Short communication

Quantitative determination of pivalic acid in dipivefrin-containing ophthalmic solutions by gas chromatography

Lon Hall

Pharmaceutical Sciences, Pharmaceutical Analysis Department, Allergan Pharmaceuticals, 2525 DuPont Drive, Irvine, CA 92715, USA

Abstract

A capillary gas chromatographic method was developed for the analysis of the degradation product pivalic acid in dipivefrin-containing ophthalmic solutions. The linear calibration range was 1.02 to 102 μg/ml ($r = 0.999$) and recoveries were greater than 98%. The relative standard deviation was less than 4.2%. The method can be used to accurately monitor pivalic acid levels for routine quality control and is simple and reliable.

1. Introduction

Dipivefrin hydrochloride (DPE) is a prodrug of epinephrine produced by the diesterification of epinephrine (EPI) and pivalic acid (PVL). It is used in ophthalmic solutions for the treatment of elevated intraocular pressure in chronic open-angle glaucoma [1]. Although the active drug is EPI, for stability purposes both EPI and PVL are considered degradation products of the active ingredient DPE. The United States Pharmacopeia (USP) XXII contains monographs for both DPE raw material and for DPE ophthalmic solutions [2]. Although the USP currently does not have a requirement to assay for degradation products of DPE, it is good science to do so. As an ester in aqueous solution, DPE degrades via hydrolysis, releasing PVL and EPI. The structures of DPE, EPI and PVL are shown in Fig. 1. A method that can quantitate PVL accurately and reproducibly in ophthalmic solutions is desirable.

Procedures for the quantitative analysis of PVL in the literature are mostly by gas chromatography (GC) [3–9], with a few by ion chromatography [10,11] and high-performance liquid
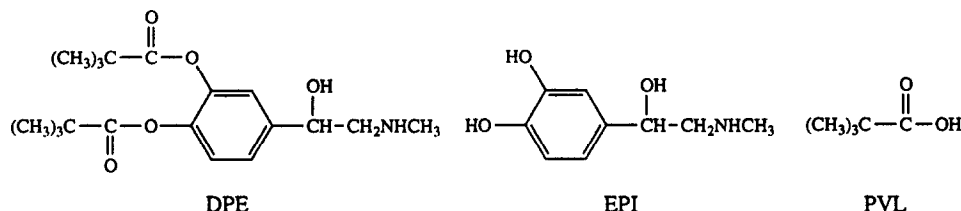

Fig. 1. Chemical structures of dipivefrin (DPE), epinephrine (EPI) and pivalic acid (PVL).

chromatography (HPLC) [12]. Most of these procedures require derivatization in non-aqueous environments or extensive organic workup.

The GC method reported herein is based on an application for volatile organic acids that appeared in the product catalog of J & W Scientific [13]. No derivatization is needed and aqueous samples can be injected directly. The temperature program was modified to slow the rate of temperature increase to allow greater resolution between compounds with similar boiling points. Additionally, the maximum oven temperature was reduced from 250 to 170°C as no further substances were observed at higher temperatures. Experimentation has determined that isovaleric acid is the best choice as an internal standard, as it does not coelute with any of the analyte or system peaks. The GC method reported herein permits quantitative determination of PVL.

2. Experimental

2.1. Apparatus

The GC system consisted of the following: an HP5890 Series II gas chromatograph with a flame-ionization detector (Hewlett-Packard, Wilmington, DE, USA); an HP7673 autoinjector (Hewlett-Packard); an HP Cyclosplitter inlet sleeve (Restek); a deactivated fused-silica guard capillary, 10 m × 0.25 mm (J & W Scientific, Folsom, CA, USA); and a DB-FFAP fused-silica capillary column (0.25 μm film), 30 m × 0.25 mm I.D. with USP Packing Type G25 (J & W Scientific). The DB-FFAP stationary phase is an acid-modified polyethylene glycol similar to the Stabilwax-DA (Restek), Nukol (Supelco) and HP-FFAP (Hewlett-Packard). PE Nelson Access*Chrom GC/LC data system (Perkin-Elmer Nelson Systems, Cupertino, CA, USA) running on a VAX 6410 computer with VMS 5.5-2 was used for data acquisition and analysis.

2.2. Materials and reagents

Acetonitrile (HPLC grade) was obtained from Baxter (McGraw Park, IL, USA). Hydrochloric

acid (ACS reagent grade) was obtained from Mallinckrodt (Paris, KY, USA). Water was distilled twice from an all-glass apparatus then deionized and filtered through activated carbon through a Milli-Q system (Millipore, Waters). A solution of 0.0018 M HCl was prepared by diluting 15 ml hydrochloric acid 1:100 000 in water. Compressed air (hydrocarbon-free grade), compressed hydrogen (high-purity grade) and compressed helium (high-purity grade) were obtained from the Linde Division of Union Carbide (Danbury, CT, USA).

2.3. Standards and samples

An internal standard solution (ISTD) was prepared of 50 ppm isovaleric acid (Aldrich, Milwaukee, WI, USA) in acetonitrile. The working standard solution was 0.01 mg/ml PVL (Aldrich) in 0.0018 M HCl. Both working standard and sample are mixed 1:1 with ISTD prior to analysis.

