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HPLC purification and separation of 5,5′-substituted-2,4-imidazolidinedithiones

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Abstract

Several 5,5′-substituted-2,4-imidazolidinedithiones, synthesized from aldehydes or ketones, have been purified by HPLC using poly(styrene–divinylbenzene) packings. Purified 5,5′-substituted-2,4-imidazolidinedithiones have been identified in column effluent by UV absorbance and corroborated by mass spectrometry. Several silica-based, polymeric, and poly(styrene–divinylbenzene)-based packings were evaluated as matrices for resolution of a mixture of purified 5,5′-substituted-2,4-imidazolidinedithiones.

1. Introduction

The 5,5′ - substituted - 2,4 - imidazolidinedithiones (2,4-dithiohydantoins) have proven to be of interest in several areas. These compounds are analogues of various drugs, such as phenytoin (5,5′-diphenyl-2,4-imidazolidinedione) [1], and have been evaluated as potential therapeutics [2]. As a synthetic tool, 5,5′-substituted-2,4-imidazolidinedithiones have been used to assist the curing of halogenated polymers [3].

Synthesis of 5,5′-substituted-2,4-imidazolidinedithiones from ketones was initially reported by Carrington [4]. This synthesis was a modification of that method developed by Bucherer and Steiner [5] for synthesis of imidazolidinediones (hydantoins) from ketones, sodium cyanide, and ammonium carbonate. Carrington observed that replacement of sodium cyanide with ammonium cyanide and replacement of ammonium carbonate with carbon disulfide lead to the synthesis of 5,5′-substituted-2,4-imidazolidinedithiones. Using this synthetic approach, many 5,5′-substituted-2,4-imidazolidinedithiones have been made from ketones [4] and aldehydes [6].

While numerous papers report on the synthesis and characterization of these compounds [4,6–8], no methods have been published for their HPLC purification. Our need for reference materials necessitated the development of an HPLC method for the purification of 5,5′-substituted-2,4-imidazolidinedithiones. The reported molar absorptivity, on the order of 27 000 at 296 nm [8], made these compounds suitable candidates for UV detection after HPLC separation. This paper reports on methods for both preparative and analytical HPLC purification of 5,5′-substituted-2,4-imidazolidinedithiones. Verification of 5,5′-substituted-2,4-imidazolidinedithiones in HPLC fractions by ^{252}Cf plasma de-

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sorption time-of-flight mass spectrometry is also described. Additionally, a mixture was formulated from the purified 5,5′-substituted-2,4-imidazolidinedithiones and then used to assess the ability of several different column packings to resolve the mixture.

2. Experimental

2.1. Materials

Aldehydes (acetaldehyde, 2-methylbutyraldehyde, 2-ethylbutyraldehyde, octyl aldehyde), ketones (acetone, 2-butanone), and other chemicals were purchased from Aldrich (Milwaukee, WI, USA) in the highest purity available. All solvents were HPLC grade. Chromatography was conducted on a Hewlett-Packard (Avondale, PA, USA) 1050 HPLC system with autosampler and variable wavelength detector. Semi-preparative (30.5 × 0.7 cm) and analytical (15 × 0.41 cm) 10-μm poly(styrene–divinylbenzene) PRP-1 columns were obtained from Hamilton (Reno, NV, USA). An analytical (15 × 0.46 cm) 3-μm polymeric C_{18} column was purchased from Interaction (San Jose, CA, USA). Analytical Cyano (20 × 0.46 cm) 5-μm, Amino (22 × 0.46 cm) 5-μm, and Velosep C_{18} (10 × 0.32 cm) 3-μm silica columns were purchased from Brownlee (San Jose, CA, USA).

2.2. Synthesis of 5,5′-substituted-2,4-imidazolidinedithiones

The 5,5′ - substituted - 2,4 - imidazolidinedithiones were synthesized from their respective aldehydes or ketones following that chemistry described by Carrington [4]. The proposed reaction scheme [8] is illustrated in Fig. 1. Equimolar amounts of the selected aldehyde or ketone, sodium cyanide, ammonium sulfate, and carbon disulfide were added to a reaction vessel and covered with an excess of ethylene glycol dimethyl ether (glyme)/1,1,2-trichlorotrifluoroethane (1:2, v/v). This mixture was refluxed overnight with mixing. The solvent was removed from the reaction mixture in a rotary evaporator. Three washes with methyl formate, followed by drying, removed excess cyanide. The reaction mixture was then suspended in methanol and filtered; the 5,5′-substituted-2,4-imidazolidinedithione was precipitated upon addition of an excess of 10 *M* HCl. The 5,5′-substituted-2,4-imidazolidinedithione was then isolated by vacuum filtration and recrystallized from methanol.

