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Short communication

A multinjection strategy for mercury speciation

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Abstract

A multiinjection strategy has been developed to increase the sampling throughput of the high-performance liquid chromatography determination of inorganic-mercury, methylmercury, ethylmercury and phenylmercury. The method involves the injection of samples each 3.5 min, in spite of the fact that phenylmercury retention time corresponds to 9.04 min. In the selected conditions, the sampling frequency was 11 h^{-1} in front of that of 6 h^{-1}, obtained by conventional injection of each sample after the complete elution of Hg species. Additionally, the analytical reagents consumption was reduced drastically in almost 50%. The main characteristics of the chromatographic separation were maintained and only the resolution of phenylmercury was reduced from 10.3 to 1.7 and that of ethylmercury from 4.6 to 3.1.

Keywords: Mercury speciation; High-performance liquid chromatography; Multiinjection

1. Introduction

Mercury is found throughout the ecosystem in trace amounts: in soil, air, water and living organisms [1]. The accumulation of monomethylmercury (MMM) in fish and the subsequent poisoning of the Minamata inhabitants, was a turning point in the analysis of environmental levels of toxic metals, because it was apparent that to provide a clear picture of toxicity, biogeochemistry and bioaccumulation, it is necessary to measure all the different physicochemical forms [2].

MMM and the other organomercury compounds are more toxic than Hg(II), because of their lipophilic nature, which allows them to permeate biological membranes and enter cells. Once present in the cell, mercury can interfere with a number of biochemical processes by binding to biomolecules containing thiol groups [3].

Methylmercury is the most commonly occurring organomercury compound in environmental and biological materials, which is of particular concern because of its accumulation as it passes through the food chain, whereas ethylmercury and phenylmercury are quite rarely present in the environment. Thus, the simultaneous determination of inorganic- and organic-mercury, is important in order to do correctly evaluate the toxicity of mercury in environmental samples [4,5].

In the last two decades, the development of species-selective analytical methodologies has made possible the identification and quantification of such species.

Nowadays, it is clear that the best strategy for metal speciation is that based on the tandem of on-line chromatographic separation methods and atomic detection [6,7]. However, in some cases the complete separation of all the species of an element, currently present in natural samples, involves long times; which in fact reduce the productivity of the laboratories and increase the cost of analyses, also increasing the analytical waste and reagent consumption.

For the determination of mercury species, like, inorganic-mercury, methylmercury, ethylmercury and phenylmercury, several methods have being proposed based on the use of gas chromatography (GC) [8–10]. However, to do these determinations strong and thermally stable derivatives are needed, thus involving tedious and time-consuming derivatization and clean-up procedures.

On the other hand, high-performance liquid chromatography offers an interesting alternative to GC providing a simplified sample preparation. Table 1 summarizes some of the previously published studies, indicating the different columns employed

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Table 1
Precedents on the use of HPLC for Hg speciation

Species	Column	Mobile phase	Time of analysis (min)	Reference
Hg^{2+}, MeHg, EtHg and PhHg PDC chelates	Spherisorb ODS (2.3 μm 15 cm × 4.6 mm)	Methanol/acetonitrile/water	16	[11]
Hg^{2+}, MeHg, EtHg and PhHg HDz chelates	Nova-pak C_{18} column (4 μm, 15 cm × 3.9 mm i.d.)	THF/methanol/acetate buffer pH 4.0, 50 μM EDTA	12	[12]
Hg^{2+}, MeHg, EtHg and PhHg PDC chelates	Zorbax ODS (5 μm, 25 cm × 4.6 mm)	Methanol/acetonitrile/water containing 1.5 mmol/L APDC	25	[13]
MeHg, EtHg and PhHg	Zorbax ODS (5 μm, 25 cm × 4.6 mm)	Methanol–0.05 M NH_4Ac containning 0.01% 2-mercaptoethanol	12	[14]
MeHg, EtHg and PhHg	Chromospher RP-18 (20 cm × 3 mm)	Methanol/water–0.1 mM 2-mercaptoethanol buffered to pH 5 with Hac/NH_4Ac	14	[15]

and the time of analyses required to completely elute all the mercury species considered [11–15]. From data in this table it can be concluded that a minimum time of 10 min is required to obtain each chromatogram, thus involving the use of 20 h to obtain 100 measurements.

In a previous study [16], a good separation of the four mercury species was obtained in only 10 min. However, there was a strong difference between the retentions time of phenylmercury, which was around 9 min, and that of the other three species which were eluted in less than 4 min. Thus, in order to increase the productivity of the analytical laboratories, a simple strategy was developed, based on the consecutive injection of samples before the complete elution of each chromatogram in order to take advantage to the fact that inorganic Hg, methylmercury and ethylmercury species can be eluted in a total time lower than the time elapsed between the elution of the latter one and that of phenylmercury.

