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Hyphenation of ultra performance liquid chromatography (UPLC) with inductively coupled plasma mass spectrometry (ICP-MS) for fast analysis of bromine containing preservatives

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Abstract

Ultra performance liquid chromatography (UPLC) was coupled to inductively coupled plasma mass spectrometry (ICP-MS) for fast analysis of three bromine-containing preservatives, monitoring the ^{79}Br and ^{81}Br isotopes simultaneously. Due to the efficiency of the 1.7 μm column packing material, the resolution of the test substances was only slightly affected when the linear flow velocity was increased from 0.5 to 1.9 mm s^{-1}. However, the sensitivity of ICP-MS detection decreased when the linear flow velocity was increased from 0.5 to 1.9 mm s^{-1}. Analytical figures of merit were determined at an intermediate and at a high linear velocity. The precision was better than 2.2% R.S.D. and regression analysis showed that a linear response was achieved at both flow rates ($R^2 > 0.9993$, $n = 36$).The analysis time was less than 4.5 min at a flow rate of 50 μL min^{-1} and limits of detection and quantification were better than 3.3 and 11 μg Br L^{-1}, respectively. The analysis time was reduced to 2.7 min when the flow rate was increased to 90 μL min^{-1} and limits of detection and quantification were better than 20 and 65 μg Br L^{-1}, respectively. The method was applied for quantitative analysis of bromine-containing preservatives in commercially available cosmetic products.

Keywords: Ultra performance liquid chromatography; Inductively coupled plasma mass spectrometry; Direct injection nebulization; Bronopol; Bronidox and methyldibromo glutaronitrile

1. Introduction

The bromine containing preservatives bronopol, bronidox and methyldibromo glutaronitrile shown in Fig. 1 are often used in cosmetics and pharmaceutical preparations and have recently attracted attention as potential allergens. The maximum allowed concentration of these preservatives in cosmetic products is 0.1% according to the European Union's Cosmetic directive [1].

Previously reported methods for the determination of bromine containing preservatives in cosmetic preparations are based on HPLC with UV [2,3] or reductive electrochemical detection [4,5]. A comparison of chromatographic methods with UV and electrochemical detection methods [6] have shown that UV-detection was less suited for analysis of cosmetic products due to the non-specific detection at 220 nm and analysis times exceeding 60 min. Electrochemical detection was superior to UV detection with respect to sensitivity and selectivity although the electrochemical detection was sensitive to oxygen/air in the HPLC-system. Bronopol has also been directly determined in pharmaceutical preparations by an enzymatic assay monitoring the inhibition of a thiol-containing protease [7].

UPLC is a fast chromatographic separation technique, which takes advantage of narrow bore columns packed with 1.7 μm porous bridged ethyl-siloxane/silica hybrid particles. According to the van Deemter equation, the theoretical plate height is reduced when the particle size is decreased. Additionally, the theoretical plate height is only slightly increasing when the linear velocity is increased. As a result, fast analysis time can be achieved at high flow rates without sacrificing the resolution. However, the back pressure increases significantly when the particle size is reduced and a specially designed hardware with a high pressure limit at 15,000 psi is required in order to operate at high linear velocities on columns packed with 1.7 μm particles. Additionally, the detector must have a minimum dispersion volume in order to preserve the resolution and an adequate sampling rate to acquire sufficient data. An introduction to UPLC

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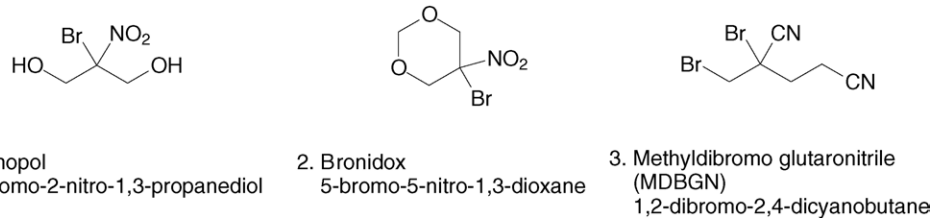

Fig. 1. Structures and trivial names of the three bromine containing preservatives.

has been published by Schwartz [8]. Very few applications of UPLC have been described in the literature [9–11] and all of them describe the coupling of UPLC to TOF-MS.

