Research Article

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DEVELOPMENT OF METHODS FOR IDENTIFICATION OF CALCIUM CHANNEL BLOCKERS IN MEDICINES

Logoyda Liliya ^{1*}, Korobko Dmutro ², Saprun Stanislav ³

¹PhD, Associate Professor of Pharmaceutical Chemistry Department, I. Horbachevsky Ternopil State Medical University, Ukraine

²PhD, Associate Professor of Pharmaceutical Chemistry Department, Dean of Pharmaceutical faculty, I. Horbachevsky Ternopil State Medical University, Ukraine

³PhD, Assistant Professor of Department of Theory and Methodology of Olympic and Professional Sport, Ternopil V. Hnatiuk National Pedagogical University, Ukraine

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*Corresponding author

E-mail: lilya-19@mail.ru

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ABSTRACT

The pharmaceutical analysis has got more emphasis to satisfy our query for better understanding of physico-chemical properties of pharmaceutical compounds, by the use of advanced instrumental methods. Calcium channel blockers are used as antihypertensive drugs, i.e., as medications to decrease blood pressure in patients with hypertension. Analyses of amlodipine, verapamil, nifedipine are described in Pharmacopeia but aim of our researches was to improve to more rapid, simple, selective, more accurate, precise, reliable, less expensive methods by TLC of amlodipine, verapamil, nifedipine in medicines and for using this methods for analysis of their metabolites in next step of researches. Methods of identification of amlodipine, nifedipine and verapamil in medicines by TLC have been developed. Established that the most optimal R_f observed using mobile phases: propanol R- water R (70:30) for amlodipine and chloroform R- propanol R (9:1) for nifedipine and n-butanol-methanol (3:2) for verapamil. The detection limits of amlodipine, nifedipine and verapamil in these systems are 0.4 mcg. However, those mobile phases are the most express. We explored the validation characteristics - specificity and suitability of the chromatographic system that met, the eligibility criteria established by the SPU. In conclusion, we have been developed chromatographic methods of identification and quantification of amlodipine, verapamil, nifedipine in medicines. The proposed methods are rapid, economical, simple, accurate, selective, precise and applicable to the analysis of pharmaceutical dosage forms. These methods can also give excellent results and can be employed for the routine analysis.

Keywords: amlodipine, nifedipine, verapamil, identification, thin layer chromatography, validation.

INTRODUCTION

Thin layer chromatography (TLC) is a chromatography technique used to separate mixtures. Thin layer chromatography is performed on a sheet of glass, plastic, or aluminum foil, which is coated with the a thin layer of adsorbent material, usually silica gel, aluminium oxide, or cellulose. This layer of adsorbent is known as the stationary phase. Thin layer chromatography finds many applications, including assaying the radiochemical purity of radiopharmaceuticals, determination of the pigments a plant contains, detection of pesticides or insecticides in food, analysing the dye composition of fibers in forensics, or identifying compounds present in a given substance, monitoring organic reactions.^{1,2}

The research of calcium channel was pioneered by Fatt and Katz³ with their work on large muscle cells of crab. Subsequent work by Fleckenstein⁴ sparked a major therapeutic advance in managing patients with heart diseases and to a new class of drug: calcium channel blockers (CCBs). This class derives its main therapeutic effects by preventing calcium ion influx through the cell membranes. It binds to L-type calcium channels, which are located on vascular smooth muscles, cardiac myocytes and cardiac nodal tissue (sinoatrial and atrioventricular nodes). Through this blockage, CCBs cause relaxation of vascular smooth muscles and vasodilation, a decrease in myocardial force output (negative inotropy), a reduction in heart rate (negative chronotropy) and a decrease in

conduction velocity within the heart (negative dromotropy). By causing vascular smooth muscle relaxation, CCBs decrease systemic vascular resistance, which in turn reduces arterial blood pressure. These drugs primarily affect arterial resistance, with only minimal effects on venous vessels. Because of their dual action on the heart and blood vessels, CCBs are widely used for managing hypertension, angina and supraventricular cardiac arrhythmias⁵.

Analyses of amlodipine, nifedipine and verapamil are described in Pharmacopeia⁶ but aim of our researches was to improve to more rapid, simple, selective, more accurate, precise, reliable, less expensive methods TLC analysis of amlodipine, nifedipine and verapamil in medicines and for using this methods for analysis of their metabolites in next step of researches.

The aim of our study was to develop methods to identify amlodipine, nifedipine and verapamil in medicines by thin layer chromatography.

MATERIAL AND METHODS

The objects of the study were tablets Amlodipine, tablets Nifedipine, tablets Verapamil, Pharmacopoeial standard samples SPU amlodipine, verapamil and nifedipine (certificate numbers 11 / 1 – 5214, 11 / 1 – 5215 and 11 / 1 – 5217), purchased by the State Enterprise Ukrainian Research Pharmacopoeial Center Quality of drugs [7,8].

