

# DEVELOPMENT OF METHODS FOR IDENTIFICATION AND QUANTITATIVE DETERMINATION OF ANALBEN IN TABLETS

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## ABSTRACT

**PURPOSE:** Analben has been created by the researchers of the National University of Pharmacy and recommended in the solid dosage form as a promising non-narcotic analgesic and anti-inflammatory drug. The aim of the work is to develop physico-chemical and chemical methods of identification and quantitative determination of the active pharmaceutical ingredient in Analben tablets.

**MATERIALS AND METHODS:** As the object of research a pilot batch of tablets “Analben, 1 mg” produced by the pharmaceutical firm “Neopharm” together with the company “Zdorovie” was selected. Analytical studies were performed by the methods of spectrophotometry, thin-layer chromatography and chemical reactions.

**RESULTS AND CONCLUSIONS:** As the result of the work performed, the spectral characteristics of the analben substance in various solvents have been studied, the optimal conditions for determining related impurities have been selected using the method of thin-layer chromatography. The spectrophotometric method for quantitative determination of Analben in the tablets under study has been developed; the solvent, concentration and wavelength have been chosen. The validation characteristics of the quantitative determination method have been studied and it has been determined that linearity is observed in the range of concentrations from 0.16 µg/ml to 0.24 µg/ml ( $\pm 20\%$ ), the systematic error of the method (0.33%) is practically insignificant, the relative confidence interval for the value Z (0.62%) is less than the critical value for convergence of results (1.66%).

**Keywords:** non-narcotic analgesic, anti-inflammatory drug, Analben, identification, quantitative determination, tablets

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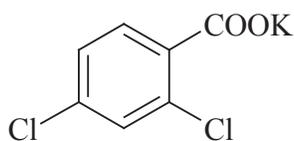
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## INTRODUCTION

Introduction of drugs of the main pharmacotherapeutic groups that are proved to be effective, safe and of high quality into medical practice remains one of the vital tasks of pharmacy.

Potassium 2,4-dichlorobenzoate under the conditional name Analben has been synthesized by the researchers of the National University of Pharmacy.



In the fundamental pharmacological research it exhibited anti-inflammatory, analgesic, antipyretic, hepatoprotective and antioxidant properties. It has been also found that the compound under study does not reveal ulcerogenic and hepatotoxic action (3,11,12). These are preconditions for creating a finished dosage form with the diversified range of the pharmacological activity on the basis of the analben substance (6,9,10).

Tablets containing analben as an active pharmaceutical ingredient are allowed for clinical trials as a non-narcotic analgesic and anti-inflammatory drug. To use the drug in medical practice it is necessary to standardize it. Standardization has been performed according to the requirements of the State Pharmacopoeia of Ukraine (SPhU) (8).

## MATERIAL AND METHODS

A pilot batch of tablets "Analben, 1 mg" produced by the pharmaceutical firm "Neopharm" together with the company "Zdorovie" was analyzed. During the research the chromatographically pure sample of the analben substance was used.

The analytical equipment was Evolution 60S (USA) spectrophotometer; AXIS ANG200 balances (Poland). Reagents (Ethanol (96 per cent); Hydrochloric acid; 0,1 M Sodium hydroxide; 0,1 M Hydrochloric acid; 0,1 M Potassium hydroxide, alcoholic; 150 g/l solution of tartaric acid R; 200 g/l solution of sodium cobaltinitrite R), measuring glassware of class A and excipients (lactose monohydrate (Pharmatos e<sup>200</sup>) s. 10681184 "FrieslandCampania-DMV B.V." Netherlands, calcium stearate s. 12, EGH.E.Pharm.6.0., starch maize s. E3208, "Roquette Freres" France, povidone (Plasdone K-17 polymer), s. 830811 Ashland, USA) meet the requirements of the State Pharmacopoeia of Ukraine (SPhU) (7) which are harmonized with the European ones (2).

Materials (filter paper blue dot TC 2642-001-42624157-98, TLC plates Merck (Germany) 60F 254 s.HX304920).

## Methods for determination

### Identification

**A.** The ultraviolet spectrum of absorption of the test solution prepared for quantitative determination in the range from 260 nm to 300 nm should have the maxima at the wavelengths of 273 nm and 281 nm, and the shoulder at 288 nm (absorption spectrophotometry) (2,7).

