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Research Article

A NOVEL RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF CODIENE PHOSPHATE, CHLORPHENIRA MINE MALEATE AND ITS PRESERVATIVE IN SYRUP FORMULATION

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ABSTRACT

An accurate, sensitive, precise and robust RP-HPLC method for quantification of codeine phosphate, chlorpheniramine maleate and sodium benzoate in syrup formulation has been developed and validated. Chromatographic separation was conducted on ZODIAC C18 (150×4.6mm, 3.5µm) column at ambient temperature using phosphate buffer (pH adjusted to 2.3, adjusted with ortho phosphoric acid), acetonitrile in gradient mode at a flow rate of 1.5 ml / min, while UV detection was performed at 254 nm. The retention time for codeine phosphate, chlorpheniramine maleate and sodium benzoate was found to be 7.169, 9.480 and 10.860, respectively. . The method was found to be linear in the range of 12.5-75 µg/ml for codeine phosphate, 5-30 µg/ml for chlorpheniramine maleate and 12.5-75 µg/ml for sodium benzoate. The % recovery of codeine phosphate, chlorpheniramine maleate and sodium benzoate was found to be 98.7, 98.5% and 99.1, respectively. The developed method was validated in terms of accuracy, specificity, robustness, precision and ruggedness. This method can be successfully used for the estimation of codeine phosphate, chlorpheniramine maleate and sodium benzoate in bulk and syrup formulation.

Keywords: Codeine phosphate, Chlorpheniramine maleate, Sodium benzoate, Simultaneous estimation. RP-HPLC.

INTRODUCTION

Combinations of two or more drugs in the pharmaceutical dosage forms are very much useful in multiple therapies. Market survey revealed that, day-by-day new drugs and their combination with another drugs are being introduced in market as they have more patient compliance than a single drug[1]. The analytical chemistry hence has challenge in developing the methods for their analysis with the help of number of analytical techniques, which are available for the estimation of the drugs and their combination[2]. Analytical monitoring of pharmaceutical product is necessary to ensure the efficacy and safety throughout the shelf life, including storage, distribution and use[3].

Codeine phosphate(CP), chlorpheniramine maleate(CPM) and sodium benzoate(SB) are recently introduced in the market as a cough syrup, which is widely used in the treatment of cough..Codeine phosphate is It is used to treat mild to moderate pain and to relieve cough, acts as antitussive. chlorpheniramine maleate is acts as antihistaminic.Sodium benzoate acts as a preservative. It acts as a bacteriostatic and fungistatic under acidic conditions.Different literature works shows that various methods have been reported for the estimation of codeine phosphate, chlorpheniramine maleate and sodium benzoate individually and in combination with other drugs. There is various methods as UV[4], LC[5,6] LC-MS[7], CZE[8-9], HPLC[10-18] for estimation of codeine phosphate, chlorpheniramine maleate and sodium benzoate individually and in combination with other drugs The present work was undertaken to develop such method of analysis, which can estimate both the drugs and preservative in combination without prior separation which is a precise, accurate, simple, reliable and less time consuming method for estimation of drugs in syrup. The need for determination of sodium benzoate in the present study is that it is physically and chemically compatible for syrup formulation which improves shelf life of product. In this respect, this method of analysis is needed for simultaneous quantification of all three combined components in one step.

MATERIALS AND METHODS

Materials

Instrument

The high pressure liquid chromatographic system consisted of Waters HPLC model (VP series) contains LC-10 AT pump variable wave length programmable UV/Visible detector Chromatographic analysis was performed using Intersil C-18 analytical column with 250×4.6 mm.

Reagents and materials

Codeine phosphate, Chlorpheniramine maleate ,Sodium benzoate, Diammonium hydrogen orthophosphate, Ortho phosphoric acid, Lichrosolv HPLC grade water, Acetonitrile, Methanol is commercially available in the market was purchased from laboratories.

Chromatographic Conditions

The mobile phase which used in this method was consisted of acetonitrile and phosphate buffer (pH 2.3, adjusted by using ortho phosphoric acid). Flow rate about 1.5 ml/min maintained throughout this analysis.The variable wavelength UV–visible detector was set at 254 nm. The analysis was performed at ambient temperature.

