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Two examples of rapid and simple drug analysis in pharmaceutical formulations using capillary electrophoresis: Naphazoline, dexamethasone and benzalkonium in nose drops and nystatin in an oily suspension

Capillary electrophoresis is a versatile tool in pharmaceutical analysis. In the course of a revision of the "Standardrezepturen", a German formula of standard dispensings for preparation in pharmacies, this technique has been applied to drug analysis in pharmaceutical formulations. The present paper deals with two different examples. First, naphazoline, dexamethasone and the preservative benzalkonium are quantified in nose drops without any sample preparation. Second, the antifungal antibiotic nystatin is quantified using nonaqueous capillary electrophoresis in methanol after sample preparation from an oily suspension.

1 Introduction

Today no pharmaceutical preparation can be marketed without analytical supervision of identity, purity and content. This is especially true for drugs produced on a larger scale, which have to meet more rigorous requirements than single preparations of an individual prescription. The "Standardrezepturen" (SR, standard dispensings) [1] is a collection of well-tried remedies for preparation in pharmacies, which originated in East Germany. After the reunification of Germany in 1990 the SR have continued to be used, especially in the eastern part of Germany, but have also found general acceptance in addition to the "Neues Rezepturformularium" (NRF, new dispensing formula). Although the SR contains detailed information about preparation, storage, durability and application, a revision is necessary to meet the changed requirements of the law and to take new scientific results into consideration.

Two of these standard dispensings were subjected to a detailed investigation at our institute: first, "Rhinoguttae Dexamethasoni 0.02% cum Naphazolino SR", a nose drop preparation containing the antiallergic and antiinflammatory glucocorticoid dexamethasone, the α_1-sympathomimetic drug naphazoline hydrochloride and the preservative benzalkonium chloride; and second, "Oleum Zinci oxydati cum Nystatino SR", a suspension of the antifungal antibiotic nystatin and zinc oxide in peanut oil. Nystatin, which is produced from cultures of *Streptomyces noursei*, is an effective drug against the yeast fungus *Candida albicans*. Its dosage is declared in International Units (IU) obtained by microbiological analysis, because the isolated and purified drug still contains (beside the effective nystatin A_1) the less effective structurally closely related substances nystatin A3 and polyfungin B. The manufacturer declares for each batch the ratio between international units and mass, which is in the range of 5050–5200 IU/mg.

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Both preparations have been investigated using modern instrumental analysis methods, among them CE, HPLC and photoacoustic spectroscopy, which are compared to the accepted methods of pharmacopeias [2, 3]. The present paper deals with the quantitative analysis of the drugs contained in these preparations using CE. Naphazoline seems to be predestined for CE; it was used as internal standard in pilocarpine analysis [4]. Dexamethasone was separated from other glucocorticoids in serum samples using mixed micellar electrokinetic capillary chromatography [5] and determined in equine urine by MEKC [6]. A capillary zone electrophoresis (CZE) microassay for dexamethasone analysis in tears was reported [7]. Benzalkonium has been analyzed using CZE [8] and capillary isotachophoresis [9]. Recently the determination of antibacterial quaternary ammonium compounds by CZE, among them benzalkonium chloride, in lozenges has been described [10]. The method development has been aimed to provide rapid, simple and highly reproducible techniques which meet the special requirements of each preparation. In the case of the nose drop preparation three substances of interest have to be separated, and the problem of the higher viscosity caused by hydroxyethylcellulose has to be circumvented. In the case of the oily suspension, nystatin has to be separated from a neutral matrix. The solubility requirements have to be met.