**B.** On the chromatogram of the test solution (a) obtained when determining related impurities the principal spot must be detected at the level of the principal spot on the chromatogram of the reference solution (a) and correspond to it by size and coloration (Fig. 4).

**C.** Shake 0.5 g of the triturated tablets with 5 ml of water for 3 min and filter through a paper filter (blue dot, fine-pored, highly dense, very slow rate of filtration, wet strength). To the filtrate obtained add 0.5 ml of hydrochloric acid; a white crystalline precipitate is formed.

**D.** Shake 1.0 g of the triturated tablets with 10 ml of water for 3 min at  $20 \pm 5^\circ\text{C}$  and filter through a paper filter (blue dot, fine-pored, highly dense, very slow rate of filtration, wet strength). Divide the filtrate into two portions. To the first portion of the filtrate add 2 ml of the freshly prepared solution of 200g/l of sodium cobaltinitrite; a yellow or orange precipitate is formed.

**E.** To another portion of the filtrate add 2 ml of 150 g/l of tartaric acid solution and leave off; a white crystalline precipitate is formed.

### Tests

**Related impurities** (thin-layer chromatography).

*Test solution.* To the accurately weighed tablet powder, which is equivalent to 5 mg of analben add 5 ml of 96% ethyl alcohol, shake for 3 min, dilute the solution to the volume of 10 ml with the same solvent, mix and filter.

*Reference solution* (a). Dissolve 5 mg of analben reference standard (RS) in 5 ml of 96% ethyl alcohol and dilute the solution to the volume of 10 ml with the same solvent. Prepare the solution immediately prior to use.

*Plate:* TLC plates (10 cm x 20 cm) with the layer of silica gel F<sub>254</sub> P.

*Mobile phase:* 96% ethyl alcohol.

*Application:* 5 mcl.

*Development:* over a path of 15 cm.

*Drying:* in the air for 15 min.

*Detection:* examine in ultraviolet light at 365 nm.

*Chromatographic system suitability:*

- ❖  $R_f$  of the principal spot on the chromatogram of the test solution: from 0.55 to 0.65;
- ❖ on the chromatogram of the reference solution (a) two clearly separated spots must be detected.

*Limit:* - any related impurities: on the chromatogram of the test solution (a) any spot, excluding the principal one, must not be more intense than the spot on the chromatogram of the reference solution (a) (0.5%) (Fig. 4).

**Assay** (absorption spectrophotometry).

*Test solution.* Place accurately weighed portion of the tablet powder equivalent to 5 mg of analben into a 50 ml volumetric flask, add 10 ml of ethyl alcohol, shake for 15 min, filter into a 25 ml volumetric flask. Shake twice the precipitate with 5 ml of ethyl alcohol and filter through the same filter into the same flask. Add 2 ml of 0.1 M solution of hydrochloric acid, dilute the solution to the volume with ethyl alcohol and mix.

*Reference solution.* Dissolve 50 mg of analben RS in ethyl alcohol and dilute the solution to the volume of 50.0 ml with the same solvent. To 5.0 ml of the solution obtained add 2 ml of 0.1 M solution of hydrochloric acid, dilute the solution to the volume of 50.0 ml with ethyl alcohol and mix.

*Blank solution.* Dilute 2 ml of 0.1 M solution of hydrochloric acid with ethyl alcohol to 25.0 ml.

The optical density of the test solution and the reference solution is measured at the wavelength of 281 nm with respect to the compensation solution.

Calculate the content of  $C_7H_3Cl_2O_2K$  in one tablet, in milligrams, equivalent to the average weight of a tablet based on the declared content of  $C_7H_3Cl_2O_2K$  in analben RS.

## RESULTS AND DISCUSSION

To develop the method of identification and quantitative determination of analben by absorption spectrophotometry we prepared 0.02% solutions of analben in water and alcohol, and studied their UV-spectra in the region from 220 nm to 300 nm (Fig. 1).

