ORIGINAL ARTICLE

Beliefs and knowledge related to nutritional supplements among pharmacy students

Názory a znalosti týkající se doplňků stravy mezi studenty farmacie

Charalampos Triantis • Evanthia Asimakopoulou • Konstantina Konidari • Anna Filotheidou • Panagiotis Theodosis-Nobelos

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Summary

A variety of supplement-drug interactions has been identified, and therefore health scientists should be aware of the proper usage of nutritional supplements. The main aim of this study was to assess beliefs and knowledge related to nutritional supplements among pharmacy students. A cross-sectional study was conducted in order to assess beliefs and knowledge related to nutritional supplements among pharmacy students. A literature review on nutritional supplements was conducted in order to develop a 24-item questionnaire, and expert indications established its face and content validity. The sample was comprised of 142 pharmacy students in Cyprus, whose results are presented. The majority of the participants was receiving nutritional supplements (66.9%) and believed that nutritional supplements may interact and may have toxic effects. Those in the higher years of study scored higher in knowledge than those in the lower ones. The mean scores (mean ± SD) on the knowledge about nutritional supplements by the years of study were 9.14 ± 2.67, 8.71 ± 2.92, 9.91 ± 2.08, 11.16 ± 3.00, and 15.76 ± 2.67 and compared using one-way ANOVA followed by Bonferroni correction. The level of statistical significance was set at $p < 0.05$. The study showed that pharmacy students are adequately informed about nutritional supplements and that this subject is essential for the curriculum of pharmaceutical studies.

Key words: beliefs • knowledge • nutritional supplements • pharmacy students

Souhrn

Byla zjištěna celá řada interakcí mezi doplňky stravy a léky, a proto by si zdravotníci měli být vědomi správného používání doplňků stravy. Hlavním cílem této studie bylo posoudit názory a znalosti týkající se doplňků stravy mezi studenty farmacie. Byla provedena průřezová studie s cílem posoudit názory a znalosti týkající se doplňků stravy mezi studenty farmacie. Byl zpracován přehled literatury o doplňcích stravy s cílem vytvořit 24položkový dotazník a na základě expertních indicií byla stanovena jeho zjevná a obsahová validita. Vzorek tvořilo 142 studentů farmacie na Kypru, jejichž výsledky jsou prezentovány. Většina účastníků užívala doplňky stravy (66,9 %) a domnívala se, že doplňky stravy se mohou vzájemně ovlivňovat a mohou mít toxické účinky. Osoby ve vyšších ročnících studia dosáhly vyššího skóre ve znalostech než osoby v nižších ročnících. Průměrné skóre (průměr ± SD) znalostí o doplňcích stravy bylo 9,14 ± 2,67, 8,71 ± 2,92, 9,91 ± 2,08, 11,16 ± 3,00 a 15,76 ± 2,67 pro jednotlivé ročníky studia a bylo porovnáno pomocí jednorozměrné ANOVA s následnou Bonferroniho korekcí. Hladina statistické významnosti byla stanovena na $p < 0,05$. Studie ukázala, že studenti farmacie jsou dostatečně informováni o doplňcích stravy a že tento předmět je nezbytný pro kurikulum farmaceutického studia.

Klíčová slova: názory • znalosti • doplňky stravy • studenti farmacie

Charalampos Triantis, PhD (✉) • A. Filotheidou • P. Theodosis-Nobelos
Pharmacy Department Frederick University
Y. Frederickou Str. 7, Nicosia 1036, Cyprus
e-mail: hsc.tc@frederick.ac.cy

E. Asimakopoulou
Nursing Department Frederick University, Nicosia, Cyprus

K. Konidari
Secondary Education, University of Athens, Greece

Introduction

Over the last few decades, the usage of nutritional supplements (NS) has been increased. Among the US

marketplaces, there were about 4,000 NS products in 1994, which increased to 29,000 by 2000, and more than 85,000 in 2018[1]. According to CRN's (Council for Responsible Nutrition) 2019 consumer survey, it is estimated that about 7 out of 10 adults in the USA take NS[2]. This growing demand for NS could be attributed to the rapid increase of lifestyle-related diseases, such as cardiovascular diseases, cancer, etc.[3–6]. People of every age and physical condition take NS in order to maintain health or prevent a possible deficiency. NS are formulations that contain in pure and concentrated form nutrients such as vitamins (multivitamin or single vitamins), minerals, amino acids, and herb supplements. Common reasons for NS consumption are good health maintenance, immune system boost, mental peace, weight management, and chronic diseases[7, 8].

Although these products intend to supplement the diet, they are not always innocent, as a variety of supplement-drug interactions have shown in clinical trials. The NS could lead to serious interactions with other drugs and implications in some diseases or in special conditions, like pregnancy. Moreover, misled beliefs about the safety, toxicity, or interactions and inadequate knowledge about NS have been observed among not only consumers but also health care professionals[9–11]. Thus, the understanding and application of the correct use of NS is a basic learning outcome of Pharmacy Schools[4].

Pharmacists, as healthcare professionals, must be informed and responsible for valid and safe consumer education on NS regarding their use and interactions[12, 13]. A recent study reported that inadequate pharmacists' knowledge of NS was the main barrier to patients' counseling[14]. The assessment of beliefs and knowledge related to nutritional supplements among health care professionals, and especially in pharmacy students, is essential for pharmacists' acknowledgment of NS and safety improvement. A valid and reliable assessment tool for the nutrition supplements, including multivitamins, minerals, herbal medicines, and their interactions, would be of critical importance for health care professional education and health care services development.

