METHOD DEVELOPMENT FOR THE ESTIMATION OF IFOSFAMIDE FOR INJECTION 1000 mg BY RP-HPLC

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Abstract:

Ifosfamide is the drug used in chemotherapy for treating varipus types of cancer including Lymphoma. The main aim of this research is to develop an RP-HPLC method for the quantification of Ifosfamide for injection 1000mg. This method is designed to be specific simple, precise, & economical featuring a lesser runtime making it ideal for routine quality control applications. Method was achieved with Welchrom XB-C18, 250 x 4.6mm 5□m column with run time 15 min at a flow rate 1.5ml/min. Retention time was obtained at 2.782 min. Detector wavelength was set at 195nm. The estimated percent of Ifosfamide in its marketed formulation was found to be 96.91%. While a limited number of analytical methods exist for estimating the combination, there remains a need for a validated and efficient RP-HPLC method that ensures Accuracy, Consistency, System Suitability speed, sensitivity, and cost-effectiveness. Consequently, the author aimed to develop a robust assay method capable of quantifying the drugs in commercial pharmaceutical dosage form.

Key words: Isosfamide, RP-HPLC, Assay method, & Anti-cancer drug.

1. Introduction:

Ifosfamide was sold under the brand **Ifex,** is a chemotherapeutic medication employed in treating various types of cancers such as testicular cancer, ovarian cancer, soft-tissue sarcoma,osteosarcoma, bladder cancer, small cell lung cancer, and cervical cancer. Administration is by intravenous route and also causes side effects that includevomiting, hair loss, blood in urine, kidney problems and infections [1-15]. Other side effects comprise suppression of bone marrow and lower levels of consciousness.

Ifosfamideworks by disrupting DNA duplication and creation of RNA. Its IUPAC name is 3-(2- Chloroethyl)-2-[(2-chloroethyl)amino)]tetrahydro-2H-1,3,2-oxazaphosphorine-2-oxide with chemical formula $C_{17}H_{15}Cl_2N_2O_2P$ and molecular weight of 361.08g/mol. It is apowder of white in colour and sparingly soluble in water, moderate in alcohol [16-22].

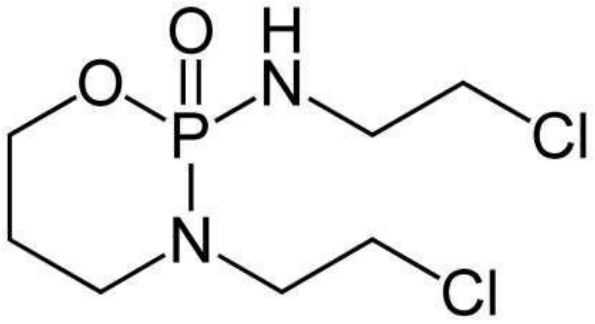

Figure 1. Chemical structure of Ifosfamide

This comprehensive literature review [23-36] investigates the physicochemical properties of Ifosfamide and presents various analytical methodologies employed for its quantification. This foundational work facilitated the validation [37] of an innovative reverse-phase high-performance liquid chromatography (RP-HPLC) method for the quantification of Ifosfamide.

2. Experimental part:

2.1 Chemicals, Reagents and Solvents:

Table 1. Chemicals, Reagents & Standards

Chemicals & Solvents Standards	Make	Grade	Batch No.	Assay
Water	Sigma Aldrich	HPLC	SHBM6418	99.5%
Acetonitrile	Merck	HPLC	A0313111410	>99.90%
Ifosfamide Working Standards	Sigma Aldrich	IHS	WS-058	100%
Ifosfamide for injection 1000mg	Baxter Healthca re Ltd.	IHS	BUY1062	-

2.2 Analytical Instrumentation & Equipments:

Table 2. Analytical Instrumentation & Equipments

S.No	Instrument	Make, Model & Details	Identification No.
1.	HPLC	Waters e2695/Alliance Series	ARD/LC/2021009
2.	HPLC-Column	Welchrom XB-C18, 250 x 4.6mm 5□m	CLL/CCC/64/23
3.	Analytical Balance	Sartorius	ARD/BAL/201806
4.	Sonicator	PCi Ultrasonic bath chiller Model	ARD/SC/2021005
5.	Shakers and Mixers	Thermo Scientific - MaxQ™ 4450	ARD/MIX/TFS/09

2.3 Chromatographic Conditions:

Table 3. Chromatographic Conditions

Column	Welchrom XB-C18, 250x4.6 mm, 5□m
Flow rate	1.5ml/min
Wavelength (□max)	195 nm
Run time	15 minutes
Column Temp.	25ºC
Sample Temp.	5ºC
Injection volume	20□L
Elution	Isocratic
Diluent	Water:Acetonitrile 70:30

2.4 Sample, Standards & Solutions Preparations

Mobile Phase: Water and acetonitrile was mixed in 70:30 ratio.

