

Semi-quantitative determination of potential migrants in food packaging materials - Part 3: Non-volatile compounds



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Introduction

The importance of analyzing non-intentionally added substances (NIAS), not clearly defined small oligomers or compounds from not specifically regulated layers increase more and more in terms of compliance evaluations. For non-volatile substances no HPLC screening method exists so far with a broad applicability to various substance groups. Also lacks a possibility to estimate semi-quantitatively the concentrations in extracts without having reference substances available.

The first challenge was to find a suitable detector which is sensitive enough and has a signal largely independent from the chemical structure of the substances. Mass spectrometric response is highly dependent on the substances so that this detector is suitable for identification but not for quantification without having a reference substance. The UV- and fluorescence detection need chromophore groups for sensitive analysis. The universal reflection index detector (RI) and the evaporative light scattering detector (ELSD) can be used as universal detectors for HPLC systems, but these detectors are lacking in sensitivity.

The charged aerosol detector (CAD) combines sensitivity and independence from UV- or fluorescence active structures. The principle of the detector is that the mobile phase is nebulized with nitrogen and then evaporated in a drying tube. The resultant non-volatile particles are charged by ionized nitrogen and finally detected by a sensitive electrometer. The detector response should be proportional to the particle number and more or less independent from physico-chemical properties of the substance. The sensitivity is high enough to get detection limits in the ppb range. Therefore, the HPLC-CAD system is expected to show new possibilities in screening of unknown non-volatile migrants.

In this study the feasibility of an 'universal' HPLC separation method with a broad applicability to different substances for screening purposes has been studied using various substances.

Method

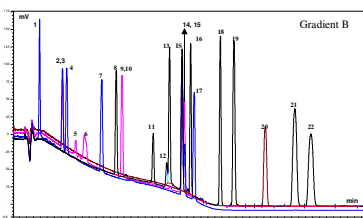
60 adhesive related substances which have various representative physico-chemical properties were selected. Standard solutions were prepared in methanol for calibration. A C18 column (HyperClone 250 × 4.60 mm, 5 µm particle size) was used for separation of the selected substances. Two compositions of acetonitrile (ACN) and water were used as the mobile phase for gradient solution (Gradient A : 10 - 0 % water in 45 min. Gradient B : 40 - 0 % water in 50 min). The standard substances have been investigated by seven calibration levels using the CAD. Sensitivity and slopes of calibration lines have been compared. The relative response factors (RRF) were determined versus the response of Tinuvin 234 as the internal standard. The CAD was set to a gas pressure of 35 psi, none filter mode and a range of 100 pA. The relative response factor (RRF) was defined as signal/concentration ratio between analyte and the internal standard Tinuvin 234. The RRF was calculated for mass related concentration (mg/l, RRF w/w) and molar concentration (mol/l, RRF mol/mol).

$$\text{Relative response factor (RRF)} = \frac{\text{Area}_s \cdot C_{is}}{C_s \cdot \text{Area}_{is}}$$

Area_s: Peak area of the analyte
C_s: Concentration of analyte (mg/l or mol/l)
Area_{is}: Peak area of the internal standard
C_{is}: Concentration of internal standard (mg/l or mol/l)

Results

23 substances (Table 1 and Figure 1) could be detected by using HPLC-CAD. The minimum molecular weight for detection on CAD was 228 g/mol. The responses of the volatile compounds were poor on the CAD. The reason for this result is the evaporation step in the CAD where a significant portion of the compound was evaporated prior to detection [1]. Volatility of chemicals is related to vapor pressure (VP) and decreases with the increase in molecular weight. The highest VP value in the detected substances on CAD was 6.29 · 10⁻⁵ mm Hg (2-Ethylhexyl diphenyl phosphate). The substances that have higher VP value than this value could not be detected in the calibration range as well as at high concentration (about 1000 mg/l). VP values of all detected substances except 2-ethylhexyl diphenyl phosphate were below 10⁻⁷ mm Hg. The magnitude of the response increases with increasing the ratio of organic solvent in the mobile phase [2]. However, there was statistically no significant difference between the mean of RRF values obtained by using the mobile phase Gradient A and B. Separation was better with mobile phase Gradient B. For retention of ionic compounds like docusate sodium or organic acids, it is necessary to adjust the pH of the mobile phase using an appropriate buffer.