Up to the present, there is not enough literature for a validated assessment tool about nutritional supplements' beliefs and knowledge, reflecting safety acknowledgment and interaction prevention issues among health care scientists. Moreover, very little information is available about the usage and knowledge of NS in the Greek and Cypriot populations. The aim of this study was to determine beliefs and knowledge among pharmacy students regarding nutritional supplements.

Experimental part

Materials and Methods

Design

Literature on nutritional supplements was reviewed in order to develop beliefs and knowledge questionnaire[7, 8, 15–19]. For the review were used the keywords nutritional supplements, vitamins, herbal medicine, interactions, and questionnaire on the electronic database including Scopus, Google Scholar, and PubMed. A 35 item pool was developed and reviewed by two experts in the field, an experienced professional pharmacist and a Pharmacy University Professor, in order to clarify their ambiguity. This study was conducted on pharmacy students in Nicosia after the approval of the Ethics Committee. Pilot interviews with 20 participants, who were not included in the present study, were conducted to test the questionnaire for its comprehensibility and the appropriateness of its items. The results were used to determine the items of the final instrument and the qualitative feedback from the respondents.

The final questionnaire consisted of 24 items. Specifically, the questionnaire was divided into 3 parts, the demographic, the beliefs (4 items), and the knowledge part (20 items). The demographic part included personal details such as place of origin/residence, sex, year of study, and some attitudes towards the supplements, like the frequency of NS usage, the category they consume, and the source of advice on which they based their decision. The beliefs part was related to their safety and included 4 items on the Likert scale. The knowledge part included 20 multiple choice type items that cover the proper way NS should be taken and some of their common interactions. The total score of the knowledge part was assessed by adding all responses from 0 to 20; incorrect answers were given 1 point, and incorrect answers 0 points.

For the sample size, it is generally accepted to enroll 5 subjects for each question, for a sample > 100, in order to strengthen the instrument validity. Therefore, the number of sample for the current study was designed to be at least 120 subjects[20–22]. A sample of 142 pharmacy students was interviewed in order to complete the questionnaire. Informed consent was obtained for all individuals who agreed to participate in the study. The participants had 15 min, sufficient time for completion of the questionnaire.

Data analysis

Descriptive statistics were used to assess demographic data. A two-sample t-test, as well as a non-parametric t-test, was used to investigate differences between the mean values of independent groups. Ordinal logistic regression was performed in order to investigate the association between ordinal and other explanatory variables. Furthermore, One-Way ANOVA and Kruskal-Wallis test were evaluated to determine the existence of differences in the mean values of the different levels of a categorical variable, while Tukey's test was used to check which groups actually differ. To adjust for multiple comparisons, a Bonferroni correction was applied. The reliability of the questionnaire was assessed separately in each category (beliefs and supplements) through Cronbach's alpha criterion. The level of statistical significance on all tests was set at $p < 0.05$, and

the analysis was applied using the statistical software R (www.r-project.org, v3.6.2).

Results

The questionnaire includes three sections: i) Demographic details and attitudes toward NS; ii) Personal beliefs about the efficacy and safety of NS (part A) and iii) knowledge of the use and interactions of NS (Part B). Part A consists of 4 questions, while part B consists of 20. In order to be established face and content validity, an extensive bibliographic search took place, as well as the items were reviewed by experts and checked during pilot interviews. In the category of personal beliefs (part A), Cronbach's reliability index was 0.65, while in the category of knowledge (part B), the value was 0.67, both corresponding to acceptable questionnaire reliability levels based on the literature, indicating a high level of internal consistency[23, 24].

The participants were also divided into 2 groups, group I for the three first years of study (1st–3rd year) and group II for the last two years of study (4th–5th year). Construct validity was confirmed for each part by t-test. The total score in beliefs about NS appeared to be of borderline significance, whilst group II had better results than group I ($t = -1.92$; $df = 139$; $p = 0.05$). On the other hand, the knowledge part gave a significantly higher mean total score for group II than group I ($t = -7.21$; $df = 77$; $p < 0.001$).

The sample consists of 142 respondents (66 men and 76 women), while the number of participating students per academic year was about the same (29, 31, 32, 25, 25). 91.5% of the participants were from Greece, while 8.5% were from Cyprus. 43.7% of the participants lived in "major urban areas", 36.6% lived in "urban", 9.2% in "suburban" and 10.6% in "rural areas". Regarding the frequency of NS consumption, 33.1% of the participants answered that they have "never" received any, 16.2% "rarely," 27.5% "sometimes," 16.2% "often," while only 7% answered "always." Regarding the source of information about NS, participants could choose up to 2 possible answers. It came up that "health care professionals" were selected by 51.4% of the participants, "personal search" by 42.3%, "advertisements – mass/social media" by 25.4%, "University" by 19.7%, and "other" by 4.2%. Regarding the main reason for receiving NS, the participants could also choose up to 2 possible options. The "wellness and health maintenance" category was chosen by 69.7% of participants, "immune system boost" by 35.2%, "mental peace" by 12.7%, "other" by 9.9%, "slimming products" by 4.2%, while "special conditions" by 0.7% (Table 1).

