THE DYES FROM MEDICINAL CAPSULES

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Abstract

Through this study we sought to identify the presence and nature of several organic dyes in some formulations that contain medicinal substances, namely operculate capsules with the aim of a potential toxicological risk.It was made an isolation of softgel by solid-phase extraction on silica gel and and then they were identified by thin-layer chromatography. Of the thirteen types of capsules was found out that the ten types are coloured with synthetic dyes and three are coloured with mineral dyes. Synthetic colorants identified are quinoline yellow, sunset yellow, azorubină, 4R, patent blue, all of them presenting a specific toxicity. Thanks to their toxicity,some countries have banned their use as food additives while in our country they are allowed.

Key words*:* operculate capsules, organic synthetic dyes, yellow quinoline, yellow azorubine, sunset, blue, patent ponceau 4R

INTRODUCTION

Food additives shall confer on the attractive organoleptic characteristics of food and also for some pharmaceuticals formulations under which medicinal substances are packed (Hurstel, O., 1994, Bungău S., 2015). Even though most drugs consumed only occasionally or short-term, some is consumed in the long-term (months or years), in chronic diseases, with risk of accumulation in the body of some additives, increasing the risk of toxicity.

As in the case of food and for medicinal products the food additives are used within the limits of laws. Towards safety studies for each excipient/additive/colouring in order of their introduction on the lists of supported by European Commission, additives for use in foods and medicines, for each medication, clinical trials shall be carried out prior to the authorization of the holder thereof (Srivastava S., 2004, Choudhary K.N. et al., 2013, Titford M., 2007, Goyal S.K., 2007, Vidotti E. C. et al., 2005).

In Romania the legislative rules regarding acceptable doses of food additives are more premisive because Acceptance Daily Intake- ADI, are not strictly determined, compared to other countries, it being sufficient that analyses to confirm or refute the presence or absence of additives in commercial products. At the same time in our country are accepted food additives which in other countries are completely banned as being harmful to health (Lungu, C.,1999, Dumitrache. S., 1986, Hajimahmoodi M., 2008).

MATERIAL AND METHOD

The objective of this paper is to obtain data on the presence of toxic colorants in some pharmaceutical formulations respectively operculated capsules, which contain medicinal substances, with the aim of assessing the toxicological risk given these colorants.

For the experimental part, there were taken into work thirteen types of capsules differently colored, empty of content, intended to be conditioned as standardized products.

There were used thirteen types of differently colored capsules (Figure 1) empty content purchased from the medicine factory Terapia Rambaxy Cluj Napoca.

Fig. 1. The medicinal capsules submitted to the study of colorants

The capsules were destined for conditioning some medicinal substances in the form of standardized products, some with still an unspecified destination use, others with a precise destination, for a product from the market, as follows:

- blue capsules noted with **A1** for the product *Diurex;* with **A2** for *Indometacin 25 mg*; with **A3** for *Paduden 200 mg;*
- green capsules noted with **V** for *Flucoric 150 mg;*
- red capsules noted with **R1** for *Glubifer complex.*

Medicinal capsules were subjected to the qualitative analysis, for the identification of the dyes from the two types of capsules, randomly chosen.

The method of analysis

The qualitative analysis of the dyes

The method for the preparation of the sample is chosen based on the matrix and the analysis technique itself, respectively the extraction of the dye is made on the solid phase followed by thin layer chromatography analysis. For this the quantitative and selective isolation of the dyes in the matrix is absolutely necessary.

Dissolution of the capsules and sample preparation for the extraction

Reagents: gelatin capsules from medicine factory Terapia Rambaxy, Cluj Napoca; solvents for the analysis: benzene, toluene, acetone, isobutylic alcohol, metanol, glacial acetic acid, ammonia and hydrogen chloride, purchased from CHIMOPAR, Bucharest; LiChrolut extraction cartridges RP-18, G 60 plates of Sil from Merck (Germany); synthetic food dyes: azorubine, patent blue, sunset yellow, ponceau 4R, quinoline yellow, brilliant blue, tartrazine from the company FLUKA- Sigma-Aldrich.

For the purpose of extraction of the dye (2) from the capsules 0.2 g of shell was shredded with the scissor and put in contact with various solvents, as follows:

- with organic solvents: chloroform, carbon tetrachloride, acetone, hexane, toluene, isobutylic alcohol, methyl alcohol; there was not observed neither the dissolving of the capsules, nor the extraction of the dye;
- with mixtures of methanol with hydrochloric acid and ammonia, in different proportions: MeOH-HCl (1N) 6:1, v/v, 1:1, v/v or 2:3, v/v; MeOH:NH_4OH 6:1, v/v , 1:1, v/v , it was not observed no dissolution of the matrix;
- with increasing the water content also increases the content of dissolved dye, and with hot distilled water, complete dissolution of the gelatin shell was observed, as well as of the dyes, due to their hydrophilic nature.

