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APPLICATION OF THERMAL ANALYTICAL METHODS TO PHARMACEUTICAL PRODUCTS. PART 2. PENICILLIN SODIUM SALTS *

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ABSTRACT

A series of penicillin sodium salts was analyzed by thermoanalytical techniques (TG, DTG and DSC). The analyses were carried out in an atmosphere of oxygen. The final residue of the thermal decomposition for all the analyzed compounds was always sodium sulphate. The purity of the examined penicillins could therefore be determined by analysis of the sodium percentage present in the compounds. Better results, concerning accuracy and precision were obtained by the addition of ammonium sulphate to the analyzed compounds.

INTRODUCTION

Thermal analytical techniques have been successfully applied in our laboratories to pharmaceutical problems [1–3], especially for the analysis of sodium salts of the cephalosporins [3]. The determination was carried out by analysis of sodium as sodium sulphate. The analysis, which allows evaluation of the purity of the commercial cephalosporin sodium salts, shows a precision and accuracy higher than flame photometry. The penicillin sodium salts analyzed by TG as cephalosporins, because of the presence of a sulphur atom in the molecule, give sodium sulphate as the final residue, and by quantitative analysis of the sodium as sodium sulphate it is possible to evaluate the purity of the antibiotic. Other information concerning the thermal stability of the compounds and the thermal characteristics of the reactions were obtained by differential scanning calorimetry.

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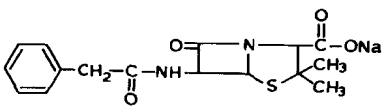
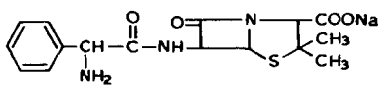
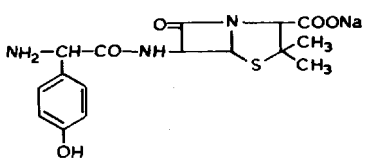
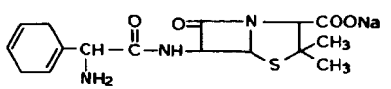
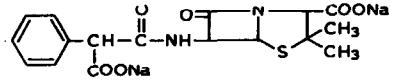
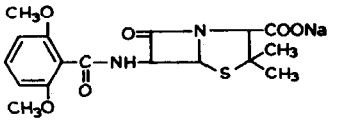
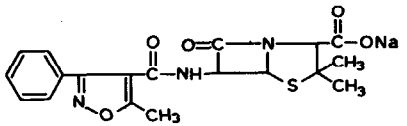
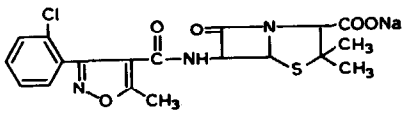
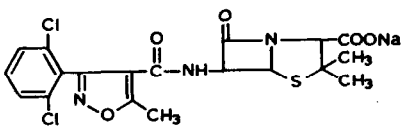
TABLE 1

The compounds examined

Antibiotic (sodium salt)	Structural formula	Empirical formula	Molecular weight
Benzylpenicillin		$C_{16}H_{17}N_2NaO_4S$	356.38
Ampicillin		$C_{16}H_{18}N_3NaO_4S$	371.39
Amoxycillin		$C_{16}H_{18}N_3NaO_5S$	387.39
Epicillin		$C_{16}H_{20}N_3NaO_4S$	373.41

Carbenicillin		$C_{17}H_{16}N_2Na_2O_6S$	422.36
Methicillin		$C_{17}H_{19}N_2NaO_6S$	402.40
Oxacillin		$C_{19}H_{18}N_3NaO_5S$	423.42
Cloxacillin		$C_{19}H_{17}ClN_3NaO_5S$	457.87
Dicloxacillin		$C_{19}H_{16}Cl_2N_3NaO_5S$	492.31

