Development of an immunochromatographic test strip for the detection of papaverine in pure ginger powder

Wenliang Ge\textsuperscript{a}, Steven Suryoprabowo\textsuperscript{b}, Qiankun Zheng\textsuperscript{c} and Hua Kuang\textsuperscript{b}

\textsuperscript{a}Wuxi No.2 people's hospital, Wuxi, People's Republic of China; \textsuperscript{b}State Key Lab of Food Science and Technology, School of Food Science and Technology, Jiangnan University, Wuxi, People's Republic of China; \textsuperscript{c}Delishi Group, Weifang, People's Republic of China

\textbf{ABSTRACT}

A lateral-flow immunochromatographic assay was developed for the detection of papaverine (PAPA) in pure ginger powder samples. We produced a sensitive monoclonal antibody against PAPA (anti-PAPA mAb) by immunizing BALB/c mice with a well-characterized PAPA-keyhole limpet hemocyanin conjugate, produced in our laboratory. The coating antigen (PAPA-ovalbumin) and goat anti-mouse IgG antibody were used as the capture reagent in the control line of the test strip. Under optimized conditions, the cut-off limits of the test strip was 1 ng/mL in 0.01 M PBS (pH 7.4) and 5 ng/mL in pure ginger powder. The results were obtained within 5 min. The results revealed that the developed method is a sensitive, rapid, and simple tool for the detection of PAPA in pure ginger powder.

\textbf{ARTICLE HISTORY}

Received 29 May 2017
Accepted 29 May 2017

\textbf{KEYWORDS}

Immunochromatographic assay; monoclonal antibody; papaverine; strip test

\section*{Introduction}

Papaverine (1-(3, 4-dimethoxybenzyl)-6,7-dimethoxyisoquinoline) (PAPA) (Figure 1) is a benzylinosquinoline alkaloid obtained from opium. PAPA was discovered in 1848 by George Merck and characterized in 1909 by Pictet and Gams (Yan et al., 2005). In humans, PAPA has multiple functions. Specifically, it has a direct spasmodyl effect on the smooth muscles of the bronchi, gastrointestinal tract, ureter, and biliary system, and a pronounced relaxant effect on blood vessels, including coronary, cerebral, pulmonary, and peripheral arteries. However, PAPA causes a drop in blood pressure when the gastrointestinal tract is relaxed (Bellia, Jacob, & Smith, 1978; Gautam, Nahum, Baechler, & Bourne, 1980; Hassouna, Issa, & Zayed, 2014). Even though it is not under international narcotics control, PAPA can be misused.

In recent years, several techniques have been developed to isolate and determine the opium alkaloids in plants, biological samples, artificial products, and food samples. These techniques include thin layer chromatography (TLC) (Habashy & Farid, 1973), gas liquid chromatography (GLC) (de Graeve, Van Cantfort, & Gielen, 1977; Guttman, Kostenbauer, Wilkinson, & Dubé, 1974), high performance liquid chromatography (HPLC) (Acevska et al., 2012; Ahmadi-Jouibari, Fattahi, Shamsipur, & Pirsaheb, 2013;
Badea, Vladescu, David, David, & Litescu, 2010; Hoogewijs, Michotte, Lambrecht, & Massart, 1981; Pierson, Hanigan, Taylor, & McClurg, 1979), capillary isotachophoresis (CE) (Válka, Walterová, & Šimánek, 1985), atomic spectrometry (Eisman, Gallego, & Varcarcel, 1994), spectrophotometry (Helgren, Chadge, & Campbell, 1957), acidimetry (Spahrl, Pernarowski, & Knevel, 1962), and immunoassays (Yan et al., 2005).

TLC, GLC, and HPLC have been routinely used for screening purposes. TLC requires minimal instrumentation, is low in cost and simple to operate, and requires less time than GLC and HPLC. However, GLC and HPLC are superior in terms of precision and sensitivity. The disadvantages of GLC and HPLC are the relatively high running costs and complicated instrumental operation. Due to unique patterns of mass fragment intensities, liquid chromatography/tandem mass spectrometry and gas chromatography/mass spectrometry are powerful qualitative tools; however, they require skilled operators and their high cost limit their applications (Khaemba et al., 2016; Kong et al., 2017; Peng, Liu, Kuang, Cui, & Xu, 2017; Zhu, Song, Liu, Kuang, & Xu, 2016).