The identification of the dyes

In the case of colored compounds the identification can be made either based on Rf values correlated with the spot color, based on the absorption spectrum UV-VIS directly on the chromatography plate or by "cutting" the spot and transferring the component into solution, the identification being accomplished later by various spectroscopic techniques: UV-VIS, IR, MS.

For the identification of the dyes from capsules, these were pulverized to shorten the dissolution time by grinding with a household electrical grinder. About 0.2 grams of the powder was dissolved in 5 ml hot distilled water in a Berzelius glass (50 mL). The sample was then diluted with 10 ml of acetate buffer (pH=4).

Solid phase extraction, on modified silica gel, of synthetic dyes

Synthetic food dyes are ionic organic compounds containing sulfonyl groupings. Therefore they can not be detained on non-polar solid sorbents such as C18 modified silica gel. Retention would be possible if by some way ionic groups would be neutralized. There are two ways to transform the ion of the dye in a non-dissociated molecule:

- the demotion of the dissociation balance - studied dyes being salts of organic sulfonic acids, in an acid medium at pH < pK_a will be found in undissociated form
- the pair of ions- consists in forming of a complex between an ionized substance (hydrophilic) and an ion of opposite charge, the resulting complex is more hydrophobic and can be extracted easier with an organic solvent; due to the presence of the negative sulfonic groups from the structure, synthetic dyes are able to form ion pairs with the organic substances that have a positive charge (quaternary ammonium salts).

The extraction cartridges were prepared by conditioning the sorbent with MeOH (5 mL) and washing with buffer (5 mL). The samples were passed through the cartridge with a flow rate of about 10 mL / min, only synthetic dyes being retained.

To remove the nonspecific adsorbed interferences (gelatin) the cartridge was washed with hot buffer, aiming that dyestuffs remain fixed to the sorbent. After drying the cartridge by passing a stream of air, the dyes were eluted with a mixture of MeOH - NH_3 (3:2, v/v).

The analysis through the thin layer chromatography

Dye extracts thus obtained were analyzed by thin layer chromatography using Sil G 60 plates.

As a mobile phase it was used the system: butanol - glacial acetic acid - ethanol - water in a ratio of 40: 8: 4: 20 (v / v). There were applied 0.5 cm strips from the colored extracts and from the standard solutions.

RESULTS AND DISSCUSIONS

In the figures 2 and 3 are presented the developed chromatographic plates 1 and 2, of the samples obtained from the capsules subjected to the analysis.

The retention factor was calculated as the ratio between the migration distance of a component (measured from start to the middle of the spot) and the migration distance of the mobile phase (from start to front).

The dyes were identified by comparing the retention factor R_f values of the real samples with the R_f of the standard solutions (tab.1. and 2) and based on the color.

