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High-performance liquid chromatography (HPLC) as a means of assessing the presence of uric acid in archeological human remains: Challenges and future directions

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Abstract

Objectives: This research aimed to replicate the Swinson, D., Snaith, J., Buckberry, J., & Brickley, M. (2010). High performance liquid chromatography (HPLC) in the investigation of gout in paleopathology. *International Journal of Osteoarchaeology*, 20, 135–143. https://doi.org/10.1002/oa.1009 method for detecting uric acid in archeological human remains to investigate gout in past populations and to improve the original High Performance Liquid Chromatography-ultraviolet (HPLC-UV) method by using HPLC-mass spectrometry (HPLC-MS), a more sensitive, compound-specific detection method.

Materials and Methods: We used reference samples of uric acid to create a dilution series to assess the limits of quantification and detection. Samples from individuals with and without gout lesions were taken from foot bones and ribs from the English cemeteries of Tanyard, Hickleton, Gloucester, and Lincoln.

Results: We could not replicate the results of Swinson and colleagues using HPLC-UV. Tests using a dilution series of uric acid showed HPLC-MS was approximately 100× more sensitive than HPLC-UV, with the additional benefit of being compound specific. A newly developed hydrophilic interaction chromatography (HILIC) method improved retention characteristics. Fourteen samples from eight individuals, five with skeletal lesions consistent with gout, were analyzed with the final method. None showed evidence of uric acid despite the newly developed method's improved sensitivity and specificity.

David Swinson: Retired.

Discussion: The lack of detectable uric acid extracted from these samples suggests that (1) urate crystals were not present in any of the bone samples, regardless of gout status; (2) urate crystals did not survive these specific archeological conditions; or (3) the concentration of uric acid in our bone extracts was low, and thus larger samples would be required.

KEYWORDS
gout, high performance liquid chromatography-mass spectrometry, high performance liquid chromatography-ultraviolet, hydrophilic interaction chromatography, paleopathology

1 | INTRODUCTION

Increased circulating uric acid (hyperuricemia) can result in gout. For gout to develop, a number of minute solid bodies or "nuclei" must be present, from which urate (monosodium urate/MSU) crystals develop (Chhana et al., 2015). Acute gout is self-limiting and after a period of inflammation and pain the concentration of uric acid decreases and these crystals are resorbed. However, if a high concentration of uric acid is sustained for a long time and certain factors are present in serum and joints, it can develop into chronic gout and the formation of MSU crystals (Chhana et al., 2015).

In skeletal human remains, chronic gout is characterized by paraarticular erosive lesions with overhanging edges (Martel hook) and sclerotic margins, which are caused by the presence of a tophus next to the bone. The lesions are often located at the distal end of the first metatarsal. However, as these lesions are only observable in chronic gout (Waldron, 2019, p. 744), the ability to identify biomarkers for MSU in skeletal human remains would offer considerable benefits for understanding patterns and the pathogenesis of gout, allowing confirmation of cases with characteristic lesions, and potentially identifying gout in individuals without skeletal changes.

In a clinical context, hyperuricemia is defined as ≥7.0 mg/dL for men and ≥6.0 mg/dL for women (blood concentration). Uric acid values higher than 7.0 mg/dL is a risk factor for the development of gout (De Oliveira & Burini, 2012). However, in chronic tophaceous gout the body load of uric acid and urate can be as high as 25× the normal level (increasing from 1200 mg to potentially 30,000 mg in males; 600 mg is considered normal for females). In chronic gout deposits of urate crystals are found not only in joints, skin and the ear pinna but also in the heart and kidneys (Rosenthal, 1998, p. 1557). Thus, it is likely that urate crystals (which revert to uric acid when dissociated in liquid) may be present in bone after death.

Uric acid ($C_5 H_4 N_4 O_3$; IUPAC 7,9-Dihydro-1H-purine-2,6,8(3H)-trione; molecular weight: 168.1103 g/mol) is an organic compound and end-product of the complex metabolism of purines, both from endogenous and exogenous sources. It is endogenously synthesized primarily in the liver and intestines, but also in muscle and vascular endothelium (Chaudhary et al., 2013). Genetic, dietary, and environmental factors influence the exogenous pool of uric acid; however, the relationship between diet and uric acid is not fully understood (De Oliveira & Burini, 2012). Bone is a very vascular tissue and *in vivo* it is well exposed to blood constituents, therefore uric acid will be present throughout bone in individuals with gout.

