cently, fluorescence polarization immunoassay (FPA) of salicylate with the TDx was introduced by Abbott Labs., North Chicago, IL 60064. In comparing it with the aca colorimetric assay, we discovered that, in two separate patients' serum samples, diflunisal (Dolobid®; Merck, Sharp and Dohme, West Point, PA 19486) significantly interfered with both assays: in the aca by 61% and in the TDx by 230%, probably owing to the structural similarity between this drug and salicylate.

Diflunisal (2',4'-difluoro-4-hydroxy-3-biphenylcarboxylic acid) is a recently introduced analgesic, antipyretic, anti-inflammatory agent (1, 2). It is indicated for mild to moderate pain, osteoarthritis, and rheumatoid arthritis. The chemical structure of diflunisal differs from aspirin by the presence of a difluorophenyl substituent at C-1 and the lack of an O-acetyl group at C-4. The drug is not metabolized to salicylic acid, and the fluorine atoms are not displaced from the difluorophenyl ring structure.

Patient I was a young man under chronic psychopharmacological support, who had a history of chronic mild to moderate pain, for which diflunisal was prescribed. There was no history of renal disease. Values for "liver" enzymes were slightly above normal. The values for serum urea nitrogen and creatinine were 150 and 12 mg/dL, respectively; aspartate aminotransferase 22 U/L (normal range 8–30 U/L); alanine aminotransferase 41 U/L (5–24 U/L); and gamma-glutamyltransferase 110 U/L (normal range in men, 3–30 U/L). A routine urine and serum screen ("Toxi-Lab"); Analytical Systems, Marion Labs., Igualda, CA 92635, revealed the presence of desipramine, doxepin, and benzodiazepine. Serum ethanol and "salicylate" concentrations were 910 and 67 mg/dL, respectively.

Patient II, a 73-year-old man, had chronic arthritis in the hip, and leg pains, for which diflunisal was prescribed. He had no history of hepatic or renal disease. The values for serum urea nitrogen and creatinine were 110 and 9 mg/dL, respectively; aspartate aminotransferase 21 U/L; alanine aminotransferase 25 U/L; and gamma-glutamyltransferase 32 U/L. A urine drug screen showed only diflunisal. The serum showed ethanol and "salicylate" concentrations of <70 and 47 mg/dL, respectively.

In evaluating the clinical efficacy of the FPIA for salicylate, we compared results by it with those by the established colorimetric aca procedure. Excluding the two patients in question, the study (n = 36) showed that the correlation coefficient was 0.987, the slope 1.064, and the y-intercept -1.866. Paired t-test showed no significant (p = 0.04) difference. For the two cases in question, we quantified each for diflunisal and salicylate in serum, using two established "high-performance" liquid-chromatographic (HPLC) methods (3, 4).

The HPLC analysis for diflunisal was modified from the method of Mood et al. (3). We prepared a methanolic standard of diflunisal (pure standard or crushed pill) by dissolving the drug in methanol. After transferring an aliquot into a volumetric flask and evaporating the methanol, we reconstituted the residual drug with "drug-free" serum (confirming the absence of salicylate and diflunisal by HPLC). We mixed the serum with acetone for precipitating protein, and injected aliquots of the supernate into the HPLC for analyses. Chromatographic conditions were: μBondapak C18 (Waters Associates, Milford, MA 01757); mobile phase, 50 mL/L acetic acid and acetonitrile (6/4 by vol); ambient temperature; flow rate, 2 mL/min; wavelength, 251 nm. We measured salicylate concentrations by the procedure of Cham et al. (4), mixing serum with acetonitrile for protein precipitation. Chromatographic conditions were: μBondapak C18; mobile phase, methanol and 50 mL/L acetic acid (18/82 by vol); ambient temperature; flow rate, 2.5 mL/min; and wavelength, 254 nm.

Using HPLC as our reference procedure, we concluded that the sera of the two patients did not contain salicylate, and that the aca and TDx measurements of "salicylate" were due to diflunisal. By HPLC, diflunisal concentrations of patients I and II were 113 and 74 mg/L, respectively. The apparent salicylate concentrations of the two patients, as measured by the aca, were 67 and 47 mg/L, and by the TDx were 260 and 170 mg/L. The interference with results [i.e., ("salicylate" concentration/diflunisal concentration) × 100] in the aca was 59% and 63% (average 61%) for patients I and II, respectively, and cross reactivity in the TDx was 230% each. The high cross reactivity in the FPIA method might result from the structural similarity of diflunisal and salicylate, e.g., the two aromatic rings, one similar to that of salicylate. The lower, but still significant, interference of 61% for the aca method might be due to retarded reactivity of the bulky "salicylate-like" moiety of diflunisal with the reagent, Fe(NO₃)₂. Further studies are needed to clarify these observations.

In addition, we checked for cross reactivities of other drugs ingested by patient I, and of another anti-arthritis drug, indomethacin, by FPIA; the results were negative. A recent AACC-TDM-TOX survey (May 1985) showed a high false-positive rate for a target value of zero, in contrast to previous

**Fig. 1.** Chromatogram of extract from plasma containing 16.9 μg of isosomin (1) per liter

The amount of diflunisal that could be detected by FPIA was 0.1 μg/mL, while the TDx was able to detect as little as 0.001 μg/mL. The aca was able to detect as little as 0.01 μg/mL. The FPIA showed no interference with aspirin, salicylate, and other drugs that might be present in patients with chronic arthritis. The TDx showed no interference with aspirin, salicylate, and other drugs that might be present in patients with chronic arthritis.

