ELSEVIER

Journal of Chromatography B, 690 (1997) 327–331

JOURNAL OF CHROMATOGRAPHY B

Short communication

Analysis of selenium in bovine liver by gas chromatography with mass-selective, electron-capture and nitrogen–phosphorus detection

Ashok K. Singh*, Thomas White, Tom Arendt, Yin Jiang

Toxicology and Endocrinology Sections, Minnesota Veterinary Diagnostic Laboratory, Department of Veterinary Diagnostic Medicine, College of Veterinary Medicine, University of Minnesota, Twin Cities Campus, St Paul, MN 55108, USA

Received 15 March 1996; revised 9 August 1996; accepted 19 August 1996

Abstract

The concentration of selenium (Se) in liver was determined by gas chromatography (GC) with mass-selective (GC–MS), electron capture (GC–ECD) and nitrogen–phosphorus (GC–NPD) detection. Liver samples were digested in a mixture containing HNO_3 and $Mg(NO_3)$. Se^{VI} was converted to Se^{IV}. Se^{IV} was derivatized with 4-nitrophenylenediamine and then extracted in toluene. A 1-μl volume of the toluene extract was analyzed by the GC–MS, GC–ECD or GC–NPD methods. The detection limits of the GC–ECD, the GC–NPD and the GC–MS methods were 25, 50 and 800 pg, respectively. The GC–NPD method was more selective for the derivatized Se than the GC–ECD method. The GC–MS method had the advantage of using the ^{76}Se isotope as the internal standard. Se concentrations in liver samples determined by the three methods were comparable.

Keywords: Selenium

1. Introduction

Selenium (Se) is an essential trace element [1] that is widely used to supplement diets designed for human or animal consumption [2]. Se acts as a cofactor for glutathion peroxidase that destroys hydrogen peroxide leading to the reduction of tissue peroxide [1]. Previous studies have shown that Se deficiency (0.004 to 0.08 μg/ml in blood, 0.02 to 0.17 μg/g in liver) may inhibit glutathion peroxidase [3] and Se overdose (0.5 to 2 μg/ml in blood, 4 to 10 μg/g in liver) may cause red cell hemolysis, nausea, vomiting, fatigue and irritability in animals [4–6]. Se has been analyzed in biological samples by using fluorimetric (FL), gas chromatographic (GC) with electron capture detection (ECD) or atomic absorption spectrometry (AAS) with Zeeman correction methods [7–14]. However, the FL and GC–ECD methods are more common than the AAS method because of the relatively higher equipment- and per sample costs of the AAS method.

The analysis of Se by FL or GC–ECD requires (1) sample digestion in a mixture of mineral acids containing HNO_3+HClO_4, $HNO_3+H_2SO_4$ or $HNO_3+HClO_4+H_2SO_4$, (2) conversion of the element to its tetravalent state (Se^{IV}) and (3) sample derivatization [15]. The digestion of tissue samples using acid mixtures may cause extensive loss of Se [16,17]. Since an internal standard is not available for the FL and GC–ECD methods, the loss of Se can not be compensated for. Capillary GC–MS, using ^{76}Se as an internal standard (IS), provided quantita-

*Corresponding author

PII S0378-4347(96)00394-5

tive recovery of Se in blood, plasma or urine samples [18,19]. However, the previously described GC–MS methods, which were developed for blood and urine samples, used a mild digestion procedure that was not suitable for liver samples.

Therefore, the aims of this study were (1) to improve the liver digestion method, minimizing Se loss, (2) to analyze Se in liver by GC–MS, GC–ECD and GC–NPD methods and (3) to compare Se concentrations obtained by the three methods.

