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Research Article

Quantification of residual elemental Sulphur present in pharmaceutical ingredients by HPLC and UPLC

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ABSTRACT

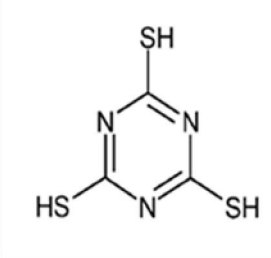
A simple, rapid and precise HPLC method was developed for the analysis of one of the Active pharmaceutical ingredient (API). Chromatographic separation of the same was performed by using a Zorbax Eclipsed XDB-C18 (150*4.6mm) 5.0µm as stationary phase with a mobile phase comprising of A : 0.05% Formic acid in water, Mobile Phase B : 0.05% Formic acid in Acetonitrile at a flow rate of 1.0ml/min and wavelength at 254nm. The column temperature was maintained at 40°C ± 2°C. The Run time and injection volume was found to be 55 min and 10µl respectively. The isolated material was injected in both HPLC and UPLC (method developed for fast analysis) for purity and it was observed to be ~97% area. Impurity was identified by studying ESI-MS, NMR, HR-MS, FT-IR, CHNSO analysis, ICP-OES and ROI analysis. A broad signal at ~3.4ppm was observed in ^{1}H-NMR spectrum. Then the CHNSO analysis is performed and the elemental Sulphur percentage is found to be 99.2 and also the ICP-OES analysis confirms the presence of residual elemental Sulphur in the API compound. The study concludes that the impurity present in above API was found to be Residual elemental Sulphur.

Keywords: HPLC, UFLC, ICP-OES, NMR and CHNSO.

INTRODUCTION

Heavy metals like Pb, Fe, Cd, Cu, Cr and Hg are used in the various forms, for the synthesis of Active pharmaceutical compounds in Pharmaceutical industry[1-3]. It is also important and critical to limit its levels in the final Active pharmaceutical product in PPM level. To meet the regulatory guidelines, pharma companies often use the precipitation techniques or Chelating agents to remove the heavy metals. TMT (Trimercaptotriazine) is used as a best chelating agent to remove the heavy metals from the synthetic compounds. In the TMT the elemental Sulphur is a possible impurity, which is very essential to remove from the TMT[4,5]. Elemental Sulphur is not toxic, but many simple Sulphur derivates are, such as Sulphur dioxide (SO_2)[8] and hydrogen sulfide are toxic[6-8]. Efforts are made to analyze the elemental Sulphur by various analytical techniques[9] and results are discussed in this paper.

Figure 1. Structure of 2,4,6-Trimercaptotriazine



Chemicals and Reagents

Trimercaptotriazine technical grade materials were purchased from Merck and Sulphur standard from Sigma Aldrich. The HPLC grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). Water was prepared using Millipore Milli-Q Plus water purification system (Bedford, MA, USA). Formic acid HPLC grades were purchased from Qualigens (Mumbai, India).

Equipment

1) Liquid chromatography was carried out on a Agilent technology HPLC system equipped with UV detector. The output signal was monitored and processed using Chemistation software. The chromatographic column used was Zorbax Eclipse XDB C18, length 150 mm, internal diameter 4.6 mm with 3.5µm particle size.
2) Liquid chromatography was carried out on a Shimadzu UFLC system equipped with UV detector. The output signal was monitored and processed using Shimadzu LC solutions software. The chromatographic column used was Shim pack XR ODS-II length 100 mm, internal diameter, 3 mm with 2.2 lm particle size.

Chromatographic conditions

In first section of study, isocratic mode of LC was used with mobile phase containing a mixture of 0.05% acidic modifier (or volatile reagent) in a mixture of water and acetonitrile. The flow rate of mobile phase was 1.0 mL/min. The column temperature was maintained at 40_ C and the detection was monitored at a wavelength of 254 nm. Samples were prepared in methanol at the concentration of 0.4 mg/mL. Injection volume was 10 µL. The run time for each set of injections was varied till last compound was eluted, but maximum 55 min in HPLC chromatogram. In second section of study, chromatographic conditions were changed as per the experimental design. Other chromatographic conditions remained as below.

