Македонски Фармацевтски билтен

Macedonian Pharmaceutical Bulletin

волумен **68** (додаток 1) 2022 / volume **68** (Suppl 1) 2022

Македонско фармацевтско друштво, ул. Маршал Тито 13б/8, Скопје, Македонија
Macedonian Pharmaceutical Association, Marshal Tito 13b/8, Skopje Macedonia

It is our great pleasure to present this Supplement Issue on "*Macedonian Pharmaceutical Bulletin*" to the scientific and professional community. This supplement includes the short communications accepted for the *Seventh Congress of Pharmacy in North Macedonia with International participation 2022*, which was held in Ohrid, October 5-9, 2022.

The main theme of the Congress was "Modern trends in Pharmacy: opportunities and challenges" A broad spectrum of topics within the pharmaceutical sciences and practice carefully selected for this special occasion in order to build up a highly interesting and comprehensive program were covered. The contributions submitted to the Congress included 6 plenary lectures, 69 section lectures, and more that 200 posters. This Congress, followed the excellent international tradition, was attended by more than 1000 domestic and foreign participants. We received more than 287 short paper submissions from more than 15 countries. These numbers show that our Congress was aiming for the highest scientific standards, and that it can be considered a well-established venue for researchers in the broad fields of Pharmaceutical sciences and practice.

Sincere thanks to the hosts of the Seventh Congress of Pharmacy in North Macedonia with International participation, Macedonian Pharmaceutical Association and Faculty of Pharmacy, Ss Cyril and Methodius University in Skopje for their vision and commitments.

We would like to thank the companies that showed interest in supporting our efforts during the organization. We acknowledge the sponsoring companies: the platinum sponsor AD ALKALOID, Skopje, the golden sponsors: PLIVA-TEVA, EUROFARM, REPLEK and KRKA, the silver sponsor HEMOFARM and the bronze sponsors: GALENIKA, SEPTIMA and SALVEO,

We would also like to thank our members of the Scientific Committee for their volunteer time and dedication to the critical peer review process. We also wish to thank all the members of the Organizing Committee, whose work and commitment was invaluable.

On behalf of the Advisory and Scientific Committees, we would like to especially thank all internationally prominent researchers, whose work was supposed to be an essential part of the Congress. The interest in publishing their short communications in this issue of the Macedonian Pharmaceutical Bulletin is of a crucial importance for reinforcing the overall quality and standards of the bulletin. They give the state of the art of the recent advances in the field of pharmacy research.

The pharmaceutical sciences continue to grow as dynamic scientific interdisciplinary fields. We believe that published short communications will be an excellent source of scientific material in the fast evolving fields in Pharmaceutical sciences and practice.

Chair of the Scientific Committee

Prof. Marija Glavas Dodov

Chair of the Organizing Committee

Prof. Rumenka Petkovska

Your hosts
Macedonian Pharmaceutical Association
and
Faculty of Pharmacy, Ss Cyril and Methodius University in Skopje

Organizing Committee

Rumenka Petkovska, Ss. Cyril and Methodius University in Skopje, Faculty of Pharmacy/Chair
Aleksandra Kapedanovska Nestorovska, Ss. Cyril and Methodius University in Skopje, Faculty of Pharmacy/ Secretary
Dushko Shalabalija, Ss. Cyril and Methodius University in Skopje, Faculty of Pharmacy
Liljana Anastasova, Ss. Cyril and Methodius University in Skopje, Faculty of Pharmacy
Blagoj Achevski, Ss. Cyril and Methodius University in Skopje, Faculty of Pharmacy
Biljana Taneva, Alkaloid AD Skopje
Snezana Petrovska, Alkaloid AD Skopje

Advisory Board

Aleksandar Dimovski – North Macedonia, Aneta Dimitrovska – North Macedonia, Atila Hinchal – Turkey, Biljana Bauer – North Macedonia, Ilkay Orhan - Turkey, Irena Mlinarič-Raščan – Slovenia, Jelena Filipovic-Grcic – Croatia, Kristina Mladenovska – North Macedonia, Katerina Goracinova – North Macedonia, Lidija Petrusevska Tozi – North Macedonia, Lilian Azzopardi – Malta, Ljubica Suturkova – North Macedonia, Renata Slaveska Raicki – North Macedonia, Rumenka Petkovska – North Macedonia, Slađana Šobajić – Serbia, Suzana Trajkovic Jolevska – North Macedonia, Svetlana Kulevanova – North Macedonia, Tatjana Kadifkova Panovska – North Macedonia, Vasia Bankova – Bulgaria, Zoran Kavrakovski – North Macedonia, Zoran Sterjev – North Macedonia

Scientific Committee

Marija Glavas Dodov / Chair
Maja Simonoska Crcarevska / Secretary

Aleksandra Grozdanova – North Macedonia
Aleksandra Kapedanovska Nestorovska – North Macedonia
Ales Mrhar – Slovenia
Ana Poceva Panovska – North Macedonia
Andreas Zimmer – Austria
Antonio Makris – Greece
Arijana Meštrović – Croatia
Beti Djurdjic – Montenegro
Betul Arica Yegin-Turkey
Brigita Djordjevic – Serbia
César Viseras Iborra – Spain
Dea Baricevik – Slovenia
Dimitar Efremov – Italy
Dinko Vitezic – Croatia
Edina Vranic – Bosnia and Herzegovina
Ela Hoti – Albania
Giangiacomo Beretta – Italy
Gjose Stefkov – North Macedonia
Hari Polenakovik – USA
Henning Blume – Germany
Ivan Stankovic – Serbia
Ivana Cvetkovikj Karanfilova – North Macedonia
Jan Tytgat – Belgium
Janina Lulek – Poland
Jasmina Tonic Ribarska – North Macedonia
Jelena Acevska – North Macedonia
Johanes Novak – Austria
Katerina Ancevska Netkovska – North Macedonia
Katerina Brezovska – North Macedonia
Kevin O'Donnell – Ireland
Liljana Anastasova – North Macedonia
Luciano Saso – Italy
Maija Dambrova – Latvia
Margarida Caramona – Portugal
Maria Halabalaki – Greece
Marija Hiljadnikova Bajro – North Macedonia
Marija Karapandzova – North Macedonia
Milen I. Georgiev – Bulgaria
Mirko Trajkovski – Switzerland
Nada Majkic-Singh – Serbia
Nadica Matevska Geskovska – North Macedonia
Natalija Nakov – North Macedonia
Nikola Geskovski – North Macedonia
Rok Dreu – Slovenia
Rubin Zareski – North Macedonia
Sanda Vladimir-Knezevic – Croatia
Sema Calis – Turkey
Slavica Ražić – Serbia
Sokol Abazi – Albania
Suela Kellici – Albania
Tanja Petreska – North Macedonia
Vilma Papajani Toska – Albania
Zorica Naumovska – North Macedonia

This issue of *Macedonian Pharmaceutical Bulletin* contains short papers accepted by the Scientific Committee for the presentation at the 7th Congress of Pharmacy in Macedonia with international participation 2022.

The authors are fully responsible for the contents of their short papers.

All reviewers that were involved in the short papers revision process are sincerely acknowledged.

Invited lectures

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 7 - 10 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.001
Short communication

Drug discovery and bio-exploration of nature: toxins, friend or foe?

Tytgat Jan

Toxicology and Pharmacology, University of Leuven (KU Leuven) Campus Gasthuisberg, O&N2, PO Box 922, Herestraat 49, 3000 Leuven, Belgium

Introduction

Nature has devoted plants, animals and microorganisms with a rich source of biologically active compounds, often for reasons of protection, but also for offensive purposes. Without any doubt, natural compounds always have played a crucial role in the development of interesting pharmaceuticals, and it is expected they still will do so. However, in the era of automated high-throughput screening techniques, the use of large libraries with purely synthetic molecules seems to become a first choice approach, rather than continuing to bio-explore nature's treasures. One of the explanations is the complexity of natural samples, necessitating lots of effort to identify and isolate the hit compound in question, and hence requiring a considerable investment in time and financial cost (Maitra et al., 2022). Indeed, since decades the complexity of natural products has resulted in a wealth of studies that focus only on assessing the activity of the entire extract (e.g. from a plant or a venomous animal), without further identifying the individual compound(s) responsible for the observed biological effects. The pharmaceutical industry has delivered more than 1200 drugs in the past 60 years that have played a key role in increasing life expectancy by an average of two months per year. However, the industry has at the same time reached a state of standstill with the output of new molecular entities (NMEs), remaining approximately constant while the cost of producing NMEs is increasing exponentially (King, 2011).

Toxins and toxinology

In general, toxins produced by plants, animal and microorganisms, have deleterious effects on a living organism and severe health concerns after being exposed to toxins, cannot be ignored. In this way, toxins are surely a foe. Also taking into account emerging toxins that are becoming increasingly prevalent in contexts of climate change and globalization of food supply chains. On the other hand, the diversity of toxins also has led to a frequent use as experimental tools for studying pharmacological mechanisms, the physiological role of the targets these toxins bind to, as well as the determination of the structure of those targets. Toxinology involves the identification, characterization, production and engineering of toxins, including their application and use as research tools and clinical products with therapeutic potential (Clark et al., 2019). Interest in toxins as tools for scientists goes back a long time, with famous examples like Claude Bernard's experiments in the 1800s with curare to demonstrate the existence of chemical communication between nerves and muscles, and Henry Dale's use of muscarine and nicotine to elucidate different subtypes of receptors for acetylcholine.

Despite the 'competition' with the screening of libraries of synthetic molecules, an increasing number of studies dealing with toxins illustrates today that we have arrived in an era where toxins have made the transition from the laboratory to the clinic. Hence the discipline of toxinology seems to shift from the classical development of anti-venoms towards real drug discovery, opening the door to consider toxins as a friend, not a foe. One justification for this phenomenon can be found in the often unique selectivity and remarkable high potency of toxins in general, which probably is a consequence of evolution during countless numbers of years. Toxic plant alkaloids are examples of the first source of toxin-derived

*jan.tytgat@kuleuven.be

therapeutics with a proof of concept: e.g. digoxin in atrial fibrillation and heart failure, and tubocurarine as a selective muscle relaxant for the use as an adjunct to classical anaesthetics in surgery. Snake venoms represent another example of a unique source that provided leads with successful therapeutic application: e.g. captopril, being the first orally active inhibitor of the angiotensin-converting enzyme (ACE), was derived from studies on small peptides known as bradykinin-potentiating peptides, isolated from the venom of the dangerous Brazilian snake *Bothrops jararaca*. It cannot be denied that captopril has led to major advances in the treatment of patients with high blood pressure and heart failure (Fischer and Riedl, 2022). Perhaps the most surprising toxin, both scientifically and economically, that has found therapeutic and cosmetic uses is botulinum toxin, produced by the bacterium *Clostridium botulinum* and related species. It is assumed to be the most potent molecule on planet earth, with applications in various movement disorders and migraine, as well as its familiar cosmetic use.

Technological developments

Technological developments in several fields of ‘omics’, have helped to increase our knowledge of toxins. Today we believe to understand how organisms like cone snails, sea anemones, spiders, scorpions and snakes, produce toxins, and how these bio-active substances have evolved. Transcriptomics and proteomics have thus far indeed enabled the identification of many (peptide) toxins, followed by the discovery of their often unique biological properties (Wangchuk, 2018).

Notwithstanding this progress, the limited availability of *in silico* systems that either identify the potential of the toxin for off-target effects, or that predict the presence of a toxin with a completely new mechanism of action, still restrict somehow the efficient valorization of these molecules. With the advent of high resolution analytical techniques, which can couple high-throughput functional screenings to compound-target identification, complex mixtures can be dealt with. The latest NMR and MS instruments provide the potential for a sensitive and accurate detection and identification of toxins, for instance in the foodstuff. The last decade in particular, cryo-EM has significantly advanced our knowledge in the field of structural biology, allowing us now to understand better how toxins interact at the molecular level. Techniques such as voltage-clamp electrophysiology with patch electrodes and high-speed atomic force spectroscopy (HS-AFM) allow the interaction of toxins with respectively targets such as ion channels-receptors, and phospholipids. These approaches allow observations in real time, enabling us to get a better insight into the dynamic nature of interactions of molecules. Thanks to several of these approaches, a significant gain in the reduction of the necessary amount of starting material required for diverse types of analyses, was achieved. The widespread use of toxins as experimental tools has been further facilitated by developments in synthetic chemistry and recombinant expression systems (e.g., bacteria, yeast and insect cells), enabling the production of lab scale quantities of toxins easier. Solid phase synthesis of peptide toxins using orthogonal protection strategies now provides a robust and efficient method to produce toxins with the specific disulfide folding networks that are essential for bioactivity. Nevertheless, solid phase synthesis remains relative expensive, is subject to trial and error and does not always accommodate for post-translational modifications (PTMs). On the other hand, toxin peptides can be easily modified (e.g. radioactive, fluorescent) to provide analogues to facilitate the characterization of cell surface ion channels and receptors. In this way, they can be considered as diagnostics.

From basic research to commercial applications

Examples of ongoing interest in the transition from basic research to commercial applications, can be found in several areas: e.g. in the treatment of pain, in immunotherapies, and for fighting microbial resistance. Several peptide toxin-derived drugs from the viper family of snakes (i.e. captopril, eptifibatide, tirofiban), one cone snail (i.e. ziconotide), and a lizard (i.e. exenatide) are currently approved by the FDA for clinical use for a diverse range of medical conditions, including blood pressure, pain, and diabetes. Other molecules are at the stage of preclinical development, such as a molecule from a sea anemone intended to use in autoimmune diseases, and snake venom-derived proteins that target clotting and coagulation malfunctions in humans.

Not surprisingly, libraries of diverse toxins for pharmaceutical screening purposes are now also available commercially, illustrating once more the fact that toxins remain interesting targets for therapeutic and diagnostic purposes.

Conclusion

As highlighted by Clark et al. (2019), technological developments in ‘omics-based’ approaches are driving the discovery of new toxins from an increasing array of organisms (e.g. from bacteria to animals). Challenges

remain in the availability of suitable (*in vitro*, *ex vivo*,or *in vivo*) models for characterizing the biological properties of these novel toxins and enabling their subsequent development within the biosciences or healthcare sectors. There is also a lack of *in silico* tools that aid the prediction and identification of completely new classes of toxins with unique or distinct mechanisms of action. Similarly, new ways to assess the potential for 'off-target' effects on the body would be of significant value when developing toxins for use in veterinary or clinical applications. Although there are a number of exceptions, the mass production of natural bio-actives (with potential therapeutic benefits) remains largely challenging given the difficulties of producing industrial scale yields of toxins through culture and/or chemical synthesis. Furthermore, work has to be done in terms of necessary regulations in the domains of food safety and environmental toxicology. Such regulations are indeed often lacking nowadays, despite emerging (aquatic) toxins as a consequence of global warming and climatic change conditions on this planet. The future therefore calls for an urgent need to link our understanding of environmental impact on toxin production with the tools and knowledge to combine improved detection and forecasting systems. Finally, it is believed that still more than 80-90 % of the world's biodiversity (including plants, animals and micro-organisms) remain under-explored for medicinal applications and therefore merits our attention in the near future.

References

Clark, G.C., Casewell, N.R., Elliott, C.T., Harvey, A.L., Jamieson, A.G., Strong, P.N. and Turner, A.D. 2019. Friends or Foe? Emerging impacts of biological toxins. TiBS 44(4), 365-379. https://doi.org/10.1016/j.tibs.2018.12.004 36

Fischer, T. and Riedl, R., 2021. Paracelsus' legacy in the faunal realm: drugs deriving from animal toxins. Drug Discovery Today 27(2), 567-575. https://doi.org/10.1016/j.drudis.2021.10.003

King, G.F., 2011. Venoms as a platform fro human drugs: translating toxins into therapeutics. Expert Opinion on Biological Therapy 11(11), 1469-1484. https://doi.org/10.1517/14712598.2011.621940

Maitra, U., Stephen, C. and Ciesla, L.M. 2022. Drug discovery from natural products – Old problems and novel solutions for the treatment of neurodegenerative diseases. J. Pharm. Biomed. Anal. 210, 114553. https://doi.org/10.1016/j.jpba.2021.114553

Wangchuk, P., 2018. Therapeutic applications of natural products in herbal medicines, biodiscovery programs, and biomedicine. J. Biologically Active Products from Nature. 8(1), 1-20. https://doi.org/10.1080/22311866.2018.1426495

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 11 – 12 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.002
Short communication

The Belgian Family Pharmacist: a review after 5 years and a preview on the future role of the community pharmacist

Hendrik De Rocker*, Jan Saevels, Koen Straetmans

Association of Pharmacists Belgium (APB), Rue Archimède 11, 1000 Brussels, Belgium

Introduction

As in numerous other countries, Belgian community pharmacists are trying to promote health of their patients. A fundamental change came back in 2010, when the remuneration model for community pharmacies was transformed from a pure margin system to a mixed system. Today the majority of pharmacists' income is composed of dispensing fees. Between 2010 and 2016, some prudent steps have been taken to develop new health promotion services in an economically sustainable way. For instance, a new medicines service for asthma patients was introduced in 2015. By early 2017, Belgian community pharmacists signed a multiannual framework with the Minister of Health. A first important milestone in the implementation of this agreement was the introduction of the concept "Family Pharmacist". Five years after the start of this service, it is time to look back, but also to look forward on expanding to other services and thus shape the future role of community pharmacists.

Family Pharmacist

Details of the service

The Family Pharmacist monitors chronic patients and accompanies them in the correct use of medicines. His main task is to keep the medication plan of his patients up-to-date and to make it available to the health care team (the family physician in the first place) and of course to the patients themselves. In order to do this, family pharmacists are required to systematically record all dispensed drugs in the patient's electronic local and shared pharmaceutical file. At each visit to the pharmacy, information is checked, consolidated, missing information (dosage, intake moment, OTC) completed, and registered in a complete, correct and up-to-date medication plan, validated and handed over to the patient.

The agreement between the patient and his family pharmacist is cemented when literally signing a contract between pharmacist and patient, hence the title of a large communication campaign "My pharmacist knows me, I sign up (chose today your family pharmacist)".

The service is covered by the compulsory universal health insurance, pharmacists receive a yearly fee per patient, without any out-of-pocket payment for the patient.

Advantages of the service

The complete, updated and patient-oriented medication plan is available to the patient himself and to other healthcare professionals who have a therapeutic relationship with him. This medication plan contains all medicines or health products dispensed to the patient, whether prescribed by the treating doctor or another prescriber (specialists, dentist), recommended by the pharmacist or taken on the patient's own initiative.

The medication plan, shared via secure platforms with the other healthcare providers, is of great importance to public health. It provides a full view of the patient's active medication. This is important when the patient consults a physician other than his usual physician, a specialist physician in a planned hospital admission or even when brought to an emergency department. On discharge from hospital, it is also helpful for the patient to take home the original medication plan that was changed within the hospital setting

The Family Pharmacist is the contact person for the patient's medication, both for the treating doctor and for other healthcare professionals who have a therapeutic relationship with the patient. This will strengthen the collaboration between pharmacist and the general practitioner who, thanks to the medication plan, has a working tool where the prescribing doctor can make any

*Hendrik.DeRocker@apb.be

necessary changes to it, verify the need for self-care medication and validate the plan.

For the patient, the medication schedule is a reminder to take all his medicines properly. It thus supports compliance, since it contains all the useful information on the posology, duration of treatment, timing of intake of each medicine and important advice on the proper use of medicines.

Results

The Family Pharmacist service officially started October 1st 2017, and turned out to be an immediate success. By the end of 2017, more than 400 000 chronic patients had signed up for the service, and over the course of just 12 months, 650 000 patients benefited from their Family Pharmacist. At present day, more than 1 million patients (on a Belgian population of 11 million citizens) have signed up for the service, meaning that implementation was extremely effective.

About 1 year after the start of the service, community pharmacists offering the service were asked about the main problems encountered when drawing up a medication plan. Wrong intake timing, wrong dosage and drug omissions were more often cited, followed by not previously detected interactions and unintended double medication, clearly demonstrating the positive impact of the service on patient's health.

Future role

Clearly this particular service is only the starting point for other cognitive services that community pharmacists can offer to patients as a strategy to improve the quality of drug therapy and health care delivery system (Melton et al., 2017). Typically, these services relate to prevention, referral and pharmaceutical care:

Prevention

Being a trusted party for the patient, preventive actions are now more easily done than before. Examples are the awareness raising for vaccination (flu, pneumococcal disease, COVID-19) and the administration of certain vaccines (COVID-19). But on top of the health-promoting activities themselves , being the family pharmacist makes it also possible to do targeted follow-up of any specific measures to promote therapy adherence.

Referral

One of the biggest advantages of having a close relationship between the pharmacist and the patient is that it makes it possible for the pharmacist to detect minor changes in the physical and mental health of the patient. Based on an established trust between the two parties, conversations on known and unknown conditions are straightforward, and if needed, a referral to a competent caregiver is easy. Screening programs for communicable and non-communicable diseases have been organised as pilot programs and could be expanded in the future.

Pharmaceutical Care

Building on patient trust in the family pharmacist, it certainly becomes easier to implement new services that are tailored to the specific needs of every individual (CoE Resolution CM/Res (2020)3). Pharmacists being highly educated professionals and experts in medicines dispensing and providing patient care, there are specific services that are being rolled late 2022: Examples are medication review for polymedicated patients (Wuyts et al.,2020), tapering programs for chronic long term benzodiazepine users, etc.

Conclusion

The visionary act five years ago to create the concept of 'family pharmacist' in Belgium not only consolidated the daily caregiving of Belgian pharmacists, but this also induced more patient centered pharmaceutical care. Next to that, the added value of the Belgian pharmacist within the care chain became more visible and recognized. This also made it more common to cooperate between pharmacists and other caregivers such as general practitioners. But it does not stop there. This collaboration means a big benefit for each patient who has no other choice than to rely on the quality of the provided pharmaceutical care. In Belgium, thanks to the concept of family pharmacist, this objective is already assured.

References

Melton, B.L., Lai, Z., 2017. Review of community pharmacy services: what is being performed, and where are the opportunities for improvement? Integr. Pharm. Res. Pract. 6, 79-89. https://doi.org/10.2147/IPRP.S107612

The Council of Europe Resolution CM/Res (2020)3 on implementation of pharmaceutical care for the benefit of patients and health services.

Wuyts, J., Maesschalck, J., De Wulf, I., Lelubre, M., Foubert, K., De Vriese, C., Boussery, K., Goderis, G., De Lepeleire, J., Foulon, V., 2020. Studying the impact of a medication use evaluation by the community pharmacist (Simenon): Drug-related problems and associated variables. Res. Social Adm. Pharm. 16(8), 1100-1110. https://doi.org/10.1016/j.sapharm.2019.11.008

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 13 - 14 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.003
Short communication

The Increasing role of health economics in the HTA of COVID19-vaccines

Maarten J. Postma[1-6], Tanja Fens[1,4], Cornelis Boersma[1,3,8]

[1]*Department of Health Sciences, University of Groningen, University Medical Center Groningen, The Netherlands*
[2]*Pharmacoeconomics Advice Groningen, Groningen, The Netherlands*
[3]*Health-Ecore Ltd., Zeist, The Netherlands*
[4]*Unit of PharmacoTherapy, -Epidemiology & -Economics (PTE2), University of Groningen, Department of Pharmacy, Groningen, The Netherlands*
[5]*Department of Economics, Econometrics & Finance, University of Groningen, Faculty of Economics & Business, Groningen, The Netherlands*
[6]*Department of Pharmacology & Therapy, Universitas Airlangga, Surabaya, Indonesia*
[7]*Center of Excellence in Higher Education for Pharmaceutical Care Innovation, Universitas Padjadjaran, Bandung*
[8]*Open University The Netherlands, Heerlen, The Netherlands*

The impact of COVID19 and vaccine implementation

As of May 2022, almost 6.5 million people died of COVID-19 globally (WHO). With the pandemic now ongoing for 2.5 years, it has typically gone through several stages, with its specific considerations. Notably and given the urgent situation in the first one-and-a-half years, health-economic aspects have not been prominent in Health Technology Assessment (HTA). Lockdowns have been installed against enormous – mostly unknown – societal costs, both concerning GDP as well as both physical and mental health. Notably, estimates have been made that countries' annual GDPs could have been affected by reductions up to 30% (Keogh-Brown et al., 2020). Subsequently, vaccines have been introduced without the general health-economic considerations that mostly apply; for example, reflected in reports of country-specific NITAGs (National Immunization Technological Advisory Groups). Notably, HTAs were focussed on efficacy, safety and delivery. With potentially unexpectedly high efficacy against severe disease, in particular, in western countries focus soon shifted to safety issues around thrombocytopenia and myocarditis as well as real-world data analyses for effectiveness.

Post-pandemic phase

Earlier this year, the emerging omikron-variant – potentially associated with less severe disease - might have precluded on new endemic/epidemic phases of the pandemic. While COVID19 vaccines were logically not immediately subject to health economics scrutiny, they will possibly be increasingly in later stages when boosters and revaccinations are considered in less-urgent situations, allowing more considerate recommendations, with potentially various brands from which to select, inclusive heterologous and homologous boosting. Notably, since the start of the COVID19 pandemic, similarities with the influenza virus, inclusive its - both seasonal and potential pandemic nature - have been sketched and influenza-like endemic/epidemic scenarios seem reasonable. In such scenarios, an increasing role for health economics in HTA of COVID19-vaccines can be expected. Considerations now eminent in COVID19-vaccines have been present for other potentially vaccine-preventable infections for decades upon which experiences can be drawn, inclusive economic impacts, specific cost-effectiveness methodologies, basic reproduction rate estimates, real-world data calibrations, discounting of long-term effects and transmission modelling (Boersma et al., 2020). Also, scarce health-economic COVID19 economic analyses already available can inform such more extensive sets of analyses.

*m.j.postma@rug.nl

Cost-effectiveness of COVID19-vaccines

Already since the beginning of the pandemic, and even in the initial sheer absence of vaccines, interest has existed in the cost-effectiveness of COVID19-vaccines. An initial paper analyzing a hypothetical COVID19-vaccine for the US found cost-effectiveness at US$8200 in the base case, using a Markov cohort static model with a one-year time horizon and the health-care perspective (Kohli et al., 2021). Notably, a 60% vaccine effectiveness against disease was used, as we now know reflecting a conservative assumption in the first months. More in line with modelling traditions in infectious diseases, next work applied dynamic transmission-dynamic models, explicitly considering the transmission of the virus in the population. Ergo, next to vaccine effectiveness on disease, also vaccine effectiveness against transmission plays an important role. For example, early 2021 Hagens et al reported cost-effectiveness of COVID19 vaccination in Turkey taking both the societal perspective with disease-related production losses and transmission dynamics into account (Hagens et al., 2021). Also, in that study favourable cost-effectiveness for vaccination was found at either low cost-effectiveness ratios or even rendering cost savings. Given the inclusion of the transmission dynamics, next to effectiveness on diseases also effectiveness on transmission was taken into the model; ergo, next to reducing the likelihood for serious disease, also the vaccination was assumed to reduce the likelihood of transmitting the virus if vaccinated. In line with later published real-world findings (Hall, 2021), it was assumed that effectiveness on transmission would be approximately half of that on disease (notably, 45 and 90%, respectively).

Implications for further discussion

Further work seems now concentrating on explicitly considering the potential novel context where COVID19 becomes endemic and will follow an influenza-like seasonal – partly predictable – pattern. This typically comes with some aspects well-known from influenza modelling (Postma and Chhatwal, 2022), such as reducing the time horizon to the 6-month winter season and relaxed non-pharmaceutical interventions (social distancing and face masks). In an analysis for Denmark (Debrabant et al,. 2021), a dynamic modelling approach was followed and continued limited social distancing and use of face masks still assumed. Both healthcare and societal perspectives were used and favourable cost-effectiveness was being identified. From the healthcare perspective, it was found that any strategy including older adults ≥60 appeared more cost-effective than strategies targeting populations <60. Inclusion of production losses in the societal perspective actually rendered strategies targeting those <60 cost-effective as well, in particular if current relatively modest vaccine prices would also be in force in endemic situations.

With cost-effectiveness issues likely getting more prominent in discussions on COVID19 vaccinations in an endemic context, the general discussion on broader impacts of vaccines will also apply (Postma et al., 2022). In particular, in the context of that discussion it is argued that for vaccines a broader view on values is needed to adequately justifying the specifics that vaccines pose. Notably, in this respect health and productivity effects for caregivers, distributive effects have been specifically mentioned, as well as providing piece of mind by reducing fears of infection, consequences of long-term complaints after COVID19 infection, macro-economic impacts and limiting capacity strains in hospitals. Some of these broader impacts have certainly been further attenuated by the COVID19 pandemic, illustrating the urgent need to take these on board in health economics of vaccines; and this not only for COVID19, but also for other vaccines. For example, hospital capacity problems with COVID19 can be exemplar for influenza and fear of COVID-infection exemplar for e.g. meningococcal B infection.

References

Boersma, C., Postma, M.J., 2021. Health Economics of Vaccines: From Current Practice to Future Perspectives. Value Health 24(1), 1-2. https://doi.org/10.1016/j.jval.2020.11.006

Debrabant et al., 2021. The Cost-Effectiveness of a COVID-19 Vaccine in a Danish Context. Clin. Drug. Investig. 41(11), 975-988. https://doi.org/10.1007/s40261-021-01085-8

Hagens et al., 2021. COVID-19 Vaccination Scenarios: A Cost-Effectiveness Analysis for Turkey. Vaccines 9(4), 399. https://doi.org/10.3390/vaccines9040399

Hall, V.J., 2021. Effectiveness of BNT162b2 mRNA Vaccine against Infection and COVID-19 Vaccine Coverage in Healthcare Workers in England, Multicentre Prospective Cohort Study (the SIREN Study). SSRN Electronic Journal.

Keogh-Brown et al., 2020. The impact of Covid-19, associated behaviours and policies on the UK economy: A computable general equilibrium model. SSM Popul. Health. 12, 100651. https://doi.org/10.1016/j.ssmph.2020.100651

Kohli et al., 2021. The potential public health and economic value of a hypothetical COVID-19 vaccine in the United States: Use of cost-effectiveness modeling to inform vaccination prioritization. Vaccine. 39(7), 1157-1164. https://doi.org/10.1016/j.vaccine.2020.12.078

Postma, M.J., Chhatwal. J., 2022. COVID-19 Health Economics: Looking Back and Scoping the Future. Value Health. 25(5), 695-696. https://doi.org/10.1016/j.jval.2022.03.008

Postma et al., 2022. Capturing the value of vaccination within health technology assessment and health economics:

Country analysis and priority value concepts. Vaccine 2022. https://doi.org/10.1016/j.vaccine.2022.04.026
WHO, https://covid19.who.int (accessed 27/5/22)

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 15 - 16 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.004
Short communication

The National Medicines Policy 2018-22 in Poland. From Creation to Implementation

Marcin Czech

Department of Pharmacoeconomics, Institute of Mother and Child, Kasprzaka str. 17A, 01-211 Warsaw, Poland

Introduction

Medicines policy play a major role in protecting, maintaining and restoring people's health. The regular provision of appropriate medicines of assured quality, in adequate quantities and at reasonable prices, is therefore a concern for all national governments (Kanji et al., 1992; Wirtz et al., 2017).

Drug policy is an integral part of state health policy. assuring access to safe and effective medicines while reducing patient participation in treatment costs. Pharmaceutical policy is a subdivision of health policy that deals with the development, provision and use of medications within a health care system. It embraces drugs (both brand name and generic), biologics (products derived from living sources, as opposed to chemical compositions), vaccines and natural health products.
Medicine Policy includes:

- Funding of Research in the Life Sciences
- Patent Law
- Licensing
- Pricing
- Reimbursement
- Formulary management
- Eligibility
- Prescribing
- Pharmacy services

Materials and methods (or other sections)

"The State Medicines Policy 2018-22" is a document of a strategic nature that defines the priorities of the Government of the Republic of Poland in the field of drug management in the indicated period (www.gov.pl). The document was created on the basis of the guidelines of the World Health Organization (WHO, 2016), it sets medium- and long-term goals for participants and decision-makers of the pharmaceutical market and identifies the main tools to achieve them.

The project was implemented through a systematic process of consultation with all interested parties, and its adoption was made through a consensus balancing the often contradictory goals and aspirations of communities widely associated with pharmacotherapy.

Results and discussion

How to assess the degree of its implementation?

In the short-term perspective, the Policy, by indicating specific solutions, highlights the ways of correcting the system's operations under the applicable legal status.

In the medium and long-term perspective, the document defines the necessary changes in the legislative environment.

The establishment of the Medical Research Agency and the reduction of bureaucratic burdens in planning and implementing clinical trials in Poland can undoubtedly be considered as effective implementation of the provisions of the document. The changes in the law have led to better control and wider cooperation of the services established

*marcin.czech@biznes.edu.pl

for this purpose in the scope of limiting parallel exports of drugs.

It should be emphasized that the level of patient copayment for drugs in outpatient treatment has decreased. The main challenges that have not yet been realized include the amendment to the Reimbursement Act. On the other hand, we are pleased with the adoption of the act on the profession of pharmacist, an act that has been awaited by the environment for many years, allowing for the redefinition of the pharmacist's tasks. It also contributed to fight against COVID-19 pandemic as pharmacist role in assisting patients and vaccinating them was crucial in this period.

The solutions under the Act on the Medical Fund are interesting and promising.

As regards purely operational tasks, it seems appropriate to continuously update the content of drug programs and the reimbursement list, transfer selected drugs from drug programs to the chemotherapy catalog / pharmacy list, and define the criteria, perhaps based on MCDA for orphan medicinal products (https://www.termedia) leading to a selection of medicinal products available under the National Program for Rare Diseases (https://www.gov.pl/).

Drug policy as an integral part of health policy should be flexible, keep pace with systemic changes in health care and changing health needs of the society.

Conclusions:

An access to innovative and generic therapies plays an important role in the treatment of severe, chronic, both common and rare diseases.

Drug reimbursement should be created in the context of epidemiology and demographic changes, and include both direct and indirect costs.

A medicines policy is based on a complex process of development, implementation and monitoring with adjustments, if necessary.

Throughout the entire process, careful planning, taking into account political and economic dynamics and the involvement of all stakeholders is needed.

Acknowledgements

I would like to thank all persons who have contributed to the development of the Policy: Jakub Adamski, Artur Falek, Anna Lech, Iga Lipska, Iwona Skrzekowska-Baran, Rafal Zysk.
I would also like to acknowledge Anna Rozalska.

References

Kanji, N., Hardon, A., Harnmeijer, J.W., Mamdani, M., Walt, G., 1992. Drugs Policy in Developing Countries. 1992, London: Zed Books

Wirtz, V.J., Hogerzeil, H.V., Gray, A.L., Bigdeli, M., Joncheere, C.P., Ewen, M.A., 2017. Essential medicines for universal health coverage. Report of the Lancet Commission on essential medicines policies. The Lancet 389, 403-476.

World Health Organization, 2016. How to develop and implement a national drug policy. Second edition, Geneva. Switzerland. Available at: http://apps.who.int/medicinedocs/pdf/s2283e/s2283.pdf (Accessed: 13 February, 2018)

www.gov.pl/Rada Ministrów przyjęła dokument „Polityka Lekowa Państwa 2018–2022" - Ministerstwo Zdrowia - Portal Gov.pL (Accessed 02.07.2022)

https://www.termedia.pl/mz/Prof-Marcin-Czech-o-priorytetach-i-wyzwaniach-polityki-lekowej-,42492.html (Accessed: 25 June 2022).

https://www.gov.pl/web/zdrowie/narodowy-plan-dla-chorob-rzadkich (Accessed: 25 June 2022).

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 17-18 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.005
Short communication

Psychopharmaca: from pharmacokinetics to clinical outcomes

Aleš Mrhar

Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, 1000 Ljubljana, Slovenia

Introduction

Clinical outcomes of active substances depend on their pharmacokinetics and pharmacodynamics. Pharmacokinetics illustrates the passage of active substances through the body in spatial and temporal terms and is defined by the processes of release from dosage forms, absorption form the site of administration to the central circulation, distribution throughout the body, metabolism and elimination from the body. Basic mechanisms of these processes are convection and diffusion with the exception of metabolism, where chemical transformation occurs. Pharmacokinetics thus ensures regardless of the mode of application the transfer of the active substance from the site of administration to the site of action, i.e. to the biophase where the targets are located. On the other hand, pharmacodynamics studies the processes that begin to take place between the active substance and the target when the active substance reaches the target. Targets are either receptors or enzymes, they may be carriers or ion channels. The result of the interaction between the active substance and the target is a measurable clinical effect such as drop in blood pressure, drop in cholesterol levels, drop in body temperature, relief of pain, recovery from infection, reduction of symptoms and signs of depression or psychosis and more (Derendorf and Schmidt, 2020).

Pharmacokinetics and pharmacodynamics are very often influenced by polymorphisms of genes encoding enzymes, carriers, receptors and ion channels which pharmacogenetics deals with.

Selected examples of psychopharmaca in the light of the influence of pharmacokinetics and pharmacogenetics on clinical outcomes will be presented below.

Pharmacokinetic and pharmacodynamic parameters

Basic pharmacokinetic information represents the relationship between concentration and time in the form of an appropriate curve showing plasma levels of active substance. From this curve one can calculate the primary and secondary parameters. Primary parameters are k_a (absorption rate constant), F (extent of absorption), Cl_p (total clearance) and V_d (volume of distribution). Secondary parameters are k_e (elimination rate constant), $t_{1/2}$ (biological half-life) and AUC (area under the curve).

Basic pharmacodynamic information is presented by E_{max} plot that shows relationship between effect and concentration of active substance. Two parameters can be identified from this plot, E_{max} (the maximal effect that an active substance can elicit, indicates efficacy) and EC_{50} (the concentration of an active substance required to produce 50% of maximum effect, indicates potency). Moreover, from E_{max} plot we can obtain therapeutic range which tells us in which range of plasma concentrations we can expect desired effect.

By merging the two curves we obtain the time course of the dependence of the effect on time, which allows us to predict the time of onset of the effect, the strength of the effect and the duration of the effect (Derendorf and Schmidt, 2020).

Examples of psychopharmaca

Midazolam is a benzodiazepine-type active substance used for anesthesia, procedural sedation, trouble sleeping and severe agitation. Its EC_{50} amounts to 0,1 mg/L and is lower than in the case of oxazepam and clobazam which are used in a similar indication. This means that midazolam shows greater potency than the

*ales.mrhar@ffa.uni-lj.si

other two benzodiazepines. Consequently, the largest dose of midazolam is 15 mg to reach therapeutic range 0,08 – 0,12 mg/L and is smaller than in the case of oxazepam and clobazam, both with maximal dose of 30 mg. Midazolam elimination half-life of 1.5–2.5 h allows duration of action up to 6 h in the case of instant release oral formulation.

Fluphenazine is a phenothiazine-type of a high-potency classical antipsychotic used for treating acute and chronic psychotic conditions, including schizophrenia and manic and hypomanic disorder. Its potency is 50 times higher than in the case of chlorpromazine, which means that a 50-fold lower daily dose of up to 10 mg in the case of peroral administration is required for approximately the same effect. Therapeutic range is 1 - 4 µg/L and can be achieved either orally in a 1-, 2- or 5-mg film coated immediate release tablet or by deep intramuscular injection into the gluteal muscle in a dose of 25 mg in the form of decanoate. As $t_{1/2}$ of elimination amounts up to 17 h oral daily dose is usually divided into two doses to maintain plasma concentrations within therapeutic range for a long period assuming absorption from the gastrointestinal tract is very rapid ($k_a>k_e$). In the case of intramuscular injection, the situation is reversed. As the rate-limiting step is hydrolysis of ester in gluteal muscle, which takes place very slowly, the absorption is significantly prolonged ($t_{1/2}$ of absorption is 231 h, $k_a<k_e$) thus allowing dosing every three weeks. Even in this case, plasma concentrations are maintained within the therapeutic range whereby concordance is ensured. In both cases, the dosage regimen should be adjusted so that plasma concentrations do not significantly exceed 2.7 µg/L due to the increased likelihood of adverse reactions.

Paliperidone (9-hydroxyrisperidone) is an atypical antipsychotic that is used in the treatment of schizophrenia and schizoaffective disorder. It is available as prolonged release tablets and intramuscular injections in the form of palmitate. The mechanism of the latter mode of application and consequent pharmacokinetics and clinical efficacy are comparable to those in the case of fluphenazine, i.e. long term hydrolysis of ester in deltoid or gluteal muscle which provides a biological half-life of tens of days, even up to 130, thus allowing application once a month or even once every three or six months with very small peak-to-trough ratio of paliperidone at steady state ranging from 1.56 to 1.70. However, paliperidone alone has a biological half-life of 23 h, which allows once a day oral application in the form of prolonged release tablets to reduce fluctuations in plasma concentrations, similar as in the case of intramuscular injections, in order to optimize clinical efficacy.

Risperidone is an atypical antipsychotic used to treat schizophrenia and bipolar disorder. Extrapyramidal disorders (parkinsonism, dystonia, tardive dyskinesia, restlessness) are the main side effects of risperidone therapy, occurring more frequently at doses higher than 4 mg daily. Moreover, the elimination rate of risperidone is highly variable, biological half-life ranges from 3 to 24 h, indicating an important role of CYP2D6 genotype in conversion of risperidone to 9-hydroxyrisperidone which is the major (90%) metabolic pathway.

A prospective study was conducted at the University Medical Centre Maribor and at the Faculty of Pharmacy Ljubljana to characterize the metabolism of risperidone to (+)- and (-)-9-hydroxyrisperidone in vivo and to evaluate the influence of CYP2D6 genotype. A population pharmacokinetic modeling approach was used to estimate the interindividual variability of the pharmacokinetic parameters in 50 hospitalized patients with acute episode of schizophrenia. CYP2D6 genotype remarkably influenced the formation clearances of the risperidone metabolites, while creatinine clearance was related to the plasma clearance of 9-hydroxyrisperidone. CYP2D6 genotype was also associated with the average plasma concentration of risperidone active moiety (a sum of all three active compounds). In comparison to the patients with CYP2D6*1/*1 genotype, average steady-state plasma concentration of risperidone active moiety was 3.3- and 1.6-fold higher in poor metabolizers (both alleles nonfunctional; CYP2D6*3 or *4) and intermediate metabolizers (one nonfunctional allele and one allele for diminished enzyme activity; CYP2D6*10 or *41), respectively. Additionally, average plasma concentration of risperidone active moiety was higher in the patients with dystonia (p=0.0066) and parkinsonism (p=0.046). The results of this study imply the potential role of CYP2D6 genotyping in personalizing risperidone therapy in patients with schizophrenia to reduce the incidence of adverse extrapyramidal disorders (Locatelli et al., 2010)

References

Derendorf, H., Schmidt, S., 2020. Rowland and Tozer's Clinical Pharmacokinetics and Pharmacodynamics, fifth ed. Wolters Kluwer, Philadelphia.

Locatelli, I., Kastelic, M., Koprivšek, J., Kores-Plesničar, B., Mrhar, A., Dolžan, V., Grabnar, I., 2010. A population pharmacokinetic evaluation of the influence of CYP2D6 genotype on risperidone metabolism in patients with acute episode of schizophrenia. Eur. J. Pharm. Sci. 41, 289-298.

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 19 - 20 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.006
Short communication

Acylcarnitines in health and disease: biomarkers and drug targets

Maija Dambrova[1,2*], Edgars Liepinsh[1]

[1]*Latvian Institute of Organic Synthesis, Laboratory of Pharmaceutical Pharmacology, Aizkraukes Str. 21, LV1006 Riga, Latvia*
[2]*Riga Stradins University, Faculty of Pharmacy, Dzirciema Str. 16, LV1007, Riga, Latvia*

Introduction

Acylcarnitines are fatty acid metabolism intermediates that emerge from the cellular energy metabolism pathways in mitochondria and peroxisomes. An acylcarnitine molecule contains mostly diet-derived biofactor L-carnitine coupled to a fatty acid moiety which can be classified as long-chain, medium-chain, short-chain, branched, hydroxylated, saturated or unsaturated. There are more than 1000 various acylcarnitines now analyzed and included in the Human Metabolome Database (Dambrova et al., 2022). The proportionally largest part of the body acylcarnitine pool consists of short-chain acylcarnitines, containing acetyl-, propionyl-moiety of fatty acids. Metabolomic profiling assays by tandem mass spectrometry frequently include acylcarnitine profile measurements in blood plasma and urine samples and identify metabolic phenotypes, which are associated with certain disease risks.

Acylcarnitines as biomarkers

Historically acylcarnitines are recognized and used as biomarkers for inborn or acquired fatty acid oxidation defects (McCann et al., 2021; Wanders et al., 2020), also as a part of newborn screening programs (Martín-Rivada et al., 2022). Accumulation of a specific acylcarnitine, especially long-chain and very-long-chain acylcarnitine, can signal about defective mitochondrial β-oxidation enzymes and mitochondrial trifunctional protein in the fatty acid metabolism pathways. The changes in acylcarnitine concentrations have been linked also to the risks of non-communicable diseases, such as diabetes mellitus, cardiovascular and neurological diseases and also some cancers (Dambrova et al., 2022). The plasma concentrations of acylcarnitines change along with the fed-and-fasted state cycles and availability of energy substrates, fatty acids and glucose. In healthy subjects, fatty acid metabolism and acylcarnitine production dominate during the fasted state, and this has to be taken into account when collecting samples for analysis. In turn, altered changes in plasma concentrations of long-chain acylcarnitines in fasted and fed states can serve as a valuable marker of tissue-specific insulin sensitivity (Makarova et al., 2019). More research is needed to establish reference levels for various acylcarnitines under different physiological states of energy metabolism in health and disease.

