# ELABORATION OF A METHOD FOR THE DETERMINATION OF ADRENALINE BY THIN LAYER CHROMATOGRAPHY

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***Abstract***

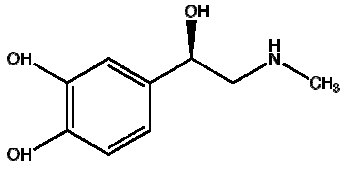
*Adrenaline is a hormone secreted by the adrenal medulla; it is the first hormone isolated in a crystalline state. In the body, adrenaline has multiple functions: it regulates blood pressure, maintains a constant blood glucose concentration, acting as an insulin antagonist (adrenaline increases and insulin decreases this concentration). Adrenaline is synthetically prepared for pharmaceutical purposes from pyrocatechin and chloride of the monochloracetic acid. Some of the methods of quantitative and qualitative determination are chromatographic methods. This paper presents a method of qualitative determination of adrenaline by thin layer chromatography. Optimal separation conditions were established: Chromatographic mobile phase acetone-water and $F_{254}$ silica gel plates, respectively, $F_{254}$ alumina plates.*

**Key words:** Hormones, Adrenaline, TLC Chromatography

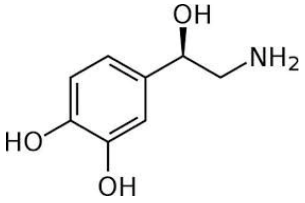
## INTRODUCTION

Adrenaline and noradrenaline, are two hormones secreted by the adrenal medulla and chromaffin tissues, resulting in a large amount from tyrosine. Biosynthesis is catalyzed by specific enzymatic systems that require the presence of copper ions. The balance between the two compounds depends on the stimuli in the environment (Niculescu et al., 2009).

Their physiological action consists of the following: they participate in the maintenance of arterial and possibly venous tone; are involved in the contraction of smooth muscles; are inducers of glycogenolysis in the liver and muscles, being in antagonism with insulin and synergism with glucagon; they are neurotransmitters of nerve information having an important role in body-ambience modulation.




Adrenaline (metilaminoetanolpirocatechina)




Noradrenaline

Epinephrine is a hormone secreted by the adrenal medulla; it is the first hormone isolated in a crystalline state. In the body, adrenaline has multiple functions: it regulates blood pressure, mantains a constant blood glucose concentration, acting as an insulin antagonist (adrenaline increases and insulin decreases this concentration) (Căpâlna et al., 1977).

In the body, adrenaline has multiple functions: it regulates blood pressure by narrowing blood capillaries, mantains a constant blood glucose concentration, acting as an insulin antagonist (adrenaline increases and insulin decreases this concentration) (Căpâlna et al., 1977).

Adrenaline is synthetically prepared for pharmaceutical purposes from pyrocatechin and monochloracetic acid chloride (Oeriu et al., 1974).

In this paper we intended to develop a chromatographic method for identifying these hormones in pharmaceutical preparations and biological products (Muthu et al., 2011; Naga et al., 2014; Oona, 2004; Alberto et al., 2002).

**MATERIAL AND METHOD**

The thin-layer chromatography method was used to identify adrenaline. These determinations were performed by thin layer chromatography on silica gel plates deposited on activated polyethylene plates, aluminum plates deposited on aluminum and polyamide plates deposited on aluminum.

*Materials and Reagents:* adrenaline - a pharmaceutical product, the concentration is 1 mg / ml and contains the following excipients: tartric acid, urea, sodium acetone bisulfite, sodium chloride and water, n-butanol, acetone, chloroform, methanol, acetic acid, distilled water; silicagel $F_{254}$ plates on plastic, aluminum $F_{254}$ plates deposited on aluminum foil, polyamide $F_{254}$ sheets deposited on aluminum foil.

*Method:* In a first step of the study, the optimum composition of the mobile phase was determined for proper separation of adrenaline from different preparations. In the chromatographic chamber, a covered Berzelius beaker, was placed a piece of filter paper on one side, which was immersed in the eluent to provide a saturated atmosphere of the developer. In these beakers the eluent mixture is introduced to a height of 0.5 cm so that the samples deposited on the chromatographic plates are not immersed.

The samples are deposited on the chromatographic plates, with calibrated capillaries, in the form of uniform and small spots. The chromatographic plates are dried and inserted into the development chamber. The development is observed and it takes place until the solvent front reaches about 1 cm from the top of the plate. The plate is removed

from the Berzelius cup and dried. Qualitative identification is achieved by comparing the value of the retention factor (Rf). Spot viewing was performed with a UV lamp.

**RESULTS AND DISCUSSION**

Separation on different stationary phases and different compositions of the mobile phase have been tested, in order to achieve a separation consistent with symmetrical and reproducible spots.

Not all solvent systems used as developers have yielded satisfactory results in separating and identifying adrenaline.

An efficient and appropriate separation was achieved using the development systems presented in Table no. 1.

*Table 1*

Composition of the mobile phase

| No | Eluent composition | Combination ratio | CSS plates | | |
|---|---|---|---|---|---|
| | | | Silicagel $F_{254}$ | Aluminum $F_{254}$ | Polyamide $F_{254}$ |
| **1** | **Acetic Acid: n-Butanol: Water** | **1:4:1** | $R_f = 0,56$ | $R_f = 0,23$ | Inadequate |
| 2 | Acetone:Chloroform: n-Butanol: Acetic Acid:Water | 6:4:4:4:3,5 | Inadequate | Inadequate | Inadequate |
| 3 | Chloroform:Metanol | 9:1 | Inadequate | Inadequate | Inadequate |

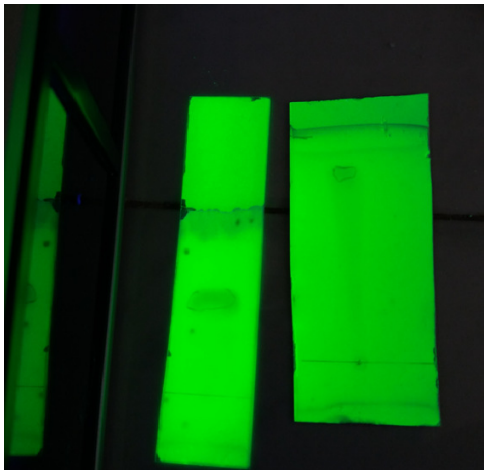


Fig. 1. Chromatographic plates resulting with the solvent system 1

The mobile phase with which adrenaline was successfully separated was Acetic Acid: n-Butanol: Water, yielding Rf separation factors of 0.51

when using silica gel plates and 0.8 for aluminum. In the other trials, the results obtained were inadequate.

## CONCLUSIONS

The experimental results obtained show a proper separation with chromatographic systems composed of acetone-water as a mobile phase and F254 silica gel plates, respectively F254 aluminum plates, allowing a good separation of these compounds.

The proposed method allows the qualitative determination of adrenaline in pharmaceutical compounds and maybe anatomical preparations.

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