Scientific Online

SOP TRANSACTIONS ON ANALYTICAL CHEMISTRY
In Press

SOP TRANSACTIONS ON ANALYTICAL CHEMISTRY

A Rapid, Green High Performance Anion-Exchange Chromatographic Assay for Saccharin and Its Determination in Environmental Water Samples

Kevin C. Honeychurch*

Centre for Research in Biosciences, Department of Biological, Biomedical and Analytical Sciences, University of the West of England, Frenchay Campus, Bristol, BS16 1QY, UK.

***Corresponding author**: kevin.honeychurch@uwe.ac.uk

Abstract:

A green, rapid and sensitive high performance anion-exchange chromatographic (HPAEC) method for the determination of saccharin in environmental water samples is reported. A linear range of 5 μg/L to 28.2 μg/mL was found with a theoretical limit of detection of 5 μg/L based on a signal-to-noise ratio of three. No interferences were seen for sodium cyclamate, acesulfame K, aspartame, nitrite, nitrate and chlorate. Following a simple filtration sample pre-treatment a percentage recovery of 92.9 % and an associated coefficient of variation of 3.8 % was found for a sample fortified with 28.2 ng/mL saccharin. Separation was achieved within 2.5 minutes using a SAP 100 mm $\times$ 4.6 mm, 8 μm strong anion exchange analytical column with a mobile phase of 0.1 M NaCl at a flow rate of 1.5 mL/min. The develop method avoids the use of potentially environmentally hazardous reagents and minimises the production of waste.

Keywords:

Saccharin; Environmental; Water; Pollution; High Performance Anion Exchange Chromatography; Green; Rapid

1. INTRODUCTION

Surface waters, such as rivers, streams, lakes and ponds are important ecosystems providing resources in the form of drinking water, food, irrigation for agriculture and recreation. Their water quality can be affected by pollution from various sources such as industry, agriculture and municipal wastewater posing a significant threat not only to the public but also their ecological health. Faecal indicator bacteria such as *Escherichia coli* (*E. coli*) have been widely used to assess water quality and to monitor possible contamination of surface waters by domestic wastewaters. The presence of *E. coli* can be used to indicate the occurrence of a variety of pathogens, responsible for a number of diseases including *Salmonella sp*., *Shigella sp*., *Yersinia sp., Leptospira sp*. and *Legionella sp*. [1].

However, the utilisation of *E. coli* as an indicator species can be time consuming as culturing techniques can take between 18 to 96 hours [2] and the relatively short survival time of faecal coliforms in the environment represents further limitation [3], as the relatively fast die-off of *E. coli* can be seen as a

poor indicator for the presence of more persistent pathogens such as *Cryptosporidium* and *Giardia* [4]. Furthermore, *E. coli* is not a single species [5]; certain genera of the coliform group such as *Proteus* and *Aerobacter* are normally found outside the human intestinal tract in soil and in fact, *E. coli* can be found in the intestines of all warm-blooded animals [6]. Consequently, the source of *E. coli* in surface waters may be from municipal sewage contamination or have its origin from wildlife (or a combination of both). It is therefore beneficial for the detection of sewage and wastewater contamination to use markers or indicators which are clearly of anthropogenic origin, ubiquitous in sewage and are persistent in the environment.

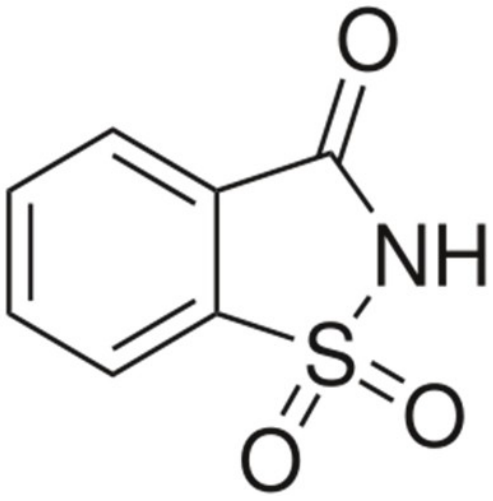
Saccharin, 1,2-Benzothiazol-3(2H)-one 1,1-dioxide (i) is an artificial sweetener commonly used in wide range products as a sugar substitute in food, beverages, pharmaceuticals, personal care products and animal feed. It is excreted from the body essential unchanged [7] and as a result detectable levels have been determined in sewage influents [8–16]. Water treatment plants have been reported to be unable to remove artificial sweeteners completely from waste water [12] and as a result investigations have shown detectable levels of saccharin in environmental water courses and soils allowing saccharin to be used as a possible marker for sewage and wastewater pollution.

