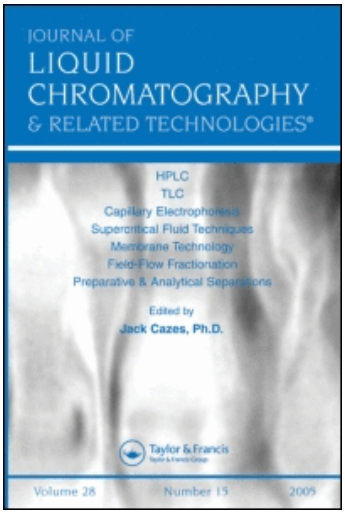


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Determination of Ivermectin Residues in Meat and Liver by HPLC and Fluorometric Detection

J. M. Degroodt[a]; B. Wyhowski De Bukanski[a]; S. Srebrnik[a]
[a] Ministry of Public Health and Environment, Brussels, Belgium

To cite this Article Degroodt, J. M. , De Bukanski, B. Wyhowski and Srebrnik, S.(1994) 'Determination of Ivermectin Residues in Meat and Liver by HPLC and Fluorometric Detection', Journal of Liquid Chromatography & Related Technologies, 17: 6, 1419 — 1426
To link to this Article: DOI: 10.1080/10826079408013774
URL: http://dx.doi.org/10.1080/10826079408013774

JOURNAL OF LIQUID CHROMATOGRAPHY, 17(6), 1419-1426 (1994)

DETERMINATION OF IVERMECTIN RESIDUES IN MEAT AND LIVER BY HPLC AND FLUOROMETRIC DETECTION

J. M. DEGROODT, B. WYHOWSKI DE BUKANSKI, AND S. SREBRNIK
Ministry of Public Health and Environment
14, J. Wytsman Street
B-1050 Brussels, Belgium

ABSTRACT

The method describes the analysis of ivermectin in meat and liver at a quantification limit of 5 µg/kg. This level is lower than the maximum residue limit (MRL) imposed for EEC countries. The absolute detection limit was 250 pg. The recoveries ranged from 70 % to 88 %. Ivermectin was extracted by a mixture of acetonitrile and water, purified on Bond Elut C_{18} columns and converted to a fluorescent derivative using trifluoroacetic anhydride and N-methylimidazol. The analysis was performed on a liquid chromatograph fitted with a µ- Bondapak C_{18} column and ivermectin was detected by fluorescence spectroscopy. The method has already been used for routine analysis.

INTRODUCTION

Ivermectin is a broad-spectrum antiparasitic agent and extensively used for food-producing animals. The formula of ivermectin is given in figure 1.

Ivermectin is a mixture of two homologous compounds (H2B1a and H2B1b) differing from each other by one methylene group. H2B1a is the major compound

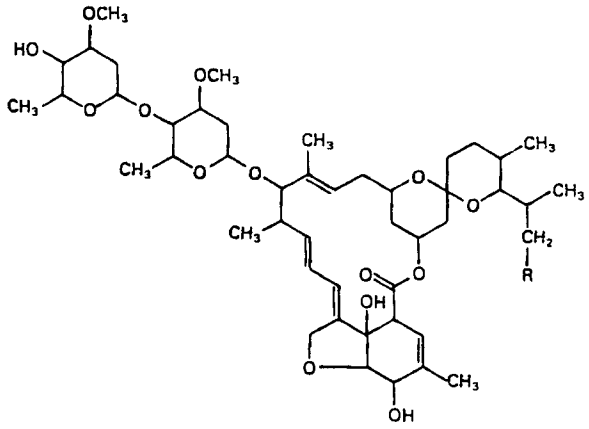


FIGURE 1. Structural Formula of Ivermectin
H2B1a: R = CH_3; H2B1b: R = H

and therefore marker substance for residue analysis. Tissue residue distribution and metabolism of tritium-labeled ivermectin have been studied in animal tissues (1) and examined thoroughly in swine (2) and have shown residues in numerous tissues and fluids, highest levels were found in bile, fatty tissues and liver.