Regarding the belief in NS safety, 4 items were answered by using a 5-point Likert-type scale. Questions 1 and 4 range from strongly disagree (1) to strongly agree (5), while questions 2 and 3 had the opposite meaning conceptually, so they range in the reverse order, from strongly agree (1) to strongly disagree (5). Lower scores represent acceptance feelings towards NS safety and lack of toxicity symptoms, whilst the higher scores reflect concern and insecurity feelings about NS safety, interactions, and possible toxicity.

In the statement that "The nutritional supplements could cause serious interactions," most participants answered that they either agree (39%) or that they are neutral (32.6%); as for if "Vitamin and mineral products are always safe," participants mostly disagreed (48.6%) or were neutral (30.3%). Similarly, the majority of the participants (47.2%) disagreed with the belief that "Herbal supplements are always safe since they are derived from natural sources," while 52.1% agreed with the belief that "Nutritional supplements could be toxic."

Table 1. Participants' demographics and attitudes

Variable	N (142)	%
Sex		
male	66	46.5
female	76	53.5
Year of study		
1st year	29	20.4
2nd year	31	21.8
3rd year	32	22.5
4th year	25	17.6
5th year	25	17.6
Place of living		
Major urban areas	62	43.7
Urban areas	52	36.6
Suburban areas	13	9.2
Rural areas	15	10.6
NS consumption		
Always	10	7
Often	23	16.2
Sometimes	39	27.5
Rarely	23	16.2
Never	47	33.1
Source of information		
Advertisements – mass/social media	36	25.4
Personal search	60	42.3
University	28	19.7
Health Care Professionals	73	51.4
Other	6	4.2
Reasons receiving NS		
Wellness and health maintenance	99	69.7
Immune system boost	50	35.2
Mental peace	18	12.7
Slimming products	6	4.2
Special condition	1	0.7
Other	14	9.9

The belief that "The nutritional supplements could cause serious interactions" did not appear to be related to the country of origin or place of stay. In addition, this belief does not differ by the frequency of NS consumption. Nevertheless, it does change significantly with gender ($p = 0.04$) and year of study ($p < 0.001$). More specifically, women had less chance to "Strongly disagree" compared to men, and students closer to graduation had fewer odds to "Strongly disagree" in comparison with 1st year.

The belief that "Vitamin and mineral products are always safe" was not related to the country of origin, year, gender, or frequency of NS consumption. However, the place of residence ($p = 0.01$) was statistically significant. More specifically, participants who lived in "urban" and "rural areas" in comparison with others who lived in "Major urban areas" had fewer odds of strongly disagreeing, while participants who lived in "Suburban areas" in comparison with others who live in "Major urban areas," had higher odds to "Strongly disagree".

The belief that "Herbal supplements are always safe since they are derived from natural sources" was not related to the country of origin, place of residence, sex, or frequency of NS consumption. However, it was related significantly ($p = 0.01$) to the year of study. All years of study had higher odds of strongly disagreeing than the 1st year.

Finally, the belief that "The nutritional supplements could be toxic" was not significantly associated with any of the above variables.

The beliefs of study respondents about NS safety are presented in detail in Table 2. The total score in beliefs questions and mean scores per year of study is presented in Table 3, whilst the score per group (I and II) of students is presented in Table 4.

In the last section (knowledge part), each subject's score was calculated as the total number of correct answers in order to calculate the average percentage. Respondents scored an average of 10.74 out of 20. The minimum score was 3, while the maximum score was 19 (Table 5). The country of origin ($p = 0.05$) and gender ($p = 0.07$) appeared to be of borderline significance, whilst students from Cyprus and women had better results. On the other hand, place of residence and frequency of NS consumption were not significant at all. Finally, the year of study was statistically significant ($p < 0.001$), with students closer to graduation performing better. Scores in knowledge questions per group (I and II) of students are presented in Table 6.

After Bonferroni correction for multiple testing, most of the variables remain statistically significant.

Table 2. Participants' beliefs about NS safety

Likert question	N	%	Mean
The nutritional supplements could cause serious interactions			3.58
Strongly agree	23	16.3	
Agree	55	39	
Neither agree nor disagree	46	32.6	
Disagree	16	11.3	
Strongly disagree	1	0.7	
Vitamin and mineral products are always safe			3.61
Strongly agree	0	0	
Agree	14	9.9	
Neither agree nor disagree	43	30.3	
Disagree	69	48.6	
Strongly disagree	16	11.3	
Herbal supplements are always safe, since they are derived from natural sources			3.81

Table 3. Total score in beliefs and mean scores per year of study

Variable	Min	Max	Median	Mean	1st quantile	3rd quantile	SD
Score in beliefs	2	5	3.75	3.74	3.25	4.25	0.60
Year	**N**			**SD**		**Mean**	
1st year	29			0.54		3.47	
2nd year	31			0.61		3.89	
3rd year	32			0.6		3.65	
4th year	25			0.67		3.8	
5th year	25			0.63		3.95	

Discussion

The study showed that pharmacy students are adequately informed about NS, and this subject is essential for the curriculum of their studies. The majority of the participants receives NS (66.9%) and believes that they may interact and have toxic effects. Those in higher years of study scored higher in knowledge than lower ones.

A limitation of this study was that the size of the sample was relatively small (n = 142), due to the participation of only students who were present in the class, even if it fits the recommended instructions per item[20–22].

