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Development and validation of HPLC technique with a 100% water mobile phase for analysing thiabendazole and its metabolite, 5-hydroxythiabendazole

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Abstract
The author describes a 100% water mobile phase HPLC technique for simultaneous analysis of thiabendazole (TBZ) and its metabolite, 5-hydroxythabendazole (5hTBZ). The chromatographic separation was performed a C1 column with a 100% water mobile phase and a diode array detector (DAD). The total run time was < 5 min. The main validation data, linearity and system suitability, were well within the international recommended criteria. The detection limits for TBZ and 5hTBZ were 0.89 and 1.02 ng/mL, respectively. The high repeatable and safety HPLC-DAD system may be further effective for the quantifying TBZ and 5hTBZ residues in animal-derived foods.

Keywords: international harmonized analytical method, water mobile phase, HPLC, thiabendazole, 5-hydroxythiabendazole

Introduction
Thiabendazole (TBZ) is widely and frequently 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].
Based on the pharmacokinetic findings of TBZ in domestic animals [5], the Codex and the Ministry of Health, Labour and Welfare of Japan set maximum residue limits (MRLs) in animal-derived foods for the sum of TBZ and its a major metabolite, 5-hydroxythiabendazole (5hTBZ), expressed as TBZ [6,7] (Fig. 1). The determination 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 current international trading, as foods are produced and distributed throughout the world, food safety have become increasing concerns for consumers. To protect the health of consumers, there is a requirement for more diligent monitoring of foods for regulators, vendors and producers. Under these circumstances, the development of international harmonized methods to determine chemical residues in foods is essential to guarantee equitable international trade in these foods. Whether in industrial nations or developing countries, an international harmonized method for residue monitoring in foods is urgently–needed. The ideal 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 detecting TBZ and 5hTBZ [8-11] have the following crucial drawbacks:

1. They consume large quantities of poisonous organic solvents, acetonitrile or methanol, in the mobile phases. Risk associated with these solvents extend beyond direct implications for the health of humans and wildlife to affect our environment and the ecosystem in which we all reside. Eliminating the use of organic solvents is an important goal in terms of environmental conservation, human health and the economy [12] ;
2. They are based on LC-MS or -MS/MS. LC-MS/MS systems are mainly available in a part of industrial nations because these are hugely expensive, and the methodologies use

Complex and specific. These systems are unavailable in a lot of laboratories for routine analysis, particularly in developing countries.

In order to establish an international harmonized method for the residue monitoring of TBZ and 5hTBZ, this paper describes an isocratic 100% water mobile phase HPLC conditions to detect the both compounds simultaneously.

Experimental

Reagents and Equipment

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

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 CO-810 column oven (Thosoh Corp., Tokyo, Japan), as well as a model SPD-M10A *VP* diode-array detector (DAD) (Shimadzu Scientific Instruments, Kyoto, Japan).

The following eleven types of C1 non-polar sorbent (the highly purified silica-based) columns (4.6 mm i.d.; 150 mm length) for HPLC analysis were used: Daisopak™ SP-200-5-C1-P and Daisopak™ SP-200-3-C1-P (Osaka Soda Co., Ltd., Osaka, Japan); Developsil® TMS-UG-3 and Developsil TMS-UG-5 (Nomura Chemical Co., Ltd., Aichi, Japan); HiQ sil™ C1-10 (KYA Technologies Corporation, Tokyo, Japan); Inertsil® TMS (GL Sciences Inc., Tokyo, Japan); Kaseisorb LC C1-300-5 (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan); Spherisorb® C1 (Waters Corporation, MA, USA); Wakopak® Wakosil 5TMS (Wako Pure Chemical Industries, Ltd., Osaka, Japan); ZORBAX® TMS (Agilent Technologies, CA, USA). Table 1 lists the particle physical/chemical specifications.

Operating HPLC conditions

The analytical column was a Wakopak® Wakosil 5TMS (5 μm, d_p, 4.6 × 150 mm) column using an isocratic 100% water mobile phase at a flow rate of 1.0 mL/min at 50 ℃. DAD was operated at 190–340 nm: the monitoring wavelengths were adjusted to 296 and 290 nm which represent maximums for TBZ and 5hTBZ, respectively. The injection volumes were 10–20 μL.

