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Quantitation of taurolidine decomposition in polymer solutions by chromotropic acid formaldehyde assay method

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Abstract

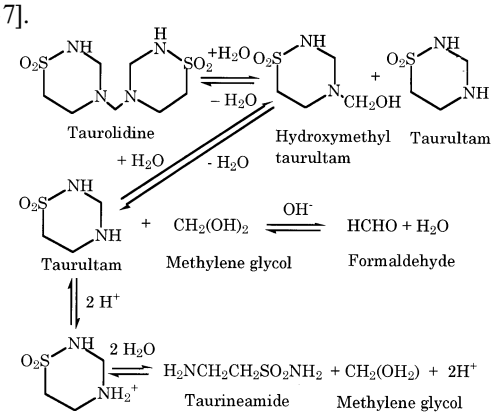
A sensitive, selective, and specific assay was needed to study the degradation kinetics of taurolidine and its stabilization by polyvinylpyrrolidone (PVP). The purpose of the present study was to evaluate the usefulness of the chromotropic acid method and other formaldehyde or amine derivatization methods. The methods evaluated included formaldehyde derivatization with chromotropic acid, acetylacetone, 4-amino-5-hydrazino-3-mercapto-1,2,4-triazole, semicarbazide hydrochloride, or 2,4-dinitrophenylhydrazine and taurolidine decomposition product derivatization with dansylchloride or 7-chloro-4-nitrobenz-2-oxa-1,3-diazole chloride. Results indicated that the chromotropic acid method provided sufficient selectivity, reproducibility and sensitivity. It was able to quench taurultam decomposition and avoided PVP interference. The method was optimized by performance based selection of reagent lots, appropriate reagent storage and preparation, and controlled derivatization conditions. In conclusion, the optimized chromotropic method was the most appropriate method for quantitating taurolidine decomposition. © 1997 Elsevier Science B.V.

Keywords: Formaldehyde; Taurolidine; Taurultam; Taurineamide; Polyvinylpyrrolidone; Chromotropic acid; Derivatization; UV spectrophotometer; HPLC; Taurutam decomposition

1. Introduction

Taurolidine is a broad spectrum, anti-microbial, anti-fungal, and anti-endotoxin agent that acts by releasing formaldehyde [1–3]. Taurolidine is marketed under the brand name Taurolin® and includes 5% polyvinylpyrrolidone (PVP) and 2% taurolidine [2]. PVP reportedly acts as a stabilizer, increasing the solubility of taurolidine and maintaining the free formaldehyde concentration below 0.004% [3,4].

In aqueous solution, taurolidine reversibly decomposes to taurultam, hydroxymethyltaurultam, formaldehyde, and taurineamide as shown below [3,5–7].



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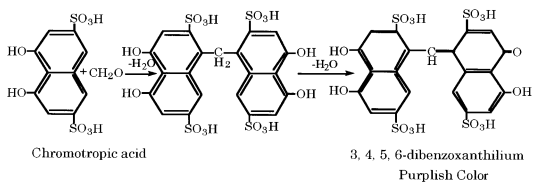
Because none of the substrates or products contained chromophores, derivitization methods were needed to quantitate taurolidine decomposition. Chromotropic acid derivatization was based on the formation of dibenzoxanthilium from formaldehyde and chromotropic acid [8–15].



In addition to this method, six other techniques were investigated, four of which were based on formaldehyde derivatization and two based on derivatization of taurultam and taurineamide. These included

1. an acetylacetone method involving the formation of 3,5-diacetyl-1,4-dihydrolutidine ($\lambda_{max} = 412$ nm) from formaldehyde and acetylacetone in the presence of an excess of ammonium salts [16–20],
2. a 4-amino-5-hydrazino-3-mercapto-1,2,4-triazole (AHMT) method involving the oxidation of the condensation product of formaldehyde and AHMT with potassium periodate to yield a tetrazine derivative [21,22],
3. an 2,4-dinitrophenylhydrazine (DNPH) method [16,23,24] based on the formation of a UV-absorbing hydrazone derivative from the reaction of DNPH and formaldehyde,
4. the derivatization of formaldehyde with semicarbazide hydrochloride to produce semicarbarzone [25,26],
5. the derivatization and HPLC separation of taurineamide and taurultam with 7-chloro-4-nitrobenz-2-oxa-1,3-diazole chloride (NBD-Cl) [27–29], and
6. the dansylation and HPLC separation of taurultam and taurineamide dervizatives [30–33].