Acylcarnitines as food supplements

Short-chain acylcarnitines, acetylcarnitine and propionylcarnitine, are proposed as useful food supplements. Some studies have shown positive effects of acetylcarnitine intake in dementia, cognitive dysfunction, neurodegenerative disease, pain, Alzheimer's disease, depressive disorder, neuralgia, type 2 diabetes and diabetic neuropathy, hyperammonemia, fatigue and dystrophy (Dambrova et al., 2022). Propionylcarnitine supplementation has been found useful in the case of some vascular diseases, including intermittent claudication, colitis, ischemia and also male sexual dysfunction (Dambrova et al., 2022). However, it has to be noted that more data are needed to study metabolic and biochemical cascade of events leading to altered levels of

*maija.dambrova@farm.osi.lv

acylcarnitines, bioavailability of supplements and concentration changes after the supplementation.

Acylcarnitine accumulation-induced disease states

At altered concentrations long-chain acylcarnitines are known to affect the activity of enzymes and ion channels, mitochondrial functionality, increase free radical production, as well as impact signaling pathways leading to various cardiovascular diseases (Dambrova et al., 2021, Dambrova et al., 2022). Since long-chain acylcarnitines induce harmful effects on mitochondria and energy metabolism pathways, their accumulation is suggested as an actor in the induction of an energetic crisis in inflammation (McCoin et al., 2015). It has been shown recently that a long-chain acylcarnitine, palmitoyl-carnitine, stimulates insulin release and induces dephosphorylation of the insulin receptor (Vilks et al., 2021). This finding provides additional molecular details to previous preclinical studies pointing at altered changes in long-chain acylcarnitine levels in case of insulin resistance (Aguer et al., 2015; Liepinsh et al., 2016). The increased levels of long-chain acylcarnitines can be proposed as a target for the treatment of inherited diseases of fatty acid oxidation, diabetes/insulin resistance, and cardiovascular diseases.

Future perspectives

In order for acylcarnitines to gain wider recognition as biomarkers and new drug target resource, bioanalytical technologies and big data analysis should be used to advance understanding of the physiological roles of acylcarnitines and their involvement in the pathogenesis of various disease states.

Conclusion

The metabolomic profiling data point to acylcarnitines as important fatty acid intermediates that signal about disturbances in the cellular energy metabolism pathways and present early signs of certain disease pathogenesis. Dietary and pharmacological means of acylcarnitine level regulation can be used to counteract pathological changes in acylcarnitine concentrations.

Acknowledgements

The authors were supported by the European Union's Horizon 2020 research and innovation programme under grant agreement No 857394.

References

Aguer, C., McCoin, C.S., Knotts, T.A., Thrush, A.B., Ono-Moore, K., McPherson, R., Dent, R., Hwang, D.H., Adams, S.H., Harper, M.E., 2015. Acylcarnitines: potential implications for skeletal muscle insulin resistance. FASEB J. 29, 336-45. https://doi.org/10.1096/fj.14-255901

Dambrova, M., Makrecka-Kuka, M., Kuka, J., Vilskersts R., Nordberg, D., Attwood, M., Smesny, S., Duygu Sen, Z., Chi Guo, A., Oler, E., Tian, S., Wishart, D., Liepinsh, E., Schiöth, H., 2022. Acylcarnitines: Nomenclature, biomarkers, therapeutic potential, drug targets and clinical trials. Pharmacol. Rev. 74, 1-50.

Dambrova, M., Zuurbier, C.J., Borutaite, V., Liepinsh, E., Makrecka-Kuka, M., 2021. Energy substrate metabolism and mitochondrial oxidative stress in cardiac ischemia/reperfusion injury. Free Radic. Biol. Med. 165, 24-37. https://doi.org/10.1016/j.freeradbiomed.2021.01.036

Liepinsh, E., Makrecka-Kuka, M., Makarova, E., Volska, K., Svalbe, B., Sevostjanovs, E., Grinberga, S., Kuka, J., Dambrova, M., 2016. Decreased acylcarnitine content improves insulin sensitivity in experimental mice models of insulin resistance. Pharmacol. Res. 113(Pt B), 788-795. https://doi.org/10.1016/j.phrs.2015.11.014

Martín-Rivada, Á., Palomino Pérez, L,, Ruiz-Sala, P., Navarrete, R., Cambra Conejero, A., Quijada Fraile, P., Moráis López, A., Belanger-Quintana, A., Martín-Hernández E., Bellusci, M., Cañedo Villaroya, E., Chumillas Calzada, S., García Silva, M.T., Bergua Martínez, A., Stanescu, S., Martínez-Pardo Casanova, M., Ruano, M.L.F., Ugarte, M., Pérez, B., Pedrón-Giner, C., 2022. Diagnosis of inborn errors of metabolism within the expanded newborn screening in the Madrid region. JIMD Rep. 63, 146-161. https://doi.org/10.1002/jmd2.12265

McCann, M.R., George De la Rosa, M.V., Rosania, G.R., Stringer, K.A., 2021. L-Carnitine and Acylcarnitines: Mitochondrial Biomarkers for Precision Medicine. Metabolites 11. https://doi.org/10.3390/metabo11010051

McCoin, C.S., Gillingham, M.B., Knotts, T.A., Vockley, J., Ono-Moore, K.D., Blackburn, M.L., Norman, J.E., Adams, S.H., 2019. Blood cytokine patterns suggest a modest inflammation phenotype in subjects with long-chain fatty acid oxidation disorders. Physiol. Rep. 7, e14037. https://doi.org/10.14814/phy2.14037

Vilks, K., Videja, M., Makrecka-Kuka, M., Katkevics, M., Sevostjanovs, E., Grandane, A., Dambrova, M., Liepinsh, E., 2021. Long-Chain Acylcarnitines Decrease the Phosphorylation of the Insulin Receptor at Tyr1151 Through a PTP1B-Dependent Mechanism. Int. J. Mol. Sci. 22, 6470. https://doi.org/10.3390/ijms22126470

Wanders, R.J.A., Visser, G., Ferdinandusse, S., Vaz, F.M., Houtkooper, R.H., 2020. Mitochondrial Fatty Acid Oxidation Disorders: Laboratory Diagnosis, Pathogenesis, and the Complicated Route to Treatment. J. Lipid Atheroscler. 9, 313-33. https://doi.org/10.12997/jla.2020.9.3.313

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 21 - 22 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.007
Short communication

Nanotechnology in medicine – our experiences

Nikola Geskovski[1]; Fatima Mraiche[3,4], Huseyin C. Yalcin[5], Filip Gorachinov[1], Diala A. Moustafa[3], Simona Dimchevska[2], Marija Glavas Dodov[1], Maja Simonoska Crcarevska[1], Katerina Goracinova*[1]

[1]*Faculty of Pharmacy, Ss. Cyril and Methodius University in Skopje, Majka Tereza 47, 1000 Skopje, North Macedonia*
[2]*Brandenburg University of Technology Cottbus – Senftenberg, Germany*
[3]*College of Pharmacy, Qatar University,* [4]*Biomedical and Pharmaceutical Research Unit, QU Health, Qatar University*
[5]*Biomedical Research Center, Qatar University, Doha P.O. Box 2713, Qatar*

Introduction

The success of design criteria for long-circulating drug delivery systems to support membrane receptor-ligand interaction and internalization has been limited and there is a need to develop smarter approaches for efficient drug tumor targeting. In order to overcome some of the current limitations, stimulus-responsive targeting has been combined with passive and active targeting strategies. More innovative nanocarriers that hold promise to optimize targeted drug delivery are systems with the ability for transformation from the stealth long-circulating form to cell interactive form in the complex tumor environment, exposing ligands at their surface for improved ligand-receptor interaction and cell internalization.

This short review will be an overview of several nanomedicines designed and characterized by our research group, and the interplay between their physicochemical characteristics and biological fate.

Discussion

Most commonly used systems to achieve enhanced permeability and retention effect are hydrophilic shell-hydrophobic core nanocarriers, so called polymer micelles, produced with self-assembly of block, grafted or branched copolymers consisting of a hydrophilic backbone and multiple hydrophobic polymer side chains. Smart synthetic linear aliphatic polyesters based on poly(lactic acid), poly(glycolic acid) and poly(ethylene glycol) play a crucial role in the development of a variety of safe, biocompatible, biodegradable therapeutic drug systems, however, their use is limited due to a low degree of functionality (hydroxyl groups). Dimchevska et al. (2017) prepared SN-38 loaded Poly(D,L-lactide-co-glycolide)-b-poly(ethyleneoxide)-b-poly(D,L-lactide-co-glycolide) (PLGA-PEG-PLGA) NPs by nanoprecipitation technique using copolymers with different Mw (LMw NPs 6,000:10,000:6,000Da and HMw NPs 70000:8000: 70000Da), chain length, PLGA/PEG or DLLA/GA ratio, and evaluated their physicochemical properties, nanobiointeractions, toxicity, efficacy as well as biodistribution of radiolabeled NPs in a healthy Wistar rat model. Various polymer properties led to diverse nucleation and growth during nanoprecipitation resulting in difference in the particle size and drug loading between LMw and HMw NPs. Another distinct feature that might also influence the biological fate, protein binding and internalization is the packing density and conformation of the PEG shell which is in control of steric hindrance, hydrophobicity and the zeta potential. Zeta potential was less negative for LMw NPs due to higher density and longer PEG chains. Cytotoxicity of empty NPs in human colon adenocarcinoma SW480 cell culture model was negligible. During internalization studies in SW480 cells, serum proteins showed profound influence in diminishing the differences in the internalization rates among LMw and HMw NPs, highlighting the fact that the protein corona is the nanobiointerface important for cell-NP interaction. In vivo biodistribution studies of radiolabeled NPs verified the efficient protection role of PEG loops directly attached to the NPs surface against immediate sequestration. Blood circulation time was significantly prolonged to several hours for LMw and HMw NPs, though for the nanoparticles with a denser hydrophilic corona (LMw NPs) the condensed packing of the loops could be clearly correlated with more efficient inhibition

*kago@ff.ukim.edu.mk

of phagocytic uptake, 30% increased blood circulation time and improved stealth effect. The long circulation time of LMw PLGA-PEG-PLGA NPs loaded with tyrosine kinase inhibitor Ponatinib was confirmed in a zebra fish model. Due to increased tumor distribution in zebrafish xenograft model developed by transplantation of K652 CML cell line into 72 hpf zebrafish embryo, the drug loaded NPs showed significantly lower cardiotoxicity compared to free drug molecules (Al-Thani et al., 2022). Similar effect of contribution of the dense "brush" conformation of PEG hydrophilic corona chains to the stealth effect was noticed for SN-38 loaded PEO-PPO-PEO/Poly(DL-lactide-co-caprolactone) NPs (Geskovski 2015; Koliqi et al. 2016). Two samples of PEO-PPO-PEO/Poly(DL-lactide-co-caprolactone) NPs, prepared by nanoprecipitation procedure with a slightly modified purification step were tested for their biodistribution in Wistar rat model. One of the samples was regularly washed in three cycles and the other was repeatedly purified by centrifugation using ultrafiltration, until no free PEO-PPO-PEO was detected in the supernatants. FTIR evaluation showed two distinctive conformations, brush and loop, of the PEO-PPO-PEO hydrophilic layer for the purified and completely purified sample. Striking similarity of the circulation time of the "brush" corona conformation of PEO-PPO-PEO/ Poly(DL-lactide-co-caprolactone) NPs to PLGA-PEG-PLGA NPs with dense hydrophilic corona was found during the biodistribution studies of radiolabeled samples. Having in mind the close values of hydrodynamic diameters and the zeta potential of the compared NPs we may assume that the Mw, density and surface conformation of PEG corona determines protein corona patterns and the abundance of different proteins in primary and secondary corona, affecting the process of phagocytosis (Djurdjic, 2015; Geskovski, 2015). Compared to the "brush" conformation the "trans" or train/loop conformation of PEG chains of the NPs resulted in almost immediate sequestration and clearance. Therefore, PEG Mw, density or ethylene glycol units per surface area to achieve efficient surface coverage and "brush" conformation should be carefully optimized during the design process if long circulation time has to be achieved. Novel studies indicate to abundance of serum albumin and apolipoprotein E in the protein corona of low-density PEG hydrophilic shell PLGA NPs and higher level of clusterin for shells with increasing density and chain length, confirming that PEG conformation mediates a specific protein adsorption and shapes the protein corona profile. Low degree of functionality of polymers like P(DLLA-CL), PLGA and PCL may be overcome by copolymerization with polyacrylic acid or polyethylene imine. Poly-ε-caprolactone-branched-polyethylene imine (PCL-b-PEI, Mw ~ 40.000-800 Da) was used for design of hyaluronic acid coated paclitaxel loaded PCL-b-PEI multifunctional smart nanocarrier capable of addressing multiple barriers (Moustafa et al. CESPT 2022). Hyaluronic acid structured into charge reversal PCL-b-PEI NP shell improves the EPR effect and tumor localization, decreases the toxicity masking the positive PEI charge and improves specific targeting for CD44 overexpressing tumors. Hyaluronidase enzyme overexpression in tumors amplifies the degradation of HA, exposing the smaller sized positively charged particles in the tumor environment which improves the diffusion through the tumor matrix, membrane interaction, internalization as well as endosomal escape. Stable charge reversal of PCL-PEI with HA and improved biocompatibility was confirmed in A549 cell lines using LDH and MTT tests. Also, higher internalization of HA decorated NPs was verified in A549 cancer cell line overexpressing the CD44 receptor. The anticancer efficacy was also significantly improved in cell lines overexpressing the cluster of differentiation receptor.

Conclusion

Multimodal characteristics of the nanoparticles such as size-tunable properties and charge reversibility induced by pH or enzyme responsive hydrolysis coupled with structural destabilization for rapid drug release, and fused with an efficacious technique for avoiding protein adsorption to minimize or mitigate the negative influence of protein corona on the internalization and sequestration should be future priorities in nanomedicine development.

References

Al-Thani, et al., 2022. Reduced Cardiotoxicity of Ponatinib-Loaded PLGA-PEG-PLGA Nanoparticles in Zebrafish Xenograft Model. Materials 15(11), 3960. https://doi.org/10.3390/MA15113960

Dimchevska, S., et al., 2017. Efficacy assessment of self-assembled PLGA-PEG-PLGA nanoparticles: Correlation of nano-bio interface interactions, biodistribution, internalization and gene expression studies. Int. J. Pharm. 533(2), 389–401. https://doi.org/10.1016/j.ijpharm.2017.05.05

Goracinova, K., et al., 2018. Design of Nanostructures for Theranostics Applications Elsevier, pp. 109–160. https://doi.org/10.1016/B978-0-12-813669-0.00004-X

Geskovski, N. Rational design of biopharmaceutical properties of nanodrug delivery systems, PhD Thesis 2015.

Koliqi, R., et al., 2016. PEO-PPO-EO/Poly(DLLA-CL) Nanoparticles as Carriers for SN-38: Design, Optimization and Nano-Bio Interface Interactions. Curr. Drug Del. 13(3), 339–352. https://doi.org/10.2174/1567201813666151130221806

Djurdjic et al., 2015. Synthesis and self-assembly of amphiphilic poly(acrylicacid)–poly(ε-caprolactone)–poly(acrylicacid) block copolymer as novel carrier for 7-

ethyl-10-hydroxy camptothecin. J. Biomater. Appl. 29(6), 867-881. https://doi.org/10.1177/0885328214549612

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 23 – 24 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.008
Short communication

Contribution of Raman chemical imaging in the analysis of falsified and substandard medical products

Hervé Rebiere*, Céline Ghyselinck, Charlotte Brenier

French National Agency for Medicines and Health Products Safety, ANSM, Laboratory Controls Division, 635 Rue de la garenne, 34740 Vendargues, France.

Introduction

Substandard and falsified medical products are a threat to the health of patients. They are products that fail to meet quality standards or that deliberately misrepresent their identity, composition or source. They affect every region of the world and are widely available over the internet. The fight against these harmful products must be efficiently organized through prevention, communication and coordination, but also action, investigation and repression. Official laboratories for the control of medicines play an active role in the fight against falsified drugs. Analyses of suspect samples provide better knowledge of these products, identify the danger to patients and help the authorities in their decisions (Rebiere et al., 2017).

Unfortunately, substandard and falsified products are becoming more and more sophisticated and sometimes field testing or conventional analyses may not be sufficient to prevent the danger. Advanced analytical methods can be used for the exhaustive assessment of a suspect sample. Mass spectrometry and Nuclear Magnetic Resonance spectroscopy are powerful methods for this task.

Raman spectroscopy is a method that has become popular due to the highly qualitative information contained in a spectrum. Modern technologies offer access to simple portable instruments that can be used in the field, as well as research instruments coupled with microscopes with high spatial/spectral resolution.

This overview will explain the basic principle of Raman spectroscopy, and will present several applications where the method appears to be successful in the characterization of substandard and falsified medical products.

Materials and methods

Samples and instrument

The suspected samples were all obtained from seizures carried out by the French police or customs services, and the analyses were performed as per a judicial request.

Measurements were carried out on a benchtop system from Renishaw (model InVia) which is composed of a 785 nm laser diode with a nominal power of 300 mW, a Leica microscope equipped with several magnification lenses, a moving tray, a rejection filter to block the Rayleigh scattering, a diffraction grating and a CCD detector. The software (Wire) is designed to control the system, perform measurements and analyze spectra (including chemometric algorithms).

Raman Chemical Imaging

Raman spectroscopy is a method that takes advantage of the vibration of molecules previously irradiated by an intense monochromatic source. As a result, two phenomena may occur: Rayleigh scattering (elastic), which is a strong signal but without any analytical interest, and Raman scattering (inelastic, i.e., with a different wavelength from that of the source), which is a signal with a very low intensity.

Conventional spectrometers use an excitation source irradiating the sample on a surface area over several mm². When coupling with a microscope, the laser may be focused on a surface area over some µm². This is more or less the size of a material particle in solid dosage form.

*herve.rebiere@ansm.sante.fr

Thus the Raman signal obtained may be that of one the chemical substances from the mixture.

Raman chemical imaging combines spatial and spectral information successively recording spectra at the surface of a sample at adjacent positions. The resulting data is called a hyperspectral image containing thousands of spectra gathered in a data-cube, i.e., a three-dimensional matrix with two spatial dimensions (x, y) and one spectral dimension (λ). These data are generally examined in a multivariate way using chemometric algorithms such as Principal Component Analysis (PCA) or Multivariate Curve Resolution Alternating Least Square (MCR-ALS). This latter method is particularly well suited to the resolution of a spectral mixture without a-priori knowledge of the chemical system. The spectrum of a sample may be considered as the weighted sum of the spectra of pure chemical species. Thus, each pixel of the image contains the same pure signals but their contributions differ from one pixel to the next. MCR-ALS decomposes the spectral dataset into the product of the matrix of pure spectra of species and the matrix of their relative contribution in the data-cube. The use of spectral libraries helps in the identification of the resulting pure spectra.

Surface Enhanced Raman Spectroscopy (SERS)

As previously mentioned, Raman scattering is a very weak signal. This signal may be drastically enhanced with a specific sample preparation using metal nanoparticles. The use of nanoparticles suitably prepared and applied over the sample surface may enhance the Raman signal by between 100 and 10000-fold. The SERS effect is explained with two mechanisms: an electromagnetic enhancement obtained with the surface plasmon generated in the gap between two close nanoparticles, and a chemical enhancement resulting from the electron transfer between analytes and nanoparticles.

Nanoparticles are commercially available in liquid form or embedded in a substrate. In our study we decided to manufacture and characterize our own nanoparticles. A specific deposition mode was optimized for further Raman chemical imaging (Cailletaud, 2018).

Results and discussion

Spectroscopic screening

Raman chemical imaging was applied on several tablets. The MCR-ALS method was used for the study of each resulting data-cube. The sample surface must preferably be flat in order to maintain the focalization of the laser. The resulting images use false colors in order to locate the different areas of the chemical pure species.

The method was applied on several kinds of samples: chloroquine tablets and anabolic tablets (Rebiere et al., 2016). Hyperspectral images exhibit the distribution map of the compounds and identify active substances and excipients (some of them are not expected in the sample). Falsification was also established on suspect Viagra© and Plavix© samples by comparing their hyperspectral images with those of authentic ones (Rebiere et al., 2018).

Detection of low dose active substances

SERS was applied on a sample called “Anabol tablet”, previously found without any active substances using conventional Raman chemical imaging. Silver nanoparticles were prepared and applied by spray coating before performing Raman measurement over the surface of the sample. Two new pure components were detected and identified as sildenafil and ciprofloxacine. Their identification was confirmed using LC-MS and their content was found at 4 µg sildenafil and 300 µg ciprofloxacine per tablet. It is assumed that the presence of these two unexpected substances is due to the use of poorly cleaned manufacturing tools in which sildenafil tablets and ciprofloxacine tablets had been previously prepared by the illegal manufacturer.

Conclusion

Raman chemical imaging was found to be useful for the screening of substandard and falsified samples whose composition is unknown. The identification of active substances and excipients was achieved. The method is complementary to separation methods giving the distribution map of the compounds in the samples. SERS is a sensitive method capable of detecting chemicals at a low dose content.

References

Cailletaud, J., De Bleye, C., Dumont, E., Sacré, P.Y., Gut, Y., Bultel, L., Ginot, Y.M., Hubert, P., Ziemons, E., 2018. Towards a spray-coating method for the detection of low-dose compounds in pharmaceutical tablets using surface-enhanced Raman chemical imaging (SER-CI), Talanta 188, 584-592.

Rebiere, H., Ghyselinck, C., Lempereur, L., Brenier, C., 2016. Investigation of the composition of anabolic tablets using near infrared spectroscopy and Raman chemical imaging Drug Test. Anal., 8, 370-377.

Rebiere, H., Guinot, P., Chauvey, D., Brenier, C., 2017. Fighting falsified medicines: The analytical approach. J. Pharm. Biomed. Anal. 142, 286–306.

Rebiere, H., Martin, M., Ghyselinck, C., Bonnet, P.A., Brenier, C., 2018. Raman chemical imaging for spectroscopic screening and direct quantification of falsified drugs – J. Pharm. Biomed. Anal., 148, 316-323.

Macedonian Pharmaceutical Bulletin, 68 (Suppl 1) 25 -26 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.009
Short communication

Orally disintegrating tablets (ODTs): An innovative approach to tablet formulations

Tansel Comoglu

Department of Pharmaceutical Technology, Faculty of Pharmacy, Ankara University, 06100 Tandogan, Ankara, Turkey

Introduction

Orally disintegrating tablets (ODTs) are solid dosage forms that disintegrate without the addition of extra water, usually less than a minute in the mouth into a paste that can be easily swallowed. ODTs have improved over the past years, in an attempt to produce a safe and efficient substitute to the conventional oral dosage forms. They are new types of dosage forms which mediate the advantages of both solid and liquid types of drug formulations such as ease of use and being stable. Thanks to these properties, the orally disintegrating tablets are considered a good alternative in both pediatric and geriatric therapy. Orally disintegrating mini-tablets (ODMTs), which combine the advantageous properties of ODTs and mini-tablets for pediatric therapy, are defined as new drug delivery systems. Therefore, ODMTs can also be defined as advanced and compact forms of ODTs for pediatric patients. In this paper, advantages, ideal properties and desired characteristics of ODTs formulations processes and future research trends in ODT technology will be told.

Advantages of ODTs

ODTs are especially convenient for patients, who have difficulties in swallowing conventional solid dosage form. ODTs include the following:

- Pediatric and geriatric populations who have complication in swallowing of tablets and capsules.
- ODTs are suitable dosage forms for during the journey, patients with permanent nausea.
- Antipsychotic drug molecules can be more easily applied to schizophrenic patients by ODTs than traditional pharmaceutical forms (Comoglu and Ozyilmaz, 2019).
- The risk of chocking or suffocation during oral administration of conventional formulations due to physical obstruction is avoided, thus providing improved safety (Indurwade and Biyani, 2000).
- Good mouth feel property of ODT helps to change the perception of medication (Parkash et al., 2011).
- Increased bioavailability and faster onset of action: Oromucosal absorption leads to pre-gastric absorption, especially for formulations where the active ingredient dissolves rapidly.

Ideal properties of ODTs

An ideal ODT should maintain the following properties.

- Should be ionizable in oral cavity.
- Be dispersible and diffusible in mouth.
- The active material should be less than 50 mg in each tablet.
- Half-life of the active material should be short and suitable for frequently dosing.
- Should not have bad taste and smell.
- Should be dispersible in oral cavity without any need of water.
- Be robust to external conditions such as humidity and temperature.
- Conventional packaging processes can be applicable.
- Be able to manufacturing with using low cost equipment (Comoglu and Ozyilmaz, 2019).

Desired characteristics of ODTs

*Tansel.Comoglu@pharmacy.ankara.edu.tr

Because administration of ODTs is different from administration of conventional tablets, ODTs should maintain several unique properties (Sresta et al., 2017),

Fast Disintegration - ODTs should disintegrate in the mouth without additional water. The disintegrated tablet should become a soft paste or liquid suspension, which can provide good mouth feel and smooth swallowing.

Drug Properties - For the ideal ODT technology, the drug properties should not significantly affect the tablet property.

Taste of Active Ingredients - Taste masking is an essential requirement for ODTs for commercial success.

Tablet Strength and Porosity - Because ODTs are designed to have a quick dissolution time, excipients should have high wettability, and the tablet structure should also have a highly porous structure.

Moisture Sensitivity - ODTs should have low sensitivity to humidity. This problem can be especially challenging because many highly water-soluble excipients are used in formulation to enhance fast dissolving properties. A good package design should be created to protect ODTs.

Formulation processes of ODT

Manufacturing techniques of ODTs can be examined as non-patented and patented technologies such as Zydis®, Flashtab®, Durasolv®.

Future research trends in ODTs

Although the ODT area has passed its infancy, as shown by a large number of commercial products on the market, there are still many aspects to improve in the ODT formulations. Despite advances in the ODT technologies, formulation of hydrophobic drugs is still a challenge, especially when the amount of drug is high. The future of ODTs also lies in the development of effective taste-masking properties. In general, the ODT formulations require large amounts of excipients, and having large doses of drug will only make the final formulation too big to handle. An ODT formulation that would require fewer excipients than the drug itself would be a breakthrough. While the problems to be solved are not easy, the history suggests that it is just a matter of time before they are solved (Fu et al., 2004).

Conclusion

ODTs have improved patient compliance, convenience, bioavailability and rapid onset of action. In future, ODTs may be the most acceptable and prescribed dosage form due to its rapid action. Their characteristic advantages such as administration without water, anywhere, anytime lead to their increased patient compliance in today's scenario of life. Considering many benefits of ODTs, a number of formulations are prepared in ODT form by most of the pharmaceutical companies.

References

Comoglu, T., Ozyilmaz, E.D., 2019. Orally Disintegrating Tablets and Orally Disintegrating Mini Tablets–Novel Dosage Forms for Pediatric Use. Pharmaceutical Development and Technology 24(7), 902-914.

Fu, Y., Yang S., Hoon J.S., Kimura, S., Park, K., 2004. Orally Fast Disintegrating Tablets: Developments, Technologies, Taste-Masking and Clinical Studies. Critical Reviews in Therapeutic Drug Carrier Systems, 21(6), 433–475.

Indurwade, N.H., Biyani, K.R., 2000. Evaluation of Comparative and Combined Depressive Effect of Brahmi, Shankhpushpi and Jatamansi in Mice. Indian Journal of Medical Sciences 54(8), 339-341.

Parkash, V., Maan, S., Deepika, S. K. Y., Hemlata, V. J., 2011. Fast disintegrating tablets: Opportunity in drug delivery system. Journal of advanced pharmaceutical technology & research 2(4), 223.

Sresta, N., Srinivasa, Babu, P., Pallavi, K., 2017. Orodispersible tablets. The Indian Pharmacist 15(1), 23-30.

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 27 -28 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.010
Short communication

The GEONs API-fingerprint program: tackling falsifications of APIs

Eric Deconinck[1,2*]

[1]*OMCL Active Pharmaceutical Ingredient (API) Working Group, GEON Network, EDQM - Council of Europe, 7 All´ee Kastner, CS 30026, F-67081, Strasbourg, France*
[2]*Service of Medicines and Health Products, Scientific Direction Physical and Chemical Health Risks, Sciensano, Brussels, Belgium*

Introduction

Falsification of active pharmaceutical ingredients (APIs) has been recognized as an important health issue since a series of health scares involving large numbers of casualties happened when altered APIs escaped detection in routine analytical testing. Any form of tampering the content or information about the content might affect the quality of the API, the finished product and therefore constitutes a direct threat to the health of patients (Position paper for OMCLs on API surveillance, EDQM). The conventional analytical approach often fails to detect falsification since the quality and quantity of the API might be within the current standards. Instead, methods that allow distinction of API samples, according to their source are needed.

Within the General European Official Medicines Control Laboratory (OMCL) Network (GEON) different initiatives were taken to augment the analysis and market surveillance of APIs used by the different manufacturers. Despite the efforts, API testing within the network is limited, compared to the surveillance of finished products. To tackle the issue of falsified API detection the API-working group (API-WG) of the GEON organizes atypical market surveillance studies (MSS), where an MSS (analysis according to the Ph. Eur.) is combined with a fingerprint study (MSSFP). An MSS, according to Ph. Eur. only gives an indication of the quality of the product on some distinct parameters, often chosen based on the manufacturing process. A MSSFP study allows to obtain a global image of the sample, to compare to batches of the (presumed) manufacturer and/or to data of fingerprint studies performed in the past. (Deconinck et al., 2022, Rebiere et al., 2022).

The aim of the paper is to highlight the challenges and pitfalls in detecting falsified APIs and to give an insight in the work of the GEONs API-WG with a special focus on the MSSFP studies. A summary is given of the MSSFP study on sildenafil citrate and this for the set-up and the obtained results.

Materials and methods

Set-up of the study

Every MSSFP study focusses on one API, selected based on the risk of falsification or incidents that occurred in one of the member states. Sildenafil Citrate is the API of one of the worlds most falsified medicines.

The idea of an MSSFP study is to select analytical techniques that allow to differentiate API samples according to their manufacturer. The selection of the techniques starts with the Ph. Eur. From the monograph infrared spectroscopy, the method for related substances and the presence of residual solvents were selected as starting point. Further these techniques were complemented with fingerprinting techniques, proven valuable in the previous studies, i.e. Raman spectroscopy, X-ray powder diffraction (XRDP) and proton-Nuclear Magnetic Resonance (^{1}H-NMR).

All samples were analyzed in the same way for each method and the data was preprocessed, where necessary, followed by unsupervised chemometric analysis using principal component analysis (PCA) and hierarchical clustering (HCA). Also the combination of data through

*eric.deconinck@sciensano.be

mid-level data fusion was explored (Deconinck et al., 2022).

Sample collection

Every participating OMCL collected samples of the targeted API, available on their national markets following their own procedures. This resulted in the collection of 79 sildenafil citrate samples from 14 different manufacturers. Samples were then centralized and dispatch to the different testing OMCLs, where each OMCL performed one technique on all samples (Deconinck et al., 2022).

Results and discussion

Of the six analytical techniques, selected to obtain fingerprint data infrared and Raman spectroscopy, as well as the test on related substance were unable to distinguish between the manufacturers. This can be explained by the fact that all samples complied with the Ph. Eur. and these techniques are not sensitive enough to detect the small differences between the manufacturers. This could be different in the case of real falsified samples.

In contrast, the results of the test on residual solvents, XRPD and ^{1}H-NMR gave valuable fingerprint information and allowed to link the differences between the samples to the different manufacturers. Together the three techniques allowed the distinction of all manufacturers, except one. Therefore, these techniques are complementary and can be used together as fingerprint techniques for sildenafil citrate and so allow the detection/identification of falsified samples.

The combination of the data of different analytical techniques did not result in significant improvement of the results, except for the combination of the residual solvent data with XRPD. In fact the fused data allowed the distinction of the manufacturer, which was not possible to differentiate using only the individual techniques (Deconinck et al., 2022).

Conclusion

From a quality point of view all samples collected in this study complied with the Ph. Eur.

Three analytical techniques (residual solvents, XRPD and ^{1}H-NMR) resulted in valuable fingerprint information, though none of the techniques allowed the distinction of all manufacturers. Therefore all three analyses are necessary, as well as three different chemometric models, i.e. the one based on residual solvents, the one on ^{1}H-NMR and the one based on the fused data of residual solvents and XRPD.

In general it can be concluded that in the case of a suspicious sildenafil citrate API sample, the GEON will proceed with analysis with each of the three techniques mentioned above in order to link the sample to a manufacturer or not. If this is not possible further analysis and research on origin and identity will be performed. In case the suspicious sample claims a manufacturer the analysis will be limited to the technique(s) able to characterize this manufacturer (Deconinck et al., 2022).

More general it should be emphasized that falsification of APIs is a real and underestimated threat, that manifests itself in the quality of finished products and health risks for the patients. Therefore it is the engagement of the GEON and the API-working group to promote API-surveillance throughout the network, to launch new MSSFP collaborative studies and to work with the European Medicine Agency to rationalize GMP inspections of API manufacturers at an international level. Further the GEON remains open for collaborative campaigns on combatting falsification, but also for cooperation with different regulatory authorities and active involvement in global campaigns in order to share the expertise and information gained during different project

References

API Surveillance: Position Paper for OMCLs, PA/PH/OMCL (12) 51 3R, EDQM,Strasbourg, France https://www.edqm.eu/en/testing-of-active-pharmaceutical-ingredients-apis-

Benefits of Chemometrics for OMCLs, PA/PH/OMCL (19) 60 DEF, EDQM,Strasbourg, France https://www.edqm.eu/documents/52006/169295/omcl-benefits-of-chemometrics-for-omcls.pdf/c3d4365a-6317-2dba-8f44-4a83a7fedd8a?t=1635169821415

Deconinck, E., Courselle, P., Raimondo, M., Grange, Y., Rebière, H., Mihailova, A., Bøyume, O., Maurin, J.K., Pioruńska-Sędłak, K., Olsen, L.S., Acevska, J., Brezovska, K., Rundlöf, T., Portela, M.J., Bertrand, M., 2022. GEONs API fingerprint project: Selection of analytical techniques for clustering of sildenafil citrate API samples. Talanta 239, 123123. https://doi.org/10.1016/j.talanta.2021.123123

Rebiere, H., Grange, Y., Deconinck, E., Courselle, P., Acevska, J., Brezovska, K., Maurin, J., Rundlöf, T., Portela, M.J., Olsen, L.S., Offerlé, C., Bertrand, M., 2022. European fingerprint study on omeprazole drug substances using a multi analytical approach and chemometrics as a tool for the discrimination of manufacturing sources. J. Pharmac. Biomed. Anal. 208, 114444. https://doi.org/10.1016/j.jpba.2021.114444

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 29 -30 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.011
Short communication

Hospital Pharmacists' Preparedness in Times of Crisis

Nenad Miljković[1,2]

[1]*Institute of Orthopaedics Banjica, Mihaila Avramovića 28, Belgrade, Serbia*
[2]*European Association of Hospital Pharmacists, Boulevard Brand Whitlock 87, Brussels, Belgium*

Introduction

The pharmacist's role in crisis response is just as essential as it is the provision of pharmaceutical care in traditional settings. (1) Pharmacists should be proactive and define their role in crisis management before others define it for them. Pharmacists' ambulatory, pharmacotherapy and critical care readiness should be delivered in line with existing and emerging supply chain and distribution tasks. Shortages of personal protective equipment (PPE), disinfectants and medicines as well as the uncertainty about available treatment options shaped the work of hospital pharmacists throughout the SARS-CoV2 pandemic. (2) One of the major risks in crisis is the occurrence of medicine shortages. Shortages of medicines have been on the rise globally this century. Factors that contribute to shortages, such as insufficient manufacturing capacity, a shortage of active pharmaceutical ingredients, and restricted distribution/allocation, are among those that are out of the scope of the pharmacist's activities. (3) Nevertheless, pharmacists effectively manage medicines shortages by implementing defined strategies ahead of the occurrence of a shortage. (2) The European Association of Hospital Pharmacists (EAHP) has worked on the issue of medicine shortages in 2014, 2018 and 2019 demonstrated that the impact that shortages have on patient care and the intensity of the work of hospital pharmacists has tremendously increased, especially in times of crisis. (3)

Material and methods

In order to further investigate problems encountered during the current SARS-CoV2 pandemic the survey on crisis preparedness among hospital pharmacies in Europe, EAHP created and conducted the survey on the future crisis preparedness of hospital pharmacies via Survey Monkey. The online questionnaire, along with its objectives and timeline, was distributed to EAHP members through a campaign carried out on social media and via the EU Monitor as to increase the engagement of individual hospital pharmacists. Throughout 17 questions aimed at collecting the general characteristics of the survey participants; medicine, disinfectant and PPE shortages; mitigating approaches adopted for medicine shortages; type, source and utility of the support received; lessons learnt; and areas of improvement for future pandemics. The survey was conducted between September and December 2020. Data on the classes of medicines affected by shortages were compared with those of the 2019 survey on medicines shortages to assess the impact of the pandemic on the type of medicine shortages. The answers to the questions regarding the medicine shortages, disinfectants, and PPE were considered as a binary response variable. Three backward stepwise logistic regression (BSLR) models were used to identify independent variables. All analyses were performed using R 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria). Statistical significance was set at $p<0.05$.

Results and discussion

Among 59% (n=861) of respondents, medicine shortages during the SARS-CoV2 pandemic posed significant problems in delivering the best care to patients and/or operating the hospital pharmacy. Anaesthetics were most affected by shortages (46%, n=670), followed by antimicrobials (37%, n=539), muscle relaxants (29%, n=425), benzodiazepine (26%, n=380) and opioids (22%, n=316). Antimalarial and antiviral drugs were reported to be in shortage by 13% (n=193) and 12.5% (n=183), respectively, of the survey respondents and were widely used and/or misused, especially during the early stages of the SARS-CoV2 pandemic. The frequencies that resulted in a statistically significantly increase in 2020 compared

*nenad.hedren@gmail.com

with 2019 were those relating to anaesthetics ($\chi2_{(1, 6117)}$ =242.37, p<0.001) and antimicrobials ($\chi2_{(1, 6117)}$=16.03, p<0.001). The top three mitigating strategies adopted to address the shortages were: therapeutic substitution (42%, n=620), creating additional strategic stock at local, regional or national level (38%, n=557) and borrowing medicines from other hospitals (35%, n=512). Importing medicines from another country (33%, n=478) and generic substitution (31%, n=448) were also reported among a variety of mitigation strategies, while the least selected methods were compounding/production of medicines in the pharmacy (28%, n=405) and using medicines from central contingency reserves kept at national level (27%, n=403). The country's National Competent Authority (NCA), was among the entities that provided the most support to overcome medicine shortages (57%, n=838) followed by manufacturers and the Scientific Societies and Healthcare Professional Organisations (SSHPO), which were reported in 39% (n=571) and 20% (n=300) of the answers, respectively. The main type of support received by the respondents was the allocation of contingency stocks to their hospital (51%, n=741), followed by feedback received from manufacturers on the availability of medicines (46%, n=675) and the expected duration of shortages (40%, n=584).

Another aspect considered in the survey was the usefulness of the help received from each of the aforementioned supporting entities. The respondents were asked to assign a 5-point Likert scale score ranging from 1 ('not useful') to 5 ('extremely useful'). The highest mean usefulness score was assigned to the NCA (mean=3.2, SD=1.15), followed by the SSHPO (mean=3.10, SD=1.21) and manufacturers (mean=3.06, SD=1.07). Handling a higher workload and stress (n=951) as well as quickly adapting the processes and practices at the hospital pharmacy (n=942) were lessons that almost 65% of participants learnt during the first peak of the pandemic, followed by working with scarce resources which was reported by 55% (n=813) of respondents. The proper handling of PPE (43%, n=627) and the assessment of therapeutic options despite the limited availability of scientific data (37%, n=543) ranked in fourth and fifth place as learning points from the pandemic. Concerning the areas of improvement to better prepare pharmacy services for future pandemics, almost half of the respondents indicated that improvements are needed in hospital stock management (49%, n=721), communication with authorities (47%, n=688), crises and surge management (47%, n=688), the use of preparedness protocols (47%, n=682) and communication with other healthcare professionals (46%, n=674). Only 28% (n=404) of respondents indicated communication with the management of the healthcare facility as an area for improvement. Moreover, the significant association between the percentages of the infected population with increased odds of all three types of shortages assessed suggests that the countries hit hardest by the pandemic were those in which the procurement of health goods was the greatest problem. Moreover, the fact that anaesthetics, antimicrobials, muscle relaxants, benzodiazepine and opioids were the most reported classes of medicines in short supply and that, for many of them, there was a significant relative increase in the frequency of shortage reporting compared with the 2019 survey. (2) The high rate of responses reporting stress management and the need to quickly adapt processes and practices at the hospital pharmacy as lessons learnt from the pandemic, as well as the need for improvements in stock management and communication with authorities and other health professionals as further areas for improvement, demonstrate the difficulties encountered during the SARS-CoV2 pandemic, characterised by a constant change in the available scientific literature and in the epidemiological situation which has produced the need for a frequent update of therapeutic protocols/guidelines and medicine inventories. (2)

Conclusion

This survey represents the perspective of the hospital pharmacists as a response to global SARS-CoV2 pandemic crisis. It shows how a global pandemic can affect the magnitude and type of medicine, disinfectants and PPE shortages. Hospital pharmacists highlighted many weaknesses in management of the pandemic, which can be considered as a starting point to plan a more resilient and overarching mitigating framework to manage future crisis.

References

Badreldin, H.A., Atallah, B., 2021. Global drug shortages due to COVID-19: impact on patient care and mitigation strategies. Res. Social. Adm. Pharm. 17, 1946–1649. https://doi.org/10.1016/j.sapharm.2020.05.017

Leonardi Vinci, D., Polidori, P., Miljković, N., et al. 2021. Lessons learnt from the COVID-19 pandemic: results of EAHP survey on the future crisis preparedness of hospital pharmacies. Eur. J. Hosp. Pharm. 2021. https://doi.org/10.1136/ejhpharm-2021-002944

Miljković, N., Batista, A., Polidori, P., et al., 2020. Results of EAHP's 2019 medicines shortages survey. Eur. J. Hosp. Pharm. 27, 202–208. https://doi.org/10.1136/ejhpharm-2020-002341

Pharmaceutical analysis/ Quality assurance/ Regulatory affairs

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 33 -34 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.012
Short communication

Static and dynamic quantum mechanical methods for exact interpretation of Infrared Multiple Photon Dissociation Spectra: current state and development perspectives

Blagoj Achevski[1*], Jasmina Tonikj-Ribarska[1], Rumenka Petkovska[1], Ljupco Pejov[2,3]

[1]*Faculty of Pharmacy, University "Ss Cyril and Methodius", 1000 Skopje, Republic of North Macedonia*
[2]*Faculty of Natural Sciences and Mathematics, Institute of Chemistry, University "Ss Cyril and Methodius", 1000 Skopje, Republic of North Macedonia*
[3]*Faculty of Science and Technology, Department of Chemistry, Bioscience and Environmental Engineering, University of Stavanger, Stavanger, Norway*

Introduction

The advances in gas phase vibrational spectroscopy of mass-selected ions, Cold Ion IR spectroscopy, especially infrared multiple photon dissociation (IRMPD) provide fundamental information for characterization and understanding of the chemical state, the structure and dynamics of gas-phase biomolecules. Additional tool for suitable and exact interpretation and band assignment of an experimental spectrum is the theoretical approaches through available quantum mechanical computational codes, based on algorithms involving the double-harmonic normal mode approximation. These are being often used in conjunction with a density functional theory (DFT) based method.

The implementation of these methods and approaches will be presented through review of articles referring to glycine as the smallest and the simplest amino acid that been continuously drawing the attention of the scientific community. The obtained experimental spectra of glycine and the attempts for their suitable interpretation and band assignment are of great interest for many scientific areas. Therefore, in this work we will highlight the current knowledge, advantages and drawbacks in the currently used experimental and theoretical methods. We will also pinpoint the aspects in which significant new contributions to this field of work can be added by implementation of our own approaches.

Review of current state

The infrared multiple photondissociation (IRMPD) spectroscopic technique has enabled numerous size-selected species to be explored in details. Such method is usually applied employing a weakly bound so called "molecular messenger" to "tag" the investigated ion, using H_2, He, Ne, Ar as typical examples of tags that attach to ions at very low temperatures and that can be detached upon absorption of one or more IR photons. Exact assignment of vibrational modes is an important step in the interpretation of spectrum obtained with these experimental techniques. Most of the band assignments, are usually based on empirical arguments and on the "chemical intuition". Any serious attempt to provide exact and in-depth understanding of molecular force fields should be based on sound theoretical analysis and argumentation (Polfer, 2011).

