International Journal of Chemical Science
Online ISSN: 2523-2843, Print ISSN: 2523-6075
Impact Factor: RJIF 5.22
www.chemicaljournals.com
Volume 2; Issue 5; September 2018; Page No. 28-31

Quantification of residual thiabendazole and its metabolite, 5-hydroxythiabendazole, in cow's milk using pipetting sample preparation with water eluent and water mobile phase HPLC coupled diode array: Method development and validation

Naoto Furusawa
Graduate School of Human Life Science, Osaka City University, Osaka 558-8585, Japan

Abstract
A quick, easy, small-scale, and safety sample preparation with water eluent followed by water mobile phase HPLC for quantifying thiabendazole (TBZ) and its metabolite, 5-hydroxythiabendazole (5hTBZ), in cow's milk was described in the present paper. Sample preparation is achieved by homogenization using a handheld ultrasonic-homogenizer with water followed by MonoTip pipette tip contains silica monolith bonded with C18 with water eluent. For determination and identification of analyte, the HPLC uses an analytical C1 column, an isocratic 100% water, and diode array detector. The procedure is harmless to both humans and the environment. The method validation data were well within the international analytical method acceptance criteria. The total analytical time was < 13 min/sample. The present method may be proposed as an international harmonized analytical method for routine residual monitoring of TBZ and 5hTBZ in cow's milk.

Keywords: international harmonized analytical method, pipette tip, 100% water mobile phase HPLC, thiabendazole, 5-hydroxythiabendazole, residue monitoring

1. Introduction

Thiabendazole (TBZ) is widely used as a fungicide and parasiticide: as an antiparasitic, it is able to control roundworms [1], hookworms, and other helminth species which attack wild animals, livestock and humans [2]; TBZ is approved as a food additive, a preservative, and is used as a post-harvest pesticide for preservation treatment of imported bananas, citrus fruits, which is a common ingredient in the waxes applied to the skins of citrus fruits [3, 4].

To ensure the safety of animal-derived foods for the consumer, the Codex Alimentarius Commission and the Japanese Health, Labour and Welfare Ministry set maximum residue limits (MRLs) for the sum of TBZ and its a major metabolite, 5-hydroxythiabendazole (5hTBZ), expressed as TBZ [5,6] (Fig. 1), based on the pharmacokinetic findings of TBZ in domestic animals [7], The monitoring of TBZ and 5hTBZ in the animal-derived foods is therefore an important job to guarantee food safety, and a validated analytical method for the simultaneous determining TBZ and 5hTBZ is presently required.

In answer to the present expansion and diversification in the international food trade, the development of international harmonized analytical methods (= universal standard methods) to determine chemical residues in foods is essential to guarantee equitable international trade in these foods and ensure food safety for consumers. Briefly, an international harmonized analytical method for residue monitoring in foods is urgently-needed. Without regard for advanced and developing countries, the applicable harmonized method must be easy-to-use, economical in time and cost, and must cause no harm to the environment and analyst.

Several previously reported methods for determining TBZ and 5hTBZ [8-11] in foods have the following three crucial drawbacks:

1) the sample preparation operations are complicated and labor intensive, which are time-and cost-consuming, do not permit the determination of large number of samples, and can give low reproducibility; 2) organic solvents are used as extraction solvents, purification eluent, and/or as LC mobile phases without fail - Risk associated with these solvents extend beyond direct implications for the health of humans and wildlife to affect our environment and the ecosystem. Eliminating the use of organic solvents is an important goal in terms of environmental conservation, human health and the economy [12]; 3) the detections/identifications are based on LC-MS or -MS/MS - The facilities that LC-MS/MS system is available are limited to part of industrial nations because these are hugely expensive, and the methodologies use complex and specific. These are unavailable in a lot of laboratories for routine analysis, particularly in developing countries. No analytical method that satisfies the aforementioned requirements has yet been identified.

