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Simultaneous determination of hydroquinone, resorcinol, phenol, *m*-cresol and *p*-cresol in untreated air samples using spectrofluorimetry and a custom multiple linear regression-successive projection algorithm

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ABSTRACT

In this study, a novel, simple, and efficient spectrofluorimetric method to determine directly and simultaneously five phenolic compounds (hydroquinone, resorcinol, phenol, *m*-cresol and *p*-cresol) in air samples is presented. For this purpose, variable selection by the successive projections algorithm (SPA) is used in order to obtain simple multiple linear regression (MLR) models based on a small subset of wavelengths. For comparison, partial least square (PLS) regression is also employed in full-spectrum. The concentrations of the calibration matrix ranged from 0.02 to 0.2 mg L^{-1} for hydroquinone, from 0.05 to 0.6 mg L^{-1} for resorcinol, and from 0.05 to 0.4 mg L^{-1} for phenol, *m*-cresol and *p*-cresol; incidentally, such ranges are in accordance with the Argentinean environmental legislation. To verify the accuracy of the proposed method a recovery study on real air samples of smoking environment was carried out with satisfactory results (94–104%). The advantage of the proposed method is that it requires only spectrofluorimetric measurements of samples and chemometric modeling for simultaneous determination of five phenols. With it, air is simply sampled and no pre-treatment sample is needed (i.e., separation steps and derivatization reagents are avoided) that means a great saving of time.

1. Introduction

Cigarette smoke is an aerosol composed of gases and heterogeneous particles formed when tobacco is burned during the smoking of cigarettes. Tobacco smoke is a complex mixture of about 5000 chemicals [1–3]. Most of these smoke components are generated by the combustion of *ca.* 4000 components of tobacco leaf [4]. During the past decades, the importance of gas-phase constituents, especially the volatile organic compounds, for the cytotoxic and carcinogenic potential of cigarette smoke has been clearly demonstrated [5–8] in various cellular and animal models.

The phenolic compounds which come from tobacco smoke, such as hydroquinone, resorcinol, and phenol itself [9–11], are irritants to the human the air tract and skin [5]. Hydroquinone has severe effects on the central nervous system and resorcinol produces a generalized eczema, urticaria and angioneurotic edema [5,12]. Phenol is considered very toxic for human beings orally. Thus, the ingestion of as little as 1 g has been reported as lethal, with symptoms including muscle weakness and tremors, loss of coordination, paralysis, convulsions, coma, and respiratory failure [6]. Cresol isomers and phenol affect the liver, kidneys, lungs, and vascular system [13,14]. Thus, the toxicity and persistence of phenols in the environment makes monitoring highly relevant, particularly in air samples of closed rooms where smoking is allowed.

The Occupational Safety and Health Agency of Argentina [7] suggests the acceptable levels for hydroquinone (4 mg m^{-3} of air), resorcinol (90 mg m^{-3} of air), *p*-cresol and *m*-cresol (22 mg m^{-3} of air) and phenol (38 mg m^{-3} of air), for short sampling intervals of 15 min in smoking designated areas.

There are several methods to determine phenols in a variety of matrices, by using, for instance, flow injection analysis with spectrophotometric detection [8], or via chromatographic means with different detections [15–18]. These methods almost always involve at least one step of sample pre-treatment in order to achieve a physical or chemical separation of the dihydroxybenzene derivates. Usually, the sample treatment is laborious and time consuming. Papers on direct determination of air samples are still rare [19–21]. Thus, the development of new method for simultaneous

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determination of phenols without pre-treatment and/or separation of these compounds is a relevant research subject.

Fluorescence spectrometry is known as a useful technique for carrying out simultaneous multianalyte determinations without pre-treatment. Moreover, the application of chemometric techniques such as PCR and PLS to the fluorescence spectral data introduces another dimension to the analysis of complex mixtures [22–24], as prior separation or sample pre-treatment is not required [19,25,26].