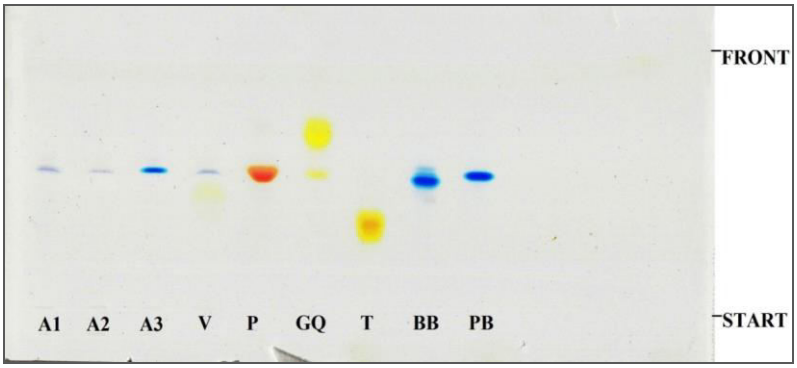

Fig. 2. Chromatogram 1

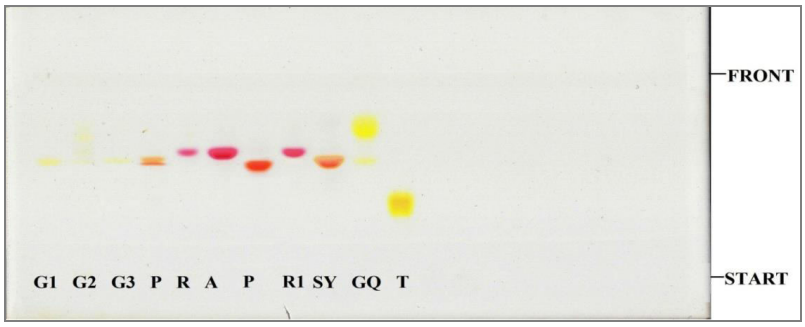

Fig. 3. Chromatogram 2

Table 1

Determination of the R_f values from the analyzed samples for chromatogram 1

Compound	**The distance of migration of the front [cm]**	**The distance of migration of the compound [cm]**	**Rf**
The A1. A2. A3 capsules	7.1	3.7	0.52
The V capsule	7.1	3.7	0.52
	7.1	3.5	0.49
Etalon P – Ponceu 4R	7.1	3.5	0.49
Etalon GQ - Quinoline Yellow	7.1	3.5	0.49
Etalon T-Tartrazine	7.1	2.2	0.30
Etalon BB - Brilliant Blue	7.1	3.5	0.49
Etalon PB - Patent Blue	7.1	3.7	0.52

***Table* 2**

Determination of the R_f values from the analyzed samples - chromatogram2

Compound	**The distance of migration of the front [cm]**	**The distance of migration of the compound [cm]**	**Rf**
The G1. G2. G3 capsules	7.0	3.5	0.50
The P capsule	7.0	3.5	0.50
	7.0	3.6	0.51

The R. R1 capsules	7.0	3.8	0.54
Etalon A - Azorubine	7.0	3.8	0.54
Etalon P- Ponceu 4R	7.0	3.5	0.50
Etalon SY - Sunset Yellow	7.0	3.6	0.51
Etalon GQ - Quinoline Yellow	7.0	3.5	0.50
Etalon T - Tartrazine	7.0	2.2	0.31

Chromatographic analysis shows the following dyes present in capsules:

- ✓ blue capsules A1, A2, A3 contain patent blue;
- ✓ green capsules V contain patent blue and quinoline yellow;
- ✓ yellow capsules G1, G2, G3 contain quinoline yellow;
- ✓ pink R and red R1 capsules contain azorubine;
- ✓ orange capsules P contain sunset yellow and ponceau 4R.

Following the experiments that were made it was found that of the 13 analyzed capsules only 10 of them contain synthetic organic dyes, the other 3 contain inorganic mineral dyes. This finding was made when the samples were passed over the cartridge, inorganic dyes were not retained on the non-polar sorbent C18.

Toxicological aspects of the dyes

Patent blue - E 131

A high intake of E 131 determines: hyperactivity syndrome and concentration deficiency (ADHD) at children; allergic reactions, dermatitis and purpura, in rare cases anaphylactic shock; hypotension, muscle contractions, bradypnea; cancer, proven experimentally by tests on laboratory animals (5, 6, 7, 8, 10). The acceptable daily intake is 15 mg / kg body (10). It is forbidden in Australia, the US and Norway. Some researchers have included it in the category of carcinogenic food additives and advise great caution in its consumption (10,11).

Quinoline yellow -E104

It is a sulfonate derived of the disulphonic acid, in toxicological terms; it is not recommended those sensitive to aspirin (salicylates) as it causes allergies, asthma, dermatitis, skin rashes, hyperactivity syndrome ADHD, concentration deficiency; people with sensitivity to aspirin should avoid it can release histamine (5, 6, 7). The acceptable daily intake is up to 10 mg / kg body (10). It belongs to the category of the " suspicious E's". It is forbidden in Norway, Japan, USA (11).

Azorubine - E122

Azorubine or carmoisina or E122, toxic azoic dye: potentially carcinogenic due to the formation of DNA adducts**;** triggers asthma attack and allergic reactions in sensitive individuals**;** is associated with ADHD syndrome; it is not recommended for pregnant and breastfeeding women, children, those with ADHD, people with allergies, asthma (1,9)**.** The

acceptable daily intake: 4 mg / kg body (10). It is forbidden in Austria, Norway, Sweden, Japan, USA**,** but allowed in Romania (11).

Ponceau 4R-E124

It is an azoic dye with the following toxic effects: allergic reactions to asthmatic patients and to the persons sensitive or allergic to aspirin; it may induce ADHD to children and adolescents; is carcinogenic, experimentally demonstrated, induces thyroid tumors (7, 9, 10); toxicity worsens when combined with sodium benzoate E211, extremely dangerous preservative; because of this in Britain it is banned in products intended for children (5, 9). The acceptable daily intake for humans: 4 mg / kg body weight (10). It is forbidden in Norway and the US, but allowed in Romania (11).

Sunset yellow-E110

It is an azoic dye with the following toxic effects: is reduced by intestinal bacteria to sulfonated aromatic aminophenols and amino sulfonic acids; allergic reactions, urticaria, asthma, bronchoconstriction, nasal congestion; chromosomal alterations evidenced by tests on animals, renal tumors; associated with growth slowing, severe weight loss, indigestion, anorexia; altered taste for food, gastric pains (1, 5, 6, 7, 9). The acceptable daily intake is 2.5 mg / kg body (10). Belongs to the dangerous E's category. It is forbidden in Norway, Austria, but allowed in Romania (11).

