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Review

Chromatographic analysis of tetracycline antibiotics in foods

Hisao Oka*, Yuko Ito, Hiroshi Matsumoto

Aichi Prefectural Institute of Public Health, Tsuji-machi, Kita-ku, Nagoya 462-8576, Japan

Abstract

Tetracycline antibiotics (TCs), such as oxytetracycline, tetracycline, chlortetracycline, and doxycycline, have for decades continued to play an important role in veterinary medicine and feed additives because of the broad spectrum antibiotics and their economical advantages. Many analysis methods of TCs, therefore, have been reported to monitor their residues in foods. We review the recent developments in chromatographic analysis methods for TCs in foods. This review involves the following techniques: thin layer chromatography, capillary electrophoresis, high-performance liquid chromatography, and sample preparation including extraction and clean up procedures.

Keywords: Reviews; Food analysis; Tetracyclines; Antibiotics

Contents

1. Introduction

Tetracycline antibiotics (TCs, Fig. 1) produced by *Streptomyces* are broad spectrum antibiotics ranging from gram-positive to negative bacterias, and are especially effective against *Staphylococcus, Streptococcus, Pneumococcus, Gonococcus, Cholera, Dysentery bacillis, Pertussis, Rickettsia, Chlamydia,* and *Mycoplasma*. TCs are actively transported into the cells of susceptible bacteria and exert a bacteriostatic effect by inhibiting protein biosynthesis after binding to the 30S ribosomal subparticle.

Since the first member of the tetracycline family, chlortetracycline (CTC) was discovered in 1948 [1], eight TCs are now commercially available (Fig. 1), of which oxytetracycline (OTC), tetracycline (TC),

*Corresponding author. Tel.: +81-52-911-3111; fax: +81-52-913-3641.

E-mail address: hisaooka@alles.or.jp (H. Oka)

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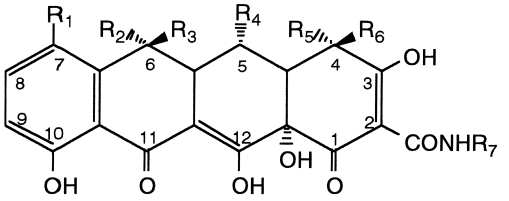

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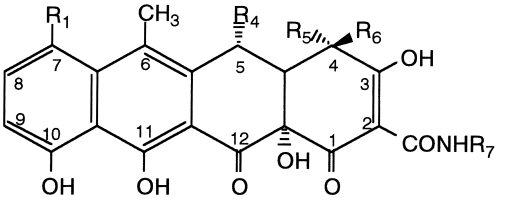

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Compound	Structure	R_1	R_2	R_3	R_4	R_5	R_6	R_7
Tetracycline (TC)	A	H	CH_3	OH	H	$N(CH_3)_2$	H	H
Oxytetracycline (OTC)	A	H	CH_3	OH	OH	$N(CH_3)_2$	H	H
Chlortetracycline (CTC)	A	Cl	CH_3	OH	H	$N(CH_3)_2$	H	H
Doxycycline (DC)	A	H	CH_3	H	OH	$N(CH_3)_2$	H	H
Minocycline (MINO)	A	$N(CH_3)_2$	H	H	H	$N(CH_3)_2$	H	H
Methacycline (MTC)	A	H	$=CH_2$		OH	$N(CH_3)_2$	H	H
Demeclocycline (DMCTC)	A	Cl	H	OH	H	$N(CH_3)_2$	H	H
Rolitetracycline (PRMTC)	A	H	CH_3	OH	H	$N(CH_3)_2$	H	[a]
4-Epioxytetracycline (EOTC)	A	H	CH_3	OH	OH	H	$N(CH_3)_2$	H
Anhydrooxytetracycline (AOTC)	B	H	-	-	OH	$N(CH_3)_2$	H	H
4-Epitetracycline (ETC)	A	H	CH_3	OH	H	H	$N(CH_3)_2$	H
Anhydrotetracycline (ATC)	B	H	-	-	H	$N(CH_3)_2$	H	H
4-Epianhydrotetracycline (EATC)	B	H	-	-	H	H	$N(CH_3)_2$	H
4-Epichlortetracycline (ECTC)	A	Cl	CH_3	OH	H	H	$N(CH_3)_2$	H
4-Epidoxycycline (EDC)	A	H	CH_3	H	OH	H	$N(CH_3)_2$	H

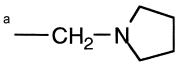
[a] 

Fig. 1. Structures of tetracycline antibiotics and their related substances.

CTC, and doxycycline (DC) are commonly applied to food-producing animals including honeybees as veterinary medicines because of the broad spectrum antibiotics and their economic advantages. However, it is a distinct possibility that other TCs may also be used for animals for the following reasons: minocycline (MINO) has the highest biological activity among the TCs and is active against tetracycline-resistant pathogens, methacycline (MTC) and demeclocycline (DMCTC) are more stable than tetracycline and give higher and more sustained blood level with smaller doses at less frequent intervals, and rolitetracycline (PRMTC) is readily soluble in water so that it is more suitable than other TCs for injection.

Maximum residue limits (MRLs) in the EU have been set for all substances belonging to the tetracycline group in various tissues, including 0.6 ppm in kidney, 0.3 ppm liver, 0.2 ppm eggs, 0.1 ppm muscle, and 0.1 ppm milk, which are expressed as the sum of parent drug and 4-epimer in the cases of OTC, TC, and CTC. Tolerances in USA are established for the sum of tetracycline antibiotic residues in animal tissues: 2 ppm in muscle, 6 ppm in liver, 12 ppm in fat and kidney.

The widespread utilization of TCs leads to an increasing resistance factor, so accurate monitoring by public health agencies is required. Microbiological assays are most commonly used for the measurement of TCs in foods, but they are time consuming, cannot identify certain TCs, and their precision appears to be variable. Therefore, a precise chro-

matographic analysis method for the TCs has been required.

Pharmaceutical preparations of TCs contain small amounts of impurities or degradation products, namely 4-epioxytetracycline (EOTC), anhydrooxytetracycline (AOTC), and α- and β-apooxytetracycline (apo-OTC) for OTC, 4-epitetracycline (ETC), anhydrotetracycline (ATC), 4-epianhydrotetracycline (EATC), and CTC for TC, 4-epichlortetracycline (ECTC), isochlortetracycline (ICTC), and 4-epiisochlortetracycline (EICTC) for CTC, and 4-epidoxycycline (EDC) for DC (Figs. 1 and 2). These degradation products may occur by undergoing dehydrogenation or epimerization under storage conditions, because of the poor stability of the TCs as described below. Therefore, a precise chromatographic analysis method for the impurities and degradation products has also been required. TCs have similar chemical and physicochemical properties. They are amphoteric compounds with characteristic pH values and form crystalline hydrates and salts with acids and bases [2]. Their UV spectra show strong absorptions around 270 and 360 nm in neutral and acidic solutions [3]. TCs are soluble in acids, bases, alcohols, and polar organic solvents and are extractable with several organic solvents such as *n*-butanol and ethyl acetate. Stability of the TCs is poor under strong acidic and alkaline conditions and form reversible epimers, 4-epi-TCs, anhydro-TCs, and iso-TCs under weakly acidic (pH 3) and strongly acidic (below pH 2), alkaline conditions, respectively [4]. TCs produce strong fluorescence with metal ions or under basic conditions [5–7]. They form chelate complexes with metal ions at β-diketones (C_{10}–C_{12}) and carboxyamide (C_2) [8–10] and bind with proteins and silanol groups [11,12] in the stationary phase.

Based on these physicochemical properties, many chromatographic analysis methods of TCs in foods have been reported over the past 20 years or more. In this paper, we review the recent developments in the chromatographic analysis methods of TCs in foods.

2. Thin-layer chromatography

2.1. Separation

As shown in Table 1 a number of workers have reported TLC methods to separate TCs using adsorbent layers of kieselguhr [13–20], silica gel [21–28], and cellulose [29–32]. In general, TLC is simple and does not require special equipment, however, most of the published methods need excessive time for the preparation of the TLC plate to avoid binding of the TCs with trace metals in the adsorbents used. In order to control the above undesirable properties, EDTA has been added to the adsorbent when the TLC plate is prepared and solvent systems containing EDTA have been used [13,15–18,20–22,32].

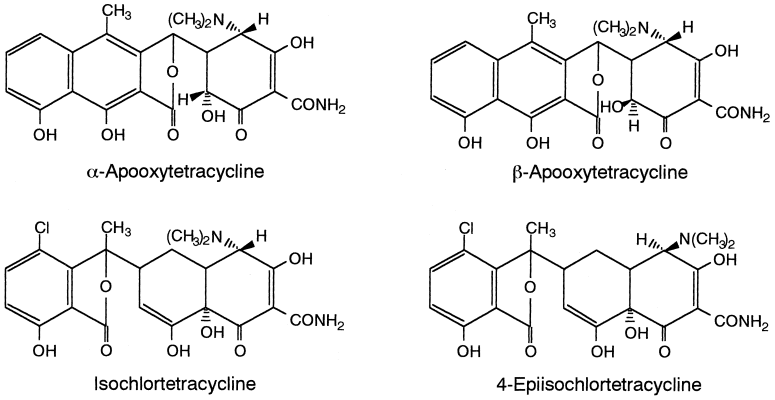


Fig. 2. Structures of apooxytetracyclines and isochlortetracyclines.

Table 1
Summary of normal-phase TLC conditions for the analysis of TCs[a]

Tetracyclines	Sample	Plate	Solvent system	Extraction and clean up	Detection	Detection limit	Ref.
OTC, TC, and CTC	–	Silica gel containing EDTA	*n*-BuOH sat. with water	Dissolution in MeOH	Spray ($FeCl_3$)	–	[21]
TC, ATC, and EATC	Tablet	Cellulose	0.1 *M* EDTA–0.1% NH_4Cl	–	FL 428 nm	–	[29]
			$CHCl_3$ sat. with EDTA–NH_4Cl				
TC, CTC, ETC, ATC, and EATC	–	Diatomaceous earth containing EDTA, glycerol, and PEG 400	Ethyl acetate sat. with EDTA	–	FL 370 nm	–	[13]
OTC, TC, and their impurities	–	Silica gel containing EDTA	*n*-BuOH–EtOH–water (4:7:4)	–	FL 370 nm	–	[22]
TC, CTC, ETC, ATC, and EATC	–	Kieselguhr containing EDTA	Me_2CO–water (10:1)	–	FL 370 nm	–	[14]
			Me_2CO–ethyl acetate–water (20:10:3)				
OTC, TC, CTC, ETC, ATC, and EATC	–	Kieselguhr containing EDTA, glycerol, and PEG 400	MEK sat. Macllvaine buffer	–	Spray (Fast Blue B)	–	[15]
TC, CTC, ETC, ATC, and EATC	–	Kieselguhr containing EDTA and glycerol	CH_2Cl_2–EtOH (9:1)	–	FL 370 nm	–	[16]
TC, CTC, ETC, ATC, and EATC	Capsule and tablet	Kieselguhr containing EDTA	Me_2CO–ethyl acetate–water (80:40:12)	Dissolution in MeOH	Spray (Fast Blue B)	1 μg/spot	[17]
OTC, TC, and CTC	Animal tissues	Kieselguhr containing EDTA, glycerol, and PEG 400	Ethyl acetate–*n*-BuOH (9:1) containing EDTA	Extraction with 0.1 N HCl and XAD-2 clean up	FL 370 nm	0.025–0.1 ppm	[18]
		Cellulose containing EDTA and NH_4Cl	*n*-BuOH sat. with water				
OTC, TC, and CTC	Milk	Precoated kieselguhr impregnated with phosphate–citric acid buffer containing glycerol	$CHCl_3$–Me_2CO–(phosphate–citric acid buffer) (5:5:2)	Extraction with Me_2CO	Bioautography	0.3 ppm	[19]
OTC, TC, CTC, DC, DMCTC, MTC, MINO, and ATC	–	Cellulose	Aq. $MgCl_2$, aq. $CaCl_2$, aq, $BaCl_2$, and aq. $ZnCl_2$	–	FL 370 nm	0.25 μg/spot	[30]
OTC, MTC, and DC	–	Cellulose containing Macllvaine buffer	Ethyl acetate sat. with water	–	Spray ($MgCl_2$) FL 370 nm	–	[31]
TC, CTC, ETC, ATC, and EATC	Capsule and powder	Cellulose containing EDTA	$CHCl_3$ sat. with EDTA	Dissolution in MeOH	FL 370 nm	–	[32]
TC	–	Precoated silica gel sprayed with EDTA	Me_2CO–ethyl acetate–water (80:35:15)	–	FL 370 nm	–	[23]

