Experimental Evaluation of Genotoxic Impurity in Risedronate by Gas Chromatography

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ABSTRACT

The pharmaceutical industry is expanding daily with the goal of creating new therapeutic compounds through chemical synthesis or from natural sources, but one crucial feature has never changed: the product must be as pure in terms of quality as possible. Therefore, purity has always been regarded as a crucial component in ensuring the quality of any pharmaceutical medicinal material or drug product. It is essential that such products are safe, effective, and of good quality as these are directly consumed by human beings. There is no drug in the world that is not harmful or even poisonous at high doses. The quality of a drug substance or drug product is impacted by the presence of contaminants. Genotoxicity is a word used to describe any adverse change to the genetic code, independent of the process that caused the change. Genotoxicity, as used in genetics, refers to a feature of chemical agents that alters the genetic material in a cell, resulting in mutations that may cause cancer. The objective of this work is to create a simple gas chromatographic analytical method for the detection of morphine and thiomorpholide in risedronate sodium at trace levels.

Keywords: *genotoxic; gas chromatography; risedronate*

INTRODUCTION

USP (United States Pharmacopeial), defined Impurity, as any component of a drug substance that is not the chemical entity defined as the drug material, a drug product, and any component that is not a formulation ingredient. Synthetic/manufacturing processes, excipients, degradation, storage conditions, and cross-pollution are the main source of impurities in API or drug products. Impurities can be recognized or unrecognized, non-volatile or volatile, and inorganic or organic moieties.

Impurity sources are categorized meticulously, to fulfil the regulatory requirements and to separate an impurity that is basically resulting from API alone or in fact resulting from excipient-API/excipient-excipient/solvent residues-API/ excipient-excipient impurity interactions.

Genotoxicity is defined as a genetic change in DNA structure. Genotoxic impurities are DNA reactive or react with genetic material through mutations. Impurities consist of functional groups which are electrophilic in their nature and can react with DNA. The mutations are similar to the rupture of chromosomes, impurity interaction with DNA during the replication process, and rearrangement of chromosomes, and this alteration leads to a potential for the root cause of cancer in humans.

During the synthesis of pharmacological compounds, chemicals built using the synthetic pathway, raw materials, reagents, catalysts, solvents, intermediates, and other processing aids may produce genotoxic contaminants. Unexpected cross-interactions between processing materials and degradants are another way that genotoxic contaminants might become more prevalent.

MATERIALS:

- Morpholine and Thiomorpholide: Sigma Aldrich
- Methanol: Rankem -GC grade
- Samples of Risedronate sodium: local market.

Instrumentation:

- GC Agilent 6890B with autosampler
- Detector- FID detector.
- Carrier Gas: Helium

Preparation of standard solutions:

Weighed and transferred 25.0 mg each of Morpholine and Thiomorpholide to ten mL volumetric flask dissolved in methanol.

Taken 1mL above solution in 50mL flask and diluted to 50mL using diluents.

Test solution:

Weighed about 500 mg of Risedronate sodium drug substance into ten mL VF, and makeup with methanol, for shake well to dissolve and filter the solution. Use a clear solution for injection.

EXPERIMENTAL RESULTS AND DISCUSSION:

System suitability and Specificity

The blank (Diluent), six standard solutions were prepared and injected for system suitability and % RSD is less than 2% as shown in Table 1. The samples, individual solvents, individual impurities solutions and spiked sample solutions were prepared to check the interference with the main analyte and found no interference as shown in Table 2.

Table 1: System suitability

Injection No.	Morpholine Area	Thiomorpholide Area
1	43768	40561
2	43677	40871
3	42799	41239
4	44239	40912
5	43898	41098
6	43781	40165
Mean	43693.7	40807.7
SD	480.21	389.64
%RSD	1.10	0.95

Table 2: Specificity

	Standard solution		Spiked sample solution	
Name	RT (minutes)	RRT (minutes)	RT (minutes)	RRT (minutes)
Methanol	1.342	1.00	1.345	1.00
Morpholine	5.007	3.73	5.002	3.72
Thiomorpholide	22.264	16.59	22.259	16.55

The solvents used in the reaction i.e. Acetone, Methanol, and Ethanol, does not show any interference with the retention time of any of the peak of interest.

Detection Limit and Quantitation Limit:

A progression of a low-level concentration of the impurity of Morpholine and Thiomorpholide solutions was prepared and injected into gas chromatography, and chromatograms were recorded. From the obtained peak area results the slope, intercept and % RSD was calculated and predicted the LOD, and LOQ values. Six injections of LOQ were injected into GC and recorded the results with chromatograms. LOD solution was injected in three replicates which were detected, and the obtained results are presented in Tables 3 and 4.

Table 3: LOD results:

Injection No.	Morpholine Area	Thiomorpholide Area
1	4465	4157
2	4378	4178
3	4413	4098
Conc (ppm)	100	100

Table 4: LOQ Precision

Injection No.	**Morpholine Area**	**Thiomorpholide Area**
1	13395	12478
2	13456	12562
3	13468	12498
4	13427	12512
5	13398	12477
6	13461	12588
average	13434.2	12519.2
standard deviation	32.4	45.9
%RSD	0.24	0.37
Conc (ppm)	300	300

Repeatability

Six sample solutions were prepared by spiking with a standard solution at 100% concentration level to show the repeatability of the sample injections. The %RSD value is represented in Table 5.

