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High-performance liquid chromatographic determination of imidazole dipeptides, histidine, 1-methylhistidine and 3-methylhistidine in equine and camel muscle and individual muscle fibres

Mark Dunnett*, Roger C. Harris

Equine Sports Medicine Unit, Royal Veterinary College, Hawkshead Lane, North Mymms, Hertfordshire, AL9 7TA, UK

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Abstract

The combined solid-phase extraction (Isolute PRS columns) and reversed-phase gradient HPLC method presented provides a sensitive, reproducible and selective quantification of carnosine, anserine, balenine, homocarnosine, histidine, 1-methylhistidine and 3-methylhistidine in equine and camel muscle and individual muscle fibres. Recoveries were 91–115%. Lower limits of detection were 0.005–0.010 mmol kg^{-1} dry muscle. The compounds were isolated from other physiological amino acids and small peptides and resolved within a single chromatographic run of 55 min. Concentrations of these compounds in equine myocardium, diaphragm, skeletal muscle, camel muscle and individual muscle fibres of both species are presented for the first time.

Keywords: Histidine; Methylhistidine; Carnosine; Anserine; Balenine; Homocarnosine

1. Introduction

High concentrations of the imidazole dipeptides, carnosine (CAR; β-alanyl-L-histidine), anserine (ANS; β-alanyl-L-1-methylhistidine) and balenine (BAL; β-alanyl-L-3-methylhistidine) occur in the skeletal muscle of many mammals [1], but particularly in those adapted to either high-speed running or to prolonged periods of hypoxia, such as the horse and whale. In these, muscle contraction is reliant upon anaerobic glycolysis for rapid ATP turnover and is associated with a large increase in muscle lactic acid concentration. The accumulation of H^+ from lactic acid dissociation within skeletal muscle would produce a critical fall in intra-cellular pH in the absence of endogenous H^+ buffers. The imidazole dipeptides (pK_a 6.8–7.1) function as important H^+ buffers over the physiological pH range [2,3]. Skeletal muscle comprises three physiologically distinct fibre types; classified as types I, IIA and IIB. Although previous studies, comparing muscle sample CAR concentration with fibre type composition, have estimated the concentration in equine type IIB fibres to be 5-fold higher than in type I fibres [4,5] direct measurements in individual muscle fibres of different types have not hitherto been attempted. Imidazole dipeptides are also present in cardiac muscle, although at amounts 50–100 times

*Corresponding author.

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lower than in skeletal muscle [6]. A fourth imidazole dipeptide homocarnosine (HCAR; β-alanyl-γ-aminobutyric acid) found in the central nervous system, appears to be absent from skeletal muscle [7]. Studies of muscle imidazole dipeptide distribution and metabolism would be facilitated by simultaneous analysis of the dipeptides and their precursors/primary metabolites; histidine (HIS), 1-methylhistidine (1MH) and 3-methylhistidine (3MH).

Previous high-performance liquid chromatographic (HPLC) methods for determining imidazole concentrations in tissues include ion-exchange elution with UV or fluorescence detection [8,9], isocratic reversed-phase (RP) elution with UV or fluorescence detection [6,10,11] and combined RP–ion-exchange gradient elution with fluorescence detection [12]. Gradient RP-HPLC of *o*-phthaldialdehyde (OPA)/2-mercaptoethanol (ME) derivatized amino acids with fluorescence detection has frequently been used [13–17]. Initially only HIS was quantifiable [14,18,19] and despite later modifications, including substitution of 3-mercaptopropionic acid (MPA) for ME, which enabled the additional measurement of 1MH and 3MH [20] and CAR [15,21,22], none of these methods encompass all the title compounds. The aim of the present investigation was to develop a selective analytical procedure combining solid-phase extraction (SPE) and gradient RP-HPLC capable of resolving all the title compounds with sufficient sensitivity to allow quantitation in muscle samples weighing less than 1 μg, whilst minimizing interference from other amino acids.

