High performance liquid chromatography of X-ray film developing baths

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A simple and fast high performance liquid chromatographic (HPLC) method for the determination of three main compounds (hydroquinone, metol and phenidone) in X-ray film developing solutions was developed. The optimal conditions for the separation were established by varying experimental parameters such as stationary phase polarity, metanol concentration, mobile phase pH. The optimized separations were performed on a Separon SGX CN column within 8 min by an isocratic elution with 25 mmol/l aqueous phosphate buffer (pH 6.8) containing 30% (v/v) CH_3OH, and UV detection at 254 nm.

The method was validated in terms of precision, linearity, limit of detection, accuracy and successfully applied for the analysis of X-ray film developing baths. The recovery tests established for two samples were within the range 100 ± 5%.

Key words: HPLC, hydroquinone, metol, phenidone, developers

INTRODUCTION

Black and white photographic processes are extensively employed by hospitals and many private medical companies to obtain X-ray plates. The main components present in a photographic developer are the developing agent (hydroquinone together with phenidone or metol) and an antioxidant (sulfite). The analysis of these baths is important in determining the quality and effectiveness of the developing process [1]. Moreover, in order to minimize unwanted environmental contamination, the effluents from used baths should be collected and the harmful compounds should be converted to alternate forms (*e.g.*, CO_2, N_2, H_2O, etc.). In the last decade various oxidation techniques have been investigated for this purpose [2–4]. The main problem of such investigations is the rapid and simple monitoring of the contaminants before and during their decomposition. Since common X-ray photographic developing baths usually contain more than one developing agent with very similar chemical properties, analytical methods allowing a simple, rapid and sensitive determination of all compounds in a single analysis are required.

Separation techniques such as high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) can offer significant advantages when considering samples containing several analytes having similar chemical properties. In the last few years, capillary electrophoresis has been successfully introduced in the analysis of various photographic solutions [5–11]. However, since most of the developing agents are neutral compounds, the conventional CE technique is not suitable for their separation.

Although many excellent applications have been performed by HPLC technique [12], to our knowledge there are no reports concerning the HPLC analysis of X-ray developing solutions.

The main aim of this study was to evaluate the HPLC technique for a rapid and simple determination of hydroquinone, metol and phenidone in common X-ray film developing solutions.

EXPERIMENTAL

The HPLC instrumentation consisted of a HPP 5001 series high-pressure pump with an injection valve equipped with a sample loop of 25 µl (Laboratorni Pristroje, Prague). The detector was a UV/Vis LCD 2563 photometer (Laboratorni Pristroje, Prague) set to absorb at 254 nm. The results and data were collected and plotted on an SP 4290 plotter/integrator (Spectrophysics CA, USA).

Separations were performed on a 10-µm Separon SGX CN (150×3 mm I.D.) column (TESSEK Ltd., Prague). The mobile phase flow rate was 0.3 ml/min.

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All mobile phase and standard solutions were prepared using doubly distilled water. HPLC grade metanol was purchased from Merck (Darmstadt, Germany). All other reagents were of analytical-reagent grade, obtained from Aldrich (Milwaukee, Wis., USA).

The stock solutions (0.005 mol/l) of the developers were prepared daily by dissolution in 0.001 mol/l HCl. All working solutions were prepared just before use by suitable dilution. Mobile phase was prepared by neutralization of 0.025 mol/l KH_2PO_4 solution in a metanol-water mixture with 0.1 mol/l KOH to pH 6.8.

All mobile phase and sample solutions were filtered through a 0.45 μm membrane filter and degassed by ultrasonication.

RESULTS AND DISCUSSION

Characterization of the developing baths

Black and white developers usually contain three main ingredients: the developing agents, the preservative (sulphite) and the alkali (carbonate, tetraborate, hydroxide, etc.) [13]. The two most frequently used developing agents are hydroquinone (1,4-dihydroxybenzene) and metol (4-methylaminophenol sulphate). Hydroquinone is an excellent electron donor but a slow developing agent, because it is not hydrophobic. Developing agents with good surfactant properties, such as metol, transfer electrons efficiently, but the oxidized forms then take time to move aside for unused molecules. When hydroquinone and metol are combined, the result is a fast-acting developer that develops to a fairly high contrast. This co-operation between two developing agents of different surfactant properties is known as super-additive development. Phenidone (1-phenyl-3-pyrazolidone) is often used as a hypo-allergenic substitute for metol. Phenidone not only acts in the same way as metol, that is, the combination of the two is super-additive, but phenidone possesses also the property of being regenerated by hydroquinone. This results in a developing solution that is not only active from the start, but also retains its activity longer.

