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THERMOGRAVIMETRIC ANALYSIS OF CONJUGATED AND UNCONJUGATED SODIUM CHOLATES

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ABSTRACT

A series of conjugated and unconjugated cholate sodium salts was analyzed by thermogravimetric (TG and DTG) techniques. The analyses were carried out in static air and oxygen atmosphere. The final residue of the thermal decomposition is sodium carbonate for the glycoconjugates and unconjugated cholates, and sodium sulphate for the tauroconjugates. The possibility of determining the purity of the cholate sodium salts examined, by analysis of the percentage of sodium present in the compounds, was evaluated. Results concerning accuracy and precision for the examined compounds are reported.

INTRODUCTION

In recent years, a research program has been developed aiming to set up potentiometric [1–3], enzymatic [4], spectrophotometric [5] and calorimetric [5,6] methods for the determination of free and conjugated cholates, which deals with an increasing interest in the field both of clinical [4,7] and of pharmacological [8] analysis. On the other hand, some of the latest results obtained in the study of the behaviour of the sodium salts of some organic molecules of pharmaceutical interest, such as cephalosporins [9] and penicillins [10,11], show that on performing an accurate thermogravimetric analysis the evaluation of the purity of these compounds can be reliably obtained on comparison of the theoretical and experimental data of the residue of alkaline sulphate, obtained at 650–800 °C in air or in oxygen stream. Such good results have induced us to try to extend the same research to the free and conjugated cholates on the basis of the extreme simplicity and speed of this technique. In Table 1 the formulae of all the examined cholates, both free and conjugated, are reported.

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TABLE 1

The compounds examined

Sodium salt	Structural formula	Empirical formula	Molecular weight
Cholate	~H3 OH H-CH2--CH2--COONQ :: CH3! HO'"'~!V ""'OH	C24H39OsNa	430.6
Deoxycholate	CH3 (~r4 CH--CH2-CH2--COONa I H3 • CHa ~ "~ "~ HO'	C24H3904Na	414.5
Chenodeoxycholate	I I oH3 CH3~H-CH2- CH2- COON0 ~ HO'"	C24H3904Na	414.5
Lithocholate	CH3I i ~ Cl~--CH2--CO0 NO HQ"	C24H3903Na	398.6
Glycoeholate	i H3 CH-CH2--CH2--CO- NH- CH2--COONO H ~ HQ"	C26H4206Na	487.6
Glychodeoxycholate	H OH t 3 I CH- CM2--CH2--CO--NH-- CH2--COONo , CH3~ HO" — H C,H~	C26H42OsNa	471.6
Glycocheno-deoxycholate	(~ CH2--CH~-CO-- N.H--CH2--COONQ " ~ HO-..	C26H42OsNa	471.6
Taurocholate	H OH ~H3 ~ CH~-CH.d-CO-NH-CH2-CH2-SO3No C.BaJJICH ~ HQ"	C26H,1407NSNa	537.7
Taurodeoxycholate	CN CH3 " CH-CH2-CH2-CO- NH--CH2--CH2-SO3No , H3 CH3 HO'"	C26H4406NSNa	521.7
Taurocheno-deoxycholate	CH- CH2--CH2--CO-NH--CH2--CH2- SOaNa CH3 Ho""	C26H4406NSNa	521.7

EXPERIMENTAL

Cholate, deoxycholate, glycocholate, glycodeoxycholate, glycochenodeoxycholate, taurocholate, taurodeoxycholate and taurochenodeoxycholate sodium salts were supplied by Sigma Chemical Company; chenodeoxycholate and lithocholate sodium salts by Calbiochem-Behring Corp.; ammonium sulphate, pro analysis, by Merck.

The TG and DTG curves of the compounds examined were obtained using a DuPont thermobalance (Model 951). The heating rate used was 10 °C min^{-1}; the furnace atmosphere was oxygen at a flow rate of 100 ml min^{-1}, or static air. The IR spectra were obtained using a Perkin-Elmer 177 IR spectrophotometer.

RESULTS

The choice of atmosphere (oxygen stream or static air) was suggested by the observation that, in these conditions, the best results were obtained in the case of the salts of antibiotics [9–11]. The TG and DTG curves, recorded for all the compounds considered, are shown in Figs. 1–3, while in Table 2 the corresponding thermogravimetric data are summarized. After an initial well-detectable loss of water, or other crystallization solvent, two main decomposition processes are observed. The first process is very fast, beginning at about 300 °C and ending at 550–600 °C for the free cholates and glycoconjugates, and at 450–550 °C for the tauroconjugates, produces a residue of carbon and sodium carbonate in the case of free cholates and glycoconjugates, and carbon and sodium sulphate, with traces of carbonate, evidenced by IR analysis, when in static air (Fig. 5) in the case of tauroconjugates. The second process is essentially the loss of carbon, in the form of carbon dioxide, so that the residue only comprises sodium carbonate or sulphate. The analysis of the two residues was performed both by IR spectrophotometry (e.g., Figs. 4 and 5) [12] and by the classical chemical methods of quantitative analysis [13].

