Evaluation and Identification of Leachables from the Container Closure System in Ophthalmic Product

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DECLARATION

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List of Abbreviations:

CEO	Chief Executive Officer
APCI	Atmospheric Pressure Chemical Ionisation
API	Active Pharmaceutical Ingredient
B	Magnetic Field Strength
BBP	Benyl Butyly Phthalate
BHA	Butylated Hydroxyanisole
BHT	Butylated Hydroxytoluene
C	Charge of an ion
CDER	Centre for Drug Evaluation and Research
CI	Chemical Ionisation
CpK	Process Capability
CQA	Critical Quality Attribute
DAP	Diamyl Phthalate
DBP	Dibutyl Phthalate
DC	Direct Current
DCHP	Dicyclohexyl Phthalate
DEHP	Di-(2-ethylhexyl Phthalate
DEP	Diethyly Phthalate

DHP	Dihexyl Phthalate
DIBP	Diisobutyl Phthalate
DIP	Direct Insertion Probe
DMGP	Dimethylglycol Phthalate
DMP	Dimethyl Phthalate
DNOP	Di-n-octyl Phthalate
DOE	Design of Experiment
DPP	Dipropyl Phthalate
DSS	Detector Sensitivity Solution
e	Electron
EFSA	European Food Safety Authority
EHA	2-ethylhexyl-4-dimethylaminobenzoate
EI	Electron Ionisation
ESI	Electrospray Ionisation
eV	Electron Volt
FAB	Fast Atom Bombardment
FD	Field Desorption
FDA	Food and Drug Administration
FI	Field Ionisation

FID	Flame Ionization Detector
GC	Gas Chromatography
HDPE	High Density Polyethylene
HPLC	High Performance Liquid Chromatography
HSGC	Headspace Gas Chromatography
HTLC	High Temperature Liquid Chromatography
ICH	International Conference on Harmization
ICP	Inductively Coupled Plasma
IE	Ionisation Energy
IPAC-RS	International Pharmaceutical Aerosol Consortium for Regulation and Science
ITX	2-isopropyl thioxanthone
IUL	Individual Unidentified leachable
LC	Liquid Chromatography
LDPE	Low Density Polyethylene
LSL	Lower Specification Limit
m/z	Mass to Charge Ratio
MALDI	Matrix Assisted Laser Desorption Ionisation
MeOH	Methanol
mins	Minutes

mL	Millilitre
mm	Millimetre
MS	Mass Spectrometry
N	Theoretical Plates
nm	Nanometres
NMR	Nuclear Magnetic Resonance
OINDP	Orally Inhaled and Nasal Drug Products
PDA	Photodiode Array
ppb	Part Per Billion
PQRI	Product Quality research Institute
PRCA	Pure Red Cell Aplasia
psi	Pounds Per Square Inch
PVC	Polyvinyl Chloride
QbD	Quality by Design
R&D	Research and Development
RF	Radio Frequency
RH	Relative Humidity
RI	Resonance Ionisation
RP	Reverse Phase

Rs	Resolution
TBA	2,4,6-Tribromoanisole
TOF	Time Of Flight
TSP	Thermospray
UPLC	Ultra Performance Liquid Chromatography
USL	Upper Specification Limit
USP	Untied States Pharmacopeia
UV	Ultra-Violet
v	Velocity
V	Voltage
v/v	Volume per volume
w/v	Weight per volume

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Abstract:

In drug product packaging, migration of components from the container closure system into final formulation can compromise the therapeutic effect of the active pharmaceutical ingredient (API) or pose a health risk to the consumer. In response to this, regulatory expectations have elevated so that pharmaceutical companies show due diligence in the detection, quantitation, identification and monitoring of leachables through the shelf life of product. This work describes methods for the screening, quantification and identification of potential leachables in ophthalmic solutions. Particular focus was given to developing analytical separations using quality by design (QbD) principles.

An ultra performance liquid chromatography (UPLC) method was developed for the analysis of 8 potential leachables in ophthalmic solutions. The method was optimized in the centre of a design space using fractional factorial design of experiments (DOE) ensuring baseline resolution while minimizing analytical run time. Repeatability validation studies were assessed using process capability (CpK) techniques. The test method was geometrically transformed to allow simultaneous analysis on high performance liquid chromatography (HPLC) test systems. Mass spectra were generated for the leachables using an electrospray ionization (ESI) ion trap mass spectrometer.

An extraction and leachable study was executed on an ophthalmic solution under long term stability conditions in Chapter 3. The screening profile and concentration of leachables in the final drug product was monitored with consideration for time, temperature and humidity variables. The structural identity of any detected leachables were unknown and were tracked using their retention time relative to the reference standard. A single leachable was detected and the unidentified leachable was qualified through extraction of the packaging components from the container closure system (Stage I) and comparison to LC profile of the long term stability analysis (Stage II). The identification was confirmed using the LC-MS analytical method developed in Chapter 2. A single leachable, diethyl phthalate, identified using MS, was found to be present due to the adhesive used on the tamper

evident seal packaging component. It was quantitated against a qualified reference standard at a maximum level of 8 ppm. The level of 8 ppm is below the allowed 10 ppm threshold level as stipulated by the FDA

Chapter 1: A Review of Analytical Techniques used for the Determination of Extractables and Leachables in Pharmaceutical Products

1. Introduction

The global pharmaceutical industry is a thriving business and the global pharmaceutical market value is expected to expand to over $975 billion by 2013.[1] Pharmaceutical companies develop new product lines in response to medical and aesthetic indications of a global population. Success for any pharmaceutical company is primarily dependent on the products efficacy towards a particular ailment. The symbiotic relationship that exists between company and customer is the driving force for new product development. Vast amounts of money are budgeted by corporate Chief Executive Officers (CEOs) towards research and development of new drug substances in the hope that the next “wonder drug” is unearthed.[2] The time and money resources required to bring a new drug product from R&D to the high street pharmacy is immense.[3] The drug substances are formulated with various raw materials such as preservatives, antioxidants and excipients and packaged for delivery to market. The formulation matrix is designed to optimize the efficacy of the drug substance and increase shelf-life of the drug product. Capital investment into early stage drug development and clinical trial investigations could be rendered worthless through the use of unsuitable packaging. Migration of components from the container closure system into final formulation can compromise the therapeutic effect of the active pharmaceutical ingredient (API) or pose a health risk to the consumer. Not only is the direct toxicity of the transferred material a concern but the interaction with it and the API and other product components may lead to harmful degradation products. The terms used for these migrating entities are Extractables and Leachables.[4]

An extractable may be defined as a chemical species which migrates under stressed conditions from any component involved in the manufacturing and packaging process. The rate of migration may be dependent upon solvent strength, exposure time and elevated temperatures. The term extractables encompasses all theoretical impurities which may elute

into the final product due to the use of stressed conditions. Leachables are a subset of extractables, as a leachable is a chemical species which can migrate into the product under normal conditions. The normal conditions are the recommended storage requirements of the final product and the extraction solvent is the actual final product. Degradants are impurities which are directly related to the breakdown products of the API. They may be a consequence of the natural stability of the API or as a result of an interaction between the API and a leachable. There are a number of sources from which a leachable may enter final product including equipment used in the manufacturing process which is in direct contact with the formulation and the final packaging. The primary source for leachables is the packaging components of the container closure system. The packaging components can be broken down into three subcategories, all of which have the potential to result in leachables.[5]

(a) Primary Packaging Components

The packaging items which are in direct contact with the final product and holds the unit formulation. These items pose the greatest risk for potential leachables. Typical examples of primary packing components are low or high density polyethylene (LDPE/HDPE) containers. Phthalate plasticizers used in the manufacture of primary containers are a typical example of migrating components.

(b) Secondary Packaging Components

The packaging components which are in direct contact with the primary packaging component or holds a number of primary packaging units together. Typical examples are labels, inks, adhesives or a LDPE overwrap to hold single units together. Secondary components are less likely to result in leachables at a significant level. Benzophenone initiator used in the UV ink curing process is an example of a component which can migrate into final product from the secondary packaging.[6]

(c) Tertiary Packaging Components

The packaging components used for the distribution of product. Typical examples are palletized product for storage and shipping. Fungicide which has been used for treatment of wood pallets can potentially migrate into the final product from the tertiary packaging.[7]

The subject area of extractables and leachables has become a focus point for the regulatory agencies recently which subsequently merits due diligence by the product manufacturer i.e. the care that a reasonable person exercises to avoid harm to other persons.[8, 9, 10] It was in 1999 that The United States Food and Drug Administration (FDA) issued a guidance document in relation to packaging requirements for new drug products.[11] The guidance within this document stipulates that the compatibility of the drug product with the primary container closure system be established. Exact procedures for establishing the compatibility are not provided for within the guidance document and this has meant that the selection process for container closure systems between pharmaceutical companies may vary. The Orally Inhaled and Nasal Drug Products (OINDP) sector of the pharmaceutical industry have taken the initiative in relation to extractables and leachables.[12] The International Pharmaceutical Aerosol Consortium for Regulation and Science (IPAC-RS) and the Product Quality Research Institute (PQRI) Leachables and Extractables Working Group have studied leachables and extractables in OINDP and submitted proposals to the FDA regarding safety and analytical thresholds.[12, 13]

The detection, quantification and characterization of leachables in drug products provides a challenge as the leachables are present at low part per million (ppm) concentrations and there are interfering matrix effects of raw materials in the final product. This challenge is often overcome by combining a number of analytical techniques and strategies. Analytical strategies will differ depending on the product matrix, whether at early stage product development or marketed product. In the case of organic extractables and

leachables, regulatory agencies have recommended the use of Gas Chromatography (GC) and Liquid Chromatography (LC) for the separation of leachable components and either Flame Ionization Detection (FID), Ultra-violet (UV) or Mass Spectrometry (MS) for their detection and quantitation.[14] The volatility (or other physical characteristics) of the chemical species will often determine the best route of analysis. Advances in analytical technology has allowed for improved separations with a high level of certainty for structure elucidation. Early day LC/UV systems permitted the basic screening of unknown compounds within the pharmaceutical product. The unknown peaks could be somewhat categorised as either a leachable or degradation substance by the use of a control sample which had no contact with the container closure system. Use of control samples, however, does not conclusively account for products which may be produced as a result of an interaction between the API and a leachable. An API- leachable degradant may elute at the same retention time as a API degradant and consequently be misidentified during analysis. The development of photodiode array (PDA) detectors meant that UV spectra of the unknown compound could be generated affording structural information about the class of compound. PDA analysis can detect compounds which have similar UV spectra and exhibit chromophores at the same wavelength, but will not give the required structural information to aid in the identification of a compound. MS was deemed a scientific breakthrough for the identification of unknown analytes across a broad spectrum of industries. MS detects molecular weight and elemental composition of substances and combined with known fragmentation patterns allows for structure elucidation with a high level of confidence. The ability for an analytical scientist to identify an unknown component is not only limited to the available MS instrumentation but also the instrument method parameters which are validated to run on the particular instrument. Consequently, there is greater focus on producing more robust methods at the initial development stage.[15] The quest for the development of robust HPLC methods is documented by Molnar.[16]

The benefits of incorporating improved statistical analysis and introducing a quality-by-design (QbD) approach to analytical method development and validation are being recognised by the authorities. Historically, QbD principles were implemented in early stage drug development to improve robustness and increase output for manufacturing processes. These principles, such as defining the critical quality attributes (CQAs) are transferable to the development, validation and control of analytical methods.[16, 17, 18]

1.1 Extractables and Leachables

The FDAs Centre for Drug Evaluation and Research (CDER) provides a definition for extractables and leachables and distinguishes between the two.[11]

"The term extractable is specific to a container/closure material. A compound is said to be an extractable if it is identified in solvents that were exposed to the virgin container material under standard conditions, as specified in the USP. A compound is said to be a leachable if it is identified in the drug product following storage of that product, but was not identifiable in the product initially (assuming the compound is not a degradation product). Therefore, an extractable is a theoretical impurity of a drug product that utilizes a container/closure known to be associated with that extractable compound, while a leachable is a known impurity of a given product."

The FDA's concern for drug product stability and purity has led to an increased focus on pharmaceutical manufacturing processes and final container closure systems. In particular, the potential for chemical species from the processing equipment or packaging components to migrate into the product and have a negative impact on drug product must be assessed. Plastic components, sterilization by-products, adhesives, fungicides, inks and accelerants are among the possible sources for leachables. Figure 1.1 illustrates a number of sources for potential leachables migration into final drug product.

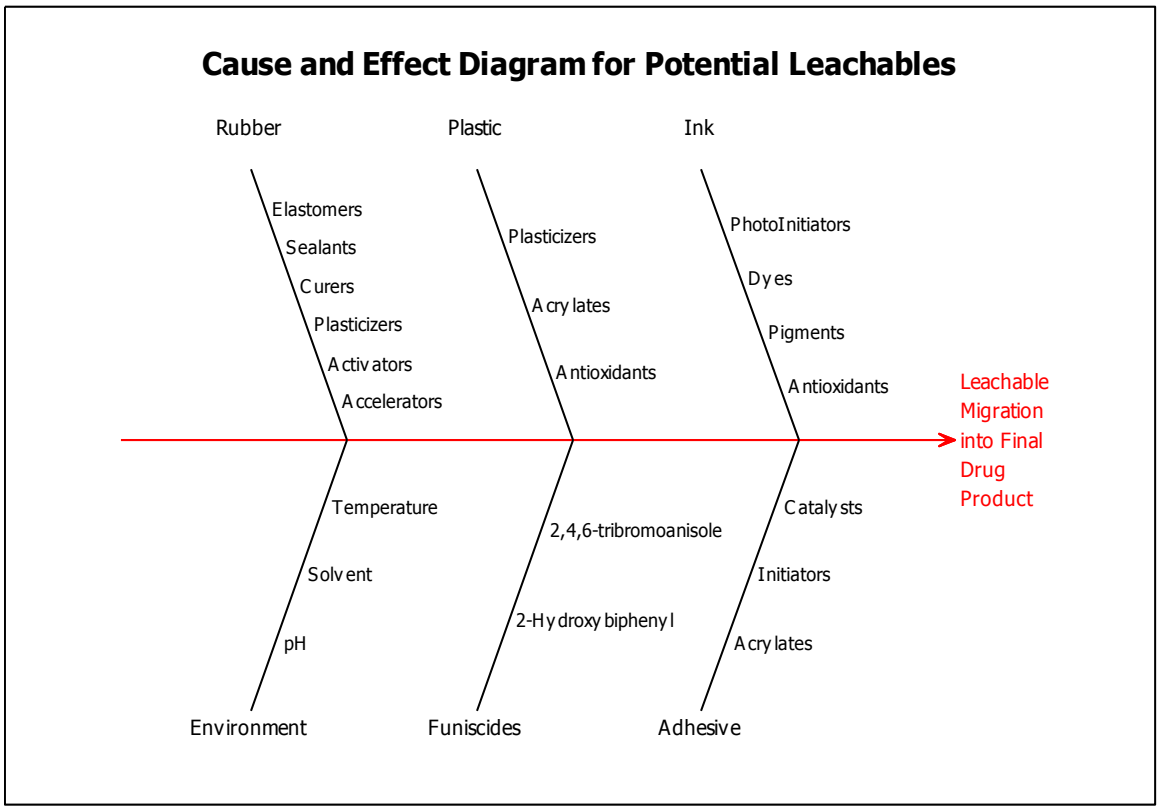


Figure 1.1: Cause and Effect Diagram for Potential Leachables in Final Drug Product

Extractables and leachables are not just bound to the final packaging configuration but also to the actual manufacture of product. Single-use process equipment including bioreactors, buffer preparation, storage of bulk formulations and filling of single unit-dose containers must all address the issue of potential extractables and leachables.[19] The FDAs commitment to this is highlighted in recent 483 observations (Warning Letters).[20] In one case, in a drug product purification process, the company had only performed extractable and leachable testing on the 0.22 µm sterilizing filter and neglected to perform the same analysis on the other equipment and materials used in the purification. The regulatory need to perform adequate extractable and leachable testing on all components is well justified. At the turn of the 21st Century, an increase was observed in the number of patients suffering from antibody-positive pure red cell aplasia (PRCA), all of whom were using the Johnson & Johnson product Eprex®.[21, 22] The formulation matrix was changed to include Polysorbate-80 as a drug product stabilizer in 1998. This change aided the leaching of organic components

such as dialkylphenol disulfide from uncoated rubber stoppers used in the Eprex® syringe and subsequently was proven to be responsible for the increased incidence of PRCA. Pfizer®, Depomed® and Johnson & Johnson McNeil® have all had product recalls after numerous customer complaints were made about unusual odours from their products Lipitor®, Glumetza® and Tylenol®, respectively. The source of the odour in all cases was attributed to 2,4,6-tribromoanisole (TBA) which is a biomethylation product of 2,4,6-tribromophenol fungicide used on wooden pallets for the shipping and storage of samples. The biomethylation reaction occurs where 2,4,6-tribromophenol is converted to 2,4,6-tribromoanisole via metabolic transformation by xerophilic fungi. In all cases the lumbar was sourced from South America where moisture content > 20% and temperature range 25 – 35°C. The TBA migrated through the tertiary, secondary and primary packaging components into the final product.[20]

In response to the need for regulatory direction, CDER produced a guidance document for industry on container closure systems for packaging human drugs and biologics.[11] This document classifies the safety concerns associated with packaging components for aerosols, injections, ophthalmic solutions and transdermal ointments and is summarized in Table 1.1.

Table 1.1: FDA Packaging Concerns for Various Classes of Drug Products[9]

Degree of Concern Associated with the route of Administration	Likelihood of Packaging Component-Dosage Form Interaction		
	High	**Medium**	**Low**
Highest	Inhalation Aerosols and Solutions; Injections and Injectable Suspensions[a]	Sterile Powders and Powders for Injection; Inhalation Powders	
High	Ophthalmic Solutions and Suspensions; Transdermal Ointments and Patches; Nasal Aerosols and Sprays		
Low	Topical Solutions and Suspensions; Topical and Lingual Aerosols; Oral Solutions and Suspensions	Topic Powders; Oral Powders	Oral Tablets and Oral (Hard and Soft Gelatin) Capsules

[a] For the purpose of this table, the term *suspension* is used to mean a mixture of two immiscible phases (e.g. solid in liquid or liquid in liquid). As such it encompasses a wide variety of dosage forms such as creams, ointments, gels, and emulsions, as well as suspensions in the pharmaceutical sense.

Inhalation aerosol represent the highest degree of concern associated with the route of administration and the highest likelihood of packaging component-dosage form interaction. Conversely, oral tablet and capsules provide the lowest degree of concern associated with the route of administration and the lowest likelihood of packaging component-dosage form interaction. The proposed packaging should be appropriate for the intended use of the product. Specifically, “Every proposed packaging system should be shown to be *suitable* for its intended use: it should adequately *protect* the dosage form; it should be *compatible* with the dosage form and the route of administration”.[13] The FDA CDER group also stipulate that

"Packaging components should be constructed of materials that will not leach harmful or undesirable amounts of substances to which a patient will be exposed when being treated with the drug product". [13]

Although the probability of migration is greatest with the material in direct contact with the final product (e.g. bottle resin), other components (e.g. inks and adhesives) should also be assessed for their potential to migrate through the barriers into the final dosage form. Use of plastic materials for pharmaceutical containers has increased with polyethylene, polypropylene, polyvinylchloride, polyester and laminated materials containing multiple polymers constituting various make-ups. Leachables can include but not limited to residual monomer building blocks or additives to make the plastic strong or malleable, colourants, UV stabilizers, initiators or catalysts. Low density plastic components pose less of a migration barrier than high density components. As a result, in low density plastics, the leachable concentration in a drug product may increase due to increased migration of chemical species from the packaging component and also a tandem loss of water through the semi-permeable membrane.

Not only does the potential for packaging component migration have to be addressed but the associated toxicology of that migration must be determined.[23, 24, 25, 26] Firstly, extraction experiments need to be carried out on the packaging components and then a toxilogical study must be performed on the extracted components to assess patient impact. Typically, the packaging component is placed in a suitable solvent at elevated temperatures (to increase rate of extraction) and the solvent is analysed for extractables. The preferred solvent is the formulation matrix (if possible) as this would reflect what is likely to occur in final marketed product. Although these extraction studies are performed in the product development phase, the only true way to assess potential extractables and leachables is over the shelf life of the product with appropriate supporting toxilogical data. The United States Pharmacopeia specifies two types of Biological Reactivity tests (in vitro and in vivo) to measure toxicity of extractables and leachables such as plastics and elastomers.[27, 28] It may

not be necessary to perform robust genotoxic, mutagenic or carcinogenic studies on every indentified leachable throughout a stability study as there may already be supporting data available. It may be sufficient to assess the safety threshold based on the molecular structure, known toxicity or the known toxicity of closely related compounds. There are various databases that a toxicologist may reference as part of a data search such as TOXNET®, INCHEM® and ExPub®. [29, 30, 31]

When developing container closure systems for a pharmaceutical product, companies will screen various vendors for each component to assess suitability. The final selection process will be based upon the analytical and toxicological data.[22 - 41] The roles and responsibilities of the final product vendor and the packaging component supplier are well outlined by Jenke *et al.* (2007).[42] He defines a collaborative strategy between both parties to enable a knowledge sharing platform while protecting the confidential information belonging to each individual group. Separately, Pan *et al.* (2008)[43] and Castner *et al.* (2009)[44] propose strategies for the determination of leachables in liquid drug products which includes LC, GC, UV, PDA and MS analytical technologies. Castner *et al.* (2009) uses Log D partitioning data based on the analyte pKa and solution pH as supporting evidence for leachable identification.[44] Whichever route of analysis that a company ultimately decides upon there are decision tree processes that must be followed to justify the use of any packaging component and supporting documentation must be sent to the regulatory authorities.[11] Figure 1.2 represents the decision tree process for data submission to regulatory agencies to support drug filings.

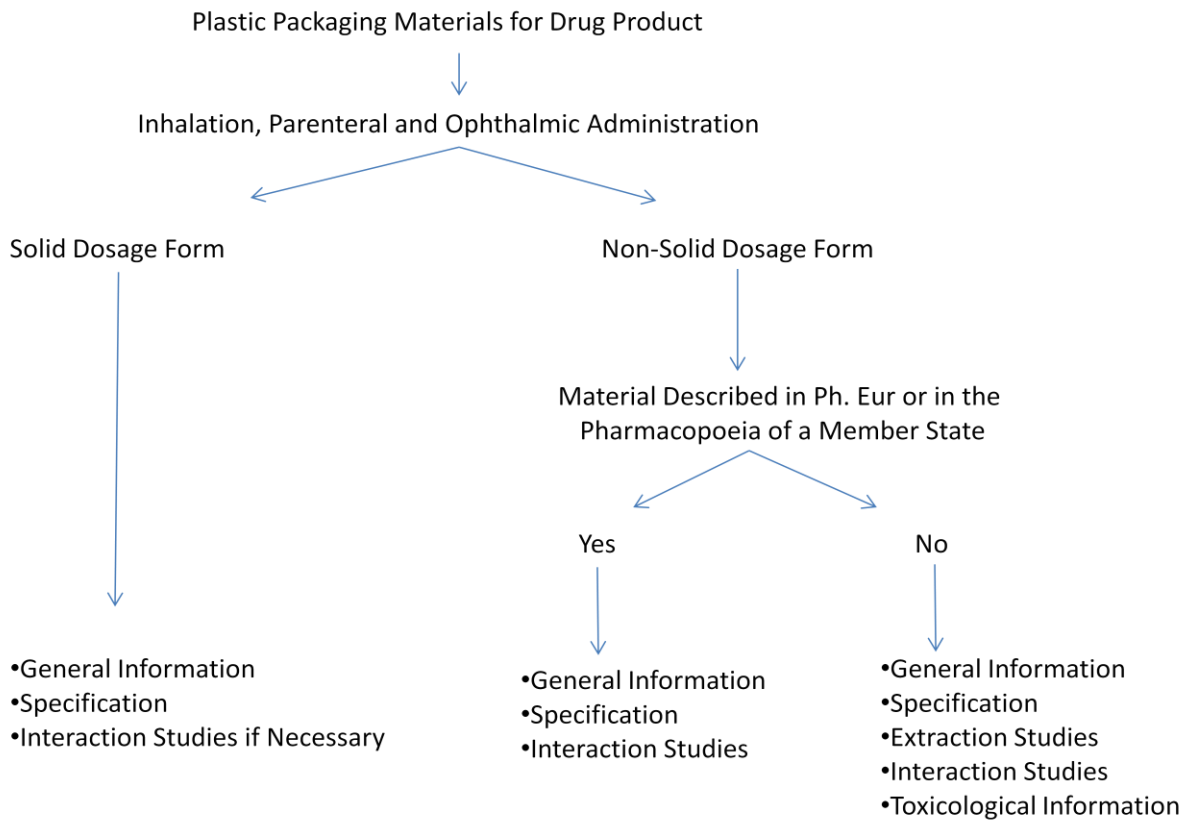


Figure 1.2: Decision Tree for Data Submission for Inhalation, Parenteral and Ophthalmic Drug Products, Adapted from Ref 9

1.2 Analysis of Extractables and Leachables

Extractables and leachables are impurities which are related to the product and result from product contact with components such as processing equipment during manufacturing of the bulk product or from the packaging components of the final container closure system.[19] Extractable and leachable profiles for each drug product must be generated and associated toxicological studies performed to assess potential patient impact.[25] The actual levels of leachables that migrate into the final product are monitored and quantitated during stability studies on the drug product over its intended shelf-life.[9, 62] The execution of the stability studies not only directly monitors leachable levels. There is the possibility of secondary interactions between the API and any migrating substance from the packaging system, resulting in the formation of adduct components or alternative degradation pathways. The impact of these moieties must also be assessed for daily exposure limits. The

concentration of leachables present within a drug product are low (approximately 1 ppm) relative to the concentration of the raw materials used in the formulation. For this reason, methods must be sufficiently sensitive to be able to detect these low level leachables in the presence of the API.

To reduce the possibility of observing adverse results at the final stage of product development, a comprehensive initial screening of packaging components should be performed.[10] This also follows FDA recommendations for a Quality by Design (QbD) approach to product development. A number of analytical techniques may be necessary to generate a total extractables profile due to the variety of potential leachables from a complete container closure system. Such analytical techniques include, but are not limited to, GC-MS, HSGC-MS, LC-MS, ICP-MS, NMR.

Limited information can be gathered using traditional LC methodology combined with UV detection to generate leachable profiles as a screening method whereby known leachable standards are used to build a profile. However, these methods do not provide structural information and as such are not reliable as characterisation tools for unidentified leachables, as retention time and relative retention to an alternative known standard is the only data obtained. The use of photodiode array (PDA) detection permits the generation of the UV spectrum of an unidentified leachable. This technique gives some additional information as to what class of compounds a particular leachable may fall within but structural elucidation is not possible. The application of sub 2 micron particle size columns and UPLC systems capable of withstanding 16,000 psi, means that screening of extractables and leachables could be achieved in a shorter time with greater resolution and sensitivity. These methods would typically be used for qualitative work against an authentic standard or for quantitative analysis for known leachables. The use of UPLC for quicker analysis is exemplified by Novakova *et al.*. for the testing of four pharmaceutical formulations. Novakova utilised a 1.7 µm particle size column instead of the traditional 5 µm or 3 µm columns. The UPLC system allows shortening analysis time up to nine times

comparing to the conventional system using 5 µm particle packed analytical columns. In comparison with 3 µm particle packed analytical columns analysis should be shortened about three times.[45]

1.2.1 Extraction Methods

For the initial assessment of container closure systems during drug development or investigations into non-conforming commercial product, the extraction methodology of the packaging components is vitally important. The design of the extraction study systematically and rationally identifies and qualifies potential leachables. The development of an extraction profile allows to some extent the identification of individual extractables from a packaging component and provides early stage knowledge as to the potential leachables in the final product. The controlled extraction studies should utilise vigorous extraction with different solvents and extraction techniques, however, the drug product formulation and the constituency of the packaging component will ultimately dictate the extraction study design.

Elastomeric closures (rubber stoppers) are critical components in the packaging of parenteral drug products. Stopper components comprise of a multitude of raw materials such as elastomers, sealant, accelerators, antioxidants, plasticizers and dyes. Paskiet (1997) developed a profile of the extractables from rubber packaging materials used in aerosol valves with a range of organic solvents and stress conditions.[46] The base rubbers were carbon black filled nitrile elastomers and the propellant system was a mixture of chlorofluorocarbons. Paskiet executed an initial extraction study to ascertain which solvent mimicked the extraction characteristics of the propellant. The analysis was conducted using 2-proponal, toluene and methylene chloride. The rubber components were refluxed for 1 h in each of the solvents and compared to a propellant soaked mixture. 2 – propanol showed the best comparison with the propellant soaked extraction. To maximise the extraction efficiency, the rubber was further refluxed in 2-propanol for 7 h (4 + 3). The data analysis indicated that the extraction was complete after the initial 4 h period. The extraction solvents

were analyzed using HPLC and size exclusion chromatography methods and 14 mg and 7 mg of glycol and oligomers were detected, respectively. Zhang *et al.* (2004) applied a strategic approach for the identification of extractables from rubber closures used in semisolid pre-filled drug applicators.[47] Some rubber extractables are known to have an adverse patient impact and as such it was necessary to screen their selected rubber component for potentially harmful extractables. The rubber was exposed to exaggerated extraction conditions as a worst case scenario by refluxing with acetonitrile for 8 h. The extracted components were separated and collected as fractions for further identification work using a semi-preparative Prodigy ODS, 5 µm, 150 mm × 10 mm HPLC column. The mobile phase system consisted of a gradient over 1-20 min from 70-90% acetonitrile. The extracted components were identified using HPLC, GC, MS and compared to authentic standards as 4-(1,1-dimethyl-propyl)-phenol, sulfur, 2,6-di-*tert*-butyl-[1,4] benzoquinone, furan-2-yl-(5-hydroxymethyl-furan-2-yl)-methanol and 2-bromo-4-(1,1-dimethyl-propyl)-phenol. The resulting LC-UV chromatogram is presented in Figure 1.18.

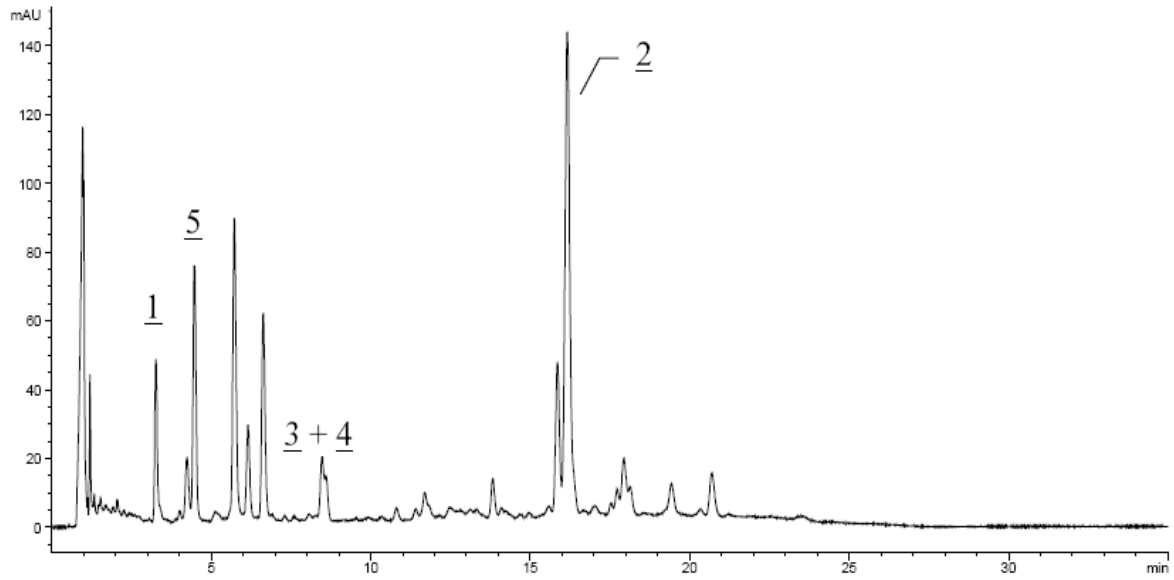


Figure 1.18: LC-UV chromatogram of extractables from rubber closure used for pre-filled semisolid drug applicators. 4-(1,1-dimethyl-propyl)-phenol (**1**), sulfur (**2**), 2,6-di-*tert*-butyl-[1,4] benzoquinone (**3**), furan-2-yl-(5-hydroxymethyl-furan-2-yl)-methanol (**4**), and 2-bromo-4-(1,1-dimethyl-propyl)-phenol (**5**), (47).

In 2009, Corredor *et al.* executed a comprehensive determination of extractables from five different brands of stoppers used for injectable products. The stoppers consisted of butyl and halobutyl rubbers, coated and uncoated with proprietary films. The stoppers were extracted using three different media, 20% w/v sulfobutylether-ß-cyclodextrin, pH 8 phosphate buffer and 50/50 v/v polyoxyethylated castor oil/dehydrated alcohol. Each medium was placed in glass vials and sealed with each of the rubber stoppers. The vials were then stored in the inverted position and stored at 40^0C/75% RH and 25^0C/60% RH. The samples were analyzed after 3 and 6 months for the elevated temperature samples and at 12 and 24 months for the lower temperature samples. The media extracts were analysed using ICP-MS, IC, HPLC and GC. An accelerated extraction study was performed to yield short term data. rubber stoppers were transferred into volumetric flasks and covered with 2-propanol. The volumetrics were stored at 70^oC for 12 hours. The 2-propanol was removed

under vacuum and the sample reconstituted in methanol. These extractions were used for method development and establishment of a baseline extractables profile. Corredor *et al.* determined 24 different extractables from the various stopper components with a quantitation limit as low as 0.03 ppm and as high as 17 ppm. A standardized procedure for the testing of extractables from rubber container closure systems was successfully developed and it has been shown that the commonly used stopper formulations yield relatively low extractable concentrations. The levels observed of the leachable components would pose no direct toxicological risk and as such would not yield any further scrutiny from regulatory agencies such as the FDA.[41] The observed extractables and associated concentrations are presented in Table 1.4.

