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Journal of Chromatography B, 668 (1995) 53–58

JOURNAL OF
CHROMATOGRAPHY B:
BIOMEDICAL APPLICATIONS

Determination of chloramphenicol in animal tissue using high-performance liquid chromatography with a column-switching system and ultraviolet detection

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First received 29 August 1994; revised manuscript received 25 January 1995; accepted 6 February 1995

Abstract

A rapid and universally applicable method for determination of chloramphenicol (CAP) residues in animal tissues using high-performance liquid chromatography with a column-switching system is presented. The clean-up procedure as well as the chromatographic conditions and detection are described. The linearity and repeatability of the data obtained by this method as well as the recovery rates of CAP in several farm animals are presented.

1. Introduction

Chloramphenicol (CAP), D-*threo*-1-*p*-nitrophenyl-2-dichloracetylamino-1,3-propanediol, is the first nitro compound that was found in nature [1]. CAP is a bacteriostatic with a broad spectrum of activity, frequently used for therapeutic and prophylactic purposes in veterinary medicine. Within the EU the interim tolerance level for CAP residues in human food has been fixed at 10.0 μg/kg [2]. In agriculture CAP is used in fattening of pig, poultry and fish. CAP is applied as aqueous solution which is injected in muscles or it is added to feed in the form of CAP-palmitate [3].

Published methods are using a combination of HPLC and GC [4], GC [5,6] or GC–MS [7] for the determination of CAP in milk, eggs, and meat. The clean-up procedures described in these methods are rather complicated and vary for the different tissues. However, there is no established HPLC method that offers the possibility for detection of CAP residues using the same rapid and simple sample procedure for all relevant tissues.

2. Experimental

2.1. Apparatus

The following apparatus were used: drying oven (Heraeus), Ultra-Turrax T25 homogenisator (Heraeus Sepatech), Labofuge A centrifuge (Heraeus Sepatech), RE-111 rotary evaporator (Büchi), LC-6A HPLC pump (Shimadzu), Model 7125 injection valve (Rheodyne), Model 7000 switching valves (Rheodyne),

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0378-4347/95/$09.50
SSDI 0378-4347(95)00072-0

SPD-10 UV detector (Shimadzu) and HP-3395 integrator (Hewlett-Packard).

2.2. Materials

A Model 51910 Sep-Pak C_{18} cartridge (Waters-Millipore), Model 51900 Sep-Pak silica cartridge (Waters-Millipore), LC-HISEP, 5 μm, 150 × 4.6 mm I.D. (Supelco), Supelcosil LC-18, 5 μm, 150 × 4.6 mm I.D. (Supelco), and Supelcosil LC-18, 5 μm, 250 × 4.6 mm I.D. (Supelco) were used.

2.3. Chemicals

Chloramphenicol, purity >99% (No. 220551, Calbiochem-Novobiochem, Bad Soden, Germany), sea sand, cleaned and treated with acid (No. 7712, E. Merck, Darmstadt, Germany) were used.

All solvents were used in pro analysi quality; water was used in double distilled quality.

A 5.00-mg amount of CAP was dissolved in 100.0 ml of water–methanol (50:50, v/v). This was used as the standard working solution.

2.4. Sample preparation

The applicability of the method was tested by analysing different tissues of pig (liver, kidney, muscle, fat with skin), turkey (liver, kidney, muscle, fat, skin) and trout (muscle, skin), each representing an important animal species for human nutrition. These tissues were cut in small pieces and homogenized with an Ultra-turrax after adding a defined amount of standard solution. For each analysis 10.0 g tissue was mixed with 5 g sand and dried for 5 h at 60°C. The dried sample was mixed with 20.0 ml acetonitrile, homogenized with an Ultra-turrax for 3 min and centrifuged for 5 min at 2980 g. At last the clear acetonitrile solution was decanted. This procedure was repeated, and the combined acetonitrile fractions were carefully evaporated to dryness. The residue was dissolved in 1 ml acetonitrile and transferred to a 5-ml syringe connected to a C_{18} cartridge. The tube was rinsed two times with 1-ml portions of the same solvent (the washings were added separately to the syringe). CAP was eluted with 4.0 ml acetonitrile, and the eluate was carefully evaporated to dryness. The whole procedure was repeated with a silica cartridge, using ethylacetate as solvent instead of acetonitrile for CAP elution.

The ethylacetate eluate was carefully rotary-evaporated to dryness and 0.5 ml *n*-hexane and 0.5 ml of the eluent were added to the residue. An aliquot (50 μl) of the lower aqueous phase was directly injected onto the HPLC system equipped with a column-switching system.