Immunoassays based on the highly specific interaction between antigen and antibody could be a sensitive tool for the detection of PAPA. The enzyme-linked immunosorbent assay (ELISA) (Yan et al., 2005) has several advantages including, (1) high analyte selectivity (specificity), (2) high throughput of samples, (3) reduced sample pre-treatment, and (4) increased cost-effectiveness (Kong et al., 2017; Peng et al., 2017). The lateral-flow immunochromatographic assay is very popular in the detection of residues because it is simple, rapid (can be performed in 5–10 min), specific, and sensitive (Liu et al., 2014; Suryoprabowo, Liu, Peng, Kuang, & Xu, 2015). The aim of this study was to develop a lateral-flow immunochromatographic test strip for the detection of PAPA in ginger powder.

**Material and methods**

**Chemicals and reagents**

PAPA was purchased from J&K Scientific (Shanghai, China). Complete Freund’s adjuvant (FCA), incomplete Freund’s adjuvant (FIA), and enzyme immunoassay-grade horseradish peroxidase-labeled goat anti-mouse immunoglobulin were obtained from Sigma (St. Louis,
MO, USA). Gelatin was supplied by Beijing Biodee Biotechnology Co., Ltd. (Beijing, China). Tetramethylbenzidine and horseradish peroxidase were acquired from Aladdin Chemistry Co., Ltd. (Shanghai, China). All reagents used for cell fusion were from Sunshine Biotechnology Co., Ltd. (Nanjing, China). Ovalbumin (OVA) and keyhole-limpet hemocyanin (KLH) were obtained from Solarbio Science & Technology, Co, Ltd. (Beijing, China). All other reagent and chemicals were supplied by the National Pharmaceutical Group Chemical Reagent Co., Ltd. (Shanghai, China).

Nitrocellulose (NC) high-flow plus membranes (Pura-bind RP) were obtained from Whatman–Xinhua Filter Paper Co., Ltd. (Hangzhou, China). Glass fiber membrane (CB-SB08) used for sample pad, polyvinyl chloride (PVC) backing material and absorbance pad (SX18) were supplied by Goldbio Tech Co., Ltd. (Shanghai, China). The conjugated coating antigens (PAPA–OVA) and specific monoclonal antibodies (anti-PAPA mAb, No. 2G8) were generated in our laboratory.

All buffer solutions were prepared using ultrapure water (Milli-Q purification system, Millipore Co., Bedford, MA, USA). The strip cutting instrument was CM 4000 (Gene, Shanghai, China), and the dispensers were The Airjet Quanti 3000™ and Biojet Quanti 3000™ (XinqidianGene-technology Co., Ltd., Beijing, China).

**Preparation and characterization of anti-PAPA monoclonal antibody**

PAPA was conjugated to OVA or KLH by the ester method (Suryoprabowo, Liu, Peng, Kuang, & Xu, 2014). Briefly, a mixture PAPA, carboxyl-reactive carbodiimide cross linker (1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride) (EDC), and N-hydroxysuccinimide (NHS) were added to N,N-dimetyl formamide (DMF) and incubated for 24 h (solution 1). KLH or OVA was mixed with 0.01 M phosphate buffer saline (PBS) solution (solution 2). Solution 1 was slowly added to solution 2 under constant stirring for 8 h at room temperature. The resulting conjugates were dialyzed against 0.01 M PBS for 3 d and subsequently distilled water for 3 d. The conjugation of protein and PAPA was assessed by UV absorption.

Female BALB/c mice (six–eight weeks of age) were used to produce anti-PAPA monoclonal antibody (mAb). The mice were immunized subcutaneously with PAPA–KLH (Figure 2). FCA was used in the first immunization, and FIA was used in subsequent immunization. The mice were immunized every three weeks with 100 µg for the first immunization and 50 µg for the remainder (2–5 times). Blood samples from mice were measured by ELISA. The mouse with the highest titer was sacrificed and its splenocytes were fused with Sp 2/0 murine myeloma cells. The hybridomas were screened by indirect ELISA. The selected hybridoma cells were expanded and injected into BALB/c mice to produce mAbs (Deng et al., 2012; Suryoprabowo et al., 2014). The ascites were harvested and purified with the caprylic acid-ammonium sulfate precipitation method (Kuang et al., 2013). The purified antibody solution was stored at −20°C until further use.

