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A Rapid UPLC Method to Determine a Migration Compound from Container Closure System to Drug Product

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Abstract

Extractable and leachable studies are playing a key role in the pharmaceutical drug product development while selecting the suitable packing material for finished dosage form. The conventional stability studies as per the ICH Q1 provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light. Force degradation studies will be conducted to understand the degradation pathway of drug related impurities under these conditions. However, there is no systematic approach or study design available to understand or predict the migration compounds into the drug product from packing material/Label compounds. In this article, a practical framework has been made on how to develop, identify, and quantify a migration compound from primary packing materials by conducting an experimentation on a model Nasal drug product containing two active pharmaceutical ingredients. This approach can be implemented to understand the potential migration compounds in the development stage to mitigate the risk and minimize the product development time. In this approach, the compatibility of drug product has been studied with Glass and HDPE packing configuration by subjecting under various stress conditions. Chromatographic and spectral techniques have been employed to identify the leachable as Diisobutyl phthalate (DIBP). Further a specific, linear, sensitive, precise, and accurate analytical method was developed using ethylene bridge hybrid octadecylsilane stationary phase to quantify DIBP in a drug device combination Nasal drug product.

Keywords

Diisobutyl Phthalate, Leachable, UPLC, Chromatography, Packing material.

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1. INTRODUCTION

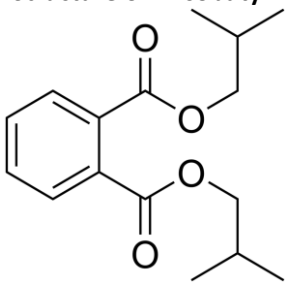
As per the Food and drug Administration (FDA) guidance for industry, a container closure system refers to the primary and secondary packaging components that protects the drug product. These include primary and secondary packaging components along with labels which will be used to display the name of the product and additional details. Since the intended use of packing material is to protect the dosage form throughout the shelf life

of the product, it is very imperative to select a suitable packing material during product development. The in-compatibility of packing material can lead to loss of potency, discoloration, degradation of active substance, degradation of excipients and leaching or migration of packing material compounds into the drug product. Nasal spray drug products are one such category where the product contain therapeutically active ingredients (drug substances) dissolved or suspended in solutions or mixtures of excipients (e.g., preservatives, viscosity modifiers, emulsifiers, buffering agents) in non-pressurized dispensers that deliver a spray containing a metered dose of the active ingredient [1]. The drug product must be evaluated for leachable compounds from elastomeric/ plastic components and printed labels from the container closure system during the development studies. Many articles are available to determine the degradation of active substance of drug product and to determine the potency or assay of active pharmaceutical ingredient. The discoloration of a product can be easily determined by visual observation. However, it is challenging to predict or determine the leachable or migration of label compounds into the drug product. Sensitive analytical methods are required to quantify these compounds as the limits for these impurities are very stringent to meet the ICH/PQRI guideline requirements. In the present work, authors have selected a Nasal spray product containing two active compounds to develop an analytical method for identifying and quantify a potential label migration compound.

Based on the extensive literature search it is observed that there are many published articles available for quantification of Active compounds in Nasal solution or spray products. However, no reported systematic studies were conducted to estimate the leachable compounds from packing materials of the Nasal drug product. Hence authors believe that there is a need to perform the migration study with systematic approach using different container closure systems using ultra high-performance liquid chromatography. During the study, an additional peak was observed when drug products packed in a different container closure systems are treated under stress conditions. The additional peak was identified and characterized using various analytical techniques like PDA (spectral identity) and LC-MS (m/z values) as Diisobutyl phthalate.

Phthalate esters are observed in a wide variety of food packing and consumer products. Hence there is a widespread exposure of these chemicals to human population [2]. Diisobutyl phthalate (DIBP) belongs to the family of phthalate ester class of chemicals and is used as a plasticizer to provide flexibility and durability to a wide variety of industrial and consumer products, including paints, lacquers, printing ink, pulp and paper, carpet, concrete, nail polish, and cosmetics. Because of its use in household products, people are exposed to DIBP via food and indoor environments. The structure of DIBP [3] is depicted in Figure-1. The results in a study [4] supports, DIBP exposure has a children's health concern, male reproductive and developmental toxicities with some evidence of female reproductive and liver toxicity.

