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Microbore liquid chromatography with electrochemical detection for the control of phenolic antioxidants in drugs and foods

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Abstract: Antioxidants are added to foods and drugs to inhibit their oxidation. As these additives are somewhat toxic, it is necessary to control the amount added to any food or drug. Liquid chromatography (LC) is a powerful tool for this purpose. Many antioxidants are electroactive molecules which enables the advantages of electrochemical detection or selectivity and sensitivity to be realized. The interest of analysts in microbore LC arises from the low mobile phase volumetric flow rates involved, the reduced on-column samples together with reduced chromatographic dilution and high efficiency. Coupling of microbore LC with electrochemical detection adds another advantage: the decrease of electrode ageing. The problem of extra-column band broadening with microbore column is discussed in the present communication. A micro LC–electrochemical detection system is constructed and tested using catecholamines. The limit of detection (LOD) for noradrenaline using a 0.7 mm bore column is found to be 0.1 pg injected in 0.2 μl (0.6 femtomoles). Three phenolic antioxidants are studied: *tert*-butyl-*p*-hydroxyanisole (BHA), di-*tert*-butyl-hydroxytoluene (BHT) and *n*-propyl gallate (3,4,5-trimethoxybenzoic acid propyl ester). The dynamic range is four orders of magnitude with LODs down to 0.1 femtomoles (20 fg injected) with a 0.3 mm bore column. No electrode response change is observed after 60 injections of 3 ng BHA over 6 days. Antioxidants are determined in different pharmaceutical preparations and foodstuffs (chewing gums, dried potato flakes). The agreement between the manufacturer stated concentrations and observed results is found to be satisfactory.

Keywords: *Microbore reversed-phase LC; electrochemical detection; antioxidants; foods and drugs.*

Introduction

Many chemicals are permitted as additives to protect foods, drugs and other materials from the effects of oxidation and/or bacterial contamination. Antioxidants are added to products containing fats or oils to prevent rancidification [1]. Preservatives also are very often added to food and drugs to slow down or to inhibit bacterial growth. In any cases, the main objective is to improve shelf-life. None of such additives are completely harmless. However, it is difficult to access the toxicity of a given additive. A chemical may be considered as safe in a country, tolerated in another country and strictly forbidden in a third one. For example, 2,6-di-*tert*-butyl-4-hydroxytoluene (BHT) is authorized as an antioxidant in France whilst it is considered to be highly toxic and is forbidden in Austria [2].

It is essential to control the quality of such additives and the exact quantity added to an edible material. Liquid chromatography (LC) is commonly used for such control purposes [3–6]. Antioxidants are molecules that react readily with oxygen and, as a result, act as inhibitors being oxidized themselves. Accordingly, antioxidants are generally found to be electroactive. The selectivity and sensitivity of electrochemical methods are important advantages in LC detection.

In this work, the advantages of using microbore LC columns coupled to electrochemical detection are demonstrated with reference to antioxidant analysis. The advantages and drawbacks of microbore LC are first summarized and electrochemical detection described. Then, the results obtained in the determination of three phenolic antioxidants by microbore LC–electrochemical detection are reported and discussed.

Microbore LC versus Classical LC

Column diameter is the distinguishing feature between classical LC and microbore

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LC. The linear velocity of the mobile phase inside the column is the most important parameter affecting the solute residence time, stationary phase–mobile phase distribution and solute diffusion. The chromatographic phenomena are particularly sensitive to the column internal diameter because, at a constant flow rate, the mobile phase linear velocity increases quadratically as the internal column diameter decreases. Usually, conventional LC columns have an internal diameter of between 2 and 5 mm, whereas, for microbore columns it is between 1 and 0.1 mm. These columns are packed with microparticulate silica or chemically bonded silica (particle diameter 3–5 μm). Below 0.1 mm i.d., the term capillary column is used. It is difficult to pack such columns which, as a consequence, are generally used as open tubes. Columns having internal diameter larger than 5 mm are used in preparative chromatography.