In order to correctly evaluate if the thermogravimetric method can be useful for purity control analytical purposes by comparing the theoretical and experimental percentages of sodium content data, in Table 3 nominal and mean sodium contents recorded of several thermogravimetric experiments, performed in oxygen or in static air, for all the compounds studied and the relative differences between these values, are reported; reproducibility data are presented in Table 4. Both tables also show the results of tests performed by the addition of ammonium sulphate to free sodium cholates and to glycoconjugates. By this technique thermogravimetric curves very similar to those found in previous papers [10,11] by adding this salt to sodium or potassium penicillins are recorded (e.g., Fig. 6). By the addition of

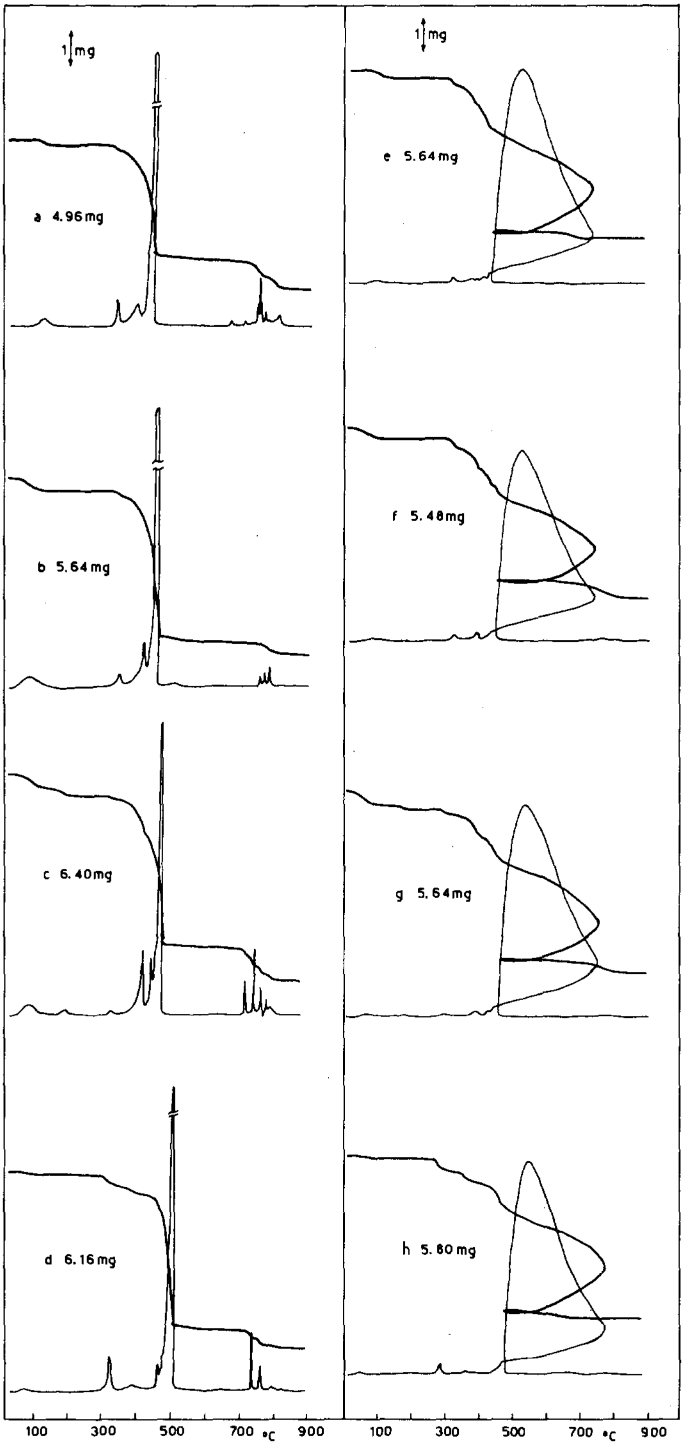

Fig. 1. TG and DTG of some unconjugated sodium cholates. Heating rate, 10 °C min^{-1}; static air or oxygen with 100 ml min^{-1} flow rate. (a) Cholate; (b) deoxycholate; (c) chenodeoxycholate; (d) lithocholate in air; (e) cholate; (f) deoxycholate; (g) chenodeoxycholate; (h) lithocholate in oxygen.