Figure-1: Structure of Diisobutyl Phthalate



Hence control of DIBP in formulation dosage forms is very much essential at a level below the acceptable limit. When the analytical evaluation threshold (AET) is calculated as per the PQRI guideline [5] considering the safety concern threshold of 1.5 µg/day, doses per device is 70 and maximum number of doses per day is 4, the analytical method shall be able to detect and quantify the DIBP at a concentration of 1.8 ppm. Analytical methods are available for quantitation of DIBP using GC-MS and micro dialysis [6-9]. However, there is no reported literature available which can be able to detect and quantify DIBP in such a trace level using UPLC equipped with Photo Diode array detector in Nasal product. Hence author has developed a rapid, specific, linear, accurate and precise analytical method to estimate the DIBP in the Nasal product.

2. EXPERIMENTAL

2.1. Chemicals and reagents

HPLC grade Acetonitrile is procured from Merck, Mumbai, India. Analytical reagent grade potassium dihydrogen phosphate was procured from Merck, Mumbai, India. Samples are taken from public domain. High pure water was obtained using TKA water purification system. Waters BEH C18 50 mm × 2.1 mm column with 1.7 µm particle size (part no. 186002350) was procured from waters corporation.

2.2. Instrumentation and software

Method development and validation studies were performed on Acquity-UPLC system (Waters

Corporation, USA) equipped with an LC pump (model ACQ-BSM), an online degasser, auto sampler (model ACQ-SM) with thermostat, and a photodiode array (PDA) (model ACQ-PDA) detector. Mass detector was used to determine the m/z value. The chromatographic data was acquired, monitored and processed using Empower 3 software from Waters Corporation.

2.3. Chromatographic conditions

The sample cooler and column oven temperature is set at 25 °C and 40 °C respectively. The injection volume of sample and standard was 5 µL. The stationary phase, Waters BEH C18 50 mm × 2.1 mm column with 1.7 µm particle size was employed in the method development and validation. A 10mM potassium dihydrogen phosphate in water was used as a mobile phase-A and Acetonitrile was used as mobile phase-B, mixture of Acetonitrile and water in the ratio 50 and 50%v/v was used as extraction solvent. The flow rate of the mobile phase is set as 0.5 mL min^{-1}. The gradient program time (in min)/% mobile phase-B is set as 0/25, 7/85, 12/85, 13/25 and 15/25. The chromatograms were acquired and monitored at wavelength 225 nm.

2.4. Standard and sample solutions

2.4.1. ***Stock solution 1***: Accurately weighed and transferred 50 mg of the Diisobutyl phthalate in a 50 mL volumetric flask and added 20 mL of the extraction solvent sonicated to dissolve and then made up to 50 mL using the same solvent. (1000 µg/mL)

2.4.2. ***Stock solution-2*:** Transferred, 2.0 mL of stock solution-1 in a 200 mL volumetric flask and volume was made up to the mark with extraction solvent. (10 µg/mL)

2.4.3. ***Working standard solution*:** Transferred, 4.0 mL of stock solution-2 in a 100 mL volumetric flask and volume was made up to the mark with extraction solvent (0.4 µg/mL*).

***Working Standard solution** 0.4 µg/mL is equivalent to 1.8 µg/g of sample with respect to test sample concentration

2.4.4. ***Extraction procedure Nasal formulation:*** Accurately weighed and transferred, 2200 mg of formulation sample in 10 mL volumetric flask. To this 7 mL of extraction solvent was added and sonicated for 5 min. Allowed the solution to attain room temperature and made up to the mark with extraction solvent. A portion of solution was centrifuged at 4000 RPM for 10 min. The resultant clear solution was collected in vial and used for the analysis.

2.4.5. Estimation of Diisobutyl phthalate in formulation samples

Injected blank, working standard solution and test samples into the chromatographic conditions mentioned in section 2.3 for the analysis.

