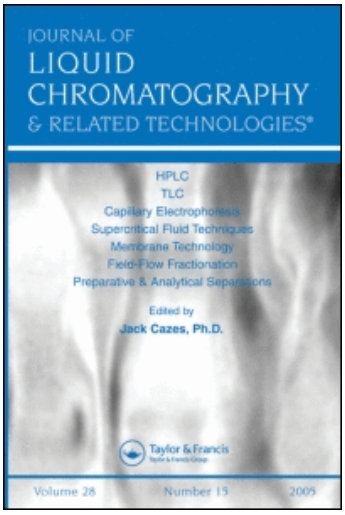

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Purpald (4-Amino-3-hydrazino-5-mercapto-1, 2, 4-triazole) as a Reagent for Post-Column Derivatization of Neutral Monosaccharides in High Pressure Liquid Chromatography

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PURPALD (4-AMINO-3-HYDRAZINO-5-MERCAPTO-1,2,4-TRIAZOLE) AS A REAGENT FOR POST-COLUMN DERIVATIZATION OF NEUTRAL MONOSACCHARIDES IN HIGH PRESSURE LIQUID CHROMATOGRAPHY

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ABSTRACT

A comprehensive study of the reaction between Purpald (4-amino-3- hydrazino-5-mercapto-1,2,4-triazole) and eight neutral monosaccharides in order to establish the optimal conditions (wavelength, oxygen supply, temperature, time, pH and concentration range) was carried out. The reaction was applied to the post-column detection in HPLC of monosaccharides eluted with water from an ion-exchange column. The detection limits and linear determination ranges thus achieved make this reaction a valid alternative to the determination of carbohydrates.

INTRODUCTION

The (4-amino-3-hydrazino-5-mercapto-1,2,4-triazole), that is comercially known as Purpald, has been used for a long time to visualize carboydrates' spots in TLC and as

a reagent for carbonyl groups which it forms violet derivatives with. Jacobsen et al.[1] used Purpald to analyse for aldehydes by UV-visible absorption spectrophotometry. More recently, the reagent has been employed for determining the number of carbon atoms in simple aldoses[2] and of inverted sugar[3], both with photometric detection.

Till now studies about the possibility of using this reagent for detection of sugars in HPLC have not been made.

After several previous assays we have considered convenient to assay it for post-column derivatization, for that, firstly, all the experimental variables that can have influence on the reaction between Purpald and several monosaccharides have been examined and optimized, after that, the optimized reaction parameters have been applied to the detection of those carbohydrates previously separated by HPLC.

EXPERIMENTAL

Reagents

Sugar standards and Purpald were supplied by Sigma Aldrich Química S.A. (Madrid, Spain). All other chemical used including oxidants and eluent solvents were pro analysis grade purchased from Scharlau (Barcelona, Spain).

Apparatus and Chromatographic Conditions

Batch measurements were made on a PU 8710 UV-visible spectrophotometer from Philips Scientific (Cambrige, UK), equiped with cells of 1-cm pathlength.

The set-up used for continuous assays was composed of the following elements (Fig. 1):

— Pump 1: a CM400 multiple solvent delivery system from Milton Roy (Riviera Beach, FL) that was used to propel the chromogenic reagent. Eluant A: a solution