2.3. Purification of 5,5′-substituted-2,4-imidazolidinedithiones

Initial purification of each 5,5′-substituted-2,4-imidazolidinedithione was achieved using a Hamilton 10 μm PRP-1 column (30.5 × 0.7 cm). Solvent A was methanol–glyme–water (3:3:44, v/v/v) and solvent B was methanol–glyme–water–acetonitrile (5:6:2:37, v/v/v/v). The elution program was: 100% A for 10 min, 0–100% B from 10 to 80 min, 100% B from 80 to 90 min, returning to 100% A from 90 to 120 min. Samples for injection were prepared by suspending recrystallized 5,5′-substituted-2,4-imidazolidinedithione in methanol such that a 1:100 dilution of the resulting solution gave an absorbance at 296 nm of 0.2 to 0.3 AU. The

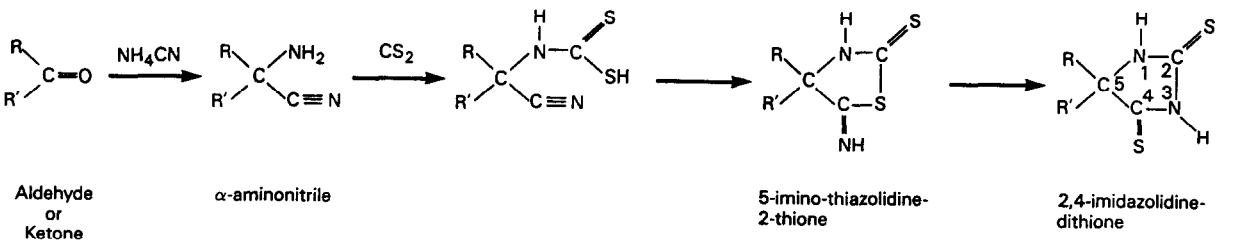

Fig. 1. Reaction scheme for synthesis of 5,5′-substituted-2,4-imidazolidinedithiones.

column was maintained at 40°C; the effluent was monitored at 296 ± 2 nm; the injection volume was 500 μl; the flow rate was 1 ml/min; and V_0 was 4 ml. Fractions were collected at 1-min intervals with a Gilson 201 fraction collector (Middleton, WI, USA). The 5,5′-substituted-2,4-imidazolidinedithione was initially identified by its absorbance at 296 nm and corroborated by mass spectrometry.

Those fractions containing the 5,5′-substituted-2,4-imidazolidinedithione were pooled, dried *in vacuo*, and further purified using a Hamilton 10-μm, PRP-1 column (15 × 0.41 cm). Solvent A was methanol–glyme–acetonitrile–water (2:3:4:41, v/v/v/v) and solvent B was methanol–glyme–acetonitrile–water (3.4:4.1:16.1:26.4, v/v/v/v). The elution program was: 100% A for 5 min, 0–100% B from 5 to 60 min, 100% B from 50 to 60 min, return to 100% A from 60 to 70 min, and equilibrate at 100% A from 70 to 80 min. Samples for injection were prepared by suspending the dried fractions in methanol such that a 1:10 dilution of the resulting solution gave an absorbance at 296 nm of 0.1 to 0.2 AU. The column was maintained at 40°C; the effluent was monitored at 296 ± 2 nm; the injection volume was 10 μl; the flow rate was 0.5 ml/min; and V_0 was 2 ml. Fractions were collected every minute; the 5,5′-substituted-2,4-imidazolidinedithione was identified by its absorbance at 296 nm and corroborated by mass spectrometry.

