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Validation of the Analytical Method for the Determination of Dexketoprofen Tromethamine as a Residual Substance. Specificity, Accuracy, Linearity, Repeatability, Detection Limit and Quantitation Limit of the Method

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Annotation: In this article, validation of the developed new analytical method by High Performance Liquid Chromatography (HPLC) method was carried out, which developed for determination of the dexketoprofen tromethamine as residual substance in the injectable pharmaceutical plant during the cleaning validation. First of all, all products of injectable plant were divided to groups according to their pharmacology and chemical characteristics. Then Maximum Allowable Carryover (MAC or MACO) was calculated for all products. Target of the scientific work was developing new analytical methods for the non-steroidal anti-inflammatory injectable products in injectable plant. One of the non-steroidal anti-inflammatory injectable products was dexketoprofen tromethamine. That is why, new HPLC method was developed for determination of MACO quantity of same substance. Validation of the new developed analytical method was done according to guidelines of European medicines agency (EMEA) and international conference on harmonization of technical requirements for registration of pharmaceuticals for human use (ICH). In accordance with the requirements of the guidelines, validation was carried out according to the following parameters for the validation of analytical methods, like specificity, accuracy, linearity, repeatability, detection limit and quantitation limit of the method.

Keywords: analytical methods, validation, specificity, accuracy, linearity, repeatability, detection limit and quantitation limit, dexketoprofen tromethamine, non-steroidal anti-inflammatory products, injectable products.

1. Introduction

In nowadays, analytical methods used in the pharmaceutical industry are developing and improving day by day. New analysis methods, new analysis equipment is being developed. At the same time, it is necessary to verify that the methods of analysis being developed are used to evaluate the quality indicators of medicines directly related to human health, the reliability of these methods of analysis and whether they achieve the expected results, and to prove it in practice. Validation of analytical methods currently used in the field of pharmaceuticals is one of the main requirements of the quality standard "Good Manufacturing Practice" (GMP).
Validation of analytical methods - by conducting experimental tests of the selected method, the expected result is achieved from a particular method and the reliability of the obtained analytical results is evaluated. Instead, validation indicators are selected based on the field of application of the method. They can be the following directions:
1. Analytical methods designed to verify identification;
2. Methods developed for quantitative analysis of impurities in the composition;
3. Methods used to check the limit quantity of impurities in the composition;
4. Established methods for quantitative analysis of the main active drug substance;
5. Methods of quantitative analysis in the solubility test.
Information on analytical methods and parameters to be validated is presented in the following table:

Table 1Analytical methods and parameters to be validated

№	Parameters of validation of analytical methods	Types of analysis methods				
		Identification	**Tests for impurities (quantity)**	**Tests for impurities (limit)**	**Assay methods (dissolution)**	**Assay methods (content)**
1	Accuracy	-	+	-	+	+
2	Specificity	+	+	-	+	+
3	Linearity	-	+	-	+	+
4	Precision (Repeatability)	-	+	-	+	+
5	Precision (Interm. Precision)	-	+	+	+	+
6	Detection Limit	-	-	+	-	-
7	Quantitation Limit	-	+	-	-	-
8	Range	-	+	-	+	+

So, based on the data presented in the above table, it can be concluded that the validation indicators of analysis methods are also different depending on the field of application.
In cases where the analytical method used is used to determine the amount of impurities, these methods should be validated in terms of accuracy, specificity, linearity, repeatability, repeatability between laboratories, detection limit, quantitation limit and range of application parameters. Based on this, the special high-performance liquid chromatography (HPLC) method developed for the determination of Dexketoprofen tromethamine as a residual substance during the cleaning process was validated in the first stage according to the following parameters.
Specificity of method – specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present.

Accuracy of method – The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.
Linearity of method – The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.
Precision (Repeatability) of method – Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.
Precision (Intermediate Precision) – Intermediate precision expresses within-laboratories variations: different days, different analysts, and different equipment.

The aim of the work is validation of the HPLC method developed for the determination of the residual quantity according to the validation parameters.

2. Materials and methods

High Performance Liquid Chromatograph
- Manufacturer country – United states of America;
- Manufacturer company - Agilent Technologies;
- Model – 1200;
- Type of column – Zorbax XDB C-18;
- size of sorbent – 5 µm;
- size of column – 150 x 4,6 mm;
- new analytical method;
- 25ml, 50ml, 100ml volume laboratory dishes;
- 1ml, 2ml, 5ml pipettes;
- Analytical balances;
- Injection water;

- 0,45 µm filters;

Method details:

- ✓ Mobile phase: buffer solution of phosphates (pH=3,0) and acetonitrile 60:40 volume ratio;
- ✓ Wavelength: 210 nm;
- ✓ Flow speed: 1,5 ml/minute;
- ✓ Sample quantity: 20 µm;

Preparation of the mbile phase: 6.6 g of accurately weighed potassium dihydrogen phosphate (KH_2PO_4) is placed into 1000 ml flask. 950 ml of purified water is added to it. The solution is shaken well and adjusted to pH 3.0 using a 0.1 M solution of orthophosphoric acid (H_3PO_4). The volume of the prepared buffer solution is make up to the mark of the flask using purified water. The prepared buffer solution is mixed with acetonitrile in a ratio of 60:40. It is filtered and degassed using 0.45µ millipore filters.