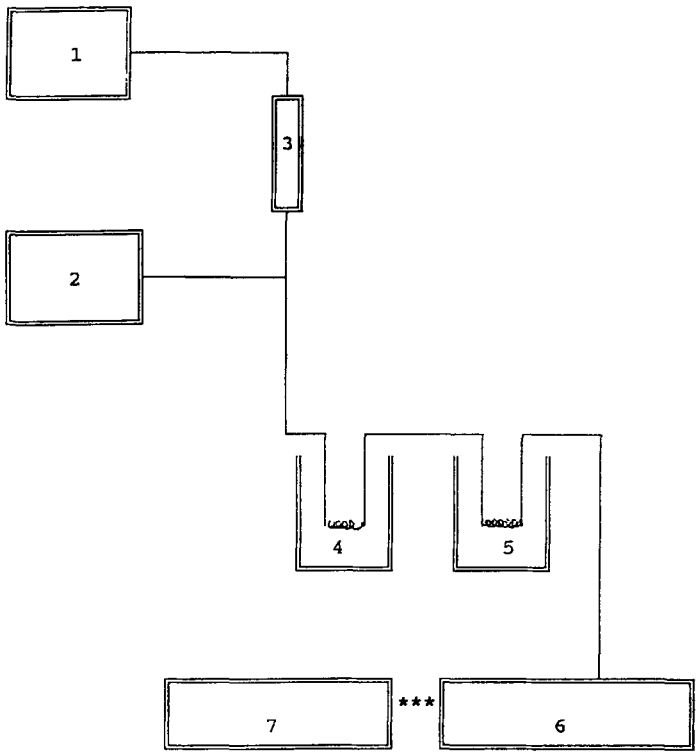

Figure 1 – Experimental set-up used. (1) Pump 1. (2) Pump 2. (3) Column. (4) Reaction bath (90°C). (5) Cooling system (0°C). (6) Detector. (7) Recorder–integrator.

containing 4000 ppm Purpald in 2 M NaOH, eluant B: 0.04 M H_2O_2; 70% A and 30% B, at a flow-rate of 0.4ml/min.

— Pump 2: a 6000A dual piston pump from Waters Associates (Milford, MA) that was employed to propel the eluent (nanopure water) at a flow-rate of 0.8 ml/min.

— Injector: a Rheodyne 7125 model with a fixed-volume loop of 20 μl.

— Column: a 30 cm × 7.8 mm Aminex HPX-87P cation-exchange column from Bio-Rad Labs (Richardson, CA) that was thermostated at 85°C in an oven from Jones Chromatography Ltd (Hengoed, UK).

— Reaction bath: sugars eluted from the column were mixed with the reagent

propelled by Pump 1 in order to have the reaction develop along a 20-m piece of Teflon tubing of 0.3 mm i.d. which was immersed in a Precis-Term S-386 controlled-temperature bath from Selecta (Madrid, Spain) that was filled with water at 90°C.

— Cooling bath: after the reaction was completed, the mixture was cooled by passage through an ice bath.

— Detector: an SM 4000 variable-wavelength UV-visible spectrophotometer from Milton Roy (Riviera Beach, FL). Detection was carried out at 550 nm.

— Recorder-Integrator: a CI 4000 model, also from Milton Roy.

RESULTS AND DISCUSSION

Purpald reacts with carbonyl compounds as shown in Fig. 2. The reaction takes place in a basic medium in the presence of oxygen and yields a violet compound that absorbs most strongly in the region of 545–550 nm[1].

The reagent is soluble in both acid and alkaline media, yet its solutions decompose with time, which calls for storage in an inert gas atmosphere — for this purpose, helium was used-.

As can be seen in Fig. 3, where the results obtained by mixing 6 mg/l formaldehyde, excess Purpald (100 mg/ml) and different concentrations of NaOH are plotted, the best pH for the reaction was that provided by 1 M NaOH.

Batch Study of the Reaction between Purpald and Carbohydrates

Mono-, di- and trisaccharides have been previously studied, but the results obtained with di- and trisaccharides were not successful due to the absence of free carbonyl groups, because of that the aim of this study is the monsaccharides.

The reaction between Purpald and carbohydrates was found to require the presence of oxygen —whether as a bubbling gas or introduced via an oxidant— and heating. The optimal working conditions for development of the reaction were as follows:

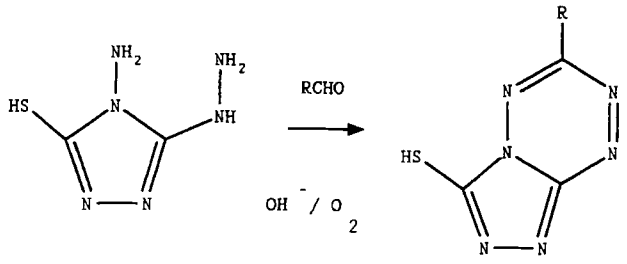

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Figure 2 – Reaction scheme between Purpald and carbonyl compounds.