Diisobutyl phthalate is calculated using below formula.

$$\frac{AT}{AS}\times\frac{WS}{100}\times\frac{2}{200}\times\frac{4}{100}\times\frac{10}{WT}\times\frac{P}{100}\times 1000000$$ ----- (Formula)

Where AT is Peak area due to ***Diisobutyl phthalate*** in the sample preparation, AS is Average peak area due to ***Diisobutyl phthalate*** in the working standard preparation, WS is the weight of ***Diisobutyl phthalate*** taken in mg, WT is the Weight of the sample taken in mg, %P is the Potency of the ***Diisobutyl phthalate.***

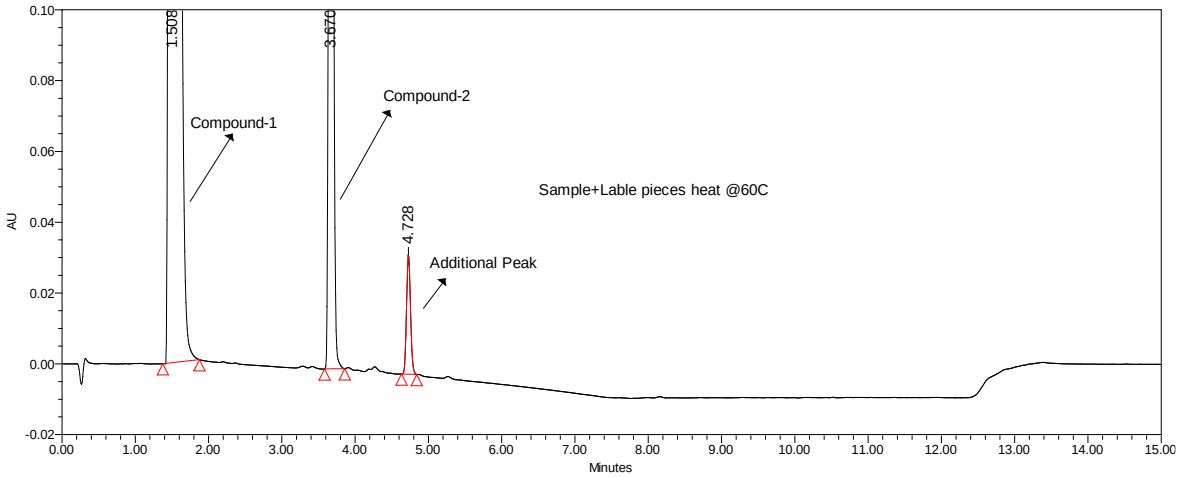
3. RESULTS AND DISCUSSION

3.1. Results

3.1.1. Method development

Analytical method development play an important role in the identification and quantification of impurities in pharmaceutical products. In first step of experiment, 7 mL of formulation samples were transferred in each of 4 different glass vials. One vial (Vial 1) used as control samples, in one vial (vial2) few amber color glass pieces were added, in another vial (vial 3) few HDPE pieces were added and in last vial (vial 4) label pieces were added. All these vials are incubated at 60 °C for about 12hrs. After the exposure, the samples were cooled to room temperature. The incubated sample were analysed for leachable impurities using experimental conditions described in Section-2. Based on the evaluation of chromatographic data (Refer Figure 2), an additional peak was observed in the chromatogram obtained from Glass vial containing label.

Figure-2: Chromatogram obtained from the analysis of sample and Label.



In step 2, experiments were conducted using Liquid chromatography instrument equipped with PDA detector and Mass detector to characterize the additional peak. LC is used as separation technique, PDA detector employed to extract the UV spectrum. Mass detector is employed to extract the Mass spectrum and its fragment. The UV spectrum, Mass spectrum and its fragment is depicted in Figure-3, 4 and 5 respectively.