So, the purpose of this study was to evaluate the effect of the multiple injections as compared with the classical injections strategy, by evaluating both classical chromatographic parameters, such as elution time and resolution, and practical aspects like reagents consumed, waste generation and sampling throughput.

2. Experimental

A chromatographic system HP 1050 model from Hewlett Packard (Waldbronn Germany) equipped with an UV detector HP 1050 Serie, 79853C model and controlled by an HP Chem Station Software HPLC 2D, reference G2170AA was employed to do the Hg speciation, using a Nucleosil 100 - 5 C8 column (5 μm, 10 cm × 4 mm) and a mobile phase composed by 0.06 M ammonium acetate, 0.005% (v/v) 2-mercaptoethanol and 3% (v/v) *n*-buthanol. In all cases a carrier flow of 1 mL min^{-1} was employed, being carried out then analytical measures at 230 nm.

All reagents were of analytical grade, obtained from commercial sources and water was purified using a Milli-Q system from Millipore S.A. (Molsheim, France).

3. Results and discussion

3.1. Chromatographic separation of mercury species

Using previously established chromatographic conditions [16], typical chromatograms, like that indicated in Fig. 1 were obtained, thus showing the good separation produced between inorganic-mercury (eluted around 1.8 min), methylmercury

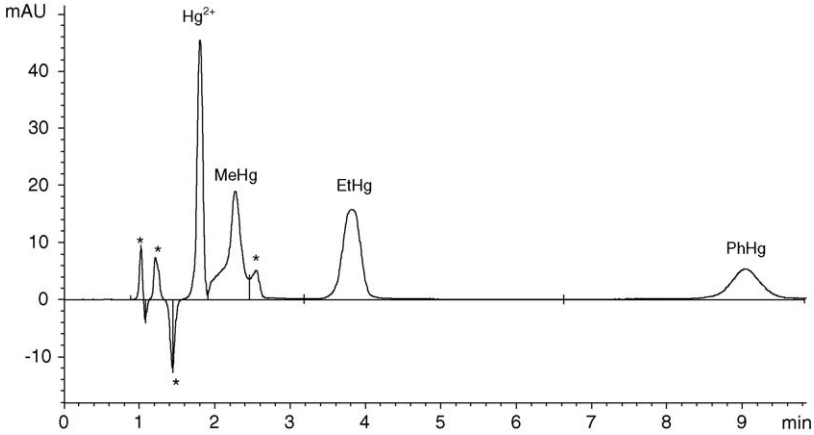

Fig. 1. A typical chromatogram obtained from Hg species separation using classical injection. Conditions: injection of a standard solution containing 50 mg L^{-1} of each Hg species. (*) Solvent and system peaks.

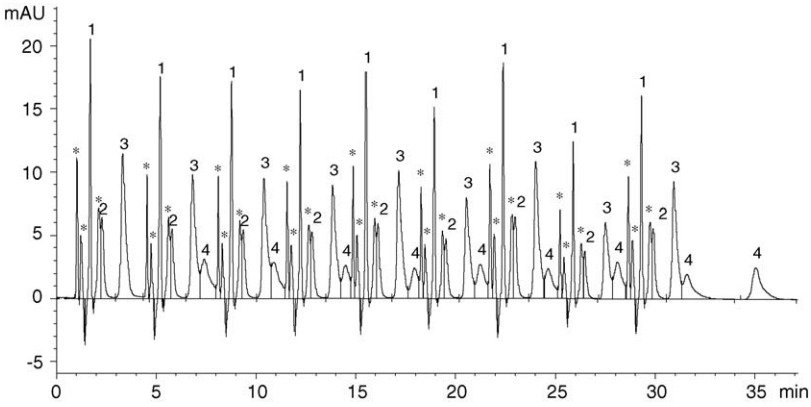

Fig. 2. Continuous recording obtained by HPLC for the multiinjection of a standard solution containing 25 mg L^{-1} of each one of the species. Injections were carried out each 3.5 minutes. Solvent and system peakes (*), inorganic-mercury (1), methylmercury (2), ethylmercury (3), and phenylmercury (4).

Table 2
Chromatographic parameters for Hg species separation by using conventional injection of samples after the elution of the previous chromatogram

Parameter	$HgCl_2$	MeHg	EtHg	PhHg
Retention time	1.804 ± 0.001	2.274 ± 0.001	3.80 ± 0.02	9.038 ± 0.005
Resolution		2.6 ± 0.3	4.6 ± 0.4	10.2 ± 1.3

(eluted around 2.3 min) and ethylmercury (eluted around 3.8 min) which are clearly separated from the system peaks and followed by an approximately 5 min dead time, after which phenylmercury is eluted around 9 min.