**References**


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**Diffusable Significantly Interferes with Salicylate Measurements by FPIA-TDX and UV–VIS aca Methods**

**To the Editor:**

Salicylate is readily quantified by several methods, of which the colorimetric method as used in the aca (Du Pont) has been most popular, according to the May 1985 AACC Therapeutic Drug Monitoring (TDM) survey. Re-
surveys in which "non-zero" target values followed gaussian distribution. This suggests a problem with false-positive results at low or zero salicylate concentrations.

From the above experience we conclude that, in general, salicylate measurements by FPIA correlated well with those by the aco method, except for samples from patients treated with diflunisal, which should be assayed for salicylate by HPLC. Thus, in interpreting the results for salicylate, one needs to know the patient's drug history. Further, diflunisal may not be detected in the Toxi-Lab A urine screen if other drugs are present with similar Rf values, as in the urine screen of patient I.

We thank Dr. J. H. Milander and Merck Sharp and Dohme Laboratories (West Point, PA) for sending diflunisal standard, and Dr. Candice Keegan of Abbott Laboratories (North Chicago, IL) for confirmation of the cross-reactivity measurements by FPIA.

References

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A spokesman for Abbott comments as follows:

To the Editor:

Diflunisal is available only by prescription at this time. Also, there is currently no recommendation by the manufacturer for coadministration of diflunisal with other non-steroidal anti-inflammatory drugs (1).

High-dose concentrations of diflunisal may produce toxicity where detection and treatment would be necessary. However, we have not done any studies on monitoring diflunisal by using the salicylate channel on the TDx and therefore do not recommend this as a method for quantitating diflunisal.

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**Table 1. Effect of Hemolysis on Results for Total Bilirubin in Serum with Normal and Above-Normal Concentrations of Bilirubin**

<table>
<thead>
<tr>
<th>Concentration, mg/L</th>
<th>Hemoglobin</th>
<th>Total bilirubin</th>
<th>Interferences, mg/L</th>
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<td>Kodak Ektachem</td>
<td>Abbott VP</td>
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<tr>
<td>3000</td>
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</table>

*Interferences were calculated as follows: concentration of TBi1 measured with hemoglobin minus concentration without hemoglobin.*

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To the Editor:

Recently the Eastman Kodak Co. has developed a multilayer dry-film slide for use in measuring total bilirubin (TBi1) in serum by an adaptation of the Jendrassik-Gröf procedure (1–3). The measurement is based on the use of dyphylline and surfactant to dissociate unconjugated and conjugated bilirubin (4) from albumin. These bilirubin fractions, together with the albumin-linked delta bilirubin (5), react subsequently with the diazoanion salt, 4-(N-carboxymethylsulfamyl)benzene-diazoniumhexafluorophosphate, to produce azobilirubin chromophores that have similar molar absorptivities and absorption maxima around 520 nm. The dry-film slides are used with the Kodak Ektachem 400 analyzer, which we calibrated once weekly with freshly prepared serum-based calibrators from Eastman Kodak. Quality-control sera to estimate precision were "Monitrol" ("Level I", cat. no. XLS-21, and "Level II", XPS-114, from American Dade, Miami, FL 33152) and "Omega Elevated Bilirubin Control" (Hyland Diagnostics, Round Lake, IL 60073).

The precision study gave the following results (mean and SD, mg/L, and CV, %). Between-day: Monitrol I: 18.79, 0.29, 1.5; Monitrol II: 61.88, 2.45, 4.0; Omega: 213.0, 5.0, 2.3; and within-day: Monitrol I: 18.69, 0.12, 0.6; Monitrol II: 62.49, 1.26, 2.0; Omega: 215.22, 1.76, 0.8; n = 30 each.

To evaluate TBi1 we compared serum samples from 92 adult patients, measuring them by both the wet-chemistry method of Malloy-Evelyn with modifications by Rand and Di Pasqua (6) in the "VP Dichromatic Analyzer" (x: Abbott Laboratories, Irving, TX 75061), using the Agent Clinical Chemistry Reagent Bilirubin of Abbott (7), and by the thin-film method (y). Linear-regression analysis gave the following equation: y = 1.080x – 0.279 mg/L; r = 0.980; S_\text{xy} = 1.432; n = 92; range = 1.36–37.9 mg/L. The agreement of the results between methods was within 2.5 mg/L (97%), the remaining 3%, pertaining to values >30 mg/L, differed by as much as 5 mg/L. The standard curve for TBi1 was linear within the dynamic range of the Ektachem, 1–270 mg/L, as tested by serially diluting human serum-based calibrator four (259 mg of TBi1 per liter) from Eastman Kodak with 70 g/L albumin solution and assaying.

To estimate the effect of hemolysis, which interferes with the measurement of TBi1 by spectral and chemical interference (3, 8), we prepared two serum pools, each containing a different amount of TBi1. We supplemented portions of each pool to give 750, 1500, and 3000 mg of hemoglobin per liter, using a hemolysate prepared from lysed erythrocytes by a freeze-thaw cycle. The serum pools, with and without added hemoglobin, were assayed in triplicate by the wet-chemistry method and in the Ektachem (Table 1).

We conclude that the accuracy of the two methods for TBi1 compares well. Excellent between-day precision, long-term stability of calibration, a low sample volume requirement (25 μL), and a 24-h per day availability demonstrate that the dry-slide test methodology for TBi1 is well suited for use in the clinical laboratory.

References
1. Jendrassik L, Gröf P. Vereinfachte pho