Rapid determination of estradiol in milchigs based on Molecular Imprinting Technology by using estradiol selective silicon magnetic composite

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Abstract: A rapid determination of estradiol is formed. Estradiol Molecular Imprinting Technology, silicon magnetic composite and UV-Visible spectrometry are well applied in this technique. Silicon magnetic composite is modified by Molecular Imprinting Technology, estradiol imprints on the layer successfully. The modified magnetic composite has a specific binding with estradiol. The complex is treated by eluent, the eluent is determined at 280 nm by UV-Visible spectroscopy. Rapid determination of estradiol is realized ultimately. In the analyses of estradiol in milk powder, limit of this research is 1.25 μg/kg, recovery reaches 99.6% in lower concentration.

1. Introduction
Estradiol is a kind of steroid estrogens which is used extensively in the animal husbandry because of it is good for animals’ growth. But it may lead to residual in the food by abuse or without withdrawal time. Those foods such as milk, meat should be detected strictly, if not sexual precocity or female human tumor may be occurred by intake. Adding estradiol in forage and water was forbidden by Ministry of Agriculture and Rural Affairs of the People’s Republic of China in 2007(No 176 Public Announcement)[1]. Current measurements of estradiol are long time-consuming[2][3], a new and quick detection method is urgently needed.

2. Materials and Methods
2.1 Instruments and Reagents
UV-Visible Spectrometer (UV-2550, Shimadzu Corporation); Liquid Chromatography (1260 Infinity, Agilent Technologies); Digital water bath oscillator (SHA-C, Guohua Instrument).
Acetonitrile (HLPC, Merck KGaA); Methanol (HLPC, Merck KGaA); Ethanol (AR, Sinopharm Chemical Reagent Co., Ltd); Ethyl acetate (AR, Sinopharm Chemical Reagent Co., Ltd); Acetone (HLPC, Merck KGaA); Cyclohexane (AR, Sinopharm Chemical Reagent Co., Ltd); n-Hexane (AR, Sinopharm Chemical Reagent Co., Ltd); Acetic Acid (AR, Sinopharm Chemical Reagent Co., Ltd); Formic acid (AR, Sinopharm Chemical Reagent Co., Ltd); 2-(N-Morpholino) ethanesulfonic acid (MES, AR, Sinopharm Chemical Reagent Co., Ltd); Dibasic sodium phosphate (AR, Sinopharm Chemical Reagent Co., Ltd); Potassium dihydrogen phosphate (AR, Sinopharm Chemical Reagent Co., Ltd); Sodium hydroxide (AR, Sinopharm Chemical Reagent Co., Ltd); Potassium chloride (AR, Sinopharm Chemical Reagent Co., Ltd); Sodium chloride (AR, Sinopharm Chemical Reagent Co., Ltd);
Ltd); Hydrochloric acid (AR, Sinopharm Chemical Reagent Co., Ltd); Glutaric dialdehyde (BR, Sinopharm Chemical Reagent Co., Ltd); Sodium hydroxide (AR, Sinopharm Chemical Reagent Co., Ltd); 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, ≥99.0%, Sigma-Aldrich Co.); N-Hydroxysuccinimide (NHS, 98%, Sigma-Aldrich Co.); Silane coupling agent KH-570 (≥98.0%, Sinopharm Chemical Reagent Co., Ltd); SiO₂ magnetic microsphere (50 mg/mL, 1 μm, Bio Mag beads); Estradiol (99.5%, Shanghai Aladdin Biochemical Technology Co., Ltd); Milk powder (900 g/can, Abbott laboratories).

2.2 Modify silicon magnetic composite
Five milliliters of SiO₂ magnetic microsphere was transferred into the tube, then it was placed on the magnetic separator to discard supernate, 200 mL of cyclohexane and 20 mL of hydrochloric acid (1 mol/L) were mixed for 30 minutes (No 1 solution). 5 mL of KH-570 and 200 mg of estradiol were dissolved in 20 mL of cyclohexane (No 2 solution). Those solutions chemically reacted at 70°C for 8 hours, modified magnetic microsphere and its supernate were separated when it was cool, it was washed by ethylalcohol and deionized water respectively until estradiol was not detected and stored at 4 °C.