As an optimal method that can be recommended as an international harmonized analytical method for the routine residue monitoring in animal-derived foods, this paper describes a quick, easy, small-scale, and organic solvent-free sample preparation followed by an isocratic water mobile phase HPLC method for determining TBZ and 5hTBZ in cow's milk. Cow's milk contains a good balance of protein, fat, and carbohydrate, is an indispensable food because it is inexpensive and readily available.

2. Materials and Methods

2.1 Reagents and apparatus

Thiabendazole (TBZ) and 5-hydroxythiabendazole (5hTBZ) standards were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Distilled water were of HPLC grade (FUJIFILM Wako).

The following apparatuses were used in the sample preparation: handheld ultrasonic-homogenizer (model HOM-100, 2 mm ID probe, Iwaki Glass Co., Ltd., Funabashi, Japan); micro-centrifuge (Biofuge® fresco, Kendo Lab. Products, Hanau, Germany); MonoTip™ C18 pipette tip (packed with silica monolith that consists of continuous through-pores and octadecyl bonding); sample throughput volume ≤ 200 µL; through-pore diameter of 10 - 20 µm; meso-pore of 20 nm; surface area of 200 m^2/g (GL Sciences Inc., Tokyo, Japan).

The HPLC system, used for method development, included a model PU-980 pump and DG-980-50-degasser (Jasco Corp., Tokyo, Japan) equipped with a model CTO-10AS $_{VP}$ column oven (Shimadzu Scientific Instruments, Kyoto, Japan), as well as a model SPD-M10A $_{VP}$ diode-array detector (DAD) (Shimadzu). A C1 non-polar sorbent (the highly purified silica-based) column, Wakopak® Wakosil 5TMS (5 µm d_P; 4.6 mm i.d.; 150 mm length) (FULIFILM Wako), was used for the HPLC analysis.

2.2 Pipette tip operating procedure

After attaching a MonoTip C18 pipette tip to a micro pipette (20 – 200 µL volume type), preconditioning of the tip was carried out by drawing and ejecting (to waste) 100 µL of distilled water to reduce background noise. A 100 µL aliquot of the sample was drew into the conditioned MonoTip C18 tip, and ejected back into another sample tube. This series of in and out operations was defined as one pipetting operation in this study.

2.3 HPLC operating conditions

The analytical column was a Wakopak® Wakosil 5TMS column using an isocratic mobile phase of water at a flow rate of 1.0 mL/min at 50℃. DAD was operated at 190 – 350 nm: the monitoring wavelength was adjusted to 293 nm which represent an average of maximum absorption spectra for TBZ and 5hTBZ. The injection volumes were 10 – 20 µL.

2.4 Preparation of stock standards and working mixed solutions

Stock standard solutions of TBZ and 5hTBZ were prepared by dissolving each of TBZ and 5hTBZ in water followed by water to a concentration of 50 µg/mL, respectively. These solutions were stored at -20℃. Working mixed standard solutions of these two compounds were freshly prepared by suitably diluting the stock solutions with water on the day of the analysis.

2.5 Preparation of calibration standards and quality control samples

For method validation studies, calibration standards and quality control samples (QCs), terms defined in the FDA guideline [13], were prepared by spiking appropriate aliquots of the mixed standard solution in blank milk samples. Calibration standards were used to construct calibration curves from which the concentrations of analytes in unknown monitoring samples are determined practically. QCs used to evaluate the performance of the proposed method. In this study, the standards were prepared in the range of 0.05, 0.1, 0.2, 0.5, 1, and 2 µg/mL for analyte. Three QC levels (for both analytes, QC1 = 0.1 µg/mL; QC2= 0.5 µg/mL; QC3 = 1 µg/mL) were prepared.

2.6 Sample preparation

An accurate 100 µL milk sample was taken into a 1.5 mL micro-centrifuge tube and homogenized with 600 µL of water with a handheld ultrasonic-homogenizer for 30 s. After being homogenized, the capped tube was centrifuged at 10,000 g for 5 min. A 100 µL aliquot of supernatant liquid was aspirated into the conditioned MonoTip C18 pipette tip and dispensed back into the sample tube. The eluate was injected into the HPLC system.