Figure-3: UV Spectrum of Diisobutyl Phthalate

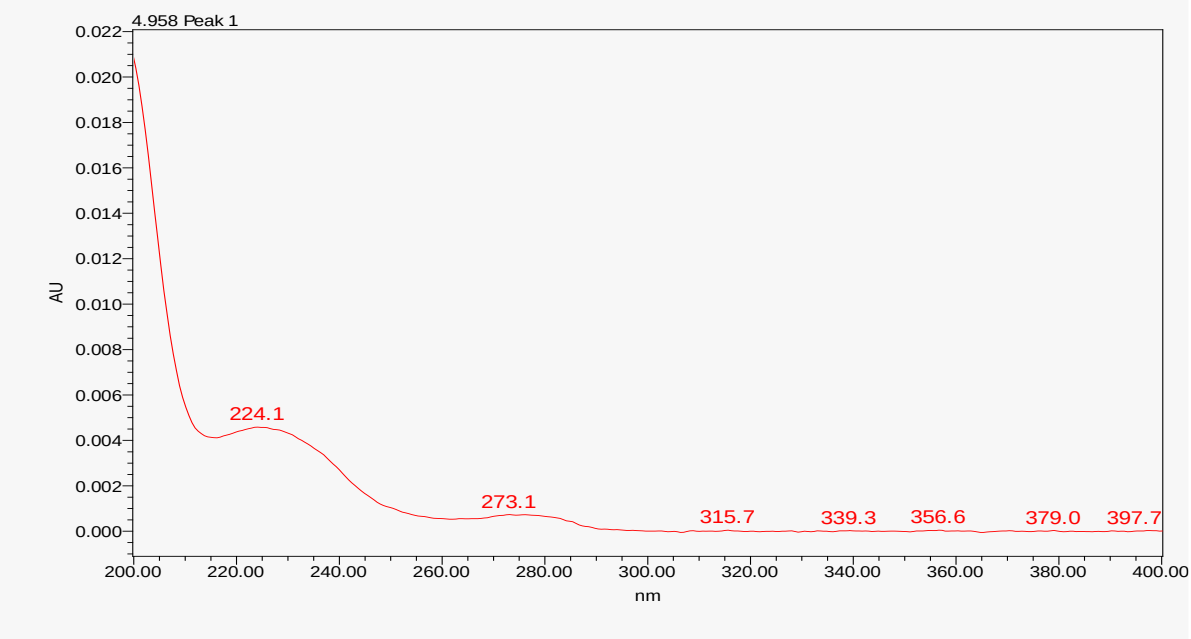


Figure-4: Mass Spectrum of Diisobutyl Phthalate

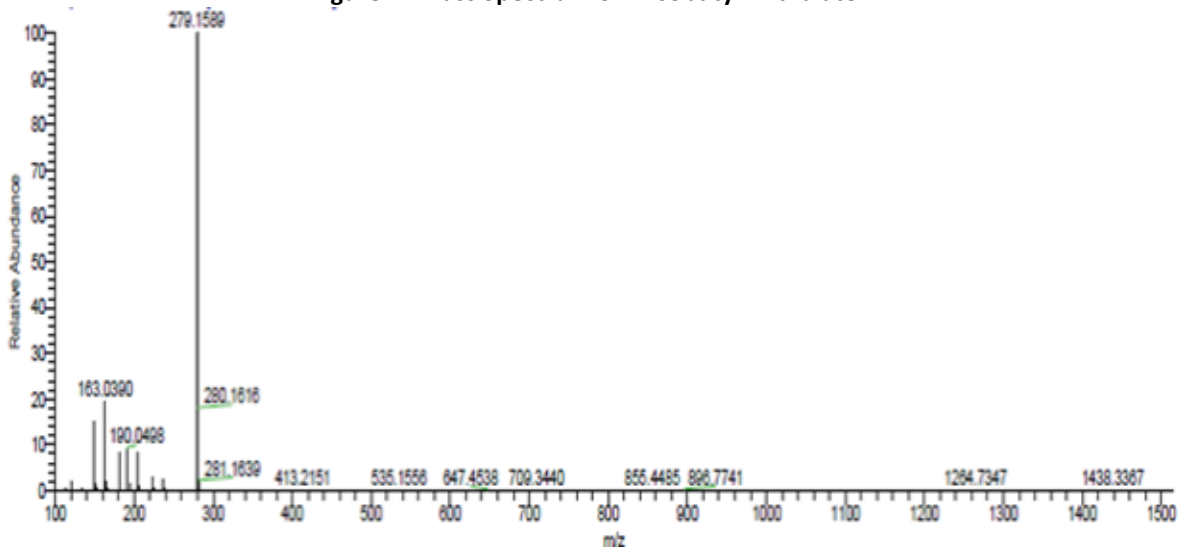
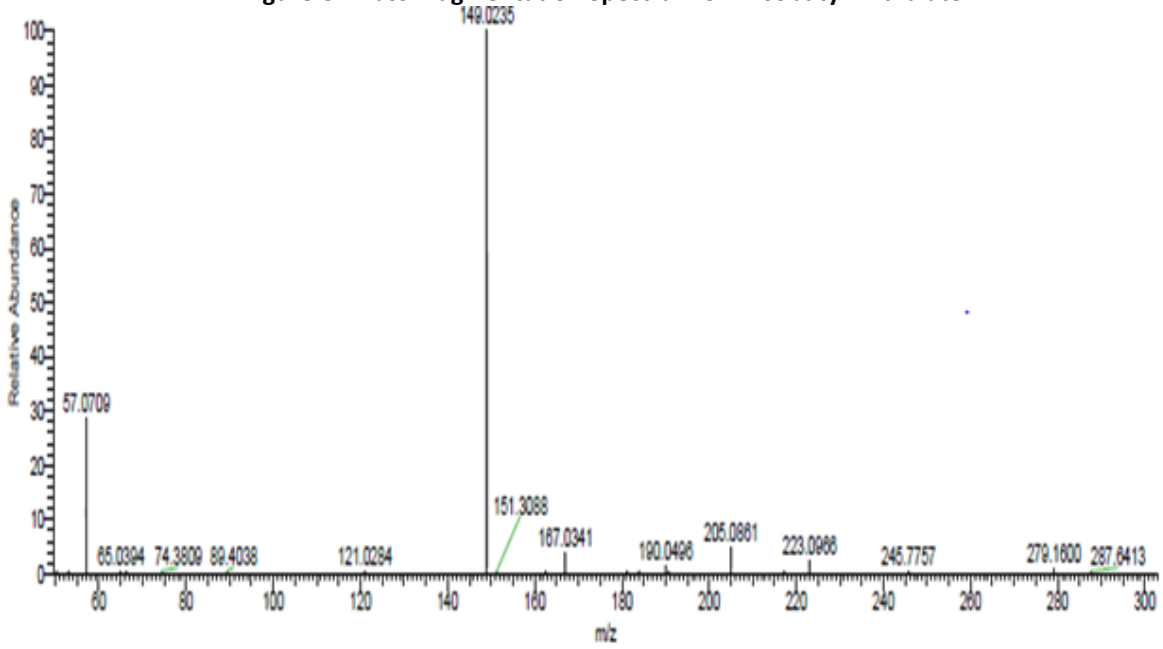


Figure-5: Mass Fragmentation Spectrum of Diisobutyl Phthalate



Based on the spectral data it is predicted that the additional compound is Diisobutyl phthalate (DIBP). For further confirmation, a standard of DIBP is procured and prepared a sample solution of 0.4 µg/mL and analyzed in the same experimental conditions. The retention time, UV spectrum and Mass spectrum are matching with that of additional peak observed in the glass vial. Thus, the addition peak is confirmed as Diisobutyl phthalate (DIBP). As control of Diisobutyl phthalate is essential in the drug product, a novel analytical method was developed and validated for its quantification.

3.1.2. Selection of stationary phase

Based on the literature and physico chemical properties suggests that C18 column or C8 is a suitable column for the separation. Based on the evaluation of these columns Acquity UPLC BEH C18, 100 x 2.1 mm, 1.7µm has provided superior separation and peak shape. Hence, it was selected for the optimization of other chromatographic parameters.

3.1.3. Selection of mobile phase and extraction solvent

Various buffers of a pH range from 2.5 to 6.5 are evaluated for the separation of active compounds in the drug product. At pH around 6.5, one compound shown peak splicting. Whereas at pH around 2.5, enough separation is not achieved between the compounds. At pH about 4.5 (10mM Potassium dihydrogen phosphate), very good separation was

observed between the compounds. Based on separation of desired compounds this buffer is selected for separation technique. Since Acetonitrile has a very low UV absorbing property at lower wavelengths, a combination of buffer and acetonitrile is finalized for the separation study. A mixture of water and acetonitrile in the ratio of 1:1 v/v is used as an extraction solvent after considering the solubility of desired components in the formulation matrix.

