Comparative Analysis of the Spectrophotometric and HPLC Method for Quantitative Determination of Fensulkal in a Substance

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Abstract

According to the World Health Organization (WHO), non-steroidal anti-inflammatory drugs (NSAIDs) are effective in treating pathologies accompanied by inflammatory and painful processes of various etiologies. More than thirty million people in the world receive NSAIDs daily, with 40% of these patients over 60 years of age, and about 20% of inpatients.

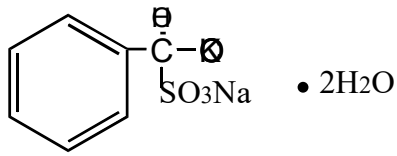
The development vector of pharmaceutical industry in the years of independence is aimed at developing and introducing import substitution drugs produced in our country.

Keywords: *Fensulkal, method, determination, chromatography.*

Introduction

In the Republic of Uzbekistan, special attention is paid to the development of pharmaceutical industry and providing the population with domestically produced products. In this regard, expanding the range of antimicrobial and anti-inflammatory drugs, using domestic resources, is a priority in the development of science and technology in the direction of modernization of production and technology to introduce domestic developments of drugs and medical products. A number of government resolutions are aimed at supporting domestic manufacturers producing pharmaceuticals, so in the production of domestic medicines equally, preference is given to drugs developed from raw materials of plant and synthetic origin. As well as standardization and quality control of medicinal products plays a crucial role in providing the population with effective and safe medicines[1].

The Uzbek Scientific Research Institute of Chemistry and Pharmaceutics named after A. Sultanov synthesized a new biologically active drug - fensulkal, which is a bisulphite derivative of phenyl glyoxylic acid. The results of patent studies have shown that potassium salt of sodium bisulphite derivative of phenyl glyoxylic acid (fensulkal) is a new, previously not synthesized and not described compound [7].



The substance is characterized by moderate toxicity and has strong anti-inflammatory and antimicrobial properties, which together is very important for pathogenetic treatment of many gynecological diseases[2]. Scientists of Tashkent Pharmaceutical Institute together with the above mentioned institute are working on creation of pharmaceutical forms and development of effective method of analysis[3].

Standardization and quality control of medicinal products plays an important role in providing the population with effective and safe medicines[4]. Nevertheless, along with the search for new medicinal products it is necessary to solve the issues of

development of method for their analysis[5],. Fensulkal in substances is quantitatively determined by several method such as chromatography spectrophotometry, spectrophotometry, HPLC[6]. Comparative analysis of the method for quantitative analysis of fensulkal in a substance is relevant as a result of the selection of a more valid one for the purpose of introducing into the quantitative analysis method the first time combined preparations containing this PAI (pharmaceutical active ingredient) are obtained[7].

Research Objective: Comparative analysis of the spectrophotometric, chromato-spectrophotometric and high-performance liquid chromatography (HPLC) method for quantitative determination of fensulkal in PAI.

The research subjects were samples of production batches of fensulkal (PS 42 Uz-0185 - 2017).

"Silufol UV-254" (Czech Republic) plates were used for thin layer chromatography (TCH) and chromatography spectrophotometric method. Various organic solvents were used as a mobile phase (MP): chloroform, acetone, ethyl acetate, hexane, etc. in different variations and ratios [5,6,7]. In work they used liquid chromatograph "Agilent Technologies 1100 series" with software "Chemstation A.09.0" with four-channel gradient pump, degasser and spectrophotometric detector. Acetonitrile, methanol as well as 1% acetic acid solution and buffer solutions with different pH values were used as organic solvents. The optical density of the investigated solution (0.001% fensulkal solution) was measured at a layer thickness of 10 mm in the range of wavelengths from 200 to 400 nm relative to the working standard (WS) of fensulkal. Purified water was used as a comparison solution. The quantification of the active substance was calculated using a formula:

$$X(\%) = \frac{D_1 * C * V_p^1 * V_p^2 * 100}{D_0 * a * V_{ali}^1} \quad (1)$$

Where, D_1 - the value of optical density of fensulkal in the test solution; D_0 - the value of optical density of working sample (WS) of fensulkal; C- the concentration of the preparation in 1 ml of standard solution g/ml; a- the quantity of the test substance; $V_p^1 * V_p^2$ – the volumes of dilution of the quantity; V^l_{ali} - the volume of aliquot part.