Diluent: Mobile phase itself was used as diluent.

Preparation of Solutions:

i) Reference solution-1: Weighed accurately 60.71 mg of Ifosfamide standard into volumetric flask of 100ml. 25ml of diluent was added to it and the final volume was made till the mark with the diluent and mixed well.

ii) Reference solution-2: 60.78g of Ifosfamide standard was weighed into volumetric flask 100ml. Later, 25ml of diluent was added and sonicated. The final volume was made upto the mark with the diluent.

iii) Sample solution-1: An accurately counted Ifosfamide for injection vials equivalent to 6.03121 g of Ifosfamide was transferred into volumetric flask of 1000ml and the final volume was made till the mark with diluent. 10ml of the above solution was transferredand diluted to 100 mL in a volumetric flask and mixed well.

iv) Sample Solution-2: An accurately counted Ifosfamide for injection vials equivalent to 6.05685 g of Ifosfamide was taken into volumetric flask of 1000ml and the final volume was made till the mark with the diluent. 10ml of the above solution was taken and diluted to 100 mL in a volumetric flask and mixed well.

3. Results and Discussion

3.1 Data summary of specificity & system suitability

Table 4. Specificity & System Suitability Summary Data

Specificity & System suitability		**Results**	**Acceptance criteria**
Specificity			
Parameter	Standard Solution		RT obtained with the sample solution should be comparable with standard.
Identification & Retention Time (RT) Conformation.	Ifosfamide1 & 2	5.996 & 5.997	
	Sample Solution		
	Ifosfamide for inj 1000 mg	5.997	
Peak purity index		Single point threshold	Peak purity should pass. Peak purity index should be greater than single point threshold.
Standard Solution Peak Purity	1.0000	0.9999	
Sample Solution Peak Purity	0.9999	0.9999	
Blank and Placebo Solution. Interference	Ifosfamide	NIL	Blank and placebo solution should not elute any peak at the RT of analyte peak.
System suitability			
% RSD for area of replicates of Ifosfamide Standard solutions 1 & 2respectively		0.051 &0.085	% RSD should be NMT 2.0%
The % RSD for RT of 2 replicate injections of the Ifosfamide samplesolution		0.007	% RSD for RT should be NMT1.0%
The Number of theoretical plates for main peak in Ifosfamide standard solutions		8300 &8368	The theoretical plates for main peak should be NLT 2000

1 & 2		
Tailing factor for Standard solutions 1 & 2 respectively	1.075 &1.075	The tailing factor for main peak in standard solution shouldbe NMT 0.8 and 2.0