3.1.4. Selection of detector wavelength

To determine the λmax of the DIPB a solution of 0.4 µg/mL is prepared in water and acetonitrile (1:1 v/v) and scanned in the HPLC equipped with PDA detector in the range from 200 nm to 400 nm. It is concluded from the experiment that the compound has wavelength maxima at 225 nm for DIBP. Refer figure-3 for the UV spectrum of DIPB. Thus 225 nm was selected as wavelength for the detection of DIBP.

3.1.5. Selection of flow rate, gradient and column temperature

The flow rate and column temperature was optimized based on system pressure, peak shape and separation between DIBP and formulation matrix.

3.2. Method Validation

Method validation is a process by which it is established that the performance characteristics of the method met intended analytical application. The analytical method was validated as per the ICH guideline [10].

3.2.1. System suitability

The system suitability parameters are evaluated for DIBP in the optimized UPLC conditions. The critical system suitability parameters like peak tailing factor and theoretical plates were established. The tailing factor, relative standard deviation (%) of area counts and theoretical plates are observed to be 0.9, 2.2 and 27584 respectively. The results are found be well within the acceptable limit.

3.2.2. Specificity

The specificity of the method was verified for interference due to blank and placebo at the retention time of DIBP. The peak homogeneity was determined for DIBP using photo diode array detector. The purity angle and Purity threshold for DIBP peak is found to 0.602 and 1.565 respectively. Based on the data it is concluded that the method is specific to DIBP analysis.

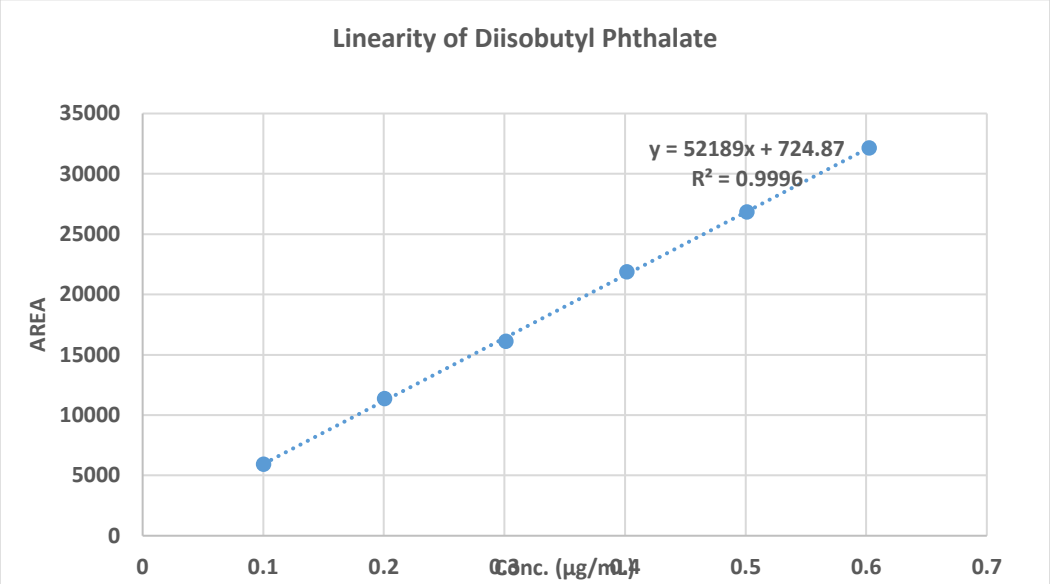
3.2.3. Linearity

For determining the linearity of DIBP, a series of solutions with different concentrations 0.10 (LOQ), 0.20, 0.30, 0.40, 0.50 and 0.60 µg/mL (0.46 ppm to 2.75ppm with respect to sample concentration) were prepared and injected into the UPLC. A linearity plot was drawn by taking the concentration on X-axis and area on Y-axis. Slope, Correlation coefficient, % Bias, were calculated using the plot. The results showed that the method is linear in the proposed range. The results are summarized in Table 1 and the linearity plot is depicted in Figure-6

