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LIQUID CHROMATOGRAPHIC DETERMINATION OF BROMIDE IN HUMAN MILK AND PLASMA

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SUMMARY

A liquid chromatographic method for the determination of bromide present in human body fluids at the level of 0.5—5.0 ppm is presented. The method involves liquid—liquid extraction of lipids and other lipophilic compounds, destruction of the aqueous phase and analysis of the residue on an aminopropyl bonded silica column with UV detection at 214 nm. The method was applied to the analysis of 278 samples of Dutch human milk. Comparison of the results obtained with those from a routinely used colorimetric procedure for plasma indicated excellent agreement. The ease of automation of the described procedure and its excellent reproducibility make it a good alternative to existing methods for bromide analysis in body fluids.

INTRODUCTION

In The Netherlands, inorganic bromide is present in foodstuffs at the level of 9.4 mg per day, the mean dietary intake [1]. In order to get an insight into the resulting levels of bromide in human milk, surveys are frequently carried out by our Institute, in cooperation with Dutch maternity centres. From previous research, bromide concentrations in human milk were known to be 1.1—9.7 ppm [2]. Data on the occurrence and toxicity of bromide residues have recently been published [3].

Currently used methods for the determination of bromide in body fluids involve either spectrophotometry after a colour reaction [4] or gas chromatography (GC) after derivatization to bromoethanol [4, 5]. Both methods are laborious and quantitation may be difficult. With spectrophotometry the difficulty is due to the extremely small linear range of the calibration curve. In GC,

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interferences may occur due to impurities in the reagent, which are formed upon aging. Liquid chromatographic methods for the determination of bromide involve ion chromatography on both weak and strong, bonded anion exchangers such as protonated aminopropylsilica [6, 7] and quaternary ammonium compounds [8–13], and ion-pair chromatography using C_8, C_{18} [14–17] or cyanopropyl [18] bonded phases or polymer resins [19, 20] and octylamine [17] or other quaternary ammonium salts [15—19] as ion-pairing reagents. Detection methods include UV detection at 200–214 nm [6—8, 12, 17, 18, 21], indirect photometry at higher wavelengths, e.g., 280 nm, using UV-absorbing eluents such as phthalates [15, 16, 22], potentiometry with ion-selective electrodes [23, 24], amperometry [25, 26] and conductometry with [9, 11, 14, 16, 19, 27, 28] and without [10, 19, 20, 22] eluent suppression.

Other references describe applications to complex matrices, such as soil and vegetables [17, 18], other foodstuffs [6] and other biological samples [29], and one deals with body fluids [11]. No high-performance liquid chromatographic (HPLC) method for the determination of bromide in milk has yet been described.

The preparation of vegetable samples, prior to ion-exchange and ion-pair chromatography and UV detection at low wavelengths, consists of aqueous extraction and clean-up by centrifugation and filtration only [17]. Flour and rice are deproteinated by Carrez clearance prior to centrifuging and filtration [6]. Sample preparation for ion chromatography with ion suppression and conductometry has been reported for oyster tissue and human serum. Oysters were lyophilized and the solutions filtered [29], serum was diluted ten-fold in eluent and analyzed without further treatment [11]. The quantification of bromide in body fluids by ion chromatography and conductometry is, however, strongly hindered by the presence of high concentrations of phosphate (ca. 1 mmol) in the samples [11, 29]. UV detection therefore seems more appropriate for these types of samples. Human milk contains a high amount of fat and proteins. Therefore, sample preparation should be directed towards removing these fouling and UV-absorbing components. Also, interference from other ions present in the samples, such as phosphate, chloride and nitrate, should be avoided.

