those obtained in our method.

The estimated PAR content in "Dafalgan Codeine" (457.88 mg and 454.79 mg) basing on micronized mixtures differs from other drugs used in the study. However, Δ(%) equal to −8.4% and −9.0% respectively, still remains in the range of errors calculated for other methods for example amperometric [4,5].

In nonmicronized mixtures of both cellulose or starch, the PAR contents ranged from 417.98 mg to 475.31 mg. The relative errors of method calculated for that group were significantly higher, typically ranging from −12.7% to −16.4%. Although, two exceptions were found for "Panadol Extra" −8.1% and −4.9% both in cellulose and starch mixtures.

"Metafen" was declared by the manufacturer to have 325 mg of PAR per tablet and relative errors observed for its determinations were similar to the ones found for other drugs. The results calculated from micronized mixtures were much closer to nominal values than from their nonmicronized analogues.

To sum up. The concentration of second API, ranging from 1.36% to 28.60% had no effect on determination of PAR in tablets under study. Although classical HPLC [2,11] and UV spectrophotometry [2,5] are the most frequently used methods in quantitative API determinations, the Differential Scanning Calorimetry is also routinely used, i.a., to investigate the mutual interactions between APIs and excipients and direct monitoring of drug polymorphic space on each stage of drug production. Considering the current application of Differential Scanning Calorimetry in the production lines we propose to extend its purpose to determinate the current and/or final contents of the API in the tablet.

4. Conclusions

Differential Scanning Calorimetry is the first line technique indispensable for industrial quality control laboratories and, next to many routine applications, could be used in quantitative assays,

Table 4. The relative errors Δ(%) of methods found in published data

references	instrumental method	nominal content	experimental content	Δ(%)
[2]	HPLC	300	299.6	−0.1
	ratio spectra derivative spectrophotometry	300	299.5	−0.2
[4]	Cyclic voltammetry	650	678	4.3
	Amperometric FIA hydrodynamic voltammetry	650	685	1.0
	Cyclic voltammetry	1000 effer.	1060	6.0
	Amperometric FIA hydrodynamic voltammetry	1000 effer.	955	−9.9
[5]	Flow injection analysis (FIA) with amperometric detection	750	760	1.3
		750	740	−1.3
		750	680	−9.3
		500	470	−6.0
		500	480	−4.0
		750	780	2.6
	UV spectrophotometry	750	750	1.4
		750	700	2.9
		500	450	−4.3
		500	460	−4.2
[11]	porous graphitized carbon column HPLC	500 (LOT 1)	493.8	−1.24
		500 (LOT 2)	495.1	−0.98

however, with several limitations. In the course of the studies it was shown that the use of calibration curves plotted from the ΔH enthalpies of micronized mixtures of PAR and commonly used excipients as starch and cellulose gives better results of estimation of the PAR content than from nonmicronized mixtures. There was no effect of the type or quantity of second API found in the tablet as ibuprofen, hyoscini butylbromidum, coffeinum or codeini phosphas on such quantitative determinations.

Resumo

La rekta Diferenciigante Skana Kalorimetrio metodo de determino de paracetamolo en komerce haveblaj medikamentoj estis evoluigita. La metodo estis bazita sur kalibrigitaj kurboj akiritaj el fandada entalpio ΔH de binaraj miksaĵoj de paracetamolo kaj kutime uzita seka aldonaĵo kiel amelo aŭ mikrokristala celulozo en kreskantaj pezokvocientoj.

Por pruvi, ke la teknologiaj procezoj de formulitaj tablojdoj influas la kvantajn determinadojn, oni uzis la mikronizitan kaj nemikronizitan miksaĵojn. La ideo pri uzado de mikronizitaj miksaĵoj estis imiti tiujn teknologiajn procezojn. La taŭga enhavo de paracetamolo en la elektitaj farmaciaj preparaĵoj estis kalkulita kaj komparita. La finrezultoj montris, ke la enhavo de paracetamolo akiritaj de mikronizitaj specimenoj estis multe pli proksima al tiuj deklaritaj de la fabrikisto kiel la nemikronizitaj. Ekskludante du medikamentojn, la influo de amelo aŭ celulozo sur kvantigado en la mikronizita grupo ne estis observita, kontraŭe al la ne-mikronizita grupo, kie estis klare videbla.

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