1. OBJECTIVE

This report describes the structural identification of one impurity present Pharmaceutical ingredient sample which elutes at very non-polar by using RP HPLC method.

2. BACKGROUND

Pharmaceutical ingredient material was manufactured by using lab optimized modified process. Process is optimized with Trimercaptotriazine treatment to remove column operations used for removal of residual palladium and enhance the quality of API. The material was analysed as per the below chromatographic conditions. During analysis, it was observed that one non-polar impurity was eluting at very late retention time (even after method run time) and interfered in next run.

3. EXPERIMENTAL

To investigate the interference of impurity in consecutive runs, the sample was run for extended time by increasing the gradient programme for organic phase. By using this modified run time, gradient method, and the sample shown an unknown impurity. The sample was then analysed by LC-MS to identify the structure/ molecular formula, but the impurity was not ionized in both the negative and positive ionization modes.

The method details for identification by LCMS are as follows-

- Column : Zorbax Eclipsed XDB-C18 (150*4.6mm) 5.0µm
- Mobile phase : A: 0.05% Formic acid in water
  B: 0.05% Formic acid in acetonitrile
- Gradient (Time/%B) : 0/45, 15/65, 30/75, 50/75, 50.1/45, 55/45.
- Runtime : 55 min
- Flow : 1.0 mL/min
- Detection wavelength : 254 nm
- Injection volume : 10µl
- Column temperature : 40°C + 2°C
- Diluent : Methanol
- Sample conc. : 0.4 mg/mL
- Retention Time : 12.8 min.

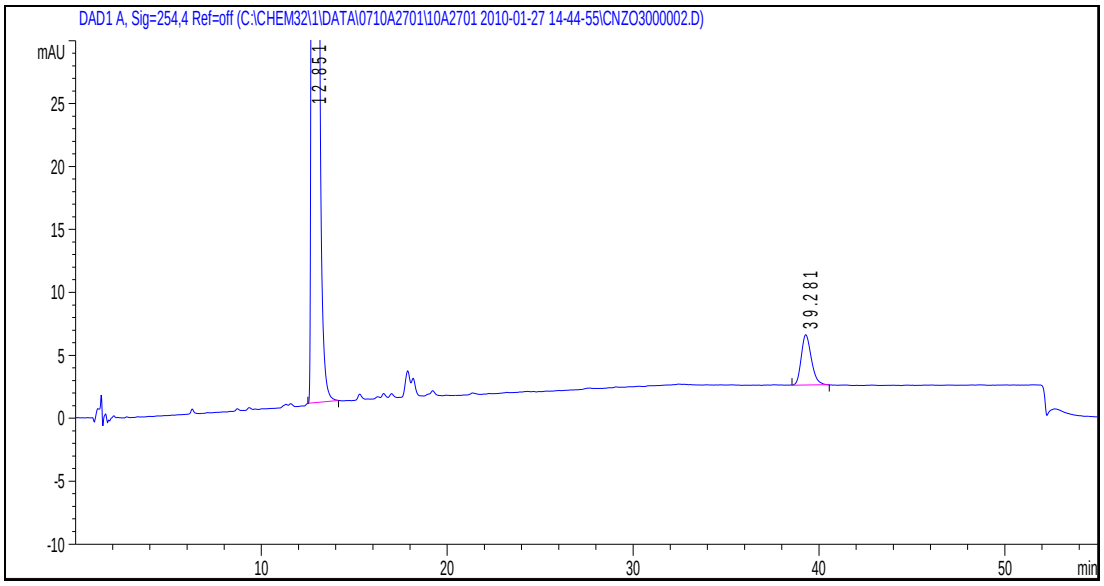


Fig. 2: HPLC chromatogram Unknown impurity was observed in the modified method

R&D has developed the method for removal of this impurity by using Hexane slurry wash, wherein it was significantly removed from ~0.9% level to less than 0.05%. This impurity was recovered from the reprocessed ML's by adding dichloromethane and isolated as solid material.

