

**DEVELOPMENT AND VALIDATION OF TANDEM
UV-SPECTROPHOTOMETRIC/EXTRACTION-PHOTOMETRIC
PROCEDURE OF ZOPICLONE QUANTITATIVE DETERMINATION**

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Realization procedure of toxicological examinations requires to give the results of analyte content determination in the sample obtained with the help of at least two methods of analysis, which are based on different principles. Therefore elaboration of so-called tandem procedures allowed to carry out substance determination in the same sample simultaneously by means of two methods of analysis is actual.

The purpose of our paper is development and validation of tandem UV-spectrophotometric/extraction-photometric procedure of zopiclone quantitative determination.

The extraction-photometric procedure of zopiclone quantitative determination using acid dye methyl orange was described before – methyl orange in the acid medium formed ionic associates with zopiclone, which were extracted by chloroform. Under these conditions the chloroform layer becomes yellow – for increasing colour intensity and method sensitivity the obtained ionic associates are decomposed by adding sulphuric acid solution in absolute ethanol to their chloroform solutions and intensive pink colour related to liberating free methyl orange appears. The amount of methyl orange is equivalent to the amount of zopiclone in ionic associates under these conditions.

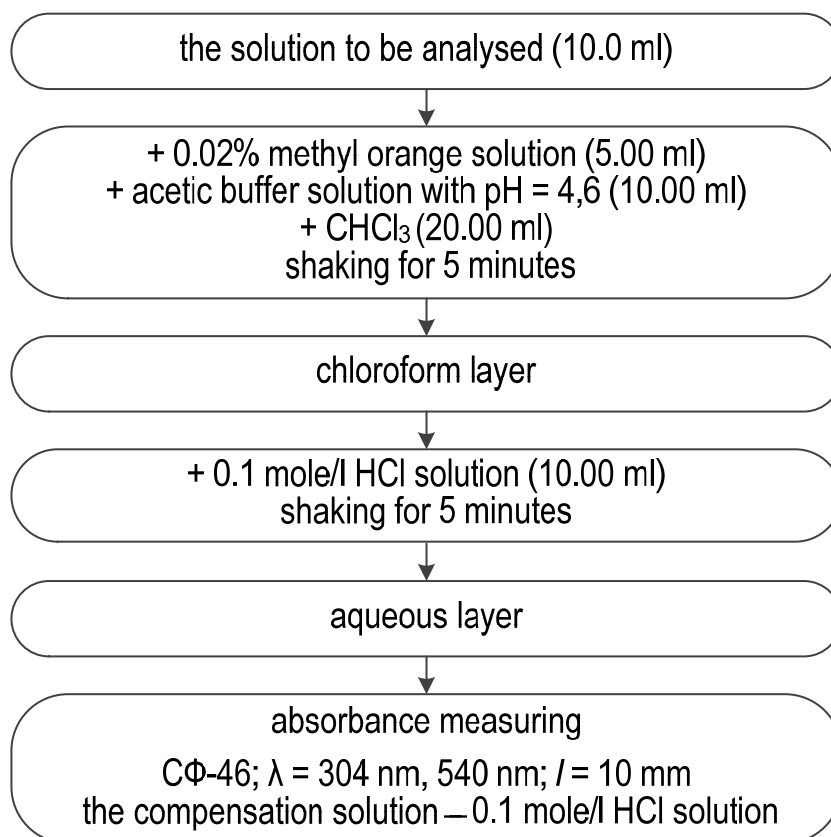
On the first stage of our researches we have modified the described procedure in the way of increasing the volumes of solutions in such a manner that it is possible to measure them with greater exactness that must considerably decrease the total uncertainty of the procedure of analysis.

On the second stage we have carried out validation of the modified procedure in the variant of the method of calibration curve using model solutions with the purpose of confirmation of its applicability for quantitative determination of zopiclone in biological liquids – the offered modified procedure is characterized by satisfactory accuracy and unsatisfactory linearity and precision for all variants of range of the methods application.

From our point of view, unsatisfactory linearity and precision of the procedure are connected, firstly, with application of photoelectrocolorimeter for absorbance measuring, and, secondly, with preparation of the solutions with application of

volatile solvent – chloroform.

With the purpose of leveling the specified defects we have developed tandem UV-spectrophotometric/extraction-photometric procedure according to the presented scheme.



I. e. it is suggested by us to carry out decomposition of ionic associates and reextraction of methyl orange and zopiclone in 0.1 mole/l hydrochloric acid solution simultaneously and to measure the absorbance of methyl orange and zopiclone in the obtained aqueous solution by spectrophotometer.

We have carried out validation of the offered tandem procedure in the variant of the method of calibration curve using model solutions. The obtained data specify that the offered tandem procedure of zopiclone quantitative determination is characterized by satisfactory linearity, accuracy and precision for all variants of range of the methods application and for both variants of the used wave length that makes it suitable for development of procedures of zopiclone quantitative determination in biological liquids.

It is necessary to point that the offered procedure allows to determine simultaneously zopiclone both by its own absorbance in UV-range of spectrum and by absorbance of methyl orange in visible range of spectrum that provides additional reliability of analysis and satisfies the requirements to researches realization in chemical and toxicological analysis – determination should be carried out with the help of at least two methods of analysis, which are based on different principles.