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Quantitative Determination of Acetaminophen in Pharmaceutical Formulations Using Differential Scanning Calorimetry. Comparison with Spectrophotometric Method

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In this paper our previous researches dealing with compatibility, thermoanalytical characterization, the kinetics of thermal degradation of acetaminophen, either pure or contained in some commercial pharmaceutical formulations, have found applications outlets. In a previous investigation the possible interactions between acetaminophen and four excipients contained in the commercial pharmaceutical formulations were tested. As a continuation of this research in the present study an analytical method based on differential scanning calorimetry (DSC) was applied to determine the acetaminophen content of four commercial pharmaceutical formulations. For a fifth drug it was shown that the method is not applicable owing to observed incompatibility with one of the excipients. Finally, the analytical results obtained were compared with those derived from two UV spectrophotometric methods (one, i.e., "direct method," recommended by the Pharmacopeia and the other based on the first-order derivative UV spectra).

Keywords acetaminophen; determination; thermal analysis; DSC; UV spectrophotometry

INTRODUCTION

4-hydroxy-acetanilide, often denoted as acetaminophen (Ac), is a non-narcotic analgesic belonging to the class of non-steroidal anti-inflammatory drugs (NSAIDs) first used in medicine in 1893. However, its clinical use goes back to 1949 when it was recognized as the main active metabolite of both acetanilide and phenacetin (4-ethoxy-acetanilide). Up to now it has been used as a substitute for acetanilide as antipyretic and analgesic because of the serious collateral effects of the latter. Having decided to use an analytical method based on differential scanning calorimetry (DSC) (Baider Ceipidor, 1981) to monitor Ac content in several commercial drug specialties, in a recent work (Tomassetti et al., 2005) we carried out an in-depth preliminary study of the compatibility between the active principle and certain excipients.

The investigation was based both on customary qualitative criteria specific to thermal analysis (Fassihi & Persicaner, 1987), and on the quantitative comparison between the values of fusion enthalpy, evaporation enthalpy, and the activation energy of the process of evaporation of pure Ac and the corresponding values obtained for the Ac contained in several commercial pharmaceutical products. Concerning this, in the early investigation it was rigorously determined whether any incompatibility exists between Ac and excipients contained in the drugs tested. In addition, it was exhaustively studied the active principle both according to a physico-chemical point of view and from the standpoint of kinetic behaviour and both when pure and when contained in the drug specialties tested (Tomassetti et al., 2005). Taking into account the obtained results we were encouraged to make the analytical determination of the Ac content of four commercial drugs using a calorimetric method. However, in the past we proposed this method in the field of drug analysis obtaining interesting results (Biader Ceipidor, 1981; Biader Ceipidor et al., 1981). On the other hand, other authors applied the same method for quantitative purposes in diagnostic studies related to the field of cultural heritage (Dei et al., 1998; Lodding & Hammel, 1960). The analytical data thus acquired were validated by means of comparison also with the results obtained using a spectrophotometric method recommended in the Pharmacopeia (Italian Pharmacopeia, 2002). Methodological variants of both methods were also taken into consideration such as the application of first derivative spectrophotometry to avoid any turbidity evidenced in some of the examined pharmaceutical formulations.

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EXPERIMENTAL

Materials and Samples

Acetaminophen (Ac), (CAS:103-90-2, lot S01005-042, purity ≥ 98%) was supplied by Sigma-Aldrich. The composition of the DF1–DF5 dosage forms tested and the percentage by weight of Ac content are given in Table 1.

Instruments

The TG/DSC measurements were carried out on a Stanton-Redcroft 625 simultaneous TG/DSC thermoanalyzer connected to a 386 IBM-compatible personal computer. Instrument calibration was performed using very pure standards. To this end, indium, gallium, lead, tin, naphthalene, and benzoic acid samples were used in the present work as their melting temperatures and enthalpies are well known (Acree, 1991; Hultgren et al., 1973). A 4–6 mg sample was weighed out and placed in an argon filled dry box to avoid oxidation and then in the TG/DSC equipment, where the gases given off were fluxed out by a continuous purge gas stream.

UV absorption spectra were performed on a Perkin-Elmer 320 UV spectrophotometer connected to a Perkin-Elmer 3600 computer Data Station using 1.0 cm quartz cells. Software such as IF-320 and CSD-13 was installed to record both direct and first-order derivative UV absorption spectra and to process experimental data.

Methods

Both for pure Ac and DF1-DF5 dosage forms rising temperature TG/DSC experiments were carried out over a temperature range from room temperature to 673 K. Different heating rates of 2.5, 5, 10, and 20 K min^{-1} were used during this study and at least three runs were performed for each heating rate. An open aluminum crucible was used to contain the sample and an identical empty aluminum crucible was used as reference material. A small sample weighing 4–6 mg, enough to uniformly cover the base of the crucible, was weighed out and placed in argon filled dry box to avoid oxidation of the sample. The simultaneous TG/DSC system was fluxed with the purge argon stream. In this way the gases given off during the thermal heating process experiment were continuously removed. From the area of the DSC peaks (corrected for the small superimposition of the initial sublimation process by a suitable deconvolution treatment) the enthalpies related to melting and subsequent vaporization (ΔH_{fus} and ΔH_{vap}) were determined respectively for both pure Ac and for Ac contained in the considered dosage forms at four different heating rates. Activation energies (E_{vap}) related to Ac vaporization were also determined according to the procedure reported in a previous paper (Vecchio et al., 2004). Taking into account that vaporization kinetics is a zero-order process (Dollimore et al., 1992), the following modified Arrhenius-type equation:

$$\ln(d\alpha / dt) = \ln k_{vap}(T) = \ln A - E_{vap} / RT \qquad (1)$$

was applied, where α is the degree of conversion, k_{vap} the specific constant rate, R is the gas constant and T the absolute temperature. The pre-exponential factor and the activation energy of vaporization (A and E_{vap}, respectively) can be obtained after a linear least square treatment of the $\ln(d\alpha / dt)$ data *vs.* $1/T$.

As far as the quantitative determination of Ac in the DF1–DF4 dosage forms by means of the calorimetric method is concerned, the melting enthalpy values derived from the corrected area of the corresponding DSC curve are plotted against the Ac content (2–20 mg) in order to construct the calibration curve.