2.4. GC analysis

Helium was used as the carrier gas. The system had the following parameters: gas flow-rates of 1.5 ml/min for helium, 200 ml/min for air and 20 ml/min for hydrogen; a 1-μl injection volume; injector temperature of 250°C; detector temperature of 250°C; an oven temperature program starting at 100°C for 2 min, followed by a 5°C/min thermal gradient to a maximum of 170°C; an analysis time of 16 min; and the purge valve set OFF at time 0.0 min, ON at 1.2 min and back OFF at 12.2 min (time must be less than run time). Calculations for the samples were based on peak area measurements.

3. Results and discussion

The analysis procedure for PVL was validated for two different DPE-containing ophthalmic solutions. Formula 1 contained DPE, benzalkonium chloride, sodium edetate and salts. Formula 2 contained DPE, levobunolol hydrochloride, polyvinyl alcohol, benzalkonium chloride, sodium edetate and salts. Typical chromato-
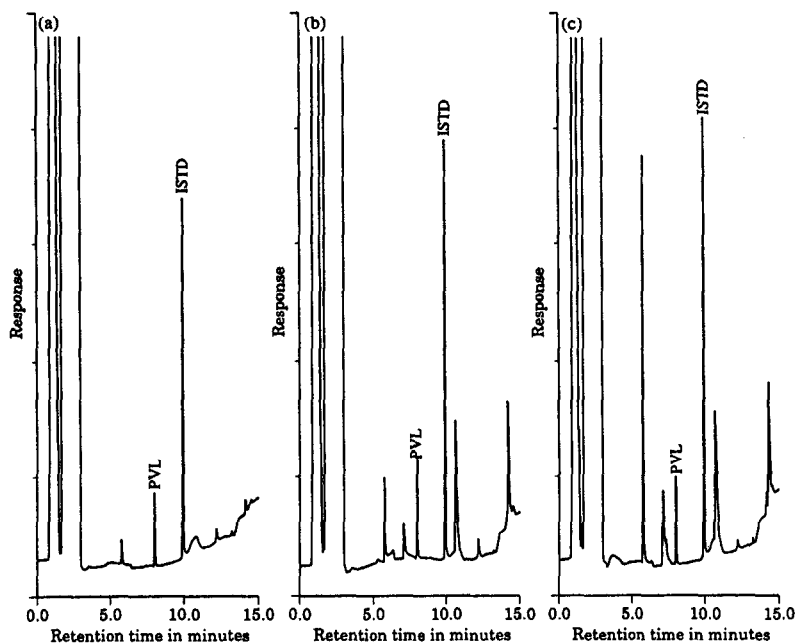
Fig. 2. Chromatograms of PVL standard (a), degraded Formula 1 (b) and degraded Formula 2 (c).

grams of PVL standard and of stability samples at expiry are shown in Fig. 2. Samples of undegraded ophthalmic solutions containing no PVL showed no interferences at the location of the PVL peak (Fig 3).

Linearity in standard diluent was checked from 1.02 to 102 μg/ml [equivalent to 0.0968% (w/w) to 9.68% (w/w) of DPE label claim after dilution for analysis]. The correlation coefficient was 0.999 for both peak area and peak height data. ANOVA results for both peak area and peak height data gave p values less than 0.0001. Therefore, a single-point standard was used.

In addition, linearity in sample matrix was checked from 5.08 to 20.3 μg/ml [equivalent to 0.484% (w/w) to 1.94% (w/w) of DPE label claim after dilution for analysis]. This range corresponds to 1.8 to 7.4% loss of DPE, consistent with the amount of degradation observed in manufactured lots during on-going stability studies. The correlation coefficient was 0.999 for both peak area and peak height data in Formula 1 and 0.998 for peak area data and 0.999 for peak height data in Formula 2. ANOVA results for both formulas and for both peak area and peak height data gave p values less than 0.0001.
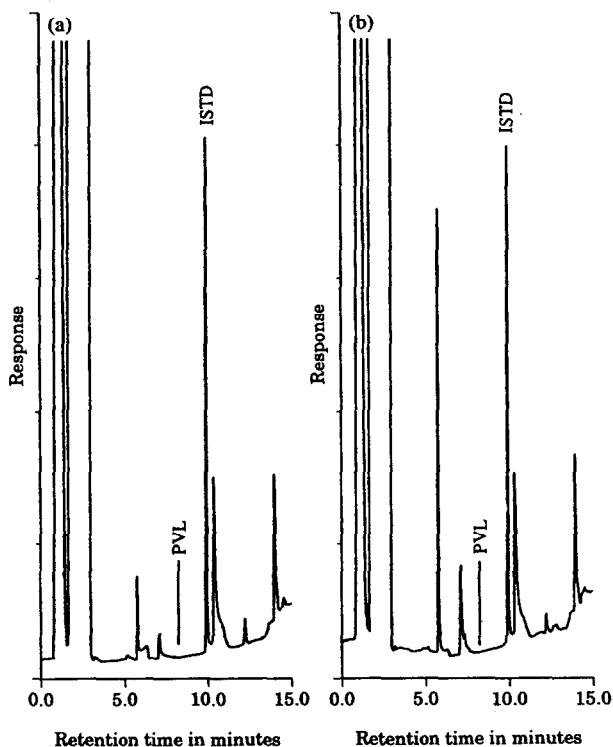

Fig. 3. Chromatograms of undegraded Formula 1 (a) and undegraded Formula 2 (b).