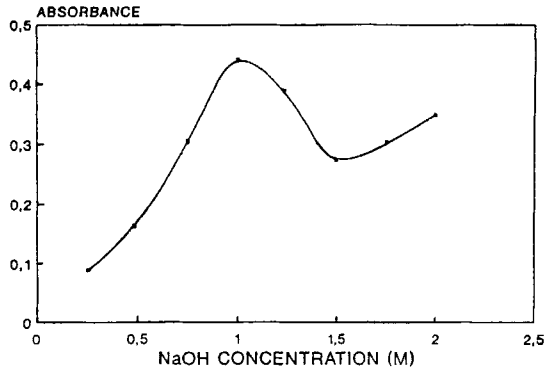

Figure 3 – Variation of the absorbance with the NaOH concentration in the reaction between Purpald (100mg/l) and formaldehyde (6mg/l).

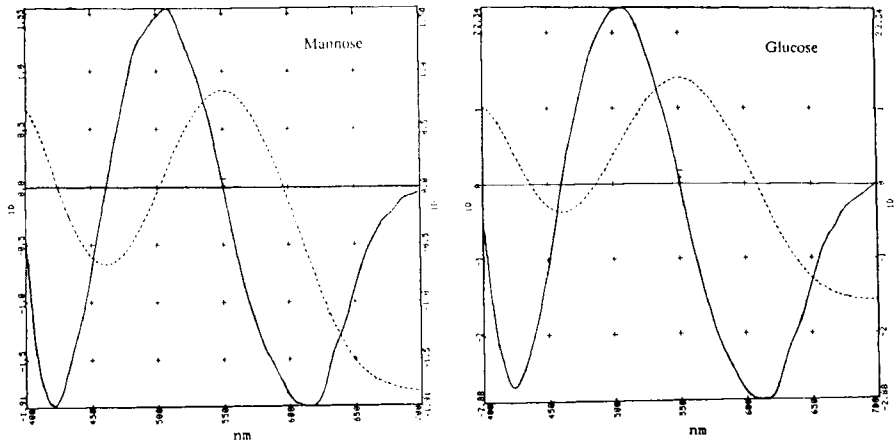

Figure 4 – UV-visible molecular absorption spectra of the derivatives of some sugars. (- - -) First derivative spectra. (---) Absorption spectra.

Wavelength: The products of the aforementioned reaction were violet coloured and showed high absorption between 500 and 600 nm, with a maximum at 550 nm (Fig. 5).

Amount of reagent: The results obtained by reacting 40mg/l of glucose in 1 M NaOH with Purpald, heating and bubbling of oxygen are shown in Fig. 4. As can be seen, the absorbance increased gradually to a constant level at Purpald concentrations above 1.5 g/l. By assaying other glucose concentrations and carbohydrates we came to the conclusion that the optimal concentration of Purpald to be used was 2 g/l.

Oxygen supply: As stated above, the reaction requires the presence of oxygen to carry out. This was initially supplied by bubbling air through the reaction medium. However, with a view to the subsequent application in HPLC post-column detection, the oxygen supply was obtained from an oxidant solution. Out of the various oxidants assayed, potassium periodate, potassium peroxydisulphate and hydrogen peroxide proved to be the most efficient —particularly the last, which provided the greatest absorbance values–.

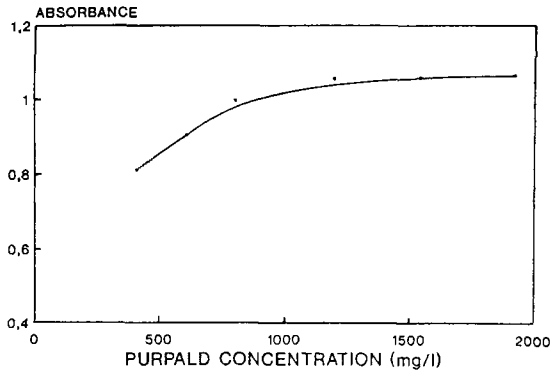

Figure 5 – Variation of the absorbance with the Purpald concentration for glucose (40mg/l in NaOH 1M).

Combined effect of the temperature, time and oxidant concentration: In order to determine the joint effect of these three variables, a series of experiments involving a sugar concentration of 40 mg/l and the amount of Purpald required to obtain a 2 g/l concentration were performed. Aliquots of 2, 5, 10 and 15 ml of a 0.04 M H_2O_2 solution were added and the mixture was completed with 1M NaOH to a final volume of 50 ml. The temperatures assayed were 70, 80 and 90°C, and the reaction times ranged between 2 and 20 min.