**Development of the lateral-flow immunochromatographic test strip device**

**Preparation of colloidal gold particles**

All solvents were prepared with ultrapure water and passed through a 0.22 µm membrane filter. Chlorauric acid (0.1 g/L; 25 mL) was heated to boiling under constant stirring and
mixed with sodium citrate tribasic dihydrate (1% w/v; 1.0 mL) under constant stirring for 30 min. When the color of the solution turned wine-red, it was cooled to room temperature, and stored at 4°C. Analysis by transmission electron microscopy revealed that gold nanoparticles (GNPs) had a nearly uniform particle size of 15 nm.

**Preparation of colloidal gold-Labeled mAb**

The half-maximal inhibitory concentration (IC$_{50}$) of anti-PAPA mAb (no. 2G8) was 0.2 ng/mL. Colloidal GNP solution (10 mL) was adjusted to pH 7 with 0.1 M K$_2$CO$_3$, and mAb (0.40 mL) was added to the solution dropwise. Thirty-five minutes later 35, 10% (w/v) bovine serum albumin (BSA; 1 mL) was added, and the mixture was stirred for 2 h and centrifuged at 8000 rpm for 45 min to remove any GNP aggregates. The solution separated into two layers; the lower layer (red gold-labeled mAb) was collected and washed with 0.02 M phosphate buffer containing 5% sucrose, 1% BSA, and 0.5% polyethylene glycol (PEG) 6000 (at pH 7.4. The conjugation products were reconstituted to 1 mL with gold-labeled resuspension buffer (0.02 M PBS, 5% sucrose, 2% sorbitol, 1% mannitol, 0.1% PEG, 0.1% tween, and 0.04% NaN$_3$) and stored at 4°C.

**Preparation of nitrocellulose capture membranes**

Coating antigen (PAPA–OVA; Figure 3) and goat anti-mouse IgG antibody were used as the capture reagent in the control line on the test strip. The coating antigen and goat anti-mouse IgG antibody were sprayed onto the NC membrane at 1 µL/cm using a dispenser, resulting in the formation of the test line and control line. The capture and control reagents were sprayed onto the glass fiber membrane to prepare the conjugate pad, which was dried at 37°C for 2 h. The NC membrane coated with capture reagents was pasted onto the center of the plastic backing plate (PVC) and the conjugate pad,

![Figure 2](image.png)

**Figure 2.** Ultraviolet spectra characterization PAPA–KLH, PAPA, and KLH.
sample pad, and absorbent pad were laminated and pasted onto the plate. Finally the plate was cut into strips (2.8 mm-wide) using a strip cutter.

Test procedure and principle

Prior to the test, 50 µL of gold-labeled mAb is mixed with 150 µL of sample solution, allowed to react for approximately 5 min, and added onto the sample pad. The solution migrates to the absorbent pad. The test results are obtained within 5 min. If the sample is PAPA-positive, PAPA competes with the PAPA–OVA conjugates embedded in the test line for the finite amount of anti-PAPA mAb. If a sufficient amount of PAPA is present, the free PAPA binds with the labeled mAb, preventing mAb from binding to the PAPA–OVA in the test line. Therefore, the more PAPA present in the sample, the weaker the color intensity of the test line. If the sample is PAPA-negative, the limited amount of colloidal gold-labeled mAb is trapped by the immobilized PAPA–OVA conjugate, and a clearly visible red test line appears.

The flow must reach the control line, which contains goat anti-mouse IgG antibody, and produces an indicator reaction. Therefore, the control line must always appear in a successful test, whereas the test line will only appear when the sample is negative (Figure 4(a)). The appearance of only the control line is a positive result (Figure 4(b)), whereas if neither the control line nor the test line appears (Figure 4(c)), the test procedure is incorrect or the strip is considered to be invalid, and the test should be repeated with a new strip.

Determination of performance

Sensitivity of the test strip

The sensitivity of the test strip was determined by testing PAPA samples. PAPA standard was diluted in 0.01 M PBS (pH 7.4) to different concentration (0, 0.05, 0.1, 0.25, 0.5, and
1 ng/mL), and the detection limit was determined. Sample solution (150 µL) was mixed with 50 µL gold-labeled mAb, allowed to react for 5 min and added onto the sample pad. After 5 min, the strip was analyzed using a test strip reader. The lowest detection limit (LDL) obtained with the naked eye was defined as the amount of PAPA that produced a color reaction on the strip that was clearly different in intensity from the PAPA-negative sample. Six replicate samples for each concentration were analyzed on the same day.

