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Journal of Chromatography B, 692 (1997) 53–59

JOURNAL OF CHROMATOGRAPHY B

Determination of total iodine in biological material by alkaline ashing and column-switching ion-pair liquid chromatography

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Received 23 July 1996; revised 22 October 1996; accepted 22 October 1996

Abstract

A method for the determination of total iodine in biological material has been developed. The method combines alkaline ashing with a selective and sensitive column-switching ion-pair HPLC technique. The ashing procedure which converts the organically bound iodine to iodide is miniaturised and requires only about 100 mg of sample. The first column of the column-switching system is polymer based and can therefore withstand the alkaline pH obtained after ashing. On the analytical column the iodide is separated as an ion-pair with tetrabutyl ammonium hydrogensulphate. The method has been applied to samples from whole blood, urine, liver, lung, carcass, and a sample throughput of at least 50 samples per day can be achieved. Validation studies by spiking experiments showed the precision to be better than 10% R.S.D. for all matrices with recoveries in the range 87–97%. The method has been applied for samples with an iodine content in the range 0.07–1060 μg/g.

Keywords: Alkaline ashing; Iodine

1. Introduction

In pharmacokinetic and toxicokinetic studies of iodine containing contrast agents for computed tomography, one important analysis is the quantitative determination of total iodine in biological material (i.e., whole blood, urine, faeces and different organs). Generally the methods available for this kind of analysis require the biological sample to be decomposed and the organically bound iodine to be converted to iodide prior to measurement. Decomposition procedures based on dry alkaline combustion or wet acid digestion have been proposed [1–5]. Several methods for the determination of the iodide formed have been published. A colorimetric assay based on the catalytic effect of iodide in the redox-reaction 2 Ce(IV)+As(III)→2 Ce(III)+As(V), was first described by Sandell and Kolthoff [6] and has been utilised in different modified forms by other authors [1–4]. Potentiometric analysis with ion selective electrodes has been employed though interference from chloride and a somewhat high limit of detection were reported [7–9].

In general, the methods suggested both for the combustion and for the final determination are manual and time-consuming. Further, rather large sample volumes are generally required. Alternative techniques not requiring sample decomposition are neutron activation analysis [10,11] and inductively coupled plasma-mass spectrometry (ICP-MS) [12,13]. These techniques have the required sensitivity and accuracy. However, none of the two tech-

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PII S0378-4347(96)00483-5

niques are easily accessible due to the high level of specialisation needed and the high cost involved.

In order to overcome the problems associated with previous methods we have developed a combination of an alkaline combustion procedure with a selective and sensitive ion-pair HPLC technique.

Ion-pair HPLC of iodide is well known [14,15]. Unfortunately, ordinary silica based C_{18} columns cannot withstand the very alkaline pH obtained in the sample solution after alkaline ashing. Therefore we have developed a column-switching system with a polymer based pre-column and a silica based C_{18} analytical column that enables the direct injection of the alkaline solution. The selectivity of the polymer column alone is insufficient, while the combination yields very good selectivity. Since iodide is a good UV absorber excellent sensitivity can be achieved at 225 nm.

2. Experimental

2.1. Chemicals

Potassium dihydrogen phosphate, disodium hydrogen phosphate, sodium hydroxide, potassium hydroxide, sodium disulphite, tetrabutylammonium hydrogen sulphate (TBA-HS) and potassium iodide were all of p.a. quality and obtained from Merck (Darmstadt, Germany). Ethanol 99.5% (v/v) was from Kemetyl (Stockholm, Sweden) and acetonitrile of HPLC grade from Lab Scan (Dublin, Ireland). The spiking experiments were made with di-iodinated octadecanoic acid ethyl ester emulsified in water (Pharmacia and Upjohn).

