profile was used: 98% A/2% B–0 min, 98% A/2% B–1 min, 2% A/98% B–20 min, 2% A/98% B–23 min, 98% A/2% B–24 min and 98% A/2% B–30 min. The flow rate was 1mL/min and injection volume was 10 μL. The column temperature was constant 30 ˚C. The chromatograms were recorded at 250 nm

HPTLC separation (CAMAG) was done using silica gel plates specifically HPTLC Silica gel 60 F_{254}(10*10cm). We used a mixture of acetonitrile : methanol : 25% ammonia solution (85:10:5 v/v/v) as a mobile phase. Migratin distance of mobile phase was over a path 80 mm. The determination of compounds was made with UV detector with wavelength 254 nm.

Results and discussion. The conditions of method were found experimentally. Best eluents from blood plasma for buspirone was acetonitrile (extraction output 90,1%), ethanol (84%); for fluoxetine was ethanol (84,96%), propanol (80%); for escitalopram was ethanol (87,12%), propanol (77,65%); for paroxetine was methanol (88,5%), ethanol (80,17%). Therefore the most appropriate eluent for the best separation and identification of the analytes ESCI, FLU, PAR, BUS, was found to be 80% ethanol in water solution, 2% acidified with concentrated formic acid (Figure 1 B). . The retention times of BUS, ESCI, PAR, FLU are 12.14, 13.82, 14.72 respectively.

The image of HPTLC (Figure 1 A). The obtained chromatogram by main spots corresponds to the typical reference solutions spots. Rf values were determinated for all four main substances. It was 0.92, 0.60, 0.72, 0.65, for BUS, PAR ESCI, FLU respectively in reference solutions and test sample of blood plasma. These results show an effective application of the methodology for BUS, PAR, ESCI, FLU analysis.

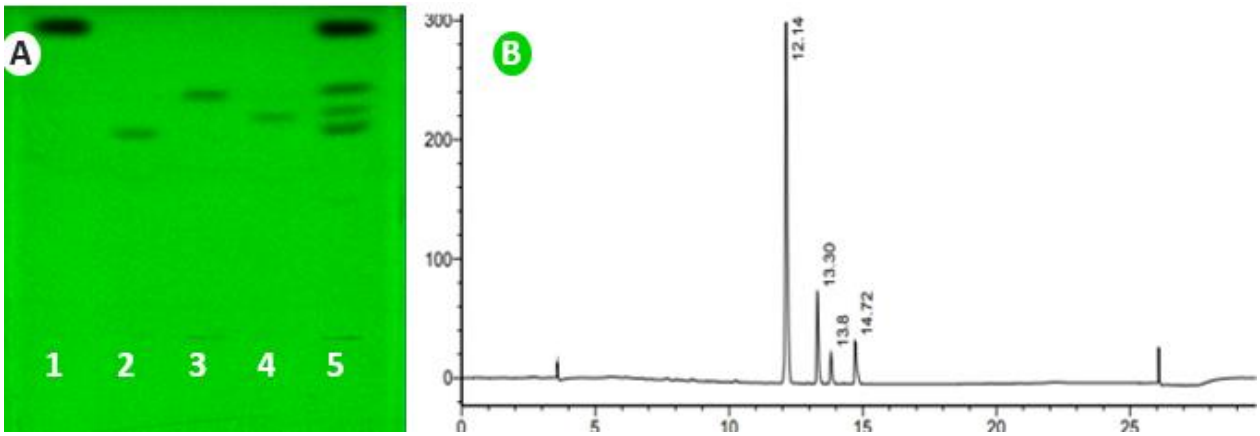

Figure 1 (A). HPTLC plate with 1-4 reference solutions of BUS, PAR, ESCI and FLU resppectively and 5-test sample of blood plasmaion. **(B).** HPLC chromatogram of compounds, extracted from blood plasma with 80% ethanol in water solution, 2% acidified with concentrated formic acid.

Conclusion. We developed sensitive and specific methods of HPLC and HPTLC wit prior solid phase extraction step for determination of SPE BUS, PAR, ESCI and FLU in blood plasma.

MODERN ANALYTICAL METHODS OF IDENTIFICATION SUBSTANCES FOR PHARMACEUTICAL APPLICATION IN LEADING WORLD PHARMACOPOEIAS

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Introduction. Methods of identification of substances for pharmaceutical use based on new advances in chemistry, physics, biology, pharmacy and other natural sciences are rapidly developing and used in a comprehensive manner.A specialist in the pharmaceutical industry, choosing a particular method of analysis, it is necessary to correctly assess their selectivity, specificity, sensitivity and informativeness.