The aqueous solution of fensulkal has a maximum absorption at a wavelength of 252±2nm. The spectrum of absorption of fensulkal is shown in Fig. 1,2.

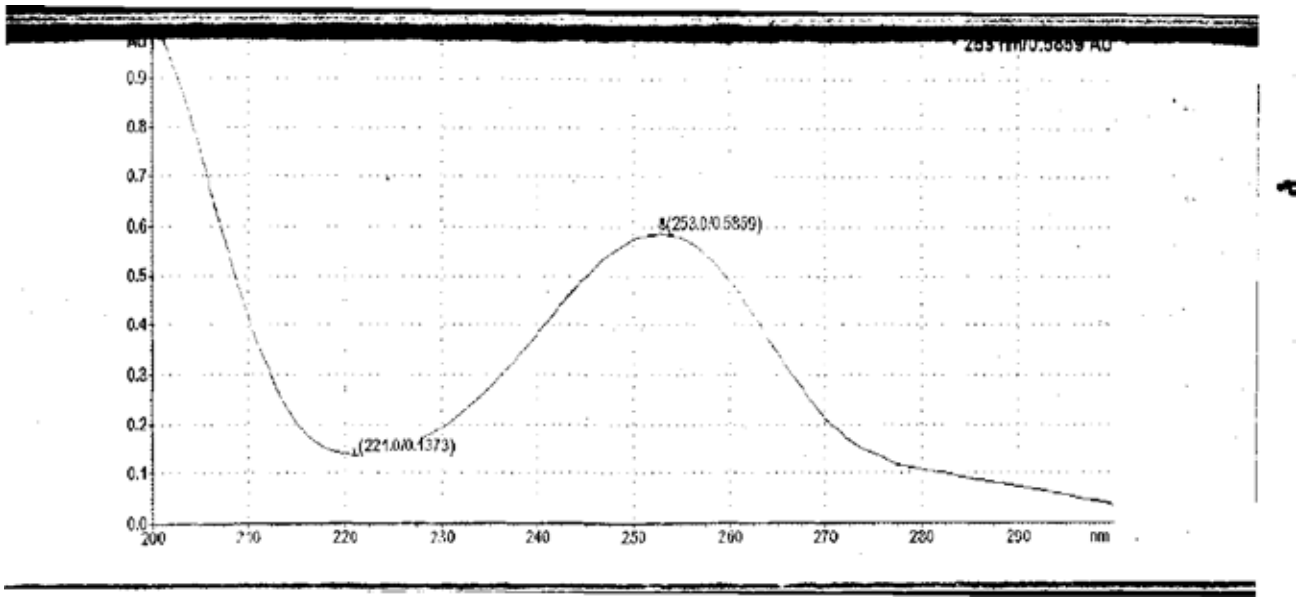


Fig. 1. UV spectrum of WS fensulkal

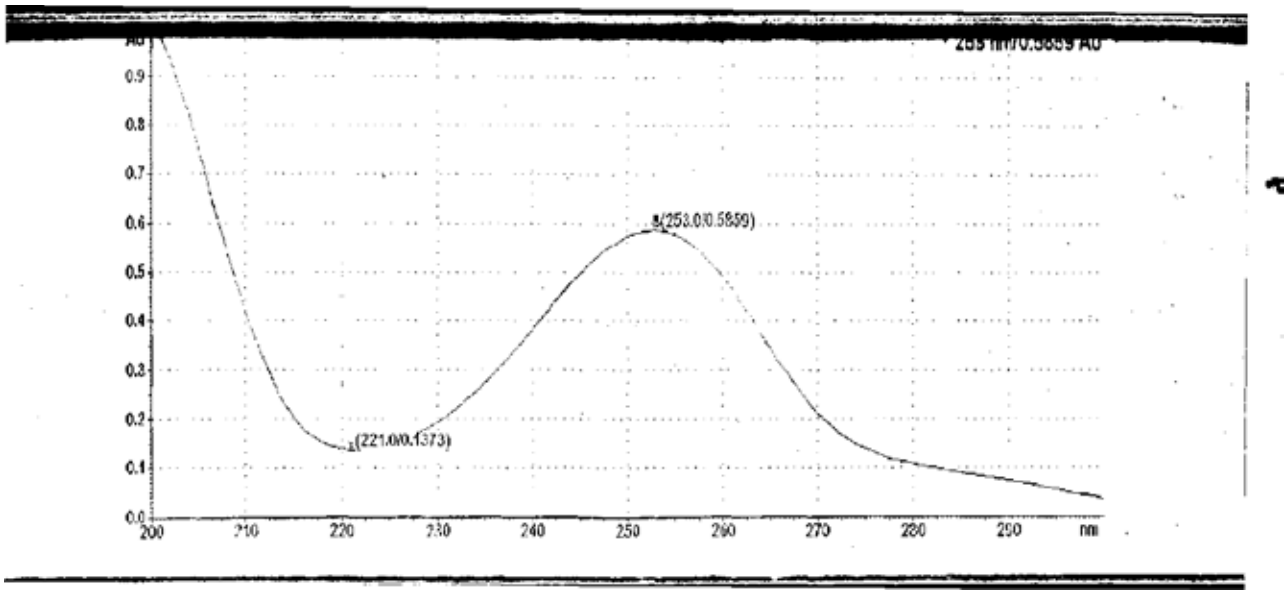


Fig. 2. UV spectrum of the test sample of fensulkal

The received data of quantitative determination of fensulkal in PAI by spectrophotometric method are given in Table 1.

Table 1: Results of the analysis of the quantitative determination of fensulkal in PAI by UV spectrophotometry

No.	Quantity of fensulkal, gr	X, %	Metrological properties
Fensulkal			
1	0,0250	98,90	X_{av}= 99,0 f =4; t(P;f)=2,78; S^2=0,078; S=0,837; ΔX_{cp}= 0,99; ε_{av}= 0,9%
2	0,0249	98,95	
3	0,0249	98,95	
4	0,0250	99,10	
5	0,0251	99,10	

Table 1 shows that the relative error of the method was 0.9%, which also confirms the accuracy of the results obtained within the method.

For the chromatography spectrophotometric determination of 0.2 g of fensulkal was placed in a measuring flask with a capacity of 100 ml and diluted with water to the mark. Then a 0.05 ml water solution of the test sample was applied to the starting line of the "Silufol-UV_{254}"chromatographic plate using a calibration capillary. At the same time, a standard sample of 0.01 ml of witness substance containing 1.0 mg of fensulkal was chromatographed. The plate was chromatographed and after the mobile phase reached the front line, the plate was dried at room temperature. The adsorption zones were detected in UV-light at λ = 254 nm. For quantitative determination of the localization zone, the adsorption zones were eluated, transferred to a paper filter and 100 ml of fensulkal was washed with purified water. Optical density of the obtained eluates was measured on spectrophotometer "Agilent 8453E" at 252 nm. The quantitative content of fensulkal was calculated using the following formula 1.