2.2. Apparatus

The liquid chromatograph consisted of two LC 10-AD Shimadzu pumps (Shimadzu, Kyoto, Japan). A Carnegie Medicine autoinjector model 200, equipped with two six-port valves for sample injection and column-switching (CMA Microdialysis, Stockholm, Sweden). The UV detector was a Shimadzu model SPD 10-A. Evaluation and quantitation was made on a Millenium chromatography data system (Waters, Milford, MA, USA). The oven used for ashing was a Carbolite Furnaces OAF 10/2 (Sheffield, UK).

2.3. Sample preparation

The preparation of blood and urine homogenates was performed in the following manner; whole blood and urine (1 volume) was diluted with 2.5 volumes of 2 *M* KOH in ethanol. This solution was then incubated over night at room temperature and after that diluted with 1.5 volumes of 50% ethanol (v/v). The final dilution was 1:5 (v/v). The preparation of organ homogenates was performed in the same manner but since the organ parts were weighed the dilution was expressed in weight per volume (w/v).

Volumes of the homogenates, typically 100–500 μl were transferred to 1.5 ml autoinjector glass vials (Chromacol, Herts, UK) and 0.2 ml of 6 *M* NaOH and 0.2 ml of 0.3 *M* $Na_2S_2O_5$ were added to the vials.

The sample vials were then transferred to the combustion oven and the ashing procedure was performed as follows: the sample was initially dried at 80°C for 12 h and after that the temperature was raised to 600°C and kept there for 2 h during the ashing step. After cooling the sample was dissolved in 500 μl of water and ultrasonicated for 5 min. The sample slurry was then transferred to a 300 μl autoinjector vial (CMA microdialysis, Stockholm, Sweden) in which it was centrifuged at 5000 rpm (2000 *g*) for 5 min. The supernatant from this centrifugation step was used as the sample solution. By means of a special injection needle with the inlet hole a few mm up from the tip, direct injection (without further transfer) from the vial was made possible.

2.4. Chromatographic conditions

The pre-column was a polymer based C_{18}, PLRP-S, 50×4.0 mm, 5 μm particles, from Polymer Labs (Amerst, MA, USA) and the analytical column was a silica based reversed-phase Zorbax, Stablebond SB-C_{18}, 150×4.6 mm, 5 μm particles (Rockland Technologies, Newport, DE, USA). The same mobile phase was used for both the pre- and analytical column, and it consisted of phosphate buffer–acetonitrile (90:10, v/v).

The composition of the phosphate buffer was 9 m*M* KH_2PO_4, 30 m*M* Na_2HPO_4 and 5 m*M* TBA-HS. The flow-rate for both pumps was 1.0 ml/min. The chromatographic separation was performed at room temperature but the samples were kept at +8°C in the autoinjector. The operative wavelength of the UV detector was 225 nm. The injected sample volumes ranged from 5–100 μl.

2.5. Column switching configuration

Injection was made with the switching valve in position A (see Fig. 1) directing the sample on to the polymer based reversed-phase pre-column and early eluting components were directed to waste. The valve was then switched to position B and the heart-cut containing the iodide was eluted into the silica based reversed-phase analytical column where the analytical separation took place. The valve was then switched back to position A, and late eluting components were directed to waste.

Quantitation was performed by means of a calibration graph made from potassium iodide standards made up in water. The standards were analysed with the same column-switching procedures as the samples. The results are expressed as iodine (I) w/v for blood and urine, and w/w for tissue material.