MATERIALS AND METHODS

Penicillin sodium salts were supplied by Istituto Biochimico Italiano (ampicillin, amoxycillin, oxacillin, cloxacillin, dicloxacillin), Squibb S.p.A. (epicillin), Farmitalia [benzylpenicillin, carbenicillin, methicillin and the drug containing ampicillin and dicloxacillin 50% (w/w)]. Ammonium sulphate was Merck pro analysis. The TG and DTG curves of the examined compounds were obtained using a Du Pont model 951 thermobalance. The heating rate used was 10°C min^{-1}; the furnace atmosphere was oxygen at a fow rate of 100 ml min^{-1}, or static air. The DSC curves were obtained using a Perkin-Elmer DSC-2 instrument. The heating rate used was 10°C min^{-1}, and the atmosphere consisted of oxygen at a flow rate of 100 ml min^{-1}. The flame photometric measurements were carried out using an Instrumentation Laboratory Flame Photometer 243, and the IR spectra were obtained using a Perkin-Elmer 177 IR spectrophotometer.

RESULTS

The examined compounds, listed in Table 1, are those commonly used for commercial drugs. The TG curves were obtained both in static air and in a stream of oxygen. The TG curves in oxygen show a more definite and reproducible behaviour. In static air some penicillins blow up, especially in the initial phase of thermal decomposition. In the stream of oxygen this problem disappears and the decomposition starts 5–10°C earlier than in static air. Figure 1 shows the thermogravimetric curves, in a stream of oxygen for all the examined compounds, and Table 2 summarizes the corresponding TG data.

Except for benzylpenicillin, which is anhydrous, the quantity of water present in these compounds is higher than in the cephalosporins. For some compounds, e.g. epicillin, the water is completely released around 100°C, while for others, e.g. dicloxacillin and methicillin, the water is lost a few degrees before the decomposition.

The thermal decomposition, as for the cephalosporins, occurs in two processes. The first, where the higher percent lost is verified, happens through a series of superimposed reactions and gives a black residue of sodium sulphate and carbon. The second process consists of carbon oxidation to give only sodium sulphate. Sometimes the second process occurs in only one step, e.g. cloxacillin, while for other compounds two well-defined steps represent the second process, e.g. epicillin and carbenicillin.

The composition of the residues has been studied by chemical assay (sulphate analysis by barium chloride) and IR techniques. The IR spectra of the residue from the first process show the disappearance of all the bands characteristic of the antibiotic, substituted by those of the sodium sulphate,

