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Development of Analytical Method by RP-HPLC Technique for Simultaneous Determination of Boric Acid and Chlorphenesin in Medicinal Powder

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ABSTRACT

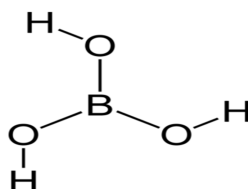
A novel reverse phase liquid chromatographic method was developed and validated for simultaneous estimation of antiseptic drug, boric acid and antifungal drug, chlorphenesin in its dosage form i.e. prickly heat powder. Refractive index detector was used for detection of eluted components. It is most suitable for quantification of boric acid because boric acid could not be detected by UV detector as it does not contain chromophore group. The reverse phase HPLC analysis was carried out on isocratic system. The column used was Inertsil C8-4 (250mm x 4.6mm, 3µm) with operating temperature at 34° C. The mobile phase consisted of water: acetonitrile in proportion 70: 30 % (v/v). The flow rate was maintained at 0.5 ml / min with detector temperature set at 34°c and sensitivity at 512. The method was validated as per ICH guidelines for system suitability, linearity, accuracy and precision. The linear ranges were 1753.2 – 5611.9 µg/ml for boric acid and 503.8 – 1612.1 µg/ml for chlorphenesin. The accuracy and precision were found to be well within the acceptable limit. The method was successfully applied for simultaneous determination of boric acid and chlorphenesin in prickly heat powder with good recoveries.

Keywords: Boric acid, Chlorphenesin, HPLC

INTRODUCTION

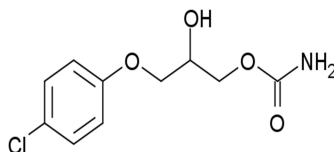
This research article provides important insight into development, validation and application of reverse phase high pressure liquid chromatographic method for the assay of boric acid and chlorphenesin in prickly heat powder. Boric acid is used as an antiseptic for minor burns or cuts and is sometimes used in dressings or salves. As an antibacterial compound, boric acid can also be used for acne treatment (Figure 1a). It exists in the form of colourless crystals or a white powder that dissolves in water.

Figure.1(a) :structure of boric acid



Chlorphenesin is a centrally acting muscle relaxant used to treat muscle pain and spasms. It is also used as antibacterial, antifungal and antiprotozoal agent in treatment of athlete's foot and other fungal and trichomonal infections of the skin administered topically. Chlorphenesin is (3-(4-chlorophenoxy)-2-hydroxypropyl) carbamate (Figure 1b).

Figure.1(b) structure of chlorphenesin



As both these ingredients i.e. boric acid and chlorphenesin are commonly used in prickly heat powder. It was a need to develop an analytical method which can accurately quantify both these ingredients simultaneously. In literature survey it was observed that no official method has been reported for simultaneous estimation of boric acid and chlorphenesin [6]. Zeng and Wang [7] have described a derivatization method which involves derivatization of boric acid with triethanolamine and then estimated it on GC/MS. Similarly Nagaoka and Matsuda[8] have described ICP-AES and ICP-MS method for estimation of only boric acid. Douglas and Stokage [9] have described a GC method for estimation of only chlorphenesin. Thus it seemed important to design a new cost effective method which could estimate both boric acid and chlorphenesin simultaneously in dosage form. In this study we report development and validation of a novel reversed phase HPLC method that allows simultaneous estimation of boric acid and chlorphenesin in prickly heat powder.

MATERIALS AND METHODS

Instrumentation

The HPLC system, Water's Alliance (2695) HPLC system equipped with separation module and refractive index detector (2414), was used. The column used was Inertsil C8-4 (250mm x 4.6mm, 3 μ m) from G. L Sciences. The chromatograms were recorded and peaks quantified by means of PC based Empower 2 software.