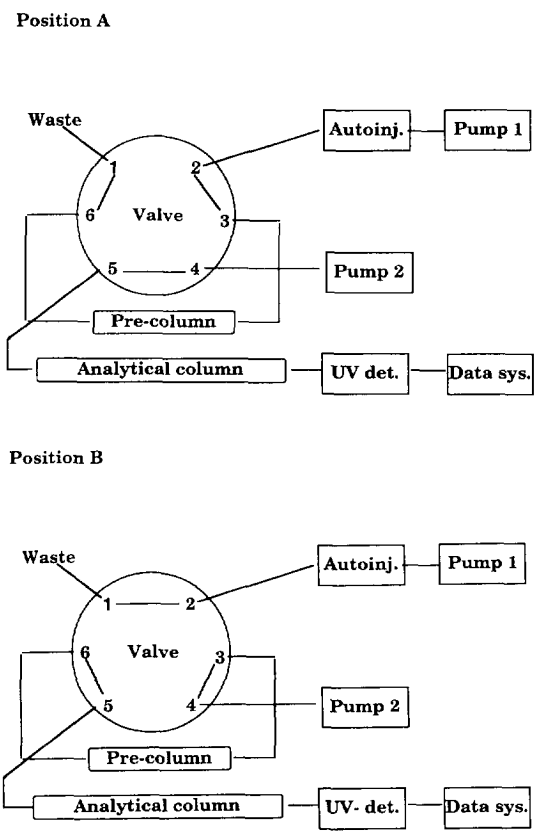

Fig. 1. Column switching configuration. The heart-cut is taken during valve position B, 6–9 min.

3. Results and discussion

3.1. Sample preparation optimisation

The treatment with the ethanolic potassium hydroxide solution was developed in order to dissolve the tissues or body fluids to obtain a homogeneous sample, prior to the alkaline ashing. This procedure was found essential for total combustion of the sample. Preliminary experiments with conventional water homogenisation with Ultraturrax equipment showed that, if the homogenate was not fully homogenised (i.e., if it contained small tissue fragments) complete combustion was not achieved which resulted in low recoveries.

The use of 6 *M* sodium hydroxide during the ashing step has been suggested [2,4] and was adopted in this work. 1 *M* sodium hydroxide was investigated but insufficient combustion with residual particles resulted.

In order to achieve a high sample throughput and a small sample consumption a miniaturised ashing procedure was developed. By performing the ashing in 1.5 ml autoinjector vials for HPLC it was possible to use only about 100 mg of biological sample. Furthermore, due to the small size of the vials each sample required only minimal space in the oven thus enabling at least 100 samples to be ashed simultaneously.

It has been reported difficult to achieve 100% recovery of iodine in the ashing step, both due to losses of iodine and due to incomplete combustion. Losses of iodine occur if too high temperatures are used [16]. On the other hand, too low ashing temperatures result in incomplete combustion [3,17]. Our optimisation of the drying and the ashing step showed that drying of the sample at 80°C for 12 h

and ashing at 600°C for 2 h gave the highest recoveries (i.e., smallest losses of iodine) and the most complete combustion. Incomplete combustion revealed itself by the presence of small black residues in the otherwise white and smooth ash. The ashing temperature of 600°C is rather close to the melting point of the glass, so it is important to use glass vials of the proper quality. The glass type we used (see Section 2) showed no signs of melting, whereas vials from some other manufacturers did.

Sodium disulphite was added to the sample prior to the combustion step in order to avoid losses due to oxidation. Amounts between 0 and 0.5 ml of the 0.3 *M* sodium disulphite solution were investigated during the combustion of whole blood to which organically bound iodine had been added to a concentration of 1000 μg/ml. The recovery of iodine was over 90% when 0.2 ml or more was used but only around 80% when smaller volumes were used. It was experienced that other matrices than whole blood yielded good recoveries also in the absence of disulphite. Components of whole blood (e.g., iron) possibly create an oxidative environment during the combustion resulting in the formation of iodate.

3.2. Chromatography system optimisation

It is well known that very alkaline sample solutions rapidly deteriorate silica based reversed-phase columns. Despite this we initially tried to inject our alkaline sample solutions directly on a Stablebond (SB-C18) column from Zorbax. This material is said to be rather stable against hydrolysis of the siloxane bonds. We achieved sufficient selectivity with this approach, but the stationary phase deteriorated after only a few 5 μl injections. Polymer based columns on the other hand can well withstand very alkaline solutions. A polymer based anion-exchange column was therefore tested. Unfortunately poor retention time reproducibility and poor resolution between iodine and chloride and hydroxy ions present in large excesses resulted.

