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Simple and sensitive ion chromatography method for the simultaneous determination of Dimethyl sulfate and Diethyl sulfate contents in Metoprolol tartrate drug substance

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ABSTRACT

A simple and sensitive ion chromatography method has been developed for the simultaneous determination of dimethyl sulfate and diethyl sulfate contents in Metoprolol tartrate drug substance. Efficient chromatographic separation was achieved on Allsep™ anion column 150 mm long with 4.6 mm i.d., 7 µm particle diameter. Mobile phase consists of 4.0 mM aqueous phthalic acid. The mobile phase was delivered in an isocratic mode at a flow rate of 1.0 mL min^{-1} at ambient temperature conditions and the analytes were monitored by conductometric detector. The drug substance was subjected to stress conditions of hydrolysis, oxidation, photolytic, thermal and humidity degradation. The method was validated for specificity, precision, linearity, solution stability and accuracy. The limits of detection (LOD) and limits of quantification (LOQ) established for dimethyl sulfate are 1.06 µg mL^{-1} and 3.20 µg mL^{-1} respectively. For diethyl sulfate LOD is 1.47 µg mL^{-1} and LOQ is 4.46 µg mL^{-1}. The average recoveries for dimethyl sulfate and diethyl sulfate are in the range of 99.5% to 102.4% and the method can be successfully applied for the routine analysis of Metoprolol tartrate drug substance.

Keywords: Ion chromatography; Metoprolol tartrate; Dimethyl sulfate; Diethyl sulfate; Validation.

INTRODUCTION

Chemically Metoprolol tartrate is (±)-1-(Isopropylamino)-3-[*p*-(2-methoxyethyl)phenoxy]-2-propanol L-(+)-tartrate (2:1) salt, is a cardio selective beta blocker. The molecular formula is $C_{34}H_{56}N_2O_{12}$ and the molecular weight is 684.82. It is used in management of hypertention, angina pectoris, cardiac arrhythmias and myocardial infraction [1]. It is classified as BCS

(Biopharmaceutics Classification System) class-I drug, as it is highly water soluble and permeable drug [2]. Metoprolol tartrate is available as 50 and 100mg tablets for oral administration and in 5mL ampoules for intravenous administration. It is marketed under the trade name Lopressor [3]. Dimethyl sulfate (DMS) and Diethyl sulfate (DES) are classified as Category-2 carcinogens. DES is reported mutagen (may cause heritable genetic damage) [4]. DMS and DES are alkylating reagents commonly used in organic syntheses and pharmaceutical manufacturing processes. In the synthesis process of Metoprolol tartrate, DMS and DES were used as process reagents. Dimethyl sulfate is anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity in experimental animals [5-8]. Both the liquid and vapour forms of these dialkyl sulfates are harmful to the skin, eyes and mucous membranes due to its potential carcinogenicity, the level of DMS in the API process needs to be monitored and controlled with appropriate methods. The primary routes of potential human exposure to diethyl sulfate are inhalation and dermal contact. Exposure to diethyl sulfate may occur during its production and use as a chemical intermediate, primarily as an ethylating agent. The potential for exposure to diethyl sulfate during its use would appear to be high because a wide variety of intermediates and products are prepared from it [9]. In the available literature, many of the analytical procedures have been identified for the determination of DMS and DES. In 1980, J.C.Gilland and et al., developed and validated the analytical method in air samples using Gas chromatography technique and they reported, the minimum detectable concentrations (LOD) in the atmosphere for DMS and DES, based on a 20 L air sample, are 0.04 ppm and 0.1 ppm respectively [10]. Low level detection of DMS 0.3 ppm was reported by using highphenated technique GC-MS in an aqueous soluble API intermediates in 2009 [11].

Subsequently, an ion chromatography (IC) method was developed and optimized to determine the contents of DMS and DES in Metoprolol tartrate drug substance with better separation of these two peaks and sufficiently low levels of detection. To the best of our knowledge no report has been published on the analysis of DMS and DES in Metoprolol tartrate drug substance in literature.

