The interference peak–*N*-acetylprocainamide peak height ratio in the deproteinized samples was 0.12–1.0 at 254 nm and 0.18–1.78 at 274 nm. The ratio was higher at the longer wavelength.

UV absorption maxima occur at 282 and 268 nm for procainamide and *N*-acetylprocainamide, respectively (1). However, monitoring the column effluent at 254 nm with a fixed-wavelength detector[3] is suitable for detecting these compounds because of the superior signal-to-noise ratio of that detector relative to the variable-wavelength detector[4].

The deproteinization sample preparation method produced a number of peaks not present in predose plasma. These peaks elute before the *N*-acetylprocainamide peak and may represent other procainamide metabolites.

Care needs to be exercised with the previously reported deproteinization plasma sample preparation method (1) to avoid overestimation of the pharmacologically active *N*-acetylprocainamide concentration. If a column is used that does not resolve the interference from the *N*-acetylprocainamide peak, the solvent extraction sequence described improves *N*-acetylprocainamide quantitation. A prudent general approach is to use the extraction method.

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Creatinine VII: Determination of Saliva Creatinine by High-Performance Liquid Chromatography

Keyphrases □ Creatinine—analysis, saliva, high-performance liquid chromatography □ Saliva—analysis, creatinine, high-performance liquid chromatography □ High-performance liquid chromatography—analysis, saliva creatinine

To the Editor:

Measurements of creatinine levels in biological fluids are important to the renal function determination and drug dosage adjustment in patients with renal insufficiency (1–4). Recently, a high-performance liquid chromatographic (HPLC) method for endogenous plasma, serum, and urine creatinine determination was developed (5, 6). This method, however, was not sensitive enough for the assay of saliva creatinine, which might be as low as 0.03 mg %, or about 3% of the plasma or serum levels determined by the less specific colorimetric method (3). Furthermore,

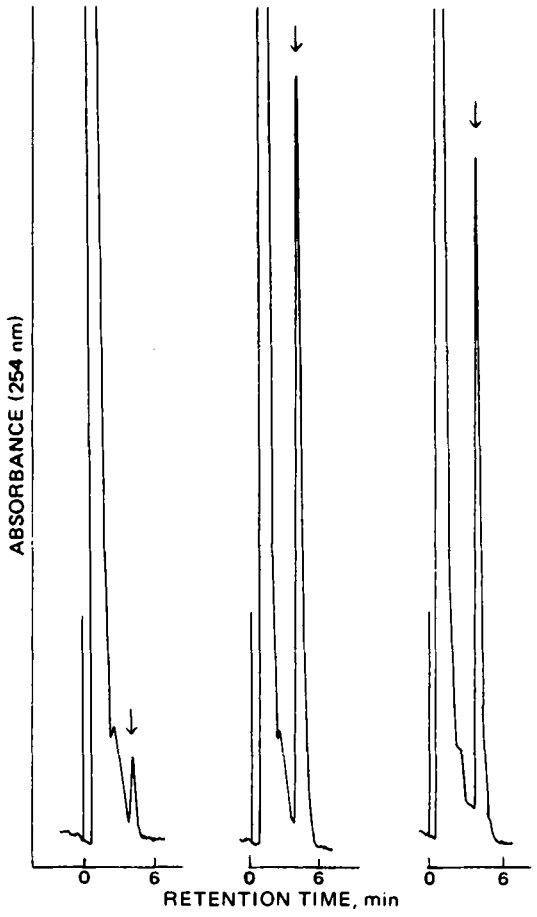

Figure 1—*Chromatograms of creatinine (↓) from various saliva samples. Key: left, pooled saliva from normal subjects; middle, pooled saliva spiked with 0.3 mg % of creatinine; and right, saliva from a normal subject with a creatinine level of 0.256 mg %. Detector sensitivity was at 0.005 A full scale.*

serious interfering peaks from the deproteinized saliva coexisted with the creatinine peak. This result is somewhat ironic because saliva composition is commonly assumed to be less complicated than that of plasma. This communication reports a modified HPLC method that is able to quantify low levels of endogenous saliva creatinine.

Sample preparation included vortex mixing of 0.3 ml of saliva with 2 ml of acetonitrile for about 10 sec in a 13 × 100-mm screw-capped glass culture tube, centrifugation at 2000 rpm for about 1 min, and direct pouring of the clear supernate into another tube containing 2 ml of methylene chloride, followed by vortex mixing and centrifugation. Exactly 100 μl of the upper aqueous layer was then injected into the chromatograph. Essentially all of the acetonitrile was partitioned into the methylene chloride layer.

The HPLC system consisted of a solvent delivery pump[1], a sample injection valve[2], and a fixed wavelength (254 nm) detector[3]. A 25.4-cm linear recorder and a 25-cm cation-exchange column[4] were used. The mobile phase was 0.012 *M* ammonium phosphate solution made basic with 0.4% (v/v) 0.1 *N* NaOH to pH 4.80. The creatinine studied at ambient temperature had an ~4.5-min retention time

[1] Model 6000A, Waters Associates, Milford, Mass.
[2] Model U6K, Waters Associates, Milford, Mass.
[3] Model 440, Waters Associates, Milford, Mass.
[4] Whatman Partisil PXS 10/25 SCX column, Whatman Inc., Clifton, N.J.