EXPERIMENTAL

Apparatus

Liquid chromatography was carried out with a Waters (Milford, MA, U.S.A.) system, consisting of an M45 pump, a Model 441 UV detector with zinc lamp and 214-nm filter or a Model 165 rapid-scanning UV detector (Beckman, Berkeley, CA, U.S.A.), a WISP 710B autosampler and a Hewlett-Packard 3380 A computing integrator. The columns used (150 × 4.6 mm I.D.) were packed in the laboratory with 5-μm Hypersil APS (Shandon, Runcorn, U.K.). Mobile phase: 0.03 *M* phosphate buffer, pH 2.8.

Chemicals

Water was twice distilled. Phosphoric acid, potassium hydroxide, potassium

bromide, acetone and petroleum ether were of analytical-reagent grade (Merck, Darmstadt, F.R.G.). Human milk substitute, Almiron M2, was obtained from Nutricia (Zoetermeer, The Netherlands).

Sample preparation

Equipment. Disposable syringes (Ribbon Pack) were obtained from Teruma (Leuven, Belgium) and 0.45-μm disposable filters (Millex, SLHA 025 BS) from Millipore/Waters (Milford, MA, U.S.A.).

Extraction procedure. A 50-ml volume of human milk was macerated for 3 min with 100 ml petroleum ether and 100 ml acetone. After centrifugation for 4 min at 3000 rpm, the liquid phases were transferred to a 500-ml separating funnel. The aqueous phase was collected in a 400-ml beaker and evaporated to about 5 ml in a bath of boiling water. The concentrate was transferred into a calibrated tube and the volume brought to 10 ml with water.

Decomposition procedure. A 2-ml aliquot of the aqueous concentrate was pipetted into a 50-ml nickel crucible, and 0.1 ml of a 2.5% (w/v) potassium hydroxide solution was added. The crucible was subsequently heated for 30 min at 120°C and for 15 min at 500°C. After cooling, 2 ml water were added to the crucible and the ashes were loosened, using a glass rod. After addition of another 8 ml of water, the mixture was taken up in a 10-ml syringe and filtered over a 0.45-μm filter. Aliquots of the clear solution were used for analysis.

RESULTS AND DISCUSSION

Chromatography of bromide

The liquid chromatographic procedure described by Cortes [7], using an amino bonded phase, acidic eluent and UV detection, was selected after some initial experiments. We preferred a chemically bonded ion exchanger rather than ion-pair chromatography, since the use of quaternary ammonium salts in the eluent is known rapidly to deteriorate chemically bonded silicas [30] and since the alternative, high pH-resistant polymeric resins of intermediate polarity are less efficient due to slow mass transfer [31]. Two other anion exchangers, of the strong-base type, were initially tested: a low capacity (0.02 mequiv./g) polymeric column and a high-capacity (1 mequiv./g) silica-based one. The first appeared to become fouled rapidly after injection of milk destruates, which led to broad peaks and a drifting baseline, thus preventing accurate quantitation. The second anion exchanger did not give the desired resolution between the bromide and the nitrate ions in a reasonable time, in the eluents tested. We were unable to optimize this separation by using, for example, a stronger eluent such as a 0.04 *M* biphthalate buffer, since we were restricted to the use of eluents not absorbing below 250 nm. The use of indirect photometry at, e.g. 308 nm, is not recommended as a detection method, since it is neither very reproducible nor quantitative [32].

The aminopropylsilica (APS) column did not pose these problems. Its only drawback was the lack of selectivity between chloride and bromide. However, due to the differences in the absorption spectra of these ions, a wavelength can be found at which only bromide is detected [12, 33]. The capacity factors