MATERIALS AND METHODS

Chemicals, reagents and samples

The standard, samples of Metoprolol tartrate drug substance and known related substances of Metoprolol tartrate, such as Metoprolol related substance-A (USP/EP), Metoprolol impurity-B (EP), Metoprolol related substance-B (USP), Metoprolol related substance-C (USP/EP), Metoprolol impurity-D (EP), Metoprolol related substance-(USP-D/EP-O), Metoprolol impurity-E (EP), Metoprolol impurity-F (EP), Metoprolol impurity-G (EP), Metoprolol impurity-H (EP), Metoprolol impurity-J (EP), Metoprolol impurity-M (EP) and Metoprolol impurity-N (EP) were procured from APL Research Centre (A division of Aurobindo Pharma Ltd., Hyderabad). Analytical reagent (AR grade) dimethyl sulfate, diethyl sulfate, phthalic acid, hydrochloric acid, sodium hydroxide, hydrogen peroxide, potassium phthalate, sodium carbonate, sodium bicarbonate, phosphoric acid, formic acid, octane-1-sulfonic acid sodium salt, pyridine-2,6-dicarboxylic acid reagents, HPLC grade methanol, 2-propanol, acetonitrile, tetrahydrofuran and acetone were procured from E Merck India. Highly purified water obtained from Millipore purification system.

Ion chromatography

An ion chromatography system Metrohm 761 Compact IC equipped with conductometric detector, Metrohm 750 auto sampler with 761 Compact IC 1.1 data handling system was used. Mobile phase consists of 4.0 mM aqueous phthalic acid. The analysis was carried out on Allsep ^{TM} anion (Alltech Associates Inc.,) 150 mm long, 4.6 mm i.d., 7 µm particle diameter column at ambient temperature. The mobile phase was delivered in an isocratic mode at a flow rate of 1.0 mL min^{-1}. The injection volume was 20 µL and run time was 25 min. The mixture of water and methanol in the ratio of 75:25 % v/v was used as diluent. The retention times of the dimethyl sulfate, diethyl sulfate peaks are at about 15.0 and 16.5 minutes respectively. The resolution between dimethyl sulfate and diethyl sulfate peaks was not less than 1.50. Relative standard deviation for the peak areas of the six replicate injections for each standard peak is not more than 5.0% .

Standard and sample solutions

Preparation of standard solution

Accurately weigh and transfer each 100 mg of dimethyl sulfate and diethyl sulfate into a 100 mL volumetric flask, add 70 mL of diluent mixed well by shaking, then solution heated to 70°C for 15 min, after that solution cooled to room temperature and make up to volume with diluent. Diluted 10 mL this solution to 100 mL with diluent and further diluted 10 mL of this solution to 50 mL with diluent. Filter through the 0.45 µ porous membrane.

Sample solution

Accurately weigh and transfer 100 mg of sample into a 10 mL volumetric flask, add 7 mL of diluent and dissolve by shaking, then solution heated to 70°C for 15 min, after that solution cooled to room temperature and make up to the volume with diluent. Filter through the 0.45 µ porous membrane.

RESULTS AND DISCUSSION

Method development and optimization

Dimethyl sulfate (DMS) and Diethyl sulfate (DES) are the process impurities during the synthesis of Metoprolol tartrate drug substance. As there was no chromophore present in dimethyl sulfate and diethyl sulfate, there was no possibility for UV or fluorescence detection. Method development for quantification of DMS and DES contents in Metoprolol tartrate drug substance was initiated with dimethyl sulfate and diethyl sulfate miscibility and drug solubility studies, based on that mixture of water and methanol in the ratio of 75:25% v/v was chosen as diluent. Preliminary experiments were carried out based on the retention of sulfate and sulfite, which were discussed in many Metrohm ion chromatography applications, using Metrosep A Supp 5, Metrosep A Supp 3, Metrosep Anion Dual 2, and Metrosep Super-Sep columns. Elution of analytes were investigated using various mobile phases by using reagents like sodium carbonate, sodium bicarbonate, formic acid, potassium phthalate, octane-1-sulfonic acid sodium salt, pyridine-2,6-dicarboxylic acid reagents and phthalic acid. In all above mentioned trials, both analytes were not separated. Separation was achieved on, Allsep ^{TM} anion (Alltech Associates Inc.,) 150 mm long, 4.6 mm i.d., 7 µm particle diameter column, with 2.0 mM aqueous phthalic acid as mobile phase**.** Several trials were made using aqueous phthalic acid as mobile phase in concentrations ranging from 1.0 mM to 6.0 mM. In 1 mM aqueous phthalic acid condition, late elution of analytes with low response was observed. Satisfactory separation was achieved at 4.0 mM aqueous phthalic acid with reasonable retention times of analytes. For better resolution

between analytes, trials were performed with 4.0 mM aqueous phthlic acid using methanol, isopropyl alcohol, acetonitrile, tetrahydrofuran and acetone as an organic modifier. In all above mentioned trials with organic modifiers, resolution between analytes was not improved and also broad peak shapes and base line disturbance were observed. For better peak shapes and good resolution, again several trials were performed with 4.0 mM aqueous phthalic acid using column temperature on separation was studied at different temperatures ranging from 10 - 60°C by increment of every 10°C interval. At 10°C, base line disturbance was observed and analyte peak shapes were broad. From 20°C upto 40°C, the analyte peaks became sharp and resolution was achieved. After that by increasing the temperature upto 60°C only dimethyl sulfate peak became sharp, while other diethyl sulfate peak base line disturbed. Finally, satisfactory separations and better peak shapes were achieved within a reasonable retention time in a mobile phase consisting 4 mM aqueous phthalic acid at a flow rate of 1.0 mL min^{-1} at ambient temperature, was used for validation study.