Methods

Preparation of Buffer solution

Weighed accurately 3.3gm of Diammonium hydrogen orthophosphate and dissolved into 1000 ml of water and adjusted the pH to 2.3 with Orthophosphoric acid.

Diluent: HPLC grade water.

Preparation of Standard Solution

Accurately weigh and transfer 50mg of Codeine phosphate, 20mg chlorpheniramine maleate & 50mg of Sodium benzoate working standards taken into a 100 ml volumetric flask, add about 70 ml of diluent and sonicate using sonicator and make volume up to the mark with the same solvent .Further pipette 5 ml of the prepared above stock solution into a 25ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter. Inject 50 µl of the standard solution into the chromatographic system and measure the area for the Codeine phosphate, Chlorpheniramine maleate and Sodium benzoate peaks and calculate the %Assay by using the assay formula.

Chromatogram of standard solution is as shown in Fig1.

Assay procedure

Preparation of Sample Solution

Accurately pipette out 5ml of the sample (equivalent to 1.264gm/ml) into a 100ml volumetric flask and 70ml of diluent was added and mixed well and made up to the mark with diluent. Mix well and filter through 0.45µm filter. Inject 50 µl of the sample solution into the chromatographic system and measure the area for the Codeine phosphate, Chlorpheniramine maleate and Sodium benzoate peaks. and calculate the %Assay by using the assay formula.

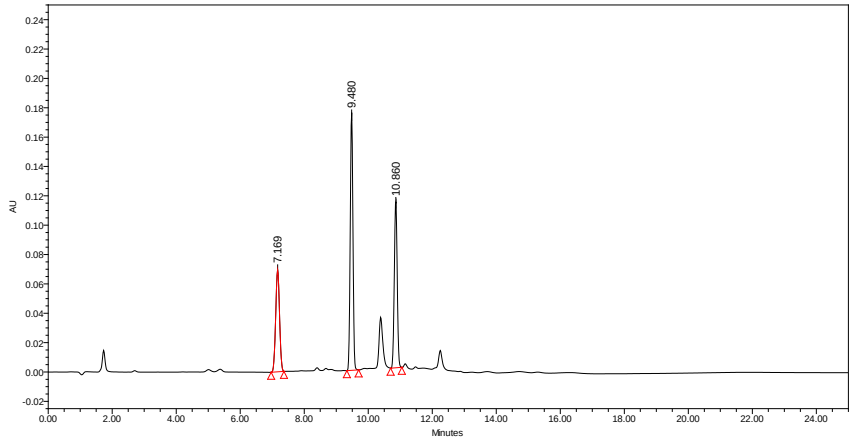

Fig. 1: It shows peaks of standard solution of Codeine phosphate, Chlorpheniramine maleate and Sodium benzoate.

Assay formula

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{Wt/ml}{\text{Label Claim}} \times 100$$

Where,

AT = Test solution area.

AS = Standard solution area.

WS = Working standard weight in mg

WT = Sample weight in mg

DS = Dilution of Standard solution

DT = Dilution of sample solution

P = Percentage purity of working standard.

Chromatogram of sample solution is as shown in Fig 2.

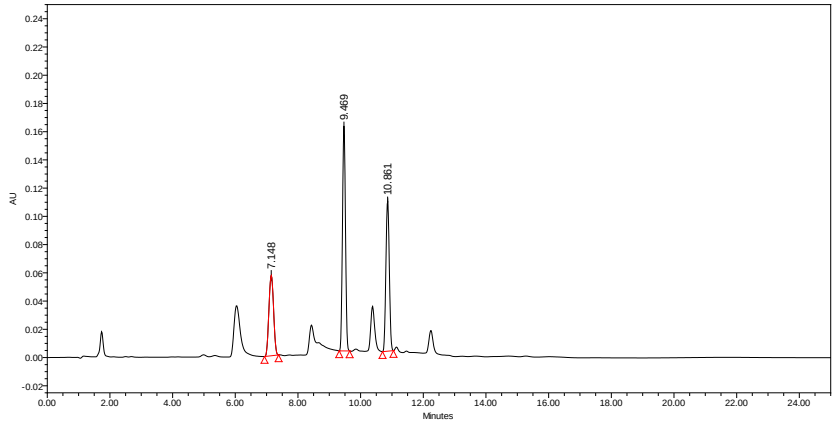

Fig. 2: It shows peaks of test solution of Codeine phosphate, Chlorpheniramine maleate and Sodium benzoate.