Table 1.4: Observed Extractables from Five Rubber Stoppers, Taken from Ref 41

		3 and 6 Months at 40 °C/75% RH		12 and 24 Months at 25 °C/60% RH	
Stopper	Vehicle	Extractable observed at >10 ppm	Amount (range in ppm)[a]	Extractable observed at >10 ppm	Amount (range in ppm)[a]
Stopper A	A	-	-	-	-
	B	Silicon	17–33	Silicon	16–24
	C	-	-	-	-
Stopper B	A	-	-	-	-
	B	Silicon	19–31	Silicon	15–23
	C	-	-	-	-
Stopper C	A	-	-	-	-
	B	Silicon	19–33	Silicon	13–23
	C	-	-	-	-
Stopper D	A	Isopropanol	17–26	Isopropanol	19–21
		t-butyl alcohol	15–31	t-butyl alcohol	13–22
		acetone	9–19	-	-
	B	Isopropanol	12–17	Isopropanol	18–35
		t-butyl alcohol	12–24	t-butyl alcohol	16–28
		Silicon	18–31	Silicon	15–23
	C	t-butyl alcohol	9–12	t-butyl alcohol	9–19
Stopper E	A	-	-	-	-
	B	Silicon	20–30	Silicon	15–24
	C	-	-	-	-

[a] The range includes results for both coated and uncoated stoppers at both timepoints.

Jenke *et al.* (2006) developed a similar screening extraction study on eight tubing materials potentially encountered in pharmaceutical production facilities. Jenke analysed 6 silicone and 2 Santoprene based tubing materials post extraction. However, a combination of static and dynamic extractions were implemented. Static extractions were executed using

water and ethanol. For the first method, lengths of tubing were autoclaved in water at 121°C for 1 hour. Alternatively, the tubing was place in glass vials with 100% ethanol and stored at 55°C for 24 h. The dynamic extractions were performed pumping various extraction solvents through a closed loop tubing system using a perstaltic pump with a flow rate to provide one complete loop every 20 min with various total extraction times. A breakdown of the dynamic extraction cycles is presented in Table 1.5. The extracts were analyzed using TIC/TOC, pH, UV, ICP-AES, LC/MS/UV, GC-MS and Headspace GC-MS.[48]

Table 1.5: Dynamic Extraction Cycles for Tubing, Taken From Ref 48

Material	Water extract		Ethanol extract			
	Weight (g)	Volume (mL)	Dimensions (length × o.d., in.)	Weight (g)	Start vol. (mL)	End vol. (mL)
Silicone tubing materials						
1	39.20	100	12 × 1 (two reps, combined)	184.3	110	90
2	17.11	50	154 × 0.375	237.8	185	140
3	53.37	100	18 × 1.375	305.6	210	190
4	62.63	100	7.5 × 1.25	108.6	70	68
5	50.17	100	13.5 × 1.25	223.1	140	125
6	51.82	100	20 × 1.365	269.8	240	210
Santoprene tubing materials						
7	29.51	100	11.5 × 1 (two reps, combined)	153.9	118	120
8	30.86	100	144 × 0.5	369.4	220	215

Jenke found that in general the levels of extractables were higher in water than in ethanol. Five of the materials had relatively high levels of calcium and magnesium. Two of the silicone based tubing materials had higher amounts of heavy metals such as Mo, Ti, Zn and Fe. The reinforcement wiring in the tubing was identified as a potential source of these metals. Of all the materials tested a silicone based tubing had the lowest level of water-soluble elements, less than 0.01 ppm. In general the level of all dynamic extracted materials was very low illustrating acceptable risk for the implementation of the tubings within a production environment.

Plastic containers are widely used in the pharmaceutical industry and plastic components are often in direct contact with the final product. Jenke *et al.* (2005) investigated the interaction between a polyolefin container material and several buffers and media used in biopharmaceutical applications.[49] A total of 9 extraction solvents were analyzed on a very low density polyethylene (VLDPE) material. The extraction solvents were:

A. 0.06 M ammonium sulfate, 0.022 M 4-morpholinesulfonic acid (MES, 0.0024 M) potassium phosphate, pH 5.4
B. 0.05 M Tromethamine (Tris), 0.15 M sodium chloride, pH 7.2
C. 2.0 M Tris
D. 0.02 M Tris-hydrochloride, 0.05 M sodium chloride, pH 8.0
E. 0.02 M sodium phosphate, 0.15 M sodium chloride, 0.02% Tween-80, pH 5.5
F. 1% Tween-80 stock solution
G. 6.0 M guanidine-hydrochloride
H. 0.15 M sodium chloride, pH 5.4
I. Binary ethanol/salt buffer mixtures (15, 30 and 45% (v/v) ethanol in 0.15 M sodium chloride, pH 5.)

The various extractions were stored at 15-30^{o}C for 3 months; 40^{o}C for 6 weeks; and storage at 40^{o}C for 3 months. The extraction mixtures were then analyzed using various analytical techniques such as pH, UV, TOC, GC, LC, MS, ICE. Hexanoic acid extracted in all solutions to its total available pool level. The quantity of extracted stearic acid was found to be greatly influenced by buffer polarity and pH as a 100 fold difference was observed across polarity and pH range. The combination of Tween based buffer at higher pH resulted in the greatest extraction of stearic acid.

Jenke *et al.* (2005) further expanded on the investigations into leachables and extractables from plastic contact materials into pharmaceutical product. Jenke determined material/water equilibrium interaction constants (E_b) for twelve organic model solutes and a non-PVC polyolefin plastic material. Plastic container were fashioned from VLDPE material and filled with 250 mL of several different solutions containing the following amounts (vol%) in ethanol (in 0.1% phosphoric acid): 0, 15, 30 and 45%. The filled containers were stored for 19 days at 40^{o}C. HPLC/UV/MS analysis was executed on the extracted solutions. The effect of solvent polarity on E_b was assessed by examining the interaction between the

plastic and selected model solutes in various ethanol/water solvents. In general, $\log E_{b,}$ could be linearly related to the polarity of the mixture.

Wang and Schnute (2010) also analyzed potential leachables from various plastic containers.[50] The leachables examined within the scope of the work were bisphenol A (BPA), 4-n-octyphenol (4-n-OP), 4-tert-octylphenol (4-t-OP) and 4-n-nonylphenol (4-n-NP). Low-cost bottle (LCB, water bottles, baby-feeding bottles and food containers) and Brand-name baby-feeding bottles (BNB) were filled with water and exposed to direct sunlight for 2 days, microwaved for 5 min and then microwaved for another 5 min. Samples were then analysed using LC/MS/MS methodology. All bottled water was found to be free from BPA. However, 4-t-OP was found in two bottles at approximately 4 and 6 ppb.

Degrazio *et al.* (2009) executed a study to evaluate a new syringe system from an extractables perspective for pharmaceuticals and biopharmaceuticals.[51] A controlled extraction study was performed using water, 2-propanol and hexane on 2 g of the cyclic olefin polymer material. he mixtures were refluxed for 4 hours. Separately, syringes prefilled with water were stored for 6 months at 40^{o}C/75% RH. Analysis of all solution was performed using HPLC/PDA/MS, GC/MS, GC/MS headspace, ICP and IC. An organic acid leachable was detected using the IC at levels of 2.2 – 2.9 µg/mL and confirmed by GC/MS and GC/MS headspace.

Akapo and McCrea (2008) developed a direct liquid immersion solid phase microextraction-gas chromatographic (SPME-GC) method for the determination of 11 potential volatile organic compounds that may leach from pre-printed foil laminate overwrap into aqueous pharmaceutical product filled in LDPE vials.[51] The target compounds were ethanol, acetone, isopropyl alcohol, ethyl acetate, 2-butanone, n-heptane, isopropyl acetate, n-propyl acetate, toluene, diacetone alcohol and 1-proxy-2-propanol. The compounds were extracted by SPME using a 100 µm polydimethylsiloxane (PDMS) fibre and desorbed inside the GC inlet port for analysis. The compounds were extracted by immersing into a 9 mL

sample solution placed in a septum capped glass vial fro 30 min at room temperature under constant mixing at 100 rpm. Three other extraction fibres were assessed; 75 µm Carboxen, 85 µm polyacrylate and 65 µm PDMS/DVB. The PDMS fibre yielded the lowest %RSD for all analytes. The method was accurate linear and precise exhibiting interlaboratory precision of less than 16%. Huang *et al.* (2012) also developed a SPME-GC/MS/MS analytical method for the determination of 2,4,6-tribromoansole (TBA), 2,4,6-tribromophenol (TBP), 2,4,6-trichloroanisole (TCA) and 2,4,6-trichlorophenol (TCP) in solid dosages as potential leachables from wooden pallets which had been treated with fungicides.[52] The solid dose tablets were transferred to a 125 mL extraction vessel with 100 mL of 0.1% formic acid extraction media for 30 minutes under sonication. A Gerstel Twister® PDMS extraction device was placed into the aqueous solutions and mixed at 1000 rpm for 90 minutes. The recovery values were between 70 and 100% for three different formulations.

The extraction technique used for the analysis of container closure systems is varied and the selection of an appropriate technique is dependent on a number of factors such as the known constituents of the material and in particular polarity and pH, available apparatus, compatibility with separation and detection system and time. Rapid analysis may be achieved through use of microwave extraction for an immediate estimation of potential extractable. However, the high energies incorporated in this technique may lead to degradation of the analytes of interest. Aggressive extraction techniques such as microwave energy, pH and polarity extremes should be treated as an absolute worst case assessment. It is advisable to accompany these studies with comparative studies of the container closure system in contact with the actual drug product or formulation under normal storage conditions for the duration of the shelf life of the product to ascertain will actually migrate into the commercial product. For an initial screening where no information is available on packaging components a mix of extraction solvents is appropriate to cover polarity and pH ranges. Table 1.6 provides a summary of extraction techniques employed for various packaging components including rubber, PVC, glass adhesives and inks.

Table 1.6: Summary of Extraction Techniques

Year	Author	Reference	Product Type	Product Component	Extraction Medium	Extraction Temperature	Extraction Time
2009	Corredor	41	Injectable	Butyl and halobutyl rubber stoppers	pH3 citrate buffer with 20% w/v sulfobutylether-ß-cyclodextrin	40	6 months
					pH 8 phosphate buffer	40	6 months
					50/50 v/v polyoxyethylated castor oil/dehydrated alcohol	40	6 months
					pH3 citrate buffer with 20% w/v sulfobutylether-ß-cyclodextrin	25	24 months
					pH 8 phosphate buffer	25	24 months
					50/50 v/v polyoxyethylated castor oil/dehydrated alcohol	25	24 months
2006	Jenke	53	Manufacturing Equipment	Silicone Tubing	Water	121	1 hr
					Ethanol	55	24 hr
				Santoprene	Water	121	1 hr
					Ethanol	55	24 hr
2006	Jenke	48	Pharmaceutical Container	Plastic component VLDPE	0.1% phosphoric acid	40	19 days
					85/15 v/v 0.1% phosphoric acid/ethanol	40	19 days
					70/30 v/v 0.1% phosphoric acid/ethanol	40	19 days
					55/45 v/v 0.1% phosphoric acid/ethanol	40	19 days
2009	Wakankar	54	Biopharmaceutical	Bioprocess bag	Water	unknown	unknown
					0.1 M phosphoric acid	unknown	unknown
					0.1 M Sodium Hydroxide	unknown	unknown
					Isopropyl alcohol	unknown	unknown
					50% isopropyl alcohol/water	unknown	unknown
					10% polysorbate 20	unknown	unknown
					10% polysorbate 80	unknown	unknown

					Hexane		
2012	USP	10	Container Plastics	Container Plastics	Water	70	24 hr
2004	Zhang	47	Injectable	Rubber Stopper	Acetonitrile	Reflux	8 hr
2008	Sanches-Silva	55	Infant Milk Formula	Photoinitiator	Amoniac and hexane liquid-liquid extractions	N/A	N/A
			Dairy Product	Photoinitiator	Acetonitrile	70	24 hr
2010	Wang	50	Bottled Water	Plastic bottle	Water	Direct sunlight	2 days
						microwave	5 min
						microwave	5 min
2005	Jenke	49	Biopharmaceutical	Polyolefin Plastic Container	0.06 M ammonium sulfate, 0.022 M 4-Morpholinesulfonic acid, 0.0024 M Potassium phosphate, pH 5.4	70	3 days
					0.05 M Tromethamine (Tris), 0.15 M sodium chloride, pH 7.2	70	3 days
					2.0 M Tris	70	3 days
					0.02 M Tris-hydrochloride, 0.05 M sodium chloride, pH 8.0	70	3 days
					0.02 M sodium phosphate, 0.15 M sodium chloride, 0.02% Tween-80, pH 5.5	70	3 days
					1% Tween-80 stock solution	70	3 days
					6.0 M guanidine-hydrochloride	70	3 days
					0.15 M sodium chloride, pH 5.4	70	3 days
					Binary ethanol/salt buffer mixtures (15, 30 and 45% (v/v) ethanol in 0.15 M sodium chloride, pH 5.4	70	3 days
2009	Degrazio	51	Injectable	Syringe Barrel	Water	40	6 months
					Water	Reflux	4 hr
					Isopropyl alcohol	Reflux	4 hr

					Hexane	Reflux	4 hr
2008	Akapo	51	Aqueous based pharmaceuticals	LDPE Foil Laminate	Solid Phase Micro Extraction using polydimethylsiloxane fibres	Room Temp	30 min
2007	Dopio-Garcia	56	Food Packaging	Polymeric films	Dichloromethane with microwave	55	1 min
1997	Tiller	57	Pharmaceutical Container	Adhesive	Water	50	3 Days
2012	Huang	52	Drug Product Packaging	cardboard, polyethylene, polycarbonate, wood pallet	10/90, v/v, Water/ Acetone PDMS extraction device placed in mixture at 1000 rpm	Sonication Room Temp	30 min 90 min
1997	Paskiet	46	Pharmaceutical Container	Rubber packaging	2-Propanol	Reflux	1 hr
					Toluene	Reflux	1 hr
					methylene chloride	Reflux	1 hr
2011	Constantinos	58	Eye Drop Solutions	Packaging Materials	Ophthalmic solutions	2 - 8	10 days
						25	20 days
						40	1 -12 months
2012	Grilli	59	Containers and closures looking for heavy metals	HDPE bottle Polypoylene cap Elastomeric closure Glass Vials	Water 0.1 N HCL 0.1 N NaOH 1% Hydrogen Peroxide	70	24 hr
					Water 0.1 N HCL 0.1 N NaOH 1% Hydrogen Peroxide	121 autoclave	1 hr

					Water 0.1 N HCL 0.1 N NaOH 1% Hydrogen Peroxide	Reflux	24 hr

1.2.2 Separation and Detection

The separation and detection of extractables and leachables can pose numerous challenges to the analytical scientist given the wide range of physical and chemical properties and the fact that they are often present in very small concentrations relative to the active pharmaceutical ingredient and other formulation components. To try and overcome this problem it is often necessary to use multiple analytical techniques to achieve a global assessment of extractables and leachables.

In 2005 the European Food Safety Authority (EFSA) Rapid Alert System uncovered traces of the photoinitiator 2-isopropyl thioxanthone (ITX) in baby milk.[60] The photoinitiator is used as a UV curing process for inks on packaging materials associated with food products. This prompted Sanchez-Silva *et al.* to develop a multi-method for the analysis of 6 photoinitiators in milk, Irgacure 184, benzophenone, Irgacure 651, Irgacure 907, Quantacure ITX, and Quantacure EHA (2-ethylhexyl-4-dimethylaminobenzoate).[55]

After extraction, The chromatographic separation was performed on a Kromasil 100 C18, 5 µm, 150 mm × 4.0 mm column. The gradient elution system consisted of 20% acetonitrile solution increasing to 80% acetonitrile over 18 min. Detection of analytes was achieved using a PDA detector, see Figure 1.19. Structural confirmation of the authentic standards were performed on an LC with TOF MS. A total of 26 various food packaging components were analysed and 9 of the test samples were clear from the presence of any of the 6 photoinitiators under investigation.

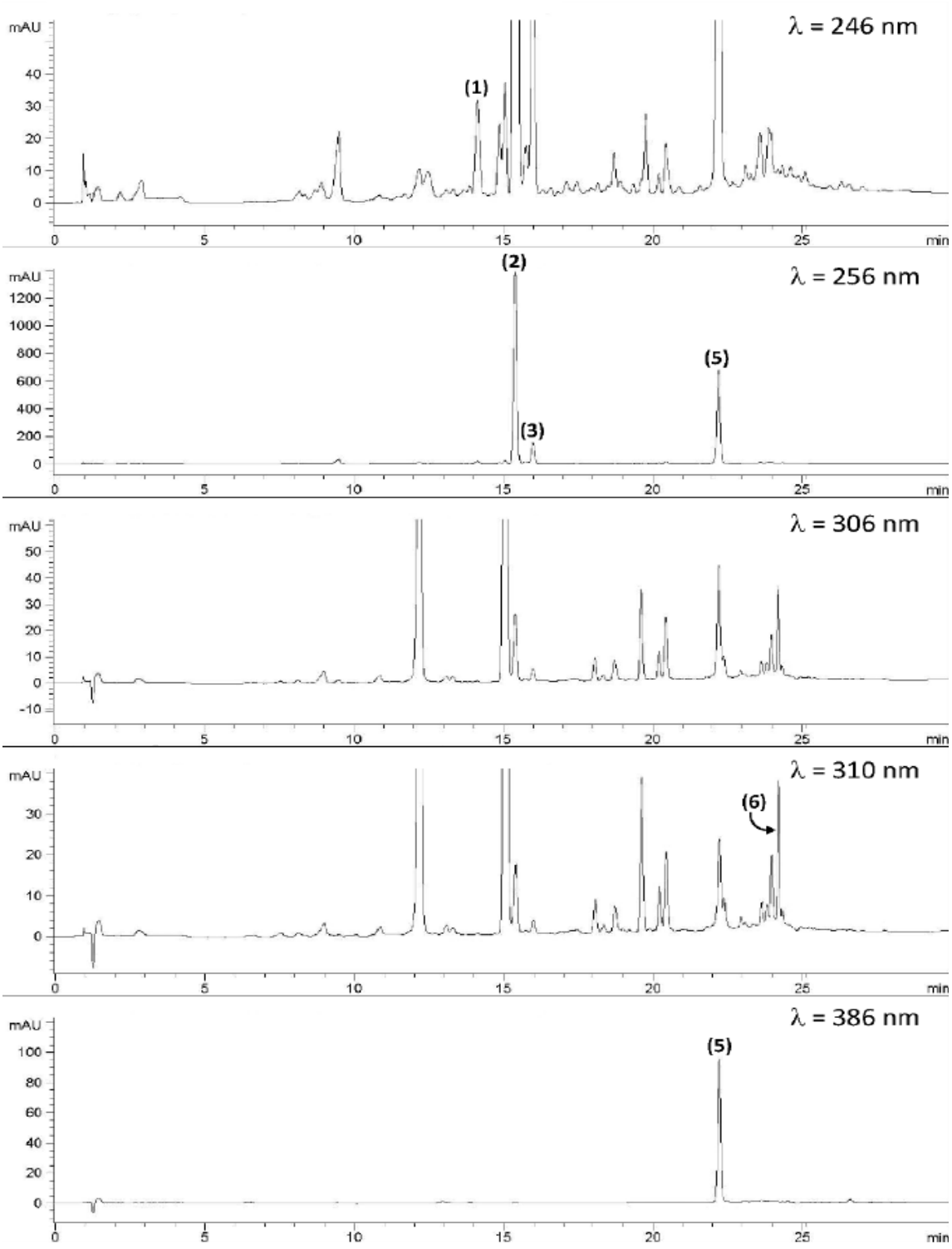


Figure 1.19: LC-UV chromatograms of packaging sample with the photoinitiators Irgacure 184 (1), Benzophenone (2), Irgacure 651 (3), Irgacure 907 (4), Quantacure ITX (5) and Quantacure EHA (6), (55).

For the MS analysis, the chromatographic separation was performed on a Zorbax Eclipse XDB C18, 150 mm × 4.6 mm, 5 µm column with a C18 pre-column. The mobile phase consisted of a water and acetonitrile gradient with formic acid at a flow rate of 1.0 mL/min. The gradient elution system consisted of 20% acetonitrile solution for the first two min and increased to 80% acetonitrile over 18 min. The organic concentration was increased to 100% at 23 min until 30 min to elute all components from the column. The MS detector was operated in positive mode under electron spray ionization (ESI) interface. The source temperature was 200^{o}C and the capillary voltage -4,500 V. The nebulizer pressure was maintained at 400 kPa and the dry gas flow rate was 11 L/min. The resulting mass spectra are presented in Figure 1.20.

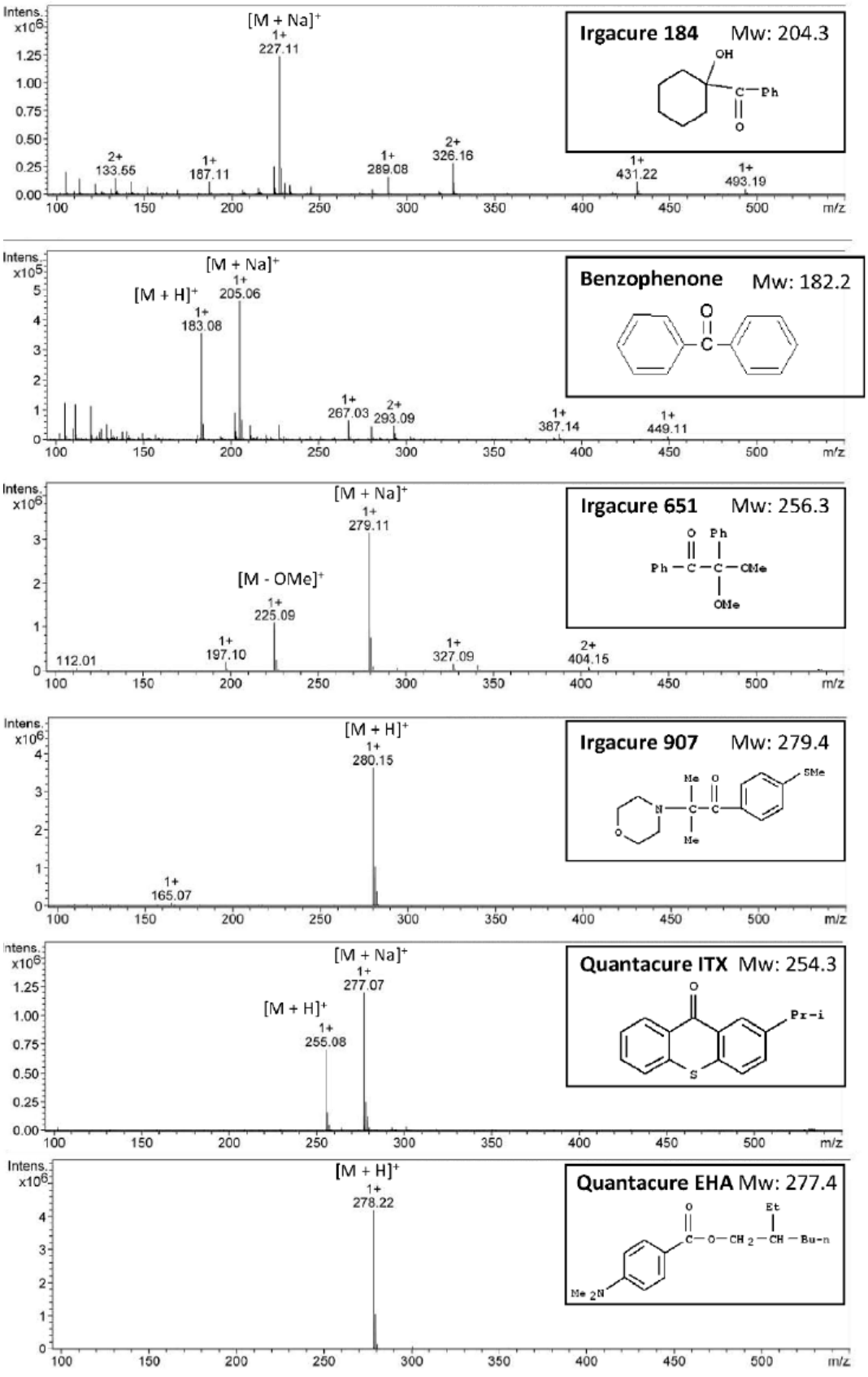


Figure 1.20 MS of photoinitiators Irgacure 184, Benzophenone, Irgacure 651, Irgacure 907, Quantacure ITX and Quantacure EHA, (55).

Dopico-Garcia *et al.* (2007) investigated the presence of phosphite and phenolic antioxidants in the presence of various food packaging material and assessed their leaching effect into water, acetic acid, 10% ethanol solution and olive oil.[61] The antioxidants under investigation were butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), AO 2246, AO 425, Ethanox 330, Irganox 1010, Irganox 1076 and Irgafos 168. Analysis was performed on a Symmetry C18, 3.5 µm, 150 mm × 3.0 mm column (flow rate of 0.5 mL/min). The injection volume was 20 µL and column temperature was maintained at 30^{o}C. A water/methanol mobile phase gradient system was employed for elution of the antioxidants. The migration test was performed using a film of sample, approximately 1 dm^{2}, with 165 mL of distilled water, 3% acetic acid, 10% ethanol and olive oil. For the aqueous stimulants, the DL of the antioxidants was reported at approximately 4.5 × 10^{-3} mg/dm^{2} with a QL of approx. 1.5 × 10^{-2} mg/dm^{2}. The migration level of the studied antioxidants was found to extremely low.

Kim *et al.* (2005) also investigated five antioxidants and seven phthalate plasticizers in polymeric materials.[62] They employed high temperature liquid chromatography (HTLC) to improve mass transfer rate and column backpressures to reduce analysis time and improve column efficiency. Utilization of diamondbond and zirconia columns was necessary to withstand the high temperatures and minimize stationary phase collapse. Each column was screened under various isothermal and temperature gradients with different concentrations of acetonitrile. Column efficiency was demonstrated to improve at higher temperatures incorporating a thermal gradient. A 1% increase in the concentration of acetonitrile was shown to be equivalent to approximately 7^{o}C increase in column temperature. The LC-UV chromatograms are presented in Figure 1.21 and Figure 1.22.

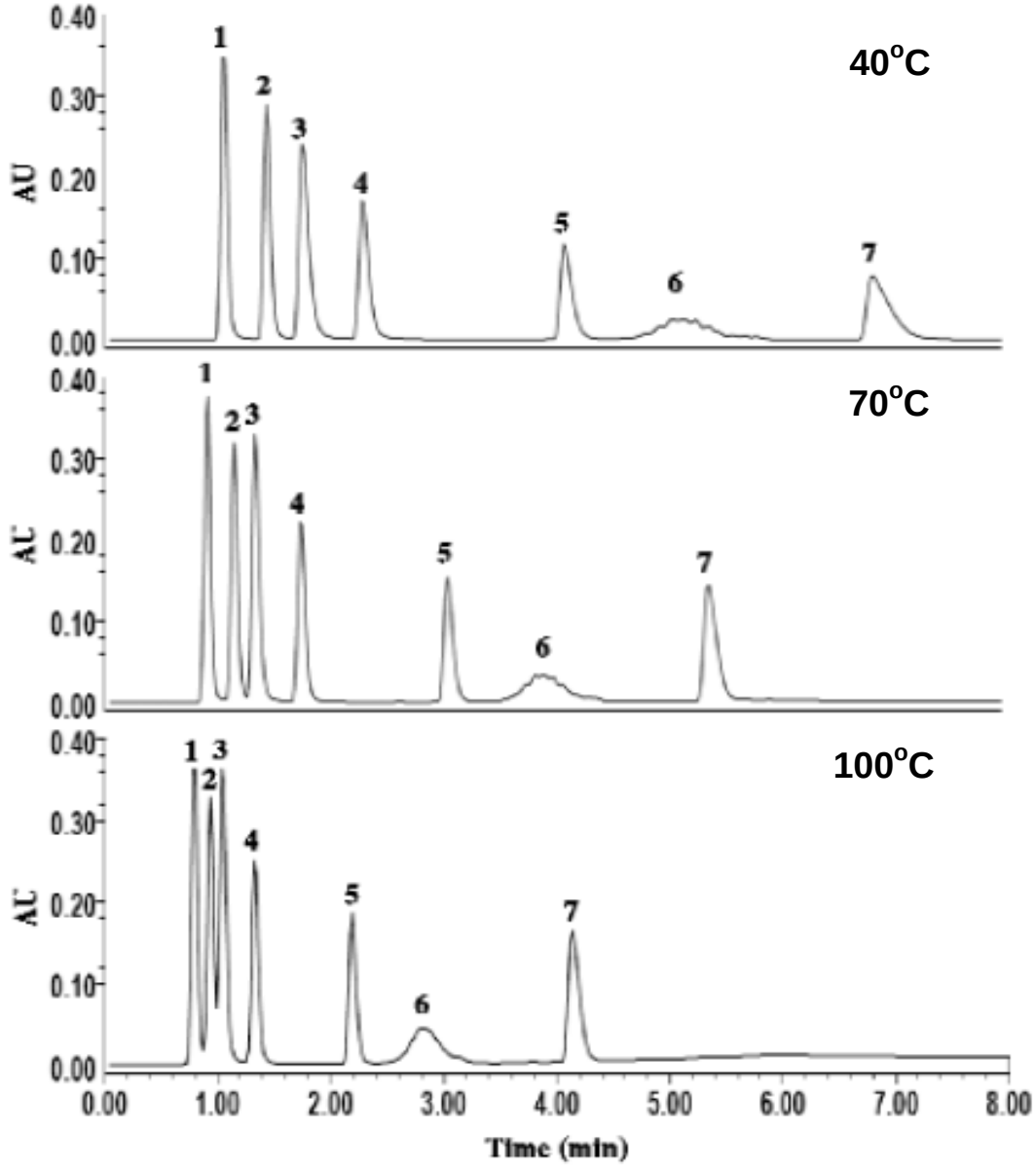


Figure 1.21: Effect of column temperature on the separation of phthalate plastizers, dipropyl phathalate (1), dibutyl phthalate (2), butyl benzyl phthalate (3), dicyclohexyl phthalate (4), dioctyl phthalate (5), diisononyl phthalate (6) and trioctyl trimellitate (7), (62).

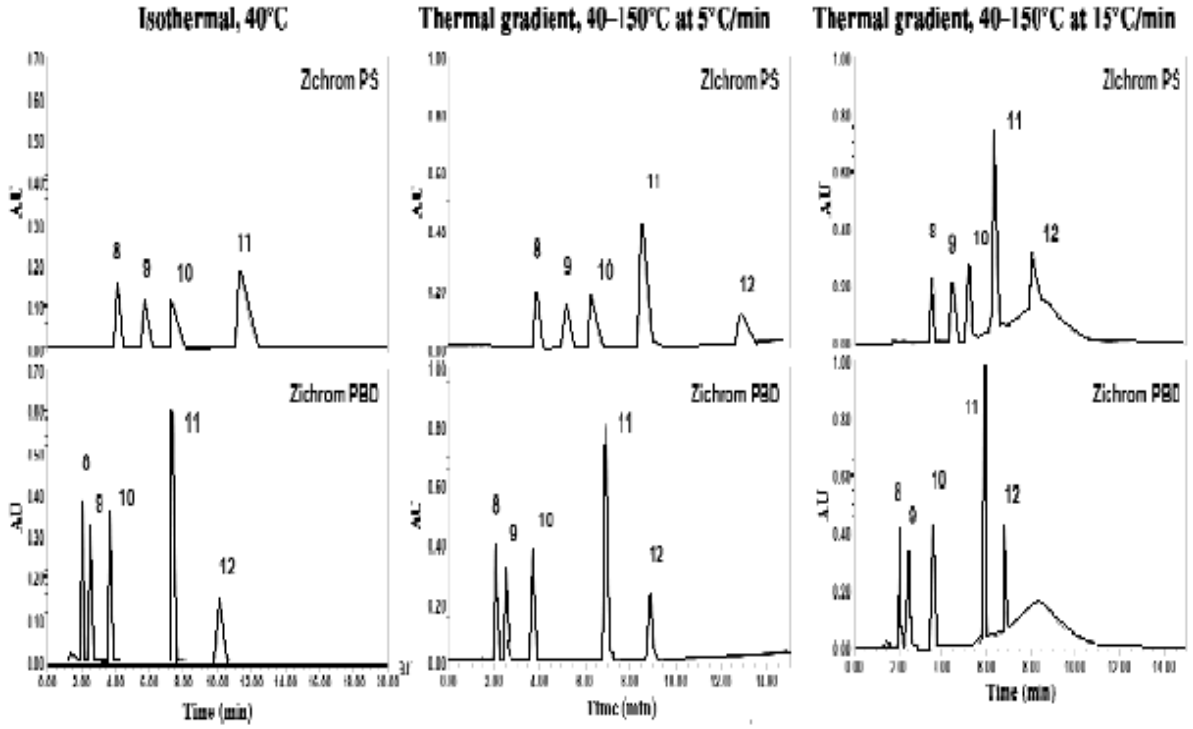


Figure 1.22: Effect of column temperature on antioxidants Irganox 245 (8), Irganox 1098 (9), Naugard XL-1 (10), Irganox 1081 (11) and Irganox 1035 (12), (62).

Noguerol-Cal *et al.* (2007) developed an UPLC method for the separation and quantitation of 10 colorants and 6 antioxidants used as polymer additives.[63] The development process was taken from 2 separate methods which already existed for the analysis of dyes and antioxidants. Traditional HPLC methods were adopted into one UPLC method which originally resulted in co-elution of peaks. Consequently, Noguerol-Cal implemented a Draped-Lin Blocked Cube-Star design of experiments for the UPLC gradient program. This involved running the test sample under various gradient conditions at previously identified critical points within the gradient parameters. The resolution of the critical pairs was measured for each run. From the data, a main effects plot was generated which affords the optimum conditions for each critical point to yield the most efficient separation. The optimized conditions for the analysis consisted of UPLC C18 column,

1.7 µm particle size, flow rate of 0.5 mL/min, wavelength of 230 nm, column temperature 30^{o}C and an injection volume of 3 µL. The LC gradient program is illustrated in Table 1.8. The use of the statistical tools by Noguerol-Cal *et al.* resulted in the development of a method in the centre of a design space i.e. a small change from the standard operating parameters (nominal) does not negatively impact the robustness characteristics of the methodology. This can be visually represented using response surface plots and optimization plots to determine most efficient nominal operating conditions. Examples optimization and response surface plots can be viewed at http://www.jmp.com/support/help/Desirability_Profiling_and_Optimization.shtml. However, Noguerol-Cal *et al.* failed to showed the expected long term process capability of the analytical separation. This could have been achieved by executing process capability analysis to estimate out of 1 million analytical runs, the expected number of assays which would yield chromatographic resolution below the acceptable value. In addition, the team have limited the analytical separation to UPLC conditions only. A geometric transformation of the analytical conditions to HPLC would allow for dual analysis. The geometric transformation consists of scaling the standard operating condition between the analytical separations taking into account the inherent difference in particle size and pressures between UPLC and HPLC. Finally, a two level fractional factorial design would allow for the analysis to be completed in a shorter timeframe. This involves testing at a high and low point but not testing every parameter, which allows for a fraction of the test conditions. The optimized nominal conditions for the UPLC separation are presented in Table 1.8, with the corresponding chromatogram presented in Figure 1.23.

