# Leaching of phthalates from Plastic packaging into Fruit Juices marketed in Pakistan and their risk assessment

**Noshad Razzaque**
National Centre of Excellence in Analytical Chemistry
**Farah Naz Talpur** ( farah.talpur@usindh.edu.pk )
National Centre of Excellence in Analytical Chemistry https://orcid.org/0000-0002-3208-7302
**Hassan Imran Afridi**
National Centre of Excellence in Analytical Chemistry
**Mehr Un-Nissa Abbasi**
National Centre of Excellence in Analytical Chemistry
**Sobia Kunbhar**
National Centre of Excellence in Analytical Chemistry
**Zaheer Chandio**
Shaheed Benazir Bhutto University Shaheed Benazirabad

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# Abstract

Phthalate esters (PAEs) also known as plasticizers, are used to improve flexibility in a variety of food packaging, including plastic containers, beverage bottles, and bags. PAEs are endocrine-disrupting compounds (EDCs) that can have serious consequences on human health. The purpose of this study is to investigate the leaching of PAEs from plastic packaging into several juice brands (n = 225), including citrus fruit juices, berry fruit and mixed fruit juices, and stone fruit juices. To clean the juices, a silica gel-packed column was used. In addition, plastic bottles were characterized by FTIR, which confirms the presence of PAEs by evaluating the aromatic esters and C-H wagging peaks at 1713 $cm^{-1}$ and 722 $cm^{-1}$ respectively. Furthermore, DSC analysis of juice bottles revealed distinct Tg and Tm values for juice bottles that are amorphous. All of the liquids were acidic, with pH levels ranging from 2.25 to 3.73. According to chromatographic analyses, mixed fruit juices had the most PAEs leaching (71.93g/L), followed by strawberry juices (31.35 g/L), citrus fruit juices (20.64 g/L), and fleshy fruit juices (18.61g/L). Different PAEs such as DPrP, DiBP, DPeP, DCHP, BBzP, DEHP, and DOP were validated by GC-MS. Estimated daily intakes (EDIs) of the PAEs from fruit juices ranged from 0.087 to 0.543 µg/kg/bw. Non-carcinogenic risks concerning PAEs in juices were found to be within safe levels (less than one). DEHP in fruit juices exhibited the potential carcinogenic effects on humans.

# Introduction

PAEs are also called the diesters of 1, 2- benzenedicarboxylic acid (phthalic acid), and have been utilized to increase the durability and transparency of plastic products (Hab et al. 2018). Since the 1930s, PAEs have been widely used as polymer additives in plastics, primarily in the production of polyvinyl chloride (PVC) and polyethylene terephthalate (PET), but they're also used in the production of rubber, cellulose, and styrene (Salazar-Beltrán et al. 2018).

These PAEs are used in many products such as medical devices, toys, cosmetics, and plastic packaging (Xue et al. 2021), which contributes 20–40% of DEHP by mass (Guo et al. 2010, Liu et al. 2008).While di-isobutyl phthalate (DBP) is commonly used in the production of cellulose plastics and dye solvents. Butyl benzyl phthalate (BBzP), di-isononyl phthalate (DiNP), and di-isodecyl phthalate (DiDP) are mostly utilized in food packaging products, floorings, and other building materials and medical devices (Sakhi et al. 2014). Moazzen et al. (2018) describe how PAEs bind to plastic materials through weak secondary molecular attraction. Hence, they are not chemically attached to polymers and can be easily leached from them (Moazzen et al. 2018). Humans are exposed to them through water, food, air, soil, and dust, and they are swallowed, breathed, or absorbed through the skin. Ingesting contaminated food, on the other hand, is one of the most common ways for humans and animals to become infected (Fan et al. 2019).

Diethyl Hexyl Phthalate (DEHP) is one of the most studied PAEs because of its detrimental health effects (Kiralan et al. 2020). PAE exposure in susceptible people such as pregnant women, babies, and toddlers has also been investigated. PAEs increase the risk of miscarriage, premature birth, and gestational hypertension during pregnancy (Mu et al. 2015). Hand-to-mouth action, mobility, food, and a variety of other factors all contribute to PAE exposure in infants and children (Huang et al. 2009, Rodrguez-Carmona et al. 2020).

In different countries like Canada and the United States, as well as the European Union, has limited or prohibited the use of PAEs in consumer items (Al-Saleh et al. 2014), still, PAES are being used in the manufacturing of plastic bottles.

PAEs in drinking water are becoming very well-known. According to studies, PAEs are consumed through drinking water and consuming food. Fromme et al. (2002) suggest that PAEs may be present in municipal drinking water if the distribution system is made of polymer materials. PAEs can also be exposed to humans through the use of a face mask, resulting in skin disorders (Xie et al. 2022).