MLR yields models are simpler and easier to interpret than PCR and PLS, since these calibration techniques perform regression on latent variables, which do not have physical meaning. In the other hand, MLR calibration is more dependent on the spectral variables selection. To overcome this problem, Araújo and co-workers [27–29] proposed a novel variable selection strategy for MLR calibration, which uses the "successive projections algorithm" (SPA) to minimize collinearity problems. SPA is a forward selection method which operates off the instrumental response. The number of variables selected can be optimized in order to maximize model prediction capability. MLR–SPA works in three stages [27–29]. First, it builds ordered chains of variables by selecting variables which share the least collinearity with previous variables; then, it constructs MLR models for each chain of variable and chooses the best model that yields the lower RMSEP values [27,28]; and finally the algorithm eliminates the variables that do not influence the model [29]. Recently a cross-validation procedure was implemented for SPA algorithm [30]. Particularly when applied for multicomponent determination of phenols, MLR–SPA was used on sea water samples and resulted in better models than PLS [26].

In the present paper, we propose a new method to determine simultaneously and directly hydroquinone, resorcinol, phenol, and *p*- and *m*-cresol in air samples by fluorescence spectroscopy (emission and excitation spectra) coupled with multivariate calibration analysis using both MLR–SPA and PLS techniques; a comparison of these chemometric analyses was also carried out. The proposed method was designed in way that air is simply sampled and no pre-treatment is needed, i.e., separation steps and derivatization reagents are avoided.

2. Materials and methods

2.1. Reagents and solutions

All reagents were of analytical grade and ultra pure water (18 MΩ) was used throughout. Hydroquinone, resorcinol, and phenol stock solutions were prepared daily by dissolving 0.00500 g of each analyte (Mallinckrodt) in 100 mL of water. The *m*-cresol and *p*-cresol stock solutions were prepared daily by dissolving 9.6 μL of each analyte (Mallinckrodt) in 100 mL of water. The standard solutions were prepared by appropriate dilutions of the corresponding stock solution.

2.2. Apparatus and acquisition of spectra

A computer-controlled spectrofluorimeter SLM Aminco Bowman series 2, equipped with a xenon discharge light source (150 W) was used to acquire all the spectra. Wavelength accuracy and wavelength repeatability were ±0.5 and ±0.25 nm, respectively. The excitation and emission slits ranged from 2 to 16 nm, but for most measurements the optimum value was of 8 nm. Fluorescence measurements were performed using a standard 1 cm × 1 cm quartz cell. The luminescence spectra were collected by scanning the emission spectrum from 290 to 370 nm, maintaining the excitation wavelength at 274 nm, whereas for the excitation spectra from 220 to 290 nm, the emission wavelength was kept at 302 nm. The spectra were saved in ASCII format, and transferred to a microcomputer.

2.3. Chemometric models

The calibration mixtures were prepared following a Brereton design [31], from which 16 calibration samples with the following analyte concentration ranges were defined: 0.02–0.2 mg L^{-1} for hydroquinone, 0.05–0.6 mg L^{-1} for resorcinol, 0.05–0.4 mg L^{-1} for phenol, *m*-cresol, and *p*-cresol.

PLS and MLR–SPA models of calibration were constructed for the five phenols by using both excitation and emission spectrum data simultaneously; all spectra of the mixtures were mean-centered prior to modeling. Full cross-validation was used to validate the models and to allow a comparative analysis of their performances. The wavelength selection was carried out by using a lab-created SPA routine implemented in Matlab® 6.5. PLS modelling was performed in The Unscrambler® 9.6 software (CAMO A/S).

2.4. Sampling

For sampling, a Dreschel-type absorption system was connected to borosilicate glass bottles of 250 mL. The external diameter of both the entry an exit tubes of the absorption bottles was 10 mm. The air intake tube had an internal diameter of 8 mm and was set at 15 mm from the bottom of the bottle. The absorption bottle was connected to a pump that operated at a constant flow rate of 80 mL min^{-1} [19,25].

