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Study of Selected Non-Deliberately Supplementary Substances in Pharmaceutical Formulations by Using Hptlc

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ABSTRACT

The safety and efficacy of pharmaceutical products along with packaging material needs to be addressed. To maintain purity of product the whole process starting from raw material to end users should be selective, as the chances of impurity entered the product at any stage of the process. These types of impurities are known as extractables and leachables among them extractables are extracting components from the packaging material while in case of leachables are migrating components from the extracting ones. According to regulatory authorities the extractables and leachables impurities data are maintained by parenteral drug associations, USFDA. In current scenario the ICH guideline has introduced guidelines on extractables and leachables under Q3E (Expert Working Group). In present work various analytes were tried to develop a selective and sensitive method by high performance thin layer chromatography, but after performing certain trials the expected results were not generated. To get a nearest idea about the analyte finest methods like LC-MS/MS for non-volatile organic compounds, GC-MS/MS for volatile organic compounds and ICP-OES for metal compounds may help appropriately.

Keywords: Extractables- Leachables, TLC, HPTLC, non-Intentionally added substances, ICH Q3(E).

1. Introduction

The impurity is defined as any element of a drug substance for therapeutic use or of a drug creation that is not the chemical object that defines the constituent, or in the case of a drug product, not an excipient in the creation[1]. Extractable studies are accomplished to identify constituents that a patient may be bare to. Extractable studies are valuations performed on a material. Substantial like extractables can be extracted with suitable diluters for example hexane, isopropanol, water, methyl chloride, and so on. Organic and inorganic chemical modules can be released from the exteriors of components used in the production and storage of drug products under test center conditions with the purpose of time and temperature. Also, extractable are properties of the elements of drug product delivery systems, container/closure systems, and manufacturing systems. Leachables studies are achieved by recognizing substances that patients are borne to. Leachables identifications are valuations performed on the drug product. Therefore, the leachables are typically a partition of extractables but it is not always possible. Leachables are chemical objects, both organic and inorganic, that travel from components of a structure into a drug product throughout the system's life. Thus, extractables are momentous because they help to sort probable leachables. The key classes of extractables

and leachables are inorganic compounds, non-volatile organic compounds, volatile organic compounds, and metal compounds. The capitals of leachables are a syringe barrels, container closure system, valve components for pressurized meter dose inhalers, and plungers in rubber stoppers and glass vials, prefilled syringes, nasal pumps, bottles, and caps. The agents like secondary packaging materials, inks and dyes, residual cleaning agents, residual monomers, phthalates, polymers and monomers, antistatic agents, antioxidants and stabilizers, lubricants, emulsifiers, molding agents, vulcanizing agents, nitrosamine and polyaromatic hydrocarbons are more effective ways through which extractable and leachables may insert the products. Drug product and transport systems- mouthpieces, administration sets, IV bags, drug delivery pumps, Secondary packaging materials like ink on labels, and fill pouches aimed at low-density polyethylene ampules[2-4]. There are some supervisory bodies like PDA (Parenteral Drug Association)[5], USFDA (United States Food and Drug Administration)[6], Indian Pharmacopoeia [1], EMEA (Europe, the Middle East and Africa)[7], Schedule M of Drug and Cosmetics Act[8] and ICH Q3E to legalize the amount of extractable and leachables in the pharmaceutical formulations[9]. Additionally, to measure the amount of extractables and leachables numerous techniques are available like primary testing methods and secondary or final testing methods. Amongst them, techniques like FT-IR, Total Organic Carbon (TOC), pH, Conductivity, NMR, Ion chromatography, Non-Volatile Residue (NVR), Differential Scanning Calorimetry (DSC) and Gel Permeation chromatography are coming under primary testing methods while techniques like GC-MS/MS, HPTLC-MS, LC-MS/MS, ICP-OES are coming under secondary or final testing methods. In the present research, a novel method of HPTLC was tried to developed and validated completely according to the ICH Q2R1 guidelines[9]. To identify various different potential extractable and leachables belonging to different classes from the pharmaceutical formulations.