2.5. HPLC with column-switching system

The purification of the extracts by SPE with C_{18} and silica cartridges was not sufficient. Therefore, to avoid interferences in the chromatograms an HPLC with a column-switching system was applied.

A HISEP column (column A: LC-HISEP, 5 μm, 150 × 4.6 mm I.D.) and two reversed-phase columns (analytical column C: Supelcosil LC-18, 5 μm, 250 × 4.6 mm I.D., and trap column B: Supelcosil LC-18, 5 μm, 150 × 4.6 mm I.D.) were combined with two switching valves, named 1 and 2, both with two possible positions, named A and B (see Fig. 1). During the whole analysis pumps A and B deliver the same eluent (acetonitrile–water–tetrahydrofurane; 80:18:2, v/v) using a flow of 0.7 ml/min (pump A) and 0.9 ml/min (pump B).

After injection of 50 μl of the extract (see section 2.4) the analysis starts with the valves in position I (valve 1 = A, valve 2 = B). In this position the sample is pre-separated at column A, while columns B and C are rinsed by pump B. The detector is connected to column A.

Just before CAP is leaving column A the valves are switched to position II (valve 1 = B, valve 2 = B). CAP eluting from column A is fixed on column B.

After the complete amount of CAP has been trapped on column B the valves are switched to position III (valve 1 = A, valve 2 = A). CAP is eluted from column B to column C and detected at the UV detector (wavelength 278 nm) which is now connected to the analytical column C.

