Study of Leachability and Interaction of Di Isononyl Phthalate with Meropenem and Leve tiracetam Using HPTLC

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***Abstract*—Aim : To develop a High Performance Thin Layer Chromatographic technique which is capable of detecting and quantifying a IV line plasticizer Diisononyl phthalate (DINP), Meropenem and Levetiracetam. The components were separated using stationary phase Merck Pre-coated TLC plates with silica gel 60F254. The mobile phase employed consisted of Ethylacetate: methanol: water: formic acid (7:4:1:3 v/v/v/v) for meropenem, toluene: acetone: methanol (6:2:2 v/v/v) for levetiracetam. IV line tubes were used to study leaching and interaction of DINP with drugs in specific time frame. Evaluation was done using Peak area. Method is validated and linearity is observed from 250 to 1500 ng/band for Meropenem and Levetiracetam. Interday and intraday precision values were within the limit of 2% RSD. The peak area of components used to evaluate. The research study ensures leaching of di isononyl phthalate plasticizer in presence of lipophilic drugs like meropenem and levetiracetam. The concentration of drugs remains unaltered, however leaching of plasticizer increased considerably while it is present with drugs lead to adverse effects.**

***Keywords*—Diisononyl phthalate, Leaching, drug interaction, HPTLC**

I. INTRODUCTION

Material vigilance [1] is a critical aspect of healthcare product safety that focuses on monitoring and assessing the potential risks associated with medical devices and their components. It involves a systematic approach to identify, evaluate, and mitigate adverse events related to medical devices. A medical device [2], in accordance with the Food and Drug Administration (FDA), is defined as a machine, implement, instrument, implant, or an in vitro component that is used for the treatment, cure, diagnosis, and prevention of a particular disease (U.S. Food and Drug Administration 2018). Intravenous (IV) line tubes are essential medical devices used for administering intravenous fluids and medications. However, they are often made of PVC, which contains plasticizers to enhance flexibility. These plasticizers can leach into drug solutions, potentially leading to drug degradation, reduced efficacy, or adverse reactions in patients [3]. Meropenem is a powerful antibiotic used to treat serious bacterial infections. It's administered through an IV line because it needs to be delivered directly into the bloodstream for rapid and effective action. Levetiracetam is an anti-seizure medication used to treat epilepsy [4]. Leaching and interaction of DINP with drugs can cause potential adverse effects, exploration of such with novel validated [5] analytical methods like HPTLC technique for simultaneous determination of DINP with meropenem [6] and Levetiracetam [7-8] would serve material vigilance and healthcare sector immensely.

II. MATERIALS AND METHODS

A Camag HPTLC CAMAG with applicator - CAMAG Linomat 5, Scanner -TLC scanner 4Software -visionCATS 3.1 version was used in study. All chemicals and reagents used were of Chromatographic grade. Drugs and IV line tubes were obtained from Sri Ramakrishna Hospital Pharmacy, Coimbatore.

III. EXPERIMENTAL

Preparation of Plasticizer solution:
A volume of 100 µl contains standard of 100 mg/ml of DINP was transferred with micro pipette and diluted in 10 ml of methanol (10000 mcg/ml). Further required dilutions were made using methanol.
Preparation of drug solutions:

A quantity of about 10 mg of meropenem and levetiracetam was taken separately in a 10ml standard flasks, dissolved and diluted in 10 ml of methanol (1000 mcg/ml). The required standards were prepared using methanol. Standard drug stocks

solutions were diluted with methanol and used for further studies.

Selection of chromatographic conditions:

The choice of wavelength directly impacts the sensitivity, selectivity and accuracy of the analytical method. The UV spectrum of DINP, meropenem, levetiracetam were recorded, wavelength selected were 226nm, 298nm, 202nm, respectively.

The DINP and drugs were separated on the Merck Pre-coated TLC plates with silica gel 60F254. The mobile phase solvents trials were made and solvents consisted of ethylacetate: methanol: water: formic acid (7:4:1:3 v/v/v/v) for meropenem, toluene: acetone: methanol (6:2:2 v/v/v) for levetiracetam was optimized as they resulted good separation.

Validation of method:

Specificity: The peak purity of DINP, meropenem, and levetiracetam, was investigated comparing their UV spectra at three different regions of the spot i.e., peak start (s), peak apex (m) and peak end (e).