Moreover, the heights of the melting DSC peaks were also tentatively taken into account, but for heuristic only, rather than analytical purposes, and so they have been treated in a separate paragraph (Appendix 1) following the Results and Discussion section. For the same heuristic rather than analytical purpose a special appendix (Appendix 2), placed at the end of section 3, illustrates the attempt to apply the thermoanalytical method also to the dosage form DF5 that, as pointed out in a previous work (Tomassetti et al., 2005), displays a non negligible degree of incompatibility with one of the excipients.

Lastly, according to the spectrophotometric method suggested by the pharmacopeia (Italian Pharmacopeia, 2002) the

TABLE 1
Components in the Examined Commercially Available Analgesics (Where Ac is Always Present as Active Component in Large Amounts, Except for DF5 Dosage Form)

Dosage Forms	Ac Content (%, w/w)	Other Components
DF1	94.1	starch potatoes, Mg stearate, polyvinylpyrrolidone
DF2	87.5	cellulose, Mg stearate, polyvinylpyrrolidone
DF3	83.6	corn starch, stearic acid
DF4	85.1	polyvinylpyrrolidone, Na carboxymethylcellulose, Mg stearate
DF5	35.5	mannitol, aspartame, Mg stearate

quantitative determination of Ac in formulations was also obtained by recording UV absorption spectra of pure Ac aqueous solution with increasing concentrations of this active component. This is a classical reference method that has been used for a long time; it is also considered a cheap and relatively quick method, being based on non-separative techniques. For this purpose, according to Italian Pharmacopeia (2002) a solution was prepared by solubilizing 75 mg of pure Ac with 25 ml of a 0.1 mol L^{-1} standard NaOH aqueous solution. This solution was diluted with 50 mL of distilled water and mixed for 15 min and subsequently diluted once again to 100 mL with distilled water. After mixing the derived solution 10 mL were taken from the derived solution and diluted to 100 mL with distilled water. Lastly, 10 mL of the final solution were added to 10 mL of 0.1 mol L^{-1} standard NaOH aqueous solution and diluted to 100 mL with distilled water. The absorbance for the final solution at constant 257 nm was then measured. The same procedure was applied using a suitable content of the examined pharmaceutical preparations. A blank was prepared by repeating the same procedure without adding Ac or any pharmaceutical preparation. About 5–6 mL of this solution was used as blank for the spectrophotometric measurements. For the analysis of pharmaceutical preparations reported in Table 1 an accurately weighed amount of several tablets for each dosage form was gently mixed and subsequently dissolved with the above-mentioned procedure until a 0.01 mol L^{-1} acetaminophen solution was obtained. The above-mentioned prepared solutions were directly analyzed using the spectrophotometric method described above. For reasons explained in the "results and discussion" section we believe that spectrophotometric readings have to be performed also on solutions after centrifugation treatment. To this end, each solution was centrifuged at 3000 r.p.m. Then, the absorbance spectra of the derived supernatant solutions were recorded between 210 and 340 nm.

Finally, first-order derivatives of absorption spectra are widely recognized as being able to reduce the interference due to non specific turbidity (Campanella et al., 2004), thus avoiding the time-consuming centrifugation process. Therefore, the first-order derivative of absorption spectra of solutions containing increasing concentrations of both pure Ac and Ac containing DF1–DF5 dosage forms were recorded according to the above-mentioned method, without previous centrifuging.

RESULTS AND DISCUSSION

Brief References to the Main Experimental Data Reported in a Previous Work

The TG/DTG and DSC curves of pure Ac and those of the main components contained in the five pharmaceutical preparations considered (Table 1) were reported in the previous paper (Tomassetti et al., 2005), in which even the TG/DTG and DSC curves of pure Ac were compared with those of Ac contained in the DF1-DF5 dosage forms examined. Lastly, the melting and vaporization enthalpies (ΔH_{fus} and ΔH_{vap}, respectively) were determined from the corrected area of the corresponding DSC peaks both for pure Ac and for Ac contained in the five dosage forms studied at selected heating rates (2.5, 5, 10, and 20 K min^{-1}). Finally, activation energies of vaporization E_{vap} of pure Ac and Ac contained in the dosage forms were determined using Eq. (1) by means of the procedure reported in (Vecchio et al., 2004). Trends of ΔH_{fus}, ΔH_{vap}, and E_{vap} values found with the scanning procedure (described in detail in ref. Tomassetti et al., 2005) are briefly represented in Figure 1, while numerical values of these quantities were tabulated in detail in the previously published paper (Tomassetti et al., 2005). Comparison of thermodynamic and kinetic values for pure Ac and for Ac contained in the DF1-DF4 pharmaceutical formulations shows (Tomassetti et al., 2005) that these values do not change appreciably (slight variations lie within the estimated uncertainties). Moreover, it was concluded (Tomassetti et al., 2005) that the presence in DF1-DF4 dosage forms of low excipients content (between 5 and about 15%, w/w) does not