IRMPD as an experimental approach provides an insight in the nature and localization of posttranslational modifications (PTMs) affecting single amino acids and peptides. The advantages of this method are presented through several PTM containing amino acids and peptides which have been characterized by IRMPD in gas phase. IRMPD spectroscopy takes advantage of the high sensitivity and resolution of MS/MS, but it is not a proteomics tool. Provides detailed structural information for the isolated species of interest in the gas phase, such as individual ions or in a carefully controlled

*bachevski@ff.ukim.edu.mk

microsolvation state. Integration with X-ray crystallography and NMR spectroscopy in the structural analysis of biomolecules and reactive intermediates, to elucidate the relationship between structure and biological function (Maitre et al., 2020).

The native-like structures of protonated glycine and peptide Gly_3H^+ were elucidated using cold ion IR spectroscopy of these biomolecules hydrated by a controlled number of water molecules. In doing so, the focus is kept on the differences in the structures of the hydrated complexes generated directly from an aqueous solution or produced by cryogenic condensation of water onto the gas-phase glycine (Saparbaev et al., 2021).

Using IRMPD is reported IR spectra of cold, protonated glycine tagged with He or between 1 and 14 H_2 molecules. Theoretical approach is conducted simultaneously with density functional theory (DFT) – based method, using second order MølIer-Plesset perturbation theory in the double-harmonic normal mode approximation. Only harmonic theory caluclations which are scaled to bring the calculated free OH stretch of protonated glycine with a helium atom attached into agreement with the free OH stretch observed in the experimental spectrum of this same ion (Masson et al., 2015).

Discussion and perspective

Experimental results obtained with most of the modern sophisticated techniques, interpreted within the quantum mechanical representation of the structure of matter, provide information about the distance between energy levels of a certain type. In the case of the IRMPD technique, these are vibrational energy levels. However, the data obtained directly from the experiment do not indicate the nature and type of these energy levels. Therefore, for the correct interpretation of the data, serious theoretical support is necessary, which in recent years is practically a necessity in the scientific literature. In the present works in the field of IRMPD spectroscopy, the theoretical support mainly consists in performing routine calculations based on the theory of function of the electron density in the so-called double harmonic approximation. Such calculations contain several assumptions inherent in all static theoretical methods (based only on the potential energy surface of the studied molecular systems).

The shortcomings of these computational methods can be approached for a further development. In the static approach, the total anharmonic vibration potentials of the relevant intra- and intermolecular modes can be calculated, and the vibration Schrödinger equation would be solved sequentially, thus obtaining the anharmonic vibration frequencies, there will be no work in harmonious approximation. Dynamic calculations, on the other hand, can be performed on a series of statistical-physical simulations (with the Monte-Carlo methods or molecular dynamics) at final temperatures, which correspond to the conditions in which the experiments are performed in reality. Sequentially, with proper mathematical processing of the generated trajectories, the total final temperature spectrograms can be calculated, which inherently contain the effects due to the anharmonicity of the movements.

Conclusion

Most of the theoretical approaches coupled with IRMPD spectroscopy are simple and straightforward, automated in the available quantum mechanical computational codes, based on algorithms involving the double-harmonic normal mode approximation, and are being used often in conjunction with a density functional theory (DFT) – based method. Such calculations contain several assumptions inherent in all static theoretical methods (based only on the potential energy surface of the studied molecular systems). There is a firm evidence that systematic errors inherent to widely used DFT methods may cancel out with those due to the harmonic approximation. Therefor an attempt will be made to make a substantial contribution to the development and implementation of new computational methods that would not have these shortcomings.

References

Maitre, P., Scuderi, D., Corinti, D., Chiavarino, B., Crestoni, M. E., Fornarini, S., 2020. Applications of Infrared Multiple Photon Dissociation (IRMPD) to the Detection of Posttranslational Modifications. Chem. Rev. 120(7), 3261–3295. https://doi.org/10.1021/acs.chemrev.9b00395

Masson, A., Williams, E.R., Rizzo, T.R., 2015. Molecular hydrogen messengers can lead to structural infidelity: A cautionary tale of protonated glycine. J. Chem. Phys. 143(10), 104313. https://doi.org/10.1063/1.4930196

Polfer, N.C., 2011. “Infrared multiple photon dissociation spectroscopy of trapped ions,” Chem Soc Rev. 40(5), 2211–2221. https://doi.org/10.1039/C0CS00171F

Saparbaev, E., Aladinskaia, V., Zviagin, A., Boyarkin, O.V., 2021. Microhydration of Biomolecules: Revealing the Native Structures by Cold Ion IR Spectroscopy. J. Phys. Chem. Lett. 12(2), 907–911. https://doi.org/10.1021/acs.jpclett.0c03678

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 35 - 36 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.013
Short communication

A regulatory perspective: Examples from microbiological quality control of non-sterile medicinal products

Sanja Milovanović*, Danijela Mikulčić, Tončica Vukman Kordić, Siniša Tomić

HALMED, Ksaverska cesta 4, 10000 Zagreb, Croatia

Introduction

The Croatian OMCL acts as a part of the national authority of the Republic of Croatia, Agency for Medicinal Products and Medical Devices (HALMED). Our key activity, regulated by the Medicinal Products Act (Official Gazette, 76/13, 90/14, 100/18), is post-marketing surveillance of the quality of medicinal products. The microbiological attributes of a medicinal product are an indispensable part of every medicinal product quality specification. For non-sterile medicinal products, the first requirement is to minimize the level of microbial contamination, i.e. to lower the total number of microorganisms present below an acceptable limit. Additionally, the emphasis of non-sterile product microbiological quality is avoiding at all costs the presence of specified (objectionable) microorganisms. The European Pharmacopeia (Ph.Eur) 10.8. (07/2022), chapter 5.1.4., defines both requirements with acceptance criteria, depending on the nature and route of administration of the product. Product compliance with the requirements for microbiological quality is of critical importance for ensuring patient safety. In this short review, we present results from routine microbiological quality control of non-sterile medicinal products, and some of the contamination findings, for the period from 2017 to 2022. Additionally, we present results from a mini marketing surveillance of microbiological quality control of selected probiotics from the market, namely of the only two probiotics registered as a medicinal product, as listed in Medicinal Products Database, HALMED [Internet], 2022. The remaining probiotics examined in this surveillance are under the Croatian national law regulated as a food supplement, and are not included in this review.

Materials and methods

Materials

Samples: The samples of medicinal products were sampled from the market, from various wholesalers. The examined samples were considered as representative, and the tested quantity was in line with the provisions of European Pharmacopeia (Ph.Eur.) 10.7. (04/2022). Total number of medicinal products tested in the period from January 2017 to January 2022, is 1074. Samples are grouped according to Ph.Eur. acceptance criteria: 987 for oral use (non-aqueous and aqueous), 69 for oromucosal, gingival, cutaneous, nasal or auricular use, 4 for vaginal use, 12 for rectal use, 1 transdermal patch and 1 medicinal product with special requirements that apply to liquid preparations for nebulization. Probiotics were sampled from a local pharmacy. Total number of samples was 2, both for oral use.

Media: all of the media used for testing is Ph.Eur. compliant media, commercially available from bioMerieux. Growth promotion is done on every batch/new shipment of media.

Reference strains: all of the strains used for testing are Ph.Eur. compliant strains, commercialy available from Microbiologics: ATCC 6538, ATCC 9027, ATCC 8739, ATCC 14028, ATCC 10231, ATCC 6633, ATCC 16404 (all EZ-CFU One Step).

Methods

The methods used for examination of medicinal products are pharmacopeial methods described in chapters 2.6.12. (*Microbial examination of non-sterile products: Microbial Enumeration Test*) and 2.6.13. (*Microbial examination of non-sterile products: Test for Specified Microorganisms*) of the European Pharmacopeia (Ph.Eur.)

*sanja.milovanovic@halmed.hr

10.8. (07/2022). Suitability of the method for each product is either performed by the manufacturer (data in Marketing Authorization) or performed by the OMCL. The method used for examination of probiotic contaminants is a pharmacopeial method described in chapter 2.6.36. (*Microbial enumeration of live biotherapeutic products: Test for Enumeration of Microbial Contaminants)* of the European Pharmacopeia (Ph.Eur.), 10.8. (07/2022). Identification of growth is done following Gram staining, by using Vitek 2 Compact and bioMerieux identification cards.

Results and discussion

All of the samples examined during the given period complied with the specification requirement for microbiological quality, i.e. we observed **no *out-of-specification* results**. However, we present examples of bioburden that was under the acceptable limits, with identified microorganisms.

Non-aqueous and aqueous medicinal products for oral use

Example 1. A medicinal product with the active substance *fosfomycin trometamol*, dosage form granules for the preparation of oral solution, was examined by membrane filtration method, dilution 1:10. Growth was observed on TSA (*Trypcase Soy agar*) plates and the result (mean for 2 plates) for TAMC (*Total Aerobic Microbial Count*) was 27 CFU/g (limit $\leq 10^3$ CFU/g). Identified microorganisms were: *Aerococcus viridans* (94%, GP), *Alloiococcus otitis* (93%, GP), *Staphylococcus lentus* (95%, GP), *Kocuria rosea* (98%, GP).

Example 2. A medicinal product with the active substance *pioglitazone hydrochloride*, dosage form tablets, was examined by direct inoculation method (surface spread), 1:100 dilution. Growth was observed on TSA plates and the result for TAMC was 693 CFU/g (limit $\leq 10^3$ CFU/g). The identified microorganism was *Acinetobacter lwoffii* (99%, GN).

Transdermal patches

Example 3. A medicinal product with the active substance *fentanyl*, was examined by direct inoculation method (surface spread), dilution 1:10. Growth was observed on TSA plates and the result for TAMC was 18 CFU/patch (limit $\leq 10^2$ CFU/patch). The identified microorganism was *Staphylococcus hominis ssp hominis* (96%, GP). All of the microorganisms identified as contamination in medicinal products in routine control are bacteria, mainly GP cocci, a part of normal human microbiota or environmental flora and infrequently pathogens in humans. There were no yeasts and moulds detected.

Probiotics

Medicinal products with the active substance *Lactobacillus acidophilus* (LA-5) and *Bifidobacterium animalis subsp. lactis* (BB-12), dosage form hard capsule, and with the active substance *Bifidobacterium animalis subsp. lactis* (BB-12), dosage form powder for preparation of oral suspension, were examined by direct inoculation (pour plate) method.

In both cases, observed growth of contaminating microorganisms was below the limits set by Ph.Eur. Identified microorganisms were *Aerococcus viridans* (87%, GP) and *Kocuria kristinae* (85%, GP), both frequent environmental and human microbiota microorganisms.

Conclusion

The Ph.Eur. provides clear requirements for microbiological quality of non-sterile medicinal products. Nevertheless, as discussed in Sandle (2016), following good practice it is up to the regulatory body, as well as the manufacturer, to perform identification and risk assessment of all microorganisms present in the medicinal product, even if the number of colonies is acceptable and species are not listed as objectionable in Ph.Eur.

As shown in Sandle (2016), the bioburden of a final product is a direct consequence of control measures implemented by the manufacturer under GMP requirements. From the pool of various samples of medicinal products examined in HALMED's OMCL for microbiological quality in the period of 2017.-2022., and lack of *out-of-specification* results, we can conclude that the measures implemented by manufacturers are efficient in assuring the microbiological quality and thus safety of medicinal products on the market.

References

Council of Europe. European Pharmacopeia (Ph.Eur.) 10.8. (07/2022); Available at: European Pharmacopoeia Online (edqm.eu)

HALMED [Internet]. Medicinal Products Database, 2022 [accessed on 01.06.2022.]. Available at: https://www.halmed.hr/Lijekovi/Baza-lijekova

Sandle, T., 2016. Pharmaceutical microbiology: essentials for quality assurance and quality control, first ed. Elsevier. pp. 93-100; 281-293.

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 37 - 38 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.014
Short communication

Possibilities and challenges of "green" chromatographic solutions

Natalija Nakov*, Jelena Acevska, Katerina Brezovska, Rumenka Petkovska, Zoran Kavrakovski, Aneta Dimitrovska

Faculty of Pharmacy, University Ss. Cyril and Methodius,Majka Tereza 47, 1000 Skopje, Republic of North Macedonia

Introduction

In the last twenty years, the focus of the scientific community has been put on the "green" analytical chemistry (GAC). The concept of GAC includes development of new effective analytical methodologies that will enable minimization and / or elimination of the hazardous chemicals and chemical waste, but at the same time will enable faster and more energy efficient analysis (Guardia & Garrigues, 2020). Given that liquid chromatography (LC) is the most widely used analytical technique in the pharmaceutical analysis, as well as the large amount of chemical waste generated during these analyzes, the development of more environmentally friendly ("green") chromatographic methods is a crucial part of the GAC concept. Several approaches could be applied during the "green" chromatographic method development in order to remove / reduce the toxic organic solvents from the classical LC mobile phases (Napolitano-Tabares et al., 2021).

Considering that there is no entirely eco-friendly organic solvent, the use of pure water as a mobile phase appears to be an ideal solution. As the name of this technique implies, the “subcritical water chromatography” (SWH) uses pressurized hot water below its critical point conditions (374°C and 218 atm). This conditions require adaptation of the conventional LC instruments, such as heating system and detectors other than UV/Vis (e.g. amperometric detector, flame ionization detector) (Dembek & Bocian, 2020). However, this strategy for greening the chromatographic methods still has no wider application because it bears a certain financial burden for the analytical laboratories.

Another “green” solution is the use of columns packed with fully porous sub-2µm particles. This approach leads to faster chromatographic separation and reduction of the solvent consumption (Shaaban, 2016). However, this approach is not quite “green” because the consumption of the toxic solvents is not eliminated. In addition, the conventional LC instruments should be converted into UPLC systems, thus this expense is sometimes not acceptable for the laboratories with low-incomes.

In this review, the focus is given on the “green” chromatographic solutions that could be easily applied on the conventional LC instruments, without a need for further investements.

Eco-friendly solvents

The replacement of acetonitrile (ACN) and methanol (MeOH) used in the LC mobile phases with eco-friendlier ones, has a big role in the GAC strategies.

Ethanol (EtOH) is the first choice for “greening” the LC mobile phase. This solvent is an effective alternative for MeOH because they belong in the same group (according the Snyder’s classification of organic solvents); EtOH has higher elution power and low UV cutoff. The drawback of EtOH mobile phase is the higher column backpressure which could be overwhelmed with higher column temperatures or with the use of columns packed with superficially porous particles (Yabre, 2018).

2-propanol (IPA), acetone and ethyl acetate are greener alternatives for ACN, but the analysts should be aware of the higher viscosity of IPA and the high UV cutoff wavelength of acetone and ethyl acetate (330 nm and 260 nm, respectively) (Olives et al., 2017).

Propylene carbonate (PC) is another green polar aprotic solvent that has emerged as an alternative for ACN. The lower miscibility with water is overwhelmed with addition of EtOH, usually in ration 7/3 (*v/v*) (PC/EtOH or water/EtOH). These mixtures, compared to

*natalijan@ff.ukim.edu.mk

the previously mentioned solvents, have low UV cutoff (around 210 nm). The mass-transfer resistance and the higher backpressure of the PC/EtOH mobile phases could be overcome with lower flow rates (Tache, 2013).

Micellar liquid chromatography (MLC)

In the recent years, the increased awareness of the GAC principles contributed MLC to be back in the focus of the analysts (Kamal & El-Malla, 2019; Ibrahim et al., 2020). The greenness of this technique is seen through the aqueous composition of the mobile phase, low toxicity and cost, and low environmental impact of the surfactants. Namely, the mobile phase contains surfactant (usually sodium dodecyl sulfate) above its critical micellar concentration, while the separation is performed on the commonly used reverse-phase stationary phases (C_{18}, C_8).

The unique characteristics of the micellar mobile phase are responsible for the diverse interactions (hydrophobic, ionic and steric) between the analyte, the surfactant-modified stationary phase and the micelles. These interactions have impact on the retention and the selectivity, thus allowing separation of ionic and neutral compounds (Rambla-Alegre, 2012). The separation of hydrophobic compounds in MCL is a challenge because of the excessive retention. This drawback could be surpassed with the addition of small concentrations of Brij-35, which as a more polar non-ionic surfactant eliminates the need of the addition of low concentration of organic solvent (Ibrahim et al., 2020). In addition, shorter chain length stationary phases could be used. Considering the wide range of interactions and retention mechanisms in MCL, it is advisable to use the design of experiments approach for the optimization of the critical method parameters.

Per aqueous liquid chromatography (PALC)

Recent literature data (Bocian & Krzeminska, 2019; Dembek & Bocian, 2020) show that pure water on ambient temperature could be used as "green" chromatographic solutions for separation of polar compounds. The separation is performed on polar-embedded stationary phases using pure water or highly aqueous mobile phase. In PALC, the selectivity is controlled through the type of the stationary phase and the ionic strength and pH value of the aqueous buffer. Considering the differences in the solvation properties of the stationary phases, the right choice of the column type is crucial for the method performance.

Concluding remarks

Finding the ideal "green" solution for particular LC application is a challenge, but still there is enough scientific knowledge that could support this process. The analysts across the laboratories should more bravely implement the "green" chromatographic solutions. These "green" solutions bear benefits to the analyst (healthier and safer working environment); to the pharma industry (significant reduce of the waste disposal costs) and to the community (reduced negative environmental impact).

References

Bocian, S., Krzeminska, K., 2019. The separation using pure water as a mobile phase in liquid chromatography using polar-embedded stationary phases. Green Chem Lett Rev. 12, 69-78, https://doi.org/10.1080/17518253.2019.1576775

Dembek, M., Bocian, S., 2020. Pure water as a mobile phase in liquid chromatography techniques. Trends Anal Chem 123, 115793. https://doi.org/10.1016/j.trac.2019.115793

Guardia, M., Garrigues, S., 2020. Past, present and future of green analytical chemistry, in Challenges in green analytical chemistry, 2nd ed. Royal Chemical Society, pp. 1-18. https://doi.org/10.1039/9781788016148-00001

Ibrahim, A., Elmansi, H., Belal, F., 2020. Solvent-free mixed micellar mobile phases: An advanced green chemistry approach for reversed-phase HPLC determination of some antihypertensive drugs. J. Sep.Sci. 43, 3224-3232. https://doi.org/10.1002/jssc.202000429

Kamal, A., El-Malla, S., 2019. Mixed micellar liquid chromatographic method for simultaneous determination of norfloxacin and tinidazole in pharmaceutical tablets. MICROC 150, 104151. https://doi.org/10.1016/j.microc.2019.104151

Napolitano-Tabares, P., Negrin-Santamaria, I., Gutierrez-Serpa, A., Pino, V., 2021. Recent efforts to increase greenness in chromatography. Curr. Opin. Green Sustain. Chem. 32, 100536. https://doi.org/10.1016/j.cogsc.2021.100536

Olives, A., Gonzales-Ruiz, V., Martin, M., 2017. Sustainable and eco-friendly alternatives for luquid chromatography analysis. ACS Sustainable Chem. Eng. 5, 5618-5633. https://doi.org/10.1021/acssuschemeng.7b01012

Rambla-Alegre, M., 2012. Basic principles of MLC. Chromatogr. Res. Int. ID 898520, https://doi.org/10.1155/2012/898520

Shaaban, H., 2016. New insights into liquid chromatography for more eco-friendly analysis of pharmaceutical. Anal. Bioanal. Chem. 408, 6929-6944. https://doi.org/10.1007/s00216-016-9726-2

Tache, F., Udrescu, S., Albu, F., Micale, F., Medvedovici, A., 2013. Greening pharmaceutical applications of liquid chromatography through using propylene carbonate-ethanol mixtures instead of acetonitrile as organic modifier in the mobile phases. J. Pharm. Biomed. Anal. 75, 230-238. https://doi.org/10.1016/j.jpba.2012.11.045

Yabre, M., Farey, L., Some, I., Gaudin, K., 2018. Greening reversed-phase liquid chromatography methods using alternative solvents for pharmaceutical analysis. Molecules 23, 1065. https://doi.org/10.3390/molecules23051065

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 39 -40 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.015
Short communication

Scientific approach and implementation of a measurement uncertainty in mass balance determination

Maja Hadzieva Gigovska[1*], Natalija Nakov[2], Jelena Acevska[2], Katerina Brezovska[2], Aneta Dimitrovska[2], Jelena Lazova[1]

[1] Research & Development, ALKALOID AD, Blvd. Aleksandar Makedonski 12, 1000 Skopje, R. Macedonia
[2] Faculty of Pharmacy, University "Ss Cyril and Methodius", Mother Theresa 47, 1000 Skopje, R. Macedonia

Introduction

Mass balance (MB) is a concept used in the pharmaceutical industry to determine the possible loss of the active pharmaceutical ingredient (API) and increase in degradation products, due consideration of the margin of analytical error (ICH, 2003). Although it is a simple concept, its determination is a real challenge because there are many critical factors that have significant influence on the results.

Forced degradation studies (FDS) are performed in order to understand the degradation pathways of API. During these studies, MB determination is performed to evaluate the correlation between the degradation of the API and the measured increase in the amount of degradation products (Hokanson et al., 2006).

Based on the literature review and the findings so far (Baertschi et al. 2013; Hokanson et al., 2006), it could be said that there is a limited number of studies presenting a systematic approach for assessing the critical factors that influence the results of the MB study (especially in FDS). Far from our knowledge, no report exists on application of measurement uncertainty (MU) in them (Baertschi et al. 2013; Hokanson et al., 2006). Therefore need of scientific approach for determination of MB in FDS that will include measurement uncertainty is recognized.

The purpose of this study is to propose a scientific approach for estimation of the MU for the MB determination in FDS, and to highlight the advantage of its application in order to obtain a valid analytical result. Statin molecule was taken as a model substance, because this API is important not only from the pharmaceutical, but also from the analytical point of view due to its proven inherent instability.

Materials and methods

Statin samples were received from Teva Pharmaceutical Industries Ltd., Israel and Ranbaxy Research Laboratory (Gurgaon, India). Reference standards were provided by the EDQM (Strasbourg, France). All reagents used were of analytical grade.

Samples were subjected to stress under acidic, alkaline, oxidative, thermal and photolytic conditions. All stress studies were performed at an initial concentration of 1 mg/mL in amber color glassware in order to protect the solutions from light degradation.

Determination of the MB was performed by: determination of API content by HPLC method (Hadzieva Gigovska et al., 2018a); determination of the water content (by Karl Fisher titration); determination of the impurities (by various techniques) before and after the forced degradation studies. The enantiomers were quantified by high performance liquid chromatography (HPLC) method proposed in the pharmacopeia monograph for the API. For residual solvent analysis, headspace gas chromatography – flame ionization detector (GC-FID) analysis was carried out as stated in Ph. Eur. 2.4.24 (European Pharmacopoeia 10.0). In this research, inductive coupled plasma (ICP) was performed to analyze inorganic impurities. Organic impurities were quantified using HPLC method (Hadzieva Gigovska et al., 2018b).

Results and discussion

The importance of the estimation of MU of the results of MB determination is seen from the definition

*mhadzieva@Alkaloid.com.mk

itself, where it is stated that the determination of the MB is performed taking into account the possibility of analytical error. MU is related to the result of the performed measurement and characterizes the dispersion of the values of the measured quantity.

In order to build a scientific multifactorial strategy following the principles of risk-based approach, during the evaluation of MB, all variables that could have an impact on the result were taken into account, ranked by the intensity and severity and the most risky variables were selected for further study. Based on the risk assessment analysis, several parameters were identified as having the largest impact to mass balance like the sample preparation, problems with quantification and relative responses. Special attention was dedicated to the development, optimization and validation of the HPLC methods used for quantification. All of them include computer-assisted approach which has involved the adaptation of diverse chemometric techniques. In addition, it can be assumed that all impurities have been detected and quantified and that results of the individual techniques have been appropriately combined. Also, chromatographic peak purity, as one of the tools mentioned by ICH to demonstrate specificity, was used (ICH, 2006). Obtained results indicated absence of co-eluting peaks with the main peaks. As additional proof for the separation power of the methods flow injection technique was used comparing the total integrated area of bolus peak with and without the column in place. Differences in relative response factor (RRfs) are perhaps the most common contributor to analytical mass imbalance, so the RRfs were determinate using the ratio of the slope of the calibration curve of each impurity to that of the API. The GC analysis of residual solvents showed that they are present in small quantities (ppm) with a low impact of the final results and are not evaluated further. The obtained results showed that contribution of the results for nonvolatile inorganic, as well as the result for the volatile organic impurities, was minimal and therefore, can be ignored in the final calculation of the MB and MU. To increase the reliability of the information obtained from the measurement results, the MU was determined according to the Eurachem/Citac Guide. In calculation of MU for assay of API “bottom up” approach and for related and degradation products “top down” approach were used. When reporting the results obtained from determining the MB with MU included, an interval (result) is obtained in which the values with a higher degree of confidence (95%) are assumed to be found. All obtained results for MB meet the generally accepted criterion and accordingly the obtained values for combined MU are within ± 2%. According to these results, the uncertainties associated with accuracy and precision were the most significant, contributing to 57% of the overall uncertainty. On the other hand, repeatability of standard peak areas was almost insignificant (less than 5% of overall uncertainty).

The analysis of MU proved to be useful in development of a well-characterized methodology, suitable for determining the MB that integrates scientific and practical knowledge. The ultimate result is an understanding of the risks, and step that need to be followed when evaluating MB, reducing the variability of the critical factors and ensuring the validity of the obtained results.

Conclusion

Implementing MU is one of the approaches that devoutly make scientist to understand the process closely. The study showed that MU can be successfully implemented in MB calculation. Analyst also gains confidence in the obtained results as this approach provides understanding between the variables and performance. The overall advantage of the approach is improved proficiency, reduced variability and gained knowledge.

References

Baertschi, S.W., Pack, B.W., Hyzer, C.S.H., Nussbaum, M.A., 2013. Assessing mass balance in pharmaceutical drug products: New insights into an old topic. TrAC 49, 126-136. https://doi.org/10.1016/j.trac.2013.06.006

Council of Europe. European Pharmacopoeia. Strasbourg: Council of Europe 2001.

Eurachem/Citac Guide CG 4. 2000. Quantifying uncertainty in analytical measurement, 2nd edn). Eurachem/Citac Working Group, London).

Hadzieva Gigovska, M., Petkovska, A., Acevska, J., Nakov, N., Antovska, P., Ugarkovic, S., Dimitrovska, A.,2018a. Comprehensive Assessment of Degradation Behavior of Simvastatin by UHPLC/MS Method, Employing Experimental Design Methodology. International Journal of Analytical Chemistry 2018. https://doi.org/10.1155/2018/7170539

Hadzieva Gigovska, M., Petkovska, A., Manchevska, B., Acevska, J., Nakov, N., Antovska, P., Ugarkovic, S., Dimitrovska, A., 2018b. Chemometrically assisted optimization, development and validation of UPLC method for the analysis of simvastatin. Macedonian pharmaceutical bulletin 64(1), 25-38. https://doi.org/10.33320/maced.pharm.bull.2018.64.01.003

Hokanson, G., 2006. Reconciling Mass Balance in Forced Degradation Studies.

ICH, 2003. Stability Testing of New Drug Substances and Products Q1A(R2).

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 41 - 42 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.016
Short communication

Extensive underreporting and insufficient quality of incident reports received from pharmacists in Croatia from 2012 to 2021

Antonela Simunovic*, Krunoslav Kranjcec, Sinisa Tomic

HALMED, Ksaverska cesta 4, 10000 Zagreb, Croatia

Introduction

Medical devices continue to gain in importance, offering fast-growing technological advances in management of a high variety of conditions. However, every medical intervention is associated with expected risks as well as unforeseen risks, which may lead to serious deterioration in state of health or even death of a patient, user or other person (Kramer et al., 2014; Zippel and Bohnet-Joschko, 2017). Medical device vigilance refers to activities related to collection, assessment, understanding of information as well as responding to new knowledge about the risks arising from the use or application of medical devices, especially adverse incidents, interactions with other substances or products, contraindications, counterfeiting, performance failures and poor construction or design of medical devices. Incident related to medical devices is defined as any malfunction or deterioration in the characteristics or performance of a device made available on the market, including use error due to ergonomic features, as well as any inadequacy in the information supplied by the manufacturer and any undesirable side-effect (MEDDEV, 2013; Medical Devices Act, 2013). Manufacturers, healthcare professionals and lay users participate in the vigilance system. There is no regulatory requirement for healthcare professionals to actively participate in the vigilance system, but their role is key to ensuring the health and safety of patients, users and others. The purpose of the research is to determine the quality of reports of incident reports received at the Croatian Agency for Medicines and Medical Devices by pharmacists from 2012 to 2021.

The quality of the report itself directly affects the course of the manufacturer's investigation of the incident and its outcome, which further has an impact on the safety of patients and users of the medical devices.

Materials and methods

Materials

Study included all incident reports received from pharmacists from year 2012 to 2021. Total number of reports included in this study is 30. All reports that were submitted in any form other than designated incident form were translated into the corresponding form fields before the assessment.

Method

HALMED assessors with relevant experience in vigilance report processing reviewed all incident form fields and assigned them with 1 or 2 points, according to importance of information. Fields were then assessed and scored based on the content of provided information which should be sufficient to allow for further processing of the incident. Final scores were translated to percentages then ranked. Report quality was categorized according to the evaluation system's five levels of classification (total score = 100%): Excellent: total incident report quality evaluation score ≥90: Good: score 80–89; Medium: score 70–79; Qualified: score 60–69; Unqualified: score <60 points.

Results and discussion

Of the 30 reports received, the designated form for incident reporting was used in only 10% of reports, most

*antonela.simunovic@halmed.hr

reports received were on the form for adverse events related to medicines (86.67%) whose fields do not fully correspond to the required data for reporting incidents related to medical devices. The quality of the report reached 100% in only one received report, two reports are at a barely satisfying level of 65% in average, and all other reports were assessed as unsatisfactory with an average score of 35.81%. Thirteen fields of the designated form were recognized as critical in reporting the incident due to the importance of the information, but only four of those fields were satisfactorily populated by an average of 94.58% (commercial name of the medical device, date of occurrence of the incident, description of the incident and patient outcome). The other nine fields falls into the unqualified level of data quality by an average of 13.81%. The outcome of the report in 90% of cases did not result in the initiation of an incident investigation for the following reasons: the report was assessed as the lowest level of risk in the risk assessment process and was recorded in the database after notification of the manufacturer (51.85%); the event does not meet the criteria for reporting according to the medical device legislation (33.33%); insufficient information in the report to initiate further investigation of the incident alongside the unresponsiveness of the reporter for further information collection (11.11%) and inadequate user knowledge (3.7%). The three reports that resulted in the initiation of the incident investigation had the following conclusions: the incident was not related to the medical device, but to the concomitant drug; the incident was due to using the medical device in a manner not in accordance with the manufacturer's specifications and unexpected, but isolated event that the manufacturer will continue to monitor. Most reports were received for medical device class risk IIb (33.33%). For the incident investigation, it is of great importance to keep the medical device involved in the event, but only in 6.67% of reports the medical device was kept in the pharmacy, while in 90% of reports the location of the medical device is unknown. Most reports were received from Zagreb 26.67%, followed by Osijek 10%, and in 30% of cases the city is unknown.

Conclusion

The poor quality of received incident reports can be attributed to the use of the incorrect reporting forms and a lack of education on vigilance and medical devices. Healthcare professionals should be further educated on the vigilance of medical devices and their role in the system, and encouraged to report suspected serious incident at national level in a harmonized manner.

References

European Commission, 2013. MEDDEV 2 12-1 rev. 8 Vigilance, European Commission, (July 2013), pp. 1–64. Available at: https://ec.europa.eu/docsroom/documents/32305/attachments/1/translations.

Kramer, D.B., Tan, Y.T., Sato, C., Kesselheim, A.H., 2014. Ensuring medical device effectiveness and safety: a cross--national comparison of approaches to regulation. Food and drug law journal 69(1), 1–26.

Medical Devices Act (Narodne novine, number 76/13).

Zippel, C. and Bohnet-Joschko, S., 2017. Post market surveillance in the german medical device sector – current state and future perspectives. Health Policy. Elsevier Ireland Ltd, 121(8), 880-886. http://dx.doi.org/10.1016/j.healthpol.2017.06.005

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 43 - 44 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.017
Short communication

Screening the capability of ATR-FTIR for simultaneous quantification of Vitamin B1, B6 and B12 in powder blend

Apostol Todorovski[1*], Marina Petreska[1], Keti Shapkovska[1],
Nikola Geskovski[2], Jelena Acevska[2].

[1]*Bionika Pharmaceuticals, Skupi 57, 1000 Skopje, R.North Macedonia*
[2]*Faculty of Pharmacy, Ss. Cyril and Methodius University, Mother Teresa 47, 1000 Skopje, R.North Macedonia*

Introduction

Fourier Transform Infrared Spectroscopy (FTIR) is a well-established analytical technique, easy-to-use for rapid and non-destructive analysis. Owing to the lack of need for hazardous solvents or reagents in sample preparation, FTIR belongs to the group of environmentally friendly techniques, known as "green analytical techniques" (Fahelelbom et al., 2020). Additionally, the quality of results that this technique produces are comparable to the most powerful techniques, while maintaining extremely low costs for routine analysis and instrument maintenance. The Attenuated total reflectance (ATR) module allows the recording of FTIR spectra of solid and liquid samples directly without any further preparations (Bunaciu et al., 2010).

Coupling FTIR-ATR with smart chemometric tools (Khoshmanesh et al., 2012) such as partial least square regression modeling provides optimum performance for quantitative analysis, besides the rich information provided about the structure of the functional groups of the sample tested. In the case of FTIR-based quantitative analysis, chemometric models are usually built upon predictor variables that are either wavelength-dependent intensities or the linear combination of wavelength-dependent spectral intensities and as such are of immense importance as process analytical tools (PAT) for monitoring content uniformity in solid dosage form production.

The aim of this research was to screen the capability of FTIR-ATR as a PAT for quantification of Vitamins B1, B6 and B12 in a powder blend, using a partial least square (PLS) regression model.

Materials and methods

Materials

The materials used in this study include pure active substances of Vitamin B1 (Thiamine hydrochloride), Vitamin B6 (Pyridoxine Hydrochloride) and Vitamin B12 (Cyanocobalamin). Target concentrations (w/w) for active substances were: 33.311 % for Vitamin B1, 66.622 % for Vitamin B6 and 0.067 % for Vitamin B12.

Equipment and method

For spectrophotometric analysis, a spectrophotometer Bruker FTIR Alpha with an ATR Platinum diamond module was used. The background spectrum within the instrument was recorded prior to the start of each measurement. The powder samples of APIs and matrix formulation samples were measured by placing an approximate quantity of each sample on the surface of the instrument's crystal using a spatula. The ATR pressure arm was lowered to provide good contact between the crystal and sample molecules. In-between each sample application, the ATR crystal was cleaned with a 70% (w/v) aqueous solution of ethanol. Each background and sample measurement was a result of 16 scans in the mid-infrared region (4000–400 cm^{-1}) with a resolution of 4 cm^{-1}. All samples were compressed directly on the ATR crystal with the aid of a standard anvil accessory and all collected data were recorded in absorbance mode, based on the additive nature of Beer's Law. Atmospheric compensation command was applied to eliminate

*apostol.todorovski@gmail.com

disturbing H_2O and/or CO_2 bands in ratio spectra due to different H_2O and CO_2 vapour concentrations in the beam path.

Spectral data were processed using control software Opus 7.5 build, while interpretation of all collected data was carried out by chemometric analysis software SIMCA. The concentration of the Vitamins was employed as a dependent variable (Y), while the corresponding FTIR spectra, as independent (X) variables. All generated spectra were pre-processed with the Standard normal variate (SNV) technique to improve the predictive ability of the chemometric model since optical layer thickness sustainably varies between these types of measurements.

Results and discussion

A series of 22 powder mixtures with varying concentrations from 80 to 120 % of target content for Vitamins B1, B6 and B12 were prepared and analyzed in triplicate (a total of 66 sample scans).

Five main components were employed for building the initial PLS quantification model (R2X=0.78, R2Y=0.86, Q2=0.57). The root mean square error of estimation (RMSEE) were 1.037 % for Vitamin B1, B6 and 0.014% for Vitamin B12. Considering the low target content of Vit. B12, the obtained RMSEE of the quantification model is inappropriate and probably occurs due to the low contact area of the ATR crystal with the analyzed sample which drastically reduces the possibility of scanning a representative sample especially when the analyte is present in such low concentrations. Therefore, it was decided to exclude the content of Vitamin B12 to eliminate its confounding effects in the quantification model. The improved model demonstrated significantly higher R2Y and Q2 (0.96 and 0.82) with RMSEE of 0.56 and 0.55% for Vit B1 and B6, respectively. The VIP plot depicts the main spectral features related to the quantification algorithms for Vit B1 and B6, which aligns with their most prominent spectral bands identified from the spectra of pure substances.

Conclusion

The initial screening experiments revealed the possibilities and drawbacks of the ATR-FTIR in conjunction with PLS modeling as a quantification tool for Vit B1, B6 and B12 in complex powder blends. The low contact area of the ATR crystal clearly poses a limitation for quantitative analysis of low-content compounds (as Vit B12), thus restricting the further development of the model towards the Vit B1 and B6 which produced favorable statistical indicators (high R2Y and low RMSEE). To establish the ATR-FTIR as PAT for powder blend homogeneity monitoring, further experiments using excipients in the powder blends (real production scenario) and a referent analytical technique for quantitative analysis, should be performed.

References

Bunaciu, A., Abou-Enein, H.Y., Fleschin, S., 2010. Application of Fourier Transform Infrared Spectrophotometry in Pharmaceutical Drugs Analysis. Appl Spectrosc Rev. 45, 206–219. https://doi.org/10.1080/05704928.2010.483520

Fahelelbom, K.M.S., Saleh, A., Mansour, R., Sayed, S., 2020. First derivative ATR-FTIR spectroscopic method as a green tool for the quantitative determination of diclofenac sodium tablets. F1000Research 9, 176. http://dx.doi.org/10.12688/f1000research.22274.2

Khoshmanesh, A., Cook, P.L.M., Wood, B.R., 2012. Quantitative determination of polyphosphate in sediments using Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy and partial least squares regression. The Analyst 137(16), .3704. http://dx.doi.org/10.1039/c2an35289c

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 45 - 46 (2022)
Online ISSN 1857 - 8969
DOI:10.33320/maced.pharm.bull.2022.68.03.018
Short communication

Electronic product information (ePI): Expanded access to information on medicines in the European Union (EU)

Izabela Stojchevska*, Natasha Vukikjevikj

Alkaloid AD Skopje, blvd. Aleksandar Makedonski 12, 1000 Skopje, Republic of North Macedonia

Introduction

Product information (PI) of the medicines is regulated, scientifically validated information that assists healthcare professionals (HCPs) in prescribing and dispensing the medicine and informs patients about its safe use. PI comprises the summary of product characteristics (SmPC, intended for HCPs), labelling (packaging information) and package leaflet (PL, intended for patients) (EMA, 2020 a). The development of ePI is intended to improve access to up-to-date PI on medicines when and where it is needed. The European Medicines Agency (EMA), in collaboration with Heads of Medicines Agencies (HMA) and the European Commission (EC), has developed key principles through stakeholder consultations to guide the development and use of ePI in the EU (EMA, 2020 b). The key principles outline: how ePI benefits public health, creates efficiency gains for regulatory systems, aligns with the existing legislative framework and complements the paper package leaflet, fits into the EU's multilingual environment, and interacts with other ongoing digital initiatives at EU and global level. (EMA, 2020 a).

Chronology

In March 2017, EC published a report, which concluded that despite ongoing efforts to make the PI easy-to-read and useful, there is a scope for improvement on how information on medicines is conveyed to patients and HCPs. Consequently, in November 2017, EMA published an action plan to improve the PI for every medicine authorized in the EU (EMA, 2017). One of the crucial areas of this plan is to explore how electronic or digital means can be used to improve accessibility to medicines information by patients and HCPs. It also includes other initiatives: making the PL easier to understand for EU citizens, updating the EU guidance available for companies to prepare the PL, and strengthening patients' input during the preparation of the PL.

Throughout 2018, EMA and the EC organized a multi-stakeholder workshop (bringing together patients, HCPs, the pharma industry, academia, etc.) to develop key principles for the use of electronic formats. The workshop's outcome was a draft proposal for 'key principles' overviewing needs, concerns and a common approach moving forward for everyone involved in ePI. These key principles were the subject of a 6-month public consultation (from January 2019, until July 2019) and now include concluded updates representing EMA-HMA-EC guidance on ePI and form the basis of follow-up implementation plans for ePI (EMA, 2020 b).

In January 2020, key principles for ePI for EU medicines were announced (EMA, 2020 a), followed by the publication of the draft Common EU Standard for ePI on GitHub at the end of May 2021. At the end of September 2021, the proof-of-concept prototype and the common EU standard for ePI were completed (EMA, 2021).

Key principles

ePI is an authorized, statutory PI for medicines in a semi-structured format created using the common EU electronic standard and adapted for electronic handling, which allows spreading via the world wide web, e-platforms, and print. The common EU electronic standard refers to the technical features (including markup language, controlled vocabulary, and interoperability

*istojcevska@alkaloid.com.mk

specifications) agreed by EMA, HMA, National Competent Authorities (NCAs), EC, pharmaceutical industry representatives, and patients and HCPs. ePI will enable the dissemination of the newest, unbiased, regulator-approved PI for all medicines in the EU. ePI will support the provision of the latest information on a medicine's safety, benefits, conditions of use etc., so that the correct information is available to the right HCP/patient at the point of need. ePI will facilitate the creation of PI accessible to everyone, including users with diverse abilities. Accessible formats like large fonts or high screen contrast will provide complete and balanced product information to users in formats suitable for their needs. ePI on the web will be accessible to screen readers, web and mobile applications, convertible to large font and amenable to other accessible formats. ePI will enable increased efficiency in the management of PI during regulatory procedures. By enabling PI changes across all relevant PI annexes and products, ePI could eliminate many manually performed tasks and redundancies that are potential sources of error. ePI will provide information on medicines that is amenable to analysis and could be used to increase knowledge by facilitating the study of characteristics of current EU medicines. ePI does not supersede or negate the pharmaceutical legislation (Article 58 of the Directive 2001/83/EC) to include a PL in the packaging of all medicines or directly convey all information required (by Articles 59 and 62 of the Directive 2001/83/EC) on the outer or immediate packaging. Since the current legislation does not require an electronic version of PI, the use of ePI will not constitute a new legal obligation. ePI is intended to deliver of the complete regulator-approved medicine PI only. It ensures maximum data protection in accordance with Regulation 2016/679 (General Data Protection Regulation) and Regulation 2018/1725 applicable to EU institutions. ePI format will be used for the PI of all medicines authorized in the EU through EMA and NCAs from submission and throughout the evaluation process providing high-level governance. All stakeholders, including pharmaceutical companies and regulators, are expected to commit to implementing the common electronic standard for creating ePI for all EU medicines. However, timelines and processes for implementation will be flexible and amenable to the available resources and priorities at a national level. ePI shall support all official EU languages and Icelandic and Norwegian so that EU citizens can read ePI in their preferred language when authorized ePI in that language is available. ePI will interface and interact with many EU and global initiatives. Related services should work together, within and across organizations or domains.

Application Programming Interface (API)

An API is a set of defined routines, protocols, and tools for building software applications. It expresses a software component in terms of its operations, inputs, outputs, and underlying types. The ePI API is built on the Fast Healthcare Interoperability Resources (FHIR) specification (EMA, 2021). FHIR is an open international modus operandi for exchanging data between different computer systems, published by developing organization Health Level Seven (HL7). FHIR enables a searchable, web-based representation of healthcare information, which can be transferred across devices (Saripalle et al., 2019).

Conclusion

The ePI initiative was launched to support the digital transformation of healthcare across the EU and the commitment laid out by the EC to prioritize innovations that will empower citizens and build a healthier society. It is also in line with EMA's current digitalization efforts to make the best use of available resources and prepare for future challenges. Digital platforms open additional possibilities to disseminate the PI electronically and better meet patients' and healthcare professionals' needs for accessible, trustworthy and up-to-date information on medicines available at the right time.

References

Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use.

EMA, 2017. EMA action plan related to the European Commission's recommendations on product information.

EMA, 2020 a. Electronic product information for human medicines in the EU: key principles.

EMA, 2020 b. Report on public consultation. 'Electronic product information for human medicines in the EU: key principles'.

EMA, 2021. Electronic Product Information (ePI) Standard and API Specification v1. (link: t.ly/llOQ)

Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, and repealing Directive 95/46/EC (General Data Protection Regulation).

Regulation (EU) 2018/1725 of the European Parliament and of the Council of 23 October 2018 on the protection of natural persons with regard to the processing of personal data by the Union institutions, bodies, offices and agencies and on the free movement of such data, and repealing Regulation (EC) No 45/2001 and Decision No 1247/2002/EC.

Saripalle, R., Runyan, C., Russell, M., 2019. Using HL7 FHIR to achieve interoperability in patient health record. Journal of Biomedical Informatics 94, 103188. https://doi.org/10.1016/j.jbi.2019.103188

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 47-48 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.019
Short communication

Optimization for method for elemental impurities in ultra-pure waters

Cvetanka Janakieva Bashevska*, Darko Bachvarovski, Bisera J. Trajkovska, Andrea Alagjozovska, Tatjana Bogovska, Hristina Babunovska

[1] *ALKALOID AD-Skopje, Pharmaceutical, Chemical and Cosmetics Company*
Aleksandar Makedonski 12, 1000 Skopje, North Macedonia

Introduction

Heavy metals are widespread pollutants of great environmental concern as they are no degradable, toxic and persistent. Heavy metals and some trace elements are biologically toxic and can affect and threaten the health of human being.

