of guaifenesin with periodic acid to o-methoxyphenoxy acetaldehyde and formaldehyde. The extreme conditions required to degrade guaifenesin indicate that its decomposition is highly unlikely in pharmaceutical products subjected to several years of aging at normal conditions or several months at 50–70°. The present study supports this assumption in that no significant loss in guaifenesin was observed for the products assayed.

Cohen (25) stated that theophylline solutions subjected to strongly alkaline pH showed decomposition and apparent ring opening after several weeks and that theophylline was also susceptible to oxidation, resulting in the formation of 1,3-dimethyluric acid.

Significant loss of theophylline was observed in liquid products subjected to 50–70° for several months. A comparison of the chromatograms from fresh liquid products and high stress samples showed a loss in theophylline as well as increases in the 5-hydroxymethylfurfural peak and several additional unidentified peaks (Fig. 2). Sample blanks subjected to similar stress contained corresponding unidentified peaks. If 1,3-dimethyluric acid is present, it is well removed from the peaks of interest.

The degradation of sucrose solutions results in a highly complex series of decomposition products, including 5-hydroxymethylfurfural. Because of the quasistable state of this strongly UV-absorbing species in solution, it is frequently regarded as an indication of vehicle decomposition (1).

The amount of 5-hydroxymethylfurfural present in the sample can readily be quantified by the addition of this component to the standard. A study conducted on a liquid theophylline product indicated that decreasing the pH over the 6–5 range decreased theophylline stability and increased the amounts of 5-hydroxymethylfurfural and a second unidentified vehicle decomposition product.

The retention time of benzoic acid was highly sensitive to the mobile phase pH (Fig. 3). When the pH of the buffer in the mobile phase was adjusted to 4.15, the benzoic acid eluted between the last vehicle decomposition product peak and the theophylline peak.

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ACKNOWLEDGMENTS

The author acknowledges the support of Dr. Eugene Brockemeyer, Director of Research, Development and Quality Assurance of Dorsey Laboratories. She also thanks Dr. Robert D. Hargens for advice during this project and Dr. V. K. Olson for guidance in the preparation of this article.

COMMUNICATIONS

Precaution in Use of High-Pressure Liquid Chromatographic Simultaneous Plasma Procainamide and N-Acetylprocainamide Determination

Keyphrases □ Procainamide—analysis, plasma, high-pressure liquid chromatography deproteinization *versus* extraction methods □ N-Acetylprocainamide—analysis, plasma, high-pressure liquid chromatography, deproteinization *versus* extraction methods □ High-pressure liquid chromatography—analysis, procainamide and N-acetylprocainamide in plasma, deproteinization *versus* extraction methods

To the Editor:

A high-pressure liquid chromatographic (HPLC) method for the simultaneous plasma procainamide and N-acetylprocainamide determination was reported recently from this laboratory (1). The method involved microvolume acetonitrile plasma protein precipitation and injection of an aliquot of the resultant supernatant solution onto a cation-exchange column. Detection was by UV absorption at 274 nm. N-Acetylprocainamide and procainamide eluted from the system with retention times of 4 and 5 min, respectively, and appeared to be symmetrical peaks that were satisfactorily resolved from each other and other plasma components. Total analysis time per sample was ~7 min. The method required extremely simple sample preparation and short analysis time. The purpose of this communication is to report some precautions in the use of this method.

Due to an irreversible loss of column performance, a new cation-exchange column obtained from the same manufacturer[1] was used as previously reported (1). This new column demonstrated that the peak in patient samples

[1] Partisil PXS 10/25 SCX, Whatman, Clifton, N.J.