2.4. Mass spectrometry

Fractions collected from HPLC effluent, which potentially contained 5,5′-substituted-2,4-imidazolidinedithiones based on their absorbance at 296 nm, were pooled, dried *in vacuo*, and suspended in methanol. As noted by Edward [8], 5,5′-substituted-2,4-imidazolidinedithiones are oxidizable, high melting anions. Based on this information we chose to screen the UV absorbing fractions for the presence of 5,5′-substituted-2,4-imidazolidinedithiones using a ^{252}Cf plasma desorption time-of-flight mass spectrometer (Foster City, CA, USA) under negative ionization. A portion (1%) of each pool was applied to an aluminum–mylar foil and allowed to dry. The foils were then loaded into the mass spectrometer. Data were acquired for 1 000 000 fissions at −10 kV acceleration potential.

3. Results

3.1. Purification of 5,5′-substituted-2,4-imidazolidinedithiones

The 5,5′-substituted-2,4-imidazolidinedithiones were synthesized following that chemistry outlined in Fig. 1. For each 5,5′-substituted-2,4-imidazolidinedithione, the starting aldehyde or ketone and the substituents at the 5- and 5′-positions are listed in Table 1. The 5,5′-substituted-2,4-imidazolidinedithiones were purified initially by recrystallization from methanol. These crystals were washed with water, dried, and resuspended in methanol.

Next, each 5,5′-substituted-2,4-imidazolidinedithione was purified by semi-preparative HPLC. A representative chromatogram for this purification of a 5,5′-substituted-2,4-imidazolidinedithione after crystallization is presented in Fig. 2A. For this example the starting material was acetone, which reacted with the ammonium cyanide and carbon disulfide to produce 5,5′-dimethyl-2,4-imidazolidinedithione. The absorbance profile, monitored at 296 nm, indicates some column fall-through prior to the initiation of the gradient. Within the gradient, three peaks (identified as peak I, II, and III, respectively) eluted from the column which could correspond to the desired product. For each peak, the corresponding fractions were pooled and sampled for evaluation by mass spectrometry.

Mass spectrometric evaluation of the three peaks was conducted to corroborate the UV absorbance data and aid in selection of that fraction which would be purified further. Of the three fractions evaluated, peak III was the only one found to contain the desired analyte (Fig. 2B). Evident in the mass spectrum is an ion intensity at m/z 159, consistent with loss of a proton from the analyte, corresponding to

Table 1
5,5′-substituted-2,4-imidazolidinedithiones synthesized from aldehydes or ketones

Derivative	Starting material	R groups[a]		Molecular mass
		5	5′	
C1	Acetaldehyde	CH_3–	H–	146
C2	Acetone	CH_3–	CH_3–	160
C3	2-Butanone	CH_3–	CH_3–CH_2–	174
C4	2-Methylbutyraldehyde	CH_3–CH_2–$\underset{\displaystyle CH_3-CH_2}{\overset{\displaystyle CH_3}{\overset{\vert}{\underset{\vert}{CH}}}}$–	H–	188
C5	2-Ethylbutyraldehyde	CH_3–CH_2–$\underset{\displaystyle CH_3-CH_2}{\overset{}{\underset{\vert}{CH}}}$–	H–	202
C7	Octyl aldehyde	CH_3–$(CH_2)_6$–	H–	230

[a] See Fig. 1 for structure of 5,5′-substituted-2,4-imidazolidinedithione.

$(M-H)^-$ (Table 1). Also evident in the mass spectrum are several intensities which correspond to unknown products of the reaction.

The fraction containing the 5,5′-dimethyl-2,4-imidazolidinedithione was further purified by analytical HPLC chromatography, as summarized in Fig. 3A. Evident in the UV tracing, monitored at 296 nm, are peaks at the initiation of the gradient which correspond to the column fall-through. Additionally, material was observed to elute from the column at 20 min. This material was collected for evaluation by mass spectrometry. Comparison of this HPLC profile to that recorded for the separation of the crude reaction mixture (Fig. 2A) shows that there has been a reduction in the complexity of the mixture.

Mass spectrometry of this material, as shown in Fig. 3B, revealed the most intense ion in the mass spectrum to be m/z 159, consistent with $(M-H)^-$ (Table 1). Also evident in the mass spectrum was an ion intensity at m/z 143, which is consistent with $(M-H)^-$ for either a 5,5′-substituted-2-, or a 5,5′-substituted-4-imidazolidinethione. Comparison of this mass spectrum to that obtained at the previous HPLC step, Fig. 2B, indicates that there has been an overall reduction in noise with a corresponding enhancement in signal for the analyte.