Preparation of stock standards and working mixed solutions

Stock standard solutions of TBZ and 5hTBZ were prepared by dissolving each compound in water followed by water to a concentration of 1,000 ng/mL. Each solution was 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.

HPLC Validation

Linearity: The calibration curve was generated by plotting peak areas ranging from 1.5 to 200 ng/mL versus their concentrations. The linearity was assessed from the linear regression with its correlation coefficient.

Detection limit: The detection limit should correspond to the concentration for which the signal-to-noise ratio. The value was defined as the lowest concentration level resulting in a peak area of three times the baseline noise.

System Suitability Test: The HPLC system suitability 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 areas and retention times calculated for 10 replicate injections of a mixed standard solution (50 ng/mL).

Results and Discussion

Optimum HPLC conditions

To optimize the separation with an isocratic 100% water and a more rapid separation, the author tested eleven types of C1 columns (Table 1). This study examined mobile phase with water, column temperatures ≥ 25 ℃, the flow rates ≥ 0.5 mL/min, and HPLC retention times ≤ 15 min (Table 1): because the HPLC separations were performed serially, the time/run was critical for routine residue monitoring. The short run time not only increased sample throughout for analysis but also affected the method-development time.

The eleven columns were compared with regard to 1) elution from the column; 2) separation between TBZ and 5hTBZ; 3) sharpness of peaks obtained upon injection of equal amounts. The resulting chromatographic profiles within the condition ranges examined were presented in Table 1.

It was difficult to elute and validly separate TBZ and 5hTBZ with 100% water mobile phase, only use of Column-(b), Wakopak Wakosil 5TMS (5 μm, 4.6 × 150 mm) (with column temperature of 50 ℃ and flow rate of 1.0 mL/min), enabled valid separation of the two target compounds, their sharp peaks, and their short retention times. Fig. 2 displays that the resulting chromatogram obtained from the HPLC. The two values (1614 for 5hTBZ and 1630 for TBZ indicating their peak areas, respectively) in Ch1 of this figure demonstrate that TBZ and 5hTBZ peaks were accurately measured with the DAD detector and its control software in this study. The two target peaks are distinguished at 3.00 min for 5hTBZ and 4.57 min for TBZ, respectively, and enabled also the multiple sequential injections. Under the extra-high polar 100% water mobile phase, raised column temperature and 4% carbon contents in the column were necessary at least to obtain the findings. The present HPLC-DAD method accomplished optimum separation in a short time without the need for a gradient mode to improve the separation and pre-column washing after an analysis. Furthermore, the DAD method easily confirmed the peak identity of the target compound. The analyte can be easily identified by its retention time and absorption spectrum without using MS or MS/MS.

HPLC validation

Table 2 summarizes the validation data for the performance parameters. The linearity and system suitability values were well within the FDA's recommended criteria [13]. The detection limits for TBZ and 5hTBZ were 0.89 and 1.02 ng/mL, respectively. The present HPLC-DAD system did not require the use of MS or MS/MS, which is very expensive, laborious to manage, and is unavailable in a number of laboratories for routine analysis.

Table 1: Physical/chemical specifications of the C1 columns[a] used and resulting chromatographic TBZ and 5hTBZ separations obtained under the HPLC condition ranges examined[b]

Column (trade name)	dp (µm)	Pore diameter (nm)	Surface area (m2/g)	Carbon content (%)	Chromatograpic TBZ and 5hTBZ peaks		
					Eluted	Separated	Peak form
(a) Inertsil TMS	5	10	450	3.5	Yes	Yes	Broadening
(b) Wakopak Wakosil 5TMS	5	12	300	4	Yes	Yes	Sharp
(c) Wakopak Wakosil 5C4-200	5	20	200	5	No	—	—
(d) Developsil TMS-UG-3	3	14	300	4.5	No	—	—
(e) Developsil TMS-UG-5	5	14	300	4.5	No	—	—
(f) Daisopak SP-200-3-C1-P	3	20	200	3	Yes	Yes	Broadening
(g) Daisopak SP-200-5-C1-P	5	20	200	3	Yes	Yes	Broadening
(h) Kaseisorb LC-C1-300-5	5	30	100	1	Yes	Yes	Rounding
(i) Spherisorb C1	5	8	220	2.2	No	—	—
(j) ZORBAX TMS	5	7	300	4	No	—	—
(k) HiQ sil C1-10	10	12	318	4	No	—	—

[a] i.d. =4.6 mm; length = 150 mm.

