ELSEVIER

Journal of Chromatography A, 798 (1998) 173–177

JOURNAL OF CHROMATOGRAPHY A

Short communication

High-performance liquid chromatographic assay of 8-hydroxyquinoline sulfate and its stability in immunobiological preparations

Aleksandar Mihajlović[a,*], Danica Agbaba[b], Dobrila Živanov-Stakić[b], Predrag Ristić[c], Mirjana Đordjević[a]

[a]*Control Department, TORLAK, Institute of Immunology and Virology, Vojvode Stepe 458, 11221 Belgrade, Yugoslavia*
[b]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Vojvode Stepe 450, 11221 Belgrade, Yugoslavia*
[c]*The Institute of Pharmacy of Serbia, Vojvode Stepe 458, 11221 Belgrade, Yugoslavia*

Abstract

Because of the ability of 8-hydroxyquinoline sulfate (8-HQS) to irreversibly bind metals from rubber stoppers, the stability of 8-HQS in tuberculin solutions was investigated. For the determination of 8-HQS, a simple and sensitive reversed-phase HPLC method with detection at 240 nm was developed and validated. Rapid decreases in concentrations of 8-HQS were found in samples stored in original vials which were exposed to different temperatures and vial positions.

Keywords: Stability studies; Pharmaceutical analysis; Hydroxyquinoline sulfate; Tuberculin

1. Introduction

According to the GMP regulations, active substances or preservatives used for human drug formulations are required to be tested for interactions with other constituents and packing [1].

8-Hydroxyquinoline sulfate (8-HQS) is often used as a preservative in immunobiological preparations of tuberculin (PPD). Its strong preservative activity was found to be based on irreversible complexing of metal ions. The same properties were found to be the probable reason for the interaction with metals from rubber stoppers, inducing a very rapid decrease in total concentration of 8-HQS in a solution [2,3]. The previous studies showed that interactions of 8-HQS with other constituents from PPD solutions were negligible [4,5].

Different methods have been used for the determination of 8-HQS: titrimetry [6,7] polarography [8], colorimetry [9,10] and chromatography [11–15]. HPLC procedures were mostly based on indirect determination of 8-HQ, preceded by formation of its metal complexes, using either mobile phases containing metal salts such as nickel acetate or nickel chloride [11,12], or formation of fluorescence metal complexes, obtained by postcolumn derivatization [13].

In order to avoid complexation or derivatization of 8-HQ, our goal was to develop a simple HPLC assay for its direct determination in PPD samples. Therefore, this work focused on assurance of an inert HPLC system (metal free) and optimisation of the conditions, including a selection of the proper re-

*Corresponding author.

0021-9673/98/$19.00
PII S0021-9673(97)01085-6

versed-phase column or mobile phase to obtain a shorter retention time of 8-HQ, reproducible and precise results.

The influence of storage conditions, such as different temperature and vial positions, on the level and kinetics of 8-HQS in samples of PPD was also investigated.

2. Experimental

2.1. Chemicals

8-HQS (purity >97%) was purchased from Fluka (Ulm, Germany). Acetonitrile was HPLC grade (Riedel-de Haen, Seelze, Germany). All other chemicals and organic solvents were of high analytical grade (Merck, Darmstadt, Germany). PPD-T is a product of the Torlak Institute of Immunology and Virology (Belgrade, Yugoslavia).

2.1.1. Composition of PPD-T tuberculin

The solution (2.5 ml) consists of 3 I.U. of tuberculo-protein (about 60 ng), 0.005% of Tween 80, and 0.01% of 8-HQS (purity >97%) dissolved in phosphate buffer (pH 7.38).

2.1.2. Composition of stoppers (type PH21/50 red-brown)

Besides a basic polymer (chlorbutyl), ZnO as an activator is present.

2.2. Equipment

2.2.1. HPLC

The system consisted of a Multisolvent pump Model ICI LC 1150 (GBC, Dandenong, Australia), an ICI LC System Organiser (GBC), an UV detector Model 1205 (GBC) and an on-line vacuum Degasser Model ERC-3415. For graphic interpretation of results WINCHROM software (version 1.2) was used. As an injector Model 9725i (Rheodyne, Cotati, CA, USA) with 10-μl polyether ether ketone (PEEK) sample loop (1/16 in. O.D.; 1 in.=2.54 cm) was used. For connection, PEEK tubing (1/16 in. O.D.× 0.010 in I.D.) were used. Injection was performed with a 100-μl syringe (SGE, Australia).

2.2.2. Column

A model 250GL2-C4-30/5 glass-lined reversed-phase column (250×2 mm, Nucleosil, 5 μm, 300 Å) preceded by a guard column type 10GLCC$_4$-C$_4$ (both from SGE) were used as the stationary phase. Separation was performed at room temperature.

2.2.3. Mobile phase

The elution was achieved with 65% of A and 35% of B where A=acetonitrile and B=water of pH 2.5 adjusted with 85% orthophosphoric acid. Both solutions were previously filtered (0.45 μm) and degassed by ultrasonic vibration. The flow-rate was 0.2 ml/min.

