Journal of Chromatography, 320 (1985) 445–449
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 17,371

Note

Rapid high-performance liquid chromatographic procedure for nitroglycerin and its degradation products

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(First received August 10th, 1984; revised manuscript received November 7th, 1984)

Given the various products and the variety of testing procedures for nitroglycerin (total assay, content uniformity, dissolution, etc.), a rapid and specific assay is highly desirable. The present procedure is designed to overcome the major shortcoming of previous analyses that reported separations may require run times in excess of 10 min and the use of an internal standard[1–4]. The following criteria were considered of paramount importance in the planning and validation of this method: specificity; short chromatographic run time; high accuracy and precision; and durability, *i.e.*, capability for intensive routine use, particularly with computer-controlled autosampler injection.

In the chromatographic separation of nitroglycerin, the efficiency of a 3-μm octadecylsilane bonded phase was exploited. Planned into the method was an allowance for column deterioration over several months of rigorous use; a significant decrease in the theoretical plate number and resolution can be tolerated while maintaining excellent separation and accuracy.

EXPERIMENTAL

Reagents

Water of high-performance liquid chromatography (HPLC) purity (> 10 MΩ resistance) was obtained with a Milli-Q Reagent Water System (Millipore); methanol and acetonitrile were of HPLC grade.

Apparatus

The HPLC system consisted of a Model 655 liquid chromatograph pump (Hitachi) equipped with a Model 638-41 UV–visible variable-wavelength detector (Hitachi); sample injection was accomplished with a Model 725 autosampler (Micromeritics) and data acquisition with a Model 4270 integrator (Spectra-Physics); the integrator was equipped for dual-channel autosampler operation through an interface (laboratory-designed) and an 8K Basic program (Autocalc; laboratory-written) for control of the chromatographic runs and subsequent calculations. The analytical column was 3.3 cm × 4.6 mm I.D. packed with 3-μm diameter spherical silica with octadecylsilane (C_{18}) bonded phase (Perkin-Elmer). A pre-column filter incorporating a 0.5-μm stainless-steel porous frit (Upchurch Scientific) was installed between

0021-9673/85/$03.30

the autosampler and the column, connected by short lengths of 0.01-in. I.D. stainless-steel tubing. A 2-μm replaceable screen filter (Supelco) was installed externally on the 0.01-in. diameter tubing between the sample injection needle and the injection valve of the autosampler. The column was eluted at ambient temperature at a flow-rate of 3.5 ml/min with a mobile phase consisting of methanol–acetonitrile–water (24:24:52); the column effluent was monitored at 210 nm (0.16 a.u.f.s., 0.5 sec time constant) in a detector flow cell of 5 mm path length.

Procedure

Depending on the sample matrix and solubility considerations, samples may be prepared in aqueous solvents containing up to about 70% of methanol or acetonitrile. The use of pure methanol or acetonitrile is not recommended as they were found to produce several negative baseline deflections appearing within the optimum k' elution range, thereby interfering with quantitation. These disturbances represent local depletion zones caused by adsorption–desorption of the organic modifier with a higher affinity for the bonded phase than the bulk eluent. This effect was encountered in this laboratory during comparison with a published method[3], but was masked by the use of concentrations ranging up to 500 μg/ml and high full-scale detector deflection (1.28–2.64 a.u.f.s.). The use of low concentration samples (*ca.* 5 μg/ml) aids in the prevention of column overloading and clogging. The use of a 0.5 μm pre-column filter was more successful than a guard column in protecting the 3-μm packing from contamination. Also, the 2-μm screen filter between the sample vial and the injection loop makes membrane pre-filtration of samples optional in many instances.

The method is calibrated with an external standard of appropriate concentration prepared from nitroglycerin triturate (10% in lactose) in the same solvent as used for samples.

RESULTS AND DISCUSSION

Linearity, precision and accuracy

Under the reported conditions the linear range of the method extends from below 1 to above 50 μg/ml. Correlation coefficients in linearity studies generally exceed 0.99, and values of 0.9999 have been achieved. The precision and accuracy of the method were tested by standard additions to a placebo and replicate injections of a standard. In a typical standard addition study, the recovery of spiked nitroglycerin was 99.4 ± 0.4%. The relative standard deviation (RSD) or coefficient of variation of replicate standard injections remain well below 1% for concentrations above 2 μg/ml and usually do not exceed 1.5% at concentrations below 2 μg/ml in a well equilibrated system.