HPLC-ICP-MS has become a well established technique within trace element speciation analysis and several reviews have been published [12,13]. However, the coupling of UPLC to ICP-MS has not been reported in the literature. From an analytical point of view, this hyphenated technique would be an attractive alternative to HPLC-ICP-MS as it combines a fast and highly efficient separation technique with one of the most sensitive and selective detectors known.

The aim of this study was to examine the performance of UPLC-ICP-MS for analysis of bromine-containing preservatives.

2. Experimental

2.1. ICP-MS

The ICP-MS instrument was a PE-SCIEX ELAN 6000 (Perkin-Elmer, Norwalk, CT, USA) equipped with a laboratory-made direct injection nebulizer [14]. The UPLC system was connected to the ICP-MS instrument by a 100 cm long 0.79 mm o.d. × 65 μm i.d. PEEK capillary tubing (Upchurch Scientific) which was inserted directly into the sample capillary of the nebulizer in order to reduce the post column dead volume to less than 3.5 μL. Data analysis was performed on a TotalChrom workstation (Perkin-Elmer). Instrumental settings are shown in Table 1.

2.2. Chromatography

The chromatographic system was a Waters Acquity UPLC system consisting of a binary solvent manager, autosampler, UV detector, column compartment, all controlled by the Empower software (Waters Corp., MA, USA). Separations were performed on a ACQUITY UPLC^{TM} BEH C_{18}, 1.7 μm, 1.0 mm × 100 mm column (Waters). The analytical column was protected by a 1.0 mm × 5.0 mm guard column packed with C18 5 μm 300 Å particles (LC Packings, Amsterdam, Netherlands) when applied for analysis of cosmetic products. Chromatographic conditions are shown in Table 1.

2.3. Chemicals and reagents

Purified water from a Millipore deionization unit was used throughout for preparation of reagents.

All reagents were of analytical grade. Stock standard solutions at concentrations of 10 mg Br L^{-1} were prepared in mobile phase from 2-bromo-2-nitro-1,3-propanediol

Table 1
Instrumental settings

ICP-MS		UPLC	
Cones	–	Analytical column	ACQUITY UPLC^{TM} BEH C_{18}, 1.7 μm, 1.0 mm × 100 mm (Waters)
Sampler	Platinum 1.1 mm i.d.	–	–
Skimmer	Platinum 0.9 mm i.d.	–	–
–	–	Guard column	300 Å, C18, 5 μm, 1.0 mm × 5.0 mm (LC-Packings)
Argon flow rates			
Auxiliary gas	15 L min^{-1}	–	–
Plasma gas	1.2 L min^{-1}	Column temperature	25 °C
Nebulizer gas	0.2 L min^{-1} [a]	–	–
		Mobile phase	0.1% formic acid in 40% methanol
RF power	1500 W	–	–
Lens voltage	5 V	Flow rate	25–90 μL min^{-1}
Data acquisition	–	Injected sample volume	5 μL
Sweeps per reading	1	–	–
Reading per replicate	300	UV-detection	214 nm
Replicates	1	–	–
Dwell time	300 ms	–	–
Monitored isotopes	^{79}Br & ^{81}Br	–	–

[a] External argon supply.

(Bronopol) (Sigma–Aldrich, Steinheim, Germany), 2-bromo-5-nitro-1,3-dioxane (Bronidox) (Sigma–Aldrich), 1,2-dibromo-2,4-dicyanobutane (Methyldibromo glutaronitrile) (Alfa Aesar, Karlsruhe, Germany). Stock standard solutions were standardized against a certified 1.000 g Br L^{-1} standard solution (Merck). Working standard solutions were prepared daily by appropriate dilutions in mobile phase.