2. Experimental

2.1. Materials and equipment

Formaldehyde reagent (37.9% in water containing 10% methanol, Fisher Scientific, Fair Lawn, NJ) was used as the primary standard. The standarization of formaldehyde content was done by iodimetric methods [23]. Dihydroxynaphthalene disulfonic acid (chromotropic acid) was purchased from Sigma and Aldrich. Sulfuric acid, acetylacetone, methanol (HPLC grade), tetrahydrofuran (THF, HPLC grade) and acetonitrile (HPLC grade) were purchased from Fisher Scientific. Glacial acetic acid and semicarbazide were analytical grade and were purchased from Mallinckrodt. 4-Amino-5-hydrazino-3-mercapto-1,2,4-triazole (AHMT) was purchased from Aldrich. Polyvinylpyrrolidone (PVP, molecular weight 10 000), 2,4-dinitrophenylhydrazine (DNPH), 7-chloro-4-nitrobenz-2-oxa-1,3-diazole chloride (NBD-Cl) and 1-dimethylaminonaphthalene-5-sulfonyl chloride (dansyl chloride, Dns-Cl) were purchased from Sigma. All chemicals were reagent grade or better except PVP.

The UV-spectrophotometer employed was a Shimadzu UV-VIS Model 2100 U spectrophotometer. The HPLC system consisted of a Shimadzu LC 6A pump, SIL-6B/9A automatic sample injector, a Shimadzu SPD 6A Spectrophotometric UV detector and Shimadzu 6A integrator.

2.2. Preparation of standard and test solutions

Formaldehyde standards were prepared by diluting 10 ml 37.9% formaldehyde reagent in an 1 l volumetric flask with deionized distilled water. This stock solution was diluted in volumetric flasks to give formaldehyde concentrations in the range 0.0732–2.195 μg ml^{-1}. A 10% methanol solution in water was prepared and diluted in the same way to serve as blank stock solution. The formaldehyde and methanol solutions were used as standards in all formaldehyde detection methods.

Solutions of taurolidine (0.000454, 0.0008, and 0.0012 M) or taurultam (0.0022 and 0.004 M) in water or buffer (pH = 4.0–7.4) were prepared and

allowed to decompose at room temperature for at least 3 days. Formaldehyde solutions (0.0732–2.195 μg ml^{-1}) in PVP and vinylpyrrolidone (1–10%) were prepared at room temperature and stored in a dark room. Taurineamide aqueous solutions (0.75×10^{-4}–0.23×10^{-2} M) were prepared at room temperature and stored in the dark.

2.3. Evaluation of the chromotropic acid method

2.3.1. General methods

The chromotropic acid reagent was prepared by dissolving 1.25 g chromotropic acid in 100 ml of distilled deionized water, then 160 ml concentrated sulfuric acid was added slowly with mixing while the solution was cooled in an ice bath.

4 ml Chromotropic acid reagent and 100 μl taurultam (0.002 or 0.004 M) and taurolidine (0.002 or 0.003 M) samples with and without PVP at pH = 4.0–7.4 or formaldehyde standard solutions were placed in screw-capped test tubes and vortex mixed for 10 s. Standard and sample tubes were derivatized at 90°C for precisely 30 min. After derivatization, the samples were cooled for 10 min in an ice bath and then stirred with a vortex mixer for 10 s. Absorbance was measured at 572 nm against the diluted methanol blank.

2.3.2. Effect of chromotropic acid storage conditions and preparation on formaldehyde derivatization analysis

Derivatization of formaldehyde (0.7452 μg ml^{-1}) was carried out in 0.2, 0.32, 0.5, 0.72, and 1.0% chromotropic acid in 75% sulfuric acid in order to evaluate chromotropic acid concentration effects.