Experimental part.

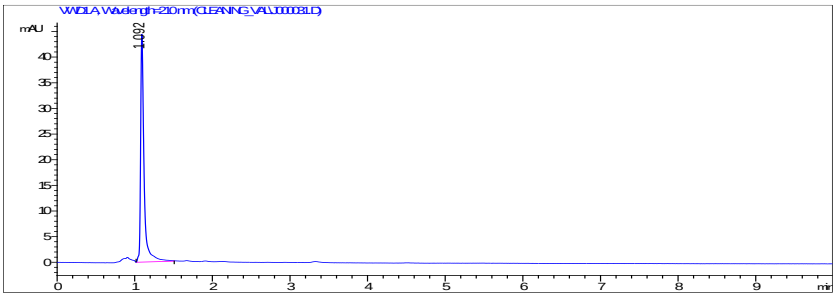
In order to validate the developed method, solutions with concentrations equal to 25%, 50%, 100%, 200% and 400% were prepared compared to the concentration to be determined (2.8µg/ml). The preparation process for each concentration is shown in Table 2.

Table 2 Information on the specific gravity and dilution value of the solutions used in the validation process

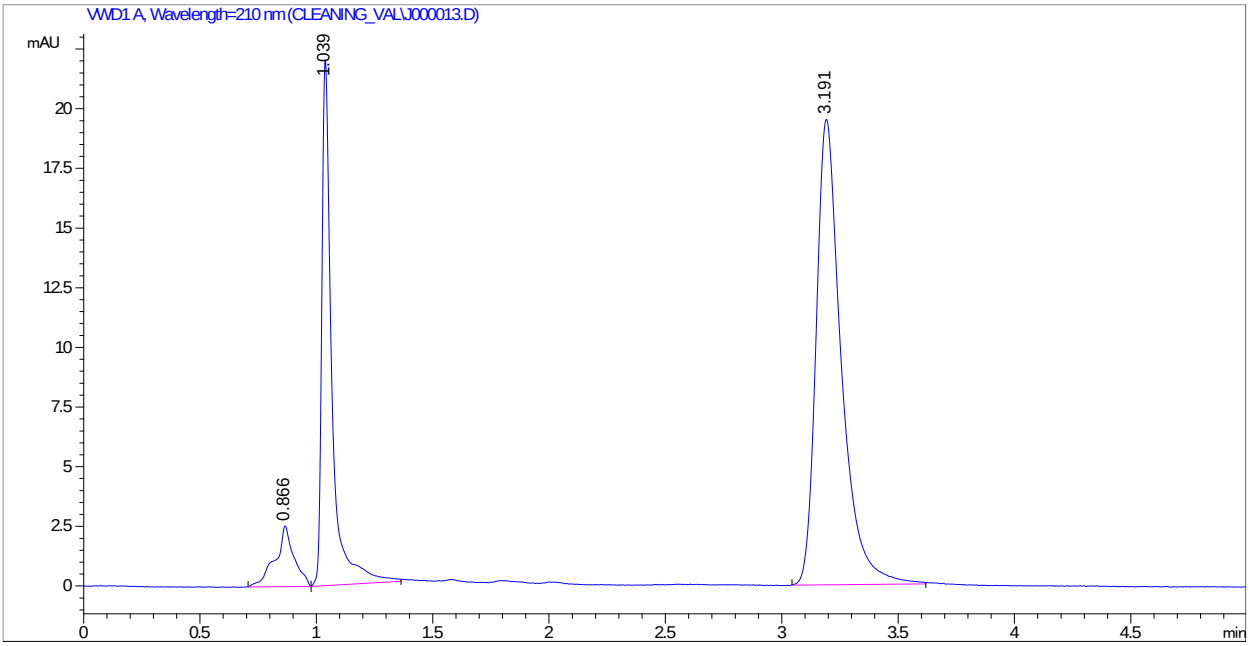
№	Concentration	The exact amount of material	Dilution level 1	Dilution level 2	Dilution level 3	Diluent
1	100% (2,8µg/ml)	700mg	till 25 ml (A solution)	0,1 ml from A solution, till 100 ml (B solution)	5 ml from B solution, till 50 ml (C solution)	Mobile phase (Buffer solution-Acetonitrile 60:40 ratio)
2	25% (0,7 µg/ml)	-	To 5 ml of C solution add 15 ml diluent	-	-	
3	50% (1,4 µg/ml)	-	To 5 ml of C solution add 5 ml diluent	-	-	
4	200% (5,6 µg/ml)		till 25 ml (A1 solution)	0,1 ml from A1 solution, till 100 ml (B solution)	10 ml from B1 solution, till 50 ml (C1 solution)	
5	400% (11,2 µg/ml)		till 25 ml (A2 solution)	0,1 ml from A2 solution, till 100 ml (B solution)	10 ml from B2 solution, till 25 ml (C2 solution)	
6	PLACEBO	Mobile phase (Buffer solution-Acetonitrile 60:40 ratio)				

