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Development of a novel method for the bioanalysis of benfotiamine and sulbutiamine in cancer cells

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Quantification of benfotiamine and sulbutiamine, synthetic thiamine analogs, in biological samples is an essential step toward understanding the role of these thiamine analogs on cancer cell proliferation. A sensitive method to quantitate benfotiamine and sulbutiamine in cells and media was successfully developed using reversed-phase HPLC. Accuracy, precision, specificity and robustness were evaluated to assess the reliability of this method in accordance with U.S. FDA guidelines. The method provided a linear range from 100–50 000 nM for benfotiamine and from 500–30 000 nM for sulbutiamine in both cells and media. The method was validated and the precision was found to be within 15% relative standard deviation (RSD), and the accuracy to be within 15% relative error (RE). Benfotiamine and sulbutiamine were used as internal standards for each other to achieve a high level of reproducibility. This method has been successfully applied to the study of benfotiamine and sulbutiamine to determine their uptake and disposition between mammalian cells and cell media. The method can contribute to future studies to determine the effect of benfotiamine and sulbutiamine as novel thiamine analogs on cancer cell proliferation.

1. Introduction

Thiamine (vitamin B1) is an important enzyme cofactor that is critical in a variety of metabolic pathways, especially glucose metabolism. Nutritional supplementation with thiamine has been widely studied especially with respect to its role in cancer cell proliferation. Thiamine has been found to influence a variety of different types of cancer. However, there is still a controversy about the role of thiamine in cancer. The general thought is that thiamine supplementation may contribute to increasing cancer cell proliferation since an increased metabolic rate would be required for this process. Supporting evidence for this mechanism is that increasing thiamine supplementation was observed to contribute toward the growth of Ehrlich's ascites tumor xenografts.[1] In addition, thiamine supplementation was shown to decrease hypoxia-mediated apoptosis in rat cardiomyocytes.[2] As a dichotomous effect of thiamine supplementation on cancer growth, an anti-proliferative effect with no increase in tumor growth in comparison with control has been observed at high doses.[1] In this scenario, high-doses of thiamine have been suggested to reduce cancer cell proliferation by inhibiting pyruvate dehydrogenase kinases (PDKs).[3]

Synthesized lipophilic thiamine derivatives can readily diffuse across plasma membranes and thereby have high bioavailability while thiamine is generally transported at low rates by high affinity carriers.[4–9] These lipid-soluble thiamine derivatives can be converted to thiamine inside cells and utilized.[9] Therefore, improvement of the pharmacokinetic properties of thiamine analogs has been considered as an effective strategy to alleviate symptoms due to thiamine deficiency. Among the lipid-soluble thiamine analogs, benfotiamine and sulbutiamine (Fig. 1) are two of the more promising synthetic derivatives. Benfotiamine and sulbutiamine have excellent bioavailability[5–8,10,11] and are known to easily cross cell membranes and increase intracellular thiamine levels.[9–12] Benfotiamine, an *S*-acyl derivate of thiamine, was discovered to stimulate transketolase (TKT) activity involved in glucose metabolism and prevent diabetic nephropathy in a similar manner to high-dose thiamine.[13–15] Sulbutiamine, a dimer of two modified thiamine molecules, is widely used clinically as a treatment for brain function including memory disorders and asthenia.[9,16–18]

Previously, HPLC methods for the analysis of benfotiamine bulk drug and in tablet dosage forms have been reported.[19,20] Adithya *et al.* developed an HPLC method with a range from 5–35 μg ml^{-1}, with a 0.1448 μg ml^{-1} of limit of detection (LOD) and a 0.4388 μg ml^{-1} of limit of quantification (LOQ).[19] In the literature, an HPLC method for analysis of sulbutiamine was reported with a range from 2–40 μg ml^{-1}, a 0.5 μg ml^{-1} LOD and a 1.51 μg ml^{-1} LOQ from a tablet dosage form.[21] However, no methods have been reported for the determination of benfotiamine and sulbutiamine from biological matrices.