2 | BACKGROUND

In 2010, Swinson and colleagues published the preliminary results of a study, which aimed at identifying uric acid from archeological human bone. Eight individuals from medieval and postmedieval English sites (Figure 1) were macroscopically assessed for the presence of gouty lesions at the head of the first metatarsal. From this analysis, five individuals were diagnosed with gout (individuals "A," "E," "F," "G," and "H") and three without (individuals "B," "C," and "D"). Bone samples were obtained from all individuals and a "white powder" observed within the erosive lesions present in individuals A and E was also sampled (Table 1). Their high performance liquid chromatography with ultraviolet detection (HPLC-UV) results found that the "white powder" samples from 'A' and 'E' were strongly positive for uric acid. The bone samples of individuals with gout "A," "E," "G," and "H" were also positive for uric acid in varying degrees of strength. In contrast, the sample of bone from individual "F" was negative despite showing erosive lesions, as were the cortical samples from the "control" individuals "B," "C," and "D." The powder samples were analyzed for crystals by polarizing microscopy: the negatively birefringent crystals found in the sample from individual "A" were assessed as typical of sodium monourate but tested negative for the murexide test and the x-ray diffraction test showed the presence of calcium apatite but not uric acid. The white powder from individual "E" was not identified as urate. The authors concluded that the correlation between observable/nonobservable erosive lesion and positive/negative identification of uric acid in HPLC-UV demonstrated the survival of this molecule and the potential of this technique.

Limbrey et al. (2011) further explored the survival of MSU crystals in the archeological record by analyzing the "white powder" from individuals "A" (Bromyard) and "E" (Lincoln) reported in Swinson et al. (2010) and a bone sample from an individual with gout from Lisbon using x-ray diffraction and polarizing microscopy. MSU crystals were not observable using x-ray diffraction, but crystals showing optical features characteristic of MSU were observed using the polarizing microscope. Only two additional studies have reported the identification of MSU crystals, both cases in mummified human remains: Elliott-

FIGURE 1 Map of England and Wales showing the sites investigated.

Smith and Dawson (1924); in Limbrey et al. (2011) found them in an Egyptian mummy and Ordi et al. (2006) in the mummified remains of the Holy Roman Emperor Charles V.

Despite the seemingly positive identification of MSU from archeological skeletal human remains, the detection of this molecule needs further consideration. This is because, while MSU is more soluble in acidic conditions, its solubility is reduced markedly in neutral/alkaline conditions (pH ≥6). Additionally, temperature and the presence of other ions (e.g., sodium, potassium, magnesium, or calcium ions) affect the solubility of urate crystals (Chhana et al., 2015). It is still unclear how MSU would respond to different archeological environments and associated bone diagenesis; however, it is more likely to be preserved in dry, cold, and/or alkaline burial conditions, based on its solubility in different environmental conditions. It is also worth noting, as Swinson et al. (2010) concluded, the identification of the crystals in the "white powder" is uncertain as the different tests produced inconsistent results.

Given these preliminary studies and standing concerns, this article has two aims: first, to replicate the methods used by Swinson et al. (2010) and, second, to develop a high performance liquid chromatography mass spectrometry (HPLC-MS) method to improve specificity and sensitivity of uric acid detection to progress research into gout in archeological human remains. As the "white powder" from individuals "A" and "E" is no longer available, this study will focus only on the analysis of bone.

3 | MATERIALS: ARCHEOLOGICAL REMAINS ANALYZED

As the initial aim of this research was to replicate the method of Swinson et al. (2010) and assess its sensitivity, we sampled the same eight individuals, plus an additional control (Table 1 and Figure 1). All individuals are from English archeological sites and are curated by the Biological Anthropology Research Centre (BARC) at the University of Bradford. Approval for destructive analysis was obtained from the BARC committee and the research was approved by Ethics at Bradford. Sampling was undertaken following the procedures outlined in the BARC Human Remains Policy (BARC, 2016).

Samples of archeological bone were prepared from all nine individuals. Codes, sample information, and the masses used in the preparation are shown in Tables 2 and 3. Samples were taken from up to three different locations per individual: foot bones (adjacent to or away from gouty lesions for individuals with gout, or any foot bone for control samples) and from ribs, to investigate if the MSU seen in bone is only deposited close to tophi or, as uric acid is a

TABLE 1 Skeletal material used in this study and summary of results from Swinson et al. (2010).