2.4. Sample preparation

The sample preparation method was adapted from reference [2] with slight modifications as this method has been shown to yield high recoveries for the compounds of interest. One hundred milligrams sample was diluted to a final volume of 10.00 mL with mobile phase containing internal standard. Bronopol and bronidox were used as internal standards for quantification of methyldibromo glutaronitrile and bronopol, respectively, after it had been verified that none of these compounds were present in the samples. The resulting suspension was mixed for 2 min on a vortex shaker, sonicated for 10 min and centrifuged for 10 min at 5600 rpm. The supernatant was filtered through a 0.45 μm regenerated cellulose acetate syringe filter when required.

3. Results and discussion

3.1. ICP-MS detection

A minimum dispersion direct injection nebulizer was used to couple the UPLC-system to the ICP-MS instrument in order to preserve the resolution. This nebulizer has previously been demonstrated to provide peak widths at base line of less than 3 s when used to couple capillary electrophoresis to ICP-MS [14]. A 65 μm PEEK capillary tubing with an internal volume of 3.5 μL was inserted directly into the sample capillary of the nebulizer in order to reduce the post column dead volume.

Bromine has a relatively high 1st ionization potential of 11.84 eV [15] and the degree of ionization in the plasma is only about 5% [16]. Both naturally occurring bromine isotopes, ^{79}Br (50.69%) and ^{81}Br (49.31%) are interfered by plasma based interferences $^{38}Ar^{40}Ar^{1}H^{+}$, $^{40}Ar^{40}Ar^{1}H^{+}$ and matrix based isobaric interferences $^{40}Ar^{39}K^{+}$, $^{31}P^{16}O_3{}^{+}$ and $^{32}S^{16}O_3{}^{1}H^{+}$, $^{33}S^{16}O_3{}^{+}$ [17]. A chromatogram from analysis of an aqueous standard solution is presented in Fig. 2 and shows that peak widths at baseline were 12 s. This was comparable to data achieved with UV-detection (data not shown) and shows that the interface did not cause a significant dispersion. Quantifications were performed on basis of the less interfered ^{79}Br isotope and both isotopes were monitored simultaneously in order to detect matrix based interferences.

3.2. Effect of the flow rate on the resolution and on the sensitivity

The effect of the flow rate on the column efficiency and sensitivity was examined by analyzing an aqueous standard solution containing 100 μg Br L^{-1} of each compound at flow rates of 25, 50, 75 and 90 μL min^{-1}. The experimental van Deemter curves

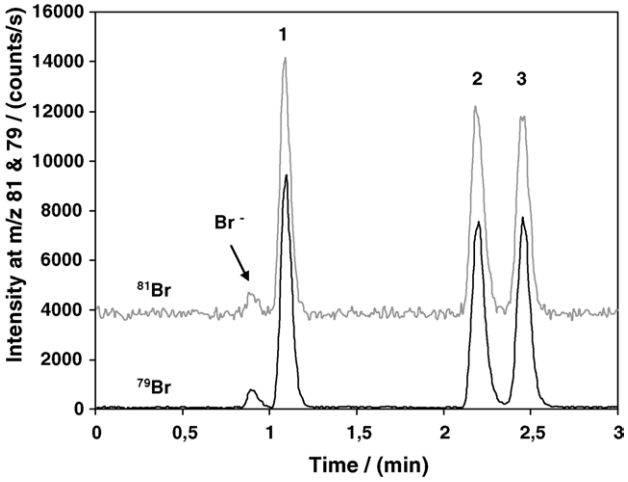

Fig. 2. Chromatogram from analysis of an aqueous standard solution containing 480 μg Br L^{-1} of each compound. Elution order: (1) bronopol; (2) bronidox; and (3) methyldibromo glutaronitrile. Conditions: column, ACQUITY UPLC™ BEH C_{18} 1.7 μm 1.0 mm × 100 mm; mobile phase, 0.1% formic acid in 40% methanol; flow rate, 90 μL min^{-1}; injected volume, 5 μL; isotopes monitored ^{79}Br and ^{81}Br.

presented in Fig. 3 show that the theoretical plate heights for the three compounds were relatively independent on the flow rates examined.