2.3 Imprinted SiO₂ magnetic microsphere couples estradiol
Imprinted SiO₂ magnetic microsphere was dispersed in 4 mL of buffer, then it was marked with 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 mL of estradiol standard solution (100 μg/L). Mixtures were vortexed for 1 min after adding 1.5 mg of NHS and 1.0 mg of EDC and shaken for 2 hrs at room temperature. The supernate and eluent were determined respectively.

2.4 Treatment and determination of sample
Four grams negative milk powder was dissolved in 4 mL of ethanol, suspensions were marked with different levels of estradiol standard solution, then 100 μL of magnetic microsphere was mixed uniformly. The supernate and eluent were determined respectively after magnetic separation.

3. Results and Discussions

3.1 Maximum absorption and standard curve
The results indicates that estradiol standard solution had maximum absorption at 280 nm, the limit of this method is 0.3 μ g/L, Signal to Noise Ratio(SNR) is three at this concentration.

![Figure 1 Standard curve of estradiol](image)
3.2 Optimization of buffers
In order to investigate influence factors of buffer, phosphate buffer (PBS), MES buffer (MES) and glutaraldehyde buffer (GA) were utilized in this section. Results in Table 1 displays that MES buffer has higher function in the determination of estradiol, PBS and GA buffer were lower. Obviously, MES is the best choice for the detection.

Table 1 Coupling Ratio of Buffers

| Marked Estradiol : 10 μg/L | PBS   | MES   | GA    |
|---------------------------|-------|-------|-------|
| Residual in supernate (μg/L) | 0.0632 | 0.041 | 0.0812 |
| Measurement of Coupled Estradiol (μg/L) | 9.251  | 9.956 | 9.033  |
| Coupling Ratio (%)        | 92.51  | 99.56 | 90.33  |

3.3 Discussion of eluants
Methanol, ethyl acetate acetonitrile, cyclohexane, hexane, acetone and their 5% formic acid-acidized solvents were used as eluant to dispose the magnetic beads which coupled with target molecule. Results in Table 2 reveals that methanol is the best one, acetone and acetonitrile are slightly lower than methanol, other solvents are not competent. All formic acid-acidized solvents are lower than theirselves.

Table 2 Recovery of the detection of estradiol by using different solvents

| Methanol/ Formic acid-acidized | Ethyl acetate/ Formic acid-acidized | Acetonitrile/ Formic acid-acidized | Cyclohexane / Formic acid-acidized | Hexane/ Formic acid-acidized | Acetone/ Formic acid-acidized |
|--------------------------------|------------------------------------|-----------------------------------|-----------------------------------|-----------------------------|------------------------------|
| Recovery (%)                   | 99.5/98.3                          | 37.8/33.2                         | 85.2/80.1                         | 23.9/20.4                   | 78.4/75.9                    | 82.6/75.5                   |

3.4 Rapid determination of estradiol in sample
Non-imprinted polymer (NIP) and estradiol molecular imprinted polymer (Estradiol MIP) have disparate adsorption of estradiol, Figure 2 shows that specific adsorption exists between Estradio MIP and estradiol, Table 3 proves that it creates good detection of estradiol, detection limit of sample is 1.25 μg/kg.
Figure 2 Dynamic test of the imprinted SiO$_2$ magnetic microsphere

| Concentration of standard solution (μg/L) | 2.5 | 5.0 | 7.5 | 10.0 | 12.5 | 15.0 |
|------------------------------------------|-----|-----|-----|------|------|------|
| Recovery (%)                             | 99.6| 87.4| 88.5| 82.0 | 83.6 | 95.5 |

4. Conclusions
This thesis creatively modify the silicon magnetic composite, the modified one has specific adsorption to target molecule, the preparation is simple and controllable. MES buffer is testify to be the optimal buffer for detection of estradiol. The experiment concludes that methanol can force efficacy elution of complex. The limit of detection can satisfy the limited requirements and recovery is high, the sample determination effect is good, it is simple, quick, accurate and suitable method for detection of estradiol in milchigs.

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