2.7 Method validation

The performance of the developed method was validated in terms of many parameters from the international guidelines for bio-analytical procedure [14-16].

3. Results & Discussion

3.1 Sample preparation and optimal HPLC conditions

The advantages to the present procedure are pretreated quickly, economically, and environment-friendly on small-scale, requiring only 100 % water as the analytical reagents. The extract obtained was easily purified by a MonoTip C18 pipette tip, which was performed by an easy pipetting operation with water eluent of 0.1 mL. The time required for the sample preparation of a single milk sample, including the centrifugation for 5 min, was < 7 min. The quick and easy procedure resulted in high recovery and reproducibility with great saving time and cost. The resulting extract was free from interference, as can be seen in HPLC traces of blank (Fig. 2B) and spiked milk sample (Fig. 2A). These findings demonstrate that the extraction and purification worked well.

Using an isocratic 100 % water mobile phase without the needs for a gradient system to improve the separation, the separations of TBZ, 5hTBZ, and interference peaks were achieved by using the C1 (Wakosil 5TMS) column with a follow rate of 1.0 mL/min and a column temperature of 50 ℃. Fig. 2A,B displays typical chromatograms for a spiked milk sample and for a blank milk sample obtained under the procedure developed here, with the DAD set at 293 nm (giving an average of maximum absorption spectra for TBZ and 5hTBZ, respectively). The HPLC-DAD system achieved optimal separation in < 6 min.

3.2 Method validation

Table 1 summarizes the main method validation parameters. The quantitative limits in milk samples were 0.024 µg/mL for 5hTBZ and 0.045 µg/mL for TBZ, respectively: the sum of both analytes = 0.069 µg/mL. The sum value is less than the Codex's MRL (0.1µ/mL). The system-suitability evaluation is an essential parameter of HPLC determination, and it ascertains the strictness of the system used. The suitability was evaluated as the relative standard deviations of peak area

and retention time calculated for 10 replicate injections of a spiked milk sample with both analytes (each 0.5 µg/mL). The values were estimated to be ≤ 0.64 % for retention times and ≤ 0.28 % for peak areas, respectively. Including this system-suitability, the linearity, accuracy, and precision were satisfied the international acceptance criteria (Table 1) [14-16].

As the other validation finding (selectivity), the present HPLC-DAD system easily confirmed the peak identity of target compound. The TBZ and 5hTBZ were identified in a sample by their retention times and absorption spectra. For example, in Fig. 2C, the TBZ spectra obtained from the milk sample was practically identical to that of the standard. The system did not require the use of MS or MS/MS, which is very expensive and is not widely available for routine work.

3.3 Cost and time performances

The total time and budget required for the analysis of a single sample were less than 13 min and approximately ＄6.4 (as of August 2nd, 2017), respectively. For sequential analysis, a batch of 24 samples could be analyzed in less than 3 h. These findings became term required for the routine assay. The short time and low-cost quantitative method increased the sample throughput for actual routine monitoring work.

3.4 Application to real milk samples

Using the complete method, 24 samples of commercial raw cow's milk purchased from convenience stores in Osaka, Japan were analyzed. No samples contained detectable concentrations of TBZ and 5hTBZ. All chromatograms were free from interferences.

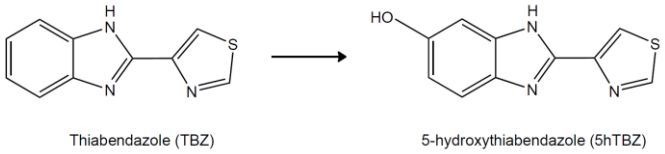

Fig 1: Chemical structures of TBZ and 5hTBZ.

