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Short communication

Analysis of pyrazine 2,5-dipropionic acid in 5-aminolevulinic acid-loaded urological and topical delivery vehicles: methodology and assay validation

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Abstract

Photodynamic therapy (PDT) using 5-aminolevulinic acid (ALA) is a novel treatment method with much potential benefit for cancer detection and eradication. Formulation into drug delivery systems, such as aqueous solutions and emulsion based creams is complicated by its rapid dimerisation to pyrazine 2,5-dipropionic acid (PY); a compound with scant documentation in terms of toxicity and effect during PDT. This degradation is especially noticeable, where pH is adjusted upwards to avoid local irritation. A good case in point is bladder instillation of ALA for treatment and diagnosis of urothelial neoplasia. This work describes a rapid and validated HPLC method designed to assess the formation of PY in ALA-loaded vehicles. PY eluted as a single peak (R_t = 5.0 min) with good intra- and inter-day reproducibility and limits of detection and quantification found to be 0.01 and 0.04 μg ml^{-1}, respectively. Sample stability for upto 16 h was demonstrated, allowing autoinjection cycles to be performed. PY formation was detected in typical buffers used for bladder instillation after 6 h of storage, emphasising the need to use these preparations immediately upon manufacture if intended for photodynamic purposes. Moreover, upto 2.35% (w/w) PY was detected in artificial urine after 6 h storage at ambient temperature indicating that formation in vivo is likely to occur once bladder instillations are in situ and exposed to endogenous urine. As a result, ALA instillation times should be kept to the minimum needed for safe and successful treatment or diagnosis. PY extraction from semi-solid devices approached 100% efficiency demonstrating that the reported assay is suitable for evaluating stability of novel dosage forms intended for ALA delivery.

Keywords: Pyrazine 2,5-dipropionic acid; 5-Aminolevulinic acid; Photodynamic therapy; Drug delivery; Assay validation; Degradation

1. Introduction

Photodynamic therapy (PDT) employs a combination of visible light with a photosensitising drug to bring about apoptotic-driven destruction of selected cells. This results from intracellular generation of reactive singlet state oxygen [1,2]. The use of 5-aminolevulinic acid (ALA) as a photosensitising prodrug in PDT has numerous advantages, being a naturally occurring precursor in the biosynthetic pathway of haem. Administration of excess exogenous ALA avoids negative feedback regulation and in combination with the limited capacity of ferrochelatase, induces intracellular accumulation of protoporphyrin IX (PpIX) [3–5]. This effect is pronounced in rapidly proliferating cells, which have not only reduced ferrochelatase activity but also enhanced porphobilinogen deaminase activity [6–8]. The end result is a rapid and selective accumulation of photosensitising concentrations of PpIX in neoplastic cells. If these cells are irradiated with red light of wavelength 635 nm, PpIX becomes electronically excited. Interaction of this excited state with molecular oxygen yields highly reactive singlet oxygen, which is believed to be the primary cytotoxic species in PDT [9,10]. Similarly, the high fluorescence yield of ALA-induced PpIX demarcates neoplastic lesions as areas of pink–red fluorescence observable under ultraviolet illumination. This technique, known as photodynamic diagno-

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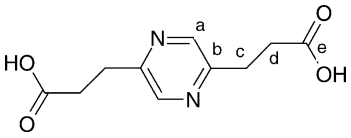
Table 1
Structure of pyrazine 2,5-dipropionic acid and summary of ^{1}H and ^{13}C NMR chemical shifts (parts per million)



Carbon position	^{1}H		^{13}C	
a	8.53		143.47	
b	–		154.38	
c	2.98		33.43	
d	2.67		30.87	
e	12.20		181.74	
1000	994.81	26.14	2.63	99.48

sis (PDD), is used to assist detection of bladder neoplasia [4].

In an attempt to lessen side effects and improve selectivity of both PDT and PDD for urological purposes, ALA is typically instilled into the bladder dissolved in aqueous solution [11,12]. ALA concentrations used, vary between 0.03 and 0.6 M and are adjusted to pH 4.8–8.4 [13,14,12]. This replicates urinary pH and avoids local irritation [13]. Similarly, topical administration of ALA in semi-solid devices has seen a considerable rise in dermatological application, especially in the eradication of superficial neoplastic lesions. However, ALA is known to degrade in solutions above pH 4, dimerising rapidly to yield cyclic compounds, which are not active in photodynamic therapy [13]. Under aerobic conditions, the major degradation product of ALA is pyrazine 2,5-dipropionic acid (PY) [15], as shown in Table 1.

