Thesis title : Application of Thin-Layer Bioautography in Analysis of the Combined Antibiotics in Feeds

Name : Rith Watthanachaiyingcharoen

Degree : Master of science (Pharmacy)

Thesis Supervisory Committee

Sumarn Saraya, Ph.D.

Weena Jiratchariyakul, Ph.D.

Cherdchai Thiratinrat, B.Sc.

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ABSTRACT

Thin-layer bioautography was used for the identification of 11 antibiotics in animal feeds. The technique was based on the selective feed extraction, and the separation was performed on the thin-layer chromatography. The antibiotics in feeds were extracted with methanol and methanol-HCl (98+2). The methanol extract was further extracted with chloroform in order to isolate groups of antibiotics. Three extracts were then obtained i.e. methanol, methanol-HCl and chloroform extracts. The methanol and chloroform extracts were spotted on the silica gel plate (Whatman LK6D), while the methanol-HCl (98+2) extract was spotted on the microcrystalline cellulose plate (Whatman PK2F). The solvent systems used for the separation on the silica gel plate was di-isopropyl ether/methanol/25% ammonia solution (75:35:2), and on the microcrystalline cellulose plate was acetone/chloroform/ n-propanol/impregnation liquid (20:20:30:20). The developed plates were placed on the TLC trough and laid by the medium inoculated with *Micrococcus lutea* ATCC 9341. The TLC plates were left overnight at room temperature. The fixing solution were then poured on to the TLC plates to make the clear

inhibition zones. The background became red while the zones of inhibition were translucent. The characteristics of inhibition zones were used to identify the antibiotics in feeds. The hRf values of tetracycline, oxytetracycline, chlortetracycline, spiramycin, erythromycin, tylosin, oleandomycin, virginiamycin, bacitracin·zinc, lasalocid·sodium, lincomycin were 43, 38, 52, 40, 63, 88, 52, 82, 0, 96 and 65.

In this study, the coefficient of variation at each concentration were less than 0.05.

The determination of antibiotics in twenty-five animal feed samples by thin-layer bioautography showed that there were seven samples containing antibiotics (four samples of chlortetracycline, one sample of oxytetracycline and two samples of virginiamycin).