The effect of the flow rate on the signal intensity for each compound is illustrated graphically in Fig. 4, which shows that sensitivity decreased at high flow rates although the mass transfer of the analyte per unit of time was increased. The effect was probably due to insufficient ionization of the analyte caused by the high solvent load to the plasma. Thus, the method could either be operated at high flow rates favouring short analysis time or at low flow rates favouring the sensitivity.

3.3. Linearity

Analytical figures of merit were determined by regression analysis performing six replicate determinations at six concen-

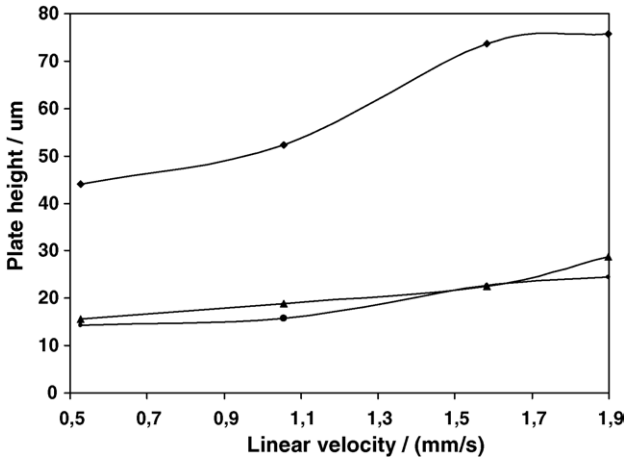

Fig. 3. Experimental van Deemter curves: (♦) bronopol; (▲) bronidox; (●) methyldibromo glutaronitrile. Solid data points corresponds to flow rates of 25, 50, 75, and 90 μL min^{-1}.

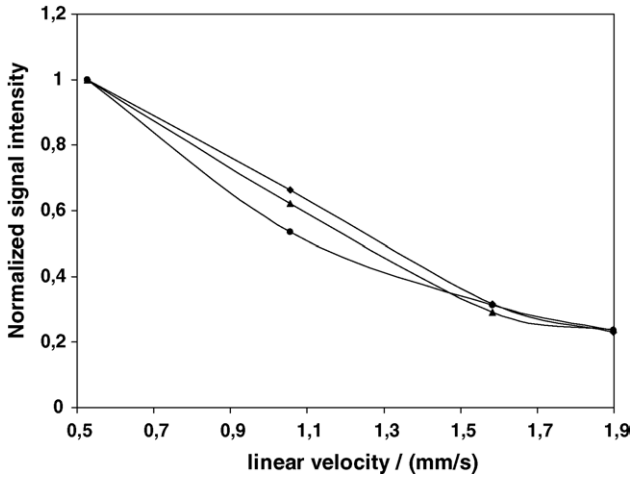

Fig. 4. Effect of the linear velocity on the normalized signal intensity: (⧫) bronopol; (▲) bronidox; (●) methyldibromo glutaronitrile. Solid data points corresponds to flow rates of 25, 50, 75, and 90 μL min^{-1}.

tration levels. Determinations were performed at an intermediate and a high linear velocity in order to favour the ICP-MS detection and the short UPLC analysis times, respectively. Results presented in Table 2 show that a linear response within the calibration range was obtained at both flow rates. Detection limits achieved at both flow rates were superior to those previously achieved by UV or electrochemical detection, Table 3 and were comparable to LODs previously achieved by HPLC-ICP-MS [18] at a flow rate of 50 μL min^{-1}.

The precision was better than 2.2% R.S.D. at both flow rates and shows that the ICP-MS instrument runs stable at high flow rates although the sensitivity was decreased (Table 2).

3.4. Application

The method was applied for analysis of six different cosmetic products. Four of the products were declared to contain bronopol or methyldibromo gluraronitrile and the other two products were declared not to contain any bromine containing preservatives. A representative chromatogram from analysis of one of the products is presented in Fig. 5 and shows that simple chromatograms were achieved despite the complex sample matrix due to the high selectivity of ICP-MS detection. The

Table 3
Comparison with previously reported detection limits

Detection	Detection limits (mg L^{-1})			Reference
	Bronopol	Bronidox	MDBGN	
Electrochemical	–	–	0.50	[6]
Electrochemical	20	20	20	[4]
UV 212 nm	0.21	0.71	–	[2]
ICP-MS[a]	0.03	0.10	0.03	This work

[a] Detection limits achieved at a flow rate of 90 μL min^{-1} were converted into units of mg preservative L^{-1} for comparison.

