DETECTION OF OXYTETRACYCLINE HYDROCHLORIDE RESIDUES AND EFFECT OF BOILING IN COW'S MILK

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ABSTRACT

A total of twenty breed cows with clinical signs of respiratory disease and mastitis from different farms in Alexandria city were involved in this study. Oxytetracyclin Hydrochloride (OTC) (Pfizer Egypt) injected intramuscular as a therapeutic dose of 20 mg/kg body weight for three successive days. Milk samples were collected immediately before first drug administration and then other 24,48,72,96,120,144,168, 216, 264, 312, 360, 408 and 456hr).

All milk samples showed positive results for presence of (OTC) 24hr. after first dose with concentration value 3.05 ±0.86 mg/ml of milk and the concentration increased at 48,72,96hr then the level lowered 120 to 408 hr. till not detected at 456hr.

As a result of boiling of milk samples at 100^{o}C for 10 minutes (OTC) residues destroyed by heat. Its level significant decreased to 2.633±0.61 mg/ml after first day from administration with reduction rate (13.8) then lowered significantly with RR (30.5, 78.9, 81.2, 80.4, 79.3, 92.9) at 48,72, 96,120 and 144 hr. from first dose as a result of boiling (OTC) level not detected from 216 hr till end of experiment with reduction rat 100% .

الملخص العربى

الكشف عن بقايا الأوكسى تتراسيكلين هيدروكلوريد فى ألبان الأبقار وتأثير الغليان عليها

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ينتج عن إستخدام المضادات الحيوية فى مقاومة وعلاج الأمراض خاصة التنفسية منها والتهاب الضرع عند الأبقار عن تواجد بقاياها فى

ألبان ولحوم الأبقار المعالجة لذا أجريت هذه الدراسة للكشف عن بقايا مركب الأوكسى تتراسيكلين وتأثير الغليان عليه فى ألبان الأبقار المعالجة، وقد تمت الدراسة على ٢٠ من أبقار التربية والتى تعانى من أعراض تنفسية والتهاب الضرع فى مدينة الإسكندرية، حيث تم إعطاء جرعة علاجية بمقدار ٢٠ ملجم/كجم من وزن الجسم لمدة ٣ أيام متتالية.

وقد تم تجميع العينات قبل الجرعة الأولى وبعد ٢٤، ٤٨، ٧٢، ٩٦، ١٢٠، ١٤٤، ١٦٨، ٢١٦، ٢٦٤، ٣١٢، ٣٦٠، ٤٠٨، ٤٥٦ ساعة من الجرعة الأولى لقياس تركيز مركب الأوكسى تتراسكيلين من خلال استخدام جهاز (HPLC) جهاز الفصل الكروماتوجرفى الدقيق حيث أظهرت الدراسة أن جميع عينات اللبن تحتوى على المضاد الحيوى بعد ٢٤ ساعة من الجرعة الأولى (٥٥ر٣±٨٦ر٥) ملجم لكل مملتر من اللبن. وقد زاد التركيز عند ٤٨، ٧٢، ٩٦ ثم انخفض التركيز إلى أن تم إنتهاء آثاره تماماً من كل العينات، وقد زاد التركيز عند ٤٨، ٧٢، ٩٦ ثم انخفض التركيز إلى أن تم انتهاء آثاره تماماً من كل العينات، وقد أظهرت النتائج أن مستوى الإوكسى نتراسيكلين تناقص تدريجياً بمعدل اختزال () ٥ر٣٠ - ٩ر٧٨ - ٢ر٨١ - ٤ر٨٠ - ٣ر٧٩ وتم إنتهاء تماماً عند ٢١٦ ساعة وذلك من خلال غليان اللبن لمدة ١٠ دقائق.

INTRODUCTION

In most of the livestock production system, antibiotic are usually used for the prevention and treatment of animal diseases and to improve the efficacy of animal production (**Dipeolu, Along 2002 and Dina, Arowolo, 1991**). The most serious objections to the presence of drug residues in food intended for human consumption arise as a consequence of human health considerations. With the extensive use of drugs in animals production, residues of the parent drug and/or metabolites have a high potential to be present in the edible animal products. The public health significance of such adulteration of food supply is determined mainly by the level of the residues and individual drugs they are originated from (**Botsoglou and Fletouris, 2001**).

