

# **Clinical and analytical aspects of the improvement of a local antifungal preparation for the treatment of nasal polyposis**

Ph.D. thesis

**András Fittler, Pharm.D.**

Program leader:

Prof. Dr. Emil Fischer

Tutors:

Prof. Dr. Lajos Botz

Prof. Dr. Imre Gerlinger



Department of Pharmaceutics,  
Medical School  
University of Pécs, Hungary

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## **General introduction**

Carefully designed pilot clinical studies occupy an important role in the development of novel medications and also in gathering information of diseases with yet unknown or debated etiological background. Especially in such cases when relatively rare or orphan diseases are in the scope of research, since patients suffering from these illnesses can hardly hope for large pharmaceutical companies (controlled by the rules of economy and competition) to develop medications for their needs. Although pilot clinical studies have the disadvantage of including only smaller amount of patients, with the meta-analysis or systematic review of several publications good, comprehensive evidence can be gathered on a certain topic.

It has to be stated, that a well organized and planned study requires thorough preparation and also the collaboration of different healthcare professionals (medical doctors, pharmacists, nurses, microbiologists, statisticians, analytical etc.). Each profession gives an added value to the work and is essential for the successful completion of the study.

As a pharmacist I would like to highlight some important topics regarding a pilot clinical study I had a chance to participate in. I hope that my work gives a good example of the diversified professional challenges that clinical pharmacists have to face.

In 2006 a double blind, randomized pilot clinical study was planned and launched to assess the efficacy of long term intranasal antifungal treatment in patients suffering from chronic rhinosinusitis with nasal polyposis (CRSwNP), because it has been hypothesized that fungal cells in the mucus exacerbate adverse immune response that result in the formation of polyps. Several studies have been published on the issue and controversial results can be found on the effectiveness of antifungal treatment. Before this well prepared study was started, the question of stability arose regarding the study medications. After the completion of a three month stability test further questions had to be faced regarding the adequate analysis of amphotericin B (AmB) solutions. Instructive and useful experience was gained while problems of microbiologic and instrumental analytical possibilities were solved.

## **Aims**

In my thesis I will aim to introduce a complex pharmaceutical view regarding a pilot clinical study organized by the Department of Otorhinolaryngology and Head and Neck Surgery in cooperation with Department of Pharmaceutics and University Pharmacy of the University of Pécs in 2006. During the preparation phase we had to face several pharmaceutically important questions.

The aims of my thesis are to:

- evaluate earlier clinical studies regarding to the antifungal treatment with AmB for chronic rhinosinusitis and also to discuss pharmaceutically important topics regarding the study sample, such as the preparation of clinical samples, optimal dosage form, proper concentration of the active ingredient, storage and stability;
- assess the efficacy of long-term AmB treatment in patients with CRSwNP;
- describe the compliance of patients in the study; and
- introduce the different chemical and microbiological analytical methods for the measurement of AmB and summarize our work in the improvement of these techniques.

Since such diversified topics and fields of research are included in my work, in the interest of transparency I divided the thesis into two sections. In the first clinical section the etiology and the therapy of CRSwNP is discussed along with the detailed presentation of the double blind pilot study of Pécs and the results of our compliance measurements. In the second analytical section the challenging problem of the analysis of AmB and the questions regarding a stability testing are introduced. Also the applied chemical and biological methods are presented and compared.

## **I. Clinical part**

### **I.a Chronic Rhinosinusitis with Nasal Polyposis and the etiology of polyp development**

Chronic rhinosinusitis (CRS) is an inflammatory disease of the nasal and paranasal sinus mucosa that has existed for more than 3 months, with typical leading symptoms such as nasal obstruction, thick mucopurulent nasal discharge, a reduction/loss of the ability to smell, facial pressure and/or pain, in some cases accompanied by an extreme degree of nasal polyposis (NP). As concomitant minor symptoms, CRS patients may complain of headaches, fever, halitosis, fatigue, dental pain and ear fullness.