TC	Capsule	Kieselguhr containing EDTA	Me_2CO–ethyl acetate–water (20:10:3)	Dissolution in MeOH	Densitometry (366 nm)		[20]
OTC, TC, CTC, DC, ETC, ATC, and EATC	–	Precoated silica gel HPTLC predeveloped with EDTA	$CHCl_3$–MeOH–EDTA (65:20:5) iso-PrOH–ethyl acetate–EDTA (3:4:7) Me_2CO–EDTA (10:1)	–	Densitometry (360 and 450 nm)	0.1 μg/spot	[33]
OTC, TC, and CTC	Fish tissues	Precoated silica gel HPTLC	$CHCl_3$–MeOH–EDTA (65:20:5)	Extraction with EDTA–Macllvaine buffer C_{18} cartridge pretreated with EDTA clean up	Densitometry (360 nm)	0.1 ppm	[146]
TC, CTC, ETC, ATC, and EATC	Capsule, syrup, powder, and tablet	Precoated silica gel HPTLC predeveloped with EDTA	$CHCl_3$–MeOH–EDTA (65:20:5)	Dissolution in MeOH	Densitometry (360 and 450 nm) Spray (Fast Violet B)	0.1 μg/spot 0.1 μg/spot	[34]
OTC, TC, CTC, DC, MINO, MTC, DMCTC, PRMTC, ETC, ATC, and EATC	–	Precoated silica gel HPTLC predeveloped with EDTA	$CHCl_3$–MeOH–Me_2CO–EDTA (5:11:25:9)	–	Densitometry (360 and 450 nm) Spray (Fast Violet B)	0.1 μg/spot 0.1 μg/spot	[24]
OTC, TC, CTC, DC, MINO, MTC, and DMCTC	Honey	Precoated silica gel HPTLC predeveloped with EDTA	$CHCl_3$–MeOH–EDTA (65:20:5)	C_{18} cartridge pretreated with EDTA and COOH cartridge clean up	Spray ($MgCl_2$) FL 370 nm	0.1 ppm	[25]
OTC, TC, CTC, and DC	Animal tissues	Precoated silica gel HPTLC predeveloped with EDTA	$CHCl_3$–MeOH–EDTA (65:20:5)	Extraction with EDTA–Macllvaine buffer C_{18} cartridge pretreated with EDTA clean up	Densitomery (360 nm) Spray (Fast Blue BB and Pyridine)	0.1 ppm	[26]
OTC, DC, and MINO	–	Precoated silica gel TLC sprayed with EDTA	CH_2Cl_2–MeOH–water (59:35:6)	–	Densitometry (280 nm)	–	[27]
CTC and DMCTC	–	Precoated silica gel TLC sprayed with EDTA	CH_2Cl_2–MeOH–water (59:35:6)	–	Densitometry (280 nm)	–	[28]
OTC, TC, CTC, DC, DMCTC, and MTC	–	Precoated silica gel TLC spray with EDTA (pH 8)	CH_2Cl_2–MeOH–water (59:35:6)	–	FL 365 nm	1 ng/spot	[151]
OTC, TC, and DC	Honey, serum, and urine	Precoated silica gel TLC impregnated with EDTA (pH 9)	$CHCl_3$–MeOH–1% NH_4OH (65:20:5)	–	FL-Densitometry (365/445 nm)	0.2 ng/spot	[35]

[a] ATC: anhydrotetracycline, EATC: 4-epianhydrotetracycline, ETC: 4-epitetracycline, MEK: methyl ethyl ketone, MINO: minocycline, MTC: methacycline, DMCTC: demeclocycline, PRMTC: rolitetracycline, EDTA: disodium ethylenediaminetetraacetate, and FL: fluorescence.

For precoated TLC plates, after spraying with EDTA and activation, it has been used [23,27,28]. These spraying techniques have been applied to precoated silica gel TLC plates, however, poor resolutions among the TCs, especially between the impurities (ETC, ATC, EATC, and CTC) of TC have been obtained.

Therefore, an improved method was designed [24–26,33,34]: the plate is predeveloped with a saturated Na_2EDTA aqueous solution and is activated before applying the sample. Using this predeveloping technique, Oka et al. have reported the successful separation of eight TCs on a silica gel high-performance TLC plate [24] with a solvent system of chloroform–methanol–5% Na_2EDTA (65:20:5, lower phase) and applied this TLC technique to the analysis of the eight residual TCs in honey. Detection limits of 0.1 ppm were accomplished by observing their fluorescence under a UV lamp (370 nm) after spraying with magnesium chloride [25].

Xie et al. have presented a TLC–fluorescence scanning densitometry method for the determination of OTC, TC, and DC in honey, serum, and urine [35]. Using the predeveloped silica gel TLC plate with 0.27 *M* Na_2EDTA and a solvent system of chloroform–methanol–acetone–1% aqueous ammonia (10:22:50:18), the TCs from the sample clearly separated. The fluorescent spots were scanned with a densitometer (λ_{ex}: 365 nm, λ_{em}: 440 nm).

In reversed-phase (RP)-TLC, as summarized in Table 2, all papers reported solvent systems containing oxalic acid to control the undesirable properties of the TCs. The TC spots show extreme tailing on RP-TLC plates when an aqueous solution of methanol, acetonitrile, and ethanol are used as the solvent systems. In several RP-HPLC systems, the use of a mobile phase containing phosphoric [36,37], citric [38,39], tartaric [39], EDTA [40,41], and malonic [39] acids gave good results for the separation of the TCs. Although these acids have been applied to RP-TLC, all TCs showed tailing spots. When TCs are purified from a culture broth, oxalate is added to prevent the formation of a chelate complex of the TCs with metal ions [42]. Therefore, use of a mixture of methanol–0.5 *M* aqueous oxalic acid solution (pH 2.0) was tried as a solvent system on a C_{18} TLC plate. Unsatisfactory separations among the TCs were obtained, but it showed no tailing spots. It was considered that aqueous oxalic acid solution is essential in the solvent system to obtain no tailing spots of the TCs [43].

In order to compare the suitability of C_8 and C_{18} TLC plates for the separation of TCs using a methanol–0.5 *M* aqueous oxalic acid solution (pH 2.0) (1:1) as the solvent system, the TCs were separated on both plates. Although poor resolution was obtained for the separation of the impurities of TC using the C_8 TLC plates, good resolution was obtained for the parent TCs. On the other hand, good resolution was obtained for the impurities using the C_{18} TLC plates, but it was unsatisfactory for the parent TCs. C_8 and C_{18} plates are suitable for the separation of the impurities and the parent TCs, respectively [43].

The separation of the eight TCs on a C_8 TLC plate with a solvent system of methanol–acetonitrile–0.5 *M* oxalic acid (pH 2.0) (1:1:4) has been reported [24]. By the combined use of these TLC techniques with a C_{18} cartridge clean up, the analyses of the residual TCs in animal tissues and honey have been successfully carried out [25,26]. Detection limits of the TCs were 0.1 ppm.

2.2. Detection

For the detection of TCs on a TLC plate, magnesium chloride [25,31], ferric chloride [44], antimony pentachloride [44], sulfuric acid [44], diazotized *p*-nitroaniline [15], modified Sakaguchi reagent [15], diphenylpicrylhydrazyl reagent [15], and diazonium salts [15,17,24,26,34] as detection reagents have been reported. Among them, the observation of the produced fluorescence under a UV lamp (370 nm) after the spraying of magnesium chloride [25] and the observation of the color produced after spraying diazonium salts [24,45] show high sensitivity for the detection of TCs on NP- and RP-TLC plates.

In order to confirm the TCs on a TLC plate, TLC–fast atom bombardment (FAB)-MS has been introduced to the residual analysis of TCs in foods [46–48]. In TLC–FAB-MS, the developed and air-dried TLC plate is inserted into the TLC–FAB-MS ion source, the FAB mass spectrum of the desired spot on the plate is directly measured, and the plate

Table 2
Summary of reversed-phase TLC conditions for the analysis of TCs[a]

Tetracyclines	Sample	Plate	Solvent system	Extraction and clean up	Detection	Detection limit	Ref.
TC, CTC, ETC, ATC, and EATC	Capsule, syrup, powder, and tablet	Precoated C_8-TLC	MeOH–MeCN–0.5 *M* oxalic acid (pH 2.0, 1:1:2)	Dissolution in MeOH	Densitometry (360 and 450 nm)	0.1 μg/spot	[34]
					Spray (Fast Violet B)	0.1 μg/spot	
OTC, TC, CTC, DC, MINO, MTC, DMCTC, PRMTC, ETC, ATC, and EATC	–	Precoated C_8-TLC	MeOH–MeCN–0.5 *M* oxalic acid (pH 2.0, 1:1:4)	–	Densitometry (360 and 450 nm)	0.1 μg/spot	[24]
			MeOH–MeCN–0.5 *M* oxalic acid (pH 2.0, 1:1:2)		Spray (Fast Violet B)	0.1 μg/spot	
OTC, TC, CTC, DC, MINO, MTC, and DMCTC	Honey	Precoated C_8-TLC	MeOH–MeCN–0.5 *M* oxalic acid (pH 3.0, 1:1:4)	C_{18} cartridge pretreated with EDTA and COOH cartridge clean up	Spray ($MgCl_2$)	0.1 ppm	[25]
OTC, TC, CTC, and DC	Animal tissues	Precoated C_8-TLC	MeOH–MeCN–0.5 *M* oxalic acid (pH 2.0, 1:1:4)	Extraction with EDTA–Macllvaine buffer	Densitometry (360 nm)	0.1 ppm	[26]
				C_{18} cartridge pretreated with EDTA clean up	Spray (Fast Blue BB and pyridine)	0.1 ppm	
OTC, TC, CTC, DC, ETC, ATC, and EATC	–	Precoated C_8-TLC	MeOH–MeCN–0.5 *M* oxalic acid (pH 2.0, 1:1:4)	–	Densitometry (360 and 450 nm)	0.1 μg/spot	[43]
		Precoated C_{18}-TLC	MeOH–MeCN–0.5 *M* oxalic acid (pH 2.0, 1:1:2)				
OTC, TC, CTC, and DMCTC	–	Precoated CN-HPTLC	MeOH–MeCN–0.5 *M* oxalic acid (2:1:6)	–	Densitometry (254 nm)	0.4 μg/spot	[152]
OTC, TC, CTC, and DC	Animal tissues	Precoated C_8-TLC	MeOH–MeCN–0.5 *M* oxalic acid (pH 2.0, 1:1:4)	Extraction with EDTA–Macllvaine buffer C_{18} cartridge pretreated with EDTA clean up	TLC–FAB-MS	0.1 ppm	[46]
OTC, TC, CTC, and DC	Milk	Precoated C_8-TLC	MeOH–MeCN–0.5 *M* oxalic acid (pH 2.0, 1:1:4)	Extraction with EDTA–Macllvaine buffer C_{18} cartridge pretreated with EDTA clean up	TLC–FAB-MS	0.05 ppm	[47]
OTC, TC, CTC, and DC	Honey	Precoated C_8-TLC	MeOH–MeCN–0.5 *M* oxalic acid (pH 2.0, 1:1:4)	Extraction with EDTA–Macllvaine buffer C_{18} cartridge pretreated with EDTA clean up	TLC–FAB-MS	0.05 ppm	[48]

[a] ATC: anhydrotetracycline, EATC: 4-epianhydrotetracycline, ETC: 4-epitetracycline, MINBO: minocycline, MTC: methacycline, DMCTC: demeclocycline, PRMTC: rolitetracycline, EDTA: disodium ethylenediaminetetraacetate, FL: fluorescence, and FAB-MS: fast atom bombardment mass spectrometry.

