Available Online at

http://www.ijcpa.in

July-September 2021

International Journal of CHEMICAL AND PHARMACEUTICAL ANALYSIS

eISSN: 2348-0726; pISSN: 2395-2466

DOI: http://dx.doi.org/10.21276/ijcpa

Research Article | Volume-8 | Issue-4 | Article ID: 0063

UPLC-HRMS BASED SCREENING METHOD FOR DETERMINATION OF NON-VOLATILE LEACHABLE IMPURITIES IN NON-AQUEOUS PARENTERAL FORMULATIONS HAVING COMPLEX MATRIX i.e., CASTOR OIL

Dhruvkumar Patel*, Dnyanoba Madrewar, Dushyantkumar B Patel, R. Suryanarayanaa, Petla Y Naidu

Department of Injectable R&D, Alembic Pharmaceuticals Limited, Vadodara, India

***Corresponding Author:** *E-mail:* **dhruv.patel@alembic.co.in**

Received date: 28 July 2021; ***Accepted date:*** 11 August 2021; ***Published date:*** 18 August 2021

ABSTRACT

As per USP guideline <1664>, assessment of leachables for parenteral formulations associated with primary packaging/delivery system is being mandatory. Leachables are foreign organic and inorganic chemical entities that are present in a packaged drug product because they have leached from either direct or indirect contact of formulation under normal conditions of storage and use or during accelerated drug product stability studies. Leachables can potentially affect drug product efficacy, safety and quality hence the quantitative determination of leachables impurities in parenteral formulation is essential. UPLC-HRMS (QToF) is used for detection and quantitation of organic non-volatile leachables at ppb level. Full scan method with scan range from 100 m/z to 1500 m/z was developed at 50% AET level with concentration of 0.075 ppm. This research paper presents the development and verification of screening method for determination of non-volatile organic leachables from complex matrix containing castor oil as excipient in parenteral formulation.

Keywords*-AET, SCT, UPLC-HRMS, MDD, Leachables, Castor oil*

INTRODUCTION

Leachables are foreign organic and inorganic chemical entities that are present in a packaged drug product because they have leached into the packaged drug product from a packaging/delivery system, packaging component, or packaging material of construction under normal conditions of storage and use or during accelerated drug product stability studies. Because leachables are derived from the packaging or delivery system, they are not related to either the drug product itself or its vehicle and ingredients[1]. Leachables are present in a packaged drug product because of the direct action of the drug product on the source of the leachable. Thus leachables[2,3] are typically derived from primary and secondary packaging, as the primary and secondary packaging can serve as a barrier between the packaged drug product and other potential sources of foreign chemical entities (such as tertiary packaging and ancillary components)[3].

Leachables, are chemical entities with wide chemical range, are of concern due to their potential safety risk to patients and potential compatibility risks for the drug product. In order to assess these risks and manage the potential issues posed by leachables, it is necessary to know their identities and the levels to which they will accumulate in the finished drug product over its shelf-life[1]. These two pieces of information can be used to establish the magnitude of patient exposure (dose) and therefore the safety risk posed by an individual leachable, as well as the likelihood of any drug product compatibility issues.

The starting action in leachable[4] method development is to establish the level at which the method must perform at to accomplish the appropriate leachables characterization functions. This level is known as the Analytical Evaluation Threshold (AET). An appropriate method must function at all levels greater than or equal to AET. AET can be based on various criteria, including Safety Concern Threshold (SCT), Label claim of the drug product (LC), and Maximum daily dose of the drug product (MDD) etc. This AET establishes the level at which unknown leachables should be identified and quantified in a particular drug product, and can therefore be

used as a basis of analytical method development[5].

AET was calculated as per below formula:

$$\text{AET} = \frac{\text{SCT} \times \text{LC}}{\text{MDD}}$$

Due to screening and semi-quantitative nature of the analytical method, 50% analytical uncertainty was considered in order to establish a Final AET for UPLC-HRMS method used for detecting and identifying leachables[6,7]. Final AET has been considered as reporting threshold for non-volatile organic compounds as leachables in drug product [7]. Final AET (i.e., 50% AET) has been derived using below formula:

$$50\%\ \text{of AET} = \frac{\text{SCT} \times \text{LC}}{\text{MDD}} \times 0.5$$

Leachable screening of parenteral formulation containing complex matrix like oil as excipient is challenging due to non-polar nature of formulation as well as matrix interference during sample analysis. The LC-MS technique has been widely adopted for determination of non-volatile leachable impurities. In this present paper, UPLC-HRMS technique was used as instrument. Use of UPLC-HRMS technique in place of HPLC-MS, provides advantage to detect and identify the leachable compounds with shorter run time. As HRMS is accurate mass technique with higher resolution, UPLC-HRMS screening method allows identification of any non-volatile organic compounds, in a given extract/solution as broad as possible.