The sampling was carried out in a 25 m^3 room in which smoking was allowed; with people randomly entering to smoke at different hours of the day (24 h). The numbers of cigarettes was not considered. Sampling time was set at 15 min, as defined by the applicable regulation as a short exposure time of the sampled air of interest. A suitable volume of the absorption solution (25 mL of water) was placed in the absorption bottles and then connected to the pump. The amount of collected sample was of 1.2 L of air. The samples were stored and kept in dark bottles at 4 °C for one week and remained stable.

The recovery studies were done on aliquots of 2 mL of the adsorption solution by adding different concentrations of all five analytes. The final volume was made up to 10 mL. The resulting mixture was analyzed by the proposed methodology.

3. Results and discussion

Hydroquinone, resorcinol, phenol, *m*-cresol, and *p*-cresol in aqueous solution show native fluorescence, as presented in Fig. 1. The overlapping spectra made it difficult for the direct determination of the analytes in the samples. Previously, hydroquinone, resorcinol, and phenol had been modeled using synchronous fluorescence [25]. So, now with two more phenols present in the system, *m*-cresol and *p*-cresol, synchronous fluorescence data were not efficient for resolving the overlap. To overcome the problem, SPA variable selection from excitation and emission spectra, followed by MLR modeling (MLR–SPA) were used. The excitation and emission spectra of a representative sample from the calibration set is shown in Fig. 2.

Both PLS and MLR–SPA strategies yielded good models for the determination of each individual phenol, but MLR–SPA models were consistently a little better than PLS, as shown by the resulting figures of merit for these models (Table 1).

Figures of merit for multivariate models have been discussed elsewhere [26,32]. The sensitivity for a given analyte *i* is defined

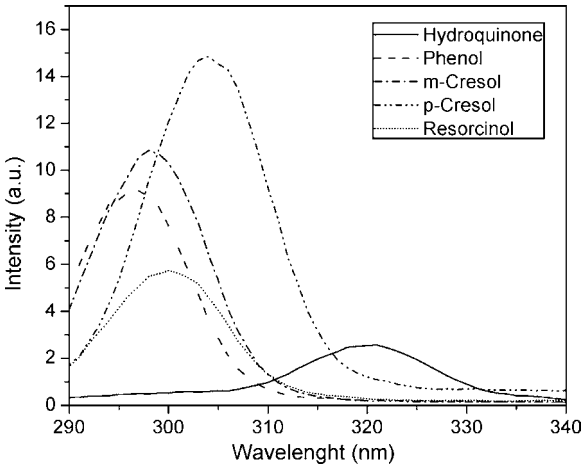

Fig. 1. Fluorescence spectra of the five individual phenols used in this study, depicting spectral overlapping.

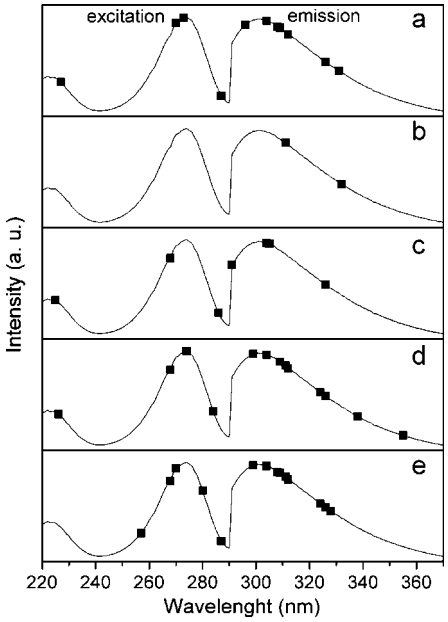

Fig. 2. Excitation and emission spectrum of a representative sample of the calibration set. SPA–MLR selected variables for (a) *m*-cresol, (b) hydroquinone, (c) *p*-cresol, (d) phenol, and (e) resorcinol.

as:

$$\mathrm{SEN}_i = \frac{1}{\left\|\mathbf{b}_i\right\|}$$

where $\|\ \|$ indicates the Euclidean norm and $\mathbf{b}_i$ is the vector of the final regression coefficients appropriate for component *i*. Sensitivity values were only obtained for the PLS models. Since it is not possible to assure that the variables selected by SPA are orthogonals, $\left\|\mathbf{b}_i\right\|$ and SEN_i were not calculated for the MLR–SPA models [26].

