Original Research Article

Extraction and characterisation of flavoured tobacco by GC-MS studies and validation of results by GC-FID to investigate hazardous flavour ingredients

Abstract: Flavoured tobacco is mainly consumed in India and a few neighbouring countries; the hazards are known. Considering the need to identify such flavouring ingredients, we selected top brands. We simply extracted the ingredients by triturating with Diethyl Ether, evaporating solvent ether and reconstituting the extract in Acetone & Ethanol, respectively, for GC-MS & GC-FID work. The flavour ingredients were identified, and hazardous ingredients, viz. Diethyl Phthalate was identified. It was validated with GC-FID analysis. Linearity, LOD & LOQ study validated the GC method.

Keywords: Flavoured tobacco, GC-MS, GC-FID, Diethyl Phthalate, Aroma compound, Nicotine, Cigarettes, Menthol.

Introduction:

Flavour refers to the biological, physical, and psychological impacts induced by the interplay of chemical stimulants, fragrances, aromas, and the olfactive systems of living organisms (Augusto).[1]Flavors that "are prominent in pyrazines including burley tobacco flavours, chocolates, nuts, and reaction/processed flavour" have a "muting influence" on menthol cooling and might generate a "flavour character that is incompatible"(Wayne).[2]It is widely known that tobacco leaf is the primary raw material for the tobacco industry; its metabolites are strongly associated with the flavour of cigarettes.[3]According to research, those who use flavoured tobacco products are more likely to become "hooked" than those who initially sample-flaflavouredbacco products. This is because flavoured tobacco products are frequently seen as "beginning" items. The flavouring disguises the harshness of tobacco products, making them more addictive and more difficult to stop. According to the Centers for Disease Control (CDC), flavoured tobacco is more addictive than traditional tobacco products.

In 2009, all flavoured cigarettes except those with menthol were banned by the Food and Drug Administration (FDA). The FDA was also granted the authority to regulate other tobacco products. Several new flavoured, smokeless tobacco products are available, such as

energy dip, dry snuff, dip and chew, nicotine dissolvable, e-cigarettes and snus. These goods are popular among teenagers because they are attractive and are considered. Because these items are new to the market, minimal rules exist.In addition, since these products are smokeless, adolescents can use them at school and other public locations without being identified as smokers. Tobacco (*Nicotiana tabacum L*.), sometimes referred to as tobacco plants, is the cworld's most commonly used drug plante are 76 species in the genus Nicotiana (Solanaceae). Still, onlyNicotiana tabacum L. (common tobacco) is economically significant. Despite this, it has enormous economic, agricultural, and social significance. It has been extensively used for smoking, chewing, and sniffing. There are around 600 recognised tobacco species, but only two are consumed by humans (Tayoub et al., 2015).[5,6]

Although it originated in South America, tobacco is now grown worldwide and in the Republic of Croatia.(Briški et al., 2003; Banožić et al., 2018),[7,8] China is the world's largest producer and consumer of tobacco, with an annual production of 4-5 million.(Hu et al.,2015).[9,10]Approximately 4000 chemicals, including particles and gases, are present in tobacco, of which 1000 are inhaled while smoking. (Tayoub et al., 2015).[5]Nicotine is the most well-known substance found in tobacco leaves and the smoke produced when they are. Our previous research has shown that tobacco includes several beneficial compounds, including nicotine and solanesol, which have strong inhibitors that stronglyinhibit *Bacillus subtilis and Micrococcus lysodeikticus*.[12]Tobacco is reprocessed due to its ability to absorb moisture to make it appropriate for storage and to meet the spot better meet better ormake cigarettes.[13]

Several studies have identified distinct classes of chemicals in tobacco, namely alkaloids (including nicotine) (Shen and Shao, 2006),[14]aroma compounds (Popova et al., 2015), [15] fatty alcohols and phytosterols (Liu et al., 2010).[16]Due to a large number of distinct chemical compt present in tobacco and tobacco-related materials that may be extracted simultaneously, it is challenging to develop selective extraction techniques for specific molecules. Severson et al. described how the major cuticular components of different commercial tobacco cultivars and tobacco introductions were found.[17]Troje et al. establish a new approach for identifying specific components from two distinct categories of chemicals in the same extract derived from tobacco material in a single step.[18]