The elements included in ICH guideline Q3D (R2) on elemental impurities are placed into three classes based on their toxicity (PDE) and likelihood of occurrence in the drug product. The classification scheme is intended to focus the risk assessment on those elements that are the most toxic. The elemental impurity classes are:

Class 1: As, Cd, Hg, and Pb;

Class 2: (*Class 2A)*: Co, Ni and V;

(*Class 2B)*: Ag, Au, Ir, Os, Pd, Pt, Rh, Ru, Se and Tl;

Class 3: Ba, Cr, Cu, Li, Mo, Sb, and Sn.

Other elements: Al, B, Ca, Fe, K, Mg, Mn, Na, W and Zn.

Inductively coupled plasma optical emission spectrometry (ICP-OES) is a technique typically used for the determination of trace metals. The inductively coupled plasma generates excited atoms which emit electromagnetic radiation at characteristic wavelengths for a particular element. These atomic emission lines are sharp and can usually be resolved from other elements. It is a type of emission spectroscopy that uses the inductively coupled plasma to produce excited atoms and ions that emit electromagnetic radiation at wavelengths characteristic of a particular element. The intensity of this emission is in direct correlation with the concentration of the element within the sample.

Water is one of the major commodities used by the pharmaceutical industry. Different grades of water quality are required depending on the different pharmaceutical uses. Control of the quality of water, is a major concern and the pharmaceutical industry devotes considerable resource to the development and maintenance of water purification systems (EMA/CHMP/CVMP/QWP/496873/2018).

This study is aimed at development and optimization of an analytical method for the determination of heavy metals from pharmaceutical water by ICP-OES. ICP-OES is an analytical technique that enables rapid, sensitive multi-elemental determinations. Most trace elements in water are present in low concentrations which approach the detection limit of the instrument.

Materials and methods

In this study several elemental impurities were determined: Class 1(As (188.980 nm), Cd (226.502 nm), Pb (220.353 nm) Hg (184.887 nm)), Class 2A (Co (228.615 nm), V (292.401 nm), Ni (231.604 nm)) and other elements (Al (396.152 nm), Cu (327.395 nm) and Fe (238.204 nm)) at their characteristic wavelengths. Instrument used was Agilent 5100/5110 VDV ICP-OES.

Sequence for analyze included blank, calibration blank, samples (samples of waters from 6 different control points from different systems), 0.01ppm Mercury Standard, 0.01ppm Vanadium standard, 0.01ppm Calibration standard and 0.01ppm Iron standard. All chemicals and reagents used were of analytical reagent grade.

Blank solution: 5% HNO_3.

*cjanakieva@alkaloid.com.mk

Calibration Blank solution: Calibration Blank Solution for ICP-OES (5% HNO_3) is intended for use as a calibration blank solution or zero concentration standard.

Standard preparation: The standard solutions at three concentration levels were prepared from high purity ICP Standards Stock solution.

0.01 ppm calibration solution: In 50 mL volumetric flask add 1 mL of *ICP-OES Wavelength Calibration Solution* (50 mg/L Al, As, Ba, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, SE, Sr, Zn and 500 mg/L K in 5% HNO_3) and dilled to the mark with 5% HNO_3. Dilute 1 mL of this solution to 100 mL with 5% HNO_3.

0.01 ppm Vanadium solution: In 10 mL volumetric flask add 1 mL of *Vanadium ICP Standard* (1000 mg/L V in 2-3% HNO_3) and diluted to the mark with 5% HNO_3. Dilute 1 mL of this solution to 100 mL with 5% HNO_3. Dilute 1 mL of this solution to 100 mL with 5% HNO_3.

0.01 ppm Mercury solution: In 10 mL volumetric flask add 1 mL of *Mercury ICP Standard* (1000 mg/L Hg in 10% HNO_3) and diluted to the mark with 5% HNO_3. Dilute 1 mL of this solution to 100 mL with 5% HNO_3. Dilute 1 mL of this solution to 100 mL with 5% HNO_3.

0.01 ppm Iron solution**:** In 10 mL volumetric flask add 1 mL of *Iron ICP Standard* (1000 mg/L Fe in 2-3% HNO_3) and diluted to the mark with 5% HNO_3. Dilute 1 mL of this solution to 100 mL with 5% HNO_3. Dilute 1 mL of this solution to 100 mL with 5% HNO_3.

Sample preparation: The samples did not require any pre-treatment. Test solution was directly injected. Direct measurement of the ultra-pure water samples.

Alkaloid AD have five systems for production ultra-pure water from different manufacturers. All our ultrapure water systems are suitable for the production of ultrapure water. Systems for production ultra-pure water are USF, Gettinge, Werner, Ion pure and WFI.

Water for analysis was taken from 6 control points from different objects. In all objects water may be present as an excipient or used for reconstitution of products, during synthesis, during production of the finished product or as a cleaning agent for rinsing vessels, equipment, primary packaging materials etc.

Method parameters

The performance characteristics of an ICP is a function of a variety of instrumental parameters. Current instrumentation has many parameters that are fixed by the manufacturer. The purpose of this section is to point out the key parameters that were require adjustment. Instrument method was made with following modifications and adjustments in parameters:

Stabilization time(s): 20, RF power (kW): 1.20, Nebulizer flow (L/min): 0.70, Plasma flow (L/min): 12.0, Aux flow (L/min): 1.00-for viewing mode Axial. Compare with modification and adjustments in parameters for Radial viewing mode: Stabilization time(s): 20, RF power (kW): 1.20, Nebulizer flow (L/min): 0.70, Plasma flow (L/min): 15.0, Aux flow (L/min): 1.20.

ICP Spectrometers are available in radial and axial viewing mode configurations, we choose axial view because that was best choose for determination low concentration recommended from manufacture. Also samples were measured with radial view for comparison. Wavelengths for elemental impurities were same for axial and radial viewing mode measurements.

Results and discussion

Control of elemental impurities is one part of the overall control strategy for a drug product that assures that elemental impurities do not exceed the permitted daily exposure (PDE), i.e the maximum acceptable intake of elemental impurity in pharmaceutical products per day.

ICP-OES can help to examine the purity of ultra-pure water (UPW) down to 10 part per billion (µg/L) and lower. This method detect and quantify elemental impurities Class 1: As, Cd, Pb, Hg; Class 2A: Co, V, Ni and other elements Al, Cu, Fe in water at low concentrations. The concentrations found in all the samples were similar like in blank and Standard solution down to 10 part per billion (µg/L) and lower.

Conclusion

ICP-OES is an excellent analytical technique for detecting a large number of elements in water. All measurements were performed on Agilent 5100/5110 VDV ICP-OES. All results from samples were below 0.01ppm compared with standard solution.

References

EMA/CHMP/CVMP/QWP/496873/2018 Guideline on the quality of water for pharmaceutical use, European Medicines Agency, 20 July 2020, Available at: https://www.ema.europa.eu/en/quality-water-pharmaceutical-use

ICH Harmonised guideline Q3D (R2) on elemental impurities, International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, 22 April 2022, Available at: https://ich.org/page/quality-guidelines

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 49 - 50 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.020
Short communication

Ongoing process verification in Alkaloid

Andrijana Markoska*, Verica Marinova, Ana Sudjukovikj, Marija Trenchevska, Katerina Aleksievska Beldedovska, Elizabeta Karadzinska, Maja Velinovska Cadinoska, Milena Dobrkovic Shotarovska, Andrea Alagjozovska, Gordana Evgenievska Mitrovska, Olivera Paneva

Alkaloid AD, Pharmaceutical,Chemical and Cosmetics Company,
Aleksandar Makedonski 12, 1000 Skopje, Republic of North Macedonia

Introduction

Ongoing process verification (OPV) is documented evidence that the process remains in control during the production of commercial batches. OPV is a program for continuous collection and analysis of in process and finished product quality attributes (QA) and critical quality attributes (CQA) from validated processes for a given product. The data collected have to be analyzed with appropriate statistical tools in order to evaluate the performance of the process. The purpose of OPV is firstly to identify the occurrence of process variability and to monitor degree of variability. Secondly based on the monitoring of deviation management to determine its impact on processes, as well as to control it, which reduces the risks in the processes, improves their stability and capability.

Ongoing process verification in Alkaloid

In order to perform Ongoing Process Verification, the process must be validated, and Quality Attributes and Critical Quality Attributes identified.

An ongoing program to collect and analyze product and process data that relate to product quality must be established.

The data should be statistically evaluated and reviewed by trained personnel. Statistical tools such as Descriptive statistics, Control Charts, and Capability Analysis should be used, where appropriate.

The control chart highlights poor quality by showing when a measurement lies outside the expected variation. More importantly, it shows when a process is trending toward failure.

The established control limits should be within the regulatory specification limits and are used to identify whether the process is in statistical control i.e. no special cause variation.

Multidisciplinary team is responsible for this process. Members of the team are from Quality Assurance Department, Production department, Research and Development Department and Quality Control Department.

The team is responsible for preparing the OPV plan and the OPV Report.

OPV Plan contains description of the process with the respective manufacturing flow charts. In OPV plan are presented parameters of the product which are going to be monitored and also quality attributes and critical quality attributes. Sampling plan as well as sampling frequency and sampling procedures, test methods and equipment used are specified in the OPV Plan.

OPV Report is prepared according previously made OPV Plan. The objective is to monitor the results, and to collect more knowledge for process performance, process variability and its trends. Statistical process analysis of QAs and CQAs is performed. The purpose is to assure that the manufacturing process remains in a state of control during the production of commercial batches and that the manufacturing process is capable of consistently yielding a product of reproducible quality. If there is need for improvement of the process, it should be noted in the conclusion of the Report.

*amarkoska@Alkaloid.com.mk

Regulatory Requirements

Regulatory requirements are specified in respective EU and FDA guidelines.

In Annex 15 Qualification and validation of EU GMP guideline is stated that *Ongoing Process Verification* should be used throughout the product lifecycle to support the validated status of the product as documented in the Product Quality Review.

There is very tight relation between Product Quality Reviews (PQRs) and OPV Reports. Although PQRs are made for all authorized medicinal products annually, the need for continuous monitoring of the process has huge meaning in detecting trends in a timely manner. In fact PQR should summarize conclusions obtained during OPV.

FDA Guidance for Industry Process Validation defines Stage 3 of Process validation — *Continued Process Verification*. The goal of the third validation stage is continual assurance that the process remains in a state of control (the validated state) during commercial manufacture.

The collection and evaluation of information and data about the performance of the process, will allow detection of undesired process variability.

Evaluating the performance of the process identifies problems and determines whether action must be taken to correct, anticipate, and prevent problems so that the process remains in control.

The production data should be collected to evaluate process stability and capability. The quality unit should review this information. If properly carried out, these efforts can identify variability in the process and/or signal potential process improvements. A process is likely to encounter sources of variation that were not previously detected or to which the process was not previously exposed. Data gathered during this stage might suggest ways to improve and/or optimize the process by altering some aspect of the process or product, such as the operating conditions (ranges and set points), process controls, component, or in-process material characteristics.

All guidelines follow a few principles:

- This process should be conducted under an approved protocol or equivalent documents and a report should be prepared to document the results obtained;
- Results of parameters identified as quality attribute or as critical quality attributes should be trended and checked to make sure they are consistent with each other;
- Out of trend results or significant atypical trends should be investigated;
- Statistical tools should be used, where appropriate;
- OPV can be established for new products and existing/legacy products.

Conclusion

Increased knowledge of the products and/or processes obtained by the collection and evaluation of OPV data provides regulatory and business value to pharma manufacturers. Implementing the OPV process gathers a large amount of data for the product, an organizational asset that leads to continuous improvement, a reliable supply chain, reduced regulatory and compliance risk, reduced cost of quality, and a better manufacturing plan.

It is very important to emphasize detecting any atypical trends through continuous monitoring in order to prevent failure in the future. The main purpose of OPV process is in detecting atypical trends in early stage.

The OPV process in fact gives our organization the opportunity to understand its production process in greater depth and to improve the quality of its products over time. Thus fulfilling the basic principle of the pharmaceutical profession, to serve patient safety.

References

EudraLex, 2013. The rules governing Medicinal Products in the European Union, Volume 4: Good Manufacturing Practice, Medicinal Products for human and veterinary use, Chapter 1 Pharmaceutical Quality System. European Commission Health and Consumers Directorate – General. Available at: https://eur-lex.europa.eu/

EudraLex, 2015. The rules governing Medicinal Products in the European Union, Volume 4: Good Manufacturing Practice, Medicinal Products for human and veterinary use, Annex 15 Qualification and Validation. Available at: https://eur-lex.europa.eu/

EMA, 2016. Guideline on process validation for finished products-information and data to be provided in regulatory submission, EMA/CHMP/QWP/BWP/70278/2012-Rev1 Corr.1.

FDA, 2011. Guidance for Industry: Process Validation: general Principles and Practices, CGMP, revision 1, Food and Drug Administration, U.S. Department of Health and Human Services

ISPE, 2019. Good Practice Guide: Practical Implementation of the Lifecycle Approach to Process Validation, International Society for Pharmaceutical Engineering, USA

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 51 - 52 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.021
Short communication

Quality management system and corporate quality assurance

Elizabeta Karadžinska[1], Marija Davčeva Jovanoska[1], Maja Velinovska Čadinoska[1], Verče Jovanovska Jankovska[1], Sonja Spirovska Burčevska[1], Nina Ekart Oman[2], Viška Miceska[3], Gabriela Gjorgjievska[4], Nada Popstefanova[1], Olivera Paneva[1]

[1] *ALKALOID AD Skopje, Blvd. Aleksandar Makedonski 12, 1000 Skopje, Republic of North Macedonia*
[2] *Alkaloid INT, Slandrova ulica 4, 1231 Crnuče, Ljubljana, Republic of Slovenia*
[3] *Alkaloid d.o.o.Beograd, Prahovska 3 11000 Belgrade, Republic of Serbia*
[4] *Alkaloid KONS dooel, Blvd. Aleksandar Makedonski 12, 1000 Skopje, Republic of North Macedonia*

Introduction

Quality Management is a wide-ranging concept, which covers all matters, which individually or collectively influence the quality of a product. It is the sum total of the organized arrangements made with the objective of ensuring that medicinal products are of the quality required for their intended use.

Quality management system (QMS) in Alkaloid as part of Integrated management system (IMS) is comprehensively designed and correctly implemented on corporate level incorporating Good Manufacturing Practice and Quality Risk Management. It is fully documented and its effectiveness continuously monitored.

Organizational structure

ALKALOID AD Skopje is a shareholding company, consisting of two profit centers – Pharmaceuticals and Chemistry Cosmetics Botanicals and the Corporate Services. The company has 2 subsidiaries in Republic of North Macedonia and 17 subsidiaries and 3 representative offices abroad.

The complex organizational structure presents challenge for maintaining and continuous improvement of a shared corporate Quality Management System.

Standards

Pharmaceutical Quality System of ALKALOID AD Skopje – PC Pharmaceuticals is based on cGMP for medicinal products for human use and quality risk management, as well as international standards ISO 9001, ISO 14001 and ISO 45001 for all products of PC Pharmaceuticals, ISO 13485 (for in-license medical devices) and HACCP (for food supplements).

Quality Management System for of all subsidiaries is based on:

- Identification and interpretation of relevant national and international laws, regulations, directives and guidelines;
- Stipulate and implement internal requirements to ensure compliance;
- Risk management;
- Information management;
- Quality improvement strategies for regulated activities and processes, products, systems and services;
- Authorization of regulated documents where required;
- Change and deviation system together with corrective actions and improvement actions;
- Trending;
- Stipulating the understanding of the processes;
- Surveillance of quality improvement activities for compliance;
- Ensure that quality aspects are addressed when cooperating with external parties;

*ekaradzinska@alkaloid.com.mk

- Defined responsibilities and quality goals throughout the whole lifecycle of the activity;
- Effectiveness of Quality Management System implemented in Alkaloid.

Corporate quality assurance

Team for corporate QA function with Quality Assurance members from the head office Alkaloid AD Skopje and the major subsidiaries: Alkaloid d.o.o. Belgrade, Alkaloid-INT d.o.o. Ljubljana, Alkaloid KONS DOOEL Skopje has the responsibility for implementation of Quality Management System of Alkaloid on corporate level in the subsidiaries through creating corporate QA Policies and support in their implementation. The team operates according to annual plan and set objectives, presented in annual Plan of activities for Quality Assurance. Achieved results (objectives and measured KPIs) are presented in annual report. The corporate QA is responsible for the performance and effectiveness of the QMS in accordance with the corporate policies.

Documentation system

Appropriate hierarchy of QMS documentation is established on four levels. Corporate (global) documents are first and second level documents.

Manual for integrated management system

Manual for IMS of Alkaloid is a first level corporate document which defines and documents the structure of the Integrated Management System and all operations which refer to the quality of Alkaloid's products.

Corporate policies for quality assurance

Corporate policies for quality assurance (Corporate QA Policy) are first level document, which in standard manner define the global framework and structural elements of a certain QA processes to be subsequently implemented on local level.

Corporate procedures

Corporate procedures are second level documents in IMS which define certain process and the process flow as well as responsibilities for all activities from the process.

Local standard operating procedures

In line with the corporate QA policies and procedures, local standard operating procedures (SOPs) are created or modified to integrate the corporate requirements but also include local specifics from the national legislation in the country the subsidiary is located.

Technical Agreements

Technical agreements are signed between the head office and each subsidiary of Alkaloid covering the responsibilities for adherence to the corporate policies and procedures, the outsourced activities, the products or operations to which they are related, and any technical arrangements

Self inspections

Self inspections are conducted by the corporate QA team in order to monitor the implementation and compliance with the corporate QA Policies and to propose necessary corrective and improvement measures.

Conclusion

Introduction of quality assurance processes on corporate level in the company is of great benefit in aligning and maintenance of a common Quality Management System in Alkaloid and compliance with all relevant regulatory requirements, applicable international standards and internal requirements.

References

Eudralex, 2013. The rules governing Medicinal Products in the European Union, Volume 4: Good Manufacturing Practice, Medicinal Products for human and veterinary use, Chapter 1 Pharmaceutical Quality System. Available at: https://ec.europa.eu/health/system/files/2016-11/vol4-chap1_2013-01_en_0.pdf

Eudralex, 2013. The rules governing Medicinal Products in the European Union, Volume 4: Good Manufacturing Practice, Medicinal Products for human and veterinary use, Chapter 9 Selfinspections. Available at: https://ec.europa.eu/health/system/files/2016-11/cap9_en_0.pdf

ICH Harmonized guideline Q10 on pharmaceutical quality system. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, 04 June 2008, Available at: https://www.ema.europa.eu/en/documents/scientific-guideline/international-conference-harmonisation-technical-requirements-registration-pharmaceuticals-human_en.pdf

ISO 9001:2015 Quality Management systems. Available at: https://www.iso.org/standard/62085.html

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 53 -54 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.022
Short communication

Risk assessment study of potential elemental impurities in montelukast film coated tablets

Stefan Angelevski[1*], Irena Slaveska Spirevska[1], Elena Lazarevska Todevska[1], Tanja Bakovska Stoimenova[1], Marija Glavash Dodov[2], Maja Simonoska Crcarevska[2], Renata Slaveska Raichki[2]

[1]Replek Farm Ltd., Kozle 188, 1000 Skopje, N. Macedonia
[2]Faculty of Pharmacy, Ss. Cyril and Methodius University in Skopje, Mother Teresa 47, 1000 Skopje, N. Macedonia

Introduction

The elements listed in the ICH guideline Q3D are classified into three main categories (1, 2A, 2B and 3) according to their toxicity (PDE - established permitted daily exposure), likelihood of occurrence in the final drug product and their toxicity considering the particular route of administration (EMA ICH Q3D Elemental Impurities, 2019). Development of risk-based control strategy for potential elemental impurities (EIs) assessment in drug development and manufacturing is challenging for the pharmaceutical industry since there are multiple potential sources of EIs contamination (Jenke et al., 2015; Li et al., 2015; Rowe et al., 2009). In general, the EIs risk assessment approach, as an integral part of the overall drug product control strategy, involves four steps: 1) identification of known and potential sources of EIs, 2) evaluation of observed or predicted levels of EIs in drug product, 3) comparison of evaluated levels of EIs with PDE values and 4) definition of the control strategy. The drug product risk assessment study is also focused on impurity levels determination and assessing the levels of EIs in relation to the established PDEs (Ph. Eur. 10th Edition, 2019; Teasdale et al., 2015).

The global impact of the COVID-19 pandemic has urged the pharmaceutical industry to provide a worldwide supply of drugs that comply with legislation and international safety standards. One of the drugs that gained attention in COVID-19 treatment management is montelukast (Khan et al., 2022).

As acknowledged, montelukast is a selective cysteinyl leukotriene 1 (CysLT1) receptor antagonists (LTRA) with bronchodilator effect. Montelukast 10 mg film-coated tablets are indicated in the treatment of mild to moderate persistent asthma and can be added to another patient's existing treatment regimen for asthma (Wermuth et al., 2021).

The aim of this research is to conduct detailed EIs risk assessment study of Montelukast 10 mg film coated tablets in accordance with the ICH guideline Q3D on EIs.

Materials and methods

The method used for determination of elements (Cd, Pb, As, Hg, Co, V and Ni) from class 1 and class 2A, was inductively coupled plasma-mass spectrometry (ICP-MS) system (Agilent 7500 Series). The analyses were carried out on montelukast sodium (active pharmaceutical ingredient-API), finished dosage form (Montelukast 10 mg film coated tablets) and placebo dosage form. Each film-coated tablet contains montelukast sodium 10.4 mg equivalent to 10 mg montelukast. Moreover, data and specific evidences were reviewed from three commercial batches of the montelukast 10 mg film coated tablets manufactured in three successive years.

The manufacturing equipment integrated in drug production process consists exclusively of stainless steel (mainly 316L grade steel). [Internal data of Manufacturer).

Results and discussion

For this risk assessment study, the potential contribution from the API, excipients, manufacturing equipment, container closure system and used utilities are

*stefan.angelevski@replek.mk

considered in order to determine the overall contribution of EIs to the finished drug product. Thus, taking into consideration the manufacturing of Montelukast 10 mg film coated tablets and life cycle management, there are several broad categories of potential sources of elemental impurities. The quality procedures that ensure control of EIs include: equipment design and installation qualification, compatibility studies for the production process, equipment cleaning verification and validation, and visual inspection/line clearance procedures.

The control of the manufacturing equipment is done according to the standard operating procedures and manuals, and the risk assessment is represented with a risk factor number to identify the critical steps in which EIs could be present in the finished drug product.

The PDE values of potential elemental impurities have been calculated for the oral routes of administration. These PDE values were established following element-specific health-based risk assessments, which are available in the ICH Q3D guideline. Since the PDE reflects only the total exposure of the drug product, for the objectives of this study, PDE values were converted into concentrations as a tool in evaluating elemental impurities in the drug product of interest. The calculations are based on the following assumptions: a) the maximum concentration level (MCL) of particular elemental impurity in 1g of the product is calculated using the maximum daily dose of product, b) the control threshold (CT) is established as 30% of the MCL value.

The obtained results show that concentration levels of all examined elements are well below the CT value which is defined as 30% of the maximum concentration level of particular EI in the drug products. Based on ICH Q3D guideline, the results for EIs Class 1 and Class 2A showed that EI levels are well below the ICH Option 1 oral and parenteral limits.

If any changes are introduced to the manufacturing process or components of the drug product across the life-cycle, the risk assessment should be reviewed and existing controls may need to be re-evaluated.

Conclusion

The outcome of this risk assessment study of potential EIs in Montelukast 10 mg film coated tablets is that no additional controls are required, since the current control strategy developed for the raw materials, finished dosage form and manufacturing process are sufficient to guarantee that the levels of EIs are consistently below their PDE values. It also confirmed that the current quality system has been designed to prevent, minimize and control any potential EIs contribution from the manufacturing equipment and utilities.

Additional considerations of the container closure system ascertain that it meets specific requirements regarding the EIs. Furthermore, it is recognized that the probability of elemental leaching into this solid dosage forms does not require additional step in the risk assessment.

Overall conclusions based on the conducted risk assessment study, as well as testing results are the following: the concentration of EIs is controlled within the acceptable limits and there is no risk associated to EIs for patients taking Montelukast 10 mg film coated tablets according to patient information leaflet.

References

EDQM, 2020. European Pharmacopoeia (Ph. Eur.) 10th Edition EDQM - European Directorate for the Quality of Medicines.

European Medicines Agency (EMA), 2015. Committee for Human Medicinal Products. ICH guideline Q3D on elemental impurities. 28 March 2019 EMA/CHMP/ICH/353369/2013.

Internal data of Manufacturer (available if necessary): statements received from suppliers, manufacturing instructions, packaging instructions, specifications, results of analysis.

Jenke, D.R., Stults, C.L.M., Paskiet, D.M., Ball, D.J., Nagao, L.M., 2015. Materials in Manufacturing and Packaging Systems as Sources of Elemental Impurities in Packaged Drug Products: A Literature Review. PDA J. Pharm. Sci. and Tech. 69, 1-48. https://doi.org/10.5731/pdajpst.2015.01005

Khan, A.R., Misdary, C., Yegya-Raman, N., Kim, S., Narayanan, N., Siddiqui, S., Salgame, P., Radbel, J., De Groote, F., Michel, C., Mehnert, J., Hernandez, C., Braciale, T., Malhotra, J., Gentile, M.A., Jabbour, S.K., 2021. Montelukast in hospitalized patients diagnosed with COVID-19. J Asthma. 59(4), 780-786. https://doi.org/10.1080/02770903.2021.1881967

Li, G., Schoneker, D., Ulman, K.L., Sturm, J.J., Thackery, L.M., Kauffman, J.F. 2015. Elemental Impurities in Pharmaceutical Excipients. J Pharm Sci. 104, 4197-4206. https://doi.org/10.1002/jps.24650

Rowe, R.C., Sheskey, P.J., Quinn, M.E. 2009. Handbook of Pharmaceutical Excipients. 6th ed.. Pharmaceutical Press.

Teasdale, A., Chéry, C.C., Cook, G., Glennon, J., Lee, C.W., Harris, L., Lewen, N., Powell, S., Rockstroh, H., Rutter, L., Smallshaw, L., Thompson, S., Woodward, V., Ulman, K. 2015. Implementation of ICH Q3D Elemental Impurities Guideline: Challenges and opportunities. Pharmaceutical Technology 39(3).

Wermuth, H., Badri, T., and Takov, V., 2021. Montelukast. Available at: https://www.ncbi.nlm.nih.gov/books/NBK459301/

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 55 – 56 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.023
Short communication

Risk evaluation and assessment for the presence of nitrosamine impurities in Alkaloid Losartan film coated tablets

Toni Camurovski, Vanja Bobevska, Sonja Spirovska Burčevska, Jasmina Kimovska, Nada Popstefanova, Verče Jovanovska Jankovska, Elizabeta Karadžinska, Olivera Paneva, Ksenija Brzilova, Maja Velinovska Cadinovska

ALKALOID AD Skopje, Blvd. Aleksandar Makedonski 12, 1000 Skopje, Republic of North Macedonia

Introduction

Nitrosamines are a class of chemical compounds, classified by the International Agency for Research on Cancer as group 2A carcinogens, i.e potential genotoxic carcinogens, that were first described in the chemical literature over 100 years ago. Since in 2018 high levels of nitrosamines were detected in valsartan, they are once again subject of research (EMA/425645/2020, EMA/526934/2019, EMA/409815/2020 Rev.9).

Currently identified risk factors for N-nitrosamine impurities in medicinal products are grouped in the following categories: Risk factors related to the manufacture of the active substance; Risk factors also related to the finished product; and Risk factors related to Good Manufacturing Practice (GMP) aspects.

According Art. 5(3) referral, Marketing Authorisation Holders (MAH) are requested to evaluate the risk of the presence of nitrosamine impurities in human medicinal products containing chemically synthesized Active pharmaceutical ingredients (APIs) or biological APIs (CMDh/412/2019, Rev.16). This evaluation should be performed in 3 sequential steps: (i) Risk evaluation, (ii) Risk confirmation test, (iii) Changes in Marketing authorisation (MA)

MAH must introduce the following specifications: limits for NDMA (96 ng/day) and NDEA (26.5 ng/day). Omission from the specification is only justified if it can be shown that the levels of the respective N-nitrosamines are consistently < 10% of the limit defined above and the root cause is identified and well-understood.

Materials and methods

PHA (Preliminary hazard analysis) tool was used for the risk evaluation to describe the risks identified through all Losartan film-coated tablets phases regarding the potential N-nitrosamine impurities formation.

The following risks were evaluated:

- Risks related to API Losartan Potassium synthetic route
- Risks related to the finished drug composition (excipients and primary packaging material)
- Risks related to the manufacturing process
- Risk of N-Nitrosamines formation during shelf life

Results and discussion

Risks related to API Losartan Potassium synthetic route

In the production of Alkaloid Losartan film coated tablets the API Losartan Potassium is used from two different manufacturers.

Both manufactures have proven that their current synthetic route has low probability to form NDMA, NDEA or other related nitrosamines. This was confirmed with test result using validated GC-MS/MS method in which nitrosamines were not detected.

According to current guidelines, the following nitrosamines are controlled in the specification of the API manufacturers: NDMA, NDEA and NDBA with a limit of Not More Than (NMT) 0.03 ppm.

*tcamurovski@Alkaloid.com.mk

Based on the reviewed data the risk for formation of N-Nitrosamines from the API was identified as low

Risks related to the finished drug composition (excipients and primary packaging material).

Regarding the possible contamination coming from excipients and the process aids, none of the used excipients or process aids does not include nitro molecular species in their structure which are prerequisite for N-nitrosamine formation and all of the used excipients are of a well-established and long history of usage in pharmaceutical products. The evaluation was corroborated by the statements and evaluation provided by the excipients manufacturers.

The possible contamination coming from the primary packaging material was also evaluated. The primary packaging used for Losartan film-coated tablets is composed of Blister: PVC and aluminum foil. Although during sealing process temperatures of 180 °C or more are applied to the outside, time period of sealing is very short, normally less than one second. In addition, aluminum foil represents an absolute migration barrier and as soon as the lidding foil is sealed onto the blister material a migration of substances through the foil is not possible anymore and formation of nitrosamines is not expected during the packaging process.

Based on the reviewed data the risk for formation of N-Nitrosamines from the excipients or the primary packaging material was identified as low

Risks related to the manufacturing process

Since there was low risk of N-Nitrosamine formation and presence in regards of the excipients used and production process, where no nitrosating compound is introduced into the process, we considered that the risk for N-Nitrosamine formation in regards to the critical manufacturing process phases and conditions is also low.
Cross-contamination is prevented by implemented organizational and technical measures and is regularly monitored. There are written procedures for cleaning of all product surfaces and cleaning validation is performed in order to confirm the effectiveness of the cleaning procedures. Cleaning agents used for the manufacturing equipment do not contain any substances which form nitrosamines. Therefore, the risk from cross-contamination was considered low.

Risk of N-Nitrosamines formation during shelf life

In regards to shelf life, since no risk of N-Nitrosamines was identified for: API, raw materials (excipients and primary packaging), process equipment, critical process conditions and cross contamination, to the best of our knowledge we considered that there is low risk of N-nitrosamines formation during shelf life of the product.

Due to the conducted risk analysis where only low risk was identified per each evaluated element (API, excipients, manufacturing process, packaging and shelf-life) and overall low risk of N-nitrosamine presence or formation is assigned for Losartan film-coated tablets.

Risk assessment (confirmation test) on the finished product

Testing of Losartan film-coated tablets was performed using very selective and sensitive Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) method. Batches produced using API from both manufacturers were used for testing. The following criteria for the limit of NDMA and NDEA were established, based on ICH M7(R1) principles: NDMA $\leq$ 0.640 ppm; NDEA $\leq$ 0.177 ppm.

The results of the analyzed batches complied with the established specification. Results for NDMA and NDEA were “not detected”. (Limit of quantification of the method was established as $<$ 0.009ppm)

Conclusion

Based on the conducted risk analysis where only low risk was identified per each evaluated element (API, excipients, manufacturing process, packaging and shelf-life) overall low risk of N-nitrosamine presence or formation is assigned for Losartan film-coated tablets.

References

CMDh/412/2019, Rev.16 (CMDh practical guidance for Marketing Authorisation Holders of nationally authorised products (incl. MRP/DCP) in relation to the Art. 5(3) Referral on Nitrosamines

EMA/425645/2020 European Medicines Regulatory Network approach for the implementation of the CHMP Opinion pursuant to Article 5(3) of Regulation (EC) No 726/2004 for nitrosamine impurities in human medicine

EMA/526934/2019 Lessons learnt from presence of N-nitrosamine impurities in sartan medicines

EMA/409815/2020 Rev.9 (Questions and answers for marketing authorization holders / applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 57 - 58 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.024
Short communication

Risk Management and Business Continuity of Alkaloid AD Skopje

Nada Popstefanova*, Verče Jovanovska Jankovska, Sonja Spirovska Burčevska, Jasmina Kimovska, Vanja Bobevska, Olivera Paneva, Elizabeta Karadžinska, Toni Camurovski, Ksenija Brzilova, Maja Velinovska Cadinovska, Sanja Uskokova

ALKALOID AD Skopje, Blvd. Aleksandar Makedonski 12, 1000 Skopje, Republic of North Macedonia

Introduction

Risk management is one of the foundations of a pharmaceutical quality system and is an integral part of the processes in a company. The purpose of quality risk management is to provide evidence-based information and analysis to make informed decisions and improve processes in the pharmaceutical quality system.

The risk management process is an essential element for establishing a proactive approach that will ensure continuity of operation and readiness of the company to anticipate, detect and respond to negative impacts that have the potential to prevent the execution of critical processes of the company, achieving its goals and meeting the requirements of stakeholders for quality.

Risk Management at Alkaloid AD Skopje

Risk management in Alkaloid AD Skopje is based on Good Manufacturing Practice, guidelines and examples of risk management tools defined in the ICH Q9 Risk Management Guide, requirements of the ISO 31000 standard Risk Management System, application guides and other standards that the company applies. The risks managed by Alkaloid AD Skopje are integrated in all business and production processes.

Risk and continuity management is under the authority of the Risk Management, Crisis Management and Continuity Management Board, the Risk and Continuity Management Team and the Heads of Organizational Units / Process Owners.

Continuity management is established by the standard ISO 22301 Continuity system requirements. The main elements according to which the process of continuity of work is based are the following:

- operational planning and control,
- business impact analysis and risk assessment,
- strategy for business continuity,
- business continuity plan,
- programs for testing and practicing the effectiveness of the strategy for continuity of work and
- evaluation of the established documentation and the ability of the established continuity in operation.

Company activities related to risk management

Risk management is a systematic process for identifying, analyzing and assessing whether the risk should be modified, eliminated or reduced to an acceptable level. This process requires a defined communication, ongoing monitoring and verification of the risks identified to establish control of the modified risks and avoid/reduce negative effects.

All activities in the company /organization include risks. Risk management can be applied throughout the organization in all areas and at all levels, at any time and for specific functions, projects and activities.

Risk determination in Alkaloid AD is performed at the level of organization, PC / program (in terms of context), process level, and activity/product. Types of risks processed in Alkaloid AD are strategic risks, process (operational) risks, and quality risks specific to the purpose of the product, process, equipment and more.

All applicable methodologies described appropriately in the quality system can be used for risk evaluation. According to the established Risk Management

*nadapopstefanova@Alkaloid.com.mk

Procedure, the methodologies used and recommended in the risk evaluation are FMEA and HACCP methodologies.

The FMEA methodology is based on three parameters: severity, probability and detection. Risk priority categorizes into four categories: low, medium, high, and critical risk. Each category has defined how to take measures to prevent and reduce risks.

The HACCP methodology is based on two parameters: severity and probability, and defining critical control points according to the decision tree. The categorization and the manner of taking measures are identical to the FMEA methodology. The Strategic Risk Register and the Quality Risk Register (process operational and product-specific risks) are used as a tool to support the process. The registers provide a comprehensive overview of risks, timely identification and management of factors that may affect the realization of the goals and strategy of the company.

By continuously reviewing the risks, the probability of occurrence of risks is reduced, the severity of the consequences of risks is reduced, and measures are taken to raise awareness, readiness, management and maintenance of risks at an acceptable level.

In 2021, at the Alkaloid AD Skopje level, 1778 risks were re-examined, of which 169 risks were reduced (30%), and 249 new risks were identified.

For PC Pharmacy in 2021, a total of 851 risks from 46 production processes were re-examined, 135 additional measures were defined, 51 risks were reduced (30%), and 41 new risks were identified.

Specific risks to the pharmaceutical industry that have been challenging in operation are the potential presence of N-Nitrosamines in the finished product, the Covid-19 pandemic, and the disrupted political, security, and economic situation in Ukraine and Russia.

For all affected products of Alkaloid AD Skopje, a risk analysis was prepared through a decision tree and a risk report by the regulatory requirements.

At the beginning of the Covid-19 pandemic, Alkaloid AD Skopje developed a Business Continuity Plan, a pandemic with a clearly defined plan of activities and measures that are taken to ensure continuity of operations. According to the Business Continuity Plan, each service illustrates its List of actions according to the processes, employees and location.

A new strategic risk has been opened for the disturbing political, security and economic situation in Ukraine and Russia. A Risk Plan has been prepared with a defined plan of activities and measures taken to ensure safety and continuity of operations.

Conclusion

Risk management is a part of the quality management processes, products and pharmaceutical quality systems. The output/results of the risk management process should be reviewed at least annually and take into account the information from other processes as context (weakness, threats), internal/external audits/inspection, the change control, product quality review (PQR), post-marketing surveillance of medical devices, clinical studies results, pharmacovigilance, medical device vigilance, complaints and recalls of the product from the market.

The identification and analysis of risks are performed until their complete reduction/elimination to an acceptable level and have a special significance in the processes related to the stakeholders.

References

Commission directive 2003/94/EC - The principles and guidelines of good manufacturing practice in respect of medicinal products for human use and investigational medicinal products for human use. Available at: https://eur-lex.europa.eu/

Commission directive 2001/83/EC - Guidelines on Good Distribution Practice of Medicinal Products for Human Use (2015/C 95/02). Available at: https://eur-lex.europa.eu/

Commission Directive 93/42/EEC on Medical Devices amended by Directive 2007/47/EC and Medical Device Regulation (EU) 2017/745. Available at: https://eur-lex.europa.eu/

Eudralex. The rules governing Medicinal Products in the European Union, Volume 4 Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use. Available at: https://health.ec.europa.eu/medicinal-products/eudralex_en

ICH Harmonized guideline Q9 on Quality Risk Management, International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, January 2006, Available at: https://ich.org/page/quality-guidelines

ISO 22301:2019 Security and resilience - Business continuity management systems. Available at: https://www.iso.org/standard/75106.html

ISO 31000 – Risk management – Principles and guidelines (valid version) Available at: https://www.iso.org/iso-31000-risk-management.html

ISO 31010 – Risk management – Risk Assessment Techniques (valid version). Available at: https://www.iso.org/standard/72140.html

ISO 14971 – Application of risk management to medical devices (valid version). Available at: https://www.iso.org/standard/72704.html

ISO Guide 73 Risk management – Vocabulary. Available at: https://www.iso.org/standard/44651.html

ISO 22716 - Cosmetics - Good Manufacturing Practices (GMP) (valid version) Available at: https://www.iso.org/standard/36437.html

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 59 - 60 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.025
Short communication

Root causes for presence of nitrosamine impurities in active pharmaceutical substances and finished pharmaceutical products

Milena Prculovska[1*], Jelena Acevska[2], Ana Poceva Panovska[2], Natalija Nakov[2], Aneta Dimtirovska[2], Katerina Brezovska[2]

[1] *REPLEK FARM Ltd., Quality Control Department, Kozle No. 188, 1000 Skopje, R.N. Macedonia*
[2]*Institute of Applied Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy, University Ss. Cyril and Methodius, Majka Tereza 47, 1000 Skopje, R.N. Macedonia*

Introduction

Nitrosamines, such as NDMA (*N*-nitrosodimethylamine), NDEA (*N*-nitrosodiethylamine) and NMBA (*N*-nitroso-*N*-methyl-4-aminobutyric acid), are organic compounds containing the nitroso functional group. According to ICH M7 (R1), nitrosamines are identified as a "cohort of concern" or Class 1 impurities (known mutagenic carcinogens). The International Agency for Cancer Research (IARC) categorized nitrosamines as "probably carcinogenic to humans" (2A-group) (ICH M7 (R1), 2018).

Detection of nitrosamines impurities in valsartan, in July 2018, actualized the possible presence of these impurities in other active substances and finished pharmaceutical products (EMA, 2018). The presence of nitrosamines impurities has also been found in other Angiotensin II receptor blocker (ARB) active pharmaceutical ingredients, containing a tetrazole ring in their structure such as losartan and irbesartan. More recently, *N*-nitrosodimethylamine have been identified in certain histamine-2-blockers (ranitidine, nizatidine) and in an antihyperglycemic medication metformin. Since then, more active substances and medicinal product batches contaminated with nitrosamines have been recalled (EMA, 2019).

Root causes for presence of nitrosamine impurities

After the recall of numerous batches of sartans and ranitidine, the question is: what are the main routes of N-nitrosamine contamination of active substances and finished products? The first route is the use of contaminated starting material in the manufacturing process, such as solvents, reagents, catalysts as well as recycled materials. The second source of contamination is formation of nitrosamine impurities during manufacturing process from an intermediate or from the active ingredient itself (EMA, 2020). Theoretically, the formation of nitrosamine impurities in pharmaceuticals is possible to occur when a precursor amine coexists with a nitrosating agent under suitable conditions for the reaction of nitrosation, such as acidic conditions for nitrite.

The tetrazole ring appear to be of key importance for desired angiotensin receptor antagonist effects of sartans at the molecular level and is present in five of eight sartans that have been widely marketed. The process of tetrazole ring formation at the very end of the synthesis has been proposed to be the root of nitrosamines contamination of sartans. The synthesis of the tetrazole ring involves the reaction of nitrile with organic azides, most commonly tributyltin azide (Bu_3SnN_3), sodium azide (NaN_3) and trimethyltin azide (Me_3SnN_3) (Baumann et al., 2011).

In case of valsartan, data show that its tetrazole ring was formed using Bu_3SnN_3 as the azide source and *N,N*-dimethylformamide (DMF) as a solvent, the other necessary factor that contributed to valsartan contamination with nitrosamines. Due to their human toxicity potential, more amount of azides remaining after the formation of the tetrazole ring was quenched with nitrite under acidic conditions. Dimethylamine as a secondary amine present either as an impurity or as a possible degradation product of DMF, reacts with nitrite and serves as a amine source for *N*-nitrosidimethylamine

*milena.prculovska@gmail.com

(NDMA), the first nitrosamine that led to valsartan recalls (Snodin and Elder, 2019). Similarly, the presence of NDEA and NMBA in valsartan is the result of a reaction of nitrites with TEA (triethylamine), or NMP (*N*-methyl-2-pyrrolidone), respectively. The synthetic pathways of losartan, irbesartan and candesartan also rely on azide-nitrile reaction to form tetrazole ring and thus are exposed to the same risk factors for nitrosamine contamination (EMA, 2020).

While in sartans nitrosamines are formed by chemical reaction during the synthesis of the active substance, as a result of the application of azide in combination with an unsuitable solvent choice, the situation with ranitidine is more complex. Ranitidine molecule is very unstable and contains both a nitrile and a dimethylamine, which combination leads to formation of *N*-nitrosodimethylamine. A potentially concerning possibility is the increasing level of NDMA in ranitidine over time, when the tablets are stored at temperatures higher than room temperature (White, 2020).

Conclusion

Knowing their mutagenic and carcinogenic effects, the presence of nitrosamines in active pharmaceutical ingredients must be limited. All this implies the need primarily for identifying the main reasons for contamination with these impurities and then developing the appropriate methods for their detection and quantification. Medicine regulatory authorities are taking steps to solve this problem, but also we need to highly improve our knowledge in this area and prevent future human exposure with these impurities through medicines.

References

Baumann, M., Baxendale, I.R., Ley, S.V., Nikbin, N. 2011. An overview of the key routes to the best selling 5-membered ring heterocyclic pharmaceuticals. Beilstein J Org. Chem. 7, 442-95.

European Medicines Agency, 2018. EMA reviewing medicines containing valsartan from Zhejiang Huahai following detection of an impurity: some valsartan medicines being recalled across the EU. Press release. Available at: https://www.ema.europa.eu/en/documents/press-release/ema-reviewing-medicines-containing-valsartan-zhejiang-huahai-following-detection-impurity_en.pdf. (Accessed May 1, 2022).

Europea Medicines Agency, 2019. EMA to review ranitidine medicines following detection of NDMA. Press release. Available at: https://www.ema.europa.eu/en/documents/press-release/ema-review-ranitidine-medicines-following-detection-ndma_en.pdf (Accessed on May 1, 2022)

European Medicines Agency, 2020. EMA Assessment report: nitrosamine impurities in human medicinal products. Procedure number: EMEA/H/A-5(3)/1490. Available at: https://www.ema.europa.eu/en/documents/referral/nitrosamines-emea-h-a53-1490-assessment-report_en.pdf (Accessed May 1, 2022)

International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). ICH M7 (R1) assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk. 2018. Available at: https://www.fda.gov/media/85885/download (Accessed May 1, 2022).