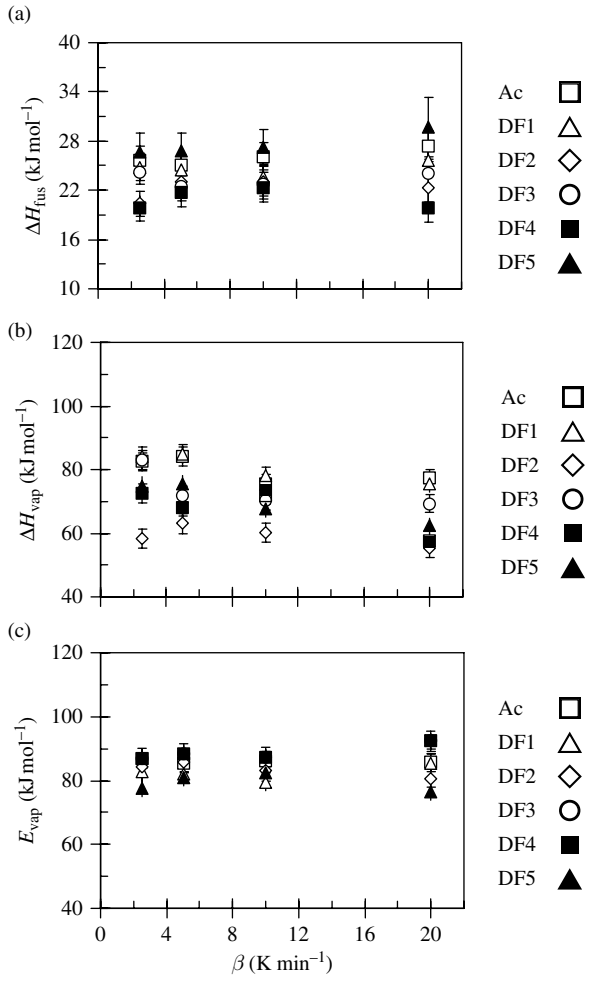

FIGURE 1. Melting (a) and vaporization (b) enthalpies as well as activation energies of vaporization (c) as a function of heating rates, β, for pure Ac, and for the five DF1-DF5 dosage forms.

significantly influence the thermal behavior or the thermodynamic and kinetic parameters for the active component investigated. However, the ΔH_{fus} value of Ac in the DF5 formulation was clearly found to be higher than that of pure Ac due to the overlapping of melting DSC peak of mannitol and Ac (Tomassetti et al., 2005); in addition it must be taken into account that this is the only dosage form with a so high excipient content: 65%, w/w. However, both the thermal behaviour shown by the DSC curves and the quantitative thermoanalytical data indicate that in this case a significant interference must be expected for one of the excipients considered (mannitol). Therefore, the application of the quantitative method was not recommended for the DF5 pharmaceutical preparation. In this study, in spite of this consequence, we decided to apply the proposed calorimetric method also to this pharmaceutical formulation, simply to verify the degree of inaccuracy that the application of the quantitative DSC analysis should be undertaken. As stated previously, the application of the thermoanalytical method to this dosage form had an exclusively heuristic, and not analytical, purpose. The results obtained are set out in a specific table and discussed in a short appendix (denoted as Appendix 2).

Determination of Acetaminophen by the Calorimetric Method

Figure 2 shows typical DSC melting peaks for increasing sample sizes of pure Ac, while in Figure 3 the melting enthalpies (ΔH_{fus}) obtained from the area of the corresponding DSC peaks were plotted against the mass (as mg) of Ac with the aim to obtain a calibration curve for the quantitative determination of Ac in the DF1–DF4 dosage forms. A linear relationship is established among the peaks area (S) and the mass data (Figure 3); the corresponding equation as well as the statistical regression parameters (such as reproducibility of data, evaluated as percent "Pooled Standard Deviation," denoted as "Pooled *SD*" (%), confidence intervals of the slope and intercept and

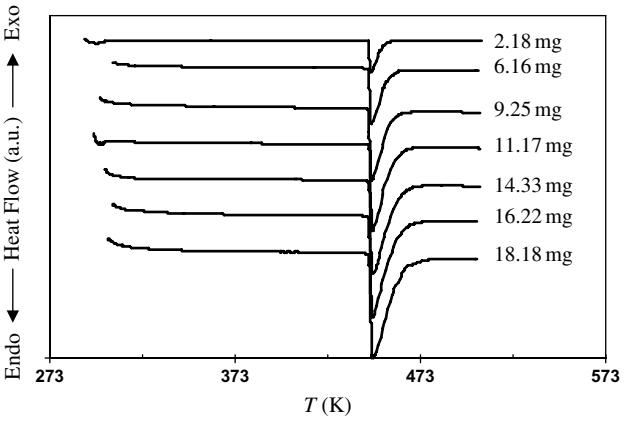

FIGURE 2. DSC of different sample sizes of pure acetaminophen at a heating rate of 5 K min^{-1} under a stream of argon.

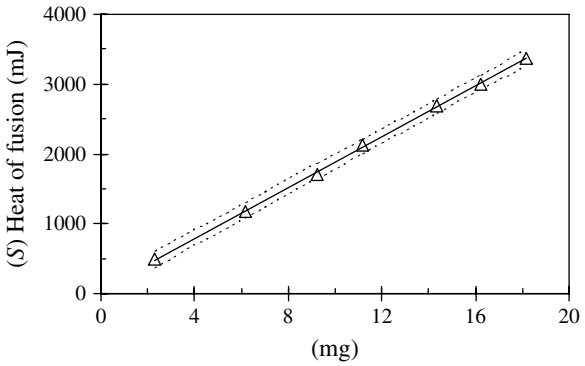

FIGURE 3. Calibration curve for pure acetaminophen based on the area of the DSC melting peak. Each point is the mean of three determinations.

significant tests of the slope and intercept) are reported in Table 2 (A). In addition, statistical regression parameters and significance tests for slope and intercept of the correlation curve representing the calculated *vs*. experimental values for the area of melting DSC peak, are displayed in Table 2 (B).

Figure 4 shows the DSC curves of the DF1–DF5 dosage forms considered. In these curves it is quite evident that the melting temperature obtained from the melting DSC peak for Ac contained in these commercial formulations, with the sole exception of the DF5 dosage form, is close to that of pure Ac (446.2 K). Confirmation of this in a previous research (Tomassetti et al., 2005) comes from the total absence of interference between Ac and the examined excipients, except the case of the DF5 dosage form. At first sight, the calibration curve associated with the proposed calorimetric method (reported in Figure 3) seems to allow the Ac content in the DF1-DF4 considered dosage forms to be quantitatively determined. The experimental percentage of Ac content values, found using the equation of the calibration curve given in Table 2 (A), compared with those declared by the commercial suppliers, denoted as nominal Ac content, are shown in Table 3.

Considering the values displayed in Table 3 obtained by using the calibration curve based on peaks area (S) values, it can be observed that the difference between the experimental and the nominal value of Ac is satisfactory (always less than about 6.5 %).