2. Experimental

2.1. Instrumentation

The HPLC system comprised 2 Constametric 3000 pumps (LDC, Stone, UK), a LDC high-pressure solvent mixer, a Waters WISP 712 autosampler with auto-addition facility (Waters, Watford, UK), a Spectrovision FD 300 dual monochromator fluorescence detector (Severn, Beds, UK), a LDC MP 3000 chromatography workstation-gradient controller and a LDC printer. A Rheodyne 0.5-μm in-line filter (Cotati, CA, USA) was connected between the high-pressure mixer and the autosampler.

2.2. Materials

Sodium acetate (anhydrous), boric acid, sodium hydroxide, perchloric acid (PCA), HIS, 1MH, 3MH, CAR, ANS, HCAR, phosphoric acid (H_3PO_4), acetic acid, tetrahydrofuran, MPA and OPA solution were from Sigma (Poole, UK). HPLC grade acetonitrile, water and methanol were from Romil (Loughborough, UK). BAL was kindly donated by Dr. H. Abe (Kyoritsu Women's University, Tokyo, Japan.). All chemicals were analytical reagent grade unless specified otherwise.

2.3. Sample preparation

Muscle samples were collected post mortem from 7 thoroughbred horses (4 male, 2 female, 1 gelding) aged 2–4 years and 5 camels (3 male, 2 female) of unknown ages, which were humanely euthanased having failed to respond to veterinary treatment. The animals were free from muscular disorders. Samples were collected within 90 min of euthanasia, swabbed to remove excess blood, frozen in liquid nitrogen and subsequently freeze-dried. Freeze-dried samples were rendered as free as possible from visible blood, fat and connective tissue, and powdered. Individual muscle fibres were dissected from non-powdered samples with the aid of a low-power microscope. All samples were stored at −85°C prior to extraction and analysis.

2.4. Tissue extraction

Powdered freeze-dried muscle (10.00±2.00 mg) was extracted in 0.5 *M* PCA (100 μl mg^{-1} tissue) on ice for 15 min with regular vortex-mixing. The extraction mixture was centrifuged at 12 000 *g* for 5 min at ambient temperature. The supernatant was collected and stored at −20°C.

2.5. Solid-phase extraction

Muscle PCA extracts (500 μl) were loaded onto 100 mg (1 ml) Isolute PRS (propylsulphonyl) SPE columns (International Sorbent, Hengoed, UK) previously conditioned with methanol (4 ml) followed by 1.0 *M* H_3PO_4 (4 ml). Interfering compounds were washed from the columns with 1.0 *M* H_3PO_4 (2×

500 μl), water (500 μl) and 0.4 *M* borate buffer, pH 9.65 with sodium hydroxide (250 μl). The sorbent was dried under vacuum for 2 min. The isolates were eluted with 0.4 *M* borate buffer, pH 9.65 (3×250 μl). A flow-rate of 2 ml min^{-1} was used at all stages. The use of borate buffer (250 μl) as the final wash had two advantages. First, it reduced the recovery of arginine in the final eluate and second, it reduced the volume of eluant necessary for full recovery of the title compounds.

2.6. Individual muscle fibre histochemistry, weighing and extraction

Three fragments were cut from each individual muscle fibre. Each of the fragments were mounted on separate microscope slides scored with a grid of 25 squares. This procedure was repeated until 25 fibres were mounted in triplicate. Fibre fragments were characterized as type I, IIA or IIB by histochemical staining for myosin ATPase activity at pH 9.6 following pre-incubation at pH 4.50 [23]. The remaining larger fibre sections were weighed on a quartz-fibre fish-pole balance as described by Lowry and Passoneau [24].