Preparation of WS solution of fensulkal: About 0.025 g (precise quantity) of fensulkal (VFS 42 Uz-0185-2007) is placed in a measuring flask with a capacity of 50 ml, add 20 ml of a mixture of purified water with methanol, shake to dissolution, bring the volume of the solution to the mark and stir. Place 1 ml of the resulting solution in a measuring flask with a capacity of 50 ml and bring the volume of solution with purified water to the mark. The solution is used freshly prepared. The resulting solutions are filtered through

a filter "Millipor" with a pore size of 0.45 µm. 20 µl of the obtained solutions are injected into the injector of the chromatograph and analyzed under the above conditions, receiving at least 5 chromatograms. The content of fensulkal, in % is calculated by formula (2):

$$\tilde{O}(\%) = \frac{S_1 * a_0 * 2 * P}{S_0 * \grave{a}_1} \quad (2)$$

Where, S_1 - the area of peak fensulkal on a chromatogram of a test solution; S_0- the area of peak fensulkal on a chromatogram WS of fensulkal; a_0– quantity of WS Fensulkal, gr; P – Fensulkal content in WS, %.Content of $C_8H_{10}O_8SNaK$ (fensulkal) should be not less than 98,5 %. The obtained results are presented in Table 2.

Table 2: Results of quantitative determination of fensulkal by chromatography spectrophotometric method

No.	Quantity of Fensulkal, gr	$X_{\%}$	Metrological properties
1	0,2001	98,7	X_{av} = 99,16%; f = 4; t(P;f) = 2,78; S^2 = 0,31; S = 0,51; ΔX_{av} = 0,69; ε_{av} = 1,9%
2	0,2000	99,0	
3	0,2004	99,2	
4	0,2000	99,3	
5	0,2001	99,6	

The data presented in Table 2 confirm the accuracy ($\varepsilon_{average}$=1.9%) of the results obtained and the reproducibility of the developed method for determination of the quantitative content of fensulkal.

HPLC analysis was performed on a liquid chromatograph by "Agilent Technologies" with a column 150 mm long, 3.0 mm in diameter, filled with Zorbax Eclipse XDB C-18 sorbent with a particle size of 5 µm, equipped with a UV detector with a variable wavelength (254 nm) and an isocratic pump. The data were processed using the "ChemStation" software. The relative standard deviation of the peak areas of the received chromatograms should not exceed 2.0% (Fig.3).

As a mobile phase several systems of organic solvents in different ratios were used.

In terms of retention time and symmetry of peaks we have chosen the following conditions for chromatography:

- 150x3.0 mm analytical column filled with Zorbax Eslipse XDBC18 sorbent with 3.5 µm particle size;
- Column temperature - indoor;
- Detection - 254 nm;
- Mobile phase degassed and filtered mixture of methanol and water in ratio (25:75);
- Injector loop volume - 20 µl;
- Flow rate - 0.6 ml/min.

The Fensulkal separation factor (K`) was 0.86, the number of theoretical plates was more than 2250 and the asymmetry factor did not exceed 1.1. The results of the quantitative determination are presented in Table 3.

Table 3: Results of the quantitative determination of fensulkal by HPLC method

No.	Quantity of Fensulkal, gr	$X_{\%}$	Metrological properties
1	0,0510	98,1	X_{av} = 99,08%; f = 4; t(P;f) = 2,78; S^2 = 0,42; S = 0,64; ΔX_{av} = 0,8; ε_{av} = 1,5%
2	0,0509	99,2	
3	0,0520	99,0	
4	0,0510	99,8	
5	0,0501	99,3	

The data presented in Table 3 confirm the accuracy (ε_{av}=1.5%) of the results obtained and the reproducibility of the developed method for determination of the quantitative content of fensulkal.

