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Short communication

Simple, cheap and effective high-performance liquid chromatographic method for determination of praziquantel in bovine muscle

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ABSTRACT

A simple high-performance liquid chromatographic method using ultraviolet detection was developed for the determination of praziquantel in bovine muscle. The sample was extracted with ethyl acetate, cleaned up by alumin B cartridge. Analyses were run at a flow-rate of 0.8 ml/min with the detector operating at a detection wavelength of 220 nm. The method is specific and sensitive, with a quantification limit of 0.02 mg/kg and a detection limit of 0.01 mg/kg. The standard calibration curve of drugs solution was linear in the range of 0.02–2.0 μg/ml (r^2 = 0.9999). At the fortified levels of 0.02, 0.05, 0.2 mg/kg, the mean recoveries of praziquantel ranged from 75% to 85%, while the intra-day and inter-day coefficient of variation (CV) of the assay were all less than 9%.

1. Introduction

Praziquantel (PZQ) [2-(cyclohexylcarbonyl)-1,2,3,6,7,11b-hexahydro-4H-pyrazino[2,1-a]isoquinoline-4-one] (Fig. 1) is a acylated isoquinoline–pyrazine used in the treatment of schistosomiasis caused by all schistosoma species pathogenic to domestic animals, especially bovines (water buffalo and cattle) [1]. Due to its efficiency as a parasiticide, praziquantel is used in American, Asiatic, African and East-European countries where infections by schistosomiasis. Later studies, including monitoring in humans and ruminants (cattle, goats and water buffalo) have shown that praziquantel induces a greater frequency of hyperploid lymphocytes as well as structural chromosomal aberrations. In vitro studies have demonstrated that praziquantel can induce micronuclei in embryonic cells and in lymphocytes [2]. The same was found about structural chromosomal aberrations [1].

Due to this interest many analytical methods for praziquantel determination have been described. These include thin-layer chromatography (TLC) [3], thin-layer electrophoresis (TLE) [4] and capillary electrophoresis (CE) [5] and HPLC [6]. These methods were mostly used to detect PZQ in plasma or urine. So far, no method is used to detect PZQ residues in bovine muscle. Previously reported methods which detected PZQ in plasma involve time-consuming, costly extraction procedures or use a fluorescence detection system at low pH conditions in plasma or urine. This article describes the development of a simple, economical, yet more sensitive HPLC method for determination of PZQ in bovine muscle for the first time. In our laboratory, a specific and sensitive HPLC method was available for the detection of PZQ in bovine muscle with a quantification limit of 0.02 mg/kg and a detection limit of 0.01 mg/kg at a signal-to-noise ratio of 3:1. Due to its high sensitivity, HPLC is well suited for determination of such analytes in bovine muscle.

Despite tendency to use LC–MS/MS techniques in the residue field, there is a lack of short, cheap and effective method for specific analytes, like PZQ. Literature review shows that methods for such compound are rather rare. The purpose of this work was to develop a simple, economical, effective, yet more sensitive determination method for PZQ residues in bovine muscle. The work presented here used HPLC with UV detector for the determination of PZQ following a solid-phase extraction (SPE). This method was validated and used to determination PZQ residues in a plan survey.

2. Experimental

2.1. Chemicals and materials

All reagents used during the extraction and analysis were analytical reagent grade or better. Methanol for preparation of the mobile phase was high-performance liquid chromatographic (HPLC) grade (Fisher Scientific Co., Pittsburg, PA, USA). PZQ standard, content of 99.1% was obtained from Dr. Ehrenstorfer (Germany). Alumin B cartridge was obtained from Fisher (Fisher Scientific Co., Pittsburg, PA, USA).

2.2. Equipment

The method was developed on a chromatographic system consisting of spectra system SHIMADZU HPLC solvent Delivery/Controller, equipped with a Hamilton 7125 injector with a 100 μl loop and an ultraviolet detector (UV SPD-10ATVP). The

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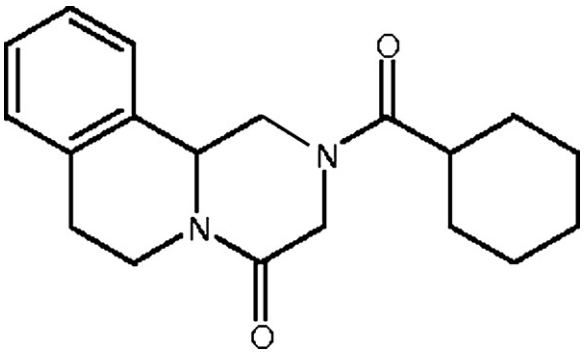

Fig. 1. Molecular structure of praziquantel.

wavelength was set at 220 nm. The separation was carried out on a reversed phase column C_{18} (250 mm × 4.0 mm, 5.0 μm particle size: Phenomenex™, USA). The mobile phase consisted of methanol and distilled water (70:30, v/v). The chromatographic analysis was operated at 25 °C. Aliquots of 20 μl samples or standard solutions were injected onto the column with a mobile phase at flow rate of 0.8 ml/min mobile phase was vacuum filtered and degassed through 0.22 μm pore size polymeric PTFE filters.

