Evolution of a Specific Fluorogenic Derivatization of Ivermectin

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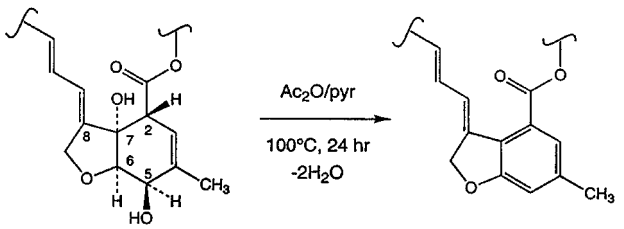
Dehydrative aromatization using non-fluorescent reagents provides a selective fluorogenic derivatization of the non-fluorescent antiparasitic agent ivermectin. This reaction does not require a separation of excess fluorescent reagent or reaction by-products from the analytical fluorophore for application to plasma samples. The two hydroxyls having protons bonded *trans* to each on the dihydroxycyclohexene ring of this drug (**I**) are stericaly positioned to allow for the facile elimination of water to produce a delocalized electron system: i.e., heating with acetic anhydride in pyridine causes the elimination of two moles of water, producing a fluorophore consisting of an aromatic ring (Mrozik *et al.*, 1982) conjugated with a diene system (**II**; λ_{excite}, 375 nm; λ_{emit}, 475 nm).



I II

The mechanism of this analytical reaction involves acetylation of the hydroxyl groups in advance of dehydration; the original development of this derivatization required a reaction time of 24 h (Tolan *et al.*, 1980). The evolution of this method has resulted in improvements in speed and sensitivity. Connors and coworkers identified several nucleophilic catalysts for acylation by acetic anhydride which are superior to pyridine, including 4-dimethylaminopyridine and some *N*-alkylated imidazoles (Pandit and Connors, 1982; Connors and Albert, 1973). Use of the more powerful nucleophilic 1-methylimidazole as acetylation catalyst in lieu of pyridine for this derivatization reduced reaction time to 1 h (Tway *et al.*, 1981). Incorporating a better leaving group by substituting trifluoroacetic anhydride for acetic anhydride as the acetylation reagent reduced reaction time to <30 s and detection limits to ca. 20 pg (S/N = 2) (de Montigny *et al.*, 1990). The use of laser-induced fluorescence detection further reduced the detection limit (Rabel *et al.*, 1993). The method was also modified by the use of solid-phase extraction from the plasma sample in lieu of liquid–liquid partitioning (Kojima *et al.*, 1987).

This review will chronicle the evolution of this analytical derivatization reaction and its numerous bioanalytical applications. In our most recent iteration, we have now simplified the method further by eliminating the chromatographic isolation step via the use of automated derivatization (as described by Rabel *et al.* (1993). This maintains an accuracy of 2% mean relative error and a precision of 5% relative standard deviation at the 1 ng measurement level in an even shorter and less cumbersome analytical procedure.

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