Snodin, D.J., Elder, D.P., 2019. Short commentary on NDMA (N-nitrosodimethylamine) contamination of valsartan products. Regul. Toxicol. Pharmacol. 103, 325-329.

White, C.M., 2020. Understanding and preventing (N-nitrosodimethylamine) NDMA contamination of medications. Ann. Pharmacother. 54(6), 611-614.

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 61 - 62 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.026
Short communication

Understanding the requirements of the MDR 2017/745 regarding person responsible for regulatory compliance

Ajra Sinanova*, Lora Atanasovska, Marija Davceva Jovanoska, Elizabeta Karadzinska, Nada Popstefanova, Olivera Paneva

Pharmaceutical Chemical Cosmetic Industry ALKALOID AD Skopje, Blvd. Aleksandar Makedonski 12, 1000 Skopje, Republic of North Macedonia

Introduction

Compliance of the medical devices has become progressively more complex with the introduction of the EU Medical Device Regulation 2017/745. One of the newly introduced requirements of the European Union's Medical Device Regulation (MDR) is appointment of a person responsible for regulatory compliance (PRRC). The focus of this regulatory requirement is mainly to improve control over manufacturing processes, post-marketing surveillance, clinical evaluation and medical device vigilance. The ultimate goal of the manufacturer is to ensure conformity and safety of the medical device.

Availability, liability and qualification requirements of the PRRC

The MDR requires every manufacturer of medical devices to appoint a PRRC within their organization. The authorized representative of a manufacturer located outside the EU should also appoint a PRRC. The PRRC for the manufacturer and its authorized representative cannot be the same. According to the MDR, the authorized representative is supposed to add additional level of scrutiny. If the same person acts as PRRC for both, the additional level of scrutiny would be compromised. The appointed PRRC for the manufacturer and for the authorized representative should be part of the organization. Exception to this is possible only for micro and small organizations. Micro and small manufacturers can subcontract the responsibilities of a person responsible for regulatory compliance to a third party.

Medical devices manufacturers and their authorized representatives must observe a multitude of legal requirements which must be fulfilled. According to the MDR, the person responsible for regulatory compliance is not personally liable. The liability requirements of the MDR refer to the manufacturer and its authorized representative as an entity. Different national legislation may enforce liability on PRRC who infringe Article 15 of the MDR, so these laws should certainly be taken into consideration.

There are two ways to fulfill the qualification requirements for PRRC. According to the MDR and the MDCG 2019-7 guidance document, the PRRC must have: expertise in the field of medical devices, university degree, diploma, or some other formal qualifications and at least one year of experience in European regulatory affairs or quality management system related to medical devices. If no university degree, diploma, or other qualification, the PRRC must have four years of professional experience in European regulatory affairs or quality management system related to medical devices. It is important to emphasize here that any qualification acquired outside the EU should be recognized by an EU Member State as equivalent to the EU corresponding qualification.

More than one PRRC can be appointed by the companies and the responsibilities can be divided, as long as the qualification requirements are met and the division of responsibilities is documented.

*asinanova@alkaloid.com.mk

Roles and responsibilities of a person responsible for regulatory compliance

The roles and responsibilities of a person responsible for regulatory compliance is mandated in Article 15, clause 3 of the EU MDR 2017/745. The regulation requires all manufactures and authorized representatives to have a designated employee in their company who is responsible for regulatory compliance with the applicable EU MDR. What are the responsibilities of a PRRC?

EU MDR outlines five major responsibilities for a PRRC, each described with detailed activities for ensuring conformity and safety of the medical devices. The PRRC is responsible for ensuring that (Jury & Pinsi, 2021):

1. the conformity of the devices is appropriately checked, in accordance with the quality management system under which the devices are manufactured, before a device is released;
2. the technical documentation and the EU declaration of conformity are drawn up and continuously updated;
3. the post market surveillance obligations are compiled. The PRRC is responsible for ensuring that post-market surveillance system is planned, established, documented, maintained and kept up-to-date;
4. the reporting obligations referred to vigilance are fulfilled. The PRRC is responsible for ensuring that vigilance system (reporting of serious incidents and field safety corrective actions) and implementing acts is established;
5. in the case of investigational devices, that the signed statement by the natural or legal person responsible for the manufacture of the investigational device that the device in question conforms to the general safety and performance requirements apart from the aspects covered by the clinical investigation and that, with regard to those aspects, every precaution has been taken to protect the health and safety of the subject.

Alkaloid AD Skopje, as a manufacturer of medical devices has allocated the PRRC responsibilities to five different persons. Each person is individually responsible for:

- ensuring compliance with QMS (batch release not included) and ensuring that EU declaration is drawn up and updated continuously;
- ensuring compliance of the medical device with batch release included;
- ensuring that the technical documentation is drawn up and ensuring that in the case of investigational devices, the statement referred to in Section 4.1 of Chapter II of Annex XV of the MDR 2017/745 is issued for clinical investigations performed with devices which do not bear the CE marking of conformity;
- ensuring that the technical documentation is kept up to date, ensuring that the post-market surveillance obligations are complied with in accordance with Article 10 of the MDR 2017/745 and ensuring that in the case of investigational devices, the statement referred to in Section 4.1 of Chapter II of Annex XV of the MDR 2017/745 is issued for clinical investigations performed with devices which bear the CE marking of conformity;
- ensuring that the reporting obligations referred to in Articles 87 to 91 of the MDR 2017/745 regarding vigilance are fulfilled.

The responsibilities of the PRRC for the EU authorized representative ALKALOID – INT d.o.o. are allocated to two persons.

Conclusion

The requirements of the MDR regarding PRRC are not completely new. Under the MDR, the tasks now outlined as responsibilities of this new role of PRRC, would already have existed as part of the Quality Management System. What is new under MDR is the requirement to designate a specific person or persons as PRRCs. It is critical that the role of PRRC is not recognized only formally, but also as a crucial role to foster a culture of compliance awareness within the organization and to ensure safety of the medical device.

References

Jury A., Pinsi, M., 2021. Person responsible for regulatory compliance (PRRC) - MDR/IVDR Article 15, British Standards Institution (BSI) StandardsLtd Available at> https://www.bsigroup.com/globalassets/meddev/localfiles/fr-fr/whitepapers/wp---person-responsible.pdf

MDCG 2019-7 Guidance on Article 15 of the Medical Device Regulation (MDR) and in vitro Diagnostic Device Regulation (IVDR) regarding a 'person responsible for regulatory compliance' (PRRC). Available at: https://ec.europa.eu/docsroom/documents/36166

Regulation (EU) 2017/745 of the European Parliament and of the Council on medical devices, 2017. Official Journal of the European Union. Available at: https://eur-lex.europa.eu/

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 63 – 64 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.027
Short communication

Systematic approach for proper control chart design for Karl-Fischer titrator

Blagoj Achevski*, Frosina Arsova, Jelena Acevska, Jasmina Tonikj-Ribarska, Katerina Brezovska, Natalija Nakov

[1]*Faculty of Pharmacy, University "Ss Cyril and Methodius", 1000 Skopje, Republic of North Macedonia*

Introduction

Statistical Process Control (SPC) is an essential part of quality assurance in many industries, including the pharmaceutical industry (Eissa & Abid, 2018; Shah et al., 2010; Velinovska et al., 2019). This methodology promotes better understanding of the source of variation of a particular process, thus contributing for its continuous improvement. Control charts (CC), as one of the main SPC tools, allow monitoring of the process variability over a period of time and detection of the systematic (common) causes of process variation (ISO 7870-1, 2019). The application of CC is expanding from the manufacturing departments of the pharmaceutical industry to the quality control analytical laboratories. CC are used for monitoring the method/instrument performance, thus may indicate the need for re-qualification of the instrument; instability of the reference material; errors in the calculation of the analytical result; insufficient training of the personal, etc. (Sengoz, 2018). The basic elements of the CC are the center line (CL) and upper and lower control limit (UCL and LCL, respectively) (ISO 7870-2, 2013). The choice of the suitable type of CC, along with the proper establishment of the UCL and LCL have great impact on the sensitivity of the CC. In case of evaluation of improperly designed CC, there is a possibility to overview a process that is really out of a statistical control, or in opposite scenario a process that is under control might be detected as an out of statistical control. Hence the need for proper design of the CC is demanded for accurate evaluation of the process variability.

The aim of this research is to establish a systematic approach for proper design of CC for monitoring the performance of instruments used in quality control analytical laboratory. The systematic approach for selection a suitable CC, establishment of CL and use of adequate rules for evaluation of CC will be shown using instrument for semi-micro determination of water i.e. Karl-Fischer (KF) titrator as an example.

Materials and methods

Materials

The semi-micro titration of water was performed on Karl Fischer Titrator DL38, Mettler Tolledo. Aquastar CombiTirtant 5mg/mL (Supelco) was used as a titrant and Aquastar CombiMethanol (Supelco) as a solvent. The Water Standard 1% (10 mg H_2O/1 g) CRM (Merck) was used for performance verification of the KF titrator.

The data for the percentage recovery (r, %) of water, obtained after addition of Water Standard 1%, were used for design of different types of CC and calculation of the control limits. Each data point in CC represents the mean r (%) of three independent additions of Water Standard 1%. The 100% recovery was chosen as a CL for the X-chart, whereas the mean value of the r (%) obtained from 26 determinations was used as a CL for J-chart. The CL of the CC were created using 26 data points of and additional 12 data points were used for further instrument performance monitoring.

Results and discussion

The first step in the systematic approach for proper CC design is selection of suitable type of CC. Suitable CC for monitoring quantitative data for grouped results (n=3)

*bachevski@ff.ukim.edu.mk

are X-chart, R-chart or J-chart (zone control chart). The evaluation has shown that X-chart is sensitive for changes of the mean and standard deviation (σ) of the determination. However, this type of chart doesn't provide information about the distribution of the individual data in the subgroups. This kind of information could be obtained with the R-chart. However, the R-chart only takes into account the within-run precision of the determination. Considering that the performance of the KF titrator is evaluated through the accuracy of the determination (recovery), the X-chart was found to be more suitable for performance monitoring of the KF titrator than the R-chart. The evaluation of the J-chart confirmed that this kind of CC incorporates the characteristics of the X-chart and the CUSUM chart. This kind of chart is efficient in detection of rapid changes of the determination, thus could be applied for the performance evaluation of the KF titrator.

The second step is defining the UCL and LCL of the CC. Therefore, several X-charts were designed using different approaches for defining the CL. The first activity is evaluation whether the data points show normal (Gaussian) distribution or not. The obtained values of the statistical parameters Skewness and Kurtosis (-0.23 and -0.76, respectively) confirmed that the data points show normal distribution, thus the use ±3σ (±3*0.91) as UCL and LCL (103.16 and 97.19, respectively) for the X-chart was justified. In addition, another X-chart using $\pm 3\sigma_{total}$ value (±3*0.85) as a UCL (102.54) and LCL (97.46) was designed. The σ_{total} includes the within-run (variance within the subgroup of results) and between-run variance (variance between all subgroup of results). The results showed that in case where X-chart is created with data points of group data, defining CL by means of σ_{total} is more appropriate. Considering the recovery tolerance limits given in the OMCL Qualification of equipment guideline (GEON, 2022), another X-chart was designed using the values of 97.5% and 102.5% as UCL and LCL, respectively. The evaluation of the sensitivity has shown that the use of the tolerance limits from the guideline as CL, reduces the sensitivity of the X-chart to raise the alarm in case the instrument gets out of a statistical control.

The σ value needed for defining the CL of the J-chart was calculated by multiplying the moving range with 0.8865 (AMCTB, 2003). The obtained values for UCL and LCL of the J-chart were 103.23 and 97.27, respectively. Although these CL are wider than the CL defined for the X-charts, the sensitivity of the J-chart was not brought in question. The reason for the better sensitivity of the J-chart compared to the X-chart with 97.5% and 102.5% as CL, might be the existence of different set of rules for evaluation of the J-chart.

The designed CC were applied for instrument performance monitoring. The results showed that WECO rules, as well as Westgard combined rules could be applied to the evaluation of the X-chart using ±3σ or $\pm 3\sigma_{total}$ value as CL. Whereas, only three WECO rules (1_{3s}, 2_{2s} and 9_x) are applicable for the evaluation of the X-chart using tolerance limits as CL. The J-chart couldn't be evaluated using WECO rules or Westgard combined rules.

Conclusion

This research showed that the proper selection of the type of CC; proper defining of the UCL and LCL; as well as evaluation of the CC using adequate rules, has a direct impact on the sensitivity of the CC and the accuracy of the evaluation of the instrument performance. The proposed systematic approach for the proper CC design for monitoring the performance of the KF titrator could be applied for designing CC for different instruments, depending of the needs of the analytical laboratories. This research could contribute towards expansion of the application of the CC as a part of the quality assurance system in analytical laboratories for quality control.

References

Analytical Methods Committee Technical Brief (AMCTB), 2003. The J-chart: a simple plot that combines the capabilities of Shewhart and cusum charts, for use in analytical quality control. Royal Society of Chemistry, 12.

ISO 7870-2. 2013. Control Charts-Part 2: Shewhart Control Charts; ISO: Geneva, Switzerland.

ISO 7870-1. Control Charts-Part 1: General Guidelines; ISO: Geneva, Switzerland, 2019.

Eissa, M., Abid, A.M., 2018. Application of statistical process control for spotting compliance to good pharmaceutical practice. Braz. J. Pharm. Sci. 54(2), e17499. https://doi.org/10.1590/s2175-97902018000217499

General European OMCL Network (GEON), Quality Management Document, PA/PH/OMCL (07) 108 R11. 2022. Qualification of equipment Annex 5: Qualification of automatic titrators.

Sengoz, N.G., 2018. Control charts to enhance quality. InTech, https://doi.org/10.5772/intechopen.73237

Shah, S., Shridhar, P., Gohil, D., 2010. Control chart: A statistical process control tool in pharmacy. Asian J. Pharm., 184-192. https://doi.org/10.4103/0973-8398.72116

Velinovska Cadinoska, M., Popstefanova, N., Ilievska, M., Karadzinska, E., Davceva Jovanoska, M., Glavas Dodov, M., 2019. Trending and Out-of-Trend results in pharmaceutical industry. Maced. Pharm Bull. 65(1), 39-60. https://doi.org/10.33320/maced.pharm.bull.2019.65.01.005

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 65 - 66 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.028
Short communication

Fc-fusion proteins: therapeutic relevance and quality assessment

Gent Misini[1], Nebija Dashor[2*], Rumenka Petkovska[1], Natalija Nakov[1]

[1]*Faculty of Pharmacy, University Ss Cyril and Methodius in Skopje, 1000 Skopje, Republic of North Macedonia*
[2]*Faculty of Medicine, University Clinical Center n.n. 10000 Pristina, Kosovo*

Introduction

Fc-fusion proteins are bioengineered polypeptides that combine a biologically active protein with the crystallizable fragment (Fc) domain of an IgG to produce a molecule with unique properties and therapeutic potential (Linderholm & Chamow, 2014). Owing to its interaction with the salvage neonatal Fc-receptor, Fc domain substantially increases plasma half-life *in vivo* and reduces the clearance of Fc-fusion proteins, which prolongs their therapeutic activity (Czajkowsky et al., 2012). In addition, Fc fragment improves the solubility and stability of the fusion protein (Carter, 2011). Fc fusion proteins are very successful class of medicines with already 13 Fc-fusion proteins approved in the EU and in the USA (EMA, 2022; U.S. FDA, 2022). Currently four therapeutic Fc-fusion proteins including aflibercept, dulaglutide, etanercept and abatacept are among the 50 globally best-selling medicines (Buntz, 2022).

Classification of Fc-fusion proteins

Based on the ligand binding domain Fc-fusion proteins are classified in four main categories.

Receptor-ECD–based Fc-fusion proteins

This category includes TNFR-FC fusion protein, etanercept, and CTLA4-Fc fusion proteins (abatacept and belatacept). Etanercept is homodimeric fusion protein consisting of ECD of the human p75 TNF receptor linked to a human Fc-IgG1 domain. Etanercept is used for the treatment of inflammatory rheumatic disorders and psoriasis. Abatacept consists of ECD of human CTLA4 linked to the modified Fc. Abatacept is used for the treatment of inflammatory rheumatic disorders. Belatacept is indicated for the prophylaxis of graft rejection (EMA, 2022).

Cytokine traps

This class of Fc-fusion proteins consist of multiple ligand-binding domains from different receptors linked to a human Fc domain. This group includes aflibercept consisting of portions of human VEGF receptor 1 and 2 ECDs fused to the Fc portion of human IgG1. Aflibercept is used for the treatment of macular degeneration (MD) and metastatic colorectal cancer. Conbercept is VEGF inhibitor fusion protein, used for the treatment of MD. The third representative, rilonacept is designed by fusing the C-terminus of the IL1 receptor accessory protein ligand-binding region to IL1R1 ECD, then this hybrid construct is fused to human IgG1 Fc. Rilonacept is used for the treatment of cryopin-associated periodic syndromes (EMA, 2022).

Peptide-Fc (peptibodies)

Romiplostim is a dimeric peptibody consisting of the IgG1 Fc-domain fused with two polypeptide mimetic sequences of thrombopoietin attached to each Fc chain. Romiplostim is used for the treatment of ITP. Dulaglutide is a glucagon-like peptide (GLP)-1 receptor agonist in which a modified GLP-1 peptide is fused to the IgG4 Fc fragment.. Dulaglutide is used for the treatment of type 2 diabetes (EMA, 2022; U.S. FDA, 2022).

Recombinant enzymes

Eftrenonacog alfa is a fusion protein consisting of human coagulation factor IX linked to the Fc fragment of

*dashnor.nebija@uni-pr.edu

human IgG1. It replaces the missing factor IX, which plays a central role in blood clotting and is indicated for the treatment of hemophilia B. Efmoroctocog alfa is a fusion protein composed of human, B-domain–deleted, coagulation factor VIII linked to the Fc domain of human IgG1. This drug is indicated for the treatment of hemophilia B. The third representative of this class is asfotase alfa, used for the tretament hypophosphatasia. Asfotase alfa is a human recombinant tissue-nonspecific alkaline phosphatase-Fc-deca-aspartate (EMA, 2022).

Biosimilar Fc-fusion proteins

Biosimilar is a highly similar version of an already authorized innovator biological medicine known as reference product. Originator of etanercept, Amgen/Pfizer's Enbrel was among the best-selling medicines and in 2021 its sales reached about 4.5 billion USD. The patent expiry in Europe and the USA led to development of biosimilar versions. Currently three biosimilars of etanercept are authorized in EU and USA. Till 2026, it is expected that the global market value of the biosimilars will rise by 25% compared to the value in 2020 (Ratih et al., 2021).

Quality assesment of Fc-fusion proteins

Fc-fusion proteins are characterized with inherent heterogeneity and complex structure. The complexity of their manufacturing process is one of the key factors affecting consistency of the product quality. Small structural alterations could possibly initiate substantial changes in drug stability, immunogenicity and efficacy. Therefore, the analytical strategies for the characterization of Fc-fusion proteins should be product specific and involve state of the art analytical techniques. A wide range of highly selective analytical techniques are applied for assessment of the critical quality attributes (CQAs) of the Fc-fusion proteins. Analytical comparability of the CQAs between different production batches is performed for the assessment of the consistency of the product quality. In addition, comparability studies of the CQAs between originator and biosimilar are inevitable for the biosimilarity assessment. High standards of the quality, safety and efficacy are required for the biosimilars. Considering the diversity of the CQAs of this class of drugs (such as physicochemical characterization, primary structure, post-translatory modifications, purity, charge and size variants, etc.), a set of analytical techniques are required for the quality assessment. The method of choice for confirmation of the primary structure of the Fc-fusion proteins (identification and quantification of protein variations) is LC-MS/MS. Size-exclusion chromatography is gold standard method for size variants analysis (Duivelshof et al., 2021). Two-dimensional gel electrophoresis is a suitable method for the assessment of identity, purity, structural integrity, charge heterogeneity and post-translational glycosylation of the Fc-fusion protein (Nebija et al., 2015). In addition, ion exchange chromatography and capillary zone electrophoresis are used for homogeneity assessment (Beck et al., 2013).

Concluding remarks

The unique functional properties of the Fc-fusion proteins, such as half-life extension and great therapeutic potential, place these medicines in the front line of drug research and development. The diversity of the Fc-fusion proteins, along with the rapid grow of their biosimilars, impose the need for implementation of specific and highly sensitive chromatographic and electrophoretic techniques for the quality assessment of the CQAs of the Fc-fusion proteins.

References

Beck, A., Diemer, H., Ayoub, D., Debaene, F., Wagner-Rousset, E., Carapito, C., Dorsselaer, A., Sanglier-Cianferani, S., 2013. Analytical characterization of biosimilarantibodies and Fc-fusion proteins. Trend Analyt. Chem. 48. https://doi.org/10.1016/j.trac.2013.02.014

Buntz, B., 2022. 50 of 2021's best-selling pharmaceuticals. Available at: https://www.drugdiscoverytrends.com/ (Last accessed on 23.05.2022).

Carter, P.J., 2011. Introduction to current and future protein therapeutics: a protein engineering perspective. Exp Cell Res 317(9), 1261-1269. https://doi.org/10.1016/j.yexcr.2011.02.013

Czajkowsky, D.M., Hu, J., Shao, Z., Pleass, R.J., 2012. Fc-fusion proteins: new developments and future perspectives. EMBO Mol. Med. 4(10), 1015-28. https://doi.org/10.1002/emmm.201201379

Duivelshof, B.L., Murisier, A., Camperi, J., Fekete, S., Beck, A., Guillarme, D., D'Atri, V., 2021. Therapeutic Fc-fusion proteins: Current analytical strategies. J Sep Sci. 44(1), 35-62. https://doi.org/10.1002/jssc.202000765

European Medicines Agency. Available at: https://www.ema.europa.eu/en

Linderholm, A., Chamow, S.M., 2014. Immunoglobulin Fc-Fusion Proteins 12(9). Available at: https://bioprocessintl.com/ (Last accessedon 23.05.2022).

Nebija, D., Noe, C.R., Lachmann, B., 2015. Charge heterogeneity study of a Fc-fusion protein, abatacept, using two-dimensional gel electrophoresis. Pharmazie 70, 527–534.

Ratih, R., Asmari, M., Abdel-Megied, A., Elbarby, F., Deeb, S., 2021. Biosimilars - Review of regulatory, manufacturing, analytical aspects and beyond. J. Microc. 165, 106143.

U.S. Food and Drug Administration. https://www.fda.gov/.

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 67 - 68 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.029
Short communication

Elucidation of molecular structure of bromazepam opened ring related compound detected in bromazepam tablets in acidic dissolution media using LC/MS

Viktorija Jakimovska Pokupec*, Katerina Janchevska, Marija Grozdanovska, Viktorija Petrovska

Research and Development, Alkaloid AD, Blvd. Aleksandar Makedonski 12, 1000 Skopje, Republic of North Macedonia

Introduction

Bromazepam or 7-bromo-1,3-dihydro-5-(2′-pyridyl)-2H-1,4-benzodiazepin-2-one belongs to the 1,4-benzodiazepines group of drugs that are used in the treatment of anxiety, insomnia, seizures, muscle spasms and similar disorders. As found in literature, bromazepam in acidic conditions undergoes reversible reaction of 4,5-azomethine bond cleavage to the opened-ring compound formation. Having this in consideration, in the process of performing comparative dissolution profiles in media pH 1.2 and pH 4.5, bromazepam would undergo this reversible ring opening reaction (Damjanović et al., 2004).

During the analysis of comparative dissolution profiles in media pH 1.2 and pH 4.5 with an in house HPLC method for dissolution, two main peaks were detected, one originating from bromazepam and other with relative retention time (RRt) 0.72. In order to evaluate the nature of the compound with RRt 0.72, observed in dissolution media pH 1.2 and pH 4.5, comprehensive Liquid Chromatography / Mass spectrometry (LC/MS) analysis and detailed literature survey were conducted.

The aim of this study was to reveal the possible nature and fragmentation pathway of the compound with RRt 0.72 (Retention time about 1.51 min) from two manufacturers of bromazepam based finished products (bromazepam tablets) and a reference standard, using advanced analytical techniques such as MS. The study as well, is aimed to justify and demonstrate that the in house High Performance Liquid Chromatography (HPLC) method is also suitable for determination of open ring compound.

Materials and methods

Materials

Samples from three tablets strengths (1.5 mg; 3 mg; 6 mg) obtained from two manufacturers of bromazepam tablets were analyzed. Bromazepam certified reference standard (CRS) (batch number 4.0), obtained from European Pharmacopeia was also used. All samples were dissolved in pH 1.2 and pH 4.5 acidic dissolution media prepared according European Pharmacopeia. For the preparation of the mobile phase, CH_3COONH_4, glacial acetic acid and acetonitrile were used.

Dissolution method

Dissolution test procedures was performed with standard apparatus II on Agilent 708-DS Dissolution apparatus. The procedure was conducted at 37°C, with 75 rpm in 900 mL volume of regulatory dissolution media pH 1.2 and pH 4.5, using sampling time point at 45 minutes.

LC method

The analysis was performed on Thermo Scientific Dionex Ultimate 3000 UHPLC-UV-DAD system coupled with Thermo Scientific LTQ XL linear ion trap mass spectrometer. LC parameters were used according to in-house analytical procedure for dissolution of Bromazepam tables where 0.01M ammonium acetate buffer with pH 5.5 and acetonitrile in ratio 60:40 (v/v%) was used as a mobile phase. An analytical HPLC column, XBridge C18, 150 mm x 4.6 mm with 5 µm particles, at

*vjakimovska@alkaloid.com.mk

temperature of 25°C with flow rate of 1.2 mL/min and injection volume of 20µL from the sample and standard solutions were used. Bromazepam peak is obtained at about 2 minutes in a run time of 6 minutes.

MS method

MS ion source parameters were employed as follows: electro-spray ionization (ESI) was used, spray voltage was set at 5.0 kV, capillary temperature was set at 275 °C, sheet and auxiliary gas flows were 30 and 8 psi respectively. MS spectra were acquired by full range acquisition covering m/z 50-800, in positive ionization mode (Kozak et al., 2008). The instrument was controlled by Chromeleon 5.0 and Xcalibur 2.0 data analysis software. Mass Frontier spectral interpretation software 7.0 was used for the fragmentation pathway prediction and elucidation of the proposed structure of the unknown compound.

Results and discussion

After dissolution of Bromazepam tablets in pH 1.2 and pH 4.5, the HPLC analysis showed peak of bromazepam on retention time 2.09 min (due to bromazepam) and unknown peak with retention time 1.51 min (RRt=0.72). The mass spectra of the peak occurring at Rt 2.09 min in all analyzed samples (in dissolution media pH 1.2 and in pH 4.5) showed protonated molecular ion at 316 m/z as parent ion of bromazepam (molecular weight, Mr 315 g/mol).

Following fragmentation ions were observed: 288 m/z, 184 m/z, 155 m/z, 127 m/z, 237 m/z, 261 m/z and 209/210 m/z. In accordance with the literature findings (Rivera et al., 2006; NIST database, 2014; Tas et al., 1986), these ions are characteristic fragmentation ions of bromazepam. The fragmentation mechanisms and characteristic fragments were confirmed using Mass Frontier spectral interpretation software 7.0.

As found in literature, bromazepam in acidic conditions (as in dissolution media pH 1.2 and pH 4.5) undergoes reversible reaction of 4,5-azomethine bond cleavage to the opened-ring compound formation, suggesting the possible origin of the unknown peak at Rt about 1.51 min (Damjanović et al., 2004). The mass spectra of the peak at Rt 1.51 min (RRt=0.72) revealed fragment ion at 334 m/z which corresponded to the protonated molecular weight of the structure of the 4,5-azomethine opened-ring compound (Mr=333 g/mol). Other fragment ions at 316 m/z, 288 m/z, 261 m/z, 237 m/z, 209 m/z, 184 m/z, characteristic for bromazepam fragmentation indicated a structure similar to bromazepam.

Comparable MS spectra and fragmentation patterns of the unknown peak were observed in bromazepam tablets from both manufacturers in all three tablet strengths and in both dissolution media. Furthermore, the findings were additionally confirmed using bromazepam CRS prepared to correspond with all three strengths as the finished product and in both dissolution media.

Conclusion

Comparable MS spectra and fragmentation pathways of bromazepam and compound at Rt about 1.5 min were obtained when analyzing bromazepam CRS and bromazepam tablets manufactured by two manufacturers, in all dosage strengths and in both acidic dissolution media. The MS elucidation of the structure of the compound at RRt=0.72 confirmed that it is an opened-ring related compound of bromazepam. It can be concluded that this related compound could be detected in bromazepam tablets after acidic dissolution and it is originating from bromazepam due to molecular rearrangement (ring opening) in acidic conditions.

Acknowledgements

The authors would like to acknowledge Alkaloid AD, Skopje for providing all the necessary materials, equipment and funding for preforming the analysis.

References

Bromazepam mass spectrum from NIST database, NIST Mass Spectrometry Data Center Collection (C) 2014 copyright by the U.S. Secretary of Commerce, N.W. Davies, Centr. Sci. Lab., Univ. Tasmania, Hobart, Australia.

Damjanović, T., Popović, G., Verbić, S., Pfendt, L., 2004. Study of acid hydrolysis of bromazepam. Can. J. Chem. NRC Canada, 1260–1265. https://doi.org/10.1139/v04-091

Kozak, M., Rezai, T., Scientific, T. F., Jose, S., 2008. A Complete Toxicology Screening Procedure for Drugs and Toxic Compounds in Urine and Plasma Using LC-MS/MS. Thermo Fischer Scientific Application Note.

Rivera, H., Walker, G. S., Stockham, A. P., Sims, D. N., Hughes, J. M., 2006. Analysis of Benzodiazepines in Blood by LC/MS/MS. Agilent Application Note.

Tas, A.C., van der Greef, J., ten Noever De Brauw, M.C., Plomp, T.A., Maes, R.A.A., Hohn, M., Rapp, U., 1986. LC/MS Determination of Bromazepam, Ciopenthixol, and Reserpine in Serum of a Non-Fatal Case of Intoxication. J. Anal. Toxicol. Oxford university press, 46-48. https://doi.org/10.1093/jat/10.2.46

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 69 – 70 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.030
Short communication

Improvement of the analytical method for content determination of Rosuvastatin film coated tablets during lifecycle

Aleksandra Damjanoska*, Cveta Dolikjoska Trajkova, Matilda Petrova, Milena Nikolovska, Mena Ivanoska Zdravkovska, Hristina Babunovska

Alkaloid AD Skopje, Blvd. Aleksandar Makedonski 12, 1000 Skopje, Republic of North Macedonia

Introduction

The lifecycle monitoring of the analytical procedures includes risk assessment and systematic experimental evaluation to gain enhanced understanding of the procedure parameters critical to the consistent delivery of reportable results. As stated in ICH Q12, the analytical method Lifecycle Management (LCM) is encompassing all activities, from method development to validation, routine use, change control and retirement of the method. This enhanced approach improves the method understanding and performance, facilitates method transfer and leads to fewer out-of-specifications results (OOS).

The in-house method for content determination of Rosuvastatin film coated tablets is adopted from an in-license partner. The European Pharmacopoeia (Ph.Eur.) Commission has adopted a new monograph on Rosuvastatin tablets (3008) at its 163rd session (March 2019). The monograph was published in the first supplement to the 10th Edition (available in October 2019) and gained effectiveness on 1st April 2020.

Rosuvastatin tablets is the first monograph on a multi-source medicinal product to have been adopted and the product itself is one of the most widely used and prescribed medicines. This monograph is the result of a close cooperation between manufacturers, experts and the EDQM. This short paper reflects how the analytical method for content determination of Rosuvastatin film coated tablets was improved during the lifecycle.

Materials and methods

Method Origin / Comparison with current Pharmacopoeia Monographs

Method 1 – Chromatographic conditions – In-house method

The chromatographic separation was performed on Symmetry C18 250 x 4.6mm, 5µm at temperature of 35 °C. The Phenomenex Luna C18 column was found suitable too. Autosampler temperature was set at 4°C and the injection volume was 10 µL. The mobile phase A consisted of Buffer 0.01M pH 3.5 (65% v/v), acetonitrile (30% v/v) and tetrahydrofuran (THF) (5% v/v). Acetonitrile was used as mobile phase B. The flow rate was set to 1.0 mL/min. Gradient program was incorporated with chromatography run time of 42 minutes with ultraviolet (UV) detection at 280 nm.

Method 2 – Chromatographic conditions – Ph Eur 10.1 04/2020:3008 Monograph

XTerra MS C18 column, 150 mm length, 3.0 mm internl diameter and 3,5 µm particle size was used and termostated at 40 °C. Autosampler temperature was set as room temperature and injection volume of 10 µL. Mobile phase A consisted of 1 per cent (v/v) solution of trifluoroacetic acid R, acetonitrile for chromatography R, water for chromatography R (1:31:68 v/v/v), whereas the mobile phase B consisted of 1 per cent (v/v) solution of trifluoroacetic acid R, acetonitrile for chromatography R in ration 1:100 (v/v). The flow rate was set to 0.7 mL/min. Gradient program was incorporated with chromatography run time of 20 minutes with UV detection at 242 nm.

*adamjanoska@alkaloid.com.mk

Results and discussion

Analytical method transfer with the in - license partner was successfully performed and the method is applied in the routine analysis. Continuously key method performance characteristics are reviewed to verify that the measurement system and the analytical operations associated with the analytical procedure are adequate during the intended time period of analysis and enable the detection of potential failures. As main characteristics that are followed during the LCM of the current method are the System suitability test (SST) parameters: similarity between standard solution1 and standard solution 2 (98,0-102,0 %), recovery (98,0-102,0 %), relative standard deviation of the areas of Rosuvastatin peak in the standard solution (≤ 2.0 %), peak asymmetry (not more than 2.0) and theoretical plates (≥ 2000). During the lifecycle of the method in the laboratories for finished products and long-term stability, issues with non-fulfillment of the criteria for SST (lack of similarity between two preparations of standard solution) with increased frequency of occurrence regardless of the sensitivity of the HPLC instrument used (UV/Vis or DAD), were detected.

Theoretically, the method described in the Ph.Eur. monograph is easier for performing, there is no use of THF in the mobile phase, the chromatography run time is much shorter (20 minutes instead of 42 minutes). Considering the above mentioned, it was decided to adapt the existing pharmacopoeial method to our product Rosuvastatin film coated tablets 5 mg, 10 mg, 20 mg and 40 mg.

After defining the analytical target profile in the development phase, several analyzes were performed in order to check the appropriateness of the pharmacopoeial method. From the obtained results it was observed that with the newly optimized method the problems with achieving SST criteria have been overcome, a better chromatography has been obtained and the duration time of one analysis has been significantly shortened. For comparison, the time needed for analysis of one batch with the in-house method was 630 minutes (about 10.5 h), while with the Ph.Eur. method the total analysis time of one batch was found to be 300 minutes (about 5 h).

After the successful optimization of the pharmacopoeial method, validation of the analytical method for content determination of Rosuvastatin film coated tablets 5mg, 10mg, 20mg, 40mg has been performed. The validation parameters include specificity, linearity and range, accuracy, precision, robustness, stability and filtration of solutions, as per internal validation guideline.

Consequently, change request was initiated for all markets where the product has a marketing authorization license.

Conclusion

Recent developments in the progression and initiation of ICH quality guidelines (ICH Q12, Q2 revision, and ICH Q14) show that the regulatory aspects of the development and lifecycle management of analytical procedures is likely to be of continuing interest in the coming years.

References

Ermer, J. and Nethercote, P., 2015. Method Validation in Pharmaceutical Analysis. A Guide to Best Practice. WILEY-VCH Verlag GmbH&Co.KGaA.

ICH Guideline Q2(R1): “Validation of Analytical procedures: Text and Methodology”, November 2005.

ICH Guideline Q12: “Technical and Regulatory Considerations for Pharmaceutical Product Lifecycle Management”, November 2019.

Ph. Eur. 10.1, 3008 (04/2020), Rosuvastatin tablets monograph, EDQM Council of Europe, 7 allée Kastner, CS 30026, F-67081 Strasbourg, France

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 71 -72 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.031
Short communication

A study on the compatibility of Ibuprofen with some essential oils used for formulation of semi-solid pharmaceutical dosage forms for topical use

Elena Cvetkovska Bogatinovska*, Monika Stojanovska Pecova, Mirjana Bogdanoska Mircheska, Marina Chachorovska

Alkaloid AD, Blvd. Aleksandar Makedonski 12, 1000 Skopje, North Macedonia

Introduction

Ibuprofen represents one of the most popular non-steroidal anti-inflammatory drugs which is widely used in treatment of acute pain and fever, as well as some chronical conditions. Thanks to its excellent analgesic and anti-inflammatory properties, ibuprofen is used as an active pharmaceutical ingredient (API) in many different pharmaceutical dosage forms, for oral and topical administration, such as tablets, oral suspensions and gels (Maswadeh, 2016). Due to the close contact of the API with one or more excipients in the formulation, any kind of interaction may result with a negative impact on the stability, physical or performance attributes of the product (Gupta et al., 2019). Therefore, for a rational selection of excipients, screening of API-excipient compatibility is a crucial aspect of the formulation development process for ensuring a safe and robust product development (Chadha & Bhandari, 2014).

Taking into consideration that ibuprofen is a highly reactive compound (contains carboxylic functional group) and the essential oils are known to have a complex chemical composition (Turek & Stintzing, 2013), the aim of this study was to investigate the solid-state compatibility of ibuprofen with Orange oil and Lavender oil which are commonly used essential oils for formulation of Ibuprofen gel for topical use.

Materials and methods

Materials

The non-steroidal anti-inflammatory drug ibuprofen was supplied by Solara Active Pharma Sciences (India), whereas the Orange oil and Lavender oil were supplied by IREKS Aroma d.o.o. (Croatia). Potassium dihydrogen phosphate (Merck), Orto-phosphoric acid 75% (Merck) and Acetonitrile (Merck) were also used.

Methods

Fourier-Transform Infrared (FTIR) spectroscopy: Varian 660 FTIR spectrometer (Varian Inc.) was employed for collection of the FTIR spectra of the binary mixtures. Attenuated total reflectance (ATR) spectra (resolution 4 cm^{-1}, 16 scans per spectrum) were obtained by MIRAcle ZnSe ATR module (PIKE technologies) with low-pressure micrometer clamp, in the 4000-550 cm^{-1-} region. The temperature-controlled FTIR ATR spectra were collected using GladiATR module with diamond crystal (PIKE technologies), coupled with variable temperature module and high-pressure micrometer clamp, in the 4000-400 cm^{-1} region.

Differential scanning calorimetry (DSC): The DSC measurements were performed on a NETZSCH DSC 204 F1 Phoenix instrument, in aluminium pans with a perforated lid (sample mass 3 mg), from 25 – 100 °C, at heating rate of 10 K/min under dynamic nitrogen atmosphere (30 mL/min).

High performance liquid chromatography (HPLC): The related and degradation products were analyzed

*ecvetkovska1@Alkaloid.com.mk

according to HPLC in-house method, using Thermo Ultimate 3000 HPLC system with diode array detector (214 nm, 220 nm, 240 nm and 260 nm) and Zorbax Eclipse XDB C18 (150 mm x 4.6 mm i.d; 5 µm) column (Agilent).

Results and discussion

The solid-state analysis of the binary mixtures of ibuprofen with the analyzed essential oils, has shown a possible solid-state interaction in the binary mixture of ibuprofen with Orange oil, in 1:1 ratio, exposed at 40 °C/75% RH. Namely, the FTIR spectrum of this binary mixture exhibits some vibrational bands in the region 1250 – 950 cm^{-1} which cannot be related neither to API, nor to the essential oil. Additionally, an appearance of new vibrational bands around 840 cm^{-1}, characteristic for the structure of limonene oxide (Pisarenko et al., 2008), were observed. Knowing that the main component of the Orange oil is limonene, which according to the literature data is unstable when exposed at elevated temperature and relative humidity, this might be an indication that the observed changes might be a result of some degradation process of the essential oil. On the other hand, changes were not observed in the binary mixture of ibuprofen and Lavender oil, neither in the initial, nor in the stressed binary mixture. However, in the DSC curves of both binary mixtures, the melting endotherm of ibuprofen was completely disappeared. This can be explained with its dissolving in the liquid essential oil at higher temperature. HPLC analysis was also performed and the results showed a continuous increase of the related impurities, with 8.25% increase of total impurities in the binary mixture of ibuprofen and Orange oil, exposed at 40 °C/75% RH, compared to 1.79% increase of total impurities in the binary mixture of ibuprofen with Lavender oil, exposed at the same stress conditions.

Based on the hypothesis that the increased impurities might originate as a result of some degradation process of the oil, the Orange oil itself was exposed to the same stress conditions for 30 days. However, no changes in the FTIR spectrum of the stressed essential oil were observed after this time. This shows that the observed changes were not due to the degradation of the oil, but as a result of an interaction that is taking place between ibuprofen and Orange oil, under certain conditions. To inspect the temperature influence on the possible interaction, temperature-controlled FTIR ATR spectra were collected from 25 – 40 °C (1 °C/minute), at constant relative humidity, for the binary mixture of ibuprofen and Orange oil in 1:1 ratio. However, the obtained spectra did not exhibit any changes. This result points out that the interaction is taking place only under certain combination of temperature and relative humidity.

To simulate storage temperature of the finished product, the same binary mixture was exposed for 30 days at 30 °C/75% RH and no changes in the FTIR spectra were observed under these conditions.

Since the ratio 1:1 is considered as worst-case scenario, the binary mixtures with both essential oils were prepared in formulation ratio 10:1, stressed under same conditions and analyzed with the same techniques. The FTIR spectra of the stressed binary mixtures, after 30 days exhibited no changes. Additionally, the HPLC analysis showed significant decrease of the total related impurities to 0.37% and 0.13% in the binary mixtures of ibuprofen with Orange oil and Lavender oil, exposed at 40°C/75% RH, respectively.

Conclusion

Summarizing the obtained results, it is evident that due to the complex nature of essential oils, some of them could easily interact with ibuprofen when used as excipients in formulations for topical administration. However, when Orange oil is used in formulation ratio and the product is properly stored, it is stable and do not interact with the API, confirming its safe application in the formulation. Therefore, detailed evaluation and careful choice of excipients is of crucial importance during formulation stage of the development in order to ensure a stable and safe finished product.

References

Chadha, R., Bhandari S., 2014. Drug-excipient compatibility screening – Role of thermoanalytical and spectroscopic techniques. Russ. Chem. Bull. 87, 82–97. https://doi.org/10.1016/j.jpba.2013.06.016

Gupta, K.R., Pounikar, A.R., Umekar, M.J., 2019. Drug-excipient compatibility testing protocols and characterization: A review. Asian J. Chem. Sci. 6, 1–22. https://doi.org/10.9734/ajocs/2019/v6i319000

Maswadeh, H.M., 2016. Incompatibility study of ibuprofen in ternary interactive mixture by using differential scanning calorimetry. J. Therm. Anal. Calorim. 123, 1963-1971. https://doi.org/10.1007/s10973-015-4773-z

Pisarenko, L.M., Krugovov, D.A., Shchegolikhin, A.N., Kasaikina, O.T., 2008. A kinetic model for limonene oxidation. Russ. Chem. Bull. 57, 83–89. https://doi.org/10.1007/s11172-008-0012-0

Turek, C., Stintzing, F.C., 2013. Stability of essential oils: A review. Compr. Rev. Food Sci. Food Saf. 12, 40–53. https://doi.org/10.1111/1541-4337.12006

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 73- 74 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.032
Short communication

A scoping review of regulatory guidelines for the assurance of medicinal product quality throughout their lifecycle

Ermira Krasniqi[1,2*], Jelena Ascevska[1], Katerina Brezovska[1], Arben Boshnjaku[3], Aneta Dimitrovska[1]

[1]*Faculty of Pharmacy, University "Ss Cyril and Methodius", Majka Tereza 47, 1000 Skopje, R. North Macedonia*
[2]*Faculty of Pharmacy, Alma Mater Europaea Campus "Rezonanca", Glloku te Shelgjet "Veternik", 10000 Prishtinë, Kosova*
[3]*Faculty of Medicine, University "Fehmi Agani" in Gjakova, 50000 Gjakovë, Kosova*

Introduction

Quality, defined as the degree to which a set of inherent characteristics fulfills requirements (ICH Q9; ICH Q10) remains a crucial part of regulatory framework, with many requirements and guidelines aiming the safe use of medicinal products by assuring highly qualitative products.

Many steps should be taken as measures and procedures to follow towards the realization of a product during product`s lifecycle, including: Pharmaceutical Development, Technology Transfer and Commercial Manufacturing up to Products Discontinuation.

World is giving much interest to the unifying of documents preparation and applying processes, while more than 40 guidelines have been harmonized to date amongst the 3 regions: Europe, United Stated of America (USA) and Japan. Including the aiming of International Pharmaceutical Federation (FIP) and the International Federation of Pharmaceutical Manufacturers Associations (IFPMA), pharmaceutical industry is considered the most regulated one for more than 50 years (Phillips et al., 2015).