PAEs have been found in beverage samples like Yin et al, investigated the leaching of DPHP, BBP, DiBP, DBP, DCHP, DEHP, DnOP, and DNNP in tea beverages (0.64 – 0.23 µg/L) (Yin et al. 2019), Bosnir et al, studied the migration of DMP, DBP, and DEHP in PET-bottled soft drink preserved with potassium sorbate (9-759 µg/L) (Bošnir et al. 2007), while Al-Salih et al, examined the leaching of DEP, DBP, BBzP, DEHP, DOP in fruit juices (0.39–0.855 µg/L) (Al-Saleh, Elkhatib and Characterization 2014). In Pakistan, the beverage industry has risen significantly in recent years and various brands of different fruit juices are now available in disposable plastic bottles. According to (Annamalai et al. 2017) some factors such as storage time, pH, and temperature influence on leaching of PAEs (4 and 37 C˚) into beverages from plastic packaging. The PAEs’ migration process depends on their concentrations in the packaging polymer, the acidity of content in contact with packages, the lipid content, and on the time and temperature of the contact (Rastkari et al. 2018). For example, n-butyl benzyl phthalate (BBP), DBP, and DEHP concentrations in drinking water increased after 10 weeks of storage in PET bottles at an outside temperature of up to 30 C, according to a case study (Casajuana and Lacorte 2003).

Annamalai et al., have determined the effect of pH and storage temperature on the leaching of phthalate esters from plastic containers. The authors have found that acidic pH (2.7) of beverages with increasing storage had significantly higher leaching of PAEs (1.3–1.6 µg/L vs. 0.59–1.09 µg/L) than the drinking water with pH 6.5 at the same storage time. In Pakistan during the summer season ( March to June), the average temperature reached 42 ºC to 44 ºC (Chaudhry 2017), which not only tiger the consumption of juices but possible leaching of PAES due to improper storage of juices avoiding cold storage. No previous work has been done on the leaching of PAEs into juices from Pakistan, hence the present study was undertaken to characterize the juice plastic bottles for the presence of PAEs by FT-IR and DSC analysis and quantification of leached PAEs by GC-FID and GC-MS techniques from plastic bottles to various brands of juices. In addition, estimated daily intakes of PAEs from juices were calculated.

# Materials And Methods

Reagents and materials

DMP, DMIP, DEP, DPrP, DBP,DiBP, BzBP, DPeP DCHP, DEHP, DOP standards of high purity (99% pure) were purchased from AccuStandard, Inc (New Haven, CT, USA), Dichloromethane and Hexane (analytical grade) were acquired from Daejung (USA). Analytical grade acetone was purchased from Sigma-Aldrich and activated silica gel was obtained from Merck (Darmstadt, Germany). A total of 15 fruit Juice brands i.e. Nestle, Polly, Maza, Cappy, Joiner, Smile, Mogu Mogu, Shezan, Fruiti-O, and Slice were purchased from local supermarts and stored at 4 ºC till analysis. Five samples of each brand were collected and analyzed in triplicate (n = 225). Fruit juices were classified into four categories (1) Citrus fruit juices, (2) stone fruit juices (3) Berry fruit & mix fruit juices, and (4) fleshy fruit juices.

## Sample Coding

To avoid any ethical issues, samples were labeled without a brand name. Citrus fruit juices were coded C-1, C-2, C-3, C-4; berry fruit and mix fruit juices were coded BS-1, BG-1, BM-1; stone fruit juices, such as mangoes and peaches, were coded M-1, M-2, P-1, P-2; and fleshy fruit juices were coded A-1, A-2, L-1, L-2 for apples and lychee.

## Ft-ir Characterization

Following the preceding procedure by Mecozzi et al. (2016), Plastic bottles were chopped into small pieces (2 cm) with scissors, rinsed with hexane, and FTIR-ATR analysis was performed on these fragments. OMNIC software was used to scan 32 scans in the mid-IR (4000–650 cm-1) with a resolution of 4 cm-1 using the FTIR-ATR with diamond ATR crystals (7.2 Version).

## Differential Scanning Calorimetric (Dsc) Analysis

The best method for determining the flow of heat and temperature of materials as a function of temperature and time is to use DSC. It can also be used to provide data on endothermic and exothermic processes. The glass transition (Tg), melting temperature (Tm), and crystallization temperature are all determined via DSC (Tc). As part of the reported approach by (Kong and Hay 2002), a DSC instrument was used to conduct thermal profiling of PET bottles. Each plastic sample was chopped into small pieces of about 5.5 mg and evaluated using a nitrogen gas flow rate of 5 ml/min and a temperature program of 25 oC to 300 oC at a rate of 10 oC/min.

## Extraction Of Leached Paes

Leached PAEs were extracted from fruit juices by using liquid-liquid extraction as per the reported method (Salazar-Beltrán et al. 2018). 20 ml of each juice sample was taken in a separating funnel and extracted with the addition of 20 ml of dichloromethane (repeated three times and pooled). Then volume was evaporated completely in a rotatory evaporator at the temperature of 40°C, Then the residue was re-dissolved in 2 mL of dichloromethane before it was cleaned up in a silica gel-packed column.

## Clean Up Procedure

A 50 mL glass chromatographic column was filled with 5 g of silica gel (50 ml, 13.5 mm ID). The column was tapped to settle the silica gel and ensure correct column packing. Following that, 1 g of Na2SO4 was added to the top of the silica gel column, which was then pre-eluted with 10 ml of hexane before the re-dissolved extract was put into the column. Non-polar components were first eluted with 15 mL of hexane to eliminate them. Following that, 20 mL of acetone was used to elute PAEs, which were then confirmed using GC-FID and GC-MS.