The etiology and pathogenesis of CRS are neither largely unknown. One of the most popular theories, which is a subject of intensive research, postulates that the causal factors are morphological variations on the lateral wall of the nasal cavity; other hypotheses include the biofilm theory and the role of superantigens. The role of fungal organisms in the development of some rare forms of CRS has long been known. Allergic fungal rhinosinusitis (AFRS) was first described by Katzenstein et al. in 1980. Ponikau et al. recently developed new mucus-collecting and culturing methods with which they were able to demonstrate the presence of mucin containing hyphae and clusters of degenerating eosinophils referring to allergy in 96% of CRS patients with polyposis. The eosinophilic granulocytes within the nasal mucus of CRS patients do not play a part in allergic reactions, but are transitory components of the nasal secretion. After destroying the hyphae, they fall apart and the major basic protein released from them exerts an extremely toxic effect on nasal mucosa. As a consequence of secondary superinfection of the nasal mucosa via an epithelial lesion, biofilm colonization and the appearance of superantigens CRS may develop. All these facts indicate the multifactorial nature of the disease.

If the fungal theory holds true, it seems obvious that, through a reduction of the amount of antigen, or its total eradication, thereby influencing the triggers of CRS, the symptoms of the patients can be relieved. Accordingly the treatment of CRS patients with intranasal lavage or a spray containing the antifungal AmB would be beneficial.

## **I.b Review and evaluation of earlier publications**

During the past 7 years, seven clinical studies have been published in which experience with local antifungal treatment regimes in CRS was discussed. Only three of the papers were based on double-blind, placebo-controlled studies and of these three studies only one was multicentre, making the interpretation of the results rather difficult. In some of the studies, the number of recruited patients was extremely low. The treatment period ranged between 4 and 80 weeks, and the form of drug/placebo application also varied since nasal lavage, nasal spray and nasal inhalation was used. The concentration and the daily amount of AmB recommended also differed. The conditions of drug storage and checking the stability of the AmB solutions was completely ignored. Patient compliance was examined in only one study. Further details can be found in my full thesis and also in the relating publication [Gerlinger et al. Laryngoscope, 2010].

## **I.c Controversial and ignored factors in previous studies**

There are several factors which need to be evaluated and discussed in detail. These are the following:

- Dosage form/application method of sprays and nasal lavages
- Preparation of spray solutions and concentration of the active substance
- Role of added glucose in the solutions
- Length of therapy
- Compliance of patients
- Storage and stability of study medication

In an attempt to clarify the situation, we conducted a double-blind, prospective, randomized, placebo-controlled clinical study. This differed from the previous ones in that, the active drug or placebo was administered postoperatively in the form of a nasal spray. We preferred the use of a nasal spray to nasal lavage because of the well-known, favourable effect of hypertonic saline solution on the symptoms of CRS. It is important from the aspect of compliance that application of a nasal spray is more convenient for patients than either nasal lavage or inhalation.

#### **I.d Results of the randomized, double blind placebo control study at Pécs**

In a double-blind, randomized, placebo-controlled study, patients received AmB (A group) or placebo (B group) nasal sprays for 12 months after polypectomy with functional endoscopic sinus surgery (FESS). Our aim was to determine whether any difference could be observed between the two groups in the rates of recurrence of nasal polyposis, in the symptoms, in the quality of life or in the endoscopic findings. Thirty-three patients with CRSwNP were recruited from among the patients presenting at our clinic for endoscopic sinus surgery between November 2005 and October 2006. The diagnosis for CRS was set up according to the criteria laid down by the “EAACI position paper on rhinosinusitis and nasal polyps, executive summary”. The AmB and placebo nasal sprays were compounded in the Central Clinical Pharmacy of Pécs. The active spray made from Fungizone (Bristol-Myers Squibb, Epernon, France) contained 5 mg/ml AmB, while the placebo was an aqueous 0.2 µg/ml acriflavin chloride solution. Patients were instructed to apply 2 times 2 doses (100 µl) daily into each nostril. Primary outcome variable was the Modified Lund-Mackay CT score, while Sinonasal Assessment Questionnaire (SNAQ-11); Quality of life test and Endoscopic assessment were used as Secondary outcome variables. Further details, methods, results and evaluation of earlier studies can be found in my full thesis and the relating publication [Gerlinger et al. Arch Otolaryngol Head Neck Surg, 2009].

Fourteen of the sixteen patients in group A, and sixteen of the seventeen patients in group B completed the study. Our results lead to the conclusion that the administration of amphotericin B nasal spray to patients operated on for nasal polyposis does not give rise to a significant alteration in either CT score, clinical symptoms, or quality of life.