2.2.4. Detection

The detection was performed at 240 nm in sensitivity range of 1.0. The integration parameters were set at: 2 (attenuation), $1.8 \cdot 10^3$ (threshold), 0.01 (peak width).

2.2.5. Mercury lamp

For the visual detection of the chelate complex of 8-HQ in stoppers a mercury table lamp (366 nm) was used.

2.3. Sample preparation

2.3.1. Standard solution

The standards were prepared by dissolving 8-HQS in phosphate buffer (pH 7.38) in concentrations of 0.005, 0.01, 0.025, 0.05, 0.075, 0.1 and 0.125 mg/ml, and filtering. Every injection was repeated five times.

2.3.2. Sample solutions

The samples were prepared by dissolving 8-HQS in prepared tuberculin solutions (dilution in buffer pH 7.38 to 3 I.U.). The samples were then closed with rubber stoppers and stored under four conditions (vertical at 4°C and 22°C, and horizontal at 4°C and 22°C) for periods of 2, 7, 10, 14, 21, 30 and 60 days. All samples were stored without shaking and those in the vertical positions were carefully closed, strictly avoiding contact with stoppers. Before injection the samples were filtered (0.2 μm).

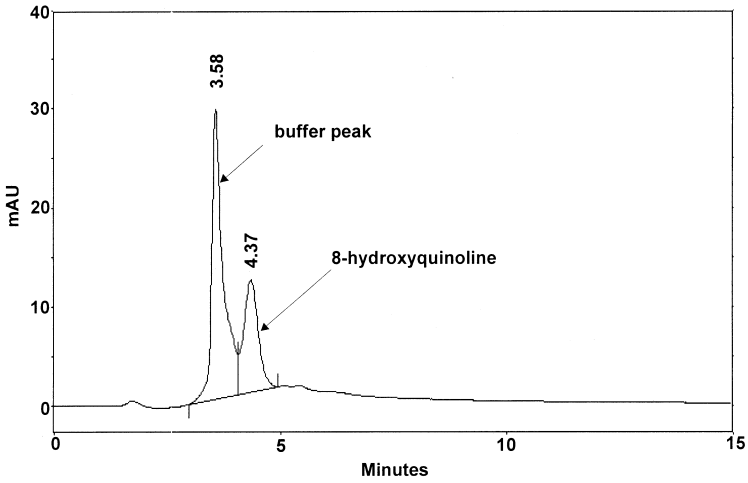

Fig. 1. Chromatogram of PPD-T (the sample stored for 7 days at 4°C in a vertical position, 0.87 μg of 8-HQS).

3. Results and discussion

3.1. HPLC optimization

Previously reported HPLC assays of 8-HQ and derivatives were carried out using different stationary (phenyl silica [11], polystyrene porous polymer [12] or microparticulate C_{18} [13]) and mobile phases containing acetonitrile–buffer or methanol–buffer mixtures. In order to obtain optimal resolution and to avoid peak tailing of 8-HQ and its derivatives, Ostaszewski and Vavrek [14] used a Hamilton PRP-1 column in a metal-free PEEK casing; the retention time of 8-HQ was about 21 min. A glass-lined column and PEEK tubing, fittings and injection loop were used in our experiments for the separation of 8-HQ.

8-HQ has two acid–base constants (pK_1=5.0 and pK_2=9.9 at 20°C) and it is necessary to use an acid mobile phase to avoid peak splitting and reduce tailing [15]. Mobile phases of various compositions of acetonitrile and water (pH range from 2.5 to 6.5), and reversed-phase columns with different kinds of packing (C_8, C_{18}, C_4) were tested. An unavoidable peak arose with PPD samples which contained 8-HQS dissolved in phosphate buffer (pH 7.38). An intensive peak at ~3 min, almost totally covered the 8-HQS peak when using either C_8 or C_{18} column. Optimal separation was achieved using a Nucleosil C_4 column (250×2 mm, 300 Å, 5 μm) and a solvent system of: A=acetonitrile, B=water, pH 2.5 (65:35, v/v). Under these conditions, 8-HQS eluted in about 4.3 min. The obtained retention time was 2–5 times shorter than had been previously reported [11–13]. The chromatogram of 8-HQS in PPD-T samples is shown in Fig. 1. The summary results of total amounts of 8-HQS (%) are given in Table 1. The statistics per day showed satisfactory accuracy and precision (Table 2). The average recovery was 97.6% and limit of detection was 10 ng which is near

Table 1
Quantities (%) of 8-HQS in PPD-T stored at different positions and temperatures

Position	Temperature (°C)	Time (days)						
		2	7	10	14	21	30	60
Horizontal	22	85.58	57.63	47.63	34.62	23.82		
Horizontal	4	89.49	76.78	71.00	59.85	56.87		
Vertical	22	94.63	74.07	72.08	56.24	49.09	30.92	22.57
Vertical	4	93.07	87.31	83.18	71.85	71.12	63.68	51.50

