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Detection of Impurities in Acetaminophen Intravenous and Oral Formulations Available on the Lebanese Market

By

Rita Rahme

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THESIS APPROVAL FORM

Student Name: Rita Rahme I.D. #: 201400934

Thesis Title: : Detection of Impurities in Acetaminophen Intravenous and Oral Formulations Available o

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The undersigned certify that they have examined the final electronic copy of this thesis and approved it in Partial Fulfillment of the requirements for the degree of:

Master in the major of Pharmaceutical Development and Management

Thesis Advisor's Name: Dr. Yolande Saab

Signature: [redacted] Date: 27 / 01 / 2022
Day Month Year

Committee Member's Name: Dr. Rony Khnayzer

Signature: [redacted] Date: 27 / 01 / 2022
Day Month Year

Committee Member's Name: Dr. Stephanie Mehanna

Signature: [redacted] Date: 27 / 01 / 2022
Day Month Year

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To my loving parents, husband, colleagues and academic supervisors

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Detection of Impurities in Acetaminophen Intravenous and Oral Formulations Available on the Lebanese Market

Rita Rahme

ABSTRACT

Introduction: The presence of impurities in pharmaceutical formulations has been a concern worldwide. Acetaminophen is a commonly used drug for its analgesic and antipyretic properties. We report here a novel method for the impurity profiling of intravenous acetaminophen formulations by head space gas chromatography. In addition, impurity profiling of oral acetaminophen tablets and capsules available on the Lebanese market and manufactured in Lebanon was not reported, as well.

Objective: The purpose of this study is to detect impurities present in intravenous and oral samples of acetaminophen on the Lebanese market and analyze the risks that these impurities pose to human health.

Methods: static headspace gas chromatography- mass spectrometry was utilized as one of the most suitable methods to detect organic volatile impurities. Samples for intravenous use where first lyophilized prior to analysis. A total of 25 samples were analyzed: 9 and 16 for intravenous and oral use, respectively. Toxicological review was performed on the detected impurities and the different risks associated were analyzed.

Results: of the 9 intravenous samples analyzed, 6 contained impurities. As for the oral

samples, 9 out of 16 samples contained impurities. A total of 14 different impurities were detected: acetaldehyde, cyclomethicone 5, cyclomethicone 6, dibutyl phthalate, diethyl phthalate, diacetamate, ethanol, ethyl stearate, formic acid, glycidol, methyl carbamate, methyl hydrazine, methyl stearate, and triethylamine. Several of the detected impurities are highly nephrotoxic, hepatotoxic, neurotoxic, carcinogenic, and/or teratogenic.

Conclusion: In view of the toxicity of the detected impurities and the negative impact they carry on the quality of the formulation as well as on human health, it is of utmost importance for the concerned pharmaceutical industries to take appropriate measures to control the source of these impurities and abide by the guidelines set by the regulatory authorities all around the world. Regulatory authorities should also take the necessary measures to preserve the safety of treated patients.

Keywords: Impurities, Volatile Organic Impurities, Impurity Profiling, Chromatography, Headspace GC-MS, Acetaminophen, Toxicology.

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List of abbreviations

ANDA	Abbreviated New Drug Application
API	Active Pharmaceutical Ingredient
cITP	Capillary Isotachophoresis
CNEMC	China National Environmental Monitoring Center
COVID	Corona Virus Disease
DAD	Diode Array Detection
DBP	Dibutyl Phthalate
DEP	Diethyl Phthalate
DNA	Deoxyribonucleic acid
EU	European Union
EMA	European Medicines Agency
FDA	Food and Drug Administration
GC	Gas Chromatography
HMLC	Hybrid Micelle Liquid Chromatography
HPLC	High-Performance Liquid Chromatography
HPTLC	High-Performance Thin Layer Chromatography
HS	Headspace
ICH	International Conference On Harmonization
IDLH	Immediately Dangerous to Life or Health.
IV	Intravenous
LC	Liquid Chromatography
MCR-ALS	Multivariate Curve Resolution Alternating Least Squares
MSDS	The Material Safety Data Sheets

MS	Mass Spectrometry
NIOSH	National Institute for Occupational Safety and Health
NMR	Nuclear Magnetic Resonance
PCA-ANN	Principal Component Analysis- Artificial Neural Networks
PDE	Permissible Daily Exposure
TDI	Total Daily Intake
TLC	Thin Layer Chromatography
UD	United States
UK	United Kingdom
USEPA	United State Environment Protection Agency
USP	United States Pharmacopeia
UV	Ultraviolet

Chapter One

Introduction

Active pharmaceutical ingredients (API) are produced by chemical synthesis. Residual solvents and trace amounts of inorganic and organic compounds are typically present, they remain in the final product and are known as impurities. Impurities are defined by the International Conference of Harmonization (ICH) as "Any component of the drug product that is not the chemical entity defined as the drug substance or an excipient in the drug product" (Pilaniya et al., 2010). The ICH Q3A(R2) "Impurities in new Drug Substances" (2006) guidance classifies impurities into three main categories:

- Organic impurities: can be either process-related or drug related. They can emerge during the manufacturing or the storage of the drug and may not always be identified. Organic impurities can be either volatile or not.
- Inorganic Impurities: usually emerge during the manufacturing process of the drug. They are usually identified.
- Residual Solvents: Inorganic or organic liquids that are used as vehicle in the synthesis of the drug product and that remain in the final product. The ICH has developed a standard guideline to control residual solvents in the final product, titled "ICH Guideline Q3C on Residual Solvents".

Impurities have chemical structures that are, to some degree, similar to the API. They can have undesirable side effects that can, in some cases, outweigh the benefits of the drug (Nageswara Rao & Nagaraju, 2003). The presence of pharmaceutical impurities in the final product can affect the safety and stability of the drug (Khandavilli et al., 2020). Thus,

the development of analytical techniques for the detection of these impurities in pharmaceuticals is of great significance. Both qualitative and quantitative profiling of impurities has taken the attention of regulatory authorities around the world.

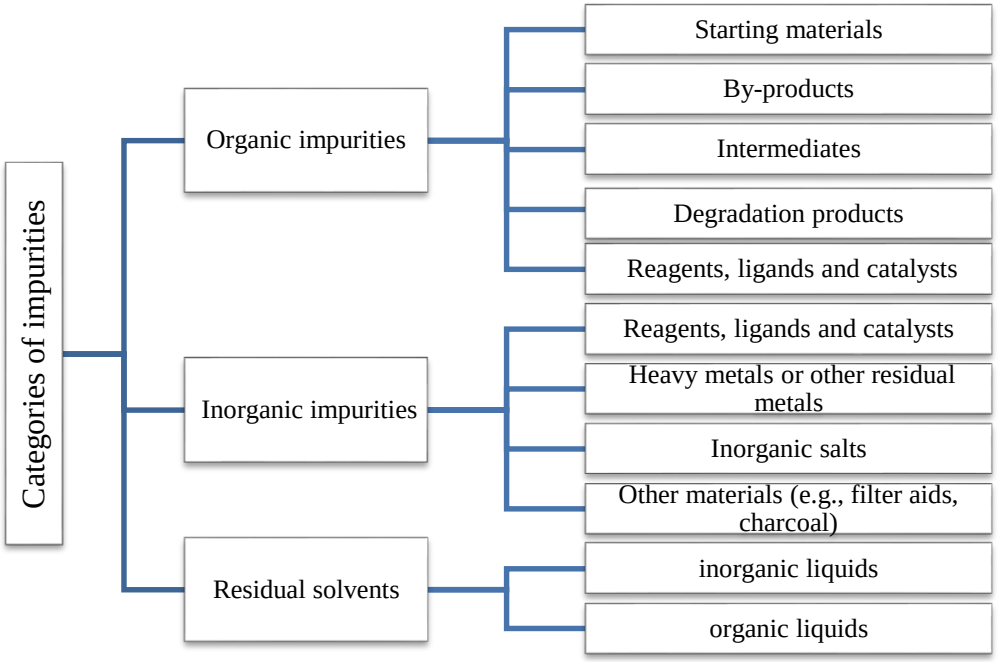

Figure 1. Classification of impurities according to the ICH Q3A (R2) Guideline.

Acetaminophen, also known as paracetamol, is a commonly used drug for its analgesic and antipyretic properties. Acetaminophen was first made in 1877. It is the most commonly used medication for pain and fever in both the United States and Europe. The use of acetaminophen became even more significant with corona virus pandemic, as it is the analgesic and antipyretic of choice in the treatment of infected patients.

A total of 14 acetaminophen impurities were identified by the European pharmacopeias. These impurities are labeled by letters, impurity A to N, however, not all of them have a well identified chemical structure and chemical name (Aqeel et al., 2019). Organic impurities that may appear in acetaminophen preparations are process-related impurities. Their profiles are influenced by the choice of synthetic method, the quality of starting

materials, reagents and solvents, the reaction conditions, the work-up and final purification, and the design of the process equipment (Kamberi et al., 2004).

1.1 Regulatory Environment

Regulatory authorities around the globe are emphasizing on the importance of identifying impurities and controlling their presence in APIs as well as in formulations. According to the Guidance for Industry Q3B (R2), titled "Impurities in New Drug Products" (2006), issued by the ICH, pharmaceutical industries should provide the regulatory authorities with a summary of the detected impurities (degradation products, impurities arising from interaction with excipients and/or the immediate container closure system) during manufacturing or storage of the API. The ICH has also established limits for reporting, identification and qualification of the impurities. The ICH additionally recommends that lower thresholds should be set from highly toxic impurities. These thresholds are expressed either as a percentage of the drug substance or as total daily intake (TDI) of the impurity, and depend on the maximum daily dose of the drug, as detailed in the table below ("Impurities in new drug products'', 2006).

