Chasing clouds, catching chemicals: Bisphenols in e-liquids analysed with SPE and LC-MS/MS

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Vaping refers to the use of an electronic device to inhale an aerosol created through heating a liquid, commonly known as an e-liquid. E-liquids typically consist of propylene glycol (PG), vegetable glycerine (VG), nicotine and various flavouring compounds. The popularity of vaping has increased significantly since 2013, growing from 1.3% of adults vaping to 10.0% in 2023. This is especially true among young adults with 23% of 18 year olds reporting long term vaping by 2023 [1]. As the prevalence of vaping continues to rise, there is an increasingly urgent need to fully understand the emerging health risks associated with it. Previous research has identified some potential harmful health effects of vaping e-liquids including cardiovascular damage [2-5], respiratory issues [6-8], reproductive issues [9, 10] and neurological damage [11, 12].

An overlooked health risk associated with the e-liquids is contamination with bisphenols, believed to migrate into the liquid from their packaging. However, research into the presence of bisphenols in e-liquids remains limited. Notably, one study detected bisphenol A in every sample tested [13], showing the need for further work to investigate both bisphenol A as well as other bisphenols which may be present.

Bisphenols are compounds composed of two phenolic rings, connected via a carbon with differing chemical groups attached. *Figure 1* shows examples of three common bisphenols. They are important in the manufacturing of plastics, where they provide the structural backbone and offer desirable properties which are difficult to achieve with alternative materials.

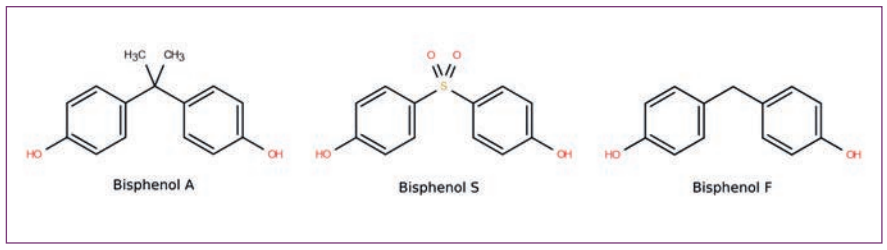

Figure 1: Chemical structures of three commonly used bisphenol compounds – bisphenol A, S and F.

Despite their usefulness within plastic production, bisphenols pose significant health risks primarily due to their endocrine disrupting properties [14]. This means that they interfere with the body's hormone systems, with research linking them to cancer (breast, prostate, ovarian, testicular and colorectal) [15], metabolism disruption [16] and harm to reproductive health [17].

The regulation of bisphenols varies significantly worldwide, with the European Union (EU) implementing the strictest regulations globally. In 2022, the European Food Safety Authority (EFSA) suggested a tolerable intake of 0.2 ng/kg bodyweight/day for bisphenol A [19]. This was made substantially lower than the EFSA's 2015 temporary tolerable intake of 4 ng/kg bodyweight/day, based on additional data demonstrating bisphenol A's greater toxicity. Following this, on the 20th January 2025, the EU banned the use and trade of bisphenol A and other hazardous bisphenol derivatives in food contact materials [18].

While e-liquids are not classified as food products and are therefore excluded from current EU bisphenol regulations, their storage in plastic bottles creates a potential source of contamination. Additionally, because exposure occurs through inhalation rather than ingestion, the health risks are less well understood and could differ significantly. For these reasons, it is important to assess the concentration of bisphenols in e-liquids, evaluate potential risks, and develop reliable sample preparation methods to enable further study.

This work utilised a polymeric solid phase extraction (SPE) technique using the Microlute® CP HLB 96 well plate to develop a sample preparation method, validate extraction of bisphenols from e-liquid solutions, and analyse 16 different brands of e-liquids for six different bisphenols - A, AP, E, F, S and Z.

