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# QUANTITATION OF ETHACRYNIC ACID BY HIGH-PRESSURE LIQUID CHROMATOGRAPHY

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## ABSTRACT

A stability-indicating assay method for ethacrynic acid based on high pressure liquid chromatography was developed. The method developed is sensitive, accurate and precise (standard deviation based on 6 readings was ± 1.5%). It was used to study the stability of ethacrynic acid in various aqueous systems. The stability tests were conducted at room temperature as well as at 65°, at various pH values and using different vehicles. The data were treated mathematically to estimate various K values. It has been shown that ammonium ion causes a fast reversible decomposition of ethacrynic acid. Formaldehyde appeared to be one of the products of decomposition.

## BACKGROUND

Ethacrynic acid (I) is used as a diuretic. It has been reported[1] that an alpha beta unsaturated ketone moiety, which is quite reactive chemically, is essential for optimum biological activity. Cohen[2] reported that ethacrynic acid can undergo dimerization under a variety of conditions, and the intact drug could be assayed in the presence of dimers by polarography. This method was later adopted by U.S.P.[3]. The purpose of these investigations was to develop a stability indicating assay method based on high pressure liquid chromatography and to study the stability of ethacrynic acid in some aqueous systems.

## MATERIALS AND METHODS

Chemicals and Reagents: All chemicals and reagents were ACS, N.F. or U.S.P. quality and used without further purification.

Aparratus: - A high pressure liquid chromatograph[4] equipped with a UV detector (254 nm), a recorder[5] and an integrator[6] was used.

Column: - A column[7] (30 cm x 4 mm i.d.) of a very nonpolar packing material consisting of a monomolecular layer of octadecyltrichlorosilane permanently bonded by silicon-carbon bonds was used.

Chromatographic Conditions: - The chromatographic solvent was 0.01M solution of $(NH_4)_2HPO_4$ in water containing 45% by volume of methanol. The temperature was ambient and the flow rate was 2.0 ml/min. The detector was set at a sensitivity of 0.16 absorbance units for full scale deflection and the chart speed was 30.5 cm per hour.

Preparation of Solutions and Dosage Forms: - All the solutions were prepared using a simple solution method and are listed in Table 1.

Assay Procedure for Ethacrynic Acid in Injections: - Dissolve the powder (the vial contained the sodium salt of ethacrynic acid

TABLE 1

List of Aqueous Solutions Prepared

| Solution No. | Conc. of I (mg/ml) | Vehicle | |
|---|---|---|---|
| 1 | 0.05 (Std. Sol.) | Water[a] | |
| 2 | 0.5 | Borate buffer | (0.04M) |
| 3 | 0.5 | $(NH_4)_2HPO_4$ buffer | (0.04M) |
| 4 | 0.5 | As above | (0.02M) |
| 5 | 0.5 | $NH_4HCO_3$ buffer | (0.04M) |
| 6 | 0.5 | As above | (0.02M) |
| 7 | 0.5 | $Na_2HPO_4$ buffer | (0.04M) |
| 8 | 0.5 | Citrate buffers of Various pH Values | (0.05M) |
| 9 | 1.0 | Phosphate buffers of Various pH Values | (0.05M) |
| 10 | 1.0 | 0.1N HCl | |
| 11 | 1.0 | 0.1N NaOH | |
| 12 | 1.0 | Sugar Solutions (10-30%) in water | |
| 13 | 1.0 | Sorbitol 50%[b] in water | |
| 14 | 1.0 | Same as above[b] except that 10% ethanol was substituted for water | |

[a] Approximately 2 ml of alcohol was used to dissolve 50.0 mg of the powder which was further diluted with water to a concentration of 50 µg/ml of ethacrynic acid.

[b] The pH was adjusted to 7.0 with a solution of NaOH (app. 0.1N). Parabens (0.005% of methyl paraben and 0.002% of propyl paraben) were added as preservatives.