Considering this complicated though important process, the main theme of this short paper is analysis of the existing guidelines for Quality, thus providing information from all aspects of pharmaceutical quality.

Materials and methods

This scoping review provides an overview of the current state of the art within the quality requirements, guidelines, practices and standards in general, and steps taken toward continual improvement of all processes. During the process, databases and guidelines of ICH, EU, WHO and ISO were analyzed. Additional published data were included from PubMed search engine.

Further analysis included discussion of the documents complements for the quality practices, requirements, standards, guidelines, auditing/inspection, staff responsibilities and training/education within the pharmaceutical industry and regulatory environment.

Results and discussion

There are many components that enable the system to function in service of the main purpose of the system itself, with quality undeniably reaching the top of the list.

Development and commercial manufacturing, up to the discontinuation of pharmaceutical products are processes subjected to government regulation, review and approval of new products and site inspection for quality management of production, packaging, storage, and distribution (Xu et al., 2016). Furthermore, all these stages of the product lifecycle need an established connection in between and sophisticated control accompanied by relevant procedures, documentation, technology, and qualified staff.

The quality of a medicinal products throughout the whole lifecycle is achieved by risk-based and science-based approaches as a result of the creation of a Quality Management System (QMS) based on the International Council of Harmonization (ICH) Guidelines, Good Manufacturing Practice (GMP) certification and ISO standards.

Since 2003 when ICH developed the Quality Vision "Develop a harmonized pharmaceutical quality system applicable across the life cycle of the product emphasizing an integrated approach to quality risk

*Ph.ermirakrasniqi@gmail.com

management and science" (Brussels, July 2003), several Guidelines (Q8-Q12) have been generated. ICH Quality Guidelines present the risk, approaches and principles towards qualitative product and a consistent product`s life from the initial development through marketing until the product`s discontinuation (Lifecycle Management, LCM). Requirements of Good Distribution Practice (GDP) and Good Pharmacy Practice (GMP) are also very important toward consistent integrity of product`s life.

Considering all the ICH guidelines for the quality assurance of the product during lifecycle such as ICH Q8, Q9, Q10, Q11 and the harmonization in between them, there were still some gaps existing in the terms of full realization of the benefits, which were intended to be met by the new guideline named ICH Q12. This guideline aims continual assurance of high-quality products, promote innovation and continual improvement, a transparent and efficient management of post-approval Chemistry (ICH Q12).

Pharmaceutical quality management and product lifecycle management remains crucial in terms of harmonizing the industry, assessors and inspectors, as well as providing a great benefit to the public health by enhancing the quality and availability of medicines worldwide. In a good quality system and approved compliance with local and international regulatory requirements (since pharmaceutical products and raw materials are manufactured and distributed worldwide) medicinal products will not be sold or supplied before certification that each production batch has been produced and controlled in accordance with the requirement of marketing authorization (MA) and other relevant requirement for the production, control, and product release.

A very complex system of quality, consisting of the concepts of Quality Assurance (QA), Quality Control (QC) and Quality Risk Management (QRM) must be in place.

It should be finally noted that the top 5 quality attributes that are related to management responsibility and continual improvement are (Patel et al., 2015): (i) management communication that quality is everyone's responsibility, (ii) site has formal quality improvement objectives and targets, (iii) clear performance criteria for feedback and coaching, (iv) quality topics included in at least half of all-hands meetings, and (v) collecting error prevention metric.

Proper documentation and records is a basic system in a good pharmaceutical quality system, enhancing visibility of the quality assurance. This comes especially because it is considered that all products and processes have an inherent element of risk, enforcing manufacturers to focus on non-conformities, possible deficiencies and planning of preventive actions in order to anticipate and prevent future possible problems (Haleem et al., 2015).

Conclusion

It is strongly believed that the concept on ICH Q12 Guideline will make possible the quality assurance of products during their full lifecycle, including stages after regulatory approval. The building of an integrated quality system from the industry will help the industry itself for the improvement toward achieving the objective of high qualitative products during the lifecycle of the product, good system documentation, measurement of performance, continual improvement, while helping the regulators for the oversight of industries and (above everything) enabling patients the usage of high qualitative products. It is of great importance that the post-approval phase should be given as much importance as pre-approval process, not just in regard to the safety but also quality of medicinal products.

The global emphasis that is given today to post-approval product lifecycle management and the changes supported by risk and science-based approaches, will help industry achieving their goals and objectives.

References

Haleem, R.M., Salem, M.Y., Fatahallah, F.A., Abdelfattah, L.E., 2015. Quality in the pharmaceutical industry – A literature review. Saudi Pharm J. 23(5), 463–469. https://doi.org/10.1016/j.jsps.2013.11.004

International Conference of Harmonization ICH Q10 Pharmaceutical Quality System. 2008. http://www.gmp-compliance.org/guidemgr/files/ICH%20Q10%20STEP%204.PDF (last accessed date June 2022).

International Conference of Harmonization ICH Q12 Life cycle Management https://database.ich.org/sites/default/files/Q12_Guideline_Step4_2019_1119.pdf (last accessed date June 2022)

International Conference of Harmonization ICH Q9 Quality Risk Management.2005. http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q9/Step4/Q9_Guideline.pdf (last accessed date June 2022).

Patel, P., Baker, D., Burdick, R., Chen, C., Hill, J., Holland, M., Sawant, A., 2015. Quality Culture Survey Report. PDA J Pharm. Sci. Technol. 69, 631-642. https://doi.org/10.5731/pdajpst.2015.01078.

Phillips, M., Kashyap, V., Cheung, MS., 2015. Increasing Product Confidence-Shifting Paradigms PDA J Pharm Sci Technol. 69(6), 736-742. https://doi.org/10.5731/pdajpst.2015.01075

Xu, W., Boehm, G., Zheng, Q., 2016. Factors to consider in developing individual pharmaceutical product quality risk profiles useful to government procurement agencies Acta

Pharm. Sin. B. 6(1), 79–92. https://doi.org/10.1016/j.apsb.2015.09.009

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 75 - 76 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.033
Short communication

Application of experimental design for determination of insulin analogs

Blerta Pajaziti[1]; Melinda Andrási[2]; Liljana Anastasova[1]; Dashnor Nebija[3]; Blin Pajaziti[3]; Rumenka Petkovska[1*]

[1] *Faculty of Pharmacy, Ss. Cyril and Methodius University, Mother Theresa 47, 1000 Skopje, North Macedonia,*
[2] *Department of Inorganic and Analytical Chemistry, University of Debrecen, Egyetem tér. 1, 4032 Debrecen, Hungary*
[3] *Department of Pharmacy, University of Prishtina "Hasan Prishtina, Bulevardi i Dëshmorëve, p.n., 10000 Prishtina, Kosovo*

Introduction

Determination of human insulin and its analogs and their related compounds is a challenge as a consequence of very similar chemical structure of these compounds. The most common methods for determination of insulin and its related compounds in pharmaceutical formulations are: radioimmunoassay, enzyme immunoassay, luminescent immunoassay, high performance liquid chromatography and capillary electrophoresis (Arcelloni et al., 1998; Dezier et al,. 1987). Capillary electrophoresis (CE) is a popular method permitting separation of analytes from small molecules to large biomolecules. Its advantages include simplicity, high separation efficiency, short analysis time and low sample and solvent consumption, making this a powerful alternative method to High performance liquid chromatography (HPLC). Consequently, there were several methods of CE developed for pharmaceutical analysis of insulin formulations (Arcelloni et al., 1998; Lamalle et al., 2015 Ortner et al., 2009).

The International Chemometrics Society (ICS) defines chemometrics as the science of relating measurements made on a chemical system or process to the state of the system via application of mathematical or statistical methods (Gemperline, 2006). Design of experiments (DoE) is a statistical method to develop optimization that is used across a variety of industries. It possesses a numerous advantages over the traditionally employed "one variable at a time" (OVAT) approach, such as increased experimental efficiency, as well as an ability to resolve factor interactions and provide detailed maps of a process's behavior (Bowden et al., 2019). To improve the separation process, CE coupled with chemometric optimization could provide a complete profile of separation, offering the useful information of factors influencing the separation as well as their interactions. The principle of DoE is arrangement of the experiments at changed combination of factors with the purpose of gaining the maximum information with minimal runs. This process is arranged by changing all the applicable factors simultaneously, according to a planned set of experiments which determine the effect of each factor and their significance (Aboul- Harnisch et al., 2018; Enein and Abdel-Megied, 2019; Orlandini et al., 2014).

Therefore, in this study, we demonstrate research of the main factors influencing migration behavior of insulin and its analogues. For this purpose, application of Response Surface Methodology (RSM) enabled the optimization of a CE method with the objective to improve the separation of insulin analogs.

Materials and methods

Chemical and reagents

Insulin and analogues were used from pharmaceutical formulations including Apidra® SoloStar® (Sanofi), Humalog® (Lilly), Humulin®R (Lilly), Lantus® SoloStar® (Sanofi), Levemir® FlexPen® (Novo Nordisk), NovoRapid® Penfill® (Novo Nordisk). The analytical reagents: sodium hydroxide, ammonium acetate, ammonia, Acetic acid and hydrochloric acid were purchased from Sigma-Aldrich.

*rupe@ff.ukim.edu.mk

Instrumentation

The instrumentation used was the CE 7100 system (Agilent, Waldbronn, Germany). For separation the fussed silica capillaries (Polymicro Technology, Phoenix, AZ, USA) with an internal diameter of 50 µm and a total length of 65 cm in positive mode using constant voltage were used. Samples were introduced with a pressure of -50 mbar for 4s at the cathodic end of capillary. Aiming to prevent the adsorption of proteins in the capillary wall, a strong preconditioning was used. This procedure included conditioning of the capillary between each injection for 18 min with 0.1M NaOH, 6 min with acetonitrile and 24 min with the running buffer. The statistical software MODDE® was used for the DoE.

Experimental design

In this study the factors with higher influence in the migration behavior of each insulin analogue were selected and analyzed using Experimental design. After a preliminary study these factors were chosen to be the pH and buffer concentration of the background electrolyte and the voltage applied. The impact of the above-mentioned factors separately and their interaction (combination of the factors) were analyzed by RSM design called CCF. The levels of factors selected for optimization were the pH value (8, 9, 10) and the concentration (40, 50, 60) of the background electrolyte (BGE) and voltage (10, 20, 30). The number of total experiments calculated for these 3 factors was 15.

Results and discussion

Six insulin analogues were determined using this Experimental design. After finishing the required CE runs in different conditions, for each insulin analog was calculated the migration time and the influence of these factors on the migration behavior was studied. The most influencing factor for migration time of each insulin analog resulted to be the voltage. Its influence was more significant than any other factor, or the combination of the factors (pH and BGE concentration, pH and voltage or BGE concentration and voltage). Other influencing factor is pH value of the BGE, which also has an important effect on the separation time. These discoveries suggest that the pH variation appears to be the most effective method of controlling a CE separation and accordingly analysis time.

Conclusion

Applying the RSM design, was able to find the optimal range of the most crucial factors including buffer pH and concentration, in relation of analysis time reduction. In consideration of the fact that when determining proteins and peptides with CE, a long preconditioning time is necessary to remove the adsorbed molecules in capillary wall, the appropriate analysis time for this determination would be 8-10 min. After several analysis for each insulin analogue, the optimal scope of specified factors to obtain acceptable analysis time were: applied voltage 20-25 kV, the buffer pH: 8.5-9.5 and the buffer concentration 40 mM ammonium acetate.

References

Aboul-Enein, H.Y., Abdel-Megied, A.M., 2019. A Chiral eneric Strategy for Enantioseparation of Acidic and Basic Drugs Using Short End Injection Capillary Electrophoresis: Application to Design of Experiment. Methods Mol. Biol. 1972, 127-136. https://doi.org/10.1007/978-1-4939-9213-3_9

Arcelloni, C., Falqui, L., Martinenghi, S., Pontiroli, A.E.,Paroni, R., 1998. Capillary Electrophoresis for Simultaneous Quantification of Human Proinsulin, Insulin and Intermediate Forms. Electrophoresis 19(8–9), 1475–1477. https://doi.org/10.1002/elps.1150190843

Bowden, G.D., Pichler, B.J., Maurer, A., 2019. A Design of Experiments (DoE) Approach Accelerates the Optimization of Copper-Mediated 18F-Fluorination Reactions of Arylstannanes. Sci. Rep. 9(1), 11370. https://doi.org/10.1038/s41598-019-47846-6

Dezier, J.F., Jouanolle, A.M., Reun, M., Poirier, J.Y,. Comparison of 2 Methods of Measuring Microalbuminuria. Immunonephelometry and Radioimmunology. Ann. Biol. Clin. 45(1), 78–84.

Gemperline, P., 2006. Practical Guide To Chemometrics, Second ed. Anal. Bioanal. Chem. 388, 511–512. https://doi.org/10.1201/9781420018301

Harnisch, H., Chien, Y-H., Scriba, G. K. E., 2018. Capillary Electrophoresis Method for the Chiral Purity Determination of Pregabalin Derivatized with Dansyl Chloride. Chromatographia 81(4), 719–725. https://doi.org/10.1007/s10337-018-3495-3

Lamalle, C., Servais, A-C., Radermecker, R., Crommen, J., Fillet, M., 2015. Simultaneous Determination of Insulin and Its Analogues in Pharmaceutical Formulations by Micellar Electrokinetic Chromatography. J. Pharm. Biomed. Anal. 111, 344–350. https://doi.org/10.1016/j.jpba.2014.12.038

Orlandini, S., Gotti, R., Furlanetto, S., 2014. Multivariate Optimization of Capillary Electrophoresis Methods: A Critical Review. J. Pharm. Biomed. Anal. 87, 290-307. https://doi.org/10.1016/j.jpba.2013.04.014

Ortner, K., Buchberger, W., Himmelsbach, M., 2009. Capillary Electrokinetic Chromatography of Insulin and Related Synthetic Analogues. J. Chromatogr. A 1216(14), 2953–57. https://doi.org/10.1007/978-1-4939-9213-3_9

Macedonian Pharmaceutical Bulletin, 68 (Suppl 1) 77 - 78 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.034
Short communication

Investigation of the effect of different flow-through cell design on the release of prolonged release tablets

Magdalena Velichkovska*, Filip Smilchevski, Ema Ilievska Vidanovska, Maja Hadzieva Gigovska, Blagica Manchevska, Sonja Dimchevska, Packa Antovska, Sonja Ugarkovic

Alkaloid AD, Aleksandar Makedonski 12, 1000, Skopje, Republic of North Macedonia

Introduction

The dissolution test is an essential tool for characterization of the performance characteristics of solid oral dosage forms and evaluation of its bioavailability.

Although basket and paddle methods are currently the most popular methods for many drug products, they do not provide a detailed picture about the in vivo behavior of the drug product.

The flow-through cell (FTC) method permits continuous extraction of the drug, simulating absorption into the systemic circulation, generating an intermittent flow of the dissolution medium into the cell where the dosage form is placed (Emara et al., 2014). It can be used as an open system, allowing release under sink conditions, which facilitates the dissolution of poorly water-soluble drugs, as well as a change of the dissolution medium within a pH range of physiological relevance (Fotaki & Reppas, 2005).

The advantage of dissolution using FTC apparatus with open loop configuration is that it provides an environment potentially closer to that of the digestive tract (Gao, 2009).

The aim of this study was evaluation of the effect of different dissolution and hydrodynamic conditions of the FTC, on the release rate of the active pharmaceutical ingredient (API) from prolonged release tablets.

Materials and methods

Materials

Prolonged release tablets containing BCS III class component from two different manufacturers were used. All products used in this study contained 80 mg of active ingredient. All reagents used were of analytical grade.

Dissolution method

Dissolution testing using FTC apparatus has been performed according to current Ph. Eur. requirements (2.9.3. Dissolution test for solid dosage forms). The FTC, USP Apparatus 4 was a Sotax CE 7smart equipped with a CP 7-35 digital piston pump (Sotax, Switzerland). A built in filtration system with 0.7 Whatman glass microfiber (GF/F) and manual filtration with Phenex 0.45 µm regenerated cellulose (RC) was used throughout the study. The temperature of the dissolution medium was kept at 37 ± 0.5 °C.

Media used were: simulated gastric fluid without enzymes (SGF-Medium 1), pH 4.5 acetic (Medium 2) and pH 6.8 phosphoric (Medium 3) prepared according to current Ph. Eur. requirements (5.17.1. Recommendations on dissolution testing).

Different cell sizes, flow rates of dissolution medium and time intervals within the FTC were considered.

The dissolution studies were performed with the tablet secured on a tablet holder, without glass beads (turbulent flow) or in the presence of glass beads, each 1 mm in diameter (laminar flow). Two flow-through cells having internal diameters of 12 (small cell) and 22.6 mm (large cell) were used. The dissolution media were pumped at flow rates of 2, 4, 8, 16 mL/min.

*mvelickovska@Alkaloid.com.mk

Medium intervals were the following: 1) 1 hour in medium 1, 1 hour in medium 2, up to 12 hours in medium 3; 2) 30 minutes in medium 1, 30 minutes in medium 2, up to 12 hours in medium 3; 3) 30 minutes in medium 1, 1 hour in medium 2, up to 12 hours in medium 3.

Chromatographic method

Quantification was performed using HPLC method. The mobile phase was comprised of sodium heptansulfonate solution adjusted to pH 2.0, acetonitrile and methanol in ratio 70: 10: 20 (v/v/v), at a flow rate of 0.8 mL/min. Kromasil C18 150 mm x 4.6 mm i.d; 5µm column was used, maintained at 55 °C. Detection was at 230 nm wavelength. Run time of 12 min is utilized with injection volume of 50 µL from the sample and the standard solution.
The amount dissolved was calculated with the following formula:

$$Amount\ dissolved\ (mg) = Concentration\ \left(\frac{mg}{ml}\right) * flow \left(\frac{ml}{\min}\right) * time\ interval (min)$$

The cumulative release profile of API has been evaluated with f_2 statistics between the test and reference product.

Results and discussion

In order to assess the influence of FTC on drug release profile, dissolution data from the test and reference product were compared. The concentration (mg/mL) of API has been determined using standard calibration curve with 4 concentration levels (0.00444-0.17776 mg/mL).

According to the obtained results, the dissolution profiles of the test and reference product was similar, but the reference product releases the API faster than the test product in the first time points. The f_2 factor demonstrates no significant difference between the products (it ranges from 64.04 to 80.26 between different experiments) showing that all evaluated effect does not significantly influence the release rate of API.

In this study, the cell size had almost no effect on API dissolution rate, as the use of glass beads in the cells. When 2 mL/min flow was applied there was a slight decrease in API release rate. Highest f_2 factor was observed when 4 ml/min was employed.

Lowest f_2 factor was observed when media intervals were set as 30 minutes in medium 1, 30 minutes in medium 2, up to 12 hours in medium 3. The other two sets had high f_2 values with no difference between them. Lower release rate of API, after the last pH change, was demonstrated with the following conditions: 30 minutes in medium 1, 1 hour in medium 2, up to 12 hours in medium 3.

Investigation of the effect of FTC parameters on the release of API from prolonged release tablets show that the most significant affect has the flow rate.

Conclusion

Evaluation of the effect of different factors in this study showed that cell size and presence of glass beads do not have significant effect on API dissolution rate. From all investigated factors, only the flow rate has demonstrated considerable effect on the release rate.

In general, the similarity between the test and reference product is maintained throughout all evaluated experiments with apparatus FTC.

When observing the cumulative release profile graphs, up to 2 hours, both products have same dissolution profiles and after 2 hours, the test product exhibits slightly faster release profile than the reference product. Concentration / time profiles, demonstrate that the reference product releases the API faster than the test product in the first time points.

References

Emara, L.H., Emam, M.F., Taha, N.F., El-Ashmawy, A.A., Mursi, N., 2014. In vitro dissolution study of meloxicam immediate release products using flow-through cell (USP apparatus 4) under different operational conditions. Int J Pharm Pharm Sci. 6, 254−260. https://innovareacademics.in/journals/index.php/ijpps/article/view/3094

Fotaki, N., Reppas, C., 2005. The flow through cell methodology in the evaluation of intralumenal drug release characteristics. Dissolution Technol. 12, 17−21. https://dx.doi.org/10.14227/DT120205P17

Gao, Z., 2009. In Vitro Dissolution Testing with Flow-Through Method: A Technical Note. AAPS PharmSciTech. 10(4), 1401-1405. https://doi.org/10.1208/s12249-009-9339-6

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 79 - 80 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.035
Short communication

Practical examples of implementation of the Unique Device Identifier (UDI) requirements for Medical Devices

Dragica Trpkoska*, Natasha Vukikjevikj

Pharmaceutical Chemical Cosmetic Industry ALKALOID AD Skopje, Blvd. Aleksandar Makedonski 12, 1000 Skopje, Republic of North Macedonia

Introduction

The new European Medical Device Regulation 2017/745 (MDR) introduces a Unique Device Identification (UDI) system for medical devices. The UDI system should allow the identification of medical devices, facilitate appropriate traceability of medical devices, enhance the effectiveness of the post-market safety-related activities for devices, improve incident reporting, enhance targeting field safety corrective actions, lead to better surveillance, reduce medical errors, and help fight against falsified devices. As such, the UDI system is intended to be incorporated into the life-cycle of the device (MDCG 2018-1 Rev.4; MDCG 2021-19).

Unique Device Identifier (‘UDI’) means a series of numeric or alphanumeric characters that is created through internationally accepted device identification and coding standards and that allows unambiguous identification of specific devices on the market (MDR (EU) 2017/745).

EUDAMED is the IT system established by the MDR and developed by the European Commission. One of the six interconnected modules in the EUDAMED is the UDI/Devices registration. This requires that manufacturers submit in EUDAMED the UDI/Device information of all devices they place on the EU market, and that they should keep the information updated (EC: MD - EUDAMED Overview).

The structure of UDI

Basic UDI-DI

The Basic UDI-DI is the main key in the database and relevant documentation (e.g. certificates, declaration of conformity, technical documentation and summary of safety and clinical performance) to connect devices with same intended purpose, risk class and essential design and manufacturing characteristics. It is independent/separate from the packaging/labelling of the device and it does not appear on any trade item (MDCG 2018-1 Rev.4).

UDI (UDI-DI and UDI-PI)

The UDI may include information on the lot or serial number and be able to be applied anywhere in the world. The production of a UDI comprises the following (EC: FAQs: UDI System):

- A UDI device identifier (‘UDI-DI’) is specific to each device. It is a unique numeric or alphanumeric code, specific to a model/variation/version. It is the device identifier used as the “access key” to all information stored in the UDI database, which is part of the EUDAMED database.
- A UDI production identifier (‘UDI-PI’) identifies the unit of device production and if applicable the packaged devices. It is a numeric or alphanumeric code. The different types of UDI-PIs include the serial number, lot number, software identification, manufacturing and/or expiry date. (EC: (EU) UDI Helpdesk).

UDI Carriers

The UDI Carrier [Automated Identification for Data Capture (AIDC) and human readable interpretation (HRI) representation of the UDI] shall be on the label or on the device itself and on all higher levels of device packaging. Higher levels do not include shipping containers (MDR (EU) 2017/745).

The UDI must appear in a plain-text version/human readable information (HRI) and in a form that uses AIDC

*dtrpkoska@alkaloid.com.mk

technology. AIDC means any technology that conveys the unique device identifier or the device identifier of a device in a form that can be entered into an electronic patient record or another computer system via an automated process. The HRI consists of legible characters that can easily be read by people. If there are significant constraints limiting the use of both AIDC and HRI on the label, only the AIDC format shall be required to appear on the label. The AIDC format can be presented as 1D barcodes, 2D barcodes, dot-matrix codes, biometrics, RFID (Radio Frequency Identification).

If linear bar codes are used, the UDI-DI and UDI-PI may be concatenated or non-concatenated in two or more bar codes. All parts and elements of the linear bar code shall be distinguishable and identifiable. If the manufacturer is using RFID technology, a linear or 2D bar code in line with the standard provided by the issuing entities shall also be provided on the label.

In the event of significant space constraints on the unit of use packaging, the UDI carrier may be placed on the next higher packaging level. Higher levels of packaging shall have their own unique UDI.

If there are significant constraints limiting the use of both AIDC and HRI on the label, only the AIDC format shall be required to appear on the label (EC: (EU) UDI Helpdesk).

The UDI carrier shall be readable during normal use and throughout the intended lifetime of the device. If the UDI carrier is readily readable or, in the case of AIDC, scannable, through the device's packaging, the placing of the UDI carrier on the packaging shall not be required.

In the case of single finished devices made up of multiple parts that must be assembled before their first use, it shall be sufficient to place the UDI carrier on only one part of each device.

Devices that are reusable shall bear a UDI carrier on the device itself.

Device contents of system or procedure packs shall bear a UDI carrier on their packaging or on the device itself. UDI carrier shall as a general rule be affixed to the outside of the packaging.

Designated Issuing Entities for UDI Provision

Only the manufacturer may place the UDI on the device or its packaging. However, the UDI must be unique, and obtained by Designated Issuing Entities for UDI Provision. The European Commission has designated 4 issuing entities: GS1 AISBL, HIBCC (Health Industry Business Communications Council), ICCBBA (International Council for Commonality in Blood Banking Automation) and IFA GmbH (Informationsstelle für Arzneispezialitäten) (EC:UDI).

Implementation period of UDI

The obligation for placing the UDI carrier on the labels of MDR certified medical devices applies according to the following timelines:

- Implantable and Class III devices: from 26th May 2021.
- Class IIa and IIb devices: from 26th May 2023.
- Class I devices: from 26th May 2025.

Conclusion

Taking into consideration the entire medical device related information that the UDI is caring, the UDI is not just an ordinary barcode. The implementation process of the UDI Requirements is still ongoing, for the majority of the medical device manufacturers. It is rather challenging process that requires constant follow up to legislation and related guidelines in order to fulfil the MDR requirements, and ultimately to offer improved protection and safety for patients and users, guaranteeing transparency and information.

References

Council of Europe, 2017. Regulation (EU) 2017/745 of the European Parliament and of the Council of 5 April 2017 on medical devices, amending Directive 2001/83/EC, Regulation (EC) No 178/2002 and Regulation (EC) No 1223/2009 and repealing Council Directives 90/385/EEC and 93/42/EEC. Official Journal of the EU. L 117, 1-175.

European Commission, (EU) UDI Helpdesk. Available at: https://eu-udi.zendesk.com/hc/en-150

European Commission, FAQs UDI System. Available at: https://ec.europa.eu/health/system/files/2020-09/md_faq_udi_en_0.pdf

European Commission, Medical Devices, EUDAMED Overview. Available at: https://ec.europa.eu/health/medical-devices-eudamed/overview_en

European Commission, Unique Device Identifier – UDI. Available at: https://ec.europa.eu/health/medical-devices-topics-interest/unique-device-identifier-udi_en

MDCG 2018-1 Rev.4 Guidance on BASIC UDI-DI and changes to UDI-DI. Available at: https://ec.europa.eu/health/system/files/2021-04/md_mdcg_2018-1_guidance_udi-di_en_0.pdf

MDCG 2021-19 Guidance note integration of the UDI within an organisation’s quality management system. Available at: https://ec.europa.eu/health/system/files/2021-07/md_2021-19_en_0.pdf

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 81 -82 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.036
Short communication

Re-validation during method transfer examples in Official medicines control laboratories

Marija Zafirova Gjorgievska*, Gabriela Petrovska-Dimitrovska, Vasil Karchev, Hrisanta Godzo, Olga Gigopulu, Liljana Ugrinova

[1]*Center for Drug Quality Control, Faculty of Pharmacy, Ss. Cyril and Methodius University, Majka Tereza 47, 1000 Skopje, Republic of North Macedonia*

Introduction

Method transfer represents a completely documented process which covers transferring of analytical methods from the originating laboratory which developed and validated the method, to the receiving laboratory for quality control of medicines. The method transfer, is an experimental demonstration that the receiving laboratory, can perform the method accordingly to its use. Successful transfer of a method should prevent the placing on the market of a medicine which does not conform to the quality specification, as well as to avoid the rejection of a medicine whose quality conforms to its specification.

The common practice of method transfer, requires verification of the method established by the manufacturer, from the competent authority/official medicines control laboratory, or in case of change of the method outside the acceptable limits, it is necessary to carry out re-validation. Depending on the scope of the changes, re-validation can be complete or partial, using appropriate validation parameters.

In this paper, two examples of method transfer including partial re-validation are presented for: dissolution using a spectrophotometric method for quantification and determination of related substances using HPLC method with gradient elution.

Examples of partial re-validation during method transfer

Example 1: Method transfer with partial re-validation for dissolution of valsartan/hydrochlorothiazide film-coated tablets (80 mg/12.5 mg and 160 mg/12.5 mg) using UV-spectrophotometric method

The method from the originating laboratory defines an on-line dissolution test using a UV-spectrophotometer with a 1 mm cell. Many of the Official medicines control laboratories are equipped with off-line disolution testing unit, and in case of spectrophotometric determination, a standard cuvette of 1 cm. With the proposed concentration of the standard and sample solutions, the obtained absorbances with the 1 cm cuvette, exceed the linearity range of the used equipment (Agilent 8453 UV-Vis spectrophotometer). In order to acquire adequate values for the measured absorbance, the concentration of the soluitions was adjusted.

According to the ICH Q2(R1) quideline for validation of analytical procedures, the nature of the change demands partial re-validation on the parameter linearity. The linearity was evaluated for both active substances in the range of 30 - 120% of the working concentration (0.053 mg/mL for valsartan and 0.0083 mg/mL for hydrochlorothiazide), on both wavelenghts, as required in the proposed method (250 nm and 272 nm).

The results from the re-validation confirm the linearity of the method in the chosen concentration range, according to the requirements of the ICH Q2(R1) quideline (for valsartan, correlation coefficents (R^2) of the regression lines were 1.0000 and 1.0000, respectively for

*marijazafirova@ff.ukim.edu.mk

wavelength 250 nm and 272 nm; for hydrochlorothiazide, R^2 of the regression lines were 0.9994 and 0.9996, respectively for wavelength 250 nm and 272 nm).

The results from the dissolution test of both dosage forms were within the specification limit.

Example 2: Method transfer with partial re-validation for determination of related substances of fingolimod in hard capsules using HPLC method with gradient elution

The method from the originating laboratory, for related substances of fingolimod, proposes usage of the chromatographic column XTerra MS C8 50 x 4.6 mm, 2.5 µm. The OMCL, Center for Drug Quality Control_MK, used an available column with similar properties of the stationary phase and similar dimensions, Poroshell 120 EC-C8 50 x 4.6 mm 2.7 µm, taking into account that due to the vast offer of chromatographic columns on the market, very often the OMCLs do not have the columns proposed in manufacturer methods at their disposal.

In order to achieve the system suitability requirements, an adjustment of the flow rate was required. The final chromatographic conditions under which system suitability was obtained, included flow rate of 1.9 mL/min instead of 1.5 mL/min, under gradient elution. According to the permited adjustments of the chromatographic conditions for gradient elution (Ph.Eur. 2.2.46), the change of the flow rate, the change from Totally Porous Particle (TPP) column to Superficially Porous Particle (SPP) column and the change of the particle size of the column, and are outside of the specified limits.

Partial re-validation due to the nature of the change was performed on the parameters: specificity, limit of quantification, system precision and accuracy on the concentration level equal to the specification limit of unknown impurity (1.0%). Specificity in regard to placebo solution was confirmed, as well as the proposed limit of quantification of 0.1%. Results for system precision were RSD = 0.5%, and for accuracy on the concentration level equal to the specification limit of unknown impurity (1.0%), recovery was 100.38% ± 1.6 % (95% level of confidence). The results from the re-validation are in line with the requirements of the ICH Q2(R1) quideline.

The results from the testing of the related substances of fingolimod in the dosage form were within the specification limits.

Conclusion

The examples for partial re-validation of the methods during method transfer are a common practice for the receiving laboratory. The laboratory work regarding re-validation is a demanding and time-consuming process. With the current practice for pharmacopoeial harmonization, regarding the analytical techniques, and the announced harmonized text for chromatographic techniques, Ph.Eur. 2.2.46, which will be officially published on January 1st of 2023, in the 11th edition of the European Pharmacopoeia, re-validation waiver of the methods is proposed, which will greatly facilitate the laboratory work.

For some analytical techniques, the need for re-validation remains necessary, as is the example with the partial re-validation of the spectrophotometric method, using a different length of the used cuvette.

References

European Pharmacopoeia (Ph. Eur.) 10th Edition, Council of Europe, Strasbourg, France, 2019.

International Conference on Harmonization Technical Requirements for Registration of Pharmaceuticals for Human Use. Guideline on Validation of Analytical Procedures: Text and Methodology Q2(R1). Geneva, Switzerland, 2005.

United States Pharmacopoeia-National Formulary (USP–NF) 2022 Issue 1, United States Pharmacopeial Convention, Rockville, USA, 2021.

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 83 – 84 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.037
Short communication

Understanding and evaluation of different degradation pathways and stability of drug product with active substance prone to chemical and physical degradation

Ana Atanasova[1*], Frosina Jovanovikj[1], Isidora Miovska[1],
Veronika Popovska Jakimovska[1], Maja Stevanoska[1];, Filip Gogu[1], Packa Antovska[1],
Jelena Lazova[1], Nikola Geskovski[2], Jasmina Tonic Ribarska[2]

[1]*Research and development, Alkaloid AD Skopje, Aleksandar Makedonski 12, 1000 Skopje, North Macedonia*
[2]*Faculty of Pharmacy, Ss Cyril and Methodius University in Skopje, Majka Tereza 47, 1000 Skopje, North Macedonia*

Introduction

Ensuring adequate stability of the active substance in its formulation in the final pharmaceutical dosage form is one of the key challenges in the pharmaceutical industry. Drug instability causes a decrease in the potency and the amount of drug substance delivered to the patient, but can also cause formation of undesirable degradation products (Sengupta et al., 2018). The possibility of predicting degradation products that may form from small-molecule organic pharmaceuticals is of a great importance. Particularly when the active component is prone to both chemically and physically induced degradation (Bhangare et al., 2022). If significant degradation takes place between manufacture and administration of the drug product then there is a risk of inadequate dosing. For treatment of hypertension and cardiovascular disease dosing accuracy is very important since ineffective treatment is likely to result in life-threatening complications. ACE inhibitors are among several antihypertensive drugs preferred for initial management of hypertension and/or cerebrovascular disease.

One of the most frequently prescribed angiotensin-converting inhibitors that has the broadest spectrum in terms of therapeutic indications relative to other drugs within the ACE inhibitor class, was subject of this study. Literature survey showed that this compound is very sensitive to light, moisture, heat, physical or chemical stress.

There are several methods described in the literature for preventing the decomposition of active substances prone to induced physical and chemical degradation (Grangeia et al., 2020; Hotha et al., 2016). In this study three approaches were tested and different degradation pathways of the active substance were observed.

Materials and methods

Tablet formulations and manufacturing

Laboratory trial N°1 for testing the first approach contains: pregelatinized maize starch (low moisture) microcrystalline cellulose (low moisture), silicon dioxide, and glycerol dibehenate. The active component was dry mixed with excipients with low water content. Laboratory trial N°2 for testing the second approach contains: hypromellose (HPMC), microcrystalline cellulose, pregelatinized maize starch, croscarmellose sodium and sodium stearyl fumarate. The polymer film can protect the active substance against external influences such as moisture and counteracts the mechanical deactivation. Laboratory trial N°3 for testing the third approach contains: hypromellose (HPMC), microcrystalline cellulose, sodium hydrogen carbonate, pregelatinized maize starch, croscarmellose sodium and sodium stearyl fumarate. In this approach, the active substance was mixed with a physiologically tolerated buffer which ensures that a pH in weakly alkaline range is set up, and is also coated with a polymeric protective coating.

*ageorgieva@alkaloid.com.mk

Laboratory trial tablets N°1 were manufactured by direct compression, and laboratory trial tablets N°2 and N°3 were manufactured by using a wet granulation process.

Tablet stability studies

Tablets were stored in cold forming aluminum/aluminum blister with proper number of tablets per blister as primary packaging and printed cardboard box as secondary packaging at 25 °C/60% relative humidity for 9 months and 40 °C/75% relative humidity for 6 months. The samples were tested for assay, related and degradation products, pH and water content.

High-performance liquid chromatography

HPLC analysis for determination of related and degradation products was performed by using a 5 µm, 250 x 4.6 mm i.d. Zorbax SB-CN column (Agilent Technologies, California, USA) at 65 °C with a mobile phase flow rate of 1.0 mL/min. The gradient elution used acetonitrile and phosphate buffer adjusted to pH 2.0 with orto-phosphoric acid. The initial mobile phase composition of 5% acetonitrile after initial hold of 5 minutes was increased to 40% linearly over 25 minutes, followed by an increase to the final composition of 60% linearly over 7 minutes and held at this composition for 7 minutes. Degradants were routinely monitored at a wavelength of 210 nm. The sample and standard solutions were dissolved in 80:20 *V/V* mixture of phosphate buffer pH 2.0 and methanol and were passed through 0.45 µm regenerated cellulose filter. The HPLC system was Thermo Ultimate 3000 HPLC System consisted of LPG-3400SD Pump, WPS-3000TSL Autosampler, TCC3000SD Column Thermostat and DAD3000SD Detector, controlled by Thermo Scientific™ Dionex™ Chromeleon™ 7 Chromatography Data System, Version 7.2 SR5 MUI.

Results and discussion

Initial analysis for presence of related and degradation products in the laboratory trials didn't reveal any significant difference in the impurity profile among the different formulation approaches. Results from the analysis of laboratory trial N°1 and N°2 after 9 months at 25 °C/60% show that the assay of the active substance decreases, with formation of specified impurity D as main degradation product (3.04% and 1.88%, respectively). Contrasting this, results obtained for laboratory trial N°3 show formation of specified impurity E as main degradation product (3.37%). Total impurities for laboratory trials N°2 and N°3 were similar (app. 4%), which can be related with the manufacturing process of wet granulation. While total impurities in laboratory trial N°1 are much lower (2.2%). Results for the samples stored at 40 °C/75% for 6 months confirm different degradation pathways among formulations, with additional increase of the amount of specified and total impurities.

The obtained results reveal that the degradation of the active substance follows parallel pathways of hydrolysis (impurity E) and intramolecular cyclization (impurity D), depending on the micro environment. Presence of sodium hydrogen carbonate in laboratory trial N°3 causes weakly alkaline environment compared to neutral environment in laboratory trial N°1 and N°2, thus catalyzing hydrolysis of the ester bond. Presence of HPMC in laboratory trial N°2 didn't show improved protection of the active substance compared to the low moisture excipients and dry mixing technology concept applied in laboratory trial tablets N°1.

Conclusion

The stability of sensitive pharmaceutical entities follows many rules defined by classic organic reaction mechanisms. It is of great importance to predict drug degradation in early steps of drug product development, increasing the knowledge about the product, minimizing the risk for patients and lowering the costs for excessive and long experiments. The first choice for a formulator to prevent hydrolysis is to avoid unnecessary or excessive contact of the active substance with water during the process of manufacture. As a result of the use of dry formulation techniques and the prevention of hydrolysis, stabile formulation can be obtained.

References

Bhangare, D., Rajput, N., Jadav, T., Sahu, A.K., Tekade, R.K., Sengupta, P., 2022. Systematic strategies for degradation kinetic study of pharmaceuticals: an issue of utmost importance concerning current stability analysis practices. J Anal. Sci. Technol. 13, 7. https://doi.org/10.1186/s40543-022-00317-6

Grangeia, H. B., Silva, C., Simões, S. P., Reis, M. S., 2020. Quality by Design in Pharmaceutical Manufacturing: A Systematic Review of Current Status, Challenges and Future Perspectives. Eur. J. Pharm. Biopharm. 147, 19-37. https://doi.org/10.1016/j.ejpb.2019.12.007

Hotha, K.K., Roychowdhury, S., Subramanian, V., 2016. Drug-Excipient Interactions: Case Studies and Overview of Drug Degradation Pathways. Am. J. Anal. Chem. 7, 107-140. https://dx.doi.org/10.4236/ajac.2016.71011

Sengupta, P., Chatterjee, B., Tekade, R. K., 2018. Current Regulatory Requirements and Practical Approaches for Stability Analysis of Pharmaceutical Products: A

Comprehensive Review. Int. J. Pharm. 543, 328-344. https://doi.org/10.1016/j.ijpharm.2018.04.007

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 85 – 86 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.038
Short communication

Introduction of major change for new premises, equipment and process of raw materials grinding in production of solid dosage forms

Ana Ivcheska*, Marija Runcheva*, Natasha Kronevska, Darko Atanasoski, Elisaveta Adamova Abrasheva, Meri Davcheva, Elizabeta Karadzinska

ALKALOID AD Skopje, Pharmaceutical, Chemical and Cosmetics Company, Aleksandar Makedonski 12, 1000 Skopje, Republic of North Macedonia

Introduction

Premises and equipment must be located, designed, constructed and maintained to suit the operations to be carried out. Their layout and design must aim to minimize the risk of errors and permit effective cleaning and maintenance in order to avoid cross-contamination. During the construction of new premises and procurement of new equipment URS are prepared. New systems and equipment should pass through all stages of qualification including design qualification (DQ), installation qualification (IQ), operational qualification (OQ) and performance qualification (PQ) as appropriate. Changes such as introducing a new premises or equipment in production must go through the change management system.

Background

The continued suitable performance of equipment is important to ensure batch-to-batch consistency Therefore, critical equipment should be qualified. The manufacturer should have a qualification policy for systems and equipment. The relevant documentation associated with qualification including standard SOPs, specifications and acceptance criteria and certificates should be maintained and the results of the qualification should be recorded and reflected in qualification reports.

Pharmaceutical quality system

The Pharmaceutical Quality System is based on International Standards Organisation (ISO) quality concepts and includes applicable Good Manufacturing Practice (GMP) regulations and complements ICH Q8 “Pharmaceutical Development” and ICH Q9 “Quality Risk Management”. The objective is to assure that manufacturing site continuously provides products with the highest standards for quality, safety and efficacy fit for their intended use.

Change management system

In order to evaluate, approve and implement changes regarding equipment and premises properly, a company should have an effective change management system to provide a high degree of assurance there are no unintended consequences of the changes. This change was evaluated by an expert team contributing the appropriate expertise from relevant areas to ensure that it is technically justified. After implementation, an evaluation of the change will be made to confirm the change objectives were achieved and that there was no deleterious impact on product quality (EU GMP Guideline Annex 15).

Critical utilities

Introducing new premises and equipment could affect the qualification status of the critical utilities such as PW, compressed air, dedusting etc. In this particular case the HVAC is subjected to PQ, new AHU unit was installed and the system for distribution of purified water was

*aivceska@Alkaloid.com.mk

extended to the new premises in production which are part of oral solid dosage forms where the new equipment for grinding of raw materials was installed.

Quality risk management

Quality risk management is a systematic process for the assessment, control and review of risks to the quality of the medicinal product across the product lifecycle (ICH quideline Q9). The scope and extent of equipment qualification and process validation was determined by using a documented risk assessment approach. QRM is applied to determine appropriate actions preceding the implementation of a change, e.g., additional testing, (re)qualification, (re)validation or communication with regulators. In this case it was determined that equipment qualification and process validation are necessary.

User requirements

During the construction of new premises and procurement of new equipment user requirement specifications are prepared in which users specify the requirments regarding the performance of the equipment/system, critical parameters of the equipment and operating rank, cleaning requiements, necessary documents from manufacturer/supplier and qualification requirements (EU GMP Guideline Annex 15). URS was prepared before precurment of the new equipment.

Premises and equipment qualification

The equipment was qualified prior to be brought into routine use to provide documented evidence that it is fit for its intended purpose (EU GMP Guideline Annex 15). The extent of the qualification was based on the criticality of the equipment. The premises is situated in an environment which presents minimal risk of causing contamination of materials or products.

Process validation

Process validation is the verification that a process meets the requirements imposed on its process results (EU GMP Guideline Annex 15). The cGMP regulations require that manufacturing processes be designed and controlled to assure that in-process materials and the finished product meet predetermined quality requirements and do so consistently and reliably (EU GMP Guideline Chapter 5). Process validation is required, in both general and specific terms, by the cGMP regulations. In this case, due to the introduction of new equipment and process of raw materials process validation will be performed.

Cleaning validation

Cleaning validation is a procedure of establishing evidence that cleaning processes for manufacturing equipment prevents contamination and cross-contamination. A pharmaceutical manufacturing plant compliant with cGMP must have cleaning validation protocol and program in place to establish documented evidence that the cleaning processes will consistently ensure that the products manufactured will meet expectations for purity, safety and quality (ICH guideline Q10). Regarding this shared product equipment, cleaning validation will be performed according company's validation strategy with a worst case product approach.

Conclusion

In conclusion, in order for a new premises, equipment and process to be introduced in a production plant they must undergo a series of activities including qualification, process validation and cleaning validation to ensure the processes carried out are compliant with cGMP and regulatory requirements and to assure the quality of the products, as well as patient's safety. All these activities should be documented and evaluated within the Change control process as part of the Pharmaceutical Quality System.

References

Eudralex - The rules governing Medicinal Products in the European Union, Volume 4: Good Manufacturing Practice, Medicinal Products for human and veterinary use, Chapter 5 Production. European Commission Health and Consumers Directorate – General.

Eudralex - The rules governing Medicinal Products in the European Union, Volume 4: Good Manufacturing practice, Medicinal Products for human and veterinary use, Annex 15 Qualification and Validation. European Commission Health and Consumers Directorate – General.