Materials and Methods

Stock and Standards

A stock solution of all six bisphenols (A, AP, E, F, S and Z) was prepared by dissolving in methanol (MeOH) at 1 mg/mL. Working solutions were prepared by diluting this stock solution in water to obtain standards of 2.5, 50, 100, 250, 2,500 and 5,000 ng/mL.

Matrix solution

For validation of the SPE procedure and preparation of matrix match standards, a solution of 70% vegetable glycerine (VG) and 30% propylene glycol (PG) (v/v) was prepared.

Samples

16 different brands of e-liquids with a composition of 70% VG 30% PG were purchased from an online UK retailer. The e-liquids were primarily packed in polyethylene terephthalate (PET) bottles, with one packaged in an low density polyethylene (LDPE) bottle and four in plastics of unknown composition. The flavours included unflavoured, mint, cherry, passionfruit, mixed berries, melon and apple pear, cream and energy drink.

SPE method

A 10 mg 96 well plate (Microlute® CP 10 mg HLB) with a positive pressure manifold (UltraPPM™ Lite) was used for processing samples. Prior to performing the SPE, samples and matrix match solutions were diluted 1:1 with ultrapure water to reduce viscosity and improve liquid flow through the SPE plate.

- **Conditioning:** 0.5 mL of methanol
- **Equilibration:** 0.5 mL of ultrapure water
- **Loading:** 1 mL diluted sample
- **Wash:** 0.5 mL of 20% methanol in water
- **Drying step:** The sorbent was dried at 20 PSI for 2 minutes using the positive pressure manifold
- **Elution:** 2 x 0.5 mL of 5% ammonium hydroxide in methanol (dried at 20 PSI for 2 minutes after each elution with the positive pressure manifold)
- **Reconstitution:** The eluate was evaporated using a nitrogen blowdown evaporator (Ultravap® Mistral) at 30°C and reconstituted with 0.1 mL of ultrapure water

Table 1: HPLC and Mass Spectrometer conditions used for analysis of bisphenols using Multiple Reaction Monitoring (MRM).

HPLC-MS/MS Method:

HPLC: ACQUITY Premier UPLC	**Mass Spectrometer:** Xevo TQ-S Micro
Solvent A: H_2O	**Needle Wash** MeOH (3 sec)
Solvent B: MeOH + 0.01% ammonia	**Syringe Draw Rate** 30 µL/min
	Needle Placement 4mm
Gradient	**Injection Volume** 2 µL
Column Waters ACQUITY UPLC-BEH C18 (2.1 x 50, 1.7 µm)	
Column Temperature 40°C	
Source Type ESI Negative	
Capillary Voltage 2500 V	
Desolvation Temperature 350°C	
Source Temperature 150°C	
Desolvation Gas Flow 1000 L/hr	
Cone Gas Flow 0 L/hr	
Detector Gain 1.00	
Minimum Points Per Peak 15	
Flow Rate 400 µL/min	
Seal Wash On (5 min)	
Sample Temperature 10°C	

Time (min)	A (%)	B (%)
0.00	94.0	6.0
0.25	94.0	6.0
0.75	65.0	35.0
3.50	30.0	70.0
3.75	30.0	70.0
3.76	5.0	95.0
4.75	5.0	95.0
4.76	94.0	6.0
5.75	94.0	6.0

MRM Transitions:

Analyte	Polarity	Precursor ion (m/z)	Product ion (m/z)	Cone voltage (V)	Collision energy (V)
Bisphenol A	Negative	226.82	211.94 (Quan)	43	17
			132.99		24
Bisphenol AP	Negative	288.84	273.97 (Quan)	38	20
			210.97		24
Bisphenol E	Negative	212.75	197.92 (Quan)	33	17
			118.92		26
Bisphenol F	Negative	198.72	92.91 (Quan)	38	20
			104.91		18
Bisphenol S	Positive	250.79	92.94 (Quan)	55	24
			156.91		15
Bisphenol Z	Negative	266.85	173.03 (Quan)	50	26
			144.96		34

Method validation

To validate the method for suitability in processing e-liquids with SPE, a blank matrix of 70% VG 30% PG (v/v) was processed through the SPE plate (following the previously outlined method), then spiked at concentrations of 0.1, 1.5, 5, 10, 100, 250 and 500 ng/mL. This was then compared to standards prepared in ultrapure water, spiked to the same concentrations, to test for matrix effects and linearity.