Aim. The purpose of our work was to compare the tendencies of the priority of choice and research of the newest methods of identification of pharmaceutical substances, which were offered recently by the leading world pharmacopoeias.

Materials and methods. We have investigated the monograph materials for pharmaceutical use by the State Pharmacopoeia of Ukraine (SPhU) 2.0, harmonized with the European Pharmacopoeia (Ph. Eur.) 8.0 (section 2 Analysis Methods, Division 2.2 Physical and Physical and Chemical Methods, in particular SPhU), the relevant sections of The United States Pharmacopoeia (USP) 38 (General Tests and Assays, where the Chemical Tests and Assays, Physical Tests and Determinations are presented), The British Pharmacopoeia (BP) (2013), a series of periodicals and electronic publications.

Results and discussion. In SPhU 2.0 and Ph. Eur. 8.0 in most of the substance monographs in the «Identification» section, there are often two parts, «First Identification» and «Second Identification». Identification tests that recommend pharmacopoeias in the «First Identification» section can be used in all cases, while «Secondary Identification» tests only on condition that the drug substance belongs to a series that has already been certified for compliance demand, such as Ph. Eur.

Or purposes of identifying pharmaceutical substances, different pharmacopoeias recommend physical, chemical and physico-chemical methods of analysis. A number of these identification methods can be characterized as non-selective. These include methods for the chemical identification of cations, anions, and certain functional groups. It is also a visual methods that evaluates aggregate state, color, crystal shape and solubility of substances. Nonselective include absorption spectrophotometry, ultraviolet and visible (UV-Vis or UV / Vis), allowing only certain functional groups to be confirmed in the test substance.

Among the selective identification methods, indirect and direct ones can be identified. In indirect methods, the substance is identified more often by a certain physical property – melting point, boiling point, viscosity (kinematic or dynamic), optical rotation for optically active substances, adsorption capacity, etc.

By indirect selection, chromatographic methods are thin-layer chromatography (TLC), gas chromatography (GC) and high performance liquid chromatography (HPLC).

The most common direct method for identifying substances for the pharmaceutical use of organic nature is spectrometry in the IR region from 4000 to 400 cm^{-1}. A variety of IR spectroscopy is Raman spectrometry where the spectra of combinational scattering are taken. The sensitivity of the spectra of the combination scattering to the nature of the chemical bonds determines the individuality of the spectrum of the substance being studied. Among the advantages of the method is the possibility of contactless analysis of solid, liquid and gaseous substances contained in a glass or polymeric shell.

Recently, methods for nuclear magnetic resonance spectrometry (NMR) and chromatographic mass spectrometry are used to identify substances without the use of standards and internal standards. The NMR method can identify both individual compounds and complex mixtures without their prior physical separation. The mass spectrometry is characterized by high sensitivity and selectivity.

When identifying drugs containing several components (multivitamins, infusion solutions, X-ray contrast media), direct selective methods such as atomic absorption spectrometry (AAS), inductively coupled plasma-atomic emission spectrometry (ICP-AES), inductively coupled plasma-mass spectrometry (ICP-MS), X-ray fluorescence spectrometry (XRF). For the identification of substances that have polymorphic modifications most often use the method of X-ray diffraction analysis.

As a result, it should be noted that the method of IR spectrometry along with others for the identification of pharmaceutical substances is included in most pharmacopoeias (more than 70% in USP, of which only the IR identification is more than 10% and more than 80% in Ph. Eur., Where only the IR identification is about 25%). Approximately 20% of monographs are used for identification using chromatographic methods. At Ph. Eur. about two-thirds of the chromatographic identification methods make TLC, while in the USP, TLC is used only in 30% chromatographic, and preferred by more specific and accurate HPLC methods.

Conclusions. An important place among modern methods of identification of pharmaceutical substances in the leading world pharmacopoeias occupy instrumental methods of analytical and physical chemistry. This is a group of methods of spectroscopic analysis (atomic emission, atomic absorption, IR, UV, Species, X-ray fluorescence spectrometry, Raman and mass spectroscopy); TLC, GC and HPLC. Future specialists in the field of pharmacy should fully master these modern methods of pharmacopoeial analysis.