For each aldehyde or ketone listed in Table 1, the synthesis, purification, and identification of the corresponding 5,5′-substituted-2,4-imidazolidinedithione were conducted in a manner similar to that described above for the acetone derivative.

3.2. Mixture analysis

A mixture was formulated which contained each of the 5,5′-substituted-2,4-imidazolidinedithiones. Separation of this mixture of 5,5′-substituted-2,4-imidazolidinedithiones on a poly(styrene–divinylbenzene)-based packing is illustrated in Fig. 4. Initiation of the gradient coincides with column fall-through. After this fall-through, the 5,5′-substituted-2,4-imidazolidinedithiones elute from the column in order of increasing aliphatic nature, reflecting the number of carbons at the 5- and 5′- positions. With the exception of a fused doublet for C1 and C2, each component is baseline resolved.

Using this mixture, a variety of column packings, under identical reversed-phase gradient and solvent conditions, were studied to identify that one best suited for the separation of 5,5′-substituted-2,4-imidazolidinedithiones. Analysis of these data, summarized in Table 2, indicates that the polymer-based packings are better at retaining the 5,5′-substituted-2,4-imidazolidinedithion-

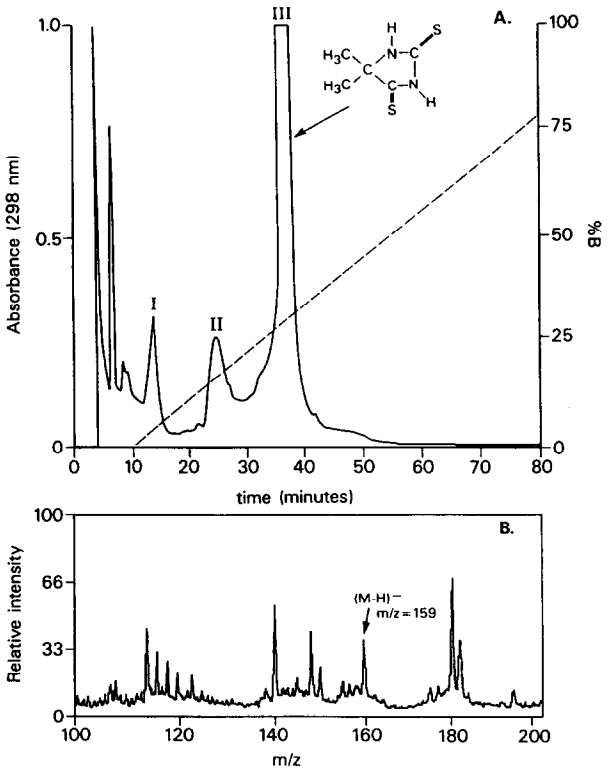

Fig. 2. Separation and characterization of recrystallized 5,5′-dimethyl-2,4-imidazolidinedithione. (A) Chromatogram from semi-preparative purification of 20 μg of 5,5′-dimethyl-2,4-imidazolidinedithione. Stationary and mobile phases, and chromatographic conditions are described in Experimental section. Indicated peaks were sampled for analysis by mass spectrometry. (B) Mass spectrum of HPLC peak III. The ion at m/z 159 is consistent with $(M-H)^-$, loss of a proton from the analyte.