There are some studies that examine attitudes, beliefs, and knowledge on NS, mainly focusing on nutrition and food-drug interactions[17–19, 25]. Our main aim was to identify the awareness about NS among pharmacy students by quantitative measurement of beliefs about NS safety and knowledge related to drug interactions from a pharmaceutical point of view. For this reason, a new valid and reliable questionnaire, with 24 items, was constructed to assess beliefs and knowledge among pharmacy students related to nutritional supplements. The questionnaire was first tested for its comprehensibility and appropriateness of its items in order to establish reliability (internal consistency). According to the literature, each part of the questionnaire was assessed separately and found acceptable according to the literature[23, 24]. Specifically, on Cronbach α reliability index for personal beliefs, the value was 0.65, and for the knowledge part, the value was 0.67. Construct validity was confirmed by a t-test comparing two students groups, divided by year of study.

Regarding demographic characteristics and attitudes of the study, some interesting findings were revealed. Since it is known from the literature, growing demand for NS consumption has been observed within the last decades. This study indicates a high percentage (66.9%) of participants who have received NS, which is in accordance with the bibliography (70% of young adults)[2, 25–26]. About the source of information, the results showed that pharmacy students trust health care professionals[27], potentially related to their professional orientation. When participants were asked where they received information about NS from, over 50% chose health care professionals.

Table 4. Scores in beliefs questions per group of students

	Group I	Group II	P value
N (%)	92 (64.8%)	50 (35.2%)	
Mean	3.67	3.88	0.05582
Min	2	2.5	
Max	5	5	

Group I: 1st–3rd, Group II: 4th–5th year
No data are missing in either group.

Table 5. Participants' knowledge about NS and mean scores by year of study

Variable	Min	Max	Median	Mean	1st quantile	3rd quantile	SD
Score in knowledge (score at 20)	3	19	10	10.74	8	12.75	3.61
Year	**N**			**SD**		**Mean**	
1st year	29			2.67		9.14	
2nd year	31			2.92		8.71	
3rd year	32			2.08		9.91	
4th year	25			3		11.16	
5th year	25			2.67		15.76	

Table 6. Grades in knowledge questions per group of students

	Group I	Group II	P value
N (%)	92 (64.8%)	50 (35.2%)	
Mean	9.26	13.46	< 0.001*
Min	3	6	
Max	16	19	

Group I: 1st–3rd, Group II: 4th–5th year
*p-value < 0.001
No data are missing in either group.

The more common reason for consuming NS was wellness and health maintenance (69.7%), followed by the immune system boost (35.2%). The most prevalent "other" option in males was protein supplements. These results are in accordance with similar studies in bibliography[3, 7, 8, 28, 29].

Regarding the beliefs of the participants about NS safety, the majority of the respondents believe that nutritional supplements, including vitamins, minerals, and herbal products, are not always safe, as well as may induce drug interactions, and could be toxic. The question "The nutritional supplements could cause serious interactions" (mean of 3.58) gathered 55.3% of positive responses (agree and strongly agree), while the question "The nutritional supplements could be toxic" had a higher mean (3.95) and gathered totally positive responses 78.2% while the negative responses were less than 10%. On the contrary, the public tends to believe that NS consumption is harmless; thus, almost 9 out of 10 did not consider it important to inform their physician or pharmacist about their usage[30]. This is accentuated by the fact that more than 20,000 citizens in the USA visit the emergency department due to consumption of NS annually[31]. Many of these were related to unsupervised consumption of NS, indicating the necessity of counseling the pharmacist about supplement usage. Therefore, NS can be safe and effective only when they are taken correctly and when interactions are accounted for. The mean level for beliefs of students per year is presented in Figure 1.

The students in higher years of study, especially in the 4th and 5th year (group II), had higher scores in the beliefs part than the younger ones. It is known that non-healthcare professionals, especially older patients, perceive NS as safe, mainly due to their facile availability (many different shops to buy these products even without a prescription, etc.)[32]. Many serious or less serious adverse interactions have been reported in clinical practice, including St. Johns wort, Ginkgo biloba, Ginger, grapefruit, etc.[33]. The knowledge of these NS interactions is partially connected with beliefs about safety and toxicity.

The patients' adherence to the medical treatment[34] as well as the pharmacists' knowledge of serious interactions are of high importance, especially due to polypharmacy and NS overconsumption[35]. The students in higher years of their study also had higher scores in the knowledge part than the younger ones. The one-way ANOVA test showed statistical significance per year of study ($F(4,137) = 30.13$, p-value < 0.001). There was a significant difference between the scores of the first three years and the last two ($p < 0.001$), while the scoring of the three first years did not vary significantly. 4th and 5th-year students gave the highest scores (11.16 and 15.76 respectively) in their knowledge of NS compared to the younger ones (Table 5). The mean level for knowledge of students per year is presented in Figure 2. These findings may lead to the assumption that the experience increases the rate of correct answers[10, 11].