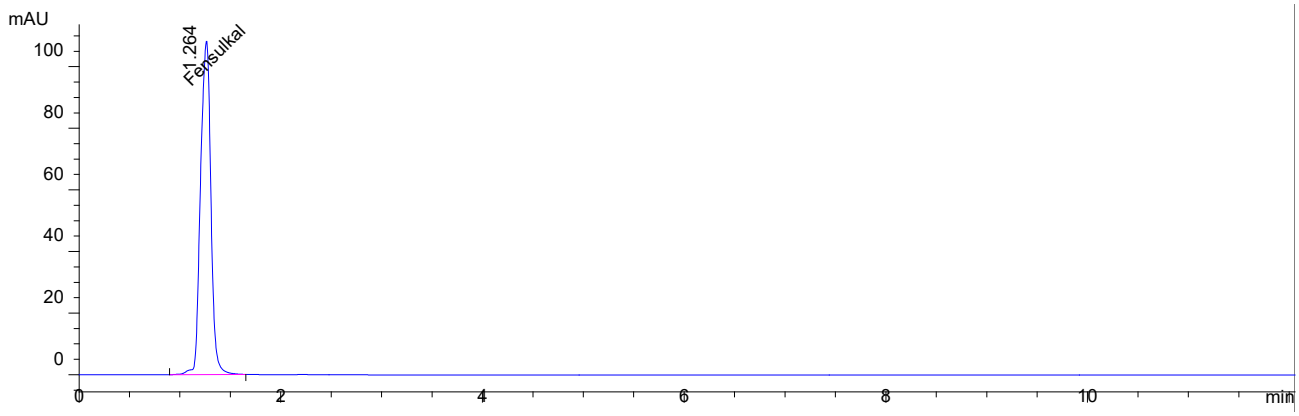


Fig. 3. Chromatogram of WS fensulkal

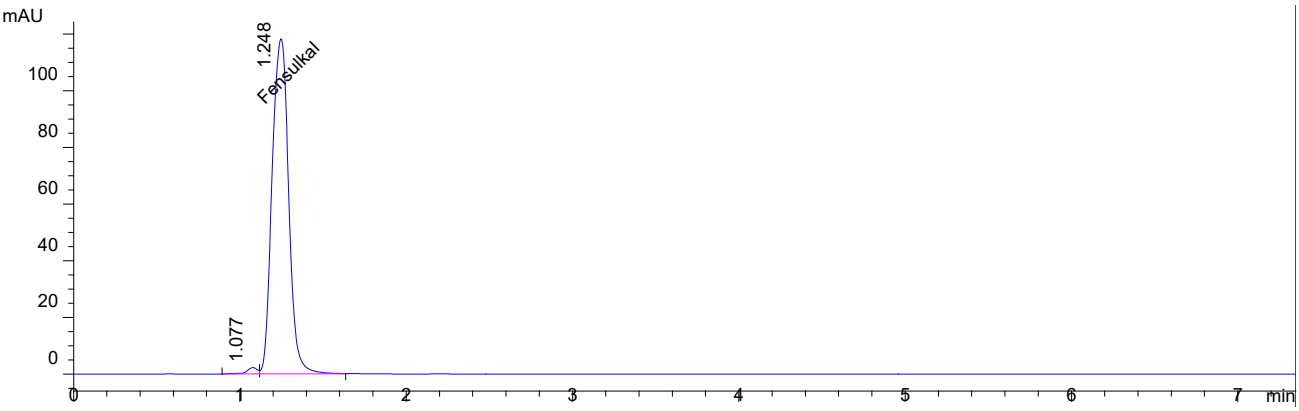


Fig. 4. Chromatogram of the test sample of fensulkal

From the above chromatograms (Fig. 3,4) we can see that at column temperature 20^0C the retention time of fensulkal is 1.248 min. The results of quantitative determination are presented in Table 3.

Since the HPLC method of determining the quantitative content of fensulkal is accurate enough to validate this method on several indicators.

Linearity was assessed by the response of the analytical instrumentation, and model solutions of different concentrations were prepared, corresponding to a range from 25% to 100% of the range specified in the respective technique. These solutions were analyzed on the "Agilent Technologies" liquid chromatograph under the conditions specified above. The results obtained indicate that the best range of linearity is observed in the range of concentrations of the preparation from 0.001 mg/ml to 0.3 mg/ml (Table 4, Fig. 5).