Table 1. Thresholds of reporting, identification and qualification according to the ICH Q3B (R2) guideline (2006).

Maximum Daily Dose	**Reporting Threshold**	**Identification Threshold***	**Qualification Threshold**
≤ 2g/day	0.05%	0.10% or 1.0 mg/day intake*	0.15% or 1.0 mg/day intake*
>2g/day	0.03 %	0.05%	0.05%

*whichever is lower.

For organic impurity reporting and qualification, the ICH recommends that the pharmaceutical industry should first start by developing a summary of actual and potential impurities of a pharmaceutical product while taking into consideration the chemical reactions involved, impurities detected in the raw material, degradation products, as well as the chemical nature of the drug (“Impurities in new drug products’’,2006).. Laboratory tests should follow to detect impurities and they should be performed on batches in the development process and batches in the commercial process; when impurities are detected and quantified, the total daily intake of the impurity should be calculated and table 1 should be used as guidance. The impurities identified should undergo laboratory tests to identify their structure, and their safety should be analyzed, when necessary (“Impurities in new drug products’’,2006).

For control of inorganic impurities, pharmaceutical manufacturers should refer to pharmacopoeial guidance on the detection of inorganic impurities, analysis of the risks imposed, and acceptance criteria (“Impurities in new drug products’’,2006).

Solvents used in pharmaceutical production should be controlled based on the class they belong to. The ICH categorizes solvents into three classes as shown in figure 2. Solvents belonging to class 1 should not be employed in the manufacturing of drug products. For solvents belonging to class 2, the ICH has set “a pharmaceutically acceptable intake of residual solvents” that can be tolerated in a formulation, otherwise known as “Permissible Daily Exposure (PDE)”. For solvents belonging to class 3, It is considered that amounts of 50 mg per day or less (corresponding to 5000 ppm or 0.5%) would be acceptable without justification, according to the “ICH Q3C(R8) impurities: guideline for residual solvents” (2021).

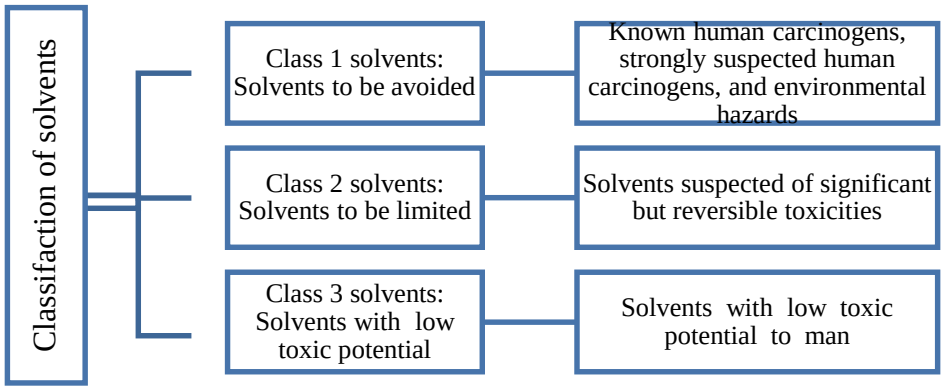

Figure 2. Classification of solvents by the ICH.

The US Food and Drug Administration (FDA) has adopted the ICH guidelines and has also developed a guidance document under the title "ANDAs: Impurities in Drug Products" (2010). The main focus of this guidance is degradation products of the active ingredient or reaction products of the active ingredient with an excipient(s) and/or immediate container/closure system in generic drug products. The FDA has recommended that manufacturers include a list of degradation products, discussion of the degradation profiles, and conform to limits specified in the United States Pharmacopeia (USP) ("ANDAs: Impurities in Drug Products", 2010).

1.2 Acetaminophen

According to the Lebanese national drug index (LNDI, n.d.), acetaminophen (brand names: Panadol®, Adol®, Doliprane®, Dolopan®...) is available on the Lebanese market in several dosage forms (tablets, capsules, effervescent tablets, granules for solution, suppository, syrup, and injectable solution) for administration via different routes (oral, rectal, and injectable). Additionally, acetaminophen is commonly available in combination with other APIs. It is an analgesic and antipyretic drug ("Paracetamol

[Acetaminophen]", 2022). The analgesic effects are believed to be due to activation of descending serotonergic inhibitory pathways in the Central Nervous System. Interactions with other nociceptive systems may be involved as well. Antipyresis is produced from inhibition of the hypothalamic heat-regulating center. It is approved for infants, children and adolescents as well as adults, including pregnant (intermittent use) and breastfeeding patients. The dose approved for intravenous use in adult patients above 50kg is 650mg every 4 hours or 1g every 6 hours; maximum single dose is 1g and maximum daily dose is 4g ("Paracetamol [Acetaminophen]", 2022). The dose should be administered by IV infusion, undiluted over 15 minutes. Intact vials and bags should be stored at 20°C to 25°C (68°F to 77°F) and should not be refrigerated or frozen. Its use is contraindicated in case of severe hepatic impairment. Healthcare providers should consider all sources of acetaminophen from different drugs that the patient might be taking, in order to avoid overdosing. Acetaminophen is primarily eliminated by hepatic metabolism by the enzymes CYP2E1 (major), CYP1A2, CYP2A6, CYP2C9, CYP2D6, and CYP3A4 ("Paracetamol [Acetaminophen]", 2022).

Frequent Side effects of Paracetamol	Hypertension/ hypotension/ peripheral edema
	Pruritus
	Hypoalbuminemia
	electrolyte imbalance
	Constipation/ diarrhea
	Oliguria
	Anemia
	Increased serum aspartate aminotransferase
	Infusion-site pain
	Agitation anxiety
	Muscle spasm
	Respiratory problems
	Nausea and vomiting*

*very frequent side effect: more than 10%.

Figure 3. Frequent side effects of acetaminophen ("Paracetamol [Acetaminophen]", 2022).

Dumarey et al. (2009) have developed methods to differentiate between synthetic pathways based on the chromatographic impurity profiles of formulations. In their research, they discuss patented synthetic pathways that were developed by pharmaceutical industries to produce acetaminophen. Several pathways of these use 4-aminophenol as an intermediate product. Once para-aminophenol is obtained, it undergoes an acetylation reaction to form acetaminophen. Conventional pathways for the manufacturing of para-aminophenol start with iron-acid multi-step processes, through the reduction of p-chloronitrobenzene or through 2 step reaction from phenol: first, nitration of phenol, then the reduction of the nitro group to an amine to obtain para-aminophenol. However, these processes have some drawbacks: the stoichiometric production of iron oxide sludge, the difficulty in separating the iron oxide sludge from the obtained products, uncontrollable reaction rate, the presence of iron particles, which can cause corrosion of the reactor, and finally, the use of mineral acids, which is not preferred from an environmental point of

view. Komatsu & Hirose (2004) have developed an alternative pathway to produce para-aminophenol, starting with nitrobenzene. The process consists of hydrogenation reaction of nitrobenzene into para-aminophenol through b-phenylhydroxylamine.

Another pathway to synthesize acetaminophen starts with phenol. Phenol is subjected to acetylation to obtain 4-hydroxyacetophenone. Acetaminophen is then obtained by reacting 4-hydroxyacetophenone with hydroxylamine to form a ketoxime. The ketoxime is then subjected to a Beckmann rearrangement in the presence of an alkyl alkanoate ester solvent and an appropriate acidic catalyst according to the "United States patent for production of acetaminophen" (1990).

In each of the above pathways, different starting materials, different catalysts, and different intermediate products are employed to finally obtain acetaminophen. These different processes are therefore expected to lead to a different set of impurities in the final product (Dumarey et al., 2009).

1.3 Impact of Impurities on Physicochemical Properties

Trace amount of organic and inorganic impurities present in a pharmaceutical formulation sometimes lack toxicological consequences and are therefore harmless from a toxicological perspective. However, we cannot undermine the impact that these impurities have on the physicochemical properties of the formulation. Variability in the amount of trace impurities leads to batch-to-batch variability in product characteristics (including, surface characteristics, crystallite size, aggregate size, surface roughness, wettability, dissolution, drug release, granulation …) which can significantly influence the processability and therefore the quality of the final product (Hulse et al., 2008). An example of how inorganic impurities lead to variability in product characteristics is

highlighted in the study conducted by Hulse et al. in 2008. Three Batches of acetaminophen containing different amount of the trace inorganic impurity aluminum were analyzed and their physicochemical properties identified. The three batches had differing surface energetic properties and dispersive free energy(Hulse et al., 2008). Organic impurities also have potential to influence the intrinsic physical properties. Keshavarz et al. (2019) concluded in their research study that the presence of 4-nitrophenol or 4′-chloroacetanilide in acetaminophen has led to reduced nucleation rate and both impurities were found to be nucleation inhibitors. In addition, the compressibility of acetaminophen is strongly dependent on the amount of 4′-chloroacetanilide (positive relationship) present in the sample. It is therefore essential to control the amount of impurities in acetaminophen in order to control its properties.