TABLE 2

Assay Results of Commercial Dosage Forms

| Dosage Form | Percent of the Label Claim Found[a] |
|---|---|
| Tablet #1 | 99.6 |
| Tablet #2 | 101.6 |
| Tablet #3 | 102.5[b] |
| Injection | 102.7 |

[a] Average of two. In the case of injection, average of three. The standard deviation based on six readings was ± 1.5%

[b] Average weight of these tablets was 103.2% of the average weight of tablets in #2.

equivalent to 50.0 mg of ethacrynic acid, 62.5 mg of mannitol and 0.1 mg of thimerosal as preservative) in 50.0 ml of water. Dilute further one in twenty with water and inject 40 µl. As a control inject an identical volume of the standard solution (Solution 1 in Table 1) after the assay sample is eluted.

Assay Procedure for Ethacrynic Acid in Aqueous Solutions: - Dilute the solution to a concentration of 50 µg/ml of ethacrynic acid and inject as described above.

Assay Procedure for Ethacrynic Acid in Tablets: - Grind one tablet (claim was 25 mg of ethacrynic acid per tablet), mix it thoroughly with approximately 3 ml of alcohol and transfer to a 500 ml volumetric flask with the aid of water. Bring to volume with water, filter and inject clear filtrate as described above.

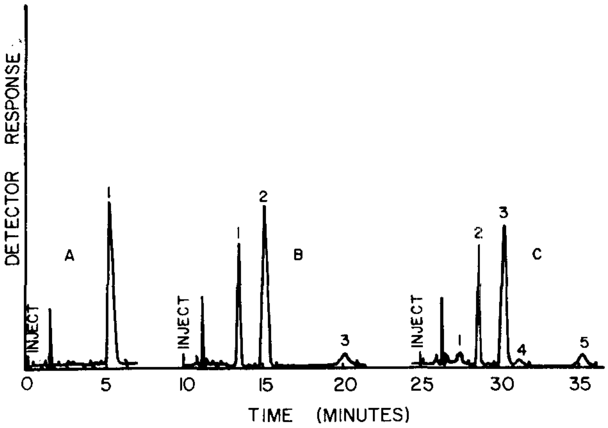


FIGURE 1

Some Sample Chromatograms; Key: A, Standard Solution of Ethacrynic Acid; B, From a Solution in 50% Sorbitol with 10% Alcohol After 21 Days of Storage at Room Temperature; Peak 2 is from Ethacrynic acid, Peaks 1 and 3 are from Methyl paraben and Propyl paraben; C, Same as B except that it was stored at 65°; Peak 3 is from Ethacrynic acid. Peaks 2 and 5 are from Methyl paraben and Propyl paraben respectively, others are from the Decomposition Products.

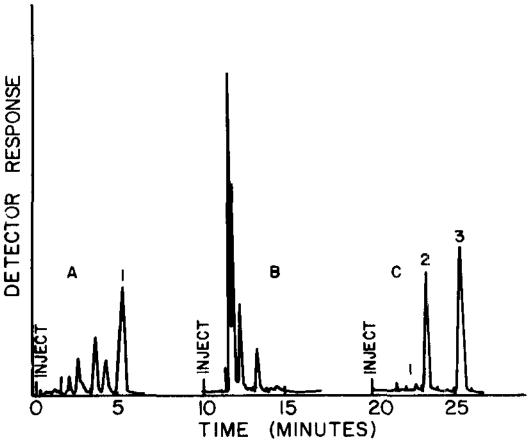


FIGURE 2

Sample Chromatograms; Key: A, From a Sample of Ethacrynic Acid After Refluxing with Solution of Ammonium Hydroxide; Peak 1 is from Ethacrynic acid; All Others are from Decomposition Products; B, Same as A except that Hydorxylamine HCl was also added, No Ethacrynic Acid was Found; C, From a Solution of Ethacrynic acid in 0.04M Solution of Ammonium Phosphate Dibasic After 34 Hours of Storage at Room Temperature; Peak 3 is from Ethacrynic acid and All Others from the Decomposition Products.