Method validation

In order to determine the contents of dimethyl sulfate and diethyl sulfate in Metoprolol tartrate drug substance, the method was validated as per the ICH guidelines [12], individually in terms of specificity, forced degradation studies (stability indicating nature), limit of detection, limit of quantification, linearity, accuracy, precision and stability of sample solution.

Specificity

Specificity is the ability of the method to measure the analyte responses in the presence of all impurities. For specificity determination, the interference of diluent and determination of dimethyl sulfate, diethyl sulfate was studied. Different solutions were prepared with known amount of drug substance and different amounts of impurities and injected separately into ion chromatograph and the chromatograms were recorded. It was observed that the peaks of impurities and diluent did not interfere with dimethyl sulfate, diethyl sulfate and well resolved. The method is also specific as most of anions like fluoride, chloride, bromide, iodide, carbonates, bicarbonates, nitrate, nitrite, sulfate, sulfite, sulfide, sulfamate, formate, acetate, citrate and tartrate. The stability indicating nature of the method was further evaluated by performing the forced degradation studies. As per International Conference on Harmonization (ICH), stress testing is to be carried out to identify the likely degradation products or to elucidate the inherent stability characteristics of the active substance [13]. Susceptibility to oxidation is one of the required tests and also hydrolytic, photolytic, thermal and humidity stress stability was required. In this study, metoprolol tartrate drug substance was subjected to stress conditions there was no interference observed for dimethyl sulfate and diethyl sulfate peak and experiment results are shown in Table 1.

LOD and LOQ

The limit of detection (LOD) and limit of quantification (LOQ), of dimethyl sulfate and diethyl sulfate were determined based on the residual standard deviation of a regression line and slope was adopted. Standard solution was injected into ion chromatograph from 2µg mL^{-1} - 30µg mL^{-1}. A plot of peak area (mV*sec) versus concentration (µg mL^{-1}) was drawn and LOD/LOQ values were predicted by residual standard on deviation response (SD) and slope (S) method using the formula 3.3 x SD/S for LOD and 10 x SD/S for LOQ. The solutions of dimethyl sulfate and diethyl sulfate for LOD and LOQ evaluation were prepared at predicted concentration levels and

precised by analyzing six times. An overlay chromatogram of blank, LOQ with standard solution chromatogram is shown in fig. 1. The achieved precised values were shown in Table 2.

Table 1: Evaluation of forced degradation studies

Type of Degradation	Degradation Condition	Dimethyl sulfate (%w/w)	Diethyl sulfate (%w/w)	Degradation of Dimethyl sulfate (%w/w)	Degradation of Diethyl sulfate (%w/w)
Sample as such	-	Not detected	Not detected	-	-
Acid degradation	5M HCl / Initial	Not detected	Not detected	Nil	Nil
	5M HCl / 85°C/ 60 min	Not detected	Not detected	Nil	Nil
	5M HCl / 85°C/ 120 min	Not detected	Not detected	Nil	Nil
Alkaline degradation	5M NaOH / Initial	Not detected	Not detected	Nil	Nil
	5M NaOH / 85°C/ 60 min	Not detected	Not detected	Nil	Nil
	5M NaOH / 85°C/ 120 min	Not detected	Not detected	Nil	Nil
Peroxide degradation	30% H_2O_2 / Initial	Not detected	Not detected	Nil	Nil
	30% H_2O_2 / 85°C/ 60 min	Not detected	Not detected	Nil	Nil
	30% H_2O_2 / 85°C/ 120 min	Not detected	Not detected	Nil	Nil
Thermal degradation	105° C / 120 Hrs	Not detected	Not detected	Nil	Nil
Photolytic degradation	10 K Lux / 120 Hrs	Not detected	Not detected	Nil	Nil
	2 W / M^2 / 120 Hrs	Not detected	Not detected	Nil	Nil
Humidity degradation[a]	90% RH / 25°C /120 Hrs	-	-	-	-

a : Sample was observed to be deliquescence

Table 2: Statistical data of linearity, LOD/LOQ for Dimethyl sulfate and Diethyl sulfate