2. Experimental

2.1. Instrumentation and reagents

Three gas chromatographs (HP-5890) equipped with nitrogen–phosphorus, electron-capture and mass-selective detectors, respectively, were used in this study. The columns used were (1) HP-5 5% phenylmethyl silicone (10 m×0.53 mm) for GC–ECD or GC–NPD analysis and (2) HP-5 (30 m× 0.25 mm) for GC–MS analysis. The flow-rate of the carrier gas (helium) was 25.0 ml/min. The temperature of the column was programmed from 150 to 250°C with increments of 10°C/min. 4-Nitro-*o*-phenylenediamine (4-NPD), EDTA and other reagents were obtained from Aldrich. ^{76}Se was obtained from Cambridge Isotope Laboratory (Boston, MA, USA). The reference liver-samples were obtained from the National Institutes of Standards and Technology (Gaithersburg, MD, USA). Other liver samples were obtained from the Minnesota Veterinary Diagnostic Laboratory.

Standards containing 0, 100, 200, 500, 750 and 1000 ng/ml of Se were prepared in 0.1 *M* HCl. A 1000 μg/ml ^{76}Se solution in 0.1 *M* HCl served as an internal standard for the GC–MS method. 4-NPD was dissolved in 0.1 *M* HCl (4 mg/ml). The derivatizing reagent was prepared fresh daily.

2.2. Sample preparation

The sample preparation method described previously [15] was modified to prevent Se losses. A 0.5–1 g amount of liver sample was transferred to a 50-ml beaker containing 4 g of $Mg(NO_3)_2$ and 10 ml of conc. HNO_3. A 100-μl volume of ^{76}Se solution was added to the samples, which were later analyzed by the GC–MS method. Each beaker was covered with a watch glass and placed on top of a hot plate. The hot plate was turned on and the sample was heated at 50°C for 12 h. Then the temperature was increased gradually to 150°C. The sample was allowed to become completely dry. The dry sample was placed in a muffle furnace and ashed for 30 min at 600°C. Then the sample was cooled to room temperature, mixed with 10 ml of conc. HCl and heated at 100°C for 30 to 60 min. This reduced Se^{VI} to Se^{IV}. The sample was cooled to room temperature and transferred to a test-tube. The beaker was washed with 6 ml of the rinsing solution (20% urea, 1% EDTA and 1% hydroxylamine–HCl in deionized water) and the wash was pooled with the previous extract. The pooled sample was mixed with 1 ml of the derivatizing reagent (NPD) and 2 ml of toluene. The tubes were capped and incubated at 60°C for 30 min. The samples were then rotoracked for 5 min and centrifuged (1000 *g* for 15 min). The toluene layer was collected and concentrated to 1 ml at 50°C under nitrogen. A 1-μl volume of the toluene extract was injected into the GC–MS, GC–ECD and GC–NPD systems. The mass-selective detector (MSD) was programmed in the SIM mode to monitor ions at *m/z* 225 and 229. The ion at *m/z* 225 represents ^{76}Se and the ion at *m/z* 229 represents ^{80}Se [20].

2.3. Calibration

A 1-ml volume of Se standard (0, 100, 200, 500, 750 and 1000 ng/ml) containing ^{76}Se (for GC–MS method only) was mixed with 1 ml of conc. HCl, 4 ml of the rinse solution, 1 ml of 4-NPD solution and 2 ml of toluene. The mixture was processed as described above. For the GC–MS method, the calibration procedure was essentially as described previously [20]. The area under the peaks representing ions at *m/z* 229 and *m/z* 225 was determined. A standard curve was constructed by plotting Se concentration on the *x*-axis and the 229/225 ratio on the *y*-axis. For the GC–ECD or the GC–NP method, the signal response was plotted against Se concentration.

The concentration of Se in liver samples was determined from the standard curve.

2.4. Recovery

Liver samples were ground and fortified with different concentrations [0 (no Se added) to 1000 ng/g] of Se ($n=5$ for each concentration). The samples were digested and derivatized as described above. The concentration of Se was determined by the three methods. Since Se is naturally present in liver samples, the blank level of Se was subtracted from the Se concentrations in the spiked samples to determine the recovery.