TABLE 3

Assay Results of Ethacrynic Acid in Various Buffer Solutions

| Solution (See Table 1) | | pH | Percentage of I Found After 3 Days | 6 Days | 13 Days |
|---|---|---|---|---|---|
| Borate | (0.04M) | 7.7 | - | 99.6 | 99.6 |
| $(NH_4)_2HPO_4$ | (0.02M) | 7.7 | 85.7 | 82.2 | 77.8 |
| As Above | (0.04M) | 7.8 | 73.5 | 70.7 | 66.1 |
| $NH_4HCO_3$ | (0.02M) | 7.8 | 91.0 | 87.5 | 87.2 |
| As Above | (0.04M) | 8.0 | 81.1 | 78.7 | 76.4 |
| $Na_2HPO_4$ | (0.04M) | 7.8 | - | 98.8 | 97.5 |
| Citrate Buffers | (0.05M) | | | After 90 Days of Storage | |
| | #1 | 7.1 | | 87.1 | |
| | #2 | 6.5 | | 88.8 | |
| | #3 | 5.4 | | 89.5 | |
| | #4 | 4.5 | | 92.8 | |

Calculations: - Since preliminary investigations indicated that the peak areas of ethacrynic acid were directly related to the concentrations (between 0.5 to 3 µg), the results were calculated using the following equation:

$$\frac{A_a}{A_s} \times 100 = \text{Percent of the label claim}$$

Where $A_a$ = peak area of the assay sample and $A_s$ = peak area of the standard sample. The results on commercial dosage forms are

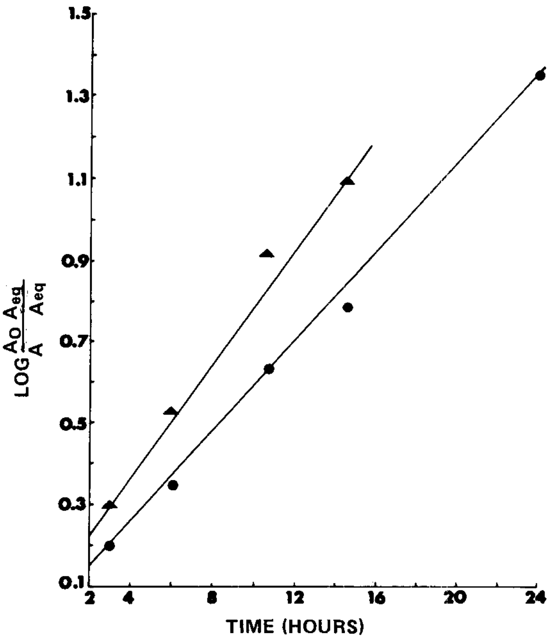


FIGURE 3

Plot of Log $\frac{A_0 - A_{eq.}}{A - A_{eq.}}$ Versus Time; Key, ▲ in 0.04M Solution of $(NH_4)_2HPO_4$ and ● in 0.02M Solution of $(NH_4)_2HPO_4$.

presented in Table 2 and some sample chromatograms are presented in Figures 1 and 2.

Since the investigations indicated (see Discussion) that the developed method was stability indicating, all the stability studies on solutions and dosage forms were conducted using this method.

Stability Tests on Dosage Forms and Solutions:

(i) Solutions of Ethacrynic Acid in Various Buffers (See Table 1, Solutions 2-8): - These solutions were stored at room temperature, 24° ($\pm$1) and assayed frequently. The results are presented in Table 3.

(ii) Further Studies on Solutions in Ammonium Phosphate Dibasic Buffer Solutions: - Since the results indicted (see Table 3) that the decomposition of ethacrynic acid was quite fast and reversible in the presence of amonium ion, these solutions were further investigated. The data were treated theoretically as follows[8]:

$$\log \frac{A_0 - A_{eq}}{A - A_{eq}} = \frac{\left(k_f + K_r\right)t}{2.3} \qquad \text{(Eq. 1)}$$

When studied on hourly basis, the equilibrium was established in about 24 hours. In Eq. 1:

$A_0$ = Initial percent concentration of ethacrynic acid.

$A_{eq}$ = Percent concentration after 24 hours (i.e. the equilibrium concentration). A = Percent concentration at time t.

$K_f$ = Decomposition constant for the forward reaction and $K_r$ = decomposition constant for the reverse reaction.

t = Time in hours.