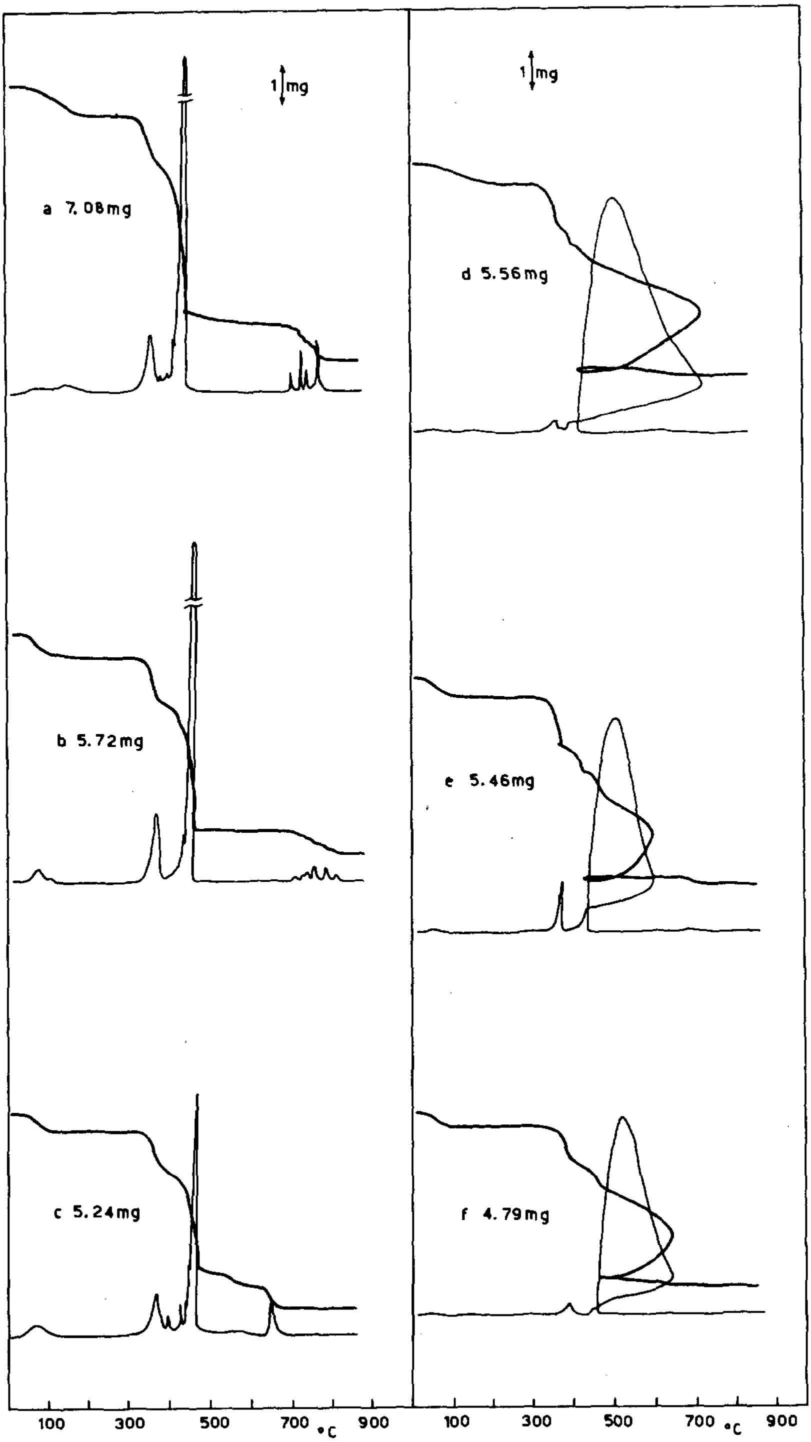

Fig. 2. TG and DTG of some glycoconjugated sodium cholates. Heating rate, 10 °C min^{-1}; static air or oxygen with 100 ml min^{-1} flow rate. (a) Glycocholate; (b) glycodeoxycholate; (c) glycochenodeoxycholate in air; (d) glycocholate; (e) glycodeoxycholate; (f) glycochenodeoxycholate in oxygen.