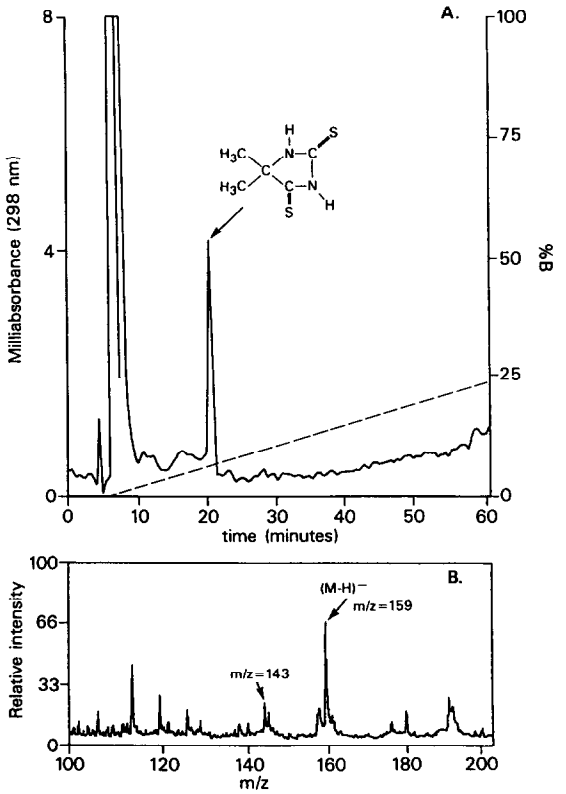

Fig. 3. Separation and characterization of purified 5,5′-dimethyl-2,4-imidazolidinedithione. (A) Chromatogram from analytical purification of 90 ng of 5,5′-dimethyl-2,4-imidazolidinedithione obtained from semi-preparative purification. Indicated peak was sampled for analysis by mass spectrometry. Stationary and mobile phases, and chromatographic conditions are described in Experimental section. (B) Mass spectrum of HPLC peak. The ion at m/z 159 is consistent with $(M-H)^-$, loss of a proton from 5,5′-dimethyl-2,4-imidazolidinedithione; the ion at m/z 143 is consistent with $(M-H)^-$ for a 5,5′-dimethyl-2- or a 5,5′-dimethyl-4-imidazolidinethione.

es than the silica-based packings. Examination of the data from the silica-based packings also indicates that, as the retentive strength of the bonded phase decreases [9], the retention of the more aliphatic 5,5′-substituted-2,4-imidazolidinedithiones also decreases. This trend is not as pronounced for the polymer-based packings. For the solvent and gradient conditions employed, the best peak shapes were obtained from the PRP-1 poly(styrene–divinylbenzene) packing; the other polymeric packing exhibited notable peak broadening and trailing. Based on analysis of these data, the PRP-1 packing appears to be best for separation of a mixture of 5,5′-substituted-2,4-imidazolidinedithiones.

4. Discussion

The available literature indicates that little has been reported about the chromatographic purification of 5,5′-substituted-2,4-imidazolidinedithiones. Carrington, in his initial studies [4], had

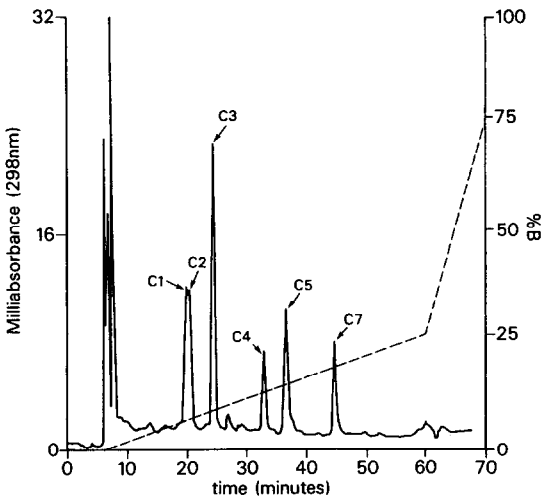

Fig. 4. Separation of a mixture of 5,5′-substituted-2,4-imidazolidinedithiones (250 ng C1, 275 ng C2, 575 ng C3, 200 ng C4, 400 ng C5, and 275 ng C7). Stationary phase: Hamilton, 10 μm, PRP-1 column (0.41 × 15 cm). Solvent A: methanol–glyme–acetonitrile–water (2:3:4:41, v/v/v/v), solvent B: methanol–glyme–acetonitrile–water (3.4:4.1:16.1:26.4, v/v/v/v).

explored the use of carbon as a means for removing contaminants from the derivatives, only to abandon this approach in later studies in favor of recrystallization from methanol [4]. Chubb and Edward [10] attempted to isolate the imine precursor of these compounds from a reaction mixture by chromatography on Florisil. This isolation attempt failed and the failure was attributed to the possibility that the precursor rearranged to form the product on the Florisil. These attempts at purifying or isolating compounds from the reaction mixture suggested that some type of phase chromatography might be useful for the purification of 5,5′-substituted-2,4-imidazolidinedithiones. Our attempts to develop normal-phase HPLC chromatographic methods for the purification of the derivatives from the reaction mixture, following the guidelines described by Snyder *et al.* [11,12], were unsuccessful at either purifying the derivatives or resolving a mixture of the derivatives (unpublished data).