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Table I—Typical Response Factors of Creatinine at Various Spiked Concentrations in Saliva Samples

Sample	Spiked Concentration, mg %	Peak Height[a], cm	Response Factor[b]
1	0	1.16	—
2	0.05	3.04	37.60
3	0.10	5.12	39.60
4	0.20	9.10	39.70
5	0.30	12.70	38.47
6	0.50	19.60	36.88
Average ± *SD*			38.45 ± 1.232

[a] The detector sensitivity was set at 0.005 *A* full scale, and a 25.4-cm recorder chart paper was used. [b] Response factor = (peak height from spiked sample—peak height from unspiked sample)/spiked creatinine concentration.

(Fig. 1). The creatinine peak was not as well resolved as that observed in plasma or serum samples using simple deproteinization (5).

Methylene chloride in the sample preparation served two purposes. It removed interfering substances from saliva through extraction. It also concentrated the aqueous supernate as compared to the previous deproteinization procedure (5), since essentially all acetonitrile remained with the methylene chloride layer in the last step. Our preliminary study indicated that most creatinine remains in the upper aqueous layer. In other words, about a threefold increase in sensitivity can be achieved by the present extraction method.

In our previous study (5), a variable wavelength detector set at 215 nm was used for creatinine quantitation. Although creatinine absorption at 254 nm in the mobile phase used here was only ~28% of that at 215 nm, the higher signal-to-noise ratio using the 254-nm fixed wavelength detector as compared to the variable wavelength detector (7) would still make the former detector more sensitive. Based on a criterion of a signal-to-noise ratio of 3, one could estimate the lower limit for quantitation with the present method as 0.0065 mg %.

The satisfactory creatinine peak height linearity with increasing concentrations was demonstrated by the response factor studies (Table I). The coefficient of variation in the range studied was 3.2%. The coefficients of variation for the intraassay and interassay studies (n = 6) using a saliva sample with 0.048 mg % creatinine were 4.2 and 6.7%, respectively. The coefficients of variation were reduced to 2 and 5.5%, respectively, with a 0.248-mg % saliva creatinine sample. Over several months, many hundred saliva samples have been satisfactorily analyzed by the present method.

In most of our saliva creatinine studies, the recorder chart speed was 10 cm/hr. The advantages of such a slow chart speed were discussed previously (8). In the present study, a micrometer[5] with its dial graduated in 0.1-mm increments was used for the measurement of peak heights.

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[5] Vernier Caliper, Fisher Scientific Co., Chicago, Ill.

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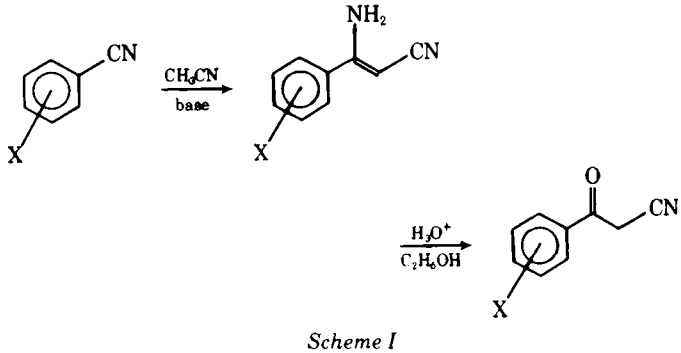
Potential Antiarthritic Agents I: Benzoylacetonitriles

Keyphrases □ Antiarthritic agents—benzoylacetonitriles, synthesis, screened in rats, structure–activity relationships □ Benzoylacetonitriles—antiarthritis activity, synthesis, screened in rats, structure–activity relationships

To the Editor:

Rheumatoid arthritis, a chronic inflammatory disorder of unknown etiology, is treated largely with agents useful only in ameliorating the acute inflammatory symptoms, *i.e.*, pain and swelling. Clearly, nontoxic drugs effective in the remission of joint and cartilage destruction are critically needed. We screened compounds in the chronic Freund's adjuvant-induced arthritis model (1) and discovered that benzoylacetonitrile (I) and its monofluorophenyl analogs (II–IV) were highly effective in this assay.

Concurrently, we found this series to be inactive against carrageenan-induced edema in rats and urate synovitis in dogs and only weakly active in suppressing UV-induced erythema in guinea pigs. When tested for the suppression of prostaglandin synthesis *in vitro*, benzoylacetonitrile had only one-third of the potency of aspirin. Additionally, I and IV displayed little or no ulcerogenic potential in rats at total daily doses as high as 800 mg/kg. These facts suggest that prostaglandin synthesis inhibition is unlikely to ac-



Scheme I