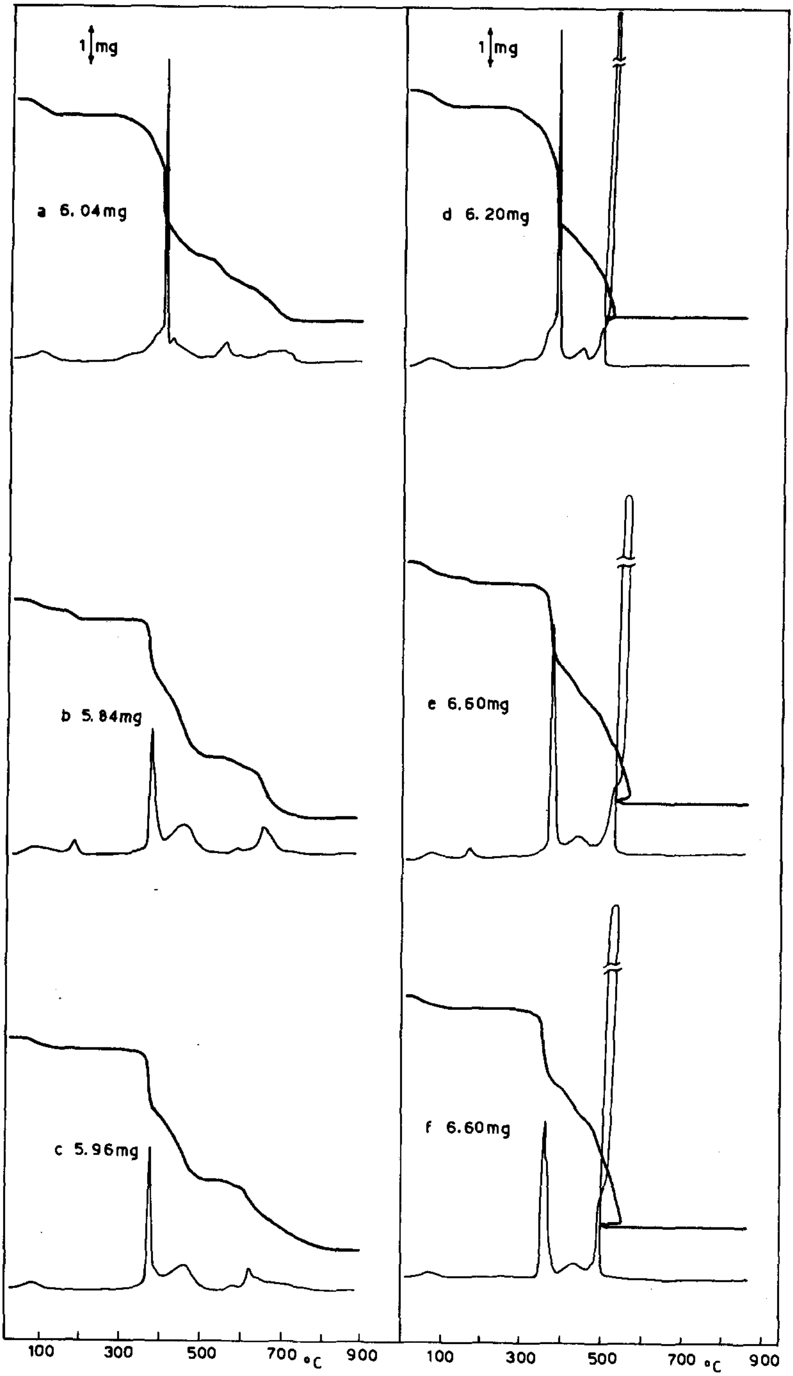

Fig. 3. TG and DTG of some tauroconjugated sodium cholates. Heating rate, 10 °C min^{-1}; static air or oxygen with 100 ml min^{-1} flow rate. (a) Taurocholate; (b) taurodeoxycholate; (c) taurochenodeoxycholate in air; (d) taurocholate; (e) taurodeoxycholate; (f) taurochenodeoxycholate in oxygen.