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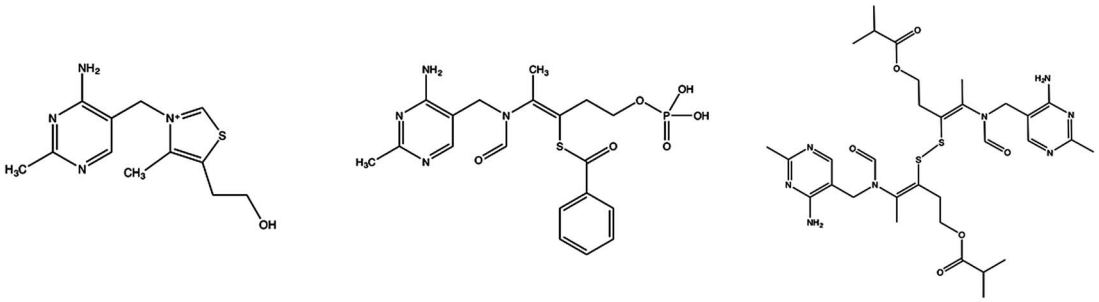

Fig. 1 Structures of thiamine, benfotiamine, and sulbutiamine.

There are many ongoing drug discovery efforts focused on the treatment of cancer, a life threatening and unconquered disease. Due to its central role in many biochemical pathways such as glycolysis, thiamine has been drawing attention as a potential therapeutic adjuvant. This has lead to the development of the lipophilic thiamine derivatives, benfotiamine and sulbutiamine, which have improved therapeutic properties including higher bioavailability. However, it is necessary to determine the effects of these two thiamine analogs on cancer cell proliferation.

In this study, we have developed and validated a bioanalytical method for the analysis of benfotiamine and sulbutiamine from cells and cell media. We have applied this method to the determination of both intracellular and extracellular levels of these compounds in order to identify their effect on cancer cell proliferation. Based on these preliminary results, we expect that the developed HPLC method will be vital toward the development of thiamine analogs as potential nutraceutical therapeutics.

2. Materials and methods

2.1. Chemicals and reagents

The reference standards for benfotiamine and sulbutiamine were purchased from MP Biomedicals (Santa Ana, CA, USA) and Toronto Research Chemicals (Toronto, Ontario, Canada), respectively. Water and acetonitrile (chromatographic grade), hydrochloric acid, di-isopropyl ether and trichloroacetic acid (TCA) were obtained from Sigma-Aldrich (St Louis, MO, USA). Dibasic sodium phosphate was supplied by J. T. Baker (Phillipsburg, NJ, USA). All standards were analytical grade with more than 90% chemical purity. Custom-formulated thiamine-deficient RPMI 1640 media and fetal bovine serum (FBS) were purchased by Mediatech (Manassas, VA, USA) and Seradigm (Radnor, PA, USA), respectively.

2.2. Preparation of the stock solutions, calibration standards and quality control (QC) samples

Stock solutions of benfotiamine (1.0 mM) and sulbutiamine (2.0 mM) were prepared in methanol. The aliquots of stock solutions were stored at −80 °C. 2 μM of benfotiamine and 4 μM of sulbutiamine were used as internal standards (ISTD) for the determination of the other analyte. Each stock solution was serially diluted with water to prepare the working solutions at appropriate concentrations. For preparation of benfotiamine and sulbutiamine (cells and cell media) calibration curves, the working solutions of benfotiamine were diluted to calibration standards over the range from 100–50 000 nM. The range of concentrations of sulbutiamine in cells and media for the calibration standards was 500–30 000 nM. Quality control (QC) samples were prepared at concentrations of 300, 1500 and 40 000 nM for benfotiamine and 1500, 4000 and 24 000 nM for sulbutiamine.

2.3. Instrumentation and chromatographic conditions

HPLC analysis was performed by using an Agilent 1100 quaternary pump HPLC system (Santa Clara, CA, USA). HPLC separation was achieved at 25 °C using a Shim-pack MAqC-ODS I column (4.6 × 150 mm, 5 μm, Shimadzu Corp. (Kyoto, Japan)). A mobile phase system containing of 15 mM sodium phosphate buffer (pH 3.6) and 100% acetonitrile were used. The injection volume of each sample was 50 μl and the mobile phase flow rate was set at 0.8 ml min^{-1}. Gradient conditions for benfotiamine analysis were as follows (time in minutes, % mobile phase B): (0, 10), (8, 30), (10, 70), (15, 80), (16, 80), (16.01, 10), (21, 10). Gradient conditions for sulbutiamine analysis were as follows (time in minutes, % mobile phase B): (0, 10), (8, 30), (10, 70), (16, 80), (17, 70), (17.01, 10), (22, 10). Benfotiamine and sulbutiamine were detected at wavelengths of 235 nm and 239 nm, respectively. Chemstation (Rev. B.01.03, Agilent) was used for instrument control, data collecting and processing. After each injection, the injection needle was washed with water.