The standard error of prediction (SEP) measures the precision of a prediction, while RMSEP measures its accuracy [33]. The SEP is defined as:

$$\mathrm{SEP} = \sqrt{\sum_{i=1}^{N_p} \frac{(\hat{y}_i - y_i - \mathrm{BIAS})^2}{(N_p - 1)}}$$

where

$$\mathrm{BIAS} = \sum_{i=1}^{N_p} \frac{(\hat{y}_i - y_i)}{N_p}$$

Concentration ranges of the analyzed phenols, sensitivity (SEN), precision (SEP), accuracy (RMSEP) and BIAS values for each analyte are also presented in Table 1.

Fig. 2 shows the variables selected by MLR–SPA strategy for the five phenols. For hydroquinone, only two variables were selected whereas for others phenols more than seven variable were selected. Thus, the hydroquinone spectrum is the least overlapping among the five phenols (Fig. 1), which makes the determination of this analyte less susceptible to interference.

In order to check the traceability of the proposed method, a recovery study with real samples was carried out for MLR–SPA models and yielded acceptable values from 92 to 104% (Table 2).

Table 3 shows the obtained results when the method was applied to real samples. The sampling by smoking was carried out as mention in Section 2.4, the collection was carried out in this area at two different hours (morning and afternoon) in the same day, and at noon in three different days. These data reveal that the concentration of all five phenols in the room air increases in the afternoon, while the room is not ventilated. The concentration of the phenols did not vary significantly when the air was sampled at the same hour in different days. It is worth noting that the concentrations of phenols measured at noon were within the range of the singular values measured in the morning and the afternoon. Although, a detailed study on air quality was out of the scope of this particular

Table 1
Results and figures of merit for comparison of models (spectral regions (nm): excitation = 220–290 and emission = 290–370).

Analytes	Hydroquinone	Resorcinol	Phenol	*m*-Cresol	*p*-Cresol
PLS models					
Factors	3	6	4	5	6
Concentration ranges ($\mathrm{mg\,L^{-1}}$)	0.02–0.20	0.05–0.60	0.05–0.40	0.050–0.40	
RMSECV ($\mathrm{mg\,L^{-1}}$)	0.009	0.085	0.026	0.024	0.045
SEN	6.4 e+005	74.1	2.4 e+003	1.2 e+003	169.3
SEP	0.009	0.087	0.027	0.024	0.046
BIAS	−6.0 e−5	0.008	5.0 e−4	−0.003	−2.6 e−4
R_{cv}	0.99	0.91	0.98	0.98	0.95
MLR–SPA models					
Analytes	Hydroquinone	Resorcinol	Phenol	*m*-Cresol	*p*-Cresol
Number of Variable	2	14	13	11	7
Concentration ranges ($\mathrm{mg\,L^{-1}}$)	0.02–0.20	0.05–0.60	0.05–0.40	0.050–0.40	0.05–0.40
RMSECV ($\mathrm{mg\,L^{-1}}$)	0.009	0.018	0.001	0.005	0.035
SEP	0.009	0.018	0.001	0.005	0.036
BIAS	5.6 e−5	−0.006	6.0 e−5	1.2 e−4	0.003
R_{cv}	0.99	0.99	0.99	0.99	0.97

Table 2
Recovery of phenols in real air samples.