## **I.e Evaluation of patient adherence and adverse events**

The knowledge of patient adherence is important both in medical research and in clinical practice. The results of clinical trials cannot be interpreted realistically without adherence information. Assessments were made of how the adherence changed during the 12-month period, which factors influenced the attitudes of the patients and what adverse events were reported.

Two indirect methods were used for the measurement of adherence: (a) recording of the amount of medication self-administered and (b) self-reporting by the patient via the Hungarian translated version of the standardized Brief Medication Questionnaire (BMQ). The combination of the two methods is considered superior to reliance on either of the single methods, since both have advantages and disadvantages. The amount of medication self-administered was recorded monthly, the cut-off point applied to define adherent patients was 80 %. The questionnaire was filled out after the last visit. The 16 questions of the BMQ measured potential non-adherence and the number of factors influencing patient behaviour. The *Regimen Screen* contained questions regarding the past week of treatment. The *Belief Screen* measured two beliefs that can be linked to non-adherence: doubts about the efficacy of the treatment, and unwanted side-effects or bothersome features. The *Recall Screen* measured potential problems in remembering all doses. The *Access Screen* analyzed barriers.

Even though a moderate decline was seen from month to month, at the end of the study the overall adherence was notably above the most commonly documented cut-off point for adherence of 80 %. The difference in compliance between the AmB ( $97,3 \pm 8,1$  %) and placebo ( $97,2 \pm 7,4$  %) groups (two-tailed independent sample t-test,  $p = 0,323$ ), or between the male ( $97,8 \pm 7,4$  %) and female ( $96,6 \pm 8,1$  %) patients (two-tailed independent sample t-test,  $p = 0,886$ ) during the 12 months was not significant. Approximately half of the patients used the prescribed amount of spray, 37 % missed not more than 20 % of the doses, and only 7,4 % were considered to be non-adherent. The most common barrier to adherence was motivational, since 48,1 % of the patients mentioned negative beliefs concerning the efficacy of the treatment or bothersome side effects. An access barrier was identified for every third patient and only 25,9 % of the patients admitted or implied the existence of recall barriers. According to our findings the incidence and also the regularity of side-effects were higher in the AmB group.

## **Analytical part**

### **II.a Introduction of the substance amphotericin B and the possibilities of analytical methods**

AmB is a polyene antifungal agent produced by *Streptomyces nodosus*. Analysis and quantitative determination of polyenes can be carried out with different methods such as chemical and microbiological analysis. The chemical measurements are based on the detection of the light absorption of the unsaturated chromophore segment of the molecule. Samples containing AmB can be measured directly with spectrophotometry or after chromatographic separation. During the past two decades several HPLC methods have been published and used. With the aid of microbiologic detection methods directly the antimicrobial effect can be measured. Such methodologies are agar diffusion and turbidimetric bioassays. Bioassay measurements or methods were rarely published even though microbiological assays are the official methods for the quantitative analysis for AmB (and also nystatin) prescribed by the American (USP. 21.) and European (Ph. Eur. 6.) pharmacopoeias. Bioassays are favoured because of the chemical instability of the molecule and the homogeneity and purity (contains minor components, such as amphotericin A) of the preparations might differ due to the fermentation and extraction procedures.

### **II.b The question of stability and optimal storage conditions for clinical study medications**

Previously published papers were screened and reviewed to evaluate the question of stability. The correct interpretation of the controversial results found is rather difficult and could be misleading, so the organization of a new stability test seemed to be the most reliable source of information. The combination of both chemical and microbiological methods was proposed since this way more detailed information could be gathered regarding the physico-chemical changes in the samples.

In our study we investigated the stability of 5mg/ml AmB solutions (Fungizone®) with chemical (spectrophotometry) and biological (bioassay) detection for 3 months. The

effect of storage temperature and the addition of 5% glucose were evaluated on the stability of the solutions for three months.

