European Journal of Pharmaceutics and Biopharmaceutics 198 (2024) 114264

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Meta-analysis guided development of a standard artificial urine

Kimberley A. Noble, Hayley K.Y. Chan, Oisín N. Kavanagh *

School of Pharmacy, Newcastle University, Newcastle upon Tyne, UK

ARTICLE INFO

Keywords:
Biorelevant media
Urinalysis
Artificial urine
Meta-analysis

ABSTRACT

In this study, we present the first meta-analysis of human urine reported in the literature, drawing data from a total of 35 articles with a combined participant count of 14,021. Through this analysis, we have developed an artificial urine (AU) composition that can be adjusted within typical physiological parameters for in vitro applications. Our findings demonstrate the utility of this AU in determining the solubility of nitrofurantoin, particularly in the context of crystalluria. Notably, we observe that in saline, nitrofurantoin solubility, within the framework of its urinary pharmacokinetics, suggests a risk of crystalluria. However, in AU, this risk is mitigated due to complexation with urea. More broadly, we anticipate that our developed formulation will serve as a foundation for translational studies across biomedical and pharmaceutical sciences.

1. Introduction

Many living things produce biofluids in quantities large and small, to digest food, excrete metabolites or to create a hostile environment for other competing species. Due to the difficulties relating to the collection of biofluids – which can be due to their rate of production, variability, or ethical considerations – a need emerges to generate artificial biofluids. If the typical composition of a biofluid is known, an artificial biofluid can be formulated reproducibly to help us understand the more complex *in vivo* system. The artificial variant has an additional advantage in that its composition can be tuned to mimic a wide variety of contexts (physiological or otherwise), improving its predictive capacity. To this end, artificial biological fluids have been made for blood, plasma,[1,2] intestinal fluid,[3] and more.[4] A few of these artificial biofluids have been well documented (e.g., FeSSIF and FaSSIF), with a standard methodology of preparation.[4,5]

Urine is a common biofluid that is produced at the end of renal filtration. During filtration, the blood is initially filtered through the Bowman's capsule and subsequent steps involve reclaiming useful substances through both passive and active tubular reabsorption.[6] At the end, urine is produced, in which we expect to see salts (e.g., NaCl) and small molecules (<40 kDa) like creatinine and urea which have not been reclaimed. The approximate glomerular filtration rate in a healthy individual is 120 mL per minute, generating approximately 2 mL of urine.[7] This equates to the kidneys processing 120 L of blood daily, with up to only 2 L of that volume excreted.

Urine composition heavily depends on the diet and health of the individual, and therefore, is highly variable.[8] If one were to use human urine as opposed to an artificial variant, then that urines composition would need to be characterised for each experiment. This is because human urine composition can change from moment to moment. Due to this variation, it is preferable to construct an artificial urine (AU) from many patient samples. However, during a literature search we noted eighteen AU preparations of various composition from fourteen separate studies, none from a patient population beyond 28 individuals. We suggest that this highlights a clear gap in the literature as two separate studies using different AU preparations may report contradictory results. Therefore, we stress the importance of defining an AU which accurately represents a true biological sample. In this work, we aim to address this gap by analysing all the available literature to enable us to propose a standard AU.

2. Materials & Methods

2.1. Search strategy

A review of studies investigating the composition of human urine was performed in accordance with the Preferred Reporting Items for Systematic Review and Meta-analyses (PRISMA) guidelines. Literature searches were conducted using the following electronic databases between July and August 2023: Google Scholar, Ovid MEDLINE, PubMed, Scopus and Web of Science. The terms used to search for the titles, abstracts, and keywords were (human urine) AND (urine composition) AND (24-hour OR 24-h). The same search terms were used for each

* Corresponding author.
E-mail address: Oisin.Kavanagh@newcastle.ac.uk (O.N. Kavanagh).

https://doi.org/10.1016/j.ejpb.2024.114264
Received 19 January 2024; Received in revised form 11 March 2024; Accepted 13 March 2024
Available online 16 March 2024

database. Date range restrictions were not applied, and the included studies were limited to humans only. All authors assessed the full-text articles with the selection of eligible studies undertaken together. Disagreements were resolved by mutual consensus, using the predefined inclusion and exclusion criteria.

2.2. Study eligibility criteria

The participants included for eligibility were healthy males and females, $\geq$ 18 years and $\leq$ 65 years old. Subjects were considered healthy if they had no past medical history of a chronic condition, which was identified by extracting data from healthy control groups. No socio-economic, race, or ethnicity limitations were applied. Only data from complete 24 h urine samples were included to reduce the contributions of natural fluctuations throughout the day. Studies focusing on patients with nephrolithiasis, chronic kidney disease, intestinal failure, hypertension, heart failure, coronary artery disease and other comorbidities were excluded. Investigations which did not include a healthy control group were omitted from the analyses. Spot urine studies were eliminated from this paper due to their variability and unreliability in comparison to 24 hr urine samples.[9]

2.3. Data analysis

Only the major urinary components excreted as by-products from normal metabolic processes were included in the analysis. These naturally occurring organic and inorganic compounds are derived from internal cellular processes and can be influenced by exogenous factors such as foodstuffs or medicines. Each urinary component from healthy participants was extracted when available. For values that were stated as weight or mmol per 24 h, the provided average urine volume for that study was used to convert values to mM. For studies which did not provide urine volume data, it was assumed that the urinary volume is 1,766 mL/day, an average from the reporting studies (n = 11,947). Additionally, both 24 h urine volume and pH were also extracted. Where urine phosphate concentrations were extracted from reported literature studies, several studies reported “phosphate” values, however, in their methods they noted that “phosphorus” concentration was measured. As this study aimed to determine free phosphate and not phosphorus, these studies were subsequently removed from this analysis. Raw and normalised data for each component were compared, normalisation was achieved using the weighted mean and weighted standard deviation, which weighted the component concentration with the number of subjects in each study.

2.4. Nitrofurantoin solubility in artificial urine

Nitrofurantoin was added in excess to 10 mL solutions of AU. The solutions were then stirred (500 rpm) with a magnetic stirrer for 48 h at constant temperature (37.5 °C) using a thermostatic water bath. Stirring was then stopped for 1 h and 1 mL aliquots were taken from the solution and filtered using pre-warmed PTFE filters (0.45 μm). Samples were then diluted (1:100) using deionised water and analysed immediately by HPLC-UV. The solution pH was determined at equilibrium and linked with the concentration data.

HPLC-UV analysis was performed using an Agilent 1260 Infinity series system (Agilent Technologies, Waldbronn, Germany) equipped with a G1311B 1260 quaternary pump, G1329B 1260 auto-sampler, vacuum degasser and G1316A 1260 temperature-controlled column compartment maintained at 25 °C. A typical injection volume was 5 μL. A C18 Kromasil 5 μm, 4.6 d, 250 mm (MZ-Analysentechnik GmbH, Mainz-Germany) column was used. Nitrofurantoin was eluted from an isocratic mobile phase of 30/70 methanol and 0.05 % v/v aqueous trifluoroacetic acid (TFA) at 7 min (254 nm) at a flow rate of 1 mL/min. This procedure was repeated in triplicate. Quantification of drug concentration was obtained by calibration curves using the area under the curve. The phase present after dissolution was determined by Powder X-Ray Diffraction (PXRD). Diffractograms were collected in Bragg–Brentano geometry on a PANalytical Empyrean diffractometer equipped with a sealed tube (Cu Kα12, λ = 1.5418 Å) an 1D X’Celerator detector between 5 and 40° 2θ.

3. RESULTS & DISCUSSION

The search identified 3,331 studies and after the removal of duplicates, 2,983 studies were screened. After title and abstract exclusion, 139 full-text studies were assessed and 104 were excluded from the analysis for reasons detailed in the predefined exclusion criteria. A total of 35 articles with a combined total of 14,021 participants were included in this review (Scheme S1).

3.1. Urea

With the highest concentration of all the components analysed, urea has a weighted mean and standard deviation (SD) of 213.85 ± 75.83 mM/24 h in 369 individuals. The absolute values ranged between 178.17 and 235.81 mM/24 h. Although urea is the largest component of human urine other than water, only four studies reported values for it. The weighted standard deviation from the mean ranged between 0.22 and 0.52 mM/24 h with an average of 0.34 (Figure S1) [10–13].

Urea is concentrated by urea recycling to create a high concentration in the inner medulla, this concentration gradient allows for counter current exchange of solutes in the loop of Henle, giving the kidney its concentrating power.[14] Therefore, due to its importance in kidney function, we expect to see large concentrations of this species in the urine.