Alongside this, ten replicates of the blank solution were injected to confirm the limit of detection (LOD) and limit of quantification (LOQ) of each bisphenol compound. This was calculated using equation 1.

$$L_X = \frac{mean_{blank} + k\sigma}{slope}$$

Equation 1: Calculation of LOD or LOQ (Lx), where meanblank is the average of 10 blank replicates, 𝜎 is the standard deviation of the blanks, slope is the gradient of the calibration curve and k is a constant of 3.3 (LOD) or 10 (LOQ).

To validate recovery, three concentrations of the bisphenols were used – 10 ng/mL (low), 40 ng/mL (medium) and 80 ng/mL (high). These three concentrations validated recovery across the calibration range - near the LOQ, the midpoint of the calibration curve and the upper part of the calibration curve.

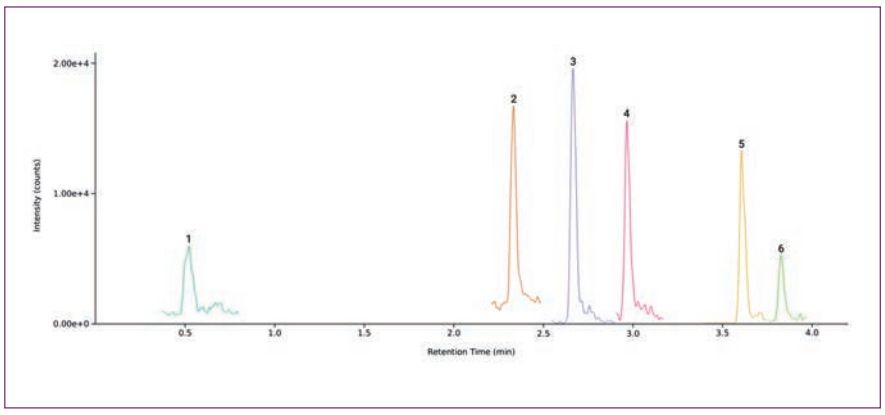

Figure 2: Chromatogram of the lowest calibration standard for each bisphenol. Peaks: (1) bisphenol S (0.5 ng/mL), (2) bisphenol F, (3) bisphenol E, (4) bisphenol A, (5) bisphenol AP, and (6) bisphenol Z (all 1.5 ng/mL).

Testing commercial e-liquids

The commercially available e-liquids were diluted and tested using the same SPE method that was validated with the blank matrix. Calibration standards were prepared as matrix matched standards, using post-spiked 70% VG 30% PG solutions.

Results and Discussion

Table 2 shows the results of the validation of the method for all six of the bisphenols.

Table 2: Validation results for six bisphenols in 70% VG / 30% PG using SPE–LC–MS/MS. R^2 = coefficient of determination, MDL = method detection limit, MQL = method quantification limit (calculated using Equation 2), ME = matrix effect

Analyte	Linearity	R^2	LOD (ng/mL)	LOQ (ng/mL)	MDL (ng/mL)	MQL (ng/mL)	ME (%)
Bisphenol A	1.5 – 500	0.9906	1.20	2.10	0.34	0.60	-13.0
Bisphenol AP	1.5 – 500	0.9866	0.50	1.23	0.28	0.70	-59.0
Bisphenol E	1.5 – 500	0.9933	0.23	0.54	0.06	0.14	-10.0
Bisphenol F	1.5 – 500	0.9900	0.42	0.87	0.10	0.20	-6.6
Bisphenol S	0.5 - 500	0.9950	0.02	0.05	0.0055	0.012	-16.6
Bisphenol Z	1.5 – 500	0.9860	0.47	1.01	0.22	0.47	-42.3