The two detection methods showed strikingly different results. According to the chemical analysis the samples are considered relatively stable under all observed conditions (loss of concentration is: 14,2% at 20°C and 4,5% at 4°C). As opposed to chemical analysis, bioassay shows complete loss of antifungal activity after 35 days of storage, at room temperature. Storage temperature had significant effect ( $p < 0,05$ ), while 5% glucose had no significant effect ( $p > 0,05$ ) on the stability of the examined solutions. We estimated the shelf life of the glucose-free solutions, being stored at 4°C, to be 30 days in accordance with our bioassay results.

This information drew our attention to the major differences that can be detected with the two different methods. Such changes occurred in the solutions that significantly affected the microbiologic effectiveness of AmB while the chemical structure (principally the polyene segment and its light absorption properties) was not altered.. After the stability test had been completed, our intention was to do the optimization of chemical and biological analytical methods in the interest of getting better understanding of the changes occurring in the solutions. The chemical analysis can be improved if a more sensitive and detailed test method is used, such as high-performance liquid chromatography (HPLC). A more precise bioassay method was needed to reduce the coefficient of variation when measuring antifungal activity. Further details, methods and results can be found in my full thesis and the relating publication [Fittler et al. Act Pharm Hung, 2007].

### **II.c Chemical methods**

As described previously, chemical methods are suitable for the identification and quantification of the antifungal substance. By the application of chromatographic methods, not only the amount of polyene molecules (containing the conjugated double-bond segments) can be determined, but also separation allows us to distinguish molecules with different physicochemical properties.

### **II.c.1 High performance liquid chromatography**

Earlier publications were reviewed and the most suitable method was selected. To improve separation, the optimization of solvent system was performed and the following observations were made: increasing the ratio of buffer or augmenting the percentage of methanol within the organic phase improves the separation of different minor components, but at the same time notably increased retention times. When lowering the pH of the buffer the separation of minor components from the main heptaene AmB is better. With our optimized eluent system and the aid of the gradient program four heptaene components can be detected besides the AmB main component, at 407 nm. Only traces of tetraenes can be detected, probably amphotericin A - the most important minor component of AmB, which does not have antifungal effect - is amongst these peaks. To our best knowledge, the separation of these components has not been published before. From the previously used internal standards we have tested 4-nitro-1-naphthylamine proved to be the best. Further details, methods and results can be found in my full thesis and the relating publications [Fittler et al. Acta Pharm Hung, 2008].

### **II.c.2 Thin Layer Chromatography**

The most detailed review regarding the TLC separation of polyenes can be found in the publication by Thomas (1976). Out of the 7 potential eluent systems we chose to test the ones with  $R_F$  values over 0.2 in hope of better separation and biologic detection. With the aid of the eluent fulfilling our previously determined criteria two spots can be observed on the plates at 366 nm. For the better separation of the components and future identification of degradation products we optimized this eluent. Increasing the amount of the inorganic component resulted in higher  $R_F$  values and an augmented distance between the spots, while raising the amount of methanol within the organic phase increased  $R_F$  values at the expense of the separation of the components. Our final eluent (Chloroform – Methanol – Borate buffer pH 8,3; 4:5:1) has the advantages of a relatively fast TLC development and the use of easily evaporating organic solvents, appropriate for microbiologic detection. At concentrations of 250 - 500 ng/spot both the major and one minor component can be seen under the UV light. Our intention was to document changes in the composition of degraded solutions and also to identify degradation products in Fungizone solutions, but we had to find, that no discrete

degradation product or minor component can be separated. Probably many different products are produced which could be causing the “tail” of the peak. Further details, methods and results can be found in my full thesis and the relating publication [Fittler et al. JPC-J Planar Chromat, 2010]. The question arose whether the degraded components possess any antifungal effect or not? This question can be answered with the aid of microbiologic detection, such as direct bioautography.

## **II.d Biological methods**

The activity of an antifungal or an antimicrobial substance can be determined by its inhibitory effect on microorganisms. Bioassay is used for quantification of such substances which cannot be measured adequately by chemical or physical methods and also when the reduction of antimicrobial activity could reveal changes which cannot be measured by chemical methods. Direct bioautography, as a post chromatographic detection for microbiologically active substances, have been used by several authors in different fields of analytical, medical or agricultural sciences.

