RESEARCH ARTICLE

Development of meloxicam and indole-3-carbinol quantification method in rectal suppositories for prevention and treatment of benign diseases of the prostate gland

V. S. Zaychenko[1], O. A. Ruban[1], V. A. Khanin[2], V. S. Kyslychenko[2], Ju. S. Masliy[1], S. A. Kutsenko[1]

[1]Department of Industrial Technology of Drugs, National University of Pharmacy, Kharkiv, Ukraine,
[2]Department of Chemistry of Natural Compounds, National University of Pharmacy, Kharkiv, Ukraine

Abstract

Aim: The aim is to study the development of a methodology for the quantitative determination of meloxicam and indole-3-carbinol in the developed rectal suppositories, which would allow the effects of the matrix, placebo, and accompanying impurities on the course of the determination to be cutoff. **Materials and Methods:** Indole-3-carbinol (Sigma-Aldrich Co., USA) and meloxicam **(**Boehringer Ingelheim GmbH, Germany) as well as developed rectal suppositories with a combination of given active pharmaceutical ingredients in the amount for 1 suppository: Indole-3-carbinol - 0.2 g and meloxicam - 0.0075 g were used In carrying out the studies, meloxicam PRS was used, corresponding to the requirements of the European Pharmacopoeia. To determine the content of indole-3-carbinol, a reagent with a purity of at least 99% was used. To calculate the quantitative content of meloxicam and indole-3-carbinol, the homogeneity values obtained for the 10 samples studied are used. The measurements were carried out by high-performance liquid chromatography (HPLC) according to SPU, supp. 1, 2.2.29, N. The validation of the methodology was carried out in accordance with the requirements of SPU according to individual validation characteristics: Specificity, linearity, repeatability, precision, accuracy, and intralaboratory precision. **Results and Discussion:** According to the developed technique, the quantitative determination of meloxicam and indole-3-carbinol in the composition of rectal suppositories has been studied. The following results were obtained: With the content of meloxicam in one dosage unit of the test preparation is 0.0078 g and the content of indole-3-carbinol - 0.205 g, which indicates the validity of the proposed procedure for the quantification of active substances in suppositories for the prevention and treatment of benign prostatic diseases. **Conclusions:** In the course of the study, a HPLC technique with a gradient elution mode has been developed. It has been established that the selected chromatographic conditions allow not only quantitatively determine the substances under investigation in the finished dosage form but also estimate the uniformity of their distribution. The validation of the developed method has been carried out, and its suitability for simultaneous quantitative determination of both meloxicam and indole-3-carbinol in a dosage form was proved.

Key words: High-performance liquid chromatography, indole-3-carbinol, meloxicam, quantitative determination, rectal suppositories, validation

INTRODUCTION

The most common benign pathologies affecting the prostate gland include adenoma and prostatitis.[1] According to statistics, over the past 20 years, the incidence of prostatitis has increased by about half, and in the 21st century, almost half of the male population of the Earth, aged 20–50, suffers from it. Untreated prostatitis has every chance to go into its chronic form, and in men over 40, prostate adenoma associated with hormonal imbalance can develop.[2]

Considering the high prevalence of these diseases, we have faced the task of developing a new domestic drug in the form of rectal suppositories for the prevention and treatment

Address for correspondence:
Ju. S. Masliy, Department of Industrial Technology of Drugs, National University of Pharmacy, Kharkiv, Ukraine. E-mail: julia.masliy@gmail.com

Received: 27-04-2018
Revised: 23-05-2018
Accepted: 08-06-2018

of benign prostatic diseases. As active pharmaceutical ingredients (APIs), we suggested using a combination of indole-3-carbinol and meloxicam.[3-5]

Cabbage broccoli (*Brassica oleracea* L. *var. Italica Plenck* family *Brassicaceae*) is widely used in folk medicine in diseases of gastrointestinal tract (GIT) as dietary product, included to the composition of dietary supplements (DS) - special food products, produced in Ukraine and abroad; "Brokkofit"(LLC" Pharmaceutical company "Vertex," Ukraine) and "Green Super Formula" (LLC "Pharmacological union of L. Pasteur," Russian Federation) - DS of general strengthening action; "Equalin"("Enrich International Inc.," USA) - DS reducing the risk of inflammatory and ulcerative processes development in GIT; "Indol-3-carbinol" ("Nittany Pharmaceuticals Inc.," USA) - DS of general strengthening action; "LOCLO" ("Nature's Sunshine Products Inc., USA") - DS improving the processes of digestion and the functional condition of GIT; "multivitamin/mineral" ("Enrich International Inc., USA) - DS source of vitamin-mineral complexes with amino acids and/or other components; and "Royal-2" ("Royal BodyCare International Inc.," USA) - DS, improving the process of digestion and functional condition of GIT.[6-8]