A number of different analytical approaches have been used for the determination of saccharin. Gas chromatographic methods [17, 18] are generally time consuming as they require derivatisation and are generally now not reported. A number of different liquid chromatographic methods have been used for its determination in water and soil samples. The majority utilised reverse phase liquid chromatography with mass spectral determination requiring a well-equipped laboratory with highly trained personal and deal of expenditure. Saccharin has been determined in plasma and urine following acidification and extracted by diethyl ether by reverse phase HPLC using fluorescence detection [19]. However, problems result from the high water solubility of saccharin which makes liquid-liquid solvent extraction difficult and means that commonly employed reverse phase chromatography techniques require low organic modifier concentrations or ion-pairing [13]. A number of other liquid chromatographic techniques have been used to overcome these issues. Recently, hydrophobic interaction liquid chromatography (HILIC) has been successfully explored [16]. However, HILIC uses mobile phases with high acetonitrile concentrations, which cannot be described as green and lead to issues with disposal.

High performance anion exchange chromatography (HPAEC) has been used by Biemer [20] to determine the concentrations of saccharin, acesulfame-K and sodium cyclamate in chewing gum, candies, mouthwash and cough syrup following acidification and solvent extraction with chloroform. Anion exchange techniques require aqueous based mobile phases which represent a green alternative to other chromatographic approaches. As a result, they present little danger to the user or in disposal. Predominantly, anion-exchange chromatography is undertaken at high pH values with buffers such as carbonate which lead to issues with incompatibility with some equipment. However, due to the very low pK_a value of saccharin (<2) this is not necessary.

This paper is divided into three main sections; in the first section the liquid anion exchange chromatographic behaviour of saccharin is investigated. The next section investigates the optimisation of the developed assay and in the third final part the possibility of determining trace levels of saccharin in an environmental water sample is investigated. This is believed to be the first report on the application of HPAEC for the determination of saccharin in environmental water samples.

2. EXPERIMENTAL



(i)

2.1 Chemical and Regents

All chemicals were obtained from Fischer (Loughborough, UK), unless otherwise stated. Deionised water was obtained from a Purite RO200-Stillplus HP System (Purite Oxon., UK). Water samples were collected from three different sites. A freshwater pond situated the grounds of the University of the West of England (Frenchay, Bristol, UK), the Caron River, Bisoe, Cornwall, UK and tap water (Frenchay, Bristol, UK). Samples were filtered with a Whatman No.1 filter paper prior to analysis. Aliquots of this sample filtrate were then introduced to the HPAEC without further treatment. Samples were stored in amber glass bottles at 4 ^{o}C in the dark.

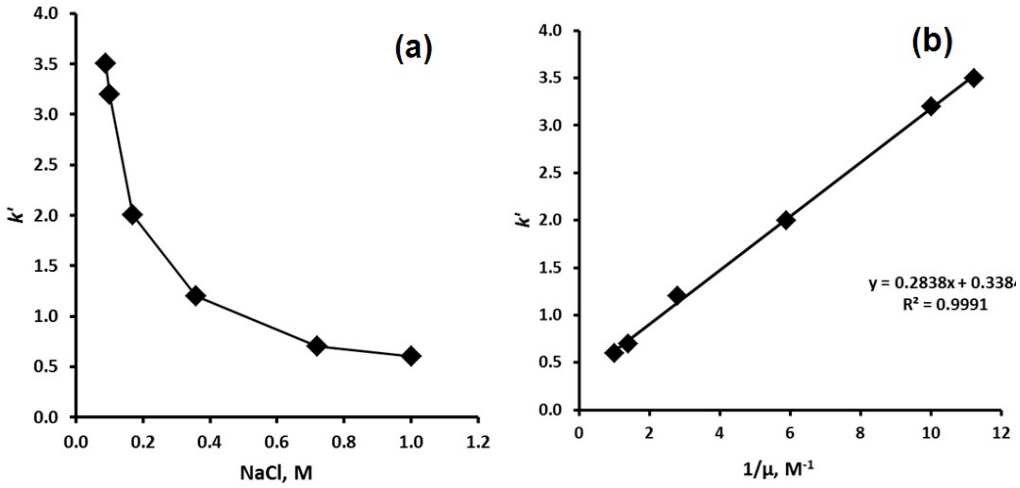
2.2 High Performance Anion Exchange Liquid Chromatography