Table 3
Summary of CE conditions for the analysis of TCs[a]

Tetracyclines	Samples	Capillary	Running buffer	Voltage or current	Extraction and clean up	Temperature	Detection	Detection limit	Ref.
OTC, TC, CTC, ETC, ATC, and EATC	–	Fused-silica	0.2 *M* phosphate buffer (pH 2.2) containing Triton X-100	12 kV	–	–	265 nm	–	[153]
OTC, TC, CTC, DC, DMCTC, MTC, and MINO	Capsule	Fused-silica	4.3 m*M* Phosphate buffer (pH 7.5, 18.2 m*M* ionic strength)	20 μA	–	25°C	260 nm	10–5 M	[154]
TC, CTC, ETC, ATC, and EATC	–	Fused-silica	80 m*M* Na_2CO_3–1 m*M* EDTA (pH 9) containing 0.5% MeOH	12 kV	–	–	270 nm	0.10%	[155]
OTC, TC, CTC, and DC	Milk, serum, and urine	Fused-silica	50 m*M* Borate and 50 m*M* phosphate buffer (pH 8.5) containing 10 mM SDS	15 kV	Metal chelate affinity column and C_{18} cartridge clean up	13°C	370 nm	1.4–5.3 ng/ml	[49]
OTC, TC, CTC, DC, DMCTC, MTC, and MINO	–	Fused-silica	30 m*M* citric acid–24.5 m*M* β-alanine (pH 3.0) containing 40% MeOH	30 kV	–	–	254 nm	5–10 μg/ml	[156]
OTC, EOTC, apo-OTC, ADOTC, and TC	Pharmaceutical preparation	Fused-silica	50 m*M* Na_2CO_3–1 m*M* EDTA (pH 11) containing 0.5% Triton-X	10 kV	–	10°C	254 nm	0.05%	[157]
OTC, TC, CTC, DMCTC, ETC, ATC, and EATC	Milk and plasma	Fused-silica	500 m*M* Magnesium acetate in *N*-methylformamide	15 kV	Precipitation with trichloroacetic acid	20°C	LIF (325/514 nm)	25 ng/ml	[50]
OTC	Fish	Fused-silica	0.2 *M* Phosphate buffer (pH 2)	8 kV	Extraction with trichloroacetic acid containing EDTA and C_{18} cartridge clean up	–	265 nm	0.05 ppm	[51]

OTC, TC, CTC, DC, DMCTC, and MINO	–	Fused-silica	15 m*M* Ammonium acetate buffer	15 kV	–	25°C	265 nm	–	[158]
CTC	–	Fused-silica	120 m*M* Borate buffer (pH 8.5) containing 1 mM EDTA	10 kV	–	25°C	280 nm	8.1 pg	[159]
CTC	–	Fused-silica	0.05 *M* Phosphate buffer (pH 7.06)	15 kV	–	–	DAD	–	[160]
TC, ETC, ATC, and EATC	–	Fused-silica	0.02 *M* Phosphate buffer (pH 3.9) containing 0.005 M EDTA	10 kV	–	–	265 nm	–	[161]
TC, ETC, ATC, and EATC	Pharmaceutical preparation	Fused-silica	MeOH–MeCN (48–52) containing 25 m*M* ammonium acetate, 10 m*M* citric acid, and 118 mM methanesulfonic acid	25 kV	–	25°C	254 nm	0.02–0.06%	[162]
DMCTC, DMTC, EDMCTC, and EDMTC	Pharmaceutical preparation	Fused-silica	0.05 *M* Phosphate buffer (pH 12.25) containing 0.35% Triton-100 and 0.001 *M* EDTA	12 kV	–	15°C	254 nm	0.30%	[163]

[a] ETC: 4-Epitetracycline, ATC: anhydrotetracycline, EATC: 4-epianhydrotetracycline, EOTC: epioxytetracycline, ADMTC: 2-acetyl-2-decarboxamidooxytetracycline, LIF: laser induced fluorescence, DMCTC: demeclocycline, DMTC: demethyltetracycline, EDMCTC: 4- epidemeclocycline, and EDMTC: 4-epidemethyltetracycline, DAD=diode array detection.

is removed from the ion source. To obtain good separation of the TCs on the TLC plate, non-volatile compounds such as oxalic acid and Na_2EDTA are added to the solvent system, as is also the case for their analysis by LC. However, these compounds do not cause any problems in the TLC–FAB-MS such as have been reported in LC–MS (clogging of the interface and deposits in the ion source) because they remain on the TLC plate and are removed with it after the measurement has been completed. Therefore, TLC–FAB-MS has been applied for the confirmation of residual TCs in animal tissues, milk, and honey using a non-volatile solvent system. This method is based on a C_{18} cartridge clean up, followed by separation of the TCs on a reversed-phase C_8 TLC plate. The detection limits were 0.05–0.1 ppm.

3. Capillary electrophoresis

CE has many advantages in comparison with HPLC: very little organic solvent in the running buffer, short run time for the separation, high separation efficiencies, 10^6 plates/m, etc. However, CE has not been applied to the analysis of drug residues in foods, because of the low sample injection volume. Typical CE studies for the analysis of TCs are summarized in Table 3, in which only three reports deal with the residual analysis of TCs in foods [49–51].

Chen and Gu have presented the simultaneous determination of OTC, TC, CTC, and DC in milk, serum, and urine by CE [49]. The samples were deproteinized by succinate buffer and the TCs in the supernatant were cleaned up by metal chelate affinity column chromatography. Salts in the eluate from the column were removed by the trimethylsilanized C_{18} cartridge. Deproteination, a metal chelate affinity column, and a C_{18} cartridge were highly efficient to eliminate the interfering substances. Recoveries of the TCs from the sample were 40–84% with a relative standard deviation (RSD) of 3.3–9.1%.

Metal complexation in non-aqueous CE was evaluated for the separation of TCs and the improved detection of TCs using laser induced fluorescence detection [50]. Magnesium ions were found to be the most suitable ions for the separation of the TCs and strongly intensified the fluorescence of TCs in dimethylformamide which is ten times that in water. This CE technique has been applied to the determination of TCs in milk and plasma.

Huang et al. have applied CE to the determination of TCs in raw and cooked channel catfish dosed at 37.5, 75.0, and 150 mg OTC/kg for 10 days [51]. The samples were extracted with trichloroacetic acid containing EDTA and cleaned up by a C_{18} cartridge. The mean recovery of OTC fortified in catfish at the levels of 0.1–25 ppm was 92.9%. The cooking procedure could only reduce the OTC residues in the catfish fillets but not completely eliminate them.

4. High-performance liquid chromatography

4.1. Separation

TCs form chelate complexes with metal ions and adsorb on the silanol group [43,52–54] in a reversed-phase (RP) column as mentioned in the Introduction, so that TCs are apt to appear as tailing peaks. In order to avoid forming chelate complexes and their adsorption on RP columns, RP column chromatography using mobile phases containing various acids (phosphoric [36,37,55–84], citric [38,39,41,54], tartaric [39], and EDTA [40,41,57,70,73,85–92]) and ion pair chromatography [54,70,74,80,82,93,94] have been reported (Table 4). However, TCs still showed extreme tailing on the RP-HPLC column even when using mobile phases containing these acids, and that only a mobile phase containing oxalic acid enabled no tailing peaks of the TCs.

An isocratic HPLC for the determination of TCs has been reported using a mobile phase containing oxalic acid on a modified silica gel column [52] The resolution and asymmetries of the TC peaks depend upon the pH of the aqueous oxalic acid solution in the mobile phase and the optimum pH is 2.0. With increasing oxalic acid concentration, the resolution and asymmetries were good, results with being obtained above 0.01 and 0.2 *M* for the parent TCs and the impurities of TC, respectively. The C_8-modified silica gel column was suitable for analyzing the parent TCs and the C_{18}-modified silica gel column is suitable for the impurities of TC. It is well known that large differences in the chromatographic

Table 4
Summary of HPLC conditions for the analysis of TCs[a]

Tetracyclines	Sample	Column	Mobile phase	Extraction and clean up	Detection	Detection limit	Ref.
TC and PRMTC	Bulk	Cation-exchange	EtOH–EDTA (4–6, pH 4.35)	Dissolution in water	250 nm	2 μg	[85]
TC, CTC, ETC, EATC, and ATC	Bulk	Zipax SCX	0.3 *M* EDTA (pH 7.0)	Dissolution in 1 *M* HCl	429 nm	0.04 μg	[86]
OTC, TC, and CTC	Urine	Anion-exchange	0.005 *M* EDTA, 0.05 *M* NaCl, and 5% MeOH in water 0.005 *M* EDTA, 0.05 *M* NaCl, and 30% MeOH in water	Extraction with ethyl acetate as calcium complexes	280 nm	4–12 μg/ml	[87]
OTC, TC, and CTC	Urine and plasma	μBondapak C_{18}	30% MeCN in phosphate buffer (pH 2.4) 40% MeCN in phosphate buffer (pH 2.4)	Extraction with ethyl acetate as calcium complexes	355 nm	1–1.5 μg/ml	[55]
OTC, TC, and CTC	Urine and plasma	μBondapak C_{18} pre-conditioned with EDTA	30% MeCN in phosphate buffer (pH 2.4) 40% MeCN in phosphate buffer (pH 2.4)	Extraction with ethyl acetate as calcium complexes	355 nm	0.25–0.5 μg/ml	[56]
OTC, TC, CTC, DC, MTC, MINO, DMCTC, PRMTC, ATC and EATC	–	Vydac TP C_{18}	2–8% iso-PrOH in 0.1 *M* ammonium EDTA –1 *M* DEA (pH 7.3)	–	254 and 405 nm	–	[57]
OTC, TC, CTC, DC, MTC, and DMCTC	Urine and serum	LiChrosorb RP-8	MeCN–0.1 *M* citric acid (24:76)	Extraction with ethyl acetate from buffered sample (pH 6.1)	350 nm	50 ng/ml	[38]
TC	Plasma	LiChrosorb RP-2	MeCN–0.01 *M* phosphoric acid (35:65)	Ion pair extraction with TBA	357 nm	200 ng/ml	[58]
OTC, TC, CTC, DC, MTC, MINO, DMCTC, PRMTC, ETC, EATC, and ATC	–	Hypersil C_1 and C_{18}	Water–DMF (9:1) containing 0.0005 *M* EDTA, 0.02 *M* KNO_3 0.015 *M* citric acid, and 0.02 M sodium citrate	–	272 nm	–	[40]
DC	Urine, plasma, and animal tissues	Nucleosil C_8	MeCN–0.01 *M* NaH_2PO_4 (pH 2.4, 3:7)	Extraction with MeCN–0.01 M NaH_2PO_4 (pH 2.4, 1:1)	270 nm	0.5 μg/ml	[59]
OTC, EOTC, and AOTC	–	LiChrosorb RP-8	MeCN–0.05% TBA (2:98) or (2:8)	–	275 nm	–	[164]
OTC, TC, CTC, DC MTC, and DMCTC	–	LiChrosorb RP-8	MeCN–0.1 *M* citric acid (76:24)	–	250 nm	–	[39]
TC, CTC, ETC, EATC, and ATC	Bulk	μBondapak phenyl	Step gradient of 12–22% MeCN in 0.2 *M* phosphate buffer (pH 2.2)	Dissolution in water	270 nm	–	[36]
OTC, TC, CTC, DC MTC, and DMCTC	–	LiChrosorb NH_2	MeCN–0.1 *M* phosphoric acid (1:9) containing 0.002 *M* HIBS	–	357 nm	–	[60]
OTC, TC, CTC, DC,	–	C_2, C_8, and C_{18}	MeCN–0.1 *M* phosphoric acid	–	357 nm	–	[61]