Determination of Acetaminophen by the Spectrophotometric Method

In order to obtain confirmation of the analytical data related to the pharmaceutical formulations tested using the calorimetric method, the Ac concentration was also determined in the same drugs using the spectrophotometric method reported in the pharmacopeia (Italian Pharmacopeia, 2002). To this end, absorption spectra of pure Ac aqueous solutions were recorded at different Ac concentrations, as shown in Figure 5. The corresponding calibration curve was obtained by reading off the

TABLE 2A
Straight Line Equation and Statistical Analysis of Calibration Curve in Figure 3. Analytical Data of Calibration Curve Equation for Pure Acetaminophen Obtained by the Calorimetric Method Using the Area (S) of the Melting DSC Peaks

Statistical parameters of calibration curve *based on the area S* (mJ) *of the melting DSC peak*	
mean regression equation[a] [S (mJ); M (mg)]	$S = (184.3 \pm 1.3) \cdot M + (23.6 \pm 15.4)$
range of linearity (mg)	2.28 ÷ 18.2
R^2	0.9991
confidence level a	0.95
confidence interval and confidence limits of the slope	4.4; (182.1÷186.5)
confidence interval and confidence limits of the intercept	53.3; (−3.1÷50.2)
$t\ (n-2)$ value; $(n = 21)$[b]	1.73
Significance test of the slope[c]	H_0 is rejected (146.1 > 1.73)
Significance test of the intercept[d]	H_0 is accepted (1.528 < 1.73)
"pooled *SD*" (%)[e]	3.8

[a]Slope and intercept values with their standard deviations. [b]n = number of points fitted to the regression line. [c]H_0 (null hypothesis): expected slope = 0. [d]H_0 (null hypothesis): expected intercept = 0. [e]"pooled *SD*" (%) = Percent pooled standard deviation.

TABLE 2B
Further Statistical Analysis of Data Used to Construct the Calibration Curve in Figure 3. Analytical Data of Correlation Curve Equation for Pure Acetaminophen Obtained by the Calorimetric Method by Plotting the Values of the Area of the Melting DSC Peaks (Calculated from the Regression Line of Table 2) (S_{calc}) vs. the Values of the Area Experimentally Determined (S_{obs})

statistical parameters of correlation curve	
mean regression equation[a] [S_{calc} (mJ); S_{obs} (mJ)]	$S_{calc} = (1.00 \pm 0.01) \cdot S_{obs} + (1.4 \pm 13.7)$
range of linearity (mJ)	477 ÷ 3498
R^2	0.9993
confidence level a	0.95
confidence interval and confidence limits of the slope	0.02; (0.99 ÷ 1.01)
confidence interval and confidence limits of the intercept	47.5; (−22.3 ÷ 25.2)
$t\ (n-2)$ value ; $(n = 21)$[b]	1.73
Significance test of the slope[c]	H_1 is accepted ($\lvert -0.115 \rvert < 1.73$)
Significance test of the intercept[d]	H_0 is accepted (0.104 < 1.73)

[a]Slope and intercept values with their standard deviations. [b]n = number of points fitted to the regression line. H_1: expected slope = 1. [d]H_0 (null hypothesis): expected intercept = 0.

absorbance values at λ = 257 nm (Figure 6a). In Table 4 the equation of the corresponding regression straight line, the confidence interval, the square of the correlation coefficient (R^2) and the "Pooled *SD*" (%) to evaluate the repeatability of the measurements, are also given. Using the regression equation related to the calibration straight line shown in Figure 6a the Ac content of the solutions for the five dosage forms considered have been checked and summarized in Table 5. These results show that all the calculated Ac percentages usually exceed the corresponding nominal values. In spite of the fact that all the prepared solutions seemed clear to the naked eye some turbidity was assumed to be present in them, probably owing to small particles of some excipients that were not completely dissolved. By way of an example absorption spectra of some of the drugs tested are compared in Figure 7, before and after centrifugation, in order to confirm the above-mentioned hypothesis. It may easily be verified that the absorbances at the maximum of the absorption spectra of the centrifuged solutions are slightly lower than those of the non centrifuged solutions. The new concentrations for the solutions of the drugs tested obtained after centrifugation were therefore calculated using new absorbances read at λ = 257 nm. Therefore, the Ac percentages in the drugs calculated by the direct spectrophotometric method, although after centrifugation were also compared in Table 5 with the nominal values. It can be observed that for DF1-DF3 drugs, the agreement with the

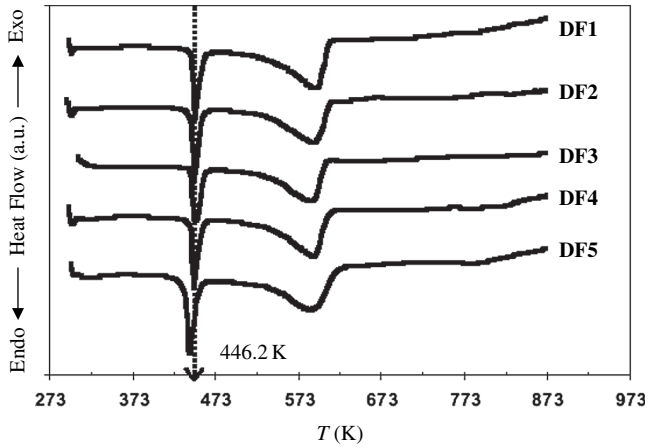

FIGURE 4. DSC curves of the five drugs tested containing acetaminophen (DF1-DF5) at a heating rate of 5 K min^{-1} under a stream of argon.

TABLE 3
Ac Content of Four Dosage Forms Found by Means of Calorimetric Method, Which Use the Area (*S*) of the Melting DSC Peaks

	Ac Content (%, w/w)		Δ (%)
Dosage Forms	(*a*)	(*b*) ± *SD*	[(*b* – *a*) / *a*]·100
DF1	94.1	102.3 ± 3.3	8.7
DF2	87.5	94.7 ± 3.1	8.2
DF3	83.6	80.3 ± 2.7	–3.9
DF4	85.1	86.7 ± 2.8	1.8

(*a*) is the nominal Ac content as provided by the supplier; (*b*) is the experimental Ac content found using the calorimetric method based on the (*S*) mean value ($n = 7$); *SD* = standard deviation.

