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Develop a Quantitative Analytical Method for low (≈ 1 ppm) levels of Sulfate

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ABSTRACT

Sulfate is used in the Pharmaceutical Industry to seal the surface of type one glass and is readily water-soluble. The widely used method for detection of Sulfate is a qualitative test based on a precipitation. New separation and detection capabilities now in use will allow the industry to use Ion Chromatography (IC) to quantitate residual sulfate at sub-ppm levels. The method presented is robust and linear from 125% to 25% of 1ppm sulfate.

Keywords: *Ion Chromatography, Anion Suppressor, Conductivity detector*

INTRODUCTION

Pharmaceutical companies utilize type 1 glass in the production of many of their drug products. This glass is sometimes treated with ammonium sulfate to seal the pores of the glass and prevent leaching of the materials within the glass into the solution. Prior to being filled with a drug product, the glass is processed by flushing with water. This process is to remove coatings and foreign materials. To assure the glass is clean enough for a drug product, it is tested for residual contaminants.

The current test method used by the majority of pharmaceutical companies is the one referenced in the United States Pharmacopoeia (USP). The USP method is based on a precipitation reaction and is subjective (clarity of solution) and is a qualitative test. One cannot quantitate, "no more turbid than the blank". A quantitative analytical method used for detection of low levels of sulfur derivatives is desirable for testing the effectiveness of the glass washing process in the production of pharmaceuticals. This would provide results that could be used to calculate what the affects would be on unbuffered product.

Some pharmaceutical products have excipients (sodium chloride) that contain known levels of sulfur contaminants and degradants while others do not. It is those products that do not contain sulfur derivatives that are of a major concern. Those products that do not contain some type of buffer, if filled in glass that contains sulfur residual, could experience changes to the compound. Changes include; pH shift, increased degradants, and precipitants (particulate matter).

MATERIALS AND METHODS

This study will involve the evaluation of the detection of Sulfate by ION Chromatography, to determine whether it will detect sulfate in the range of 1ppm or less and be suitable for use in a commercial laboratory environment.

This IC method will be evaluated to determine if it will detect the sulfate ion, and provide reproducible/ validatable results.

Materials

Ion Chromatography
Mobile Phase - Isocratic
1.8 mM Sodium Carbonate
1.7 mM Sodium Bicarbonate
Regeneration Solution - 43.2 mN Phosphoric Acid
Column – DIONEX IonPac AS9-SC (250 x 4 mm)
Detector – Alltech 350 Conductivity
Suppressor: Anion MicroMembrane Suppressor - AMMS-II
Regenerating solution Pump - Waters Model 510
HPLC 0 - 6000 psi
Mobile Phase pump - Spectra SYSTEM model P1500, by Thermo Separation
Auto Sampler - Spectra System model AS3000 by Thermo Separation products
Flow Rate – 1.5 mL/min for nominal conditions both solutions
Sample injection – 100µ L

Data was collected, processed, and printed using a PIONEX 50 MHz 486 PC, loaded with OS/2 for the operating system and PC1000 ver. 2.5 software for data analysis. All chromatograms were obtained at room temperature, and the temperature control in the unit was turned off. The target retention time is approximately 10 minutes, as listed in the DIONEX PRODUCT SELECTION GUIDE for determination of the anion, Sulfate. This retention time, during analysis, ranged from ≈ 10 to 14 minutes, depending upon the flow rate and concentrations of mobile phase and regeneration solution.

Reagents and Solutions

All reagents were analytical grade. All mixtures were prepared using high-purity deionized water. Water was prepared using a Milli-Q system (Millipore, Bedford, MA) and filtered through a 0.2 µm membrane filter. Deionizer readout at the time of preparation was NLT 18Ωcm.

Mobile phase and regeneration solutions were degassed under vacuum with stirring for NLT 15 minutes. A stock solution was prepared at a

concentration of 200ppm SO_4, from $NaSO_4$. Dilutions were made from this stock solution for Linearity, Level of Detection, Robustness, and Level of Quantitation analysis. Separate solutions were prepared for the accuracy/recovery analysis.