[b] Isocratic mobile phase of water; flow-rates ≥ 0.5 mL/min; column temperatures ≥ 25 ℃; HPLC retention times ≤ 15 min.

Table 2: Chromatographic method validation data

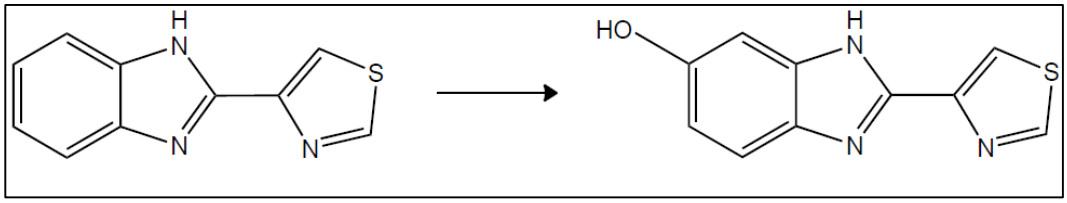
		TBZ	5hTBZ	Acceptance criterion[a]
Linearity (r)[b]		0.9992	0.9994	≥ 0.999
Range (ng/mL)		1.5 - 200		
Detection limit[c] (ng/mL)		0.89	1.02	
Injection repeatability[d] (RSD, %):	Retention time	0.78	0.54	≤ 1
	Peak area	0.41	0.35	≤ 1

[a] Recommendations in the FDA guidelines [13].

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

c Detection limit as the concentration of analyte giving a signal-to-noise ratio = 3.

[d] Data as the relative standard deviations calculated for 10 replicate injections (10 µL) of a mixed standard solution (50 ng/mL of TBZ and 5hTBZ, respectively).



Thiabendazole (TBZ) **5-hydroxythiabendazole (5 hTBZ)**

Fig 1: Chemical structures of TBZ and its metabolite, 5hTBZ

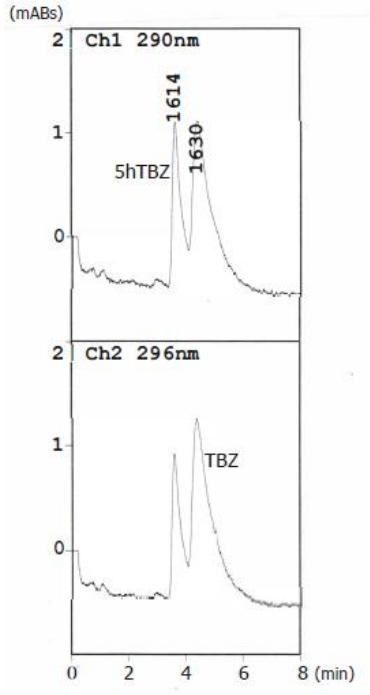


Fig 2: Chromatograms obtained from the present HPLC system for a standard mixture (each compound 100 ng/mL): DAD set at 290 nm (Ch l) for 5hTBZ peak (Retention time, Rt = 3.00 min) and 296 nm (Ch 2) for TBZ peak (Rt = 4.57 min).

Conclusion

A validated HPLC-DAD method for analysing TBZ and its metabolite, 5hTBZ, using an isocratic 100% water mobile phase and C1 column has been successfully established. The method is harmlessness to the environment and to humans and has a short run time and high system suitability. The HPLC system may be proposed as an international harmonized method for simultaneous detection of TBZ and 5hTBZ. For the quantification in animal-derived foods, the proposed HPLC method will be applicable enough by performing a suitable sample preparation technique.

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, 3251.
5. 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.
6. Codex Alimentarius. Pesticide Residues in Food and Feed, 2017, 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
7. 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), 2017, http://www.m5.ws001.squarestart.ne.jp/foundation/search.html
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. Green Chemistry-Theory and Practice, Oxford University Press, 1998.
13. FDA. Reviewer Guidance, Validation of Chromatographic Methods, Center for Drug Evaluation and Research (CFDER), 1994.