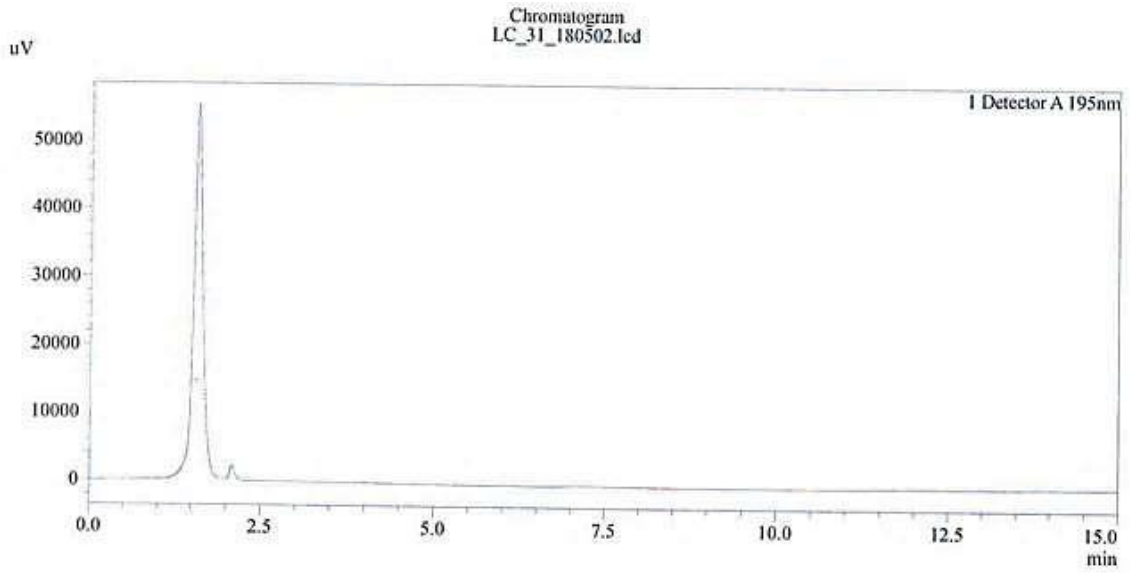

Figure 2. Chromatogram for Blank

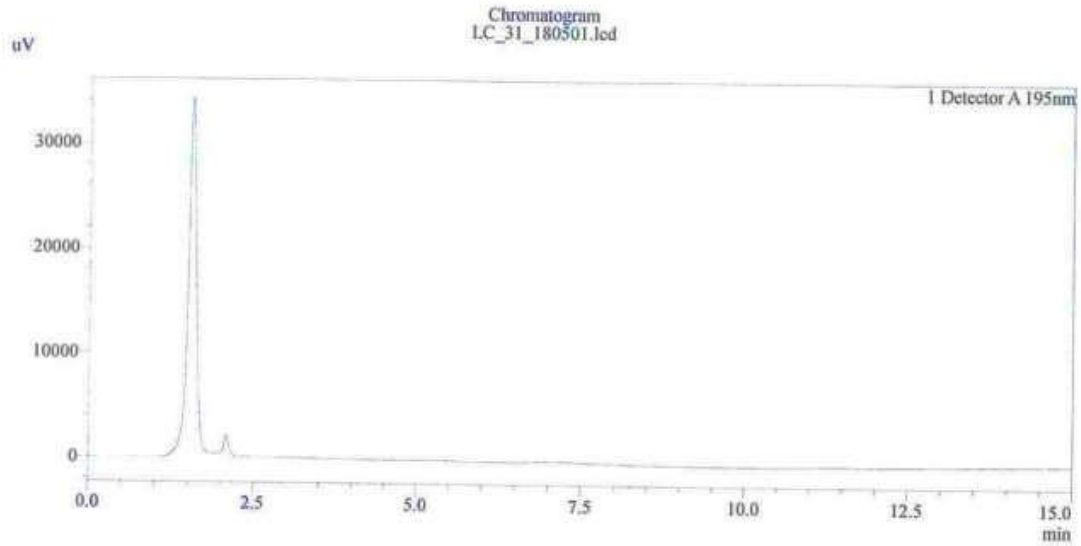

Figure 3. Chromatogram for Placebo

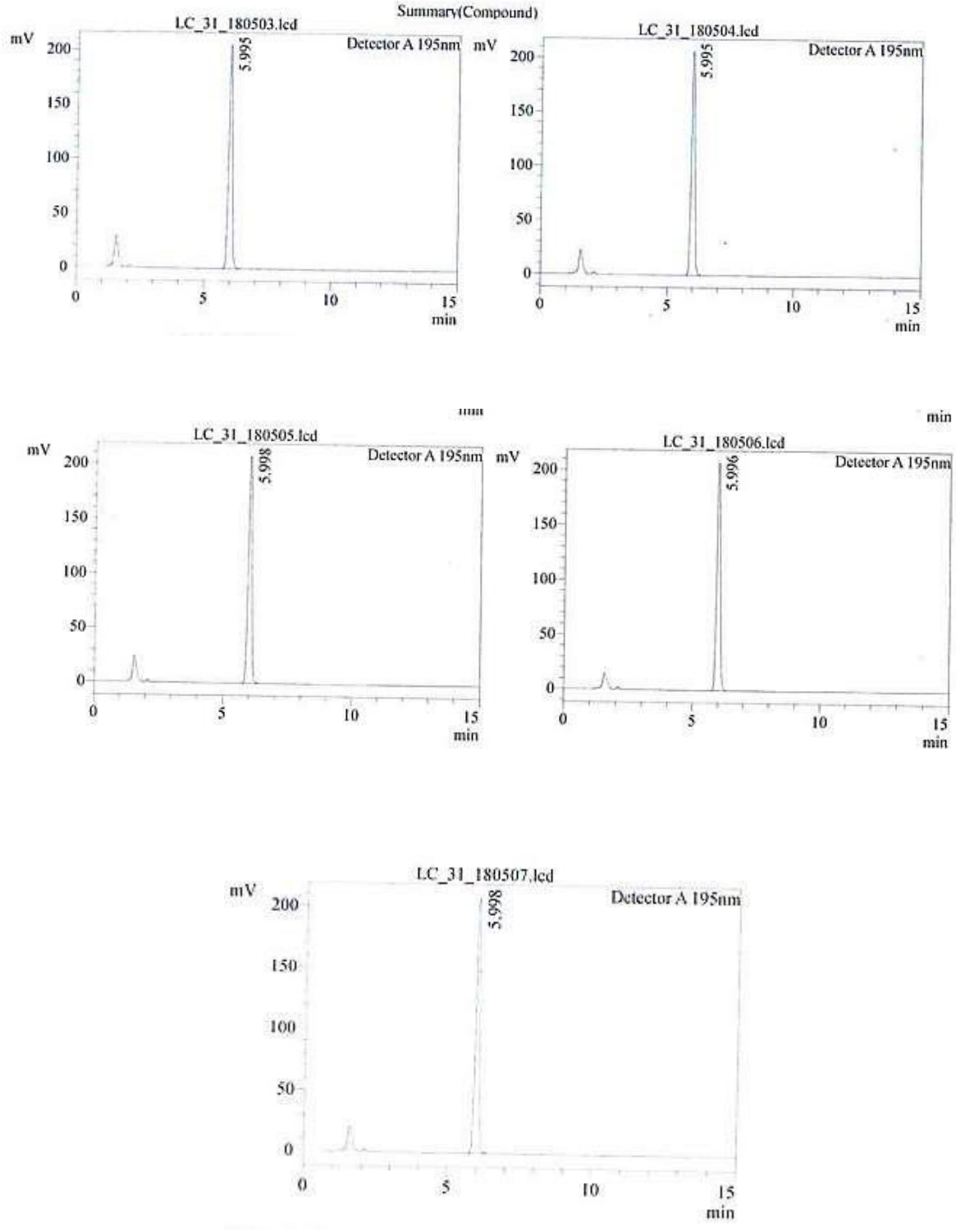

Figure 4. Chromatograms for Standard solution-1

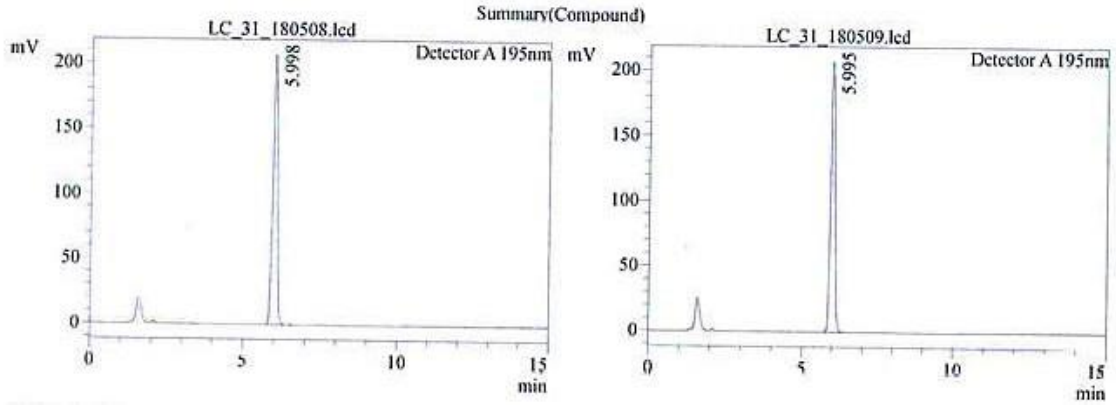

Figure 5. Chromatograms for Standard solution-2

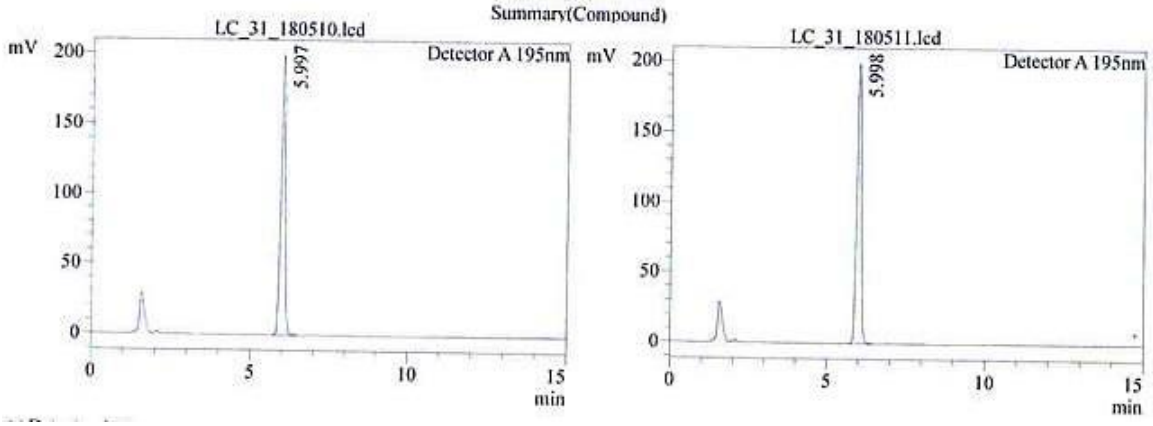

Figure 6. Sample Solutions 1&2 chromatograms

Table 5. System Suitability of Standard Solution-1

Solutions	RT	Area	Tailing factor	Theoretical plates
Standard solution injection-1	5.995	1993869	1.074	8218
Standard solution injection-2	5.995	1996282	1.075	8261
Standard solution injection-3	5.998	1996354	1.075	8336
Standard solution injection-4	5.996	1995901	1.076	8317
Standard solution injection-5	5.998	1995505	1.075	8346
Mean	5.996	1995582	1.075	8300
Standard deviation	0.002	1016	0.001	52
% Relative Standard deviation	0.027	0.051	0.063	0.626
Bracketing Standard solution	6.004	1994022	1.078	8406

Table 6. System Suitability of Standard Solution-2

Solutions	RT	Area	Tailing factor	Theoretical plates
Standard solution injection-1	5.998	1992044	1.074	8343
Standard solution injection-2	5.995	1989662	1.076	8392
Mean	5.997	1990853	1.075	8368
Standard deviation	0.002	1684	0.001	34
% Relative Standard deviation	0.028	0.085	0.129	0.412

Table 7. Ifosfamide Sample Solution Specificity