1.4 Toxicity Profile of Major Impurities

A total of 14 acetaminophen impurities were detected by the European pharmacopeias. However, not all of them have well identified chemical structure and known toxicity profile(Aqeel et al., 2019). In this section, we will discuss the most toxic acetaminophen process-related impurities.

p-aminophenol, also known as 4-Aminophenol, p-hydroxyaniline or paramidophenol, is a primary impurity of acetaminophen (“4-aminophenol’’,2022). It exists in acetaminophen pharmaceutical preparations from two different sources: excess starting material (since acetaminophen is obtained through an acetylation reaction between 4-aminophenol and acetic anhydride) and as a degradation product of acetaminophen. p-Aminophenol can be found in oxidative hair dyes as well (Directorate General for Health and Consumers, 2011). It can cause nephrotoxicity, mainly due to selective necrosis of

renal proximal tubules, hepatotoxicity (at higher doses) as well as teratogenic toxicity at maternal toxic doses, with effects on gestation, embryonic development, and lactation. It can also cause methemoglobinemia even at the lower concentrations (Li et al., 2005; Lock et al., 1993). p-Aminophenol is cytotoxic by itself and also has a cytotoxic glutathione metabolite (Li et al., 2005). Recent studies on possible techniques to remove p-aminophenol from pharmaceutical formulation concluded that salt formation by the addition of salicylic acid, oxalic acid, l-tartaric acid, and camphorsulfonic acid can help remove p-Aminophenol through filtration and washings(Khandavilli et al., 2020).

4-Nitrophenol, also known as para-nitrophenol, p-Nitrophenol or 4-Hydroxynitrobenzene, is a well-known acetaminophen process-related impurity (‘‘4-nitrophenol’’,2022). It is also widely used in the manufacture of pesticides, fungicides, paints, dyes and leather preservative. 4-Nitrophenol is classified as an ‘‘organic priority pollutants’’(Lam et al., 2013). It is an endocrine disruptor that exhibits estrogenic as well as anti-androgenic activities (Zhang et al., 2013). It is capable of disrupting the estradiol-to-testosterone balance, inducing hyperplasia of Leydig cells and reduction of aromatase expression. It can also cause acute hepatotoxicity (dark, enlarged, and thicker liver lobes). It also exhibits hematologic toxicity as it can cause methemoglobenemia (Zhang et al., 2013).

Acetanilide, also known as N-phenylacetamide, is a by-product in acetaminophen formulations and is also found in cosmetic products as fragrant and as an H_2O_2 stabilizer, according to the risk profile of acetanilide (*2011*).. Of interest, Acetanilide was previously marketed as an analgesic and antipyretic drug. It was withdrawn from the market due to its toxic adverse effects: methemoglobenemia, hepatotoxicity and nephrotoxicity. Studies

have shown that N-phenylacetamide, when ingested, causes hyperplasia of both the spleen and the bone marrow. It can also cause cyanosis (“risk profile of acetanilide”, 2011).

Another impurity that can be found in acetaminophen starting material is 4-chloroacetanilide, also known as n-(4-chlorophenyl)-acetamide (‘’4’-Chloroacetanilide’’,2022). A study was performed on rats to evaluate the hepatotoxic and the nephrotoxic potential of monochloroacetanilides. The in-vivo nephrotoxic potential of 4-chloroacetanilide was the highest among all tested monochloroacetanilides with the potential to cause elevation in blood urea nitrogen concentration and in kidney weight. 4-chloroacetanilide also had the highest hepatotoxic potential, by causing elevation in liver enzymes (Rankin et al., 1993).

1.5 Impurity Detection Methods

Several analytical techniques have been employed to detect and quantify acetaminophen impurities in acetaminophen active ingredient, acetaminophen formulation as well as when acetaminophen is present in combination with other APIs. Recent studies have also identified potential separation techniques between acetaminophen and its highly similar impurities.

1.5.1 High-Performance Liquid Chromatography

High-performance liquid chromatography, or HPLC, is a commonly used technique for analysis of bulk drugs as well drugs in formulation. It is used to separate, identify, and quantify components of a mixture. It offers the advantages of simple sample preparation with a low risk of errors (Nageswara Rao & Nagaraju, 2003). HPLC can be coupled with several detection modes. The choice of a proper detection mode is very crucial, in order to ensure that all components of a mixture are detected (APIs as well as impurities)

(Siddiqui et al., 2013). One option is to use UV detectors, with multiple wavelength scanning programs, in order to make sure that all UV-absorbing components are detected. Photodiode-array detectors, Fluorescence, electrochemical, refractive-index, and conductivity detectors have also been used in impurity detection (Nageswara Rao & Nagaraju, 2003). However, one major drawback of HPLC is its high cost (for both the columns and the solvents) (Nageswara Rao & Nagaraju, 2003). In addition, studies performed with HPLC on patented column packing may not be reproducible(Nageswara Rao & Nagaraju, 2003).

Thomis et al. (1984) analyzed multi-component effervescent tablets. The two main impurities that were detected are salicylic acid and Diacetyl-p-aminophenol.

Ibrahim et al. (2020) conducted a study on a commonly prescribed drug combination: acetaminophen, propyphenazone, and caffeine. The primary purpose of this research was to detect the presence of 4-aminophenol and 4-nitrophenol, two principal process-related impurities of acetaminophen. The group developed two methods that can serve as purity indicating methods and that can be applied to acetaminophen alone or in combination with the above mentioned APIs: one HPLC method and one Thin Layer Chromatography (TLC) method. For the purpose of the HPLC, a “dual-mode” gradient was applied with a ternary mobile phase consisting of methanol: acetonitrile: water for improved resolution, shorter analysis time and better separation. The HPLC was coupled with a UV detector set at 220 nm. In both methods, the authors achieved optimum separation between the impurities and the APIs. Ibrahim et al. (2021), applied the same concept to a different combination of APIs: acetaminophen and methionine. The authors again succeeded in developing an HPLC method as well as a TLC method for the simultaneous determination

of acetaminophen and methionine in the presence of two major impurities.

El-Yazbi et al. (2020) combined HPLC with diode array detection (DAD) on combination capsules of acetaminophen and chlorzoxazone. Seven peaks eluted and 5 different impurities were detected and their retention time identified in the following order (from shortest to longest retention): 4-aminophenol, 4-nitrophenol, acetanilide, 2-amino-4-chlorophenol, and 4-chloroacetanilide. The developed method is reliable for separation and purity detection

Vojta et al. (2015) applied HPLC to a suppository dosage form, combining acetaminophen, codeine pitophenone, and fenpiverinium, marketed under the brand name Spasmopan®, used as analgesic and spasmolytic for the gastrointestinal system or the bladder. Suppositories are difficult to analyze using HPLC since they contain non-polar placebo components that may damage the column if not removed. Therefore, sample preparation required several steps: the suppositories were first put in a freezer for 30 min, then, they were grated and homogenized by stirring, solvents were added, the sample was subjected to heating and sonication was performed. The final concentration of the APIs was calculated. Ion-pair reversed phase liquid chromatography was coupled with UV detection. The authors succeeded in developing a method for the quality control of Spasmopan® and for the detection of impurities of acetaminophen as well as the APIs(Vojta et al., 2015).

1.5.2 Spectrofluorometry

Spectrofluorometry can be combined with a separation technique or could be used alone, otherwise known as direct Spectrofluorometry (GH et al., 2009). It can be used to determine the presence of drugs/impurities that contain fluorophores, components in

molecules that cause them to fluorescence (GH et al., 2009). One of the oldest studies that were performed to detect acetaminophen impurities was conducted by Street and Schenk in 1979. The study aimed at detecting p –Aminophenol by a direct spectrofluorometric method in acetaminophen and acetaminophen containing tablets(Street & Schenk, 1979). Street and Schenk (1981) later developed a spectrofluorometric method that allows a rapid and accurate analysis for acetylsalicylic acid content in any pharmaceutical preparation containing acetaminophen and caffeine.

1.5.3 Thin Layer Chromatography

Thin layer chromatography (TLC) is another commonly used technique in impurity profiling. TLC's primary purpose is to separate mixtures. It is performed on a sheet coated with adsorbent material (could be silica gel, aluminum oxide or cellulose) that serves as a stationary phase. The mobile phase can be either one solvent or a mixture of solvents. Analytes are drawn up the plate via capillary action at different rates, and therefore separate. Recent improvements of the technique have led to High performance TLC, commonly known as HPTLC, to allow for better resolution and accurate quantification(Pilaniya et al., 2010). One major advantage of TLC over HPLC is its lower cost and faster process(Pilaniya et al., 2010).

In 2013, Abdelaleem and Abdelwahab(2013) developed a TLC method for the detection 4-Aminophenol as well as 2-Amino-4-chlorophenol (a degradation product of chlorzoxazone). The same combination of APIs and impurities were previously tested for under reverse phase HPLC in 2007. The developed TLC method offered several advantages: lower limit of detection, lower limit of quantification, faster resolution and lower cost. The authors concluded that it could be a resort for developing countries(Ali et

al., 2007).

Farid and Abdelaleem (2015), developed an HPTLC method coupled with UV detectors for the detection of an acetaminophen impurity with major toxicity, 4-Aminophenol, in combination tablets containing acetaminophen, pseudoephedrine and loratadine. The stationary phase employed was made of aluminum plates pre-coated with silica gel. The mobile phase consisted of acetone–hexane–ammonia. The authors succeeded in developing a cost and time effective method in order to detect the mentioned APIs and impurity, as an alternative to HPLC(Farid & Abdelaleem, 2016).

1.5.4 Other Methods

El Sherbiny and Wahba (2020), published a research that analyzes some pharmaceuticals, among which acetaminophen, in the presence of their synthetic impurities (p-amino phenol and p-nitro phenol) using hybrid micelle liquid chromatography (HMLC) coupled with UV detection(Sherbiny & Wahba, 2020). HMLC is a reversed-phase separation technique that uses an aqueous-organic solvent containing a surfactant above its critical micelle concentration as a mobile phase and a surfactant coated stationary phase. HMLC is an alternative method to reversed phase HPLC that offers several advantages: it allows direct injection of physiological fluids, enhanced detection (particularly for fluorescence) and the low cost and low toxicity of mobile phases, supporting the international trend of green chemistry (Poole, 2003). However, the technique is not commonly used because of its two major limitations: limited elution strength and poor chromatographic efficiency (Poole, 2003).