of some ions, which may occur in the samples, on the APS column are given in Table I. Phosphate ions cannot be detected up to concentrations of at least 10 mg/ml, due to the use of a phosphate buffer as the eluent. The efficiency of the coumn expressed as the number of theoretical plates, measured with Br^-, was 7700. The peak asymmetry, measured at 10% peak height, was 1.3. The measurements apply to a 10-μl loop injection. Upon injection of larger volumes, the peak asymmetry increased considerably, e.g. $A_{0.1} = 2.0$ with a 50-μl injection. The resolution, R_s, between Br^- and its nearest neighbour, BrO_3^-, is 1.6 [34]. BrO_3^- may occur as an oxidation product of Br^-, but it will be well resolved from the latter. In our samples no BrO_3^- was detected. From the UV spectra of Br^- and Cl^-, 214 nm was selected as the wavelength for detection. At 214 nm the maximum output of the zinc lamp is obtained and the absorption of Br^- is about 60% of its maximum, while that of the interfering chloride is only 5%. The detectability of Br^- and Cl^- at different wavelengths between 205 and 229 nm was also determined in an HPLC system, using a variable-wavelength UV detector. The results are given in Table II. It can be seen that even a chloride concentration of 1400 mg/l does not hinder the determination of 2.6 mg/ml of bromide. Normal values of chloride and bromide in body fluids are known to be 88—739 mg/l [35] and 1.1—9.7 mg/l respectively.

TABLE I

CAPACITY FACTORS (k) OF SOME SELECTED IONS ON HYPERSIL APS

Mobile phase: 0.03 M phosphate buffer, pH 2.8.

Ion	k	Ion	k
Cl^-	1.85	NO_2^-	2.11
Br^-	1.82	NO_3^-	2.05
BRO_3^-	2.06	I^-	2.11

TABLE II

DETECTABILITY OF 10-μl INJECTIONS OF AQUEOUS SOLUTIONS OF Br^- AND Cl^- AT DIFFERENT WAVELENGTHS

Concentrations: Br^-, 2.6 mg/ml; Cl^-, 1400 mg/ml.

Wavelength (nm)	Relative detector response		Response ratio* Br^-/Cl^-
	Br^-	Cl^-	
205	142	84	2000
210	80	14	7000
214	47	6	9000
229	2.5	0	—

*Calculated for equimolar concentrations.

TABLE III

RELATIVE RESPONSES AND COEFFICIENTS OF VARIATION FOR DIFFERENT INJECTED AMOUNTS OF BROMIDE

Injection volume: 5 μl. Other conditions: see text. $n = 4$.

Amount injected (ng)	Relative response	Coefficient of variation
5.3	14	5.9
26.5	66	1.5
42.5	105	0.7
53.0	132	0.8

A calibration curve was constructed for bromide standards in the range 0—50 ng per injection, using an injection volume of 5 μl. Excellent linearity was observed ($r = 0.99996$; $n = 4$). The detection limit, defined as three times the peak-to-peak noise level, is 1 ng. The coefficients of variation for different concentrations of bromide are given in Table III. They are low (1.5%) at concentrations of up to some 25 ng per injection. At lower concentrations the coefficient increases dramatically, e.g. to 5.9% for 5.3 ng Br^-. Therefore, the limit of accurate determination is about 25 ng, which means that, when using an injection volume of 10 μl, the theoretical value for human milk is 2.5 ppm. Lower values can be detected, down to some 0.2 ppm, but the accuracy will be low. The reproducibility of the HPLC analysis was determined using 10-μl loop injection of standards containing 2.88 μg Br^- per ml. The coefficient of variation was 2.2% ($n = 12$).

Application to human milk

Three methods of sample preparation of human milk were investigated. Direct injection of the concentrated aqueous phase and injection of the aqueous phase after clean-up over a Sep-Pak C_{18} cartridge resulted in chromatograms which contained too many interfering peaks to permit the accurate and sensitive analysis of bromide. Destruction of the aqueous phase as described in Experimental led to clean chromatograms (Fig. 1B). The recovery, calibration curve and coefficients of variation for the total procedure were determined using Almiron M2 baby food, a human milk substitute. The results are given in Table IV. The mean recovery is 79%, comparable to that achievable in other procedures for the determination of bromide in which ashing is applied for sample preparation [4]. The coefficient of variation is again low (3%) for concentrations down to 2 ppm, while below 1 ppm the accuracy of the method is low (coefficient of variation = 25%). The mean coefficient of variation for the procedure in the concentration range of interest (0.7—5.8 ppm) is 14%, which is acceptable for our purposes.