Owing to the extremely low sample weights, typically 0.8–8.0 μg, it was necessary to maximize detector response by minimizing the sample extract volume. Sample extracts were therefore concentrated by freeze-drying and re-dissolution. As PCA cannot be removed by freeze-drying an alternative solvent for tissue extraction had to be found. Comparative extractions of freeze-dried powdered camel muscle (1 mg ml^{-1}) between PCA and 3 alternative solvents were performed ($n=4$ for each solvent). Relative recoveries of CAR and ANS, respectively, in the three alternative solvents compared with PCA were; 100.9% and 100.3% (methanol–0.4 *M* borate buffer, 75:25, v/v, pH 9.65), 87.3% and 83.2% (acetonitrile–0.4 *M* borate buffer, 75:25, v/v, pH 9.65) and 101.0% and 101.4% (4% sulphosalicylic acid). Comparative extractions between PCA and methanol–0.4 *M* borate buffer (75:25, v/v) were made in 12 individual equine muscle fibres. Each fibre was halved; one half was extracted with PCA the other with methanol–borate buffer. Mean (±S.D.) CAR concentrations in the PCA extracted fibres and the methanol–borate buffer extracted fibres, 46.94±9.66 mmol kg^{-1} DM and 47.21±11.44 mmol kg^{-1} DM, were not significantly different ($p>0.05$).

Individual weighed fibre sections were placed in 200-μl glass microvials (Chromacol, London, UK) and extracted with 100 μl methanol–0.4 *M* borate buffer (75:25, v/v, pH 9.65). Extractions were performed on ice with regular vortex-mixing for 15 min whilst ensuring that the muscle fibres were permanently submerged. Extracts were freeze-dried, re-dissolved in 50 μl 0.4 *M* borate buffer, pH 9.65 and stored at −85°C until analysis.

2.7. HPLC conditions

Chromatography was performed on a Hypersil ODS (3 μm, 150×4.6 mm I.D.) analytical column (Shandon, Runcorn, UK) protected by a Sepralyte ODS (40 μm, 20×2.1 mm I.D.) guard column (Upchurch, Oak Harbor, WA, USA), at ambient temperature, utilizing a binary gradient formed from solvent A [12.5 m*M* sodium acetate, pH 7.2–tetrahydrofuran (995:5, v/v)] and solvent B [12.5 m*M* sodium acetate, pH 7.2–methanol–acetonitrile (500:350:150, v/v)]. The 12.5 m*M* pH 7.2 acetate buffer was prepared by mixing isomolar solutions of sodium acetate (1.026 g l^{-1}) and acetic acid (0.751 g l^{-1}). Solvents were filtered to 0.45 μm (HVLP 047 PTFE filters, Millipore, UK, Watford, UK) and degassed by helium sparging prior to and throughout the analytical run. Mobile phase was freshly prepared for each new sample batch. The gradient composition was: 0 min, 0% solvent B; 3 min, 0% B; 20 min, 35% B; 36 min, 60% B; 40 min, 100% B; 43 min, 100% B; 45 min, 0% B; 55 min, 0% B. The flow-rate was 1.0 ml min^{-1} and analysis time was 55 min per sample. Detector excitation and emission wavelengths were 340 nm and 450 nm, respectively. The derivatization reagent was prepared by mixing MPA (80 μl) with 1 mg ml^{-1} OPA solution (borate buffer, pH 9.65) (4 ml) and stored in the dark at 2°C for 24 h before use. During the automated derivatization, extract (25 μl) and reagent (25 μl) were reacted for 90 s prior to injection. Fresh reagent was used with each new sample batch. Tissue concentrations were calculated by comparison of the integrated peak areas with those from a range of external standards.

2.8. Standard preparation

A mixed stock standard (10 m*M*) solution containing the title compounds was prepared by dissolving the required weight of each compound in HPLC grade water (10 ml). Working standard solutions were prepared over the concentration range 0.010–1.000 m*M* by dilution of the stock standard with borate buffer. The stock standard solution was stored at −20°C when not used.

