ON THE FLUORIMETRIC DETERMINATION OF ADRENALINE AND NORADRENALINE

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A modification of the fluorimetric method for the determination of adrenaline and noradrenaline in pharmaceutical solutions is described. In this method sulphite present is destroyed with iodine, the added excess of which is reduced with arsenous acid, a manipulation which does not affect the fluorescence obtainable.

Adrenaline and noradrenaline in low concentrations are most suitably determined by the fluorimetric method[1,2]. In pharmaceutical solutions, sulphite is usually present as a stabiliser. This introduces a difficulty in the determination of the catechols as these cannot be quantitatively transformed into the corresponding adrenochromes by potassium ferricyanide or by manganese dioxide if the sulphite present is not destroyed before the oxidation of the catechols.

Adrenaline, however, can be determined *ad modum* Ehrlén[3], even in the presence of sulphite by measuring the transient fluorescence obtained by oxidation with air in strongly alkaline solution. In this procedure there seems to be no interaction between the adrenochrome formed and the sulphite. A possible explanation of this phenomenon is that this reaction is performed at such a high pH that all sulphite is present as SO_3^{--} being non-reactive, while the oxidation with MnO_2 or $Fe(CN)_6^{---}$ is performed in neutral or slightly acid solution, where the sulphite is present partly as HSO_3^- which is reactive forming a bisulphite compound with the adrenochromes.

Noradrenaline cannot effectively be determined by the method of Ehrlén[3] as the sensitivity is much lower than for adrenaline. It is, therefore, necessary first to oxidise noradrenaline with ferricyanide or MnO_2 to noradrenochrome and then to transform it with sodium hydroxide to the fluorescent adrenolutine. This procedure may sometimes be preferable in the determination of adrenaline as a higher fluorescent energy is obtained.

As mentioned before, the sulphite present has to be destroyed in such a way that the fluorescence obtained is not affected. Several methods described are based on the separation of the catechols from the sulphite or on the destruction of the latter. The separation methods are tedious and not well suited to routine work. The destruction of the sulphite ions is usually brought about by oxidation with iodine in an acid solution such that the reaction between iodine and the catechols proceeds very slowly. The excess of iodine added has to be removed in order not to suppress the fluorescence later on. This can be done by addition of thiosulphate, an excess of which, however, also has a strong quenching influence on the fluorescence. The influence of thiosulphate is rather peculiar; added to a solution of the adrenochrome it has no influence on

the fluorescence but if added before the oxidation of the catechol a concentration of 10^{-5} equiv./1. thiosulphate diminishes the fluorescence by about 40 per cent.

Because of the difficulties in obtaining reproducible results in this way, the use of the very small excess of iodine which is obtained when starch is used as an indicator for reaching the equivalent point in the oxidation of the sulphite has been proposed[4]. The best result with this method is reached when MnO_2 is used as oxidant for the catechols as MnO_2 seems to diminish the influence of the free iodine on the fluorescence. Nevertheless in our opinion it is difficult to obtain reproducible results this way even in careful work and a method, where the influence on the fluorescence is still smaller, therefore, seems to be highly desirable. In this paper a method is described where the excess of iodine is destroyed with arsenious acid before the oxidation of the catechols.

Experimental

Reagents

Iodine solution containing 12 g. of iodine and 20 g. of potassium iodide in 1 l. of water.

Arsenious acid solution containing 2 g. of As_2O_3 in 1 l. of water.

Phosphate buffer pH 7 made up from one part of M/15 potassium dihydrogenphosphate and two parts of M/15 disodium hydrogenphosphate.

Manganese dioxide (Baker A.R.) purified according to Crawford and Law[5].

Ascorbic acid solution 0·1 *per cent* in boiled and cooled water.

Sodium hydroxide 1·1 *M*. If the sample also contains a local anaesthetic, this can be kept in solution if the NaOH-solution is made up in 50 per cent ethanol.

As *fluorescence standard* a solution of quinidine sulphate in 0·005 M sulphuric acid is suitable. A suitable fluorimeter is the Photovolt filter fluorimeter Model 540, equipped with a photomultiplier as detector, using a primary filter with maximum transmission at 365 mμ and a secondary filter transmitting maximally over 505 mμ.

Method

Into a 15 ml. volumetric flask is pipetted an aliquot of the sample containing about 25 to 50 μg. noradrenaline or adrenaline. 2 ml. of 0·1N hydrochloric acid and 2 drops of starch indicator are added. Iodine solution is added drop by drop with shaking until a blue colour develops. Then 0·5 ml. of arsenous acid solution is added and the mixture diluted to the mark with water. After mixing, 5·0 ml. is pipetted into 5·0 ml. buffer solution in a centrifuge tube. When mixing the blue colour should disappear completely but the pH of the resulting solution should not be less than 6·5 to 6·7. About 100 mg. of MnO_2 is added to the tube and this is closed with a rubber stopper and shaken for about 90 seconds. The mixture is then centrifuged 1 to 2 minutes at 3000 r.p.m. 5 ml. of ascorbic acid solution is pipetted into a 25-ml. measuring flask. To this solution 5·0 ml. of the clear solution from the centrifuge tube is

added and with mixing immediately diluted to the mark with sodium hydroxide solution. The whole procedure from the addition of MnO_2 should not exceed 4 to 5 minutes. If a suitable manganese dioxide is used a perfectly clear solution is obtained after centrifugation making filtration unnecessary.

The solution is pipetted into the fluorescence measuring cell and the fluorescence continuously measured. A slightly pronounced fluorescence maximum appears about 5 minutes after the addition of the sodium hydroxide and the maximum reading is recorded.

The background fluoresence is determined by adding the ascorbic acid solution when the sodium hydroxide and the adrenochrome solution have reacted for at least fifteen minutes. The background fluorescence can usually be neglected.

A standard graph is constructed from standard adrenaline or noradrenaline solutions containing all the ingredients in the unknown solution.

Discussion

The method described is essentially similar to the methods which Crawford and Law[5] and Lund[2] used on biological samples. The reason why MnO_2 was chosen as oxidant instead of ferricyanide is that ferricyanide in itself a strong fluorescence quencher could be a possible source of errors in the determination.

It was shown that standard graphs of good linearity could be obtained for adrenaline as well as for noradrenaline. The reproducibility was tested for both substances, by calculation of the standard deviation. For the method applied to pure solutions of adrenaline and noradrenaline without introducing iodine or arsenous acid, SD = $\pm 1{\cdot}9$ was found for adrenaline ($n = 5$) and SD = $\pm 1{\cdot}2$ for noradrenaline ($n = 9$). When sulphite (50 mg./ml.) was added to the test solution the corresponding figures was $\pm 1{\cdot}5$ ($n = 6$) and $\pm 0{\cdot}6$ ($n = 6$). In all these experiments the instrument showed about 75 scale deflections. In a separate series the standard deviation was determined in ten experiments each with varied sulphite and noradrenaline content.

Sulphite mg./100 ml. ..	0	50	100
Noradrenaline μg. ..	10·0	14·1	5·03
Scale deflections, mean ..	52	72	27
$\pm$ SD	1·7	1·1	0·8

It was also shown that the final concentration of arsenous acid could be increased several times without influencing the results.

This method cannot be applied to pharmaceutical combinations containing procaine and noradrenaline. In such solutions it is necessary to separate the catechol before determination[6].

References

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