"Placebo" and 100% solutions (prepared by the above method) were used to study the specificity of the developed method. Initially, a sample of the "Placebo" solution was included in the analysis. Next, a 100% concentrated solution was sent. As a result of the analysis, the following chromatograms were obtained.

Criteria for evaluating the specificity*: according to this, no peak should be observed in the chromatogram obtained from the "Placebo" solution in the interval corresponding to the retention time of the substance to be determined.*



1 – photo. Chromatogram obtained from the analysis of the "Placebo" solution



2 – photo. Chromatogram from a 100% (relative to the selected concentration) solution analysis.

As a result of the analysis, in the chromatogram obtained from the "Placebo" solution, it was observed that there was no other peak at the retention time of dexketoprofen tromethamine. The specificity of the developed method was confirmed.

In order to study the linearity of the method, 25%, 50%, 100%, 200% and 400% solutions were used.
Criteria for evaluating linearity: The correlation coefficient determined by comparing the theoretical amounts of solutions with different concentrations to the amounts determined in practice should be in the range of 0.99-1.01.

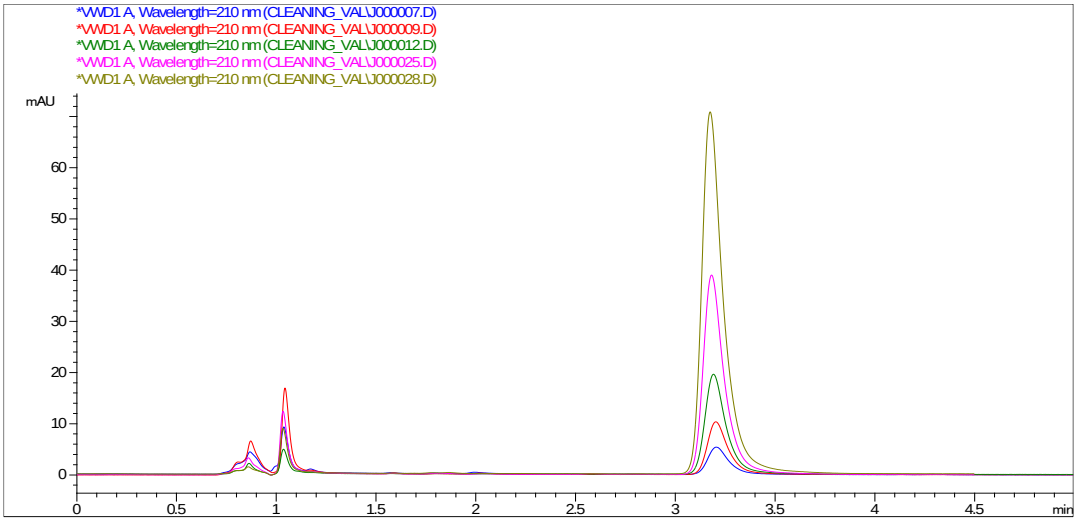
Table Results of the analysis to determine the linearity of the method

Relative equations	**Theoretically calculated concentrations**	**Practical prepared concentrations**	**The surface of the peaks in the chromatogram**
25%	0,7 µg/ml	0,7001 µg/ml	41,37209
50%	1,4 µg/ml	1,4002 µg/ml	77,17941
100%	2,8 µg/ml	2,8004 µg/ml	145,76787
200%	5,6 µg/ml	5,6008 µg/ml	294,94766
400%	11,2 µg/ml	11,2016 µg/ml	541,95709