With this procedure 278 human milk samples were analysed. The bromide concentrations found range from 0.3 to 4.4 mg/l. The frequency distribution was characterized by an arithmetic mean of 1.56 mg/l and a median of 1.40 mg/l.

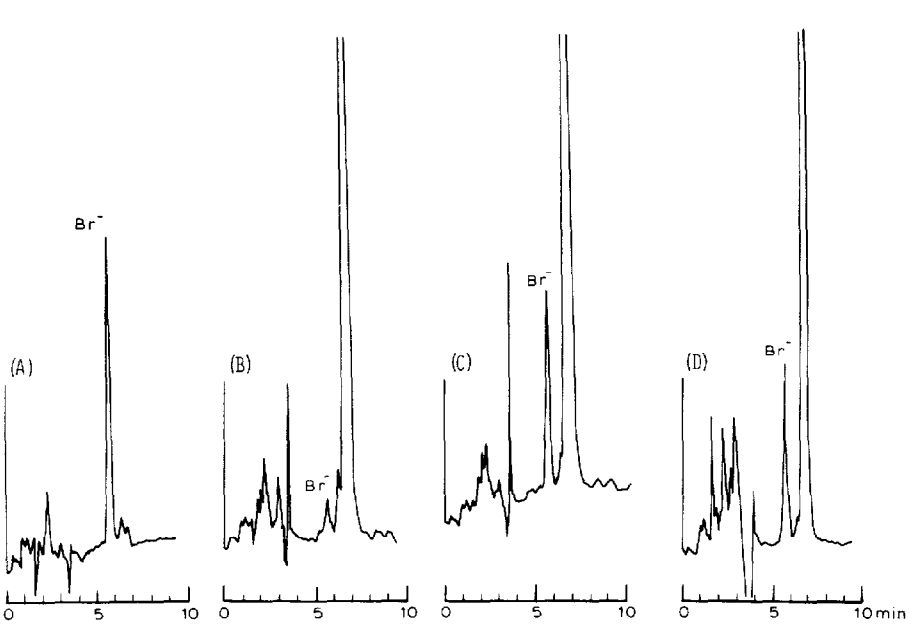

Fig. 1. HPLC analysis of 5 μl of (A) bromide standard (4.2 μg/ml), (B) human milk substitute blank, (C) Br^--spiked (2.3 mg/l) human milk substitute and (D) human milk sample. UV detection: 214 nm, 0.01 a.u.f.s. Sample treatment and HPLC conditions: see Experimental.

The method described is in principle also suited to the determination of other inorganic anions, such as NO_3^-. If the determination of chloride is also needed, this can be done simultaneously with the bromide analysis, by use of multiwavelength detection. Fig. 2 shows the analysis of both these ions in human milk, detection being at both 195 and 214 nm. The signal at 195 nm is the sum for Br^- and Cl^-. From the signal at 214 nm, the bromide concentration can be calculated, and from the combined data the concentration of chloride can be obtained.

TABLE IV

MEAN RECOVERIES AND COEFFICIENTS OF VARIATION FOR BROMIDE, SPIKED IN HUMAN MILK SUBSTITUTE, AFTER APPLICATION OF THE TOTAL PROCEDURE DESCRIBED IN EXPRIMENTAL

Spiked level (mg/l)	Number of experiments	Mean recovery (%)	Coefficient of variation
0.72	8	79	25
1.4	8	77	11
2.3—2.8	6	83	3
5.3—5.8	6	78	3
2.3—5.8	12	81	4
0.72—5.8	28	79	14