Table 1.8: Optimized UPLC Gradient Program for the analysis of 10 colourants and 6 antioxidants, Adapted from Ref 63.

Time (min)	Water (%)	Methanol (%)	Curve
0	40	60	Linear
0.8	30	70	Linear
0.9	10	90	Linear
4.0	0	100	Linear
4.5	0	100	Linear
5.0	40	60	Convex
6.0	40	60	Linear

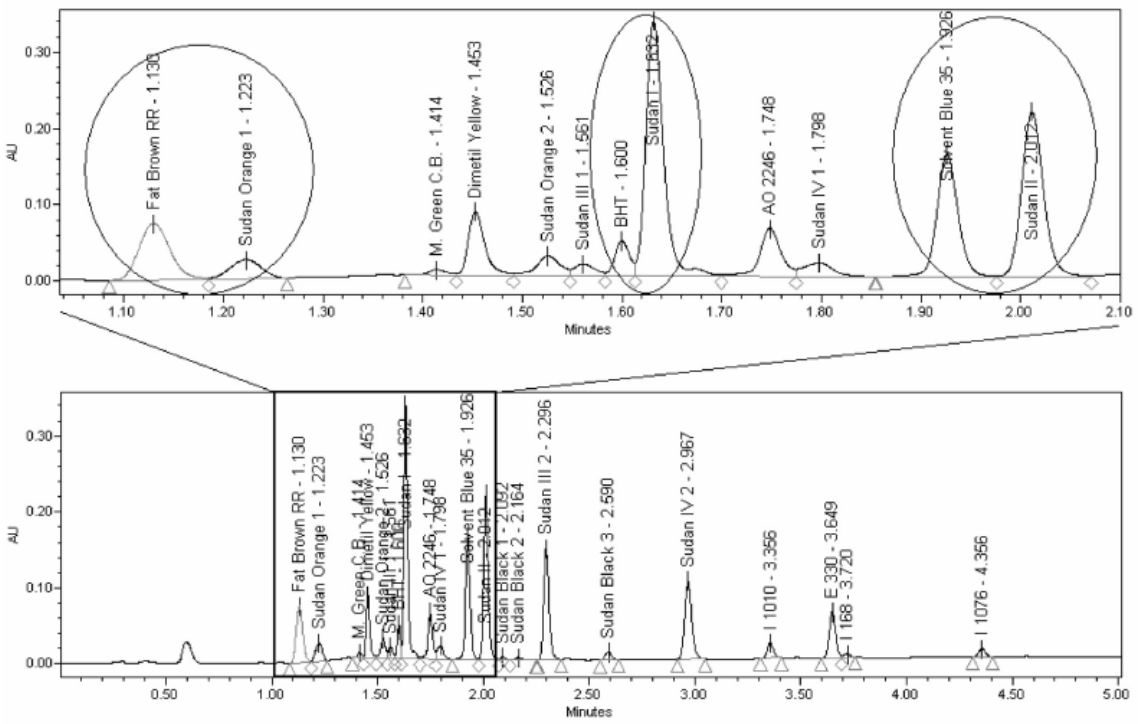


Figure 1.23: UPLC separation of 10 colourants and 6 antioxidants used as polymer additives, Adapted from Ref 63.

There is a responsibility on manufacturers to identify, characterise and toxicologically assess major leachable compounds. It is a regulatory expectation that laboratories would use GC-MS or LC-MS or a combination of both to qualitatively analyse leachables. Tiller *et al.*. (2006) qualitatively assessed leachables from a custom made proprietary adhesive using LC-MS methodology.[57] A combination of an Alliance 2690 HPLC system and a Finnigan LCQ MS allowed for the structural characterisation of 2 major aromatic compounds as shown in Figure 1.24.

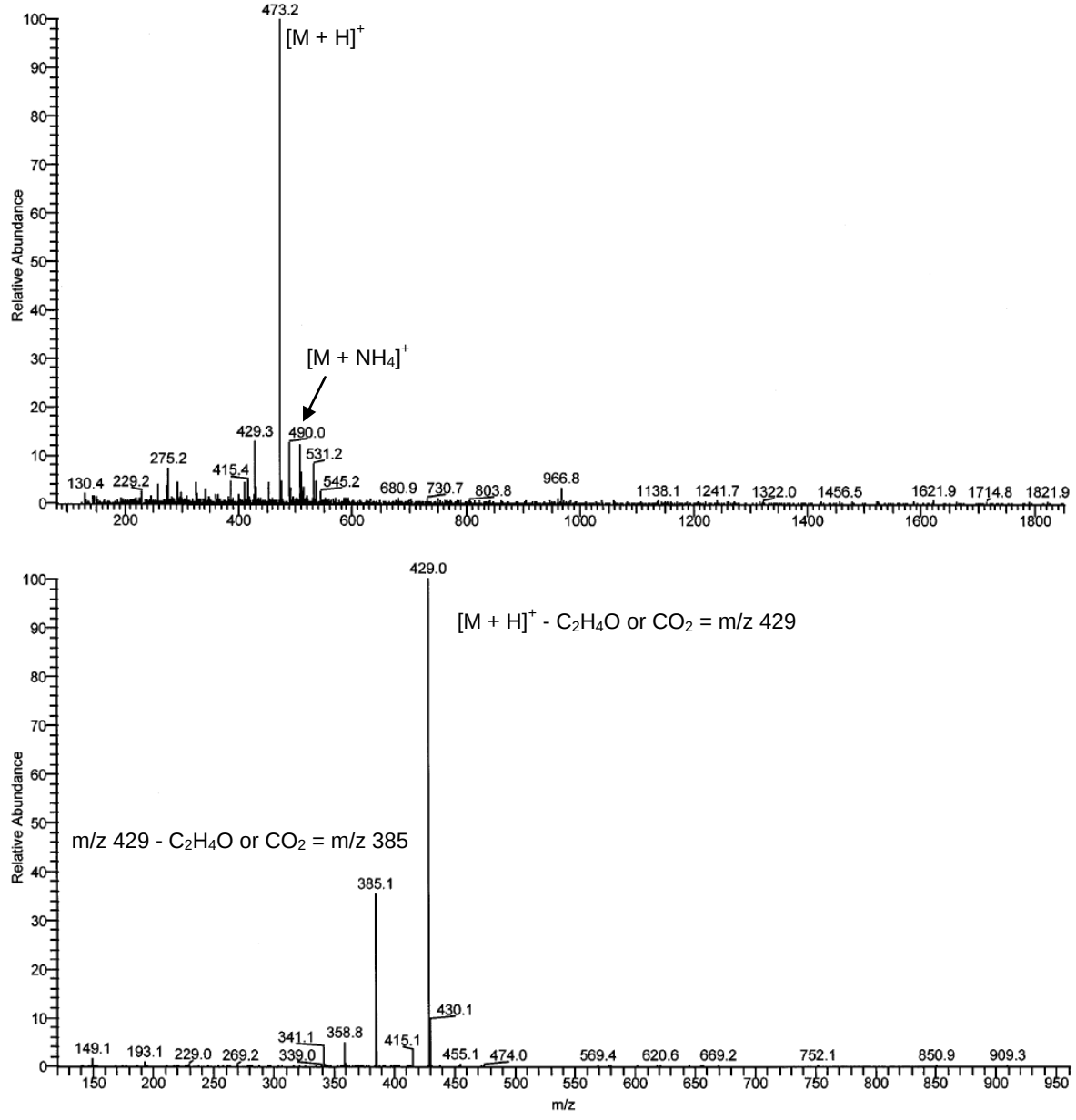


Figure 1.24: Mass Spectra of potential leachables from an adhesive, Ref 57.

Fichtner *et al.* (2004) developed an LC-MS method for the identification and quantification of extractables of polypropylene and polyethersulfone namely, 4-hydroxybenzoic acid, ethyl ester, diethyl phthalate, bis-(-4-chlorophenyl)-sulfone, benzoic acid-*p*-tolylester, butylated hydroxytoluene and 1,3-di-tert-butylbenzene. The extractables were associated with sterile-grade filtration cartridges.[64] Degrazio (2009) also utilized LC-MS technology to identify an organic acid leachable in a Crystal Zenith® syringe barrel for biopharmaceutical applications.[65] Ito *et al*. (2005) quantitated the leaching of di-(2-ethylhexyl)phthalate (DEHP) and mono-(2-ethylhexyl)phthalate (MEHP) from polyvinyl chloride (PVC) tubing using LC-MS/MS in various drug solutions. The level of DEHP and MEHP ranged from 0.1 – 50 ppm and 0.1 – 5 ppm, respectively. The levels were dependent on the levels of drug additives such as castor oil.[66] Wang (2012) described his UPLC-MS analysis of leachables from bottled water, specifically bisphenol A, octylphenols and nonylphenols.[67] LODs were reported at approx. 0.05 ppb and a correlation co-efficient of greater than 0.995 for each. In the bottles under analysis, bisphenol A, octylphenols, and nonylphenols were detected at 0.4, 7.1 and 0.2 ppb, respectively. Kopperud *et al.* (2011) used LC-MS applications to identify leachables from acrylate polymers used for orthodontic base-plate materials.[68]

Jenke (2005) identified extractables associated with polyolefin containers using LC-MS. The investigation was centred on the effect of several different buffers and media on extractables in biopharmaceutical containers.[48] The interaction between buffer/media and container was permitted for 6 weeks at 40^{o}C and 3 months at 25 or 40^{o}C. LC-MS analysis was performed on the extracted solutions. A Phenomenex Prodigy C8 column, 150 mm × 4.6 mm, 5 µm particle size and a 10 mM ammonium acetate and methanol mobile phase gradient was employed for separation of components. The MS settings consisted of API-ES (positive ion): gas temp, 325^{o}C; fragmentor, 65; drying gas, 11.0 L/min; nebulizer pressure, 35 psig; Vcap, 5000V. Specific ions monitored included caprolactam, 114; Extractable A, 229; Extractable C, 296; Extractable B, 271. API-ES (negative ion): gas temp, 325^{o}C;

fragmentor, 65; drying gas, 11.0 L/min; nebulizer pressure, 35 psig; Vcap, 5000V. Specific ions monitored included hexanoic acid, 115; Extractable C, 277; stearic acid, 283. The mass spectra are presented in Figure 1.25 and Figure 1.26.

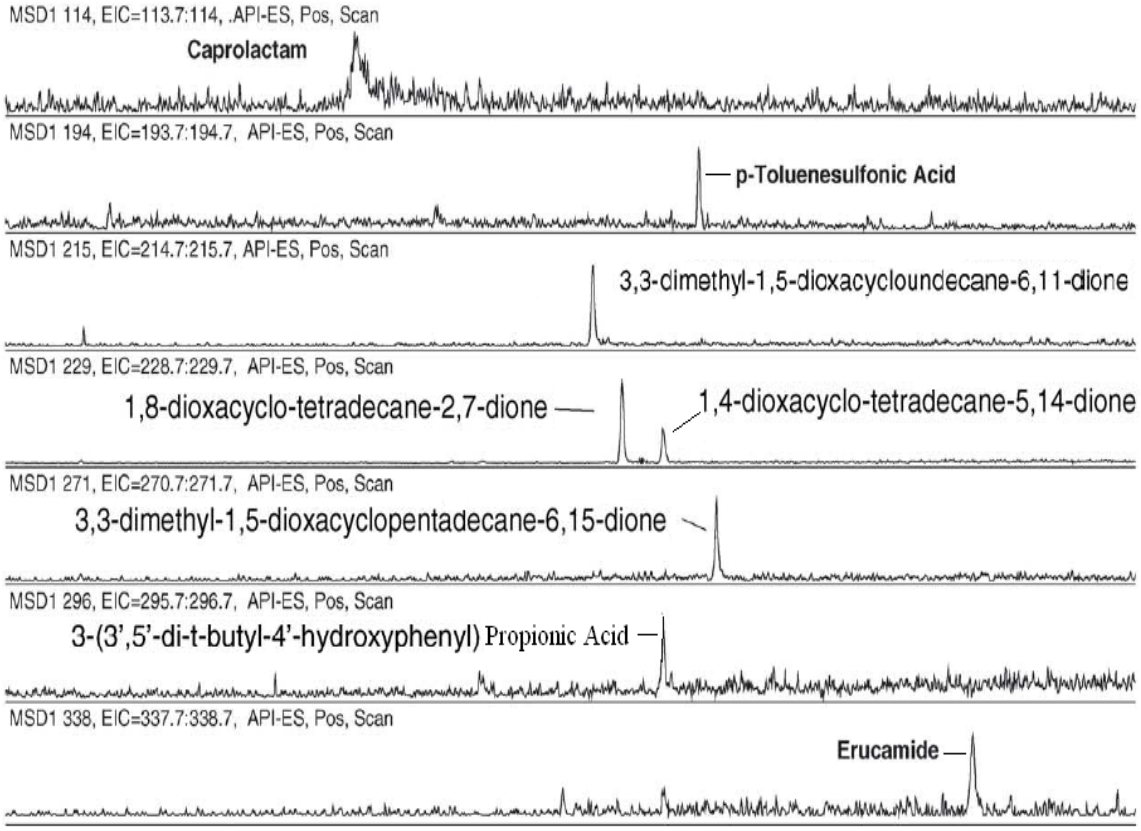


Figure 1.25 LC-MS Positive Ion Mode Water Extract of Polyolefin Material taken from Ref 48

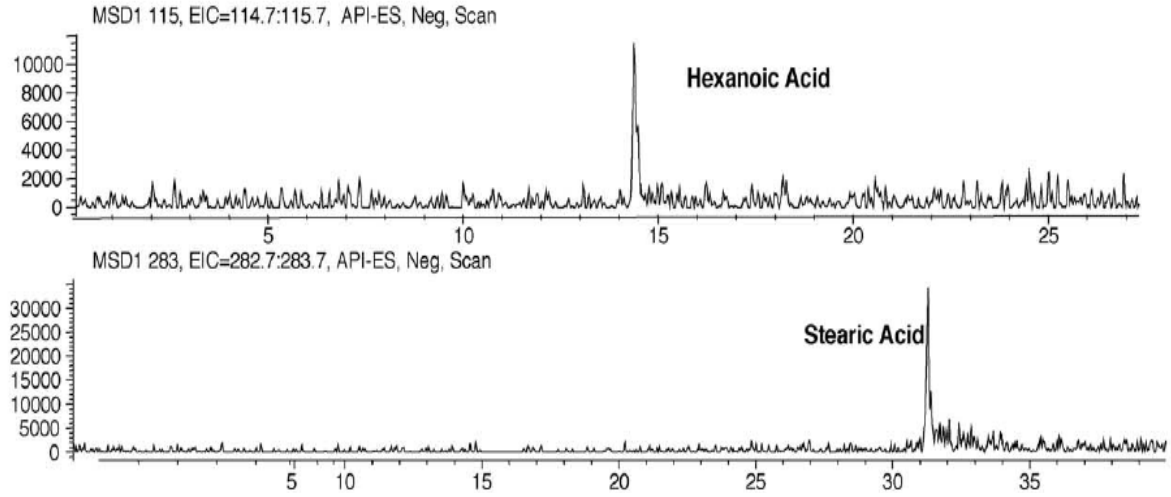


Figure 1.26: LC-MS Negative Ion Mode Water Extract of Polyolefin Material taken from Ref 48.

Table 1.7 Summary of Separation and Detection Techniques

Year	Author	Reference	Product Type	Product Component	Analytes	Technique	Separation Mode	Detection Method	Quantitation Limit
2009	Corredor	41	Injectable	Butyl and halobutyl rubber stoppers	2-dibutylamine-4, 6-dithiol-s-triazine (DBAT), 4-pentylphenol, BHT, sulfur, Irganox 1010	HPLC	Waters Xterra MS C18 100 × 4.6 mm, 5 µm Water:Methanol gradient with 0.05% TFA	UV: 220 nm	0.1 ppm, 0.3 ppm, 0.3 ppm, 0.1 ppm, 0.3 ppm
					2,4,4-trimetyl-2-pentene (TMP), 4-hydroxy-4-metyl-2-pentanone, acetone, hexanes, isopropanol, t-butyl alcohol	HS-GC	RTX-624, 30 m × 0.32 mm, 1.8 µm Helium gas: Temperature 40 degrees for 3 min, increase at 20 degree/min to 235 degrees	Flame ionization Detector	0.19 ppm, 15.8 ppm, 0.4 ppm, 0.17 ppm, 0.14 ppm, 0.15 ppm
					Fluoride, Chloride, Bromide	IC	Dionex AS 19, 250 × 4 mm Water:Methanol gradient with 100 mM sodium hydroxide	ED-40 Conductivity Mode	1.0 ppm, 2.0 ppm, 2.0 ppm
					Aluminium, Calcium, magnesium, Silicon, Titanium, Zinc	ICP-AES	Plasma Power, 1550 watts: nebulizer gas flow, 0.75 L/min	UV: 396.152 nm Aluminium 154.065 nm Bromine 317.933 nm Calcium 280.270 nm Magnesium 251.612 nm Silicon 334.941 nm Titanium 213.856 nm Zinc	2 ppm for all

2006	Jenke	53	Manufacturing Equipment	Silicone Tubing, Santoprene Tubing	Cyclic Oligomers [(CH3)2SiO]n where n=5 through n=11	GC-MS	J&W DB-5HT, 30 m × 0.25 mm, 0.1 µm Helium gas: Temperature 70 degrees for 3 min, increase at 10 degrees/min to 250 degrees	MS 70eV (+), mass range of 45 - 550 amu	Unknown
					Di-t-butyl phenol	LC/MS/UV	Waters Xterra MS C18 100 × 4.6 mm, 3.5 µm 10 mM Ammonium Foramte:Acetonitrile gradient	UV: 210, 230, 250 and 280 nm MS: API-ES, positive and negative ion, mass range 80 - 800	UV: 380 ppb MS: Not detected
					di-octyl adipate, DEHP, dibutyl phthalate, Irganox degradate	LC/MS/UV	Agilent Eclipse DB C8, 150 × 4.6 mm, 5 µm 10 mM Ammonium Foramte:Acetonitrile gradient	UV: 250 nm MS: API-ES, positive ion, single ion monitoring 371 m/z di-octyl adipate 391 m/z DEHP 279 m/z Dibutyl phthalate 296 m/z Irganox degradate	UV: 410 ppb, Not Detected, 350 ppb, 720 ppb MS: 10 ppb, 10 ppb, 20 ppb, 90 ppb
					29 elements	ICP-AES	Cyclonic, double pass glass spray chamber and V-groove nebulizer	Unknown	Lowest: Ca 0.004 ppm Highest: Mg 1.799 ppm
2006	Jenke	48	Pharmaceutical Container	Plastic component VLDPE	Caprolactam, 1,4-Dioxacyclotetradecane-5-14-dione (CE1), 3,3-Dimethyl-1,5-dioxacycloundecane-6,11-dione (CE2), 3-(3,50Di-t-butyl-4-	LC-MS	Phenomenex Prodigy C8, 150 × 4.6 mm, 5 µm 10 mM Ammonium Foramte:Acetonitrile gradient	MS: ES (+) 114 m/z Caprolactam ES (+) 229 m/z CE1 ES (+) 271 m/z CE2	0.01 ppm for all except HA which is 0.05 ppm

					hydroxyphenyl) propanoic acid (ID2), Hexanoic acid (HA), Stearic acid (SA)			ES (+) 279 m/z ID2 ES (-) 115 m/z HA ES (-) 283 m/z SA	
2004	Zhang	47	Injectable	Rubber Stopper	4-(1,1-dimethyl-propyl)-phenol, sulfur, 2,6-di-tert-butyl-[1,4] benzoquinone, furan-2-yl-(5-hydroxymethyl-furan-2-yl)-methanol and 2-bromo-4-(1,1-dimethyl-propyl)-phenol.	HPLC	Phenomenex Prodigy C18, 150 × 3.2 mm, 3 µm Water:Acetonitrile gradient	UV: 220 nm	Unknown
					4-(1,1-dimethyl-propyl)-phenol, sulfur, 2,6-di-tert-butyl-[1,4] benzoquinone, furan-2-yl-(5-hydroxymethyl-furan-2-yl)-methanol and 2-bromo-4-(1,1-dimethyl-propyl)-phenol.	GC-MS	Restek Rtx-5, 30 m × 0.25 mm, 10 µm Helium gas: Temeperture gradient 100 degrees for 1 min, increase at 10 degrees/min to 250 degrees	MS 70eV EI ionization MS Methane CI ionization	Unknown
2008	Sanches-Silva	55	Infant Milk Formula	Photoinitiator	Irgacure 184, Benzophenone, Irgacure 651, Irgacure 907, Quantacure ITX, Quantacure EHA	HPLC	Kromasil 100 C18, 150 mm × 4.0 mm, 5 µm Water: Acetonitrile gradient	PDA: Irgacure 184 - 246 nm Benzophenone - 256 nm Irgacure 651 - 256 nm Irgacure 907 - 306 nm Quantacure ITX - 386 nm Quantacure	30 µg/L for all

								EHA - 310 nm	
						HPLC -MS	Zorbax Eclipse XDB C18, 150 mm × 4.6 mm, 5 µm Water:Acetonitrile with 0.1% Formic acid gradient	ESI in positive mode	Unknown
2010	Wang	50	Bottled Water	Plastic bottle	bisphenol A (BPA), 4-n-octyphenol (4-n-OP), 4-tert-octylphenol (4-t-OP) and 4-n-nonylphenol (4-n-NP)	uHPLC/MS/MS	Dionex Acclaim PolarAdvantage II RSLC column 50 mm × 2.1 mm, 2.2 µm Water:Methanol gradient	APCI Negative MRM mode. Detection optimized for each analyte using "compound optimization" manual	BPA - 0.12 ppb 4-n-OP - 0.12 ppb 4-t-OP - 0.13 ppb 4-n-NP - 0.17 ppb
2005	Jenke	49	Biopharmaceutical	Polyolefin Plastic Container	Caprolactam, 1,4-Dioxacyclotetradecane-5-14-dione (A), 3,3-Dimethyl-1,5-dioxacycloundecane-6,11-dione (B), 3-(3,5-Di-t-butyl-4-hydroxyphenyl) propanoic acid (C), Hexanoic acid (HA), Stearic acid (SA)	IEC	Dionex HPICE-AS1 Mobile phase 1 mM Hydrochloric acid with 5 mM tetrabutylammonium hydroxide regenerant.	Unspecified	0.2 ppm for all

					Caprolactam, 1,4-Dioxacyclotetradecane-5-14-dione (A), 3,3-Dimethyl-1,5-dioxacycloundecane-6,11-dione (B), 3-(3,5-Di-t-butyl-4-hydroxyphenyl) propanoic acid (C), Hexanoic acid (HA), Stearic acid (SA)	HPLC/MS/UV	Phenomenex Prodigy C8, 150 × 4.6 mm, 5 µm 10 mM Ammonium Formate:Acetonitrile gradient	UV: 230 MS: ES (+) 114 m/z Caprolactam ES (+) 229 m/z A ES (+) 296 m/z C ES (+) 271 m/z B ES (-) 115 m/z HA ES (-) 277 m/z SA	0.2 ppm for all
2009	Degrazio	65	Injectable	Syringe Barrell	Screening run - none specified	HPLC/PDA/MS	Unspecified	Unspecified	Unknown
					25 elements	ICP-AES	Unspecified	Unspecified	1.0 ppm for all
					Fluoride, Chloride, Nitrite, Bromide, Nitrate, Phosphate, Sulfate	IC	Unspecified	Unspecified	0.5 - 1.0 ppm
2008	Akapo	51	Aqueous based pharmaceuticals	LDPE Foil Laminate	Ethanol, acetone, ispropyl alcohol, ethyl acetate, 2-butanone, n-heptane, isopropyl acetate, n-propyl acetate, toluene, diactone alcohol, 1-propoxy-2-propanol, isobutyl alcohol	GC	J&W DB-1701, 30 m × 0.53 mm, 1.5 µm Helium gas: Temperature 35 degrees for 20 min, increase at 10 degrees/min to 200 degrees	Flame ionization Detector	4.2 ppm, 0.4 ppm, 1.5 ppm, 0.05 ppm, 0.2 ppm, 0.01 ppm, 0.02 ppm, 0.01 ppm, 0.005 ppm, 1.2 ppm, 0.05 ppm

2007	Dopio-Garcia	56	Food Packaging	Polymeric films	Butylated hydroxyanisole (BHA), 2,4,bis(tert-butyl)phenol (DBP), 2,6-di-tert-butyl-p-cresol (BHT), 2,2-methylenebis(4-methyl-6tert-butylphenol) (AO2246), ,2-methylenebis(4-ethyl-6tert-butylphenol) (AO425), Irganox 1010, Ethanox 330, Irgafos 168 ox, Iganox 1076.	HPLC	Symmetry C18 150 × 3.0 mm, 3.5 µm Water: Methanol gradient aqueous samples Water:Methanol:Acetonitile:THF gradient for oil samples	UV: 276 nm Aqueous sample UV: 220 nm Oil Sample	1.4 × 10-2 mg/dm2 1.5 × 10-2 mg/dm2 1.7 × 10-2 mg/dm2 1.5 × 10-2 mg/dm2 1.4 × 10-2 mg/dm2 1.2 × 10-2 mg/dm2 1.2 × 10-2 mg/dm2 1.6 × 10-2 mg/dm2 1.5 × 10-2 mg/dm2
1997	Tiller	57	Pharmaceutical Container	Adhesive	Adhesive screening run - none specified	HPLC- MS	Waters Novapak C18 150 × 2.1 mm Water:Acetonitrile gradient with 0.1% acetic acid	UV:220 nm MS: APCI positive mode MS: ESI poisitive mode	Unknown
2012	Huang	52	Drug Product Packaging	cardboard, polyethylene, polycarbonate, wood pallet	2,4,6-tribromoansole (TBA), 2,4,6-tribromophenol (TBP), 2,4,6-trichloroanisle (TCA) and 2,4,6-trichlorophenol (TCP)	GC-MS/MS	DB5 ultra inert (5% phenylmethyl-siloxane) capillary 20 m × 0.18 mm, 0.36 µm Helium gas: Temperature 65 degrees for 1 min, increase at 25 degrees/min to 285 degrees	Multiple reaction monitoring (MRM): TBA: 346 - 331 qunatifier 346 - 303 qualifier TCA: 212 - 197 quantifier 212 - 169 qualifier TCP: 196 - 132	2,500 ng/tablet for halophenols and 100 pg/tablet for halanisols

								quantifier 196 - 160 qualifier TBP: 330 - 222 quantifier 330 - 250 qualifier	
1997	Paskiet	46	Pharmaceutical Container	Rubber packaging	Acenphthylene, Phenanthrene, Anthracene, Fluoranthene, Pyrene	SEC HPLC	Unspecified	SEC - RI HPLC - PDA	Unspecified
2011	Constantinos	58	Eye Drop Solutions	Packaging Materials	1,3-Butadiene	GC	W125-9134 DB-ALCI capillary 30 m × 0.53 mm, 3.0 µm Helium gas: Temperature 85 degrees for 11 min	FID	0.5 ppm
2012	Grilli	59	Containers and closures looking for heavy metals	HDPE bottle Polypoylene cap Elastomeric closure Glass Vials	Arsenic, Cadmium, Mercury, Lead, Iron, Copper, Zinc	AA ICP-OES ICP-MS	Unspecified Unspecified Unspecified	Flame atomization Wavelengths unspecified m/z unspecified	ppb range

1.3 Summary/Concluding remarks

The strategy taken for the extraction, separation and detection of extractables and leachables is very much dependent on the overall goal of the investigation. For the qualification of a new packaging material whereby a number of potential candidates have been identified and very little information is known about the chemical composition of the individual components it would be important to execute a comprehensive extraction study to establish an extractable profile of each. The assessment should be performed using solvents across the polarity range such as water, 2-propanaol and hexane at defined temperature and time periods. A typical extraction study on individual packaging components would be for 70^{o}C for 24 hours. The temperature may be lowered but it is recommended to extend the extraction time accordingly. If information is required immediately regarding the extractable profile and time does not allow for extraction studies to be executed over a number of days, a microwave extraction may be used to extract leachables from packaging components in minutes. The microwave extraction method may lead to primary and secondary degradation of the leachable component due to the microwave energies. Alternatively, if time allows, a corresponding leachable study should be executed using the finished product and final packaging configuration to determine which potential extractables actually leach into the product. Ideally, these studies can be extended out to 6 months at 40^{o}C or to 24 months at 25^{o}C.

Once the extractions have been completed it is important to assess the most appropriate analytical technique for separation and identification. If assessing a target extractable or leachable which is known and exhibits a chromaphore then simple HPLC-UV may suffice to complete the analysis. Alternatively, if the extractable or leachable is unknown then tandem LC-MS or GC-MS may be necessary to aid in the structural identification of the compounds of interest. MS may also be necessary if the DL or QL is very low, in the region

of ppb. These limits may be of critical importance when dealing with components which have a high level of toxicological concern.

The objectives of this thesis is to present a HPLC and UPLC liquid chromatography analytical method for the separation of eight potential leachable components and the subsequent identification of those compounds by MS. The 8 leachables were specifically chosen as they represented constituents of the packaging materials associated with ophthalmic formulations. Ophthalmic formulations typically consist of an active ingredient, preservative, antioxidants and buffering agents. The ophthalmic formulations are typically stored in low density polyethylene containers. The contents of the container are described using ink on an adhesive backed label. The bottle is then placed in a cardboard box and an adhesive based tamper evident seal is placed over the lip of the carton. The initial separation was developed using statistical design of experiment techniques using UPLC technology. Upon generation of an analytical methodology centred within a robust design space, the operational parameters were geometrically transformed to classical HPLC conditions. The dual methodology for the separation allows for the screening analysis to be performed irrespective of the LC technology within an analytical laboratory. The leachable analytes were identified on an ion trap mass spectrometer using electrospray ionization (ESI) in the positive mode. The most common approach to MS detection for liquid samples is atmospheric pressure ionization. Of these atmospheric pressure ionization techniques, ESI is the most frequently used as it can be used for both high and low molecular weight compounds, making it suitable for a great diversity of compounds. The formation of ions in ESI is highly dependent on the pKa of the analytes under analysis and the pH of the mobile phase. As the mobile phase used is acidified at low pH and typically ophthalmic formulations are amine, amide or antibiotic based the positive ion mode was selected for analysis.