3.2. Chloride

The weighted mean and SD of chloride from the 24 h urine collections of 1,438 participants is 112.67 ± 55.07 mM/24 h. The absolute values ranged between 75.99 and 140.60 mM/24 h. The normalised standard deviations ranged between 0.06 and 0.90 mM/24 h with an average of 0.29 (Figure S2). The study with the largest normalised standard deviation (0.84), suggests that chloride is more variable than other studies indicate. However, this could be explained by methodological choices in determining chloride concentrations, namely: potentiometric titration by Vinayagamoorthy, coulometry by Hesse and Seiner, ion-selective electrodes by Wang, “standard laboratory techniques” by Rodgers, unicel DxC 600 synchronic biochemical detecting system by Cai and no method mentioned by Sui as analysis was outsourced to another laboratory. We note that for several components, namely chloride and sodium, the study from Cai’s laboratory reports a much larger spread of data than other studies.

The chloride ion is abundant within the body, accounting for, on average, 0.15 % total body weight, or 115 g.[15] The body utilises this important ion for acid-base balance and maintaining osmotic pressure, it seems reasonable that this species, acquired from the diet, should be present in such large quantity.

3.3. Sodium

A relatively large number of studies (24 of 35) included sodium in their analysis. The weighted mean and SD of sodium from the 24 h urine collections of 12,463 individuals was 89.62 ± 37.14 mM/24 h. The absolute values ranged between 54.30 and 139.74 mM/24 h. The normalised standard deviation is 0.36, ranging between 0.05 and 0.86 mM/24 h (Figure S3).[10,11,13,16–18,18–42] This component shows the second highest range of normalised standard deviation, again an analysis by Cai is significantly more variable in comparison to the other studies.

One study observed the effect of a ‘low’ and ‘high’ salt diet (<3.5 g and 14.7 g per day) on urine composition.[13] However, both diets

contained a reasonable amount of salt, for example, the authors noted that in the average adult Croatian diet 11.6 g/day is consumed. As other studies allowed individuals to eat and drink 'freely', both diets were included in this *meta*-analysis as they were deemed within reason. As sodium chloride symporters transport both sodium and chloride ions simultaneously back from the filtrate and into the blood, and sodium and chloride are often consumed together through the diet as table salt, it seems logical that these ions should be seen in roughly similar quantities within the urine. Fluctuations are probably explained by variation in diet between individuals.

3.4. Potassium

The weighted mean and SD of potassium from the 24 h urine collections of 12,545 individuals was 35.50 ± 12.56 mM/24 h. The absolute values ranged between 23.32 and 56.68 mM/24 h. The average normalised standard deviation from the mean is 0.32, with a range between 0.04 and 0.50 mM/24 h (Figure S4).[10–13,16–18,18–37,39–43].

As one of the most abundant cations in body, potassium is involved in nerve and muscle cell excitation through membrane electrical potentials.[44,45] A potassium-sodium exchange occurs primarily in the collecting duct where there are numerous K^+ channels. For K^+ to be secreted there must be an overall negative charge in the lumen of the nephron to provide an electrochemical gradient for potassium to travel down. This negative charge is provided by the reabsorption of Na^+ which occurs at a faster rate than the Cl^- ion, which often follows Na^+.[44].

3.5. Ammonium

The weighted mean and SD of ammonium from the 24 h urine collections of 474 individuals was 17.83 ± 3.07 mM/24 h. The absolute values ranged between 5.17 and 25.71 mM/24 h. The average normalised standard deviation to the mean is 0.24, ranging between 0.08 and 0.38 mM/24 h (Figure S5) [11,12,17,18,25].

Ammonium excretion by the kidneys into the urine is essential to maintaining a healthy serum CO_2 concentration. Disruption of this mechanism can result in an increase of CO_2, metabolic acidosis and chronic kidney disease, therefore, as this mechanism is rather tightly controlled to prevent acidosis, it could be expected that variation is relatively limited.[46] Urine ammonium has been suggested to be an effective marker for predicting acidosis.[46] Bingham proposes a urine ammonium concentration much lower than the other studies by using a colorimetric assay as opposed to Seiner and Hesse who both use an ion selective electrode, and Fittschen, who detected ammonium enzymatically. Possibly, this colorimetric test may have been less sensitive in comparison to the ion selective electrode and enzymatic methodologies.

3.6. Phosphate

The weighted mean and SD of phosphate from the 24 h urine collections of 505 individuals was 12.16 ± 4.44 mM/24 h. The absolute values ranged between 11.80 and 21.54 mM/24 h. The average normalised standard deviation is 0.18, ranging between 0.05 and 0.39 mM/24 h (Figure S6).[18,20–23,26–28,30,32,33,42].

Phosphate is a particularly important ion, helping form nucleic acids within DNA and RNA, adenosine triphosphate to provide cellular energy and phospholipids to form membranes and compartmentalise cells.[47] As excessive phosphate levels can be detrimental and dangerous to an individual, causing death by cardiovascular and chronic kidney diseases, then phosphate regulation must be tightly controlled, as can be seen by the low deviation of the data.[47] Robertson has reported several components considerably higher than all other studies; this applies for creatinine, uric acid, sodium, potassium, phosphate, calcium and magnesium. For every component that Robertson has reported, the concentration has been the highest of all studies, because of this, his studies have been removed from our *meta*-average.

3.7. Sulphate

The weighted mean and SD of sulphate from the 24 h urine collections of 809 individuals was 9.98 ± 2.07 mM/24 h. The absolute values ranged between 8.21 and 13.80 mM/24 h. The average normalised standard deviation is 0.17, the lowest of all components, with a range between 0.06 and 0.38 mM (Figure S7).[17,18,25,30,33].

Intake of protein from the diet is reflected in sulphate presence in the urine, however, sulphate also has the potential to be used as a marker for the progression of kidney disease.[48] A low level of hydrogen sulphide (H_2S), a precursor of urinary sulphate, has previously been identified in individuals suffering from chronic kidney disease.[48].

3.8. Creatinine

The weighted mean and SD of creatinine from 12,131 individuals was 6.49 ± 1.57 mM/24 h. The absolute values ranged between 0.86 and 14.29 mM/24 h. A study conducted by Athanasatou in 2018 reported a daily creatinine excretion of approximately ten-fold below that of all other studies, we believe this may be a ten-fold error arising from a typographic error. Although Athanasatou's study has been included in the supplemental figures for comparison, it was excluded from our *meta*-analysis average. The normalised standard deviations relative to the mean ranged between 0.05 and 0.42 mM/24 h, with an average of 0.25 (Figure S8).[10,11,17–28,30–34,37,38,40,42,43,49–54].

The consistency of creatinine is to be expected and it is often utilised to normalise other species found in the urine to counter variation in urine volume. For example, when testing the urine for the misuse of drugs, a certain threshold of creatinine must be detected to confirm that the sample has not been manipulated by excessive fluid intake.[55].

3.9. Calcium

The weighted mean and SD of calcium from the 24 h urine collections of 9,226 individuals was 2.58 ± 1.28 mM/24 h. The absolute values ranged between 1.43 and 3.00 mM/24 h. The standard deviations across the twelve studies normalised to the mean ranged between 0.08 and 0.58 mM/24 h with an average of 0.40, the second highest of all components (Figure S9).[17,18,20–30,32,33,42,53].

Calcium plays a role in producing action potentials during nerve signalling. Calcium is rarely freely unbound in the plasma, as it is stored within the bone, and the 1 % found in the extracellular fluid is often bound to albumin (or contained within enzymes or other storage proteins).[56] Because of this, we are unsurprised by our observation that calcium excretion is relatively low.

3.10. Magnesium

The weighted mean and SD of magnesium from the 24 h urine collections of 9,038 individuals are 2.38 ± 0.87 mM/24 h. The absolute values ranged between 1.36 and 3.24 mM/24 h. The magnitude of dispersion relative to the mean averaged at 0.29 with a range between 0.07 and 0.59 mM/24 h (Figure S10).[17,18,20–30,32,33,42,57].

Only 3 % of serum magnesium is excreted into the filtrate, high retention of magnesium may be due to its role in carbohydrate metabolism, enzyme active sites and ion pump function.[58].

3.11. Citrate

The weighted mean and SD of citrate from the 24 h urine collections of 2,654 individuals was 1.84 ± 0.87 mM/24 h. The absolute values ranged between 1.22 and 2.12 mM/24 h. The magnitude of dispersion relative to the mean was the highest of all studies, averaging at 0.56 with a range between 0.12 and 0.67 mM/24 h (Figure S11).[17,18,20–26,28,30,32,33].

Citrate forms a complex with calcium, resulting in an increase in

solubility and a reduction in the likelihood of calcium stone formation. [59] Diet and acid-base status will significantly impact and fluctuate urinary citrate. This high variability is reflected in this study, with citrate the most variable, of all 13 components identified.