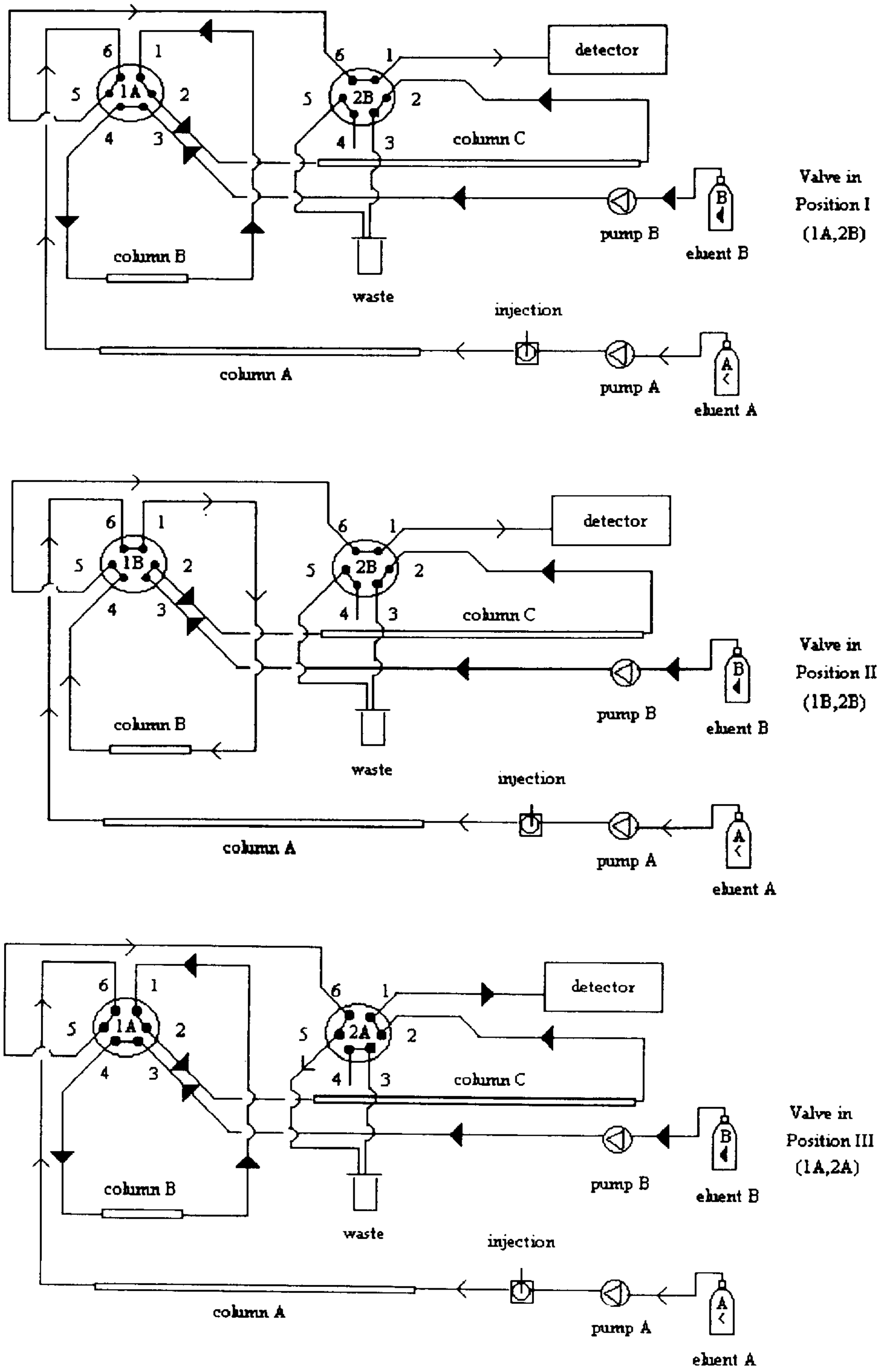

Fig. 1. Column-switching system for the HPLC determination of CAP residues in animal tissues.

Column A is rinsed by pump A in valve position III.

The HISEP column is an HPLC phase especially developed for removing proteins and other compounds with high molecular mass from biological samples.

The hydrophobic surface of the HISEP phase is protected by a hydrophilic polymer. Therefore, only smaller molecules can reach the hydrophobic phase of the column.

Other materials, such as proteins, are eluted near the eluent front. Substances with lower molecular mass, e.g. CAP, are effectively retained [8].

3. Results

3.1. Linearity and repeatability of the method

Solutions (50 μl) containing different concentrations of CAP were directly injected onto the HPLC system to check the linearity and repeatability of the HPLC with column-switching system.

Each concentration of CAP was analysed six times. Fig. 2 shows a typical chromatogram obtained of a CAP standard solution using the conditions described above. The peak heights were used for evaluation. The linearity and repeatability of the data determined in this analysis are shown in Table 1.

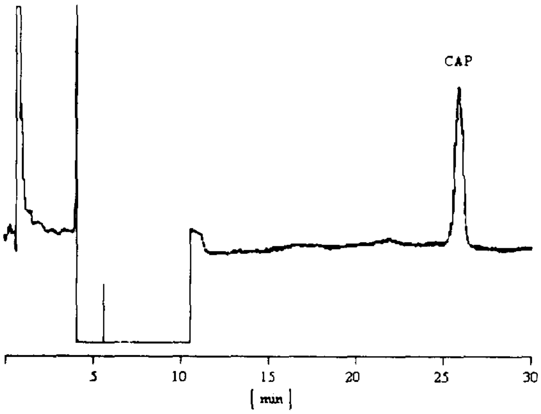

Fig. 2. Determination of CAP by HPLC with a column-switching system (20.0 ng CAP) (injection 50 μl CAP standard solution, containing 0.4 μg/ml).

Table 1
Linearity and repeatability ($n = 6$) of the data obtained by the HPLC with column switching

Amount of CAP injected [a] (ng)	Peak height (mm)			
	Mean	Median	Variance	S.D.
1.0	3.17	3.0	0.06	0.24
2.0	7.5	7.5	0.0	0.0
6.0	20.5	20.75	0.33	0.58
8.0	27.96	28.0	0.03	0.19
10.0	35.0	35.0	0.0	0.0
12.0	41.42	41.5	0.2	0.45
14.0	47.17	47.0	0.06	0.21
20.0	63.17	63.0	0.22	0.17

Resulting linear regression line: $y = 3.20x + 1.54$; standard deviation: 2.28; correlation coefficient: 0.998.

[a] Injected in 50 μl standard solution.

3.2. Recovery of CAP in different tissues of pig, turkey and trout

Prior to the analysis of the different animal tissues, samples of definitely CAP-free materials were checked (blank) for several times using the extraction, clean-up procedure and chromatographic conditions described above. Samples of these untreated materials were fortified with CAP in the range 2.0–20.0 μg/kg and analysed in a recovery study [9].

The different tissues were homogenized, extracted and centrifuged as described above followed by clean-up with C_{18} and silica cartridges. The purified extracts were injected onto the HPLC with column-switching system.

During the validation 1400 samples were analysed over a period of 12 weeks. The sample extraction, sample clean-up and the measurement for each tissue and each fortified concentration were repeated at least six times, in some cases ten or twenty times [9].

Examples of the results obtained are shown in Tables 2–4. The standard deviation and the coefficients of variation for different fortified tissues are shown in Table 5. Typical chromatograms are presented in Fig. 3.