The results are presented in Figure 3. In order to determine various K values, the data were further treated as follows:

$$\frac{K_f + K_r}{2.3} = \text{slope of the line in Figure 3 and}$$

$$\frac{K_f}{K_r} = \frac{B_{eq}}{A_{eq}} = K_{obs} \quad \text{where } B_{eq} = A_0 - A_{eq}$$

Since two different concentrations of $(NH_4)^+$ were studied, two $K_{obs}$ values could be determined. Furthermore, $K_{obs} = K_0 +$

TABLE 4

List of Various Decomposition Constants as Determined by Using Equations 1 and 2

| Decomposition Constant | Value ($hour^{-1}$) | Remarks |
|---|---|---|
| $K_r$ | 0.101 | When $(NH_4)^+$ Conc. was 0.04N |
| $K_f$ | 0.026 | |
| $K_{obs}$ | 0.256 | |
| $K_r$ | 0.108 | When $(NH_4)^+$ Conc. was 0.08N |
| $K_f$ | 0.052 | |
| $K_{obs}$ | 0.485 | |
| $K_1$ | 5.742 liter-mole$^{-1}$ | |
| $K_o$ | 0.026 | |

$K_1 \ (NH_4)^+$ ------------- (Eq. 2)

By solving two simultaneous equations it was possible to determine $K_o$ (decomposition constant without the effect of ammonium ion) and $k_1$ (decomposition constant in the presence of ammonium ion). The results are presented in Table 4.

(iii) Solutions in Phosphate Buffers, 0.1N NaOH, 0.1N HCl, Sorbitol 50% and Sugar (Varying Concentrations): - These solutions were stored at 65° and assayed after appropriate intervals. Solutions in sorbitol were also studied at room temperature. The results are presented in Table 5.

TABLE 5

Assay Results of Various Solutions When Stored at 65°[a]

| Solution or Dosage Form (See Table 1) | pH (± 0.1) | Percent Retained After | |
|---|---|---|---|
| | | 21 Days | 62 Days |
| 0.1N NaOH | 13.0 | 0.0 | - |
| 0.1N HCl | 1.3 | 28.4 | - |
| Phosphate Buffers | | | |
| (0.05M) #1 | 3.0 | 85.7 | - |
| #2 | 3.4 | 84.1 | - |
| #3 | 3.8 | 86.3 | - |
| #4 | 4.2 | 84.0 | - |
| #5 | 4.6 | 87.6 | - |
| Sugar Solution | | | |
| #1 - 10% | 3.1 | - | 67.8 |
| #2 - 20% | 3.1 | - | 47.2 |
| #3 - 30% | 3.1 | - | 50.4 |
| Sorbitol 50% | 7.0 | 80.6 | 71.9[b] |
| Sorbitol 50% With 10% Alc. | 7.0 | 85.3[c] | 73.3[b] |
| | | Results at Room Temperature | |
| Sorbitol 50% | 7.0 | 98.3 | 97.1[b] |
| Sorbitol 50% With 10% Alc. | 7.0 | 100.0 | 97.6[b] |

[a] Solutions containing sorbitol were also studied at room temperature.

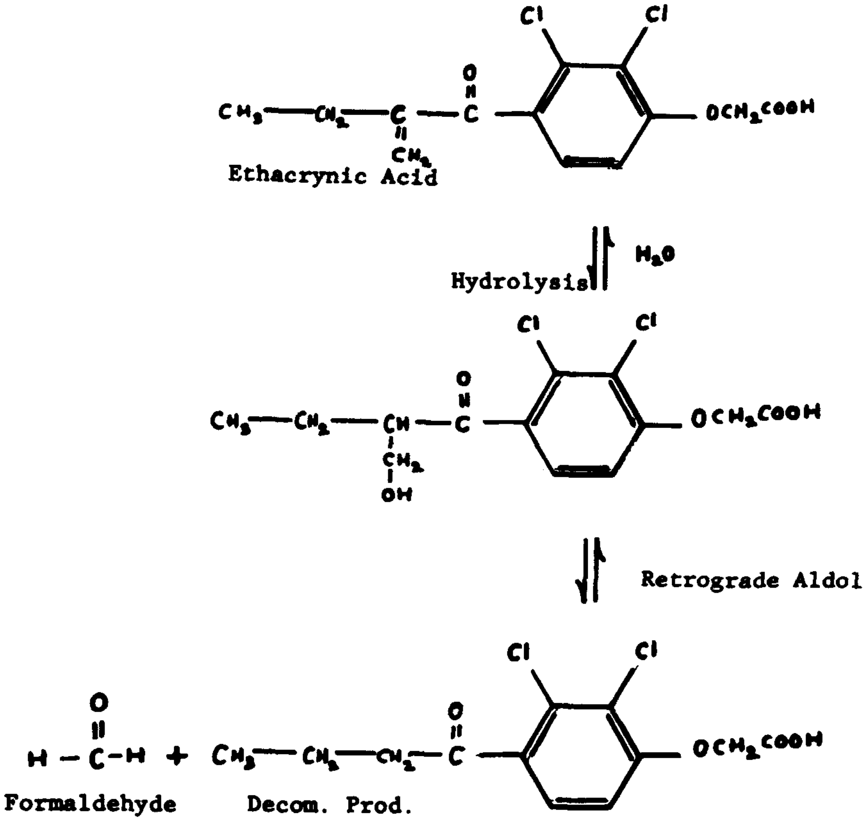
[b] The final pH values of the solutions have changed to 4.6 and 4.9 from an original of 7.0 (see Table 1) for sorbitol solutions stored at higher temperature. The first one is for 50% sorbitol and the second one for 50% sorbitol containing 10% of alcohol. The corresponding decreases at room temperature were 5.2 and 5.8 (from an original of 7.0)

