The Establishment of a Method of Analysis of Tetracycline Residues in Meat

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Morenike Dipeolu and Yoshihiro Kanamaru

Department of Food Science, Faculty of Agriculture, Gifu University.

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SUMMARY

The need to establish a method of analysis of tetracycline residues in meat has been reported. One of the published methods was selected and tested. The method using solid-phase extraction (Bakerbond Octadecyl (C_{18}) cartridge) was found to be simple, accurate and sufficiently rapid for the analysis of a substantial number of samples per day. Recovery of tetracyclines from beef, beef liver, pork and chicken is high (50.4 - 81.7%). Although the peak pattern of tetracyclines differs in different tissues, this analytical method can be considered to be appropriate for the quantitative analysis of tetracycline residues in meat and meat products in a developing country like Nigeria.

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INTRODUCTION

Food adequacy, supply and related issues have been a major concern to consumers all over the world. While food quality is a major concern in developed countries of the world, concerns about the food supply and consumer access to food is more of an issue in developing countries.

Food standards and regulations are minimal and in some cases even non-existent, because the technological means to monitor food quality is often inadequate due to lack of funds. Food adulteration, therefore, constitutes a danger to the health of consumers.

In Nigeria, the danger of food adulteration is further exacerbated by the potential danger of drug residues present in animal products for human consumption. There, as in many other developing countries, antibiotics, vaccines and various other drugs and pesticides are freely used to combat animal diseases. Antibiotics have been and are still being freely used at low and subtherapeutic levels in poultry and livestock feeds as growth promoters. These agents, which ultimately end up in the food chain for human consumption, may occur as residues at undesirable levels, constituting a risk to consumers. There are two prime concerns hypersensitivity reactions and effects on human flora.

On a limited number of persons with hypersensitivity to antibiotics as penicillins, exposure to minute quantities of these drugs can have an immunologic impact. There is also the problem of the animals themselves acquiring resistant enteric flora which may in turn contribute to the human reservoir of coliforms and salmonellae resistant to antibiotics[1].

In Nigeria, quality control measures for meat and meat products are inadequate and, as there are no available data on drug residues in meat sold for human consumption, there is the need for surveillance of veterinary drugs in foods of animal origin.

Numerous methods have been discovered for the analysis of drugs in animal tissues. While microbiological assay methods lack specificity [2,3] and are difficult to quantify accurately, immunochemical methods are susceptible to interference from either related compounds or from a matrix constituent [4].

Chromatographic methods of analysis of drugs in food, however, offer a promising approach to the detection and identification of specific antibiotic residues. [5] The HPLC method has been found useful for the qualitative and quantitative analysis of drugs in food products of animal origin. However, according to

Petz (1990) [6], an ideal method for the analysis of veterinary drugs in animal tissues should be specific, sensitive, accurate, precise, inexpensive and suitable for automation. As there doesn't seem to be an existing method that fulfils all these requirements, the analyst's primary task is to select a method which meets as many of the analytical criteria as possible. The use of advanced and sophisticated technology such as that found in Japan for the surveillance of antibiotic residues in meat sold for human consumption would have been useful in Nigeria, however laws prohibiting the importation of meat and meat products into the country made this impossible. This necessitated the need for the establishment of an analytical method which is easy, not cumbersome, practical and appropriate for a developing country like Nigeria. To this end, we selected and tested a quantitative method from among many of the published works on the quantification of tetracycline residues in meat.

METHODS

The method of analysis of tetracycline in animal tissues described by Oka *et al*. (1985)[7] was adopted. Commercially available beef liver, beef, chicken and pork were used for the study. The tetracyclines used were oxytetracycline (OTC) as dihydrate, tetracycline (TC) and chlortetracycline (CTC) as hydrochloride (Sigma Chemical Co.). Standard tetracycline solutions are obtained by diluting each tetracycline (5 mg) in 1000ml of distilled water. Meat samples weighing 5g each were spiked with the tetracyclines (1 and 10 ppm) and blended three times with 20, 20 and 10 ml of 0.1 M Na_2 EDTA - McIlvaine buffer (pH 4.0) using a high speed blender. The sample was then centrifuged at 4000 rpm for 10min and filtered. The filtrate was applied on a Baker 10 C_{18} cartridge (No 7020-03, J.T. Baker Inc., Phillipsburg, NJ, USA), activated with methanol and water, and then washed with 20 ml of water. The tetracyclines were eluted with 10 ml of 0.01 M methanolic oxalic acid solution. For the determination of tetracyclines, 100 ml of each sample and the standard solution were injected into HPLC. HPLC equipped with a constant - flow pump (CCPD, Tosoh Co., Ltd.) was used, together with a variable wavelength UV detector (UVIDEC- 100 - IV Japan Spectroscopic Co., Ltd.) operated at 350 nm and 0.2 range. The procedure was performed at room temperature on a Lichrosorb RP - 18 (5 mm, 250 x 4.0 mm I.D., GL Science) column with a methanol-acetonitrile-0.01M oxalic acid solution of pH 2.0 (1:1.5:2.5) as the mobile phase, at a flow rate of 1.2 ml/min.