MATERIALS AND METHODS

Reagents and material

Acetic acid (100%, LC-MS LiChropur), formic acid (98-100%, LC-MS LiChropur), Ammonium acetate (LC-MS LiChropur) were purchased from Merck (Darmstadt, Germany). Acetonitrile (99.9%, v/v, Optima-LC-MS grade) was purchased from ThermoFischer Scientific (Fair Lawn, NJ, USA). Methanol (99.9%, v/v, LC-MS Reagent) was purchased from J.T.Baker (Avantor Performance Materials, LLC and Radnor, PA 19087). EDTA (Emprove Bio grade) was purchased from Merck (Merck KGaA, Darmstadt, Germany). n-Hexane (99%, v/v, ULTRA RESI-ANALYZED) was purchased from J.T.Baker (Avantor Performance Materials, LLC, Radnor, PA 19087). Ultrapure water used in the experiments was prepared by passing purified water through a Milli-Q Advantage A10 water system (EMD Millipore, Billerica, MA, USA). Standards used for system suitability and recovery assessment were procured from sigma Aldrich, Switzerland and Alfa Aesar, ThermoFisher Scientific. Test samples provided for this study consisted formulation development batch from R&D and one submission batch from manufacturing facility (Alembic Pharmaceuticals Limited, Vadodara, INDIA).

Standard preparation

Standard solutions were prepared by weighing about 25 mg of each standard and diluting with 5 mL of dichloromethane and 45 mL of methanol to achieve desired concentration level of 500 ppm. Further diluted mixed standard stock solution to achieve desired concentration of 0.0375 ppm with methanol. This solution was used as a working solution (100%). Concentration ranges of standards from 50% level to 150% level were prepared considering calculation of working concentration.

Sample preparation

2 mL of sample solution (Finished product) was taken in to glass test tube and 1 mL of 1% w/v EDTA in water was added into it and vortex to mix, then 4 mL of n-Hexane added and vortex to mix. Allowed it to separate the layer, removed 2 mL of n-Hexane from supernatant layer and evaporated under N_2 current. Reconstituted it with 2 mL of methanol. Spiked samples were prepared with same procedure except reconstituted with 2 mL of standard solution.

Methods

UPLC H-Class and Xevo G2 XS QToF from waters were employed for sample analysis for non-volatile leachable impurities(Table 1).

Table 1: Typical instrument parameters for UPLC-HRMS

Column chemistry and column manager				
Column name	Acquity UPLC BEH C18, 2.1*100 mm, 1.7 µ			
Column temperature	30.0 ℃			
Quaternary solvent manager				
Solvent Name A	Acetonitrile			
Solvent Name B	0.1% v/v Acetic Acid in 5 mM ammonium acetate in water			
Flow rate	0.250 mL/min			
Run time	35.00 min			
Gradient table	**Time (min)**	**Composition**	**Composition B**	**Curve**
	0.00	92.0	8.0	Initial
	10.00	98.0	2.0	6
	30.00	98.0	2.0	6
	32.00	92.0	8.0	6
	35.00	92.0	8.0	6
Sample temperature	10.0°C			
Injection volume	6 µL			
Xevo G2-XS QToF				
Function 1	MS^E			
Ionisation mode	APCI (Atmospheric Pressure Chemical Ionisation)			
Polarity	Positive/Negative			
Start mass	100.00 m/z			
End mass	1500.00 m/z			
Analyzer mode	Sensitivity			
Corona mode	Voltage			
Voltage	5 kV			
Source temperature	150°C			
Probe temperature	400°C			
Cone gas flow	50 L/h			
Desolvation gas flow	600 L/h			

RESULTS AND DISCUSSION

Test sample contains commercial alcohol, benzyl alcohol, benzyl benzoate and castor oil as excipients along with 50 mg/ml of an API. Castor oil is the major component of the drug product. Presence of higher amount of matrices in the drug product was major challenge to extract leachable impurities into the final solution. Due to higher amount of oil matrix in test article, the sample could not be injected directly into the LC-MS system. Different method development trials were taken for sample preparation which can give consistent output. Among this, 1% w/v EDTA in water was used during preparation of sample as it has good chelating power. Due this nature, the tiny quantity of oil was extracted into the n-Hexane and followed by methanol and thus help to reduce the matrix impact in the analysis by UPLC-HRMS.