The successful approach to overcome these problems was to develop the heart-cut column-switching system described in Section 2. It was found to be beneficial to use the same mobile phase in both pumps since this meant that no disturbance of the equilibrium in the analytical column occurred when the mobile phase of the pre-column was directed into the analytical column. The ion-pairing mobile phase was therefore optimised to give sufficient selectivity on the analytical column. However, with this mobile phase the selectivity on the pre-column towards chloride and hydroxy ions was too poor if a pre-column was used which was too short. It was found that at least a 50 mm column was needed. Both 10 and 25 mm column were tested and found insufficient. However, even with a 50 mm column the resolution was not complete, but by taking a heart-cut over the iodide peak, excellent resolution was achieved on the analytical column. In Fig. 2 a chromatogram taken by monitoring the pre-column outlet shows the iodide peak on the slope of the huge front peak, and the heart-cut taken. Fig. 3 then shows the chromatogram obtained on the analytical column. Both figures are from a blood sample containing 75 ng/ml of iodine.

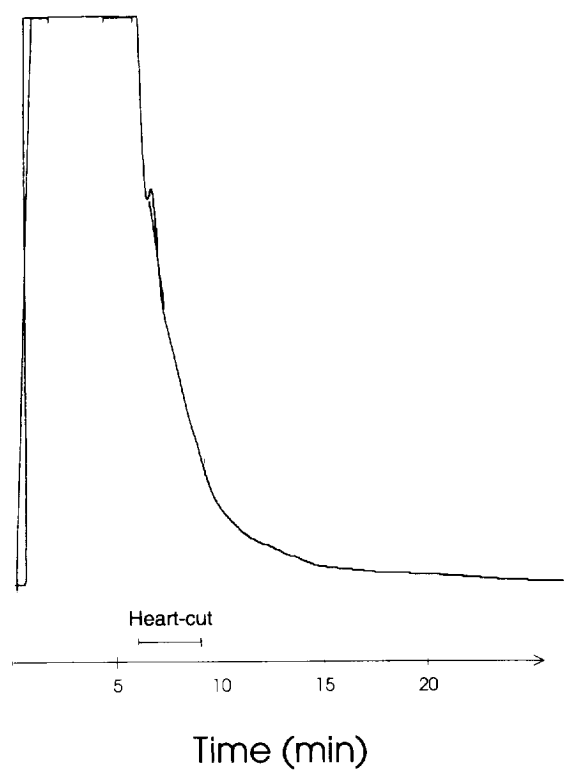

Fig. 2. Monitoring of the pre-column. Chromatogram of a blood sample at 75 ng/ml level. 100 μl injected.

Table 1
Between-day method repeatability and accuracy from spiked biological samples

Day	Found iodine concentration														
	Whole blood			Urine			Liver			Lung			Carcass		
	Added concentration (μg/ml)			Added concentration (μg/ml)			Added concentration (μg/g)			Added concentration (μg/g)			Added concentration (μg/g)		
	Endog.	10.6	1060	Endog.	10.6	1060	Endog.	5.30	53.0	Endog.	53.0	53.0	Endog.	5.30	53.0
1	0.065	9.6	1000	1.92	11.7	1026	0.065	4.60	47.6	0.055	4.65	48.1	0.185	5.00	50.5
2	0.080	9.0	1101	1.98	11.1	1093	0.075	4.60	49.2	0.080	4.55	48.1	0.240	4.75	50.0
3	0.075	9.0	1043	2.02	11.1	959	0.080	4.65	47.8	0.065	4.65	48.9	0.220	4.95	52.0
4	0.060	9.2	1162	1.85	11.4	993	n.a.	5.20	49.4	0.085	5.25	47.8	0.225	4.50	51.5
5	0.085	8.8	888	1.81	10.6	880	0.070	4.75	50.5	0.085	4.95	44.0	0.220	5.10	46.5
6	0.095	9.9	1015	2.05	12.1	934	0.105	5.05	50.5	0.075	n.a.	n.a.	0.245	5.55	55.5
7	0.075	9.8	1016	2.04	12.6	995	0.095	5.65	52.5	0.055	n.a.	n.a.	0.220	5.55	52.0
Mean	0.076	9.3	1032	1.95	11.5	983	0.082	4.93	49.6	0.071	4.81	47.4	0.222	5.06	51.1
R.S.D. (%)	15.4	4.8	8.3	4.9	5.9	6.9	18.8	8.0	3.4	18.4	6.0	4.1	8.7	7.7	5.4
Recovery (%)		87.1	97.4		91.6	92.6		91.6	93.4		89.6	89.3		91.6	96.0

