Specificity of Nitroglycerin Assays

Keyphrases □ Nitroglycerin—analysis, stability, various procedures compared □ Vasodilators, coronary—nitroglycerin, stability analysis, various procedures compared □ Stability—nitroglycerin, analysis, various procedures compared

To the Editor:

Recently, intravenous nitroglycerin was shown to be beneficial in the management of acute myocardial infarction and of left ventricular failure (1, 2). This therapeutic use has sparked renewed interest in the determination of nitroglycerin stability in solutions. Several studies (3–5) demonstrated that while nitroglycerin infusion is stable when stored in glass containers, significant drug adsorption onto plastic intravenous bags does occur.

Suphajettra *et al.* (6) examined nitroglycerin stability at elevated temperatures in various nonaqueous solvents and found appreciable drug degradation only in polyethylene glycol 400. These investigators employed the Bell (7) and kinetic (8, 9) assays for their stability study and found that the former method gave an apparently slower degradation rate. They proposed that a reaction complex sequestered nitroglycerin from detection when using the kinetic assay but that this reaction complex was broken down during the workup in the Bell method. If this hypothesis is correct, then the Bell procedure is not stability indicating. Since neither of these assays involved a separation step, it is also possible that the difference in the observed rates was due to a difference in the extent of interference produced by nitroglycerin degradation products such as glyceryl dinitrates, glyceryl mononitrates, inorganic nitrate, and nitrite ions. Thus, the assay that is subject to interference from degradation products will produce an artifactually slower degradation rate.

Since comparative information on the specificity of nitroglycerin assays is not available, we examined the three most used methods—*viz.*, the Bell (7), the kinetic (8, 9), and the USP (10) procedures, for potential interference by nitroglycerin degradation products. Aqueous nitroglycerin solutions (~300 μg/ml), standardized by the USP procedure, were assayed in duplicate in the separate presence of approximately equimolar concentrations of the glyceryl dinitrates[1] and mononitrates[1]. Inorganic nitrate[2] and nitrite[2] ions were present at three times this concentration. Since the USP procedure requires a rather tedious column separation for each sample, some workers (6) have omitted it. This modified USP method was examined also for potential interference by nitroglycerin degradation products.

The degree of interference is expressed as an apparent molar interference factor, defined as the fractional change in spectrophotometric absorbance seen in the presence of an equimolar concentration of the potentially interfering substance. For example, an apparent molar interference factor of +0.50 indicates that when the interfering substance is present at equimolar concentration, the nitroglycerin absorbance would be artifactually increased by 50%. Ideally then, a stability-indicating assay should have an apparent molar interference factor close to zero for all possible degradation products.

Table I—Interference with Nitroglycerin Determination by Different Nitroglycerin Degradation Products

	Apparent Molar Interference Factor[a]			
	Kinetic[b] Assay	Bell[c] Assay	USP[d] Procedure	Modified USP Procedure
1,2-Glyceryl dinitrate	+0.01	+0.01	+0.02	+0.70
	+0.01	0.00	−0.05	+0.46
1,3-Glyceryl dinitrate	+0.02	+0.03	−0.02	+0.60
	+0.03	+0.02	−0.07	+0.50
1-Glyceryl mononitrate	−0.03	−0.01	−0.07	+0.25
	−0.03	−0.05	−0.04	+0.28
2-Glyceryl mononitrate	−0.02	−0.03	−0.09	+0.22
	−0.02	+0.01	−0.07	+0.19
Nitrite ion	0.00	+0.36	+0.01	+0.39
	0.00	+0.36	0.00	+0.40
Nitrate ion	0.00	−0.02	−0.01	+0.38
	0.00	−0.01	+0.01	+0.40

[a] By duplicate determinations. [b] References 8 and 9. [c] Reference 7. [d] Reference 10.

Table I shows the apparent molar interference factors produced by each nitroglycerin degradation product as measured by duplicate determinations in each assay. Within the limits of assay variation, the kinetic and USP assays were stability indicating. The Bell assay, which is effected *via* hydrolysis and subsequent reaction of the nitrite ion produced, showed predictable interference from inorganic nitrite. This finding is relevant if nitroglycerin degradation in solution produces nitrite ion. Ayres *et al.* (11) studied base-catalyzed nitroglycerin hydrolysis and found ~2 moles of nitrite ion formed for each mole reacted. Under the hydrolysis and reaction conditions of the Bell assay, the glyceryl dinitrates, mononitrates, and inorganic nitrate did not interfere. The modified USP method was nonspecific for nitroglycerin, and all substances tested gave positive interference in this procedure. This finding emphasizes the necessity of the column separation step in the USP procedure when specificity for nitroglycerin is desired, particularly when assaying a primary standard upon which all subsequent determinations are based.