## Gas Chromatographic Analysis

For phthalate separation, a gas chromatograph (Perkin Elmer Model GC-8700) with a DB-5 column (30m length, 0.25 mm ID) was utilized. Nitrogen was used as the carrier gas, and 2 liters of each sample volume were injected for analysis. The oven temperature was maintained at 130°C to 220 ºC at a ramp rate of 3 ºC/min and held for 8 minutes. The injector temperature and detector temperatures were 260 °C and 270 °C.

## Quantification Of Paes By Calibration On Gc-fid

For PAE quantification, a standard mixture of PAEs such as DMP, DMIP, DEP, DPrP, DiBP, BzBP, DPeP, DCHP, DEHP, DOP was preserved in the range of 6.25–100 g/ml. For all analytes, a good coefficient of determination ($R^2 > 0.99$) was observed, as shown in Table 1.

Table 1
Analytical figures of merits for calibration of PAEs

| **PAEs** | **Linear equation** | **$R^2$** |
|---|---|---|
| DMP | 0.0484 × + 0.226 | 0.999 |
| DMIP | 0.0493 × + 0.336 | 0.995 |
| DEP | 0.0508 × + 3.136 | 0.996 |
| DPrP | 0.5078 × + 1.136 | 0.996 |
| DBP | 0.4551 × + 2.681 | 0.978 |
| DiBP | 0.4632 × + 2.381 | 0.991 |
| BBzP | 0.5321 × + 3.451 | 0.989 |
| DPeP | 0.478 × + 2.783 | 0.993 |
| DCHP | 0.4556 × + 2.791 | 0.992 |
| DEHP | 0.4358 × + 2.837 | 0.994 |
| DOP | 0.4247 × + 2.919 | 0.990 |

[Insert Table 1 about here]

## Conditions For Gc-ms Analysis

TRACETM GC from Thermo Scientific PAEs leached from plastic materials into juices were identified using ultra gas chromatography with a Thermo Scientific ISQTM GC single quadruple pole mass spectrometer. At the column, helium with high purity was utilized as the carrier gas, with a flow rate of 1.0 ml/min, an oven temperature of 80°C (1 minute), and a ramp of 10°C/min to 320°C with an 8-minute hold. The temperature of the MS transfer line was 300°C, whereas the temperature of the sources was kept at 260°C, 70 EV was employed as electron ionization energy with a mass range of 40–400 m/z.

## Estimation Of Exposure To Paes

The following equation was used to calculate the daily intake of PAEs by the Pakistani population through the use of fruit juices (Guo et al. 2011).

$EDI = CQ/bw$

Where $C$ is the concentration of PAEs in Juices, $Q$ is the average amount of daily intake of juices while bw (kg) is average body weight. For Pakistani adults, 66 kg and 0.5 L were used as the *bw* and Q values respectively.

# Risk Assessment

The following equation was used to calculate the hazard index (HI), which was used to estimate non-cancer risks:

$HI = EDI/RfD$

where $RfD$ is the reference dose for the non-carcinogenic health risk of a chemical proposed

by the guidelines. The $RfD$ of DiBP, BBzP, DPeP, DEHP and DOP is 28.7, 200, 66.6, 20 and 40 μg/kg/b (Abdolahnejad et al. 2019, Vimalkumar et al. 2022). HI of less than one shows the safety concerns (Wang et al. 2021). The carcinogenic risks (CRs) of the PAEs for adults from the fruit juices were conducted with the following equation:

# CR = EDI × CFS

Where CFS is a slope factor of exposure to the PAEs. The CFS value for DEHP is 0.014 $(\text{mg/kg-bw/day})^{-1}$ (Xie et al. 2022)

# Results And Discussion

FTIR-ATR characterization of plastic bottles

FTIR-ATR was utilized to assess all plastic juice bottles in the region of 650–4000 cm1 in the current study (Fig. 1). This method is used to provide data on the functions of plastic materials. Figure 2 shows a peak at 1713 cm-1 in the characterization of plastic bottles, indicating the presence of carbonyl compounds in plastic bottles.

[Insert Fig. 1 and Fig. 2 here]

Furthermore, as shown in Fig. 2, the presence of PAEs was confirmed by peaks such as C–H wagging at 722 cm-1, aromatic C–H out of a plane at 871 cm-1, C–H bending in a plane at 1015–1017 cm-1, and a C = O ester peak at 1713 cm-1. Similarly, (Vijayakumar et al. 2012) investigated the carbonyl group with an aromatic ring at 1713 cm-1 and aromatic C-H wagging at 722.6 cm-1 for the validation of PAEs in trash PET bottles. Table 2shows the details of the characteristic bands found in each brand of juice plastic bottle evaluated in the current experiments.

Table 2
FTIR characteristics peaks in all plastic bottles of juices.