Reproducibility

The reproducibility of the method was ascertained by replicate assays of low-potency solid dosage forms and showed RSDs averaging below 1% with no evidence of interference. A new analytical column will generally yield a count of about 40 000 theoretical plates per meter, ensuring excellent resolution of all component peaks. Further, the method was so designed as to retain ample resolution in spite of column

deterioration after several thousand sample injections. Columns exhibiting a decreased peak height and less than half the original plate number retain more than adequate resolution and reproducibility as defined in the system suitability tests[5] for this method.

Interference

During the course of method development, it was noted that a small interfering peak coeluted with nitroglycerin when acetonitrile was used as the sole organic modifier. This situation seemed to be general to C_{18} bonded phases, also being apparent with several other packing materials (Whatman ODS-3, μBondapak C_{18}, etc.). The source of this interference was traced to a terpene contained in a flavoring agent. It was found that a mobile phase modified with equal volumes of acetonitrile and methanol allowed excellent resolution while retaining a run time of under 1 min. Fig. 1

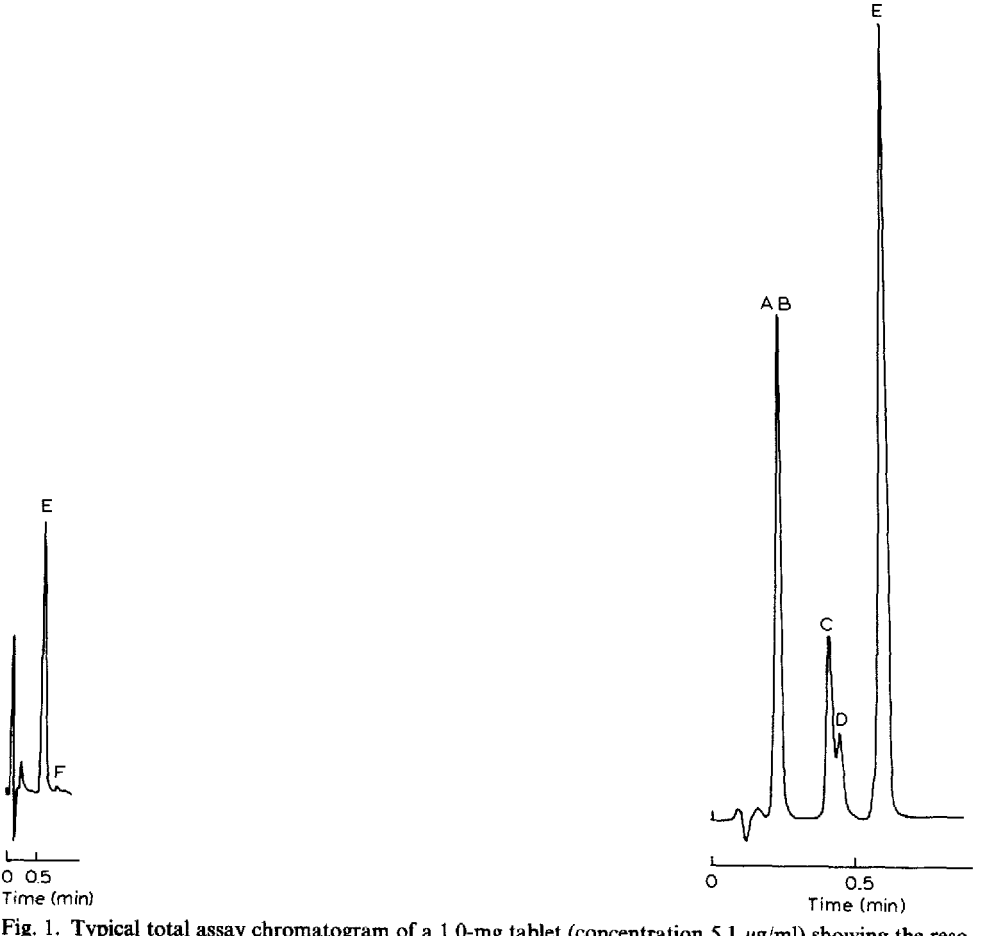

Fig. 1. Typical total assay chromatogram of a 1.0-mg tablet (concentration 5.1 μg/ml) showing the resolution of the terpene component. Peaks: E, nitroglycerin (k' = 5.67); F, terpene (k' = 8.67).