Studies were undertaken using a system consisting of an IsoChrom pump (Spectra Physics), with a 100 mm $\times$ 4.6 mm, 8 μm SAP strong anion exchange column (Polymer Laboratories, Shropshire, UK) connected to a 7125 valve manual injector fitted with a 10 μL sample loop (Rheodyne, Cotati, USA). Sample filtrates were examined using a mobile phase of 0.1 M NaCl, at a flow rate of 1.5 mL/min. UV detection was undertaken using a Spectra Physics UV/Vis detector at 210 nm. Chromatograms were recorded using a Siemens Kompensograph X-T C1012 chart recorder. All experiments were undertaken at room temperature.

3. RESULTS AND DISCUSSION

3.1 Mobile Phase

The majority of anion exchange mobile phase utilise a high pH buffer which can be problematic with certain instrumentation. This is generally used in conjunction with sodium chloride or a similar salt. This present study has sort to avoid the use of the high pH buffer as to avoid issues with instrument compatibility, subsequent disposal and the health and safety of the user. In ion exchange chromatography the ionic strength of the mobile phase can be used to manipulate the retention of a particular solute by completing for the ion exchange sites [21]. Thus the solvent strength increases as the ionic strength



(a) (b)

Figure 1. (a) Plot of *k'* verses the NaCl concentration of the mobile phase; (b) Plot of *k'* verses reciprocal of ionic strength of mobile phase.

increases. Concentrations of NaCl were investigated between 89 mM and 1.0 M in order to optimise the chromatographic separation of saccharin. Column capacity factor (k) Equation 1 for saccharin was calculated for each NaCl concentration. Equation 2 shows the relationship of k and mobile phase ionic strength (μ) for a monovalent ion based system. Where t_0 is the time of the unretained fraction, t_R is the retention time of the analyte and μ is the ionic strength of the mobile phase.

$$k' = \frac{t_R - t_0}{t_0} \tag{1}$$

$$k' = \frac{1}{\mu} \tag{2}$$

Figure 1(a) shows that *k'* increases with decreasing concentrations of NaCl. **Figure 1(b)** shows a plot of k versus the ionic strength (1) of the mobile phase. A near linear relationship was obtained demonstrating the near theoretical relationship saccharin shows under the conditions explored. A mobile phase of 0.1 M NaCl was chosen as balance between k and assay time. A typical chromatogram of a standard solution is shown in **Figure 2** . A retention time of 2.1 minutes was obtained.

3.2 Calibration

Calibration standards were prepared in the concentration range from 10.2 ng/mL to 28.2 μg/mL in deionised water. **Figure 3** shows representative chromatograms obtained, with the resulting calibration curve. Peak height was found to be linear over the entire concentration range investigated ($R^2 = 0.999$). Coefficient of variation of 1.2 % and 3.2 % were found for concentrations of 28.2 μg/mL and 0.282 μg/mL saccharin respectively. A theoretical detection limit of 5 ng/mL (equivalent of 50 pg on column) was obtained based a signal-to-noise ratio of three.