3.12. Urate

The weighted mean and SD of urate from the 24 h urine collections of 9,209 individuals was 1.72 ± 0.54 mM/24 h. The absolute values ranged between 1.36 and 2.30 mM/24 h. The overall magnitude of dispersion relative to the mean averaged 0.27, ranging between 0.08 and 0.35 mM/24 h (Figure S12).[11,17,18,20–30,32,33,42] This component showed the lowest range of normalised standard deviations. Unlike other mammals, humans do not possess hepatic uricase enzymes. [60] This results in humans having a higher uric acid serum concentration, a product of purine metabolism. The kidneys are capable of coping with the excretion of this toxic product, increasing renal elimination after excess intake through diet.

3.13. Hippurate

The weighted mean and SD of hippurate from the 24 h urine collections of 98 individuals over three studies was 1.59 ± 0.16 mM/24 h, the lowest of all the components analysed in this study. The absolute values ranged between 0.71 and 1.93 mM/24 h (Figure S13). However, of these three studies, only one reported a standard deviation which was weighted to 0.15 mM/24 h.[49,51,61].

Hippurate is increased after the consumption of tea and fruits.[62] Presence of gut microbiota is essential in the initial cleaving of these precursor polyphenols, found in tea.[63] As two of only three studies did not provide standard deviation, it is difficult to assess variability. The two studies which employed HPLC to determine the urinary concentration (Mulder, 2005 and Ogawa, 2011) were much closer than Ikeda's study, which described determination by manual chromatography.

3.14. Urine volume

Of the 35 studies identified within the *meta*-analysis, 24 provided total 24 h urine volume. The weighted mean and standard deviation were also calculated from these 11, 934 individuals at 1,766.53 ± 712.96 mL per 24 h with the absolute mean variation between the studies at 1,116 and 2,479 mL/24 h (Figure S14). The average normalised standard deviation was 0.32 ranging between 0.03 and 0.47 mL/24 h.[11–13,16–34,37,38,40–42,52,57].

3.15. Urine pH

We found 13 studies which provided total 24 h urine pH. The weighted mean and standard deviation is pH 6.07 ± 0.49 (Figure S15). The absolute values of pH ranged between 5.89 and 6.77 with an average normalised standard deviation of 0.06, which ranged from 0.01 to 0.10.[11,12,17,18,20–30,32,33,42,64] As urine pH was taken at the end of the 24 h collection, this does not reflect the variability and fluctuation of pH throughout the day, but only reports one average value of the whole day.

3.16. Artificial urine proposal

We have summarised these above findings in Fig. 1 and Table 1, below, with a comparison of the weighted means and standard deviations of all the components in human urine obtained from this review from a total of 14,021 individuals. These findings have been converted to a list of readily available chemicals and their weights, in mg/L for the reader to reproduce the proposed AU (Table 3). Raw data detailed in Figures S1-15.

3.17. Previously reported artificial urine compositions

An additional literature search for reported AU compositions found 18 different proposals from 14 separate studies. These compositions

Table 1
Comparison of the weighted means (mM) and weighted standard deviations for each urinary component.

Component	Weighted mean (mM)	Weighted SD (mM)	Normalised standard deviation
Urea	213.85	75.83	0.34
Chloride	112.67	55.07	0.29
Sodium	89.62	37.14	0.36
Potassium	35.50	12.56	0.32
Ammonium	17.83	3.07	0.24
Phosphate	12.16	4.44	0.18
Sulphate	9.98	2.07	0.17
Creatinine	6.49	1.57	0.25
Calcium	2.58	1.28	0.40
Magnesium	2.38	0.87	0.29
Citrate	1.84	0.87	0.56
Urate	1.72	0.54	0.27
Hippurate	1.59	0.16	0.15
pH	6.07	0.49	0.06
Volume (mL)	1766.53	712.96	0.32

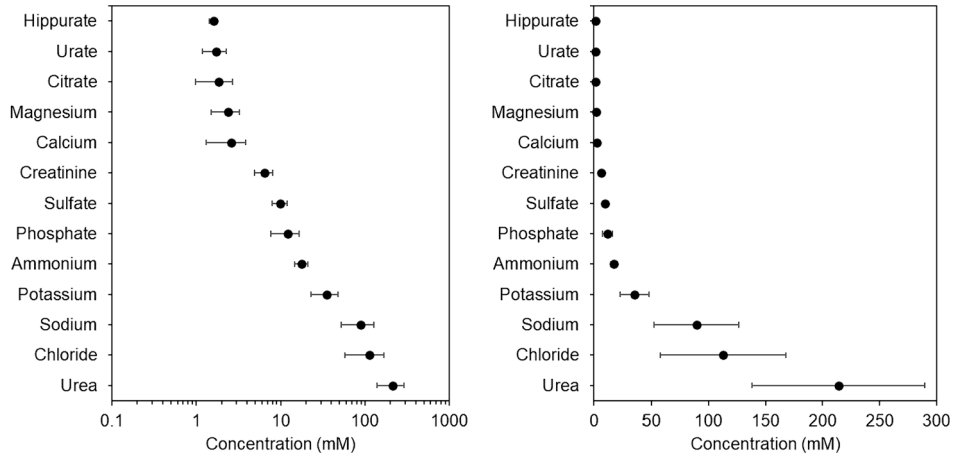

Fig. 1. Weighted mean and standard deviations of human urinary components on a log (left) and linear (right) scale, according to the 24-h urine collections of 35 literature references.

Table 2
Comparison of previously reported AU compositions to the weighted mean and standard deviations of the 14,021 individuals from this *meta*-analysis. Values which fall within 1 SD of the weighted mean reported in this study are in bold, values which fall outside of this range are in italic.

	Sarigul, 2019[8]	Brooks, 1997 [77]	Shmaefsky, 1990[78]	Chutipongtanate, 2010[79]	Brown, 1989 [80]	Opalko, 1997 [81]	Grases, 1998 I [82]	Grases, 1998 II [82]	Grases, 1998 III [82]	Grases, 1998 IV [82]	Grases, 1998 V [82]	Mayrovitz, 2001[83]	Christmas, 2002[84]	McCurdy, 2001[85]	Shafat, 2013 [86]	Malpass, 2002[87]	Sigg, 2022 [88]	Putnam, 1971[89]
Urea (mM)	**249.75**	**170**	*404*	**200**	–	–	–	–	–	–	–	*416*	–	**266.4**	–	–	**166.5**	**222.96**
Chloride (mM)	**88**	**120**	*251*	**105**	*208.3*	*169.2*	*473.9*	*473.9*	*473.9*	*473.9*	*473.9*	*210*	*205.5*	**114.1**	*177.5*	*177.2*	**118.32**	**159**
Sodium (mM)	**92.63**	*135*	*288*	**93.6**	*147.7*	*152.26*	*318.42*	*342.82*	*365.82*	*391.62*	*416.02*	*213.02*	*171.7*	**120.68**	*153.9*	*153.86*	*133.7*	*137*
Potassium (mM)	**31.33**	*21*	*80*	**30**	*63.7*	*63.7*	*162.5*	*162.5*	*162.5*	*162.5*	*162.5*	–	*63.7*	**46**	*64*	*63.7*	*21.13*	*62.1*
Ammonium (mM)	*23.67*	*25*	–	**15**	*27.6*	–	*86.9*	*86.9*	*86.9*	*86.9*	*86.9*	*56.1*	*36.92*	**19.82**	–	–	*24.3*	–
Sulphate (mM)	*18*	**12.01**	–	**11**	*38.5*	*20.8*	*40.1*	*40.1*	*40.1*	*40.1*	*40.1*	–	*20.8*	*34.16*	*120.9*	*20.8*	**11.93**	*21.6*
Phosphate (mM)	*23.33*	**14**	*39*	*4*	*32.3*	*3.23*	**13.9**	*27.8*	*40.9*	*55.6*	*69.5*	*17.61*	*32.3*	*19.8*	*3.2*	*3.23*	**14.24**	*1.5*
Creatinine (mM)	**7.79**	**7**	–	*4*	–	–	–	–	–	–	–	*17.7*	–	*9.72*	–	–	*0.62*	*13.31*
Calcium (mM)	**1.66**	**2.5**	–	**3**	*5.75*	–	*6*	*6*	*6*	*6*	*6*	–	–	*4.29*	*4*	**3.5**	**2.52**	*0.6*
Magnesium (mM)	*4.39*	**2**	–	**2**	*3.85*	*3.85*	*5.9*	*5.9*	*5.9*	*5.9*	*5.9*	–	*3.85*	*3.82*	*3.9*	*3.85*	**2.03**	*8.2*
Hippurate (mM)	–	–	–	–	–	–	–	–	–	–	–	–	–	*3.52*	–	–	–	*6.37*
Citrate (mM)	**2.45**	**2**	–	*5*	*3.21*	*3.21*	–	–	–	–	–	–	–	*2.81*	*3.2*	*3.21*	**2.08**	*3.34*
Urate (mM)	**1.49**	*0.4*	–	*1*	–	–	–	–	–	–	–	–	–	–	–	–	–	*2.8*

Table 3
Proposed AU that reproducibly produces a solution of pH 6.4, if important, a total volume of 1743 mL would be appropriate.