2.9. Recovery study

Samples of pooled PCA extracts of freeze-dried powdered diaphragm muscle were spiked with a mixed standard containing the title compounds. These samples were spiked to simulate tissue concentrations of 100 mmol kg^{-1} DM ($n=5$), 50 mmol kg^{-1} DM ($n=5$), 5 mmol kg^{-1} DM ($n=5$) and 1 mmol kg^{-1} DM ($n=5$). Recoveries following SPE were calculated by comparison with equivalent standards. Concentrations of endogenous compounds prior to spiking were determined and subtracted when calculating recoveries.

2.10. Reproducibility study

A PCA extract of pooled diaphragm muscle was spiked with a mixed standard of the title compounds to simulate muscle concentrations at the higher and lower limits of quantitation; HLQ, 100 mmol kg^{-1} DM and LLQ, 1 mmol kg^{-1} DM. The spiked extract was divided into 30 aliquots. Within-day variation of the combined SPE–HPLC analysis was determined by repeated injections ($n=5$) of the SPE eluates on 3 occasions within day 1. Between-day variation of the combined SPE–HPLC analysis was determined by repeated injections ($n=5$) of the SPE eluates on days 1, 5 and 10.

2.11. Lower detection limits

The lowest levels of detection for the title compounds were quantified by injecting a mixed standard (100 μl) which resulted in full-scale deflection (FSD) at a detector sensitivity setting of 20 nA FSD. The minimum area reliably measurable was 3000 area units which approximated to a 5 mm peak at a signal-to-noise ratio of 3:1. The concentration of the minimum peak was interpolated from the mixed standard.

3. Results and discussion

The aim of the present work was to develop a sensitive and reproducible SPE–HPLC technique which would enable the selective isolation and quantification of the title compounds across a broad concentration range in muscle samples weighing <1 μg–10 mg.

3.1. HPLC conditions

Previously published chromatographic methods [14,15,22] applied to the determination of the title compounds in equine and camel muscle extracts failed to resolve all the compounds of interest. Various combinations of columns (Apex Phenyl 5 μm, Apex ODS 5 μm, Spherisorb ODS 2 5 μm and 3 μm, Hypersil ODS 3 μm, Hypersil ODS HC 3 μm and Primesphere ODS 3 μm) and mobile phases (10 m*M*, 12.5 m*M* and 25 m*M* acetate, phosphate and mixed acetate–phosphate buffers) were evaluated to optimize the resolution of the imidazole compounds from interfering amino acids. Acceptable resolution of the seven imidazole standards alone could not be achieved using 5 μm packed columns. Optimal separation of the title compounds from 22 amino acids (including taurine, β-alanine and GABA) was achieved using the Hypersil ODS 3 μm column in conjunction with the 12.5 m*M* acetate buffer binary gradient, as described. The final chromatographic parameters resulted in a good separation of the title compounds, although base-line separation was not quite achieved between 1MH and 3MH (Fig. 1). Retention times for the individual compounds were: HIS, 20.19 min; 1MH, 21.82 min; 3MH, 22.23 min; ANS, 26.43 min; CAR, 27.45 min; BAL, 30.08 min; HCAR, 31.71 min

3.2. Solid-phase extraction

During the analysis of physiological samples for low levels of the title compounds there is the possibility of impaired resolution from citrulline,