MTC, and DMCTC			containing TBA				
TC	Urine	LiChrosorb RP-8	iso-PrOH–DEA–tetraammonium EDTA–water (11:5:1:83)	Direct injection	365 nm	0.1 μg/ml	[88]
OTC, TC, and CTC	Honey	μBondapak phenyl	MeCN–0.05 *M* phosphoric acid (1:9)	Extraction with MeCN–0.05 *M* phosphoric acid (2:8)	272 and 375 nm	0.1 ppm	[62]
TC, CTC, ETC, EATC, and ATC	Bulk	μBondapak C_{18}	MeCN–water (pH 2.5, 24:76) containing 0.5% ethanolamine	Dissolution in MeOH	280 nm	–	[165]
TC	Urine and plasma	μBondapak C_{18}	MeCN–phosphate buffer (pH 2.2) (16:84)	Direct injection after addition of TFA	357 nm	0.05 μg/ml	[63]
TC, CTC, ETC, EATC, and ATC	Bulk	μBondapak phenyl	Gradient elution (20% A–50% A) A: MeOH–water–1 *M* phosphoric acid (75:20:5) B: MeOH–water–1 *M* phosphoric acid (5:90:5)	Dissolution in MeOH	280 nm	–	[37]
OTC, TC, CTC, DC MTC, DMCTC, and their impurities	Bulk	LiChrosorb RP-8	MeCN–0.1 *M* NaH_2PO_4 (3:7 pH 8.0) containing 0.0194 *M* DOA MeCN–0.05 *M* NaH_2PO_4 (35:65, pH 8.0) containing 0.0097 *M* DOA MeCN–0.1 *M* NaH_2PO_4 (20:80 pH 8.0) containing 0.0194 *M* DOA MeCN–0.1 *M* NaH_2PO_4 (28:72, pH 8.0) containing 0.0194 *M* DOA	Dissolution in MeOH	280 nm	–	[64]
OTC, TC, CTC, DC, MTC, DMCTC, ETC, EATC and ATC	Bulk	Vydac C_{18}	Various mixtures of MeOH–0.001 *M* EDTA (pH 6.6)	Dissolution in water	380 nm	–	[89]
CTC	Premix	Vydac 201 TP (C_{18})	Water–DMF (88:12) containing 0.001 *M* EDTA, 0.05 *M* citric acid, 0.013 *M* sodium citrate, 0.1 *M* KNO_3	Dissolution in 4 *M* HCl–Me_2CO–water (1:13:6)	280 or 340 nm	–	[41]
OTC, TC, and CTC	Meats and fish	Wako gel DMS-10H (dimethyl silica) Shimadzu PSG-100 (polystyrene)	MeCN–0.05 *M* phosphate buffer (pH 2.5) (1:9) MeOH–0.04 *M* KH_2PO_4–0.08 M EDTA (8:1:1)	Extraction with 1 *M* HCl XAD-2 clean up	370 nm	0.1–0.3 ppm	[65]
OTC, TC, CTC, ETC, EATC and ATC	–	Cosmosil C_8	MeOH–MeCN–0.01 *M* oxalic acid (pH 2.0) (1:1.5:5)	–	360 nm	5 ng	[52]
		Cosmosil C_{18}	MeOH–MeCN–0.2 *M* oxalic acid (pH 2.0) (1:1:3.5)		400 nm	20 ng	
TC, CTC, ETC, EATC, and ATC	Capsule, powder, syrup, and tablet	Nucleosil C_{18}	MeOH–MeCN–0.2 *M* oxalic acid (pH 2.0) (1:1:5)	Dissolution in MeOH	400 nm	–	[34]
OTC, TC, and CTC	Animal tissues	LiChrosorb RP-8	25% aq. DMF containing 0.02 *M* phosphoric acid	Extraction with 5% perchloric acid C_{18} cartridge clean up	340 nm	0.1–0.2 ppm	[66]

Table 4. Continued

Tetracyclines	Sample	Column	Mobile phase	Extraction and clean up	Detection	Detection limit	Ref.
OTC, TC, and CTC	Animal tissues	Nucleosil CN	MeOH–0.01 *M* NaH_2PO_4 (pH 2.5, 75:25)	Extraction with 0.5% *m*-phosphoric acid–MeOH (8:2) C_{18} cartridge clean up	268 nm	0.05–0.1 ppm	[67]
OTC, TC, CTC, and DC	Animal tissues	LiChrosorb RP-8	MeOH–MeCN–0.01 *M* oxalic acid (pH 2.0) (1:1.5:2.5)	Extraction with McIlvaine buffer (pH 4.0) containing 0.1 *M* EDTA C_{18} cartridge clean up	350 nm	0.05–0.1 ppm	[95] [96]
DC and its impurities	Capsule, syrup, and tablet	Hamilton PRP-1	THF–0.2 *M* phosphate buffer (pH 8.0)–0.2 *M* TBA–0.1 *M* EDTA–Water (6:10:5:1:78)	Dissolution in mobile phase	254 nm	–	[68]
OCT	–	Spherisorb ODS	MeCN–0.025 *M* H_2SO_4–DMF (19.5:74:6.5)	–	354 nm	–	[166]
OTC and its impurities	Bulk	LiChrosorb RP-8	0.2 *M* Ammonium oxalate–0.1 M EDTA–DMF (55:20:25)	Dissolution in 0.1 *M* HCl in MeOH–water (15:58)	280 nm	–	[90]
OTC, TC, CTC, DC, MTC, DMCTC, ETC, EATC and ATC	–	Vydac RP C_{18}	15–35% MeOH in 0.001 *M* EDTA (pH 6.6)	–	380 nm	–	[91]
OTC and TC	Fish tissues	Nucleosil C_{18}	MeCN–0.05 *M* NaH_2PO_4	Extraction with 0.5% *m*-phosphoric acid–MeOH (8:2) C_{18} cartridge clean up	265 nm	0.05–0.1 ppm	[69]
OTC	Honey	Partsil C_8	Water–DMF (17:3) containing EDTA (pH 3.5)	Dissolution in water	355 nm	0.5 ppm	[92]
OTC, EOTC, TC, and CTC	Premix	Econosphere C_8	MeCN–phosphate buffer (pH 8.0) containing EDTA and DOA	Extraction with acidic MeOH	280 nm	–	[70]
OTC, TC, DC, and MINO	Serum	Spheri-5 phenyl	MeOH–MeCN–TEA–0.08 M phosphate buffer (pH 2.4) (100:15:5:880)	Dilution with mobile phase containing 2% phosphoric acid	267 nm	0.2 μg/ml	[71]
OTC, TC, CTC, DC, MTC, DMCTC, ETC, EATC, ATC, PRMTC, ICTC, and apo-OTC	Plasma	Copolymer of divinyl–benzene with styrene	MeCN–CH_2Cl_2–0.2 *M* acetate buffer (pH 3.6) (15:1:85) containing EDTA	Extraction with ethyl acetate	268 or 357 nm	0.5 μg/ml	[167]
OTC, TC, and DC	Animal tissues	Polymer Lab. PLRP-S	0.01 *M* Phosphoric acid–MeCN–MeOH (gradient, 80:0:20 to 30:50:20)	Extraction with 1 *M* HCl Liquid–liquid partition clean up	355 nm	0.1 ppm	[88]
TC, ETC, EATC, and ATC		Polymer Lab. PLRP-S	*tert*.-BuOH–0.2 *M* phosphate buffer (pH 9.0)–0.02 *M* TBA–0.1 *M* EDTA (55:100:50:10)	–	254 nm	–	[73]
OTC, TC, CTC, and DC	Honey	Bakerbond C_8	MeOH–MNeCN–0.01 *M* oxalic acid (1:1.5:3)	C_{18} cartridge pretreated with EDTA and COOH cartridge clean up	350 nm	0.05–0.1 ppm	[97]

OTC, TC, CTC, DC, MTC, DMCTC, MINO, and PRMTC	–	Chemcosorb C_8	MeOH–MeCN–0.01 *M* oxalic acid (pH 3.0) (1:1.5:7)	–	350 nm	–	[24]
OTC, TC, CTC, DC, MTC, DMCTC, MINO, and MINO	Honey	Chemcosorb C_8	MeOH–MeCN–0.01 *M* oxalic acid (pH 3.0) (1:1.5:7)	C_{18} cartridge pretreated with EDTA and COOH cartridge clean up	350 nm	0.05–0.1 ppm	[25]
OTC, EOTC, AOTC, and apo-OTC	–	Polymer Lab. PLRP-S	*tert.*-BuOH–0.2 *M* phosphate buffer (pH 8.0)–0.02 *M* TBA–0.0001 *M* EDTA (55:100:50:10)	–	254 nm	–	[74]
OTC, TC, CTC, and DC	Animal tissues	LiChrosorb RP-8	MeOH–MeCN–0.01 *M* oxalic acid (1:1.5:3)	Extraction with McIlvaine buffer (pH 4.0) containing 0.1 *M* EDTA C_{18} cartridge pretreated with EDTA clean up	350 nm	0.01 ppm	[26]
OTC	Fish tissues	Hypersil ODS	0.5% $(NH_4)_2HPO_4$–0.5% DEA–MeCN–DMF (40.5:40.5:19.6, pH 2.5)	Extraction with 1 *M* HCl–50% TCA (15:1) C_{18} cartridge clean up	365 nm	0.005 ppm	[75]
OTC, TC, and CTC	Feeds	Nova-Pak C_{18}	MeOH–MeCN–0.01 *M* oxalic acid (1:1.5:6.5)	Extraction with McIlvaine buffer (pH 2.0 and 4.5) containing EDTA Sephadex LH-20 clean up	370 nm	–	[98]
OTC	Fish tissues	Supelcosil LC-18DB	0.005 *M* phosphate buffer (pH 2.0)–MeCN–THF (81:10:9)	Extraction with phosphate buffer (pH 4.2) containing 0.1 *M* EDTA C_8 cartridge clean up	357 nm	0.005 ppm	[76]
OTC, TC, CTC, and DC	Fish tissues	Spheri C_{18}	MeCN–DMF–0.01 *M* oxalic acid (22:6:72)	Extraction with McIlvaine buffer (pH 4.0) C_{18} cartridge pretreated with EDTA clean up	355 nm	0.05–0.1 ppm	[99]
DC	Urine and serum	Nova-Pak C_{18}	MeCN–AcOH–0.1 *M* KH_2PO_4 (75:150:125 and 65:150:125)	Extraction with McIlvaine buffer (pH 4.0) containing 0.1 *M* EDTA C_{18} cartridge clean up	340 nm	0.025 ppm	[77]
OTC	Fish plasma	Polymer Lab. RLRP-S	MeCN–THF–0.02 *M* H_3PO_4 (63:47:890)	Direct injection	350 nm	0.1 μg/ml	[78]
OTC and CTC	Milk	C_{18} column	0.005% H_3PO_4–MeCN (1:1 and 13:7)	Amberlite XAD-2	355 nm	0.5 ppm	[79]
OTC, TC, and CTC	Milk	Nova-Pak C_{18}	0.01 *M* oxalic acid–MeOH–MeCN (gradient, 100:0:0 to 70:8:22)	Dilution with McIlvaine buffer (pH 4.0) containing 0.1 *M* EDTA and ultrafiltration	360 nm	0.015–0.05 ppm	[147]

Table 4. Continued

Tetracyclines	Sample	Column	Mobile phase	Extraction and clean up	Detection	Detection limit	Ref.
OTC, TC, CTC, and ECTC	Feeds	Nova-Pak C_{18}	MeOH–MeCN–0.01 *M* oxalic acid (1:1.5:6.5)	Extraction with McIlvaine buffer (pH 2.0 and 4.5) containing EDTA Sephadex LH-20 clean up	370 nm	–	[100]
CTC	Tissues	Polymer Lab. PLRP-S	MeCN–0.1 *M* glycine (125:875)	Extraction with 1 *M* HCl–0.1 *M* glycine Cyclohexyl cartridge clean up	Conversion to ICTC FL; 340/420 nm	0.02–0.05 ppm	[134]
OTC	Fish plasma	Cyano Spheri-5	MeOH–0.02 *M* oxalic acid–DMF (5:95:5)	Addition of TFA	350 nm	4 ng	[101]
CTC	Capsule, tablet, and ointment	Zorbax C_8	DMSO–$HClO_4$–water (32:5:63)	Dilution with 0.01 *M* HCl	280 nm	0.05%	[168]
OTC, TC, CTC, and DC	Animal tissues	Nova-Pak phenyl	MeOH–MeCN–0.02 *M* oxalic acid (gradient, 5:15:80 to 13:27:60)	Extraction with McIlvaine buffer (pH 4.0) containing 0.1 *M* EDTA C_{18} cartridge deactivated by silylation clean up	355 nm	0.005–0.01 ppm	[53]
OTC, TC, and CTC	Animal tissues	YMC-PACK ODS	1 *M* Imidazole buffer–MeOH (77:23)	Extraction with McIlvaine buffer–MeOH (3:7) C_{18} cartridge clean up	Formation of metal chelate with Mg FL; 380/520 nm	0.01–0.25 ppm	[139]
TC, ETC, EATC, and ATC	–	Polystyrene–divinylbenzene	*tert.*-BuOH–0.2 *M* KH_2PO_4–0.02 M–TBA–0.01 *M* EDTA–water (6:10:15:10:59)	–	254 nm	–	[80]
CTC and its impurities	–	Polystyrene–divinylbenzene	*tert.*-BuOH–1 *M* $HClO_4$–water (5:5:10)	–	254 nm	–	[169]
OTC and TC	Honey	Pecosphere C_{18}	0.01 *M* SDS containing 0.01 M oxalic acid–MeCN (7:3)	Dilution with MeCN and water	285 nm	1.2–3.0 ppm	[93]
OTC and TC	Milk	Nucleosil C_{18} pretreated with CTC	MeCN–0.02 *M* H_3PO_4 (24:76)	Extraction with TBA into CH_2Cl_2	355 nm	0.01 ppm	[81]
OTC, TC, and CTC	Milk	Micro Pak ODS	MeCN–0.01 *M* oxalic acid (3:7)	Matrix solid-phase dispersion (C_{18} with EDTA and oxalic acid)	365 nm	0.1 ppm	[102]
OTC, TC, and CTC	Salmon muscle	LiChroCART RP-18	MeOH–MeCN–0.01 *M* oxalic acid (10:17:73)	Extraction with McIlvaine buffer C_{18} cartridge clean up	355 nm	0.08–0.5 ppm	[103]
OTC	Fish tissues	Micro Pak ODS	MeOH–MeCN–0.01 *M* oxalic acid (2.5:27.5:70)	Matrix solid-phase dispersion (C_{18} with EDTA and oxalic acid)	365 nm	0.05 ppm	[104]
OTC, TC, and CTC	Animal tissues	LiChrosorb RP-8	MeCN–0.01 *M* oxalic acid (1:1)	Extraction with succinate buffer After formation of metal chelate with copper, XAD-2 clean up	350 nm	0.01–0.05 ppm	[105]
OTC and CTC	Honey	Asahipak Gel ODP-50	OTC; MeOH–0.1 *M* glycine buffer (1:20) containing SHS CTC; MeOH–0.1 *M* glycine buffer (1:5)	C_{18} cartridge and ion pair extraction clean up	Formation of metal chelates FL; 370/505 nm	0.005–0.02 ppm	[94]