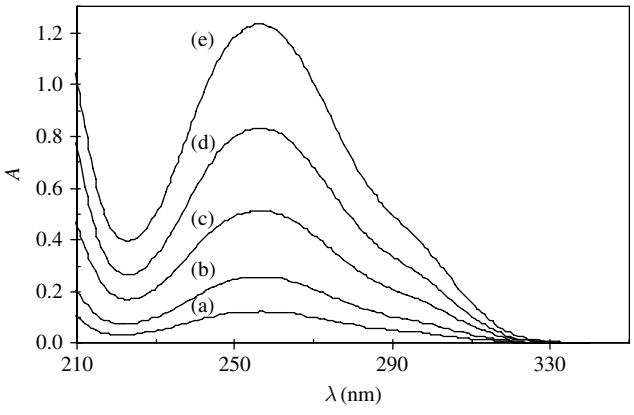

FIGURE 5. Absorption spectra of Ac aqueous solution at several concentrations: (a) $1.15 \cdot 10^{-2}$ mol L^{-1}, (b) $2.10 \cdot 10^{-2}$ mol L^{-1}, (c) $4.21 \cdot 10^{-2}$ mol L^{-1}, (d) $7.02 \cdot 10^{-2}$ mol L^{-1} and (e) $10.5 \cdot 10^{-2}$ mol L^{-1}.

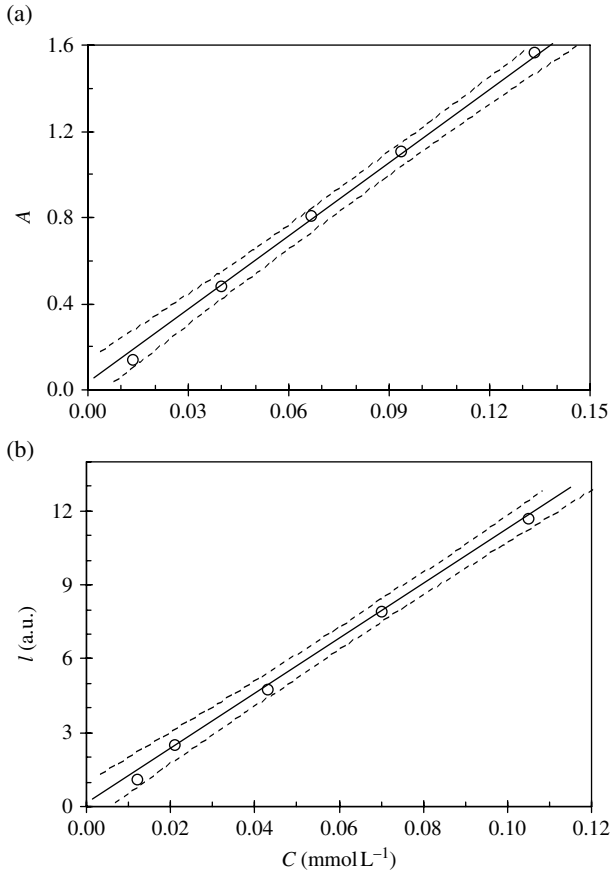

FIGURE 6. Calibration curve for pure acetaminophen based on: (a) the direct spectrophotometric method; (b) the first-order derivative absorption spectra. Each point in both curves is the mean of three determinations.

nominal values is better than that obtained by considering the absorbances of non centrifuged solutions. In the case of the DF4 drug this agreement remained almost unchanged while for the DF5 drug, the agreement is worse. In seeking to account for this, it was observed that in spite of the other centrifuged solution that of DF5 is the only one consisting of a not completely negligible precipitate. Therefore, it was supposed that a non negligible part of the active component remained adsorbed on the precipitate; to recover Ac adsorbed on the precipitate, the latter was once again dispersed in fixed volumes of aqueous solution, according to the procedure described in the "Methods" section. However, after the new centrifugation the absorbance of supernatant was read off and related to the quantity of Ac previously left adsorbed on the precipitate. This extraction-centrifugation process was repeated three times; after the third extraction, absorbance at 257 nm and consequently the Ac concentration "recovered" in the supernatant were practically equal to 0. The concentration values of three "recoveries" were added to the value obtained after the first drug dissolution followed by the relative centrifugation. New value of the Ac concentration for the DF5 drug thus obtained is also given in

TABLE 4
Straight Lines Equations and Statistical Analysis of the Calibration Curves in Figure 6. Analytical Data of Calibration Curves Equations for the Pure Acetaminophen, Obtained by the Two Spectrophotometric Methods: Direct and First-order Derivative Method

statistical parameters of calibration curve	
based on absorbance A vs. concentration C (mmol L^{-1}) (i.e., direct method)	
mean regression equation[a]	$A = (11.81 \pm 0.09)\cdot C - (0.006 \pm 0.007)$
range of linearity (mmol L^{-1})	$(1.15 \div 10.5)\cdot 10^{-2}$
R^2	0.9993
confidence level *a*	0.95
confidence interval and confidence limits of the slope	0.30; (11.66 ÷ 11.96)
confidence interval and confidence limits of the intercept	0.02; (–0.018 ÷ 0.006)
t (*n*–2) value ; (*n*=15)[b]	1.77
Significance test of the slope[c]	H_0 is rejected (136 > 1.77)
Significance test of the intercept[d]	H_0 is accepted (|–0.831| < 1.77)
"pooled SD" (%)[e]	0.4
based on (*l*) (a.u.) *vs. concentration C* (mmol L^{-1}) (i.e., first-order derivative method)	
mean regression equation[a]	$L = (112.3 \pm 0.6)\cdot C - (0.015 \pm 0.047)$
range of linearity (mmol L^{-1})	$(1.15 \div 10.5)\cdot 10^{-2}$
R^2	0.9996
confidence level *a*	0.95
confidence interval and confidence limits of the slope	2.0; (111.3 ÷ 113.3)
confidence interval and confidence limits of the intercept	0.16; (–0.096 ÷ 0.067)
t (*n* – 2) value ; (*n* = 15)[b]	1.77
Significance test of the slope[c]	H_0 is rejected (192.6 > 1.77)
Significance test of the intercept[d]	H_0 is accepted (|–0.309| < 1.77)
"pooled SD" (%)[e]	3.0

[a]Slope and intercept values with their standard deviations. [b]*n* = number of points fitted to the regression line. [c]H_0 (null hypothesis): expected slope=0. [d]H_0 (null hypothesis): expected intercept = 0. [e]"pooled *SD*" (%) = Percent pooled standard deviation.

brackets in Table 5. After carrying out this procedure for the DF5 dosage form a better agreement was found between the calculated and the nominal value: the Δ% decreases from −14.0 to −2.4%.