Another less commonly used technique in impurity detection and structure elucidation is Nuclear Magnetic Resonance spectroscopy, known as NMR. NMR can be used as a

quantitative technique in mixtures of known compounds or as qualitative technique to identify unknown compounds against a spectral library (Eldridge et al., 2007). In impurity detection and profiling, NMR is usually coupled with a separation technique such as HPLC (Eldridge et al., 2007). Limitations of this technique explain its rare use in impurity profiling: relatively poor sensitivity versus other techniques, column overloading because of the high concentration of active ingredient and low concentration of the impurity (in LC-NMR). Eldridge et al. (2007), have tried coupling capillary isotachophoresis (cITP) to NMR (cITP-NMR) as a method to increase sensitivity. They have applied their technique on an acetaminophen sample, after they have exposed to heat, to detect 4-aminophenol, a product of acetaminophen thermal degradation. The researchers succeeded in detecting 4-aminophenol in spiked samples. Forshed et al. (2002), have employed quantitative NMR in the detection 4-aminophenol in acetaminophen using Bayesian regularized neural network regression. The method developed requires minimal sample preparation and is relatively fast and simple.

Yehia and Mohamed (2016) used the chemometrics approach on a quaternary mixture of Acetaminophen, Guaifenesin, Phenylephrine and p-aminophenol(Yehia & Mohamed, 2016). Chemometrics is the "chemical discipline that uses mathematical, statistical, and other methods employing formal logic to design or select optimal measurement procedures and experiments". The chemometrics approach considers all variables at the same time. The model is fit to the data (Héberger, 2008). The researchers developed three advanced chemometric methods: Concentration Residuals Augmented Classical Least Squares (CRACLS), Multivariate Curve Resolution Alternating Least Squares (MCR-ALS) and Principal Component Analysis- Artificial Neural Networks (PCA-ANN). The

three methods showed to be successful, easy to apply, resource-efficient for acetaminophen formulation analysis.

In 2012, headspace gas chromatography coupled with mass spectrometry (sHS\GC-MS) was used for the detection of organic volatile impurities in acetaminophen. It was applied to tablets of acetaminophen. The researchers succeeded in detecting five major volatile organic impurities: 1-methanone, Acetic acid, Menthol, N-(2-hydroxyphenyl)acetamide, N-phenylacetamide, and Phenol. The method developed was automated, simple, requires no solvent use and minimal sample preparation (Pietraś et al., 2012).

Chapter Two

Objectives and Aims

Acetaminophen is one of the most commonly used medications, and with the coronavirus pandemic, its use has become even more significant. To the best of our knowledge, up to this day, intravenous acetaminophen formulations were never analyzed by gas chromatography techniques for their impurity profiling. In addition, Oral acetaminophen tablets and capsules available on the Lebanese market and manufactured in Lebanon were never analyzed for impurity profiling. This study was designed in order to detect organic volatile impurities in formulations available on the Lebanese market.

Objective 1: detect impurities present in acetaminophen IV products.

Since an intravenous product has 100% bioavailability, one can be concerned about the impurities that might be present in an acetaminophen bottle or bag, and that are administered to the patient, along with acetaminophen. The primary objective of this research is to develop, optimize, and validate a headspace Gas Chromatography method for the detection of acetaminophen impurities that are organic and volatile.

Objective 2: detect impurities present in oral acetaminophen products.

Orally administered acetaminophen might have a lower bioavailability, however, it does not carry a lower risk of exposure to impurities, since its use is significantly more extensive, and more frequent, when compared to intravenous acetaminophen. The second objective of this research is to apply the developed method to oral acetaminophen samples and detect impurities, with major focus on locally manufactured acetaminophen products.

Chapter Three

Materials and Methods

3.1 Sample collection

The experiment was performed on acetaminophen products available on the Lebanese market. Samples were collected from different sources to be representative of what is currently used in Lebanon: registered suppliers, local manufacturers, hospitals, non-governmental organizations in charge of distributing medical supplies to hospitals/clinics. For the acquisition of the acetaminophen samples, a scan of the Ministry of Public Health Lebanese national drug index was performed, suppliers of registered acetaminophen samples were contacted and the brands/generics currently available on the market were ordered. In addition, leading hospitals were contacted. Major hospitals in the country provided samples of the acetaminophen brands/generics they use. Non-governmental organizations, which might have some brands of acetaminophen not registered in the Ministry of public health data, were also contacted and samples collected. A Scan of acetaminophen products manufactured in Lebanon by the 11 registered Lebanese manufacturers was performed. Some of the local manufacturers were found to produce oral or rectal acetaminophen, but none of these produce intravenous acetaminophen.

3.2 Methods

Headspace is a separation technique used to separate volatile components from a heavier sample matrix. The volatile compounds are then injected in a gas chromatography for analysis. Components of a mixture that are more volatile will be more concentrated in the

headspace phase, while components that are less volatile will remain in the liquid phase. Extraction of vapor from the headspace phase will follow, by a headspace sampling system, and the vapor will be injected in the GC column (Bicchi et al., 2012). This process allows an efficient separation and therefore only the more volatile compounds will enter the GC system. The migration of components between the headspace phase and liquid phase is not only dependent on volatility (Bicchi et al., 2012). Molecules distribute themselves between the two phases differently depending on each molecule's thermodynamic properties. This distribution is represented through the partition coefficient, also known as distribution ratio, or K. The value of K depends on both the compound as well as the sample matrix, and it is affected by several factors including temperature. The analysis of a sample happens after equilibrium has been reached between the two phases (equilibrium between the liquid/solid sample and its vapor), thus the name Equilibrium Headspace Sampling also called Static Headspace Sampling (Bicchi et al., 2012). Headspace Gas chromatography is a one-step gas extraction technique, and it has several advantages including simplicity, versatility and ease of automation. Another advantage of the technique is that typically, the ionization energy supplied in GC–MS studies is stronger compared to other techniques like LC–MS, and this leads to a large number of fragment ions, which allows an easier and more accurate match with compounds in the National Institute of Standards and Technology database, a well-established database for identification of the mass spectrum obtained from GC−MS (Misra et al., 2015). A major limitation, however, is the absence of analyte enrichment or accumulation, therefore its low sensitivity, according to a report by "*PerkinElmer*"(n.d.).

3.2.1 Sample preparation

Oral tablets and effervescent tablets of acetaminophen were crushed, along with their tablet coating (when present), using a mortar and pestle and 1g of tablet weight was transferred to chromatography vials for analysis.

IV acetaminophen samples were prepared by freeze-drying. In summary, the freeze-drying process is able to remove water from the material being processed while keeping it frozen. Freeze-drying was previously evaluated as a sample preparation method for the analysis of samples using headspace (Aggio et al., 2016), however, it was not previously performed on acetaminophen samples. Figure 4 explains the protocol adopted for freeze drying samples.

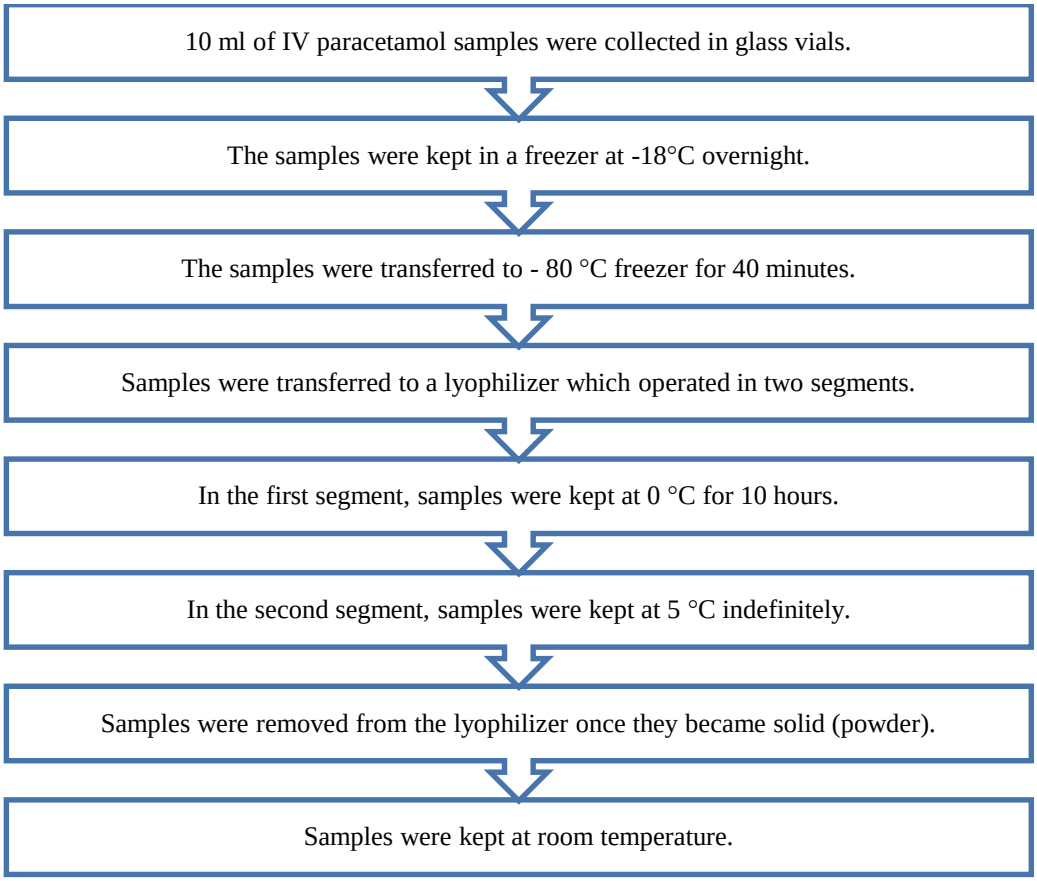

Figure 4 Protocol for freeze drying intravenous acetaminophen sample.