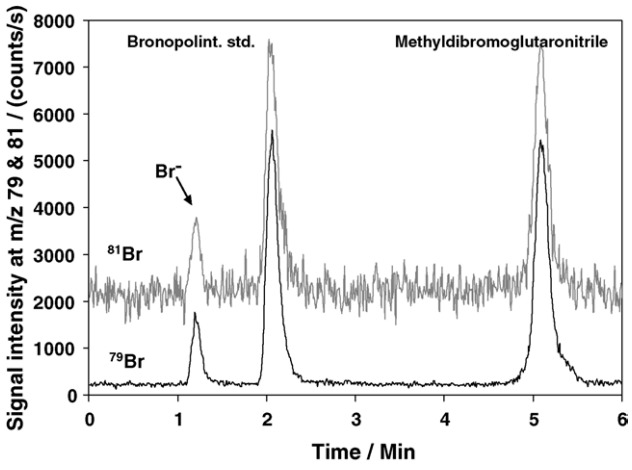

Fig. 5. Chromatogram from analysis of a commercially available hair shampoo declared to contain methyldibromo glutaronitrile. Bronopol was added as an internal standard at a concentration of 100 μg Br L^{-1}, flow rate 50 μL min^{-1}. The trace for the ^{79}Br isotope has been offset by 12,500 counts s^{-1}.

first eluting compound was present in all the products analyzed and co-eluted with bromide when the samples were spiked. All the peaks in the chromatogram had a bromine isotope ratio which was close to the theoretically value, showing that the detected compounds contained bromine and not were due to interferences. Results presented in Table 4 shows that the content of the bromine containing preservatives in the analyzed products were in accordance with the European Union's Cosmetic directive.

Interestingly, in one of the products, declared to contain methyldibromo glutaronitrile, only a large peak eluting in the void volume was detected. When the sample was spiked with

Table 2
Data from regression analysis, $n = 36$, monitored isotope ^{79}Br.

	Flow rate (μL min^{-1})	Range (μg Br L^{-1})	R^2	LOD (μg Br L^{-1})	LOQ (μg Br L^{-1})	Precision (% R.S.D.)[a]
Bronopol	50	2.5–160	0.9999	2.18	7.28	2.12
	90	10–480	0.9997	12.85	42.82	2.12
Bronidox	50	2.5–160	0.9998	3.12	10.41	1.64
	90	10–480	0.9993	19.31	64.38	2.08
Methyldibromo glutaronitrile	50	2.5–160	0.9998	3.30	10.99	1.92
	90	10–480	0.9994	17.67	58.91	1.68

[a] Precision expressed as % relative standard deviation was calculated on the basis of six replicate analyses of a 100 μg Br L^{-1} standard solution.

Table 4
Data from analysis of commercially available soaps and shampoos

Product	Declared reservative	Results (%)		
		Bronopol	Bronidox	MDBGN
Shampoo #1	Bronopol	0.0028	–	–
Shampoo #2	MDBGN	–	–	0.0200
Shampoo #3	MDBGN	–	–	–
Hand soap #1	MDBGN	–	–	0.0022
Hand soap #2	none	–	–	–
Body wash	none	–	–	–

methyldibromo glutaronitrile a peak was observed at 5.2 min, indicating that the preservative was not present or had decomposed completely during storage.

4. Conclusion

The results presented shows that UPLC-ICP-MS is an attractive analytical technique combining a highly efficient separation technique with a very sensitive and element specific detector. Separations could be performed at a high linear velocity if a short analysis time is desired or at an intermediate linear velocity if a high sensitivity is desired. Short analysis times are advantageous when using ICP-MS detection due to the reduced argon consumption.

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