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DEVELOPMENT AND EVALUATION OF DIMETHYL SULPHATE AS A GENOTOXIC IMPURITY IN SALBUTAMOL SULPHATE DRUG SUBSTANCE BY USING HPLC

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ABSTRACT A simple and accurate pre-column derivatization method was developed for the determination of Dimethyl sulphate (DMS) in the Salbutamol sulphate bulk drug. Alkylation of 2-MercaptoPyridine with Dimethyl sulphate gives corresponding 2-(Methylthio) Pyridine which is analyzed by reverse phase liquid chromatographic method by using UV detector. Chromatographic separation between Dimethyl sulphate and Salbutamol sulphate was achieved using a C18 column using a mobile phase containing buffer and acetonitrile in gradient mode. The resolution between the between Dimethyl sulphate and Salbutamol sulphate was found to be more than 5.0. The limit of detection (LOD) and limit of quantification (LOQ) of the Dimethyl sulphate was 0.231 and 0.463 µg mL-1, respectively, for 20 µL injection volume. The percentage recoveries of the Dimethyl sulphate ranged from 94.4 to 98.1 in the samples of Salbutamol sulphate. The developed method was validated as per International Conference on Harmonization guidelines in terms of specificity, limit of detection, limit of quantification, precision, linearity, accuracy and ruggedness.

KEYWORDS : Development, Validation, Salbutamol Sulphate, Dimethyl Sulphate, 2-(Methylthio) Pyridine, Derivative, HPLC

INTRODUCTION

Salbutamol sulphate [1,2] (trade name Ventolin), ((*RS*)-4-[2-(*tert*-Butylamino)-1-hydroxyethyl]-2-(hydroxymethyl)phenol (Figure 1), is a medication that opens up the medium and large airways in the lungs [2]. It is used to treat asthma, exercise-induced bronchospasm, and chronic obstructive pulmonary disease (COPD). It may also be used to treat high blood potassium levels [2, 3]. It is usually used by inhaler or nebulizer but is also available as a pill and intravenous solution [2, 3].

Salbutamol sulphate involves the use of Methanol and Sulphuric acid as process solvents. Dimethyl sulphate is one of the possible impurity during the manufacturing process of Salbutamol sulphate. So the study is proposed and conducted for the method development and further validation of method for determination of Dimethyl sulphate in Salbutamol sulphate drug substance. The results obtained after completion of validation are all within the set acceptance criteria. As per the IARC monograph of Dimethyl sulphate, it is listed in 2A group (Probably carcinogenic to humans) and also it is genotoxic as per the final evaluation [4, 5]. Recommended maximum daily dose for Salbutamol sulphate is 32 mg. Based on the genotoxic impurity guideline, daily dose and threshold of toxilogical concern (TTC) approach, the limit for the Dimethyl sulphate is decided as 46.88 µg/mL [6]. Various approaches are utilized while developing method for the content of Dimethyl sulphate like derivatization [8] as well as extraction [10]. The analytical tools used during these analytical approaches are GC-MS as well as LC-MS [9-11] as limit of evaluation is very low. In the present work we have developed a simple precise method for determination of dimethyl sulphate along with Salbutamol sulphate and its known impurities using C18 column by high performance liquid chromatography. The developed method was validated according to International Conference on harmonization (ICH) guidelines [7] for the quantitative determination of the Dimethyl sulphate in Salbutamol sulphate.

EXPERIMENTAL SECTION

Chemicals and Reagents

Samples of Salbutamol sulphate were obtained from R & D synthetic Department of FDC Ltd, Mumbai, India. HPLC-grade acetonitrile and Potassium dihydrogen orthophosphate was procured from Merck, Darmstadt, Germany. Derivative reagent used was 2-Mercapto Pyridine procured from Rankem. Also, Dimethyl sulphate with certified purity procured from Rankem.

Instrumentation

HPLC system used was Agilent (LC 1200 series, US) system equipped with auto sampler, quaternary pump, degasser and a UV Detector. The output signal was monitored and processed using Chromeleon software.