Component	Quantity (mg/L)	Aqueous solubility at 15 °C
Urea	12,843.64	900 g/L[91]
NaCl	2,891.31	353 g/L† [92]
$NaHCO_3$	679.16	82.7 g/L† [93]
KCl	2,646.76	336 g/L† [92]
NH_4Cl	953.63	356.8 g/L† [94]
Na_2SO_4	1,416.49	120 g/L[95]
Creatinine	734.08	13.33 g/L‡
$NaH_2PO_4{\cdot}2H_2O$	1,884.88	850 g/L‡
$CaCl_2$	286.38	ca. 800 g/L[96]
$MgCl_2$	226.13	548.5 g/L† [97]
Citric acid	353.57	23.61 g/L† [98]
Uric acid	289.36	47 mg/L[99]
Hippuric acid	285.68	3.75 g/L‡

† Determined by interpolation.
‡ Merck index at 20 °C

were compared to the weighted means of the constituents identified in this study. In 10/18 studies, none of the component concentration values lay within 1 SD of the weighted mean of this study. In 3/18 reports, compositions contained between 1 and 2 constituents and 5/18 contained between 5 and 8 constituents within 1 SD of the weighted mean of this study (Table 2).

Only two of these papers, Sarigul et al. and Chutipongtanate et al. produced an AU with reference to a human sample (n = 28 and 5 respectively). However, in the study by Sarigul et al., only morning urine was analysed after an 8-hour fasting period and in Chutipongtanate et al. the urine of the 5 individuals were pooled before analysis. In total, six studies provided a proposed artificial composition without any referenced rationale. Of the 18 AU preparations identified, six reference papers which we are unable to access, with one study referencing the work of Burns and Finlayson.[65] Although this publication is often cited, the manuscript cannot be accessed as the journal has been discontinued, both authors have passed and none of their colleagues or the librarians at the University of Florida can locate the publication (personal communication). Additionally, two studies reported that pH was adjusted using NaOH, NH_4OH or HCl but did not detail how much was added. Notably, several studies included components which we have not selected in this study (Table S1).

Clinical values of healthy urine compositions have also been extracted from UK resources: NHS trusts (South Tees,[66] York and Scarborough,[67] North West London,[68] North Bristol,[69] Royal Berkshire,[70] North West Anglia[71]), Pathology Harmony[72] and US sources: Merk[73], Mayo Clinic[74] and the International Association of Providers of AIDS Care[75] (IAPAC) and, additionally, the Medical council of Canada[76] (MCC) (Figure S18-S30). Notably, there is variation of the defined healthy ranges of urine components between different NHS trusts on several occasions (urea, chloride, sodium, creatinine, phosphate, magnesium, citrate and urate). Most notably, Royal Berkshire reports that a healthy urine chloride concentration should not exceed 108 mmol/24hr, however, South Tees and Scarborough report the healthy maximum at over double this value (250 mmol/24hr). Mayo Clinic reported the largest range for sodium, ammonium, creatinine, magnesium, citrate, urate and hippurate and was second highest for a further three components: urea, chloride and calcium. This may introduce diagnostic variability in different sites but might also reflect demogaphic differences. None of the UK, US or Canadian sources provided detail for their proposed healthy urine values. Despite the variability of ranges between different sources, the data range acquired from this *meta*-analysis overlaps with each component from all sources.

3.18. Proposed artificial urine

We have consolidated the urinary composition of over 14,000 individuals in the largest pooled analysis of urine composition to date. This understanding has enabled us to propose a practical and representative AU of 13 major components. The major components of human urine, other than water, are urea (213.85 ± 75.83 mM/24 h), followed by chloride (112.67 ± 55.07 mM/24 h), sodium (89.62 ± 37.14 mM/24 h), potassium (35.50 ± 12.56 mM/24 h), ammonium (17.83 ± 3.07 mM/24 hr), phosphate (12.16 ± 4.44 mM/24 h), sulphate (9.98 ± 2.07 mM/24 h), creatinine (6.49 ± 1.57 mM/24 h), calcium (2.58 ± 1.28 mM/24 h), magnesium (2.38 ± 0.87 mM/24 h), citrate (1.84 ± 0.87 mM/24 h), urate (1.72 ± 0.54 mM/24 h) and hippurate (1.59 ± 0.16 mM/24 h).

To allow readers to easily reproduce the proposed composition of AU, we have provided a table of readily available chemicals and their weights in mg/L (Table 3). Components can be purchased in bulk from a variety of chemical suppliers to make 1 L solutions working out at around £50 (ca. €60) per formulation. By tuning the amounts of NaCl, KCl and sodium bicarbonate, one can achieve a degree of pH control using mineral acids or bases to achieve the same quantities across the pH scale. We typically buy these items in bulk and prepare multiple batches for storage in airtight containers. This base formulation can be spiked with additional components if desired. We use $NaHCO_3$ to reach the target sodium concentration as this allows the user to increase the sodium icontent while carbon dioxide will bubble out. Additionally, we note that uric acid is present above its solubility limit, however, we suggest initially dissolving all the components except uric acid. Then add the uric acid and immediately autoclave the solution. When dissolved in solutions of high ionic strength uric acid is known to sustain high levels of supersaturation (up to 2200 mg/L) for up to 7 days.[90] Experiments should consider the precipitation of uric acid.

Our formulation is intended to be dynamic and different groups may choose to develop an AU where certain components (or indeed, every component) is at one extreme or the other. Fundamentally, we provide a reliable dataset from which a reasonable AU composition can be developed. We expect that some teams might increase the complexity of this formulation by adding additional components like proteins, peptides, or cells.

3.19. An application of artificial urine

Crystalluria is a poorly understood phenomenon that is identified following the appearance of crystals in the urine alongside reduced kidney function and pain. These precipitates can cause direct damage to the kidneys – a sensitive apparatus – which can be irreversible, as such there is a critical need to understand the underlying chemistry of precipitation in the urinary tract to prevent crystalluria. Fundamentally, precipitates will only nucleate when their solubility limit has been exceeded (i.e., they become supersaturated), but the challenge is finding out where that limit lies, particularly in complex solvents such as urine. Drug aqueous solubility is often reported as a single unit and commonly without solution pH, ionic strength, or temperature data. The solid phase remaining after these measurements is also seldom reported. Solubility data must be contextualised in this way to develop quantitative mathematical relationships which can enable the prediction of drug behaviour. At its most basic, solution pH, complexation, pK_a and the crystallising drug can all influence the phase solubility diagram, and critically, all these characteristics are expected to change dramatically in the kidneys.

We have previously identified [100] that nitrofurantoin is a good false positive to test an AU formulation due to its apparent risk of crystallisation in aqueous media, which is reversed in AU. These theoretical analyses have been confirmed experimentally in this work and we find that nitrofurantoin solubility is increased in AU due to the presence of urea and creatinine with which it forms complexes (Fig. 2). PXRD diffractograms confirm the presence of a new phase which matches that of nitrofurantoin-urea (Figure S16). This increased solubility in our AU is contrasted with the solubility in a simple urine mimic. As illustrated by Chen, Cadwallader and Jun [101], urea initially increases nitrofurantoin solubility up to around 2 % w/v urea, then solubility rapidly decreases. Our proposal contains 12.8 g urea, while one quarter of AUs reporting urea in the literature exceed 20 g per litre.

This solubility data is then contextualised by taking the highest concentration of nitrofurantoin that has been observed clinically in the urine (Figure S17) and calculating the expected supersaturation of that solution in various pH environments (between 4.5 and 8). This context reveals that our AU predicts that nitrofurantoin is highly unlikely to cause crystalluria while alternative AUs do not enable us to come to that conclusion.

3.20. Limitations

The kidneys maintain urine pH by excreting or conserving substances in the urine filtrate. Such that the urinary pH will reflect (to a degree) a difference in composition. Therefore, an AU with fixed composition may not capture these subtle changes to urine composition and although all the preceding studies detail an AU whose pH can be adjusted, the composition may not reflect the *in vivo* state. Our values reflect urine composition at pH 5–7.