4. IDENTIFICATION OF IMPURITY

The isolated material was injected in HPLC for purity and it was observed to be ~97% area. It was then submitted to several other tests for identification. The details and observations are mentioned below

4.1. ESI-MS

ESI-MS analysis was carried out for the isolated impurity in positive and negative ionization modes, the data was not observed conclusive.

4.2. NMR

^{1}H-NMR analysis was performed for this isolated non-polar impurity. No significant signals were observed in $CDCl_3$ solvent with D_2O exchange.

4.3. HR-MS

HR-MS analysis was carried out for the isolated impurity as well as acetylated derivative in positive and negative ionization modes, the data was not observed conclusive in negative mode and there was no ionization observed in positive mode.

4.4. FT-IR

FT-IR analysis is performed for this isolated impurity by making pellet with KBr and dissolving the material in $CHCl_3$. There was no significant signal observed in FT-IR spectrum.

4.5. CHNSO analysis

As there was no clear information observed from above all techniques, this material was analyzed for CHNSO analysis. The results are as follows-

Table 1: Results from CHNSO analysis

Sample Name	%N	%C	%H	%S	%O
Sample - 1	NF	NF	NF	99.2	NF

Based on the above data it was concluded that only the elemental Sulphur might be present in isolated impurity.

4.6. ICP-OES Analysis

Simultaneously, the isolated impurity was analyzed for the trace metal analysis by using ICP-OES to get the information of all trace metals along with the Sulphur. The ICP-OES data is captured below-

Test Results

Table 2: Results from ICP-OES analysis

S. No.	Elements Analysed	Unit	Non polar impurity
1	Aluminium	ppm	13.7
2	Arsenic	ppm	<0.25
3	Boran	ppm	0.7
4	Cadmium	ppm	<0.25
5	Cobalt	ppm	<0.25
6	chromium	ppm	0.5
7	Copper	ppm	1.0
8	Iron	ppm	18.2
9	Mercury	ppm	<0.25
10	Potassium	ppm	0.5
11	Lithium	ppm	<0.25
12	Magnesium	ppm	9.5
13	Manganese	ppm	<0.25
14	Molybdenum	ppm	<0.25
15	Sodium	ppm	30.6
16	Nickel	ppm	8.0
17	Lead	ppm	<0.25
18	Palladium	ppm	<0.25
19	Platinum	ppm	<0.25
20	Ruthenium	ppm	<0.25
21	Sulphur	ppm	43745.5
22	Antimony	ppm	<0.25
23	Tin	ppm	<0.25
24	Strontium	ppm	0.8
25	Thallium	ppm	<0.25
26	Zinc	ppm	3.6

Based on the above data, Sulphur is majorly present in sample matrix and all other trace metals are below 35ppm.

4.7. Residue on Ignition (ROI) analysis

In parallel to CHNSO analysis, the material was subjected to ROI test to confirm the possibility of any residual inorganic metals in the material. The results shown that there was no residue present after ignition at 600°C.

RESULTS AND DISCUSSIONS

Based on all above tests, it was concluded that the material might be containing only or majorly residual Sulphur. It was obvious from CHNSO, and ICP-OES analysis. The ROI data confirms that the Sulphur might have oxidized in SO_2 and evaporated during heating at 600°C, due to which no residue was observed.

To confirm above conclusion, the process and the analytical data for various experiments was re-looked. It was observed that there is a change in process with respect to treatment in place of column purification to remove palladium to acceptable level. This impurity was observed in API samples only after treatment. To know the presence of non-polar impurity in TMT, it was injected in extended HPLC gradient conditions and it was observed that this impurity is present in TMT itself.

This was confirmed by performing the spiking studies using isolated non-polar impurity with TMT in the UFLC technique for can minimize the analysis time (*3 times lesser) and consumption of solvents for analysis (*6 times lower) than standard HPLC conditions, which subsequently will improve the productivity of the resources.

The UFLC chromatographic conditions method details are given below. This method can be used for the quantification of elemental sulphur after any laboratory experiment.

