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A flow injection system with in-series ultraviolet and electrochemical detection for the simultaneous determination of lovastatin and butylated hydroxyanisole in a tablet

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Abstract: A flow injection (FI) method has been developed to determine simultaneously a drug with an ultraviolet chromophore, lovastatin, and butylated hydroxyanisole (BHA) in a tablet. The system involves ultraviolet (UV) absorbance detection for the drug and oxidative amperometric electrochemical detection for BHA. The method has been shown to be reproducible for routine determinations with an accuracy of ±1% for lovastatin and ±4% for BHA. Precision for both analytes was approximately ±1% (RSD). The use of FI compared with HPLC for these determinations has led to a dramatic decrease in the time required per assay. The method with UV detection has been shown to be specific for the drug in the presence of potential autoxidation products as well as BHA and its oxidation products. The use of electrochemical detection in series allows the method to be simultaneously specific for low levels of BHA (40 μg/tablet) in the presence of its oxidation products as well as the drug and its potential autoxidation products.

Keywords: *Flow injection analysis; lovastatin; butylated hydroxyanisole; electrochemical detection.*

Introduction

Many pharmaceutical compounds are susceptible to oxidation by a variety of mechanisms [1]. In addition, a large number of these compounds contain an ultraviolet (UV) chromophore which is altered as a result of oxidation. Butylated hydroxyanisole (BHA) is a frequently used pharmaceutical antioxidant which impedes the autoxidation of drugs by acting as a radical-scavenging chain terminator in the autoxidation process [2].

The amounts of BHA and other phenolic antioxidants that are allowed in food, cosmetic and pharmaceutical products are subject to government regulation [3, 4]. Because of such regulations and the potencies of most drugs, any method developed to

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measure simultaneously BHA and a drug would be required to detect microgram quantities of BHA in the presence of milligram quantities of the pharmaceutically active compound.

Ideally this method should be rapid, sensitive, specific, accurate and precise for both analytes. To meet these objectives, the use of a flow injection system employing, in series, a UV absorbance detector for a drug (lovastatin) and an oxidative amperometric electrochemical detector for BHA was investigated.

Experimental

Apparatus

The FI-detector system comprised an isocratic HPLC pump (model SP8770, Spectra-Physics, San Jose, CA, USA) and an electronically actuated 10-port loop injection valve (Valco, Houston, TX, USA) [5, 6] or an HPLC pump and injector system (model HP1090, Hewlett–Packard, Avondale, PA, USA) operated with a shunt of 10 × 0.005 in i.d. stainless steel tubing in place of a column. Detection was accomplished using a photodiode array UV detector at 254 nm (10 milliabsorbance units full scale) (model HP1040A, Hewlett–Packard Co., Avondale, PA, USA) and an amperometric electrochemical detector at +0.60 V versus Ag/AgCl (100 nA full scale) (Model LC-4B Potentiostat, Bioanalytical Systems, West Lafayette, IN, USA). The electrochemical (EC) flow cell (Bioanalytical Systems, model TL-8A) was operated in the direct current oxidative mode with a glassy carbon working electrode, a Ag/AgCl reference electrode, and a platinum auxiliary electrode. The EC detector was enclosed in a Faraday cage which was grounded through the potentiostat ground to prevent stray electromagnetic interference. Regardless of the FI system used, the detectors were always arranged in series with the UV detector encountered first in the direction of the carrier flow. The sequence of the detectors was chosen to ensure the selectivity of the UV detector for the drug in the presence of the products of electro-oxidation formed at the EC detector. Output from each detector was collected on a Hewlett–Packard model 3392A integrator. Peak measurement using area or height yielded equivalent results.

"Coating" or "fouling" of the glassy carbon electrode due to electro-oxidation of BHA was observed with time and caused the EC detector response to diminish. Whereas this phenomenon resulted in a decrease in sensitivity of approximately 50% over 8 h, the decrease observed during the course of a typical analytical run bracketed by standards (approximately 30 injections at 4 injections min^{-1}) was insignificant. EC detector sensitivity was regained by cleaning the working electrode surface according to the manufacturer's instructions. Since bias due to fouling did not interfere with quantification and because electrode response could be easily restored, pretreatment of the electrode surface was not deemed necessary and thus was not attempted.

All sample and standard solutions were injected in triplicate using an injection volume of 4 μl. The carrier solvent flow-rate was 1.0 ml min^{-1}. All determinations were performed under ambient laboratory conditions of temperature (approximately 24°C) and light.

Reagents

All salts and solvents were HPLC grade. All solutions were de-aerated with helium prior to use. The FI carrier solvent was 25 mM NaH_2PO_4 (pH 4.0 with H_3PO_4)–acetonitrile–methanol (33:55:12, v/v/v). The choice of the carrier solvent was based upon the

successful use of this mixture as a sample solvent/diluent in the preparation for analysis by HPLC of tablets containing lovastatin and BHA.

