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Analytical studies on the hydrolysis of Chinoin-102 and Chinoin-127, candidate compounds for stability studies*

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Abstract: The isolation and structural elucidation of some products of hydrolytic studies with two drugs, Chinoin-102 and Chinoin-127, are described.

CH-127 is 1,6_{ax}-dimethyl-4-oxo-1,6,7,8,9,9a-hexahydro-4H-pyrido-[1,2-a]-pyrimidine-3-carboxamide. CH-102 is 8,9-dimethoxy-3-imino-5,6-dihydro-3H-thiazolo-[4,3-a]-isoquinoline-1-carbonitrile. Degradation of the active compounds was studied by hydrolysing them in acidic, neutral and alkaline media. The isolated degradation products were identified by MS and IR techniques. The products identified were: for CH-127, 1,6-dimethyl-4-oxo-1,6,7,8-tetrahydro-4H-pyrido-[1,2-a]-pyrimidin-3-carboxamide; for CH-102, acid amide and acid derivatives. For the selective determination of the drugs and their degradation products, UV spectrophotometric and densitometric methods were applied.

Keywords: *Drug stability; degradation; quality control; ultraviolet spectrophotometry; densitometry.*

Introduction

The manufacturer of a new medicine must ensure that its quality remains acceptable during the shelf-life [1, 2]. The task of establishing the shelf-life can be divided into two parts: development of stability-indicating methods; and determination of the shelf-life with the aid of those analytical methods using long-term stability tests or accelerated stability tests in which different mathematical models can be applied.

There have been many articles and seminars in the past two decades on various aspects of stability. However, the literature on the development of stability-indicating assays, from preparation of the degradation products to quantitative analytical methods, is limited. The present paper describes the development of analytical methods suitable for stability studies of two new drugs, Chinoin-102 and Chinoin-127 (Fig. 1).

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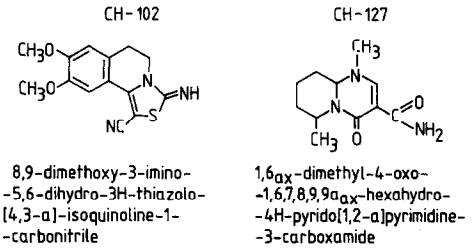

8,9-dimethoxy-3-imino--5,6-dihydro-3H-thiazolo-[4,3-a]-isoquinoline-1--carbonitrile

$1,6_{ax}$-dimethyl-4-oxo--1,6,7,8,9,$9a_{ax}$-hexahydro--4H-pyrido[1,2-a]pyrimidine--3-carboxamide

Figure 1
The structural formulae of CH-127 and CH-102.

Experimental

Apparatus

Ultraviolet spectra were recorded on a SP8-100 spectrophotometer (Pye–Unicam, Cambridge, UK). Densitometric measurements were made using a Shimadzu High Speed TLC Scanner CS-920 (Shimadzu Corp., Kyoto, Japan). MS spectra were recorded on a JEOL MS-D-300 GC–MS instrument (Japan). Sample introduction was by direct inlet. Sample heating was programmed to rise 20°C/min. Temperature of ion source was 150°C. Mode of ionization was electron impact. Electron energy was 70 eV. Ion current was 300 μA. Accelerating voltage was 3 kV. For the high-resolution measurements, R = 10 000. IR-spectra were recorded using a Spectromom 2000 instrument (MOM, Budapest, Hungary).

Materials

CH-102, CH-127 and their degradation products were prepared in the research laboratories of Chinoin Pharmaceutical and Chemical Works, Ltd, Budapest. The two drugs have been subjected to pharmacological and clinical investigations.

CH-102 is intended for the treatment of angina whereas CH-127 [3] has analgesic and anti-inflammatory effects.

Heat treatment

For CH-102: 2-g samples were boiled for 5 h in 50 ml of 1 M HCl, 50 ml of 1 M NaOH and 50 ml of distilled water, respectively, using a reflux condenser.