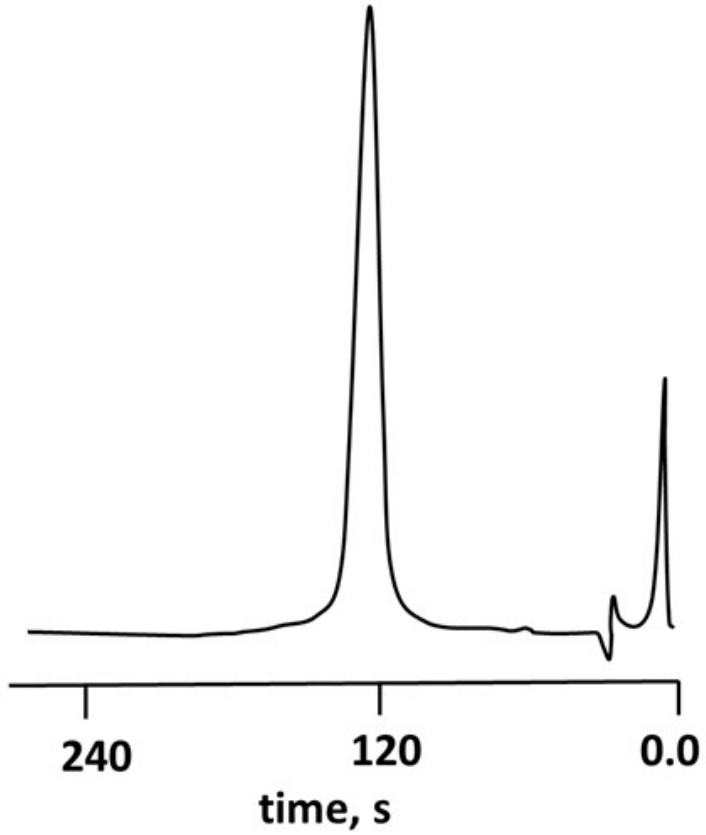


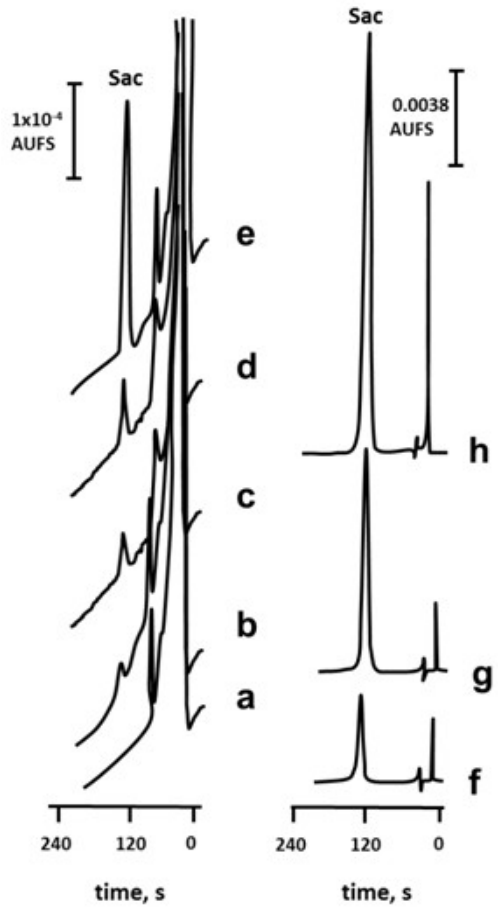
Figure 2. Typical chromatogram obtained for a 28.2 μg/mL saccharin standard. Mobile phase 0.1 M NaCl, flow rate 1.5 mL/min detection wavelength 210 nm.

3.3 Interferences

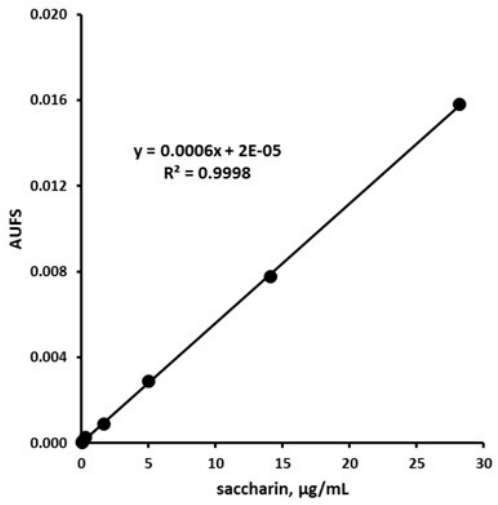
Nitrate, nitrite, chlorate, sodium cyclamate, acesulfame K and aspartame were investigated as possible interferences for the determination of saccharin. The artificial sweetener acesulfame K (Ace) (**Figure 4(a)**), nitrate (**Figure 4(b)**) and nitrite (not shown) were found to give well defined chromatographic responses, but with retention times removed from that of saccharin and consequently did not interfere. **Figure 4(a)** shows the resulting chromatogram obtained for 28.2 μg/mL saccharin in the presence of 22.0 μg/mL acesulfame K. As can be clearly seen, both compounds are fully resolved from each other, with a 100 % baseline separation. Similarly, **Figure 4(b)** shows the chromatogram obtained for a representative filtered pond water sample fortified with saccharin near the theoretical detection limit of the assay, at 5.68 μg/L, in the presences of native concentrations of nitrate (7.0 mg/L). As can be seen the peak for saccharin is well resolved from that of the preceding nitrate peak. Incidentally, it was found possible to determine the concentrations of nitrate present in the same filtered water sample utilising the same chromatographic conditions as that optimised of saccharin.