International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; ICH guideline Q10 Pharmaceutical Quality System, Step 4, June, 2008.

International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; ICH guideline Q9 Quality Risk Management, Step 4, November, 2005.

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 87 – 88 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.039
Short communication

Simultaneous HPLC determination of Metronidazole, Lidocaine and Miconazole in a combined intravaginal semi solid dosage form

Tanja Bakovska Stoimenova*, Marjan Piponski, Irena Slaveska Spirevska, Kristina Grncharoska, Marika Bogoevska Nikolovska, Kristina Varsamovska, Maksim Osman Nikolov

Replek Farm Ltd., Kozle 188, 1000 Skopje, N. Macedonia

Introduction

There are number of pharmacopoeial (Ph. Eur., BP, USP) and non-pharmacopoeial methods available for determination of metronidazole, miconazole and lidocaine, individually, but only few non-pharmacopoeial methods for their simultaneous determination in some combined pharmaceutical dosage forms (Akay et al., 2002; Belal and Haggag, 2012). The pharmaceutical formulation of interest that was subject of this research, was combined semi solid preparation for intravaginal use, containing metronidazole, miconazole and lidocaine as active substances.

Lidocaine is local anesthetic of amide type. It interacts with voltage-gated Na^+ channels in the nerve cell membrane and blocks the transient increase in permeability of excitable membranes to Na^+. It is used to provide local anesthesia by nerve blockade at various site in the body and works by causing temporary numbness or loss of feeling in the skin and mucous membranes.

Metronidazole belongs to a class of medications called nitroimidazole antimicrobials. It is antibiotic and antiprotozoal medication and is used to treat infections by stopping the growth of certain bacteria and parasites. With wide range of antibacterial activities and antiparasitic properties this antimicrobic medication is set apart from other antibiotics to treat a wide variety of infections.

Miconazole nitrate is a synthetic imidazole derivative antifungal agent which has a wide spectrum of activity and is particularly effective against pathogenic fungi, including Candida albicans. Also it is effective against gram-positive bacteria. It shows effect during ergosterol synthesis in the cytoplasmic membrane and changes permeability of the mycotic cell of Candida species and inhibits glucose utilization *in vitro*.

The aim of our work was to develop and validate a method for simultaneous quantification of these three active substances in a combined pharmaceutical formulation. For that purpose, simple and selective high performance liquid chromatography (HPLC) method was developed and validated, for simultaneous determination of lidocaine, metronidazole and miconazole in combined vaginal suppositories.

Materials and methods

Materials

The reagents that were used are 85% o-phosphoric acid, 70-72% perchloric acid and acetonitrile. The o-phosphoric acid and perchloric acid were purchased from Merck, Germany, while acetonitrile was purchased from Sigma Aldrich, USA. Deionized water that was used was prepared in Replek Farm by use of Simplicity UV System, with conductivity of 0.05 µS/cm. The reference standards such as Metronidazole CRM, Lidocaine CRM and Miconazole CRM were obtained from Sigma Aldrich, USA and the tested product, vaginal suppositories containing these three active substances were obtained from Replek Farm Ltd., Skopje, N. Macedonia.

The following instruments were used during the research: analytical balance AG285 from the producer Mettler Toledo, US bath TP690/H, optical shaker KS 260 basic and HPLC system Shimadzu Nexera XR UPLC

*tanja.bakovska@replek.mk

system with LPG quaternary pump with degasser, auto sampler, column oven, PDA detector and controller. Data acquisition, analysis and reporting were performed by Lab solution version 5.97. The type of column used was Zorbax RX C8 purchased from Agilent Technologies, Santa Clara, United States.

Syringe filters Nylon 0.45 µm that have been used to filter the solutions before transferring into vial, were purchased from Agilent Technologist, United States.

Chromatographic method

The separation was carried out on Zorbax RX-C8 250 mm x 4.6 mm, 5 µm column with mobile phase A (MFA) consisting of 0.1% (V/V) o-phosphoric acid with 0.1% (V/V) of perchloric acid and mobile phase B (MFB) consisting of acetonitrile. Flow rate of 1.2 mL/min, injection volume of 5 µL, the column temperature of 35ºC and detector at wavelength of 215 nm were set as other parameters of the method. The gradient is initiated with 80% (V/V) MFA and 20% (V/V) MFB, continued with linear variation to 6th minute when MFA drops to 30% (V/V) and MFB increases up to 70% (V/V). From 6 to 10 minutes the ratio between mobile phases remains the same as previous. The MFA returns to 80% (V/V) and MFB to 20% (V/V) accordingly in 1 minute and the column is re-equilibrated for 4 minutes.

Results and discussion

These three active substances are quite different in their hydrophobicity and solubility in water and in organic solvents. Therefore, it is impossible to achieve their separation by use of isocratic mode of elution, on reversed phase bonded matrix filled column.

Due to the significant difference in the physico-chemical properties of these three active substances, the positive outcome in their separation was achieved by using gradient elution method with a total run of 15 minutes where the ratio of MFA and MFB is constantly changing.

The gradient elution of the analytes was achieved in 15 minutes, with retention time of metronidazole, lidocaine hydrochloride and miconazole nitrate of about 3.2 minutes, 5.5 minutes and 9.3 minutes, respectively. The three peaks were well separated from each other, with resolution for more than 20 between each of the neighboring peaks. The obtained values of theoretical plates for metronidazole, lidocaine and miconazole were 15641, 54165 and 106051, respectively.

The method was validated in accordance to ICH guideline Q2(R1). During selectivity testing, no interference from the formulation excipients was observed. The linearity of the method was proved in five concentration levels, for each substance, and the following results were obtained by regression analysis: correlation coefficient > 0.9990 and relative standard deviation of the response factors for each concentration level < 2 %, in all cases. The precision of the system and of the method were also evaluated and the obtained relative standard deviation of the responses was less or equal to 2 %, in both cases, for each substance. Accuracy of the method was studied by recovery investigation. The obtained recovery values were within the range of 100 ± 2 %, for each substance.

Conclusion

The developed reverse phase HPLC method provides simple, specific, accurate, precise and reproducible simultaneous quantitative analysis of metronidazole, lidocaine and miconazole in combined semi solid pharmaceutical formulations. The established method was validated and proved as suitable for its intended use, in accordance to ICH guideline Q2(R1). The proposed method, by use of simple sample preparation, low-cost reagents, and relatively short run time, provides reproducible simultaneous quantification of all active substances of interest and could be successfully used for routine analysis of intravaginal semi solid dosage formulations containing this combination of active substances, especially in pharmaceutical industry.

References

Akay, C., Ozkan, S.A., Senturk, Z., Cevheroglu, S., 2002. Simultaneous Determination of Metronidazole and Miconazole in Pharmaceutical Dosage Forms by RP-HPLC. Il Farmaco, 57(11), 953–957. https://doi.org/10.1016/S0014-827X(02)01296-X.

Belal, T.S., Haggag, R.S., 2012. Gradient HPLC-DAD Stability Indicating Determination of Miconazole Nitrate and Lidocaine Hydrochloride in their Combined Oral Gel Dosage Form. J. Chrom. Sci. 50, 401–409. https://doi.org/10.1093/chromsci/bms019.

British Pharmacopoeia, 2022. British Pharmacopoeia Commission - The Stationery Office: London.

European Pharmacopoeia, 10th ed., 2019-2020. European Directorate for the Quality of Medicines & Healthcare – Council of Europe: Strasbourg.

International Conference on Harmonization, 2005. Validation of analytical procedures: Text and methodology Q2(R1). ICH, Geneva, Switzerland.

The United States Pharmacopeia / The National Formulary (USP 43-NF 38), 2020. The United States Pharmacopeia Convention: Rockville.

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 89 – 90 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.040
Short communication

Development of powerful chromatographic methods for improved separation of tetrahydrocannabinol isomers during HPLC analysis of cannabis flowers and extracts

Irena Slaveska Spirevska[1]*, Marjan Piponski[1], Gordana Trendovska Serafimovska[2], Tanja Bakovska Stoimenova[1], Elena Lazarevska Todevska[1], Martina Miloshevska[1]

[1] *Replek Farm, st. Kozle 188, 1000 Skopje, N. Macedonia*
[2] *HAPA Pharm Ltd, N. Naumovski-Borce, 79b, 1000 Skopje, N. Macedonia*

Introduction

THC (tetrahydrocannabinol) usually refers to the naturally existing isomer of Δ^9-THC, but may also include Δ^8-THC (Grotenhermen & Russo, 2002). Out of approximately 500 components of Cannabis, Δ^9-THC is the primary and perhaps the only compound (Δ^8-THC is also active but its concentration is very low) responsible for its psychoactive effects. Most of the pharmacological effects of this natural product are due to activation of two types of G-protein-coupled receptors, the cannabinoid CB_1 receptors (distributed in the brain) and CB_2 receptors (present almost uniquely in the immune system). This fact explains the actions of Δ^9-THC on cognitive and motor functions, as well as its immune-modulatory effect (Di Marzo, 2004).

The review of scientific literature and application notes of chromatographic equipment and columns, Shimadzu, Waters (Aubin et al., 2018), Agilent (Storm et al., 2019), Knauer (Loxterkamp et al., 2020) etc., reveals different methods for separation of cannabinoids. These methods achieve separation of eight, up to seventeen different cannabinoids, most commonly and dominantly present in the cannabis flowers and extracts. They focus on achieving maximal resolution between peaks of cannabinoids, using gradient and/or isocratic elution, obtaining maximal resolution of about 1.2 between the critical separation pairs, CBGA/CBDA, CBG/CBD, and most important Δ^9-THC/Δ^8-THC. Our experience in the analysis of different cannabis flowers and extracts showed a necessity for much higher resolution between the psychoactive cannabinoid Δ^9-THC and its positional isomer Δ^8-THC, as well as between other peaks of cannabinoids that might eventually co-elute with them, since they are psychoactive and their content in extracts and products is strictly limited.

The aim of this work was to develop powerful RP-HPLC methods for optimal separation of tetrahydrocannabinol isomers during chromatographic analysis of cannabis flowers and extracts, in order to achieve accurate determination of strictly regulated psychoactive cannabinoid Δ^9-THC.

Materials and methods

The following reagents were used: methanol, acetonitrile, isopropanol, 85% *o*-phosphoric acid, 70-72% perchloric acid, 99 % formic acid and 99 % trifluoroacetic acid purchased from Merck Darmstdat, Germany and Sigma-Aldrich, USA. The deionized water was "in house" product prepared with conductivity of 0.05 µS/cm.

The following instruments were used: analytical balance Mettler Toledo AG285, pH–metter Metrohm 827 pH Lab, US bath Branson 3510 and IKA orbital shaker KS 260 basic. The regenerated cellulose (RC) 0.45 µm syringe filters for sample filtration were purchased from Agilent Technologies (USA).

The following HPLC systems were used: Shimadzu Prominence LC2030-i Cannabis Potency Analyzer, Shimadzu Prominence LC2040-i 3D, Dionex Ultimate 3000 UHPLC system and Agilent HPLC 1260 system.

The following chromatographic columns were used: Zorbax ODS (250 mm × 4.6 mm, 3.5 µm), Shimadzu

*irena.slavevska@replek.mk

Nex-Leaf SH-SPP ODS (150 mm × 4.6 mm, 2.7 µm) and Poroshell ODS HPLC (150 mm × 4.6 mm, 2.7 µm).

Certified reference materials containing cannabinoids of interest were purchased from Cerilliant, Sigma-Aldrich, USA and Cayman Chemical, USA.

The test samples were prepared from cannabis flowers and extracts obtained from Replek Farm Ltd.

Results and discussion

In our routine analytical experience, we clashed with cannabis extracts yielding unknown peaks overlapping with the peaks of THC isomers, which we found very hard to resolve using existing, published methods. This was the main reason for development of simple and fast RP-HPLC-UV/DAD method, for better separation of THC positional isomers in order to additionally separate them from the other co-eluting peaks.

We developed simple methods with increased resolution of critical elution pair, Δ^9-THC and Δ^8-THC, up to 3.2, by use of C18 column with core shell particles with 25000 NTP (number of theoretical plates), and up to 4.3 by use of C18 column with standard fully porous bead particles with 35700 NTP, and mobile phase composed only of methanol, acetonitrile and acidified water.

The results from our experiments showed that the polar C18 (ODS) matrixes, as expected, have better separation power because of the higher number of heterogenic interactions between the analyte and the stationary phase. The columns used, Poroshell and Nex-Leaf columns contained superficially porous particles (SPP) type, thus expected to yield highest resolution per unit length, but their separation power is compromised by the highly hydrophobic biological nature of the samples making them prone to clogging and having shorter column life.

The acid used for preparation of the acidified water did not significantly influence the separation of the peaks of the nonionic compounds of interest. By use of all four tested acids: *o*-phosphoric acid, perchloric acid, formic acid and trifluoroacetic acid, suitably diluted in water, satisfying resolution between Δ^9-THC and Δ^8-THC was obtained.

Mobile phase composition and flow rate, column temperature and characteristics, injection volume, and finally the equipment characteristics and quality, contribute to separation quality and method selectivity.

Conclusion

Various combinations of chromatographic conditions were tested and optimized to be used for this purpose.

Obtaining the best possible separation between the psychoactive Δ^9-THC and its positional isomer Δ^8-THC is crucial in order to avoid co-elution of other possibly interfering peaks that might contribute to false higher content of strictly regulated Δ^9-THC in the tested samples.

This was achieved by use of proper combination of simple chromatographic conditions: L1 (ODS) HPLC column and simple ternary mobile phase composed only of acidified water, methanol and acetonitrile.

References

Aubin, J.A., Layton, C., Helmueller, S., 2018. Separation of 16 Cannabinoids in Cannabis Flower and Extracts Using a Reversed-Phase Isocratic HPLC Method. Waters Corporation, Milford, USA.

Di Marzo (Ed.), 2004. Cannabinoids. Kluwer Academic/ Plenum Publishers, New York, USA, pp. 4.

Grotenhermen, F., Russo, E. (Eds.), 2002. Cannabis and Cannabinoids, Pharmacology, Toxicology, and Potential. The Haworth Integrative Healing Press, New York, pp. 27.

Loxterkamp, L., Stephan, S., Monks, K., 2020. (C)an(n)alyze: Determination of 16 Cannabinoids Inside Flowers, Oils and Seeds. Knauer Wissenschaftliche Geräte GmbH.

Shimadzu Corporation, 2018. The Potency Determination of 16 Cannabinoids by UHPLC with Diode-Array Detection. Application News, No. SSI-HPLC-020.

Storm, C., Zumwalt, M., Macherone, A., 2019. Dedicated Cannabinoid Potency Testing Using the Agilent 1220 Infinity II LC System. Agilent, USA.

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 91 - 92 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.041
Short communication

Regulatory Opportunities Investigations -requirements and procedures for medicines registration in the EAEU and opportunities

Ljuba Karanakov*, Katerina Zafirova Georgievska, Ana Zisovska Beleva, Julijana Dimovska, Svetlana Neceva

Regulatory Affairs Department, Replek Farm Ltd. Skopje, Kozle str. 188, 1000 Skopje, Republic of North Macedonia

Introduction

The regulatory intelligence (RI) function continues to develop and increase in importance as companies operate globally. While the scope of RI varies by geography, resources and company size, it has become an essential component of maintaining awareness of and remaining compliant with the ever-evolving regulatory landscape.

However, RI often operates in the background of a company, with limited awareness of its value and contribution to the company's success. The ability to determine the return on investment (ROI) of RI remains elusive for many companies, leaving senior leadership still to determine its value (Clarivate, 2021).

Global harmonization has brought steady method in regulatory submission. Asia is predicted to overtake Europe in pharmaceutical marketplace within the next decade and sales are driven by using increase in key rising markets. The term "rising marketplace economy" was first utilized in 1981 by "Antoine W. Van Agtmael" of the International Finance Corporation of the World Bank. Emerging markets are economies of countries that are within the process of becoming a developed country. More than 85 % population lives in the emerging market and so the real financial boom has come from these markets. This promotes many multinational corporations switched to those rising international locations particularly in China, India, Euro Asian Economic Union (EAEU), Korea and Mexico (Singam et al., 2020).

The EAEU consists of Kazakhstan, Russia, Belarus, Armenia, and Kyrgyzstan and has a combined population of more than 185 million people. The EAEU was formed in 2014 and is headed by the Eurasian Economic Commission (EEC). In recent years, the EAEU has taken steps to unify the pharmaceutical market, and in the future might include a single pharmaceutical regulator similar to the European Medicines Agency (EMA). The EEC has introduced the 'Agreement on common principles and rules of circulation of medicines within the Eurasian Economic Union' at the end of 2014, and the 'Rules of registration and expertise of medicinal products for human use' (EEC Decision No. 78) on November 3rd, 2016. The decision describes two pathways of registering medicines in the EAEU, which have become the official procedures at the start of 2021. Medicines registered before December 31st, 2020; need to comply with the EAEU requirements by the end of 2025 (Grata International, 2022).

Materials and methods

The materials of the study were available publications in peer-reviewed journals on thematic queries based on keywords of the selected topic, official websites, regulatory legal acts, regulating the procedure for registering medicinal products in the EU and different countries, in order to investigate the requirements and procedures for registration of medicinal products in EAEU as a potential opportunity for accelerated registration and marketing authorization procedures of medicines.

Results and discussion

Replek Farm Ltd. Skopje, as an EU GMP certified pharmaceutical manufacturer and Marketing Authorization Holder of numerous Marketing Authorization certificates of medicines in different EAEU countries, has considered new approaches to registration within the framework of the Eurasian Economic Union from the perspective of new opportunities and emerging

*ljuba.karanakov@replek.mk

problems for foreign manufacturers of generic medicines. A comparative analysis of the mutual recognition procedure and the national procedure for registration of medicinal products revealed a number of advantages for domestic and foreign manufacturers, favoring the introduction of medicinal products into circulation on the whole territory of the EAEU.

The rules for registration and expert appraisal of medicines for medical use within the EAEU were approved by the Decision of the EEC Council No. 78 dated 3 November 2016 (the 'Rules') and entered into force on 6 May 2017. Medicines registered under the Rules can be circulated and offered for sale throughout the EAEU, without undergoing registration procedures in each of these member states (Grata International, 2022).

Pathways for Medicine Registration in the Eurasian Economic Union (EAEU) are:

1. The mutual recognition procedure is carried out by the reference Member State – The MAH first selects a reference state in the mutual recognition procedure and submits the registration dossier to the competent authority (CA) there. The overall process has a maximum duration of 210 calendar days, with an extension possible if a CA requests additional information. After market authorization in the reference state, the MAH provides access to the eCTD and the expert report in the EAEU unified register for the other member states. The recognition process has a maximum duration of 90 calendar days, making the maximum duration of the whole mutual recognition procedure 300 calendar days (Biomapas, 2021; Foteeva et al., 2022).

2. The decentralized marketing authorization procedure is carried out simultaneously by several Member States where the application for the marketing authorization has been submitted and the reference Member State is need to be chosen. (Grata International, n.d.) The decentralized procedure has a maximum duration of 210 calendar days and is thus faster than the mutual recognition pathway (Biomapas, 2021).

3. Procedure for Previously Approved Medicines in the EAEU: Medicinal products that have received market authorization before December 31st, 2020, are required to comply with the new EAEU standards by December 31st, 2025. MAHs largely follow the mutual recognition procedure: they select a reference state to submit the eCTD and other documents to, harmonized with the new regulations. It is important to note that this harmonization process should not include new information on safety, efficacy, or technical details of the medicinal product; i.e., any variations should first be submitted and processed for the old eCTD. The reference state performs the evaluation and potential inspections and then provides an expert opinion (Biomapas, 2021).

The procedure for bringing a medicinal product in compliance with the new requirements is accelerated and has a maximum duration of 100 calendar days. If the medicine was already registered in at least three member states for five years or more, a registration certificate without an expiration date would be issued. In other cases, the authorities grant the standard validity period of five years (Biomapas, 2021).

Furthermore, a maximum of 210 days before the expiry of a registration certificate, a re-registration request can be submitted, after which a certificate without an expiration date can be obtained (Biomapas, 2021).

Conclusion

A comparison of registration requirements for different pathways for medicine registration in the Eurasian Economic Union has been done to understand the difference in current regulatory requirements, define a clear regulatory strategy for Replek Farm Ltd. Skopje by looking at the target EAEU regions. It was evaluated that the procedure for EAEU registration of previously approved medicines will lead to harmonized and efficient regulatory approval for current registrations, avoiding unnecessary duplication of work and eventually disabling medicines lag. The review of the process of harmonization of the registration practice of medicinal products in the EAEU speaks of regulatory intelligence (RI) possibilities. The future of developing new potential within EAEU for foreign generic medicines manufacturers, as Replek Farm Ltd. Skopje, shows facilitated access and potential benefit regarding the registration export of effective and safe medicines.

References

Biomapas, 2021. Drug Registration in the Eurasian Economic Union (EAEU). https://www.biomapas.com/drug-registration-in-the-eurasian-economic-union-eaeu/ (accessed May, 2022)

Clarivate, 2021. Showing the value of regulatory intelligence in pharma – Measuring and communicating the RO, Clarivate. https://clarivate.com/lp/showing-the-value-of-regulatory-intelligence-in-pharma/ (accessed May, 2022)

Grata International (n.d.): Registration of medicines in the Eurasian Economic Union, 3. Grata International https://gratanet.com/publications/registration-of-medicines-in-the-eurasian-economic-union (accessed May, 2022)

Foteeva, A.V., Barshadskaya O.S., Rostova N.B., 2022. Mutual recognition procedure for the registration of medicines: new challenges or opportunities. Razrabotka I registratsiya lekarstvennykh sredstv = Drug development & registration. 11(1), 159-164. (In Russ.) https://doi.org/10.33380/2305-2066-2022-11-1-159-164

Singam, S.R.S.L., Yetukuri, K., Nadendla, R.R., 2020. Drug Registration requirements for Pharmaceuticals in Emerging markets, International Journal of Drug Regulatory Affairs 8(4), 73-82 https://doi.org/10.22270/ijdra.v8i4.442

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 93 - 94 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.042
Short communication

Assessing imatinib mesylate in two different solid dosage forms

Ela Hoti[1*], Leonard Deda[2], Viola Gjylameti[1]

[1]*Department of Pharmacy, Faculty of Medicine, UMT, Tirana, Albania*
[2]*Applied Pharmacokinetics Lab, Dep. of Biomedical Sciences, Faculty of Medicine, UMT, Tirana, Albania*

Introduction

Imatinib mesylate (IM), is a tyrosine kinase inhibitor (TKI), which is used to treat chronic myeloid leukaemia (CML) and other malignant diseases related to over expression of tyrosine kinases protein (Savage and Antman, 2002). Imatinib is one of the first molecular therapies for clinical use that has shown impressive efficacy for CML, advanced gastrointestinal stromal tumours (GISTs), and myeloproliferative disorders associated with PDGFR. It is approved as a first-line therapy for Ph+ ALL (Iqbal and Iqbal, 2014).

Several RP-HPLC methods can be found in the literature (Rele and Patil, 2019; Rosasco et al., 2005) for its determination in various pharmaceutical forms. The purpose of this work was to evaluate imatinib mesylate in two different solid dosage forms with a new RP-HPLC method developed in our laboratory.

Materials and methods

Chemicals and equipment

Working standard: Imatinib mesylate (gift from AKBPM). Reagents: metanol (MeOH) (Carlo Erba Reagents), acetonitril (ACN) HPLC grade (Sigma-Aldrich), orthophosphoric acid 85%, distilled water, samples from two different imatinib solid dosage forms.

Chromatographic data were obtained using an Agilent 1200 reversed phase chromatographic system consisting of a High Pressure Mixing Binary Pump, DAD detector and equipped with a ChemStation PC-controlled. The pH measurements were made with a pH meter (Denver Instruments). Centrifuges, mixers and ultrasonic baths were also used. Nylon membrane filters were used for mobile phase filtration (type: Hydrophilic Nylon membrane filter) (EMD Millipore) (47 mm; 0.45 µm pore).

Preparation of standard solutions

100 mg of standard Imatinib was dissolved in methanol in a 100 mL volume flask and was made up to the volume mark with methanol. The obtained solution, at a concentration of 1 mg/mL, was filtered through 0.45-mm porous filters, and stored in suitable containers, refrigerated at 4-8 °C. Solutions with concentrations of 5, 10, 20, 30 and 40 µg/mL, were prepared daily for the calibration curves by diluting the stock solution with distilled water. All the solutions obtained were filtered through 0.45 µm pore filters, prior to injection.

Development and optimization of analytic conditions

Many tests were performed to attain optimum conditions. Different column types: LiChrospher® 100 RP-18 (5µm) LiChroCART® 125-4, ODS HYPERSIL C18 (250 × 4.6) (5µm), LiChrospher® 60 RP-select B C8 (5µm) LiChroCART® 250 were tested. Different compositions of the mobile phase components, different pH, as well as changes in column temperature.

Chromatographic conditions

The flow rate was adjusted at 1.0 mL/min and the detection was carried out at 276 nm. The volume of injection was 20 µL.

Method validation parameters

*ela.hoti@umed.edu.al

The method was validated according to the ICH validation parameters and was assured to meet the conditions of linearity, accuracy and precision.

Preparation of the samples for the uniformity content

Each tablet is poured into a pre-filled Erlenmeyer flask with 100 mL of distilled water and placed in an ultrasound bath to disintegrate for 45 minutes. A 3 mL sample from each container is transferred in a test tube, which is centrifuged for 5 minutes. An aliquot of 2 mL from the supernatant liquid is further diluted in a 1 to 5 ratio with distilled water until the theoretical concentration reaches 20 µg/mL. The same procedure was applied for 10 tablets of each of the pharmaceutical products, which contain 100 mg of Imatinib. Samples were injected into the apparatus and the data obtained were plotted in graphs to compare the different products.

Results and Discussions

Literature review on the characteristics of Imatinib, revealed various evaluations with HPLC (Rele and Patil, 2019; Rosasco et al., 2005). Unfortunately, none of the published methods could be carried out in our laboratory, hence to evaluate imatinib in solid dosage forms a new method was developed. The tests revealed that the LiChroCART® 250-4 LiChrospher® 60 RP-select B C8 (5µm) column resulted the most suitable one. The chosen mobile phase consisted of Acetonitrile / H_2O adjusted to pH 2.2 with orthophosphoric acid in the ratio (32.5: 65.5 v/v). Tests were carried out at room temperature. The analysis time was 6.5 minutes with imatinib eluting at 4.8 minutes. The method proved sensitive to the pH changes of the mobile phase.

The data obtained showed a consistent and practical method. DAD was used to confirm peak purity, and the result showed a peak with no interference with excipients, degradation products, or impurities.

Method validation

The linearity of the method was estimated through the correlation coefficient of the linear regression line, and regression equation was $y = 68.83x + 17.18$ with $r^2 = 0.999$. Repeated injections ($n = 5$) of each standard solution of 10 µg/mL, 20 µg/mL and 30 µg/mL were performed daily to determine accuracy and precision within day and $n = 18$ between days. All values of RSD for within day and between day tests were below 2.5%.

Uniformity content of Imatinib tablets

10 tablets from each pharmaceutical product were analysed as defined in European Pharmacopeia 10. The method demonstrates that all the tablets tested are within the specified range respectively between 85% and 115% of the average content.

Conclusions

The new method used to evaluate imatinib mesylate was accurate and precise. The results of the uniformity content for the two pharmaceutical products were within the specified range according to ICH and Ph. Eur. Determination of Imatinib in pharmaceutical dosage forms with this method enables the opportunity for further studies regarding the dissolution test.

References

Iqbal, N. and Iqbal, N., 2014. Imatinib: A Breakthrough of Targeted Therapy in Cancer. Chemotherapy Research and Practice 2014. https://doi.org/10.1155/2014/357027

Rele, R.V. and Patil, S.P., 2019. Development of Analytical Method by RP-HPLC Technique for Determination of Imatinib mesylate in Bulk Drug and Pharmaceutical Dosage Form. Asian J. Research Chem. 12(2), 79-83. http://dx.doi.org/10.5958/0974-4150.2019.00018.X

Rosasco, M., Moyano, M.A., Pizzorno, M.T., Segall, A.I., 2005. Validation of an HPLC Method for the Determination of Imatinib Mesylate in Pharmaceutical Dosage. J. liquid chromatography and related technologies 28(20), 3283-3292. https://doi.org/10.1080/10826070500330976

Savage, D. and Antman, K., 2002. Imatinib Mesylate-A New Oral Targeted Therapy. New England Journal of Medicine 346(9), 683-693. doi: 10.1056/NEJMra013339

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 95 – 96 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.043
Short communication

Extraction and Interaction study by HPLC-DAD method for screw cap PP 28 child-resistant used in packaging of drug product

Marija Mojsovska*, Marija Arsova, Elena Trajchova Kovachovska, Natasha Anevska - Stojanovska

Institute for Research and Development, Alkaloid AD-Skopje, Blv. Aleksandar Makedonski 12, 1000 Skopje, North Macedonia

Introduction

One of the essential before one pharmaceutical product is approved from regulatory agencies and to be released on the market, is to determine its purity, through determination of its impurity profile. Nowadays, migration and sorption of mobile chemical species from components used in the manufacture and storage of pharmaceutical products must be assessed.

The aim of the presented study is to evaluate the suitability and to justify the choice of the plastic material screw cap PP 28 child-resistant (CR) with tamper evident ring, embossed at the top and with PE-liner (pressure seal disc).

The compatibility of the screw cap PP 28 CR is tested by performing extraction and interaction studies for detection and identification of organic and inorganic compounds, possibly deriving from the screw cap, which potentially may interact with the final drug product during storage and use.

Materials and methods

Extraction and migration study

The samples for Polypropylene (PP) outer cap, high-density polyethylene (HDPE) inner cap and polyethylene (PE) foam liner extractable testing were prepared from new, unused PP outer and HDPE inner caps and rings and PE foam liner, which were cut into small pieces (~ 0.5-1 cm^2 in size) before extraction. Molded screw caps were used instead of HDPE and PP granulate so that manufacturing influence can be taken into account (thermo-processing, molding, lubrication etc.). The extraction was performed by using ultra-sonic (US) bath and two specially selected solvent systems (placebo for oral solution adjusted to pH 3 and pH 5). The choice of solvents was made in accordance to the minimum and maximum specified pH values in the specification of the finished drug product, thus covering the extreme pH values at which the plastic components can be exposed during shelf life. The US (ultrasonic) extraction was performed by extracting ~ 27 g Polypropylene (PP) outer cap and ~ 35 g high-density polyethylene (HDPE) inner cap with tamper evident ring with 100 mL of placebo. Accordingly, same type of extraction was performed on ~ 2 g polyethylene (PE) foam liner with 50 mL of placebo. The extraction was conducted for about 2 hours, taking care that the ultrasonic (US) bath temperature did not exceed 35 °C as a result of the constant application of energy to the system.

After the extraction, the extracting solvents were filtered from the plastic pieces through 0.2 µm Regenerated Cellulose (RC) membrane filter before injecting. The samples for HPLC-DAD evaluation were analyzed directly without further processing. The HPLC-DAD screening was performed on Agilent 1260 HPLC system equipped with binary pump and diode-array detector. The following method parameters were used for the screening of samples and blanks: Column: Waters XTerra RP18 150 x 3.0 mm; 3.5 µm; Temp. 25 °C; Mobile phase A: 10 mM ammonium acetate B: acetonitrile; Flow 0.3 mL/min; Injection volume 20 µL;

*mmojsovska@Alkaloid.com.mk

Wavelength: 210, 220, 230, 250 nm + UV spectra. The method is gradient (Petruševski et al., 2016).

Sorption study

Sorption studies were performed in order to investigate possible interactions between the selected screw cap and the formulation due to possible sorption of the active substance and preservative. For that purpose, initial analysis were preformed, afterwards bottles of finished product oral solution were stored for about three months at 25 °C/60% RH and 40°C/75% RH in inverted and non-inverted position.

Validated HPLC method was used for determining the content of API and Preservative in the drug product formulated as oral solution.

5 mL from oral solution were diluted to 50 mL with mobile phase, then filtered through 0.2 µm GHP filter into HPLC vial and analyzed. The nominal concentration of test and standard solution is 0.3 mg/mL of preservative and 0.1 mg/mL of API. The content is calculated from peaks area ratio in the chromatograms of the test and the standard solution respectively, at detection wavelength of 210 nm, on Thermo Ultimate Dionex system equipped with quaternary pump and diode-array detector. The following method parameters were used: Column Zorbax SB C8, 250 mm x 4.6 mm, 5 µm; Temp. 25 °C; Mobile phase A: Solution A (1.244 g pentane-1-sulfonic acid sodium salt in 1000.0 mL volumetric flask, dissolve with 600 mL water. Add 28 mL 85% ortho-phosphoric acid and dilute with water R) B: acetonitrile; Flow 1.8 mL/min; Injection volume 10 µL; Wavelength: 210 nm. The method is isocratic.

Results and discussion

Representative UV chromatograms of analyzed placebo solutions, standards and tests solutions were obtained end evaluated after extraction and migration method. Values for Assay of API and preservative were collected after sorption method and compared to data values of untreated samples.

From the obtained data of sorption studies it can be concluded that the assay of API and preservative in finished product oral solution, as well as related and degradation products, are not significantly changed after storage of the original bottles for three months at 25 °C/60% RH and 40 °C/75% RH in inverted position.

The HPLC-DAD screening of the generated results for presence of extractable and leachable compounds revealed that all screw cap components do not contain any extractable compounds above the method detection limit, for any of the generated extracts, thus the selected packaging material does not affect the efficacy of the drug product.

The outcome from the analysis of inverted bottles with placebo and API oral solution is that there are no detected compounds deriving from the plastic screw cap PP 28 CR.

Conclusion

The collected data demonstrate that the selected material is suitable in regards to the integrity and compatibility with the oral drug product (EMA, Guideline on Plastic Immediate Packaging Materials CPMP/QWP/4359/03). It can be concluded that there aren't any changes in the quality of the drug product.

References

EMA, Guideline on Plastic Immediate Packaging Materials CPMP/QWP/4359/03. Microsoft Word - Guideline on Primary Plastic Packaging Materials.doc (europa.eu)

Petruševski, V., Jolevska, S.T., Ribarska, J.T., Chachorovska, M., Petkovska, A., Ugarković, S., 2016. Development of complementary HPLC-DAD/APCI MS methods for chemical characterization of pharmaceutical packaging materials, J. Pharm. Biomed. Anal. 124, 228–235. https://doi.org/10.1016/j.jpba.2016.03.005

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 97 – 98 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.044
Short communication

Development and Validation of HPLC method for determination of Methylprednisolone aceponate in cream

Paulina Apostolova[1*], Dino Karpicarov[1*], Marija Arev[1], Emilija Janevik-Ivanovska[1], Biljana Gjorgjeska[1], Zorica Arsova-Sarafinovska[1,2]

[1]*Faculty of Medical Sciences, Department of Pharmacy, Goce Delchev University of Shtip, Krste Misirkov No. 10-A, 2000 Shtip, Republic of North Macedonia*
[2]*Institute of Public Health of Republic of North Macedonia, 50-ta Divizija 6, 1000 Skopje, Republic of North Macedonia*

Introduction

Methylprednisolone aceponate (MPA) is an active pharmaceutical ingredient (API), used as a potent topical glucocorticoid in the treatment of various types of eczema and psoriasis. Compared to other glucocorticoids, MPA has high efficiency and reduced application (once a day) (Ruzicka, 2006).

The need to develop and validate a method for routine content determination of MPA in MPA cream, arose due to the lack of individual monograph, both for the active ingredient and for the pharmaceutical dosage form, in any of the official editions of different pharmacopoeias.

Therefore, the aim of our study was to develop and validate a simple and rapid reversed-phase HPLC method for the routine determination of MPA in MPA cream.

Materials and methods

The method was performed using Waters - Alliance HPLC system equipped with quadruple pump, separation module e-2695, and automatic sampler (Waters corporation, USA). The detection wavelength was optimized with Waters 2489 UV/Vis Detector. All data were processed with the Empower 3® software.

The separation was achieved using the LiChrospher® RP-18 100 mm x 4 mm, 5 µm column, at 40 °C, and with isocratic elution. Mobile phase consisted of 55 volumes of acetonitrile (ACN, Fischer Chemical) and 45 volumes of ultra-pure water produced in a laboratory on our department. The flow rate was 1 mL/min and the injection volume was 10 µL. The temperature of the injector was set at 2 5ºC. The run time was 15 minutes and the detection was performed at 240 nm.

We used two standards, one for the API – MPA Reference standard (99.4%), and one for the antimicrobial preservative present in the dosage form – Benzyl alcohol, puriss. p.a., ACS reagent, ≥ 99.0%. The standards were purchased from Sigma Aldrich. The commercial cream samples were purchased from a local pharmacy.

Standard and sample preparation

The standard solution was prepared by dissolving the API in a mixture of equal volumes of ACN and methanol. The working concentration of the standard and the sample solution was 0.1 mg/mL. To extract MPA from the cream, a quantity of the cream containing equivalent of 5 mg MPA, together with the solvent, was added to a volumetric flask and treated on vortex mixer and ultrasonic bath. The solutions were cooled down to room temperature and the rest of the solvent was added. Before the injection in the HPLC system, the standard and sample solutions, were filtered through a 0.45 µm polytetrafluoroethylene (PTFE) filter.

Results and discussion

A variety of mobile phases and columns were investigated during the development of the HPLC method for analysis of MPA in MPA cream. The proposed method was defined on a basis of the system suitability test.

*paulina.apostolova@ugd.edu.mk

The method performance was fully validated according to the ICH Q2(R1) Guideline by a determination of accuracy, precision, specificity, linearity, and range.

The impact of the system or method changes on the obtained results, was evaluated through the robustness of the method (ICH Q2(R1), 2019).

System suitability

System suitability test is designed to evaluate the components of the analytical system to show that the performance of the system meets the standards required by the method. In our case, the system suitability was evaluated through these parameters: capacity factor, resolution, symmetry factor and selectivity. Each of these test parameters was determined by injecting six consecutive replicas of the standard solution, prepared in the working concentration, and after calculating the arithmetic mean of the results obtained for each test parameter, we concluded that this HPLC system was suitable for content determination of MPA in MPA cream (USP <1225>, 2018).

Specificity (selectivity)

The specificity of the method was demonstrated with acceptable resolution between the peaks from the API and the antimicrobial preservative (Rs = 13.81), and without any interference from placebo peaks.

Linearity and range

Linearity was determined using six standard solutions with concentrations ranging from 80 – 120% of the working concentration. By processing the data using the least squares method, we obtained the following equation: $y = 19162x - 41164$, and concluded that the method is linear in the given range, with a correlation coefficient of 0.9999 ($R^2 = 0.9999$).

Precision

The precision was determined in terms of system precision, method precision, and intermediate precision. On each level of precision, we calculated the standard deviation (SD) and the relative standard deviation (RSD) of the retention time, area under the curve (AUC), and percentage of content. Based on the obtained results for SD and RSD (RSD ≤ 2%), we concluded that the system precision and method precision meet the acceptance criteria. The intermediate precision was determined using six sample solutions, prepared in the same manner, with the same concentrations, and analyzed under the same conditions, on two different days. Based on the results from the F-test, we concluded that there is less than 5% probability that the difference in the results will be significant. Thus, we confirmed the acceptable intermediate precision of this method.

Accuracy

The accuracy of the method was determined by spiking the sample solution with known amounts of the standard solution, in order to produce three solutions with concentrations of 120%, 150% and 180% of the working concentration. The average recovery was 99.41% (98.84% – 99.84%), which confirmed the method accuracy.

Robustness

The robustness of the method was determined by monitoring the response of the method to the changes we delibrately introduced on the column temperature, flow rate, and composition of the mobile phase. We calculated the plate number and RSD for the tailing of the main peak. Considering that the above mentioned, parameters meet the acceptance criteria (RSD ≤2%), we concluded that the method is robust.

Conclusion

The validation results show that the method is accurate, precise, robust, selective, and linear in the given range. It is easily applicable because it does not require complex sample preparation, or special preparation of the working environment. Also, due to the easy availability of the organic solvents used as a mobile phase, the method is economically affordable. This method offers important contribution to scientific knowledge and it can be routinely used for content determination of MPA in MPA cream.

References

International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, 2019. ICH Harmonised Tripartite guidelines, Validation of Analytical Procedures: Text and Methodology Q2(R1).

Ruzicka, T., 2006. Methylprednisolone aceponate in eczema and other inflammatory skin disorders – a clinical update. Int. J. Clin Pract. 60(1), 85–92. https://doi.org/10.1111/j.1368-5031.2005.00754.x

United States Pharmacopoeia NF 36, 2018. <1225> Validation of compendial procedures. The USP Convention, Rockville, 7665–7666.

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 99 - 100 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.045
Short communication

A validated isocratic RP-HPLC method for determination of linezolid in pharmaceutical dosage forms

Zana Mustafa[1*], Shaban Memeti[1,2,3], Dragica Doneva[1], Katerina Starkoska[1], Maja Shishovska[1], Zharko Tanturovski[1], Sanija Zulfikjari[1], Aleksandra Dimitrovska[1], Zorica Arsova-Sarafinovska[1,4]

[1]*Institute of Public Health of Republic of North Macedonia, 50-ta Divizija 6, 1000 Skopje, Republic of North Macedonia*
[2]*Medical Faculty, University Ss. Cyril and Methodius, 50-ta Divizija 6, 1000 Skopje, Republic of North Macedonia*
[3]*University Clinic for Pediatric Surgery, 50-ta Divizija 6, 1000 Skopje, Republic of North Macedonia*
[4]*Faculty of Medical Sciences, University Goce Delchev, Krste Misirkov No 10-A, 2000 Stip, Republic of North Macedonia*

Introduction

Linezolid is an oral and parenteral antibiotic that belongs to a new group of synthetic antibiotics known as fluorinated oxazolidinones. It is indicated for Gram–positive infections and has been approved for vancomycin-resistant enterococcal infections, including bacterial pneumonia, skin and skin tissue infections, and infections related to susceptible organisms complicated by bacteremia (Hashemian et al., 2018).

Linezolid has been assayed in dosage forms by spectrophotometry, liquid chromatography, high–performance thin–layer chromatography and micellar electrokinetic chromatography (Mohapatra et al., 2011). However, there is yet no monograph on linezolid in the current European Pharmacopoeia. Therefore, we aimed to develop simple, fast and reliable RP-HPLC method for determination of linezolid in dosage forms in the presence of its degradation products. The method performance was further fully validated according to requirements in the ICH Q2(R1) Guideline (ICH, 2019).

Materials and methods

The method was developed using Shimadzu Nexera–I LC-2040C 3D Plus Ultra-High-Performance Liquid Chromatography system equipped with quadruple pump, automatic sampler and PDA detector. All data was processed with the LabSolutions 5.106 Version software.

Chromatographic separation was performed on a reversed-phase column Agilent ZORBAX SB C18 (250 x 4.6 mm I.D., particle size 5 µm), in an isocratic mode. The mobile phase consisted of a mixture of methanol and water acidified with o-phosphoric acid, pH 2.6, 50:50 (*V/V*). The flow rate was kept at 1.0 mL/min. Wavelength was selected by scanning a standard solution of linezolid over 200–400 nm using Model Lambda 12 (Perkin Elmer) UV-visible spectrophotometer and the wavelength of 254 nm was chosen for detection of linezolid. The injection volume was 20 µL. All separations were performed at a temperature of 30°C ± 2°C.

Linezolid USP reference standard and Linezolid related compound C were used in the study. Methanol and o-phosphoric acid were purchased from Merck (Darmstadt, Germany). Double-distilled water was used to prepare the solutions. Samples of Linezolid 2 mg/mL solution for infusion were obtained commercially.

Standard and sample preparation

The working concentration of the standard and the sample solution was 0.12 mg/mL. The standard solution was prepared by dissolving the linezolid reference standard with mobile phase. To prepare the sample solution, 3 mL volume of Linezolid 2 mg/mL solution for infusion was transferred into a volumetric flask and diluted with the mobile phase up to 50 mL. Before the injection in the HPLC system, both the standard and

*zanahaxhijaha@yahoo.com

sample solutions were filtered through a 0.45-µm nylon syringe filter.

Results and discussion

The selection of chromatographic conditions and mobile phase composition were based on system suitability parameters (resolution, tailing factor), run time and ease of preparation.

The method specificity was demonstrated with the resolution (Rs = 4.01) between the peaks obtained from the linezolid active substance and its closest eluted impurity, linezolid related compound C ((S)-5-(Aminomethyl)-3-(3-fluoro-4-morpholinophenyl) oxazolidin-2-one

The linearity of the method was evaluated by linear regression analysis and calculated by the least-square regression method. The calibration curve was constructed with 8 concentration levels ranging from 27.11 – 325.32 µg/mL. We obtained the following equation: y = 58922x – 28075, and concluded that the method is linear in the given range, with a correlation coefficient of 0.9999 ($R^2 = 0.9999$).

The precision of the methods was determined by repeatability (intraday precision) and intermediate precision (interday precision) and was expressed as relative standard deviation (RSD, %) of a series of measurements. The repeatability was evaluated by assaying 6 replicates of sample solution at the working concentration (0.12 mg/mL) on the same day. The intermediate precision was studied by comparing the results obtained on three different days. Based on the results for RSD (0.06% and 0.11%, for repeatability and intermediate precision, respectively), we concluded that the proposed method has acceptable repeatability and intermediate precision (RSD < 2%).