Materials and reagents

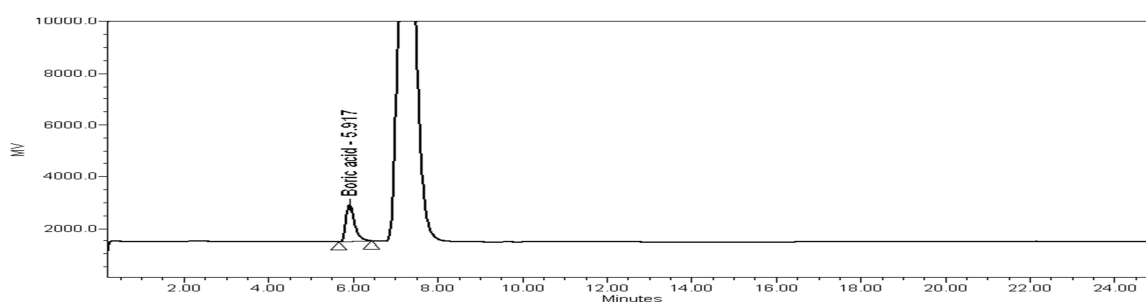
Reference standards of boric acid and chlorphenesin were obtained from reputed firms with certificate of analysis. Acetonitrile and water used were of analytical grade from Merck and the HPLC grade water was obtained by using Millipore water system.

Procedures

Standard stock solution of boric acid

About 35 mg of boric acid was accurately weighed and transferred to 10 ml volumetric flask. About 7 ml of diluent [water: acetonitrile (30: 70 % v/v)] was added to it and sonicated for 20 minutes. Further the volume was adjusted up to the mark with diluent to give concentration of 3500 μ g/ml of boric acid. A 10 μ l was injected for analysis. The typical chromatogram is given in figure 2.

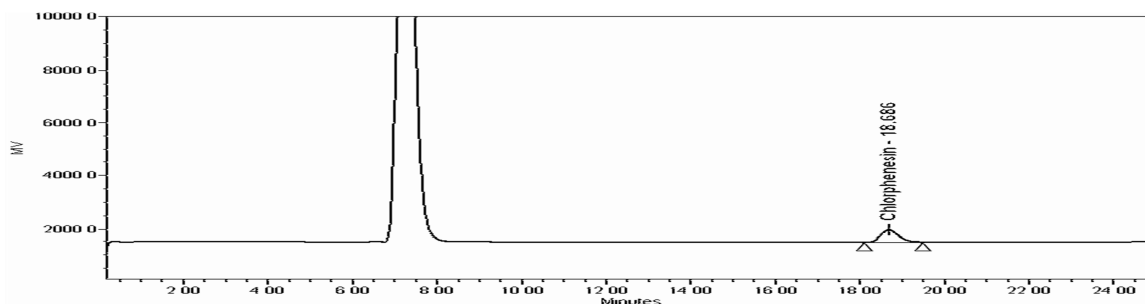
Figure.2 Typical chromatogram of Boric acid (standard stock)



Standard stock solution of chlorphenesin

About 10 mg of chlorphenesin was accurately weighed and transferred to 10 ml volumetric flask. About 7 ml of diluent [water: acetonitrile (30: 70 % v/v)] was added to it and sonicated for 20 minutes. Further the volume was adjusted up to the mark with diluent to give concentration of 1000 μ g/ml of chlorphenesin. Further 10 μ l was used for injection. The typical chromatogram is given in figure 3.

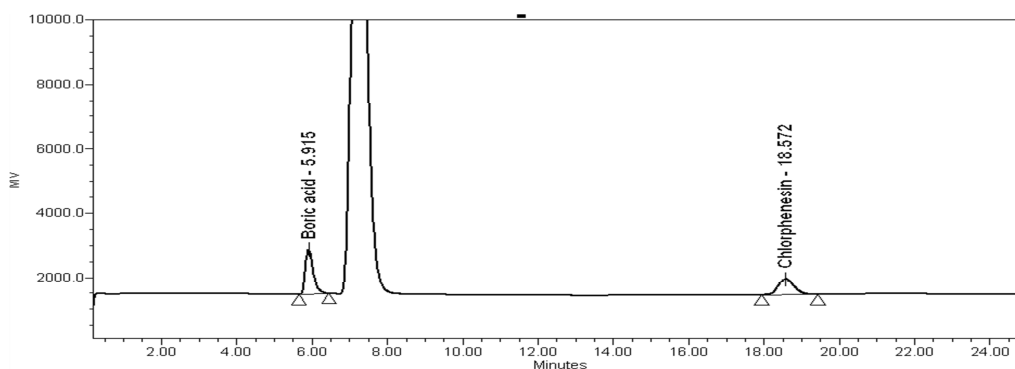
Figure.3: Chromatogram of chlorphenesin (standard)