References:

1. IMS Health, IMS Forecasts Global Pharmaceutical Market Growth of 4 - 6% in 2010; Predicts 4 - 7% Expansion Through 2013, available at http://www.imshealth.com/portal/site/ims/menuitem.d248e29c86589c9c30e81c033208c22a/?vgnextoid=500e8fabedf24210VgnVCM100000ed152ca2RCRD&vgnextchannel=5687ce9e0a99f210VgnVCM10000071812ca2RCRD&vgnextfmt=default
2. Fiercepharma, Top 15 Pharma R&D Budgets, available at http://www.fiercepharma.com/special-reports/top-15-pharma-rd-budgets
3. Forbes, The Truly Staggering Cost of Inventing New Drugs, available at http://www.forbes.com/sites/matthewherper/2012/02/10/the-truly-staggering-cost-of-inventing-new-drugs/
4. FEILDEN, A., 2008. Extractables and leachables: Issues and challenges. *American Pharmaceutical Review,* 11(3),.
5. Capsule Google ebooks, Design Matters: Packaging 01: An Essential Primer for Today's Competitive Market.
6. FANG, X., CHERICO, N., BARBACCI, D., HARMON, A.M., PISERCHIO, M. and PERPALL, H., 2006. Leachable study on solid dosage form. *American Pharmaceutical Review,* 9(6), pp. 58-63.
7. KOSCHIERoschier, F., GALLO, M.A., FENG, X., BAXTER, G.E., PRESTON, R. STEVENS;, K., POWERS, W. Toxicological studies on 2,4,6-tribromoanisole, Food and Chemical Toxicology, Volume 49, Issue 9, September 2011, Pages 2074-2080, ISSN 0278-6915
8. Q3A (R2) 'Impurities in New Drug Substances' , ICH Harmonised Tripartite Guideline; International Conference on Harmonisation of Technical requirements for Registration of Pharmaceuticals for Human Use; October 25, 2006
9. Q3B (R2) 'Impurities in New Drug Products' , ICH Harmonised Tripartite Guideline; International Conference on Harmonisation of Technical requirements for Registration of Pharmaceuticals for Human Use; June 2, 2006
10. <661> Containers – Plastics. The United States Pharmacopeia, USP 31; USP Convention , Inc.: Rockville, MD, 2008; 251-255
11. U.S. Department of Health and Human Services Food and Drug Administration Centre for Drug Evaluation and Research (CEDER); Rockville, MD, Container Closure Systems for Packaging Human Drugs and Biologics; Guidance for Industry; May 1999 1-41
12. NORWOOD, D.L., PASKIET, D., RUBERTO, M., FEINBERG, T., SCHROEDER, A., POOCHIKIAN, G., WANG, Q., DENG, T.J., DEGRAZIO, F., MUNOS, M.K. and NAGAO, L.M., 2008. Best practices for extractables and leachables in orally inhaled and nasal drug products: An overview of the PQRI recommendations. *Pharmaceutical research,* 25(4), pp. 727-739.
13. Leachables and Extractables Testing: Points to Consider, IPAC-RS, March 2001, available at http://www.ipacrs.com/leachables.html.
14. Norwood, Daniel L. , Jenke, Dennis , Manolescu, Cristina , Pennino, Scott and Grinberg, Nelu 'HPLC and LC/MS Analysis of Pharmaceutical Container Closure System Leachables and Extractables', Journal of Liquid Chromatography & Related Technologies, 32:11, 1768 – 1827
15. International Conference on Harmonization Tripartite Guideline, Validation of analytical Procedures: Text and Methodology, ICH Q2(R1).
16. MOLNÁR, I., 2005. Searching for robust HPLC methods - Csaba Horváth and the Solvophobic Theory. *Chromatographia,* 62(SUPPL. 13), pp. S7-S17.
17. DEJAEGHER, B. and VANDER HEYDEN, Y., 2011. Experimental designs and their recent advances in set-up, data interpretation, and analytical applications. *Journal of pharmaceutical and biomedical analysis,* 56(2), pp. 141-158.
18. TSANG, P.K.S., LAREW, J.S.A., LAREW, L.A., MIYAKAWA, T.W. and HOFER, J.D., 1998. Statistical approaches to determine analytical variability and specifications:

Application of experimental design and variance component analysis. *Journal of pharmaceutical and biomedical analysis,* 16(7), pp. 1125-1141.
19. MARTIN, J., 2010. Regulatory expectations and consensus industry recommendations for extractables testing of single-use process equipment. *BioPharm International,* 23(11 SUPPL.), pp. 6-10.
20. U.S. Department of Health and Human Services Food and Drug Administration Centre for Drug Evaluation and Research (CEDER); Rockville, MD, Inspections, Compliance, Enforcement, and Criminal Investigations, Enforcement Actions, Warning Letters
21. Sharma B, Bader F, Templeman T, Lisi P, Ryan M, Heavner GA. 2004. Technical Investigations into the Cause of the Increased Incidence of Antibody Mediated Pure Red Cell Aphasia Associated with EPREX®. Eur J Hosp Pharm5: 86-91
22. PANG, J., BLANC, T., BROWN, J., LABRENZ, S., VILLALOBOS, A., DEPAOLIS, A., GUNTURI, S., GROSSMAN, S., LISI, P. and HEAVNER, G.A., 2007. Recognition and identification of UV-absorbing leachables in EPREX® pre-filled syringes: An unexpected occurrence at a formulation-component interface. *PDA Journal of Pharmaceutical Science and Technology,* 61(6), pp. 423-432.
23. NICHOLAS, K., 2006. Extractables and leachables determination: A systematic approach to select and qualify a container closure system for a pharmaceutical product. *American Pharmaceutical Review,* 9(2),.
24. OSTERBERG, R.E., 2005. Potential toxicity of extractables and leachables in drug products. *American Pharmaceutical Review,* 8(2), pp. 64-67.
25. BEIERSCHMITT, W.P., 2009. Toxicology issues in extractables and leachables. *American Pharmaceutical Review,* 12(6), pp. 122-126.
26. NORTHUP, S.J., 2008. Pharmaceutical containers: Safety qualification of extractables/leachables. *American Pharmaceutical Review,* 11(2),.
27. United States Pharmacopeia, General Chapters: <87> Biological Reactivity Tests, In Vitro.
28. Untied States Pharmacopeia, General Chapters: <88> Biological Reactivity Tests, In Vivo.
29. TOXNET, Toxicology Data Network, Untied States National Library of Medicine. Available to view at http://toxnet.nlm.nih.gov/
30. INCHEM, International Programme on Chemical Safety (IPCS), Chemical Safety Information for Intergovernmental Organizations. Available to view at http://www.inchem.org/
31. ExPub, Chemical Hazard Information for EH&S Professionals. Available to view at www.expub.com
32. ALARCON, A., BARCELO, B., CAIRE-MAURISIER, F., DELAIRE, M., FEUILLOLEY, M., CENOT, S., LACAZE, C., LASCHI, A., PISARIK, L., SENHAL, N. and SMATI, C., 2007. Container-content interaction I. Regulation. *S.T.P.Pharma Pratiques,* 17(3), pp. 131-141.
33. ALARCON, A., BARCELO, B., CAIRE-MAURISIER, F., DELAIRE, M., FEUILLOLEY, M., GENOT, S., LACAZE, C., LASCHI, A., PISARIK, L., SENHAL, N. and SMATI, C., 2007. Container-content interaction II. Methodology. *S.T.P.Pharma Pratiques,* 17(3), pp. 143-160.
34. LASCHI, A., SEHNAL, N., ALARCON, A., BARCELO, B., CAIRE-MAURISIER, F., DELAIRE, M., FEUILLOLEY, M., GENOT, S., LACAZE, C., PISARIK, L. and SMATI, C., 2009. Container-content compatibility studies: A pharmaceutical team's integrated approach. *PDA Journal of Pharmaceutical Science and Technology,* 63(4), pp. 285-293.
35. MARKOVIC, I., 2009. Risk management strategies for safety qualification of extractable and leachable substances in therapeutic biologic protein products. *American Pharmaceutical Review,* 12(4),.
36. VEGA-MERCADO, H., SCHULTZ, T., CONDER, M. and DEKLEVA, M., 2004. Container closure validation: Technical and compliance aspects. *American Pharmaceutical Outsourcing,* 5(2), pp. 8-20

37. BALL, D.J., NORWOOD, D.L. and NAGAO, L., 2007. Utility and application of analytical and safety thresholds for the evaluation of extractables and leachables in drug products. *American Pharmaceutical Review,* 10(4),.
38. PEARSON, R., 2010. Extractables and leachables: What to do and why to do it? 2010, , pp. 2241-2244.
39. FEILDEN, A., 2008. Extractables and leachables: Issues and challenges. *American Pharmaceutical Review,* 11(3),.
40. WAKANKAR, A.A., WANG, Y.J., CANOVA-DAVIS, E., MA, S., SCHMALZING, D., GRIECO, J., MILBY, T., REYNOLDS, T., MAZZARELLA, K., HOFF, E., GOMEZ, S. and MARTIN-MOE, S., 2010. On developing a process for conducting extractable-leachable assessment of components used for storage of biopharmaceuticals. *Journal of pharmaceutical sciences,* 99(5), pp. 2209-2218
41. CORREDOR, C.C., HABY, T.A., YOUNG, J.D., SHAH, P.A. and VARIA, S.A., 2009. Comprehensive determination of extractables from five different brands of stoppers used for injectable products. *PDA Journal of Pharmaceutical Science and Technology,* 63(6), pp. 527-536.
42. JENKE, D., 2007. An extractables/leachables strategy facilitated by collaboration between drug product vendors and plastic material/system suppliers. *PDA Journal of Pharmaceutical Science and Technology,* 61(1), pp. 17-23
43. PAN, C., HARMON, F., TOSCANO, K., LIU, F. and VIVILECCHIA, R., 2008. Strategy for identification of leachables in packaged pharmaceutical liquid formulations. *Journal of pharmaceutical and biomedical analysis,* 46(3), pp. 520-527.
44. CASTNER, J., BRESNICK, M. and BENITES, P., 2009. A strategy for determining leachables in liquid drug products. *Rubber World,* 240(3), pp. 32-40.
45. NOVÁKOVÁ, L., MATYSOVÁ, L. and SOLICH, P., 2006. Advantages of application of UPLC in pharmaceutical analysis. *Talanta,* 68(3), pp. 908-918
46. PASKIET, D.M., 1997. Strategy for determining extractables from rubber packaging materials in drug products. *PDA Journal of Pharmaceutical Science and Technology,* 51(6), pp. 248-251.
47. ZHANG, F., CHANG, A., KARAISZ, K., FENG, R. and CAI, J., 2004. Structural identification of extractables from rubber closures used for pre-filled semisolid drug applicator by chromatography, mass spectrometry, and organic synthesis. *Journal of pharmaceutical and biomedical analysis,* 34(5), pp. 841-849.
48. JENKE, D.R., JENE, J.M., POSS, M., STORY, J., TSILIPETROS, T., ODUFU, A. and TERBUSH, W., 2005. Accumulation of extractables in buffer solutions from a polyolefin plastic container. *International journal of pharmaceutics,* 297(1-2), pp. 120-133.
49. JENKE, D., GARBER, M.J. and ZIETLOW, D., 2005. Validation of a liquid chromatographic method for quantitation of organic compounds leached from a plastic container into a pharmaceutical formulation. *Journal of Liquid Chromatography and Related Technologies,* 28(2), pp. 199-222.
50. WANG, J. and SCHNUTE, W.C., 2010. Direct analysis of trace level bisphenol A, octylphenols and nonylphenol in bottled water and leached from bottles by ultra-high-performance liquid chromatography/tandem mass spectrometry. *Rapid Communications in Mass Spectrometry,* 24(17), pp. 2605-2610.
51. AKAPO, S.O. and MCCREA, C.M., 2008. SPME-GC determination of potential volatile organic leachables in aqueous-based pharmaceutical formulations packaged in overwrapped LDPE vials. *Journal of pharmaceutical and biomedical analysis,* 47(3), pp. 526-534.
52. HUANG, J.-., ALQUIER, L., KAISA, J.P., REED, G., GILMOR, T. and VAS, G., 2012. Method development and validation for the determination of 2,4,6-tribromoanisole, 2,4,6-tribromophenol, 2,4,6-trichloroanisole, and 2,4,6-trichlorophenol in various drug products using stir bar sorptive extraction and gas chromatography-tandem mass spectrometry detection. *Journal of Chromatography A,* 1262, pp. 196-204.
53. JENKE, D.R., STORY, J. and LALANI, R., 2006. Extractables/leachables from plastic tubing used in product manufacturing. *International journal of pharmaceutics,* 315(1-2), pp. 75-92.
54. WAKANKAR, A.A., WANG, Y.J., CANOVA-DAVIS, E., MA, S., SCHMALZING, D., GRIECO, J., MILBY, T., REYNOLDS, T., MAZZARELLA, K., HOFF, E., GOMEZ, S. and MARTIN-MOE, S.,

2010. On developing a process for conducting extractable-leachable assessment of components used for storage of biopharmaceuticals. *Journal of pharmaceutical sciences,* 99(5), pp. 2209-2218.
55. SANCHES-SILVA, A., PASTORELLI, S., CRUZ, J.M., SIMONEAU, C., CASTANHEIRA, I. and PASEIRO-LOSADA, P., 2008. Development of an analytical method for the determination of photoinitiators used for food packaging materials with potential to migrate into milk. *Journal of dairy science,* 91(3), pp. 900-909.
56. DOPICO-GARCÍA, M.S., LÓPEZ-VILARIÑÓ, J.M. and GONZÁLEZ-RODRÍGUEZ, M.V., 2007. Antioxidant content of and migration from commercial polyethylene, polypropylene, and polyvinyl chloride packages. *Journal of Agricultural and Food Chemistry,* 55(8), pp. 3225-3231.
57. TILLER, P.R., EL FALLAH, Z., WILSON, V., HUYSMAN, J. and PATEL, D., 1997. Qualitative assessment of leachables using data-dependent liquid chromatography/mass spectrometry and liquid chromatography/ tandem mass spectrometry. *Rapid Communications in Mass Spectrometry,* 11(14), pp. 1570-1574.
58. PISTOS, C., KARAMPELA, S., VARDAKOU, I., PAPOUTSIS, I., SPILIOPOULOU, C. and ATHANASELIS, S., 2012. Migration study of 1,3-butadiene in eye-drop solutions. *Drug and chemical toxicology,* 35(3), pp. 293-299.
59. GRILLI, A., 2012. Drug container closure testing: Impact of extraction and analysis methodology on metal contaminant quantification. *Pharma Times,* 44(8), pp. 18-20.
60. European Food Safety Authority, 2005, Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) related to 2-Isopropyl thioxanthone (ITX) and 2-ethylhexyl-4-dimethylaminobenzoate (EHDAB) in food contact materials.
61. DOPICO-GARCÍA, M.S., LÓPEZ-VILARIÑÓ, J.M. and GONZÁLEZ-RODRÍGUEZ, M.V., 2007. Antioxidant content of and migration from commercial polyethylene, polypropylene, and polyvinyl chloride packages. *Journal of Agricultural and Food Chemistry,* 55(8), pp. 3225-3231.
62. KIM, B.-., YANG, D.-. and JONG, H.O., 2007. Analysis of polymer additives in high-temperature liquid chromatography. *Journal of chromatographic science,* 45(1), pp. 16-21.
63. NOGUEROL-CAL, R., LÓPEZ-VILARIÑO, J.M., GONZÁLEZ-RODRÍGUEZ, M.V. and BARRAL-LOSADA, L.F., 2007. Development of an ultraperformance liquid chromatography method for improved determination of additives in polymeric materials. *Journal of Separation Science,* 30(15), pp. 2452-2459.
64. FICHTNER, S. and GIESE, U., 2004. Identification of "Extractables" on Polymer Materials: Application of LC-MS-Technology. *KGK-Kautschuk und Gummi Kunststoffe,* 57(3), pp. 116-121.
65. DEGRAZIO, F., RUNKLE, J., SMYTHE, J. and MILLER, A., 2009. Analysis of biopharmaceutical market-appropriate plastic syringe barrel for extractables. *PDA Journal of Pharmaceutical Science and Technology,* 63(4), pp. 360-367.
66. ITO, R., SESHIMO, F., MIURA, N., KAWAGUCHI, M., SAITO, K. and NAKAZAWA, H., 2005. High-throughput determination of mono- and di(2-ethylhexyl)phthalate migration from PVC tubing to drugs using liquid chromatography-tandem mass spectrometry. *Journal of pharmaceutical and biomedical analysis,* 39(5), pp. 1036-1041.
67. WANG, H.-., ZHOU, Y., WANG, X., QU, W.-. and JIANG, Q.-., 2012. Screening and assessing the phenols in water environment of Shanghai city. *Fudan University Journal of Medical Sciences,* 39(3), pp. 231-237.
68. KOPPERUD, H.M., KLEVEN, I.S. and WELLENDORF, H., 2011. Identification and quantification of leachable substances from polymer-based orthodontic base-plate materials. *European journal of orthodontics,* 33(1), pp. 26-31.

Chapter 2: Development of LC-MS Methods for the Analysis of Potential Leachables in Pharmaceutical Packaging Components

2. Introduction

Historically, investment into investigational new drug development eased once the therapeutic effect of the active was sufficiently proven through clinical trial studies.[1] The final challenge was then to bring the product to market. Much of the emphasis has been placed upon logistics and marketing campaigns once the product was packaged and ready for sale. The container closure system of the final product was chosen for its aesthetic properties and overall functionality for the consumer. It was not until recently that the chemical and physical properties of the packaging components has come under scrutiny. The potential for analytes from the final packaging to migrate into the final product and the impact to the consumer must now be assessed.[2] A toxicological assessment must be performed on any potential leachables and any degradants associated with interactions between the leachables and the active pharmaceutical ingredient. It is not only the moral obligation of the pharmaceutical companies to show this due diligence but in 1999 The United States FDA issued a guidance document in relation to packaging, requirements for new drug products.[3] In accordance with this guidance document it is the lawful obligation of the pharmaceutical companies to establish the compatibility of the drug product with the primary container closure system.

The challenge of detecting and qualifying potential leachables within formulation matrices is often overcome by combining a number of analytical methodologies. Currently, chromatographic techniques in tandem with MS analysis are seen as the industry standard for the separation of leachables. The volatility (or other physical characteristics) of the chemical species will ultimately determine the most appropriate method of analysis. The relative low level concentration (ppm) of leachables within pharmaceutical formulations requires instrumentation with a significant dynamic range of operation to detect these leachable species in the presence of the active pharmaceutical ingredient. The transition is being made from LC-UV detection of leachable components to LC-MS technology with the increased demand for qualitative analysis of these compounds for toxicological purposes.

[14,15] Jenke *et al.* have utilised LC-MS analytical techniques to identify extractables including caprolactam, p-toluenesulfonic acid, propionic acid and stearic acid associated with polyolefin containers. The interaction between a polyolefin container material and several test solution representative of buffers and media used in biopharmaceutical applications was investigated under various storage conditions.[16]

This work presents a HPLC and UPLC liquid chromatography analytical method for the separation of eight potential leachable components and the subsequent identification of those compounds by MS. The initial separation was developed using statistical design of experiment techniques using UPLC technology and subsequently transformed to HPLC conditions. The leachable analytes were identified on an ion trap mass spectrometer using electrospray ionization (ESI) in the positive mode.

2.2 Experimental

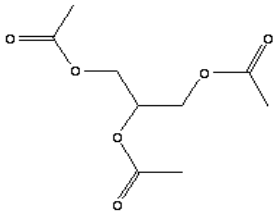
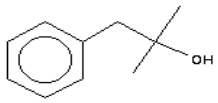
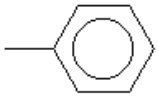
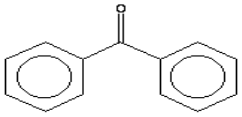
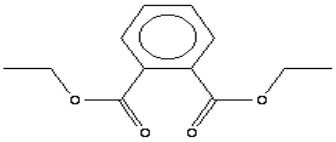
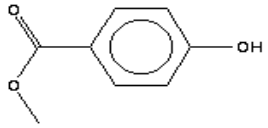
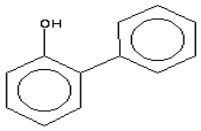
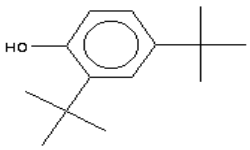
2.2.1 Reagents and Preparation of Standards

Analytical grade (all purity ≥ 99%) glyceryl triacetate, darocur 1173, toluene, benzophenone, diethyl phthalate, methylparaben, 2-phenylphenol and 2,4-ditertbutylphenol were purchased under license from Sigma-Aldrich (Poole, UK). The chemical structure of each drug is provided in Table 2.1.

All solvents were HPLC grade or better. Acetonitrile and methanol were obtained from Labscan (Dublin, Ireland). Ultrapure water was obtained from a Millipore Milli-Q water purification unit (Millipore, Bedford, MA, USA) with specific resistance of 18.2 MΩ/cm or better. Analytical grade formic acid was purchased from BDH Chemicals (Poole, UK). All glassware utilised for the storage of leachable stocks and standards were silanised prior to the preparation to prevent the leachables from adhering to the glass surfaces. This was executed by rinsing the glassware with 10% dichlorodimethylsilane in dichloromethane. This was followed by rinsing with dichloromethane and rinsing with methanol twice each. Stock solutions of the potential leachables were prepared in methanol to a concentration of 200

ppm and were stored in the refrigerator at 5 ^{o}C ± 3 ^{o}C. Working solutions were prepared freshly before analysis in methanol. The chemical structure and common uses of potential leachables are presented in Table 2.1.

Table 2.1: The Chemical Structure of Potential Leachables

Analyte	Structure	Common Use
Glyceryl Triacetate		• Plasticizer • Antifungal agent • Gelatinizing agent
Darocur 1173		• Photoinitiator
Toluene		• Adhesives • Paper coating • Printing • Rubber Manufacture • Paint Manufacture
Benzophenone		• Photo-initiator • UV blocker
Diethyl phthalate		• Plasticizer • Resin solvent • Varnish • Cosmetic Softener
Methylparaben		• Antifungal Agent • Preservative
2-Phenylphenol		• Fungicide • Food additive • Disinfectant
2,4-Ditertbutylphenol		• Antioxidant

2.2.2 Liquid Chromatography

Initial chromatographic separations were performed on Waters Acquity H-Class UPLC with a vacuum degasser, quaternary solvent manager, autosampler and UV detector. Separations were performed using a Waters Acquity UPLC BEH C18, 2.1 × 50 mm i.d., 1.7 µm column. A multistep gradient was employed with mobile phases of (A) water with 1% (v/v) trifluoroacetic acid and (B) methanol with 1% (v/v) trifluoroacetic acid. Gradient conditions were 80% mobile phase A with an increase of mobile phase B to 55% over 5 min. The mobile phase B concentration was increased to 90% over the next 2 min (total 7 min). The initial gradient conditions were reemployed at 7.1 mins at re-equilibrate to a total run time of 11 min. The nominal H-Class LC conditions are described in Table 2.2 and the Gradient Program is described in Table 2.3.

Table 2.2: UPLC Conditions for the Separation of Leachables

Pump flow rate	0.6 mL/min
Detector	210 nm
Column Temp.	45°C
Run time	11 min
Injection volume	2 µL
Needle Wash	50% (v/v) Acetonitrile
UPLC Column	Waters BEH C18
Column Dimensions	50 × 2.1 mm
Particle Size	1.7 µm

Table 2.3: Gradient Program for the Separation of Leachables

Minutes	%A	%B	Curve No.
Initial	80	20	*
5.0	45	55	6
7.0	10	90	6
7.1	80	20	6
11.0	80	20	6

2.2.3 Method Optimization

To ensure method robustness a design of experiments (DOE) was employed to define the critical parameters which affect the separation of potential leachables and to optimize each of those conditions. The purpose of the DOE was to generate a method which separates the components as quick as possible within a robust design space. The critical method parameters which affect the separation were defined as: initial concentration of methanol (MeOH1), intermediate concentration of methanol (MeOH2), the final concentration of methanol (MeOH3), time for the first gradient step (t_1), time for the second gradient step (t_2).

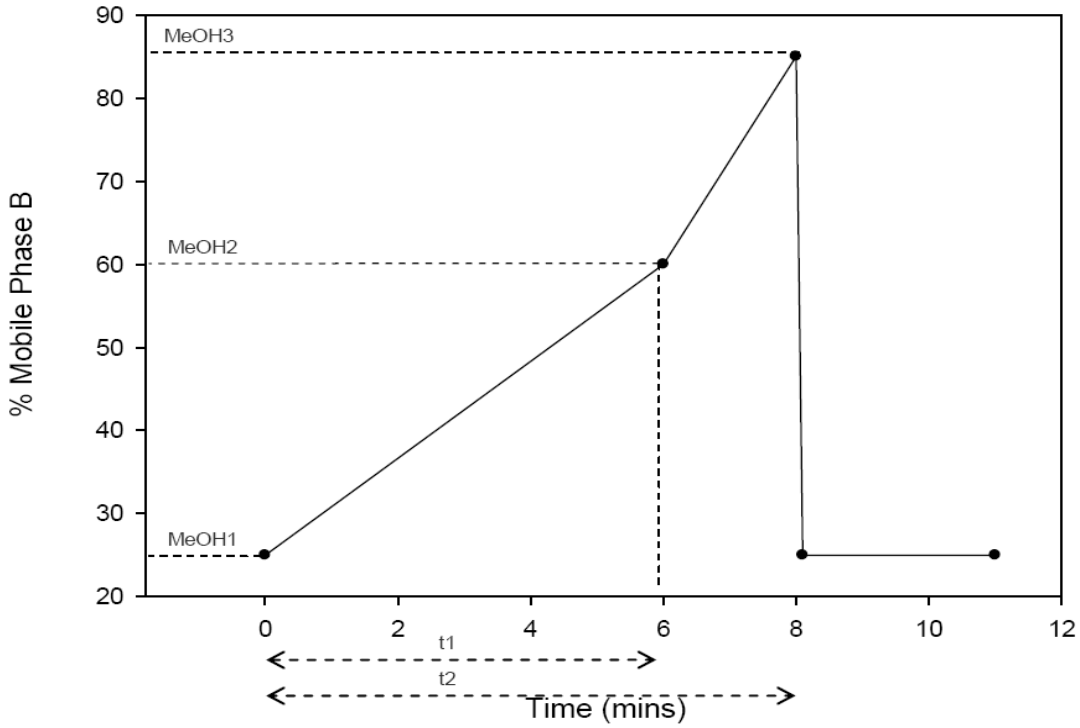


Figure 2.1: Gradient Profile for Ultra Performance Liquid Chromatography (UPLC)

A two level fractional factorial DOE was applied to the UPLC process examining the critical parameters identified with resolution between the critical pairs in the separation as the output. The DOE is outlined in Table 2.4.

Table 2.4: Design of Experiment for Separation of Leachables

Run Order	t1 (mins)	t2 (mins)	MeOH1 (% B)	MeOH2 (% B)	MeOH3 (% B)
1	5	7	20	55	90
2	7	7	20	55	80
3	5	9	20	55	80
4	7	9	20	55	90
5	5	7	30	55	80
6	7	7	30	55	90
7	5	9	30	55	90
8	7	9	30	55	80
9	5	7	20	65	80
10	7	7	20	65	90
11	5	9	20	65	90
12	7	9	20	65	80
13	5	7	30	65	90
14	7	7	30	65	80
15	5	9	30	65	80
16	7	9	30	65	90
17	6	8	25	60	85

2.2.4 Method Validation

The precision of the method was obtained by calculating the standard deviation and relative standard deviation of the area and peak height data for each leachable. The method was assessed via process capability analysis which ensures the method delivers the required resolution. The statistical analysis was executed using Minitab® software.

2.2.5 Geometric Transformation of UPLC Method to HPLC

The optimized parameters for the UPLC method were geometrically transferred to HPLC conditions to allow the method to be run on Acquity (H-Class) and Alliance systems. The nominal Acquity (H-Class) conditions are described in Table 2.2.

2.2.6 Liquid Chromatography – Mass Spectrometry

Chromatographic separations were performed on an Agilent 1100 series HPLC with a liquid degasser, binary pump, liquid sampler (LS) autosampler, and diode array detector. The LC was coupled to a Bruker Daltonics Esquire-LC ESI-ion trap mass spectrometer. Agilent Chemstation version A.09.03 (Agilent Technologies, USA) and Bruker Daltonics esquire control version 4.0 (Bruker Daltonics, UK) were employed to control the system and data analysis was performed using Bruker Data Analysis 2.0 (Bruker Daltonics, UK). Separations were performed on a Waters XBridge^{TM} C18 100 × 4.6 mm, 3.5 µm column. A multistep gradient was employed with mobile phases of (A) water with 1% (v/v) formic acid and (B) methanol with 1% (v/v) formic acid. Gradient conditions were 77.5% mobile phase A with an increase of mobile phase B to 62.5% over 22 min. The mobile phase B concentration was increased to 85% over the next 8 min (total 30 min). The initial gradient conditions were re-employed at 32 min at re-equilibrate to a total run time of 40 min.

Table 2.6: ESI-MS Operating Parameters

Parameter	Positive Mode
Capillary Voltage	4500
End Plate Offset (V)	-500
Skimmer 1 (V)	40.0
Cap. Exit Offset (V)	117.3
Octopole 1 (V)	12.0
Octopole 2 (V)	1.70
Octopole RF (Vpp)	162.2
Lens 1 (V)	-5.0
Lens 2 (V)	-60.0
Trap Drive (Arbitrary units)	37.3
Dry Gas Flow (N_2; L/min)	10.0
Nebulizer Pressure (psi)	25.0
Dry Gas Temp. (^oC)	340
Scan (m/z)	50 – 2000
MS(n)	Auto MS(2)
No. of Precursor Ions	2

2.3 Results and Discussion

2.3.1 LC and Method Optimization

For the development of the analytical method, 8 model leachable compounds were selected to represent the various sources from which extractables and leachables have been known to migrate from. Glyceryl triacetate and diethyl phthalate are plasticizers used in the manufacture of plastic overwraps or films to bind single units together for distribution to market. Darocur 1173 and benzophenone are photoinitiators and are constituents of inks to aid in the drying (curing) of ink to paper or plastic substrates. Toluene and 2-phenylphenol are used in the manufacture of rubber components and some inks and varnishes. Methylparaben is a preservative and antifungal agent used to maintain the integrity of natural products such as paper. Finally, 2,4-ditertbutylphenol is an antioxidant used to protect packaging components from oxidative degradation. A gradient LC method was developed for the initial separation of the 8 potential leachables on a Waters H-Class system and BEH column technology. To assess the performance of the separation, resolution data was calculated for each of the components. Resolution was calculated using Empower Software and manually calculated using Eqn. 2.1 and the results are shown in Table 2.7

$$R_S = \frac{2\left(t_{R(2)} - t_{R(1)}\right)}{W_1 + W_2} \qquad \textbf{(Eqn. 2.1)}$$

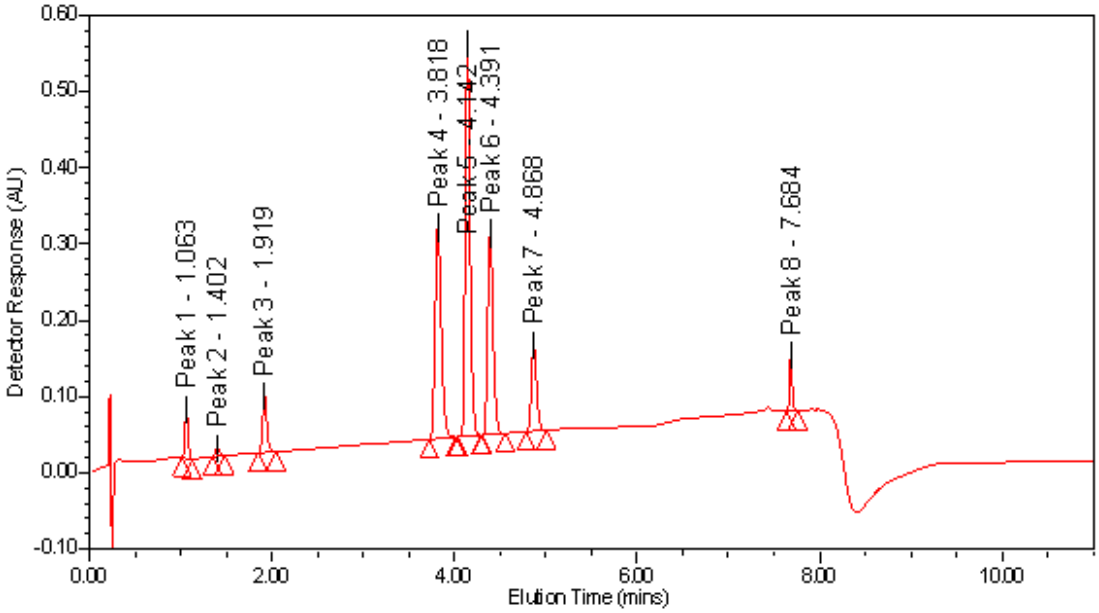


Figure 2.2: UPLC chromatogram of a mixture of potential leachables. Peak Identity: (1) Triacetin, (2) Darocur 1173, (3) Methylparaben, (4) Diethyl Phthalate, (5) Toluene, (6) Benzophenone, (7) Phenyl Phenol, (8) 2,4-Ditertbutylphenol.

Table 2.7: Resolution Data for Eight Potential Leachables

Leachable	t_R (min)	Rs
Triacetin / Darocur 1173	1.06 / 1.40	4.95
Darocur 1173 / Methylparaben	1.40 / 1.92	6.47
Methylparaben / Diethyl Phthalate	1.92 / 3.82	18.33
Diethyl Phthalate / Toluene	3.82 / 4.14	2.99
Toluene / Benzophenone	4.14 / 4.39	2.47
Benzophenone / Phenyl Phenol	4.39 / 4.87	4.55
Phenyl Phenol / 2,4-Ditertbutylphenol	4.87 / 7.68	36.77

All values were significantly above 1.5 which indicates complete separation of analytes and is satisfactory in accordance with the literature.[17] This was deemed a good starting point for method optimization. The critical pairs in the separation are Diethyl Phthalate / Toluene (Peaks 4 & 5) and Toluene / Benzophenone (Peaks 5 & 6) with regard to resolution. A critical pair represents the two components of the chromatogram with the lowest calculated resolution between them. The resolution between Phenyl Phenol and 2,4-Ditertbutylphenol (Peaks 7 & 8) was also deemed critical as an indirect measurement of assay run time. The resolution of these critical pairs were monitored during DOE method optimization techniques. The resolution results are presented in Table 2.8

Table 2.8: Design of Experiments and Resolution Results for Critical Pairs

Run Order	t1 (mins)	t2 (mins)	MeOH1 (% B)	MeOH2 (% B)	MeOH3 (% B)	Peak (4,5) (resolution)	Peak (5,6) (resolution)	RS (7,8) (resolution)
1	5	7	20	55	90	2.893	2.404	35.514
2	7	7	20	55	80	2.963	2.392	26.127
3	5	9	20	55	80	1.928	2.657	32.731
4	7	9	20	55	90	3.088	2.456	35.676
5	5	7	30	55	80	5.126	1.987	30.756
6	7	7	30	55	90	2.25	2.669	33.544
7	5	9	30	55	90	0	2.924	32.941
8	7	9	30	55	80	2.195	2.618	35.19
9	5	7	20	65	80	3.625	2.354	38.68
10	7	7	20	65	90	1.697	2.688	32.016
11	5	9	20	65	90	1.231	2.808	31.114
12	7	9	20	65	80	1.694	2.679	31.568
13	5	7	30	65	90	3.615	2.315	37.745
14	7	7	30	65	80	1.079	3.057	36.69
15	5	9	30	65	80	1.021	3.165	34.966
16	7	9	30	65	90	1.076	3.102	33.034
17	6	8	25	60	85	2.22	2.686	42.621

To assess the impact of each vital operational parameter on the resolution of the critical pairs, response surface plots were generated. Each critical pair was monitored in isolation without consideration for the impact on the remaining two critical pairs. This approach allows for direct in-depth analysis on the effect of changing any of the 5 operational factors on the

resolution of each of the critical pairs. The response surface plots for resolution between diethyl phthalate (DEP) and (TOL) are presented in Fig. 2.3. As T1 increases the resolution between DEP and TOL decreases. Maximum resolution is attained if T1 is set to 5 min and T2 is set to 7 min. Minimum resolution is attained if T1 is set to 7 min, irrespective of any other setting within the design space. As T2 increases, the resolution between DEP and TOL decreases. In order to maintain baseline resolution for DEP and TOL, the concentration of methanol at point MeOH1 needs to be set as low as possible. An additional increase in the concentration of methanol at MeOH2 further reduces the resolution between the critical pair. The response surface plots for resolution between TOL and benzophenone (BEN) are presented in Fig. 2.4. T1 has minimal impact on the resolution between TOL and BEN. As T2 increases resolution increases marginally. However, the concentration of methanol at MeOH2 at T2 and the concentration of methanol at MeOH2 relative to MeOH1 has a significant impact on the resolution of the critical pair The response surface plots for resolution between phenyl phenol and 2,4-ditertbutylphenol are presented in Fig. 2.5. Resolution between phenyl phenol and 2,4-ditertbutylphenol should ideally be as low as possible as this is an indication of run time. This can be achieved by minimising the methanol concentration at MeOH3. It must be noted that this is a secondary consideration as resolution between the previously described critical pairs will take precedence over a reduction in run time.