Site name, site code and details	Skeleton ID	Age and sex	Skeletal co-morbidities	Evidence of gout	Previous HPLC-UV result (Swinson et al., 2010)	References
Tanyard, Bromyard, West Midlands (AA04/29) Quaker cemetery (1750 to 1890 AD)	TY84/ HB5	Mature adult male	Calculus, PD, EH, OA spine, 2 rib fractures	R MT1 head, large undercut erosion with WP on medial side. R MT5 small erosion. L MT1 head small erosion with WP. L MT1 cup erosion. Martell hook on radiograph	(A) WP positive ++ Bone positive ++ (vertebral)	Brickley (2004); Lewis (2004)
	TY138/ HB14	Child	-	None	(B) Bone negative (L cortical MT1)	
	TY120/ HB11	Young adult female	Calculus, PD	None	(C) Bone negative (L cortical tarsal)	
	TY64/ HB2	Middle adult female	Calculus, PD, abscess	None	(D) Bone negative (L cortical tarsal)	
	TY65/ HB4	Middle adult female	Calculus, PD, EH, abscess, fusion SI joint	None	N/A	
St Mark's Railway Station, Lincoln (ZEB95) Carmelite Friary (1242–1260 to 1538 AD)	L6560	Mature adult male	Fracture R fibula, PNBF L&R tibiae, L fibula, OA spine and acromio-clavicular joints, DDD, SN	R MT 3, 4 & 5 proximal erosions. L MT1 head large medial erosion. L MT 4 & 5 healed erosions onto shafts. WP in L MT5 shaft erosion	(E) WP positive +++ Bone positive ++	Trimble (1998); Isaac and Roberts (1997)
Blackfriars, Gloucester (19/91) Dominican Friary (1246 to 1538 AD)	BF128	Mature adult male	PD, fusion SI joint, OA spine and R elbow	R MT1 head, large erosion on medial side. R MT5 head small marginal erosion	(F) Bone negative (trabecular R MT heads erosions)	Atkin (1983); Wiggins et al. (1993)
St Wilfrid's Hickleton, Doncaster, South Yorks (HK83) Medieval parish churchyard	HK10	Mature adult male	DISH, DDD, OA spine, 2 rib fractures	R MT1 head, medial erosion with secondary bone proliferation	(G) Bone positive + (trabecular RMT head erosion)	Sydes (1984); Stroud (n. d.)
	HK34	Mature adult female	OA spine, SN, supernumerary vertebra (T13)	L M1T head, deep medial erosion, undercut. L MT5, similar deep erosion on lateral side	(H) Bone positive ++ (trabecular L MT head erosion) Bone positive + (trabecular L MT head erosion)	

Note: HPLC-UV results from Swinson et al. (2010). + indicates a positive test result, − indicates a negative result, the number of symbols is an expression of quantity.
Abbreviations: DDD, discarthrosis; DISH, diffuse idiopathic skeletal hyperostosis; EH, enamel hypoplasia; L, left; MT, metatarsal; OA, osteoarthritis; PD, periodontal disease; PNBF, sub-periosteal new bone formation; R, right; SI, sacro-iliac; SN, Schmorl's nodes; WP, white powder.

TABLE 2 Samples used for method development.

Site name, code and details	Skeleton ID	Age and sex	Bone(s) sampled in present study	Sample number(s)	References	Sample weight (mg)
Tanyard, Bromyard, West Midlands (AA04/29)	TY84/ HB5	Mature adult male	L MT1 (near lesion)	9	180615_UA_84	1.29
			R rib (no lesion)	10	TY_HB5_C	1.78
			L MT1 (near lesion)	16	16_TY5_MT	7.10
			L MT1 (near lesion)	17	TY_HB5_B	11.4
	TY138/ HB14	Child	L MT1 (no lesion)	13	TY138_A	4.12
			MT (no lesion)	24	TY137[a]	5.08
	TY120/ HB11	Young adult female	R talus (no lesion)	14	TY_120_A	1.68
			MT (no lesion)	22	TY120_MT	4.55
			Rib (no lesion)	23	TY120_RIB	8.05
	TY64/ HB2	Middle adult female	L MT3 (no lesion)	12	TY64_A	2.68
			L MT3 (no lesion)	20	TY64_MT	3.24
			Rib (no lesion)	21	TY64_RIB	6.27
	TY65/ HB4	Middle adult female	L talus (no lesion)	11	180615_UA_65	3.72
St Mark's Railway Station, Lincoln (ZEB95)	L6560	Mature adult male	LMT1 (near lesion)	1	L6560_LMT1	1.24
			RMT3 (away from lesion)	2	L6560_RMT3	1.37
			Foot[b]	18	L6560_A	3.03
			Foot[b]	19	L6560_B	5.07
Blackfriars, Gloucester (19/91)	BF128	Mature adult male	R MT1 (near lesion)	7	BF128_A	2.82
			L rib (no lesion)	8	BF128_B	4.20
St Wilfrid's Hickleton, Doncaster, South Yorks (HK83)	HK10	Mature adult male	Left talus (away from lesion)	3	HK10_A	1.73
			Rib (no lesion)	4	HK10_B	2.30
	HK34	Mature adult female	L MT1 (away from lesion)	5	HK34_A	1.87
			R rib (no lesion)	6	HK34_B	2.64

Abbreviations: L, left; MT, metatarsal; R, right.
[a]Sample mislabeled as TY137 instead of TY138.
[b]Bone not specified in lab book, but only foot bones were available.

TABLE 3 Samples fortified with uric acid for spiked sample experiment.