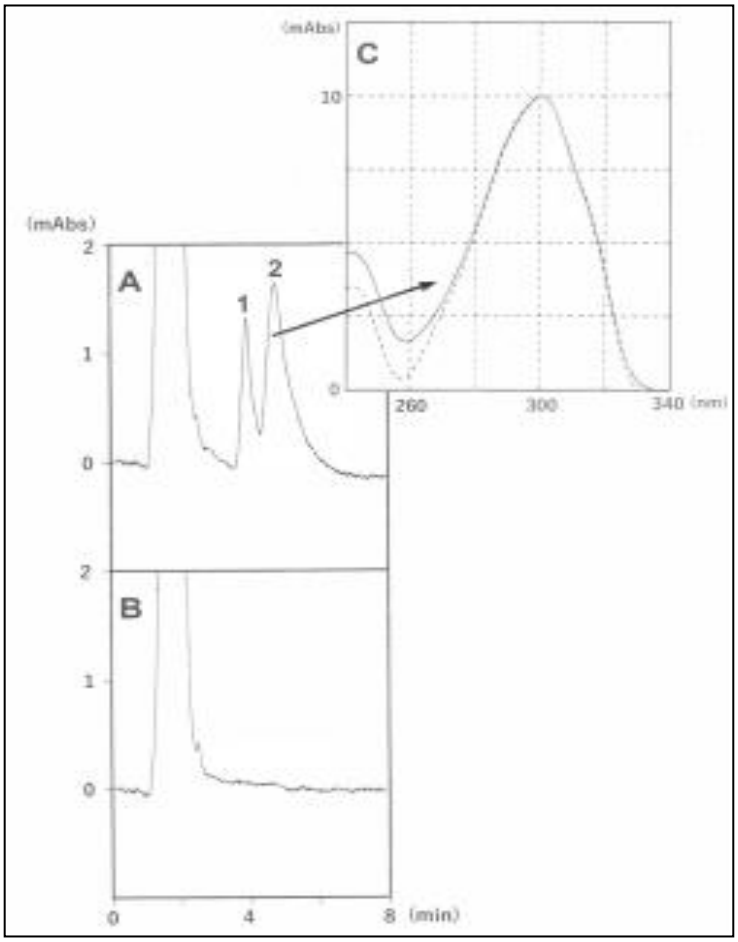

Fig 2: A chromatogram obtained from a spiked (at 0.5 µg/mL for each compound) milk sample (A), a blank milk sample (B), and absorption spectra for peak 2 (TBZ) in the chromatogram-A (C). A, B: DAD set at 293 nm (an average of maximum absorption spectra for TBZ and 5hTBZ). Peaks: 1 = 5hTBZ (Retention time, Rt = 3.93 min); 2 = TBZ (Rt = 4.75 min). C: Solid line = peak 2 in a chromatogram for a spiked milk sample; dashed line = TBZ standard.

Table 1: Method validation data

Parameter		5hTBZ	TBZ	Acceptance riterion[a]	Recommended value[b]
Linearity (r)[c]		0.9993	0.9992		≥0.999
Range (µg/mL)		0.05 - 2			
Accuracy[d] (%)		85.3	90.6	70 - 110	
Precisione[e] (%)		2.9	2.1	≤ 20	
Sensitity[f] (µg/mL)		0.024	0.045		
System suitability[g] (%)	Retention time	0.59	0.64		≤ 1
	Peak area	0.33	0.47		≤ 1

[a] Codex alimentarius commission [15,16].

[b] Recommended data in the FDA guidelines [14].

[c] r is the correlation coefficient ($p < 0.01$) for calibration curve.

[d] Average recoveries from 18 replicates (= six replicates at thee spiked levels: 0.1, 0.5, and 1 µg/mL for 5hTBZ and TBZ, respectively).

[e] Values are relative standard deviations (RSDs, n= 18).

[f] Quatitative limit as the concentration of analyte giving a signal-to-noise ratio = 10.

[g] Data as the RSDs calculated for 10 replicate injections of the prepared eluate for a milk sample spiked with 5hTBZ and TBZ (each 0.5 µg/mL).