Antibiotic represent the most important group of exogenous inhibitory factors in milk. The most common being tetracyclines antibiotic are produced by Streptomyces spp. is known as broad-spectrum antibiotic with a bacteriostatic effect (**Pavlina, et al. 2003**). Oxytetrocycline has good activity against acute diseases cause by gram-positive and gram-negative bacteria, included spirochete species, Actinomyces species and myco plasma species. The antimicrobial drug widely used for the treatment of many acute diseases in cows, sheep and goat, like Oxytetracycline are likely to be lift as residues in milk or meat. Long acting formulation is an alternative to diseases therapy which normally require two or three daily parental treatment to maintain an efficacy level of antibiotic and to prolong is circulating antibiotic concentration of the acting agent.

Antibiotic residues had great side effects on public health which cause carcinogenicity. **Thomas, (1994)** suggested that there is a close association between antibiotic residues in food and breast cancer. **Qnifade and Babatude (1996)** declared that tetracycline residues was suspected to be carcinogenic by reaction with nitrile producing nitrosamine. One study explained the mechanism of carcinogenicity of antibiotic residues via damaged DNA may amplify the abnormality of (**Brookets, 1980**).

Clubb (1986) revealed that severed antibiotic residues has immunosuppression effect in human by interference with phagocytosis and immunoglobalin synthesis beside elimination of antigen.

Continuous injection of milk or dairy products with high or even low doses of antibiotic residues might induce emergency resistant strains of microorganism drug resistance has a definite complication factor in medical treatment especially young children **(Martin et al, 1996 and FAO/WHO, 1999)**.

Recent studies suggested that contamination of milk with a little antibiotic residues inhibit starter cultures of cheeses fermented dairy products, interfere with acid production, a high moisture content which results in poor quality body and texture, rapid proteolysis and off flavour **(Jensen, 1995, Heeschen & Harding 1995 and Hubbert et al, 1996)**.

Kurittu et al. (2002) used a luminescent Escherichia coli strain for the specific detection of tetracycline residues in raw borine milk. The detection limits for tetracycline, oxytertracycline, chlortetracycline, doxycline, methacycline, democlocycline were between 2 and 35 mg/ml.

Analyzed (OTC) by **Capolongo et al., (2002)** in edible tissues (muscle, liver, and kidney), of 18 turkeys were determined after continuous administration of the drug for 3 days in drinking water at maximum recommended concentration of 400 mg/L by using metal chelate affinity chromatography. They reported that after 1 day of withdrawal, OTC residues were detected in muscle and liver after 3 days after end of treatment.

Uno, (2002) studied the tissue distribution and residues depletion of (OTC) and oxolanic acid (OA). The author concluded that, residual (OTC) was reduced to 50-70 ug/ml in muscles by usual method of cooking, baking and frying. These results confirm that the cooking procedure could only reduce but not completely eliminate these drug residues in prawn.

The purpose of this study was to determine the presence of (OTC) oxytetracycline residues in milk from cows with clinical signs of respiratory and mastitis disease, being treated with (OTC). Milk sample were tested for (OTC) residues within the whole period of antibiotic intra muscular treatment (3d) as well as period after drug administration, in order to determine (OTC) residues in milk and also, to compare the effect of boiling of milk on these residues. HPLC method were used.

MATERIAL AND METHODS

Twenty breed cows with clinical signs of respiratory diseases and mastitis from different farms in Alexandria city were involved in this study.

oxytetracycline hydrochloride (OTC) long acting (Pfizer Egypt) injected intramuscular at does of 20mg/kg body weight for three successive days.

Milk samples were collected immediately before the first drug administration (0 hr) and then after (24, 48, 72, 96, 120, 144, 168, 216, 264, 312, 408 and 456 hr) the withdrawal period of OTC is 5 days.

Equal volumes of fore milk from each quarter of the same cow were taken during milking in the morning and in the afternoon and then mixed together, each sample was classified into 2 portion one part for detection of (OTC) residues, and other part were boiled at (100^{o}C) for 10 minutes.

Method

Oxytetracycline residues in milk samples, were detected according to **Jarunee, (2002)** using high performance liquid chromatography (HPLC).

Reagent

I. Standard oxytetracycline 100%.
II. Oxytetracycline hydrochloride purchased (Pfizer Egypt).
III. Solvents:

a- A cetonitric and methanol were HPLC grade.
b- Mcllvaine-EDTA-NACl buffer prepared according to the method (AOAC, 1997) (1997).