0022-3549/ 79/ 0400-0532$01.00/ 0

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Table I—Apparent Concentration of *N*-Acetylprocainamide and Procainamide in Plasma Collected from Patients as Determined by the Deproteinization Method and the Extraction Method [a]

Sample	*N*-Acetylprocainamide Concentration, μg/ml		Percent Deviation [b]	Procainamide Concentration, μg/ml		Percent Deviation [b]
	Deproteinization	Extraction		Deproteinization	Extraction	
1	1.44	1.28	12.5	20.11	— [c]	—
2	1.59	1.18	34.8	6.87	6.74	1.93
3	1.55	1.16	33.6	5.20	5.02	3.60
4	1.42	1.13	25.7	4.87	4.97	−2.00

[a] The HPLC column did not resolve the interference peak from the *N*-acetylprocainamide peak. Detection was at 254 nm with the fixed-wavelength detector. [b] (Concentration by deproteinization method − concentration by extraction method/concentration by extraction method) × 100. [c] Above the upper limit of the calibration curve.

Table II—Concentration of *N*-Acetylprocainamide and Procainamide in Plasma Collected from Patients and Analyzed by the Deproteinization Method and the Extraction Method with UV Detection at 254 and 274 nm [a]

Sample	*N*-Acetylprocainamide Concentration, μg/ml				Procainamide Concentration, μg/ml			
	Deproteinization		Extraction		Deproteinization		Extraction	
	254 nm [b]	274 nm [c]	254 nm	274 nm	254 nm	274 nm	254 nm	274 nm
5	0.24	0.22	0.28	0.28	6.72	6.70	6.67	7.00
6	0.47	0.47	0.43	0.43	4.09	4.25	3.57	3.91
7	2.50	2.77	2.31	2.58	2.55	2.81	2.29	2.56
8	3.67	3.93	3.75	3.94	5.57	5.82	5.60	5.98
9	3.07	3.26	3.13	3.29	2.24	2.21	2.14	2.28
10	1.52	1.40	1.40	1.43	5.44	5.50	5.45	5.79
11	1.28	1.31	1.24	1.17	3.87	4.08	3.68	3.76
12	0.68	0.63	0.63	0.70	3.61	3.62	3.51	3.70
13	1.70	1.80	1.74	1.90	4.69	5.09	4.66	4.90

[a] The HPLC column resolved the interference peak from the *N*-acetylprocainamide peak. [b] Fixed-wavelength detector. [c] Variable-wavelength detector.

formerly attributed entirely to *N*-acetylprocainamide was not homogeneous since its leading edge was deformed. Other cation-exchange columns of the same make demonstrated various degrees of peak resolution from the *N*-acetylprocainamide peak. This resolution was achieved with the mobile phase as originally reported (1) or with slightly modified acetonitrile or salt concentrations so that *N*-acetylprocainamide and procainamide eluted with retention times less than 6 min. The interference peak was not present in plasma samples collected just prior to initial procainamide dose administration, suggesting that the compound responsible may have been another procainamide metabolite.

Patient plasma samples were also prepared for chromatography by a solvent extraction method. The extraction sequence used was as follows. A mixture of 0.1 ml of plasma, 0.5 ml of water, and 0.1 ml of 1 *M* NaOH was vortexed for 1 min with 3 ml of ethyl acetate. After centrifugation, most of the organic layer was transferred to a tapered tube and vortexed (1 min) with 0.1 ml of 0.5% phosphoric acid. The tube was centrifuged, and an aliquot (20–40 μl) of the aqueous phase was injected. With this sample preparation, the *N*-acetylprocainamide and procainamide peaks appeared as symmetrical peaks with no interference.

Therefore, plasma samples were prepared for quantitative chromatography by the extraction method. The internal standard was *p*-amino-*N*-(2-dipropylaminoethyl)benzamide hydrochloride[2]; 0.2 ml of an aqueous solution (2 μg/ml) was included in the first sample preparation step described in place of water. To resolve procainamide and the internal standard, the mobile phase acetonitrile concentration was reduced. The mobile phase was composed of 18 parts of acetonitrile and 82 parts of 0.1 *M* ammonium phosphate (monobasic), which had been acidified with 0.2% phosphoric acid. The percentage of phosphoric acid (0.05%) in the mobile phase reported previously (1) was in error. With a 3-ml/min flow rate, the *N*-acetylprocainamide, procainamide, and internal standard retention times were 5.1, 7.1, and 8.7 min, respectively.