Standard solution of boric acid and chlorphenesin

About 35 mg of boric acid and 10 mg of chlorphenesin were accurately weighed and transferred to 10 ml volumetric flask. About 7 ml of diluent [water: acetonitrile (30: 70 % v/v)] was added to it and sonicated for 20 minutes. Further the volume was adjusted up to the mark with diluent to give concentration of 3500 µg/ml of boric acid and 1000 µg/ml of chlorphenesin. Further 10 µl was injected. The typical chromatogram is given in figure 4.

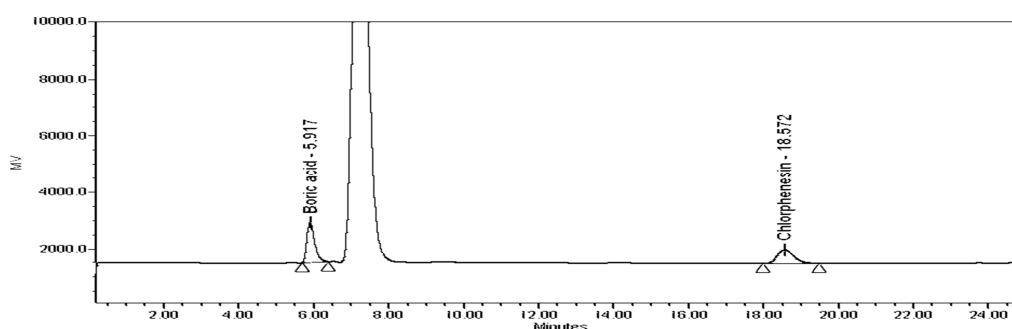
Figure.4:Chromatogram of boric acid and chlorphenesin (standard)



Sample Preparation

About 5 gm of sample i.e. equivalent to 175 mg of boric acid and 50 mg of chlorphenesin was accurately weighed and transferred to 50 ml volumetric flask, about 35 ml of diluent [water : acetonitrile (30 : 70 % v/v)] was added to it and sonicated for 20 minutes. The volume was adjusted to mark with diluent to give a concentration of 3500 µg/ml of boric acid and 1000 µg/ml of chlorphenesin. Further 10 µl was used for injection. The typical chromatogram is given in figure 5.

Figure.5 Typical chromatogram of the sample with boric acid and chlorphenesin



Chromatographic Conditions

The reverse phase HPLC analysis was carried out on isocratic system. The column used was Inertsil C8-4 (250mm x 4.6mm, 3µm) with operating temperature at 34° C. The mobile phase consisted of water: acetonitrile in proportion 70: 30 % v/v. The flow rate was maintained at 0.5 ml / min with detector temperature at 34°c and sensitivity at 512. The diluent used was water: acetonitrile (30: 70 % v/v). The injection volume of the standard and sample solutions was set at 10 µl.