Site name, code and details	Skeleton ID	Age and sex	Bone(s) sampled in the present study	Sample number(s)	References	Sample weight (mg) and uric acid concentration
Tanyard, Bromyard, West Midlands (AA04/29)	TY120/ HB11	Young adult female	Rib (no lesion), spiked sample	A1	TY120_B_A1	1.0, 100 ppm
				B1	TY120_B_B1	1.0, 50 ppm
				C1	TY120_B_C1	1.0, 10 ppm
				D1	TY120_B_D1	1.0, 5 ppm
				E1	TY120_B_E1	1.0, 1 ppm

circulating molecule, MSU can be deposited throughout the skeleton, remaining in Haversian systems and around trabecular bone. Samples weighing between 1.2 and 16.6 mg were scratched from broken surfaces, and, where possible, from areas with extant post-mortem damage, using a metal dental tool. The tool was cleaned in acetone between samples.

4 | METHODS

4.1 | Method validation

A dilution series of uric acid in mobile phase was made and analyzed using HPLC-UV-MS (UV and MS monitored in series—that is,

TABLE 4 Samples used for final method.

Site	Skeleton ID	Age and sex	Bone(s) sampled in the present study	Sample reference number(s) F = foot, R = rib	Sample weight (mg)
Tanyard, Bromyard, West Midlands (AA04/29)	TY84/HB5	Mature adult male	L MT1 (near lesion)	TYHB5_F_C	4.5
			R rib (no lesion)	TYHB5_R_C	15.4
	TY138/HB14	Child	L MT1 (no lesion)	TY138_F_C	7.6
	TY120/HB11	Young adult female	R talus (no lesion)	TY120_F_C	3.5
			Rib (no lesion)	TY120_R_C	12.8
	TY64/HB2	Middle adult female	L MT3 (no lesion)	TY64_F_C	4.0
			Rib (no lesion)	TY64_R_C	7.9
St Mark's Railway Station, Lincoln (ZEB95)	L6560	Mature adult male	LMT1 (near lesion)	L6560_F_C	5.6
Blackfriars, Gloucester (19/91)	BF128	Mature adult male	R MT1 (near lesion)	BF128_F_C	4.8
			L rib (no lesion)	BF128_R_C	7.2
St Wilfrid's Hickleton, Doncaster, South Yorks (HK83)	HK10	Mature adult male	Left talus (away from lesion)	HK10_F_C	4.2
			Rib (no lesion)	HK10_R_C	16.6
	HK34	Mature adult female	L MT1 (away from lesion)	HK34_F_C	6.5
			R rib (no lesion)	HK34_R_C	4.6

Abbreviations: L, left; MT, metatarsal; R, right.

sequential to each other in the same instrument) allowing comparison of UV and MS results, to assess the limits of detection and quantification of uric acid using both methods.

4.2 | Replication of the Swinson et al. (2010) method

Following Swinson et al. (2010), bone samples were crushed with a clean pestle and mortar. 1 mL of deionized water was added to the bone powder, mixed, and then syringed into a vial, which was sonicated and centrifuged. These were then analyzed using HPLC-UV-MS allowing comparison of UV and mass spectrometry results.

4.3 | Development of sample preparation and the HILIC method

Several methods to extract uric acid from archeological bone were assessed using samples listed in Table 2 and weighing between 1.2 and 11.4 mg. In brief, these involved uncrushed and crushed samples, different sonication durations, and extraction into deionized water (neutral) or mobile phase (acidic). Extraction into mobile phase was more likely to dissociate MSU crystals, reverting them to uric acid, as it is acidic. In the final extract procedure bone samples were ground in a mortar and pestle and extracted into 1 mL of HPLC mobile phase (30% ammonium formate and 70% acetonitrile) (see below and Table 4).

A series of experiments were undertaken to establish a validated HPLC-UV-MS method to assess the presence of MSU in bone with the added specificity gained by using mass spectrometry. We also aimed to increase retention in the HPLC stationary phase to allow adequate separation from interfering species. The strategy was to develop a hydrophilic interaction chromatography method (HILIC) capable of retaining uric acid, which is highly polar in nature and hence elutes very close to the solvent front in reversed phase methods. The final method used an amino stationary phase (Phenomenex Luna NH_2, 250 × 4.6 mm, 5 µm) with an isocratic flow of 30 mM ammonium formate and acetonitrile (30:70) at 1 mL/min (Alliance 2790, Waters LLC). The column eluent was monitored by UV at 284 nm in series with a triple quadrupole mass spectrometer (Quattro Ultima, Waters LLC) fitted with an electrospray source operating in negative mode (ESI-MS). The MS used a cone voltage of 35 V, capillary voltage of 0.5 kV and source/desolvation temperatures of 120 and 360°C, respectively, and monitored the signal in multiple reaction monitoring (MRM) mode using two transitions specific to uric acid (167–124 Da and 167–96 Da achieved with a collision energy of 22 eV). Flow into the source was reduced to 200 µL/min using a splitter.