Linearity: From the standard solution of DINP, Meropenem and levetiracetam (500 µg/mL) aliquots of 0.5 to 3µL was applied on TLC plate to obtain standard densitograms. The calibration curve was obtained by plotting peak area against concentration of the samples. Linear calibration curve was assessed by using linear least square-regression analysis.

Precision

Intraday Precision: Intraday precision assessment for the determination of DINP and meropenem /levetiracetam was performed on a single day at different concentration levels: 500 to 1000 (ng/band) for DINP, meropenem, levetiracetam. Subsequently, the Relative Standard Deviation (RSD %) values were computed. Precision and repeatability for the developed analytical method was within the limits (RSD<2) according to ICH guidelines.

Interday: Interday precision assessment for determining DINP, meropenem, levetiracetam, conducted across distinct days. Concentration levels of 500 and 1000 (ng/band) were utilized for DINP, meropenem, Levetiracetam. The RSD (%) values were computed for each of the analyte.

Repeatability: The repeatability sample of DINP, meropenem, levetiracetam,1000 (ng/band) was injected thrice and the RSD (%) of peak areas was determined.

Determination of presence of DINP in IV line tube by extraction procedure

To evaluate the presence of DINP in tube, it was cut into pieces, and extraction was carried out based on the literature [9]. Approximately 30 mg of each sample was cut and 2 mL of acetone was added, and the mixture was extracted for 30 minutes at room temperature using a reflux condenser. Subsequently, it was evaporated at room temperature. The remaining residue were reconstituted with methanol and evaluated by HPTLC. DINP peak were observed at Rf 0.82.

Evaluation of leachability:

The selected IV line tube (which was confirmed for presence of DINP) was used for the leachability study. The size of the tube was selected based on trial and error to fix the length of tube required to fill the study sample. The tube cut size 35cm in length was chosen for the study. The IV tubes were filled with matrix and they were kept at room temperature and air condition room during study. Study timing was framed as 2 hours, every time sample was taken for analysis, tubes were cut into 4 pieces (35cm) and sampled at 0 ,30, 60, 120 minutes. For every sampling time, a sample was taken from the respective tubes and evaluated by HPTLC. Matrix for the study was normal saline as it is commonly administered fluid for patients, either with or without drugs. During the study samples are collected and analyzed by the HPTLC method.

Interaction study:

To evaluate interaction, different set of tubes were filled with DINP and Meropenem as well as DINP with Levetiracetam and subjected to condition as mentioned in leachability study. The samples taken at different time intervals were analysed by HPTLC method and results were evaluated based on peak area of drugs remaining.

IV. RESULTS AND DISCUSSION

The HPTLC method developed for separation of DINP from Meropenem and Levetiracetam were validated and found to have good accuracy and precision. Their %RSD values were within the limit (<2). The method is said to be specific as the peak purity index value for all individual components were close to 1. An ideal densitogram of DINP with meropenem and levetiracetam are shown in figure 1-2. The Rf value of meropenem is 0.2 and

Levetiracetam is 0.5 and DINP is 0.8. The method is validated as per ICH guidelines and parameters in Table 1. The linearity was found to be 250-1250 ng/band for both drugs and for a fixed concentration (10%) of DINP was used in the study for interactions. The correlation values were 0.998 and 0.997, respectively for Meropenem and Levetiracetam The linear calibration curve obtained is shown in fig 3-4. DINP extracted from tube confirms its presence in selected IV line tube and used in leaching study. DINP leached into matrix and leaching was more in room temperature than in air conditioned room. The leaching of plasticizer DINP steadily increases to matrices, 2-3% at air condition 5-7% at room temperature while the drug solutions were present in tube.

Interaction study of DINP with two drugs Meropenem and Levetiracetam assessed with a sampling period of 0-2hr. In all the study samples were done triplicate and results were consolidated (Table 2). The leaching of DINP from tubes was higher (7 times) while in presence of two drugs than without drugs. However, the concentration of drugs remains unaltered during the study. The study ensures increased leaching of DINP plasticizer in presence of lipophilic drugs like Meropenem and Levetiracetam which was confirmed by HPTLC analysis which in turn may result adverse events in future.