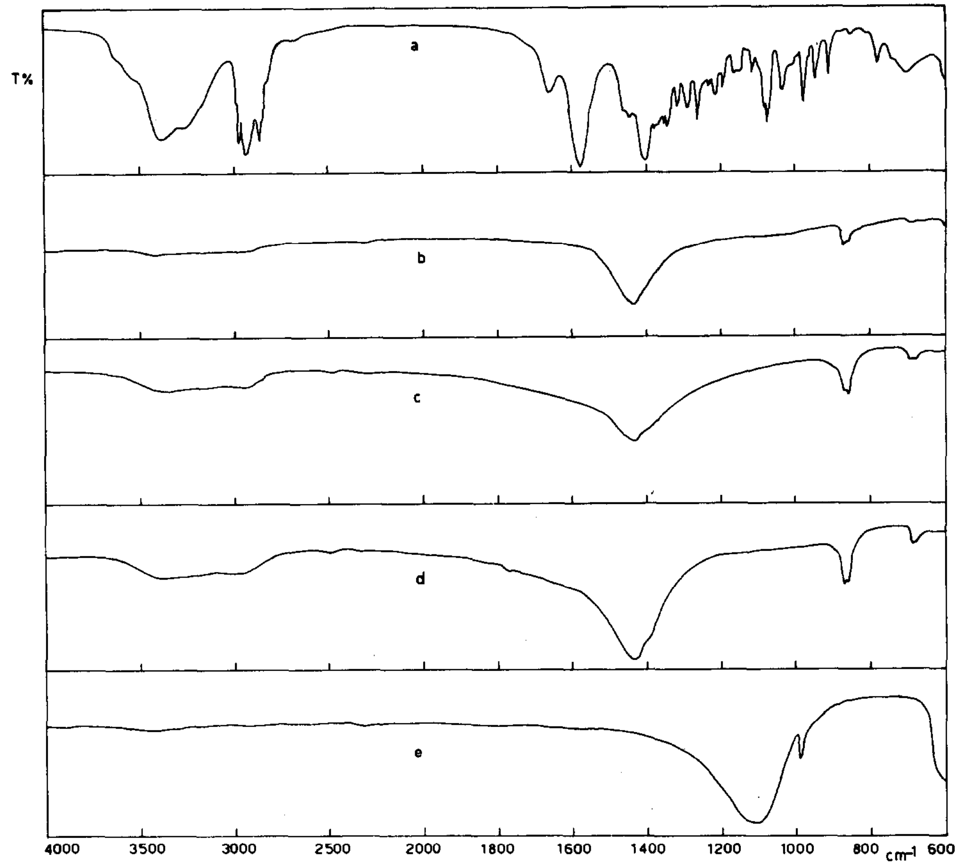

Fig. 4. IR spectra in KBr of sodium cholate. (a) Before heating; (b) at the end of the first decomposition process (600 °C); (c) at the end of the second decomposition process (820 °C); (d) IR spectrum of pure sodium carbonate after heating at ~ 800 °C in static air; (e) at the end of the second decomposition process, but by operating with addition of ammonium sulphate. All the decomposition processes occur in static air.

ammonium sulphate, also in the case of free and glycoconjugated sodium cholates, the final residue is only sodium sulphate, as evidenced for example by the IR spectrum of the final thermogravimetric residue, so obtained, starting from sodium cholate (Fig. 4).

DISCUSSION

The main differences between the thermogravimetric curves, recorded in oxygen and in static air, are easily detected by comparison of the curves of Figs. 1–3: in oxygen, contrary to static air, the first decomposition process produces a strongly exothermal reaction (especially for free and glycocon-