For CH-127: 3-g samples were boiled for 32 h in 50 ml of 0.1 M HCl and in Britton–Robinson buffers of pH: 3, 7 and 11, respectively, using a reflux condenser.

Thin-layer chromatography (TLC)

A number of chromatographic systems have been published for isoquinoline, pyridine and pyrimidine compounds. The main components of the systems are chloroform and methanol [4, 5].

For CH-102. Various neutral solvents were tried; a mixture of chloroform–methanol (70:30, v/v) effected separation, but the spot of the drug itself was not compact enough. An acidic system was ineffective for separation, R_f values of the degradation products were low and close. A chloroform–methanol mixture containing a base gave good separation and compact spots.

For CH-127. The chloroform–methanol mixtures containing an acid or a base were ineffective for separation; the R_f values were high and too close. A polar solvent was

Table 1
Thin-layer chromatography* of Chinoin-102 and Chinoin-127

TLC system	CH-127	CH-102
Adsorbent	Kieselgel $60F_{254}$	Kieselgel $60F_{254}$
Solvent	Chloroform–methanol–acetic acid–acetone (90:10:1:20, v/v/v/v)	Chloroform–methanol–25% ammonium hydroxide (70:20:2.5, v/v/v)
Presaturation time (min)	30	30
Running height (cm)	15	15
Running time (min)	90	90
Visualization	$UV_{254\ nm}$ I_2 vapour	$UV_{254\ nm}$ I_2 vapour
Amount applied (μg)	600	200

*Before application of the solutions, the pH was adjusted to 7.

added to the system containing acetic acid; this gave lower R_f values and improved separation. The final conditions for TLC are outlined in Table 1.

Isolation of degradation products

CH-102. Both degradation products were isolated from the solution containing 1 M NaOH that had been boiled for 5 h. The suspension was extracted with chloroform, then evaporated to dryness at room temperature. The R_f of the residue subjected to TLC was 0.74.

The pH of the aqueous phase was adjusted to pH 7 with 1 M HCl. The suspension was evaporated to dryness, extracted with methanol, then again evaporated to dryness. The R_f of the residue subjected to TLC was 0.20.

CH-127. The sample was hydrolysed for 32 h in Britton–Robinson buffer (pH 3) then 0.02 ml of the solution was transferred to a preparative layer. Development was effected as described above; the spot of the main degradation product (R_f 0.66) was eluted with methanol and dried under vacuum. The degradation product with an R_f of 0.37 could not be isolated.

Spectrophotometric determination of CH-127

The UV-absorption spectrum of the drug was measured at 318 nm in a 1-cm cell using a solution containing 10 μg/ml in 0.1 M HCl.

Results and Discussion

Isolation and identification of the structure of the degradation products

Induction of degradation of the active compound. Four types of activation can bring about chemical change: thermal, photochemical, electrochemical and radiochemical activation [1, 2]. Under normal conditions only thermal and photochemical activation

are important. Photochemical sensitivity has to be investigated in the preliminary experiments. It is advisable to use a xenon lamp because its spectrum is similar to that of natural light [1]. Photochemical change is often accompanied by organoleptic change. Photochemical sensitivity can be avoided by proper packaging. Thermal effects should be taken into account and experiments carried out by appropriate heat treatment. The degree of heat treatment needed to cause degradation depends upon the molecule to be studied. In order to explore the degradation of the molecule, it is advisable to carry out heat treatment in acidic, neutral and alkaline media; the results provide information about the ideal pH range for the formulation of a solution.

CH-127 and CH-102 were heat-treated as described in the experimental part. On the basis of earlier experiments CH-102 seemed to be relatively stable, so that strong conditions (1 N acid or base) were chosen for heat treatment. CH-127 was heat-treated at different pH values.