To date, the majority of stability studies involving ALA have evaluated its pharmaceutical loss and consequential effects on the clinical outcome of PDT or detection rate of PDD. Rarely has PY formation per se been evaluated in delivery systems. Importantly, the toxicity of PY is as yet unknown and its presence may cause adverse drug reactions, both locally and systemically. For this reason, its quantification is desirable. This paper describes the validation of a HPLC assay for the analysis of PY in aqueous solutions, as used in PDT and PDD of bladder tumours, for example. Therefore, such an assay may be useful in the selection of appropriate formulation and storage conditions for ALA solutions and also in the determination of PY formed in the bladder after instillation. Moreover, given the increasing interest in ALA PDT of skin lesions, the method is applied to the determination of PY in other ALA-based drug delivery systems, such as topical semi-solid dosage forms.

2. Experimental

2.1. Chemicals

5-Aminolevulinic acid hydrochloride salt and Porphin® cream (20%, w/w ALA in Unguentum Merck®) were obtained from Crawford Pharmaceuticals, Milton Keynes, UK. Acetylacetone and formaldehyde 37% reagent were obtained from Sigma Aldrich, Dorset, UK. All other chemicals were of analytical reagent grade. Buffer solutions were prepared according to details in the British Pharmacopoeia (BP 2001) and the *Pharmacopoeia Helvetica* (Ph. Helv.VI).

2.2. Synthesis and determination of pyrazine 2,5-dipropionic acid

Pyrazine 2,5-dipropionic acid (PY) was synthesised by the method of Bunke et al. [15]. The structure of PY was confirmed by nuclear magnetic resonance spectroscopy (NMR, General Electric QC500) and the melting point determined using differential scanning calorimetry (DSC, DSC2920, TA Instruments, Surrey, UK). PY was analysed using HPLC (Waters Nova-Pak® column, Waters associates, Harrow, UK) running acetonitrile–acetate buffer (pH 2.8; BP; 0.1 M)(3:97, v/v) as mobile phase. Detection was by UV absorbance at 275 nm (Shimadzu SPD-6A UV spectrophotometric detector, Dyson Instruments Ltd., Tyne & Wear, UK) and data capture by Shimadzu Class VP™ software.

Least squares linear regression analysis and correlation analysis were performed on all daily calibration plots giving determination of the line of best fit, coefficient of determination and the residual sum of squares (RSS), as recommended [16]. Limits of detection (LoD) and quantification (LoQ) of the method were determined as recommended [17]. Inter- and intra-day variation of the analysis was investigated over a 5-day duration using high and low PY concentrations. PY stability, whilst awaiting injection was investigated by injecting samples, of known PY concentration, from the same vial, onto the column at hourly intervals over a defined time period (16 h). The autoinjector operated under ambient conditions of temperature and lighting.

2.3. Specificity for PY and method robustness

Method specificity and the identification of potential interferences from other compounds formed during ALA degradation, was verified using solutions of protoporphyrin IX and pseudo-porphobilinogen, mixed and injected with a standard solution of PY. The presence of additional peaks in the chromatograms or changes in shape or size of the PY peak was monitored.

Robustness was investigated by making deliberate alterations in injection volume, mobile phase composition and flow rate. Changes in peak areas and retention times were reported as the means of five replicate measurements. Where flow rate or mobile phase was altered, flow was allowed to proceed for at least 5 h before the experiment.

2.4. Method application

ALA was dissolved, in concentrations appropriate for PDT and PDD of bladder tumours, in phosphate buffers (BP)

of different pH values. In each case, ALA was dissolved, the pH adjusted to the required value with 5 M KOH and the solution made up to the appropriate volume. The drug was also dissolved in artificial urine (British Standard BSEN 1616:1997, Annex A, A.2.1, pH 6.6 [18]) at the same concentrations. Solutions were placed in closed vials with 2 cm^3 air gaps. The vials were then stored at ambient conditions of light and temperature. The formation of PY in each system was monitored by removing 0.1 ml samples from each vial at hourly intervals upto 6 h. Each removed aliquot was adjusted to 10 ml with 0.1 M borate buffer (*Pharmacopoeia Helvetica*) and the diluted samples stored at 5 °C until analysed. This borate buffer was used since it allows maintenance of ALA stability for prolonged periods of time; even when high ALA concentrations (8 mg ml^{-1}) and temperatures upto 37 °C is used.