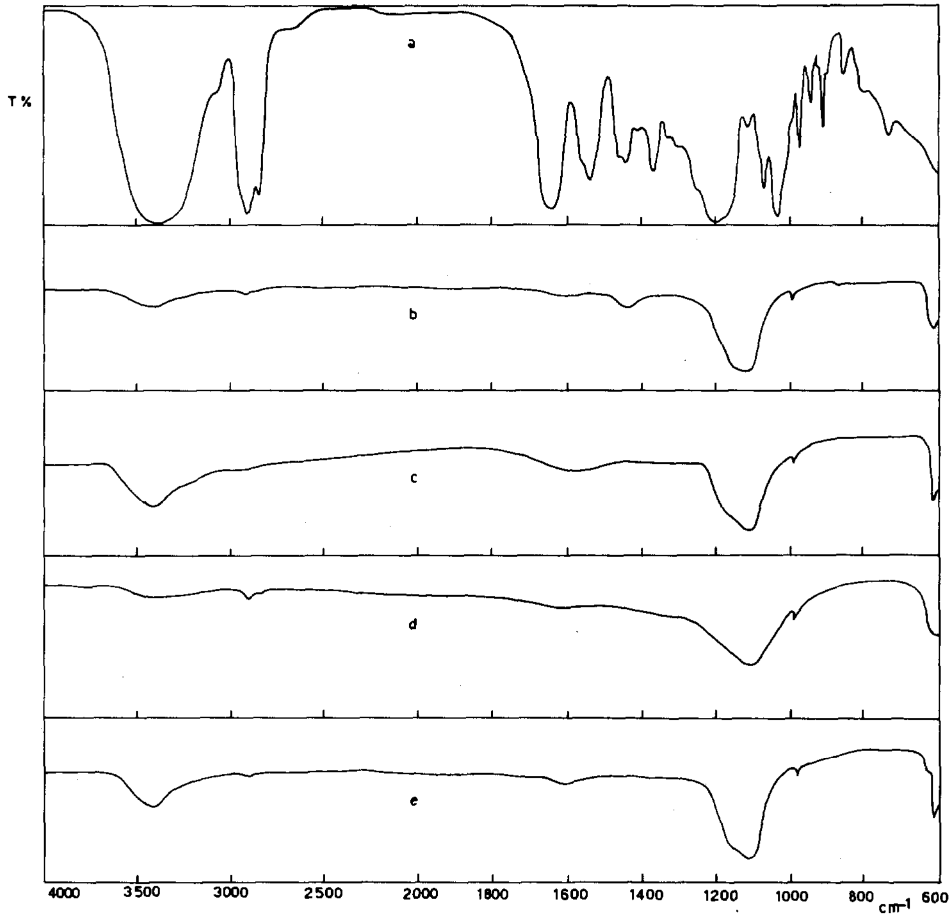

Fig. 5. IR spectra in KBr of sodium taurocholate. (a) Before heating; (b) at the end of the first decomposition process (480 °C) in static air; (c) at the end of the first decomposition process (460 °C) in oxygen flux; (d) at the end of the second decomposition process (720 °C) in static air; (e) IR spectrum of pure sodium sulphate after heating at ~ 800 °C in static air.

jugated cholates, behaving similarly) which leads to a residue of sodium carbonate or sulphate with little associated carbon. In oxygen atmosphere for tauroderivatives, at the end of the first decomposition process, the residue does not contain carbonate traces associated with sulphate and carbon, as occurring in static air (Fig. 5). Nevertheless, the analytical data (Tables 3 and 4) show that the results obtained in static air are generally slightly better than those obtained in oxygen stream; this is probably due to the markedly high speed and exothermicity of the first decomposition process in oxygen, thus inducing small movements of the crucible with splashes and sputtering of the substance, which give the method a lower accuracy and precision. Analogous problems have been previously documented in communications concerning the thermogravimetry of some anti-