Statistical parameters	Dimethyl sulfate	Diethyl sulfate
Correlation coefficient	0.9994	0.9990
Intercept	-0.053	0.147
Residual standard on deviation response	0.277	0.313
Slope	0.877	0.745
Concentration range (µg mL^{-1})	2 - 30	2 - 30
Limit of detection(µg mL^{-1})[a]	1.06	1.47
Limit of quantification(µg mL^{-1})[a]	3.20	4.46
Precision for Limit Of Detection (%R.S.D)	19.1	17.5
Precision for Limit Of Quantification (%R.S.D)	9.1	6.7

a : Precised LOD and LOQ values

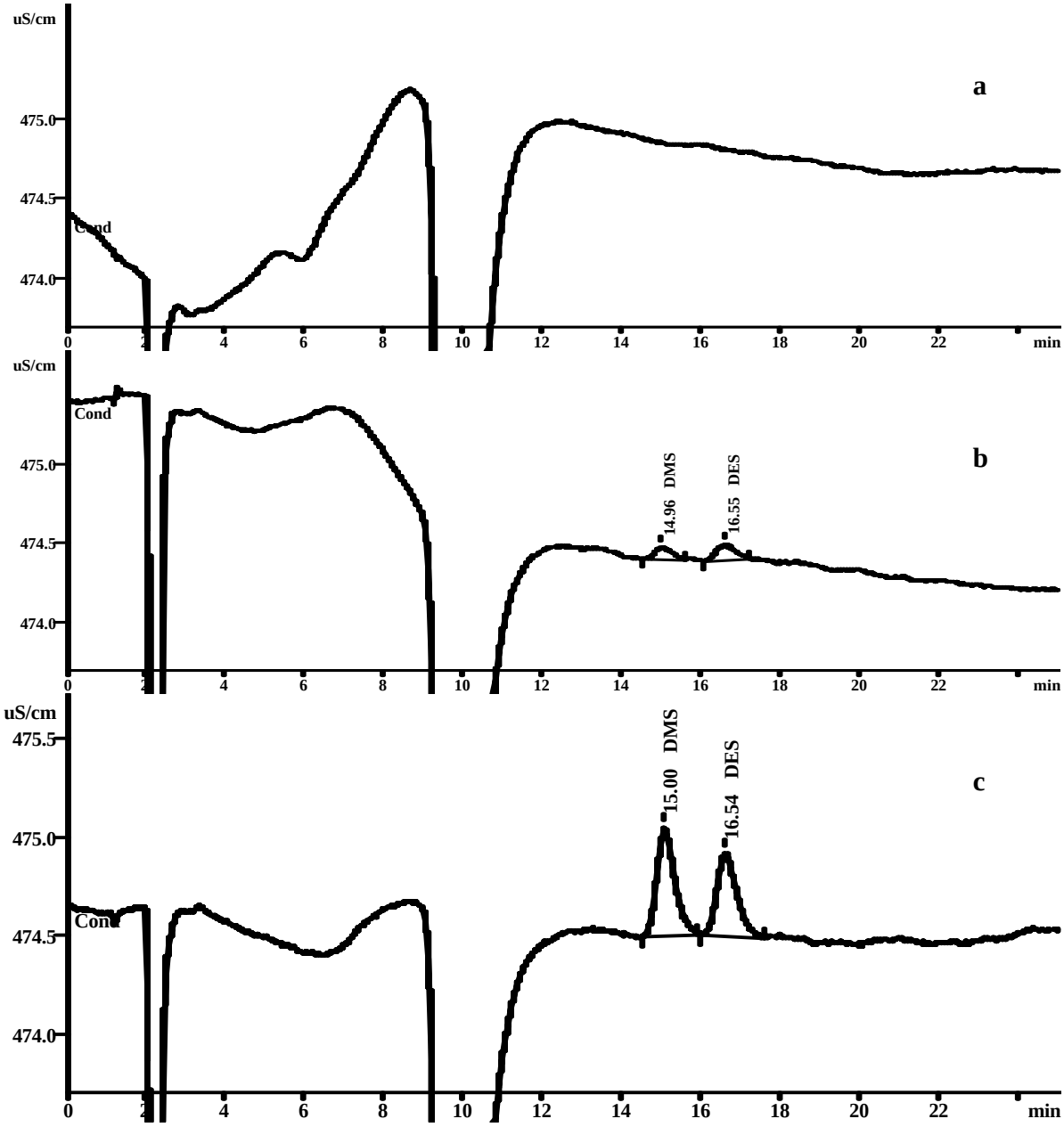

Fig 1. A typical representative overlay ion chromatograms of (a) Blank, (b) LOQ, (c) standard

Linearity

The linearity of the method was determined by taking the same data obtained in LOD and LOQ. The data was subjected to statistical analysis using a linear-regression model. The statistical parameters slope, intercept, residual standard on deviation response and correlation coefficient values are calculated and shown in Table 2.