CONCLUSIONS

Through this study we found that in a large number of gelatin capsules taken into work, intended to be used for conditioning some medicinal substances, are found various potentially toxic organic dyes.

The presence of these dyes may cause toxic effects of the worst, which is why many countries have banned their as food additives and however they are not banned in Romania.

Their toxicity resides in the fact that using them even temporarily, in short cure, dyes from capsules have a strong allergic potential, possibly even wrong attributed to the medicinal substance in a pharmaceutical product, or empowering it. Administered to persons with attention deficiency may worsen the disease through the presence of the dyes from the pharmaceutical forms or can trigger ADHD in young children and adolescents.

The administration in chronic diseases, of some pharmaceutical forms colored with these synthetic dyes, may lead through accumulation or long-term exposure to severe toxic effects such as chromosomal changes, tumors at various sites (kidney, thyroid etc.)

Administrated to pregnant women, can cross the placenta and could reach toxic concentrations in the fetus.

REFERENCES

1. Banu, C., Butu C., Lungu C., Alexe P., Răsmeriţă D.,Vizireanu, C., 2000, Aditivi şi ingrediente pentru industria alimentara, Ed. Tehnică, Bucuresti, pp. 13-16
2. Bungău, S., Copolovici, D., Copolovici, L., 2015, Instrumental Analitical Methods/Metode instrumentale de analiză, Ed. Italian Academic Publishing
3. Bungău S., Bungău C, Ţiţ D. M., 2015, Studies about last stage of product lifecycle management for a pharmaceutical product, Journal of Environmental Protection and Ecology, Vol. 16, No. 1, pp. 56-62
4. Cîmpan Gabriel, 2001**,** Encyclopedia of Chromatography, Ed. Jack Cazes, Marcel Dekker, New York, 624-626
5. Choudhary K.N., Soni P.P., Sao D.K., Murthy R., Deshkar A.M. and Nanda B.R., 2013, Role of gentian violet paint in burn wound management: a prospective randomised control trial, J Indian Med Assoc. 111(4); 248–50
6. Dumitrache. S., Popa,G., 1986, Toxicologia produselor alimentare, Ed Academiei, Bucuresti, pp12-18,22-39
7. Goyal S.K., 2007, Use of rosaniline hydrochloride dye for atmospheric SO2 determination and method sensitivity analysis". Journal of Environmental Monitoring, 3; 666–670
8. Hurstel, O., Meldie, J**.,** 1994, Les colorants alimentaires, Ind. Alim. Et Agr.9, pp.234
9. Hajimahmoodi M., Oveisi M. R., Sadeghi N., Jannat B., E. Nilfroush, 2008, Simultaneous determination of carmoisine and Ponceau 4R, Food Analytical Methods, vol. 1, no. 3, pp. 214–219
10. Keser Karaoglan G., Gumrukcu G., Ustun Ozgur M., Bozdogan A., 2007, Abilities of partial least-squares (PLS-2) multivariate calibration in the analysis of quaternary mixture of food colors (E-110, E-122, E-124, E-131), Analytical Letters, vol. 40, no. 10, pp. 1893–1903
11. Lungu, C., 1999, Nutriţie umană şi toxicologia produselor alimentare, Ed, Evrika, Brăila, pp 9-14, 67-103
12. Mencinicopschi Ghe.**,** 2000, Biblia Alimentelor, Ed. Litera Internaţional, pp.48-55
13. Sercan, E., 2001, Efectele devastatoare ale unor E-uri periculoase, Curierul National 07.02;
14. Minioti K. S., Sakellariou C. F., Thomaidis N. S., 2007, Determination of 13 synthetic food colorants in water-soluble foods by reversed-phase high-performance liquid chromatography coupled with diode-array detector,"Analytica Chimica Acta, vol. 583, no. 1, pp. 103–110
15. Srivastava S, Sinha R, Roy D., 2004, Toxicological effects of malachite green, Aquatic Toxicology 66(3); 319–29
16. Titford M., 2007, Comparison of historic Grübler dyes with modern counterparts using thin layer chromatography, Biotech. Histochem. 82(4–5); 227–234
17. Vidotti E. C., Cancino J. C., Oliveira C. C., Rollemberg M. D. C. E., 2005, Simultaneous determination of food dyes by first derivative spectrophotometry with sorption onto polyurethane foam, Analytical Sciences, vol. 21, no. 2, pp. 149–153
18. *** www.controleuri.ro/euri-detalii/ html
19. *** www.food-info.net/ro/e/e122.htm
20. *** www.chemweb.com