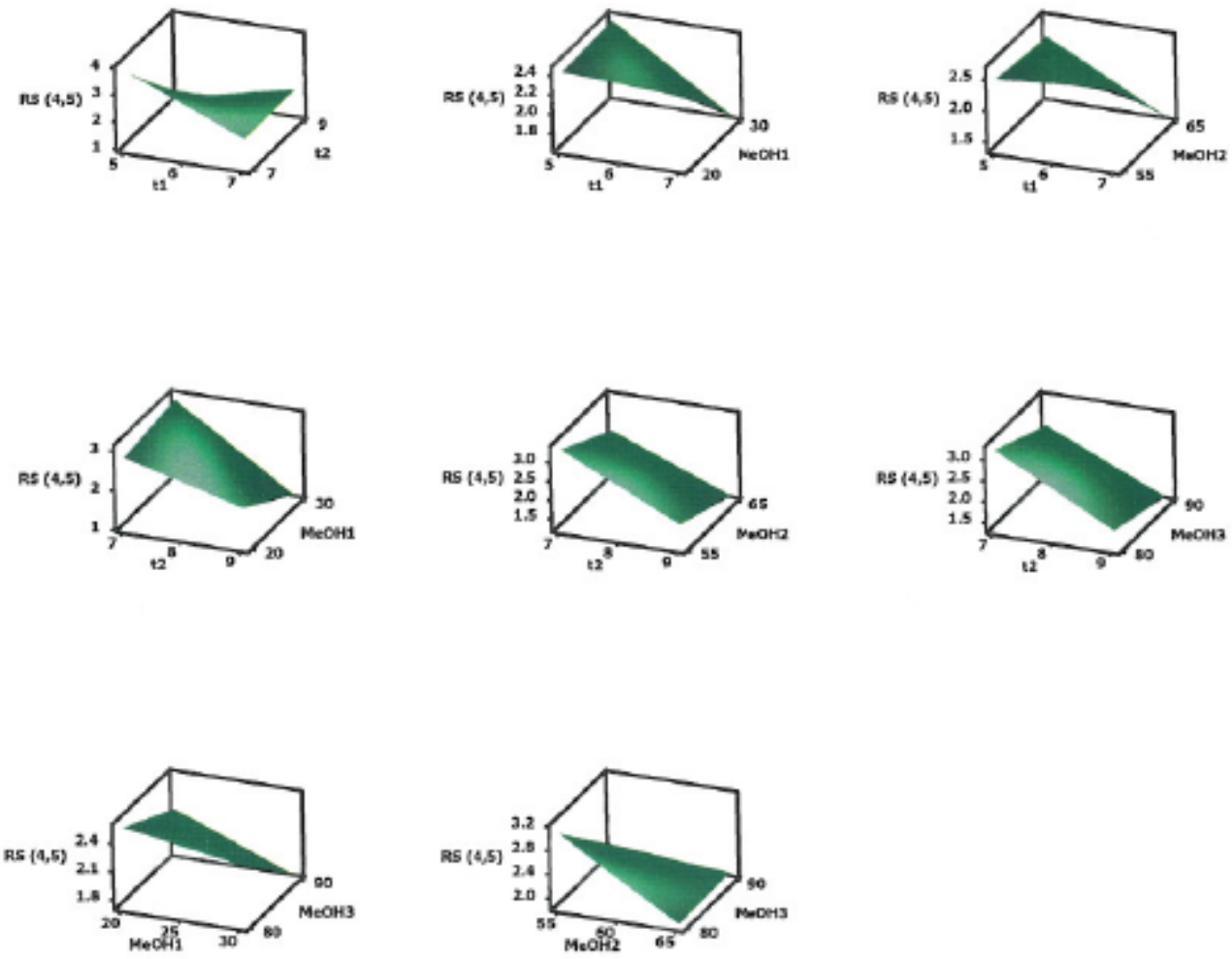


Figure 2.3: Response Surface Plots for Resolution between Diethyl Phthalate and Toluene

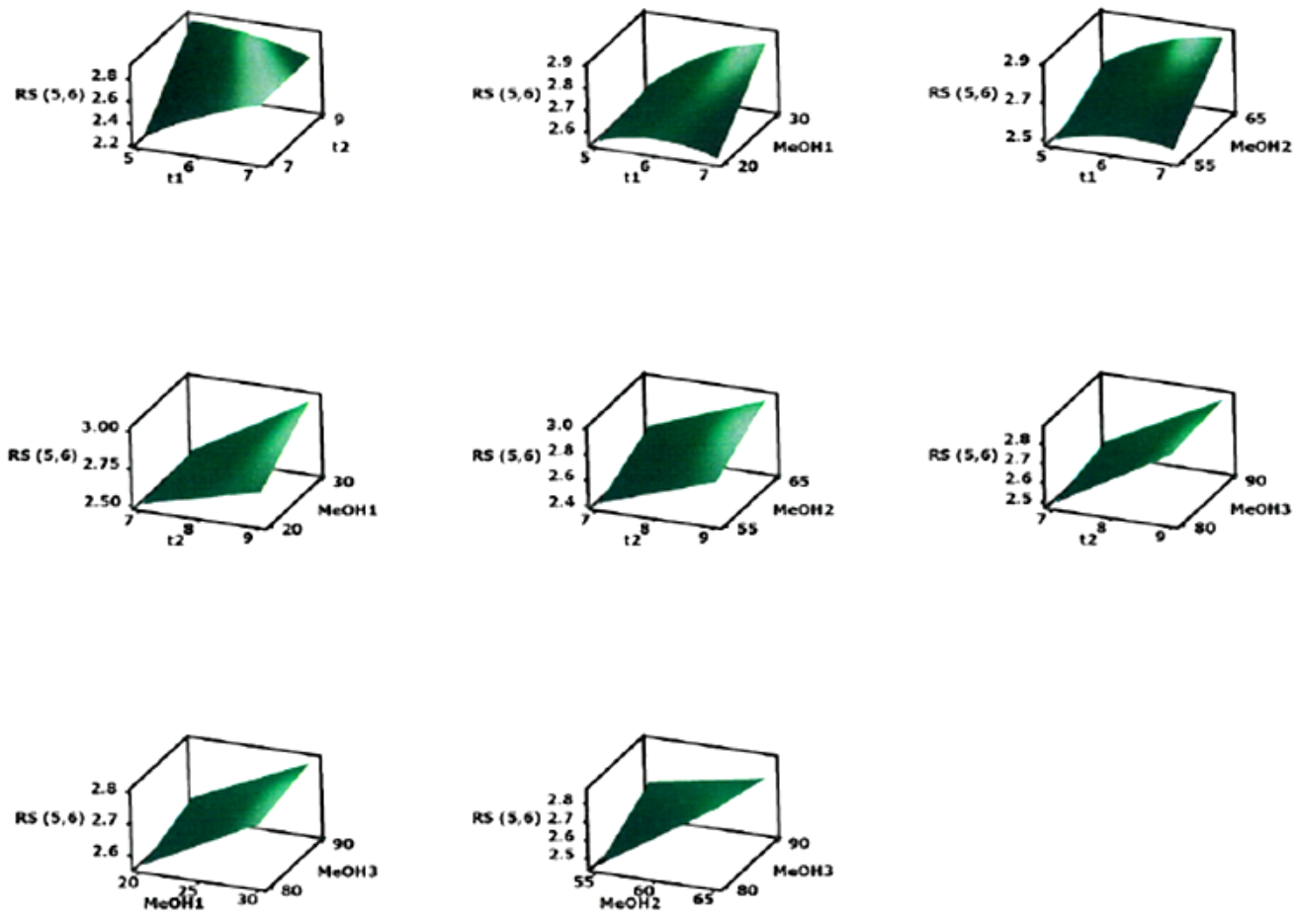


Figure 2.4: Response Surface Plots for Resolution between Toluene and Benzophenone

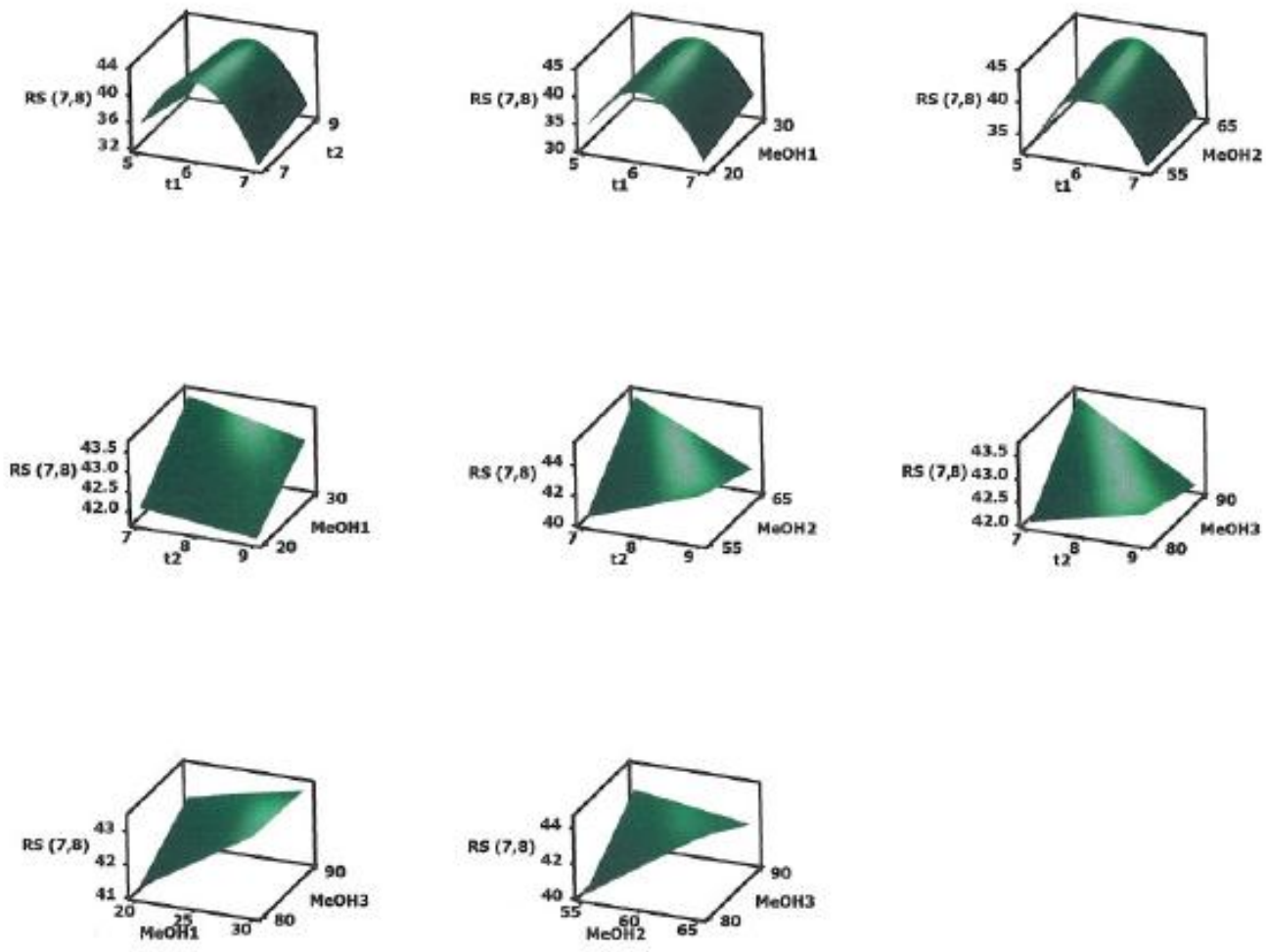


Figure 2.5: Response Surface Plots for Resolution between Phenyl Phenol and 2,4-ditertbutylphenol

While there is merit in evaluating each method parameter in isolation, a true optimization of the analytical separation can only be achieved by assessing all variables simultaneously. This approach accounts for the singular effects of adjusting any of the critical variables and the combination effect of that change with any other variations in the system e.g. a change in the initial concentration of methanol may have little impact on separation but if that change is combined with a modification to the gradient time, it may have a significant effect. To enable a global assessment of all the method parameters and allow for optimization of the analytical method an optimization plot was utilized. See Fig. 2.6 for the optimization plot for the separation of leachables.

Using the data generated from the DOE this tool allows the user to modify each method parameter and assess its impact on the separation system. Each point is modified so that it operates on a plateau of composite desirability ensuring that the method will operate within the centre of a design space while still maintaining optimal resolution between the critical pairs. Consequently, small variations in each of the settings which may occur from day-to-day, system-to-system or analyst-to-analyst variation will not adversely affect the resolution of the critical pairs. The red lines within the optimization plot which define the settings can be moved to the left or right (decreased or increased, respectively) and resolution is still maintained. The final optimized method parameters for each of the critical method variables as determined from the DOE and optimization plot are presented in Table 2.9. A comparison of the chromatography pre- and post- optimization is presented in Fig. 2.7. There is a 10% reduction in the run time of the analysis with acceptable resolution maintained for the critical pairs. This reduction in run time is of critical importance to a pharmaceutical company whereby real time data is required to support rapid response to Ministry of Health's or other government regulators. It is never a case that a single sample is analyzed within and QC or stability testing environment. Often there are at least 15-20 samples analysed in the one assay and as such the savings in efficiency can be significant when run time is minimised. The 10% reduction in run time in this instance is applicable to

both UPLC and HPLC operating conditions. There is further method assurance that the operational parameters exist within the centre of a design space. Consequently, the method is sufficiently robust to withstand additional external variation which may be associated with day-to-day, system-to-system or analyst-to-analyst factors without compromising the resolution of components. To support baseline resolution of 1.5 under normal operating conditions in different laboratories, it is expected to develop the method with resolution between the critical pairs to be no less than 2.0.[17]

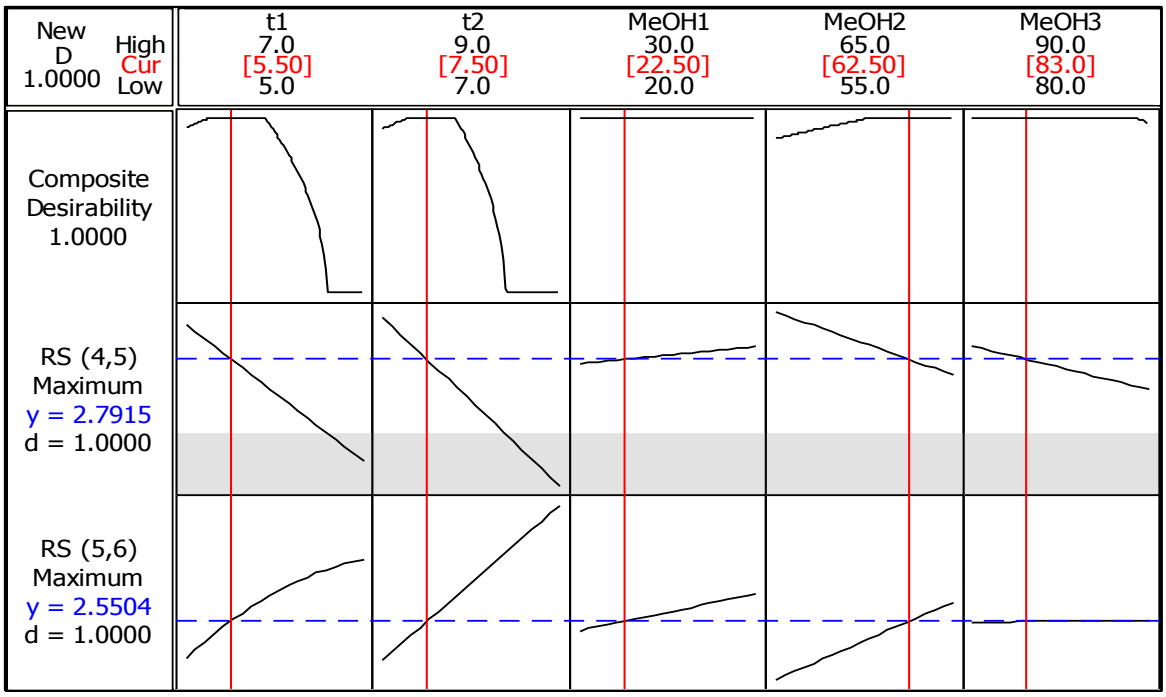


Figure 2.6: Optimization Plot for the UPLC Separation of Leachables.

Table 2.9: UPLC Optimal Gradient Program for the Separation of Eight Leachables

Minutes	%H_2O	%MeOH	Curve No.
Initial	77.5	22.5	*
5.50	37.5	62.5	6
7.50	17.0	83.0	6
7.60	77.5	22.5	6
10.0	77.5	22.5	6

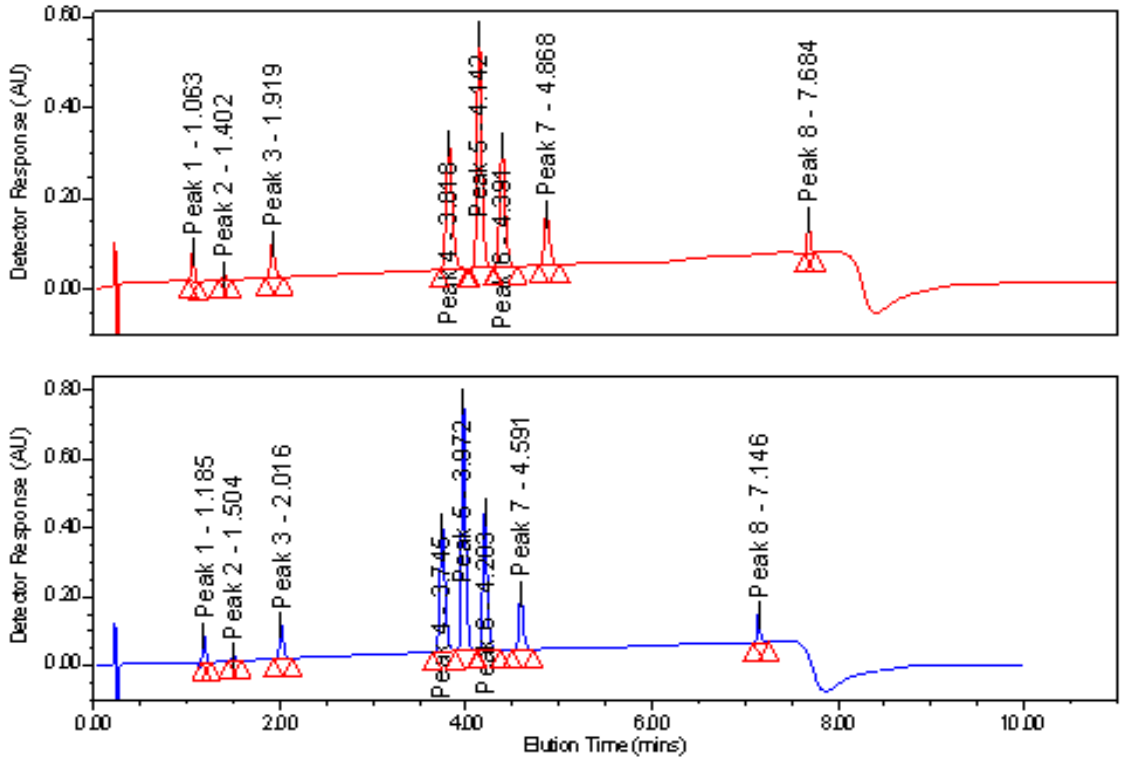


Figure 2.7: Top: UPLC chromatogram for the separation of 8 leachables before optimization Bottom: UPLC chromatogram for the separation of 8 leachables after optimization

The process capability (CpK) refers to a range of Key Performance Indicators (KPIs) that measure the ability of the method to deliver the resolution that the analyst requires. Process capability is a comparison between the total variation of the process and range of acceptability. The Lower Specification Limit (LSL) for the resolution parameters is 1.5 and the normal distribution in this case represents the variation of the method with regard to resolution. CpK values are used to determine the actual capability of the method using Eqn. 2.2

$$CpK = \frac{\text{LSL - Average Resolution}}{3\ \text{Sigma}} \qquad \textbf{(Eqn. 2.2)}$$

Typically a CpK value above 1.25 is acceptable for a one side specification and a CpK value above 2 is considered excellent as the specification window is twice as wide as the process variation. The resolution between diethyl phthalate and toluene is normally distributed and

centred on an average of 2.54. There is excellent repeatability with a standard deviation (sigma) of 0.011. The CpK value is 30.38 illustrating that short and long term method robustness is highly probable. The resolution between toluene and benzophenone is normally distributed and centred on an average of 2.83. There is excellent repeatability with a standard deviation of 0.007. The CpK value for the long term resolution of the toluene and benzophenone is 57.19. The resolution between these two critical pairs was previously identified as a measure of success for the development and optimization of the method. The part per million (ppm) total for both critical pairs is 0.0. This means that if the analytical test was run 1 x 10^6 times as described by the analytical procedure the resolution of the critical pairs would never fall below the baseline resolution of 1.5. This is critical for a QC laboratory whereby timelines and project milestones do not allow for invalid analytical assays due to poor resolution of chromatographic peaks. The data indicates that the method is centred in a design space of method robustness.

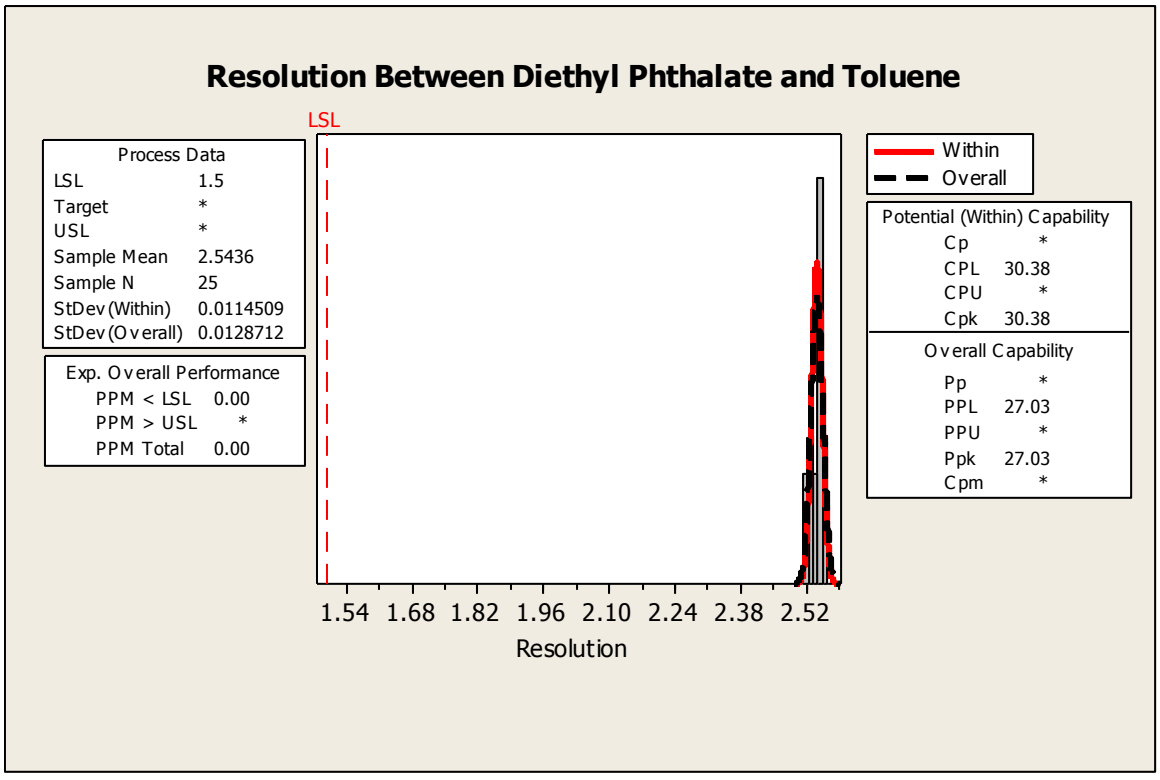


Figure 2.8: Process Capability graph for resolution between diethyl phthalate and toluene using optimized UPLC method

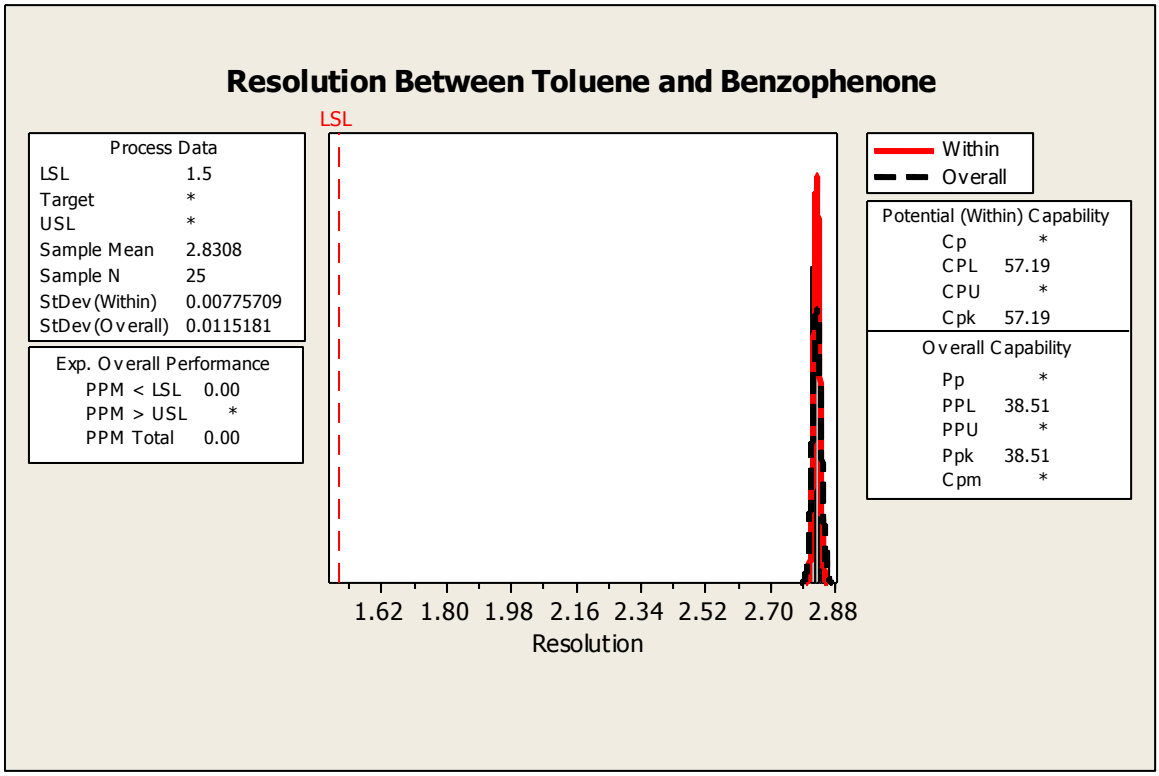


Figure 2.9: Process Capability graph for resolution between toluene and benzophenone using optimized UPLC method

The UPLC method was geometrically transferred to HPLC conditions using a Waters® calculator, whereby the method parameters of the UPLC assay were inputted into the data system such as column chemistry, length diameter, particle size, flow, and current gradient. Following the basic principles of the Van Deemter curve the program calculates the relevant length, diameter and particle size, flow and gradient profile under HPLC conditions. The geometric transformations can pose some problems especially when working with gradient methods as differences in void volumes within HPLC and UPLC methods can pose a lot of troubleshooting issues. Many routine stability testing laboratories would still be in the process of transitioning from the traditional HPLC systems to alternative technology which can operate at much higher pressures i.e. UPLC. The transformation includes changes to injection volume, run time, dwell volumes, injection volume, column technology and particle size of the packing.

Table 2.10: Optimal UPLC and HPLC Conditions.

Parameters	UPLC Conditions	HPLC Conditions
Pump flow rate	0.6 mL/min	1.4 mL/min
Detector	210 nm	210 nm
Column Temp.	45°C	45°C
Run time	11 min.	40 min.
Injection volume	2 µL	20 µL
Needle Wash	50% (v/v) Acetonitrile	50% (v/v) Acetonitrile
UPLC Column	Waters BEH C18	Waters XBridge C18
Column Dimensions	50 × 2.1 mm	100 × 4.6 mm
Particle Size	1.7 µm	3.5 µm

Table 2.11: Optimal Gradient Program for UPLC and HPLC Conditions

Minutes (UPLC)	Minutes (HPLC)	%A	%B	Curve No.
Initial	Initial	77.5	22.5	*
5.50	22.5	37.5	62.5	6
7.50	30	17.0	83.0	6
7.60	31	77.5	22.5	6
10.0	40	77.5	22.5	6

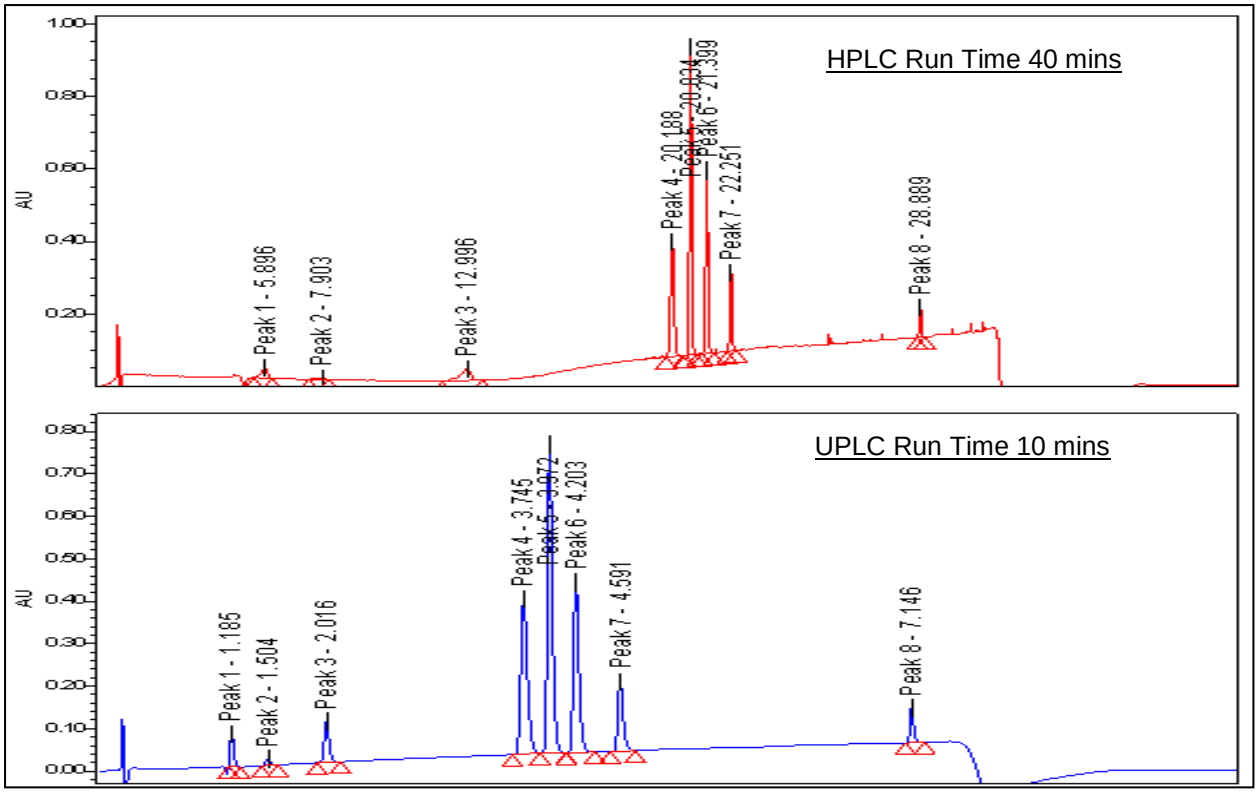


Figure 2.10: (a) HPLC chromatogram for leachables. Peak Identity: (1) Triacetin, (2) Darocur 1173, (3) Methylparaben, (4) Diethyl Phthalate, (5) Toluene, (6) Benzophenone, (7) Phenyl Phenol, (8) 2,4-Ditertbutylphenol.
b) UPLC chromatogram for leachables. Peak Identity: (1) Triacetin, (2) Darocur 1173, (3) Methylparaben, (4) Diethyl Phthalate, (5) Toluene, (6) Benzophenone, (7) Phenyl Phenol, (8) 2,4-Ditertbutylphenol.

2.3.4 LC-MS

Simple molecular ion spectra were recorded for the leachable components that yielded an effective response for the protonated molecular ion [M+H$^+$]. Table 2.12 summarises the parent and product ion transitions for each of the leachable components.

Table 2.12: Precursor and Product ions recorded for LC-MS of Leachables

Leachable	m/z	Precursor Ion	m/z	Product Ion
Triacetin	219	$[M+H^+]$	241	$[M+H^+]$ + [Na]
	219	$[M+H^+]$	159	$[M+H^+] – [C_2H_4O_2]$
	159	$[M+H^+] – [C_2H_4O_2]$	181	$[M+H^+] – [C_2H_4O_2]$ + [Na]
Methylparaben	153	$[M+H^+]$	122	$[M+H^+] – [CH_3O]$
	153	$[M+H^+]$	154	$[M+H^+] + [H^+]$
Daracur 1173	165	$[M+H^+]$	187	$[M+H^+]$+ [Na]
	165	$[M+H^+]$	147	$[M+H^+] – H_2O$
	165	$[M+H^+]$	119	$[M+H^+] – [C_2H_7O]$
	119	$[M+H^+] – [C_2H_70]$	91	$[M+H^+] – [C_2H_7O] – [CO]$
Diethyl Phthalate	223	$[M+H^+]$	245	$[M+H^+]$ + [Na]
	223	$[M+H^+]$	177	$[M+H^+] – [C_2H_5O]$
	177	$[M+H^+] – [C_2H_5O]$	149	$[M+H^+] – [C_2H_5O] – [C_2H_5]$
Benzophenone	183	$[M+H^+]$	206	$[M+H^+]$ + [Na]
	183	$[M+H^+]$	184	$[M+H^+]$ + [H]
	183	$[M+H^+]$	106	$[M+H^+]$ - $[C_6H_5]$
2,4-ditertbutylphenol	207	$[M+H^+]$		
Phenylphenol	171	$[M+H^+]$		

The MS signals for each leachable were generally due to loss of distinct groups from each structure. Simultaneously, addition of sodium adducts (23 mass units) to the parent molecule are also confirmation of the parent structure, as is the case for triacitin, darocur 1173, diethyl phthalate and benzophenone resulting in product ions of 241,187, 223 and 206, respectively. The molecular ion of triacetin loses an acetic acid group with a m/z 60, resulting in a product ion of m/z 159. This product ion binds with sodium to yield a further product ion of 181. The precursor ion of methylparaben loses a methoxide group (m/z 31) to yield a product ion of m/z 122. The product ion also exhibits double protonation with a signal at 154. Daracur 1173 loses a water moiety (18 mass units) from the parent ion of m/z 165. This parent ion also fragments by losing an ethoxonium ion to give a product ion of 119. Further subsequent loss of a carbonyl group affords the ion at m/z 91. The diethyl phthalate precursor ion (223) loses the ethoxide group into a product ion with m/z 177. This product ion further fragments with the loss of an ethyl group to give the new product ion with m/z 149. Benzophenone also exhibits double protonation and loss of a benzene group (m/z 77) to give the product ion at 106 m/z. The leachables with contain benzene rings exhibit π-π stacking and result in signals at much higher m/z regions than the molecular ion. This is particularly evident in the spectrum for daracur 1173, benzophenone and 2,4-ditertbutylphenol. The major challenge in the analysis of phenolic compounds is to attain the high sensitivity required for determination of these compounds, at ppm level, present in many pharmaceutical samples. Compounds with phenolic groups have been determined by a variety of methods including gas chromatography/MS (GC/MS) after solid phase extraction (SPE) and derivatization by trimethylsilation and HPLC with fluorescence detection.[18] Recently, LC electrospray ionization mass spectrometry (LC-ESI-MS) and tandem MS (LC-ESI-MS/MS) in the negative ion mode have been employed to analyze these compounds.[19] However, many phenols are not strong acids and therefore show low ionization efficiency in ESI-MS.