The numerical values of the apparent molar interference factors were in general accord with stoichiometric considerations. Thus, when interference occurred, the glyceryl dinitrates gave about two-thirds of the absorbance value generated by an equimolar amount of nitroglycerin while the glyceryl mononitrates, inorganic nitrite, and nitrate ions gave approximately one-third of this value. Comparison of the kinetic and USP procedures showed that there was more apparent assay variability with the latter. This result was reflected by the larger coefficient of variation found for the USP procedure (CV = 4.6%, n = 14) compared with that for the kinetic method (CV = 1.6%, n = 14).

Since the kinetic and USP assays were shown to be free from interference from potential degradation products of nitroglycerin, stability studies may be conducted with either procedure.

[1] Supplied by Arnar-Stone Laboratories, McGaw Park, IL 60085.
[2] As potassium nitrate and sodium nitrite, respectively, both Baker analyzed reagents, J. T. Baker Chemical Co., Phillipsburg, NJ 08865.

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Specificity of Nitroglycerin Assays: A Response

Keyphrases □ Nitroglycerin—analysis, stability, various procedures compared □ Vasodilators, coronary—nitroglycerin, stability analysis, various procedures compared □ Stability—nitroglycerin, analysis, various procedures compared

To the Editor:

This response will attempt to clarify several points raised by Morrison and Fung (1) concerning a report by Suphajettra *et al.* (2), which had discussed the possible formation of an interaction compound or complex between nitroglycerin in solution and polyethylene glycol 400. It was then suggested (2) that a loss of nitroglycerin "stability" had occurred due to the sequestering effect on it, which prevented its analysis by both UV (3) and colorimetric (4) techniques. As a result, the different apparent nitroglycerin degradation rates due to polyethylene glycol 400 (Fig. 1) as determined by these two methods simply reflected the relative recoveries of intact nitroglycerin or the "assayable" compound, attributable on one hand to "the more drastic hydrolysis procedure employed in the Bell method, which resulted in a relatively greater breakdown of the reaction compound . . ." (2). It was not our intention to imply that a basic deficiency existed with the UV–kinetic assay for determining free nitroglycerin molecules.

Furthermore, the term stability was used generically in the context of the report to describe not only degraded molecules but also those firmly bound or sequestered and, consequently, not available for analysis. Inasmuch as colorimetric nitroglycerin determination depends on the successful breakdown, in some stoichiometric fashion, of the molecule to a decomposition product, *i.e.*, nitrites, this method obviously cannot be regarded as stability indicating. It is precisely because of such a premise that the formation of an interaction compound, which effectively sequestered nitroglycerin from analysis by these two methods, was proposed. Furthermore, had no nitroglycerin "complex" been formed, the presence of nitrite ions, regardless of source (*i.e.*, as a chemical contaminant or as a nitroglycerin decomposition product), would produce linear curves closely parallel to the abscissa (Fig. 1) following the Bell colorimetric assay.

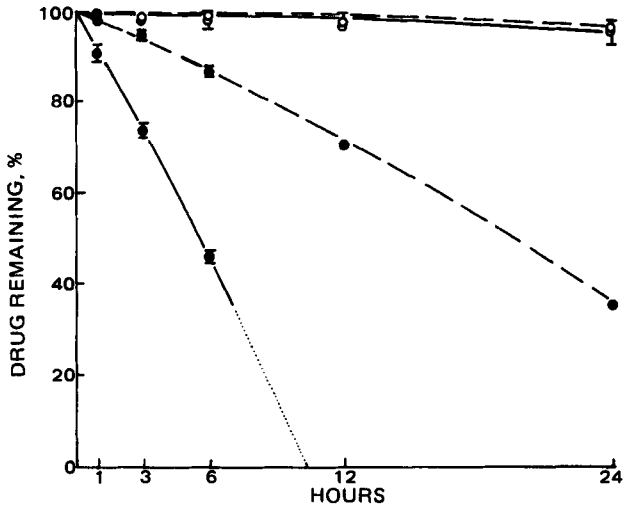

Figure 1—*Percentage of nitroglycerin remaining in povidone* (○) *and polyethylene glycol 400* (●) *solutions as a function of time at 80 ± 0.5° compared using the spectrophotometric* (—) *and colorimetric* (– –) *methods.*

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Percutaneous Butyrolactone Absorption in Rats

Keyphrases □ Butyrolactone—percutaneous absorption, with and without depilation, rats □ Hypnotics—butyrolactone, percutaneous absorption, with and without depilation, rats

To the Editor:

Butyrolactone (I) is a relatively nontoxic hypnotic agent when administered intravenously and orally to rats (1, 2). When I is introduced into the systemic circulation, it is instantaneously and completely converted to γ-hydroxybutyric acid (II). The latter is an endogenous substance in

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