[c] Similar results were obtained on increasing the concentration of alcohol to 20%.

## RESULTS AND DISCUSSION

The method developed for the quantitative determination of ethacrynic acid appears to be stability indicating (Figures 1A-C and 2A-C). The method is accurate and precise (standard deviation based on six readings was ± 1.5%) and sensitive (as small as 0.05 μg can be assayed using 0.02 absorbence unit for full scale deflection). The peak areas were directly related to concentrations (range tested was 0.5 to 3 μg of ethacrynic acid). In the two commercial dosage forms (injection and tablets) no decomposition was noticed in samples which were about one year old. Ethacrynic acid is very unstable in both highly acidic and basic media (Table 5). Weakly acidic or neutral media appears to be relatively safer for the stability of ethacrynic acid in aqueous solutions (Table 3 and 5). Ammonium ion causes the decomposition of ethacrynic acid very rapidly (Figure 2C). This is in direct contrast to borate, phosphate and sodium ions which do not have adverse effects (Table 1 and 3). The decomposition process in ammonium ion appears to be complex and reversible (Figures 2A, 2C and 3). An equilibrium was established in about 24 hours. The $K_f$ value appears to be directly related to the concentration of ammonium ion present (Table 4). In 0.04$\underline{N}$ concentration of ammonium ion, the $K_f$ was 0.026 versus 0.052 at a concentration of 0.08$\underline{N}$. In contrast, $K_r$ values were 0.101 and 0.108 respectively (Table 4). The $K_1$ value due to the presence of ammonium ion was estimated to be 5.742 liter-mole$^{-1}$ per hour.

One process of decomposition of ethacrynic acid in the presence of ammonium ion is probably according to Scheme I. The presence of



**SCHEME I: Hydrolysis of Ethacrynic Acid And Retrograde Aldol.**

formaldehyde as one of the decomposition products was determined as follows[9]:

(i) There was no decomposition of ethacrynic acid in 0.04M ammonium phosphate dibasic in 24 hours in the presence of 0.1N concentration of formaldehyde. Apparently, the equilibrium was shifted to the left due to the presence of formaldehyde.

(ii) A 25.0 mg of quantity of ethacrynic acid was mixed with 20 ml of 0.5N ammonium hydroxide solution and 21.8 ml of water and refluxed for one hour. The mixture was cooled, neutralized with 0.1N solution of sulfuric acid, brought to volume (500 ml) with water and assayed for ethacrynic acid. The results showed 60.2% ethacrynic acid still intact (Figure 2A).

(iii) The above experiment was conducted by replacing 21.8 ml of water with 21.8 ml of a solution of hydroxylamine HCl (2%) in water. The results were 0% retained (Figure 2B). Since hydroxylamine HCl reacts with formaldehyde[9], the equilibrium shifted to the right.

Considering the above discussion and the results in Table 5, it is possible to formulate a liquid dosage form (with limited stability) containing sorbitol, alcohol (10% by volume) and preservatives (methyl paraben 0.005% and propyl paraben 0.002%). The pH should be adjusted to about 7 with a 0.1N solution of sodium hydroxide.

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