Table 2
Recovery rates of CAP in different tissues of pigs

CAP concentration (μg/kg)	Recovery (%)			
	Liver	Kidney	Muscle	Fat/skin
2	74	101	105	97
6	96	104	113	82
8	90	98	96	99
10	91	90	98	93
12	92	93	91	95
14	89	96	88	93
20	99	99	90	94

Table 3
Recovery rates of CAP in different tissues of poultry

CAP concentration (μg/kg)	Recovery (%)				
	Liver	Kidney	Muscle	Fat	Skin
8	97	86	88	101	99
10	98	99	98	97	93
12	102	93	90	88	95
14	103	99	95	82	99

Table 4
Recovery rates of CAP in different tissues of trout

CAP concentration (μg/kg)	Recovery (%)	
	Muscle	Skin
8	100	90
10	106	99
12	107	101
14	104	96

Interferences in the chromatograms near the retention time of CAP were not observed in any of the matrices. Quantitation was performed by the external standard calibration technique whereby the peak heights obtained by injection of dilutions of the calibration solution were plotted vs. ng CAP.

4. Discussion

The application of HPLC with a column-switching system permits a significant simplification of the clean-up procedure. Furthermore, loss of CAP can be minimized.

The linearity and reproducibility of the data obtained by this method are satisfactory (Table 1, Fig. 2). The method is suitable for all mentioned tissues of pig, turkey and trout (Tables 2–4, Fig. 3), and the recovery rates for these matrices are sufficient (Table 5).

Thus, a rapid and selective HPLC method for the determination of CAP in all relevant animal tissues used for human nutrition is available [9].

By using a computer with an HPLC-manager and a laboratory data system it would be possible to work with electric switching valves to run the method automatically.

Acknowledgement

We thank the Union of the Pharmaceutical Industry of Germany (VDPI) and the Supelco-

Table 5
Standard deviation and variation coefficient for the recovery of CAP in different tissues for 10.0 μg/kg (6 measurements of each tissue)

Tissue	Pig		Turkey		Trout	
	S.D. (%)	C.V. (%)	S.D. (%)	C.V. (%)	S.D. (%)	C.V. (%)
Liver	3.48	12.14	3.09	9.56	–[a]	–[a]
Kidney	2.14	4.58	2.14	4.58	–[a]	–[a]
Muscle	2.0	4.0	2.52	9.4	2.19	4.81
Fat/skin	1.95	3.81	1.37	1.89	1.11	1.22

[a] Not analysed.

Sigma-Aldrich Chemical Company for their support.

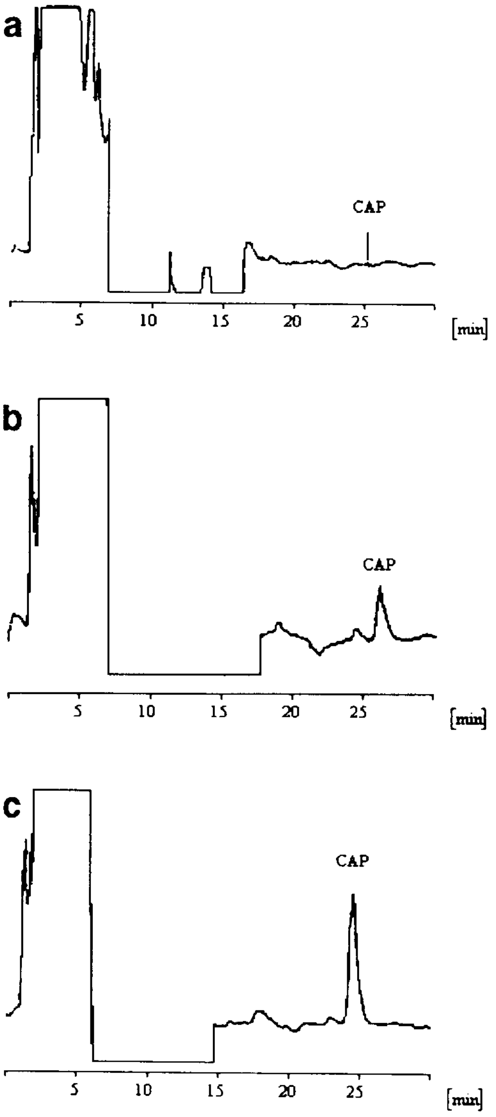

Fig. 3. Determination of CAP in pig liver by HPLC with a column-switching system: (a) without CAP contamination; (b) fortified with 6.0 μg CAP/kg; (c) fortified with 20.0 μg CAP/kg.

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