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Short Communication

Development and use of a stability-indicating high-performance liquid chromatographic assay for Meldrum's acid

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ABSTRACT

A high-performance liquid chromatographic method was developed that separates Meldrum's acid from its primary decomposition products, malonic acid and acetone. The method uses a reversed-phase column under isocratic conditions, with detection by ultraviolet absorption at 210 nm. Quantitation of the parent molecule and the acid decomposition product was possible over concentration ranges of 0.1–10.0 and 0.1–2.0 mg/ml, respectively. Acetone could be determined only at much higher concentrations. Using the malonic acid concentration as a measure of decomposition, this method was used to determine the hydrolytic stability of Meldrum's acid and its skin penetration properties.

INTRODUCTION

Meldrum's acid (Fig. 1) is a unique compound formed by the condensation of malonic acid and acetone [1,2]. The structurally induced acidity of the protons in the bridging methylene of this compound produces unusually strong reactivity with such compounds as amines, aldehydes, and alcohols [3]. Hydrolysis essentially reverses the condensation reaction, producing malonic acid and acetone.

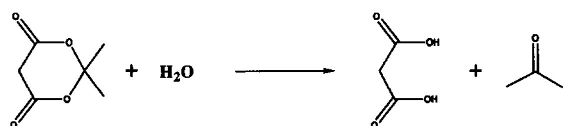

Fig. 1. Hydrolytic decomposition of Meldrum's acid.

This compound was being considered as a possible ingredient in a topical product. To help dimension potential safety concerns, we needed to determine if Meldrum's acid penetrated skin and if it decomposed in doing so. Therefore, analytical methodology for tracking the extent of its passage through skin with time became necessary. In order to assess the acid's stability as it passed through skin, the ideal analytical method would be able to quantify both the parent compound and at least one of its primary decomposition products.

This paper describes the development of a reversed-phase high-performance liquid chromato-

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graphic (HPLC) method that separates the three analytes of interest and can be used to quantitate both Meldrum's acid and malonic acid. This method was used to examine the hydrolytic stability of Meldrum's acid in several matrices and its *in vitro* penetration through human skin.

EXPERIMENTAL

Chemicals and reagents

Meldrum's acid, malonic acid, and tris(hydroxymethyl)aminomethane (Tris) buffer were purchased from Aldrich (Milwaukee, WI, USA). HPLC-grade water was purified in our laboratory (purification system by Barnstead/Thermolyne, Dubuque, IA, USA). HPLC-grade acetonitrile was purchased from Burdick & Jackson (Muskegon, MI, USA). Phosphoric acid (ACS reagent grade) was purchased from Mallinckrodt (Paris, KY, USA).

Chromatographic conditions

The liquid chromatographic system used consisted of a Model 840 chromatography data station, a Model 510 pump, WISP 712B autoinjector and a Model 490 multiwavelength UV–VIS detector (all from Waters Assoc., Milford, MA, USA). Waters Expert software was used for integration in all analyses. Meldrum's acid, malonic acid, and acetone were separated by isocratic elution. The column used was an LC-18 5-μm column (25 cm × 4.6 mm I.D., Supelco, Bellefonte, PA, USA) with an LC-18 5-μm guard column (2-cm cartridge type, also from Supelco). The mobile phase was an aqueous solution containing 5% acetonitrile and 0.5% phosphoric acid, at a flow-rate of 1 ml/min. A 25-μl injection volume was used, with UV detection at 210 nm.

Solution stability

Samples of Meldrum's acid at 1 mg/ml concentration in either distilled, deionized water or 20 m*M* Tris buffer were prepared. The pH of each sample was adjusted with either 0.5 *M* H_2SO_4 or 1 *M* NaOH prior to final dilution. Samples in each solvent at pH values of 3, 7.5 and 9 were prepared.

Samples were stored at room temperature (uncontrolled) and analyzed immediately after preparation and after 2, 4, 6, 28, 30, 56, 72, 96 and 120 h storage. To compensate for the time required for each analysis, samples were run in a staggered fashion. Elapsed analysis times reported in the data tables are approximate ($\pm$ 30 min). The matrix in which Meldrum's acid was most stable was then used in the skin penetration experiment described in the next section.