### **II.d.1 Bioassay with agar diffusion**

Because the U.S., European and Hungarian pharmacopoeias recommend bioassay for the quantification of polyenes, the application of agar diffusion method seemed to be an appropriate analytical method. We observed that using the conditions proposed in Ph. Eur. 6. (indicator organism and assay medium), the reproducibility of the assay was difficult: limit of detection was relatively high; inhibition zone borders were indistinct. These factors lead to the inaccuracy of the assay. We tested, which assay parameters would be the most optimal for the quantitative measurement of AmB during a stability test, where a wide concentration range is aimed to be measured.

In our study we compared five commonly used assay media and as indicator organisms we chose *Candida albicans* (ATCC 90028) and *Saccharomyces cerevisiae* (ATCC 9763). Lowest limit of detection (0,25 µg/ml AmB) can be achieved using MHA-E and *C. albicans*. For the other observed media *S. cerevisiae* increases the sensitivity of the method,

however *C. albicans* enables to measure an approximately 6 times wider concentration range, which condition is much suitable for a stability test. Also clear and distinct zones of inhibitions were documented. Our analytical method (MHA–GMB and *C. albicans*) was validated. Limit of detection (LOD) was 1,54 µg/ml and the limit of quantification (LOQ) was 15 µg/ml. The linear segment of the log dose-response curve was between 1,54-60,0 µg/ml and the inhibition zones could be read easily and accurately. Further details, methods and results can be found in my full thesis and the relating publication [Fittler et al. Mycoses, 2010].

### **II.d.2 Direct bioautography**

Our aims were to separate and detect the microbiological activity of main and minor components in fresh and degraded AmB solutions. The direct bioautography of AmB has not been published before. Degradation of the observed substance makes the separation and the microbiologic visualization troublesome.

With the aid of our optimized eluent (Chloroform – Methanol – Borate buffer pH 8,3; 4:5:1) two components can be separated on silica gel layers in AmB samples. The observed components both have antifungal effect. According to our experiments a mixture of degradation products are produced during storage of AmB solutions. It can be stated that direct bioautography proved to be a sensitive method with the detection limit of 0.8 ng/spot. In our judgment direct bioautographic detection method can be useful for future measurements since apart from being sensitive for a commonly used polyene antifungal of clinical practice, it is relatively fast, cheap and easy to perform. Further details, methods and results can be found in my full thesis and the relating publication [Fittler et al. JPC-J Planar Chromat, 2010].

## General summary

Various topics from different fields of pharmaceutical sciences have been discussed in the thesis. Certainly a drawback of such diversity can be that no single project was specified in full detail, on the other hand, as an advantage, the thesis introduced a pharmaceutical approach which evolved during my work and my personal professional ideology.

Earlier clinical studies regarding the efficacy of antifungal treatment for chronic rhinosinusitis were summarized and evaluated. Emphasis was laid on pharmaceutically important factors such as stability and adherence. It was concluded, that chronic rhinosinusitis with nasal polyposis has an unclarified pathogenesis and the effectiveness of local antifungal therapy is far from clear.

For the evaluation of long-term efficacy of amphotericin B treatment, a prospective randomized placebo controlled trial was conducted, involving 33 patients. Patients with nasal polyposis were operated on with an endoscopic technique and were treated with a nasal spray containing 5 mg/ml amphotericin B, while the placebo group received a nasal spray lacking amphotericin B. We evaluated our results with the aid of a modified Lund-Mackay CT score, the SNAQ-11 test, a quality of life test and endoscopy. Our results lead to the conclusion that the administration of amphotericin B nasal spray to patients operated on for nasal polyposis does not give rise to a significant alteration in either CT score, clinical symptoms, or quality of life.

The second part of the thesis summarizes and compares chemical (spectrophotometry, HPLC) and microbiological (bioassay, direct bioautography) methods for the quantitative measurement of amphotericin B. Optimized HPLC, TLC, bioassay and direct bioautography methods were developed for the qualitative and quantitative measurement of amphotericin B, while several important observations were made regarding these techniques. The stability test of amphotericin B nasal spray solutions was performed. With the aid of our improved HPLC method several minor components can be detected in AmB samples. Earlier TLC methods for amphotericin B were tested and a new eluent system was documented, which can be a useful marker of degradation. Direct bioautography further improved the sensitivity of the TLC method. Because the bioassay parameters recommended by the Ph. Eur. 6. were less sensitive,

an optimized method was improved capable of the quantitative measurement of amphotericin B at the concentration range of 1,54-60,0  $\mu\text{g/ml}$ .