Table 4: Dependence of areas of chromatographic peaks on solution concentration (Linearity)

Solution concentration, mg/ml	Chromatographic peaks area
0,001	51,27
0,10	3649,44
0,15	7350,09
0,20	11039,68
0,30	14765,40
Correlation coefficient	0.999422
Standard deviation	1315.841881
Linear Angular Coefficient	7.01688E-06

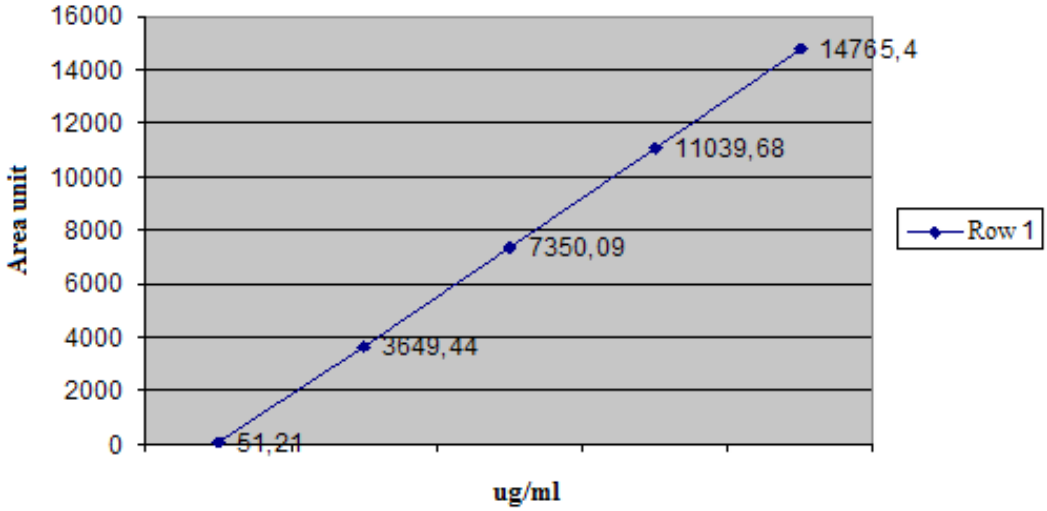


Fig. 5. Fensulkal Linearity Graph

Correctness of the technique was determined by three times determination of 7 analytical concentrations of the substance under study (Table 5). The quantitative content of fensulkal was calculated by the above formula.

Table 5: Evaluation of the correctness of the developed HPLC methodology of the fensulkal

Number of active component from declared, %	Quantity of fensulkal, gr	Found, gr	Regeneration, %
70	0,0350	0,0348	99,4
80	0,0401	0,0398	99,2
90	0,0451	0,0446	98,8
100	0,0502	0,0497	99,0
110	0,0551	0,0547	99,2
120	0,0601	0,0595	99,0
130	0,0650	0,0647	99,5
Average regeneration rate			99,15

From the results in the table we can see that the average regeneration rate is 99.15%, which indicates the correctness of the technique. So, we have developed a new method of separation, detection and determination of the quantitative content of the substance fensulkal by HPLC. The validation of the technique has been carried out; such parameters as sensitivity, linearity, accuracy, reproducibility, selectivity and correctness have been established.

Similarity (e.g. the same spectrophotometer, the same laboratory assistant, the same day, identical reagents) was shown by analyzing 6 prepared samples at 100% concentration of the pharmaceutical substance in the solution under study. The results were evaluated and calculated by calculating the mean value, standard deviation, coefficient of variation and confidence interval. The number of tests required for routine control of each sample was established in the specification depending on a certain coefficient of variation and boundaries of the norm. With the help of intermediate precision it was shown that the analytical technique ensures the obtaining of consistent results regardless of changes in external circumstances provided the homogeneity of the sample under study. All conditions for the laboratory have been taken into account in the validation process. Typical variables were variability, staff, day of analysis and instrument.

Conclusion

Method have been developed to control the quality of fensulkal in substances using UV-, chromatography spectrophotometric, and HPLC method of analysis, the

relative error of which was 0.9 percent for the UV-, chromatography spectrophotometric method; 1.9 percent for the chromatography method; and 1.5 percent for the HPLC method. The developed method are included in the pharmacopoeia article of fensulkal and can be used for quality control not only of fensulkal in PAI, but also in medicinal forms with its content.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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