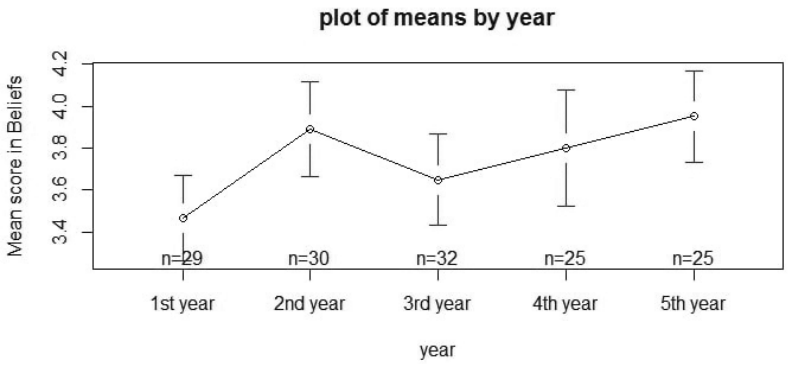

Fig. 1. Plot of mean scores in beliefs by year of study

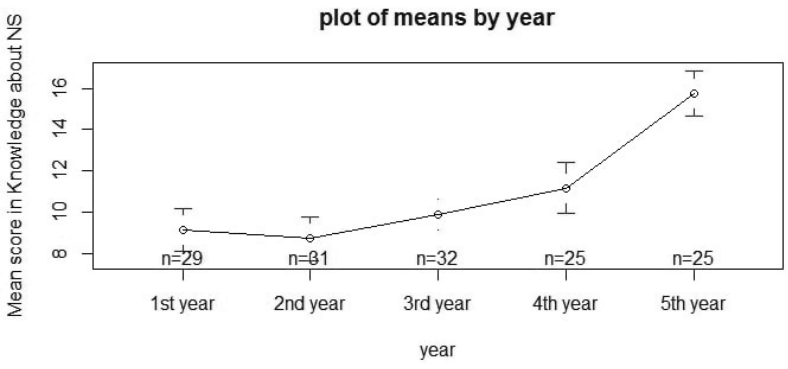

Fig. 2. Plot of mean scores in knowledge about NS by year of study

The belief and knowledge scores did not differ between participants who received supplements and those who did not (p-values > 0.05). However, there has been a difference between years of study, indicating the role of University studies. The last two years of studies include most pharmaceutical courses such as pharmacology, pharmacognosy, and toxicology. The mean scores, both in beliefs and in knowledge, were higher in the 5th year of study, which could attribute to the clinical education on public or private pharmacies that is added to the last year of study. A correlation study took place by using Pearson's product-moment correlation. For group I, the correlation coefficient between beliefs and knowledge scores was 0.236, demonstrating no correlation (< 0.3). On the other hand, the total sample and higher year of study (group II) showed a low correlation that the beliefs' score is increased by the improvement of knowledge, while the correlation coefficient was 0.337 and 0.399, respectively.

Conclusion

Over the last decades, the consumption of nutritional supplements is increasingly expanded. It is crucial that vitamins, mineral products, herbal supplements, etc., should be recognized as an essential part of the curriculum of Pharmacy Schools. It is necessary to assess the beliefs and level of knowledge of health care students and professionals, providing advanced primary health care services in the institutional and the community area, addressing the matter of non-prescription supplementation intakes with a critical scope and up to date knowledge. This study showed that pharmacy students receive NS, having acknowledged their potential toxicity and interactions. This is of importance since the knowledge does not have only a sterile role, but is able to transform the beliefs of health care advisors, with potent linkage to an advance in the professional practice, intensifying their vocational role.

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PŮVODNÍ PRÁCE

Kapilárna zónová elektroforéza v spojení s UV detekciou pre simultánne stanovenie tramadolu a paracetamolu vo vzorkách farmaceutického a biologického charakteru

Capillary zone electrophoresis in combination with UV detection for simultaneous determination of tramadol and paracetamol in pharmaceutical and biological samples

Andrea Horniaková • Ondrej Štefánik • Ivana Čižmárová • Michaela Matušková • Peter Mikuš • Juraj Piešťanský

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Súhrn

Prezentovaná práca sa zameriava na vývoj a validáciu analytickej metódy na báze kapilárnej zónovej elektroforézy v spojení s UV detekciou pre simultánne stanovenie tramadolu a paracetamolu vo farmaceutických a biologických vzorkách. Základný elektrolyt bol tvorený 50 mM uhličitanom amónnym, ktorý predstavuje pomerne atypický elektrolytový systém. Vyvinutá metóda disponuje vhodnými validačnými parametrami ako linearita (koeficient determinácie $r^2 \geq 0{,}995$), selektivita alebo medza dôkazu na úrovni 0,25 – 0,5 µg/ml. Metóda bola charakterizovaná adekvátnymi hodnotami presnosti a správnosti, ktoré boli v súlade s kritériami validačných smerníc, či už pre matrice farmaceutického alebo biologického charakteru. Detekcia bola uskutočnená pri vlnovej dĺžke 200 nm. Daná metóda bola úspešne použitá pre potreby stanovenia tramadolu a paracetamolu v rozličných liekových formách a v biologickej matrici moču. Dosiahnuté výsledky indikujú potenciál začlenenia metódy do procesov kontroly kvality liečiv a/alebo bioanalýzy.

