A Validated method for determination of Metabisulfite content in Cetrizine Dihydrochloride and Ambroxol Hydrochloride 5+30mg/5mL syrup by Ion exchange chromatography.

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Abstract: *A commercial, particular and strong Ion Chromatography method was developed for the quantitative determination of metabisulfite content in Cetrizine Ambroxol syrup. The method was developed using Ion pac AS11HC Column, 250 X 4.6mm X 5.0 µm column with mobile phase containing 18mM sodium hydroxide in water. The eluted compounds were monitored using conductivity detector. The developed method was validated as per ICH guidelines with respect to limit of detection(LOD), limit of quantification(LOQ), exactness, reproducibility, ruggedness and robustness. The LOD, LOQ values of metabisulfite were 0.3PPM and 1.0PPM respectively.*
Keywords: *validation, quantitative determination, Ion Chromatography, Limit of detection, Limit of quantification, Sodium Metabisulfite.*

I. Introduction

Sodium metabisulfite also referred as Sodium pyrosulfite is with the chemical formula $Na_2S_2O_5$. It is the antioxidant used in Cetrizine Ambroxol relief syrup. Sodium metabisulfite is used for stabilizing the relief syrup. It is the major excipient and preservative used in the syrup. Ambroxol (Fig.1) is a secretolytic mediator used in the healing of respiratory diseases related with extreme mucus. Ambroxol is indicated as "secretolytic therapy in bronchopulmonary diseases associated with abnormal mucus secretion and impaired mucus transport. It promotes mucus clearance, facilitates expectoration and eases productive cough, allowing patients to breathe freely and deeply". Cetrizine (Fig.2) is an antihistamine used in allergy treatment.

It is felt compulsory to have an Ion chromatography method for the quantitative estimation of Metabisulfite in Cetrine relief syrup. Currently, the determination of impurities and monitoring their level during the stability and the possible degradants or by-products during the stability is one of the most difficult tasks for pharmaceutical analysis during method development . Hence a stable and reproducible Ion exchange method was developed for the quantitative determination of Metabisulfite The samples were supplied by Dr Reddy's analytical research and development. International Conference on Harmonization (ICH) of analytical requirements guidelines has been followed for validating this method.

II. Experimental

2.1 Materials and reagents

Cetrizine Ambroxol syrup samples were provided by Dr. Reddy's Laboratories Limited, IPDO, Hyderabad, India. The analytical reagent (AR) grade Sodium metabisulfite and Sodium hydroxide were brought from Merck, Darmstadt, Germany.

2.2 Chromatographic Conditions and Equipment

IC was carried out on a Dionex Ion chromatography system equipped with Electrochemical detector model ICS5000DC, Autosampler model AS-AP, Gradient pump ICS5000SP with conductivity detection. This instrument is equipped with Variable Wavelength Detection, Electrochemical Detection consisting of Conductivity and Amperometry. Chromeleon software is utilized to detect the output signal. The chromatographic column used was Ion pac AG11HC(guard) and AS11HC(Analytical), 250X 4.6mm, and 5µm particle size. The resolution was achieved on an isocratic method. The mobile phase composition contained 18mM Sodium hydroxide in IC grade water.
The flow rate of mobile phase was 1.20 mL/min . The column temperature was kept at ambient and the detection was performed utilising electrochemical detector. The diluent selected was 0.05% Formaldehyde .

2.3 Preparation of Stock Solutions

A solution of Metabisulfite standard (1000ppm) was prepared by dissolving an appropriate amount of

Sodium metabisulfite in diluent. An individual stock solution (10ppm) of Metabisulfite was prepared in diluent.

III. Method development and optimization

The core aim of this effort is to build up a constancy indicating Ion chromatography method for determination of Metabisulfite content in Cetrizine Ambroxol relief syrup within shorter run time.

Initially attempts were made by using different Ionpac columns (AG12A, AS12A, AS11, AG11) using different buffers (Sodium tetraborate, Sodium hydroxide). In all above columns and intended experimental conditions, Metabisulfite was giving two peaks. The problem for the question why single standard is giving two peaks was not solved. Attempts were made with column and mobile phase optimization.

Experimentation proceeded as follows :Suspected column contamination, followed column washing procedure as per Dionex recommened manual and analysed the standard but the problem persists same. Suspected Metabisulfite standard contamination, hence looked for alternative lot but the same problem continued. Reveiwed the literature and found that " Metabisulfite is stable in solid state but in aqeous solution it converts to sulfite, which on oxidation convets to sulfate." Practically injected the Metabisulfite 10ppm standard, Sulfite 10ppm standard and Sulfate 10ppm standard into ion chromatography system. It is interestingly found out that first peak retention time of Metabisulfite is matching with Sulphite and second peak retention time of Metabisulfite is matching with Sulphate. Discussed regarding this with Dionex expert, and he suggested to use " diluent as Formaldehyde, where the oxidation of metabisulphite to sulfate can be restricted."