Sample	Phenol	Hydroquinone	Resorcinol	*m*-Cresol	*p*-Cresol
Added (mg m^{-3} air)					
1	–	–	–	–	–
2	9.40	4.80	12.09	5.40	5.80
3	12.30	6.10	14.85	7.30	7.18
4	14.85	7.60	16.41	8.85	9.10
Found (mg m^{-3} air)[a]					
1	2.10 ± 0.31	2.34 ± 0.10	3.37 ± 0.48	1.30 ± 0.15	1.58 ± 0.27
2	8.84 ± 0.75	4.89 ± 0.33	12.15 ± 0.42	4.98 ± 0.59	5.46 ± 0.37
3	12.58 ± 0.81	6.18 ± 0.24	15.04 ± 0.38	7.15 ± 0.45	6.97 ± 0.39
4	14.23 ± 0.60	7.54 ± 0.46	17.11 ± 0.23	8.81 ± 0.32	9.12 ± 0.29
Recovery (%)					
1	–	–	–	–	–
2	94.0	101.9	100.5	92.2	94.1
3	102.3	101.3	101.3	97.9	97.1
4	95.8	99.2	104.3	99.5	100.2

[a] $n = 3$.

Table 3
Analysis of phenols real air samples.

Sample	Phenol	Hydroquinone	Resorcinol	*m*-Cresol	*p*-Cresol
		(mg m^{-3} air)			
1[a]	1.71	1.50	2.95	1.11	1.23
2[b]	2.44	2.68	6.28	1.75	1.98
3[c]	2.10 ± 0.31	2.34 ± 0.10	3.37 ± 0.48	1.30 ± 0.15	1.58 ± 0.27

[a] Sample collected in the morning.
[b] Sample collected in the afternoon.
[c] Mean of 3 replicates collected in different days, at noon.

work, the data given in Table 3 demonstrated the applicability of this methodology on real air samples.

4. Conclusion

The proposed method has been successful for simultaneous prediction of concentrations of up to five phenols in air samples from a smokers environment. The determination is simple, fast and low cost, owing to the fluorimeter which is an available technique in many laboratories of chemical analysis. It is important to underline that this is a direct method for simultaneous determination of phenols without sample treatment which means great time savings. The calibration models obtained for the MLR–SPA presented the best results that the calibration model obtained for the PLS. Thus, the proposed method could be used in the control of the phenols presence in air, while, for example, the private rooms to the smokers.