Analytical equipment: Scales AVT-120-5D, measuring vessel glass and reagents that meet the SPU requirements. TLC test was carried out using Silica gel, chromatographic plates 60 F254 «Merck» (Germany) and «Sorbfil» (Russia).

Sample preparation for amlodipine

Investigation solutions from tablets Amlodipine. To sample powder tablets or powder, equivalent to 10 mg amlodipine, add 5.0 ml of methanol R and dilute with methanol R to 10.0 ml, mix and filter.

Reference solution. 10 mg Pharmacopoeial standard sample SPU of amlodipine dissolved in methanol R and dilute with the same solvent to 10.0 ml.

Mobile phase: propanol R- water R (70:30).

Samples that are applied: 20 µl, applied the test solution and investigation solutions.

Over a path of 10 cm from the starting line.

Detection: examination in ultraviolet light at 254 nm and 366 nm.

Developer: Dragendorff reagent.

Results: the chromatogram obtained with the test solution is detected at the main spot spots basic substance in the chromatogram obtained with reference solution, corresponding in size and color.

Sample preparation for nifedipine

Investigation solutions from tablets Nifedipine. To sample powder tablets or powder, equivalent to 10 mg nifedipine, add 5.0 ml of methanol R and dilute with metanol R to 10.0 ml, mix and filter.

Reference solution. 10 mg Pharmacopoeial standard sample SPU of nifedipine dissolved in methanol R and dilute with the same solvent to 10.0 ml.

Mobile phase: chloroform R- propanol R (9:1).

Samples that are applied: 10 µl, applied the test solution and investigation solutions.

Over a path of 10 cm from the starting line.

Detection: examination in ultraviolet light at 254 nm and 366 nm.

Developer: Dragendorff reagent.

Results: the chromatogram obtained with the test solution is detected at the main spot spots basic substance in the chromatogram obtained with reference solution, corresponding in size and color.

Sample preparation for verapamil

Investigation solutions from tablets Verapamil. To sample powder tablets or powder, equivalent to 10 mg verapamil, add 5.0 ml of metanol R and dilute with metanol R to 10.0 ml, mix and filter.

Reference solution. 10 mg Pharmacopoeial standard sample SPU of verapamil dissolved in methanol R and dilute with the same solvent to 10.0 ml.

Mobile phase: n-butanol R-methanol R (3:2).

Samples that are applied: 5 µl, applied the test solution and investigation solutions.

Over a path of 10 cm from the starting line.

Detection: examination in ultraviolet light at 254 nm.

Results: the chromatogram obtained with the test solution is detected at the main spot spots basic substance in the chromatogram obtained with reference solution, corresponding in size and color.

Table 1: Chromatographic characteristics of amlodipine in different solvent systems

Mobile phase	Mobile phase (plate) Rf on «Sorbfil»	The limit of detection, mcg	Detection in ultraviolet light at 254 nm	Detection in ultraviolet light at 365 nm	Dragendorff reagent (color)
chloroform-methanol (9: 1)	0,37	0,4	violet	blue	orange
chloroform-methanol ammonia (25%) (4: 4: 2)	0,86	0,4	violet	blue	orange
n-butanol-methanol (3:2)	0,65	0,4	violet	blue	orange
ammonia (25%)-propanol (30: 70)	0,67	0,4	violet	blue	orange
formic acid-isopropanol-water (40: 2: 10)	0,80	0,4	violet	blue	orange
propanol-water (70:30)	0,60	0,4	violet	blue	orange
n-butanol-acetic acid-water (40: 10: 10)	0,62	0,4	violet-blue	blue	orange
n-butanol-acetic acid-water (40: 10: 20)	0,64	0,4	violet-blue	blue	orange

Table 2: Chromatographic characteristics of nifedipine in different solvent systems

Mobile phase	Mobile phase (plate) Rf on «Sorbfil»	The limit of detection, mcg	Detection in ultraviolet light at 254 nm	Detection in ultraviolet light at 365 nm	Dragendorff reagent (color)
chloroform-methanol (9: 1)	0,30	0,4	blue	violet	orange
chloroform-methanol ammonia (25%) (4: 4: 2)	0,93	0,4	violet	yellow-orange	orange
n-butanol-methanol (3:2)	0,72	0,4	violet	yellow-orange	orange
ammonia (25%)-propanol (30: 70)	0,70	0,4	violet	yellow	orange
formic acid-isopropanol-water (40: 2: 10)	0,80	0,4	yellow	yellow-green	orange
propanol-water (70:30)	0,72	0,4	violet	yellow	orange
n-butanol-acetic acid-water (40: 10: 10)	0,77	0,4	violet	yellow-green	orange
n-butanol-acetic acid-water (40: 10: 20)	0,78	0,4	violet	yellow-green	orange