4.4 | Investigating chemical interactions between uric acid and bone

A series of synthetic hydroxyapatite and archeological bone samples (Table 3) fortified with uric acid (100–1 ppm w/w) were prepared and

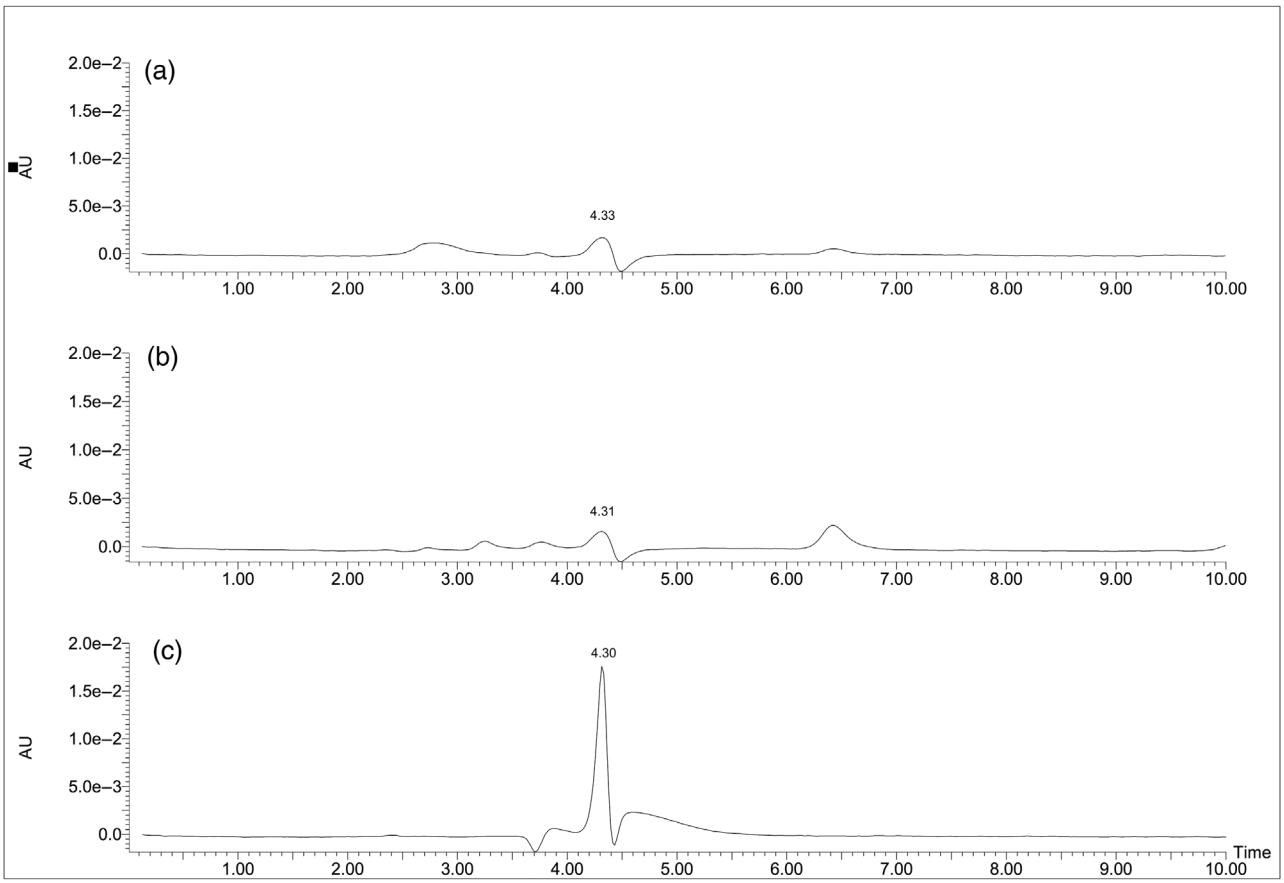

FIGURE 2 HPLC-UV showing samples TY84/HB5 with gouty lesions (a) and TY65/HB4 without gouty lesions (b) with uric reference (c). The bone samples both show additional minor peaks at different retention times (eluting before 4 min and at c. 6.4 min), indicating additional material in the bone extracts, which is not seen in the reference sample.

extracted in the same manner to assess potential chemical interactions that could complicate the extraction or produce interfering peaks in the HPLC methods. These tests were performed to confirm the adequacy of the approach for examining archeological bone.

4.5 | Testing the new method using new samples from individuals analyzed by Swinson et al. (2010)

Finally, a series of new samples from individuals investigated by Swinson et al. (2010) were extracted into mobile phase using between 3.5 and 16.6 mg of sample from the feet and ribs of eight individuals (Table 4), to compare their results with those using the newly developed method.

5 | RESULTS AND DISCUSSION

5.1 | Method validation

The dilution series of uric acid in mobile phase run using HPLC-UV indicated uric acid had a retention time of c. 4 min (previously observed at c. 9 min by Swinson et al., 2010), with a limit of quantification of 1.734 μg/mL and limit of detection of 0.504 μg/mL. The same dilution series produced a limit of quantification of 0.01734 μg/mL using HPLC-MS and a limit of detection of 0.00504 μg/mL. Thus, HPLC-MS was shown to be approximately 100× more sensitive than HPLC-UV.