Investigation of the degraded samples. The aim of the investigation of heat-treated samples was to separate, isolate and identify the degradation products. The heat-treated samples were examined using TLC. Chromatograms of the two heat-treated samples are shown in Figs 2–4. It can be seen that CH-127 was relatively stable in a neutral medium but degraded in acidic and alkaline media. The main degradation product in HCl had an

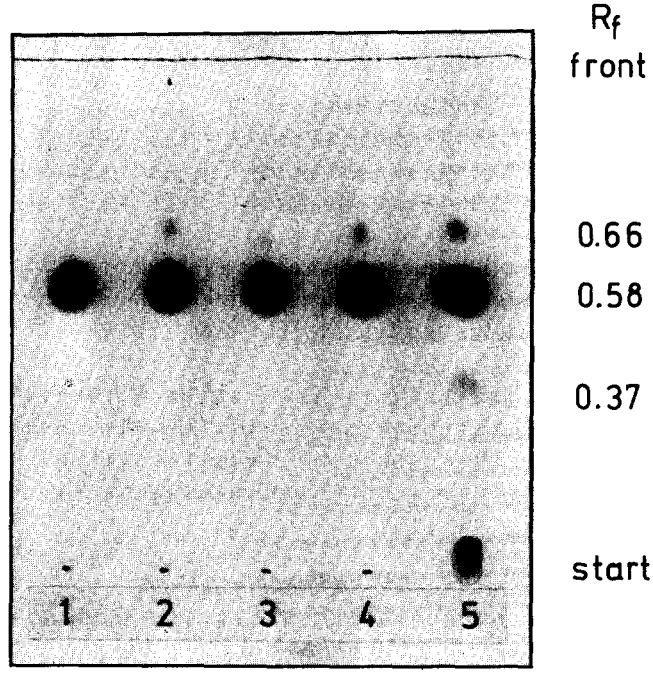

Figure 2
TLC chromatogram of CH-127.
Samples applied:
(1) CH-127 control, 600 μg;
(2) CH-127 hydrolysed in buffer (pH 11), 600 μg;
(3) CH-127 hydrolysed in buffer (pH 7), 600 μg;
(4) CH-127 hydrolysed in buffer (pH 3), 600 μg;
(5) CH-127 hydrolysed in 0.1 M HCl, 600 μg.

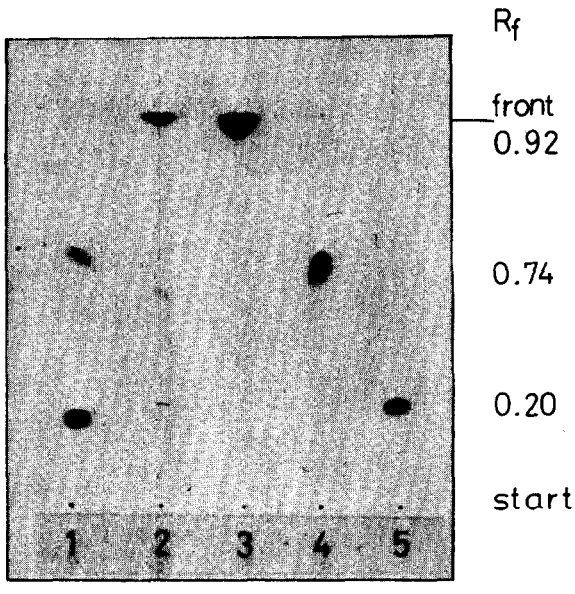

Figure 3
TLC chromatogram of CH-102.
Samples applied:
(1) CH-102 hydrolysed in 1 M NaOH, 0.01 μl of filtrate;
(2) CH-102 hydrolysed in 1 M NaOH, precipitate dissolved in methanol, 200 μg;
(3) CH-102 control, 200 μg;
(4) CH-102 carboxamide derivative, 20 μg;
(5) CH-102 carboxylic acid derivative, 20 μg.

