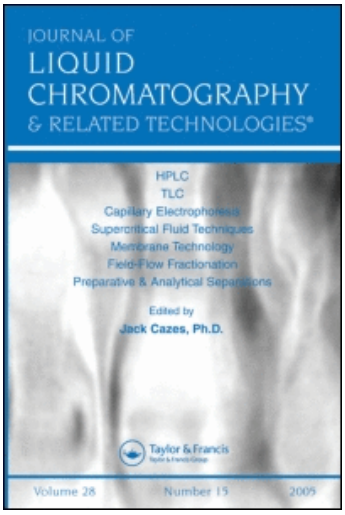

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Determination of Chloramphenicol Residues in Meat Samples by High Performance Liquid Chromatography

I. Caniou[a]; E. Nikolaides[a]; H. Tsoukali[b]; J. A. Stratis[c]; G. A. Zachariadis[c]

[a] Institute of Food Hygiene Ministry of Agriculture Thessaloniki, Greece [b] Laboratory of Forensic Medicine and Toxicology Faculty of Medicine, Aristotole University of Thessaloniki Thessaloniki, Greece [c] Laboratory of Analytical Chemistry Department of Chemistry Aristotole, University of Thessaloniki Thessaloniki, Greece

To cite this Article Caniou, I. , Nikolaides, E. , Tsoukali, H. , Stratis, J. A. and Zachariadis, G. A.(1995) 'Determination of Chloramphenicol Residues in Meat Samples by High Performance Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 18: 17, 3519 — 3527

To link to this Article: DOI: 10.1080/10826079508010467

URL: http://dx.doi.org/10.1080/10826079508010467

JOURNAL OF LIQUID CHROMATOGRAPHY, 18(17), 3519-3527 (1995)

DETERMINATION OF CHLORAMPHENICOL RESIDUES IN MEAT SAMPLES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

I. CANIOU[1], E. NIKOLAIDES[1], H. TSOUKALI[3], J. A. STRATIS[2]*, AND G. A. ZACHARIADIS[2]

[1]*Institute of Food Hygiene*
Ministry of Agriculture
Thessaloniki, Greece
[2]*Laboratory of Analytical Chemistry*
Department of Chemistry
Aristotole University of Thessaloniki
Thessaloniki 54006, Greece
[3]*Laboratory of Forensic Medicine and Toxicology*
Faculty of Medicine
Aristotole University of Thessaloniki
Thessaloniki 54006, Greece

ABSTRACT

The results of a comparative study of chloramphenicol (CAP) determination in meat, by high performance liquid chromatography are presented. A spectrophotometrical detector at 278 nm was used for measurement of CAP absorbance and subsequent quantitative determination. Two different mobile phases, were tested. The first phase consisted of acetonitrile - water (30:70 v/v) and the second of acetonitrile - di-ammonium hydrogen phosphate 0.005M (25:75 v/v). The two procedures were compared by means of the retention time and the sensitivity achieved. Recoveries of CAP from meat samples ranged between 63-79 %. The detection limits for the two procedures were 14.1 $\mu g \cdot kg^{-1}$ and 18.0 $\mu g \cdot kg^{-1}$ respectively.

INTRODUCTION

Chloramphenicol (CAP D(-)threo-2,2-dichloro-N-[2-hydroxy-1-(hydroxymethyl)-2-(4-nitrophenyl) ethyl] acetamide, CAS No. 56-75-7) has a molecular weight of 323.1 and a chemical structure described in figure 1 (the molecular formula $C_{11}H_{12}C_{12}N_2O_5$).

It is a broad-spectrum antibiotic, with clinical applications similar to tetracyclines, sulfonamides, nitrofurans etc. It acts as an inhibitor of aminoacids incorporation in the peptides, because of its greater affinity to bound with microbe ribosomes (1).

During the period of 1950-1980 it was extensively used in animal disease treatment, specially in respiratory or intestinal infections, caused by various microbes such as *Salmonella typhosa, Hemophilus influenzae, Streptococcus pneumoniae, Staphylococcus epidermidis* and *Staphylococcus aureus*. Its use in meat-, milk- or egg-producing animals was banned in USA (1984) and in European Union (1986) (2,3) because of its toxicity to humans. However, it is still one of the therapeutic agents of last resort for some pathogens with resistance to other antimicrobial drugs (4).