OTC, TC, and CTC	Milk	Nova Pak C_{18}	MeOH–MeCN–0.01 *M* oxalic acid (30:50:30 or 0:60:40)	C_{18} cartridge clean up	Particle beam MS	0.1 ppm	[106]
OTC, TC, CTC, and DC	Animal tissues	LiChrosorb RP-8	MeOH–MeCN–0.01 *M* oxalic acid (pH 2.0) (1:1.5:2.5)	Extraction with McIlvaine buffer (pH 4.0) containing 0.1 *M* EDTA C_{18} cartridge clean up	350 nm	0.05–0.1 ppm	[107]
OTC	Blood and serum	Polymer Lab. PLRP-S	MeCN–0.02 *M* phosphoric acid (23:77) containing SHS	Automatic dialysis	350 nm	0.05 ppm	[82]
MTC, EMTC, OTC, DC, EOTC, and ADMTC	–	Polymer Lab. PLRP-S	2-Methyl-2-PrOH–0.2 *M* phosphate buffer–0.01 *M* EDTA–water (2.5:10:10:77.5)	–	254 nm	–	[83]
OTC	Salmon muscle	Ultrasphere ODS	MeOH–0.02 *M* phosphate buffer (pH 2.25) (60:190)	Extraction with phosphate buffer Sephadex G-25 clean up	365 and 254 nm	0.05 ppm	[84]
OTC	Animal serum	Hisep shielded hydrophobic phase	MeOH–0.2 *M* oxalic acid (pH 7.0) (1:9)	Direct injection	360 nm	0.05 ppm	[170]
OTC, TC, and CTC	Animal tissues	Nova-Pak C_{18}	(Phosphate–citric acid buffer)–MeCN (72:28) containing 0.005 *M* TBA	Extraction with McIlvaine buffer (pH 4.0) containing 0.1 *M* EDTA C_{18} cartridge clean up	365 nm	0.01 ppm	[54]
OTC	Salmon muscle	Ultrasphere ODS	MeCN–THF–0.025 *M* oxalic acid (225:25:750) containing 0.01 M SOS	Extraction with 1% meta-phosphoric acid	355 nm	0.05 ppm	[108]
OTC, TC, CTC, DC, MINO, MTC, and DMCTC	Milk	Polymer Lab. PLRP-S	MeOH–MeCN–0.01 *M* oxalic acid (gradient, 0:0:100 to 8:22:70)	Metal chelate affinity column clean up	355 nm	0.005 ppm	[109] [110]
OTC, TC, and CTC	Milk	Polymer Lab. PLRP-S	0.05 *M* Oxalate buffer (pH 2.0) containing 0.005 *M* octanesulfonate –MeCN (gradient, 80:20 to 62:38)	Deproteinization by HCl and MeCN	365 nm	0.005 ppm	[136]
OTC, TC, and CTC	–	Polymer Lab. PLRP-S Supelco LC-18 Supelco LC-18-DB	Mixture of MeOH, MeCN, DMF, and 0.05 *M* buffer at various ratios	–	355 nm	–	[171]
OTC, TC, CTC, and DC	Milk	Bakerbond C_8	MeOH–MeCN–0.01 *M* oxalic acid (pH 2.0) (1:1.5:2.5)	Extraction with McIlvaine buffer (pH 4.0) containing 0.1 *M* EDTA C_{18} cartridge clean up	350 nm	0.01 ppm	[47]
OTC, TC, CTC, and DC	Honey	Inertsil phenyl	MeOH–MeCN–0.005 *M* TFA (2:2:11)	C_{18} cartridge pretreated with EDTA clean up	Frit FAB-MS	0.1 ppm	[129]
OTC, TC, and CTC	Animal tissues	LiChrosorb RP18	14% iso-PrOH in 0.05 *M* aq. DEA containing 0.001 *M* EDTA (pH 7.3)	Extraction with 0.02 *M* HCl–0.01 *M* EDTA After conversion to anhydro-form reextraction with CH_2Cl_2	280 nm	–	[172]

Table 4. Continued

Tetracyclines	Sample	Column	Mobile phase	Extraction and clean up	Detection	Detection limit	Ref.
MINO and its metabolites	Serum and urine	C_{18}	Gradient of 10–40% MeCN in 0.2% aq. formic acid	Mixture with 0.025 n*M* EDTA	352 nm	–	[173]
OTC, TC, CTC, DC, and MINO	–	Vydac 201H554 (C_{18})	MeCN–0.01 *M* EDTA (pH 6.6) (27:73) MeCN–0.1% TFA (24:76)	–	Eupropium phosphorescence	1–10 nm	[174]
OTC, TC, and CTC	Animal tissues	Spherisorb ODS 2	MeOH–MeCN–0.01 *M* oxalic acid (20:35:45)	Extraction with EDTA–McIlvaine buffer C_{18} cartridge clean up	360 nm	50 ng/g	[114]
OTC and CTC	Animal tissues	Asahi Pak ODP-50	MeCN–Sorensen buffer (pH 12) (1:9)	Extraction with EDTA–McIlvaine buffer containing 70% MeOH C_{18} and SCX cartridge clean up	Degradation under alkaline conditions FL; 350/420 nm	–	[138]
OTC, TC, CTC, and DMCTC	Milk	Polymer Lab. PLRP-S	MeOH–MeCN–0.01 *M* oxalic acid (10:15:60)	Metal chelate affinity chromatography clean up	FL; 406/515 nm post-column addition of zirconium ion	1–4 ng/ml	[115] [116]
OTC	Fish tissues	Lichrosorb RP-8	MeCN–0.05 *M* oxalic acid (14:86)	Extraction with EDTA–McIlvaine buffer C_{18} cartridge clean up	355 nm	10 ng/g	[117]
OTC	Fish tissues	Hypersil ODS	MeCN–THF–0.01 *M* oxalic acid (15:3:82)	Extraction with 0.01 *M* EDTA C_{18} cartridge clean up	365 nm	10 ng/g	[118]
OTC, TC, and CTC	Milk	Polymer Lab. PLRP-S	(0.02 *M* H_3PO_4–0.01 *M* SDS)–MeCN (70:30)	Extraction with 1 *M* HCl and MeCN	380 nm	2–4 ppb	[137]
OTC	Fish tissues	Ultrasphere ODS	MeCN–0.02 *M* phosphate buffer (pH 2.3) (17:83) containing 1.3 m*M* EDTA	Extraction with trichloroacetic acid, HCl, and EDTA C_{18} cartridge clean up	353 nm	0.05 ppm	[175]
OTC	Swine tissues	Wakosil II 5C18 RS	MeCN–0.05 *M* NaH_2PO_4 (12:88)	Extraction with MeOH–0.3% *m*-phosphoric acid (3:7) C_{18} cartridge clean up	360 nm	0.05 ppm	[130]
OTC, TC, and CTC	Animal tissues	Chromspher C_8	MeCN–0.01 *M* oxalic acid gradient	Extraction with glycine–HCl buffer Cyclohexyl cartridge clean up	FL; 390/490 nm, post-column addition of aluminium ion	20–230 ng/g	[119]
OTC, EOTC, and apo-OTC	Animal tissues	Inertsil C_8	MeCN–THF–0.1 *M* ammonium acetate buffer (pH 3)	Extraction with EDTA–McIlvaine buffer C_{18} cartridge clean up	250 nm	–	[131]
OTC, TC, CTC, and DMCTC	Animal tissues	Polymer Lab. PLRP-S	0.1 *M* KH_2PO_4–0.01 *M* citric acid–0.01 *M* EDTA (gradient)	Extraction with succinate buffer Metal chelate affinity chromatography clean up	350 nm	0.01–0.02 ppm	[135]

DC	Plasma	LiChrospher RP-18	MeCN–water–HCl_4 (298.5:699: 2.5, pH 2.5) containing 0.6 mM Na_2EDTA and 5 m*M* oxalic acid	Extraction with ethyl acetate after adjustment of pH at 6	350 nm	0.1 ppm	[120]
OTC	Swine tissues	L-column ODS	MeCN–1 *M* imidazole buffer (pH 7.2) containing 50 mM magnesium acetate and 10 mM Na_2EDTA (1:9)	Extraction with MeCN–1 M imidazole buffer (pH 7.2) containing 50 m*M* magnesium acetate and 10 m*M* Na_2EDTA (15:85)	Formation of metal chelate with magnesium ions FL; 380/520 nm	0.04 ppm	[140]
TC and MINO	Serum	Symmetry Shield RP8	Water containing 0.1% TFA, 2% MeOH, and 7% MeCN	Porous polymer cartridge clean up after acidification	350 nm	–	[132]
OTC, TC, and CTC	Milk, meat and cheese	Lichrosorb RP-18	MeOH–MeCN–0.01 *M* oxalic acid (17.5:17.5:65)	Extraction with EDTA–McIlvaine buffer C_{18} cartridge clean up	DAD	12–22 ng/g	[121]
OTC, TC, and CTC	Milk, meat, and cheese	Lichrosorb RP-18	MeOH–MeCN–0.01 *M* oxalic acid (17.5:17.5:65)	Matrix solid-phase dispersion (C_{18} with EDTA and oxalic acid)	DAD	30 ng/g	[121]
OTC, TC, CTC, DC, DMCTC, MTC, and MINO	Pharmaceutical preparations	μBondapak C_{18}	0.05 *M* MeCN–phosphate buffer (pH 2.5) (16:84)	Dissolution with mobile phase	Amperometric detection (1.2 V)	0.1–1.0 ppm	[176]
OTC	Animal tissues, fish tissues, milk, and egg	YMC-PACK ODS	MeOH–1 *M* imidazole buffer (pH 7.2) (1:9)	Extraction with EDTA–McIlvaine buffer Porous polymer cartridge clean up	Formation of metal chelate with magnesium ions FL; 380/520 nm	0.02–0.04 ppm	[141]
OTC, TC, CTC, and DC	Milk	L-column ODS	MeCN–0.02 *M* oxalic acid (31:69) containing 0.01 M SOS	On line clean up (C_{18})	365 nm	0.01 ppm	[122]
OTC, TC, CTC, and DC	Animal tissues and egg	Polymer Lab. PLRP-S	MeCN–0.01 *M* oxalic acid gradient (15:85 to 40:60)	Extraction with succinate buffer metal chelate affinity chromatography clean up	FL; 406/515 nm, post-column addition of zirconium ion	0.42–1.38 ng/g	[123]
OTC, TC, and CTC	Animal tissues	Prodigy ODS 2	MeCN–0.01 *M* oxalic acid containing 0.04% HFBA and 0.01 *M* EDTA gradient (1:9 to 9:1)	Extraction with EDTA–McIlvaine buffer C_{18} cartridge clean up	APCI-MS (SIM)	0.01–0.02 ppm	[142]
OTC, TC, CTC, and DC	Animal tissues	TSK Gel Super Octyl	MeCN–0.005 *M* TFA (1:4)	Extraction with EDTA–McIlvaine buffer C_{18} cartridge clean up	ESI-MS–MS	0.1 ppm	[133]
OTC	Shell	Lichrospher RP-18E	MeCN–0.02 *M* *o*-phosphoric acid (24:76)	Extraction with 0.013 M methanolic oxalic acid	355 nm	0.008 ppm	[177]
OTC, TC, and CTC	Animal tissues	Polymer Lab. PLRP-S	MeCN–0.01 *M* oxalic acid (25: 75)	Extraction with oxalic buffer Porous polymer cartridge clean up	360 nm	10–25 ng/g	[124]

