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# HPTLC and MS for Separation and Identification of Some β-Blockers in Urine

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Using high performance thin layer chromatography (HPTLC) coupled with mass-spectrometry (MS) we separated and identified some β-blocker drugs *i.e.*, labetalol and metoprolol in urine. The separation was performed on silica gel 60 HPTLC (Merck) with toluene-ethyl acetate-acetone-$NH_4OH$ (25 %), 10 + 20 + 20 + 1 (v/v) as mobile phase. Identification of the components was performed in ultraviolet light (UV) and by mass-spectrometry. After one dose of drug (metoprolol or labetolol) intake, urine samples were taken from which the eliminated drug is extracted with ethyl acetate. After processing the extract was separated and identified by HPTLC and MS. Quantitative measurement were performed by photodensitometry of the developed plates at 265 nm by using a Camag photodensitometer.

**Key Words: HPTLC, MS, Drugs, Separation, β-Blockers.**

## INTRODUCTION

A series of β-blocker drugs (metoprolol, atenolol, propanolol) were separated on silica gel layers impregnated with L-aspartic acid as chiralic selector. The acetonitrile-water-methanol mixture was used in different proportions as mobile phase. Detection was performed by exposure in iodine vapor atmosphere, the detection limits being of 0.26 μg for atenolol and of 0,23 μg for the rest of the separated drugs[1]. Propanolol was separated from other blockers on silica gel layers using as mobile phase the acetonitrile-methanol-water mixture (36:36:28, v/v) and detection was performed by UV light after derivatization with dabisil chloride[2].

A mixture of acebutolol, alprenolol, atenolol, butnitrolol, bupranolol, carazolol, labetolol, metipranolol, metoprolol, natolol, oxprenolol, penbutolol, pindolol,

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propanolol, timolol and toliprolol has been separated on silica gel layers using bidimensional chromatography after derivatization with dansil chloride[3]. Other mixtures of β-blocker drugs were separated by thin-layer chromatography using chiralic stationary phases[4-7]. Thus different modified celulloses: celullose *tris*-(phenyl carbamate), celullose *tris*-(2,6-dichlorphenylcarbamate), celullose *tris*-(3,4-dichlorophenylcarbamate), celullose *tris*-(2,3-dichlorphenylcarbamate) have been used. The hexane-propane-2-ol (8:2, v/v) mixture has been used as mobile phase. Celullose 2-ethoxyphenylcarbamate and celullose 3-buthoxyphenylcarbamate has been used as stationary phase and hexane-propane-2-ol (8:2, 7:3, v/v) as mobile phase in order to separate the alprenolol, acebutolol, metoprolol and propranolol mixture. The separated drugs were visualised by spraying the developed plates with anisaldehyde solution[8].

Atenolol, celiprolol, metoprolol, propafenone, propanolol, talinol compounds were extracted from plasma and separated on silica gel layers by ascendent technique. The drugs isolation was performed using liquid-liquid extraction after alkalization of blood plasma. The samples obtained after plasma processing were separated on silica gel using ethyl acetate-acetone-ammonia (5:4:1, v/v) and the separated compounds were identified by UV light irradiation (254 nm) or by different reagents. The visual limits after the reaction with specific indicators range[9] are between 100-300 ng.

A β-blockers method of separation and identification in urine has been elaborated using these data.

## EXPERIMENTAL

**Solutions of β-blockers:** Bisohexal, labetolol, tenox, fosipril, sotahexol, metoprolol, cardin, vasocardin were prepared. These were separated on chromatographic plates using high performance silica gel 60 $F_{254}$ (Merck). Separations were performed in normal chromatograpic chambers using ascendent technique. Toluene-ethyl acetate-acetone-ammonium hydroxide (25 %) mixtures were used in different proportions as mobile phase. The best separations were obtained for toluene-ethyl acetate-acetone-ammonium hydroxide (25 %) (10:20:20:1, v/v) mixture as mobile phase.

This method has been used when separating and identifying metoprolol and labetolol in urine and blood. Thus, urine and blood samples have been left for 2 h after the intake of a drug dose (labetolol and/or metoprolol).

Urine sample (100 mL) was treated with ammonia up to pH = 9 and then extracted with two 50 mL ethyl acetate (diethyloxide) portions. The organic phase was separated and dried on anhydrous sodium sulphate and then it was evaporated at 60 °C under vacuum. The obtained residue was dissolved in 0.2 mL methanol and it was analyzed near the studied drugs.

The plates development was performed in normal chromatographic chamber using the toluene ethyl acetate-acetone-ammonium hydroxide (25 %) (10:20:20:1,

v/v) mixture as mobile phase. The compounds visualization was performed by UV light examination at 254 nm. Labetolol and amlodipine in tenox form intense blue fluorescent spots and the rest of the drugs form dark spots. The same idea was tried for the extract from blood with metoprolol, but the metoprolol was not identified.

For quantitative determinations, there were applied on the chromatographic plates volumes of 1, 3, 5, 7 and 10 μL from the samples of solutions 0.1 % of metoprolol and 20 μL urine extracts and, respectively, the same volumes of labetolol 0.1 % and urine extracts 20 μL. After the photodensitometry of the plates with the help of a Camag photodensitometer, the peaks for metoprolol and labetolol were obtained (figures not shown).

The obtained curves are illustrated in Figs. 1 (for metoprolol) and 2 (for labetolol) and the regression functions are illustrated in set number 1 and set number 2.