3.2.2 GC-MS conditions

sHS\GC-MS analysis was performed using SHIMADZU Nexis GC-2030 combined with MS-QP2020 NX and a SHIMADZU headspace sampler. Helium of high purity was used as a carrier gas, at a pressure of 102.4 kPa, total flow of 21.1 mL/min, and column flow of 1.92 mL/min. The separation was performed using DB-5 ms column, which is a nonpolar and low bleed column with a maximum temperature of 325 °C, length of 30.0m, inner diameter 0.25 mm ID, and a film thickness of 0.25 μm. Blank (empty vial) was injected at least once at the beginning of a sequence and between samples. Acetaminophen samples were injected in triplicates in order to ensure repeatability and validity of results. The results were compared and averaged. Detailed parameters are listed in table 2.

Table 2. sHS\GC-MS detailed parameters

Headspace Parameters	
Oven temperature	150.0 °C
Sample line temperature	170.0 °C
Transfer line temperature	180.0 °C
Pressurizing Gas Pressure	50 kPa
Equilibrating time	30.00 min
Pressurizing time	2.00 min
Pressure Equilibrating Time	0.10 min
Load time	0.50 min
Load Equilibrating Time	0.10 min
Injection time	1.00 min
Needle Flush time	5.00 min
GC cycle time	45.00 min
Gas Chromatography Parameters	
Injection mode	Split
Split ratio	10
Temperature	30.0 °C
Equilibrating time	1.0 min
Oven Temperature Program	30.0 °C for 2 minutes, then increase to 250 °C at a rate of 10 °C per minute. Hold on 250 °C for 5 minutes.
Total Program time	29 min
Column ID	DB-5 ms
Mass Spectrometry Parameters	
Ion Source Temperature	280 °C
Interface Temperature	250 °C
Solvent Cut time	0.5 min
Acquisition Mode	Scan
Scan Range	30.00 – 500.00 m/z

Chapter Four

Results

4.1 Sample distribution

25 samples of intravenous as well as oral acetaminophen available on the Lebanese market were collected and tested. The samples were distributed as follows: 5 intravenous bags, 4 intravenous bottles, 12 oral tablets, 4 effervescent tablets.

As for the manufacturing countries of these samples, five of the intravenous samples were manufactured in France, three in Italy, and one in Spain. Six of the oral samples were manufactured in Lebanon, four in France, three in Belgium, one in Australia, one in the United Arab Emirates and one in Ireland. Sample distribution based on manufacturing country for both oral and intravenous products is presented in figure 5. Overall, the majority of the samples are manufactured in France (36%), followed by Lebanon (24%), Belgium and Italy (12% each).

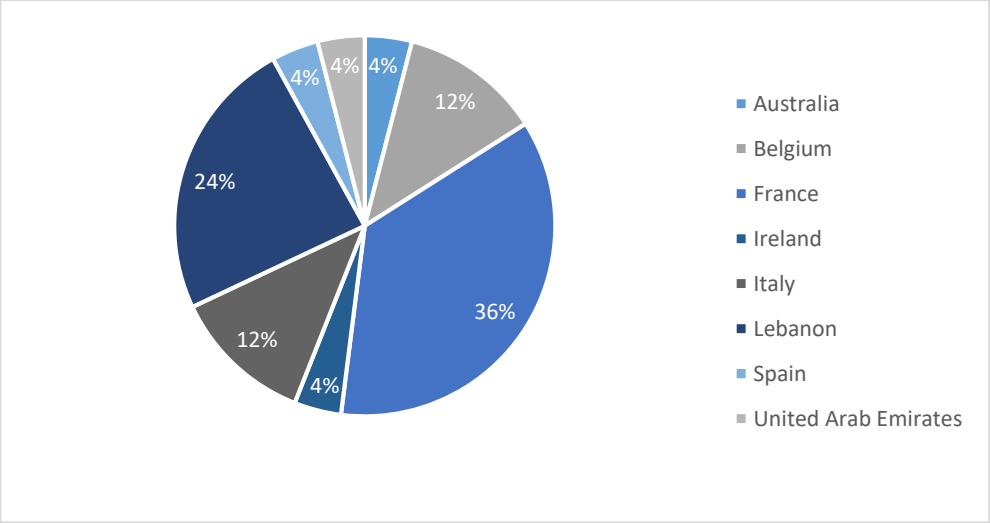

Figure 5. Distribution of Samples based on manufacturing country.

4.2 Results from intravenous samples

Of all intravenous samples tested, impurities were detected in 6 samples out of 9. In addition to the detected impurities, other components were detected including the active ingredient acetaminophen and some excipients such as acetic acid. According to the handbook of pharmaceutical excipients (2006), Glacial acetic acid is widely used as an acidifying agent in a variety of pharmaceutical formulations. It is also combined with an acetate salt (such as sodium acetate) to create a buffer system. It is thus generally regarded as relatively nontoxic and nonirritant. It is included in the FDA Inactive Ingredients Guide for injectable solutions, and in parenteral preparations licensed in the UK (Rowe et al., 2006). Since the primary purpose of this research is to investigate and detect impurities, only ingredients not listed on the products leaflet are presented in table 3.

Impurities detected in IV acetaminophen samples were: alcohol (ethanol), aldehyde (acetaldehyde), tertiary amine (Triethylamine), cyclomethicones 5 and 6, phthalates (DEP and DBP), and hydrazine (methylhydrazine). These impurities appeared on the chromatogram in the following order (from the shortest retention time to the longest): acetaldehyde, methylhydrazine, ethanol, triethylamine, cyclomethicone 5, cyclomethicone 6, DEP and DBP. Figure 6 shows the chromatogram of sample 8.

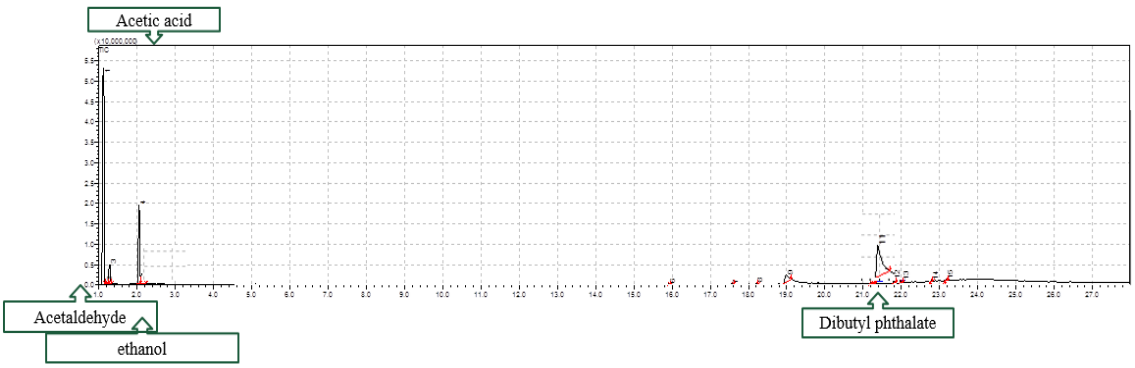

Figure 6. Chromatogram obtained from the first run of sample 8.

Table 3. Impurities detected in intravenous acetaminophen samples.

Sample ID	Impurity detected	Retention time	Area %	Similarity index
Sample 3	Ethanol	1.29	5.66	94.5
Sample 5	Ethanol	1.29	3.36	97.0
Sample 6	Cyclomethicone 5	10.94	2.39	96.5
	Cyclomethicone 6	13.56	5.33	95.0
	Diethyl phthalate (DEP)	17.55	15.02	96.0
Sample 7	Triethylamine	2.89	17.79	92.5
Sample 8	Acetaldehyde	1.19	3.61	98.0
	Ethanol	1.28	3.49	98.0
	Dibutyl phthalate (DBP)	21.80	0.74	94.5
Sample 9	Methylhydrazine	1.20	14.17	90.5

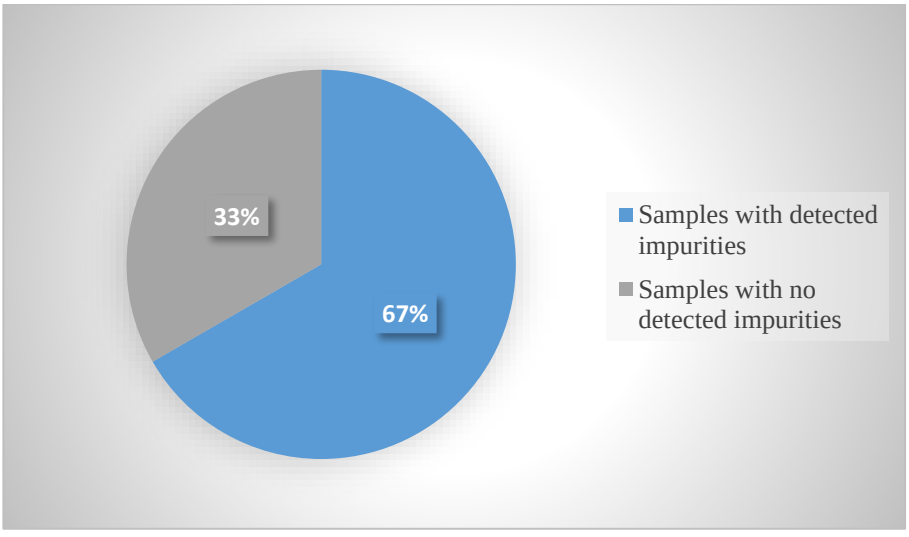

Figure 7. Percentage of intravenous samples with impurities detected.