R_f of 0.66. A spot with an R_f of 0.37 was also detected (Fig. 2). CH-102 was stable in acidic and neutral media. The degradation products in an alkaline medium produced spots with R_f values of 0.74 and 0.20 (Figs 3 and 4).

Isolation of the degradation products. The isolation of degradation products can be effected by extraction, preparative TLC or preparative HPLC. In the present work extraction and elution were used as described in the experimental part. For the degradation of CH-127, the spot with an R_f of 0.37 could not be isolated.

Identification of the structure of the degradation products. For the identification of the structure of degradation products different methods are available. Mass spectroscopy, NMR and IR spectroscopy are mostly used; X-ray diffraction is applied occasionally. The structure is unequivocally established if the spectroscopic and TLC behaviour of the synthetic product is identical with that of the isolated product.

MS and IR spectroscopic methods were used in the present work. Evaluation of the spectra for the unknown products established their structures unequivocally in all instances. MS and IR spectra of the degradation products of CH-127 and CH-102 are shown in Figs 5, 6 and 7–10, respectively. These figures illustrate the interpretation of the low resolution spectra, as well as the formulae derived from the molecule ion and from the high resolution spectra of the selected fragments.

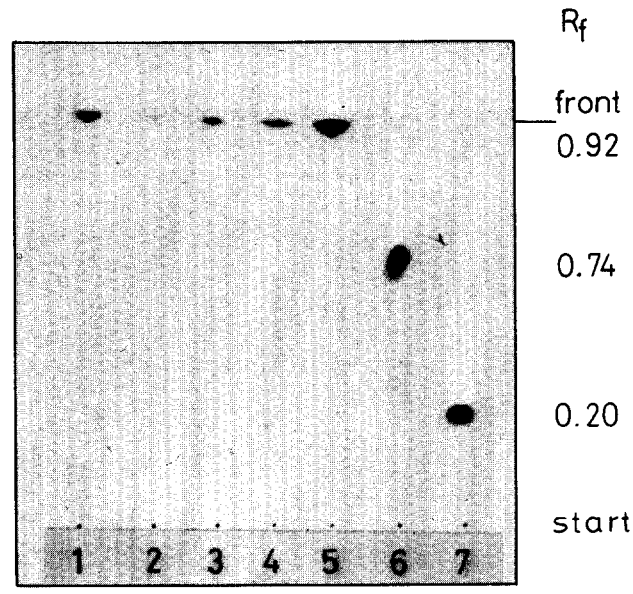

Figure 4
TLC chromatogram of CH-102.
Samples applied:
(1) CH-102 hydrolysed in 1 M HCl, precipitate dissolved in methanol, 200 μg;
(2) CH-102 hydrolysed in 1 M HCl, 0.01 μl of filtrate;
(3) CH-102 hydrolysed in distilled water, precipitate dissolved in methanol, 200 μg;
(4) CH-102 hydrolysed in distilled water, 0.01 μl of filtrate;
(5) CH-102 control, 200 μg;
(6) CH-102 carboxamide derivative, 20 μg;
(7) CH-102 carboxylic acid derivative, 20 μg.

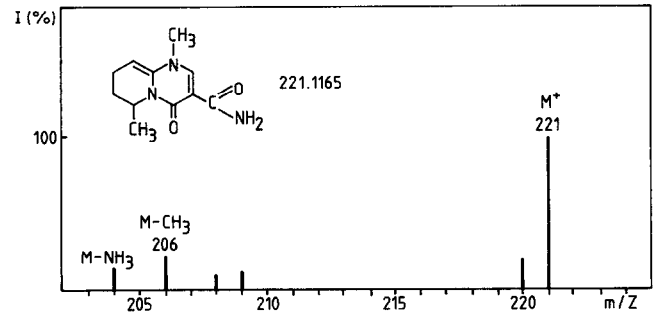

Figure 5
MS spectrum of CH-127 degradation product R_f 0.66.