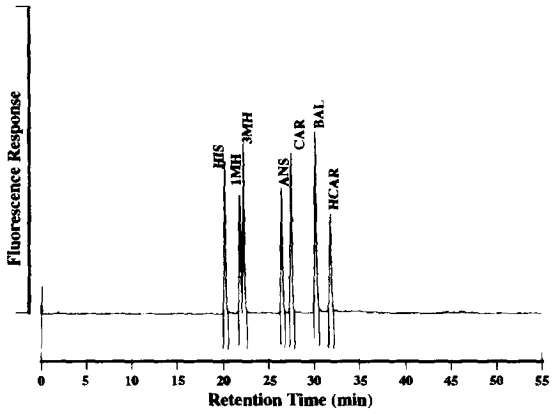

Fig. 1. HPLC separation of a mixed standard containing 500 μ*M* HIS, 1MH, 3MH, ANS, CAR, BAL and HCAR. Column: Hypersil ODS (3 μm, 150×4.6 mm I.D.). Mobile phase: Solvent A, 12.5 m*M* sodium acetate pH 7.2–tetrahydrofuran (995:5); Solvent B, 12.5 m*M* sodium acetate pH 7.2–methanol–acetonitrile (500:350:150). Flow-rate, 1.0 ml min^{-1}. Detector wavelengths, EX 340 nm, EM 450 nm. Detector sensitivity, 1000 nA FSD. Injection volume, 25 μl.

alanine, taurine and arginine when these amino acids are present at relatively much higher concentrations. SPE employing a benzene propylsulphonyl bonded phase has been used for the extraction of amino acids from urine [25]. However, this procedure did not discriminate between different classes of amino acids. The presence of the benzene ring in the bonded phase may also induce secondary non-polar interactions thus complicating the adaptation of this method. A propylsulphonyl strong cation-exchange bonded phase has been shown to effectively retain biogenic imidazoles from acidic plasma extracts [26]. SPE was adopted to provide a selective extraction of the title compounds from the other amino acids and small peptides present in muscle. The SPE method described in the experimental section proved highly selective for the title compounds. Most amino acids, including citrulline, alanine and taurine, were absent from the final extract, however arginine (26.01 min) was partly recovered (approx. 70%). Chromatograms of a PCA extract of equine myocardium pre- and post-SPE are shown in Fig. 2A and B respectively. The SPE results in an effective concentration of the analytes as separate neutralization and buffering (pH 9.65) of the PCA extracts are avoided. The overall recoveries as

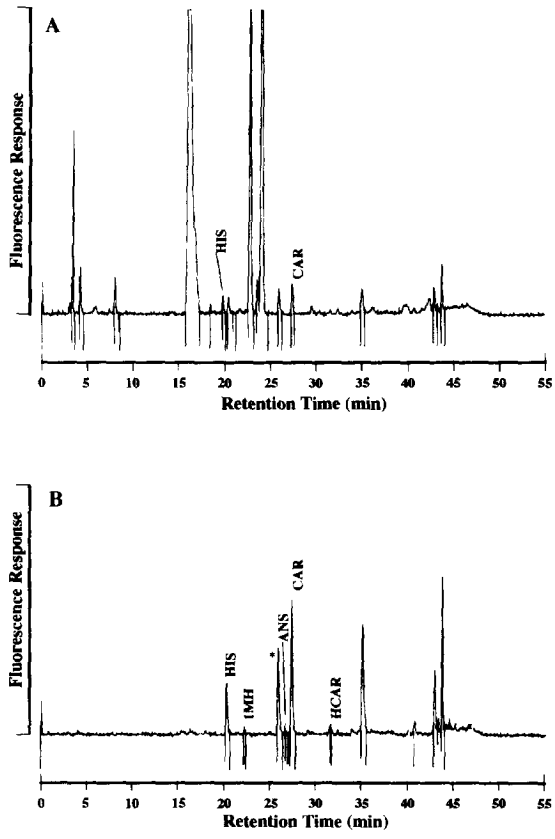

Fig. 2. HPLC separation of a PCA extract of equine myocardium: (A) Pre-SPE. (B) Post-SPE. Conditions as given in Fig. 1. Detector sensitivity: 50 nA FSD. * Indicates arginine peak.

means±coefficient of variation, C.V.(%), of the title compounds from spiked tissue samples following SPE are shown in Table 1. Excellent recoveries were obtained for all the title compounds.

Post-SPE extracts of equine myocardium, and equine and camel middle gluteal muscle were spiked with a mixed standard of the authentic compounds. Subsequent HPLC produced only single peaks at the retention times for the putative compounds.

