Journal of Chromatography, 537 (1991) 494–496
Elsevier Science Publishers B.V., Amsterdam

CHROM. 22 788

Note

Localization of amino acids on thin-layer chromatograms with acetylacetone–formaldehyde reagent

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(First received November 16th, 1989; revised manuscript received August 31st, 1990)

Various reagents have been suggested for revealing amino acids on thin-layer chromatogram [1]. Some of these are specific whereas others give coloured spots with most amino acids.

Although ninhydrin comes closest to the ideal detection agent, showing reactivity and sensitivity towards most amino acids, the colour formed is unstable [2,3]. It gives a faint yellow colour with proline and hydroxyproline [1,4]. Only under controlled conditions of pH and temperature and in the presence of organic solvents having maximum water content does the colour intensity approach 100% [5,6]. It was therefore, considered of interest to develop a new reagent for the localization of amino acids on thin-layer chromatographic (TLC) plates.

It was reported earlier that primary amines react with acetylacetone–formaldehyde reagent to produce yellow products in aqueous medium [7]. This observation has been utilized to detect the amino acids on thin-layer chromatograms.

EXPERIMENTAL

Glass plates (10 × 20 cm) were coated with an aqueous slurry of silica gel [BDH, Glaxo Laboratories (India)] using a Desaga spreader. The plates were dried in an oven at 105 ± 2°C for 30 min.

Reagent

Formaldehyde [37% (w/v); 15.0 ml] was mixed with freshly distilled acetylacetone (7.8 ml) and diluted to 100 ml with acetate buffer solution (pH 4.7) [8]. A freshly prepared reagent solution was used.

Standard solution of amino acids

Amino acids (0.1 mmol) were weighed accurately and dissolved in and diluted to 100 ml with water. A freshly prepared solution was used.

0021-9673/91/$03.50

TABLE I

DETECTION OF AMINO ACIDS ON A TLC PLATE WITH ACETYLACETONE–FORMALDEHYDE REAGENT

Amino acid	Detection limit (10^{-4} mmol)		
	Proposed reagent		Ninhydrin reagent [1]
	Visible light	UV light	
Glycine	1.3	0.26	0.13
L-Lysine·HCl	1.6	0.27	0.27
L-Arginine·HCl	2.3	0.47	0.47
L-Tyrosine	5.5	0.55	1.6
L-Leucine	2.2	0.60	0.76
DL-Isoleucine	6.0	0.68	–
DL-Valine	6.8	0.76	0.85
DL-Serine	4.7	0.95	0.76
L-Cystine	2.0	1.2	–
L-Glutamic acid	2.7	1.3	2.7
DL-Methionine	3.3	1.3	0.67
L-Histidine·HCl	3.1	1.5	2.6
DL-Tryptophan	9.7	1.9	2.4
DL-Alanine	6.7	2.2	1.0
DL-Threonine	4.1	2.5	4.1
L-Ornithine·HCl	17.0	2.9	–
Hydroxyproline	7.6	3.0	3.8
L-Aspartic acid	15.0	6.0	7.5
L-Asparagine	22.0	6.0	7.5
L-Proline	26.0	6.9	8.6

Thin-layer chromatogram

Appropriately diluted standard amino acid solution (10.0 μl) was spotted on the plate. After evaporation of solvent, it was developed with *n*-butanol–acetic acid–water (40:5:7) [9] and dried at room temperature. The plate was sprayed evenly with the reagent solution and heated in an oven at 100 ± 2°C for 10 min. The spots were examined under both visible and UV light (UVK-125-254). The results are recorded in Table I.

RESULTS AND DISCUSSION

The proposed reagent gives well defined, stable, yellow spots with all the amino acids. The spots are more easily detected under UV than visible light. The limit of detection of amino acids was found to be in the range $0.26 \cdot 10^{-4} - 6.9 \cdot 10^{-4}$ mmol and $1.3 \cdot 10^{-4} - 26 \cdot 10^{-4}$ mmol amino acid under UV and visible light, respectively.

The sensitivity of the reagent was compared with that of ninhydrin reagent [1] (Table 1). The reactivity of the proposed reagent with tyrosine, leucine, valine, glutamic acid, histidine, tryptophan, threonine, proline, hydroxyproline, asparagine and aspartic acid is distinctly superior to that of ninhydrin.

A study of the effect of pH on reaction showed that the maximum colour yield is obtained at pH 4.7.

The effect of temperature on rate of colour development was studied. The colour intensity increased with increase in temperature. Heating of the TLC plate (after spraying with the reagent) at $100 \pm 2°C$ for 10 min was found to be most suitable. The spot was remained stable for more than 24 h.

In the proposed procedure, amino acids seem to react with acetylacetone–formaldehyde reagent to form N-substituted 3,5-diacetyl-1,4-dihydrolutidine, which gives a yellow colour in visible light and fluorescence under UV light.

The reagent is also applicable to the detection of small peptides. The product is suitable for their densitometric determination.

ACKNOWLEDGEMENT

The authors thank Cadila Labs., Ahmedabad, India, for the gift of samples of amino acids.

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