Table 1: Results of Linearity

Linearity	
Compound Conc (ppm)	**Area**
0.1004	5928
0.2008	11391
0.3012	16126
0.4017	21900
0.5012	26852
0.6025	32156
Correl. Coeff.	0.9998
Slope	52188.9
Intercept	724.9
%Bias	3.3
Acceptance criteria: **1.Correl. Coeff. Must be Not less than 0.999.** **2. %Bias must be Not more than 5.0%**	

Figure-6: Linearity Plot of Diisobutyl Phthalate



3.2.4. Accuracy

An accuracy study was conducted by the intentional addition of known amounts of DIBP in the formulation matrix at four different levels LOQ, 50%, 100% and 150% of target AET level (1.8ppm). The samples were analyzed, and recovery is calculated using a standard of DIBP prepared in extraction solvent. The results of accuracy study are represented in Table 2. From the data it is evident that the average recovery from accuracy study ranged from 98.8-107.3% which indicates that the selected extraction solvents are suitable for the accurate determination of DIBP in the formulation matrix.

Table 2: Results of Accuracy

Accuracy				
	LOQ (%)	50%	100%	150%
	100.61	102.56	105.91	103.15
	98.79	108.22	104.73	98.14
	109.19	111.07	103.92	99.35
	87.95		105.01	
	102.70		105.47	
	93.38		105.90	
Average	98.8	107.3	105.2	100.2
Std.dev	7.4	4.3	0.8	2.6
%RSD	7.5	4.0	0.7	2.6
Acceptance criteria:				
The individual and average recovery must be with 85.0 and 115.0%				

3.2.5. Method Precision

Precision of the method is demonstrated by preparing six samples containing DIBP at target AET level by intentional spiking into the product. The samples were analyzed as per the method and measured the area of DIBP. The relative standard deviation is calculated for the results obtained from six preparations. The %RSD for content is found to be 0.7%. The results are represented in the Table-3

Table 3: Results of Method Precision

Method Precision	
Sample Solution No.	**Content (ppm)**
1	1.94
2	1.92
3	1.91
4	1.93
5	1.94
6	1.94
Average	1.9
Std.dev	0.0
%RSD	0.7
Acceptance criteria: %RSD must be less than 2.0	

3.2.6. Limit of detection and limit quantification

The limit of detection (LOD) and limit of quantitation (LOQ) are established for DIBP by diluting the standard stock solution. At concentration 0.05 µg/mL and 0.10 µg/mL, the signal to noise ratios of above 3 and 10 are achieved. Hence these concentrations were finalized as LOD and LOQ respectively. Further the average recovery and Precision at LOQ level was found to be 98.8% and 7.5 % RSD respectively. The results are summarized in Table 2 and 4.

Table 4: Results of LOQ Precision

Precision at LOQ	
Sample prep No.	**Content (ppm)**
1	0.46
2	0.45
3	0.50
4	0.40
5	0.47
6	0.43
Average	0.5
Std.dev	0.0
%RSD	7.5
Acceptance criteria: %RSD must be less than 15.0	

3.3. DISCUSSION

A combination drug product was selected for this research work. During the evaluation an additional peak was observed in the sample containing label is exposed to temperature. Upon the characterization it is identified as Diisobutyl Phthalate and it is migrating into the drug product from the labeled material. As literature supports this compound has reproductive and liver toxicity, an analytical method was developed and validated for the determination of DIBP in the Nasal drug product. As per the PQRI guideline the AET values for this compound was derived to be 1.8 ppm. Hence this concentration was considered as specification during the development and validation.

The validated method further used for the analysis of aged sample packed in the Glass container as well HDPE container which are affixed with Label. Based on the data it is evident the samples stored in Glass container does not have this compound. A content of about 0.9 ppm is observed in samples stored HDPE packing configuration. However, further investigation may be required to understand the migration of DIBP from the container-closure system into the drug solution.

4. CONCLUSION

Developed UPLC analytical method for quantification of DIBP is specific, sensitive, accurate, precise, and reproducible. Glass packing configuration is found to be better choice for Nasal products over HDPE packing configuration. The chromatographic method was validated according to the ICH guidelines. The validation parameters indicate that the proposed method can be suitable for the quantification of DIBP in drug products.

5. CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

6. ACKNOWLEDGEMENTS:

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