Method validation

The method was validatedby using different parameters.

System suitability testing

This is an important parameter of many analytical procedures. System suitability test parameters like tailing factor, retention time, theoretical plates per unit, resolution factor are determined.

Specificity

Specificity was tested against standard compounds and against potential interferences in the presence of placebo. No interference was detected at the retention time of Codeine phosphate, Chlorpheniramine maleate and Sodium benzoate in sample solution.

Linearity

Linearity is studied to determine the range over which analyte response is a linear function of concentration. This study was performed by preparing standard solutions at seven different concentrations and analyses were performed in triplicate. The responses were measured as peak area. The calibration curves were obtained by plotting peak area against concentration.

Precision

Precision was considered at two levels, i.e. repeatability and intermediate precision, in accordance with ICH recommendations. Repeatability, or intra-day precision, was determined by performing nine analyses at three concentrations on the same day. Intermediate

precision was determined by analyzing the same sample in the same way on different days. Results were expressed as SD and RSD.

Accuracy

The accuracy expresses the closeness of agreement between the value which is accepted and the value found. It is expressed as recovery (%), which is determined by the standard addition method. Samples were spiked with 50, 100, and 150% of the standard and analyzed. The experiment was performed in triplicate. Recovery (%)) was calculated for each concentration.

Ruggedness

It is a measure of the reproducibility of a test result under normal, expected operating condition from instrument to instrument and from analyst to analyst.

Robustness

A method is robust, if it is not changed by small changes in operating conditions. To determine the robustness of this method, the experimental conditions were deliberately altered at two different levels and chromatographic response was evaluated.

RESULTS AND DISCUSSION

In the present work a new method development and validation was carried out for the estimation of Codeine phosphate, Chlorpheniramine maleate and Sodium benzoate by RP-HPLC technique. The wavelength selection was made at 254 nm since all the selected drugs reported in various literatures were having a maximum absorbance at around 254 to 258 nm. Hence the wavelength was selected at 254 nm for the detection of the three compounds.

Method Development

For the method development several trials were carried out and reported. These lead to the optimized chromatographic conditions for the separation and estimation of Codeine phosphate, Chlorpheniramine maleate and Sodium benzoate in the bulk and marketed formulation. Preliminary studies involved trying ODS-silica columns and several mobile phase compositions for the effective separation of these three drugs. By Zodiac C-18, 3.5µ,150×4.6mm column eluted with Solvent-A: Phosphate buffer pH-2.3, Solvent-B: Acetonitrile by gradient elution pattern at a flow rate of 1.5 ml/ min and a detection wavelength of 254 nm with injection volume of 50 µl at Ambient(30°C) temperature used the best separation of these analytes. The chromatograms of trial methods were as shown in Fig.3-5 and optimized method were as shown in Fig 6-8. The assay results were as shown in Table 1.

Results of Trial-1: RT'S were observed at 11.236(CP), 15.568(CPM) and the third peak is not eluted in 20 min runtime. Due to assymetry in peaks and longer RT's another trial is made with change in mobile phase-buffer pH.

Results of Trial-2: RT's were observed at 10.236(CP), 13.568(CPM) and the third peak is not eluted in 20 min runtime. Due to tailing in peaks and to further reduce RT's another trial is made with change in mobile phase-buffer pH, flow rate and elution pattern.

Results of Trial-3(Final Optimized Method): RT's were observed at 7.169(CP), 9.480(CPM) and 10.860(SB). The peaks are sharply resolved with less tailing and hence the trial-3 method is optimized for analysis.

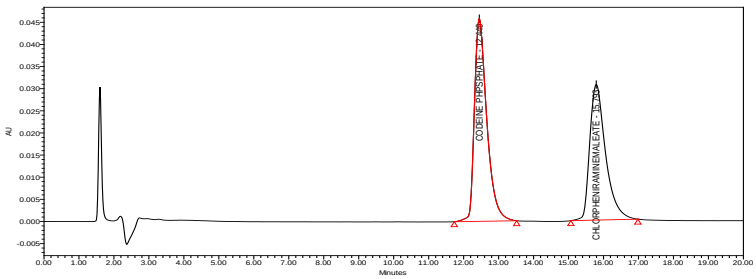

Fig. 3: It shows Trial-1.