Detection of PAPA in ginger powder
Pure ginger powder was purchased from local market. Pure ginger powder (1 g) was diluted in 10 mL distilled water, vortexed for 5 min, and allowed to stand at room temperature before being used. The concentration of PAPA was determined using the immunochromatographic strip.

![Figure 4](image1.png)

**Figure 4.** Illustration of typical strip test results. If the sample is negative (a), a positive result could be indicated only if the control line appears (b), and if the control and test line does not appear (invalid) (c).

![Figure 5](image2.png)

**Figure 5.** Optimization of the immunochromatographic strip. Concentration of coating antigen (a) 0.1 mg/mL; (b) 0.25 mg/mL. The dosage of the mAb that add in GNP: (1) 8 µg/L; (2) 10 µg/L. The standard concentration: (a) 0 ng/mL; (b) 5 ng/mL.
temperature for 10 min. Two layers formed; the resulting supernatant was spiked with
PAPA standard solution (10 µg/mL, prepared with 0.01 M PBS, pH 7.4). The final
PAPA concentrations were 0.1, 0.25, 0.5, 1, 2.5, and 5 ng/mL. An un-spiked (blank)
sample was used as the control. Six replicates of each concentration were analyzed on
the same day with the test strips.

**Results and discussion**

**Optimization of strip test**

The sensitivity of the lateral-flow immunochromatographic strip is mainly affected by the
concentration of coating antigen and GNP-mAbs. The coating antigen was synthesized in
our laboratory and its concentration was optimized. Strips with different coating antigen
concentrations (0.1 and 0.25 mg/mL) on the test line were evaluated with 0.01 M PBS pH
7.4 containing either 0 or 5 ng/mL PAPA. Both coating antigen concentrations resulted in
color development in both the control and test lines. There were significant differences in
color intensity between 0.1 and 0.25 mg/mL coating antigen. Specifically, 0.25 mg/mL
coating antigen resulted in a deeper color intensity than 0.1 mg/mL coating antigen. At
0.25 mg/mL coating antigen, we did not obtain any color on the test line with 5 ng/mL
PAPA, suggesting that a more sensitive detection could be achieved. Therefore,
0.25 mg/mL coating antigen was selected for the preparation of the test line (Figure 5).

**Figure 6.** Colloidal gold immunochromatography assay for PAPA in 0.01 M PBS (pH 7.4). PAPA concent-
tration: 1= 0 ng/mL; 2= 0.05 ng/mL; 3= 0.1 ng/mL; 4= 0.25 ng/mL; 5= 0.5 ng/mL; and 6= 1 ng/mL.
PAPA, papaverine; C, control line; T, test line.
The concentration of mAb that is added to GNP affects the sensitivity of the immuno-chromatographic test strip. The reaction system (50 µL), which contained different concentrations of mAb (8 or 10 µg/L), was allowed to react with PAPA-negative (0 ng/mL) and PAPA-positive (5 ng/mL) samples. Figure 5 shows that there were significant differences in color intensity between 8 and 10 µg/L mAb. Even though 8 and 10 µg/L mAb resulted in adequate color intensities in the control and test line, we observed that the optimum concentration of mAb in GNP was 8 µg/L of colloidal gold because it contributed to strong color intensities on both lines with the negative sample and more sensitive detection with the positive sample. Therefore, the optimal system for our developed immunochromatographic test strip consisted of 0.25 mg/mL coating antigen and 8 µg/L of mAb in GNP.

The sensitivity of the assay was investigated using a series of PAPA standards diluted in 0.01 M PBS (pH 7.4). The LDL obtained with the naked eye was defined as the amount of PAPA that produced a color reaction on the strip that was clearly different in intensity from the PAPA-negative samples. Figure 6 shows that the color intensity on the test line changed from strong (0 ng/mL) to weak and finally disappeared completely at 1 ng/mL.