4.3 Results from oral samples

Of all oral samples tested, impurities were detected in 8 samples out of 16 (figure 8). In addition, some excipients such as methylparaben were detected. Methylparaben is commonly used as an excipient for its antimicrobial properties (preservative) (Rowe et al., 2006). It is a common ingredient in cosmetics, food, and pharmaceutical formulations; it can be used alone or in conjunction with other parabens or antimicrobial agents(Rowe et al., 2006). Parabens are antimicrobials that work across a wide pH range and have a broad spectrum of activity, though they are particularly efficient against yeasts and molds. In oral solutions and suspensions, it use is limited to a concentration of 0.015–0.2%(Rowe et al., 2006).

Impurities detected in oral acetaminophen products are: stearates (methyl and ethyl stearate), alcohol (ethanol), organic acid (formic acid), glycidol, and diacetamate. These impurities appeared on the chromatogram in the following order (from shortest to longest retention time): Ethanol, Formic acid, Glycidol, Diacetamate, Methyl and Ethyl Stearate.

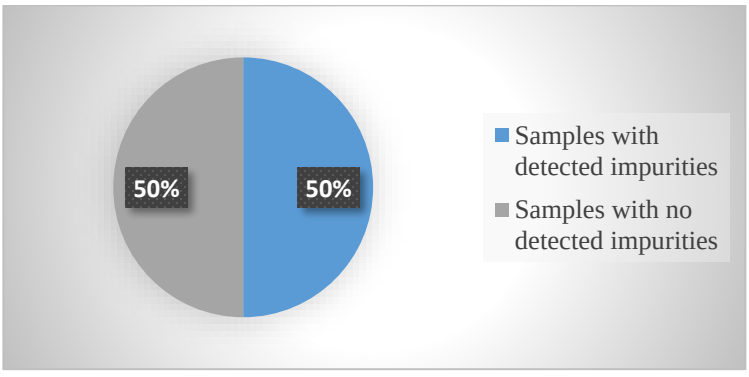

Figure 8. Percentage of oral samples with impurities detected.

Table 4. Impurities detected in oral acetaminophen samples.

Sample ID	Impurity detected	Retention time	Area %	Similarity index
sample 12	Ethanol	1.28	1.63	98.0
sample 13	Formic acid	2.30	3.83	96.0
sample 15	Methyl stearate	23.51	1.11	96.0
	Formic acid	2.26	2.86	92.0
Sample 17	Methyl stearate	23.52	1.16	97.0
	Ethyl Stearate	24.17	0.73	96.0
sample 19	Diacetamate	19.90	0.96	93.5
sample 22	Glycidol	3.17	4.29	90.0
sample 23	Diacetamate	19.87	4.25	91.0
sample 24	Formic acid	2.29	1.63	94.0

4.4 Results from both oral and IV

There is only one impurity that was detected in both oral and IV products: ethanol, which was detected in 4 different samples. Other impurities that were commonly detected (i.e. detected in more than one sample) included formic acid, detected in three oral products, methyl stearate, detected in two IV products and diacetamate, detected in two oral products (figure 9). Table 5 shows a list of the 14 detected impurities in both oral and IV products.

Table 5. List of detected impurities, their alternative names and molecular formula.

Impurity	Alternative Names	Molecular Formula
Acetaldehyde	ethyl aldehyde ethanal	CH_3CHO
Cyclomethicone 5	Decamethyl cyclopentasiloxane Dimethylsiloxane pentamer	$C_{10}H_{30}O_5Si_5$
Cyclomethicone 6	Dodecamethyl cyclohexasiloxane Cyclomethicone 6	$C_{12}H_{36}O_6Si_6$
DBP	n-Butyl phthalate Polycizer DBP	$C_6H_4(COOC_4H_9)_2$
DEP	phthalic acid diethyl ester 1,2-Diethyl phthalate	$C_6H_4(COOC_2H_5)_2$
Diacetamate	4-acetamidophenyl acetate p-acetoxyacetanilide	$C_{10}H_{11}NO_3$
Ethanol	ethyl alcohol Methylcarbinol	C_2H_6O
Ethyl Stearate	Octadecanoic acid ethyl ester Ethyl octadecanoate	$C_{20}H_{40}O_2$
Formic acid	Methanoic acid Aminic acid	CH_2O_2
Glycidol	Oxiranemethanol 2,3-Epoxy-1-propanol	$C_3H_6O_2$
Methylhydrazine	Monomethylhydrazine 2-methylhydrazine	CH_3NHNH_2
Methyl stearate	Methyl octadecanoate Stearic acid methyl ester	$C_{19}H_{38}O_2$
Triethylamine	N,N-diethyl-Ethanamine Triethylamine	$(C_2H_5)_3N$

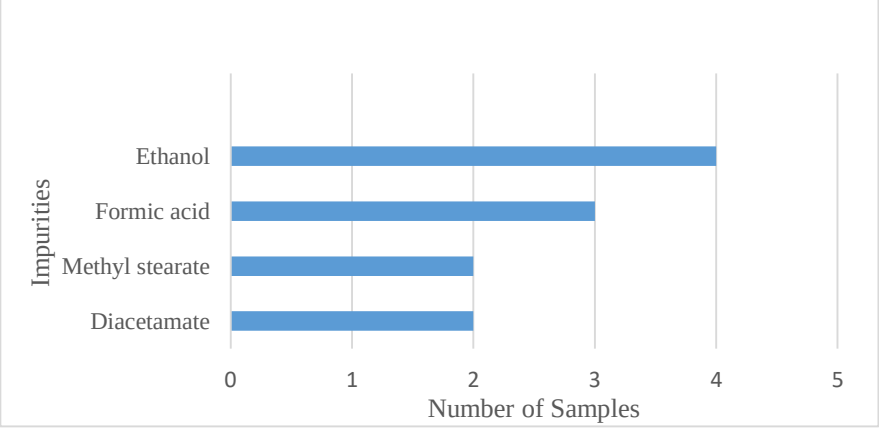

Figure 9. Commonly detected impurities.

Chapter Five

Discussion

5.1 Impurities in IV samples

5.1.1 Phthalates

Two different phthalates were detected in IV samples: DEP and DBP. It is suspected that these phthalates have leaked from the plastic container (bag or bottle) into the solution. In fact, phthalates can easily leach from plastic, as they are not part of the chain of the polymers chain that makes up the plastic (Boberg et al., 2018). Phthalates are important industrial chemicals used in a wide variety of plastic products. They are added to plastic to confer flexibility, pliability and elasticity (Boberg et al., 2018). Although phthalates and their metabolites have a short-half life in living organisms and do not accumulate in the environment, they still pose a risk to humans since they have *constant* presence in all human exposure pathways (Chou & Wright, 2006). Humans are exposed to phthalates by numerous different pathways, including: food, ambient air, indoor air, drinking water, soil, toys, aerosol sprays, cosmetics, automobile interior, pharmaceutical products, and medical devices(Chou & Wright, 2006). They are classified as top-priority pollutants by several regulatory agencies worldwide, including China National Environmental Monitoring Center (CNEMC), the United State Environment Protection Agency (USEPA), and the European Union (EU). Researchers have associated Phthalate exposure with developmental and reproductive toxicity, allergy/asthma, and carcinogenicity (Yousefzadeh et al., 2017).

Some phthalates are listed as excipients for oral dosage forms and are allowed in limited

quantities (Rowe et al. 2006). However, hospitalized patients' exposure to phthalates parenterally is of concern worldwide. Common sources of hospital exposure to phthalates include tubing products (infusion tubing system, feeding tubes, catheters ...), IV bags (medicated or saline bags), and nutrition bags(E. Erickson, 2021). Recently in February 18, 2021, members of the American congress have written a letter to the Food and Drug Administration (FDA) urging it to update its guidelines regarding phthalates in medical devices including bags and containers for intravenous use. Some pharmaceutical industries have taken the initiative to develop IV solution products that are manufactured using non-toxic plastic materials. Although phthalate free medical devices exist, these only account for less than 40% of the bags used in US hospitals(E. Erickson, 2021).

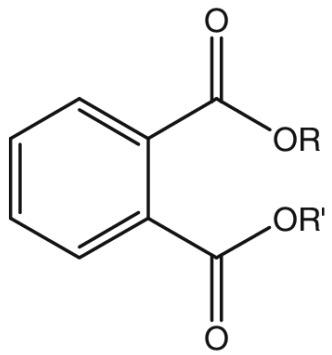

Figure 10. General Structure of Phthalates.

DEP is an important industrial chemical, used in perfumery, as a solvent, denaturant and in the manufacturing of varnishes and dopes (Rowe et al., 2006). In the pharmaceutical industry, DEP is commonly used as an excipient in oral pharmaceutical formulations (Rowe et al., 2006). It is included in the FDA Inactive Ingredients Guide (oral capsules, delayed action, enteric coated, and sustained action tablets) and is included in non-parenteral medicines licensed in the UK, according to the handbook of pharmaceutical excipients (Rowe et al., 2006). It is generally used as a plasticizer in the film coating of

tablets, beads and granules at concentrations of 10–30% by weight of polymer. At these levels, exposure to DEP is regarded as harmless (Rowe et al., 2006). However, toxicities have been identified on higher levels of exposure. In a literature review on the toxicity of DEP, researchers have concluded that DEP can cause androgen-independent male reproductive toxicity (i.e., sperm effects) as well as developmental toxicity and hepatic effects, with some evidence of female reproductive toxicity (Weaver et al., 2020). It is also proven that consumption in high quantities can have a narcotic effect and causes paralysis of the central nervous system (Rowe et al., 2006). Repeated exposure to DEP may cause damage to the nervous system, leading to numbness and weakness in the extremities.