2. Materials and Methods

To develop a sensitive and accurate HPTLC method stationary phase and mobile phase combinations plays an important role. Various analytes have been used to develop a HPTLC method along with various mobile phase combinations. Silica gel G have been used as a stationary phase on plate-based aluminum as a plate material. Precoated aluminum plate coated with silica gel G were used. Analytes like stearic acid, Pentaerythritol tetrakis [3-(3,5-di- tert butyl- 4- hydroxyphenol) propionate], 2-mercaptobenzothiazole, Tris (2,4- di-tert-butylphenyl) phosphite, Nonyl Phenol etc. were used to develop a method. These analytes have belonged to the categories rubber lubricants, antioxidants in polyethylene and polypropylene, rubber materials, antioxidants in polyethylene and polypropylene and additives in plastic and rubber materials respectively. Procurement of these analytes have been done from TCI, Tokyo, Japan and nonyl phenol were procured from CDH, New Delhi, India. All the analytes are having purity of >98%, >95%, >98%, >98%, and analytical standard grade respectively. As a mobile phase various solvents like n-Hexane, Acetone, Ethyl acetate, Petroleum Ether, Dichloroethane, Ethyl ether, Acetonitrile, Phosphate buffer (6pH), Formic acid, Chloroform, Methanol, etc. were used to develop a method. Other reagents used were of HPLC grade. The glassware used was of borosilicate according to the appropriate volume size. Different solvents were tried to check the solubility of all standards, also melting point has been tested for all of them.

2.1. Preparation of rinsing solution

Precisely measured capacity of methanol (HPLC grade) and water (Milli-Q-Water) filled used at room temperature as a rinsing solution.

2.2. Preparation of Stalk Solutions

It is a concentrated solution that will be diluted to some lower concentration for actual use. All the standards were accurately weighed 10 mg dissolved in 10 ml acetone (HPLC grade) individually and sonicated for 10 min (if required).

2.3. Preparation of the final solution

From the above solution, accurately 0.15ml solution was withdrawn from all four standards and made up the volume with acetone to produce 10ml of the total solution.

3. Results

3.1. Chromatographic trials results

To develop a method various mobile phase has been tried. Capillary tube was used for a sample application on TLC plate and spotting method was used to apply a sample. Initially normal TLC has been tried to optimize a chromatographic condition. In accordance with method development various mobile phase combination trials with ratios are as mentioned here. Starting with the very first trial Petroleum ether: Acetone in the ratio of 9:1 respectively, resultant in no spot separation was observed. Next trial was taken in N-Hexane: Ethyl Acetate in the ration of 7:3 respectively was tried and resulted again no spot separation. After that Dichloroethane: Ethyl Ether were used as a mobile phase in the ration of 1:1 and observed two analytes spot but not clearly. Furthermore Acetonitrile: 0.01M Phosphate buffer solution at 6pH was prepared as a mobile phase in the ration of 8:2 and any analyte spot were not observed. Next to that N-Hexane: Ethyl acetate: Formic acid combination was tried as a mobile phase in the ratio of 8:2:0.1, in this trial three analytes were observed but not clearly. At last Chloroform: Methanol was tried as a mobile phase in the ratio of 5:1 and observed two analytes but not clearly. To carried out the study continue more solvents have been used as a mobile phase. Next trial was done by using Cyclohexane: Ethyl acetate: Ammonium hydroxide in the ratio of 8:2:0.1, in that any spot was not observed. Subsequently Cyclohexane: Ethyl acetate: Formic acid was tried in a combination at 8:2:0.1 ratio in that any spot was not observed. Moreover n-Hexane: Isopropyl Alcohol was applied as a mobile phase with 1:0.25 ratio and any spot observation were not found. Moving further Methanol: Water: Acetic acid trial was done as a mobile phase in the ratio of 1:1:0.1, in this one analyte were applied and more than one spot was observed as a part of impurity or might some other reason. After that Methanol: Water in the ratio of 7.4:2.6 were established as a mobile phase but any spot was not found on plate. Finally at last Acetonitrile: Water was used as a mobile phase in the ratio of 6.7:3.3 but spot separation was not observed clearly.