Detection of PAPA in ginger samples

To evaluate the feasibility of the developed method, we measured PAPA in pure ginger powder. One of the major advantages of the immunochromatographic test strip assay is that it is rapid and easy to use.
Weight 1 g pure ginger powder that bought at local market, then diluted 10 mL with distilled water, vortex for 5 min. After vortex, stand it in room temperature for 10 min, it will become two layers, then get the supernatant for analyzed. The liquid was spiked with the PAPA standard (10 µg/mL, prepared with 0.01 M PBS pH 7.4), at final concentration of PAPA in pure ginger powder of 0.1, 0.25, 0.5, 1, 2.5, and 5 ng/mL. The spiked series were prepared and analyzed with the optimized test strips. The color intensity decreased as the PAPA concentration increased (Figure 7). The signal color changed from strong to weak, and disappeared completely at 5 ng/mL.

**Conclusions**

A sensitive, simple, and rapid analytical method for the detection of PAPA in pure ginger powder was developed by colloidal gold-based lateral-flow immunochromatographic assay. Our results demonstrate that the cut-off limits of the semi-quantitative test strip for PAPA can be as low as 1 ng/mL in 0.01 M PBS pH 7.4 and 5 ng/mL in pure ginger powder.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**Funding**

This work is financially supported by the National Natural Science Foundation of China [21631005], National Key R&D Program [2016YFD0401101, 2016YFD0501208, 2016YFF0202300], the grants from Natural Science Foundation of Jiangsu Province and MOF [BE2016307, BK20150145, BX20151038, CMB21S1614, CLE02N1515, 201513006], and Taishan Industry Leading Talent Special Funds.

**Notes on contributors**

*Wenliang Ge* graduated from Shanghai Medical University, school of medicine, clinical laboratory medicine in 1988. He has been working in Second People’s Hospital of Wuxi City since 1989 up to now. For a long term, he has been engaged in clinical testing and immunization research and now he is working as the chief physician in the hospital.

*Mathew Suryoprabowo* was born in Indonesia and got his bachelor degree in Pelita Harapan University (Indonesia). He got his master degree in food science (2014) from Jiangnan University, Wuxi, China. His research interests are monoclonal antibodies development and immunochromatographic strip test and applications.

*Qiankun Zheng* graduated from Nanjing Agricultural University in 1996. Currently, he is working as a senior engineer in Delicious food company, China. He is good at food quality control and assurance.

*Hua Kuang* got her Ph.D from China Agricultural University in 2009 and then began to work as a faculty in the college of Food science and technology of Jiangnan University. She is currently a full professor in food safety. Her research interests are biosensor development.

**References**

Acevska, J., Dimitrovska, A., Stefkov, G., Brezovska, K., Karapandzova, M., & Kulevanova, S. (2012). Development and validation of a reversed-phase HPLC method for determination of
alkaloids from \textit{Papaver somniferum} L. (Papaveraceae). \textit{Journal of Aoac International}, 95(2), 399–405.

Ahmadi-Jouibari, T., Fattahi, N., Shamsipur, M., & Pirsaheb, M. (2013). Dispersive liquid–liquid microextraction followed by high-performance liquid chromatography-ultraviolet detection to determination of opium alkaloids in human plasma. \textit{Journal of Pharmaceutical and Biomedical Analysis}, 85, 14–20.

Badea, I. A., Vladescu, L., David, I. G., David, V., & Litescu, S. C. (2010). Development of a new HPLC method for determination of papaverine in presence of its photooxidation products. \textit{Analytical Letters}, 43(7–8), 1217–1229.

Bellia, V., Jacob, J., & Smith, H. T. (1978). Determination of papaverine in blood samples using a flame-ionization and a nitrogen-phosphorus detector is described: The method is quite simple and permits the determination at blood levels at 5–500 ng/ml. \textit{Journal of Chromatography A}, 161, 231–235.

de Graeve, J., Van Cantfort, J., & Gielen, J. (1977). Determination of papaverine in blood samples by gas-liquid chromatography and mass fragmentography. \textit{Journal of Pharmaceutical and Biomedical Analysis}, 23(3), 265–272.

Eisman, M., Gallego, M., & Varcarcel, M. (1994). Determination of papaverine and cocaine by use of a precipitation system coupled online to an atomic-absorption spectrometer. \textit{Journal of Pharmaceutical and Biomedical Analysis}, 12(2), 179–184.

Gautam, S. R., Nahum, A., Baechler, J., & Bourne, D. W. (1980). Determination of papaverine in plasma and urine by high-performance liquid chromatography. \textit{Journal of Chromatography B: Biomedical Sciences and Applications}, 182(3–4), 482–486.