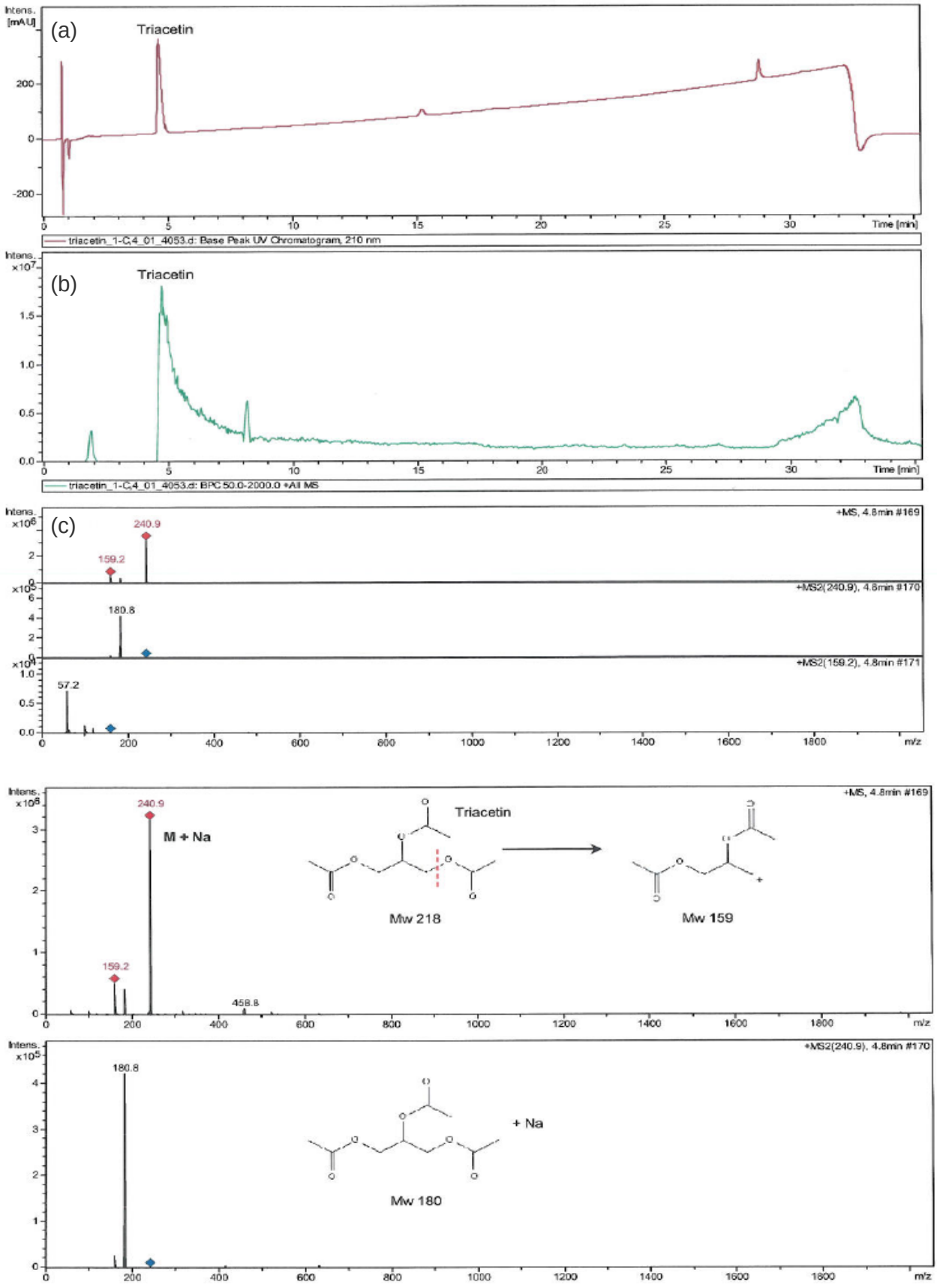

(a)
Triacetin
Intens. [mAU]
Time [min]
triacetin_1-C,4_01_4053.d: Base Peak UV Chromatogram, 210 nm
(b)
Triacetin
Intens. x10^7
Time [min]
triacetin_1-C,4_01_4053.d: BPC 50.0-2000.0 +All MS
(c)
+MS, 4.8min #169
240.9
159.2
+MS2(240.9), 4.8min #170
180.8
+MS2(159.2), 4.8min #171
57.2
m/z
+MS, 4.8min #169
240.9
M + Na
159.2
458.8
Triacetin
Mw 218
Mw 159
+MS2(240.9), 4.8min #170
180.8
+ Na
Mw 180

Figure 2.11: (a) UV Chromatogram, (b) Extracted Ion Chromatogram and (c) Mass Spectra of Triacetin.

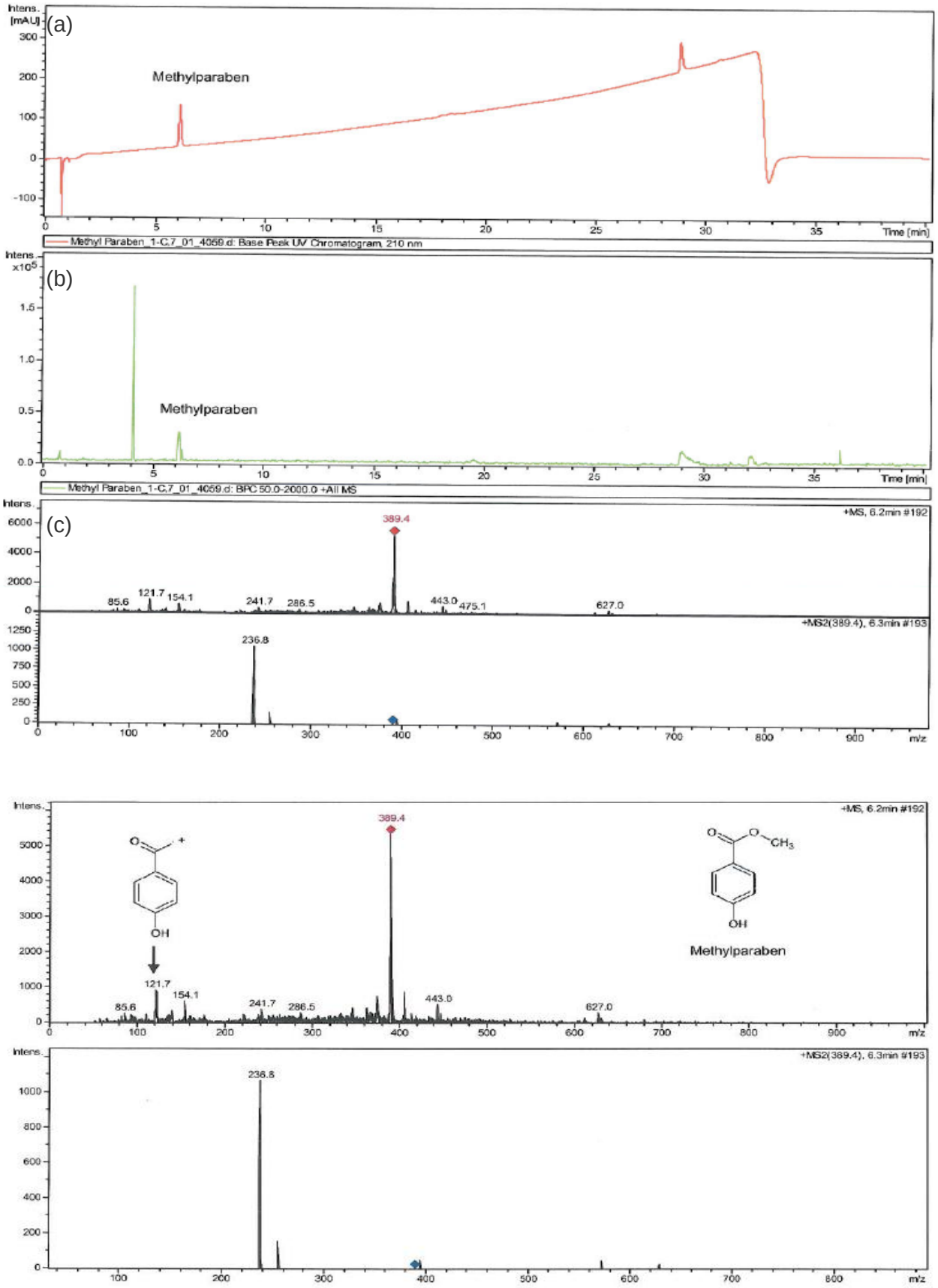


Figure 2.12: (a) UV Chromatogram, (b) Extracted Ion Chromatogram and (c) Mass Spectra of Methylparaben.

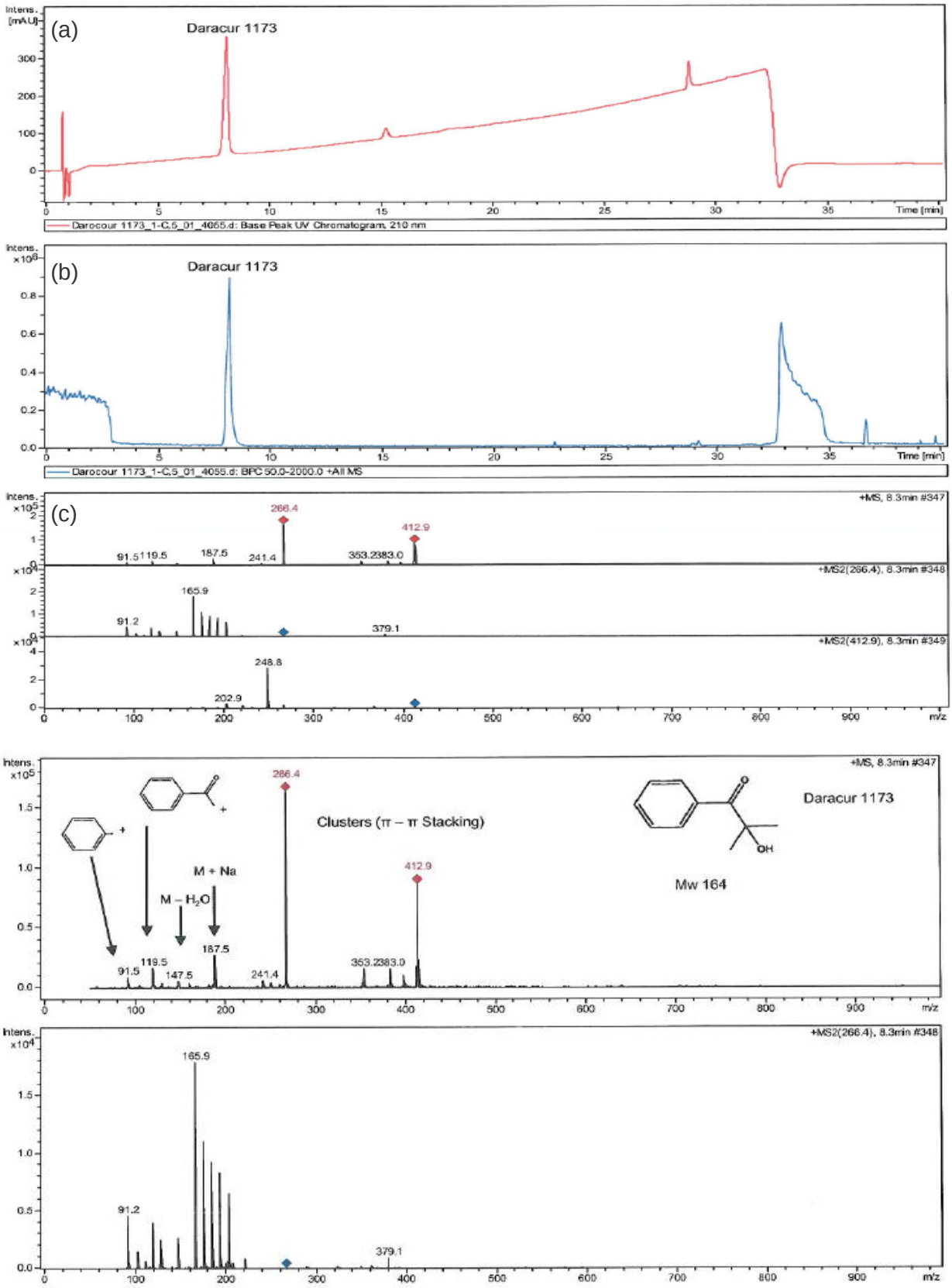


Figure 2.13: (a) UV Chromatogram, (b) Extracted Ion Chromatogram and (c) Mass Spectra of Daracur 1173.

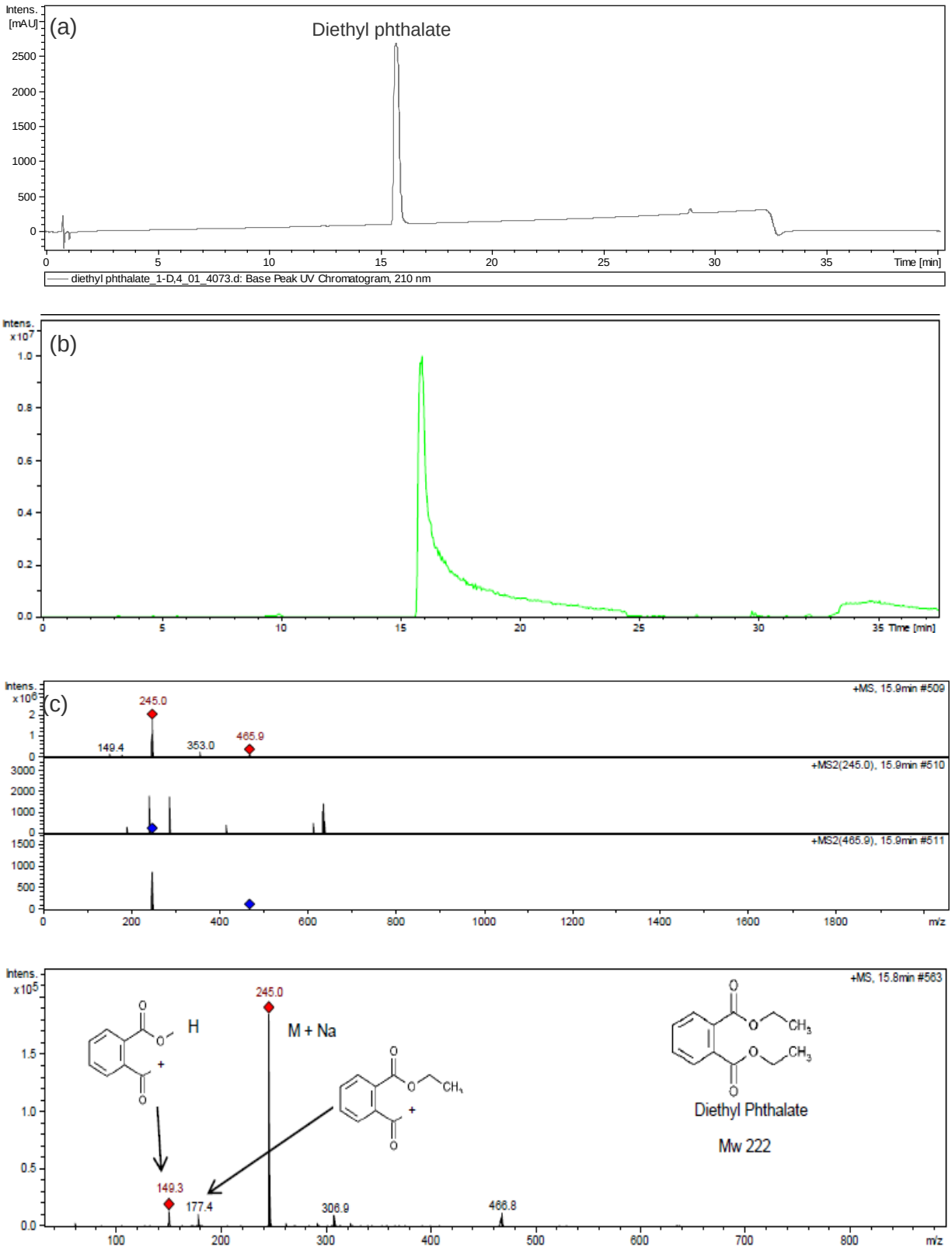


Figure 2.14: (a) UV Chromatogram, (b) Extracted Ion Chromatogram and (c) Mass Spectra of Diethyl phthalate

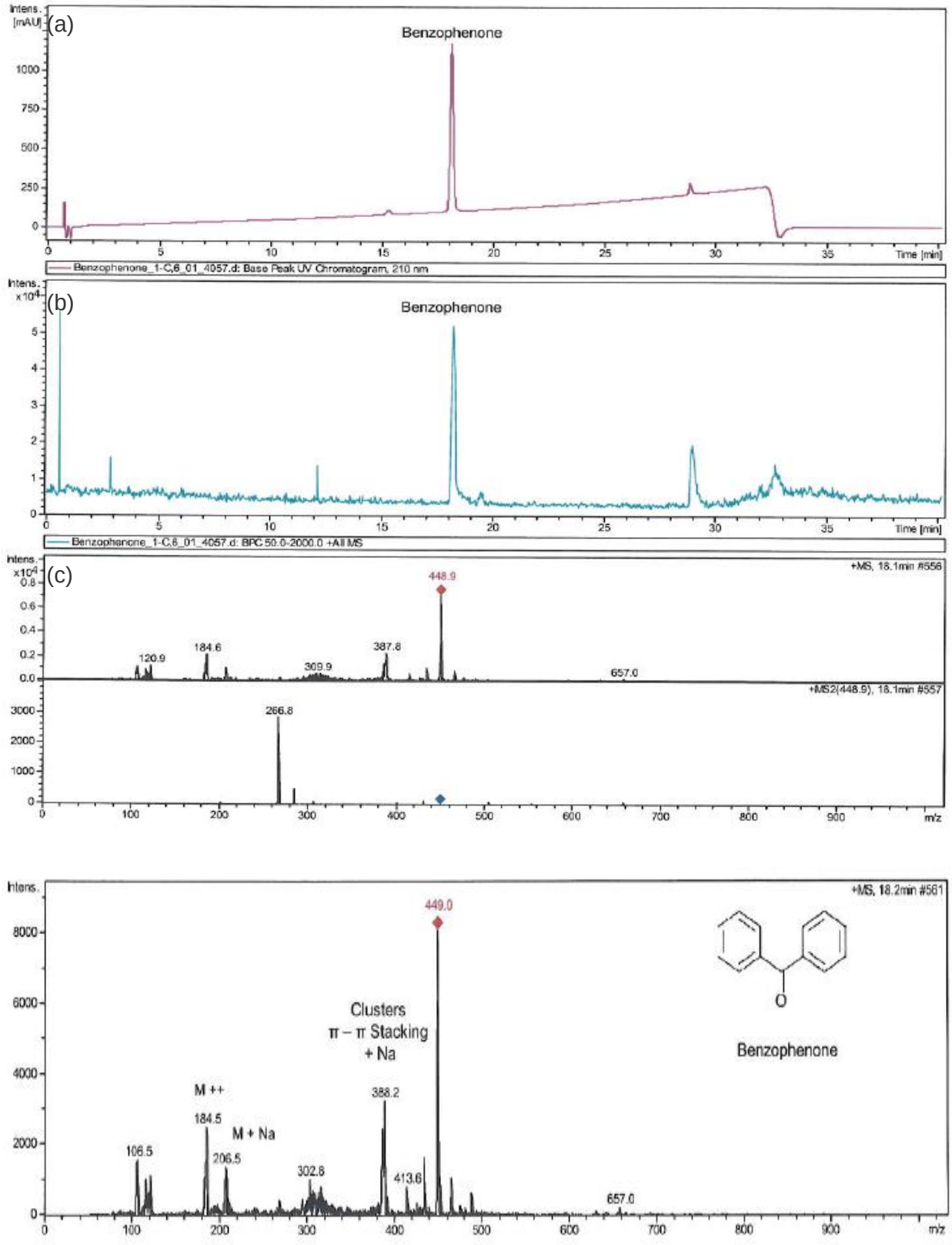


Figure 2.15: (a) UV Chromatogram, (b) Extracted Ion Chromatogram and (c) Mass Spectra of Benzophenone

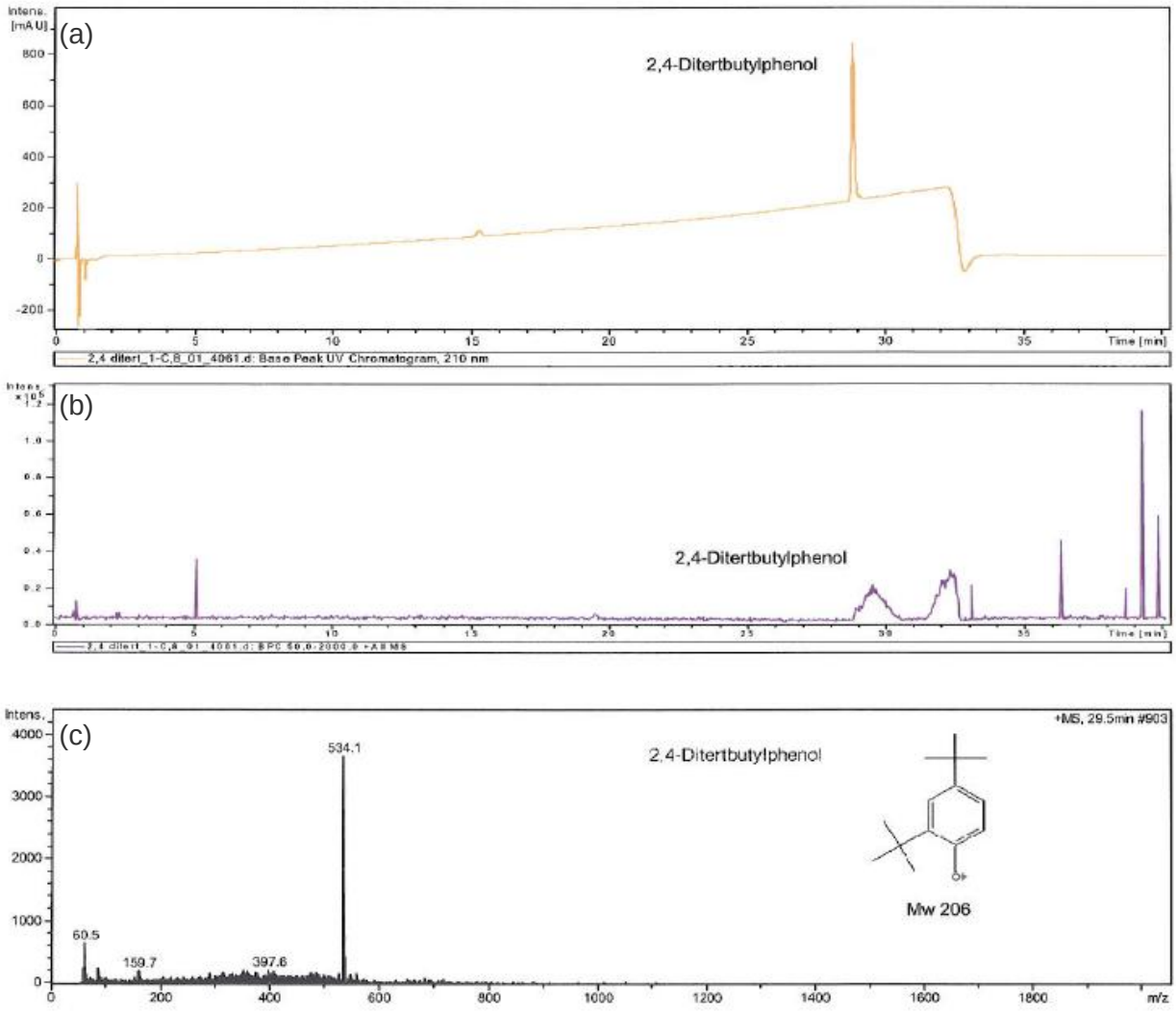


Figure 2.16: (a) UV Chromatogram, (b) Extracted Ion Chromatogram and (c) Mass Spectra of 2,4-Ditertbutylphenol

Conclusion

An UPLC method was developed for the separation of eight potential leachables from container closure systems for pharmaceutical products. The 8 leachables were separated in 11 min with baseline resolution for all components. The analytical method was further optimized using design of experiment (DOE) techniques. The critical parameters which affect resolution were identified and assessed using a two level fractional factorial design. Response surface plots and optimization plots were employed to optimize each of the 5 factors. The runtime of the analytical method was subsequently reduced by 9% while maintaining resolution between the critical pairs. The method was further validated using process capability analysis whereby long term and short term resolution of the critical pairs was proven to be sufficient with CpK values of 30.38 and 38.51. The optimized analytical method was geometrically transformed to HPLC operating conditions with comparable chromatography achieved. The same chromatography profile was achieved with acceptable baseline resolution of greater than 1.5. The potential leachables were analyzed using MS and structural identification confirmed for 5 of the compounds. However it was not possible to structurally identify 3 of the compounds as the phenolic structure meant poor ionization. Future work would involve examining a larger number of potential leachables using the optimised method. The analytical method should also be assessed for its application to detect and resolve leachables from active pharmaceutical ingredients and other final product excipients. Further MS work would be executed to ascertain suitable MS methodology for the 3 remaining leachables for phenolic compounds. A possible strategy for this analysis would be the derivatisation of the phenolic compounds with 1,2-dimethylimidazole-4-sulfonyl chloride (DMISC) to improve the ionisability of the compounds. .

References:

1. Fiercepharma, Top 15 Pharma R&D Budgets, available at http://www.fiercepharma.com/special-reports/top-15-pharma-rd-budgets
2. Forbes, The Truly Staggering Cost of Inventing New Drugs, available at http://www.forbes.com/sites/matthewherper/2012/02/10/the-truly-staggering-cost-of-inventing-new-drugs/
3. United States, Food and Drug Administration, New Drug Application (NDA), available to view at http://www.fda.gov
4. EBELING, M., 2011. 'Get with the Program!': Pharmaceutical marketing, symptom checklists and self-diagnosis. *Social Science and Medicine,* **73**(6), pp. 825-832.
5. GU, P., WILLIAMS, K.A., ASLANI, P. and CHAAR, B.B., 2011. Direct-to-consumer advertising of prescription medicines on the internet: An Australian consumer perspective. *Journal of Pharmacy Practice and Research,* **41**(3), pp. 196-202.
6. NABOKO, D., PETKOVA, V. and DIMITROVA, Z., 2008. Combination of marketing strategies for increase of anti-smoking drugs sales. *Journal of Medical Marketing,* **8**(2), pp. 144-150.
7. SWEET, M., 2009. Pharmaceutical marketing and the internet. *Australian Prescriber,* **32**(1), pp. 2-4.
8. HEUER, R., 1986. CHILD-RESISTANT CLOSURES ADDRESS SPECIFIC NEEDS. *Packaging Boston, Mass.,* **31**(6), pp. 32-34.
9. KALEIDO, L., 2003. Bright future for caps. *Packaging Magazine,* **6**(12), pp. 26.
10. FEILDEN, A., 2008. Extractables and leachables: Issues and challenges. *American Pharmaceutical Review,* **11**(3),.
11. <661> Containers – Plastics. The United States Pharmacopeia, USP 31; USP Convention , Inc.: Rockville, MD, 2008; 251-255
12. U.S. Department of Health and Human Services Food and Drug Administration Centre for Drug Evaluation and Research (CEDER); Rockville, MD, Container Closure Systems for Packaging Human Drugs and Biologics; Guidance for Industry; May 1999 1-41
13. Waters Mass Spectrometry on-line Primer, 2012, available to view at http://www.waters.com/waters/nav.htm?cid=10073244&locale=en_IE
14. OSTERBERG, R.E., 2005. Potential toxicity of extractables and leachables in drug products. *American Pharmaceutical Review,* **8**(2), pp. 64-67.
15. BEIERSCHMITT, W.P., 2009. Toxicology issues in extractables and leachables. *American Pharmaceutical Review,* **12**(6), pp. 122-126.
16. JENKE, D., GARBER, M.J. and ZIETLOW, D., 2005. Validation of a liquid chromatographic method for quantitation of organic compounds leached from a plastic container into a pharmaceutical formulation. *Journal of Liquid Chromatography and Related Technologies,* **28**(2), pp. 199-222.
17. <621> Chromatography. The United States Pharmacopeia, USP 31; USP Convention , Inc.: Rockville, MD, 2008; 251-255
18. Fatemeh S. Mirnaghi, Fatemeh Mousavi, Sílvia M. Rocha, Janusz Pawliszyn, Automated determination of phenolic compounds in wine, berry, and grape samples using 96-blade solid phase microextraction system coupled with liquid chromatography–tandem mass spectrometry, Journal of Chromatography A, Volume 1276, 8 February 2013, Pages 12-19, ISSN 0021-9673
19. Leandro N. Francescato, Silvia L. Debenedetti, Thiago G. Schwanz, Valquiria L. Bassani, Amélia T. Henriques, Identification of phenolic compounds in Equisetum giganteum by LC–ESI-MS/MS and a new approach to total flavonoid quantification, Talanta, Volume 105, 15 February 2013, Pages 192-203, ISSN 0039-9140

Chapter 3: Identification of Unknown Leachable in Ophthalmic Solution and Influence of Migration Rate

3.1 Introduction

Extractables are defined as compounds which can be extracted from the container/closure component during a Stage I testing process.[1] Stage I testing may utilize organic solvents, elevated temperature or other extraction techniques to aggressively extract compounds for materials qualification and investigational purposes. Extractable compounds may or may not be present in product stored in the approved container system under International Conference on Harmonization (ICH) environmental conditions.[2,3,4] Stage I Extractable Testing, for a given container/closure component or system, can be considered a one-time qualification which may serve as a reference database. Future products, utilizing the same container/closure component or system, may then refer to this database and associated technical reports to support its qualification for commercial use.

Leachables are compounds which are detectable in product when stored in the approved container system under ICH guidelines. Testing of leachables from product in approved container systems is known as Stage II Testing. This stage of testing consists of stability studies in the final marketed container closure system. Stage II testing requires that all regulatory product shelf-life specifications be monitored with validated analytical procedures and be conducted under approved protocols. This stage of testing is also known as a Product Compatibility Test.

Various analytical technologies have been used to try and identify these migrating species from the packaging components such as direct UV and photodiode array (PDA).[5,6,7] Currently, chromatographic techniques in tandem with mass spectrometry analysis are seen as the industry standard for the separation of leachables.[8,9,10] The volatility (or other physical characteristic) of the chemical species will ultimately determine the most appropriate method of analysis.[11,12] The relative low level concentration (ppb - low ppm range) of leachables within pharmaceutical formulations requires instrumentation with a

significant dynamic range of operation to detect these leachable species in the presence of the active pharmaceutical ingredient. The transition is being made from LC-UV detection of leachable components to LC-MS technology with the increased demand for qualitative analysis of these compounds for toxicological purposes. Jenke *et al.* have utilised LC-MS analytical techniques for Stage II testing to identify extractables such as caprolactam, p-toluenesulfonic acid, propionic acid and stearic acid associated with polyolefin containers at a concentration level of 0.2 ppm.[7]

Ophthalmic solutions typically consist of a mixture of salts and buffers to control pH and a natural polymeric material to serve as a wetting/lubricant agent on the eye. Multidose containers utilise a preservative agent within the formulation to maintain product integrity over the consumer shelf-life period, approximately 30 days. Alternatively, if the product is for single use only, then no preservative is necessary. Finally, if the ophthalmic solution is for the treatment of a medical indication, then an API will be incorporated into the formulation matrix. These ophthalmic solutions are typically stored in low density polyethylene (LDPE) containers. A pre-printed label is placed on the outside of the container using adhesive and then stored in a cardboard box with a pre-printed insert. A tamper evident seal is place on the carton using adhesive. 12 carton units are bound together using LDPE overwrap film and placed in cardboard shippers ready for market distribution. There is potential for leachables from plastic, adhesive and inks within this packaging design. This work presents a Stage I and Stage II testing study for an ophthalmic solution and associated packaging components and the identification of an observed leachable in the final product.

3.2 Experimental

3.2.1 Reagents and preparation of standards and samples

Analytical grade (all purity ≥ 99%) glyceryl triacetate, darocur 1173, toluene, benzophenone, diethyl phthalate, methylparaben, 2-phenylphenol and 2,4-ditertbutylphenol were purchased under license from Sigma-Aldrich (Poole, UK). The chemical structure of each drug is provided in Table 2.1.