3. Results and discussion

GC–MS, GC–ECD and GC–NPD all provided sensitive analyses of Se in liver samples. The chromatograms obtained by the GC–MS (Fig. 1) and the GC–NPD (Fig. 2A,B) methods were relatively cleaner than those obtained by the GC–ECD method (Fig. 2C,D) that exhibited several non-specific peaks. Se concentration was determined by using the standard curve (Fig. 3). All three methods exhibited greater than 90% recovery of Se from liver samples (Table 1).

The detection limits of the GC–ECD, the GC–NPD and the GC–MS methods were 25, 50 and 800 pg, respectively. The GC–MS detection limit ob-

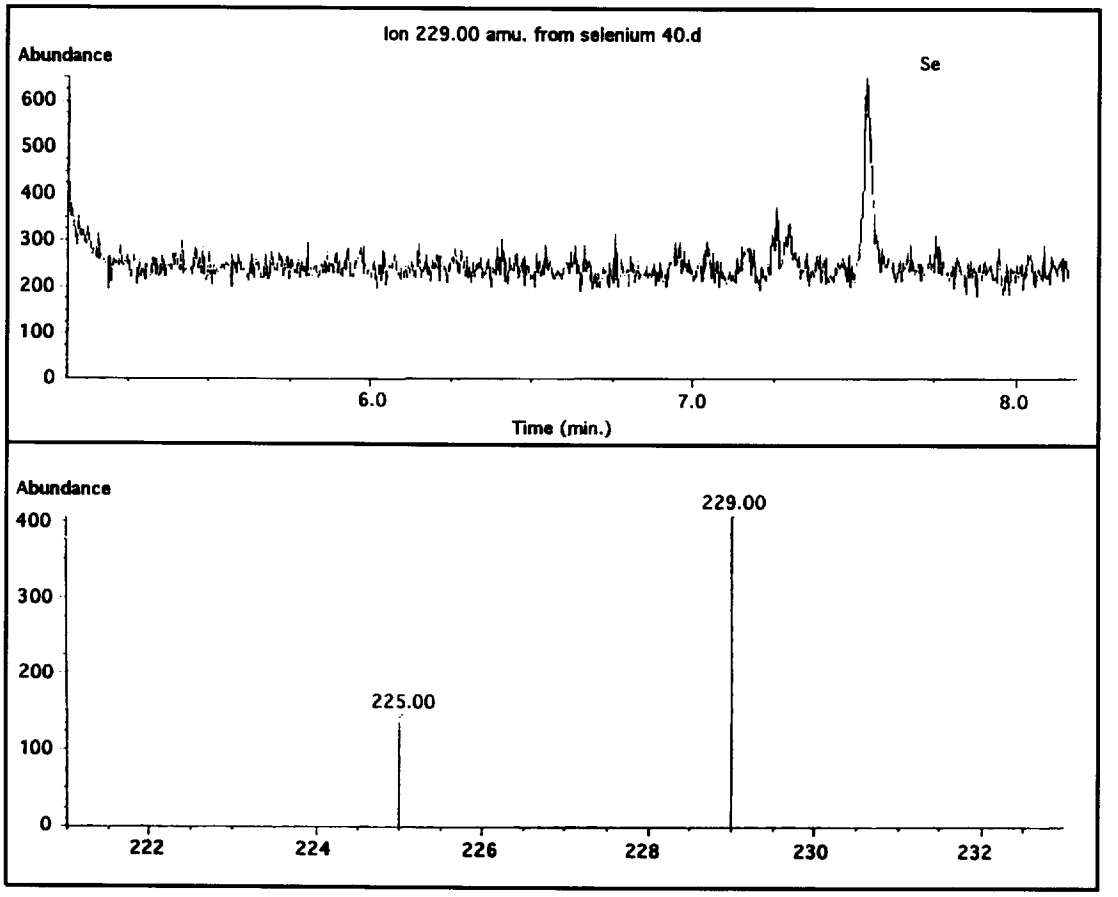

Fig. 1. Analysis of Se in a liver sample by GC–MS. Liver samples were fortified with 200 ng/g Se and analyzed by GC–MS. (Top) Chromatographic separation of the Se–NPD complex; (bottom) mass spectrum of the peak.