V. CONCLUSION

The medical devices play crucial role in treating simple aliment to complex one therefore their efficiency and safety ultimately carries great value. The compatibility of medical devices during drugs administration very vital. The HPTLC technique developed for evaluation of selected drugs and DINP are of the first one of the similar kinds and serves as important role in the assessment of leaching and interaction studies. The present work ensures leaching of DINP plasticizer in present lipophilic drugs like Meropenem and Levetiracetam. The drugs concentrations remain unaltered in the study period, however DINP leaching increased considerably while it is present with drugs lead to adverse effects to patients in future.

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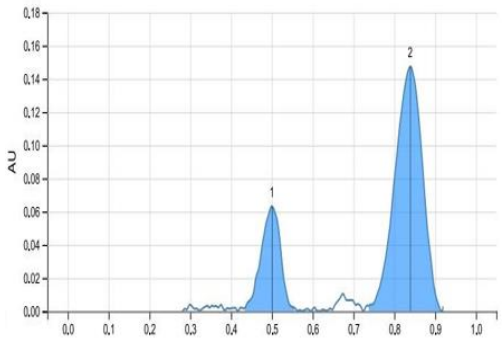

Fig 1. Densitogram of Levetiracetam and DINP

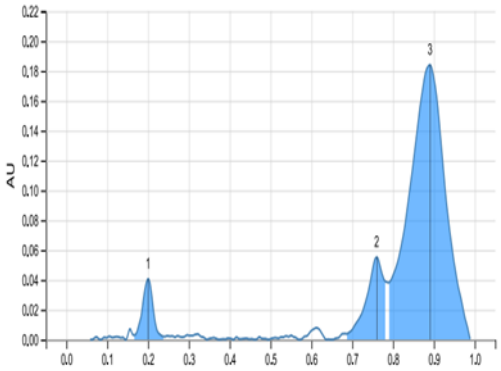

Fig 2. Densitogram of Meropenem and DINP

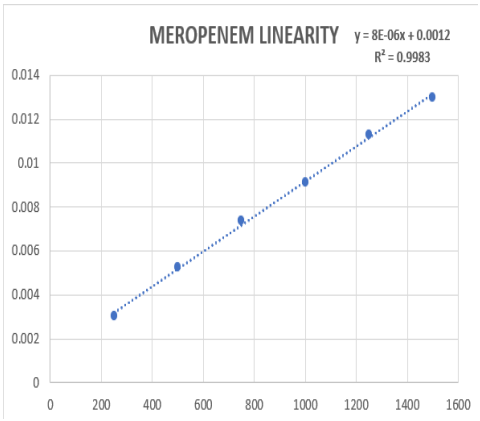

Fig 3. Calibration curve of meropenem

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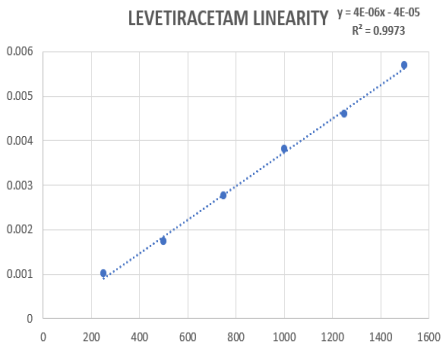

Fig 4. Calibration curve of levetiracetam

Table No.1 Validation parameters

Parameter	DINP	Meropenem	Levetiracetam
Detection wavelength (nm)	226	298	202
Linear concentration range (µg/mL)	0.5- 3µL		
Regression equation (Y)[a]	Y=8E-06x+0.00027	Y=8E-06x+0.0012	Y=4E-06x-4E-05
Correlation coefficient (r^2)	0.9982	0.9983	0.9973
precision % RSD	0.6	0.9	1.4
Interday			
Intraday	1.2	0.9	1.3
Repeatability	1.66	1.3	1.7

Table No.2 HPTLC result for DINP interaction and drugs

Time (min)	Peak Areas			
	DINP	Meropenem	DINP	Levetiracetam
0	0.01799	0.00184	0.01905	0.00195
30	0.02065	0.00178	0.02640	0.00196
60	0.02197	0.00171	0.02706	0.00197
120	0.02397	0.00186	0.02877	0.00194