All these observations contribute to the better – although still debatable – understanding of the role of local antifungal therapy in chronic rhinosinusitis. Certainly the summarization and improvement of analytical methods will be highly beneficial for future clinical studies.

## **Summary of new, novel results and conclusions in the thesis**

### **Evaluation of earlier publications regarding the use of Amphotericin B in Chronic Rhinosinusitis with Nasal Polyps**

1. Chronic rhinosinusitis with nasal polyposis has an unclarified pathogenesis and the effectiveness of local antifungal therapy is debatable.
2. In the previously published studies different dosage forms were used, adherence measurement was neglected, storage temperature and stability of study medications was not evaluated and the length of therapy differed dramatically.
3. Glucose should be omitted from the nasal solutions because of the assumption that it might facilitate the growth of fungi.
4. Further research and better study design is needed to draw final conclusions.

### **Clinical efficacy of AmB in patients with Chronic Rhinosinusitis with Nasal Polyps**

1. Our results lead to the conclusion that the administration of amphotericin B nasal spray to patients operated on for nasal polyposis does not give rise to a significant alteration in either CT score, clinical symptoms, or quality of life.
2. There is no evidence that AmB is effective.

### **Adherence to Amphotericin B sprays**

1. Patient adherence was relatively good; only a moderate decline was seen during the 12 months.
2. The most common barrier to adherence was motivational.
3. The incidence and also the regularity of side-effects were higher in the AmB group. Local mucosal irritation, that is the most common side-effect of AmB treatment, clearly exerts a negative influence on adherence.

4. Nasal sprays are presumably a more convenient dosage form than nasal lavages, and a higher adherence is to be expected.
5. Two indirect methods were used for the measurement of adherence: (a) recording of the amount of medication self-administered and (b) self-reporting. The combination of the two methods is considered superior to reliance on either of the single methods, since both have advantages and disadvantages.

### **Analysis of Amphotericin B**

1. In our study we investigated the stability of 5mg/ml AmB solutions (Fungizone®) with chemical (spectrophotometry) and biological (bioassay) detection. We estimated the shelf life of the glucose-free solutions, being stored at 4°C, to be 30 days in accordance with our bioassay results.
2. The two detection methods showed strikingly different results. With chemical analysis the samples are considered relatively stable under all observed conditions, while bioassay showed complete loss of antifungal activity after 35 days
3. The assay parameters (F-medium with *S. cerevisiae* as test organism) recommended by the Ph. Eur. 6. were less sensitive and were only applicable for the measurement of a narrow concentration range.
4. Our optimized bioassay method is capable of the quantitative measurement of amphotericin B at the concentration range of 1,54-60,0 µg/ml.
5. With the aid of our optimized eluent (Chloroform – Methanol – Borate buffer pH 8,3; 4:5:1) two components can be separated on silica gel layers in AmB samples. The observed components both have antifungal effect..
6. Direct bioautography (TLC separation plus microbiologic detection with *C. albicans*) of AmB proved to be a very sensitive method with the detection limit of 0.8 ng/spot.

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## Publications, posters and oral presentations