Table 4. Continued

Tetracyclines	Sample	Column	Mobile phase	Extraction and clean up	Detection	Detection limit	Ref.
DC and EDC	Turkey tissues	Polymer Lab. PLRP-S	MeCN–MeOH–0.01 *M* oxalic acid gradient (15:5:80 to 20:40:20)	Extraction with succinate buffer Metal chelate affinity chromatography clean up	FL; 406/515 nm, post-column addition of zirconium ion	1–1.2 ng/g	[125]
OTC, TC, CTC, DC, MTC, and MINO	Milk and shrimp	Polymer Lab. PLRP-S	MeOH–0.005 *M* oxalic acid (58:42)	Extraction with succinate buffer Metal chelate affinity chromatography clean up	Particle-beam MS (negative Cl)	30–100 ng/g	[126]
OTC, TC, DC, and DMCTC	Animal tissues and egg	Polymer Lab. PLRP-S	MeCN–MeOH–0.1 *M* KH_2PO_4–0.01 *M* citric acid–0.01 *M* EDTA gradient	Extraction with ethyl acetate On line metal chelate affinity chromatography clean up	305 nm	10 ppb	[178]
CTC, ICTC, and IECTC	Egg	Polymer Lab. PLRP-S and Chromspher C_8	MeCN–0.1 *M* glycine (pH 12) (12.5:87.5) and MeCN–0.02 M oxalic acid gradient	Extraction with glycine–HCl buffer Cyclohexyl cartridge clean up	FL; 420/340 nm) and FL (390/490 nm, post-column addition of aluminium ion	–	[127]
MINO	Plasma	Nucleosil 5-CN	MeOH–(20 m*M* HCl_4–4 m*M* TEA) (1:4)	Extraction with ethyl acetate	350 nm	30 ng/ml	[179]
OTC	Fish tissues	YMC C_{18}	MeCN–(0.01 *M* oxalic acid–0.03 *M* SOS) (29.5:70.5)	Extraction with EDTA–McIlvaine buffer Pheny cartridge clean up	355 nm	6–22 ng/g	[148]
OTC, TC, and CTC	Urine	Brownlee RP-18	MeCN–0.1% TFA gradient (0:100 to 80:80)	Addition of 0.2 *M* KH_2PO_4	ESI-MS–MS	10 ppb	[145]
OTC, TC, CTC and DC	Animal tissues, fish tissues, honey, milk, and egg	Bakerbond C_8	MeCN–MeOH–0.01 m*M* oxalic acid (27:18:55)	Extraction with EDTA–McIlvaine buffer C_{18} cartridge clean up	APCI-MS–MS (SRM)	1–4 ppb	[128]
OTC	Plasma	LiChrosorb RP8	MeOH–MeCN–0.01 *M* oxalic acid (10:15:75)	Extraction with ethyl acetate–iso-PrOH (6–0.5)	357 nm	5 ng/ml	[111]
MINO	Bulk	Chemospher C_8 Rosil C_8 Hypersil C_8 LiChrosorb C_8 Partisil C_8 Zorbax C_8	DMF–0.2 *M* ammonium oxalate–0.1 *M* EDTA (25:55:20) DMF–0.2 *M* ammonium oxalate–0.1 *M* EDTA–water (15:55:20:10)	Dissolution in 0.01 *M* HCl	280 nm		[112]
		Nucleosil C_8	DMF–0.2 *M* ammonium oxalate–0.1 *M* EDTA–water (10:55:20:15)				
DC	Serum	Ultrabase C_{18}	MeCN–phosphoric acid (pH 2.5) (28:72)	Extraction with ethyl acetate from buffered sample (pH 6.1)	350 nm	25 ng/ml	[180]
OTC, TC, CTC, DC, DMCTC, and MINO	Animal tissues	Chromospher C_8	MeCN–0.1 *M* oxalic acid (pH 2.0) (2:8)	Metal chelate affinity column clean up	365 nm	10 ng/g	[113]

[a] DEA: diethanolamine, FAB-MS: fast atom bombardment mass spectrometry, EDTA: disodium ethylenediaminetetraacetate, FL: fluorescence $\lambda_{ex}/\lambda_{em}$, TBA: tetrabutylammonium, THF: tetrahydrofuran, SOS: sodium octanesulfonate, SHS: sodium 1-heptanesulfonate, ATC: anhydrotetracycline, EATC: 4-epianhydrotetracycline, ETC: 4-epitetracycline, DMSO: dimethyl sulfoxide, ICTC: isochlotetracycline, TFA: trifluoroacetic acid, DMF: *N*,*N*-dimethylformamide, TCA: trichloroacetic acid, DOA: *N*,*N*-dimethyloctylamine, HIBS: 1-hydroxy-3,5-diisobutylbenzenesulfonic acid, SCX: strong cation-exchange, DAD: diode array detection, APCI: atmospheric pressure chemical ionization, SIM: selected ion monitoring, HFBA: heptafluorobutyric acid, ESI: electrospray ionization, MS–MS; tandem mass spectrometry, TEA: triethylamine, and SRM: selected reaction monitoring.

behavior of TCs have been observed using packing materials from different suppliers. So, the capability of the mobile phase containing oxalic acid for the separation of TCs have been ascertained using packing materials from different suppliers. As a result, even though packing materials from different suppliers were employed, variation in the ratio of organic solvent and aqueous oxalic acid solution in the mobile phase enabled good separation. The mobile phase containing oxalic acid has appeared in many papers on the HPLC analysis of TCs in foods since the late 1980's [25,26,47,53,95–128].

In order to separate TCs without a reduction in peak resolution on the conventional modified silica gel column, it is essential to add oxalic acid into the mobile phase as described above. However, the use of a well end-capped modified silica gel synthesized from 99.99% pure silica gel column enabled us to separate TCs without the addition of oxalic acid [129–133]. The peak resolution of TCs on the well end-capped modified silica gel column has not been reduced without oxalic acid, because silanol groups have been mostly end-capped and the metals have been completely removed. In most studies [129–133], volatile mobile phases which are applicable to direct interfaced LC–MS without causing clogging problems at the interface have been used.

To avoid the addition of oxalic acid to the mobile phase without a reduction in peak resolution of the TCs, an alternative way is the use of a polystyrene–divinylbenzene copolymer (PS–DVB) column [68,72–74, 78,82,83,109,134–137] as the stationary phase. This packing material is prepared by the polymerization of styrene and divinylbenzene and does not possess silanol groups, therefore, it is not essential to add oxalic acid to the mobile phase to obtain good separation of TCs on this column.

4.2. Detection

As mentioned in the Introduction, TCs show strong UV absorption around 270 and 360 nm in neutral and acidic solutions [3], so the most conventional detection method for TCs was the use of a UV detector (Table 4). Because TCs produce strong fluorescence with metal ions or under basic conditions [5–7], the highly sensitive detection of TCs in HPLC has been carried out by detecting fluorescence after degradation of the TCs under alkaline conditions [134,138] and the formation of a metal chelate [94,139–141].

The degradation of TCs under alkaline conditions was achieved by the combined use of a PS–DVB column and alkaline mobile phase, because the PS–DVB column is stable under alkaline conditions [134,138]. This method showed detection limits five to ten times as much as those of UV detection.

For the formation of a metal chelate, two methods have been reported: one is the formation of metal chelates of TCs with magnesium ion in the mobile phase, and another is the complexation of TCs with zirconium ion [115,116] or aluminum ion [119,127], which is added post-column to the HPLC eluate. The detection limits increased more than ten times as much as those of UV detection, and in recent reports, the post column method using zirconium ion showed 50–100-fold higher detection limits in animal tissues [123,125].

Mass spectrometry (MS) is also used as a highly sensitive detection method of TCs [125,126,128, 133,142,143]. The mass spectrometric techniques, which have been applied to tetracycline analysis in foods, are as follows: thermospray (TSP) MS [144], frit FAB MS [129], atmospheric pressure chemical ionization (APCI) MS [128,142], electrospray (ESI) MS [133,145], and particle beam (PB) MS [106,126]. Detection limits of the TCs in LC–TSP-MS, frit LC–frit FAB-MS, and LC–PB-MS were the same as those of UV detection, however, LC–ESI-MS and LC–APCI-MS showed over 10-fold higher detection limits.

Mass spectrometric techniques can confirm the residual TCs with high sensitivity and selectivity; therefore, a method combining a simple and precise chromatographic separation with an appropriate mass spectrometric determination technique would offer a significant advantage for the absolute confirmation of the residual TCs. Although LC–MS appears best suited for this purpose, most previously reported LC conditions cannot be directly applied to existing LC–MS systems, because they require mobile phases containing such non-volatile compounds as oxalic and citric acids to improve the chromatographic resolution of the TCs (Table 4). However, mobile phases containing non-volatile compounds, when used in LC–MS, have been observed to cause

clogging at the interface and a build-up of deposits in the ion source, so that the LC–MS cannot be operated for a prolonged period. In order to overcome this problem, a combination of a well end-capped modified silica gel synthesized from a 99.99% pure silica gel column (TSK Gel Super Octyl) and a volatile mobile phase (acetonitrile–0.05% trifluoroacetic acid) has been successfully applied to the confirmation of TCs in animal tissues without a clogging problem, because the peak resolution of TCs on the well end-capped alkyl-bonded silica gel column has not been reduced without oxalic acid as described above. More recently, Nakazawa et al. found that a mobile phase containing oxalic acid can be applied to LC–APCI-MS without a clogging problem at the interface by setting the nebulizer probe temperature at 475°C, since oxalic acid decomposes to carbon dioxide and water at high temperature. They successfully applied this LC–APCI-MS technique to the determination of TCs in animal and fish tissues, milk, honey, and egg [128].

5. Extraction and clean up

As TCs form a chelate complex with metal ions [8–10] and bind with proteins, it has been considered that strong acid and acidic deproteinizing agents are suitable to extract TCs from biological samples [18,65–67,69,70,72,75,86]. However, TCs are decomposed to the anhydro-form under acidic conditions (<pH 2.0) and converted to their epimers by heating for a long time. Therefore, extraction has been achieved using a mild acidic solvent containing EDTA (0.1 *M* EDTA–McIlvaine buffer, pH 4.0) [25,26,46, 47, 53, 54,77,95,98–100,107,114,117,118, 121,129–131,133,138,141,142,145–148]. Extraction efficiencies of the buffer from animal tissues are estimated to be 87, 84, 80 and 68% for OTC, TC, CTC, and DC, respectively [95].

For a clean up procedure, Amberlite XAD-2 [18,65,105] and chelate extraction [87,149,150] have been employed, however, these methods require complicated procedures and gave poor recovery and reproducibility. These problems during the extraction and clean up steps are caused by the undesirable properties of TCs mentioned above. In order to overcome these undesirable properties, pretreatment of the C_{18} cartridge with EDTA [25,26,46,47, 59,99,129,146] or a silylation reagent [53], the addition of oxalic acid to the eluent of the cartridge [95,103,106,107], and matrix solid-phase dispersion using a C_{18} adsorbent with EDTA and oxalic acid [102,104] have been tried. Sufficient separations of TCs and co-existing substances in foods have been obtained using the above techniques. Among them, the C_{18} clean up method combined with McIlvaine buffer containing EDTA for extraction has been introduced in 1983 for the first time [146] and appears to be the current standard for the extraction and clean up of TCs from foods. The AOAC International Official Analysis Method for TCs in edible animal tissues has been established based on this extraction and clean up method [96].

As described above, TCs have a chelating ability [8–10]. This ability has been used to establish a clean up method, and interesting clean up methods of TCs in foods have been reported, such as metal chelate affinity chromatography (MCAC) [105,109, 110,115,116,123,125,126,135]. The principal of MCAC is as follows: Agarose gel containing immobiled iminodiacetic acid is loaded with copper(II) ions, which bind to the diacetate group. Once bound, the gel becomes active and capable of binding chelators such as TCs. Chelated compounds (TCs) are removed by the passage of a stronger chelating agent such as EDTA through the gel. Although the RSD was below 10%, the recoveries of the TCs from animal tissues were relatively lower ranging from 40 to 80%. The metal chelate chromatography clean up combined with HPLC analysis has been adopted as an AOAC International Official Analysis Method for TCs in milk [110].