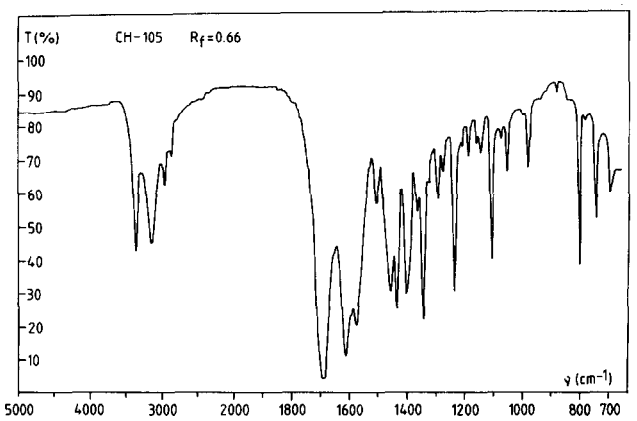

Figure 6
IR-spectrum of CH-127 degradation product R_f 0.66.
Evaluation:
3360 cm^{-1}
3155 cm^{-1} $\upsilon - NH_2$

2960 cm^{-1}
2970 cm^{-1} $\upsilon - CH_3; CH_2$

1685 cm^{-1} $\upsilon - C = O$ and $-C(=O)-NH_2$
1612 cm^{-1} $\upsilon - NH_2$

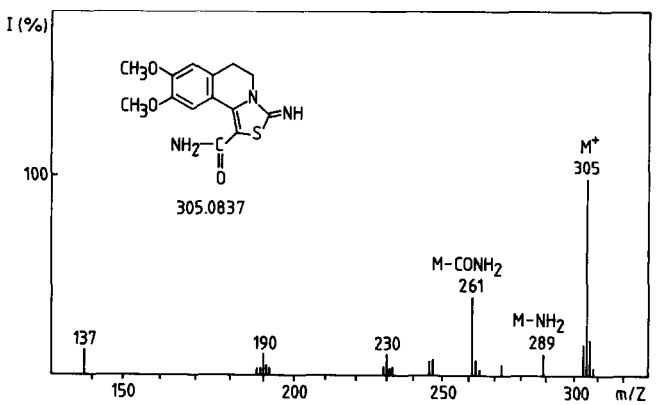

Figure 7
MS spectrum of CH-102 degradation product R_f 0.74.

The compounds identified by their IR and MS spectra were synthesized. The R_f values and the MS and IR spectra of the synthesized and isolated products were identical. On the basis of the structures established for the degradation products, reaction schemes for degradation of the active compounds are illustrated in Figs 11 and 12. It can be seen that the degradation of the two molecules follows different reaction pathways. The most common pathway of the degradation of drugs is hydrolysis and this was found with CH-102 (Fig. 12). Oxidation is a second possibility, as was found for CH-127 (Fig. 11).

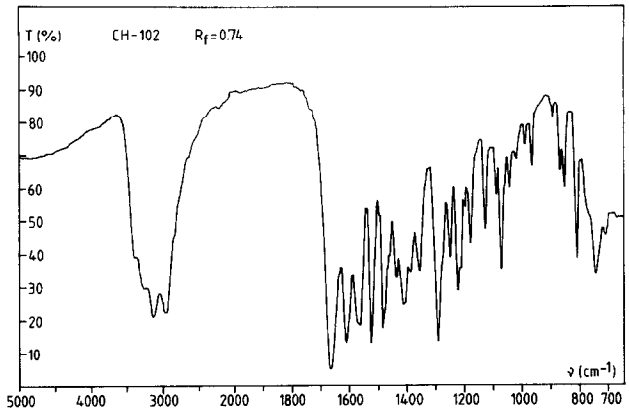

Figure 8
IR spectrum of CH-102 degradation product R_f 0.74.
Evaluation:
3400 cm^{-1} / 3260 cm^{-1} $\upsilon - NH_2$