n.a. = not analysed.
Recovery % = (Found conc. × 100)/(added conc. + endog. conc.).

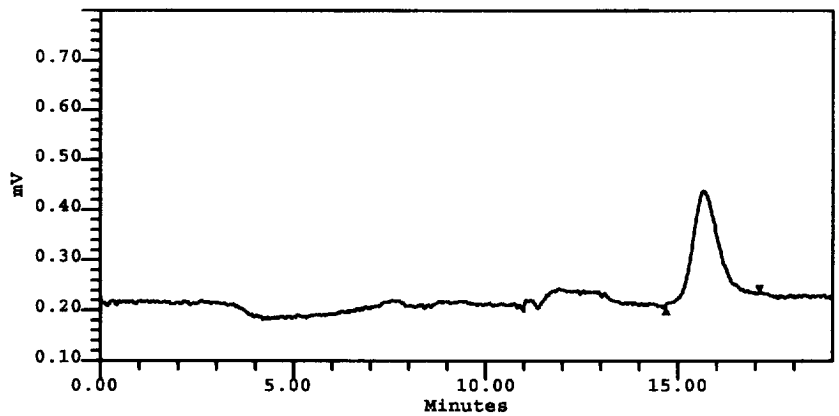

Fig. 3. Monitoring of the analytical column. Chromatogram of a blood sample at 75 ng/ml level. 100 μl injected.

3.3. Method performance

The detector response was linear for iodide standards over the entire range 0.01–15 μg/ml with r^2>0.999. However, by employing different injection volumes as well as different sample amounts, a wide concentration range could be covered. The range of the method could be set to 0.07–1060 μg/g, considering the good method repeatability obtained on different levels within this range as shown in Table 1.

Method precision and accuracy were investigated from a set of non-spiked and spiked biological samples from the rat. The examined matrices were whole blood, urine, liver, lung and carcass. Spiking was performed directly into whole blood and urine, but for the lung, liver and carcass, the homogenate was spiked.

The results obtained are tabulated in Table 1. In summary, the between-day method repeatability was in all cases, except for whole blood, liver and lung at endogenous levels, better than 10% R.S.D., and the recoveries ranged from 87 to 97%. The concentrations obtained after spiking as shown in Table 1, have been calculated back to the concentration in the intact biological sample.

Generally the limit of quantification is set to the concentration where the between-day method repeatability has an R.S.D. of about 20%. This limit was reached at about 70 ng/g for liver and lung.

4. Conclusions

The combination of a miniaturized alkaline ashing step and column-switching HPLC has been shown to be powerful for the determination of total iodine in biological material. By employing over-night combustion of many samples and an auto-injection HPLC system the method can be made very efficient with respect to sample throughput. In addition, excellent sensitivity and good precision has been achieved. Furthermore, the method exhibited excellent robustness during hundreds of injections without any loss in performance. The recovery was less than 100%, a feature the procedure seems to share with most combustion procedures for iodine. However, it might be possible to compensate for this by preparing calibration standards in the same matrix as the sample.

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