2.3. Sample preparation

Added 15 ml of ethyl acetate into 5 g portions of homogenized bovine muscle samples which have been spiked appropriate amounts of PZQ standard in 50 ml polypropylene centrifuge tubes. After vortexing (2 min) and centrifugation (3000 × *g*, 5 min), supernatant was collected. Repeated the extraction with 15 ml of ethyl acetate, combined with the first portion and then evaporated to dryness at 45 °C. The residue was dissolved in 3 ml of methanol and transferred to 10 ml centrifuge tubes. After centrifugation at 3000 × *g* for 5 min, the methanol phase was filtered through 0.22 μm pore size polymeric PTFE filters and applied to alumin B cartridge which was previously conditioned with 5 ml of methanol and 5 ml of water. Analytes were eluted and collected with 15 ml of 95% methanol. After evaporation, each residue was reconstituted in 1.0 ml of mobile phase and then filtered through 0.22 μm pore size polymeric PTFE filters, 20 μl was injected into the HPLC system for analysis.

3. Results and discussion

3.1. Method development

The PZQ residues are usually extracted from plasma with an organic solvent like dichloromethane, toluene, acetonitrile and further clean-up is made by solid-phase extraction (SPE) with C_{18}, silica or strong cation-exchange (SCX) cartridges [6–9]. Ethyl acetate was chosen as extraction solvent because it can be evaporated rapidly under vacuum at 45 °C. This evaporation step is critical. Excessive heat and drying reduced recovery [6]. Some organic solvents such as dichloromethane, chloroform and hexane were tried for extraction. The recoveries were found to be much less and very high processing losses were encountered compared

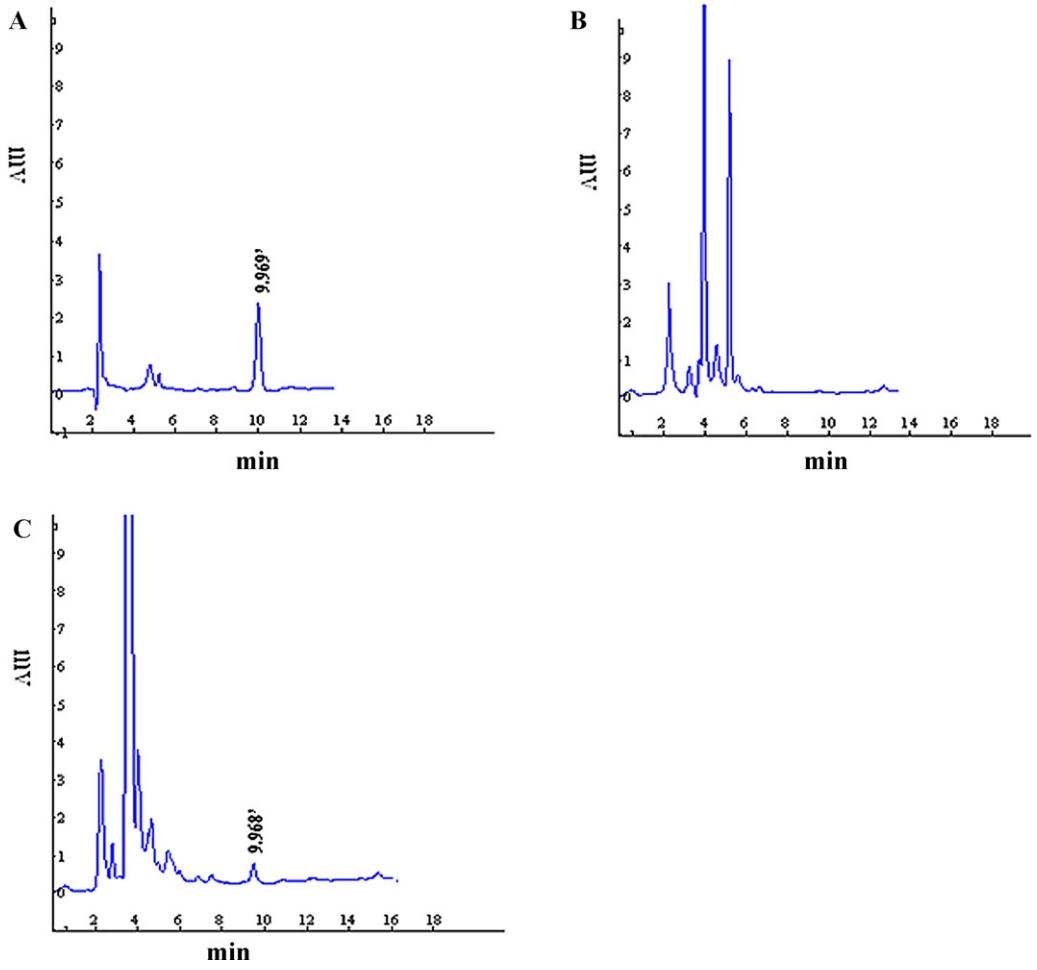

Fig. 2. Chromatograms of (A) praziquantel standard (1.0 mg/kg); (B) control bovine muscle; (C) fortified bovine muscle (0.02 mg/kg).