Apparatus

a- Refrigerating centrifuges, (Ijniversal I6R, Hettich Germany).
b- Vortex mixer (model G 6560 8. Genie scientific USA).
c- Water bath (model FEDIIS, WTB Binel-er, Germany).
d- Thermocouple.
e- High performance liquid chromatograph consists of a spectral system binary pump model P2000, vacuum membrane Pegasser, Aspecteral Detector model UV 2000 set at 350 Rheodyne 7125 sampling valve with a loop and a computer for control and handing.

RESULTS AND DISCUSSION

The results of HPLC analysis shown in Table (1), Fig. (1), revealed that all milk samples give positive results for presence of (OTC) residues twenty-four hours after first dose of drug administration. (3.05±0.86 mg/ml of milk) however average (OTC) concentration in milk increased at (48,72,96hr) from first dose with mean value of (4.60±0.90, 11.12±0.82 and 11.6±1.24 mg/ml of milk. Then, (OTC) level lowered at 120,144,168,216,264,312,360and 408 from first dose till not detected at 456 hr. with respectively mean values (8.31±0.96, 4.03±1.01, 3.32±2.02, 2.73±1.75, 1.66±1.06, 1.08±0.68, 0.65±0.39, 0.56±0.32 mg/ml) of milk. Form Table (1), Figs. (1,2,3) the highest concentration level was observed at 96 hr after beginning of therapy (withdrawal time 5 days). Our results were inagreement with that of **Pavlina, et al. (2003)** who reported that the highest average concentration of 195.86 mg OTC mg kg was detected by HPLC method 5 days after the first drug administration. **Jarunee (2002)**, reported that all milk samples showed positive results for presence of antibiotic residues (OTC) twenty four hours (7.3 mg/ml) after first drug administration and showed the highest concentration level at 96-120 hr (withdrawal 5 days). **Cinquina et al., 2007** reported that the withdrawal time for long acting (OTC) in goat (6 days) and in sheep milk (7 days).

Dinsmore et al (1996) revealed that (OTC) was detected in milk of all cases during treatment at maximum concentration ranging from 47 to 1.800 j 91.kg (ppb). ruination of (OTC) residues after the last infusion ranged from zero to144hours (6 days). milk obtained from cows that are treated for retained fetal

membranes by intra uterine infusion of (OTC) may contain the drug for variables lengths of time. Milk should be discarded to avoid illegal residues.

Rule, et al. (2001) determined the two commercial preparations of oxytetracycline (OTC) the pharmacokinetic behaviour, the presence of detectable milk residues and the penetration of milk of OTC administered by intravenous (IV) and intramuscular (IM) routes in goats producing milk. The authors concluded that, the presence of detectable residues in milk indicates that milk should not be used for human consumption for 2 and 3 days after administration of conventional and long acting formulations, respectively.

Schollibaum (1990) investigated the factors responsible for antibiotic contamination of milk he found that 59% and 41% of tested samples due to post secretion and secretory contamination, respectively. **Both (1982)** observed that the use of intrmamary infusion antibiotics constituted 92% of secretary contamination. Treatment of mastitic cases with penicillin G intramammary associated withdrawal time ranged from 2-18 days due to slow elimination of antibiotic by certain lactating cases.

Kurek et al., (1982) noticed that quantitative detection of penicillin in bulk milk samples (0.025-3-1/u/ml) was more than those obtained from individual

Jacques et al. (1998) reported that one of 973 milk sample was suspect for tetracycline residues by means of B. Calidolactis tube test as well as by the receptor assay 8 other samples were also considered to be positive.

Boiling it is of some interest to determine if residues of antibiotic can be destroyed by cooking procedures. The effect of heating at $100^{O}C$ for 10 min. on OTC residues was shown in Table (2), Fig. (1,3).

Our result showed that there were significant deference at ($p<0.05$) between non heated and heated samples. OTC concentration in heated milk was (2.63 ± 0.61 mg/ml) of milk after 24hr from drug administration with reduction rat 13.8%. Level of (OTC) residue was 3.2 ± 0.83 at 48 hr and 2.62 ± 0.44 at 72 hr, 2.31 ± 0.32 at 96hr after boiling with highly significant reduction rat (RR) 30.5, 78.9, 81.2, respectively. Level was decreased to $1.63\pm$ 0.35 at 120 hr, 90.0 ± 0.54 and 0.22 ± 0.13 at 144 and 198 hr. till not detected at 216 hr. from first day of drug administration, with significant reduction rate (80.4, 79.3, 92.9) Table (2), Fig. (4).