Eighty-three molecules, including 45 cembranoids, 15 adenoids, 20 sucrose esters, and 3 glucose esters, were identified (or fructose esters). There may be three novel cembranoidsand

seven new labdanoids. Glucose esters (or fructose esters) are also described for the first time in Nicotiana tobacco.[19]Menthol cigarettes are far more addictive than regular cigarettes. Menthol is more popular among youth, women, and minority groups. The California Department of Public Health reports that African American smokers have the greatest prevalence of menthol cigarette use (82.6%). In a few nations, flavour compounds in electronic cigarette (EC) fluids that may harm human health have been examined.[20]Alternatives to traditional cigarettes (CC) have been offered to the public in recent years. The market has seen the introduction of electronic cigarettes (ECIG) and heated tobacco products (HTP). [21]However, this research only examines particular chemical components considered essential to fluctuating tobacco's aroma. Clue-cured cigarettes are manufactured differently. Cigars are rolled tobacco leaves, whereas cigarettes are tobacco encased in paper with a filter. According to the National Cancer Institute, when cigars are smoked, nicotine is absorbed via the lungs more rapidly than when cigarettes are smoked. Cigarettes and cigars pose relative risks tothe oral cavity and oesophageal cancer.Ground tobacco leaves are used to craft dry snuff. It may be sniffed or breathed and is available in many flavours. When sniffed or inhaled, the nicotine from snuff is absorbed through the mucous membranes. The National Cancer Institute reports that dry snuff is connected with mouth cancer. Snus is comparable to dry snuff but is kept in a little pouch.It is swallowed by placing it beneath the lip. Snus is not a safer substitute for smoking. Nicotine addiction and dependence can result from this. Snus can cause malignancies of the mouth, pancreas, and oesophagus.

Dip and chew are examples of wet snuff. They are positioned between the lip and the gums. Most users use them by sucking on them and spitting tobacco fluids. A popular brand is 'Energy Dip,' which contains caffeine and nicotine. Dip and chew users are more likely to get mouth cancer. Mints, strips, and sticks that dissolve in the mouth are examples of nicotine dissolvable products.Dissolvable nicotine products are linked to oral cancer, pancreatic cancer, and cardiovascular illness. The candy-like look and extra flavouring may appeal to children. Children who consume it can get nicotine toxicity. Electronic cigarettes are battery-powered devices that provide nicotine and flavourings to the user by transforming chemicals into vapour. According to the World Health Organization, no studies demonstrate that electronic cigarettes are a safer and more effective alternative to traditional cigarettes. In addition, the Food and Drug Administration has not certified any e-cigarette brand as a

smoking cessation aid since manufacturers have failed to demonstrate the safety and effectiveness of these devices for quitting.

Flavoured tobacco may appear to be a safer alternative to smoking, but it can cause major health issues in adolescents and adults. Potentially more hazardous than conventional tobacco is flavoured tobacco. New flavoured tobacco products are far less conspicuous and easier to use in public. Young individuals are more prone to use flavoured tobacco products due to the flavourings' ability to hide the strong tobacco taste.

Waterpipe tobacco smoking (WTS, also known as hookah, shisha, narghile, and other names) is passing tobacco smoke through water before inhaling it. [22]Hookah is a communally smoked water pipe withvarious delicious flavours. The hookah has the same carcinogens as cigarettes and has been linked to lung cancer, respiratory sickness, low birth weight, and periodontal disease. According to the CDC, an hour of hookah smoking is comparable to inhaling 100–200 times as much smoke as one cigarette. The three types of chewing tobacco are loose leaf, plug, and twist. It has carcinogens and raises the possibility of getting mouth cancer. The CDC says there is a strong link between chewing tobacco, precancerous white patches in the mouth, and irritation-caused gum recession. The County and Statewide Archive of Tobacco Statistics (C-Stats) says that in Sacramento, men (14%) are more likely to chew tobacco than women (7.7 %). [23]As one of the most important non-food crops, tobacco plays a significant role in global agriculture. Tobacco processing enterprises create a vast quantity of tobacco trash as a by-product, often discarded owing to its nicotine concentration; only a tiny portion of this garbage gets recycled. [24]