3.3. Standards

Standard curves demonstrated a linear relationship between integrated peak area and concentration in the range 0.010–1.000 m*M*. Linear regression equations for each compound were: HIS, $y=65\,510x+103$ ($r=0.998$); 1MH, $y=103\,497x+1164$ ($r=$

Table 1
Recoveries of imidazole dipeptides, HIS, 1MH and 3MH from equine muscle tissue spiked at different concentrations

Compound	100 mmol kg^{-1} DM Recovery±C.V.(%)	50 mmol kg^{-1} DM Recovery±C.V.(%)	5 mmol kg^{-1} DM Recovery±C.V.(%)	1 mmol kg^{-1} DM Recovery±C.V.(%)
HIS	106.0±5.5	101.8±5.0	99.3±3.1	96.0±8.3
1MH	105.1±5.0	109.1±10.8	100.9±11.9	96.9±3.9
3MH	101.7±4.1	103.6±11.1	101.0±11.9	100.8±9.5
ANS	99.5±10.2	111.1±6.8	99.2±3.2	101.4±6.7
CAR	91.1±10.2	109.5±9.2	98.4±11.5	100.8±9.2
BAL	99.1±8.8	105.1±7.0	99.3±3.3	103.1±6.6
HCAR	100.8±8.1	112.7±9.5	102.5±7.4	115.6±9.6

C.V.=coefficient of variation.

0.997); 3MH, $y=100\,599x+3682$ ($r=0.996$); ANS, $y=257\,649x+2977$ ($r=0.997$); CAR, $y=247\,476x+833$ ($r=0.997$); BAL, $y=211\,263x+5845$ ($r=0.995$) and HCAR, $y=294\,440x+2597$ ($r=0.995$). (y= integrated peak area in arbitrary units, x= concentration, mM).

3.4. Reproducibility study

Within-day and between-day reproducibilities as means±standard deviation (S.D.) and C.V.(%) for the combined SPE–HPLC analysis are given in Table 2. Within-day mean coefficients of variation in muscle extracts spiked at 100 mmol kg^{-1} DM (HLQ), ranged from 1.2 to 6.3% for multiple determinations ($n=5$) on three occasions within day 1. The between-day mean coefficients of variation ranged from 1.7 to 2.9% for multiple determinations ($n=5$) on three occasions within a period of 10 days. The method demonstrated good within-day and between-day reproducibility.

3.5. Lower detection limits

The lower limits of detectability determined for 100 μl injected at a detector sensitivity setting of 20

Table 2
Within-day and between-day reproducibility of the determination of imidazole dipeptides, HIS, 1MH and 3MH in equine muscle spiked at HLQ and LLQ

Compound	Concentration	Within-day		Between-day	
		Mean±S.D.	(C.V.)	Mean±S.D.	(C.V.)
HIS	100.00	98.09±1.84	(1.7)	100.03±2.43	(2.4)
	1.00	0.99±0.03	(3.0)	1.01±0.04	(4.0)
1MH	100.00	98.35±6.57	(6.3)	101.36±2.99	(2.9)
	1.00	1.02±0.04	(3.9)	1.03±0.05	(4.9)
3MH	100.00	100.77±1.85	(1.9)	100.65±2.03	(2.0)
	1.00	1.01±0.01	(1.0)	1.02±0.01	(1.0)
ANS	100.00	101.29±2.21	(2.2)	99.87±1.86	(1.9)
	1.00	1.01±0.04	(4.0)	1.03±0.06	(5.8)
CAR	100.00	102.91±1.34	(1.3)	100.55±1.73	(1.7)
	1.00	1.00±0.05	(5.0)	0.99±0.07	(7.1)
BAL	100.00	98.08±1.24	(1.2)	98.93±2.13	(2.2)
	1.00	1.00±0.03	(3.0)	1.02±0.03	(2.9)
HCAR	100.00	97.47±1.90	(1.8)	98.42±2.70	(2.7)
	1.00	1.03±0.09	(8.7)	1.08±0.07	(6.5)