Glucosinolates make at some types of cabbage, up to 1% of dry residue. In 100 g of fresh broccoli contains 50–100 mg of glucosinolates, at this broccoli sprouts contain ten times more, then a mature plant. Glucosinolates also disintegrate 35–60% at heating. Indole compounds forming at the decomposition possess lower anticarcinogenic effect, than those contained in fresh broccoli.[9,10]

Biological activity of food indoles is due to their ability of inducing the activity of monooxygenase system and some enzymes of the second phase of metabolism of xenobiotics (glutathione transferase). Phytoestrogenic action of indole compounds - indole-3-carbinol is manifested in the prophylaxis of hormone-dependent types of breast and prostate cancer, as this glucosinolate affects the exchange of estrogenic substances in the body. It is able to inhibit the activity of androgenic and estrogen receptors, which play an important role in the pathogenesis of benign prostatic hyperplasia.[6] There was a significant correlation established between the high incidence of broccoli cabbage consumption and the incidence of stomach and duodenal ulcers, as well as small and large intestine tumors, a reduction in the risk of developing breast and uterine tumors in women and prostate cancer in males.[11,12]

Considering the etiopathogenesis of prostatitis and adenoma of the prostate gland, as an anti-inflammatory and anesthetic, we have proposed to use a non-steroidal anti-inflammatory drug meloxicam.[3-5]

It is known that the quantification of APIs in the combination drugs is one of the most difficult tasks of pharmaceutical analysis. [13] The aim of this study was the development of a methodology for the quantitative determination of meloxicam and indole-3-carbinol in the developed rectal suppositories, which would allow the effects of the matrix, placebo, and accompanying impurities on the course of the determination to be cutoff.

MATERIALS AND METHODS

Indole-3-carbinol (Sigma-Aldrich Co., USA) and meloxicam **(**Boeh4inger Ingelheim GmbH, Germany) as well as developed rectal suppositories with a combination of given APIs in the amount for 1 suppository: Indole-3-carbinol - 0.2 g and meloxicam - 0.0075 g were used.

In carrying out the studies, meloxicam PRS was used, corresponding to the requirements of the European pharmacopoeia. To determine the content of indole-3-carbinol, a reagent with a purity of at least 99% was used.

The measurements were carried out by high-performance liquid chromatography (HPLC) according to SPU, supp. 1, 2.2.29, N[14,15] using the following equipment: A 2695 chromatograph with a diode-array detector 2996 by Waters Co., USA, and scales ER-182 by A&D, Japan, measuring dishes of Class A.

Before the main validation tests, the presence of documents demonstrating the suitability of the equipment, raw materials, and reagents used was monitored.

The validation of the methodology was carried out in accordance with the requirements of SPU.[14,15]

Method of Quantitative Determination

To calculate the quantitative content of meloxicam and indole-3-carbinol, the homogeneity values obtained for the 10 samples studied are used.

The quantitative content of meloxicam and indole-3-carbinol in grams (X), in one dosage unit of the test preparation, is calculated by the formula:

$$x = \frac{\Sigma xi \cdot a}{10 \cdot 100}$$

Where,

$\sum x_i$ - the value of the sum of the homogeneity values obtained for 10 samples of meloxicam or indole-3-carbinol tested, in percentage.

a - the content of meloxicam or indole-3-carbinol in the preparation, in gram.

The content of $C_{14}H_{13}N_3O_4S_2$ (meloxicam) in one dosage unit of the test preparation should be from 0.00675 to 0.00825 g.

The content of C_9H_9NO (indole-3-carbinol) in one dosage unit of the test preparation should be from 0.18 to 0.22 g.

Determination of Uniformity of Dosage

The test is conducted in parallel for 10 samples.

One dosage unit of the test drug was placed in a 50 ml beaker. 50.0 ml of dimethylformamide P was added and held in an ultrasonic bath for 15 min at 60°C until the dosage form completely disintegrated. The resulting solution was cooled and quantitatively with three portions of dimethylformamide P 10.0 ml each transferred to a 100.0 ml volumetric flask, and the volume of the solution was taken to the mark with dimethylformamide P and mixed. The resulting solution was filtered through a POR-16 glass filter, discarding the first 5 ml of the filtrate (test solution for quantitation).

The measurements were carried out by liquid chromatography as described below.

The total standard sample solution was chromatographed, obtaining from 2 to 6 chromatograms. The volume of injection was 20 µl. For the peak areas of meloxicam and indole-3-carbinol, the relative standard deviation (RSD) was calculated from the resulting chromatograms.