Skin penetration

The cells used in the skin penetration experiment were ground-glass Franz-type static diffusion cells [4], slightly modified to remove the individual circulating water-bath feature. Temperature control was maintained by placing the cells in a Reacti-Therm heating–stirring module (Pierce, Rockford, IL, USA) fitted with an aluminum block. Each block contained six wells for receiving reservoirs of the cells. A small magnetic stirring bar (2 × 7 mm) was placed in each receiving reservoir. Skin samples were dermatomed shaves of human cadaver skin obtained at autopsy. The shaves consisted of stratum corneum, epidermis, and a portion of the upper dermis. Punches 16 mm in diameter were taken from the shaves and used in the penetration experiments.

Twelve cells were used, ten for replicate treatments and two for vehicle controls. Skin samples were clamped, dermis down, between the donor (upper) and receiving (lower) reservoirs. The exposed skin area was *ca.* 0.8 cm^2. The receiving reservoirs were all filled with 4 ml of distilled, deionized water (pH 7.5). Based on the previous stability work, water at this pH provided reasonable Meldrum's acid stability over 24 h, while still approximating physiological fluid. All of the receiving solutions were heated in the aluminum block to 37°C to reflect body temperature. The donor reservoir of each treatment cell was charged with 400 μl of a 1% (w/v) solution of Meldrium's acid in distilled, deionized water (pH 7.5). The remaining two cells were charged with 400 μl of distilled, deionized water (pH 7.5) (control). All of the donor reservoirs were kept at ambient temperature and covered with Parafilm to prevent evaporation.

Complete collections of receiving solutions from the treatment and control cells were made after exposure at 37°C, with stirring, for 4 h. The receiving reservoirs were replenished and collected after a further 4-h exposure (8 h total). The lower reservoirs were again replenished, and a final collection was made after a total of 24-h exposure. Residual solution from each of the donor reservoirs was also collected at the end of the 24-h exposure period, so that mass-balance determinations could be performed. Each of the donor and receiving solutions was analyzed via HPLC for Meldrum's acid and malonic acid. The malonic acid present was used to estimate the amount of decomposition of Meldrum's acid during the experiment (where one molecule of the parent compound produces one molecule of malonic acid). Receiving solutions were run as received, whereas the residual donor solutions were diluted 1:10 with pH 7.5 water prior to assay.

RESULTS AND DISCUSSION

HPLC of Meldrium's acid and decomposition products

An example chromatogram, demonstrating the excellent resolution of the three analytes of interest, is shown in Fig. 2. The small peak eluting at *ca.* 14 min was not identified. The area of this peak did not change in any of the stability studies, indicating that it is not a decomposition product of Meldrum's acid. It is most likely an impurity from the parent material.

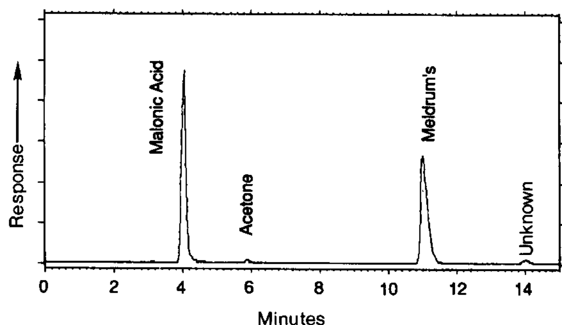

Fig. 2. Chromatographic separation of Meldrum's acid, malonic acid, and acetone. Conditions as in Experimental.

Calibration curves were prepared on three separate days using standards of Meldrum's acid and malonic acid. The chromatographic response, in terms of peak area, for Meldrum's acid was linear with concentration from 0.1 to 10 mg/ml, with a mean correlation coefficient greater than 0.999. The peak-area response for malonic acid was linear with concentration from 0.1 to 2 mg/ml, with a mean correlation coefficient greater than 0.999. The day-to-day reproducibility of the calibration curves was excellent provided that the water used for sample preparation was maintained in the optimum pH range. For both compounds, relative standard deviations for the analysis of multiple samples on a single day was always less than 3% at any concentration.