To ensure that PY was, indeed, the single degradation product formed during ALA degradation in aqueous solution, all diluted samples were assayed simultaneously for ALA. ALA was derivatised by reaction with acetyl acetone and formaldehyde by slight modification of the method of Oishi et al. [19]. To a HPLC vial, 3.5 ml acetylacetone reagent, 50 μl of sample and 0.45 ml 10% (w/w) formaldehyde solution were added, mixed for approximately 5 s and heated for 20 min at 100 °C. After cooling on ice, the solution containing ALA–acetyl acetone/formaldehyde reagent derivative was injected (10 μl) onto a Waters Spherisorb® column (Waters associates, Harrow, UK). The mobile phase was methanol–water–glacial acetic acid (49.5:49.5:1%, v/v/v) flowing at 1.5 ml min^{-1}. Detection was by fluorescence (Shimadzu RF-535 fluorescence detector, Dyson Instruments Ltd, Tyne & Wear, UK) with excitation at 370 nm and emission at 460 nm. The amounts of PY formed were compared to the amounts of ALA lost.

The extraction and determination of PY from semi-solid pharmaceutical dosage forms containing ALA were evaluated using both proprietary and bespoke formulations. These contained clinically relevant ALA loadings of 20% (w/w). Porphin® cream (20%, w/w, ALA in Unguentum Merck®) was used as received. Organogels, consisting of 5% (w/w) Carbopol® ETD 2050 dissolved in glycerol or poly(ethyleneglycol) 400 (PEG 400), were prepared by addition of the dry polymer to the vigorously stirred solvents before loading with ALA (20%, w/w). The analysis was carried out immediately after ALA and PY addition so that any degradation of ALA to PY would be negligible. Samples (50 mg) were then dissolved or dispersed in 12.5 ml of 0.1 M borate buffer pH 5 (*Pharmacopoeia Helvetica*) prior to analysis.

2.5. Statistical analysis

Mathematical characterisation of the relationships between the *x* and *y* variables in the representative calibration plots was performed using least squares linear regression, following analysis of residuals. Confirmation of the validity of the linear regression was performed using ANOVA and correlation analysis.

3. Results and discussion

The instability of ALA has been a problematic aspect in the design and evaluation of drug delivery systems intended for photodynamic therapy. It has prompted numerous strategies for maintaining the stability of ALA, such as addition of ethylene diamine tetra acetic acid [20,21] or antioxidants [22] to ALA solutions. PY is recognised as the major degradation product and its evaluation within dosage forms, such as bladder instillations is important and one objective of this study. In addition to this, the development of bioadhesive gels [23], pressure-sensitive patches [24] and bioadhesive patches [25] for topical ALA delivery to numerous anatomical sites has been described recently. Given that regulatory authorities require full documentation on potential degradation pathways, such as PY formation, validated analytical methods are required, such as those described by the ICH [17]. This paper describes a validated HPLC method for the determination of PY, allowing assured determination of the degradation product in a variety of semi-solid dosage forms.

3.1. Synthesis of pyrazine 2,5-dipropionic acid

Synthesis of (PY), as described in Section 2.2, resulted in an orange–brown, hygroscopic powder. The yield of PY obtained was approximately 97% of the theoretical yield. Differential scanning calorimetry showed that PY decomposed at 178.00 ± 2.05 °C and did not melt, in a similar fashion to that exhibited by the closely related compound, pyrazinoic acid, which decomposes around 227 °C [26]. Table 1 summarises the data derived from nuclear magnetic resonance (^1H and ^{13}C NMR) analysis of PY dissolved in D_2O. The chemical shifts presented are in close agreement with those reported by Bunke et al. [15] and confirm the structure of the expected pyrazine derivative. The spectrum shows a singlet signal for the aromatic protons at 8.53 ppm and additional signals characterising the methylene and carboxylic acid protons related to the propionic acid chains. ^{13}C NMR of PY clearly indicates the presence of five non-equivalent carbons associated with pyrazine 2,5-dipropionic acid. The upfield signals are associated with the two carbons (c and d) in the propionic acid chain, whereas other carbons (a, b and e) are positioned at much higher frequencies (further downfield) due to the electronegativity of neighbouring atoms. The carbonyl carbon is situated to the extreme left of the spectrum (187.74 ppm) due to the presence of the electronegative oxygen atom.