### Publications

1. **Fittler A.**: Amfotericin B tartalmú orrspray orrpolyp kezelésére. *Gyógyszerészet* 51, 280-284. (2007)
2. **Fittler A.**, Mayer A., Kocsis B., Gerlinger I., Fónay F., Botz L.: Amfotericin B orrspray oldat stabilitásvizsgálata biológiai és kémiai detektálással. *Acta Pharmaceutica Hungarica* 77, 159-164 (2007)
3. Gerlinger Imre dr., **Fittler András.**, Mayer Anna., Fónay Fruzsina dr., Pytel József dr., Botz Lajos dr. Amphotericin B tartalmú orrspray posztoperatív alkalmazása orrpolypózissal járó krónikus rhinosinusitis eseteiben – megelőzhető-e a recidíva? *Fül-, Orr-, Gégegyógyászat* 53(4), 150-159 (2007)
4. **Fittler A.**, Matus Z., Kocsis B., Botz L. Amfotericin B tartalmú készítmények kvantitatív analízisének kémiai és mikrobiológiai vonatkozásai *Acta Pharmaceutica Hungarica* 78. 69-74. (2008)
5. Gerlinger I., **Fittler A.**, Mayer A., Patzkó Á., Fónay F., Pytel J., Botz L. Amphotericin B-tartalmú orrspray posztoperatív alkalmazása orrpolypózissal járó krónikus rhinosinusitis eseteiben. Megelőzhető-e a recidíva? *Orvosi Hetilap* 149(37), 1737-1746. (2008)
6. Imre Gerlinger; **András Fittler**; Fruzsina Fónai; Ágnes Patzkó, MD; Anna Mayer; Lajos Botz, PhD: Postoperative application of amphotericin B nasal spray in chronic rhinosinusitis with nasal polyposis, with a review of the antifungal therapy. *European Archives of Oto-Rhino-Laryngology* 266(6), 847-855 (2009). (IF:0,843/2008)
7. **András Fittler**, Lajos Botz. Clinical and analytical aspects of the improvement of a local antifungal preparation for the treatment of nasal polyposis.(abstract) *European Journal of Pharmaceutical Sciences* 38(1),Suppl. 142-143 (2009) (IF:3,65/2008)
8. **András Fittler**, Béla Kocsis, Imre Gerlinger, Lajos Botz: Optimization of bioassay method for the quantitative microbiologic determination of amphotericin B. *Mycoses* 53(1), 57-61 (2010) (IF: 1.529/2008) Times cited:1
9. Imre Gerlinger, **Andras Fittler**. Fungal theory in the pathogenesis of chronic rhinosinusitis (CRS) - pros and cons (In Reference to The Effect of Topical Amphotericin B on Inflammatory Markers in Patients with Chronic Rhinosinusitis: A

Multicenter Randomized Controlled Study), *The Laryngoscope* 120(1), 210-212 (2010) (IF:1,877/2008)

10. **András Fittler**, Béla Kocsis, Zoltán Matus, Lajos Botz. A sensitive detection method for the thin layer chromatography of amphotericin B. *Journal of Planar Chromatography* 23(1), 17–21 (2010) (IF:0,982/2008)

### Posters

1. **Fittler A.**, Mayer A., Botz L., Kocsis B., Gerlinger I.: Amphotericin B tartalmú vizes oldat stabilitásvizsgálata: biológiai és kémiai vonatkozások Gyógyszerkutató Szimpózium, Debrecen, 2006. november 24-25.
2. Tóvölgyi Zs., **Fittler A.**, Gerlinger I., Botz L.: Beteg-együttműködés orrpolyposisban szenvedő betegek klinikai vizsgálata során. Gyógyszerkutató Szimpózium, Szeged, 2007. november 9-10.
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4. Matus Z., **Fittler A.**, Kocsis B., Botz L. Chemical and microbiological aspects of the quantitative analysis of amphotericin B. Is HPLC in itself enough for quantification? 14th International Symposium on Separation Sciences New Achievements In Chromatography September 30.-October 3. 2008. The Zora Hotel, Primošten Croatia
5. Matus Z., **Fittler A.**, Kocsis B., Botz L.: Kromatográfiai és mikrobiológiai módszerek alkalmazhatósága a gombaellenes hatású amfotericin B meghatározására (Mit is mérünk valójában?) Magyar Elvásztástudományi Társaság, Elvásztástudományi Vándorgyűlés 2008. november 5-7. Sárvár
6. Matus Z., **Fittler A.**, Kocsis B., Botz L. Chemical and microbiological aspects of the quantitative analysis of amphotericin B. Is HPLC in itself enough for quantification? 8th Balaton Symposium on High-Performance Separation Methods and 15<sup>th</sup> International Symposium on Separation Sciences Hotel Azúr, Siófok, 2009. szept 2-4
7. **András Fittler**, Lajos Botz. Clinical and analytical aspects of the improvement of a local antifungal preparation for the treatment of nasal polyposis. 3rd BBBB-Bosphorus International Conference on Pharmaceutical Sciences, Antalya-Turkey 26. October 2009.

8. **Fittler András**, Botz Lajos, Intranazális gombaellenes készítmény fejlesztésének klinikai és analitikai vonatkozásai. Congressus Pharmaceuticus Hungaricus XIV. Budapest 2009. október 13-15.

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