Many studies provided 24 hr urine volume of the participants, however, there were some studies which did not report this data; therefore mM/L (mM) could not be accurately calculated for these studies. To retain these data points, we averaged the 24 h urine output of the 11,947 individuals whose data was provided in the remaining studies. Using the average value of 1,766 mL per 24 hr we were then able to calculate the concentrations for the studies which did not provide 24 hr urine volume. Some studies contained additional components outside of the thirteen included in this study with oxalate, lactic acid and iron II included in twelve, four and two studies, respectively. Oxalate presence in the urine is primarily determined by ingestion of oxalate and oxalate precursors in the diet,[102] lactic acid from metabolism and bacterial production[103] and iron also through diet, with the majority being reabsorbed in the nephron.[104] These were not included in this work as we decided to include thirteen major and well reported compounds to allow the proposed AU to be practical and cost effective as well as representative of a human urine sample. Certain study groups were more likely to contain a bias within their sampling, for example, only sampling one gender or one ethnicity, as was seen in several papers analysed in this *meta*-analysis.[10,16,22,24,26,30,33,34,36,37,40,43,49,105,106].

It is possible that the storage conditions prior to analysis affected the determined concentrations of the supersaturated uric acid species. Many teams refrigerated their urine before subsequent analysis which can further reduce the equilibrium solubility of substances, resulting in precipitation. The variety of methods employed would suggest that we should have observed wide and dramatic variation in uric acid content, but it appears that uric acid can sustain a supersaturated state such that the values obtained may reflect a true uric acid concentration.

There are several ways to look at the composition of the urine, for example, by weight of the solid over 24 h or by concentration. We converted a variety of units of measurement into mM for ease of understanding. While we acknowledge that, for example, two people who excrete the same weight of creatinine over a 24 h period may show a slight difference in concentration based on the amount of liquid they each have consumed, we felt that it was important to use universal unit of measurement (with appropiate context, like volume/24h) to avoid confusion. We also suggest that this difference in weight per 24 h vs mM would only become evident with excess liquid consumption and as the data taken from these studies allowed the participants to eat and drink freely, and urine volume from each study was within a healthy range, then mM is more than adequate to use as the unit of measurement to determine composition.

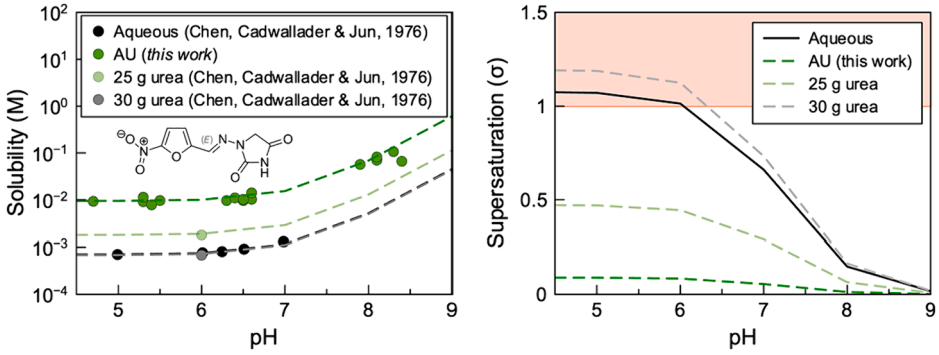

Fig. 2. pH solubility plot of nitrofurantoin in aqueous and artificial urine media (left). The maximum concentrations of nitrofurantoin found in the urine (200 mg/L, 0.000839 M) are divided by the equilibrium solubility of this drug in aqueous and AU solutions to find the theoretical supersaturation at that pH (right).

4. Conclusion

By combining over 14,000 individuals' urine composition data, this has allowed us to characterise the major components of human urine more accurately than any previous study we have found. We have tried to strike a balance between a fundamental composition which incorporates the available data on urine composition and practicality – bearing in mind that users can spike this composition with less abundant components should the need arise – we propose an AU containing thirteen of the major compounds found in urine: urea, creatinine, urate, hippurate, citrate, sodium, potassium, chloride, sulphate, phosphate, ammonium, calcium, and magnesium.

By combining these datasets, we have enabled a better understanding of a healthy human urine sample which could potentially be influential in diagnostics, pathogenic cellular and crystal growth, urinary tract infection therapeutic testing and potentially much more. We have leveraged this knowledge to develop a proposal for a standardised AU (between pH 5–7) which can be formulated using cheap and readily available constituents. This AU can serve as a foundation for future work in this area and can be spiked to increase its complexity.

CRediT authorship contribution statement

Kimberley A. Noble: Writing – review & editing, Writing – original draft, Formal analysis. **Hayley K.Y. Chan: . Oisín N. Kavanagh:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Formal analysis, Data curation, Conceptualization.

Data availability

Data will be made available on request.

Acknowledgements

The authors wish to thank Rose Williams, Beatrice Guttridge-Smith and Nikita Binu for conducting some preliminary work in this area during their MRes studies. This study was funded, in part, by an Engineering and Physical Sciences Research Council grant (EP/Y014596/1).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejpb.2024.114264.

References

[1] W.O. Reece, H.H. Erickson, J.P. Goff, E.E. Uemura, Dukes' Physiology of Domestic Animals, John Wiley & Sons, 2015.
[2] H.A. Krebs, Chemical composition of blood plasma and serum, Annual Rev. Biochem. 19 (1) (1950) 409–430, https://doi.org/10.1146/annurev.bi.19.070150.002205.
[3] K. Pyper, J. Brouwers, P. Augustijns, I. Khadra, C. Dunn, C.G. Wilson, G. W. Halbert, Multidimensional analysis of human intestinal fluid composition, Eur. J. Pharm. Biopharm. 153 (2020) 226–240, https://doi.org/10.1016/j.ejpb.2020.06.011.
[4] M.R.C. Marques, R. Loebenberg, M. Almukainzi, Simulated biological fluids with possible application in dissolution testing, Dissolution Technol. 18 (3) (2011) 15–28, https://doi.org/10.14227/DT180311P15.
[5] A. Fuchs, M. Leigh, B. Kloefer, J.B. Dressman, Advances in the design of fasted state simulating intestinal fluids: FaSSIF-V3, Eur. J. Pharm. Biopharm. 94 (2015) 229–240, https://doi.org/10.1016/j.ejpb.2015.05.015.
[6] P.B. Rehberg, Studies on kidney function, Biochem. J 20 (3) (1926) 447–460.
[7] G. Sterner, B. Frennby, S. Mansson, U. Nyman, D. Van Westen, T. Almén, Determining, "true" glomerular filtration rate in healthy adults using infusion of inulin and comparing it with values obtained using other clearance techniques or prediction equations, Scand. J. Urol. Nephrol. 42 (3) (2008) 278–285, https://doi.org/10.1080/00365590701701806.
[8] N. Sarigul, F. Korkmaz, İ. Kurultak, A new artificial urine protocol to better imitate human urine, Sci. Rep. 9 (1) (2019) 20159, https://doi.org/10.1038/s41598-019-56693-4.
[9] C. Ji, L. Sykes, C. Paul, O. Dary, B. Legetic, N.R.C. Campbell, F.P. Cappuccio, Systematic review of studies comparing 24-hour and spot urine collections for estimating population salt intake, Rev. Panam. Salud Publica 32 (4) (2012) 307–315, https://doi.org/10.1590/S1020-49892012001000010.
[10] D.R.R. Williams, S.A. Bingham, Sodium and potassium intakes in a representative population sample: estimation from 24 h urine collections known to be complete in a Cambridgeshire village, Br. J. Nutr. 55 (1) (1986) 13–22, https://doi.org/10.1079/BJN19860005.
[11] S.A. Bingham, R. Williams, T.J. Cole, C.P. Price, J.H. Cummings, Reference values for analytes of 24-H Urine collections known to be complete, Ann. Clin. Biochem. 25 (6) (1988) 610–619, https://doi.org/10.1177/000456328802500603.
[12] I. Fittschen, H.H. Hahn, Characterization of the municipal wastewaterpart human urine and a preliminary comparison with liquid cattle excretion, Water Sci. Technol. 38 (6) (1998) 9–16, https://doi.org/10.2166/wst.1998.0231.
[13] L. Barić, I. Drenjančević, A. Matić, M. Stupin, L. Kolar, Z. Mihaljević, H. Lenasi, V. Šerić, A. Stupin, Seven-day salt loading impairs microvascular endothelium-dependent vasodilation without changes in blood pressure, body composition and fluid status in healthy young humans, Kidney Blood Press. Res. 44 (4) (2019) 835–847, https://doi.org/10.1159/000501747.
[14] B. Yang, L. Bankir, Urea and urine concentrating ability: new insights from studies in mice, American Journal of Physiology-Renal Physiology 288 (5) (2005) F881–F896, https://doi.org/10.1152/ajprenal.00367.2004.
[15] K. Berend, L.H. van Hulsteijn, R.O.B. Gans, Chloride: the queen of electrolytes? Eur. J. Intern. Med. 23 (3) (2012) 203–211, https://doi.org/10.1016/j.ejim.2011.11.013.
[16] T. Vinayagamoorthy, 24-hour urine composition of Sri Lankan adults and their response to 100 Mg dihydrochlorothiazide, Ann. Clin. Biochem. 17 (4) (1980) 212–213, https://doi.org/10.1177/000456328001700410.
[17] A. Hesse, A. Classen, M. Knoll, F. Timmermann, W. Vahlensieck, Dependence of urine composition on the age and sex of healthy subjects, Clin. Chim. Acta 160 (2) (1986) 79–86, https://doi.org/10.1016/0009-8981(86)90126-9.
[18] R. Siener, A. Jahnen, A. Hesse, Influence of a mineral water rich in calcium, magnesium and bicarbonate on urine composition and the risk of calcium oxalate crystallization, Eur. J. Clin. Nutr. 58 (2) (2004) 270–276, https://doi.org/10.1038/sj.ejcn.1601778.
[19] C.-Y. Wang, M.E. Cogswell, C.M. Loria, T.-C. Chen, C.M. Pfeiffer, C.A. Swanson, K. L. Caldwell, C.G. Perrine, A.L. Carriquiry, K. Liu, C.T. Sempos, C.D. Gillespie, V. L. Burt, Urinary excretion of sodium, potassium, and chloride, but not iodine, varies by timing of collection in a 24-hour calibration study1–3, J. Nutr. 143 (8) (2013) 1276–1282, https://doi.org/10.3945/jn.113.175927.
[20] A.L. Rodgers, D. Webber, B. Hibberd, Experimental determination of multiple thermodynamic and kinetic risk factors for nephrolithiasis in the urine of healthy