Even though a practical method for the normal-phase HPLC purification and separation of 5,5′-substituted-2,4-imidazolidinedithiones was not found, some important information about the solubility of the derivatives was obtained. The derivatives were observed to be soluble primarily in alcohols, non-aqueous organic acids, organic bases, and ethers. Attempts to mix various other solvents (*i.e.*, water or non-polar compounds) with solutions of the derivatives resulted in precipitation of the derivatives. The optimum solubility of the derivatives was empiri-

Table 2
Adjusted retention volumes for 5,5′-substituted-2,4-imidazolidinedithiones separated from a mixture using different HPLC column packings

Derivative[a]	Retention volume, V'_R (ml)[b]				
	Si-C_{18} [c]	PS-C_{18} [d]	Si-CN [e]	Si-NH_2 [f]	PS [g]
C1	1.2	6.6	2.8	1.3	6.3
C2	1.2	7.8	2.8	1.4	6.9
C3	1.8	10.1	3.5	1.5	8.9
C4	5.0	12.6	5.5	1.6	13.4
C5	7.3	14.6	7.3	1.7	15.0
C7	12.6	20.8	9.8	1.9	18.7

[a] See Table 1 for definition of derivative code.
[b] Solvents and gradient, held constant for all columns, as described in Experimental.
[c] Brownlee Velosep C_{18} 3 μm, 100 × 3.2 mm.
[d] Interaction Polymeric ACT-1 3 μm, 150 × 4.6 mm.
[e] Brownlee Cyano 5 μm, 200 × 4.6 mm.
[f] Brownlee Spheri-5 amino 5 μm, 220 × 4.6 mm.
[g] Hamilton poly(styrene–divinylbenzene) PRP-1 10 μm, 150 × 4.1 mm.

cally observed to lie within a narrow range of polarity. Based on these observations, attempts were made to develop a reversed-phase HPLC method for the purification of these derivatives.

Initially, reversed-phase separations were hindered by the tendency of the derivatives to precipitate as solvent polarity increased. Using solvents with a polarity such that the derivatives remained soluble, the less aliphatic derivatives were observed to fall through silica packings but retain on polymer packings. Based on this observation, the initial solvent composition was optimized such that the C2 derivative was retained on a semi-preparative, poly(styrene–divinylbenzene)-packed HPLC column. After optimization of the initial solvent, the C7 derivative was used as a reference analyte for formulating a solvent that, under gradient conditions, eluted the more aliphatic derivatives from the poly(styrene–divinylbenzene) packing. These solvents and gradient conditions were used for the semi-preparative purification of 5,5′-substituted-2,4-imidazolidinedithiones. Slight modifications of the solvents and gradient were made to optimize the separation obtained from the analytical, poly(styrene–divinylbenzene)-packed HPLC column.

Development of an HPLC method for purification and identification of 5,5′-substituted-2,4-imidazolidinedithiones was facilitated by the use of mass spectrometry. The 5,5′-substituted-2,4-imidazolidinedithiones exhibit maximum molar absorptivities at 296 nm [8]. The 5,5′-substituted-2- or 5,5′-substituted-4-imidazolidinethiones, reaction side products, exhibit maximum molar absorptivities at 264 nm [8]. High concentrations of 5,5′-substituted-2- or 4-imidazolidinethiones in the crude reaction product can contribute measurable absorbance at 296 nm, complicating correct identification of 5,5′-substituted-2,4-imidazolidinedithiones based on UV absorption data (see Fig. 2A). Mass spectrometric evaluation of collected fractions was used to corroborate those fractions containing 5,5′-substituted-2,4-imidazolidinedithiones based on the theoretical mass of the analyte ion, $(M-H)^-$. Measurable absorbance at 296 nm coupled with a correct mass for the $(M-H)^-$ ion, served to distinguish 5,5′-substituted-2,4-imidazolidinedithiones from 5,5′-substituted-2- or 4-imidazolidinethione side products.