All solvents were HPLC grade or higher. Acetonitrile and methanol were obtained from Labscan (Dublin, Ireland). Ultrapure water was obtained from a Millipore Milli-Q water purification unit (Millipore, Bedford, MA, USA) with specific resistance of 18.2 MΩ/cm or better. Analytical grade formic acid was purchased from BDH Chemicals (Poole, UK). All glassware utilised for the storage of leachable stocks and standards were silanised prior to the preparation to prevent the leachables from adhering to the glass surfaces. This was executed by rinsing the glassware with 10% dichlorodimethylsilane in dichloromethane. This was followed by rinsing with dichloromethane and rinsing with methanol twice each. Stock solutions of the potential leachables were prepared in methanol to a concentration of 200 ppm and were stored in the refrigerator at 5 ^{o}C ± 3 ^{o}C. Working solutions were prepared fresh before analysis in methanol.

3.2.2 Stage I Extraction Study

Final packaging components for an ophthalmic solution were extracted with appropriate solvent mixtures in accordance with USP <661> Plastic Containers.(REF) A list of the packaging components is provided in Table 3.1. Each packaging component was placed into silanised glass vials with Teflon screw caps containing 20 ml of extraction solvent (50:50, methanol:water) and stored at 70^{o}C for 48 h. The samples were vortexed after 24 h. The samples were removed from the oven and stored at 5 ± 3^{o}C until time of analysis. A control sample consisting of extraction solvent (50:50 methanol:water) was stored under identical conditions as the test samples to eliminate any system related or extraction solvent related observations.

Table 3.1: List of Packaging Components for Extraction

Carton Related	***Label Related***	***Adhesive Related***	***Ink Related***
Blank Carton	Bottle Label	Label Adhesive	Carton Ink Yellow
Blank Carton Insert	Blank Bottle Label	Carton Adhesive	Carton Ink Magenta
Carton Flood-Coat	Label Ink		Carton Ink Cyan
Tape Seal			Carton Ink Black
Printed Carton			Label Ink
Carton Insert			

3.2.3 Stage II Leachable Study

A batch of the ophthalmic formulation was manufactured, filled and packaged in its final configuration. This test sample was named as “Batch”. A quantity of the same batch of final product was filled into glass vials which acted as control samples for final analysis to identify leachable artefacts. This glass control sample was named as “Batch-GL”. Simultaneously, a quantity of the batch was filled into the low density polyethylene bottles. These bottles were unlabelled and no other packaging component was used on the bottles. These served as additional controls during analysis. This unlabelled control sample was named as “Batch-

UL". The test samples and associated controls were stored at three different conditions and tested at various time points.

One set of samples and associated controls were placed in a stability chamber set at 25 ± 2°C and 40 ± 5% relative humidity (RH) and tested at the time points outlined in Table 3.2.

Table 3.2: Stage II Testing Schedule at 25°C ± 2°C and 40% ± 5% RH

		Time Point (months)				
Test	**Initial**	**3**	**6**	**9**	**12**	**18**
HPLC Leachable	X	X	X	X	X	X
Test samples	Batch, Batch-GL & Batch-UL at each timepoint					

A second set of samples and associated controls were placed in a stability chamber set at 30°C ± 2°C and 25% ± 5% relative humidity (RH) and tested at the time points outlined in Table 3.3.

Table 3.3: Stage II Testing Schedule at 30°C ± 2°C and 25% ± 5% RH

		Time Point (months)				
Test	**Initial**	**3**	**6**	**9**	**12**	**18**
HPLC Leachable	X	X	X	X	X	X
Test samples	Batch, Batch-GL & Batch-UL at each timepoint					

The final set of samples and associated controls were placed in a stability chamber set at 40°C ± 2°C and 20% ± 5% relative humidity (RH) and tested at the time points outlined in Table 3.4.

Table 3.4: Stage II Testing Schedule at 40°C ± 2°C and 20% ± 5% RH

		Time Point (months)			
Test	**Initial**	**1**	**2**	**3**	**6**
HPLC Leachable	X	X	X	X	X
Test samples	Batch, Batch-GL & Batch-UL at each timepoint				

3.2.4 Liquid Chromatography

Chromatographic separations were performed using a Waters Alliance HPLC with a vacuum degasser, quaternary solvent manager, autosampler and UV detector. Separations were performed using a Waters Sunfire® C18, 100 × 4.6 mm i.d., 3.5 µm column. A multistep gradient was employed with mobile phases of (A) water/acetonitrile (90/10, v/v) containing 0.03% perchloric acid and (B) acetonitrile with 0.03% perchloric acid. Gradient conditions were 100% mobile phase A for 5 min with an increase of mobile phase B to 30% over 11 minutes. There was a hold in the gradient for 10 min with a subsequent increase in the mobile phase B concentration to 80% over the next 17 min. A final clean up step was employed by increasing the mobile phase B concentration to 100% for 3.9 min hold. The initial gradient conditions were reemployed at 47.1 mins at re-equilibrate to a total run time of 53 min. The nominal Alliance HPLC conditions are described in Table 3.5 and the Gradient Program is described in Table 3.6.

Table 3.5: HPLC Conditions for Leachable Analysis

Pump flow rate	1.5 mL/min
Detector	210 nm
Column Temp.	30°C
Run time	53 min
Injection volume	50 µL
Needle Wash	Acetonitrile/Water (90/10, v/v)
HPLC Column	Sunfire C18
Column Dimensions	100 × 4.6 mm
Particle Size	3.5 µm

Table 3.6: Gradient Program for Leachable Analysis

Minutes	%A	%B	Curve No.
Initial	100	0	*
5.0	100	0	6
16	70	30	6
26	70	30	6
43	20	80	6
43.1	0	100	6
47	0	100	6
47.1	100	0	6
53	100	0	6

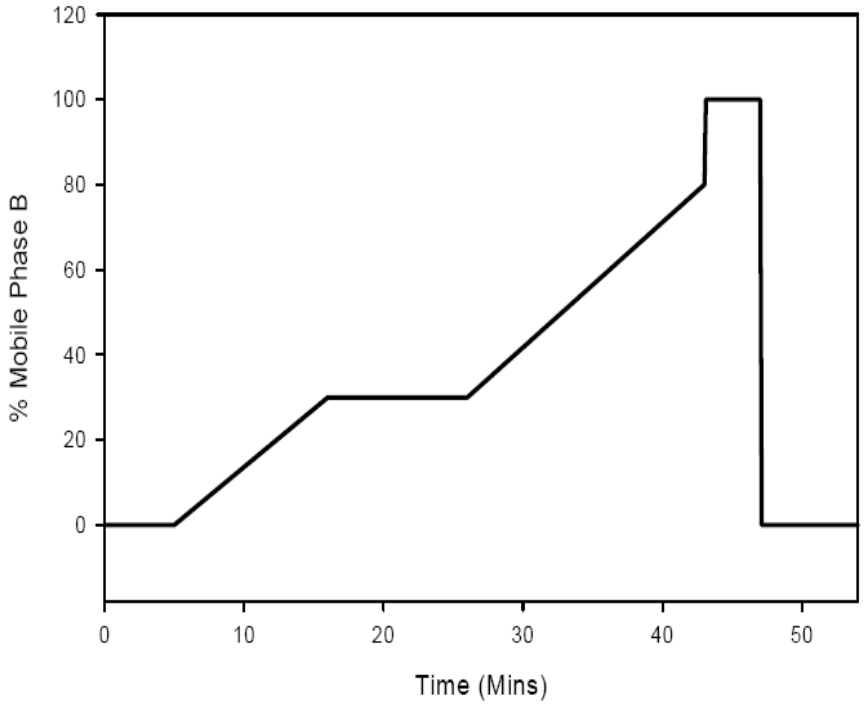


Figure 3.1: Gradient profile for HPLC analytical method

3.2.4.1 Benzophenone Stock Standard (250 ppm)

25 mg of benzophenone reference standard was quantitatively transferred into a 100 mL volumetric flask. 75 mL of acetonitrile was added to the flask and mixed using a magnetic stirrer for 1 h. The stir bar was removed and the volumetric brought to volume with acetonitrile. The stir was re-introduced to the solution and mixed for a further 20 min. The stock standard was stored under ambient laboratory conditions.

3.2.4.2 Benzophenone Working Standard (5 ppm)

Using a Class A pipette, 2 mL of the benzophenone stock standard was transferred into a 100 mL volumetric flask and brought to volume with a diluent of water/acetonitrile (90/10, v/v). The solution was mixed for 20 min using a magnetic stir bar. The working standard solution was stored under ambient laboratory conditions. This standard was used a bracketing standard to quantitate unknown leachables during the Stage II testing.

3.2.4.3 Benzophenone Detector Sensitivity Solution (DSS) (0.1 ppm)

Using a Class A pipette, 2 mL benzophenone working standard was transferred into a 100 mL volumetric flask and brought to volume with a diluent of water/acetonitrile (90/10, v/v) and mixed using a magnetic stir bar for 20 min. The DSS solution was used to confirm that the system was capable of quantitating low level leachables in the part per million range.

3.2.4.4 Blank

A vial of the water/acetonitrile (90/10, v/v) diluent used to dilute the standard and sample solutions was transferred into an amber HPLC vial and analysed simultaneously with the standards and samples. This blank was used to identify any system related peaks unrelated to the sample.

3.2.4.5 Sample Preparation

Samples were pooled into amber glass jars to protect from light to a total volume of 30 mL. The samples were vortexed gently to ensure homogeneity. Using a Class A pipette, 1 mL of test sample was transferred to a 10 mL amber volumetric flask and brought to volume with a diluent of water/acetonitrile (90/10, v/v). The test solutions were mixed using magnetic stir bars for 20 min at ambient laboratory conditions. Each test sample was prepared in duplicate. In order for any peaks in a specific final container closure configuration to be considered a leachable, it must have been observed in both preparations. This eliminated spurious, trace level peaks that were not consistently observed which could be due to trace - level glassware contaminates. The associated glass and unlabelled controls were prepared in the same manner and were analyzed with the test sample. If a peak at the same retention time was observed in the control sample and the final packaged batch, and their differences in concentration was greater than 30%, a subtraction procedure was used to quantify leachable peaks. If the difference was less than 30% then the peak was not deemed a leachable and was final product related.

3.2.5 Liquid Chromatography – Mass Spectrometry

Chromatographic separations were performed on an Agilent 1100 series HPLC with a liquid degasser, binary pump, ALS autosampler, and diode array detector. The LC was coupled to a Bruker Daltonics Esquire-LC ESI-ion trap MS. Agilent Chemstation version A.09.03 (Agilent Technologies, USA) and Bruker Daltonics esquire control version 4.0 (Bruker Daltonics, UK) were employed to control the system and data analysis was performed using Bruker Data Analysis 2.0 (Bruker Daltonics, UK). Separations were performed on a Waters XBridge^{TM} C18 100 × 4.6 mm, 3.5 µm column. A multistep gradient was employed with mobile phases of (A) water with 1% (v/v) formic acid and (B) methanol with 1% (v/v) formic acid. Gradient conditions were 77.5% mobile phase A with an increase of mobile phase B to 62.5% over 22 minutes. The mobile phase B concentration was increased to 85% over the

next 8 min (total 30 min). The initial gradient conditions were reemployed at 32 mins at re-equilibrate to a total run time of 40 min.

Table 3.7: ESI-MS Operating Parameters

Parameter	Positive Mode
Capillary Voltage	4500
End Plate Offset (V)	-500
Skimmer 1 (V)	40.0
Cap. Exit Offset (V)	117.3
Octopole 1 (V)	12.0
Octopole 2 (V)	1.70
Octopole RF (Vpp)	162.2
Lens 1 (V)	-5.0
Lens 2 (V)	-60.0
Trap Drive (Arbitrary units)	37.3
Dry Gas Flow (N_2; L/min)	10.0
Nebulizer Pressure (psi)	25.0
Dry Gas Temp. (^oC)	340
Scan (m/z)	50 – 2000
MS (n)	Auto MS(2)
No. of Precursor Ions	2

3.2 Results and Discussion

3.2 Stage I Extraction

To investigate the potential extractables which may migrate into the extraction medium, the analytical chemistry aspect of the investigation necessarily drives the type of extraction testing methodology which is selected. The overall goal of such an investigation is to separate and identify unknown extractables which are, most probably, present in low concentrations (e.g. low ppm) within the container/closure systems. HPLC, however, may detect many compounds relating to the extraction medium. Unfortunately, compounds from the extraction medium are present in high concentrations relative to the extractable compounds which may migrate into it. The extraction medium, therefore, poses a significant potential interference to the detection of ppm concentrations of extractables. To overcome this potential obstacle, extraction media must be chosen to minimize their interference with the detection of extractables. For these reasons an analytically ‘clean’ extraction medium must be chosen for the detection of extractables in Stage I testing. Hence a 50:50 methanol:water analytically clean extraction medium was chosen to mimic the solubility characteristics of ophthalmic formulations and not pose an interference to MS studies.

Table 3.8: Individual Unidentified Leachables Detected from the Extracted Packaging Components

Packaging Component	Leachable*	Average Peak Area (n=2)
Carton Related		
Blank Carton	Not Detected	Not Detected
Blank Carton Insert	Not Detected	Not Detected
Carton Flood-Coat	Not Detected	Not Detected
Tape Seal	IUL (0.93)	3.42×10^6
Printed Carton	IUL (0.93)	2.08×10^6
Carton Insert	IUL (0.93)	5.61×10^5
Label Related		
Bottle Label	IUL (0.93)	1.01×10^6
Blank Label	Not Detected	Not Detected
Adhesive Related		
Label Adhesive	Not Detected	Not Detected
Carton Adhesive	Not Detected	Not Detected
Ink Related		
Carton Ink Yellow	Not Detected	Not Detected
Carton Ink Magenta	Not Detected	Not Detected
Carton Ink Cyan	Not Detected	Not Detected
Carton Ink Black	Not Detected	Not Detected
Label Ink	Not Detected	Not Detected

* IUL is an Individual Unidentified Leachable with a relative retention time when compared to the benzophenone working standard.

High levels of an Individual Unidentified Leachable (IUL) at relative retention time 0.93 were observed from the tape seal. The tape seal is a tamper evident seal which is placed on the outside of the final product container to alert the customer if the product has been opened

previously. The printed carton also contained the aforementioned IUL(0.93) upon extraction. The IUL was not detected in the blank carton or any of the carton inks. IUL(0.93) was detected in the carton insert but was not detected in the blank carton insert or associated inks. Finally, IUL(0.93) was extracted from the bottle label but was not detected in the blank label or associated inks. This indicates that the offending IUL has migrated from the tape seal through the outer packaging component of the carton to contaminate the carton, carton insert and bottle label.

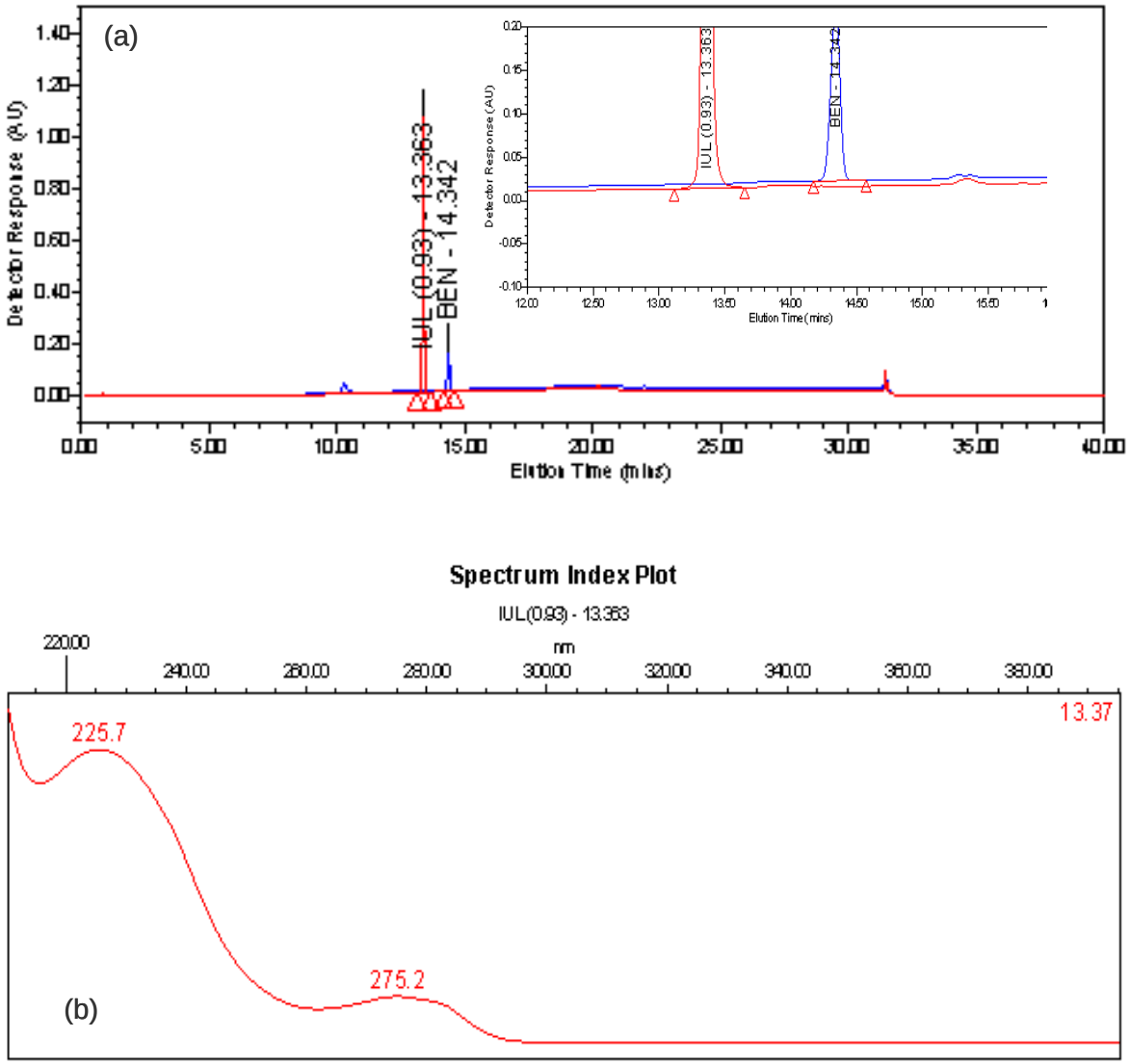


Figure 3.2: (a) HPLC overlay of tape seal extraction and benzophenone working standard.(b) Photodiode array spectrum of leachable IUL (0.93) extracted from tape seal

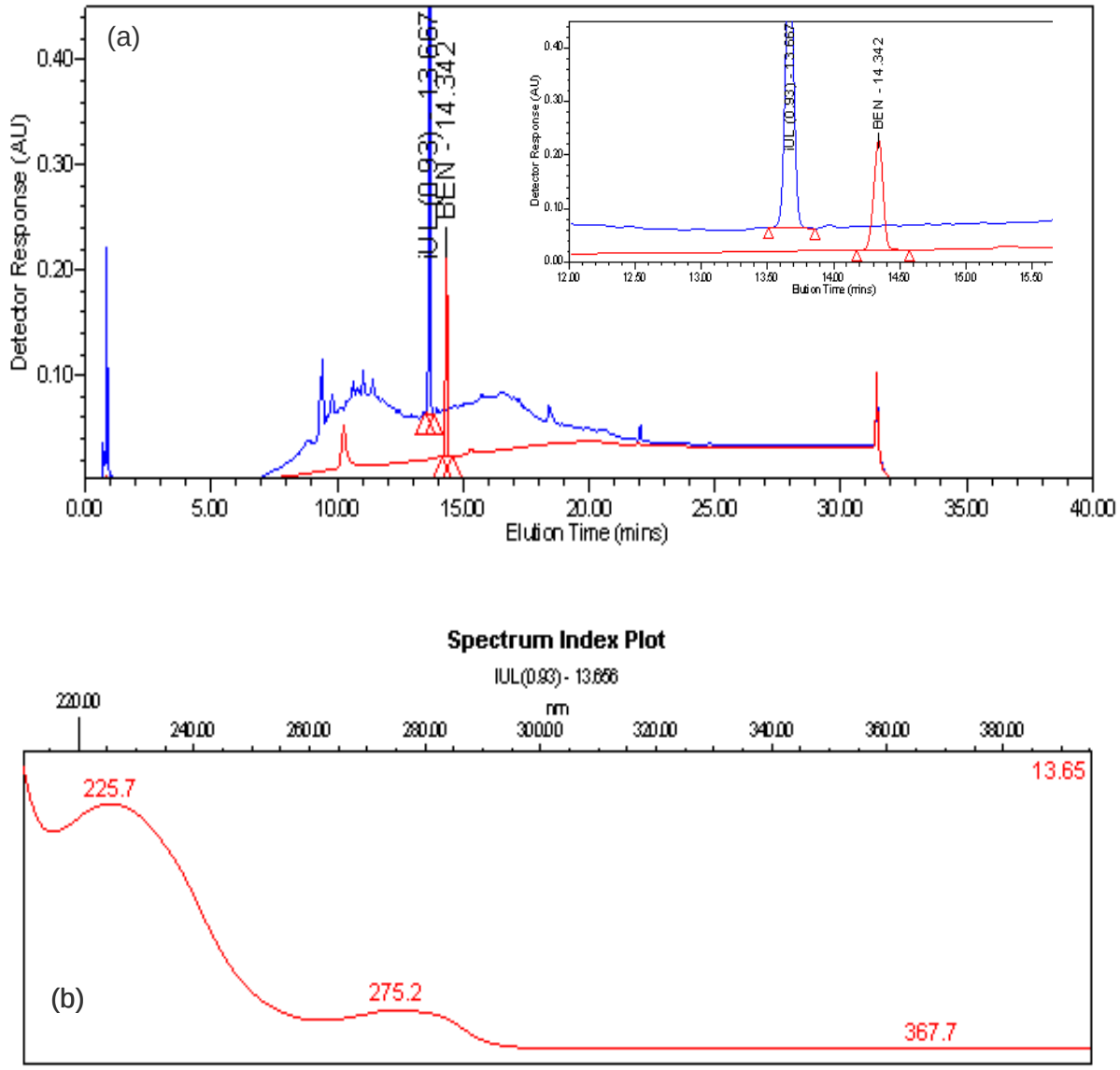


Figure 3.3: (a) HPLC overlay of carton extraction and benzophenone working standard. (b) Photodiode array spectrum of leachable IUL (0.93) extracted from carton

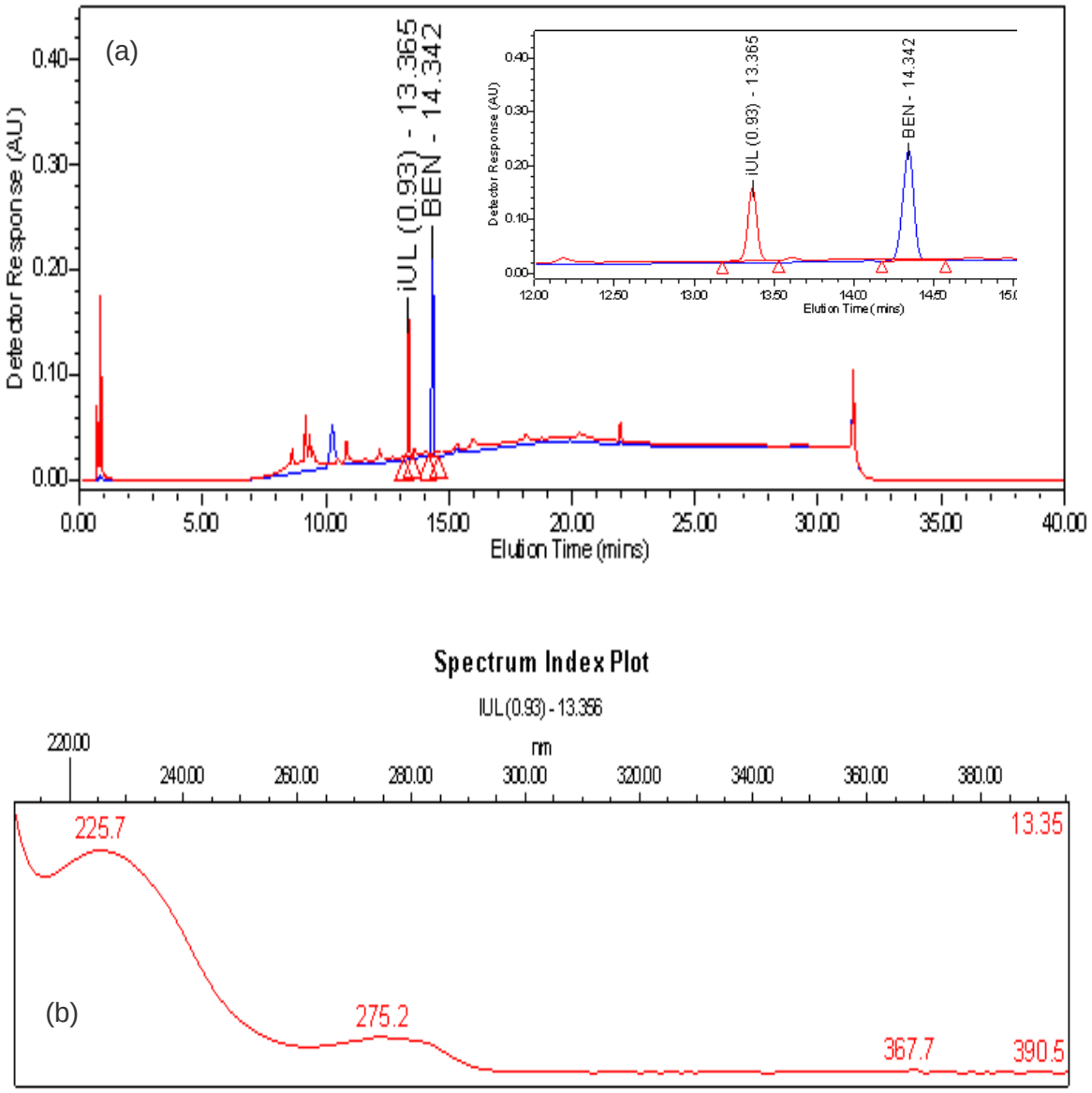


Figure 3.4: (a) HPLC overlay of carton insert extraction and benzophenone working standard.
(b) Photodiode array spectrum of leachable IUL (0.93) extracted from carton insert

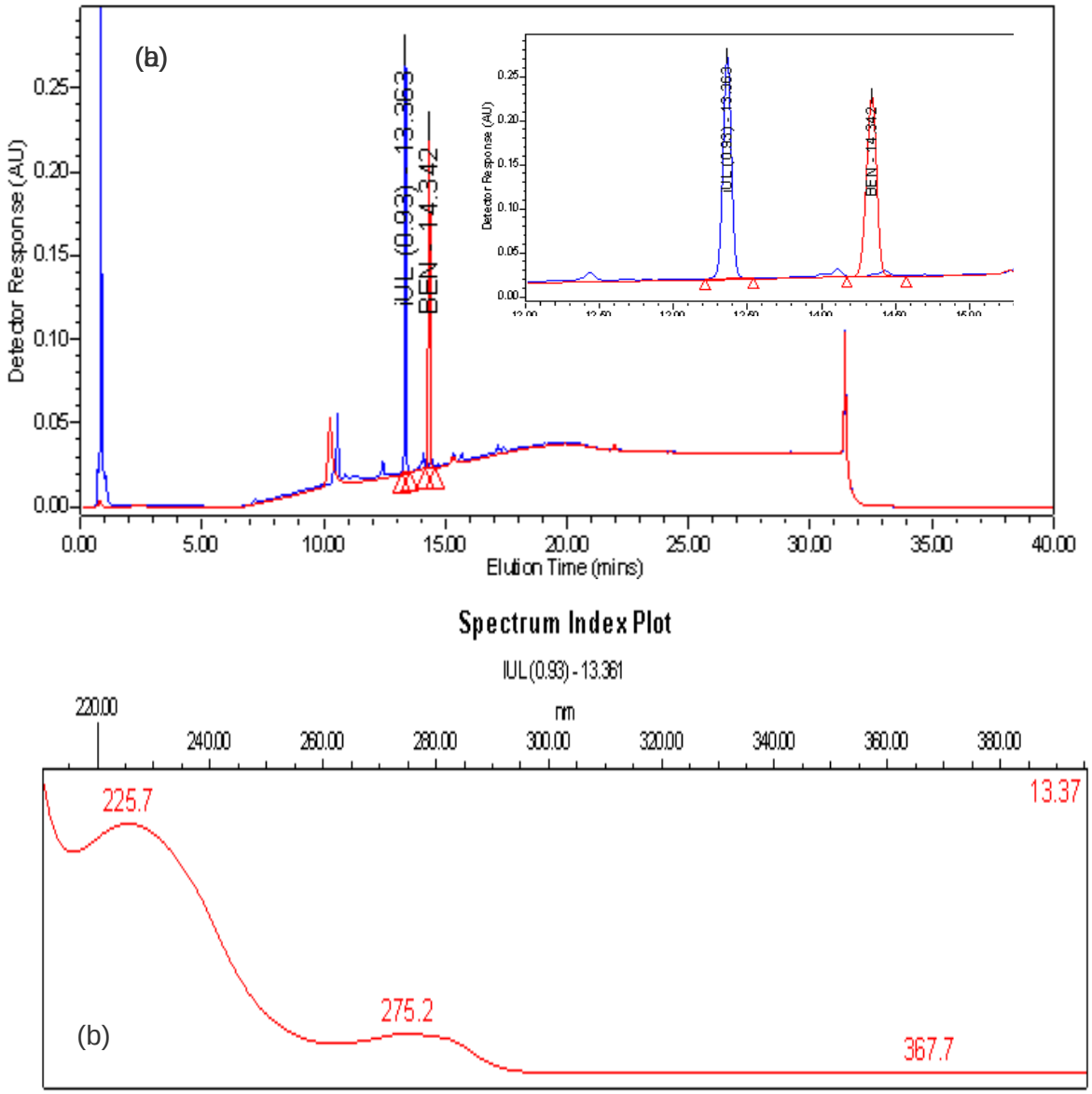


Figure 3.5
(a) HPLC overlay of bottle label extraction and benzophenone working standard.
(b) Photodiode array spectrum of leachable IUL (0.93) extracted from bottle label.

3.3 Stage II

IUL (0.93) was observed in all ophthalmic solutions stored in the final packaging configuration at the storage conditions for the routine, intermediate and accelerated stability storage conditions. The chromatographic peak named as IUL (0.93) was not observed in either the glass control or unlabelled control samples. Therefore, the resulting peak is a consequence of a leachable from the container closure system and is not directly related to any of the raw materials or reaction by-products of the ophthalmic solution itself. The HPLC profile from the stability studies is consistent with the Stage I extraction profile of the tamper evident seal, as it elutes an extractable/leachable peak at a relative retention time of 0.93 when compared to a benzophenone working standard. The UV spectra of IUL (0.93) from the Stage I extraction study and IUL (0.93) from the Stage II leachable study are a direct match with λ_{max} = 225.7 nm and a secondary chromaphore at 275.2 nm. This indicates that the source of the leachable is a constituent of the tamper evident seal.

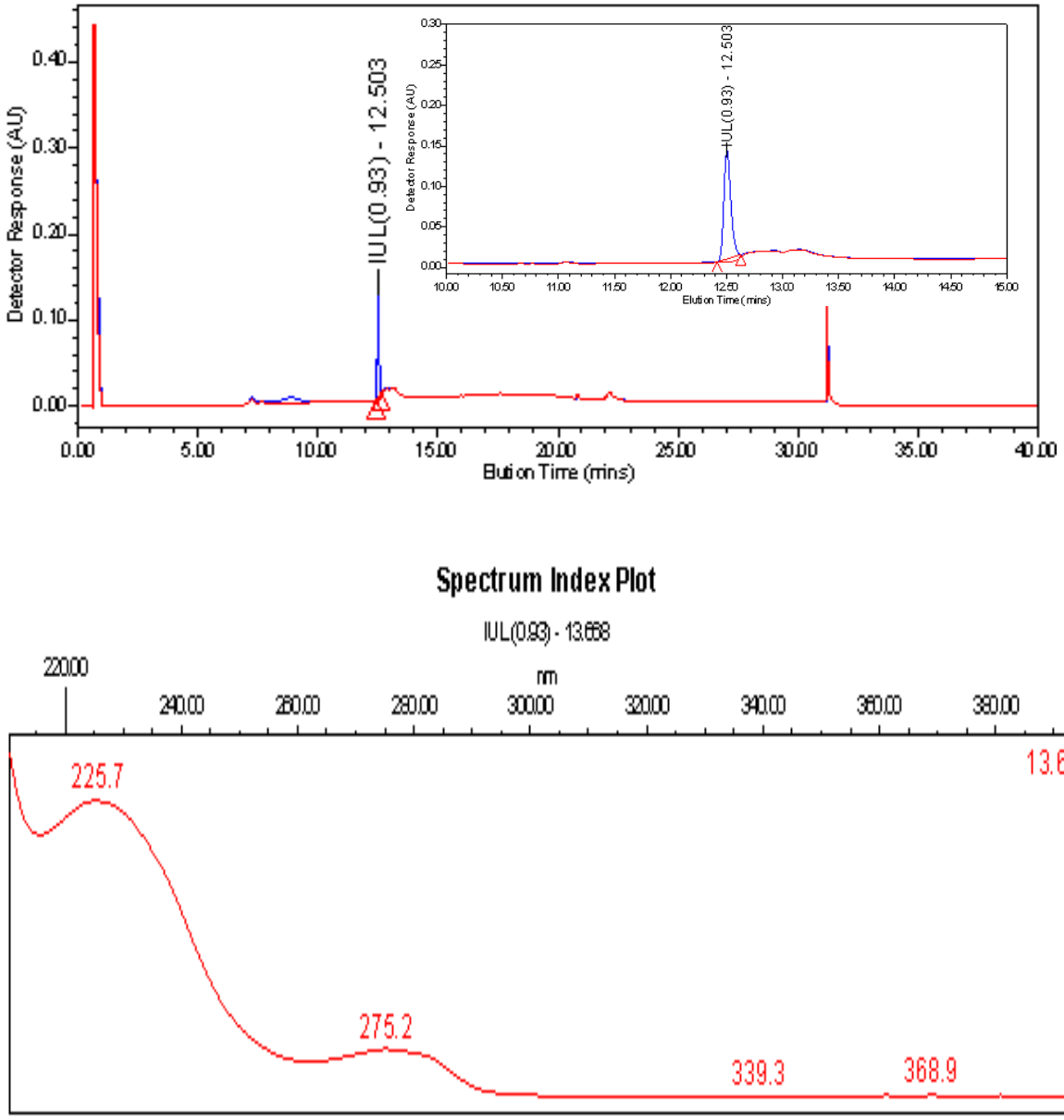


Figure 3.6: (a) HPLC overlay of final product and associated glass control which had been stored at 25°C and 40% RH for 12 months.
(b) Photodiode array spectrum of leachable IUL (0.93) found in final product formulation which had been stored at 25°C and 40% RH for 12 months.