Table 1
Recovery of PVL from spiked samples

		1.94% (w/w)		0.968% (w/w)		0.484% (w/w)	
		Peak area	Peak height	Peak area	Peak height	Peak area	Peak height
Formula 1	Mean (%)	100.9	104.5	99.5	101.9	100.1	100.3
	S.D.	1.1	1.3	3.2	3.5	4.2	1.8
	R.S.D. (%, ±)	1.1	1.3	3.2	3.5	4.2	1.8
	n	3	3	3	3	3	3
Formula 2	Mean (%)	99.2	98.0	99.0	100.7	98.2	100.5
	S.D.	2.9	1.3	1.2	1.9	3.3	2.1
	R.S.D. (%, ±)	2.9	1.4	1.2	1.9	3.4	2.1
	n	3	3	3	3	3	3

Recovery studies to show method accuracy were completed at levels equivalent to 0.484% (w/w), 0.968% (w/w) and 1.94% (w/w) of DPE label claim. Results are summarized in Table 1.

The limit of PVL quantitation is less than 0.255 μg/ml [equivalent to 0.0242% (w/w) of DPE label claim after dilution for analysis]. An average of three peak area measurements at this concentration gave a recovery of 131%, a signal-to-noise ratio greater than 10:1 and a relative standard deviation (R.S.D.) of ± 3.6%.

Three replicates of each 0.968% (w/w) spike used in the accuracy studies were obtained to determine single-day precision. The PVL as % (w/w) of DPE label claim was calculated. On a second day, three replicates each of the same samples were obtained to provide information concerning day-to-day precision values. Person-to-person precision experiments were run on the same samples to provide information about precision values between different analysts as well as providing feedback with respect to clarity of method write-up. Peak area data are summarized in Table 2.

The following parameters for system suitability are guidelines: tailing factor ≤ 1.25 for PVL and ≤ 1.65 for ISTD; and a resolution of ≥ 1.0 between PVL and system peak 1 (SP1) and between ISTD and system peak 2 (SP2) [2]. See Fig. 4 for a chromatogram illustrating the relevant peaks.

Plate counts and capacity factors are not

Table 2
Summary of day-to-day and operator-to-operator precision

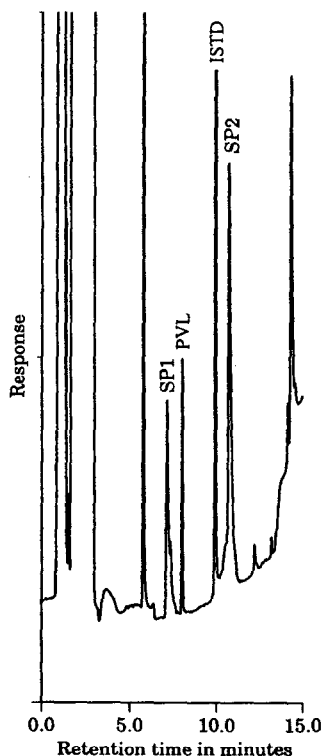|		Operator A		Operator B
		Day 1	Day 2	
Formula 1	Mean (%, w/w)	0.962	0.972	0.977
	S.D.	0.031	0.010	0.021
	R.S.D. (%, ±)	3.2	1.0	2.2
	n	3	3	3
Formula 2	Mean (%, w/w)	0.958	0.985	0.968
	S.D.	0.011	0.030	0.041
	R.S.D. (%, ±)	1.2	3.0	4.2
	n	3	3	3

Fig. 4. Potential interfering system peaks in a chromatogram of PVL standard.

critical system suitability parameters. Both will vary considerably over the lifetime of a particular capillary as ends are cut off to removed oxidized or contaminated portions. Because the stationary phase is a stable nitroterephthalic acid-modified polyethylene glycol and both PVL and isovaleric acid (ISTD) are volatile free fatty acids with the same molecular mass, any variation in the system will result in similar changes in plate count and capacity factor for both substances. The tailing factor is a good control for capillary functionality as increases in this parameter are indicative of the formation of activated sites in the capillary. The tailing factor for the ISTD peak is particularly important, as values above 1.65 are likely to indicate that the system has not resolved ISTD and SP2. The resolution factors are essential controls to assure adequate separation of PVL and ISTD from interfering peaks. These interfering peaks, SP1

and SP2, were shown to be from the Milli-Q water used.

4. Conclusions

A simple, accurate, sensitive and precise GC method was developed to determine PVL in DPE-containing ophthalmic solutions. With this method, the PVL resulting from the degradation of DPE can be monitored accurately at concentrations representing 0.0968 to 9.68% (w/w) of DPE label claim.

Acknowledgements

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