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Development and Validation of a HPLC Method for Chlorphenamine Maleate Related Substances in Multicomponents Syrups and Tablets

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Research Article

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Abstract

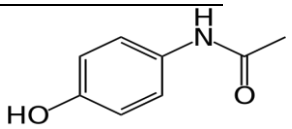
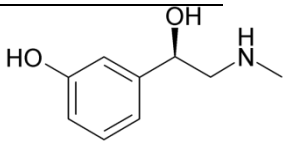
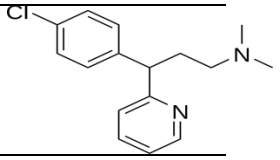
Chlorphenamine maleate is a first-generation alkylamine antihistamine used in the prevention of the symptoms of allergic conditions such as rhinitis and urticaria. Because of that it is frequently used in multicomponents pharmaceutical formulations against the cold. Since the method for determination chlorphenamine related substances in pharmaceutical products is not described in current pharmacopoeias, the aim of this work was to develop and validate a precise, accurate and robust method. HPLC method for determination related substances of chlorphenamine maleate has several problems, how to separate all active ingridients and their impurity in this formulation. Also, problem was to separate placebo in syrup because of presented flavor. Separation was achieved on a reversed-phase C-18 column (5 µm, 25 cm x 0.46 cm, kept at 30°C), using a mixture of potassium dihydrogen phosphate and octane sulphonate sodium salt in water and acetonitrile as mobile phase, at flow rate 1.0 ml/min and UV detection at 214 nm. The method was validated following ICH guidelines and validation parameters showed that it could be used as stability indicating method for determination of related substances of chlorphenamine maleate in multicomponents syrups and tablets.

Keywords: *Chloprphenamine maleate; HPLC; Multicomponents syrups; Multicomponents tablets; Related substances*

Introduction

Determination of related substances in multicomponents pharmaceutical formulations is very difficult job. Analytical method has to ensure good separation of active compounds and their degradation products. Especially, problem is big amount of anti-inflammatory active compounds with regard to antihistaminics such as case in our formulations with chlorphenamine maleate. Also, problem is how to separated placebo, especially presented flavor in placebo of multicomponents syrups. All these compound, active and placebo, have different properties, and they have to be separated, and this method has to be developed as stability-indicating method. The aim of this study was the development and validation according to ICH quidelines of a HPLC method for determination of related substances of chlorphenamine maleate in pharmaceutical formulation such as multicomponents syrups and tablets, including good separation between all active ingridients and excipients [1, 2, 3, 4, 5].

Table 1: Chemical structures of the assayed compounds

Name	Molecular form	Molecular wieght	Structural form
Acetaminophen	$C_8H_9NO_2$	151.17	
Phenylephrine	$C_9H_{13}NO_2$	167.21	
Chlorphenamine	$C_{16}H_{19}ClN_2$	274.99	

Material and Method

Standards of acetaminophen, phenylephrine and chlorphenamine maleate are all of 99% purity. Chlorphenamine maleate Impurity A and Impurity B

were LGC referents standards and Chlorphenamine maleate Impurity C was Ph. Eur. referent standard. Chemicals, acetonitrile was from J. T. Baker, gradient grade, and other chemicals, potassium dihydrogen phosphate, octane-1-sulphfonate sodium salt, hydrochloric acid 37%, sodium hydroxide pellets, hydrogen peroxide 30% and maleic acid were from Merck, pro analysi grade. Water was purified with a Milli-Q system from Millipore. The HPLC system was Waters Acquity UPLC H-class. All excipients used in our formulation are tested according their monograph and they meet the specifications and quality.

Apparatus and chromatographic conditions

In HPLC method we used column Gemini C-18 5 µm, 25 cm x 0.46 cm kept at 30°C. The mobile phase content 3.4 g potassium dihydrogen phosphate and 1.5 g octane sulphonate sodium salt in 450 ml of water mixed with 550 ml of acetonitrile. Flow rate was 1.0 ml/min. UV detection was performed at 214 nm. Injection volume was 50 µl. Solvent solution for standard, resolution and sample solution was water / acetonitrile, 80:20 (v:v).