3.4 Analytical Application

Samples of river, tap and pond water were examined by using the optimised HPAEC method. No interferences were found with any of these samples. Native saccharin levels were found to be below the

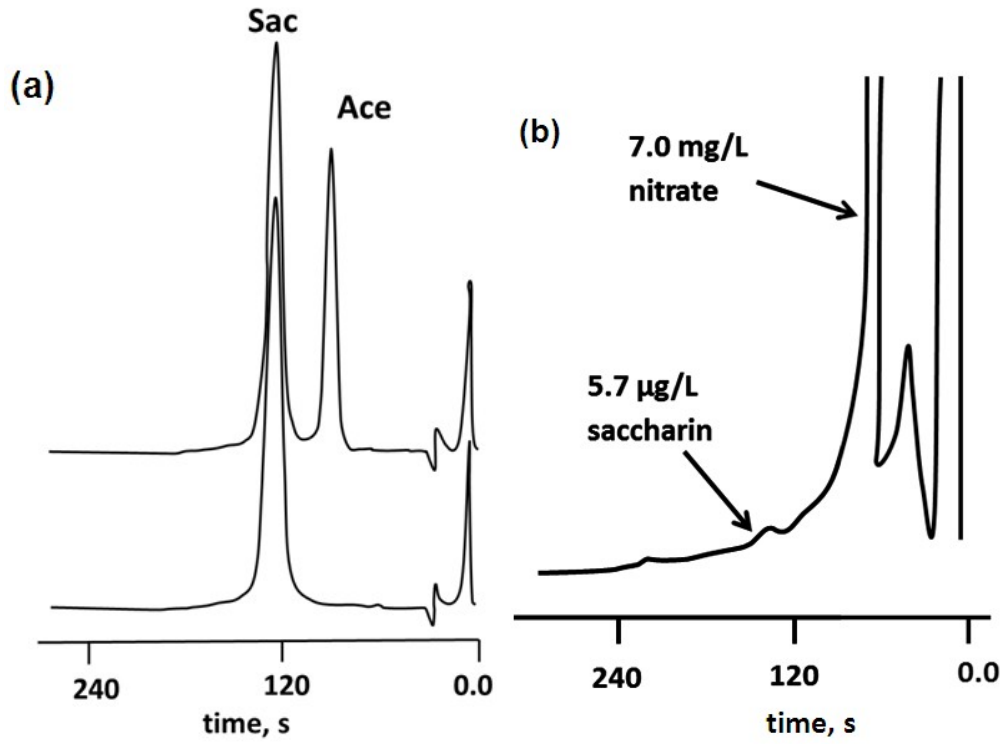


(a)



(b)

Figure 3. Top: typical chromatogram obtained for (a) 0 ng/mL, (b) 28.2 ng/mL, (c) 56.4 ng/mL, (d) 112.8 ng/mL (e) 282 ng/mL, (f) 5.00 μg/mL, (g) 14.1 μg/mL and 8.2 μg/mL saccharin (Sac). Below: resulting calibration curve. Injection volume 10 μL, mobile phase 0.1 M NaCl, flow rate 1.5 mL/min detection wavelength 210 nm.



(a) (b)

Figure 4. Typical chromatogram obtained for (a) 28.2 μg/mL saccharin (Sac) in the presence of 22.0 μg/mL acesulfame K (Ace) (b) filtered pond water sample fortified with 5.7 μg/L saccharin in the presence of 7.1 mg/L nitrate. Injection volume 10 μL, mobile phase 0.1 M NaCl, flow rate 1.5 mL/min detection wavelength 210 nm.

Table 1. Precision data for the HPAEC determination of saccharin in pond water fortified with 28.2 μg/L.