Solutions	RT	Area	Tailing factor	Theoretical plates
Sample solution injection-1	5.997	1909461	1.076	8394
Sample solution injection-2	5.998	1910647	1.076	8381
Mean	5.997	1910054	1.076	8387
Standard deviation	--	838	--	9
% Relative Standard deviation	0.007	0.044	0.003	0.102

3.2 Precision

Precision expresses the closeness of agreement between a series of results that wereobtained from multiple sampling of the homogeneous sample as prescribed.

System Precision:

This indicates the performance of the system when multiple injections of homogeneous standard solution were given under prescribed chromatographic conditions. System precision studies were carried out by standard solution single preparation and injecting the sample under the same conditions with six determinations.

Method Precision:

Method Precision studies were carried out with test solution of six preparations andinjecting the sample under the same chromatographic conditions.

Table 8. Precision summary data

System precision	**Results**	**Acceptance limits**
% RSD for area of the standard replicate injections	0.056	% RSD should be NMT 2.0%
No. of theoretical plates for main peak in standard solution	8406	Theoretical plate count should be NLT 2000
Tailing factor of standard solution	1.078	The tailing factor for main peak in standard solution should be NMT 0.8 and 2.0
Method precision		
Calculated %RSD for % Assay content of Ifosfamide from six samples preparations	0.055	%RSD should be NMT 2.0 with all the individual values within limit.

3.3 Proposed procedures for marketed Pharmaceutical Formulation:

The Marketed Formulation (Ifosfamide for Injection 1000mg **– Holoxan-1**) was analysed separately by injecting 10µL of standard and sample solutions into the HPLC system. The quantity of drug presented in formulation was calculated by comparison of peak area of standard to that of the sample.

Table 9. Results of Assay in dosage form

Formulation	**Batch No**	**Label claim(mg)**	**% Assay***	**Estimated amount**
Holoxan – 1	IFUY 1090	Ifosfamide for injection 1000mg	96.916	969.16 mg

*= Average assay % of 3 replicate injections of individual batch.

Conclusion

The precision results reflected minimal variability, underscoring the method's reliability for routine analysis. Key parameters such as specificity and precision were within acceptable limits, confirming the method's efficacy in quantifying Ifosfamide accurately. Overall, the HPLC method provided a comprehensive analytical tool for the quality control of Ifosfamide for injection 1000mg ensuring that pharmaceutical products meet regulatory standards.

Conflict of Interest

The authors declared that there was no conflict of interest.

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