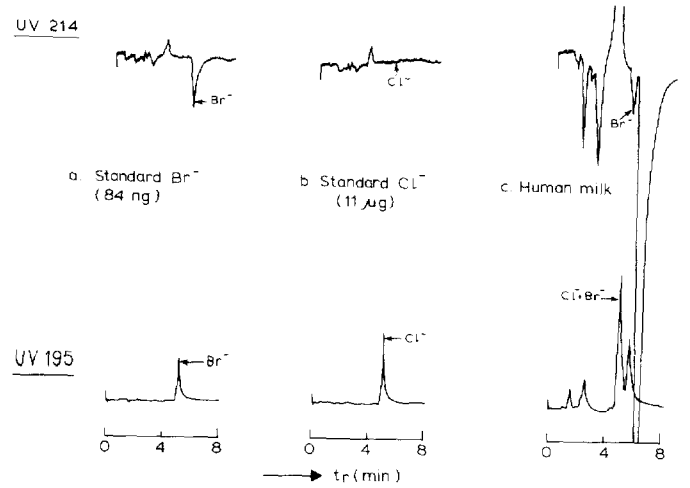

Fig. 2. Chromatograms of 5 µl of (a) standard bromide (84 ng), (b) standard chloride (11 µg) and (c) human milk sample, recorded at 214 nm (upper trace) and 195 nm (lower trace). For explanation see text.

Application to human blood plasma

The method developed for human milk was also applied to bromide analysis in human plasma. Initial experiments were carried out in order to try to simplify the sample preparation procedure. Centrifugation and filtration of the samples was tried, but this led to a loss of 50% of the material to be analyzed. Therefore destruction, as carried out in the case of milk, was applied to plasma as well. After destruction, sixteen samples were analysed by two procedures: HPLC, and spectrophotometry as described by Van Logten et al. [4]. The latter procedure is used routinely in our institute. The results are given in Fig. 3.

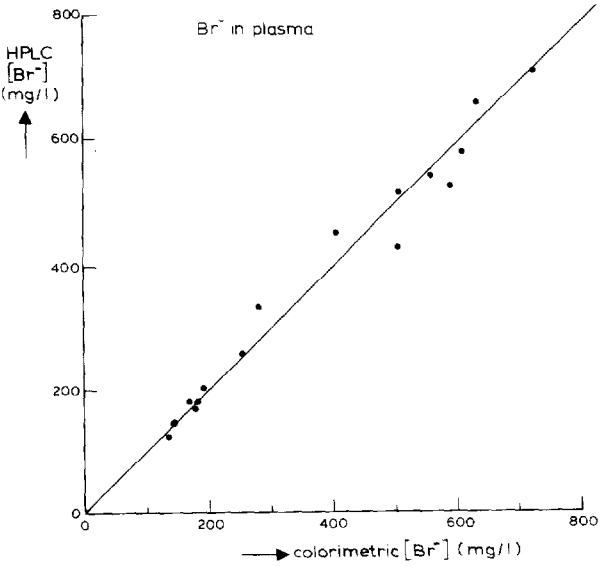

Fig. 3. Results of sixteen analyses of bromide in human plasma by HPLC and colorimetry.

Application of Student's t-test showed that any differences were insignificant ($t_{0.05}$ = 0.398; n − 1 = 15). The HPLC method is preferred to colorimetry because of its longer linear range and ease of automation. Sample preparation is the same in each case.

CONCLUSIONS

A method is presented for the analysis of bromide in human milk and in plasma, using a chemically bonded aminopropylsilica column with phosphate buffer as the eluent and UV detection at 214 nm. The sensitivity and reliability of the procedure are suitable for the determination of naturally occurring levels of bromide in human body fluids. The data obtained are in good agreement with those obtained from a routinely used colorimetric procedure. The method is in principle also suitable for the determination of other common anions present in body fluids, such as nitrate and chloride.