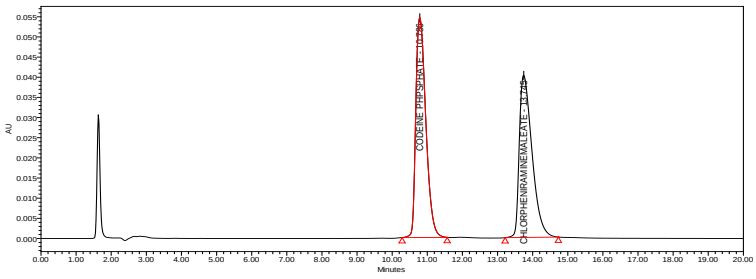

Fig. 4: It shows Trial-2.

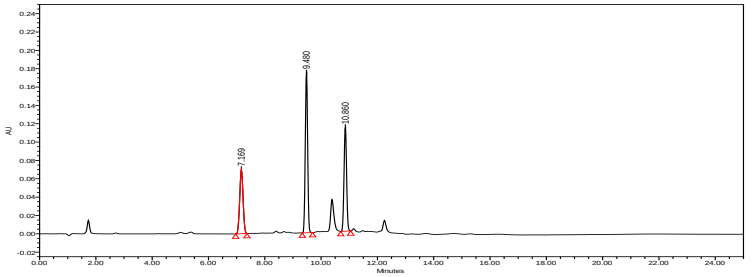

Fig. 5: It shows Trial-3.

Table 1: Table shows assay results of Codeine Phosphate, Chlorpheniramine Maleate and Sodium Benzoate.

S. No.	Compound name	Assay value
1.	Codeine phosphate	99.2%
2.	Chlorpheniramine maleate	101.2%
3.	Sodium benzoate	99.8%

Method Validation

After completion of development, the validation of the method has been performed for assay determination which includes accuracy, selectivity, precision, linearity and range, ruggedness and robustness.

System Suitability Testing:

This is an importent parameter of many analytical procedures. System suitability test parameters like tailing factor, retention time, theoretical plates per unit, resolution factor are determined and the results are tabulated and are as shown in Table 2.

Accuracy (recovery)

To the required quantity of placebo, a known quantity of the three active ingredients (codeine phosphate, chlorpheniramine maleate and sodium benzoate) with the same proportion as in the drug formulation was added to get simulated drug formulation. The results are as shown in the Table 3.

Precision

The system precision of this method was evaluated by calculating the %RSD of the peak areas of six replicate injections of the standard solution, which were found to be 0.41%, 0.35% and 0.37% and for method precision evaluated with six sample replicate injections were found to be 0.43%, 0.36% and 0.39% for codeine phosphate, chlorpheniramine maleate and sodium benzoate respectively and it was found to be less than 1.0% shown in the Table 4.

Specificity

Specificity showing the good separation of the three analytes from each other,Furthermore, excipients of the formulation did not interfere with the active ingredients.The average and relative standard deviation of area from standard chromatograms was calculated. and the results are as shown in Table 5.

Linearity and range

Linearity was studied from different concentrations of the three analytes in the range of 12.5-75 µg/ml for codeine phosphate, 5–30 µg/ml for chlorpheniramine maleate and 12.5-75 µg/ml for sodium benzoate. The results obtained are shown in the Table 6-8 and show that the current method was linear for the three analytes in the range specified above with a correlation coefficient of better than 0.999.

Table 2: Table shows list of system suitability parameters of Codeine Phosphate, Chlorpheniramine Maleate and Sodium Benzoate.

Parameters	Codeine phosphate	Chlorpheniramine maleate	Sodium benzoate
Tailing factor	1.2	1.2	1.1
Retention time	7.18	9.39	10.92
Theoretical plates per unit	17779.2	52624.9	71472.4
Resolution	8.5	11.6	9.2

Table 3: Table shows results of % recovery studies for Codeine Phosphate, Chlorpheniramine Maleate and Sodium Benzoate.