Diethyl phthalate was one of the powerful allelochemicals in barnyard grass root exudates.[25]Diethyl phthalate (DEP) is one of the phthalate esters with a short chain and low molecular weight. [26]Diethyl phthalate (DEP), an odourless, colourless, greasy chemical, is utilised to enhance the performance and durability of various products.[27]Because phthalates, including DEP, are not covalently attached to goods, they are easily discharged into the environment and can be taken orally, inhaled, or dermally. (Clark et al. 2011; Wormuth et al. 2006).[28,29] Two studies assessed foetal survival following exposure to DEP during gestation. (Furr et al. 2014; Howdeshell et al. 2008; NTP 1988)[30,31]Two further studies assessed the number of newborn pups that survived after gestation. (Gray et al. 2000; Hardin et al. 1987)[32,33]

Origin of the research problem:

Cigarette smoking and chewing tobacco have negative health impacts, such as cancer and lung and cardiovascular illnesses. A significant variety of compounds and brand-specific flavours add hundreds of volatiles, making tobacco smoke a very complex combination. Many of these substances have been recognised and classified as carcinogens or poisons. The number of people who use Ghutka has gone down in 140 countries. One reason is that more people are learning about cigarette smoking and chewing Gutka's health risks. From 2000 to 2011, the number of cigarettes smoked per person went down by 40.7%, but the amount of other combustible products smoked went up by 96.9%. Hookah (also called waterpipe, shisha, or narghile) tobacco is one of these products that can be burned but isn't a cigarette. Its use among young people is on the rise. 18% of high school seniors in the United States said they used hookah tobacco at least once a year.The smoking technique of hookah tobacco is unique. First, the tobacco is indirectly cooked with charcoal, then the smoke travels through a water bowl, and lastly, the smoker inhales through a rubber hose with a mouthpiece. Hookah tobacco products include apple, mint, cherry, chocolate, coconut, liquorice, cappuccino, and watermelon flavours, among others. Even though hookah tobacco poses the same health risks as cigarettes, it is common to misunderstand that it is safer and less addictive. One of the primary reasons for hookah tobacco's appeal is its presentation in various pleasant flavours with different alluring scents.

The scenario in India is also becoming worst, Flavoured tobacco brands are becoming popular, and Ghutka is prepared by rubbing catechu, tobacco and flavouring ingredients. This is more hazardous than chewing plain tobacco with calcium hydroxide (Chuna) because manufacturers use a natural essential oil combination for flavouring, and manufacturers use Aroma Chemicals,whicharetoo of cheap quality.

Significance of the study:

The flavour ingredients in Tobacco are of two types –

a) Natural essential oils which are non-hazardous or less hazardous and

b) Synthetic analogues, which are aroma chemicals.

The synthetic aroma chemicals have side effects - allergic disorders affecting the lung, hepatic toxicity, renal toxicity, Neurotoxicity etc. In the proposed study, we will try to identify the flavouring ingredients in tobacco products available on the market. This survey

will probe the hazardous chemicals used along with the impurities carried with them. This will help to make the aware FDA and concerned public health officials.

Objectives:

The study's primary purpose is to identify dangerous compounds used in flavoured tobacco and provide a straightforward approach for quantitative analysis. This will help FDA to establish the SOP of the analysis of tobacco & related products.

It was observed and noted that these manufacturers are not blending the required flavours for manufacturing the finished tobacco products but outsourcing the flavours required. We, therefore,ose blending units and collected samples of flavour concentrate to investigate ingredients in these flavours.

Experimental Work done:

The experimental work done is divided into three phases:

1)Extraction of Flavoured Tobacco & similar products

2)GC-MS analysis of Tobacco Extracts, Flavour Concentrates & Identification of hazardous compounds used

3)GC-FID analysis and Limit of Detection, Linearity study by using the reference standard

1)Extraction of Flavoured Tobacco:

Reagent and Materials:

Among several market samples of flavoured tobacco, 3 samples were shortlisted. BABA, VIMAL & TULSI. BABA 120 was selected as being the most popular & having pleasant & high intensity. The intensity of the flavour was very high and could be smelled from a long distance (20-25 feet).