2 Materials and methods

2.1 Chemicals and preparations

Dexamethasone, naphazoline hydrochloride, ammonium acetate and benzalkonium chloride are obtained from Sigma (Deisenhofen, Germany). Nystatin according to DAB 10 (German pharmacopeia) was purchased from Fährhaus Pharma (Hamburg, Germany). Fifty mM phosphate buffer, pH 7.0, and 0.1 M sodium hydroxide solution are obtained from Fluka (Buchs, Switzerland). Acetic acid, 96%, methanol gradient grade and acetone are supplied by Merck (Darmstadt, Germany). The nose drops "Rhinoguttae Dexamethasoni 0.02% cum Naphazolino SR" are composed of: 0.02 g dexamethasone, 0.03 g naphazoline hydrochloride, 0.01 g benzalkonium chloride, EDTA, 0.1 g sodium salt, 0.2 g sodium chloride, 1.16 g boric acid, 0.13 g sodium tetraborate, 2.0 propylene glycol, 3.0 g hydroxyethylcellulose, water to 100.0 g. This gives an isotonic solution, buffered to pH 6.8. The oily suspension "Oleum

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Zinci oxydati cum Nystatino SR" is composed of: 7 000 000 IU Nystatin and 50:50 m/m zinc oxide/peanut oil to 100.0 g. Both preparations, manufactured by the PKH (Pharmazeutisches Kontroll- und Herstellungslabor GmbH Halle, Germany) according to [1], obtained from the PKH.

2.2 Quantification of naphazoline, dexamethasone and benzalkonium in nose drops

2.2.1 Sample and buffer preparation

Phosphate buffers of different pH values in concentrations from 5 to 100 mM are used for optimization. The 50 mM phosphate buffer, pH 7.0, used in analytical runs can be prepared or obtained ready for use. All buffers are filtered through a 0.2 μm membrane filter and degassed for 10 min using ultrasound. The nose drops are used as samples as received. The standard solutions of naphazoline HCl and benzalkonium chloride are obtained simply by dilution of a stock solution in water. In the case of dexamethasone a stock solution in propylene glycol is diluted with water as performed for the preparation of "Rhinoguttae Dexamethasoni 0.02% cum Naphazolino SR".

2.2.2 Capillary electrophoresis

All CE operations were carried out using the HP^{3D}CE system (Hewlett-Packard, Waldbronn, Germany) equipped with an integrated diode array detector. A bulkware fused silica CE capillary with 50 μm ID (CS, Langerwehe, Germany) is cut to 330 mm length (245 mm to detection window). The capillaries are flushed for 10 min with 0.1 M NaOH prior to first use but for 3 min with 0.1 M NaOH and for 5 min with the run buffer before each subsequent run. The capillary temperature is set to 50°C using the air cooling of the capillary cassette by the built-in Peltier element. The sample solutions are injected by pressure (50 mbar, 4 s). The separation voltage is +15 kV at the inlet electrode (outlet set to ground). The UV detection is performed at three wavelengths corresponding to the UV maxima of the substances: 242 nm for dexamethasone, 221 nm for naphazoline and 207 nm for benzalkonium. The UV wavelength λ = 200 nm as well as the electrical current are also acquired for controlling the system stability.

2.2.3 Data analysis

The peak areas are obtained by integration of the electropherograms using the HP Chemstation software. The peak areas are corrected by dividing by the migration times. Every sample was run twice. Calibration curves for each substance with at least five concentrations from 50 to 500 μg/mL are set up. The calculation of linear regression, correlation coefficients and sample concentrations was performed using Microsoft Excel.

2.3 Quantification of nystatin in an oily suspension

2.3.1 Sample and buffer preparation

The run buffer is 20 mM ammonium acetate in methanol with 1% acetic acid, which is filtered and degassed using ultrasound as described in Section 2.2.1. The sample preparation is performed as follows: 100 mg of "Oleum Zinci oxydati cum Nystatino SR" are weighed into a centrifuge glass. Two mL acetone are added to dissolve the peanut oil. After centrifugation for 3 min at 3000 *g*, nystatin and the zinc oxide sediment and the acetone fraction are removed. Five mL methanol are added to solve nystatin. The mixture is subjected to an ultrasonic bath for 30 min and shaked every 10 min. After renewed centrifugation the supernatant gives a solution of nystatin in methanol (approximately 0.293 mM for 100% recovery). These solutions are subjected to CE after addition of 10 μL glacial acetic acid, which secures the protonation of nystatin and prevents the formation of an isolating sample plug resulting in current breakdown. Calibration curves are set up by dissolving nystatin in methanol (at least five concentrations from 0.05 to 1.0 mM). For the determination of recovery rates of the sample preparation, calibration samples (1–24 million IU per 100 g zinc oxide/peanut oil, 50:50 m/m) are subjected to the sample preparation procedure described above and the obtained values are compared to the values of the calibration samples obtained without sample preparation.

