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DETERMINATION OF PANTOTHENATE IN PHARMACEUTICAL PREPARATIONS (1)

DETERMINAÇÃO DE PANTOTENATO DE CÁLCIO EM MEDICAMENTOS

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R E S U M O

Descrevemos neste trabalho um método simples para a dosagem de pantotenato de cálcio, sódio ou ácido pantotênico em preparados farmacêuticos.

Utilizamos a propriedade que têm os compostos acima de formarem, na hidrólise ácida, beta-alanina, que é então cromatografada em papel circular. O cromatograma é revelado com ninhidrina e o composto formado é eluído e determinado espectrofotomètricamente a 520 μ, como preconizado por Giri, Radhakrishnam & Vaidyanathan.

Estabelecemos as condições ideais de hidrólise, a possibilidade de interferência de aminoácidos presentes em preparados farmacêuticos e a concentração ideal de trabalho. Fizemos, também, testes de recuperação.

INTRODUCTION

One of the products of the acid hydrolysis of calcium pantothenate is beta-alanine. Most of the literature published on calcium pantothenate analysis involves purification of the compound by means of column chromatography followed by hydrolysis and determination of beta-alanine. SCHMALL & WOLLISH [1] separated calcium pantothenate followed by alkaline hydrolysis. YAMAGISHI & YOSHIDA [2] determinated pantothenates after purification by means of paper chromatography and acid hydrolysis, with ninhydrin. RODRIGUES & SILVA [3] separated the vitamins from complex B, by descendent chromatography and detected each component by specific reactions. They studied a few solvents and found that 80% ethanol gave the best separation. They did not attempt, though, a quantitative determination. SICHE & KAKAK [4] described a method for the determination of pantothenates using paper chromatography. Both authors condensated the butirolactone formed by acid hydrolysis of pantothenate with hydroxylamine and separated the resulting hydroxamic acid by paper chromatography. Detection of this compound was achieved with $FeCl_3$. Nevertheless this method involves too many steps to be suitable for routine laboratory work. HUBBARD *et alii* [5] described a method for the determination of calcium pantothenate in pharmaceutical preparations, which is a modification of ZAPPALA & SIMPSON [6]'s method. Column chromatography which was used for the separation of pantothenate from other components of the B complex is a somewhat painful process and also not suitable for daily routine.

Our finality is to render the determination of pantothenic acid and its salts in pharmaceutical preparations a simple method, which may be used in the daily routine of our laboratory. We find it simpler

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to make the hydrolysis first and purify the resulting beta-alanine by paper chromatography.

Pharmaceutical preparations may deteriorate on storage and its pantothenate may be decomposed producing beta-alanine. To avoid any errors in the determination of the pantothenate actually present, an aqueous extract of sample not hydrolysed must be chromatographed simultaneously to verify the possible presence of any beta-alanine resulting from decomposition of pantothenate on storage. The method involves:

1) Acid hydrolisis of the mixture containing pantothenate.

2) Separation of the beta-alanine formed by circular paper chromatography.

3) Reaction of beta-alanine with ninhydrin.

4) Elution and spectrophotometric determination of the compound formed.

METHOD

Reagents

a) D-calcium pantothenate — E. Merck AG.

b) Trichloroacetic acid solution — 10% aqueous solution.

c) Ethanol 80% v/v.

d) Ninhydrin solution — 0,4% p/v ninhydrin in acetone 95% (aqueous v/v).

e) Copper sulphate solution — 0,02% aqueous p/v solution of $CuSO_4.5H_2O$.

f) Copper sulphate solution — 0,005% p/v dilute solution e 1:4 with absolute ethanol (prepare daily).

Determination

a) *Hydrolysis* — Transfer to separate 10 ml glass stoppered flasks a quantity of samples containing 3-10 mg of calcium or sodium pantothenate and approximately the same quantity of pantothenate standard. Add 0,5 ml of 10% trichloracetic acid solution per mg of pantothenate.

Stopper the flasks and let them stay at the temperature of 90°C, in an oven. Release the pressure within the flasks a few times, at the beginning of the heating. After heating for at least 12 hours, take the mixtures from the oven, allow them to cool and transfer them quantitatively into 10 ml volumetric flasks, filtering if necessary.

b) *Chromatography* — Circular chromatography is done on Whatman paper n. 1, 40 x 40 cm. Transfer to the paper, with micro-pipets, quantities of hydrolysed sample and standard containing 3.5-11 micrograms of beta-alanine, corresponding to approximately 10-30 micrograms of calcium pantothenate. Transfer to the same chromatographic paper an equivalent amount of non hydrolysed sample. Develop the chromatogram with 80% ethanol, let it dry and soak it by means of a brush, with 0,4% ninhydrin. Heat the chromatogram in an oven at exactly 65°C for 30 minutes. Eluete the stain formed (Rf 0,77-0,83) with 4 ml of 0,005% copper sulfate solution, letting the eluest in contact with the paper, for 30 minutes. Filter and read in spectrofotometer at 520 milimicra, setting the 100% transmitance, with the eluent solution.

RESULTS AND DISCUSSION

Beer's law is followed over a suitable working range for analysis of pharmaceutical preparations. It is advisable not to use a standard curve though, but to run simultaneously a standard treated as for the samples, due to the small variations on the intensity of the color resulting from variations of the heating temperature after spraying with ninhydrin.

Ten per cent trichloroacetic acid was satisfactory for hydrolysis. Beta-alanine was completely decomposed by a final solution of 3 N HCl, after heating for 30 minutes in an oven at 90°C.

From all the solvents studied for the development of the chromatogram, 80% ethanol gives the best results. We tried also butyl alcohol-acetic acid-water (4:1:1); 40% fenol; sec. butyl alcohol-88% formic acid-water (75:15:10); water saturated butyl alcohol.

Pharmaceutical preparations containing methionine, besides pantothenate, may be analysed for this amino-acid by the same method.

The method described in this report measures total pantothenate (d and l forms).

Recovering tests were made, by adding a known amount of standard to pharmaceutical preparations which did not contain pantothenic acid in any form. Volumes of the hpdrolysates theoretically containing 11 micrograms of pantothenate were chromatographed. The results obtained from these tests are given in the table below:

Type of sample	Amount of standard (recovered — mcg)	Recovery %
Trivitamin injection	11,1	101
Liver injection	11,9	108
Liver tablet	12,0	109
Vitamin and mineral elixir	9,9	90
Multivitamin elixir	11,0	100
Average	11,2 ± 0,76	102 ±

The figures given represent an average of at least two determinations. The method described in this report is being used in our laboratory routine work for the last five months.

SUMMARY

A simple method for the determination of calcium or sodium pantothenate and pantothenic acid in pharmaceutical preparations is presented. The property of the mentioned compounds of forming beta-alanine by a acid hydrolysis is used. The beta-alanine formed is purified by paper chromatography and detected with ninhydrin. The stain is eluted and the absorbance measured according to Giri, Radhakrishnam & Vaidyamathan. Hydrolysis conditions were studied as well as the possible interference of other amino-acid which may be present. Several kinds of solvent for development of the chromatogram were also studied.

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