Finally, to improve the results of spectrophotometric analysis without the time-consuming centrifugation process the first-order derivative absorption spectra of pure Ac at several concentrations were considered for increasing Ac concentrations (see Figure 8). A new calibration straight line was constructed by plotting the difference (*l*) between the maximum at 240 nm and the minimum at 278 nm of the first-order derivative absorption spectra against the Ac concentration. The mentioned calibration curve is shown in Figure 6b while the main analytical data related to this curve are also reported in Table 4. After recording also the first-order derivative of absorption spectra for non-centrifuged solutions of the DF1-DF5 drugs, the calibration curve given in Figure 6b was used to obtain the Ac content in the above-mentioned drugs. The results are summarized in Table 5. The values obtained from the derivative method are seen to be in better agreement with the nominal values than those obtained with the direct method applied to the non-centrifuged solutions. However, the agreement between the values obtained by the direct method after centrifugation and the nominal values is found to be significantly better than that obtained both by using the direct method and the method based on the first-order derivative absorption spectra.

Discussion on the Validation of the Calorimetric Method

If we observe the validation of the calorimetric method, the precision of measurements on the Ac standard and of Ac in dosage forms is seen to be quite satisfactory (see the "Pooled

TABLE 5
Ac Content of the Five Dosage Forms Found by Means of the Spectrophotometric Methods, i.e., the Direct Method, With and Without Centrifugation, and the First-order Derivative Method

	Ac Content (%, w/w)				Δ (%)·100		
Dosage Forms	(*a*)	(*b*) ± *SD*	(*c*) ± *SD*	(*d*) ± *SD*	(*b* – *a*) / *a*	(*c*– *a*) / *a*	(*d*– *a*) / *a*
DF1	94.1	98.2 ± 3.2	96.6 ± 9.9	97.2 ± 13.0	4.3	2.6	3.3
DF2	87.5	93.6 ± 3.1	88.1 ± 9.7	90.4 ± 12.9	7.0	0.7	3.3
DF3	83.6	93.6 ± 3.2	84.5 ± 9.3	91.6 ± 13.1	11.9	1.0	9.5
DF4	85.1	92.7 ± 3.2	92.7 ± 9.8	89.7 ± 12.9	9.0	9.0	5.4
DF5	35.5	37.4 ± 1.4	30.1 ± 3.4	36.6 ± 5.3	5.3	–15.2	3.2
DF5	35.5	–	(35.0 ± 3.8)	–	–	(–1.5)	–

(*a*) is the nominal Ac content as provided by the supplier; (*b*) is the experimental mean value of Ac content found using the direct spectrophotometric method ($n = 5$); (*c*) is the experimental mean value of Ac content found using the spectrophotometric method after centrifugation ($n = 5$) and (*d*) is the experimental mean value of Ac content found using the first-order derivative spectrophotometric method ($n = 5$); *SD* = standard deviation. Value in brackets was obtained after three subsequent extraction-centrifugation procedures.

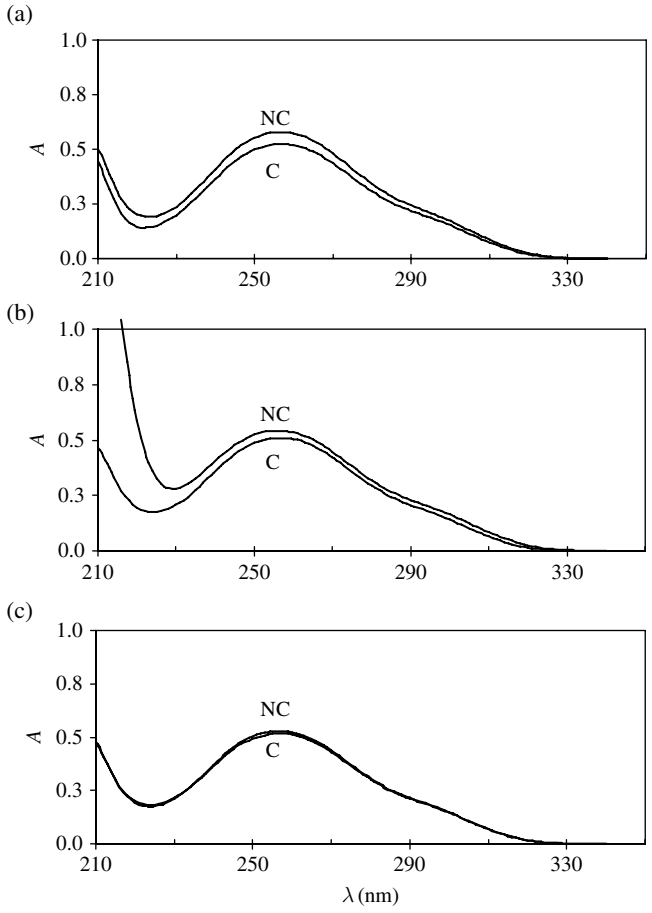

FIGURE 7. Absorption spectra of centrifuged (C) and non-centrifuged (NC) 0.105 mol L^{-1} aqueous solutions of Ac containing formulations. Spectra of (a) pharmaceutical formulation DF1, (b) pharmaceutical formulation DF2 and (c) pharmaceutical formulation DF3.