Inj. sample	Spike level	Amount present	Amount recovered	% recovered	Mean recovery	Acceptance Criteria
Codeine Phosphate	50 %	25mg	24.8mg	99.2%		
	100 %	50mg	49.8mg	99.6%	98.7%	100 ± 2.0%
	150 %	75mg	75.07mg	100.1%		
Chlorpheniramine Maleate	50 %	10mg	9.85mg	98.5%	98.5%.**5**	
	100 %	20mg	19.7mg	98.5%		100 ± 2.0%
	150 %	30mg	29.16mg	98.6%		
Sodium Benzoate	50 %	25mg	24.72mg	98.9%		
	100 %	50mg	49.35mg	98.7%	99.1%	100 ± 2.0%
	150%	75mg	74.4mg	99.2%		

Table 4: Table shows results of precision for codeine phosphate, chlorpheniramine maleate and sodium benzoate

Parameter	System Precision			Method Precision		
Average Area	Codeine phosphate	Chlorpheniramine maleate	Sodium benzoate	Codeine phosphate	Chlorpheniramine maleate	Sodium benzoate
	582985	1036039	719061	580118	1036157	713987
SD	2390.2	3724.3	2719.3	2494.5	3812.9	2834.8
%RSD	0.41	0.35	0.37	0.43	0.36	0.39

Table 5: Table shows results of specificity of Codeine Phosphate, Chlorpheniramine Maleate and Sodium Benzoate.

Parameter	Codeine phosphate	Chlorpheniramine maleate	Sodium benzoate
Average area	582964	1036045	719072
SD	2389.5	3724.7	2719.5
%RSD	0.40	0.38	0.39

Table 6: Table shows linearity result for Codeine Phosphate.

Linearity Level	Concentration (µg/mL)	Area
1	25	146517
2	50	294820
3	75	446999
4	100	595115
5	125	748334
6	150	898406
Correlation Coefficient		0.999991

Table 7: Table shows linearity results for Chlorpheniramine Maleate.

Linearity Level	Concentration (µg/ml)	Area
1	5	252347
2	10	524063
3	15	792940
4	20	1063971
5	25	1342834
6	30	1618163
Correlation Coefficient		0.999980

Table 8: Table shows linearity results for sodium benzoate

Linearity Level	Concentration (µg/ml)	Area
1	12.5	147150
2	25	306740
3	37.5	470036
4	50	626825
5	62.5	779389
6	75	936540
Correlation Coefficient		0.999943

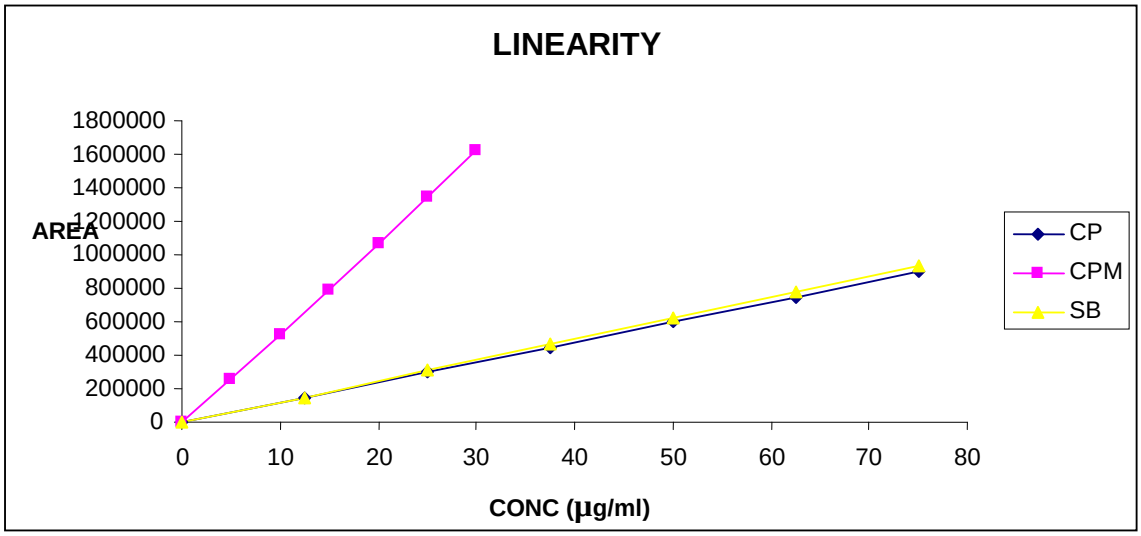

Fig. 6: It showsLinearity graph of Codeine Phosphate, Chlorpheniramine Maleate and Sodium Benzoate in comparison.