Column	: Shimpak ODS-II (100*3.0mm) 2.2µm
Mobile phase	: A:Water:Acetonitrile (45:55) B : Acetonitrile
Gradient (Time/%B)	: 0/50, 5/80, 8/100, 9.5/100, 10/50, 10.5/50.
Runtime	: 10.5 min
Flow	: 0.7mL/min
Detection wavelength	: 254 nm
Injection volume	: 4.0µl
Column temperature	: Ambient
Diluent	: Methanol
Sample conc.	: 0.4 mg/mL

It was concluded that the impurity present in TMT and pharmaceutical ingredients were eluting at same

retention time. The spiked UFLC chromatogram is attached below-

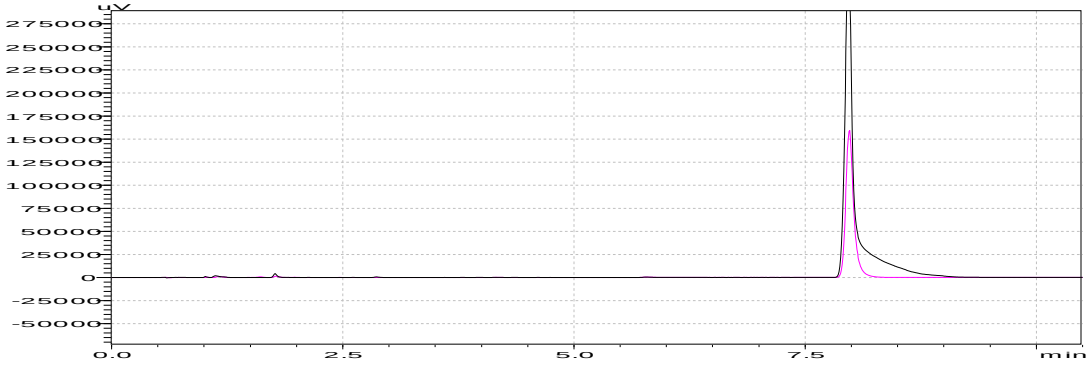
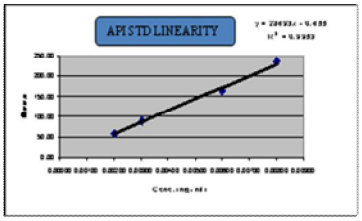


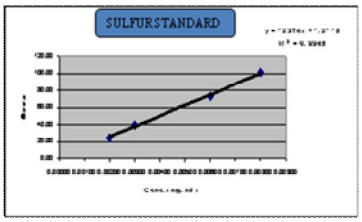
Fig. 3: Overlayed UFLC chromatogram of Unknown impurity and TMT impurity

The inset in Table 3 is the calibration curve for a series of elemental sulfur standards and the API. The points represent the area of the elemental sulfur peak for standard solutions with concentrations of 20, 30, 60 and 80 mg/L. The line through the points represents the best linear fit to these data using the method of least squares. The excellent fit to the data (*r*2) 0.999 is an indication of the highly quantitative nature of the HPLC analysis. The relationship between analyte concentration and peak area is linear throughout the investigated range from approximately 20 to 400 mg/L.

Table 3: the calibration curve for a series of elemental sulfur standards and the API

Linearity of API STD			
Conc.(mg/mL)	Level(%)	Area	Average
0.00200	50	58.46	58.65
		58.85	
0.00305	75	87.84	87.61
		87.37	
0.00601	150	164.17	164.14
		164.12	
0.00801	200	234.46	234.24
		234.03	
Linearity of Sulfur standard			
Conc.(mg/mL)	Level(%)	Area	Average
0.00201	50	25.58	25.44
		25.30	
0.00300	75	40.89	40.13
		39.38	
0.00600	150	72.92	72.92
		72.93	
0.00801	200	101.86	101.77
		101.68	





Quantification studies were performed by using UFLC for the impurity present in ingredient samples against standard Sulphur (Aldrich Make) and the results were captured in following table.