Chloramphenicol may be very toxic to humans and should not be used for any purpose that might result in the presence of its residues in food for human consumption. Some of the undesirable effects it causes to humans are blood dyscrasias, erythropenia, thrombocytopenia, aplastic anaemia and in higher doses (>75 mg $\cdot$ kg^{-1} $\cdot$ day^{-1}) the so called "gray syndrome" (1, 5).

Residues of CAP in edible meat products must be lower than 10 $\mu g \cdot kg^{-1}$, according to EC directive 675/92 (6), but in the near future, almost zero tolerance levels must be in force, following the development of more sensitive analytical methods.

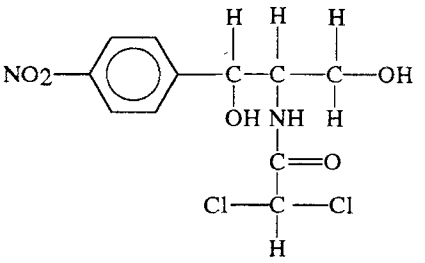

FIGURE 1. Chemical structure of chloramphenicol (CAP)

Because of its special clinical interest, various methods have been developed, based on almost all types of chromatography (GC, LC, TLC), with sufficient recoveries (7-10). The detection limits of these methods are strongly depended on the chromatographic system applied i.e. the mobile and stationary phase, detectors, flow characteristics, etc. The range of detection limits for GC and LC methods is 1-10 $\mu g \cdot kg^{-1}$ (11-15) while for TLC the level of conentrations is higher, 10-200 $\mu g \cdot kg^{-1}$ (16, 17). The sensitivity is significantly improved by the use of electron capture or fluorescence detectors.

In this work, high performance liquid chromatography was applied for the analysis of chloramphenicol in pig and ovine meat. A spectrophotometrical detector was used for measurement of CAP absorbance at λ_{max}=278 nm and subsequent quantitative determination. Two different mobile phases were tested. The first phase (A) consisted of acetonitrile - water (30:70 v/v) while the second (B) of acetonitrile - di-ammonium hydrogen phosphate 0.005M (25:75 v/v). The two procedures were compared by means of the retention time and the sensitivity achieved.

MATERIALS AND METHODS

Reagents and Solvents

Chloramphenicol (Sigma, C-0378) was used without further purification. CAP stock solution was prepared by dissolving 100 mg of CAP in 100 ml methanol and the calibration standard solutions were prepared by diluting the stock solution in double distilled water.

Meat samples (5g) were treated and extracted to ethyl acetate (Merck, p.a.) according to the sample preparation procedures published in the frame of BCR programme, by the Commission of the European Communities [18]. A Moulinette meat homogenizer, a vortex mixer and a table centrifuge (Heraeus) were also used for sample preparation and extraction procedures. Two sequential extractions were performed, and the combined extracts were evaporated to almost dryness in a rotary evaporator. The residues were reconstituted by a mixture of hexane-chloroform (50:50 v/v), and then by water. After stirring and centrifuging, the supernatant liquid was injected to the valve.

HPLC grade acetonitrile (Merck) and ammonium dihydrogen phosphate (Merck, p.a.) were used for the preparation of the eluents. Water was purified by demineralization (conductivity ‹1 μS/cm). These eluents were filtered through 0.22 millipore membrane filters (47 mm diameter) and degassed in ultrasonic bath prior to their use. The two mobile phases prepared as eluents were: (A) acetonitrile - water (30:70 v/v) and (B) acetonitrile - 0.005M di-ammonium hydrogen phosphate (25:75 v/v).

Apparatus and Conditions

The above phases were tested using the following analytical instumentation, respectively:

A) Liquid chromatograph GILSON model 303 (isocratic) combined with a GILSON UV-Vis detector at 278 nm (λ_{max} of CAP).

The analytical column was a Lichrospher RP-18, 250X4 mm, and 5 μm particle size. The eluent flowrate was 1 ml·min^{-1}.

B) Liquid chromatograph JASCO 880-PU (isocratic), combined with a JASCO 870 UV-Vis detector at 278 nm.

The analytical column was also a Lichrospher RP-18, 250X4 mm, and 5 μm particle size. The eluent flowrate was 1 ml·min^{-1}.

RESULTS AND DISCUSSION

Series of typical chromatograms of chloramphenicol, obtained during calibration study with the two mobile phases are given in figures 2 and 3.

CAP is eluated in significantly smaller retention times with mobile phase A (3.8 min) than with mobile phase B (10.5 min), because the latter contains ammonium dihydrogen phosphate and is more polar than the first. The rest of the peaks are due to elution of other substances with similar pK to CAP, but they don't interfere with its determination.