A few reports have been published about the effect of heating on the stability of OTC in milk. **Jarunee, (2002)** evoked that there were significant deference in (OTC), (TC) concentration as a result of milk heated to 63^{o} for 30 minutes with reduction rate of (OTC) spiked in milk at 200-300 ppb were 86.77 and 19.63 respectively and OTC was Completely reduced by 190 min. at $71^{O}C$ indicating that longer heat or sever heating was required to reduce antibiotic residues in milk.

(OTC) are similar to other antibiotics in their distribution and activity. **Scheibner (1972)** concluded that high concentration of penicillin G and oxytetracycline were com-

pletely in activated by an hour heating at 20°C.

El-Sherbini, et al., (2003), showed that thermal degradation of penicillin by pasteurization (63°C/30 min), boiling (100°C/5min) and sterizations 121°C/min were 40%,60% and 91% respectively.

The rate of inactivation of antibiotic by heat and pasteurization nearly similar that was reported by **Konecy (1978)**. Other investigators found that thermal inactivation rate of penicillin ranged from 8 to 26.5% (Tropilo et al., 1985 and Abd El-Alim et al., 1994). On the other hand few studies concluded that penicillin was not in activated by pasteurization or drying.

Adel and William, (1994): Reported that when meat was packed in a sausage casing and cooking in boiling water, (OTC) was reduced 95% in 30 minutes. Microwave cooking to well done (no red color) required of minutes gave a final temperature 98-102° and reduced level 60% from control. Frying required 8 minutes with final temperature 81°C residues reduced to 17.3%. Degeneration was related to final temperature reached, which was higher from microwave cooking or for boiling than for frying.

Our result of thermal inactivation of antibiotic by boiling nearly similar those reported by **Hamann et al., (1978)**. They observed that boiling was inactivated penicillin with high percentage (85%) a lower percentage of thermal inactivation was stated by **tropilo et al., (1985)**.

Administraion of antibiotic to lactating cows for treatment or prophylaxis lead to transferring of drug residues or their metabolites to human through consumption of contaminated milk or dairy products. These antibiotics may be found as it is or may present as metabolites with a modified structure and quit different in pharmaco-toxicological properties of antibiotic. Tetracyclines mainly absorbed from upper small intestine. Divalent calcium of milk and dairy products interfere with their absorption. As a result of chelation with calcium they tend to be deposited in the growth zone of teeth causing unpleasant pigmentation (fluorescence, yellow, brown discolouration).

This result also suggested that boiling for 10 min. of milk could caused a reduction of drug residue but it did not completely eliminated.

Shahani (1997), studied heat stability of drug residues in milk and reported that OTC was less heat stable than CTC and both were not completely reduced by 30 min. at 62 and 71°C only 23.6-35.6% reduction for OCT.

Table (1): Oxytetracycline residues (mg/ml of milk) in cow's milk after intramuscular administration at a dose of (20 mg/Kg B.W.) for three successive days.

Period after OTC Administration	Before boiling		
	min	max	Mean±S.E
24 hr.	1.561	4.541	3.05±0.86
48 hr.	2.96	6.045	4.60±0.90
72 hr.	9.706	12.53	11.12±0.82
96 hr.	9.89	14.01	11.61±1.24
120 hr.	6.65	9.96	8.31±0.96
144 hr.	2.28	5.79	4.03±1.01
168 hr.	0	6.982	3.32±2.02
216 hr.	0	5.99	2.73±1.75
264 hr.	0	3.643	1.66±1.06
312 hr.	0	2.32	1.08±0.68
360 hr.	0	1.345	0.65±0.39
408 hr.	0	1.09	0.56±0.32
456 hr.	0	0	0±0

* Statistically significant at $p < 0.05$ ** Statistically significant at $p < 0.01$

Table (2) : Effect of boiling on tetracycline residue (mg/ml of milk) after intramuscular administration at dose of (20 mg/kg B.W) for three successive days.