Sample	Native, μg/L	Added, μg/L	Found, μg/L	% Recovery
1	nd	28.2	25.47	90.3
2	nd	28.2	25.48	90.4
3	nd	28.2	27.29	96.8
4	nd	28.2	25.46	90.3
5	nd	28.2	27.28	96.7

Mean recovery = 92.9 %, Coefficient of variation = 3.8 %, nd = not detected.

detection limit of the method. To evaluate the possible analytical application of the developed method a sample of pond water was taken and fortified with 28.2 μg/L saccharin. A 100 mL aliquot was taken and filtered with a Whatman No. 1 filter paper. Aliquots for this were then examined using the optimised HPAEC method. Quantification was achieved by external calibration. The analytical percentage recovery for a typical pond water was found to be 92.9 % with an associated coefficient of variation of 3.8 % (n=5) for a sample fortified at 28.2 μg/L. **Table 1** summaries the concentrations of saccharin detected. These data demonstrate that the proposed method has promise for the determination of saccharin in such samples.

4. CONCLUSIONS

A green alternative liquid chromatographic method for the determination of saccharin in environmental water samples has been developed. Using high performance anion chromatography successful separation of saccharin was successfully obtained using a mobile phase of 0.1 M NaCl at a flow rate of 1.5 mL/min. A run time of only 2.5 minutes was obtained with successful separation of saccharin from other sample compounds. The developed method avoids the use of potentially environmentally hazardous reagents allowing for a green alternative for the determination of saccharin. The method offers several advantages over previously described HPAEC assays for saccharin [20] as it does not require an internal standard; it is faster and more sensitive. Future studies will focus on exploring the application of high performance anion chromatography for the determination of other artificial sweeteners [10] and other possible sewage pollution markers [5, 22]. Although the developed method is able to determine saccharin levels comparable to those reported in a several studies [23, 24] solid-phase extraction will be explored to isolate and concentrate these analytes.

ACKNOWLEDGMENT

I am grateful to the University of the West of England. I like to thank Paul Bowdler for the gift of the anion exchange column.

References

[1] R. M. Leal-Bautista, G. Hernandez, N. Jaime, G. Cuevas, and G. Velazquez, "Pathogens and pharmaceuticals pollutants as indicators of contamination at the northeasthern aquifer of quintana roo.," *Tropical and Subtropical Agroecosystems*, vol. 13, no. 2, pp. 211–219, 2011.

[2] T. A. Young, J. Heidler, C. R. Matos-Pérez, A. Sapkota, T. Toler, K. E. Gibson, K. J. Schwab, and R. U. Halden, "Ab initio and in situ comparison of caffeine, triclosan, and triclocarban as indicators of sewage-derived microbes in surface waters," *Environmental Science & Technology*, vol. 42, no. 9, pp. 3335–3340, 2008.

[3] I. J. Buerge, T. Poiger, M. D. Müller, and H.-R. Buser, "Caffeine, an anthropogenic marker for wastewater contamination of surface waters," *Environmental Science & Technology*, vol. 37, no. 4, pp. 691–700, 2003.

[4] S. T. Odonkor and J. K. Ampofo, "Escherichia coli as an indicator of bacteriological quality of water: an overview.," *Microbiology Research*, vol. 4, no. 2, 2013.

[5] A. Daneshvar, K. Aboulfadl, L. Viglino, R. Broséus, S. Sauvé, A.-S. Madoux-Humery, G. A. Weyhenmeyer, and M. Prévost, "Evaluating pharmaceuticals and caffeine as indicators of fecal contamination in drinking water sources of the greater montreal region," *Chemosphere*, vol. 88, no. 1, pp. 131–139, 2012.

[6] J. Wiley, L. M. Sherwood, and C. J. Woolverton, *Microbiología de Prescott*. Harley y Klein, 7a ed., MacGraw-Hill, 2008.

[7] J. L. Byard, E. W. McChesney, L. Golberg, and F. Coulston, "Excretion and metabolism of saccharin in man. II. Studies with ^{14}C-labelled and unlabelled saccharin," *Food and Cosmetics Toxicology*, vol. 12, no. 2, pp. 175–184, 1974.

[8] F. T. Lange, M. Scheurer, and H.-J. Brauch, "Artificial sweetenersa recently recognized class of emerging environmental contaminants: a review," *Analytical and Bioanalytical Chemistry*, vol. 403,

no. 9, pp. 2503–2518, 2012.