Various concentrations of the standard solutions were injected and the values of the peak areas were used in obtaining standard curves for the tetracyclines. These standard curves were used in calculating the concentration (c), of tetracyclines in the samples injected. The recovery of tetracyclines from the tissues was then calculated using the following formula :

$$\text{Recovery} = \frac{\text{Total volume of tissue extract (ml)}}{\text{quantity injected to HPLC (ml)}} \times \text{concentration (c)}$$

$$\text{Recovery (\%)} = \frac{\text{Recovery}}{\text{amount of tetracycline injected into tissue}} \times 100$$

RESULTS AND DISCUSSION

When a standard solution containing the authentic tetracyclines was analysed by HPLC, a satisfactory peak resolution was observed, as shown in Fig. 1. The figure also shows that the peaks of the tetracyclines (OTC, TC and CTC) in the tissues responded properly to the increase in the concentration of these antibiotics in the fortified tissues. These results indicate that this method is capable of analysing tetracycline residues in meat.

Since the original paper does not give any information on the effect of tissues on the HPLC profiles of extracted antibiotics, we examined four different kinds of tissues and compared their elution patterns. The chromatograms show that the peak patterns of these tetracyclines differ among the tissues tested. Peaks in the

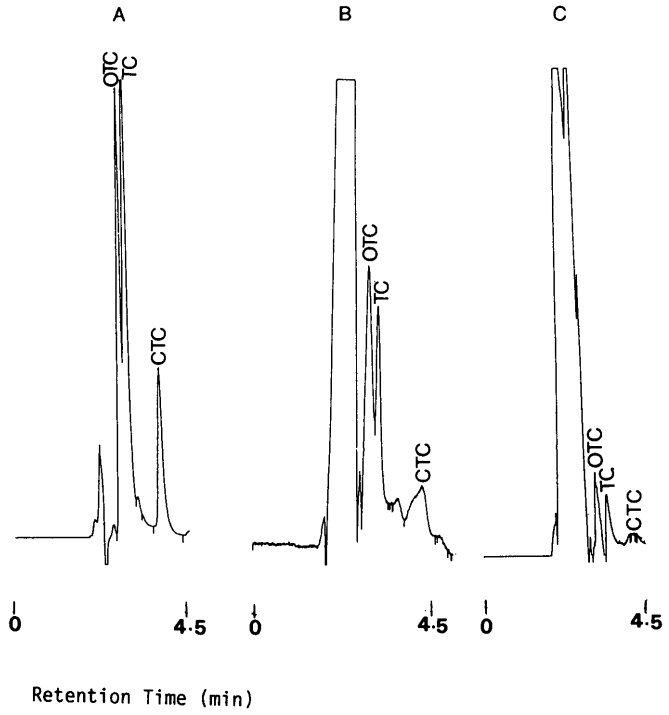

Fig. 1 Typical high-performance liquid chromatograms of meat extracts. (A), Standard of TCs (50ng); (B), fortified (10 ppm) chicken extract; (C), chicken extract (1 ppm).