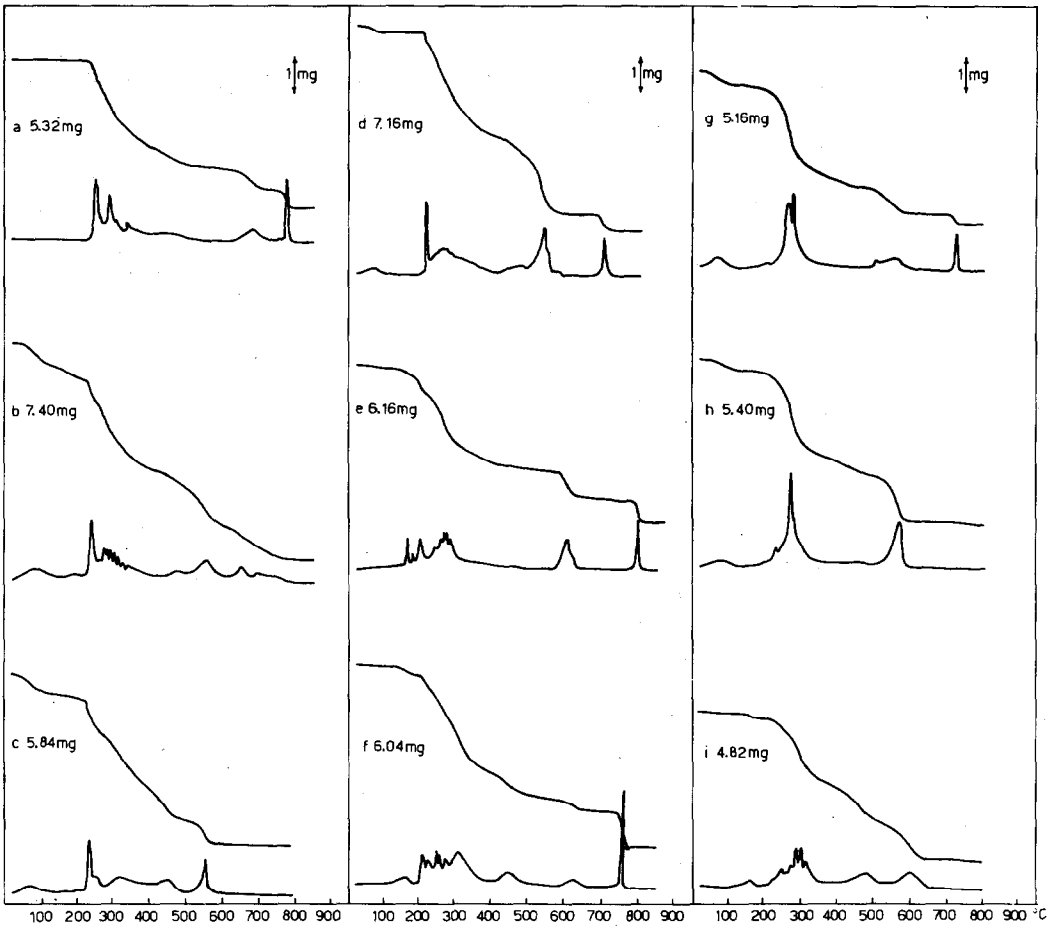


Fig. 1. TG and DTG of some sodium penicillins. Heating rate, 10°C min^{-1}; oxygen flow rate, 100 ml min^{-1}. (a) Benzylpenicillin, (b) ampicillin, (c) amoxycillin, (d) epicillin, (e) carbenicillin, (f) methicillin, (g) oxacillin, (h) cloxacillin, (i) dicloxacillin.

particularly a very strong one corresponding to the asymmetric stretching near 1100 cm^{-1} and a very sharp weak one near 1000 cm^{-1} corresponding to the symmetric SO_4^{2-} stretching [3,4]. During the second decomposition process dioxide evolution was checked by bubbling the gas flowing from the furnace over barium hydroxide solution. Previous results have shown that the sodium found as sodium sulphate was less than the theoretical value (Tables 3 and 4), especially for these molecules where the ratio sulphur/sodium is lower.

The problem was obviated by adding ammonium sulphate to the antibiotic. The addition of a known quantity of ammonium sulphate, whose thermal decomposition in a stream of oxygen does not produce a residue (Fig. 2), increases the sulphur/sodium ratio, so allowing sodium sulphate to be

TABLE 2

Thermal analysis of the compounds in the oxygen stream. The wt.% of the residues at the end of each process are referred to the anhydrous compound. Water is expressed as a fraction of the anhydrous compound.

Sodium salt	H_2O loss		First process			Second process		
	Found %	pdt	Calcd.%	Found%	pdt	Calcd.%	Found%	pdt
Benzylpenicillin				41.4	230 260 630	19.9	20.0	630 690 750 750 790 820
Ampicillin	11.8	30 80 170		49.1	180 240 500	19.1	18.5	500 550 615 615 650 790
Amoxycillin	6.1	30 75 150		31.7	190 235 500	18.3	18.2	500 555 620
Epicillin	2.9	30 65 100		49.1	190 265 490	19.0	18.5	490 535 600 670 700 745

Carbenicillin	2.3	35 90 135	51.6	155 275 570	33.6	27.7	570 615 660 780 810 835
Methicillin	4.2	40 160 180	38.9	200 315 570	17.7	14.6	570 620 670 720 750 780
Oxacillin	7.6	30 75 125	37.4	160 270 500	16.8	15.9	500 565 615 710 730 755
Cloxacillin	6.3	30 75 130	39.9	175 275 500	14.9	14.5	500 565 630
Dicloxacillin	3.4	60 160 180	34.5	215 300 535	14.4	13.2	535 600 670

TABLE 3

Precision of sodium analysis in the sodium salts of some penicillins by TG, with and without the addition of ammonium sulphate, in the oxygen stream

Antibiotic (sodium salt)	% Sodium						
	Calcd.	Found by TG (mean of seven values)	S.D.	% Relative S.D.	Found by TG with A.S. added (mean of seven values)	S.D.	% Relative S.D.
Benzylpenicillin	6.45	6.46	0.27	4.2	6.46	0.24	3.7
Amoxycillin	5.93	5.89	0.06	1.0	5.97	0.09	1.5
Epicillin	6.16	5.99	0.23	3.8	6.14	0.08	1.3
Cloxacillin	5.02	4.89	0.21	4.3	5.00	0.02	0.4

A.S. = Ammonium sulphate.