3130 cm^{-1} $\upsilon = NH$
2910 cm^{-1} $\upsilon - CH_3$
1664 cm^{-1} $\upsilon - C = O$ and $= NH$
1290 cm^{-1} / 1070 cm^{-1} $\upsilon - OCH_3$

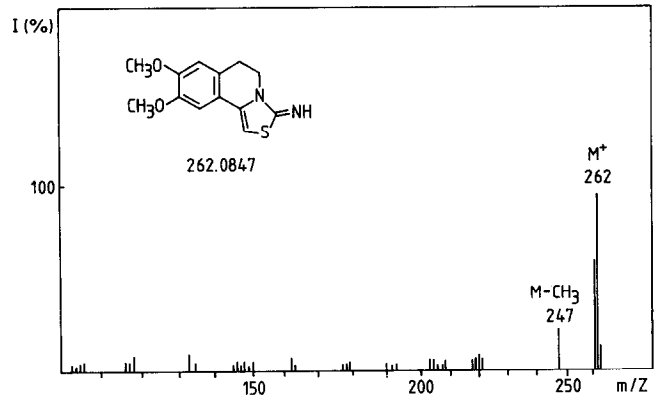

Figure 9
MS spectrum of CH-102 degradation product R_f 0.20.

The development of analytical methods which are specific for the degradation products or the active ingredient

Choice of methods. The main desirable characteristics of analytical methods used in stability studies are selectivity, routine applicability, sensitivity and economy. It is advisable to develop an analytical method for the determination of degradation products since this approach results in an error that is smaller by one order of magnitude than that for methods used for the determination of the active ingredient. Spectroscopic,

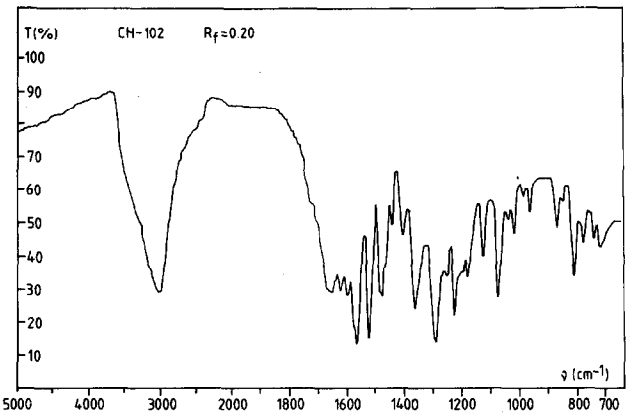

Figure 10
IR spectrum of CH-102 degradation product R_f 0.20.
Evaluation:

3500–3050 cm^{-1}	υ – OH, = NH
	υ – C = O and = NH
1660 cm^{-1}	
1290 cm^{-1}	υ – OCH_3
1075 cm^{-1}	

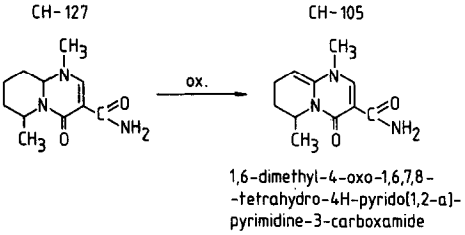

Figure 11
Degradation scheme for CH-127.

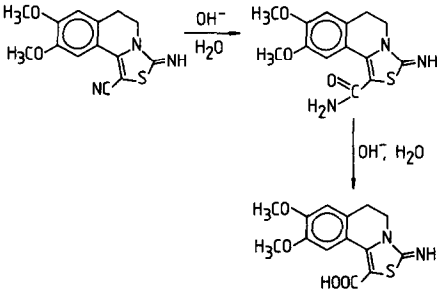

Figure 12
Degradation scheme for CH-102.

chromatographic and electrometric methods are now used in most cases [6]. In addition to UV and visual spectroscopy, fluorimetric methods [7, 8] and quantitative NMR [9] have been reported. Of the chromatographic methods available, HPLC [10] and gas chromatography [11] are analytical methods which can be used routinely. Even nowadays polarography and voltammetry should be considered for some drugs instead of photometry and chromatography [12, 13].