The reference standard for lovastatin was obtained in-house. Lovastatin (mevinolin) contains a conjugated double bond system which provides a UV chromophore with λ_{max} = 247 nm and A (1%, 1 cm) (in acetonitrile) = 418 [7]. Data are provided in respect of a typical tablet containing lovastatin and BHA, for which the method was validated and routinely used. The BHA used in the formulations was a mixture of two isomers containing approximately 99% of 3-tert-butyl-4-hydroxyanisole and approximately 1% of 2-tert-butyl-4-hydroxyanisole. The BHA reference standard was obtained from the United States Pharmacopeial Convention (No. 831, 3-tert-butyl-4-hydroxyanisole, USP, Rockville, MD, USA).

A stock standard solution (100 μl ml^{-1}) containing solely BHA was prepared by dissolving 20 mg of BHA reference standard in 200 ml of carrier solvent; both BHA isomers exhibit identical electrochemical behaviour in the carrier solvent. The resulting solution (5.0 ml) was further diluted to 100 ml with carrier solvent (BHA solution A, 5 μg ml^{-1}).

A working standard solution containing both analytes was prepared by adding 12 ml of BHA solution A to a 200-ml volumetric flask containing 20 mg of lovastatin reference standard. The flask was filled to volume with carrier solvent so that the working standard solution contained 300 ng ml^{-1} of BHA and 100 μg ml^{-1} of drug. Sample and standard solutions were stable for at least 24 h when stored under ambient laboratory conditions.

Procedure

Tablets for analysis varied in their content of lovastatin, but all contained 40 μg of BHA. Samples for testing were prepared by first adding a tablet to an appropriately sized volumetric flask to which carrier solvent was added. The size of flask was chosen so that the final drug concentration was approximately 100 μg ml^{-1}. The system was sonicated for approximately 5 min to cause disintegration of the tablet matrix and dissolution of the analytes. An aliquot of the resulting solution was filtered through a 0.45-μm Nylon-66 filter and the analytes were assayed. Calculation of the sample concentration for both analytes was based upon the detector response compared with that of a standard solution.

Results and Discussion

Using a flow injection system, a non-chromatographic and thus non-separating technique, to determine simultaneously two analytes in a single solution presented a major problem in terms of the selectivity of any measurements made. In order to overcome this difficulty, two selective detectors were employed in series; each detector was exclusive of response to one of the analytes.

The choice of operating conditions for the two detectors was made to maximize signal response for one analyte while minimizing interference. Near 254 nm, the UV absorbance of lovastatin (and many other drugs) is near a maximum whereas the UV absorbance of BHA and its degradation products is near a minimum. This, coupled with the concentration difference of about three orders of magnitude between BHA and lovastatin in the standard and sample solutions, resulted in a UV response that was virtually free of BHA contribution. In order to ensure a $\leq$1% UV absorbance

contribution due to BHA under these wavelength and concentration conditions, the ratio of molar absorptivities of drug to BHA must be ≥0.1.

The response for the electrochemical detector, on the other hand, is due entirely to electro-oxidation of BHA. For the method to be applicable, the drug, its potential degradation products and the oxidative products of BHA must not be electrochemically active. Such was the case for lovastatin in this study. The choice of a working potential of +0.60 V (versus Ag/AgCl) was based upon the hydrodynamic voltammogram shown in Fig. 1; this figure demonstrates that a maximum current for the oxidation of BHA in the flowing stream occurs at potentials ≥ +0.70 V (versus Ag/AgCl). However, at these higher potentials, the background current due to oxidation of trace impurities in the mobile phase and also perturbations caused by the movement of the injection valve were enhanced. As a result, an applied potential of +0.60 V (versus Ag/AgCl) was chosen as a compromise between maximizing current reponse and minimizing interference.

Prior to the routine application of this method to the measurement of lovastatin and BHA in tablet formulations, the method was validated to ensure accuracy, precision, specificity and linearity of detector response over the anticipated concentration range. Three-point recovery studies of both lovastatin and BHA from spiked placebo tablets (75–125% of a formulation dose in 25% increments) indicated that the assay is accurate to within 1% for lovastatin and 4% for BHA (Table 1). The experiment was performed with tablets containing the smallest (5 mg:40 μg) and largest (40 mg:40 μg) ratios of drug to antioxidant. Precision of the detector response was determined by making 10 replicate injections of a solution containing the analytes at 100% of the expected potency concentration level. Precision as indicated by the relative standard deviation (RSD) of the results was approximately 1% for both analytes.