In European countries the reglementation 675/92 EEC (3) allows a maximum residue limit (MRL) of 15 µg H2B1a/kg liver and of 20 µg H2B1a/kg fatty tissue. Recent analytical methods used high-performance liquid chromatography (HPLC) to analyze and to quantify ivermectin in feeds, serum, blood, plasma, milk and animal tissues, for the detection ultraviolet spectrophotometry (4-10) and fluorometry (11-14) were used.

Our aim was to develop a method allowing us to execute the surveillance program imposed to each member state of the European Community by the Council Directive 86/469/EEC (15). It was important to quantify ivermectin with accuracy in positive samples down to 15 µg/kg tissue and to have a method suitable for routine-analysis. Sample preparations were adapted following the method described by Th. Reuvers and al. (4) combined with the fast derivatization procedure of ivermectin to a fluorescent derivative from P. De Montigny and al. (14).

EXPERIMENTAL

Apparatus

A Vortex super mixer from Lab-Line Instruments (Illinois, USA) was used for the extraction procedure. Centrifugations were achieved with a centrifuge GLC-2B from Sorvall (Dupont, USA). The solvents were evaporated using a Rotavapor-R from Büchi (Switzerland) and a Reacti-Therm heating module from Pierce (Illinois, USA). Purification of the extracts on Bond Elut C_{18} columns were achieved using a Baker-10 extraction system (J.T. Baker, NJ., USA). HPLC analyses were performed with a 5000 liquid chromatograph from Varian (USA). Ivermectin was detected with an LS-4 fluorescence spectrometer from Perkin-Elmer (USA). The chromatograms were registered with a recorder A-41 from Ankersmit (The Netherlands) (paper speed: 2 mm/min).

Solvents and reagents

Acetonitrile and methanol were delivered by Labscan (Dublin, Ireland), N-methylimidazol GC 99% by Aldrich (Steinheim, Germany) and trifluoroacetic anhydride 99 % by Sigma (St. Louis, USA).

Standard and standard solutions

Ivermectin standard was supplied by Merck, Sharp and Dohme B.V. (The Netherlands) (purity: 90.07%). The working standard solution contained 1.8 µg ivermectin/ ml methanol and was used for the standard curves and to spike the samples.

Columns

For the purification procedure Analytichem Bond Elut C_{18} columns (6 ml) from Varian (CA, USA) were used. The HPLC column was a µ- Bondapak C_{18}, 10 µm column (3.9 mm X 300 mm) from Millipore-Waters (USA).

Sample preparation

Extraction procedure of ivermectin from meat and liver

4 g minced meat or liver were mixed with 40 ml acetonitrile and 3.5 ml water in a closed 100 ml glass centrifuge tube. The mixture was shaken on a Vortex during 2 minutes and then centrifuged at 2000 rpm during 10 minutes. The solvent was transferred into a 100 ml brown round bottom flask. The extraction procedure was repeated once more with 20 ml acetonitrile and 3.5 ml water. Both extracts were joined together and evaporated until 6 ml using the Rotavapor-R. No acetonitrile may be left in the flask. Finally 6 ml water were added to the extract.

Purification procedure of ivermectin from meat and liver

The Analytichem Bond Elut C_{18} columns were conditioned successively with 4 ml acetonitrile and 4 ml acetonitrile/water (1:9). The extracts were passed through the columns and the columns were dried over a period of 10 minutes by air aspiration. Ivermectin was then eluted with 5 ml acetonitrile which were evaporated afterwards under a stream of nitrogen.

Derivatization of ivermectin

To the dried residue were added successively:

1. 150 µl trifluoroacetic anhydride / acetonitrile (1:2; v/v)
2. 100 µl N- methylimidazol / acetonitrile (1:1; v/v)

The tubes were shaken, closed and stored in the dark, since the fluorescent derivative of ivermectin is very sensitive to light.