Guttman, D. E., Kostenbauder, H. B., Wilkinson, G. R., & Dubé, P. H. (1974). GLC determination of papaverine in biological fluids. \textit{Journal of Pharmaceutical Sciences}, 63(10), 1625–1626.

Haba, G. M., & Farid, N. A. (1973). Thin-layer chromatographic method for separation and determination of papaverine and its oxidation products. \textit{Talanta}, 20(7), 699–702.

Hassouna, M. E. M., Issa, Y. M., & Zayed, A. G. (2014). Determination of residues of Acetaminophen, caffeine, and Drotaverine hydrochloride on swabs collected from pharmaceutical manufacturing equipment using HPLC in support of cleaning validation. \textit{Journal of Aoac International}, 97(5), 1439–1445.

Helgren, P. F., Chadde, F. E., & Campbell, D. J. (1957). The determination of theophylline, pentobarbital, and papaverine in tablets by spectrophotometric analysis. \textit{Journal of the American Pharmaceutical Association (Scientific ed.)}, 46(11), 644–646.

Hoogewijs, G., Michotte, Y., Lambrecht, J., & Massart, D. L. (1981). High-performance liquid chromatographic determination of papaverine in whole blood. \textit{Journal of Chromatography B: Biomedical Sciences and Applications}, 226(2), 423–430.

Khaemba, G. W., Tochi, B. N., Mukunzi, D., Joel, I., Guo, L., Suryobrobowo, S., … Xu, C. (2016). Development of monoclonal antibody and lateral test strip for sensitive detection of clenbuterol and related beta(2)-agonists in urine samples. \textit{Food and Agricultural Immunology}, 27(1), 11 1–127.

Kong, D., Xie, Z., Liu, L., Song, S., Kuang, H., Cui, G., & Xu, C. (2017). Development of indirect competitive ELISA and lateral-flow immunochromatographic assay strip for the detection of sterigmatocystin in cereal products. \textit{Food and Agricultural Immunology}, 28(2), 260–273.

Kuang, H., Xing, C., Hao, C., Liu, L., Wang, L., & Xu, C. (2013). Rapid and highly sensitive detection of lead ions in drinking water based on a strip immunosensor. \textit{Sensors}, 13(4), 4214–4224.

Liu, L. Q., Luo, L. J., Suryobrobowo, S., Peng, J., Kuang, H., & Xu, C. L. (2014). Development of an immunochromatographic strip test for rapid detection of Ciprofloxacin in milk samples. \textit{Sensors}, 14(9), 416785–16798.

Peng, J., Liu, L., Kuang, H., Cui, G., & Xu, C. (2017). Development of an icELISA and immunochromatographic strip for detection of norfloxacin and its analogs in milk. \textit{Food and Agricultural Immunology}, 28(2), 288–298.
Pierson, S. L., Hanigan, J. J., Taylor, R. E., & McClurg, J. E. (1979). Simple and rapid high-pressure liquid chromatographic determination of papaverine in plasma. *Journal of Pharmaceutical Sciences, 68*(12), 1550–1551.

Spahr, J. L., Pernarowski, M., & Knevel, A. M. (1962). Direct acidimetric analysis of papaverine in concentrated lithium chloride solution. *Journal of Pharmaceutical Sciences, 51*, 749–752.

Suryoprabowo, S., Liu, L. Q., Peng, J., Kuang, H., & Xu, C. L. (2014). Development of a broad specific monoclonal antibody for fluoroquinolone analysis. *Food Analytical Methods, 7*(10), 2163–2168.

Suryoprabowo, S., Liu, L. Q., Peng, J., Kuang, H., & Xu, C. L. (2015). Antibody for the development of specific immunoassays to detect nadifloxacin in chicken muscles. *Food and Agricultural Immunology, 26*(3), 317–324.

Válka, I., Walterová, D., & Šimánek, V. (1985). Determination of papaverine by capillary isotachophoresis. *Journal of Chromatography A, 350*(1), 336–338.

Yan, J., Mi, J.-Q., He, J.-T., Guo, Z.-Q., Zhao, M.-P., & Chang, W.-B. (2005). Development of an indirect competitive ELISA for the determination of papaverine. *Talanta, 66*(4), 1005–1011.

Zhu, Y., Song, S., Liu, L., Kuang, H., & Xu, C. (2016). An indirect competitive enzyme-linked immunosorbent assay for acrylamide detection based on a monoclonal antibody. *Food and Agricultural Immunology, 27*(6), 796–805.