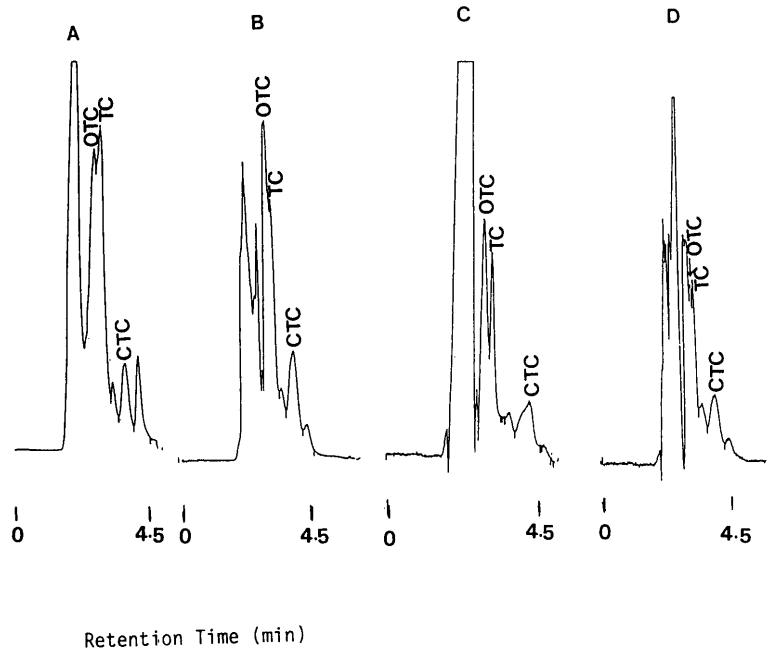

Fig.2 Typical high-performance liquid chromatograms of meat extracts. (A), fortified (10 ppm) beef liver extract; (B), beef extract (10 ppm); (C), chicken extract (10 ppm); (D), pork extract (10 ppm).

Table 1. Recovery of TCs from Fortified Tissues

Sample	Concentration (ppm)	Recovery (%) (standard deviation)		
		OTC	TC	CTC
Beef liver	1	68.8 (7.1)	64.9 (10.7)	76.4 (5.8)
Beef liver	10	71.5 (2.5)	81.7 (4.5)	79.2 (2.3)
Beef	1	66.8 (4.9)	72.2 (8.9)	63.8 (4.4)
Beef	10	60.9 (4.3)	69.0 (7.9)	66.6 (5.4)
Pork	1	61.6 (5.2)	72.3 (18.6)	80.0 (10.0)
Pork	10	68.3 (10.8)	58.3 (3.8)	64.1 (9.0)
Chicken	1	72.0 (8.4)	50.4 (4.5)	60.2 (1.8)
Chicken	10	77.6 (7.2)	78.9 (11.4)	76.5 (3.9)

Each value is the average for 3 samples.

liver samples seem to be higher than peaks recorded in other tissues (Fig. 2). This can be attributed to the structure of the liver. The liver parenchyma, which is less complicated than muscle matrix [8], may have contributed to facilitating the elution of tetracyclines from this tissue. It was also observed that there was a slight interference in the peaks of oxytetracycline and tetracycline. This interference, which was present in samples of liver, pork and beef was not observed in chicken samples.

While the peak of chlortetracycline is distinctly visible, it is very small. Chlortetracycline and deoxytetracyline have been shown to have relatively small peaks when compared to oxytetracycline and tetracycline [9]. In addition Onji *et al*. (1984) [3] used chlortetracycline at a concentration three times that of oxytetracycline and tetracycline to produce comparable peaks.

Tetracycline recovery from the tissues is high (50.4 - 81.7%), as summarized in Table 1. This is similar to the result of Oka *et al*. (1985)[7], who recorded a recovery of 67.5 - 94.9%.

In view of the high recovery of tetracyclines from fortified tissues and the strong absorption bands recorded (the differences in the peak patterns notwithstanding), this method can be said to be adequate for the analysis of tetracycline residues in meat.

The method was found to be simple and sufficiently rapid for the analysis of a substantial number of samples per day. The clean-up and extraction method is efficient and not cumbersome, which is highly desirable as these procedures are very important for the analysis of drug residues in meat in view of the complicated and delicate nature of the meat matrix [4].The analytical conditions of HPLC can also be considered to be favourable, and while the UV detector is highly sensitive, it is not overly sophisticated when compared with flourescence, mass spectometry and other forms of spectroscopic detection.

In view of the above, the described method for the analysis of tetracycline residues in meat appears appropriate for the quantitative analysis of tetracycline residues in meat and meat products in a developing country like Nigeria.

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肉に残存するテトラサイクリンの分析法について

ディオペル，M. A. ・金丸義敬

岐阜大学農学部食品科学講座

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要　　約

ナイジェリアのような発展途上国では，肉に残存している抗生物質の量を簡単，かつ迅速に調べる方法がきわめて重要である。われわれはテトラサイクリンについてすでに報告されている方法の中から一つを選択し，その有用性について調べてみた。この方法では，固相の抽出法を用いて抗生物質を肉から分離するが，その方法は単純で，しかも迅速であり，一度に多数の試料を正確に分析できると考えられる。牛肉，牛の肝臓，豚肉，鶏肉からの回収率も高かった（50.4－81.6%）。組織によってテトラサイクリンのピークのパターンは幾分異なっていたが，この方法はナイジェリアのような発展途上国での肉や肉製品中のテトラサイクリンの定量分析に適したものであると考えられる。

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