The production of parallel chromatograms (n_0) was stopped when the requirements for (RSD) specified in SPU 2.2.46, N, Table 2.2.46–2 were reached.[14,15]

The test solution and the solution of the total reference sample were alternately chromatographed, obtaining the number of parallel chromatograms (n) for each of the solutions no less than when testing the suitability of the chromatographic system under the following conditions:

- A column of size 250 mm × 4.6 mm, filled with an octadecylsilica gel, a particle size of 5 µm (Symmetry Shield C18, Waters Corp.);
- Mobile phase A: 0.5 ml of formic acid (conc.) was mixed with 500 ml of water, degassed by any convenient method;
- Mobile phase B: 0.5 ml of formic acid (conc.) was mixed with 250 ml of acetonitrile, degassed by any convenient method;
- The elution mode is gradient, according to the following scheme:

Time, min	Mobile phase A	Mobile phase B	Elution mode
0–20	80→35	20→65	Linear gradient
20–21	35→80	65→20	Linear gradient
21–30	80	20	Isocratic

- The wavelength is 270 nm;
- Flow rate - 1 ml/min;
- The temperature of the column thermostat is 50°C;
- Injection volume 20 µl.

The order of release of the substances to be determined: (1) systemic peaks, (2) meloxicam (about 15 min), and (3) indole-3-carbinol (about 17.5 min).

The content of meloxicam and indole-3-carbinol in percent (X), in one dosage unit of the test preparation, was calculated by the formula:

$$x = \frac{S_i \cdot m_o \cdot 100 \cdot P \cdot 100}{S_o \cdot 1 \cdot 100 \cdot 100 \cdot a}$$

Where

- S_i - the average peak area of meloxicam or indole-3-carbinol, calculated from the chromatograms of the test solution of the preparation;
- S_0 - the mean value of the peak area of meloxicam or indole-3-carbinol, calculated from the chromatograms of the TRS solution;
- m_0 - the weight of the sample of meloxicam or indole-3-carbinol in TRS, gram;
- P - the content of meloxicam or indole-3-carbinol in TRS, percentage;
- a - the content of meloxicam or indole-3-carbinol in the preparation, gram.

The acceptance value ARV (SPU 1.2, Table 2.9.40–2) was calculated using the case 1 for calculating the reference M.[14,15]

The requirements are considered fulfilled if the acceptance value for 10 samples of the preparation is ≤15% or the final acceptance value calculated for 30 samples is ≤15 and any individual value of the content in one dosage unit of the test drug is at least (1–0.25)*M and not more than (1+0.25)*M.

The results of the analysis are considered reliable if the requirements of the "chromatographic system suitability test" are met.

Notes:

1. Preparation of TRS solution: 0.02 g (accurate sample) of meloxicam (EPCRS) and 0.1 g (exact sample) of indole-3-carbinol (RS SPU) are placed in a 100.0 ml volumetric flask, dissolved in 50.0 ml of dimethylformamide P brought to the mark with the same solvent and stirred. The solution is used freshly prepared.
2. Checking the suitability of the chromatographic system: A chromatographic system is considered suitable if the following conditions are met:
   - The efficiency of the chromatographic column, calculated from the peak of meloxicam on the chromatogram of TRS, should be not <15000 theoretical plates (SPU 2.0);[15]
   - The peak symmetry factor calculated from the

peak of meloxicam or indole-3-carbinol from the chromatograms of the TRS solution should be no more than 1.5 (SPU 2.0).[15]

RESULTS AND DISCUSSION

The validation of the developed method was carried out, and its suitability for simultaneous quantitative determination of both meloxicam and indole-3-carbinol in a dosage form has been proven.

Under the above conditions, chromatograms of the test solution and the reference solution and blank were obtained. The order of the substances release was observed as follows: (1) Meloxicam and (2) indole-3-carbinol. A typical chromatogram of the detected substances is shown in Figure 1.

The results of the quantitative determination of meloxicam and indole-3-carbinol in the developed preparation according to the above procedure testify to its reproducibility.

The validation of the procedure for the quantification of meloxicam and indole-3-carbinol was carried out according to individual validation characteristics: Specificity, linearity, repeatability, precision, accuracy, and intralaboratory precision.