DBP is another commonly used phthalate. It is used in cosmetics, as an insect repellant among other uses (Rowe et al., 2006). It is employed in pharmaceutical formulations as a plasticizer in film coating. It is included in Included in the FDA Inactive Ingredients Guide (oral delayed action, enteric coated, tablets) and in non-parenteral medicines licensed in the UK (Rowe et al., 2006). In Europe, DBP is classified as a reprotoxic substance and its use is prohibited in cosmetic products, restricted in toys and childcare articles as well as food containers. With respect to medical devices, the directive encourages the replacement of phthalates in medical devices. If DBP is to be used, all devices are to be labelled and the manufacturers should justify its presence in devices intended for use in children, pregnant or nursing women (Rowe et al., 2006). According to the “Guideline on the use of phthalates as excipients in human medicinal products” (2014) , Studies have shown that DBP is associated with reduced fertility due to testicular

atrophy and reduced sperm production, as well as external, skeletal and visceral malformations.

5.1.2 Cyclomethicones

Two other major impurities that were detected in IV samples are cyclomethicones 5 and 6. Cyclomethicone is a generic name for several cyclic dimethyl polysiloxane compounds with a general structure shown in figure 11. They are widely used in cosmetics and in topical pharmaceutical formulations. they are also employed in oral pharmaceutical formulations, but to a lesser extent. Different cyclomethicones (with different n) are usually combined together, which explains that both cyclomethicone 5 and cyclomethicone 6 were detected in the same sample (sample 6). Cyclomethicones are included in the FDA inactive ingredients guide (oral powder for reconstitution), in nonparenteral medicines licensed in the UK, as well as in the Canadian List of Acceptable Non-medicinal Ingredients. the safety of cyclomethicones has been extensively assessed by several routes including oral, inhalation, dermal, and ocular. In these studies, cyclomethicones had very minimal absorption and were found to be safe with only minimal or no toxicity. However, to out knowledge, no studies were conducted on intravenous cyclomethicones. Therefore, it remains ambiguous whether these impurities found in sample 6 pose a risk to human health or not. Consequently, the source of the

impurities and their levels should be controlled (Toxicology and Industrial Health, 2017; Johnson et al., 2011).

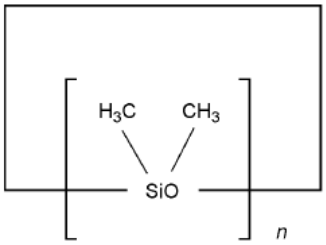

Figure 11. General Structure of cyclomethicones.

5.1.3 Methylhydrazine

Methylhydrazine is largely employed in military applications as a high-energy fuel, as a rocket propellant and thruster fuel, and as a fuel for small electrical power generating units. Methylhydrazine is also employed as a solvent and a chemical intermediate (‘’Methylhydrazine’’, 2022). Regulatory authorities around the world have already warned from occupational exposure to methylhydrazine, which can happen via different routes including inhalation, skin absorption, ingestion, skin and/or eye contact (‘’Methylhydrazine’’, 2022). Exposure to methylhydrazine is associated with irritation to the eyes and skin, irritation to the respiratory system manifested as vomiting and diarrhea, tremor, ataxia, anoxia, cyanosis, as well as convulsions (‘’Methylhydrazine’’, 2022). Moreover, according to the National Institute for Occupational Safety and Health (NIOSH) (2019), methylhydrazine is classified as a potential occupational carcinogen in humans. Animal studies have associated methylhydrazine with lung, liver, blood vessel & intestine tumors. Methylhydrazine is listed on the NIOSH list of compounds immediately dangerous to life or health (IDLH). No doubt, IV exposure to methylhydrazine can be very toxic to humans. The presence of such a genotoxic impurity

in IV products cannot be tolerated and immediate interventions should be taken by the respective manufacturer.

5.1.4 Triethylamine

Triethylamine is an aliphatic amine that is utilized as a solvent in the manufacturing of many pharmaceutical products (Raghuram et al., 2010). The presence of organic residual solvents in the final pharmaceutical product should be kept to a minimum and must be controlled. The European Directorate of Quality Medicines Document (EDQM) has set limits to the amount of solvent that can be tolerated by on toxicity data. For triethylamine, it should be kept to a limit of 320 ppm (Raghuram et al., 2010).

5.1.5 Acetaldehyde

Acetaldehyde was detected in sample 8 along with Ethanol and Dibutyl phthalate (DBP). Several researchers have developed methods for the detection and quantification of acetaldehyde in biological fluids. Acetaldehyde exposure is of particular importance since it has been linked to a variety of diseases, including diabetes, cardiovascular disease, neurodegenerative disorders, as well as cancer. Aldehydes are strong electrophiles and readily react with the nucleophilic sites in the Deoxyribonucleic acid (DNA) and proteins to cause reversible and irreversible modifications (Fang & Vaca, 1997). These modifications, if not eliminated or repaired, can lead to alteration in cellular homeostasis, cell death and ultimately contribute to disease pathogenesis (Fang & Vaca, 1997). Acetaldehyde exposure can happen through a variety of sources: it is the major component in cigarette smoke, an oxidative by-product of ethanol in alcoholic beverages, it is emitted from residential fireplaces and wood stoves, bush fires, and agricultural burning (Guan et al., 2012). Since acetaldehyde is readily volatile, inhalation exposure poses a significant

risk. Acetaldehyde has been classified as Group 1 human carcinogens by the International Agency for Research on Cancer (IARC) (Fang & Vaca, 1997; Guan et al., 2012).

5.1.6 Ethanol

Ethanol is used in medicine for a variety of reasons. It could be a solvent, preservative, or dermal penetration enhancer, for example. It is possible that ethanol is present in IV acetaminophen samples because it was used during the manufacturing or as a contaminant during the preparation of the samples before testing. Despite the fact that ethanol can impair cognitive and psychomotor functioning, ethanol exposure when used as an excipient in pharmaceuticals is usually minimal (*Information for the package leaflet*, 2018). Ethanol intake in medicines normally results in significantly lower exposures than drinking alcoholic beverages (*Information for the package leaflet*, 2018). Despite the fact that exposure is usually limited, the impact of long-term exposure to even low amounts of ethanol in medicines on children's health and development has not been studied. Excessive alcoholic beverage consumption during pregnancy can lead to fetal alcohol syndrome (FAS), which can cause learning and behavioral issues in the baby. This raises the possibility that chronic ethanol consumption during childhood may pose a developmental risk, at least theoretically. After exposure to ethanol from pharmaceuticals, increased levels of the metabolite acetaldehyde have been detected in neonates (Pandya et. al, 2016). Although the clinical relevance of this finding is unknown, it is a cause for caution. The EMA has set a guidance on the reporting of ethanol content as an excipient(*Information for the package leaflet*, 2018).

5.2 Impurities in oral samples

5.2.1 Formic acid

Formic acid was one of the most commonly detected impurities: it was detected in three oral samples; samples 13, 15 and 24. Formic acid is an organic acid. A review on “reactive impurities in excipients” determined the presence of formic acid in polyethylene glycol, hydroxypropyl methylcellulose, povidone and polyvinyl alcohol (Wu et al., 2011). These are of the most commonly used excipients, and they are used in the samples where formic acid was detected. Therefore, the source of formic acid might be the starting material. Formaldehyde, also present as an impurity in excipients, can be air oxidized into formic acid at high temperatures (for example during accelerated stability testing). Several studies were conducted to test the interaction of formic acid with the API and how its presence can affect the stability of the drug product (Wu et al., 2011). Formic acid can react with the amine group in the API and cause *N*-formylation or *N*-methylation, it can accelerate the degradation of the API, or cause nonspecific degradation. These interactions between impurities in the excipients and the API can have detrimental effect on the quality of the drug product (Wu et al., 2011). It is therefore essential to conduct compatibility and stability studies and avoid the use of excipients that might affect the quality of the drug product.

5.2.2 Stearic acid esters

Methyl stearate was detected in sample 15 ad 17, while ethyl stearate was detected in sample 17. According to the handbook of pharmaceutical excipients (Rowe et al., 2006), stearic Acid, otherwise known as octadecanoic acid, is a saturated long-chain fatty acid with an 18-carbon backbone. Stearic acid is frequently utilized in pharmaceutical formulations, both oral and topical. It's mostly employed as a tablet and capsule lubricant in oral formulations, though it can also be used as a binder or in combination with shellac

as a tablet coating. Stearic acid has also been suggested as a possible drug carrier for sustained-release formulations. As a tablet lubricant, it is employed in a concentration of 1-3% (Rowe et al., 2006).

Stearic acid can undergo Esterification, a reaction between the carboxylic acid group with an alcohol, to form stearic acid esters, such as methyl and ethyl stearate (figure 12). Methyl stearate was detected in samples 15 and 17, while ethyl stearate was detected in sample 17. In both samples 15 and 17, stearic acid is listed as an excipient. Therefore, we expect the source of stearate esters to be an esterification reaction between stearic acid and methanol/ethanol.

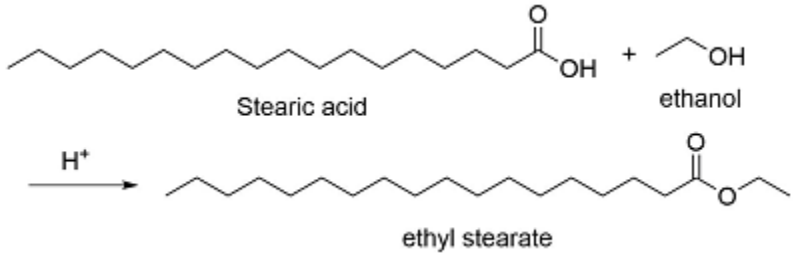

Figure 12. Esterification of stearic acid reaction.