2.3.2 Capillary electrophoresis

All CE runs were performed with the apparatus described in Section 2.2.2. A fused-silica capillary 645(560) × 0.05 mm with extended lightpath (ID 0.15 mm) is used (Hewlett-Packard). The capillary is conditioned for 5 min with 0.1 M NaOH prior first use and for 4 min only with methanolic run buffer before each subsequent run. The capillary temperature is set to 25°C. The separation voltage is +30 kV, which yields a current of approximately 16 μA. Additional pressure of 50 mbar is applied to speed up the analysis. Nystatin shows UV maxima at 304 and 318 nm. UV

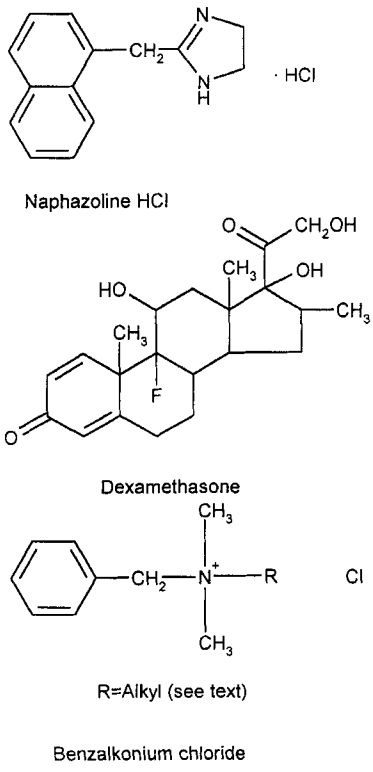


Figure 1. Chemical formulas of naphazoline HCl, dexamethasone and benzalkonium chloride.