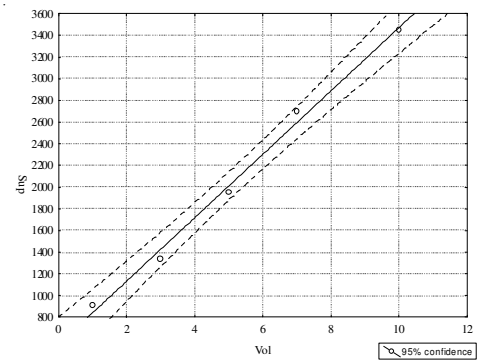



Fig. 1. Graphical representation of the area according to the concentration for the metoprolol samples

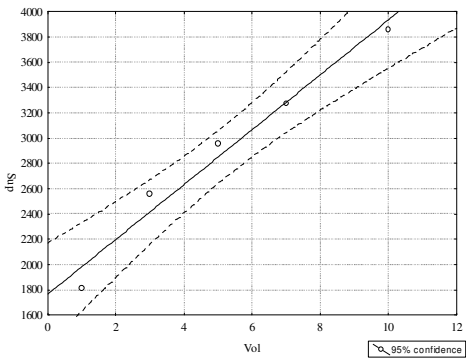


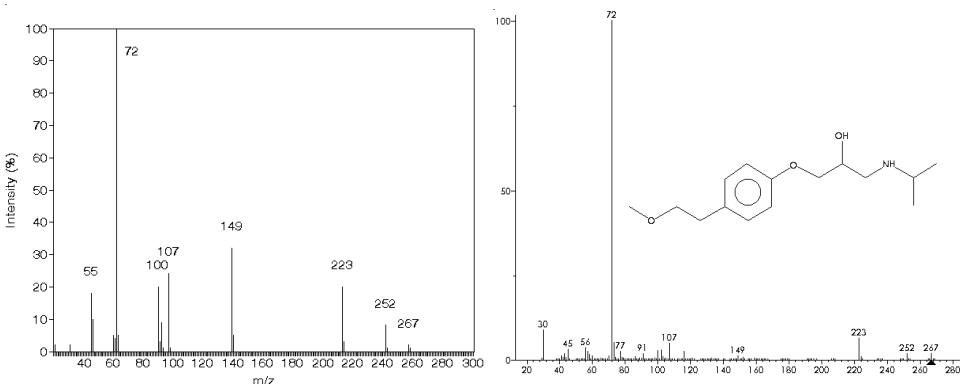

Fig. 2. Graphical representation of the area according to the concentration for the labetolol samples

## RESULTS AND DISCUSSION

In order to increase the urine extracts identification safety of the two drugs (metoprolol and labetolol), the spots on the chromatographic plate were scraped, extracted with methanol and analyzed with a double focalization Finnigan MAT 311 model mass spectrometer. The sample introduction system is heated, programmed from 25-330 °C at 50 °C/min speed, the impact energy of 70 eV and the scavenging domain ranging from 25-500 (Daltons).

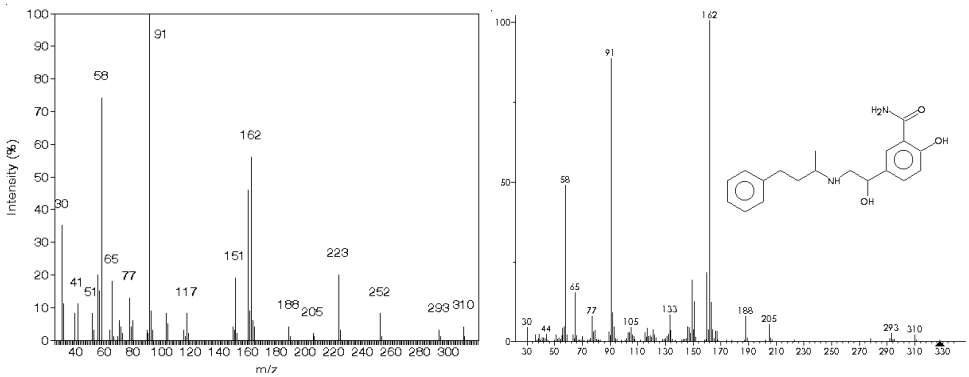
The obtained spectrum was analyzed and compared with the spots from the data library for metoprolol. The obtained results showed (Fig. 3) that the analyzed substance is metoprolol. The same thing was done for labetolol (Fig. 4). It can be affirmed that in the extracts, the presence of both metoprolol and labetolol.

In blood sample the presence of metoprolol was not noticed, probably because its concentration is under the detection limit.




(a) (b)

Fig. 3. Mass spectrum for metoprolol (a-urine sample; b-spectra library)




(a) (b)

Fig. 13. Mass spectrum for metoprolol (a-urine sample; b-spectra library)

The spots from the urine extract belong to metoprolol and labetolol, this can be confirmed by $R_f$ values, the colours of the spots as well as the mass spectra which are identical with those in the spectra library (Figs. 3 and 4). Another clue for labetolol is the intense blue fluorescence of the spot in UV light at 254 nm. The detection limit for labetolol and tenox is 0.01 μg/spot and for the rest of the drugs it is 0.1 μg/spot.

## Conclusion

High performance thin layer chromatography (HPTLC) coupled with mass-spectrometry can be used to separate and identify some β-blocker drugs in biological samples.

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