TABLE 4

Sodium contents of the compounds examined

Comparison of the results obtained by TG, with and without the addition of ammonia sulphate, and flame photometry. Values are the mean of three determinations.

Antibiotic (sodium salt)	% Sodium				% Difference between found and calcd. values		
	Calcd.	Found by TG	Found by TG with A.S. added	Found by flame photometry	TG	TG with A.S. added	Flame photometry
Benzylpenicillin	6.45	6.46	6.46	6.53	+0.2	+0.2	+1.2
Ampicillin	6.19	5.99	6.31	6.46	−3.2	+1.9	+4.4
Amoxycillin	5.93	5.88	5.95	6.29	−0.8	+0.3	+6.1
Epicillin	6.16	5.99	6.16	6.13	−2.8	0	−0.5
Carbenicillin	10.89	8.97	10.84	10.57	−17.6	−0.5	−2.9
Methicillin	5.71	4.72	5.78	5.54	−17.3	+1.2	−3.0
Oxacillin	5.43	5.15	5.42	5.48	−5.2	−0.2	+0.9
Cloxacillin	5.02	4.89	5.00	5.21	−2.6	−0.4	+3.8
Dicloxacillin	4.67	4.27	4.67	4.37	−8.6	0	−6.4
Drug containing sodium ampicillin and sodium dicloxacillin 50% (w/w)	5.43	5.15	5.48	5.47	−5.2	+0.9	+0.7