Period after OTC Administration	After boiling			R.R.	t-test
	min	max	Mean±S.E		
24 hr.	1.57	3.69	2.63±0.61	13.8	1.696
48 hr.	1.763	4.63	3.20±0.83	30.5	11.977**
72 hr.	2.031	3.469	2.62±0.44	78.9	20.149**
96 hr.	1.92	2.95	2.31±0.32	81.2	10.136**
120 hr.	1.021	2.239	1.63±0.35	80.4	11.056**
144 hr.	0	1.864	0.90±0.54	79.3	6.574*
168 hr.	0	0.450	0.22±0.13	92.9	1.640
216 hr.	0	0	0±0	100	1.558
264 hr.	0	0	0±0	100	1.562
312 hr.	0	0	0±0	100	1.602
360 hr.	0	0	0±0	100	1.668
408 hr.	0	0	0±0	100	1.765
456 hr.	0	0	0±0	0	-

* Statistically significant at $p < 0.05$ ** Statistically significant at $p < 0.01$ R.R: Reduction rat.

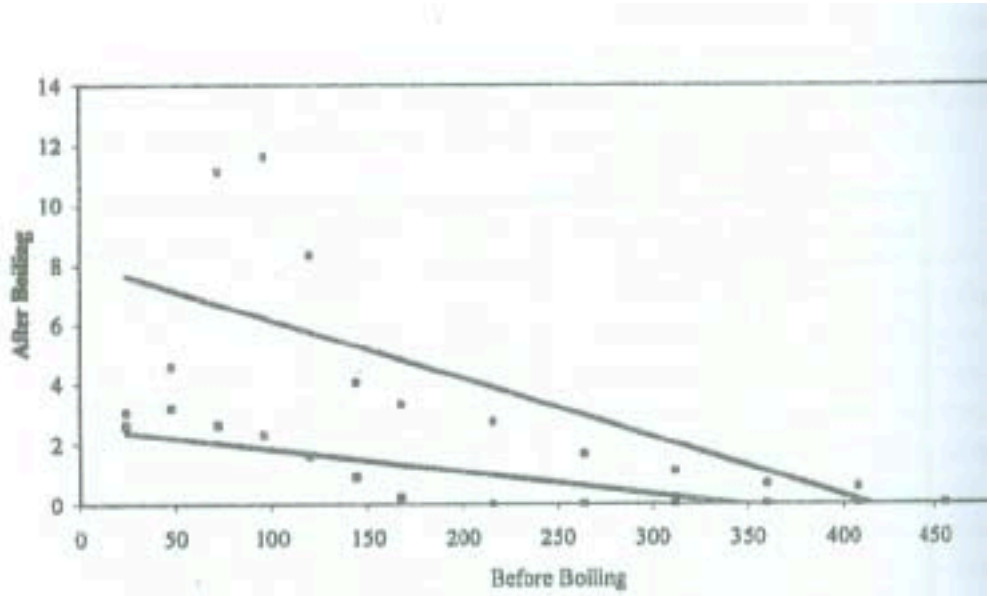

Figure (1): Oxytetracycline residues and the effect of boiling in cow's milk after intramuscular administration at a dose of (20 mg/Kg B.W.) for three successive days

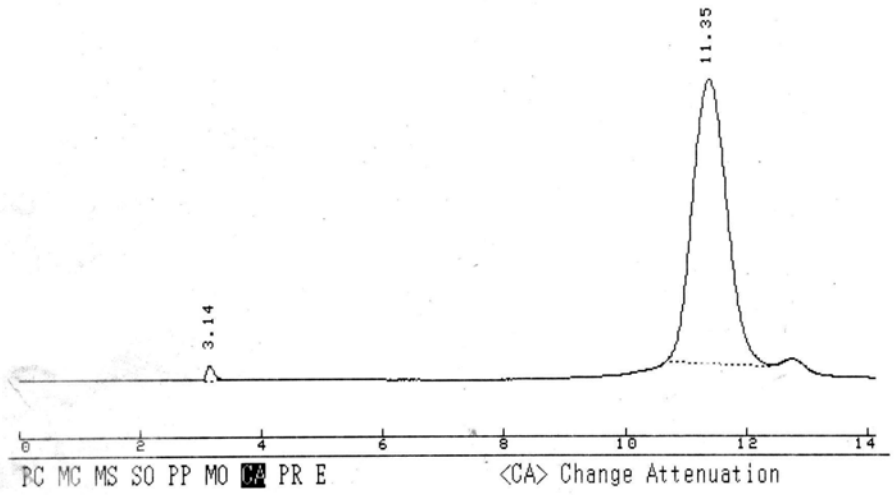

Figure (2): Chromatograph of standard oxytetracyclin (mg/ml).