Formaldehyde (0.0732–2.195 μg ml^{-1}) derivatization was conducted with three different lots of chromotropic acid (two lots from Sigma and one from Aldrich). The linearity and sensitivity (slope:S.D.) of the calibration curves were compared. The effects of chromotropic acid reagent storage were evaluated by storing chromotropic acid reagent in a dark cabinet at room temperature for 11 days, in a desiccator at room temperature for 5 days, and in a 100°C oven for 2 days. Then the reagents were used to derivatize formaldehyde solutions (0.0732–2.195 μg ml^{-1}). Again, linearity and sensitivity of the resultant calibration curves were compared. The effect of chromotropic acid preparation on formaldehyde analysis was evaluated by comparing calibration curves generated from chromotropic acid solutions that were prepared by dissolving chromotropic acid in sulfuric acid followed by dilution in water to those prepared by dissolving chromotropic acid in water and then diluting in sulfuric acid. The stability of solutions of chromotropic acid–formaldehyde derivatives prepared with 0.5% chromotropic acid reagent and 0.0745 μg ml^{-1} formaldehyde was assessed by measuring the absorbance of samples stored under ambient conditions for 5 days. The effect of storing 0.5% chromotropic acid reagent solution at room temperature on formaldehyde (0.0745 μg ml^{-1}) derivatization was evaluated by measuring the absorbance of chromotropic acid–formaldehyde derivative solution as a function of reagent storage time.

2.3.3. Effect of derivatization conditions on formaldehyde analysis

Derivatization of formaldehyde (1.1316 μg ml^{-1}) with chromotropic acid 0.5% in 75% sulfuric acid was conducted at 70, 90 and 100°C for 5–358 min. The derivatization kinetics were evaluated by comparing absorbance time profiles at each temperature. The effects of sample volume (50, 100, 200 μl) and pH (4.02, 5.03, 6.05, 7.04) on chromotropic acid derivatization were evaluated by comparing the absorbances of various formaldehyde samples (75.44 μg) derivatized at 90°C for precisely 30 min in 0.5% chromotropic acid–sulfuric acid solution.

2.4. Evaluation of the alternate derivitization methods

2.4.1. Acetylacetone methods

100 ml Samples (0.000454, 0.0008 and 0.0012 M taurolidine and 0.00217 M taurultam aqueous solutions or formaldehyde standard solutions) were added to 4.0 ml of a solution containing 0.02 M acetylacetone, 0.05 M acetic acid, and 2.0 M ammonium acetate in screw-capped tubes. Reaction mixtures were hand mixed and placed in a

60°C bath for 10 min. Absorbance was measured at 412 nm.

2.4.2. AHMT method

1 ml 2.0 N NaOH was placed in 10 ml screw-capped test tubes and 50 μl samples (formaldehyde standard solutions, 0.004 M taurultam aqueous solutions at pH 4.0–7.4, or 1–10% PVP or 1–10% vinylpyrrolidone aqueous solutions) were added along with 1.0 ml 1.0% AHMT in 0.5 N HCl. After 30 min, 1 ml 0.5% potassium periodate solution in 0.2 N NaOH was added and mixed followed by 2 ml 0.2% sodium borohydride in 1.0 N NaOH. The solutions were analyzed at 550 nm.

2.4.3. DNPH derivatization method

100 ml Samples (taurultam or formaldehyde solutions) were added to 0.5 ml 0.005 M 2,4-DNPH solution in 2.0 N HCl. The derivitization was quenched by adding 0.4 ml 0.13 M phosphate buffer (pH = 6.8), 0.7 ml 1.0 N sodium hydroxide solution, and 1.0 ml THF–water mixture (9:1). Quantitation was carried out by HPLC using a Waters Novapak® C_{18} column (4 μm, 3.9 mm × 150 mm i.d.) with acetonitrile–water (1:1) as mobile phase at a flow rate of 1.0 ml min^{-1} and UV detection at 345 nm.

2.4.4. Semicarbazide method

5 μl samples (0.122 M taurultam, 0.0644 M taurolidine or 0.134 M formaldehyde) were added to 3 ml 0.01 M semicarbazide solution in 0.05 M phosphate buffer (pH = 7.4) in a UV-quartz cuvette and mixed. The absorbance was measured at 235 nm.

2.4.5. NBD-Cl derivatization method

1 ml NBD-Cl reagent was mixed with 1 ml 0.75×10^{-4}–0.23×10^{-2} M taurineamide or 0.0022 M taurultam aqueous solution and heated for 5 min at 90°C. The derivitization was quenched in an ice bath and the derivatives were analyzed by HPLC on a Micropak-MCH 10 column (ODS, 30 mm × 4.0 mm i.d., 10 μm, Varian) at 465 nm using acetonitrile–water (55:45) mobile phase at a flow-rate of 1.0 ml min^{-1}.