Advantages and drawbacks of microbore columns

Flow rates in classical LC are in the ml min^{-1} range. As a consequence in routine analysis, the daily solvent consumption can be a few litres. A typical flow rate of microbore LC is 50 μl min^{-1} which means that mobile phase consumption is substantially lower. Depending on the bore diameter, the flow rate may be even lower. The very reduced solvent consumption in microbore LC is the first advantage compared to conventional LC. This leads to reduced solvent costs, concomitant waste disposal costs and improvements in safety standards. Also, more reproducible analytical data may be expected because the same batch of mobile phase can be used for months in routine analysis. The other most important advantage is the very reduced solute dilution which improves sensitivity. However it is necessary to reduce sample size, a typical injection volume being of the order of 0.5 μl. This is more than 10 times lower than the injection volume required in conventional LC. The major drawback of microbore LC is that conventional pumps, injectors and detectors cannot be used. Their volumes are such to produce too high a band broadening with microbore columns.

Efficiency and limit of detection

Band broadening is linked to column efficiency which is defined by the plate number, N, via

$$N = (V_R/\sigma)^2 = 4\,(V_R/W_{0.6h})^2 \qquad (1)$$

where V_R is the retention volume, σ is the peak standard deviation, σ^2 is the peak variance, both expressed in volume units and $W_{0.6h}$ is the peak width measured at 60% of the peak height. The concentration, C_{max}, at the maximum peak height, is given by the expression

$$C_{max} = \frac{m}{\sigma\sqrt{2\,\pi}} = \frac{m\sqrt{N}}{V_R\sqrt{2\pi}} \qquad (2)$$

in which m is the mass of solute injected. Both equations (1) and (2) assume a Gaussian profile for the peak. Equation (2) shows that the peak height is proportional to the injected mass and that it is dependent upon retention volume and column efficiency. The retention volume is linked to the square of the column diameter. Thus a reduction of the column diameter by a factor of two reduces V_R by four whilst the peak height becomes four times higher. This illustrates the main advantage of the use of microbore columns, reduced solute dilution. Equation (2) shows that the higher the efficiency, the higher the concentration at the peak maximum. For example, the injection of 10 μg of a solute in a 20,000 plate column produces a peak of exactly the same height that the injection of 20 μg of the same solute in a 5000 plate column with the same diameter. Often, limits of detection (LOD) are expressed in terms of the mass of solute injected [7]. Equation (2) and the above example show that the LOD is dependent upon column efficiency. The LOD also depends on the response of the solute. Due to the reduced solute dilution with microbore columns, it is essential to reduce dead volumes and extra-column band broadening to maximize the chromatographic system efficiency.

Band broadening

Each part of the chromatographic system, injector, column, detector, and connecting tubes, contributes to band broadening. It is essential that pre- and post-columns band broadening are minimized. Peak widths and variances are linked. Variances are additive so that the final measured variance, σ^2, is the sum

of the contribution of each chromatographic step:

$$\sigma^2 = \sigma^2_{inj} + \sigma^2_{col} + \sigma^2_{det} + \sigma^2_{tube}. \quad (3)$$

To obtain maximum efficiency, each variance must be minimized. Consider for instance the detector variance σ^2_{det}. If it is assumed that a loss of 10% of peak efficiency is the maximum acceptable and assuming a suitable injector is used and the length and internal diameter of the connecting tubing are reduced to a minimum, the injector and tube variance contribution are minimized and negligible compared to the column variance, then the total variance is given by:

$$\sigma^2 = \sigma^2_{col} + \sigma^2_{det}. \quad (4)$$

The maximum tolerated detector variance is defined as:

$$\sigma^2_{det} = 0.1\ \sigma^2_{col} \quad (5)$$

or

$$\sigma_{det} = 0.32\ \sigma_{col}. \quad (6)$$

Equation (1) shows that solute retention volumn and variance increase in proportion. Introducing the solute capacity factor, k',

$$k' = (V_R - V_0)/V_0, \quad (7)$$

in which V_0 is the column dead volume, the column variance can be expressed by:

$$\sigma_{col} = V_R/N^{1/2} = V_0\ (1 + K')N^{1/2}. \quad (8)$$

The maximum acceptable detector variance becomes

$$\sigma_{det} = 0.32\ V_0\ (1 + k')/N^{1/2}. \quad (9)$$

The parameters affecting the detector variance are its time constant, the mobile phase flow rate, and the cell volume and its geometry [8–10]. For microbore and capillary LC columns, the detector volume is the key factor.