The following series of test will show that the method is acceptable to be used in the pharmaceutical industry. The pharmaceutical industry is required to Validate or certify the method will provided the required results accurately and repeatedly. The validation will consist of testing for, Linearity, Robustness, Limit of Detection, Limit of Quantitation and Accuracy /Recovery.

Accuracy of the analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Sometimes termed trueness. Accuracy for the test will demonstrated by adding known amounts of analyte to sample or placebo formulation representing approximately75%, 100% and 125% of the total theoretical concentration of the analyte. The analysis will be performed in triplicate at each interval. The target average recoveries should be 98% to 102% of the theoretical concentration at each interval tested. The RSD should be <2%.

The Linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. To determine method linearity, at least 5 sample or standard solutions ranging from approximately 50% to 150% of the working concentration should be analyzed. The test results should be linear with respect to the concentration of the analyte with a correlation coefficient of not less than 0.99.

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. To establish the LOD, sequential dilution and analysis of samples of a known concentration. The LOD is the lowest concentration of analyte which gives a signal to noise ratio of not less than three.

The limit of Quantitation of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The Quantitation limit is a parameter of quantitative assays for low levels of compounds. Samples will be prepared to contain target concentrations of approximately 0.05%, 0.1% and 0.5% of the analyte and tested in triplicate.

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. The solution concentrations will be shifted by ≈ 1%, the flow rates will be adjusted by ± 2 tenths.

RESULTS

The IC Suppressed conductivity method used in this experiment for the detection of SO_4 produced the following results.

TABLE 1. Linearity/LOQ/LOD - tests were performed under nominal conditions (flow rate 1.5mL/min; regeneration solution 43.2 mN; mobile at phase Na_2CO 1. 7mM and $NaHCO_3$ 1. 8 mM, Linearity results
Correlation coefficient = 0.990668
Y-intercept = 0.10002

Target conc ppm	Calc Conc Ppm	Avg. Resp	% RSD
1.25	1.2019	2657570	4.0
1.0	0.9118	2016187	1.07
0.75	0.7608	1682374	2.41
0.50	0.5958	1311337	6.49
0.25	0.2785	612955	10.96

TABLE 2. ACCURACY/RECOVERY - tests were performed under nominal conditions (flow rate 1.5mL/min; regeneration solution 43.2 mN; mobile at phase Na_2CO 1. 7mM and $NaHCO_3$ 1. 8 mM, a the specified % of 1ppm. Sample chromatograms; See Figure 1 for 75%, Figure 2 for 100%, Figure 3 for 125%.

Prep	%	RSD	% Recovery
A	125	0.21	153
B	125	0.88	195
C	125	0.97	153
A	100	1.51	222
B	100	0.58	208
C	100	0.29	180
A	75	0.48	226
B	75	0.89	302
C	75	0.49	224

TABLE 3. Robustness - each test required variations in sections of the methodology (the most efficient/accurate results were obtained from the slower flow rate at nominal solution concentrations). (RRT = Relative Retention Time; REC = Recovery) Where indicated, mobile phase (MP) is Na_2CO_3 and $NaHCO_3$ regeneration solution (RS) H_3PO_4. Data in table 3 are averages of three replicate injections. For Sample chromatograms, see figure 4 - MP @ 1.7/1.6 and RS @ 42.78; figure 5 -MP @ 1.9/1.8 and RS @ 43.63, figure 6 - flow @ 1.7ml/min, figure 7 - flow @ 1.3 mL/min.

Method change	RRT	%RSD	%REC
All at nominal	11.862	1.07	91.8
Flow@1.7mL/ min Sol at nominal	10.499	1.38	89.0

Method change	RRT	%RSD	%REC
Flow@1.3mL/ min Sol at nominal	13.73	10.0	94.9
Solutions @ MP 1.7/1.6 mM and RS 42.78 mN	12.319	1.66	104
Solutions @ MP 1.9/1.7 mM and RS 42.78 mN	14.273	0.216	104

