



Determination of sedatives and adrenergic blockers in blood meal using accelerated solvent extraction and Orbitrap mass spectrometry

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ABSTRACT

The detection of veterinary drugs in blood meal is needed since it is used as an environment-friendly agricultural material despite its origination from animal blood. A method using accelerated solvent extraction and liquid chromatographic linear ion trap quadrupole Orbitrap mass spectrometry was developed to determine sedatives and adrenergic blockers in blood meal. The determination method was established following optimizations of accelerated solvent extraction, dispersive solid-phase extraction and high resolution mass spectrometric detection. Linearity, sensitivity, accuracy, repeatability and reproducibility of the method were fully validated. The method was applied to commercial blood meal products.

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1. Introduction

Raising animals in the contemporary livestock industry requires various veterinary drugs including antibiotics, anthelmintics, growth promoters (including anabolic steroids, thyrostats, β -agonists, corticosteroids, etc.), coccidiostats, anti-inflammatory drugs, sedatives, insecticides and disinfectants [1]. The use of xenobiotic compounds in animal husbandry has given rise to consumer anxieties regarding residual risk and food safety. Maximum residue limits (MRLs) and withdrawal times of veterinary drugs were established to minimize these potential problems. Sedatives and adrenergic blockers are injected right before transport of the animals to reduce the stress of animals generated during handling and transport to the slaughterhouse [1,2]. Thus, animal tissues have become main samples for residue analysis and food safety for sedatives and adrenergic blockers [2–5]. However, it cannot be ignored that the waste, not only from animal living spaces, but also from the slaughterhouse is reused in agricultural practices following its processing.

The use of agrochemicals, such as pesticides, fertilizers and veterinary drugs, has raised concerns for contamination of foods as well as of the environment. The public hope for healthy living has raised demand for healthy and safe foods, and organic agriculture has received much attention as a new alternative to help achieve these goals. Organic agriculture is a holistic production management system which promotes and enhances agroecosystem health, including biodiversity, biological cycles and soil biological activity. It is performed over all stages from production, preparation, storage and transport to marketing [6]. The production in organic agriculture is closely related to environmentally friendly agriculture, which encourages the use of the byproducts of agriculture, stockbreeding, or forestry instead of chemical fertilizers. However, the reuse of the byproducts polluted by synthetic chemicals is liable to lead to secondary environmental pollution. Even though blood derived from animals which were injected with sedatives and/or adrenergic blockers has a relatively high potential for residues, it is processed into blood meal and used as an environment-friendly agricultural material (EFAM). The Republic of Korea has legislated relevant regulations (Environmentally friendly agriculture fosterage act, Act No. 9623) for safe and dependable environmentally friendly agriculture. It is also noted that raw materials for the EFAMs should not contain synthetic chemicals. However, more specific regulations and active pre- and post-inspection activities are still necessary. It is important to determine the amounts of sedatives and adrenergic blockers in blood meal so that it can be used as a