Table 4: Quantification of sulphur with %area after RRF correction

Quantification of Sulfur impurity in samples								
		Area						
	Standard-1	16416.00		Standard potency (%)	99.50	**STD conc**		
	Standard-2	16316.00		Wt. of STD	10.43	0.0042		
	Standard-3	16359.00						
	AVERAGE	16363.67		**RRF at 254nm**	**0.43**			
	SD	50.16						
	%RSD	0.31						
S.No.	**B.No**	**Injection**	**AREA**	**Weight (in mg)**	**Assay on as is basis (% w/w)**	**Avg.**	**Sample conc.**	**%area after RRF correction**
1	Batch-1	1	28986	39.78	1.85	**1.84**	0.3978	**1.95**
		2	28828	39.78	1.84		0.3978	
2	Batch-2	1	424	40.16	0.03	**0.03**	0.4016	**0.03**
		2	416	40.16	0.03		0.4016	
3	Batch-3	1	547	19.78	0.07	**0.07**	0.1978	**0.07**
		2	536	19.78	0.07		0.1978	
4	Batch-4	1	729.00	19.72	0.09	**0.09**	0.1972	**0.10**
		2	721.00	19.72	0.09		0.1972	
5	Batch-5	1	12179.00	40.78	0.76	**0.76**	0.4078	**0.77**
		2	12181.00	40.78	0.76		0.4078	

CONCLUSION

Based on all above results and discussions, it was concluded that the material might be containing only or majorly residual elemental sulfur. It was obvious from CHNSO and ICP-OES analysis. This HPLC and UPLC methods can be directly used for LC-MS analysis on need basis. The use of UFLC has proved that the method can minimize the analysis time (*3 times lesser) and consumption of solvents for analysis (*6 times lower) than standard HPLC conditions, which subsequently will improve the productivity of the resources. This impurity was observed in API samples only after treatment with TMT. To know the presence of non-polar impurity in TMT raw material, it was injected in extended HPLC gradient conditions and it was observed that this impurity is present in TMT raw material itself.

REFERENCES

1. Magnetic susceptibility of the elements and inorganic compounds, in Handbook of Chemistry and Physics. CRC press. 2000. ISBN 0849304814.
2. Henke KR. Chemistry and Environmental implications of Thio-Red and 2,4,6 Trimercaptotriazine compounds, Dissertations. Department of Geology and Geological engineering, University of north Dakota, Grand forks, ND, 1997:172.
3. Henke K and Atwood DA. Inorganic chemistry. 1998;37:224-227.
4. Matthew M. Matlock, Kevin R. Henke and David A. Atwood University of Kentucky Effectiveness of Commercial Heavy Metal Chelators with New Insights for the Future in Chelate Design. Journal of hazardous materials. 2002;92(2):129-142.
5. Kevin R. Henke, Aaron R. Hutchison, Matthew K. Krepps, Sean Parkin, and David A. Atwood. Chemistry of 2,4,6-Trimercapto-1,3,5-triazine (TMT): Acid Dissociation Constants and Group 2 Complexes. Inorganic Chemistry. 2001;40(17):4443-4447.
6. Victor W. Rosso, David A. Lust, Peter J. Bernot, John A. Grosso, Sandeep P. Modi, Andrew Rusowicz, Thomas C. Sedergran, James H. Simpson, Sushil K. Srivastava and Michael J. Humora, Transition. 1997;1(4):311-314.
7. Evangelou VP and Zhang YL. Crit Rev Environ Sci Technol. 1995;25:141-199.
8. Giraud E, Ivanova A, Gordon CS, Whelan J and Considine MJ. Sulphur dioxide evokes a large scale reprogramming of the grape berry transcriptome associated with oxidative signalling and biotic defence responses. Plant Cell Environment. 2011 Ju1 18.
9. Mathieu Verbeken, Sultan Suleman, Bram Baer, Elien Vangheluwe, Sylvia Van Dorpe, Christian Burvenich, Luc Duchateau, Frans H Jansen and Bart De Spiegeleer. Stability-indicating HPLC-DAD/UV-ESI/MS impurity profiling of the anti-malarial drug lumefantrine. Malaria Journal. 2001;10:51.