| Sample ID | Aromatic C – H wagging | Aromatic C – H out of the plane | C – H bend in the plane | C – C – O Asymm | C – H def. in alkane | C = O ester |
|---|---|---|---|---|---|---|
| C-1 | 722.6 | 871.9 | 1016.7 | 1241.6 | 1408.1 | 1713.29 |
| C-2 | 722.6 | 871.8 | 1016.7 | 1241.8 | 1408.6 | 1713.70 |
| C-3 | 722.5 | 871.9 | 1016.7 | 1242.6 | 1407.9 | 1713.04 |
| C-4 | 722 | 871.5 | 1016.4 | 1240.6 | 1408.1 | 1712.53 |
| BS-1 | 722.6 | 871.7 | 1016.9 | 1242.4 | 1408.4 | 1713.10 |
| BG-1 | 722.6 | 871.8 | 1017 | 1242.2 | 1408.6 | 1713.40 |
| BM-1 | 722.6 | 872.6 | 1016.2 | 1240.2 | 1407.9 | 1713.30 |
| M-1 | 722.3 | 872.2 | 1015.8 | 1239.3 | 1407.6 | 1713.64 |
| M-2 | 722.2 | 871.8 | 1016.5 | 1241.5 | 1408.2 | 1714.54 |
| P-1 | 722.3 | 871.7 | 1016.5 | 1240.9 | 1408.3 | 1713.91 |
| P-2 | 721.4 | 871.1 | 1016.3 | 1239.8 | 1408.1 | 1712.33 |
| A-1 | 722.9 | 872.2 | 1016.8 | 1241.9 | 1408.2 | 1713.94 |
| A-2 | 722.7 | 872.3 | 1016.7 | 1241.6 | 1408.4 | 1713.73 |
| L-1 | 722.1 | 871.9 | 1016 | 1239.2 | 1407.8 | 1712.86 |
| L-2 | 722.1 | 871.7 | 1016.8 | 1241.5 | 1408.6 | 1713.31 |

[Insert Table 2 about here]

## Thermal profile of PET juice bottles by DSC analysis

For PET juice bottles, the glass transition temperature (Tg) was calculated to be between 38.30 and 97.55 ºC. The temperature at which polymers transition from a glassy to a rubbery state is known as Tg. Glassy states are described as brittle and unyielding, whereas rubbery states are described as flexible and squishy. Plasticizers in polymer materials diminish it while intermolecular forces of attraction such as hydrogen bonding, dipole-dipole interactions, induction forces, and others increase it. Rand co-polymerization in polymers generates chaos and diminishes molecular packing, so it's also diminished.

Cross-linking increases Tg, causing polymer materials to become more restricted and rigid. The chemical structure of epoxy resin, the type of hardener, and the degree of curvature are all elements that go into determining the Tg. Scientists previously discovered Tg values in the range of 67 to 81 ºC during studies on the crystallization behavior of PET materials (Demirel et al. 2011, Shabafrooz et al. 2018), which agrees with our findings. Tm refers to the temperature at which plastic materials begin to melt. Tm values for polymers are in the range of 100-400 ºC, according to Riaz et al (Riza et al. 2020). In the current study, Tm values were determined in the range of 251.71 to 261.27 ºC. Juice bottles are classified as semi-crystalline polymers because of their clear Tg and Tm values. Table 3 shows the Tg and Tm values of the samples.

**Table 3.** Glass transition and melting temperature of Juices plastic sample analyzed.

| Sample ID | Glass transition (Tg) $^{O}$C | Melt Temperature (Tm) $^{O}$C |
|---|---|---|
| M-1 | 62.44 | 253.01 |
| M-2 | 68.09 | 251.71 |
| P-1 | 40.58 | 258.32 |
| P-2 | 78.39 | 261.27 |
| A-1 | 86.14 | 255.33 |
| A-2 | 90.23 | 252.58 |
| L-1 | 91.18 | 254.82 |
| L-2 | 77.68 | 262.61 |
| BS-1 | 81.93 | 257.78 |
| BG-1 | 76.08 | 253.80 |
| BM-1 | 38.30 | 255.11 |
| C-1 | 97.55 | 252.89 |
| C-2 | 87.12 | 256.71 |
| C-3 | 82.32 | 255.28 |
| C-4 | 71.51 | 253.44 |

[Insert Table 3 about here]

## Leaching Of Paes From Plastic Bottles Into Fruit Juices

Results for PAEs leaching into citrus and stone fruit juices are depicted in Table 4. DPrP and DOP were found in all citrus fruit juices. In the C-2 sample, there was a higher concentration of DPrP and DOP, ranging from 9.66 to 11.37 g/L. Furthermore, the pH of all citrus fruit juices was found to be acidic, ranging from 2.25 to 3.33, suggesting that PAEs leaching is a possibility. DPrP, DiBP, DPeP, DCHP, DEHP, and DOP were found in stone fruit juices. DiBP, DPeP, and DCHP were found in sample M-1 at concentrations of 7.23, 11.36, and 11.36 g/L, respectively, whereas only DPrP and DOP were found in M-

2, P-1, and P-2 samples. In the P-1 sample, there was a higher concentration of DPrP and DOP. All stone fruit juices had a pH of 2.84 to 3.56, which was considered acidic.

Table 4
Quantification of leached phthalates in Citrus fruit and Stone fruit Juices.

| Sample ID | pH | Phthalates detected | Concentration (µg/L) |
|---|---|---|---|
| C- 1 | 3.33 ± 0.09 | DPrP | 11.09 ± 0.92 |
| | | DOP | 9.48 ± 1.15 |
| C-2 | 3.26 ± 0.15 | DPrP | 11.37 ± 1.20 |
| | | DOP | 9.66 ± 1.30 |
| C-3 | 2.25 ± 0.06 | DPrP | 11.09 ± 0.86 |
| | | DOP | 9.49 ± 0.75 |
| C-4 | 3.25 ± 0.14 | DPrP | 11.11 ± 1.20 |
| | | DOP | 9.47 ± 1.30 |
| M-1 | 2.84 ± 0.05 | DiBP | 7.23 ± 1.15 |
| | | DPeP | 11.36 ± 1.36 |
| | | DCHP | 8.64 ± 1.06 |
| M-2 | 3.23 ± 0.27 | DPrP | 11.09 ± 0.75 |
| | | DOP | 9.47 ± 1.20 |
| P-1 | 3.51 ± 0.20 | DPrP | 11.52 ± 0.85 |
| | | DOP | 9.73 ± 1.15 |
| P-2 | 3.56 ± 0.03 | DPrP | 11.19 ± 0.81 |
| | | DOP | 9.48 ± 0.85 |