Validation of the method showed that all six compounds were linear. Bisphenol AP and Z had R^2 values slightly below 0.99, however the residuals at each calibration level were within ±20%. LOQ values ranged from 0.05 ng/mL (bisphenol S) to 2.10 ng/mL (bisphenol A). The method detection limit (MDL) and method quantification limit (MQL) were calculated using recovery at a low concentration (10 ng/mL results in table 3), matrix effect and concentration factor (equation 2) to give the true limits of detection and quantification in a sample processed via the SPE method.

$$M_X = \frac{L_x}{Recovery \times ME} \; / \; concentration\ factor$$

Equation 2: Calculation of MDL or MQL (M_x), where L_x is the LOD (for MDL) or LOQ (for MQL), recovery is the value at 10 ng/mL (as a decimal), ME is the matrix effect (as a decimal), and the concentration factor is 5

Matrix effect was classed as significant if it was >±20%, this was only the case for bisphenol AP and Z. Therefore, to correct for this matrix effect, matrix match calibration was used for the recovery and repeatability validation and sample testing.

Recovery and repeatability validation results for the three concentrations (10, 40 and 80 ng/mL, n=8) are in table 3. All compounds met recovery criteria of 80 – 120% at all three concentrations. All repeatability values were <20%RSD at the lowest concentration and <15%RSD at the other two concentrations, ranging from 1.5 – 18.6%RSD. This showed that the SPE method was repeatable at the three different concentrations.

Table 3: Recovery and repeatability of bisphenols (10, 40, 80 ng/mL, n=8) in 70% VG / 30% PG using the SPE combined with LC–MS/MS. SD = standard deviation.

Compound	10 ng/mL			40 ng/mL			80 ng/mL		
	Recovery (%)	SD	%RSD	Recovery (%)	SD	%RSD	Recovery (%)	SD	%RSD
Bisphenol A	96.3	4.8	5.0	93.8	1.7	1.8	97.0	6.7	6.9
Bisphenol AP	93.4	17.4	18.6	105.4	14.9	14.1	107.8	6.6	6.1
Bisphenol E	106.0	3.9	3.7	92.3	1.5	1.6	96.5	3.9	4.1
Bisphenol F	93.2	2.3	2.5	92.3	2.2	2.4	96.8	2.2	2.3
Bisphenol S	102.0	7.7	7.6	94.7	4.9	5.1	91.0	5.8	6.4
Bisphenol Z	102.3	17.8	17.4	110.7	11.3	10.2	107.9	4.7	4.4

Table 4: Bisphenol concentrations (mean ± SD, n=4) in commercial e-liquids analysed using the validated method of SPE combined with HPLC–MS/MS.

Sample	Flavouring	Analyte Concentration (ng/mL)					
		Bisphenol A	Bisphenol AP	Bisphenol E	Bisphenol F	Bisphenol S	Bisphenol Z
1	Unflavoured	<LOQ	<LOD	<LOQ	<LOD	<LOD	<LOD
2	Mint	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD
3	Mint	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
4	Passionfruit	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
5	Mixed berries	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
6	Mixed berries	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
7	Mixed berries	<LOD	<LOD	<LOQ	<LOQ	<LOD	<LOD
8	Melon	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
9	Mixed berries	4.65 ± 0.29	<LOD	<LOQ	<LOQ	<LOD	<LOD
10	Mixed berries	<LOD	<LOD	0.30 ± 0.02	<LOD	<LOD	<LOD
11	Apple Pear	<LOD	<LOD	0.23 ± 0.01	<LOD	<LOD	<LOD
12	Mixed berries	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD
13	Mixed berries	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
14	Cream	<LOD	<LOD	0.26 ± 0.04	<LOD	<LOD	<LOD
15	Energy drink	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
16	Mixed berries	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

The validated SPE and LC-MS/MS method was applied to sixteen commercial e-liquids, each prepared in quadruplicate. Four samples (9, 10, 11 and 14) contained quantifiable levels of bisphenols. Bisphenol A was detected in sample 9, while bisphenol E was present in samples 10, 11 and 14.

