Journal of Chromatography A, 1324 (2014) 238–241

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Short communication

Conventional high-performance liquid chromatography versus derivative spectrophotometry for the determination of 1,3,6-pyrenetrisulfonic acid trisodium salt and 1,3,6,8-pyrenetetrasulfonic acid tetrasodium salt in the color additive D&C Green No. 8 (Pyranine)☆

Simion Jitian[a], Samuel R. White[b], H.-H. Wendy Yang[b], Adrian Weisz[b,*]

[a] Faculty of Engineering Hunedoara, "Politehnica" University of Timisoara, Romania
[b] Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, College Park, MD 20740, USA

ARTICLE INFO

Article history:
Received 30 September 2013
Received in revised form
13 November 2013
Accepted 14 November 2013
Available online 22 November 2013

Keywords:
D&C Green No. 8
Pyranine
1,3,6-Pyrenetrisulfonic acid sodium salt
1,3,6,8-Pyrenetetrasulfonic acid sodium salt
HPLC
Derivative spectrophotometry

ABSTRACT

Specifications in the U.S. Code of Federal Regulations for the color additive D&C Green No. 8 (Colour Index No. 59040) limit the levels of the subsidiary colors 1,3,6-pyrenetrisulfonic acid trisodium salt (P3S) and 1,3,6,8-pyrenetetrasulfonic acid tetrasodium salt (P4S). The present paper describes a comparative study of two possible methods to replace the currently used multi-step TLC/spectrophotometry method of separating and quantifying the minor components P3S and P4S in G8. One of the new approaches uses conventional high-performance liquid chromatography (HPLC) and the other, derivative spectrophotometry. While the derivative spectrophotometric method was shown to be inadequate for the analysis of minor components overwhelmed by components of much higher concentration, the HPLC method was proven highly effective. The closely related, very polar compounds P3S and P4S were separated by the new HPLC method in less than 4 min using a conventional HPLC instrument. P3S and P4S were quantified by using five-point calibration curves with data points that ranged from 0.45 to 7.63% and from 0.13 to 1.82%, by weight, for P3S and P4S, respectively. The HPLC method was applied to the analysis of test portions from 20 batches of D&C Green No. 8 submitted to the U.S. Food and Drug Administration for certification.

Published by Elsevier B.V.

1. Introduction

D&C Green No. 8 (G8, Pyranine, Colour Index No. 59040, mainly the trisodium salt of 8-hydroxy-1,3,6-pyrenetrisulfonic acid, HP3S in Fig. 1) is a color additive used in externally-applied drugs and cosmetics in the U.S. [1]. It is manufactured by sulfonating pyrene to form 1,3,6,8-pyrenetetrasulfonic acid (P4S), followed by hydrolyzing one of the four sulfonic acids to yield HP3S, which is isolated as a trisodium salt [2] (Fig. 1). During manufacture, the intermediate P4S and the synthetic by-product 1,3,6-pyrenetrisulfonic acid trisodium salt (P3S in Fig. 1) may be carried over into the final product. Prior to use as a color additive, G8 is batch-certified by the U.S. Food and Drug Administration (FDA) to ensure compliance with specifications in the Code of Federal Regulations (CFR) [1]. Among the CFR specifications for G8 are limits of "not more than 6 percent" and "not more than 1 percent" for the trisodium and tetrasodium salt of P3S and P4S, respectively. Two analytical methods, both using thin-layer chromatography (TLC), have been developed in the past for analyzing P3S in G8 [3,4]. For certification purposes, P3S and P4S are currently determined by a multi-step procedure that includes streaking the dye solution on a semipreparative TLC plate containing a mixed layer of silica gel and cellulose, developing and drying the plate, scraping the subsidiary color bands, extracting the subsidiary colors from the adsorbent, and, finally, quantifying them by visible spectrophotometry [3,5].

In the pursuit of a simpler method for analyzing P3S and P4S in G8, two approaches were considered: (i) the development of a high-performance liquid chromatography (HPLC) method and (ii) the simultaneous determination of P3S and P4S by derivative spectrophotometry. HPLC has been used successfully for the determination of intermediates and/or subsidiary colors in various color additives [6–11]. Methods using derivative spectrophotometry have the significant advantage of allowing quantification of two or more components of a mixture that have overlapping

☆ Presented at the 244th ACS National Meeting, Philadelphia, PA, August 19–23, 2012.

* Corresponding author. Tel.: +1 240 402 1145; fax: +1 301 436 2961.
E-mail address: adrian.weisz@fda.hhs.gov (A. Weisz).

0021-9673/$ – see front matter. Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.chroma.2013.11.033

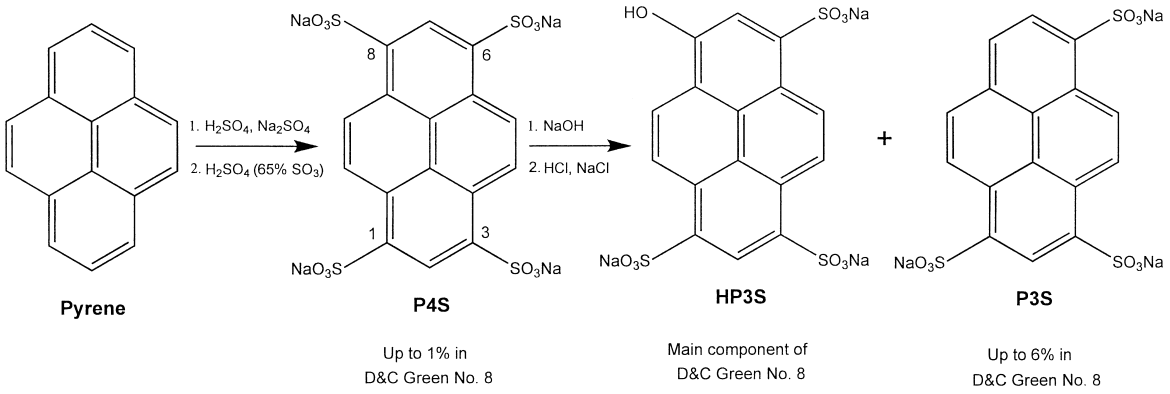

Fig. 1. Manufacture of D&C Green No. 8 by sulfonation of pyrene.