The existence of six PAEs, DPrP, DPeP, DCHP, DOP, BzBP, and DEHP, was confirmed in berry fruit juices and berry mix fruit juices. Three PAEs were found in the BS-1 sample, namely DPrP, DPeP, and DOP, with concentrations ranging from 8.64 to 11.40 g/L, but only two PAEs were found in the BG-1 sample, namely DPrP and DOP, with concentrations of 11.32 and 9.61 g/L, respectively. Four PAEs were found in the BM-1 sample: DPrP, BBzP, DEHP, and DOP. All of the berry and mixed fruit juices had a pH of 3.28 to 3.54, which was considered acidic. DPrP and DOP were detected in fleshy fruit juices from A1, A-2, and L-1 samples, but only DPrP at a concentration of 11.56 g/L was found in L-2 samples. In the L-1 sample, there was a higher concentration of DPrP and DOP (Table 5). Apart from that, all samples of fleshy fruit were acidic (pH 3.34 to 3.73).

Table 5
Quantification of leached PAEs into berry & mix fruit juices and Flesh fruit juices.

| Sample ID | pH | Phthalates | Concentration (μg/L) |
|---|---|---|---|
| BS-1 | 3.28 ± 0.14 | DPrP | 11.31 ± 1.30 |
| | | DPeP | 11.40 ± 1.10 |
| | | DCHP | 8.64 ± 1.06 |
| BG-1 | 3.54 ± 0.11 | DPrP | 11.32 ± 1.01 |
| | | DOP | 9.61 ± 1.30 |
| A-1 | 3.34 ± 0.08 | DPrP | 11.24 ± 0.70 |
| | | DOP | 9.53 ± 1.15 |
| A-2 | 3.38 ± 1.27 | DPrP | 11.18 ± 0.95 |
| | | DOP | 9.49 ± 1.01 |
| L-1 | 3.42 ± 0.11 | DPrP | 11.78 ± 0.70 |
| | | DOP | 9.66 ± 1.25 |
| L-2 | 3.73 ± 0.07 | DPrP | 11.56 ± 1.15 |

[Insert Table 4 and Table 5 here]

Total PAEs leaching into all types of fruit juices varied from 7.23 to 43.4 g/L, with higher concentrations in BM-1 samples than in all other juices tested (Fig. 3). Furthermore, the PAE concentrations identified in the current study (7.23–43.4 g/L) were compared to other reported data from various locations across the world (Table 6), revealing that the current findings are comparable to milk beverages (8.18–34.64 g/L). For non-alcoholic drinks (0.02–466 g/L) and fruit juices (22–126 g/L), the results from Vietnam and China were higher than those from the current study, while the data from New York revealed decreased PAE leaching in apple juices from plastic packaging.

Table 6
Comparison of phthalates leaching from food/drinking stuff with current study results.

| **Beverages** | **Phthalates detected** | **Concentration(μg/L)** | **Country** |
|---|---|---|---|
| Non-alcoholic beverages | DPrP, BzBP, DEHP, DiBP, DCHP, DOP | 0.02–466 | Vietnam |
| Milk beverages | DPrP,BzBP, DCHP, DOP | 8.18–34.64 | China |
| Apple juice | DBP, BzBP, DEHP, DOP | 0.39–0.885 | Newyork |
| Fruit juices | DPrP, BzBP, DEHP, DOP | 22–126 | China |
| Fruit juices | DPrP, DiBP, DPeP, BBzP, DCHP, DEHP, DOP | 7.23–43.4 | Current study |

These differences may be attributed to different storage conditions and PAEs content in PET bottles (Bach et al. 2012).

[Insert Fig. 3 and Table 6 here]

# Identification Of Paes By Gc-ms

The presence of PAEs in juices was confirmed using the GC-MS technique, which was based on their molecular ion peak and mass fragments described in the literature (Yin et al. 2014). A total of 7 PAEs were identified based on their molecular ion peak and mass fragments namely DPrP, DiBP, BBzP, DPeP, DCHP, DEHP, and DOP. Protonated phthalic anhydride has a mass fragment of 149 m/z and can be found in a variety of PAE compounds. PAEs with a base peak of 149 m/z included DPrP, DiBP, DPeP, BBzP, DCHP, DEHP, and DOP. DPrP was one of the most abundant PAEs detected with a molecular ion peak at 250 m/z, followed by mass fragments of 149 m/z, 121 m/z, and 104 m/z. Another most abundant PAE is DOP was detected with a molecular ion peak at 390 m/z with mass fragments of 279, 177, and 149 m/z. Similar mass fragments for presence of DOP are reported by (Viñas et al. 2015). DOP is included on the list of priority substances to be assessed under the Canadian Environmental Protection Act (Walker et al. 2008). DEHP was also identified with a molecular ion peak at 390 m/z in the berry mix fruit juice sample with a high concentration. DEHP is characterized as a developmental and reproductive toxicant by several studies (Ye et al. 2014). The leached PAEs identified in the present study are given in Table 7.