RESULTS AND DISCUSSION

Chromatographic Method Development

The method development study was initiated by understanding the physico chemical properties of both the active ingredients like molecular weight, structural formula, chemical formula, pKa, UV absorptivity, solubility etc and also understanding the chemical nature of excipient matrix so that a scientific approach can be used to develop a suitable method. The structural analysis (Figure 1) revealed that it is a weak acid of boron showing polar characteristic and has no UV absorptivity as per Woodward-Fieser rules[1] as it has inorganic in nature. Hence it was not possible to develop a UV spectroscopic or a HPLC-UV method. The structural analysis of chlorphenesin (Figure.1, b) revealed that it is an homo annular conjugated organic compound which can show UV absorptivity as per Woodward-Fieser rules[5]. But as there was a need to develop a simultaneous estimation method to reduce analysis time and cost. A refractive index detector hyphenated with HPLC method was selected for this purpose. As the refractive index detector is a universal detector it was capable of detecting both boric acid and chlorphenesin in one single chromatograph. Further the chromatographic conditions were carefully evaluated so as to minimize the method development time. Hence a proper scientific approach was used for selection of various chromatographic conditions like stationary phase, mobile phase, column temperature, flow rate, detector temperature and sensitivity, injection volume and run time. From the structural formula (figure 1) it can be seen that both the active ingredients have polar functional groups, hence a C 8 column was selected as it relatively more polar than the conventional C18 column which would help in retention of both the ingredients. Different brands of columns like Hypersil-C8, Ultracarb-C 8, Waters C 8 were tried out but Inertsil C 8-4 showed excellent chromatographic behavior i.e. sufficient retention time, Gaussian peaks and resolution. The Gaussian peaks can be attributed to excellent column specifications i.e. surface area of 450 m²/gm, pore diameter of 100°A and carbon loading of 5%. A series of changes in mobile compositions were tried i.e. water: acetonitrile (40 : 60, 50 :50, 60 : 40 % v/v). Finally the composition of 70: 30 % (v/v) water: acetonitrile was selected as it gave good chromatographic pattern. This made quantification of the boric acid peak non erroneous and fit statistically. Further optimization of flow rate, injection volume, detector sensitivity and temperature, column temperature were done to get the best chromatographic pattern. On completion of the method development study, the overall possible capabilities of the analytical procedure were investigated by carrying out validation⁴ of the method. The validation characteristics like system suitability, specificity, linearity, accuracy, ruggedness and robustness were evaluated.

Method Validation

System Suitability

The integrity of any analytical procedure is subject to fulfillment of system suitability criteria. Hence system suitability was demonstrated on standard solutions of boric acid and chlorphenesin for parameters like number of theoretical plates, tailing factor and relative standard deviation. The results are shown in table 1 which indicates good performance of the system.

Table 1. System suitability parameters

Drug	Retention Time (Mins)	Symmetry factor	Number of theoretical plates
Boric acid	5.911	1.38	3816.5
Chlorphenesin	18.564	1.18	8549.85

Specificity

Specificity is the measure of analytical capabilities of a method to assess unequivocally the analyte in the presence of components which may be expected to be present. Hence blank (diluent), placebo, boric acid standard, chlorphenesin standard, mixed standard containing both the active ingredients and sample was injected individually to prove specificity. The typical chromatograms of individual boric acid and chlorphenesin standard are given in figure 2 and 3 respectively.

Linearity

A linear relationship for both the active ingredients was evaluated across the range of analytical method. To understand the degree of linearity the test data was subjected to regression analysis like correlation coefficient, residual sum of squares, y-intercept and slope. For the establishment of linearity five concentration levels were evaluated for both the active ingredients. The linear range for boric acid was found to be 1753.2 – 5611.9 µg/ml and for chlorphenesin it was found to be 503.8 – 1612.1 µg/ml. The statistical evaluation of linearity is presented in table 2.

Table 2. Statistical evaluation of the test data subjected to regression analysis

Level	Boric acid		Chlorphenesin	
	µg/ml	Response	µg/ml	Response
50 %	1753.2	10481881	503.8	7470306
80 %	2805.0	17052354	806.0	11912642
100 %	3506.3	21689955	1007.6	15246821
120 %	4208.9	26317147	1209.1	18560416
160 %	5611.9	35124508	1612.1	24677076
Correlation Coefficient (r)	0.9999		0.9998	
Residual sum of squares (r²)	0.9999		0.9996	
% Intercept (y)	-1.52		1.34	
Slope (m)	6411		15643	

Accuracy

The accuracy of the analytical procedure expresses the closeness of the experimental value to the true value. This was demonstrated by applying the proposed method to synthetic mixture containing known amount of drug corresponding to 50%, 100% and 150% of the label claim for both the active ingredients. The results of the same are presented in table 3.