The concentration of leachable within the ophthalmic solution increased with time across each storage condition and began to plateau at approximately 8 ppm. This indicates that maximum intake of the unidentified leachable to the end consumer would potentially be less than 10 ppm which is below the guidelines of not more than 10 ppm as stipulated by the US FDA. The data for each of the storage conditions was evaluated using linear regression analysis to ascertain the effect that the storage condition has on the rate of leaching. See Table 3.10 for linear equations. The rate of leaching is determined by the slope of the regression line. The slope in all 3 cases is positive indicating an increase in the concentration of leachable with time. The routine (25^{o}C/40% RH), intermediate (30^{o}C/75% RH) and accelerated conditions (40^{o}C/25% RH) afforded slopes of 0.25, 0.43 and 0.93, respectively. The highest slope of 0.93 for the accelerated condition indicates that the rate of leaching is dependent upon temperature i.e. as the temperature is increased the migration rate of the unidentified leachable increases. Therefore it can be concluded that the concentration of IUL (0.93) within the ophthalmic solution is dependent on the storage temperature and the length of time the product is exposed to those conditions. This theory is further substantiated by the execution of ANOVA (Analysis Of Variance). The statistical analysis show that variation in the concentration of the unidentified leachable with the formulation is significantly impacted by timepoint and condition i.e. Prob > F is less than 0.05 for 95% confidence. Approximately, 54% of the concentration of leachable is dependent on the age of the product, 20% is dependent on the storage condition and 9% is the dependent on the interaction between the two. The final 17% of variation in leachable concentration is dependent on with sample variation or variation of the analytical method. Reference Table 3.12 for partition of variation analysis.

Table 3.9: Results for IUL(0.93) in Batches Maintained at Various Storage Conditions

Sample Condition	Timepoint (months)	Replicate A (ppm)	Replicate B (ppm)	Glass Control (ppm)	Unlabelled Control (ppm)
Batch-25	0	0.00	0.00	0.00	0.00
	3	1.03	1.00	0.00	0.00
	6	2.30	2.67	0.00	0.00
	9	3.50	3.35	0.00	0.00
	12	2.79	5.74	0.00	0.00
	18	4.20	4.51	0.00	0.00
	24	6.12	5.34	0.00	0.00
Batch-30	0	0.00	0.00	0.00	0.00
	3	4.81	3.03	0.00	0.00
	6	5.24	4.75	0.00	0.00
	9	7.43	7.27	0.00	0.00
	12	7.80	7.78	0.00	0.00
	18	7.92	8.63	0.00	0.00
	24	7.66	8.14	0.00	0.00
Batch-40	0	0.00	0.00	0.00	0.00
	1	3.21	3.25	0.00	0.00
	2	4.18	4.50	0.00	0.00
	3	4.99	5.08	0.00	0.00
	6	6.79	6.01	0.00	0.00

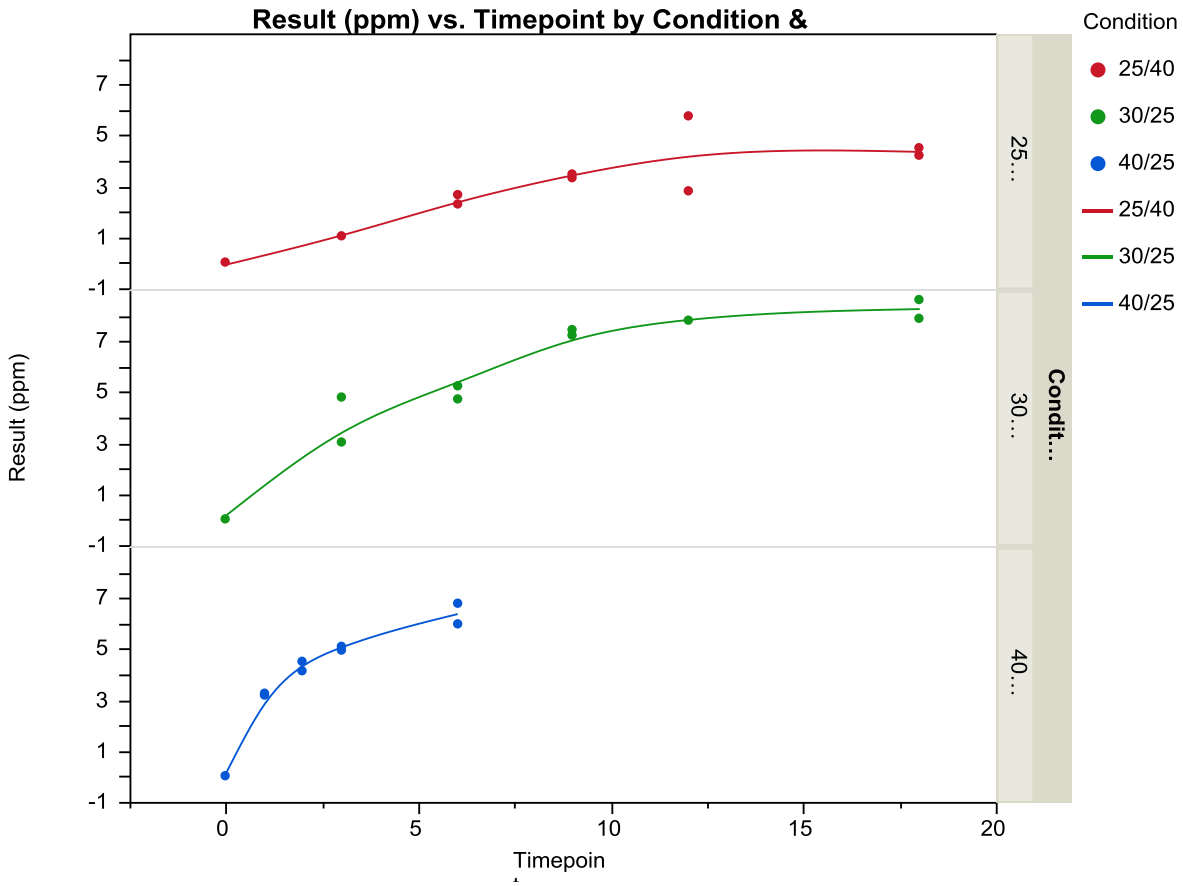


Figure 3.7: Trend Graphs for leaching rate of IUL(0.93) at three different storage conditions

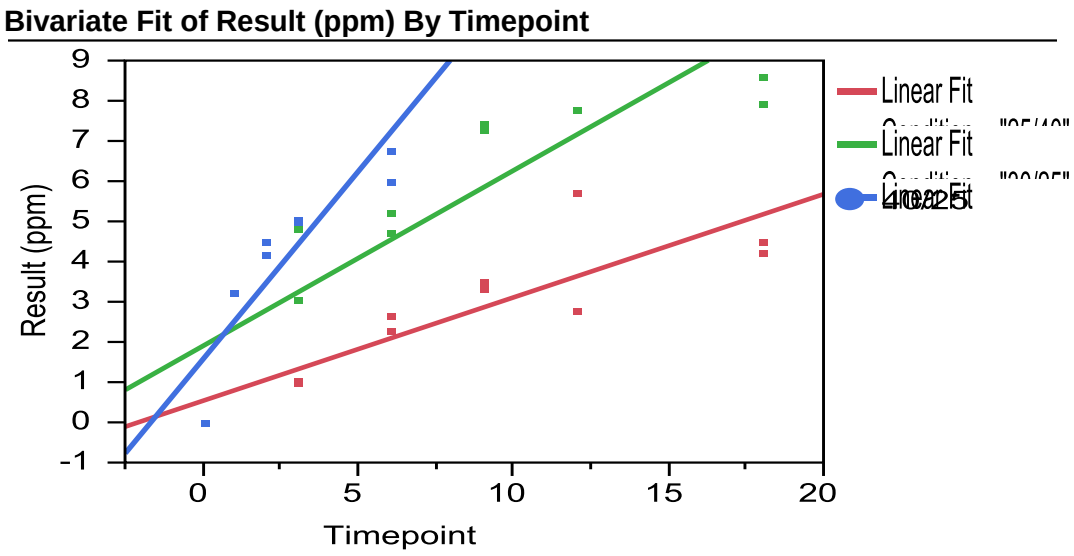


Figure 3.8: Bivariate fit of results (ppm) by timepoint grouped by storage condition.

Table 3.10 Linear Fit Equations for the Leaching Rate of IUL into Ophthalmic Product

Condition	Linear Fit Equation
25/40	Result (ppm) = 0.5340714 + 0.2570952*Timepoint
30/25	Result (ppm) = 1.8961429 + 0.4365238*Timepoint
40/25	Result (ppm) = 1.5591509 + 0.9341038*Timepoint

Table 3.11 Summary of Linear Regression Fit for Diethyl Phthalate Leaching

Parameter	**25/40**	**30/25**	**40/25**
Square	0.768	0.795	0.786
RSquare Adj	0.745	0.775	0.759
Root Mean Square Error	0.913	1.432	1.120
Mean of Response	2.590	5.388	3.801
Observations (or Sum Wgts)	12	12	10

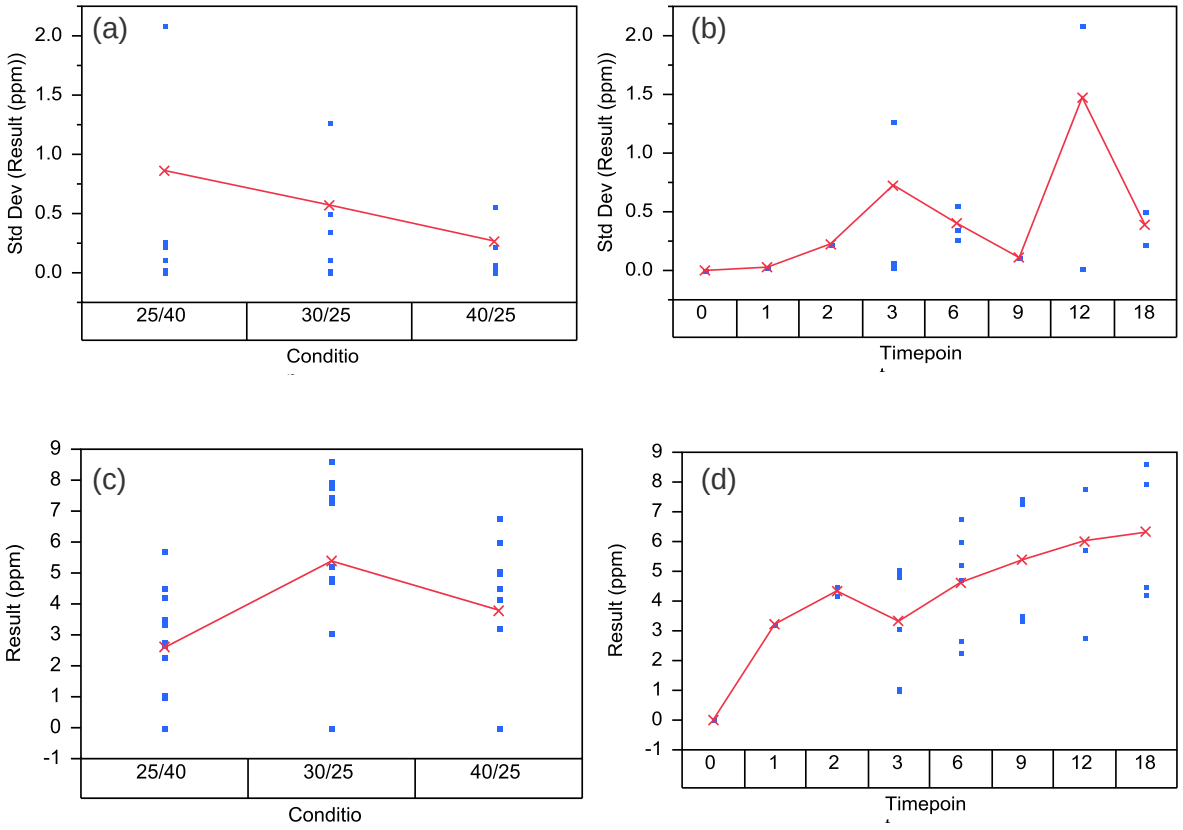


Figure 3.9: Graphical representation of standard deviation by (a) condition and (b) timepoint and concentration of the unidentified leachable (ppm) by (c) condition and (d) timepoint.

Table 3.12 Partition of Variation (POV) Crossed, Independent Analysis

Component	Pop Variance	% of Total	Sqrt(Var Comp)	F Ratio	Prob>F	Sig.
	.	.	.	.	.	
Between Total	5.647	83.14	2.376	27.6210	0.0000	*
Between Condition	1.388	20.44	1.178	16.9783	0.0000	*
Between Timepoint	3.650	53.74	1.911	89.2714	0.0000	*
Between Condition*Timepoint	0.608	8.96	0.780	7.4386	0.0026	*
	.	.	.			
Within Total	1.145	16.86	1.070			
Within Condition	0.138	2.03	0.371			
Within Timepoint	0.076	1.12	0.276			
Within Condition*Timepoint	0.139	2.04	0.373			
Common	0.000	0.00	0.000			
	.	.	.			
Total	6.792	100.00	2.606			

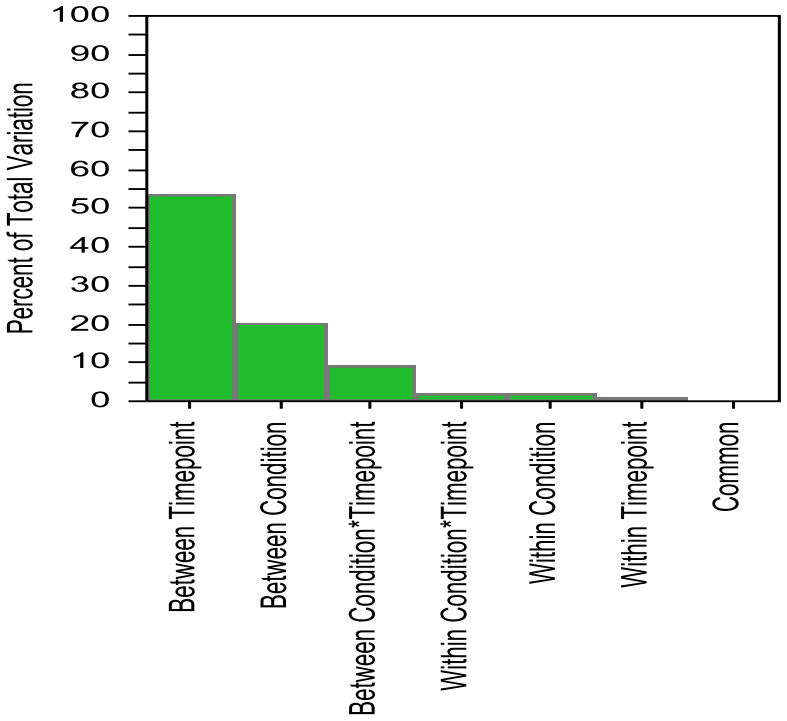


Figure 3.10: Graphical representation of percent of total variation

3.4 MS Analysis

The tape seal on the outer packaging of the final product uses an adhesives which has high concentrations of diethyl phthalate. It was hypothesised that the leaching IUL (0.93) was indeed diethyl phthalate. Simple molecular ion spectra were recorded for the diethyl phthalate standard, extracted tape seal and the final product which contained the unidentified leachable component at relative retention time (0.93). Table 3.11 summarises the parent and product ion transitions for the leachable components.

Table 3.11: Precursor and product ions recorded for LC-MS of diethyl phthalate standard and IUL (0.93) from tape seal extraction and IUL (0.93) form ophthalmic product

Leachable	**m/z**	**Precursor Ion**	**m/z**	**Product Ion**
Diethyl Phthalate	223	$[M+H^+]$	245	$[M+H^+]$ + [Na]
	223	$[M+H^+]$	177	$[M+H^+] – [C_2H_5O]$
	177	$[M+H^+] – [C_2H_5O]$	149	$[M+H^+] – [C_2H_5O] – [C_2H_5]$
IUL (0.93) from Extraction of Tape Seal	223	$[M+H^+]$	245	$[M+H^+]$ + [Na]
	223	$[M+H^+]$	177	$[M+H^+] – [C_2H_5O]$
	177	$[M+H^+] – [C_2H_5O]$	149	$[M+H^+] – [C_2H_5O] – [C_2H_5]$
IUL (0.93) from Ophthalmic Product	223	$[M+H^+]$	245	$[M+H^+]$ + [Na]
	223	$[M+H^+]$	177	$[M+H^+] – [C_2H_5O]$
	177	$[M+H^+] – [C_2H_5O]$	149	$[M+H^+] – [C_2H_5O] – [C_2H_5]$

The MS signals for each leachable were generally due to loss of distinct groups from each structure. The diethyl phthalate precursor ion (223) loses the ethoxide group into a product ion with m/z 177. This product ion further fragments with the loss of an ethyl group to give

the new product ion with m/z 149. The addition of sodium adducts (23 mass units) to the parent molecule are also confirmation of the parent structure i.e. 245 mass units.

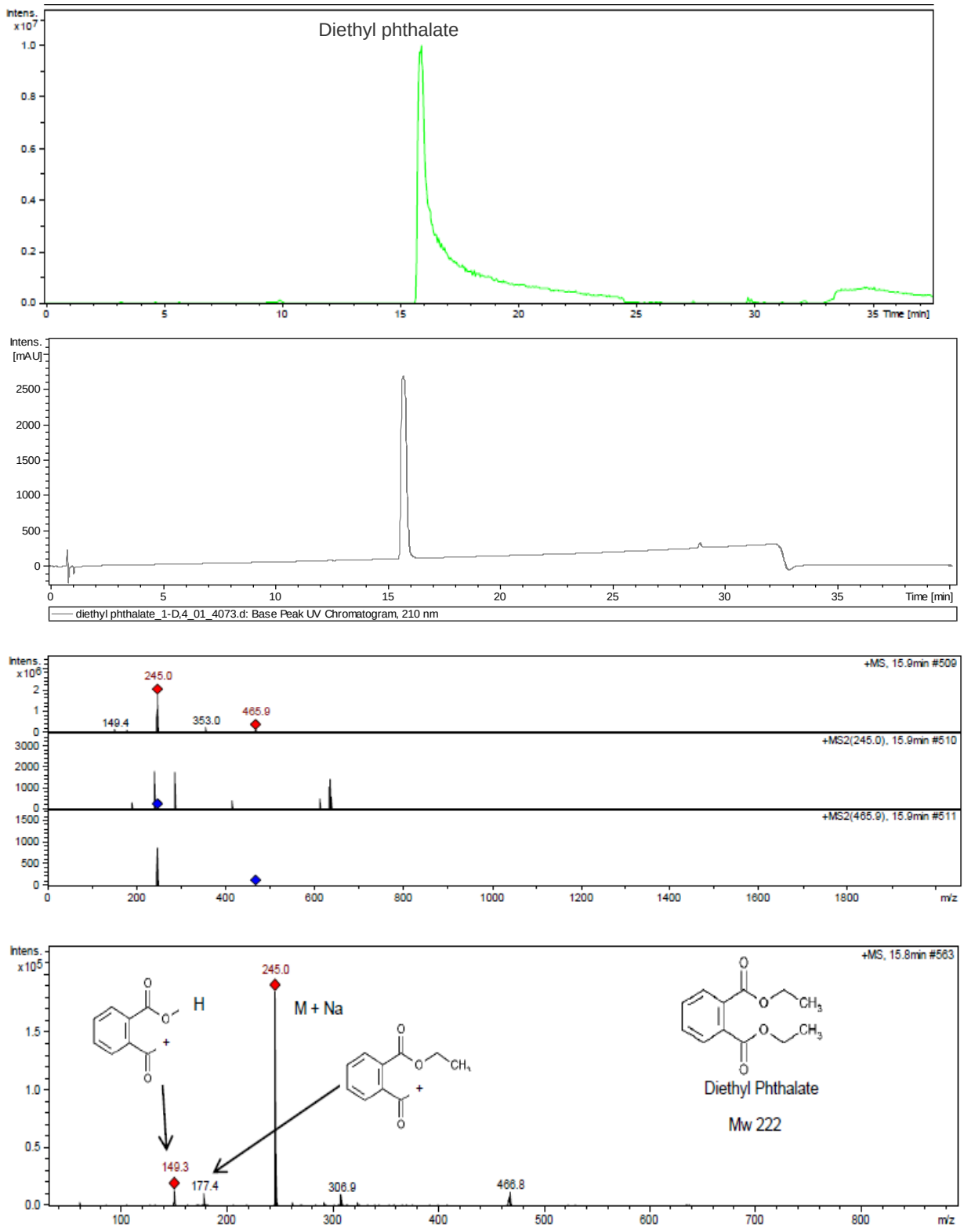


Figure 3.11 UV Chromatogram, Extracted Ion Chromatogram and Mass Spectra of Diethyl phthalate

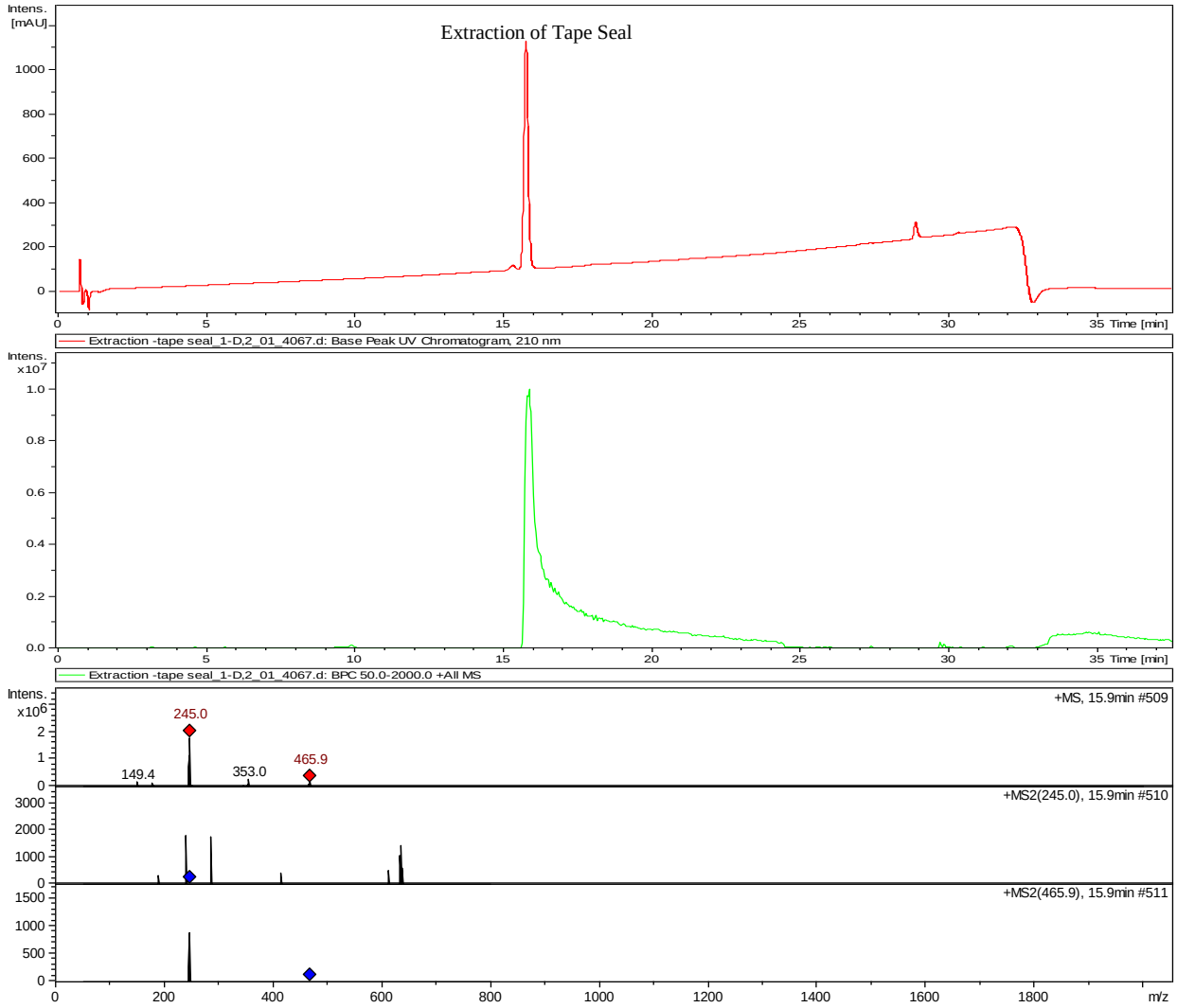


Figure 3.12: UV Chromatogram, Extracted Ion Chromatogram and Mass Spectra of Extraction of Tape Seal.

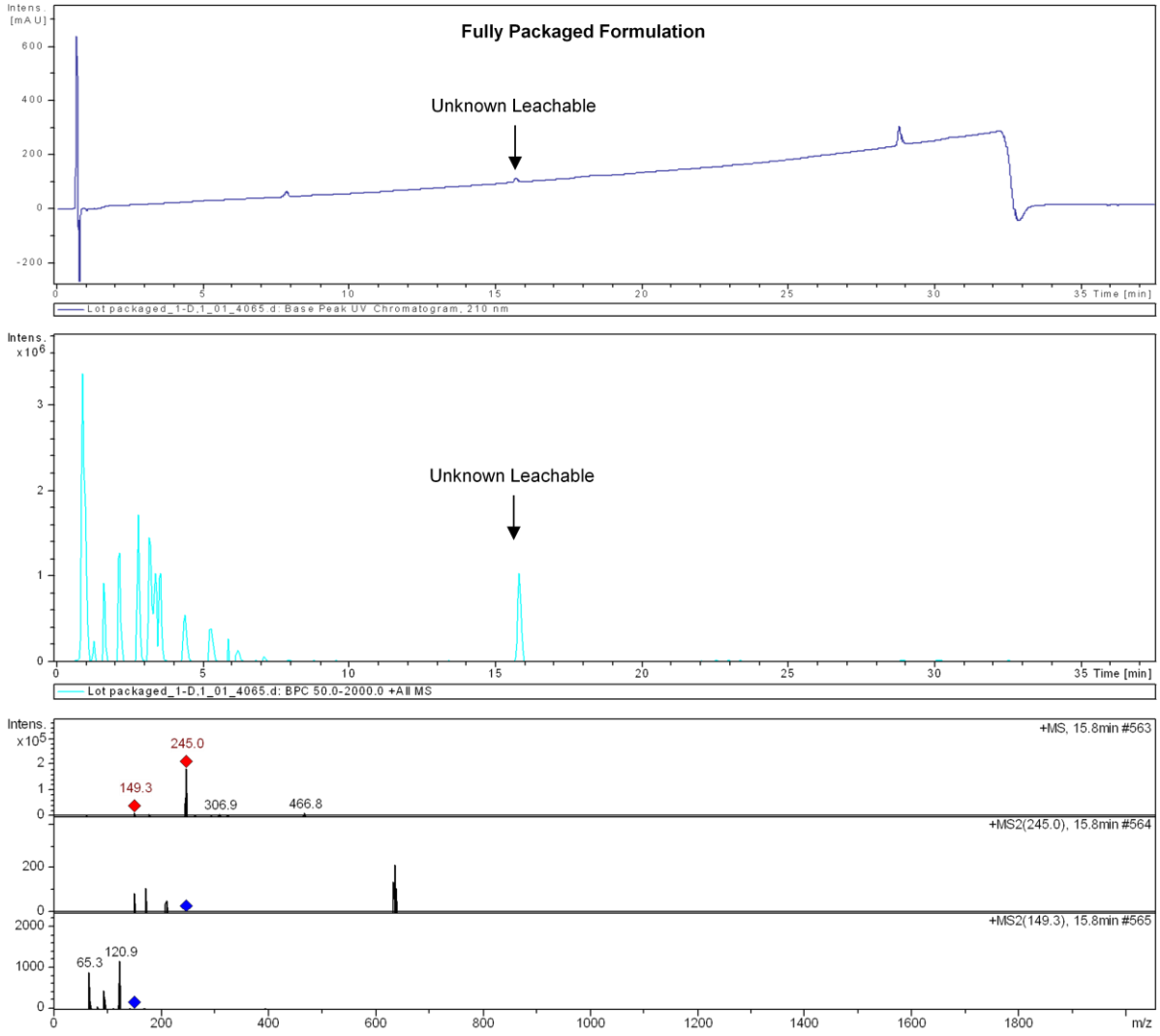


Figure 3.13: UV Chromatogram, Extracted Ion Chromatogram and Mass Spectra of Final product.

Conclusion

A Stage I extraction study was executed on final packaging components for an ophthalmic solution with appropriate solvent to ascertain an extractables profile for the various components. An extractable at relative retention time 0.93 when compared to a benzophenone standard was observed in the tape seal, printed carton, carton insert and bottle label. The extractable was shown to have migrated from the tape seal through the outer packaging of the product to contaminate the carton, carton insert and bottle label. A Stage II study was performed to determine if the extractable leaches into the final product and if so, determine what conditions affect the rate of leaching. A corresponding leachable at relative retention time 0.93 when compared to a benzophenone working standard was observed in the final product under routine, intermediate and accelerated conditions. The UV spectra and HPLC profiles indicate that the leachable is a consequence of an analyte migration from the tape seal into the final product. Migration rates were analysed using linear regression and ANOVA analysis. The rate of migration is dependent primarily on time and storage conditions. As time and temperature increases the migration rate of the leachable increases. The maximum observed concentration of the leachable was approximately 8 ppm which is below the allowed limits of less than 10 ppm as directed by the US FDA. HPLC-MS analysis was performed to identify the leachable component. The leachable was successfully identified as diethyl phthalate which is a plasticizer used in the manufacture of the tape seal. Future work in this area would include selection of 3 alternative tamper evident seals to be used on the ophthalmic solution. Alternate plastic based models and paper based models would be selected. Further Stage I extraction studies would be executed to determine the presence of diethyl phthalate or other volatile phthalate plasticizers. The affect of diethyl phthalate has on the active pharmaceutical ingredient (API) within the formulation should also be investigated to ascertain if the degradation pathway of the API is altered due to the presence of the leachable.

References:

1. U.S. Department of Health and Human Services Food and Drug Administration Centre for Drug Evaluation and Research (CEDER); Rockville, MD, Container Closure Systems for Packaging Human Drugs and Biologics; Guidance for Industry; May 1999 1-41.
2. Q3A (R2) 'Impurities in New Drug Substances' , ICH Harmonised Tripartite Guideline; International Conference on Harmonisation of Technical requirements for Registration of Pharmaceuticals for Human Use; October, 2006.
3. Q3B (R2) 'Impurities in New Drug Products' , ICH Harmonised Tripartite Guideline; International Conference on Harmonisation of Technical requirements for Registration of Pharmaceuticals for Human Use; June, 2006.
4. Q1A(R2), 'Stability Testing of New Drug Substances and Products', ICH Harmonised Tripartite Guideline; International Conference on Harmonisation, February 2003.
5. CORREDOR, C.C., HABY, T.A., YOUNG, J.D., SHAH, P.A. and VARIA, S.A., 2009. Comprehensive determination of extractables from five different brands of stoppers used for injectable products. *PDA Journal of Pharmaceutical Science and Technology,* **63**(6), pp. 527-536.
6. PAN, C., HARMON, F., TOSCANO, K., LIU, F. and VIVILECCHIA, R., 2008. Strategy for identification of leachables in packaged pharmaceutical liquid formulations. *Journal of pharmaceutical and biomedical analysis,* **46**(3), pp. 520-527.
7. JENKE, D., GARBER, M.J. and ZIETLOW, D., 2005. Validation of a liquid chromatographic method for quantitation of organic compounds leached from a plastic container into a pharmaceutical formulation. *Journal of Liquid*
8. ZHANG, F., CHANG, A., KARAISZ, K., FENG, R. and CAI, J., 2004. Structural identification of extractables from rubber closures used for pre-filled semisolid drug applicator by chromatography, mass spectrometry, and organic synthesis. *Journal of pharmaceutical and biomedical analysis,* **34**(5), pp. 841-849.
9. ITO, R., SESHIMO, F., MIURA, N., KAWAGUCHI, M., SAITO, K. and NAKAZAWA, H., 2005. High-throughput determination of mono- and di(2-ethylhexyl)phthalate migration from PVC tubing to drugs using liquid chromatography-tandem mass spectrometry. *Journal of pharmaceutical and biomedical analysis,* **39**(5), pp. 1036-1041.
10. FLISZAR, K.A., WALKER, D. and ALLAIN, L., 2006. Profiling of metal ions leached from pharmaceutical packaging materials. *PDA Journal of Pharmaceutical Science and Technology,* **60**(6), pp. 337-342.
11. PASKIET, D.M., 1997. Strategy for determining extractables from rubber packaging materials in drug products. *PDA Journal of Pharmaceutical Science and Technology,* **51**(6), pp. 248-251.
12. ZUCCARELLO, D.J., MURPHY, M.P., MEYER, R.F. and WINSLOW, P.A., 2009. A comprehensive approach for the determination of extractable and leachable metals in pharmaceutical products by inductively-coupled plasma. *PDA Journal of Pharmaceutical Science and Technology,* **63**(4), pp. 339-352.

Chapter 4: Final Conclusion

A screening LC method with UV and MS detection methodologies has been developed for 8 potential leachables from packaging components associated with the container closure system of ophthalmic formulations. The method was developed using statistical techniques such as fractional factorial DOE and process capability analysis. The test methodologies are geometrically transferable from HPLC to UPLC instrumentation.

A stage I extraction study was executed on the packaging components of an ophthalmic product to ascertain potential leachables which were observed in final product. Extracts of the tape seal identified the offending leachable as diethyl phthalate and this was confirmed via MS analysis.

A stage II leachable study was performed at various temperature and humidity conditions to determine the stability profile of the product over a significant period of time. The rate of leaching was determined to be dependent on temperature.

Future work in this area would focus on screening a larger number of potential leachables on the newly developed screening method and subsequent derivatization techniques to improve sensitivity of phenolic compounds for ESI analysis. Possible future work would also focus on screening alternate adhesives to be used on the tamper evident seal and assess leaching rates of constituents under the stability storage conditions.