Tables 1: Selected Analytes

Analytes	**Manufacturer/ Supplier**	**Purity**
1) Stearic Acid	TCI	>98%
2) Pentaerythritol tetrakis [3-(3,5-di- tert butyl- 4-hydroxyphenol) propionate]	TCI	>95%
3) 2-Mercaptobenzothiazole	TCI	>98%
4) Tris (2,4- di-tert-butylphenyl) phosphite	TCI	>98%
5) Nonyl Phenol	CDH	Ana. Std.

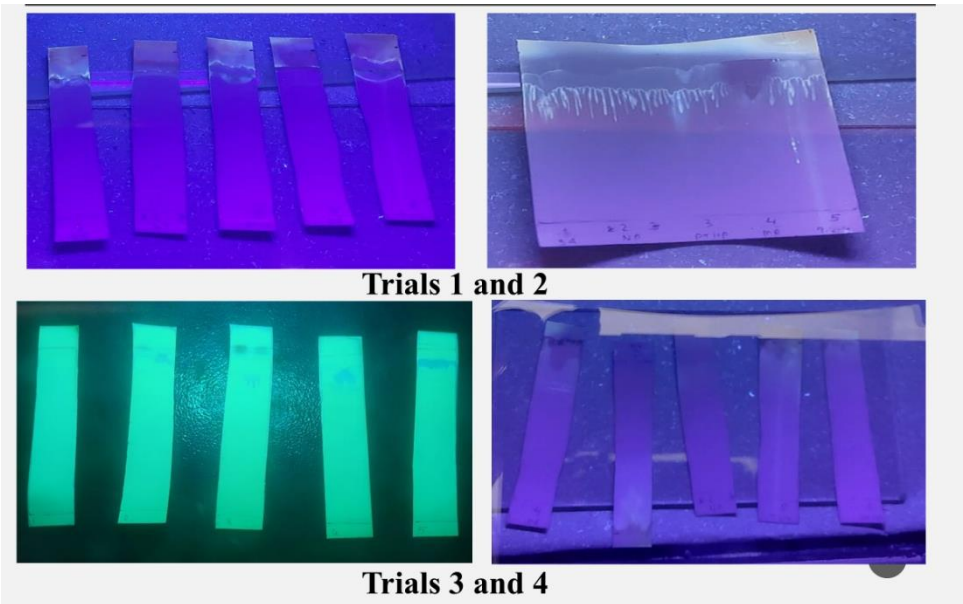
Table 2: Description of Analyte

Analytes	**Category**
1) Stearic Acid	Rubber-Lubricant
2) Pentaerythritol tetrakis [3-(3,5-di- tert butyl- 4-hydroxyphenol) propionate]	Antioxidants in polyethylene and polypropylene

3) 2-Mercaptobenzothiazole	Rubber materials
4) Tris (2,4- di-tert-butylphenyl) phosphite	Antioxidants in polyethylene and polypropylene
5) Nonyl Phenol	Additives in plastic and rubber material

Table 3: Observation of the trials

Sr no.	**Mobile Phase Trials**	**Observation**
1	Petroleum ether: Acetone (9:1)	Not observed any spot
2	N-Hexane: Ethyl Acetate (7:3)	Not observed any spot
3	Dichloroethane: Ethyl Ether (1:1)	Observed spot in two analytes but not clear
4	Acetonitrile: 0.01M Phosphate buffer-6pH (8:2)	Not observed any spot
5	N-Hexane: Ethyl acetate: Formic acid (8:2:0.1)	Spot observed in three analytes but not clear
6	Chloroform: Methanol (5:1)	Spot observed in two analytes but not clear
7	Cyclohexane: Ethyl acetate: Ammonium hydroxide (8:2:0.1)	Not observed any spot
8	Cyclohexane: Ethyl acetate: Formic acid (8:2:0.1)	Not observed any spot
9	n-Hexane: Isopropyl Alcohol (1:0.25)	No clear spot observed
10	Methanol: Water: Acetic acid (1:1:0.1)	More than one spot observed in single plate
11	Methanol: Water (7.4:2.6)	Not observed any spot
12	Acetonitrile: Water (6.7:3.3)	Not observed any spot