Method development

The chemical structure of the study compounds is shown in Fig. 1. Since all the analytes studied have UV chromophores, UV detection at 254 nm was selected for this investigation. At this wavelength all three developing agents exhibit an absorbance sufficient for sensitive detection.

Due to the strongly hydrophylic nature of the analytes studied, the influence of the column pack-
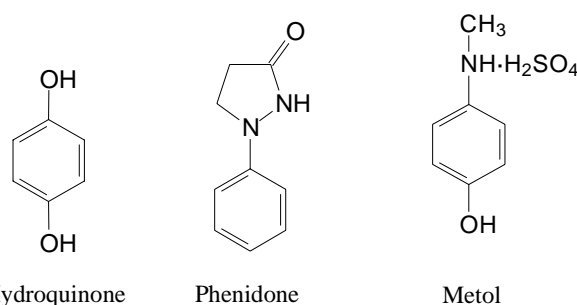

Fig. 1. Structure of the compounds studied

ing polarity (Separon C_{18} and Separon SGX CN) on the chromatographic behaviour of the developers was briefly investigated. The results showed that for all three analytes the peaks obtained with a polar Separon SGX CN stationary phase were slightly sharper and more symmetrical than those obtained with the unpolar Separon C_{18} phase. As an example, Fig. 2 compares the chromatograms of phenidone standard obtained with both columns. Similar behaviour was also observed for hydroquinone and metol (results not shown). Therefore, in all further experiments the Separon SGX CN stationary phase was used.

Initially, several mobile phases consisting of KH_2PO_4, H_2O and CH_3OH (pH 6.0), were tested for their ability to separate the analytes. The total concentration of KH_2PO_4 was kept constant (5 mmol/l), while the ratio of the two solvents (H_2O:CH_3OH) varied from 80:20 to 50:50 (v/v). As expected, by increasing the metanol concentration, the retention time of all analytes decreased. Unfortunately, none of the mobile phase systems tested resolved metol and phenidone. The best overall resolution was obtained with a mobile phase containing 30% of metanol.

Another important parameter affecting the separation selectivity is the pH of the mobile phase. Figure 3 illustrates the effect of the mobile phase
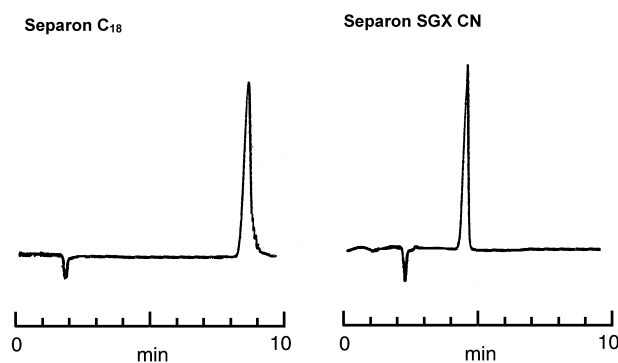

Fig. 2. Chromatograms of phenidone standard obtained with two different stationary phases. Mobile phase: 5 mmol/l KH_2PO_4, 40% (v/v) CH_3OH, pH 6.0. UV detection at 254 nm
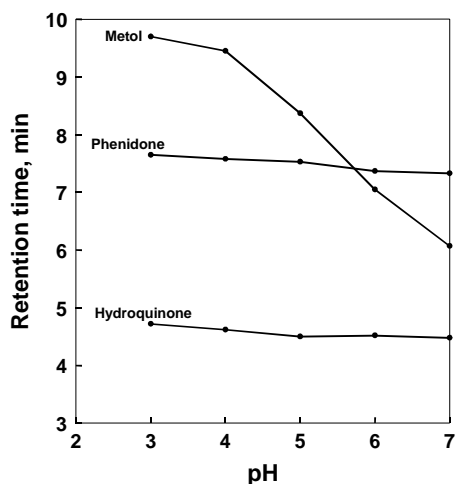
Fig. 3. Effect of mobile phase pH on the retention time of developers. Mobile phase: 5 mmol/l H_3PO_4, 30% (v/v) CH_3OH. UV detection at 254 nm