Increasing temperatures decreased the time required for the absorption maximum to be reached. This was so for all eight monosaccharides studied, but the higest signals for an identical amount of sample were given by fructose, ribose, arabinose and xylose.

Figure 6 shows the three-dimensional plots obtained for arabinose at the three temperatures assayed. The best results were obtained at 90°C on account of the fairly short heating time required to obtain high signals (3–5 min) by using 2ml of a 0.04M H_2O_2 solution.

Quantitative variables: A calibration graph for each carbohydrate was obtained, by using different amounts of the monosaccharide that were added 2 ml of 0.04 M H_2O_2 and the amount of Purpald required to obtain a concentration of 2 g/l, the mixture being

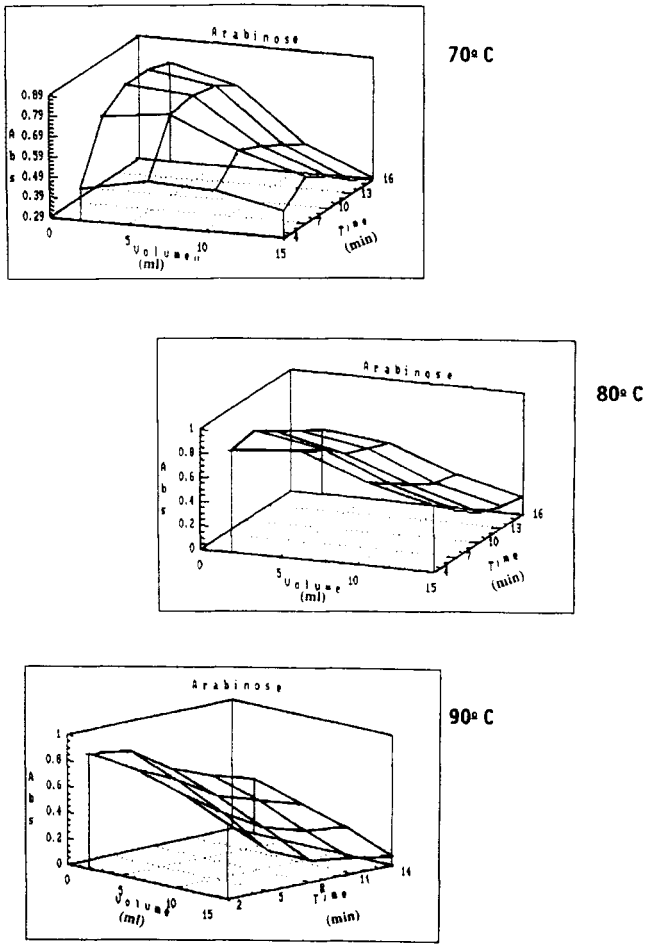

Figure 6 – Variation of the absorbance with the heating time and oxidant (H_2O_2) concentration at various temperatures.

TABLE 1
Detection Limits and Linear Determination Ranges for some Carbohydrates

Carbohydrates	Determination limits (ng)	Linear range of concentrations (ng)
Xilose	30	20-500
Mannose	80	60-500
Rhamnose	80	70-500
Glucose	70	50-500
Fructose	20	20-500
Galactose	50	40-500
Arabinose	30	20-500
Ribose	15	20-500

made to 50 ml with 1 M NaOH. After heating at 90°C for 5 min, the mixture was allowed to cool and its absorbance measured at 550 nm.The results obtained are listed in Table 1. As can be seen, the detection limits are fairly low and the linear determination ranges are quite wide. We should emphasize that Beer's law is obeyed throughout the ranges, so the absorbances of the different carbohydrates are additive and the overall carbohydrate content of a mixture can be readily determined.

Application of the Reaction to Post-column Derivatization in HPLC

After the reaction between carbohydrates and Purpald was optimized, it was applied to the post-column derivatization in order to evaluate the eluted carbohydrates by molecular absorption spectrophotometry at 550 nm.

