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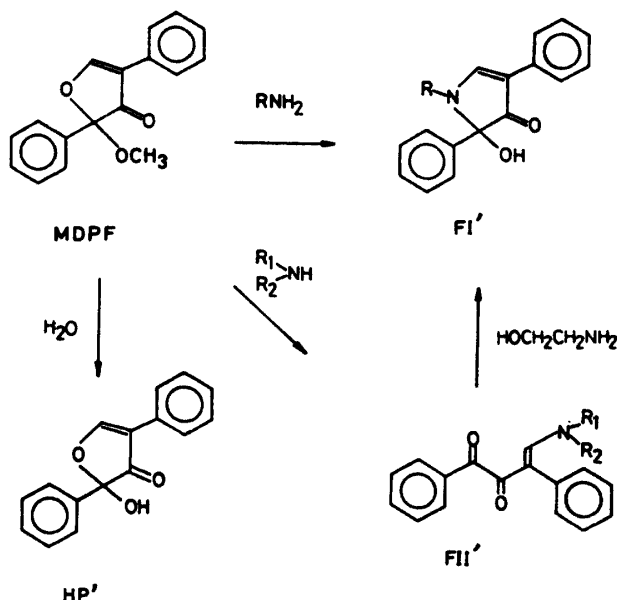
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**Stepwise Fluorometric Determination of Primary and Secondary Amines by Liquid Chromatography after Derivatization  
With 2-Methoxy-2,4-diphenyl-3(2H)-furanone\***

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A high-performance liquid chromatographic (HPLC) method was developed for the stepwise fluorometric determination of primary and secondary amines. Amines were reacted with 2-methoxy-2,4-diphenyl-3(2H)-furanone (MDPF) at pH 9.6 and 20°C for 30 min to produce fluorescent pyrrolinones (FI') from primary amines and nonfluorescent aminodienones (FII') from secondary amines. The MDPF-adducts of amines were separated on a reversed-phase C<sub>18</sub> (TSK LS-410 K) column with a mixture of methanol and 50 mM phosphate buffer (pH 7.0) (70 : 30). After the detection of FI' with the first fluorescence monitor ( $\lambda_{ex}$  360 nm,  $\lambda_{em}$  > 405 nm), the eluate was mixed with 12 M ethanolamine hydrochloride (pH 10.5) to convert FII' to fluorescent MDPF-ethanolamine which was detected with the second fluorescence monitor ( $\lambda_{ex}$  390 nm,  $\lambda_{em}$  480 nm).



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The present method permits the determination of 3 pmol of lower *n*-alkylamines and 50 pmol of lower di-*n*-alkylamines. The relative standard deviations were 2.3—2.7% for 50 pmol of the *n*-alkylamines and 2.9—3.4% for 1 nmol of the di-*n*-alkylamines.