6. Conclusions

OTC, TC, CTC, and DC are most often applied to livestock animals including honeybees as veterinary medicines because of their economic advantages. Many chromatographic analysis methods of TCs in foods have been reported for 25 years. However, most of them require complicated procedures, because the properties of TCs to form a chelate complex with metal ions and to bind with proteins

and silanol groups in the stationary phase interfere with the establishment of a simple chemical analysis method for TCs. These undesirable properties have been completely controlled by the use of EDTA and oxalic acid during each analytical step (extraction, clean up, and separation). On the other hand, the chelating ability of TCs has been splendidly used for the development of a clean up method and interesting clean up methods of TCs in foods have been established, namely metal chelate affinity chromatography. These techniques remarkably improved the analysis method of TCs in foods, however, there is still some room for improvement from the view point of recovery and reproducibility. We are hoping to continue our efforts toward the goal of establishing an analysis method of TCs used in foods.

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References

[1] B.M. Dugger, Ann. New York Acad. Sci. 51 (1948) 177.
[2] L.A. Mitscher (Ed.), The Chemistry of the Tetracycline Antibiotics, 1st Edition, Marcel Dekker, New York, 1978, p. 52.
[3] L.A. Mitscher (Ed.), The Chemistry of the Tetracycline Antibiotics, 1st Edition, Marcel Dekker, New York, 1978, p. 91.
[4] L.A. Mitscher (Ed.), The Chemistry of the Tetracycline Antibiotics, 1st Edition, Marcel Dekker, New York, 1978, p. 123.
[5] L.A. Mitscher (Ed.), The Chemistry of the Tetracycline Antibiotics, 1st Edition, Marcel Dekker, New York, 1978, p. 110.
[6] K.W. Kohn, Anal. Chem. 33 (1961) 862.
[7] H. Piger, C. Schlatter, Analyst 101 (1976) 808.
[8] M. Ishidate, T. Sakaguchi, Chem. Pharm. Bull. 6 (1958) 1.
[9] J.Y. Lee, W. Evrett, J. Am. Chem. Soc. 103 (1981) 5221.
[10] S. Ishino, T. Sakaguchi, I. Morimoto, T. Okutsu, Yakugaku Zasshi 101 (1981) 118.
[11] H. Zia, J.C. Price, J. Pharm. Sci. 65 (1976) 226.
[12] C. Bogert, A.M. Kroon, J. Pharm. Sci. 70 (1981) 186.
[13] P.P. Ascione, J.B. Zagar, G.P. Chrekian, J. Pharm. Sci. 56 (1967) 1393.
[14] A.A. Fernandez, V.T. Noceda, E.S. Carrera, J. Pharm. Sci. 58 (1969) 443.
[15] N.D. Gyanchandani, I.J. McGilveray, D.W. Hughes, J. Pharm. Sci. 59 (1970) 224.
[16] I.C. Dijkhuis, M.R. Brommet, J. Pharm. Sci. 59 (1970) 558.
[17] C. Radecka, W.L. Wilson, J. Chromatogr. 57 (1971) 297.
[18] J.J. Ryan, J.A. Dupont, J. Assoc. Off. Anal. Chem. 57 (1974) 828.
[19] R. Bossuyt, R.V. Renterghem, G. Waes, J. Chromatogr. 124 (1976) 37.
[20] C.D. Gatsonis, C.A. Ageloudis, Pharmazie 37 (1982) 649.
[21] G.J. Kapadia, G.S. Rao, J. Pharm. Sci. 53 (1964) 223.
[22] Y. Nishida, E. Tsuchiya, S. Toyoshima, Yakugaku Zasshi 87 (1967) 516.
[23] U. Valcavi, A. Brandt, G.B. Corsi, F. Minoja, G. Pascucci, J. Antibiot. 34 (1981) 34.
[24] H. Oka, Y. Ikai, N. Kawamura, K. Uno, M. Yamada, K.-I. Harada, M. Uchiyama, H. Asukabe, M. Suzuki, J. Chromatogr. 393 (1987) 285.
[25] H. Oka, Y. Ikai, N. Kawamura, K. Uno, M. Yamada, K.-I. Harada, M. Suzuki, J. Chromatogr. 400 (1987) 253.
[26] Y. Ikai, H. Oka, N. Kawamura, M. Yamada, K.-I. Harada, M. Suzuki, J. Chromatogr. 411 (1987) 313.
[27] W. Naidong, S. Geelen, E. Roets, J. Hoogmartens, J. Pharm. Biomed. Anal. 8 (1990) 891.
[28] W. Naidong, C. Hauglustaine, E. Roets, J. Hoogmartens, J. Planar Chromatogr. 4 (1991) 63.
[29] D.L. Simmons, H.S.L. Woo, C.M. Koorengevel, P. Seers, J. Pharm. Sci. 55 (1966) 1313.
[30] E. Ragazzi, G. Veronese, J. Chromatogr. 132 (1977) 105.
[31] A. Szabó, M.K. Nagy, E. Tömörkény, J. Chromatogr. 151 (1978) 256.
[32] A.I.H. Omer, E.A.G. Kariem, R.B. Salama, J. Chromatogr. 205 (1981) 486.
[33] H. Oka, K. Uno, K.-I. Harada, Y. Kaneyama, M. Suzuki, J. Chromatogr. 260 (1983) 457.
[34] H. Oka, M. Suzuki, J. Chromatogr. 314 (1984) 303.
[35] H. Xie, C. Dong, Y.- L Fen, C.-S. Liu, Anal. Lett. 30 (1997) 79.
[36] N. Muhammad, J.A. Bodnar, J. Pharm. Sci. 69 (1980) 928.
[37] K. Dihuidi, E. Roets, J. Hoogmartens, H. Vanderhaeghe, J. Chromatogr. 246 (1982) 350.
[38] A.P. De Leenheer, H.J.C.F. Nelis, J. Pharm. Sci. 68 (1979) 999.
[39] H.J.C.F. Nelis, A.P. De Leenheer, J. Chromatogr. 195 (1980) 35.
[40] J.H. Knox, J. Jurand, J. Chromatogr. 186 (1979) 763.
[41] H.R. Howell, L.L. Rhodig, A.D. Sigler, J. Assoc. Off. Anal. Chem. 67 (1984) 572.
[42] L.A. Mitscher (Ed.), The Chemistry of the Tetracycline Antibiotics, 1st Edition, Marcel Dekker, New York, 1978, p. 58.
[43] H. Oka, K. Uno, K.-I. Harada, M. Suzuki, J. Chromatogr. 284 (1984) 227.
[44] E. Stahl (Ed.), Thin-Layer Chromatography, 2nd Edition, Springer–Verlag, Berlin, Heidelberg, 1969, p. 573.

[45] H. Oka, K. Uno, K.-I. Harada, M. Hayashi, M. Suzuki, J. Chromatogr. 295 (1983) 129.
[46] H. Oka, Y. Ikai, J. Hayakawa, K. Masuda, K.-I. Harada, M. Suzuki, V. Martz, J.D. MacNeil, J. Agric. Food Chem. 41 (1993) 410.
[47] H. Oka, Y. Ikai, J. Hayakawa, K. Masuda, K.-I. Harada, M. Suzuki, J. AOAC Int. 77 (1994) 891.
[48] H. Oka, Y. Ikai, J. Hayakawa, K.-I. Harada, K. Masuda, M. Suzuki, R. Himei, M. Horie, H. Nakazawa, J. Food Hyg. Soc. Jpn. 34 (1993) 517.
[49] C.-L. Chen, X. Gu, J. AOAC Int. 78 (1995) 1369.
[50] J. Tjørnelund, S.H. Hansen, J. Chromatogr. A 779 (1997) 235.
[51] T.S. Huang, W.X. Du, M.R. Marshall, C.I. Wei, J. Agric. Food Chem. 45 (1997) 2602.
[52] H. Oka, K. Uno, K.-I. Harada, K. Yasaka, M. Suzuki, J. Chromatogr. 298 (1984) 435.
[53] E.J. Mulders, D.V. De Lagemaat, J. Pharm. Biomed. Anal. 7 (1989) 1829.
[54] J.R. Walsh, L.V. Walker, J.J. Webber, J. Chromatogr. 596 (1992) 211.
[55] J.P. Sharma, E.G. Perkins, R.F. Bevill, J. Chromatogr. 134 (1977) 441.
[56] J.P. Sharma, R.F. Bevill, J. Chromatogr. 166 (1978) 213.
[57] G.D. Mack, R.B. Ashworth, J. Chromatogr. Sci. 16 (1978) 93.
[58] S. Eksborg, H. Ehrsson, U. Lönroth, J. Chromatogr. 185 (1979) 583.
[59] R. Böcker, J. Chromatogr. 187 (1980) 439.
[60] S. Eksborg, J. Chromatogr. 208 (1981) 78.
[61] S. Eksborg, B. Ekqvist, J. Chromatogr. 209 (1981) 161.
[62] U. Jürgens, Z. Lebensm, Unters. Forsch. 173 (1981) 356.
[63] J. Hermansson, J. Chromatogr. 232 (1982) 385.
[64] J. Hermansson, M. Andersson, J. Pharm. Sci. 71 (1982) 222.
[65] Y. Onji, M. Uno, K. Tanigawa, J. Assoc. Off. Anal. Chem. 67 (1984) 1135.
[66] H. Terada, M. Asanoma, Y. Sakabe, Eisei Kagaku 30 (1984) 138.
[67] Y. Hoshino, M. Horie, N. Nose, H. Iwasaki, J. Food Hyg. Soc. Jpn. 25 (1984) 430.
[68] K. Dihuidi, M.J. Kucharski, E. Roets, J. Hoogmartens, H. Vanderhaeghe, J. Chromatogr. 325 (1985) 413.
[69] M. Horie, Y. Hoshino, N. Nose, H. Iwasaki, H. Nakazawa, Eisei Kagaku 31 (1985) 371.
[70] G.S. Chappell, J.E. Houglum, J. Assoc. Off. Anal. Chem. 69 (1986) 28.
[71] K. Tyczkowska, A.L. Aronson, J. Assoc. Off. Anal. Chem. 69 (1986) 760.
[72] W.A. Moats, J. Chromatogr. 358 (1986) 253.
[73] K. Wolfs, E. Roets, J. Hoogmartens, H. Vanderhaeghe, J. Chromatogr. 358 (1986) 444.
[74] N.H. Khan, E. Roets, J. Hoogmartens, H. Vanderhaeghe, J. Chromatogr. 405 (1987) 229.
[75] I. Nordlander, H. Johnsson, B. Osterdahl, Food Addit. Contam. 4 (1987) 291.
[76] A. Rogstad, V. Hormazabal, M. Yndestad, J. Liq. Chromatogr. 11 (1988) 2337.
[77] M.E. Sheridan, G.S. Clarke, J. Chromatogr. 434 (1988) 253.
[78] K. Eivindvik, K.E. Rasmussen, J. Liq. Chromatogr. 12 (1989) 3061.
[79] V.L. Ellerbroek, Arch. Lebensmittelhyg. 40 (1989) 7.
[80] N.H. Khan, P. Wera, E. Roets, J. Hoogmartens, J. Liq. Chromatogr. 13 (1990) 1351.
[81] D.J. Fletouris, J.E. Psomas, N.A. Botsoglou, J. Agric. Food Chem. 38 (1990) 1913.
[82] T. Agasøster, K.E. Rasmussen, J. Chromatogr. 570 (1991) 99.
[83] W. Naidong, K. Verresen, E. Roets, J. Hoogmartens, J. Chromatogr. 586 (1991) 61.
[84] R.G. Aoyama, K.M. McErlane, H. Erber, J. Chromatogr. 588 (1991) 181.
[85] A.G. Butterfield, D.W. Hughes, W.L. Wilson, N.J. Pound, J. Pharm. Sci. 64 (1975) 316.
[86] R.F. Lindauer, D.M. Cohen, K.P. Munnelly, Anal. Chem. 48 (1976) 1731.
[87] J.P. Sharma, G.D. Koritz, E.G. Perkins, R.F. Bevill, J. Pharm. Sci. 66 (1977) 1319.
[88] B.G. Charles, J.J. Cole, P.J. Ravenscroft, J. Chromatogr. 222 (1981) 152.
[89] A. Aszalos, C. Hanneke, M.J. Hayden, J. Crawford, Chromatographia 15 (1982) 367.
[90] W.N. Barnes, A. Ray, I.J. Bates, J. Chromatogr. 347 (1985) 173.
[91] A. Aszalos, Chromatographia 20 (1985) 313.
[92] P. Sporns, S. Kwan, L.A. Roth, J. Food Prot. 49 (1986) 383.
[93] T. Galeano-Diaz, A. Guiberteau-Cabanillas, F. Salinas, Anal. Lett. 23 (1990) 607.
[94] K. Nakaya, Y. Kobayashi, N. Tanahashi, J. Food Hyg. Soc. Jpn. 32 (1991) 43.
[95] H. Oka, H. Matsumoto, K. Uno, K.-I. Harada, S. Kadowaki, M. Suzuki, J. Chromatogr. 325 (1985) 265.
[96] J.D. MacNeil, V.K. Martz, G.O. Korsrud, C.D.C. Salisbury, H. Oka, R.L. Epstein, C.J. Barnes, J. AOAC Int. 79 (1996) 405.
[97] H. Oka, Y. Ikai, N. Kawamura, K. Uno, M. Yamada, K.-I. Harada, M. Uchiyama, H. Asukabe, Y. Mori, M. Suzuki, J. Chromatogr. 389 (1987) 417.
[98] E. Martinez, W. Shimoda, J. Assoc. Off. Anal. Chem. 71 (1988) 477.
[99] H. Björklund, J. Chromatogr. 432 (1988) 381.
[100] E. Martinez, W. Shimoda, J. Assoc. Off. Anal. Chem. 72 (1989) 848.
[101] B. Iversen, A. Aanesrud, A.K. Kolstad, J. Chromatogr. 493 (1989) 217.
[102] A.R. Long, L.C. Hsieh, M.S. Malbrough, C.R. Short, S.A. Barker, J. Assoc. Off. Anal. Chem. 73 (1990) 379.
[103] G.J. Reimer, L.M. Young, J. Assoc. Off. Anal. Chem. 72 (1989) 813.
[104] A.R. Long, L.C. Hsieh, M.S. Malbrough, C.R. Short, S.A. Barker, J. Assoc. Off. Anal. Chem. 73 (1990) 864.
[105] W.H.H. Farrington, J. Tarbin, J. Bygrave, G. Shearer, Food Addit. Contam. 8 (1991) 55.
[106] P.J. Kijak, M.G. Leadbetter, M.H. Thomas, E.A. Thompson, Bio. Mass Spectrom. 20 (1991) 789.