A.S. = Ammonium sulphate

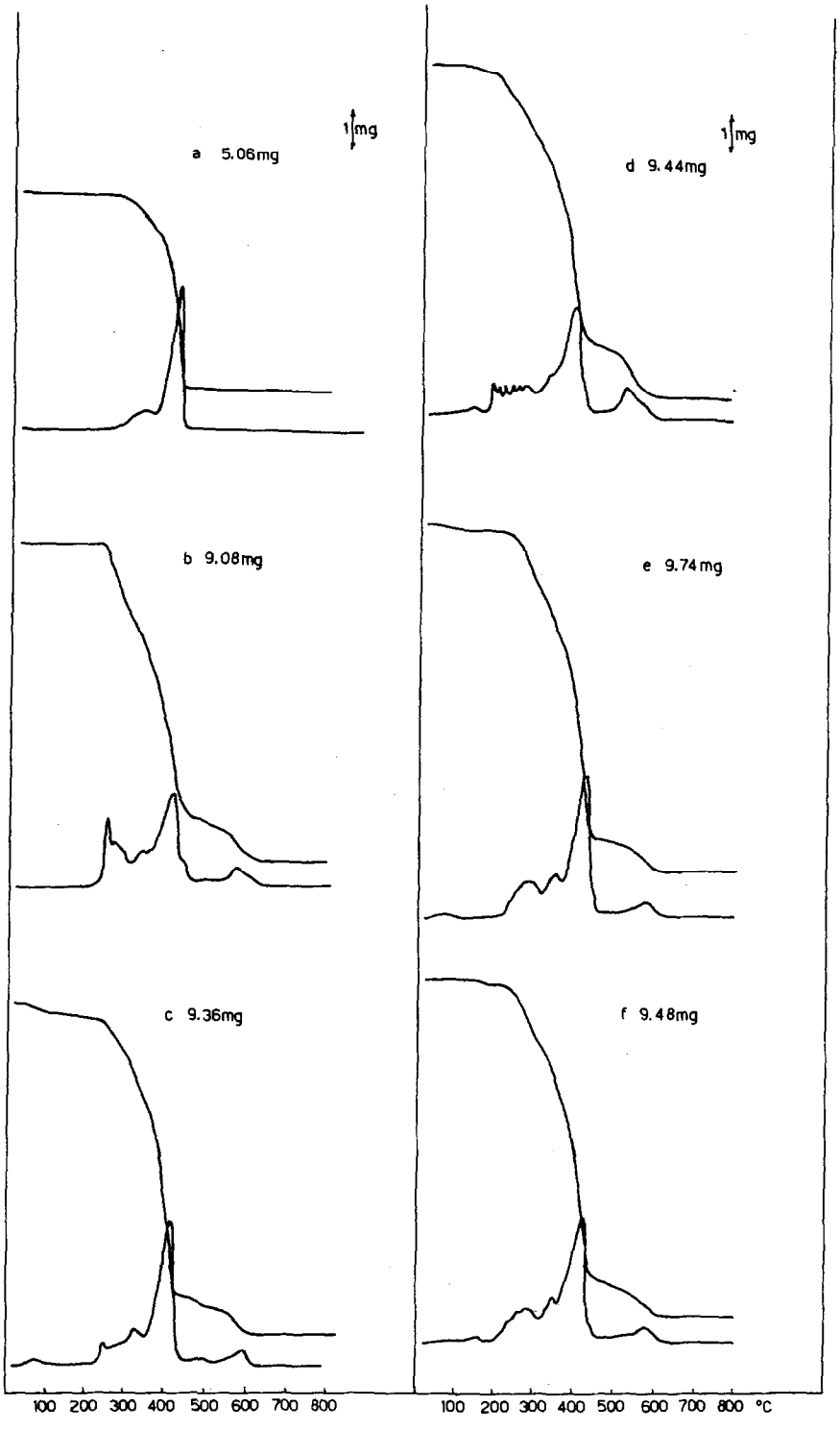


Fig. 2. TG and DTG of ammonium sulphate (A.S.) and some sodium penicillins with the addition of ammonium sulphate. Heating rate, 10°C min^{-1}; oxygen flow rate, 100 ml min^{-1}. (a) Ammonium sulphate; (b) benzylpenicillin with 61.7% (w/w) A.S., (c) ampicillin with 71.9% (w/w) A.S., (d) methicillin with 57.2% (w/w) A.S., (e) cloxacillin with 68.0% (w/w) A.S., (f) dicloxacillin with 65.0% (w/w) A.S.

TABLE 5

Thermal analysis, in the oxygen stream, of the compounds to which sodium sulphate was added

The weight of the residue at the end of each process is referred to the anhydrous antibiotic.

Sodium salt with A.S. added	H_2O loss	First process			Second process		
	pdt	Calcd.%	Found%	pdt	Calcd.%	Found%	pdt
Benzylpenicillin			46.0	220	19.9	20.0	520
				410			565
				520			640
Ampicillin	30		45.5	180	19.1	19.5	520
	70			410			590
	150			520			640
Amoxycillin	30		33.3	190	18.3	18.4	525
	75			410			580
	140			525			620
Epicillin	30		42.5	210	19.0	19.0	520
	70			425			605
	110			520			640
Carbenicillin	30		45.7	165	33.6	33.5	530
	90			400			580
	130			530			630
Methicillin	50		49.5	190	17.7	17.9	490
	160			415			540
	170			490			640
Oxacillin	30		38.8	160	16.8	16.7	530
	70			430			590
	130			530			640
Cloxacillin	30		38.5	180	14.9	14.9	500
	70			430			580
	120			500			630
Dicloxacillin	60		41.0	195	14.4	14.4	500
	155			425			575
	170			500			640

A.S. = Ammonium sulphate.

obtained quantitatively as the residue of the thermal decomposition of the different antibiotics. Some TG curves of antibiotics plus ammonium sulphate are collected in Fig. 2. The thermal decomposition always occurs in two processes and the second process is always a single step process and commonly occurs at a lower temperature than that corresponding to the antbiotic alone.