Solvents& Reference Standards:

Diethyl Ether (MERCK), Absolute Ethanol (MERCK), Sodium Sulfate Anhydrous (MERCK), Diethyl Phthalate (Sigma Aldrich, Lot # LRAC4368), Whatman filter paper No 41

Method of Extraction:

The tobacco leaf samples were triturated/powdered in a granite mortar & pestle. 25 gm of the sample was extracted 6 times with 50 ml of Diethyl Ether, filtered on a multi-folded Whatman filter paper no 41, the fractions were collected together, and the solvent ether was evaporated slowly ina water bath at around 45-50^{o} C. The dark brown-coloured extract was viciousand oily. It was further diluted with Acetone /Absolute Alcohol (Ethanol):

1) For GC-MS analysis with dilution factor 1:10 (Acetone)

2) For GC-FID analysis with dilution as required for Limit of Detection & Linearity study.

The respective diluted solutions were used for:

1) GC-MS analysis for investigation of flavour ingredient molecules

2) GC-FID analysis for quantitative analysis of the ingredient molecules

2)GC-MS Analysis work:

GC-MS analysis of the volatile constituents of tobacco flavour was performed with Agilent GC 7890A series & GC-MS 5977 (Single quadruple). The volatile constituents were separated on a 30 m 0.25 mm i.d., df = 0.25 µm, Rxi-5 Sil (fused silica column). Helium was used as carrier gas at a 1.0 ml/ min flow rate.

The column temperature was held at 50^{o} C for 2 min then programmed as follows:

1)@ 4^{o} C per min to 120^{o} C; this was held for 2 min. Then,

2)Second @ 5^{o} C per min to 260^{o} C, which was also held for 2 min.

The input and ionisation source temperatures were 230° C and 150° C, respectively. In contrast, the temperature of the GC-MS transfer line was 250° C. By comparing spectra with those in the NIST11.L (USA) and Agilent.Mass spectrally identified L spectral libraries, the volatile components of tobacco taste. Identities were confirmed by contrasting the retention indices and mass spectra of discovered compounds with those of genuine reference substances. An HP personal computer with a Pentium processor was used for data analysis. Each sample received 0.1 µL of sample injection.

3) GC-FID analysis, Limit of Detection and Linearity study:

For GC-FID analysis, Limit of Detection (LOD) & Linearity study, the hazardous marker compound identified was ‘Diethyl Phthalate’. Reference standard for Diethyl Phthalate from Sigma-Aldrich (Sigma Aldrich, Lot # LRAC4368) was procured.

A) Preparation of Standard solutions:

Standard solutions from the above reference standard were prepared by using AR grade Absolute Alcohol (Ethanol) as a dilution solvent shown in table 1:

Sr. No.	Standard Solutions
1	DEP Ref Std 0.01 % in Ethanol w/v
2	DEP Ref Std 0.02 % in Ethanolw/v
3	DEP Ref Std 0.05 % in Ethanol w/v
4	DEP Ref Std 0.10 % in Ethanol w/v
5	DEP Ref Std 0.25 % in Ethanol w/v
6	DEP Ref Std 0.50 % in Ethanol w/v

Table 1 Preparation of standard solutions from a reference standard.

B) Preparation of Sample solutions:

The dark brown-coloured extract of Flavoured Tobacco after the evaporation of Diethyl Ether (solvent) was viciousand oily; it was diluted 10 times (1:10) with AR Grade Absolute Alcohol (Ethanol). This sample solution was injected after the Standard solutions.

Chromatographic Conditions:

GC-FID analysis of the Standard & Sample solutions was performed with ShimadzuGC 2014 series.The column used was the same for GC-MS analysis: 30 m 0.25 mm i.d., df = 0.25 µm, Rxi-5 Sil (fused silica column). Nitrogen was used as carrier gas at a 1.0 ml/ min flow rate.

The column temperature was held at 50^{o} C for 2 min then programmed as follows:

1) @ 4^{o} C per min to 120^{o} C; this was held for 2 min. Then,

2) @ 5^{o} C per min to 260^{o} C, which was also held for 2 min.