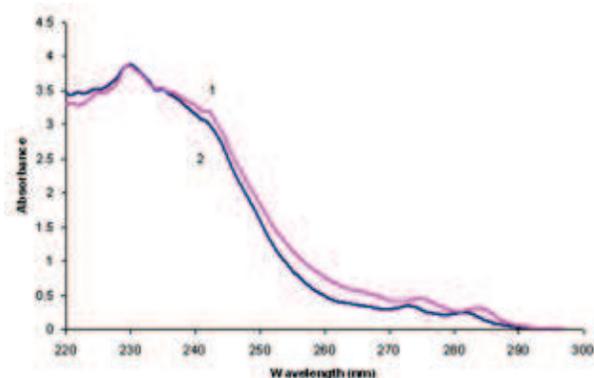


Fig. 1. The UV-spectra of 0.02% solution of analben: 1 – in ethyl alcohol; 2 – in water

As it is seen from the given spectra, in water the absorption maxima were observed at the wavelengths of 222 nm, 230 nm, 235 nm, 273 nm and 281 nm that correspond to absorption of the benzene ring. When replacing the solvent to ethyl alcohol the absorption maxima were observed at 221 nm, 225 nm, 230 nm, 235 nm, 275 nm and 284 nm and were similar by intensity.

The flattest absorption maxima were observed at the wavelengths of 273 nm and 281 nm in water, and 275 nm and 284 nm in ethyl alcohol. It was expedient to study the effect of pH on the character of the analben spectrum when adding acid or alkali. For this purpose the absorption spectra of analben in water and alcohol, as well as with addition of 0.1 M solution of hydrochloric acid or 0.1 M solution of sodium hydroxide were registered (Fig. 2, 3).

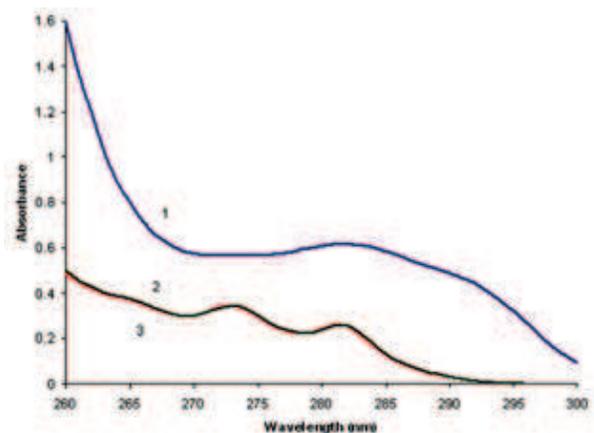


Fig. 2. The UV-spectra of 0.02% solution of analben: 1 – in water with addition of 0.1 M solution of hydrochloric acid; 2 – in water; 3 – in water with addition of 0.1 M solution of sodium hydroxide

As seen from the given spectra, when adding 0.1 M solution of sodium hydroxide or alcoholic solution of potassium hydroxide to the aqueous (Fig. 2) or alcoholic (Fig. 3) solutions of analben, respectively, the character of the spectra does not change; it can testify that the solutions are not hydrolyzed and there is no process of hydrolysis suppression. In case of addition of 0.1 M solution of hydrochloric acid a hypsochromic shift is observed, the intensity of the spectrum almost doubles and there is one rather flat maximum at the wavelength of 281 nm (Fig. 2) or two maxima at the wavelengths of 273 nm and 281 nm and the shoulder at 288 nm (Fig. 3).

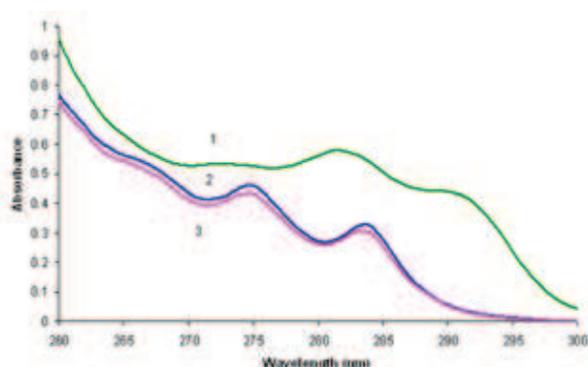
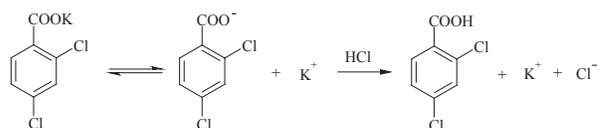
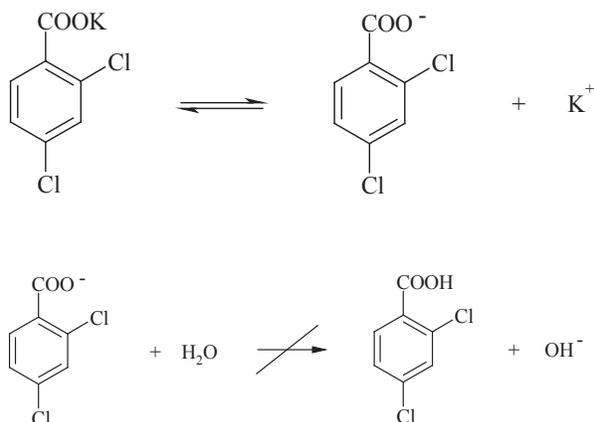