Check samples of each compound were independently prepared each day from stock solutions and were assayed against their respective calibration curves. The mean recovery for Meldrum's acid check samples ($n = 27$) over a three-day validation period was 98.6%. The mean recovery for malonic acid check samples ($n = 27$) over the same period was 99.1%.

The peak-area response for acetone was poor (note the small acetone peak in Fig. 2), and a linear relationship could be obtained only over concentration ranges much higher than those for either Meldrum's acid or malonic acid. The poorer response for acetone is probably due to the combination of a lower response factor at 210 nm and irreproducible volatilization losses at low concentrations. Since acetone could not be effectively analyzed by this method, only the malonic acid concentration was used to quantify the amount of decomposition of Meldrum's acid.

Stability study

Data from the stability experiment are presented in Table I. Meldrum's acid was found not to be completely stable at any pH, showing nearly 10% loss after five days in the best case. The data are consistent with a hydrolytic decomposition mechanism, since both of the expected products from such a reaction (malonic acid and acetone) were identified in the chromatograms. Stability was found to improve with increasing pH. This

TABLE I

MELDRUM'S ACID STABILITY IN WATER AND TRIS BUFFER

Amount of material remaining determined by comparison with HPLC peak response at time zero. Times are ± 30 min. Samples had to be run in staggered fashion to facilitate analysis.

pH	Percentage Meldrum's acid remaining								
	2 h	4 h	6 h	28 h	30 h	56 h	72 h	96 h	120 h
Water									
3	96	89	–	–	52	24	14	5	0
7.5	100	100	100	–	98	97	96	93	89
9	100	100	–	98	–	97	97	94	91
Tris buffer									
3	93	85	–	–	39	14	7	0	–
7.5	100	99	98	93	–	88	84	78	71
9	100	100	–	97	–	95	94	90	87

result would seem to indicate that hydrolysis is occurring predominantly at the acetal functionality of the molecule, rather than the ester groups. Acetals are hydrolyzed readily under acidic conditions, but are extremely resistant to base hydrolysis [5]. Ester hydrolysis would probably be catalyzed by both acidic and basic conditions, meaning neutral pH should have been best. Therefore, in this experiment, ester hydrolysis should be the sole source of decomposition at pH 9. The stability of Meldrum's acid is better in water than in Tris buffer, regardless of pH. The reasons for this are not completely clear, but stabilization of the carbocation acetalysis intermediates by lone-pair electrons from the Tris nitrogens may be partially responsible.

TABLE II

MASS BALANCE OF MELDRUM'S ACID FROM SKIN PENETRATION STUDY

Values are the average of ten samples.

Time (h)	Distribution (percentage of original dose)			
	Receiving reservoir	Donor reservoir		
		Meldrum's	Malonic	Total
4	0.0	–	–	–
8	0.0	–	–	–
24	29.8	38.7	13.1	81.7

Skin penetration experiment

The mean skin penetration data from the ten replicate Meldrum's acid treatments are shown in Table II. These data clearly show that Meldrum's acid does not appreciably penetrate skin, even after 8 h of contact. The skin barrier is breached between 8 and 24 h, with substantial penetration occurring during this period. The mean penetration of Meldrum's acid after 24 h was *ca.* 30%. Neither Meldrum's acid nor malonic acid was found in any of the control samples (donor or receiving solutions).

Malonic acid was found only in the residual donor solutions, indicating that Meldrum's acid can remain intact as it passes through skin, and that malonic acid penetrates only poorly, if at all. The absence of malonic acid in the receiving solutions also implies that Meldrum's acid is stable at 37°C for at least 24 h under the conditions of this experiment. The overall mass balance for Meldrum's acid was fairly quantitative, with *ca.* 18% of the material being unaccounted for. Most likely, the missing Meldrum's acid was retained in the skin as either the parent molecule or the malonic acid decomposition product.

CONCLUSION

The HPLC method developed provided an excellent means to determine the relative stability of

Meldrum's acid under different conditions and to track its skin penetration *in vitro*.

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