Kľúčové slová: kapilárna zónová elektroforéza • UV detekcia • tramadol • paracetamol • kontrola kvality • bioanalýza

A. Horniaková • O. Štefánik • I. Čižmárová • M. Matušková
Katedra farmaceutickej analýzy a nukleárnej farmácie FaF UK
Bratislava, SR

P. Mikuš[1,2], PharmDr. Juraj Piešťanský, PhD.[1,2] (✉)
[1]Katedra farmaceutickej analýzy a nukleárnej farmácie
Farmaceutická fakulta Univerzity Komenského
Odbojárov 10, 832 32 Bratislava, SR
e-mail: piestansky@fpharm.uniba.sk
[2]Toxikologické a antidopingové centrum FaF UK, Bratislava, SR

Summary

The aim of the present study is the development and validation of a simple method based on capillary zone electrophoresis coupled with UV detection for simultaneous determination of tramadol and paracetamol in pharmaceutical and biological samples. The background electrolyte was composed of 50 mM ammonium carbonate, which is a type of a non-conventional electrolyte system. The developed method was characterized by suitable validation parameters, such as linearity (coefficient of determination $r^2 \geq 0{,}995$), selectivity or the limit of detection at the level of 0.25 – 0.5 µg/ml. Acceptable values of accuracy and precision were obtained, which were in good agreement with the recommended validation guidelines for analysis of pharmaceutical and biological samples. Detection was performed at a wavelength of 200 nm. The developed method was successfully applied to determine tramadol and paracetamol in various dosage forms and in urine biological samples. Achieved results indicate a potential of the method to be integrated in the common quality control processes of drugs and/or in bioanalysis.

Key words: capillary zone electrophoresis • UV detection • tramadol • paracetamol • quality control • bioanalysis

Úvod

Bolesť predstavuje subjektívny nepríjemný pocit v dôsledku skutočného alebo možného poškodenia tkaniva a patrí medzi najčastejší sprievodný jav širokého spektra akútnych či chronických ochorení[1)]. Z tohto dôvodu je tlmenie bolesti jednou z hlavných

terapeutických priorít súčasnosti. Rozoznávame tri základné typy bolesti, a to bolesť nociceptívnu (poškodenie najmä muskuloskeletálne), neuropatickú (poškodenie nervov) a psychickú[2]. Hlavnými skupinami liečiv uplatňovanými v terapii bolesti sú analgetiká, nesteroidné antiflogistiká, opioidy a v špeciálnych prípadoch, najmä pri tlmení neuropatickej bolesti, sa môžu použiť aj tricyklické antidepresíva alebo antiepileptiká[1].

Tramadol, chemicky *trans*-(±)-2-(dimetylaminometyl)-1-(metoxyfenyl)cyklohexanol, patrí do skupiny syntetických opioidných analgetík. Tieto zlúčeniny pôsobia na centrálny nervový systém a spôsobujú supresiu vnímania bolesti. Tramadol je indikovaný na liečbu akútnej a chronickej bolesti miernej až stredne silnej intenzity. Pôsobí dvoma hlavnými mechanizmami účinku, a to plnou neselektívnou agonizáciou opioidných μ, δ a κ receptorov (vykazuje však vyššiu afinitu k μ-opioidným receptorom) a inhibíciou spätného vychytávania noradrenalínu a sérotonínu[3].

Zo stereochemického hľadiska je tramadol chirálna molekula s dvoma chirálnymi centrami. Stereoizoméry sa mierne líšia aj mechanizmom pôsobenia, avšak prítomnosť oboch enantiomérov, čiže ich racemickej zmesi, je dôležitá pre zachovanie synergie terapeutického efektu. Aktívna zlúčenina tramadolu predstavuje zmes (+) (1R,2R)-tramadolu, ktorý prednostne inhibuje spätné vychytávanie sérotonínu a (–) (1S,2S)-tramadolu, ktorý aktivuje α-2 adrenergné receptory a tým znižuje spätné vychytávanie adrenalínu zo synaptickej štrbiny[4].

Tramadol vykazuje desaťnásobne nižší analgetický účinok oproti morfínu, ale má bezpečnejší terapeutický profil a nižšie riziko vzniku závažných nežiadúcich účinkov. Je indikovaný hlavne pri bolestiach svalov, kĺbov, pooperačných rán, chronickej bolesti chrbta, dentálnej alebo neuropatickej bolesti. Kontraindikáciou je vek pod 16 rokov, tehotenstvo, laktácia, alebo deficiencia enzýmu CYP2D6 – zodpovedného za metabolizáciu tramadolu. Abúzus tramadolu vedie k závažným prejavom, ako sú výrazné zníženie srdcovej frekvencie alebo tlmenie centra dýchania v predĺženej mieche. Pri dlhodobom užívaní, ktoré si často manažment terapie chronickej bolesti vyžaduje, sa môže objaviť fyzická závislosť, tolerancia či abstinenčný syndróm po vynechaní. V praxi sa často môžeme stretnúť s fixnou kombináciou tramadolu s ďalším analgetikom, a to paracetamolom. Výhody danej fixnej kombinácie pramenia zo synergie účinku týchto dvoch liečiv. Podanie dvoch analgeticky pôsobiacich liečiv s rozdielnym mechanizmom účinku vyvolá rýchlejší nástup účinku a jeho dlhšie trvanie[5].