1. Bisphenol A	2. Diethylene glycol dibenzoate	3. Triethylene glycol dibenzoate	4. BADGE
5. Propylene glycol dibenzoate	6. Dipropylene glycol dibenzoate	7. 4,4'-Bis(2-ethylhexyloxy) benzophenone	8. Irganox 1098
9. 2-Ethylhexyl diphenyl phosphate	10. 2,2,4-Triethyl-1,3-pentanediol dibenzoate	11. Irganox 1081	12. Chlarnox B1
13. Irganox 1035	14. Diethylene glycol adipate	15. Diethylene glycol phthalate	16. Irganox 3114
17. Ukon OB	18. Irganox 1010	19. Irganox 1330	20. Irganox PS-800
21. Irganox 1076	22. Irgaflex 168	23. Tinuvin 234	

Figure 1: Representative chromatograms of the adhesive related substances detected on HPLC-CAD by using gradient condition B (standard solution containing 50 µg/ml).

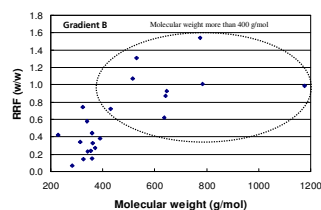


Figure 2: Relationship between molecular weight and RRF (w/w) of the substances detected on HPLC-CAD by using gradient condition B.

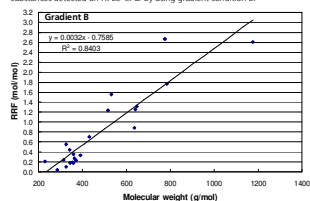


Figure 3: Relationship between molecular weight and RRF (mol/mol) of the substances detected on HPLC-CAD by using gradient condition B.

Table 1: Relative response factor (RRF), LOD and correlation coefficient R values of various substances analyzed with HPLC-CAD under two elution conditions.

Classification	Nr.	Substances	MW (g/mol)	Vapor pressure mmHg (25 deg C)	LOD (µg/ml)		Correlation coefficient R		RRF (w/w)	
					Gradient A	Gradient B	Gradient A	Gradient B	Gradient A	Gradient B
Group B Plasticizers	1	Diethylene glycol dibenzoate	390.56	1.42E-07	1.6852	ND< 5 ppm	0.9990	ND< 5 ppm	0.54	0.38
	2	Diethylene glycol adipate	370.57	8.50E-07	1.5692	ND< 10 ppm	0.9989	ND< 10 ppm	0.42	0.27
	3	2-Ethylhexyl diphenyl phosphate	362.44	6.29E-05	1.9676	ND< 5 ppm	0.9984	ND< 5 ppm	0.39	0.33
	4	Diethylene glycol dibenzoate	314.34	7.22E-07	1.2381	ND< 5 ppm	0.9994	ND< 5 ppm	0.60	0.34
	5	Triethylene glycol dibenzoate	358.40	4.75E-08	0.6217	0.9263	0.9998	0.9263	0.71	0.44
	6	Dipropylene glycol dibenzoate	342.42	4.66E-07	2.6110	ND< 5 ppm	0.9976	ND< 5 ppm	0.32	0.23
	7	Propylene glycol dibenzoate	284.30	1.90E-07	ND< 5 ppm	ND< 10 ppm	ND< 5 ppm	ND< 10 ppm	0.13	0.07
	8	2,2,4-Triethyl-1,3-pentanediol dibenzoate	354.45	8.72E-09	2.6915	ND< 10 ppm	0.9974	ND< 10 ppm	0.29	0.24
Group F Antioxidants	9	Irganox 1076	531	3.38E-13	1.1318	0.5698	0.9995	0.9999	1.25	1.31
	10	Irgaflex 168	646.93	1.84E-13	0.9041	1.2594	0.9997	0.9993	1.05	0.93
	11	Irganox 1330	775.21	3.14E-22	1.5254	1.2902	0.9989	0.9992	1.54	1.54
	12	Irganox 1010	1177.7	1.16E-33	1.5294	1.1558	0.9992	0.9996	0.97	0.99
	13	Chlarnox B1	326	5.25E-09	ND< 10 ppm	ND< 25 ppm	ND< 10 ppm	ND< 25 ppm	0.21	0.14
	14	Irganox 1081	358	4.90E-08	1.4376	ND< 5 ppm	0.9990	ND< 5 ppm	0.40	0.15
	15	Irganox PS 800	515	1.76E-13	1.5266	0.9197	0.9992	0.9997	1.00	1.07
	16	Irganox 1098	697	1.19E-22	1.6625	0.9855	0.9988	0.9996	0.88	0.62
	17	Irganox 1035	642	5.38E-18	1.6356	2.1303	0.9989	0.9982	0.97	0.87
	18	Irganox 3114	784	8.89E-24	1.6167	1.5366	0.9989	0.9990	0.98	0.99
Group G Others	19	Bisphenol A	228.29	3.91E-07	NR	3.5687	NR	0.9955	NR	0.42
	20	4,4'-Bis(2-ethylhexyloxy) benzophenone	324.46	3.25E-09	1.8164	4.1938	0.9988	0.9926	0.61	0.74
	21	BADGE	340.42	1.08E-07	2.0444	1.3744	0.9981	0.9992	1.02	0.58
	22	Ukon OB	430.06	1.72E-12	1.4780	0.6613	0.9991	0.9998	0.89	0.72
	23	Docusate sodium	445.63	2.17E-11	NR	NR	NR	NR	NR	NR
	Mean								0.72a	0.61a