controls and calcium oxalate stone formers: does a universal discriminator exist? Urolithiasis 43 (6) (2015) 479–487, https://doi.org/10.1007/s00240-015-0802-4.
[21] W. Zhu, Z. Mai, J. Qin, X. Duan, Y. Liu, Z. Zhao, J. Yuan, S.P. Wan, G. Zeng, Difference in 24-hour urine composition between diabetic and non-diabetic adults without nephrolithiasis, PLoS One 11 (2) (2016) e0150006.
[22] T. Deng, Z. Mai, C. Cai, X. Duan, W. Zhu, T. Zhang, W. Wu, G. Zeng, Influence of weight status on 24-hour urine composition in adults without urolithiasis: a nationwide study based on a chinese han population, PLoS One 12 (9) (2017) e0184655.
[23] C. Cai, Z. Mai, T. Deng, Z. Zhao, W. Zhu, Y. Wen, X. Duan, W. Wu, G. Zeng, Impact of dyslipidemia on 24-h urine composition in adults without urolithiasis, Lipids Health Dis. 17 (1) (2018) 250, https://doi.org/10.1186/s12944-018-0899-x.
[24] Z. Mai, X. Li, C. Jiang, Y. Liu, Y. Chen, W. Wu, G. Zeng, Comparison of metabolic changes for stone risks in 24-hour urine between non- and postmenopausal women, PLoS One 14 (1) (2019) e0208893.
[25] W. Sui, J. Hancock, J.R. Asplin, E.R. Gould, R.S. Hsi, Nephrolithiasis and elevated urinary ammonium: a matched comparative study, Urology 144 (2020) 77–82, https://doi.org/10.1016/j.urology.2020.05.063.
[26] T. Deng, Z. Mai, X. Duan, Z. Zhao, W. Zhu, C. Cai, W. Wu, G. Zeng, Association between hypertension and 24-h urine composition in adults without urolithiasis in China, World J. Urol. 39 (2) (2021) 589–595, https://doi.org/10.1007/s00345-020-03189-w.
[27] W.G. Robertson, A. Hodgkinson, D.H. Marshall, Seasonal variations in the composition of urine from normal subjects: a longitudinal study, Clin. Chim. Acta 80 (2) (1977) 347–353, https://doi.org/10.1016/0009-8981(77)90043-2.
[28] G.C. Curhan, W.C. Willett, F.E. Speizer, M.J. Stampfer, Twenty-four–hour urine chemistries and the risk of kidney stones among women and men, Kidney Int. 59 (6) (2001) 2290–2298, https://doi.org/10.1046/j.1523-1755.2001.00746.x.
[29] M.A. Cameron, N.M. Maalouf, B. Adams-Huet, O.W. Moe, K. Sakhaee, Urine composition in type 2 diabetes: predisposition to uric acid nephrolithiasis, J Am Soc Nephrol 17 (5) (2006) 1422, https://doi.org/10.1681/ASN.2005121246.
[30] E.N. Taylor, G.C. Curhan, Differences in 24-hour urine composition between black and white women, J Am Soc Nephrol 18 (2) (2007) 654, https://doi.org/10.1681/ASN.2006080854.
[31] K. Murakami, S. Sasaki, Y. Takahashi, K. Uenishi, T. Watanabe, T. Kohri, M. Yamasaki, R. Watanabe, K. Baba, K. Shibata, T. Takahashi, H. Hayabuchi, K. Ohki, J. Suzuki, Sensitivity and specificity of published strategies using urinary creatinine to identify incomplete 24-h urine collection, Nutrition 24 (1) (2008) 16–22, https://doi.org/10.1016/j.nut.2007.09.001.
[32] G.C. Curhan, E.N. Taylor, 24-h uric acid excretion and the risk of kidney stones, Kidney Int. 73 (4) (2008) 489–496, https://doi.org/10.1038/sj.ki.5002708.
[33] B.H. Eisner, S.P. Porten, S.K. Bechis, M.L. Stoller, The role of race in determining 24-hour urine composition in white and asian/pacific islander stone formers, J. Urol. 183 (4) (2010) 1407–1411, https://doi.org/10.1016/j.juro.2009.12.033.
[34] J. Xu, M. Wang, Y. Chen, B. Zhen, J. Li, W. Luan, F. Ning, H. Liu, J. Ma, G. Ma, Estimation of salt intake by 24-hour urinary sodium excretion: a cross-sectional study in Yantai, China, BMC Public Health 14 (1) (2014) 136, https://doi.org/10.1186/1471-2458-14-136.
[35] Trijsburg, L., Vries, J. H. M. de, Boshuizen, H. C. , Hulshof, P. J. M. , Hollman, P. C. H. , Veer, P. van 't, Geelen, A. Comparison of Duplicate Portion and 24 h Recall as Reference Methods for Validating a FFQ Using Urinary Markers as the Estimate of True Intake. Brit. J. Nutrit. 114(8) (**2015**) 1304–1312. 10.1017/S0007114515002871.
[36] C. Campino, C. Hill, R. Baudrand, A. Martínez-Aguayo, M. Aglony, C.A. Carrasco, C. Ferrada, C. Loureiro, A. Vecchiola, R. Bancalari, F. Grob, C.A. Carvajal, C. F. Lagos, C. Valdivia, A. Tapia-Castillo, C.A. Fuentes, C. Mendoza, H. Garcia, R. Uauy, C.E. Fardella, Usefulness and pitfalls in sodium intake estimation: comparison of dietary assessment and urinary excretion in chilean children and adults, Am. J. Hypertens. 29 (10) (2016) 1212–1217, https://doi.org/10.1093/ajh/hpw056.
[37] A. Athanasatou, A. Kandyliari, O. Malisova, A. Pepa, M. Kapsokefalou, Sodium and potassium intake from food diaries and 24-h urine collections from 7 days in a sample of healthy Greek adults, Frontiers Nutrition 5 (2018), https://doi.org/10.3389/fnut.2018.00013.
[38] S.J. Mann, L.M. Gerber, Addressing the problem of inaccuracy of measured 24-hour urine collections due to incomplete collection, J. Clin. Hypertension 21 (11) (2019) 1626–1634, https://doi.org/10.1111/jch.13696.
[39] N. Shinozaki, K. Murakami, K. Asakura, K. Uechi, S. Kobayashi, S. Masayasu, S. Sasaki, Dietary phosphorus intake estimated by 4-day dietary records and two 24-hour urine collections and their associated factors in Japanese Adults, Eur. J. Clin. Nutr. 72 (4) (2018) 517–525, https://doi.org/10.1038/s41430-018-0114-1.
[40] S.S. Yeung, R.S. Chan, L. Li, J. Leung, J. Woo, Urinary sodium excretion and dietary sources of sodium intake in community-dwelling Chinese adults aged 50 years and over, Public Health Nutr. 24 (7) (2021) 1861–1868, https://doi.org/10.1017/S1368980021000811.
[41] A. Buch, R. Goldsmith, L. Nitsan, M. Margaliot, G. Shefer, Y. Marcus, N. Stern, 24-h potassium excretion is associated with components of the metabolic syndrome: results from a national survey based on urine collection in adults, Nutrients 13 (8) (2021) 2689, https://doi.org/10.3390/nu13082689.
[42] P.M. Ferraro, E.N. Taylor, G.C. Curhan, Factors associated with sex differences in the risk of kidney stones, Nephrol. Dial. Transplant. 38 (1) (2023) 177–183, https://doi.org/10.1093/ndt/gfac037.