Pooled final muscle extract was spiked at HLQ and LLQ which represented tissue concentrations of 100 mmol kg^{-1} DM and 1 mmol kg^{-1} DM for each compound.
Values (mmol kg^{-1} DM) represent the mean±S.D. of 15 determinations; 5 on each of 3 sessions for the within-day reproducibility and 5 on each of 3 days for the between-day reproducibility.
C.V.=Coefficient of variation (%).

nA FSD were 0.010 mmol kg^{-1} DM (1.6 pmol on column) for HIS, 1MH and 3MH and 0.005 mmol kg^{-1} DM (0.8 pmol on column) for CAR, ANS, BAL and HCAR. Detector sensitivity settings at 10 nA FSD or less resulted in unacceptable signal-to-noise ratios. Injection volumes greater than 100 μl resulted in peak broadening and reduced resolution between 1MH and 3MH.

3.6. Application to tissue samples

Samples were taken from equine middle gluteal muscle (skeletal locomotive muscle), internal intercostal muscle (skeletal non-locomotive muscle), diaphragm (non-skeletal non-locomotive muscle), myocardium and camel middle gluteal muscle. Concentrations of the title compounds determined in equine and camel muscle are given in Table 3. Typical HPLC separations of post-SPE PCA extracts of equine and camel middle gluteal muscle are shown in Fig. 3A and B.

The CAR concentration in equine middle gluteal muscle is in close agreement with previously published values (108.3–125.3 mmol kg^{-1} DM) [2,5,16,27]. The ANS concentration in equine middle gluteal muscle is comparable with previous values of 0.20–0.37 μmol g^{-1} wet muscle (WM) (0.80–1.48 mmol kg^{-1} DM) [9,28]. BAL has not been detected previously in equine muscle [9,28]. The HIS concentration in equine middle gluteal muscle is close to that reported previously (0.85±0.04 mmol kg^{-1} DM) [29]. The low 1MH concentration probably

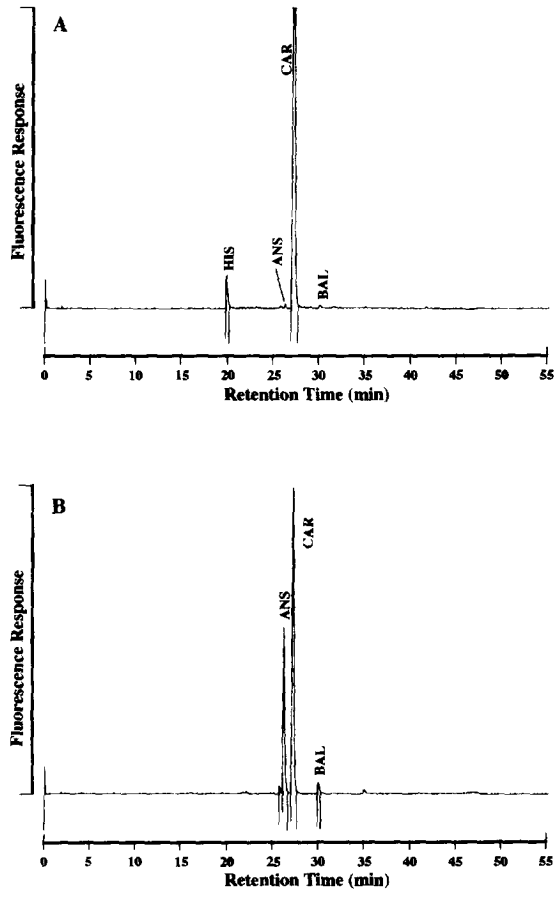

Fig. 3. HPLC separations of post-SPE PCA extracts of (A) equine middle gluteal muscle, (B) camel middle gluteal muscle. Conditions as given in Fig. 1. Detector sensitivity: 500 nA FSD.