3.2. Determination of pyrazine 2,5-dipropionic acid

PY has been quantified previously by Bunke et al. [15] using a validated capillary electrophoresis method employ-

ing UV absorption and diode array detection to monitor ALA degradation in aqueous solution. Using this instrumental configuration, it was confirmed that PY was the major degradation product formed under aerobic conditions in aqueous solution. Unfortunately, the analytical method struggled with the excipients found in emulsion type systems and required modification to a micellar electrokinetic running mode. Work by Dalton et al. [27] has described a method based on HPLC and UV detection, which is similar to that detailed in this study. However, the work focused on in vivo formation of PY after intravesical administration of ALA and did not present details of validation nor applicability to drug delivery systems.

3.3. Chromatographic evaluation

Fig. 1(a) shows a typical chromatogram obtained after injection of 10 μl of a pyrazine 2,5-dipropionic acid (PY) standard solution in 0.1 M borate buffer pH 5 (*Pharmacopoeia Helvetica*). A single prominent peak with good symmetry is present in the chromatogram with a retention time (R_t) of approximately 5 min. Its identity was mostly likely that of PY, given its absence from the chromatogram in Fig. 1(b) following an injection containing only buffer and no PY.

Analytical response was shown to be linear ($r^2 = 0.9997$; $R_{SS} = 5.95 \times 10^{10}$) over the concentration range investigated, which was from 50 to 1000 μg ml^{-1} of PY. The LoD was