Sample 9 contained 4.65 ng/mL of bisphenol A, which with a median daily e-liquid consumption of 4.6 mL [20], this corresponds to a daily intake of 21.39 ng. For an average adult (73.7kg [21]), this exceeds the EFSA tolerable daily intake of 0.2 ng/kg bodyweight/day by approximately 1.45 fold.

The remaining three e-liquids contained a lower level of bisphenol (<0.30 ng/mL), specifically bisphenol E. However, as toxicological data for bisphenol E is limited, its health effects remain unclear. These findings highlight the need to identify the sources of bisphenols in e-liquids and help better evaluate potential risks to the consumers.

Conclusion

A sensitive SPE method combined with LC-MS/MS was developed and validated for six bisphenols in e-liquids (70% VG 30% PG), showing good linearity, recoveries of 91.0 to 110.7% and good repeatability ranging from 1.6 to 18.6 %RSD.

Application of the method to sixteen commercial e-liquids identified bisphenol A in one sample and bisphenol E in three samples. The sample containing bisphenol A would exceed the EFSA tolerable daily intake under typical use. These results highlight consumer exposure to bisphenols and the need for further investigation of their presence in e-liquids. This method, using the Microlute® CP HLB 96 well plate, provides a reliable tool for monitoring and supporting risk assessment of bisphenols in e-liquids.

References

1. S. E. Jackson, H Tattan-Birch, L. Shahab, J. Brown, Trends in long term vaping among adults in England, 2013-23: population based study, BMJ, 2024, 386

2. L. Mohammadi, D. D. Han, F. Xu, A. Huang, R. Derakhshandeh, P. Rao, A. Whitlatch, J. Cheng, R. J. Keith, N. M. Hamburg, P. Ganz, J. Hellman, S. F. Schick, and M. L. Springer, Chronic E-Cigarette Use Impairs Endothelial Function on the Physiological and Cellular Levels, Arteriosclerosis, Thrombosis, and Vascular Biology, 2022, 42, 11

3. A. Moshensky, C. S. Brand, H. Alhaddad, J. Shin, J. A. Masso-Silvalra, I. Advani, D. Gunge, A. Sharma, S. Mehta … L. E C. Alexander, Effects of mango and mint pod-based e-cigarette aerosol inhalation on inflammatory states of the brain, lung, heart, and colon in mice, 2022, eLife, 11, 67621

4. X. Meng, X. Guo, Z. Peng, C. Wang, R. Liu, Acute effects of electronic cigarettes on vascular endothelial function: a systematic review and meta-analysis of randomized controlled trials, European Journal of Preventive Cardiology, 2023, 30(5), 425-435

5. M. J. Mears, H. L. Hookfin, P. Bandaru, P. Vidal, K.I. Stanford, and L. E. Wold, Electronic Nicotine Delivery Systems and Cardiovascular/Cardiometabolic Health, Circulation Research, 2023, 132, 9

6. L. Honeycutt, K. Huerne, A. Miller, E. Wennberg, K. B. Filion, R. Grad, A. S. Gershon, C. Ells, G. Gore, A. Benedetti, B. Thombs, M. J. Eisenberg, A systematic review of the effects of e-cigarette use on lung function, NPJ Prim. Care Respir. Med., 2022, 32, 45

7. D. N. Bhatta, S. A. Glantz, Association of E-Cigarette Use With Respiratory Disease Among Adults: A Longitudinal Analysis, Am. J. Prev. Med., 2020, 58(2), 182-190