Chromatographic condition

The chromatographic column used was X-Select CSH C18 column (250 mm x 4.6 mm, 5µm), (Waters Ltd., USA). The mobile phase used was 0.02M phosphate buffer, pH adjusted was 3.0. Buffer adjusted pH 3.0 was used as a mobile phase A and acetonitrile was used as a mobile phase B. The gradient program time (minutes) % mobile phase B (T%B) was set as 0/30, 15/30, 20/60, 25/60, 25.1/30 and 35/30 respectively. The flow rate of the mobile phase was 1.0 mL/min. The column temperature was maintained at 25°C, and the eluent was monitored at a wavelength of 245 nm. The injection volume used was 20 µL.

Preparation of Diluent (Derivatization solution)

Weigh accurately about 10.0 g of 2-MercaptoPyridine in 1000 ml volumetric flask. Dissolve it in sufficient amount of acetonitrile and water in the ratio (65:35 v/v) with sonication and make up to the mark. (Conc. of 2-Mercaptopyridine 1.0%).

Preparation of Standard Solutions

The stock solution of the Dimethyl suphate were prepared by dissolving an appropriate amount of the standard in diluent. For

quantitation of Dimethyl sulphate in Salbutamol sulphate a solution of 2.34 µg/mL concentration was used. The target analyte concentration was fixed as 50.0 mg mL-1.

RESULTS AND DISCUSSION

Method Development

A solution of Salbutamol sulphate and Dimethyl sulphate (50mg/mL & 2.34µg/mL) prepared in diluents for method establishment. To develop a rugged and suitable HPLC method for the separation, different stationary phases and mobile phases were employed. Preliminary column screening involved different types of C18, C8, Cyano, Amino and Phenyl columns were employed. On X-Select CSH C18 (250 mm x 4.6 mm, 5µm) column provided selectivity between the Salbutamol sulphate peak and the Dimethyl sulphate peak using a mobile phase consisting of buffer-methanol (70:30, v/v), but the retention times of Salbutamol sulphate, known impurities and Dimethyl sulphate were both longer than 40 min and the peaks were broad. We continued to select the best mobile phases that would give optimum resolution and selectivity for the Dimethyl sulphate and Salbutamol sulphate. Good separation was achieved on C18 column and buffer-acetonitrile (70:30 v/v) as the mobile phase. There was less separation when phosphate buffer of (pH 5.0) and methanol used as mobile phase on C18 column.

Optimized Chromatographic Conditions

Due to the better chromatographic results obtained on the C18 column, further method optimization and quantification of the Dimethyl sulphate were carried out on this column. Based on the data obtained from method development and optimization activities, the X-Select CSH C18 (250 mm×4.6 mm, 5µm) column with the mobile phase of buffer-acetonitrile in gradient was selected for the final method. The flow rate of the experimental method was 1.0 mL/min with an injection volume of 20µL. The column temperature was 25°C, and the detection wavelength was 245 nm. Under these conditions, Dimethyl sulphate and Salbutamol sulphate were separated well and the peak of the Dimethyl sulphate eluted after the peak of Salbutamol sulphate. In the optimized method, the typical retention times of the Salbutamol sulphate and Dimethyl sulphate were approximately 2.56 and 8.77 min, respectively. Baseline separation of Salbutamol sulphate and Dimethyl sulphate was obtained with a total run time of 35 min. The system suitability results were given in Table 1.

TABLE 1: System suitability criteria

Component	Retention time (min)	Relative retention time (min)	Resolution	Tailing factor
Salbutamol Sulphate	2.56	1.00	--	—
2-(Methylthio) Pyridine	8.77	3.44	9.5	1.1

The structure of Dimethyl sulphate, 2-(Methylthio) Pyridine and Salbutamol sulphate are displayed in Fig. 1. The typical chromatogram of the Salbutamol sulphate spiked with Dimethyl sulphate displayed in Fig. 2.