Table 7
Mass fragments of leached PAEs obtained by GC-MS analysis of Juice samples.

| PAEs | Molecular formula | Molecular weight | Mass fragments |
|---|---|---|---|
| DPrP | $C_{14}H_8O_4$ | 250.29 | 209, 191, 149 |
| DPeP | $C_{18}H_{26}O_4$ | 306.40 | 237, 219, 149 |
| DiBP | $C_{16}H_{22}O_4$ | 278.35 | 205, 190, 149 |
| BBzP | $C_{19}H_{20}O_4$ | 312.36 | 238, 206, 149 |
| DCHP | $C_{20}H_{26}O_4$ | 330.41 | 249, 167, 149 |
| DEHP | $C_{24}H_{38}O_4$ | 390.56 | 279, 177, 149 |
| DnOP | $C_{24}H_{38}O_4$ | 390.55 | 279, 167, 149 |

[Insert Table 7 about here]

## Estimation Of Exposure To Paes

The estimated daily intakes (EDIs) of PAEs from all fruit juice samples were calculated based on the concentration of PAEs measured and the results are listed in Table 8. The total EDIs of the 7 PAEs detected in the range of 0.087 μg/kg/bw to 0.543 μg/kg/bw, with a median value of 0.157 μg/kg/bw. EDI values of PAEs were found higher in berry and mix fruit juice (0.543 μg/kg/bw), followed by stone fruit juices (0.206 μg/kg/bw), fleshy fruit juices (0.162 μg/kg/bw), and citrus fruits (0.159 μg/kg/bw).

Table 8
Estimated daily intakes (EDIs) of phthalates from the 15 fruit juices (unit: μ/kg-bw/day)

| Samples | DPrP | DiBP | BBzP | DPeP | DCHP | DEHP | DOP | Total |
|---|---|---|---|---|---|---|---|---|
| C-1 | 0.084 | 0 | 0 | 0 | 0 | 0 | 0.071 | 0.155 |
| C-2 | 0.086 | 0 | 0 | 0 | 0 | 0 | 0.073 | 0.159 |
| C-3 | 0.084 | 0 | 0 | 0 | 0 | 0 | 0.071 | 0.155 |
| C-4 | 0.084 | 0 | 0 |  | 0 | 0 | 0.071 | 0.155 |
| M-1 | 0 | 0.054 | 0 | 0.086 | 0.065 | 0 | 0 | 0.206 |
| M-2 | 0.084 | 0 | 0 | 0 | 0 | 0 | 0.071 | 0.155 |
| p-1 | 0.087 | 0 | 0 | 0 | 0 | 0 | 0.073 | 0.16 |
| p-2 | 0.084 | 0 | 0 | 0 | 0 | 0 | 0.071 | 0.155 |
| BS-1 | 0.085 | 0 | 0 | 0.086 | 0.065 | 0 | 0 | 0.236 |
| BG-1 | 0.085 | 0 | 0 | 0 | 0 | 0 | 0.072 | 0.157 |
| BM-1 | 0.084 | 0 | 0.060 | 0 | 0 | 0.328 | 0.071 | 0.543 |
| A-1 | 0.085 | 0 | 0 | 0 | 0 | 0 | 0.072 | 0.157 |
| A-2 | 0.084 | 0 | 0 | 0 | 0 | 0 | 0.071 | 0.155 |
| L-1 | 0.089 | 0 | 0 | 0 | 0 | 0 | 0.073 | 0.162 |
| L-2 | 0.087 | 0 | 0 | 0 | 0 | 0 | 0 | 0.087 |

# Risk Assessment

The risk of exposure to PAEs in adults by consumption of fruit juices was estimated by calculating the hazard index (HI). In Table 9 results showed that HI for DiBP, BBP, DPeP, DEHP, and DOP were $1.8\times10^{-3}$, $3\times10^{-4}$, $1.2\times10^{-3}$, $1.6\times10^{-2}$, and $5.4\times10^{-3}$. These values are less than 1, indicating that these PAEs in fruit juices collected in this study possesses non-carcinogenic health risks to human health (Škrbić et al. 2005).

Table 9
Hazard Index of phthalate exposure from fruit juices.

| Sample ID | Phthalates | HI |
|---|---|---|
| C-1, C-2, C-3, C-4 | DnOP | 0.0072 |
| M-2, P-1, P-2 | DnOP | 0.0054 |
| M-1 | DiBP | 0.0019 |
| M-1 | DPeP | 0.0013 |
| BM-1, BG-1 | DnOP | 0.0036 |
| BM-1 | BBzP | 0.0003 |
| BS-1 | DPeP | 0.0013 |
| BM-1 | DEHP | 0.0164 |

[Insert Table 8 and Table 9 here]

Human cancer risk caused by DEHP through the consumption of fruit juices was assessed by calculating the carcinogenic risk (R). Based on the concentration of DEHP detected in the BM-1 sample (berry mix fruit juice), the cancer risk of DEHP for berry and mix fruit juices was $4.5\times10^{-3}$, which is higher than the maximum acceptable risk level i.e.$1.6\times10^{-6}$ (Škrbić, Đurišić-Mladenović, Cvejanov and chemistry 2005). Thus, the potential carcinogenic risk attributable to DEHP present in the fruit juices should be a concern for the Pakistani population. Consumption of fruit juices for a long-duration could be harmful to human health.