8. L. P. Hariri, M.D., Ph.D., B. M. Flashner, M.D., D. J. Kanarek, M.D., W. J. O'Donnell, M.D., A. Soskis, M.D., D. R. Ziehr, M.D., A. Frank, M.D., D. C. Christiani, M.D., E-Cigarette Use, Small Airway Fibrosis, and Constrictive Bronchiolitis, 2022, NEJM Evid., 1, 6

9. M. W. Marbrey, S. M. Cripps, R. Huang, B. M. Kistner, A. Somany, E. S. Douglas, K. M. Caron, Flavored e-cigarettes modulate embryo development, fetal growth, and potentiate early fetal demise without nicotine, 2025, Communications Medicine, 5, 373

10. A. Vallée, M. Eid, A. Feki, J-M. Ayoubi, Maternal vaping and pregnancy adverse outcomes: A systematic review and meta-analysis, 2025, 38(5), 101951

11. M. L. Novak, G. Y. Wang, The effect of e-cigarettes on cognitive function: a scoping review, Psychopharmacology (Berl), 2024, 241(7), 1287-1297

12. M. Yuan, S. J. Cross, S. E. Loughlin, F. M. Leslie, Nicotine and the adolescent brain, J Physiol., 2015, 593(16), 3397-3412

13. P. Kubica, D. Osiecka, A. Kabir, N. P. Kalogiouri, V. F. Samanidou, Comparative study of bisphenols in e-cigarette liquids: Evaluating fabric phase sorptive extraction, ultrasound-assisted membrane extraction, and solid phase extraction techniques, Talanta, 2025, 283, 127096

14. P. Fenichel, N. Chevalier, F. Brucker-Davis, Bisphenol A: An endocrine and metabolic disruptor, Annales d'Endocrinologie, 2013, 74(3), 211-220

15. N. G. Khan, J. Correia, D. Adiga, P. S. Rai, H. S. Dsouza, S. Chakrabarty, S. P. Kabekkodu, A comprehensive review on the carcinogenic potential of bisphenol A: clues and evidence, Environ. Sci. Pollut. Res. Int., 2011, 28(16), 19643-19663

16. I. Cimmino, F. Fiory, G. Perruolo, C. Miele, F. Beguinot, P. Formisano, F. Oriente, Potential Mechanisms of Bisphenol A (BPA) Contributing to Human Disease, 2020, 21(16), 5751

17. E. A. Salami, O. A. Rotimi, The impact of Bisphenol-A on human reproductive health, Toxicology Reports, 2024, 13, 101773

18. Commission Regulation (EU) 2024/3190 of 19 December 2024 on the use of bisphenol A (BPA) and other bisphenols and bisphenol derivatives with harmonised classification for specific hazardous properties in certain materials and articles intended to come into contact with food, amending Regulation (EU) No 10/2011 and repealing Regulation (EU) 2018/213, https://eur-lex.europa.eu/eli/reg/2024/3190/oj/eng (Accessed September 2025)

19. EFSA Panel on Food Contact Materials, Re-evaluation of the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs, EFSA Journal, 2023, 21(4), 6857

20. A. Aherrera, A. Aravindakshan, S. Jarmul, P. Olmedo, R. Chen, J. E. Cohen, A. Navas-Acien, A. M. Rule, E-cigarette use behaviors and device characteristics of daily exclusive e-cigarette users in Maryland: Implications for product toxicity, Tob. Induc. Dis., 2020, 18, 93

21. C. Stival, A. Lugo, A. Odone, P. A. van den Brandt, E. Fernandez, O. Tigova, J. B. Soriano, M. J. López, S. Scaglioni, S. Gallus, Prevalence and Correlates of Overweight and Obesity in 12 European Countries in 2017–2018, Obes. Facts, 2022, 15(5), 655-665