

antioxidant fixed for each individual person, we can be more resistant to stress, focus and greater power of concentration and mental activity. The aim was to evaluate research conducted medicinal plants with antioxidant action, given the increasing incidence and prevalence of many diseases in Moldova pathologies such as cardiovascular, endocrine and cancer are influenced by the presence of oxidative processes.

**Materials and methods:** Medicinal plants with antioxidant activity were selected based on scientific publications, species were identified by Flora Identification Manual for the Republic of Moldova and reference pharmacopoeias. Were characterized antioxidant active principles responsible for the action and assessed phytopreparations by State Drug Nomenclature Moldova: vegetable drugs, medicinal species, phyto antioxidant action. Was evaluated, also, legal status on their release from pharmacies (OTC / Rx) and their presence in pharmacies in Moldova.

**Results:** Antioxidants herbs that was evaluated contains: vitamins, flavonoids and tannins in amounts large or small, exhibiting antioxidant by trapping free radicals. Antioxidants act by giving electronic and completing the last layer of free radicals, which are not deficient in electrons, loses its harmful action. Also, flavonoids potentiate the action of other antioxidants, including vitamins E, A and C. We note that among medicinal plants with antioxidant action, rank: *Rosa canina* L.- 14 medicinal products, *Taraxacum officinale* Web. - 10, *Phaseolus vulgaris* L. - 6, *Cynara scolimus* L. - 5, *Vaccinium myrthillus* L. - 4, *Hippophae rhamnoides* L. - 4, *Cichorium intybus* L. - 4, *Centaurea cyanus* L. - 3, *Potentilla erecta* L. - 2 and *Aronia melanocarpa* - 1.

**Conclusion:** The revaluation result phytopreparations after Nomenclature, we find that in 6350 registered products, 53 exhibit antioxidant, of which: 2 vegetable products, 17 medicinal species, 10 phytopreparations monocomponent and 24 phytopreparations multicomponent. After the release status of the pharmacy, we find that 95% are part of the OTC, 5% is released under doctor's prescription, and their presence in pharmacies is 53%.

**Keywords:** Antioxidants, phytopreparations, medicinal plants

## 9. SPECTROPHOTOMETRIC ASSAY OF PROTEIN CARBONYLS IN HUMAN PLASMA

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**Introduction:** The oxidative stress represents the aggression produced at the molecular level by the imbalance between pro-oxidant and antioxidant agents, with severe functional consequences in all organs and tissues. An overproduction of reactive oxygen species (ROS) results in oxidative damages especially in proteins (the main target of ROS), as well as in lipids, or DNA. A great effort has been undertaken to assess the biomarkers of protein oxidative injury, most cited being the quantitative assay of protein carbonyls, nitrotyrosine levels, GSH level and/or GSH/GSSH ratio. The present study aims at finding a robust spectrophotometric method to quantify the protein carbonyls in human plasma. The study model was represented by bovine serum albumin (BSA), which was subjected to different oxidative damage conditions, in order to be carbonylated.

**Materials and methods:** Three hydroxyl radical generating systems were investigated: potassium ascorbate / ferric chloride, ascorbic acid / ferrous sulfate and hydrogen peroxide / copper sulfate. All systems were applied to BSA solutions for 10 to 24 h at 37 Celsius degrees. After degradation, the protein carbonyl levels were evaluated by means of a modified Levine's method, using the derivatisation with 2,4-dinitrophenylhydrazine. The carbonyl content was expressed in mg carbonyls/mg proteins. An ABL&E-JASCO model V 530 spectrophotometer was used throughout the experiments.

**Results:** The best yield for carbonyl generation was induced by potassium ascorbate / ferric chloride system, after 24 h degradation time. The carbonyl proteins pellets are separated only at centrifuge speeds higher than 5000 rpm, with an optimum at 7000 rpm, at 4 Celsius degrees. The protein carbonyls are stable in the BSA solution only for a short period (hours). Levine's method was modified by replacing the solubilisation in guanidine hydrochloride with 1 M NaOH solution.

The total protein content was assessed using Lowry method, in order to avoid nucleic acids interferences. The method was validated according to the

EMA/CHMP/EWP/192217/2009 "Guideline for bioanalytical method validation".

**Conclusion:** A controlled method for the generation and a validated method for the quantification of protein carbonyls using BSA as a model, with spectrophotometric assay were developed. The methods can be further used for human plasma protein assay. Also, it could be useful for the development of a new capillary electrophoresis method for protein carbonyls assay.

**Keywords:** Protein carbonyls, oxidative stress, spectrophotometry

## 10. SPECTROPHOTOMETRIC DOSING OF BETA CAROTENE IN TABLETS METHOD VALIDATION

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**Introduction:** Validation is a verification methodology, compliance and accreditation of validity as a method of scientific analysis. This methodology aims to demonstrate that the analysis method corresponds to the utilization for which it was foreseen, and to the parameters of validation in accordance with regulatory organizations. It is now recognized that no drug substance or drug can enter the European and American market without being accompanied by corresponding validation folder. The results of this study will serve as support in concluding validation methods report for the determination of the active principles of EUROSEPT product.

**Materials and methods:** The study was conducted on lozenges EUROSEPT, series 041211 made in December 4th, 2012 and valid until 12.2014. 1 tablet contains: active substances: ascorbic acid, eucalyptus essential oil, Tolu balsam,  $\beta$ -carotene; auxiliary substances: aerosil, corn starch, talc, magnesium stearate, stearic acid, citric acid, aspartame, sorbitol, lactose. In the process of dosage methods validation the following parameters were analyzed: specificity, precision (repeatability, reproducibility), linearity, accuracy. Spectrophotometric dosage method was performed by using scanning spectrophotometer double perchin Elmer Lambda 25 UV-VIS.

**Results:** Comparative analysis performance of the validation parameters were done, according with the European and international regulatory organizations: ISO, FDA, ICH, USP. Empirical results expressed through absorbance value were used for reconstruction of five calibration graphs used for the completion of the final one. The correlation coefficient of linear regression model is 0.9997, which shows that the experimental points approximate and arrange around the regression line according to experimental conditions stipulated in the MFT. The calibration graph is an average of all data points, which is characterized by a relative error less than 2%. Standard deviation of residuals, standard deviation of intercept and slope values are less than 0.5%, which indicates that the calibration chart can be used to determine the specificity of the dosage method. At the maximum absorption of 454 nm, for the solution obtained in the placebo-controlled trial were not observed significant absorbance values, which indicates that the secondary constituents do not interfere the procedure of the active substances quantification (ascorbic acid, eucalyptus oil, balm Tolu) of the reconstitute pharmaceutical form. Statistical obliquity value does not exceed the 1.5%, which demonstrates the accuracy of the spectrophotometric dosage method of beta-carotene in the visible domain. The correlation coefficient  $r^2$  is 0.9984, which indicates that with the change in the amount of beta-carotene concentration of 80-120%, the dependence of absorbance versus concentration signal is one almost linear.

**Conclusion:** Spectrophotometric UV-VIS validation method of  $\beta$ -carotene dosage within the preparation EUROSEPT was performed, according to validation parameters: linearity, repeatability, reproducibility, specificity, accuracy, according to regulatory organizations.

**Keywords:** Validation, Spectrophotometry UV-VIS, Eurosept,  $\beta$ -Carotene