A modern conventional 25 cm LC column can easily have 10,000 plates with a dead volume of 1 ml. For a moderately retained solute ($k' = 5$), equation (9) gives σ_{det} = 19 μl (variance σ^2_{col} = 3600 μl^2). 10,000 plate is a typical efficiency for a 25 cm microbore column, but the dead volume can be as low as 50 μl producing, with the same compound, a 1 μl σ_{det} value (variance σ^2_{col} = 9 μl^2). Clearly, the same detector cannot be used with the two columns. Table 1 lists the characteristics of the different LC techniques. It is interesting to note the mobile phase volume occupied by an eluted solute with a k' value of 5. This volume varies from 170 ml in preparative chromatography to 150 nl with an open tubular capillary column. This emphasizes the fact that the same detector cell cannot be used in preparative LC and in microbore LC. Each LC technique requires its own detector cell. The peak volumes listed in Table 1 do not depend on the injected mass. Of course, the mass which is possible to inject is several orders of magnitude lower in a capillary column than in a preparative column. For microbore LC, 0.2–0.4 μl cell volumes produce band broadenings lower than 10% of the column band broadening.

Experimental

Chromatographic system

A modular LC system was used consisting of a Hitachi L6000 pump (Merck Instruments, Paris, France), a Rheodyne 7520 valve (Touzart et Matignon, Vitry, France) with a 0.2 μl internal loop, five different columns characteristics of which are listed in Table 2, a

Table 1
Characteristics of different LC techniques

Technique	Column*				Flow rate		Peak volume	
	Length (m)	Diameter (μm)	Plates per col.	V_0 (ml)	(ml min^{-1})	(mm min^{-1})	$k' = 1$	$k' = 5$
Preparative	0.5	50,000	4000	350	50	70	55 ml	170 ml
Conventional	0.25	4600	10,000	1.5	1.0	160	0.15 ml	0.45 ml
Microbore	0.25	1000	10,000	0.07	0.05	180	7 μl	21 μl
Open capillary	10	20	400,000	0.003	0.001	3200	50 nl	150 nl

*Typical values, columns with other lengths and diameters are marketed [7].

Table 2
Analytical data for BHA

Column					280 nm UV detection (μmol inj.)		+0.9 V Electrochem. detection (μmol inj.)	
Phase*	$L \times$ i.d. (mm × mm)	Flow rate (μ min^{-1})	Effic. plates per col.	HETP (μm)	Dyn. range	LOD	Dyn. range	LOD
Hypersil	100 × 4.6	700	8000	12.5	10^{-7}–10^{-11}	10^{-11}	10^{-7}–10^{-11}	3×10^{-12}
Lichrospher	250 × 2.1	400	6700	37.3	10^{-8}–10^{-12}	10^{-12}	5×10^{-8}–5×10^{-13}	5×10^{-13}
Kromasil	300 × 1.0	50	16,500	18.2	10^{-9}–10^{-13}	10^{-13}	5×10^{-9}–5×10^{-14}	10^{-14}
Lichrospher	250 × 0.7	30	9000	27.8	10^{-10}–10^{-14}	10^{-14}	5×10^{-10}–5×10^{-15}	10^{-15}
Lichrospher	200 × 0.3	10	8200	24.4	10^{-10}–10^{-14}	10^{-14}	10^{-11}–10^{-16}	10^{-16}

* All stationary phase were 5 μm octadecyl (C18) bonded silica particles.
Solute *tert*-butyl-*p*-hydroxyanisole (BHA), mobile phase: methanol–aqueous $LiClO_4$ 0.01 M, pH 5.5 (25:75, v/v).

2000 UV detector (Spectra Physics, Darmstadt-Kranichstein, Germany) with a 0.25 μl cell (2 mm optical path length), a Chromatofield Eldec 201 electrochemical detector (Precision Instruments, Marseille, France) and a Shimadzu CR5A integrator (Touzart et Matignon). The UV detector was operated at 280 nm. The five columns were slurry packed in the laboratory with 5 μm C18 silica particles. All connections were made with 125 μm internal bore tubing. All experiments were performed at room temperature (20°C ± 2°C).

Electrochemical detector

The Chromatofield electrochemical cell used had a three electrode arrangement with a working electrode consisting of glassy carbon. The cell body was made of two parts, a stainless steel block housing the Ag–AgCl reference electrode and the mobile phase inlet, and a fluorinated polymer block with the carbon working electrode and the mobile phase outlet. The stainless steel block acts as the counter-electrode. The two blocks were arranged in such a way that the internal cell volume was about 0.2 μl. The reference electrode is filled with a 3 M NaCl solution, each week and it was checked and refilled as necessary. The conditioning of the cell is easy: the Ag–AgCl reference electrode is removed when the mobile phase is flowing. This eliminates air bubbles trapped in the cell. The voltage is set at +0.8 V (Ag–AgCl) in the oxidation mode, unless otherwise indicated, and the current is monitored. The current stabilizes in 5–10 min. The electrode response was found to be reproducible. In case of electrode contamination, it is possible to clean the carbon electrode with a 0.4 μm particle alumina paste (Chromatofield).