Standards and sample preparation

According to maximum daily dose, specification limits for known impurity A, B and C are 0.5%, for unknown 0.2% and total impurity 1.0%. From Active Substances Master File (ASMF), only chlorphenamine maleate Impurity C is degradation impurity. Since chlorphenamine maletae Impurity C is only degradation impurity, we prepared resolution solution which contains 0.1 mg/ml chlorphenamine maleate and 0.001 mg/ml chlorphenamine maleate impurity C. Request was to have resolution between these two peaks minimum 1.5. In this solution we also have impurity A and B in same concentration (0.001 mg/ml) to confirm their relative retention times. For chlorphenamine maleate standard stock solution 10 mg were made up 100 ml solvent solution. For chlorphenamine maleate reference solution pipetted 1 ml of standard stock solution in 100 volumetric flask and dilute with solvent solution to volume. Test solution for multicomponent tablets weighed mass of tablet powder containing 1 mg of chlorphenamine maleate in 10 ml volumetric flask, dissolve in ultrasonic bath for 15 minutes and dilute with solvent solution to volume, centrifuge solution at 4000 rpm for 10 minutes, and filtrate solution through 0.45 µm nylon filter discarding first ml of filtrate. Test solution for multicomponent syrup was performed pipetted 5 ml of syrup (5 ml contains 1 mg of chlorphenaime maleate) in 10 ml volumetric flask and dilute with solvent solution to the volume.

Validation

The selectivity was tested by running solution containing all excipients for tablets and for syrups in the same concentrations and conditions like samples. We have achieved good separation of peaks and good selectivity because all excipients eluated to about 5 minutes and chlorphenamine about 21 minutes.

Table 2: Order of eluation peaks

Name	Relative retention time
Placebo for multicomponents tablets	0.10
Placebo for multicomponents syrups	0.11
Maleic acid	0.11
Acetaminophen	0.13
Phenylephrine	0.14
Impurity B	0.27
Impurity A	0.48
Chlorphenamine	1.0
Impurity C	1.1

We also did forced degradation of standard solution, placebo solutions and sample solutions. Forsed degradation was carried out by oxidation of the solution (with 3% hydrogen peroxide, kept 2 hours at 90°C), alkaline hydrolysis of the solution (with 1 mol/l sodium hydroxide solution, kept 2 hours at 90°C), acid hydrolysis of the solution (with 1 mol/l hydrochloric acid solution, kept 2 hours at 90°C), thermal decomposition of the solution (with solvent solution, kept 2 hours at 90°C), thermal decomposition at solid state (heating to early melting) and photolysis of the solution (solution exposed to daylight for 72 hours).

The linearity was tested by preparing standard solution of chlorphenamine maleate and chlorphenamine impurity C from 5 to 200% of the target analyte concentration. We also did response factor for chlorphenamine impurity C in target concentration on six injections. The linearity of the sample solutions was also tested in the same concentrations as linearity for standard solutions.

The accuracy of the method was tested by applying it to mixture of chlorphenamine maleate and excipients by triplicate in three levels (80, 100 and 120%). The percent recovery, RSD and confidence limit are calculated. The precision was tested like system repeatability and method repeatability. In system repeatability we tested by running 6 replication of test solution in target concentration. In method repeatability we tested 6 different sample solutions in target concentration. We also tested reproducibility on two different batches of tablets and syrups, analysing from two different analysts. The limit of detection (LOD) is the lowest concentration of analyte that we can detect and it was 3 times the noise level. The quantification limit (LOQ) is the lowest concentration of analyte that can be accurately and precisely measured and it was 10 times signat-to-noise ratio. The robustness of the

method was tested by changing the UV detection, flow rate, column temperature and mobile phase ratio