2.4.6. Dansylation method

100 μl 0.75×10^{-4}–0.23×10^{-2} M taurineamide or 0.0022 M taurultam aqueous solution was mixed with 1.0 ml dansyl chloride reagent in 0.025 M borate buffer (pH = 9.0) at 40°C. After 30 min the reaction was quenched in an ice bath, and the derivatives were analyzed by HPLC on a Micropak-MCH 10 column (ODS, 30 mm × 4.0 mm i.d., 10 μm, Varian) at 254 nm using acetonitrile–water (55:45) mobile phase.

3. Results

3.1. Evaluation of the chromotropic acid method

3.1.1. Sensitivity and recovery

Analysis of formaldehyde by the chromotropic acid derivatization method resulted in linear calibration curves in the concentration range 0.0988–1.972 μg ml^{-1} (Fig. 1). The detection limit of the assay was 0.0741 μg ml^{-1} in the sample (signal-to-noise ratio 2) which corresponded to 2.5×10^{-6} M taurultam in solution. The ruggedness of this method was demonstrated by a mean recovery of 101% for daily repetitive analysis of a 0.741 μg ml^{-1} formaldehyde standard solution over 6 days. The coefficient of variation (C.V.) was 1.73%.

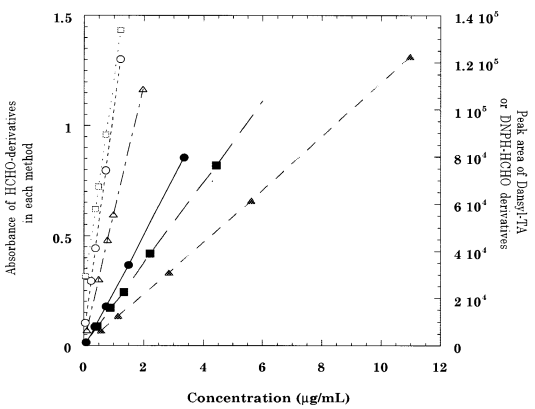


Fig. 1. Calibration curves for formaldehyde using AHMT in the presence (□) or absence (○) of 3% PVP, chromotropic acid (△), acetylacetone (●) and DNPH (■). Taurineamide calibration curve with dansyl chloride (▲).

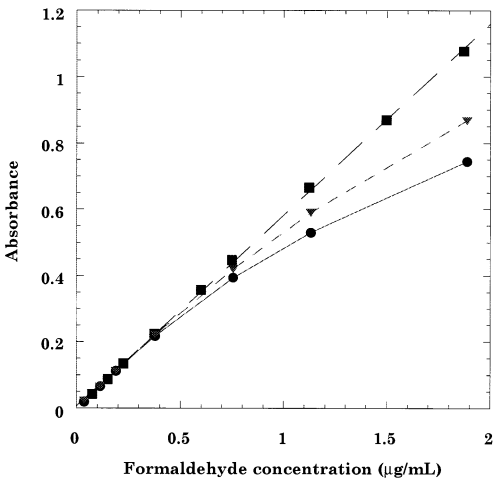


Fig. 2. Calibration curves resulting from the use of various chromotropic acid lots: Lot 1 (●,○), Lot 2 (■) and Lot 3 (▼).

The recovery of formaldehyde (1.132 μg ml^{-1}) from PVP solutions (0–17%) was $100 \pm 2.5\%$ and the slopes of formaldehyde calibration curves in the presence and absence of 3% PVP were 0.59 absorbance (μg $ml^{-1})^{-1}$ ($R^2 = 0.99985$) and 0.6 absorbance (μg $ml^{-1})^{-1}$ ($R^2 = 0.99997$), respectively. $A2 \times 10^{-5}$ M concentration change for taurolidine or taurultam corresponded to an 0.0223 absorbance (μg $ml^{-1})^{-1}$ change which was well within the discriminatory capability of the method. Thus the chromotropic acid method was suitably sensitive and specific.

Formaldehyde–chromotropic acid derivatives were stable for at least 5 days at room temperature. The absence of complete conversion of taurultam or taurolidine to formaldehyde during or after derivatization suggested that decomposition was quenched.