Trials 1 and 2

Trials 3 and 4

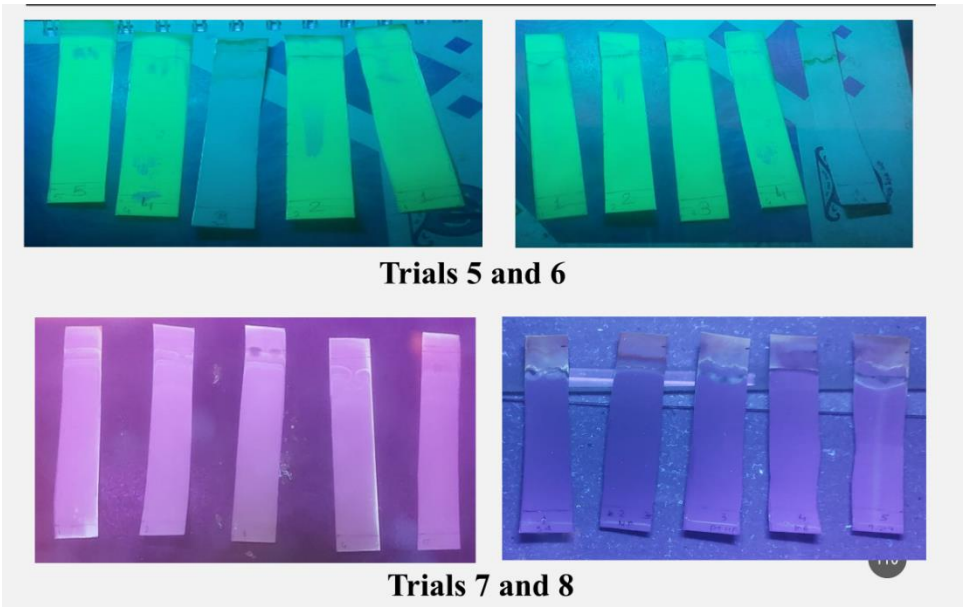


Figure 1: various Mobile Phase Trials

4. Discussions

4.1 Discussions

After performing numerous trials, the results are not falls as expected. Some of the above-mentioned analytes are showing positive result but the optimization was not possible with the same. The analytes were procured in the form of high purity and analytical grade, so the chances of impurities presented are very less. The solvents for mobile phase were tried with different combinations and different ratio to get the expected results. The figure shows that the separation of analytes is not clear. It may consider that the stalk solutions are prepared with acetone as a solvent because mostly the analytes are dissolves easily in acetone.

5. Conclusion

From the trials it may conclude that the use of thin layer chromatography for the development of selective and sensitive method for extractables and leachables should be as a primary analytical technique, for the secondary or final technique use of LC-MS, GC-MS and ICP-OES and HPTLC-MS techniques are more advisable. The use of mobile phase for the mentioned analytes are not giving any precise result so, be ensure while selecting mobile phase for the selected extractables and leachables. Acetone is appropriate as a solvent in which analytes can dissolves directly or by applying sonication.

The risk of toxicity is higher in case of leaching it may affect the functions of the product in terms of reduction in stability, alter impurity profiles, inactivate the active ingredients, alter the smell, taste or color.

The conclusions derived from the study that all the selected substance samples were found reported as leachables from the literature survey. The use of flexible material is increasing day by day but with the use of such type of material the quality of the product, safety and efficacy need to be talked. It was observed that pharmaceutical formulations particularly parenteral and ocular drug products are available in such materials as a packaging material and elastic has the disadvantage of leaching that is not intentionally added but comes to the product throughout the life span of the product early from its manufacturing to its use. So, it may cause impairment the patients who are the consumers of the products or the end users.

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7. Conflict of Interest

The Authors have no conflict of interest.

8. Informed Consent Statement

There are no participants involved in this study.

9. Authors' Contributions

All authors have contributed equally to this work and have reviewed and approved the final manuscript for publication.

10. Financial Disclosure

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11. Consent for Publication

There are no participants involved in this study.

12. Ethical Statement

This project was exempt from IRB review as it did not qualify as human subject research under federal regulations.

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