III. ESTIMATION OF SRC-820

CHAPTER III

ESTIMATION OF SRC-820

Introduction:

Several methods including gas-liquid chromatography have been described for the determination of methaqualone - a quinazolinone derivative having o-tolyl substituent at 3 position (Douglas, 1973; Mordbo, 1977; Cf. Kar and Umeokafor, 1979). Also, a non-aqueous titration method is cited in the British Pharmacopoeia (1973) for its estimation. Maggiorelli and Gangemi (1964) described another method based on alkaline hydrolysis of methaqualone followed by titration with sodium nitrite. The fluorimetric method of Brown and Smart (1969) based on the reduction of methaqualone with lithium borohydride is also available which can be applied for the estimation of SRC-820. However, adaptation of the alkaline hydrolysis method for the estimation of SRC-820 [2-methyl-3(3'-methyl-2'-pyridyl)-4 (3H) quinazolinone] was found to be unsatisfactory as regards to linearity and reproducibility. It was felt, therefore, necessary to devise a simple and reliable method for the estimation of SRC-820,—a method within the competence of small laboratories.

Results:

At the outset, acid hydrolysis of the compound was studied. The acids used were: hydrochloric, acetic and sulphuric. When hydrochloric or acetic acid was used followed by diazotization of the hydrolysate, the results were found to vary widely from one set of experiment to another. However, such variations were not observed when sulphuric acid was employed.

With sulphuric acid, the optimum time of hydrolysis of SRC-820 and the optimum concentration of the acid were determined and they were found to be 30 min. and 3.5 N respectively. Further increasing the time of hydrolysis or the strength of acid resulted in reduction in the intensity of final colour, obtained after diazotization and coupling with β-naphthol.

It was thought desirable to find out whether any change in the absorption spectrum of SRC-820 has occurred during acid hydrolysis with or without prior reduction with lithium borohydride. For this, ultraviolet absorption of pure and reduced SRC-820 were followed in 0.01 N sulphuric acid before as well as after the acid hydrolysis, using 'UVISPEK' spectrophotometer (Model-H, Hilger and Watts).

Characteristic UV absorption spectra were obtained for both pure and lithium borohydride reduced SRC-820 (Fig.2A-a and Fig.2B-a). Three peaks of pure SRC-820 could be identified at 225 nm, 260 nm and 300 nm, respectively. However, on reduction, there is a hypsochromic shift of 5 nm in the first two peaks (now at 230 nm and 265 nm) whereas the peak at 300 nm remained unchanged (Fig.2B-a).

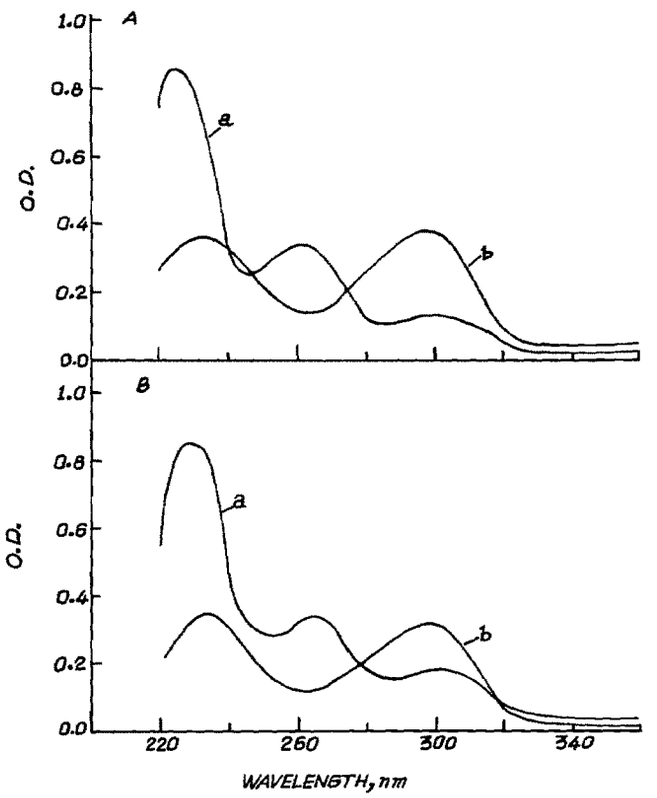
After acid hydrolysis (without the preliminary reduction with $LiBH_4$) in 4.5 N sulphuric acid for 30 min, absorption spectrum of SRC-820 was found to have changed significantly (Fig.2A-b). About 60% reduction in O.D. at 225 nm peak and the three-fold increase at 300 nm peak could be observed. A similar pattern of change in the absorption was observed when SRC-820 was first reduced with lithium borohydride and then subjected to acid treatment (Fig.2B-b).