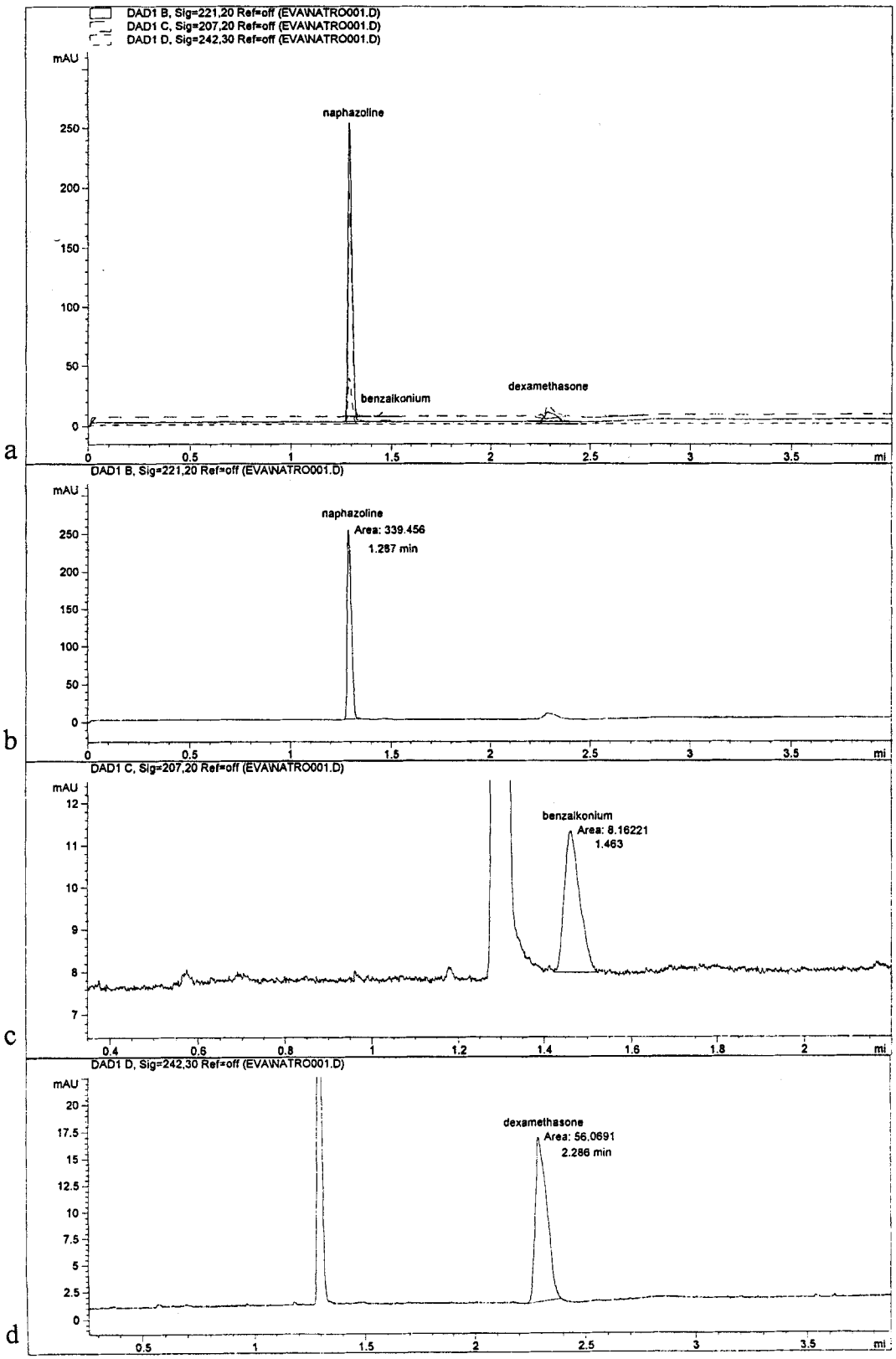


Figure 2. Electropherograms of one analysis run of a nose drop preparation of "Rhinoguttae Dexamethasoni 0.02% cum Naphazolino SR". (a) Overlay of the signals at 207, 221 and 242 nm; (b) detailed view of the naphazoline peak, $\lambda = 221$ nm; (c) detailed view of the benzalkonium peak, $\lambda = 207$ nm; (d) detailed view of the dexamethasone peak, $\lambda = 242$ nm.

detection at λ = 304 nm results in best sensitivity. The UV wavelength λ = 318 nm as well as the current are also acquired for controlling the system stability.

2.3.3 Data analysis

The data analysis is performed as described Section 2.2.3.

3 Results and discussion

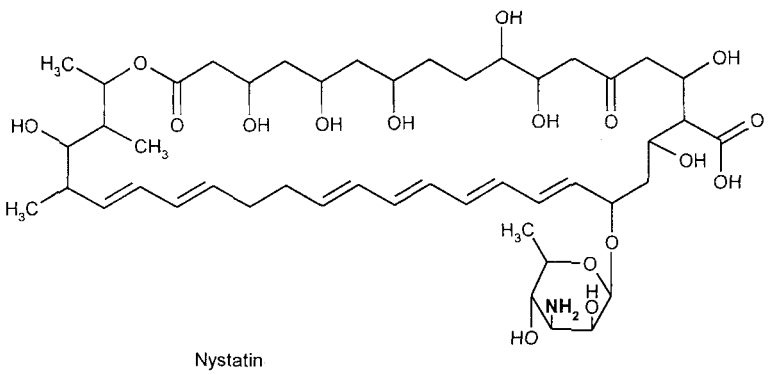
3.1 Quantification of naphazoline, dexamethasone and benzalkonium in nose drops