Fig. 2. Separation of nitroglycerin and degradation products using the present procedure. Peaks: A, B, mononitroglycerins (k' = 1.56); C, 1,3-dinitroglycerin (k' = 3.56); D, 1,2-dinitroglycerin (k' = 4.00); E, nitroglycerin (k' = 5.67).

shows a typical total assay chromatogram of a tablet performed under the given conditions.

The resolution of nitroglycerin in the presence of its four major degradation products is shown in Fig. 2. The mononitrates elute as a single peak and the dinitrates are partially resolved, whereas excellent resolution between these isomer pairs and nitroglycerin itself is achieved.

The present method does not afford the complete resolution of all five glyceryl nitrate esters, which may be desirable for certain applications. This can be accomplished, however, using an alternative procedure. The analytical column for this procedure is 7.5 cm × 4.6 mm I.D. packed with 3-μm octylsilane (C_8) bonded phase (Supelco), elution is performed at 2 ml/min with a mobile phase of methanol-water (40:60) and all other conditions are as given above (see Apparatus). The choice of the bonded phase and the mobile phase modifier is an important consideration. In contrast with previous reports, a C_8 bonded phase is more successful than a C_{18} phase in compressing the time axis of the chromatogram[6] while offering selectivity among isomer pairs (*i.e.*, mono- and dinitrates) not found for a phenyl phase[7]. While both methanol and acetonitrile are capable of resolving the glyceryl mononitrates and dinitrates from nitroglycerin, methanol further enhances the selectivity among isomer pairs. The chromatogram in Fig. 3 shows the separation achieved using the alternative procedure (nitroglycerin concentration *ca.* 15 μg/ml). Full resolution occurs within 4 min in the presence of the otherwise difficult to resolve mononitrates.

Column maintenance

The major threats to good column performance have been identified as par-

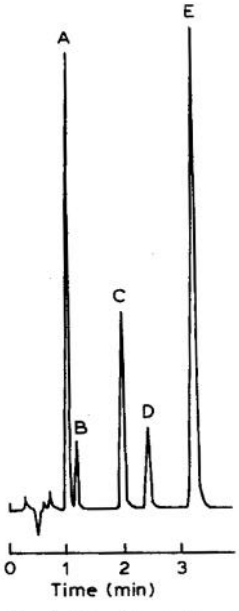

Fig. 3. Results of alternative procedure for full resolution of nitroglycerin and its degradation products. Peaks: A, 2-mononitroglycerin (k' = 2.61); B, 1-mononitroglycerin (k' = 3.25) C; 1,3-dinitroglycerin (k' = 6.21); D, 1,2-dinitroglycerin (k' = 7.86): E, nitroglycerin (k' = 10.82).

ticulate matter and additives. The former have been mainly eliminated through the placement of appropriate filters within the sample flow path (see Apparatus). The on-column accumulation of partially soluble residues may be circumvented by back-flushing the column periodically with filtered HPLC-grade water at 1 ml/min followed by normal flushing with acetonitrile or methanol. Despite the small column dimensions, the inlet and outlet frits can be replaced without mishap, and even stubborn viscous materials can be removed manually from the column head.

CONCLUSIONS

The procedure described has been applied to the assay of nitroglycerin solid dosage forms manufactured by Forest Laboratories. The ternary mobile phase has proved efficient in eliminating interference, thereby allowing a single method to be used for all solid dosage forms. The procedure has further proved to be extremely fast, accurate and amenable to automated injection; using appropriate sample preparation techniques, it may also be applied successfully to the quantitation of nitroglycerin in lactose triturate and other matrices. In all instances, the procedure has met or exceeded expectations.

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