3.1.2. Effect of chromotropic acid storage conditions and preparation on formaldehyde derivatization analysis

The absorbances resulting from derivatization of formaldehyde (0.7452 μg ml^{-1}) with 0.2, 0.32, 0.5, 0.72, or 1.0% chromotropic acid in 75% sulfuric acid were equivalent (0.351, 0.354, 0.357, 0.347, and 0.340, respectively).

Formaldehyde calibration curves were dependent on the chromotropic acid lot (Fig. 2). For example, the calibration curve from two lots were linear with squared correlation coefficients greater than 0.9996, however for a third lot, the curve was distinctly nonlinear ($R^2 < 0.967$), and a nonzero intercept was obtained (0.06145). Furthermore the slope was about 50% less than that obtained for other lots. Thus, chromotropic acid lots were deemed unsuitable if calibration curve nonlinearity was observed.

The linearity of formaldehyde–chromotropic acid calibration curves was maintained when the chromotropic acid reagent was stored in a dark cabinet at room temperature for 11 days prior to use, but decreased after storage in a desiccator at room temperature for 5 days, and additional nonlinearity was observed for reagent stored in a 100°C oven for 2 days (Fig. 3).

Formaldehyde calibration curves showed some nonlinearity when the chromotropic acid reagent solution was stored under ambient conditions for 1 day prior to derivatization. Thus, fresh chromotropic acid reagent solution was prepared on the day of use.

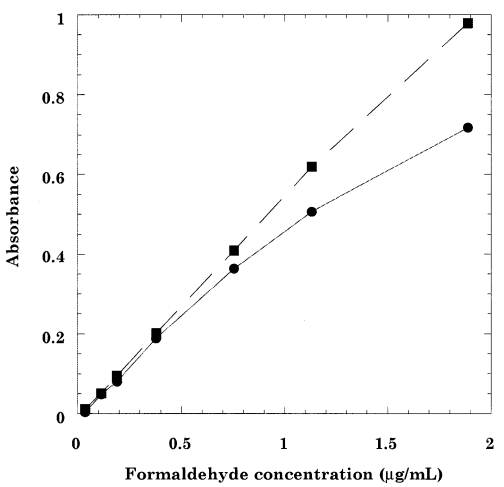


Fig. 3. The effect of chromotropic acid storage on chromotropic acid derivatization with formaldehyde. Reagent stored in the dark at room temperature (●), in a desiccator at room temperature (▼) and in a drying oven at 100°C for 2 h (■).

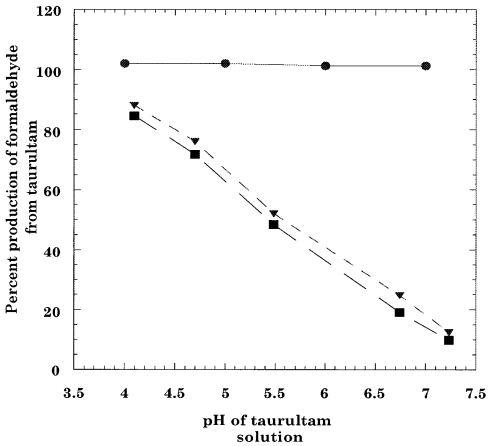


Fig. 4. The effect of pH on the apparent formaldehyde concentrations in 0.004 M taurultam solutions at room temperature using acetylacetone (●), AHMT (▼) and chromotropic acid (■) methods.

The order of addition in chromotropic acid reagent solution preparation affected the linearity of the calibration curve. For example, nonlinearity was observed when the reagent was dissolved in sulfuric acid and then diluted with water, whereas linear calibration curves were observed when the reagent solution was prepared by dissolving the reagent in water followed by dilution with concentrated sulfuric acid.

3.1.3. Effect of derivatization conditions on formaldehyde analysis

Formaldehyde derivatization at 70, 90 and 100°C was completed after 130, 30 and 20 min, respectively. Thus a 30 min derivitization at 90°C was appropriate.

The absorbance of 0.745 μg formaldehyde decreased by about 10% when a 100 μl sample volume was used versus 50 μl. No absorbance differences were observed when sample pH was varied (pH = 4.02, 5.03, 6.05, or 7.04).

3.2. Assessment of alternate derivitization methods

Analysis of formaldehyde by the acetylacetone method resulted in a linear calibration curve (Fig. 1); however, taurultam solutions completely degraded to formaldehyde (Fig. 4). Thus, this method apparently failed to quench taurultam decomposition.