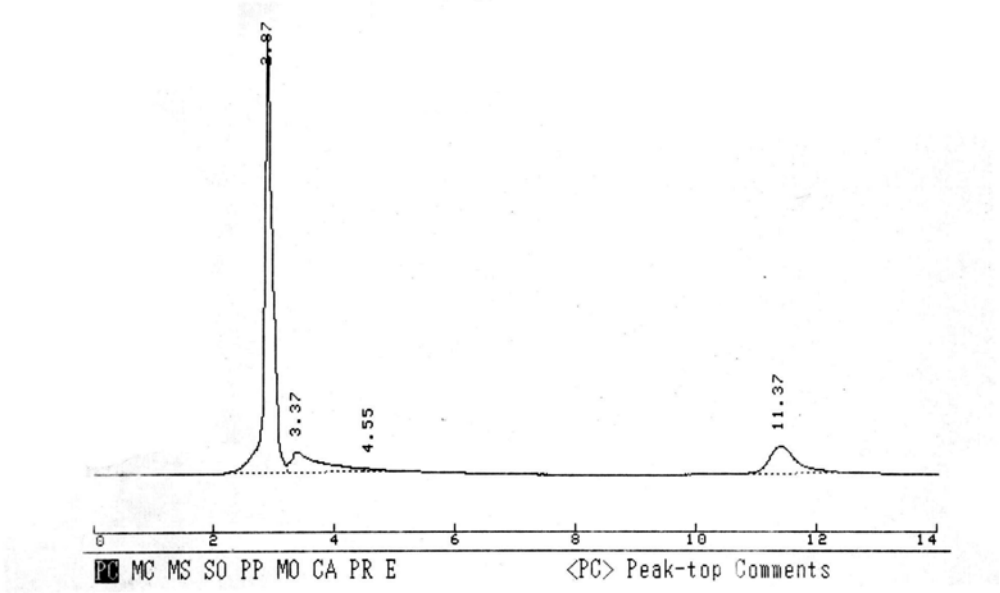

Figure (3): Chromatograms of oxytetracycline concentration mg/ml of milk after 24 hr from first day of drug administration .

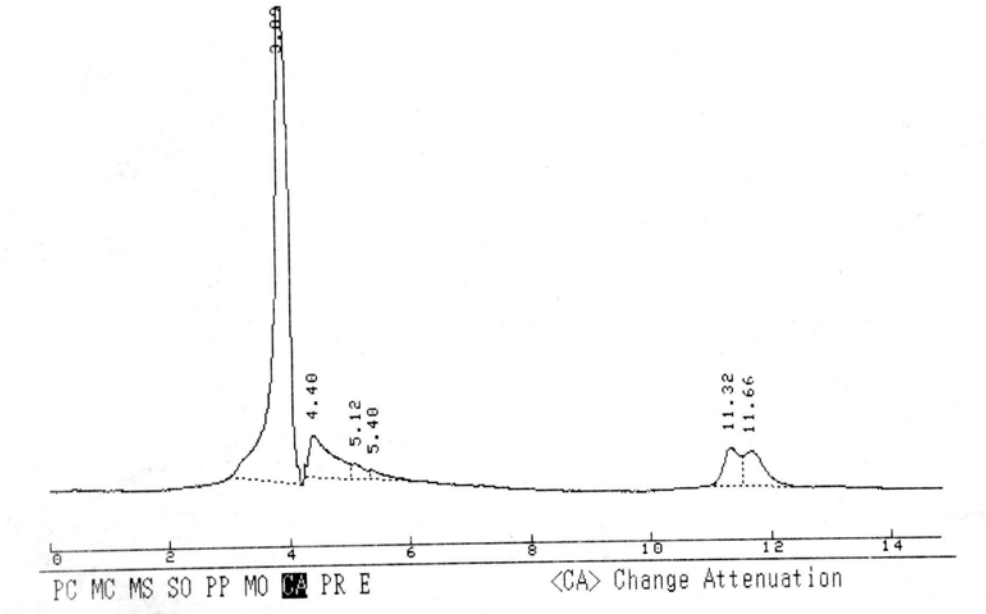

Figure (4): Chromatograms of oxytetracyclin concentration after boiling of milk at 24 hr from drug administration.

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