Paracetamol (alebo acetaminofén), chemicky *N*-(4-hydroxyfenyl)acetamid, je jedným z najpoužívanejších voľnopredajných liečiv, určených na terapiu bolesti a pyrexie. Mechanizmus účinku nie je úplne objasnený ale predpokladá sa nekompetitívna reverzibilná inhibícia enzýmu cyklooxygenáza (COX) s vyššou selektivitou v mozgu, taktiež dochádza k znižovaniu tkanivových koncentrácií prostaglandínov a iných prozápalových mediátorov. Na rozdiel od nesteroidných antiflogistík (NSAID) sa ale nevyznačuje protizápalovým účinkom a nemá ani vplyv na zrážanlivosť krvi, lebo nepôsobí ako inhibítor syntézy tromboxánu. Výhodou však je, že vykazuje výrazne menšie riziko vzniku žalúdočného krvácania a vredov v porovnaní s NSAID[1, 6]. Jedným z metabolitov paracetamolu je *N*-arachidonylaminofenol (AM404), ktorý vykazuje analgetický účinok. Predpokladá sa viacero mechanizmov analgetického pôsobenia tohto metabolitu v organizme, ako napríklad blokáda neuronálneho vychytávania anandamidu či blokáda neuronálnych sodíkových kanálov[1, 6].

Paracetamol je určený na terapiu miernej akútnej aj chronickej bolesti, v kombinácii s NSAID alebo opioidmi aj na liečbu stredne silnej bolesti. Štandardná jednorazová dávka u dospelých by mala byť od 500 mg do 1000 mg, pričom denná dávka by nemala prekročiť 3 – 4 g[7]. Pri dlhodobom užívaní vysokých dávok hrozí zvýšené riziko kardiovaskulárnych, gastrointestinálnych a renálnych nežiadúcich účinkov či akútne zlyhanie pečene[6].

Metabolizmus paracetamolu je spriahnutý s enzýmom CYP2D6, pričom na základe rôznej expresie tohto enzýmu v organizme je možné jedincov rozdeliť na extenzívnych, ultrarýchlych a pomalých metabolizérov paracetamolu. Z toho vyplýva i variabilita tolerancie dávky alebo rôzna náchylnosť na prejavenie nežiadúcich účinkov[6]. Paracetamol nie je odporúčaný osobám s akútnou alebo chronickou hepatopatiou, pri abúze alkoholu či malnutrícii[8].

Medzi najnebezpečnejšie nežiadúce účinky na úrovni gastrointestinálneho traktu (GIT) patrí jeho hepatotoxicita, ktorá je dôsledkom nadmerného hromadenia metabolitu *N*-acetyl-*p*-benzochinónimínu. Ten sa v prípade vyčerpania dostupného glutatiónu nevyhnutného k jeho inaktivácii viaže na mitochondriálne proteíny, kde dochádza k formovaniu cytotoxických aduktov, čo vedie k mitochondriálnej dysfunkcii a závažnej hepatocelulárnej nekróze. Ako primárne antidotum sa využíva *N*-acetylcysteín[6, 9].

Tramadol spolu s paracetamolom patria medzi frekventovane predpisované liečivá a s tým sa spája aj potreba vývoja efektívnych analytických metód pre ich kvalitatívne aj kvantitatívne hodnotenie v biologických matriciach, akými sú predovšetkým krv a moč. Takéto metódy sú schopné prispieť k zefektívneniu samotnej liečby v dôsledku možnosti monitorovania compliance pacienta (zvýšenie terapeutického účinku) a eliminácie/minimalizácie nežiadúcich účinkov. Odhliadnuc od hodnotenia liečiv v biologických matriciach je nevyhnutné disponovať vhodnými analytickými metódami aj v procese zabezpečovania ich kontroly kvality. Medzi štandardné a rutinne aplikované analytické prístupy pre potreby identifikácie a kvantifikácie daných liečiv vo vzorkách farmaceutického aj biologického charak-

teru patria chromatografické techniky (kvapalinová a plynová chromatografia) v spojení s rôznymi typmi detekcie[10–19]. Je tomu tak z dôvodu ich kváziuniverzality, vysokej presnosti, správnosti, robustnosti, schopnosti analýzy širokého spektra zlúčenín a relatívnej jednoduchosti obsluhy[20].

Avšak v súčasnosti sa začínajú čoraz častejšie v oblasti analýzy farmaceutických a klinických vzoriek presadzovať techniky kapilárnej elektroforézy (CE), ktoré v porovnaní so štandardnými chromatografickými prístupmi prinášajú značné výhody[21, 22]. Sú nimi napr. vysoká separačná účinnosť, relatívne krátky čas analýz, jednoduchosť prevedenia, spotreba minimálneho množstva vzorky a organických rozpúšťadiel a minimálna až absentujúca potreba rozsiahlej externej predúpravy vzorky[22, 23].

Z hľadiska CE módov aplikovaných do prostredia analýzy tramadolu a/alebo paracetamolu vo vzorkách farmaceutického a/alebo biologického charakteru v doteraz publikovaných vedeckých prácach zaujíma dominantné postavenie kapilárna zónová elektroforéza (CZE). Tramadol bol vo vzorkách liekových foriem alebo modelových vodných vzorkách analyzovaný CZE v spojení s detekciou na báze DAD[24, 25], laserom indukovanej fluorescencie (LIF)[26], vodivostnej – C^4D[27] alebo elektrochemiluminiscenčnej detekcie[28]. Jedna z prác naviac popisuje využitie CE realizovanej v nevodnom prostredí[25]. Zmienenými prístupmi bolo možné dosiahnuť hodnoty medze dôkazu (LOD) na úrovni 0,008 – 2,05 μg/ml.