Results and Discussion

In Figure 1 and 2, we can see very good separation between all components presented in multicomponent syrups and tablets. We also have good resolution between chlorphenamine and impurity C (see figure 3). Some of the characteristics of column are that offer extended lifetime under extreme pH conditions and excellent stability for reproducible, high efficiency separations. Also, take full advantage to high and low pH conditions (pH 1-12) to manipulate selectivity, and it is ideal for analytical and preparative separations of basic and acid components [4]. We can also observe that octane sulphonate sodium salt as ion pairing reagens was needed to ensure good separation all compounds in these conditions. Low wavelength was necessary to get enough sensitivity for small concentration of chlorphenamine maleate in our formulations. Main validations parameters are shown in Tables 3, 4, 5 and 6.

Table 3: Linearity and range

Parameters	**Results**
Standard linearity for chlorfenamine maleate	
Linearity range (%)	5-200
Linearity range (mg/ml)	0.000053-0.00214
Intercept (%)	1.00
Slope	6998.8456
r	0.9998
LOD (%)	0.04
LOQ (%)	0.14
Sample linearity for chlorfenamine maleate	**Results**
Linearity range (%)	5-200
Linearity range (mg/ml)	0.000052-0.00208
Intercept (%)	1.06
Slope	6961.9793
r	0.9998
LOD (%)	0.05
LOQ (%)	0.16
Standard linearity for chlorfenamine impurity C	**Results**
Linearity range (%)	5-200
Linearity range (mg/ml)	0.000059-0.00235
Intercept (%)	0.42
Slope	70767.7069
r	0.9995
LOD (%)	0.07
LOQ (%)	0.22

Table 4: Accuracy and recovery

Standard chlorphenamine maleate	**Results**
RSD (%)	1.1
Sample chlorphenamine maleate	**Results**
Mean Recovery (%)	99.93
Bias (%)	3.12
RSD (%)	1.58

Table 5: Precision

System repeatability	**Results**		
RSD (%)	2.91		
Method repeatability	**Results**		
Recovery (%)	103.47		
RSD (%)	1.61		
C.L.	±1.75		
Reproducibility	**Results RSD (%)**		
	Impurity C	**Any other unknown impurity**	**Total impurity**
Multicomponent syrup batch 1 (analyst 1 and 2)	0.00	0.004	0.002
Multicomponent syrup batch 2 (analyst 1 and 2)	0.00	0.003	0.004
Multicomponent tablets batch 1 (analyst 1 and 2)	0.008	0.002	0.005
Multicomponent tablets batch 2 (analyst 1 and 2)	0.008	0.002	0.005

Table 6: Robustness

Parameter		
UV detection	**Mean Recovery (%)**	**Resolution**
Low	107.21	2.8
Target	98.09	2.8
High	105.52	2.9
Flow rate	**Mean Recovery (%)**	**Resolution**
Low	96.53	2.8
Target	98.09	2.8
High	101.45	2.7
Column temperature	**Mean Recovery (%)**	**Resolution**
Low	106.06	2.8
Target	98.09	2.8
High	108.76	2.8
Mobile phase ratio	**Mean Recovery (%)**	**Resolution**
Low	106.12	3.1
Target	98.09	2.8
High	92.78	2.5

Peak purity results (purity angle has to be less than purity threshold) for forsed degradation of standard

solution and sample solutions are presented in tables 7, 8 and 9.