ND: Not detected, NR: Not retained, ¹ Means with the same letter are not significantly different (p > 0.05).

Nine substances that have a molecular weight of more than 400 g/mol, could be detected with enough and/or consistent mass concentration related response between 0.6 and 1.5 (Table 1 and Figure 2). The mean value was 0.72 with standard deviation of 0.37 in Gradient A and 0.61 with standard deviation of 0.41 in Gradient B. The frequency of the relative response factors in 0.2 steps are shown in figure 4. The molarity related relative response mol/mol shows a linear correlation increasing with the molecular weight (figure 3).

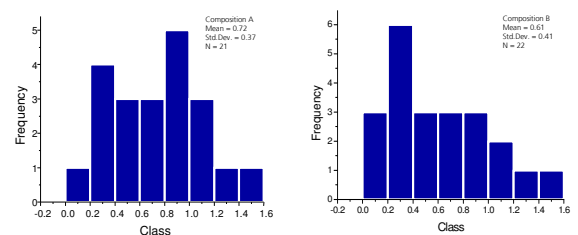


Figure 4: Distribution of RRF of all selected substances obtained in the mobile phase composition A and B.

Conclusions

- (1) The CAD showed good sensitivity for substances with molecular weight greater than 400 g/mol or vapour pressure smaller than 10⁻¹¹ mm Hg (25 °C).
- (2) The mobile phases water-acetonitrile gradient are suitable for separating most of the test substances, highly polar and ionic compounds need specific mobile phases with adjusted pH.
- (3) Substances with molecular weight >400 g/mol show relative response (w/w) to Tinuvin 234 between 0.6 and 1.5. This means, a Tinuvin-equivalent of 1 mg/l estimates a real substances concentration between 1.7 mg/l (RRF = 0.6) and 0.7 mg/l (RRF = 1.5). Therefore, semi-quantification via Tinuvin 234 equivalent is possible with an acceptable range of uncertainty.
- (4) CAD coupled with a reversed-phase HPLC system shows a powerful feasibility to develop a screening method for unknown non-volatile migrants.

References

- [1] R. McCarthy, P. Gamache, D. Asa. Development and evaluation of corona charged aerosol detection (CAD): a new universal detector for HPLC, LabPlus international, June 2005.
- [2] T. Görecki, F. Lynen, R. Szucs, P. Sandra. Universal response in liquid chromatography using charged aerosol detection, Anal. Chem, 78, 2006.

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