Based on the nature of possible leachables from the components used in the parenteral formulations i.e., anti-oxidants, filters, plasticizers, polymerization or hydrogenation catalysis and other polymer additives, standard with different nature i.e., BHT, Irganox 1010, Irganox 1330, Irganox 1076, Ionol 46, Irgafos 168, Pyrene, Dioctyl phthalate, 2-Mercaptobenzothiazole and Oleamide have been selected. Among these compounds, BHT, Irganox 1010, Irganox 1330, Irganox 1076, Ionol 46 were analysed in negative polarity while Irgafos 168, Pyrene, Dioctyl phthalate, 2-Mercaptobenzothiazole, Oleamide were analysed in positive polarity in APCI mode. APCI mode has been selected over ESI mode, due to varying nature of compounds in terms of polarity for better and consistent ionization,

where APCI provide good ionisation of non-polar compounds.

Due to explorative nature of the analytical method full method validation was not executed. However, the analytical method has been partially validated for parameters/experiments mentioned in Table 2 to check suitability for the intended purpose of leachable compounds screening in proposed formulation. Due to semi quantitative nature, parameters/experiments have been partially evaluated to check suitability of fit for purpose method for intended use. This developed and partially validated method has been used for the leachable screening study of proposed formulation (Table 2).

Table 2: Method verification parameters

Sr. No.	Method verification parameters	Acceptance criteria
1	Specificity	Any interference in blank should be less than 20.0% of the working standard concentration
2	Linearity and quantitation limit and detection limit	$R \geq 0.95$
3	System precision	% RSD $\leq$ 20.0% of Working standard concentration
4	Accuracy (Recovery check)	Average recovery across all compound should be between 50% to 150%

Specificity

It is the ability of the method to measure specifically the analyte of interest in the presence of other components such as impurities, degradation products or excipient that may be expected to be present in the sample preparation. Blank solution and working standard solution (100% level) were injected to check specificity. There was no any interference observed more than 20% of the working standard solution for each standard (Table 3).

Table 3: Result for specificity

Compound name	Blank solution response	Standard response	% Interference
2-Mercaptobenzothiazole	0	548521	0.00
Dioctyl Phthalate (DOP)	9200	334301	2.75
Irgafos 168	0	868684	0.00
Oleamide	5931	2148740	0.28
Pyrene	0	315895	0.00
BHT	0	1212791	0.00
Ionol 46	0	2086832	0.00
Irganox 1010	8130	2188529	0.37
Irganox 1076	0	2561795	0.00
Irganox 1330	0	3583291	0.00

Linearity and LOD/LOQ determination

Linearity has been performed to check linear response of the analytical standards used in analytical technique. Linearity experiment has been performed at three levels i.e., 50%, 100% and 150% level of the working level concentration. Correlation coefficients (R) calculated by extrapolating response (Area) against standard concentrations and the linearity were plotted through simple linear regression. LOQ and LOD are calculated from the linearity experiment. Results from the calibration curves obtained within acceptance criteria for all the targeted standards (acceptance Criteria: $R \geq 0.95$). Calculated R values are observed between 0.9954 to 1.0000. Calculation of LOQ and LOD determination from Linearity is summarized in below tables. Hence, it could be summarise that the instrument response is linear throughout the entire concentration range defined for this method (Table 4).

Table 4: Results for linearity, LOQ and LOD

Compound name	R value	LOQ (ng/mL)[$]	LOD (ng/mL)[&]
2-Mercaptobenzothiazole	0.9999	0.0002	0.0001
Dioctyl Phthalate (DOP)	0.9999	0.0030	0.0010
Irgafos 168	0.9999	0.0078	0.0026
Oleamide	0.9999	0.0036	0.0012
Pyrene	1.0000	0.0050	0.0016
BHT	0.9998	0.0014	0.0005
Ionol 46	0.9999	0.0028	0.0009
Irganox 1010	1.0000	0.0065	0.0022
Irganox 1076	0.9954	0.0258	0.0085
Irganox 1330	0.9981	0.0167	0.0055

[$]: LOQ (Limit of Quantification) Calculation: 10* SD/Slope.
[&]: LOD (Limit of Detection) Calculation: 3.3* SD/Slope.