Analysed the standard using 0.05% Formaldehyde as diluent, observed metabisulfite
as major peak (peak area around 0.48) but sulfate peak is seen still but area of the paek was negligible (around 0.01 area). The negligible sulfate peak is attributed to the presence of minute traces of sulphate present in Metabisulfite standard solid itself.

From practical experience found that it is necessary to prepare the diluents freshly before starting the analysis and will be stable for 24hours only.

Now the method is developed and injected the Cetrizine relief syrup samples but again the problem was standard is giving single paek for metabisulphite but samples are giving two peaks. The reason for this behavior was attributed to the explanation that as these samples are stability samples and aqeous solutions of Metabisulfite is already converted to Sulphite and Sulphate in the solution itself before injecting into IC system. Though Formaldehyde was used as diluent standard gave single peak but samples gave two peaks.
This was further supported by practically injecting the placebo of Cetrizine sample with out metabisulfite and the placebo sample of fresh addition of Metabisulfite just before injecting into the IC system. The placebo without Metabisulphite shown peak absence and the placebo with fresh addition of Metabisulphite gave single peak using Formaldehyde diluent.

Finally method was optimised with good peak shape using AS11HC, AG11HC column using Sodium carbonate mobilephase and 0.05% Formaldehyde as diluent.

IV. Method Validation
The described method has been extensively validated.

4.1 Precision
The repeatability of IC method was checked by six-fold analysis of Cetrine placebo samples spiked with 1000 ppm of specification limit of Metabisulfite. The RSD% of the % area of Metabisulfite was calculated. The intermediate precision (ruggedness) of the method was evaluated by performing the analysis with different analyst on different day. The chromatogram of standard represented in Figure-3

4.2 Limit of Detection (LOD) and Quantification (LOQ)
The LOD and LOQ for Metabisulfite were determined at a signal-to noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentrations. The precision study was also determined at the LOQ level by injecting six (n=6) individual preparations and calculating the **% RSD** of the area for Metabisulfite.

4.3 Accuracy
The accuracy of an analytical procedure expresses the closeness of agreement between the true value and the value found. Accuracy of the IC method was established by standard addition and recovery experiments. Recovery was calculated for each added concentration. The study was carried out for Metabisulfite in triplicate using four concentration levels from LOQ, 50%, 100% and 150% of the specification limit and recovery of the Metabisulfite was calculated.

4.4 Linearity of Response

The linearity of the detector response to different concentrations was evaluated for Metabisulfite by injecting each separately prepared solutions covering the range LOQ to 200% of the specification limit. The correlation coefficients, slopes and Y-intercepts of the calibration curve were determined.

4.5 Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. To determine the robustness of the method the experimental conditions were deliberately changed. The mobile phase flow rate was 1.20 mL/min; to study the effect of flow rate, it was changed to 1.3 and 1.1 mL/min

V. Results and Discussion

Validation of the Method

5.0.1 System suitability

System suitability is established by injecting six standards of Brmoacetate(1000ppm) and calculated the % RSD for six injections of Metabisulfite peak area.

5.0.2 Precision

In this study the RSD% of the area of Metabisulfite in samples spiked with Metabisulfite was within 1.7%. The RSD% in the intermediate precision study was 0.9%. The RSD% values are presented in Table –I.

5.0.3 Limit of Detection and Quantification

The determined limit of detection, limit of quantification and precision at LOQ values for Metabisulfite are reported in Table-I.

5.0.4 Linearity

Linearity calibration plot for the assay method was obtained over the calibration ranges tested and correlation coefficient obtained was greater than 0.999. Linearity calibration plot for the related substances method was obtained over the calibration ranges tested i.e., LOQ to 150% (LOQ, 25%, 50%, 75%, 100%, and 150% of specification limit). The correlation coefficients, slopes and Y-intercepts of the calibration curve were determined. The values are represented in Table-I

5.0.5 Accuracy

The recovery of Metabisulfite was ranged from 99.4 to 99.9%. The recovery of The percentage recovery of the Metabisulfite are listed in Table -II.

5.0.6 Robustness

In all the deliberately varied chromatographic conditions (Different flow rate), the system suitability acceptance criteria was within the allowable range. The values are presented in Table-III.

5.0.7 Specificity

As per synthetic scheme, chloride and citrate may interfere with Metabisulfite. Hence specificity has been carried out with choride and citrate. The values are presented in Table-IV.