Methaqualone when treated under identical conditions gave two peaks: one at 230 nm and another at 265-270 nm. After acid hydrolysis in 4.5 N sulphuric acid even upto 1 hour, no such change in the UV spectrum of methaqualone could be observed. Therefore, further experiments with methaqualone were not carried out.

In the case of SRC-820, attempts were made to separate and identify the products after sulphuric acid

Fig.2

Absorption spectra of SRC-820



A. Pure SRC-820

a) before acid hydrolysis
b) after acid hydrolysis

B. Reduced SRC-820

a) before acid hydrolysis
b) after acid hydrolysis

treatment using thin layer chromatography (TLC).

Of the three solvent systems tried, good resolution was achieved in n-butanol:glacial acetic acid:water (4:1:5) solvent system. The components were detected by viewing the plates under UV as well as using Dragendorff's reagent (Block et al, 1958). Two major components having Rf values 0.86 (Component-I) and 0.80 (Component-II) could be detected by viewing the TLC plates in UV light. Anthranilic acid, under these conditions was found to have an Rf value of 0.86. Component-I having the higher mobility exhibited an intense fluorescence when viewed under UV light and reacted with Dragendorff's reagent. Component-II had a weak fluorescence and did not react with Dragendorff's reagent.

The Component-I after elution in 95% ethanol showed a major absorption peak at 240 nm and a minor one at 260 nm. The same substance in dilute hydrochloric (HCl) or sulphuric acid (H_2SO_4) had an absorption maximum in the range of 290-295 nm. Component-II in 95% ethanol showed a single peak at 270 nm.

Component-I on diazotization and coupling with β-naphthol yielded a dye with an absorption maximum at 380 nm. Component-II, on the other hand, did not undergo diazotization.

After acid hydrolysis of SRC-820, diazotization was carried out treating the hydrolysate with freshly prepared sodium nitrite (0.1 M) in icebath (0-4°C) for 1 min. The diazo derivative thus formed (yellowish in colour) was coupled with β-naphthol (7.2 mg%) under an alkaline condition. The violet colour of the dye was estimated at 380 nm (the absorption maximum, as observed) in the 'UVISPEK' x spectrophotometer.

Under the optimum conditions of assay, the colour-formed obeys Beer's law and is linear even upto 200 µg of SRC-820. The method as finally adopted which does not involve preliminary reduction with $LiBH_4$ is described as follows:

Method for the Estimation of SRC-820:

An accurately weighed amount of SRC-820 was dissolved in 1 N sulphuric acid and diluted further to give a concentration of 100 µg/ml. This formed the stock standard and an acid strength of 0.01 N. One ml of 7 N sulphuric acid was added to 1 ml of an appropriately diluted standard solution and mixed. Blanks contained 1 ml of 0.01 N sulphuric acid and 1 ml of 7 N sulphuric acid. Hydrolysis was carried out in a boiling waterbath for 30 min. Care was taken to minimise evaporation by covering the mouths of the test tubes with glass bulbs. Then the tubes were cooled, transferred to an icebath, 0.2 ml of freshly prepared 0.1 M sodium nitrite ($NaNO_2$)

added and mixed, and exactly after one min, 1 ml of 8 N sodium hydroxide was added. This was followed by the addition of 4 ml of 7.2 mg% (w/v) solution of β-naphthol in 2 N sodium hydroxide. The colour was read after 15 min. at 380 nm, against the blank.

Reproducibility of the method was tested on four different days as shown in the Table-II.

The mean O.D. for 10 µg of SRC-820 was found to be 0.093 ± 0.004.

Change in the O.D. at 300 nm after acid hydrolysis was also followed for the estimation of SRC-820. For this, after acid hydrolysis, different aliquots were taken and the total volume made to 5 ml with water giving a final concentration of 2 to 16 µg per ml and then their O.D. determined at 300 nm.