System precision

System precision carried out by continuous aspirations of working standard (100% level). Six consecutive aspirations from a six different standard preparations were monitored and % RSD for the response (Area) for all the standard aspiration for individual standard found within acceptance criteria. From % RSD calculation, results obtained in the range of 1.16%-8.89% (Acceptance Criteria: % RSD ≤ 20%) This proves the method consistency and suitability (Table 5).

Table 5: Results for system precision

Compound name	System precision (%RSD)[#]
2-Mercaptobenzothiazole	2.67
Dioctyl Phthalate (DOP)	1.95
Irgafos 168	8.89
Oleamide	1.16
Pyrene	3.61
BHT	5.39
Ionol 46	3.74
Irganox 1010	2.43
Irganox 1076	1.96
Irganox 1330	1.63
#: Six aspirations of working standard (100% Level)	

Accuracy (Recovery)

The accuracy was performed at working standard concentration (100% level) considering three set preparation of spiked samples. Average recovery across all compounds was found between 50% to 150% (Table 6).

Table 6: Results for recovery (Accuracy)

Compound name	Avg. recovery (%)	Avg. recovery across all compounds (%)
2-Mercaptobenzothiazole	71.08	73.85
Dioctyl Phthalate (DOP)	80.26	
Irgafos 168	74.05	
Oleamide	67.11	
Pyrene	76.76	
BHT	100.81	91.09
Ionol 46	88.68	
Irganox 1010	87.03	
Irganox 1076	88.16	
Irganox 1330	90.79	

Analysis of test sample

The submission batch of test sample at stability time points (5℃ ± 3℃ (Horizontal Placement), 3 months, 14 months) has been analysed along with freshly prepared control sample with above screening method and differential peaks between test sample and control sample have been evaluated. No non-volatile organic leachable compounds observed above reporting threshold at any time point of test formulation.

CONCLUSION

Due to complex nature of drug product, it was difficult to separate and identify leachables at ppb and sub-ppb level. The adopted UPLC-HRMS technique has higher sensitivity and reproducibility along with mass accuracy and resolution. Herein, the results of all the parameters performed as a part of partial validation are within acceptance criteria. The primary requirement of any analytical method used for leachable evaluation is that the methods should be sensitive and precise enough to detect and quantitate at least at the level of Analytical Evaluation Threshold (AET) of drug product. Based on the results, it can be inferred that the analytical method used for leachable screening study are sensitive and precise enough to achieve required analytical evaluation threshold of proposed formulation. Therefore, the analytical method used for leachable screening study is suitable for the intended purpose of leachable screening in the proposed formulation.

ACKNOWLEDGMENT

The authors are grateful for the R&D Facilities from the Alembic Pharmaceuticals Limited, Vadodara, INDIA

REFERENCES

1. General information chapter: Assessment of drug product leachables associated with pharmaceutical packaging/delivery systems, USP, 1664.
2. Phadke R, Mali R, Gosar A. Extractables and leachables-pharmaceutical review. European Journal of Pharmaceutical and Medical Research. 2019; 6(3): 474-483.
3. General information chapter: Assessment of extractables associated with pharmaceutical packaging/delivery systems, USP, 1663.
4. Ball DJ, Norwood DL, Stults CLM, Nagao LM. Leachables and extractables handbook: Safety evaluation, qualification, and best practices applied to inhalation drug products, John Wiley and Sons, 2012.
5. Paskiet D, Jenke D, Ball D, Houston C, Norwood DL, Markovic I. The Product Quality Research Institute (PQRI) leachables and extractables working group initiatives for Parenteral and Ophthalmic Drug Product (PODP). PDA Journal of Pharmaceutical Science and Technology. 2013; 67(5): 430-447.
6. Guideline IH. Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk M7. International conference on harmonization of technical requirements for registration of pharmaceuticals for human use (ICH): Geneva 2014 Jun.
7. Johnson C. A Practical Approach to Extractables & Leachables. Drug Development and Delivery. 2019; 19(1).