2.4. Cell culture conditions

Human glioblastoma astrocytoma cells (U-87 MG) were purchased from ATCC (Manassas, VA, USA). Custom-formulated thiamine-deficient RPMI 1640 media supplemented with 10% heat-inactivated FBS was used for cell culture. Cells were incubated at 37 °C in a humidified atmosphere of 5% CO_2. Cultured cells in T-175 flasks were treated with trypsin/EDTA to be harvested, followed by washing three times using ice-cold phosphate-buffered saline (1× PBS). Cells were counted using a TC20 Automated Cell Counter (Bio-Rad, Hercules, CA, USA)

and divided into aliquots of 1×10^6 cells by centrifugation at 4 °C for 5 min at 500 g.

2.5. Sample preparation

Sample preparation was done as outlined in the procedure by Basiri *et al.* for thiamine.[22] Cell pellets were treated with 500 μl of 15% trichloroacetic acid (TCA) solution to precipitate proteins in the samples. Likewise, 500 μl of media was treated with 100 μl of 72% TCA solution. Afterwards, different concentrations of benfotiamine and sulbutiamine were spiked into both cells and media. Internal standards were added to each sample to a final concentration of 2 μM and 4 μM for benfotiamine and sulbutiamine, respectively. Benfotiamine and sulbutiamine were used as an internal standard for each other. Following a 2 min vortex, the mixture was kept on ice for 30 min. The mixture was treated with 6 volumes of di-isopropyl ether to remove the TCA and mixed for another 1 min. After centrifugation at 13 000 g at 10 °C for 6 min, the supernatant (organic layer) of the mixture was discarded. Evaporation of the remaining ether was done in the hood and the mixture was filtered through 0.22 μm hydrophilic nylon membrane syringe filters, prior to analysis by HPLC.

2.6. Method development and validation

This method was validated in accordance with the current U.S. FDA guidance for bioanalytical method validation. Selectivity, linearity, sensitivity, reproducibility, and stability tests were carried out to validate the method.

3. Results and discussion

3.1. Method development

Factors that establish a selectivity and sensitivity were optimized in HPLC with UV detector for quantification of benfotiamine and sulbutiamine. The gradients that provided good separation of these compounds were established step-by-step. Detection wavelengths for each compound were found to improve sensitivity using the variable wavelength detector. Fluorescence detection was also applied to these compounds in an effort to achieve higher sensitivity because detection based on fluorescence is generally more sensitive than UV absorption. However, it did not improve the detection ability for these compounds. The potential risk that this inorganic salt may precipitate in higher levels of organic solvents was avoided by using a 15 mM sodium phosphate buffer. Both methanol and acetonitrile were tested as organic solvents and acetonitrile provided a more stable baseline for the chromatogram at both 235 nm and 239 nm.

3.2. Sensitivity and selectivity

In Fig. 2 and 3, the representative chromatograms demonstrate the selectivity and sensitivity of the method from cells and media. Fig. 2 shows the chromatograms for benfotiamine and