# Conclusion

In this study, the occurrence, exposure, and risks of PAEs from fruit juices were investigated. Seven PAEs were detected in 15 fruit juice samples. The total concentration of PAEs in fruit juices ranged from 11.56 to 71.93 µg/L. In all juices, DPP was the most commonly leached phthalate (93.33 percent of samples studied), followed by DOP (80 percent of samples). Based on the results of the determination, estimated daily intakes (EDIs) of PAEs from fruit juices were calculated with a median value of 0.157 µg/kg/bw. In the risk assessment, the hazard index of PAEs exposure from all fruit juices was located at acceptable levels. However, one fruit juice sample could exhibit carcinogenic risks to humans posed by DEHP exposure. Authorities need to take measures to control the contents of DEHP present in bottled juices.

### Competing Interests

**and Funding**: All Authors declare that they have no conflict of interest related to this publication. Moreover, the authors did not receive support from any organization for the submitted work.

# Declarations

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**Availability of data and material**

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors Contribution

Farah Naz Talpur and Noshad Razzaque prepared work plan and experimental setup. Noshad did the experimental work under the guidance of Farah N. Talpur and Hassan Imran Afridi·. Mehr-Un-Nissa Abbasi, Sobia Kunbhar and Zaheer Chandio helped in instrumental analysis and in compilation of statistical data.

Consent to publish

All authors reviewed the manuscript and consented to publish with Dr. Farah Naz Talpur as a corresponding author.

Consent to participate

Current studies does not involve any human data, hence does not require approval for participation.

Ethical approval

Study does not invlove any human or animal data, hence does not require ethical approval.