4. Conclusion

A pipetting sample preparation with a 100 % water eluent followed by an isocratic 100 % water mobile phase HPLC-DAD method for quantification of residual TBZ and 5hTBZ in cow's milk has been successfully established. The method validation data were satisfied the international method acceptance criteria. The present procedure provided an easy-to-use, fast, and environment/analyst-friendly and resulted in high recovery and repeatability with considerable saving of analysis time/cost. In particular, the present technique may be proposed as an international harmonized method for routine residue monitoring TBZ and 5hTBZ in cow's milk.

5. References

1. Igual-Adell R, Oltra-Alcaraz C, Soler-Company E, Sánchez-Sánchez P, Matogo-Oyana J, Rodríguez-Calabuig D. Efficacy and safety of ivermectin and thiabendazole in the treatment of strongyloidiasis. Expert Opin. Pharmacother. 2004; 5(12):2615-9.
2. Portugal R, Schaffel R, Almeida L, Spector N, Nucci M. Thiabendazole for the prophylaxis of strongyloidiasis in immunosuppressed patients with hematological diseases: a randomized double-blind placebo-controlled study. Haematologica. 2002; 87(6):663-4.
3. Rosenblum C. Non-Drug-Related Residues in Tracer Studies. J. Toxicol. Environ. Health. 1977; 2(4):803-14.
4. Sax NI. Dangerous Properties of Industrial Materials. Vol. 1-3 7th ed. New York, NY: Van Nostrand Reinhold. 1989; pp.3251.
5. Codex Alimentarius. Pesticide Residues in Food and Feed.http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCAC%2BMRL%2B2%252FMRL2e.pdf
6. Ministry of Health, Labour and Welfare of Japan, Positive List System for Agricultural Chemical Residues in Foods, MRLs Data Base (The Japan Food Chemical Research Foundation). http://www.m5.ws001.squarestart.ne.jp/foundation/search.html
7. Chukwudebe C, Wislocki PG, Sanson DR, Halls TDJ, Vanden Heuvel WJA. Metabolism of thiabendazole in laying hen and lactating goats. J Agric. Food Chem. 1994; 42:2964-2969.
8. Ekman E, Faniband MH, Littorin M, Maxe M, Jonsson BAG, Lindh CH. Determination of 5-hydroxythiabendazole in human urine as a biomarker of exposure to thiabendazole using LC/MS/MS. J Chromatogr. B. 2014; 973:61-67.
9. Shang N, Zhang J, Shao B. Simultaneous Determination of Azoles Antifungal Drugs in Chicken Tissues by Ultra-performance Liquid Chromatography Tandem Mass Spectrometry. Anal. Sci. 2013; 29(12):1183-1188.
10. Jedziniak P, Szprengier-Juszkiewicz T, Olejnik M. Determination of benzimidazoles and levamisole residues in milk by liquid chromatography-mass spectrometry: Screening method development and validation. J. Chromatpogr. A 2009; 1216(46):8165-8172.
11. Kai S, Akaboshi T, Kishi M, Kanazawa H, Kobayashi S. Analysis of benzimidazole anthelmintics in livestock foods by HPLC/MS/MS. BUNSEKI KAGAKU 2005; 54(9):775-782.
12. Anastas PT, Warner JC. 1998. Green Chemistry: Theory and Practice, Oxford University Press, Oxford, United Kingdom.
13. FDA/CDER/CVM. Guidelines for Industry – Bioanalytical Method Validation, 2001.
14. FDA/CDER. Reviewer Guide, Validation of Chromatographic Methods, 1994.
15. Codex alimentarius. CAC/GL 37-2001: Harmonized IUPAC Guidelines for the use of Recovery Information in Analytical Measurement, 2001.
16. Codex alimentarius. CAC/GL 71-2009: Guidelines for the Design and Implementation of National Regulatory Food Safety Assurance Programme Associated with the Use of Veterinary Drugs in Food Producing Animals, 2009.