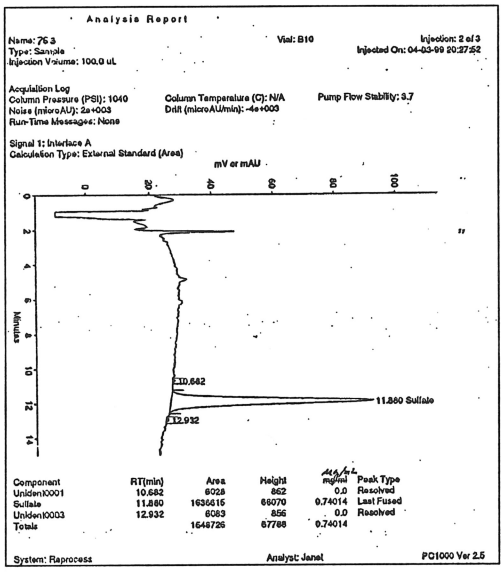

Figure 1. Accuracy/Recovery test = 75% of a 1ppm solution, nominal flow rates, MP, and RS.

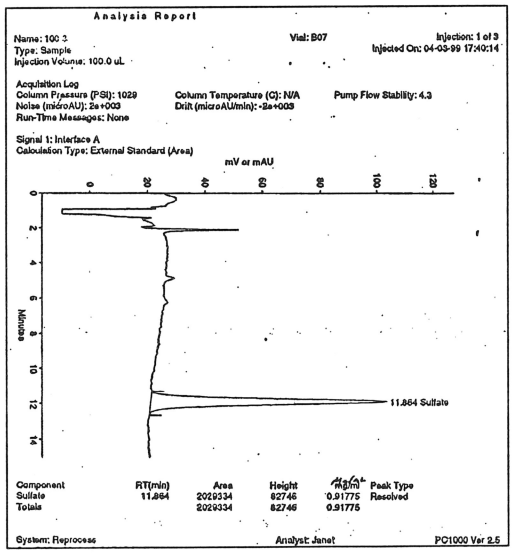

Figure 2. Accuracy/Recovery test = 100% of a 1ppm solution, nominal flow rates, MP, and RS.

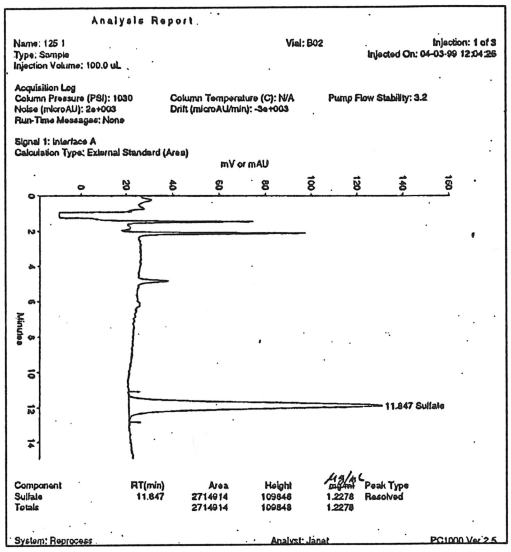

Figure 3. Accuracy/ Recovery test - 125% of 1 ppm solution, nominal flow rates, MP, and RS.

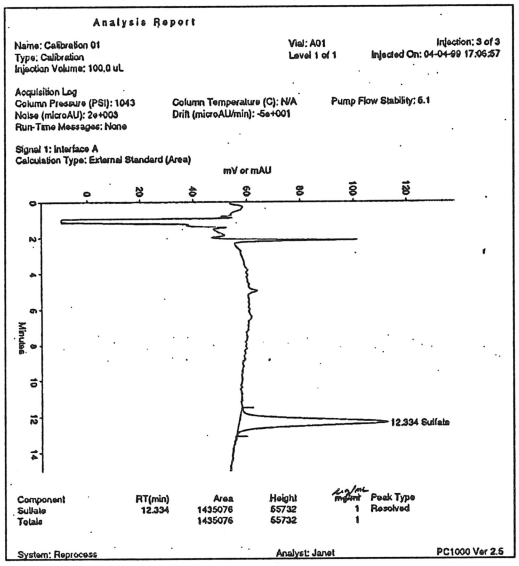

Figure 4. MP @ 1.7,1.6, and 42.7mN. Flow rate was at 1.5 ml/min.

DISCUSSION

The purpose of this project was to determine whether the stated method was capable of producing results in the range of 1 ppm of sulfate. The data collected show that the method is accurate, reproducible and validatible. Although results collected during the robustness showed variations, the statistics show the tests are acceptable, and these variations could be managed procedurally.