Because of the dilution resulting from joining the column eluate and the reagents, somewhat higher reactant concentrations, *viz.* 0.08 M H_2O_2, 4 g/l Purpald and 2 M NaOH were used.

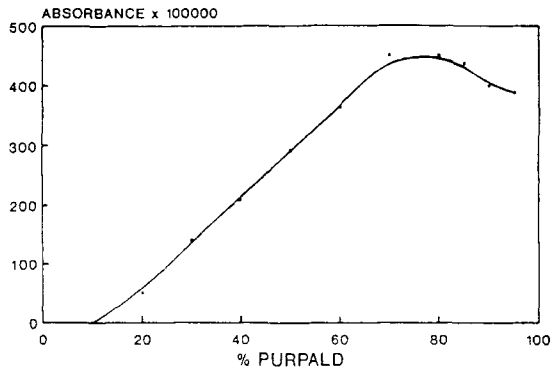

Figure 7 – Variation of the absorbance for ribose (50mg/l) as a function of the chromogenic reagent composition.

The results obtained by injecting a 50 mg/l ribose solution and varying the composition of the chromogenic reagent are shown in figure 7. As can be seen the highest signal was obtained using a 70% Purpald in NaOH 2M and 30% H_2O_2.

The appropiate reaction temperature and time were accomplished by using an eluent and reagent flow-rate of 0.8 and 0.4 ml/min, respectively. The mixture was conveyed into a teflon capillary 20m long and 0.3 mm i.d. that was stitched through a steel net with mesh openings to avoid peak broadening as far as possible[4]. It was immersed in a thermostated bath at 90°C. Then, it was cooled and driven to the detector cell.

We should note that the initial column used to separate the carbohydrates (an Aminex A-25 anion-exchange column in borate form) was eventually discarted because of the difficulty involved in obtaining an appropriate pH for the reaction by using a buffer of pH 9.35 as eluent. Such an optimal pH was most readily obtained by using an Aminex HPX 87-P column in Pb form - although mannose and rhamnose are not separated in this column-, which was employed with nanopure water as eluent.

Application of the proposed procedure to a standard mixture of sugars (50mg/l in each) provided good resolution and sensitive detection (Fig. 8).

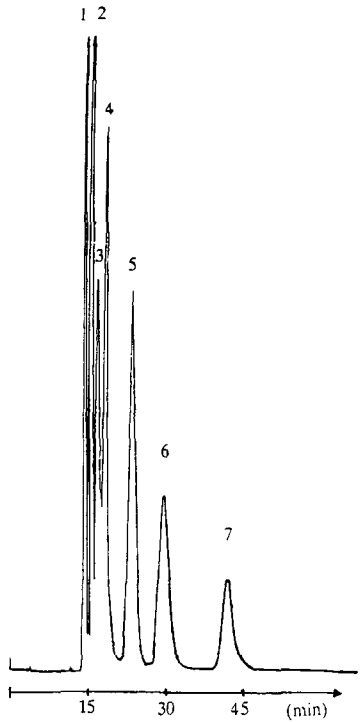

Figure 8 – Chromatogram obtained for a mixture of monosaccharides (50mg/l in each). 1-Glucose, 2-Xylose, 3-Galactose, 4-Mannose, 5-Arabinose, 6-Fructose, 17-Ribose

CONCLUSIONS

The reaction between Purpald and carbohydrates can be carry out quantitatively, which allows the analyses of both individual sugars and carbohydrate mixtures. The reaction can be applied to the post-column derivatization of carbohydrates previously isolated by HPLC using an appropriate ion exchange column. The results obtained in this respect make Purpald a useful alternative to other reagents employed for this purpose.

ACKNOWLEDGEMENT

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REFERENCES

1. N.W. Jacobsen and R.G. Dickinson, Anal. Chem., 46: 298-299 (1974).

2. E. Humeres, F. Nome and R. Aguirre, Carbohydr. Res., 46 284-288 (1976).

3. E. Reinefeld, K.M. Bliesener, H. Van Malland and C. Reichel, Zucker, 29 308-316 (1976).

4. B. Lilling and E. Henz, "Reaction Detection in Liquid Chromatography", in "Chromatographic Science" I.S. Krull, eds., Marcel Dekker, INC., N.Y.(1986), Vol. 34, pp. 27

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