Table 3
Concentrations of imidazole dipeptides, HIS, 1MH and 3MH in equine and camel muscle

Compound	Concentration (mean±S.D., mmol kg^{-1} DM)				
	Equine				Camel
	Middle gluteal (n=7)	Intercostal (n=3)	Diaphragm (n=4)	Myocardium (n=4)	Middle gluteal (n=5)
HIS	0.25±0.05	0.39±0.01	0.60±0.36	0.56±0.06	ND
1MH	0.03±0.05	0.02±0.04	0.13±0.03	0.18±0.04	ND
3MH	ND	ND	ND	ND	ND
ANS	0.66±0.27	0.66±0.13	0.82±0.10	0.07±0.07	37.45±12.56
CAR	107.81±12.87	63.70±16.16	38.66±5.09	3.05±1.31	29.66±16.32
BAL	0.20±0.06	0.10±0.05	0.08±0.03	ND	0.46±0.30
HCAR	ND	ND	ND	(0.06)[a]	ND

ND=Not detected.
[a] Only detected in one sample.

arises through ANS hydrolysis. The CAR concentration in equine myocardium is similar to the value reported in frogs; <1 mmol kg^{-1} WM (<4 mmol kg^{-1} DM) [6], but higher than the values reported in other species, including humans; 10.12–25.11 μg g^{-1} WM (0.18–0.44 mmol kg^{-1} DM) [30]. The ANS concentration in equine myocardium is lower than reported in rats; 43.9±11.0 μg g^{-1} WM (0.73±0.18 mmol kg^{-1} DM) [11]. The HIS concentration in equine myocardium is similar to values reported in other species, including humans; 27.91–67.95 μg g^{-1} WM (0.72–1.75 mmol kg^{-1} DM) [30]. The presence of HCAR in only 1 sample is probably anomalous, as earlier studies in several species did not detect its presence in skeletal muscle or myocardium [7,11,31]. Lower CAR concentrations in equine intercostal muscle and diaphragm are consistent with their lower glycolytic capacity, resulting in lower lactic acid production, and hence a reduced need for H^+ buffering.

The title compounds were determined in individual middle gluteal muscle fibres from a single horse (n=86 fibres) and camel (n=41 fibres). HIS, 1MH, 3MH, BAL and HCAR were not detected in these. CAR concentrations (mean±S.D.) in equine type I, IIA and IIB muscle fibres were 27.27±5.89, 89.69±10.27 and 96.62±12.93 mmol kg^{-1} DM, respectively. CAR concentrations in camel type I, IIA and IIB muscle fibres were 11.96±5.71, 25.65±4.86 and 23.86±7.31 mmol kg^{-1} DM, respectively. ANS concentrations in camel type I, IIA and IIB muscle fibres were 24.11±7.33, 38.91±8.60 and 34.26±8.85 mmol kg^{-1} DM, respectively. Higher CAR and ANS concentrations in type II muscle fibres are consistent with their higher capacity for H^+ production and thus greater buffering requirement.

4. Conclusions

A selective, sensitive and reproducible SPE–HPLC method has been developed and validated for the isolation and quantification of the title compounds in muscle and individual muscle fibres. This method avoids the interference from other physiological amino acids and small peptides associated with earlier techniques and for the first time enables the resolution of all 7 compounds within a single chromatographic run. It has been used successfully to determine the title compounds in equine muscle and, for the first time, in camel muscle and individual muscle fibres. The selectivity of the method makes it a valuable technique for the study of imidazole dipeptide metabolism and distribution in muscle which may be important to aetiological studies of exertional rhabdomyolosis and exercise induced muscle damage. Although the present method was developed for the analysis of muscle tissue it is probable that it could be used equally well for the analysis of plasma, urine and other physiological fluids. This could be of value in the clinical investigation of metabolic disorders such as, homocarnosinosis, imidazole aciduria and serum carnosinase deficiency.

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