The results collected during the Accuracy/Recovery study indicate that there could have been a solution preparation error resulting in recovery of greater than 100%. This test should be repeated.

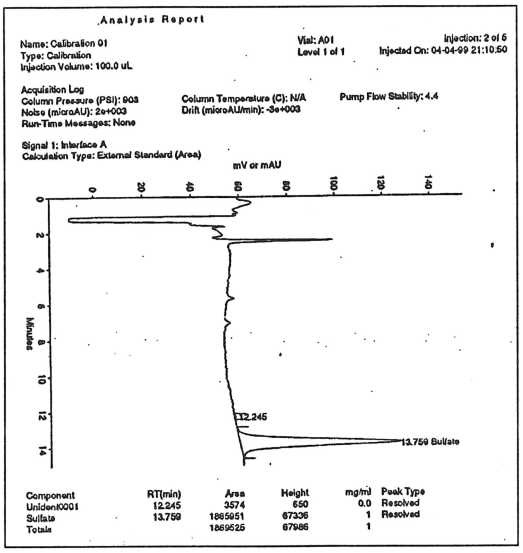

Figure 5. MP @ 1.9,1.8, and 43.63. Flow rate was at 1.5 ml/min.

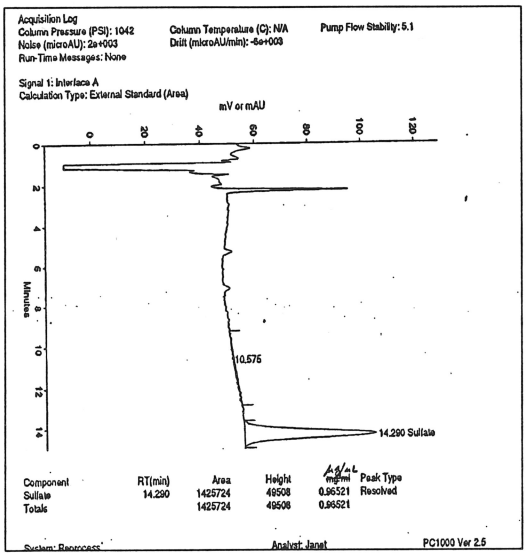

Figure 6 - MP @ 1.8,1.7mM, and 43.2mN. Flow rate was at 1.7 ml/min.

The results collected during the Linearity study indicate the method is linear from 125% to 25% of 1ppm SO_4 from a 200 ppm SO_4 stock solution.

The method appears to have an LOQ/LOD of ≅ 0.25ppm SO_4 , although with slight adjustments in the sensitivity of the instrument lower quantities could possibly be detected accurately.

The method has proven robust with modifications to increased/decreased flow rate, increase/decrease mobile phase concentration, and increase/decrease regeneration solution concentration. To optimize the method, an increase in flow to ≈ 1.7 or greater, and a lower concentration of mobile phase/regeneration solution could be implemented. It is not recommended

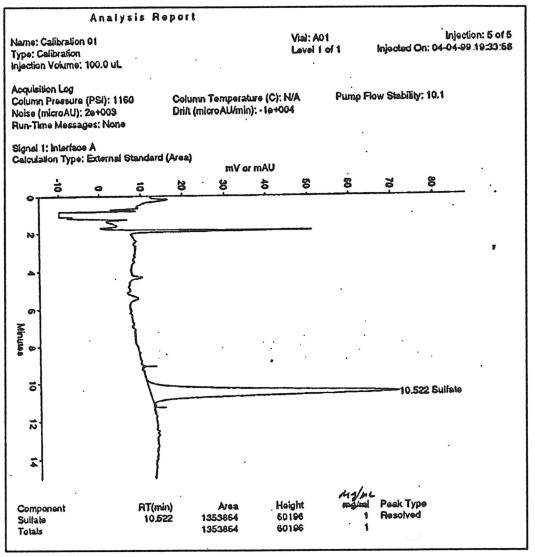

Figure 7. MP @ 1.8,1.7mM, and 43.2mN. Flow rate was at 1.3 ml/min.

to reduce the flow rate, because of the high % RSD calculated during that portion of testing.

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