The increase in O.D. at 300 nm was found to be proportional (Table-III) to the amount of SRC-820 taken (2 to 16 µg).

Thus, by means of the colourimetric method, SRC-820 could be estimated upto as high as 200 µg whereas, the increase in absorption at 300 nm after acid treatment alone could be applied for determining small amounts of SRC-820 in the range of 2 to 16 µg. Recovery of SRC-820 added to serum ranged between 101-114.4%.

TABLE-II

ESTIMATION OF SRC-820 (COLOURIMETRIC METHOD)

Concentration of SRC-820 (µg)	Range of O.D. at 380 nm (n = 4)	Mean O.D. ± S.D.
10	0.088 - 0.098	0.093 ± 0.004
20	0.181 - 0.192	0.189 ± 0.005
30	0.282 - 0.293	0.286 ± 0.004
40	0.382 - 0.391	0.387 ± 0.003
50	0.471 - 0.480	0.477 ± 0.004
60	0.585 - 0.606	0.596 ± 0.008
70	0.684 - 0.698	0.690 ± 0.005
80	0.780 - 0.801	0.792 ± 0.006
100	0.977 - 0.988	0.983 ± 0.004

Time of hydrolysis, 30 min; acid : H_2SO_4, 3.5 N. The hydrolysate was diazotized and coupled with β-naphthol. The colour of the dye was estimated at 380 nm; n = number of experiments.

TABLE-III

ESTIMATION OF SRC-820 (SPECTROPHOTOMETRIC METHOD)

Sr. No.	Concentration of SRC-820 (µg)	Increase in O.D. at 300 nm (after acid treatment)
1.	Nil	0.000
2.	2	0.035
3.	4	0.075
4.	6	0.111
5.	8	0.152
6.	10	0.183
7.	12	0.222
8.	14	0.273
9.	16	0.307

Time of hydrolysis; 30 min; acid 3.5 N H_2SO_4

Prior adjustment of the serum pH to values ranging from 6 to 8 before extraction resulted in 88.8% to 108.6% recovery, thus showing that the pH of serum in the range of 6-8 had little effect on recovery of SRC-820.

SUMMARY

1. SRC-820 in 0.01 N HCl or H_2SO_4 has three absorption maxima at 225 nm, 260 nm and 300 nm.

2. Reduction of SRC-820 with lithiumborohydride ($LiBH_4$) results in a hypsochromic shift of about 5 nm, in the first two absorption peaks, 225 nm and 260 nm.

3. Absorption spectrum of SRC-820 changes significantly after acid (H_2SO_4) treatment. About 60% reduction in the O.D. at 225 nm with a simultaneous three-fold increase in that at 300 nm could be seen. A similar pattern of change occurs when SRC-820 was first reduced and then treated with sulphuric acid.

4. Absorption maxima of methaqualone are not changed after acid treatment.

5. Acid hydrolysate, when subjected to thin layer chromatography (TLC), revealed the presence of two components (Rf. values 0.8 and 0.86).

6. The faster moving component (Rf. 0.86) exhibits intense fluorescence under UV light and has, in ethanol, a major absorption peak at 240 nm and a minor one at 260 nm. In dilute acid, it has a single absorption maximum at 290 nm. It can

be identified with Dragendorff's reagent. This component, after diazotization and coupling with β-naphthol, yields a yellow dye which has an absorption maximum at 380 nm.

7. The slower moving component (Rf. 0.8) shows weak fluorescence under UV light. It does not react with Dragendorff's reagent and has a maximum absorption at 260 nm in ethanol. It does not undergo diazotization reaction.

8. A new colourimetric method for the estimation of SRC-820 is devised, based on preliminary acid hydrolysis and diazotization reaction. The details are described in the text. This method can be used for estimating upto 200 µg of SRC-820.

9. The increase in O.D. at 300 nm (after acid hydrolysis and before diazotization) is also linear from 2 to 16 µg of SRC-820 and this property can also be utilized for the determination of SRC-820 in the lower ranges. Also, this increase in O.D. at 300 nm correlates well with the colour obtained after diazotization and coupling.

10. The method is also applicable for estimating SRC-820 in biological fluids such as serum. Recovery experiments show that SRC-820 can be extracted almost completely within a pH range of 6-8 of serum.