Fig. 3. The UV-spectra of 0.02% solution of analben: 1 – in alcohol with addition of 0.1 M solution of hydrochloric acid; 2 – in alcohol; 3 – in alcohol with addition of 0.5 M alcoholic solution of potassium hydroxide

This is due to the fact that the process of the substance transfer into the acidic form occurs and the dissociation process of the acid obtained is suppressed, the carboxy group of analben is in the protonated form.



Thus, we recommend to perform identification of analben in tablets using spectrophotometry in the range from 260 nm to 300 nm in alcoholic medium with addition of 0.1 M solution of hydrochloric acid indicating positions of maxima and the shoulder.

The method of thin-layer chromatography was used for identification of the active pharmaceutical ingredient in tablets and the test for purity. The research was conducted on “TLC 60F254” plates in alcohol. The chromatogram was detected with in the ultraviolet light at the wavelength of 365 nm. On the chromatogram of the test solution the principal spot is observed; by size, coloration and location it corresponds to the principal spot on the chromatogram of the reference solution (the analben sample of chromatographic grade).  $R_f$  for analben is approximately 0.65 (Fig. 4).



Fig. 4. TLC of analben identification and related impurities

To prove analben to be the salt of a weak organic acid the reaction of interaction with a strong mineral acid was carried out. As a result, a precipitate of 2,4-dichlorobenzoic acid identified by the melting point (160-164°C) was formed. The presence of potassium cation was proven by pharmacopoeias reactions (7).

Quantitative determination of analben in tablets was performed by the method of spectrophotometry after extraction of the active substance from the tablet mass with the help of ethylic alcohol. 0.1 M solution of hydrochloric acid was added to increase the intensity of the absorption spectrum, and the spectrophotometric study was conducted at the wavelength 281 nm. During the spectrophotometric method development the effect of excipients on the nature of the spectrum was studied

According to the standardized procedure (1,4,5), the following validation characteristics of the recommended method for quantitative determination of analben – robustness, specificity, linearity, convergence, accuracy and the range of application have been studied.

*Robustness* was determined by studying stability of solutions over time and the effect of pH. It has been found that the test solution of analben is stable for an hour. When studying the influence of pH changes on the character of the spectrum different amounts of 0.1 M solution of hydrochloric acid were added to the same weighed portion of analben and the optical density of the solution obtained was measured at the wavelength of 281 nm. As can be seen from the data obtained (Table 1), with increase of the acid concentration the optical density does not practically change, but after addition of 2 ml of 0.1 M solution of hydrochloric acid the more constant values

are reached, that is why this volume is added to the reaction mixture.

To confirm *specificity* of the method the relative systematic error introduced by the solvent and excipients of tablets was calculated. For this purpose the optical density ( $A_{\text{blank}}$ ) of the placebo solution and the optical density ( $A_{100\%}$ ) of the test solution were determined three times with removing the cuvette. The mean values found, such as  $A_{\text{blank}}=0.001$ ;  $A_{100\%}=0.619$ , indicate the absence of a significant impact on the results of measurements since the contribution of placebo in the total absorption of the analytical solution is  $\delta_{\text{exc}}=100 \cdot 0.001/0.619=0.16\%$ .

*Linearity, convergence, accuracy and the range* of the method application were studied on the model mixtures within the range of concentrations from 80% to 120% in relation to the nominal value. Solutions with the known concentration were obtained by diluting the initial model solution. The working concentration of the test solution and the reference solution was about 0.20 mg/ml. The linear dependence of the optical density of analben solutions from the concentration in the range from 0.16 mcg/ml to 0.24 mcg/ml ( $\pm 20\%$ ) was found. The results are given in the normalized coordinates (Fig. 5).