[43] E. Rodríguez-Rodríguez, R.M. Ortega, P. Andrés Carvajales, L.G. González-Rodríguez, Relationship between 24 h urinary potassium and diet quality in the adult Spanish population, Public Health Nutr. 18 (5) (2015) 850–859, https://doi.org/10.1017/S1368980014001402.
[44] M.L. Halperin, K.S. Kamel, Potassium, Lancet 352 (9122) (1998) 135–140, https://doi.org/10.1016/S0140-6736(98)85044-7.
[45] S.A. Lanham-New, H. Lambert, L. Frassetto, Potassium1, Adv. Nutr. 3 (6) (2012) 820–821, https://doi.org/10.3945/an.112.003012.
[46] K.L. Raphael, D.J. Carroll, J. Murray, T. Greene, S. Beddhu, Urine ammonium predicts clinical outcomes in hypertensive kidney disease, J Am Soc Nephrol 28 (8) (2017) 2483–2490, https://doi.org/10.1681/ASN.2016101151.
[47] H. Komaba, M. Fukagawa, Phosphate—a poison for humans? Kidney Int. 90 (4) (2016) 753–763, https://doi.org/10.1016/j.kint.2016.03.039.
[48] G. Andrésdóttir, S.J.L. Bakker, H.P. Hansen, H.-H. Parving, P. Rossing, Urinary Sulphate excretion and progression of diabetic nephropathy in type 1 diabetes, Diabet. Med. 30 (5) (2013) 563–566, https://doi.org/10.1111/dme.12131.
[49] M. Ikeda, H. Ohtsuji, Hippuric Acid, Phenol, and trichloroacetic acid levels in the urine of Japanese subjects with no known exposure to organic solvents, Occup. Environ. Med. 26 (2) (1969) 162–164, https://doi.org/10.1136/oem.26.2.162.
[50] A.C. Baxmann, M.S. Ahmed, N.C. Marques, V.B. Menon, A.B. Pereira, G. M. Kirsztajn, I.P. Heilberg, Influence of muscle mass and physical activity on serum and urinary creatinine and serum cystatin C, Clin. J. Am. Soc. Nephrol. 3 (2) (2008) 348–354, https://doi.org/10.2215/CJN.02870707.
[51] M. Ogawa, Y. Suzuki, Y. Endo, T. Kawamoto, F. Kayama, Influence of coffee intake on urinary hippuric acid concentration, Ind. Health 49 (2) (2011) 195–202, https://doi.org/10.2486/indhealth.MS1164.
[52] M.-Y. Rhee, S.-H. Jo, J.-H. Kim, K.-I. Kim, D.-Y. Nah, S.-W. Kim, N. Gu, K.-C. Sung, K.-S. Hong, E.-J. Cho, S.-Y. Lee, Difference in 24-hour urine sodium excretion between controlled and uncontrolled patients on antihypertensive drug treatment, J. Clin. Hypertension 21 (8) (2019) 1057–1062, https://doi.org/10.1111/jch.13610.
[53] L.M. Smith, J.C. Gallagher, Reference range for 24-h urine calcium, calcium/creatinine ratio, and correlations with calcium absorption and serum vitamin d metabolites in normal women, Osteoporos Int. 32 (3) (2021) 539–547, https://doi.org/10.1007/s00198-020-05615-6.
[54] T.T. Pieters, W.B. Veldhuis, P. Moeskops, B.D. de Vos, M.C. Verhaar, S. Haitjema, A.D.R. Huitema, M.B. Rookmaaker, Deep learning body-composition analysis of clinically acquired CT-scans estimates creatinine excretion with high accuracy in patients and healthy individuals, Sci. Rep. 12 (1) (2022) 9013, https://doi.org/10.1038/s41598-022-13145-w.
[55] E.J. Cone, Y.H. Caplan, F. Moser, T. Robert, M.K. Shelby, D.L. Black, Normalization of urinary drug concentrations with specific gravity and creatinine, J. Anal. Toxicol. 33 (1) (2009) 1–7, https://doi.org/10.1093/jat/33.1.1.
[56] K.F. Foley, L. Boccuzzi, Urine calcium: laboratory measurement and clinical utility, Lab. Med. 41 (11) (2010) 683–686, https://doi.org/10.1309/LM9SO94ZNBHEDNTM.
[57] R. Siener, A. Hesse, Influence of a mixed and a vegetarian diet on urinary magnesium excretion and concentration, Br. J. Nutr. 73 (5) (1995) 783–790, https://doi.org/10.1079/BJN19950081.
[58] C.G. Musso, Magnesium metabolism in health and disease, Int. Urol. Nephrol. 41 (2) (2009) 357–362, https://doi.org/10.1007/s11255-009-9548-7.
[59] M. Zacchia, P. Preisig, Low urinary citrate: an overview, J. Nephrol. 23 (Suppl 16) (2010) S49–S56.
[60] J. Maesaka, S. Fishbane, Regulation of renal urate excretion: a critical review, Am. J. Kidney Dis. 32 (6) (1998) 917–933, https://doi.org/10.1016/S0272-6386(98)70067-8.
[61] T.P. Mulder, A.G. Rietveld, J.M. Van Amelsvoort, Consumption of both black tea and green tea results in an increase in the excretion of hippuric acid into urine, Am. J. Clin. Nutr. 81 (1) (2005) 256S–S260, https://doi.org/10.1093/ajcn/81.1.256S.
[62] H.J. Lees, J.R. Swann, I.D. Wilson, J.K. Nicholson, E. Holmes, Hippurate: the natural history of a mammalian-microbial cometabolite, J. Proteome Res. 12 (4) (2013) 1527–1546, https://doi.org/10.1021/pr300900b.
[63] M.R. Olthof, P.C.H. Hollman, M.N.C.P. Buijsman, J.M.M. Van Amelsvoort, M. B. Katan, Chlorogenic acid, quercetin-3-rutinoside and black tea phenols are extensively metabolized in humans, J. Nutr. 133 (6) (2003) 1806–1814, https://doi.org/10.1093/jn/133.6.1806.
[64] M.A. Shafiee, P. Shaker, S.F. Hosseini, M. Alavinia, M. Aarabi, A.J. Rezaee, M. L. Halperin, Are individual analyses of multiple short urine collections throughout the 24 hours superior to a standard 24-hour urine collection in precipitation risk assessment of healthy subjects? Nephrology 26 (3) (2021) 234–238, https://doi.org/10.1111/nep.13842.
[65] J. Burns, B. Finlayson, A proposal for a standard reference artificial urine in in vitro urolithiasis experiments, Invest. Urol. 18 (2) (1980) 167–169.
[66] *Pathology tests*. South Tees Hospitals NHS Foundation Trust. https://www.southtees.nhs.uk/services/pathology/tests/ (accessed 2024-03-08).
[67] *York and Scarborough Teaching Hospitals NHS Foundation Trust - Test Directory*. https://www.yorkhospitals.nhs.uk/our-services/a-z-of-services/lab-med/test-directory/.
[68] *Tests - North West London Pathology*. https://www.nwlpathology.nhs.uk/tests-database/.
[69] *Test Information | North Bristol NHS Trust*. https://www.nbt.nhs.uk/severn-pathology/requesting/test-information.
[70] Royal Berkshire NHS Trust. *Pathology Handbook*. http://pathology.royalberkshire.nhs.uk/ranges.asp.
[71] North West Anglia NHS Trust. *Home*. NW Anglia Website. https://www.nwangliaft.nhs.uk/.