5.2 | Replication of the Swinson et al. (2010) method

The results of these investigations were inconsistent with the previously published work (Swinson et al., 2010); that is, using the HPLC-UV methods, we see a peak at c.4.3 min in individuals with skeletal lesions and in individuals with no skeletal lesions characteristic of gout. This is illustrated by individuals TY84/HB5 (individual with gout, chromatogram 2A) and TY65/HB4 (individual with no gouty lesions, chromatogram 2B) (Table 1; Figure 2). The presence of a peak in TY65/HB4 (as well as TY84/HB5) was unexpected, as this individual (a middle adult female) is demographically unlikely to have gout in addition to having no skeletal lesions, although we cannot rule gout out from these data alone. However, the peaks observed may not be uric acid, as this method is not compound specific, that is the peak could relate to a different compound. Thus, we explored the presence

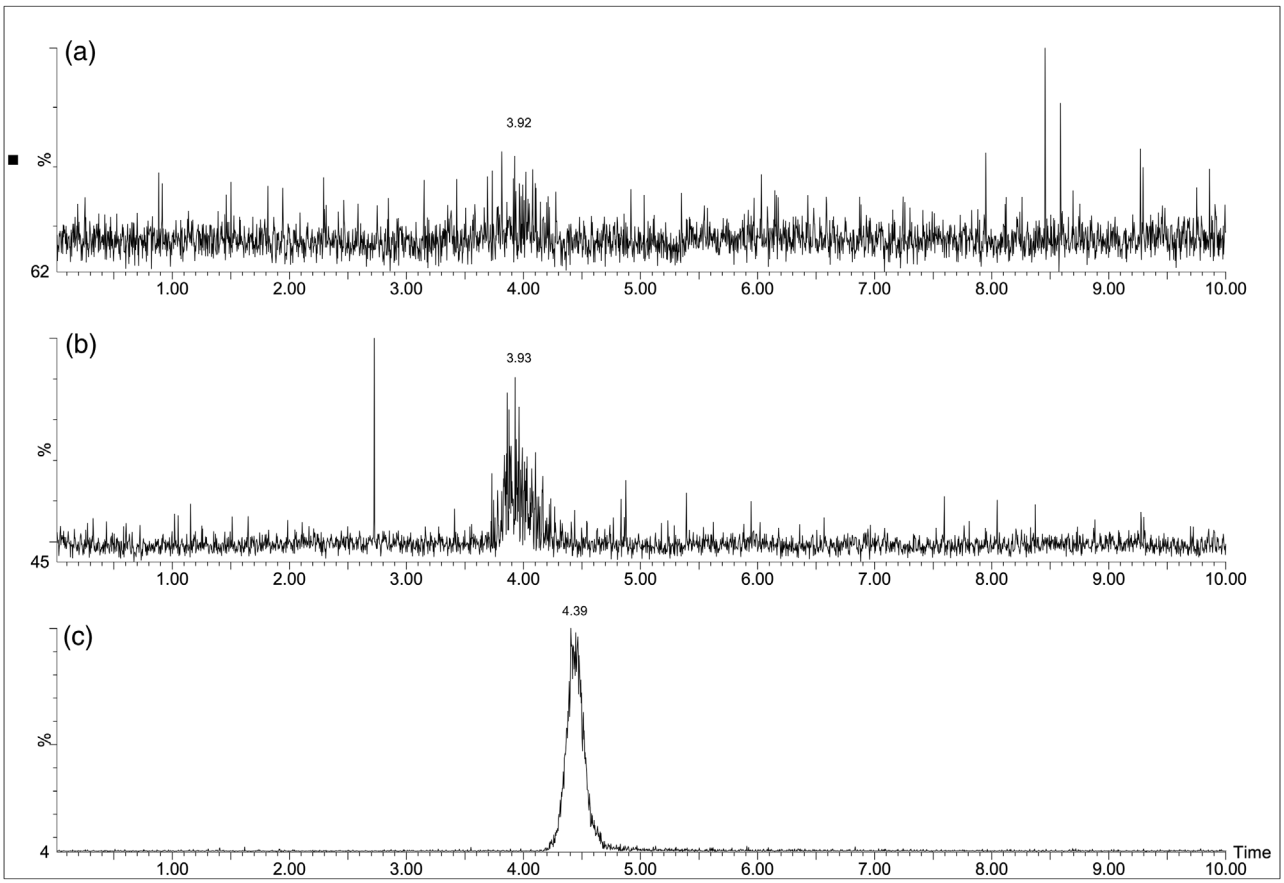

FIGURE 3 HPLC-MS showing samples TY84/HB5 with gouty lesions (a) and TY65/HB4 without gouty lesions (b) with uric acid reference (c).

of uric acid using the MS detected chromatogram, which (unlike UV detection) is compound-specific (Figure 3). This confirms the presence of uric acid in the reference sample (chromatogram 3C), but no corresponding peaks are visible in the bone extracts (chromatograms 3A and 3B) at 4.4 min. We attribute the minor fluctuations in HPLC-MS chromatograms of the bone extracts to interfering compounds (i.e., not uric acid), noting that they are at a different retention time (3.9 min) to the uric acid (4.4 min).