The sensitivities achieved with the above two procedures were compared by means of the calibration curves obtained for a concentration range of CAP 0.5-5 mg·kg^{-1}. The calibration curves obtained for the two procedures are described by the intercepts and

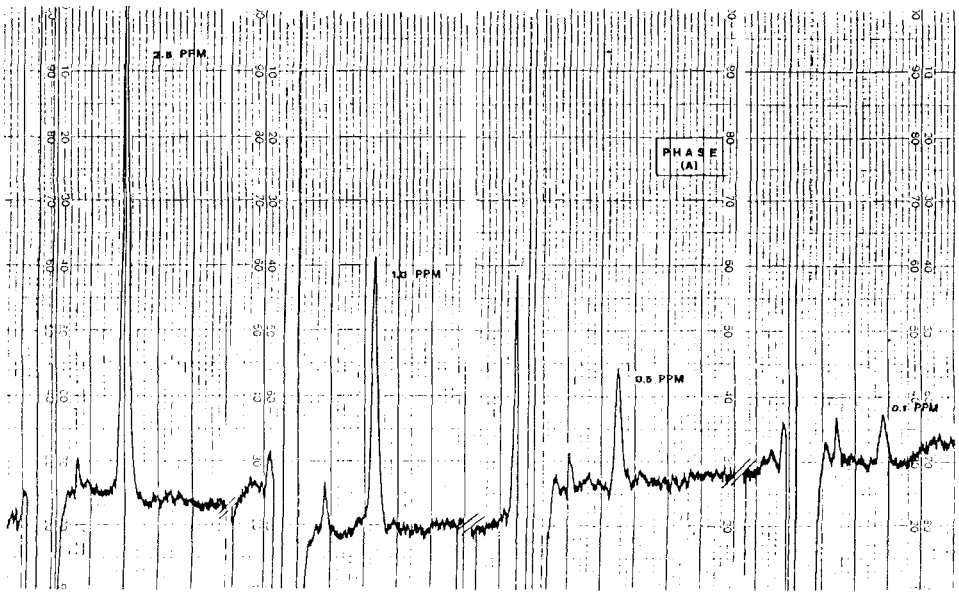

FIGURE 2. Series of typical chromatograms of chloramphenicol, obtained during calibration study with mobile phase (A) acetonitrile - water, (30:70 v/v). Attenuation 2mV, recorder chart speed 1 $cm \cdot min^{-1}$.

slopes, calculated by the regression analysis and given in Table 1. According to these results, better sensitivity (b=8.05) was gained by mobile phase A, than by method B (b=5.52).

This, together with the fact that the retention time for the elution with mobile phase A (acetonitrile-water) is significantly smaller, leads to the conclusion that this is the preferred procedure for a rapid and sensitive determination.

The reproducibilities of the two methods are comparable, and they are expressed by a relative standard deviation of 17%, for six succesive determinations of a standard solution at the 1.0 mg l^{-1} concentration level. The detection limits of the two methods