Mobile phases and chemicals

The mobile phases were methanol–water mixtures. For the catecholamine separation, water-rich mobile phases (water 95%, v/v) with ethylenediaminetetraacetic acid, disodium salt (2.5 g l^{-1}, Merck, Darmstadt, Germany) were used. A counterion, sodium heptylsulphate (Kodak, Rochester, NY, USA), was added (60 mg l^{-1}). The supporting electrolyte was phosphoric acid (10 g l^{-1}, Merck) with the pH adjusted to 3.0 with a 0.1 M sodium hydroxide solution. For the antioxidant analysis, methanol-rich mobile phases were used (methanol 75%, v/v) without counterion. The pH was adjusted to 5.5 with phosphoric acid. Lithium perchlorate (Merck) was added to the mobile phases (0.01 M) as the supporting electrolyte. Methanol was obtained from BDH (Poole, Dorset, England). The antioxidants were *tert*-butyl-*p*-hydroxyanisole (BHA), 2,6-di-*tert*-butyl-4-hydroxytoluene (BHT) and propyl gallate (3,4,5-trimethoxybenzoic acid propyl ester). The European Community code numbers are E310, E320 and E321 for propyl gallate, BHA and BHT, respectively. The antioxidants were obtained from Fluka (Buchs, Switzerland).

The commercial foods and drugs tested for antioxidants were bought in local pharmacy and grocery stores. The antioxidants were extracted according to the following procedure. A weighed amount (~1 g) of material to be analysed was placed in a 10 ml vial, 5 ml of methanol added and the vial sonicated for 2 h. After 8 h standing, the supernatant methanol phase was transferred to another vial, 5 ml of fresh methanol added to the remaining material and the extraction repeated. The 10 ml methanol extract was filtered on a Sep-Pak silica cartridge (1 cm × 0.8 cm o.d., Sep-Pak, pure silica, ref. 51900, Waters Associates, Milford, MA, USA). The 1 cm cartridge was directly connected to a 10 ml syringe containing the methanol extract. The filtered solution was injected without further dilution.

Catecholamines were used to test the electrochemical system. They were adrenaline (A), noradrenaline (NA), 3,4-dihydroxyphenyl ethylene glycol (DOPEG), 1,3-(3,4-dihydroxyphenyl)alanine L-DOPA), 3,4-dihydroxybenzylamine (DHBA), 3,4-dihydroxyphenylacetic acid (DOPAC), dopamine (DA) obtained from Sigma (St Louis, MO, USA).

Results and Discussion

System performances

Catecholamines are often used as test solutes to check the performances of an electrochemical LC detector [11–12]. The counterion reversed-phase LC method described by Kissinger [8, 10] was used. Figure 1(A) shows the separation of seven catecholamines with baseline resolution using a microcolumn (250 mm × 0.7 mm i.d.) filled with 5 μm Lichrospher RP 18 silica. The efficiency is about 10,000 plate range for the seven peaks indicating that the external band broadening contributions were minimal. The system ef-