Determination of CH-127. It was supposed that the spot with an R_f of 0.37 represented a secondary degradation product. To confirm this, CH-105 was heat-treated for 80 h at 100°C in 0.1 M HCl; the samples were examined by TLC as discussed before.

Figure 13 illustrates that this assumption was correct and the spot with an R_f of 0.37 was formed both from CH-127 and from CH-105. Since the primary degradation product at the neutral pH of the formulated product is CH-105, which does not degrade further (even after strong heat-treatment), the stability tests were based on the simultaneous determination of CH-127 and CH-105. The difference in the spectroscopic properties of the two molecules suggested the use of a spectrophotometric assay. The spectra shown on Fig. 14 show that the active ingredient can be selectively measured at 318 nm.

Determination of CH-102. Since the spectral parameters of the drug and the degradation products were almost identical, a degradation-specific TLC method was

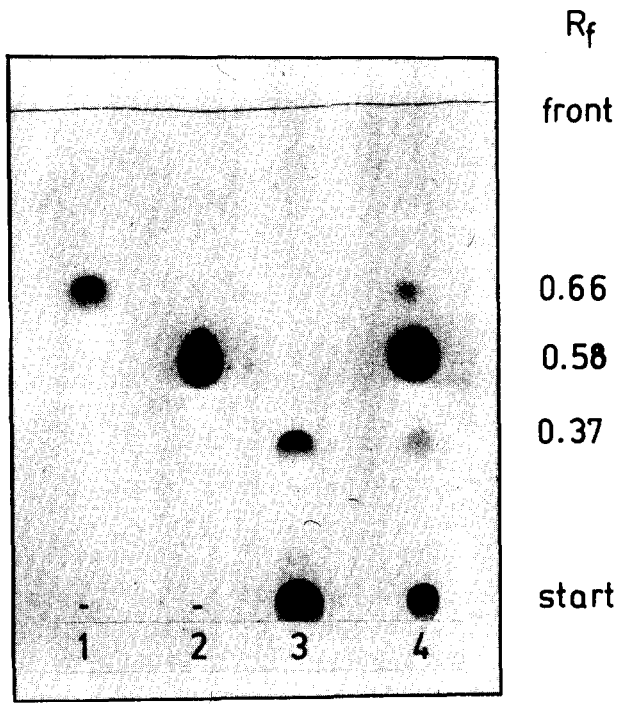

Figure 13
TLC chromatogram of CH-105 and CH-127.
Samples applied:
(1) CH-105 control;
(2) CH-127 control;
(3) CH-105 hydrolysed in 0.1 M HCl;
(4) CH-127 hydrolysed in 0.1 M HCl.

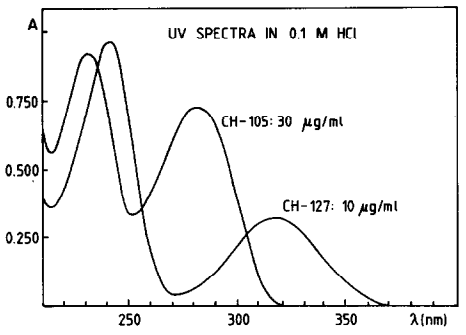

Figure 14
UV spectra of CH-105 and CH-127 in 0.1 M HCl.

developed utilizing the difference in their polarity; the acid amide and acid derivatives were determined using densitometry.

Concluding Remarks

As in other branches of science, there is now a tendency for specialization in the field of analytical chemistry. There are experts in techniques such as electrochemistry, chromatography and spectroscopy and within these techniques further specialization can be observed. This might lead to a situation where there would be no analytical chemist to choose the right method for a given task.

In the present paper the authors have tried to demonstrate that stability investigation is a field which needs a non-specialized analytical chemist who approaches the problem from the point of view of the task and not from the methods.

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