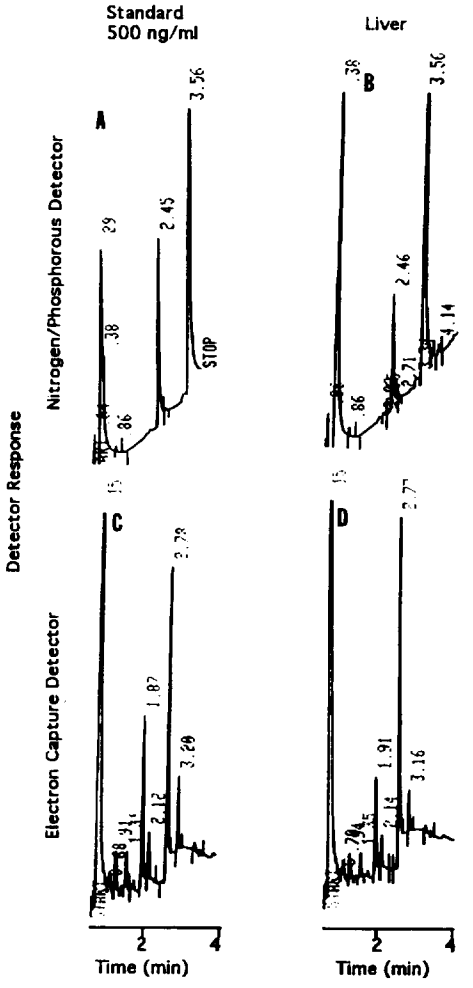

Fig. 2. Analysis of Se by GC–NPD (retention time, 2.4 min) or electron-capture detection (retention time, 1.9 min). (A) Se standard analyzed by the electron capture detector; (B) Se extracted from liver samples analyzed by the electron capture detector; (C) Se standard analyzed by the nitrogen–phosphorus detector; and (D) Se extracted from a liver sample and analyzed by the nitrogen–phosphorus detector.

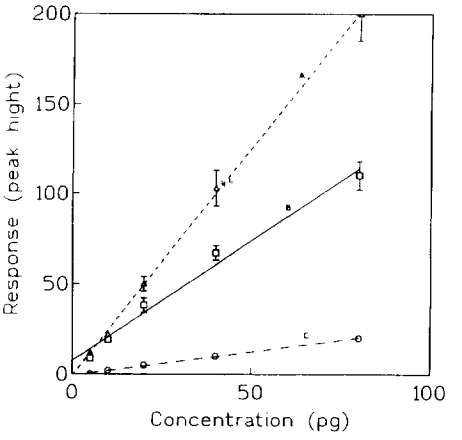

Fig. 3. Standard curve for the Se–NPD complex analyzed by the three methods (values are mean±S.D., $n=5$). A=Electron capture detector; B=nitrogen–phosphorus detector and C=mass spectrometer detector.

served in this study for liver samples was comparable to those reported previously for other biological samples [20,21]. A previous study has reported the detection limit for Se by GC–ECD to be 2 ng/ml [22], which was higher than the values reported in this study. The GC–ECD and the GC–NPD methods, because of their lower detection limits, were suitable for detecting Se deficiency in liver samples. The GC–MS method, however, may be more suitable for (1) analyzing liver samples with normal- or high Se concentrations and (2) determining recovery from a digestion procedure. The GC–NPD method was more selective than the GC–ECD method for Se analysis.