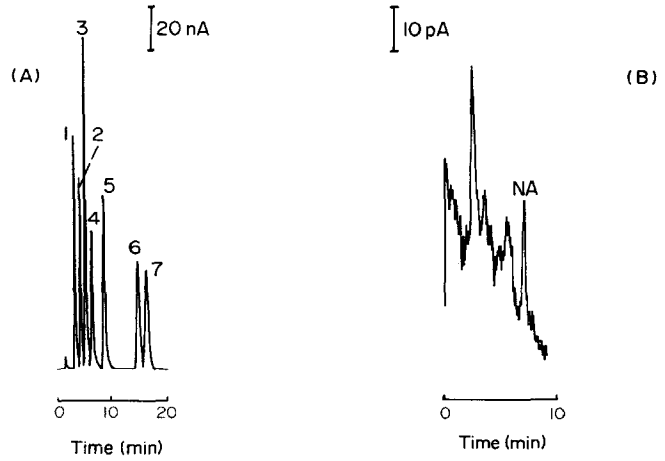

Figure 1
(A) Separation of seven catecholamines: 1, DOPEG; 2, NA; 3, L-DOPA; 4, A; 5, DHBA; 6, DOPAC; 7, DA (100 pg each injected in 0.2 μl). Column 250 mm × 0.7 mm, Lichrospher C18, 5 μm; mobile phase: water–methanol (95:5, v/v), 0.1 M H_3PO_4, 2.5 g l^{-1} EDTA, 0.06 g l^{-1} $C_7H_{15}SO_3Na$, pH 3.05, 30 μl min^{-1}. Electrochemical detection, oxidation potential +0.8 V vs Ag–AgCl. (B) Chromatogram of 0.1 pg injected of noradrenaline (NA) corresponding to the minimum NA detectable (LOD). Flow rate 25 μl min^{-1}. All other experimental conditions were identical to (A).

ficiency is about 40,000 plates m^{-1} with a HETP of 25 μm. The HETP corresponds to five times the particle diameter. This is a fair value although HETPs corresponding to three particle diameters have been obtained by previous workers [13]. The LOD of the system was measured for noradrenaline. Figure 1(B) shows the chromatogram corresponding to 0.1 pg (10^{-13} g or 0.6 femtomoles) injected. This LOD compares favourably with the 0.2 pg LOD obtained with a similar system [13], it is two orders of magnitude lower than LODs obtained using conventional LC columns with electrochemical detection [12].

Analytical performance data for antioxidants

The catecholamine analysis demonstrated that the electrochemical detector coupled with the microcolumn LC system was performing satisfactorily. The next step was to determine the analytical performance data, LOD (signal to noise ratio = 3), linearity range and reproducibility of the analysis for BHA, BHT and propyl gallate in synthetic mixtures. This was done using four different microbore columns. The two detection modes, namely UV and electrochemistry, were tested with BHA. The electrochemical detector was connected in series after the UV detector. The results obtained are listed in Table 2. The same experiments were performed using a conventional analytical column for comparison.

The electrochemical detector is one to two orders of magnitude more sensitive than the UV detector. The LOD difference between the two detectors increases when the column diameter decreases. This is due to the decrease of the linear mobile phase velocity in the detector cell. With the 0.3 mm i.d. column, the flow rate was 10 μl min^{-1}. The time to flush one detector cell volume (0.2 μl) was 1.2 s. With the 2.1 mm i.d. column and a 0.4 ml min^{-1} flow rate, the detector flush time was only 30 ms. This means that the residence time of the solutes on the electrode surface was 40 times lower with the 2.1 mm column than with the 0.3 mm column. Clearly, the amount of solute electrolysed depends on its residence time on the working electrode. These results show that the microcell is well-suited for use with microbore columns. It may have too low a volume to be used with conventional LC columns.

Table 2 gives the efficiency and the HEPT, measured for the BHA peak, obtained with the columns. There is no apparent connection between the observed efficiencies and HEPT values and the column diameter. The columns were slurry packed in the laboratory. It is very difficult to obtain the same efficiency for two microbore columns of identical geometry, when packing successively with the same hardware and the same batch of stationary phase. It is easier to pack conventional LC

columns. The highest efficiency (the lowest HETP) was obtained with the conventional LC column. This is due to the packing and also to the fact that external peak dispersion was negligible in the chromatographic set-up designed for microcolumns.

Figure 2(A) shows the chromatograms obtained with a 300 × 1 mm column using UV and electrochemical detection. Figure 2(B) shows the chromatograms obtained when the minimum detectable amounts (1.8 pg) were injected. The repeatability and reproducibility may be expressed in terms of the standard deviation calculated on several measurements done successively or at different times. For the four microbore columns tested, the relative standard deviation on five successive measurements (repeatability) was lower than 3%. To check the electrode stability and/or ageing, 10 chromatograms of 3 ng BHA were performed per day on each of 6 days. The average areas for the 10 daily BHT peaks were 275289, 290292, 302042, 281961, 282435 and 284251. The corresponding relative standard deviations were 2.2, 6.6, 3.3, 0.8, 1.2 and 1.1%, respectively. These results indicate that there is no electrode ageing. The relatively high RSD obtained on Day 2 (6.6%) was due to a mobile phase change. The repeatability and reproducibility tests were not performed near the LOD (1.8 pg in 0.2 μl is 0.9 ppb) because such an antioxidant concentration added to drugs or foods would be ineffective. Common amounts are in the 10–100 ppm range or more as shown by the analysis of real samples.