The TG data obtained by adding to each antibiotic a mass of ammonium sulphate about double that of the antibiotic are reported in Table 5. Table 4 summarizes the results obtained for the analysis of the sodium content by

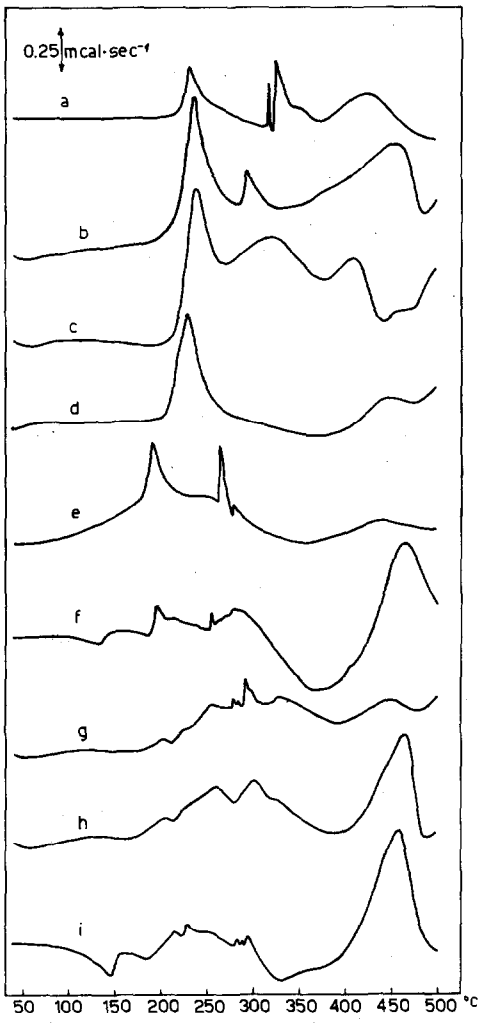


Fig. 3. DSC of sodium penicillins. Heating rate, 10°C min^{-1}; oxygen flow rate, 100 ml min^{-1}. (a) Benzylpenicillin, (b) ampicillin, (c) amoxycillin, (d) epicillin, (e) carbenicillin, (f) methicillin, (g) oxacillin, (h) cloxacillin, (i) dicloxacillin.

TG with and without the addition of ammonium sulphate, and by flame photometry. Finally, Fig. 3 shows the DSC curves, obtained in a stream of oxygen, for all the examined penicillins. Besides the initial endothermic process corresponding to the release of water (when present), the curves carried out up to 500°C show a complex thermal behaviour corresponding to many unresolved, mainly exothermic reactions.

DISCUSSION

The DSC curves show that is impossible to identify melting processes, and generally there are no well-defined isolated peaks useful for quantitative purposes. At the same time the DSC curves, being quite different to each other, can be useful for qualitative analysis (calorimetric "finger prints"). Concerning thermogravimetric analysis, it is better to operate in a stream of oxygen because a better reproducibility is obtained with a precision in the range 1–4.5%. The addition of ammonium sulphate improves the reproducibility (0.5–3.7%; Table 3). The obtained precision is always higher than that obtained by flame photometry, which is about 5–6% [3]. The accuracy obtained with and without the addition of ammonium sulphate is referred to in Table 4. While without ammonium sulphate the obtained accuracy notably changes for the examined penicillins, better for benzylpenicillin and amoxycillin, but worst for carbenicillin and methicillin, by addition of ammonium sulphate the difference between the found and calculated values for all the examined penicillins is always $\pm 2\%$. The addition of ammonium sulphate notably improves the accuracy and the precision of the analytical method. Nevertheless, it is better to run a TG analysis without ammonium sulphate first in order to determine exactly the water percent. Finally, the addition of ammonium sulphate avoids sputtering and allows better defined curves to be obtained in the final process (which is sometimes expected to take place above 100°C, e.g. methicillin).

CONCLUSIONS

Using the described procedure it is possible to evaluate by TG the sodium content of the single sodium penicillins and homogeneous defined mixtures of two or more salts of these antibiotics (e.g. Table 4, commercial drug containing the same weight of sodium ampicillin and sodium dicloxacillin). The accuracy and precision of the method are good, especially if compared with those obtained by flame photometry. It must also be considered that the results obtained by flame photometry are the results corrected for the undeclared water content, data obtained by thermal analysis. Considering the uncorrected data the accuracy would be less considering also that the water content is not disregarded in some compounds (Table 2). The method is easy, does not require sample pretreatment and the operational time is very short, so in this case it can also represent a good alternative to traditional quality control methods.

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