[9] A. Zygler, A. Wasik, and J. Namieśnik, "Analytical methodologies for determination of artificial sweeteners in foodstuffs," *TrAC Trends in Analytical Chemistry*, vol. 28, no. 9, pp. 1082–1102, 2009.

[10] I. J. Buerge, H.-R. Buser, M. Kahle, M. D. Muller, and T. Poiger, "Ubiquitous occurrence of the artificial sweetener acesulfame in the aquatic environment: an ideal chemical marker of domestic wastewater in groundwater," *Environmental Science & Technology*, vol. 43, no. 12, pp. 4381–4385, 2009.

[11] M. G. Kokotou, A. G. Asimakopoulos, and N. S. Thomaidis, "Artificial sweeteners as emerging pollutants in the environment: analytical methodologies and environmental impact," *Analytical Methods*, vol. 4, no. 10, pp. 3057–3070, 2012.

[12] M. Scheurer, H.-J. Brauch, and F. T. Lange, "Analysis and occurrence of seven artificial sweeteners in German waste water and surface water and in soil aquifer treatment (SAT)," *Analytical and Bioanalytical Chemistry*, vol. 394, no. 6, pp. 1585–1594, 2009.

[13] Z. Gan, H. Sun, R. Wang, and B. Feng, "A novel solid-phase extraction for the concentration of sweeteners in water and analysis by ion-pair liquid chromatography–triple quadrupole mass spectrometry," *Journal of Chromatography A*, vol. 1274, pp. 87–96, 2013.

[14] D. Van Stempvoort, J. Roy, J. Grabuski, S. Brown, G. Bickerton, and E. Sverko, "An artificial sweetener and pharmaceutical compounds as co-tracers of urban wastewater in groundwater," *Science of The Total Environment*, vol. 461, pp. 348–359, 2013.

[15] Z. Gan, H. Sun, B. Feng, R. Wang, and Y. Zhang, "Occurrence of seven artificial sweeteners in the aquatic environment and precipitation of tianjin, china," *Water Research*, vol. 47, no. 14, pp. 4928–4937, 2013.

[16] M. G. Kokotou and N. S. Thomaidis, "Determination of eight artificial sweeteners in wastewater by hydrophilic interaction liquid chromatography-tandem mass spectrometry," *Analytical Methods*, vol. 5, no. 16, pp. 3825–3833, 2013.

[17] D. T. Heitkemper, D. S. Jackson, L. A. Kaine, K. A. Mulligan, and K. A. Wolnik, "Determination of saccharin in shrimp by ion chromatography and capillary gas chromatography-mass spectrometry," *Journal of Chromatography A*, vol. 671, no. 1, pp. 323–329, 1994.

[18] P. Hartvig, O. Gyllenhaal, and M. Hammarlund, "Determination of saccharin in urine by electron-capture gas chromatography after extractive methylation," *Journal of Chromatography A*, vol. 151, no. 2, pp. 232–236, 1978.

[19] D. Bekersky and W. A. Colburn, "Determination of saccharin in plasma and urine by high performance liquid chromatography," *Analytical Letters*, vol. 13, no. 10, pp. 805–816, 1980.

[20] T. A. Biemer, "Analysis of saccharin, acesulfame-k and sodium cyclamate by high-performance ion chromatography," *Journal of Chromatography A*, vol. 463, pp. 463–468, 1989.

[21] A. Fallon, R. Booth, and L. Bell, *Laboratory techniques in biochemistry and molecular biology*, vol. 17. North-Holland Publishing Company, 1987.

[22] J. J. Harwood, "Molecular markers for identifying municipal, domestic and agricultural sources of organic matter in natural waters," *Chemosphere*, vol. 95, pp. 3–8, 2014.

[23] J. Spoelstra, S. L. Schiff, and S. J. Brown, "Artificial sweeteners in a large canadian river reflect human consumption in the watershed," *PloS one*, vol. 8, no. 12, p. e82706, 2013.

[24] D. R. Van Stempvoort, W. D. Robertson, and S. J. Brown, "Artificial sweeteners in a large septic plume," *Ground Water Monitoring & Remediation*, vol. 31, no. 4, pp. 95–102, 2011.