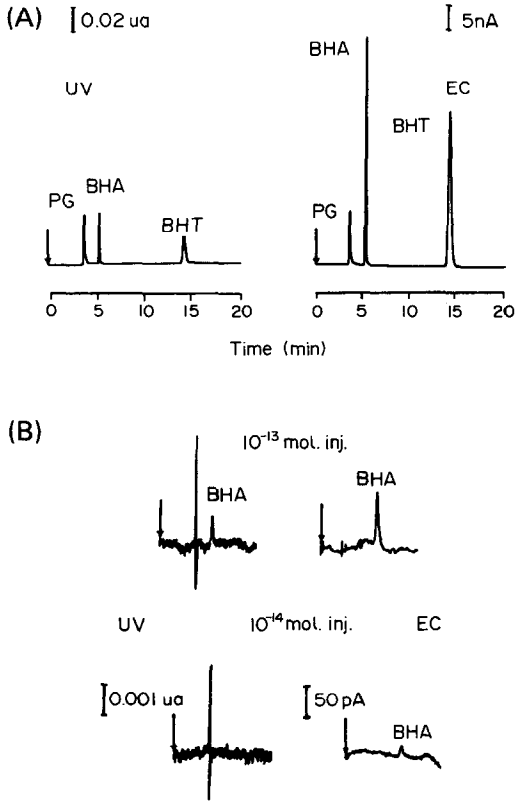

Figure 2
(A) Antioxidant (PG 3 ng, BHA 15 ng, BHT 50 ng, injected in 0.2 μl) chromatograms. Left: UV 280 nm detection, right: electrochemical +1.1 V vs Ag–AgCl detection. The UV detector was connected first. Column: 300 mm × 1 mm, Kromasil C18, 5 μm; mobile phase: water–methanol (10:90, v/v), 0.01 M $LiClO_4$, pH 5.5, 50 μl min^{-1}. (B) Chromatograms corresponding to the minimum detectable amount of BHA. Left: UV 280 nm detection, right: electrochemical +1.1 V vs Ag–AgCl detection. No UV signal was recorded when 1.8 pg of BHA (electrochemical LOD) were injected. The other chromatographic conditions were listed above.

Real sample analysis

Different drugs and foods were tested for the antioxidants BHA and BHT. Table 3 lists the results obtained with two pharmaceutical specialities, a sample of dry potato flakes and five different chewing gums. The first pharmaceutical preparation was a bactericidal cream to treat Gram + bacteria infected vaginitis. The second one was a gel for external use prescribed to treat dry hyperkeratosis, psoriasis, ichthyosis or non-oozing eczema. The amounts of antioxidant found in the two preparations were about 10% different than the quoted amounts (Table 3).

The maximum amount of antioxidants authorized in the base gum used for chewing gum preparation is 1000 ppm (1 g kg^{-1}). Five samples, produced by two different companies, one, noted A in Table 3, used BHT, the other one, B, used BHA, were analysed. In the five samples, the concentrations found were to be close to 170 ppm, a relatively high concentration for an edible product. For two samples, the amount of antioxidant remaining in the gum after it has been chewed for about 30 min (no sweet taste remaining) was analysed again. It was found that the main part (66% for sample no. A2 and 85% for sample no. B1) of the antioxidant remained in the gum (Table 3). The weight of a chewing gum tablet is about 2.8 g containing about 0.47 mg of antioxidant. For sample no. A2, one tablet contained 0.52 mg of BHT from which less than 0.16 mg are ingested.

Two antioxidants were found in the commercial sample of dry potato flakes analysed. The sum of the BHA and BHT concentrations

Table 3
Antioxidants in real samples

Sample		Antioxidant	Quoted ppm (mg kg^{-1})	Found ppm (mg kg^{-1})
Pharmaceuticals				
Gyno-Pevaryl®	bactericidal cream	BHT	52	49.7 ± 1.6
Keratosane®	skin care gel	BHA	10	11.3 ± 0.4
Foods				
chewing gum no. A1	natural flavour	BHT	—	164 ± 4
chewing gum no. A2	chlorophyll	BHT	—	184 ± 5
chewing gum no. B1	chlorophyll	BHA	—	178 ± 3
chewing gum no. B2	raspberry	BHA	—	175 ± 3
chewing gum no. B3	menthol	BHA	—	176 ± 3
chewing gum no. A2	chewed 30 min	BHT	184 ppm (packed tablet)	121 ± 2 (66%)
chewing gum no. B1	chewed 30 min	BHA	178 ppm (packed tablet)	152 ± 2 (86%)
dry potato flakes	—	BHA	total	9.9 ± 0.3
		BHT	BHA + BHT	15.3 ± 0.5
			25	