Our assessment of the Swinson et al. (2010) method found the limit of detection/limit of quantification with HPLC-UV for a dilution series of reference uric acid is c. 0.2 μg/mL (200 ng/mL). Extracted bone samples (TY65/HB4 and TY84/HB5) displayed a peak in the chromatogram with a similar retention time to the reference uric acid (c. 4.3 min), which could easily be interpreted as a positive uric acid response with no further qualifying information (see Figure 2). Sample TY84/HB5 had previously been identified as "positive" in Swinson et al. (2010) and TY65/HB4 had been identified as "negative"; however, further interrogation of the samples prepared at the University of Bradford using the more specific HPLC-MS method suggests the peak observed in the UV is not uric acid (that is, the bone extracts contain a UV active compound with similar retention characteristics in the HPLC method, but that is not uric acid). Chromatograms showing the HPLC-MS responses of the two bone extracts alongside a reference uric acid are presented in Figure 3.

5.3 | Detection of uric acid using the HILIC method

The HILIC method was shown to retain the uric acid on column strongly ("moving" it away from early eluting interferences) with a retention time of >15 min (capacity factor, $k' = 14.7$) and give a linear response in the UV and MS monitored dilution series between 21 and 0.0011 μg/mL (1.1 ng/mL), with a limit of detection of 5 μg/mL for UV and 0.05 μg/mL for MS. As noted previously, the MS MRM monitored method is highly specific to uric acid and has the advantage of being c. 100 times more sensitive. It is capable of detecting 10 ppm uric acid in prepared bone using the sample masses employed in this study. This developed method was applied to samples listed in Table 4. Representative chromatograms are presented in Data S1 and S2.

5.4 | Investigating chemical interactions between uric acid and bone

Studies to assess extraction of uric acid from spiked samples of synthetic hydroxyapatite and spiked samples of archeological bone were undertaken to investigate the extraction procedure used and explore potential interactions between hydroxyapatite/bone and uric acid. These experiments showed uric acid was successfully extracted and