The Material Safety Data Sheets (MSDS) of methyl stearate indicates that acute accidental ingestion, in large quantities, may produce localized irritation of the oral or gastrointestinal lining and induce vomiting and mild diarrhea ("*Material Safety Data Sheet, Ethyl Stearate"*, 2007; "*Methyl stearate"*, 2012). Long-term exposure to the product is not thought to produce adverse health effects (as classified using animal models). Ethyl stearate is also expected to have a low ingestion hazard. Therefore, the presence of stearic acid ester can be regarded as non-dangerous in oral acetaminophen formulations ("*Material Safety Data Sheet, Ethyl Stearate"*, 2007; "*Methyl stearate"*, 2012).

5.2.3 Diacetamate

Paracetamol Diacetamate

Figure 13. Chemical structure of diacetamate versus acetaminophen.

Diacetamate, also known as 4'-Acetoxyacetanilide or Acetaminophen Related Compound A, has a chemical structure similar to acetaminophen, with an acetoxy group attached. It is one of the main process-related impurities found in acetaminophen (Kamberi et al., 2004). The synthesis of acetaminophen can happen from a reaction between 4-aminophenol and acetic anhydride, as previously discussed in section 1.2. Acetic anhydride present in the media then reacts with acetaminophen, to form diacetamate as a by-product (Payne, 2020).

5.2.4 Glycidol

Glycidol is an epoxide used as a chemical intermediate in the production of functional epoxides, glycidyl urethanes, pharmaceuticals and other products. Exposure to glycidol can happen via different routes: inhalation, ingestion, and dermal (Foroumadi & Emami, 2014). Exposure to glycidol has been a concern worldwide due to its carcinogenic potential: it is classified as Group 2A, or probably carcinogenic in humans (‘Glycidol’, 2000). Carcinogenicity resulted from oral administration of glycidol in mice, rats as well as hamsters. After oral administration to mice, it produced increase in tumors of the Harderian gland in both males and females, of the forestomach, lung, liver and skin in males, and of the mammary gland and subcutaneous tissue in females. In rats, it produced increases in the incidence of gliomas of the brain and forestomach tumors in both males

and females. Mesotheliomas of the tunica vaginalis/peritoneum, as well as tumors of the intestine, skin, thyroid gland and Zymbal gland were increased in males (‘Glycidol’, 2000). Tumors of the clitoral gland, mammary gland and oral mucosa as well as leukemia were increased in females. In hamsters, there was a marginal increase in the incidence of splenic haemangiosarcomas after oral administration. Intra-amniotic injection of glycidol in rats increased the frequency of resorptions and, at high doses, limb malformations (‘Glycidol’, 2000).

The ICH has issued a guidance for the industry, titled “M7 (R1) Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk” in which glycidol was one of the mutagenic impurities discussed (2018). It is expected that the primary source of glycidol in pharmaceuticals is a reaction between glycerol and sugars, catalyzed by heating. The ICH has set an acceptable lifetime intake of 4 µg/day while exposure from food *alone* is expected to be between 20 and 80 µg/day. Based on the risk that glycidol poses to human health, pharmaceutical industries should take an immense effort to control its presence.

5.3 Interactions in co-existent impurities

The toxicological review discussed in the previous slides analyzes the risks imposed by each impurity alone. However, our results show that some of the formulations contain several impurities along with the API. Exposure to impurities happens from several different sources other than the analyzed formulation: polypharmacy, environmental exposure, air pollution, cigarette smoke, food…These impurities might have additive or synergistic effects. therefore, several questions are yet to be answered: Is there an

interaction between co-existent impurities? Is there an interaction between impurities and API? What is the impact on formulation stability/quality/safety?

Chapter six

Conclusion

The research study performed is the first to develop an analytical technique for the detection of volatile organic impurities in IV acetaminophen samples. In addition, samples available on the Lebanese market, including oral samples manufactured in Lebanon, have never been previously analyzed. The limitations of this research paper are: possibility of sample contamination with ethanol (from the sample preparation process); the ability to detect volatile impurities only, a limitation related to the analytical technique used; finally, due to the lack of materials and resources, we were unable to do quantitative analysis. However, many of the detected impurities are highly dangerous and are not allowed in the formulations even in trace amounts. Therefore, we were able to draw robust conclusions from the qualitative analysis performed. The results of this research have highlighted the importance of impurity profiling as a guide for pharmaceutical industries to limit the presence of impurities in their final product. Impurities were detected in 8 out of 16 oral samples, and in 6 out of 9 intravenous samples. Although regulatory authorities from all around the world have established robust guidance for the industry, the results of this study suggest that immense effort is still needed to ensure safe medication delivery to patients. Several of the detected impurities can cause serious damage to human health and are highly toxic, including reproductive toxicity, nephrotoxicity, hepatotoxicity, neurotoxicity (convulsions), carcinogenesis, and teratogenicity. We therefore present this thesis project as a call to the pharmaceutical industry to limit impurities in their end product in an attempt for medicine to be the road to cure rather cause illness.

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Appendix 1: Sample Datasheet

Sample #	Expiry date	Man. Country	dosage form	Strength	Excipients
1	2022-05	France	injectable solution	10 mg/ml	Sodium acetate, Acetic acid, Sodium hydroxyde, water for injection
2	2022-10	France	injectable solution	10 mg/ml	Sodium acetate, Acetic acid, Sodium hydroxyde, water for injection
3	2022-10	France	injectable solution	10 mg/ml	Sodium acetate, Acetic acid, Sodium hydroxyde, water for injection
4	2022-10	France	injectable solution	10 mg/ml	Sodium acetate, Acetic acid, Sodium hydroxyde, water for injection
5	2022-05	France	injectable solution	10 mg/ml	Sodium acetate, Acetic acid, Sodium hydroxyde, water for injection
6	2022-06	Spain	injectable solution	10 mg/ml	Mannitol, sodium citrate dihydrate, acetic acid glacial, water for injections.
7	2021-06	Italy	injectable solution	10 mg/ml	Mannitol, cysteine hydrochloride monohydrate, disodium phosphate dihydrate, sodium hydroxide, hydrochloric acid, water for injections.
8	2021-06	Italy	injectable solution	10 mg/ml	Mannitol, cysteine hydrochloride monohydrate, disodium phosphate dihydrate, sodium hydroxide, hydrochloric acid, water for injections.
9	2021-06	Italy	injectable solution	10 mg/ml	Mannitol, cysteine hydrochloride monohydrate, disodium phosphate dihydrate, sodium hydroxide, hydrochloric acid, water for injections.
10	2023-01	Lebanon	tablets	500 mg	Starch, stearic acid, povidone, hydroxypropyl methylcellulose, titanium dioxide, talc, polyethylene glycol.

11.0	2023-02	Belgium	tablets	1000 mg	Hydroxypropylcellulose, croscarmellose sodium, glyceryl behenate, magnesium stearate, colloidal anhydrous silica, hypromellose, titanium dioxide, proplylene glycol
12	2024-01	Lebanon	tablets	500 mg	Methylparaben, Mannitol, cysteine hydrochloride monohydrate, disodium phosphate dihydrate, sodium hydroxide, hydrochloric acid.
13	2024-05	Lebanon	tablets	500 mg	Maize starch, Purified talc, Pregelatinised starch, povidone, stearic acid, potassium sorbate
14	2023-05	Australia	tablets	500 mg	Maize starch, Purified talc, Pregelatinised starch, povidone, stearic acid, potassium sorbate
15		Ireland	tablets	500 mg	Maize starch, potassium sorbate, purified talc, stearic acid, polyvidone, starch pregelatinised, hypromellose, triacetin and carnauba wax.
16	2023-10	France	tablets	1000 mg	Sodium starch glycolate (Type A Primogel) Povidone K30 (E1201) Pregelatinized maize starch Stearic acid (E570)
17	2024-05	Lebanon	tablets	500 mg	Maize starch, potassium sorbate, purified talc, stearic acid, polyvidone, starch pregelatinised, hypromellose, triacetin and carnauba wax
18	2023-08	France	tablets	1000 mg	Hydroxypropylcellulose, croscarmellose sodium, glycerol behenate, magnesium stearate, colloidal anhydrous silica.
19	2020-11	France	Effervescent tablets	500 mg	OPADRY OY-S-38901, hypro-mellose (E464), titanium dioxide (E171), propylene glycol (E1520).
20	2024-06	Lebanon	tablets	500 mg	Maize starch, potassium sorbate, purified talc, stearic acid, polyvidone, starch pregelatinised, hypromellose, triacetin and carnauba wax

21	2022-05	Belgium	Effervescent tablets	1000 mg	Anhydrous citric acid, sodium bicarbonate, sodium carbonate, sorbitol (E420), sodium docusate, povidone, sodium benzoate, aspartame (E951), potassium acesulfate, 7/8 grapefruit flavor, orange flavor
22	2023-03	Belgium	effervescent tablets	1000 mg	Anhydrous citric acid, sodium bicarbonate, sodium carbonate, sorbitol (E420), sodium docusate, povidone, sodium benzoate, aspartame (E951), potassium acesulfate, 7/8 grapefruit flavor, orange flavor
23	2023-02	France	effervescent tablets	500 mg	anhydrous citric acid, sodium bicarbonate, sorbitol, anhydrous sodium carbonate, sucrose for compression, crospovidone, sodium benzoate, orange flavor, aspartame, acesulfame potassium.
24	2022-11	United Arab Emirates	tablets	500 mg	Maize starch, potassium sorbate, purified talc, stearic acid, polyvidone, starch pregelatinised, hypromellose.
25	2022-02	Lebanon	tablets	500 mg	Maize starch, potassium sorbate, purified talc, stearic acid, polyvidone, starch pregelatinised, hypromellose, triacetin.