The chemical formulas of the drugs are shown in Fig. 1. Optimization was carried out with real nose drop samples of "Rhinoguttae Dexamethasoni 0.02% cum Naphazolino SR". To circumvent the problems related to the higher viscosity of the solution caused by hydroxyethylcellulose, it was decided to elevate the capillary temperature to 50 °C. A relatively short capillary was used to keep the analysis time short. The buffer substance was chosen with respect to UV absorption, mobility, availability in analytical purity, and price. Phosphate buffer was tested with different pH values and concentrations from 5 to 100 mM. Optimization resulted in the choice of a commonly used 50 mM phosphate buffer at pH 7.0. Naphazoline yielded the largest peaks under the given conditions. Although the peak shape had to be optimized, the naphazoline analysis showed a notable robustness over a wide range. As expected, it was more difficult to determine the small amounts of the preservative benzalkonium chloride. This quaternary ammonium salt (M_r 926.1, see Fig. 1) is a mixture of components varying in length by one alkyl chain (predominantly C_{12}, but also C_{14} and C_{16} homologs). The intention of this approach was a joint quantification of benzalkonium without separation of the components, which is not important for pharmaceutical quality control. The steroidal drug dexamethasone (see Fig. 1) is a neutral substance which cannot be ionized under CE conditions. Therefore it migrated with the electroosmotic flow. Nevertheless, a quantifiable peak can be obtained, since no other substance of the nose drop preparation shows a UV absorption at λ = 242 nm. The peak shape was an important criterion of optimization. Sample overload effects visible at dexamethasone concentrations higher than 0.03% and result in peak broadening.

Figure 2 shows an electropherogram of a batch of "Rhinoguttae Dexamethasoni 0.02% cum Naphazolino SR" recorded at 207, 221 and 242 nm. The analysis is complete after only 3 min. The overlay presentation in the same scale (Fig. 2a) is dominated by the large naphazoline peak (shown again in Fig. 2b). Figures 2c and 2d illustrate that benzalkonium and dexamethasone also show quantifiable peaks. The peaks can be identified by the UV spectra. The presented method allows the quantification of naphazoline, dexamethasone and benzalkonium at the same time. Calibration curves for the three components are performed before each series of nose drop testing. The calibration curves show good linearity with correlation coefficients typically better than 0.995; the concentrations contained in the nose drop preparation are in the linear range. The achieved limits of detection (S/N 3:1) are: naphazoline, 1.6 μmol/L (0.3 μg/mL), dexamethasone, 4.1 μmol/L (1.6 μg/mL); and benzalkonium, 117 μmol/L (40 μg/mL). The reproducibility was high with average RSD values of 1.5% for naphazoline hydrochloride and 6% for benzalkonium chloride and dexamethasone.

3.2 Quantification of nystatin in an oily suspension

This analytical problem is of completely different nature when compared to the application described above. A sample preparation is necessary to separate nystatin from zinc oxide and peanut oil. The described relatively simple procedure provides recovery rates of approximately 92%; these are also verified by HPLC. The water solubility of nystatin is poor. Therefore a nonaqueous approach is chosen using the suitable solvent methanol. Ammonium acetate is selected as run buffer because of its good solubility in methanol. Nystatin is a relatively large molecule (see Fig. 3) which has acidic (because of the carboxyl group) as well as basic properties. Under acidic conditions (1% acetic acid) nystatin is protonated at the amino group of the basic sugar. As a cation it can be separated from a neutral matrix. Since the EOF is completely neutralized under nonaqueous, acidic conditions, it has been decided to speed up the analysis by the application of 50 mbar hydrodynamic pressure applied from the inlet side. The loss of resolution caused by the parabolic flow profile has no detrimental effects. The UV spectrum of nystatin shows two maxima at 304 and 318 nm. A capillary with extended lightpath (bubble cell) is used to improve sensitivity according to Beer's law. The system



Nystatin

Figure 3. Chemical formula of nystatin.