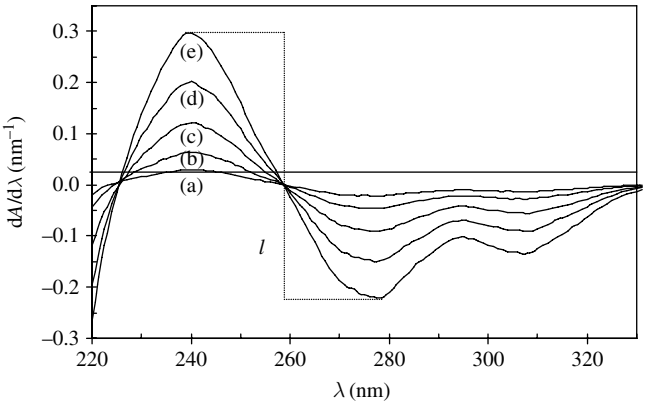

FIGURE 8. First-order derivative absorption spectra of Ac aqueous solution at several concentrations: (a) $1.15 \cdot 10^{-2}$ mol L^{-1}, (b) $2.10 \cdot 10^{-2}$ mol L^{-1}, (c) $4.21 \cdot 10^{-2}$ mol L^{-1}, (d) $7.02 \cdot 10^{-2}$ mol L^{-1} and (e) $10.5 \cdot 10^{-2}$ mol L^{-1}.

SD" (%) value in Table 2 along with the standard deviation (*SD*) values reported in Table 3). The linear range is generally good (see Table 2). In this instance, selectivity essentially depends on the fact that no further DSC peaks related to other active components or excipients contained in the dosage form considered must overlap the Ac melting peak; in this case the selectivity of the method is very good. This is what was found, for instance, in the determination of Ac in DF1-DF4 dosage forms. Otherwise, the method is not selective, as was found, for instance, with the dosage form DF5. Moreover, as already explained, it is worth mentioning that if an overlapping of Ac melting peak with that of other active components or excipients occurs, this could easily be detected by comparing their respective DSC curves. Thus, the selectivity level of the calorimetric method can be evaluated by clearly observing the

thermal behaviour revealed by the DSC curves for each particular application. It is, therefore, possible to decide in advance whether it would be reasonable to apply the reported method or to abstain from its application.

As far as the accuracy is concerned, since certified reference samples of the considered dosage forms were not available, the actual values of Ac content in dosage forms were unknown, and only the nominal values were available. Therefore, it was not possible to calculate the accuracy of the method according to the classical principle. Alternative criteria were used to estimate the accuracy.

First of all, in order to evaluate if the pure standard Ac used could be considered a reference standard, the average experimental ΔH_{fus} value (26.1 ± 1.8 kJ mol^{-1}) was compared with the ΔH_{fus} values reported in literature by different authors (26.5 kJ mol^{-1} [Szelagiewicz et al., 1999] and 27 kJ mol^{-1} [Boldyreva et al., 2004]). An excellent agreement of 98.5 and 96.7 % was found between the experimental value and each literature value, respectively.

In addition, some recovery trials were carried out by adding known weights of pure standard Ac to each of the dosage forms studied and applying the calorimetric method based on the area of the melting DSC peak after gentle homogenization of the final powder. The experimental recovery values were found in all cases to be not less than 96.5%, w/w with respect to the calculated values. All these results certainly represent a positive indication of the accuracy of the method. Moreover, also the comparison with the spectrophotometric method described in the text was originally meant as an attempt to determine the accuracy of the calorimetric method. The aim was actually to evaluate the correlation of the values obtained using it with the results of a reference method published in the pharmacopeia (Italian Pharmacopeia, 2002).

Unfortunately this study was partly nullified by the discovery that the spectrophotometric method itself is subject to a number of drawbacks when applied to real samples. At this stage we consequently attempted to improve the spectrophotometric method itself by introducing a centrifuging operation or else by performing a first derivative. In spite of this, for the purpose of the method accuracy estimation we consider it a positive fact that the Ac content values found for the four dosage forms analysed using the calorimetric method are in sufficiently good agreement with those obtained using the spectrophotometric method (first derivative) in at least three cases out of four.

APPENDIX 1

For heuristic purposes only also the calibration curve obtained by plotting the heights of DSC melting peaks vs. the mass of Ac was also constructed and is set out in Figure 9, while the corresponding analytical data and the statistical parameters of regression are shown in Table 6. The square of the correlation coefficient value is found to be reasonably acceptable also

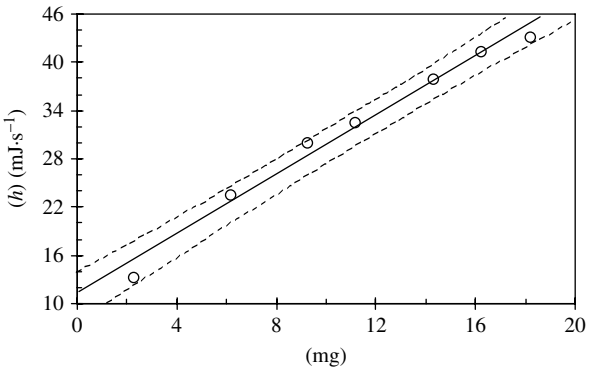

FIGURE 9. Calibration curve for pure acetaminophen based on the height of DSC melting peak. Each point is the mean of three determinations.

in this case, even if, of course, it is worse than that found with the ΔH_{fus} mentioned method reported in Figure 3, as well as the "Pooled *SD*" (%). However, this was only to be expected as the height of a melting DSC peak is not only a function of the sample size, but also depends on other parameters selected for the thermal analysis experiment (e.g., heating rate, geometry of the sample cells, and the particle size and packing density). We decided, however, although only for heuristic and not for analytical purpose, to consider this approach not with a view to using it extensively, but to experimentally evaluate also in this case the degree of agreement with the nominal values of Ac in the four dosage forms expected to be obtained using this parameter for quantitative purposes. Results are summarized in Table 7. Moreover, in spite of this there were other sources of errors; it is therefore of some interest to know the extent to which the use of the latter procedure can affect the degree of agreement between the experimental Ac percentages found for the dosage forms considered and their nominal values.

APPENDIX 2

Examining the data reported in Table 8, as expected for the DF5 dosage form, the difference between the experimental method based on the area of the melting DSC peak and the nominal value is quite large. On the other hand, as mentioned above, the DF5 dosage form is the only one in which mannitol is present, the melting peak temperature of which, as previously evidenced (Tomassetti et al., 2005), is practically identical to that of Ac. This is the reason why the experimental Ac content found using the calorimetric method for this drug is much higher than the nominal one (Δ = + 51%) (Table 8). However, this result was to some extent expected on the basis of the value of peaks area (S) found for the DF5 drug, which was decidedly higher than that of pure Ac (see both Figure 1 and the ΔH_{fus} values reported in the previous work [Tomassetti et al., 2005]).