Table 5: Precision results

Inj. No.	Morpholine (%)	Thiomorpholide (%)
1	0.1	0.1
2	0.1	0.1
3	0.1	0.1
4	0.1	0.1
5	0.1	0.1
6	0.1	0.1
Mean	0.1	0.1
SD	0.00	0.00
% RSD	0.00	0.00

Ruggedness:

The test was carried out by another day of method precision with another analyst using another instrument and another column. Six sample solutions were prepared by spiking with a standard solution at a 100% concentration level. The %RSD value is represented in Table 6.

Table 6: Intermediate results

Inj. No.	Morpholine (%)	Thiomorpholide (%)
1	0.1	0.1
2	0.1	0.1
3	0.1	0.1
4	0.1	0.1
5	0.1	0.1
6	0.1	0.1
Mean	0.1	0.1
SD	0.00	0.00
% RSD	0.00	0.00

Table 7: Cumulative RSD

Solvent	% RSD in Repeatability	% RSD in Intermediate precision	% Cumulative RSD (Twelve preparations)
Morpholine	0.00	0.00	0.00
Thiomorpholide	0.00	0.00	0.00

Batch Analysis and Accuracy:

As such sample prepared and injected into GC system and the results showed in Table 8. The sample was spiked at LOQ, 50%, 100% and 150% levels of the standard concentration. Each level was spiked in triplicate and %

recovery was calculated. The obtained results were in the range of 97 to 99 for Morpholine and 95 to 99 for Thiomorpholide % and a result represented in Table 9 and 10.

Table 8: Batch analysis (sample without spiking) Area in as is sample

Sample	Morpholine Area	Thiomorpholide Area	Morpholine Content (ppm)	Thiomorpholide Content (ppm)
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
Mean			0	0

Table 9: Morpholine Recovery results

Accuracy level	**Amount added**	**Amount present**	**Amount found**	**% Recovery**
	7.48	0.0	7.33	98.0
LOQ	7.48	0.0	7.35	98.3
	7.48	0.0	7.31	97.7
	12.47	0.0	12.33	98.9
50	12.47	0.0	12.27	98.4
	12.47	0.0	12.15	97.4
	25.01	0.0	24.87	99.4
100	25.01	0.0	24.76	99.0
	25.01	0.0	24.66	98.6
	37.56	0.0	37.23	99.1
150	37.56	0.0	37.11	98.8
	37.56	0.0	37.05	98.6
		Mean		98.5

Table 10: Thiomorpholide Recovery results

Accuracy level	**Amount added**	**Amount present**	**Amount found**	**% Recovery**
LOQ	7.51	0.0	7.22	96.1
	7.51	0.0	7.17	95.5
	7.51	0.0	7.27	96.8
50	12.52	0.0	12.29	98.2
	12.52	0.0	12.22	97.6
	12.52	0.0	12.18	97.3
100	25.1	0.0	24.76	98.6
	25.1	0.0	24.59	98.0
	25.1	0.0	24.66	98.2
150	37.49	0.0	37.22	99.3
	37.49	0.0	37.04	98.8
	37.49	0.0	36.99	98.7
Mean				97.8

Robustness

The Gas chromatographic analysis carried out for system suitability by altering some of the chromatographic conditions as follows:

1. Change in carrier gas flowrate;

a. Flowrate altered to 3.6 mL/min instead of 4.0 mL/min b. Flowrate changed to 4.4 mL/min instead of 4.0 mL/min

2. Change in initial column oven temperature;

a. Oven programme altered to 81°C instead of 90°C b. Oven programme altered to 99°C instead of 90°C.

Table 11: Robustness results

Conditions	% RSD
Unaltered	1.16
Flow (3.6 mL/min) Morpholine	1.78
Flow (3.6 mL/min) Thiomorpholide	1.82
Flow (4.4 mL/min) Morpholine	1.91
Flow (4.4 mL/min) Thiomorpholide	1.85
Oven programme 81°C (Morpholine)	1.55
Oven programme 81°C (Thiomorpholide)	1.45
Oven programme 99°C (Morpholine)	1.62
Oven programme 99°C (Thiomorpholide)	1.56

Solution stability

The Spiked Sample prepared and kept at room temperature for 24 hours and injected the solution in GC system for every 12 hrs to check the solution stability. The difference is not observed to the initial value and the results tabulated in Table 12.

Table 12: Solution stability results

Time interval	Results obtained (%)	Results obtained (%)
	Morpholine	Thiomorpholide
Initial	0.1	0.1
12hrs	0.1	0.1
24hrs	0.1	0.1

CONCLUSION

Based on the results generated for developed analytical methods and followed by validation for Nitrofurantoin, Aripiprazole, Febuxostat, Levodropropizine, Risedronate, and Dronedarone for genotoxic impurities are precise, rugged, linear, accurate and robust and can be used for intended purpose.

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