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REFERENCES

1 R.H. de Vos, W. van Dokkum, P.D.A. Olthof, J.J. Quirijns, T. Muys and J.M. van der Poll, Food Chem. Toxic., 22 (1984) 11.
2 R.C.C. Wegman and P.A. Greve, Med. Fac. Landbouww. Rijksuniv. Gent, 39 (1974) 1301.
3 Proceedings of the International Symposium on Residues and Toxicity of Bromide, Food Chem. Toxic., 21 (1983) 357.
4 M.J. van Logten, M. Wolthuis, A.G. Rauws and R. Kroes, Toxicology, 1 (1973) 321.
5 Panel on Fumigant Residues in Grain, Analyst (London), 101 (1976) 386.
6 U. Leuenberger, R. Gauch, K. Rieder and E. Baumgartner, J. Chromatogr., 202 (1980) 461.
7 H.J. Cortes, J. Chromatogr., 234 (1982) 517.
8 R.S. Bowman, J. Chromatogr., 285 (1984) 467.
9 H. Small, T.S. Stevens and W.C. Bauman, Anal. Chem., 47 (1975) 1801.
10 D.T. Gjerde, G. Schmuckler and J.S. Fritz, J. Chromatogr., 187 (1980) 35.
11 P. de Jong and M. Burggraaf, Clin. Chim. Acta, 132 (1983) 63.
12 G.P. Ayers and R.W. Gillet, J. Chromatogr., 284 (1984) 510.
13 M.J. van Os, J. Slanina, C.L. de Ligny, W.E. Hammers and J. Agterdenbos, Anal. Chim. Acta, 144 (1982) 73.
14 I. Molnár, H. Knauer and D. Wilk, J. Chromatogr., 201 (1980) 225.
15 M. Dreux, M. Lafosse and M. Pequinot, Chromatographia, 15 (1982) 653.
16 W.E. Barber and P.W. Carr, J. Chromatogr., 260 (1983) 89.
17 A.M.P. van Wees, M.A.H. Rijk, M.W. Rijnaars and R.H. Vos, in A. Frigerio and H. Milon (Editors), Chromatography and Mass Spectrometry in Nutrition Science and Food Safety, Elsevier, Amsterdam, 1984, pp. 19—25.
18 J.P. de Kleyn, Analyst (London), 107 (1982) 223.

19 R.M. Cassidy and S. Elchuk, J. Chromatogr. Sci., 21 (1983) 454.
20 R.M. Cassidy and S. Elchuk, J. Chromatogr., 262 (1983) 311.
21 J.P. Ivey, J. Chromatogr., 267 (1983) 218.
22 S.A. Wilson and E.S. Yeung, Anal. Chim. Acta, 157 (1984) 53.
23 W. Röhse, G. Roewer and R. Boran, Z. Chem., 22 (1982) 226.
24 K. Suzuki, H. Aruga and T. Shirai, Anal. Chem., 55 (9183) 2011.
25 R.D. Rocklin and E.L. Johnson, Anal. Chem., 55 (1983) 4.
26 C.Y. Wang, S.D. Bunday and J.G. Tartar, Anal. Chem., 55 (1983) 1617.
27 J.P. Ivey, J. Chromatogr., 281 (1983) 314.
28 P.K. Dasgupta, Anal. Chem., 56 (1984) 103.
29 W.F. Koch, J. Res. Natl. Bur. Stand., 84 (1979) 241.
30 A. Wehrli, J.C. Hildenbrand, H.P. Keller, R. Stampfli and R.W. Frei, J. Chromatogr., 149 (1978) 199.
31 C.E. Werkhoven-Goewie, W.M. Boon, A.J.J. Praat, R.W. Frei and U.A.Th. Brinkman, Chromatographia, 16 (1982) 53.
32 N. Vonk, Eur. Spectrosc. News, 53 (1984) 25.
33 K.J. Stetzenbach and G.M. Thompson, Ground Water, 21 (1983) 36.
34 L.R. Snyder and J.J. Kirkland, Introduction to Modern Liquid Chromatography, Wiley, New York, 1979, pp. 37—50.
35 A.A. Jenssen, Residue Rev., 89 (1983) 6.