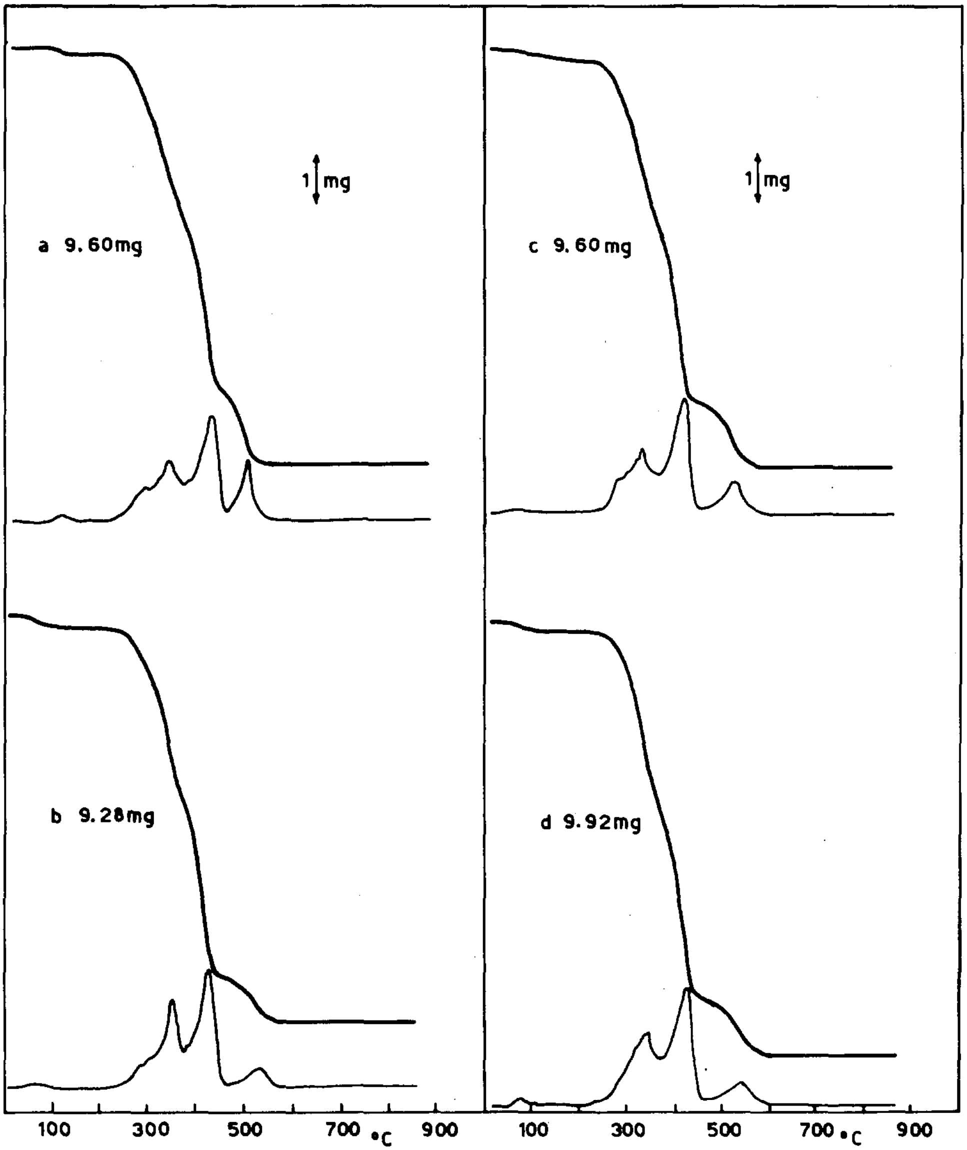

Fig. 6. TG and DTG of some unconjugated and glycoconjugated sodium cholates with the addition of ammonium sulphate (A.S.). Heating rate 10 °C min^{-1}, static air. (a) Cholate with 52.0% (w/w) A.S.; (b) deoxycholate with 50.3% (w/w) A.S.; (c) glycocholate with 46.2% (w/w) A.S.; (d) glycodeoxycholate with 49.7% (w/w) A.S.

biotics [9,11]. Under the analytical profile the results of Tables 3 and 4 show that for the tauroconjugated cholates accuracy and precision are undoubtedly good, even when compared with the corresponding values in the previous analyses of the antibiotics. For the unconjugated cholates, precision is 4.5% in static air, 6.1% in oxygen, and 4.7% with the addition of

TABLE 2

Thermal analysis of the compounds in static air and in oxygen stream. The wt.% of the residues at the end of each process refer to the anhydrous compounds. Humidity (or crystallization solvent) are expressed as a fraction of the anhydrous compounds

Sodium salt	Humidity or crystallization solvent loss				First process					Second process				
	Static air		Oxygen		Calcd.%	Static air		Oxygen		Calcd.%	Static air		Oxygen	
	Found %	pdt	Found %	pdt		Found %	pdt	Found %	pdt		Found %	pdt	Found %	pdt
Cholate	3.6	80	3.6	70	—	29.8	320	16.3	310	12.3	12.3	600	12.8	580
		110		110			450		525			745		665
		160		150			600		580			820		710
Deoxycholate	7.1	30	6.9	30	—	19.9	310	22.3	310	12.8	13.3	630	12.4	590
		75		75			450		525			770		760
		140		140			630		590			830		820
Chenodeoxycholate	9.8	30	9.7	30	—	28.8	300	18.4	280	12.8	12.7	610	12.2	610
		70		60			460		535			725		775
		200		180			610		610			800		820
Lithocholate	2.0	30	2.0	25	—	23.4	280	17.6	260	13.3	13.3	610	14.2	565
		50		45			490		540			715		640
		100		80			610		565			810		700

Glycocholate	10.1	40	10.0	30	—	24.9	310	14.4	310	10.9	11.3	610	11.9	540
		150		150			445		505			765		630
		200		210			610		540			780		700
Glycodeoxycholate	10.4	40	10.3	30	—	22.7	315	14.7	315	11.2	12.2	600	11.1	600
		75		60			465		510			755		685
		190		160			600		600			820		730
Glycochenodeoxy-cholate	8.5	40	8.4	30	—	22.8	320	15.9	320	11.2	11.4	620	11.7	570
		70		50			465		525			650		635
		130		120			620		570			700		700
Taurocholate	7.3	35	7.2	30	—	41.1	270	39.4	260	13.2	13.2	480	13.1	460
		75		65			390		390			535		535
		140		140			480		460			720		570
Taurodeoxycholate	8.6	35	8.5	30	—	39.7	315	50.3	310	13.6	13.7	535	13.6	480
		165		170			365		380			635		560
		190		200			535		480			760		600
Taurochenodeoxy-cholate	5.0	35	4.9	25	—	43.6	305	56.4	280	13.6	13.8	535	13.8	460
		70		70			365		365			610		535
		150		130			535		460			830		600