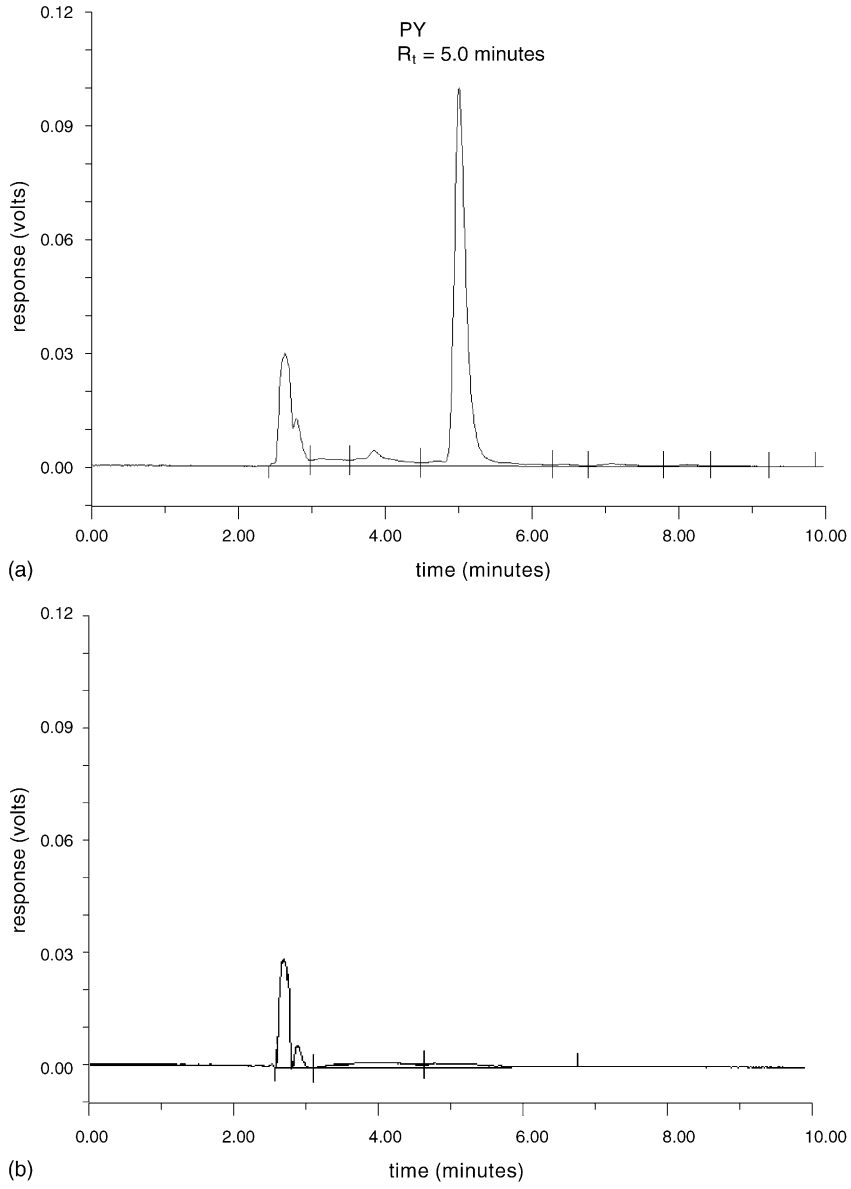


Fig. 1. (a) Chromatogram obtained by injection of a 10 μl aliquot of a 250 μg ml^{-1} sample of pyrazine 2,5-dipropionic acid in 0.1 M borate buffer pH 5 (*Pharmacopoeia Helvetica*). (b) Chromatogram obtained by injection of a 10 μl aliquot of a sample containing no pyrazine 2,5-dipropionic acid only 0.1 M borate buffer pH 5 (*Pharmacopoeia Helvetica*).

0.01 μg ml^{-1} and the LoQ was 0.04 μg ml^{-1}, which can be compared to those determined by Bunke et al. [22] who reported a limit of quantification of 1.00 ng ml^{-1}. Although that may be approximately 40 times smaller than found in this study, it must be borne in mind that it arises from the greater sensitivity of CE compared to HPLC. It must be appreciated also that most drug delivery systems containing ALA, such as the topical cream, contain high loadings, such as 20% (w/w), to enhance drug penetration. A limit of quantification of 0.04 μg ml^{-1}, as found in this study, is perfectly adequate to detect small amounts of degradation emanating from such high loadings. The high and low concentrations of the samples containing PY were 250 and 1000 μg ml^{-1} PY, respectively. The method described was found to be reproducible with a coefficient of variation of 6.17 and 2.63% for inter-day variation and 0.16 and 0.11% for intra-day variation for high and low, respectively. Levels of accuracy were 99.48 and 101.60% for inter-day variation and 98.54 and 100.71% for intra-day variation for high and low, respectively.

Guidelines published by Shabir [16] recommend an acceptable limit of 2% change in analytical response for samples stored for protracted periods of time in an autoinjector. Multiple injections of a PY standard (250 μg ml^{-1}) onto the column at hourly intervals produced a coefficient of variation of 0.54%. It was shown that 99.48% of the peak area captured at the start of the experiment remained after 16 h, well within the recommended 2% tolerance limit.

The primary contaminating compounds likely to be encountered are protoporphyrin IX and pseudo-porphobilinogen but neither was detectable using this method. Adjusting the detection wavelength to match the λ_{max} of each compound and using solutions containing 1 mg ml^{-1} of the respective contaminant produced no additional peaks in the chromatograms after 1 h of surveillance. No alterations in the shape or size of the PY peak were observed when either contaminant was present in the injection solution. Therefore, this analytical method is adaptable for in vivo use, since PpIX leaking out of damaged neoplastic tissue will not interfere with the detection of PY in sample solutions.

Table 2
Influence of forced variation in analytical parameters on peak area and retention time of a peak corresponding to a sample containing 250 μg ml^{-1} pyrazine 2,5-dipropionic acid

Parameter	Response
Injection volume (μl)	PY peak area (±S.D.) $n = 3$
5	$1.67 \times 10^6 \pm 2.27 \times 10^4$
10	$2.66 \times 10^6 \pm 1.82 \times 10^5$
20	$6.23 \times 10^6 \pm 5.28 \times 10^5$
Flow rate (ml min^{-1})	PY retention time (min) (±S.D.) $n = 3$
0.25	12.75 ± 0.31
0.50	5.00 ± 0.01
1.00	2.66 ± 0.11
Mobile phase composition (%) acetate buffer/% acetonitrile (v/v)	PY retention time (min) (± S.D.) $n = 3$
100/0	5.79 ± 0.02
97/3	5.00 ± 0.01
90/10	3.15 ± 0.01