Table 2
Precision in determination of 8-HQS by HPLC method

Time (days)	Temperature (°C)	Position	Linearity (R^2)	Amount[a] (%)	R.S.D. (%)	S.E.
2	4	V		93.07	2.10	0.79
	4	H	0.9978	89.49	2.23	0.84
	22	V		94.63	2.69	1.02
	22	H		85.58	2.14	0.81
7	4	V		87.31	2.24	0.85
	4	H	0.9975	76.78	3.03	1.14
	22	V		74.07	2.38	0.89
	22	H		57.63	0.65	0.25
10	4	V		83.18	3.07	1.16
	4	H	0.9981	71.00	1.96	0.74
	22	V		72.08	0.66	0.25
	22	H		47.69	3.05	1.15
14	4	V		71.85	1.32	0.49
	4	H	0.9961	59.85	1.28	0.48
	22	V		56.24	2.75	1.04
	22	H		34.62	2.65	1.00
21	4	V		71.12	1.04	0.39
	4	H	0.9983	56.87	2.01	0.76
	22	V		49.09	0.88	0.33
	22	H		23.82	1.93	0.73
30	4	V	0.9968	63.68	1.12	0.42
	22	V		30.92	2.30	0.87
60	4	V	0.9988	51.50	1.88	0.71
	22	V		22.57	1.83	0.69

[a] $n=7$.

experimental value (25 ng). Analysis of seven calibration curves ($n=5$) over a 2-month period indicated that the correlation coefficient was always >0.995. The day-to-day relative standard deviation of the slopes of the correlation curves was 7.1%.

The significant decrease in 8-HQS concentration was noticed in PPD samples of the same batch. The missing of secondary peaks at chromatograms showed that there was no possible degradation of 8-HQS in the solution. Regarding the properties of phenol-like compounds, such as 8-HQ which possess high volatility, we noticed the presence of preservative in the rubber stoppers. The fluorescent yellow-coloured area on the stoppers was visually detected under a mercury lamp (366 nm) in all PPD samples, even ones stored in the vertical position without any contact between the stopper and the solvent. Therefore the kinetics of preservative loss from PPD solutions was further investigated. The linear dependence of variables t (days) and log C (%) enabled the calculation of kinetic parameters $t_{1/2}$ (half-time of reaction) and k (coefficient of velocity) by the equations: $\log C=-k/2.303t+\text{constant}$ and $t_{1/2}=0.693/k$. The kinetic parameters are shown in Table 3.

The results showed very rapid decrease in 8-HQS in both vial positions of sample solutions at 22°C. Almost similar decreases were shown in samples stored in a horizontal position at 4°C and in a vertical position at 22°C ($t_{1/2}=25$–27 days). Even without

Table 3
Kinetic parameters of 8-HQS in PPD

Position	Temperature (°C)	k (day^{-1})	$t_{1/2}$ (days)
Horizontal	22	0.0693	10
Vertical	22	0.0258	27
Horizontal	4	0.0276	25
Vertical	4	0.0106	65

contact between solvent and stopper, the results showed a significant decrease in 8-HQS per week (up to 50%) for samples stored in a vertical position at 4°C. The stability results confirmed that the expiry date of PPD could be significantly reduced considering the loss of preservative in immunobiological preparation.

4. Conclusion

The described HPLC method was found to be simple, accurate and reproducible for assay and stability studies of 8-HQS in PPD preparation.

Acknowledgements

The authors are grateful to the Institute of Pharmacy of Serbia for help with HPLC measurements and the Institute Torlak for financial support.

References

[1] K. Krummen, W. Grimm, Stability Testing in Japan, the EEC and the USA, WVA, Stuttgart, 1993.
[2] H.R. Held, S. Landi, H. Pivnick, Bull. WHO 39 (1968) 809.
[3] H.R. Held, S. Landi, Bull. WHO 33 (1965) 395.
[4] H.R. Held, S. Landi, J. Pharm. Sci. 57(4) (1968) 605.
[5] H.R. Held, S. Landi, J. Pharm. Sci. 63(8) (1974) 1205.
[6] The US Pharmacopoeia 23rd Revision, US Pharmacopoeial Convention, Rockville, MD, 1995.
[7] Pharmacopoeia Helvetica, Département Fédèral del' Interieur, Berne, 7th ed., 1993.
[8] V. Mejstrik, L. Drzleva, Z. Sagnes, Chem. Prum. 38(7) (1988) 361.
[9] S.S. Atemchenko, V.V. Petrenk, N.P. Zhovna, V.A. Tsilinko, Zh. Anal. Khim. 40(4) (1985) 744.
[10] N.P. Yavors'kii, E.M. Babich, E.P. Koren'kova, Farmatseut. Zh. 19(4) (1986) 29.
[11] E.J. Wojtowicz, J. Pharm. Sci. 73 (1984) 1430.
[12] F.W. Ezzedeen, S.J. Stohs, M. Stublar, J. Chromatogr. 276 (1981) 121.
[13] K. Miura, H. Nakamura, H. Tanaka, Z. Tamura, J. Chromatogr. 210 (1981) 536.
[14] I. Nagiel-Ostaszewski, M.T. Vavrek, J.H. Weisburger, Xenobiotica 6 (1991) 751.
[15] D. Agbaba, A. Mihajlović, P. Beljanski, D. Živanov-Stakić, S. Vladimirov, Chromatographia 45 (1997) 145.