Chromatography

A 10 µl or 50 µl portion of the extract was injected into the liquid chromatograph at room temperature using a C_{18} µ-Bondapak reversed-phase

column and a flow rate of 1.5 ml/min. The eluent was methanol/water (95:5; v/v). Ivermectin was detected with a fluorescence detector (excitation wavelength: 364 nm; emission wavelength: 470 nm; slits: 15 and 20; fixed scale: 2).

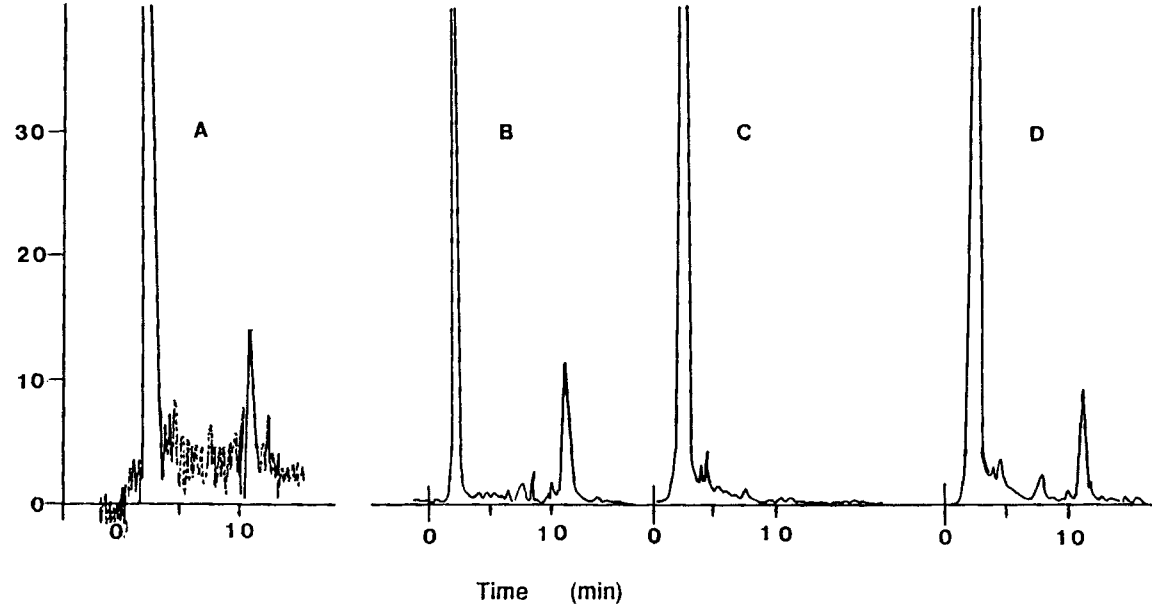
RESULTS AND DISCUSSION

Meat and liver samples were collected in slaughterhouses. Meat from cattle and pigs was taken randomly, not only fatty tissues, since all kinds of meat will be eaten by the consumer.

The HPLC analyses of the ivermectin standard resulted in a linear response within the range of 2 to 30 ng. The correlation coefficient was 0.9980. Table 1 summarizes the recoveries, standard deviations and coefficients of variation of ivermectin in meat and liver. Each result is the mean of five different extractions. The recovery studies were equally good for both matrixes, 70% to 88% could be reached. The quantification limit was 5 µg ivermectin/kg meat or liver.

The absolute detection limit was 250 pg without being disturbed by a higher background (fixed scale : 20). This is shown in figure 2 A. The same figure shows a typical chromatogram of a standard solution (2 B), a blank meat sample (2 C) and a spiked meat sample (2 D). The retention time for ivermectin was 11 minutes under the described LC conditions.