Table 2 demonstrates changes in the size and retention time of the PY peak upon modification of injection volume, flow rate and mobile phase composition. As expected, increases in injection volume caused corresponding enlargements in peak area. The retention time was influenced strongly by the flow rate, with a flow rate of 0.5 ml min^{-1} representing an acceptable balance between total time for the analytical run and avoidance of solvent front aberrations. Increasing the proportion of acetonitrile (AcCN) in the mobile phase reduced the R_t of PY. Small changes, such as those between 3 and 10% of AcCN in the mobile phase, had a marked influence in bringing the PY peak off earlier.

3.4. Determination of pyrazine 2,5-dipropionic acid in aqueous vehicles

It is well known that as ALA concentration and pH rise, the extent of ALA degradation increases over time [13,20,28]. Table 3 details the loss of ALA and the formation of PY in the solutions studied after 6 h of storage. No ALA loss or PY for-

Table 3
Amounts of ALA lost and PY formed in aqueous buffer systems after 6 h storage under aerobic conditions and at ambient lighting and temperature

ALA concentration (M)	Buffer system	% ALA lost	Remaining ALA concentration (M)	Theoretical yield of PY (mg)[a]	Actual yield of PY (mg)	% PY in formulation
0.075	Phosphate BP pH 4.0	0.00	0.075	0.00	0.00	0.00
0.150	Phosphate BP pH 4.0	0.00	0.150	0.00	0.00	0.00
0.300	Phosphate BP pH 4.0	0.00	0.300	0.00	0.00	0.00
0.075	Phosphate BP pH 6.0	20.20 ± 0.11	0.060	16.97 ± 0.08	19.33 ± 2.08	0.19 ± 0.02
0.150	Phosphate BP pH 6.0	35.43 ± 0.29	0.100	59.52 ± 0.48	58.00 ± 2.65	0.58 ± 0.03
0.300	Phosphate BP pH 6.0	65.80 ± 1.06	0.100	221.09 ± 3.56	216.67 ± 15.28	2.17 ± 0.15
0.075	Phosphate BP pH 8.0	57.56 ± 0.06	0.030	48.35 ± 0.04	43.00 ± 3.61	0.43 ± 0.04
0.150	Phosphate BP pH 8.0	73.15 ± 0.10	0.040	122.89 ± 0.17	126.67 ± 20.82	1.267 ± 0.21
0.300	Phosphate BP pH 8.0	80.65 ± 0.08	0.060	270.98 ± 0.26	277.33 ± 12.50	2.77 ± 0.13
0.075	Artificial urine pH 6.6	25.03 ± 1.65	0.060	21.03 ± 1.39	21.83 ± 2.85	0.22 ± 0.03
0.150	Artificial urine pH 6.6	42.17 ± 1.22	0.090	70.84 ± 1.59	73.67 ± 4.51	0.74 ± 0.05
0.300	Artificial urine pH 6.6	69.20 ± 2.33	0.090	232.51 ± 7.82	234.67 ± 5.51	2.35 ± 0.06

[a] Calculated from the ALA loss data on the basis that two molecules of ALA hydrochloride (M_r = 167.6) react to give one molecule of PY (M_r = 224).

mation was detected in phosphate buffer BP pH 4.0, regardless of the initial ALA concentration. Substantial ALA loss occurred in all of the other solutions studied. None of these solutions were still within the pharmaceutically acceptable range of 90–100% (w/w) ALA after 6 h storage (ICH guidelines Q6A and Q6B, 2003) [29,30] The percentages of ALA lost increased with increasing initial ALA concentration and also with increasing pH. Around 20.2% of the original ALA loading had been lost from a 0.075 M solution at pH 6.0, while 80.65% of the original loading had been lost from a 0.3 M solution at pH 8.0. In each case, the amount of PY actually formed was not significantly different to the theoretical yields based on ALA loss. For example, the theoretical yield of PY in a 0.3 M ALA solution at pH 6.0, stored for 6 h, was 221.09 ± 3.56, which was not significantly different ($p = 0.3995$) from the actual yield, which was 216.67 ± 15.28. In addition, the theoretical yield of PY in artificial urine containing 0.3 M ALA, stored for 6 h, was 232.51 ± 7.82, which was not significantly different ($p = 0.4955$) from the actual yield, which was 234.67 ± 5.51. Similar comparisons were made for all of the other solutions studied. With the exception of the solutions at pH 4.0, appreciable percentages of PY had developed in each of the solutions, including artificial urine, studied after 6 h of storage. In fact, the solution at pH 8.0 with an initial ALA concentration of 0.3 M contained as much as 2.7% PY after 6 h.