The method is specific for each analyte in the presence of the other as noted earlier. Interference due primarily to flow perturbations caused by the movement of the injection valve as well as to small detector responses caused by the tablet excipients was noted. This interference was manifested in the form of a constant positive assay bias for each drug-antioxidant ratio in the tablet combination. This bias was 0.2% and 0.7% for

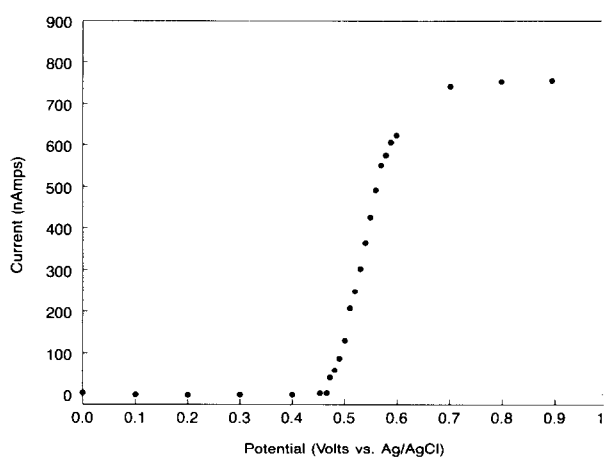

Figure 1
Hydrodynamic voltammogram of a 300 ng ml^{-1} solution of BHA in a carrier solvent of 25 mM NaH_2PO_4 (pH 4.0 with H_3PO_4)–acetonitrile–methanol (33:55:12, v/v/v).

Table 1
Recovery of lovastatin and BHA from spiked placebo tablets

% Dose (%)	Lovastatin added (mg)	BHA added (μg)	Lovastatin found (mg)	BHA found (μg)	% Recovery Lovastatin	BHA
75	3.75	30.00	3.76	30.42	100.3	101.4
100	5.00	40.00	5.04	40.24	100.8	100.6
125	6.25	50.00	6.24	50.20	99.8	100.4
				Mean ± RSD	100.3 ± 0.5%	100.8 ± 1.0%
75	30.00	30.00	30.24	29.94	100.8	99.8
100	40.00	40.00	40.00	38.88	100.0	97.2
125	50.00	50.00	50.20	52.00	100.4	104.0
				Mean ± RSD	100.4 ± 0.5%	100.3 ± 3.4%

the drug and 2% and 4% for BHA in tablets containing 40 mg and 5 mg drug, respectively. Where such a bias is unacceptable, it is anticipated that it may be eliminated by inserting a short (approximately 10 mm) column packed with an inert material (e.g. glass beads) between the injection valve and the detectors to act as a pulse dampener. Generally, pressure increases due to valve movement can be eliminated or minimized through the use of a pulse dampener. Since the biases were constant in this work, however, a pulse dampener was not employed. The FI system provided linear detector responses for lovastatin (UV) and BHA (EC) over concentration ranges from 50% of the least concentrated sample solution to 150% of the most concentrated sample solution (25 μg ml^{-1} to 300 μg ml^{-1} for lovastatin and 100 ng ml^{-1} to 600 ng ml^{-1} for BHA). Linear regression analysis on the calibration curve for the analytes yielded correlation coefficients (R) >0.999 for each.

A typical set of results for a tablet formulation containing 20 mg of lovastatin and 40 μg of BHA indicated a mean (N = 10) assay of 19.7 ± 0.28 mg for the drug and 34.2 ± 0.68 μg for BHA. Assay values ranged from 19.3 to 20.2 mg and 33.0 to 35.5 μg for lovastatin and BHA, respectively. The low assay of BHA is typical, since it has been demonstrated that in this case approximately 15% of the initial content of BHA is lost during manufacture of the tablets.

These results were tested for statistical equivalence to assay results for lovastatin and BHA gnerated through the use of validated HPLC and/or manual spectrophotometric or electrochemical methods. A parametric t-test on paired values was conducted at a significance level (P) of 0.05 on data generated by FI and the other methods from identical samples (N = 10). It was shown that the mean and standard deviation of all sets of results were equivalent.

Conclusions

The simultaneous determination of lovastatin, a drug containing a UV chromophore, and BHA in tablets has been successfully demonstrated using a flow injection system with in-series specific detectors. This technique is generally applicable to a large number of drugs which contain a UV chromophore that may be altered by oxidation, but which are not electrochemically active under the described conditions. The major advantage of the FI procedure is the speed at which assays can be performed (approximately 4 assays per min). The specificity afforded by in-series specific detectors eliminates the need for

more time-consuming chromatographic separation techniques. The use of this method has increased productivity among the analysts performing this analysis while allowing laboratory personnel to make more efficient use of HPLC equipment. This method has been shown to be reproducible and has prompted further development in the applications of FI analysis.

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