Fig. 5. Chromatogram of a standard solution under optimum conditions. Mobile phase: 25 mmol/l KH_2PO_4, 30% (v/v) CH_3OH, pH 6.8

pH on the retention time of the analytes studied. In the pH range studied, hydroquinone and phenidone exist in the molecular forms. On this account, the varying of pH has but little effect on their retention. In contrast, in the case of metol, due to the deprotonation its net positive charge decreases with pH. This deprotonation process results in reducing the polarity of metol and in a significant decrease in retention time. Moreover, the mobile phase pH strongly affects the detectability of metol. The results presented in Fig. 4 show that the peak area of metol significantly increases by increasing the pH, indicating that the deprotonated form of metol as compared to protonated one exhibits a much higher absorptivity at 254 nm.

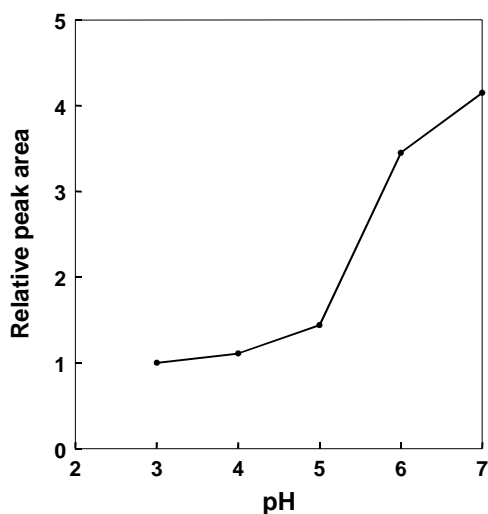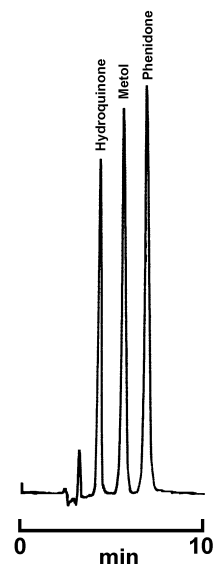Based on these results, pH 6.8 was selected for further separations, because it provided the shortest

separation time with acceptable resolution and detectability. The chromatogram obtained under optimum conditions for a standard solution is shown in Fig. 5. As one can see, an excellent separation of three compounds was obtained in less than 8 min.

Validation

Using the optimal experimental parameters described above (a mobile phase of 25 mmol/l KH_2PO_4, 30% (v/v) CH_3OH, pH 6.8) several analytical performance characteristics important for quantitative analysis were measured. The preliminary validation of the method included assessment of stability of the solutions, precision, linearity, detection limits and accuracy.

Being strong reducing agents, all developers are very sensitive to oxygen, especially in alkaline solutions. Although the stability test is often considered as part of the ruggedness of the procedure, it should be carried out at the beginning of the procedure validation, because it predetermines the validity of the data of the other tests. As expected, the stability of aqueous solutions ($1 \cdot 10^{-3}$ mol/l) of hydroquinone, metol and phenidone strongly depended upon the pH. These solutions were stable in the dark, at room temperature and pH values lower than 5, for at least 10 hours. At basic pH values the concentrations of these developers gradually decreased. For example, at pH 9, hydroquinone concentration decreased to about 84% after 10 h. A similar behaviour was observed also for phenidone and metol. Therefore, all standard solutions were prepared daily by dissolution in 0.001 mol/l HCl.

Fig. 4. Effect of mobile phase pH on the metol peak area. Conditions as in Fig. 3

The accuracy of the ethod was determined by measuring the repeatability and intermediate accuracy (between-day precision). In order to determine the repeatability of the method, five replicate injections of a mixed standard solution of analytes (5 · 10^{-4} mol/l each) were done. The intermediate accuracy was evaluated over 3 days by performing five successive injections daily. The obtained relative standard deviations (RSD) of the retention time and peak area are summarized in Table 1.