Rovnako v prípade analýzy paracetamolu vo vzorkách farmaceutického charakteru bola preferovaným separačným módom CZE[29–36], použitá však bola i micelárna elektrokinetická chromatografia – MEKC[37]. CE v spojení s UV[29–32], DAD[33], MS[34], C^4D[27, 35] alebo chemiluminiscenčnou[36] detekciou poskytovala hodnoty LOD na úrovni 0,085 ng/ml až 5,0 μg/ml. Pri analýze paracetamolu vo farmaceutických vzorkách bola využitá aj metóda CZE s ampérometrickou detekciou v prevedení na mikročipe[38]. V prípade vzoriek farmaceutického charakteru je dôležité podotknúť, že žiadna z vyššie uvedených metód nevyžadovala extenzívnu predúpravu vzorky.

Z doteraz publikovaných štúdií zameraných na bioanalýzu tramadolu a paracetamolu vyplýva, že preferovanými matricami boli biologické vzorky moču[8, 34, 36, 39–44], plazmy[38] a krvného séra[39, 45, 46]. Testované však boli i vzorky slín[47] alebo pečeňového a obličkového tkaniva[48]. Popísané boli CZE metódy s UV[39–41, 45–49], MS[8, 34, 39, 50], elektrochemiluminiscenčnou[42, 43] alebo chemiluminiscenčnou[36] detekciou. Pre biologickú matricu moču sa hodnoty LOD pohybovali na úrovni 0,004 – 0,1 μg/ml pre tramadol a na úrovni 0,085 ng/ml až 5,2 μg/ml pre paracetamol. Hodnoty LOD pre tramadol a paracetamol v krvnej plazme a sére boli 0,1 μg/ml (tramadol) a 0,3 – 5,2 μg/ml (paracetamol). Drvivá väčšina vyvinutých metód vyžadovala pomerne rozsiahlejšiu a viackrokovú predúpravu vzorky, ktorá zahŕňala napr. extrakciu na tuhej fáze – SPE[41, 44], mikrodialýzu[49], elektromembránovú extrakciu – EME[40], mikroextrakciu na kvapalnej fáze – LPME[44], extrakciu podporenú mikrovlnným žiarením – MAE[50], precipitáciu proteínov a následnú centrifugáciu[38, 45, 47].

Z doteraz publikovaných prác vyplýva, že na separáciu tramadolu a paracetamolu v CZE móde sú zvyčajne používané konvenčné základné elektrolyty (BGE) reprezentované prevažne fosfátovými[28, 30–33, 37, 40, 42, 43] alebo borátovými[24, 26, 29, 36, 39, 46, 48] tlmivými roztokmi. Výber BGE je však vo veľkej miere determinovaný použitou detekčnou technikou. Elektrolytové systémy vhodné pre CZE-UV sú častokrát nevhodné pre CZE-MS. Cieľom predkladanej práce bol preto vývoj relatívne rýchlej a jednoduchej CZE-UV metódy pre potreby simultánneho hodnotenia tramadolu a paracetamolu vo vzorkách farmaceutického a biologického charakteru, ktorá by súčasne poskytovala z hľadiska zloženia BGE vysokú mieru kompatibility s MS detekciou. Takéto prístupy k analýze liečiv v rozličných typoch matríc sú zaujímavé z hľadiska kontroly kvality liečiv a liekov, a súčasne i z hľadiska bioanalytických aplikácií.

Pokusná časť

Inštrumentácia

Elektroforetické experimenty boli prevedené s použitím systému Agilent 7100 CE vybaveného UV detektorom (Agilent Technologies, Santa Clara, California, USA). Separácia bola realizovaná v kremennej kapiláre s vnútorným priemerom 50 μm, vonkajším priemerom 300 μm, celkovou dĺžkou 70 cm a efektívnou dĺžkou kapiláry 62,5 cm. Pred prvým použitím bola kapilára premytá roztokom 1 M NaOH po dobu 30 minút, demineralizovanou vodou (10 minút) a nosným elektrolytom – BGE (10 minút). Každý deň pred začiatkom meraní bola kapilára preplachovaná 0,1 M NaOH 10 minút, demineralizovanou vodou 10 minút a BGE 10 minút. Na konci každého dňa sa kapilára prepláchla demineralizovanou vodou po dobu 10 minút. Vzorky boli injektované do kapiláry hydrodynamicky tlakom 50 mbar počas 10 sekúnd. Separácia bola prevedená v móde pozitívnej polarity aplikáciou napätia o hodnote +20 kV, ktoré sa zvyšovalo na začiatku separácie z 0 kV na +20 kV po dobu 20 sekúnd. Pred každým injektovaním vzorky bola kapilára prekondicionovaná aplikáciou negatívneho napätia –25 kV počas 15 sekúnd s následným premytím nosným elektrolytom 1 minútu, aby sa predišlo „carry-over" efektu. Merania boli realizované pri teplote 20 °C a detekcia bola uskutočnená pri vlnovej dĺžke 200 nm.

Chemikálie a vzorky

Štandardy analytickej čistoty – hydrochlorid tramadolu a paracetamol – boli získané od spoločnosti Zentiva (Praha, Česká republika). Chemikálie použité na prípravu elektrolytových systémov uhličitan amónny ($(NH_4)_2CO_3$) a hydrogenuhličitan amónny