The Injector temperature was 250^{o} C, and the Detector temperature was 280^{o}C.The injection volume for six standard solutions & sample solution was 1 µL.

RESULTS & DISCUSSION:

GC-MS Analysis: Interpretation of the NIST library & Internal Libraryis shown in table 2.

Sr.No	Retention Time	Area %	NIST identification
1	1.45	38.23	Acetone
2	3.52	0.74	Methyl Hexyl Ketone
3	6.46	0.76	**Mono hydroxy Benzene (Phenol)**
4	7.93	1.40	Benzyl Alcohol
5	9.39	0.65	Phenyl Ethyl Methyl Ether
6	10.37	1.29	Phenyl Ethyl Alcohol
7	10.60	0.79	(4-tert-butylcyclohexyl) acetate
8	11.79	0.79	Iso Borneol
9	12.34	11.81	Menthol
10	12.91	0.64	Beta Pinene
11	14.15	3.33	Citronellol
12	15.02	2.95	GeraniolFormate
13	18.05	0.81	Methyl Anthranilate
14	18.33	16.59	Nicotine
15	18.60	1.01	Citronellyl Propionate
16	22.80	1.57	Alpha Guide
17	25.82	0.92	Delta Guaiene
18	29.36	2.43	**Diethyl Phthalate**
19	31.74	0.95	Carotol
20	31.85	3.31	Patchouli Alcohol
21	37.14	5.65	**Benzyl Benzoate**
22	40.96	3.38	Musk Tetralin (Tonalid)

Table 2GC-MS Analysis

After omitting the solvent peak, the total compounds (molecules) identified are 22. Among these 21 molecules, Diethyl Phthalate is undesirable and causes many health hazards. It aggravates pulmonary function and inflammation of the airway in asthma patients, develops infertility, and its carcinogenic activity is suspicious. Diethyl Phthalate is mainly used for the dilution of fragrance oil. In food flavours, Triethyl Citrate is preferred due to its non-hazardous nature. The trend of using Diethyl Phthalate could be because of the price difference and high solubility of the ingredients in DEP.

GC-FID Analysis: Linearity research, Limit of Detection (LOD), and Limit of Quantification (LOQ) Limit of Quantification (LOQ) was the lowest concentration of analytes that the technique could consistently identify with acceptable precision and accuracy. In this analysis, LOQ was based on the lowest concentration in the calibration curve, that is, 0.01% (~ 0.1 mg/ml or 100 ppm). LOD is three times lower than LOQ; this LOD is 0.0033% (~0.033 mg/ml or 33.33 ppm)

Linearity of Calibration Curve and Working Range

The ability of a technique to achieve an analyte concentration that was proportionate to the measured signal within the operating range was known as linearity. The method's linearity was confirmed by measuring the instrument signal vs the concentration data. The variance of the regression line slope was used to summarise the obtained data. The intercept and slope of a straightforward linear regression equation applied to the data should be used to compute the correlation coefficient. The following is the linear regression equation:

$$Y = mX + C$$

m = Slope of the equation/coefficient

C = y intercept

Y =dependent variable

X =independent variable

Before sample analysis, the instrument performed and analysed six calibration standard points for Diethyl Phthalate concentration. The concentration range of the SIM mode calibration curve for the six phthalate chemicals was between 0.01% (0.1 mg/ml) to 0.5 % (5mg/ml).The various amounts of the acquired signals were plotted, and a linear curve was formed for each phthalate molecule. Table 2 displays the R2 achieved for each of the linear curves. All R2 values are more than 0.990, indicating that the calibration curves demonstrate strong linearity across the calibration range of 0.1 to 5 mg/ml shown in table 3.

% Concentration	Area under peak	% Area
0.01	11829	0.0103

0.02	26088	0.0218
0.05	65674	0.0534
0.10	124753	0.1001
0.25	302196	0.2593
0.50	617363	0.5075

Table 3 The concentration range of the SIM mode calibration curve of the six phthalate chemicals.

Linear Regression

1. Y and X relationship

R Square (R2) equals 0.9998. It means that 100% of the variability of Y is explained by X.

correlation (R) equals 0.9999. It means a very strong direct relationship between X and Y is shown in fig1.