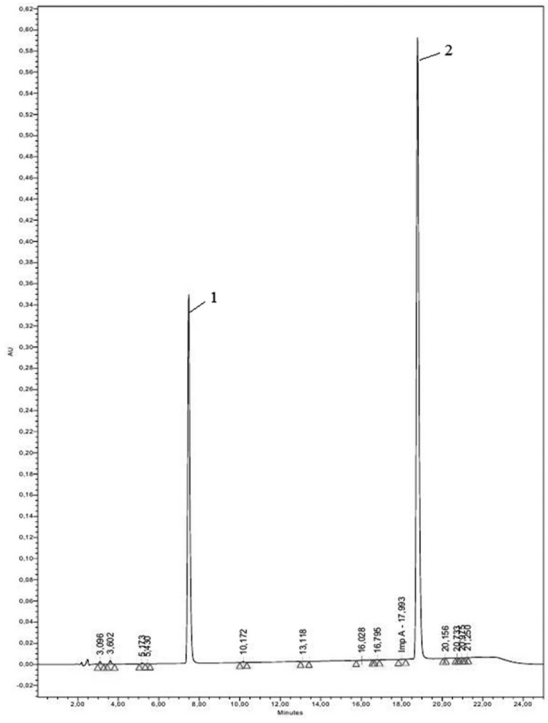

Figure 1: Chromatogram of the reference solution: 1 - meloxicam and 2 - indole-3-carbinol

To estimate the error in sample preparation of model solutions and a standard sample solution, theoretical values of the uncertainty of the analytical operation were calculated, which amounted to Δ_{sp} = 0.5%.

During the validation, it was proved that sample preparation has insignificant effect on the result of quantitative determination; therefore, sample preparation is not analyzed below.

Since the HPLC method used in the technique is specific, it is sufficient to fulfill all the requirements for linearity, repeatability, precision, accuracy, and intralaboratory precision criteria to prove the specificity of the technique.

The linearity was evaluated over the entire range of the method application by the standard method. The study of the nature of the optical density dependence on the concentration was carried out using 9 model solutions for analysis with concentrations of 80, 85, 90, 95, 100, 105, 110, 115, and 120%. The results of the linear dependence of the peak area on the concentration of indole-3-carbinol and meloxicam are shown in Figures 2 and 3, respectively. Characteristics of the linear dependence for indole-3-carbinol and meloxicam are presented in Tables 1 and 2.

The results were statistically processed by the least squares method according to SPU requirements.[15]

The requirements for the parameters of linear dependence in our case are satisfied on the whole range of the technique application (80–120%).

The Graph of the Dependence of the Given Values $S_r = a+b*C_r$

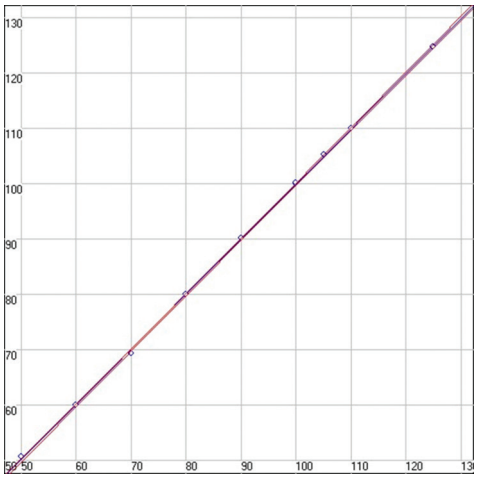

Figure 2: The linear dependence of the peak area on the concentration of indole-3-carbinol in normalized coordinates

The Graph of the Given Values Dependence $S_r=a+b*C_r$

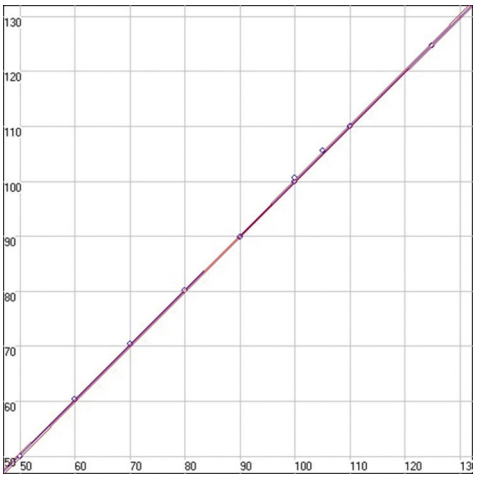

Figure 3: The linear dependence of the peak area on the concentration of meloxicam in normalized coordinates

To carry out measurements and calculate the metrological evaluation of the repeatability and accuracy of the procedure, three values of peak areas for the reference solution and 27 peak areas for model solutions were obtained. The actual values (Xi_{fact}) were calculated, the ratios of the average peak areas for each of the 27 solutions to the average peak area of the reference solution, yielding the values $X_i = (C_i/C_{st})\cdot 100\%$, $Y_i = (S_i/S_{st})\cdot 100\%$, and the value of $Z_i = (Y_i/X_i)\cdot 100\%$, which is the concentration found as a percentage of the input. The results of calculations for the investigated APIs are given in Tables 3 and 4.