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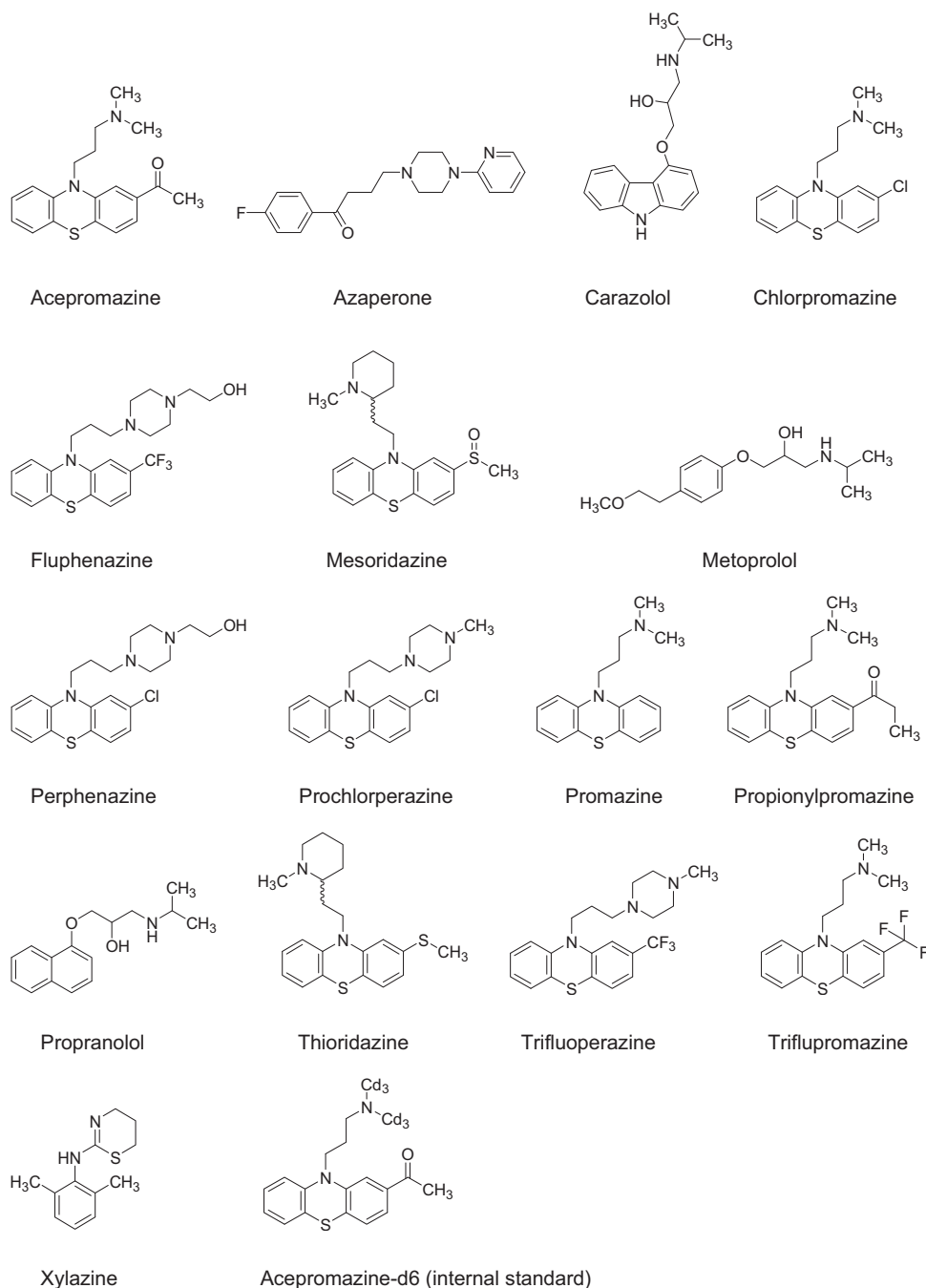


Fig. 1. The chemical structures of sedatives, adrenergic blockers and internal standard tested.

real EFAM. In this study, accelerated solvent extraction (ASE) and liquid chromatography (LC) coupled to linear ion trap quadrupole Orbitrap mass spectrometry (MS) have been employed to determine the sixteen sedatives and adrenergic blockers in powdered blood meal formulas.

2. Experimental

2.1. Chemicals and reagents

The sixteen compounds tested were as follows, and their chemical structures are drawn in Fig. 1—sedatives: acepromazine, azaperone, chlorpromazine, fluphenazine, mesoridazine, perphenazine,

prochlorperazine, promazine, propionylpromazine, thioridazine, triflupromazine, trifluoperazine; α -adrenergic blocker: xylazine; β -adrenergic blockers: carazolol, metoprolol, propranolol. Acepromazine maleate (purity 98%), carazolol ($\geq 98.5\%$), chlorpromazine hydrochloride (95%), fluphenazine dimaleate ($\geq 90\%$), mesoridazine benzenesulfonate ($\geq 98\%$), perphenazine, prochlorperazine dimaleate, promazine hydrochloride (99.9%) propionylpromazine hydrochloride (99.9%), ($\pm$)-propranolol hydrochloride ($\geq 99\%$), thioridazine hydrochloride ($\geq 99\%$), trifluoperazine dihydrochloride ($\geq 99\%$), triflupromazine hydrochloride (99.7%), xylazine (99%) and acepromazine-d6 hydrochloride (99.6%; internal standard, IS) were purchased from Sigma-Aldrich (Taufkirchen, Germany). Azaperone (98.5%) and metoprolol fumarate were supplied from Dr. Ehrenstorfer (Augsburg, Germany) and US Pharmacopeial

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