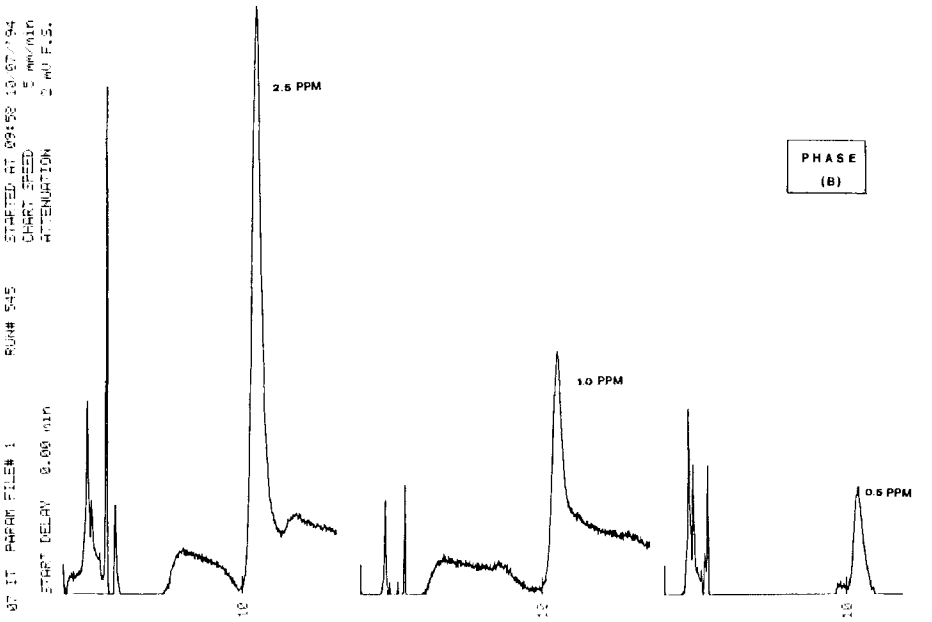

FIGURE 3. Series of typical chromatograms of chloramphenicol, obtained during calibration study with mobile phase (B) acetonitrile - di-ammonium hydrogen phosphate 0.005 M, (25:75 v/v).
Attenuation 2mV, recorder chart speed 0.5 cm·min^{-1}.

TABLE 1.

Regression Analysis Data for CAP Determination with Two Different Mobile Phases.

Mobile phase	Slope b	Inter-cept a	Estimation error	Correlation coefficient r	Squared coefficient r^2
A	8.05	+0.05	0.71	0.98	0.96
B	5.52	-0.21	1.36	0.96	0.93

TABLE 2.

Recovery Study of Chloramphenicol from Fortified Meat Samples.

Sample	CAP added (ng)	CAP found (ng)	Recovery (%)
1	300	235	78.3
2	300	230	76.6
3	200	140	70.0
4	200	135	67.5
5	150	100	66.6
6	150	95	63.3

calculated as the mean of six determinations of control samples with negligible CAP concentration ± three times the standard deviation of the six results, are 14.1 $\mu g \cdot kg^{-1}$ and 18.0 $\mu g \cdot kg^{-1}$ respectively.

In Table 2, the results of the recovery study from fortified meat samples are listed. Recoveries between 63-79 were calculated using mobile phase A. The corresponding recoveries for method B are slightly higher than these and could be further improved by increasing the ratio of acetonitrile in the eluent mixture.

REFERENCES

1. H.M. Feder, Jr, C. Osier and E.G. Maderazo, Rev. Infect. Dis., **3:** 479-491 (1981)

2. Food and Drug Administration clamps down on chloramphenicol use in food animals, J. Am. Vet. Med. Assoc., 184: 1041 (1984)

3. Comite des Medicaments Veterinaires, Groupe de travail pour "Securite des residus", 2e reunion, Bruxelles, (1986)

4. A.G. Grilman, L.S. Goodman and A. Grilman, Goodman and Gilman: The Pharmacological Basis of Therapeutics, 6th Ed. MacMillan Publishing Co. Inc., New York, NY, (1980)

5. H.C. Meissner and A.L. Smith, Pediatrics, 64: 348-356 (1979)

6 . Commission of the European Communities, 675/92

7. E.H. Allen, J. Assoc. Off. Anal. Chem., 68: 990-999 (1985)

8. J.M. Degroodt, B. Wyhowski de Bukanski, J. De Groof, H. Beernaert and S. Spebrnik, J. Liq. Chrom., 15: 2355-2371 (1992)

9. V.M. Moretti, C. van de Water and N. Haagsma, J. Chrom., 583: 77-82 (1992)

10. C. van de Water, D.Tebbal and N. Haagsma, J. Chrom., 478: 205-215 (1989)

11. G. Samouris, Ph.D.Thesis, Aristotle University, Thessaloniki (1990).

12. R. Malisch, U. Sandmeyer and K. Kypke-Hutter, Lebensmittelchem. Gerichl. Chem., 38: 11-12 (1984)

13. H. Holtmannspotter, H.P. Thier, Deutsch. Lebensm. Rundsch., 78: 347-350 (1982)

14. M. Becheiraz, A. Haldemann and R. Etter, Mitt. Geb. Lebensmittelunters Hyg., 74: 147-155 (1983)

15. M. Petz, Z. Lebensm. Unters Forsch., 176: 289-293 (1983)

16. I. Karkocha, Chem. Abstr., 89: 127809p (1978)

17. I. Karkocha, Chem. Abstr., 91: 37562q (1979)

18. Commission of the European Communities, Agriculture, Veterinary Drug Residues, Residues in Food-Producing Animals and their products: Reference Materials and Methods, Edited by R.J. Heitzman, Brussels pp. 153-172 (1992)

Received: April 21, 1995
Accepted: May 19, 1995