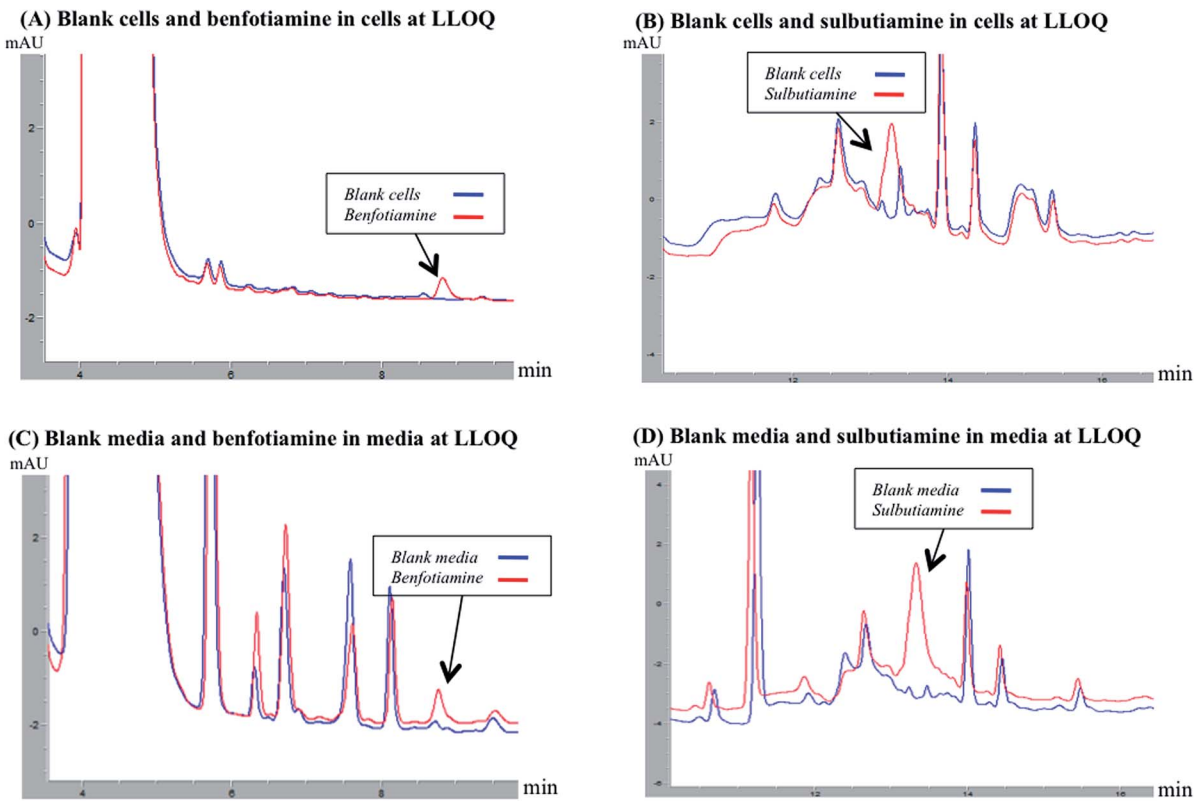

Fig. 2 Representative chromatograms of benfotiamine (A and C, red) and sulbutiamine (B and D, red) at LLOQ levels in cells and media. The chromatograms are overlaid with chromatograms of blank cells (A and B, blue) and blank media (C and D, blue).