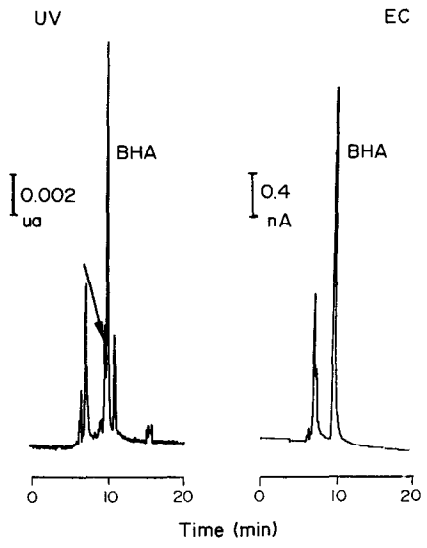

Figure 3
UV (280 nm) and electrochemical (+1.1 V vs Ag–AgCl) detection chromatograms of a methanol extract of the chewing gum tablet no. B2 (Table 3). Column: 300 mm × 1 mm, Kromasil C18, 5 μm; mobile phase: water–methanol (10:90, v/v), 0.01 M $LiClO_4$, pH 5.5, 20 μl min^{-1}. The arrow pinpoints the peak of an unknown non-electroactive compound in extracts. It introduced an error in the BHA UV quantitation.

corresponded to the quoted figure of 0.0025%. Figure 3 shows the UV and electrochemical chromatograms of a chewing gum extract. The high selectivity of the electrochemical detection is a great advantage. The peak of interest, BHA, is easy to determine in the electrochemical chromatogram. The UV chromatogram shows a great number of peaks corresponding to extracted UV adsorbing molecules which are not electroactive. In particular, an unknown compound (peak indicated by an arrow) interferes with the BHA peak producing an error on the integrated UV peak area. The relative standard deviation was found to be 1.5% on the antioxidant determination performed using the same extraction. It increased to 3% for the determinations made on three different extractions with the same material.

References

[1] G. Pascal, *Cahiers de Nutrition et Dietetiques* **XIV**, **4**, 271–290 (1980).
[2] G. Full, G. Krammer and P. Schreier, *PIC Hewlett Packard* **3**, 6–7 (1991).
[3] L. Gagliari, A. Amato, A. Basili, G. Cavazutti, E. Gattavechia and D. Torrelli, *J. Chromatogr.* **315**, 465–469 (1984).
[4] B.J.F. Hudson (Ed.), *Food Antioxidants*. Elsevier, London (1990).
[5] S.P. Kochhar and J.B. Rossell in *Food Antioxidants* (B.J.F. Hudson, Ed.), pp. 19–64, Elsevier, London (1990).
[6] J. Loeliger in *Free Radicals Food Additives* (O.I. Aruoma and B. Halliwell, Eds), pp. 121–150. Taylor & Francis, London (1991).
[7] A. Berthod, *Spectrochim. Acta Rev.* **13**, 11–25 (1990).
[8] R.P.W. Scott, *Liquid Chromatography Detectors*, *J. Chromatogr. Lib.*, Vol. 11. Elsevier, Amsterdam (1977).
[9] T.M. Vickrey (Ed.), *Liquid Chromatography Detectors*, *Chromatogr. Sci. Series*, Vol. 23. Marcel Dekker, New York (1983).
[10] M. Novotny, *Anal. Chem.* **60**, 500A–510A (1988).
[11] P.T. Kissinger, *J. Chromatogr.* **488**, 31–52 (1989).
[12] V.F. Reeban, A.B. Belenkii, A.Y. Gurevich and B.B. Belenkii, *J. Chromatogr.* **467**, 41–47 (1989).
[13] T. Huang, R.E. Shoup and P.T. Kissinger, *Bioanalytical Systems*, *Current Separations* **9**, 139–143 (1990).

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