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Study on screening and confirming the novel beta agonists abused in animal production

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Illegal use of beta agonist drugs as grow promoter and lean maker threatens safety of animal borne food in China and other countries. In most countries beta agonist drugs are banned for feed additives in animal production. In China, screening and confirming methods for detecting known beta agonist drugs such as clenbuterol, ractopamine, salbutamol, have been published as national standards. Survey for those drugs is covered in national residue plan, more than 20000 samples are token from farmers, feed mills and slaughterhouses for analysis. In order to avoid detected and punished, novel drugs are triggered to study and use in animal production, since there is no method for detecting them.

In 2010, a novel beta agonist drug was suspected in more than 100 swine farmers, where urines had positive results with ractopamine ELISA kit, but no known agonists were confirmed by HPLC tadem mass, compared to all drugs in mass bank. A preparative HPLC was used to purified a doubt feed premix, a component was got with purification of 98.5%, molecular ion peak showed its weight of 344.15 D. Element analysis showed that it consisted

of C, H, O, N, and its molecule was considered as C19H24N2O4. A ion trap HPLC-MS was used to study their fragments from first to eighth grade ions, finally two possible molecular structure were figured out. NMR was used to fit the possible molecular structure, and confirmed that its structure of 2-(4-(nitrophenyl)butan-2-ylamino)-1-(4-methoxyphenyl) thanol and named as . phenylethanolamine A. After identifying its structure, a quantity method of HPLC tadem mass spectrometry was developed, their recoveries ranged 74.4%~97% for spiked samples with 1ug/kg to 50ug/kg, its LOQ was 1ug/kg.

In order to develop a screening method for detecting all beta agonist drugs, we expressed ß2 Adrenergic receptor in E.coli or mammalian cells and purified it, then develop a screening kit similar to ELISA, by using ß2AR instead of antibody. A fusion protein with hydrophilic maltose-binding protein(MalE) at N termimal and His tag at C terminal, constructed three expressed vectors pMAL-p2x-ß2AR(SX), pMAL-p2x-ß2AR(EX), pMAL-p2x -ß2AR(EX-62), which were expressed in E. coli with activity of binding adrenergic ligands. For increasing expression level, a mammalian system, HKE293 cells, was used for expressed pCDNA3.1+-ß-G, its activity was measured by saturation binding assay using the ß2-AR antagonist iodocyanopindolol ([3H]ICYP). The purified ß2AR and crude membranc protein were coated to a plate, then blocked with BSA, meanwhile clenbuterol conjugated with HRP was synthesized, then screening kit was established. The ranges of recovery of spiked urines with concentration of 1ng/mL,10ng/mL,100ng/mL were between 40% and 70%.