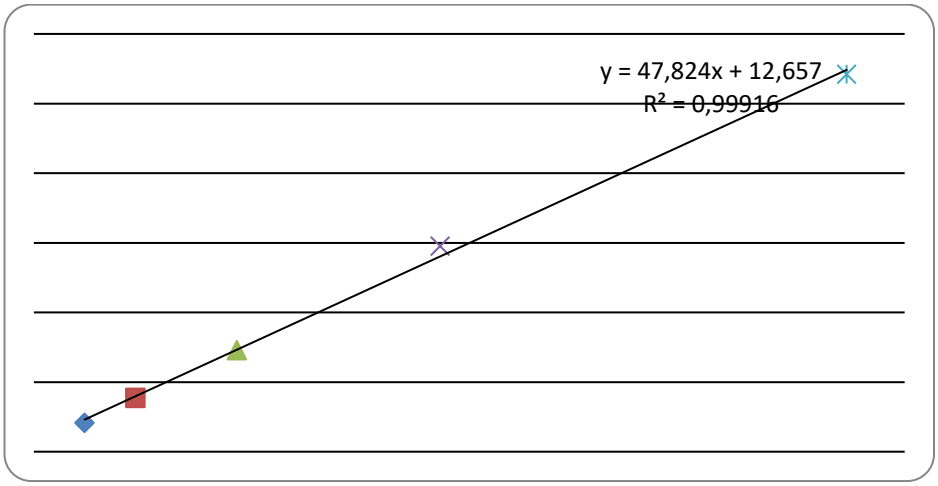
Based on the obtained results, a graph was drawn of the ratio of the concentration of the solutions to the areas of the peaks in the chromatograms obtained from their analysis. A correlation coefficient was calculated based on the results.

Table 4 Linearity parameters

Range of concentrations	**Correlation coefficient**	**The slope of the calibration curve**	**Intersection**
0,7 – 11,2 µg/ml	0,99916	47.82	12.65



3 – photo. Chromatograms of solutions of different concentrations obtained from linearity analysis.



4 – photo. A graph obtained based on the results of linearity

The correlation coefficient calculated based on the results of the analysis was equal to 0.99916. This result satisfies the requirement of the linearity criterion, and the linearity of the method is confirmed.

Separate 50%, 100%, and 200% solutions were prepared by two researchers to study the accuracy of the method. Each prepared solution was quantitatively analyzed 3 times. The precision of the method was evaluated by comparing the peaks of dexketoprofen tromethamine solutions determined as a result of the analysis with the theoretical amounts.

Accuracy assessment criteria: When the amounts determined as a result of the analysis are considered as 100% of the theoretical amounts (individually for each solution), the results should lie in the interval from 95% to 105% (for the analysis of foreign substances).

The following tables show the results of the analysis performed to assess the accuracy of the method.

Table 5Accuracy parameters

Theoretical concentration	Researcher 1		Researcher 2	
	Surface of peak	Percentage of accuracy	Surface of peak	Percentage of accuracy
50%	76,91942	95,99%	75,99965	97,15%
	77,17941	95,67%	76,59843	96,39%
	76,84325	96,10%	77,12985	95,73%
100%	146,22124	100,99%	145,74892	101,32%
	146,76518	100,62%	145,76787	101,30%
	147,22441	100,30%	146,86026	100,55%
150%	210,97513	104,99%	212,49823	104,24%
	211,80757	104,58%	213,16598	103,91%
	210,99655	104,98%	211,95468	104,50%

The results of the analysis revealed that the accuracy (accuracy level) of the method was in the range of 95-105% at different concentrations, thereby confirming the accuracy of the method.
In order to study the reproducibility (reproducibility) of the method, 100% (relative to the selected concentration) solutions were used.
Reproducibility (reproducibility) evaluation criteria: the relative standard deviation of the results obtained from several analyzed (in most cases, it consists of 10-fold analysis) analysis should not exceed 2% in order for the analytical method being validated to be satisfactorily evaluated according to the reproducibility indicator.

Table 6 Repeatability parameters

№	Concentration	Test solution peak surface	Weight	RSD
1	100%	146,86026	0,7001 g	0,901523%
2		147,22441	0,7001 g	
3		146,76518	0,7001 g	
4		146,22124	0,7001 g	
5		147,66826	0,7001 g	
6		148,59988	0,7001 g	
7		145,76787	0,7001 g	
8		145,24635	0,7001 g	
9		145,74892	0,7001 g	
10		149,43343	0,7001 g	

The results of the analysis show that the relative standard deviation of the method does not exceed 2%. In this case, the relative standard deviation is 0.901523%. This proves that the method is reproducible.

3. Conclusion.

Based on the results of the experimental experiments, the specificity, accuracy, linearity and reproducibility of the HPLC method developed for determining the residual amounts of the dexketoprofen tromethamine drug substance were confirmed by validation, and given that the obtained results fully correspond to the requirements of the validation criteria, the determination of the residual amount of the dexketoprofen tromethamine drug substance in the validation of the purification processes of this method was found suitable for use.

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