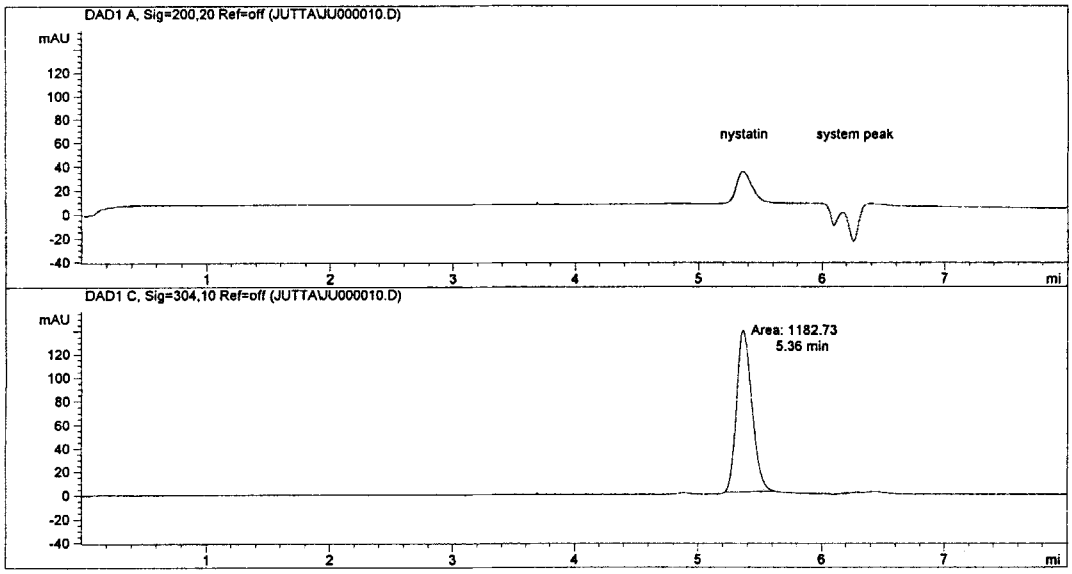


Figure 4. Electropherogram of a nystatin sample in methanol (1 mM) according to the sample preparation described under Section 2.3.1.

peak detected at 200 nm is not seen at 304 nm. Figure 4 shows an electropherogram of a nystatin sample in methanol under the described conditions.

The calibration curves show good linearity with correlation coefficients better than 0.995. The quantification yields a limit of detection (S/N 3:1) of 6.6 μmol/L (6 μg/mL). The RSD of calibration as well as test samples is approximately 1% in the optimum range from 0.2 to 0.8 mmol/L and 2.4% in the range from 0.05 to 1.0 mmol/L. Older batches of "Oleum Zinci oxydati cum Nystatino SR" sometimes produce analytical problems by causing current breakdowns. This observation has been tentatively ascribed to possible degradation products with a different solubility behavior.

4 Concluding remarks

These two applications demonstrate that CE is a versatile tool in the analysis of pharmaceutical formulations. In the nose drop preparation three drug substances are quantified at the same time. Absolutely no sample preparation is necessary. The method is rapid, simple and extremely reliable. It is expected that a number of similar aqueous preparations, especially nose drops as well as eye drops containing α-sympathomimetic drugs of the imidazoline type, can be analyzed this way. The oily suspension requires a sample preparation, which yields a recovery of 92%. This step, which is also required for most of the other analytical methods (*e.g.*, HPLC, microbiological analysis), contributes much more to the standard deviation than CE. The nonaqueous CE method has been developed as a rapid and simple separation of nystatin from a neutral matrix and enables a reliable quantification. Although the application of methanol as CE medium shows a number of disadvantages compared to the commonly used aqueous systems, it will play an important part in the analysis of poorly water-soluble ionic substances, especially because of its high UV transmission.

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