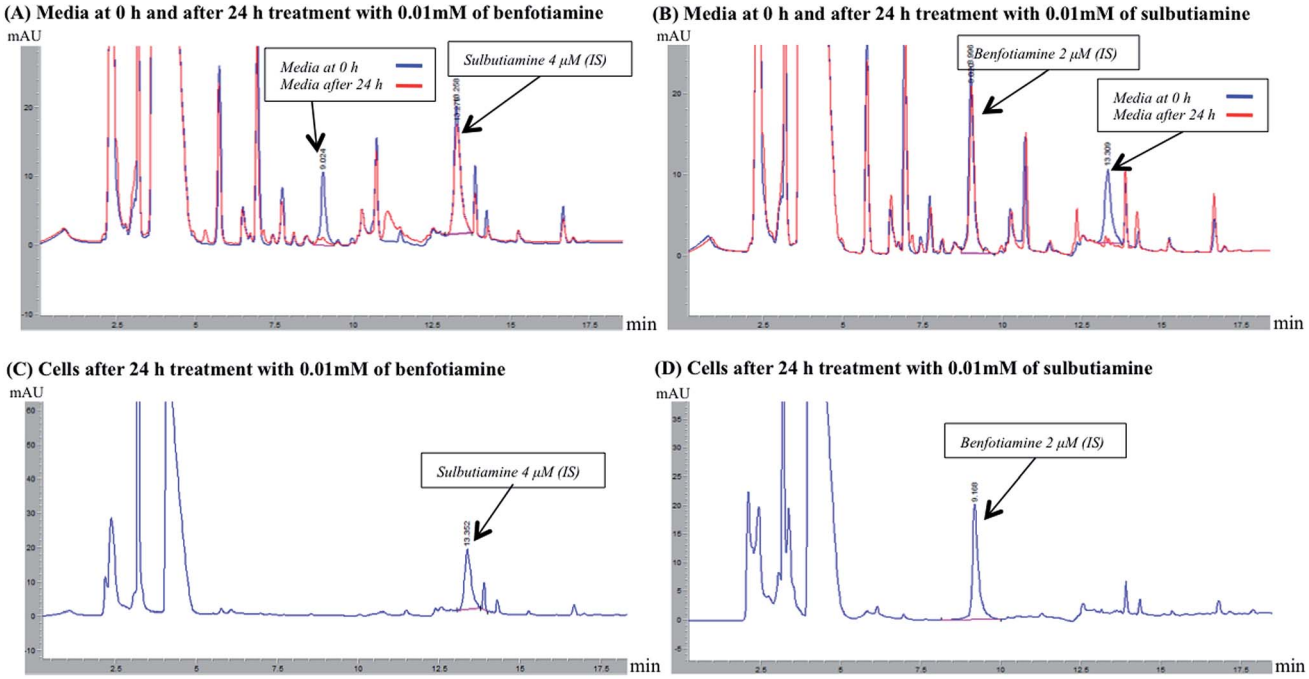

Fig. 3 Representative chromatograms of benfotiamine (A and C) and sulbutiamine (B and D) in media and cells. Media at 0 h and after 24 h treatment with 0.01 mM of benfotiamine and sulbutiamine (A and B). The chromatograms are overlaid with chromatograms of media at 0 h (blue) and media after 24 h (red). Cells are shown after 24 h treatment with 0.01 mM of benfotiamine and sulbutiamine (C and D).

Table 1 Calibration curves for benfotiamine and sulbutiamine in cells and cell media ($n = 3$)

	Cells			Cell media		
Analyte	Slope	Intercept	R^2	Slope	Intercept	R^2
Benfotiamine	0.8686 ± 0.0251	−0.0074 ± 1.7968	0.9972 ± 0.0011	0.8801 ± 0.0268	0.0064 ± 0.6024	0.9958 ± 0.0045
Sulbutiamine	1.0921 ± 0.0499	0.0330 ± 2.9314	0.9975 ± 0.0004	1.0362 ± 0.0464	0.0886 ± 1.9746	0.9966 ± 0.0021

Table 2 The intra-day ($n = 5$) and inter-day ($n = 15$) precision (RSD) and accuracy (RE) for the LC-UV method used to quantitate benfotiamine in cells and cell media

			Intra-day ($n = 5$)			Inter-day ($n = 15$)		
Matrix	Analyte	Nominal conc. (nM)	Measured conc.	RSD (%)	RE (%)	Measured conc.	RSD (%)	RE (%)
Media	Benfotiamine	100 (LLOQ)	111	5.51	10.50	101	12.21	1.18
		300 (LQC)	274	4.67	−8.80	282	7.28	−5.93
		1500 (MQC)	1443	9.73	−3.82	1443	6.94	−3.79
		40 000 (HQC)	41 003	7.96	2.51	41 832	6.43	4.58
Cells		100 (LLOQ)	107	3.30	6.73	103	7.11	2.60
		300 (LQC)	272	2.99	−9.37	284	5.19	−5.44
		1500 (MQC)	1372	1.21	−8.56	1437	5.82	−4.20
		40 000 (HQC)	41 977	5.02	4.94	41 819	5.44	4.55

sulbutiamine at the LLOQ levels in both cells and media. Limit of detection (LOD) and lower limit of quantification (LLOQ) that define the sensitivity of the method were determined for each analyte at signal-to-noise (S/N) ratios of greater than 3 and 10, respectively. The LOD was 50 nM (22.3 ng ml^{-1}) and LLOQ was 100 nM (210.9 ng ml^{-1}) for benfotiamine in both cells and cell media. The LOD was 300 nM and LLOQ was 500 nM for sulbutiamine in these biological matrices. As shown in Table 2, the precision and accuracy of the method at the LLOQ level was validated within 20% of the relative standard deviation (RSD) and relative error (RE). Selectivity of the method helps to differentiate and quantify the analytes of interest in samples that include other compounds. The chromatograms were acquired following sample extraction to investigate any

Table 3 The intra-day ($n = 5$) and inter-day ($n = 15$) precision (RSD) and accuracy (RE) of the LC-UV method used to quantitate sulbutiamine in cells and cell media

			Intra-day ($n = 5$)			Inter-day ($n = 15$)		
Matrix	Analyte	Nominal conc. (nM)	Measured conc.	RSD (%)	RE (%)	Measured conc.	RSD (%)	RE (%)
Media	Sulbutiamine	500 (LLOQ)	444	8.89	−11.15	456	6.92	−8.87
		1500 (LQC)	1497	7.73	−0.21	1492	6.10	−0.56
		4000 (MQC)	4319	2.46	7.98	4032	6.77	0.79
		24 000 (HQC)	22 585	2.71	−5.90	23 042	3.55	−3.99
Cells		500 (LLOQ)	515	8.96	3.10	506	9.66	1.16
		1500 (LQC)	1400	1.69	−6.65	1410	8.22	−6.02
		4000 (MQC)	4125	3.06	3.13	3990	5.85	−0.25
		24 000 (HQC)	24 701	1.16	2.92	23 384	6.38	−2.57