From Fig. 1, it can be seen that four mercury species considered can be separated and determined in one sample in less than 10 min (see Table 2 which summarizes the main chromatographic parameters). However, the dead time found between ethylmercury and phenylmercury could offer a way for increasing the sampling throughput, by doing the injection of a new sample after the elution of inorganic-mercury, methylmercury and ethylmercury of the first injection, and before the elution of phenylmercury, in such a way which could permit the first three peaks of the second chromatogram to appear before the peak for phenylmercury.

3.2. Effect of multiinjection on the separation of Hg species

Preliminary attempts were carried out by injecting sequentially samples each 3.7 min, which provided an appropriate separation of the four mercury species considered. However, after several injection sessions, the efficiency of the column was modified reducing the retention time of phenylmercury from 9.0 to 7.4 and, because of that, an injection time of 3.5 min was finally chosen.

Fig. 2 shows a series of chromatograms found by carrying out successive injections of a standard solution containing 25 mg L^{-1} of each Hg species and, as it can be seen the sampling frequency was clearly increased with only a small reduction of the chromatographic resolution. The detection limits (DL) obtained were: 50 ng mL^{-1} for PhHg, 79 ng mL^{-1} for MeHg, 108 ng mL^{-1} for EtHg and 110 ng mL^{-1} for $HgCl_2$.

Good precision of retention times, peak area and resolution was obtained for all mercury species using multiinjection as

Table 3
Chromatographic data found from the separation of four Hg species by doing nine successive injections of a standard containing 25 mg L^{-1} of each species, each 3.5 min

Injection	$HgCl_2$			MeHg			EtHg			PhHg		
	t_r	P_a	R	t_r	P_a	R	t_r	P_a	R	t_r	P_a	R
First	1.705	0.66		2.304	0.22	2.96	3.338	0.74	3.10	7.419	0.36	1.08
Second	1.716	0.68	7.01	2.310	0.22	2.94	3.338	0.70	3.14	7.411	0.34	0.93
Third	1.705	0.65	3.23	2.297	0.22	2.78	3.334	0.68	3.05	7.422	0.34	1.26
Fourth	1.705	0.62	3.30	2.296	0.21	2.89	3.332	0.68	3.05	7.422	0.32	1.58
Fifth	1.705	0.68	2.76	2.299	0.21	2.88	3.335	0.74	3.07	7.426	0.32	1.27
Sixth	1.704	0.66	2.69	2.294	0.22	2.93	3.319	0.67	3.05	7.412	0.33	1.13
Seventh	1.705	0.67	3.17	2.298	0.23	2.59	3.340	0.68	2.89	7.420	0.35	1.05
Eighth	1.702	0.62	3.03	2.281	0.21	2.78	3.318	0.62	2.83	7.405	0.32	1.19
Ninth	1.706	0.63	2.83	2.293	0.21	2.93	3.325	0.68	3.70	7.431	0.35	4.92
Average	1.706	0.65	3.5	2.298	0.22	2.85	3.331	0.69	3.10	7.418	0.34	1.6
S.D.	0.004	0.02	1.4	0.01	0.007	0.1	0.008	0.04	0.2	0.01	0.01	1.3

Note: Data of retention time (t_r) were corrected, by considering the injection time for each chromatogram. Resolution values (R) were calculated from consecutive peaks obtained in each series. Peak area (P_a) of analytes. S.D.: standard derivation of nine data.

Table 4
Comparison of practical parameters of the Hg species separation by using both, conventional injection and multiple injection

Parameters	Conventional injection	Multiinjection
Waste generated for 100 analysis	960 mL	530 mL
Reagent consume for 100 analysis	NH_4Ac 4.440 g, 2-mercarpoethanol 48 μL, *n*-butanol 28.80 mL	NH_4Ac 2.450 g, 2-mercarpoethanol 27 μL, *n*-butanol 15.90 mL
Sampling frequency	$6\,h^{-1}$	$11\,h^{-1}$

demonstrated by repeated injections of standard solutions in Table 3.

3.3. Comparation of classical and multiple injection for Hg species determination

Table 4 summarizes the practical parameters found by working in both, classical injection and multiinjection mode, regarding sampling frequency, waste generation and reagent consume. It can be concluded that multiinjection provides a strong reduction of the time of analysis, thus involving a 50% reduction of waste generation and reagent consume, and because of that, it offers an environmental friendly alternative to the classical injection.

4. Conclusions

Studies carried out by developing a multiinjection strategy in HPLC determination of Hg species, which permits the injection of samples each 3.5 min instead of waiting for the complete elution of Hg species, clearly permits improvements in the time of analyses with a clear reduction of waste generation and reagent consume and without affecting the chromatographic parameters, such as retention time, repeatability and chromatographic resolution.

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