The AHMT method resulted in a linear calibration curve for formaldehyde concentration from 0.096 to 2.404 μg ml^{-1} (Fig. 1). When derivatization was conducted in PVP solutions (1–10%) without formaldehyde, the absorbance increased from 0.15 to 1.3 with PVP concentration. When the derivatization reaction was conducted in the presence of 1-vinyl-2-pyrrolidone (the PVP subunit), no absorbance was observed. Thus PVP impurities might be responsible for background absorption.

The DNPH method of formaldehyde derivatization resulted in linear calibration curves for formaldehyde standards (0.5–10 μg ml^{-1}, Fig. 1), however the DNPH–formaldehyde derivatives degraded with a 1 h half-life at neutral pH under ambient conditions. Furthermore, DNPH derivatization failed to quench taurultam decomposition.

The analysis of formaldehyde in taurultam or taurolidine solutions by the semicarbazide method resulted in measured formaldehyde concentrations that were 100 or 300% of the initial concentrations of taurultam and taurolidine, respectively. These results suggest complete conversion of taurultam or taurolidine to formaldehyde during derivitization.

Analysis of taurineamide and taurultam by the NBD-Cl derivatization method failed because the NBD–taurineamide and NBD–taurultam derivatives could not be separated.

Analysis of taurineamide and taurultam by dansylation methods had two disadvantages: taurultam decomposition was not quenched and the apparent extent of dansylation was affected by the pH of the derivatized sample solution.

4. Discussion

Analytical methods for characterizing taurolidine decomposition involved derivatization and detection of formaldehyde, taurultam and/or taurineamide. Method utility depended on meeting four criteria: (1) a degree of sensitivity consistent

with the ability to discriminate decomposition reactant concentration changes of about 2×10^{-5} M; (2) specificity (selectivity) that is consistent with the ability to measure taurolidine, taurultam, taurineamide and/or formaldehyde in the presence of the related compounds and buffers (e.g. acetate, phosphate), PVP, vinylpyrrolidone (PVP monomer), PVP impurities (e.g. acetaldehyde), and polyethylene glycol (PEG); (3) the lack of instability during analysis due to either the decomposition of the analyte under the analysis conditions or the failure of these conditions to quench taurolidine or taurultam decomposition; and (4) precision that is consistent with a coefficient of variation of 2% for repeated analyses.

The colorimetric measurement of derivatized formaldehyde with chromotropic acid has been previously reported as a simple and sensitive derivatization method for formaldehyde detection. Although it might not be suitable for bioanalytical purposes, this method met the criteria for studying taurolidine decomposition in aqueous solutions in the presence of potential formulation and buffer components. Our results on derivatization time and temperature are consistent with previous reports [26,28,29]. We have found the quality of chromotropic reagent to vary somewhat with reagent lot and storage. The order of solvent addition was found to be critical for successful derivatization. However the sample pH, volume, age and the presence for extraneous sample components (e.g. PVP, PEG, buffer salts, etc) did not affect the assay.

None of the alternate analysis methods met all four of these criteria. The acetylacetone, DNPH, semicarbazide and dansylation methods failed to quench taurolidine and/or taurultam decomposition. AHMT and the semicarbazide methods were affected by the presence of PVP. The NBD-Cl method failed to distinguish taurineamide and taurultam.

Some of these analytical methods have been reported for measuring taurolidine decomposition products in biological fluid and aqueous solutions. The acetylacetone method was found to detect the equivalent of 3 molecules of formaldehyde per molecule of taurolidine [31]. Thus, this method is a measure of the total formaldehyde residues in taurolidine and the derivitization conditions failed to quench the taurolidine decomposition.

Dansylation methods have been reported to measure taurultam and taurineamide in plasma and blood samples using various separation columns (Micropak MCH 10 [31] or 5 mm silica Hypersil column [32]). The instability of dansyl–taurultam and dansyl–taurineamide observed herein has not been previously reported.

The NBD-Cl derivatization method was also reported for taurultam in plasma and aqueous solutions [27]. Surprisingly, taurineamide was not observed to react with NBD-Cl. These results were in contrast to our observation of taurineamide derivatization and the inability to separate taurineamide and taurultam derivatives. Furthermore, NBD-Cl has been known to react with primary amines [28,29]

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