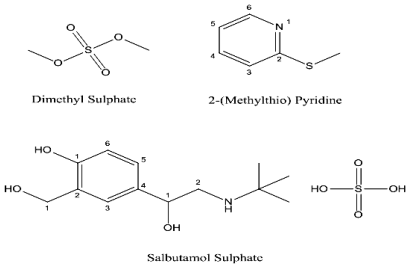

Fig.1 Chemical structures of Dimethyl Sulphate, 2-(Methylthio) Pyridine and Salbutamol Sulphate

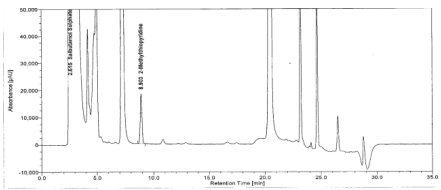

FIG. 2 A typical HPLC chromatogram of Salbutamol Sulphate spiked with Dimethyl Sulphate at specification level

Method Validation

Precision

The precision of an analytical procedure expresses the closeness of agreement among a series of measurements obtained from multiple samplings of the same homogenous sample under prescribed conditions. The system and method precision for the Dimethyl sulphate were checked at its specification level (i.e. 2.34µg/ml with respect to analyte concentration, 50.0 mg mL-1). The percentage RSD of method repeatability and system repeatability for the Dimethyl sulphate were found to be 0.56% and 0.80%, respectively, which confirms good precision of the method.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results,which are directly proportional to the concentration of the analyte in the sample. The linearity of the method for the Dimethyl sulphate was checked at six concentration levels, i.e. from limit of quantitation (LOQ) (50%) to 150% of the Dimethyl sulphate specification level (2.34µg/ml),which is with respect to of Salbutamol sulphate analyte concentration. The coefficient of regression of the calibration curve was found to be 1.00, thus confirming the excellent correlation between the peak area and concentration of the Dimethyl sulphate.

Limit of Detection and Limit of Quantitation

The limit of detection (LOD) and limit of quantification were achieved by injecting a series of dilutions of Dimethyl sulphate [7]. The precision of the developed method for Dimethyl sulphate at LOD and LOQ was checked by analyzing standard solutions prepared at the LOD and LOQ level and calculating the percentage relative standard deviation of area. The limit of detection and quantification for Dimethyl sulphate was found to be 0.231 µg mL-1 and 0.463 µg mL-1 respectively.

Ruggedness and Robustness

The ruggedness [7] of a method was defined as degree of reproducibility of results obtained by analysis of the same sample under a variety of normal test conditions such as different analysts, different instruments and different days. The recovery experiments carried out for the Dimethyl sulphate in Salbutamol sulphate samples at the same concentration levels tested. The data obtained from both the experiment was well in agreement with each other, thus proving the method ruggedness. The robustness [7] of an analytical procedure is measured by its capability to remain unaffected through small, but deliberate, variations in method parameters and provide an indication of its reliability during normal usage. In the varied chromatographic conditions like flow rate, mobile phase ratio and pH, the resolution between the peaks of Dimethyl sulphate and Salbutamol sulphate was found to be >5.0 illustrating the robustness of the method.

Recovery of Dimethyl sulphate

The standard addition and recovery experiments were conducted for the Dimethyl sulphate in bulk samples of Salbutamol sulphate in triplicate at LOQ (0.46µg/mL), 50% (1.17µg/mL), 100% (2.34µg/mL) and 150% (3.51µg/mL) with respect to test concentration. The percentage recovery ranged from 94.4% to 98.1 % (Table 2).

TABLE 2 Summary of method validation data

Parameter	µg mL-1	r	% Mean recovery	% RSD
LOD	0.231	-	-	3.28
LOQ	0.463	-	-	1.79
Linearity				
(LOQ to 150%)	-	1.0000	-	-
Accuracy				
LOQ % spiking	-	-	98.1	0.67
50% spiking	-	-	95.7	0.18
100 % spiking				
150 % spiking			96.0	0.23
Precision				
System precision	-	-	-	0.80
Method precision	-	-	-	0.56
Intermediate pre (Ruggedness)	-	-	-	1.94

CONCLUSION

A simple, rapid and accurate pre-column derivatization High

Performance Liquid Chromatography (HPLC) method developed in ordered to separate Dimethyl sulphate and Salbutamol sulphate. Method validation was carried out using a C18 column due to the better chromatographic results achieved on the column. The validated method was demonstrated to be specific, accurate, precise, sensitive and rugged. The developed and validated method can be implemented for the determination and quantitative of Dimethyl sulphate in Salbutamol sulphate bulk drug.

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