The calculation of the linear dependence parameters  $Y_i=b \cdot X_i+a$  for analben (Table 2) was performed by the least square method (according to the data of Table 3); its results were compared with the acceptance criteria given in the SPhU (8).

Table 1. The effect of pH on the optical density of alcoholic solutions of analben

Optical density	Amount of 0.1 M solution of hydrochloric acid, ml				
	0.50	1.00	1.50	2.00	2.50
A	0.572	0.577	0.579	0.576	0.576

Table 2. Statistical characteristics of the linear dependence

Parameters	Values	Criteria (for tolerances – 90-110%, g=9)	Conclusion
b	0.9882		
$S_b$	0.0094		
a	0.9662	1) $1.8595 \times S_a = 1.77$ ; 2) if it is not satisfied 1), then $\leq 5.1$	satisfied
$S_a$	0.9505		
$S_o$	0.3651		
r	0.9997	$\geq 0.9924$	satisfied

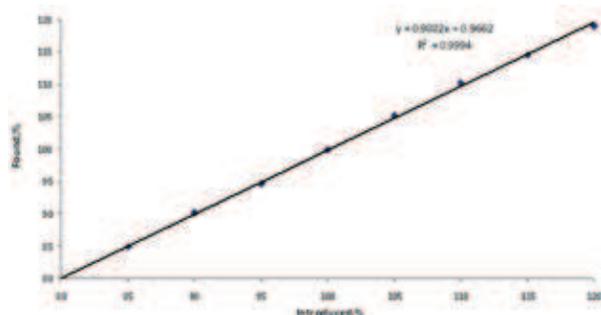


Fig. 5. The linear dependence of the optical density from the concentration of analben in the normalized coordinates

The data of Table 2 testify that all requirements to the linear dependence parameters are met, i.e. linearity of the method is confirmed within the range of the concentrations selected (80-120%).

The results obtained (Table 3) testify that the criteria of the systematic error insignificance are performed. The systematic error of the method (0.33%) is practically insignificant, i.e. the method of analy-

sis is characterized by satisfactory correctness within the range of concentrations from 80% to 120% and precision (convergence) since the relative confidence interval for the value Z (0.62%) is less than the critical value for convergence of results (1.66%).

Therefore, the complex physico-chemical and chemical investigations conducted allowed to develop the project of methods for quality control on the dosage form under research – Analben tablets.

## CONCLUSIONS

1. The methods of identification of Analben in tablets have been developed by absorption ultraviolet spectrophotometry, thin-layer chromatography and chemical reactions.
2. The conditions have been selected and the method of spectrophotometric quantitative determination of Analben in tablets has been developed. The validation characteristics of the quantitative determination method for analben have been studied using the accep-

Table 3. The results of analysis for test solutions and their statistical processing

No. of the test solution	Introduced in % to the concentration of the reference solution ( $X_i = C_i/C_{st}$ , %)	Average optical densities $A_i$ ( $A_{st}=0.621$ )	Found in % to the concentration of the reference solution ( $Y_i = A_i/A_{st}$ , %)	Found in % to the introduced $Z_i=100$ ( $Y_i/X_i$ )
1	80	0.495	79.71	99.64
2	85	0.527	84.86	99.84
3	90	0.560	90.18	100.20
4	95	0.588	94.69	99.67
5	100	0.620	99.84	99.84
6	105	0.653	105.15	100.15
7	110	0.684	110.14	100.13
8	115	0.711	114.49	99.56
9	120	0.739	119.00	99.17
Mean, Z%				99.80
Relative standard deviation, Sz%				0.33
Relative confidence interval $\Delta as\% = t(95\%, 8) \times Sz$				0.62
Critical value for convergence of results $\Delta as\%$				2.24
Systematic error $\delta$				-0.20
Criterion of the systematic error insignificance 1) $\delta \leq \Delta as / (g)^{0.5} = 0.72 / \sqrt{9}$ , 2) if it is not satisfied 1), then $\delta \leq 0.72$				0.24
The overall conclusion of the method				correct

tance criteria for tolerances of the active ingredient content of  $\pm 10\%$ , and they confirm specificity, linearity, convergence, accuracy and the range of the method application.

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