Table 4 Autosampler stability ($n = 4$), bench-top stability ($n = 4$), and freeze–thaw stability ($n = 4$) of benfotiamine and sulbutiamine at LQC and HQC in cells and cell media

Matrix	Analyte	Conc. (μM)	Autosampler stability (% ± SD)	Bench-top stability (% ± SD)	Freeze–thaw stability (% ± SD)
Media	Benfotiamine	300 (LQC)	112 ± 6.76	106 ± 1.66	106 ± 1.91
		40 000 (HQC)	102 ± 0.43	101 ± 0.62	101 ± 2.27
	Sulbutiamine	1500 (LQC)	108 ± 9.83	92.4 ± 13.70	110 ± 11.98
		24 000 (HQC)	100 ± 5.81	101 ± 1.03	94.0 ± 1.99
Cells	Benfotiamine	300 (LQC)	114 ± 7.07	—	—
		40 000 (HQC)	103 ± 2.50	94.6 ± 0.72	101 ± 2.12
	Sulbutiamine	1500 (LQC)	101 ± 5.78	—	58.8 ± 5.36
		24 000 (HQC)	99.8 ± 7.83	93.0 ± 0.22	94.2 ± 1.21

interference. Fig. 3A and B shows the chromatograms for benfotiamine and sulbutiamine in media at 0 h and after 24 h treatment with 0.01 mM of each compound. Fig. 3C and D shows the chromatograms for benfotiamine and sulbutiamine in cells after 24 h treatment with 0.01 mM of each compound.

3.3. Linearity and calibration curve

Nine increasing concentrations of benfotiamine with 4 μM of sulbutiamine as an internal standard were added to the cells and thiamine-deficient RPMI 1640 media to generate calibration curves. The spiked concentrations were 100, 200, 500, 1000, 2000, 5000, 10 000, 50 000 nM. Likewise, calibration curves for sulbutiamine quantification with 2 μM of benfotiamine were plotted with the range of 500, 700, 1000, 3000, 5000, 7000, 10 000, 30 000 nM. Calibration curves with $1/x$ weighted linear regression were obtained using the peak area ratios between the analyte and the ISTD *versus* the analyte concentration. Slopes, *y*-intercepts and *R*-squared values of the regression lines are shown in Table 1. As shown in Table 1, the method showed good linearity with the values for the correlation coefficients (R^2) > 0.995 for each analyte.

Table 5 Absolute recovery ($n = 3$) and relative recovery ($n = 3$) of the method

Matrix	Analyte	Conc. (nM)	AR (%)	RR (%)
Media	Benfotiamine	300 (LQC)	113 ± 1.01	100 ± 0.34
		1500 (MQC)	104 ± 8.56	92.1 ± 0.14
		40 000 (HQC)	103 ± 0.24	96.1 ± 0.48
	Sulbutiamine	1500 (LQC)	110 ± 3.90	98.2 ± 9.20
		4000 (MQC)	101 ± 1.58	101 ± 3.34
		24 000 (HQC)	97.6 ± 1.42	102 ± 0.34
Cells	Benfotiamine	300 (LQC)	106 ± 4.71	103 ± 2.11
		1500 (MQC)	106 ± 1.08	97.3 ± 0.87
		40 000 (HQC)	106 ± 0.03	96.3 ± 0.33
	Sulbutiamine	1500 (LQC)	111 ± 3.96	98.1 ± 0.64
		4000 (MQC)	106 ± 1.87	99.6 ± 1.26
		24 000 (HQC)	99.8 ± 1.29	98.3 ± 0.59

3.4. Precision and accuracy

Precision and accuracy of the method were validated at the LLOQ, LQC, MQC and HQC levels of benfotiamine and sulbutiamine in both cells and cell media. The precision, defined as the closeness of measurements acquired from multiple sampling at the same concentration, was calculated by assessing the % RSD for repeatability (intra-day precision) and reproducibility (inter-day precision) of the method. The accuracy, determined as the closeness between measured concentrations and nominal concentrations, was acquired by assessing the % RE. According to the FDA requirements, the % RSD and % RE values should be less than 15% for all QC samples and less than 20% for the LLOQ.[23,24] RSD and RE values for benfotiamine and sulbutiamine in cells and cell media are shown in Tables 2 and 3, respectively. The intra-day ($n = 5$) and inter-day ($n = 15$) precision and accuracy for benfotiamine were