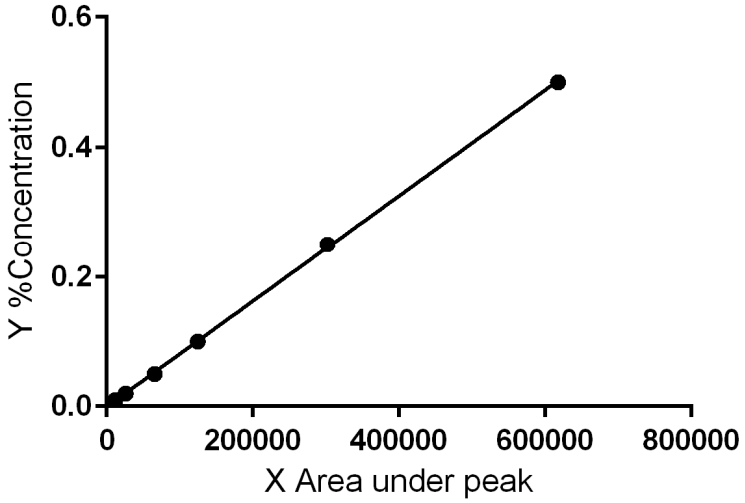

Fig 1 Relationship between concentration and area under the peak.

Thus, the sample concentration was calculated from the above calibration curve. The Area under peak was 255529, and the concentration calculated using the curve was 0.21 % (2.1 mg/ml).Considering the dilution factor, DEP content is 2.1 %of DEP (Diethyl Phthalate).

The method is validated concerning resolution, reliability & reproducibility. **Y and Xrelationship** R Square (R2) equals **0.9998**. It means that 100% of the variability of Y is explained by X. correlation (R) equals **0.9999.** It means that there is a **very strong direct**

relationship between X and Y.This method can be used for the estimation of Diethyl Phthalate in Flavoured Tobacco

Conclusion:

Looking at the analysis of GC-MS & GC-FID, we can conclude that the GC method for estimating Diethyl Phthalate is validated. It is noticed that apart from Diethyl Phthalate, no other hazardous ingredient in the sample analysed was found. We are happy to note that the concentration of Diethyl Phthalate in the sample is not exceeding 2.5 %. The toxicity data ofthe ingredient, Benzyl Benzoate, needs to be verified because it can cause ataxia, convulsions & respiratory paralysis.This extraction and GC analysis method can be used to estimate Diethyl Phthalate.

References

1 F. Augusto, A. Leite e Lopes and C. A. Zini, *TrAC - Trends Anal. Chem.*, 2003, **22**, 160–169.

2 G. Ferris Wayne and G. N. Connolly, *Nicotine Tob. Res.*, DOI:10.1080/14622203310001649513.

3 L. Zhang, X. Wang, J. Guo, Q. Xia, G. Zhao, H. Zhou and F. Xie, *J. Agric. Food Chem.*, 2013, **61**, 2597–2605.

4 V. Popova, T. Ivanova, A. Stoyanova, V. Nikolova, T. Hristeva, M. Docheva, N. Nikolov and I. Iliev, *J. Appl. Biol. Biotechnol.*, 2019, **7**, 45–49.

5 G. Tayoub, H. Sulaiman and M. Alorfi, *Herba Pol.*, 2015, **61**, 23–30.

6 N. Moghbel, B. M. Ryu and K. J. Steadman, *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.*, 2015, **997**, 142–145.

7 F. Briški, N. Horgas, M. Vuković and Z. Gomzi, *Clean Technol. Environ. Policy*, 2003, **5**, 295–301.

8 M. Banožić, I. Banjari, M. Jakovljević, D. Šubarić, S. Tomas, J. Babić and S. Jokić, *Molecules*, DOI:10.3390/molecules24081611.

9 B. Yan, S. Zhang, W. Chen and Q. Cai, *J. Anal. Appl. Pyrolysis*, 2018, 248–254.

10 R. S. Hu, J. Wang, H. Li, H. Ni, Y. F. Chen, Y. W. Zhang, S. P. Xiang and H. H. Li, *Sep. Purif. Technol.*, 2015, **146**, 1–7.