Table 1
Absolute recovery, intra-day and inter-day precision and accuracy ($n = 5$).

Spike level (mg/kg)	Recovery		Intra-day[a]		Inter-day[b]	
	Mean (%)	CV (%)	Precision (CV%)	Accuracy (%)	Precision (CV%)	Accuracy (%)
0.02	79.01 ± 3.33	4.21	5.50	80.13 ± 3.57	8.17	83.59 ± 3.41
0.05	80.07 ± 2.30	2.87	4.59	81.79 ± 2.73	7.30	82.27 ± 2.96
0.2	81.20 ± 2.25	2.77	3.15	81.22 ± 2.40	3.36	80.66 ± 2.35

[a] Intra-day variation was determined by 5 replicates on a single day.
[b] Inter-day variation was determined by 5 replicates on 5 different days.

to using the above-described method which involves a single step and simple procedure, resulting in good recoveries. The PZQ were concentrated and cleaned up by alumin B cartridge. The matrix interference effect was reduced by eluting with 95% methanol. These extracts give relatively clean PZQ chromatograms shown in Fig. 2.

3.2. Method validation

The chromatographic method was validated on five different validation days to find out the accuracy precision of the present HPLC method for detection of PZQ in bovine muscle samples. The recovery of PZQ from bovine muscle was determined by comparing peak areas obtained from muscle to which PZQ (0.02–2.0 mg/kg) had been added to that of the peak areas obtained from corresponding unspiked standards. The standard calibration curve of drugs solution was linear in the range of 0.02–2.0 mg/kg ($r^2 = 0.9999$). As shown in Table 1, at the fortified levels of 0.02, 0.05, 0.2 mg/kg, the mean recoveries of PZQ ranged from 75% to 85%, while the intra-day and inter-day coefficient of variation (CV) of the assay were all less than 9%. The method is sensitive, with a quantification limit of 0.02 mg/kg and a detection limit of 0.01 mg/kg at a signal-to-noise ratio of 3:1. We have also determined 20 blank samples to verify presence of matrix interferences. No peaks interfered with the detection of PZQ in spiked bovine muscle samples with that of the muscle components as can be seen in representative chromatograms in Fig. 2.

4. Conclusion

Despite tendency to use LC–MS/MS techniques in the residue field, there is a lack of short, cheap and effective method for specific analytes, like PZQ. Literature review shows that methods for such compound are rather rare. A simple high-performance liquid chromatographic method using ultraviolet detection was developed for the determination of PZQ in bovine muscle. Analyses were run at a flow-rate of 0.8 ml/min with the detector operating at a detection wavelength of 220 nm. The sample was extracted with ethyl acetate, cleaned up by alumin B cartridge. The standard calibration curve of drugs solution was linear in the range of 0.02–2.0 μg/ml ($r^2 = 0.9999$). At the fortified levels of 0.02, 0.05, 0.2 mg/kg, the mean recoveries of PZQ ranged from 75% to 85%, while the intra-day and inter-day coefficient of variation (CV) of the method were all less than 9%. A simple, economical and effective sample preparation protocol including solid-phase extraction has led to a sensitive method, with a quantification limit of 0.02 mg/kg and a detection limit of 0.01 mg/kg. The achieved detection limits proved the efficiency of this methodology for control of trace levels of these substances.

References

[1] M. Giorgi, A.P. Salvatori, G. Soldani, M. Giusiani, V. Longo, P.G. Gervasi, G. Mengozzi, J. Vet. Pharmacol. Ther. 24 (2001) 251–259.
[2] J. Obermeir, H. Frohberg, J. Arch. Toxicol. 38 (1977) 149–161.
[3] A. Högemann, K. Kiec-Kononowicz, F. Westhoff, G. Blaschke, J. Arzneimittel Forschung 40 (1990) 159–1162.
[4] M.L. Renata, A.D.F. Maria, J. Chromatogr. B 877 (2009) 3083–3088.
[5] S. Xiao, B. Catto, Quinn, J. Chromatogr. B 275 (1983) 127–132.
[6] J.W. Kelly, L. He, J. Pharm. Biomed. Anal. 11 (1993) 1141–1144.
[7] P.S. Bonato, A.R.M. Oliveira, F.J.M. Santana, B.J.D. Fernandes, V.L. Lanchote, A.E. Gonzalez, H.H. Garcia, O.M. Takayanagui, J. Pharm. Biomed. Anal. 44 (2007) 558–563.
[8] R.M. Lima, M.A.D. Ferreira, T.M.J. Ponte, M.P. Marques, O.M. Takayanagui, H.H. Garcia, E.B. Coelho, P.S. Bonato, V.L. Lanchote, J. Chromatogr. B 877 (2009) 3083–3088.
[9] J. Li, Y. Wang, A. Fenwick, T.A. Clayton, Y.Y.K. Lau, C.L. Quigley, J.C. Lindon, J. Utzinger, E. Holmes, J. Pharm. Biomed. Anal. 45 (2007) 263–267.