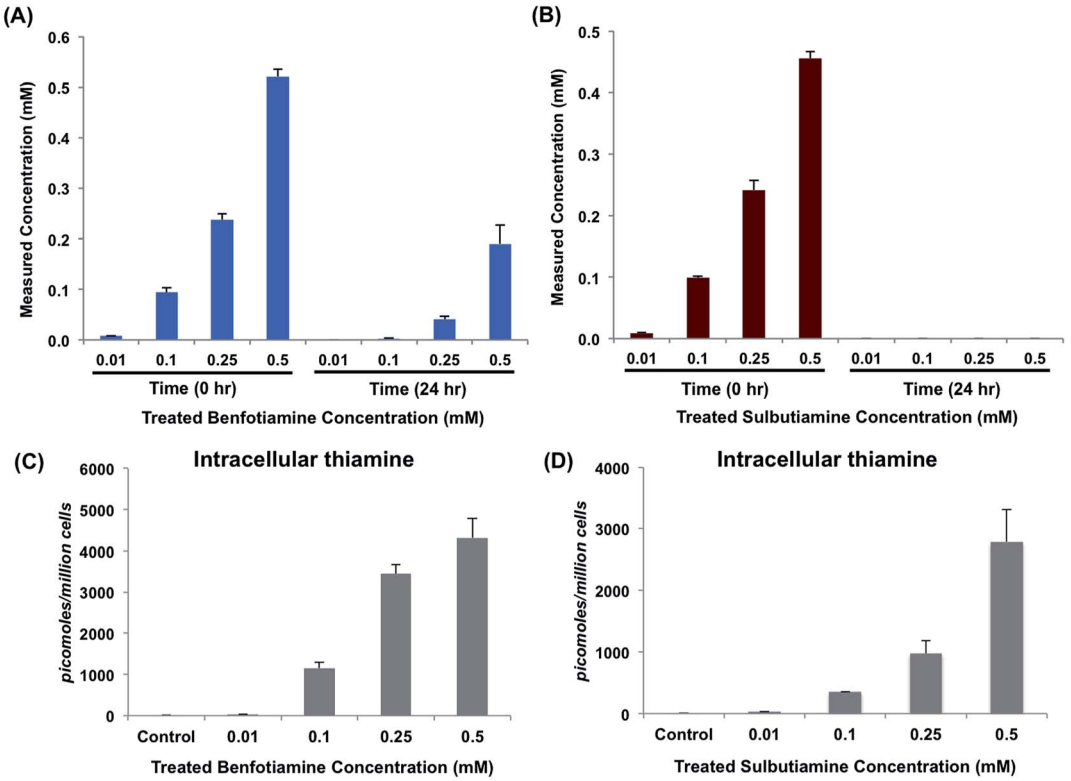

Fig. 4 Determination of benfotiamine (A) and sulbutiamine (B) in media after 24 h treatment with 0.01, 0.1, 0.25 and 0.5 mM of compounds in human colon carcinoma cells (HCT 116). HCT 116 cells were treated with RPMI 1640 containing different concentrations of benfotiamine and sulbutiamine, and the experiment was done in duplicate. After 24 h, fresh cell media (before cell treatment) treated with compounds and the spent media (after cell culture) were prepared and analyzed. The samples were injected into the HPLC and the concentrations of benfotiamine in both media 0 h and 24 h were determined as shown in (A). The results for sulbutiamine are depicted in (B). After 24 h treatment with each compound, intracellular thiamine levels were determined in cells (C and D). The concentrations shown in the graphs are mean ± S.E.M (standard error of mean) values.

determined at three different concentrations: 300 nM (low quality control, QC), 1500 nM (mid-QC) and 40 000 nM (high-QC). The intra-day ($n = 5$) and inter-day ($n = 15$) precision and accuracy for sulbutiamine were evaluated at 1500 nM (LQC), 4000 nM (MQC) and 24 000 nM (HQC). All values met the requirements found in the U.S. FDA Guidance.