UV–vis absorption spectra without requiring their prior physical separation. Such methods thereby save multiple steps required in chromatographic techniques. The principles of derivative spectrophotometry have been reviewed, and its many applications to individually quantifying color additives present in foods and pharmaceutical preparations have been reported [12–14]. Use of the technique has not been attempted, however, for the analysis of minor color components present in a specific color additive.

The present study compares two possible methods to replace the currently used multi-step TLC/spectrophotometry method of separating and quantifying P3S and P4S in G8. One of the new approaches uses conventional HPLC and the other uses derivative spectrophotometry.

2. Experimental

2.1. Materials

The samples of G8 used in this study were from 18 batches submitted to the FDA for certification in 2009–2011 and two in 2003. The reference materials used were P3S and HP3S, which were prepared previously at over 99% purity [15], and P4S (>98%, Sigma–Aldrich, St. Louis, MO, USA), which was used as received. Methanol, water, and ammonium acetate (NH_4OAc) were of chromatography grade. The hydrochloric acid used (HCl ~37%, Fisher Scientific, Fair Lawn, NJ, USA) was an A.C.S. Plus reagent.

2.2. Analytical HPLC

HPLC analyses were performed with a Waters Alliance 2690 Separation Module (Waters, Milford, MA, USA) and an Agilent Technologies 1200 Series HPLC system (Agilent, Santa Clara, CA, USA). The eluents were (A) 0.1 M aqueous NH_4OAc adjusted to pH 3.54 by addition of HCl, and (B) 0.1 M NH_4OAc in methanol. The column (Kinetex PFP 2.6 μ, 100A, 100 mm × 4.60 mm i.d., Phenomenex, Torrance, CA, USA) was eluted by using consecutive linear gradients of 0–20% methanol in 8.80 min and 20–100% methanol in 0.2 min, followed by 100% methanol for 3 min. The column was re-equilibrated with 0% methanol for 3 min. The effluent was monitored with a detector set at 280 nm, either a Waters 996 photodiode array detector or an Agilent G1315C DAD SL detector. Other conditions included: flow-rate, 1 ml/min; column temperature, 30 °C; injection volume, 3 μl.

Each solution of G8 was prepared for HPLC analysis in a 10-ml volumetric flask by dissolving in water approximately 100 mg of dye. Prior to chromatography, a portion (~1.5 ml) of the color solution was filtered through a syringeless AUTOVIAL 5 filter device (Whatman, Sanford, ME, USA) with a 0.45-μm pore size.

2.3. Method validation

P3S and P4S were quantified by using five-point calibration curves prepared according to an external-standard procedure that involved analyzing separate test portions of a sample of G8 spiked with analytes. The G8 sample used as the matrix (sample 18 in Table 1) had been previously found by HPLC to contain undetectable amounts of the analytes. The data points ranged from 0.45 to 7.63% and from 0.13 to 1.82%, by weight, for P3S and P4S, respectively. The instruments' response was linear over these ranges (correlation coefficients ranged between 0.9997 and 0.9999 for both analytes). Based on the calibration data, the limit of detection (LOD) was 0.15% for P3S and 0.04% for P4S using the Waters instrument, and 0.02% for P3S and 0.01% for P4S using the Agilent instrument. The limit of

Table 1
P3S and P4S found in certified batches of D&C Green No. 8[a] using HPLC, compared with results obtained by using TLC/spectrophotometry.

Sample no.	Manufacturer	P3S found (%)			P4S found (%)		
		HPLC[b]		TLC[c]	HPLC		TLC
		Agilent	Waters		Agilent	Waters	
1	A	1.86	1.80	2.06	0.32	0.28	0.56
2	A	2.00	2.00	2.37	0.17	0.13	0.21
3	A	1.77	1.76	2.26	0.12	<0.12	0.42
4	A	1.15	1.20	1.22	0.24	0.20	0.55
5	A	0.89	0.87	0.71	ND[d]	ND	0.50
6	A	1.25	1.22	1.23	0.04	ND	NF[e]
7	B	1.60	1.58	1.34	1.39	1.40	1.31
8	B	1.96	1.94	3.02	0.19	0.15	0.42
9	B	1.82	1.79	3.04	0.07	ND	NF
10	B	1.19	1.14	1.58	0.13	<0.12	0.37
11	B	1.20	1.17	1.44	ND	ND	0.11
12	B	1.33	1.28	2.00	0.11	<0.12	0.21
13	B	1.40	1.40	1.37	2.65	2.76	2.42
14	B	1.08	1.05	1.44	ND	ND	0.22
15	B	3.34	3.33	3.54	0.07	<0.12	NF
16	C	ND	ND	NF	ND	ND	NF
17	C	ND	ND	NF	0.13	<0.12	NF
18	C	ND	ND	NF	ND	ND	NF
19	C	ND	ND	NF	0.07	<0.12	0.35
20	D	3.05	3.02	3.00	ND	ND	NF

[a] Batches certified 2009–2011, except for samples 15 and 20, certified 2003.
[b] Analyzed at 280 nm.
[c] Thin-layer chromatography/spectrophotometry.
[d] Not detected (Agilent: <0.02% P3S, <0.01% P4S; Waters: <0.15% P3S, <0.04% P4S).
[e] Not found.