[72] Pathology Harmony Group. Harmonisation of Reference Intervals, 2011.
[73] *Urine Tests: Normal Values - Resources*. MSD Manual Professional Edition. https://www.msdmanuals.com/en-gb/professional/resources/normal-laboratory-values/urine-tests-normal-values.
[74] *SUP24 - Overview: Supersaturation Profile, 24 Hour, Urine*. https://www.mayocliniclabs.com/test-catalog/overview/616180#Clinical-and-Interpretive.
[75] *Normal Laboratory Values – International Association of Providers of AIDS Care*. https://www.iapac.org/fact-sheet/normal-laboratory-values/.
[76] *List of normal lab values | Medical Council of Canada*. https://mcc.ca/examinations-assessments/resources-to-help-with-exam-prep/normal-lab-values/.
[77] T. Brooks, C.W. Keevil, A Simple artificial urine for the growth of urinary pathogens, Lett. Appl. Microbiol. 24 (3) (1997) 203–206, https://doi.org/10.1046/j.1472-765X.1997.00378.x.
[78] B.R. Shmaefsky, Artificial urine for laboratory testing, Am. Biol. Teach. 52 (3) (1990) 170–172, https://doi.org/10.2307/4449071.
[79] S. Chutipongtanate, V. Thongboonkerd, Systematic comparisons of artificial urine formulas for in vitro cellular study, Anal. Biochem. 402 (1) (2010) 110–112, https://doi.org/10.1016/j.ab.2010.03.031.
[80] P. Brown, D. Ackermann, B. Finlayson, Calcium oxalate dihydrate (weddellite) precipitation, J. Cryst. Growth 98 (3) (1989) 285–292, https://doi.org/10.1016/0022-0248(89)90143-7.
[81] F.J. Opalko, J.H. Adair, S.R. Khan, Heterogeneous nucleation of calcium oxalate trihydrate in artificial urine by constant composition, J. Cryst. Growth 181 (4) (1997) 410–417, https://doi.org/10.1016/S0022-0248(97)00222-4.
[82] F. Grases, A. Llobera, Experimental model to study sedimentary kidney stones, Micron 29 (2) (1998) 105–111, https://doi.org/10.1016/S0968-4328(98)00006-7.
[83] H.N. Mayrovitz, N. Sims, Biophysical effects of water and synthetic urine on skin, Adv. Skin Wound Care 14 (6) (2001) 302.
[84] K.G. Christmas, L.B. Gower, S.R. Khan, H. El-Shall, Aggregation and dispersion characteristics of calcium oxalate monohydrate: effect of urinary species, J. Colloid Interface Sci. 256 (1) (2002) 168–174, https://doi.org/10.1006/jcis.2002.8283.
[85] D. McCurdy, Intercomparison study of inductively coupled plasma mass spectrometry, thermal ionization mass spectrometry and fission track analysis of μBq quantities of 239Pu in synthetic urine, J. Radioanal. Nucl. Chem. 249 (1) (2001) 121–131, https://doi.org/10.1023/A:1013263909665.
[86] M. Shafat, K. Rajakumar, H. Syme, N. Buchholz, M.M. Knight, Stent encrustation in feline and human artificial Urine: does the low molecular weight composition account for the difference? Urolithiasis 41 (6) (2013) 481–486, https://doi.org/10.1007/s00240-013-0608-1.
[87] C.A. Malpass, K.W. Millsap, H. Sidhu, L.B. Gower, Immobilization of an oxalate-degrading enzyme on silicone Elastomer, J. Biomed. Mater. Res. 63 (6) (2002) 822–829, https://doi.org/10.1002/jbm.10410.
[88] A.P. Sigg, M. Mariotti, A.E. Grütter, T. Lafranca, L. Leitner, G. Bonkat, O. Braissant, A method to determine the efficacy of a commercial phage preparation against uropathogens in urine and artificial urine determined by isothermal microcalorimetry, Microorganisms 10 (5) (2022) 845, https://doi.org/10.3390/microorganisms10050845.
[89] D.F. Putnam, Composition and concentrative properties of human Urine, NASA Contractor Reports (1971).
[90] H. Iwata, S. Nishio, M. Yokoyama, A. Matsumoto, M. Takeuchi, Solubility of uric acid and supersaturation of monosodium urate: why is uric acid so highly soluble in Urine? J. Urol. 142 (4) (1989) 1095–1098, https://doi.org/10.1016/S0022-5347(17)39003-1.
[91] L.A. Pinck, M.A. Kelly, The solubility of Urea in water, J. Am. Chem. Soc. 47 (8) (1925) 2170–2172, https://doi.org/10.1021/ja01685a502.
[92] S.P. Pinho, E.A. Macedo, Solubility of NaCl, NaBr, and KCl in water, methanol, ethanol, and their mixed solvents, J. Chem. Eng. Data 50 (1) (2005) 29–32, https://doi.org/10.1021/je049922y.
[93] Hill, A. E.; Bacon, L. R. TERNARY SYSTEMS. VI. SODIUM CARBONATE, SODIUM BICARBONATE AND WATER. *J. Am. Chem. Soc.* **1927**, *49* (10), 2487–2495. 10.1021/ja01409a018.
[94] S. Sawamura, N. Yoshimoto, Y. Taniguchi, Y. Yamaura, Effects of pressure and temperature on the solubility of ammonium chloride in water, High Pressure Res. 16 (4) (1999) 253–263, https://doi.org/10.1080/08957959908200298.
[95] II. On the Solubility of Salts in Water at High Temperatures. Phil. Trans. R. Soc. **1884**, 175, 23–36. 10.1098/rstl.1884.0003.
[96] Rard, J. A.; Clegg, S. L. Critical Evaluation of the Thermodynamic Properties of Aqueous Calcium Chloride. 1. Osmotic and Activity Coefficients of 0−10.77 Mol·kg-1 Aqueous Calcium Chloride Solutions at 298.15 K and Correlation with Extended Pitzer Ion-Interaction Models. J. Chem. Eng. Data 1997, 42 (5), 819–849. 10.1021/je9700582.
[97] J.-T. Li, J.-K. Wang, Y.-L. Wang, Solubility of KCl and MgCl2 in binary solvents formed by acetone and water in the temperature range between (293.15 and 323.15) K, J. Chem. Eng. Data 52 (3) (2007) 1069–1071, https://doi.org/10.1021/je700017b.
[98] Oliveira, M.L.N., Malagoni, R.A., Franco, Moilton. R. Solubility of Citric Acid in Water, Ethanol, n-Propanol and in Mixtures of Ethanol+water. Fluid Phase Equilibria **2013**, 352, 110–113. 10.1016/j.fluid.2013.05.014.
[99] Z. Wang, E. Königsberger, Solubility equilibria in the uric acid-sodium urate–water system1dedicated to professor heinz Gamsjäger on the occasion of his 65th birthday12presented at the Twelfth Ulm-Freiberg Conference, Freiberg, Germany, 19–21 March 19972, Thermochim Acta 310 (1) (1998) 237–242, https://doi.org/10.1016/S0040-6031(97)00230-X.
[100] O.N. Kavanagh, Alkalising agents in urinary tract infections: theoretical contraindications, interactions and synergy, 20420986221080794, Therapeutic Advances in Drug Safety 13 (2022), https://doi.org/10.1177/20420986221080794.
[101] L.-K. Chen, D.E. Cadwallader, H.W. Jun, Nitrofurantoin solubility in aqueous urea and creatinine solutions, J. Pharm. Sci. 65 (6) (1976) 868–872, https://doi.org/10.1002/jps.2600650617.
[102] J.J. Crivelli, T. Mitchell, J. Knight, K.D. Wood, D.G. Assimos, R.P. Holmes, S. Fargue, Contribution of dietary oxalate and oxalate precursors to urinary oxalate excretion, Nutrients 13 (1) (2021) 62, https://doi.org/10.3390/nu13010062.
[103] H. Henry, N. Marmy Conus, P. Steenhout, A. Béguin, O. Boulat, Sensitive determination of d-lactic acid and l-lactic acid in urine by high-performance liquid chromatography-tandem mass spectrometry, Biomed. Chromatogr. 26 (4) (2012) 425–428, https://doi.org/10.1002/bmc.1681.
[104] S. Nakatani, A. Nakatani, E. Ishimura, N. Toi, A. Tsuda, K. Mori, M. Emoto, Y. Hirayama, A. Saito, M. Inaba, Urinary iron excretion is associated with urinary full-length megalin and renal oxidative stress in chronic kidney disease, Kidney Blood Press. Res. 43 (2) (2018) 458–470, https://doi.org/10.1159/000488470.
[105] C. Cao, X. Jin, Q. Ding, J. Zhu, D. Yang, B. Fan, The altered composition of gut microbiota and biochemical features as well as dietary patterns in a southern chinese population with recurrent renal calcium oxalate stones, Urolithiasis 51 (1) (2023) 95, https://doi.org/10.1007/s00240-023-01467-x.
[106] K. Oginawati, A.A.H. Anka, S.H. Susetyo, S.A. Febriana, I. Tanziha, C.R. S. Prakoeswa, Urinary hippuric acid level as a biological indicator of toluene exposure on batik workers, Heliyon 7 (8) (2021) e07775.