The mobile phase composition may need some adjustment when different columns are used due to column differences in chromatographic behavior. An increased salt or acetonitrile concentration in the mobile phase decreased resolution of the interference peak from the *N*-acetylprocainamide peak and vice versa.

Table I summarizes a comparison between the deproteinization method published previously (1) and the method described here, which involves solvent extraction prior to HPLC. The column used on this occasion did not resolve the interference peak from the *N*-acetylprocainamide peak. The plasma samples were collected from inpatients who were receiving procainamide intravenously. The *N*-acetylprocainamide concentration was substantially overestimated by the deproteinization method.

A column that did resolve the interference from the *N*-acetylprocainamide peak in deproteinized samples was used in another experiment. The column effluent was passed through a fixed-wavelength UV detector[3] (254 nm) and a variable-wavelength UV detector[4] (274 nm), which were connected in series. Plasma samples collected from inpatients who were receiving oral or intravenous procainamide were prepared for analysis by both deproteinization and solvent extraction (Table II). There was general good agreement among the four concentration estimates for each compound quantitated. Therefore, the deproteinization method appears to be suitable for rapidly quantitating plasma *N*-acetylprocainamide and procainamide provided that an efficient HPLC column is used.

[2] E. R. Squibb & Sons, Princeton, N.J.

[3] Model 440, Waters Associates, Milford, Mass.

[4] Model LC55, Perkin-Elmer Corp., Norwalk, Conn.

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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Roger L. Nation
Myung G. Lee
Shiew-Mei Huang
Win L. Chiou ˣ
Clinical Pharmacokinetics Laboratory and Department of Pharmacy
College of Pharmacy
University of Illinois at the Medical Center
Chicago, IL 60612

Received September 25, 1978.
Accepted for publication January 10, 1979.

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, 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.

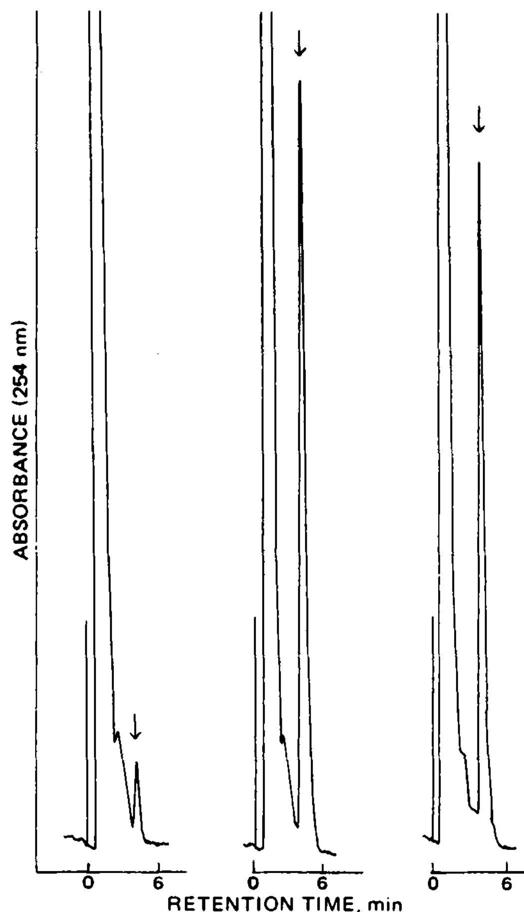

Figure 1—*Chromatograms of creatinine (↓) from various saliva samples. Key: left, pooled saliva from normal subjects; middle, pooled saliva spiked with 0.3 mg % of creatinine; and right, saliva from a normal subject with a creatinine level of 0.256 mg %. Detector sensitivity was at 0.005 A full scale.*

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.

0022-3549/79/0400-0534$01.00/0
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