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References

[1] C.R. Green, A. Rodgman, Rec. Adv. Tob. Sci. 22 (1996) 131.
[2] T.A. Perfetti, W.M. Coleman, W.S. Smith, Beitr. Tabakforsch. Int. 18 (1998) 95.
[3] R.R. Baker, C. Proctor, Where there's smoke, in: Chemistry in Britain, London, Chemical Education Trust Fund for the Chemical Society and the Royal Institute of Chemistry Royal Society of Chemistry, 2001, pp. 38–41.
[4] J.C. Leffingwell, Basic chemical constituents of tobacco leaf and differences among tobacco types, in: D.L. Davis, M.T. Nielson (Eds.), TOBACCO Production, Chemistry and Technology, Blackwell Science, London, 1999, pp. 265–284 (Chapter 8).
[5] Physicians for smoke-free Canada, 1999. http://www.smoke-free.ca (Cited March 1999).
[6] Agency for Toxic Substances and Disease Registry (ATSDR), Toxicological Profile for Phenol, US Public Health Service, US Department of Health and Human Services, Atlanta, GA, 1989.
[7] Decreto N° 351/97 reglamentario en la Ley Nacional 19587, Seguridad e Higiene Laboral.
[8] K.O. Lupetti, F.R.P. Rocha, O. Fatibello-Filho, Talanta 62 (2004) 463.
[9] W.S. Schlotzhauer, O.T. Chortyk, J. Anal. Appl. Pyrol. 12 (1987) 193.
[10] K. Torikaiu, Y. Uwano, T. Nakamori, W. Tarora, H. Takahash, Food Chem. Toxicol. 43 (2005) 559.
[11] H. Sakuma, S. Matsushima, S. Munakata, S. Sugawara, Agric. Biol. Chem. 46 (1982) 1311.
[12] A. Barbaud, P. Modiano, M. Cocciale, S. Reichert, J.L. Schmutz, J. Dermatol. 135 (1996) 1014.
[13] Criteria for a Recommended Standard Occupational Exposure to Phenol, USDHEW, PHS, CDC, NIOSH, 1976, pp. 76–196.
[14] Criteria for a Recommended Standard Occupational Exposure to Cresol, USDHEW, PHS, CDC, NIOSH, 1978, pp. 78–133.
[15] I. Rodriguez, M.H. Bollain, R. Cela, J. Chromatogr. A 750 (1996) 341.
[16] G. Boattoa, M. Nieddua, A. Cartaa, A. Paua, S. Lorenzonib, S.P. Manconia, D. Serra, Forensic Sci. Int. 139 (2004) 191.
[17] M. Kojima, S. Tsunoi, M. Tanaka, J. Chromatogr. A 1042 (2004) 1.
[18] C.H. Risner, J. Liquid Chromatogr. Related Technol. 16 (1993) 4117.
[19] M. Pistonesi, M.E. Centurión, M. Pereyra, A.G. Lista, B.S. Fernández Band, Anal. Bioanal. Chem. 378 (2004) 1648.
[20] A. Avramescu, S. Andreescu, T. Noguer, C. Bala, D. Andreescu, J.L. Marty, Anal. Bioanal. Chem. 374 (2002) 25.
[21] Y. Hanada, I. Imaizumi, K. Kido, T. Tanizaki, M. Koga, H. Shiraishi, M. Soma, Anal. Sci. 18 (2002) 655.
[22] M. Del Olmo, C. Díez, A. Molina, I. De Orbe, J.L. Vílchez, Anal. Chim. Acta 335 (1996) 23.
[23] A. Espinosa-Mansilla, A. Muñoz de la Peña, F. Salinas, D. Gonzalez Gómez, Talanta 62 (2004) 853–860.
[24] D. Cozzolino, M.J. Kwiatkowski, M. Parker, W.U. Cynkar, R.G. Dambergs, Anal. Chim. Acta 513 (2004) 73–80.
[25] M.F. Pistonesi, M.S. Di Nezio, M.E. Centurión, M.E. Palomeque, A.G. Lista, B.S. Fernández Band, Talanta 69 (2006) 1265–1268.
[26] M.S. Di Nezio, M.F. Pistonesi, W.D. Fragoso, M.J.C. Pontes, H.C. Goicoechea, M.C.U. Araujo, B.S. Fernández Band, Microchem. J. 85 (2007) 194–200.
[27] M.C.U. Araújo, T.C.B. Saudanha, R.K.H. Galvão, T. Yoneyama, H.C. Chame, V. Visani, Chemom. Intell. Lab. Syst. 57 (2001) 65–73.
[28] R.K.H. Galvão, M.F. Pimentel, M.C.U. Araújo, T. Yoneyama, V. Visani, Anal. Chim. Acta 443 (2001) 107–115.
[29] R.K.H. Galvão, M.C.U. Araújo, W.D. Fragoso, E.C. Silva, G.E. José, S.F.C. Soares, H.M. Paiva, Chemom. Intell. Lab. Syst. 92 (2008) 83–91.
[30] R.K.H. Galvão, M.C.U. Araújo, E.C. Silva, G.E. José, S.F.C. Soares, H.M. Paiva, J. Braz. Chem. Soc. 18 (2007) 1580–1584.
[31] R.G. Brereton, Chemometrics: Data Analysis for the Laboratory and Chemical Plant, Wiley, Chichester, West Sussex, Hoboken, NJ, 2003.
[32] A.C. Olivieri, N.M. Faber, J. Ferré, R. Boqué, J.H. Kalivas, H. Mark, Pure Appl. Chem. 78 (2006) 633–661.
[33] T. Naes, T. Isaksson, T. Fearn, T. Davies, A User-Friendly Guide to Multivariate Calibration and Classification, NIR Publications, Chichester, UK, 2002.