Mass spectrometric evaluation of collected HPLC fractions also allowed for qualitative evaluation of purity. The intent of these evaluations was not to identify reaction side products, but to identify 5,5′-substituted-2,4-imidazolidinedithiones by correlation of expected masses, calculated from the structure of the derivative, with those masses for ions observed in the mass spectra. Purity was assessed as an overall reduction of the number of peaks within the mass spectra, relative to the $(M-H)^-$ ion of interest, as a result of sequential chromatographic steps. As illustrated in Fig. 2A, several peaks eluted from the semi-preparative, poly(styrene–divinylbenzene)-packed HPLC column after the fall-through. All of these peaks exhibited absorbance at 296 nm, especially peak III. Corroboration of peak III as that containing the desired species was made based on its mass spectrum, Fig. 2B. Evident in this mass spectrum is a peak which is consistent with the $(M-H)^-$ ion of the desired compound. Also present in the mass spectrum are numerous other peaks, attributed to products from side reactions. Mass spectrometric evaluation after an additional step of purification (Fig. 3B) indicates that the greatest ion intensity is consistent with the $(M-H)^-$ ion of the desired compound; the number of additional ion intensities is notably reduced.

Development of a reversed-phase purification method using a poly(styrene–divinylbenzene)-packed HPLC column allowed for a comparison of several silica and polymer packings. The objective was to determine which packing afforded the best separation of a mixture of 5,5′-substituted-2,4-imidazolidinedithiones. As summarized in Table 2, packings were selected which covered the full range of reversed-phase interactions [9]. In all cases a mixture, composed of the indicated derivatives, and each individual derivative were analyzed. Retention times were determined from the mixture analysis for all packings except the amino-silica packing, where retention times were determined from the analysis of the individual derivatives.

In the first two columns of Table 2, silica and polymeric C_{18} (highly retentive) packings are compared. Due to the constraints on solvent polarity to maintain the derivatives in solution, the derivatives, especially the less aliphatic ones, are not retained on the silica C_{18} packing to the extent that they are retained on the polymeric C_{18} packing. For the more aliphatic derivatives an increase in retention volume follows increasing methylene substitution at C5 of the 5,5′-substituted-2,4-imidazolidinedithiones (Table 1). The third column in Table 2 lists data for a cyano-silica packing, which is somewhat less retentive than a C_{18} packing. Analysis of the data shows that overall retention of the derivatives is less, reflecting the decreasing retentive strength; however, the less aliphatic derivatives were retained to a greater extent than on the silica C_{18} packing. The fourth column in Table 2 represents a further reduction of the retentive strength of the packing material. For the amino-silica packing, the retention volumes for the derivatives are nearly similar. Finally, the fifth column in Table 2 contains data from a poly(styrene–divinylbenzene) packing. Retention of the less aliphatic compounds is similar to that observed for the polymeric C_{18} packing, but the retention volumes are slightly less for the more aliphatic derivatives. From these data one can speculate that the 5,5′-substituted-2,4-imidazolidinedithiones perhaps interact with the poly(styrene–divinylbenzene) packings through the 5-membered ring, elution of the less aliphatic derivatives from the packing being strongly influenced by this interaction; whereas elution of the more aliphatic derivatives is less influenced by this interaction and more influenced by their aliphatic side chain. The crossover between these two modes of interaction appears to occur between the C3 and C4 derivatives.

5. Conclusion

Using the chemistry described by Carrington [4], several 5,5′-substituted-2,4-imidazolidinedithiones have been synthesized. Following synthesis, these compounds were purified by reversed-phase HPLC using poly(styrene–divinylbenzene)-packed HPLC columns, following the methods described in this paper. The identity of each purified 5,5′-substituted-2,4-imidazolidinedithione was established by UV absorbance at 296 nm and corroborated by time-of-flight ^{252}Cf plasma desorption mass spectrometry. This is the first time that methods for HPLC purification and separation of 5,5′-substituted-2,4-imidazolidinedithiones have been reported. The chromatographic procedures described in this paper should facilitate further analysis and characterization of this class of compounds.

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