TABLE 3

% Sodium content of examined cholates, glycocholates and taurocholates sodium salts. Comparison of the results obtained by TG in oxygen stream and in static air with and without the addition of ammonium sulphate (reported values are the mean of three determinations)

Sodium salt	% Sodium						
	Calcd.	Found by TG (static air)	Found by TG (in oxygen)	Found by TG with A.S. added (static air)	% Difference between found and calcd. values		
					TG (static air)	TG (oxygen)	TG with A.S. (static air)
Unconjugated							
Cholate	5.34	5.33	5.56	5.34	−0.2	+4.1	0.0
Deoxycholate	5.55	5.78	5.38	5.20	+4.1	−3.1	−6.3
Chenodeoxycholate	5.55	5.52	5.30	5.61	−0.5	−4.5	+1.1
Lithocholate	5.77	5.77	6.17	5.80	0.0	+6.9	+0.5
Glycoconjugated							
Glycocholate	4.71	4.90	5.15	5.01	+4.0	+9.3	+6.4
Glycodeoxycholate	4.87	5.29	4.81	5.05	+8.6	−1.2	+3.7
Glycochenodeoxycholate	4.87	4.94	5.07	4.85	+1.4	+4.1	−0.4
Tauroconjugated							
Taurocholate	4.28	4.28	4.25		0.0	−0.7	
Taurodeoxycholate	4.41	4.44	4.40		+0.7	−0.2	
Taurochenodeoxycholate	4.41	4.47	4.47		+1.4	+1.4	

A.S. = ammonium sulphate.

TABLE 4

Precision of sodium analysis in two typical unconjugated and tauroconjugated sodium cholates by TG in oxygen stream and in static air with and without addition of ammonium sulphate

Sodium salt	% Sodium									
	Calcd.	Found (in oxygen)	Mean	SD %	Found (static air)	Mean	SD %	Found (static air with A.S. added)	Mean	SD %
Sodium cholate	5.34	5.53			5.21			5.34		
		5.34			5.77			5.28		
		5.23	5.56	6.1	5.21	5.33	4.5	5.80	5.34	4.7
		6.21			5.38			5.05		
		5.51			5.08			5.21		
Sodium tauro-cholate	4.28	4.31			4.24					
		4.47			4.47					
		4.18	4.25	3.7	4.05	4.28	3.5			
		3.99			4.41					
		4.28			4.21					

A.S. = Ammonium sulphate.

ammonium sulphate (Table 4), while the accuracy varies greatly with the specific compound considered and with the experimental conditions (Table 3). For these compounds, independent of the experimental conditions, a purity control can, however, be performed by thermoanalysis, but with lower values of accuracy and precision when compared with those of the analyses of antibiotics and taurocholates. These analytical results can be easily interpreted in light of the results of the analysis of the residue from thermogravimetry of the compounds examined. A residue of sodium sulphate, stable up to > 900 °C is obtained with taurocholates [14], however, in the case of free and glycoconjugated cholates, a final residue is obtained at a temperature ranging from 700 to 800 °C, according to the particular compounds considered and the experimental conditions. This residue is formed by sodium carbonate, which slowly starts to decompose at ~ 700°C [14] and is much less stable than sodium sulphate, so that accuracy and precision of the analytical data are lowered. When ammonium sulphate is added, a final residue of sodium sulphate may be obtained instead of carbonate, but real improvement of the analytical quality of the results was not obtained. The sources of error are obviously different in this case, they are not related to the thermal stability of the residue, but ascribed to the experimental problems, as these compounds do not contain a sulphur atom in their molecules, therefore it is more difficult to get all of the sodium contained in the molecule completely transformed into sodium sulphate so that a more laborious preparation of the sample for analysis is required. The humidity content or the crystallization solvent of the considered compounds can be determined easily, precisely and accurately by thermogravimetric analysis and the purity control (yield of the method) is only limited to the presence of substances which affect the found percentage of sodium content, in comparison with the nominal value (for example impurities of sodium carbonate, or of a cholanic acid not salted, contained in the analysed sodium cholates). The presence of substances, such as impurities and isomers of those substances analysed (for example cheno- and urso-sodium cholates, contained in the deoxycholate, or vice versa) cannot be detected.

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