The performances of the three methods were also evaluated by analyzing a reference sample with a certified Se concentration of 710±70 ng/g. The values obtained using the GC–MS, GC–ECD and GC–NPD methods were 770±30, 750±40 and 730±35 ng/g, respectively. This suggests that the liver Se concentration obtained by the GC–MS method did not differ significantly from those obtained by the GC–ECD and the GC–NPD methods. Also, the three methods provided comparable results for the Se concentration in liver samples that were suspected of being poisoned by Se (Table 2). This further suggests that the digestion procedure de-

Table 1
Recovery of Se from liver samples (values are mean±S.D., $n=3$)

Se added (ng)	Measured (ng/g) by GC–MS	Recovery %		
		GC-MS	GC-ECD	GC-NPD
0	250±30	–	93	95
200	440±25	95	90	97
300	545±40	98	95	98
500	755±35	105	97	101
750	950±50	93	91	98

Table 2
Se concentration in different liver samples submitted to the Minnesota Diagnostic Laboratory

Liver sample	Se concentration (ng/ml)		
	GC–MS	GC–ECD	GC–NPD
1	310±30	290±40	330±20
2	1100±70	1090±80	1150±50
3	850±40	800±30	900±50
4	700±70	720±40	650±40
5	510±40	490±30	525±40

scribed in this study does not cause significant loss of Se from liver samples.

References

[1] R.F. Burk and J.M. Lane, Fund. Appl. Toxicol., 3 (1983) 218.
[2] A. Shenkin, G.S. Fell, D.J. Halls, P.M. Dunbar, I.B. Holbrook and M.H. Irving, Clin. Nutr., 5 (1986) 91.
[3] R.F. Carter, Med. J. Aust., 1 (1966) 525.
[4] I.D.S. Civil and M.J.A. McDonald, N.Z. Med. J., 87 (1978) 354.
[5] A.W. Halverson, I.S. Palmer and P.L. Guss, Toxicol. Appl. Pharmacol., 9 (1966) 477.
[6] A.W. Halverson, D. Ding-Tsay and K.C. Triebwasser, Toxicol. Appl. Pharmacol., 17 (1970) 151.
[7] N.T. Crosby, Analyst, 29 (1977) 1029.
[8] J.H. Watkinson, Anal. Chem., 38 (1966) 92.
[9] Analytical Methods Committee, Analyst, 104 (1979) 778.
[10] L.R. Harrison, B.M. Colvin and H.S. Gosser, Proc. 42th Annual AAVLD Meeting, St. Louis, MO, 1981, p. 31.
[11] G. Alfthan and J. Kumpulainen, Anal. Chim. Acta, 140 (1982) 221.
[12] A.A. Brown, J.M. Ottaway and G.S. Fell, Anal. Proc., June (1982) 321.
[13] J. Alexander, K. Saeed and Y. Thomassen, Anal. Chim. Acta, 120 (1980) 377.
[14] K. Saeed, Y. Thomassen and F.J. Langmyhr, Anal. Chim. Acta, 110 (1979) 285.
[15] C.F. Poole, N.J. Evans and D.B. Wibberley, J. Chromatogr., 136 (1977) 73.
[16] T.W. May, J. Assoc. Off. Anal. Chem., 65 (1982) 1140.
[17] H.J. Robberecht, R.E. van Grieken, P.A. van den Bosch, H. Deelstra and D. van den Berghe, Talanta, 19 (1982) 1025.
[18] S.A. Slavin and D.C. Manning, Prog. Anal. Atom. Spectrosc., 5 (1986) 1272.
[19] D.C. Reamer and C.A. Veillon, Anal. Chem., 53 (1981) 2166.
[20] V. Ducros and A. Favier, J. Chromatogr., 583 (1992) 35.
[21] C.A. Swanson, B.H. Patterson, O.A. Leavander, C. Veillon, P.R. Taylor, K. Helzlsouer, P.A. McAdam and L.A. Zech, Am. J. Clin. Nutr., 54 (1991) 917.
[22] A.F. Al-Attar and G. Nickless, Analyst, 115 (1990) 1441.