According to the calorimetric procedure described in Appendix 1, in which the calibration curve in Figure 9 utilizes

TABLE 6
Straight Line Equation and Statistical Analysis of the Calibration Curves in Figure 9. Analytical Data of Calibration Curve Equation for Pure Acetaminophen Obtained by the Calorimetric Method Using the Height (*h*) of the Melting DSC Peaks

statistical parameters of calibration curve *based on the height* (*h*) (mJ s^{-1}) *of the melting DSC peak*	
mean regression equation[a] [*h* (mJ s^{-1}); *M* (mg)]	$h = (1.90 \pm 0.06) \cdot M + (11.2 \pm 0.7)$
range of linearity (mg)	2.28 – 18.2
R^2	0.9821
confidence level *a*	0.95
confidence interval and confidence limits of the slope	0.2; (1.8 ÷ 2.0)
confidence interval and confidence limits of the intercept	2.5; (9.9 ÷ 12.4)
t (*n* – 2) value; (*n* = 21)[b]	1.73
Significance test of the slope[c]	H_0 is rejected (32.3 > 1.73)
Significance test of the intercept[d]	H_0 is rejected (15.5 > 1.73)
"pooled SD" (%)[e]	4.3

[a]Slope and intercept values with their standard deviations. [b]*n* = number of points fitted to the regression line. [c]H_0 (null hypothesis): expected slope = 0. [d]H_0 (null hypothesis): expected intercept = 0. [e]"pooled *SD*" (%) = Percent pooled standard deviation.

TABLE 7
Ac Content of Four Dosage Forms Found by Means of Calorimetric Method, Which Use the Height (*h*) of the Melting DSC Peaks

	Ac content (%, w/w)		Δ (%)
Dosage Forms	(*a*)	(*b*) ± *SD*	[(*b* – *a*) / *a*]·100
DF1	94.1	65.2 ± 5.5	−30.7
DF2	87.5	66.7 ± 5.4	−23.8
DF3	83.6	96.5 ± 8.0	15.4
DF4	85.1	72.7 ± 6.1	−14.6

(*a*) is the nominal Ac content as provided by the supplier; (*b*) is the experimental Ac content found using the calorimetric method based on the (*h*) mean value (*n* = 7); *SD* = standard deviation.

the height of the melting DSC peaks, it is odd to observe that while, as expected, the agreement between the experimental and the nominal value of Ac is usually less good for the first four dosage forms examined; indeed, the average value of the differences between these two values is actually constant at about 17–18%. However, as far as the dosage form DF5 is concerned, the experimental Ac content derived from this procedure is closer to the nominal value than that obtained using the curve given in Figure 3. In fact, the difference between the experimental and the nominal value in this case is about 16% (Table 8), i.e. comparable to that found for the other four drugs.

CONCLUSIONS

On the basis of the reported results it may be concluded that first of all the application of the calorimetric analytical method to determine acetaminophen (Ac) contained in some commercially available pharmaceutical formulations entails verifying the compatibility between the active component and the excipients found in the formulations. On the other hand, it is important to stress the fact that this compatibility study can be performed by the same technique, i.e. thermal analysis, above all following the classical criteria based on the disappearance of certain peaks, or the appearance of new peaks and on the control of the invariance of the "onset" temperatures associated with different observed processes (Tomassetti et al., 2005). However, it is also better to suggest to further validate previous data concerning possible shifts of peaks temperature to compare thermodynamic data (such as ΔH values) and kinetic data (such as activation energy values), both determined by thermal analysis for the pure active component and for the active component contained in the examined commercial pharmaceutical formulations (Tomassetti et al., 2005). From the results obtained in this preliminary thermoanalytical study it can be evaluated if the calorimetric method examined actually represents a useful tool for the analytical determination of a certain active component contained in commercial pharmaceutical preparations.

The main advantages of the calorimetric method are represented by the complete absence of any need for pre-treatment or manipulation of the sample. Therefore, the method is simple, quick, and cheap and needs only a very small amount of sample for the purpose of analysis. However, its accuracy, at least in this research, proved to be satisfactory even if probably slightly lower than that of the spectrophotometric method,

TABLE 8
Ac Content of the DF5 Dosage Form Found by Means of Calorimetric Methods, which Use the Area (*S*) or the Height (*h*) of the Melting DSC Peaks

	Ac Content (%, w/w)			Δ (%)	
Dosage Forms	(*a*)	(*b*) ± SD	(*c*) ± SD	$[(b - a) / a]\cdot 100$	$[(c - a) / a]\cdot 100$
DF5	35.5	54.3 ± 1.8	29.1 ± 2.4	52.8	−18.1

(*a*) is the nominal Ac content as provided by the suppliers; (*b*) is the experimental Ac content found using the calorimetric method based on the (*S*) mean value ($n = 7$) and (*c*) is the experimental Ac content found using the calorimetric method based on the (*h*) mean value ($n = 7$); *SD* = standard deviation.

while its precision seems to be better than that of the spectrophotometric method. As far as the considered spectrophotometric method is concerned (at least for the commercial formulations examined in this work) the careful procedure seems to require accurate centrifugation of the solutions of the examined drugs before measuring the absorbance. Even if this procedure is time-consuming the accuracy of the measurements increases after centrifugation especially in the case of perceptible turbidity of the examined solutions. In this case it is also necessary to make at least other two successive dissolutions-centrifugations of the precipitate and afterwards to carry out the spectrophotometric determination on the supernatant, thus increasing analysis time. First-order derivative spectrophotometric determination only partially improves the accuracy of the method, without the need for an extended analysis time. Therefore, it represents a compromise between the demand for sufficient accuracy of the method and the need for short analysis times. As far as the analysis time is concerned, however, the calorimetric method tested always enables the analysis to be performed using shorter times than for the spectrophotometric method, whatever the applications procedure used for the latter.

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