Table 3: Chromatographic characteristics of verapamil in different solvent systems

Mobile phase	Stationary phase (plate) Rf on «Sorbfil»	The limit of detection, micrograms	Detection in ultraviolet light at 254 nm	Detection in ultraviolet light at 365 nm
chloroform-methanol (9: 1)	0,79	0.4	violet	—
chloroform-methanol ammonia (25%) (4: 4: 2)	0,95	0.4	violet	—
n-butanol-methanol (3:2)	0,43	0.4	violet	—
ammonia (25%)-propanol (30: 70)	0,75	0.4	violet	—
formic acid-isopropanol-water (40: 2: 10)	—	—	—	—
propanol-water (70:30)	0,47	0.4	violet	—
n-butanol-acetic acid-water (40: 10: 10)	0,64	0.4	violet	—
n-butanol-acetic acid-water (40: 10: 20)	0,63	0.4	violet	—

RESULTS

Authors had investigated various mobile phases (solvent system) in order to identify the optimal choice of amlodipine, nifedipine and verapamil investigation by TLC in medicines. The factors of mobility in the studied of amlodipine, nifedipine and verapamil in mobile phases, are listed in Table 1-3.

DISCUSSION

In order to develop methods of identification of amlodipine, verapamil and nifedipine in different solvent system, we investigated the sensitivity of detection amlodipine, nifedipine and verapamil. The detection limits of amlodipine, nifedipine and verapamil are given in table 1-3. We found that amlodipine, nifedipine and verapamil identification by TLC using a sensitive of all investigated solvents. Established that the most optimal Rf observed using mobile phases: propanol R- water R (70:30) for amlodipine and chloroform R- propanol R (9:1) for nifedipine. The detection limits of amlodipine and nifedipine in these systems are 0.4 mcg. However, both mobile phases are the most express. Established that the most optimal Rf observed using rmobile phases for verapamil: propanol-water (70:30) and n-butanol-methanol (3:2). The detection limit of verapamil in these systems is 0.4 mcg. However, the most express mobile phases is n-butanol-methanol (3:2). Thus, identification of verapamil in medicines we offer TLC-method using mobile phases n-butanol-methanol (3:2).

The analysis considered probable, though the test requirements «Check suitability chromatographic system».

Checking the suitability of the chromatographic system. Chromatographic system is considered appropriate when:

- The chromatogram obtained with reference solution is a clearly visible spot;
- Rf principal spot in the chromatogram obtained with reference solution to be about 0.6.

We previously studied the behavior of placebo tablets in terms of methods of identification of amlodipine, nifedipine and verapamil. It was established that the excipients are part of pills and do not affect the sensitivity and specificity of amlodipine and nifedipine detection.

Validated analytical methods play a major role in achieving this goal. The results obtained from method of validation can be used to judge the quality, reliability and consistency of analytical results. It is an integral part of any good analytical practice. Validation of analytical methods is also required by most regulations and quality standards that impact laboratories. Analytical methods of validation is essential for adherence to Current Good Manufacturing Practice and Good Laboratory Practice regulations.

According to the SPU and Note for guidance on validation of analytical procedures [9,10]: text and methodology (CPMP/ICH/381/95) to test the Identification must be validated, to determine such characteristics as specificity and suitability of the chromatographic system^{7,8}.

To investigate the specificity, it is necessary to confirm the selected mobility of amlodipine, nifedipine and verapamil system to ensure proper Rf stability of solutions in time. The maximum difference of Rf values in the same plate (for two series of plates) must not exceed the value of 0.02. Originally, plates were tested according to the requirements of SPU on chromatographic resolution.

When checking for the stability of the solution at the time we started chromatography of amlodipine, nifedipine and verapamil freshly prepared test solution sustained, over time for 30 min. Visual assessment of spots on the size and intensity of staining confirms that they clearly appear as freshly cooked and seasoned in time solutions (for plates of different series). The solutions were stable over time and new areas, had been identified.

Thus, we explored the validation characteristics - specificity and suitability of the chromatographic system that met, the eligibility criteria established by the SPU. The objective of any analytical measurement is to obtain consistentreliable and accurate data.

CONCLUSION

In conclusion, we developed TLC methods of identification of amlodipine, nifedipine and verapamil in medicines. Established that the most optimal Rf observed using mobile phases: propanol R- water R (70:30) for amlodipine and chloroform R- propanol R (9:1) for nifedipine and n-butanol-methanol (3:2) for verapamil. The validation study of the characteristics of both specificity and suitability of the chromatographic system, confirmed that they meet the eligibility requirements under the SPU. Prospects for future research will be aimed at developing methods of quantitative determination of amlodipine, nifedipine and verapamil in medicines and for analysis their metabolites.

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8. ICH Topic Q2 (R1) Validation of Analytical Procedures: Text and methodology.

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