[107] H. Oka, Y. Ikai, N. Kawamura, J. Hayakawa, J. Assoc. Off. Anal. Chem. 74 (1991) 894.
[108] G. Carignan, K. Carrier, S. Sved, J. Assoc. Off. Anal. Chem. 76 (1993) 325.
[109] M.C. Carson, J. AOAC Int. 76 (1993) 329.
[110] M.C. Carson, W. Breslyn, J. AOAC Int. 79 (1996) 29.
[111] H.J. Nelis, J. Vandenbranden, A. de Kruif, F. Belpaire, A.P. De Leenheer, J. Pharm. Sci. 81 (1992) 1216.
[112] W. Naidong, K. Vermeulen, I. Quintens, E. Roets, J. Hoogmartens, Chromatographia 33 (1992) 560.
[113] J.M. Degroodt, B. Wyhowski de Bukanski, S. Srebrnik, J. Liq. Chromatogr. 16 (1993) 3515.
[114] J. Sokol, E. Matisova, J. Chromatogr. A 669 (1994) 75.
[115] S. Croubels, C. Van Peteghem, W. Baeyens, Analyst 119 (1994) 2713.
[116] S. Croubels, W. Baeyens, C. Van Peteghem, Anal. Chim. Acta 303 (1995) 11.
[117] V.M. Moretti, G.L. Maggi, A. Albertini, F. Bellagamba, U. Luzzana, G. Serrini, F. Valfré, Analyst 119 (1994) 2749.
[118] M. Touraki, P. Rigas, P. Pergandas, C. Kastritsis, J. Chromatogr. B 663 (1995) 167.
[119] R.J. McCracken, W.J. Blanchflower, S.A. Haggan, D.G. Kennedy, Analyst 120 (1995) 1763.
[120] M.D.F. Santos, H. Vermeersch, J.P. Remon, M. Schelkens, P. De Backer, R. Ducatelle, F. Haesebrouck, J. Chromatogr. B 682 (1996) 301.
[121] E. Brandstetetová, P. Kubalec, L. Bovanová, P. Simko, A. Bednáriková, L. Macháckova, Z. Lebensm. Unters Forsch A 205 (1997) 311.
[122] S. Taguchi, S. Yoshida, N. Fukushima, S. Hori, J. Food Hyg. Soc. Jpn. 38 (1997) 259.
[123] S. Croubels, K.E.I. Vanoosthuyze, C. Van Peteghem, J. Chromatogr. B 690 (1997) 173.
[124] A. Posyniak, J. Zmudzki, S. Semeniuk, J. Niedzielska, R. Ellis, Biomed. Chromatogr. 12 (1998) 294.
[125] S. Croubels, H. Vermeersch, P. De Backer, M.D.F. Santos, J.P. Remon, C. Van Peteghem, J. Chromatogr. B 708 (1998) 145.
[126] M. Carson, M.A. Ngoh, S.W. Hadley, J. Chromatogr. B 712 (1998) 113.
[127] D.G. Kennedy, R.J. McCracken, M.P. Carely, W.J. Blanchflower, S.A. Hewitt, J. Chromatogr. A 812 (1998) 327.
[128] H. Nakazawa, S. Ino, K. Kato, T. Watanabe, Y. Ito, H. Oka, J. Chromatogr. B 732 (1999) 55.
[129] H. Oka, Y. Ikai, J. Hayakawa, K.-I. Harada, M. Suzuki, R. Himei, M. Horie, H. Nakazawa, J.D. MacNeil, J. Agric. Food Chem. 42 (1994) 2215.
[130] M. Horie, K. Saito, N. Nose, H. Nakazawa, J. Food Hyg. Soc. Jpn. 36 (1995) 293.
[131] R.W. Fedeniuk, S. Ramamurthi, A.R. McCurdy, J. Chromatogr. B 677 (1996) 291.
[132] Y.-R. Cheng, D.J. Phillips, U. Neue, Chromatographia 44 (1997) 187.
[133] H. Oka, Y. Ikai, Y. Ito, J. Hayakawa, K.-I. Harada, M. Suzuki, H. Odani, K. Maeda, J. Chromatogr. B 693 (1997) 337.
[134] W.J. Blanchflower, R.J. McCracken, D.A. Rice, Analyst 114 (1989) 421.
[135] G. Stubbings, J.A. Tarbin, G. Shearer, J. Chromatogr. B 679 (1996) 137.
[136] C.R. White, W.A. Moats, K.L. Kotula, J. AOAC Int. 76 (1993) 549.
[137] W.A. Moats, R. Harik-Khan, J. Agric. Food Chem. 43 (1995) 931.
[138] S. Horii, J. Liq. Chromatogr. 17 (1994) 213.
[139] Y. Yoneda, N. Furushiro, R. Andoh, J. Food Hyg. Soc. Jpn. 30 (1989) 42.
[140] S. Kawata, K. Sato, Y. Nishikawa, K. Iwama, J. AOAC Int. 79 (1996) 1463.
[141] K. Fujita, K. Ito, E. Araki, K. Tanno, M. Murayama, Y. Saito, J. Food Hyg. Soc. Jpn. 38 (1997) 12.
[142] W.J. Blanchflower, R.J. McCracken, S.A. Haggan, D.G. Kennedy, J. Chromatogr. B 692 (1997) 351.
[143] H. Oka, Y. Ito, Y. Ikai, T. Kagami, K.-I. Harada, J. Chromatogr. A 812 (1998) 309.
[144] H. Oka, J. Patterson, in: H. Oka, H. Nakazawa, K.-I. Harada, J.D. MacNeil (Eds.), Chemical Analysis of Antibiotics Used in Agriculture, AOAC International, Arlington, VA, 1995, pp. 333–406, Chapter 10.
[145] A. Weimann, G. Bojesen, J. Chromatogr. B 721 (1999) 47.
[146] H. Oka, K. Uno, K.-I. Harada, M. Suzuki, Yakugaku Zasshi 103 (1983) 531.
[147] M. Thomas, J. Assoc. Off. Anal. Chem. 72 (1989) 564.
[148] J.R. Meinertz, G.R. Stehly, W.H. Gingerich, J. AOAC Int. 81 (1998) 702.
[149] M. Fluop, A. Drapkin, New England J. Med. 19 (1965) 986.
[150] European Pharmacopoeia, Part II, Maisonneuve, France, 2nd Edition, 1993, p. 211.
[151] W. Naidong, S. Hua, E. Roets, J. Hoogmartens, J. Planar Chromatogr. 7 (1994) 267.
[152] J.S. Kang, S. Ebel, J. Planar Chromatogr. 2 (1989) 434.
[153] S. Croubels, W. Baeyens, C. Dewaele, C. Van Peteghem, J. Chromatogr. A 673 (1994) 267.
[154] M.F.M. Tavares, V.L. McGuffin, J. Chromatogr. A 686 (1994) 129.
[155] A.V. Schepdael, J. Saevels, X. Lepoudre, R. Kibaya, N.Z. Gang, E. Roets, J. Hoogmartens, J. High Resolut. Chromatogr. 18 (1995) 695.
[156] J.J. Pesek, M.T. Matyska, J. Chromatogr. A 736 (1996) 313.
[157] Y.M. Li, A. Van Schepdael, J. Hoogmartens, J. Liq. Chromatogr. 20 (1997) 273.
[158] Y.-C. Chen, E.-E. Lin, J. Chromatogr. A 802 (1998) 95.
[159] Y.M. Li, H. Moons, A. Van Schepdael, J. Hoogmartens, Chromatographia 48 (1998) 576.
[160] S.K. Yeo, H.K. Lee, S.F.Y. Li, J. Chromatogr. 585 (1991) 133.
[161] C.-X. Zhang, Z.-P. Sum, D.-K. Ling, Y.-J. Zhang, J. Chromatogr. 627 (1992) 281.
[162] J. Tjørnelund, S.H. Hansen, J. Chromatogr. A 737 (1996) 291.
[163] Y.M. Li, A. Van Schepdael, E. Roets, J. Hoogmartens, J. Chromatogr. A 740 (1996) 119.
[164] D. Mourot, B. Delépine, J. Boisseau, G. Gayot, J. Chromatogr. 190 (1980) 486.

[165] J.Y.C. Hon, L.R. Murry, J. Liq. Chromatogr. 5 (1982) 1973.
[166] J. Torel, J. Cillard, P. Cillard, M. Vie, J. Chromatogr. 330 (1985) 425.
[167] H.J.E.M. Reeuwijk, U.R. Tjaden, J. Chromatogr. 353 (1986) 339.
[168] N.H. Khan, E. Roets, J. Hoogmartens, H. Vanderhaeghe, J. Pharm. Biomed. Anal. 7 (1989) 339.
[169] W. Naidong, E. Roets, J. Hoogmartens, Chromatographia 30 (1990) 105.
[170] R. Ueno, K. Uno, T. Aoki, J. Chromatogr. 573 (1992) 333.
[171] C.R. White, W.A. Moats, K.L. Kotula, J. Liq. Chromatogr. 16 (1993) 2873.
[172] R.B. Ashworth, J. Assoc. Off. Anal. Chem. 68 (1985) 1013.
[173] R.H. Böcker, R. Peter, G. Machbert, W. Bauer, J. Chromatogr. 568 (1991) 363.
[174] J.X. Duggan, J. Liq. Chromatogr. 14 (1991) 2499.
[175] W.X. Du, M.R. Maeshall, W.B. Wheeler, M. Mathew, D. Gatlin, S.D. Rawles, D.-H. Xu, W.A. Rodgers, C.I. Wei, J. Food Sci. 60 (1995) 1220.
[176] A.G. Kazemifard, D.E. Moore, J. Pharm. Biomed. Anal. 16 (1997) 689.
[177] H. Pouliquen, D. Gouelo, M. Larhantec, N. Oilet, L. Pinault, J. Chromatogr. B 702 (1997) 157.
[178] A.D. Cooper, G.W.F. Stubbings, M. Kelly, J.A. Tarbin, W.H.H. Farrington, G. Shearer, J. Chromatogr. A 812 (1998) 321.
[179] H. Mascher, J. Chromatogr. A 512 (1998) 339.
[180] I. Gastearena, M.C. Dio-Viéiez, E. Segura, M.M. Goñi, M.J. Renedo, D. Fos, Chromatographia 35 (1993) 524.