Table 1 Method Validation results- LOD, LOQ, Regression, Repeatability and Intermediate Precision
(Linearity range is LOQ to 200% with respect to specification of Metabisulfite)

Parameter	Metabisulfite
LOD(ppm)	0.3
LOQ(ppm)	1.0
Y-Intercept at 100%	-1.112
Correlation coefficient	0.999
Repeatability (RSD%)	1.69
Intermediate precision (RSD%)	0.91

Table 2 Method Validation –Accuracy (Recovery) data

Spike level %	% Recovery
LOQ	89.32
50%	109.62
75%	116.03
100%	102.70
150%	87.08

Table 3 Method Validation – Robustness data

System suitability at 1.10mL/min		System suitability at 1.20mL/min		System suitability at 1.30mL/min	
Sr. No	Area	Sr. No	Area	Sr. No	Area
1	0.5310	1	0.4752	1	0.4623
2	0.5325	2	0.4838	2	0.4703
3	0.5281	3	0.4632	3	0.4503
4	0.5357	4	0.4798	4	0.4594
5	0.5310	5	0.4810	5	0.4673
6	0.5250	6	0.4834	6	0.4760
Avg	0.5306	Avg	0.4777	Avg	0.4643
%RSD	0.69	%RSD	1.63	%RSD	1.94

Table 4 Method Validation – Specificity data

Sample	Retention time
Blank	Not detected
Metabisulfite standard	7.957
Citrate standard	ND
Chloride standard	4.42
Sample as such	Not detected
Sample spiked	Metabisulfite -8.31 Chloride-4.42

Figure-1 **Ambroxol :** trans-4-(2-Amino-3,5-dibromobenzylamino)-cyclohexanol

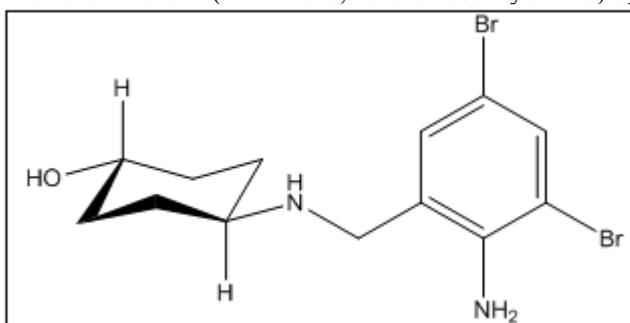

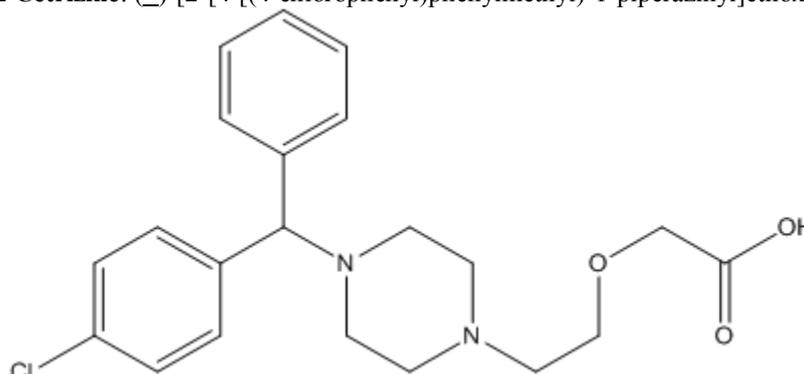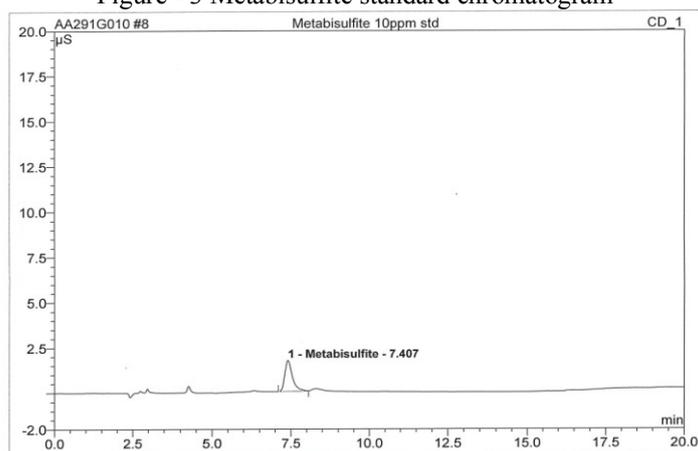Figure-2 **Cetrizine**: (+)-[2-[4-[(4-chlorophenyl)phenylmethyl)-1-piperazinyl]ethoxy]acetic acid

Figure - 3 Metabisulfite standard chromatogram

VI.   Conclusion

The rapid gradient IC method developed for the simultaneous trace level quantitative determination of Metabisulfite in Cetrine Ambroxol relief syrup is precise, accurate, linear, rugged and robust. Satisfactory results were obtained from validation of the method. This method exhibited an excellent performance in terms of sensitivity. This method can be used for routine analysis of trace level quantitative determination of Metabisulfite in Cetrine Ambroxol relief syrup.

Acknowledgements

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