Table 1. **Accuracy of the HPLC method expressed as RSD (%) values for retention time and peak area (n = 5)**

Analyte	Repeatability		Intermediate accuracy	
	Retention time	Peak area	Retention time	Peak area
Hydroquinone	0.31	2.28	0.36	2.80
Metol	0.28	1.59	0.34	2.02
Phenidone	0.25	1.45	0.31	1.75

Detector response linearities were assessed for each analyte with six mixed standard solutions of the analytes. Each concentration level was injected three times. The regression curves were obtained by plotting the peak areas *versus* concentration using the least square method. To determine the limit of detection (LOD), solutions with low concentrations of analytes were injected in order to find a concentration corresponding to a signal-to-noise ratio of 3. The data obtained are summarized in Table 2. These data show the suitability of the proposed HPLC method for its application to real samples. A significantly higher detection limit for hyd-

Table 2. **Linearity data and LOD values**

Parameter	Hydroquinone	Metol	Phenidone
Linear range (mol/l)	$2 \cdot 10^{-4}$–$2 \cdot 10^{-3}$	$1 \cdot 10^{-5}$–$1 \cdot 10^{-3}$	$1 \cdot 10^{-5}$–$1 \cdot 10^{-6}$
r^2	0.9987	0.9999	0.9995
LOD (mol/l)	$7 \cdot 10^{-5}$	$2 \cdot 10^{-6}$	$3 \cdot 10^{-6}$

roquinone is caused by its poorer absorptivity at 254 nm.

Sample analysis

To evaluate the proposed HPLC system on real samples, it was applied to the analysis of a commercial hydroquinone–metol (DK-50) and hydroquinone–phenidone (XAD-3) X-ray film developing solutions. The only sample
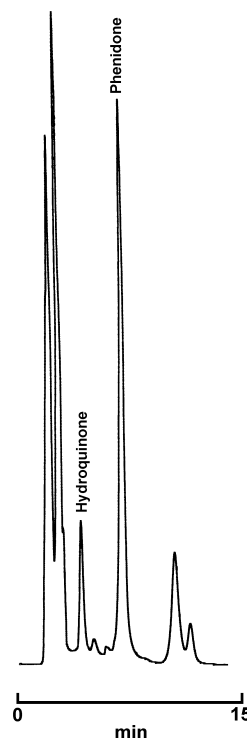

Fig. 6. Chromatogram of 1:100 diluted spent hydroquinone–phenidone developer sample

pre-treatment stage involves filtration of the sample through a 0.45 μm pore filter and appropriate dilution. Figure 6 shows the chromatogram obtained for a 1:100 diluted hydroquinone–phenidone developer sample.

The accuracy of the method was evaluated by adding known amounts of each component to X-ray film developing solutions and analyzing the spiked samples with the proposed HPLC method. The results are given in Table 3: the recoveries in all the cases were within the range 100 ± ± 5%. These results suggest that interferences by the other matrix components are not significant and the HPLC conditions are suitable to obtain an adequate of the accuracy method.

Table 3. **Results of determination of developing agents (g/l) in X-ray developer solutions (n = 3)**

Sample	Analyte	Found, g/l	Added, g/l	Found total, g/l	Recovery, %
DK-50	Hydroquinone	2.85	2.00	4.91	103
(fresh)	Metol	1.43	1.00	2.39	96
XAD 3	Hydroquinone	2.16	1.00	3.11	95
(spent)	Phenidone	0.98	0.50	1.47	98

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RENTGENO FOTOGRAFINIŲ RYŠKALŲ ANALIZĖ EFEKTYVIOSIOS SKYSČIŲ CHROMATOGRAFIJOS METODU

S a n t r a u k a

Optimizuotos trijų ryškiklių (hidrochinono, metolo ir fenidono) atskyrimo ir nustatymo efektyviosios skysčių chromatografijos metodu sąlygos: kolonėlė – 150 × 3 mm Separon SGX CN (10 µm); judrioji fazė – 25 mmol/l fosfatinis buferis, 30% (v/v) CH_3OH, pH 6,8; UV detektavimas esant 254 nm bangos ilgiui. Išmatuotos pagrindinės analizinės charakteristikos: kalibracinės kreivės yra tiesinės koncentracijų intervale $\cdot 10^{-5}$–1 $\cdot 10^{-3}$ mol/l metolui ir fenidonui bei 2 $\cdot 10^{-4}$–2 $\cdot 10^{-3}$ mol/l hidrochinonui; aptikimo ribos yra lygios 7 $\cdot 10^{-5}$ mol/l hidrochinonui, 3 $\cdot 10^{-6}$ mol/l fenidonui ir 2 $\cdot 10^{-6}$ mol/l metolui. Metodas pritaikytas ryškikliams nustatyti rentgeno fotografiniuose ryškaluose.