Accuracy

Accuracy of the method was performed by recovery experiments using standard addition technique. The recoveries were determined by spiking the dimethyl sulfate and diethyl sulfate at three different levels ranging from 50% to 150% (with respect to 0.2% level) into Metoprolol tartrate drug substance. These samples were prepared as per the procedure, analyzed in triplicate and the percentage recoveries were calculated. The average recovery values were 101.3% and 101.0% for dimethyl sulfate and diethyl sulfate respectively. The completely validated accuracy results are shown in Table 3.

Table 3: Accuracy data of Dimethyl sulfate and Diethyl sulfate

S.No	Dimethyl sulfate			Diethyl sulfate		
	Level-I (50%)	Level-II (100%)	Level-III (150%)	Level-I (50%)	Level-II (100%)	Level-III (150%)
*Added (%w/w)	0.099	0.198	0.297	0.099	0.199	0.298
*Found (%w/w)	0.100	0.201	0.302	0.101	0.198	0.301
Recovery (%)	101.0	101.5	101.7	102.0	99.5	101.0
* %RSD	1.1	1.3	2.3	3.5	1.3	1.6

**Average of 3 replicates*

Precision

The precision was the study of the method using repeatability and reproducibility (ruggedness). The performance of the method was evaluated with replicate injections of standard and sample solutions. Standard solution was analyzed six times for checking the performance of the ion chromatography system under the chromatographic conditions on the day tested (System precision). The relative standard deviation for dimethyl sulfate and diethyl sulfate are 2.2% and 2.3% respectively. Repeatability was the intra-day variation (Method precision) and the relative standard deviation for the content of dimethyl sulfate and diethyl sulfate are 1.0% and 2.0% respectively. The intermediate precision was the inter-day variation (Ruggedness) and the relative standard deviation for the content of dimethyl sulfate and diethyl sulfate are 2.0% and 2.6% respectively. The repeatability and reproducibility of the method was studied by analyzing six sample solutions separately by adding dimethyl sulfate and diethyl sulfate at known concentration levels. The ruggedness of the method was defined as the degree of reproducibility obtained by the analysis of the same sample (which is used in the Method precision) under a variety of conditions using different series of column, with different analyst on different day by preparing new standards and new mobile phase. The experiment results of the precision (System precision, Method precision and Ruggedness) are shown in Table 4.

Solution stability

The sample solution was prepared by the addition of dimethyl sulfate and diethyl sulfate with known concentration level into Metoprolol tartrate drug substance. The stability of the solution was tested by recording the chromatograms freshly prepared and at different intervals with the gap of every one hour up to 24 hours at ambient temperature. The stability of solution was determined by comparing results of the freshly prepared sample solution. The results indicate that the sample solution was stable for 24 hours at ambient temperature.

Table 4: Statistical data of precision for Dimethyl sulfate and Diethyl sulfate

Repeatability (System precision) Area (mV*sec)		
	Dimethyl sulfate	**Diethyl sulfate**
1	17.146	14.881
2	18.076	14.512
3	17.028	15.466
4	17.138	14.764
5	17.196	14.613
6	17.185	14.738
Avg	17.295	14.829
SD	0.387	0.337
%RSD	2.2	2.3
Reproducibility (Method precision) *(%w/w)*		
1	0.197	0.195
2	0.199	0.197
3	0.202	0.201
4	0.202	0.201
5	0.203	0.201
6	0.199	0.191
Avg	0.200	0.198
SD	0.002	0.004
%RSD	1.0	2.0
Reproducibility (Ruggedness) *(%w/w)*		
1	0.198	0.201
2	0.197	0.199
3	0.197	0.192
4	0.206	0.197
5	0.202	0.188
6	0.196	0.199
Avg	0.199	0.196
SD	0.004	0.005
%RSD	2.0	2.6

CONCLUSION

A simple and sensitive ion chromatography method was developed and validated for the simultaneous determination of dimethyl sulfate and diethyl sulfate in Metoprolol tartrate drug substance. The results of various validation parameters demonstrated that the method is specific, stability indicating, sensitive, linear, precise and accurate. The proposed method is sensitive, simple and userfriendly, for the determination of dimethyl sulfate and diethyl sulfate contents in Metoprolol tartrate drug substance.

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