Table 3. Accuracy regression analysis data

Drug	Level (%)	Amount of drug added (mg)	Amount of drug recovered (mg)	% Error	% Recovery	% RSD
Boric acid	50% - 1	1650.0	1643.8	0.38	99.6	0.74
	50% - 2	1670.0	1669.2	0.05	100.0	
	50% - 3	1676.0	1660.6	0.92	99.1	
	100% - 1	3413.2	3392.3	0.61	99.4	
	100% - 2	3443.2	3384.5	1.70	98.3	
	100% - 3	3424.6	3424.4	0.01	100.0	
	150% - 1	5391.6	5409.6	0.33	100.3	
	150% - 2	5311.6	5295.4	0.30	99.7	
150% - 3	5351.6	5399.2	0.89	100.9		
Chlorphenesin	50% - 1	562.0	557.455	-0.81	99.2	0.70
	50% - 2	552.0	549.499	-0.45	99.5	
	50% - 3	544.0	540.264	-0.69	99.3	
	100% - 1	1102.8	1116.23	1.22	101.2	
	100% - 2	1116.8	1110.66	-0.55	99.5	
	100% - 3	1118.8	1125.13	0.57	100.6	
	150% - 1	1662.2	1670.28	0.49	100.5	
	150% - 2	1638.2	1646.66	0.52	100.5	
150% - 3	1660.8	1662.67	0.11	100.1		

Precision

The degree of scatter between a series of measurements was obtained from multiple sampling (six samples) of the same homogenous batch. The relative standard deviations for boric acid and chlorphenesin were found to be 0.35% and 0.94 % respectively. The results of the same are presented in table 4

Table 4. Statistical evaluation of the test data subjected to method precision

Sample Number	Sample weight	% Assay	
		Boric acid	Chlorphenesin
1	5.00325	93.0	109.9
2	5.01452	92.2	106.9
3	5.00852	92.6	108.7
4	5.01478	92.2	108.0
5	5.02365	92.4	109.0
6	5.00145	92.6	108.3
Mean		92.5	108.5
Standard deviation		0.32	1.02
% RSD		0.35	0.94

Robustness

The robustness of the method was determined to check the reliability of an analysis with respect to deliberate variations in method parameters.

The typical variations are given below:

Variation in mobile phase composition ± 2 %

Variation in the flow rate by ± 0.2 ml/min

The results of analysis of standard solution under the variations in above parameters indicated robustness of the method.

Stability of solution

The stability of the solutions under study was established by keeping the solutions at room temperature for 24 hours. The results indicated no significant change in the assay results of the solutions. It confirmed the stability of the drug in the solvents used for the analysis.

Method application

About 5 gm of sample was accurately weighed and transferred to 50 ml volumetric flask, about 35 ml of diluent was added to it and sonicated for 20 minutes. The volume was adjusted to mark with diluent to give a concentration of 3500 $\mu\text{g/ml}$ of boric acid and 1000 $\mu\text{g/ml}$ of chlorphenesin. Further 10 μl was injected into chromatograph under specified conditions. The analyte peak was identified by comparison with that of respective standard. The percentage (%) assay results are expressed in table 4 which indicates that the amount of boric acid and chlorphenesin in the product meets the requirement.

CONCLUSION

From the relevant data collected during evaluation of the validation characteristics like system suitability, specificity, linearity, accuracy, ruggedness and robustness the statistics obtained determine the overall capabilities of the analytical procedure. The reproducibility and repeatability of the proposed method were evident from the low values of the standard deviation and % RSD. The proposed method was capable of detecting both inorganic and organic compound in a single chromatographic run thereby reducing the analysis time and cost effectively. Thus a novel method was developed and successfully applied for simultaneous determination of boric acid and chlorphenesin in prickly heat powder.

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