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# Figures

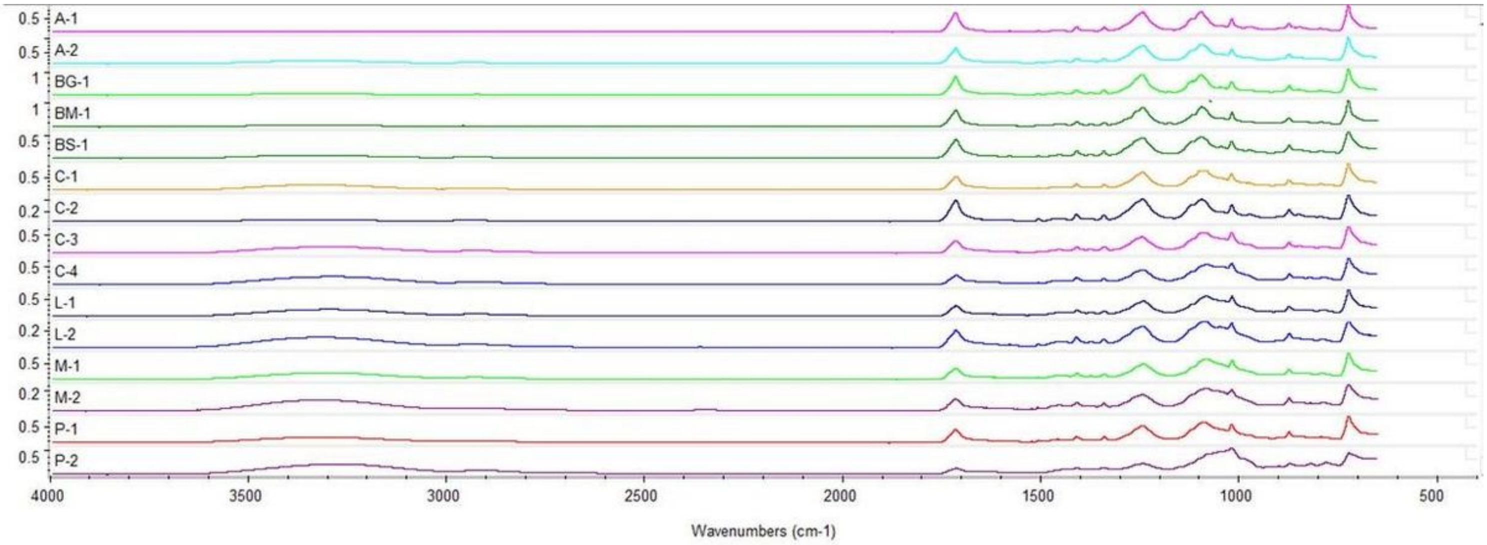

**Figure 1**

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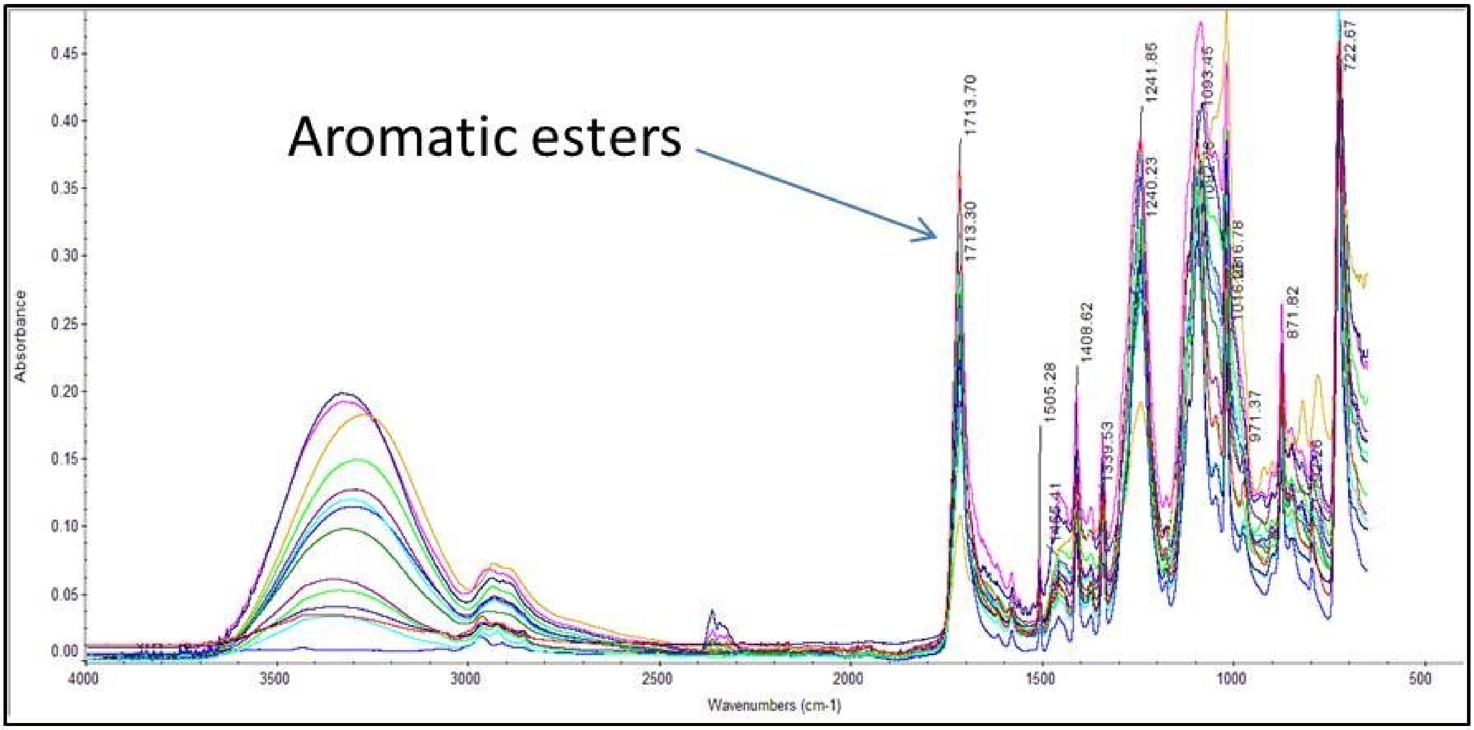

**Figure 2**

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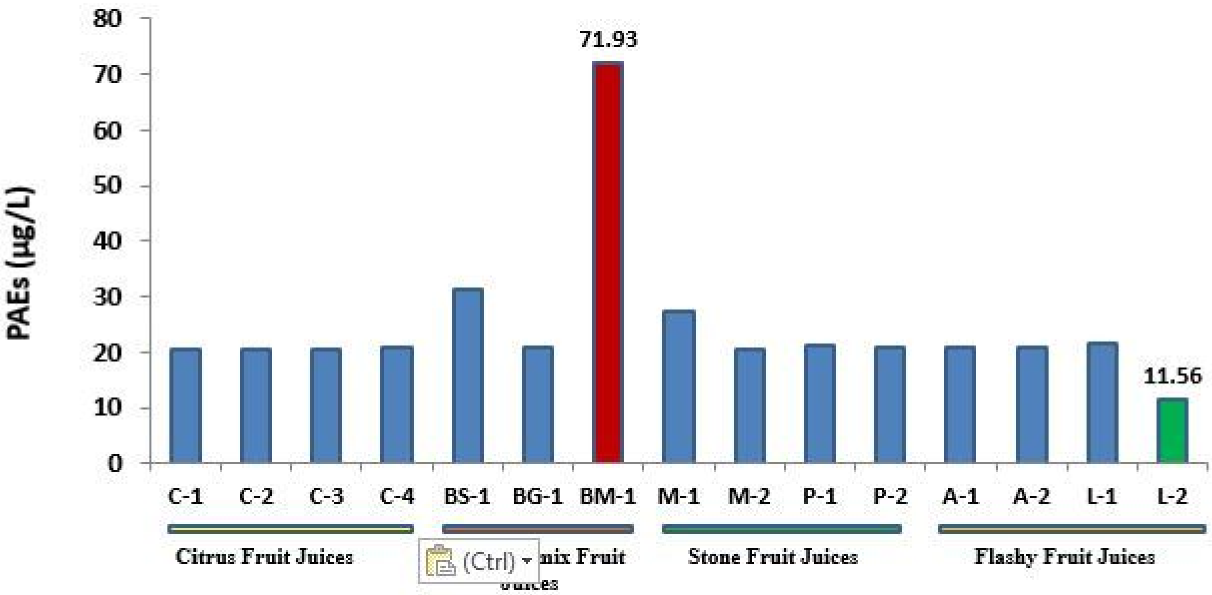

**Figure 3**

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