Accuracy is the degree of agreement between a measured value and the accepted reference value. Accuracy of the method was determined by calculating recovery of linezolid by standard addition method. Known amount of linezolid (54.2 – 277.8 µg/mL) was added to pre quantified sample solution and the amount of linezolid was determined. The average recovery was 100.77% (99.31 % – 101.43%) which confirmed the method accuracy.

Robustness is the ability to provide accurate and precise results under a variety of conditions. In order to measure the extent of method robustness, the most critical parameters (column temperature and flow rate) were deliberately changed while keeping the other conditions constant. We calculated the RSD of the tailing factor and retention time of the linezolid in the chromatograms after deliberately changing the working temperature (30°C - 2°C) and flow rate (1.0 mL/min ± 0.1 mL/min). The results of robustness study (RSD ranging from 0.12 % - 0.69%) indicated that the proposed method is robust (RSD ≤ 2%).

System suitability testing is an integral part of liquid chromatographic method validation performed to check and ensure on-going performance of a chromatographic system. It was estimated by 6 repeated injections of working standard solution at 100% of test concentration (0.12 mg/mL linezolid) and evaluated through the folowing parameters: capacity factor, tailing factor and theoretical plate. The results obtained for capacity factor (5.5), tailing factor (0.967) and theoretical plate (7040) confirmed that the proposed HPLC system is suitable for determination of linezolid in the phramaceutical dosage forms.

Conclusion

The proposed RP–HPLC method allows simple, accurate and precise determination of linezolid in pharmaceutical dosage forms, in the presence of its degradation products and related compounds. The advantages of the method include short run time, simple sample and mobile phase preparation, isocratic mode of elution, and excellent peak symmetry. Therefore, the developed method can be applied for the routine analysis for determination of linezolid in pharmaceutical dosage forms in quality control laboratories.

References

Hashemian, S.M.R., Farhadi, T., Ganjparvar, M., 2018. Linezolid: a review of its properties, function, and use in critical care. Drug Des. Devel. Ther. 12, 1759. https://doi.org/10.2147/DDDT.S164515

International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, 2019. ICH Harmonised Tripartite guidelines, Validation of Analytical Procedures: Text and Methodology Q2(R1).

Mohapatra, S., Annapurna, M.M., Ravi Kumar, B.V.V., Anwar, M., Warsi, M.H., Akhter, S., 2011. Validated stability indicating RP-HPLC method for the estimation of linezolid in a pharmaceutical dosage form. J. Liq. Chromatogr. Relat. Technol. 34(18), 2185-2195. https://doi.org/10.1080/10826076.2011.585548

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 101 – 102 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.046
Short communication

Evaluation of chromatographic conditions for simultaneously determination of Emtricitabine and Tenofovir

Dragica Doneva[1], Maja Shishovska[1], Zana Mustafa[1*], Shaban Memeti[1,2,3], Katerina Starkoska[1], Sanija Zulfikjari[1], Aleksandra Dimitrovska[1]; Zorica Arsova-Sarafinovska[1,4]

[1]*Institute of Public Health of Republic of North Macedonia, 50-ta Divizija 6, 1000 Skopje, Republic of North Macedonia*
[2]*Medical Faculty, University Ss. Cyril and Methodius, 50-ta Divizija 6, 1000 Skopje, Republic of North Macedonia*
[3]*University Clinic for Pediatric Surgery, 50-ta Divizija 6, 1000 Skopje, Republic of North Macedonia*
[4]*Faculty of Medical Sciences, Goce Delchev University, Krste Misirkov No 10-A, 2000 Stip, Republic of North Macedonia*

Introduction

The nucleoside analogue reverse transcriptase inhibitor emtricitabine and the nucleotide analogue RTI tenofovir disoproxil fumarate are antiviral drugs. They have each shown antiviral activity against a number of HIV clinical isolates and cell lines. A fixed-dose combination of two antiretroviral drugs (tenofovir and emtricitabine) used for the treatment of HIV is already present on the European market (Dando and Wagstaff, 2004)

However, monographs of emtricitabine and tenofovir are not included in the actual European Pharmacopoeia (Ph. Eur. 10th Edition). There is no official method for quality control of pharmaceutical dosage forms containing both emtricitabineand tenofovir. Therefore, reliable HPLC methods for simultaneous analysis of emtricitabine and tenofovir are needed for quality control of the approved fixed-dose combination of two the antiretroviral drugs.

The mostly used RP-HPLC columns in the quality control of the pharmaceuticals are those packed with silica particles with irregular or spherical shape, or could be monolith rods. On their surface there is octa-decyl-silyl (ODS, C18) reverse-phase coating. However, the chromatography performance obtained from defferent brand of the RP-HPLC columns differs significantly between each other, due to the quality of the column silica. The content of the metal ions residues in the silica could significantly influence the column performance (Bhavsar et al., 2012).

Few UV, RP-HPLC, high performance thin layer chromatography (HPTLC), and liquid chromatography with tandem mass spectrometry (LC/MS/MS) methods have been published for simultaneous estimation of emtricitabine and tenofovirdisoproxil fumarate in pharmaceutical formulation. However, most of them require sofisticated equipment and time consuming sample preparation (Bhavsar et al., 2012).

Therefore, the aim of this study was to optimize and to propose chromatography conditions for simultaneous identification and determination of emtricitabine and tenofovir active compounds in pharmaceutical dosage forms suitable for routine analysis in the quality control laboratories.

Materials and methods

Materials and reagents

HPLC analyses were performed using an Agilent Technologies chromatographic system (Hewlett Packard, Avondale, USA) consisting of a binary pump with DAD detector and auto sampler controlled by Agilent Technologies HPLC 1100 software.

Chromatographic separation was achieved on following analytical HPLC columns: Hypersil BDS C18 (125 mm x 4 mm); Zorbax SB C18 (250 mm x 4.6 mm); Lichrospher 100 C18 (250 mm x 4 mm); Purospher C18 endcapped (150 mm x 4.6 mm); Purospher C18 endcapped (250 mm x 4.6 mm), X-Select HBB C18 (250

*ssavovska@yahoo.com

mm x 4.6 mm), all with particle size 5 µm and Chromolith 18e performance (100 x 4.6), rod.

The mobile phase was composed of mixture of acetonitrile and water acidified with o-phosphoric acid (pH 2.6) in ratio 30/70 (V/V), with flow rates ranging from 1 to 3 mL/min, filtered through 0.45 µm nylon filter. The column temperature was set at 30 °C, injection volume 10 µL, with operating wavelengths at 260 nm and 280 nm.

Preparation of standard solutions

The stock solutions of standards were prepared with quantities of 16.35 mg emtricitabine and 24.53 mg tenofovirdisopronil succinate accurately weighted, transferredin separate volumetric flasks with volume of 25 mL and dissolved in a mixture of acetonitrile and water acidified with o-phosphoric acid (pH 2.6) in ratio 40/60 (*V/V*). We mixed the obtained solutions at an ultrasonic bath for 5 min. Then, for preparing the test solution the volumes of 3 mL from each of two stock solutions were mixed in 10 mL volumetric flask and filled up to the volume with the same solvent.

Results and discussion

In this study we used seven different ODS columns and we evaluate their efficiency in emtricitabine and tenofovir analysis.

Testing for identification, specificity, selectivity, resolution and suitability was according to requirements in the ICH Q2(R1) Guideline (ICH, 2019).

UV spectra scanned in mobile phase during the chromatographic analysis showed absorption maximum at 220 nm, 237 nm and 284 nm for emtricitabine and at 210 nm and 260 nm for tenofovir.

From the evaluated HPLC columns only two did not separatethe two analysed chromatographic peaks, at the applied chromatographic conditions: (Hypersil BDS C18 (125 mm x 4 mm) and Zorbax SB C18 (250 mm x 4.6 mm)). All other columns showed satisfactory resolution, over 4.3.

The best separation factor (ά) values are obtained for Purospher C18 endcapped (150 mm x 4.6 mm) and Purospher C18 endcapped (250 mm x 4.6 mm) (4.3 and 6.1, respectively).

The highest number of theoretical plates (N) were also estimated for the Purospher C18 endcapped (150 mm x 4.6 mm) columns and Purospher C18 endcapped (250 mm x 4.6 mm) columns (11677 and 16812 for the shorter and 20736 and 13114 for the longer column, respectively). We also obtained the best values for the height of theorethical plates withPurospher C18 endcapped columns.

The best peak symmetry was achieved on columns: Lichrospher 100 C18 (250 mm x 4 mm) and Purospher C18 endcapped (150 mm x 4.6 mm) at flow-rate of 1 mL/min and on Chromolith 18e performance (100 x 4.6), rod, at flow-rate 3 mL/min. These three columns showed retention times for emtricitabine (2.03; 1.81; 0.52 min) and for tenofovir (2.62; 5.77; 1.17 min) consequently.

The analysis run-timewith the studied columns ranged from6 min to 13 min maximum, which is optimal run-time for the routine control in the pharmaceutical laboratories.

Conclusion

The emtricitabine and tenofovir can be successively separated withRP–HPLC octadecylsylil columns.

Our experimental results showed that the optimal chromatographic conditions for simultaneous HPLC analysis of emtricitabine and tenofovir are achieved with mobile phase composed of 30% acetonitrile and 70%, water acidified with o-phosphoric acid at pH 2.6, at flow-rate 1 mL/min, column oven set at 30ºC, with specific UV detection at 260 nm for tenofovir and at 284 nm for emtricitabine.

Optimal results were obtained withLichrospher 100 C18 (250 mm x 4 mm) and Purospher C18 endcapped (150 mm x 4.6 mm) columns.

References

Bhavsar, D.S., Patel, B.N., Patel, C.N., 2012. RP-HPLC method for simultaneous estimation of tenofovirdisoproxilfumarate, lamivudine, and efavirenz in combined tablet dosage form. Pharmaceutical methods 3(2), 73-78. https://doi.org/10.4103/2229-4708.103876

Dando, T.M., Wagstaff, A.J., 2004. Emtricitabine Tenofovir Disoproxil Fumarate. Drugs 64, 2075–2082. https://doi.org/10.2165/00003495-200464180-00005

European Pharmacopoeia 10th Edition, European Directorate for the Quality of Medicines and Health Care, Strasbourg, 2019 - 2022.

International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, 2019. ICH Harmonised Tripartite guidelines, Validation of Analytical Procedures: Text and Methodology Q2(R1).

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 103 -104 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.047
Short communication

A review of published cases of severe cutaneous reactions associated with the use of the most frequently prescribed antiepileptic drugs

Simona Atanasovska*, Vladimir Kostovski, Irina Dukovska Teova, Sofija Dimkoska, Emilija Filipovska, Kristina Najdova, Ivona Trpenoska Aleksovska, Marija Panajotovikj Pop-Nikolova, Ace Kuzmanovski, Sandra Chomovska – Madevska

Global Pharmacovigilance, Alkaloid AD Skopje, Blvd. "Aleksandar Makedonski" 12, 1000 Skopje, North Macedonia

Introduction

Epilepsy is one of the most common neurologic disorders with an annual incidence of 50 per 100,000 persons per year in developed countries (Kim et al., 2020). The first-generation antiepileptic drugs (AEDs) which were introduced between 1912 and 1978 include carbamazepine, phenobarbital, phenytoin, primidone, and valproate. Gabapentin, lamotrigine, levetiracetam, oxcarbazepine, pregabalin, tiagabine, topiramate or vigabatrin are members of the second generation and the newest AEDs (or the third generation) are brivaracetam, eslicarbazepine acetate, lacosamide and perampanel.

In more than 25% of the patients, adverse drug reactions (ADRs) are the main reason for discontinuation of the treatment with antiepileptic drugs after the initial antiepileptic drugs are administrated. Up to 33% patients are refractory to multiple AEDs and such cases may potentially lead to recurrent adverse drug reactions and drug interactions (Mohanraj and Brodie, 2005). Hypersensitivity to AEDs is less common, but the risk of severe allergy is higher in this group (Fowler et al., 2019). Adverse skin reactions occur in 3% of individuals who receive antiepileptic drugs and are the most common reason for discontinuation of the treatment. Although most of them are usually mild, occasionally they may be severe when occurring as part of the syndromes of erythema multiforme, such as Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), and drug reaction with eosinophilia and systemic symptoms (DRESS) (Kim et al., 2020).

Materials and methods

For the purpose of this review, electronic literature search was conducted in through comprehensive screening using built logical sequences. Case reports or case series which reported detailed clinical description of the patients diagnosed with SJS, TEN and DRESS which were suspected or caused by the antiepileptic drugs levetiracetam, lamotrigine and lacosamide were included in the review. The review of the published case reports or case series covered period of 10 years starting from 01.05.2012 until 01.05.2022.

Results and discussion

44 publications describing a total of 52 patients which fulfilled the inclusion criteria for severe cutaneous reactions were included in the review. The age of the patients ranged from 2 years to 73 years (median: 32.7 years) and for 1 patient age was not reported. 32 of the patients were female (61.53%) and 20 male (38.47%) patients. DRESS, SJS and TEN were diagnosed in 15 (28.85%), 15 (28.85%) and 22 (42.30%) patients, respectively.

Levetiracetam

9 case reports (17.31%) related with levetiracetam use induced severe cutaneous reactions were identified during the review. 4 patients were diagnosed with DRESS and all of them completely recovered. 1 patient diagnosed with SJS completely recovered. 4 case reports related with levetiracetam induced TEN were identified. From them, 3 patients fully recovered and 1 was still recovering at the time when the article was published.

*satanasovska2@alkaloid.com.mk

Lamotrigine

42 case reports (80.77%) related with lamotrigine use were detected during the search of the PubMed/MEDLINE database. 10 patients (23.81%) were diagnosed with DRESS, and only one of the patients was with unknown outcome, while the other patients completely recovered. 14 patients (33.33%) were diagnosed with SJS. 9 of them have fully recovered, 1 patient recovered with sequelae, 1 patient had fatal outcome and 3 patients had unknown outcome. 18 patients with TEN (42.86%) were associated with lamotrigine use. 7 patients completely recovered, 4 patients were with status recovering at the period when the articles were published, 2 patients recovered with sequelae, 2 patients had fatal outcome and for 3 patients there was not specified the outcome from the ADRs.

Lacosamide

1 case report (1.92%) with DRESS related with lacosamide use was identified during the review. The patient fully recovered after the adverse drug reaction. In the retrieved case reports and case series, the onset of the severe cutaneous drug reactions was in range from 1 day to 6 months after initiating the anticonvulsant therapy. In most of them, the onset was approximately 2 weeks after the initiation of the AEDs. The therapy used for the patients with severe cutaneous drug reactions consisted mainly of corticosteroids, cyclosporine, immunoglobulins and antibiotics. The choice of antiepileptic drug depends on a variety of factors, including the type of seizure, drug response, side effects, and patient comorbidities (Man Kei Fong and Sheng, 2017). Aromatic AEDs such as phenytoin, carbamazepine and phenobarbital as well as some newer drugs, including lamotrigine have been related with eliciting a whole spectrum of hypersensitivity reactions, ranging from simple maculopapular skin eruptions to a severe life-threatening condition (Scaparrotta et al., 2011). During our literature review for published case reports and case series of the PubMed/MEDLINE database, 80.77% of the case reports with severe cutaneous reactions were associated with lamotrigine use. Severe cutaneous reactions due to lamotrigine have been shown to be associated with human leukocyte antigen (HLA) alleles in the population and the chances of these adverse effects are higher when lamotrigine is combined with valproate (Srivastava et al., 2017). These data are in line with the data obtained during our review as from the 42 identified case reports associated with lamotrigine, valproate was identified as concomitant drug in 16 cases. For levetiracetam, which is AED that does not contain an aromatic ring in their molecule, nine case reports of severe skin reactions were identified.

According to Li et al (2020), lacosamide is safe and effective in antiepileptic treatment, and its common side effects are dizziness, headache, drowsiness, diplopia, and cardiovascular abnormalities. Only one case report with lacosamide was identified during our review in which cross-reactivity with the drug was suspected in view of the rapid onset of DRESS syndrome after the initial rash resolution and soon after the introduction of the lacosamide (Man Kei Fong and Sheng, 2017).

Conclusion

The risk for an adverse event represents the probability for the event to occur among a defined exposed population and cannot be determined solely by literature screening. The conducted literature review for period of ten years suggested that when hypersensitivity skin reactions occurred, the aromatic AED lamotrigine was associated with higher risk of SJS/TEN/DRESS compared with levetiracetam and lacosamide. The frequency of lamotrigine induced DRESS and TEN is very rare (<1/10000) and for the lamotrigine induced SJS the frequency is defined as rare (≥1/10000 to <1/1000) (SmPC, Lamal, 2021). Levetiracetam as non-aromatic AED and lacosamide as one of the newest third-generation AED were associated with lower number of severe cutaneous reactions. These literature data also correlated with the data obtained from the EudraVigilance database regarding AEDs induced severe cutaneous reactions.

The benefits provided by the use of the antiepileptic drugs lamotrigine, levetiracetam and lacosamide far outweigh the risks that are related with them and therefore are safely used in everyday clinical practice for treatment of epilepsy.

References

Fong, K.M., Sheng B., 2017. DRESS syndrome: A case of cross-reactivity with lacosamide?. Epilepsia Open 2(2), 273–275. https://doi.org/10.1002/epi4.12053

Kim, K.H., Kim, Y.D., Bae, K.E., Kim, W.D., 2020. Adverse Skin Reactions with Antiepileptic Drugs Using Korea Adverse Event Reporting System Database, 2008–2017. J. Korean Med. Sci. 35(4). https://doi.org/10.3346/jkms.2020.35.e17

Li, J., Sun, M., Wang, X., 2020. The adverse-effect profile of lacosamide. Expert Opin. Drug Saf. 19(2), 131-138. https://doi.org/10.1080/14740338.2020.1713089.

Mohanraj, R., Brodie, M.J., 2005. Pharmacological outcomes in newly diagnosed epilepsy. Epilepsy Behav. 6(3), 382-387. https://doi.org/10.1016/j.yebeh.2005.01.008

Scaparrotta, A., Verrotti, A., Consilvio, P.N., Cingolani, A., Pillo, D.S., Gioacchino, D.M., Verini, M., Chiarelli, F., 2011. Pathogenesis and clinical approaches to anticonvulsant hypersensitivity syndrome: current state of knowledge. Int. J. Immunopathol. Pharmacol. 24(2), 277-284. https://doi.org/10.1177/039463201102400201

Srivastava S., Ramanujam B., Ihtisham K., Tripathi M.,2017. Cutaneous Adverse Drug Reactions to Lamotrigine and Human Leukocyte Antigen Typing in North Indian

Patients: A Case Series. Ann. Indian Acad. Neurol. 20(4), 408-410. https://doi.org/10.4103/aian.AIAN_234_17

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 105 – 106 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.048
Short communication

Challenges in the development and registration of generic topical products

Belma Asanova*, Emilija Davidova*, Natasha Vukikjevikj

Alkaloid AD Skopje, Blvd. Aleksandar Makedonski 12, 1000 Skopje, Republic of North Macedonia

Introduction

Topical delivery of active substances is most widely used in the treatment of skin diseases. It offers advantages such as non-invasiveness, direct drug delivery at the site of action, patient compliance and lower cost of treatment.

The marketing authorization approval of a topical generic product until recently required evidence on therapeutic equivalence in relation to a reference product documented through clinical/pharmacodynamic endpoint studies. However, regulatory bodies have updated the regulation relevant to topical generic products and became open to accept new surrogate methods for topical bioequivalence assessment.

The Draft Guideline on quality and equivalence of topical products by European Medicines Agency (EMA) offers guidance on the quality of topical products, not covered by other general quality guidelines, when developing a generic topical product. The guideline clearly states that regulatory applications based in literature to demonstrate safety and efficacy should be supported by equivalence data of the formulation. At the same time, provides details of *in vivo* and *in vitro* models that may substitute clinical data and when biowaivers are applicable.

Regulatory aspects in obtaining and maintaining marketing authorization for generic topical drug product

Proving equivalence of generic topical drug products vs. reference medicinal product

Proving topical bioequivalence between a generic and reference medicinal product is a very complex process, which is basically dependent on the formulation of the product. The basis of the topical generic products development is, understandably, an in-depth characterization of the reference product. The Regulatory agencies, such as EMA and U.S. Food and Drug Administration (FDA), have improved the topical generic products development process by promoting the extended pharmaceutical equivalence concept. According to the draft EMA guideline, for simple formulations, bioequivalence may be demonstrated by documenting the qualitative (Q1), quantitative (Q2), microstructure (Q3) and performance (Q4) equivalence. Nevertheless, when addressing complex semisolids, equivalence regarding local availability should also be demonstrated which may be an extremely difficult and challenging goal. To optimize the regulatory requirements for the therapeutic equivalence of topical generic drug products, as the "one size fits all" approach will not work for BE determination of all types of topical dosage forms, the Strawman decision tree and the Topical Drug Classification system (TDS) are common features that facilitate product development, reduce the regulatory burden and assure product quality (Miranda et al., 2018).

Challenges in proving Q1, Q2, Q3 and Q4 equivalence

Since the qualitative composition of the comparator product is always included in the available product information documents, proving Q1 equivalence, seems to be relatively simple. However, matching the grades of the excipients used is an important rule for achieving the product performance and Q3 similarity. If the information of the grade is not available, analysis of different grades and relations with the product performance is considered beneficial however, it is a quite demanding process.

The approach for obtaining the Q2 equivalence is performance of reverse engineering of the comparator product, or to have information of the full qualitative and quantitative composition of the product via publicly

*basanova@alkaloid.com.mk; edavidova@alkaloid.com.mk

available databases which is almost impossible to obtain, and it is recommended to be accompanied by the reverse engineering approach anyways. Another issue, beside the difficulties in acknowledgment of the quantitative composition of the comparator product is the patent pendings, variability of the excipients in their grades, batch-to-batch variabilities of the excipients or the unsatisfactory outcome of the reverse engineering leading to non-similarity in the surrogate tests. Therefore, accomplishing the Q1/Q2 equivalence could be a quite challenging task (Shah, et al., 2016).

The IVR (in-vitro release) (Q3) reflects the microstructural arrangement of the dosage form and the state of aggregation of dispersed particles. Q3 mainly follows and it depends on the success of the Q1 and Q2 sameness and reflects the similarity in the in-vitro release between the generic and the reference product. The *in vitro* release characteristics of the drug from its dosage form is an excellent indicator that enables identifying the microstructural arrangement of the dosage form. However, there are cases where even with successful Q1, Q2 and even Q4 sameness, Q3 equivalence was not achieved. Therefore, it remains unclearly defined in the guidelines whether some rheology endpoints can be waived or if there are some acceptable and completely safe criteria for the patients or if there are additional bridging data that could mitigate this (Miranda et al., 2022).

Q4 (performance) equivalence challenges arise mainly from the established criteria in the EMA guideline, which are very demanding. Even when no clinically significant difference is obtained, the strict criteria in the guideline may lead to a conclusion that there is no Q4 equivalence between the test and the reference product not considering the intrinsic variability of topical semisolid dosage forms. With regards to Q5 similarity (equivalence with respect to efficacy), additional challenge is the condition in each IVPT (In vitro permeation testing) 12 donors with at least 2 sections per donor to be used, due to the inter- and intra-individual variability of human skin. This significant increase in the requirements compared to the pre-existent guidelines could be extremely difficult since human skin is usually retrieved from plastic surgery, with ethical consent being required. (Shin, et al., 2020).

Post-authorization changes

For any change during the life-cycle of the product, a risk assessment should be performed to determine its impact on quality, safety, or efficacy of the product. The guideline states that if the proposed change does not meet the extended pharmaceutical equivalence acceptance criteria, or qualitative and quantitative composition, then equivalence should be demonstrated using an appropriate clinical study. Description of different situations and exceptions where waivers in respect to post-authorization changes are applicable, as well as inclusion of the possibility of measuring equivalence by other physical and chemical means than IVRT (in cases where IVRT is not discriminative) are not very clearly stated. Due to the lack of experience and close recommendations in the guideline post-authorization changes remain a big challenge for the topical generic products.

Conclusion

The EMA guideline on quality and equivalence of topical products following the FDA approach for topical products is a refreshment for the pharmaceutical industry, since it promotes a valuable input on the standardization and definition of strict criteria regarding the definition, as well as validation of the extended pharmaceutical equivalence. However, the presented approach by EMA also bears some relevant limitations which should be carefully debated in a near future in order to allow a successful translation into the practice. Sufficient and clear guidance available to the industry, will lead to a facilitated development and registration of a generic topical product, still maintaining the ultimate requirements for high quality, safety and efficacy, and finally their availability to patients and consumers at a more reasonable cost.

References

Committee for Medicinal Products for Human Use (CHMP), EMA. Draft Guideline on Quality and Equivalence of Topical Products, CHMP/QWP/708282/2018. 2018.

Miranda, M., Sousa, J.J., Veiga, F., Cardoso, C., Vitorino, C., 2018. Bioequivalence of topical generic products. Part 2. Paving the way to a tailored regulatory system. Eur. J. Pharm. Sci. 122, 264–272. https://doi.org/10.1016/j.ejps.2018.07.011

Miranda, M., Veloso, C., Brown, M., Pais, A., Cardoso, C., Vitorino, C., 2022. Topical bioequivalence: Experimental and regulatory considerations following formulation complexity. International Journal of Pharmaceutics. 620. https://doi.org/121705. 10.1016/j.ijpharm.2022.121705

Shah, V.P., Rădulescu, F. S., Miron, D., Yacobi, A., 2016. Commonality between BCS and TCS. Int. J. Pharm. 509, 35–40. https://doi.org/10.1016/j.ijpharm.2016.05.032

Shin, S.H., Rantou, E., Raney, S.G., Ghosh, P., Hassan, H., Stinchcomb, A., 2020. Cutaneous pharmacokinetics of acyclovir cream 5% products: Evaluating bioequivalence with an in vitro permeation test and an adaptation of scaled average bioequivalence. Pharm. Res. 37, 210. https://doi.org/10.1007/s11095-020-02821-z

US FDA Product-Specific Guidances for Generic Drug Development.

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 107 - 108 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.049
Short communication

Change in the primary packaging of tablets from glass bottle to blister

Ana Ivcheska*, Ivana Trajkova*, Natasha Kronevska, Elizabeta Karadzinska, Olivera Peneva

ALKALOID AD Skopje, Pharmaceutical, Chemical and Cosmetics Company,
Aleksandar Makedonski 12, 1000 Skopje, Republic of North Macedonia

Introduction

All medicinal products need to be appropriately protected and packaged in containers that conform to prescribed standards. It is of utmost importance that the products are protected from moisture and light and that the leaching of extractable substances into the packaging/containers is prevented. There should be no chemical interaction between the container and the product.

Primary packaging is the material that is used for the containment, protection, handling, delivery and presentation of a product that is provided to a patient at the point of sale. It is in direct contact with the product and is often referred to as retail packaging or POS (Point-of-Sale) Packaging. The main purpose of primary packaging is to preserve the product as well as provide key information to the patient.

Packaging is used in order to provide appropriate protection and containment of a medicinal product during it's shelf-life. The product should be protected during storage, distribution and until it is consumed by the patient. The packaging should also provide identification information regarding the product.

Packaging must provide protection against climatic conditions biological, physical and chemical hazards and must be economical. The package must ensure adequate stability of the product throughout the shelf life. During a product's life cycle, a change in the primary packaging may be required due to various reasons. In this particular case a change was required due to disposal of equipment used for packaging in a glass bottle. The change was documented and evaluated within the company's change management system and appropriately justified.

Change control process

Change control is a systematic approach to all changes of the product, process or both that can have direct or indirect impact on the quality of the finished medicinal product/medical device and quality system. In order for this change to be successfully implemented a series of activities must be done such as: stability testing under ICH and long-term conditions and monitoring of the results, packaging validation, regulatory activities etc.

Stability testing

Stability of a pharmaceutical product could be defined as the capability of the product to remain within it's physical, chemical and microbiological specifications while contained in a specific container/closure system. (Kommanaboyina and Rhodes, 1999). Stability studies are required to be conducted in a planned way following the guidelines issued by ICH, WHO and or other agencies. (Bajaj et al., 2012). In order to change immediate packaging of the product, relevant stability studies should be started under ICH/VICH conditions and relevant stability parameters should be assessed in at least two pilot scale or industrial scale batches and the manufacturer should have at least 3 months satisfactory stability data at the disposal before submission of variation. In this particular case two industrial batches were placed under accelerated and long-term conditions for stability testing.

*aivceska@Alkaloid.com.mk

Packaging validation

Packaging validation is establishing documented evidence, which provides a high degree of assurance that a specific packaging process, will consistently provide a product which meets regulatory approved specification criteria. For the Packaging validation process, a Blister packaging validation matrix exists, with product grouping and risk assessment, for evaluating the critical product and process parameters, and identifying representative worst-case product. Upon introduction of new product in the blister packaging process, re-evaluation of the risk assessment is performed, and decision is made for re-validation.

Regulatory activities and requirements

In order to change immediate packaging of a product a variation should be submitted to all concerned regulatory agencies. This type of change entails change in the Summary of Product Characteristics (SmPC), Patient Information Leaflet (PIL) and mock-up as well as change in relevant parts of the dossier. Additional data that is required prior submission is appropriate data on the new packaging (comparative data on permeability e.g. for O2, CO2 moisture) and proof that no interaction between the content and the packaging material occurs (e.g. no migration of components from the container into the product, as well as no loss of components from the product into the proposed container/primary packaging). (Guidelines 2013/c 223/01). Confirmation should be provided that the proposed new material complies with relevant pharmacopoeial requirements, as well as other regulatory requirements such as the legislation of the Union on plastic material and objects in contact with foodstuffs. This variation was classified as Type IB variation, meaning that the change can be implemented after approval of variation.

Conclusion

In order for this change to be successfully implemented and to maintain/improve the quality of the product as well as ensure the safety of the patients, all described activities must be carried out in accordance to cGMP and regulatory requirements.

References

Bajaj, S., Singla, N., and Sakhuja, N., 2012. Stability Testing of Pharmaceutical Products. Journal of Applied Pharmaceutical Science 2(3), 129-138.

Guidelines 2013/c 223/01 Guidelines on the details of the various categories of variations, on the operation of the procedures laid down in Chapters II, IIa, III and IV of Commission Regulation (EC) No 1234/2008 of 24 November 2008 concerning the examination of variations to the terms of marketing authorisations for medicinal products for human use and veterinary medicinal products and on the documentation to be submitted pursuant to those procedures (2013/C 223/01). Official Journal of the European Union 2013, ISSN 1977-091X.

Kommanaboyina, B., Rhodes, C.T., 1999. Trends in Stability Testing, with Emphasis on Stability during Distribution and Storage. Drug Development and Industrial Pharmacy 25, 857-868.

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 109 - 110 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.050
Short communication

Cleaning validation in production area – development of analytical method for quantitative determination of Cefixime residues

Miona Manasova[1*], Elena Bilbilovska[1], Biljana Markoska Janevski[1], Jelena Acevska[2], Gordana Mitrovska[1], Hristina Babunovska[1]

[1] *Alkaloid AD Skopje, Blvd.Aleksandar Makedonski 12, 1000 Skopje, North Macedonia*
[2] *Faculty of Pharmacy, Ss. Cyril and Methodius University, Mother Teresa 47, 1000 Skopje, North Macedonia*

Introduction

Cleaning is considered a critical process in the manufacturing of pharmaceutical products. The cleaning process must reduce residues of previous product to levels that ensure patient safety and result in visibly clean equipment.

Cleaning validation is documented evidence that an approved cleaning procedure will reproducibly remove the previous product or other residues on product contact equipment surfaces below the scientifically set acceptance levels.

An analytical method is one of the deciding factors in establishing the cleanliness of pharmaceutical manufacturing equipment. It is, therefore, important that there be a high level of confidence in the results obtained using the method. A properly developed cleaning validation strategy includes analytical method validation, which defines the method parameters necessary in providing a high level of confidence in the cleaning results (Kaiser and Ritts, 2004).

Measuring cleanliness is a difficult task. Essentially, trace residues in surfaces are the target analytes. The residue must first be extracted from a surface, recovered from the extraction medium, and then suitably quantitated (Kaiser and Ritts, 2004).

API residue is typically tested, as it may be the high hazard component in the formulation. The primary consideration for the test method is that it is sensitive to levels lower than the cleaning limit for the analyst of interest. If the test method is not sensitive enough to test residue levels lower than the cleaning limit, the method sensitivity must be enhanced, a different test method must be employed, or the manufacturing equipment must be dedicated.

Product grouping is a way to reduce validation activities in sites with multiple products and processes. If a product in a group requires a more aggressive cleaning process, that product becomes a worst-case product in that group. The worst-case product should be determined by performing a risk assessment considering solubility, toxicological effect and cleanability. All the products in the group should be cleaned using the worst-case product parameters (Govind et al., 2018).

Cefixime granules for oral suspension 100mg/5mL was chosen on the basis of a worst-case rating approach.

The aim of this study was to validate simple analytical method for verification of cefixime residues on equipment after production of Cefixime granules for oral suspension 100mg/5mL.

Materials and methods

The HPLC-UV method for determination of cefixime residues on stainless steel surface was developed and validated in order to control a cleaning procedure after manufacturing of Cefixime granules for oral suspension 100mg/5mL. The HPLC method has been validated to show specificity, linearity and range, accuracy, precision, limit of quantification (LOQ) and limit of detection (LOD), as per ICH guideline Validation of analytical procedures: Text and Methodology Q2(R1).

*mmanasova@alkaloid.com.mk

Chemicals and reference standards

Potassium dihydrogen phosphate, Tetrabutylammonium hydroxide, Sodium hydroxide, o-Phosphoric acid 85%, Acetonitrile, Methanol and Purified water were supplied from Merck KGaA, Darmstadt, Germany. Reference standard for Cefixime trihydrate was supplied from Alkaloid, AD Skopje.

Instrumentation and analytical conditions

The analysis was performed on Thermo Ultimate DAD 3000 and was controlled by Chromeleon CDS software version 7.2 SR5. The method was optimized by using column Symmetry C18, 150mm x 3.9mm, 5µm; with column temperature of 40 ºC; autosampler temperature of 23 ºC; at a 1.5 mL/min flow rate and 254 nm detection. Mobile phase: mixture od acetonitrile and buffer solution pH=6.5 in ratio 23 : 77% (*V*/*V*). The injection volume was 10 µL.

Results and discussion

The method was specific and distinguished the specific analyte of interest (cefixime) from the other ingredients of the formulation, potential degradants and the cleaning agent.

It exhibited good linearity between the responses of cefixime related to the concentrations of standards in the range of 0.4 µg/mL to 20 µg/mL (r=1.00).

The precision of the method was verified by repeatability and method precision. The repeatability was shown by six replicate injections of standard solution containing cefixime in the working concentration of 10 µg/mL (RSD=0.3%). The method precision was evaluated using six samples which are first extracted from a stainless steel coupons, recovered from the extracted medium and then suitably quantitated (RSD=0.9 %).

The LOD and LOQ were determined at a signal-to-noise ratio of 3:1 and 10:1, respectively. The limit of detection was 0.12 µg/mL and the limit of quantification was 0.4 µg/mL.

For cleaning validation, accuracy is measured through recovery of samples from equipment surface e.g. stainless steel and extraction of the recovered samples into testing solutions.

The test solution with known concentration was applied to the surface of the equipment (stainless steel coupons, size 25 cm^2). The residue was removed by two Texwipe TX715 swabs (one methanol wetted swab and the other one dry). The swabs were thereafter extracted with mobile phase for 15 minutes with sonication (Yang et al., 2005).

Accuracy was reported as % recovery of the amount of analyte in the recovered samples measured against the amount of analyte spiked onto the sample recovery surface.

The accuracy was performed at three concentration levels and the obtained mean recovery was 90.63% and RSD=3.61%. Acceptance criteria for recovery was 70.0-110.0% and RSD $\leq$ 15.

This parameter should be performed on all materials from which the production equipment is made.

It is necessary to use recovery factor to all individual results in cleaning validation of pharmaceutical manufacturing equipment. Recovery factor is the recovery of swabbed material from the equipment to the solution.

Conclusion

Proper development and validation of the analytical method helps assure that the cleaning procedure is effective and reproducible in preventing contamination and cross-contamination in production area.

Simple analytical method for quantitative determination of cefixime residues on equipment after production of Cefixime granules for oral suspension 100mg/5mL was validated. The linearity of the method covers the required sensitivity for residue detection and the accuracy of the method was proved by recovery of swabbed samples from equipment. The method can be applied to routine control of pharmaceutical equipment cleanliness by sampling from stainless steel surface areas of 25 cm^2.

References

Kaiser, H.J., Ritts, B., 2004. Validation of Analytical Methods Used in Cleaning Validation. Analytical Methods Validation 15-30. Available at: https://asset-pdf.scinapse.io/prod/2526683963/2526683963.pdf

International Conference of Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, 2005. Validation of Analytical Procedures: Text and Methodology, Q2 (R1).

ISPE Guide: Cleaning validation lifecycle - Applications, Methods and Controls, 2020, 99-135.

Yang, P., Burson, K., Feder D., Macdonald, F., 2005. Method Development of Swab Sampling for Cleaning Validation of a Residual Active Pharmaceutical Ingredient. Pharmaceutical Technology 29(1), 84-94.

Govind, R.P., Kant, A.R.K., Tanuj, J., Dheeraj, B., 2018. A Review On Cleaning Validation In Pharmaceutical Industry. Jorunal of Drug Delivery and Therapeutics 8(3), 138-146. https://doi.org/10.22270/jddt.v8i3.1695

Macedonian pharmaceutical bulletin, 68 (Suppl 1) 111 - 112 (2022)
Online ISSN 1857 - 8969
*DOI:*10.33320/maced.pharm.bull.2022.68.03.051
Short communication

Consultation with target patient groups: User (Readability) testing/bridging procedure Regulatory experience with Agency for medicinal products and medical devices of Bosnia and Herzegovina (ALMBiH)

Gordana Damjanovska*

REPLEK FARM Ltd, Kozle 188, 1000 Skopje, Republic of North Macedonia

Introduction

All medicinal products placed on the market in the European Union are required by EU law to be accompanied by labeling and package leaflet which provide a set of comprehensible information for appropriate and safe use of the product.

European Commission's "Guideline on the readability of the labeling and package leaflet of medicinal products for human use", Revision 1, 12 January 2009, and "Guidance concerning consultations with target patient groups for the package leaflet Article 59(3), Article 61(1) and 63(2) of Directive 2001/83/EC as amended by Directive 2004/27/EC" require that the package information leaflet (PIL) reflects the results of consultations with target patient groups to ensure that it is legible, clear and easy to use, that these results are provided to the competent authority, and that the package leaflet must be written and designed to be clear and understandable, enabling the users to act appropriately, when necessary with the help of health professionals.

In addition, CMDh guidance "Consultation with target patient groups -meeting the requirements of article 59(3) without the need for a full test -recommendations for bridging" (CMDh/100/2007, Revision 3, December 2017), indicates that not every leaflet needs to be subject to a full test. Applicants and marketing authorisation holders (MAH) may be able to rely on testing applied to PILs for similar products. Bridging is used when leaflets are sufficiently similar in both content and layout and successful user consultation on one leaflet can be used to demonstrate that another leaflet meets the requirements of article 59(3) of Council Directive 2001/83/EC. Evidence of successful user test on the PILs used for bridging purpose (e.g. a copy of the relevant Public Assessment Report (PAR) or European Public Assessment Report.

The aim of this paper is to present the regulatory outcome for the User testing/ Bridging procedures submitted to ALMBiH in module 1 (section 1.3.4) of the application for obtaining or renewal of marketing authorization (MA) for medicinal products manufactured by Replek Farm Ltd. Skopje in the period 2019-2022.

Material and methods

User (Readability) testing was performed as one to one, face to face interviews with selected participants using structured Questionnaire 30 up to 45 minutes. Interviews were divided in the following stages: preliminary test (pilot test) for identification of major changes to the leaflet; 1st and 2nd round of testing that involved 25 participants; revision of PIL after 1st and 2nd round of testing if necessary, in order to achieve better understanding and 3th round of testing that was to be performed, if necessary. Interviews were conducted with healthy volunteers that could be potential users of the medicinal products subject of user testing, recruited by placing an announcement in pharmacy shops by marketing team of Replek Farm Ltd. Skopje) who have signed Informed Consent Form. Total number of 25 participants was tested for each User testing. Five participants were tested in the preliminary stage, 10 participants were tested in the 1st round, and 10 participants in the 2nd round. Interviews were conducted in accordance with protocols for conducting studies during COVID-19. Interviewers were previously informed for performing of Readability testing with Instructions for the Interviewer and obtained structured

*gordana.damjanovska@replek.mk

Questionnaire based on prioritized data from final revised version of PIL. The Questionnaire was developed to include 15 questions specific to the medicine, 3 specific to the layout and 3 for overall feedback regarding the PIL. The questions addressed key safety issues and concerns of the medicine.

Results and discussion

Six User (Readability) testing were performed by REPLEK FARM Ltd Skopje, for these medicinal products: NEURO-VIT, film coated tablets 100mg/200mg/50mcg submitted for first registration; Paracetamol Syrup 100mg/5ml; Paracetamol Tablets 500mg; REKONAZOL Shampoo 2%; NORFLOKSACIN Film-coated tablets 400mg and VENTOR tablets 100mg submitted for renewal of marketing authorization to ALMBiH. Revision of PIL between two rounds of testing was assessed as necessary for NEURO-VIT, film coated tablets to achieve better understanding.

According to “Guideline on the readability of the labeling and package leaflet of medicinal products for human use”, Revision 1, 12 January 2009, a satisfactory test outcome is when, for each question, 90% of all participants are able to find the information requested within the PIL and 90% can show that they understand and can act upon it. All six User (Readability) testing met these criteria and were accepted as satisfactory by ALMBiH with subsequent granting of MA or renewals of exsisting MA for all submissions untill 2021. Submissions in 2022 are under review.

Nine Bridging User (Readability) testing were performed by REPLEK FARM Ltd Skopje, for these medicinal products: Karvedilol Replek Farm Tablets 6.25mg; 12.5mg; MHRA Public Assessment Report: Procedure No: UK/H/1170/001-4/DC; UK Licence No: PL 32256/0004-7). Ibuprofen Replek Farm Cream 100mg/g; MHRA/UKPAR /PL 10972/0089. IBUPROFEN REPLEK FARM Suryp 100mg/5ml; MHRA Public Assessment Report/UK Licence No. : PL 00037/0677. Ibuprofen Replek Farm Film-coated tablets 400mg; MHRA Public Assessment Report/UK Licence No. : PL 00037/0674. Klaritromicin Replek Farm Film-coated tablets 500 mg; Public Assessment Report (Scientific discussion) Procedure No NL/H/3682/001-002/DC). REFALGIN Tablets 500 mg; Public Assessment Report: (Decentralised Procedure No. DE/H/5204/001/DC, 03.09.2018). Atorvastatin Replek Farm Film-coated tablets 10mg; 20mg; Public Assessment Report (BASG - Public Assessment Report and Scientific discussion. Procedure No. AT/H/0667/001-004/DC). Folic acid Replek Farm Tablets 5mg; Public Assessment Report (Public Assessment Report Scientific discussion. Procedure No. SE/H/1793/01-02/DC). Olanzapin Replek Farm Film coated tablets 5 mg, 10mg; Public Assessment Report and Scientific discussion. Procedure No. IS/H/0140/001/DC; IS/H/0143/001/DC.

All nine Bridging user testing were submitted for renewal of Marketing Authorisation and were accepted as satisfactory by ALMBiH with subsequent renewal of MA.

Conclusion

User consultation and user testing is the most applied form mostly valued type of consultation with target patient groups. At the same time this procedure is time and resources consuming, usually takes 1,5 month, that may be challenging when several studies need to be performed in short period of time. Recruitment of participants may also be challenging taking into consideration voluntarily participation as well as inclusion of children. Regarding the questions, profound analysis of key safety issues and careful choice should be done, as well as suitable questionnaire. If preliminary results are not satisfactory, revision of PIL might be necessary with accompanying variation of existing PIL.

The most challenging part of this procedure is obtaining available PAR/EPAR (user consultation included) on official website of HMA or EU Medicinal Agency and even more, obtaining Parent PIL that is updated and in English. Translating Parent PIL from one of the languages used in EU to English may sometimes substantially change the context of the key safety information; hence the whole procedure may be invalid. Additionally Parent PIL may not be updated and accompanying variation of existing Daughter PIL might be necessary.

References

CMDh Consultation with target patient groups -meeting the requirements of Article 59(3) without the need for full test-recommendations for bridging. CMDh/100/2007, Rev. 3 December 2017.

DIRECTIVE 2001/83/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 6 November 2001 on the Community code relating to medicinal products for human use.

EUROPEAN COMMISSION GUIDELINE ON THE READABILITY OF THE LABELLING AND PACKAGE LEAFLET OF MEDICINAL PRODUCTS FOR HUMAN USE Revision 1, 12 January 2009.

EUROPEAN COMMISSION Guidance concerning consultations with target patient groups for the package leaflet Article 59(3) and 61(1) of Directive 2001/83/EC as amended by Directive 2004/27/EC, May 2006