The sample preparation procedure provided clean extracts. For the purification we chose Bond Elut C_{18} columns, with which a very good reproducibility could be reached. The conversion of ivermectin to a fluorescent derivative was very fast and resulted in a high sensitivity. The derivative has to be protected from light. The stability was checked and showed a degradation of 50% in three hours when tubes containing the fluorescent derivative of ivermectin were exposed to daylight. Quantifications were done using a standard curve; samples with high concentrations were diluted or less extraction material was used. Daily analysis of 12 samples could be done without difficulties. To keep the HPLC column in good conditions, this one was rinsed afterwards with 30 ml methanol and reconditioned before reuse. 100 meat samples were analyzed until now, 3 samples were positive for ivermectin ranging from 15 to 30 µg/kg. 30 liver



RE 2. Chromatograms of Ivermectin. -A. Absolute Detection Limit (250 pg Ivermectin); -B. Standard
ion (3.6 ng Ivermectin); -C. Blank Meat Sample; -D. Spiked Meat Sample (22.5 µg Ivermectin/kg Meat);
ion volume : 10 µ l; Fixed Scale of the Fluorescence Spectrometer : 20 (A), 2 (B, C, D).

TABLE 1

Recovery Data of Ivermectin in Meat and Liver by HPLC and Fluorometric Detection

Added quantity (µg/kg)	Mean recovery (n = 5) (µg/kg)		Standard deviation		Coefficient of variation (%)		Recovery (%)	
	Meat	Liver	Meat	Liver	Meat	Liver	Meat	Liver
11.25	-	07.95	-	1.15	-	14.43	-	70.68
22.50	17.91	17.67	0.67	1.03	3.74	05.85	79.59	78.53
45.00	36.10	39.79	2.64	4.47	7.31	11.23	80.22	88.42
90.00	77.32	-	4.14	-	5.36	-	85.91	-

samples were analyzed, 1 sample contained 5.6 µg ivermectin/kg liver.
The method has proved to be suitable as a routine analysis method for ivermectin residues at a level below the maximum residue limit imposed by the EEC reglementation.

ACKNOWLEDGEMENT

We wish to thank Mrs. L. Vifquin for technical assistance and Mr. J. De Groof for statistical analyses.

REFERENCES

1. S.H.L. Chiu, R.P. Buhs, E. Sestokas, R. Taub, T.A. Jacob, J. Agric. Food Chem., 33: 99-102 (1985)

2. S.H.L. Chiu, E. Sestokas, R. Taub, M.L. Green, F.P. Baylis, T.A. Jacob, A.Y.H. Lu, J. Agric. Food Chem. 38: 2079-2085 (1990)

3.Reglementation (EEC) N° 675/92 of 18 march 1992, Off. J. of the Eur. Comm. N° L 73/8 of 19.03.1992

4. Th. Reuvers, R. Diaz, M.M. Depozuelo, M. Ramos, Anal. Chem. Acta 275: 353-358 (1993)

5. A. Fox, D.W. Fink, Analyst, 110: 259-261 (1985)

6. D.D. Oehler, J.A. Miller, J. Assoc. Off. Anal. Chem., 72: 59-61 (1989)

7. S.J. Doherty, A. Fox, D.W. Fink, J. Assoc. Off. Anal. Chem., 73: 931-934 (1990)

8. C.M. Dickinson, J. Chromatogr., 528: 250-257 (1990)

9. S.H.L. Chiu, E. Sestokas, R. Taub, J. Chromatogr. 433: 217-224 (1988)

10. J.V. Pivnichny, A.A. Lawrence, J.D. Stong, J. Chromatogr. Sci. 25: 181-186 (1987)

11. P.C. Tway, J.S. Wood, G.V. Downing, J. Agric. Food Chem., 29: 1059-1063 (1981)

12. R. Chiou, R.J. Stubbs, W.F. Bayne, J. Chromatogr., 416: 196-202 (1987)

13. S.V. Prabhu, T.A. Wehner, P.C. Tway, J. Agric. Food Chem., 39: 1468-1471 (1991)

14. P. De Montigny, J.S.K. Shim, J.V. Pivnichny, J. Pharm. Biomed. Analysis, 8: 507-511 (1990)

15. Council Directive (86/469/EEC) of 16 september 1986 on the determination of residues in animals and in fresh meat. Off. J. of the Eur. Comm. N° L 275/36 of 26.09.1986

Received: July 12, 1993
Accepted: October 5, 1993