Table 7: Peak purity for standard solution of chlorphenamine maleate

Forced degradation conditions	Purity angle	Purity threshold
Oxidation of the solution	0.046	0.255
Acid hydrolysis of the solution	0.102	0.279
Alkaline hydrolysis of the solution	0.106	0.281
Thermal decomposition of the solution	0.091	0.274
Thermal decomposition at solid state	0.089	0.274
Photolysis of the solution	0.074	0.277

Table 8: Peak purity for sample solution of multicomponent tablets

Forced degradation conditions	Purity angle	Purity threshold
Oxidation of the solution	0.050	0.256
Acid hydrolysis of the solution	0.049	0.257
Alkaline hydrolysis of the solution	0.051	0.258
Thermal decomposition of the solution	0.049	0.255
Thermal decomposition at solid state	0.050	0.244
Photolysis of the solution	0.037	0.248

Table 9: Peak purity for sample solution of multicomponent syrups

Forced degradation conditions	Purity angle	Purity threshold
Oxidation of the solution	0.044	0.268
Acid hydrolysis of the solution	0.048	0.297
Alkaline hydrolysis of the solution	0.062	0.269
Thermal decomposition of the solution	0.045	0.269
Thermal decomposition at solid state	0.050	0.273
Photolysis of the solution	0.501	0.846

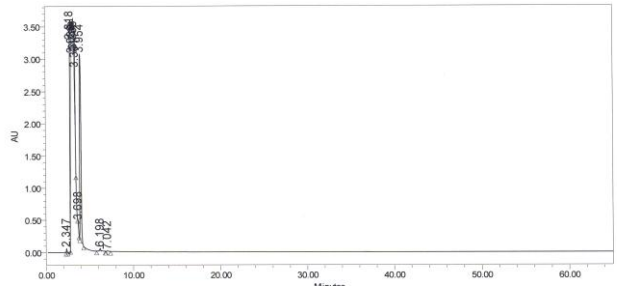


Fig. 1 Chromatogram of the placebo solution for multicomponent tablets

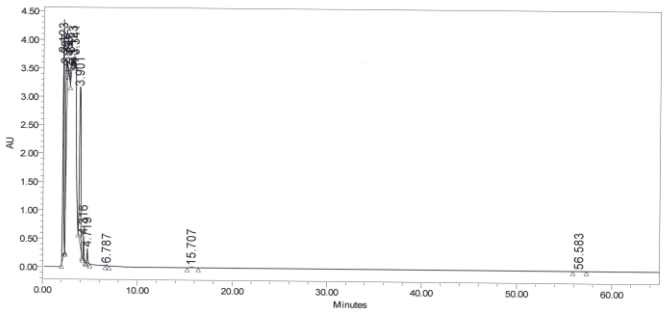


Fig. 2 Chromatogram of the placebo solution for multicomponent syrups

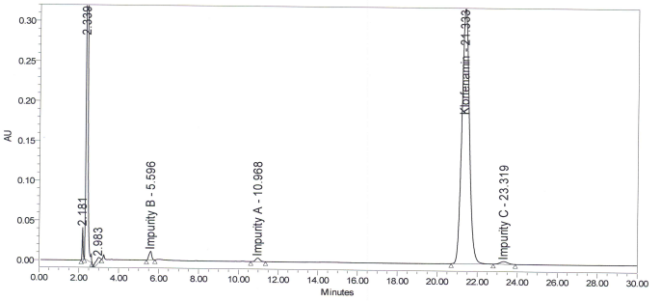


Fig. 3 Chromatogram of the resolution solution

Conclusion

HPLC method has been development and validated for determination of related substances of chlorphenamine maleate in multicomponent syrups and tablets with isocratic eluation. The method is selective, because we have very good separation between large concentrations of presented anti-inflammatory active compounds, and specially presented flavour in syrups. The method described in this study is suitable to determine concentrations of chlorphenamine maleate and chlorphenamine maleate impurity C in range 0.00005 to 0.002

mg/ml. These parameters showed a good linearity with correlation coefficients equal to 0.999 for three solutions. We have shown that the method is robust with little change critical chromatographic parameters. Validation parameters have proved that our method can used as stability indicating method for determination of related substances of chlorphenamine maleate in multicomponent syrups and tablets.

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AUTHORS' CONTRIBUTIONS

Authors contributed equally to all aspects of the study.

PEER REVIEW

Not commissioned; externally peer reviewed.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.