To assess the intralaboratory precision, a relative confidence interval was used for five parallel determinations of the quantitative content of substances, which should be less than the maximum permissible uncertainty of the analysis results: $\Delta_z \leq 1.6$ %. The tests were carried out using one series of the drug by different analysts on the same chromatograph on different days using different measuring utensils.

Intralaboratory precision was confirmed by the fact that the value of the relative confidence interval for five parallel determinations of one series of the drug satisfies the eligibility criterion.

According to the developed technique, the quantitative determination of meloxicam and indole-3-carbinol in the composition of rectal suppositories has been studied. The following results were obtained: With the content of $C_{14}H_{13}N_3O_4S_2$ (meloxicam) in one dosage unit of the test preparation is 0.0078 g and the content of C_9H_9NO (indole-3-carbinol) - 0.205 g, which indicates the validity of the proposed procedure for the quantification of active substances in suppositories for the prevention and treatment of benign prostatic diseases.

Table 1: Characteristics of linear dependence for indole-3-carbinol

Parameter	Value	Requirements 1	Requirements 2	Conclusion
b	0.99893827			
Sb	0.0044602136			
a	|0.10837913|	<=|0.76126664	<=|1.1538051|	Are maintained on the first criterion
Sa	0.40939319			
RSD_0	0.31664376			
RSD_0/b	|0.3169803|	<=|0.86044636|		Maintained
RSD_y	26.589123			
r	|0.99992909 |	>|0.99947625 |		Maintained

Student (95, 1, 8) = 1.8595. Conclusion: The linearity requirements are maintained. RSD: Relative standard deviation

Table 2: Characteristics of linear dependence for meloxicam

Parameter	Value	Requirements 1	Requirements 2	Conclusion
b	0.99790381			
Sb	0.0043819478			
a	|0.3399173|	<=|0.74790827|	<=|1.1538051|	Are maintained on the first criterion
Sa	0.40220934			
RSD_0	0.31108743			
RSD_0/b	|0.3117409|	<=|0.86044636|		Maintained
RSD_y	26.589123			
r	|0.99993155|	>|0.99947625|		Maintained

Student (95, 1, 8) =1.8595. Conclusion: The linearity requirements are maintained. RSD: Relative standard deviation

Table 3: The results of analysis of model solutions of indole-3-carbinol and their statistical processing

Test solutions	Name	Average S_i	C_i	S_{ir}	C_{ir}	d_i
1	RS	3311730	100	100	100	100
2	M1	1674320	50	50.557262	50	101.11452
3	M2	1984238.5	60	59.915467	60	99.859112
4	M3	2297405	70	69.371748	70	99.102498
5	M4	2646979.5	80	79.927394	80	99.909243
6	M5	2983807.5	90	90.098151	90	100.10906
7	M6	3316020.5	100	100.12955	100	100.12955
8	M7	3483734	105	105.19378	105	100.18455
9	M8	3645820	110	110.08808	110	100.08007
10	M9	4134940.5	125	124.85742	125	99.885933

Table 4: The results of analysis of model solutions of meloxicam and their statistical processing

Test solutions	Name	Average S_i	C_i	S_{ir}	C_{ir}	d_i
1	RS	2482945.5	100	100	100	100
2	M1	1238473.5	50	49.879206	50	99.758412
3	M2	1499375.5	60	60.386968	60	100.64495
4	M3	1747948	70	70.398162	70	100.5688
5	M4	1990958	80	80.185328	80	100.23166
6	M5	2231055	90	89.855174	90	99.839082
7	M6	2496673.5	100	100.55289	100	100.55289
8	M7	2620227	105	105.52898	105	100.50379
9	M8	2731419	110	110.00721	110	100.00655
10	M9	3097217.5	125	124.73965	125	99.791719

CONCLUSIONS

1. In the course of the study, a HPLC technique with a gradient elution mode has been developed.
2. It has been established that the selected chromatographic conditions allow not only quantitatively determine the substances under investigation in the finished dosage form but also estimate the uniformity of their distribution.
3. The validation of the developed method has been carried out, and its suitability for simultaneous quantitative determination of both meloxicam and indole-3-carbinol in a dosage form was proved.
4. The reproducibility of the above procedure has been proved, in which conditions meloxicam and indole-3-carbinol were analyzed for the studied preparation in the form of suppositories for the prevention and treatment of benign prostatic diseases.

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Source of Support: Nil. **Conflict of Interest:** None declared.