Of interest, as seen in Table 3 was that the theoretical amounts of PY formed could be predicted reasonably well based on ALA loss. This confirms that PY is, indeed, the major degradation product of ALA formed in aerated solutions. In several of the solutions studied the quantities of PY present after 6 h storage were greater than 1% of the final weight of the formulation. When used in PDT or PDD, the dose, or amount of solution instilled into the bladder, of each of the solutions studied, is likely to exceed 10 mg. Therefore, the International Conference on Harmonisation recommends that the biological safety of the degradation product be established (ICH guideline Q3B(R), 2003) [31]. No safety data is currently available for PY. Therefore, the pH and concentration of ALA solutions for bladder instillation should be kept as low as possible. Solutions with pH values above 4.0, particularly those of high ALA concentration should ideally be used immediately upon preparation. Sizable amounts of PY were formed in artificial urine at ambient temperature. This vehicle was included in the study to mimic the situation, where an ALA solution has been instilled and is being diluted with endogenous urine flow. As seen in Table 3, ALA loss is substantial, approaching 70% for 0.3 M ALA solutions after 6 h. Although systemic acidification of the urine can alleviate this problem, the work of Dalton et al. [27] showed that pyrazine 2,5-dipropionic acid was only detectable in two from seven dogs who received ALA bladder instillations and no urine pH modification. The results from this work suggest that ALA loss was sizeable around pH 6, even when the diluting effect of urine is not a contributing factor. Moreover, long retention times, such as 6 h, will further reduce ALA stability in vivo. Bladder retention times of ALA solutions should, therefore, be kept to the minimum needed to produce the desired result.

Table 4
Extraction efficiencies of PY from pharmaceutical formulations containing defined loadings (6 and 12%, w/w) of ALA (means ± S.D., $n = 5$)

Formulation	Theoretical PY loading (%, w/w)	Determined PY loading (% w/w)	Extraction efficiency (%)
Porphin® cream	6.57	6.69 ± 0.55	101.86 ± 4.40
Glycerol gel	5.79	5.70 ± 0.20	98.40 ± 3.41
PEG 400 gel	6.18	6.05 ± 0.19	97.85 ± 3.03
Porphin® cream	12.55	13.21 ± 0.95	105.28 ± 7.56
Glycerol gel	12.63	12.90 ± 0.27	102.15 ± 2.14
PEG 400 gel	12.46	12.85 ± 1.10	103.13 ± 3.82

3.5. Extraction efficiencies

Table 4 shows the PY extraction efficiencies from the various formulations investigated in this study. As ALA is extremely unstable when not in an acidic environment, large amounts of PY may be formed. Therefore, formulations were spiked with two relatively high loadings of PY. Furthermore, ALA was included in the formulation so that potential difficulties with the extraction could be exposed. It can be seen that the extraction efficiencies of PY from the formulations studied was high in all cases. Variabilities in the extraction efficiencies were low, with standard deviations below 5% in all cases.

In conclusion, a rapid and validated assay procedure for the determination of PY in both semi-solid dosage forms and aqueous solutions was described. It has been shown that if a buffer is selected for intravesical instillation using an elevated pH, such as 6.0, in an attempt to alleviate local irritation, then significant amounts of PY are to be expected in the formulation. It is, therefore, important that these solutions are used immediately after preparation. Bearing in mind the formation of sizable amounts of PY in artificial urine, even at ambient temperature, bladder retention times should be reduced to the minimum needed for successful photodynamic diagnosis or therapy. Additional work in this laboratory has produced results (to be reported shortly) that PY formation is particularly problematic in topical dosage form design. It is important, therefore, that PY extraction is optimal.

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