3.5. Stability

Table 4 shows the autosampler stability (25 °C, 24 h), bench-top stability (4 °C, 8 h) and freeze–thaw stability (three freeze–thaw cycles, −80 °C, 72 h) data for benfotiamine and sulbutiamine in both cells and cell media. All samples were stable on the autosampler for 24 h at the LQC and HQC levels. The samples were kept in the 4 °C refrigerator for 8 h to test bench-top stability. This simulated all sample preparation processes which were conducted on ice. After 8 h, the compounds were extracted and analyzed. For freeze–thaw stability testing, the samples were frozen at −80 °C overnight and thawed at room temperature. Subsequently, samples were taken through two more freeze–thaw cycles before being analyzed. In media, both benfotiamine and sulbutiamine at the LQC and HQC levels were stable for 8 h at 4 °C and during all three freeze–thaw cycles. On the other hand, both compounds were degraded at the LQC concentration when incubated with cells for the evaluation of bench-top stability (4 °C) and freeze–thaw stability even though they were stable at the HQC. Additionally, it appears as though sulbutiamine under such conditions was degraded to a derivative compound as shown by the chromatograms (data not shown).

3.6. Recovery

Recovery reported as a percentage (%) indicates the extraction efficiency of the analytical process.[23,24] Absolute recovery (AR) and relative recovery (RR) from cells and cell media were evaluated by comparing the peak areas between standard solutions and spiked samples or spiked samples and post-preparation spiked samples at the LQC, MQC and HQC levels. Spiked samples were prepared by adding standard solutions to the biological matrix prior to sample preparation; the post-preparation samples were made by spiking them with standard solutions following sample extraction. Both spiked samples ($n = 3$) in biological matrices and standard solutions ($n = 3$) with equivalent concentrations were prepared at the LQC, MQC, and HQC for benfotiamine and sulbutiamine to evaluate the absolute recovery. Likewise, spiked samples ($n = 3$) in the biological matrices and post-preparation spiked samples ($n = 3$)

at same concentrations were prepared for the evaluation of relative recovery.

Table 5 shows the absolute recovery and relative recovery for the two thiamine analogs from biological matrices at the LQC, MQC, and HQC points. Acquired data for benfotiamine was within the range from 91.95% to 113.62% with an average of 101.93% in cells and cell media. The recovery for sulbutiamine was within the range from 88.04% to 115.05% with an average of 101.84% at three concentration levels in both matrices. These results show the complete recovery of all analytes with excellent efficiency.

3.7. Application of the method

The validated method was applied to biological samples to examine the impact of the two thiamine analogs on intracellular thiamine levels in cancer cells. After treating the cell media with each compound at different concentrations, the levels of benfotiamine and sulbutiamine were determined by harvesting 5 million cells per sample, respectively. The results showed that benfotiamine and sulbutiamine were not detected in the cell samples after 24 h treatment with each compound (Fig. 3C and D). As shown in many studies,[9–12] these analogs easily diffuse across plasma membranes leading to increases in intracellular thiamine levels. Therefore, this result suggested that the thiamine analogs were converted into thiamine after crossing the cell membrane. This conclusion was supported by this work which measured intracellular thiamine levels (Fig. 4C and D) using a previously developed and validated method by Basiri *et al.*[22] in order to confirm the utilization of benfotiamine and sulbutiamine. The cells dosed with higher concentrations of the thiamine analogs contained correspondingly higher amounts of intracellular thiamine (Fig. 4C and D). These results provide valuable insight regarding the behavior of these thiamine analogs in cells. Interestingly, while the levels of benfotiamine decreased in cell media after 24 h following cell treatment (Fig. 4A), Fig. 4B shows that sulbutiamine was not detected in cell media after 24 h. Sulbutiamine, which consists of two modified thiamine molecules (Fig. 1) appears to be transformed into two modified molecules of thiamine. This was confirmed by the fact that these thiamine derivative peaks were detected in both media and cell samples after 24 h treatment with sulbutiamine derivatives that were generated by reducing the disulfide bond in sulbutiamine with dithiothreitol (DTT) (data not shown). Taken together these experiments show that sulbutiamine is reduced under cell culture conditions and the derivatives are further converted into thiamine.

4. Conclusions

A new robust and sensitive HPLC-UV method to determine benfotiamine and sulbutiamine has been developed and fully validated for the first time using cells and cell media as biological matrices in following regulatory guidance. The developed method has good precision, accuracy, and linearity for the determination of benfotiamine within the range from 100–50 000 nM and for sulbutiamine within the range from 500–30 000 nM in cells and cell media. The LLOQ is 100 nM and 500 nM for benfotiamine and sulbutiamine, respectively. Benfotiamine and sulbutiamine performed well as internal standards for each other. The sample preparation method provided for the complete recovery of these compounds. As a result, this method was successfully applied to *in vitro* studies of benfotiamine and sulbutiamine in cancer cells to determine the impact of these thiamine analogs. This study has established a new bioanalytical method to identify the effect of thiamine analogs on cancer cell proliferation.

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