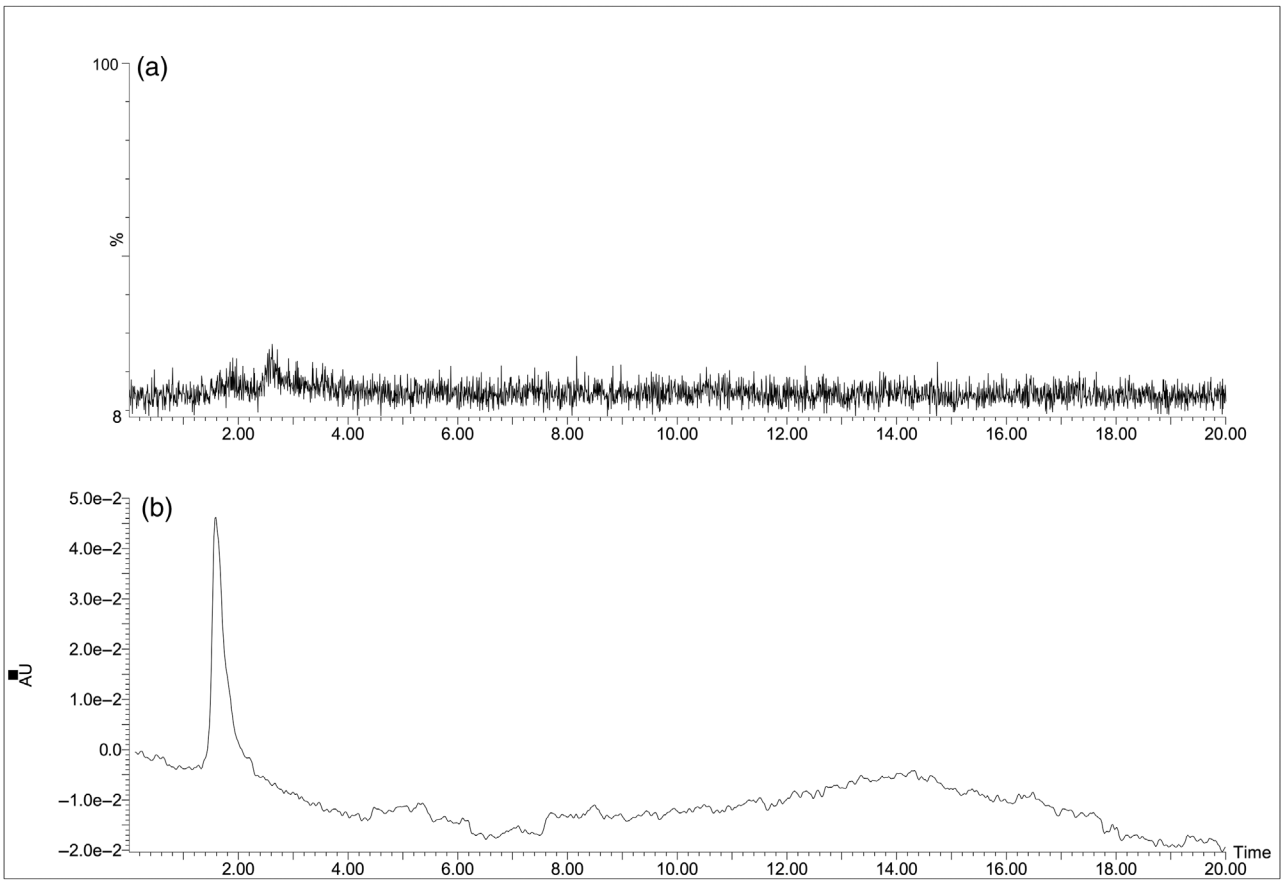

FIGURE 4 HPLC-UV-MS (UV bottom, MS top) of extract from individual TY84/HB5 (sample TYHB5-F-C). This individual displayed gouty lesions but there is no peak c. 15.7 min (which would be indicative of uric acid if present).

the HPLC-MS method could detect uric acid with no interferences down to c. 10 ppm w/w. Representative chromatograms (HPLC-MS and HPLC-UV) are presented in Data S1 to show this positive result (S3 and S4).

5.5 | Testing the new method using new samples from individuals analyzed by Swinson et al. (2010)

Newly prepared bone samples from individuals used by Swinson et al. (2010) were analyzed using the newly developed HPLC-MS (HILIC) method (Table 1). Despite being from the same individuals/bones as previously studied (Swinson et al., 2010), none of these extracts from archeological yielded results that indicated the presence of uric acid. UV and MS chromatograms for each analyzed bone sample are presented in Data S5–S17 with individual TY84/HB5 also shown in Figure 4. This is from a new sample from the foot bones of the same individual discussed in Section 4.1, who displayed an erosive lesion consistent with gout and associated white deposits (see below).

We do, however, note that there are early eluting positive peaks (c. 2 min) observed in the UV monitored method from the bone extracts indicating the presence of a compound or compounds other than uric acid. While these may correlate to those observed previously in the studies by Swinson et al. (2010) and in our initial work, these were also found in nongouty individuals (see Section 4.1). Further work could investigate what these peaks represent.

6 | CONCLUSION

The developed HPLC-UV-MS method is robust, providing compound specific, highly sensitive detection with adequate retention characteristics for assessment of uric acid in bone. Using this highly developed method, we have not observed positive uric acid responses in extracts from archeological human bone. The lack of uric acid detected using HPLC-MS could be explained in several ways. First, it is possible that the MSU crystals are not deposited in Haversian systems or on the surface of trabecular bone following decomposition, making this approach impossible. Second, MSU is highly soluble under certain conditions (Wang & Königsberger, 1998), and therefore may not always survive the archeological record, particularly in areas with high rainfall percolating through acidic soils (as is the case in some parts of Britain). While this may seem discouraging, it is important to consider that this may not be the case in alkaline or arid environments. Third, MSU may be present in bone, but was extracted at smaller concentrations than detected here. This means that larger sample sizes or more

concentrated extracts would be required for detection. We have shown the advantages of the specificity of using a MS monitored HPLC method, to avoid the potential misinterpretation of nonspecific data. We recommend the developed HPLC-UV-MS method presented should be used over HPLC-UV methods for the detection of uric acid in archeological bone and similar approaches be developed for the analysis of other molecules relating to pathology in the future.

AUTHOR CONTRIBUTIONS

Jo Buckberry: Conceptualization (equal); formal analysis (equal); funding acquisition (supporting); investigation (equal); methodology (equal); project administration (equal); writing – original draft (equal); writing – review and editing (equal). **Richard Telford:** Formal analysis (equal); investigation (equal); methodology (equal); writing – original draft (equal); writing – review and editing (equal). **Laura Castells Navarro:** Investigation (equal); writing – original draft (equal); writing – review and editing (equal). **John Snaith:** Writing – review and editing (equal). **David Swinson:** Writing – review and editing (equal). **Andrew Healey:** Formal analysis (equal). **Megan B. Brickley:** Conceptualization (equal); funding acquisition (lead); investigation (equal); project administration (equal); writing – original draft (equal); writing – review and editing (equal).

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DATA AVAILABILITY STATEMENT

Most of the data that supports this article are provided in the tables, figures and Supplementary Information. Additional chromatic data are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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