The correlation coefficient values were found to be within the acceptance limits for Codeine Phosphate, Chlorpheniramine Maleate and Sodium Benzoate.

Robustness

This current method was investigated by analyzing samples using the same chromatographic conditions set forth in method development but with a small change in the following chromatographic parameters:

(a) Flow rate: 1.4 and 1.6 ml/min instead of 1.5 ml/min,

(b) pH of the buffer preparation in mobile phase: 2.2 and 2.4 instead of 2.3.

% RSD of codeine phosphate, chlorpheniramine maleate and sodium benzoate assay under these conditions is calculated and the results are shown in Table 9.

Ruggedness

This method was demonstrated by analyzing three samples (assay) of syrup formulation by two analysts in the same laboratory on two different days. The %RSD values for the 12 samples are calculated to be 0.42%, 0.62%, and 0.49% for codeine phosphate, chlorpheniramine maleate and sodium benzoate respectively. This method results are shown in the Table 10.

Table 9: Table shows robustness results for change in flow rate and pH of Codeine Phosphate, Chlorpheniramine Maleate and Sodium Benzoate.

Flow rate	Inj. sample	Plate count	Tailing	RT	%RSD
	Codeine phosphate	16098.54	1.0	7.148	0.42
1.4ml/min	Chlorpheniramine maleate	58143.60	1.04	9.469	0.36
	Sodium benzoate	63871.82	1.1	10.86	0.38
	Codeine phosphate	13652.01	1.23	7.105	0.40
1.6ml/min	Chlorpheniramine maleate	51711.86	1.0	9.420	0.35
	Sodium benzoate	57803.38	1.0	10.85	0.37
pH variation					
	Codeine phosphate	17779.21	1.2	7.180	0.41
2.2	Chlorpheniramine maleate	52624.93	1.2	9.394	0.37
	Sodium benzoate	71472.43	1.1	10.923	0.39
	Codeine phosphate	17768.98	1.1	7.165	0.45
2.4	Chlorpheniramine maleate	52628.56	1.0	9.35	0.33
	Sodium benzoate	71470.75	1.0	10.910	0.40

Table 10: Table shows ruggedness results of analysts-1&2 on days-1&2 of Codeine Phosphate, Chlorpheniramine Maleate And Sodium Benzoate.

	Analyst-1					
	Day-1			**Day-2**		
A.I	**CP**	**CPM**	**SB**	**CP**	**CPM**	**SB**
1.	593892	1024792	727778	584035	1047999	725842
2.	593436	1023254	735642	592456	1036824	724689
3.	593945	1025256	731028	586945	1047642	723688
Mean	593757	1024434	731482	587812	1044155	734739
SD	2796.98	1047.9	3159.6	4276.9	6351.3	1077.8
%RSD	0.40	0.103	0.54	0.72	0.61	0.15

	Analyst-2					
	Day-1			**Day-2**		
A.I	**CP**	**CPM**	**SB**	**CP**	**CPM**	**SB**
1.	590077	1053039	723222	591486	1052786	725846
2.	584628	1048958	724674	587628	1058462	725486
3.	587463	1035478	723654	584968	1059466	735644
Mean	587389	1045825	723850	588027	1056904	728992
SD	2725.2	9190.15	745.57	3277.3	3602.02	5763.6
%RSD	0.46	0.87	0.103	0.55	0.34	0.79

CONCLUSION

A novel RP- HPLC method was developed for the simultaneous estimation of codeine phosphate, chlorpheniramine maleate drugs and sodium benzoate preservative. In this method preparation of sample is simple and the time of analysis is short. This method was validated as per guidelines. It was found to be new, simple, sensitive,accurate, precise and This method showing high degree of precision with less than 2%RSD. This method can be used for fast analysis of codeine phosphate, chlorpheniramine maleate drugs and sodium benzoate.

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