11 A. Charlton, *J. R. Soc. Med.*, 2004, **97**, 292–296.

12 S. Duan, Y. Du, X. Hou, N. Yan, W. Dong, X. Mao and Z. Zhang, *Molecules*, DOI:10.3390/molecules21121743.

13 Y. N. Xin, J. W. Zhang and B. Li, *J. Therm. Anal. Calorim.*, 2018, **132**, 1347–1358.

14 J. Shen and X. Shao, *Anal. Chim. Acta*, 2006, **561**, 83–87.

15 V. Popova, V. Gochev, T. Girova, I. Iliev, T. Ivanova and A. Stoyanova, *Curr. Bioact. Compd.*, 2015, **11**, 31–37.

16 Y. Liu, G. Yong, Y. Xu, D. Zhu, H. Tong and S. Liu, *Chromatographia*, 2010, **71**, 727–732.

17 R. F. Severson, R. F. Arrendale, O. T. Chortyk, A. W. Johnson, D. Michael Jackson, G. Richard Gwynn, J. F. Chaplin and M. G. Stephenson, *J. Agric. Food Chem.*, 1984, **32**, 566–570.

18 Z. Švob Troje, Z. Fröbe and D. Perović, *J. Chromatogr. A*, 1997, **775**, 101–107.

19 L. Ding, F. Xie, G. Xu, K. Liu, S. Wang and J. Xie, *J. Sep. Sci.*, 2010, **33**, 3429–3436.

20 E. E. Omaiye, W. Luo, K. J. McWhirter, J. F. Pankow and P. Talbot, *Chem. Res. Toxicol.*, 2020, **33**, 2972–2987.

21 J. K. Aranyosi, E. Galgoczi, A. Erdei, M. Katko, M. Fodor, Z. Ujhelyi, I. Bacskay, E. V. Nagy and B. Ujhelyi, *Molecules*, 2022, **27**, 3001.

22 K. H. Kim, E. Kabir and S. A. Jahan, *J. Hazard. Mater.*, 2016, **317**, 229–236.

23 M. A. Farag, M. M. Elmassry and S. H. El-Ahmady, *Sci. Rep.*, 2018, **8**, 1–12.

24 M. Banožić, J. Babić and S. Jokić, *Ind. Crops Prod.*, , DOI:10.1016/j.indcrop.2019.112009.

25 T. S. Cheng, *Aquat. Toxicol.*, 2012, **124**–**125**, 171–178.

26 L. J. Cheng, M. J. Hung, Y. I. Cheng and T. S. Cheng, *Aquat. Toxicol.*, 2013, **144**–**145**, 124–132.

27 J. A. Weaver, B. E. J. Beverly, N. Keshava, A. Mudipalli, X. Arzuaga, C. Cai, A. K. Hotchkiss, S. L. Makris and E. E. Yost, *Environ. Int.*, 2020, **145**, 105848.

28 K. E. Clark, R. M. David, R. Guinn, K. W. Kramarz, M. A. Lampi and C. A. Staples, *Hum. Ecol. Risk Assess.*, 2011, **17**, 923–965.

29 M. Wormuth, M. Scheringer, M. Vollenweider and K. Hungerbühler, *Risk Anal.*, 2006, **26**, 803–824.

30 J. R. Furr, C. S. Lambright, V. S. Wilson, P. M. Foster and L. E. Gray, *Toxicol. Sci.*, 2014, **140**, 403–424.

31 K. I. Howdeshell, V. S. Wilson, J. Furr, C. R. Lambright, C. V. Rider, C. R. Blystone, A. K. Hotchkiss and L. E. Gray, *Toxicol. Sci.*, 2008, **105**, 153–165.

32 L. E. Gray, J. Ostby, J. Furr, M. Price, D. N. R. Veeramachaneni and L. Parks, *Toxicol. Sci.*, 2000, **58**, 350–365.

33 B. D. Hardin, R. L. Schuler, J. A. R. Burg, G. M. Booth, K. P. Hazelden, K. M. MacKenzie, V. J. Piccirillo and K. N. Smith, *Teratog. Carcinog. Mutagen.*, 1987, **7**, 29–48.